Observations in man and animals on the distribution and excretion of sodium and potassium.

Published papers submitted for the degree of Doctor of Science

by

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NOTE ON AUTHORSHIP OF PAPERS

The work described in the series of published papers was started by me in 1950 in Sheffield when radioactive isotopes of sodium and potassium first became available in Britain. It was continued during the academic year 1952-53 in Boston where I was a visiting research worker in the Harvard Medical School and resumed in Sheffield in 1953 on appointment to my present post.

The research was carried out with the assistance of several different colleagues over a period of thirteen years. This co-operation has been essential in work involving short half life isotopes, particularly in man when the doses are restricted and the numerous chemical manipulations and radioactive counting have to be completed within a few hours. All the investigations carried out in Sheffield were initiated and supervised by me and I have played a full part in all the laboratory work.

The research described in papers 10, 11, 12, 18 and 19 represents work done in conjunction with American colleagues. In papers 10, 11, 18 and 19 mine was the major contribution and I was responsible for writing the first draft. I played an extensive practical part in the investigation described in paper 12 but did not write the first draft.

SUMMARY OF PUBLISHED WORK

The work described in the papers submitted was begun in 1950 when radioactive isotopes of sodium and potassium first became available for biological research. The initial investigations were made with ^{24}Na and methods were developed leading to the measurement of the amount of sodium in the human body that was free to exchange with the isotope (1). This afforded a means of ascertaining the amount of metabolically active sodium in the body and is commonly referred to as "exchangeable sodium" or Na_E. During the course of this work it was found that there was a large amount of sodium in bone but that about 75 per cent was not available for exchange (2, 3). This was confirmed by the use of the long half-life isotope ^{22}Na (4). The significance of the large amount of sodium in bone could not be investigated in man but studies in rats under various experimental conditions showed that in states of acute depletion sodium was

withdrawn from the bones especially in young animals (5, 6). This work demonstrated that ^{22}Na is not safe for use in man as a small amount may be retained in bone for a long period (7).

The amount of exchangeable potassium in the body can likewise be measured with the isotope ^{42}K. Practically all the potassium in animals was available for exchange (8). When exchangeable sodium and potassium are measured simultaneously, as is often required, special techniques must be developed because the half lives of the isotopes ^{24}Na and ^{42}K (15.0 and 12.3 hours) are close (9). The introduction of tetraphenyl boron proved extremely satisfactory for the separation of potassium from sodium in biological fluids. Rubidium was thought to have a similar distribution to potassium in the body but investigations with the isotope ^{86}Rb showed that it was not a reliable alternative to ^{42}K (8).

Previous to the use of sodium and potassium radioisotopes the only way to study the metabolism

of these electrolytes in the human body was by cumulative metabolic balance methods. In long term investigations this is extremely laborious. However, measurements of Na_E and K_E at intervals can demonstrate cumulative changes, are simpler and less time consuming, and gave results in good agreement with the balance method (10). Furthermore, the isotope techniques yielded a measure of the amounts of sodium and potassium in the body (11). The potassium content of the body was related to the lean tissue mass; in comparison with healthy males the amount of potassium was reduced in females and in those with wasting diseases. In chronic disease there was an increase in the proportion of sodium in the body and this was seen to the greatest extent in oedematous states.

Characteristic changes in sodium and potassium metabolism take place after the infliction of an injury or a surgical operation (12, 13). These consisted in a retention of sodium and an enhanced

excretion of potassium lasting over a few days. These changes are probably related to the levels of adrenocortical hormones in the blood as trauma increases adrenal secretion, but this response was detected in patients undergoing bilateral adrenalectomy and in patients with Addison's disease (adrenal insufficiency) given a constant exogenous supply of adrenal steroids during an operation (14, 15). It is now known that the blood levels are elevated by surgical operation even in these circumstances as the metabolism and excretion of the steroids are delayed.

Electrolyte metabolism may be deranged in thyroid disorders and accordingly measurements were made of exchangeable sodium and potassium in hypothyroidism and hyperthyroidism before and after treatment (16). Treatment of hypothyroidism with thyroxine led to a decrease in both Na_E and K_E due probably to a loss of myxoedematous tissue. Successful therapy of hyperthyroidism was associated with an increase in K_E

due to restoration of lost muscular tissue. Changes in Na_E were variable and not readily interpreted. In some patients there was little change but in many there was a moderate decrease in Na_E on return to health. Decalcification of the skeleton may occasionally occur in hyperthyroidism and it was considered possible that some of the difficulties in interpretation of Na_E changes might be due to alteration in bone sodium content. However, experimental studies in rats given large doses of thyroxine did not demonstrate any effects on bone sodium metabolism (17). A mild degree of cardiac failure occurs in many thyrotoxic patients and the decrease in Na_E is probably related to a loss of a small amount of latent oedema. This has been confirmed in subsequent unpublished observations.

A similar pattern of changes in body electrolyte composition, namely an excess of sodium and a loss of potassium, has already been noted in cardiac

patients (11). It was particularly evident in patients with severe mitral stenosis and after successful surgical treatment serial measurements showed a gradual restoration of the body composition towards normal (18). A delay in the excretion of ingested sodium is a recognised early feature of congestive cardiac failure and it was thought that this might be related to the increased sodium content of the body. This was investigated in dogs with experimental valvular lesions of the heart (19). Considerable changes in the ability to excrete sodium developed without any gross alterations in the amount of exchangeable sodium in the body. The reduction in sodium excretion rate could not be attributed to the dilution of infused sodium in an expanded body sodium pool. The abnormality was apparently due to a direct effect of the cardiac lesions on renal function.

The introduction of chlorothiazide, an effective oral diuretic, constituted a considerable advance in aiding the excretion of the excess of sodium present

in cardiac failure. However, chlorothiazide often caused a considerable excretion of potassium as well as sodium which is particularly disadvantageous in the depleted cardiac patient. This potassium loss was attributed to the carbonic anhydrase inhibitor activity of chlorothiazide. However, a later derivative, hydroflumethiazide, which was a negligible carbonic anhydrase inhibitor, nevertheless under certain circumstances caused a marked loss of potassium (20). The liability to lose potassium was thought not to be related so much to the choice of the thiazide diuretic as to the circumstances under which it was given. This hypothesis was tested experimentally in normal men and it was demonstrated that the extent of potassium loss following the administration of a thiazide diuretic was related to adrenal mineralocorticoid activity (21). Excessive potassium loss may be prevented by giving an aldosterone antagonist. Triamterene, a recently introduced oral diuretic enhances sodium

excretion but depresses potassium excretion; this was probably due to a direct action on the distal renal tubule (22). It may be used in conjunction with a thiazide and together they may promote a large sodium diuresis without excessive potassium loss. This recent work on the action of diuretics in relation to the excretion of sodium and potassium has been reviewed in the Bradshaw lecture (23).

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[*Reprinted from "Clinical Science."*
Vol. 12, No. 2, May, 1953.]

THE MEASUREMENT OF EXCHANGEABLE SODIUM IN MAN USING THE ISOTOPE ^{24}Na.

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Received August 5, 1952.

THE isotope ^{24}Na was originally used in clinical investigation in an attempt to measure extracellular fluid volume, and its distribution after intravenous injection was studied from this point of view by Kaltreider and his associates (14). They pointed out, however, that the radiosodium entered bone and it obviously does not afford a reliable method of measuring extracellular fluid volume, giving figures greater than those obtained by more accurate later methods such as inulin, sucrose and thiosulphate (1, 5, 22).

In 1946 Moore showed that it is possible to calculate the total amount of an element in the body with which an injected isotope exchanges (17). This method of studying body constituents by the dilution of a radioisotope has been fully described in the case of potassium (2) and its general application has recently been reviewed (9). The investigation now reported deals with the extent to which radiosodium exchanges with the normal ^{23}Na present in various fluids and tissues of the human body, the period required for reaching equilibrium and the range and reproducibility of measurements of exchangeable sodium in normal subjects. Since this work was begun other reports on this subject have been published (6, 10) and the present work confirms and extends many of these observations.

Methods.

Radio-active sodium, ^{24}Na, of half life 14·8 hr. was obtained by irradiating sodium carbonate in the nuclear reactor at Harwell. This material was free from any significant radio-active contaminants. It was dissolved in sterile pyrogen-free water to give an activity of approximately 500–700 μc. per ml. and the total sodium carbonate content varied from 0·02 to 0·17 g. per ml.. The solution was injected from a micrometer syringe† which was shown by gravimetric determinations to deliver 0·1 ml. to an accuracy of ± 0·0005 ml.. After filling the syringe the needle tip was dipped in 0·9% saline to remove any active material on its outer surface and to allow exchange within the tip. As the ^{24}Na may exchange with sodium in the glass of the syringe the standards and the injection into the subject were made in succession from the same filling of the syringe. Three standards were prepared by adding 0·1 ml. to 20 ml. of saline. For counting, the standards were further diluted

* We wish to thank Prof. E. J. Wayne for his kindly advice and interest; Dr. A. Jordan and his staff in the Biochemistry Department, Sheffield Royal Infirmary, and the staff of the Physics Department at the Radiotherapy Centre for their assistance; and Drs. R. E. Davies and H. L. Kornberg for their co-operation in the determination of sodium in bone. Part of the expense was defrayed by grants from the Tuberculosis and Medical Research Funds of the University of Sheffield, and from the Research Fund of the Sheffield Regional Hospital Board.

† Burroughs Wellcome Agla syringe.

a thousandfold so that the final dilution was 1 : 20,100. Geiger-Muller liquid counters of the type described by Veall (24) were used throughout the observations.

The dose of 0·1 ml. (50–70 μc.) was given to the subject by the following technique. A 10 ml. syringe filled with 0·9% saline was connected by a short length of rubber tubing to a needle which was introduced into the vein. The fine micrometer syringe needle was inserted through the rubber and the point passed into the base of the needle in the vein. The radio-active material was in this way washed into the vein with the 10 ml. of saline. Subsequently saline washings from the 10 ml. syringe and its rubber connection and needle were collected and any small leak at the needle mount and venepuncture site was soaked up on filter paper. The activity on these papers was estimated either by an end window counter or by soaking the activity into the saline washings which were subsequently counted by liquid counter. The total loss at injection was at most 0·2% of the intended dose.

Blood withdrawn for counting was placed in dry heparinised tubes and the plasma separated by centrifuging. Commercial samples of heparin contain sodium and accordingly serum was preferred for the total sodium estimations which were carried out on the flame photometer. No significant difference, however, was found in the sodium content of serum and plasma when a correction was made for the sodium content of the added heparin.

Plasma, urine and other fluid samples were counted directly undiluted. Portions of skin, muscle and viscera obtained at surgical operations were weighed wet and dissolved in concentrated nitric acid of the same volume in ml. as the weight of the specimen in g.. No rinsing of the operation field was carried out and no sodium-containing solution was applied to the skin or tissues before the specimens were secured. The excess of nitric acid was driven off by heating and the final volume made up to 12–15 ml. for counting. The solution was later further diluted for flame photometry. Strong acids may affect flame photometer readings in the estimation of sodium (7, 19) but in the concentration in the final solution this effect was negligible. This was confirmed by the close agreement in total sodium content found between tissue prepared in this way or minced finely in a blender without the addition of acid. Bone was dissolved in nitric acid and made up to 15 ml. for counting. Owing to the presence of excess calcium the total sodium could not be estimated directly with the available flame photometer and a more elaborate technique was developed (4). The principle is that a known amount of ^{24}Na is added to the bone solution, interfering substances are removed prior to flame photometry by precipitation and sublimation, and a correction is made by measurement of the residual radio-activity for the sodium lost in these procedures.

The radiation hazard involved with a dose of 60 μc. of ^{24}Na is small; in a patient of 60 kilogram weight it is approximately 0·11 e.r..

The counting rates of the plasma samples and of the diluted injection standards were usually between 1,000 and 2,000 counts per minute at 24 hours after the injection. These specimens were counted at least three times and often up to six times for periods of five to ten minutes each, and the results of repeated counts generally agreed to within less than $\pm$ 2% of the mean. In the urine samples the counting rates were usually lower and the reproducibility attained from similar counting times was slightly less. In the examination of tissue digests approximately 100 μc. of ^{24}Na were injected but the counting rates did not usually exceed 300 counts per minute and were often much lower, particularly when tissues containing relatively little sodium were investigated. In these cases the estimates showed greater variability but usually fell within $\pm$ 7% of the mean value.

Results.

Excretion of radiosodium.

Before any calculations can be made of the extent to which the injected isotope has been diluted in the body, allowance must be made for the fraction which has been excreted. In normal subjects the ^{24}Na is excreted chiefly in the urine which is readily collected and assayed for radio-activity. The quantity lost in this way varies considerably in subjects in sodium balance on routine hospital diet. In 50 experiments the excretion of ^{24}Na in 24 hours was between 3·8 and 12·2% of the injected dose, with a mean of 7·3%. The total sodium excretion in the same period varied between 74 mEq. and 296 mEq., with a mean of 173 mEq.. In health, fæcal excretion of radiosodium in a similar period should be negligible and this was confirmed in two experiments. In the first, the excretion was measured in a healthy subject after intravenous injection of ^{24}Na; in 24 hours 0·01% of the dose and in 48 hours 0·12% was excreted in the fæces. In the second experiment an attempt to assess the maximal probable loss by this route was made by investigating a case of ulcerative colitis passing liquid, but not blood-stained, stools. In this case in spite of the diarrhœa the excretion during the 24 hours after the injection was only 0·22% of the dose.

The loss of radiosodium through the skin cannot so readily be estimated. In the investigation of Freyberg and Grant (11) the excretion of sodium through the skin in two subjects lightly clad and carrying out laboratory duties but taking care to avoid any visible sweating was between 2·6 mEq. and 9·1 mEq. in 24 hours. The higher figure represents approximately 5% of the mean urinary excretion of sodium in our experiments. If it is assumed that sweat and urine have a similar specific activity, that is radio-activity per mEq. of sodium, then the cutaneous loss in 24 hours is less than one-twentieth of 7·3% of the dose, provided that sweating is minimal. This affords an approximate indication of the loss that might be anticipated under cool conditions.

It is of greater importance to estimate the largest error that is likely to arise as a result of neglecting cutaneous loss during hot weather. Accordingly an attempt was made to measure the excretion in the sweat on a warm summer day (highest day temperature 85°F.) in one normal subject who for the 24 hours after the injection remained clothed in socks and pyjamas, refrained from washing, and absorbed on a towel any sensible perspiration. He then bathed in 11·2 l. of water in which his clothing, towel and bed linen were also soaked. A sample of the water measured for radio-activity showed that the loss in the sweat in this case was 0·6% of the dose.

It is apparent that the extrarenal loss of radiosodium in the 24 hours after the injection is extremely small and in the calculations that follow account has only been taken of radio-activity lost through urinary excretion. The error introduced in this way in a 24-hour equilibration period is negligible.

Rate of establishment of equilibrium of distribution of ^{24}Na.

Intravenously injected ^{24}Na rapidly leaves the vascular system and the concentration in the plasma falls precipitately during the initial phase of spread into the extracellular fluid and later more slowly as the ^{24}Na penetrates into the less readily accessible fluid compartments and tissues. It is apparent that progressive dilution of the ^{24}Na that is not excreted will continue until an equilibrium is established with that portion of the body sodium which is exchangeable. Thereafter, apart from loss by radio-active decay for which allowance is made in all the calculations,

decreases in the concentration of ^{24}Na are due to excretion alone. The amount of sodium with which the ^{24}Na has exchanged at any time after the injection may be calculated as:—

$$\text{Sodium exchanged} = \frac{^{24}\text{Na injected} - ^{24}\text{Na excreted}}{^{24}\text{Na}/^{23}\text{Na per unit volume of plasma}}$$

Measurements made in this way at intervals of between 5 minutes and 24 hours after injection are shown in Fig. 1. There is a rapid increase in the amount of sodium exchanged during the first hour and thereafter a slower rise for 12 hours. During the 12 to 24-hour period there is relatively little change in the distribution,

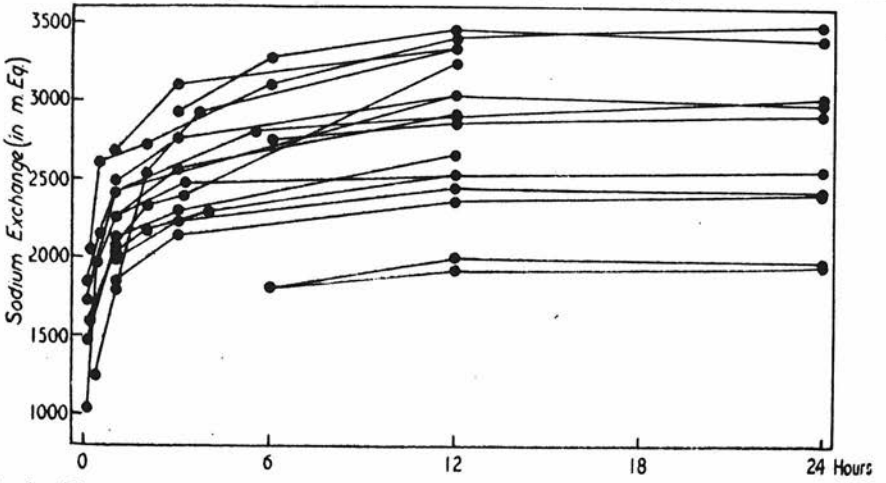

Fig. 1. Measurements of the amount of body sodium with which ^{24}Na has exchanged between 5 minutes and 24 hours after intravenous injection. Exchange is rapid during the first 3 hours and continues slowly up to 12 hours. Thereafter there is little change.

suggesting that equilibrium has been practically established after 12 hours. To confirm this, the exchangeable sodium has been measured both at 12 and 24 hours in 50 subjects either healthy or suffering from conditions in which no disturbance of sodium distribution might be anticipated. The divergences between the 12- and 24-hour observations were both positive and negative and distributed about a mean of + 0·65% with a standard deviation of ± 3·3%. It is evident that though an increase in the total exchangeable sodium between 12 and 24 hours may be real, the errors in our measurement are too big to reveal this and the difference between the readings at 12 and 24 hours is, in our case, not significantly different from zero.

In a small group of 7 cases the observations were extended up to 48 hours. In these cases (Table I) the difference between 12 and 24 hours averaged + 0·2% with a standard deviation of ± 2·75%, while between 12 and 48 hours the average change was + 1·6% with a standard deviation of ± 4·55%. Again it is not possible to detect in our results a significant increase in the total exchangeable sodium over the 12 to 48-hour period.

Exchange in tissues and body fluids.

The results so far suggest that after 12 hours the penetration of the radiosodium into the fluids and tissues of the body is practically complete. The extent to which exchange has occurred is determined by comparing their specific activities with that of plasma. The specific activity is the ratio $\frac{^{24}Na}{^{23}Na}$ per unit volume of fluid or

TABLE I.

Comparison of measurements made 12, 24 *and* 48 *hours after intravenous administration of* ^{24}Na, *of plasma sodium and total exchangeable sodium (TES).*

Subject	Sex	Diagnosis	12 hr.		24 hr.		48 hr.	
			Plasma sodium mEq./l.	TES mEq.	Plasma sodium mEq./l.	TES mEq.	Plasma sodium mEq./l.	TES mEq.
G.W.	M	Normal	144	3120	144	3160	141	3140
M.H.	F	,,	138	1920	142	1960	138	1880
W.W.	M	,,	141	3090	144	3160	141	3120
H.C.	M	,,	135	2520	135	2520	138	2650
E.C.	F	,,	137	2420	140	2460	137	2510
L.M.	M	,,	138	2940	138	2900	140	2800
A.W.	M	Chr. iridocyclitis	143	2800	140	2670	141	3010

tissue digest. If complete exchange has taken place the two specific activities are equal. The relative specific activity is calculated as:—

$$\frac{\text{tissue or fluid specific activity}}{\text{plasma specific activity}} \times 100.$$

This affords a measure of the percentage of sodium in the tissue or fluid which has exchanged with the ^{24}Na in the plasma. A series of results obtained in this way is shown in Table II and Fig. 2.

TABLE II.

Relative specific activity of fluids and tissues.

Sex	Age Years	Fluid or tissue	Time after ^{24}Na injection Hr.	Plasma sodium mEq./l.	Fluid or tissue sodium mEq./l. or mEq./kg.	Relative specific activity %
F	53	Gastric juice	12	142	69	103
M	50	,, ,,	12	136	78	96
M	60	,, ,,	12	133	35	111
F	47	,, ,,	24	130	98	93
F	35	,, ,,	24	134	68	97
F	65	,, ,,	24	139	68	95
F	37	,, ,,	24	142	58	112
F	34	,, ,,	24	130	54	85
M	55	Skin	12	134	57·7	98
F	32	,,	12	130	56·5	85
M	35	,,	14	133	28·3	95
F	8	,,	16	136	19·4	97
M	67	,,	21	133	61·0	100
M	66	,,	24	137	57·3	89
M	65	,,	24	132	80·5	94
F	32	Muscle	12	130	38·4	105
M	55	,,	12	134	29·6	97
M	35	,,	14	134	17·7	112
F	8	,,	16	136	20·1	98
F	65	,,	18	135	27·2	94
M	67	,,	21	133	23·8	101
M	65	,,	24	132	29·9	83
M	66	,,	24	137	22·1	105
F	65	Spleen	18	134	41·7	95
M	66	,,	24	137	43·7	93
M	55	Rib	12	134	216	26
F	36	,,	12	140	249	24
M	35	,,	14	134	284	23
F	8	,,	16	136	200	32
F	65	,,	18	134	341	23
M	67	,,	21	133	283	22
M	65	,,	24	132	240	25
M	66	,,	24	137	254	24
M	26	,,	24	134	208	31
M	36	CSF	12	140	144	63
F	52	,,	12	140	140	80
M	56	,,	12	140	146	66
M	40	,,	12	132	145	53
F	48	,,	17	139	140	84
F	34	,,	24	138	152	88
F	16	,,	24	140	145	96
M	36	,,	24	135	142	84
F	32	,,	24	138	141	94
M	55	,,	28	140	140	90
F	62	,,	36	141	148	94
F	53	,,	36	140	142	105

Gastric juice.

This was obtained either 12 or 24 hours after the intravenous injection of the ^{24}Na, the subject fasting for the twelve hours previous to the passage of the stomach tube. It will be seen that exchange is complete within the experimental error of the method at both times of examination.

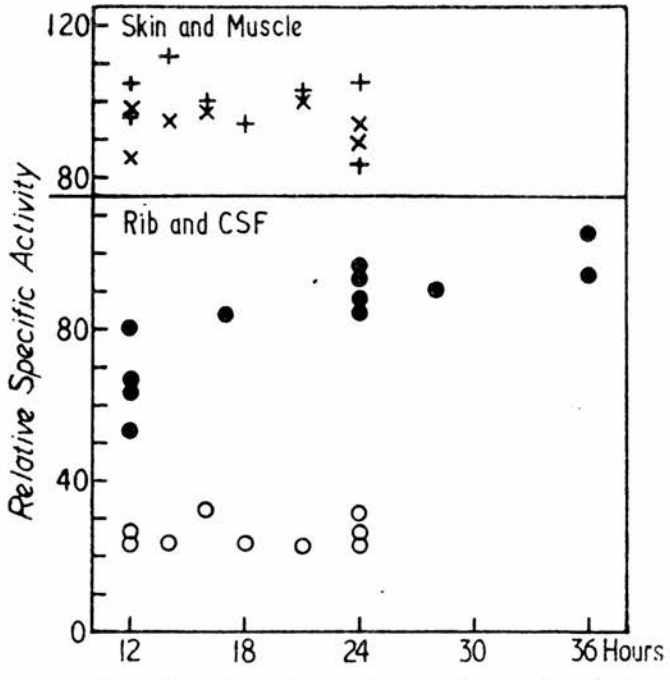

Fig. 2. The relative specific activity of muscle +, skin ×, lumbar cerebrospinal fluid ● and rib ○ after intravenous injection of ^{24}Na. Only in the cerebrospinal fluid is there a progressive increase after 12 hours.

Skin and muscle.

These tissues, in about 2 g. amounts, were obtained at surgical operations carried out during the period 12 to 24 hours after the injection. The counting rates were low, especially in the muscle digests, but here again the evidence suggests that exchange does not progressively increase in the 12 to 24-hour period and is complete 12 hours after the injection. The results in two specimens of normal spleens removed in high gastrectomy operations are similar.

Bone.

The ribs, obtained at thoracotomy operations, were split open and the marrow completely removed by scraping and wiping with filter paper. About 4 g. of the cortical portion of the rib were taken for solution in nitric acid. The results show that during the 12 to 24-hour period only between 22 and 32% exchange occurred. Furthermore, with the passage of time during this period the extent of the exchange did not increase and it appeared that after 12 hours equilibrium had been established with all the bone sodium available for exchange. It will be seen that though the total sodium content varied between 200 and 341 mEq./kg. of bone, the proportion exchangeable was remarkably constant.

TABLE III.

Measurements of total exchangeable sodium in normal subjects.

Authors	Males				Females			
	No. of subjects	Mean mEq./kg.	Range mEq./kg.	SD	No. of subjects	Mean mEq./kg.	Range mEq./kg.	SD
Miller and Wilson	10	43·7	39·5–47·9	3·16	6	42·3	39·4–44·8	2·26
Forbes and Perley (10)	25 (29 observations)	41·9	32·3–54·1	5·60	7	39·5	35·7–41·6	2·29
Edelman *et al.* (9)	11	41·4	36·1–46·0	—	3	41·0	31·4–45·9	—

Cerebrospinal fluid

This was obtained at lumbar puncture 12 to 36 hours after the injection of ^{24}Na in patients being investigated for suspected disease of the nervous system. No cases have been included in which either the pressure or chemical composition of the fluid was abnormal. Exchange proceeds slowly in comparison with the other tissues and fluids so far examined, being approximately 65% complete at 12 hours and 90% at 24 hours.

Measurement of total exchangeable sodium.

The evidence so far presented suggests that, apart from the cerebrospinal fluid, an equilibrium of distribution has been established twelve hours after the injection of the isotope. At this time the amount of sodium unexchanged in the cerebrospinal fluid is extremely small in relation to the amount of sodium in the body. Measurements of the total exchangeable sodium in a healthy subject may accordingly be made at any convenient time longer than 12 hours after the injection. The mean of the 12- and 24-hour observations has been considered in this report as the best value for total exchangeable sodium. The 48-hour observations are technically more difficult on account of the rapid decay of the tracer material.

The results in a series of normal male and female subjects calculated from the means of the values obtained from the 12- and 24-hour plasma samples are shown in Table III, and are compared with two other published series. The standard deviations of the observations are quoted where available. These results do not reveal a statistically significant difference between males and females, or between the results of the separate observers except possibly between the results on females reported here and those reported by Forbes and Perley ($t = 2{\cdot}28$, $p = 0{\cdot}04$).

The reproducibility of the results was determined by making the measurements in the same subject on two separate occasions. The normal subjects continued with their usual activities in the hospital after the radiosodium injection. The reproducibility of the observations was also determined in a series of in-patients suffering from mild chronic iridocyclitis without any apparent general constitutional disturbance. When the measurements were repeated within a few days, a specimen of plasma was taken immediately before the second injection and assayed for total sodium and ^{24}Na activity in order that the necessary blank corrections might be made. For injections following each other at two-day intervals the correction for the first dose amounted to about 10% of a second dose of similar activity. After more than five days the correction was negligible.

The error in a single observation of the mEq. per kilogram includes both counting errors and flame photometer errors. The former is less than 2% and the latter of the order of 2%. Additional errors are present in comparing the values obtained at 12 and 24 hours but the standard deviation of the difference between the two is, as has been shown, 3·3%. In a series in which duplicate tests were made over a period on one individual (Table IV) the total exchangeable sodium deduced from the two tests did not differ by more than 6·7%, the range of these differences being from 4·7% to 6·7%.

The total exchangeable sodium may be calculated from the specific activity of any body fluid in which exchange between the two forms of sodium is complete. Urine is a convenient alternative to plasma and the results have accordingly also been calculated from the radio-activity and sodium concentration in the 12 to 24-hour urine. To compare them with the mean values obtained from the 12- and 24-hour plasma samples, the total renal excretion of ^{24}Na up to 18 hours has been taken as (^{24}Na in 0–12-hour urine) + ½ (^{24}Na in 12–24-hour urine). This introduces a slight error as the rate of radiosodium excretion is not constant during the second 12-hour period. As, however, the total excretion during this nocturnal period

TAB

Reproducibility of measurements

Subject	Date	Diagnosis	Sex	Age yr.	Weight kg.	Calculations from mean of 12- and 24-hr. plasma samples: Plasma sodium mEq./l.	TES mEq.	TES mEq./kg.
J.C.	20.11.51	Normal	M	21	63·5	139	2880	45·4
	13.12.51				63·5	140	2970	46·8
M.T.	22.11.51	Normal	M	21	58·5	139	2310	39·5
	6.12.51				58·5	136	2330	39·9
R.A.	4.12.51	Normal	M	23	78·0	136	3280	42·1
	20.12.51				78·0	138	3440	44·1
G.W.	19.2.52	Normal	M	34	76·7	144	3140	40·9
	13.3.52				77·1	140	2940	38·1
L.M.	25.6.52	Normal	M	26	60·2	139	2920	48·4
	1.7.52				60·6	141	2860	47·4
T.H.	13.11.51	Chronic	M	30	61·0	137	2560	41·9
	15.11.51	iridocyclitis			61·0	136	2570	42·0
A.W.	7.2.52	Chronic	M	32	57·6	141	2690	46·7
	12.2.52	iridocyclitis			57·3	142	2740	47·8
A.S.	4.9.51	Chronic	M	47	74·5	137	3450	46·3
	6.9.51	iridocyclitis			74·5	137	3390	44·5
J.B.	14.2.52	Normal	F	29	53·5	140	2420	45·2
	21.2.52				53·5	140	2380	44·5
M.H.	14.2.52	Normal	F	16	41·1	140	2000	48·5
	19.2.52				40·5	140	1950	48·1
M.B.	11.3.52	Normal	F	39	57·4	141	2310	40·4
	18.3.52				57·2	138	2240	39·2
E.W.	19.6.51	Chronic	F	40	66·6	140	2940	44·1
	21.6.51	iridocyclitis			67·1	136	2900	43·2

is usually only about 2–3% of the dose the error is insignificant. In general, good agreement is found between values calculated from plasma and urine in this way as may be seen in Table IV. If reliance has to be placed on measurements made from urine alone it is more satisfactory to take two or more spot specimens after 24 hours rather than to use the bulked 12–24-hour urine. Measurements with plasma are, however, preferable, as the sodium content is more readily measured, the counting rates are higher, and the degree of reproducibility is greater.

While these tests were carried out the subjects ate a normal diet and did not modify their usual salt intake. Variations in sodium turnover as indicated by urinary excretion of sodium in the 24-hour equilibration period did not appear to have any effect on the reproducibility of the measurements of total exchangeable sodium (Table IV). The mean 24-hour urinary sodium excretion in this series of 18 observations represented 6·5% of the total exchangeable sodium; the greatest excretion was 10%. Even when there was a considerable difference in sodium excretion on the two occasions of the test the measurements of total exchangeable sodium did not differ significantly and demonstrated that an increased output of sodium did not interfere with the diffusion of the radiosodium in the body.

A comparison has been made in four normal subjects between the oral and intravenous routes of administration of ^{24}Na. The radiosodium was given in the morning after the subject had fasted overnight and measurements were made 12 and 24 hours later. No significant difference between the two routes was found in the results which are summarised in Table V.

IV.

total exchangeable sodium (TES).

Calculations from urine in 12 to 24-hr. period			Total sodium excretion in 24 hours	Difference of 2nd plasma TES calculation from 1st	Difference of 2nd urine TES calculation from 1st	Difference of mean urine TES from mean plasma TES
Urine sodium mEq./l.	TES mEq.	TES mEq./kg.	mEq.	%	%	%
96	3110	49·1	221			
110	3060	48·1	294	+ 3·1	1·6	+ 5·4
81	2400	41·0	154			
86	2370	40·5	128	- 1·0	1·2	+ 2·5
124	3230	41·5	280			
100	3460	44·3	254	- 4·7	- 7·1	— 0·7
134	3110	40·6	234			
91	2800	36·4	200	6·7	10·0	— 3·5
120	2800	46·6	296			
86	2750	45·6	157	2·1	- 1·8	— 4·0
101	2540	41·6	237			
97	2510	41·2	225	+ 0·2	1·2	— 0·7
93	2520	43·7	96			
80	2800	48·8	142	+ 2·4	+ 11·0	— 1·9
—	—	—				
—	—	—		1·7	-	
54	2210	41·3	114			
72	2230	41·8	138	1·6	- 1·0	- 7·2
107	1900	46·3	174			
81	1990	49·1	84	— 0·8	- 4·7	— 1·0
—						
—				— 3·0		
—						
—				- 1·4		

Discussion.

The isotope ^{24}Na affords a convenient method of measuring the total amount of sodium in the body which is available for exchange. Equilibrium in the distribution of radiosodium between the plasma and the body fluids and tissues is nearly complete in 12 hours and after this period there is relatively little subsequent increase in total exchangeable sodium measurements. Observations cannot satisfactorily be extended beyond 48 hours with the isotope ^{24}Na in doses within the accepted tolerance limits.

The isotope ^{22}Na (half life 2·6 years) has been used for more prolonged observations by Ray and his colleagues (20) who concluded that a period of 3 to 5 days was required for complete exchange in normal subjects. There are, however, difficulties associated with long equilibration periods particularly with regard to the extrarenal loss of radiosodium and an isotope with the half life of ^{22}Na is obviously not suitable for repeated use in human subjects. A comparison of the results with ^{24}Na at different intervals after injection suggests that 12 hours is the shortest period sufficient for equilibration. In this work the calculation of exchangeable sodium has been based on the mean of two determinations made at 12 and 24 hours, the results at these time intervals in healthy subjects being in close agreement. In patients with pathological collections of fluid a 12-hour equilibration period is insufficient; in cases of pleural effusion investigated in these laboratories, exchange was not complete until 24 hours after the injection. In all cases it is clearly advantageous to base the calculations on two specimens as this affords a check

TABLE V.

Comparison of measurements with ^{24}Na given intravenously and orally. The results are the means of observations made at 12 and 24 hours.

Subject	Sex	Age years	Route	Date	Weight kg.	Plasma sodium mEq./l.	TES mEq.	Difference in TES %
M.T.	M	21	Intravenous*	22.11.51, 6.12.51	58·5	138	2320	
			Oral	13.12.51	58·5	142	2480	+ 6·9
J.C.	M	21	Intravenous*	20.11.51, 13.12.51	63·5	140	2930	
			Oral	6.12.51	63·5	136	2720	7·2
M.R.	F	30	Intravenous	16.10.51	52·1	135	1900	
			Oral	23.10.51	52·2	135	1940	+ 2·1
G.W.	M	34	Intravenous*	19.2.52, 13.3.52	76·9	142	3010	
			Oral	20.11.51	77·2	138	2890	4·0
			Oral	29.11.51	77·2	137	2920	3·1

* Means of observations on two separate occasions are shown.

that equilibrium has been attained and increases the accuracy of the determination. When only one specimen of blood is to be drawn, 24 hours is probably the equilibration period of choice as this allows a margin if exchange is unduly slow, the extrarenal loss of sodium is still small and the counting rates are sufficiently high to give accurate results.

The extent to which the sodium has exchanged in the 12 to 24-hour period has been investigated in a number of fluids and tissues. In cerebrospinal fluid taken from the lumbar region, equilibrium is not attained until at least 36 hours after the injection. Exchange in ventricular fluid is more rapid and may be complete at 11 hours (10). In the other fluids and tissues available for examination there is no evidence that further exchange takes place in the 12 to 24-hour period. In skin and muscle there is some variability in the relative specific activities but the evidence suggests that exchange is complete after 12 hours. In the muscle digest, intracellular sodium probably formed at the most about 15% of the total sodium in the specimen (18) and the results are not of sufficient accuracy to warrant definite conclusions regarding the exchange in this fraction. Bone was the only structure in which exchange was far from complete. At 12 hours, only 25% of the sodium in human rib had exchanged and this fraction did not alter significantly in the 12 to 24-hour period. The results presented here suggest that of the sodium in bone, between 70 and 80% is not available for exchange. In dogs studied with ^{22}Na over periods of several weeks Moore and his associates have demonstrated that slightly over half of the sodium in ribs is not exchangeable (8). In our results the portion of non-exchangeable sodium is rather greater than that reported by other workers (10). Measurement of the total sodium content of bone is technically difficult, but the method used in these investigations has been designed to overcome the major sources of error (4). The marrow, in which complete exchange may be anticipated, has been entirely removed from the human ribs but this is not so readily done in the ribs of smaller animals and may account for the higher exchange values reported elsewhere. In man it is probable that the only significant amount of sodium not measured by the isotope dilution technique after a 24-hour equilibration period is that firmly bound in the apatite structure of the skeleton in an insoluble mineral form (12, 13). The skeleton forms about one-fifth of the body weight (3) and contains roughly 250 mEq. of sodium per kg. of bone, of which approximately one-quarter is exchangeable. Thus, using mean values, in a man weighing 70 kg. there is 2,900 mEq. of exchangeable sodium of which 875 mEq. or just under one-third is in the bone. Little is known about the function of the sodium in the bone and its availability for general metabolism in the body in abnormal states. The proportion of non-exchangeable sodium does not appear to change in acute adrenal insufficiency in animals (23) but there is no information so far available regarding the effect of sodium depletion in other conditions.

In their measurements of total exchangeable sodium, Perley and Forbes (10) commented that radiosodium given by the oral route gave results considerably lower than those obtained by intravenous injection. They did not state whether the ^{24}Na was given on an empty stomach nor did they compare the results of oral and intravenous administration in the same subject. The sodium ion diffuses rapidly and 12 hours after intravenous injection of radiosodium complete exchange in gastric juice has taken place. The results reported here show that there is no significant difference between measurements of total exchangeable sodium made after intravenous injection or after administration by mouth with the subject fasting. Furthermore, it is apparent that by using the oral route and measuring the radioactivity and sodium content of urine, estimations of total exchangeable sodium may be made without the necessity of any venepuncture.

The free diffusion of radiosodium into the alimentary canal is of significance when an attempt is made to ascertain what portion of the exchangeable sodium is intracellular. Inulin, for example, does not enter the gastric juice or bile (15, 16) and measurements of intracellular sodium based on a subtraction of the inulin space from the sodium space are obviously inaccurate (21). Furthermore, a large part of the " intracellular " sodium measured in this way must be in the bone. More information is clearly required about the position and metabolic importance of the exchangeable sodium in bone.

SUMMARY.

The distribution of the isotope ^{24}Na in the human body after intravenous injection has been studied in relation to measurements of total exchangeable sodium.

The isotope is excreted chiefly in the urine; the extrarenal loss of ^{24}Na in the fæces and through the skin in normal subjects is less than 1% of the dose in the 24 hours after the injection.

Exchange of the ^{24}Na with the available ^{23}Na in the body is practically complete after 12 hours as shown by a comparison of measurements made after 12, 24 and 48 hours in the same individual. After 12 hours the sodium in gastric juice, skin, muscle and spleen has completely exchanged with the injected ^{24}Na. Exchange in cerebrospinal fluid obtained at lumbar puncture is slower and is approximately 65% complete at 12 hours and 90% at 24 hours. In rib only about one-quarter of the bone sodium is available for exchange. In man it is estimated that slightly under one-third of the total exchangeable sodium is situated in bone.

The mean total exchangeable sodium in males is 43·7 mEq./kg., and in females 42·3 mEq./kg.. Calculations of total exchangeable sodium made by determining the specific activity of either plasma or urine are in close agreement. The reproducibility on separate occasions was investigated in 12 subjects; the largest discrepancy in measurements using plasma specific activity was 6·7%. There is no significant difference between the results obtained after intravenous injection or oral administration of the isotope with the subject fasting.

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[*Reprinted from the Proceedings of the Biochemical Society*, 19–20 *September* 1952.
Biochem. J., 1952, Vol. 52, p. xv.]

The Determination of Sodium in Bone. By R. E. DAVIES, H. L. KORNBERG and G. M. WILSON.
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The sodium content of dissolved bone cannot be measured directly with a flame photometer. Measurements with this instrument are vitiated by an excess of strong acid, ammonia, calcium, phosphate or oxalate. Furthermore, co-precipitation of sodium occurs with the methods commonly used for the removal of calcium or phosphate; this source of error has been largely overlooked in previous methods for the determination of sodium in bone (Butler & Tuthill, 1931; Harrison, 1937; Forbes & Perley, 1951).

The principle of our method is: ^{24}Na is added to the bone solution, interfering substances are removed by precipitation and sublimation and a correction made, by measurement of the residual radioactivity, for the loss of sodium.

One part of marrow-free bone was dissolved in 2 vol. 70% A.R. HNO_3 and diluted with water to a final concentration of approx. 0·3 g. bone/ml. solution. To a sample (1 ml.) was added 0·01 ml. $^{24}Na_2CO_3$ solution corresponding to approx. 12 000 counts/min., the solution neutralized with ammonia (sp.gr. 0·88) and adjusted to pH 7 with 3 N-HCl. Excess (5 ml.) 4% (w/v) ammonium oxalate was added and, after 1 hr., the solution was filtered through Whatman no. 40 filter paper into a 25 ml. Pyrex flask. The filtrate was evaporated to dryness and heated in a muffle furnace at 280° until all ammonium nitrate and oxalate had sublimed (approx. 1 hr.). The charred residue was extracted twice with water and the extract filtered into a measuring tube (vol. of filtrate approx. 7 ml.). Approx. 2 ml. of this solution were diluted to 10 ml. with KCl (20 mg. K^+/100 ml.) and the resultant solution analysed with the flame photometer for the sodium content. The rest of the filtrate was diluted with 0·5 ml. 3 M-NaCl (as carrier) and water to 10 ml. The radioactive material was assayed with a liquid-counter tube. Analysis of a solution containing all reagents was carried out to determine the blank correction.

The loss of sodium in the analysis was determined by comparison of the initial and final radioactivities of the solutions; the results obtained by flame photometry were accordingly corrected.

By the use of this technique on pure sodium solutions and on known mixtures of calcium and sodium salts, results correct to ± 5% were obtained. The exchange of ^{24}Na with hard glass was negligible. Considerable co-precipitation of sodium with calcium occurred: over 20% of the sodium was still retained even after three washings of the precipitate, showing that the use of labelled sodium was essential to this determination.

The sodium content of human rib (10) was 216–254 (average 239) m-moles/kg. Human femur (6) contained 290–320 (average 300) m-moles/kg., and rabbit femur (16) 267–370 (average 312) m-moles/kg.

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(*Reprinted from Nature, Vol.* 170, *p.* 979, *December* 6, 1952)

Relation between Total and Exchangeable Sodium in the Body

EARLY workers, who studied the fate of sodium-24 administered to animals, showed that the isotope exchanged with the sodium of the tissues[1]. They assumed that all the body sodium participated in this exchange. That this assumption is incorrect was suggested by recent results obtained with dogs[2] and humans[3–5], in which 20–35 per cent of the body sodium was estimated to be non-exchangeable with plasma sodium-24.

In order to study directly the relationship between total sodium and exchangeable sodium, sodium-24 was injected intravenously into six adult, well-nourished rabbits. Blood samples were taken periodically and analysed for their radioactivity and sodium content. After varying times (6–96 hr.), the rabbits were killed and small samples of tissues were taken. The bodies were then minced. The tissue samples and portions of the well-mixed minces were dissolved in nitric acid and their radioactivity and sodium content determined[6].

The exchangeable sodium, obtained from analysis of blood samples, was 48 ± 0·75 (s.e.) m.mol. sodium/kgm. body-weight, the value given by analysis of the minces being 46 ± 1·13 m.mol. sodium/kgm. body-weight. These figures are not significantly different, and show that the usual clinical method of measuring exchangeable sodium, as the ratio of the total radioactivity in the patient to the specific activity of the plasma, gives valid results. The total sodium of the rabbits was 62 ± 1·5 m.mol. sodium/kgm. body-weight, that is, the exchangeable sodium was 69 ± 1·7 per cent of the total sodium. The non-exchangeable sodium of the rabbits therefore was 16 ± 1·9 m.mol./kgm. body-weight.

Studies of the exchange of plasma sodium-24 with the sodium of bone in dogs[2] and humans[4,5] have shown that 55–70 per cent of the bone sodium is non-exchangeable. In rabbits[5] this figure is 61–68 per cent. Since the average sodium content of the rabbit bones was 270 m.mol./kgm. bone, the non-exchangeable sodium of bone was about 170 m.mol./kgm. bone; and, as direct measurements exclude the presence of any other major store of non-exchangeable sodium in the rabbit, this means that the bones constituted approximately 10 per cent of the total body-weight, a figure in good agreement with direct determinations[7].

These experiments therefore show that virtually all the non-exchangeable sodium of the rabbit is located in bone and is about 28 per cent of the total body-sodium.

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Printed in Great Britain by Fisher, Knight & Co., Ltd., St. Albans.

Reprinted from *Radioisotope Conference, 1954—Volume I*

15

OBSERVATIONS ON THE MEASUREMENT AND DISTRIBUTION OF EXCHANGEABLE SODIUM IN MAN*

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Exchangeable sodium in man has been measured with ^{24}Na *and* ^{22}Na. *Human bones have a high sodium content though only about one quarter is available for exchange. Little is known of the metabolic importance of this sodium. Attempts have been made to follow the exchange of sodium in bone by external counting. The physical problems of making such a measurement have been investigated. Counts recorded at various sites in amputated lower limbs invariably included a large proportion from radioactive sodium in soft tissues. With the counter directly over the patella* 50 per cent *of the counts came from soft tissues; nevertheless this was the most suitable site. The ratio of these counts to the serum specific activity was determined. The exchange of sodium in bone and other tissues was measured directly in samples removed from amputated limbs. Studies on four subjects with* ^{22}Na *have shown that equilibrium of distribution is not obtained until 96 or more hours after the injection. The results of external counting over the patella were consistent with the view that the delay in attaining equilibrium was due to slow exchange with bone sodium.*

The measurement of body composition by radioisotope dilution is now a well established technique[1, 2]. In this manner estimations of the amounts of sodium and potassium in the human body available for exchange have been made[3–5] with the isotopes ^{24}Na and ^{42}K. The method requires that an equilibrium of distribution of the injected material should be obtained within the time when these relatively short lived radioisotopes can be measured. The recent availability of ^{22}Na has afforded an opportunity of more prolonged observations.

The present paper describes some observations using both the isotopes ^{22}Na and ^{24}Na in an attempt to study the exchange of radiosodium in the human body. Attention has been directed to those compact tissues rich in sodium such as cartilage, tendon and, in particular, bone in which the time for equilibrium may possibly be prolonged. In man it has been estimated that approximately one third of the exchangeable sodium is in bone and previous work has suggested that only 20 to 30 per cent of the bone sodium is available for exchange[4]. The total amount of sodium in bone represents nearly half of the sodium in the human body[6], bone being the only tissue

* Paper read, and answers given, by D. S. Munro.

containing significant quantities of non-exchangeable sodium[7]. Little is known about the importance of the different bone fractions in sodium metabolism.

Information about the exchange of bone sodium is given from observations on four amputated limbs, but its value is limited because of the individual variations in chemical structure. It is more informative to study the process of exchange in a single subject with the passage of time. As it is impossible to take repeated bone biopsies from the same person the feasibility of studying the exchange in bone by external counting has been examined.

THE EXTERNAL COUNTER AND ITS RELATION TO THE BODY

An end window counter was used (G.E.C. Type GM4) enclosed in a thick lead shield and suspended on a mobile stand.

A lead filter 0·5 mm thick was inserted between the counter window (7 mg/cm^2) and the tissues, to eliminate the effect of β-particles from skin and superficial tissues. The filter cut down the counting rate by 30 per cent with the counter on the skin in the region of the knee. Greater thicknesses of lead had no further significant effect on the counting rate and it was therefore apparent that the observed counts were due to γ-ray emission from the active sodium.

The contribution of the radiation from the rest of the body to the counts recorded by the external counter is high, even when a site on the lower limb is used. For this reason lead of thickness 3·5 cm was arranged laterally round the counter and this was thickened to 5·5 cm on one side to reduce the background due to the radioactive sodium content of the trunk.

In using the external counter it is necessary to adopt a technique in which as large a fraction of the observed counting rate as possible is due to radioactive sodium in bone rather than in neighbouring soft tissues. Two possible methods of approach may be adopted to obtain such a ratio. The counter may be set at a distance away from the skin and shielded extensively so that it receives radiation only from a narrow cylindrical volume arranged to include a large proportion of bone. Alternatively, the counter may be placed on the skin as close to bone as possible relying on the inverse square law to reduce to a minimum the contribution of counts from tissues beyond the bone.

Attempts to use a collimated type of counter gave a great increase in the ratio of the body background to total tissue counts and made heavy lateral screening necessary. The counting rate obtained with the end window counter was too low to allow much collimation. The only available scintillation counter could not easily be screened to give a sufficiently low background with the crystal several centimetres from the skin. The better arrangement was to place the counter in contact with the skin at some site where the bone was covered only by a thin layer of tissue.

THE SITE

An ideal site for counting radioactive sodium in bone is a prominent anatomical point where the bone is superficial and where the counter can be accurately replaced on later occasions. The bone itself must be sufficiently thick to make the contribution of counts from other tissues negligible. It

was thought that, though no perfect site exists in the body, some areas, notably in the leg near the knee joint, might be suitable for external counting in the manner described.

As a preliminary study the distribution of counting rate was investigated in a medium of density approximating to bone using point sources of both ^{24}Na and ^{22}Na. Two different materials were tried, *Catalin* (density 1·24 and approximate atomic number 6·2), and glass (density 2·51 and approximate atomic number 12·4). These measurements do not give a true estimate of the distribution of sensitivity obtained during *in vivo* counting since the site chosen in the body would not be homogeneous in density and atomic number. They did, however, allow the following conclusions to be drawn:

(*a*) that the proportion of the count due to a cylindrical block of tissues (of 4 cm radius and 4 cm deep) near the counter would be appreciably higher with the counter itself in contact with the skin than when it was set back 3 cm inside the shield to give greater collimation.
(*b*) That at least 60 per cent of the counting rate observed when the counter was in contact with the skin would come from tissues at a depth greater than 4 cm below the skin or outside a radius of 4 cm from the central axis of the counter.
(*c*) That only 55 per cent of the counts received from the top layer of tissues 4 cm thick would come from the cylinder of 4 cm radius underneath the counter window.
(*d*) That the proportion of the counts coming from such a block would be appreciably higher for ^{22}Na than for ^{24}Na; this is clearly due to the smaller energy of the γ-radiation from the former source.

TECHNIQUE OF EXTERNAL COUNTING IN THE LIVING SUBJECT

For reasons to be given later external counts were recorded over the patella. The measurements were made on one limb with the other leg at a fixed distance. The counter was placed in contact with the skin over the patella with the leg extended. It was then moved laterally and lowered 4·0 cm keeping the face parallel to the initial position and with the shield in contact with the skin so that it remained as near its original position as possible. A count in this position included the body background and contributions from the tissues lying more than 4 cm below the skin. The difference in counting rate was taken as proportional to the active sodium content of a block of tissue immediately under the end window approximately 4 cm thick; this included the patella and adjacent portions of the femur. This value was divided by the count from a standard radium source and by the specific activity of serum obtained at the time of the external count. The specific activity of the serum was expressed as microcuries of radioactivity per milli-equivalent of sodium. This bone/serum ratio is calculated thus:

$$\text{Bone/serum ratio} = \frac{\text{Patella count} - \text{Background count}}{\text{Radium count}} \times \frac{1}{\text{Serum specific activity}}$$

STUDIES ON AMPUTATED LIMBS

The last step in the preliminary studies was to undertake experiments on legs amputated from subjects who had received radioactive sodium. Counts were recorded at various sites on these limbs before and after the removal of the soft tissues by dissection and an estimate obtained of the proportion of counts due to radioactive sodium in bone. The exchange in bone and soft tissues was also studied in these limbs.

Three limbs were examined in this way: in two ^{24}Na was used and in the other ^{22}Na. The legs were amputated because of distal dry gangrene of minimal extent. The following considerations showed that the arterial disease had not interfered with the penetration of radioactive sodium into the tissues in the region studied. The bone samples were taken from the proximal part of the limb where the circulation was adequate as shown by the subsequent healing of the amputation stump. Furthermore, exchange was complete in cartilage and tendon in close proximity to bone. On one occasion tissues such as tendon, muscle and nerve taken in regions far distal to the bone specimens and to the point at which the external counts were made showed complete exchange.

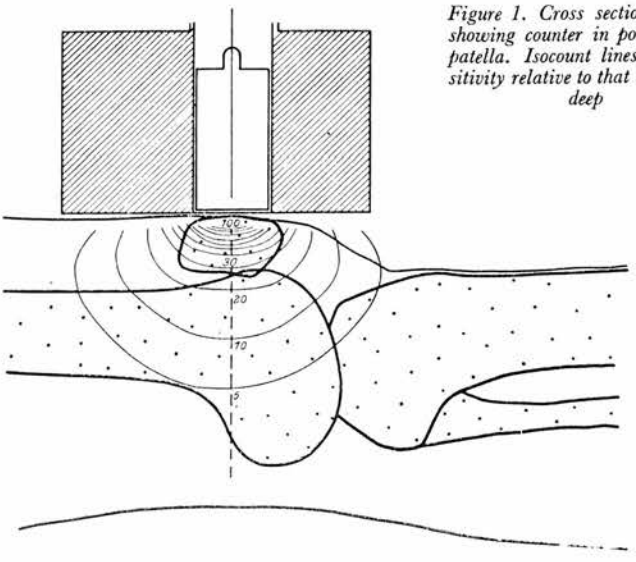

Figure 1. Cross section of knee showing counter in position over patella. Isocount lines give sensitivity relative to that at 0·5 cm *deep*

In the amputated limbs containing ^{24}Na, external counts directly over the patella with the leg extended showed that about 45 per cent of the total was due to radioactive sodium in bone; the patella itself contributed 27 per cent. A measurement across the femoral condyles with the leg flexed gave about 50 per cent of counts from radioactive sodium in bone. Using the isotope ^{22}Na observations over the patella showed that 53 per cent of counts came from the dissected bones whilst tests down the shaft of the tibia gave a rather lower proportion (45 per cent).

Counts made over the dissected bones included contributions from marrow and extracellular fluid in the bone. There are also small portions of tissue not successfully removed by dissection such as cartilage and the firmly adherent insertions of tendons near the knee. Under normal conditions it was not possible to obtain counts from any site where the bone sodium contribution was more than 50 per cent. It was decided for routine observations to count over the patella with the knee extended because this site could be acccurately located and the patient was comfortable.

Figure 1 shows a diagram of the knee joint taken from a lateral radiograph and superimposed on this is a line diagram of the counter with isocount lines in the plane containing the central axis of the counter. The isocount lines are those measured in a *Catalin* block using the isotope ^{22}Na, and they indicate the effectiveness of the counter in receiving radiation originating in the bone.

SODIUM EXCHANGE IN THE AMPUTATED LIMBS

The sodium exchange was estimated in weighed portions of each tissue after solution in nitric acid as previously described[4]. Bone samples present

Table I. Sodium Content and Exchange of Tissues in Amputated Limbs

Patient	*Isotope and route of administration*	*Equilibration time*	*Tissue*	*Percentage exchange**	*Sodium content of tissue* m.equiv./kg*
5. F.	*Oral* ^{24}Na	19 h	*Tibia*	25	208
			Femur	18	295
			Patella	37	210
			Tendon	38	—
6. M.	*Oral* ^{24}Na	21 h	*Tibia*	17	252
			Femur	18	278
			Patella	30	204
			Tendon	103	94
7. M.	*Oral* ^{24}Na	3 d	*Tibia*	15	302
			Tibia	27	190
			Femur	17	257
			Femur	22	240
			Patella	17	238
			Patella	14	139
			Rect. fem. muscle	88	33
			Gastrocnemius	98	49
			Soleus	89	56
			Patellar tendon	87	88
			Skin over tibia	89	56
			Articular cartilage	98	179
			Quadriceps tendon	99	66
8. F.	*Intravenous* ^{22}Na	6 d	*Tibia*	33	206
			Fibula	29	415
			Tendocalcaeneus	90	101
			Muscle	87	52
			Calf tendon	96	81
			Post tibial nerve	93	94

* The sodium content and exchange values for all bone samples refer to dry weight; deductions have been made for sodium contained in the extracellular fluid. The sodium content of the other specimens is calculated as m. equiv./kg of wet tissue.

special difficulties because the calcium content enhances the sodium estimations obtained by flame photometry[8]. Accordingly, the calcium content of the bone was estimated chemically[9], and the photometer used with a special filter (Barr and Stroud interference filter Type A997, band width: 140 Å, peak: 5860 Å). The contribution due to calcium was estimated and the true sodium content of the bone solution was calculated by applying the necessary correction[10]. The exchange in bone and soft tissues in these limbs is shown in *Table I.* The bone exchange is lower than that reported in dogs by I. S. EDELMAN and others[11].

RESULTS OF OBSERVATIONS ON PATIENTS

The earlier work was done with ^{24}Na in 20 normal subjects on an ordinary diet. Doses of the order of 50–80 μc of ^{24}Na were given. After 24 hours the smaller dose gave a counting rate on the patella of 400 c.p.m. and a background of about 80 c.p.m. The counting time was normally about 12 minutes on the patella and somewhat less on the background.

The ratio of patella counts to serum specific activity was calculated as described and it was evident that the individual variation was great—a range of 2·0 to 1 being observed in 35 tests. The following experiment tested the effect of variations in the extracellular fluid in the limb on the count over the patella. A volunteer who had been given a dose of ^{24}Na had external counts performed over both patellas at 24 hours. A pressure cuff inflated to 90 mm of mercury was then placed on one thigh for 103 minutes. Though considerable congestion was produced in the limb and pitting oedema developed over the tibia no change was detected in the count over the patella on the congested limb, the rate being the same, within experimental error, as obtained on the opposite limb.

In studies with ^{24}Na it was apparent that equilibrium of distribution was not always obtained in 24 hours. In some cases a slow rise in the measurements of total exchangeable sodium occurred up to 48 hours—the time limit of satisfactory measurements after the use of tracer doses of ^{24}Na up to 80 μc. The use of the isotope ^{22}Na was then adopted so that information on the time of equilibration could be obtained.

STUDIES WITH ^{22}Na

The physical half-life of this isotope is 2·6 years; the biological half-life depends on the turnover of sodium in the body. With salt restriction sufficient to reduce the daily urinary sodium excretion to 15 m. equiv. the elimination of the active sodium was such as to reduce the serum activity to half its value in about 30 days. With an unrestricted salt intake determined only by the subject's choice this biological half-life fell to approximately 11 days. These are comparable with results observed by S. THREEFOOT and others[12].

After a dose of 70 μc of ^{22}Na in a patient who was on salt restriction for 8 days and thereafter unrestricted the radiation exposure was approximately 1·3 rep. At the conclusion of these observations the radiation was readily reduced by increasing the sodium intake.

The active material was given by intravenous injection of 70 μc of ^{22}Na dissolved in 50 ml of 0·9 per cent sodium chloride solution. This was

delivered from a calibrated burette to 4 patients with cardiovascular disease in whom detailed observations on sodium metabolism were being made. The excretion of ^{22}Na in urine and in faeces was measured. In all four the loss of activity in the urine during the 7 days on salt-restricted diet was approximately 4 per cent of the dose while the faecal loss was very much smaller than this, being negligible in three cases and in the fourth about 0·4 per cent of the administered activity. No allowance was made for loss of ^{22}Na through the skin. One subject refrained from taking a bath or washing during the period of observation. He then bathed in a small volume of water and his pyjamas, bedclothes and towel were all soaked in the bath water. This water contained only 0·7 per cent of the injected dose. This agrees with some earlier observations of a similar nature made[4] with ^{24}Na.

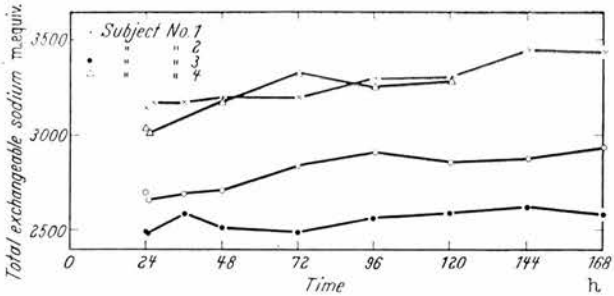

Figure 2. Variation of total exchangeable sodium with time

Total exchangeable sodium (Na_E) was calculated at 24, 25, 36 and 48 hours after injection and subsequently at 24-hour intervals for a period of one week by the following formula.

$$Na_E = \frac{^{22}Na \text{ injected} - {}^{22}Na \text{ excreted}}{^{22}Na/\text{total sodium per unit volume of serum}}$$

The total sodium content of the serum was measured by flame photometry. The results are shown in *Figure 2*. There is an apparent slow rise in Na_E with the passage of time. Taking the mean of the values obtained at 24 and 25 hours as 100 per cent the later measurements may be expressed as follows:

Table II. Total Exchangeable Sodium

Expressed as per cent of mean at 24 and 25 hours

Patient	*Age* y	*Wt* kg	Hours							
			24/25	36	48	72	96	120	144	168
1. M.	41	75·0	100	100·4	101·1	103·0	104·6	104·3	109·1	108·8
2. M.	43	68·8	100	100·1	100·8	105·7	108·5	106·4	107·1	109·1
3. F.	43	65·8	100	104·0	101·2	100·3	103·2	104·4	105·5	103·5
4. M.	45	76·0	100	—	104·9	109·9	107·6	108·4	—	—
Mean	—	—	100	101·5	102·0	104·7	106·0	105·9	107·2	107·1

The statistical errors in the individual measurements of the total exchangeable sodium are small, the standard deviation in each one being approximately 0·8 per cent. When the Na_E is expressed as a percentage of the mean value at 24 and 25 hours, the mean value of all the readings at 72 hours and later is 106·1 per cent with a standard deviation of 2·3 per cent.

It is important to emphasize that these observations were made whilst the subjects were on low sodium diets. The total daily excretion of ^{22}Na was only 0·7 per cent of the dose. As far as can be ascertained collection of all specimens of urine and faeces was complete but in any case the excretion rate was so low that the observed increase in Na_E with time cannot be attributed to loss of excreted ^{22}Na. The results suggest therefore that exchange of injected ^{22}Na continues at least up to 96 hours after administration, a view also expressed by G. E. BURCH and his associates[13].

The possibility that this slow rise from 24–96 hours in these cases was due to slow exchange in the bones was investigated by external counting over the patella as already described. The results are shown in *Figure 3.*

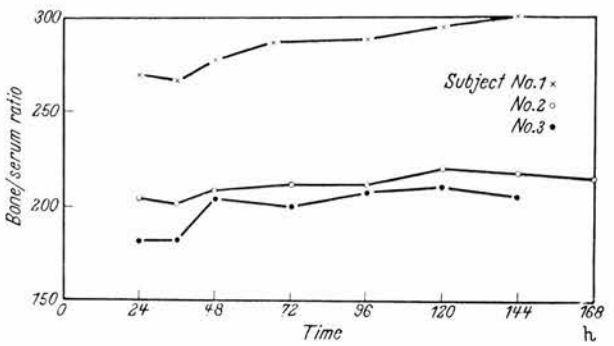

Figure 3. Variation by bone/serum ratio with time

It can be seen that this ratio follows the same trend as the total exchangeable sodium. The statistical error in making the bone counts is greater than in measurement of serum activity. The mean value of all the readings at 72 hours and later expressed as a percentage of the 24 hour value is 109·3 per cent with a standard deviation of 4·1 per cent. It should be emphasized that the counting rate over the patella remained constant within experimental error and the increase in the bone/serum ratio was due to a reduction in the serum specific activity with time.

This is important because the patella count is derived from two sources—soft tissue and bone. As the specific activity of sodium in the soft tissues is mainly due to extracellular fluid sodium it may be assumed to follow that of the serum. The fact that counts over the patella remain constant as the serum specific activity diminishes may therefore be associated with increasing radioactivity in the bones due to progressive exchange of the ^{22}Na with bone sodium.

This work was supported throughout by grants from the Endowment Fund of the United Sheffield Hospitals, the Tuberculosis Research Fund of the University of Sheffield and the Sheffield Regional Hospital Board.

Dr H. E. Renschler holds a British Council Scholarship.

We are indebted to the Physics Staff of the Sheffield Radiotherapy Centre, the Staff

of the Department of Pharmacology and Therapeutics in the University of Sheffield, and the Nursing Staff of the Sheffield Royal Infirmary for their help.

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DISCUSSION

JANET VAUGHAN (Oxford). What was the age of subjects injected with radioactive sodium and was there any variation in the patellar content with age?

Answer. The subjects whose amputated limbs we studied were all elderly; we have no information about the sodium content or sodium exchange in younger people.

JANET VAUGHAN (Oxford). Would Dr Munro care to speculate as to the state of deposition of radioactive sodium in the bone or the method of its incorporation.

Answer. I am sure that the problem of determining the exact localization of sodium in bones could readily be solved by microautoradiography with ^{22}Na. The work would presumably have to be done with animals where larger doses could be employed. I would not care to speculate further on this point at the present stage.

J. GOVAERTS (Liége, Belgium). I want to ask a question concerning the toxicity of ^{22}Na. Most tracer work has been done with ^{24}Na. The doses of ^{22}Na you employed are those indicated by Marinelli as tolerance doses. Have you ever observed toxic effects in human subjects?

Answer. Using doses up to 70 μc of ^{22}Na we have not observed any toxic effects on our subjects. The relatively rapid biological turnover soon eliminates the isotope.

Answer (G. M. WILSON). These experiments were carried out with a view to investigating ultimately the metabolic significance of the exchangeable fraction of the bone sodium in man. The fact that some sodium in bone is available for exchange with a sodium isotope does not necessarily mean that it is metabolically available. There is evidence in animals that sodium can be drawn from bone to meet a body sodium deficiency. We have been developing the external counting technique in the hope that it may be possible to study sodium metabolism in human sodium deficiency states.

Published by Butterworths Scientific Publications
88 Kingsway, London, W.C.2.

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[*Reprinted from the Journal of Physiology,*
1957, Vol. **139**, No. 3, p. 474.]
PRINTED IN GREAT BRITAIN

J. Physiol. (1957) 139, 474–488

THE EXCHANGE OF BONE SODIUM WITH ISOTOPES IN RATS

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(*Received* 2 *August* 1957)

Bone is the only tissue in which the sodium concentration is greater than in extracellular fluid (Harrison, Darrow & Yannett, 1936) and it contains a large proportion of the total amount within the body (Bergstrom, 1955; Cheek, West & Golden, 1957; Davies, Kornberg & Wilson, 1952*a*). The sodium in the extracellular fluid of bone only accounts for a small proportion of the total. The greater part lies in the bone crystals (Neuman & Neuman, 1953; Nichols & Nichols, 1956). The bone content of calcium and phosphorus increases with age and there is some evidence that there is a corresponding alteration in sodium (Bergstrom & Wallace, 1954).

The effects of sodium loss have been studied by following the alterations in total body content by balance techniques and correlating these with alterations in serum sodium level. From time to time discrepancies have been revealed in such experiments which were greater than could be accounted for by the errors of the measurement (Atchley, Loeb, Richards, Benedict & Driscoll, 1933; Flanagan, Davis & Overman, 1950; Gamble, Wallace, Eliel, Holliday, Cushman, Appleton, Shenberg & Piotti, 1951). It is now known that the bone sodium content falls after loss of sodium from the body (Bergstrom, 1955; Nichols & Nichols, 1956; Levitt, Turner, Sweet & Pandiri, 1956), and it has been suggested that bone may act as a sodium reservoir for the rest of the body (Bergstrom, 1956). Renwick, Robson & Stewart (1955) did not observe any drop in serum sodium level, even after 30 days of severely restricted sodium intake in man.

Ever since the earliest studies with radio-sodium it has been realized that bone sodium does not exchange completely within periods adequate for complete exchange in all other tissues (Kaltreider, Meneely, Allen & Bale, 1941). For example, in rats only 30–40% of bone sodium exchanged with injected radio-sodium within 24 hr (Bauer, 1954). Similar values have been reported

in rabbits (Davies, Kornberg & Wilson, 1952*c*), in dogs (Edelman, James, Baden & Moore, 1954), and in man (Edelman, Olney, James, Brooks & Moore, 1952; Miller & Wilson, 1953; Miller, Munro, Renschler & Wilson, 1954). The most readily available radioactive isotope, ^{24}Na, has a physical half-life of 15·4 hr which has limited that duration of many observations. Experiments on rats with the isotope ^{22}Na, which has a physical half-life of 2·6 years, have suggested that some radio-sodium may be incorporated into bone sodium and remain there for many weeks (Bauer, 1954). Similarly, in a human subject given ^{22}Na, measurements with a whole body counter have shown that some radioactivity remained in the body long after there was none detectable in serum samples (Miller, Munro & Wilson, 1957).

In the present work various factors influencing the exchange of radio-sodium with the sodium in the bones of rats have been investigated. The effect of age on the bone composition and penetration of radio-sodium has been studied and long-term experiments with ^{22}Na have been made, while the rats have been on a sodium-deficient diet. Particular attention has been paid to the changes in bone composition following acute and chronic sodium depletion. Bergstrom (1956) has suggested that the fall in bone sodium content may be due to release of the rapidly exchangeable fraction. However, little so far is known about the effects of sodium depletion on bone sodium exchange.

METHODS

Male albino rats were used. They were of the same strain and reared under identical conditions. In a few experiments they were given ^{22}Na immediately after weaning. Otherwise they were divided into groups on a weight basis (*a*) between 50 and 100 g, (*b*) between 230 and 280 g, and (*c*) between 400 and 450 g. These weights correspond to ages of 5 weeks, 24 weeks and over 1½ years respectively.

The rats were normally fed on a standard diet containing about 15 m-equiv sodium/100 g. Rats weighing about 250 g consumed on the average 20 g of the diet a day. A reduced sodium intake was achieved by giving a diet of rice and distilled water with supplements of yeast and olive oil. On this diet the mean daily urinary excretion of sodium in 250 g rats was 0·08 m-equiv/day.

Doses of either 20–40 μc ^{24}Na or 10 μc ^{22}Na in 0·5 ml. 0·9% (w/v) NaCl were given by intraperitoneal injection. The radioactivity of blood and bone samples containing ^{24}Na was measured in liquid counters (Veall, 1948). In some experiments, double tracer techniques were employed and initially the solutions of blood and bone contained both ^{24}Na and ^{22}Na. They were counted as soon as possible, and again a week later, when the short-half-life ^{24}Na had decayed to a negligible quantity. The concentrations of the two isotopes were thus readily calculated. A scintillation counter (Type N550, Ekco Electronics Ltd.) was used to measure samples containing only small amounts of ^{22}Na.

The radioactivity within living rats was estimated in a specially designed system. Eight matched G.-M.counting tubes (Type G. 26, 20th Century Electronics Ltd.) were arranged symmetrically around a central cylinder of brass with an internal diameter of 7·5 cm. The counters were connected in parallel through a probe unit (Harwell catalogue type 1014 A) to an automatic scaler (Type 530A, Ekco Electronics Ltd.). The rats were placed in a large glass tube, perforated for ventilation, and fitted with an adjustable plastic disk, which could be taken out to allow the rat

to enter and then moved along to confine the animal at the far end. Two plastic rings were cut to the size of the brass cylinder and fitted to the outside of this tube to ensure that the rat was always placed at the centre of the counter array. The whole apparatus was shielded with lead 2 cm thick. A diagram of the apparatus and of the rat inside the glass chamber is shown in Fig. 1.

During measurements, the rat remained inside the counter for 5 min. Alternate counts were made from a standard solution of ^{22}Na in a bottle held inside a Perspex container and inserted in the same way as the rats. Tests on samples of ^{22}Na up to 0·1 μc showed a linear relationship between the amount of radioactivity and the counting rate recorded.

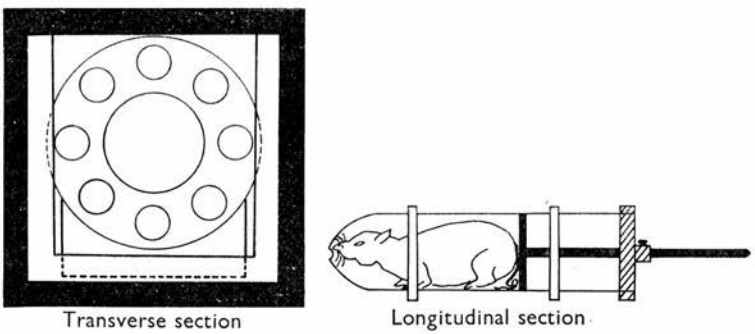

Fig. 1. The arrangement of 8 G.-M. counting tubes around the central cylinder into which the rat chamber is inserted. The whole is enclosed in lead 2 cm thick.

Blood samples were removed by cardiac puncture. Owing to the difficulty of getting sufficiently large serum samples in the smallest rats, a comparison was made of the sodium specific activity of whole blood and serum. Twelve rats between 240 and 260 g were given an intraperitoneal injection of ^{24}Na and blood samples obtained 24 hr later. After clotting, the serum was separated and the blood clot transferred to distilled water, in which it was broken into small fragments with a glass rod. The cellular debris from the clot was removed by centrifuging and the supernatant fluid removed and diluted to 12 ml. The serum was diluted to the same volume and the sodium specific activity measured in both solutions. There was close agreement between the two values ($P > 0{\cdot}7$). Thereafter either haemolysed whole blood or serum samples were used in estimating blood sodium specific activity.

The rats were killed by anaesthetizing with ether and opening the thoracic cage. Immediately after death, the femora, tibiae and humeri were dissected free of all adherent muscle and tendon. The periosteal membranes were removed and the shafts separated from the bone ends by saw cuts made between the epiphyseal plates at constant anatomical landmarks. The bone shafts were split with forceps and all the marrow and cancellous bone removed. The three bones from each side were pooled so that two separate bone samples were obtained from each animal.

The bone fragments were transferred to crucibles and their water content measured by the loss of weight after drying in an oven at 150° C for 3·5 hr. This method of estimating bone water was compared in duplicate samples from 10 animals, with drying at 90° C to a constant weight. There was no significant difference ($P > 0{\cdot}8$). The more rapid method was accordingly used to facilitate counting with the short half-life isotope. The dried bone samples were ashed in a furnace at 700° C for 1 hr. The ash was dissolved in 2–5 ml. 2 M-HCl, according to the weight of the sample, and the volume made up to 15 ml. with resin-filtered distilled water.

The sodium content of the bone solutions after 1:100 dilution was estimated in the EEL flame photometer, using an interference filter for estimating sodium in the presence of calcium (Barr & Stroud, Type A. 997; band width 150Å, peak 5860 Å).

In the presence of calcium the estimate of sodium concentration by flame photometry is too

high because of interference effects. Calcium solutions free of sodium give deflexions which correspond to an apparent sodium content. This effect obtained with calcium solutions measured in the EEL flame photometer before and after the addition of 0·2 m-equiv Na/l. is shown in Fig. 2.

The concentrations of sodium and calcium in this experiment cover all the estimations made on bone solutions. Hence, a correction for the calcium interference effect was applied when spraying diluted bone solutions in which the calcium content was invariably below 20 m-equiv/l.

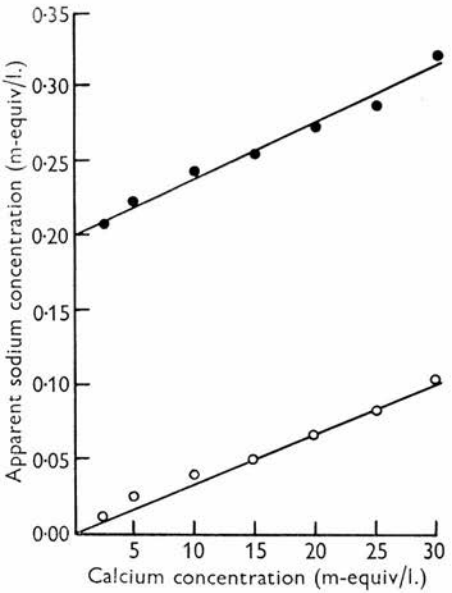

Fig. 2

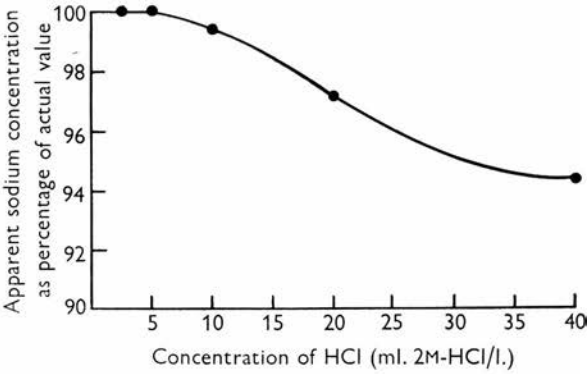

Fig. 3

Fig. 2. The apparent sodium content of calcium solutions before and after the addition of sodium. ○, calcium solutions; ●, calcium solutions with 0·2 m-equiv sodium/l. added.

Fig. 3. The effect of increasing concentrations of HCl on the estimate of the sodium in a standard solution containing 0·1 m-equiv/l.

The presence of chloride ions has been shown to depress the estimate of sodium concentration by flame photometry (Collins & Polkinhorne, 1952). Accordingly, the effect of chloride concentration on the measurements of the sodium concentration in a standard solution was investigated (Fig. 3). It is evident that no effect was detected with concentrations below 5 ml. 2M-HCl/l., which was more than twice the maximum concentration in the diluted bone solutions.

The calcium content of the diluted bone solutions was measured by flame photometry (Powell, 1953). These estimates were compared with an established chemical method (King, 1946) on forty-five bone solutions and five prepared standards. No significant difference was demonstrated ($P = 0{\cdot}3$).

Finally, the accuracy of measuring bone sodium was tested by recovery experiments. Sodium, in concentrations varying between 0·05 and 0·20 m-equiv/l. was added to fifteen different bone solutions of known sodium content. The mean recovery was 101·9 % of the value calculated from the dilution (S.D. $\pm 4{\cdot}3$ %).

RESULTS

Effect of age on bone composition and sodium exchange. Three groups of rats of different weights were studied. The values for the water, sodium, calcium and exchangeable sodium contents of bone, measured 24 hr after the injection of ^{24}Na, were obtained by taking the mean of the estimates on the two bone samples from each animal. The differences between the duplicate samples taken from each side were small, the standard error of the difference being $\pm 1{\cdot}5\%$ for bone sodium and $\pm 1{\cdot}2\%$ for bone calcium, both expressed as m-equiv/kg dry bone. The corresponding figure for the relative specific activity of bone sodium was $\pm 2{\cdot}2\%$, which included the errors of sodium and calcium estimation in addition to the error in measuring radioactivity. The mean values, the standard deviation, and the standard error for each group are shown in Table 1. Within each group the variations in bone mineral content and the exchangeable sodium are small.

TABLE 1. Composition of bones of young, adult and old rats

No. of rats and age	Wt. (g)	Water content of wet bone (%)	Calcium (m-equiv/kg dry bone)	Sodium (m-equiv/kg dry bone)	Na:Ca ratio	Specific activity relative to blood sodium (%)	Exchangeable bone sodium (m-equiv/kg dry bone)
15, young	77	18·9	12,146	246·6	0·0202	64·0	157·8
S.D.	11	2·5	434	11·1	0·0013	5·0	11·4
S.E.	3	0·6	112	2·9	0·0003	1·3	2·9
15, adult	249	15·8	12,825	253·3	0·0197	54·7	138·7
S.D.	22	2·4	249	8·5	0·0007	2·8	9·4
S.E.	6	0·6	64	2·2	0·0002	0·7	2·4
8, old	427	13·6	13,313	275·0	0·0205	50·0	137·2
S.D.	22	0·6	220	9·6	0·0005	2·9	5·3
S.E.	8	0·1	78	3·4	0·0002	1·0	1·9

However, the differences between the three groups are striking. The water content of the bones decreased with age. Thus, the amount of water in these cortical bone samples expressed as percentage of wet bone fell from 18·9% in young rats to 13·6% in old rats. The bone calcium content, however, showed a considerable rise with age. The bones from old animals contained 9·6% more calcium/kg dry bone than those from the young rats.

The amount of sodium in bone increased conspicuously with age. This rise in sodium content followed calcium in the same proportion and the Na:Ca ratio remained constant throughout the three age groups.

This increase in bone mineral content was associated with a considerable decrease in the specific activity of bone sodium relative to blood 24 hr after injecting radio-sodium. The relative specific activity of bone sodium fell from 64% in the young rats to 50% in the old animals. However, if the amount of sodium which had exchanged with ^{24}Na was expressed in the same units as the total sodium content (m-equiv/kg dry bone) then the fall was much smaller,

though it was still evident and the difference between the young and adult rats remained highly significant ($P < 0{\cdot}001$). It should be emphasized that this drop in exchangeable sodium occurred in spite of an increase in the total sodium content of bone (Fig. 4). The fall in exchangeable bone sodium with age occurred mainly between the young and adult rats; there was no appreciable difference between the adult and old rats in this respect. In contrast, the total bone sodium content increased steadily with age.

A regression analysis confirmed that the fall in water content, the increase in bone sodium and calcium, the drop in the relative specific activity of bone sodium, and in exchangeable bone sodium are all highly significant ($P < 0{\cdot}001$).

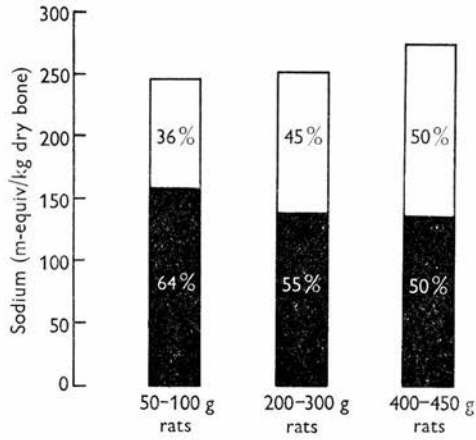

Fig. 4. The effect of age on bone sodium content and the specific activity of bone sodium relative to blood 24 hr after injecting ^{24}Na. Lower black portions of columns represent exchangeable bone sodium.

Acute sodium depletion and bone sodium exchange. Two groups of fifteen rats weighing between 50 and 100 g, and 230 and 300 g were used (Table 2). Sodium depletion was carried out as described by Bergstrom & Wallace (1954). A solution, representing in volume 10% of the body weight and containing 90 m-mole ammonium chloride and 50 g glucose/l. was injected intraperitoneally. The rats were allowed only distilled water from the time of this injection until death. After 4 hr the fluid in the peritoneal cavity was withdrawn, thus removing sodium from the body. On the morning of the following day, 20–40 μc ^{24}Na were injected intraperitoneally. Twenty-four hours later the animals were killed under ether anaesthesia.

The effects of sodium depletion on bone composition and serum levels in the young and adult rats are shown in Table 2. The results in an equal number of rats not depleted of sodium, but allowed distilled water and no food for a similar period, are shown for comparison. The amount of sodium removed on a body-weight basis was similar in the two age groups. The weight loss in the

sodium-depleted animals was slightly greater than in the rats subjected to starvation alone. The serum sodium concentration was not altered by removal of sodium from the body in the young rats but showed a significant fall in the adults.

The bone water and calcium contents did not change, but the bone sodium was decreased by the depletion experiments ($P < 0{\cdot}001$). This decrease was greater in the young rats. The specific activity of the bone sodium relative to blood was higher in the depleted rats, particularly in the younger group (Fig. 5).

TABLE 2. The effect of sodium depletion on serum sodium and bone composition in young and adult rats

Group	No. of rats and age	Wt. (g)	Sodium removed (m-equiv/kg rat wt.)	Body wt. loss (%)	Serum sodium (m-equiv/l.)*	Water content of wet bone (%)	Calcium (m-equiv/kg dry bone)	Sodium (m-equiv/kg dry bone)	Na:Ca ratio	Specific activity relative to blood sodium (%)	Exchangeable bone sodium (m-equiv/kg dry bone)
Control	15, young	77	0	20·2	150·7 (12)	18·9	12,146	246·6	0·0202	64·0	157·8
S.E.		3	—	1·1	0·9	0·6	112	2·9	0·0003	1·3	2·9
Depleted	15, young	74	14·9	21·7	150·6 (12)	18·6	12,189	209·7	0·0171	76·8	161·0
S.E.		4	0·7	1·2	1·1	0·4	135	3·1	0·0002	1·4	4·1
Control	15, adult	249	0	12·0	149·3 (15)	15·8	12,825	253·3	0·0197	54·7	138·7
S.E.		6	—	0·9	0·8	0·6	64	2·2	0·0002	0·7	2·4
Depleted	15, adult	254	13·9	15·8	138·6 (14)	14·3	12,791	229·1	0·0179	57·6	132·0
S.E.		4	0·8	0·8	2·0	0·5	71	3·7	0·0003	1·5	4·1

* An adequate specimen for serum sodium determination was not obtained in a few animals; in them whole blood specific activity only was measured. The number of rats on which the mean is based is shown in parentheses.

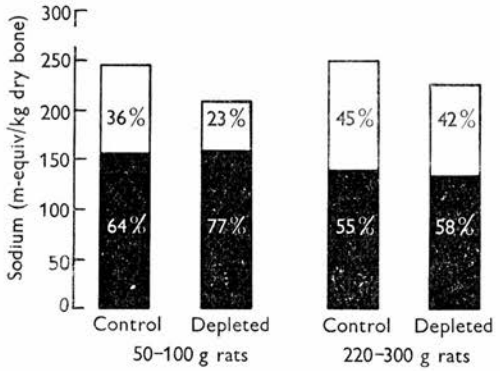

Fig. 5. The mean bone sodium content and exchange with ^{24}Na after 24 hr in control and sodium-depleted rats. Lower black portions of columns represent exchangeable bone sodium.

In spite of the decreases in total sodium content of the bones, the amount of sodium available for exchange in a 24 hr period with ^{24}Na showed no significant change ($P = 0{\cdot}6$).

Long-term exchange of ^{22}Na *with bone sodium.* Rats weighing between 230 and 280 g were placed on the rice diet, and after 4 days were given injections of ^{22}Na. Thereafter they were killed in groups at intervals up to 14 weeks. Twenty-four hours before death they received injections of ^{24}Na. The rats

lost about 15% of their body weight after 14 weeks on this diet, but no obvious signs of nutritional deficiency were observed. The effect on the total calcium and sodium content of bone and the exchange of bone sodium with radio-sodium are shown in Table 3. The calcium content did not change, but both the water and sodium contents of the bones fell gradually while on the restricted intake of sodium (Fig. 6). After 14 weeks the total sodium content

TABLE 3. Bone composition of rats maintained on a rice diet

Weeks after ^{22}Na injection	No. of rats	Wt. at death (g)	Water content of wet bone (%)	Calcium (m-equiv/kg dry bone)	Sodium (m-equiv/kg dry bone)	Na:Ca ratio	Bone specific activity relative to blood (%)	
							^{24}Na	^{22}N
1	3	253	17·1	13,171	251	0·0191	52·7	58·7
S.E.		3·3	0·7	243	5·0	0·0006	1·2	2·7
2	3	240	15·3	13,309	253	0·0190	52·7	63·0
S.E.		10·0	0·8	138	8·0	0·0006	2·2	4·6
4	3	218	16·6	12,992	224	0·0173	54·7	73·0
S.E.		0·5	0·8	247	8·4	0·0009	1·8	3·6
6	3	210	16·4	12,792	236	0·0184	55·3	82·7
S.E.		15	0·8	139	6·9	0·0004	0·4	1·8
7	3	206	14·5	13,001	238	0·0182	57·0	87·0
S.E.		3·3	0·4	353	4·8	0·0004	0·6	2·7
9	2	195	14·0*	12,697	217	0·0171	50·5	77·5
S.E.		10·6	—	61	7·8	0·0007	1·1	4·6
11	3	203	12·7	12,618	233	0·0184	51·3	75·0
S.E.		0·5	0·4	123	3·2	0·0004	1·3	2·0
14	3	212	13·3	12,714	222	0·0185	52·3	83·7
S.E.		4·4	0·4	105	7·3	0·0004	1·5	1·7

* One sample only.

The rats were given intraperitoneal injections of ^{24}Na 24 hr before death.

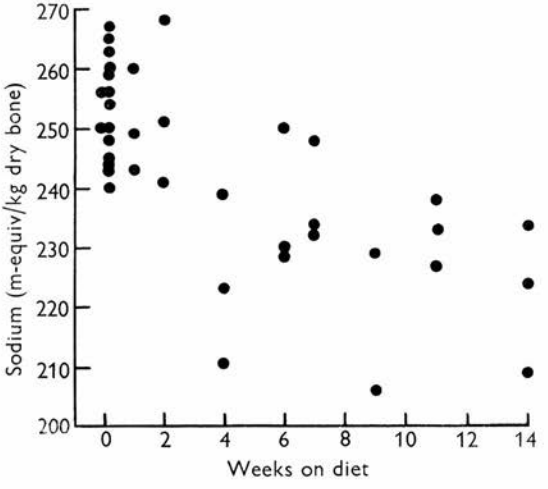

Fig. 6. The sodium content of bones from normal rats and from rats fed on a rice diet for periods up to 14 weeks.

was 14% lower than the mean value of 257 m-equiv/kg dry bone in fifteen normal rats. The fall in sodium content without any change in calcium is further reflected by the decrease in the Na:Ca ratio.

In spite of this decrease in sodium content, the relative specific activity of bone sodium, measured 24 hr after injecting ^{24}Na, remained within the normal range described above. However, the measurements with ^{22}Na showed that there was a progressive increase in the relative specific activity of bone sodium up to 7 weeks. By this time the specific activity of bone sodium was 75–87% of the specific activity of the sodium in blood samples. Thereafter no further increase was demonstrated.

Serum sodium concentration while on rice diet. The mean serum sodium level in twenty-three rats maintained on a standard diet and weighing 230–280 g was $150 \pm 1{\cdot}0$ (S.E.) m-equiv/l. After 7 weeks on the rice diet in a group of six rats there was no change in the serum sodium, which was $150 \pm 0{\cdot}8$ m-equiv/l., but after 13 weeks in a further group of six rats there was a fall to $145 \pm 0{\cdot}8$ m-equiv/l. The difference is significant ($P < 0{\cdot}02$).

Persistence of ^{22}Na *in bone.* Six rats were placed on the rice diet and given ^{22}Na as described above. After 6 weeks on the restricted diet they were allowed the standard diet for 1 week. Two rats were then killed. The remaining four were again placed on the rice diet and were killed in pairs 3 and 5 weeks later. The specific activity of the bone sodium relative to blood following the change in diet was 391, 196 and 257% respectively in the three pairs. The bone sodium content and the exchange with ^{24}Na after 24 hr were within the normal range. These results suggested that some of the ^{22}Na had entered a part of the bone sodium not exchanging freely with the sodium of the extracellular fluid and further investigations using counting *in vivo* were carried out.

Eight rats were placed on a rice diet shortly after weaning. Four days later they were given 10 μc ^{22}Na and kept on the rice diet for a further week. Thereafter they were allowed the standard diet and grew normally. The first measurements of whole-body radioactivity were made 11 weeks after the injection. After some preliminary observations over the course of a week, daily intraperitoneal injections of 25 ml. of 0·9% (w/v) NaCl were given for 6 days. In all animals there was prompt fall in radio-activity following the first injection but after subsequent injections the whole-body radioactivity showed no significant change. During the injections the animals did not gain weight as the excess saline was quickly excreted in the urine. After the period of saline injections the whole-body radioactivity was followed in two of the rats for a further 4 weeks, while they continued on a standard diet (Fig. 7). They showed a slight, slow decline in total radioactivity over this period. These two rats were then killed 17 weeks after the injection of ^{22}Na. At this time approximately 0·05 μc ^{22}Na remained in each animal. The blood contained no detectable radioactivity. In the shafts of the long bones there was 8·5 μc/kg dry bone in

each animal. The sodium content of the bones was respectively 256 and 266 m-equiv/kg dry bone.

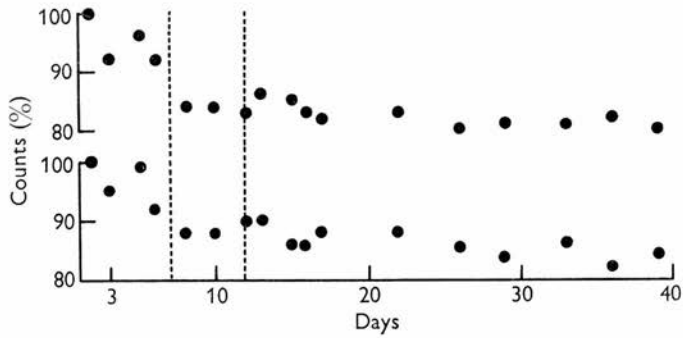

Fig. 7. Whole body radioactivity measurements on two rats begun 11 weeks after injection of ^{22}Na. During the period between the dotted lines daily intraperitoneal injections of sodium chloride were given.

TABLE 4. Estimates of sodium content of rat bones

Rat wt. (g) or age	Site of bone sample	Type of bone sample	Sodium content (m-eqiuv/kg)	Authors
333	Shaft with marrow	Wet bone	206	Bergstrom & Wallace (1954)
218	Shaft with marrow	Dry bone	308	Bauer (1954)
Adult	Shaft with marrow	Dry, fat-free, solid	229*	Nichols & Nichols (1956)
Old	Shaft with marrow	Dry, fat-free, solid	273*	Nichols & Nichols (1956)
Adult	Shaft with marrow	Wet bone	165	Woodbury (1956)
300	Shaft without marrow	Dry tissue	288	Levitt *et al.* (1956)
220	Shaft with marrow	Dry, fat-free, solid	297	Cheek *et al.* (1957)
249	Shaft without marrow	Dry bone	253	This paper
427	Shaft without marrow	Dry bone	275	This paper

* In these values a deduction has been made for the sodium in the extracellular fluid.

DISCUSSION

There have been considerable variations in the estimates of rat bone sodium content by other groups of workers. Some of these are summarized in Table 4. Before estimating bone sodium content it was usual to remove calcium by precipitation, though simultaneous loss of sodium frequently occurred (Harrison, 1937; Forbes & Perley, 1951; Bergstrom & Wallace, 1954). When radiosodium became available it was possible to correct for loss of sodium with greater accuracy (Davies, Kornberg & Wilson, 1952*b*; Forbes & Lewis, 1955). The introduction of flame photometry did not immediately simplify the problem because of interference effects between calcium and sodium. In some experiments the calcium content has been measured separately on one half of a sample and a

correction applied for the interference with the sodium estimation on the remainder (Miller *et al.* 1954). Others prepared special standard solutions containing calcium as well as sodium and hoped to make this correction automatically (Bauer, 1954; Edelman *et al.* 1954). In these studies the interference filter minimized the effect of the calcium present and the calcium content of the same dilution was measured subsequently. The preliminary tests and recovery experiments confirm that the methods are satisfactory.

Apart from the difficulties of earlier chemical techniques, confusion has also arisen from the preparation of bone samples. The region from which the samples were taken has not always been specified and it is not always clear whether the marrow and cancellous bone have been removed. Cancellous bone has a higher mineral content than compact bone (Engstrom, 1956) and in the rapidly growing region of the epiphyseal plate the sodium content and the rate of sodium exchange is higher (Bauer, 1954). Because of the diminishing water content of older bones, it is misleading to express bone sodium and calcium content per unit of wet bone weight when comparing animals of different ages.

Others have shown that the calcium content of bone increases with age as the water content decreases (Hammett, 1925; Logan, 1935; Neuman & Neuman, 1953). Bergstrom & Wallace (1954), who calculated bone sodium and calcium content per kilogram of wet bone weight, also observed that bone sodium increased with age, but found that the Na:Ca ratio varied from 0·014 in young rats to 0·022 in their oldest animals. The values for bone sodium and calcium obtained in this study are in agreement with the recent reports of Nichols & Nichols (1956).

The results of the measurement of bone sodium exchange are in keeping with current conceptions of bone structure. It is known that bone mineral is not inert but undergoes constant turnover throughout life (Shohl, 1939; Hevesy, 1955). The processes governing ionic transfer between bone and extracellular fluid are related to the bone growth and the resorption and remodelling of osteones (Neuman & Neuman, 1957). Young osteones have a high uptake of 'bone-seeking' radio-isotopes (Jowsey, Owen & Vaughan, 1953). In young animals, whose bones contain a higher proportion of newly formed crystals, a relatively greater surface is available for exchange processes. Furthermore, the more highly hydrated, imperfect crystals are capable of more rapid exchange. With increasing age the bone crystals become larger, less hydrated and more compact. Thus a larger proportion of their surface is effectively isolated from the body fluids (Neuman & Neuman, 1957).

The sodium removed by the depletion procedure amounted to 25–30% of the total body content (Bergstrom & Wallace, 1954; Woodbury, 1956). The alterations in bone structure may account for the smaller release of sodium from the bones of the older rats and the apparently more effective action of

the bones as a sodium reservoir in the young. The latter, with the greater loss from the bones, showed no depression of serum sodium level, whereas there was a conspicuous fall in the serum of adults. When sodium is transferred from bones to the extracellular fluid after acute sodium depletion it is probable that the loss is from the exchangeable rather than the non-exchangeable bone sodium. However, in spite of the drop in bone sodium following acute sodium depletion, the amount of exchangeable bone sodium remains the same as in control rats. These results suggest therefore that, after depletion, some sodium in bone which was not originally available for exchange has become exchangeable so that the drop in bone sodium appears to be entirely at the expense of the non-exchangeable fraction.

The change in bone sodium content was not associated with a corresponding loss of calcium. The calcium content has been calculated as m-equiv/kg dry bone and therefore will not necessarily reflect loss of calcium from the body if a reduction in total bone weight has occurred. The Na:Ca ratio does, however, indicate a disproportionate loss of sodium. Similar results have been reported by other workers (Bergstrom, 1955; Levitt *et al.* 1956). As sodium is thought to be limited to the surface of bone crystals, it is likely that acute sodium depletion removes some of the superficial sodium, leaving the calcium undisturbed within the interior of the crystal (Neuman & Neuman, 1957).

Up to 24 hr there is a relatively rapid exchange of radio-sodium in the extracellular fluid with sodium in the bones and by the end of that time the specific activity of the bone sodium is 55% of the blood value. The duration of persistence of radio-sodium in the extracellular fluid and the rapidly exchanging portion of bone sodium depends on the intake of sodium. When this is low, as on the rice diet, the specific activity of the extracellular fluid remains high and further slow penetration of radio-sodium into the bones continues. If the intake of sodium is then increased, the radio-sodium is quickly lost from the extracellular fluid and the rapidly exchanging portion of bone sodium. On the other hand, any radio-sodium that has entered the slowly exchanging part in the bone is not affected by this rapid turnover. Following the elimination of the radio-sodium from the extracellular fluid the specific activity of the bone sodium relative to blood becomes extremely high (Green, Reynolds & Girerd, 1955).

The measurements made in rats fed with an increased amount of sodium after a prolonged period of restriction and in the rats treated with intraperitoneal saline injections showed that ^{22}Na had entered a part of the bone that was effectively isolated from the extracellular fluid. In their work with dogs, Edelman *et al.* (1954) did not observe any continuing entry of ^{22}Na into the ribs during a period of one month. This may be too short an interval for the detection of any increase in bone sodium exchange in a larger animal.

The persistance of ^{22}Na in man for some years, described by Miller *et al.* (1957), is most likely due to incorporation of the isotope in bone in a manner comparable to the process in rats.

SUMMARY

1. The composition of bone has been investigated in rats weighing between 50 and 450 g. The sodium and calcium content increased with age and the water decreased.

2. The proportion of bone sodium which exchanged with isotopes during the 24 hr after injection fell with increase in age, but because of the rise in total bone sodium content the fall in the absolute amount of exchangeable bone sodium was small.

3. After acute sodium depletion in both young and adult rats the total bone sodium content dropped, but the specific activity of bone sodium relative to blood increased. The absolute amount of exchangeable bone sodium remained constant. The changes in the bones were all greater in the younger animals, in which the serum sodium did not fall. In the older group the loss of sodium from bone was less and there was a decrease in serum sodium concentration.

4. In chronic experiments rats were kept on a rice diet for some weeks. The total bone sodium content fell steadily on the restricted diet, but the serum sodium only declined slightly after several weeks. There was a continued penetration of ^{22}Na into the bones. The calcium content was unaffected.

5. Some of the ^{22}Na entered a fraction of the bone which was not in free communication with the extracellular fluid. The excretion of ^{22}Na trapped in this way was not enhanced by the administration of large amounts of saline.

During this work R.S.S. was holding a research fellowship granted by Glaxo Laboratories (India) Priv. Ltd. We should like to thank Dr G. H. Jowett and Dr H. Miller for their advice and assistance. We are grateful for grants towards the purchase of isotopes and apparatus from the Tuberculosis Research Fund of the University of Sheffield, the Endowment Fund of the United Sheffield Hospitals and Glaxo Laboratories Ltd. Mr E. Salvin provided skilled technical assistance.

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[*Reprinted from the Journal of Physiology*,
1958, Vol. **142**, No. 3, p. 438.]
PRINTED IN GREAT BRITAIN

J. Physiol. (1958) 142, 438–446

THE EFFECT OF ADRENALECTOMY ON BONE SODIUM METABOLISM

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(*Received* 4 *February* 1958)

It has been appreciated for many years that the adrenal cortex plays an important role in sodium metabolism (Baumann & Kurland, 1927; Loeb, Atchley, Benedict & Leland, 1933). Many have shown that urinary sodium excretion increases after adrenalectomy because of diminished renal tubular reabsorption (Harrop, Soffer, Ellsworth & Trescher, 1933; Harrop, Weinstein, Soffer & Trescher, 1933; Harrison & Darrow, 1939). Others believe that this may not be the only route by which sodium is lost from the extracellular fluid and that intracellular sodium rises in adrenal insufficiency (Swingle, Parkins, Taylor & Hays, 1937; Grollman, 1954). Flanagan, Davis & Overman (1950) have speculated about bone as a possible site in which sodium might be sequestered after adrenalectomy as they did not find any increase in intracellular sodium in the soft tissues. In a later investigation (Stern, Cole, Bass & Overman, 1951) no conclusive evidence was obtained concerning the role of bone and the effect of adrenalectomy on bone sodium metabolism remained uncertain. The effect of acute sodium depletion on bone sodium metabolism in rats with intact adrenal glands has already been studied (Munro, Satoskar & Wilson, 1957), and this work has now been extended by investigating the changes brought about by adrenalectomy.

METHODS

Male albino rats weighing between 230 and 280 *g* were used throughout the experiments. Adrenalectomy was carried out under ether anaesthesia (Griffith & Farris, 1942). All the rats received a standard diet and NaCl 0·9 %(w/v) solution to drink for a week after the operation. Thereafter the rats were divided into groups maintained on different regimes so that they received saline solution, a rice diet with distilled water, or cortisone acetate by intramuscular injection, in various combinations. The details of the diets and the methods for chemical and radioactivity measurements have been described previously (Munro *et al.* 1957). Either ^{22}Na or ^{24}Na was used, depending on availability at the time of the measurements. In all cases when bone sodium exchange was being measured, the rats were allowed distilled water only from the time of intraperitoneal injection of radiosodium up to the time of killing.

RESULTS

In the first series of experiments, radiosodium was administered after adrenalectomy, and the effect of the operation on bone composition and on the exchange of bone sodium in a 24 hr period was studied in groups of rats maintained on different types of replacement therapy (Table 1). The results are compared with those in rats having intact adrenal glands.

Maintenance on saline. These rats were given 0·9% NaCl solution to drink, appeared healthy and were killed 10 days after the operation. During the final 24 hr, following radiosodium injection, distilled water only was provided. In comparison with normal animals, the serum sodium was slightly reduced and this difference was significant ($P < 0{\cdot}01$). The water, sodium and calcium contents of the bone were not altered. The relative specific activity of bone sodium was decreased slightly but significantly ($P < 0{\cdot}01$). The decrease in exchangeable bone sodium was not significant.

Maintenance on saline and cortisone. The rats in this group received saline as drinking water and cortisone acetate, 2 mg daily by intramuscular injection, for 10 days after adrenalectomy and were then killed. At the commencement of the final 24 hr, when radiosodium was injected, they also received the final injection of cortisone, but thereafter were only allowed distilled water. These rats appeared entirely healthy and showed no biochemical abnormalities. In comparison with the previous group, the addition of cortisone restored the serum sodium concentration and the bone radiosodium exchange to the normal levels.

Maintenance on rice diet. For the first week after operation the rats were maintained on a normal diet and saline solution. They were then changed to a rice diet and distilled water, and within 2 or 3 days the majority became ill. The chief features were inactivity and coldness. They were killed up to 145 hr after the change of treatment. The results in Table 1 refer to rats maintained 36 hr or longer on the rice diet. Radiosodium was given 24 hr before the anticipated time of killing but some rats deteriorated rapidly and were killed before the radiosodium was given or before the full period of 24 hr had elapsed. All the rats on the restricted sodium intake lost weight; the mean loss during this period was 4·9 g/day. Those which were active at the time of death had higher serum sodium concentrations than those which were sluggish, but in all cases the values were depressed and the mean was significantly lower than in the other groups (in all cases $P < 0{\cdot}001$). The bone water and sodium were significantly lower than in normal animals ($P < 0{\cdot}001$). The bone sodium fell rapidly in the 24–48 hr period following withdrawal of saline, but thereafter little further decrease was apparent, even though the serum sodium continued to fall (Fig. 1).

The exchange of bone sodium with radiosodium after a 24 hr equilibration

TABLE 1. Bone composition of rats after adrenalectomy in comparison with normal controls. The mean values and standard errors are shown

Condition	Diet	Cortisone acetate (mg/day)	No. of rats	Wt. at death (g)	Serum sodium (m-equiv/l.)*	Bone water content (% of wet wt.)	Bone calcium (m-equiv/kg dry wt.)	Bone sodium (m-equiv/kg dry wt.)	Na/Ca quotient	Specific activity of bone sodium relative to blood (%)	24-hr exchangeable bone sodium (m-equiv/kg dry wt.)
Normal	Standard and water	0	10	246	149·3 (10)	16·8	12847	256·3	0·0200	54·7	142·0
S.E.	—	—	—	8	0·8	0·6	91	2·2	0·0002	0·7	4·3
Adrenalectomized	Standard and 0·9% saline	0	10	261	143·6 (5)	16·3	13193	261·2	0·0195	51·1	133·4
S.E.	—	—	—	6	1·8	0·6	128	4·5	0·0004	0·9	3·6
Adrenalectomized	Standard and 0·9% saline	2·0	10	252	147·0 (5)	16·8	13084	260·2	0·0198	53·8	139·7
S.E.	—	—	—	5	1·4	0·7	157	4·7	0·0002	1·0	3·0
Adrenalectomized	Rice diet and distilled water for 36 hr	0	3	245	141·0 (3)	14·6	12568	237·7	0·0189	47·0	111·6
S.E.	—	—	—	2	1·0	0·3	8	1·2	0·0001	2·5	5·4
Adrenalectomized	Rice diet and distilled water for 48 hr or more	0	19	239	131·7 (18)	13·2	12701	222·1	0·0175	42·0	93·1
S.E.	—	—	—	2	1·8	0·2	56	2·1	0·0002	2·0	3·3
Adrenalectomized	Rice diet and distilled water for 72 hr	2·5	9	252	146·0 (8)	15·1	12765	236·1	0·0185	53·8	126·6
S.E.	—	—	—	7	0·9	0·2	107	4·3	0·0003	1·6	2·5
Adrenalectomized	Rice diet and distilled water for 108 hr	1·25	3	235	137·0 (3)	12·8	12663	230·3	0·0182	43·6	100·7
S.E.	—	—	—	5	2·1	0·6	168	1·2	0·0001	3·8	9·0

* The figures in parentheses refer to the number of rats in which serum Na was determined.

period was conspicuously reduced in the adrenalectomized rats given the rice diet and distilled water (Table 1). Four adrenalectomized rats were killed whilst still active at shorter intervals after the radiosodium injection, which was given 48 hr after the change of diet, and the progress of bone exchange during these shorter equilibration periods was compared with that in normal rats of similar size injected at the same time (Fig. 2). In the adrenalectomized animals the whole process was much slower.

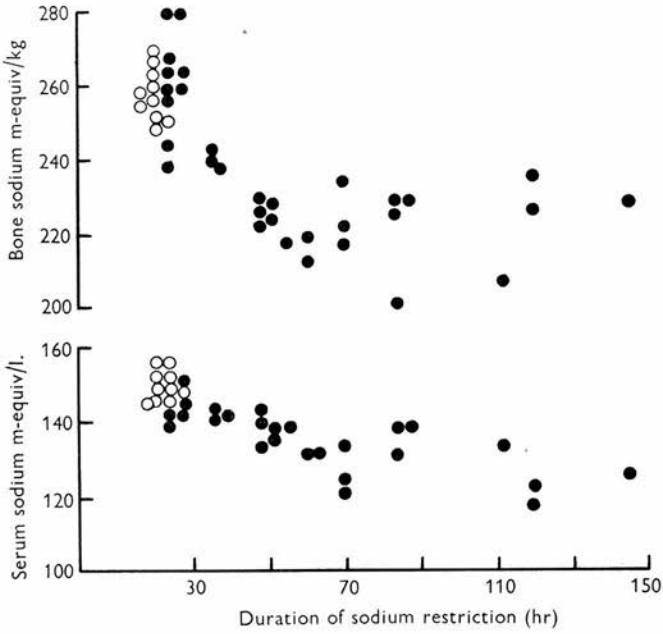

Fig. 1. The fall in bone sodium and serum sodium in adrenalectomized rats on rice diet and distilled water: ●, adrenalectomized rats; ○, normal rats.

Maintenance on rice diet and cortisone. In this group the rats were given three daily intramuscular injections of 2·5 mg cortisone. The first injection was given when the rice diet and distilled water were substituted for the standard diet and saline solution. The animals remained active and were killed after 72 hr on the restricted saline intake. The radiosodium was injected 24 hr before death. The serum sodium was almost at the normal level. The bone sodium content was significantly reduced ($P < 0{\cdot}001$). As the relative specific activity of bone sodium remained within the normal range there was also a significant decrease in the exchangeable bone sodium ($P < 0{\cdot}01$).

Three rats placed on the rice diet and distilled water were given 1·25 mg cortisone daily and were killed after 108 hr. They remained active and in fair general condition while alive, and showed biochemical changes intermediate between those seen in the two previous groups.

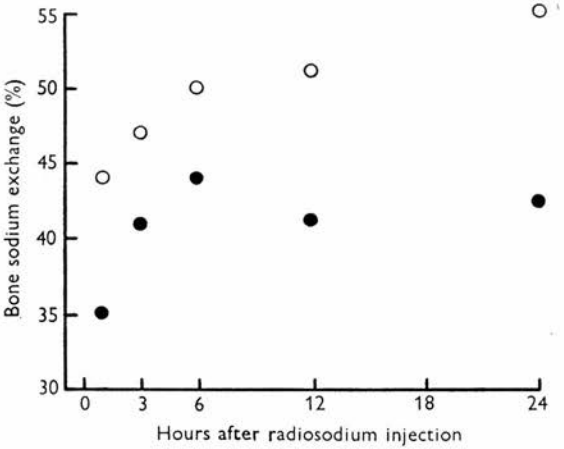

Fig. 2. Bone sodium exchange in normal (○) and adrenalectomized (●) rats shown as a percentage of bone specific activity relative to blood. The rats had been on rice diet and distilled water for 48 hr preceding intraperitoneal injection of radiosodium given at zero time on the graph.

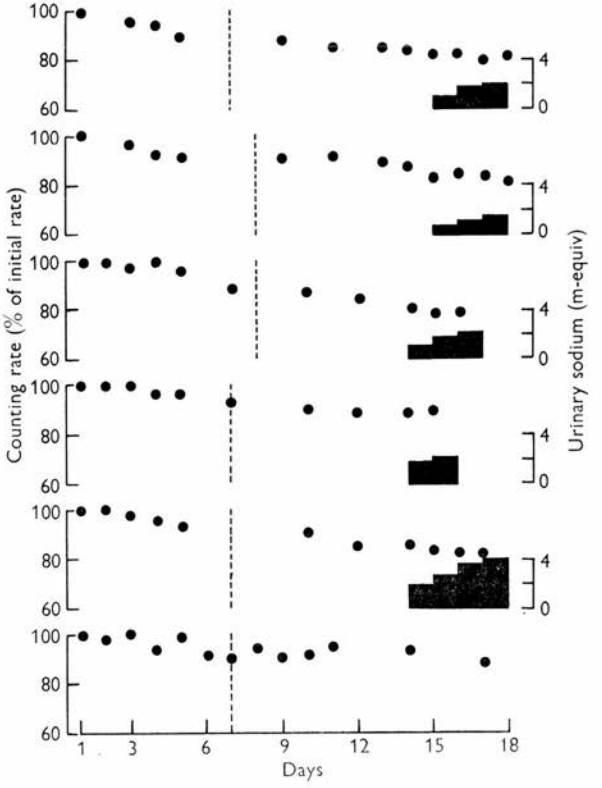

Fig. 3. Total body radioactivity in rats with ^{22}Na incorporated into bones. Measurements begun 16 weeks after intraperitoneal injection of ^{22}Na. The dotted line indicates the day of adrenalectomy. Five rats received rice diet for the last 3–4 days. The cumulative loss of sodium in the urine during this period is shown.

Release of ^{22}Na from bones after adrenalectomy

In the second series of experiments, the effect of adrenalectomy was studied in twelve rats previously given ^{22}Na so that it was incorporated in their bones. Shortly after weaning these rats were placed on a diet of rice and distilled water, and 4 days later were injected with ^{22}Na. The restricted sodium intake was maintained for a further 7 days. Thereafter they were given a normal diet.

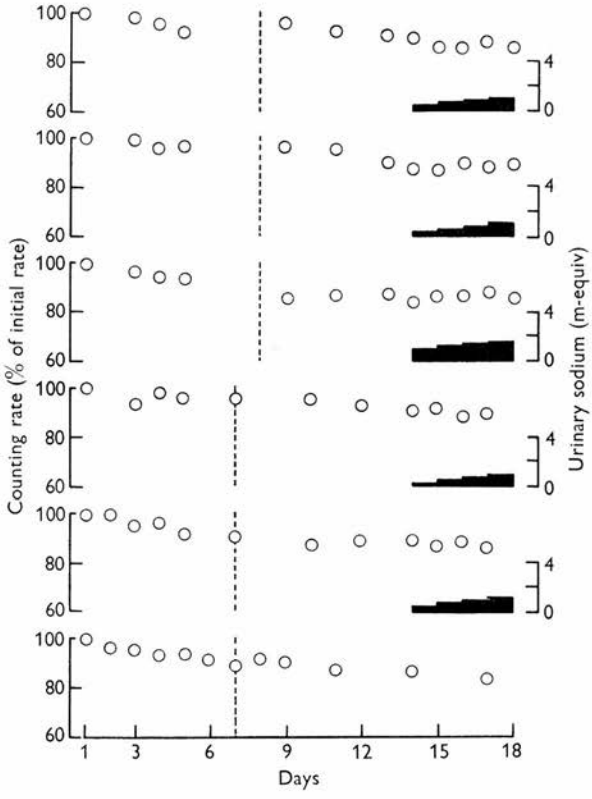

Fig. 4. Total body radioactivity in control rats with ^{22}Na incorporated into bones. Measurements begun 16 weeks after intraperitoneal injection of ^{22}Na. The dotted line indicates the day of sham operation. Five of the rats received rice diet for the last 4 days. The cumulative loss of sodium in the urine during this period is shown.

Earlier work has shown that under these circumstances some ^{22}Na is retained in a slowly exchanging fraction of bone sodium (Munro *et al.* 1957). Measurements of whole-body radioactivity were begun 16 weeks after the radiosodium injection (Figs. 3, 4). After some preliminary observations, bilateral adrenalectomy was carried out in six rats and in the remainder similar incisions were made in the back and the adrenals were exposed but left intact. All the rats were then given NaCl 0·9% (w/v) solution as drinking water and the

measurements were continued for a week. The rates of loss of radioactivity from the body after operation were not significantly different in the two groups; over the week the decrease in total body counts in the adrenalectomized rats was 16·8% ± 1·6 (S.E.) and in the sham-operated rats 13·8% ± 1·0. Finally, five rats from each group were placed on the diet of rice and distilled water. The adrenalectomized animals excreted more sodium in the urine and their condition rapidly deteriorated. During this period the rate of loss of ^{22}Na was still similar in the two groups. Measurements made on blood and bone samples after killing the animals confirmed that the radiosodium was confined to the skeleton.

DISCUSSION

Changes in bone composition were only seen in rats receiving a low sodium intake after adrenalectomy. These developed quickly in contrast to the slow changes, developing over several weeks, seen in normal rats placed on a salt-deficient diet (Munro *et al.* 1957). Rats receiving cortisone and saline showed no abnormalities. In those receiving saline only, a slight drop in serum sodium and in bone sodium exchange occurred, but they were given distilled water only, during the 24 hr period following the injection of radiosodium, and a minor degree of sodium depletion may have developed during this time. In the rats receiving a severely restricted sodium intake and cortisone, the changes resembled, but were not quite so severe as, those produced in rats with intact adrenal glands depleted of sodium by peritoneal lavage (Munro *et al.* 1957). The total bone sodium, the bone water and the serum sodium were all reduced, but the bone sodium specific activity relative to blood remained within the normal range. Adrenalectomy followed by sodium restriction and no replacement therapy produced the most severe decreases in serum sodium and in bone sodium content. The striking feature in this group was the fall in bone sodium exchange with radiosodium, and this contrasts sharply with the observations in the cortisone-treated, salt-deprived group, and in the salt-depleted rats with intact adrenals. The reason for this difference is not immediately apparent but a similar finding was reported in an adrenalectomized dog (Stern *et al.* 1951). An early decrease in total bone sodium content occurred, but after 48 hr on the sodium-deficient diet there was no further release of sodium from the bone. In most cases the radiosodium was only injected during this latter period after the fall in bone sodium content had occurred. The diminished entry of radiosodium into the bone, the cessation of release of sodium from the bone and the considerable decrease in bone water suggest that circulatory deficiency in the bone may be an explanation for the observed changes. The cold and sluggish condition of the rats confirmed that a considerable depression of the peripheral circulation had developed.

Previous work had shown that the release of ^{22}Na embedded in infancy in the slowly exchanging fraction of bone sodium was not increased in intact rats

by giving large amounts of saline (Munro *et al.* 1957). Similarly, adrenalectomy did not alter significantly over relatively short periods the rate at which ^{22}Na was set free from the bones, even though urinary sodium excretion was greatly increased.

The available evidence does not suggest that adrenalectomy has any primary effect on bone sodium metabolism. However, if salt replacement is inadequate, sodium may be withdrawn from the bone in an attempt to meet the deficiency, as in rats with intact adrenal glands. If the condition of the rat is allowed to deteriorate, the exchange of bone sodium with the sodium in the extracellular fluid is depressed and further withdrawal of sodium from bone cannot readily occur.

The effect in man of adrenal insufficiency on bone sodium exchange is not known, but in health the amount of bone sodium that exchanges readily with sodium isotopes is fairly constant (Miller, Munro, Renschler & Wilson, 1954). Measurements of exchangeable sodium, made in a patient recovering from acute adrenal insufficiency following the administration of cortisone, showed an increase which exceeded the alterations observed in the extracellular fluid (Wilson & Miller, 1953). This may have been due partly to an increase in the amount of bone sodium available for exchange with radiosodium and suggests that the features observed in the rat and dog may have their human counterpart.

SUMMARY

1. No abnormalities in bone composition or bone sodium exchange with radiosodium were found in rats maintained after adrenalectomy on saline and cortisone acetate. The serum sodium concentration remained normal.

2. A decrease in serum sodium concentration and in bone sodium and water content occurred in all rats not given saline to drink. Rats given a salt-deficient diet supplemented with cortisone acetate remained active and the bone sodium exchanged normally with radiosodium. Rats given a salt-deficient diet without cortisone became inactive and cold. They showed a decrease in the rate of exchange of bone sodium with radiosodium.

3. Adrenalectomy did not alter the rate of release of ^{22}Na previously incorporated into the slowly exchanging fraction of bone sodium.

4. Alterations in bone sodium metabolism only occurred after adrenalectomy, when sodium deficiency was allowed to develop.

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Letters to the Editor

THE HUMAN USE OF ^{22}Na

SIR,—Dr. Martin and his associates in your last issue draw attention to the great advantages of the use of ^{22}Na in the study of sodium metabolism. There is no doubt that it could be of the greatest assistance in the investigation of many clinical problems. They rightly comment on misgivings about the use of this long-half-life isotope in man. Clearly, the danger depends on the extent to which it may be retained in the body.

In the course of an experiment in September, 1955, on one of ourselves, after a dose of 50 microcuries of ^{22}Na, we followed changes in sodium metabolism using both the cumulative balance technique and the whole-body counter. In addition we attempted to study sodium exchange in the patella by an external counting technique. The results suggested that a small portion of the dose was taking part in an extremely slow exchange with a fraction of the sodium in bone.

The external counting technique indicated that the effective half-life of the ^{22}Na in the body at the end of fifty days was approximately ten days. This method was not sensitive enough to give an accurate measurement of the residual activity of the body after a long period. Observations have been made, however, in the high-pressure ionisation chamber system of the Department of Medical Physics at Leeds. The following figures have been obtained with this instrument by Dr. P. R. J. Burch, Medical Research Council fellow:

Days after injection of ^{22}Na	*Body content of ^{22}Na*
256	0·055 μc.
290	0·052 μc.
469	0·031 μc.

We are grateful to Dr. Burch for allowing us to quote his observations.

The results suggest that the body retained over a long period about 0·1% of the injected dose, which has a biological half-life of approximately one year

The radiation dose involved in this test is of the order of 0·8 rads to the whole body from the radiosodium undergoing turnover in the extracellular fluid. For the very slow phase in bone, the dose is bigger, but if it be assumed that the specific activity of that sodium in the bone involved in the long-term exchange approximates to that of the plasma at its highest value, a dose of 14·0 rads appears to be the upper limit in the small volume of bone in which the ^{22}Na is deposited.

This radiation dose is in the range which may be hazardous, though the matter is at present controversial. Nevertheless, the dangers associated with long-continued irradiation of bone are well recognised.

In view of these results it would seem advisable in future to limit the use of this isotope in clinical investigation to special circumstances where information essential for treatment of the patient is not otherwise available.

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[*Reprinted from the Journal of Physiology*,
1956, Vol. **133**, No. 1, p. 194.]
PRINTED IN GREAT BRITAIN

J. Physiol. (1956) 133, 194–201

A COMPARISON OF THE DISTRIBUTION OF ^{42}K AND ^{86}Rb IN RABBIT AND MAN

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(*Received* 12 *March* 1956)

Many investigations of potassium metabolism with the isotope ^{42}K have been hampered by the short half-life of 12·5 hr. Nevertheless, its distribution throughout animal tissues has been examined extensively (Noonan, Fenn & Haege, 1941; Fenn, Noonan, Mullins & Haege, 1941) and the exchangeable potassium content of the body has been measured by the dilution method (Corsa, Olney, Steenburg, Ball & Moore, 1950). However, in some tissues potassium exchange is slow and an equilibrium of distribution is not attained within the period of study possible after injection of a tracer dose. Similar problems in studying sodium metabolism with the short half-life ^{24}Na have been solved with the isotope ^{22}Na (Burch, Ray & Threefoot, 1952; Miller, Munro, Renschler & Wilson, 1954). In the case of potassium no suitable long half-life isotope is available.

Rubidium, another alkali metal, is widely distributed in animal tissues (Sheldon & Ramage, 1931; Bertrand & Bertrand, 1946) and can be substituted for potassium in some structures without impairing their function (Ringer, 1883; Sandow & Mandel, 1951). The alkalosis occurring in rats deprived of potassium can be corrected by the administration of rubidium (Relman, Roy & Schwartz, 1955). The isotope ^{86}Rb has a convenient half-life of approximately 19 days and is suitable for tracer work as it emits β and γ radiation (β 1·82, 0·72 MeV, γ 1·08 MeV). Its distribution and turnover in human blood and urine have been studied (Zipser, Pinto & Freedberg, 1953). It is taken up by red blood corpuscles in a similar proportion to potassium and at the same rate (Love & Burch, 1953). The rates of disappearance of the two isotopes from the plasma after intravenous injections in man are similar

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(Burch, Threefoot & Ray, 1955). The extent to which ^{86}Rb may act as a substitute for ^{42}K in tracing potassium throughout the body has accordingly been studied at first in rabbits and subsequently less extensively in man.

METHODS

^{86}Rb was obtained from the nuclear reactor at Harwell, the target material being rubidium carbonate. The counting rate from a solution was measured in a liquid counter (Veall, 1948) over 168 days. A small proportion of the activity came from a contaminant of longer half-life, probably ^{134}Cs. Subsequent calculations showed that this contaminant accounted for 0·3% of the counts recorded at the time of the experiments. The radioactive half-life of ^{86}Rb from these measurements was 18·4 days in contrast to 19·5 days as previously quoted (Atomic Energy Research Establishment, 1954). A period of 18·66 days has recently been found by Emery, Bradley & Veall (1955).

^{42}K, also from Harwell, was prepared from potassium carbonate. Mixtures of the two isotopes, containing approximately 20 μc of ^{86}Rb and 50–200 μc of ^{42}K, were injected intravenously into sixteen rabbits, the higher doses of ^{42}K being used for the longer periods of study. In four animals the isotope mixture was delivered by tube into the stomach.

The animals were killed in groups of five, at intervals after administration of the isotopes. Blood samples were obtained immediately before death from the marginal vein of the ear not injected. Portions of tissues were taken, weighed and dissolved by gentle heating in about 15 ml. of distilled water, to which was added 1 ml. of concentrated nitric acid per gram of tissue. The final volume was measured and the solution filtered. The filtrate was used for counting and for potassium estimation by flame photometry. The addition of this quantity of nitric acid did not affect the latter determination. The radioactivity of the solutions containing a mixture of the two isotopes was measured immediately in a Veall liquid counter and 10 days later when the ^{42}K content had decayed to a negligible proportion. Twelve thousand counts were recorded or, in examining samples of low activity such as bone, brain and spleen, counting was continued for 20 min. The effect of the specific gravity of the solutions on the counting rates of the two isotopes was investigated as described by Rose & Emery (1951). The measurements from ^{86}Rb were depressed to a greater extent than those from ^{42}K by increases in the specific gravity. The specific gravity of all the tissue solutions was measured and the necessary correction applied. This was less than 10% for ^{86}Rb and less than 5% for ^{42}K.

In the human observations, 30 μc of ^{86}Rb and 100 μc of ^{42}K were delivered simultaneously from calibrated burettes into a funnel connected to an intravenous infusion apparatus. Samples of urine were collected at intervals after the injection and examined in the same way as the rabbit tissue solutions.

RESULTS

Distribution of ^{42}K *in rabbits*

After intravenous injection ^{42}K exchanges rapidly with the potassium in the body. The potassium content of several tissues is shown in Table 1; the wide variation in the spleen is conspicuous. The bulk of the body potassium lies in skeletal muscle and the exchange in other tissues and fluids has accordingly been compared with it (Table 2). The specific activity of each tissue, that is the counting rate per m-equiv of potassium, was calculated. From 20 hr onwards the values from most tissues were in close agreement. Brain and red cells gave consistently lower results but slowly approached the levels of the other tissues up to 62 hr, the time limit of the observations. After administration of ^{42}K by stomach tube the same distribution was achieved. The urine samples

were taken from the bladder at dissection. Often the bladder was greatly distended and contained urine secreted many hours previously. In such circumstances close agreement between urine and other specific activities could not be expected.

TABLE 1. Potassium concentration in tissues of the rabbit

Tissue	m-equiv/kg wet tissue	S.D.	No. of observations
Muscle	109·1	9·3	15
Spleen	97·0	79·4	19
Brain	87·4	8·3	19
Ileum	75·9	10·5	19
Lung	74·5	10·5	8
Liver	74·4	8·5	15
Heart	71·7	6·0	16
Kidney	63·3	9·6	16

TABLE 2. Mean specific activity of ^{42}K in rabbit tissues and fluids relative to skeletal muscle

Time after administration ...	8 hr	20 hr		39 hr		62 hr
Route	Intra-venous	Intra-venous	Intra-gastric	Intra-venous	Intra-gastric	Intra-venous
No. of rabbits	5	3	2	3	2	5
Muscle	1·00	1·00	1·00	1·00	1·00	1·00
Plasma	1·29	—	1·62	—	1·62	1·00
Brain	0·25	0·37	0·38	0·52	0·56	0·63
Red blood cells	0·42	0·64	0·68	0·70	0·81	0·89
Urine from bladder	1·06	—	1·38	—	1·05	1·22
Spleen	1·64	1·12	1·36	0·97	0·98	0·90
Lung	1·33	—	1·31	—	1·05	0·95
Heart	1·36	1·13	1·31	0·95	1·00	0·98
Intestine	1·57	1·04	1·47	0·89	1·10	0·98
Kidney	1·30	1·25	1·39	1·09	1·00	1·19
Liver	1·49	0·85	1·40	0·89	1·01	0·97

Distribution of ^{86}Rb *in rabbits*

The radioactivity due to ^{86}Rb has been measured in the same tissue and fluid samples and the results have been expressed as the counting rate per m-equiv of potassium. When the ^{86}Rb concentration in the various samples is compared with that of skeletal muscle there is a wide range of variation (Table 3). These differences persist with the passage of time. In the four rabbits receiving the isotopes through the stomach the ultimate distribution of ^{86}Rb resembled that in the other animals.

The distribution of ^{86}Rb has also been examined in relation to ^{42}K. The ratio $^{86}Rb/^{42}K$ was determined in the tissues and in the injection mixture. When the ratio in the various samples is divided by the injection ratio the result is a measure of the relative concentration of the two isotopes. A value greater than unity indicates that ^{86}Rb is concentrated relative to ^{42}K and the reverse holds for values below one. The results are shown in Table 4. The pattern of distribution of ^{86}Rb and ^{42}K is already achieved by 8 hr. An analysis of variance

shows that the differences between tissues are highly significant throughout the whole series ($F = 244$, $P \ll 0{\cdot}001$). The differences between the groups killed at the different times are also significant ($F = 4{\cdot}3$, $P < 0{\cdot}01$) except between 39 and 62 hr where no difference is apparent ($F = 2{\cdot}8$, $P > 0{\cdot}1$). The last two sets of values have accordingly been taken together and are shown in Table 5.

TABLE 3. ^{86}Rb activity per m-equiv of potassium in rabbit tissues relative to skeletal muscle

Time after administration ...	8 hr	20 hr		39 hr		62 hr
Route	Intra-venous	Intra-venous	Intra-gastric	Intra-venous	Intra-gastric	Intra-venous
No. of rabbits	5	3	2	3	2	5
Muscle	1·00	1·00	1·00	1·00	1·00	1·00
Plasma	1·37	1·04	1·46	0·86	0·97	0·90
Brain	0·21	0·22	0·30	0·31	0·34	0·40
Red blood cells	0·48	0·58	0·74	0·69	0·78	0·91
Urine from bladder	1·04	—	1·37	—	1·00	0·79
Spleen	2·39	1·45	1·95	1·22	1·28	1·13
Lung	2·16	—	2·18	—	1·33	1·23
Heart	2·21	1·38	1·98	1·24	1·21	1·19
Intestine	2·26	1·41	2·38	1·30	1·45	1·40
Kidney	2·88	1·66	3·25	2·11	2·14	2·22
Liver	4·60	2·10	4·46	2·30	2·66	2·38

TABLE 4. Ratio: $\frac{^{86}\text{Rb}/^{42}\text{K in tissues or fluids}}{^{86}\text{Rb}/^{42}\text{K in injection mixture}}$

Time after administration ...	8 hr	20 hr		39 hr		62 hr
Route	Intra-venous	Intra-venous	Intra-gastric	Intra-venous	Intra-gastric	Intra-venous
No. of rabbits	5	3	2	3	2	5
Brain	0·67	0·60	0·64	0·62	0·61	0·63
Urine from bladder	0·77	—	0·77	—	0·92	0·65
Bone (femur)	0·82	0·58	0·68	0·59	0·76	0·81
Plasma	0·87	—	0·71	—	0·59	0·90
Muscle	0·79	1·09	0·79	1·04	0·97	0·99
Red blood cells	0·91	0·94	0·86	1·03	0·95	1·02
Heart	1·30	1·26	1·20	1·37	1·20	1·20
Spleen	1·16	1·34	1·14	1·30	1·22	1·23
Lung	1·30	—	1·31	—	1·22	1·26
Intestine	1·15	1·42	1·30	1·52	1·27	1·41
Kidney	1·79	1·49	1·85	2·00	2·08	1·88
Liver	2·46	2·55	2·57	2·70	2·56	2·43

^{86}Rb is concentrated more than ^{42}K in liver, kidney, intestine, heart and spleen. The two isotopes are present in equal proportions in muscle and red blood cells. The concentration of ^{86}Rb is less in brain, bone and urine.

In the four animals given the isotopes by stomach tube the dose was measured and fully washed in. Several of the viscera were removed intact and their content of ^{42}K and ^{86}Rb has been calculated as a percentage of the dose. The results again demonstrate the uneven distribution of the two isotopes in the body (Table 6). Lungs, spleen and liver contain 8·2% of the administered ^{86}Rb as against 3·8% of the ^{42}K.

Observations in man

In two subjects in whom measurements of potassium exchange were being made, ^{86}Rb was given simultaneously with ^{42}K. Frequent specimens of urine were collected separately up to 48 hr.

TABLE 5. Ratio: $\frac{^{86}Rb/K^{2} \text{ in tissues}}{^{86}Rb/^{42}K \text{ in injection mixture}}$ determined in samples taken 39 and 62 hr after administration of the isotopes

Tissue	Distribution ratio	S.D.	No. of samples
Brain	0·62	0·05	10
Urine from bladder	0·65	0·15	6
Bone	0·73	0·27	10
Muscle	1·01	0·06	10
Red blood cells	1·02	0·05	10
Spleen	1·26	0·09	9
Heart	1·27	0·15	10
Intestine	1·45	0·16	10
Kidney	1·92	0·21	10
Liver	2·53	0·24	10

TABLE 6. Percentage of intragastric dose of ^{86}Rb and ^{42}K in organs of four rabbits

	Rabbit 1		Rabbit 2		Rabbit 3		Rabbit 4	
Time (hr) ...	19		19		39		39	
Weight (kg)...	2·35		2·00		2·40		2·00	
	^{86}Rb	^{42}K	^{86}Rb	^{42}K	^{86}Rb	^{42}K	^{86}Rb	^{42}K
Heart	0·42	0·37	0·41	0·32	0·28	0·24	0·31	0·25
Liver	9·53	4·36	10·02	3·39	3·97	1·66	7·06	2·63
Kidneys	1·30	0·75	1·63	0·84	1·00	0·44	1·14	0·60
Brain	0·07	0·10	0·13	0·20	0·13	0·19	0·14	0·26
Spleen	0·09	0·08	0·16	0·13	0·06	0·50	—	—
Lungs	0·95	0·72	0·74	0·57	0·51	0·42	0·54	0·45

The ^{42}K specific activity was measured in each sample and the exchange of the isotope with the potassium in the body was estimated by the formula

$$\text{Potassium exchange m-equiv} = \frac{^{42}K \text{ injected} - ^{42}K \text{ excreted}}{^{42}K \text{ per m-equiv of potassium in urine sample}}.$$

If ^{86}Rb were distributed in the same proportion as ^{42}K throughout the potassium of the human body a similar result would be obtained by substituting the values for ^{86}Rb in the above equation. This has been done and the results with both isotopes are shown in Figs. 1 and 2. The curves are conspicuously different, the values calculated from ^{86}Rb being higher than the true potassium exchange measured with ^{42}K. When the ratio $^{86}Rb/^{42}K$ in the urine samples is divided by the ratio in the injection mixture the mean value in the first subject is $0·66 \pm 0·055$ (S.D.) and in the second $0·64 \pm 0·11$. These results are similar to those found in rabbit urine (Table 5), and show that in man also a smaller proportion of ^{86}Rb than of ^{42}K is excreted in the urine. Furthermore, in Fig. 2

there is a swing in the ^{86}Rb curve not observed with ^{42}K, indicating that the excretion of the two isotopes is not rigidly related. A less conspicuous swing is also apparent in Fig. 1.

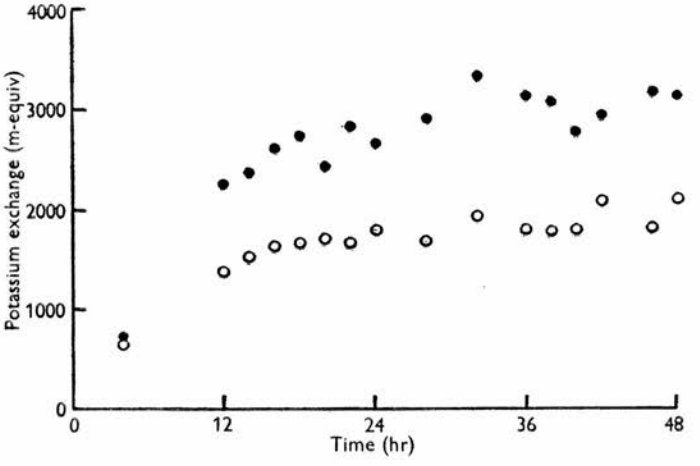

Fig. 1. Potassium exchange in a male 32 years weighing 56·4 kg. ○, values from ^{42}K; ●, values from ^{86}Rb. The results from ^{86}Rb are consistently above those obtained with ^{42}K.

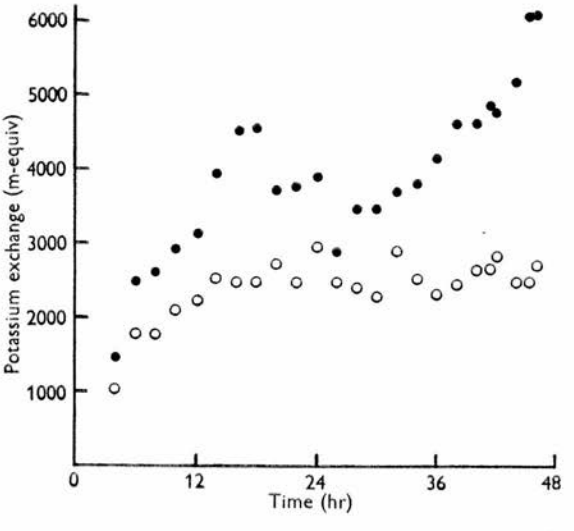

Fig. 2. Potassium exchange in a female 33 years weighing 40·9 kg. ○, values from ^{42}K; ●, values from ^{86}Rb. The results from ^{86}Rb are higher and show conspicuous variations not apparent in the ^{42}K measurements.

DISCUSSION

After intravenous injection the concentrations of ^{42}K are at first higher in the viscera than elsewhere but thereafter the isotope gradually becomes more evenly distributed throughout all the organs. At 62 hr the ^{42}K specific activities of the rabbit tissues, except for brain and red blood cells, are in close agreement. The slower exchange of the isotope with the potassium in these tissues has been reported previously (Fenn *et al.* 1941; Corsa *et al.* 1950; Katzman & Leiderman, 1953). The ^{86}Rb is at this time unevenly distributed in relation to both the potassium of the body and the simultaneously administered ^{42}K. It is concentrated more than ^{42}K in liver, kidney and intestine and less in brain, bone and urine. The two isotopes follow a similar course of distribution only in skeletal muscle and red blood cells. In the dog the distribution of ^{86}Rb has been investigated in relation to the body potassium but not simultaneously with ^{42}K (Love, Romney & Burch, 1954). Concentration occurred in the liver and kidney but was less than that seen in these experiments in the rabbit.

In both human and rabbit urine the proportion of ^{86}Rb being excreted was less than that of ^{42}K. The changing pattern of excretion of ^{86}Rb showed that it did not necessarily follow potassium. Attempts have been made to calculate the exchangeable potassium mass of the body from measurements of the potassium and ^{86}Rb content of erythrocytes (Threefoot, Ray & Burch, 1955). However, the conspicuously uneven distribution of ^{86}Rb in relation to the potassium of the viscera shows that rubidium does not follow the same course as potassium in the body. Though in some ways the two cations may behave similarly, rubidium has its own characteristic behaviour and clearly cannot be regarded as a reliable tracer for potassium in the whole organism.

SUMMARY

1. The distribution of mixtures of ^{42}K and ^{86}Rb injected intravenously into rabbits was investigated in various tissues.

2. ^{42}K exchanged completely within 62 hr with the potassium in all tissues except brain and red blood cells. ^{86}Rb was not evenly distributed throughout the potassium in the body. It was concentrated more than ^{42}K in liver, kidney, intestine, heart and spleen and less in brain and bone. In muscle and red cells the two isotopes were present in equal proportions.

3. Similar results were obtained after giving the mixture by stomach tube.

4. In two human studies the urinary excretion of ^{42}K and ^{86}Rb was disproportionate. The latter was unsuitable for use in the measurement of the exchangeable potassium mass of the body.

We should like to thank Dr H. Miller for his advice and assistance in this work. We are grateful for research grants towards the purchase of the isotopes from the United Sheffield Hospitals Endowment Fund and from the Research Fund of the Sheffield Regional Hospital Board.

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Reprinted from
PHYSICS IN MEDICINE AND BIOLOGY, Vol. 2, No. 3, p. 239, January 1958

The Assay of Mixtures of Sodium-24 and Potassium-42 in Clinical Tracer Studies; with Particular Reference to the Measurement of Exchangeable Sodium and Potassium

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§ 1. INTRODUCTION

IN metabolic studies in man an investigation of the distribution of sodium and potassium ions is often required at the same time, as in the simultaneous measurement of exchangeable sodium and potassium (Edelman *et al.* 1952). This involves the measurement of the radioactivity of mixtures of sodium and potassium isotopes in various biological fluids. The available isotopes are ^{42}K (half-life 12·4 hours), ^{22}Na (half-life 2·6 years) and ^{24}Na (half-life 15·0 hours). It is a simple matter to carry out tracer studies using ^{42}K and ^{22}Na at the same time, as the amounts of these two isotopes can be determined by recounting the samples after the short half-life potassium isotope has decayed to a negligible quantity. However, repeated measurements may be required as the condition of the patient changes and many investigators may be reluctant to use the long half-life sodium isotope (Veall *et al.* 1955, Miller *et al.* 1957). It is accordingly of some importance in clinical investigation to develop convenient methods for the simultaneous use of the two short half-life isotopes, ^{24}Na and ^{42}K.

The individual measurement of the activities of the two isotopes has been achieved by chemical precipitation of potassium, first as cobaltinitrite and then as tartrate (James *et al.* 1954), or by separation on an ion exchange column (Arons *et al.* 1954). A more rapid method depending on the single precipitation of potassium with sodium tetraphenylboron has been developed. A preliminary account of this work has been published (Munro *et al.* 1955) and it is here presented in detail.

Physical methods of separation depend on the differences which exist between the radioactive properties of isotopes (Keynes and Lewis 1951, Tait and Williams 1952). ^{42}K emits more energetic β particles, which have greater penetrating properties than those of ^{24}Na. Two counting systems can be constructed with a G.M. tube arranged so that a filter may be placed between the counter and sample. This principle has been applied to end-window counting of samples dried on planchettes (Keynes and Lewis 1951, Tait and Williams 1952, James *et al.* 1954),

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and its extension to use in liquid counter tubes is described here. Scintillation counters measure chiefly γ radiation. ^{24}Na emits more γ-rays of greater energy than ^{42}K and the isotopes may be separated on this basis. It is, however, more satisfactory to use a scintillation counter in combination with a G.M. tube (Robinson *et al.* 1955). These physical methods have been compared from the point of view of theoretical advantage, accuracy in practice, and convenience.

§ 2. Experiments on Mixtures

2.1. *Preparation of Mixtures*

^{24}Na and ^{42}K, prepared from spectroscopically pure sodium and potassium carbonate, were obtained from Harwell. For liquid counting, a solution of each isotope was made containing initially 5·5 μc ^{24}Na/litre and 1·8 μc ^{42}K/litre. In addition each solution contained 15 m.equiv/litre of both sodium and potassium and the target material added was less than 1/100 of the total. Portions of these solutions were mixed by volume in the ratios 1 : 9, 1 : 1, and 9 : 1, each in duplicate. More highly active solutions were prepared for end window counting; they contained 120 μc ^{24}Na/litre and 140 μc ^{42}K/litre and were mixed in the same proportions.

2.2. *Counting Equipment*

The following types of counter were used :

1. Geiger Müller liquid counter tubes of wall thickness 30 mg/cm^2, as described by Veall (1948).

2. G.M. liquid counter tubes of a similar type, but with the wall thickness increased to 200 and 300 mg/cm^2 (20th Century Electronics).

3. A G.M. liquid dipping counter (20th Century Electronics, B6) mounted centrally in a lead castle so that a cylindrical aluminium filter could be inserted between the counter tube and the annular vessel containing the fluid sample. The capacity of the vessel was 18 ml and aluminium filters of thickness 30 and 270 mg/cm^2 were used.

4. A scintillation counter (Ekco N 550), as described by Haigh (1954), with crystal housing thickness 130 mg/cm^2.

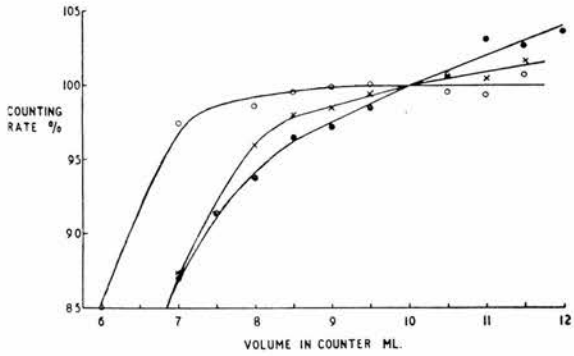
5. The same scintillation counter with the crystal housing increased to 925 mg/cm^2.

6. An end window counter (G.E.C., M4, aluminium window 7 mg/cm^2) housed in a lead castle and arranged to measure the radioactivity of 0·5 ml samples dried on steel planchettes. An aluminium filter of thickness 260 mg/cm^2 could be placed between the sample and the counter tube.

2.3. *Characteristics of Liquid Counter Tubes*

These tubes are designed to contain up to about 11 ml of fluid but in practice, when two tubes are used in combination, it is most convenient

to pipette 10 ml into a thick walled tube for the first count and then to pour the fluid directly into the thin walled tube for the second count. A slight loss inevitably occurs on transfer and the volume sensitivity of the different tubes has accordingly been investigated (see figure). In all three tubes the counting rate obtained with 10 ml of a solution containing ^{24}Na was calculated as 100%. In the thin walled (30 mg/cm^2) tube adding further fluid above 8·5 ml did not significantly increase the counting rate. There was a small increase in counting rate with volume seen in the 200 mg/cm^2 tube, but this did not exceed 1% for each 1 ml added above 8·5 ml. A slightly larger increase, about 2·5% per ml added, was observed in the 300 mg/cm^2 tube. A significant increase of counting rate with volume was not found when similar experiments were done with ^{42}K. These differences are determined by the relative contribution to the counting rate of the β particles and of γ radiation.



The relationship between the volume of ^{24}Na solution in liquid counter tube and the counting rate expressed as a percentage of the value obtained with 10 ml. Three tubes of different wall thickness were used. o, 30 mg/cm^2 ; x, 200 mg/cm^2 ; •, 300 mg/cm^2.

The effect of specific gravity was also investigated as this affords a measure of the extent of self-absorption of β particles, and biological fluids may vary greatly in composition. Both ^{42}K and ^{24}Na were used and the two thicker walled tubes were compared with the standard thin walled liquid counter (table 1). Increasing the specific gravity of the solution up to 1·29 had no significant effect on the counting rate of ^{42}K in the thick walled tubes relative to the thin walled tube. Similarly, with ^{24}Na, no change was seen in the 200 mg/cm^2 tube but in the 300 mg/cm^2 tube the counting rate relative to the thin walled tube rose significantly with increasing specific gravity. Clearly, in the latter tube the γ radiation of ^{24}Na was making an appreciable contribution to the counting rate.

In counting samples of urine and serum, the specific gravity does not exceed 1·050, and corrections are not required.

Table 1. The effect of specific gravity on the ratio of the counting rates in liquid counters of varying wall thickness

Wall thickness 200 mg/cm²			
^{24}Na		^{42}K	
Specific gravity	200 mg/cm² wall / 30 mg/cm² wall	Specific gravity	200 mg/cm² wall / 30 mg/cm² wall
1·000	100·0 ± 0·6 (S.E.)	1·000	100·0 ± 0·5 (S.E.)
1·051	99·6 ± 0·9	1·057	100·4 ± 0·8
1·102	101·4 ± 0·9	1·105	98·8 ± 0·8
1·210	101·6 ± 0·9	1·204	100·1 ± 0·9
1·291	102·4 ± 0·9	1·288	99·2 ± 0·9
Wall thickness 300 mg/cm²			
^{24}Na		^{42}K	
Specific gravity	300 mg/cm² wall / 30 mg/cm² wall	Specific gravity	300 mg/cm² wall / 30 mg/cm² wall
1·000	100·0 ± 0·5 (S.E.)	1·000	100·0 ± 0·5 (S.E.)
1·049	103·8 ± 0·6	1·061	100·2 ± 0·8
1·103	106·9 ± 0·9	1·094	100·1 ± 0·8
1·192	107·9 ± 0·9	1·201	100·3 ± 0·9
1·286	110·2 ± 0·9	1·289	99·2 ± 0·9

2.4. *Relative Efficiency of Different Counting Systems*

Equal counting rates from the standard solutions of ^{24}Na and ^{42}K prepared for liquid counting were obtained in the 30 mg/cm² G.M. tube. In the other systems the relative counting rates were greatly altered (table 2). In the G.M. counters increasing the thickness between the sample and the sensitive region of the tube depresses disproportionately the contribution of ^{24}Na to the counting rate. The greatest discrimination was obtained with the 300 mg/cm² tube. In scintillation counters, the efficiency for ^{24}Na was similar to that obtained with the thin walled G.M. liquid counter, but the efficiency for ^{42}K was much less. The greatest differentiation can be achieved by a combination of the 300 mg/cm² liquid counter and the scintillation counter with the 925 mg/cm² crystal housing, but this is at the expense of a great reduction in the counting rates.

Table 2. Relative counting rates of solutions of ^{24}Na and ^{42}K in liquid counting systems.

Counting system	Volume of solution (ml)	^{24}Na standard (%)	^{42}K standard (%)	Ratio $^{42}K/^{24}Na$	Ratio $^{24}Na/^{42}K$
G.M. liquid counter. Wall 30 mg/cm^2	10	100	100	1·00	1·00
G.M. liquid counter. Wall 200 mg/cm^2	10	27	53	1·96	0·51
G.M. liquid counter Wall 300 mg/cm^2	10	13	41	3·15	0·34
Scintillation counter. Crystal housing 130 mg/cm^2	10	98	32	0·33	3·06
Scintillation counter. Crystal housing 925 mg/cm^2	10	90	8	0·09	11·25
G.M. liquid counter for use with filter. No filter	18	51	78	1·53	0·65
G.M. liquid counter with 270 mg/cm^2 filter	18	26	61	2·35	0·43

2.5. *Calculations of Proportions of ^{24}Na and ^{42}K in Mixtures*

The contribution of the two isotopes in a mixture to the total counting rate can be calculated if the mixture is measured in two counting systems with different efficiencies for ^{24}Na and ^{42}K. In using these short half-life isotopes it is preferable to count the mixture first in the system which gives the slower total counting rate. In the case of the G.M. equipment this means using the thick walled tube or tube with aluminium filter first, and in the former case the convenience of this arrangement for the transfer of fluid from one tube to the other has already been mentioned.

When a combination of two liquid G.M. tubes with different wall thickness is used, the counting rates from the two isotopes are given by the formulae :

$$^{42}\mathrm{K}=\frac{Y-X\,[m\exp(\lambda_{\mathrm{Na}}t)]}{[n\exp(\lambda_{\mathrm{K}}t)-m\exp(\lambda_{\mathrm{Na}}t)]} \quad . \quad . \quad . \quad . \quad . \quad (1)$$

and

$$^{24}\mathrm{Na}=\frac{Y-X[n\exp(\lambda_{\mathrm{K}}t)]}{[m\exp(\lambda_{\mathrm{Na}}t)-n\exp(\lambda_{\mathrm{K}}t)]} \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where X is the recorded counting rate of the mixture in the thin walled tube and Y in the thick walled tube : m is the efficiency for ^{24}Na of the thick walled tube relative to the thin walled tube, and n a similar factor for ^{42}K : t is the time interval between the midpoint of the two counting times : and λ_{Na} and λ_{K} are the decay constants for ^{24}Na and ^{42}K respectively. The formulae give counting rates obtained in the thin walled counter at the time of counting in this tube.

As m and n are constant for a given combination of systems and $\exp(\lambda t)$ varies only with the interval t, the values in the brackets in (1) can be easily calculated and a table constructed for each system. If $m\exp(\lambda_{\mathrm{Na}}t)=a$ and $n\exp(\lambda_{\mathrm{K}}t)=b$, the relationship becomes

$$\left.\begin{aligned} ^{42}\mathrm{K}&=\frac{Y-aX}{b-a}\\ \text{and}\quad ^{24}\mathrm{Na}&=X-{}^{42}\mathrm{K}. \end{aligned}\right\} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

These formulae can be adapted for use with the other combinations of counting systems.

An attempt was also made to separate the two isotopes on the basis of the difference in their decay rates. The mixture was counted in the thin walled liquid counter tube and recounted several times in a period 18–68 hours after the initial count. The proportions of the two isotopes were calculated using the same formulae where m and $n=1$.

2.6. *Separation of Mixtures by Combination of Counting Systems*

The proportions of ^{42}K and ^{24}Na in the three mixtures were measured by nine different combinations of counting systems and the results are shown in table 3. The estimates of the composition of the mixtures and their standard errors have been expressed as a percentage of the value determined from the known dilution. The most unsatisfactory method was that based on the decay rates of the two isotopes, which is not surprising in view of the small difference between their half-lives. The liquid counting systems with the aluminium filter required an unduly large amount of fluid (18 ml) to be suitable for use with plasma and, even with this volume, the counting rates were low. As only 0·5 ml dried samples could be measured with the end window counter, the counting rates were low with the concentrations of isotopes used in the liquid counters and larger amounts of ^{42}K and ^{24}Na were accordingly used with

Table 3. Results of separation of ^{24}Na and ^{42}K by physical methods

Combination of counting systems		Mixture 1 (10% ^{24}Na, 90% ^{42}K)		Mixture 2 (50% ^{24}Na, 50% ^{42}K)		Mixture 3 (90% ^{24}Na, 10% ^{42}K)	
Liquid counter Wall 30 mg/cm²	Liquid counter Wall 200 mg/cm²	96±10·5 (8)	100±1·1 (8)	97±1·1 (8)	102±0·8 (8)	99±0·7 (8)	112†±4·5 (8)
Liquid counter Wall 30 mg/cm²	Liquid counter Wall 300 mg/cm²	97±5·2 (6)	100±0·8 (6)	99±1·2 (6)	100±0·7 (6)	97±1·3 (5)	106±3·9 (5)
Liquid counter for use with filter							
No filter	30 mg/cm² aluminium filter	79±22·7 (8)	102±1·0 (8)	100±4·5 (8)	107†±2·3 (8)	110†±0·8 (8)	98±4·2 (8)
Liquid counter for use with filter							
No filter	270 mg/cm² aluminium filter	113 ; 132 (2)	98 ; 99 (2)	91 ; 102 (2)	105 ; 99 (2)	94 ; 100 (2)	108 ; 92 (2)
End-window counter							
No filter	260 mg/cm² aluminium filter	97±9·5 (8)	100±1·4 (8)	98±1·4 (10)	99±0·9 (10)	99±1·8 (8)	81±11·1 (8)
Scintillation counter Crystal housing thickness 130 mg/cm²	Liquid counter Wall 30 mg/cm²	129†±8·2 (4)	94†±0·6 (4)	99±1·5 (4)	100±1·5 (4)	102±1·8 (4)	83±22·3 (4)
Scintillation counter Crystal housing thickness 130 mg/cm²	Liquid counter Wall 200 mg/cm²	128†±2·4 (4)	98±0·9 (4)	102±1·9 (4)	98±3·0 (4)	101±2·2 (4)	94±5·0 (4)
Scintillation counter Crystal housing thickness 925 mg/cm²	Liquid counter Wall 300 mg/cm²	97±1·6 (8)	101±1·1 (8)	99±0·5 (8)	101±1·0 (8)	99±0·5 (8)	109±2·2 (8)
Differential decay							
Liquid counter Wall 30 mg/cm²	Recount after 18–68 hr in same counter	84±21·9 (11)	101±2·8 (11)	91†±1·8 (11)	109†±2·2 (11)	93†±1·1 (6)	178†±8·9 (6)

The means and their standard errors are shown as percentages of the calculated value.
The figures in brackets after the standard error indicate the number of replicates.
† Means significantly different from calculated value.

this equipment. In clinical practice, this would mean giving increased doses of isotopes to the patients. Accordingly attention was concentrated on the systems using 10 ml of fluid. The combination of scintillation counter with thick crystal housing and liquid counter tube with 300 mg/cm^2 wall thickness gave satisfactory estimates except in the measurement of the third mixture, which contained only 10% ^{42}K. The combinations of liquid counters with different wall thicknesses were equally satisfactory in the first two mixtures but again showed a significant error in the measurement of the third mixture. They were in practice the most rapid methods.

2.7. *Chemical Methods of Separation*

In these experiments the potassium in the mixture was separated by chemical precipitation, washed and again brought into solution. The potassium specific activity of the redissolved precipitate was determined by counting in the thin walled liquid counter tube and by flame photometry.

Two different methods were used :

1. The double precipitation method for the separation of potassium from sodium, as described by James *et al.* (1954). The potassium was first precipitated as potassium cobaltinitrite and then as potassium tartrate. The final precipitate was redissolved and the potassium specific activity measured.

2. A single precipitation method using tetraphenylboron (Wittig and Raff 1950). Details of the technique are given in Appendix 1. The potassium content of the redissolved precipitate was determined by flame photometry : the reagents used did not interfere with this estimation.

Table 4. Results of ^{42}K specific activity from chemical separation.

Method	^{42}K counts as percentage of total counts in mixture			
	10%	50%	90%	100%
Sodium cobaltinitrite and tartaric acid precipitation	98 ± 1·3 (4)	97 ± 1·3 (4)	99 ± 0·8 (4)	98 ± 0·6 (2)
	Mean 98 ± 0·6 (14)			
Sodium tetraphenylboron precipitation	101 ± 0·5 (4)	99 ± 1·7 (4)	102 ± 0·9 (4)	99 ± 1·4 (2)
	Mean 100 ± 0·6 (14)			

The means and their standard errors are shown as percentages of the calculated value. The figures in brackets after the standard error indicate the number of replicates.

2.8. *Efficiency of Chemical Separation of ^{42}K*

The potassium in the standard ^{42}K solution and in the mixtures has been separated by the two different chemical methods. The specific activity of the isolated potassium has been expressed as a percentage of the value calculated from the known dilution. The results are shown in table 4. There is close agreement between the observed and calculated values for potassium specific activity both in the ^{42}K solution and in all the mixtures. Both counting and flame photometry contribute to the standard errors. In six experiments precipitation of the potassium present in the standard ^{24}Na solution was done by both methods and on no occasion was the counting rate of the separated material significantly above background.

§ 3. Application of Methods to the Simultaneous Measurement of Exchangeable Sodium and Potassium

The determination of the amount of exchangeable sodium or potassium in the body is now an established procedure in clinical investigation (Edelman *et al.* 1952). The calculation depends on the extent of the dilution of the isotope in the corresponding natural element after a suitable interval has been allowed for mixing in the body. During this period allowance must be made for any of the administered isotope that has been lost from the body. Thus, after equilibrium of distribution has been attained

$$\text{Exchangeable potassium in m.equiv} = \frac{^{42}\text{K injected} - ^{42}\text{K excreted}}{^{42}\text{K per m. equiv of body potassium}}$$

A similar formula applies for the calculation of exchangeable sodium. The measurement of the amount of isotope injected presents no difficulties. The main route of excretion is in the urine and in the usual equilibrium periods up to 48 hours other routes can be neglected. The amount of radioisotope per m.equiv of cation can be determined in any representative sample of body fluid after distribution equilibrium has been attained. In practice, this means the determination of the specific activity of serum or urine. These measurements are easily made if a single isotope is used but the separate determination of the variable mixtures of ^{24}Na and ^{42}K in the fluid samples is more troublesome. These problems have been examined in the light of the information gained from the studies on the mixtures.

3.1. *Dose of ^{42}K and ^{24}Na*

In clinical investigation it is important to reduce the exposure to radioactivity to the least possible amount. In combined measurements the dose of ^{24}Na can readily be restricted to under 100 μc but in the past when simultaneous measurements with both isotopes have been made, larger doses of ^{42}K, up to 350 μc, have been employed (James *et al.* 1954, Arons *et al.* 1954, Robinson *et al.* 1955). This is due to the small amount of potassium in serum in comparison with sodium.

Attention has been paid to developing a method by which reliable measurements can be made when the dose of each isotope is kept below 70 μc.

3.2. *Urinary Excretion of ^{42}K and ^{24}Na during Equilibrium Period*

In the 24 hour period following the injection, only a small fraction (under 10%) of the dose of each isotope is excreted in the urine. Usually there is not a gross discrepancy between the proportions of the two isotopes excreted, but in oedematous patients the excretion of ^{24}Na may be almost negligible. If the doses of the two isotopes are similar, the situation does not arise in which ^{42}K represents only a small fraction of the radioactivity in urine. The conditions are thus entirely suitable for using a physical method of separation. In practice, the most rapid and convenient method is to pipette 10 ml of urine into a 200 mg/cm^2 G.M. liquid counter tube and, after counting, to pour this directly into a 30 mg/cm^2 tube for recounting. As the urinary excretion is small, sufficient accuracy can be assured with this technique.

3.3. *Measurement of ^{42}K Specific Activity*

This requires the determination of the ^{42}K activity and chemical potassium content of a sample of serum or urine taken after equilibrium of distribution has been attained. Serum has the great disadvantage that the potassium content is low and if concentration methods are used an inconveniently large amount of blood has to be withdrawn, particularly if duplicate measurements are made. Urine has a higher, though more variable content of potassium. Small urine samples passed after the equilibrium period are thus preferable to serum for the determination of potassium specific activity. The separation of the two isotopes in these samples may be made either by physical or chemical methods, but the accuracy of the measurement of exchangeable potassium largely depends on this determination. In practice, the chemical method using sodium tetraphenylboron is preferable. It is more reliable, and quicker and provides a more suitable specimen for flame photometry. If a physical method is used, prolonged counting is required to achieve the necessary accuracy and interference effects from other variable constituents of the untreated urine make the flame photometry less reliable.

A possible objection to the use of urine is that it may yield different results from serum taken at the time when the specimen is passed. This has been investigated in a series of ten patients in whom exchangeable potassium alone was being measured. During the last two or three hours of a 24-hour equilibration period the patient was encouraged to drink water or tea freely so that a high rate of urine flow was achieved. The bladder was completely emptied at the end of the period. Thereafter two ' spot ' urine samples were passed at about hourly intervals. Blood samples were withdrawn at approximately the midpoint of the collection period. An analysis of variance showed no significant difference

between the two sets of specimens. The two urine specimens showed a much smaller scatter than the sera (table 5). The potassium content of the redissolved urinary potassium precipitates varied from 41 to 88 m.equiv/l in contrast with a range of serum determinations between 3·6 and 5·8 m.equiv/l. This was accompanied by correspondingly higher counting rates from the urinary potassium concentrates.

Table 5. Comparison of ^{42}K specific activity of corresponding urine and serum samples in 10 patients. The potassium in the urine was separated by the tetraphenylboron method. The values are expressed as a percentage of the first urine specimen and the standard errors are shown.

	Urine samples (%)	Serum samples (%)
1	100·0	107·9±4·9
2	101·0±0·7	106·1±4·8

3.4. *Measurement of ^{24}Na Specific Activity*

The fluid of choice for this determination is clearly serum, in which there is a high concentration of sodium easily measured by flame photometry. The serum potassium concentration is determined and the contribution of ^{42}K to the total serum counting rate is calculated from the known potassium specific activity as described by James *et al.* (1954). When equal doses of ^{24}Na and ^{42}K are given the contribution of ^{42}K to the counting rate in a 30 mg/cm^2 wall thickness liquid counter G.M. tube is usually about 5% of the total.

3.5. *Comparison of Methods in Clinical Practice*

In one patient with myxoedema in whom measurements of exchangeable sodium and potassium were required, sufficient samples were obtained to allow a comparison between entirely physical methods of separation and a combination of physical and chemical methods, as suggested above. A dose of approximately 50 μc of each isotope was given. The 24-hour excretion in the urine was measured by the combination of thin and thick walled liquid G.M. tubes. The ^{42}K specific activity in two spot urine samples was determined both by tetraphenylboron precipitation and counting in the 30 mg/cm^2 liquid G.M. tube and by differential counting in the 300 mg/cm^2 liquid G.M. tube and in the scintillation counter with 925 mg/cm^2 crystal housing, which was theoretically the most satisfactory combination.

The ^{24}Na specific activity was calculated from the serum counts in the usual manner. The results obtained by the two methods are shown in table 6. The striking difference is in the time taken by the

two methods. The thick screening in the differential physical method greatly reduces the counting rates and prolongs the measurements required to obtain sufficient accuracy. This difference is even more important when several patients are being investigated at the same time. The precipitation of several specimens with tetraphenylboron can be carried out in parallel and the counting in the thin walled tube, which gives a rapid rate, can quickly be started. On the other hand, with many samples to count in the two heavily screened systems the delay becomes very great as the isotopes decay.

Table 6. Comparison of two methods of measuring simultaneously exchangeable sodium and potassium in the same patient. The time refers to the interval between taking the last sample from the patient and obtaining the results.

Method	Exchangeable sodium (m.equiv)	Exchangeable potassium (m.equiv)	Time taken for determinations (hr)
G.M. liquid counters and tetraphenylboron precipitation	2016	1862	5
G.M. liquid counters and scintillation counter	1981	1796	8

Details of the method which appears quickest and most reliable for the simultaneous measurement of exchangeable sodium and potassium in clinical investigation are given in Appendix 2.

§ 4. Discussion

The comparison of the various methods of estimating ^{24}Na and ^{42}K in mixtures shows clearly that with the equipment readily available at the present time, the physical methods of separation cannot achieve the same accuracy as chemical ones within an equal period of time. Tetraphenylboron precipitation is more rapid than the cobaltinitrite tartaric acid method and equally effective. In working with short life isotopes in man, speed of method is of considerable importance as it permits a reduction in the dose of the isotopes. The previously described physical methods (Arons *et al.* 1954, Robinson *et al.* 1955) are both slower in practice and require higher doses of ^{42}K than the method described here. Robinson and his associates administer 10 μc ^{24}Na and 350 μc ^{42}K and state that this gives a radiation dose to the patient of 0·45 rep. Moreover, much of the ^{42}K is concentrated initially in the liver (McArdle and Merton 1952).

The use of urine for the determination of ^{42}K specific activity was first described by Corsa *et al.* (1950). The main objection to using urine is that in certain conditions, especially post-operatively, the patient may have difficulty in providing samples. It is also essential that throughout the test the rate of urine flow should be high so that the spot samples taken after 24 hours are not heavily contaminated by urine formed at an earlier stage. If this precaution is taken there is good agreement between plasma and urine potassium specific activity. When urine samples are not available the tetraphenylboron method can be applied to serum, though large samples are required as described in the cobaltinitrite precipitation method of James *et al.* (1954).

Measurements of exchangeable sodium and potassium may be of great assistance in the investigation of certain clinical problems. Increasing attention is being paid to the importance of reducing as far as possible human exposure to ionizing radiations and for this reason it is important to develop methods using small doses of short half-life isotopes, particularly if repeated observations are required. When a dose of not more than 50 μc of ^{24}Na and ^{42}K is given, the calculated radiation dose to the patient does not exceed 0·15 rad.

Acknowledgments

We are grateful to Dr. H. Miller for his advice. The work was supported by grants from the Tuberculosis Research Fund of the University of Sheffield and the Endowment Fund of the United Sheffield Hospitals.

Summary

1. Combinations of different counting systems have been investigated for their efficiency in assaying mixtures of ^{24}Na and ^{42}K. The advantages of a combination of thin and thick walled liquid G.M. counter tubes of the Veall pattern are described. Physical methods are unreliable when only a small proportion of the counts is derived from one isotope.

2. The separation of potassium by precipitation with sodium tetraphenylboron is described. Methods dependent on chemical separation are reliable even when ^{42}K makes only a small contribution to the counting rate of a mixture.

3. The application of these methods to the simultaneous measurement of exchangeable sodium and potassium is discussed and a rapid method for carrying out these measurements is described.

Résumé

1. On a étudié les combinaisons de différents systèmes de comptage afin de trouver leur efficacité dans l'analyse des mélanges de ^{24}Na avec ^{42}K. On décrit les advantages de la combinaison de tubes compteurs G.M. liquides, modèle Veall, à parois minces et épaisses. Les méthodes physiques ne sont pas exactes lorsque seulement une petite partie de comptage provient d'un isotope.

2. On décrit la séparation de potassium par précipitation avec du sodium tétraphénylebore. Les méthodes basées sur la séparation chimiques donnent des résultats exacts même quand ^{42}K ne contribue que très peu à la vitesse de compte du mélange.

3. On discute l'application de ces méthodes à la mesure simultanée de sodium et potassium échangeables et l'on décrit une méthode rapide d'éxecuter ces expériences.

ZUSAMMENFASSUNG

1. Man hat den Wirkungsgrad von Kombinationen verschiedener Zählungssysteme in der Analyse der Gemische von ^{24}Na mit ^{42}K untersucht. Man beschreibt die Vorteile der Kombination von dünne—und dickwandigen Flüssigkeits-G.M.-Zählern des Veall-Typs. Die physikalischen Methoden sind unverlässlich, wenn nur ein kleiner Zählungsanteil von einem Isotopen herrührt.

2. Man beschreibt die Trennung von Kalium durch Fällung mit Natrium-Tetraphenylbor. Die auf chemischer Trennung basierenden Verfahren sind verlässlich, sogar wenn ^{42}K nur in einem kleinen Masse der Zählungsrate eines Gemisches beiträgt.

3. Man erörtert die Anwendung dieser Verfahren zur gleichzeitigen Messung von austauschbarem Natrium und Kalium und man beschreibt eine Schnellmethode zur Ausführung dieser Messungen.

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APPENDIX 1

The Qualitative Separation of Urinary Potassium with Sodium Tetraphenylboron for the Estimation of ^{42}K Specific Activity

Reagents

1. Sodium tetraphenylboron 3% w/v (approx. 0·1 M) solution in water. Dissolve 3 g in 100 ml of water and add 0·5–1·0 g of alkali-free aluminium hydroxide. The solution is stirred for 5 min and then filtered, the first 20 ml being refiltered. The solution keeps for some weeks at room temperature and can be used so long as no turbidity develops.
2. Concentrated HCl.
3. 1% w/v NaCl checked for potassium impurity.
4. 40% w/v $HgCl_2$ in aqueous solution (prepared in boiling water bath).
5. 0·1% w/v freshly prepared solution of NaI.

Procedure

1. Put 40 ml urine into large centrifuge tube (75 ml) and add 0·5 ml conc. HCl.

2. Add, at room temperature, 3% sodium tetraphenylboron solution from a pipette, stirring slowly until no further precipitation occurs. It is sometimes necessary to centrifuge as the turbidity of the solution makes it impossible to detect further precipitation. One ml of the sodium tetraphenylboron solution precipitates approximately 3·5 mg potassium. 5–20 ml are sufficient in normal urines. Note volume of reagent used.

3. Centrifuge at 3000 r.p.m. for 5 min. Discard supernatant.

4. Resuspend the precipitate in approx. 10 ml. NaCl solution ; acidify with two drops conc. HCl and add 0·2 ml of sodium tetraphenylboron. Fill the tube with NaCl solution, stirring continuously so that no lumps of precipitate are left. Wash down stirring rod and repeat centrifuging.

5. Decant and discard supernatant. Repeat washing with NaCl solution twice, adding each time 0·2 ml sodium tetraphenylboron after acidifying with conc. HCl.

6. Add 12 ml distilled water to the precipitate and place the centrifuge tube in hot water bath.

7. Add hot $HgCl_2$ solution in excess. The appropriate volume may be calculated, each ml sodium tetraphenylboron solution used requiring 0·2 ml $HgCl_2$. Alternatively excess $HgCl_2$ may be demonstrated by the addition of one drop of the supernatant to a solution of 0·1% w/v NaI. A yellow colour, quickly changing to red, will be seen in the presence of excess $HgCl_2$.

8. Keep the tube for 5 min in boiling water. The new precipitate that forms subsides quickly leaving a clear supernatant.

9. Cool thoroughly and filter. The filtrate, which contains ^{42}K and potassium, can be used for flame photometry and counting.

The small quantities of precipitate which may appear after filtering do not interfere with counting or flame photometry.

Appendix 2

The Simultaneous Measurement of Exchangeable Sodium and Potassium with ^{24}Na and ^{42}K

The isotopes should be prepared from spectroscopically pure target material. The solutions of ^{24}Na and ^{42}K are made separately by dissolving the radioactive material in 0·9% w/v NaCl and are sterilized by boiling. The concentration of radioactivity should be 50–70 μc in 50 ml, as this is the amount of each isotope to be administered to the

patient. Sufficient surplus material must be prepared for subsequent 1 : 200 dilution as standards.

No special preparation of the patient is required before giving the isotopes. At about 9 a.m. 50 ml of each solution are given from sterile 50 ml burettes delivering into an intravenous infusion funnel, and are washed in with 150 ml of dextrose saline solution.

During the subsequent 24 hours the patient collects all urine in a bottle provided. On the morning of the next day the patient is restricted to tea and toast for breakfast but is encouraged to drink freely to provide a large urine flow. Exactly 24 hours after the injection of the radioactive material the bladder is emptied to complete the 0–24 hour urine collection.

Thereafter two ' spot ' urine samples are collected at 45 min intervals into chemically clean containers. These specimens are treated as described in Appendix 1 for the determination of potassium specific activity. At the midpoint of each ' spot ' urine collection a 30 ml blood sample is withdrawn and allowed to clot. The serum is separated and used for counting and flame photometry.

The volume of the 0–24 hour urine is recorded and the radioactivity is measured by counting ^{24}Na and ^{42}K standards and a sample of the urine in 30 mg/cm^2 and 200 mg/cm^2 liquid counter tubes.

The radioactivity of all other samples is measured only in the 30 mg/cm^2 tube. The ' spot ' urines treated with tetraphenylboron contain ^{42}K only and provide a measurement of the potassium specific activity. The sera contain both ^{24}Na and ^{42}K but the contribution of ^{42}K to the total counting rate is calculated from the potassium concentration in the serum and the potassium specific activity. The difference represents the radioactivity due to ^{24}Na. The sodium content of the sera is measured by flame photometry.

Reprinted from METABOLISM
Vol. III, No. 4, July, 1954
Printed in U.S.A.

Body Sodium and Potassium

II. A Comparison of Metabolic Balance and Isotope Dilution Methods of Study

By GRAHAM M. WILSON, JOHN M. OLNEY, LAURA BROOKS, J. ALLAN MYRDEN, MARGARET R. BALL AND FRANCIS D. MOORE

UNTIL recently the investigation of changes in body composition has been largely dependent on the measurement of intake and output and the calculation of the resultant "balance." The principles of this method are well appreciated, their practice has stood the test of time and the sources of error, though numerous, are widely realized.[1] The availability of radioactive isotopes suitable for human use has introduced a new method of measuring body composition, with the use of the dilution principle.[2, 3] Serial determinations of the total content of sodium and potassium in the body available for exchange with the corresponding isotope may be made in this way, and the changes may be compared with the cumulative balance results. An investigation of this type has accordingly been carried out to determine to what extent the two methods agree.

METHODS

The metabolic balance techniques are essentially those described by Moore and Ball.[1] The cumulative changes have been estimated by adding algebraically the daily balances. The measurements of total exchangeable sodium and potassium[4-7] have been made by dilution in the body of the isotopes Na^{24} and K^{42}. A 24-hour equilibration period has been used in all cases. The differences found between the two methods of study have been expressed as a cumulative total for each period analyzed.

CLINICAL MATERIAL

Over a six-year period, studies by both methods have been carried out in 16 patients under circumstances permitting a direct comparison of the two methods of measurement of change in body electrolyte (table 1). During this time hundreds of K_e-Na_e measurements have been carried out in these laboratories, and approximately 80 metabolic balance studies; but only when the two were complete and simultaneous (largely a matter of coincidence) could the comparison be made. It is these paired observations which form the basis for this comparative analysis.

One of these patients was a normal student volunteer. One patient had suffered a mid-shaft fracture of the femur, another a fracture of both bones of the lower

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This work was supported throughout by the Atomic Energy Commission. We wish also to acknowledge the support of the Upjohn Company and Winthrop-Stearns, Inc.

Received for publication November 23, 1953.

TABLE 1.—*Clinical Data*

Case No.	Age sex	Diagnosis	Clinical information
1	24 M	Normal student	
2	51 M	Fracture tibia and fibula; Colles' fracture	Convalescing normally, marked stress response
3	17 M	Fracture femoral mid-shaft	Convalescing normally, very marked stress response
4	67 M	Partial thickness burns arms and face	"Flash burn" convalescing normally; minimal stress response
5	56 M	Hiatus hernia; duodenal ulcer	Operation: repair hernia, P.G.E., vagotomy; normal course
6	56 F	Carcinoma of the breast	Operation: mastectomy; minor wound sepsis
7	65 M	Carcinoma of the rectum	Operation: Miles resection; intestinal obstruction; recurrence
8	36 M	Perforated duodenal ulcer; duodenal fistula	Neglected case; advanced sepsis; recovery
9	71 M	Carcinoma of the stomach	Operation: thoracic gastrectomy; very sluggish recovery
10	66 F	Carcinoma of the stomach	Operation: thoracic gastrectomy; normal convalescence
11	77 F	Carcinoma of the rectum	Operation: excision, normal convalescence
12	45 F	Mitral stenosis	Operation: valvuloplasty; normal convalescence
13	40 F	Mitral stenosis	Operation: valvuloplasty; normal convalescence
14	50 F	Mitral stenosis	Operation: valvuloplasty; slow convalescence
15	38 F	Common duct stricture	Operation: repair; marked jaundice
16	27 M	Cushing's syndrome	Operation: adrenalectomy; slow recovery

leg. There was one extensive second-degree burn and one patient with carcinoma of the breast. Six patients had peritoneal cavity surgery for cancer or ulcer and one for common duct stricture. There were three with severe mitral stenosis undergoing surgical treatment. One patient had Cushing's disease. Since we are interested here in comparing two methods of study under the widest variety of conditions, the heterogeneity of the group is favorable to our purposes. Relationship of the absolute values to the pathology and clinical course of these and other patients is discussed elsewhere.[1, 12]

In the majority of these patients, after accidental trauma or surgery, there was a steady loss of weight and usually a gain of sodium and a loss of potassium during their stay in the hospital. Patient G. S. (case 8), who was in negative sodium balance owing to drainage from an intestinal fistula, was the only exception.

TABLE 2.—*Change in Body Sodium Balance Data and Isotope Dilution (Na_e)*

Case No.	Dates	Days	Wt. kg.	Na_e		Balance change (mEq.)	Difference mEq.	Difference per week		Move positivity shown by
				mEq.	change (mEq.)			mEq.	% of 1st Na_e	
2	12–6–52 (Frac)									
	12–12–52		88.8	3411						Balance
	1–2–53	21	86.4	3504	+93	+182	89	29	0.85	
3	9–23–52 (Frac)									
	9–26–52		72.7	2792						
	10–2–52	7	69.3	3060	+268	+371	103	103	3.7	Balance
	10–23–52	28	68.3	2992	+200	+678	478	119	4.3	Balance
4	11–3–52 (Burn)									
	11–6–52		45.9	2367						
	11–21–52	15	43.9	2173	−194	+16	210	105	4.4	Balance
5	2–12–53		70.7	2861						
	2–14–53 (Op)									
	2–19–53	7	66.9	2749	−112	−10	122	122	4.3	Balance
6	3–25–53		70.7	2342						
	3–26–53 (Op)									
	4–2–53	7	67.0	2447	+105	+187	82	82	3.5	Balance
	4–9–53	14	68.4	2412	+70	+568	498	259	11.0	Balance
7	3–13–52		60.9	2524						
	3–15–52 (Op)									
	3–20–52	7	58.5	2391	−133	+82	215	215	8.5	Balance
	4–4–52	21	56.9	2844	+320	+495	175	59	2.3	Balance
8	9–25–51 (Perf)									
	9–27–51		68.4	3421						
	11–1–51	35	52.5	3054	−367	−146	221	44	1.3	Balance
12	5–15–52		34.2	1542						
	5–19–52 (Op)									
	5–22–52	7	34.6	1645	+103	+25	78	78	5.0	Na_e
	5–29–52	14	33.0							
	6–5–52	21	33.7	1750	+208	+267	59	19	1.2	Balance
13	10–18–51		41.2	1932						
	10–25–51	7	41.1	2085	+153	+108	45	45	2.3	Na_e
	10–31–51 (Op)									
	11–14–51	28	39.8	2130	+198	+122	76	19	1.0	Na_e
14	11–27–52		60.8	2695						
	11–29–52 (Op)									
	12–4–52	7	62.3	2717	+22	+41	19	19	0.7	Balance
	12–11–52	14	58.0	2782	+87	+60	27	14	0.5	Na_e
	12–18–52	21	57.8	2884	+189	+76	113	38	1.4	Na_e

RESULTS

Serial measurements of total exchangeable sodium and potassium in the 16 cases are shown and compared with metabolic balance changes in Tables 2 and 3.

In assessing these results certain orders of magnitude should be borne in mind. The daily intake of sodium ranges around 150 mEq., and of potassium 100 mEq. in the normal adult male; balance changes of 100 mEq. per day of either ion are large, but do occur in the dramatic sweeps of surgical metabolism. Average

TABLE 3.—*Change in Body Potassium Balance Data and Isotope Dilution (K_e)*

Case No.	Dates	Days	Wt. kg.	K_e		Balance change mEq.	Difference mEq.	Difference per week		More positivity shown by
				mEq.	change mEq.			mEq.	% of 1st K_e	
1	1–4–52		78.0	3761						
	1–11–52	7	77.4	3729	−32	−36	4	4	0.1	K_e
3	9–23–52 (Frac)									
	10–2–52		69.3	3100						
	10–23–52	21	68.3	3215	+115	+53	62	20	0.65	K_e
5	2–12–53		70.7	2875						
	2–14–53 (Op)									
	2–19–53	7	66.9	2589	−286	−156	130	130	4.5	Balance
6	3–25–53		70.0	2220						
	3–26–53 (Op)									
	4–2–53	7	67.0	1803	−417	+42	459	459	21.0	Balance
	4–9–53	14	68.4	1955	−265	+51	316	158	7.1	Balance
	4–16–53	21	68.6	2120	−100	+100	200	66	2.9	Balance
7	3–13–52		60.9	2238						
	3–15–52 (Op)									
	3–20–52	7	58.5	2190	−48	−253	205	205	9.2	K_e
	4–4–52	21	56.9	1954	−284	−399	115	38	1.7	K_e
9	5–13–47		43.0	1830						
	5–14–47 (Op)									
	5–27–47	14	41.8	1425	−405	−338	77	38	2.1	Balance
10	4–29–47		42.8	1170						
	4–30–47 (Op)									
	5–13–47	14	41.5	935	−235	−141	94	47	4.0	Balance
	5–27–47	28	42.0	1220	+50	+215	165	41	3.5	Balance
11	3–19–47		59.0	1230						
	3–20–47 (Op)									
	4–1–47	14	57.5	1170	−60	−141	81	40	3.2	K_e
	4–15–47	28	59.8	1410	+180	+352	172	43	3.5	Balance
12	5–15–52		34.2	1259						
	5–19–52 (Op)									
	5–22–52	7	34.6	1154	−105	−120	15	15	1.2	K_e
	5–29–52	14	33.0	1177	−82	−81	1	0.5	0.04	Balance
	6–5–52	21	33.7	1169	−90	+31	121	40	3.2	Balance
13	10–18–51		41.2	1565						
	10–25–51	7	41.1	1488	−77	−30	47	47	3.0	Balance
	10–31–51 (Op)									
	11–14–51	28	39.8	1424	−141	−47	94	23	1.5	Balance
14	11–27–52		60.8	1965						
	11–29–52 (Op)									
	12–4–52	7	62.3	1930	−35	+34	69	69	3.5	Balance
	12–11–52	14	58.0							
	12–18–52	21	57.8	1628	−337	−33	304	101	5.1	Balance
15	9–23–47		53.0	1607						
	10–22–47	28	53.8	1630	+27	−98	125	31	1.9	K_e
16	12–17–47		54.0	1530						
	1–4–48 (Op)									
	1–22–48	35	54.0	1285	−245	−59	186	37	2.4	Balance

weekly changes in chronic disease range around 50 to 100 mEq. The normal total exchangeable sodium and potassium is about 2800 to 3000 mEq. (of each ion) in the adult male. Errors in balance measurement are both random and systematic; when the latter, they are cumulative with the passage of time. Errors in the K_e and Na_e methods[3, 4, 7, 12] are random and are not cumulative, regardless of the passage of time between measurements.

Study of the results show there are many points of agreement and considerable discrepancies between paired readings. Circumstances of agreement and of divergence will be examined for each ion.

Sodium

There are 27 paired observations, permitting 17 comparisons of change in 10 patients (Table 2).*

Similarities. In 15 of the 17 comparisons the two methods show changes which agree to within 125 mEq. per week. Several of the comparisons are for periods of two weeks or more, and here the total discrepancy is divided by the number of weeks to derive the figures for average weekly agreement or discrepancy.

Divergences. In 2 of the 17 comparisons, differences are more than 125 mEq. per week.

Altogether there are 5 observations out of 17 in which the total divergence (regardless of the time interval) is greater than 200 mEq. These will be examined in detail:

In Case 3 (fracture of the femur), on the 28th day the balance method showed a difference of +478 mEq. over the isotope method. The previous measurements on the seventh day showed the same trend, so that over the subsequent three-week interval the mean deviation was at the rate of 125 mEq. a week. In Case 4 (second degree burns) a difference of 210 mEq. occurred in 15 days; again the balance showed the larger positive value. Dressing losses not completely collected could well account for a part of this. In Case 6 (carcinoma of the breast) on the 14th day there was a difference of 498 mEq.; a week previously the measurements were in good agreement. The patient then gained 381 mEq. according to the balance, but by the isotope measurement lost 35 mEq. No satisfactory explanation was found for this considerable discrepancy, but it is possible that an error in balance may have arisen through inadequate collection of transudate from the wound, which may be copious after radical mastectomy. In Case 7 there was an abdominoperineal resection of the rectum, and during the next week there was a difference of 215 mEq., the balance showing a gain in sodium and the isotopes a small loss. Sodium lost in the plasma fraction of the hemorrhage at operation was included in the output, but no account was taken of the amount of sodium removed with the resected tissue, conceivably as much as 100 mEq. In Case 8 (perforated ulcer; sepsis) in which there were numerous complications involving large extrarenal losses of sodium as mentioned above, there was a discrepancy of 221 mEq. after five weeks, that is, at a mean rate of 44 mEq. per week, isotope methods showing the larger loss. This

* When repeated measurements were made in the same patient, the first is, of course, used as the starting reference point; subsequent changes are then referred back to this first observation, cumulatively.

is a small weekly discrepancy for such a complex case; extrarenal collections were laborious.

Trend. In the 17 paired comparisons there are 5 instances wherein the balance technique shows a smaller gain of sodium (or larger loss); in 12 instances the balance technique shows a greater tendency to positive gain of body sodium than the isotope method. If positive, the balance is more strongly positive. If negative, the balance shows less loss. This systematic trend of the differences (whether large or small) is striking.

Potassium

There are 35 paired observations, permitting 22 comparisons of change in 13 patients (Table 3).

Similarities. In 18 of the 22 comparisons the two methods show changes which agree to within 125 mEq. per week. Several of the comparisons are for periods of two weeks or more, and here the total discrepancy is divided by the number of weeks to derive the figure for average weekly agreement or discrepancy.

Divergences. In 4 of the 22 comparisons the differences are more than 125 mEq. per week.

Altogether there are only 4 instances out of 22 in which the total divergence (regardless of the time interval) is greater than 200 mEq. These will be examined in detail:

Two of these divergences, greater than 200 mEq., occurred in Case 6 on the 7th and 14th days, when the differences were 459 and 316 mEq., respectively, the isotopes showing a loss while in both instances the balance showed a small gain. No account was taken of the potassium content of the amputated breast and pectoral muscles (estimate 150 mEq.), though this alone cannot account for the large differences. The isotope measurements showed a gradual restoration of exchangeable potassium to 100 mEq. below the preoperative level whereas the balance showed an over-all gain of 100 mEq. at the end of the study, at a time when the patient was still 1.47 kg. below her initial weight. This is a clear instance of "presumptive error" in the balance. It is to be recalled that in this case there was also a considerable discrepancy in the sodium measurements. In Case 7, in contrast to the others, the balance method showed a greater loss of potassium after operation than the isotope determinations, even though no account was taken of the potassium in the excised rectum and lower colon. The difference between the two methods on the seventh day was 205 mEq.; this was greatly reduced in the later measurement. In Case 14 (mitral stenosis) a difference of 304 mEq. was present on the 21st day; this had developed over a period of two weeks, as no substantial deviation had been noted in the previous measurements on the seventh day. The patient was losing weight, having lost 4.5 kg. between the 7th and 21st day. The change in body potassium shown by isotopes is closer to the predicted magnitude of potassium lost with 4.5 kg. of lean tissue than the balance figure. This does not constitute any validation of the method, however.

Trend. In the 22 paired comparisons there are 7 instances wherein the balance technique shows a smaller gain of potassium (or larger loss); in 15 instances the

balance technique shows a greater tendency to positivity of body potassium than the isotope. If positive, the balance is more strongly positive. If negative, there is less loss. This systematic trend of the differences (whether large or small) is striking in the potassium series just as it was with sodium.

Discussion

In a review of these results, it is found that in 9 instances in the 39 comparisons made there was a discrepancy greater than 200 mEq., regardless of the time interval. On five of these occasions the interval between the isotope measurements was two weeks or more, and when the differences were expressed on a mean weekly basis they were considerably less than 200 mEq. per week, being in the range of 40 to 150 per week. On the other 4 occasions differences greater than 200 mEq. arose within a week. These discrepancies were confined to two cases (Cases 6 and 7).

In considering the comparisons between the two methods, the fact emerges that the cumulative balance shows a greater retention of both cations within the body. This is examined more fully in Table 4, where the mean changes shown by both methods are summarized. As explained above, the condition of these patients for the most part led to a retention of sodium and a loss of potassium, and the measurements by both methods demonstrated these anticipated changes. The cumulative balance shows a more rapid gain in sodium and less rapid loss of potassium in comparison with the isotope measurements. The differences between the two techniques, however, are small, especially in the case of potassium, and the scatter of the measurements on which the means are based is considerable. In neither case does the excess in the body shown by the cumulative balance attain statistical significance when analyzed on the basis of weekly differences.

The reproducibility of the isotope dilution measurements has been examined in previous investigations both in this laboratory and elsewhere. In the case of measurements with K^{42} alone, the reproducibility in healthy subjects was within 4% of the mean.[4] A similar figure applies in the case of Na^{24} alone.[6] When the two isotopes are used simultaneously, there is a slight loss of accuracy, but the reproducibility of the measurements is within 4% to 5% of the mean.[7] In the whole series of observations reported here the mean total exchangeable sodium is 2574 mEq. and potassium 1856 mEq. When the variability ascertained in healthy subjects is applied to these figures, the range when the two isotopes are used together is ±129 mEq. for sodium and ±95 mEq. for potassium.

Table 4.—*Comparison of Changes in Amounts of Sodium and Potassium in the Body Measured by Isotope Dilution and Metabolic Balance*

	Total duration of comparisons, weeks	No. of comparisons	Mean change by isotope dilution method per week mEq. ± S.E.	Mean change in cumulative balance per week mEq. ± S.E.	Difference between means, i.e. excess in body shown by balance method per week mEq.
Sodium	30	17	+40 ± 26	+101 ± 29	+61
Potassium	41	22	−57 ± 20	−31 ± 12	+26

In balance measurements the reproducibility cannot be ascertained experimentally, but the over-all accuracy may be less than that of the isotope techniques because of unmeasured gains or losses. Under these circumstances the extent of the agreement between the two methods is surprisingly great: in 33 of the 39 comparisons the weekly agreement was within 125 mEq.

Large differences can occasionally be ascribed to incomplete collection of excreted material in the balance work, particularly when the cumulative balance shows a considerably greater accumulation of both cations over the same period. In Case 6 this might apply, as there was disagreement in both the sodium and potassium measurements over the same period from the 7th to 14th day and in the same positive direction. However, in the same patient in the previous week, there was an unexplained large discrepancy only in the potassium measurements. In Case 7, also, the disagreement between the two methods occurred over the same week both with sodium and potassium, but as the deviations were in opposite directions incomplete collections clearly cannot be the explanation. The balance method ignores loss of sodium in sweat, but these studies were carried out during the cooler months of the year. Freyberg and Grant[8] estimated the daily loss of sodium through the skin in lightly clad subjects doing laboratory work as between 2.6 and 9.1 mEq. a day. It is tempting to suggest that the greater accumulation of sodium over potassium in the body at the rate of about 5 mEq. a day shown by the balance method is partly due to neglect of the cutaneous sodium loss, but in view of the considerable scatter in the determinations this cannot be fully substantiated by statistical analysis.

It is important to emphasize the cumulative nature of systematic errors in the balance method: the error accumulates to an unlimited extent as long as the study is carried on. Sources of such error are (1) erroneous intake figures based on errors in food analysis, unrecognized rejections, or unmeasured feedings and (2) erroneous output data based on unmeasured loss (sweat, discards, exudates), or on errors in output analysis (sparingly soluble fecal salts, for instance).

It is conceivable that over the period of a year even a tiny systematic error (1 mEq. per day) could add up to a very significant fraction of the total body electrolyte. There is theoretically no limitation on this cumulative error in the balance technique.

In this laboratory, in "control balance studies" of healthy adults on light activity in the hospital, there has been a definite tendency toward a continuously slightly positive potassium balance. An exact correlation of balance changes with weight cannot always be made. Studies of potassium balance and nitrogen metabolism in children reveal a maintained positive balance for potassium with a potassium:nitrogen ratio over 3 to 5 mEq. per kilogram.[1, 10] No rational explanation can be offered for this apparently excessive retention of potassium in the body. Whatever the source of these errors, it seems clear that the metabolic balance as a technique for long-term study composition change has distinct limitations. By contrast, the errors and limitations of the isotope measurements are more readily ascertained, and are not cumulative. The errors are random and are not magnified by the passage of long time intervals.[11]

For *short-term study* of changes in body composition the metabolic balance

is of usefulness directly proportional to the care with which it is accomplished. The balance method demonstrates changes in body composition far smaller than the confidence limits of the isotope dilution methods and will, of course, continue to be the basis of sound biochemical study in a wide variety of states. The isotope dilution methods are more suitable for *serial, sequential observations* over a long period of time, in which application they are far superior to the balance methods. In addition, the isotope dilution methods provide a measure of *starting total body composition* quite beyond the scope of metabolic balance.

The significance of the non-exchangeable fraction of body electrolyte remains to be examined. The isotope dilution methods do not measure all the sodium and potassium in the body, but only that available for exchange within 24 hours. About 55% of the sodium in human bone does not exchange within this period.[6, 9] There is thus a large amount of non-exchangeable sodium in the body, a "reservoir" amounting to about one-quarter of the total body sodium. In the case of potassium the isotope measures approximately 95% of the total amount in the body.[4]

If, with either of these ions, a "reservoir effect" were to occur, we should expect to see a large and systematic discrepancy between the balance and isotope dilution methods. By "reservoir effect" is meant that in positive balance a significant amount of the ion would be "salted away" in non-exchangeable form, yielding a positive balance much larger than the change in exchangeable ion by isotope dilution. A "reservoir effect" in negative balance would, by the same token, be indicated by the "appearance" of large amounts of ion from a non-exchangeable pool, yielding a negative balance much larger than the change in exchangeable ion by isotope dilution. One purpose of this study has been to search for these phenomena. We have not found them. Although the balance is more positive for sodium (while both methods show positivity), possibly attributable to "storage" in a "reservoir," the same is also true for potassium, where both methods show negativity. The latter is the exact reverse of a "reservoir effect." Even in Case 8, in which there was a large drain of sodium from the body, there was no evidence of any mobilization of previously non-exchangeable sodium. Search for a "reservoir effect" in these data is unrewarding, despite our initial suspicion that such would be found. Such a conclusion is not to be interpreted as meaning that "reservoir effects" will not be found in other circumstances; under these varying clinical conditions they were not seen.

Summary

Changes in body composition with respect to sodium and potassium have been determined in the same subjects both by isotope dilution methods and by metabolic balance studies. The differences between the total exchangeable sodium and potassium measurements made at intervals have been compared with the cumulative metabolic balance totals over the same periods. There was agreement within 125 mEq. a week in 33 of the 39 comparisons. The significant discrepancies are described and discussed in detail.

The balance method showed a slightly greater accumulation of sodium in the body than the isotope method. This difference, though possibly real and due to neglect of cutaneous loss, attains only a low degree of statistical signifi-

cance in short-term studies. The difference in the potassium measurements also showed a greater tendency to retention by the balance method, but here the difference was even smaller and certainly not significant in short-interval studies.

Serial sequential determinations of total exchangeable sodium and potassium afford reliable evidence of changes in the body content of these cations. They are more rapidly and easily performed than balance studies and in suitable circumstances may be used as an alternative method for studying changes in body composition. Where long time intervals are involved, the isotope dilution is much more reliable than the balance method, does not lead to cumulative error, is less expensive, and does not require prolonged hospitalization.

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CMW & H.M. 0·6% loss in 24 hrs

or $\frac{1}{20}$ of 7·3% i.e. 0·36% loss.

Reprinted from METABOLISM
Vol. III, No. 4, July, 1954
Printed in U.S.A.

Body Sodium and Potassium

III. Inter-related Trends in Alimentary, Renal and Cardiovascular Disease; Lack of Correlation between Body Stores and Plasma Concentration

By FRANCIS D. MOORE, ISIDORE S. EDELMAN, JOHN M. OLNEY, ANTHONY H. JAMES, LAURA BROOKS AND GRAHAM M. WILSON

IN THE previous papers of this series[1, 2] methods were described for the measurement simultaneously of the total exchangeable sodium and potassium. These methods were compared with the metabolic balance as a means of study of body electrolyte. When studies of total electrolyte are combined with measurements of total body water and body weight,[3-11] considerable insight is gained into the body composition and its changes in disease. The total exchangeable potassium is proportional to the size of the lean body mass, those tissues (muscle and parenchyma) which contain approximately 98% of the body potassium. The total exchangeable sodium is an expression of the size of the "extracellular mass" and includes all the sodium in plasma, the interstitial phase, transcellular water[4] and the exchangeable fraction of bone sodium.[12] It is therefore a happy coincidence that readily available isotope dilution methods provide a direct measure of three of the most important parameters of body composition: cell substance, extracellular substance and water.

The total exchangeable electrolyte measurements describe the weight of a mass of electrolyte. The dimension is *mass*, not *volume*. When the denominator of the isotope dilution formula[3, 4] is the specific activity of a radioisotope at equilibrium, and the numerator is the radioactivity remaining in the subject, the dividend has the dimension of *mass*. Failure to understand this basic principle is responsible for reference to the total exchangeable potassium (K_e) as the "potassium space."

When a solid is diluted in a liquid (rather than in isotopic solid) and the denominator of the dilution formula is concentration of solid in liquid (or solute in solvent), then the dimension of the dividend is *volume*. This is true of the inulin space, thiocyanate space, blue dye volume, etc.

When radiosodium is employed, either mode of expression may be used, depending on the biologic measurement determined for the denominator of the equation. If the concentration of radiosodium in plasma (radioactivity per cubic centimeter) is determined, one has measured the sodium "space," a volume expressed in milliliters or liters. If the equilibrium specific activity (Na^{24}/Na^{23} ratio) is determined, one has measured the total exchangeable sodium (Na_e), a *mass* expressed in milligrams or milliequivalents.

It is of interest in this connection that the total body water method based on

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This work was supported throughout by the Atomic Energy Commission. We wish also to acknowledge the help of the Upjohn Company and Winthrop-Stearns, Inc.

Received for publication November 23, 1953.

deuterium dilution is in actuality the "total exchangeable hydrogen," expressed as an equivalent water volume.

Total exchangeable sodium and potassium have now been measured and reported extensively in healthy subjects.[4-11] Some studies have been made in pathological conditions.[13-15] It is becoming apparent that simple malnutrition alone considerably changes the body content of sodium and potassium and that changes in serum concentration do not necessarily reflect corresponding alterations in body content.[16] The concept that lowering of the serum potassium or sodium concentration is due to a "deficiency" of one or another of these ions has often been regarded as firmly established. The studies described herein have helped to define the limited extent to which "deficiencies" are associated with decreases in plasma concentration of sodium or potassium.

These studies have also shown that a variety of diseases tend to *decrease* the cell mass and *increase* the extracellular phase and that coincident with this one may observe in the plasma an *elevated* potassium and *depressed* sodium concentration. This common but paradoxical situation (which frequently occurs with a high water fraction and a loss of body fat) has certain definite implications relative to trauma and acute disease, and may well comprise a compositional and biochemical "depletion syndrome."

Methods and Materials

Methods

Total exchangeable sodium and potassium have been measured by dilution of the isotopes Na^{24} and K^{42} utilizing techniques previously described.[1] A 24-hour equilibration period has been used in all the studies. No measurements have been reported which were made in the immediate postoperative period.

Methods will not be reviewed here in detail save to point out the following facts:

1. The total exchangeable potassium (K_e) measures virtually all the potassium in the body.
2. The total exchangeable sodium (Na_e) measures all the "available" sodium, the nonexchangeable fraction of bound bone sodium not being included.
3. The methods are reproducible to $\pm 4\%$; differences less than $\pm 8\%$ of the mean of two successive measurements should be viewed with reservation as being of borderline significance.
4. Repeated measurements with the passage of long time intervals appear to be a more accurate reflection of changing body composition than the metabolic balance, because of the cumulative nature of errors in balance measurement.[2]

Materials

1. *Healthy subjects* have previously been studied and reported. They are mentioned here only as reference points for the pathological series.
2. *Obese Subjects.* Four obese males and three obese females in good general health were studied. The males weighed from 90 to 125 kg. and the females from 70 to 95 kg.
3. *Alimentary Disease.* Twenty-eight patients with chronic gastrointestinal

disease were studied. These were surgical patients, either preoperative or several weeks or months postoperative. Fifteen were suffering from malignancy uncomplicated by primary renal or cardiac disease. The remainder were suffering from benign inflammatory disease (ulcer, colitis, chronic pancreatitis). All had lost weight.

4. *Renal Failure.* Fourteen patients suffering from renal failure were studied. Of these, seven were suffering from acute renal shutdown (transfusion, postpartum, postoperative) and seven were suffering from chronic parenchymatous renal disease (pyelonephritis, hypertensive, chronic glomerulonephritis). In these patients we were particularly interested in the relationship of a high serum potassium to the total exchangeable potassium.

5. *Nonedematous Valvular Heart Disease.* Of a large series of patients with mitral stenosis studied and reported elsewhere,[13] a small group of five representative patients and their preoperative measurements are described here; none was edematous at the time of the measurement, all were chronic invalids.

6. *Hypertension.* Seven patients with severe hypertension, but without edema, were investigated with regard to the total exchangeable sodium. Of these, four had malignant hypertension and one benign. One had Cushing's disease and one chronic glomerulonephritis. In these patients the relationship of the body sodium stores to hypertension is of particular interest.

7. *Edema.* Measurements were made in 11 patients with edema resulting from a wide variety of causes. Four had rheumatic valvular disease with chronic congestive failure, three were nephrotics, one had idiopathic edema, one carcinomatosis and one glomerulonephritis. Many of these patients had low serum sodium concentrations; the relationship of this finding to the total exchangeable sodium is of particular importance.

Results

Healthy Subjects

For convenience of reference the mean values for total exchangeable potassium and sodium obtained in normal adults of both sexes are summarized in Table 1. These values will be used for comparison with the results found in the pathological

Table 1.—*Total Exchangeable Potassium and Sodium in Healthy Adults**

	Number	Age yrs. mean	Range	Weight kg. mean	Range	Exchangeable cation mEq./kg.		
						Mean	S.D.	Range
Potassium								
Males.....	30	25.8	22–31	72.6	63.1–81.0	46.3	±4.3	35.6–53.6
Females....	14	23.6	19–29	55.4	48.0–61.6	39.0	±5.4	28.0–47.2
Sodium								
Males.....	12	28.1	21–49	74.8	66.6–83.2	42.1	±4.0	36.1–48.3
Females....	12*	27.6	20–52	62.5	51.8–81.6	39.6	±3.2	34.4–45.9

* These figures for exchangeable sodium are a combination of the results in seven females reported by Forbes and Perley[8] and in five females studied in this laboratory. There was close agreement between the two series. The other figures are all results obtained in this laboratory.[4, 7]

TABLE 2.—*Exchangeable Potassium in Obese Subjects*

	Subject	Age	Weight kg.	Exchangeable potassium	
				mEq.	mEq./kg.
Males	F. G.	57	90.9	2819	31.0
	R. W.	31	91.0	4040	44.3
	C. J.	40	93.4	3546	38.0
	A. G.	30	125.5	4470	35.6
	Mean	39.5	100.2	3719	37.2
Females	A. B.	26	76.6	2762	36.1
	K. C.	31	71.2	2313	32.5
	M. R.	46	94.5	2583	27.3
	Mean	34.3	80.8	2553	32.0

TABLE 3.—*Exchangeable Sodium in Obese Subjects*

	Subject	Age	Weight kg.	Exchangeable sodium	
				mEq.	mEq./kg.
Males	F. G.	57	90.4	3540	39.2
	C. J.	40	93.4	3094	33.1
	Mean	48.5	91.9	3317	36.2
Females	K. C.	31	71.2	2237	31.4
	M. R.	46	95.0	3018	31.8
	Mean	38.5	83.1	2628	31.6

conditions subsequently described. It will be noted that the values for females for both sodium and potassium are lower than in males. In healthy adult males the ratio $Na_e:K_e$* is 0.91, indicating that more potassium than sodium is normally exchangeable with the tracers. In females there is disproportionately less potassium, so that the ratio $Na_e:K_e$ rises to 1.02.

The lesser total body water in females has been described.[5] There is good evidence that there is more fat and less lean tissue in the female, as an explanation of this reduced total body water. The lower exchangeable potassium would appear to be on the same basis.

Obese Subjects

As depot neutral fat contains neither sodium nor potassium, it is not surprising that the values for total exchangeable potassium and sodium are low in obese subjects (Tables 2 and 3). It is important to note that the values for both cations are about equally depressed. Here again the relationship to the low total body water of the obese patient[5] should be stressed.

* The symbols Na_e and K_e are used to denote total exchangeable sodium and potassium respectively.

TABLE 4.—*Exchangeable Potassium in Patients with Alimentary Disease*

Subject	Diagnosis	Date	Age yrs.	Weight kg.	Exchangeable potassium		Serum potassium mEq./L.
					mEq.	mEq./kg.	
Males							
F. P.	Duodenal ulcer; gastroenterostomy; persistent vomiting	11-22-52	20	52.4	2098	40.0	3.0
A. B.	Carcinoma of rectum	3-13-52	65	60.9	2238	36.1	4.9
	3 wks. after abdominoperineal excision	4-4-52		52.6	1954	34.0	4.3
T. O'C.	Carcinoma of stomach	9-28-49	73	51.6	1510	29.3	—
A. N.	Jejunal fistula following gastrectomy	7-1-47	62	63.0	1780	28.3	—
B. K.	Carcinoma of esophagus	7-19-50	62	69.0	1810	26.2	—
W. H.	Carcinoma of stomach	1-11-51	82	74.0	2102	28.4	4.6
A. H.	Duodenal ulcer; pyloric stenosis, preoperative	11-9-50	54	52.6	1682	31.9	4.2
	After partial gastrectomy	4-5-51		56.0	2164	44.0	—
A. J.	Carcinoma of stomach	5-13-47	71	43.0	1830	42.6	—
M. S.	Chronic pancreatitis; carcinoma of the prostate	7-9-53	73	63.0	1681	26.7	5.4
G. S.	Duodenal fistula following perforation	11-1-51	36	52.5	1582	30.1	4.3
J. D.	Lymphoma of jejunum; diarrhea following jejunal resection	6-15-50	44	63.0	1885	29.9	3.9
L. S.	Partial gastrectomy for ulcer; persistent vomiting & anorexia	9-14-50	69	54.7	1210	22.2	—
J. D.	Chronic duodenal ulcer	2-6-47	35	71.0	2954	41.6	—
W. J.	Carcinoma of stomach	5-13-47	71	43.0	1617	37.6	—
J. B.	Inoperable carcinoma of stomach	3-16-47	70	47.0	1654	35.2	—
L. W.	Inoperable carcinoma of stomach	4-30-47	51	46.5	1279	27.5	—
J. W.	Ulcerative colitis	3-31-48	27	37.0	1173	31.7	—
Mean of initial readings			56.8	55.5	1770	32.1	4.3
Comparison with Table 1 $t = 9.6$, $p < .0001$							
Females							
L. P.	Chronic pancreatitis	1-8-53	65	52.4	1440	27.5	3.5
J. H.	Carcinoma of stomach	10-5-50	75	61.1	1410	23.1	—
F. H.	Carcinoma of esophagus	8-17-50	54	40.4	1262	31.3	3.0
E. W.	Pericolic abscess	10-5-50	64	42.2	1033	24.6	3.1
L. T.	Duodenal ulcer; pyloric stenosis	7-18-50	50	37.2	994	26.7	—
	12 wks. after gastroenterostomy	10-12-50		43.6	1837	42.1	3.9
M. R.	Gastric ulcer	2-7-50	67	38.1	1202	31.6	3.2
	14 mos. after gastrectomy	4-12-51		34.8	1200	34.5	4.0
A. H.	Carcinoma of stomach	4-29-47	66	42.8	1170	27.3	—
A. C.	Carcinoma of rectum	3-19-47	77	59.0	1230	20.9	—
E. D.	Carcinoma of rectum	7-9-53	79	52.9	1238	23.4	4.6
A. B.	Inoperable carcinoma of rectum	3-19-47	70	52.0	1362	26.2	—
C. C.	Obstructive jaundice	9-23-47	40	53.0	1611	30.4	—
Mean of initial readings			64.3	48.3	1268	26.6	3.6
Comparison with Table 1 $t = 6.5$, $p < .0005$							

Alimentary Disease

Measurements of total exchangeable potassium in a group of 20 patients with lesions of the alimentary system are shown in Table 4. In all, this illness had caused considerable loss of weight through interference with appetite or digestion or by extrarenal loss. The exchangeable potassium was conspicuously reduced in comparison with normal healthy subjects. The measurements of serum potassium concentration fell within the range of 3.0 to 5.4 mEq. per liter in this group. In two patients with pyloric stenosis due to chronic duodenal ulceration, the measurements of total exchangeable potassium were repeated several months after uncomplicated surgery (Patients A. H. and L. T., Table 4). In both there was a substantial gain in weight and a conspicuous rise in total exchangeable potassium. One female patient (M. R.) continued to lose weight after a gastrectomy for gastric ulcer, but in her case there was no definite change in exchangeable potassium. In one male patient (A. B.) measurements were repeated three weeks after excision of a carcinoma of the rectum. Recurrence was soon evident, his condition deteriorated, he lost weight and as he entered his terminal phase the exchangeable potassium was further reduced.

TABLE 5.—*Exchangeable Sodium in Patients with Alimentary Disease*

Subject	Diagnosis	Date	Age	Weight kg.	Exchangeable sodium		Serum sodium mEq./L.
					mEq.	mEq./kg.	
Males							
F. P.	Duodenal ulcer; gastroenterostomy, persistent vomiting	11-22-52	20	52.4	3126	59.7	140
G. S.	Duodenal fistula following perforation	11-1-51	36	52.5	3054	58.0	137
J. P.	Dysphagia due to bulbar palsy	7-22-53	65	53.5	3058	57.2	144
J. W.	Duodenal ulcer, multiple gastric ulcers	4-4-51	51	53.0	2545	48.0	133
A. B.	Carcinoma of rectum	3-13-52	65	60.9	2524	42.1	140
	3 wks. after abdominoperineal excision	4-4-52		52.6	2844	54.1	133
M. S.	Chronic pancreatitis; carcinoma of prostate	7-9-53	73	63.0	2913	46.2	132
P. B.	Carcinoma of colon	7-22-53	48	59.1	2796	47.3	136
Mean of initial readings			51.1	56.3	2859	51.2	137
Comparison with Table 1 $t = 4.0$, $p < .0001$					s.d.	6.1	
Females							
A. K.	Carcinoma of pancreas	11-15-52	83	50.9	2700	53.0	116
	Repeat after sodium load	11-28-52		53.6	3103	57.9	139
L. P.	Chronic pancreatitis	1-8-53	65	52.4	2586	49.4	138
E. D.	Carcinoma of rectum	7-9-53	79	52.9	2166	40.9	142
Mean of initial readings			75.7	52.1	2484	47.8	132
Comparison with Table 1 $t = 2.4$, $p < .02$					s.d.	6.7	

Measurements of exchangeable sodium in a similar group of 10 patients are presented in Table 5. These patients all show high values. They may be exceptionally high when a severe degree of wasting has developed as was apparent in the first three patients (F. P., G. S. and J. P.). This is particularly true of the *relative* sodium content (results expressed as mEq./kg.). As further loss of weight occurs the *absolute* amount of exchangeable sodium may also increase (A. B). It is to be emphasized that none of these patients showed any trace of edema at the time of the measurements.

TABLE 6.—*Exchangeable Potassium in Renal Failure*

Subject	Diagnosis	Date	Age	Weight kg.	Exchangeable potassium		Serum potassium mEq./L.	Blood urea nitrogen mg./100 ml.
					mEq.	mEq./kg.		
Males								
J. W.	Chronic pyelonephritis	11-21-50	52	56.2	1320	23.5	8.2	99
W. R.	Postoperative anuria	9-14-50	51	74.6	2530	34.0	7.0	132
	Repeat; diuresis beginning	9-21-52		74.8	2370	31.0	5.0	97
	Repeat; considerable improvement	10-5-50		73.0	2380	32.6	4.7	17
W. A.	Carcinoma of prostate; nephrostomy	3-22-51	71	54.8	1910	34.8	6.1	19
K. M.	Malignant hypertension	1-25-51	41	64.5	1960	30.4	4.9	28
D. S.	Chronic glomerulonephritis	11-21-50	33	54.0	2250	41.6	6.1	78
J. L.	Lower nephron syndrome; gross sepsis	8-10-49	65	68.8	1960	28.5	3.5	100
D. K.	Anuria after prostatectomy	10-12-50	72	61.3	2890	47.2	6.3	118
	Repeat after diuresis	11-7-50		53.2	2040	38.5	4.0	24
Mean of initial readings			55.0	62.0	2127	34.3	6.0	82
Comparison with Table 1 $t = 4.1$, $p < .0001$					s.d. 7.4			
Females								
M. R.	Post-transfusion anuria	5-10-51	25	58.2	2145	36.9	8.5	290
H. M.	Postpartum anuria; septic abortion	3-22-51	32	64.6	1829	28.2	5.0	170
M. F.	Bilateral renal cortical necrosis	6-9-49	35	53.8	1130	21.0	5.5	98
B. B.	Polycystic kidneys	4-19-51	53	62.0	1645	26.5	6.5	105
F. W.	Chronic glomerulonephritis	4-19-51	30	45.5	1810	39.8	5.5	101
D. P.	Chronic glomerulonephritis	5-31-51	29	50.2	1695	33.6	5.5	100
M. M.	Chronic glomerulonephritis	5-26-49	32	50.0	1600	32.0	5.0	92
Mean			33.7	54.9	1707	31.1	5.9	137
Comparison with Table 1 $t = 2.9$, $p < .005$					s.d. 5.96			

Renal Failure

An elevation of the serum potassium concentration is a feature commonly associated with either acute or chronic renal disease. A series of measurements of total exchangeable potassium has been made in such a group of 14 cases (Table 6). In both male and female patients with severe renal disease (as manifested by the elevation of the blood urea) the total exchangeable potassium is considerably lower than in healthy subjects, even though a high serum potassium concentration coexists. Furthermore, in those cases in which measurements were possible after a fall in the serum potassium level the changes in total exchangeable potassium were variable. In one case of anuria that followed operation (W. R.), recovery was associated with a fall in blood urea and serum potassium concentration. The body weight fell slightly, and there was a small decrease in total exchangeable potassium. In a second similar case (D. K.) there was also a fall in blood urea and serum potassium but a much greater loss of weight and decrease in exchangeable potassium.

Nonedematous Valvular Heart Disease

The results in a series of patients with severe mitral stenosis but without edema have been described elsewhere.[13] There was initially a small but significant elevation in exchangeable sodium and a slight depression of exchangeable potassium. After a successful operation these changes were reversed (Tables 7 and 8).

Hypertension

In view of the known effect of a restriction of dietary sodium intake on the blood pressure and the implication of body sodium in blood pressure variations, a group of seven patients with severe hypertension but without any edema were

TABLE 7.—*Exchangeable Sodium in Patients with Advanced Mitral Stenosis, Preoperative*

Subject	Sex	Date	Age	Weight	Exchangeable sodium		Serum sodium mEq./L.
					mEq.	mEq./L.	
H. H.	F	5-14-52	45	34.2	1542	45.0	139
M. B.	F	10-17-52	40	41.2	1932	46.9	143
M. M.	F	11-19-52	47	47.3	1985	42.0	135
A. S.	F	1-14-53	41	47.1	1575	33.5	133
H. T.	M	1-8-53	42	38.6	2507	64.9	136

TABLE 8.—*Exchangeable Potassium in Patients with Advanced Mitral Stenosis, Preoperative*

Subject	Sex	Date	Age	Weight kg.	Exchangeable potassium		Serum potassium mEq./L.
					mEq.	mEq./L.	
H. H.	F	5-14-52	45	34.2	1249	36.8	4.6
M. B.	F	10-17-52	40	41.2	1565	38.0	5.1
M. M.	F	11-19-52	47	47.3	1581	33.4	4.0
A. S.	F	1-14-53	41	47.1	1600	34.0	5.5
H. T.	M	1-8-53	42	38.6	1645	42.6	5.0

TABLE 9.—*Exchangeable Sodium in Nonedematous Hypertensive Patients*

Subject	Diagnosis	Date	Age	Weight kg.	Exchangeable sodium		Serum sodium mEq./L.	Blood pressure mm. Hg
					mEq.	mEq./L.		
Males								
E. M.	Malignant hypertension	4-5-51	42	65.7	2650	40.3	140	220/150
	Bilateral adrenalectomy	4-10-51						
	Cortisone 25 mg./day	5-10-51		62.0	2629	42.4	142	180/120
E. G.	Malignant hypertension	6-7-51	33	63.0	2992	47.5	134	220/140
	Bilateral adrenalectomy	6-19-51						
	Cortisone 25 mg./day	6-27-51		59.8	2733	45.7	122	200/140
J. M.	Malignant hypertension	2-14-51	31	96.6	3806	39.4	140	220/150
G. W.	Cushing's disease with hypertension	4-1-52	30	81.9	2652	31.2	141	180/120
	Bilateral adrenalectomy	4-3-52						
	Cortisone 25 mg./day	10-8-52		80.3	2618	32.6	140	130/80
Mean of Initial Readings			34.0	76.8	2869	39.6	138.8	210/140
Comparison with Table 1 $t = 0.89$, $0.3 < p < 0.4$					s.d. 5.8			
Females								
P. K.	Malignant hypertension	6-7-51	38	47.2	1680	35.6	130	225/140
G. C.	Benign hypertension	11-29-51	52	77.7	2677	34.4	135	150/100
D. P.	Chronic glomerulonephritis	5-31-51	29	50.2	2159	43.0	120	220/120
Mean			39.7	58.4	2202	37.7	128	198/120

investigated with regard to total exchangeable sodium (Table 9). The group is not large enough for detailed statistical analysis, but it is clear that the results all fall well within the normal range. In three of these patients the measurements were repeated after bilateral adrenalectomy. In E. M., with malignant hypertension, and in G. W., with Cushing's disease, the blood pressure fell substantially, but in neither was there any significant alteration in total exchangeable sodium. In E. G. there was a reduction in serum sodium concentration and in exchangeable sodium but no change in blood pressure. In these cases of hypertension there is no direct correlation between exchangeable sodium and the blood pressure level.

Edema

A series of measurements of total exchangeable sodium and potassium has been made in patients with well-marked edema. The results are shown in Tables

TABLE 10.—*Exchangeable Sodium in Edematous Patients*

Subject	Diagnosis	Age	Weight kg.	Exchangeable sodium		Serum sodium mEq./L.
				mEq.	mEq./kg.	
Males						
W. B.	Congestive cardiac failure; mitral stenosis	44	57.6	3254	57.6	135
M. L.	Congestive cardiac failure; myocardial fibrosis	15	33.6	1957	58.2	120
K. M.	Congestive cardiac failure; hypertension	41	66.8	4560	68.3	139
F. C.	Congestive cardiac failure; hypertension	61	58.3	3787	65.0	137
J. P.	Nephrosis	42	66.8	4020	60.2	137
A. S.	Nephrosis	10	29.1	2320	79.7	140
Mean		35.5	52.0	3316	64.8	134.7
Comparison with Table 1 $t = 7.0$, $p < .0001$					s.d. 7.65	
Females						
C. G.	Congestive cardiac failure; rheumatic heart disease	40	48.2	2521	52.3	140
R. C.	Congestive cardiac failure; mitral stenosis	29	46.5	3041	64.5	123
E. S.	Congestive cardiac failure; mitral stenosis	24	37.2	2742	73.8	138
M. L.	Idiopathic edema; obesity	33	96.8	5230	54.0	141
L. P.	Ovarian carcinomatosis	60	54.8	2652	48.4	135
Mean		37.2	56.7	3237	58.6	135.4
Comparison with Table 1 $t = 4.5$, $p < .0001$					s.d. 9.28	

10 and 11. In all cases the exchangeable sodium is greatly increased even when the serum sodium is low. On the other hand, the exchangeable potassium is considerably diminished. These changes are both absolute and relative; in some of the edematous patients the sodium figures are higher and the potassium lower than is seen in any other group.

Relationship Between Serum Concentration and Total Exchangeable Measurements of Sodium and Potassium

It has frequently been observed in these laboratories that there is not a close correlation between the serum concentration of either sodium or potassium and the amount available in the body as measured by isotope dilution. This has now been further confirmed in a wide variety of cases; the results are shown in Figures 1 and 2. In spite of ranges in serum sodium concentration between 117 mEq. and 145 mEq. per liter and in potassium concentration between 2.9 mEq. and 8.5 mEq. per liter, there is not a corresponding alteration in total exchangeable sodium and potassium. Indeed, there is frequently an inverse correlation: eleva-

TABLE 11.—*Exchangeable Potassium in Edematous Patients*

Subject	Diagnosis	Age	Weight kg.	Exchangeable potassium		Serum potassium mEq./L
				mEq.	mEq./kg.	
Males						
W. C.	Congestive cardiac failure; hypertension	34	61.2	1347	23.4	5.0
K. M.	Congestive cardiac failure; hypertension	41	64.5	1960	30.4	4.2
W. C.	Congestive cardiac failure; hypertension	29	83.8	3153	37.6	
A. B.	Congestive cardiac failure; hypertension	34	57.6	1347	23.4	
W. B.	Congestive cardiac failure; mitral stenosis	44	57.6	2090	37.0	5.2
M. A.	Glomerulonephritis	29	72.5	3450	47.6	4.0
J. P.	Nephrosis	42	64.6	2545	39.4	5.4
Mean		36.1	66.0	2251	34.1	4.76
Comparison with Table 1 $t = 3.8$, $p < .0002$				s.d. 8.25		
Females						
E. S.	Congestive cardiac failure; mitral stenosis	24	37.1	1041	28.0	3.6
M. L.	Idiopathic edema; obesity	33	95.0	2180	22.9	4.3
Mean		28.5	66.1	1610	25.4	3.9

tion of serum potassium often occurs during severe stress when large amounts of potassium are being excreted; this accounts for the low exchangeable potassium often observed in the presence of a high serum potassium. Similarly, low serum sodium concentrations were most frequently seen in patients with congestive cardiac failure or after major trauma. In such circumstances the body is strongly retaining sodium, and exchangeable sodium measurements are usually high.

DISCUSSION

The results of all the measurements have been expressed both in absolute terms and as milliequivalents per kilogram of body weight. Although many other correlations have been investigated, that with body weight has been found the most satisfactory basis for assessment in normal subjects.[7, 8] When measurements are expressed in this manner in pathological conditions, certain difficulties immediately arise. These are chiefly related to the nature of changes in body weight. An increase in body fat clearly reduces total cation content when expressed as a function of body weight (mEq./kg.). By the same token, a decrease in fat alone increases relative cation content. Changes in body fat content have recently been investigated in conjunction with measurements of total body water, and it has clearly been shown that an increased mobilization and oxidation of fat is a prominent feature in the response to many acute pathological states.[6] A "pure" fat gain or loss is, however, a relatively uncommon occurrence, and there are

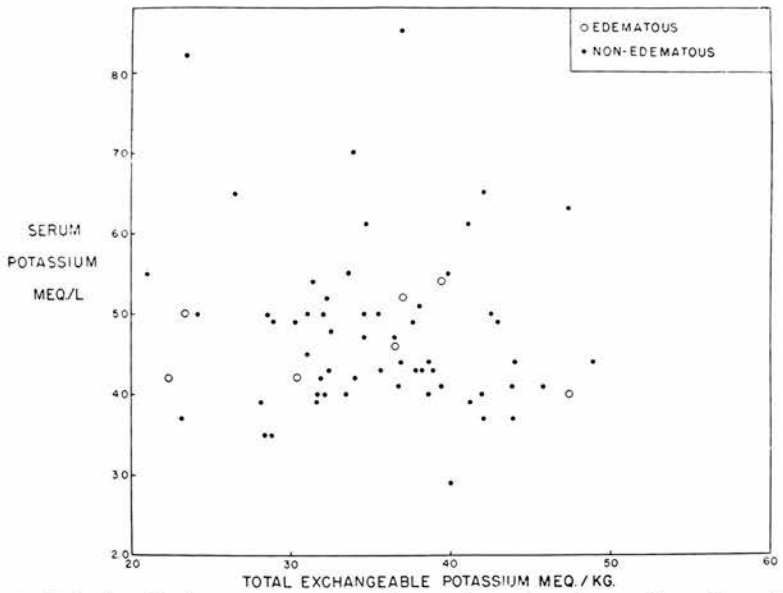

FIG. 1.—Relationship between serum sodium and total exchangeable sodium. There is no definite correlation, but in many instances the low serum concentrations are associated with high exchangeable sodium values. It will be noted that many serum sodium concentrations fall below normal whereas elevations are rare. By contrast, the total exchangeable sodium values tend frequently to be high. There are very few below normal.

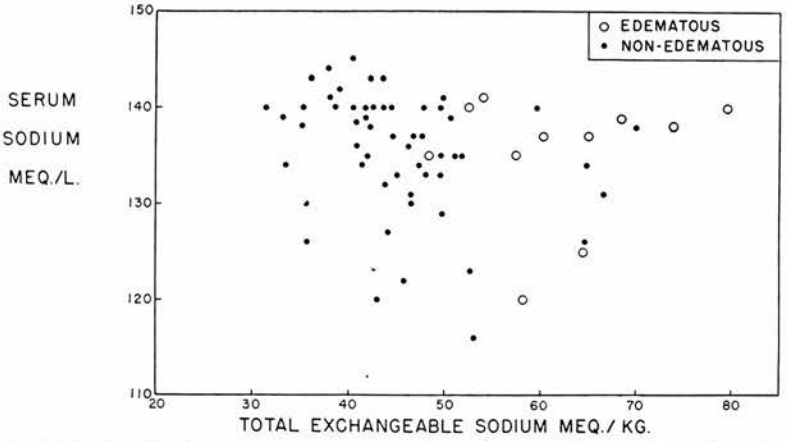

FIG. 2.—Relationship between serum potassium concentration and total exchangeable potassium. There is no definite correlation, but in many instances high serum concentrations are associated with abnormally low total exchangeable potassium values. It will be noted that the serum concentrations tend to be above normal, only a few were observed below normal. By contrast, the total exchangeable potassium values tend to be low in disease. Only a few were observed in the high normal range.

usually associated changes in lean body mass and in extracellular fluid volume. The complex nature of these changes in body weight must always be remembered in the expression and interpretation of measurements of exchangeable potassium and sodium.

The normal female subject has a lower total exchangeable potassium than the male. This correlates with the observation that the total body water is also

lower.[5] Both findings are presumably traceable to the relatively greater fraction of body weight occupied by fat and the smaller fraction occupied by lean tissue (chiefly muscle). A similar phenomenon is evident in the obese subject of either sex.

The patients with chronic disease of the alimentary system show the effect of undernutrition and wasting on body composition with respect to the cations potassium and sodium. The former is almost entirely intracellular in situations, and in amount is related to the lean tissue of the body. Selective loss of fats tends to increase the exchangeable potassium (expressed on a body weight basis), but selective loss of lean tissue (as in paralysis) results in bone, fat and extracellular water becoming a greater fraction of body weight; therefore the exchangeable potassium falls. In chronic disease states it is thus not surprising to find that total exchangeable potassium is usually reduced in amount. At the same time there is an increase in total exchangeable sodium. This is probably to be attributed to at least two factors. First is the loss of fat and lean tissue which reduces body weight and leaves plasma and interstitial water as a larger fraction. Second is the expansion of extracellular fluid volume. This latter is known to occur in chronic starvation, wherein considerable sodium retention is observed.[17, 18] Extracellular fluid seems to fill at least partially the space previously occupied by fat and lean tissue[19] in starvation.

Chronic disease of many systems frequently leads to malnutrition and wasting. In these circumstances a loss of exchangeable potassium and a gain of exchangeable sodium may be anticipated quite apart from any more specific changes due to the disease. This is clearly seen in the cases of renal failure, in which the total exchangeable potassium was reduced owing to loss of lean tissue, in spite of the fact that the serum potassium was elevated. The extracellular fluid contains about 2% of the total body potassium. Thus, it is not surprising that the changes in serum concentration are completely overshadowed by the alterations in the amount of potassium in lean tissue.

The role of sodium in the development of hypertension has been investigated from several points of view. It has been clearly established that severe sodium restriction leads to a decline in the blood pressure level.[20, 21] It has been shown that sodium plays an essential part in the maintenance of renal hypertension.[22, 23] More recently it has been claimed that there may be an excess of sodium in the arteries of hypertensive animals and humans[24] and that the extracellular fluid volume is increased.[25] It is accordingly of interest to find that in a small group of subjects with severe hypertension the measurements of total exchangeable sodium fall well within the normal range. Furthermore, changes in blood pressure could not be correlated with any alteration in total exchangeable sodium. It has also been shown that decreases in blood pressure regularly occur on low sodium diets at a time when the patient is no longer in negative sodium balance.[26] Exchangeable sodium is located in several compartments of the body, chiefly the extracellular fluid, bones and gastrointestinal tract.[12, 27] While the total amount of exchangeable sodium in these grossly hypertensive patients was clearly not significantly altered, it is not possible from these measurements to exclude an alteration in the relative distribution of this sodium.

In edematous patients the expansion of the extracellular fluid leads to a great excess of exchangeable sodium as previously described.[28] In these measurements an equilibration period of 24 hours was used, and it is possible that equilibrium of distribution was not fully attained after this interval in some of the cases.[29] Exchangeable potassium was reduced in consequence of lean tissue wasting and the addition of fluid low in potassium content.

For the most part, chronic disease, be it alimentary, renal or cardiac, induces alterations in body composition characterized by a relative depletion of body potassium and a relative excess of body sodium. Contrary to the situation with sodium, potassium contents do not seem to reach abnormally high levels. In the 54 subjects studied, not a single instance of excess body potassium content was noted. This suggests that "potassium intoxication" is not due to the presence in the body of an absolute excess of potassium, but instead to an increase in the extracellular portion of the total.

The lack of correlation between serum concentrations and the accompanying total exchangeable cation measurements has been described previously,[13] and confirmed in patients with cirrhosis of the liver.[30] In many of the cases reported here this point is further emphasized. Serum levels are affected by many other factors than the amount in the body. The rate of lean tissue catabolism, renal function and acid-base balance[31] may alter the concentration of potassium in the plasma. Excessive retention of water may depress serum sodium concentration even though there is an adequate absolute amount of sodium in the body. It is clearly unwise to diagnose a cation deficiency or excess on the basis of an abnormality of the serum level alone.[16] Similarly many factors alter the amount of cation in the body when this is assessed in terms of body weight. Loss of lean tissue or increase of fat will decrease exchangeable potassium. Furthermore, in view of the wide normal range of total exchangeable sodium and potassium, considerable caution must be exercised in interpreting the results in pathological states, particularly when the value for the individual in good health is not known. The interpretation of any single observation must always be made against the background of the clinical history and condition of the patient.

From examination of the whole series, it is clear that in good health a generous mass of lean tissue is maintained with only a minimum surrounding milieu of "ancient sea water"; yet the toxic potassium ion concentration in that milieu is kept low and the circulation-maintaining osmotic determinant, the sodium concentration, is kept high. This entire balance is the result of vertebrate evolution and of individual growth; it requires energy, particularly at the cell surface and in the kidney, for its maintenance. A wide variety of diseases cause it to "slip away," the energy necessary is not available and the organism tends to revert to a more primitive composition. The cell mass (total exchangeable potassium) drops, the toxic ion concentration (plasma potassium) increases, the tide of salty milieu (total exchangeable sodium) rises, and its osmotic determinant (plasma sodium concentration) falls.

It is extremely rare to observe an elevated total exchangeable potassium. Only under some condition of muscular hypertrophy would one expect to find it. Conversely, a low total exchangeable sodium is of extreme rarity in chronic

disease. Only in the Addisonian or in true acute dehydrating sodium deficiency[16] might one expect to find it.

The organism rarely climbs "up the ladder" of lean tissue dominance; when sick, it quickly "backslides" to an increasing fraction of salt and water.

Summary

1. Measurements of total exchangeable potassium and sodium have been made in a variety of pathological conditions and compared with the values found in healthy adults.

2. In obesity the values for both cations were reduced.

3. In chronic disease of the alimentary system leading to body wasting there was a decrease in exchangeable potassium and an increase in exchangeable sodium.

4. In renal failure accompanied by an elevation in the serum potassium concentration, exchangeable potassium was reduced.

5. In advanced mitral stenosis a slight elevation of total exchangeable sodium with a slight lowering of total exchangeable potassium was seen.

6. In severe hypertension exchangeable sodium was within the normal range. Variations in blood pressure following adrenalectomy were not accompanied by alterations in exchangeable sodium, and no correlation between the two was evident.

7. In edema exchangeable sodium was greatly increased and exchangeable potassium reduced.

8. In the whole series there was no definite correlation between serum levels and the corresponding measurements of exchangeable sodium and potassium. The two clearly varied independently, and at times inversely.

9. Therefore, when depression or elevation of the serum concentration of these ions is observed clinically, one must be guarded in concluding that a deficiency or excess is present in the body.

10. Depletion due to a variety of conditions produces a compositional "syndrome" characterized by (*a*) a high total exchangeable sodium with a low serum sodium concentration, (*b*) a low total exchangeable potassium with a high serum potassium concentration, (*c*) a high water fraction[6] a large proportion of which appears to be extracellular, and (*d*) a low fat fraction.

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REPRINTED FROM
ANNALS OF SURGERY
227 South Sixth Street, Philadelphia, Penna.
Vol. 141 FEBRUARY, 1955 No. 2

STUDIES IN SURGICAL ENDOCRINOLOGY

I. THE URINARY EXCRETION OF 17-HYDROXYCORTICOIDS, AND ASSOCIATED METABOLIC CHANGES, IN CASES OF SOFT TISSUE TRAUMA OF VARYING SEVERITY AND IN BONE TRAUMA*

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I. INTRODUCTION

CHANGES IN THE FUNCTION of the hypothalamus, anterior and posterior pituitary, gonads, thyroid, adrenal medulla and adrenal cortex have been described or hypothecated as endocrine features of recovery from injury and surgery.[1-3, 8, 9, 12, 21, 28, 30, 45] In many instances data are fragmentary, evidence indirect and conclusions suppositional. Nonetheless the unfolding picture is that of convalescence as an extensive endocrine adjustment analogous in scope if not in duration to such other life situations as growth, puberty, pregnancy, or senescence. Of the various endocrine influences bearing on the metabolism of convalescence, those pertaining to the steroid hormones of the adrenal cortex are certainly the most familiar. Yet even here, despite the accumulation of data during the past 15 years and much theoretical speculation—often based on observations in laboratory rodents—there is a pressing need for more facts based on everyday traumatic experience in the normal human being.

Such facts should be collected from the study of a variety of normally convalescent surgical patients as well as from abnormal states of shock or special problems such as third-degree burns. Endocrine studies of surgical patients are most informative if accompanied by metabolic data adequate to illuminate interpretation, and if the observations are carried on through the hospital course of the patient. Small bits of data based on studies lasting only two or three days may be extremely misleading. And finally, clinical interpretation of the events recorded is an important aspect of each study. Metabolic study without clinical interpretation omits one of the most important observations to be made: the patient's course.

There have been several studies reported of the urinary excretion of 17-ketosteroids in man after soft tissue trauma and after burns.[2, 10, 20, 38, 49] Studies in our laboratories over the past four years have disclosed few findings of additional interest by this method, and are largely unreported. We have observed, as have others, that the urinary excretion of 17-ketosteroids after trauma is variable and unpredictable. Some individuals in good health (and in young or middle age) undergoing fairly extensive

* This work was supported by a grant from the United States Atomic Energy Commission to the Peter Bent Brigham Hospital (AT(30-1)-733), and sponsored by the Commission on Liver Diseases, Armed Forces Epidemiological Board, and supported by The Surgeon General, Department of the Army, through a contract (DA-49-007-MD-472) with Harvard Medical School. The assistance of grants from the Upjohn Company and Winthrop-Stearns, Inc., is gratefully acknowledged.

Submitted for publication August, 1954

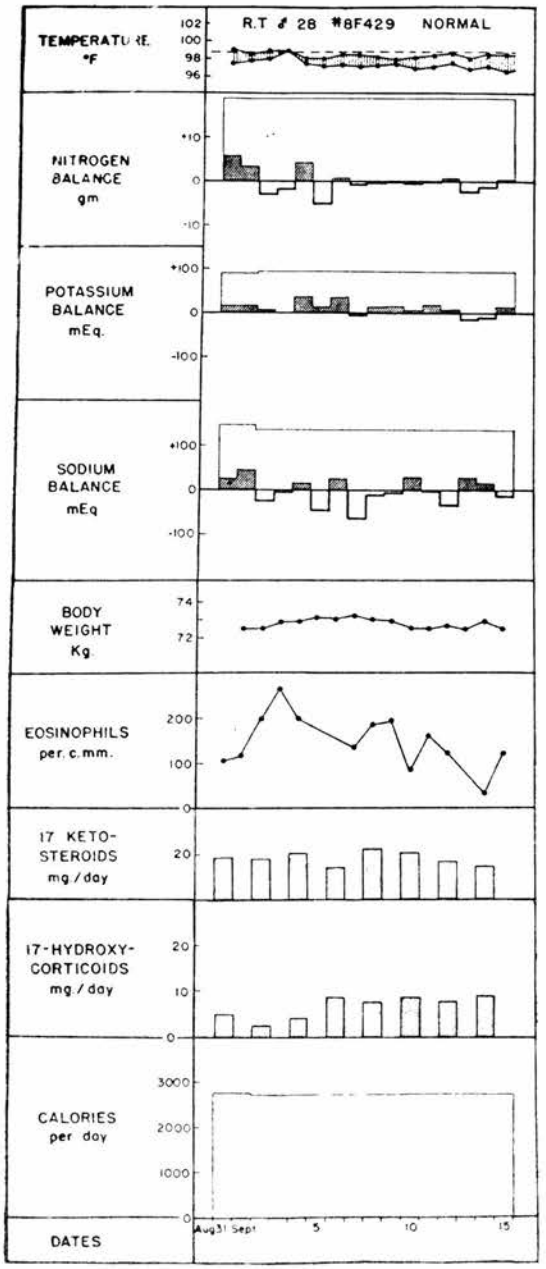

FIG. 1. "Control Balance." Normal volunteer. Normal diet and ward activity. After two days of stabilization, nitrogen balance becomes zero, with minor daily variation. Sodium balance is zero for the period, with apparently random daily fluctuations around 60 mEq. Potassium balance is slightly positive for the period (about 10 mg. per day or 10 per cent of intake), an apparently systematic error previously discussed.[33] Body weight fluctuates insignificantly (200 to 500 Gm.). The eosinophile counts reflect the systematic inaccuracies of the method; the low count on 9/14 was correlated with a period of apprehension. The steroid values were all within the normal range.

The charting convention is essentially that of Moore and Ball[33] save that negative balance is black-enclosed white below the zero line (rather than solid black). In subsequent charts the cross-hatched caloric intake is that of intravenous glucose; operative blood loss, and red blood replacement nitrogen and potassium are indicated as in Moore and Ball.

trauma may show no change in the urinary excretion of 17-ketosteroids. We have been unable consistently to correlate such 17-ketosteroid changes as are observed either with catabolic or anabolic phases of convalescence, or with changes in the eosinophile count. The same has been true of other methods for the measurement of the urine corticoids, some of which even fail to show consistent changes following the administration of ACTH or cortisone in normal individuals.[31] This field of work is clearly in a phase of development where methods and interpretations are changing constantly, and where new technics should be explored to see what further light they cast on the endocrinology of trauma. Studies carried out by new technics may provide interesting comparisons with gluco-corticoid data previously reported from surgical patients and based either on biologic or chemical assay.[5, 6, 22, 46, 50, 52, 53]

With the development of a fairly simple colorimetric method for the measurement of 17-hydroxycorticoids in the urine,[41] a quick and reproducible method for study of the urinary excretion of specific adrenal hormones became available.* The present studies were undertaken to compare the urinary excretion of 17-ketosteroids with the urinary excretion of 17-hydroxycorticoids, and the relation of both to the clinical and metabolic events in surgical patients. It was hoped that such facts, collected from a variety of patients, would illuminate some of the many unsettled

* The final color reaction of this method yields quantitative results *in vitro* with compounds E, F and S, of the known active adrenal steroids.[40]

problems in surgical metabolism and endocrinology. Questions of special importance relate to:

1. The correlation of metabolic and endocrine changes with the nature and magnitude of the trauma.

2. The correlation amongst various indices of endocrine activity.

3. The differentiation between changes in secretory rate on the one hand and changes in visceral inactivation or excretion of steroid hormones on the other, as the mechanism by which the recorded changes occur.

4. The differentiation between "primary" and "permissive" roles of adrenal function in post-traumatic metabolism.

5. The alleged occurrence of "adrenal exhaustion" on the one hand or "deleterious overactivity of the pituitary-adrenal axis" on the other, as complications of everyday surgical disease.

In this and the succeeding manuscript[48] are described studies of clinical metabolism and the urinary excretion and blood levels of 17-hydroxycorticoids in surgical patients. The data indicate some tentative conclusions in regard to the above questions. In this manuscript, metabolic and urine hormone data will be presented; in the second, blood curves will be presented. Detailed discussion of endocrine mechanisms is reserved for the second paper.

II. Materials and Methods

1. Patients

The patients were studied on the private and public wards of the Peter Bent Brigham Hospital. A variety of traumatic types was sought. Three patients were suffering from appendicitis and were studied following appendectomy. One of these patients had a perforated appendicitis with spreading peritonitis; a pelvic abscess developed and was later drained. One patient was studied after inguinal herniorrhaphy, as an example of minimal soft-tissue trauma. One patient underwent repair of a hiatus hernia with vagotomy and posterior gastroenterostomy, and was studied as an example of more extensive intraperitoneal manipulation. There was one patient undergoing radical mastectomy for carcinoma of the breast and one patient with an extensive second-degree burn. We have not included any of our studies of third-degree burns in this paper as they raise an additional series of metabolic problems and interpretations, particularly those of exudate loss[32] and prolonged infection.[27] One patient underwent two-stage bilateral thoracodorsal sympathectomy for hypertension, permitting contrast of primary and secondary trauma. As examples of bone trauma there were three patients with fractures. In one, there was a mid-shaft fracture of the femur in a vigorous young man; in another a comminuted fracture of the tibia in a middle-aged man and in a third an intracapsular femoral neck fracture in a middle-aged woman.

2. Metabolic Methods

Balances of nitrogen, potassium, sodium (and in some instances chloride) were measured. The intake data are based on analyses of our food stocks by lots, and by weight of intakes and refusals. Duplicate diet analyses were done as a check on the method, but not routinely. Outputs were based on analyses of all urine, stools, drainage, vomitus or other discharge from the patient. Operative blood loss was measured by total nitrogen on the sponge and drape rinsings. The patient's body weight was measured daily with an accuracy of ± 25 Gm., using a Toledo bed scale.

3. Analytic Methods

a. Chemical. The analyses for nitrogen, sodium and potassium in urine, food and feces were carried out by methods previously described.[33] Feces are analyzed by dry ashing in a platinum crucible.[39] Foods were analyzed by wet ashing. Accurate data on chloride intake have been difficult to establish. Our chloride analyses have been done by the method of Wilson and Ball,[54]

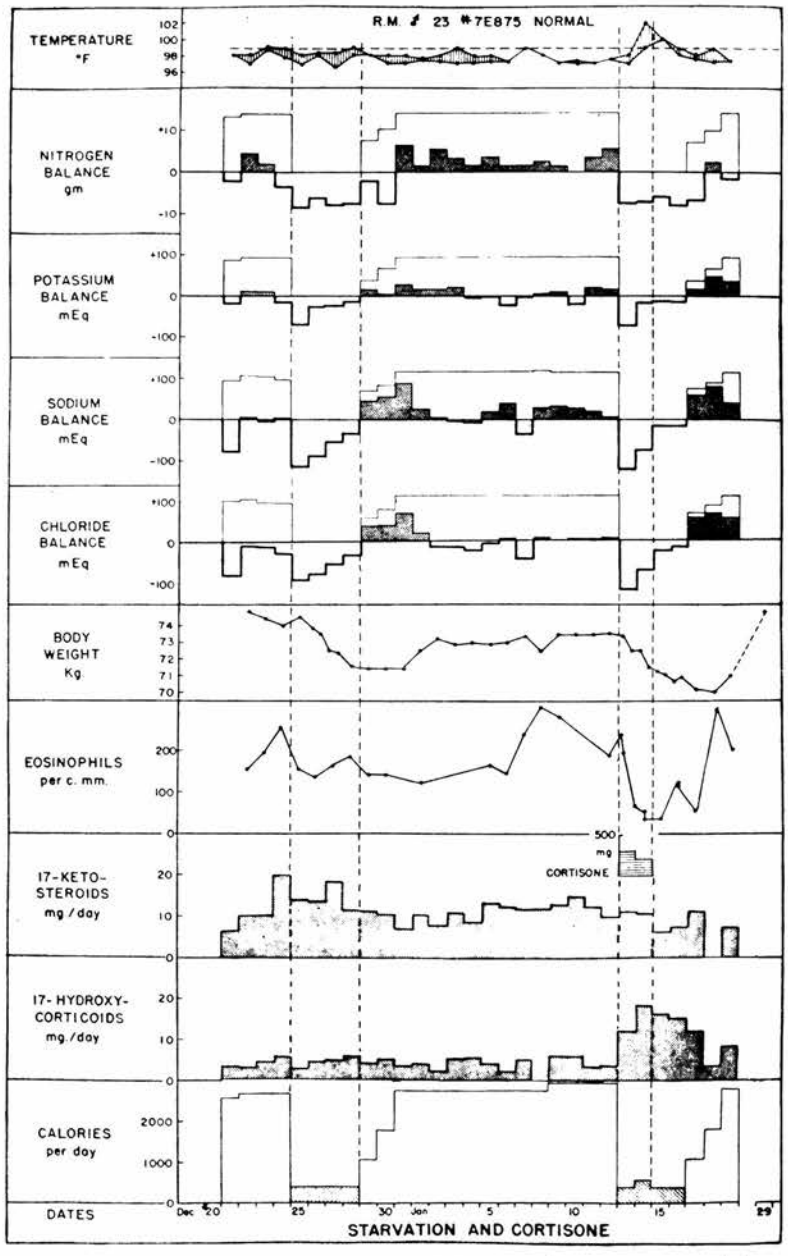

FIG. 2. Starvation in a normal subject, followed by a 15-day recovery period, and then a similar period of starvation with the administration of (intramuscular) cortisone. The loss-rates of starvation were typical of results obtained in a group of such studies. Note that total weight loss was not materially affected by cortisone in this instance. The initial weight gain after starvation was almost wholly due to reloading of sodium and chloride; initial weight was not regained in 15 days. Steroid values were all normal up to the start of cortisone, although there was an interesting increase in 17-ketosteroid excretion during the first starvation period. The increase in excretion of 17-hydroxycorticoids which accompanies the administration of cortisone persists for three days after discontinuance of the drug, an observation of interest with respect to the rate of destruction and conjugation of the steroids.

using nitric acid digestion. Refinement in technics of food analyses have enabled us to obtain satisfactory chloride balance data. The flame photometer used for sodium and potassium determinations was a Baird instrument, and on occasions a Barclay.

b. Endocrine. Eosinophile counts were done by the method of Dunger.[16] The urine excretion of 17-ketosteroids was measured by Talbot's modification[51] of the method of Callow and Callow.[7] The urinary excretion of 17-hydroxycorticoids was measured by the method of Reddy, Jenkins and Thorn,[41] with minor modifications. In our laboratories, normal values for 17-ketosteroids range from 8 to 16 mg. per day in the adult male and from 4 to 12 mg. a day in the adult female. The normal daily urine excretion of 17-hydroxycorticoids ranges from 5 to 10 mg. in the adult with little or no sex differential. In some instances we have observed that elderly or depleted patients have diminished daily excretion of 17-ketosteroids, whereas their excretion of 17-hydroxycorticoids is normal. These findings have been corroborated in a study of the endocrinology of the normal aging male carried out by Harrison and Leman.[24] It should be emphasized that this method for urine 17-hydroxycorticoids, employing butanol extraction, is a technic which measures both the free and conjugated steroids.

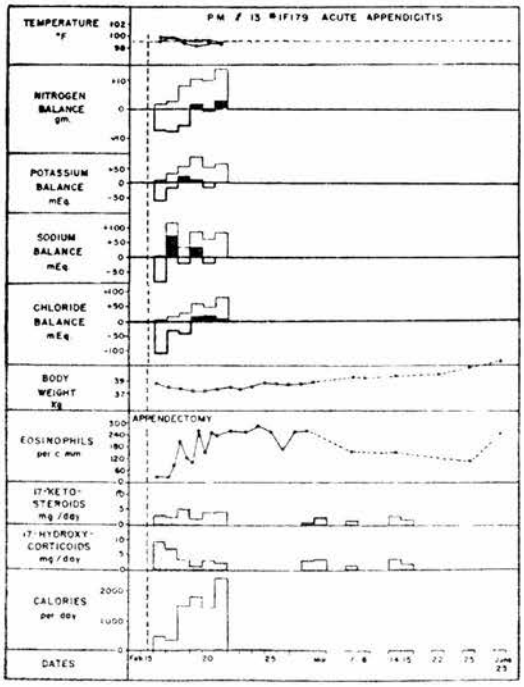

FIG. 3. Case 1. Appendectomy for appendicitis. Elevation of urine 17-hydroxycorticoids for two days was accompanied by minor metabolic changes and catabolic weight loss. Over a four-month period normal growth and steroid excretion is resumed.

c. Body Composition. Measurements of total body water, total exchangeable sodium and potassium were carried out by methods extensively described in the literature.[11, 17, 18, 34, 35, 42, 43, 55] The total body water determinations are based on equilibrium samples taken at two, three or four hours, and for each measurement at least two equilibrium samples must check before equilibrium is considered as established and dilution measured. The total exchangeable sodium and potassium are based on simultaneous determination of these two isotopes in a 24-hour serum sample.[26] In some of the instances of minor trauma, where these findings were within the normal range, they have not been included in the presentation of the cases, in the interest of brevity.

4. *Charting and Presentation*

The charting convention is that of Moore and Ball.[33] The uppermost line indicates the intake. A positive balance appears above the zero line and is cross-hatched; a negative balance appears below and is enclosed by heavy black lines. Therefore, the entire white area subtended below the intake line indicates the total excretion or loss for the day.

For brevity of presentation in this paper, the charts will not be described in detail. They are largely self-explanatory. There will be a short clinical account of each case and a summarizing statement of the metabolic and endocrine changes.

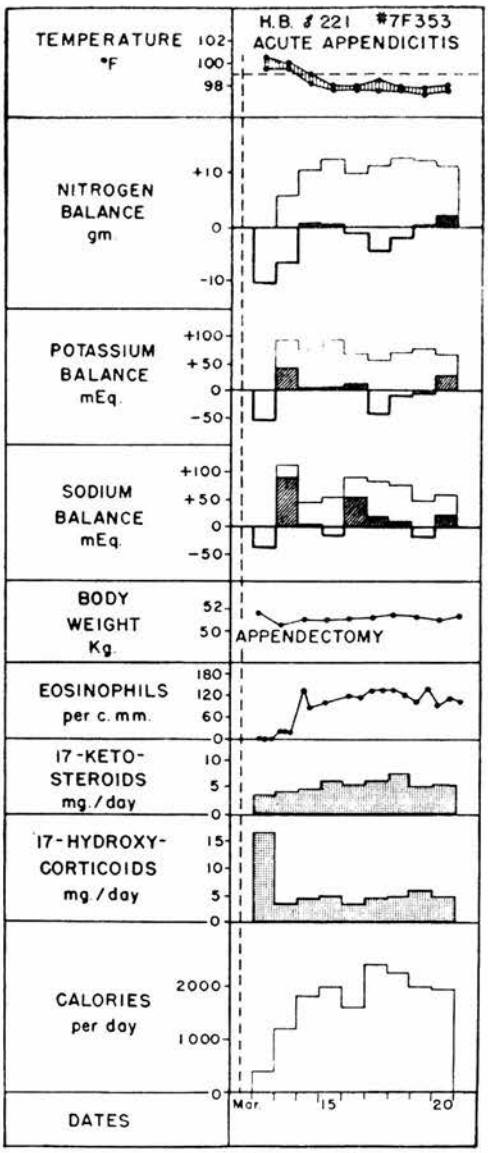

FIG. 4. Case 2. Appendectomy for appendicitis. There was an increase for one day in the excretion of 17-hydroxycorticoids; other changes were minor and transient. Sodium conservation was not evident on the day of operation and was more marked the next day.

5. *Control Studies in the Normal Subject*

One rigorous test of the accuracy of metabolic balance lies in a determination of its ability to measure changes which correlate with alterations in body composition. The simplest setting in which to make such observations is the so- contro- e." By this is meant the - of a no- -er- son in the hospital on- -plete met- -n, in whom no procedure o- disease is p- -nt, and weight is constant While the - -l balance study does not no- -te the o- -ll accuracy of measureme- -f extra- losses, since none are pres- it is a check on the accuracy of the -ard te- for urine collections and the a- -racy of the intake data and food analyses In a nor- individual whose weight is -nstant, the balance calculations for each da- based on multiple analyses of intake, urine and stools should add up to a net of zero. Such a study is shown in Figure 1. This will not be analyzed here in detail save to point out the relative consistency of nitrogen and potassium balance and the tendency to daily variability of sodium balance. In the case of nitrogen and sodium, the net for the period is zero. For potassium there is a small net positive balance previously described[33] and again seen in Figure 2 (see below). The accuracy of intake data may be expressed precisely on a statistical basis; this is not possible with output data since there is no check on output information, particularly where there are large amounts of exudate, sweat or extrarenal losses. When intake is entirely intravenous, the intake calculation is most accurate; when weather is cool and output wholly by urine and stools, the output is most accurate. It is difficult to ascribe an accuracy parameter for metabolic balance studies on surgical patients because of the variability of conditions surrounding any one patient on successive days, and between patients. We believe that changes within the limits of ±10 per cent should be viewed with extreme reservation.

In describing alterations in nitrogen and potassium balance, the reader should carry a mental picture of the normal balance in a normal adult male, as shown in Figure 1. In addition, it is well to have in mind the changes produced by starvation alone since post-traumatic changes are frequently con-

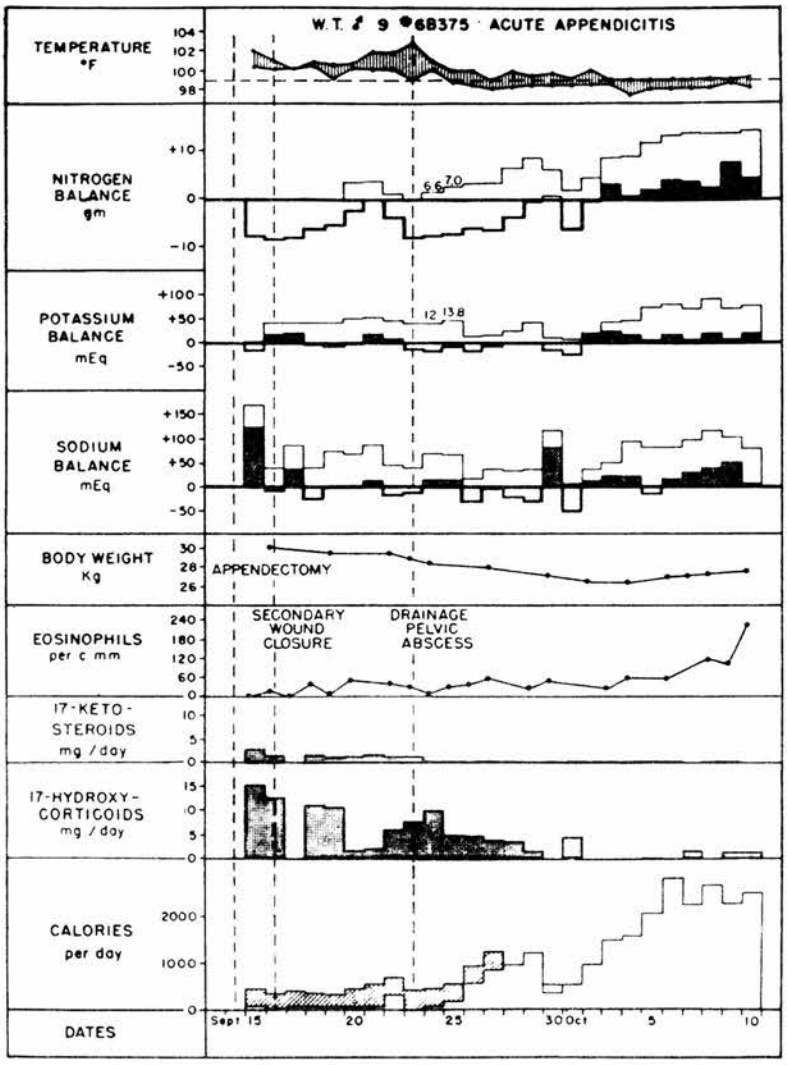

FIG. 5. Appendectomy for ruptured appendicitis, with complications. The high rate of nitrogen loss for a child of this weight is evident. Nitrogen loss and 17-hydroxycorticoid excretion paralleled the clinical course, showing an increase with formation of the pelvic abscess. Subsequently, repeated determinations of 17-ketosteroids in the urine showed none present, and 17-hydroxycorticoids were at a low level.

trasted with those of starvation. If nitrogen excretion on a day of zero intake is greater than it was on the previous day with normal intake, this is interpreted as being a negative balance greater than that which would be produced by starvation alone. In the case of potassium, an excretion greater than 70 mEq. per day on zero intake is interpreted as being a negative balance greater than would be observed in starvation. The varying response of individuals to starvation, possibly depending upon body composition and stress factors, makes any such interpretation a first approximation only.

In addition, when employing a method for urinary steroids, it is important to visualize the changes which would be produced

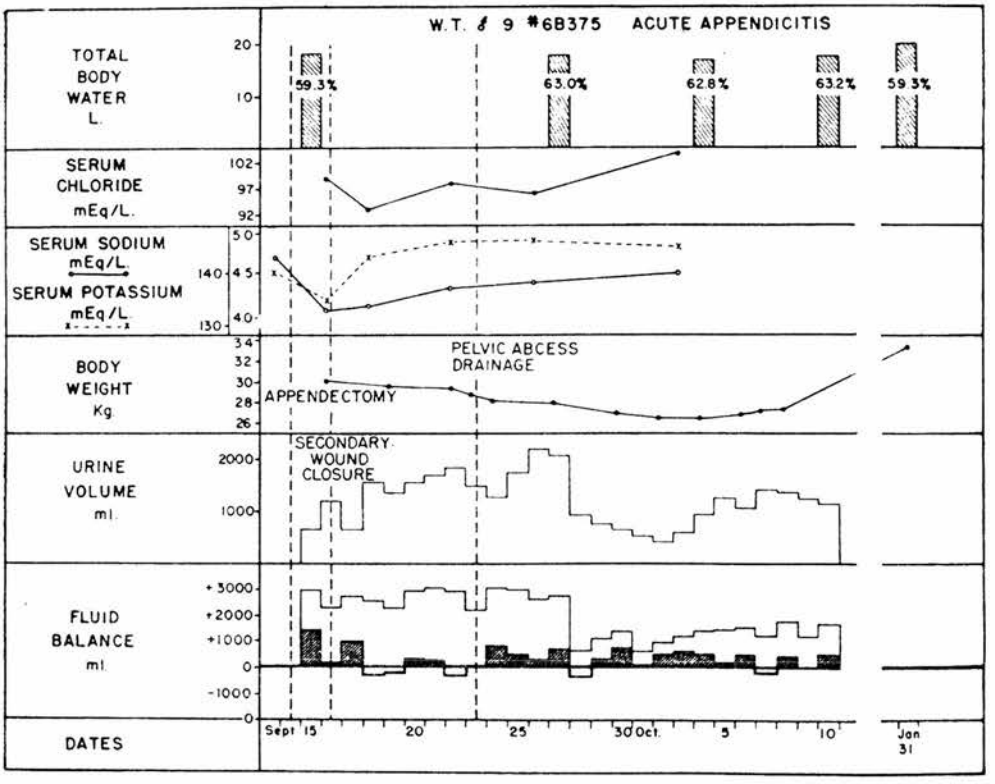

FIG. 6. Case 3. Body composition chart. In this and Figures 9, 13 and 16 the total body water (and in some instances total electrolyte) is shown above; each bar represents total water in liters, the figures the fraction of body weight occupied by water. The other indices are self-explanatory. Fluid balance is charted as the other balances, intake by all routes and measurable output being charted. By such calculation, the normal adult fluid balance is about +750 ml. daily. The increasing water fraction with falling weight was evident until the late recovery phase when weight and water rose, but water fraction fell as normal body composition was resumed four months later. The reader is referred to the bibliography for detailed discussion of the interpretation of body compositional changes.

by pharmacologic administration of related hormones.

For the above reasons, Figure 2 is shown. It is a study of a normal male volunteer. After four days of equilibration on control balance, the patient underwent a four day period of starvation with administration of intravenous dextrose and water. After a 15-day period of recovery, the patient underwent starvation again, this time with the intramuscular administration of cortisone for the first two days. The magnitude of changes produced by starvation is clearly seen. It will be observed that in the starvation state, large losses of sodium were sustained for the first three or four days, in marked contrast to the post-trauma state, as previously described.[33] It is of interest that during 15 days of recovery this patient's body weight was not restored and that his initial weight-gain after starvation was wholly due to salt retention, as he compensated for his previous sodium and chloride losses. The sensitivity of the urine 17-hydroxycorticoid method is apparent. An increased excretion of these substances in the urine continued for three days after the intramuscular administration of cortisone had been stopped. This is a finding of especial importance relative to the continuation

of urine 17-hydroxycorticoid excretion long after the peak in the blood curve has been passed, as described in the subsequent paper.[48]

III. Results

A. Three Cases of Appendectomy for Appendicitis

Case 1. *Acute appendicitis with appendectomy (Pentothal-ether anesthesia).* Patient P. M., male, age 13, Unit No. 1F179, was admitted February 15, 1953, and discharged February 22, 1953 (Fig. 3).

Clinical Summary. This 13-year-old schoolboy entered the hospital with a 12-hour story of generalized abdominal crampy pain which was persistent, although it did not keep him awake. The pain was increasingly referred to the right lower quadrant and was accompanied by the physical findings of acute unruptured appendicitis confirmed at appendectomy, which was carried out under Pentothal-ether anesthesia. The postoperative course was entirely uneventful. There was no significant elevation of temperature although tachycardia persisted for about 3 days.

Metabolic Summary. Changes in the excretion and balance rates of nitrogen, potassium, sodium and chloride are virtually indistinguishable from the changes to be predicted from a short period of starvation. Sodium conservation was evident only on the first day, when urine excretion was limited to 9.7 mEq., the rest of the loss being extrarenal. Body weight fell significantly but started to rise again after the fifth day. Caloric intake was below 500 for 2 days and was rapidly increased thereafter.

Endocrine Summary. The eosinophile count fell to 30 for 2 days, thereafter rising to a level around 240 per cubic mm., where it remained for almost 2 weeks before resuming a level near 120.

There was no alteration in excretion of 17-ketosteroids. The urinary excretion of 17-hydroxycorticoids showed elevated levels on the first and second days (9.5 and 7.5 mg.), thereafter falling to the level expected for a child of this age (2 to 4 mg.) and observed over the next month.

The patient was studied intermittently for another 3 months during which his weight continued to ascend as a manifestation of his normal growth curve. The urinary excretion of 17-ketosteroids and 17-hydroxycorticoids remained in the normal range.

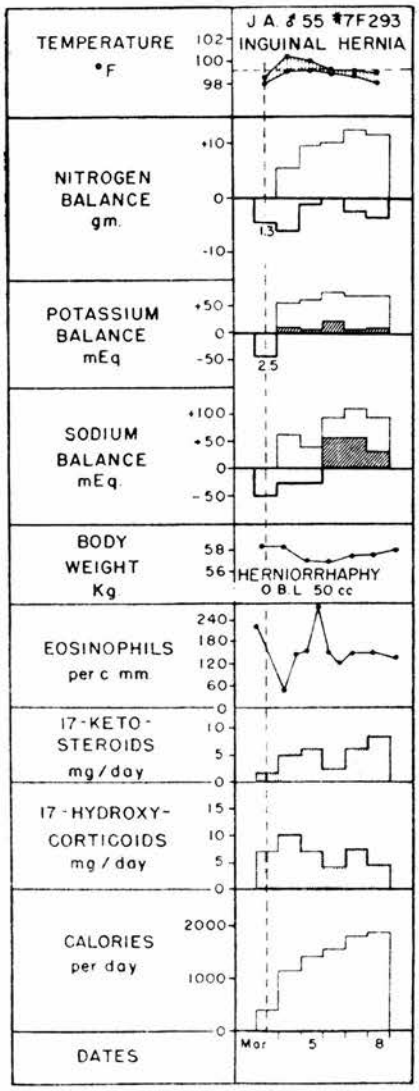

FIG. 7. Case 4. Inguinal herniorrhaphy. Save for a transient eosinophile fall, there were no changes beyond those expected with one day of starvation.

Case 2. *Acute appendicitis with appendectomy (Pentothal-ether anesthesia).* Patient H. B., male, age 21, Unit No. 7F358, was admitted March 14, 1953, and discharged March 21, 1953 (Fig. 4).

Clinical Summary. This 21-year-old Harvard medical student entered the emergency ward with a 5-hour story of abdominal pain and tenderness, becoming localized in the right lower quadrant. There were typical physical signs of acute appendicitis confirmed by appendectomy, which was carried out under Pentothal-ether anesthesia. The postoperative course was entirely uneventful.

Metabolic Summary. The changes in nitrogen, potassium and sodium balance are indistinguish-

able from those occurring with a short period of starvation. The body weight fell approximately 1.5 Kg. but rapidly resumed its trend towards normal. The caloric intake was restricted for one day, thereafter rapidly returning to normal.

Endocrine Summary. Eosinophile count remained low for two days, thereafter returning to a constant and normal range.

Urinary excretion of 17-ketosteroids showed no significant change. Urinary excretion of 17-hydroxycorticoids showed an elevation to 16 mg. per day on the first postoperative day, thereafter returning to a constant and normal range.

Case 3. *Acute appendicitis, peritonitis and pelvic abscess formation; operations: appendectomy, secondary wound closure, drainage of pelvic abscess (Pentothal and Pentothal-ether anesthesias).* Patient W. T., male, age 9, Unit No. 6B375, was admitted September 14, 1952, and discharged October 11, 1952 (Figs. 5 and 6).

Clinical Summary. This 9-year-old schoolboy was admitted to the hospital with a story of increasing abdominal discomfort without localization for 2½ days. During 13 hours of this time he was riding in an automobile and it was noticed that the peri-umbilical component of the pain became more severe. He had a chill, and his abdomen became rigid.

Physical examination on admission showed a temperature of 102.6°, pulse, 108; there was a rigid abdomen with involuntary spasm throughout and tenderness most marked in the right lower quadrant. There was some tenderness in the cul-de-sac bilaterally on rectal examination. The patient was operated upon under Pentothal-ether anesthesia and an acutely inflamed appendix with perforation was removed. There was a foul-smelling fecal peritonitis without localization. The peritoneal cavity was not drained, but the subcutaneous tissues were left open and on the second postoperative day this wound was drawn together under light Pentothal anesthesia. The patient was placed on penicillin and streptomycin.

The patient's postoperative course was characterized initially by a continuous fever in the range of 100 to 103° by rectum. On the ninth postoperative day a pelvic abscess which had been observed to be forming had softened sufficiently to permit drainage. A copious, foul-smelling collection of purulent material in the cul-de-sac was drained by rectum.

The postoperative course was uneventful from that time forward, although naso-gastric suction which had been present intermittently throughout the early period had to be continued for a few days. The patient gradually was able to increase his dietary intake and was discharged home on the 27th day.

Metabolic Summary. The excretion of nitrogen showed a marked elevation throughout most of the first week. The absolute nitrogen excretion rate was 9 Gm. per day, or approximately 20 Gm. per day per 70 Kg. of body weight. By the seventh postoperative day there was a tendency towards reduction of nitrogen excretion rate but as the pelvic abscess became more marked and temperature again was elevated, and with drainage of the abscess, nitrogen excretion rate was again elevated and remained so for another week. As caloric and nitrogen intake was then resumed, positive balance was finally achieved on the 20th hospital day but still with rather high absolute excretion rates.

The balance of potassium was not remarkable save for the very small losses observed. He was given daily parenteral potassium during the long period of intravenous maintenance.

The sodium balance showed a marked renal conservation of sodium for the first few days. The total balance is shown in the chart; much of the loss was by naso-gastric suction. The absolute urinary sodium excretion rate averaged less than 5 mEq. per day for the first 3 postoperative days. By careful adjustment of intake and output a strongly positive or negative balance was consistently avoided save for the first and 20th postoperative days; the clinical objective was a zero sodium balance, and this was virtually achieved.

The patient's body weight fell from 30 Kg. to 26 Kg. (a loss of 12 per cent of body weight) during a prolonged catabolic phase incident to surgery and sepsis. For the first 2 weeks the caloric intake was entirely composed of intravenous glucose with a very low total. After this, oral intake rapidly increased.

Endocrine Summary. The eosinophile count was remarkable for its very prolonged low level between zero and 60 per cu. mm. This level was maintained for approximately 3 weeks, after which time only one normal count was observed prior to discharge.

The urinary excretion of 17-ketosteroids showed measurable amounts of these substances in the urine for the first 10 days, and thereafter none was present.

The urinary excretion of 17-hydroxycorticoids showed a configuration closely paralleling the patient's course. The 17-hydroxycorticoids were elevated to 15 mg. per day on the first postoperative day and remained elevated for 4 days during the most acute illness. They came sharply down to normal on the sixth postoperative day (the day that nitrogen excretion was reduced), but rose as

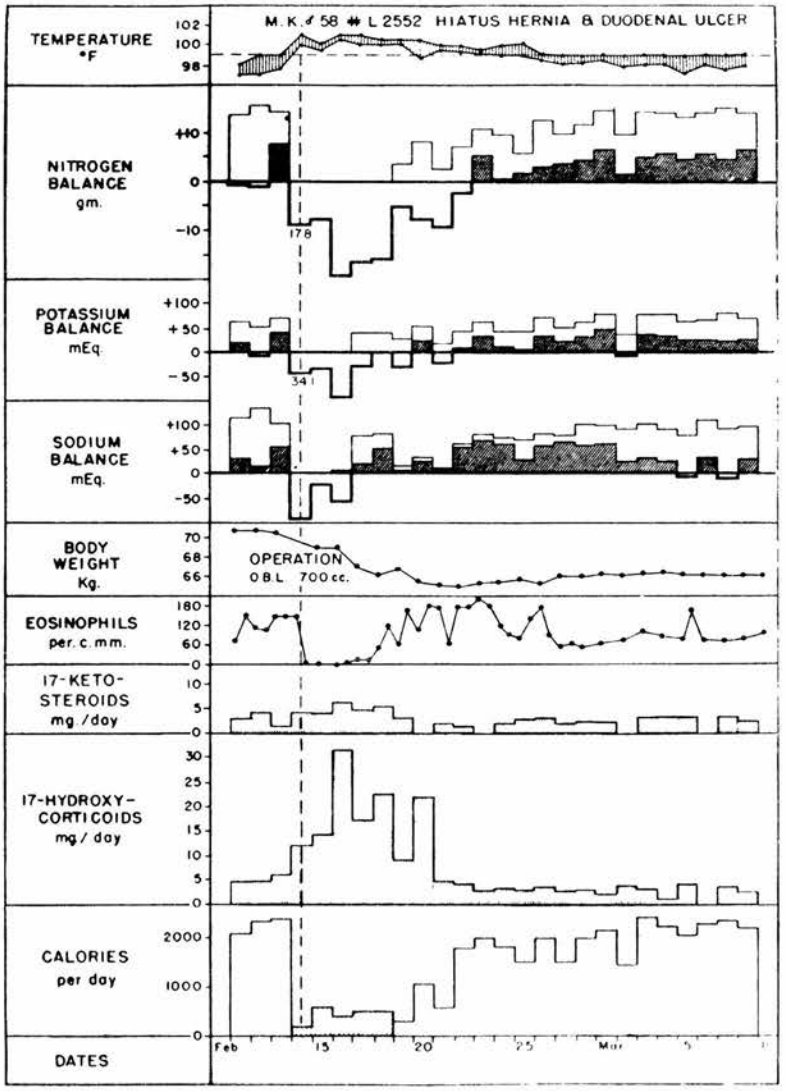

FIG. 8. Case 5. Vagotomy, repair hiatus hernia and posterior gastro-enterostomy. A prolonged and intense metabolic and endocrine adjustment was apparent. Although there was no change in urinary excretion of 17-ketosteroids, there was a prolonged increase in the urinary 17-hydroxycorticoids. The catabolic aspects were suddenly reversed on February 22nd and 23rd and a period of strongly positive nitrogen balance ensued. Sodium conservation was prolonged (two weeks) and its duration does not correlate with any of the measured endocrine indices.

the pelvic abscess formed and was drained. There was measurable 17-hydroxycorticoid in the urine as late as the 17th postoperative day, but on subsequent occasions when it was measured, the excretion had fallen almost to zero.

Body Compositional Change. The total body water decreases in absolute volume but increases in percentage of body weight as weight declines, indicating loss of fat in the most acute and febrile phase of the disease. There is a fall in serum sodium concentration early in the course. The serum potassium concentration is roughly equivalent in its changes. The water balance shows no striking alteration save for the rather high urine volume maintained during the period of parenteral feeding and disappearance of body fat. On re-study 4

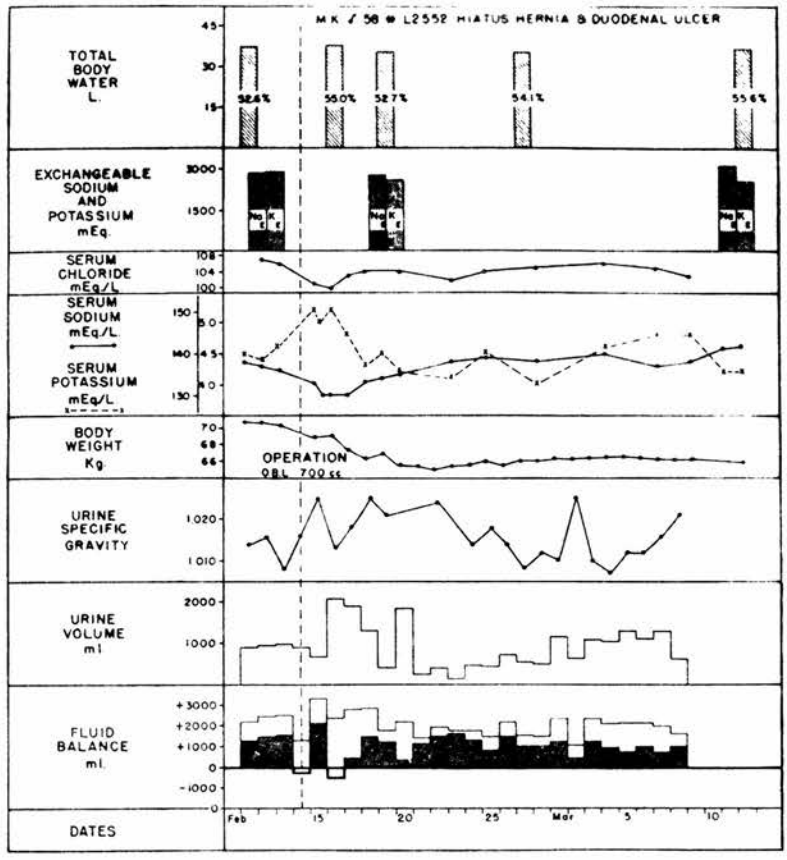

FIG. 9. Case 6. Body composition chart. Gain of water fraction and sodium, with loss of potassium were evident immediately after operation. The serum Na-K changes were pronounced and classical for post-traumatic alterations in the absence of extrarenal losses or oliguria. There was no azotemia.

months after the illness, weight gain was recorded with reduction in relative water volume, indicating resumption of normal growth with deposition of fat.

Summary of the Three Appendicitis Cases. The two cases of unruptured appendicitis are of interest in demonstrating metabolism and urinary steroid changes under circumstances of minimal trauma, superimposed on a spontaneous inflammatory process. There were no changes in 17-ketosteroids in either of the two cases but clearcut changes in 17-hydroxycorticoids. There was a reasonably good inverse correlation between urinary excretion of 17-hydroxycorticoids and the blood eosinophile level in the first two cases.

The case of perforated appendicitis with peritonitis demonstrates the changes in metabolism produced by sepsis after appendectomy. The degree of tissue trauma was comparable to that in the other cases but the septic process was vastly greater. It produced a very high nitrogen excretion rate and a prolonged catabolic phase with a loss of 12 per cent of the body weight. This weight loss was at the expense of both fat and lean tissue as shown by the balance and the compositional changes.

Coincident with these metabolic and compositional changes was a prolonged elevation of excretion of 17-hydroxycorticoids in the urine, although alterations in 17-

ketosteroid excretion were inconsequential. The configuration of 17-hydroxycorticoid excretion closely paralleled the septic process. It was initially intense, then subsiding, with relapse during the period of reformation of the pelvic abscess. In this case the inverse correlation between urinary excretion of 17-hydroxycorticoids and blood eosinophile levels was entirely absent: the blood eosinophiles were low throughout the entire period of hospitalization during periods of both high and low rates of urinary 17-hydroxycorticoid excretion.

B. Five Cases of Soft Tissue Trauma of Varying Severity

Case 4. *Inguinal herniorrhaphy performed (spinal anesthesia).* Patient J. A., male, age 55, Unit No. 7F293, was admitted March 2, 1953, and discharged March 9, 1953 (Fig. 7).

Clinical Summary. This was a 55-year-old factory worker with a right inguinal hernia of 2 months' duration. This was a small, 2 x 2 cm. bulge, transmitting a cough impulse. Repair of a mixed direct and indirect hernia was carried out under spinal anesthesia. Postoperative course was entirely uneventful.

Metabolic Summary. Changes in balance of nitrogen, potassium and sodium were minor. There was not a return to positive nitrogen balance during the six-day period of study. There was a loss of 1 Kg. of weight, with a later return to upward trend. Caloric intake was only reduced the day of operation, thereafter returning rapidly to a level of approximately 1700 calories per day.

Endocrine Summary. There was a fall in eosinophile count to 40 per cubic mm. the day of operation.* There was an early return to normal count with, on one occasion, an elevated count.

Urinary excretion of 17-ketosteroids was reduced the day of operation and on the third postoperative day, but at other times was in the low normal range for a man of this age. The urinary excretion of 17-hydroxycorticoids was normal on the day of operation, slightly higher the following day, thereafter returning to a lower range. These changes were all within the normal range for an adult male. This was the most minor trauma studied and the only one which does not show abnormalities of the urine 17-hydroxycorticoid excretion.

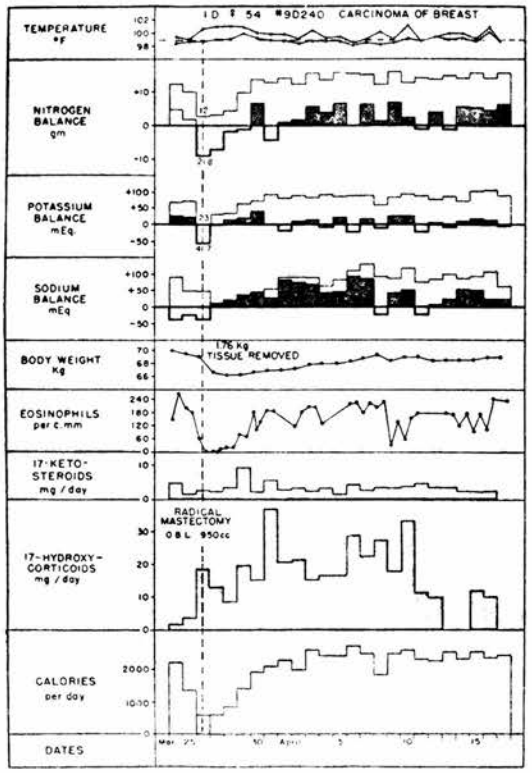

FIG. 10. Case 6. Radical mastectomy. The increased urinary excretion of 17-hydroxycorticoids was more prolonged than in any other case. Metabolic changes were less pronounced, save for sodium conservation which was prolonged (ten days), and which correlated in duration with the elevation of 17-hydroxycorticoid excretion, a very unusual finding in our experience.

Case 5. *Hiatus hernia; duodenal ulcer. Operation: vagotomy and gastroenterostomy; transperitoneal repair of hiatus hernia (Pentothal-ether anesthesia).* Patient M. K., male, age 58, Unit No. L2552, was admitted February 4, 1953, and discharged March 9, 1953 (Figs. 8 and 9).

Clinical Summary. This 58-year-old bus driver had been a patient in the hospital on many occasions because of digestive complaints; a cholecystectomy had been carried out 4 years previously. He had had attacks of pain suggesting pancreatitis and in addition had been shown to have a double kidney pelvis and double ureter on the right which had produced intermittent attacks of acute pyelitis.

* In studies yet to be reported, we have shown that under Pentothal-ether anesthesia alone, without trauma, the eosinophiles fall essentially to zero. Under spinal anesthesia alone, there is no drop in eosinophiles. The transient nature of the eosinophile drop here may be related to the use of spinal anesthesia.

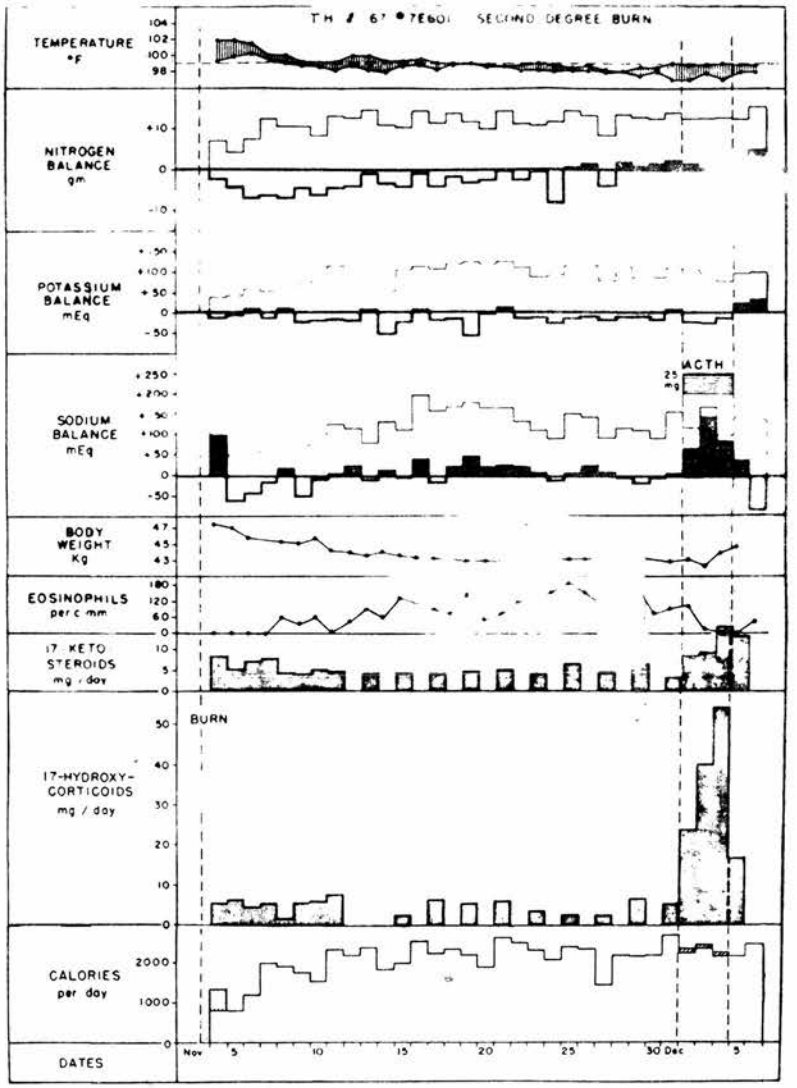

FIG. 11. Case 7. Second-degree burns. Nitrogen loss was prolonged despite normal intake levels. Eosinophile depression was transient. There were no changes in urinary steroid excretion. One month after the burn ACTH was administered to study adrenal responsiveness in the face of the previous low urinary steroid values. The response elicited was quite normal.

In 1951 a diagnosis of hiatus hernia and duodenal ulcer had been established by roentgenogram. Careful study was undertaken in this complex situation to determine the primary symptom-producing mechanism. The pancreatitis was quiescent, the pyelitis had cleared up. At operation, minimal evidences of pancreatitis were found. There was a moderate-sized hiatus hernia and a duodenal ulcer with considerable stenosis. A gastroenterostomy and transperitoneal vagotomy were carried out, and at the same time a repair of the hiatus hernia. The patient's postoperative course showed a mild febrile elevation (100° by rectum) for about 4 days. It was thereafter uneventful. The patient's dietary intake became significant by the eighth day.

Metabolic Summary. Balances of nitrogen, potassium and sodium showed changes characteristic of extensive trauma; they were somewhat more marked than one might have predicted; the patient was obese and the peritoneal manipulation was ex-

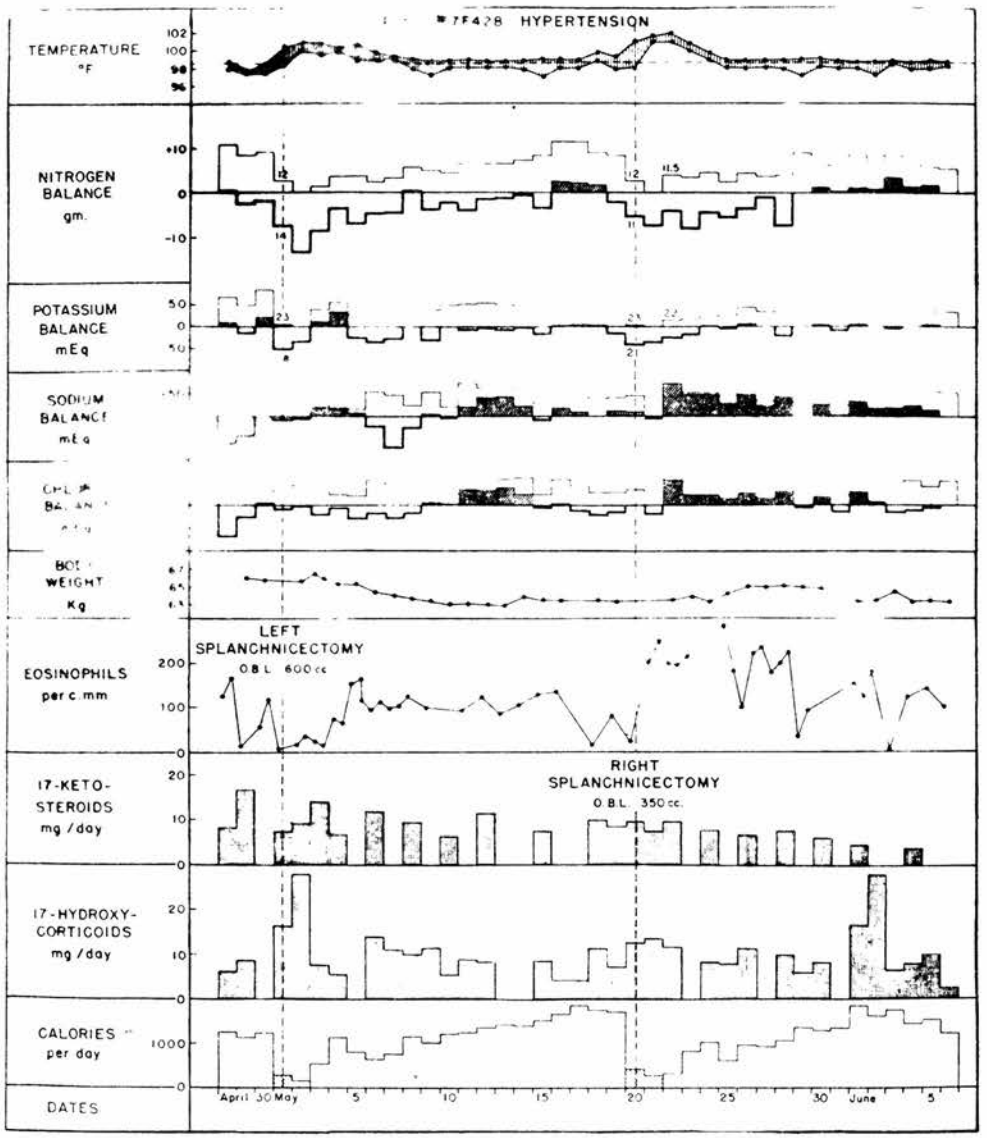

FIG. 12. Case 8. Two-stage splanchnicectomy. There were many contrasts between the response to the two operations, most noteworthy of which were the eosinophile counts, urine 17-hydroxycorticoids, weight curve and sodium-chloride metabolism.

tensive. The nitrogen excretion rate showed the "delayed peak" phenomenon, reaching its maximum on the second postoperative day with an excretion of 20 Gm. in the face of zero intake. This excretion rate was rapidly reduced during a clearcut "corticoid withdrawal phase" on the eighth to tenth days during which nitrogen excretion rate fell from 17 to 7 Gm. a day, reaching its lowest level on the ninth postoperative day when less than 5 Gm. was excreted on an increasing intake. Thereafter the balance was consistently positive in the range of 2 to 7 Gm. per day.

The potassium balance changes were similar. Again the maximum excretion rate was achieved on the second postoperative day.

Sodium showed a negative balance the first 3 days due to losses by nasogastric suction. The urinary excretion rate on these days was less than 30

mEq. per day. There was thereafter a 13-day period of very consistent sodium conservation (average less than 10 mEq. per day urinary excretion), and this was not followed by any clearcut diuresis during the entire period of observation. The patient's body weight underwent an initial fall from 70 Kg. to 65.5 Kg., a loss of approximately 7 per cent of body weight in six days. The caloric intake was entirely on the basis of intravenous glucose for four days. There was then a rapid return to a level around 2000 calories, this being achieved orally on the ninth postoperative day.

Endocrine Summary. The eosinophile count fell abruptly to near zero and remained there for four days, after which it returned to a normal level.

The urinary excretion of 17-ketosteroids showed essentially no change throughout and was in the low normal range for a man of this age. In sharp contrast, the urinary excretion of 17-hydroxycorticoids showed a very marked alteration. This consisted in an elevation on the day of operation to approximately twice normal, and somewhat higher the following day. On the second postoperative day the excretion was 32 mg., an extremely high rate, and this occurred on the same day that the nitrogen balance and potassium loss achieved their maximum negativity. There was thereafter a sudden reduction in the urinary excretion of 17-hydroxycorticoids, reaching normal values again on the seventh postoperative day. In this case the rapid reduction in excretion rate of 17-hydroxycorticoids was concomitant with the reduction in nitrogen excretion rate and the achievement of positive balance.

Body Compositional Changes. After operation the total body water was reduced in absolute volume but increased in relative percentage as weight fell, a change characteristic of fat loss. The total exchangeable sodium was increased, the total exchangeable potassium was reduced; these changes are characteristic of depletion of any sort, [55, 56] in this instance following an operation. There was a marked "shift" of sodium and potassium concentrations in the serum. The sodium fell to 130 mEq. per liter, and potassium rose to 5.3 mEq. per liter, changes most marked on the second postoperative day. Values then returned to normal. There was a rather prolonged period of urine specific gravity over 1020 although there was no clinical evidence of dehydration.

Case 6. *Carcinoma of the breast; radical mastectomy (Pentothal-ether anesthesia).* Patient I. D., female, age 56, Unit No. 9D240, was admitted March 23, 1953, and discharged April 18, 1953 (Fig. 10).

Clinical Summary. This 56-year-old hospital maid examined her own breasts after reading a public cancer bulletin. Finding a mass on the right, she reported to the Out-Patient Department and was admitted for surgery. On physical examination a 2 x 4 cm. mass, with a few palpable nodes high in the axilla, was found. At operation a colloid carcinoma with node involvement was removed. Considerable wound fluid collected and was aspirated in the postoperative period; following discharge a course of roentgen-ray therapy was carried out.

Metabolic Summary. Nitrogen balance showed a rapid loss for 2 days after operation, followed by consistent anabolism in the range of 5 Gm. per day. Potassium balance showed a negative balance the day of operation but thereafter was intermittently positive, adding up for the period to a slightly positive net. Sodium balance showed a marked alteration: on the day of operation there was a small negative sodium balance but for the 4 succeeding days total sodium excretion was only slightly above zero, and for the first 12 postoperative days there was a strongly positive net balance and at no time did the patient show a clearcut diuresis. This prolonged sodium conservation was correlated with a consistent weight gain, beginning immediately after the operation. The weight loss on the day of operation is to be attributed largely to the removal of 1.76 Kg. of tissue.

Endocrine Summary. Eosinophile count fell essentially to zero, remained there for 3 days, following which a normal postoperative course was observed.

Urinary excretion of 17-ketosteroids was low throughout, except for the third postoperative day when there was a transient elevation of urinary 17-ketosteroids.

The urinary excretion of 17-hydroxycorticoids showed a remarkable pattern, not seen in any other patient. On the day of operation the excretion rose to 18 mg. and remained elevated for the next 15 days. On 4 occasions, as shown in the chart, this excretion rose to a level of 25 mg. or higher. Suddenly, on the 15th postoperative day, the excretion dropped down to a high normal level where on 4 occasions it was observed prior to discharge. The meaning of this urinary excretion of 17-hydroxycorticoids is not clear.* The eosinophiles pursued a normal course and the nitrogen and potassium metabolism were not in any way remarkable. Of the balances, sodium metabolism is the only one

* It has been shown[29] and we have corroborated the fact that certain types of pharmacologic agents may produce an increase in the apparent urine 17-hydroxycorticoid concentration as measured by this method, of which iodides are the most important. Reviewing the medications given to these patients, no such drugs were found.

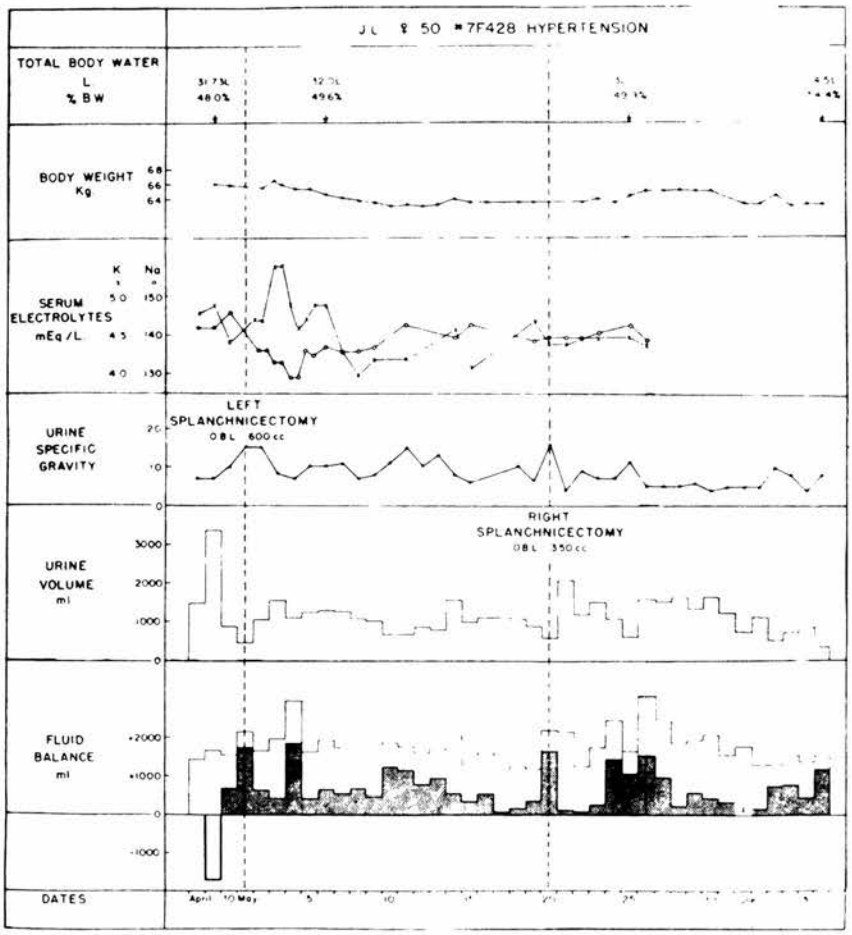

FIG. 13. Case 8. Body composition chart. The serum Na-K shift after the first operation was in marked contrast to the lack thereof after the second operation. The weight gain and water gain after the second operation is shown. Oliguria with slight increase in specific gravity was noted at each operation.

which shows any correlation with the urine steroids, there being a rather unusually prolonged retention of sodium.

Case 7. *Second-degree burns of 22 per cent of body surface.* Patient T. H., male, age 67, Unit No. 7E601, was admitted November 3, 1952, and discharged December 7, 1952 (Fig. 11).

Clinical Summary. This 67-year-old man was admitted approximately 6 hours following an electrical burn incurred while he was investigating a 550-volt switch box. The burn was a flash burn rather than a direct electrical contact burn. The burned area was confined to the exposed parts of face, hands and forearms. A short-sleeved sweater protected the chest and upper arms. On admission the patient was placed on constant catheter drainage and was given minor intravenous therapy of plasma, dextrose in water and saline, with oral treatment sufficing for the majority of his fluid requirements. He was placed on open treatment, and with very little event the lesions gradually healed and closed. He was discharged home with the wound fully epithelialized on the 35th burn day.

Metabolic Summary. There was negative nitrogen balance intermittently for the first 22 days, despite intakes over 10 Gm. per day and caloric intake in the range of 2000 to 3000 calories. The peak nitrogen excretion rate was not reached until the fourth postburn day. Although fever was present for the first 3 days, there was thereafter a normal temperature. Potassium balance followed a similar

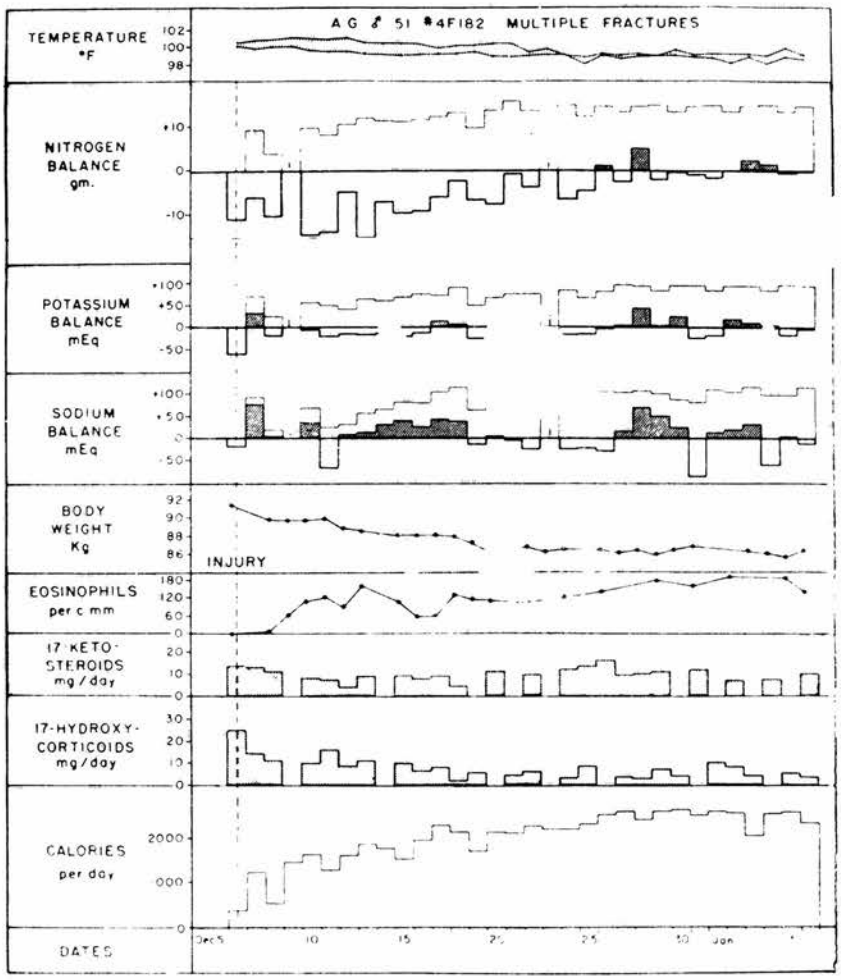

FIG. 14. Case 9. Multiple fractures and cerebral concussion. A prolonged nitrogen catabolism was out of proportion to the electrolyte, eosinophile and endocrine changes, and persisted in the face of normal intakes.

pattern, intermittently negative throughout the entire period with a net negative value.

Sodium balance showed a transient gain of 100 mEq. followed by diuresis of almost exactly the same amount, and then a balance which for the period is a small net positive figure. At no time was renal sodium conservation marked.

Weight curve showed a gradual loss of 4 Kg., this amounting to slightly more than 8 per cent of body weight and being well correlated with the nitrogen changes. For the last two weeks of hospitalization, weight was constant.

Endocrine Summary. The eosinophile count remained essentially zero for 4 days, after which there was a gradual resumption of normal values in a stepwise fashion. At no time was there a sudden rise. The urinary excretion of 17-ketosteroids was noteworthy for its lack of any change throughout the period of study, and the same was true of the urinary excretion of 17-hydroxycorticoids. At no time was the urinary excretion observed to be increased. Because of this anomalous situation, the patient was given a test dose of ACTH just before discharge in an attempt to determine if there was any abnormality of the adrenal cortex. The response to this dose was within the normal range, there being an increase in the excretion of urinary 17-hydroxycorticoids to 54 mg. per day (a ten-fold

increase), with a fall in the eosinophile count. There was a small increase in 17-ketosteroid excretion.

Case 8. *Essential hypertension; bilateral (two-stage) transthoracic thoraco-lumbar splanchnicectomy and ganglionectomy (Pentothal-ether anesthesia).* Patient J. L., female, age 50, Unit No. 7F428, was admitted April 20, 1953, and discharged June 7, 1953 (Figs. 12 and 13).

Clinical Summary. This was the second admission of a 50-year-old housewife who was admitted for evaluation and treatment of hypertension. She was shown to have good renal function and relatively little evidence of cerebral damage. Her eyegrounds were normal. She had some cardiac enlargement with auricular fibrillation and had suffered from recurrent attacks of angina pectoris. She was fully digitalized but had never been in congestive failure. The patient had a two-stage transthoracic thoracolumbar sympathectomy and ganglionectomy. The approach was through the open chest, removing the sympathetic chain from D-4 through L-3. The operations were carried out with a 19-day interval. The postoperative convalescence was uneventful in both instances. Some pleural fluid developed on the right side after the second operation. Postoperative blood pressure at 120/80 was somewhat lower than the level of 175/110 previously recorded. At no time did she exhibit refractory hypotension.

Metabolic Summary. The difference in response between the two operations was quite evident as regards nitrogen and sodium. After the first operation there was a noticeable increase in nitrogen excretion rate despite low intake; after the second operation there was little change in nitrogen excretion rate. After the first operation a short period of sodium conservation was followed by diuresis with weight loss; after the second operation sodium conservation was intense and prolonged and there was a gain in weight; there was no clearcut diuresis.

Potassium metabolism was not remarkable; there was a tendency to lose potassium (and gain sodium) throughout.

Endocrine Summary. Again, contrasts between the two operations are apparent. After the first operation the eosinophiles fell to near zero and remained there for two days, later returning to normal; after the second operation the eosinophiles were only depressed on a single observation and rose in less than 24 hours to a high level where they remained for several days, well above the normal for this patient. These changes correlate well with the 17-hydroxycorticoid data: after the first operation there was an increase in urinary excretion of 17-hydroxycorticoids, whereas after the second there was none.

After neither operation was there a significant change in the urinary excretion of 17-ketosteroids.

On the 12th and 13th days after the second operation there was a fall in eosinophiles to zero and rise in urine 17-hydroxycorticoids associated with a rather painful chest tap.

Body Compositional Summary. The serum electrolyte alterations were quite different after the two operations: there was a marked shift of sodium and potassium concentration in the serum after the first operation, the potassium rising to 5.5 mEq./L and the sodium falling to 130 mEq. L.; after the second operation there was no change whatsoever.* There were no extra-renal losses. There were no significant alterations in [illegible] urine specific gravity [illegible] balance, save for [illegible]arked tendency to [illegible] tention on the day [illegible] operation on both occasions. Total body water rises in both relative and absolute terms over the whole period of study.

Summary of the Five Cases of Soft Tissue Trauma of Varying Severity. Metabolic patterns and composi[illegible]al changes in these five cases show a nice correlation between magnitude of trauma and magnitude of chemical alteration. The hernia repair is the most minor trauma we have studied and shows little biochemical response. The hiatus hernia-duodenal ulcer repair elicited a remarkably intense response with a very clear metabolic reversal or "corticoid withdrawal" phase.[30] The splanchnicectomies make a very clear contrast between two similar operations carried out in sequence in the same patient. Three of the patients (those of Cases 5, 6 and 8) show very prolonged periods of sodium conservation in uneventful convalescence.

The 17-ketosteroid excretion patterns are irregular, and not correlated with any clinical or chemical indices.

All the traumata except the hernia and second-degree burn show an increased excretion of 17-hydroxycorticoids early in their course, and this correlates with nitrogen balance (see below). The low hormone

* The occurrence of this change only with a clearcut urine hormone increase may be of considerable significance.

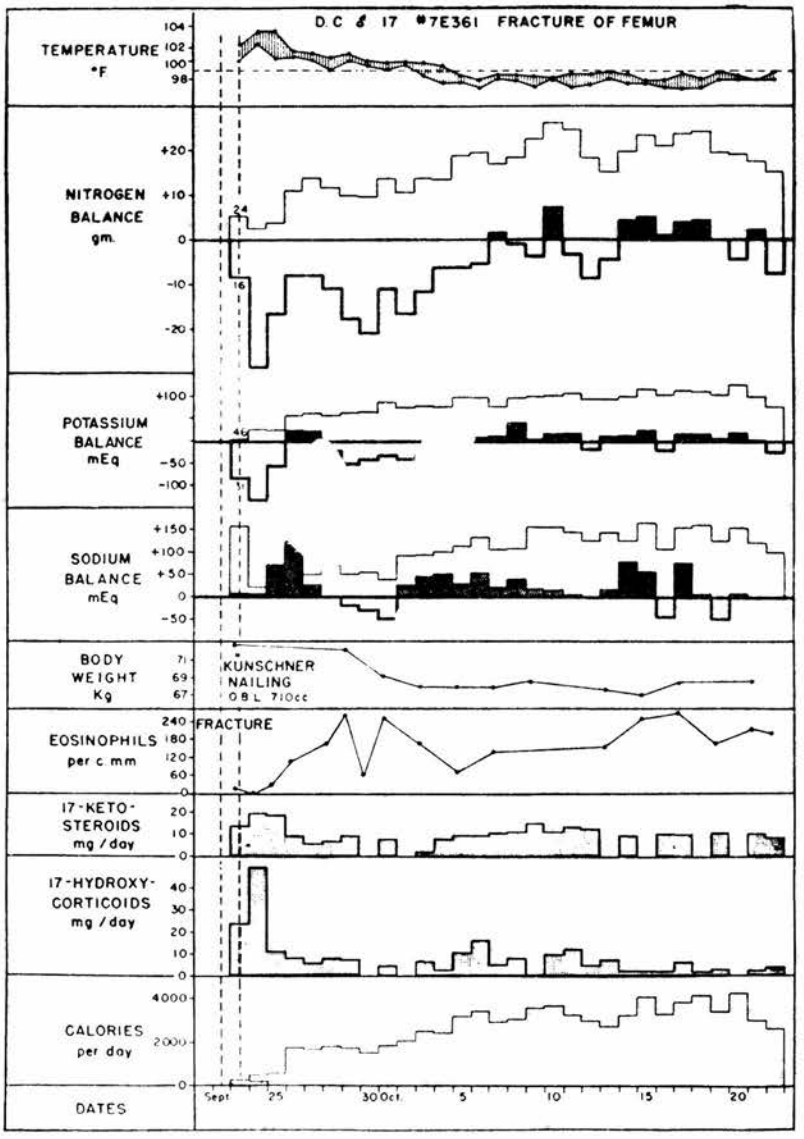

FIG. 15. Case 10. Fracture of femur and nailing. A prolonged and intense nitrogen catabolism was accompanied by a brief but intense endocrine adjustment. Early sodium conservation and potassium excretion were marked; sodium diuresis was accompanied by marked weight loss on September 28th and 29th. Consistent positive nitrogen balance was not achieved during the period of observation.

excretion in the first day or two of the second-degree burn was unexpected and remains unexplained. The continued high excretion rate in the case of breast cancer is an unique observation; we have failed to corroborate this subsequently in cases of local or widespread malignancy.

The inverse correlation between eosinophile count and 17-hydroxycorticoid excretion is usually clear. In the hypertensive there is very clear inverse correlation, particularly as regards the contrast between the two operations.[23] There are several instances of lacking correlation, however,

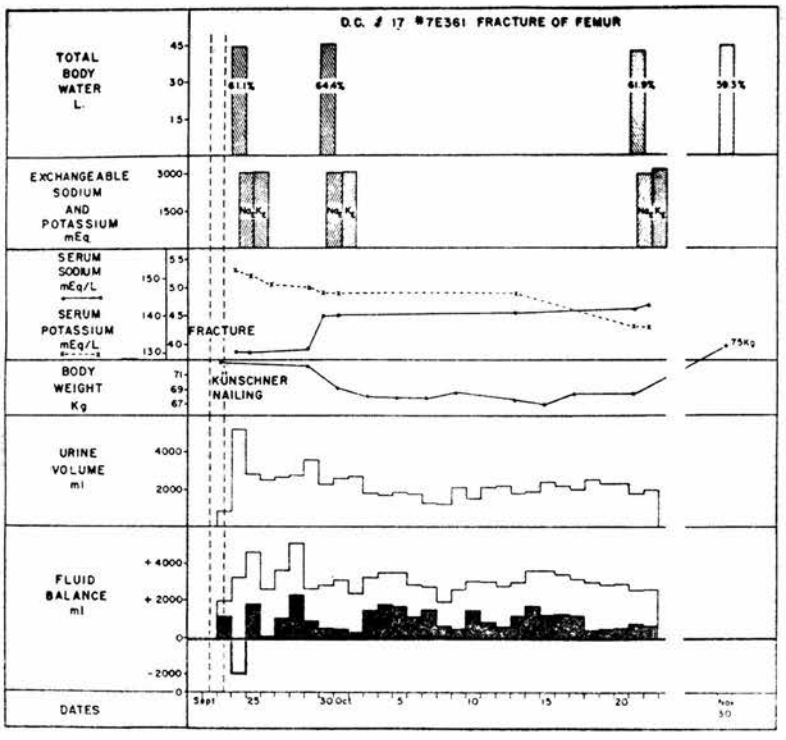

FIG. 16. Body composition chart. Increase in water fraction after injury, with a marked Na-K shift in the serum were the outstanding features.

most marked in the superficial burn where low eosinophiles coexisted with low 17-hydroxycorticoid excretion, and in the breast case where the anomalous high excretion was accompanied by a normal postoperative eosinophile curve. In general, there is a correlation of clinical severity, nitrogen loss, eosinophile fall and 17-hydroxycorticoid excretion.

C. THREE CASES OF BONE TRAUMA

Case 9. *Multiple fractures (right tibia and fibula, left radius and ulna). Closed reduction and application of plaster (local anesthesia).* Patient A. G., male, age 57. Unit No. H-182 [illegible] admitted December 6, 1952, and discharged [illegible] 1953 (Fig. 14).

Clinical Summary. This 57-[illegible] admitted to the hospital hypotensive and [illegible] traumatic shock, following an automobile accident in which his car had struck a tree. The patient was [illegible] and sustained multiple fractures on [illegible] sisting of a compound fracture of the tibia and fibula and a fracture of the left radius and ulna. A large hematoma of the scalp appeared; there were no neurological findings to suggest severe intracranial injury. He had a mild cerebral concussion and rapidly regained consciousness with no further neurological deficit. The patient was given blood transfusions totalling 1000 ml.

Shortly after admission the patient was taken to the operating room where a closed reduction of the compound fracture of the tibia and fibula was carried out with plaster fixation. A closed reduction of the Colles' fracture was also carried out. The patient had an uneventful postoperative course with slight elevation of temperature for about 10 days, thereafter returning to normal. The patient was discharged [illegible] bone appeared [illegible] after [illegible] time [illegible] ambulatory.

[illegible] Nitrogen Metabolism was noteworthy for the prolonged period of negative balance. Absolute excretion rates [illegible] with high intake levels and adequate calorie [illegible] for approximately 3 weeks after which a state [illegible] constant intake was achieved [illegible]

of reduction in nitrogen excretion rate. There was a loss of approximately 6 per cent of body weight over a 2 weeks' period, after which weight stabilized

Endocrine Summary. Eosinophile count was low for 3 days, thereafter rising gradually to a normal level. No period of overswing was observed.

There was no detectable abnormality of 17-ketosteroid excretion. The 17-hydroxycorticoid excretion was elevated for 2 days, thereafter returning to normal.

Body Compositional Changes (chart not shown). A rise in body water was observed, most marked on the fifth post-trauma day when an increase from 48.7 to 56.1 per cent of body weight had occurred (3.75 liters). Body weight fell gradually a total of 5 Kg. The net compositional change (loss of fat and lean tissue) was very marked in view of transient nature of the endocrine respon

Case 10. *Fracture of right femur; insertion of intramedullary rod (Pentothal-ether anesthesia).* Patient D. C., male, age 17, Unit No. 7E361, was admitted September 23, 1952, and discharged October 24, 1952 (Figs. 15 and 16).

Clinical Summary. This 17-year-old boy was hurt while carrying an injured teammate back from the football field. He fell, heard a snap, and was unable to rise because of pain. Upon admission to the hospital he was shown to have a mid-shaft fracture of the right femur with no break in the skin. There was no marked swelling of the overlying soft tissues. The patient was placed on bed rest, received 1000 ml. of blood, and showed fever with tachycardia. Approximately 18 hours after injury the patient was taken to the operating room. An open reduction and intramedullary nailing was carried out and a very considerable blood loss encountered; 1000 ml. of blood transfused. The patient was placed on balanced suspension postoperatively and remained febrile with some tachycardia, for approximately 10 days. By the 11th postoperative day the patient was up on crutches without weight bearing. He was discharged on the 32nd hospital day.

Metabolic Summary. Nitrogen balance was noteworthy for an extremely high excretion rate, over 30 Gm. on the second day. An elevated nitrogen excretion rate persisted with negative balance for about 2 weeks despite good oral intakes. After 15 days intermittent positive balance was recorded although excretion rate was still high and never showed a sudden reduction of the "corticoid withdrawal" type.

Potassium balance was strongly negative for the first 3 days, during which time a total of 250 mEq of potassium was lost. After this there was a transient period of loading with very low excretion rate, then a return of higher excretion rate before positive balance was finally attained on the 15th postoperative day.

The sodium balance was remarkable for a considerable renal excretion of sodium on the days of the trauma and operation. For the next 4 days renal sodium conservation was evident despite high intakes of sodium, these intakes being mostly by mouth. The urine sodium excretion during this phase averaged 7 mEq. per day. This positive sodium balance totaled approximately 250 mEq. and almost exactly equaled the simultaneously negative potassium balance and was followed, on the seventh, eighth and ninth post-trauma days, by a definite sodium diuresis.

The patient's weight curve showed only a very slight fall during the first 6 days. Then, during the period of sodium diuresis there was an abrupt weight loss to a level approximately 5 per cent lower than that at the outset.

The caloric intake was low for approximately 3 days, thereafter being above 2000 calories per day for the next 12 days, and thereafter above 3000 calories per day. Caloric intakes over 2000 per day were recorded for a full 16 days before nitrogen balance became positive.

Endocrine Summary. The eosinophile count remained low for only 3 days, thereafter returning to levels slightly higher than those observed subsequently.

The urinary excretion of 17-ketosteroids showed a slight elevation on the second and third days of study but was still in the normal range, thereafter being in the low normal range.

The urinary 17-hydroxycorticoids showed, on the day of operation, an excretion of 25 mg. per 24 hours. On the following day the excretion was over 50 mg. per 24 hours, this being the highest spontaneous excretion of 17-hydroxycorticoids observed in this series of cases. Thereafter the excretion was normal save on the 14th postoperative day when there was a transient elevation to 14 mg.

Body Compositional Changes. There was a rise in body water (relative and absolute) as weight fell. There was a marked Na-K shift (sodium down to 130 mEq/L, potassium up to 5.2 mEq. per L). The return to normal electrolyte levels occurred on the sixth day when body weight and water balance both indicated a diuresis of accumulated water.

Case 11. *Intracapsular fracture of the right femoral neck; insertion of Smith-Petersen nail (Pentothal-ether anesthesia).* Patient L. C., female, age 54, Unit No. N3959, was admitted March 3, 1953, and discharged April 6, 1953 (Fig. 17).

Clinical Summary. This 54-year-old mother had fallen backwards off low steps, sustaining immediate pain and inability to move the right hip. She

was brought to the hospital where films revealed a fracture of the right femoral neck. The fracture was subcapital, very close to the head. The characteristic deformity of the right leg was present. There was virtually no systemic disturbance on admission. On the afternoon of admission a nailing of the right hip was carried out. The position of the nail, although not ideal, was considered satisfactory. The patient initially did well. Convalescence was uneventful as regards ability to take food. There was some low grade temperature elevation, however, and on the 11th postoperative day roentgenograms showed that the pin had slipped out of place and that reoperation would later be indicated. Metabolic study was carried to the 18th postoperative day.

Metabolic Summary. The nitrogen changes were transient, with loss most marked the first postoperative day, true also of potassium changes. Potassium balance was transiently negative, less than 50 mEq., thereafter positive. A marked tendency to retain sodium was maintained until about the 12th postoperative day. There was loss of about 6 per cent of body weight. Caloric intake was slow to reach a normal level.

Endocrine Summary. The eosinophiles remained low for 2 days, the day of trauma and the day of operation. They thereafter commenced to rise, and rose gradually to an unsteady equilibrium at about 120 per cubic mm. The urinary excretion of 17-ketosteroids was normal throughout. The urinary excretion of 17-hydroxycorticoids showed an increase on the day following the nail insertion, although the excretion did not exceed 11 mg. per day. Thereafter the excretion rate was in the range of 5 mg. per day.

Summary of the Bone Trauma Cases. The most evident contrast is brought out between the two cases of midshaft long-bone fracture on the one hand, and the intracapsular fracture on the other. The former, involving pain, hematoma and soft tissue disruption produced large metabolic and endocrine changes most noteworthy for the prolonged high excretion rate of nitrogen in the face of normal diet, and a very high 17-hydroxycorticoid excretion, whereas the intracapsular fracture (and this not in an elderly or feeble person) elicited a response scarcely more noticeable than trifling soft-tissue trauma.

The total nitrogen excretion of the femoral shaft fracture (Case 10) approximates that of a severe third-degree burn.

In the three fractures there is a consistent tendency to inverse correlation between 17-hydroxycorticoid excretion and eosinophile count. The excretion of 17-ketosteroids does show a significant rise in the midshaft femoral fracture, not in the others.

IV. Discussion

It has been our purpose here to explore the metabolic inter-relationships in a variety of surgical cases, and particularly to examine the changes in urinary excretion of 17-hydroxycorticoids as related to the rest of the clinical and metabolic picture.

A. Metabolic Changes

Changes in metabolism of nitrogen, potassium and sodium are essentially similar to those previously reported from these laboratories and elsewhere. The range of traumas studied is wide; there have been few opportunities in the recent literature to examine bone and soft tissue trauma in the same frame of reference. A phase of nitrogen negativity is followed by one of positivity in most of the cases. In certain instances, particularly the burn and the fractures, there is a prolonged phase wherein nitrogen balance continues to be negative despite adequate intakes of nitrogen and calories and few evidences of continued endocrine imbalance. This "disproportionate" nitrogen loss in long-bone fractures is reminiscent of Cuthbertson's original cases[13, 14] wherein nitrogen loss seemed so great for the degree of soft tissue trauma.[33] It is attractive to offer the hypothesis that the initial trauma excites a certain response, and that an unhealed wound then maintains the nitrogen catabolism; hence, a wound which takes a long time to complete its local anabolism (fracture, burn) is accompanied by a prolonged nitrogen loss.

All these patients were in moderate or very good health prior to trauma, even Case 6 (the hypertensive) having minimal visceral disease. In a recent paper from these laboratories[35] the metabolic and endocrine

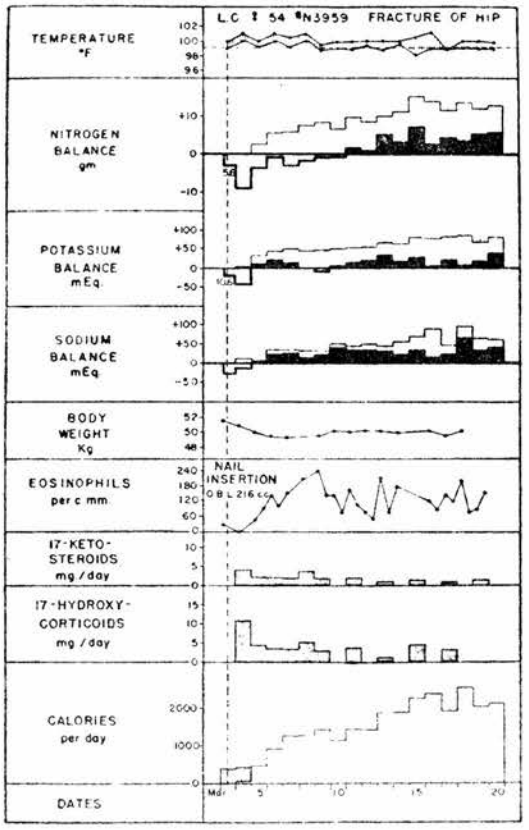

FIG. 17. Case 11. Fractured femoral neck and nailing. The entire metabolic and endocrine adjustment was minor. Only sodium conservation was prolonged.

findings in three cases of late mitral stenosis undergoing operation are described. The reader is referred to that description for examples of metabolic and endocrine findings in patients with depletion and visceral disease far more pronounced than any considered here. Depletion appears to accentuate the electrolyte changes (both in urine and serum) and to diminish the nitrogen losses; water conservation is more pronounced; endocrine changes are comparable to those seen here.

Sodium conservation is marked in many of the cases described here and is much longer in duration than one might predict for uneventful traumatic episodes in well-nourished people going on to complete recovery. In the study of post-traumatic sodium balance, we are increasingly impressed by the lack of correlation between sodium changes on the one hand, and nitrogen and potassium changes on the other. In several instances in these cases (Cases 5, 6, 8 and 11) sodium conservation persists for almost two weeks while the potassium and nitrogen changes are much more transient.

LeQuesne and Lewis[28] have described "primary" and "secondary" sodium retention after trauma. In our studies the urine collections have not been split up sufficiently to corroborate this finding but there are several cases herein where the urine sodium excretion on the day of trauma was maintained at a higher level than it was subsequently. This correlates with the observation of Dudley[15] that immediately after trauma there is a clearcut antidiuretic phase in which urine osmolarity is high but volume low, sodium contributing considerably to this osmolarity.

On the day of operation, and for two or three days thereafter, depending on the magnitude of trauma, potassium is lost in high ratio to nitrogen. But the potassium balance becomes positive before nitrogen in most. Therefore, if one considers the negative-nitrogen period as a whole the K:N ratio of the negative balance is quite low. In Table I are shown the K:N ratios in this group of 11 patients. A K:N ratio of negative balance in the range of 2.5 to 3.0 mEq. per gram is considered to represent "balanced tissue loss." A higher ratio suggests loss of cell electrolyte faster than protoplasmic matrix; high ratios are usually encountered in the early phases of post-traumatic metabolism.[33] As shown in the table, the K:N ratios on the day of operation are high in all instances except Case 3. For the first two days (day of operation and first postoperative day) it is lower. When the first four days are considered as a period, the ratio is low in nine of the cases and in Case 2, 3, 4 and 5 the net potassium balance has become positive while nitrogen is still negative. If calculations are based on

a longer time, the potassium balance will be found almost invariably to have become positive while nitrogen is still negative; cell electrolyte is lost before matrix and reloaded before the matrix is entirely rebuilt. Therefore, K:N ratios calculated for the entire nitrogen-negative period are very low, or the balances are of opposite sign.

B. CHANGES IN BODY COMPOSITION

In cases of minor injury, body compositional changes are inconsequential. In the more major traumata, particularly Cases 3, 5, 8, 9 and 10, a characteristic change is observed, consisting in a relative increase of total body water as nitrogen and weight are lost. This suggests fat mobilization during a period of lean tissue loss. From these changes the approximate rates of fat oxidation may be calculated, from the nitrogen loss the lean tissue changes may be deduced and a complete picture of the compositional change reconstructed.[34] These data are not tabulated here.

The lowering of serum sodium concentration and rise of serum potassium concentration which immediately follow major injury are most marked in the case of hiatus hernia and in the fracture of the femur. In these patients, it is important to emphasize that there were no unusual extrarenal losses and that the lowering of sodium and rise of potassium appeared to occur as a feature of compositional alteration following trauma and not due to changes in external balance. Indeed, the balance frequently is in an inverse direction to that predicted from the serum concentration alterations. This "post-traumatic Na:K shift" is described more fully elsewhere.[37] In Case 8 the "post-traumatic Na:K shift" occurred only after the first operation, when endocrine changes were marked. After the second operation the endocrine adjustment was minimal and there was no "Na:K shift."

C. ENDOCRINOLOGIC CHANGES

The changes in eosinophile count will not be reviewed in detail since they are evident on the charts. The very minor traumata induce very transient eosinophile changes and in one instance (herniorrhaphy under spinal anesthesia) the eosinophiles did not fall lower than 50 per cu. mm. A tendency to swing back after two or three days to a level higher than the norm for that individual was not a regular observation in this series, although suggestive changes of this type were seen in patients 1, 2, 4, 5, 10 and 11. The correlation of this eosinophile "backswing" with a reduction in urinary nitrogen excretion rate and an increase in sodium excretion rate was usually poor, the correlation of eosinophile changes with nitrogen excretion changes is generally closer and more predictable than is any relationship between eosinophile change and [illegible] excretion. The usefulness [illegible] count lies in its qualitative rather than in quantitative accuracy.[34]

The excretion of 17-ketosteroids in the urine demonstrates very few changes which we are able to interpret significantly. Only in the femoral shaft fracture is a significant post-traumatic rise seen.*

By contrast, the excretion of urinary 17-hydroxycorticoids demonstrates clearcut alterations which are of extreme importance because they are correlated with the magnitude of the trauma and the patient's course. The excretion of 17-hydroxycorticoids in the urine shows a correlation with (a) the clinical magnitude of the trauma; (b) the magnitude of nitrogen excretion and of negative nitrogen balance; and (c) the lowering, and duration of lowering, of the eosinophile count. The nitrogen correlation is shown in Figure 18.

In the light of the configuration in the blood levels of free hydroxycorticoids de-

* Since hormone precursors of the 17-ketosteroids are both adrenal and testicular in origin, the possibility arises that stimulation of the former and inhibition of the latter might lead to no change in total excretion. Although this seems to be only a remote possibility it must be borne in mind until 17-ketosteroid fractionation settles the point.

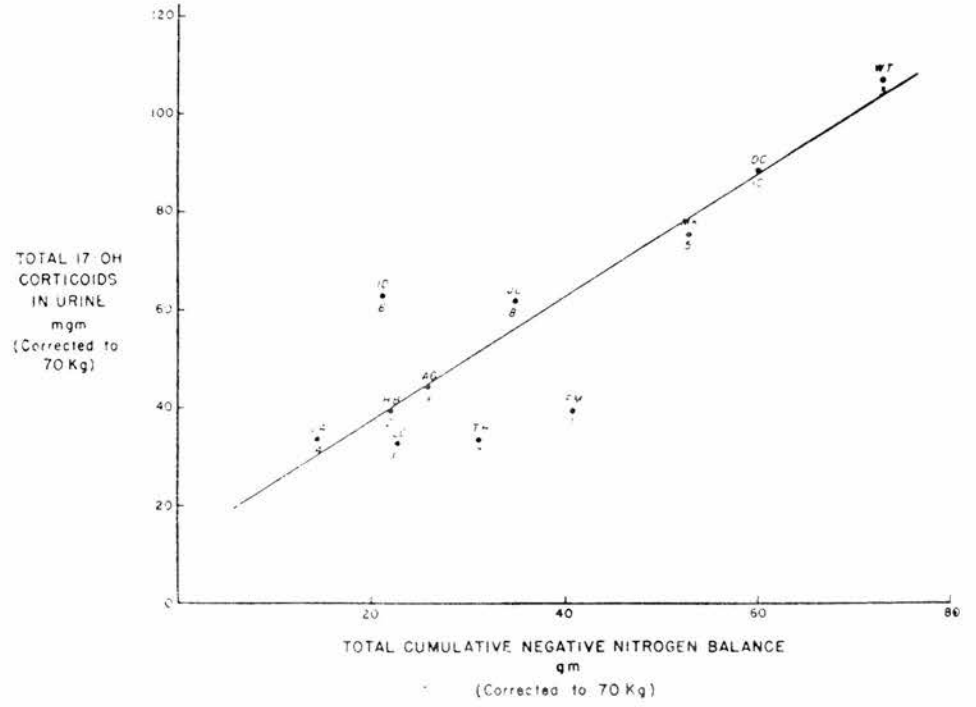

FIG. 18. Correlation between urinary 17-hydroxycorticoid excretion and total cumulative negative nitrogen balance, both for the first four days following trauma, and corrected to 70 Kg. body weight. Each dot indicates a single patient by number and initials.

Cases 1, 6 and 7 fall well away from the line drawn by sight through the other points. Cases 6 and 7 (cereinoma of the breast and second-degree burns) are discussed in the text as regards their unusual departure from nitrogen-steroid correlation.

scribed in greater detail in the succeeding paper,[18] the duration of elevated urinary excretion of these hormones after trauma must be interpreted tentatively as being due to the continued excretion of a hormone which is being conjugated and destroyed in the organism, probably in the liver. For this reason, the duration of elevation of urinary excretion of total hormone is much greater than that of blood elevations of the free hormone. The total urinary excretion of 17-hydroxycorticoids may turn out to have a closer correlation with the magnitude of tissue trauma than the absolute height of elevation of the free blood corticoids, although the latter curve is, of course, of much greater interest with respect to the mechanisms involved.

The second operation on Case 8 raises the evanescent question of "adrenal exhaustion." There is only a most transient eosinophile drop, no increase in hormone excretion, and no increase in nitrogen excretion rate. It would be our interpretation that there is no adrenal insufficiency and that this reflects some metabolic effect of the previous operation. In support of this are the facts that blood pressure was not lowered to shock levels, that convalescence was smooth, and that salt retention was marked and prolonged. Adrenal denervation was an improbable cause for "under-reaction" here, as the painful chest tap two weeks later was able to evoke a transient response. The concept of "adrenal exhaustion" is also brought up by the events in Cases 3 and 7. In Case 3, after the subsidence of the peritoneal infection there was a long period when urinary 17-ketosteroids were so depressed as to be unmeasurable, and 17-hydroxycorticoid excretion was very low. Yet the patient was maintaining blood

TABLE I. *K:N Ratios of Negative Balance.*

Case	Operation	Day of Operation	1st 2 Days	1st 4 Days
		mEq. K per gram N		
1. P. M.	Appendectomy	11.7	8.4	3.4
2. H. B.	Appendectomy	5.2	0.54	– – (K +)
3. W. T.	Appendectomy	2.0	0.18	– – (K +)
4. L. A.	Herniorrhaphy	9.3	2.1	– (K +)
5. M. K.	Hiatus Hernia	4.7	4.5	– (K +)
6. L. D.	Mastectomy	5.4	3.2	1.2
7. F. H.	2nd° Burns	6.2	3.1	1.4
8. J. L.	Splanchnicectomy	7.1	4.1	1.1
9. A. G.	Fractures	5.5	1.7	0.8
10. D. C.	Fracture	9.7	5.8	4.0
11. L. C.	Fracture	6.1	4.8	1.4

pressure well, convalescing normally, exhibiting normal sodium balance and keeping his eosinophile count low. Adrenal function was evidently at a low ebb, yet we have no suggestion that this was an abnormality of convalescence; recovery was complete. In Case 7 the urine steroid excretions were low throughout, but the adrenal was virtually ruled out as the cause of this anomaly by the events when ACTH was given. In our experience adrenal "exhaustion" or "surgical adrenal failure" is a rarity outside of frank endocrine disease, adrenal removal, or cortisone withdrawal. And when it does occur hypotension and unregulated renal salt loss are its most ominous features; the diagnosis cannot be based on the occurrence of isolated abnormalities of the eosinophile count or urine hormone assay.

"Deleterious (or pathological) over-activity of the pituitary adrenal axis" is a difficult concept to support on the basis of any of these cases. In Case 10 (fracture of femur) we see the highest urine excretion of 17-hydroxycorticoids, the greatest nitrogen loss and the most intense sodium retention. Yet this is the case of a young man with severe injury, and convalescence was gratifying. It is difficult to regard the process as deleterious or abnormal.

V. SUMMARY AND CONCLUSIONS

1. A series of 11 cases has been studied and reported with respect to clinical events, metabolic balances and certain indices of endocrine activity.

2. The pattern of metabolism and endocrinology after trauma is further detailed. Of special interest in this series of cases is the correlation of certain indices with the magnitude of the trauma, of which the most outstanding are as follows:

a. The duration and the magnitude of nitrogen negativity correlates closely with the extent of the trauma.

b. The duration and magnitude of elevation in urinary excretion of 17-hydroxycorticoids also correlates closely with the nitrogen loss and the magnitude of the trauma.

c. Even minor traumata show an increase in the urinary excretion of 17-hydroxycorticoids; the method, while quantitative and sensitive, is still not as sensitive as the purely qualitative eosinophile fall.

3. Certain observations are notable for their lack of correlation as follows:

a. The magnitude and duration of sodium conservation after trauma correlates poorly with the nitrogen changes, or with any of the endocrine observations made. In several instances, continued sodium conservation is notable with eosinophiles returned to normal and with the urinary excretion of 17-hydroxycorticoids returned to a low value. This may possibly be related to the presence of an electrolyte-active steroid[19, 17] which we are unable to detect at the present time.

b. The mid-shaft bone traumata are noteworthy for the prolonged and profound nature of the nitrogen changes observed, in contrast to the transient endocrine and electrolyte changes. In these fractures, the potassium, sodium, eosinophile, urinary steroid and weight changes are about what one would predict for the amount of tissue trauma involved. The magnitude and duration of increased nitrogen excretion rate is large and is "out of proportion." Further study of calcium and phosphorus metabolism must be done before this phenomenon is clearly understood.

4. In one case of a second-degree burn and in a patient with radical mastectomy for

carcinoma of the breast, the characteristic pattern of excretion of urinary 17-hydroxycorticoids was markedly distorted. In the burned patient there was no elevation until ACTH had been administered, and in the breast case there was prolonged alteration despite only transient changes in nitrogen balance or [illegible]hiles.

In [illegible] these cases is "adrenal exhaustion" [illegible] "pathological over-activity" acceptable as either a clinical or laboratory interpretation of the recorded events.

6. Of 37 cases so far studied in this laboratory, of which 11 are herein reported, the urinary ex[illegible] 17-hydroxycorticoids shows consistent correlation with the magnitude of clinical trauma and total nitrogen loss. This fact, while of importance in itself, does not constit[illegible] conclusive evidence as to whether such steroidal changes are the primary cause of the post-traumatic metabolism, are "permissive" or are merely "associated."

7. A tendency to lose fat and gain relative water after trauma is again noted.

8. A tendency to develop hyperkaliemia and hyponatremia is noteworthy soon after the trauma in several of the cases.

ACKNOWLEDGMENT

The authors wish to express their gratitude to Miss Gertrude Lanman and Mrs. Barbara Mangini for technical assistance, and to Miss Marcia Foster, research dietitian.

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13.

The Electrolyte and Metabolic Response to Trauma

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Reprinted from
Lectures on the Scientific Basis of Medicine, Volume IV: 1954–55

XI

The Electrolyte and Metabolic Response to Trauma

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THE infliction of an injury immediately sets in motion a train of events which have as their purpose the healing of the wound and the restoration of health. The changes that occur are not confined to the immediate vicinity of the wound, for local repair is associated with a more general reaction of the body. Cuthbertson in 1932 first drew attention to the considerable breakdown of protein that occurred after a fracture of bone and since then the further studies of Cuthbertson (1942, 1954) and of Howard (1945) and Moore (1953) have added greatly to our knowledge. The nature and significance of these general bodily changes in convalescence forms the substance of this lecture.

In order to acquire a detailed picture of the biochemical changes that follow the receipt of an injury several methods of study are required. The intake and output of substances from the body may be measured by the familiar metabolic balance technique (Moore and Ball, 1952). At the same time the concentration of substances in the body fluids, particularly the plasma, may be investigated. These methods are limited to the demonstration of changes and afford no information regarding the initial total body composition. The metabolic response to trauma is influenced profoundly by at least two factors, namely the nutritional state of the patient and the nature and severity of the inflicted injury. The well-nourished, healthy adult and the patient depleted by disease show conspicuously different patterns of response after injuries of equal severity.

A detailed study of the general bodily reaction accordingly requires a knowledge both of the initial body composition and of the changes that are subsequently superimposed on this background.

Recent Methods of Study

It is only within recent years that it has been possible to attempt measurements of the amounts of water, fat, sodium, potassium and chloride within the living human body. This has been largely due to the ready availability of suitable isotopes. The principle of the method is simple and has been fully described by Moore and his associates (Edelman *et al.*, 1952a). A known amount of the isotope is added to the body and the quantity excreted during the establishment of equilibrium of distribution is measured. The dilution of the isotope in the corresponding natural element in the body is measured and the total amount of the element with which the isotope has exchanged is calculated. For example, in the case of sodium, the isotope ^{24}Na with a half life of 15 hours may be used. An accurately measured amount of the radioisotope is introduced intravenously either from a calibrated syringe or burette. After a suitable interval, usually about 24 hours, a sample of blood is withdrawn. Then:

$$\text{Total exchangeable sodium in m.eq.} = \frac{\text{Radiosodium injected—radiosodium excreted}}{\text{Radiosodium per m.eq. of sodium in plasma}}$$

Similar estimations of the amounts of potassium and chloride in the body may be made with radioisotopes.

The same principle is utilized in the measurement of total body water with deuterium oxide. The deuterium exchanges rapidly with the hydrogen of water but is only incorporated to an insignificant extent in organic molecules during the two to three hours required for the measurement in healthy subjects (Schloerb *et al.*, 1950). In this manner considerable information has been gathered about the amount of water in the human body at different ages and in the two sexes (Edelman *et al.*, 1952b).

There are several limitations to isotope dilution measurements. The attainment of an equilibrium of distribution of an

isotope is difficult in an essentially dynamic system divided into several compartments with different rates of exchange (Burch *et al.*, 1953). The total exchangeable mass of the element is not necessarily the total amount in the body. The sodium isotopes do not measure a large proportion of the sodium in bone (Davies *et al.*, 1952; Edelman *et al.*, 1954; Miller *et al.*, 1954) though it is improbable that this non-exchangeable sodium is of any importance from the point of view of the rapid metabolic changes after surgery (Bauer, 1954). The potassium in red cells exchanges slowly and is not all included in measurements of exchangeable potassium (Corsa *et al.*, 1950); here again the red cell potassium is not of great metabolic significance. These and other associated problems are being extensively explored, but in spite of the difficulties there can be little doubt that these methods offer the most reliable picture of body composition at present available.

Certain additional information may be deduced from measurements of total body water and exchangeable potassium. When the water content of the body is known, the fat content may be calculated on the assumption that lean tissue contains about 73 per cent of water (Rathbun and Pace, 1945).

$$\% \text{ fat} = 100 - \frac{\% \text{ water}}{0{\cdot}73}$$

Clearly the assumption regarding the extent of hydration of lean tissue is not justified in many pathological conditions and in the immediate post-operative period. On the other hand in the majority of cases these calculations, though not precise, offer a first approximation of changes in body fat in the presence of disease. The problems associated with calculations of this nature have recently been fully reviewed (Moore *et al.*, 1952; Keys and Brozek, 1953).

The bulk of potassium is in the cellular mass of the body, mostly in the muscles—only about 2 per cent is in the extracellular fluid. Apart from periods of acute stress, measurements of exchangeable potassium reflect changes in the cellular mass of the body. In the healthy female in comparison with the male, there is more fat and less muscle. Fat is anhydrous and much

of the potassium resides in muscles. Accordingly, it is found that the percentage of water and the exchangeable potassium are both less in the female. The difference in relative muscle mass between the male and female is also reflected in a consideration of the ratio of exchangeable potassium and sodium. In the male this is commonly above, and in the female, below unity. In any form of wasting disease the body loses potassium and the ratio consistently falls (Moore *et al.*, 1954).

The isotope dilution method is not only of value in ascertaining the body composition before surgery. Interval determinations may be made while the patient is in hospital and the changes revealed in this manner usually are in good agreement with those shown by metabolic balance techniques (Wilson *et al.*, 1954a). Furthermore, the observations may be extended far out into convalescence after the patient has left hospital when balance methods are no longer applicable. On the other hand, isotope dilution studies cannot be readily repeated more often than once a week so that the immediate changes after trauma are best followed by metabolic balance techniques. It is important to emphasize the advantages that accrue from a combination of the measurements of body composition and of metabolic balance. The latter only affords a limited picture of the biological changes in progress and even in the best conditions available for the study of surgical patients is liable to considerable error. On the other hand, the initial body composition measurements reveal the background on which these changes are imposed and the later measurements can be used as a check of the cumulative changes shown by the balance studies. In this way it is possible to elucidate the rôles played by previous disease and by trauma and to show how changes in body composition alter the response to injury.

The Physiological Response to Injury

The physiological response to an injury in man is best studied in an adult of normal body composition who receives a major surgical operation. It is important that the condition for which the operation is carried out should not have impaired his nutritional state. In such circumstances observations can be

made preoperatively and the whole sequence of events followed. The assessment of the severity of the injury inflicted by a surgical operation is clearly difficult. A loose semi-quantitative scale has been employed (Moore and Ball, 1952). A third degree burn of 25 per cent or more of the body surface is at the top with scale 10. Multiple severe wounds and extensive multi- visceral operations are scale 9 to 7. Scale 5 is represented by such operations as gastrectomy. Appendicectomy and repair of a hernia may be about scale 3 and so on down to scale 1 which may be represented by simple ligation of a vein.

We shall consider in the first instance the metabolic response to a fairly severe injury, about scale 7, in an adult male in good nutritional condition.

Nitrogen balance. In a healthy adult the mean daily intake and output of nitrogen is equal and is about 12 gm. per day. Most of the body nitrogen exists as protein and clearly if lean tissue is being added to the body the patient will show a positive nitrogen balance; conversely if protein is being broken down the patient will show a negative nitrogen balance. If a person is starved the loss of nitrogen is initially about 10 gm. a day but settles down to a rate of about 7 gm. a day after the first 12 days (Benedict, 1915). After an injury of the severity that we are discussing the nitrogen intake for the first day or two is negligible but the excretion rate is unduly high, commonly about 12–15 gm. a day. It is this excessive excretion of nitrogen that characterizes the first phase of the metabolic response to trauma. It represents a breakdown of protein in the body. This accelerated catabolism of protein cannot be prevented by ensuring a high protein intake immediately after the receipt of an injury. Nitrogen taken at this stage is in general only excreted and there is accordingly a negative nitrogen balance which cannot be corrected by any dietetic measures. This stage of accelerated catabolism of protein persists for a few days—perhaps about five days in the type of injury being considered. Thereafter the rate of excretion is reduced and the intake is increased, so gradually balance is achieved. This stage clearly represents the 'turn of the tide'. The rapid ebb of nitrogen from the body is

checked and after a few more days the patient's appetite returns and the body is ready to receive an inflow of nitrogen. Provided an adequate diet with respect to protein and calories is given he then passes into positive nitrogen balance and the lost lean tissue is reconstituted. This restoration of body protein proceeds for several weeks depending on the extent of the original breakdown of lean tissue.

Potassium metabolism. There is a conspicuous loss of potassium on the day of injury and a negative balance usually persists for two or three days, though not for so long as the negative nitrogen balance. In lean tissue there is a fairly constant ratio of about 2·5–3·5 m.eq. of potassium per gram of nitrogen (K : N ratio) (Hastings, 1941). The potassium loss after injury is more than would be predicted on the basis of the nitrogen loss and the K : N ratio is high, usually over 5 m.eq. per gram. As convalescence progresses positive potassium balance is soon achieved and the potassium lost in excess of nitrogen is rapidly restored. Thereafter there is a slower retention of potassium in proportion to the lean tissue restored with a K : N ratio of about 3 m.eq. per gram. There is often relatively little change in the serum potassium concentration but after a severe injury a rise may occur at the time of rapid potassium excretion and a subsequent fall to the preoperative value during the period of potassium retention.

Sodium metabolism. After injury urinary excretion of sodium is extremely low and the amount lost in the sweat and faeces is normally slight. The net balance effect depends largely on the amount of sodium given intravenously or by mouth. Provided that there are no undue extrarenal losses sodium given in the early stage is retained in the body. Usually after a few days any excess sodium is excreted in the urine, but the period of sodium retention is very variable and may be prolonged after a severe injury. If there are large losses through exudate from a wound or by drainage from the alimentary canal a negative balance may develop. The usual features after injury are, however, a stage of sodium retention followed by excretion. A fall in serum sodium concentration commonly occurs after operation. Paradoxically, it is frequently observed that the

serum sodium concentration decreases during the period of sodium retention and rises during the sodium diuresis.

Water metabolism. Immediately after operation there is oliguria and water retention. This water retention develops irrespective of the amount of sodium given and cannot generally be controlled by sodium restriction. If a great excess of water is given by any route a dangerous dilution of body contents occurs (Wynn and Rob, 1954). After two or three days there is normally a diuresis of any excess water taken during the early stage.

Body weight and fat metabolism. There is a rapid drop in weight after an extensive injury and this proceeds for several days. It is greater than can be accounted for in terms of tissue excised and protein catabolism. In these calculations it is assumed that a gram of nitrogen represents 30 gm. of wet lean tissue (Moore and Ball, 1952). The weight loss that cannot be accounted for in these balance studies is presumably due to fat oxidation in the early stage. Serial observations made with deuterium oxide have also shown a reduction in body fat and have confirmed that rapid fat oxidation is a prominent feature of the response to severe injury (Edelman *et al.*, 1952c). Positive nitrogen balances later in convalescence, on the other hand, usually account fully for weight changes, suggesting that replacement of fat does not occur until much later—an interpretation substantiated by clinical observations.

Adrenocortical activity. Immediately after injury there is an increase in the excretion rate of the 17 hydroxycorticoids in the urine. This is usually maximal on the day of injury and the succeeding day and thereafter declines to the pre-trauma level within about seven days. Such a general pattern has been described by several groups of workers using different methods for the determination of urinary corticoids (Venning *et al.*, 1944; Norymberski *et al.*, 1953, Moore *et al.*, 1955). By contrast there is often little change in the excretion of urinary ketosteroids which may show a small rise on the day of injury but thereafter little change (Bennet and Moore, 1951). More recently the blood levels of 17 hydroxycorticoids have been studied after severe injury. In those with uncomplicated courses high levels

have been recorded only for the first two days (Franksson *et al.*, 1954). The blood levels apparently fall before the urinary excretion returns to the normal rate.

The behaviour of the blood eosinophils has been frequently investigated after injury and surgical operations. It is well recognized that the count rapidly falls to zero at which level it remains for a day or two and then climbs to the preoperative level or higher—an 'overshoot' of this nature is frequently observed a week or so after injury. Frequently these fluctuations in the eosinophil count correlate closely with the alterations in 17 hydroxycorticoid excretion. However, it is now widely recognized that the eosinophil count is an unreliable index of adrenocortical activity. Indeed, after the operation of bilateral adrenalectomy when the patient is kept on a fixed daily intake of cortisone the characteristic changes in eosinophil count are seen with a drop to zero and subsequent temporary rise to a high level. Clearly in these circumstances the eosinophil count does not reflect a change in the supply of adrenal cortical hormone.

The Influence of the Nature and Severity of the Trauma

The typical metabolic response has been described in a well-nourished adult receiving a major surgical operation involving only soft tissues. The response is considerably affected by the extent of a soft tissue injury.

Minor soft tissue injuries. Operations such as repair of an inguinal hernia or uncomplicated appendicectomy elicit a response which is smaller and of shorter duration than that seen in major trauma. Indeed, with the simplest operations the phase of accelerated catabolism is difficult to detect and there are often few features that cannot be explained simply by the enforced reduction of diet. Definite weight loss is demonstrable and there is an elevation in the urinary 17 hydroxycorticoid excretion not usually lasting more than a day. Changes in serum electrolytes are not conspicuous but if frequent serial readings are made a drop of about 1–3 m.eq./l. may be demonstrated in the sodium and chloride determinations.

Major soft tissue injuries. In these cases if the patient before injury was in good health there is a profound metabolic response. The duration and extent of protein catabolism is increased and a considerable quantity of fat is burnt. A prolonged period of sodium retention is frequently noted. The urinary excretion of 17 hydroxycorticoids is increased over a longer period. It is to be noted that this type of reaction also occurs after relatively minor trauma if sepsis subsequently develops, for example appendicectomy complicated by the development of a pelvic abscess.

Bone fractures. Cuthbertson in his early studies (1932) showed that there was a profound nitrogen loss after fracture of long bones. This has been confirmed in later studies. In injuries of this type there is a prolonged phase of protein catabolism despite adequate intakes of nitrogen and calories. By contrast the 17 hydroxycorticoid excretion is similar to that seen in soft tissue injury and only lasts a few days. The excessive protein catabolism may continue for a month—far longer than there is any evidence of increased corticoid excretion. In one series of cases an interesting contrast has been made between midshaft long bone fracture on the one hand and intracapsular fracture of the neck of the femur on the other. The former produced the typical large metabolic disturbance whereas the intracapsular fracture—and this not in an elderly or feeble person—elicited a response no greater than that of trifling soft tissue trauma (Moore *et al.*, 1955).

Burns. A description of some of the changes occurring in burns occurs in Dr. J. P. Bull's lecture printed earlier in this volume.[1] It will only be noted here that this constitutes an extremely severe form of injury. Problems of water and electrolyte metabolism are complex owing to the development of considerable inflammatory oedema. There is a long period of negative nitrogen balance corresponding in duration to what is seen in long bone fractures rather than to other types of soft tissue injury. Here again in our experience evidence of increased adrenocortical activity disappears some time before the increased protein catabolism declines.

[1] See pp. 167–81 above.

Nutritional Depletion and the Metabolic Response to Injury

Most forms of chronic disease lead to characteristic changes in body composition. Clinical examination reveals a loss of fat and muscular tissue and this is confirmed by more detailed measurements of body composition. The proportion of water by weight in the body rises indicating a reduction of the relatively anhydrous fatty tissue. There is an increase in body sodium relative to body weight and there is often an increase in the absolute measurements indicating a replacement of fat by extracellular fluid (McCance and Widdowson, 1951). Exchangeable potassium is reduced consequent on loss of lean tissue, especially skeletal muscle.

These gross changes in body composition not unnaturally affect the response to an inflicted injury. It has been fully demonstrated that the patient in poor nutritional state shows a relatively inconspicuous response. The catabolism of nitrogen is less in the first stage and the loss of weight often not so steep but there may be a more pronounced tendency to salt and water retention.

The excretion of 17 hydroxycorticoids in the urine is less than in healthy subjects.

Response to a second injury. Some of the most interesting demonstrations of the effect of a change of the nutritional state on the metabolic response have been in studies of two-stage surgical operations. The first operation is carried out on a patient in good nutritional health, the second two to three weeks later when he has not fully recovered from the depletion caused by the previous injury. If the two operations are of strictly equal severity, for example a lumbodorsal sympathectomy on each side, the contrast is striking. In one such patient studied recently the first operation produced the typical response with a rapid catabolism of protein and fat and the characteristic serum electrolyte changes, namely a fall in sodium and a rise in potassium concentrations. There was also a considerable increase in the output of 17 hydroxycorticoids in the urine. On the occasion of the second operation performed 19 days later the metabolic disturbance was slight. The changes in nitrogen

excretion were less conspicuous and no change in serum electrolytes and output of 17 hydroxycorticoids was seen. There was, however, more marked sodium retention after the second operation (Moore *et al.*, 1955, case 8).

General Discussion

The changes after injury have been widely studied during recent years, particularly with regard to the chief body constituents such as protein, fat, sodium, potassium and water and several fairly definite patterns of response have been established. At the present time, however, relatively little is known regarding the mechanisms, control and significance of these metabolic changes.

The rapid catabolism of protein and fat presumably has as its primary purpose the provision of raw materials and energy for the healing of the wound. Browne (1950) introduced the concept that a 'loosening' of body nitrogen occurred to make it available for wound healing. It is significant that the healthy person who heals his wounds well after a single trauma and thrives clinically shows this catabolism most vigorously and the depleted patient who is more apt to fail in healing does not show a conspicuous catabolic reaction. The mechanism of control of this catabolic phase is obscure. A supply of adrenal cortical hormone is essential for its development but the metabolic changes cannot be attributed solely to a hypersecretion of the adrenal cortex (Ingle, 1953). In the bone injuries the period of protein catabolism clearly far exceeded the duration of increased corticoid excretion and appeared to be related rather to the time required for new tissue formation. Indeed, in those types of injury in which there is a long period of repair, such as fractures and burns, it is tempting to postulate that the wound itself functions almost as an endocrine organ and releases some substance that sustains the catabolic reaction.

The interpretation of the changes in water and electrolyte metabolism seen after injury presents many problems. It seems probable that the conspicuous water retention may be related to the release of antidiuretic hormone. If this concept is true the hormone is being liberated in spite of a falling concentration of

sodium and chloride in the plasma. The mechanism of this reduction in electrolyte concentration is also as yet undetermined. The fall is not due to any loss of these ions from the body. Dilution of the body electrolytes by the water retention may clearly be an important factor but the fall cannot be entirely eliminated by fluid restriction. Other possible explanations are a movement of water from the cells or alimentary canal into the extracellular fluid or a movement of sodium and chloride in the opposite direction. More detailed study of these points is still required.

The description and discussion have been almost entirely confined to the metabolic disturbances seen after injury in healthy adults. No attempt has been made to describe the variations in the metabolic response to injury seen in those with various types of disease. For instance, after cardiac operations gross disturbances in water and electrolyte metabolism may be seen (Wilson *et al.*, 1954b). The importance of understanding in the first place the sequence of events that occur in the healthy adult as a result of trauma needs no elaboration. An appreciation of what we may term the physiology of convalescence must form the scientific basis for the study of the abnormal response and for the intelligent care of the postoperative patient.

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Reprinted from ANNALS OF SURGERY, Vol. 145, No. 1, January 1957

Metabolic Response to Adrenalectomy *

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CUTHBERTSON [1, 2] first drew attention to the marked urinary loss of body nitrogen, sulphur and phosphorus which follows injury, a loss beginning shortly after the infliction of the trauma and reaching a maximum within ten days. His findings have been substantiated by later investigators and further amplified particularly with regard to electrolyte, calorie, nitrogen and water behaviour. The mechanism by which these widespread changes are initiated and controlled remains undecided. It has been suggested that the adreno-cortical steroids play an essential part and there is undoubtedly a rise in plasma steroids following major trauma (Franksson *et al.;* [4] Sandberg *et al.*[16]). Many of these steroids are known to have profound effects on electrolyte, protein and carbohydrate utilization and the essential part played by the adrenal cortex is further emphasized by the inability of the adrenalectomized animal to survive after trauma of moderate severity. It remains in doubt, however, whether the metabolic response to operation depends on a general increase of plasma steroids above the basic preoperative level, or whether, as Ingle [7] suggests from his studies on small mammals, that a sustained and non-augmented ("permissive") steroid secretion is sufficient.

In this paper metabolic and steroid studies are presented in eight female patients, who were submitted to bilateral adrenalectomy for advanced mammary carcinoma. On seven patients full studies were performed; data on the eighth case is limited to showing electrolyte and eosinophil changes; this case will not be discussed fully in the text and only introduced under "electrolyte and eosinophil behaviour." Before, during and after operation these patients were maintained on a constant intramuscular dose of cortisone.

PLAN OF STUDY

The intention of this study was to estimate the gain or loss in terms of electrolytes, nitrogen and water of patients undergoing bilateral adrenalectomy and oophorectomy and maintained on a constant dose of cortisone. The operation, which was performed in one or two stages, inflicted considerable operative stress and at the same time was thought to remove any internal source of adreno-cortical steroids.

The results of these investigations are open to the criticism that many of the patients had frequently been restricted in ac-

* Submitted for publication March 21, 1956.

TABLE I. *Serum Sodium, Potassium and Chloride Expressed as mEq./L. Normal Range: Sodium 133–152 mEq./L.; Potassium 3.5–5.6 mEq./L.; Chloride 99–108 mEq./L.* (*Wootton and King*[17])

Day	E. W.			N. G.			W. F.			P. L.			E. E.			F. F.			I. F.		
	Na	K	Cl	Na	K	Cl	Na	K	Cl	Na	K	Cl	Na	K	Cl	Na	K	Cl	Na	K	Cl
−4																					
−3				128	—	102				144	4.2	94									
−2	140	4.5	106	—	—	—				143	5.2	101				140	4.7	102	145	4.2	96
−1	140	—	102	124	—	103	133	—	100.6	—	—	—	143	4.5	101	155	6.7	107	148	4.5	96
Operation																					
+1	138	—	109	125	—	104	125	—	99	143	4.8	98	—	—	—	145	6.5	98	144	4.2	93
+2	138	4.7	97	131	—	101	120	—	91.5	—	—	—	144	3.8	101	142	5.2	93	—	—	—
+3	135	4.9	97	124	—	103	123	—	96.1	142	4.8	106	—	—	—	141	5.5	97	141	3.9	98
+4	137	—	95	126	—	95	126	—	96.6	—	—	—	—	—	—	141	5.8	92	—	—	—
+5	—	—	—	134	—	94	123	—	93.2	—	—	—	150	6.1	110	140	5.1	98	—	—	—
+6	135	4.8	100	126	—	93	126	—	95.2	142	4.2	97	147	3.9	110	143	5.5	94	146	5.3	98
+7	134	5.2	92	133	—	95	141	—	100.4	—	—	—	—	—	—	136	5.0	95	—	—	—
+8													145	4.0	104						
+9																					

tivity or in one case, bedridden for some time due to advanced mammary carcinoma and widespread secondary deposits, and would not respond, therefore, as a fit patient to operative trauma. In addition five of the studies were made on patients undergoing a second operation within an interval of ten to 14 days. It is well recognized that the metabolic response in such instances is depressed; with these reservations we believe that some instruction may be gained from these studies.

The seven patients (excluding Case 8, B. W., from whom limited data only were obtained) were women, whose ages ranged from 30 to 58 years. All had widespread soft tissue, and often bony, metastases, following a previous radical mastectomy for mammary carcinoma. Only one patient, however, was restricted to bed prior to operation. For one to three days before the operation patients were allowed a measured quantity of milk. On the day of operation, only water was given and thereafter milk or water, according to the patient's choice, for up to a week after operation. In the majority of cases sodium chloride and potassium chloride was added to the milk mixture. Portions of the milk mixture were routinely analysed and a record kept of the amount of fluid taken. Urine was collected in 24-hourly specimens by catheter. Vomitus and gastric aspirate were analysed separately when obtained. Sweat and stools were not assayed; none of the patients had postoperative diarrhea or marked perspiration. Blood samples were taken before and after operation and these, together with the sodium, potassium and chloride concentrations of the ingesta and excreta were analyzed by standard methods. The urinary steroids were assayed by Norymberski's method (Gibson and Norymberski [15]) and are expressed as mg. of 17-ketogenic and 17-keto-steroids/24 hr. This method affords a satisfactory index of the activity of the adrenal cortex; its reliability has been previously discussed by Norym-

berski *et al.*,[5] Hubble [6] and Jepson *et al.*[8] Intravenous infusions of sugar solutions were avoided as the resultant glycosuria interferes with the steroid assays.

Eosinophil counts were prepared using Manners [12] technic. Details of the individual patients will be found in Appendix I.

COMMENTS

Two of the patients, E. W. and N. G., underwent a "one-stage" operation involving the removal of both adrenal glands and ovaries. This required three separate abdominal incisions and the operation lasted approximately two hours. The remaining five patients (W. F., P. L., E. E., F. F., and I. F.) were studied only during the final operation for the removal of one adrenal gland; the other adrenal and the ovaries having been extirpated 10 to 14 days previously. This operation only required one incision and lasted 30 to 40 min. E. W. and N. G. received for the "one-stage" procedure 100 and 150 mg./24 hr. of cortisone respectively, the remaining five patients 200 mg./24 hr. It is evident, therefore, that E. W. and N. G. had greater operative stress and smaller maintenance doses of cortisone than the remaining five patients.

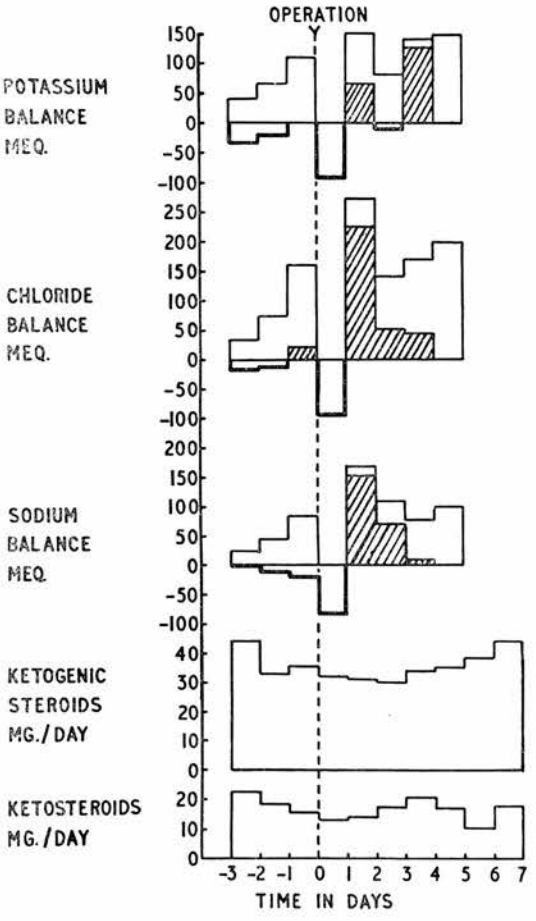

FIG. 1. Patient P. L.—Day to day balance following total adrenalectomy.

CHEMICAL DATA

Serum Electrolytes. The results of the serum sodium, potassium and chloride estimations are shown in Table I. The preoperative levels fall within the normal range, with the exception of N. G. who had a low serum sodium. Following operation the general trend is for the serum sodium and chloride levels to fall slightly (Fig. 1). This fall in serum sodium and chloride is of a degree similar to that associated with operations of a comparable magnitude in "normal" patients (Jepson and Wilson [9]). The serum potassium readings do not show any constant postoperative changes.

Electrolyte Balance (Table II). These balance studies were designed to calculate the amount of electrolyte excreted in urine or vomitus compared to the intake in the post-operative period. In the series of balances undertaken in the adrenalectomized patients the average cumulative balances for 5 to 7 postoperative days were sodium + 13 mEq., potassium − 102 mEq., and chloride + 39 mEq. For the five patients (E. W., P. L., F. F., E. E. and I. F.) on whom both sodium and chloride balances were performed and who had preoperative serum sodium and chloride levels within normal limits, the cumulative balance was sodium + 23 mEq. and chloride + 59 mEq. In a previous study carried out under similar conditions on patients undergoing partial gastrectomy for simple peptic ulceration, without a previous history of vomiting

TABLE II. *Data Collected for Five to Seven Days Postoperatively: (1) The Cumulative Balance of Sodium, Potassium, Chloride (mEq.) and Nitrogen (Gm.); (2) The Retention of Chloride in Excess of Base (mEq.); (3) Relationship of the Urinary Potassium (mEq.) and Nitrogen (Gm.)*

		Na	K	Cl	N	Cl−(Na+K)	K/N Ratio
		Milliequivalents			Gm.	Milliequivalents	M.equiv. : Gm.
	E. W.	−141	−169	−130	−26.7	+180	5.9
	N. G.	− 94		− 63	−53.8		
	W. F.	+ 69			−21.0		
	P. L.	+151	− 3	+234	− 6.7	+ 86	11.1
	F. F.	+ 87	− 83	+ 52	−12.1	+ 48	9.6
	E. E.	+214	−161	+241	−29.0	+188	10.7
	I. F.	−195	− 93	−103	−33.5	+185	6.6
	Average	+ 13	−102	+ 38	−26.1	+137	
	"95% range"	−189	−155	−236	−30		
Davies *et al.*[3]	for partial	to	to	to	to		
	gastrectomies	+389	+139	+526	−100		
	Average	+105	− 9	+145	− 65.6	+ 49	

or excessive intake of anti-acids (control group), the cumulative postoperative balances over a similar period for sodium, potassium and chloride averaged + 105 mEq., − 9 mEq. and + 145 mEq. respectively.[3] These figures show the same trend in "adrenal-intact" and adrenalectomized patients, that is, an overall sodium and chloride retention and a potassium loss. These is, however, in the two groups a quantitative difference; the average sodium and chloride retention is considerably less and the urinary potassium loss greater in the adrenalectomized patients as compared with the control group (Table II), although these differences in sodium balance are not significant when Students' "t" test is applied. The retention of chloride over base [Cl − (Na + K)] is consistently present in the adrenalectomized patients and averages some 88 mEq. higher than the control group. Although the 95 per cent range (Table II) for the balance studies on patients undergoing partial gastrectomy is great, for example the potassium ranges from − 155 to + 139 mEq., it appears that in the adrenalectomized patients the overall sodium and chloride retention is less and the potassium loss greater than that which follows operations of an approximately similar magnitude. If the patients E. W. and N. G., who underwent the most severe procedure of bilateral oophorectomy and adrenalectomy and in whom the postoperative salt intake was small, are eliminated then the balance studies for the remaining five unilateral operations (cortisone 200 mg./24 hr.) are sodium + 65, potassium − 85 and chloride + 106 mEq. respectively. The nitrogen balance was determined in the seven patients and ranged from − 6.7 to − 53.8 Gm. with an average figure − 26.2. This is substantially less than the mean balance of − 65.6 Gm. (95 per cent range − 30 to − 100) reported by Davies *et al.*[3] for similar studies in patients undergoing partial gastrectomy. This is probably due to the inclusion in the average figures of five patients undergoing a second major operation within a period of ten to 14 days; under such circumstances the second operation has long been recognized to result in a smaller negative nitrogen balance. E. W. and N. G. ("one-stage" operations) developed negative balances of − 26.7 and − 53.8 respectively, even though in the case of E. W. the intake of nitrogen was nil. As these patients were receiving the smaller dose of cortisone, the greater negative balance is unlikely to reflect increased plasma steroid levels. In none of the patients was a marked increase in

TABLE III. *Urinary Keto (KS), Ketogenic (KGS), Steroid and Eosinophils (EOS)*

Day	E. W.			N. G.			W. F.			P. L.			E. E.			F. F.			I. F.		
	KS (mg./24 hr.)	KGS (mg./24 hr.)	EOS (per ml.)	KS (mg./24 hr.)	KGS (mg./24 hr.)	EOS (per ml.)	KS (mg./24 hr.)	KGS (mg./24 hr.)	EOS (per ml.)	KS (mg./24 hr.)	KGS (mg./24 hr.)	EOS (per ml.)	KS (mg./24 hr.)	KGS (mg./24 hr.)	EOS (per ml.)	KS (mg./24 hr.)	KGS (mg./24 hr.)	EOS (per ml.)	KS (mg./24 hr.)	KGS (mg./24 hr.)	EOS (per ml.)
−3										22.4	44.6		9.4	34.7							
−2										18.5	33.1		9.4	49.4		4.3	39.6	30	4.8	18.6	1
−1	5.5	9.3	100	13.3	24.5	80	7.6	60.2	0	15.6	35.8		8.4	46.6	40	5.2	44.1	50	3.4	5.0	1
Operation															0						
+1	3.5	23.0	20	5.8	25.2	30	3.7	41.3	5	13.2	32.2					4.7	43.8	10	8.0	23.4	1
+2	2.6	26.0	0	5.9	25.5	5	12.2	56.8	5	14.1	31.2		4.6	58.3		8.4	34.0	5	9.4	24.3	1
+3	2.5	27.0	90	11.1	22.8		6.0	55.4		17.3	30.1		8.1	93.2	20	13.3	29.6	0	10.9	21.8	
+4	3.0	17.0		13.4	27.8		7.7	62.2	0	20.9	33.6		7.2	55.8		11.0	30.6	5	10.8	21.2	1
+5	4.9	16.0	250	10.8	31.9	20	4.4	40.1	0	17.0	35.0		4.5	62.6	0	5.6	43.5	35	11.9	20.2	5
+6	4.0	15.0		11.4	34.1	70	6.1	58.0		10.5	38.1		7.0	56.6	50	5.6	36.8	1	14.0	35.5	1
+7			70	9.4	21.5				1	17.8	44.2		7.6	48.0	5	4.6	38.8	1			10
+8															30	9.2	35.6	1			0
Mean	3.7	19.0		10.1	26.6		6.8	53.4		16.7	35.8		6.6	51.0		7.2	37.6			29.0	
Corr. for 100 mg. Cortisone i.m.	3.7	19.0		6.7	17.7		3.4	26.7		8.3	17.9		3.3	25.5		3.6	19.8			14.5	

urinary nitrogen evident for the first few post-operative days, and in many cases the excretion fell to very low levels. It would, however, be incorrect to interpret too closely these findings, inasmuch as the nitrogen response is known to be markedly decreased in the debilitated (Moore and Ball[14]). The urinary K/N ratio (5.9 to 11.1) was far in excess of that to be expected from the breakdown of tissue alone and was largely dependent upon the renal potassium "leak" following trauma.

If no allowance is made for insensible perspiration and feces the patients remained in positive fluid balance throughout the study, showing a moderate oliguria in the immediate postoperative period with a return to larger urinary volumes toward the third or fourth postoperative day. As intravenous and oral intake were not excessive the postoperative diuresis which often succeeds the oliguric period was not to be expected and indeed failed to occur. The composite findings in patient P. L. are shown in Fig. 1.

Steroid Studies. The urinary excretion of the adreno-cortical hormones and their degradation products was estimated as mg./24 hr. of "ketogenic" steroids (Norymberski *et al.*[5]). In addition, urinary ketosteroid estimations were made. Table III gives both the daily and the mean steroid excretion for the individual patients, which must depend partly on the amount of cortisone given daily. In order to make the figures easier to compare the mean absolute steroid excretion has been corrected by dividing it by two-thirds or one-half if the daily cortisone was 150 or 200 mg. respectively. This allows the urinary steroid excretion following a "standard" dose of 100 mg. cortisone to be evaluated.

The preoperative ketogenic steroid excretion varied according to the amount of cortisone administered: thus N. G. (cortisone 100 mg./24 hr.) excreted 24.5 mg. on the day before operation, while in E. E. (cortisone 200 mg./24 hr.) the amount re-

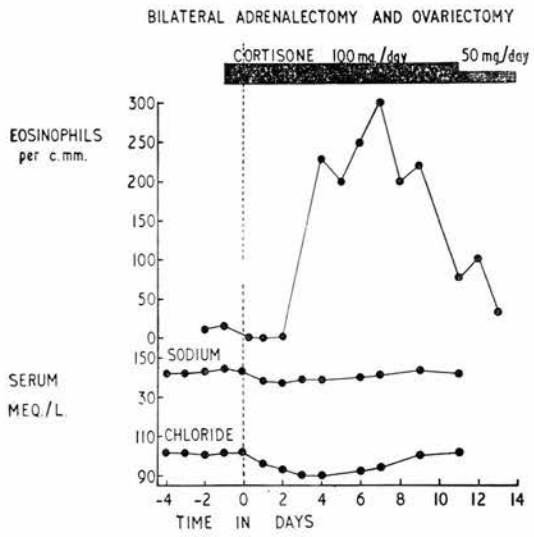

FIG. 2. Patient B. W.—Changes in eosinophil count and serum sodium and potassium following total adrenalectomy and oophorectomy (one stage operation).

covered was 40.6 mg. as 17-ketogenic steroids. It is nevertheless apparent that factors other than size of cortisone dose influence the ketogenic excretion. Five of the patients (W. F., P. L., E. E., F. F. and I. F.) received 200 mg. cortisone/24 hr. and were submitted to a standard operative procedure. The mean daily postoperative output of ketogenic steroid varied, however, from 29.0 to 53.4 mg. It appears that following the commencement of intra-muscular cortisone therapy the absorption from the depot increases to reach a plateau of urinary excretion after many days and the low urinary ketogenic excretion in the preoperative period of patient E. W. may be explained by this factor. In the five patients who received 200 mg./24 hr. the injections were, however, commenced six to 12 days preoperatively. No relationship between ketogenic excretion, weight of patient, daily volume of urine, or general condition was discovered and it is probable that the varying urinary recovery rate of 14.5 to 26.7 mg. of ketogenic steroid per 100 mg. of intramuscular cortisone is an expression of an individual metabolic pattern or varying ability to mobilize cortisone from the intramuscular depot. None of the patients had clinical evidence of hepatic impairment. The ability of patients to excrete large amounts of ketogenic steroid in small urinary volumes has been previously commented upon (Jepson *et al.*[8]). The ketosteroid urinary excretion diminished somewhat in the first 24 to 48 hours postoperatively. The daily eosinophil counts show two distinct patterns. In patients W. F. and I. F. the intra-muscular cortisone suppressed the eosinophil count preoperatively and following the operation low or zero counts continued. In the remaining patients, however, the eosinophil counts remained high, became depressed or were zero following operation and for the succeeding few days, and then climbed to the preoperative level, or in the case of E. W. exhibited a rebound. This phenomenon of eosinophil count rebound, together with the changes in serum sodium and chloride, which followed bilateral adrenalectomy in a patient (not included in balance studies) maintained on constant intramuscular dose of cortisone is shown in Figure 2.

DISCUSSION

The pattern of the metabolic response to operation in terms of electrolyte, fluid and nitrogen balance is now well recognized.[14] In the first postoperative week there is a tendency for sodium and chloride to be retained by the body, and for potassium and nitrogen to be lost, the potassium loss being in excess of that predicted for the breakdown of body tissue. Quantitative balance studies available on "normal" patients[3] undergoing a standard operation indicate, however, that the range of the balance for sodium, potassium, chloride and nitrogen varies greatly from individual to individual. Thus the average postoperative sodium retention following a partial gastrectomy was + 105 mg. while the 95 per cent range was − 189 to + 389 mg. Similar wide variations in "normal" response

were found for nitrogen, chloride and potassium.[3]

These metabolic responses consequent to trauma are influenced by several known factors such as immobilization, anxiety, starvation and hormonal secretion. Endocrine control is known to be excreted by the posterior pituitary anti-diuretic hormone (Lewis[10]), aldosterone (Llaurado[11]) and 17α-hydroxycorticosteroid or hydrocortisone (Sandberg *et al.*[16]). Increased amounts of these steroids have been detected postoperatively in either plasma or urine, and it has been suggested that the nitrogen, electrolyte and carbohydrate response to operation may be modified or determined by the increased secretion of 17α-hydrocorticosterone. There is general agreement that the presence of an adequate amount of this hormone is necessary for the survival of the animal or human following major trauma. Ingle,[7] however, has brought evidence, derived from rat experiments, to suggest that the presence of a "permissive" quantity of 17α-hydrocorticosterone will without any further rise at the time of operation, allow the animal to respond normally to trauma. Jepson *et al.*[8] have studied a patient who failed to respond to a partial gastrectomy and to subsequent corticotrophin stimulation by any rise in urinary 17-ketogenic steroid output while making a smooth recovery to operation and they have suggested that the 17α-hydroxycorticosterone response to operation depends not only on the magnitude of the trauma but on age and the patient's general condition and disease. That a concomitant rise in adrenal secretion of 17-hydroxycorticosterone is not an essential for normal convalescence is further supported by the present series of patients. It is unlikely that significant amounts of hormone could be secreted from the already cortisone-suppressed adrenal between the time of anesthetic induction and the ligation of the adrenal vein. Response to operation has been modified by the general condition of the patients who were suffering from local or widespread carcinomatosis and by the fact that in five of them the study involved the second of two major operations undergone at a short interval. With these reservations the metabolic response to trauma on the part of patients sustained by intra-muscular cortisone seems to fall within the pattern of "normal" response. There may, however, be quantitative differences in response between the "cortisone-supported" and the "adrenal intact" patient, differences which may reflect the absence of hormones such as alderostone which are known to have marked effects on excretion of electrolytes. This similarity of response applies in addition to the eosinophil response, which generally showed a postoperative suppression followed by a rise or "rebound." It is unlikely that these findings can be attributed to variations in plasma 17-hydroxycorticosterone levels as the urinary excretions were, with the exception of E. W., relatively constant. A similar conclusion, that variations in plasma steroid level are not essential for the metabolic response to surgical procedures, was reached by Mason[13] following studies on patients undergoing hypophysectomy or adrenalectomy. We have been unable to find any quantitative correlation between the amount of assayable ketogenic steroid in the urine and the negative nitrogen balance, nor electrolyte and chloride behaviour. The greatest negative nitrogen balance was in the two patients undergoing a "one-stage" bilateral adrenalectomy and oophorectomy; in the remainder of the patients the urinary excretion of nitrogen was less and varied irrespective of the ketogenic steroid excretion.

There are many reasons for believing that although the presence of a quantity of 17α-hydrocorticosterone is necessary for the body to support "stress," variations in the level of steroid are not essential for either the initiation, the maintenance and probably the magnitude of the metabolic re-

sponse as described in terms of nitrogen, eosinophil or electrolyte behaviour.

The mechanism by which these impressive chemical changes are brought about following operation remains undetermined. The only known source of aldosterone is the adrenal glands and the hormone is therefore unlikely to play any part. The post-pituitary anti-diuretic hormone may be responsible, together with the decreased fluid intake and postoperative renal blood flow, for the postoperative oliguria. The means by which the serum sodium and potassium levels and the renal excretion of nitrogen and electrolytes are ordered is not at present clear.

SUMMARY

1. Levels of serum electrolyte, eosinophil behavior, excretion of 17-keto- and of 17-ketogenic urinary steroid have been studied in eight patients undergoing bilateral adrenalectomy for mammary carcinomatosis. In addition to these, balance studies of sodium, potassium, chloride and nitrogen were performed.

2. The postoperative metabolic behaviour of these patients appeared to fall within the normal pattern, nor could any quantitative relationship be found between the magnitude of the metabolic response and the level of steroid excretion.

3. It is concluded that the normal pattern of metabolic response may be initiated, maintained and terminated without any variation in adrenocortical steroid level.

ACKNOWLEDGMENTS

We wish to acknowledge the technical help and advice received from Dr. E. K. Blackburn and the Department of Haematology, The Royal Infirmary, Sheffield.

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APPENDIX

1a. **Patient E. W.** (aged 42). This patient had undergone a radical mastectomy and postoperative radiotherapy four years previously. Twelve months prior to the present admission both ovaries were irradiated and a three months' course of testosterone given. Neither procedure halted the spread of the recurrent carcinoma which at the time of inspection was fungating from the anterior chest wall and invading 'en cuirasse.' Her estimated weight loss was 14 Kg. Cortisone i.m. 50 mg. b.i.d. (100 mg./24 hr.) was commenced 72 hours prior to operation. No other therapy was given, with the exception of small doses of morphine postoperatively. Oral cortisone was substituted at the end of the study. This patient underwent a bilateral adrenalectomy through extra-peritoneal exposures, together with a bilateral oophorectomy at a single operation lasting 125 min. Blood loss was estimated at 400 ml. The adrenal glands together weighed 20 Gm. Their thin cortices were well filled with lipid, the zona fasciculata being narrower than normal, while the glomerulosa showed cellular hypertrophy. The ovaries (right + left = 30 Gm.) were invaded by extensive metastatic deposits. Her postoperative course was uneventful, the blood pressure remaining 100–130 mm. Hg systolic, 70–80 diastolic throughout the operation and postoperative period.

	Intake				Serum				Output				
Day	Vol. (ml.)	Na (mEq./L.)	K (mEq./L.)	Cl (mEq./L.)	N (Gm.)	Na (mEq./L.)	K (mEq./L.)	Cl (mEq./L.)	Alkaline Reserve	Vol. (ml.)	Na (mEq./L.)	K (mEq./L.)	Cl (mEq./L.)	N (Gm.)
−1	2550	51	90	91	20	140	4.5	106		1150	81	46	48	5
Operation														
+1	0	0	0	0	0	140		102		350	15	23	14	1
+2	550	0	0	0	0	138		109		750	88	72	88	1.5
+3	1300	14	0	14	0	138	4.7	97	23	530	25	27	21	4.2
+4	1550	0	0	0	0	135	4.9	97	28	845	8	27	8	11.0
+5	1400	0	0	0	0	137		95		710	20	20	13	9.0
+6														
+7														

1b. **Patient N. G.** (aged 51). This lady was referred from the Orthopedic Department. Nine months previously she had noticed a painless lump in the right breast and retraction of the nipple. This had been neglected until she began with severe continuous lumbar back-ache six months later. This had continued in a more exaggerated fashion so that for a month before admission she was largely compelled to accept absolute bed rest. The lumbar and thoracic vertebrae and pelvis were grossly invaded by carcinomatous tissue and 900 ml. of clear fluid was aspirated from the pleura. Lung films demonstrated lung secondaries. This patient commenced, 96 hr. prior to the operation, a dose of 75 mg. of cortisone i.m. b.i.d. (150 mg./24 hr.). The operation and postoperative course were similar to that of the patient E. W.

	Intake				Serum			Output			
Day	Vol. (ml.)	Na (mEq.)	Cl (mEq.)	N (Gm.)	Na (mEq.)	Cl (mEq.)	Vol. (ml.)	Na (mEq.)	Cl (mEq.)	N (Gm.)
−2	4500	94	140	24	128	192	3600	149	176	103
−1	3000	116	93	15.9	—		2000	133	137	9.1
Operation										
+1	800	0	0	0	125	104	730	21	22	5.6
+2	2500	0	0	0	128	103	1000	39	43	8.5
+3	1500	0	0	0	126	95	1015	32	30	7.8
+4	1650	37	40	6.7	134	94	1160	32	28	12.7
+5	1098	30	30	0	126	93	703	19.0	5.8	11.6
+6	1402	3	3	0	133	95	740	21.5	7.4	14.3
+7										

1c. **Patient W. F.** (aged 32). This patient had a radical mastectomy and postoperative radiation 3 years previously. For the last year, notwithstanding hormone therapy, secondary deposits had continued to develop in the chest wall and very extensively in all the red-marrow bones. On inspection the patient was wasted, with a proptosis of the left eye due to retro-orbital deposit. Her hemoglobin was 40%. At the first operation 14 days later the left adrenal was resected. Cortisone was begun 10 days prior to the second operation, 50 mg. 6 hourly (200 mg./24 hr.) and continued at such a level through the metabolic studies. Her convalescence following the second operation was untoward.

		Intake			Serum			Output		
Day	Vol. (ml.)	Na (mEq.)	Cl (mEq.)	N (Gm.)	Na (mEq.)	Cl (mEq.)	Vol. (ml.)	Na (mEq.)	Cl (mEq.)	N (Gm.)
−2	1635	33	56	8.5			1300	9	11	9.0
−1	1332	21	31	6.0			1120	16	17	8.3
Operation										
+1	715	16	28	4.0	133	100.6	495	17.5	17	4.7
+2	1465	25	40	7.0	137	99	840	3.8	5	11.0
+3	1511	16	28	5.0	120	92	735	1.0	1.0	10.6
+4	1481	24	34	6.3	123	96	1200	1.0	1.0	9.8
+5	1930	23	33	6.0	126	96	1040	3.4	4.0	7.4
+6	1886	4	6	1.1	123	93	1400	11.5	12.0	6.9
+7										

1d. **Patient P. L.** (aged 46). This patient underwent radical mastectomy for a poorly differentiated papillary adeno-carcinoma in 1954. In March 1955 an x-ray of the chest revealed a large secondary deposit at the apex of the left lung together with erosive lesions in the spine and ribs. The pain from the lumbar deposits had largely restricted her to bed for the two months prior to operation. First stage operation: right adrenalectomy and bilateral oophorectomy followed by left adrenalectomy 11 days later. Cortisone started 12 days prior to final operation and continued at 200 mg. i.m./24 hr. throughout the study. No postoperative complications or hypotension.

		Intake				Serum					Output			
Day	Vol. (ml.)	Na (mEq./L.)	K	Cl	N (Gm.)	Na (mEq./L.)	K	Cl	A.R.	Vol. (c.m.m.)	Na (mEq./L.)	K	Cl	N (Gm.)
−5														
−4														
−3	1250	25	40	33	6.6	144	4.2	94		740	27	72	49	10.3
−2	1450	43.5	64	73	7.7	143	5.2	101		930	54	84	85	13.4
−1	1650	86	109	159	8.7				27	1160	104	107	137	14.0
Operation														
+1	950	0	0	0	0	143	4.8	98	28	770	81	89	91	8.1
+2	2500	170	150	272	13.2					730	18	83	47	8.4
+3	2000	110	82	142	10.6	142	4.8	106	26	890	39	91	89	12.9
+4	2000	78	140	170	12.3					1030	69	109	126	12.3
+5	2300	100	148	200	12.3					1200	100	151	197	13.4
+6						142	4.2	97	26					

1e. **Patient E. E.** (aged 55). Radical mastectomy and radiotherapy (1950) for mammary carcinoma. Four years later there were nodules over the front of the chest which proved resistant to further radiotherapy and oral testosterone. Had lost approximately 10 Kg. of weight in past year. First stage operation: right adrenalectomy and bilateral oophorectomy, followed by left adrenalectomy 14 days later. Cortisone 200 mg. i.m./24 hr. started 7 days prior to final operation and continued throughout the study. Untoward convalescence.

Day	Intake Vol. (ml.)	Intake Na (mEq./L.)	Intake K (mEq./L.)	Intake Cl (mEq./L.)	Intake N (Gm.)	Serum Na (mEq./L.)	Serum K (mEq./L.)	Serum Cl (mEq./L.)	Serum A.R. (mEq./L.)	Eos. (per c.m.m.)	Output Vol. (ml.)	Output Na (mEq./L.)	Output K (mEq./L.)	Output Cl (mEq./L.)	Output N (Gm.)
−5															
−4															
−3	1671	62	88	97	8.5						1560	34.5	61	57	9.9
−2	1500	66	93	115	8.0						1990	141	104	187	12.4
−1	1500	66	93	111	8.0	143	4.5	101			1460	110	92	137	9.8
Operation															
+1	200	9	12	13	1.1						None passed	—	—	—	—
+2	535	17	31	29	2.7	144	3.8	101	27		790	48	96.5	57	9.6
+3	1170	33	50	85	5.8					20	880	2.9	95	9	10.8
+4	1500	63	93	104	8.0						590	1.9	71	8	14.0
+5	1500	123	132	129	8.0	150	6.1	110		0	860	42	196	118	15.9
+6	2000	76	116	122	10.6	147	3.9	110	27	50	720	49	144	103	13.6
+7	2000	73	108	122	10.6					5	580	36	100	68	11.1
+8						145	4.0	104	24	30					

1f. **Patient F. F.** (aged 30). Carcinoma of breast developed during pregnancy two years previously. Removed locally on two occasions. Had again recurred locally but no evidence of widespread deposits. First stage operation: right adrenalectomy and bilateral oophorectomy, followed by left adrenalectomy 18 days later. Cortisone 200 mg./24 hr. i.m. begun 10 days prior to second operation and continued throughout study. Smooth convalescence.

Day	Intake Vol. (ml.)	Intake Na (mEq./L.)	Intake K (mEq./L.)	Intake Cl (mEq./L.)	Intake N (Gm.)	Serum Na (mEq./L.)	Serum K (mEq./L.)	Serum Cl (mEq./L.)	Serum A.R. (mEq./L.)	Output Vol. (ml.)	Output Na (mEq./L.)	Output K (mEq./L.)	Output Cl (mEq./L.)	Output N (Gm.)
−3														
−2	2000	100	104	116	10.6	140	4.7	102	25	2100	156	111	172	11.2
−1	2000	104	98	94	10.5	155	6.7	107		1220	71.5	98	119	8.2
Operation														
+1	250	7	—	7	—	145	6.5	98		530	50	54	64	5.5
+2	1200	70	69	71	6.4	142	5.2	93	25	560	35	127	56	9.6
+3	1500	87	90	106	7.5	141	5.5	97		560	51	67	60	10.8
+4	2000	110	118	140	10.0	141	5.8	92	26	740	52	77	68	11.5
+5	2000	78	86	83	11.0	140	5.1	98		1530	86	119	118	12.1
+6	2000	86	102	114	11.0	143	5.5	94	24	1020	77	104	103	8.3
+7						136	5.0	95		1190				
+8										1520				

1g. **Patient I. F.** (aged 58). This patient underwent radical mastectomy for carcinoma of the right breast in 1950. Developed secondary deposits in lumbar vertebrae, for which she received x-ray therapy. 1955 large axillary mass caused swollen right arm. Did not respond to oral stilbestrol. First operation: right adrenalectomy and bilateral oophorectomy, followed by left adrenalectomy 12 days later. Cortisone commenced 6 days prior to second operation 200 mg./24 hr. i.m. and continued throughout the study. Convalescence uninterrupted.

Day	Intake Vol. (ml.)	Intake Na (mEq./L.)	Intake K (mEq./L.)	Intake Cl (mEq./L.)	Intake N (Gm.)	Serum Na (mEq./L.)	Serum K (mEq./L.)	Serum Cl (mEq./L.)	Serum A.R. (mEq./L.)	Eos. (per c.m.m.)	Output Vol. (ml.)	Output Na (mEq./L.)	Output K (mEq./L.)	Output Cl (mEq./L.)	Output N (Gm.)
−2	1040	28	52	58	5.5	145	4.2	96	29	1	1290	108	43	107	8.3
−1	600	23	38	45	2.0	148	4.5	96		1	520	63	16	63	3.9
Operation															
	(i.v.)														
+1	2890	40	0	40	0	144	4.2	93	24	1	1790	134	68	158	7.4
+2	466	0	0	0	0					1	930	46	71	62	7.7
+3	1500	52	84	100	7.9	141	3.9	98	24		940	25	62	40	11.2
+4	1500	55	85	107	8.0					1	630	43	47	48	11.0
+5	1500	59	85	135	7.9					5	950	115	82	146	13.1
+6	1038	38	59	72	5.5	146	5.3	98		1	980	76	86	113	12.4
+7										10					
+8										0					

Metabolic Response to Trauma and Operation

G. M. WILSON *(Sheffield)*

The infliction of an injury sets in motion a characteristic train of metabolic events which are designed to provide the energy and raw materials necessary for repair of the damage. These changes were first fully described by Cuthbertson (1932), who drew attention to their significance and importance. Subsequently, Howard (1945) and Moore (1953), among many others, have made notable contributions in the study of the metabolic response to injury. My own interest was stimulated by working with Dr. Moore in Boston and later work was continued in conjunction with Professor Jepson in Sheffield.

The investigation of the metabolic disturbances caused by injury in man is often complicated. The nature and severity of the injury, the development of infection, changes in the diet, and the administration of analgesic drugs and anaesthetics are all variable factors making interpretation difficult.

The pattern of response is best studied in a healthy adult who receives a severe injury or an extensive surgical operation. Figure 1 is taken from Howard's (1945) work. The characteristic feature

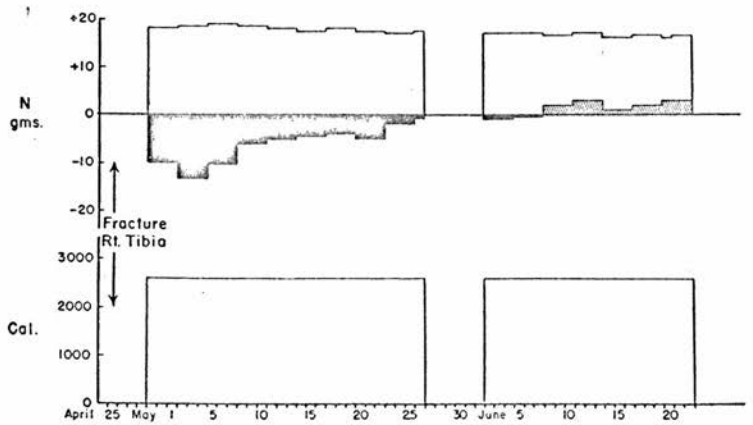

Figure 1 Nitrogen balance following a fracture. The N intake is plotted upwards from the zero line and the excretion downwards from the intake. The area under the zero line represents negative nitrogen balance and the cross-hatched area above, positive balance.

of the reaction to trauma, in this case the fracture of a long bone, is that the rate of nitrogen excretion is greatly increased. This excess nitrogen excretion arises from the rapid catabolism of protein, probably chiefly muscular tissue. The negative nitrogen balance during this stage cannot be prevented by the administration of large amounts of protein as this only adds further to the urinary excretion of nitrogen. The duration and extent of the catabolic phase is related to the nutrition of the individual, the severity of the injury, and the length of the healing process. It is most conspicuous in the well-nourished, but this reaction is much less noticeable in those who have lost weight as a result of malnutrition or disease (Moore and Ball, 1952). The more severe the injury, the greater is the nitrogen loss. The catabolic phase is particularly striking after fractures and burns, in which much new tissue has to be formed, and may be prolonged for three or four weeks. The patient then passes into an anabolic phase, the nitrogen balance becomes positive and the lost lean tissue is restored. At this stage it is most important that the patient should receive a high calorie diet with abundant protein, which is now readily retained in the body.

In many cases the intake of food is interrupted as a result of operation or injury. The effects of relative starvation and of injury are then superimposed. If a healthy adult suddenly stops eating, urinary excretion of nitrogen continues, at about the rate of 10 g. a day (Moore and Ball, 1952). As intake is resumed he quickly passes into positive balance. The effects of starvation alone and of a surgical operation involving soft tissues and followed by relative starvation are shown in Figures 2 and 3. After the operation the rate of nitrogen excretion is much increased and is greater than can be accounted for by starvation alone. It will be noted in Figure 3 that the duration of protein catabolism after the extensive soft tissue injury is only a few days in contrast with the prolonged period seen after the fracture.

Other changes are associated with the protein catabolism and some are illustrated in Figure 3. There is rapid oxidation of fat which contributes to the fall in body weight characteristically seen after injury. There is an increased excretion of phosphorus and sulphur consistent with the breakdown of muscular tissue. Potassium is also excreted in excess; this occurs promptly after injury and does not persist for so long as the increased nitrogen excretion. In contrast, there is usually retention of sodium. Urinary volume is decreased and water is retained in the body (Le Quesne and Lewis, 1953; Wynn and Rob, 1954; Wynn, 1956).

Changes in plasma electrolytes commonly accompany this metabolic response. There is a fall in the concentration of sodium and chloride, which occurs within the first 24 hours after operation

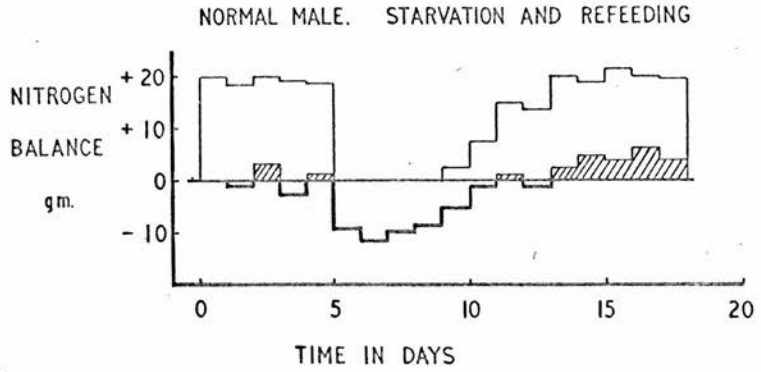

Figure 2 Nitrogen balance following withdrawal of food and gradual refeeding, as might occur following a surgical operation. The negative nitrogen balance during the period of starvation is approximately 10 g. a day.

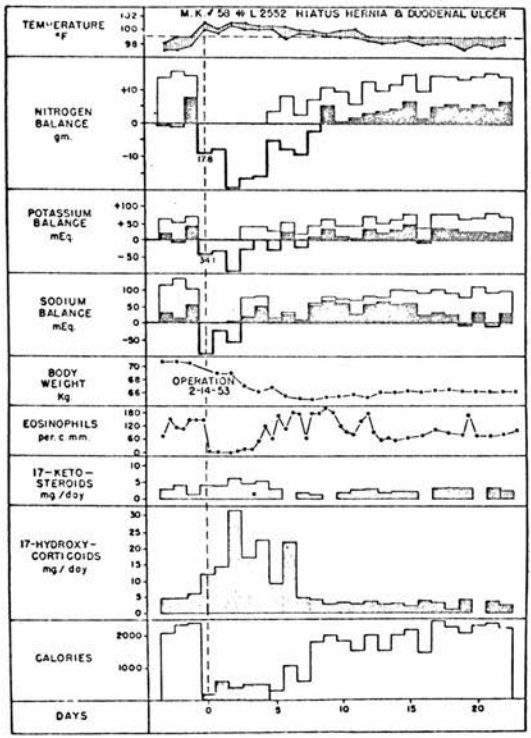

Figure 3 Balance chart from a patient undergoing repair of hiatus hernia and gastroenterostomy and vagotomy. The negative nitrogen balance after operation is greater than seen with starvation (cf. Figure 2). Note also the negative potassium balance, the loss of weight and enhanced secretion of 17-hydroxycorticoids. The transient negative sodium balance was largely due to wound exudate as urinary excretion was slight.

(Figure 4). These decreases are not associated with a loss of these electrolytes from the body: indeed, they occur at a time when sodium and chloride are being retained in the body. On the other hand, the plasma potassium concentration either remains steady or actually rises, even though there is an increased potassium excretion in the urine. These changes are particularly conspicuous in

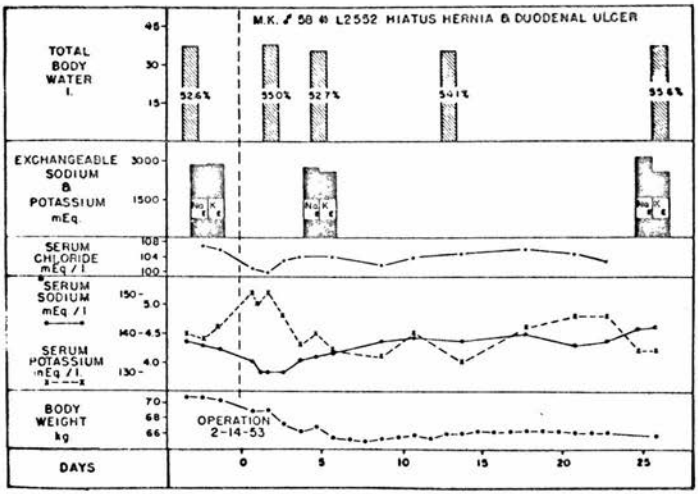

Figure 4 Water and electrolyte changes following operation. The serum sodium and chloride levels and exchangeable potassium fall after operation. For the first few days after operation the total body water remains constant but the weight drops and thus the percentage of water in the body rises.

patients undergoing cardiac operations such as mitral valvotomy (Figure 5) (Wilson et al., 1954). The decrease in plasma sodium concentration is related to the loss of intracellular electrolytes, particularly potassium, and to water retention (Edelman, 1956). Wynn (1957) has pointed out that these changes preserve the equilibrium between intra- and extracellular osmolarity.

These are but a few of the many metabolic reactions to injury and time does not permit reference to any others in detail. It should be mentioned that changes occur in the plasma proteins (Peters, 1948). The serum albumin falls precipitately after operation and this rapid response cannot be attributed to general depletion of protein. The plasma iron concentration also falls abruptly after injury (Feldthusen et al., 1953; Baird et al., 1957). Similarly, this change is not due to bleeding or external loss of iron.

The mechanisms by which these characteristic reactions are initiated and sustained have been widely discussed. Trauma certainly leads to an increased secretion of adrenal cortical hormones. Elevated plasma concentration of hydroxycorticoids and increased urinary excretion of adrenal steroids have been reported by many

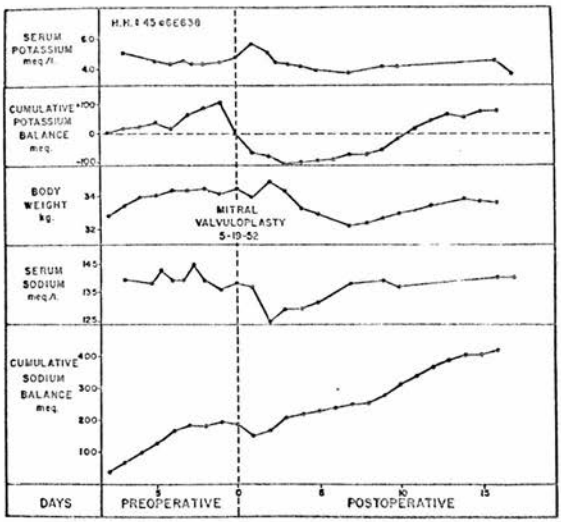

Figure 5 Sodium and potassium metabolism in association with an operation on the mitral valve. The cumulative balance shows steady retention of sodium but nevertheless a drop in serum sodium concentration occurs. The body weight does not drop promptly after operation and this indicates water retention. There is a considerable potassium deficit during the period of hyponatraemia.

workers (Franksson et al., 1954; Sandberg et al., 1954; Jepson, Jordan and Levell, 1956). There is also an increased excretion of aldosterone (Llaurado, 1955) and of antidiuretic hormone (Eisen and Lewis, 1954). It is thus tempting to relate the metabolic changes to these alterations in hormone secretion, particularly as they are consistent with many of the known actions of these hormones. However, certain difficulties arise. In severe injuries, particularly fractures of the long bones, the period of protein catabolism may exceed considerably the duration of increased adrenocortical activity as assessed by steroid excretion in the urine (Figure 6) (Moore et al., 1955). In other patients an entirely normal metabolic reaction mayoccasionally follow a major surgical operation or injury without any evidence of increased adrenocortical secretion, even though it is subsequently demonstrated that the patient has adrenal glands that respond fully to A.C.T.H. stimulation (Figure 7) (Jepson, Edwards and Reece, 1956). Metabolic changes associated with total adrenalectomy or hypophysectomy have been studied by several groups (Mason, 1955; Robson et al., 1956; Jepson et al., 1957); it is agreed that even though the supply of hormone is kept constant throughout the period of study, before, during and after operation, a normal response develops. However, even if large doses of hormone are given before operation, it is not certain that some additional secretion has not been supplied from the glands in the period between induction of anaesthesia and removal of the adrenals or

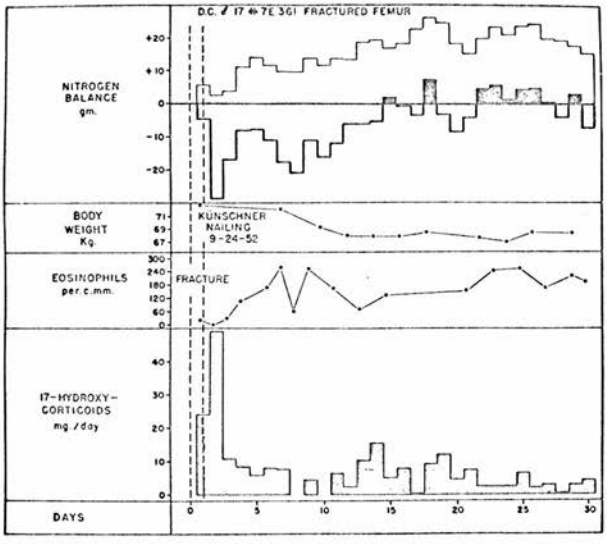

Figure 6 Nitrogen balance and 17-hydroxycorticoid excretion after fracture of shaft of femur and nailing. There is a high excretion of corticoids after injury and operation but this does not persist throughout the catabolic phase.

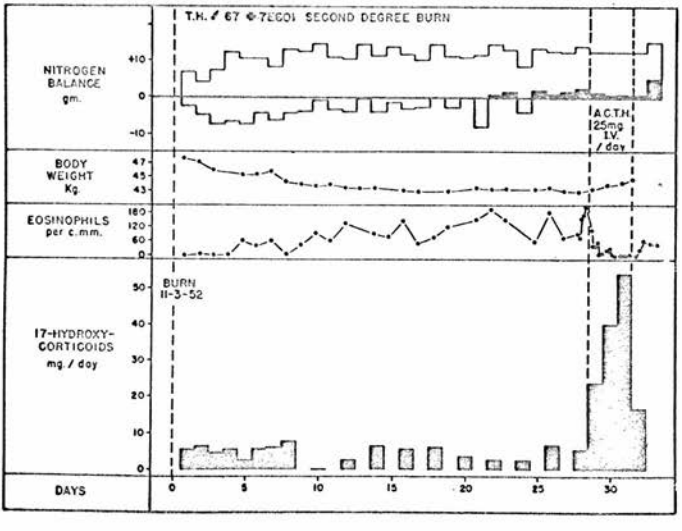

Figure 7 The 17-hydroxycorticoid excretion in the urine is not increased after an extensive burn which gave rise to enhanced nitrogen excretion for a prolonged period. The adrenal glands subsequently responded adequately to A.C.T.H. stimulation.

pituitary, though measurements of urinary excretion of both hydroxycorticoids and aldosterone make this seem unlikely (Dudley et al., 1957). This difficulty does not arise in patients with completely defective adrenals undergoing surgery. Recently in Sheffield we had the opportunity of studying the metabolic response to a major operation of a patient with Addison's disease. He presented the typical clinical features of this disease; his basal level of ketogenic steroid excretion in the urine was negligible and there was no response to A.C.T.H. stimulation. After four years on maintenance therapy, a tuberculous cavitating lesion developed in the right upper pulmonary lobe, which was removed. A constant dose of cortisone and fludrocortisone was given throughout the period of study. A normal reaction to operation ensued (Figure 8). These

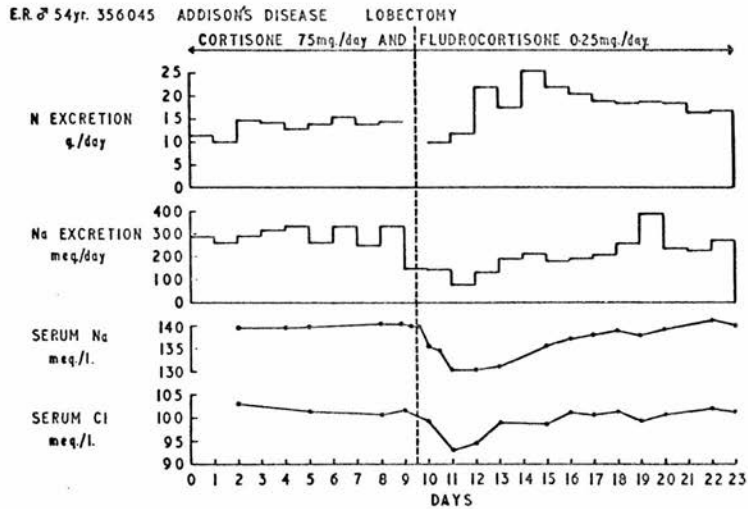

Figure 8 Metabolic changes following excision of a tuberculous right upper pulmonary lobe in a patient with Addison's disease maintained on a constant dose of cortisone and fludrocortisone. The serum sodium and chloride concentrations and urinary sodium excretion fell after operation. The characteristic rise in nitrogen excretion developed.

observations in man are in agreement with Ingle's (1952) animal experiments and confirm that a variable secretory response or supply of hormone is not required to initiate or maintain the metabolic response to injury. However, a constant supply does not necessarily mean that the blood and tissue concentrations of cortical hormones remain unchanged. The mechanism whereby the metabolic response is initiated and controlled is still uncertain.

Attention has mainly been directed to the physiology of the healing process and no attempt has been made to recount the numerous abnormalities that may be superimposed on this physiological response. An understanding of the general reaction to injury and

of the metabolic aspects of recovery is clearly of fundamental importance in the clinical care of patients. The gaps in our knowledge are still large but even in these days of the rapid development of specific remedies the metabolic reaction of the patient as a whole is perhaps still worthy of the most detailed study.

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Reprinted from METABOLISM
Vol. VII, No. 2, March, 1958
Printed in U.S.A.

Exchangeable Potassium and Sodium in Hyperthyroidism and Hypothyroidism

By D. S. MUNRO, H. RENSCHLER AND G. M. WILSON

IN 1934, Byrom showed that the administration of thyroxine to normal subjects increased the urinary excretion of potassium, though in patients suffering from myxedema a diuresis with an increased urinary loss of sodium resulted.[1] There are several reports of abnormalities of sodium metabolism in myxedema. Profound hyponatremia has been described in primary myxedema,[2] and the treatment of hypothyroidism is associated with a temporary expansion of the extracellular fluid volume followed by an increased urinary loss of both sodium and chloride.[3, 4] These studies were made chiefly with metabolic balance technics and were necessarily confined to a relatively short period after beginning treatment. However, clinical observation suggests that the response to the treatment of thyroid disorders develops gradually over a period of many weeks. The isotope dilution method of measuring body composition affords an opportunity of studying changes over long intervals,[5] and it is clearly advantageous in investigating slowly developing alterations. In view of the evidence that thyroxine may influence sodium and potassium metabolism both in the subcutaneous tissue and in the cells[6-8] the body content of these electrolytes has been investigated in hyper- and hypothyroidism. As both conditions are reversible, serial measurements have been made in patients until approximately normal thyroid balance was restored.

METHODS

Twenty-five patients with hyperthyroidism and fourteen with myxedema have been studied. In all, the clinical diagnosis was not in doubt and was confirmed by laboratory tests which included measurements of basal metabolic rate, serum cholesterol concentration and sleeping pulse rate. In addition, radioactive iodine tracer studies were carried out in the majority using the technics previously described from this department.[9] These methods were also used, where applicable, to follow the response to treatment. In the hyperthyroid group 18 were treated with I^{131}, four with antithyroid drugs and three by operation. The hypothyroid patients were given *l*-thyroxine sodium in doses between 0.1 and 0.3 mg. daily.

Exchangeable sodium and exchangeable potassium were either measured separately or in combination. All the observations in an individual were made by the same technic. The isotopes Na^{24} and K^{42} were prepared in a nuclear reactor from spectroscopically pure sodium and potassium carbonate. They were dissolved in 0.9 per cent sodium chloride and the solutions were sterilized by boiling. Between 50 and 70 microcuries of each isotope were administered intravenously. In most cases the fluid was delivered from a sterile 50 ml. burette into an intravenous infusion funnel, but in a few of the earlier cases the micrometer syringe technic of Miller and Wilson was used.[10] Standard solutions were prepared in

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We should like to thank Dr. S. Oleesky for assistance with some of the initial measurements, Dr. H. Miller and Dr. G. H. Jowett for help and advice, Mr. D. Gow for technical assistance, and the Endowment Fund of the United Sheffield Hospitals for a grant for the purchase of isotopes.

Received for publication April 30, 1957.

triplicate and counted with the samples of body fluids in liquid counters.[11] Every third count was a standard, and in all samples counting was continued until 6,000 counts were recorded or until 20 minutes had elapsed. The minimum counting time was three minutes and all samples were counted twice. All the urine passed after administering the isotopes was collected, and the bladder was emptied exactly 24 hours after the injection to complete the collection. Thereafter the specific activity of sodium was estimated in serum samples, and that of potassium was estimated in urine samples.

In the measurement of exchangeable sodium, two 30 ml. blood samples were withdrawn and the serum separated by centrifuging, and in the measurement of exchangeable potassium four "spot" urine samples were collected at consecutive intervals of 45 minutes. In the simultaneous measurement of exchangeable sodium and exchangeable potassium, two urine samples were collected at 45 minute intervals and a blood sample withdrawn at the midpoint of each period. In this combined technic all samples contained both Na^{24} and K^{42}. In the 0 to 24 hour urine collection the relative contributions of the two isotopes were calculated[12] by counting the urine in two liquid counters, one of standard wall thickness (30 mg./cm.^{2}) and the other with the counter wall thickness increased to 300 mg./cm.^{2} As the radiations from the two isotopes differ greatly in their energies, the relative counting rates of each under these different conditions also vary. By counting the 0 to 24 hour urine and the standard solutions in both counters, two simultaneous equations were derived to give the proportions of the two isotopes in the mixture. This principle has frequently been applied to end-window counting, where absorbers of varying thickness can be interposed between the sample and the G.M. counting tube.[13] To estimate the potassium specific activity in the urine samples, a chemical method of separating potassium by a double precipitation, first with sodium cobaltinitrite and then with alcoholic tartaric acid, has been used. This is essentially the same method as applied by James and others[14] to serum samples. Urine has been chosen because its potassium content is almost invariably higher than serum, and larger volumes are readily available. After obtaining the potassium specific activity in this way, the appropriate deduction is then made from the serum counting rate. In all these three methods the final calculations are similar.

The exchangeable sodium is calculated from the formula:

$$Na_e = \frac{\text{Radiosodium injected} - \text{radiosodium excreted}}{\text{Serum sodium specific activity}}$$

and, similarly, the exchangeable potassium:

$$K_e = \frac{\text{Radiopotassium injected} - \text{radiopotassium excreted}}{\text{Urinary potassium specific activity}}.$$

Measurements of the concentration of serum sodium and potassium and of urine potassium were made by flame photometry. The patient was weighed at the time of each isotope dilution measurement.

Results

A total of 181 observations has been made. All the patients were studied at least once before treatment and thereafter at intervals during the response to therapy.

Hyperthyroidism

The measurements of exchangeable potassium made before and after successful treatment are shown in table 1. Among the sixteen patients studied, only two showed a decrease in this value. In the remainder there was a rise and in the majority this was substantial. In all these patients there was a gain in body weight. The mean increase in potassium in relation to body weight was 19 mEq. per Kg. The serum potassium concentration showed no consistent or significant change.

TABLE 1.—*Exchangeable Potassium and Sodium in Hyperthyroidism*

Patient no.	Age (yr.)	Sex	Before treatment					After treatment				
			Weight (Kg.)	Exchangeable potassium (mEq.)	Serum potassium (mEq/L.)	Exchangeable sodium (mEq.)	Serum sodium (mEq./L.)	Maximum weight (Kg.)	Exchangeable potassium (mEq.)	Serum potassium (mEq./L.)	Exchangeable sodium (mEq.)	Serum sodium (mEq./L.)
1	24	F	57.7	2400	4.0	2495	140	63.0	2802	4.0	2745	141
2	32	F	67.2	2987	4.5	3115	146	68.5	3352	3.7	3009	139
3	35	F	62.6	2469	4.2	2426	142	68.4	2337	4.7	2762	139
4	38	F	57.2	2071	3.8	2423	140	65.4	2386	3.4	2702	141
5	41	F	51.8	1978	4.3	2555	145	55.1	2181	3.9	2196	139
6	42	F	54.1	1895	3.7	2318	140	59.1	2162	3.9	2470	142
7	44	F	63.5	1890	4.0	2645	142	72.7	2305	3.9	2638	137
8	52	F	44.4	1500	4.3	2040	140	49.4	1816	4.4	2259	142
9	53	F	53.8	2015	4.6	1936	144	55.8	1885	4.5	1927	142
10	55	F	48.8	1894	4.1	2064	143	52.2	1907	4.1	2095	143
11	56	F	52.1	1960	5.0	2098	143	53.0	2018	4.0	2116	140
12	59	F	41.9	1500	3.9	1895	140	58.4	1825	4.0	1788	141
13	66	F	50.4	1697	4.5	2414	147	64.3	2176	3.8	2316	145
14	67	F	55.9	1835	4.4	2452	141	62.2	1906	3.8	2389	140
15	45	F	50.2	2041	4.1	—	—	59.2	2274	4.4	—	—
16	51	F	50.3	1720	—	—	—	55.6	1866	4.4	—	—
17	23	F	36.6	—	—	1966	143	48.6	—	—	1900	141
18	26	F	65.9	—	—	2535	147	71.3	—	—	2737	139
19	26	M	78.9	—	—	3422	142	97.3	—	—	3420	141
20	27	F	57.2	—	—	2602	138	67.2	—	—	2469	142
21	32	F	55.4	—	—	2294	142	65.8	—	—	2195	143
22	38	F	51.2	—	—	2482	140	54.1	—	—	2395	139
23	45	F	47.3	—	—	2258	140	59.1	—	—	2484	144
24	50	F	39.4	—	—	1766	131	50.6	—	—	1920	138
25	53	F	42.0	—	—	1783	136	53.1	—	—	2072	141

The alterations in exchangeable sodium are also shown in table 1. These were extremely variable and no consistent pattern emerged. They were not related to the age of the patient or the cardiac state. None of the patients had congestive failure before treatment, and the loss of sodium observed in some patients was not caused by loss of any demonstrable edema fluid. The changes in exchangeable sodium were not related to the extent of gain in potassium following treatment (fig. 1). There were no consistent changes in serum sodium concentration.

In fourteen of the patients with hyperthyroidism, simultaneous measurements of exchangeable sodium and potassium were made before treatment and at intervals throughout the period of recovery. The Na_e/K_e ratio has been calculated and the serial changes are shown in figure 2. All these patients were females and in this sex the normal Na_e/K_e ratio is 1.02.[15] In ten of the thyrotoxic cases this value was definitely exceeded but fell in the majority with the passage of time after treatment.

Hypothyroidism

Measurements of exchangeable potassium have been made in six patients who all presented definite features of hypothyroidism (table 2). In five, a loss of potassium occurred after treatment with thyroxine. There were no definite changes in

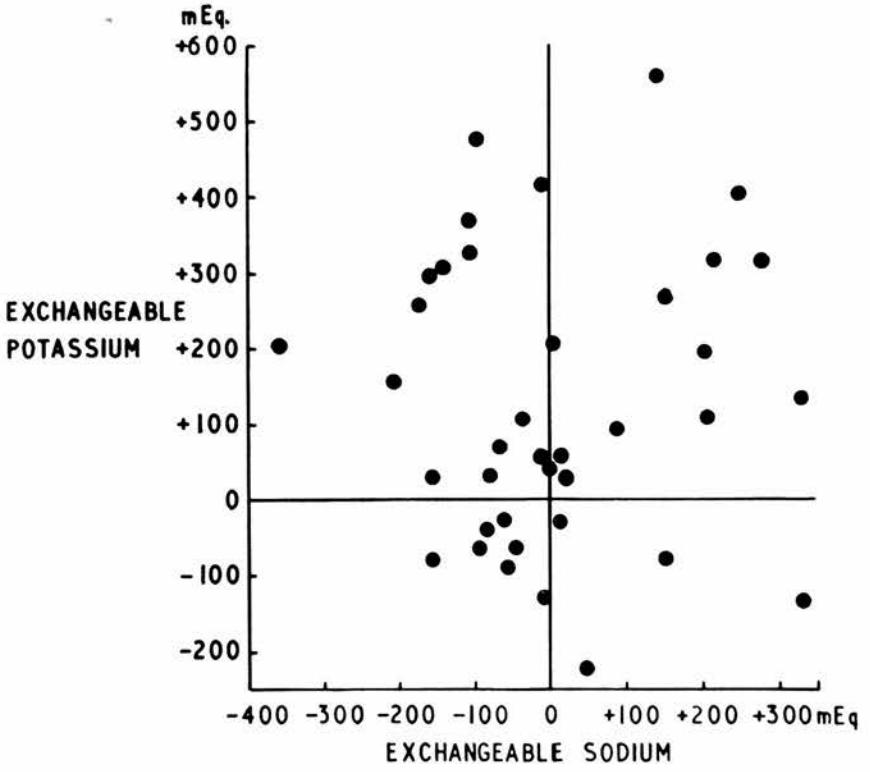

FIG. 1.—Scatter diagram showing absence of correlation between alterations in exchangeable potassium and sodium in hyperthyroidism. The measurements were made before treatment and during recovery and show the differences from the values obtained when the patients were euthyroid.

serum potassium. The alterations in exchangeable sodium have been studied in 12 patients (table 2). All showed a loss of sodium after treatment. In two patients (36 and 38) the serum sodium was depressed below the normal range before treatment but subsequently rose. This increase in serum concentration occurred in spite of a diminution in the exchangeable sodium content of the body.

Weight Changes in Relation to Thyroid Function and Exchangeable Cation

In this series of studies the hyperthyroid patients gained weight as thyroid function returned to normal, and the hypothyroid patients lost weight when thyroxine was given. The alterations in exchangeable cation in both groups of patients have been examined in relation to those weight changes throughout the period of response to treatment. In the hyperthyroid patients the maximum weight has been regarded as the normal, and its attainment usually coincided with complete recovery. The measurements of weight and exchangeable cation made in each individual during the period of recovery have been calculated retrospectively as changes from the values in health. In the patients with hypothyroidism the lowest weight has been taken as the normal value and the changes produced by the disease have been calculated from this reference point.

A single scatter diagram has been constructed in this way showing all the

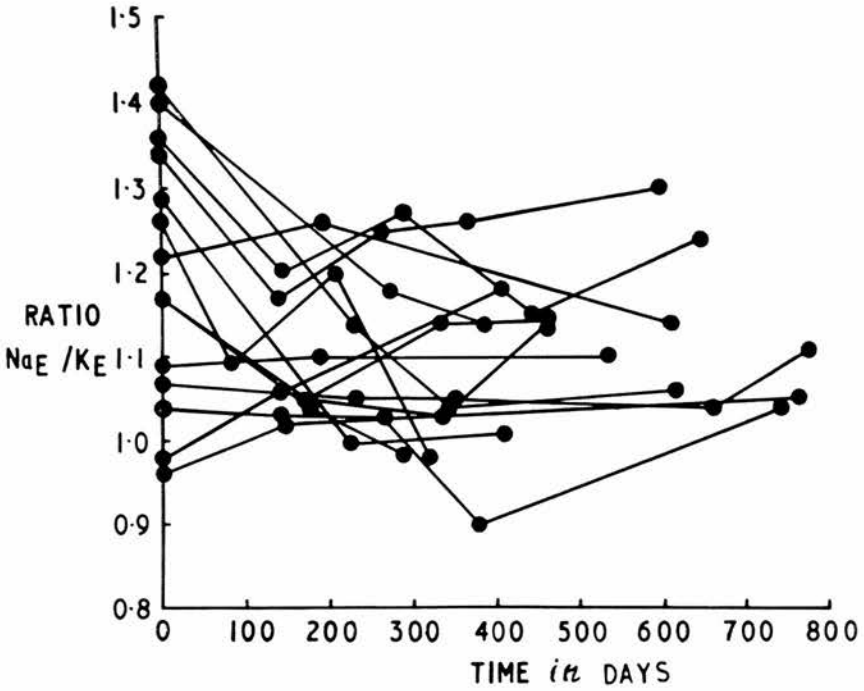

FIG. 2.—Alterations in the Na_e/K_e ratio following treatment of hyperthyroidism.

measurements of exchangeable potassium made throughout the whole range of thyroid function (fig. 3). The slopes of the two regression lines fitted to the hyper- and hypothyroid patients were significant (respectively, $0.01 > P > 0.001$ and $P < 0.001$), and there was no difference between them. The changes in exchange-

TABLE 2.—*Exchangeable Potassium and Exchangeable Sodium in Hypothyroidism*

Patient no.	Age (yr.)	Sex	Before treatment					After treatment				
			Weight (Kg.)	Exchangeable potassium (mEq.)	Serum potassium (mEq./L.)	Exchangeable sodium (mEq.)	Serum sodium (mEq./L.)	Minimum weight (Kg.)	Exchangeable potassium (mEq.)	Serum potassium (mEq./L.)	Exchangeable sodium (mEq.)	Serum sodium (mEq./L.)
26	50	F	74.0	2192	4.1	2806	141	65.2	2259	4.0	2321	140
27	54	F	54.7	2107	3.7	2372	136	52.3	1764	4.3	2058	141
28	59	F	83.0	2510	4.6	3330	141	60.5	2097	4.4	2856	144
29	69	F	66.0	2265	3.9	2590	144	63.5	2188	4.5	2421	142
30	35	F	57.5	2277	—	—	—	53.2	2191	3.7	—	—
31	62	F	74.3	2299	—	—	—	63.8	2075	4.6	—	—
32	26	F	80.8	—	—	3002	142	77.6	—	—	2798	144
33	36	F	74.7	—	—	2624	139	69.3	—	—	2574	—
34	42	F	46.7	—	—	2322	144	40.5	—	—	2110	142
35	49	F	54.5	—	—	2670	143	47.8	—	—	2036	139
36	54	F	73.0	—	—	2475	131	61.4	—	—	2403	141
37	56	F	67.5	—	—	2624	140	60.1	—	—	2336	142
38	59	F	55.9	—	—	2122	134	46.4	—	—	1966	143
39	68	F	67.6	—	—	2802	142	60.0	—	—	2609	142

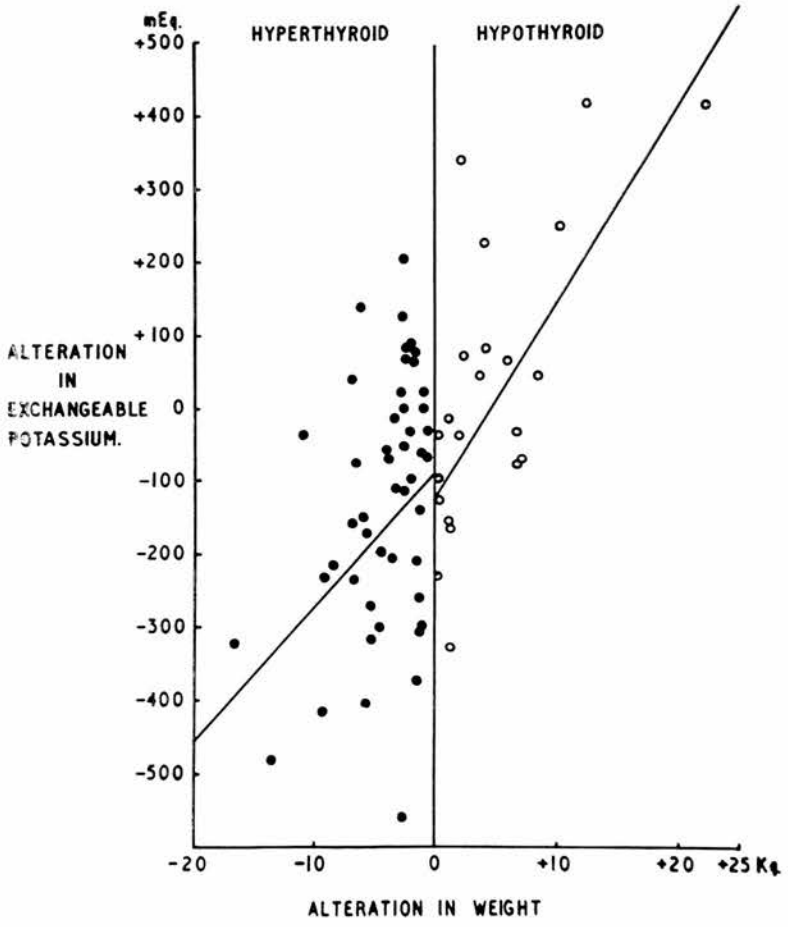

FIG. 3.—The alterations in exchangeable potassium in hyperthyroidism and hypothyroidism in relation to weight changes following treatment. Regression lines have been fitted to both sets of results.

able sodium have also been plotted in the same way (fig. 4). However, in the sodium measurements a significant regression line could be fitted only to the obserservations made in the hypothyroid patients ($P < 0.001$).

DISCUSSION

Two reports of exchangeable sodium and potassium in thyroid disease have already been published. Aikawa[16] confined his study to myxedema. In six patients in whom the exchangeable sodium was measured, four exceeded the mean normal value for his laboratory and in two of the three patients a single repeat measurement showed a decrease in exchangeable sodium. In his method of measurement, a single blood sample was withdrawn only three hours after injecting Na^{24}, though Miller and Wilson[10] showed that this interval was too short. In four out of eight patients the serum sodium level was below 135 mEq. per liter. Aikawa also measured the exchangeable potassium in four further cases and,

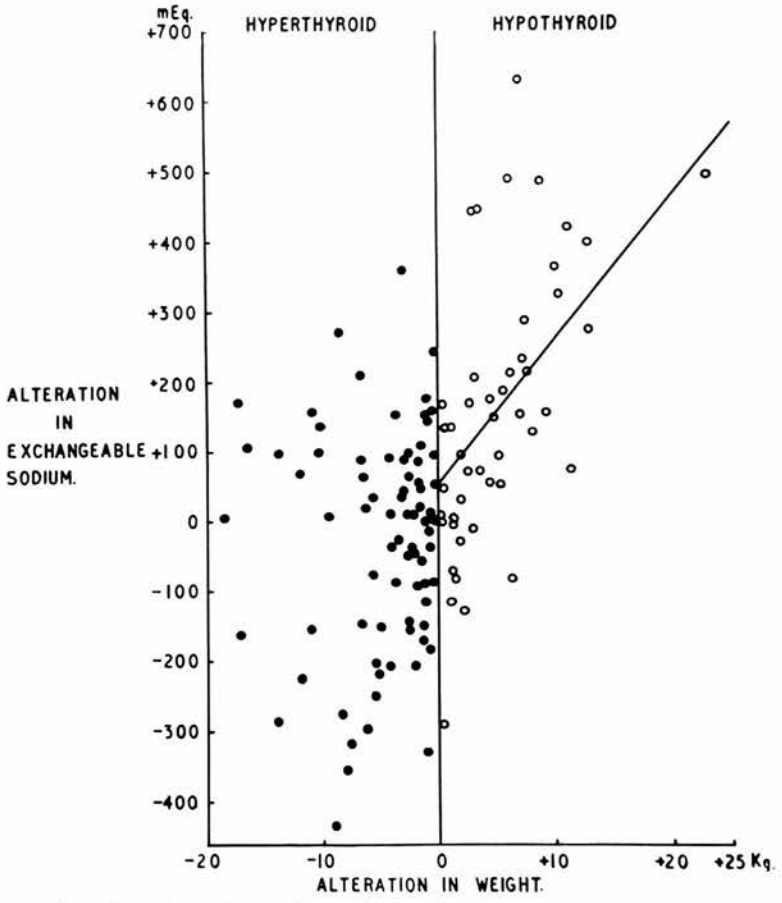

FIG. 4.—The alterations in exchangeable sodium in hyperthyroidism and hypothyroidism in relation to weight changes following treatment. The slope of the regression line is significant only in the hypothyroid patients.

though all were within his normal range, when repeated after treatment with thyroid extract, three cases showed lower values.

Arons, Vanderlinde and Nusimovich[17] made simultaneous measurements of exchangeable sodium and exchangeable potassium in nine cases of hypothyroidism and found that the exchangeable potassium was low in five. In two cases, treatment with thyroid extract was followed by a temporary decrease in exchangeable potassium, but no change occurred in the others. No consistent change was noted in exchangeable sodium. They extended their study to four patients with hyperthyroidism; two of these patients showed an increase in exchangeable potassium during the first two months after treatment. In the other two, who were treated with I^{131}, little change was demonstrated in two months.

The information which we have obtained in the study of hypothyroidism agrees with the report of Aikawa.[16] We have not found the exchangeable potassium to be low in hypothyroid patients, as observed in five of the nine patients of Arons, Vanderlinde and Nusimovich.[17] Our method of making a series of

measurements and following each patient from the diseased state back to normal has obvious advantages over isolated or paired observations. The range of normal values is wide,[18] and in any condition in which weight has been altered it is of little value to compare the values as mEq. per Kg. of body weight with the range in health.

In hypothyroid patients there is an increased amount of metachromatic mucoprotein in collagenous fibres, which disappears after treatment.[7] An increase in mucopolysaccharide in connective tissue in animals is associated with a rise in sodium and potassium content.[8] The decrease in the body content of both cations observed on treatment of hypothyroidism may thus be due, at least in part, to a loss of myxedematous tissue.

The loss of weight in thyrotoxicosis may be due to loss of body fat or lean tissue. Release of cell nitrogen from the body is inevitably accompanied by negative balances of potassium.[19] Therefore, if weight loss in thyrotoxicosis is due to a loss of lean tissue, the exchangeable potassium would fall. Though this has in fact been found in our subjects, the mean loss of 19 mEq. per Kg. of body weight is far lower than would be found if the decline in weight had been solely owing to loss of lean tissue. It is probable, therefore, that loss of both fat and lean tissue occur in thyrotoxicosis.

The alterations in exchangeable sodium in thyrotoxicosis do not follow any clear pattern, though Cachera and others[20] found that extracellular fluid volume was increased in thyrotoxicosis and that it decreased after successful treatment. We have not been able to demonstrate consistently any increase in exchangeable sodium in thyrotoxicosis, nor any decrease with treatment. Though this sequence was seen in some patients, others showed the opposite trend. No satisfactory explanation can be offered for the wide variation.

It is important to remember that the isotope dilution method measures not only the sodium in the extracellular fluid but also includes a considerable fraction of the large quantity of sodium in the bone.[10] Hyperthyroidism may cause decalcification of bone[21] but nothing is known of the effect of excess of thyroxine on bone sodium content. This clearly may be a factor of importance in the interpretation of these measurements.

Summary

Exchangeable potassium and sodium have been measured with K^{42} and Na^{24} in cases of hyperthyroidism and hypothyroidism before and after treatment.

In untreated hyperthyroidism the proportion of sodium to potassium in the body was low. This ratio rose after treatment, because of a gain in body potassium content. There were no consistent alterations in exchangeable sodium.

In hypothyroidism, treatment with thyroxine caused a loss of both sodium and potassium from the body.

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[*Reprinted from the Journal of Physiology*,
1958, Vol. **142**, No. 3, p. 447.]
PRINTED IN GREAT BRITAIN

J. Physiol. (1958) 142, 447–452

BONE CALCIUM AND SODIUM CONTENT AND THE EXCHANGE OF RADIOSODIUM IN BONES FROM RATS TREATED WITH THYROXINE AND PARATHORMONE

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(*Received* 4 *February* 1958)

In healthy rats the proportion of sodium relative to calcium in bone is constant (Munro, Satoskar & Wilson, 1957). Nevertheless, after removal of sodium from the body by intraperitoneal dialysis or following adrenalectomy, the amount of sodium in bone falls although there is no change in the calcium content (Munro, Satoskar & Wilson, 1957, 1958). However, little is known about the influence of calcium loss on bone sodium metabolism. Skeletal decalcification can be produced by parathormone, which increases the urinary excretion of calcium (Albright, Bauer, Ropes & Aub, 1929; Woods & Armstrong, 1956). This method has been used in the present work to determine whether there is any associated change in bone sodium.

Decalcification of the skeleton has been reported in cases of thyrotoxicosis (Albright *et al.* 1929; Aub, Bauer, Heath & Ropes, 1929; Logan, Christensen & Kirklin, 1942; Puppel, Gross, McCormick & Herdle, 1945; Krane, Brownell, Stanbury & Corrigan, 1956). Measurements of exchangeable sodium in patients suffering from hyperthyroidism before and after treatment have shown variable results difficult to interpret (Munro, Renschler & Wilson, 1958). The suggestion was made that some of the results might be due to alterations in the composition of bones produced by the disease, although there is no direct information with regard to sodium. Accordingly, in the present series of experiments, the action of thyroxine and parathormone on the calcium content of bone has been studied in rats, and measurements have also been made of sodium content of bone and exchange with radiosodium.

METHODS

Male albino rats were used and were weighed regularly throughout the experimental period. The methods of measuring the water, sodium, calcium and the radioactivity in the bones have been described previously (Munro *et al.* 1957). Serum concentrations of sodium and calcium were

measured by flame photometry. Parathormone was given by intramuscular injection in a dose of 100 U.S.P. units daily. L-Thyroxine sodium was given in drinking water in a concentration of 500 μg/100 ml., or by intramuscular injection in a dose of up to 55 μg/day.

RESULTS

Effect of thyroxine on bone composition. Thyroxine was given in increasing doses to six rats over a period of 9 weeks. For the first 5 weeks L-thyroxine sodium was added to the drinking water in a concentration of 500 μg/100 ml. This was then supplemented by intramuscular injections of 55 μg of L-thyroxine sodium on alternate days for 2 weeks, and finally daily for the last 2 weeks. At the same time another six rats, initially of similar weight, received corresponding injections of water. Definite evidence of hyperthyroidism appeared in the treated rats. Their mean weight was 83% of that of the control rats by the end of the treatment. The rats receiving thyroxine kept discretely apart in their cage while the control animals commonly huddled closely together in one corner. At death the hearts of the treated rats were noticeably larger and were approximately 50% greater in weight. However, this degree of hyperthyroidism did not produce any definite changes in bone composition (Table 1).

Effect of parathormone on bone composition and the release of ^{22}Na. The first observations were carried out on twelve rats each given 10 μc of ^{22}Na shortly after weaning. After a further 10–12 weeks, intraperitoneal injections of NaCl solution 0·9% (w/v) were given. This greatly increased the turnover of sodium in the extracellular fluid and ensured that any ^{22}Na subsequently retained was exclusively in the bones. The rats were then divided into two groups of six and kept in two separate metabolism cages. After some preliminary measurements of whole-body radioactivity the rats in one group received five daily injections of 100 U.S.P. units of parathormone, while the others were given injections of distilled water. At the end of this period all the rats were given an intraperitoneal injection of ^{24}Na and were killed 24 hr later. The results of the studies of bone composition are shown in Table 2.

The administration of parathormone did not alter body weight. The total urinary excretion of calcium from the treated rats during the period of injections was 4·5 m-equiv, but only 0·65 m-equiv from the controls. There was also an increase in the urinary loss of sodium from the treated rats, which excreted 82 m-equiv in comparison with 55 m-equiv in the controls. The serum calcium level, measured on a pooled sample, was greater in the treated group but there was no difference in the serum sodium concentrations. The water and sodium contents of the bones in the two groups were not significantly different. The bone calcium was lower in the treated rats ($P < 0{\cdot}001$). There was no change in the ^{24}Na relative specific activity of bone sodium and the loss of ^{22}Na from the bones was not increased by the parathormone injections. The

TABLE 1. The effect of thyroxine on bone composition and sodium exchange

Group and no.		Wt. at death (g)	Serum sodium (m-equiv/l.)	Water content of wet bone (%)	Calcium (m-equiv/kg dry bone)	Sodium (m-equiv/kg dry bone)	Na:Ca ratio	^{24}Na bone specific activity relative to blood (%)	Exchangeable bone sodium (m-equiv/kg dry bone)
Control, 6	Mean	313	149·2	13·0	12,779	257	0·0202	49·0	126
	S.E.	6	0·7	0·3	37	1·2	0·0002	0·5	1·0
Thyroxine-treated, 6	Mean	259	151·6	12·5	12,658	263	0·0207	50·7	133
	S.E.	8	1·0	0·3	89	3·2	0·0002	1·0	3·7

TABLE 2. Effect of parathormone on bone composition and the release of ^{22}Na from bone

Group and no.		Wt. at death (g)	Serum sodium (m-equiv/l.)	Serum calcium* (m-equiv/l.)	Water content of wet bone (%)	Calcium (m-equiv /kg dry bone)	Sodium (m-equiv /kg dry bone)	Na:Ca ratio in bone	^{24}Na bone specific activity relative to blood (%)	^{24}Na exchangeable bone sodium (m-equiv/kg dry bone)	Decrease of whole-body ^{22}Na content during injections (%)	^{22}Na retained (μc/kg dry bone)
Control, 6	Mean	234	152·5	5·0	14·0	12,641	258	0·0203	52·8	137	11·8	10·6
	S.E.	11	0·5	—	0·4	69	1·1	0·0002	0·8	2·1	1·4	0·5
Parathormone-treated, 6	Mean	238	153	6·3	14·0	12,249	259	0·0212	51·2	132	9·7	11·1
	S.E.	9	0·6	—	0·3	74	3·7	0·0003	1·0	2·7	1·5	0·7

* Pooled samples of serum used.

TABLE 3. The delayed effect of parathormone on bone composition and release of ^{22}Na

Group and no.		Wt. at death (g)	Water content of wet bone (%)	Calcium (m-equiv/kg dry bone)	Sodium (m-equiv/kg dry bone)	Na:Ca ratio	Decrease of whole body ^{22}Na content during and after injections (%)	^{22}Na retained (μc/kg dry bone)
Controls, 3	Mean	350	13·6	13,189	259	0·0198	18	8·3
	S.E.	20	0·3	188	6·9	0·0004	1·2	0·4
Parathormone-treated, 4	Mean	309	13·0	12,610	247	0·0195	17	8·1
	S.E.	12	0·3	137	5·8	0·0003	1·7	0·4

^{22}Na remaining in these animals was located in the bones and no ^{22}Na radioactivity could be detected in the serum samples.

A second series of observations with parathormone was then carried out on seven rats given ^{22}Na after weaning. The design of the experiments was similar except that the rats were not killed until 5 weeks after the last of the six daily injections of parathormone. Measurements of the loss of ^{22}Na with the whole body counter were continued up to the time of death. The weights of the rats were slightly greater than in the first series and those treated with parathormone were lighter at death. Throughout the longer period of study the rate of loss of ^{22}Na from the body was similar in the two groups. There was a significant decrease in bone calcium in the rats receiving parathormone ($P < 0{\cdot}02$) and a slight, insignificant decrease in bone sodium. Measurements of blood and bone radioactivity after death again established that the ^{22}Na was confined to bone.

DISCUSSION

Some connexion between the thyroid gland and calcium metabolism has long been suspected (Thomson & Collip, 1932). A greatly increased excretion of calcium and rarefaction of bones have been noted in cases of long-standing thyrotoxicosis (Aub *et al.* 1929; Puppel *et al.* 1945), although skeletal decalcification demonstrable radiologically is rare in this disease (Williams & Morgan, 1940). The administration of thyroid hormone to normal dogs produced an increase in calcium excretion without any changes in serum calcium levels (Logan *et al.* 1942). However, severe hyperthyroidism in adult rats, brought about by feeding desiccated thyroid, did not cause decalcification of bones, nor was there any evidence of lack of calcification in rapidly growing animals (Smith & McLean, 1938). Similarly, from comparison of the skeletons of twin sheep, one of which was thyroidectomized, Todd, Wharton & Todd (1938) found no modification of bone texture, weight or thickness. Bell & Cuthbertson (1942) observed in their experiments in rats that administration of thyroid gland (thyroideum siccum, B.P.) produced a greater reduction in soft tissue than in mineral matter, the quality of the bone being unaffected. Furthermore, in a chronic experiment, they did not observe any significant difference in the percentage of calcium in the femurs of control and thyroid-treated rats. Similarly, in the present experiments, there was reliable evidence that a severe degree of hyperthyroidism was produced in the rats, but no abnormalities in calcium or sodium metabolism were detected.

It is known that the administration of parathyroid extract to rats increases the urinary excretion of calcium and decreases the bone calcium content (Thomson & Collip, 1932; Logan, 1940; Talmage, Lotz & Comar, 1953; Bacon, Patrick & Hansard, 1956; Woods & Armstrong, 1956). However, the results in rats are somewhat variable and the rat can develop resistance to the action

of parathormone within a few days of injection (Thomson & Collip, 1932; Woods & Armstrong, 1956). Following injections of parathyroid extract rapid resorption of bone has been demonstrated, both organic bone matrix and its associated bone salts being resorbed simultaneously (McLean & Bloom, 1941). In the rats used in the present studies the bone calcium fell after parathormone administration but, though the urinary loss of both sodium and calcium rose, there was no alteration either of bone sodium content or of the exchange of bone sodium with ^{22}Na or ^{24}Na. Increased urinary sodium loss after parathormone has also been reported by Ellsworth & Nicholson (1935). The observations of Taylor & Moore (1956), on pullets depleted of calcium by their first period of egg production, showed that after severe losses of calcium the bone sodium content increased. The disturbance of calcium metabolism was far greater than in the present experiments on rats. It is evident that under the conditions of these experiments administration of neither parathormone nor thyroxine is followed by any striking change in bone sodium metabolism.

SUMMARY

1. Thyroxine given to rats in sufficient dosage to produce hyperthyroidism caused no definite changes in bone calcium or sodium metabolism.

2. Parathormone produced a significant decrease in bone calcium content but there were no associated alterations in bone sodium content. The availability of bone sodium for exchange with sodium isotopes remained within the normal range after the loss of calcium from the bone.

The work was done during tenure by R.S.S. of a research fellowship granted by Glaxo Laboratories (India) Priv. Ltd. We are grateful for grants for the purchase of isotopes and apparatus from the Tuberculosis Research Fund of the University of Sheffield, the Endowment Fund of the United Sheffield Hospitals, and Glaxo Laboratories Ltd.

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Reprinted from CIRCULATION
Vol. IX, No. 2, February 1954
Printed in U.S.A.

Metabolic Changes Associated with Mitral Valvuloplasty

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Patients with chronic congestive heart failure operated upon for the surgical correction of mitral stenosis have been observed repeatedly to have low plasma sodium concentrations and elevated plasma potassium concentrations after operation. This study was primarily directed at an understanding of these abnormalities. The authors have shown that the preoperative patient has a characteristic disorder of body composition noteworthy for a high total body water, a high total body sodium, a low total exchangeable potassium and a low plasma sodium concentration. The effects of surgery on this abnormal body composition, and the therapeutic implications are discussed.

DURING recent years increasing attention has been paid to the metabolic response to a wide variety of surgical operations.[1] It has been shown that many factors influence this response. Of these, the previous health and nutritional state of the patient, and the severity of the inflicted trauma, are probably the most important. Coincidentally with these studies, technics have been developed for investigating the total amounts of water, sodium and potassium in the body available for exchange with suitable isotopes.[2–5] Already some knowledge of the amounts of these substances present in normal individuals has been gained and is available for comparison with the measurements made in patients before and after surgical operations.[6, 7]

By combining the balance and isotope dilution technics a detailed study may be made of some of the biochemical changes arising after major surgery. It is important to emphasize the dynamic character of these changes; isolated observations reveal only a static disorder, while sequential observations by these methods reveal the rapidly changing picture as each day passes. These methods are particularly valuable in investigating the complex problems that are seen after operations on the mitral valve.

In patients with mitral stenosis of such severity as to warrant surgical intervention, abnormalities in the metabolism of water and electrolytes are already present and the nutritional state has frequently considerably deteriorated as the result of longstanding heart failure. The stress of operation is then superimposed, and it is not surprising that in the postoperative period gross biochemical disturbances may become apparent. The present study is an attempt to elucidate the nature and mechanisms of some of these disturbances.

METHODS AND MATERIAL

The metabolic balance studies were carried out following the principles described by Moore and Ball.[1] The intake of sodium, potassium and nitrogen in the diet has been calculated from food analyses carried out in this laboratory. The intravenous intake given therapeutically or for the performance of various investigations has also been measured. In the case of blood transfusions, which were frequently large, only the readily available nitrogen, sodium and potassium in the plasma have been entered in the balance chart. Similarly only the plasma fractions of these constituents in the operative blood loss have been entered. The total amount of whole blood transfusions and blood loss have been indicated, however, in the legends of the charts. In calculating the total excretion, account has been taken of loss in the urine, feces, wound exudate and fluid drained from the chest.

The method of charting the metabolic balance is essentially that described by Moore and Ball.[1]

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The intake is charted upwards from the zero line and the output downwards from the top of the intake line. A positive balance is indicated by a shaded area above the zero line, a negative balance by an area below the zero line and enclosed by heavy lines.

The body content of water, sodium and potassium has been measured by dilution of deuterium oxide, sodium[24] (Na^{24}) and potassium[42] (K^{42}). The details, accuracy and reproducibility of these technics have been described previously.[2, 5, 8] The measurements of total exchangeable sodium and potassium have, for the most part, been carried out simultaneously.[9]

The chemical methods used in the metabolic balance studies have been previously described.[1] Urinary excretion of 17-ketosteroids was measured by the method of Talbot[10] and of 17-hydroxycorticoids by the method of Reddy, Jenkins, and Thorn.[11]

Clinical Details

The study is based essentially on the investigations of three patients by metabolic balances (cases 1, 2, and 3) and on isotope dilution measurements made at intervals on these and nine other patients (cases 4 through 12). In case 12 only preoperative measurements were available as the patient died shortly after operation from cerebral embolism. The clinical details are mentioned in the text where applicable. In addition, studies of water metabolism and changes in blood chemistry as a result of operation have been carried out in an additional 81 patients. All were relatively severe cases of mitral stenosis falling into groups III and IV.[12] Mitral stenosis was the predominant lesion in all. In some, minor degrees of aortic and mitral incompetence were also present. Except for one of the balance patients (case 1) all have been studied during the relatively cool period of the year from October to March. The patients were brought into the best possible condition for surgery by medical treatment with digitalis, mercurial diuretics and low salt diets before the preliminary preoperative measurements were made. Blood transfusions were given liberally in an attempt to cover the loss at operation and later. The volume of these transfusions and of the other fluids given to the patients immediately after operation was changed at definite intervals. The nature and effects of these alterations in treatment policy will be described later. During the immediate pre- and postoperative periods the dietary sodium intake was restricted to approximately 9 mEq. a day except in cases 1 and 2 where the intake was larger, as described in the metabolic balance studies.

General Metabolic Changes

The general metabolic course of patients undergoing mitral valvuloplasty is best illustrated by the balance studies. Cases 1, 2, and 3 are accordingly described briefly in this section; certain aspects are discussed later in greater detail in the next sections along with the findings in the larger groups.

Case 1 (fig. 1 *A*, *B*, *C*). The patient, a woman 45 years of age, underwent operation May 19, 1952. She had rheumatic fever at the age of 12; at the age of 35 she suffered a right hemiplegia, apparently embolic in origin. In the ensuing 10 years her course was one of gradual deterioration; digitalis, diuretics and sodium restriction were employed. Dyspnea, orthopnea and ankle edema followed; she was fibrillating on admission and showed marked enlargement of liver and spleen, with wasting of the body. Her peripheral edema had disappeared when the study was begun. Clinical and radiologic signs of mitral stenosis were typical. Operation was technically satisfactory and convalescence uneventful save for a small pulmonary embolus on the sixteenth postoperative day. She was readmitted for follow-up study four months later (Sept. 23, 1952) at which time her exercise tolerance was vastly improved; there was no further orthopnea. She was still fibrillating; liver and spleen were still enlarged.

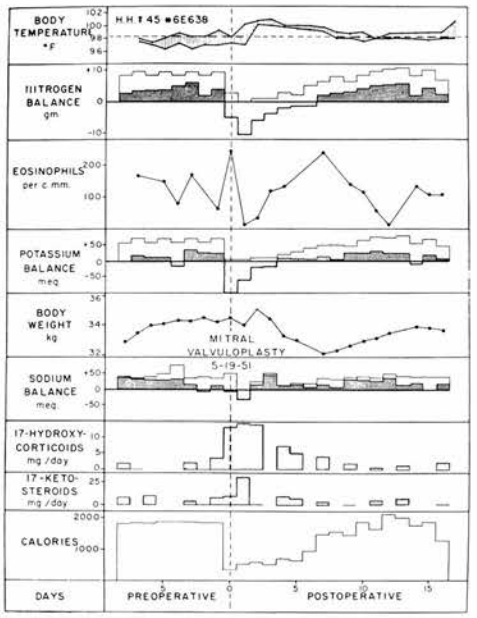
The balance of nitrogen demonstrated a transient negative phase with high excretion on one day and a rapid return to positive balance as her intake improved with resumption of caloric intake. Potassium followed a similar pattern. Sodium intake was restricted throughout, but the balance was consistently positive except for the first day after operation. Body weight showed an increase for three days postoperatively, then a sharp reduction followed by a later rise. Eosinophile count was near zero for two days after operation, then returned sharply to normal or above normal values save for two days immediately preceding her pulmonary embolus. Urinary steroid analyses showed elevation of the 17-ketosteroids on the first postoperative day; there was an increase in 17-hydroxycorticoids persisting three days and then falling slowly to the subnormal starting values.

Intake-output fluid balance (fig. 1*C*) was not remarkable; the initial total body water and total exchangeable sodium were elevated; the total exchangeable potassium was slightly low. Following operation, body water and sodium further increased as potassium decreased. On late follow-up (128 days), a return of total body water to the lowest recorded fraction of body weight (55.4 per cent) was accompanied by a fall in total exchangeable sodium and a rise in total exchangeable potassium.

The serum concentrations of sodium and potassium showed changes in opposite directions, the sodium falling and potassium rising postoperatively, and later returning towards normal. Both were normal initially.

Case 2 (fig. 2 *A*, *B*, *C*). The patient, a 40 year old woman, was operated upon Oct. 31, 1951. With no previous history of rheumatic fever, this patient was first told that she had a cardiac murmur in 1948. Within a year she noted increasing dyspnea and palpitation, and was digitalized. In 1950 she had a cerebral embolus, with left hemiplegia and gradual recovery. Disability increased although she maintained her occupation as a typist; digitalis, ammonium chloride and diuretics were used. She was thin and emaciated with a residual left-sided weakness. Typical signs of mitral stenosis were present with hepatomegaly, and cardiac enlargement. Operation was technically satisfactory; convalescence was uneventful. Her first readmission for follow-up was on Feb. 10, 1952, 105 days after operation. There was no edema. Exercise tolerance was greatly improved. Cardiac signs were unchanged. She was again studied on Oct. 31, 1952 (365 days after operation). There was still further conspicuous improvement. She was leading a normal life, symptom-free on digitalis.

Balance of nitrogen was consistently positive; there was no increase in excretion rate after operation. Potassium balance was close to zero save for a considerable period after surgery. Sodium balance showed a positive trend save for a spontaneous diuresis beginning on the fifth postoperative day,



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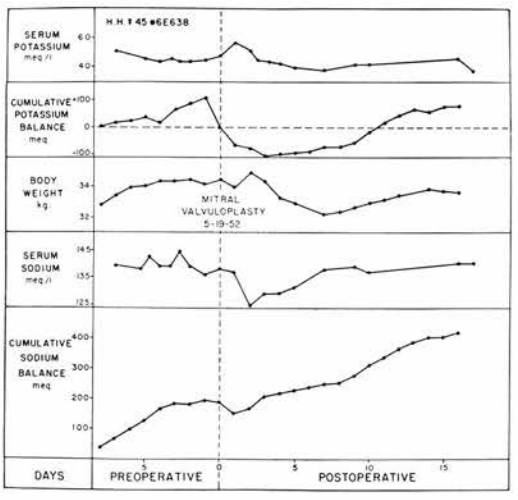

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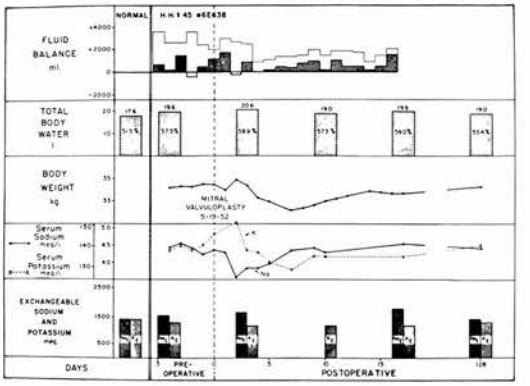

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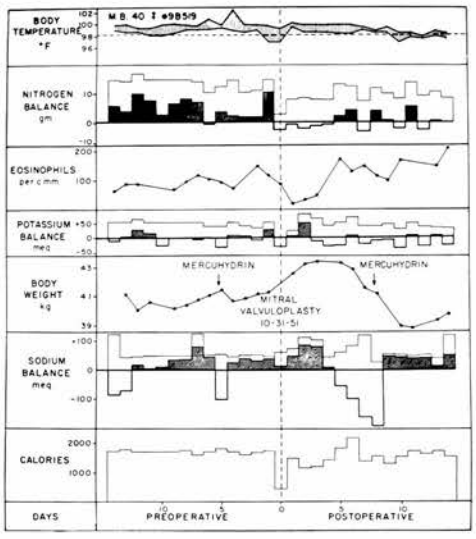
FIG. 1 (*A*) Case 1. Metabolic balance chart. In this and in figures 2*A* and 3 the balances of nitrogen, potassium and sodium are charted as previously described.[1] Body temperature indicates the maximum and minimum for each day. Eosinophils, body weight, caloric intake and 24-hour excretions of 17-hydroxycorticoids and 17-ketosteroids are shown. The operative blood loss was 240 ml., and blood transfusion at operation was 500 ml.

(*B*) Case 1. Cumulative balances and serum electrolyte changes. Here and in figures 2*B* and 4 each day's balance of potassium and sodium is added to the previous figure and the resultant cumulative net change charted.

(*C*) Case 1. The fluid balance is charted as in the other balances, measurable output is subtracted from total intake; in normal individuals this "intake-output balance" shows a net positive figure (+750 ml.) which represents the sum of other unmeasured losses. Total body water is charted as a column at the top of which the absolute figure (in liters) is shown. In the center of the column the water fraction of body weight is indicated as per cent. Total exchangeable sodium and total exchangeable potassium are charted as columns, the height of which represents the absolute figure as shown on the ordinate. At the left of the double line are shown normal values for body water, total exchangeable sodium and total exchangeable potassium for a female of this body weight.



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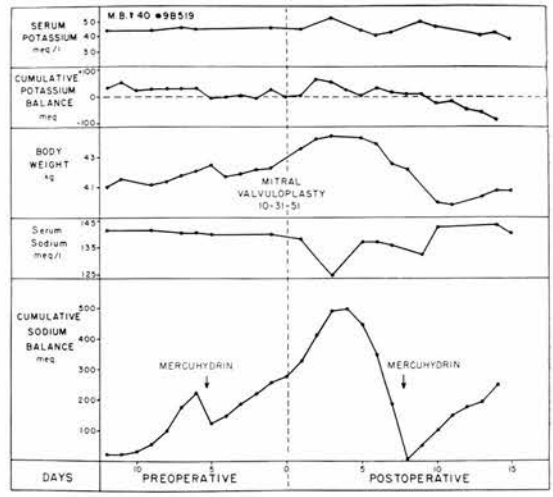

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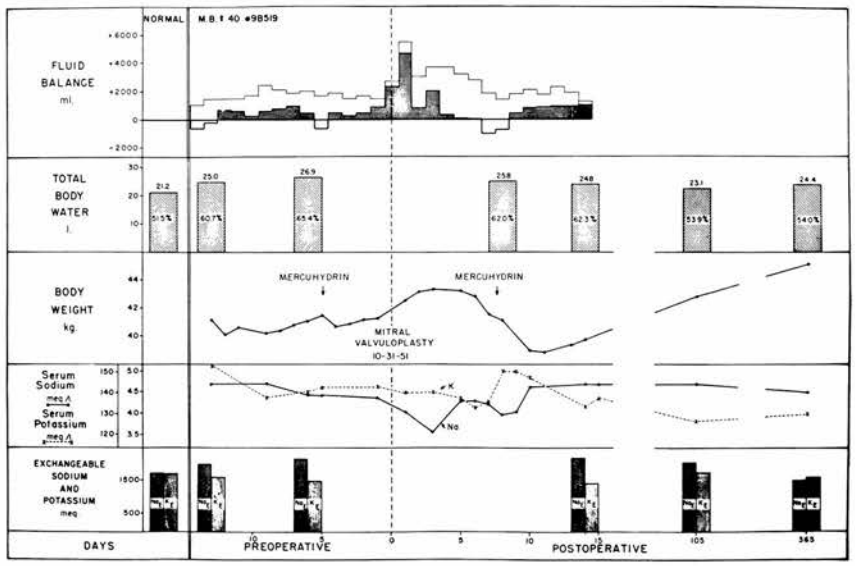

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FIG. 2. (*A*) Case 2. Metabolic balance chart. The operative blood loss was 225 ml. and the blood transfusion at operation 500 ml. Further details are described in the text. (*B*) Case 2. Cumulative balances and serum electrolyte changes. (*C*) Case 2. Changes in fluid balance, body composition and serum electrolytes.

lasting four days, accounting for 500 mEq. of sodium, and towards the end of which Mercuhydrin was administered. Following this episode excretion rates again became very small. There had previously been (five days before operation) a one-day sodium diuresis (100 mEq.) on Mercuhydrin. Body weight changes followed the sodium balance; there was again in this case a postoperative weight gain, dropping abruptly during diuresis, and then rising slowly during the ensuing year. Eosinophil count was moderately low (75 to 125 cu. mm.) until operation when it dropped to near zero for three days, and then rose to values higher than those seen preoperatively. Caloric intake was never seriously impaired. Urinary steroids were not measured.

Intake-output fluid balance showed the postoperative water loading with large fluid intakes,

and later diuresis. The starting total body water and total exchangeable sodium were elevated; the total exchangeable potassium was low. These measurements were not repeated until after her diuresis and from that time a gradual and consistent approach of all these measurements toward normal was seen.

The serum concentration of sodium was initially near normal and fell abruptly to 125 mEq. per liter during the postoperative period of water and salt loading; there was then a rise and later a small dip at the very end of diuresis; only at this later time did potassium rise. These values were later normal.

Case 3 (figs. 3, 4 and 5). This patient, a woman aged 50 years, was operated upon Nov. 29, 1952. She recalled having had chorea in childhood; at the age of 15 she noticed dyspnea and palpitation on exertion; these symptoms gradually progressed. At the ages of 22 and 24 she had two normal pregnancies, and was adjusted well to her disease until the age of 42 when orthopnea and paroxysmal nocturnal dyspnea began. During the 18 months prior to admission there was rapid deterioration so that walking on the level was difficult despite digitalis and a low salt diet. She was a thin, slightly wasted woman, without edema. Auricular fibrillation, enlarged heart and liver and murmurs both systolic and diastolic were present. Operation was technically satisfactory. There was a six-day febrile period after operation during which the patient was anorexic; thereafter improvement was uninterrupted although her appetite remained poor. She was readmitted June 19, 1953 (156 days later), for

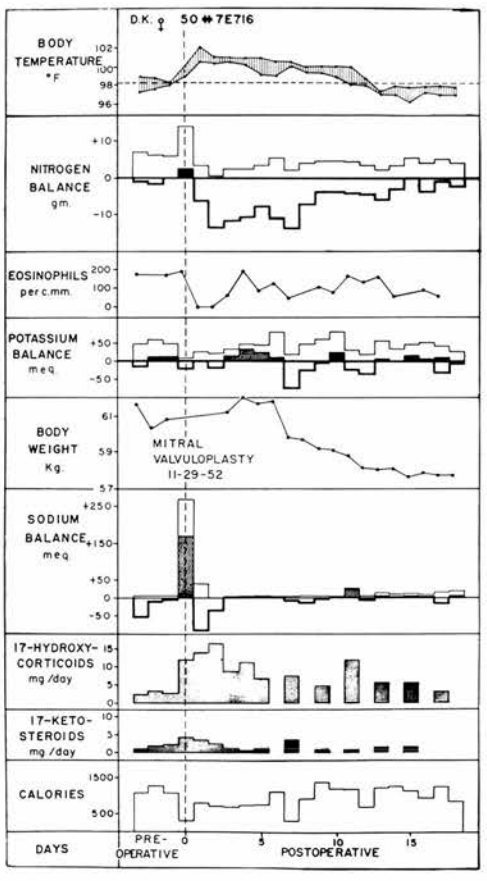

FIG. 3. Case 3. Metabolic balance chart. The operative blood loss was 334 ml. and the blood transfusion at operation 2500 ml. Further details are described in the text.

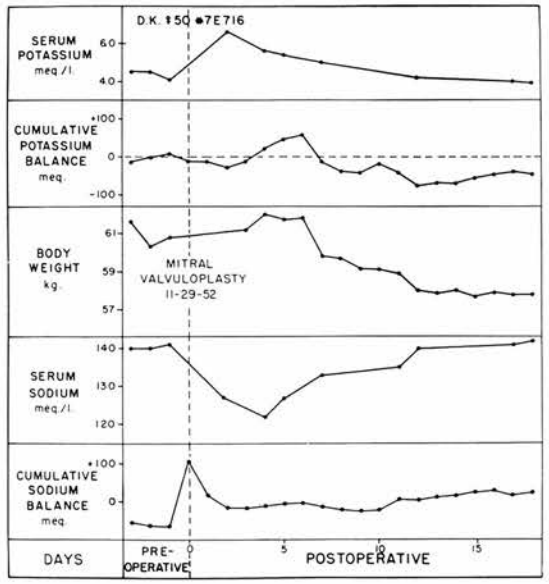

FIG. 4. Case 3. Cumulative balances and serum electrolyte changes.

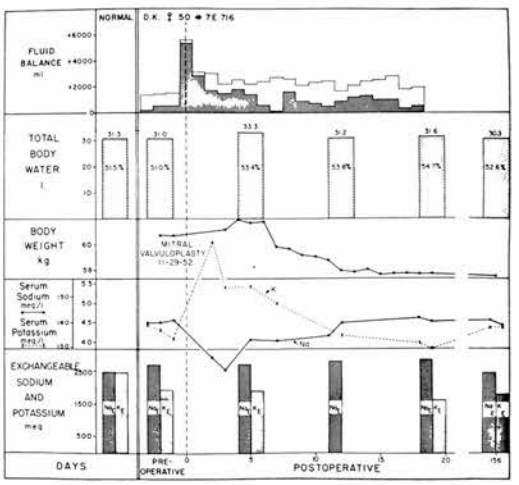

FIG. 5. Case 3. Changes in fluid balances, body composition and serum electrolytes.

study. Exercise tolerance was tremendously improved and she was leading a normal life. She had not regained her preoperative weight and was anorexic, possibly because of digitalis overdosage; cardiac signs were unchanged.

Balance of nitrogen demonstrated a marked increase in excretion rate, low intake, relative caloric starvation and marked negative balance for eight days after operation. Thereafter, excretion was less marked but balance was still negative up to discharge; daily caloric intake rarely exceeded 1000. Potassium balance was essentially zero for the duration of study. Sodium balance was a spectacular example of conservation with zero intake. Save for the day of operation when blood transfusions increased sodium intake and the following day when some sodium was lost through drainage from the chest there was a remarkable lack of sodium intake or loss. For a period of 16 days there was a total of only 160 mEq. flux with the environment, and net zero balance. Body weight rose in the early postoperative period, then fell suddenly with a large water, nitrogen and potassium loss. Thereafter, it slowly declined during the period of observation. Eosinophile count showed an operative fall. It did not at any time thereafter rise to high levels. Urinary steroid analyses showed a slight rise in 17-ketosteroid excretion over the otherwise abnormally low levels. The 17-hydroxycorticoid excretion was greatly increased for six days and intermittently elevated for eight more days.

Intake-output fluid balance (fig. 5) showed water loading for six days after operation, then a diuresis for two days, followed by normal balance. The initial total body water was not elevated, but on the fifth postoperative day it was increased by 2.3 liters, which was later excreted in the urine. At follow-up five months after operation the total body water was within the normal range. Total exchangeable sodium was elevated at the outset, and remained about the same until follow-up when it was reduced to normal. The total exchangeable potassium was low throughout.

The serum concentrations of sodium and potassium showed the conspicuous "diamond-shaped" configuration of inverse changes seen previously. The sodium, initially normal, fell to 122 mEq. per liter. This change was maximal by the second and third postoperative day, later returning to normal values.

In summary it is apparent from the metabolic studies that the response with regard to nitrogen balance, eosinophile counts and urinary steroid excretion is not beyond the range of expectation for surgical operations of similar severity.[1, 25] On the other hand, there are conspicuous abnormalities in body composition before operation and considerable disturbances in salt and water metabolism in the early postoperative period. The tendency towards lowering of the plasma sodium concentration is truly remarkable; it is often accompanied by an elevation of the plasma potassium concentration. This disorder has been the source of considerable difficulty in treatment and speculation as to pathogenesis. While a lowering of the plasma sodium concentration during positive sodium balance is a normal feature of post-traumatic metabolism (the "sodium paradox"), it does not usually occur to such a marked extent and is ordinarily not accompanied by such a marked hyperkaliemia. These aspects have accordingly been studied in greater detail in a larger group of patients.

Preoperative Body Composition

The clinical study of body composition has been greatly extended by the development of the isotope dilution methods.[7, 8] The measurement of total body water,[2, 6, 20] total exchangeable sodium and potassium[3, 4, 5, 9, 15, 17] permit the observer a remarkable insight into body composition as regards four of its most significant parameters: water, extracellular "mass" (sodium), lean tissue mass (potassium) and fat (by inverse proportion to water content).

Measurements of total body water, exchangeable potassium and exchangeable sodium were made in 12 patients before and at intervals after operation. The results are shown in table 1. In all cases except case 12 there was no peripheral edema at the time of the first measurements. The results in the female patients before operation are compared with those obtained in healthy adults in table 2. The mitral stenosis patients have a definite excess of total water and sodium in their body composition. The total exchangeable potassium is slightly diminished, but this decrease only attains a low degree of significance by statistical comparison. In case 6 no preoperative determination of total potassium was made. Only three male patients with mitral stenosis were studied with isotopes, and in them the changes in body composition were similar to those seen in the females, but the group is too small for detailed statistical analysis. The total exchange-

TABLE 1.—*Isotope Dilution Measurements before and after Mitral Valvuloplasty*

Case No.	Sex	Age *Yrs.*	Days before (−) or after (+) operation	Weight *Kg.*	Sodium			Potassium			Body Water		Fluid Intake
					Serum *mEq./L.*	Exchangeable		Serum *mEq./L.*	Exchangeable		*L.*	*% Body Weight*	
						mEq.	*mEq./Kg.*		*mEq.*	*mEq./Kg.*			
1	F	45	−4	34.2	139	1542	45.0	4.4	1259	36.8	19.6	57.5	Unrestricted
			+3	34.6	130	1645	47.6	4.4	1154	33.4	20.6	58.9	
			+10	33.0	137	—	—	4.2	1174	35.7	19.0	57.3	
			+17	33.7	141	1750	51.8	4.2	1169	34.6	19.9	59.0	
			+128	34.78	139	1423	41.5	4.5	1292	37.7	19.0	55.4	
2	F	40	−13	41.2	143	1932	46.9	5.1	1565	38.0	25.0	60.7	Unrestricted
			−6	41.1	139	2085	50.8	4.5	1488	36.2	26.9	65.4	
			+8	41.2	132	—	—	5.0	—	—	25.8	62.0	
			+14	39.8	143	2130	53.5	4.3	1424	35.8	24.8	62.3	
			+105	42.9	143	2018	47.0	3.9	1716	40.1	23.1	53.9	
			+365	45.20	140	1500	42.7	4.0	1564	34.6	24.4	54.0	
3	F	50	−2	60.75	140	2695	44.4	4.3	1965	32.3	31.0	51.0	Unrestricted
			+5	61.70	127	2717	44.0	5.4	1930	31.3	33.3	53.4	
			+12	58.05	140	2782	47.9	4.2	—	—	31.2	53.8	
			+19	57.78	141	2884	49.9	3.9	1628	28.1	31.6	54.7	
			+154	57.62	140	2487	43.2	4.4	1781	30.9	30.3	52.6	
4	F	22	−7	45.68	134	2144	47.2	4.3	1624	35.6	25.3	55.3	Unrestricted
			+6	46.05	129	2206	47.9	4.7	1594	34.6	29.0	64.3	
			+13	44.30	139	2328	50.4	4.4	—	—	26.0	58.7	
			+174	49.91	141	2181	43.7	4.2	2186	43.8	28.9	57.9	
5	F	47	−1	47.30	135	1985	42.0	4.0	1581	33.4	25.1	53.1	Unrestricted
			+1	48.98	124	—	—	6.1	—	—	28.1	57.4	
			+7	46.40	132	2040	43.9	4.7	—	—	—	—	
			+14	45.98	136	2125	46.2	5.0	1561	34.0	25.2	54.8	
			+167	50.69	136	2039	40.2	4.6	1914	37.8	26.6	52.5	
6	F	54	−6	45.80	137	2040	44.5	4.7	—	—	24.1	52.5	Unrestricted
			+1	46.11	130	2143	46.5	4.7	1684	36.5	26.1	55.8	
			+9	44.45	137	2086	46.9	4.7	1555	35.0	24.4	54.9	
			+15	43.92	137	2066	47.0	4.3	1418	32.3	23.4	53.3	
			+155	38.75	133	1917	49.5	4.8	1467	37.9	23.3	60.1	
7	F	40	−5	51.00	140	2127	41.7	4.9	1919	37.6	26.9	52.9	Restricted to
			+2	49.10	134	2033	41.4	4.9	1728	35.2	25.9	52.7	1500 ml.
			+9	48.78	136	1978	40.6	4.4	1884	38.6	26.4	54.5	
			+120	51.52	143	2111	41.0	4.5	1923	37.3	26.3	51.1	
8	F	41	−1	47.05	133	1575	33.5	5.5	1600	34.0	21.5	45.7	Restricted to
			+2	47.50	126	—	—	6.0	—	—	21.7	45.7	1500 ml.
			+7	46.35	126	1653	35.7	4.9	1317	28.4	22.5	48.5	
			+14	44.80	138	1855	41.3	4.4	1461	32.6	23.4	52.2	
			+118	57.30	140	1915	33.4	4.8	1730	30.2	23.4	40.8	
9	F	50	−7	44.70	140	2219	49.6	4.3	1658	37.1	24.8	55.4	Restricted to
			−2	44.00	—	—	—	—	—	—	25.0	56.9	1500 ml.
			+2	42.60	126	—	—	5.3	—	—	23.6	55.4	
			+7	41.25	130	1871	45.4	5.0	1396	33.8	23.6	56.5	
			+14	41.40	135	1955	47.2	5.0	1468	35.5	23.3	56.8	

TABLE 1.—*Continued*

Case No.	Sex	Age Yrs.	Days before (−) or after (+) operation	Weight Kg.	Sodium			Potassium			Body Water		Fluid Intake
					Serum mEq./L.	Exchangeable		Serum mEq./L.	Exchangeable		L.	% Body Weight	
						mEq.	mEq./Kg.		mEq.	mEq./Kg.			
10	M	32	−2	58.60	137	2774	47.3	4.4	2829	48.3	35.2	60.1	Restricted to 1500 ml.
			+5	57.00	131	2655	46.6	5.0	2707	47.5	36.8	64.6	
			+12	56.02	138	2827	50.5	4.7	2336	41.7	35.8	65.2	
11	M	42	−11	38.62	134	2507	64.9	5.0	1645	42.6	26.1	67.6	Unrestricted
			−4	39.70	126	2501	64.6	5.0	—	—	29.4	74.7	Restricted to 1000 ml.
			+3	36.88	131	2462	66.5	5.0	1311	35.4	26.3	71.1	Restricted to 1500 ml.
			+10	36.08	133	2415	66.9	4.2	1395	38.7	—	—	
			+17	37.12	138	2595	69.9	3.8	1290	34.7	26.8	72.2	Unrestricted
			+38	39.00	139	2691	69.0	4.6	1540	39.5	27.4	70.3	
			+114	49.12	143	2643	53.8	4.3	2097	42.7	30.3	61.6	
12	M	44	−5	57.60	135	3245	57.6	4.6	2090	37.0	36.6	64.8	Unrestricted

able sodium on a milliequivalent per kilogram basis was exceptionally high in cases 11 and 12. No peripheral edema was present in case 11, but this patient was emaciated. In case 12 the high total sodium was associated with slight pitting edema at the ankles, but considerable wasting was also present.

In all those with mitral stenosis the normal ratio between total exchangeable sodium and potassium was lost. In healthy females the ratio of exchangeable sodium to exchangeable potassium is 1.02 while in healthy males it is 0.90. The corresponding ratios in the female and male patients with mitral stenosis were, respectively, 1.25 and 1.33, indicating a relative excess of sodium over potassium in their body composition.

In the comparisons shown in table 2 the only figures available for healthy adult females refer for the most part to a group with a mean age younger than that of the mitral stenosis patients. However, in the case of total body water there is probably a slight decrease with advancing years,[6] which renders the differences more striking. Little is known about the alterations in total exchangeable sodium and potassium with increasing age, but the available evidence does not suggest that there is any definite change between the third and fifth decades. For ease of reference the normal body composition of a healthy adult of similar weight to the patient with mitral stenosis has been calculated and is shown on the chart for comparison with the preoperative and later results (figs. 1*C*, 2*C*, 5 to 8, 11 and 12). The values shown in table 2 have been used for this purpose in females. The corresponding values for healthy young adult males are: total body water 62.0 per cent of body weight, exchangeable sodium 41.4 mEq. per kilogram, exchangeable potassium 46.8 mEq. per kilogram.[7]

POSTOPERATIVE CHANGES

Changes in Body Weight and Total Body Water. In the first six cases in which total body water was measured (cases 1 to 6 in table 1) the fluid intake in the immediate postoperative period was unrestricted. In these circumstances there was regularly an increase in weight after operation in spite of the negative nitrogen balance. Normally patients lose weight after surgery as a result of a number of factors.[1, 21] It is an outstanding abnormality of surgical metabolism for body weight to increase after operation. This was clearly due to water retention as was confirmed by the observations with deuterium oxide (figs. 1*C*, 5, 6, 9, and table 1). In cases 1, 3, 4, 5, and 6 measurements made within six days of operation all showed an increase in the total body water over the preoperative value. In

TABLE 2.—*Body Composition of Female Mitral Stenosis Patients before and after Operation in Comparison with Healthy Adult Females*

	Body Water					Exchangeable Sodium					Exchangeable Potassium				
	No.	Age	% Body Weight ± s.d.	*t*	*p*	No.	Age	mEq. Kg. ± s.d.	*t*	*p*	No.	Age	mEq./Kg. ± s.d.	*t*	*p*
Healthy females*	24	29.7	50.5 ± 4.7	—	—	12	27.6	39.6 ± 3.2	—	—	14	23.6	39.0 ± 5.4	—	—
Mitral stenosis before operation	9	43.2	54.5 ± 5.1	2.13	<.05 >.02	9	43.2	44.3 ± 5.1	2.70	<.02 >.01	8	41.9	35.4 ± 1.3	1.84	<.10 >.05
Mitral stenosis 3–6 mos. after operation	7	41.0	52.0 ± 5.4	.72	<.5 >.4	7	41.0	41.4 ± 4.2	1.05	<.4 >.3	7	41.0	36.8 ± 4.8	.91	<.4 >.3

* The figures for total body water and for exchangeable potassium in healthy females are taken from previously published results obtained in this laboratory.[6, 7] The figures for exchangeable sodium are a combination of the results in seven females reported by Forbes and Perley[3] and in five females studied in this laboratory; there was close agreement between these two series.

case 2 the measurement was made eight days after operation following a large water diuresis and an increase was not seen (fig. 2*C*). It is thus clear that in patients allowed unrestricted access to fluids, water retention readily occurred after operation even though the sodium intake was severely restricted. For example in case 3 there was a gain in total body water of 2.3 liters as measured on the fifth postoperative day, but the isotope studies showed an increase in exchangeable sodium of only 22 mEq. and by cumulative balance an increase of 55 mEq. Similarly, in all the other cases studied water was retained in considerable excess of sodium. The water retention persisted in these cases for the first week after operation but thereafter subsided. The measurements had all fallen to about the preoperative value by the time of discharge from the hospital which was usually between two and three weeks after operation.

In cases 7 through 11 the fluid intake was restricted to 1500 ml. a day for the day of operation and the early postoperative days. This figure does not include restoration of the operative blood loss and chest drainage fluid which were approximately covered by blood transfusions. In these cases with a strictly regulated fluid intake there was a steady loss of weight after operation and the total body water measurements no longer showed any definite increase but remained either approximately unchanged or slightly decreased (table 1 and fig. 7).

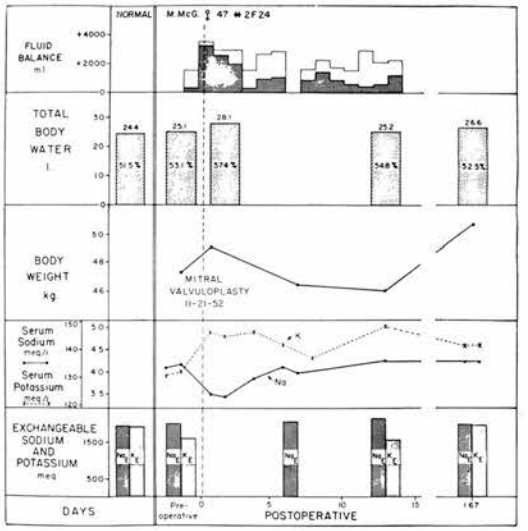

FIG. 6. Case 5. Charted as in figure 1*C*. The postoperative water loading, later excreted, with associated weight change is shown. The inverse sodium-potassium relationship in the serum is characteristic. The initial body composition shows an elevation in body sodium and a lowering of body potassium. In late convalescence (167 days after operation) body weight has increased significantly with a fall in total exchangeable sodium, and a rise in total exchangeable potassium. Although the absolute body water content has increased, the relative fraction (52.5 per cent of body weight) is at its lowest observed value, suggesting that body fat has been deposited.

Water Excretion in Relation to Total Body Water. The changes in total body water occur-

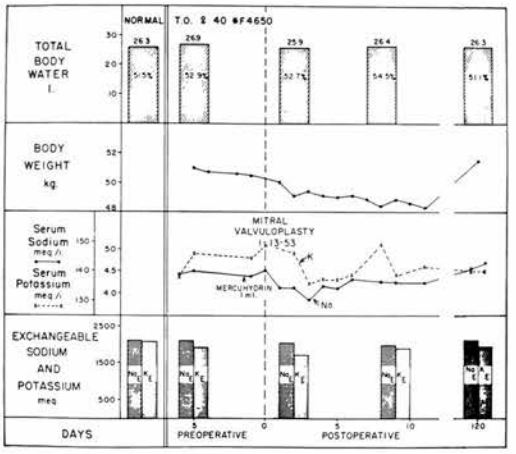

FIG. 7. Case 7. Charted as in figure 1*C*. Fluid intake was restricted to 1500 ml. There is no weight gain in the early postoperative period. Serum electrolyte changes are similar to those observed in other cases, but much less marked. Starting body composition shows a normal body sodium, and a lowered body potassium. Study in late convalescence (120 days) shows a return of body potassium towards normal. The total body water has now returned to normal in both absolute and relative terms.

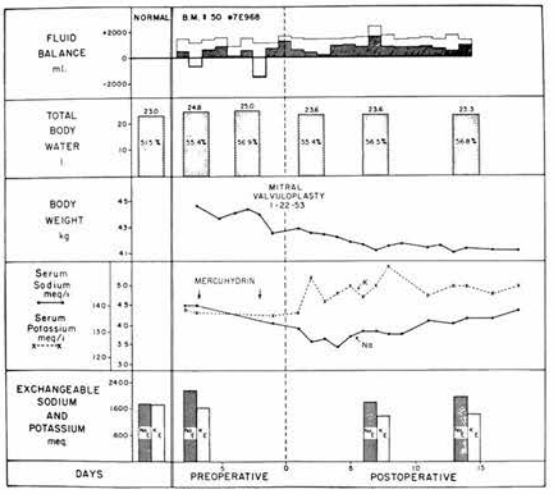

FIG. 8. Case 9. Charted as in figure 1*C*. See text for details.

ring shortly after operation can be correlated with water retention as shown by plotting the fluid intake and the urine output as a "balance." This clearly takes no account of extrarenal fluid loss, but nevertheless it shows that in those cases with considerable increases in total body water after operation there was a great excess of fluid intake over urine output

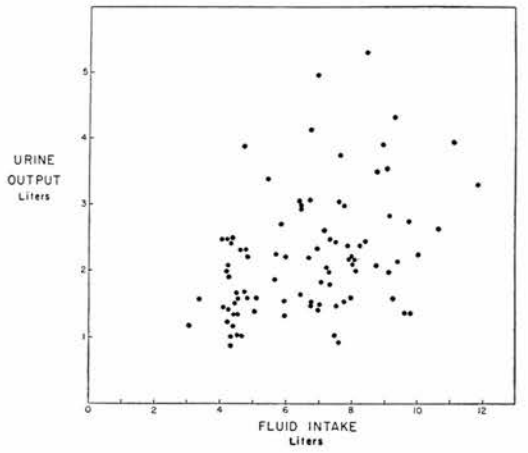

FIG. 9. Relationship between fluid intake exclusive of blood (abscissa, in liters) and urine output (ordinate, in liters). Each dot represents the total for the day of operation plus the two succeeding days, for a single patient. Data are based on study of 90 patients in all three of the fluid regimes discussed in the text. It will be noted that as fluid intake for these three days is increased over 4500 ml. a larger urine output (over 3000 cc.) results in only 12 instances. In all others the excessive fluid intake resulted only in excessive fluid retention. It should be noted that oliguria has been very rare in our experience with mitral stenosis surgery and therefore is but a very rare cause of the commonly observed hyperkaliemia.

(figs. 5 and 6). This was most noticeable during the day of operation and the first postoperative day when the urine output was always low even though the intake was extremely large. On the other hand the patients with a restricted fluid intake still maintained in this immediate postoperative period a urine volume similar to that seen in the unrestricted patients. In consequence there was no gross excess of fluid intake over output and no rise in total body water (figs. 7, 8, 9).

The ability of the kidney to excrete water during the first few days after a mitral valvuloplasty was studied more extensively by comparing three groups (30 cases each) in whom varying policies were followed with regard to fluid administration (table 3). All were investigated during the winter months when large cutaneous losses of water due to hot weather may be excluded. In one group of cases studied during this period (January to March, 1953), the mean total fluid intake exclusive of blood

TABLE 3.—*Changes in Urine Output and in Blood Chemistry Compared with Different Fluid Intakes. The Fluid Intakes and Urine Outputs Represent the Totals for the Day of Operation and the Two Succeeding Days*

Period of Study		No. of cases			Age Yrs.	Fluid intake for 3 days *ml.*	Urine output 3 days *ml.*	Blood transfusion. *ml.*	Blood and Serum Chemistry							
									Before Operation				On 2nd day after Operation			
		F	M	Total					Na *mEq./L.*	K *mEq./L.*	Cl. *mEq./L.*	BUN *mg./100 ml.*	Na *mEq./L.*	K. *mEq./L.*	Cl *mEq./L.*	B.U.N. *mg./100 ml.*
Gr. 1. 1/53 to 3/53	Mean	24	6	30	40.0	4400	1750	841	137.7	4.64	107.5	11.9	131.4	5.15	100.3	32.3
	s.d.				5.7	410	630	634	4.6	0.38	4.1	4.4	4.9	0.59	4.6	21.0
Gr. 2. 10/52 to 1/53	Mean	25	5	30	41.5	7610	2340	1172	137.4	4.54	106.4	11.9	127.8	5.21	97.2	31.4
	s.d.				8.4	1240	930	668	2.6	0.33	2.1	4.3	1.4	0.66	3.8	18.7
Gr. 3. 1/52 to 3/52	Mean	20	10	30	36.7	7860	2560	575	138.4	4.65	105.8	11.3	128.0	5.06	94.7	17.3
	s.d.				6.7	1594	908	173	3.8	0.56	3.1	4.8	7.0	0.66	5.0	10.7

transfusion for the day of operation and the two succeeding days was 4400 ml., and the urine output 1750 ml. In the other two groups the patients were allowed to drink freely; intravenous infusions of 5 per cent dextrose solution were frequently given and the fluid intakes were consequently much larger. In the period October 1952 to January 1953, the mean intake of 30 cases was 7610 ml. over the same three days while the urine output was 2340 ml. Thus an increase of 3210 ml. in intake was only associated with a gain of 850 ml. in urine volume. Similar results were obtained in a group of 30 cases with unrestricted fluid intake studied a year earlier in the months January to March, 1952. In comparison with the restricted cases in the first group the intake in this third group was greater by 3460 ml. but the urine output only by 810 ml. The individual figures in these 90 cases are shown in figure 9, where it is apparent that in the majority of cases the kidneys were not able to respond proportionately to a large water load in the immediate postoperative period.

Sodium Metabolism. This was investigated by balance studies, by frequent measurements of serum sodium concentrations and by determinations of total exchangeable sodium at approximately weekly intervals. The sodium intake in all the cases was restricted, though to a variable degree. In cases 1 and 2, studied by both balance and isotope dilution technics, the sodium intake was approximately 40 mEq. a day (figs. 1*A*, and 2*A*). In case 3, also studied by both methods, the intake was only 9 mEq. a day (fig. 3) and a similar restriction was applied to cases 4 to 11 in whom isotope studies only were carried out.

In case 1 the patient was steadily retaining sodium both before and after operation as is shown in the cumulative balance chart (fig. 1*B*). This sodium retention continued up to the time of discharge from the hospital. In case 2 there was similarly preoperative retention of sodium, interrupted transiently by an injection of Mercuhydrin (fig. 2*B*). After operation sodium retention continued at approximately the same rate, but on the fifth day after operation a spontaneous sodium diuresis began and 500 mEq. were excreted in five days. Towards the end of the sodium diuresis an injection of Mercuhydrin was given, but this was clearly only responsible for a small part of the sodium excretion. Subsequently up to the time of discharge from the hospital there was a further period of steady sodium retention. In both these cases the isotope measurements confirmed the increase of sodium in the body (table 1). The initial preoperative measurements of total exchangeable sodium were in the upper part of the normal range and the further sodium retention accordingly led to unduly high values. In spite of this evidence of a growing excess of sodium in the body, the serum sodium concentration fell significantly after operation (figs. 1*B*, 2*B*). In case 1 this fall was from 138 mEq. per liter to 125 mEq. per liter, and in case 2 from 140 mEq. per liter to 125

TABLE 4.—*Comparison of Decreases in Serum Sodium and Chloride Concentrations on Second Postoperative Day in Three Groups of Patients Receiving Different Fluid Intakes*
(See table 3 for details)

	Changes in Serum Sodium			Changes in Serum Chloride		
	Mean decrease in serum sodium ± s.d. *mEq. L.*	*t*	*p*	Mean decrease in serum chloride ± s.d. *mEq. L.*	*t*	*p*
Group 1. Restricted fluids	6.3 ± 4.4		—	7.2 ± 4.9	—	—
Group 2. Unrestricted fluids	9.6 ± 3.4	3.27	<.005 >.001	9.2 ± 4.9	1.59	<.2 >.1
Group 3. Unrestricted fluids	10.4 ± 6.3	2.93	<.005 >.001	11.1 ± 6.6	2.60	<.02 >.01

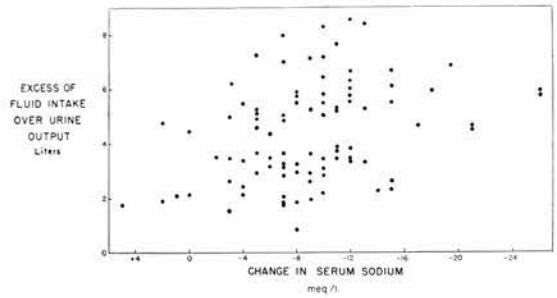

FIG. 10. Relationship between serum sodium change (abscissa, in mEq. per liter) and excess of fluid intake over output (ordinate, in liters) based on the same three-day periods, indicated in figure 13. Data are based on the same 90 patients shown in figure 13. It will be noted that as fluid intake exceeds output by greater volumes, there is a tendency to lower the serum sodium more markedly. However in the lower intake ranges (excess over output being 2.5 liters or less) considerable drops may still be seen and correlation is less clear cut.

mEq. per liter. The levels were restored slowly after operation. In case 2 it is of considerable interest that the rise in serum sodium concentration began immediately before and continued during the large sodium diuresis (fig. 2*B*).

In the other cases with the more severely restricted sodium intake there was no opportunity for any significant degree of sodium retention. In case 3 the cumulative balance (fig. 4) and the isotope dilution studies (table 1) showed no sodium depletion. Nevertheless, after operation the serum sodium concentration fell sharply from 141 mEq. per liter to 122 mEq. per liter (fig. 5). Thereafter it rose slowly and was finally restored to the preoperative level. The large changes in serum sodium concentration observed in this case clearly occurred without any significant changes in the amount of sodium in the body. Similar observations were made in cases 4, 5 and 6 in whom definite postoperative decreases in serum sodium developed with, at the same time, slight increases in the total exchangeable sodium (table 1 and fig. 6).

In cases 1 to 6 fluid was given freely, but even when the fluid intake was restricted so that there was a progressive fall in body weight and no increase in total body water after operation the serum sodium concentration still fell (fig. 7). Further evidence was sought by comparing measurements made preoperatively and on the second postoperative day in the three groups of cases, 90 in all, described above (table 3). In the two groups with unrestricted fluid intakes the decreases in serum sodium were greater than in the group with restricted fluid intake. Specifically, the unrestricted groups showed mean postoperative serum sodiums of 127 and 128 mEq. per liter while the restricted group was 131 mEq. per liter. These are averages of three groups of 30 cases each. Statistical analysis shows that these differences are significant (table 4), and in both instances the comparisons contrast unrestricted fluid regimens with the restricted group. There is thus some relation between the excess of fluid intake over urine output and the extent of the decrease in serum sodium concentration. The larger falls in serum sodium were usually seen in those patients showing considerable fluid retention (fig. 10). It is, how-

ever, quite clear that decreases in serum sodium concentration still occurred even in the presence of a low fluid intake and adequate urine volume.

Persistent Low Serum Sodium Concentrations. It is to be emphasized that in the majority of cases the transient fall in serum sodium characteristically observed after operation was not associated with any loss of sodium from the body and occurred in patients having adequate amounts of total exchangeable sodium. Usually the serum sodium began to rise on the fourth or fifth postoperative day and thereafter steadily climbed to the normal range. However, in a certain number of cases the serum sodium level either before or after operation was persistently low. This condition was particularly seen in severely disabled patients kept strictly on a low sodium diet and treated vigorously with mercurial diuretics. In them the measurements of total exchangeable sodium often showed some evidence of salt depletion. The following case is illustrative of those few which showed sodium depletion prior to operation.

Case 8 was that of a female with severe mitral stenosis and auricular fibrillation who had been treated with a low salt diet for six months prior to operation. On admission to the hospital there was no peripheral edema but there was hepatomegaly and considerable pulmonary congestion. She was accordingly given several injections of Mercuhydrin before operation (fig. 11). On the day before operation the serum sodium was only 133 mEq. per liter and the total exchangeable sodium was 1575 mEq. or 33.5 mEq. per kilogram, a low figure for a person of her slim build. After operation the fluid intake was restricted to 1500 ml. a day; there was a slow loss of weight and a slight gain in total body water. The serum sodium fell to 125 mEq. per liter in which region it remained for nine days in spite of a highly successful valvuloplasty. A total exchangeable sodium measurement on the seventh postoperative day showed an increase of 78 mEq., confirming that the losses of sodium from hemorrhage at operation and in chest drainage fluid had been adequately restored. During this period she was clearly not making good progress. She could only think and speak slowly though there was no evidence of any definite paralysis. She was largely indifferent to her surroundings and was frequently incontinent of urine.

On the tenth postoperative day she was given an infusion of 3 per cent sodium chloride yielding 240 mEq. of sodium. During the 24 hours from the beginning of this infusion the total fluid intake was 1250 ml., and the urine output of 650 ml. contained a total of 7 mEq. of sodium. Thus 237 mEq. of sodium was retained in the body after 24 hours; at the same time the serum sodium had risen from 125 mEq. per liter to 131 mEq. per liter. Thereafter there was a great improvement in her general condition and a slow steady rise in the serum sodium concentration to 142 mEq. per liter even though the sodium intake was kept at 9 mEq. a day. The total exchangeable sodium, measured five days after the infusion, when the serum sodium had reached 138 mEq. per liter, was 1855 mEq., an increase of 202 mEq. over the reading before the infusion. At the same time the total body water had increased by 0.9 liters. Further measurements were made 118 days after operation, when she was

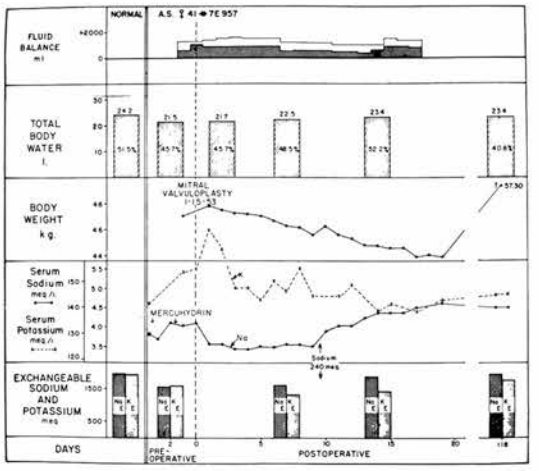

FIG. 11. Case 8. Charted as in figure 1*C*. Fluid intake was restricted and is remarkably constant. There is weight gain for one day only, followed by a steady loss until discharge. Serum electrolytes show several significant points. Sodium, low to begin with, falls after operation and is persistently low. On the ninth postoperative day a sodium load of 240 mEq. was given as 3.0 per cent sodium chloride. The effect on serum sodium is initially very small, but is followed by a rise which persists to normal values. The potassium rises rapidly to high levels (6.5 mEq. per liter), falls abruptly without specific therapy, and later falls further as the sodium rises. Body composition is initially remarkable in that there is a low total exchangeable sodium as well as the expected low total exchangeable potassium. Study in late convalescence shows a return of all values toward normal along with a gain of 10 Kg., one of the largest observed. It is of great significance that with this weight gain the water fraction of body composition falls to 40.8 per cent, indicating a large accumulation of fat.

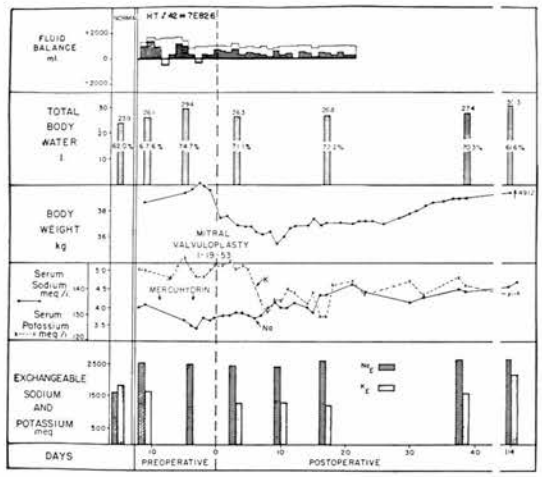

FIG. 12. Case 11. Charted as in figure 1*C*. Clinical details are described in the text. Serum electrolytes show change similar to case 8, figure 11; sodium is low preoperatively and goes lower as weight and water are gained; postoperative changes are not marked. The potassium is initially high but postoperatively does not rise further. Starting body composition is remarkable for its large excess of sodium, and the remarkably low body potassium as postoperative changes become apparent. The total body water at 74.7 per cent of body weight is among the highest observed, and was borne out by the remarkable cachexia seen clinically. In late convalescence (114 days) weight gain has been marked (11 Kg.); the water fraction has fallen to 61.6 per cent of body weight. Although the progression of body composition toward normal has been great, the patient at this time still has a relatively high total body water, and his body sodium-potassium ratio is still elevated.

in excellent health, had gained 12.5 Kg. in weight and had an enormously improved exercise tolerance. The results at this time are shown in table 1, and, using these figures for comparison, there is evidence that there was a deficiency of sodium in the body when she came to operation. At that time she had 1575 mEq. of exchangeable sodium in contrast to 1915 mEq. when she was in good health. Normal serum sodium concentrations were only attained when this deficiency was restored.

Similar features were seen in case 9, a female with a tight mitral stenosis, minimal aortic regurgitation and auricular fibrillation. On admission the serum sodium concentration was 140 mEq. per liter and the total exchangeable sodium 2219 mEq. or 49.6 mEq. per kilogram. She was treated preoperatively on a 9 mEq. a day sodium diet, mercurial diuretics and ammonium chloride. On this regime the total body water did not change but the serum sodium concentration fell to 133 mEq. per liter before operation (fig. 8). The estimated loss of sodium through hemorrhage and chest drainage was about 150 mEq. but she only received about 90 mEq. in the transfusions given at operation. The postoperative measurement of total exchangeable sodium was only 1871 mEq., a decrease of 348 mEq. The serum sodium concentration fell to 125 mEq. and was unduly slow in returning to the normal range, reaching only 135 mEq. per liter on the fourteenth postoperative day; at this time the sodium deficiency was still 264 mEq. in comparison with the preoperative reading. In this case the large sodium loss resulting from the mercurial diuretics and operation apparently impeded the restoration of the serum sodium concentration.

In the two cases described above fluid intake was restricted to 1500 ml. a day, and there was no evidence of water retention when the serum sodium concentration was persistently low. Retention of water without sodium may, however, occur preoperatively as is illustrated in the following case.

Case 11 was that of a male who had been severely disabled with mitral stenosis and auricular fibrillation for several years. During the last two years his condition deteriorated rapidly and for some time before study he was confined to bed with extreme respiratory distress even at rest. He had lost a large amount of weight. On admission, he was grossly emaciated, there was some peripheral edema and the serum sodium was 138 mEq. per liter. After treatment with mercurial diuretics and restriction of sodium intake to 9 mEq. per day the peripheral edema disappeared but the serum sodium fell. When the detailed preoperative studies were begun (fig. 12) the serum sodium was 134 mEq. per liter, the total exchangeable sodium 2507 mEq., or 64.9 mEq. per kilogram and the total body water 26.1 liters. His condition began to deteriorate further and he became more breathless, apathetic and disorientated. He slowly gained weight. A week later the serum sodium was 126 mEq. per liter, the total body water 29.4 liters and the total exchangeable sodium 2501 mEq. Thus he had gained 3.3 liters of water without any increase in body sodium. After this measurement his fluid intake was restricted to 1 liter a day, he lost weight and the serum sodium concentration rose slightly. There was some improvement in his general condition and a successful valvuloplasty was carried out under local anesthesia despite the apparently preterminal state of the patient. The fluid restriction was continued, there was no further fall in serum sodium concentration and there was a conspicuous improvement in his condition. The total body water on the third postoperative day was 26.3 liters. Subsequently the serum sodium concentration rose

steadily to normal, and there were small increases in total body water and exchangeable sodium. On the thirtieth postoperative day he was changed to a high calorie diet with a sodium intake of 35 mEq. a day. Thereafter, while in the hospital, he gained weight steadily with only slight further increases in total body water and exchangeable sodium but a considerable rise in total exchangeable potassium. In this case the results suggest that there was a small deficiency of sodium in the body before operation when the serum sodium was low despite the apparently high figure for exchangeable sodium on a body weight basis. The striking feature, however, was the ease with which he retained water at this time, leading to a further depression of the serum sodium.

Potassium Metabolism. This was investigated by the same methods as in the sodium studies. In all cases there was a loss of potassium from the body after operation clearly shown in the balance studies and confirmed by the isotope dilution measurements (figs. 1*A*, 2*A*, 7 and table 1). Coincident with this large excretion the serum potassium concentration rose. This was a constant finding in all the cases studied (figs. 1*C*, 2*C*, 5–8). The increased potassium excretion was at its greatest on the day of operation despite the oliguria and did not persist for so long as the increased nitrogen excretion. The extent of the rise in serum potassium concentration could not be correlated with the amount of blood transfused at operation, the fluid intake in the immediate postoperative period or the volume of urine excreted (table 3). There appeared to be a definite inverse relationship to the serum sodium concentration, as the largest falls in the latter were invariably accompanied by considerable increases in the serum potassium concentration. Furthermore in those cases where the serum sodium concentration was persistently low either before or after operation the serum potassium remained consistently high (figs. 8, 11, 12) and only fell when the serum sodium concentration rose.

Correlation of Isotope and Balance Measurements of Sodium and Potassium. In cases 1 to 3 the results of the isotope dilution studies may be checked against the cumulative metabolic balances for sodium and potassium. This comparison is summarized in table 5 where the changes from the preoperative to the last measurement before discharge from the hospital are shown. During this period of three to four weeks there is good agreement between the sodium measurements. In two of the potassium studies the correlation is also reasonably close, but in case 3 there is some discrepancy between the two methods, the isotope measurements showing a greater loss of potassium than can be accounted for in the balance study. The extent of agreement that may be anticipated between the two methods has been more fully reviewed elsewhere.[13, 14, 15]

Chloride Metabolism. This has been studied only by measurement of serum concentration before and after operation. In general the changes ran parallel to those found in sodium concentrations. After operation there was a considerable fall (table 3), and this was affected in the same way as the sodium by restricting the fluid intake (table 4). The scatter in the chloride determinations was, however, greater and the changes do not attain such a high degree of significance.

Changes in Blood Urea Nitrogen. These were studied in the three groups of cases shown in Table 3. There was invariably a rise in blood urea nitrogen after operation related to at least two factors, namely the size of the blood transfusions given at operation and the fluid intake and urine output after operation. The rise was least in group 3 where the smallest blood transfusions were given and fluid intake was unrestricted; in both groups 1 and 2 the rises were larger and approximately similar, but in the group 2 cases the blood transfusions and fluid intake were greater than in the group 1 cases.

TABLE 5.—*Comparison of Changes in Sodium and Potassium in the Body as Measured by Metabolic Balance and Isotope Dilution*

	Interval, days	Change in Sodium		Change in Potassium	
		Balance *mEq.*	Isotope *mEq.*	Balance *mEq.*	Isotope *mEq.*
Case 1.....	21	+250	+208	+55	−90
Case 2.....	28	+250	+198	−75	−141
Case 3.....	22	+76	+189	−44	−337

Changes in Late Convalescence

Nine patients returned for further studies between three and six months after discharge from the hospital. In all a good functional result had been obtained. Their exercise tolerances were greatly improved. In the majority (cases 1, 2, 4, 5, 7, 8, and 11) there was a corresponding improvement in nutritional state following the operation. The striking features were the gain in weight and the return of the body composition toward normal (table 1 and figs. 1*C*, 2*C*, 5–7, 11, 12). In case 6, however, convalescence had been complicated by the development of two attacks of pneumonia and pleurisy. She had lost weight and her general condition had deteriorated in spite of considerable relief of her dyspnea. Her body composition was still abnormal. In case 3, although the cardiac symptoms had greatly improved so that she was able to lead a practically normal life, there was persistent anorexia after operation, possibly due to high digitalis dosage, and she had failed to gain weight.

The results in seven female patients, three to six months after operation, are shown compared with the healthy adults and the preoperative measurements in table 2. Case 6 has been excluded from this group as she was not fully recovered from the pneumonia when the measurements were made. These patients now show no significant differences as regards body composition in comparison with the healthy adults. In case 1 a further study was possible one year after operation; she continued to gain weight and lose sodium over this period. In the male patient, case 11, who had been greatly emaciated, some restoration of body composition towards normal had occurred after 114 days (fig. 12). Even though he had gained over 10 Kg. in weight the process was still clearly incomplete. With improved cardiac function he was rebuilding muscle mass rapidly and in 114 days had increased his total exchangeable potassium from 1290 mEq. to 2097 mEq., almost double the earlier figure. His water fraction had lowered from its high value of 74.7 per cent to 61.6 per cent, as clear evidence of fat accumulation. At this time his clinical appearance was that of a most dramatic "filling out" of subcutaneous fat, and muscular masses; no longer bedridden, he was active about the house.

His total exchangeable sodium was still elevated as evidence of some further metabolic convalescence yet to be completed.

Discussion

Disordered Biochemistry. The body composition of the patients with mitral stenosis before surgery showed certain abnormal features when contrasted with that of healthy adult women, namely a relative excess of sodium and water. Such features are, of course, commonly seen in edematous patients,[16, 17, 18] but in these cases no clinically detectable edema was present at the time of the measurements. These changes may also be seen as a result of undernutrition[17, 19] and of various chronic wasting diseases[20] which lead to a loss of cell mass and of fat. Chronic cardiac disease undoubtedly falls into this category and the preoperative body composition is probably due to the wasting rather than latent edema. In this respect it is of interest that with the gain in weight seen over the course of several months after a successful valvuloplasty the proportions of water and sodium in the body decrease, as the exchangeable potassium rises. This is clear evidence of an increasing muscle mass, and an increasing store of body fat. Taken together, these indicate a reversal of the "syndrome of depletion" and a rebuilding of those energy-exchanging and energy-storing tissues vital to an active existence.[8] Taken together these changes are a most eloquent witness to the effectiveness of the valvuloplasty itself; where cardiac function is slow to return, or fails to return, such restorative changes are not seen. Similar changes are seen in the rehabilation of those suffering from chronic undernutrition[19] and in convalescence from a successful surgical operation.[20, 21]

Immediately after operation several complex changes develop, which are common to all types of trauma and are in no way peculiar to those undergoing mitral valvuloplasty. These features, which are also regularly seen in patients without cardiac disease, include the increased nitrogen excretion,[1, 21] the loss of potassium in excess of nitrogen,[1] the fall in eosinophil

count[22] and the rise in urinary excretion of 17-ketosteroids and 17-hydroxycorticoids.[23] In noncardiac patients also, postoperative retention of water, decrease in serum sodium and elevation of serum potassium concentration frequently occur.[1, 24, 25] However, in patients with mitral stenosis undergoing surgery these changes are often extremely conspicuous and merit further consideration.

The mechanism of the changes in serum sodium concentration seen postoperatively is obscure. The results reported here clearly show that the serum sodium concentration falls postoperatively even though there is no loss of sodium from the body and, more strikingly, even where sodium loading is occurring. Retention of water may play a part, but when the fluid intake is restricted so that no apparent increase in total body water occurs a definite decrease in sodium concentration still develops. Figure 10 offers graphic evidence that in general there is a poor correlation between water retention and postoperative hyponatremia. Thus the evidence suggests that "external dilution" is not the only significant factor, if it is assumed that the retained water is evenly distributed over the body. Clearly, if fluid were held preferentially in the extracellular fluid, only a small amount would be required to produce a definite decrease in the sodium concentration. Changes in total body water of under 1 liter cannot be detected with confidence by the deuterium oxide method.

Other factors that may be of importance are movements of electrolytes and water between the intracellular and extracellular compartments. After operation potassium leaves the cells in considerable quantity and is rapidly excreted at a time when there is marked oliguria. It is possible that at the same time water moves out of the cells into the extracellular fluid where it is retained and dilutes the sodium and chloride ("internal dilution"). Thirst is a prominent postoperative symptom and may possibly be associated with such a cellular dehydration.[26] Alternatively sodium may move into the cells replacing potassium, though direct measurements have not yet shown such a transfer in patients with medically treated heartfailure,[27] or may enter bones or the gastrointestinal tract which both contain large stores of sodium.[28, 29, 30]

In patients with a persistently low serum sodium concentration, either before or after operation, several factors may be at work. Sodium depletion may develop as a result of a low sodium diet and treatment with mercurial diuretics[31, 32] or from inadequate restoration of sodium lost in hemorrhage at operation and through drainage of fluid from the chest. Excessive retention of water readily occurs in sick patients placed on a low sodium diet, particularly if they are subjected to the stress of an operation or respiratory infection. Certain of the features seen in the mitral stenosis patients after operation may be reproduced by the administration of antidiuretic hormone. This leads to retention of water, depression of serum sodium concentration and the development of symptoms associated with water intoxication.[33,34] In patients with heart disease and a low serum sodium concentration there is persistent antidiuretic activity.[35]

Therapeutic Considerations. From the practical therapeutic point of view certain general principles emerge from this study. Patients undergoing mitral valvuloplasty show all the postoperative features commonly seen in general surgical cases. Their significance and therapeutic implications have been described elsewhere.[1] However, the disturbances of fluid and electrolyte metabolism tend to be unduly large in comparison with noncardiac patients and it is in dealing with them that additional care is often required.

In one of the groups, the majority, the serum sodium concentration is normal before operation and the total exchangeable sodium is high. The postoperative fall in serum sodium is not due to body sodium depletion and constitutes part of the physiologic response to trauma. It is transient, is not harmful to the patient and is not an indication for the administration of extra sodium. After operation the patient will readily retain water even in the absence of salt. Excessive fluid intake at this stage depresses still further the serum sodium concentration and may aggravate the patient's condition. Thirst is not a reliable indication of dehydration; it may be present even though there has

been fluid retention of over 3 liters. The most valuable indications in regulating the fluid intake are the patient's weight and urine volume. A series of preoperative weight measurements is of great assistance as a loss normally occurs after operation due to metabolism of fat and lean tissue.[1] If the weight remains steady or increases, excess water is clearly being retained. In general, in a patient of average size the fluid intake in the days immediately after operation should not exceed 2000 ml. a day unless there are special indications such as for example high fever, unduly hot weather, or large drainage from the chest. Similarly the daily excess of fluid intake over urine volume should not be greater than about 1500 ml. unless an exceptionally large extrarenal loss of fluid can be demonstrated.

In another group of patients, a minority, the serum sodium concentration is persistently low. Before operation in the nonedematous patient this is suggestive of salt depletion due to a combination of low sodium diet and administration of mercurial diuretics.[31] The condition may be aggravated or appear later if the sodium loss at operation is not fully replenished. In the postoperative period sodium depletion may be suspected if the serum sodium has not begun to rise after a week from the date of the valvuloplasty and the case is otherwise uncomplicated. In these circumstances administration of hypertonic saline and fluid restriction are indicated to return the body composition to normal. It is very important to emphasize that a persistent low plasma sodium either before or after operation may occur in the absence of sodium depletion (an example is found in case 11, figure 12) and with the "high body water, high body sodium, low body potassium" syndrome of depletion. In such patients water restriction and caloric intake are of much greater importance than giving sodium. High dosage of the latter even over short periods may lead to disastrous pulmonary edema *while the plasma sodium still remains low*. In this second group with a persistent low plasma sodium, therefore, one must approach sodium therapy with great caution and search diligently for evidence of true sodium depletion before giving over 150 mEq. of sodium. The urine sodium concentration should be determined in all such cases to avoid overlooking the rare "urinary salt loser" in whom energetic sodium replacement is vital.

In none of these patients was the elevation of serum potassium associated with indisputable evidence of potassium toxicity. Were such to occur in the postoperative period, the administration of hypertonic saline intravenously might be of benefit. Such a benefit has been observed in other cardiac surgical patients and in surgery undertaken during the uremic state.

When edema is present, even in minimal amounts, restriction of fluid intake to 1200 to 1500 ml. a day often alleviates hyponatremia, and sodium administration with its hazard of increasing already expanded stores of salt and water may be avoided. However, if hyponatremia exists with symptoms such as disorientation, delirium, muscular weakness, apathy or stupor, hypertonic saline and fluid restriction are indicated for the rapid relief of these manifestations.

A quick restoration of the serum sodium level should not be anticipated or attempted. Measurements of total body sodium have shown that after an adequate amount of sodium has been given there may be only a relatively small immediate rise in serum sodium as readjustment of the balance between sodium and water in the body apparently requires several days. In brief:

1. *Where surgery has been successful and caloric intake can be resumed* the electrolyte pattern will be observed to restore itself gradually to normal if water and salt loading are avoided. The reciprocal nature of the changes in body sodium and potassium and in plasma sodium and potassium will be noted. This restoration is merely a biochemical index of general convalescent rehabilitation.

2. *Where surgery has not been successful and caloric intake remains restricted* the electrolyte pattern remains distorted, the syndrome of depletion is unrelieved and, in our experience, a wide variety of maneuvers designed for "passive" restoration of water and electrolyte to normal are quite unavailing.

3. Finally there is a third or intermediate group where *preoperative depletion has been*

maximal, surgical convalescence is slow and caloric resumption hesitant. In these cases, water and salt restriction, daily weight measurement and caloric forcing are indeed life-saving measures. In addition, as mentioned above, hypertonic saline may be given intravenously under the following circumstances, using 250 cc. of 3.0 per cent sodium chloride: (a) Where plasma potassium is dangerously high and electrocardiographic evidences of toxicity are manifest. (b) Where there is a persistent low sodium without edema, fluid is being restricted, caloric intake has begun, and weight is constant or falling. Here a small dose of hypertonic saline will occasionally initiate a gradual restoration of the plasma electrolyte pattern to normal. This indication is clear if true sodium deficiency can be established by history or Na_E, as in case 8, fig. 11. (c) Where an acute depression of plasma sodium after operation progresses to 120 mEq. per liter or below, with associated symptoms. Here again a single dose of hypertonic saline given with caution may be of emergency value even though it appears to exaggerate the high body sodium aspect of the patient's disorder.

Summary

The effect of severe mitral stenosis on body composition has been studied before and after valvuloplasty.

In nonedematous patients immediately before operation there was a slight excess of sodium and water, and a depression of body potassium.

Immediately after operation the general features of a response to severe trauma were seen, such as an increased nitrogen and potassium excretion, retention of sodium, a fall in eosinophil count and an increased urinary steroid excretion. The changes in water and electrolyte metabolism were of the same nature but frequently greater in degree than those commonly seen in noncardiac patients after operation. Water retention readily occurred in spite of severe restriction of sodium intake. The serum sodium and chloride concentrations fell and the potassium rose. The fall in serum sodium was not due to loss of sodium from the body; it was greater in those showing considerable water retention but this was not the sole cause. In a few cases a combination of low salt diet, mercurial diuretics and incompletely restored operative loss led to salt depletion and a persistently low serum sodium concentration.

Body composition slowly returned towards normal over a period of many months after a successful operation. The gain in weight was due to the restoration of lean tissue and fat.

Acknowledgments

This work was supported throughout by the Atomic Energy Commission. We wish also to acknowledge the generous support of the American Heart Association, The New England Heart Association, The Upjohn Company and Winthrop Stearns, Inc.

Sumario Español

Pacientes con decompensación cardíaca crónica operados para la correción quirúrgica de la estenosis mitral han sido repetidamente observados tener una concentración baja de sodio plasmático y una concentración alta de potasio plasmático luego de la operación. Este estudio fué principalmente encaminado a investigar estas anormalidades. Los autores han demostrado quel el paciente preoperatorio tiene un desorden característico de composición orgánica, notable por una cantidad grande total de agua, una cantidad alta total de sodio, una cantidad total baja de potasio intercambiable y una concentración de sodio plasmático baja. Los efectos de la cirugía en esta composición anormal del cuerpo y las implicaciones terapéuticas se discuten.

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19.

Relationship Between Exchangeable Sodium and Rate of Sodium Excretion in Dogs With Experimental Valvular Lesions of the Heart

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Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 180, No 2, February, 1955
Printed in U.S.A.

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Relationship Between Exchangeable Sodium and Rate of Sodium Excretion in Dogs With Experimental Valvular Lesions of the Heart[1]

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AFTER surgical operations the amount of sodium in the body is frequently found to be increased (1). This sodium retention is usually transitory, but may be prolonged in patients or animals with heart disease (2, 3). Furthermore, the excretion rate of sodium following an infusion of isotonic saline is reduced in dogs with mild valvular heart damage (3). The possibility of a causal relationship between the increased sodium pool and the decreased rate of sodium excretion was suggested. If the total body sodium were increased the effectiveness of the same stimulus (i.e. size of infusion) might be reduced since the load would be a smaller fraction of the total body sodium.

In order to test this hypothesis measurements of the total exchangeable sodium (Na_E) in dogs have been made before and after the surgical production of right-sided valvular damage, which reduced the ability to excrete sodium. Observations have also been made before and after sham cardiac operations which did not affect the heart valves and did not result in a diminished sodium excretion.

METHODS

The procedure used for determining the rate of sodium excretion was identical with that described in the previous paper (3).

Total exchangeable sodium was measured by dilution of the isotope Na^{24} (4). Between 100 and 150 μc of Na^{24} were administered intravenously to dogs from a calibrated 50-ml syringe. The dogs were then placed in metabolic cages and the urine collected. Twenty-four hours later the bladder was catheterized, and a venous blood sample was withdrawn. The concentration of sodium in the serum was determined by lithium internal standard flame photometry. The radioactivity of the serum, urine and injection standards was measured with a Geiger-Muller counter. The error involved in the counting was approximately 2%, in the flame photometry approximately 2% and in the intravenous injection about 1%. The loss of radioactivity during the period of equilibration was measured only in the urine; fecal loss during this period is negligible (5). Total exchangeable sodium was then calculated as:

$$Na_E \text{ in mEq} = \frac{\text{counts injected} - \text{counts excreted}}{\text{counts/l. of serum} \div \text{sodium mEq/l. of serum}}$$

Measurements of total exchangeable sodium were made as close to the times of the diuresis studies as possible. In order to avoid the acute effects of surgery no observations were made until at least 2 weeks after the operations.

In one dog, balance studies were performed and the cumulative sodium balance was compared with the changes in total exchangeable sodium. This animal was kept in a metabolic cage and was fed on a constant daily diet containing sodium 40 mEq, potassium 32 mEq and chloride 35 mEq. Feces and urine were collected daily. The cage was washed three times with distilled water and the washings were added to the urine. The urine was analyzed for sodium by the internal standard flame photometer and for chloride by the potentionetric method of Kellogg (6). The feces were wet ashed and then analyzed for sodium as above and for chloride by the method of Wilson and Ball (7).

The lesions produced in the dogs were the same as those in the previous study, namely tricuspid insufficiency, pulmonary insufficiency and pulmonary artery stenosis (3). The techniques for producing these lesions were identical with those previously described.

RESULTS

Equilibration of injected Na^{24} with the body sodium was studied in one normal dog, one

Received for publication September 7, 1954.

[1] Aided by a grant from the Life Insurance Medical Research Fund and by the Eugene Higgins Trust through Harvard University. This work was also aided by a grant from the U. S. Atomic Energy Commission to the Peter Bent Brigham Hospital.

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TABLE 1

Date and Condition	Wt., kg	Mean Right Atrial Press, cm H_2O	Na_E, mEq (av)	Range and No. of Observations	8-Hr. Na Excretion, % of Load	Range and No. of Observations	Na Load as % of Na_E
Dog 1							
Control	17.5	2.5–3.0	717	710–728 (2)	81	77–88 (3)	9.8
TI[a]	15.8	12.0–13.5	785	737–825 (4)	48[h]	34–58 (3)	8.9
TI + PS[b]	16.3–19.3	31.0	1274[f]	1052–1519 (3)	1.4[h]	0.15–3.8 (4)	5.5
Dog 5							
Control	20.1	1.0–1.5	860	850–870 (2)	91	85–95 (4)	8.4
Sham[c]	19.3	1.5	856	856 (1)	93	82–103 (3)	8.3
PS	18.7–20.1	3.5	864	824–887 (4)	92	74–107 (4)	8.2
Mild TI + PS[d]	20.1	11.0	897	845–973 (5)	46[h]	36–60 (8)	7.8
Dog 8							
Control	23.6	−1.5	943	943 (1)	95	92–98 (3)	15.4
PI[e]	22.2	−1.5	928	919–937 (2)	86[g]	80–84 (4)	15.1
Dog 9							
Control	18.6	−0.5	939	939 (1)	82	71–99 (7)	15.7
PI	18.4	0.0	952	936–968 (2)	68[g]	55–80 (7)	15.3
Dog 11							
Control	20.5	2.0	964	964 (1)	53	51–59 (4)	15.2
Sham-PI	19.8–20.7	6.5	1069[f]	1025–1114 (2)	54	40–69 (5)	13.5
Sham-TI	19.6	7.5	961	961 (1)	53	48–58 (3)	15.2
Dog 15							
Control	19.6	0	986	980–992 (2)	86	82–89 (2)	15.4
Mild TI	18.9–20.2	5.0	1005	965–1045 (2)	68	44–79 (5)	14.6

[a] TI = tricuspid insufficiency. [b] PS = pulmonary stenosis. [c] Pericardotomy. [d] No clinical evidence of frank failure. [e] PI = pulmonary insufficiency. [f] Na_E change > 9%. [g] P between 0.05 + 0.01. [h] $P < 0.01$.

dog with pulmonary stenosis and in one dog in congestive failure as the result of tricuspid insufficiency and pulmonary stenosis. In the normal dog equilibration of injected radiosodium with the body sodium was substantially complete after 1 hour as judged from the fact that measurements of Na_E made after 1 hour and up to 24 hours after the injection were the same within the error of the method. A similar result was obtained in the dog with pulmonary stenosis. In the dog in failure, with gross edema and ascites, equilibration was complete 4 hours after the injection of Na^{24}. The volume of the ascitic fluid in this animal, as estimated by the dilution of Evans blue, was 2.4 liters.[7] These studies indicated that equilibrium of distribution of the Na^{24} had certainly been attained 24 hours after injection in all cases and that measurements made after this interval represented the total exchangeable sodium.

The reproducibility of the Na_E measurements was studied by making duplicate determinations in three normal dogs kept at an approximately constant weight and which showed no apparent change in their clinical condition. The coefficient of variation was ±3%. In view of these results and of experience with the dilution method elsewhere (8) changes in the Na_E greater than 9% are regarded as probably significant.

[7] The 2.4 liters of ascitic fluid was obtained by paracentesis at the conclusion of the experiment.

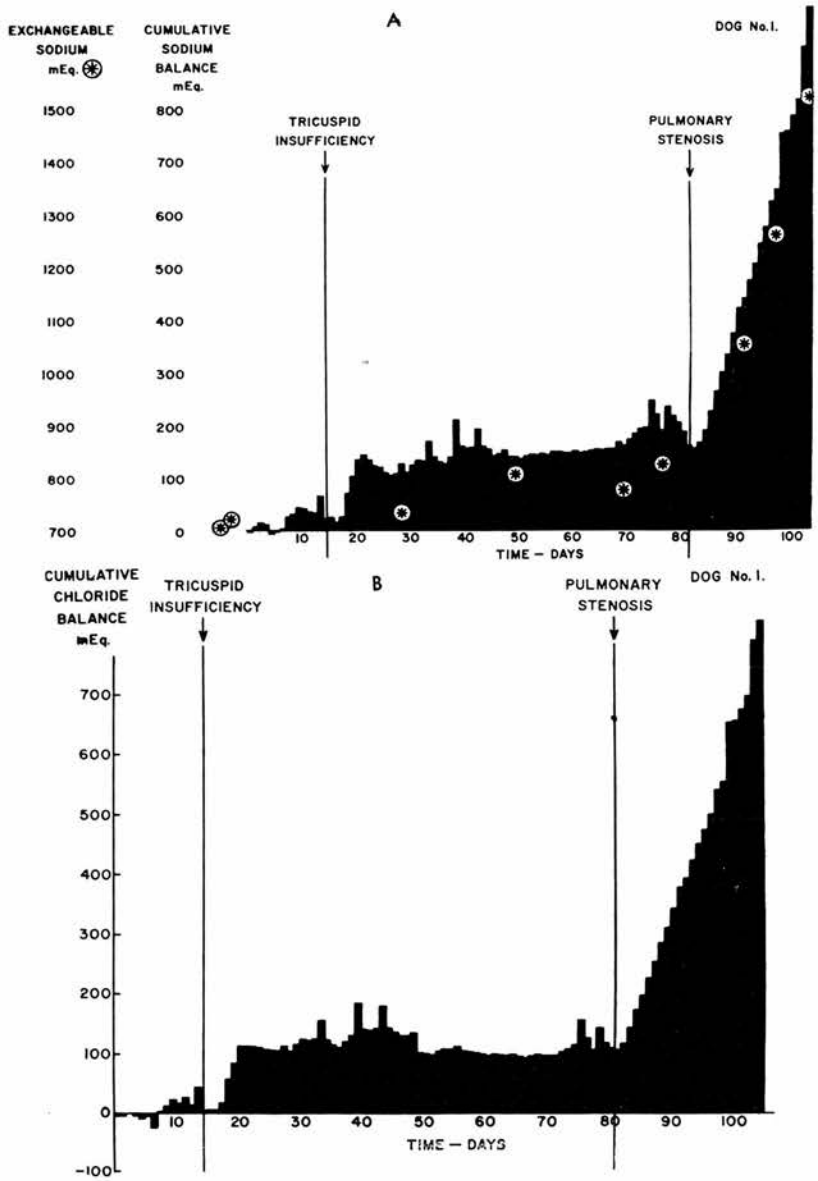

FIG. 1. *A:* Comparison of cumulative sodium balance and total exchangeable sodium in (*1*) normal dog, (*2*) same dog after tricuspid insufficiency, and (*3*) after superimposed pulmonary stenosis and the development of frank congestive failure. *B:* Cumulative chloride balance in the same dog.

The effect of cardiac valvular damage on Na_E and sodium excretion is summarized in table 1. Tricuspid insufficiency was produced in two dogs as an initial lesion (*dogs 1* and *15*). In *dog 1* changes in body sodium were studied both by balance measurements and by repeated determinations of Na_E. Both methods showed an increase in the body sodium. By the cumulative balance (fig. 1) the mean increase was 140 mEq of sodium and 100 mEq of chloride. This increase began three days after operation, when the restricted postoperative diet was changed to the regular diet, and continued in a stepwise fashion for three more days. After this time the cumulative balance remained relatively stable at the new level. The mean increase in body sodium measured by isotope dilution was 68 mEq

during the same period. The comparison of the two methods is illustrated in figure 1 where the points determined by isotope dilutions are plotted in relation to the corresponding balance figures. The discrepancy of 72 mEq between the two methods occurred in a balance period of over 2 months and is clearly within the experimental errors of the two methods. The agreement here is considerably closer than was obtained in studies on surgical patients (9). In the second dog with tricuspid insufficiency the elevation of the Na_E was not significant (table 1). However, after saline infusion a reduced rate of sodium excretion was noted in both animals.

Two dogs were studied before and after pulmonary insufficiency. These animals showed no conspicuous change in Na_E but the 8-hour sodium excretion was reduced (P between 0.01 and 0.05).

Pulmonary stenosis was performed as the first operation in one dog. There was no change in either the Na_E or the 8-hour sodium excretion after the production of the stenosis.

In two dogs (*dogs 1* and *5*) combined tricuspid insufficiency and pulmonary stenosis were produced. In *dog 1* frank congestive failure, with gross ascites, developed after the second operation. There was a large increase in Na_E and a great reduction in the 8-hour sodium excretion which fell to 2% of the preoperative value. During the period of gross sodium retention the cumulative balance and isotope dilution measurements were in close agreement (fig. 1*A*). In *dog 5* the valvular damage was less severe, ascites was not observed and the Na_E was not significantly increased. The 8-hour sodium excretion, however, was diminished to about 50% of the preoperative values.

In two dogs (*dogs 5* and *11*) control operations not involving the valves, namely pericardotomy and myocardotomy were performed. Neither operation altered the 8-hour sodium excretion following saline infusion. In *dog 5* there was no change in Na_E. In *dog 11* there was an increase of 10% in Na_E after right ventricular myocardotomy but after a second operation, at which a right atrial myocardotomy was performed, the Na_E was the same as the preoperative reading.

DISCUSSION

A considerable decrease in the rate of sodium excretion following saline infusion may occur following the cardiac valvular operations with either no demonstrable change in Na_E or only a slight increase. Pulmonary insufficiency consistently reduced the rate of sodium excretion slightly but the Na_E was unchanged. In the dogs with severe tricuspid insufficiency the rate of sodium excretion was more conspicuously reduced. The Na_E in one of the dogs with TI remained unchanged; in the other it was increased by approximately 10%, a figure which is on the border-line of significance.

After single valvular lesions it is clear that small alterations in total exchangeable sodium may occur. In all cases except one these changes were less than the error of the method. Nevertheless, in the cases with the lesions of either pulmonary insufficiency or tricuspid insufficiency a reduction in the rate of sodium excretion occurred. This reduction cannot be attributed to any gross alteration in the ratio of the infused sodium load to the total body sodium.

No consistent relation between Na_E and sodium excretion is apparent, but it is not clear whether or not the body may be sensitive to small changes in Na_E which the method fails to detect. It does appear, however, that large changes in the size of the body sodium pool are not responsible for the altered response to saline infusion seen after certain cardiac operations in dogs.

SUMMARY

The effect of experimental valvular lesions of the heart on total body sodium of the dog has been studied by metabolic balance and isotope dilution methods. These changes have been correlated with the ability to excrete an infused sodium load.

Minimal valvular lesions, such as either tricuspid or pulmonary insufficiency which consistently reduced the rate of sodium excretion produced a slight rise or no change in the amount of exchangeable sodium in the body. No relationship was apparent between these small changes in body sodium and the rate of excretion of a sodium load.

More severe valvular damage (combined tricuspid insufficiency and pulmonary stenosis) greatly reduced the sodium excretion rate. In one dog the rate was halved without any change in total exchangeable body sodium. In another sodium excretion was minimal and gross edema and ascites developed.

Pulmonary stenosis and sham operations on the myocardium and pericardium produced no significant alterations in body sodium or sodium excretion.

Considerable changes in the ability to excrete a sodium load clearly developed without any gross alterations in the amount of sodium in the body. The reduction in sodium excretion rate could not be attributed to the dilution of the infused sodium load in an expanded body sodium pool.

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Reprinted from THE LANCET, *September* 12, 1959, *pp.* 303–308

HYDROFLUMETHIAZIDE
A NEW ORAL DIURETIC

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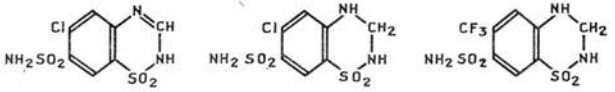
DURING the past few years many substances have been examined in an attempt to find an effective oral diuretic. Mercurial compounds given by injection have dominated diuretic therapy for nearly three decades, and it is only recently that their supremacy has been challenged by chlorothiazide. This substance was first synthesised by Novello and Sprague in 1957, and much clinical experience (Bayliss et al. 1958, Magid and Forsham 1958, Watson et al. 1958) has since shown its value. It is not, however, without untoward side-effects—in particular, the frequent production of hypokalæmia—and consequently the search for other effective agents has continued.

Two substances related to chlorothiazide—hydroflumethiazide and hydrochlorothiazide—have recently been produced, and are effective in much smaller quantity. Both these new compounds differ from chlorothiazide in having a saturated bond at position 3 : 4 in the molecule (fig. 1). Moreover, hydroflumethiazide has a trifluoromethyl group substituted for the chlorine atom in position 6. Two preliminary clinical studies of hydroflumethiazide have already been published (Hobolth et al. 1958, Sele 1958).

Evaluation of diuretics is difficult (Spencer and Lloyd-Thomas 1953): fluctuations in the patient's response occur for no obvious reason. Initially treatment may



Chlorothiazide. Hydrochlorothiazide. Hydroflumethiazide.

Fig. 1—Chemical structure of chlorothiazide, hydrochlorothiazide, and hydroflumethiazide.

TABLE I—CONSTANCY OF URINARY EXCRETION ON CONTROL DAYS IN TWO NORMAL MEN

Subject	Age (yr.)	Weight (kg.)	Number of experiments	Urine volume (ml.)	Total sodium excretion (mEq.)	Total potassium excretion (mEq.)
1	29	65	8	522 ± 201	60·3 ± 16·6	41·1 ± 6·43
2	41	78·5	10	761 ± 151	65·4 ± 16·1	47·0 ± 16·1

The figures are the means and standard deviations and relate to the 10-hour period from 7 A.M. to 5 P.M.

produce a diuresis and then refractoriness ensues, and this may be unassociated with any change in serum-electrolytes or urea. Thus in the comparison of diuretics, the great variability of the background has to be considered, and, although an attempt is made to obtain a " steady " state, the steadiness is always suspect. Hence we have first studied the action of hydroflumethiazide in normal subjects, and compared it with chlorothiazide and hydrochlorothiazide. Later the drug was given to a group of patients who would ordinarily have been treated with mersalyl.

Methods

The action of hydroflumethiazide, chlorothiazide, and hydrochlorothiazide was studied in two normal men. Urine was collected at 2-hourly intervals up to 10 hours after giving the drugs. Similar observations were also made on other days when no drugs were given (table I). The subjects carried out normal hospital and laboratory duties, but their fluid and food intake was kept as constant as possible. On this regime the two subjects had a similar sodium and potassium excretion. After giving a diuretic at least 3 days were allowed to elapse before a further experiment was carried out, to allow body composition to return to normal.

Thirty patients were studied, and all but three were initially treated in hospital. Where maintenance diuretic therapy was required, treatment was continued with hydroflumethiazide after discharge. As far as possible the patients were in a steady state before the drug was started, and all were on a normal diet without added salt (about 5 g. NaCl daily) and unrestricted fluid. The inpatients were weighed daily and urine was collected over 24-hour periods. Blood was taken for determination of electrolytes and urea before the diuretic was given, and at weekly intervals thereafter. Hæmoglobin estimation, white-cell count, and urine examination for albumin, cells, and casts were done weekly. The dose of hydroflumethiazide used in the clinical study was 50 mg. three times daily, reduced to 50 mg. daily for maintenance therapy.

Urinary sodium and potassium were estimated by flame

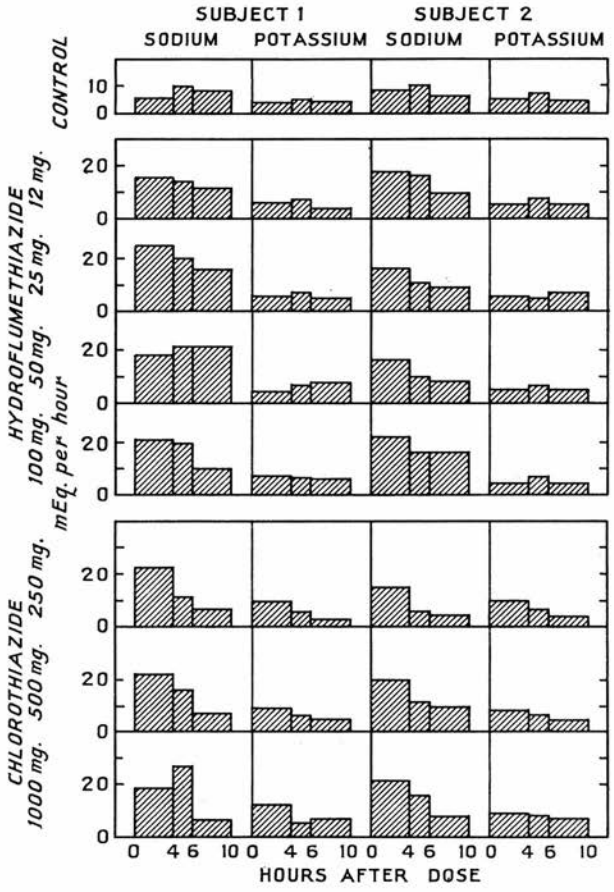


Fig. 2—Excretion of sodium and potassium by 2 normal men after various doses of hydroflumethiazide and chlorothiazide. The control excretion-rates are the means of observations made on 8 days in subject 1 and on 10 days in subject 2.

photometry, chloride by the potentiometric titration with silver nitrate (Sanderson 1952), bicarbonate by a microdiffusion method (Conway 1957), titratable acidity by titration to pH 7·4 with 0·1 N NaOH, and pH by a 'Marconi' pH meter. Serum-electrolytes and urea were determined by routine laboratory methods.

Results in Normal Subjects

Response to Increasing Doses of Hydroflumethiazide

Hydroflumethiazide considerably increased sodium

TABLE II—TOTAL 10-HOUR EXCRETION OF URINARY ELECTROLYTES ON A CONTROL DAY AND AFTER HYDROFLUMETHIAZIDE, HYDROCHLOROTHIAZIDE, AND CHLOROTHIAZIDE

—	Control day	Hydroflumethiazide (100 mg.)	Hydrochlorothiazide (100 mg.)	Chlorothiazide (1000 mg.)
Sodium (mEq.) ..	53·3	168·1	185·2	144·5
Potassium (mEq.)	50·0	64·5	57·6	67·7
Chloride (mEq.) ..	73·7	204·3	216·6	147·1
Bicarbonate (mEq.)	3·5	4·6	10·6	29·2
Titratable acid (mEq.)	9·8	11·7	10·4	5·4
Water (ml.) ..	703	1299	1152	1207

excretion, and even with the lowest doses some effect was still apparent up to 10 hours after giving the drug (fig. 2). A comparison was made with the response to three different doses of chlorothiazide. The most conspicuous difference between the two drugs lies in the duration of action, which was considerably longer with hydroflumethiazide. With chlorothiazide even at the higher dose levels the sodium diuresis had ceased after 6 hours.

Potassium excretion was increased by both agents (fig. 2). The action of chlorothiazide was more intense, as during the first 4 hours the potassium excretion was greater with the lowest dose of chlorothiazide than with the highest dose of hydroflumethiazide.

Thus the different duration of action of the two drugs made a quantitative comparison difficult. Clearly the results and any calculated dose-response curves would depend largely on the duration of urine collection. The time relation suggested that the mechanism of action and the effect on electrolyte excretion might also differ.

Comparison of Action of Hydroflumethiazide, Chlorothiazide, and Hydrochlorothiazide

In the two normal subjects the effect of 100 mg. of hydroflumethiazide on the volume and composition of the urine was compared with the effect of 1000 mg. of chlorothiazide and of 100 mg. of hydrochlorothiazide. The results were similar in the two subjects and have therefore been presented as a mean (fig. 3 and table II).

Urine volume.—With chlorothiazide the urine flow was maximal during the 2–4 hr. collection, and by the end of the 8th hour had returned to normal. Hydrochlorothiazide and hydroflumethiazide also considerably increased urine flow, but the maximal flow was less than with chlorothiazide, and the action continued longer.

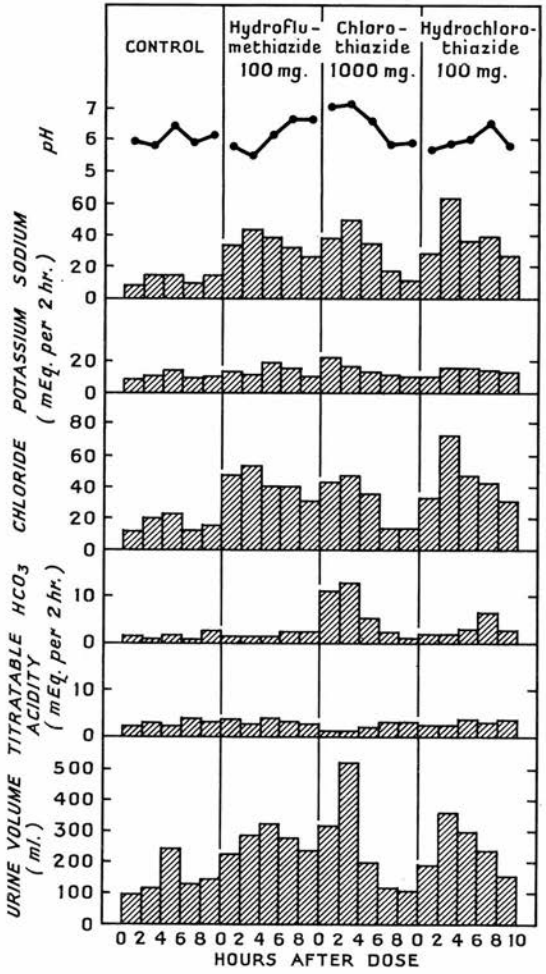


Fig. 3—Effect of a single oral dose of hydroflumethiazide, 100 mg., chlorothiazide, 1000 mg., and hydrochlorothiazide, 100 mg., on urine volume and composition in 2 normal men (results given as the mean). The first column records excretion on a day on which no drug was taken.

Sodium excretion.—Chlorothiazide produced a considerable increase in sodium excretion which was confined to the first 6 hours. The effect of hydrochlorothiazide and hydroflumethiazide was again more prolonged. The maximal excretion-rate of sodium for the three substances was similar, although one of the subjects produced a high peak in the 2–4 hr. collec-

tion after hydrochlorothiazide, and this resulted in a high excretion-rate for this substance (fig. 3). The total excretion over the 10 hours was substantially greater with hydrochlorothiazide and hydroflumethiazide than with chlorothiazide, a result of the briefer action of the latter.

Potassium excretion.—This was increased by all three drugs. With chlorothiazide potassium excretion was at nearly twice the control level during the first 4 hours, but later was at the normal rate. With the other two substances, the potassium excretion-rate was elevated for the first 8 hours, but it did not reach the level seen with chlorothiazide at any time.

Chloride excretion.—Changes in chloride excretion closely paralleled changes in sodium excretion (fig. 3). Chlorothiazide increased chloride excretion during the first 6 hours only, while hydrochlorothiazide and hydroflumethiazide increased it throughout the 10-hour collection period. The proportion of chloride ions to sodium ions was higher with hydrchlorothiazide and hydroflumethiazide than with chlorothiazide (table II).

Bicarbonate excretion.—The most characteristic difference among the three compounds was seen with bicarbonate excretion. This was considerably increased during the first 4 hours after chlorothiazide administration, but was not significantly altered by either hydrochlorothiazide or hydroflumethiazide.

Titratable acidity.—The excretion pattern was unaffected by hydrochlorothiazide or hydroflumethiazide. With chlorothiazide no titration was possible during the first 4 hours, as the urine was alkaline. Thereafter the excretion was as on the control day.

pH changes.—These paralleled the changes in bicarbonate excretion. Chlorothiazide differed from hydrochlorothiazide and hydroflumethiazide in producing an alkaline urine during the first 4 hours. The urinary pH changes with the latter two substances were similar to those of the control day, the urine being acid throughout with the pH rising towards the end of the collection period.

Clinical Trial

The results are summarised in table III. When the drug was effective, diuresis generally began as soon as treatment was started and continued until œdema had disappeared.

Pulmonary Heart-disease

Seven cases were studied. There was a good response in three patients, none in three, and no response in one until ammonium chloride was given.

The blood-urea was elevated in all those cases failing to

TABLE III—CLINICAL RESPONSE TO HYDROFLUMETHIAZIDE

Patient no.	Sex and age	Diagnosis	Response to hydroflumethiazide	Weight loss (kg.)	Initial serum-electrolytes and urea					Response to other diuretics	Side-effects
					Na	K	Cl	HCO_3	Urea		
1	M 64	Pulmonary heart-disease	Good	5	135	4·0	92	32	32	No comparison made	None
2	M 64	,,	Poor	0	135	4·0	94	31	50	,,	Hypokalæmia
3	F 55	,,	Good	3	141	4·5	94	34	25	,,	None
4	M 56	,,	Fair	4	136	4·3	88	30	54	Diuresis was slow until NH_4Cl 2 g. t.d.s. was given	None
5	F 68	,,	Poor	0	136	5·6	84	36	90	Mersalyl; poor	None
6	M 58	,,	Poor	0	137	3·9	88	31	68	Mersalyl; fair Chlorothiazide; poor	Hypokalæmia
7	F 46	,,	Good	2	140	4·9	87	32	32	No comparison made	None
8	F 81	Ischæmic heart-disease	Good	3	142	5·1	99	33	50	,,	None
9	M 65	,,	Good	3	148	4·3	109	..	29	,,	Hypokalæmia
10	M 74	,,	Good	5	141	4·8	97	29	25	,,	None
11	F 64	,,	Good	1	149	5·0	100	..	26	,,	None
12	M 64	,,	Poor	0	138	3·6	97	25	47	Mersalyl; good Chlorothiazide; poor	None
13	M 62	,,	Good	0	135	3·3	89	..	35	No comparison made	None
14	F 56	,,	Poor	0	134	3·6	105	25	46	Mersalyl; fair Chlorothiazide; poor	Hypokalæmia
15	M 54	,,	Good	4	132	4·6	97	..	24	No comparison made	None
16	M 54	,,	Good	0	142	5·0	101	26	39	Mersalyl; good	None
17	M 55	,,	Good	3	147	3·6	97	28	32	No comparison made	None
18	M 59	,,	Good	4	136	3·6	99	24	24	,,	None
19	F 44	Cardiac failure associated with thyrotoxicosis	Good	2	138	3·6	104	..	23	,,	None
20	F 54	,,	Good	3	145	4·0	104	24	25	,,	Hypokalæmia
21	M 52	,,	Good	4	144	3·5	100	24	22	,,	None
22	F 54	Chronic rheumatic heart-disease	Good	7	139	3·8	99	..	30	,,	None
23	F 46	,,	Good	10	139	4·4	96	23	25	,,	Transient irritating erythema
24	F 43	,,	Good	2	133	4·4	97	..	32	Mersalyl; good	None
25	F 36	,,	Good	1	138	4·0	101	..	28	No comparison made	None
26	F 34	,,	Good	0	132	3·5	97	..	24	Mersalyl; good	None
27	F 44	,,	Good	0	138	4·2	98	25	35	Mersalyl; good	None
28	F 64	,,	Poor	0	136	4·7	89	30	30	Mersalyl; poor Chlorothiazide; poor	None
29	F 46	Nephrosis	Poor	0	138	4·0	96	24	61	Chlorothiazide; poor	Hypokalæmia
30	F 26	Cirrhosis of the liver and ascites	Poor	0	130	4·0	102	23	27	Mersalyl; fair	Hypokalæmia

respond. In patient no. 6, however, the blood-urea gradually fell to normal without an increase in response to hydroflumethiazide, although mersalyl produced a fair diuresis. Chlorothiazide was also tried in this man, but it failed to produce an effect.

Patient no. 2 did not respond to hydroflumethiazide at first, but after about 6 days urinary sodium excretion increased, and diuresis began. Blood-urea had fallen to normal during this time, and the patient's general condition had improved.

Patients no. 4 and 6 were given ammonium chloride, 2 g. three times daily, when hydroflumethiazide failed to act. Patient no. 4 had a fairly good diuresis with combined hydroflumethiazide and ammonium chloride, but in patient no. 6 the response was not improved. We do not know at present whether the administration of ammonium chloride when the serum-chloride is low will improve the response to hydroflumethiazide.

Ischæmic Heart-disease

Of the ten cases studied, the response was good in all but two.

A typical response is shown in fig. 4. This patient developed mild congestive failure with œdema while at rest in bed after a myocardial infarction. He was treated with hydroflumethiazide only, and there was immediately a brisk diuresis and fall in weight as œdema was lost. Hydroflumethiazide was later stopped, but œdema developed again, and maintenance therapy was necessary.

Patients no. 11 and 13, who were having attacks of left ventricular failure, had a small diuresis, and symptomatically were improved. Dyspnœa diminished, and acute attacks of dyspnœa did not recur while they were on maintenance hydroflumethiazide.

Patient no. 16 was an outpatient who had severe ischæmic heart-disease and chronic œdema controlled by two injections of mersalyl weekly. Treatment was changed to hydroflumethiazide, 50 mg. daily, and this was equally effective in controlling œdema, and he has been maintained on this now for 5 months.

Patients no. 12 and 14 both failed to respond to hydroflumethiazide, even when dosage was raised to 300 mg. daily. Patient no. 12 developed mild congestive failure after a cardiac infarction, and hydroflumethiazide did not produce a diuresis. No reason was found for this, and mersalyl produced a good diuresis and clearing of the œdema. Patient no. 14, with advanced ischæmic heart-disease, had no diuresis with hydroflumethiazide or with chlorothiazide, but showed a fair response when mersalyl was given. She later also became refractory to mersalyl, and died some 2 weeks later.

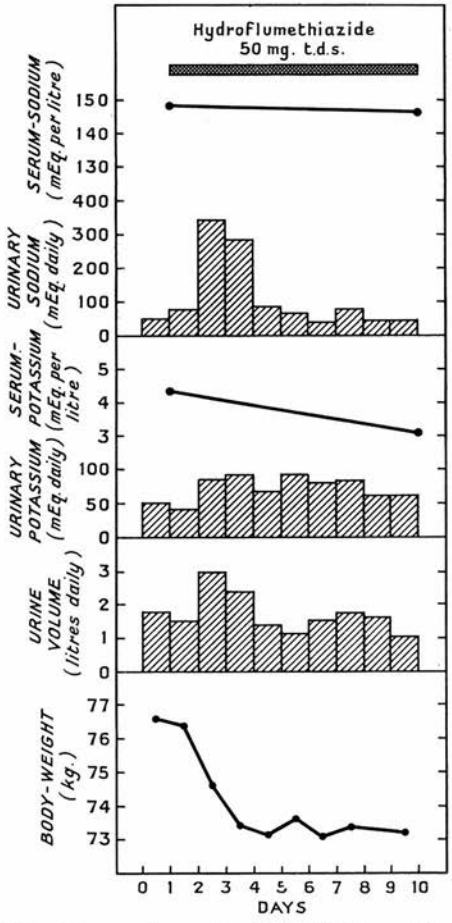


Fig. 4—Case 9 (congestive cardiac failure in ischæmic heart-disease). Effect of hydroflumethiazide on body-weight, urine volume, and urinary and serum sodium and potassium.

Cardiac Failure Associated with Thyrotoxicosis

Three cases were treated before or immediately after treatment with ^{131}I and all responded well.

Thus patient no. 19 had received ^{131}I therapy 4 weeks previously, but was still mildly hyperthyroid; on admission she was in congestive failure with considerable œdema and uncontrolled auricular fibrillation. Digoxin and hydroflumethiazide were started immediately. There was a good response (fig. 5), but evaluation of the effect of the diuretic was not

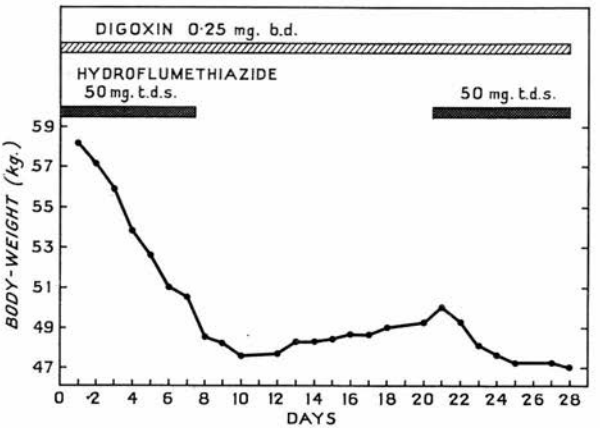


Fig. 5—Case 19 (congestive cardiac failure associated with thyrotoxicosis). Response of body-weight to two courses of hydroflumethiazide.

possible, because of the simultaneous digoxin therapy. Hydroflumethiazide was discontinued when the œdema had disappeared. Her weight gradually rose again, however, and œdema and venous congestion reappeared. On restarting hydroflumethiazide there was a good diuresis with remission of symptoms and signs, and she was subsequently kept on a maintenance dose of 50 mg. daily.

Chronic Rheumatic Heart-disease

With one exception, all these patients responded well and no other diuretic therapy was required.

The results in an illustrative case are shown in fig. 6. This patient had chronic rheumatic heart-disease with auricular fibrillation and mitral stenosis, and was in severe cardiac failure with considerable œdema. She was in hospital for 4 days preceding the administration of hydroflumethiazide, and during this time her weight was increasing. Hydroflumethiazide produced a good diuresis and her weight fell rapidly.

Patients no. 26 and 27 were treated as outpatients. They had previously required mersalyl three times a week, but satisfactory control of œdema formation was achieved by hydroflumethiazide 50 mg. twice daily.

One patient failed to respond. She was in chronic congestive cardiac failure with gross œdema. Mersalyl and chlorothiazide were ineffective. Hydroflumethiazide produced a moderate diuresis on the first 2 days, but then ceased to act. It was found then that the patient was in an hyponatræmic state (serum-electrolytes were Na 125 mEq. per litre, K 3·2 mEq. per litre, Cl 84 mEq. per litre, and urea 35 mg. per

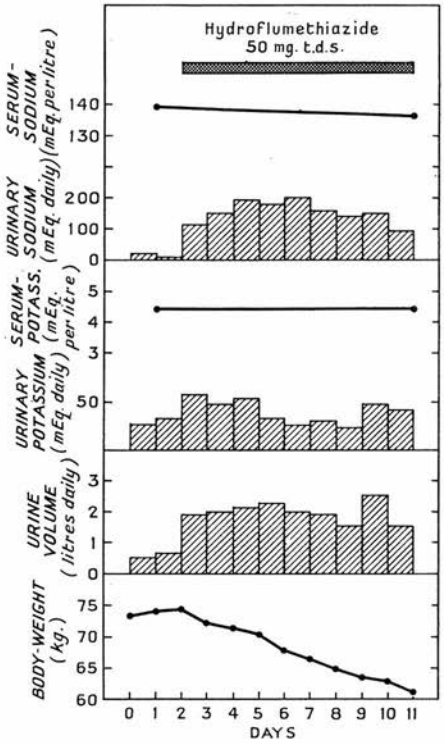


Fig. 6—Case 23 (congestive cardiac failure in chronic rheumatic heart-disease). The effect of hydroflumethiazide on body-weight, urine volume, and urinary and serum sodium and potassium.

100 ml.). She proved refractory to all further measures and died 10 days later.

Nephrosis

The action of hydroflumethiazide was examined on one outpatient.

She had previously been on chlorothiazide, 500 mg. three times a day, but despite this had considerable œdema. Hydroflumethiazide, 50 mg. three times daily, was substituted for the chlorothiazide, but this did not produce a diuresis and weight was unchanged. On stopping hydroflumethiazide, there was no increase in œdema or in weight, and, during the 2 months in which she received no diuretic therapy, her condition has been unchanged. It seems that neither chlorothiazide nor hydroflumethiazide was capable of removing the persistent œdema in this patient, but also they were not apparently necessary to prevent further accumulation, although her

dietary salt intake was only moderately restricted (about 5 g. NaCl daily).

Hepatic Cirrhosis with Ascites

The one patient studied had severe disturbance of liver function and low serum-albumin.

There was considerable ascites but no œdema. At first her urine was practically sodium-free (excretion of about 2 mEq. per 24 hr.). On the 1st day of hydroflumethiazide administration there was a moderate diuresis, and sodium excretion rose to 140 mEq. per 24 hr. On the following days sodium excretion fell, but was still considerably greater than the pretreatment level. Ascitic fluid continued to accumulate, however, and paracentesis was required. Moreover, the serum-potassium fell rapidly, and it was necessary to give potassium-chloride supplements. Subsequently an attempt to control ascites formation with hydroflumethiazide and a very low salt diet (1 g. sodium chloride daily) also failed. Mersalyl was tried, and produced a greater diuresis than hydroflumethiazide, but was also ineffective in preventing reaccumulation of ascites, and paracentesis at intervals was necessary.

Side-effects

Hydroflumethiazide was remarkably free of disturbing side-effects other than a tendency to produce hypokalæmia. There were no complaints of digestive upset, no changes in hæmoglobin or white-cell count, and no albuminuria or other urinary abnormality.

Hypokalæmia occurred in seven of our patients. It appeared within a few days or gradually over a period of several weeks. Even when the serum-potassium was less than 3 mEq. per litre, it produced no apparent effect other than a feeling of lassitude in some patients. In all cases it was easily corrected by giving potassium chloride, 1 g. three times daily, and with this addition hydroflumethiazide could be continued.

Two patients complained of transient skin irritation, but this disappeared despite continuation of treatment.

Discussion

Hydroflumethiazide produced a considerable diuresis in normal subjects, and, as might be expected from its chemical structure, its action was apparently similar to that of chlorothiazide, the diuresis being associated with a high excretion of sodium and chloride.

The differences between hydroflumethiazide and chlorothiazide are in the amount required to produce a diuresis, in the action on bicarbonate excretion, and in the period of action. Hydroflumethiazide is at least ten times as effective, weight for weight, as chlorothiazide. The

maximal rate of sodium excretion, however, is similar for the two drugs. Thus the only result of this increased efficiency is that a smaller quantity of hydroflumethiazide is required in treatment.

Bicarbonate excretion was increased by chlorothiazide, a well-recognised effect of the drug (Ford and Spurr 1957, Matheson and Morgan 1958), but this was not affected by hydroflumethiazide. Acetazolamide and chlorothiazide have a similar sulphonamide group and exert carbonic-anhydrase inhibitory activity. Presumably this is why bicarbonate excretion increases after chlorothiazide. Hydroflumethiazide also possesses the sulphonamide group, and probably its lack of effect on bicarbonate excretion results from the much smaller quantity used. The change in molecular configuration has enhanced activity in respect of sodium and chloride excretion without improving the carbonic-anhydrase inhibitory properties.

Hydroflumethiazide has a more prolonged action than chlorothiazide. The reason for this is unknown. The excretion pattern seen with hydrochlorothiazide was very similar to that of hydroflumethiazide, and probably their modes of action are similar.

There is no doubt from the clinical study that hydroflumethiazide is an effective diuretic in circumstances where mersalyl is ordinarily used. The response was least good in cases of pulmonary heart-disease, an observation similar to that made by Watson et al. (1958) with chlorothiazide. In those failing to have a diuresis, renal function was generally impaired, as shown by an elevated blood-urea, and this may have been partly responsible.

Only one patient with nephrosis and chronic œdema was available for study, and this patient had no diuresis with hydroflumethiazide; chlorothiazide was also ineffective. Slater and Nabarro (1958) obtained a good response using chlorothiazide in three patients with the nephrotic syndrome, and the failure of our patient to respond either to chlorothiazide or hydroflumethiazide was not explained.

The results obtained in the patient with cirrhosis of the liver with ascites were also disappointing.

In this patient, however, the severity of the condition was such that mersalyl and a very low salt diet were also incapable of controlling ascites formation. When hydroflumethiazide was first given sodium output considerably increased, and it seems likely, therefore, that a less severe case would be controlled by treatment with this diuretic.

Some cases of cirrhosis develop symptoms of hepatic precoma with chlorothiazide treatment, and, since similar results may follow acetazolamide, this untoward effect may be partly related to the carbonic-anhydrase inhibitory properties of chlorothiazide (Magid and Forsham 1958, Read et al. 1958). If so, hydroflumethiazide would probably be the preferable oral diuretic, since its carbonic-anhydrase inhibitory action is negligible. Our patient certainly showed no evidence of cerebral disturbance on 50 mg. hydroflumethiazide three times daily.

From their similar mode of action and chemical structure, one would expect that where chlorothiazide failed to produce a diuresis, hydroflumethiazide would fail also, and, in the limited number of cases where comparison was possible, this was in fact confirmed. This was not so in relation to mercurial diuretics, however, for the latter produced a good diuresis in several patients when the response to hydroflumethiazide was poor or absent. On the other hand the reverse was not true, and in all our cases where mercurial diuretics had failed, hydroflumethiazide was also ineffective. Hence mersalyl is more effective in producing a diuresis in some cases, and should be tried if hydroflumethiazide fails.

Throughout the present study, a dose of 50 mg. three times daily was used as standard initial treatment. This was sufficient to produce a good response in the majority, and in those in whom the response was poor an increase in dose had no effect. In view of the long action of the drug, however, a single dose of 100–150 mg. in the morning should be equally effective.

The only side-effect encountered with hydroflumethiazide was hypokalæmia in some patients, necessitating the administration of supplementary potassium, generally as potassium chloride, 1 g. three times daily.

Hypokalæmia is also a complication of chlorothiazide treatment and has been attributed to its carbonic-anhydrase inhibitory activity. This phenomenon also occurs, however, with hydroflumethiazide, which in the dosage used has a negligible effect on carbonic anhydrase; so it therefore seems unlikely that the hypokalæmia can be explained in this way. It is essential that the serum-potassium should be watched in patients on continuous oral diuretic therapy. In cirrhosis with fluid retention especial care has to be taken, as a considerable fall in serum-potassium may occur within a few days. In our cases a low serum-potassium was not associated with any obvious harm, but it is a hazard, especially if other drugs (such as digitalis) are being used.

Summary

Hydroflumethiazide is an oral diuretic related chemically to chlorothiazide. In comparison with chlorothiazide in normal subjects it was active in about one-tenth the dose, caused a more prolonged diuresis of water and sodium, and produced less bicarbonate but more chloride excretion. Its action closely resembled that of hydrochlorothiazide.

In a clinical study of 30 patients hydroflumethiazide was an effective diuretic in the majority. Where it failed chlorothiazide was also ineffective, but mersalyl was occasionally successful. Failure to respond was usually associated with impaired renal function, as shown by an elevated blood-urea.

Hypokalæmia in a few cases was the only side-effect encountered.

The hydroflumethiazide (' Hydrenox ') was kindly supplied by Boots Pure Drug Company, and the hydrochlorothiazide (' Esidrex ') by Ciba Laboratories.

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THE ACTION OF HYDROFLUMETHIAZIDE IN RELATION TO ADRENAL STEROIDS AND POTASSIUM LOSS

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Reprinted from THE LANCET, *March* 5, 1960, *pp.* 505–509

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AN increase of potassium excretion associated with a fall in the serum-potassium level is one of the more serious problems encountered with diuretic therapy.

Considerable potassium loss may sometimes be produced by mercurial diuretics (Cort and Matthews 1954), but the introduction of the benzothiadiazine diuretics has brought this hazard into prominence (Bayliss et al. 1958, Read et al. 1958). A similar phenomenon has been described with acetazolamide, and was attributed to diminished production of hydrogen ions—resulting from carbonic anhydrase inhibition (Counihan et al. 1954)—which led to potassium instead of hydrogen ions being exchanged for sodium in the distal renal tubules. As chlorothiazide is chemically similar to acetazolamide and can inhibit carbonic-anhydrase, the potassium loss it produces has been explained in the same way (Pitts et al. 1958). However, this cannot be the only mechanism involved, since some of the new oral diuretics—in particular, hydrochlorothiazide and hydroflumethiazide—may cause hypokalæmia when used in doses which do not increase urinary bicarbonate (Fleming et al. 1959, Kerr et al. 1959, Edmonds and Wilson 1959).

The well-known influence of adrenal steroids on sodium and potassium excretion, together with the frequent finding of abnormality in aldosterone excretion in clinical states in which diuretics are used, suggested that the level of circulating steroids may partly determine the relative amounts of sodium and potassium lost during the diuresis. To test this hypothesis, we have studied the composition of the urine of two normal subjects after administration of hydroflumethiazide, under conditions designed to vary the degree of renal stimulation by salt-retaining hormones. This variation was achieved either

with diets high or low in salt, which are known to alter the endogenous secretion of aldosterone (Axelrad and Luetscher 1954, Mulrow et al. 1956), or by giving large doses of fludrocortisone with a normal diet. Fludrocortisone was used because a sufficiently large supply of aldosterone was not obtainable; but the action of the two substances is similar.

Additional evidence was obtained by using a steroidal spirolactone, SC-9420 (Searle). The spirolactones probably antagonise the action of aldosterone and related steroids on the renal tubular cells (Liddle 1958). We used SC-9420 because it is well absorbed when taken by mouth and is without untoward side-effects. Our results support the view that its most important effects are due to antagonism of sodium-retaining steroids and that the activity of these steroids is chiefly responsible for the excess potassium loss observed with hydroflumethiazide under certain conditions.

Methods

Most of the observations were made on two normal men, aged 29 and 42; but in addition the course of action of the spirolactone was studied in two other healthy men aged 22 and 23. They continued their customary hospital and laboratory duties during the experiments.

Either a normal diet was taken, or a special diet providing only about 1 g. of sodium chloride daily. When a high salt intake was required, 20 g. of sodium chloride daily was added to the normal diet. Fluid intake was unrestricted. The subjects were weighed daily.

The following substances were taken by mouth during the experiments: hydroflumethiazide 50 mg. thrice daily, at 8 A.M., 4 P.M., and midnight; spirolactone SC-9420 either 100 mg. or 200 mg. four-hourly beginning at 6 A.M. to a daily total of 400 mg. or 800 mg.; and fludrocortisone acetate either 0·5 mg. twice daily or 1 mg. thrice daily, the doses in both cases being evenly spaced over the twenty-four hours beginning at 8 A.M.

Urine collections were made over twenty-four-hour periods beginning at 8 A.M.

Sodium and potassium in serum and urine were measured by flame photometry, chloride by potentiometric titration (Sanderson 1952) and creatinine in serum and urine by the method of Owen et al. (1954).

Results

Normal Salt Intake and Fludrocortisone

The aim of these experiments was to determine the effect of a standard dose of hydroflumethiazide on the excretion of electrolytes when the subjects were on a

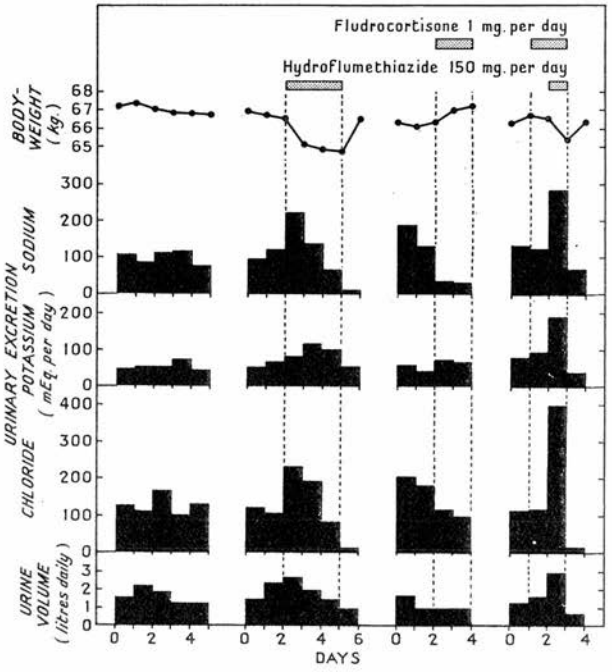

Fig. 1—Action of hydroflumethiazide and fludrocortisone acetate on urine volume and sodium, potassium, and chloride excretion in subject 1 on a normal diet.

normal diet and when a potent sodium-retaining steroid was administered. Spontaneous variations in electrolyte excretion were studied during a week before any treatment was given and subsequently on control days before the administration of drugs (fig. 1 and table I). The changes induced by the diuretic and by fludrocortisone greatly exceeded the spontaneous fluctuations.

After urine collections on two control days, hydroflumethiazide 150 mg. daily was started and continued for three days. On the first day that the drug was given, both subjects showed a great increase in water, sodium, and chloride excretion, accompanied by a fall in body-weight; but potassium excretion was only slightly enhanced. On the subsequent days, sodium and chloride excretion fell to a level similar to that seen before the diuretic was given, but potassium excretion remained slightly elevated (fig. 1). On stopping the diuretic there was sodium and chloride retention and a rise in body-weight.

Two weeks later, the effect of fludrocortisone was studied. Urine was collected for two days without any treatment and

TABLE I—INFLUENCE OF FLUDROCORTISONE ON DAILY SODIUM, POTASSIUM, AND CHLORIDE EXCRETION WHEN HYDROFLUMETHIAZIDE WAS GIVEN TO SUBJECT 2 ON A NORMAL DIET

The fludrocortisone was given in two-day courses and when hydroflumethiazide was added it was given on the second day. The general plan of the experiments is shown in figs. 1 and 2 (mean ±S.D.).

Treatment (doses per day)	Sodium (mEq.)	Potassium (mEq.)	(Na+K) (mEq.)	Na/K	Chloride (mEq.)
Mean of 15 control days	133 ±31·9	83 ±16·8	216	1·6	133 ±41·9
Fludrocortisone 1 mg. (2nd day)	14·0	51·6	66	0·27	42·8
Fludrocortisone 3 mg. (2nd day)	4·5	117	121	0·04	40·5
Hydroflumethiazide 150 mg.	343	113	456	3·03	340
Hydroflumethiazide 150 mg. Fludrocortisone 1 mg.	224	185	409	1·21	347
Hydroflumethiazide 150 mg. Fludrocortisone 3 mg.	174	240	414	0·73	355

then fludrocortisone 0·5 mg. twice daily was given for two days. Both sodium and chloride excretion fell but the fall of chloride was appreciably less than that of sodium. Potassium excretion increased slightly and body-weight rose. After a further two weeks, the experiment was repeated but on this occasion hydroflumethiazide 150 mg. was given on the second day of the fludrocortisone administration. The action of the diuretic on chloride excretion was not inhibited by the fludrocortisone but there was a definite change in proportions of the two cations. Potassium loss was consistently increased and the Na/K ratio altered.

The experiments were then repeated with the amount of fludrocortisone raised to 3 mg. daily (fig. 2 and table I). This dose reduced sodium and chloride excretion to a very low level although again the depression of chloride was less than that of sodium. When hydroflumethiazide was given along with the bigger dose of fludrocortisone a large diuresis of chloride again ensued. This chloride was accompanied by sodium and potassium, but the excretion of the latter was even further enhanced.

Action of the spirolactone, SC-9420.—The experiments using fludrocortisone 3 mg. daily were repeated to determine whether the effects on cation excretion could be reversed by the administration of the spirolactone.

The course of action of a single dose of spirolactone 200 mg. given during fludrocortisone administration is shown in fig. 3.

Sodium and chloride excretion rose slightly within two to four hours and then rapidly to a maximum between six and eight hours. The effect had disappeared after twenty-four hours. Similar results were obtained in three other subjects.

When the spirolactone was given alone in a daily dose of either 400 mg. or 800 mg. to the subjects on a normal diet,

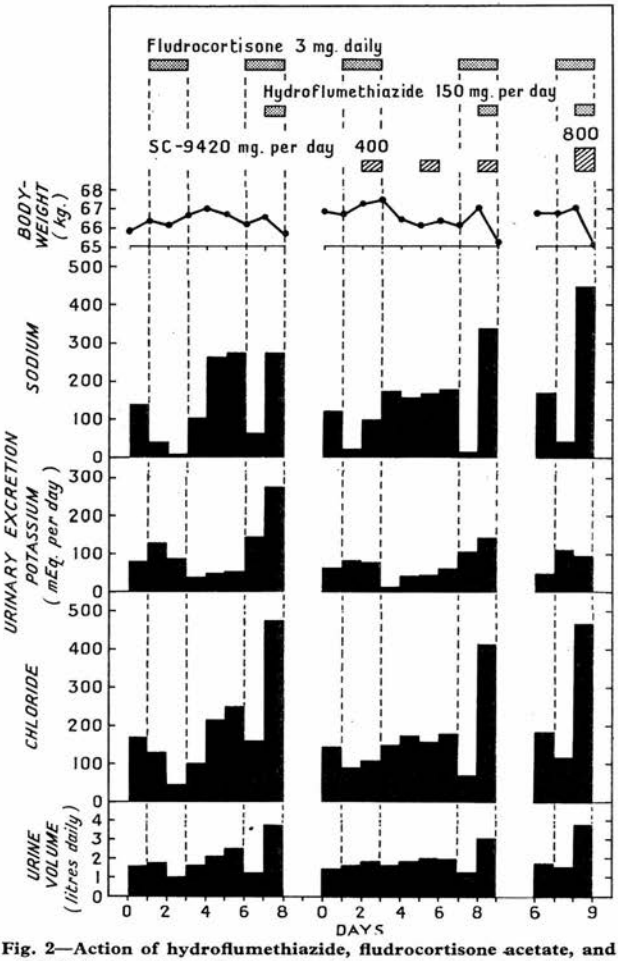

Fig. 2—Action of hydroflumethiazide, fludrocortisone acetate, and SC-9420 on urine volume and sodium, potassium, and chloride excretion in subject 1 on a normal diet. (Only the final three days of the last experimental period are shown.) Note that chloride excretion was similarly increased by hydroflumethiazide in each experiment but that the proportions of sodium and potassium accompanying the chloride were altered by concurrent spirolactone.

TABLE II—EFFECT OF SPIROLACTONE SC-9420 ON DAILY SODIUM, POTASSIUM, AND CHLORIDE EXCRETION WHEN GIVEN ALONE OR WITH FLUDROCORTISONE AND HYDROFLUMETHIAZIDE (SUBJECT 2).

The general plan of the experiment is shown in fig. 2.

Treatment (doses per day)	Sodium (mEq.)	Potassium (mEq.)	(Na+K) (mEq.)	Na/K	Chloride (mEq.)
SC-9420 400 mg... ..	189	83	272	2·28	172
SC-9420 800 mg.	192	79	271	2·43	176
SC-9420 400 mg. Fludrocortisone 3 mg. ..	57	79	136	0·72	78
SC-9420 800 mg. Fludrocortisone 3 mg. ..	113	83	196	1·36	115
SC-9420 400 mg. Fludrocortisone 3 mg. Hydroflumethiazide 150 mg.	347	159	506	2·18	426
SC-9420 800 mg. Fludrocortisone 3 mg. Hydroflumethiazide 150 mg.	301	144	445	2·09	376

there was only a small rise of sodium and chloride excretion (fig. 2 and table II). When, however, 400 mg. of the spirolactone was taken on the second day of fludrocortisone administration, sodium excretion rose considerably and chloride moderately. The effect of the fludrocortisone had been largely reversed. On the second day of a further course of fludrocortisone 3 mg. daily, hydroflumethiazide 150 mg. and spirolactone 400 mg. were taken. Sodium, potassium, and chloride excretion rose, but the rise of sodium compared with potassium was much greater than that observed when the spirolactone was omitted (fig. 2 and table II). The total (Na+K) excretion and chloride excretion, however, were not significantly altered.

As the effect of the fludrocortisone was not completely reversed by 400 mg. of spirolactone, the experiments were done once more in the same way but with the dose of spirolactone increased to 800 mg. In these circumstances, in subject 1, the potassium loss following the diuretic was even further reduced so that sodium and chloride were excreted in nearly equivalent amounts. In subject 2, however, this dose was still insufficient to reverse completely the effect of fludrocortisone on the hydroflumethiazide-induced diuresis.

High Salt Intake

With a high salt intake the findings in the two subjects were essentially similar.

The addition of sodium chloride 20 g. daily to the normal diet produced an increase in body-weight and a rise in urine volume and in sodium and chloride excretion (fig. 4). Subject 1 showed a slight increase in potassium excretion as sodium excretion rose during the first two days on the increased salt intake, but thereafter it fell to the previous level. Subject 2 had no comparable increase of potassium excretion.

TABLE III—CREATININE CLEARANCE AND SERUM ELECTROLYTES OF TWO NORMAL SUBJECTS ON VARIOUS DIETS AND TREATED WITH HYDROFLUMETHIAZIDE

Diet was started on day 0 in all cases

—	Treatment (doses per day)	Diet	Day	Creatinine clearance (ml. per min.)	Sodium (mEq. per l.)	Potassium (mEq. per l.)	Chloride (mEq. per l.)
Subject 1	None	Normal	−1	110	139	4·2	106
	Hydroflumethiazide 150 mg. on days 4–7	High-salt	1	124	143	4·2	111
			3	120	140	4·3	103
			6	115	140	4·3	102
			9	110	138	4·5	108
			11	117	140	4·5	110
	Hydroflumethiazide 150 mg. on days 3–8	Low-salt	2	93	138	4·0	104
			5	102	136	3·7	100
			8	88	136	3·4	99
Subject 2	None	Normal	−1	115	140	4·2	105
	Hydroflumethiazide 150 mg. on days 4–8 and 16–18	High-salt	2	127	143	4·2	111
			7	132	143	4·1	108
			18	123	144	4·4	106
	Hydroflumethiazide 150 mg. on days 3–5	Low-salt	2	101	140	3·9	99
			5	91	138	3·1	97

When hydroflumethiazide was given there was a considerable increase in urine volume and in sodium and chloride excretion; body-weight fell to a level a little below that recorded in the period preceding the high salt intake. After the first day of hydroflumethiazide, sodium and chloride excretion returned to a level balancing intake, and it continued unchanged during the rest of the period of diuretic administration. Body-weight also remained constant after the initial fall. There was no alteration in daily potassium excretion when the diuretic was given. As soon as the diuretic was stopped, there was a fall in urine volume, and in sodium and chloride excretion associated with a rise in body-weight. Again, potassium excretion was unchanged.

Serum electrolytes altered only slightly during these experiments (table III). Both subjects showed a slight increase in serum sodium and chloride levels when the salt intake was increased, with a tendency to fall again during the diuretic administration. The serum-potassium level showed no significant changes in either subject.

Endogenous creatinine clearance was used as a measure of glomerular filtration rate and showed only small changes. In both subjects, glomerular filtration rate was greater during the period of high salt intake but fell slightly when the diuretic was given. Daily creatinine excretion showed little variation.

Low Salt Intake

In these experiments, each subject spent three periods on a low salt diet.

On the morning of the first day of the diet, a single dose of hydroflumethiazide 100 mg. was given, and as a result the body-weight fell by about 1 kg. Subsequently, it was maintained at this level by the low-salt diet. The urinary excretion of sodium and chloride became very low. On the first occasion, subject 1 remained on the diet for nine days and subject 2 for six days. After the first three days, hydroflumethiazide 150 mg. daily was begun. The results were similar in the two subjects, and details of subject 1 only are shown (fig. 5). Chloride excretion rose considerably, but this rise was accompanied mainly by potassium and the rise of sodium excretion was small. The excretion of sodium, potassium, and chloride then gradually fell despite continued administration of the diuretic. The decrease in sodium loss was particularly obvious, but the daily excretion of potassium and chloride remained above the control level throughout the period of treatment with hydroflumethiazide. When the diuretic was stopped, there was an immediate conservation of potassium, the excretion-rates falling well below those observed during the initial two control days on the low-salt diet (fig. 5). Body-weight fell when the diuretic was given and then stabilised at a new level. It rose rapidly when salt was reintroduced into the diet.

Serum sodium and chloride concentrations were slightly depressed during the period on the low salt intake. In both subjects there was a conspicuous fall in serum-potassium level during the diuretic treatment (table III). Creatinine clearance was depressed during the low-salt regime and fell further when the diuretic was given. Daily creatinine excretion showed little variation.

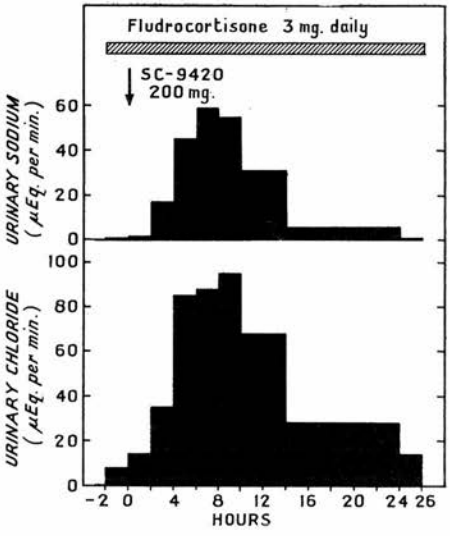

Fig. 3—Effect of a single oral dose of SC-9420 on sodium and chloride excretion in subject 1 on the second day of fludrocortisone treatment.

At intervals of several weeks, the experiments were repeated in the same way except that the period on the low-salt diet was reduced to four days. The pattern of body-weight changes and of sodium, potassium, and chloride excretion on the first three days was similar in all the experiments. In the second experiment, the subjects took the spirolactone alone on the fourth day (fig. 5). Sodium excretion increased but body-weight and potassium and chloride excretion were unchanged. The course of action of the spirolactone was also examined while the subjects were on a low-salt diet. It was similar in the two subjects, and the results for subject 1 are reproduced in fig. 6. Sodium excretion increased after a delay of four to six hours and reached a maximum in ten to twelve hours.

In the final experiment, hydroflumethiazide 150 mg. was taken during the fourth day in addition to the spirolactone (fig. 5). The increase in chloride excretion and the fall in body-weight closely resembled that observed when hydroflumethiazide was taken alone, but the pattern of excretion of the cations was strikingly different. The proportion of sodium was considerably greater than previously and of potassium much less. The total excretion (Na+K), however, was similar in the two experiments and balanced the increase of chloride ions.

Discussion

There was considerable variation in the relative proportions of sodium and potassium accompanying the

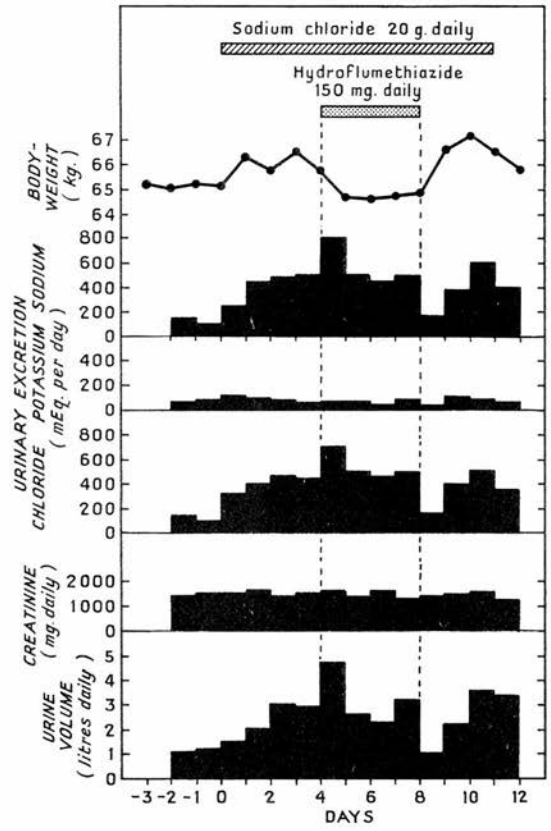

Fig. 4—Action of hydroflumethiazide on urine volume, sodium, potassium, chloride, and creatinine excretion of a normal subject on a high salt intake (sodium chloride 20 g. daily added to a normal diet).

increased excretion of chloride induced by hydroflumethiazide. The ratio between the two cations was chiefly influenced by the amount of endogenous or exogenous sodium-retaining steroids present at the time. It is generally considered that most, if not all, of the potassium filtered by the glomerulus is reabsorbed, and that the excreted potassium comes from exchange with sodium ions in the distal renal tubule (Berliner et al. 1951). This exchange depends on the presence of sodium ions within the tubular lumen, the availability of potassium, and the supply of adrenal steroids. On a low-salt diet, potassium excretion is not excessive although secretion

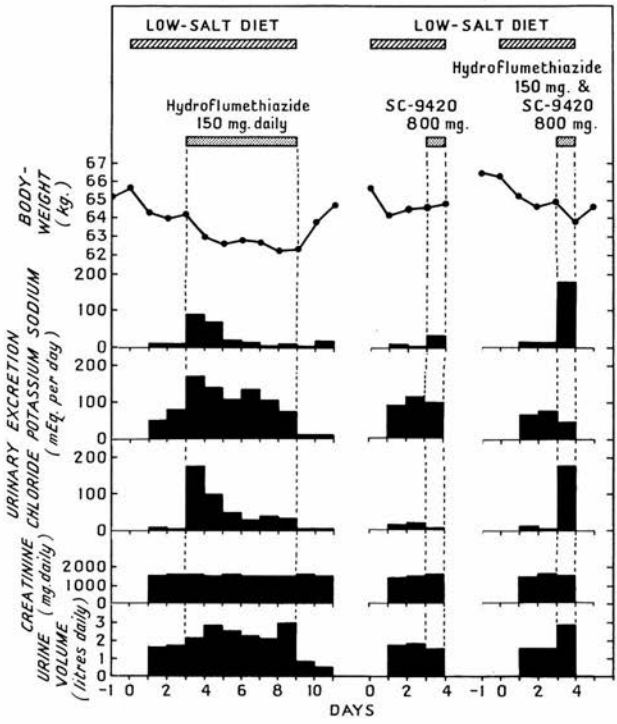

Fig. 5—Effect of hydroflumethiazide and SC-9420 on urine volume, sodium, potassium, chloride and creatinine excretion of subject 1 during three periods on a low-salt diet (sodium chloride 1 g. daily). At the beginning of each period on the diet, hydroflumethiazide 100 mg. was taken to produce moderate sodium depletion; urine was not collected on this day.

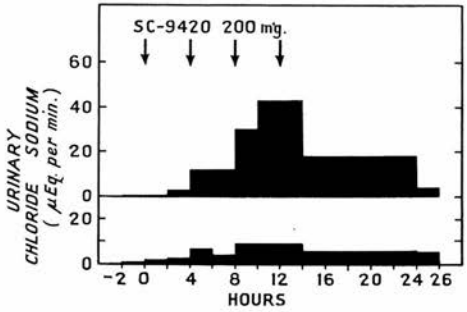

Fig. 6—Effect of the spirolactone, SC-9420, on sodium and chloride excretion in subject 1 on a low-salt diet (about 1 g. sodium chloride daily).

of aldosterone is considerably increased. Moreover, administering large quantities of sodium-retaining steroids to animals on a low-salt intake does not produce potassium depletion (Seldin et al. 1951, Relman and Schwartz 1952), in contrast to their action in animals on a normal salt intake. The probable explanation of these findings is that when sodium-chloride excretion is very low, as on a reduced salt intake, little sodium and chloride reaches the distal tubular region where cation exchange occurs, and consequently the exchange is limited.

There is experimental evidence that the benzothiadiazine diuretics act proximally to the cation-exchange region, probably on the proximal renal tubule (Vander et al. 1959). Our results are consistent with this view. The action on the proximal site results in the delivery of increased quantities of sodium and chloride to the distal region. When the level of circulating sodium-retaining steroids is low, as on a high salt intake, or when their action is antagonised by spirolactone, there is little increase in the exchange and potassium excretion is unaltered. When, however, the exchange mechanism is stimulated by sodium-retaining steroids, potassium loss becomes excessive. These conditions obtain when the diuretic is given during a period of salt restriction or when fludrocortisone is given concurrently.

Our results show that fludrocortisone has a dual effect on sodium metabolism in the kidney. It causes an increase in sodium and chloride reabsorption and also encourages the exchange of sodium for potassium ions. The extent of the potassium loss will depend on the balance of these two actions. If the action of fludrocortisone on sodium and chloride reabsorption predominates, few of these ions reach the region of the renal tubule where the cation exchange takes place. Thus only limited exchange of sodium for potassium ions can occur, even though the mechanism is under stimulation by fludrocortisone.

The increase in chloride excretion induced by hydroflumethiazide in our experiments was unaffected by the simultaneous administration of fludrocortisone. This suggested that, although the diuretic antagonised or masked the sodium and chloride reabsorbing action of fludrocortisone, it had no effect on the sodium-potassium exchange mechanism, which remained under the influence of the steroid.

When the spirolactone was given alone to a subject on a normal diet, it had little effect. When, however, it

was given with fludrocortisone, the effects of the latter were reversed. This supported the evidence of other workers (Liddle 1958, Ross and Bethune 1959) that the effects of this substance are principally, if not entirely, the result of antagonism of the actions of sodium-retaining steroids.

The reciprocal changes in sodium and potassium ions observed in our experiments strongly suggested that the phenomena resulted from a sodium-potassium exchange and that this was considerably influenced by sodium-retaining steroids.

It was consistently observed that, despite continued administration of the diuretic over several days, the excessive loss of sodium and chloride always ceased and the body-weight stabilised at a new lower level. This level, however, was appreciably lower on the low-salt diet than on the high-salt diet. When the diuretic was discontinued, the excretion of sodium and chloride fell abruptly. Apparently some factor antagonised the action of the diuretic on sodium and chloride excretion and persisted after the diuretic was withdrawn. The explanation is still uncertain. The changes in serum electrolytes and glomerular filtration rate were small, and it is difficult to believe that they were responsible for the diminished excretion. Nor did it seem likely that adrenal steroids were responsible, since the potent sodium-retaining steroid, fludrocortisone, did not antagonise the diuretic even in large doses.

Summary

The action of a benzothiadiazine diuretic, hydroflumethiazide, has been studied in two normal subjects. With the subjects on a normal diet, hydroflumethiazide increased the excretion of water, sodium, and chloride and to a small extent of potassium. When fludrocortisone was given concurrently, the potassium loss was greatly increased. The spirolactone SC-9420, which is an aldosterone antagonist, reversed this effect of fludrocortisone.

With the subjects on a high salt intake (20 g. sodium chloride added daily to the normal diet), which leads to a low endogenous secretion of aldosterone, hydroflumethiazide produced a large diuresis of sodium, chloride, and water and a fall of body-weight. Potassium excretion was unaffected and the serum-potassium level was unchanged.

With the subjects on a low salt intake (about 1 g. sodium chloride daily), which causes a high endogenous secretion of aldosterone, hydroflumethiazide produced a rise of chloride excretion accompanied mainly by potas-

sium; the rise of urinary sodium was small. The serum-potassium level fell. If the aldosterone antagonist was given concurrently with the diuretic, potassium excretion was much less and sodium appeared in its place.

The extent of the potassium loss following the administration of hydroflumethiazide is related to the activity of sodium-retaining steroids. If the exogenous supply or the endogenous secretion is high, the diuretic causes a large excretion of potassium. This can be counteracted by the administration of an aldosterone antagonist.

We wish to thank Dr. G. R. Venning of G. D. Searle & Co. Ltd. for supplies of SC-9420 (' Aldactone ') and Dr. E. V. Morton of Boots Pure Drug Co. Ltd. for the hydroflumethiazide.

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The Lancet Office,
7, Adam Street, Adelphi, London, W.C.2

Reprinted from the BRITISH MEDICAL JOURNAL
September 22, 1962, *vol. ii, pp.* 756–764

TRIAMTERENE, A NEW DIURETIC DRUG

BY

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I. STUDIES IN NORMAL MEN AND IN ADRENALECTOMIZED RATS

Triamterene (2,4,7-triamino-6-phenylpteridine) represents a new type of diuretic drug. It has been shown to have a diuretic effect in normal subjects (Hild and Krueck, 1961; Laragh *et al.*, 1961; Crosley *et al.*, 1962), producing increased excretion of sodium and water but a decrease in potassium excretion. This effect was at first attributed mainly to aldosterone antagonism (Crosley *et al.*, 1961; Wiebelhaus *et al.*, 1961). Hild and Krueck (1961) suggested that, in addition to aldosterone antagonism, triamterene probably had an independent direct effect on the kidneys.

We have studied the effect of triamterene in normal subjects and in adrenalectomized rats. Our results suggest that the natriuresis and potassium retention are due to direct action on renal tubular cells.

Studies in Normal Men

The observations were made on eight healthy men aged 26–44 years. During the studies the subjects carried out their normal hospital and laboratory duties and a normal diet was taken. When a high salt intake was required 12 g. of sodium chloride daily was added to the normal diet.

The following substances were taken by mouth during the experiments: triamterene (as capsules), hydroflumethiazide and fludrocortisone acetate (both as tablets). The dosage regime is indicated below.

Urine was collected at two-hourly intervals for ten hours during the period 8 a.m. to 6 p.m., after giving the drug at 8 a.m. At least two days were allowed to elapse before a further dose was given. Similar collections were also made on other days when no drugs were given.

Studies in Adrenalectomized Rats

Male albino rats weighing initially 250–300 g. were used. Adrenalectomy was performed under ether anaesthesia, and the rats were kept in a controlled-temperature room (25° C.). They were given a constant diet of commercial rat cake and were allowed to drink 1% sodium chloride solution freely. All experiments were performed at least two weeks after adrenalectomy.

The rats were studied in groups of three in each metabolism cage, and the urine collection from each group was pooled. At the beginning of each experiment the bladder was emptied by gentle suprapubic pressure. An intragastric tube was passed and the required dose of triamterene or spironolactone suspended in 4 ml. of water was introduced into the stomach. Control groups of rats received 4 ml. of water by the same route. Urine collections were made from each group of rats for five hours and then the bladders were emptied again.

Chemical Procedures

The concentrations of sodium and potassium in all urine samples were determined by flame photometer. Chloride was estimated by potentiometric titration with silver nitrate (Sanderson, 1952) and bicarbonate by a microdiffusion method (Conway, 1950). Titratable acidity and *p*H of urine were measured by a Marconi *p*H meter.

Measurement of triamterene in urine samples was carried out by fluorimetry, using the Hilger H 730 fluorescent attachment in conjunction with Hilger H 700 Uvispek spectrophotometer. The highest concentration of triamterene that could be measured by the fluorimeter was 0.1 μg./ml. A stock solution of 400 μg./ml. of triamterene was prepared in 98% formic acid. From this, two series of seven dilutions of triamterene ranging

from 0.1 μg./ml. to 0.005 μg./ml. were made, the first series by diluting with water and the second series by diluting with water containing 1:2,000 parts normal urine. The concentration of triamterene was plotted against the percentage transmission scale reading of the spectrophotometer, and two parallel straight lines were obtained, the dilutions containing urine giving consistently slightly higher readings. In preparation of urine samples for fluorimetry, 0.1 ml. of urine was made up to 200 ml. with 0.02% formic acid. A blank was prepared from urine passed by the same subject just before triamterene had been given. As a reference standard, triamterene solution 0.1 μg./ml. was used. The spectrophotometer scale was set to 100% transmission with the reference standard, and the blank and the unknown solutions were measured. After subtracting the reading of the blank, which was always less than 3%, the concentration of triamterene in the urine was determined from the calibration curve. To check the reproducibility of the method, the same urine sample was estimated twelve times and the coefficient of variation was 1%.

Results in Normal Men

Response to Increasing Doses of Triamterene

The effect of triamterene in doses of 50–300 mg. was studied in four subjects while on an ordinary diet. All the doses used increased sodium excretion and produced considerable decrease in potassium excretion. The effect of the oral dose was apparent in the first two hours and the maximal effect occurred after four to six hours. In each case a faint blue colour of the urine was noticed after the ingestion of triamterene.

The detailed estimations from the two-hourly urine collections with each dose of triamterene in one subject on a normal diet are shown in Fig. 1. Similar results were obtained in the other three subjects. The total ten-hourly volumes of urine and excretions of sodium and potassium in the four subjects are summarized in Table I. In four normal subjects, triamterene (200 mg.) produced considerable increases in the amounts of bicarbonate and chloride excreted, the former being rather larger, particularly during the first four hours. During the same period there was a conspicuous increase in urinary *p*H. The results in one subject are shown in Fig. 2, and similar results were obtained in each case.

TABLE I.—*Urinary Excretion of Sodium and Potassium During 10 Hours in Four Normal Men, Showing Effect of Increasing Doses of Triamterene*

Subject	Age (yr.)	Weight (kg.)	Control				Triamterene 50 mg.			Triamterene 100 mg.			Triamterene 200 mg.			Triamterene 300 mg.		
			No. of Observations	Volume (ml.) Mean ± S.D.	Sodium mEq Mean ± S.D.	Potassium mEq Mean ± S.D.	Volume (ml.)	Sodium (mEq)	Potassium (mEq)	Volume (ml.)	Sodium (mEq)	Potassium (mEq)	Volume (ml.)	Sodium (mEq)	Potassium (mEq)	Volume (ml.)	Sodium (mEq)	Potassium (mEq)
I	26	74	7	481 ± 37	75·5 ± 13·9	44·3 ± 10·2	510	126	24	560	128	34	670	150	28	970	208	21
II	30	74	5	801 ± 396	80·0 ± 31·0	38·0 ± 4·8	575	116	34	940	157	27	1,071	120	19	1,232	168	13
III	38	77	4	774 ± 153	98·4 ± 21·2	56·3 ± 9·9	840	131	36	1,035	159	24	1,026	164	20	2,270	135	22
IV	44	78	4	765 ± 214	57·3 ± 15·3	49·4 ± 9·2	940	95	35	820	88	26	1,200	134	39	1,235	154	32

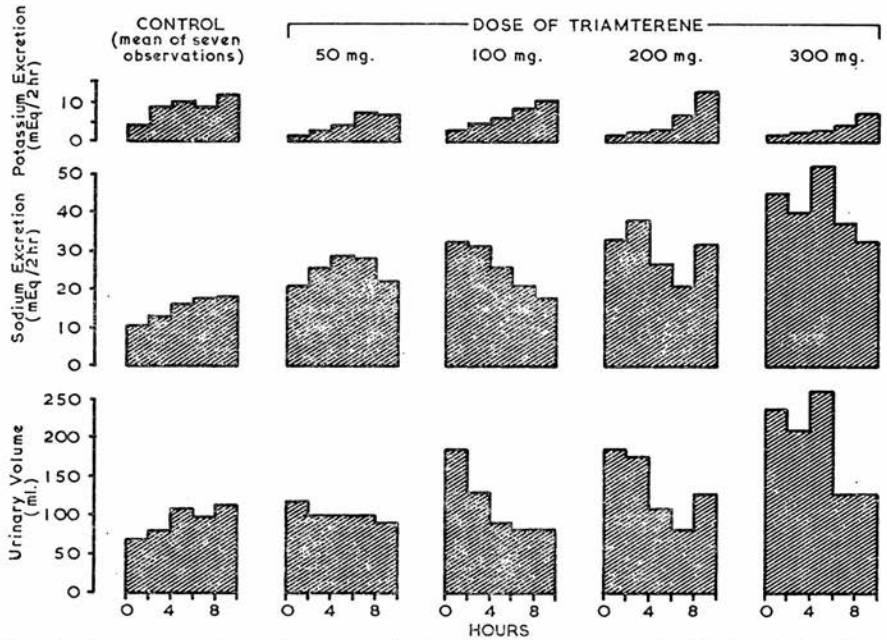

FIG. 1.—Response to increasing doses of triamterene in one normal subject, showing the two-hourly urine excretion of water, sodium, and potassium for 10 hours.

Effect of Fludrocortisone

Fludrocortisone acetate was given to two normal subjects on an ordinary diet, 1 mg. at 12 midnight and 1.5 mg. at 8 a.m. Similar results were obtained in both subjects (Fig. 3). Sodium excretion was reduced and the potassium excretion increased after fludrocortisone. Hydroflumethiazide given in addition at 8 a.m. led to natriuresis and the potassium loss was increased still further. Triamterene, however, reversed the action of fludrocortisone and considerably reduced the potassium excretion, particularly during the first four hours.

High Sodium Chloride Intake

To the normal diet of four subjects 12 g. of sodium chloride daily was added for five days. Urine was collected at two-hourly intervals for ten hours, on the third, fourth, and fifth days. A single dose of 200 mg. of triamterene was given at 8 a.m. on the fifth day.

Triamterene produced an increase in urine volume and sodium excretion, and a considerable decrease in potassium excretion. The detailed results for one

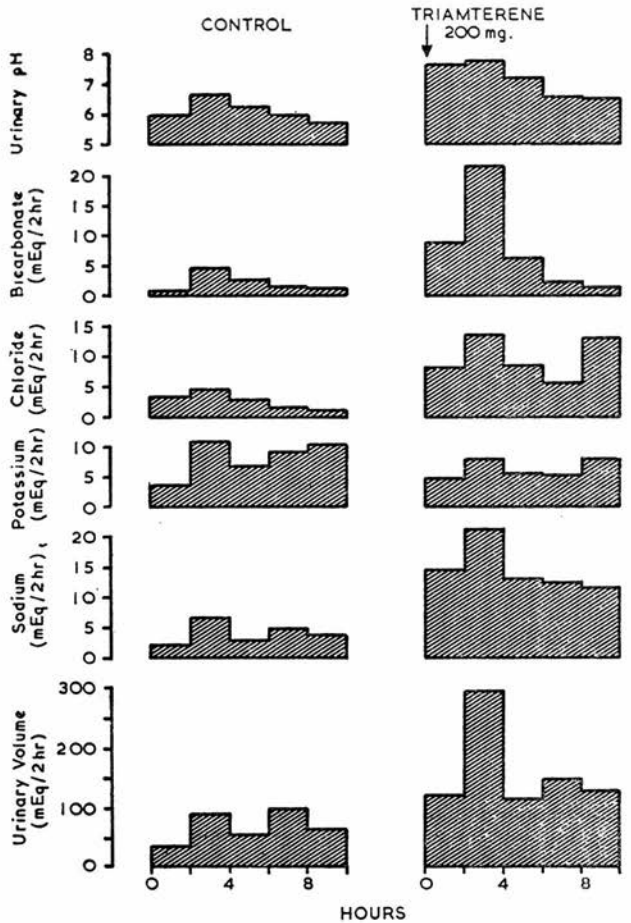

FIG. 2.—Effect of triamterene on the urinary *p*H and on sodium, potassium, chloride, and bicarbonate excretion in one normal subject on an ordinary diet. Observations refer to two-hourly collections of urine.

subject are shown in Fig. 4 and similar results were obtained with the other three subjects. There was no consistent difference between the extent of potassium retention when on high sodium intake and on a normal diet (Table II).

Excretion of Triamterene in Urine

A capsule containing 200 mg. of triamterene without excipient was given to four normal subjects at 8 a.m.

TABLE II.—*Potassium Excretion After Administration of Triamterene to Healthy Subjects Receiving Normal and High Sodium Diets*

Subject	Normal Diet				Normal Diet with Addition of 12 g. NaCl Daily			
	K Excretion mEq/10 Hours		Decrease in K Excretion		K Excretion mEq/10 Hours		Decrease in K Excretion	
	Control (Mean)*	Triamterene 200 mg.	mEq/10 Hours	% of Control	Control†	Triamterene 200 mg.	mEq/10 Hours	% of Control
I	44·3	27·9	16·4	37·0	42·2	18·4	23·8	56·4
II	38·0	19·0	19·0	50·0	39·6	17·6	22·0	55·6
III	56·3	20·5	35·8	63·6	41·6	22·2	19·4	46·6
IV	49·4	39·4	10·0	20·2	46·1	38·0	8·1	17·6

* Figures as in Table I. † Mean of two observations.

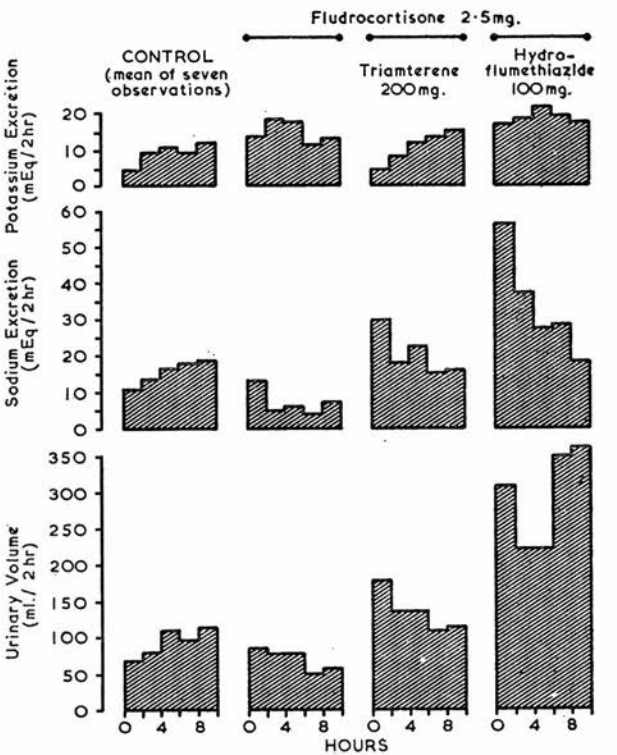

FIG. 3.—Influence of triamterene and hydroflumethiazide on the effect of fludrocortisone on urine volume and on sodium and potassium excretion in one normal subject.

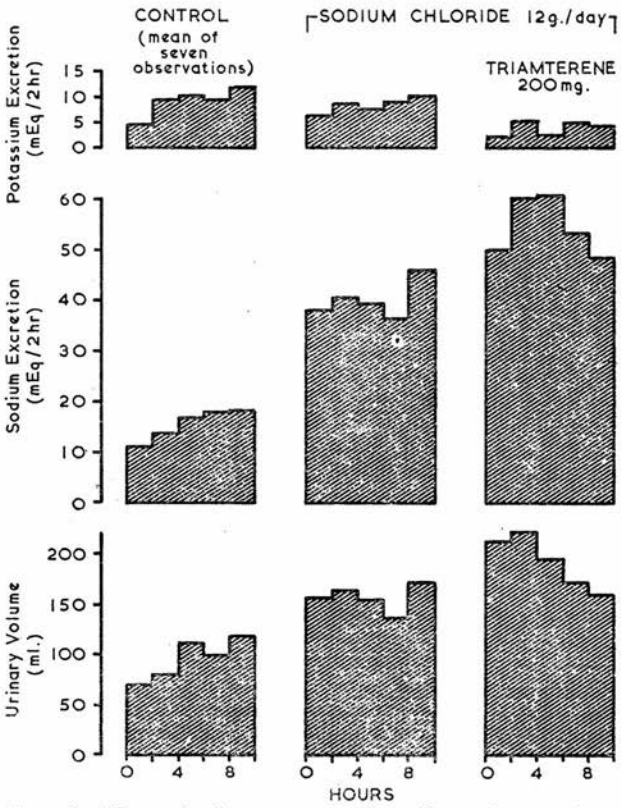

FIG. 4.—Effect of triamterene on the urine volume and on sodium and potassium excretion in one normal subject who was receiving a high sodium-chloride intake.

Urine was collected for 24 hours in the following manner: hourly collections for the first six hours, two-hourly collections for the next six hours, and a single twelve-hour collection. The maximum excretion of the drug occurred during the second and third hours after ingestion (Fig. 5). The total excretion in 24 hours was 15–30% of the oral dose.

Results in Adrenalectomized Rats

Triamterene in the range of 0.5–32 mg. and spironolactone 2–16 mg. were given, as single doses by intragastric tube, to the adrenalectomized rats.

The doses of 1 mg. of triamterene and 2 mg. of spironolactone (in the rat) are equivalent on a weight

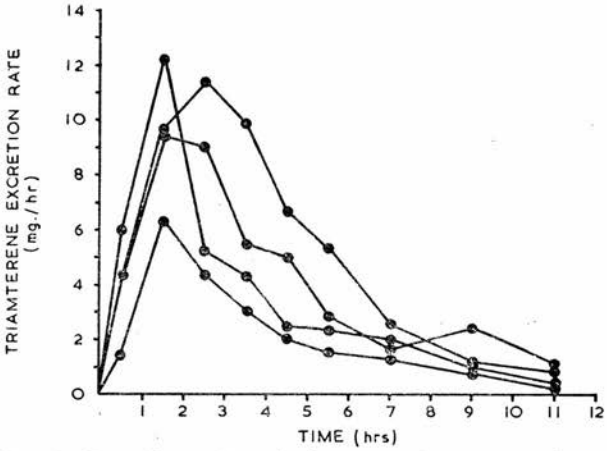

FIG. 5.—Rate of excretion of triamterene after oral administration of 200 mg. in four normal men on a normal diet.

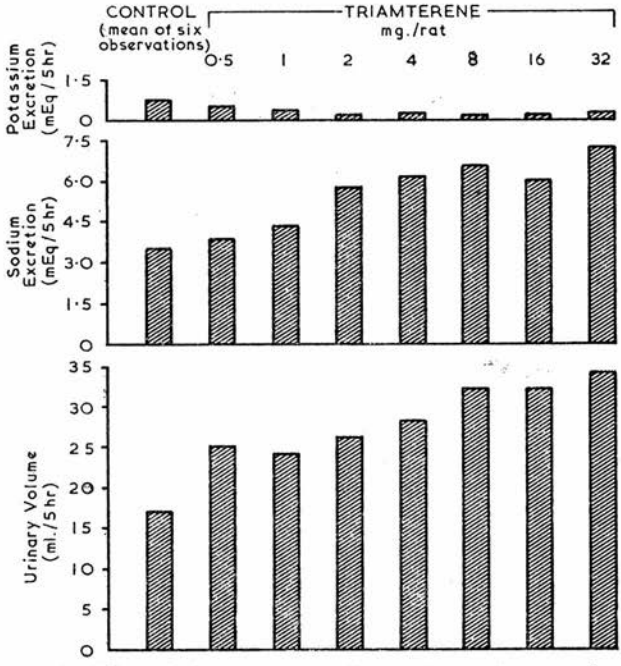

FIG. 6.—Effect of triamterene on sodium and potassium excretion in adrenalectomized rats. All observations refer to urine pooled from groups of three rats.

basis to 200 mg. of triamterene and 400 mg. of spironolactone respectively in a man of 70 kg. (triamterene 3 mg./kg. and spironolactone 6 mg./kg. body weight approximately).

Triamterene enhanced the excretion of sodium and produced a considerable decrease in potassium excretion. This effect was apparent with the smallest dose used (0.5 mg./rat) and increased with the increasing dosage (Fig. 6). In contrast spironolactone produced no appreciable change in sodium or potassium excretion in the adrenalectomized rats (Table III).

TABLE III.—*Comparison of the Effect of (A) Triamterene and (B) Spironolactone on Adrenalectomized Rats*

A. Triamterene

Group	Triamterene (mg./Rat)	Urine Vol. (ml.)	Sodium Excretion (mEq/V.)	Potassium Excretion (mEq/V.)
Control (6 groups) mean±S.D. ..	0	17±3·67	3·6±0·64	0·74±0·08
I	0·5	25	3·8	0·40
II	1	24	4·4	0·34
III	2	26	5·7	0·21
IV	4	28	6·1	0·20

B. Spironolactone

Group	Spirono-lactone (mg./Rat)	Urine Vol. (ml.)	Sodium Excretion (mEq/V.)	Potassium Excretion (mEq/V.)
Control (6 groups) mean±S.D. ..	0	26±7·6	4·4±1·24	0·74±0·18
I	2	20	3·4	0·81
II	4	28	3·8	0·76
III	8	18	3·3	0·83
IV	16	36	5·8	0·72

Discussion

Triamterene produced an increase in sodium, chloride, and bicarbonate excretion and a considerable decrease in potassium excretion in normal subjects. The natriuretic effect of triamterene in normal subjects, when given an ordinary diet, a high salt diet, or when under the influence of fludrocortisone was less than that of hydroflumethiazide. Urinary bicarbonate was increased after administration of triamterene. However, the natriuresis produced by triamterene does not resemble that due to a carbonic anhydrase inhibitor, as with the latter there is usually an associated increase in potassium excretion (Counihan *et al.*, 1954 ; Pitts, 1958).

The decrease in potassium excretion produced by triamterene in the normal subjects given an ordinary diet

is difficult to explain on the basis of aldosterone antagonism. In health the level of endogenous aldosterone secretion is low (Axelrad and Luetscher, 1954), and it has been found that spironolactone has little effect on electrolyte excretion in normal men receiving an ordinary diet (Edmonds and Wilson, 1960). Triamterene depressed potassium excretion in normal subjects given additional sodium, which reduces the level of endogenous aldosterone to a minimum (Mulrow *et al.*, 1956 ; Ulick *et al.*, 1958). In normal subjects the potassium retention resulting from triamterene was as great when a high sodium intake was given as with an ordinary diet ; this is further evidence that the potassium-conserving action of triamterene does not depend mainly on aldosterone antagonism.

Triamterene, like spironolactone, when given with fludrocortisone reverses the effects of the latter on sodium and potassium excretion. It does not necessarily follow that its mode of action is due to competitive inhibition of the mineralo-corticoids. Similar results would be obtained if triamterene had a direct effect on the renal tubules.

Spironolactone, even in high dosage, did not influence potassium excretion in the adrenalectomized rats, as had been already reported (Kagawa, 1960). On the other hand, triamterene produced a considerable reduction in urinary potassium output in these adrenalectomized rats.

The diuretic effect of triamterene is similar to that of chlorazanil, a triazine derivative. Williamson *et al.* (1959) showed that chlorazanil antagonized the salt-retaining effect of desoxycorticosterone and could inhibit its kaliuretic effect ; also they found it to be effective in adrenalectomized rats.

It is clear that triamterene has a natriuretic and potassium-retaining action, demonstrable both in normal men and in adrenalectomized rats. It influences potassium excretion by a mechanism independent of aldosterone antagonism. It is probable that one of the actions of triamterene is on distal renal tubular cells. leading to a depression of exchange of sodium for hydrogen and potassium ions.

Summary

Triamterene (2,4,7-triamino-6-phenylpteridine) is effective as a diuretic agent, increasing sodium, chloride, and bicarbonate excretion and reducing potassium

excretion in normal men. The degree of potassium retention was similar in normal subjects, both when on a normal diet and when receiving a high sodium intake. In adrenalectomized rats triamterene produced an increase in sodium excretion and a decrease in potassium excretion.

The results indicate that the action of triamterene is not due to aldosterone antagonism, but is probably a direct effect on the renal tubules.

W. I. B. is in receipt of a grant from the Iraqi Ministry of Education. We thank Dr. A. J. Smith and Mr. D. Gow for their willing co-operation and Messrs. Smith Kline and French for the supply of triamterene.

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II. CLINICAL TRIAL IN OEDEMATOUS PATIENTS

Triamterene has a diuretic effect in oedematous patients (Hild and Krueck, 1961 ; Laragh *et al.*, 1961 ; Donnelly *et al.*, 1962 ; Crosley *et al.*, 1962), producing an increased excretion of sodium and water but a decrease in potassium excretion. Our findings in normal subjects and in adrenalectomized rats suggested that the diuretic action of triamterene is not due to aldosterone antagonism but probably to a direct action on the renal tubular cells (Baba *et al.*, 1962).

The present work describes and compares the various types of responses encountered when hydroflumethiazide, spironolactone, and triamterene were given alone and in combination to patients with chronic salt and water retention.

Patients and Methods

Clinical studies of the action of triamterene were made in 42 patients ; 27 were studied as in-patients and 15 as out-patients.

In-patient Study.—Hydroflumethiazide (100 mg.), spironolactone (400 mg.), and triamterene (200 mg.) were given alone or in combination, according to the plan illustrated in Fig. 1. All the drugs were given at 10 a.m. and urine was collected over 24-hour periods, starting at 10 a.m. each day. Before giving each dose, urine collections were made for two days, during which no drugs were given. Patients admitted to the trial were assigned alternately to Group A or Group B, the grouping determining the order of administration of the drugs. Body-weight was measured daily. All the patients took a normal diet and fluid intake was unrestricted. Blood was taken at least twice weekly for the determination of electrolytes and urea. Urine and serum creatinine levels were estimated at the beginning and end of the period of study.

Out-patient Study.—The effects of prolonged administration of triamterene alone and when combined with hydroflumethiazide or spironolactone were studied in 15 patients with chronic oedema who had all been

previously treated for at least three months with oral diuretics. Observations of body weight, degree of oedema, serum electrolytes, and blood urea were made at two-week intervals, and each drug or combination of drugs was given either daily or on alternate days for periods of two weeks. Normal diet and unrestricted fluids were allowed during the trial.

Chemical Methods.—Urinary sodium and potassium were estimated by flame photometry; triamterene in urine was estimated by fluorimetry (Baba *et al.*, 1962); serum electrolytes and urea were determined by routine laboratory methods; serum and urine creatinine levels were estimated by the method of Owen *et al.* (1954).

Results

Response to Single Doses of Triamterene and of Hydroflumethiazide.—The effects of single doses of triamterene (200 mg.) and of hydroflumethiazide (100 mg.) were compared in patients in hospital. Patients of Group A received triamterene first and hydroflumethiazide second; in Group B the order was reversed. In both groups the sodium excretion was moderately increased after triamterene, but less so than after hydroflumethiazide in the doses used. Urinary potassium loss, which was increased after hydroflumethiazide was greatly reduced after triamterene (Tables I and II; Fig. 1).

Response to Combination of Triamterene and Hydroflumethiazide.—The response of hydroflumethiazide was considerably modified in both groups when triamterene was given simultaneously. There was a further increase in sodium and water excretion, whereas potassium excretion was greatly reduced. The sodium excretion and weight loss were greater than with either drug alone (Tables I, II; Fig. 1).

Comparison of Triamterene with Spironolactone.—In 20 patients the trial was continued by giving triamterene 200 mg. daily for four days, and on the fourth day hydroflumethiazide (100 mg.) was given in addition. In six of these oedematous patients spironolactone (400 mg.) was then given daily for four days, and on the fourth day hydroflumethiazide was also given. Three other patients were studied in a similar manner except that the four days of spironolactone administration preceded those of triamterene. A typical example of the results obtained is shown in Fig. 1; similar results

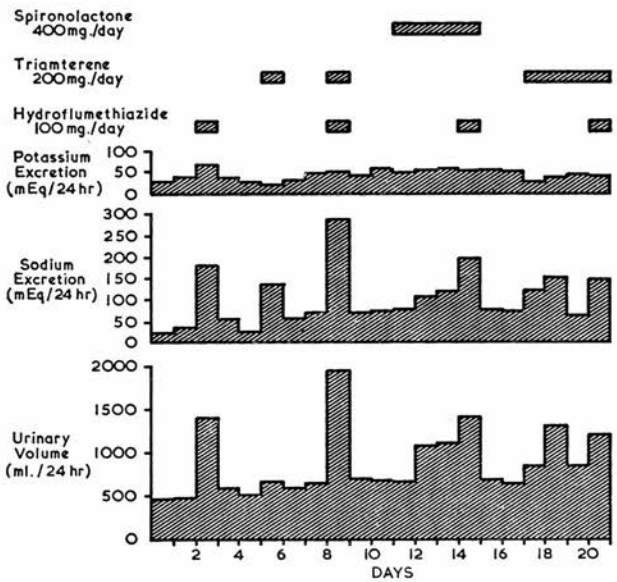

FIG. 1.—Case 4 (Group B, Table I), chronic rheumatic heart disease. Effect of hydroflumethiazide, triamterene, and spironolactone on daily urinary volume and excretion of sodium and potassium.

were found with the other patients in this group. The increase in sodium excretion after the first dose of triamterene was found to be greater than that produced by spironolactone. Repeated doses of triamterene were associated with a reduction in sodium excretion in most cases, whereas daily spironolactone produced a progressive increase in sodium excretion (Fig. 1). The average total loss of weight after three days of triamterene treatment was 0.5 kg., and after spironolactone it was 0.43 kg. When hydroflumethiazide was added after three days of either triamterene or spironolactone there was an increase in sodium excretion and weight loss (mean loss of 0.62 kg. with triamterene and hydroflumethiazide and of 0.55 kg. with spironolactone and hydroflumethiazide). With both combinations the potassium excretion was less than that produced by hydroflumethiazide alone (Fig. 1). Serum electrolytes and urea showed no significant changes. After three consecutive doses of triamterene creatinine clearance was unchanged in two patients and reduced by 40–50% in five.

TABLE I.—*Effect of Triamterene, Hydroflumethiazide, and Triamterene+Hydroflumethiazide on Urinary Sodium and Potassium Excretion in Patients with Chronic Oedema Receiving a Normal Diet*

Group A

Case No.	Age and Sex	Diagnosis	Control. Mean±S.D. of Four Untreated Days		Triamterene 200 mg.		Hydroflumethiazide 100 mg.		Triamterene 200 mg. Hydroflumethiazide 100 mg.	
			Na (mEq/day)	K (mEq/day)	Na (mEq/day)	K (mEq/day)	Na (mEq/day)	K (mEq/day)	Na (mEq/day)	K (mEq/day)
1	F 33	Chronic rheumatic heart disease	5±2·4	25±4·9	39	16	65	109	103	33
2	F 34	,, ,, ,, ,,	37±11·0	64±5·1	52	47	75	47	155	40
3	M 59	,, ,, ,, ,,	27±9·4	49±7·9	38	18	90	86	144	38
4	M 55	Chronic cor pulmonale	125±21·7	52±9·8	285	43	430	134	353	76
5	M 68	,, ,, ,,	80±7·3	46±2·6	149	33	227	67	282	24
6	M 65	,, ,, ,,	110±16·1	44±4·5	113	29	153	70	261	48
7	F 65	,, ,, ,,	14±4·7	22±6·5	30	16	76	34	99	24
8	M 68	,, ,, ,,	10±6·5	17±5·4	21	7	46	43	113	31
9	F 71	,, ,, ,,	22±19·6	28±2·2	53	9	228	66	173	47
10	F 54	Nephrotic syndrome and femoral venous thrombosis	13±5·3	32±8·2	56	32	156	33	235	32
11	F 37	Nephrotic syndrome	38±18·4	53±14·2	95	43	133	115	122	87
12	F 70	Hypertensive heart failure	28±9·5	30±8·9	50	26	92	32	125	17

Group B

			Control. Mean±S.D. of Four Untreated Days		Hydroflumethiazide 100 mg.		Triamterene 200 mg.		Triamterene 200 mg. Hydroflumethiazide 100 mg.	
			Na (mEq/day)	K (mEq/day)	Na (mEq/day)	K (mEq/day)	Na (mEq/day)	K (mEq/day)	Na (mEq/day)	K (mEq/day)
1	F 60	Chronic rheumatic heart disease	96±31·0	62±8·0	210	65	145	53	261	59
2	F 65	,, ,, ,, ,,	75±22·0	55±9·6	294	79	95	36	332	63
3	F 76	,, ,, ,, ,,	18±4·4	32±6·7	26	35	15	8	41	11
4	F 57	,, ,, ,, ,,	37±14·1	31±7·4	189	68	134	19	291	47
5	M 59	Ischaemic heart disease	83±40·3	59±19·5	327	76	50	32	250	56
6	M 75	,, ,, ,,	71±12·2	31±6·0	168	49	173	18	395	31
7	F 56	,, ,, ,,	35±9·0	29±5·1	198	66	76	11	307	40
8	M 72	,, ,, ,,	79±36·6	42±6·5	124	45	121	28	233	48
9	M 68	Femoral venous thrombosis	48±8·5	34±3·6	238	69	139	35	271	35
10	M 54	Nephrotic syndrome	142±36·4	44±11·0	370	62	264	35	502	67
11	F 68	Chronic cor pulmonale	6±2·2	17±0·3	26	25	8	3	70	21

TABLE II.—*Mean Daily Excretion of Sodium and Potassium (% Control) and Loss of Weight (kg.) Produced by Triamterene, Hydroflumethiazide Alone and in Combination, in Patients of Groups A and B (Table I)*

Group		Triamterene 200 mg.	Hydroflu-methiazide 100 mg.	Triamterene 200 mg. Hydroflu-methiazide 100 mg.
A	Sodium Potassium Loss of weight	+158% −33% 0·15 kg.	+441% +93% 0·6 kg.	+647% +14% 0·80 kg.
B	Sodium Potassium Loss of weight	+73% −41% 0·12 kg.	+240% +54% 1·01 kg.	+419% +11% 1·02 kg.

Triamterene Excretion in Urine.—The urine contained 10–88% of the administered dose (200 mg.) of triamterene in 24 hours. A correlation was found between the excess sodium excreted in 24 hours and the total amount of triamterene in the urine (Fig. 2).

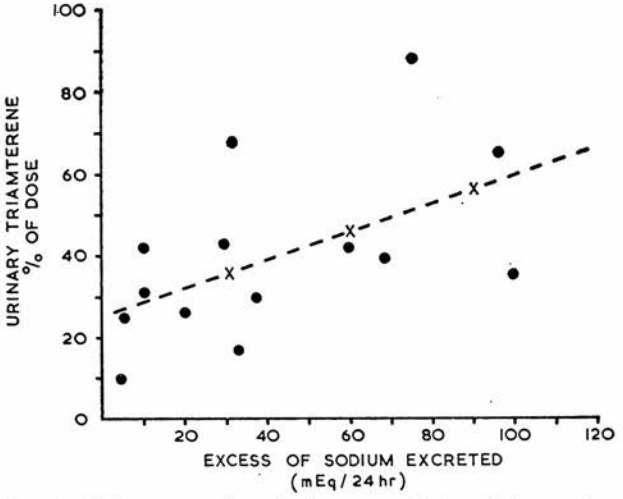

FIG. 2.—Urinary excretion of triamterene during 24 hours after a single dose of 200 mg., showing the correlation between the excess sodium excreted and the amount of triamterene in the urine ($r=0.54$).

Prolonged Treatment

Nineteen oedematous patients were studied. All had been treated previously with oral diuretics for several months, and according to their response to this previous treatment the patients were included in one of three

groups. Comparisons of the various regimes were made over periods of a fortnight. Examples of the results are shown in Fig. 3.

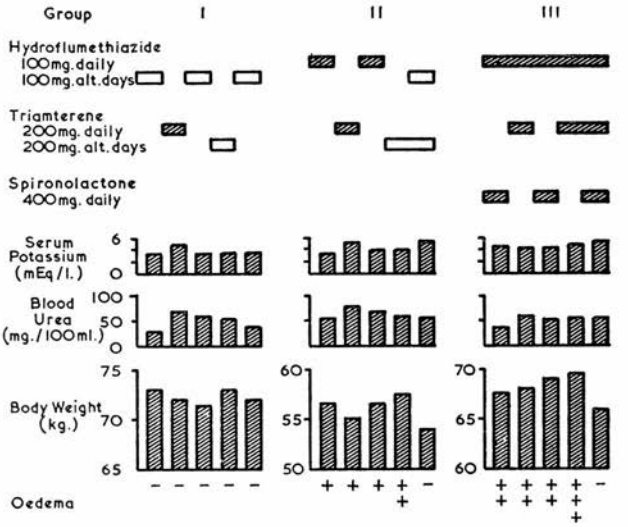

FIG. 3.—Examples of responses to prolonged treatment. Each therapeutic regime was given for a period of two weeks. Group I: Case 6—hypertensive heart disease; Group II: Case 12—thyrotoxic heart disease; Group III: Case 18—chronic cor pulmonale.

Group I

The seven out-patients had previously been controlled satisfactorily with hydroflumethiazide 100 mg. on alternate days and daily potassium supplements.

When triamterene 200 mg. on alternate days without potassium supplements was substituted there was a rise in weight in all the patients although at the end of the fortnight pitting oedema was not usually evident. With this dose of triamterene no rise in blood urea occurred and the serum potassium remained at about the level observed during treatment with hydroflumethiazide and potassium supplements.

When the dose of triamterene was increased to 200 mg. daily there was no increase in weight, and from the point of view of oedema control this was as satisfactory as with hydroflumethiazide on alternate days. However, the serum potassium showed a mean rise of 21%, although it did not go above the normal range, the

highest reading being 5.3 mEq/l. In all patients there was an increase in serum chloride (mean +6.4 mEq/l.) and a decrease in serum bicarbonate (mean −4.0 mEq/l.). The blood urea rose on average by 48%, and by the end of the fortnight six of the seven patients had readings above 40 mg./100 ml. In some cases the raised blood urea persisted during the subsequent two-week period of hydroflumethiazide therapy.

Group II

The six out-patients had been treated previously with hydroflumethiazide 100 mg. daily and daily potassium supplements. This regime had not kept them entirely oedema-free and minimal pitting was usually detectable at the ankles.

Triamterene 200 mg. daily was also not effective in relieving their oedema, and again there was a rise in serum potassium and blood urea. Further increase in oedema developed when triamterene was given only on alternate days. Similar changes in serum potassium and blood urea occurred as in the Group I patients.

A combination of hydroflumethiazide 100 mg. and triamterene 200 mg. both together on alternate days with no potassium supplements was then tried. In all cases there was a conspicuous loss of weight and at the end of the fortnight no pitting oedema was detectable. The serum potassium was in the range 3.3–4.0 mEq/l. and there was no rise in blood urea.

Group III

These six patients had persistent oedema in spite of prolonged daily treatment with hydroflumethiazide, spironolactone, and potassium supplements. Two were studied as out-patients and four in hospital. Continuation of the hydroflumethiazide but substitution of triamterene 200 mg. daily in place of spironolactone and potassium led to an increase in weight, oedema, and blood urea. However, when hydroflumethiazide, spironolactone, and triamterene were all given together daily there was a reduction in weight and loss of oedema. The blood urea remained above the normal range. More prolonged therapy has been continued in these patients.

Two patients with hitherto intractable oedema had shown persistently low serum sodium values.

Case 16.—A housewife aged 33 with chronic rheumatic heart disease had previously taken daily hydroflumethiazide 100 mg., spironolactone 800 mg., and potassium supplements for 10 months, but, despite this, she had considerable oedema and the sodium concentration in the serum was reduced to 127mEq/l. Triamterene (200 mg.) daily was added to her previous therapy including the potassium supplements. After one week on this regime she lost 7 kg. in weight and the oedema cleared completely (Fig. 4). There

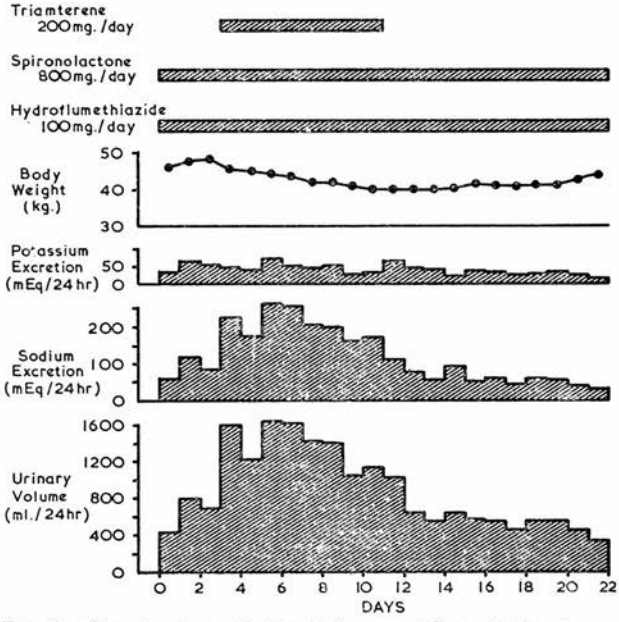

FIG. 4.—Chronic rheumatic heart disease. Effect of triamterene +hydroflumethiazide+spironolactone on daily body weight, urinary volume, and excretion of sodium and potassium.

was a rise in the serum sodium level to 140 mEq/l; however, there was also a rise in serum potassium to 9.6 mEq/l. and in blood urea to 82 mg./100 ml. After triamterene and potassium supplements were withdrawn, on account of the hyperkalaemia and impending uraemia, there was a decrease in sodium and water excretion and the weight began to increase. The serum sodium dropped to 123 mEq/l. and there was a reduction in potassium level to 4.9 mEq/l.

A similar diuretic response was obtained in Case 17, a housewife aged 50, with chronic cor pulmonale and severe congestive heart failure. She had a considerable degree of oedema and ascites. She had previously received hydro-

flumethiazide, spironolactone, and potassium supplements, but, despite this, the oedema was persistent and her serum sodium concentration was reduced to 124 mEq/l. In this case, triamterene 200 mg. daily was given in addition to spironolactone and hydroflumethiazide, but in view of the experience with the previous case potassium supplements were stopped. After three weeks on this regime the oedema had cleared completely, and the serum sodium level was remaining steady at about 138 mEq/l., the serum potassium at 3.6 mEq/l., and the blood urea at 40 mg./100 ml.

Side-effects

Apart from the rise in serum potassium and blood urea, no serious undesirable effects have so far been noticed. No change has been found in haemoglobin concentrations, leucocyte count, and blood film. No albuminuria or haematuria has been produced, but the faint blue colour of the urine has been consistently observed. A few patients have complained of looseness of bowel motions, which rapidly reverted to normal on discontinuing triamterene administration.

In addition to the studies described above, six patients received triamterene in the larger dose of 300 mg. daily for 14 days. Four of these developed diarrhoea and two had nausea and vomiting after four days of this dosage. The symptoms were rapidly relieved after stopping the drug. As with the other patients receiving daily triamterene, there was a rise in the level of the blood urea in all these cases, but no other toxic manifestations were found.

However, it is obviously too early to assess the potential toxicity of this drug in man.

Discussion

Triamterene produced an increase in sodium excretion and a considerable decrease in potassium excretion in oedematous patients. The increase in water and sodium excretion was found to be much less than that produced by hydroflumethiazide in the doses used. However, the two drugs produce their effect by different mechanisms, so that triamterene potentiates the action of hydroflumethiazide when given simultaneously, producing a further increase in water and sodium excretion and a decrease in potassium excretion.

Our results in oedematous patients support our finding that the mechanism of action of triamterene is different from that of spironolactone. The effect of repeated

doses of spironolactone produced a progressive increase in sodium excretion, whereas in most cases repeated doses of triamterene led to progressive decrease during the three-day period. The potassium-retaining action of triamterene was found to be greater than that of spironolactone. Prolonged administration of triamterene produced an increase in serum potassium level, and additional potassium should not be given when patients are treated with triamterene either alone or in combination with hydroflumethiazide. Triamterene was found to potentiate the action of spironolactone and hydroflumethiazide when all three were given together, which further suggests that triamterene has a different mechanism of action from that of spironolactone.

Prolonged administration of triamterene produced a rise in the serum levels of potassium and chloride and in blood urea and a decrease in serum bicarbonate level. The rise in blood urea may be explained by reduced glomerular filtration; repeated doses of triamterene over a three-day period produced a decrease in creatinine clearance in most patients.

It appears from our studies that administration of triamterene and hydroflumethiazide on alternate days produces no rise in blood urea. This regime was of considerable value in the treatment of patients whose oedema was persistent when treated with hydroflumethiazide alone. Daily administration of triamterene and hydroflumethiazide again produced a rise in level of blood urea.

The combination of triamterene, hydroflumethiazide, and spironolactone has proved of value in the treatment of refractory cases of oedema, but the level of serum potassium must be watched carefully.

It is evident from our studies that triamterene when given alone is a weak diuretic agent. Its main therapeutic use is in combination with a thiazide, or with spironolactone and a thiazide in treatment of refractory cases of oedema.

Summary

The action of triamterene (2,4,7-triamino-6-phenylpteridine) has been studied in 42 oedematous patients—27 as in-patients and 15 as out-patients. Triamterene produced an increase in sodium and water excretion. Its natriuretic effect was found to be less than that of hydroflumethiazide. Like spironolactone, it potentiates

the action of hydroflumethiazide, producing a further increase in sodium excretion and a decrease in potassium excretion. Its potassium-retaining action was greater than that of spironolactone, and potassium supplements should not be given when patients are treated with a combination of triamterene and hydroflumethiazide. Triamterene alone was not effective in patients whose oedema was persistent when treated with hydroflumethiazide alone, but in these cases improvement followed when the drugs were given together. Prolonged daily administration of triamterene alone or with hydroflumethiazide produced a rise in blood urea; however, this effect was not observed when the combined therapy was given on alternate days. This has proved the most satisfactory regime. Triamterene, hydroflumethiazide, and spironolactone given together were found effective in patients with otherwise persistent oedema.

W.I.B. is in receipt of a grant from the Iraqi Ministry of Education. We are grateful to Dr. K. Hardy, Medical Superintendent, Wharncliffe Hospital, and to the nursing staffs of the Royal Infirmary, Sheffield, and of Wharncliffe Hospital, Sheffield, for their willing co-operation, and to the technicians of the Department of Therapeutics, University of Sheffield, for their assistance. We thank Messrs. Smith Kline and French for the supply of triamterene.

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Printed in Great Britain by Fisher, Knight & Co., Ltd.,
The Gainsborough Press, St. Albans.

Reprinted from the BRITISH MEDICAL JOURNAL
February 2, 1963, *vol. i, pp.* 285–292

DIURETICS*

BY

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W. W. Bradshaw, in whose memory this lecture was founded, was initially in practice in Andover, where he married a rich widow. Thereafter he became practically independent of his profession, and, it is reported, enjoyed ease and leisure for the cultivation of his mind (Poore, 1881). He was thus a complete contrast to the lecturer who is endeavouring to commemorate him to-day. However, at one time he showed an interest in therapeutics and published an article entitled "On the Use of Cod-liver Oil in Chronic Rheumatism" (Bradshaw, 1845). He reported seven cases successfully treated. In six the cod-liver oil was given along with gin or brandy. In the seventh patient, an old parish midwife aged 71, "the stomach revolted if the dose was given on any vehicle except that of strong whisky," but on this medication there was remarkable improvement. "She certainly appeared to grow younger . . . and spent many years afterwards in the glories of longevity." This was attributed unreservedly to the effects of the oil! I fear that neither the design of his investigation nor his interpretation of the results brings us any closer together.

Introduction of Modern Diuretics

At a time when so much attention is being paid to the undesirable effects of drugs it is noteworthy that the development of the two most powerful and extensively used groups of diuretics to-day stemmed from the clinical observation of unexpected side-effects. Vogl (1950) has described how as a third-year medical student in Vienna unable to procure the mer-

* The Bradshaw Lecture delivered to the Royal College of Physicians of London on November 27, 1962.

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curial preparation ordered by his chief he gave an organic mercurial compound, merbaphen, to a non-oedematous girl as treatment for congenital syphilis and noted in the beautifully kept charts of the nurses a conspicuous rise in urine volume after each injection. At first this was regarded as a side-effect peculiar to syphilitic patients, but the drug was then tried successfully in a case of rheumatic heart disease, and so the organic mercurial diuretics were introduced (Saxl and Heilig, 1920). Subsequently mersalyl was synthesized in 1924 and the structure activity relationships of many organic mercurial compounds have been studied (Kessler *et al.*, 1957).

The thiazide group of diuretics descend from the observation of Southworth (1937) that treatment with sulphanilamide produced a metabolic acidosis and an alkaline urine. Subsequent investigation showed that sulphanilamide acted as a mild diuretic (Strauss and Southworth, 1938 ; Schwartz, 1949), and this was related to its carbonic-anhydrase-inhibitor activity (Mann and Keilin, 1940 ; Pitts and Alexander, 1945). This led to the synthesis of many related compounds, including acetazolamide (Roblin and Clapp, 1950) and finally chlorothiazide (Novello and Sprague, 1957) and its numerous derivatives.

Functional Organization of the Nephron

Disorders in several different organs may lead to generalized oedema, but the ultimate common denominator is a disturbance in the function of the nephron resulting in an inadequate excretion of sodium. The aim of treatment with diuretics is to correct this derangement, and an understanding of how they may achieve this result requires some appreciation of the function of the various segments of the nephron (Fig. 1). This will be described only briefly, as there have recently been several comprehensive reviews of renal physiology and pharmacology (Pitts, 1959 ; Wirz, 1961 ; Orloff and Berliner, 1961 ; Beyer and Baer, 1961 ; O'Connor, 1962).

Urine formation begins with the ultrafiltration of the plasma in the glomerular capillary tufts. The volume and sodium content of the filtrate depend on the condition of the glomeruli, the blood-pressure within them, and the composition of the plasma. About 80% of the filtered sodium is actively reabsorbed in the proximal convoluted tubule. Chloride probably diffuses passively

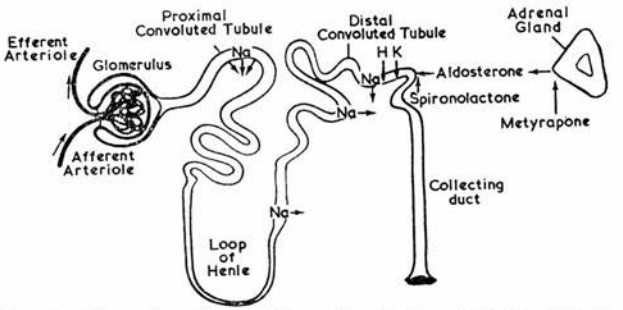

FIG. 1.—Sites of sodium reabsorption in the nephron and of the action of aldosterone and aldosterone antagonists.

secondary to an electrical potential gradient, and water accompanies this reabsorption of solutes so that the fluid within the tubular lumen remains iso-osmotic with plasma.

The loop of Henle has been the subject of much recent interest since it has been proposed that it acts as a counter current multiplier (Wirz *et al.*, 1951 ; Wirz, 1961). This hypothesis suggests that sodium is pumped out of the lumen of the ascending limb into the medullary interstitial fluid and that some then enters the descending limb. The osmotic pressure in the cortical portion of the kidney around the convoluted tubules is equal to that of the plasma, but it increases progressively in the medulla to reach a maximal value in the region of the tips of the medullary papillae. Water diffuses out of the descending limb into the hypertonic surroundings. The mechanism for the active transport of sodium in the loop is unknown.

In the distal convoluted tubule there is some further active reabsorption of sodium with accompanying anion and also an exchange of sodium for potassium and hydrogen ions. This exchange mechanism is under the influence of adrenal mineralocorticoids, and in the presence of excess aldosterone there is increased retention of sodium and excretion of potassium.

The collecting ducts may also be a site for sodium and potassium exchange (Jaenike and Berliner, 1960 ; Sullivan *et al.*, 1960 ; Hierholzer, 1961). They are relatively impermeable to water in the absence of pituitary antidiuretic hormone, but in its presence water passes freely out of the ducts into the hyperosmotic renal medulla.

Site of Action of Diuretics

A rise in the excretion of sodium with accompanying anions and water may be obtained by increasing the glomerular filtration of sodium or by reducing the reabsorption of sodium from the lumen of the nephron. When a diuresis occurs in an oedematous patient both mechanisms are often at work. Most primary diuretic agents probably have little or no direct influence on glomerular filtration, though this may be enhanced by other drugs, such as digoxin, which improve the circulation to the kidney. Xanthine derivatives are often considered to act by elevating the glomerular filtration rate, and, given intravenously in man, theophylline may have some effect in this way (Nielsen, 1961 ; Kleeman *et al.*, 1962). However, these drugs can act as diuretics without increasing glomerular filtration and have an important effect elsewhere in the nephron (Davis and Shock, 1949 ; Beyer, 1958a). The commonly employed clinically useful diuretics all act at one or more of the sites where sodium is removed from the lumen of the nephron.

Several different methods have been employed in attempts to determine the site of action of different diuretics, but they have not yielded consistent results. There is no agreement on where even the most commonly employed ones, such as mersalyl and chlorothiazide, have their principal effect. Every region in the nephron where active transport of sodium takes place, from the proximal tubules to the collecting ducts, has been proposed. The "stop-flow" method of investigation has placed their principal site of action in the proximal tubules (Kessler *et al.*, 1958, 1959 ; Vander *et al.*, 1958, 1959). The validity of this method has been criticized (Berliner, 1960), and it is probably unreliable in the elucidation of disturbances of proximal tubular function. However, both organic mercurials and thiazides may interact with transport systems known to be located in the proximal tubules, such as those for *p*-aminohippurate and probenecid (Berliner *et al.*, 1948 ; Beyer and Baer, 1962). The interpretation of changes in urine composition following the administration of these drugs in terms of current concepts of the function of different segments of the nephron has suggested that both organic mercurials and thiazides have predominantly distal actions (Januszewicz *et al.*, 1959 ; Au and Raisz, 1960 ; Earley *et al.*, 1961 ; Lambie and Robson, 1961 ; Levitt and Goldstein, 1962).

Histochemical and autoradiographic studies have shown that the mercurials and thiazides are widely distributed in the nephron (Cafruny *et al.*, 1955a, 1955b ; Cafruny and Farah, 1956 ; Darmady *et al.*, 1962). The presence of a drug in the cells of a particular segment does not necessarily mean that it has its effect on electrolyte transport there. It may be excreted into the tubular fluid, pass down the nephron, and act on a more distal luminal-membrane transport system (Orloff and Berliner, 1961). Nevertheless it is becoming difficult to accept that the action of either of these two groups of diuretics is confined to a single site, and it seems probable that they may well alter the function of various segments where sodium transport is taking place (Farah and Miller, 1962). Furthermore, the direct action of these drugs is not necessarily confined to the nephron : the possibility of an action on the renal vascular system has been little explored.

The exchange of sodium for potassium takes place in the distal part of the nephron and there is general agreement that drugs particularly affecting this mechanism—for example, the aldosterone antagonists —have a predominantly distal site of action (Malvin and Wilde, 1960 ; Vander *et al.*, 1960), though the locality has not yet been precisely defined. Evidence from experiments on potassium excretion suggests that the thiazines have an action proximal to the sodium and potassium exchange region (Edmonds and Wilson, 1960).

Clinical Assessment of Diuretics

The action of diuretics in man may be studied in either normal subjects or oedematous patients. Investigations in the former group are simpler to arrange and interpret, as repeated observations can be made in the same individual under reproducible conditions. It is important to standardize time of taking the drugs and of collection periods, diet, and posture. The last is of considerable importance. Recumbency increases urinary volume and the excretion of sodium (Thomas, 1957, 1959) and potentiates the action of a diuretic (Grossman, 1960). Indeed, in our experience rest in the horizontal position may produce as great a diuresis as a potent drug taken when the subject is up and about (Fig. 2).

While preliminary studies in healthy subjects are always valuable, final assessment of the value of a new

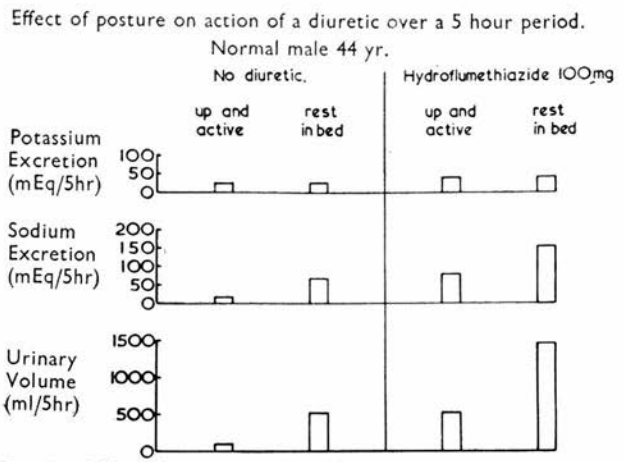

FIG. 2.—Effect of posture and activity on the action of a diuretic in a normal male. The results are the means of duplicate observations.

diuretic must be made in oedematous patients. Many difficulties can arise in determining its efficacy under these conditions (Spencer and Lloyd-Thomas, 1953). A spontaneous diuresis may occur at any time, and this may be precipitated by such measures as rest in bed, treatment of pulmonary infections, and administration of cardiotonic drugs. The extent of the response to a diuretic depends in part on the amount of oedema present. The first dose, when fluid retention is maximal, produces the largest result and subsequently the response gradually diminishes. The fluid loss on the first occasion may precipitate a further continued spontaneous diuresis which may obscure the duration of action of the drug and the effect of later doses. Nevertheless it is easier to assess the value of a diuretic than of many other drugs in properly designed clinical trials, as objective measurements of the results in terms of weight loss and urinary excretion of water and electrolytes are readily made.

As several potent diuretics are now available a clinical trial of a new one should be designed not only to show that it is effective but also to compare it with those already established. The type of trial designed by Gold *et al.* (1960) is the most satisfactory in this respect, and we have extensively used slightly modified versions (Figs. 3 and 6). The trial should not be started until the patient is in a fairly stable condition after a few days in hospital.

The essential feature is the order of administration of the drugs in the sequence ABBA or BAAB and the allowance of a day without treatment between each dose. It is most important that different diuretics should be compared whenever possible in the same patient. Claims, for example, that some recent thiazides cause less potassium loss than their predecessors are not convincing if the different drugs have all been given to separate groups of patients (Ford, 1960, 1961). As will be described later, the condition of the patient rather than the choice of thiazide is a more important factor in determining how much potassium is excreted. A diuretic such as spironolactone has a cumulative effect and in comparing agents of this type continuous administration for several days may be advisable. Even so, the same general principle of alternating the periods of treatment in the same patient should be followed.

Organic Mercurial Diuretics

Although these have now been in use for over 40 years almost the only point on which there is general agreement is that given parenterally they are extremely effective agents. Indeed, in most circumstances mersalyl or a related compound is still the drug of choice for speedy relief of oedema. Accordingly an organic mercurial is appropriately used as a reference standard in determining the potency of the newer diuretics. Gold *et al.* (1960) showed that 2 ml. of meralluride was more effective than 2 g. of chlorothiazide, and we also have found that thiazide diuretics usually produce a smaller response than 2 ml. of mersalyl (Fig. 3).

In studying the action of mercurials much confusion has been caused by assuming that the small amount of theophylline contained in such preparations as mersalyl and meralluride is without pharmacological effect. This is not the case as shown by recent investigations. Free water clearance is not increased by pure organic mercurials (Wesson and Anslow, 1952; Miller and Riggs, 1961), whereas theophylline has this effect (Goldstein *et al.*, 1961). Mixed preparations show a biphasic response which has been misinterpreted in the past (Farah and Miller, 1962).

Mercurials probably act at many sites in the nephron by inhibiting intracellular enzymes concerned with active transport processes, though the precise mechanisms are uncertain (Kessler, 1960). The effect of full doses of a

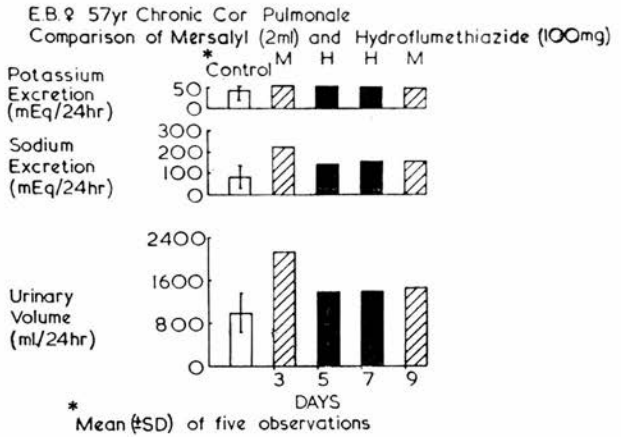

FIG. 3.—Comparison of diuretic activity of mersalyl 2 ml. and hydroflumethiazide 100 mg.

mercurial can be increased by giving thiazides or aminophylline (Weston and Escher, 1948 ; Weston *et al.*, 1952 ; Pitts *et al.*, 1958 ; Domenet *et al.*, 1961 ; Edmonds, 1960). These agents may affect either different mechanisms of sodium reabsorption or different sources of energy for a single mechanism. A decrease in plasma chloride concentration commonly follows the use of mercurial diuretics, and this may be corrected by the administration of ammonium chloride, which also acts as an acidifying agent. A reduction in the *p*H of the renal tubular cells is now regarded as probably of considerable importance in enhancing the effect of mercurial diuretics (Mudge and Weiner, 1958 ; Orloff and Berliner, 1961). Acidosis with renal failure and hepatic precoma are clear contraindications to the use of ammonium chloride. Recently dibasic amino-acid hydrochlorides have been used as adjuvants in place of ammonium chloride, but their value has not yet been established (Milne, 1962).

Toxic reactions to organic mercurials are rare, are seldom serious if the intravenous route is avoided, and usually take the form of allergic skin rashes. Organic mercurials should be avoided in cases of primary renal disease with oliguria and azotaemia. It is important not to confuse the effects of renal venous congestion secondary to cardiac disease with those of primary renal disease, as in the former case the use of mercurial

diuretics will improve the function of the kidneys. On the other hand, repeated doses of mercurials should not be given to patients who fail to respond, as this may lead to a high concentration of mercury in the cortex and possible renal damage.

The chief disadvantage of the mercurials is that they are only really satisfactory agents when given intramuscularly. Many elderly patients, though appreciating the relief that they afforded, dreaded the twice-weekly injections, and for them the advent of the thiazides was an immense boon.

Chlorothiazide and Related Drugs

The effectiveness of chlorothiazide was demonstrated in numerous clinical trials, and its success led to the development of a long and still-growing series of heterocyclic sulphonamide diuretics. Originally the action of chlorothiazide was attributed to its carbonic-anhydrase inhibitor activity, but this is relatively weak and it produces a greater loss of chloride than bicarbonate in the urine (Beyer and Baer, 1962 ; Kessler, 1962). Its diuretic activity is not significantly modified by acidosis or alkalosis (Beyer, 1958b). The later derivatives, though potent diuretics, have a negligible carbonic-anhydrase-inhibitor action when given in the usual therapeutic doses and do not significantly increase bicarbonate excretion (Edmonds and Wilson, 1959 ; Fleming *et al.*, 1959). They are effective in smaller doses by weight, and this greater potency is associated with increasing lipid/water solubility characteristics (Beyer and Baer, 1961).

Chlorothiazide is incompletely absorbed from the gut in man, but hydrochlorothiazide, hydroflumethiazide, and later derivatives are fully absorbed (Young *et al.*, 1959 ; Milne, 1962). The action of hydrochlorothiazide and hydroflumethiazide is usually over in under 12 hours, and this is probably the ideal duration of activity for a diuretic, particularly in the elderly and incontinent. More recent products such as trichlormethiazide and polythiazide act over a longer period (Ford, 1961). From the therapeutic point of view no one has any outstanding advantage and there is no evidence that the smaller dose of the more recent compounds reduces the liability to toxic effects. Chlorthalidone, in which the thiazide group has been replaced by a phthalamide group, closely resembles the thiazides in its pharmacological effect, the only noticeable difference being in its

duration of action, which may last up to 48 hours or more (Stewart and Constable, 1961 ; Douglas *et al.*, 1961).

The thiazides have been very extensively used and have caused remarkably few serious toxic reactions. Skin rashes may occur in a few patients and thrombocytopenic purpura is occasionally seen. More serious blood dyscrasias are fortunately extremely uncommon (Laragh, 1962a). Apart from these injurious effects there are other pharmacological actions of this group of drugs which are of considerable interest.

Action in Reducing Blood-pressure

Sodium depletion has long been recognized as an effective method of reducing the arterial pressure in hypertensive patients (Allen and Sherrill, 1922 ; Kempner, 1948 ; Medical Research Council, 1950). So long as the only way to attain this was the imposition of a highly unpalatable diet it was an unpopular method of treatment. However, the introduction of the thiazide diuretics afforded an alternative more acceptable approach and they proved effective hypotensive agents either alone or in association with other drugs (Tapia *et al.*, 1957 ; Juel-Jensen and Pears, 1960 ; Veterans Administration Cooperative Study, 1962). Chlorthalidone similarly has a hypotensive action (Cottier *et al.*, 1960 ; Bryant *et al.*, 1962a). Their action has been attributed entirely to loss of sodium from the body with a consequent diminution in extracellular fluid and plasma volume (Dustan *et al.*, 1959 ; Johnson *et al.*, 1962), but there were soon reports that the hypotensive effect persisted despite restoration of the sodium loss and plasma volume (Wilkins, 1957 ; Conway and Lauwers, 1960 ; Varnauskas *et al.*, 1961). This suggested that the thiazides when given over a period of several weeks might have a direct effect in reducing peripheral vascular resistance (Pickering *et al.*, 1961 ; Villarreal *et al.*, 1962), a view greatly strengthened by recent experiments using diazoxide. This thiazide derivative has sodium-retaining properties, but nevertheless decreases arterial blood-pressure (Rubin *et al.*, 1961, 1962 ; Hutcheon and Barthalmus, 1962 ; Dollery *et al.*, 1962). Experimentally, chlorothiazide reduces the response to the vasoconstrictor action of noradrenaline (Aleksandrow *et al.*, 1959 ; Eckstein *et al.*, 1962). The balance of evidence now favours the view that this group of

drugs has a direct action on the blood-vessels, quite apart from any hypotensive effect that may result from sodium depletion.

Antihypertensive drugs have been introduced much more rapidly than they can be evaluated therapeutically and the quick succession of new agents has not encouraged the essential long-term assessment. The thiazide diuretics alone are insufficient treatment for severe hypertension but are useful adjuvants permitting reduction in dose of the powerful drugs which are more liable to cause unpleasant side-effects (Pickering *et al.*, 1961). They are usually given twice or thrice daily in rather smaller doses than those employed for the relief of oedema. A longer-acting preparation such as chlorthalidone may be given in a single daily dose (Cottier *et al.*, 1960; Bryant *et al.*, 1962a). Tolerance to the antihypertensive action does not apparently develop, and with relatively small doses side-effects such as potassium depletion are rare. Whether treatment with these substances alone will improve the survival and decrease morbidity in patients with moderate hypertension is quite unknown.

Diabetes Insipidus

It is at first sight a surprising paradox that a thiazide diuretic may be effective in reducing the volume of urine excreted by patients suffering from diabetes insipidus. This was first demonstrated by Kennedy and Crawford (1959) and has subsequently been extensively confirmed both in pituitary and in nephrogenic cases (Crawford *et al.*, 1960; Havard and Wood, 1960; Calesnick and Brenner, 1961; Alexander and Gordon, 1961; Cutler *et al.*, 1962).

Administration of 1 g. of chlorothiazide daily or an equivalent amount of one of the later derivatives may decrease the urine volume by up to half (Fig. 4). There is as yet no agreement on the mechanism of this antidiuretic action. This effect may be produced by other diuretics, such as mersalyl and spironolactone, and may be maintained by salt-restriction alone; however, the thiazides apparently cause a greater reduction in urine volume than the other diuretics (Havard and Wood, 1961). Chlorothiazide reduces free water clearance, probably by suppressing sodium reabsorption at those sites in the distal part of the nephron where sodium is normally reabsorbed without water. Goodman and Carter (1962) suggest that the reduction in urine volume

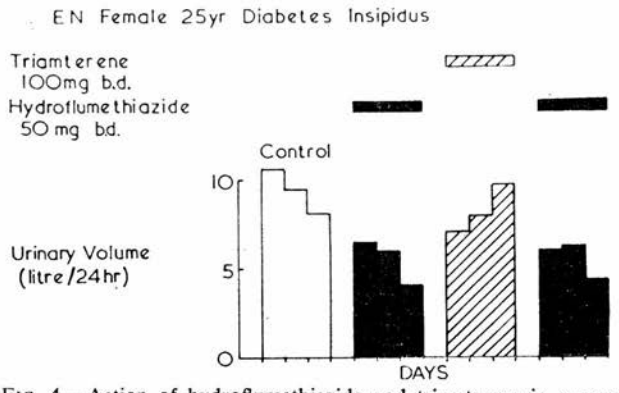

FIG. 4.—Action of hydroflumethiazide and triamterene in a case of diabetes insipidus.

is due to a decrease in the amount of urine reaching the distal part of the nephron secondary to the sodium deficit induced by the diuretic, and in their case of nephrogenic diabetes insipidus administration of chlorothiazide did not change serum osmolality.

On the other hand, Robson and Lambie (1962) point out that chlorothiazide may produce a good therapeutic response even though there is no fall in glomerular filtration rate. In their detailed study of three cases of vasopressin-sensitive diabetes insipidus they reported that chlorothiazide invariably produced an immediate and significant reduction in serum osmolality. This preceded any diminution in polyuria and persisted for as long as the drug was given. It is suggested that thirst was consequently reduced and that this was an important factor in diminishing the polyuria when chlorothiazide was given. Dies *et al.* (1962) have reported that hydroflumethiazide increased water transport in the toad bladder like vasopressin, and accordingly the suggestion was made that these drugs increased the permeability of the human collecting tubule to water (Dies and Rivera, 1962), but this requires further confirmation.

Whatever the precise mechanism may be there is no doubt that this is a useful oral method of treating diabetes insipidus, especially the nephrogenic type in which vasopressin is ineffective.

Metabolic Disturbances

Chlorothiazide and its derivatives may aggravate established diabetes mellitus (Goldner *et al.*, 1960;

Becket and Lewis, 1960), and they have produced acidosis when given to diabetics during pregnancy (Sugar, 1961). Using the intravenous tolbutamide test, Runyan (1962) has shown that a thiazide given in large dosage to mild diabetics invariably had an adverse effect. These drugs may also bring to light latent diabetes in those with a family history (Shapiro *et al.*, 1961 ; Hollis, 1961). The symptoms of diabetes mellitus provoked by thiazides usually appear after two to three months of treatment, and the disturbance is reversible on stopping the drug. Diazoxide, the related antihypertensive drug, may precipitate severe diabetes. Dollery *et al.* (1962) have reported the acute development of severe diabetes in two patients without any family history during the fourth week of treatment with both diazoxide and hydrochlorothiazide. There was a prompt recovery after withdrawal of the drugs. The results of plasma-insulin studies in one of the patients suggested that there was a direct inhibitory effect on the pancreatic islet cells.

This hyperglycaemic action should not preclude the use of thiazide diuretics in diabetic patients with oedema, but the dose should be kept low. Most diabetics are not adversely affected to any significant degree ; loss of control with hyperglycaemia was noted in 6 out of 20 patients in the report of Goldner *et al.* (1960). It is important to remember this hazard, as the polyuria and weight loss may mistakenly be regarded as the result of diuretic therapy and their relation to hyperglycaemia be overlooked (Shapiro *et al.*, 1961).

Prolonged treatment with thiazide diuretics leads to a rise in plasma-uric-acid concentration (Healey *et al.*, 1959). This usually produces no symptoms but rarely may precipitate attacks of acute gouty arthritis (Oren *et al.*, 1958 ; Aronoff, 1960). Chlorothiazide and related drugs in the concentration achieved with oral administration probably block urate secretion by the renal tubule (Bryant *et al.*, 1962b ; Demartini *et al.*, 1962). The effect is quickly reversed on stopping the drug.

Potassium Depletion Due to Diuretics

Excessive potassium loss may occur during treatment with diuretics. It is not usually such a prominent feature when mersalyl is used, but carbonic-anhydrase inhibitors considerably increase the excretion of potassium (Counihan *et al.*, 1954). As chlorothiazide also inhibits

carbonic anhydrase to some extent this was regarded as the cause of the potassium loss often seen when the drug was given. The more recent thiazide derivatives have little carbonic-anhydrase-inhibitor activity and in normal subjects cause little or no increase in potassium excretion (Edmonds and Wilson, 1959). However, clinical studies showed that the serum-potassium concentration sometimes decreased significantly when these agents were given, and it was apparent that carbonic-anhydrase inhibition was not the major factor in determining the amount of potassium that was excreted. The loss was greatest in certain conditions such as hepatic cirrhosis. It was also conspicuous in patients with severe congestive failure, particularly those who showed hepatic enlargement and ascites and had received intensive treatment with diuretics (Edmonds, 1960). Under these circumstances there is an enhanced secretion of aldosterone (Laragh, 1962b ; Venning *et al.*, 1962), and in the presence of liver disease the metabolism of the hormone is slowed (Hurter and Nabarro. 1960 ; Ayers *et al.*, 1962 ; Coppage *et al.*, 1962).

Experimentally when a thiazide diuretic is given to normal subjects the amount of potassium excreted in the urine is related to the amount of salt-retaining steroid being secreted by the adrenals or supplied exogenously (Edmonds and Wilson, 1960). In experiments on adrenalectomized rats Gantt and Synek (1961) found that hydrochlorothiazide caused a potassium diuresis only when a mineralocorticoid hormone was given. The most widely accepted explanation is that thiazide diuretics have a major effect in preventing sodium reabsorption proximal to the region in the nephron where the exchange of sodium for potassium takes place. When a diuretic with this type of action is given a large amount of sodium becomes available for exchange (Fig. 5). The exchange mechanism is stimulated by aldosterone and if the level in the blood is high there is a considerable potassium loss. Hence the clinical condition of the patient is the main factor in determining how much potassium is excreted and not the choice of any particular thiazide.

Mersalyl and other organic mercurial diuretics usually produce less potassium loss than the thiazides (Edmonds, 1960). When mersalyl is given to normal subjects on an ordinary diet the amount of potassium excreted in the urine is decreased (Dale and Sanderson,

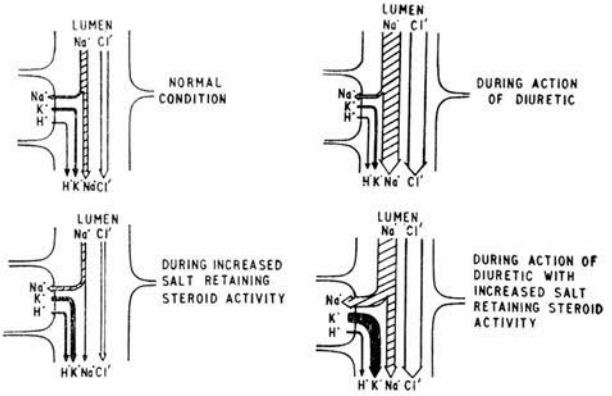

FIG. 5.—Effect of salt-retaining steroid on the exchange of sodium for potassium and hydrogen ions in the distal part of the nephron. When a diuretic acting proximally is given, a large amount of sodium becomes available for exchange, and if the exchange mechanism is stimulated by aldosterone or related compounds there is excessive potassium loss.

1954), and this is attributed to a direct depressant effect of mercurials on the secretion of potassium (Berliner *et al.*, 1950, 1951). However, in the presence of excess aldosterone this depressant effect may be partially overcome. Normal subjects taking a low-sodium diet show an increased potassium excretion when given mersalyl (Dale and Sanderson, 1954). Similarly in clinical conditions associated with increased aldosterone secretion potassium depletion may complicate prolonged treatment with mersalyl. It is, however, usually not as great as may be seen with the thiazides, which have no direct action on the sodium and potassium exchange mechanism.

Aldosterone Antagonists

Excessive aldosterone secretion may to some extent contribute to the abnormal salt-and-water retention seen in oedematous patients (Nelson and August, 1959). Bilateral adrenalectomy in cases of hepatic cirrhosis with intractable ascites may be followed by relief of oedema (Marson, 1954; Giuseffi *et al.*, 1957; Henley *et al.*, 1960). On the other hand, oedema is not produced by excessive aldosterone secretion alone, as in Cohn's syndrome, or by continued administration of large amounts to healthy subjects (August *et al.*, 1958a, 1958b; Lieberman, 1958). In many early cases of congestive cardiac failure with oedema aldosterone secretion is not significantly increased (Laragh, 1962b).

It is thus not surprising that aldosterone antagonists alone are not powerful diuretics but are valuable adjuvants to other diuretics in those conditions in which aldosterone is present in excess. In these cases drug therapy is aimed at either inhibiting its effect on the nephron or preventing its synthesis in the adrenal cortex (Fig. 1).

Spironolactone has a structure resembling that of aldosterone and acts as a competitive antagonist at the site of action in the distal part of the nephron. In this region aldosterone enhances the absorption of sodium with accompanying chloride and also the exchange of sodium for potassium ions. Administration of an aldosterone antagonist in suitable cases accordingly increases the excretion of sodium and decreases that of potassium. Spironolactone in the form first introduced (" aldactone ") was incompletely absorbed from the gut, but in the more recent finely dispersed small particle form (aldactone A) is much better absorbed and is effective in about a quarter of the original dose (Noel and Leahy, 1962). This is an excellent example of how pharmaceutical formulation may greatly alter the activity of a drug (Gantt *et al.*, 1962). While spironolactone alone may produce a diuresis in some cases (Gantt and Eckland, 1962; Chey and Shay, 1962) delivery of a relatively large amount of sodium to the distal part of the nephron is essential for the full effect of spironolactone, and this is achieved by the concomitant administration of a mercurial or thiazide diuretic (Edmonds, 1960; Shaldon *et al.*, 1960). The spironolactone ensures that the sodium is not absorbed or exchanged for potassium under the influence of aldosterone but is passed on for excretion. Spironolactone has a cumulative action and the full diuretic effect is usually not seen until it has been given for several days. In many patients it is unnecessary to continue potassium supplements along with spironolactone after any initial existing potassium depletion has been corrected. In others, particularly those with hepatic cirrhosis and ascites, some excess potassium loss may persist when combined therapy is given, and supplements must be continued (Shaldon, 1961). Careful clinical and biochemical control is essential when this type of treatment is being given.

The production of both cortisol and aldosterone in the adrenal cortex may be inhibited by the administration of metyrapone (" metopirone," SU 4885) which

prevents hydroxylation at the 11β position. The resultant fall in the level of circulating cortisol leads to pituitary release of corticotrophin. In these circumstances the adrenal secretes deoxycortone, which is strongly salt-retaining and resembles aldosterone in its action on the kidney. A reduction in salt-retaining steroid production can only be achieved if along with the metyrapone prednisolone is also given to suppress pituitary activity. In this way some diuretic effect may be obtained in suitable cases (Holub and Jailer, 1960; Shaldon and McLaren, 1960). However, a relatively large dose of prednisolone must be given, at least 30 mg. a day in our experience, and this is certainly a disadvantage. This method is not so satisfactory in cutting down potassium loss, and direct comparisons have not demonstrated that the metyrapone and prednisolone combination possesses any special advantages.

Triamterene

Triamterene (2,4,7-triamino-6-phenylpteridine: SKF 8542; "dytac") is a recently introduced diuretic which increases the excretion of sodium but depresses that of potassium (Wiebelhaus *et al.*, 1961; Hild and Krueck, 1961; Laragh *et al.*, 1961; Crosley *et al.*, 1962). On this account it was originally regarded as an aldosterone antagonist, but further investigations showed that it exerted this effect after adrenalectomy when no mineralocorticoid was given and also in men taking an excess of sodium chloride to depress the adrenal secretion of aldosterone (Liddle, 1961; Baba *et al.*, 1962a; Cattell and Havard, 1962). The effect is presumably directly on the nephron, and one site of action must lie in the distal tubule where the drug depresses the absorption of sodium and the exchange of potassium for sodium. It also raises the *p*H of the urine and increases the excretion of bicarbonate. We have found no evidence that it is a carbonic-anhydrase inhibitor (Baba, unpublished observations). In comparison with a thiazide diuretic it has a weaker natriuretic action when given in maximal tolerable doses (Fig. 6). When a thiazide and spironolactone are given in full dosage triamterene produces an additive effect, and this provides further evidence that it has a pharmacological action distinct from that of the other two drugs (Baba *et al.*, 1962b; Donnelly *et al.*, 1962; Shaldon and Ryder, 1962). Triamterene did not possess the action of a thiazide in reducing the polyuria of diabetes insipidus in the only

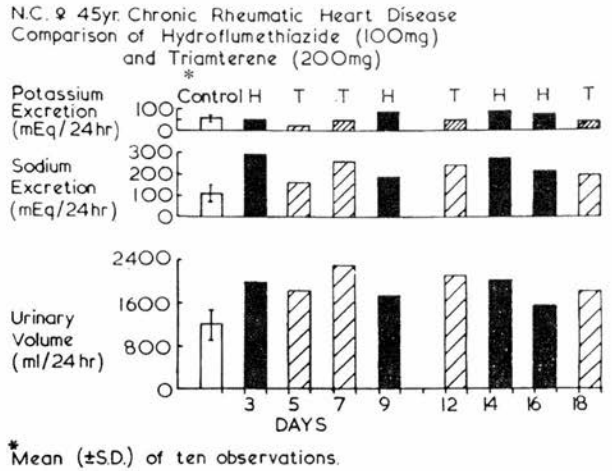

FIG. 6.—Comparison of the diuretic activity of triamterene 200 mg. and hydroflumethiazide 100 mg.

patient with diabetes insipidus in whom so far we have been able to make a comparison (Fig. 4). Unlike the thiazides it increases the excretion of uric acid (Cattell and Havard, 1962).

Triamterene is not a diuretic of first choice but it may be a useful adjuvant to the thiazides, particularly in treating patients in whom excessive potassium loss is prone to occur. So far it has not been associated with any serious toxic effects, but if given daily in doses of 200 mg. or more it may occasionally produce slight diarrhoea. The main use of the drug is to supplement the action of other diuretics in patients not otherwise responding, and in these circumstances it may produce a fall in creatinine clearance and a rise in blood urea. There is at present some diversity of opinion regarding the necessity of giving potassium supplements when triamterene is employed in addition to other diuretics. Most investigators treating cases of severe congestive failure have found them unnecessary, and indeed they may precipitate a dangerous rise in serum-potassium concentration (Baba *et al.*, 1962b; Cattell and Havard, 1962). On the other hand, Shaldon and Ryder (1962), treating patients with hepatic cirrhosis and ascites in whom excessive potassium loss is particularly liable to occur, found that they were required. Clearly, until further experience has been gained this new

diuretic should only be used under conditions where close clinical and biochemical supervision is possible.

Conclusion

Triamterene is at present the most recent of a wide range of powerful diuretics available for the relief of oedema. They have different mechanisms of action and their effects are thus additive. As a result there are now relatively few patients resistant to treatment. If there is failure to achieve a response this is probably due to diminished glomerular filtration, and it is important to look to extrarenal factors that may require attention, such as the treatment of pulmonary infection, the adequacy of bed rest, the correction of potassium depletion, and the proper use of digoxin.

Diuretics can only relieve a symptom. They do not remove the underlying cause, though the dispersal of oedema may in turn have secondary beneficial effects such as better arterial oxygenation and improvement of the circulation. Nevertheless it is possible to pay too high a price in the attempt to gain complete symptomatic relief. Excessive diuretic therapy may, in spite of the persistence of some oedema, lead to a rising blood urea, serious electrolyte disturbances, hepatic coma, and the hyponatraemic state with its associated gross derangement of cellular function. The physician must display understanding and judgment in his employment of these agents and ensure that his own wisdom does not fall short of that of the body.

Though it has been my privilege to deliver this lecture it includes work done in my department which would certainly never have been undertaken or completed without the loyal assistance of many colleagues, and to them I am most grateful.

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Printed in Great Britain by Fisher, Knight and Co., Ltd.,
Gainsborough Press, St. Albans.