FLUID RETENTION IN PATIENTS WITH CHRONIC LIVER DISEASE.

A Critical Evaluation of the Factors responsible for the development of fluid retention in patients suffering from Laennec's Cirrhosis of the Liver, with reference to the inadequacies of commonly accepted theories, and including in particular the proposal of a new hypothesis with evidence in its support.

by

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PREFATORY NOTE.

The investigations incorporated in this thesis were carried out in the metabolic laboratories of New York University under the tenure of a Rockefeller undergraduate scholarship and subsequently of a New York University postgraduate fellowship. The study was aided by grants from the Rockefeller Foundation and Nutrition Foundation. This work was part of a concerted effort by a group of physiologists and physicians into the nature, physiology and pathology of Laennec's Cirrhosis of the Liver, carried out under the supervision of Dr Charles Hoagland, member of the Rockefeller Institute for Medical Research and Dr E.P. Ralli, associate professor of medicine in New York University. I am indebted to Dr Hoagland and Professor Ralli for their permission to present in this form that part of the investigation for which I was personally responsible.

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INTRODUCTION.

Manifestations of fluid retention are amongst the most common signs of cirrhosis of the liver, and they take the form either of ascites or oedema affecting the lower extremities in the majority of cases. The association of ascites with liver disease was apparently first recognised and recorded by Erasistratus (310-250 B.C.) (1) and in recent years many workers have reported large series of cases of this condition in which ascites and oedema were the most commonly found features. In a group of 386 cases collected by Ratnoff and Patek (2) 301 were found to possess ascites, while oedema of the ankles was present, in addition, in 201 of their cases. In 1929 Rolleston and McNee (3) recorded the incidence of 80 patients with ascites out of a total of 85 suffering from cirrhosis of the liver; and Klopstock (4) reported an incidence of 69% in a series of 250 cases of this condition.

The occurrence of peripheral oedema in patients with cirrhosis of the liver is common clinical knowledge. Chapman, Snell and Rowntree (5 , 6) found oedema to be present in 20 out of 58 patients who were suffering from cirrhosis without ascites, and in 83 out of 112 patients with ascites. In a series of cases described by Hendrickson (7) 44% of a group of 162 patients with hepatic/

hepatic cirrhosis suffered from oedema. Ratnoff and Patek (2) noted oedema without ascites in 16 out of their series of 386 patients. Austin Flint (8) reported the appearance of peripheral oedema, before ascites was observed in 11 out of 21 cases; but in regard to these cases it is well recognised that ascitic fluid, when present in small quantities is not easily detectable.

The oedema is usually confined to the lower extremities, but may also involve the scrotum and abdominal wall, or become generalised. Facial oedema in patients with cirrhosis of the liver, who were not suffering from detectable renal conditions has also been described, but is a rarity (9. 10. 11.)

The factors which have been considered responsible for this fluid retention have been numerous and varied. Depending upon the author, emphasis has been laid on such forces as diminished colloid osmotic pressure of the plasma, hydrostatic pressure in the portal vein, increased permeability of capillary walls, an elusive water soluble fraction of meat and the activities of histamine-like substances. As will be seen, in recent years the first two of these forces have received greatest attention and the onus for the development of ascites has been almost entirely ascribed to them.

In the following presentation, each of these factors/

factors is reviewed, and particular attention is paid to the osmotic pressure theory and the mechanical portal hypertension hypothesis. With regard to the latter, criticisms of a theoretical nature are advanced which throw doubt on the proposal that this force is an important determining cause of ascites; while with regard to the former, experimental and clinical evidence is presented which indicates that although a diminution of plasma colloid osmotic pressure does exist in these cases, it cannot be considered to be the sole factor or even one of great moment. The subsequent and larger part of the thesis purports to offer an alternative explanation of the phenomenon under discussion and it includes experimental evidence in its support.

COLLOID OSMOTIC PRESSURE.

An inquiry into the factors responsible for aberrations in fluid balance naturally begins with a consideration of osmotic pressure. For this is the one force that has received the greatest attention because it is the one susceptible of most accurate measurement.

Osmotic pressure may be considered as the expression in solutions of the same phenomena which gives rise to pressure in gases and obeys comparable laws. The total osmotic pressure of a solution, biological or other, is determined by the concentration of dissolved chemical particles of all kinds, and it varies, as in the case of gases, with temperature and volume.

The osmotic pressure is a property inherent in a solution, but it is measured by the energy which becomes manifest when the solution is placed in such a machine that this energy is measurable in terms of work done. This machine usually consists of a semi-permeable membrane, in which case the work done may be measured in centimeters of water. In the body the membranes which allow the osmotic pressure to manifest itself are of varying degrees of porosity and permeability, but in the main, as is the case of the capillary endothelium or serous surface, are permeable to electrolytes and normally not (or only slightly so) to protein molecules. (12) In these circumstances it becomes clear that the effective osmotic pressure of biological fluids (e.g. plasma/

plasma) is dependent upon and will vary with the concentration of these particles which are to be found on the one side of the membrane only - namely those of albumin and globulin.

In the experiments which follow the osmotic pressure of the plasma of patients suffering from cirrhosis of the liver was calculated (vide infra) from estimations of the plasma albumin and globulin. In those cases in whom ascites was present the osmotic pressure of the ascitic fluid was not taken into account, as it was found that the protein content of the fluid was less than 0.1% and would neither differ significantly from the film of peritoneal fluid which would be present in those cases without ascites nor would it exert significant osmotic effects.

The colloid osmotic pressure has, since Starling (13, 14) been recognised as one of the most important variants controlling the balance and equilibrium between intra and extra vascular fluid. The demonstration by Barker and Kirk (15), Darrow and Hopper(16) and Leiter (17) of the oedema and ascites which ensues when the proteins in the plasma are reduced by plasmapheresis or by deprivation of protein (Frisch et al(18) and Weech et al (19)) has lent support to Starling's hypothesis. And the relationship has been made more convincing by the prevention of plasmapheretic oedema by/

by the intravenous injection of acacia (Fahr and Kerckoff (20)). Claims have been made in these experiments that there are critical levels of plasma colloid osmotic pressure below which oedema and ascites make their appearance.

Naturally these theories have been applied to pathological conditions in man which are associated with gross disturbances in fluid balance, and an endeavour has been made to correlate water retention with the plasma colloid osmotic pressure.

Critical levels of serum albumin and globulin below which oedema is stated regularly and predictably to occur have been described by various observers in nephrosis and nephritis (21. 22); and highly relevant to our attention are those which claim similar levels for the development of ascites and oedema in cirrhosis of the liver and purport to place almost the entire burden upon the plasma proteins for the development of ascites in this condition.

A low plasma albumin and a normal or elevated plasma globulin in cases of cirrhosis of the liver has been established by many workers. (23-27) Myers and Keefer (28) confirmed these estimations and further state that "the ascites would appear to result from increased hydrostatic pressure in the portal vein and a low plasma protein level" and a similar explanation was offered for the dependent oedema. Post and Patek (29)/

(29) following the idea of Loeb and his associates(30) that ascitic fluid may also be regarded as a transudate, report the levels of plasma albumin and globulin in a series of 43 patients with ascites and 13 without this phenomenon. Apparently only one plasma determination was performed on each patient. The two groups were then compared, and it was claimed that the values for serum albumin was strikingly different in the two groups. They make the statement "that ascites rarely appears when the level of serum albumin is greater than 3.5 gms %." They reach a conclusion similar to that of Meyers and Keefer that "the lowering of the serum albumin and therefore of the oncotic pressure is ample explanation for water retention in most cases of cirrhosis of the liver." (2)

Studies on Patients.

The first part of this report is concerned, therefore, with an examination of the colloid osmotic pressures of the plasma in patients suffering from established cirrhosis of the liver. The patients selected for this study were those in whom severe liver damage was unquestionably present as evidenced by physical findings and laboratory tests. A history of chronic alcoholism extending over years was obtained from each patient. Their diet consisted of 450 gms of carbohydrate, 100-120 gms protein and 85 gms. fat.

Meat/

Meat was given once daily, and the rest of the protein was obtained from milk, eggs, cheese and gelatin.* An aqueous extract of liver specially prepared by the Rockefeller Institute for Medical research was given diluted with 40 ccs normal saline intravenously. Two or three injections were given intravenously each week.** The plasma levels of albumin and globulin were determined at regular intervals of 3–4 weeks by the method of Howe (31) and the total proteins were performed by the micro-Kjeldahl method.*** Plasma cholesterol and cholesterol ester levels were also determined by a modification of the Schoenheimer and Sperry method (32). These offered undisputed evidence of liver damage, and are here not reported. Five mgms of Bromsulphalein per kgm. body weight were injected for this dye retention test, and the percentage retention was determined on a sample of plasma withdrawn after one half hour. The readings were made in a photo-electric colorimeter/

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* Knox gelatin. Charles B. Knox. Gelatin Co. Johnstown, N.Y.

** Details of preparation and results obtained by its use will, I understand, appear in a paper to be published shortly by Drs. Hoagland and Ralli.

*** I am indebted to Prof. Ralli for permission to quote some of her determinations of plasma albumin and globulin. These are mainly the earlier figures given; and they were obtained before this particular aspect of the work on these patients was underway.

colorimeter. Normal values were found to vary up to 6%. The colloid osmotic pressure was calculated from the plasma albumin and globulin determinations by the formula of Weiss and Peters (33) and correction was made for water content of plasma using Eisenman's formula(34).

Repeated Calculations of Colloid Osmotic Pressure were made on three groups of patients.-

(1) Patients who suffered from severe cirrhosis of the liver with fluid retention (ascites and oedema), but in whom with therapy a normal fluid balance was restored and in whom ascites and oedema subsided. This arrest occurred after varying periods (3 mos. - 2½ years). In this group it was possible to determine plasma levels of albumin and globulin both prior and subsequent to the cessation of fluid retention and ascitic accumulation; and comparison of the respective colloid osmotic pressures during these two phases was made.

(2) Patients in whom massive oedema and fluid re-accumulation in the abdomen continued during the period of observation.

(3) Patients who in spite of severe liver damage never at any time suffered from either ascites or peripheral oedema.

Results/

TABLE I.

Data on Patients.

Cases.	Age.	Sex.	Duration of Liver Symptoms.	Jaundice.	Oedema and Ascites.	Spider Angiomata.	Bromsulphalein retention on admission.
	Years						per cent.
1.	46	M.	24 months	No	Yes	No	21
2.	47	F.	7 months	Slight	Yes	Yes	20
3.	66	M.	2 weeks	No.	Yes	No	26
4.	39	F.	6 months	Yes	Occasionally	Yes	28
5.	53	M.	18 months	Yes	Yes	Yes	20
6.	59	M.	6 weeks	No	Yes	No	30
7.	57	M.	12 months	Yes	Yes	Yes	30
8.	54	M.	4 months	No	Yes	No	20
9.	43	M.	6 weeks	Yes	Yes	Yes	35
10.	72	M.	4 years	No	No	No	28
11.	40	M.	2 months	No	No	No	23
12.	36	F.	18 months	Yes	No	Yes	50
13.	68	F.	3 months	Slight	No	Yes	23
14	53	M.	2 months	Slight	No	Yes	35

Results on Patients.

In this study, a series of 14 patients were investigated and relevant data are given in Tables 1 - IV. In Table I are recorded brief notes of clinical relevance and the results of the Bromsulphalein test. The levels of albumin and globulin in the plasma during the period in which ascites and oedema were present, and at varying intervals after this had subsided, in conjunction with the calculated osmotic pressure are given in Tables II and III. Table IV presents the albumin and globulin levels and colloid osmotic pressure at intervals in patients in whom ascites or oedema was not present on clinical examination, and in whose history there was no suggestion that such had occurred.

In all patients the bromsulphalein retention was increased. In all three groups of patients the levels of albumin were low on admission to hospital, never exceeding 2.4 grams per cent. This was true whether or not ascites was present. The levels of globulin were elevated in all cases. In patients Nos. 1-6 (Table II-III) ascites was present for periods of 1-22 months. Cases No.1 and 2 are remarkable in that 19 and 33 paracenteses respectively were performed and the amounts of fluid removed on each occasion ranged from 8 to 25 litres. As is shown in Table III, ascitic fluid ceased to reaccumulate prior to any increase in the levels/

TABLE II.

Data on patients with Ascites.

Case No.	Nos. of Para-centesis	Range of Albumin & Globulin levels during period of Ascites.					
		Alb.	Glob.	C.O.P.	Alb.	Glob.	C.O.P.
		Grams	%	mm. H_2O	Grams	%	mm. H_2O
1.	19	1.7	4.9	179	1.9	3.9	166
2.	33	1.9	2.8	138	2.7	3.6	214
3.	3	1.7	3.9	161	1.4	4.0	148
4.	3	2.4	5.6	246	1.8	4.9	185
5.	1	1.6	4.6	168	1.8	3.9	160
6.	1	1.8	3.6	153			
7.	5	1.7	3.8	151	1.8	3.8	157
8.	15	2.1	3.7	176	2.0	3.2	154
9.	0	1.6	3.7	143	1.6	3.6	141

TABLE III.

Data on patients shown in Table II after cessation of accumulation of Ascitic Fluid.

Case No.	Period of no ascites	No. of plasma determin-ations.	After 4 weeks.			After 8 weeks.			After 6 months.			After 12 months.		
			Alb.	Glob.	C.O.P.	Alb.	Glob.	C.O.P.	Alb.	Glob.	C.O.P.	Alb.	Glob.	C.O.P.
	Months		Grams	%	mm. H_2O	Grams	%	mm. H_2O	Grams	%	mm. H_2O	Grams	Per cent	mm. H_2O
1.	20	20	1.4	5.4	174	1.8	5.2	194	2.0	4.2	188	1.9 (8 months)	4.4	179
2.	9	8	2.6	3.8	224	2.6	3.6	208	3.1	3.2	229	2.6	3.0	194
3.	20	14	1.7	4.0	166	2.6	4.2	224	3.0	3.7	237	3.4	3.2	247
4.	*	14	2.3	5.3	228	2.6	5.2	244						
5.	4	4	2.0	4.3	183	1.9	4.0	167	2.1	3.9	178			
6.	2	2	2.7	3.9	221	2.4	4.7	223						
7.	Died while still accumulating.													
8.	Still accumulating.													
9.	Died while still accumulating.													

* Occurred at intervals, following bleeding episodes.

levels of albumin in the plasma. Plasma levels of albumin, determined at intervals of about 4 weeks, failed to show any significant change until 6 months or more after ascites was absent. In Case I, for example, followed for a period of 20 months without ascites, no change was observed in the level of albumin in the plasma, the latest determination by me in August 1944 being 1.9 gms per cent. Cases 7 and 9 died while still accumulating ascitic fluid. In Case 8, a total of 15 paracenteses were performed (up to Aug. 1944). Ascites only developed in Case 4 following episodes of severe bleeding. At such times, it was necessary to transfuse the patient and to give 5 per cent glucose infusions. The ascites was so severe as to require paracenteses on 3 occasions. The levels of albumin in the plasma were as low when no ascites was present as during the periods when it occurred.

The levels of plasma albumin in Cases 10 to 14, in whom ascites or oedema was never present, were as low as in Cases 1 to 9. The patients, with the exception of Case 14, were followed for periods of 5 to 17 months and the levels of albumin and globulin are reported at intervals corresponding to those of the previous groups. In Case 10 and 11 who were followed for the longest period, 12 - 17 months, the albumin levels rose to 3.5 and 3.1 grams per cent.

These/

TABLE IV.

Data on patients with no Ascites clinically detectable.

Case No.	Total time observed	Control Period.			After 8 weeks.			After 6 months.			After 12 months.			Total No. Plasma Determinations.
		Alb.	Glob.	C.O.P.	Alb.	Glob.	C.O.P.	Alb.	Glob.	C.O.P.	Alb.	Glob.	C.O.P.	
	Months	Grams	%	mm. H_2O	Grams	%	mm. H_2O	Grams	%	mm. H_2O	Grams	%	mm. H_2O	
10.	17	2.4	3.9	205	2.6	4.0	219	2.4	4.1	219	3.1 (10 months)	3.5	232	14
11.	12	2.4	4.1	209	2.3	3.3	176	2.9 (3 months)	3.5	226	3.5	2.7	252	10
12	5	1.7	4.5	190	2.2	4.6	208	2.2	4.3	201				5
13	8	2.3	4.3	208	2.7	3.9	221	2.4	3.7	202				9
14	2	2.2	4.3	201										

These observations suggest that the levels of albumin and globulin, and the colloid osmotic pressure exerted by these substances are not the sole determining factors in the production of ascites in patients with cirrhosis of the liver. I suggest that the discrepancy, between these findings and those of Post and Patek (28) particularly, is due to a more careful and more regular examination of the colloid osmotic pressure during that critical time when the patients were passing from the phase in which fluid retention was marked, to the phase in which this had ceased to occur.

From a study of the colloid osmotic pressure of Case No.3 Table II-III, it will be seen that if comparison is made between the level of albumin determined when seen first while he was rapidly accumulating and reaccumulating ascitic fluid, and the albumin level 12 months after this had ceased, a rise of 1.7 gms per cent ~~is seen to have~~ occurred with an increase in colloid osmotic pressure of 86 mm. of water. This is undoubtedly a significant rise, and compares favourably with the reports of these workers. But it is not permissible to suggest a casual relationship between this rise in osmotic pressure and the restoration of a normal fluid balance, when it is a fact that the level of albumin in the plasma 4 weeks after ascites had subsided and failed to recur is seen to be similar to the level/

level prior to this cessation - namely 1.7 grams per cent, and when no appreciable change had occurred in the colloid osmotic pressure. A similar observation could be made on all the cases if studied in this fashion.

HYDROSTATIC PRESSURE IN THE PORTAL VEIN.

The mechanical explanation that ascites is the result of an egress of fluid from the radicles of the mesenteric veins which is produced by compression of the portal vein by slowly contracting fibrous tissue was suggested by Morgagni (35), and has been popular since that time. There is, indeed, no doubt that the venous pressure in the portal system is elevated in patients suffering from cirrhosis of the liver. This has been established by direct manometric measurements by Bellis, Thompson and others (36 37); and, further, McIndoe (38) has demonstrated that transfusion of the liver by saline requires a higher head of pressure when that organ is cirrhotic than in its healthy state.

The existence of elevated hydrostatic pressure should not be accepted too readily as adequate explanation of the phenomenon of ascites. And in this brief section there is enumerated some experimental evidence which tends to contradict the thesis, and also simple observations of a clinical nature which are not explained by it.

Bolton (39) in experiments on monkeys has shown that slight constriction of the portal vein does not result in the development of ascites. Some ascites was produced by greater constriction, but this persisted only a few weeks and subsequently subsided nor did/

did it recur. Complete obliteration of the inferior vena cava was also performed on Rhesus maccus. The animal soon recovered and seven days later considerable ascites was present without oedema of the legs. Examination of the animal after one year revealed complete reabsorption of ascitic fluid had occurred.

It is common clinical experience that advanced cirrhosis of the liver in which the organ may be either grossly enlarged, or small and shrunken, may occur without ascites being present. Four out of the series of 14 cases reported here fall into this group. And it is a further clinical fact that ascites is usually absent when the portal venous pressure is high as evidenced by the occurrence of haematemesis and melaena.

My experience is in agreement with that of Ratnoff's and Patek's (2) when they state that "ascites appears after an episode of haematemesis only to recede as the patient recovers from the blood loss". Those patients who suffer from the burden of massive and persistently recurring ascites escape the dangers of a vascular accident, while the latter, the result of the rupture of veins dilated by high portal pressure, tends to occur in those who show no evidence of fluid retention. The lack of correlation of these two events in the life history of the patient with cirrhosis cannot be neglected in any attempt to explain the problem of ascitic accumulation.

A/

A similar line of reasoning is followed by Hendrickson (7) when he maintains that if ascites was due to transudation of fluid as a result of portal hypertension, the fluid would pass into the mucosa of the stomach and intestine rather than into the peritoneum.

These considerations, and the fact of the extreme rapidity with which ascitic fluid is produced, taken in conjunction with the successful stopping of ascitic fluid accumulation when presumably marked anatomical changes have not occurred (See Cases 1-6 Table II) and the successful control in some cases by the use of organic mercurial diuretics (40) which can in no way be conceived to influence the mechanics, reveal that increased portal hypertension cannot be considered as a determining factor in the development of ascites. I think it is safe to conclude that while ascites is not caused by portal hypertension, such hypertension might well be responsible for the diversion of the fluid, retained for other reasons, into the abdominal cavity.

MISCELLANEOUS OTHER FACTORS.

Various other factors have from time to time been considered responsible for the development of ascites. It is proposed merely to mention these theories here; for they have been investigated by other workers, and most of them have been found wanting.

Fiessinger and Diaconescu (41) were among the first to consider that increased permeability of the capillary wall might be an important factor in the development of ascites and oedema in patients with liver disease. This possibility has now been extensively studied particularly by White and Jones (42). These two workers measured the permeability of the capillaries of patients suffering from various types of intrahepatic disease. They used the plethysmographic method of Landis and Gibbon (43) whereby the increase in volume of a part of the body (e.g. the forearm and hand) which occurs when the veins draining the part are partially occluded, is taken as an index of the permeability of the capillaries of the part to a given rise in intravascular pressure. They found that the rate of filtration of fluid through the capillary walls was no greater in patients with hepatic dysfunction than in normal individuals; and they concluded that abnormal accumulations of fluid in the tissues and serous cavities which occurs in certain liver diseases was in no way dependent upon alterations in/

in capillary permeability.

The conception that Histamine or histaline-like substances might be the active agents in producing a massive effusion of fluid into the peritoneal cavity is quoted by Lichtman (40). It was first proposed by Loeper and has subsequently been disproved by Fiessinger who found no trace of such substances in the ascitic fluid of these patients.

The curious finding by Bollman (44) that ascites can be precipitated at will by the feeding of a water soluble fraction of meat to animals whose livers have been damaged by carbon tetrachloride is not yet explained. The only conclusion that may be drawn from this fact, at the present time, is that the causes of ascitic accumulation under these experimental conditions must be more elusive than would at first sight appear, and that the phenomenon will not be finally explained in terms of crude mechanics or simple physico-chemical postulates.

THE CONTROL OF WATER EXCRETION.

It is a surprising fact that there is an almost complete absence in the medical literature of investigations of renal function in conditions which are associated with abnormalities in water balance. This is the more remarkable when it is recognised that water is the substance present in greatest amount in urine and that its excretion is one of the most vital functions of the kidney.

It seems obvious that the ultimate analysis of the problem of water retention will depend upon our knowledge of renal physiology. The retention of water in the body in pathological amounts can only result from a diminution in the requisite fluid loss in perspiration, expiration or in renal excretion; and the last of these is the one most actively controlled. It is only under such circumstances of lessened excretion relative to fluid intake that a positive water balance would be established. The problem of the determination of the causes of fluid retention in a body actively accumulating fluid reduces itself to the determination of the causes of inadequate excretion of water from the body, particularly these of inadequate renal excretion.

The/

The observation that there is a decrease in total urine output in patients with cirrhosis of the liver is not a new one, but one deserving greater attention than given to it hitherto. In 1901 Gilbert and Lereboiullet (45) reported this fact, and noted what they called "opsiurie" (i.e. "qui arrive ou se fait tard") as a common symptom of serious hepatic disease. Greene (46) in a review of the treatment of patients with cirrhosis of the liver with infusions of ascitic fluid, presents the typical picture in regard to water balance. It is seen in the case quoted in Table V that during the period of water retention the urine excretion is small in relation to the fluid intake, and is smaller than the fluid intake itself.

Comparison between the urine output and urine specific gravities of the cases shown in Tables VII and VIII offer further evidence of this phenomenon. The fluid intake in all these individuals was unrestricted; and the urine output in these cases with liver disease and fluid retention is seen to be significantly lower than in the other cases.

Adlersberg and Fox (47) recalling the axiom of Hanot that "Il y a un oedème hépatique comme il y a un oedème renal", investigated the rate of excretion of water given to normal individuals, to those with serious/

TABLE V.

From "Physiologic considerations in the Treatment of Portal Cirrhosis." by Carl H. Greene.

Date	Fluid Intake cc.	Urine Output cc.
9 May.	1000	600
10 "	880	500
11 "	1320	830
12 "	1520	750
13 "	1560	1340

Water balance in a patient with cirrhosis of the liver actively accumulating fluid.

serious parenchymatous liver damage and to those with obstructive jaundice.

In the group of normal individuals the total amount of water administered (1500 cc.) was eliminated in 5 hours, and a maximum rate of excretion was attained in 2-3 hours. On the other hand, in cases with severe liver damage, as evidenced by clinical signs, impaired galactose and sodium benzoate tolerance tests and abnormal cholesterol: cholesterol ester ratios, total excretion in 5 hours was considerably diminished and varied between 300 and 1000 cc. The maximum rate of excretion, though slower than in the previous group, was reached in 1-2 hours. They indicate that the retention of water roughly paralleled the severity of the disease. The results obtained in those cases with obstructive jaundice were within normal limits.

The relationship between water metabolism and liver has also been explored by Pick and his co-workers. (48). In a Harvey lecture delivered in 1929 he discussed this relationship under what is termed the hydrodynamic function of the liver. He quoted the work of Molitor and Pick by whom oedema was produced in cold-blooded animals when their livers were extirpated; and he noted also the diminished urinary excretion which ensues/

ensues when a reversed Eck fistula is constructed in animals - i.e. when blood from the Inferior Vena Cava is directed into the portal stream and produces liver damage. A similar diminution of urine flow is reported by Kunz and Molitor (49) by the use of synthalin which causes hepatic damage.

The diuretic effect of liver extract when given to cases of nephrosis has been reported by Pick (48). This has also been recorded by two Australian workers, Hicks and Mitchell (50). In addition they produced hepatic enlargement due to parenchymatous cell proliferation in animals in which ~~excessive~~ polyuria was produced by the ingestion of saliferous food.

The experiments and findings reported above, though few in number, are sufficient to confirm the old clinical impression that hepatic function is closely linked to water metabolism.

With the elucidation of the process of urine production in the kidney, it is now recognised that variations in urine flow are only brought about by alterations in tubular or glomerular activity (51). In view of the diminished urine flow and diminished water tolerance which is now established in conditions of parenchymatous damage of the liver, it would seem highly desirable that measurements of glomerular filtration/

filtration rate and tubular reabsorptive activity be made in patients with signs of liver dysfunction. Whether the methods available are sufficiently accurate to detect the slight changes in renal function which would be enough to cause inadequate excretion and create a positive water balance is open to question. In view of the large normal variation in the rate of glomerular filtration (51-52), the lack of a direct method of measuring tubular reabsorption, and the difficulties which would be encountered in obtaining a sufficiently high rate of urine production in patients with considerable fluid retention necessary for the clearance technique, the problem of obtaining accurate and significant results would be considerable.

A low urine output, or an inadequate one relative to fluid intake can only result from one of two causes (51.) }, namely a diminished glomerular filtration rate or an elevated rate of tubular reabsorption of water.

Although the evidence is not sufficient definitely to exclude the possibility (51. 53), it is generally accepted that the regulation of urine flow is controlled by variations in tubular reabsorption rather than by variations in the volume of glomerular filtrate. The latter is believed to be maintained at a ~~constant~~ level of between 90 and 130 cc./min. (51).

Although/

Although no measurements have been made, there is no reason to suppose that the rate of glomerular filtration in patients with hepatic cirrhosis is diminished. The glomerular filtration rate has been shown to be directly proportional to the blood pressure (54-59) and inversely to the plasma colloid osmotic pressure (57, 60, 61, 51). The blood pressure in cases with cirrhosis of the liver shows no deviation from the normal, other than that found in the occasional case associated with essential hypertension or with chronic nephritis, so that the presumptive evidence favours the supposition that the glomerular filtration rate in these patients is normal, or at least is not diminished. Indeed, if any deviation from the normal does obtain, it would be likely to be an increase in the filtration rate by virtue of the lowered colloid osmotic pressure of the plasma.

As has been suggested above, and recognised by workers in this field (51) the extent of the reabsorption of water in the tubules is most difficult to determine. And yet from the argument, it would seem that such an increased tubular reabsorption of fluid must occur if a diminished urine output and delayed diuresis to a given dose of water is to be explained. It is now recognised that the most important mechanism by which changes in the tubular reabsorption of water are/

are mediated is the action of the antidiuretic hormone of the posterior pituitary. This is believed to exert its effect directly upon the renal parenchyma stimulating the absorption of water and increasing the excretion of chlorides (62-71).

With these considerations, investigations were accordingly directed to discover whether in patients with cirrhosis of the liver there was any abnormality in the 24 hour urinary excretion of this hormone. Urine estimations were undertaken in preference to blood analyses because of the greater reliability of the method. It was hoped that by so doing more adequate information could be obtained in regard to the tubular activity in these patients, than by endeavouring to calculate the actual volume of water reabsorbed by the tubules from determinations of the inulin clearance (glomerular filtration rate) and the urinary concentration of glucose obtained with different plasma levels of that substance (72).

PROCEDURE.

Urine was collected from 4 normal individuals and from 8 of the patients with cirrhosis of the liver. All subjects received an adequate fluid intake during the period of urine assay.

Preparation/

Preparation of urine.

The urine was extracted, with slight modification, by the method reported by several workers (73, 74, 75, 76, 77). Twenty-four hour specimens were collected under toluene and acidified with 3 per cent acetic acid. Evaporation at room temperature to volumes of about 80 ml. was accomplished by suspension of the urine in cellophane sacks* ($1\frac{3}{4}$ inches x 2 to 3 feet) before electric fans. This reduction in volume was completed in from 36 to 48 hours. Electrolytes were removed by dialysis in a stream of running water, the process being facilitated by constant oscillation through an angle of 30°. The urine became chloride-free in 6 to 8 hours. The final volume was brought to 100 ml. One ml. of this solution was used for each assay, an amount which represented just under 15 minutes urine output.

Method of Assay.

Assay of the antidiuretic activity was performed by the method of Burn (78). The rats used were adult males from the laboratory stock weighing 120 to 240 grams. They had been maintained on a standard Nu Chow diet/

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* Visking Cellulose Sausage Casings - 36/32 "NoJax" Casing.

diet and water had been given ad lib. Food was removed 12 to 18 hours before the assay was performed. Water was allowed up to the time of assay. Series of 4 rats were placed in metabolism cages. Two series were used for each assay and the difference in total rat weight in each series was not greater than 10 per cent. The animals were hydrated to 5 per cent of their body weight with lukewarm tap water, given by means of a gastric tube. Immediately after hydration an intraperitoneal injection of 1 ml. of the solution to be assayed was given to each rat. The urine was collected and the volumes recorded, after the first 30 to 45 minutes, at 15-minute intervals. Fecal contamination was prevented by use of a fine wire mesh and a small amount of glass wool in the neck of the funnel. In a certain number of experiments, determinations of the total chloride excretion in each cage for a 2-hour period were made by the open Carius method of the Volhard titration as applied by Van Slyke (79). Pitressin (Parke Davis), as commercially prepared, was assayed for its antidiuretic activity and for its ability to stimulate the excretion of chlorides. The effect of pitressin added to the urine of normal individuals and dialyzed for 6 to 8 hours was studied in a similar manner.

A/

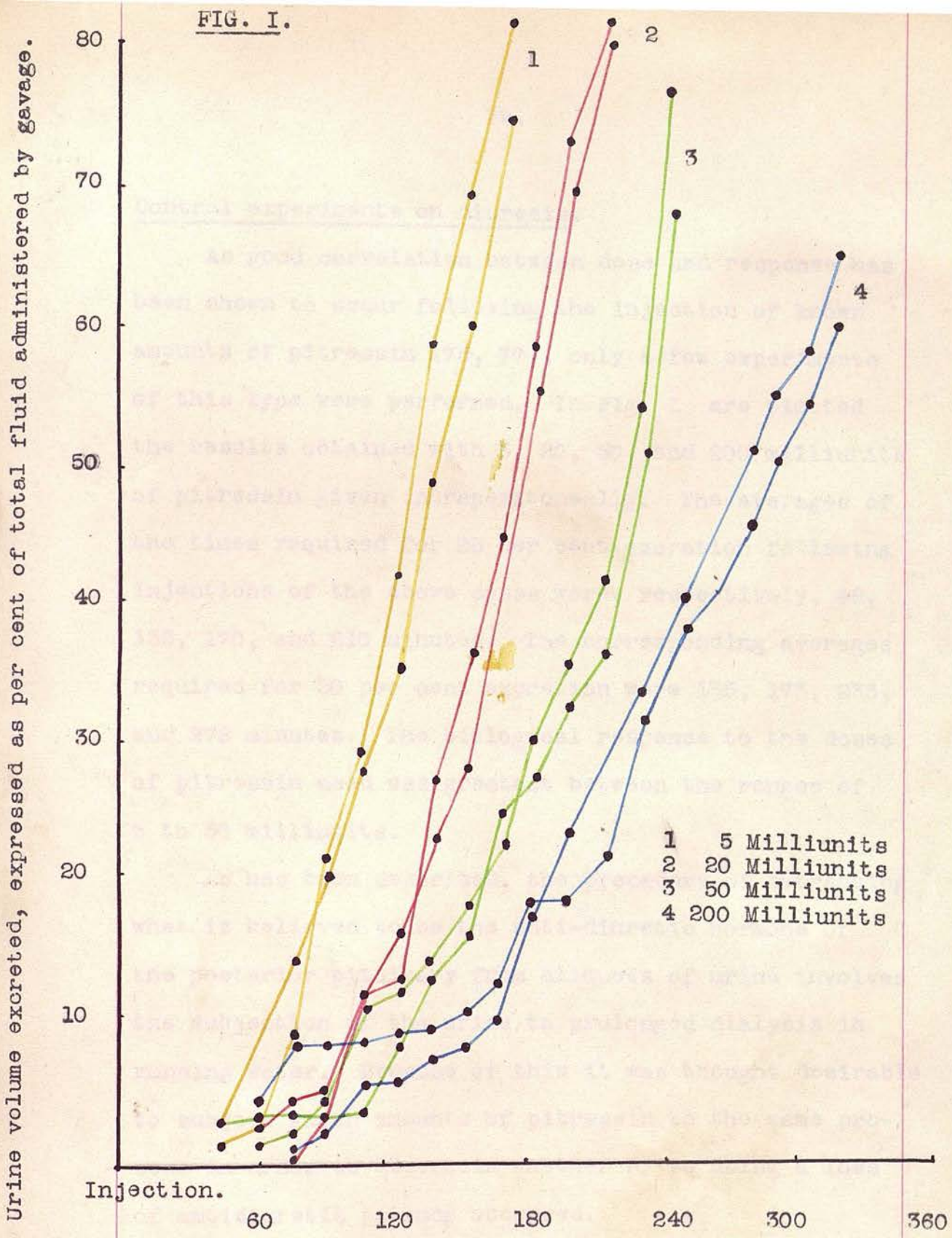
A total of 200 rats was used in the determinations. Six types of experiment were performed: (1) Assay of known amounts of pitressin (Parke Davis) for antidiuretic activity. (2) Assay of known amounts of pitressin subjected to dialysis for antidiuretic activity. (3) Assay of urine from normal individuals for antidiuretic activity. (4) Assay of urine from patients with cirrhosis of the liver for antidiuretic activity. (5) Establishment of diuretic curves for the rat group when hydrated and given 1 ml. of distilled water intraperitoneally. (6) The total chloride excretion was determined in the urines of the rats in experiments 1, 2, 4, and 5.

RESULTS.

In each assay, the diuretic response of 4 hydrated rats was recorded in the form of a graph, plotting the time taken for excretion against the volume of urine excreted. This latter was expressed as the percentage of the total amount of water administered. To facilitate comparison, results are expressed as the time in minutes for the excretion of 25 per cent and 50 per cent of the volume of water administered in the hydration. Each assay was done in duplicate.

Control/

FIG. I.



The antidiuretic effect in hydrated rats of graded doses of pitressin (Parke Davis) given intraperitoneally.

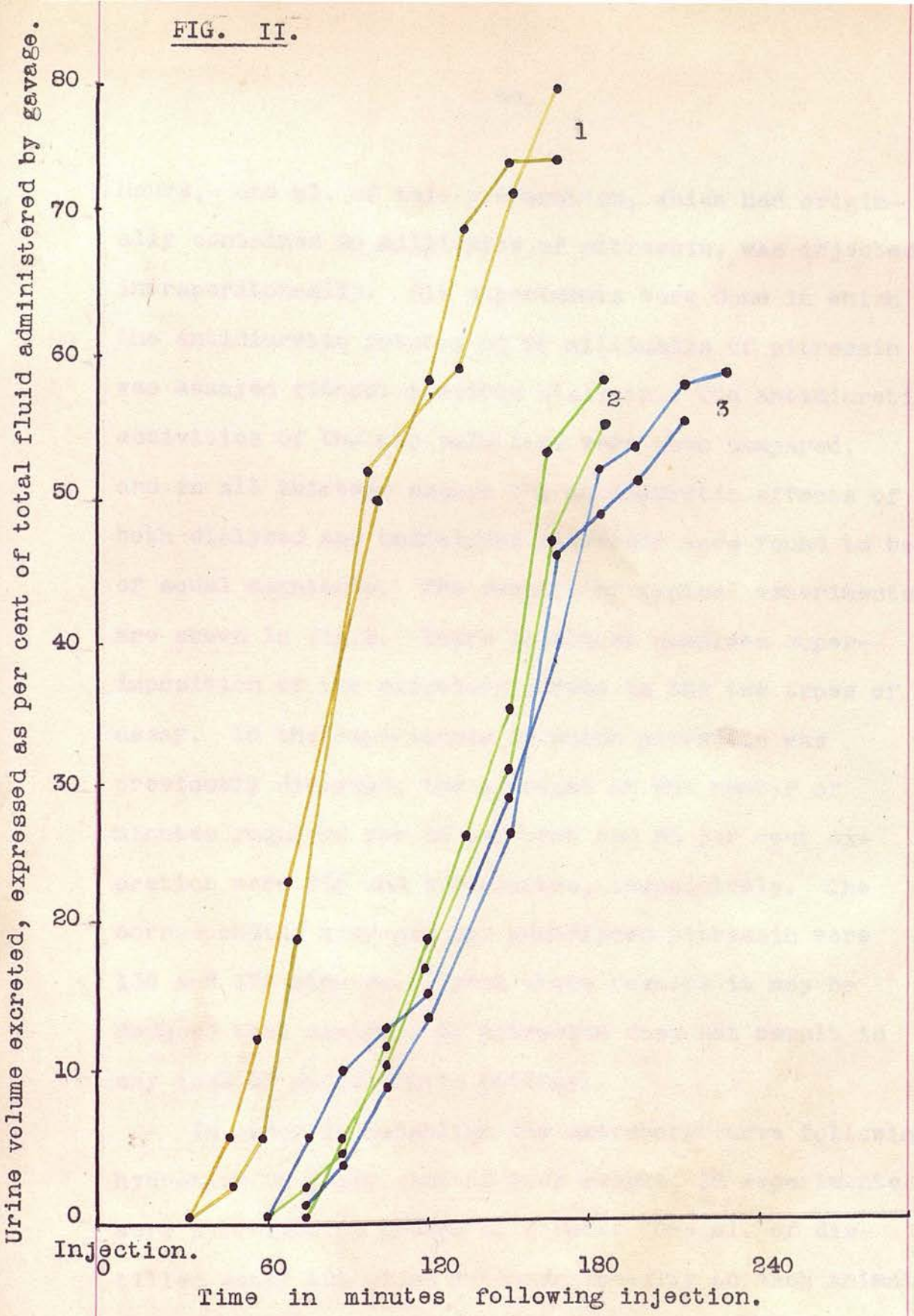
Control experiments on diuresis.

As good correlation between dose and response has been shown to occur following the injection of known amounts of pitressin (75, 77), only a few experiments of this type were performed. In Fig. I are plotted the results obtained with 5, 20, 50, and 200 milliunits of pitressin given intraperitoneally. The averages of the times required for 25 per cent excretion following injections of the above doses were, respectively, 98, 135, 170, and 210 minutes. The corresponding averages required for 50 per cent excretion were 135, 173, 233, and 278 minutes. The biological response to the doses of pitressin used was greatest between the ranges of 5 to 50 milliunits.

As has been described, the procedure of extracting what is believed to be the anti-diuretic hormone of the posterior pituitary from aliquots of urine involves the subjection of the urine to prolonged dialysis in running water. Because of this it was thought desirable to subject known amounts of pitressin to the same process in order to ascertain whether by so doing a loss of antidiuretic potency occurred.

Seven experiments of this type were performed. Known amounts of pitressin were added to about 100 ml. of normal urine and the mixture dialyzed from 7 to 8 hours/

FIG. II.



Effect of Dialysis upon antidiuretic activity of pitressin (Parke Davis).

1. Hydrated rats given 1 ml. distilled water intraperitoneally.
2. Hydrated rats given 20 milliunits pitressin intraperitoneally.
3. 20 milliunits pitressin, dialysed in urine for 7 hours given intraperitoneally to hydrated rats.

hours. One ml. of this preparation, which had originally contained 20 milliunits of pitressin, was injected intraperitoneally. Six experiments were done in which the antidiuretic potency of 20 milliunits of pitressin was assayed without previous dialysis. The antidiuretic activities of the two solutions were then compared, and in all thirteen assays the antidiuretic effects of both dialyzed and undialyzed pitressin were found to be of equal magnitude. The results of typical experiments are shown in Fig.2. There is almost complete superimposition of the excretory curves in the two types of assay. In the experiments in which pitressin was previously dialyzed, the averages of the number of minutes required for 25 per cent and 50 per cent excretion were 135 and 173 minutes, respectively. The corresponding averages for undialyzed pitressin were 135 and 172 minutes. From these results it may be deduced that dialysis of pitressin does not result in any loss of antidiuretic potency.

In order to establish the excretory curve following hydration to 5 per cent of body weight, 12 experiments were performed on groups of 4 rats. One ml. of distilled water was given intraperitoneally to each animal after hydration to simulate the conditions of the experiments with urine. The results are shown on

Table/

TABLE VI.

Effect of hydration (5 per cent of body weight) on urine output in rats.

Rat groups No.	Time in minutes for excretion of 25 per cent.	50 per cent.
1.	75	95
2.	75	95
3.	60	100
4.	60	95
5.	65	100
6.	75	110
7.	85	115
8.	85	110
9.	85	115
10.	85	110
11.	80	110
12.	75	110
Average.	75	106
Range.	60 to 85	95 to 115.

TABLE VII.

Effect on urine output in hydrated rats of the intraperitoneal injection of the extract of urine of normal subjects.

Normal subjects.					
Case No.	Sex.	Sp. gr.	24-hour urine volume	Time for excretion of 25 per cent.	50 per cent.
			ml.	Minutes.	
R.	M.	1.010	1350	95 110	130 135
D.	F.	1.010	1600	75	150
S.	M	1.025	1100	90 95	180 185
G.1	M	1.015	1080	85 105	120 135
G.2	M.	1.014	1200	100 115	125 145
		Average.		97	145
		Range.		75 to 115.	120 to 185.

Table VI. The time required for 25 per cent excretion ranges from 60 to 85 minutes, and for 50 per cent excretion from 95 to 115 minutes, with averages of 75 minutes and 106 minutes respectively.

Results of assay of human urines.

Twenty-four hour specimens of urine from 4 normal individuals were assayed for their antidiuretic activity. In one case (G1 and G2, Table VII), the assay was performed on two separate urine samples. The results of the experiments are shown in Table VII, and indicate that normal urine has a slight antidiuretic activity. The time taken for 25 per cent excretion averaged 97 minutes, and for 50 per cent excretion, the average was 145 minutes. The ranges of the two were 75 to 115 and 125 to 185 minutes, respectively. Assay of antidiuretic activity was performed on twenty-four hour urine samples from eight cases with cirrhosis of the liver. The results are shown in Table VIII. In one case (8A and 8B), two twenty-four hour samples were assayed. Cases 7, 8, and 9 were patients who had massive ascites and considerable peripheral oedema at the time the urine was assayed. Cases 1, 2, and 4 were cases in whom massive ascites and oedema had been present for a considerable time, but in whom there was no ascites demonstrable at the time of assay. In these cases,/

TABLE VIII.

Effect on urine output in hydrated rats of the intraperitoneal injection of the extract of urine of cirrhotic subjects.

	Case No.	Sex.	Sp.gr.	24 hour urine volume ml.	Degree of ascites	Pereph. oedema.	Time for excretion of 25 per cent.	Time for excretion of 50 per cent
							Minutes.	
Cases with cirrhosis of the liver.								
Patients with massive ascites.	8A	M	1.020	1000	4 +	2+	195 215	360 370
	8B	M	1.020	585	4 +	2+	190 175	320 305
	9.	M	1.020	800	3 +	2+	195 190	300 330
	7	M	1.030	410	3 +	2+	180 175	225 245
Patients with no clinical ascites at time of assay.	2	F	1.015	1600	0	0	150 145	200 205
	4	F	1.015	1420	0	0	150 155	215 205
	1	M	1.010	1650	0	0	130 150	160 190
Ascites never present.	14	M	1.014	1500	0	0	125 110	160 165
	10	M	1.014	1320	0	0	75 85	105 120

cases, ascitic fluid had not accumulated for periods of 3 to 16 months prior to the assay. Cases 10 and 14 were patients with severe hepatic cirrhosis in whom ascites and oedema had never been present to a demonstrable extent. In Cases 7, 8, and 9, the time required for 25 per cent excretion ranged from 175 to 215 minutes. In Cases 1, 2, and 4, excretion of 25 per cent of administered water occurred in from 130 to 155 minutes, while in Cases 10 and 14, the time required was 75 to 125 minutes. The corresponding values for 50 per cent excretion were 225 to 370 minutes, 160 to 215 minutes, and 105 to 165 minutes, respectively.

Discussion of Results of Assay of human urine.

It is clear from the above results that the urine of patients with cirrhosis of the liver and ascites contains a substance which produces a delay in the diuresis of rats hydrated to 5 per cent of their body weight. Furthermore this substance is present in the urine of patients with cirrhosis of the liver without ascites in amounts considerably less than in those in whom fluid retention is marked. In these cases of cirrhosis of the liver in whom fluid retention had never occurred, the antidiuretic effect of the urine approached that produced by the urine from normal individuals./

individuals. The antidiuretic activity of the urine of patients 10 and 14 (Table VIII) can be seen to fall within the range of antidiuretic activity established for the urine of normal persons. Expressed in units of pitressin, the average twenty-four hour excretion of antidiuretic substance by the normal person is in the region of 5 milliunits. The twenty-four hour excretion of antidiuretic substance of the cases with cirrhosis of the liver and ascites ranges between 50 and 200 milliunits; and the twenty-four hour excretion of cases of cirrhosis of the liver without ascites but with a previous history of fluid retention lies between 20 and 50 milliunits. The corresponding figure for those two cases with cirrhosis in whom ascites had never been present is around 5 milliunits.

It is unnecessary, however, to transcribe the results obtained and expressed in minutes necessary for 25 and 50 per cent excretion (Tables VII-VIII) into milliunits of pitressin to see that the magnitude of the antidiuretic effect of the various aliquots of urine parallels the degree of fluid retention in these patients.

THE NATURE AND MODE OF ACTION OF THE ANTIDIURETIC SUBSTANCE IN THE URINE OF PATIENTS WITH CIRRHOSIS OF THE LIVER.

The nature of the antidiuretic effect which has been observed by injecting preparations of the urine from patients with cirrhosis of the liver cannot be stated at the present time with absolute certainty. Gillman and Goodman have confirmed that an antidiuretic hormone is secreted by the posterior pituitary, that this is a true hormone, that it passes into the circulation, and that it acts directly on the kidney through which it filters into the urine where it is stable and easily detectable (74). The method used by them for the detection of this hormone was essentially similar to the one described in this thesis and used for the extraction of human urine. It has also been applied to urine by Ingram et al. (76) and Boylston et al. (77) who investigated the concentration of an antidiuretic substance in the urine of animals in which dehydration had been induced. They found a good correspondence between the severity of the dehydration and the concentration of an antidiuretic substance in the urine; and they were of the opinion that this substance was the antidiuretic hormone of the posterior pituitary. All these investigators found that the substance could not be detected in the urine of animals unless/

unless the posterior pituitary or hypothalamic-hypophyseal system was intact.

In 1939 Robinson and Farr, using this method in the Rockefeller Institute for Medical Research, reported that an antidiuretic substance could be recovered from the urine of normal individuals following injection of pitressin. The degree of antidiuretic effect which resulted when preparations of the urine were injected into hydrated rats was comparable to the amount of pitressin administered. (73).

From the standpoint of methodology, therefore, it would appear certain that if there was an excessive amount of pituitary antidiuretic hormone in the urine of patients with hepatic cirrhosis and fluid retention, this would be detected by the use of the method described. It is permissible to accept the fact of antidiuretic activity having been demonstrated by its use, as evidence that the activity is a manifestation of the presence of the antidiuretic hormone.

Teel and Reid (75) reported the presence of an antidiuretic substance in the urine of patients suffering from pre-eclampsia and eclampsia. They suggested that this substance might be posterior pituitary in origin; and a comparison between its pharmacological properties and those of pituitrin (pitressin) was made by Ham in 1942 (80). Pituitrin or pitressin has been shown/

shown by numerous workers to possess the ability of considerably increasing the urinary excretion of chlorides. (68-71). This was confirmed by Ham who pointed out that the antidiuretic substance present in the urine of patients with preeclampsia and eclampsia did not possess this chloruretic property, and when injected into hydrated rats produced no specific effect upon chloride excretion. Because of this difference in pharmacological action he argued that the antidiuretic substance was not the antidiuretic hormone of the posterior pituitary.

Observations on chloride excretion.

In view of the evidence presented by Ham, the effects upon chloride excretion of the extract of urine from patients with cirrhosis of the liver was examined. The total chloride excretion of groups of four rats hydrated to 5 per cent of their body weight was determined. A total of twenty-four such estimations were made, each of which was performed in duplicate. The results of the experiments are shown in Table IX and Fig.3. The total chloride excretion is expressed in micro-equivalents per 100 grams of rat weight excreted in the 2-hour period following the intraperitoneal injection. In Fig.3 the total chloride excretion is expressed in micro-equivalents per 100 grams of rat weight/

TABLE IX.

Observations on chloride excretion.
Total chloride excreted per 100 grams rat weight in 2-hour period, after hydration to 5 per cent of body weight, and injected as indicated.

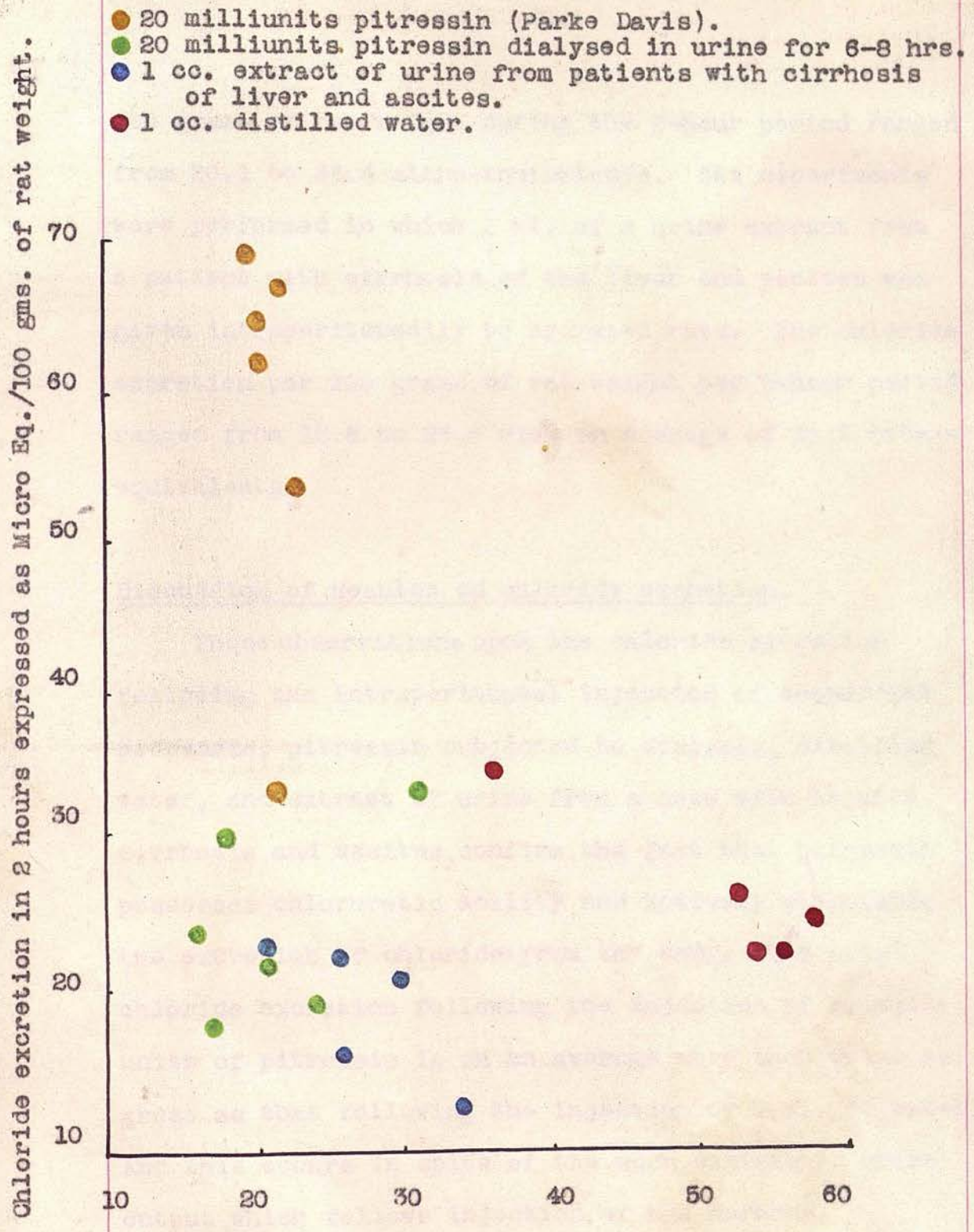
Chlorides are expressed in micro-equivalents.

Exp. No.	1 ml. distilled water injected intraperitoneally	20 milliunits of Pitressin(Parke Davis) injected intraperitoneally	20 milliunits of pitressin dialysed and injected intra-peritoneally.	1 ml. urine extracted from patients with cirrhosis of liver and ascites injected intra-peritoneally.
1.	27.0 27.4	67.6 67.2	34.1 34.4	23.5 23.1
2.	25.6 26.7	51.8 51.3	29.6 30.1	21.9 21.2
3.	23.8 24.3	63.1 63.5	22.5 22.0	19.0 19.0
4.	34.5 35.0	65.7 65.1	20.1 20.6	21.9 21.2
5.	23.9 25.1	54.5 53.5	24.4 24.7	12.6 13.0
6.		60.3 59.1	34.2 34.3	15.4 15.8
Range	23.8 to 35.0	51.3 to 67.6	20.1 to 34.4	12.6 to 23.5
Average	27.3	60.2	27.6	19.2

weight for the 2-hour period and is plotted against the antidiuretic effect obtained with the substance injected. The urine output in the same two hours was measured, and the volume, expressed as a percentage of the total amount of water administered by gavage, is used to indicate the antidiuretic response.

Five experiments were performed in which 1 ml. of distilled water was injected intraperitoneally into hydrated rats. The total chloride excretion per 100 grams of rat weight in the ensuing 2 hour period ranged from 23.8 to 35.0 micro-equivalents, with an average of 27.3 micro-equivalents. In six experiments in which hydrated rats were given 20 milliunits of Pitressin (Parke Davis) in a volume of 1 ml. of water, the chloride excretion per 100 grams of rat weight in that period ranged from 51.3 to 67.6 micro-equivalents, the average being 60.2 micro-equivalents. Commercial pitressin was added to the urine of a normal individual and dialyzed for a period of seven hours. Six experiments were performed in which 1 ml. of this solution which originally contained 20 milliunits of pitressin, was assayed for its antidiuretic activity and its ability to stimulate the excretion of chlorides. As previously mentioned, there is no loss of antidiuretic activity, when pitressin is treated in this way. When injected intraperitoneally into hydrated rats, the total chloride excretion per

FIG. III.



Water and chloride excretion in hydrated rats following the intraperitoneal injection of commercial pitressin, dialysed pitressin, extract of urine from patients with hepatic cirrhosis, and distilled water.

100 grams of rat weight during the 2-hour period ranged from 20.1 to 34.4 micro-equivalents. Six experiments were performed in which 1 ml. of a urine extract from a patient with cirrhosis of the liver and ascites was given intraperitoneally to hydrated rats. The chloride excretion per 100 grams of rat weight per 2-hour period ranged from 12.6 to 23.5 with an average of 19.2 micro-equivalents.

Discussion of Results on chloride excretion.

These observations upon the chloride excretion following the intraperitoneal injection of commercial pitressin, pitressin subjected to dialysis, distilled water, and extract of urine from a case with hepatic cirrhosis and ascites, confirm the fact that pitressin possesses chloruretic ability and actively stimulates the excretion of chlorides from the body. The total chloride excretion following the injection of 20 milli-units of pitressin is on an average more than twice as great as that following the injection of 1 ml. of water. And this occurs in spite of the much diminished urine output which follows injection of the hormone.

In addition it is seen that a similar amount of pitressin when subjected to dialysis loses this ability to increase chloride excretion while it retains its antidiuretic/

antidiuretic powers. This is well illustrated in Fig. 3. The total chloride excreted in the 2-hour period following injection by 20 milliunits of pitressin dialyzed for seven hours is practically the same as that excreted following injection of 1 ml. of water and less than half the amount excreted by rats given the same amount of pitressin which has not been previously dialyzed.

The quantity of total chloride excreted by rats given injections of extract of urine from a patient with cirrhosis of the liver reveal that this substance does not possess chloruretic ability. The total chloride excretion following injection of this preparation approximates to the amount excreted when distilled water is injected intraperitoneally.

In this respect these findings are in agreement with those of Ham. His argument, however, is invalidated. The fact that pitressin possesses chloruretic properties, which are not possessed by the antidiuretic substance present in the urine of patients with cirrhosis of the liver cannot be accepted as evidence to support the thesis that the latter is not pitressin nor pituitary in origin. If, indeed, this antidiuretic substance were the pituitary hormone, its chloruretic property would be lost by the method used for its extraction from the urine, as it is when pitressin is dialyzed/

dialyzed. Dialysis was used by Ham in his investigations on the antidiuretic substance reported by Teel and Reid. The separation by dialysis of the antidiuretic and chloruretic properties of pitressin is an interesting, if incidental, finding. It suggests that pitressin is in reality two separate hormones, or at least that the chloruretic fraction may be destroyed without interference with the properties of the antidiuretic.*

Information regarding the mode of action of the antidiuretic substance present in the urine of patients with cirrhosis of the liver may be obtained from a further consideration of the findings upon chloride excretion. In the first column of Table IX the results of the estimation of total chloride (each in duplicate) excreted in the urine of five groups of four rats in the/

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* I was unable myself to pursue further this interesting finding. On the termination of the Fellowship, however, Dr Ralli was informed of the fact and she kindly agreed to endeavour to recover the chloruretic fraction from the water used in the dialysis of known amounts of pitressin. Personal communications from her indicate that this has been done, and that the chloruretic fraction "lost" by dialysis is to be found outside the cellulose sac containing the pitressin.

the two hour period following injection with 1 ml. of distilled water and hydration to five per cent body weight is expressed in micro-equivalents per 100 grams of rat weight. The sum total of chlorides excreted by these rats (five groups of four animals) was measured and was found to be 1,092.0 micro-equivalents. This was contained in the total urine output by the twenty rats excreted during the two hour period following injection, and reached a total of 109.6 cc. These figures represents an overall concentration of chlorides of 996.3 micro-equivalents per 100 cc. of urine.
A similar calculation was made from data obtained in the experiments with six groups of four rats which were hydrated and injected with 1 cc. of a preparation of urine from a patient with cirrhosis of the liver and ascites. The total chloride excreted during the two hour period following injection was 825.6 micro-equivalents. This was contained in 58.4 cc. of urine and represents a concentration of 1413.7 micro-equivalents per 100 cc. of urine.

As has already been stated the injection of urine from patients with cirrhosis and ascites, prepared as described above, results in a delayed diuresis in rats hydrated to five per cent of their body weight. Furthermore from a perusal of the results given in Table IX it may be seen that there is no absolute increase in the/

the excretion of chlorides when this preparation is injected. But it is clear from the figures just given that the urine excreted by rats under these conditions contains a higher concentration of chlorides when the animals are injected with the electrolyte free urine preparation as compared with distilled water.

Chlorides, with other electrolytes, are treated as a threshold substance by the kidney. Their clearance is less than that of inulin, which may be taken as a measure of glomerular filtration; so they are presumed to be reabsorbed by the tubules, when the plasma level is low, and excreted in the urine when this is high. Given a constant plasma level, and a constant glomerular filtration rate, variations in urine concentration can only be brought about by variations in tubular reabsorption of water. (51, 52, 81). It may be fairly assumed that the average plasma levels of chlorides of two groups of rats, twenty and twenty-four strong, kept under uniform conditions, of similar age and weight and from the same stock, are of the same or very nearly the same amount. If that is conceded the marked difference in the urine concentrations of chlorides which have been described may only be due to differences in the extent of tubular reabsorption of water in the kidneys of these animals. The possibility that this could/

could be due to a diminished rate of glomerular filtration is precluded by virtue of the fact that the total chloride output in each group was approximately equal.

It may be concluded from these experiments that the antidiuretic substance present in the urine of patients with cirrhosis of the liver and fluid retention produces its effect by a direct action on the kidney. The fact that its injection is followed by a diminished output of concentrated urine is evidence of this; and the available data suggest that this is attained by an increase in tubular reabsorption of water.

RECAPITULATION AND SPECULATION.

The observations which are included in this thesis on the plasma levels of albumin and globulin in patients with cirrhosis of the liver with and without ascites suggest that ascites is not determined by the level of plasma proteins or by the oncotic pressure thereby excreted. Moreover it would seem that too much emphasis has been attached to the critical level of albumin in the plasma as a determining factor in the production of ascites. The evidence presented by Post and Patek (2, 29) does not withstand critical analysis. Not only does the data presented here fail to support the theory, but there are reports in the literature which are detrimental to it. Although not specifically discussed by them, an analysis of the work by Butt, Snell and Keys (83) reveals a lack of correlation between plasma oncotic pressure and the degree of fluid retention. They report the plasma oncotic pressures of patients with cirrhosis of the liver and obstructive jaundice. Ascites was present in all of the cases of cirrhosis of the liver and in only two of the cases with obstructive jaundice. The osmotic pressures of the plasma, however, were as low in patients with obstructive jaundice as with cirrhosis.

In the patients reported here in whom ascites was controlled/

controlled the plasma levels of albumin remained below the normal value for at least six months. Until more cases are studied one cannot know whether the plasma levels of albumin will return to or remain above the lower level of normal, 3.8 grams per cent (84). It is equally significant that the plasma levels of albumin were below normal value in the patients in whom ascites was never present.

It has been the custom of clinical workers to explain the low urine output in patients with hepatic cirrhosis on the basis that the fluid which is retained in the abdominal cavity results in a reduced amount of body fluid being available for renal excretion. The ascites is considered to be the cause of the diminished urinary flow. This theory has been somewhat uncritically accepted. From the present day conception of renal physiology a diminished urine flow can result only from a reduction in the rate of glomerular filtration or from an increase in the rate of tubular reabsorption of water. The mechanism by which ascites could accomplish these alterations in renal function is difficult to imagine. It has indeed never been described; and I feel it is not likely to be. Arguing from first principles, it is clear that if there is such a relationship between ascites and oedema and diminished urine output, the latter must be responsible for the former rather than vice versa. A low urine output

is conceivably a cause, but inconceivably the result of ascitic accumulation.

The investigations which were undertaken in an endeavour to substantiate these theoretical considerations have been described. It is admitted that much work, involving accurate measurements of renal functions, and more detailed investigations of the chemical nature and physiological action of the substance present in the urine of patients with fluid retention, should be performed. The presence of a substance in the urine of these patients which when injected into hydrated rats results in a diminished excretion of water containing a normal amount of chloride for the conditions of the experiment is suggestive that tubular reabsorption in the patients is in excess of normal values. And the explanation proposed that this is the antidiuretic hormone of the posterior pituitary gland is more intelligible than the appeal of the clinician to what may be superficially plausible but what is fundamentally unsound.

It is not possible from the experiments undertaken to estimate to what extent the presence of an antidiuretic substance in the urine of patients with cirrhosis of the liver is responsible for fluid retention. It is not the purpose of this thesis to claim that the colloid osmotic pressure of the plasma plays no part in the control of the distribution of body water in these patients. The method of physiological/

physiological thought is based upon the philosophy of mechanism and materialism and occurrences are interpreted in terms of preceding events which we are pleased to designate as causes. Under these circumstances it is all but impossible to evaluate the relative importance of one event in the activation of a system which is motivated by two or more forces. It is only permissible therefore for me to indicate yet another force in the system under consideration and to leave the final determination of the extent of the part played by it to future investigations which are profusely suggested by its discovery and by the findings here reported.

There is little danger, and perhaps some gain, in speculation if it is realised when the demarkation line between the realm of proved fact and untested hypothesis has been crossed. But the not inconsiderable evidence obtained from the methodology employed, and the experiments which suggest a specific renal action, make it more than likely that the antidiuretic substance present in the urine of patients with hepatic cirrhosis and fluid retention is the antidiuretic hormone of the posterior pituitary.

Under the circumstances, it becomes pertinent to inquire how it is that an excessive amount of this hormone is found in the over-hydrated patient with oedema and ascites and hydrothorax when it has been repeatedly and conclusively shown in experiments on animals/

animals that this hormone is secreted in response to dehydration and that its production is diminished when this is alleviated. (74, 76, 77).

Whether the resolution of the paradox is to be found in the theory that a cirrhotic liver has lost the capacity to inactivate the hormone is unknown. It is, however, a likely proposal. There is evidence that the liver does inactivate various hormones; and Heller and Urban have shown that a suspension of liver tissue inactivates the antidiuretic hormone of the posterior pituitary gland (85).

The parallelism between the severity of fluid retention and the amount of antidiuretic substance present in the urine of these patients has already been indicated. In the cases in which no fluid retention occurred, and in whose urine no antidiuretic substance was found, the severity of the liver dysfunction, as measured by laboratory tests (i.e. Bromsulphalein retention test, and Albumin: Globulin ratios Cholesterol:Cholesterol ratios) was no less than that in patients with ascites. If it is the case that the hormone is inactivated by the liver, it would appear that all hepatic functions are not equally impaired in every patient. The recent review of liver physiology given by Mann favours this conception.(82).

SUMMARY.

1. The orthodox theories of the causes of fluid retention in patients with cirrhosis of the liver are discussed.

2. Observations on the plasma level of albumin and globulin, and the colloid osmotic pressure exerted by them, in patients with cirrhosis of the liver with and without ascites are reported.

3. Following treatment reaccumulation of ascitic fluid ceased in six patients with cirrhosis of the liver. Determinations of the level of plasma proteins and the colloid osmotic pressure thereby exerted performed at monthly intervals prior to the cessation of accumulation and for a period up to six months after it had occurred, do not support the contention that the level of plasma proteins is an important determinant in the development of ascites.

4. The plasma colloid osmotic pressure is shown to be as low in patients without ascites as in those with it.

5. The importance of considering the factors which modify renal excretion in a discussion of fluid retention is stressed.

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6. The presence of an antidiuretic substance in the urine of patients with liver disease and ascites is reported. The fact that its concentration varies with the extent of fluid retention is noted.

7. The nature of the antidiuretic substance in the urine of patients with cirrhosis of the liver is discussed. Evidence is put forward to support the suggestion that it is the antidiuretic hormone of the posterior pituitary gland and criticisms that a similar substance reported in the urine of patients with eclampsia is not this hormone, are invalidated.

8. Evidence is put forward to support the hypothesis that the antidiuretic substance present in the urine of patients with cirrhosis of the liver and water retention causes the delay in diuresis in hydrated rats by a specific kidney action.

9. The chloruretic and antidiuretic properties of commercial pitressin are confirmed. It is shown that the latter is not affected when the pitressin is dialyzed. There is a considerable loss in chloruretic power with this procedure.

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