THE ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN THE NEWBORN.

A THESIS PRESENTED BY

HYLTON SMITH, B.Sc.,

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PREFACE

In infants suffering from haemolytic disease of the newborn death from cardiac failure consequent upon prolonged anoxia can occur within the first twenty-four hours of life. If the infants survive this period the anoxia is relieved as blood oxygenation mechanisms become more efficient. Unfortunately, improvement is often temporary and after about forty-eight hours rapid destruction of blood cells, which commences "in utero", can result in deep jaundice. This is followed by damage to the central nervous system (known as kernicterus) with medullary failure and death in the more severe cases. Thus even though some infants are in imminent danger of cardiac failure in the immediate post-natal period, it is considered best to attempt exchange transfusion in order to reduce the risk of kernicterus.

The development of exchange transfusion has greatly altered the prognosis of haemolytic disease in the newborn and the procedure is now accepted as being essential in cases of any severity. The difficulties associated with blood transfusion have received increasing attention in recent years and its employment is beset with problems

not only of cytology and serology, but of micro-biology, biochemistry, allergy, dynamics, religion and race. Some of these problems are significantly affected by the volume of blood used in exchange transfusion. Considering that the infant may be perfused with stored blood of electrolyte content greatly different from that of the recipient's blood and in quantities up to three times his own blood volume, it is surprising that so little clinical change is observed. Hazards such as sepsis, air embolism, portal vein thrombosis and perforation of the umbilical vein have been described but are fortunately uncommon. Circulatory overloading is an ever present danger in the profoundly anaemic infant and it may be precipitated by conducting the exchange transfusion too rapidly. Deterioration and even death however, may occur in infants whose pre-operative condition gives rise to no concern. An example of such deterioration prompted this investigation. During the exchange transfusion of a male infant, the infant suddenly collapsed and appeared pale and unresponsive. Calcium gluconate did not affect an improvement and administration of glucose only caused a temporary improve-

-ment. The transfusion was carried to a satisfactory conclusion in negative balance of blood. Previous work on citrate intoxication was recalled and it was decided to undertake a combined clinical and biochemical study of infants during exchange transfusion.

C O N T E N T S

INTRODUCTION

THE IMPORTANCE OF CITRATE IN TRANSFUSION OF CITRATED BLOOD

One of the factors suggested as a cause of electrolyte disturbance during exchange transfusion is the toxicity of citrate. The dangers in the use of citrated blood were first emphasized by Lewisohn (1915) but so few transfusion reactions unequivocably attributable to citrate intoxication have been observed that citrated blood is regarded by many as being non-toxic. The rapid transfusion of large volumes of blood would certainly be necessary in the adult before the adverse effects of citrate became apparent, and Mollison (1952) maintained that it was safe to administer blood as long as the rate of infusion of citrate was below two hundred and sixty milligrams per kilogram of body weight per hour. Recent studies (Wexler, Pincus, Natelson, Lugovoy 1949, Ames, Sylles, Rapoport 1950) in the technique of exchange transfusion in newborn infants have indicated that the recommended safe rate of administration is usually exceeded. In fact during an exchange transfusion it is routine procedure to administer up to a litre of blood in a period of ninety minutes, which in a normal infant is equivalent

to a citrate infusion rate of five hundred and eighty milligrams per kilogram of body weight per hour (Wexler etal, 1949).

The Influence of Citrate Ion upon Calcium Ion.

Laboratory experiments have indicated that the citrate ion combines with a calcium ion forming a soluble, dialysable weakly ionised complex and it is believed that the convulsions which are produced upon the intravenous administration of sodium citrate solution to animals are the result of the reduction of actively ionised calcium in serum (Shelling & Mallow, 1928). During an exchange transfusion up to four grams of sodium citrate are introduced into the circulation and it is theoretically possible that such an amount would remove all the free calcium ions from plasma, thus profoundly altering muscle and nerve irritibility.

THE PARTITION OF CALCIUM IN SERUM.

The distribution of calcium in serum has been speculated upon for many years and it is commonly believed that two forms of calcium exist, termed diffusible and non-diffusible, according to whether or not they can pass through

a dialysing membrane, and presumably through living cell membranes. The non-diffusible fraction is thought to be in combination with protein, particularly albumin, while the diffusible fraction consists of ionised calcium, whether dissociated (i.e. active) or not, plus the small amount of calcium which is bound to citrate (McLean & Hastings, 1935).

Mc Lean and Hastings (1935) suggested that the concentration of actively ionised calcium in plasma depends largely upon the degree of dissociation of the non-diffusible calcium protein complex, which ionises as a weak electrolyte. They calculated the dissociation constant for this proteinate complex. Hopkins et al (1952) also observed a constant relationship between calcium proteinate and calcium ion upon adding varying amounts of calcium salts to plasma, and calculated a dissociation constant which was significantly lower than the value obtained by McLean and Hastings (1935). Evidence in favour of the presence of a weakly dissociated, soluble ultrafilterable complex of calcium and citrate in plasma was reviewed by Greenberg (1939) and there is little doubt that such a complex exists. The discovery by Dickens (1940) that bone is relatively rich in citrate is very interesting. The

citrate is thought to be present as a superficial deposit on the crystal lattice of apatite by virtue of its property of complexing with calcium, and since the enzymes citrogenase and aconitase have been demonstrated in bone tissue, it is suggested by Dixon and Perkins (1952) that deposition of citrate in the skeleton is the result of metabolic activity in bone cells. Because of the two phase equilibrium between bone and tissue fluid it is possible that citrate, which is normally present in plasma to the extent of 1 - 6 mg. per 100 ml., could arise from bone.

Calcium citrate dissociates in two stages. The primary dissociation is considered to be virtually complete while the secondary dissociation is feeble. In the secondary dissociation the equilibrium is almost entirely in favour of the CaCit ion.

A. $Ca_3Cit_2 \rightleftharpoons Ca(Ca\ Cit)_2 \rightleftharpoons Ca^{++} + 2\ Ca\ Cit'$

B. $Ca\ Cit' \rightleftharpoons Ca^{++} + Cit'''$

It is believed that calcium citrate exists in serum largely in the form of the negatively charged complex anion. Within the hydrogen ion concentration range of blood, McLean and Hastings (1935) calculated the secondary dissociation of the complex to be 3.22 at 22°C. The amount of citrate ion which is normally present in serum would

be sufficient to combine with the whole of the calcium which exists in the un-ionised, diffusible fraction.

The state of calcium in the serum of newborn infants is not completely known. Total serum calcium concentrations range from 7.3 to 16.9 milligrams per hundred millilitres (Todd, 1939), while the actively ionised calcium concentration, measured by the frog heart method, is 5.7 to 6.1 milligrams per hundred millilitres and the ultrafilterable fraction 5.3 to 5.9 milligrams per hundred millilitres (Smith, 1951). The discrepancy between ionised and ultrafilterable calcium was believed by Smith (1951) to be due to an unusual ratio between serum albumin and globulin in neonatal blood, but it is the concentration of albumin and not the albumin to globulin ratio which influences calcium ion concentration. In fact the albumin concentration in neonatal blood is similar to that observed in normal adults. I suggest that the true explanation of the discrepancy probably lies in the fact that the normal range of ultrafilterable calcium quoted by Smith (1951) is not valid since control of hydrogen ion concentration during ultrafiltration was not attempted, and this factor has been shown to be very

important by Hopkins et al (1952).

Changes in serum calcium during the infusion of citrate have been observed. Bruneau & Graham (1943), Krautwald & Dorow (1940), Wexler et al (1949) reported a decrease, no change and an increase respectively in total serum calcium concentration.

Evidence that calcium is mobilised during the infusion of citrate was presented by Scott & Lensing (1942). They demonstrated by a micro-incineration technique that intracellular calcium migrated into the extracellular fluid as citrate entered the extracellular fluid.

The intravenous injection of citrate into bi-laterally nephrectomised dogs or the injection of parathyroid hormone resulted in a marked hypercalcaemia, but injection of citrate into nephrectomised, thyro-parathyroidectomised dogs did not. The role of parathyroid hormone is obscure in this respect but Atwell (1945) thought that it stimulated the release of calcium citrate from the skeleton. Whatever the mechanism, profound changes occur during citrate infusion and this is reflected in an increase in ultrafilterable calcium, and a decrease in ionised

(i.e. active) calcium (Shelling & Maslow (1928), Ames et al (1952)).

It is apparent from the foregoing evidence that there is normally present in serum a citrate complex which is in combination with calcium ion. The infusion of citrate solution causes an increase in the concentration of this complex with a consequent lowering of the actively ionised calcium concentration although the diffusible fraction of calcium remains normal or even elevated. The lowering of the actively ionised calcium concentration will alter the equilibrium between the diffusible and non-diffusible fractions and by some mechanism possibly involving parathyroid hormone, calcium ions are mobilised from skeletal and intracellular spaces. It may be that lowering of calcium ion concentration in serum is the direct stimulus for the liberation of parathyroid hormone.

The physiological effect of the infusion of citrate solution, which is clinically of great importance, is essentially due to reduction in calcium ion concentration which causes increased neuro-muscular irritibility with a resulting tetany and cardiac failure. Wexler, Pincus, Natelson & Lugovoy (1949) studied the changes in serum citrate

concentration during exchange transfusion in newborn infants and observed marked increases. Ames, Symmes and Reppoport (1951) reported that electro-cardiograph tracings consistent with hypocalcaemia were recorded during exchange transfusion. Gustafson (1951) also observed similar changes including a persistent tremor along the iso-electric line which disappeared after the intravenous injection of calcium gluconate solution. Nakasone et al (1954) attributed the phenomenon to a citrate-induced hypocalcaemia because transfusion of blood containing no citrate did not cause any cardiac changes.

Theoretically the infusion of citrate in the amounts used in exchange transfusion should lower actively ionised calcium concentration to levels associated with tetany although this is rarely observed in practice. It is possible that the metabolism of infused citrate is so rapid that the calcium ion concentration in serum is maintained within the normal range, although in the present work it will be shown that lowering of actively ionised calcium concentration does occur.

THE METABOLISM OF CITRATE

Citrate is oxidised via the tri-carboxylic acid cycle which is also the common pathway for the final oxidation of glucose (Canterow & Trumper 1955). Administration of glucose causes a decrease in the fasting serum citrate concentration coinciding with the most active phase of glucose metabolism (Natelson et al 1948). Since citric acid lies directly in the tri-carboxylic acid cycle, the injection of glucose or citrate should theoretically catalyse the oxidative process. Carbohydrate undergoes metabolism to pyruvic acid which then yields acetic acid by means of oxidative decarboxylation. The acetic acid fragment combines with co-enzyme A forming active acetyl-coenzyme A which condenses with oxaloacetate yielding citric acid (Thunberg 1953). Since oxaloacetate is regenerated with each complete cycle, any substance lying in the cycle will act as a catalyst in the oxidation of pyruvate.

Langeck (quoted by Thunberg,1953) measured the breakdown of citrate in various minced tissues and concluded that liver contained the highest concentration of citric acid dehydrogenase. Kidney was also a rich source of enzyme,

but muscle tissues contained much less.

Wexler et al (1949) studied the rate of citrate metabolism in infants during exchange transfusion and formed the opinion that citrate was rapidly removed from the blood, presumably by metabolic routes. It appeared that infants suffering from erythroblastosis foetalis were less able than normal infants to oxidise citrate, but except in those cases where severe liver damage was present, the serum citrate concentration after reaching a certain level tended to fall spontaneously, even though citrated blood was still being injected.

The Role of the Liver.

Evidence confirming the oxidation of citrate by liver tissue was presented by Sjostrom (1937) who perfused citrate solution into a cat liver which had been functionally isolated by means of section of the blood vessels. The perfusion fluid contained over one hundred milligrams of citrate per hundred millilitres of solution but a single passage through the liver was sufficient to reduce the citrate concentration to below two milligrams per hundred millilitres. Up to twenty grams of citrate could be removed by this method but when the liver was poisoned with allyl formate solution, much less citrate

was oxidised. Martensson (1940) criticised this experiment on the grounds that Sjostrom did not oxygenate the liver tissue adequately, thereby causing cell damage with release of citric acid dehydrogenase, but there is no doubt that liver can oxidise citrate. It is postulated that the presence of an efficient liver appears to be important during the exchange transfusion of citrated blood since infants with obvious hepatic cell impairment are unable to remove citrate from their blood at a rate sufficient to prevent a toxic hypercitraemia.

Mollison & Cutbush (1949), Billing, Cole & Lathe (1954) suggested that hepatic function is deficient in the neonatal period when compared to the normal adult but Billing et al (1954) believe the low excretary capacity is concerned specifically with the metabolism of bilirubin. It may be of significance that elevated serum citrate concentrations are characterestic of liver disease and that in the present series newborn infants present a high serum citrate level compared to normal adults.

However the liver in infants suffering from erythroblastosis foetalis shows only minor histological

changes and rarely a mild to moderate degree of fatty change is seen. This does not exclude the possibility that metabolic impairment is present.

The Role of the Kidneys.

Martensson (1940) perfused citrate solution into an animal whose liver had been removed and observed that the citrate was effectively removed from the perfusion fluid. On perfusing renal tissue he observed that the citrate concentration of the fluid in the renal view was thirty per cent lower than the citrate concentration of the fluid in the renal artery. In this experiment renal excretion was negligible so that citrate must have been removed by the renal parenchyma. Martensson suggested that under normal conditions the kidney was the principal site of citrate metabolism but such a hypothesis which ignores the metabolic activity of the liver cannot be entirely accepted.

The role of the kidney in the metabolism of citrate was also investigated by Freeman and Chang (1950). Bilateral nephrectomy in dogs resulted in a post-operation elevation of serum citrate concentration and the intravenous injection of citrate solution resulted in a

further elevation of serum citrate concentration which only slowly decreased. On the other hand bilateral ligation of the ureters failed to cause any increase in serum citrate concentration and only a transient increase after the intravenous injection of citrate solution. This experiment emphasizes the fact that renal excretion of citrate is less important than the oxidation of citrate in the renal parechyma. Renal excretion of citrate is a factor which can be varied according to dietary intake and it is profoundly influenced by the hydrogen ion concentration of the glomerular filtrate (Homer Smith 1949).

The state of renal function in the newborn infant has been reviewed by Smith (1951). Measurement of urea clearance, creatinine and inulin clearance, diodone clearance and p-animohippurate clearance all indicate that renal function in the newborn infant is immature compared to the normal adult. Glomerular development is more advanced than tubular development at full term but because of epithelial cells covering the glomerulus, glomerular filtration rate is low. Even so circulation is predominantly glomerular and as proximal tubular tissue develops more post-glomerular blood is presented

for clearance.

It is important to realise that the evaluation of renal function in the newborn infant is difficult. Accurate specimen collection is a major problem and the complex relation existing between hormonal, respiratory, nervous, circulatory and alimentary functions must influence any of the functional tests employed. The volume of urine excreted during the first twenty four hours of post-natal life is only about twenty millilitres. Thus if the kidneys participate in the removal of citrate during exchange transfusion, their role must be almost entirely metabolic.

In conclusion, citrate infused during the transfusion of citrated blood is rapidly removed from the plasma, oxidation occuring via the tri-carboxylic acid cycle. The oxidative process may be accelerated by the simultaneous infusion of glucose and citrate since they have a common metabolic pathway. Liver and kidneys are involved in the oxidation of citrate. Normal newborn infants are apparently able to oxidise citrate almost as quickly as it is infused but infants suffering from erythroblastosis foetalis are deficient in this respect.

The inability of these infants to oxidise citrate as rapidly as normal infants may be related to the mild degree of liver dysfunction associated with erythroblastosis foetalis.

THE IMPORTANCE OF POTASSIUM IN TRANSFUSION OF CITRATED BLOOD.

Another factor which has been suggested as a cause of electrolyte disturbance, during exchange transfusion is that of potassium intoxication (Miller, McCord, Joos, Clausen 1954). What influences the response of an individual to a given degree of hyperpotassaemia is not fully understood but it is dependant upon a delicate equilibrium between sodium, potassium, calcium, magnesium and hydrogen ions in the extracellular fluid. A decrease in the concentration of calcium or magnesium or an increase in that of sodium, potassium or hydrogen ions causes increased excitibility of tissues and vice versa. Ringer first observed that antagonism between various cations can occur. He demonstrated this relation between calcium and potassium ions in terms of myocardial contractibility. Clinically, Brown et al (1955) observed that in the presence of a high serum potassium concentration

cardiac failure could be precipitated by the induction of an experimental hypocalcaemia, and in fact, Govan & Weiseth (1946) relieved cardiac failure caused by hyperpotassaemia by the intravenous administration of calcium gluconate solution.

Winkler, Hoff and Smith (1938) attempted to correlate serum potassium concentration during the infusion of a potassium chloride solution with changes in the electrocardiograph recordings. At a serum potassium concentration of 20 to 27 mg. per 100 ml., an increase in the amplitude of the T wave was observed; at 30 to 39 mg. per 100 ml., a depression in the ST segment; at 39 mg. per 100 ml., intraventricular block; and finally, above 39 mg. per 100 ml., disappearance of the P wave.

THE INFLUENCE OF POTASSIUM ION UPON CARDIAC MUSCLE DURING THE TRANSFUSION OF CITRATED BLOOD.

During storage of blood for transfusion, probably because of cessation of intracellular metabolic processes, potassium ion diffuses into plasma. Loutit, Mollison & Young (1943) investigated this phenomenon and observed significant increases in the plasma potassium concentration. Mollison (1951) is of the opinion that the amount of

potassium ion injection during an exchange transfusion is well within the limits of safety. He quotes the example of a newborn infant weighing 3.25 kilograms who, during an exchange transfusion, gained 20 milligrams per kilogram of body weight in a period of forty minutes. The plasma potassium concentration of the infant's blood rose from 19 mg. per 100 ml. before transfusion to 23 mg. per 100 ml. immediately after the transfusion. The plasma potassium concentration of the donor blood was 55 mg. per 100 ml.

Miller, McCord, Joos & Clausen (1954) investigated the changes in serum potassium concentration which occurred during the transfusion of citrated blood to newborn infants suffering from erythroblastosis foetalis. In a series of eight transfusions three infants developed a toxic hyperpotassaemia; one of these cases presented marked cardiac changes when the plasma potassium concentration was 36 mg. per 100 ml., while the donor blood had a plasma potassium concentration of 72 mg. per 100 ml. Miller et al (1954) concluded that most full term infants are able to maintain their serum potassium below levels associated with cardiac abnormalities, even

though high concentrations of potassium ion may beinfused during exchange transfusion.

It is significant that one infant in the series was found to have a co-existent hypocalcaemia and hyperpotassaemia at the instant when ventricular failure occurred, but cardiac signs disappeared after the intravenous injection of calcium gluconate solution. It is probable that the injection of calcium ion restored electrolytic equilibrium and thus normal myocardial irritibility.

RELEVANT ASPECTS OF THE "METABOLISM" OF BODY POTASSIUM.

When a solution of a suitable potassium salt is administered by intravenous route it is rapidly removed from the plasma. Eichelberger (1941) observed a sixty per cent retention of potassium by the liver after injection under conditions similar to those encountered in exchange transfusion, but the liver appears to be a temporary site. After reaching a maximum concentration in the intracellular fluid of liver cells, potassium migrates into the extracellular fluid and thence into the intracellular fluid of red blood corpuscles and muscle cells.

Movement of Potassium Ion between Intracellular and Extracellular Spaces.

Potassium ion is predominantly intracellular in the body, muscle cells being the major source, but movement of potassium and other cations occurs accross cellular membranes, migration being dependant upon the equilibrium between intracellular and extracellular electrolytes. For example potassium ion moves into extracellular fluid when excessive quantities of water and sodium ion are lost from the body. Potassium migration appears to be influenced by different phases of the metabolism of carbohydrate - glycogenolysis is followed by an increase in extracellular potassium and glycogenesis by a decrease in extracellular potassium. Such an influence would account for the observation that extracellular potassium concentration decreases during the infusion of glucose and might explain, in part, the ability of newborn infants to maintain serum potassium concentration within normal limits during transfusion with citrated blood containing glucose.

The Influence of Adrenal Hormones.

The distribution of sodium, potassium, chloride and water between intracellular and extracellular fluids is

modified by adreno-cortical hormones. Steroids of the 17 - hydroxy - 11 - oxy - corticosterone series appear to enhance the effect of anterior pituitary hormone in antagonising the oxidation of glucose, thereby increasing glycogen stores, and this is associated with increase in intracellular potassium ih liver cells.

The mineralo-corticoids consists of steroids not possessing an oxygen atom at C-11 and those belonging to the "amorphous fraction". They appear to alter cell wall permeability and also to act upon the distal tubules causing increased re-absorption of sodium and water and decreased re-absorption (or increased tubular secretion) of potassium.

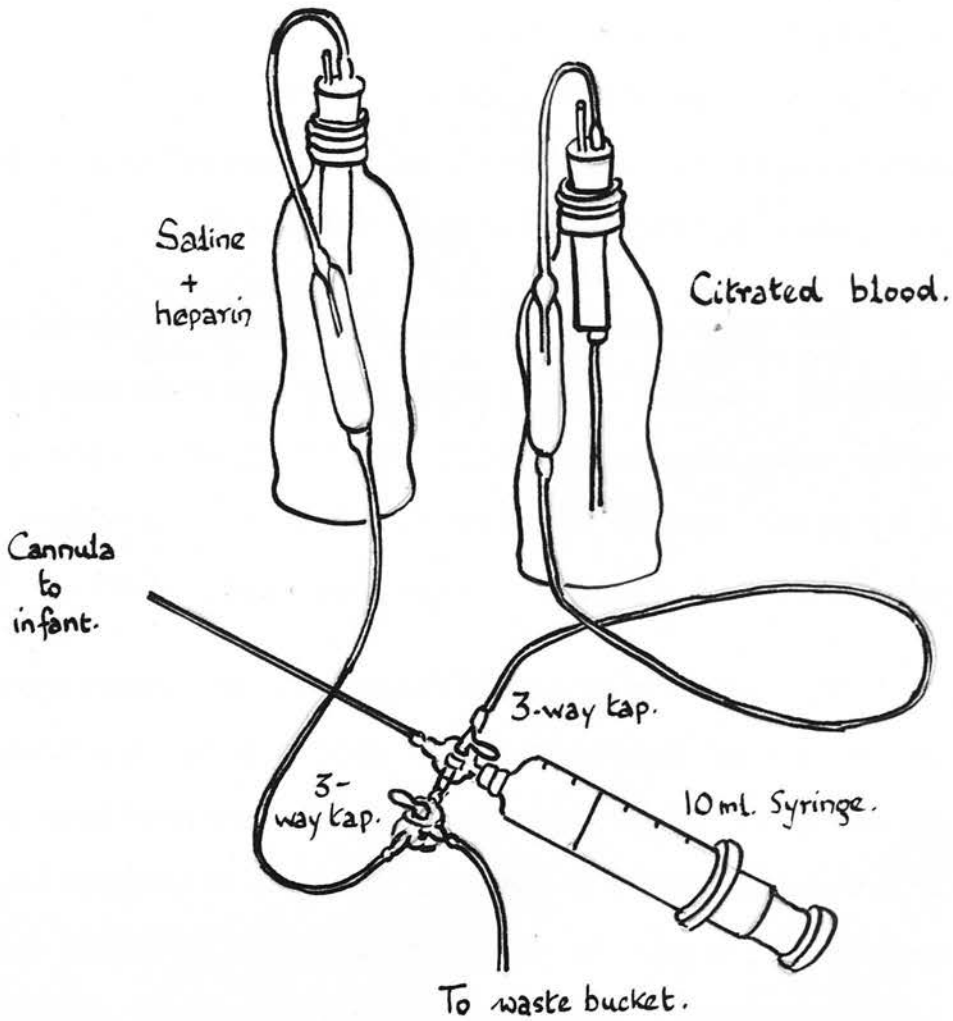
The stimulus for the renal excretion of potassium is obscure but it is possible that intracellular potassium concentration is a vital factor. Thus increased renal excretion follows cellular dehydration, and the intravenous infusion of potassium does not result in increased renal excretion of potassium until the intracellular potassium concentration reaches a critical concentration (Tarail & Elkington, 1949).

Evidence suggests that the adrenal cortex is functional in the newborn infant and that adreno-cortical hormones from maternal and foetal sources are circulating in the blood. (Farquhar 1953). This being the case efficient homeostatic mechanisms exist to cope with the infusion of exessive amounts of potassium.

As a working hypothesis for the present series of experiments, it is suggested that potassium entering the plasma during exchange transfusion is transferred firstly to the intracellular fluid of the hepatic cells. From here it migrates to the intracellular fluid of muscle cells and red blood corpuscles, where a distribution of cations occurs to re-establish electrolytic equilibrium. This process involves the extrusion of intracellular sodium ion. Renal excretion of potassium, involving adreno-cortical homones, may then proceed at a rate at which the immature kidney can excrete, but renal excretion of excess potassium in the prevention of a toxic hyperpotassaemia cannot be an important homeostatic mechanism during the immediate post-natal transfusion period.

FIG 1.

Diagrammatic arrangement of equipment for exchange transfusion.



PLAN OF WORK.

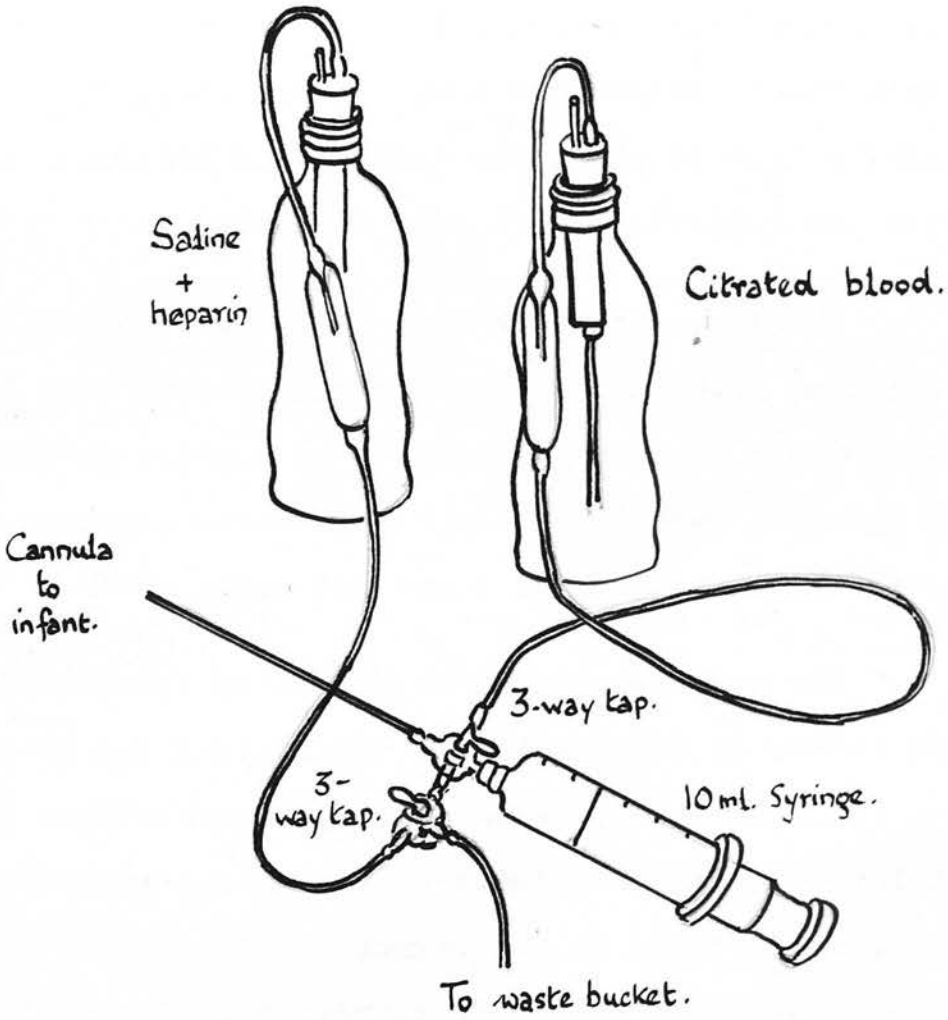
In the belief that analysis of serum taken at regular intervals during exchange transfusion would reflect the ability of the newborn infant suffering from erythroblastosis foetalis to maintain a normal serum electrolyte picture, electrolyte balance, particularly in reference to calcium and potassium ions, has been evaluated.

The serum concentration of sodium, potassium, total calcium, calcium ion, protein and citrate were determined after each hundred millilitres of blood exchanged, and a clinical record of apex beat, venous pressure and occurrence of abnormal signs was kept.

The purpose of this thesis is to present evidence in favour of the hypothesis that during the transfusion of citrated blood, serum citrate concentration rises to a level where serum ionised calcium concentration is depressed, owing to the formation of a weakly ionised, physiologically inactive, ultrafilterable complex. This hypocalcaemia may or may not be associated with a hyperpotassaemia but if such a phenomenon occurs in the presence of imminent cardiac failure due to anoxia, an electrolyte disturbance of any magnitude might precipitate cardiac failure.

FIG 1.

Diagrammatic arrangement of equipment for exchange transfusion.



METHODS

CLINICAL METHODS

TECHNIQUE OF EXCHANGE TRANSFUSION.

At the time of birth the umbilical cord was clamped some inches from the infant and the attached portion was surrounded with gauze swabs moistened with sterile saline in order to prevent desiccation pending the results of haemoglobin estimation, nucleated red cell count, serum bilirubin estimation, and Coomb's test.

If it was decided to proceed to an exchange transfusion the cord was cut not more than three-quarters of an inch from the umbilicus, the umbilical vessels recognised and the vein held open by the application of two fine pairs of mosquito artery forceps to its walls. The risk of air embolism is negligible.

The assembled transfusion apparatus (figure I) was flushed with heparinised saline (5,000 I.U. per pint) and the end of the polythene tubing which was to be inserted was cut to form as short a bevel as practicable. With the umbilical cord held downwards and towards the operator's left, the polythene tubing was inserted into

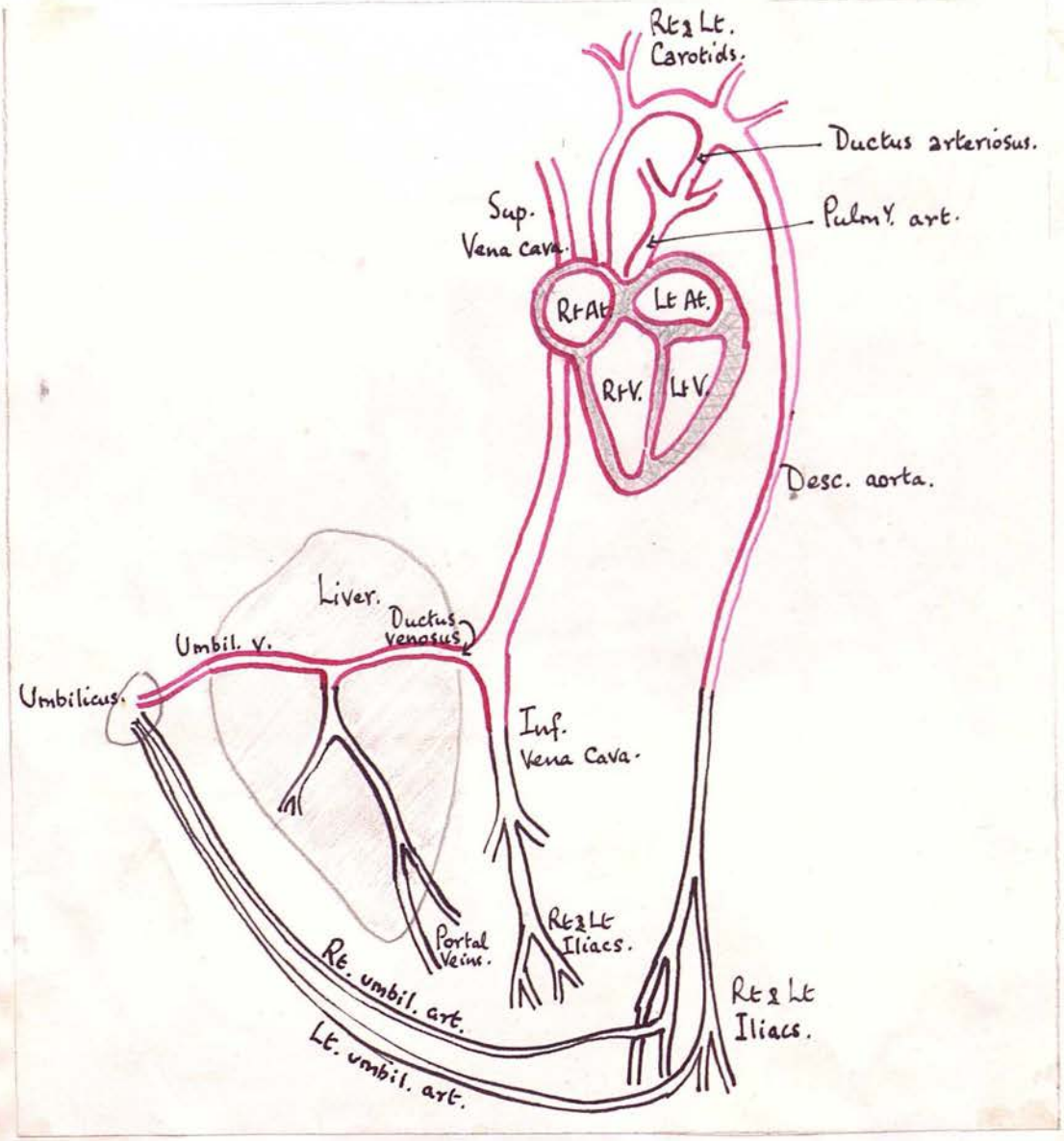


FIG II

Cardio-vascular system in the newborn infant (diagrammatic.).

the umbilical vein to a distance of about seven and a half centimetres. Careful suction was then applied to the syringe and if the end of the catheter was at the correct point in the vein there was a ready return of the infant's blood. If the blood did not flow back freely, careful adjustment of the catheter was necessary. This entailed moving it by a number of centimetres or simply a millimetre or two or even by retaining it in the same position but rotating it through 90^{o} to 180^{o}. There was no easy explanation for the remarkable differences in the ease of obtaining blood with a minimum of change in the position of the catheter. In fortunate cases the catheter passed from the portal sinus into the inferior vena cava via the ductus venosus (figure II) but in some cases the catheter apparently passed into the portal circulation where it was increasingly difficult to obtain blood. It was by no means the case that when the correct position for the catheter tip was found blood continued to flow back readily during the course of the transfusion, and manipulation was required from time to time.

A catgut ligature was tied tightly around the polythene tube and another around the stump of the umbilical cord,

and the two ligatures were clipped together with a pair of artery forceps - in this way the position of the catheter did not change appreciably with movement of the infant or back pressure of blood.

The exchange transfusion was carried out by alternate withdrawal of ten millilitres of blood from the infant and replacement with ten millilitres of donor blood. It is apparent that the longer the length of polythene tube extending from the apparatus to the infant then the greater the dead space and the less efficient the exchange. For this reason the tube was kept as short as practicable.

At intervals of one hundred millilitres of blood withdrawn the whole apparatus was flushed with heparinised saline, but it was necessary to prevent this entering the circulation of the infant.

At intervals of one hundredmillilitres of blood exchanged one millilitre of ten per cent calcium gluconate was injected intravenously.

The total volume of blood exchanged was calculated roughly as being about three times the infant's expected blood volume, that is, about one hundred and twenty millilitres per pound of body weight. Stress has been

placed in the literature on methods of calculating the volume of blood required and the bonus of blood which may be given during the exchange to correct the infant's anaemia. It must be remembered that exchange transfusion is a method of removing sensitised cells and antibody rather than of correcting the anaemia for in the severely affected cases there is no doubt that an abrupt rise in haemoglobin concentration throws an extra and possibly fatal burden on the heart.

A careful clinical record of progress was kept during the transfusion. This included recording the volume of the blood withdrawn and replaced, heart rate, and occurrence of cyanosis, twitching or crying. The exchange was carried out slowly and if the infant was not tolerating the procedure, the apparatus was flushed with heparinised saline and the infant was rested for fifteen or twenty minutes. As many as three or four rest periods were necessary for readjustment in some badly affected babies.

At the end of the transfusion, two catgut ligatures were inserted through the cord, one being tied around the vein and the other around the arteries.

Vitamin K and systemic streptomycin were given and the infant nursed naked in the incubator room for twelve hours in order that bleeding from the cord stump could be immediately observed.

If the infant was very restless, it was found of value to inject half grain phenobarbitone intramuscularly at any stage during the transfusion.

COLLECTION OF BLOOD SPECIMENS DURING EXCHANGE TRANSFUSION.

Two sets of tubes were provided. One set contained no preservative while the other contained sodium fluoride, 50 mg. per 5 ml. blood.

A. Ten millilitres of blood were withdrawn from the bottle of donor blood. Five millilitres of this blood were placed in a plain tube and five millilitres in a fluoride tube. The specimens were labelled "DONOR I".

B. Before commencement of the transfusion ten millilitres of blood were collected from the infant and divided equally between a plain and a fluoride tube as in A.

C. In the first series of cases blood was collected for analysis immediately before the administration of calcium gluconate. For this purpose one hundred millilitres of blood were withdrawn from the infant. The gluconate was then injected and the withdrawal of the blood continued. The ten millilitres of blood withdrawn immediately prior to the administration of gluconate were retained for analysis. This procedure was repeated for each hundred millilitres volume of blood withdrawn. The specimens collected in this way were labelled "100" to "800" at intervals of one hundred corresponding to the

volume of blood removed.

D. A second series of cases were studied in order to determine the effect of calcium gluconate. In these cases two hundred millilitres of blood were exchanged from the infant. The gluconate was injected and the withdrawal of blood was continued. The ten millilitres of blood removed immediately prior to, and (the ten millilitres) immediately subsequent to, the administration of gluconate were retained for analysis. The procedure was repeated for each two hundred millilitre volume of blood withdrawn.

E. When bottles of donor blood were changed, procedures A and B were repeated and the specimens placed in tubes labelled "DONOR II" and "CHANGE-OVER".

F. In the event of any abnormal incident during the transfusion a specimen of blood was collected immediately.

During specimen collection, contamination of blood samples with the contents of the polythene catheter was avoided by the following procedure:-

A few millilitres of the infant's blood were sucked into the syringe and rejected into the waste bucket. By

this procedure any residual donor blood was removed from the polythene cannula. The actual blood specimen was then withdrawn and placed in the centrifuge tube. Specimens were centrifuged soon after clot retraction and the sera were stored in the refrigerator prior to analysis.

ANALYTICAL METHODS

Standard Methods

Sodium: Reference - Varley H. Practical Clinical Biochemistry 1954, 1st edition, p. 338. Dilution of Serum 1/500.

Potassium: Reference - as for sodium. Dilution of serum 1/50.

Glucose: Reference - Hagedorn H.C., Jensen B.N., Biochem Z. 135, 46 (1923).

Total Protein: Reference - King E.J. Micro-analysis in Medical Biochemistry, 1951. 2nd edition p.45.

Total Calcium

METHOD A.

Principle.

Calcium was precipitated as calcium oxalate and this was oxidised to the carbonate. The latter was dissolved in hot boric acid solution and titrated with N/50 hydrochloric acid.

Technique.

0.20 ml of serum was transferred to a specially

made centrifuge tube (described by the authors). Serum was dried at 100°C and ashed overnight at 500°C in a muffle furnace. This procedure removed citrate ion which might have interfered with the oxalate precipitation.

The ashed material was dissolved in 0.2 ml of distilled water, and the hydrogen ion concentration was adjusted to pH 5 using BDH universal indicator and N/50 HCl and NaOH. 0.2 ml of saturated ammonium oxalate solution was added and the tube rotated to mix the contents.

After standing overnight, the tube was centrifuged at 2000 r.p.m. for fifteen minutes. The supernatant fluid was carefully aspirated to within one millimetre of the surface of the precipitate. 0.3 ml. of 0.5% ammonium oxalate was added and the tube was again rotated to wash the precipitate. After centrifuging the supernatant fluid was aspirated as before.

The calcium oxalate precipitate was dried at 110°C and oxidised in a muffle-furnace at 475° to 525°C for thirty minutes. When cool, 0.1 ml of hot 10% boric acid was added to the carbonate. The temperature of the tube was maintained at 100°C in a boiling water bath for five minutes to dissolve the carbonate.

0.2 ml of indicator solution (see below) was placed in the tube and the carbonate was titrated with N/50 sulphuric acid from a Conway micro-burette.

A blank consisting of 0.1 ml of 10% boric acid and 0.25 ml of indicator solution was titrated to the same end point. During the titration, the contents of the tubes were mixed by bubbling air through the solution.

1 ml of N/50 sulphuric acid is equivalent to 200 mg. of calcium.

The indicator consisted of five parts of 1% alcoholic bromo-cresol green and one part of 1% alcoholic methyl red. For use, six drops of this mixture were diluted to five millilitres with distilled water. This indicator was also used for the ammonia titration in the estimation of serum proteins.

Reference.

Sobel A.E., Sobel B.A., J. Biol. Chem. 129, 721 (1939).

METHOD B.

Principle.

Calcium was chelated with ethyl diamine tetra-acetic acid at pH 11.6. At this hydrogen ion concentration

magnesium does not interfere. Murexide was used as the indicator.

This method was used only as a check on method A.

Reference.

Kibrick A.C., Ross M., Rogers H.E., Proc. Soc. Exper. Biol & Med. 81, 353 (1952).

Citric Acid.

INTRODUCTION.

Three procedures have been in general use for the estimation of citric acid concentration in serum..

One of the methods was introduced by Thunberg (1929) and has since been modified (Ostberg 1934, Hagelstain, 1945). The method depends upon the measurement of the time necessary to decolorise methylene blue solution in the presence of a citric dehydrogenase system. The method is subject to interference from substances other than citric acid, it requires elaborate technique, is time-consuming and thus unsuitable for determinations on large numbers of blood samples.

The second procedure depends upon the oxidation of citric acid with an acid solution of permanganate and its subsequent bromination to yield pentabromacetone. The pentabromacetone is then determined either by its colorimetric reaction with a solution of sodium sulphide (Pucher et al, 1936), or with sodium iodide (Taussky & Shorr, 1947), potassium iodide (Wolcott & Boyer, 1948), thiorea (Natelson et al, 1948) or pyridine in alkaline solution (Ettinger, 1952.). Oxidation of citric acid with an acid solution of permanganate is a sensitive

reaction which is influenced by changes in hydrogen ion concentration and the rate of addition of permanganate (Krog. 1945). Weil-Malherbe and Bone (1949) overcame this difficulty by employing an acid solution of ammonium vanadate as an oxidising agent. Bromination of the acetone is also a sensitive reaction and it is essential that excess bromine be removed since it interferes with colour development. Thiosulphate and ferrous sulphate have been used for this purpose (Taylor 1953).

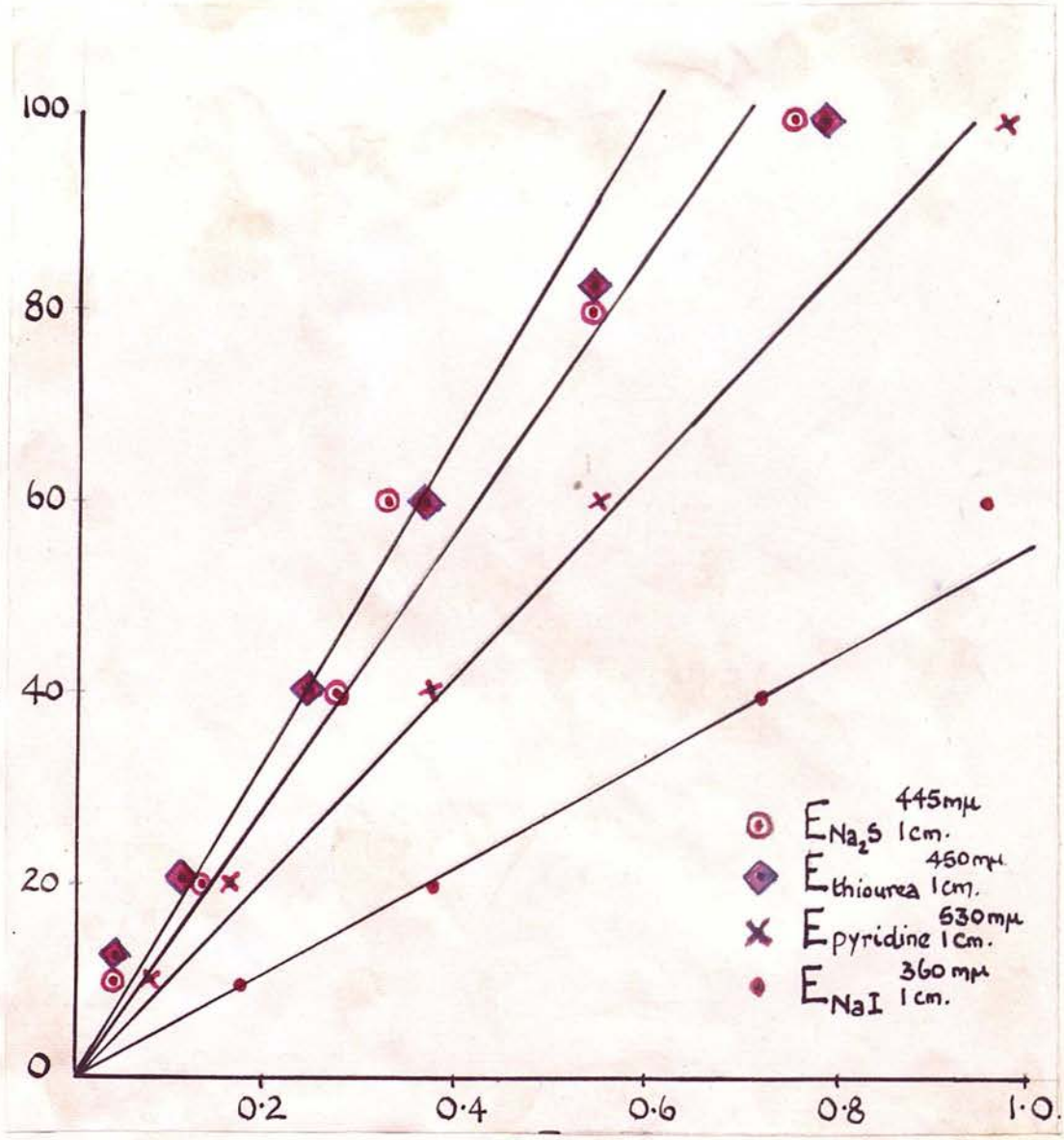
The third procedure depends upon the oxidation of citric acid to acetone di-carboxylic acid, followed by thermal decomposition of the latter substance to yield acetone. This is distilled into an alkaline solution of salycylaldehyde to form a red coloured complex, the potassium salt of acetone di-salicylaldehyde (Krog, 1945). In the original method oxidation of citric acid was carefully controlled and the volatile acetone was isolated from interfering chromogens. However, relatively large volumes of blood were required for analysis, acetone was distilled without proper precautions to prevent losses and allowance was not made for the thermo- and photo-sensitive properties of acetone di-salicylaldehyde.

Thin and Robertson (1952) overcame the distillation problem by allowing acetone to distil into alkaline salycylaldehyde solution in a Conway micro-diffusion unit, while Bahner (1952), using the same principle, distilled acetone in a thermal gradient micro-diffusion unit. Acetone was distilled at 100°C and collected in an alkaline solution of salicylaldehyde maintained at a temperature below 50°C.

EXPERIMENTAL.

Evaluation of the Pentapromacetone Methods.

In order to assess the merits of each method, a series of standard solutions of citric acid were subjected to analysis by each original technique referred to in the introductory section. The results of analysis using the pentabromacetone principle are summarised in Table I and Graph.A. It is concluded that the sodium sulphide and thiorea reactions give low extinction coefficients and show a susceptibility to the development of turbidity in the final coloured solution, necessitating the use of a correction factor. The pyridine reaction is also unsuitable because of the toxic nature of the solvent, whilst the sodium iodide reaction is affected by



GRAPH A.

Comparison of calibration curves of four pentabromacetone methods.

atmospheric oxidation of the iodide to iodine. The modifications proposed by Weil-Malherbe and Bone (1949) and Taylor (1953) are not satisfactory, recoveries being unreliable.

Table I. The relative merits of the pentabromacetone methods for the estimation of citric acid, expressed in terms of extinction coefficients.

Citric Acid µg. per 0.1 ml.	E 445mµ 1 cm. Na S	E 450mµ 1 cm. thiourea	E 530mµ 1 cm. pyridine	E 360mµ 1 cm. NaI
10	0.048	0.042	0.080	0.180
20	0.140	0.125	0.170	0.380
40	0.278	0.258	0.380	0.720
60	0.330	0.369	0.550	0.950
80	0.540	0.540	0.780	-
100	0.750	0.780	0.970	-

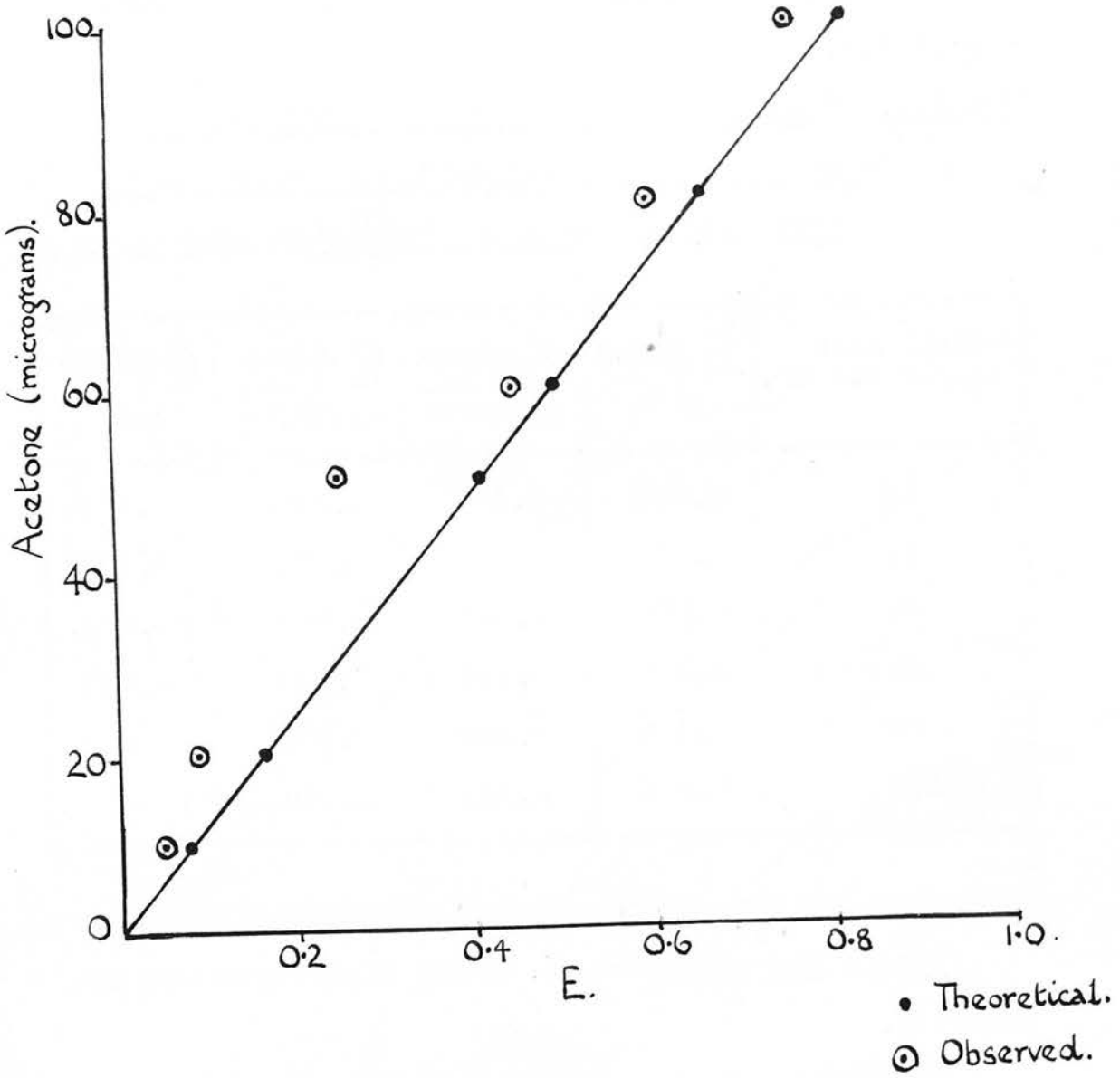
Evaluation of the Acetone Di-salicylaldehyde Methods.

Krog's Method proved to be too time-consuming in practice.

The method of Thin & Robertson (1952), which was used originally for the estimation of acetone bodies in blood, was assessed in the following way. One millilitre

Graph B.

Recovery of acetone. Method of Thin & Robertson.



of each of a number of acetone solutions, containing ten to one hundred micrograms per millilitre, was placed in the outer chamber of a series of Conway units. One millilitre of alkaline salicylaldehyde solution (3% in 3N KOH) was placed in the inner chamber. Acetone was liberated by the addition of 0.5 millilitres of 9N sulphuric acid to the outer chamber, and diffusion and colour development were allowed to proceed for seventeen hours, in the dark, at 37°C. The acetone di-salicylaldehyde solutions were transferred quantitatively to ten millilitre volumetric flasks, made up to the mark with alkaline salicylaldehyde solution and extinction coefficients determined at 490 millimicrons in a Unicam Model SP600 spectrophotometer.

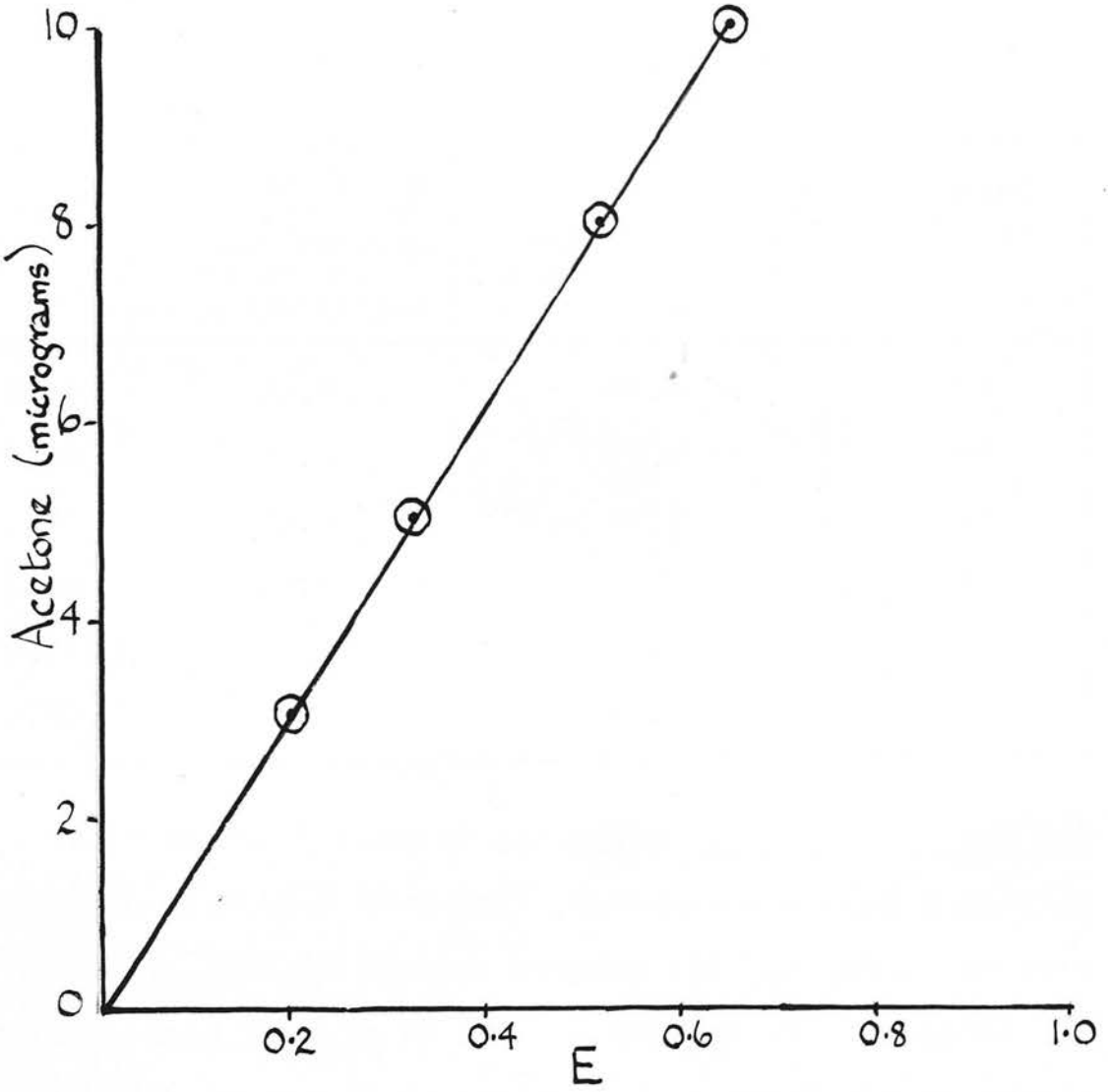
In order to determine the degree of recovery of acetone, one millilitre of each acetone solution was placed in a series of ten millilitre volumetric flasks, together with one millilitre of alkaline salicylaldehyde solution. These were placed in the same environment as the Conway units, and the volume was adjusted to the mark with 3N KOH solution prior to the determination of extinction coefficients. Table II and Graph B summarise the result. It is apparent that quantitative recoveries

of acetone are unreliable at concentrations below sixty micrograms per millilitre.

Table II. Recovery of acetone using the method of Thin & Robertson.

Acetone µg. per ml.	E 490 mµ 1 cm. Acetone added to Conway units.	E 490mµ 1 cm. Acetone added direct to salicylaldehyde.	% Recovery
10	0.05	0.08	62.5
20	0.09	0.17	53.0
50	0.25	0.41	61.0
60	0.45	0.50	90.0
80	0.60	0.66	91.0
100	0.76	0.82	9216

The Method of Bahner, using the thermal gradient micro-diffusion unit was repeated. Standard solutions of acetone were prepared, and the acetone content of each solution was estimated, as follows:- 0.1 ml of each solution was placed in the buckets of a series of Bahner units. Acetone was liberated by the addition of 0.1 ml. of 34% phosphoric acid olution and distilled into 5 ml. of alkaline salicylaldehyde solution contained in the



GRAPH C.

Calibration curve using Bahner's method.

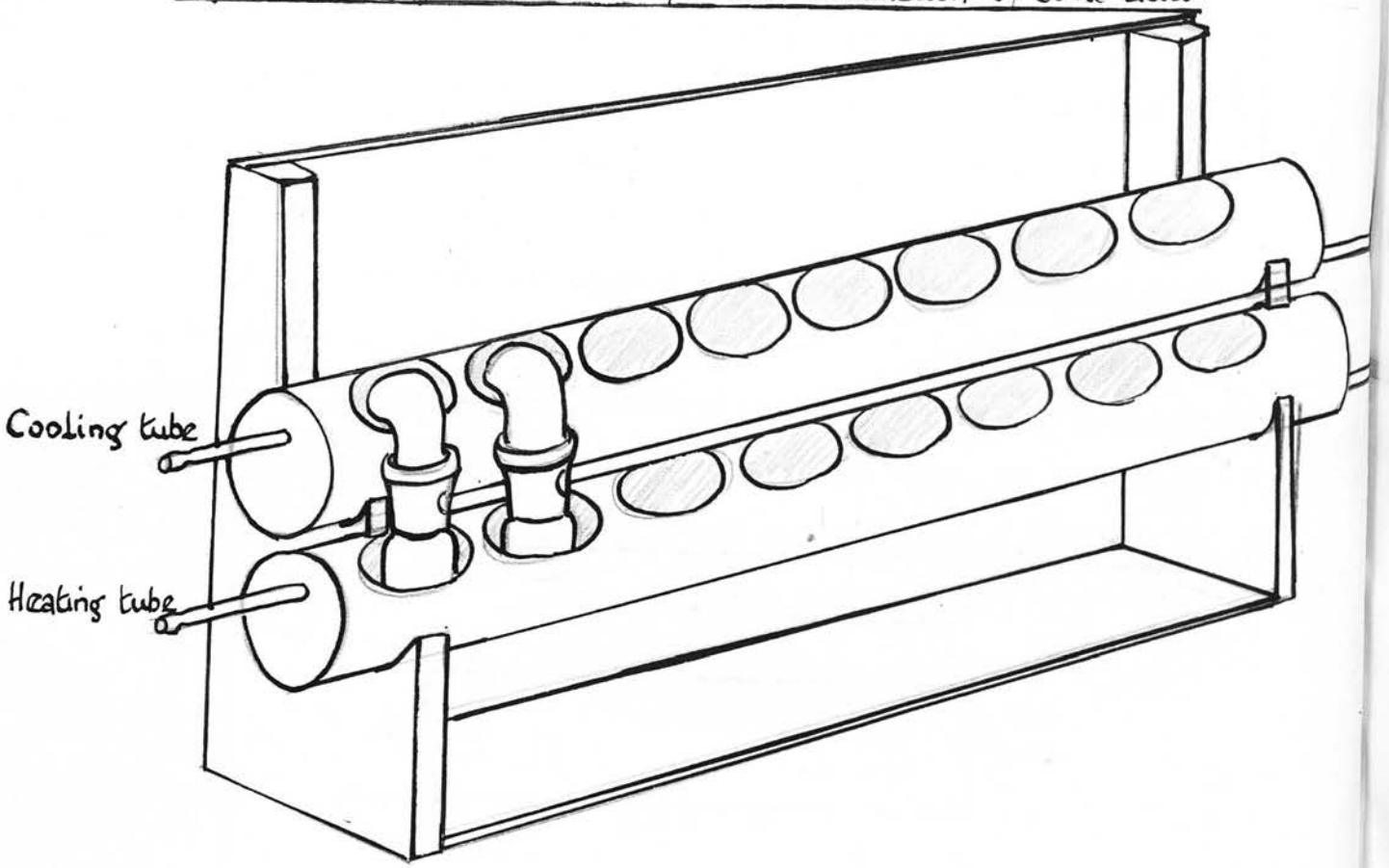
"mushroom" of the unit. Details of the technique are described by Bahner. The results, tabulated in Table III, indicate that the method obeys Beer's Law, and on adding acetone to blood, quantitative recoveries are obtained. Unfortunately the elaborate nature of the apparatus makes it inapplicable to routine analysis. A modified thermal gradient micro-diffusion apparatus was therefore designed to meet the needs of the present investigation.

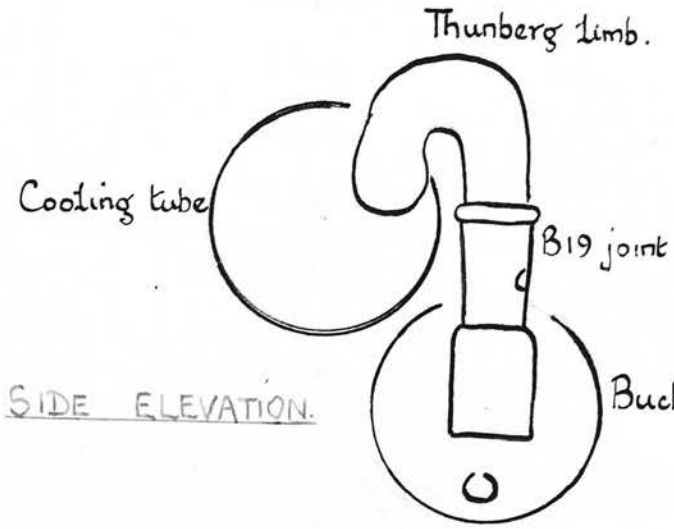
Table III. Calibration curve using Bahner's micro-diffusion unit.

Acetone µg / 0.1 ml.	E 490 mµ 1 cm.
3	0.200
5	0.322
8	0.520
10	0.647
Serum	0.250
" + 5	0.570
" + 10	0.902

FIG III

The thermal gradient bath for the estimation of citric acid.





SIDE ELEVATION.

FIG IV.

Micro-distillation unit.

("in situ").

A Modified Method for the Estimation of Citric Acid

DESCRIPTION OF APPARATUS

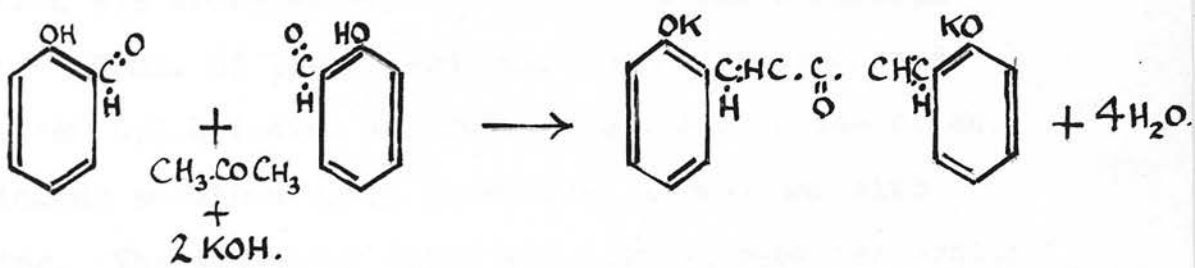
A distillation unit was designed which is simpler than the Bahner unit but which retains the principle of distillation in a closed unit across a temperature gradient. It consists of a pyrex bucket of about 10 ml. capacity which is attached by a Quickfit joint (B19) to the limb of a Thunberg tube. Acetone or citric acid solution is introduced into the bucket, and citric acid is oxidised by means of an acid solution of ammonium vanadate. The acetone is then distilled into an alkaline solution of salicylaldehyde contained in the "limb". A temperature gradient was attained in the following way. Two 14 inch lengths of $1\frac{1}{2}$ inch diameter copper tubing were plugged at each end with rubber bungs. Holes of 1 inch diameter were bored along the tubes at intervals of $1\frac{1}{2}$ inches between centres. The tubes were supported in retort stands in such a way that the assembled micro-distillation unit already described rested with the bucket in the lower tube and the Thunberg limb in the upper tube. Tap water was allowed to circulate through the upper tube and steam heated water maintained at 85° to 100°C was

circulated through the lower tube. The number of estimations capable of being carried out at any one time was governed only by the length of the temperature gradient bath and the number of micro-distillation units available. Twelve was found to be a convenient number to handle at one time.

KINETICS OF THE ACETONE DI-SALICYLALDEHYDE REACTION.

A. Reaction.

In alkaline solution acetone combines with salicylaldehyde forming a salt of acetone di-salicylaldehyde.



B. Absorption Curve of Acetone Di-salicylaldehyde and Salicylaldehyde.

Preparation of Alkaline Salicylaldehyde Solution.

Salicylaldehyde, obtained fromBDH Ltd., was distilled in vacuo below 80°C. The middle fraction of distillate was stored in a brown reagent bottle at room temperature. It was an almost colourless liquid. 3.0 N potassium

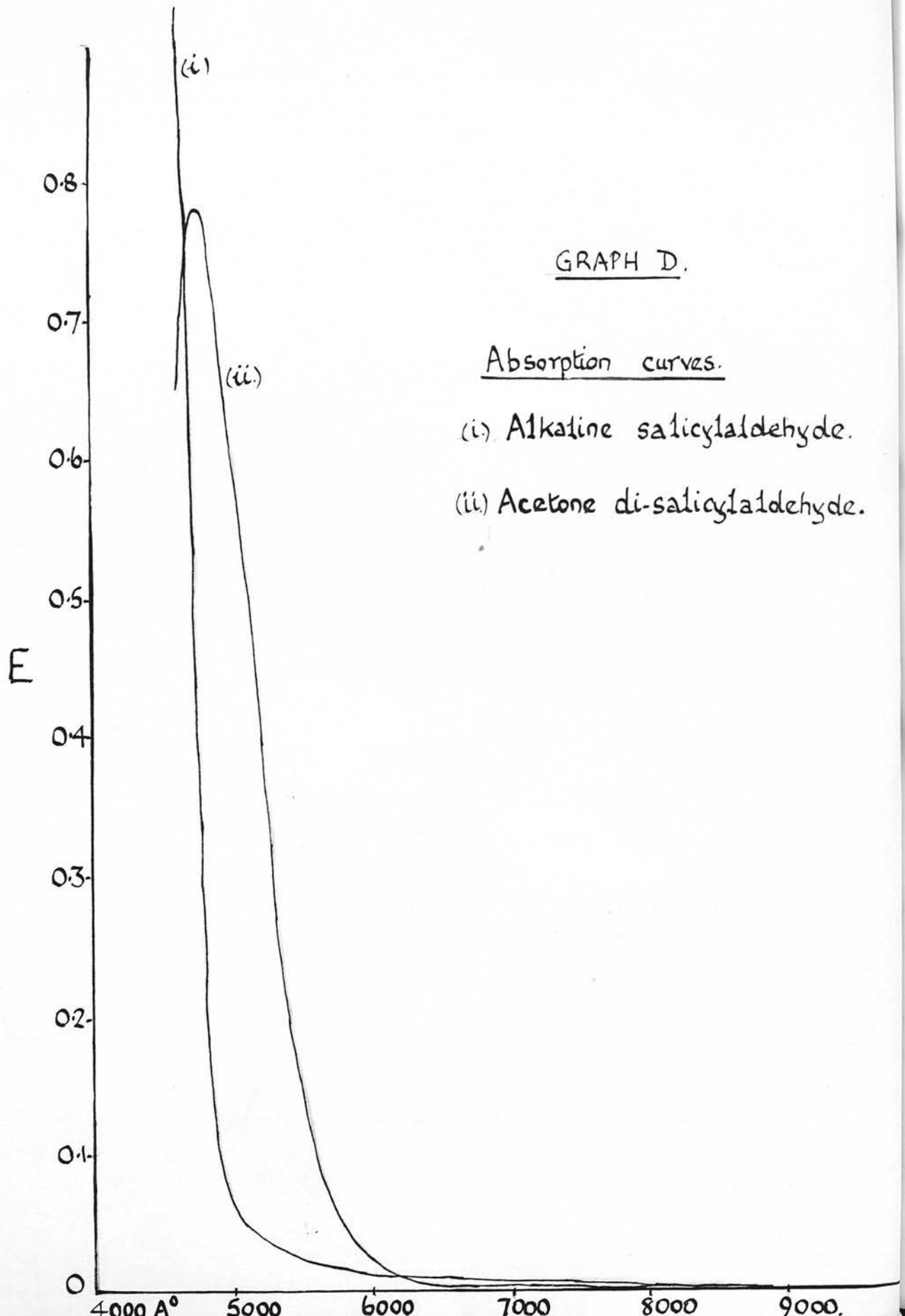
hydroxide solution, carbonate free, was prepared by a standard technique and stored in an aspirator bottle, fitted with a soda-lime guard. Except where otherwise stated alkaline salicylaldehyde solution was freshly prepared on each occasion before use by mixing 3 ml. of re-distilled salicylaldehyde with 97 ml. of 3.0 N potassium hydroxide solution.

Preparation of Acetone Solution. 5 ml. of Analar acetone was diluted to 500 ml. with carbon dioxide-free distilled water. To standardise this solution, 5 ml. of the stock solution was transferred to a 250 ml. glass-stoppered flask. 25 ml. of 1.0 N sodiumhydroxide solution and 50 ml. of 0.1 N iodine solution were added to the flask, the iodine solution being introduced slowly and with shaking. The stoppered flask was left at room temperature for fifteen minutes, and an excess of normal sulphuric acid was added - usually 25 to 30 ml. Excess iodine was estimated by titration with 0.1 N sodium thiosulphate, using starch solution as indicator. 1 ml. of 0.1 N iodine = 0.9675 mgm. of acetone. From this stock acetone solution, which is stable for one month, dilute working standards were prepared.

TABLE IV.

Absorption curves of alkaline salicylaldehyde and acetone di-salicylaldehyde.

mμ	E 1 cm. Sal.	E 1 cm. AD Sal.
460	00	0.646
470	0.780	0.780
480	0.196	0.780
490	0.080	0.730
500	0.056	0.630
510	0.046	0.530
520	0.040	0.422
530	0.035	0.307
540	0.028	0.216
550	0.026	0.158
560	0.019	0.087
570	0.018	0.053
580	0.015	0.030
590	0.015	0.016
600	0.015	0.010
625	0.015	0.003
650	0.012	0.004
675	0.012	0.005
700	0.010	0.004
725	0.007	0.000
750	0.006	0.005
775	0.006	0.004
800	0.006	0.007
850	0.006	0.004
900	0.000	0.000



GRAPH D.

Absorption curves.

(i.) Alkaline salicylaldehyde.

(ii.) Acetone di-salicylaldehyde.

Absorption Curves.

1) 9.9 ml. of alkaline salicylaldehyde solution were added to 0.1 ml. of a solution containing 22.8 micrograms of acetone. The reaction was carried out in an all-glass stoppered test tube and was allowed to proceed for eighteen hours in the dark at room temperature. The absorption curve was then determined in a Unicam Spectro-photometer (Model SP600) using one centimetre cells, and water as a zero blank.

2) The absorption curve of a freshly prepared solution of alkaline salicylaldehyde was determined in a similar manner.

The results are tabulated in Table IV. Although maximum absorption of acetone di-salicylaldehyde occurs at 475 millimicrons it appears that minimum interference from salicylaldehyde occurs at 490 millimicrons. This then, is the optimum wave-length for the determination of extinction values.

C. Influence of Time and Light upon the Acetone Di-salicylaldehyde Reaction.

1) 9.9 ml. of alkaline salicylaldehyde solution were added to 0.1 ml. of solution containing 15.2 micrograms of acetone. The reaction was allowed to

proceed for six hours at room temperature in the dark, and at hourly intervals during this period extinction values were determined. The acetone di-salicylaldehyde solution was left in the same environment for a further twelve hours and the extinction value again determined. Results are recorded in Table V. Maximum colour is attained in six hours, and remains stable under the above conditions for at least eighteen hours.

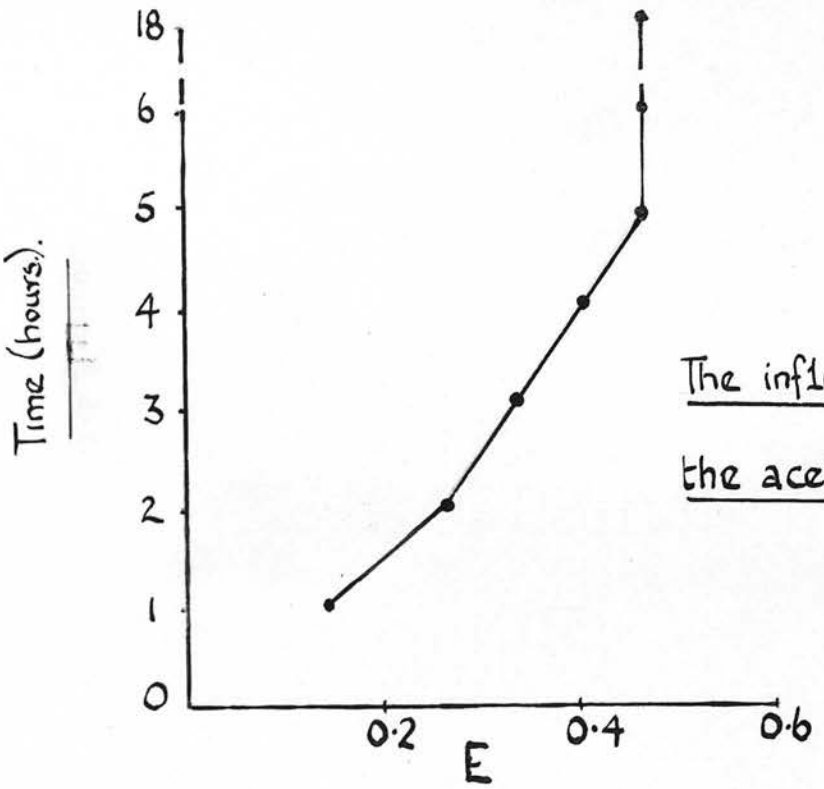
Table V.

The influence of time upon the acetone di-salicylaldehyde reaction.

Time in hours.	1	2	3	4	6	18
E 490 mμ 1 cm.	0.143	0.265	0.335	0.400	0.465	0.465

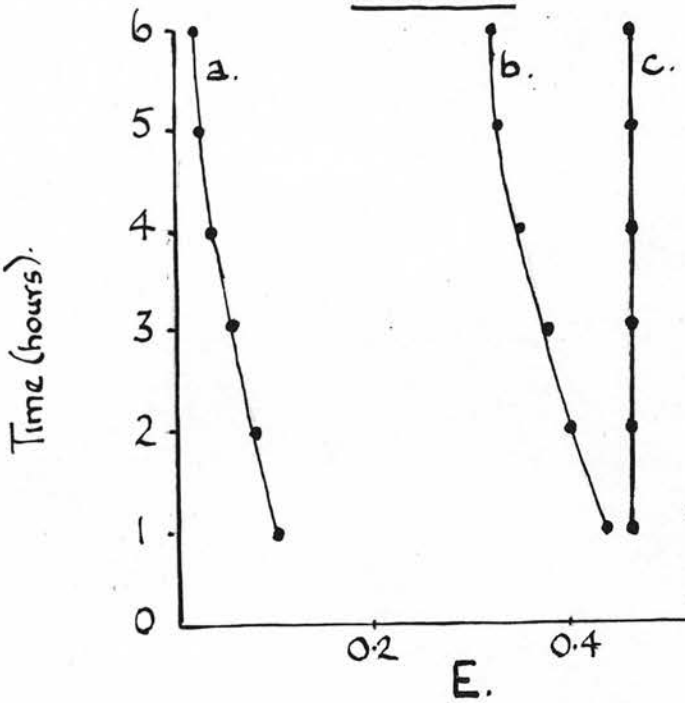
2) Another series of glass stoppered test tubes containing 9.9 ml. of alkaline salicylaldehyde solution and 15.2 micrograms of acetone in 0.1 ml. of solution were maintained at room temperature in the dark for six hours. After this period the tubes were divided into three groups. One of these groupswas kept in the dark at room temperature, a second group in diffuse daylight

GRAPH E.



The influence of time upon the acetone~salicylaldehyde reaction.

GRAPH F.



The influence of light upon the acetone~salicylaldehyde reaction.

a ~ sunlight.
b ~ diffuse daylight.
c ~ darkness.

at room temperature, and the third group in direct sunlight at room temperature. The results, recorded in Table VI show that, while the colour remains stable in the dark, it is profoundly affected by direct sunlight and, to a much smaller degree, by diffuse daylight.

Table VI

The influence of light upon the acetone di-salicylaldehyde reaction.

Time in Hours.	1	2	3	4	5	6
$E^{490\ m\mu}_{1\ cm.}$ (dark)	0.465	0.465	0.463	0.465	0.465	0.465
$E^{490\ m\mu}_{1\ cm.}$ (diff. daylight)	0.435	0.400	0.382	0.351	0.329	0.320
$E^{490\ m\mu}_{1\ cm.}$ (sunlight)	0.100	0.080	0.065	0.038	0.030	0.020

D. The Influence of Concentration of Salicylaldehyde and Potassium Hydroxide upon the Acetone - di-salicylaldehyde Reaction.

By varying the volume of salicylaldehyde and the normality of potassium hydroxide solution it is possible to determine the optimum combination of the two factors in the preparation of alkaline salicylaldehyde solution.

Each stoppered test tube contained 9.9 ml. of alkaline salicylaldehyde solution and 15.2 micrograms of acetone in 0.1 ml. of solution. Blank tubes containing alkaline salicylaldehyde solution were put up with each variation of salicylaldehyde and potassium hydroxide. Colour was allowed to develop for seventeen hours in the dark at room temperature.

Reference to Table VII will show the various combinations used and the extinction coefficients obtained. Maximum difference in extinction values between salicylaldehyde and acetone di-salicylaldehyde occurred in a 3% solution of salicylaldehyde using 3 N potassium hydroxide as diluent.

Table VII

Influence of variation of the concentration of salicylaldehyde and the normality of potassium hydroxide upon the acetone di-salicylaldehyde reaction.

ml. Salicyl.	ml. IN.KOH	E 490 mu 1 cm.	ml. 2N.KOH	E 490 mu 1 cm.	ml. 3N.KOH	E 490mu 1 cm.
0.1	9.9	0.370 Blank 0.089	9.9	0.385 Bl.0.096	9.9	0.392 Bl.0.099
0.2	9.8	0.391 Bl. 0.094	9.8	0.420 Bl.0.108	9.8	0.438 Bl.0.113
0.3	9.7	0.410 Bl. 0.099	9.7	0.450 Bl.0.127	9.7	0.480 Bl.0.132
0.5	9.5	0.435 Bl. 0.120	0.5	0.478 Bl.0.156	9.5	0.505 Bl.0.165

E. The Influence of Temperature upon the Acetone Di-salicylaldehyde Reaction.

9.9 ml. of alkaline salicylaldehyde solution were added to 7.6 micrograms of acetone in 0.1 ml. of solution. The stoppered tubes were placed in the dark for seventeen hours at various temperatures, after which extinction coefficients were determined. A freshly prepared solution of alkaline salicylaldehyde was used as a blank. Results are recorded in Table VIII. Maximum colour development appears to be at 37°C. Bahner (1952) observed at decomposition of the acetone di-salicylaldehyde colour at higher temperatures. The fact that there is little difference in the extent of reaction below 20°C, yet a marked rise at 37°C suggests there may be a critical temperature between 20° and 37°C at which a maximum reaction takes place.

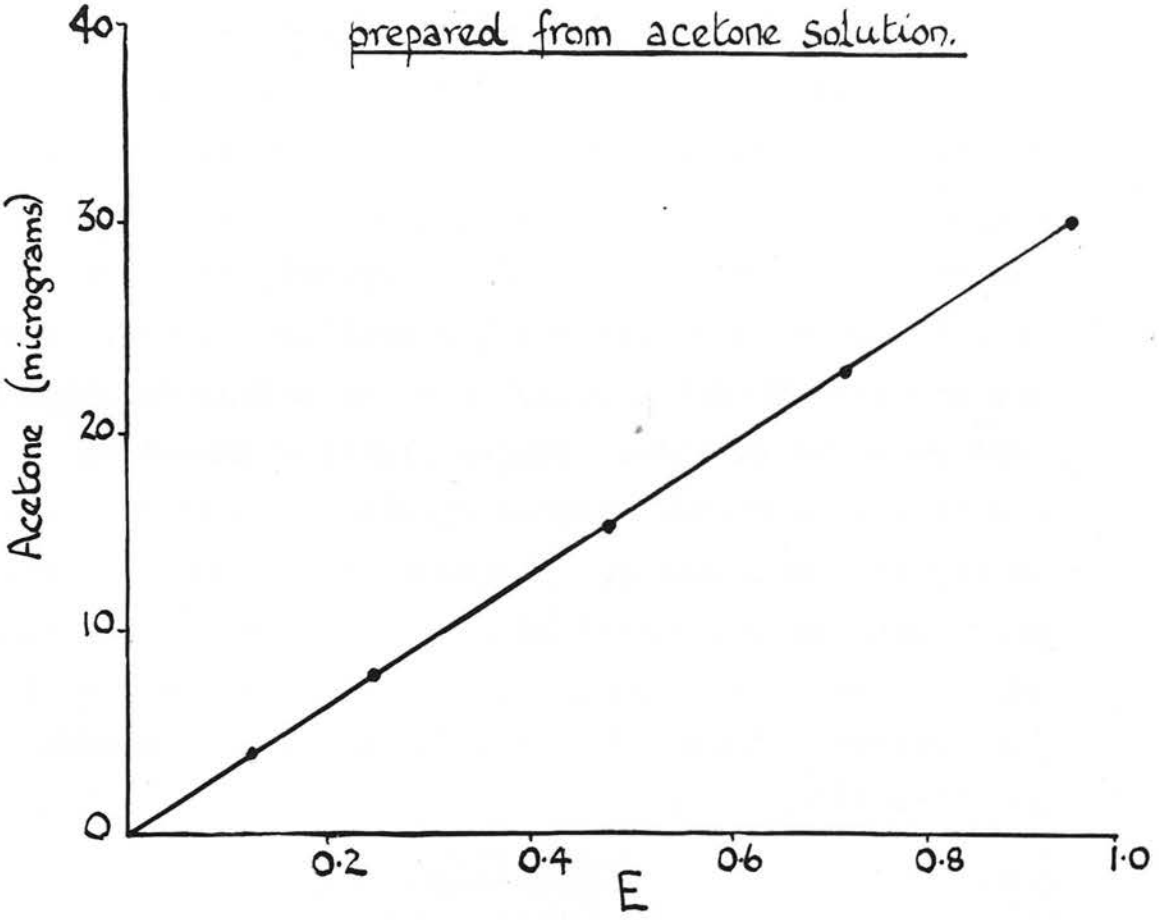
Table VIII

The influence of temperature upon the Acetone Di-salicylaldehyde reaction.

Temperature °C	E 490 mμ 1 cm.
4	0.246
15	0.240
20	0.246
37	0.435

GRAPH G.

Calibration curve of acetone di-salicylaldehyde prepared from acetone solution.



F. Calibration Curve of Acetone Di-salicylaldehyde prepared from Acetone Solution.

Acetone solutions containing 3.8, 7.6, 15.2, 22.8 and 30.4 micrograms per 0.1 ml. of solution were prepared. 0.1 ml. of each solution was added to 9.9 ml. of alkaline salicylaldehyde solution. The stoppered test tubes containing the solution were placed in the dark at room temperature for seventeen hours. Extinction coefficients were determined in a spectrophotometer at a wavelength of 490 millimicrons. Alkaline salicylaldehyde solution was used as a zero blank. Reference to Table IX will show that the calibration curve was linear.

Table IX

Calibration curve of acetone di-salicylaldehyde prepared from acetone solutions.

Acetone µg per 0.1 ml.	E 490 mu 1 cm.
3.8	0.128
7.6	0.246
15.2	0.480
22.8	0.718
30.4	0.950

G. Calibration Curve of Acetone Di-salicylaldehyde prepared from Citric Acid Solutions.

Citric acid solutions containing 10 to 100 micrograms per 0.1 ml. were prepared. 0.1 ml. of each solution was transferred to the appropriate member of a series of micro-distillation buckets. 0.1 ml. of 9 N sulphuric acid and 0.1 ml. of 2% ammonium vanadate were added to each bucket, and after assembling the micro-distillation unit, the contents of the bucket were thoroughly mixed by rotation. The Thunberg limb contained 1 ml. of alkaline salicylaldehyde solution. The assembled unit was put in the temperature gradient bath where the citric acid was oxidised for a period of three hours at a temperature of 100°C. The technique and the precautions to be observed are described later. Units were transferred to a dark cupboard and remained there overnight at room temperature. The acetone di-salicylaldehyde was then quantitatively transferred with freshly prepared alkaline salicylaldehyde solution to 10 ml. volumetric flasks and the volume was adjusted to the mark. Extinction coefficients were determined as before. The results, recorded in Table X, indicate a linear calibration curve. It will be observed that the method is sensitive to one microgram of citric acid.

GRAPH H.

Calibration curve of acetone di-salicylaldehyde prepared from citric acid solution.

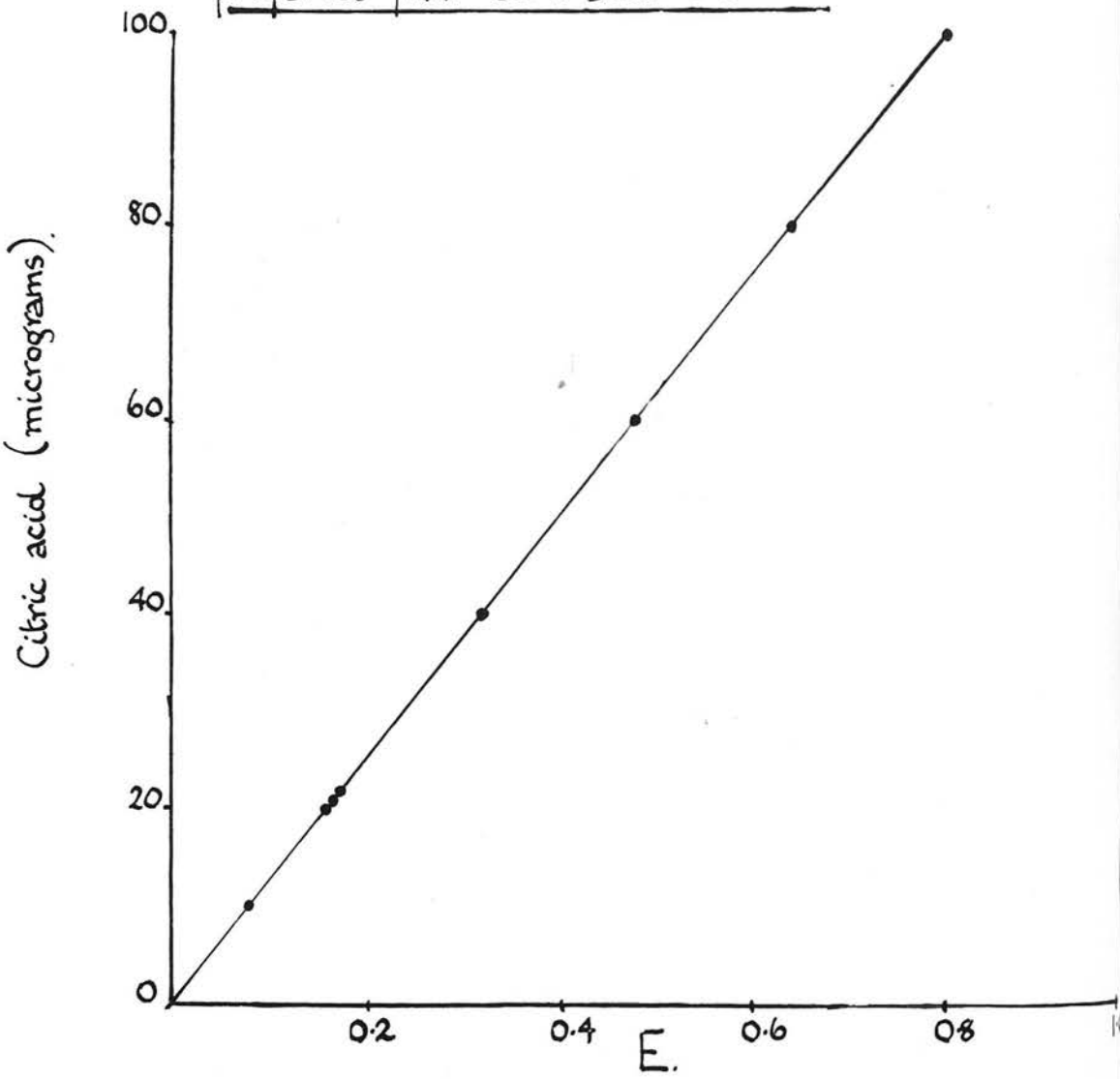


Table X

Calibration curve of acetone di-salicylaldehyde prepared from citric acid solutions.

Citric Acid µg per 0.1 ml.	E 490 mu 1 cm.
10	0.080
20	0.160
21	0.170
22	0.180
40	0.318
60	0.480
80	0.635
100	0.800

H. Removal of Acetone Bodies.

One of the disadvantages of existing methods for the estimation of citric acid in serum is that acetone and related substances such as aceto-acetic acid, β-hydroxybutyric acid interfere with the reaction unless they are removed prior to citric acid oxidation. The efficiency of removal varies with the method.

Citric acid solution containing 40 micrograms per 0.1 ml. was prepared, and 0.1 ml. was transferred to a

series of micro-distillation buckets.

Solutions containing approximately 20 and 40 micrograms of acetone, 2 0 and 40 micrograms of aceto-acetic acid, and 20 and 40 micrograms of β-hydroxybutyric acid per 0.1 ml. were prepared.

0.1 ml. of each solution was mixed with the citric acid solution in each bucket.

2 ml. of 10% trichloracetic acid was added to each bucket and the volume of solution was reduced to approximately 0.5 ml. by evaporation in a hot air oven at 100°C.

Oxidation and distillation of residual citric acid were performed as described in section G.

From the results in Table XI it will appear that acetone bodies are completely removed by preliminary treatment with heat and trichloracetic acid. It is doubtful of the part played by tri-chloracetic acid in affecting the removal of acetone bodies, β-hydroxybutyric acid has a much higher boiling point that acetone or aceto-acetic acid (B.P. 130°C in vacuo) and is not likely to distil over in the micro-diffusion units. The other two are volatile at well below 100°C and heating to 100°C in the absence of tri-chloracetic acid might well be

effective. This of course, is irrelevant as regards the actual estimation in blood, where tri-chloracetic acid is necessary for protein precipitation.

Table XI

Removal of acetone bodies.

Citric Acid µg.	Acetone Bodies µg.	E 490 mu 1 cm.
40	Nil	0.320
40	20 acetone	0.318
40	40 acetone	0.323
40	20 aceto-acetic	0.321
40	40 aceto-acetic	0.324
40	20 β-hydroxy-b	0.322
40	40 β-hydroxy-b	0.325
40	20 of each	0.322

I. The Influence of Acid as an Oxidising Agent.

It has been claimed by Pozzi-Escot (1946.) that vanadate is not an oxidising agent in reference to citric acid. It merely acts as a catalyst and in fact it is the acid which oxidises citric acid.

40 micrograms of citric acid were transferred to

each of two micro-distillation buckets. To one unit was added 0.1 ml. of 9 N sulphuric acid, to the other 0.1 ml. of 9 N sulphuric acid and 0.1 ml. of 2% ammonium vanadate solution. The citric acid was oxidised and the acetone distilled by the technique described in section G.

The results are recorded in Table XII and show that presence of vanadate was necessary before oxidation of citric acid occurred.

Table XII

The influence of acid as an oxidising agent.

	E 490 mu 1 cm.
Citric acid without vanadate	0.002
Citric acid with vanadate	0.320

DISCUSSION.

From the evidence presented it appears:-

a) that acetone bodies, even in concentrations far beyond those of the physiological range of serum, are efficiently removed in the pre-oxidation stage.

b) the presence of acid does not by itself cause oxidation of citric acid.

c) the method obeys the Beer-Lambert laws within the range of five to a hundred micrograms of citric acid.

d) the method is sensitive to a variation in concentration of one microgram of citric acid.

e) non-volatile interfering substances do not come into contact with the alkaline salicylaldehyde reagent.

CONCLUSION.

A modified method for the estimation of citric acid in serum.

Principle:

Proteins are precipitated by trichloracetic acid and centrifugation. An aliquot of the supernatant fluid is heated to effect concentration to a smaller volume and to remove acetone bodies. The citric acid in an aliquot of the concentrate is oxidised to acetone by an acid solution of vanadate and the acetone distilled into a cooled alkaline solution of salicylaldehyde. After colour development the extinction coefficient of the acetone di-salicylaldehyde thus formed is measured at 490 mu. Serum citric acid is determined from a calibration curve prepared at the same time and under the same conditions as the unknown.

Reagents.

1. Trichloracetic acid :- 10% W/V
2. Sulphuric acid :- approx. 9 N. Concentrated acid diluted one in four.
3. Ammonium vanadate :- 2% W/V.
4. Alkaline salicylaldehyde solution :- 3 ml. of re-distilled salicylaldehyde are mixed with 97 ml. of 3.0 N potassium hydroxide. Freshly prepared before use.
5. Stock citric acid solution :- 200 micrograms per millilitre.

Apparatus.

Micro-distillation units and temperature gradient bath. (Figures III and IV)

Method

1. Precipitation of proteins and removal of acetone bodies. 1 ml. of serum from the pre-transfusion specimen is added, slowly and with shaking, to 2.0 ml. of 10 per cent trichloracetic acid.

After centrifugation 2.0 ml. of supernatant fluid are transferred to a graduated centrifuge tube which is then placed in a hot air oven at 100°C. The tubes are left in the oven until the volume of fluid is

is reduced to 0.5 ml. If the volume should fall below this point it is re-adjusted at room temperature by the addition of distilled water.

2. Determination of citric acid concentration.

Approximately 1 ml. of freshly prepared alkaline salicylaldehyde solution is placed in each of the required number of Thunberg limbs by means of a Pasteur pipette with a curved end (See Figure IV).

It is essential to ensure that none of the alkaline reagent is allowed to run down into the micro-distillation bucket. This danger is avoided by introducing the tip of the pipette as far into the Thunberg limb as possible and then suspending the limb in such a say that the fluid drains into the bulb of the limb.

The required number of micro-distillation buckets is set out and into the first of them is introduced 0.25 ml. of concentrate from the pre-transfusion specimen.

The second and subsequent buckets are used for the concentrates from the specimens taken while transfusion was in progress, but in the case of these specimens a volume of 0.1 ml. of concentrate is used.

At the same time a further series of buckets for the calibration curve is prepared from citric acid solutions covering a range of concentrations from 0 to 100 micrograms per 0.1 ml. in steps of ten micrograms. A volume of 0.1 ml. of each concentration is taken for analysis.

To all buckets is now added 0.1 ml. of 2 per cent ammonium vanadate.

Finally, each bucket receives 0.1 ml. of 9 N sulphuric acid. It is necessary that the acid be added to one bucket at a time and the unit assembled before passing on to the next.

The assembled units are placed on the temperature gradient bath so that the bulbs containing the alkaline salicylaldehyde are immersed in the water flowing through the cooling jacket. In this situation the bulbs are in almost total darkness. Oxidation and distillation are carried out for three hours by maintaining a flow of hot water through the lower jacket.

Occasionally, the pressure developed within the unit during distillation is sufficient to blow it open. This may be prevented by the use of hooks and springs or by very lightly smearing the joint with silicone grease.

As distillation proceeds condensation of water

containing acetone occurs on the inner walls of the unit. This serves to increase the surface area of the acetone solution and thus facilitates the diffusion process.

The units are removed from the bath and placed in a dark cupboard at room temperature, overnight, until diffusion of acetone into the alkaline salicylaldehyde and colour development of acetone di-salicylaldehyde are complete.

The individual units are dis-assembled and the acetone di-salicylaldehyde solution is washed into a ten millilitre volumetric flask with three two millilitre washings of freshly prepared alkaline salicylaldehyde solution. The volume is then adjusted to ten millilitres with alkaline salicylaldehyde solution.

The extinction coefficient is determined at 490 mu, a reagent blank serving as zero control.

THE DETERMINATION OF CALCIUM ION CONCENTRATION.

INTRODUCTION

The concentration of calcium ion in serum has been measured by means of electrometric (Josephs 1928, Leblanc & Harnapp 1930), ultra centrifuge (Chanutin, Ludewig & Masket 1942), solubility product (Weir & Hastings, 1936, Brinkman & Van Dam, 1920, Nordbö, 1939), and biological methods (McLean & Hastings 1934), but none of these procedures is entirely satisfactory. There has been a certain amount of confusion about what is meant by calcium ion and the term has been used as if it were synonymous with diffusible, dissociated or physiologically active calcium. In this thesis, calcium ion will be defined as that fraction of calcium in serum to which the isolated frog heart is sensitive in terms of contractility.

ELECTROMETRIC MEASUREMENTS. Amalgam electrodes have the disadvantage that they can only be used satisfactorily in the presence of single cations. If more that one cation is present in the "substrate", electrode potential is lowered. Protein, too, influences the apparent ionic concentration owing to build up on the electrode surface. Josephs (1938) overcame this difficulty by interspercing

a dialysing membrane between the electrodes and the protein: cation solution so that only non-colloids traversed the membrane. In the investigation of the kinetic interactions between separate cations and protein the method has proved invaluable in determining the binding capacity of protein solutions, but again because of a multiple cation system and changes in carbon dioxide content, the method is not suitable for the estimation of calcium ion in serum. Electrodes of the third order (lead amalgam: lead oxalate: calcium oxalate) have been used by Leblanc et al (1930), but Josephs (1938) found them to be unsatisfactory because of artificial lowering of the activity coefficient. The author found this to be so in the present investigation. Eventually it may be possible to prepare a pure calcium electrode but at present it is impractical because of the high reactivity of the element.

THE ULTRACENTRIFUGE has been used as a means of determining calcium ion in serum. It, in fact, is a measure of calcium not bound to protein. The principle depends upon removal of protein by ultracentrifugation and the determination of calcium in the supernatant (Chanutin et al, 1942). The method would not have been suitable

for the present investigation as it measures total diffusible calcium which of course, includes calcium bound to citrate as well as active calcium ion.

MEASUREMENTS OF SOLUBILITY PRODUCT as a means of determining calcium ion is sound in principle. It depends upon the fact that at a given temperature, when a solid salt is in equilibrium with its saturated solution, the activity of the ions of that salt in solution is fixed, and is not influenced by the addition of other ions. The measurement of the solubility products of calcium carbonate (Weir et al, 1936), calcium oxalate (Brinkman et al 1920, Stewart & Percival, 1927) and calcium picrolonate (Nordbö 1939) have been used as a means of assessing calcium ion concentration in serum.

A BIOLOGICAL METHOD FOR THE DETERMINATION OF CALCIUM ION CONCENTRATION was first used quantitatively by Stewart and Percival (1927). The principle depends upon the sensitivity of the frog heart to calcium ions in solution and it is a measure of that fraction of calcium in serum which is physiologically active as regards frog heart muscle. In the belief that citrate ion during exchange transfusion may depress myocardial activity it was felt that a physiological method would best indicate whether

inhibitary factors existed in the serum obtained from infants undergoing exchange transfusion.

It is known that if excess of calcium ion is present in the perfusing fluid, the frog heart contracts well, but relaxes progressively less; the beats become steadily smaller in extent, and finally the heart stops in a condition of extreme contraction. If, on the other hand, a deficiency of calcium ion exists, the heart will stop in diastole.

EXPERIMENTAL.

THE TECHNIQUE OF ISOLATING THE FROG HEART.

Healthy medium sized toads of the Bufo species were pithed and the heart exposed. A straight glass cannula five centimetres long, internal diameter six millimetres, capacity one millilitre and with a tip external diameter less than two millimetres, was inserted into the ventricle via the left branch of the aorta. Some difficulty was occasionally experienced in passing through the aortic valve, but in such cases the cannula was gently manoeuvred without damaging the heart. When in position a ligature was tied around the aorta to hold the camula firmly in place.

The heart was raised from the body and a second ligature was tied around the tissues to which the heart was attached. It is important to avoid damage to the auricles. The heart was separated from the body by excising the tissue distal to the ligature.

A fine steel hook was passed through the apex of the heart and this was attached to a recording lever. Care was taken to ensure that the hook did not puncture the ventricle. The preparation was suspended in a moist chamber.

By careful counterbalancing and eliminating as much friction as possible, it was found that a magnified contraction of about eight centimetres amplitude could be obtained with a good preparation. Some were only capable of five to six centimetres and a fair percentage were discarded as unsatisfactory. In many cases it was a question of patience. One essential point for a successful preparation was frequent changing of the bathing fluid during the entire operation. This was done by means of a Pasteur pipette inserted into the cannula.

PREPARATION OF SOLUTIONS OF REFERENCE.

On the assumption that the isolated frog heart is sensitive only to calcium in the ionised form McLean & Hastings (1934) directly compared biological fluids with artificial solutions of reference and measured calcium ion concentration in terms of myocardial contractility.

The solutions of reference for the present experiments were prepared by suitable dilution from stock solutions in such a way that they were isotonic with human serum at a pH value of 7.35 at 38°C. These solutions contained known amounts of all the serum electrolytes. It was the procedure to analyse the serum obtained during exchange transfusion for the concentration of potassium, sodium and glucose and to prepare the reference solution accordingly. An example is given below.

The following stock solutions were prepared :-

Sodium Chloride,	NaCl	Analar	200	Millimols	per litre.
Potassium Chloride,	KCl	"	154	"	"
Sodium Bicarbonate,	$NaHCO_3$	"	154	"	"
Magnesium Chloride,	$MgCl_2$	"	103	"	"
Calcium Chloride,	$CaCl_2$	"	100	"	"

Working solutions containing all the electrolytes except calcium were prepared from the stock solutions.

To quote an example a litre of working solution was made up to a definite electrolyte concentration as follows:-

Salt	Desired concentration in MM/litre	Millilitres of stock solution required per litre
NaCl	135	675
KCl	4.5	29.2
$MgCl_2$	1.0	9.7
$NaHCO_3$	27.4	178

When saturated with 5% CO_2 in oxygen this solution had a pH value of 7.35.

In order to obtain the final solutions of reference containing calcium ion, the working solution was transferred to a series of volumetric flasks and the appropriate volume of stock calcium chloride solution was added to each flask in order to contain calcium ion within the range 0.2 to 1.5 MM/litre at intervals of 0.1 MM per litre.

PROCEDURE FOR THE DETERMINATION OF CALCIUM ION CONCENTRATION IN SERUM.

After isolating the frog heart by the technique described previously, contractions were stabilised with

a solution of reference containing 1.2 MM of calcium per litre.

Once stabilised, the sensitivity of the preparation was determined by exposing the heart to a full range of solutions of reference. The technique used to change the solutions in the cannula was to empty the cannula with a Pasteur pipette and wash the heart out with two volumes of the fresh solution of reference. Solutions were removed from the cannula while the heart was in systole.

When the sensitivity of the heart was established the test serum was placed in the cannula and the amplitude of contraction was compared with those obtained from the reference solutions. When identical contractions were produced, the next higher concentration should cause an increase, the next lower concentration a decrease, in the magnitude of contraction. All results were checked in duplicate.

Factors in serum most likely to influence the heart preparation were hypocalcaemia, hyperkalaemia, and the presence of toxic or pressor substances. The first two were measurable. Human serum appeared to be non-toxic,

although invariably, when donor blood was added to the cannula, the heart stopped contracting immediately. This may have been due to the high concentration of citrate ion. The effect of pressor substances, assuming they were present in the sera, was negligible.

A typical calibration curve is tabulated below:-

Concentration of Calcium Ion in Solutions of Reference. MM/litre.	Amplitude of Contraction of Frog Heart in cm. when exposed to reference solutions.
1.30	8.6
1.20	8.4
1.10	8.0
1.00	7.6
0.90	7.0
0.80	6.4
0.70	5.7
0.60	4.0
0.50	3.1
0.40	2.1
0.30	1.0
0.20	0.5

THE INFLUENCE OF ELECTROLYTES OTHER THAN CALCIUM ION UPON THE FROG HEART.

It was necessary to determine to what degree the presence of certain substances in serum influence the sensitivity of the frog heart to calcium ion. These included the cations potassium, magnesium, hydrogen and sodium, and the anions citrate and phosphate.

McLean & Hastings (1934) pointed out the importance of adjusting glucose and sodium in concentration in the solutions of reference to approximately those in human serum. If the ionic strength of the reference solution is reduced by substituting glucose for sodium, increased contraction of the heart occurs. The procedure in this investigation was to analyse sera for potassium, sodium, glucose and to prepare the solutions of reference according to the figures obtained. However in determining the influence of cations upon the frog heart, the concentration of glucose was adjusted to the mean glucose observed during the exchange transfusion, viz. 200 mg. per 100 ml.

Variation of Hydrogen Ion Concentration.

Solutions of reference with a hydrogen ion concentration varying from 7.0 to 7.8 were prepared and

the effect of these solutions upon the sensitivity of the frog heart was determined. The results, tabulated in Table XIII, indicate that the amplitude of contraction of the frog heart is not affected by changes in hydrogen ion concentration between pH 7.3 and 7.8

TABLE XIII

The influence of variation in pH upon frog heart contractility.

Hydrogen ion concentration of reference solution pH	Amplitude of frog heart contraction in cm.
7.0	7.0
7.1	7.2
7.2	7.3
7.3	7.9
7.4	8.0
7.5	8.0
7.6	8.0
7.7	8.0
7.8	8.0

Variation of Magnesium Ion Concentration.

A solution containing 135 MM per litre of sodium, 4.5 MM per litre of potassium, 27.4 MM per litre of bicarbonate, 1.2 MM per litre of calcium and 2 gm. per litre of glucose was saturated with a gaseous mixture of 5% CO_2 in oxygen. Magnesium chloride was then added to this solution in order to obtain a range of reference solutions containing 0.0 to 2.0 MM per litre of magnesium. A frog heart was exposed to these solutions and the results, tabulated in Table XIV, indicate that the sensitivity of the heart is not influenced by change in magnesium ion concentration.

Table XIV

The influence of magnesium ion upon frog heart contractility.

Concentration of Magnesium ion in reference solutions in MM/litre	Amplitude of contraction of frog heart when exposed to reference solutions (cm.)
0.0	7.8
0.5	7.8
1.0	7.9
1.5	7.9
2.0	7.9

Variation in Phosphate Ion Concentration.

A solution containing 135 MM per litre of sodium, 4.5 MM per litre of potassium, 27.4 MM per litre of bicarbonate, 1.2 MM per litre of calcium, 1.0 MM per litre of magnesium and 2 gm per litre of glucose was saturated with a gaseous mixture of 5% of CO_2 in oxygen. A series of reference solutions were prepared from the above solution containing 0 to 3.0 MM per litre of phosphate ion. Reference to Table XV will indicate that within the range stated, phosphate ion does not affect the sensitivity of the frog heart.

Table XV

The influence of phosphate ion upon frog heart contractility.

Concentration of phosphate ion in reference solutions MM/litre	Amplitude of contraction of frog heart (in cm.) when exposed to reference solutions.
0.0	7.6
0.5	7.6
1.0	7.6
1.5	7.6
2.0	7.6
3.0	7.6

Variation in Potassium Ion Concentration.

A solution containing 135 MM per litre of sodium, 27.4 MM per litre of bicarbonate, 1.2 MM per litre of calcium, 1.0 MM per litre of magnesium and 2.0 gm. per litre of glucose was saturated with 5% CO_2 in oxygen. A series of reference solutions containing 2 to 10 MM per litre of potassium were then prepared from this solution. The results, tabulated in Table XVI, indicate that potassium ion is antagonistic to calcium ion in terms of myocardial contractility within the range used.

Table XVI

The influence of potassium ion upon frog heart contractility

Concentration of potassium ion in reference solutions MM/litre.	Amplitude of contraction of frog heart in cm. when exposed to reference solutions
10.0	6.1
8.0	6.7
6.0	7.5
5.0	8.0
4.0	8.4
3.0	8.7
2.0	8.9

Variation in Sodium Ion Concentration.

A solution containing 27.4 MM per litre of bicarbonate, 1.2 MM per litre of calcium, 4.5 MM per litre of potassium, 1.0 MM per litre of magnesium and 2.0 gm per litre of glucose was saturated with 5% CO_2 in oxygen. A range of reference solutions containing sodium ion within the range 130 to 150 MM per litre were prepared from the stock solution. Table XVII, tabulating the results, indicates that the frog heart is sensitive to changes in sodium ion.

Table XVII

The influence of sodium ion upon frog heart contractility.

Concentration of sodium ion in reference solutions MM/litre	Amplitude of contraction of frog heart in cm. when exposed to reference solutions.
130	7.2
135	7.0
140	6.6
145	6.2
150	6.0

Variation in Citrate Ion Concentration.

A solution containing 135 MM per litre of sodium, 27.4 MM per litre of bicarbonate, 1.2 MM per litre of calcium, 4.5 MM per litre of potassium, 1.0 MM per litre of magnesium and 2.0 gm per litre of glucose was saturated with 5% CO_2 in oxygen. Citrate ion was then added to this solution so that a range of reference solutions containing citrate ion were prepared. Reference to Table XVIII will show that the sensitivity of the frog heart is influenced by the presence of citrate ion in the reference solutions.

Table XVIII

The influence of citrate ion upon frog heart contractibility.

Concentration of citrate ion in the reference solutions MM/litre	Amplitude of contraction of frog heart in cm. when exposed to reference solutions.
0.00	7.2
0.25	6.8
0.50	4.0
1.00	3.0
2.00	2.5
3.00	2.0
4.00	1.0
5.00	1.0

CONCLUSION

It has been confirmed experimentally that the frog heart is a sensitive indicator of the physiological activity of calcium ion, and it is concluded that certain factors present in serum do alter the sensitivity of the heart to calcium ion. It is essential to adjust hydrogen ion concentration, sodium ion concentration and potassium ion concentration in the solutions of reference so that their concentration is identical with those in the biological test fluid. Provided the method is restricted within the range of 0.2 to 1.5 MM per litre of calcium ion, then the method has been shown to be quantitative.

RESULTS.

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE A

Ml. of blood exchanged before spec.	Serum Na mg. per 100 ml.	Serum K mg. per 100 ml.	Total Serum Calcium mg. per 100 ml.	Serum Citrate (as Cit''') mg. per 100 ml.	Serum Calcium Ion mg. per 100 ml.	Serum Protein gm. per 100 ml.
0	315	16.2	8.4	22.0	4.0	5.4
100	306	20.5	8.4	93.0	2.9	5.4
200	-	-	-	-	-	-
300	304	20.8	9.5	129.0	3.0	5.4
400	304	20.4	8.9	130.0	2.7	5.5
500	305	22.7	10.0	102.0	2.5	5.4
600	314	24.0	9.5	126.0	2.3	5.6
700	320	25.7	10.0	140.0	2.0	5.7
Donor 1	382	32.0	9.5	500.0	> 2.0	-
Donor 11	360	31.5	7.4	550.0	> 2.0	-

Table XX

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE B

Ml. of blood exchanged before spec.	Serum Na mg. per 100 ml.	Serum K mg. per 100 ml.	Total Serum Calcium mg. per 100 ml.	Serum Citrate (as Cit''') mg. per 100 ml.	Serum Calcium Ion mg. per 100 ml.	Serum Protein gm. per 100 ml.
0	310	17.0	9.7	2.0	5.2	5.2
100	340	34.0	10.0	58.	3.9	5.0
200	335	25.0	10.2	90	4.0	5.0
300	333	20.0	11.2	85	3.8	5.1
400	336	26.5	12.0	105	3.6	5.1
500	345	30.0	12.6	120	4.0	5.0
600	344	30.0	12.9	130	4.2	5.2
Donor 1	420	32.0	-	640	> 2.0	6.0
Donor 11	450	33.5	-	665	> 2.0	5.8

Table XXI

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE C

Ml. of blood exchanged before spec.	Serum Na mg. per 100 ml.	Serum K mg. per 100 ml.	Total Serum Calcium mg. per 100 ml.	Serum Citrate (as Citrate) mg. per 100 ml.	Serum Calcium Ion mg. per 100 ml.	Serum Protein gm. per 100 ml.
0	345	13.3	9.8	4.2	5.2	5.2
100	350	13.6	10.1	50	4.8	5.1
200	360	19.0	10.4	75	3.0	5.3
300	375	14.3	10.3	82	2.6	5.6
400	350	14.0	11.2	90	2.4	5.6
500	340	14.0	12.0	98	2.0	5.8
600	370	12.7	12.1	110	1.8	5.8
700	397	17.7	12.3	115	2.0	5.9
Donor 1	-	22.6	-	470	2.0	-
Donor 11	-	19.8	-	452	2.0	-

Table XXII

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE D

Ml. of blood exchanged before spec.	Serum Na mg. per 100 ml.	Serum K mg. per 100 ml.	Total Serum Calcium mg. per 100 ml.	Serum Citrate (as Citrate) mg. per 100 ml.	Serum Calcium Ion mg. per 100 ml.	Serum Protein gm. per 100 ml.
0	355	20.5	13.8	21	4.8	5.2
100	356	20.5	13.0	130	1.6	5.2
200	355	21.4	14.4	95	1.6	5.1
300	360	23.2	14.0	123	2.4	5.1
400	352	38.0	15.0	138	2.0	5.1
500	365	35.4	14.0	145	1.2	5.2
600	365	26.0	10.8	111	1.6	5.2
700	369	24.4	16.8	90	1.6	5.2
Donor 1	-	16.2	12.6	640	> 2.0	-
Donor 11	-	28.1	9.4	527	> 2.0	-

Table XXIII

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE E

Ml. of blood exchange d before spec.	Serum Na mg. per 100 ml.	Serum K mg. per 100 ml.	Total Serum Calcium mg. per 100 ml.	Serum Citrate (as Cit'''') mg. per 100 ml.	Serum Calcium ion mg. per 100 ml.	Serum Protein gm. per 100 ml.
0	328	16.1	8.4	8.1	4.6	6.4
100	315	16.1	9.6	64	1.8	6.3
200	336	13.8	9.4	72	2.3	6.3
300	315	17.4	11.8	60	2.0	6.3
400	313	17.5	11.0	121	3.5	6.3
500	326	15.4	9.2	160	1.3	6.4
Donor 1	334	24.8	7.0	557	> 2.0	-
Donor 11	-	31.7	7.0	526	> 2.0	-

Table XXIV.

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE F.

Ml. of blood exchanged before spec.	Serum Na mg. per 100 ml.	Serum K mg. per 100 ml.	Total Serum Calcium mg. per 100 ml.	Serum Citrate (as Citrate) mg. per 100 ml.	Serum Calcium ion mg. per 100 ml.	Serum Protein gm. per 100 ml.
0	296	23.1	9.0	3.8	3.6	5.6
50	285	20.4	8.0	3.9	4.0	5.7
100	297	18.8	7.0	85	2.4	5.7
150	297	21.9	7.4	129	1.6	5.8
200	320	22.7	12.8	152	1.2	5.6
300	321	20.6	11.2	120	1.6	5.6
402	326	25.1	15.2	92	2.4	5.6
500	325	26.0	13.6	98	2.0	5.6
Donor 1	364	35.6	6.8	-	> 2.0	-
Donor 11	372	35.7	6.8	-	> 2.0	-

Table XXV.

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE G

Ml. of blood exchanged before spec.	Serum Na mg. per 100 ml.	Serum K mg. per 100 ml.	Total Serum Calcium mg. per 100 ml.	Serum Citrate (as Cit'''') mg. per 100 ml.	Serum Calcium Ion mg. per 100 ml.
0	322	37.0	9.5	4.7	4.0
100	342	37.5	9.7	96	2.8
200	342	38.0	10.0	91	2.4
300	332	38.4	10.5	140	1.6
400	330	38.2	10.5	103	2.0
500	334	36.2	11.0	72	1.6
600	332	35.7	11.0	79	1.8
700	332	37.0	11.3	100	2.0
Donor 1	400	50.0	9.0	582	> 2.0
Donor 11	450	55.0	9.5	606	> 2.0

Table XXVI.

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE H.

Ml.of Blood exchanged before spec.	Serum Na mg.per 100 ml.	Serum K mg.per 100 ml.	Total Serum Calcium mg. per 100 ml.	Serum Citrate (as Cit'''') mg.per 100 ml.	Serum Calcium Ion mg.per 100 ml.
0	-	-	-	-	-
100	308	23.0	9.9	75	2.8
200	325	23.1	10.2	94	3.2
300	325	22.0	10.4	120	1.8
400	327	17.8	10.7	115	1.6
500	336	14.4	10.6	92	1.6
Donor 1	470	18.5	-	560	> 2.0
Donor 11	395	24.5	-	593	> 2.0

Table XXVII

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE I

Ml. of Blood exchanged before spec.	Serum Na mg. per 100 ml.	Serum K mg. per 100 ml.	Total Serum Calcium mg. per 100 ml.	Serum Citrate (as Citric) mg. per 100 ml.	Serum Calcium Ion mg. per 100 ml.	Serum Protein gm. per 100 ml.
0	355	12.4	8.6	40	2.8	4.4
100	350	14.0	6.6	102	1.7	4.5
200	350	18.0	7.0	150	1.8	4.5
300	355	20.0	10.4	282	> 2.0	4.9
400	357	28.0	6.8	346	> 2.0	5.1
500	319	36.0	9.4	319	> 2.0	5.1
Donor-1	415	111.0	-	490	> 2.0	-

Table XXVIII

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE J.

Ml.of blood exchanged before spec.	Serum K mg.per 100 ml.	Total Serum Calcium mg. per 100 ml.	Serum Citrate (as Citrate) mg.per 100 ml.	Serum Calcium Ion mg. per 100 ml.	Serum Protein gm. per 100.ml.
0	-	8.3	1.4	4.0	3.9
100	27.7	9.2	62	3.5	4.4
200	31.6	10.9	95	2.8	4.4
300	28.3	12.2	79	2.9	4.2
400	39.5	13.7	114	2.3	4.3
500	38.0	13.6	155	1.7	4.5
600	34.8	13.1	75	2.8	4.7
700	34.8	15.2	6 5	2.9	4.8
Donor 1	38.5	-	925	> 2.0	-
Donor 11	-	-	473	> 2.0	-

Table XXIX

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE K

Ml. of blood exchanged before spec.	Serum Na mg. per 100 ml.	Serum K mg. per 100 ml.	Total Serum Calcium mg% per 100 ml.	Serum Citrate (as Cit!!!) mg. per 100 ml.	Serum Calcium Ion mg per 100 ml.	Serum Protein gm. per 100 ml.
0	400	31.0	10.6	4.0	4.4	5.1
100	375	36.0	23.2	52	2.8	5.2
150	364	25.0	10.6	98	2.0	5.4
200	387	30.0	21.8	110	1.7	5.4
270	380	30.0	21.6	140	1.6	5.5
300	387	50.0	15.4	153	1.4	5.5
Donor 1	512	32.0	-	520	> 2.0	5.8

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE I

Ml.of blood exchanged before spec.	Serum Na mg.% per 100 ml.	Serum K mg.% per 100 ml	Total Serum Calcium mg%	Serum Citrate (as Cit''') mgm%	Serum Calcium Ion mg%
0	-	-	8.4	2.5	4.2
100	340	20.0	10.5	59	3.2
200	315	24.5	9.5	90	2.6
300	310	23.5	10.0	70	2.3
400	313	18.0	13.1	120	2.9
500	315	18.0	12.0	110	2.4
Donor 1	385	20.0	7.4	894	> 2.0
Donor 11	-	-	-	521	> 2.0

Table XXXI

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE M

Ml. of blood exchanged before spec.	Serum Na mg. per 100 ml.	Serum K mg. per 100 ml.	Total Serum Calcium mg. per 100 ml.	Serum Citrate (as Cit''') mg. per 100 ml.	Serum Calcium Ion mg. per 100 ml.	Serum Protein gm. per 100 ml.
0	310	16.5	12.8	8.4	4.8	5.8
100	315	15.7	14.2	27	4.0	5.9
200	323	18.2	17.5	194	2.5	6.1
300	340	17.0	13.5	225	2.0	6.1
400	341	16.0	15.6	240	1.7	6.0
500	348	16.5	17.4	225	1.8	6.2
600	347	16.5	17.2	203	1.8	6.1
700	349	17.1	17.6	255	1.6	6.1
Donor 1	414	16.8	-	533	> 2.0	-
Donor 2	420	17.0	-	540	> 2.0	-
Change over	337	15.7	15.8	278	1.8	6.1

Table XXXII

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE N

Ml. of blood exchanged before spec.	Serum Na mg. per 100 ml.	Serum K mg. per 100 ml.	Total Serum Calcium mg. per 100 ml.	Serum Citrate (as Cit''') mg. per 100 ml.	Serum Calcium Ion mg. per 100 ml.	Serum Protein gm. per 100 ml.
0	352	28.0	6.4	24.5	4.0	4.4
200 Before	338	28.0	8.2	55.	3.6	4.4
200 After gluc	-	-	-	-	-	-
400 Before	338	29.0	7.4	45	3.0	4.5
400 After gluc	335	30.2	10.6	40	3.0	4.4
500 Before	335	33.0	10.0	35	2.8	4.5
500 After gluc	325	33.5	14.0	20.7	2.9	4.5
Donor 1	402	28.5	11.6	625	> 2.0	4.5
Donor 11	400	48.0	7.0	.653	> 2.0	4.1

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE O

Ml. of blood exchanged before spec.	Serum Na mg. per 100 ml.	Serum K mg. per 100 ml.	Total Serum Calcium mg. per 100 ml.	Serum Citrate (as Cit''') mg. per 100 ml.	Serum Calcium Ion mg. per 100 ml.	Serum Protein gm. per 100 ml.
O	335	18.7	8.4	17.4	5.2	5.5
200 Before	335	18.1	10.8	45	3.6	4.7
After gluc	343	20.0	12.4	129	3.2	4.7
400 Before	355	21.7	11.6	183	0.8	4.3
After gluc	343	20.6	15.2	88	3.2	4.3
600 Before	355	22.4	12.6	131	2.8	4.5
After gluc	342	23.6	15.2	118	3.2	4.5
Donor 1	430	28.5	8.2	602	> 2.0	4.1
Donor 11	425	30.5	8.6	650	> 2.0	4.2

Table XXXIV

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE P.

Ml. of blood exchanged before spec.	Serum Na mg. per 100 ml.	Serum K mg. per 100 ml.	Total Serum Calcium mg. per 100 ml.	Serum Citrate (as Cit'''') mg. per 100 ml.	Serum Calcium Ion mg. per 100 ml.	Serum Protein gm. per 100 ml.
0	327	17.7	11.6	23.4	4.8	6.8
200 Before	336	24.3	9.0	32	5.6	6.8
After gluc	338	20.7	9.8	61	4.4	6.9
Donor 1	410	21.4	8.6	575	2.0	4.5

Table XXXV

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE Q

Ml. of Blood exchanged before spec.	Serum Na mg. per 100 ml.	Serum K mg. per 100 ml.	Total Serum Calcium mg. per 100 ml.	Serum Citrate (as Citrate) mg. per 100 ml.	Serum Calcium Ion mg. per 100 ml.	Serum Protein gm. per 100 ml.
0	327	19.9	10.0	5.8	3.6	5.3
200 Before	323	21.8	8.8	93	1.6	4.8
200 After gluc.	341	15.7	9.8	167	0.8	4.9
400 Before	303	17.8	9.0	63	2.8	4.5
400 After gluc.	310	16.2	10.0	29	2.8	4.4
600 Before	318	17.3	9.2	172	1.0	5.0
600 After gluc.	329	19.4	10.8	167	0.8	4.9
Donor 1	342	28.5	16.4	494	2.0	4.6
Donor 11	386	29.6	10.0	543	2.0	4.5

Table XXXVI

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE R

Ml. of blood exchanged before spec.	Serum Na mg. per 100 ml.	Serum K mg. per 100 ml.	Total Serum Calcium mg. per 100 ml.	Serum Citrate (as Citrate) mg. per 100 ml.	Serum Calcium ion mg. per 100 ml.	Serum Protein gm. per 100 ml.
0	320	40.8	8.6	11.7	4.8	5.9
50	347	46.8	11.8	36	4.4	6.0
200 Before	333	53.2	12.0	128	2.4	6.0
After gluc	349	47.8	14.0	112	2.4	5.6
400 Before	351	50.1	9.6	114	2.2	5.8
After gluc	337	46.7	12.4	144	2.2	5.8
Donor I	-	85.4	-	563	> 2.0	-
Donor II	400	52.8	17.0	550	> 2.0	4.6

Table XXXVII

SERUM ELECTROLYTE CHANGES DURING EXCHANGE TRANSFUSION IN ERYTHROBLASTOSIS FOETALIS

CASE S

Ml. of blood exchanged before spec.	Serum Na mg. per 100 ml.	Serum K mg. per 100 ml.	Total Serum Calcium mg. per 100 ml.	Serum Citrate (as Cit''') mg. per 100 ml.	Serum Calcium Ion mg. per 100 ml.	Serum Protein gm. per 100 ml.
0	332	18.0	8.8	22	5.2	5.5
200 Before	337	17.6	11.4	62	3.6	5.6
After gluc	325	15.3	12.8	33	4.1	5.6
400 Before	342	24.8	15.8	65	4.0	5.6
After gluc	332	19.0	19.6	79	4.0	5.7
600 Before	332	21.4	17.2	119	3.4	5.7
After gluc	352	23.1	19.6	157	3.2	5.8
800 Before	330	19.0	20.2	158	3.2	5.8
After gluc.	328	19.1	29.8	197	3.4	5.8
Donor I	420	28.6	15.0	897	> 2.0	4.2
Donor II	410	26.2	10.4	830	> 2.0	4.3

FIG V. Electrolyte changes during seven clinically normal transfusions.

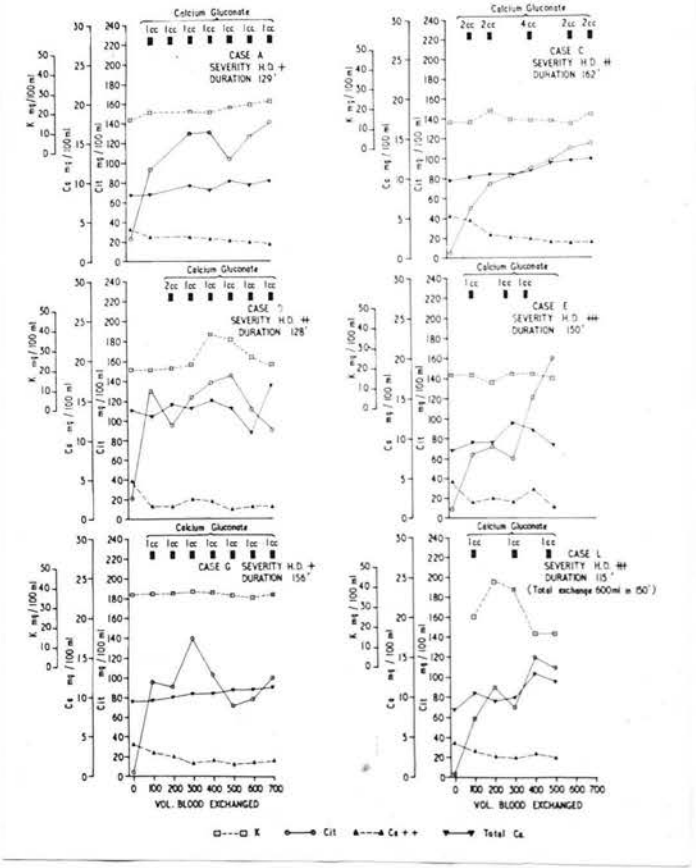
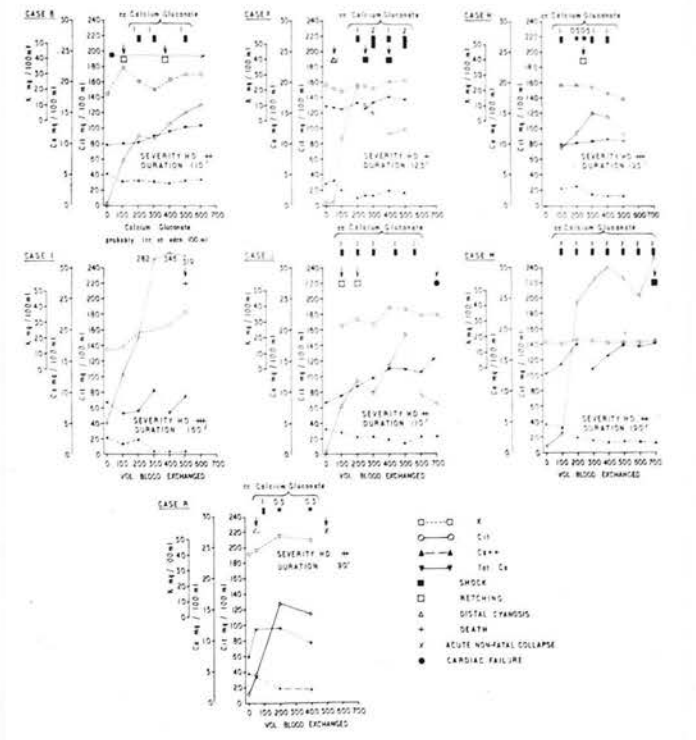


FIG VI. Electrolyte changes during seven clinically abnormal transfusions.



INTERPRETATION OF RESULTS

INTRODUCTORY REMARKS

In a series of nineteen exchange transfusions performed on newborn infants suffering from erythroblastosis foetalis, specimens of blood were collected regularly during the transfusion. Blood was allowed to coagulate and the serum, separated soon after clot retraction, was stored at 0°C until analysis was possible. An aliquot was preserved with sodium fluoride. Specimens were analysed for total calcium, active calcium ion, total protein, citrate, sodium and potassium. The results of the analyses are recorded in Tables XIX to XXXVIII. Of the nineteen cases investigated, analytical data has been charted in figures V and VI. Figure V shows the individual results obtained from seven normal transfusions, while figure VI shows the individual results from seven transfusions at which clinical abnormalities were observed. By reference to the appropriate figures it will be apparent that they depict only the general trend of serum electrolytes during transfusion. It will be appreciated that since specimens were collected at intervals during transfusion, it is only at these points that an assessment of electrolytes can be made. Fluctuation must have occurred in the periods

between collection of specimens, this fluctuation being influenced by movement of electrolytes in and out of the plasma compartment and, of course, by metabolic factors. Nevertheless if this point be borne in mind in the evaluation of the results, it is believed that the general pattern which emerges from this investigation adequately portrays the effect of infusion of citrated blood upon serum electrolytes.

PATTERN OF CLINICAL BEHAVIOUR OBSERVED.

Of the nineteen infants studied one died during transfusion. Six others showed some clinically recognisable disturbance at some stage of the procedure. The remaining twelve infants were normal throughout. Different patterns of clinical behaviour were observed and a description of these is essential to the further understanding of the investigation.

Two babies produced a clinical picture which, probably as a result of modification in technique, was not observed in subsequent transfusions. In both cases (F, R) difficulty was experienced in withdrawing blood during the first hundred millilitres of blood exchanged. Persistent efforts were made to overcome this by partial

withdrawal and re-introduction of the catheter. In both cases the catheter tip was advanced along the umbilical vein for about 11 cm. and repeatedly encountered some resistance which was thought at the time to be within the portal circulation. Both babies developed a deep cyanosis of the lower half of the body and this contrasted dramatically with the pallor of the upper half. Increased venous filling became apparent over the trunk yet no pitting oedema of the legs was noted. A spontaneous disappearance of the symptoms was observed after about half an hour. This phenomenon has been designated "Distal cyanosis".

Three infants (B, H, J,) suffered from repeated retching of mucus. This is a common observation in exchange transfusion and because of this milder degrees of it have not been recorded.

Three infants, (B, F, M) presented a clinical picture to which the term "shock" has been loosely applied. These infants showed some or all of the following features in varying degree. After crying lustily initially, on restraint they became unduly quiet and pale during operation. At least one infant was so pale that the possibility of internal bleeding was considered. The babies were cool and their abdomens became distended yet the latter was not

due to their liver enlargement. They became "hypotonic" and unresponsive. Little change in heart rate was observed and there was no increase in venous pressure. Case B showed this clinical picture within the first hundred millilitres of blood exchanged but survived an exchange of six hundred millilitres.

Signs of congestive heart failure appeared in one case only (J). He suffered from repeated retching during the exchange of the first two hundred millilitres of blood. He remained irritable and after an exchange of seven hundred millilitres, was beginning to show evidence of deterioration. Respiration became a little distressed and dyspnoea was obvious after eight hundred millilitres of blood. At this stage the heart rate had increased from a resting rate of 138 to 164 per minute. The venous pressure in the portal circulation rose from a resting level of 7.5 cm. to 30 cm. The baby was a little cyanosed, the superficial veins became more obvious and the liver enlarged. Unfortunately the blood specimen taken at this point was lost but the values found in the preceding one were consistent with early cardial failure.

One infant (R) collapsed suddenly and unexpectedly but he fortunately recovered. He had been well until 740 m.l. of blood had been exchanged. He then retched and became

apnoeic and deeply cyanosed. The heart sounds were faint but his condition gradually improved over a ten minute period. His has been broadly termed "acute non - fatal collapse". Samples of this infant's blood were being taken only at intervals of two hundred millilitres and the nearest specimen to the incident was taken after 400 ml. of blood had been exchanged.

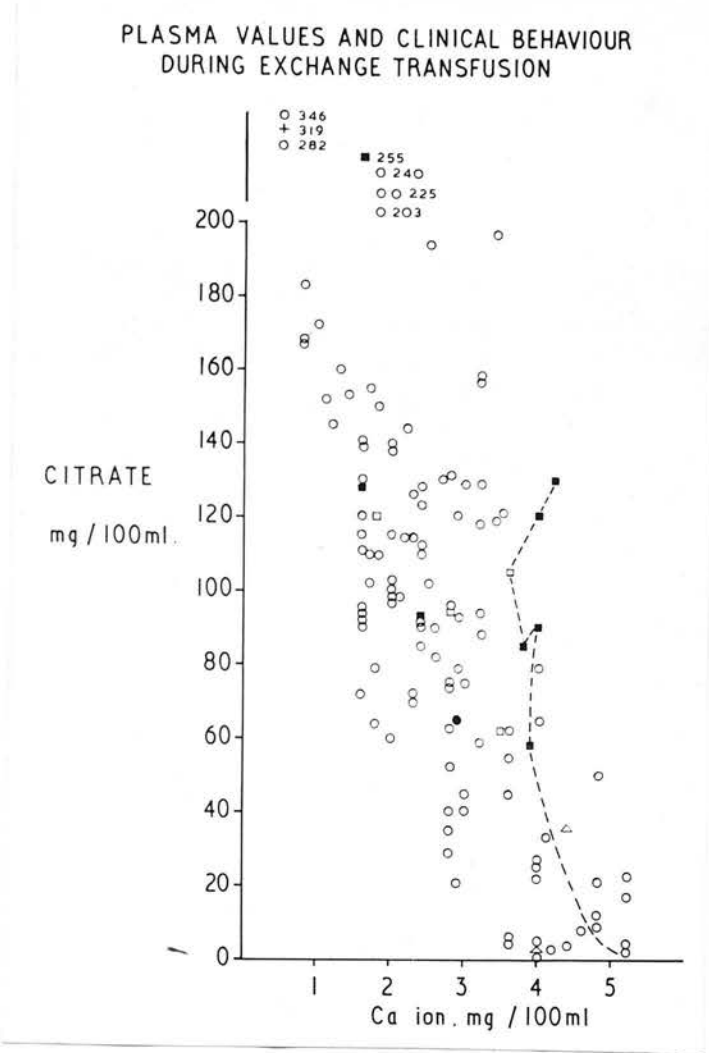
One infant (1) died during transfusion. His liver and spleen were palpably enlarged before transfusion, and during transfusion no abnormality other than pallor was observed until the infant collapsed and died after the exchange of 500 ml. of blood. Autopsy revealed the cause of death as sub-dural haemorrhage with some intra-peritoneal haemorrhage, the latter being due, presumably, to traumatic tear of the umbilical vein.

PATTERN OF SERUM ELECTROLYTES

1. The group as a whole.

From the outset of the investigation attention was focussed on the relationship of four biochemical situations to the clinical behaviour of the baby. These were the influence of high citrate levels, of calcium ion depression of citrate, of high serum potassium levels and the mutually reinforcing influence of a high potassium and a low calcium ion upon the heart. These are depicted in

FIG VII.

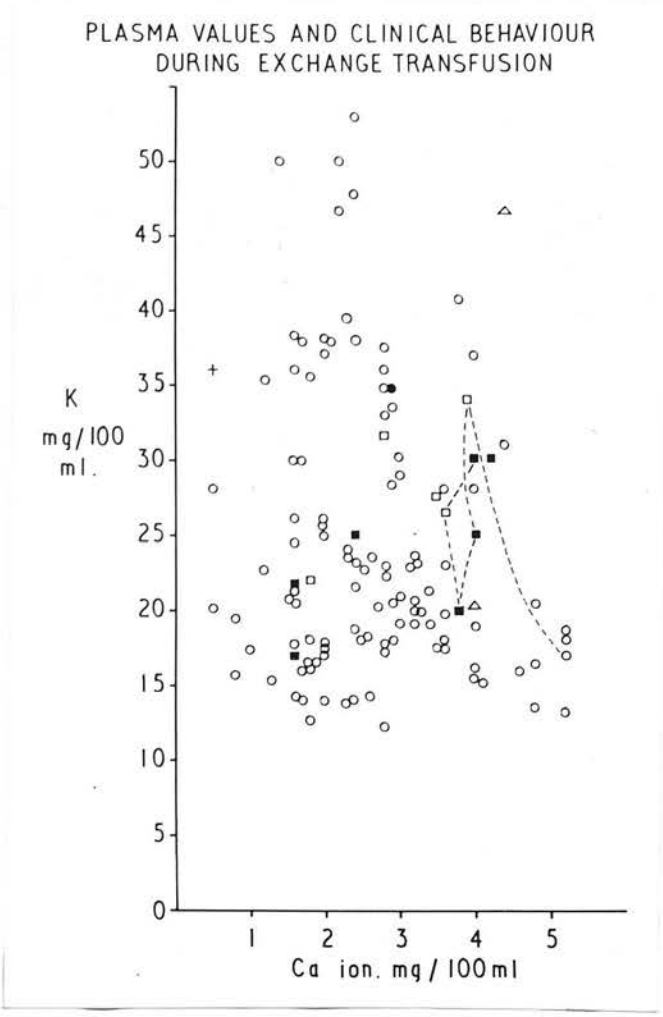


Clinical symbols.

- ■ Shock.
- □ Retching
- △ Distal cyanosis
- \+ Death.
- X Acute non-fatal collapse.
- ● Cardiac failure.

The significance of citrate and calcium ion concentrations.

FIG VIII



Clinical symbols.

- ■ Shock.
- □ Retching
- △ Distal cyanosis
- + Death.
- × Acute non-fatal collapse
- ● Cardiac failure.

The significance of potassium and calcium ion concentrations.

figures V11 and V111. In each graph every relevant analytical result in the series is included and marked by a symbol which denotes the clinical state of the patient at the time when the sample was taken. In figure V11 the co-existing citrate and calcium ion levels are entered against each other. If high citrate levels "per se " were responsible for clinical abnormality then the symbols denoting abnormal behaviour should be across the top of the graph. If clinical abnormality was the result of high citrate levels depressing calcium ion, however, then the abnormal cases should be at the top of the graph and towards the left. The upper part of the graph does not in fact, contain the abnormal cases nor do they lie predominantly to the left of the picture. The one death does lie in the top left hand corner but otherwise there is reasonable dispersal of the abnormal symbols among those which imply normal behaviour when the specimen was taken. In figure V111 the serum potassium levels were plotted against the co-existing levels of calcium ion. If hyperpotassaemia were responsible then the abnormal cases should lie across the top of the picture whereas if depression of calcium ion were responsible then they should lie to the left of the picture. If the offending biochemical upset were

co-existing hyperpotassaemia and hypocalcaemia then the abnormal cases should lie in the top left hand quadrant. Apart from the important exception that the only death did in fact represent the highest serum potassium level and the lowest calcium ion level, the other abnormal symbols are scattered across the picture and fairly evenly disposed among the normal cases. From these two pictures it is possible to state that with the exception of the fatal case there was no relationship in this series of cases between the co-existing biochemical values and the clinical behaviour at the moment of taking the specimen.

It was apparent that a pattern of serum electrolytes during transfusion emerged from this investigation, this pattern being common to all infants whether or not clinical incident was observed. In summary there appeared to be an increase in total calcium and citrate, a decrease in active calcium ion, and either no change or an increase in serum potassium. These changes will be discussed in detail.

2. Serum electrolyte changes in individual cases.

(a) Variation in total serum calcium.

Bruneau and Graham (1945), Wexler et al (1949) and Ames et al (1951) have all observed increases in total serum calcium during exchange transfusion, which they

considered to be a physiological response to the infusion of citrate. Although this was an important point to confirm, it was thought unjustifiable at the commencement of this investigation and in our state of knowledge at that time, to omit the use of calcium gluconate as a precautionary measure against hypocalcaemia.

In the first series of cases studied, however, the concentration of total serum calcium was determined at intervals throughout transfusion immediately prior to the injection of gluconate solution. In this way it was thought that the specimens would reflect that level of calcium in serum which would be least influenced by exogenous calcium. Reference to tables XIX to XXXVIII and figures V and VI shows that, as would be expected, there was a gradual rise in total serum calcium during transfusion. Table XXXIX illustrates the progressive changes after the replacement of each hundred millilitres of blood.

TABLE XXXIX

Changes in total serum calcium during exchange transfusion.

Series of 19 Cases

VOLUME (ml) OF BLOOD EXCHANGED.	MEAN VALUE OF ALL TOTAL CALCIUMS RECORDED AFTER EXCHANGE OF EACH 100 ml. OF BLOOD.	RANGE OF TOTAL CALCIUM OBSERVED AFTER EXCHANGE OF EACH 100 ml. OF BLOOD
0	9.50	6.4 - 13.8
100	10.95	8.4 - 23.2
200	11.05	7.0 - 21.8
300	11.17	7.0 - 15.4
400	11.41	8.9 - 15.8
500	11.87	8.2 - 17.4
600	12.41	9.5 - 17.2
700	14.01	10.0 - 17.6

(All results expressed in mgm. per 100 ml.).

In the second series of cases the immediate effect of calcium gluconate upon total serum calcium was investigated. Blood was collected at intervals of 200 ml. during the exchange, immediately after the injection of calcium gluconate. It was the usual procedure to inject 1ml. of 10% solution of calcium gluconate, equivalent to 9 mgm. of calcium ion, after the exchange of every hundred millilitres of blood. If this amount of calcium was confined to the plasma, it should have been sufficient to increase the total serum calcium by 5 mg. per 100 ml. assuming an infant of average weight with a plasma volume of 175 ml.(Hill, 1954).

However, two factors were operating simultaneously which were bound to modify the plasma calcium. Firstly, the infant's blood was being replaced continuously by donor blood with a plasma calcium concentration of about 7.0 mg. per 100 ml. Secondly, rapid transfer of electrolytes in and out of the plasma compartment must have been taking place. However, if even distribution of injected calcium throughout the extracellular water had been allowed to occur, each 9 mgm. would have been transferred into about 1400 ml. of fluid (Hill, 1954). This would have resulted in an increase in the pre-transfusion plasma calcium level by about 0.7 mgm. per 100 ml. It can be anticipated, therefore, that the extent of the rise in serum calcium after the injection of calcium gluconate will vary, depending upon the state of transfer of the exogenous calcium between interstitial and plasma water at the time of collection of the specimen. Table XXXX shows the changes in total serum calcium in specimens collected after gluconate. It is concluded that the observed increase could be accounted for by the calcium derived from the injected gluconate. Furthermore, on analysing the results obtained in the first series of cases, it is suggested that the gradual rise in serum calcium as transfusions progressed could have been due to the accumulation of exogenous, (i.e. injected) calcium in the extracellular water. There was no evidence that rapid

mobilisation of calcium from skeletal or other body sources occurred during exchange transfusion.

TABLE XXXX

The influence of intravenous calcium gluconate upon total serum calcium.

	Series of six cases	
Volume (ml) of Blood Exchanged	Mean increase of Total Calcium above pre-transfusion level	Maximum increase of Total Calcium above pre-transfusion level
200	1.36	2.00
400	2.88	3.80
600	2.65	4.00
800	9.60 x	9.60 x

All results expressed in mg. per 100 ml.

x One observation.

Variations in calcium ion and citrate

Unlike the total serum calcium level, the concentration of active calcium ion decreased during exchange transfusion, even in the presence of regular injections of calcium gluconate. Table XXXXI records the changes in the series of cases. As a general rule, it appeared that an initial fall in calcium ion occured during the first two hundred millilitres of blood exchanged, and thereafter, no further significant decreased followed, except in the

fatal case. (I).

Table XXXXI

Changes in serum calcium ion concentration during exchange transfusion.

Series of 19 Cases.

Volume (ml) of Blood Exchanged	Mean of all Serum Calcium ion values after each 100 ml. of blood exchanged	Range of Serum Calcium ion observed after each 100 ml. of blood exch--anged
0	4.4	2.8 - 5.2
100	3.0	1.6 - 4.8
200	2.7	1.2 - 5.6
300	2.4	1.4 - 3.8
400	2.5	0.8 - 4.0
500	2.9	1.2 - 4.0
600	2.4	1.0 - 4.2
700	2.0	1.6 - 2.9

(All results expressed in mgm. per 100 ml.).

Conversely, serum citrate levels increased dramatically during the infusion of the first two hundred millilitres of blood, then fluctuated around these high levels. Table XXXXII records the changes observed in serum citrate concentration.

Table XXXXII

Changes in serum citrate during exchange transfusion. Series of nineteen cases.

(All results expressed in mgm. per 100 ml)

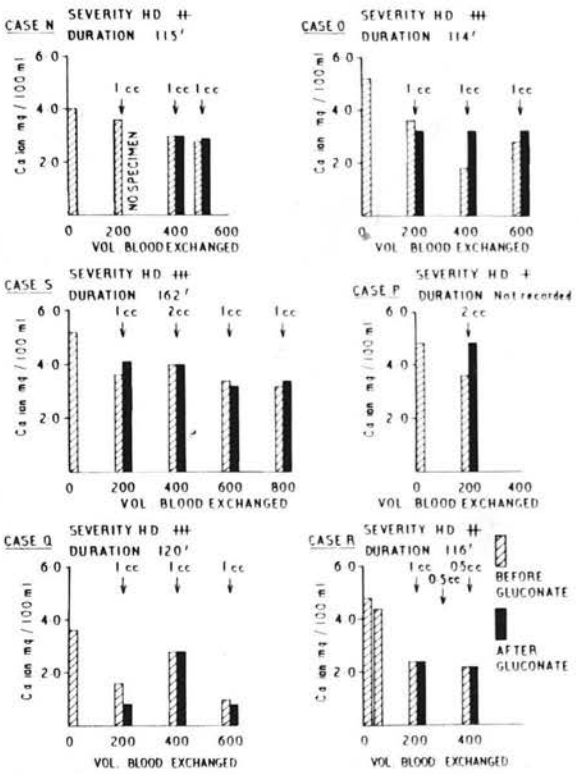
Volume (ml) of blood exchanged	0	100	200	300	400	500	600	700	Maximum
Case A	22	93	-	129	130	102	126	140	140
B	2	58	90	85	105	120	130	-	130
C	4	50	75	82	90	98	110	115	115
D	21	130	95	123	138	145	111	90	145
E	8	64	72	60	121	160	-	-	160
F	4	85	152	120	92	98	-	-	152
G	5	96	91	140	103	72	79	100	140
H	-	75	94	120	115	92	-	-	120
I	40	102	150	282	346	319	-	-	346
J	1	62	95	79	114	155	75	65	155
K	4	52	110	153	-	-	-	-	153
L	3	59	90	70	120	110	-	-	120
M	8	27	194	225	240	225	203	255	255
N	25	-	55	-	45	35	-	-	45
O	17	-	45	-	183	-	131	-	183
P	23	-	32	-	-	-	-	-	32
Q	6	-	93	-	63	-	172	-	172
R	12	-	128	-	114	-	-	-	128
S	22	-	62	-	65	-	119	158	158
Mean value @ each 100 ml.	12.6	73.5	96	130	129	133	126	132	

Table relating rate of transfusion, rate of citrate infusion, and the incidence of clinical abnormality.

Case	Severity of Haemolytic Disease	Birth Wt. lbs.	oz.	kgm.	Vol. Blood Exchanged (ml)	Time taken for Exchange (mins)	Citrate infused mg / kgm /how	Incident
A	+	7	1	3.2	700	129	286-	-
B	++	6	8	2.9	600	107	397	Shock, retching
C	++	82	2	3.3	700	162	202	-
D	++	7	0	3.2	700	128	400	-
E	+++	5	10	2.5	500	150	356	-
F	+	5	12	2.6	500	125	-	Distal cyanosis
G	+	7	7	3.5	700	156	293	-
H	+++	5	0	2.3	500	195	242	Retching
I	+++	6	0	2.7	500	160	218	Death
J	++	7	7	3.3	850	110	542	Retching-heart failure
K	+	7	6	3.2	300	240	80	-
L	+++	6	10	3.0	600	150	385	-
M	++	6	3	2.7	700	190	240	Shock
N	++	5	12	2.6	500	115	404	-
O	+++	8	3	3.8	600	114	302	-
P	+	9	6	-	200	Not recorded	-	-
Q	+++	7	0	3.2	560	120	303	-
R	++	8	0	3.6	470	81	298	(Distal cyanosis (Acute collapse
S	+++	8	9	3.8	800	162	334	-

FIG IX.

EFFECT OF CALCIUM GLUCONATE UPON SERUM CALCIUM ION CONCENTRATION



Several conclusions have been drawn from these results. Great excess of citrate was found in the serum during exchange transfusion, and if, in fact, the formation of calcium citrate were dependent only upon chemical kinetic factors, then all available calcium ion in the plasma of these infants, except for the small amount liberated in the primary dissociation, should have been combined as calcium citrate. That this situaion did not exist was apparent from the clinical state of the infants. The physiological environment then, must be more complex than "in vitro" experiments would suggest, although all the calcium supplied in the form of calcium gluconate appeared to be in active immediately after injection if it can be presumed that calcium gluconate is completely ionised. The results are illustrated in figure IX. The absence of the classical manifestations of hypocalcaemia during transfusion, even though subsequent analysis of serum showed a profound depression of calcium ion concentration is difficult to explain. Since the formation of calcium citrate is known to be a slow reaction, it is suggested that complex formation may have been incomplete during transfusion but on storage of the serum in the absence of active metabolic factors, the reaction between citrate and calcium ions was able

to reach completion.

It is claimed that the rate of transfusion is the most important factor during exchange transfusion in the development of hypercitraemia. Table XXXXlll shows that infusion rate of citrate in this series, and it is apparent that the safety rate recommended by Mollison (1951) was commonly exceeded. There did not appear to be any correlation between the hypercitraemia and the citrate infusion rate and it is considered significant that hypercitraemia of equal severity developed in both the clinically normal and abnormal groups. All infants showed considerable ability to "metabolise" infused citrate, even in the presence of alleged hepatocellular immaturity.

Although the citrate infusion rate may be important, it cannot be doubted that the speed of transfusion must influence the cardio-vascular system. In all of the present transfusions the speed of exchange was leisurely by intention but in some cases it was prolonged by the technical difficulties encountered. A total of 6.36 litres of blood was exchanged in the normal group ln 1386 minutes (excluding cases K and P) - this is an

average rate of 4.6 ml. per minute. In the abnormal group a total of 4.12 litres of blood was exchanged in 968 minutes, an average rate of 4.3 ml. per minute. The cases showing no clinical disturbance were, therefore, exchanged slightly more quickly but the fact that, in the abnormal group, intentional delays followed the occurrence of incidents and so increased the duration of the operation a little, must be kept in mind.

It has been postulated that a high serum citrate level in the resting state is indicative of hepetocellular dysfunction. Hypercitraemia was commonly observed in the pre-transfusion state in this investigation.

TABLE XXXXIII

Changes in serum potassium during exchange transusion. Series of 19 Cases.

The results expressed in mg. per 100 ml.

Vol. of Blood exchange ml.	0	100	200	300	400	500	600	700	Maximum recorded at each transfusion.
Case									
A	16.2	20.5	-	20.8	20.4	22.7	24.0	25.7	25.7
B	17.0	34.0	25.0	20.0	26.5	30.0	30.0	-	34.0
C	13.3	13.6	19.0	14.3	14.0	14.0	12.7	17.7	19.0
D	20.5	20.5	21.4	23.2	38.0	35.4	26.0	24.4	38.0
E	16.1	16.1	13.8	17.4	17.5	15.4	-	-	17.5
F	23.1	18.8	22.7	20.6	25.1	26.0	-	-	26.0
G	37.0	37.5	38.0	38.4	38.2	36.2	35.7	37.0	38.4
H	-	23.0	23.1	22.0	17.8	14.4	-	-	23.1
I	12.4	14.0	18.0	20.0	28.0	36.0	-	-	36.0
J	-	27.7	31.6	28.3	39.5	38.0	34.8	34.8	39.5
K	31.0	36.0	30.0	50.0	-	-	-	-	50.0
L	-	20.0	24.5	23.5	18.0	18.0	-	-	24.5
M	16.5	15.7	18.2	17.0	16.0	16.5	16.5	17.1	17.1
N	28.0	-	28.0	-	29.0	-	33.0	-	33.0
O	18.7	-	18.1	-	21.7	-	22.4	-	22.7
P	17.7	-	24.3	-	-	-	-	-	24.3
Q	19.9	-	21.8	-	17.8	-	17.3	-	21.8
R	40.8	-	53.2	-	50.1	-	-	-	53.2
S	18.0	-	17.6	-	24.8	-	21.4	19.0	24.8
Mean value for each 100 ml. of blood exchanged	21.6	22.9	24.9	24.3	26.0	25.2	26.7	25.0	

Natelson (1948) quoted the normal range of serum citrate as 1.5 to 6.0 mg. per 100 ml., with a mean value of 3.8 mg. per 100 ml, and in this series the range was 1.4 to 40.0 mg per 100 ml., with a mean value of 12.6 mg. per 100 ml. The fatal case had a resting serum citrate level of 40.0 mg. per 100 ml., yet post-mortem examination revealed no gross hepto-cellular damage. Further-more, liver enlargement was not a common feature in any of the other cases but this does not exclude the possibility of a "metabolic" immaturity" existing. Confirmatory evidence of high resting citrate values has been provided by the low level of resting serum calcium ion observed in this series.

Variation in serum potassium

Changes observed in serum potassium during exchange transfusion were unpredictable. Hill(1954) quoted the normal range of serum potassium in the newborn infant as 17.5 to 21.1 mgm. per 100 ml. In this series the normal pre-transfusion range was 13.3 to 40.0 mgm. per 100 ml. Table XXXXiii shows that five infants (F,G,K,N,R,) were found to have

pre-transfusion serum potassium levels above normal and in these instances, either no change or a rise accompanied the transfusion. Of those infants who were found to have a normal pre-transfusion serum potassium level, five cases (B,D,I,J,L,) developed a hyperpotassaemia during transfusion. All these infants were transfused with donor blood containing over 30.0 mgm. per 100 ml. of potassium in the plasma. Except in the fatal case, however, the hyperpotassaemia was not relatable to any particular clinical abnormality since the phenomenon was observed in both clinically normal and abnormal infants. Furthermore two infants classified as clinically abnormal (H,M,) had normal serum potassium levels throughout transfusion. One point is worthy of note. The plasma potassium level of the donor blood used in the fatal case was 110 mg. per 100 ml, and at death co-existing high potassium and low calcium ion concentrations were recorded. Whether the high potassium level influenced the onset of cardiac failure could not be ascertained since electrolyte imbalance was not considered to be the cause of death.

Summary of biochemical changes in abnormal cases

Figure VI illustrates serum electrolyte changes during exchange transfusion in the abnormal cases. Clearly, there is no particular pattern which can be associated with the clinical picture of distal cyanosis (F,R,). Symptons appeared early in the exchange and at a time when serum citrate was beginning to rise and serum calcium ion was beginning to fall. Both infants had a pre-transfusion hyperpotassaemia which persisted throughout transfusion. Retching (B,H,J,) was associated with a hyperpotassaemia, a rising serum citrate level and a serum calcium ion which was by no means unduly low when compared with other apparently normal transfusions. Shock in case B appeared before any marked change in either serum citrate or calcium ion, but a severe hyperpotassaemia was present at the time of clinical evidence of shock. Case F presented only a mild hyperpotassaemia. Signs of shock appeared towards the end of the transfusion in case M. At this time, the serum citrate level had suddenly increased to 250 mg. per 100 ml. but serum potassium was within the normal range. Serum calcium ion level was apparently uninfluenced by the sudden change in citrate, remaining as it had done

throughout the transfusion at about 2 mgm. per 100 ml.

Cardiac failure appeared in case J at a point in the transfusion when the serum electrolytes were beginning to return towards the normal. The citrate level had fallen substantially, total calcium had reached a peak of 15 mgm. per 100 ml, calcium ion had risen by 1.0 mgm. per 100 ml. and serum potassium had fallen by 5 mgm. per 100 ml. to a level of 30 mgm. per 100 ml.

Acute non-fatal collapse in case R occurred after 500 ml. of blood had been exchanged, and the nearest sample of blood to this incident, taken at 400 ml., indicated a profound hyperpotassaemia although the serum citrate level was falling and the serum calcium ion was relatively stable at 2 mgm. per 100 ml. It is significant that the bottle of donor blood had been changed, after 400 ml. of blood from the first bottle had been transfused, and the plasma potassium concentrations in these bottles were 52.8 mg. per 100 ml. and 85.4 mg. per 100 ml.

Finally in the fatal case (I) serum citrate had reached by far the highest level in the series.

Yet death took place when the citrate level was beginning to decrease. Serum calcium ion concentration was not measurable nor had it been for some time before death. At the time of death, serum potassium had reached a peak value of 36 mg. per 100 ml. Thus, there existed a profound hypocalcaemia and hyperpotassaemia.

The only constant feature of the abnormal cases is the hyperpotassaemia which was severe in the cases of cardia failure, acute non-fatal collapse and death. There is some suggestion of potassium intoxication in these cases but unfortunately three apparently normal infants (D,G,I,) exhibited hyperpotassaemia of similar magnitude.

In conclusion it is believed that none of the clinical incidents was due entirely to electrolytic imbalance, since similar biochemical changes were common to all the infants of the series. Serum electrolytes reflect the total electrolyte pattern to some degree, but clinical abnormality need not necessarily be the result of a sudden change in plasma electrolytes. Rather it is the result of prolonged exposure of the intracellular environment to a gradually changing extracellular environment. Considered from this point of view,

a study of serum electrolytes throughout a transfusion is more important than the assessment of electrolytes at any particular instant in a transfusion. Critical evaluation of the results of this investigation has failed to show a correlation between electrolytic inbalance and clinical abnormality during transfusion but there is no doubt that all infants undergoing exchange transfusion are exposed to an abnormal biochemical state over a prolonged period.

GENERAL DISCUSSION

The possible importance os rising levels of plasma citrate in the production of symptons during the infusion of citrated blood was emphasized by Wexler et al (1949) and Ames et al (1951). The level of citrate was found to rise to a figure ten to thirty times greater than the pre-transfusion value. This was accompanied by a decrease in plasma calcium ion concentration. Prolongation of the Q T interval on E C G tracings was recorded by Ames et al (1951). Although the latter could be attributed to the depression of calcium ion concentration by citrate, no constant relationship existed between the plasma citrate concentration and the E C G change. Furthermore the Q T interval could return to normal in the presence of continued high levels of citrate. These studies were made on convalescent infants by Ames et al. Plasma citrate, total calcium, and total protein were determined and plasma calcium ion concentration was assessed indirectly by employing the nomogram of McLean and Hastings. Several of the babies became irritable during the infusion, their skins were noted to be pale and mottled and a normal skin temperature was not restored by the use of blankets alone.

One infant had a severe reaction. He became dyspnoeic then apnoeic, rigid and showed opisthotonus. The period of apnoea lasted for two minutes and during the normal incident the pulse was weak and irregular. Artificial repiration and oxygen were administrated "bloody frothy sputum" welled up from the nose and mouth, spasmodic respiration recommenced in six to seven minutes and breathing returned to normal in ten minutes. The face was visibly oedematous, but two hours after the transfusion the infant appeared to be normal. The plasma citrate of the donor blood was about 450 mg. per 100 ml. and six and a half minutes after transfusion the infant's plasma level was 34 mg. per 100 ml. The latter compares with a pre-transfusion value of 3.4 mg. per 100 ml. He had, therefore, sustained a tenfold increase in the plasma citrate but other babies of the series showed greater increases without clinical incident. Other factors than alleged citrate intoxication may have operated. The infant was a six week old prematurely born male child weighing five pounds. He was convalescent from "atelectasis pneumonitis and mild diarrhoea". He had received 40 ml. of citrated plasma in 14 minutes

which is equivalent to 160 ml. per hour. It is also stated tha the plasma used was obtained from a bottle which had been previously opened so that the risk of contamination is introduced as a factor. A further infant did die during the transfusion. He was given 80 ml. of plasma in 3 hours and was found dead in bed. The citrate concentration of the donor blood was 1600 mg. per 100 ml. and the infant's plasma citrate level immediately after death was 56 mg. per 100 ml. Post-mortem examination revealed only a dilated heart in a poorly nourished infant. The authors concluded that the two reactions were almost certainly instances of acute lowering of calcium ion concentrations in the plasma. They believed that the absence of reactions in the majority of cases might be explained on the basis of their being forces capable of counteracting the calcium ion lowering effect of citrate. However they provide no explanation of why these two less fortunate individuals should have been deficient in this homeostatic mechanism. More recently, Bunker et al(1955) have shown in a series of 130 adults who received moderate

to large volumes of citrated blood that many patients developed hypercitaemia and theoretically calcium ion concentration in the plasma ought to have been seriously depressed. More marked elevation of plasma citrate occurred when transfusion was extremely rapid, prolonged or repeated. Higher levels also occurred where the liver was damaged and this may be important in the newborn infant with or without haemolytic disease.

It has been demonstrated conclusively in the present investigation that a profound increase in serum citrate occurs during the infusion of citrated blood and it is important to consider the facts which influence this phenomenon.

The acid -citrate -dextrose mixture used as an anti -coagulant in this country has been criticised and praised for many years. When first instituted, the dangers of citrate were emphasized but until the technique of replacement transfusion and massive blood transfusion became common, little attention was focussed upon the possibility of citrate intoxication in medical practice. In the infants in this series amounts of

citrate ranging from 738 to 3413 mgm. were infused into a plasma compartment of average volume less than 200 ml, so that accumulation of citrate under these circumstances could be dangerous. It is believed that citrate in stored donor blood is confined essentially to the plasma. Thus is the recommended ratio of volume of anti-congulant to blood is strictly adhered to, then the plasma citrate content of the donor blood should be about 600 mg. per 100 ml. It will be appreciated that any marked deviation from the standard technique may result in significant changes in the plasma citrate. Ames particularly emphasizes this point when describing an alleged case of citrate intoxication, following the infusion of plasma containing 1536 mg. per 100 ml. of citrate. With this in mind, Walker and Neligan (1954) recommend the use of packed cells in exchange transfusion.

Although the plasma citrate level in the donor blood is the first consideration, the rate at which it is infused is obviously important. It is desirable that the rate of transfusion be adjusted so that thehomeostatic mechanisms controlling citrate are able to deal with the citrate as fast as it is being infused. Mollison (1956)

stipulates a maximum rate of 260 mgm. of citrate per kilogram of body weight per hour, but Wexler et al (1949) and Walker et al (1954) freely admit to exceeding this rate without clinical incident. In the presentseries, similar conditions prevailed. Nakasone et al (1954) performed an interesting experiment in which citrate solutions of varying strengths were infused into dogs and the response of cardiac muscle was measured by E C G tracings. They suggested that it was not the actual level of citrate in the perfusate which was the factor precipitating cardiac changes but rather the cumulative dose of citrate in the animal's body. In other words, hypocalcaemia was induced after the infusion of a critical amount of citrate whether this was attained over a short period by the infusion of a solution containing a high concentration of citrate or after a longer period with a solution containing less citrate. For this reason the slower the rate of transfusion the less severe the hypercitraemia which will develop. It is suggested that the clinical incidents in this series were not related to the infusion rate of citrate. Reference to Table XXXXlll will show that the safety rate was often exceeded in cases who appeared

normal throughout transfusion, yet in other abnormal cases the infusion rate was below the recommended safety rate.

It should be borne in mind that reports describing rapid metabolic breakdown of citrate during the infusion of citrated blood are based upon observations of plasma citrate concentration during a period when rapid diffusion out of the plasma compartment is occurring. Transfer of citrate to other compartments of body water would account, at least in part, for the disappearance of exogenous citrate out of the plasma water. The average amount of citrate contained in 100 ml. of citrated donor blood is 370 mgm. Assuming that no transfer occurred out of the plasma compartment into interstitialwater then the amount of this citrate which would be retained after the exchange of 100 ml. of blood by the technique described in clinical methods would be about 320 mgm. In practice, however, a proportion of this would be transferred to interstitial water, and assuming even distribution throughout the extracelluler water, about a seven-fold reduction in the theoritical maximum plasma concentration could be expected. The actual level

in the plasma would depend upon the degree of transfer of the citrate at the time of collection of the specimen, but it should be within the range 46 - 320 mg. per 100 ml. Thus the levels recorded in this series indicate that a considerable portion of infused citrate migrated out of the plasma compartment after each hundred millilitres of blood exchanged.

Increased intracellular metabolism of citrate was likely to occur under the stimulus of the infusion of citrate. Sites of metabolism have been discussed in the introductory section of this thesis. Suffice to record that Wexler observed delayed removal of citrate from plasma in one fatal case and as post-mortem examination revealed gross liver damage, he postulated that impaired liver function might seriously delay citrate metabolism. In the fatal case of the present series, a similar delayed removal of citrate was observed, yet post-mortem examination revealed no severe liver damage. It is quite possible that a mild degree of metabolic immaturity exists in newborn infants, especially those suffering fromerythoblastosis foetalis. This would explain the resting hypercitraemia observed in some cases in this series. It can only be concluded,however,

that all infants appeared to have efficient homeostatic mechanisms for removing infused citrate from plasma.

It is suggested as a hypothesis that all the factors discussed must operate during the infusion of citrated blood. Initially, the prevention of severe hypercitraemia by careful adjustment of the donor plasma citrate level and the rate of transfusion can be controlled by the operator. Presumably, any infused citrate enters the plasma and from there it is transferred to interstitial water and finally the intra cellular water, where it is metabolised. Reference to figures VII and VIII will indicate the general pattern of serum citrate during transfusion. The initial, dramatic rise to a level between 100 and 160 mgm. per 100 ml. may represent a phase in which citrate is essentially confined to the plasma. Once mechanisms controlling transfer out of the plasma compartment become operative it is reasonable to expect a fall in the plasma citrate if the rate of transfer exceeds the rate of citrate infusion in the plasma. This has been confirmed in the present investigation. Fluctuations observed at later stages in the transfusion may reflect the efficiency of intracellular metabolic pathways. Thus a rising citrate in the presence of a

constant rate of citrate infusion suggests some impairment of function (cases I,M.). Conversely a falling serum citrate level suggests efficient metabolism, while a fluctuating citrate level may be indicative of sudden changes in the citrate intake with which the intracellular metabolic processes are unable to cope. All except two infants (I,M.), were able to "metabolise" infused citrate very efficiently. If citrate intoxication was the causative factor in some or all of the clinical incidents in this series, then it is difficult to explain the fact that serum citrate levels of a similar magnitude did not precipitate incidents in the normal infants.

The high serum citrate recorded in this investigation should have induced severe hypocalcaemia. The theoretical implications are that all available calcium ions in the plasma of infants undergoing transfusion should be inactivated, but the results indicate that although significantly reduced, this reduction was not complete except in the fatal case. Two explanations are proposed. Firstly, citrate was present in the plasma in high concentration but there was no evidence to show whether the whole or part of it was present in an active ionic form. In fact, an inactive molecule, possibly

bound to protein may have existed. Such complexes are known to exist in the case of protein and glucose, and the lipopoteins. Under such circumstances, only ionised citrate would be available for reaction with calcium and othercations. Secondly, the formation of calcium citrate is not an instantaneous reaction and this might explain the apparent discrepancy between the absence of the clinical manifestations of tetany, even though subsequent analysis of serum showed a profound depression of calcium ion. During transfusion, calcium citrate formation may have been incomplete, whereas on storage of the serum in the absence of enzyme action, reaction was allowed to reach completion in the presence of excess of citrate ion. Whatever the explanation, analysis revealed that complete inactivation of calcium ion in serum did not occur. There was no evidence to confirm the finding of others that significant mobilisation of calcium ion occurred from bone or other sources in response to an induced hypercitraemia, nor did it appear that the injection of calcium gluconate was effective in elevating calcium ion in serum. On clinical grounds, Walker and Neligen (1953) abandoned the use of gluconate and they did not observe tetany in a series of 272 transfusions.

Herlitz (1943) observed active tetany in infants whose plasma calcium ion level had fallen below 3.5 mgm. per 100 ml., yet even with serum calcium ion levels below 2.0 mgm. per 100 ml., classical tetany was not seen during this investigation. The absence of clinical abnormality is not inconsistent with a reduction in calcium ion as the factors governing neuro-muscular irritability are complex and the maintenance of a normal state of irritability may have been due to compensatory mechanisms. The exact means by which tetany is brought about has not yet been elucidated, but the original work done by Ringer and Loeb discussed in any standard textbook of physiology, might elucidate the problem. They postulated a delicate equilibrium between the cations of the serum, and any disturbance of this equilibrium such as a decrease in calcium, magnesium or hydrigen ions, or an increase in sodium, potassium or hydroxyl ions causes hyper-irritibility of the tissues. It has been shown that an increase in potassium and a decrease in calcium ion occurred in serum, thus favouring the development of neuro-muscular hyperirritibility.

It has been demonstrated that citrated blood is weakly acid in reaction and the infusion of such a medium would favour the production of a metabolic acidosis. This

problem is at present under investigation by Walker (personal communication) but no results have been reported to date. Increase in hydrogen ion concentration would tend to compensate for the co-existing hypocalcaemia and hyperpotassaemia in terms of neuro-muscular irritability. For example Mudge and Vislocky (1949) observed the acidosis of chronic renal disease was not related to specifically to a change in acid-base equilibrium but was associated with a decrease in intracellular potassium and a rise in intracellular sodium. In this investigation perfusion of donor blood into the infants did not significantly alter their serum sodium level in the face of a virtual replacement of the infants' blood and a donor plasma concentration around 400 mg. per 100 ml. Since renal excretion of sodium must have been negligible during transfusion, diffusion or transfer of excess sodium out of the plasma compartment must have occurred. Is it reasonable to postulate that at least some of this sodium entered the intracellular water, possibly reflecting that some movement of potassium into the extracellular water had occurred ?

The leakage of potassium from red blood cells

during storage is a well known phenomenon (De Gowin et al 1940) and the significance of this was emphasized by Mollison (1951). Toxic levels of potassium in blood plasma are associated with cardiac arrest and in fact, injection of potassium citrate solution into the coronary circulation has been used experimentally to produce elective temporary cardiac arrest (Melrose et al., 1955). In the adult the transfusion of one or two pints of blood does not greatly affect the recipient's plasma potassium concentration but the situation is different during exchange transfusion, in a newborn infant where one pint of blood is equivalent to about twice the infant's blood volume. Miller et al (1954) studied the influence of transfusion upon serum potassium concentration in eight infants. One infant with a pre-transfusion serum potassium level of 31.8 mgm. per 100 ml. was transfused with donor blood containing 72.5 mgm. per 100 ml. potassium in the plasma. He developed clinical signs of hyperpotassaemia which were confirmed by electrocardiographic changes and at this point his serum potassium level was 35.7 mgm. per 100 ml. However as no classical signs of hyperpotassaemia developed in any of the other babies studied, they could not be certain that the high serum potassium concentration induced in two of the patients and present in a third

were necessarily harmful to these infants. They state that they observed two deaths during exchange transfusion but in neither was potassium intoxication considered as a likely cause. As in the case of Miller, sudden unexplained death in two infants prompted the investigation carried out by Campbell (1955). He found that donor plasma potassium levels of 40.0 mgm. per 100 ml. were not uncommon even in blood stored for less than seven days. After a careful study he concluded that "hyperpotassaemia is not infrequent in the course of an exchange transfusion for haemolytic disease of the newborn, and that the levels reached are potentially lethal". No proof could be provided, however, to show that the deaths in his series of cases were the result of hyperpotassaemia. Attempts on his part to reduce the severity of the hyperpotassaemia with insulin were fairly successful.

Serum potassium levels recorded in this investigation indicated that 60% of infants developed a hyperpotassaemia, either before or during transfusion, but these changes were common, even in the asymptomatic infants. Rise in plasma potassium level during

exchange transfusion is influenced by the intake of potassium in the donor plasma, the efficiency of the renal mechanism to excrete excess potassium and the efficiency of intracelluler "metabolic" sites in removing potassium from the plasma. It was apparent in this investigation that the development of hyperpotassaemia was closely related to the infusion of donor blood. The incidence of hyperpotassaemia was high when the donor plasma potassium level exceeded 30.0 mgm. per 100 ml. As in the case of citrate though, the high serum potassium levels were not relatable, surprisingly, to the rate of transfusion of donor blood. Thus the development of hyperpotassaemia may be the result of accumulation of potassium, rather than the potassium concentration of the perfusate.

In the adult, renal excretion of potassium is a most important homeostatic mechanism preventing potassium intoxication but since renal immaturity is an accepted phenomenon in the newborn, their ability to excrete excess potassium is doubtful. In fact, the normal urine output in the first twentyfour hours is about 20 ml. so that the maintenance of a normal serum level during transfusion must depend upon mechanisms other than renal. One of these must be the transfer of potassium from the plasma

to other compartments of body water. Simple distribution within the extracelluler water will result in a sevenfold decrease in the concentration of any exogenous potassium which is confined initially to the plasma. The serum potassium concentration "per se" does not necessarily reflect the state of cellular potassium, and it is not justifiable to automatically associate a high serum potassium level with a toxic accumulation of potassium in body water. In fact Taraal and Elkington (1949) have shown that a cellular deficiency of potassium may exist in the presence of high serum potassium levels. Although this would explain the absence of potassium intoxication in the cases in this investigation with high serum potassium levels, it is difficult to imagine in infants suffering from erythroblastosis foetalis. These infants are exposed to a severe haemolytic process which commences "in utero" and it is possible that in the absence of renal homeostatic mechanism, these infants are in a state of positive potassium balance. This fact tends to be confirmed by the high incidence of resting hyperpotassaemia.

Experiments (Josephs et al, 1939, Fenn, 1939, Houssay et al 1939, Eichelberger, 1941) have shown that

the intravenous injection of soluble potassium salts results in the accumulation of potassium within the liver cells and this process is enhanced by the simultaneous infusion of glucose solution. Presumably, potassium enters the intracelluler space of the hepatic cells during hepatic glycogenesis. This is suggested as a working hypothesis in the present investigation. The liver is only a temporary site but it serves as a means of preventing potassium intoxication in the absence of renal regulating mechanisms. The liberation of potassium during glycogenolysis inevitably occurs but this process occurs at a rate which allows adequate renal excretion of potassium by the rapidly developing renal tissue.

The biochemical abnormality observed in this investigation was the co-existing hyperpotassaemia and hypocalaemia. The danger of simultaneously rising potassium and falling calcium ion levels in the blood was described by Guest (1952). Protection of dogs against normally lethal concentrations of potassium may be provided by the intravenous injection of calcium. (Winkler et al, 1939). Modification of the effects of potassium intoxication in children by the administration of calcium salts was claimed by Govan and

Weiseth (1946). Latent tetany in a group of adults with co-existing hypopotassaemia and hypocalcaemia was rendered manifest by the slow intravenous infusion of potassium salts even though the final plasma potassium level did not exceed the usual normal range. (Engel et al 1949). None of these facts were confirmed in this investigation. There is some suggestion that the hyperpotassaemia may have precipitated heart signs in the cases of acute non-fatal collapse, cardiac failure and death but completely asymptomatic infants presented a hyperpotassaemia of similar magnitude. The evidence is strong is favour of profound electrolytic imbalance in the fatal case, although the actual cause of death was found to be haemorrhage.

It appears that there is no association between the clinical disturbance observed during exchange transfusion and the changes which undoubtedly occur in plasma electrolytes. The various factors which might be responsible for clinical deterioration are outside the scope of this thesis but these have been discussed in a paper by Farquhar and Smith (in press). Briefly they suggest that operation is being carried out upon an immature subject whose cardiac vitality may be impaired and whose

cardiovascular system is undergoing major physiological readjustment. There is, therefore, every reason for conducting exchange transfusions slowly and gently. Cooling of the infant must be prevented and there is some evidence that the injection of cold blood may give rise to a state of shock and possibly cardiac disturbance. Furthermore, excessive manipulation of the umbilical vein during transfusion and interference within the portal circulation may provoke retching, shock and possibly some reflex spasm of the great veins. These factors are considered to be more important in precipitating the clinical incidents observed in this investigation than the electrolytic imbalance which was induced by the transfusion of blood.

C O N C L U S I O N

The evidence presented in this investigation suggests that two factors are involved in the electrolytic imbalance which occurs during exchange transfusion. Firstly, hypocalcaemia following the infusion of high concentrations of citrate in the donor blood, and secondly, hyperpotassaemia which is influenced by the infusion of donor plasma containing high concentrations of potassium.

Transfusion of citrated donor blood invariably caused a profound increase in the plasma citrate level, yet all the infants in this series displayed an amazing ability to remove infused citrate from their plasma. The factors governing citrate metabolism and the effect of transfer of citrate out of the plasma compartment have been discussed and the relative importance of each has been critically examined.

The severity of the hypercitraemia should have been sufficient theoretically to inactivate all the calcium ions in the plasma, yet although significantly reduced, only in one instance, the fatal case, was

calcium ion activity not measurable. Explanations have been presented to account for the apparent discrepancy between the absence of the clinical manifestations of hypocalcaemia during transfusion and the observed depression of calcium ion in the serum specimens collected for analysis.

The absence of clinical abnormality is not inconsistent with a reduction in calcium ion in the serum. There is no evidence to suggest that citrate during transfusion is circulating in an ionic form. Furthermore the factors governing neuro-muscular irritability are complex, and the maintenance of a normal state of irritability in the face of a reduced calcium ion concentration may be due to compensatory mechanisms.

The injection of calcium gluconate solution had no effect upon serum calcium ion concentration. The results tend to confirm the opinion of other workers that calcium gluconate is of no value in maintaining calcium ion concentration in the serum

during exchange transfusion.

The incidence of hyperpotassaemia is high in newborn infants suffering from erythroblastosis foetalis and they are in potential danger of developing hyperpotassaemia during exchange transfusion. This is influenced by the potassium content of the donor plasma.

The state of potassium in the body and the mechanisms responsible for maintaining plasma potassium within normal limits have been discussed. It is suggested that the intravenous infusion of glucose in the donor blood is effective in laying down potassium during hepatic glycogenesis.

An attempt has been made to determine if a correlation existed between clinical incident during exchange transfusion and the induced electrolytic imbalance. There appeared to be no correlation. Electrolytic imbalance of similar magnitude existed in infants irrespective of their clinical condition. The only fatal case of the series showed the most

profound changes in serum electrolytes.

In the evaluation of the possible causes of clinical deterioration during exchange transfusion, it is emphasized that severe electrolytic imbalance apparently exists without clinical incident. It may be that the incidence of symptoms is dependant upon the degree of severity of the imbalance, but there was no evidence to suggest this to be the case. It is recognised that the infants undergoing transfusion were critically ill, and various factors other than electrolytic imbalance are believed to be the cause of clinical disturbances.

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