There is no convincing evidence in the present series or in published cases to suggest that a longer remission of symptoms occurs after A.C.T.H. therapy than after any other treatment which succeeds.

A.C.T.H. in no way avoids the expediency of all the other established methods in the assessment, treatment, and management of the asthmatic case.

Summary

The use of adrenocorticotrophic hormone is described in 10 asthmatics. In eight of these there was a remarkable relief of severe acute respiratory distress, resistant to other forms of therapy.

It is suggested that this substance should at present be used in asthma only as an emergency measure.

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EXCHANGE TRANSFUSION AS A MEANS OF REMOVING BILIRUBIN IN HAEMOLYTIC DISEASE OF THE NEWBORN

BY

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Damage to the brain occurs with varying frequency in haemolytic disease of the newborn. Exchange transfusion, as ordinarily carried out, is not entirely effective in preventing it.

It is now known that brain damage is found in association with a great excess of plasma bilirubin in the following conditions: (1) haemolytic disease of the newborn from iso-immunization relative to blood-group antigens D, E, A, and S; (2) prematurity without haemolytic disease; and (3) some types of "familial" hyperbilirubinaemia. Claireaux *et al.* (1953) have shown that in the first two conditions the yellow pigment in affected brains is the indirect-reacting plasma bile pigment, bilirubin. They have reviewed the evidence that this pigment is damaging to brain tissue, and that the direct-reacting bile pigments are innocuous. Some further indications of the toxic character of bilirubin have been published by Lathe (1954).

A more detailed study of the pigment changes in haemolytic disease of the newborn was undertaken with a view to developing methods of avoiding its accumulation. It seemed possible that the role of bilirubin would be made clear from the clinical results of preventing high plasma bilirubin concentrations in infants with haemolytic disease. An encouraging report by Hsia, Allen, Gellis, and Diamond (1952) and Hsia (1953) has now come to our attention. The work described here confirms their observations regarding the pattern of bile pigment changes in the plasma in haemolytic disease,

and shows that it is possible to control the plasma bilirubin concentration by exchange transfusion.

Methods and Materials

Clinical Material.—Observations have been made on a series of infants affected with haemolytic disease at this hospital. The brief clinical descriptions of six of these have been drawn from the case records. Serological data have not been included individually, since there was a common basis for serological diagnosis of haemolytic disease in all six cases: (1) mother's group was Rh-negative, (2) maternal antibodies were present, (3) infant's group was Rh-positive, (4) infant cells gave a positive test with rabbit anti-human globulin serum (Coombs +). The blood specimens removed at exchange transfusion of three infants at the Hammett Hospital have also been available for study. **Blood specimens** were taken from heel pricks when not otherwise stated. **Haemoglobin** estimations were made with a visual colorimeter (M.R.C. grey wedge) which was suitably calibrated—100% haemoglobin being taken as 14.8 g. per 100 ml. **Bile pigment** estimations were made on serum and plasma by the method of King (1951). In Cases 1 and 2 the relative proportions of direct- and indirect-reacting pigments were estimated semi-quantitatively by the appearance of pigment bands on chromatography columns (Cole and Lathe, 1953). In Cases 4, 5, and 6 the proportions of the two types of pigment were determined quantitatively by the method of Malloy and Evelyn (1937).

Plasma Bilirubin in Haemolytic Disease

No detailed study of bile pigment metabolism in a series of untreated cases of haemolytic disease of the newborn has yet been published. That such a study would have shown a wide variation from case to case is suggested by the data of Hsia, Allen, Diamond, and Gellis (1953), and of Mollison and Cutbush (1954). In this hospital there has been an opportunity of studying a number of affected infants who were not given an exchange transfusion because the cord blood contained more than 105% haemoglobin. Some of these infants later gave evidence of a considerable haemolytic process as shown by the changes in haemoglobin and plasma bile pigments. Two contrasting cases are described. Case 1 is an instance of haemolytic disease in which no exchange transfusion was given. In Case 2 only a simple transfusion was possible.

FIG. 1.—Changes in concentration of blood haemoglobin and of plasma bile pigments in Case 1.

FIG. 2.—Changes in concentration of blood haemoglobin and of plasma bile pigments in Case 2.

Case 1 (Fig. 1).—Two previous siblings were unaffected. The gestation period was estimated at 39 weeks. The birth weight was 8 lb. 5 oz. (3.7 kg.) and the cord haemoglobin was 109%. Cord serum bile pigment was 5 mg. per 100 ml. The infant looked slightly yellow at birth, but its condition was otherwise satisfactory. Jaundice deepened within 24 hours and lasted about a week. The blood haemoglobin concentration dropped at the rate of 8% per day for 11 days. The plasma bile pigment concentration reached a peak of 21 mg. per 100 ml. on the fourth day. No neurological signs appeared. A simple transfusion was later given to correct the anaemia.

Case 2 (Fig. 2).—There were two previous unaffected siblings. The period of gestation was estimated at 37 weeks. At birth the infant weighed 7 lb. 2 oz. (3.2 kg.) and was pale and limp. The cord blood contained 90% haemoglobin. The serum contained 6.3 mg. of bile pigment per 100 ml. Attempts at exchange transfusion failed and 128 ml. of blood was given as a simple transfusion. Following this, the blood haemoglobin fell consistently at the rate of 4.2% per day for 15 days. The plasma bile pigment rose rapidly, and when it was between 36 and 38 mg. per 100 ml. the neurological behaviour became abnormal, with irritability, head retraction, arms held stiffly extended, increased muscle tone, and disinterest in feeding. The plasma bile pigment began to drop about the third day, and fell below 20 mg. about day 6. Although much improved, the baby had not recovered normal neurological behaviour in eight months.

Course of Plasma Bile Pigment in Exchange Transfusion

It is apparent from examining a number of graphs of the rise and fall of plasma pigment in neonatal haemolytic disease that high concentrations of bile pigment are not sustained for long. Not infrequently the period of exposure to high concentrations does not exceed 36 or 48 hours. This encouraged us to seek a means of maintaining a low pigment concentration during this critical period. In the course of studying the amount of bile pigment removed at exchange transfusion it was observed that in large transfusions the blood withdrawn during the procedure contained more bile pigment than the baby's blood volume was calculated to contain at the beginning. This could be explained only by a passage of pigment from the tissues into the circulation during the transfusion. To examine this point, the bile pigment was quantitatively estimated in each specimen of blood removed in a number of exchange transfusions.

The course of plasma bile pigment concentration, together with the calculated extent of blood replacement, at each stage throughout one exchange transfusion, is plotted in Fig. 3. The replacement of the infant's blood did not lower the plasma pigment as much as was calculated on the basis of blood exchanged. It was concluded then that the plasma pigment concentration was partly sustained by removal of pigment from the tissues. The overall effect on bile pigment concentration of 10 exchange transfusions, given im-

FIG. 3.—Changes in concentration of plasma bile pigment, and the extent of replacement of the infant's blood, throughout an exchange transfusion immediately after birth. (Birth weight, 6 lb. 10 oz.—3 kg.)

mediately after birth for the purpose of removing infant red cells and maternal antibodies, and studied in this way, is given in the Table (A).

Change in Plasma Bile Pigment During Perfusion with Blood

Birth Weight		Exchange	Plasma Bile Pigments			
			Initial (mg./100 ml.)	Final (mg./100 ml.)	Decrease %	
A. Exchange Transfusion at Birth						
lb.	kg.	ml./lb.	ml./kg.			
5.9	2.67	68	150	7.0	4.8	31
6.6	2.99	51	112	9.2	5.3	42
5.4	2.45	41	90	7.8	4.4	44
6.7	3.09	63	139	3.2	2.2	31
6.5	2.95	75	165	10.9	2.6	76
5.8	2.63	51	112	5.1	3.6	29
5.7	2.58	62	136	10.2	5.6	45
7.0	3.18	62	136	5.0	4.0	20
7.1	3.22	81	178	8.2	6.0	27
7.7	3.49	96	211	10.8	4.8	56
B. Transfusions Because of Hyperbilirubinaemia						
9.1	4.13	39	86	16.0	14.5	9
7.0	3.18	71	156	38.0	26.0	32
6.5	2.95	35	77	17.3	9.1	47
5.7	2.58	61	134	24.0	11.6	51
5.7	2.58	42	92	20.4	10.5	48
7.5	3.40	54	119	23.3	14.0	40
7.1	3.22	105	231	21.9	8.0	60
6.4	2.90	71	156	20.2	12.1	40
6.5	2.95	61	134	21.0	15.7	25
7.4	3.36	60	132	21.3	10.8	49

Treatment of Hyperbilirubinaemia

Since it seemed that exchange transfusion could be used as a means of washing bilirubin out of the tissues of affected infants, it was next applied to babies that had not been given exchange transfusions at birth (cord blood haemoglobin greater than 105%), but in whom a marked rise of plasma bilirubin was noted during the neonatal period. In the first instance of this (Case 3) the exchange transfusion was not made until neurological signs of damage had appeared.

Case 3.—Two previous siblings were unaffected. The mother had had one miscarriage. The gestation period was estimated at 41 weeks. At birth the infant weighed 7 lb. ½ oz. (3.2 kg.). The concentration of haemoglobin in the cord blood was 111%. The cord serum bile pigment was 1.9 mg. per 100 ml. The liver and spleen were not palpable. The plasma bile pigment at 12 hours was 7.7 mg. per 100 ml. Icterus appeared at 18 hours and became severe at 24 hours. The plasma bile pigment was 19.3 mg. per 100 ml. at 36 hours. It continued to rise, and on the third day head retraction appeared, and there were periods of limb extension, grimaces, etc. Plasma bile pigment was then 38 mg. per 100 ml. An exchange transfusion of 71 ml. per lb. (156 ml. per kg.) was made. During it, opisthotonos developed. The transfusion reduced the bile pigment from 38 to 26 mg. per 100 ml. The pigment rose again to a peak of 32.5 mg. on day 5; it then dropped to 12 mg. during the ensuing two days. The neurological signs had meanwhile improved. At four months a diagnosis of cerebral palsy was made.

The following case is a more typical example of an exchange transfusion which was undertaken when the pigment concentration reached 20 mg. per 100 ml.

Case 4 (Fig. 4).—The mother had one previous infant without any sign of haemolytic disease. The membranes were ruptured artificially during the 38th week because

FIG. 4.—Changes in concentration of blood haemoglobin and of plasma bile pigments in Case 4.

of diminished foetal movements. Caesarean section was undertaken three days later because labour had not started. The birth weight was 6 lb. 6 oz. (2.9 kg.), and cord blood haemoglobin was 128%. Cord serum bile pigments were 3.4 mg. per 100 ml., of which 2.6 were bilirubin. Jaundice appeared in 24 hours and deepened as the bile pigment rose to 20.2 mg. on the third day. An exchange transfusion of 71 ml. per lb. (156 ml. per kg.) reduced the pigment to 12.1 mg. per 100 ml. On the following (fourth) day it rose again, but after the fifth day it fell consistently. No neurological signs appeared.

This pattern of change in plasma pigment concentration is probably the one most often observed among infants of substantial weight which are not seriously affected at birth and for whom an exchange transfusion is later performed because of rising bilirubin. The rise of pigment may be much more abrupt, however, reaching 20 mg. per 100 ml. in 24 hours. The Table (B) shows the overall effect on bile pigment concentration of 10 transfusions given because of the elevated plasma pigment. Each of these was studied throughout the whole of the transfusion. Some of these were repeat transfusions.

Obstruction Due to Haemolytic Disease

Note must be taken of the type of bile pigment present in each individual case of haemolytic disease of the newborn. In early studies of haemolytic disease, and of jaundice of the newborn in which there is no excessive haemolysis (Billing *et al.*, 1954), the type of pigment present in the plasma was examined in many cases. The predominating pigment was bilirubin, and direct-reacting pigment I and II (Cole *et al.*, 1954) were present in much smaller amounts. For a period, because of the small amounts of these direct-reacting pigments, the relative proportions of the direct pigments and bilirubin were not estimated. That this practice may lead to serious error is indicated in Case 5 and Case 6.

Case 5.—Two previous siblings showed jaundice at the third and first days of life, respectively, and the second infant died. The pregnancy was complicated by toxæmia, which necessitated a surgical induction of labour at the 37th week. The weight at birth was 5 lb. 11 oz. (2.6 kg.), and cord haemoglobin was 75%. Cord serum bile pigment was 6.3 mg. per 100 ml. The liver and spleen were greatly enlarged. The infant became jaundiced within three hours. It was given an immediate exchange transfusion of 62 ml. per lb. (136 ml. per kg.), which reduced the plasma bile pigment from 10.2 to 5.6 mg. per 100 ml. Within 24 hours the pigment had risen to 19 mg., and the rise continued to 24 mg. at 48 hours, when a second exchange transfusion of 61 ml. per lb. (134 ml. per kg.) was made. At the conclusion the bile pigment had dropped to 11.6 mg., but it rose to 20.4 mg. in another 12 hours. Some indefinite neurological signs (grimacing and twitching) were reported by the nursing staff. A third exchange transfusion of 42 ml. per lb. (92 ml. per kg.) at 75 hours reduced the pigment to 10.5 mg. It rose again 12 hours later to 18.4 mg. and remained between 18 and 20 mg. for another 36

hours, when it rose to 24 mg. At this point the character of the bile pigments was examined for the first time, and it was found that, of a total bile pigment of 24 mg. per 100 ml., only 5.3 mg. was bilirubin. In the light of this evidence that obstruction was the dominant feature further transfusions were not made. The total bile pigment remained between 22 and 24 mg. per 100 ml. (bilirubin 5.6 mg.) until the middle of the sixth day, when it dropped within 24

hours to 13.2 mg. per 100 ml., of which bilirubin was 3 mg. The infant's condition at three months was satisfactory.

The amount and type of pigments present in the early stages of this case are not known. The only specimen which had been preserved was the cord serum (total pigment 6.3 mg.), which contained equal parts of bilirubin, pigment I, and pigment II, as determined by Billing (1955).

Case 6 (Fig. 5).—The mother had three previous infants. The third, which was affected with haemolytic disease, was given an exchange transfusion, following which it developed marked signs of brain damage, which persisted until death at 10 months. The present infant was 7 lb. 2 oz. (3.2 kg.) at birth. The gestation period was uncertain. The cord blood haemoglobin was 39% and plasma bile pigment 9.2 mg. per 100 ml., of which 6.8 mg. was bilirubin. The liver and spleen were greatly enlarged. Although the clinical condition at birth was precarious, it improved greatly during the course of an exchange of 81 ml. of blood per lb. (178 ml. per kg.). Because of the desire at all costs to avoid a repetition of the tragedy of the previous sibling, and because of uncertainty concerning the significance of the direct-reacting pigment, a second exchange—105 ml. per lb. (231 ml. per kg.)—was given on the second day, when the plasma bile pigment concentration was rising rapidly. It never again reached dangerous concentrations. No abnormal neurological signs appeared.

Discussion

Liver Function

The cases which have been presented illustrate some of the fundamental aspects of bile pigment metabolism in the newborn infant affected with haemolytic disease. The plasma bilirubin concentration is determined partly by the rate of pigment formation in the reticuloendothelial system, but also by the rate at which the pigment is removed by the liver. In all newborn infants this latter capacity is probably very much reduced, and in many of the small infants the reduction is extreme—down to 1 or 2% of normal (Billing *et al.*, 1954). These small infants also take much longer to develop an adequate excretory ability.

Among infants of normal weight there is considerable variation in the excretory capacity of the liver at birth and immediately after it. This may be seen by comparing Cases 1 and 2. Although the haemoglobin concentrations fell in the first case at approximately twice the rate of the second, the peaks of bile pigment concentration are of an inverse order. The apparent difference in liver capacity may arise in part from the disparity in birth weights—8.3 and 7.1 lb. (3.8 and 3.2 kg.) respectively—and in the estimated gestation periods (39 and 37 weeks respectively). The changes of plasma bile pigment concentration in these cases also illustrate the way in which the capacity of the liver to excrete bilirubin develops rapidly during the neonatal period, since in both cases the loss of haemoglobin appears to have taken place at a relatively constant rate. It is this change in liver capacity which gives the dramatic character to the rise and fall of bile pigment (Fig. 2), and not any sudden acceleration of the haemolytic process in the neonatal period, as is often inferred. Indeed, in small premature infants unaffected with haemolytic disease the same thing takes place (on a reduced scale).

Removal of Bilirubin by Perfusion

The bilirubin produced from haemoglobin does not remain free in the body fluids, but attaches itself to various constituents, and in particular to plasma albumin, which has a marked affinity for it. At abnormally high plasma pigment concentrations appreciable amounts of bilirubin pass out of the circulation and become attached to other body constituents. This process may be reversed by the continuous replacement of the pigment-laden plasma proteins of the infant with protein which is free of pigment. This is demonstrated by the course of plasma bilirubin concentration throughout an exchange transfusion (Fig. 3). The extent of this shift of pigment from the tissues is indicated by the area between the two curves in Fig. 3. The Table shows that a perfusion of 50–60 ml. per lb. (110–132 ml. per kg.) fairly consistently results in a drop of plasma pigment concentration of 30–50%.

FIG. 5.—Changes in concentration of blood haemoglobin and of plasma bile pigments in Case 6.

After an exchange transfusion the bile pigment of the plasma nearly always rises again for a variable period. Although this may partly be due to a movement of further pigment out of the tissues, it seems that the previous trend of rising pigment concentration is continued in some degree, but at a lower level, until the liver capacity becomes sufficiently developed to remove bilirubin at the rate at which it is being formed. However, another aspect must also be considered. The baby receives at transfusion a very large amount of preserved blood. In the presence of an already overtaxed ability to excrete bilirubin any breakdown of the transfused blood will add to the accumulated pigment. Recent papers reveal that 10–20-day-old blood is still being used for exchange transfusion. Breakdown of 10% of such blood would lead to the accumulation of another 25 mg. of bilirubin per lb. (55 mg. per kg.) of body weight, from which a marked rise of plasma bilirubin would follow. Because of the limited capacity of the liver to dispose of bilirubin, newborn infants require a more rigorous criterion for adequate preservation of blood than do adults. For this reason some blood depots now adopt the highly desirable procedure of issuing the blood drawn during the previous 24 hours for exchange transfusions.

“Inspissated Bile Syndrome”

The predominant plasma pigment of haemolytic disease of the newborn (Claireaux *et al.*, 1953), as well as that of non-haemolytic neonatal jaundice (Billing *et al.*, 1954), is bilirubin, which is indirect-reacting in the van den Bergh test. The occasional appearance of large amounts of direct-reacting pigments I and II due to obstruction resulting from haemolytic disease is demonstrated in Case 5 and Case 6 (Fig. 5). The simultaneous appearance of direct-reacting pigments in the plasma and of bile thrombi was noted by Aschoff (1924) in experimental haemolytic anaemia in dogs. The same relation probably holds in some severe cases of haemolytic disease of the newborn. Bile thrombi have been described (Claireaux, 1950), and direct-reacting pigment may predominate as seen above. Hsia, Patterson, Allen, Diamond, and Gellis (1952) have called attention to the fact that haemolytic disease of the newborn is a frequent cause of the “inspissated bile syndrome.” It may be significant that the three cases of haemolytic disease in which we observed considerable direct-reacting pigment at birth, or shortly after, had hepatomegaly at birth.

The occasional appearance of much direct-reacting pigment emphasizes the great need for further work to establish unequivocally whether or not the direct-reacting pigments are damaging to the brain, since the evidence on this point is not yet conclusive enough to allow one to withhold exchange transfusion with equanimity.

Factors Affecting Transfusion

The facility with which high concentrations of plasma bilirubin can be prevented by exchange transfusion will depend on (1) the rate at which bilirubin is produced in the infant, (2) the effectiveness of the individual exchange operation, (3) the concentration of pigment which is considered to be a serious threat to the baby, and (4) the length of time required for the liver to attain adequate excretory function. In an affected infant the rate at which bilirubin is produced is greatly reduced by removing the infant's own cells, and where there are indications for doing this at birth it is the single most effective measure which can be taken to protect the infant. The limitation of a single exchange transfusion lies in the fact that as it proceeds it becomes increasingly inefficient in the removal of affected red blood cells. However, as shown in Fig. 3, this limitation does not apply to the same extent to the removal of bilirubin, the amount of which will be determined in large measure by the volume of blood with which the infant is perfused. Thus, under some circumstances, where the infant is quite robust and the plasma bilirubin is dangerously high, there

may be some justification for increasing the amount perfused to 100 ml. per lb. (220 ml. per kg.), as was done at this hospital on one occasion.

Toxic Level of Bilirubin

Treatment is directed toward preventing a rise of plasma bilirubin above the level at which the incidence of brain damage becomes appreciable. Mollison and Cutbush (1954) state that at plasma concentrations of total bile pigment of 19–24 mg. per 100 ml. 1 baby in 13 suffered brain damage, and that this increased to 4 out of 12 if the amount of pigment rose to 24–29 mg. per 100 ml. Above that amount the majority of infants were affected. Similar results have been observed by Hsia, Allen, Gellis, and Diamond (1952) in a series of 229 infants affected with erythroblastosis: “Kernicterus occurred in 18% of the infants whose serum bilirubin was recorded at a level between 16 and 30 mg. per 100 c.cm., and in 50% of those in whom levels above 30 mg. per 100 c.cm. were seen.”

It has been suggested that, in addition to other indications, an exchange or re-exchange transfusion should be done each time the plasma bilirubin concentration rises to 20 mg. per 100 ml. or above (*British Medical Journal*, 1954; Crosse *et al.*, 1954). Hsia (1953) reported that in 250 cases of erythroblastosis in which exchange transfusions were performed each time bilirubin exceeded 20 mg. there were no cases of kernicterus. The experience of undertaking exchange transfusions when the bilirubin reaches 20 mg. per 100 ml. shows that, in general, an exchange transfusion maintains the pigment concentration below the danger level for one or two days. Thereafter perfusion has to be repeated unless the pigment excretory capacity of the liver has developed to an adequate extent.

Small Infants

Small infants are more likely to require repeated transfusions, since, on the average, they need a longer period for the development of the capacity to excrete bilirubin (Billing *et al.*, 1954). It may be noted, too, that the occurrence of brain pigmentation in jaundice of premature infants that are unaffected with haemolytic disease, as well as the greater incidence of brain damage among premature than mature infants with haemolytic disease (Allen *et al.*, 1950; Armitage and Mollison, 1953), suggest that small infants may be more sensitive to bilirubin, and thus may need to be protected against bilirubin concentrations of less than 20 mg. per 100 ml. of plasma. These two aspects of the small newborn infant should be taken into account when premature delivery of infants (Watson *et al.*, 1954) is being considered.

The possibility of successfully treating infants that are severely affected with haemolytic disease (and even of premature infants) is considerably extended by the fact that the concentration of plasma bilirubin can be controlled. Of course, this does not provide a reason for delaying an initial exchange transfusion for the purpose of removing the infant's sensitized cells. On the contrary, it emphasizes the necessity of doing this as soon as there is an indication of a serious haemolytic condition. In the first instance the cord haemoglobin is of major importance, but a later rapid rise of plasma bilirubin may be a significant warning.

Summary

The changes in concentration of plasma bile pigments have been followed in cases of haemolytic disease of the newborn and throughout exchange transfusions. A drop of 30–50% in the concentration of plasma bile pigment is usually brought about by exchange transfusion, during which bile pigment is washed out of the tissues. By one or more transfusions the bilirubin concentration may be kept below the level at which the incidence of brain damage becomes appreciable. The number of times

perfusion has to be repeated will be influenced by the speed with which the liver develops the capacity to excrete bilirubin.

Because of the underlying hepatic deficit in the newborn baby the importance of using recently drawn blood for transfusion is stressed.

The significance of the large amounts of direct-reacting bile pigment that have been shown to occur in the plasma of some cases of haemolytic disease is discussed.

This study has been made possible by the close co-operation of Miss M. Ashforth in following the clinical condition of the infants and in securing specimens for analysis. She has also been responsible for most of the exchange transfusions. I am grateful to Dr. A. White Franklin for opportunities of studying infants under his care. The serological and haematological examinations have been supervised by Dr. John Murray. Specimens from the Hammersmith Hospital have been obtained with the help of Professor J. McClure Browne and Dr. J. Dean. I am also grateful to P. G. Cole, C. R. J. Ruthven, and S. Bebbington for skilled laboratory assistance, and to Dr. John Murray, Dr. A. E. Claireaux, and Dr. Barbara H. Billing for frequent discussions and suggestions.

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Believed to be the only unit of its kind ever to be formed in the British Army, the Parachute Medical Team is described by Dr. J. S. Macdonald in the *Journal of the R.A.M.C.*, January, 1955. It is staffed by personnel of the 16th Independent Parachute Brigade Group and operates in the Middle East. On receiving a signal that an aeroplane has crashed in the desert, the team sets off in a "Valetta" aircraft standing by and fitted out specially for the dropping of men and containers. On finding the crashed aircraft, a suitable dropping zone is sought, a smoke canister thrown out to determine wind speed and direction, and then the medical officer and nursing orderly jump. If they find that further help is needed, they fire a green Very cartridge, and the rest of the team follow—namely, three privates R.A.M.C., and one corporal and two signalmen Royal Signals. (The main duty of one of the signalmen is to carry a radio set down, since this is still the surest way of getting these delicate instruments to the ground intact.) If the first two to jump find they do not need any assistants, they fire a red Very light, and the aircraft drops food and water. The team's task is to give such first-aid treatment as may be required until the R.A.F. ground rescue team can arrive and take them all to safety. The equipment parachuted includes intravenous fluids, splints, morphine, anaesthetics, surgical instruments, antibiotics, and a bivouac tent to provide that most longed-for solace in the desert—shade.

ALDOSTERONE IN URINE OF NORMAL MAN AND OF PATIENTS WITH OEDEMA

ITS INCREASED RECOVERY AFTER HYDROLYSIS WITH ACID AND WITH BETA-GLUCURONIDASE*

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Neutral lipid extracts of acidified urine from patients with the nephrotic syndrome, in heart failure, and in some other oedematous states cause sodium retention and potassium elimination when injected into adrenalectomized rats (Deming and Luetscher, 1950; Luetscher *et al.*, 1952; Luetscher and Johnson, 1954b; Chart and Shipley, 1953; Singer and Wener, 1953; Gordon *et al.*, 1954). The substance responsible for this mineralocorticoid activity has been separated by paper chromatography from urine extracts (Luetscher and Johnson, 1954a). Further work (Luetscher, 1955; Luetscher, Neher, and Wettstein, 1954) has shown that this sodium-retaining corticoid is identical with aldosterone (Simpson and Tait, 1953; Simpson *et al.*, 1954) in the following ways: (1) its high sodium-retaining potency in bioassay; (2) its absorption of ultra-violet and of infra-red radiations; (3) its reducing activity; (4) its melting point, and (5) the chromatographic mobility of the native substance, of its mono- and di-acetates, and of its oxidation products after treatment with periodate.

Cope and Garcia-Llaurado (1954) found a substance of similar chromatographic behaviour in the urine of a woman suffering from a severe potassium-losing condition. Their extracts of neutral urine from normal men yielded only traces of activity. Luetscher and Axelrad (1954) obtained a much larger yield of aldosterone from normal urine which had stood for a day at pH 1. Venning *et al.* (1954) found increased amounts of sodium-retaining substances in urine treated with beta-glucuronidase. Thus the amount of active material extracted depends partly upon the method of hydrolysis. This report compares the yields, after various methods of hydrolysis, from urine of normal subjects on their usual diet and after salt restriction. The present study also includes data on the hydrolysis of urine of patients showing an increased urinary output of sodium-retaining material.

Methods

Urine was immediately stored in a refrigerator without preservative. The total 24-hour specimen was mixed, the volume was recorded, and a small aliquot was set aside for chemical analysis. The remaining urine was frozen and stored at -20° C. until extraction. Immediately before extraction, urine was brought to 20° C. and the pH was adjusted as indicated.

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