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CHANGES IN THE BLOOD COMPOSITION OF UN-ANÆSTHETIZED RABBITS FOLLOWING THE INGESTION OF WATER AND SALINE[1].

With special reference to the distribution of fluid between plasma and corpuscles and to the relationship between blood composition and diuresis.

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Introduction.

For some time it has been a matter of dispute whether, following upon the administration of water by the alimentary tract, there is or is not a blood dilution. Thus Haldane and Priestley [1916], Priestley [1916, 1921], Engel and Scharl [1906] consider that no appreciable change in blood concentration takes place and in contrast Blix [1916], Marx and Mohr [1927], Bayliss and Fee [1930], Rioch [1930] detect considerable dilutions. Haldane and Priestley [1916] note a constancy of the hæmoglobin percentage in man, but in a subsequent paper Priestley [1916] describes a decrease in the electrical conductivity.

In these results there may be nothing essentially contradictory. The type and breed and diet of the animal used, the dosage of water, the substance used as an index of blood dilution, and the times after water administration at which the blood samples are taken may determine these differences. Further, the differences in physiological response induced by such procedures as the administration of anæsthetics and decortication or decerebration are of at least equal importance.

Dilution of the blood is usually estimated by observing the changes in percentage concentration of some substance which is selected as a measure of dilution. In this paper the percentage of solid substance and the hæmoglobin content of whole blood, the hæmatocrit reading, the protein content of plasma, and the chlorine content of plasma have been used for this purpose. Although it is not methodically difficult to detect even small changes in the concentration of these substances it is by no

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means so easy to decide that such changes are the result of water absorption. Thus, Barcroft has shown that during muscular exercise or a state of anger or fright contractions of the spleen occur with an outpouring of additional red cells into the circulation. In addition, changes have been found in the chlorine and protein contents of the plasma and in the hæmoglobin content of whole blood during exercise in men even after splenectomy (Smirk—unpublished observations). The chlorine content of the plasma may vary independently of the plasma protein or the hæmoglobin content of whole blood either by movements of chlorine ions to and from the erythrocytes (the Hamburger phenomenon) or the tissues [this paper and unpublished observations]. Since the lymph may contain much protein the formation and storage in tissues or serous cavities of additional lymph as a result of water absorption would give rise to a fall in the plasma protein concentration even if the blood volume remained constant. It will be shown in a subsequent paper with Heller that this source of alteration in the plasma protein percentage is not purely theoretical. Again venous congestion of the part from which the blood sample is taken may alter the concentration of hæmoglobin and the distribution of chlorine between corpuscles and plasma; and even heating the source of the blood sample to obtain a more liberal flow may produce small alterations in man [Smirk, 1928 *a*].

It may be suggested that since exercise, emotional changes and many other factors may alter the concentration of various substances in the blood it will be well to avoid them by some such device as anæsthesia, decortication or decerebration.

The object, however, of this work is firstly to enquire whether it is indeed true that the tissues react rapidly and quantitatively to small changes in concentration of the blood in such a manner as to keep constant the blood volume, and secondly, to determine what relation, if any, there may be between blood concentration and normal water diuresis. The first object is of importance since if attained it may help to reveal whether the partition of water between blood and tissues is or is not mainly determined by physico-chemical changes. The second consideration should show whether in all animals the kidney is extremely sensitive to changes in blood concentration, or whether a biased view point has so far been obtained by considering mainly those animals where the renal response is most active.

Since most anæsthetic substances alter, usually diminishing, the urinary output, they are clearly unsuitable for the study of changes in blood concentration during the progress of a normal water diuresis. In addition Hicks and I [1930] have shown directly on rabbits that the blood which became diluted after giving water by the alimentary tract was reconcentrated if chloretone or morphine were then administered. This perhaps indicates an altered distribution of water between blood and tissues under the influence of at least two narcotic drugs.

Although the decorticated or decerebrated preparations described by Fee [1929] gave responses to water administration as gauged by the urinary output, it does not appear safe to assume from this that the "pre-renal" distribution of water about the body, particularly its partition between the blood and tissues, must have remained normal. Especially is this so while many schools of thought emphasize the importance of the nervous system in determining water interchange and excretion.

For this reason it has been considered most desirable to work upon intact unanæsthetized animals and man and to eliminate the effects of struggling and emotion by means of controls in which the entire experimental procedure is repeated with the exception of water administration. Also it has been thought desirable to use many different substances as indices of blood dilution. In this paper are described only the experiments on rabbits.

Experimental procedure.

The rabbits received 4 p.c. of their body weight of warm water by stomach tube, their previous dietic state being controlled.

Samples of blood were taken without congestion, and as far as possible without the production of fright or struggling, from the marginal ear veins.

Estimations of the plasma protein were made by Kjeldahl's method, no correction being made for non-protein nitrogen. Hæmatocrit observations were made by a method previously described [Smirk, 1928 *b*]. The percentage solid substance in whole blood was determined by receiving a small quantity of blood into a weighing bottle and determining the loss on drying to a constant weight by heat in an oven with a steady temperature of 107° C. for 36 hours. Hæmoglobin changes were detected by preparing solutions of 0·1 cm. of the bloods in 25 c.c. of tap water and passing coal gas in order to convert hæmoglobin to carboxyhæmoglobin. Using one of the samples before water administration as the standard the changes in concentration were determined by colorimetry and were expressed as a percentage fall below the original concentration without determination of the actual amount of hæmoglobin. In some experiments the iron content of the blood, which depends almost solely on the hæmoglobin, was determined by a method already described [Smirk, 1927 *a*]. The plasma chlorides were determined by rapid destruction of the proteins with ammonium persulphate and nitric acid in the presence of silver nitrate followed by back titration with alcoholic ammonium thiocyanate in the presence of acetone as an end point intensifier [Smirk, 1928 *b*].

Samples of blood were shaken with air before analysing in order to correct any changes in the distribution of chlorine ions due to differences in the CO_2 contents of the samples.

Heparine was usually used as anticoagulant, but where only small samples of blood were taken for plasma protein analysis neutral potassium oxalate was employed to avoid errors due to the nitrogen content of heparine.

The amounts of blood removed were in all cases controlled by the removal of similar quantities of blood in the control animals, and the necessity of adopting this procedure was made evident.

Urine samples were obtained by expression.

Results.

The changes in blood concentration after water administration have been expressed as a percentage of the original concentration. In other words, the initial blood concentration of any substance used as an index of blood dilution is called 100 p.c., and if the concentration of this substance falls to, say, 96 p.c. of this in the course of an experiment it is recorded simply as a 4 p.c. dilution. In all the charts the horizontal line represents the initial blood concentration—a line drawn vertically downwards from this represents a test in which the blood has become diluted: vertically upwards a test where the blood has been concentrated. Each vertical line, therefore, represents a separate experiment and the length of the line the degree of dilution or concentration.

(*a*) Control observations on the effect upon the haemoglobin concentration of removing 2 c.c. or 3 c.c. of blood.

In a 2-kilogram rabbit one would anticipate a blood volume of not less than 120 c.c., and theoretically the change in hæmoglobin percentage resulting from the removal of 2 c.c. or 3 c.c. should not exceed 1·7 p.c. to 2·5 p.c. In actual practice this is not so. A second sample of blood removed 1½ hours later may show a dilution of as much as 8 p.c., usually about 3 p.c. or 4 p.c. It is unlikely that this finding depends on any methodical error, since estimations of the plasma protein and hæmatocrit observations are in agreement with the hæmoglobin determinations: and it will be seen from subsequent control experiments where only 0·5 c.c. to 0·8 c.c. of blood have been removed (Diagrams 1, 2, 4) that such changes are not then observed. It is not proposed to pursue this matter further, but it may well be that compensatory adjustments of blood volume in response to small hæmorrhages are not accurate to 3 p.c.

(*b*) The effect of the administration of 4 p.c. of the body weight of water upon the haemoglobin concentration of a sample of blood taken 1½ hours later together with control observations in which the experimental procedure was repeated: water administration excepted. See Diagram 1.

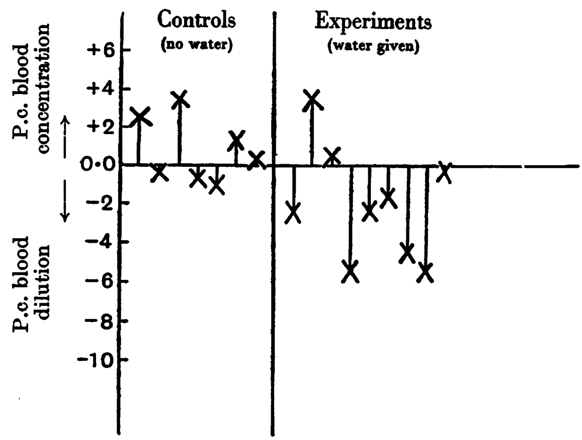

Diagram 1. Changes in hæmoglobin.

Diagrams 1–7 show the effect of water ingestion on the concentration of various blood constituents in the rabbit. Each vertical line represents a separate experiment. A fall of the vertical line below the base line represents a blood dilution.

Only 0·5 c.c. to 0·8 c.c. of blood were removed per sample and the full period of 1½ hours was allowed to elapse before the second blood sample was taken in the control observations. The previous diet and treatment of the control animals was identical with that of animals receiving water and in no case were animals deprived of water before the experiments for a time long enough to cause appreciable blood concentration, the correction of which after water administration would simulate blood dilution. Rabbits whether on diets of oats and unlimited water or cabbage and unlimited water showed dilution of the blood after the additional dose of water by stomach tube as contrasted with the absence of blood dilution in the controls where no additional water had been given.

It will be observed that the hæmoglobin concentration in the controls is not steady, but this might be expected from the presence of variations introduced by factors such as splenic contraction which have been referred to in the introduction. The average of all the control observations would suggest about 1 p.c. concentration of the blood which is approximately the same as the control observations for the plasma protein.

In one experiment where water has been given, a blood concentration was observed and this likewise may be due to variations in the amount of circulating hæmoglobin induced by the spleen. The remaining experiments suggest that there is a definite dilution of the blood as estimated by the hæmoglobin concentration.

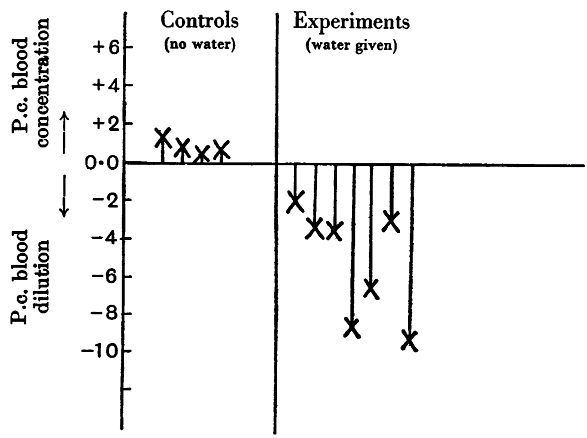

Diagram 2. Changes in plasma protein.

(*c*) The effect of the administration of 4 p.c. of the body weight of water upon the plasma protein concentration, together with control observations. See Diagram 2.

The procedure and precautions in these experiments have been exactly similar to those of the last series (Section (*b*)). The estimations of nitrogen by Kjeldahl's method were made in duplicate or triplicate on 0·1 c.c. of plasma each.

The concentration of protein tends to remain steady in the controls: being concentrated about 1 p.c. as would be expected from a urine loss which is not replaced by additional water given. It is probable that variations in the size of the spleen do not affect the protein concentration to the same degree as the hæmoglobin.

In contrast to the controls, animals receiving water showed in all cases a definite plasma dilution although the degree of dilution was variable.

(*d*) The effect of the administration of 4 p.c. of the body weight of water on the percentage of solid matter in whole blood. See Diagram 3.

Again the experimental procedures of Sections (*b*) and (*c*) have been repeated with the addition that a series of five observations were made on blood samples taken ½ hour after water administration in addition to eleven observations at 1½ hours. Further controls were considered

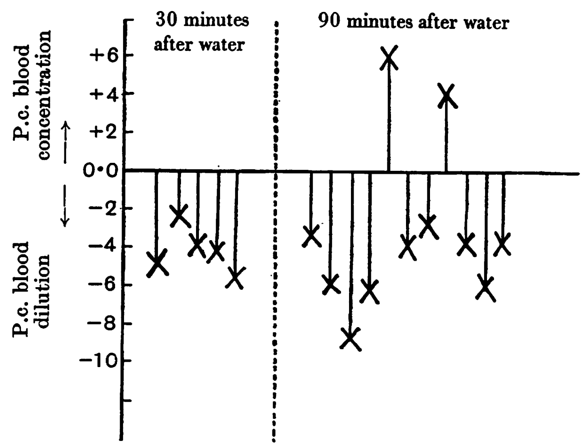

Diagram 3. Changes in the dried weight of whole blood.

unnecessary here since the percentage solid matter depends largely on the hæmoglobin and protein in which the absence of any important variation has been demonstrated.

Both at ½ and 1½ hours after giving water the blood was diluted. The two experiments in which a concentration of the blood was observed and some of the experiments where the dilution appears unusually great are probably explained by the splenic and other errors outlined in the introduction.

(*e*) The effect of the administration of 4 p.c. of the body weight of water on the percentage volume of red cells, together with control observations. See Diagram 4.

It will be observed that any difference there may be between the control animals and the animals that received water is much smaller when the hæmatocrit readings are studied than in experiments where the hæmoglobin or plasma protein is used as an index of blood dilution. In order to study this in detail a series of results were collected together in which both hæmatocrit observations and hæmoglobin determinations were made on the same blood samples. These results are described in Section (*g*). For the moment it will suffice to state that the dilution estimated by the change in the percentage volume of corpuscles is as an average much less than the dilution estimated by the hæmoglobin percentage, the solid matter in blood or by the plasma protein. (See Diagrams 1, 2, 3, 4.)

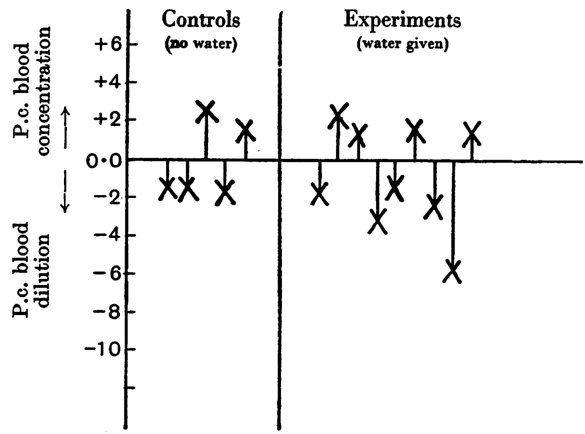

Diagram 4. Changes in the hæmatocrit.

(*f*) The effect of the administration of 4 p.c. of the body weight of water on the chloride content of plasma. See Diagram 5.

It will be observed that a slight fall in the plasma chlorine is the rule, and that this fall is less than the fall in plasma protein.

(*g*) A comparison of the degrees of blood dilution as determined by haematocrit observations and haemoglobin estimations on the same blood samples after the administration of 4 p.c. of the body weight of water. See Diagram 6.

Changes in the hæmoglobin percentage may be used as indications of the total amount of additional fluid which enters a given volume of blood, whereas the hæmatocrit readings inform us as to changes in the percentage volume of red cells; and therefore indicate the manner in which water is distributed between the plasma and the corpuscles: since upon this distribution depend changes in their proportional volumes.

In Diagram 6 are collected the results of experiments in which hæmatocrit observations were made and a dilution of the hæmoglobin was observed.

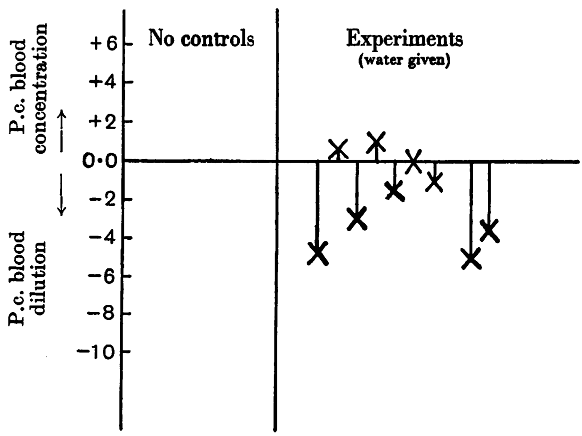

Diagram 5. Changes in the plasma chloride.

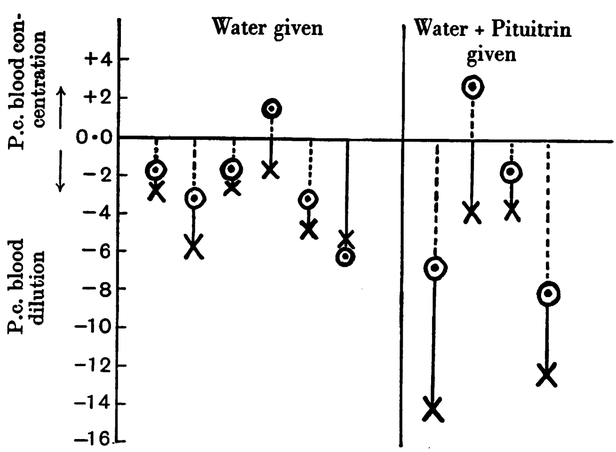

Diagram 6. Comparison of hæmatocrit and hæmoglobin dilutions in blood after water administration.

× Hæmoglobin dilution. ⊙ Hæmatocrit dilution.

In addition are included hæmatocrit and hæmoglobin determinations from four experiments in which the excretion of urine was prevented by pituitrin and a larger blood dilution resulted.

Of ten observations, each the average of two or three hæmoglobin determinations and six to eight hæmatocrit readings, nine showed that the percentage blood dilution appeared greater when hæmoglobin was used as an index of dilution, and in the remaining experiment the two fell within experimental error. Of the nine results showing greater dilutions with the hæmoglobin two showed a slight concentration by the hæmatocrit. This may well be due to some such factor as splenic contraction, which by throwing additional blood into the circulation would tend to diminish the dilution effect of the water without greatly disturbing the relationship which has been described between the hæmatocrit and the hæmoglobin readings. In other words both points would be moved vertically upwards on the diagram.

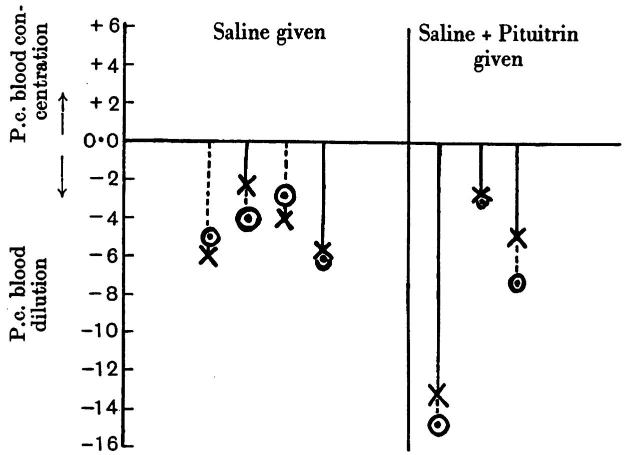

Diagram 7. Comparison of hæmatocrit and hæmoglobin dilutions in blood after 0·9 p.c. saline administration.

× Hæmoglobin dilution. ⊙ Hæmatocrit dilution.

One may briefly conclude from these observations that most of the entering water remains in the plasma but that some must enter the corpuscles and cause an increase in their average size.

(*h*) A comparison of the degrees of blood dilution as determined by haematocrit observations and haemoglobin estimations on the same blood samples after the administration of 4 p.c. of the body weight of 0·9 p.c. saline. See Diagram 7.

It was thought that a possible explanation of the distribution of added water between plasma and corpuscles which has been described in Section (*g*) might be, that the water enters the blood as hypotonic saline, or at least that adjustments take place which render the net result an equivalent of this. Therefore it was decided to examine the distribution of the added water when isotonic saline was administered, since from *in vitro* experiments one would then expect to find the whole of the added fluid in the plasma. This anticipation was realized since the dilutions as estimated by the hæmoglobin and the hæmatocrit were then approximately equal, and this was especially evident when large blood dilutions were produced with the help of pituitrin.

Thus where water was given the average of all the results (including pituitrin experiments) gives a dilution of 5·5 p.c. for the hæmoglobin and 2·6 p.c. for the hæmatocrit, but where saline is given although the dilution is still 5·5 p.c. for the hæmoglobin it is 6·0 p.c. for the hæmatocrit.

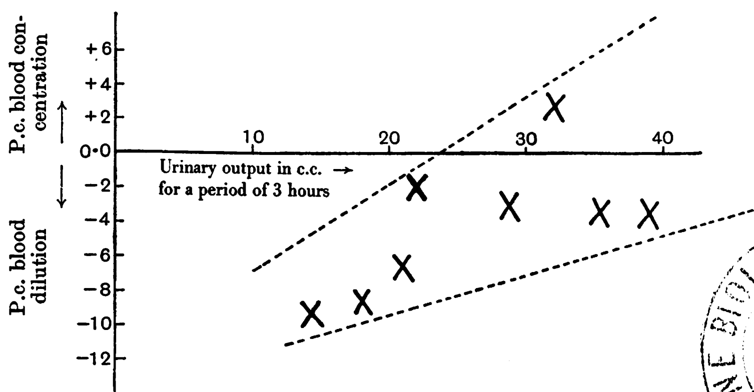

Diagram 8. The relation between the dilution of plasma proteins and diuresis (rabbit).

This can only be the case if where water was given a proportion of the water entèred the corpuscles and where isotonic saline was given all the water detained in the blood remained in the plasma.

(*i*) The relationship between diuresis and the dilution of the plasma protein.

In Diagram 8 has been represented the relationship of the output of urine over the 3 hours following water administration to the dilution of the plasma protein expressed as a percentage. The abscissa represents the urinary output in the 3-hour period per kg. of the animal's body weight and the blood samples were taken shortly before and $1\frac{1}{2}$ hours after water administration. It will be observed that as a rule the large blood dilutions are met in animals with a small diuresis and conversely the animals with a relatively large diuresis have the smaller blood dilution. It can at least be said that the diuresis over the full period of 3 hours is not proportional to the degree of plasma dilution as estimated by the plasma protein, and it would even seem probable that the cause of blood dilution may sometimes be the inadequacy of the renal response.

It may be suggested that one cannot fairly relate the urine output for 3 hours after the administration of water to the plasma composition at the mid-way point (*i.e.* $1\frac{1}{2}$ hours after).

Similar charts have been constructed, however, for the period $1\frac{1}{2}$ hours before the second blood sample and also for the $1\frac{1}{2}$ hours afterward, and both show the same distribution of results. The 3-hour chart may, therefore, be given as being representative.

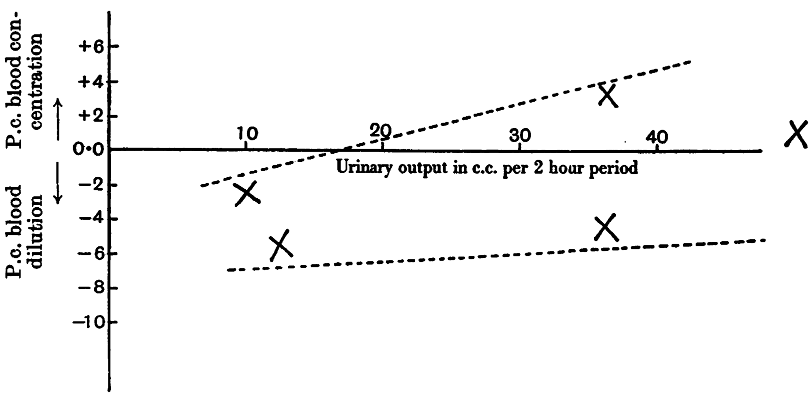

Diagram 9. The relation between the dilution of hæmoglobin and diuresis (rabbit).

(*j*) The relationship between diuresis and blood dilution as measured by the hæmoglobin of whole blood.

In precisely the same manner as in Section (*i*), Diagram 9 relates the hæmoglobin dilution at $1\frac{1}{2}$ hours after water administration to the urinary output during the 2 hours after giving water. Similar relationships are obtained if the blood dilution is related to the output of urine during the $\frac{1}{2}$ hour following the taking of the second blood sample or to the urinary output during the $1\frac{1}{2}$ hours after giving water.

The chart shows that the degree of blood dilution does not determine the degree of diuresis, and again, as with the plasma protein, results are perhaps more compatible with the idea that it is the absence of an adequate renal response to water administration which may determine the blood dilution.

(*k*) The relationship between diuresis and the dilution of the total solid content of whole blood.

Diagram 10 which is constructed on the same principle as Diagrams 8 and 9 relates the dilution of the total solids $1\frac{1}{2}$ hours after water administration to the output of urine in the second hour.

No relationship can be made out between dilution of the blood and diuresis. If the urinary output up to the time of taking the blood sample, or the urinary output for the full 3-hour period are studied, the same absence of relationship is observed.

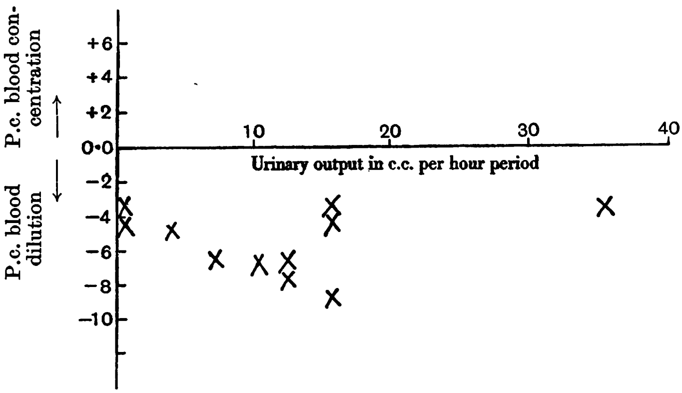

Diagram 10. The relation between the dilution of the total solids of blood and diuresis (rabbit).

Discussion.

By blood dilution is here meant a diminished concentration in blood of any of its main constituents: by general blood dilution a dilution of all of these or at least of such representative constituents as hæmoglobin, plasma proteins and plasma chloride.

From the consideration of certain fallacies outlined in the introduction it will be evident that changes in the concentration of any one substance in the blood do not justify the conclusion that there has been an alteration in the blood volume, since the salts, hæmoglobin and other proteins may vary independently. But dilution of two or three representative substances including those most abundant in blood must be regarded as very strong evidence of an increased water content of blood and strong evidence also of an increased blood volume.

The degree of the changes in blood concentration is always small both relative to the quantities of water given and to the resulting diuresis, and the part played by methodical errors in analysis and sampling of blood must, therefore, receive careful consideration. As far as possible analytical errors have been controlled by duplicate and triplicate estimations, and sampling errors by avoidance of congestion and procedures liable to alter blood concentration.

The most important controls in my opinion are firstly the frequent comparisons with animals in which the whole experimental procedure is repeated with the exception of water administration, and secondly the use of several different indices of blood dilution involving as many different analytical processes.

Comparison of the results obtained in the animals which received water with the results in animals where no water was given make it, I think, certain that under the conditions of my experiment a general blood dilution was indeed the rule, and that this dilution was not occasioned by the removal of the first blood sample or by any other procedure than the giving of water.

It would not be difficult, however, to obtain results of this kind by depriving animals of water, thus producing a blood concentration which would be corrected only in those animals which subsequently received water, thus giving rise to an apparent blood dilution.

This, however, is not the explanation of the results since the rabbits were allowed free access to water up to or to within an hour of the experiment. The previous diet may be of importance since animals fed on cabbage have much larger outputs of urine than animals receiving oats, although the latter animals have free access to water. This also remains true when the rabbits have been deprived of food for 24 hours, and is not due to the additional water content of the cabbage. The general impression conveyed by my results is that animals fed on oats show slightly greater dilutions of the blood. The effect of a previous diet of cabbage upon diuresis persists some days even if oats have been given in the interval. It is not so likely therefore that the difference in urinary output operates through any alteration in the composition of the fluid absorbed from the alimentary tract. The chlorine contents of random samples of cabbage and oats (expressed as NaCl) were found to be respectively 0·298 and 0·057, and since a larger weight of cabbage can be eaten by a rabbit there is little doubt that on this diet its tissues and urine will be richer in salts.

Since, however, the animals were allowed water but deprived of food about 15 hours before the experiment it is not probable that the salt content of the water in the alimentary canal prior to its absorption is greatly different under the two diets.

The animals used to study the relationships of protein and of the total solids of plasma to diuresis were both on uniform diets.

Inspection of Diagrams 1, 2, 3 and 5 reveals that although the same dose of water is given for each kilogram of the animals' body weight the degree of blood dilution differs from one animal to another to an extent which cannot be explained by chemical error. Although splenic contractions induced by fright or struggling might possibly explain the variability in the degree of blood dilution as estimated by the hæmoglobin and the total solids of whole blood it is unlikely that this would explain the varying degrees of protein dilution. The constancy of the protein content of plasma in the control observations also supports the contention that these results indeed represent varying degrees of change in the blood volume. Bayliss and Fee [1930] have suggested from their work on decerebrated dogs that the amount of additional water in plasma is such as would be expected if the extra water were uniformly distributed throughout the tissues. This does not appear to be the case in rabbits, and two reasons why a non-uniform distribution is to be expected at 1½ hours after water administration in rabbits may be stated.

Firstly, whether and to what degree a blood dilution takes place depends partly upon a time factor operating through a system which may be represented diagrammatically as follows:

Alimentary canal

↑↓

Tissues ⇄ Plasma → Kidneys

↓↑

Corpuscles

It will be clear in such a system as the above that either all water entering the blood from the alimentary tract must be quantitatively and immediately removed by renal excretion or tissue absorption (very rarely by re-excretion into the lower alimentary tract) or a general blood dilution must result. The degree of a general blood dilution produced by a time lag in the disposal of water is not specially likely to be such as would be obtained by the uniform distribution of added water in the body.

Secondly, it would appear from these results that we must consider the effect of the salt content of the entering water upon its distribution. It is highly probable that water entering the blood stream from the alimentary canal has already become hypotonic saline or rapidly attains equilibrium with the tissues, the net result being the addition to the blood of hypotonic saline.

It will be evident from Diagrams 7 and 6 that when the administration of isotonic saline produces blood dilution the degrees of dilution as measured by the hæmoglobin and by hæmatocrit readings are equal; whereas if a blood dilution results from the administration of water the change in the hæmoglobin percentage is greater than the change in percentage cell volume.

The only reasonable interpretation of these findings is that isotonic saline remains in the plasma and does not penetrate the corpuscles to an appreciable degree, but water to which no salt is added outside the body is distributed between the corpuscles and the plasma, the larger part, however, entering the plasma. These relationships remain true when larger blood dilutions are produced with the aid of pituitrin which diminishes the formation of urine.

Thus averaging all the seven experiments using isotonic saline the hæmoglobin dilution is 5·5 p.c. and the average cell volume dilution is 6·0 p.c. When water is given the hæmoglobin dilution averages 5·49 p.c., but in contrast the dilution of the cell volume is only 2·6 p.c., showing that a proportion of water has entered the corpuscles and increased their size: thereby the relative volumes of plasma and corpuscles are not altered to the same degree as is the hæmoglobin percentage.

It would appear justifiable to conclude from this that the distribution between the plasma and the corpuscles of extra water added to the blood is at least partly dependent upon the concentration of salt dissolved therein. It is also likely that water either enters the blood as hypotonic saline or rapidly takes up salt from the tissues so that the final result is equivalent to this. Variations in the salt reserves might easily account in this way for differences in the distribution of added water between corpuscles and plasma.

Nor is it unlikely that the effective salt content of added fluid normally influences its distribution between plasma and tissues. Thus hypertonic saline will cause dilution of the blood and a withdrawal of fluid from tissues, and conversely it is likely that the degree or at least the rate of penetration of water into the tissues will depend upon the degree of hypotonicity.

Although the experiments were carried out on different animals it is significant that the dilution of plasma as estimated by the plasma proteins is greater than the dilution as estimated by the plasma chloride.

This must mean either that protein has left the blood or salt has entered it, but not in a sufficient amount to render the extra water isotonic.

Observations on man also suggest that movements of salt occur after water administration, but in the direction blood to tissues. It is desired to treat of this in a subsequent paper.

It will be clear from Diagrams 8, 9 and 10 that the degree of diuresis is not proportional to the degree of blood dilution of the substances under consideration, and that where any relationship exists, as would appear in Diagram 8 and to a lesser degree in Diagram 9, it suggests that dilution follows upon inadequate diuresis. It is certain that a good diuresis may be present with little or no general dilution of the blood, and in apparently normal rabbits a definite general blood dilution may be accompanied by a poor diuresis. It does not appear in this animal that diuresis is causally related to general blood dilution. This observation is not easy to reconcile with Cushny's theory that the composition of the blood is the determinant of urinary composition and diuresis.

On the other hand, the absence of parallelism between blood composition and urine formation is compatible with the production of increased urine, being due either to the mobilization of some diuretic substance from the tissues or to a diminished content of the blood in the hormone of the pituitary body [Verney, 1926, 1929].

SUMMARY.

1. The percentage of solid substance in whole blood, the hæmoglobin of whole blood, the percentage cell volume (by the hæmatocrit), the protein content of plasma and the chlorine content of plasma have been used to study changes in blood concentration.

2. In most experiments on rabbits each one of the above yields evidence of some degree of blood dilution when 40 c.c. of water per kilogram of body weight are administered, and it is concluded from this that both the water content of the blood and the blood volume have been increased.

3. The average degrees of dilution in similarly conducted experiments vary according to which substance is used as an index of dilution, and reasons for these differences are suggested. Thus:

Index of dilution	Degree of dilution
(1) Percentage solid substance in whole blood	3·5
(2) Hæmoglobin of whole blood	2·0
(3) Percentage cell volume	1·9
(4) Protein of plasma	5·2
(5) Chlorine of plasma	1·6

4. In individual rabbits the degrees of blood dilution as estimated by various constituents are not uniform and may be in excess of or less than would be expected from an equal partition of added water among the water-bearing tissues of the body.

5. The administration of water or water and pituitrin produces chiefly a dilution of the plasma, but the red cells also take up some water and are increased in size.

6. Blood dilution produced by 0·9 p.c. saline or saline + pituitrin is entirely a plasma dilution.

7. It is probable in rabbits that the dilution of blood following the alimentary absorption of water is in fact a dilution with hypotonic saline. It is likely that the effectual salt content of the added water determines its distribution between corpuscles and plasma, and may also affect the rate at which it can be taken up for temporary storage in the tissues.

8. The degree of hæmoglobin, plasma protein and total solid dilution has played no significant part in determining the urinary output during these experiments. On the contrary, it is probable that general dilution of the blood is partly determined by inadequacy of the rate of diuresis relative to the absorption rate from the alimentary canal.

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