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THE EFFECT OF WATER DRINKING ON THE BLOOD  
COMPOSITION OF HUMAN SUBJECTS  
IN RELATION TO DIURESIS.

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INTRODUCTION.

THE results of experiments on animals [Smirk, 1932; Heller and Smirk, 1932 *a, b*] and on the rate of water absorption in man (see the previous paper) taken together afford evidence as to the relation between water absorption, water storage, blood composition and diuresis in man. The problem, however, of the relation between the water content of blood and diuresis is complex, and its experimental investigation is difficult.

In experiments on water diuresis the study of blood dilution is usually concerned with:

- (1) How much of the additional water given is stored in the blood?
- (2) Does any change take place in the water content of the blood which would be likely to affect the kidney? (on the supposition that the kidney may be directly influenced by small changes in the water content of blood).

These problems are different, and the determination of the percentage by weight of water in blood is no certain measure of the amount of ingested water retained in the circulation.

For example, if a change takes place in the proportion of corpuscles and plasma as a result of splenic contractions [Barcroft, Harris, Orahovats, Weiss, 1925], posture [Thompson, Thompson and Dailey, 1928], or local passive congestion [Smirk, 1928], then, since the proportion of solid is greater in corpuscles than in plasma, the percentage water content of a whole blood sample will decrease as the percentage by volume of the corpuscles increases. Again, if for any reason the amount of transudation from blood into lymph spaces is increased, then the water

content of the blood will depend upon the percentage of solids in the transudate which has been lost. In other words, an increase in the water content of blood, as determined by the percentage of solid residue after drying, may merely represent an increase in the proportion of plasma or a loss of one of the solid constituents of plasma such as the salt or protein. Moreover, two solutions each containing 95 p.c. of water and in one case 5 p.c. of protein and in the other 5 p.c. of NaCl are physically and physiologically very different solutions.

To give "blood dilution" a precise meaning it is, therefore, necessary to state the substance used as an index of dilution and the nature of the diluent.

#### THE SIGNIFICANCE OF VARIOUS INDICES OF BLOOD DILUTION.

##### *The plasma chloride.*

Dilution of the plasma chloride is for most practical purposes an approximate index of increase in the proportion of water molecules to total molecules in a protein-free filtrate of plasma. If the protein of plasma is also diluted analysis of the plasma chloride would give a slight underestimate of the increased water content of protein-free filtrate. Changes in the CO<sub>2</sub> content of blood will alter the distribution of Cl between plasma and corpuscles.

##### *The whole blood chloride.*

The whole blood chloride is uninfluenced by the CO<sub>2</sub> content of blood, but, since the plasma contains twice as much Cl as the corpuscles, it depends as much upon variations in the proportion of plasma and corpuscles as upon changes in the chlorine content of plasma. If blood is diluted with saline of Cl concentration less than plasma (0.6 p.c.) and greater than the whole blood chlorine (0.45 p.c.) then the plasma chloride falls and the whole blood chloride rises. A fall in whole blood chloride is, therefore, not necessarily an index of a diminished chlorine content in protein-free filtrate of plasma.

##### *The conductivity of plasma.*

The conductivity of whole blood depends largely upon the relative volume of plasma and red blood cells, but the conductivity of plasma depends for the most part upon the amount of dissociated electrolytes. The effect of plasma protein *per se* upon the electrical conductivity is slight in view of the large molecular weight and feeble dissociation.

A protein-free filtrate of plasma, however, will have a greater electrical conductivity than that of plasma owing to the presence of the same ionic concentration in a smaller volume of fluid.

*The total osmotic pressure of blood.*

This is the pressure required to prevent the diffusion of water into plasma, the water and plasma being separated by a membrane impermeable to all ions or molecules other than water. The pressure exerted by electrolytes will depend upon their molar concentration and the degree of electrolytic dissociation. The effect of non-electrolytes such as urea depends only upon their molar concentration. The total osmotic pressure may be ascertained indirectly by its effect upon the vapour pressure [Hill, 1930 a].

*The protein content of plasma.*

The protein content of plasma is probably the most reliable single index of general blood dilution and of the storage of water in blood. It is liable, however, to this error: in so far as additional water is stored as lymph the blood plasma will be deprived of the protein contained in that lymph. If the volume of transuded lymph is exactly replaced by the water entering from the gut, then a dilution of the plasma protein would result which would not be a true indication of the storage of additional water in blood.

Under certain circumstances this objection is not merely theoretical [Heller and Smirk, 1932 c].

*The percentages of hæmoglobin or of iron in whole blood* have a similar significance since the iron is almost entirely present as hæmoglobin. They depend upon the number of circulating red cells and, therefore, are probably influenced by splenic contractions, postural changes in blood volume, alterations in blood-pressure and local changes in the circulation of the part from which the blood sample is taken.

*The water content of whole blood as determined by drying* is largely controlled by the percentages of hæmoglobin and protein.

Since all the ordinary blood constituents are capable of independent variation, one cannot quite confidently deduce the amount of additional water stored in blood from change in the blood composition. On the other hand, when, after drinking water, a dilution of all the main blood constituents is present, the general blood dilution is most probably due to additional water stored in the circulating blood.

The remaining object was to ascertain if any change in the water content of blood was likely to affect the kidney directly.

Histologically it appears that the blood supplying the kidneys is contained in vessels. There is, so far as I am aware, no evidence that blood is in direct contact with renal tubule cells. The tubule cells are presumably reacting to lymph filtered from blood plasma; alternatively to the constituents of plasma which diffuse through the blood-vessel wall, or possibly to the changes in the composition of glomerular filtrate. If, then, water diuresis is the result of a change in the composition of blood, it must be a change in its diffusible constituents. Again, if the change in composition is one of dilution, rather than the addition or subtraction of some hormone-like substance, then the change is probably a dilution of the salts or electrolytes, or as Haldane expressed it, an increase in the diffusion pressure of water.

Dilution of the blood with a protein-free filtrate of blood would diminish the percentage of hæmoglobin in whole blood and of protein in plasma, and would probably increase slightly the amount of fluid bathing the renal cells—it would not, however, change appreciably the saline composition of this fluid.

On the other hand the withdrawal of salt from the blood—there is abundant evidence that this may occur—would leave the protein-free filtrate of that blood hypotonic; using Haldane's term, the diffusion pressure of water would be increased. Yet the removal of salt from the blood would not necessarily be accompanied by any blood dilution as estimated by the hæmoglobin percentage or the plasma protein, and is not inconsistent with concentration of these latter constituents.

A fall in the salt or electrolyte concentration does not necessarily imply a storage in the blood of any of the water given—it suffices that in its passage through the blood or as a result of the temporary storage of water in tissues some salt should pass out from the circulation.

#### *Experimental procedure.*

Doses of drinking water, 1000 to 1500 c.c., were administered by mouth to normal subjects.

Samples of blood were taken before and after the administration of the water. Capillary blood samples were taken from the fingers without compression and venous samples were taken without congestion. The absence of congestion is of considerable importance, since it has been already shown [Smirk, 1928] that changes in the blood composition are induced by congestion alone. Urine samples were taken at intervals, which varied with the nature of the test. The subjects were either

recumbent throughout the tests or else they were engaged in laboratory work and were seated only for short periods.

The following methods of analysis were used:

(1) Changes in the hæmoglobin percentage of whole blood were measured in two ways: (a) By colorimetric comparison of blood samples taken before and after the administration of water. 0.1 c.c. of each blood sample was diluted with 25 c.c. of tap water and converted to carboxy-hæmoglobin by passing coal gas. Using the diluted first sample as standard, the degree of dilution in the second sample was expressed as a percentage of the original blood concentration. (b) The iron content of blood was estimated by the methods described by Smirk [1927 a].

(2) Estimations of the plasma protein were made by Kjeldahl's method without correction for non-protein nitrogen.

(3) 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 thiocyanate in the presence of acetone as an end-point intensifier [Smirk, 1927 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<sub>2</sub> contents of the samples.

Heparine was usually employed as anti-coagulant.

## RESULTS.

### (1) *Control observations.*

#### (a) *Capillary blood samples.*

Capillary blood samples (0.1 or 0.2 c.c.) were taken at short intervals from pricks in the finger-nail beds made by a bayonet-pointed needle or by Clark's blood gun. The blood should flow freely. The iron p.c. was determined and the results have been recorded graphically on Fig. 1. It will be observed that most results agree closely, but that eight aberrant values are met in thirty-four determinations.

It is a common practice to obtain a more liberal supply of capillary blood by immersion of the hand in hot water. As a control on this procedure samples of blood were taken from the fingers shortly after immersion for 2-5 min. in hot or in cold water and the composition compared with that of samples taken before the hand was either heated or cooled.

Heating or cooling the hand almost always causes some increase or decrease in the hæmoglobin concentration of capillary samples. The

direction of the change is much more frequently an increased hæmoglobin percentage after heating and a decreased percentage after cooling the hand. In one subject the reverse was obtained, and in a few people the results have been irregular.

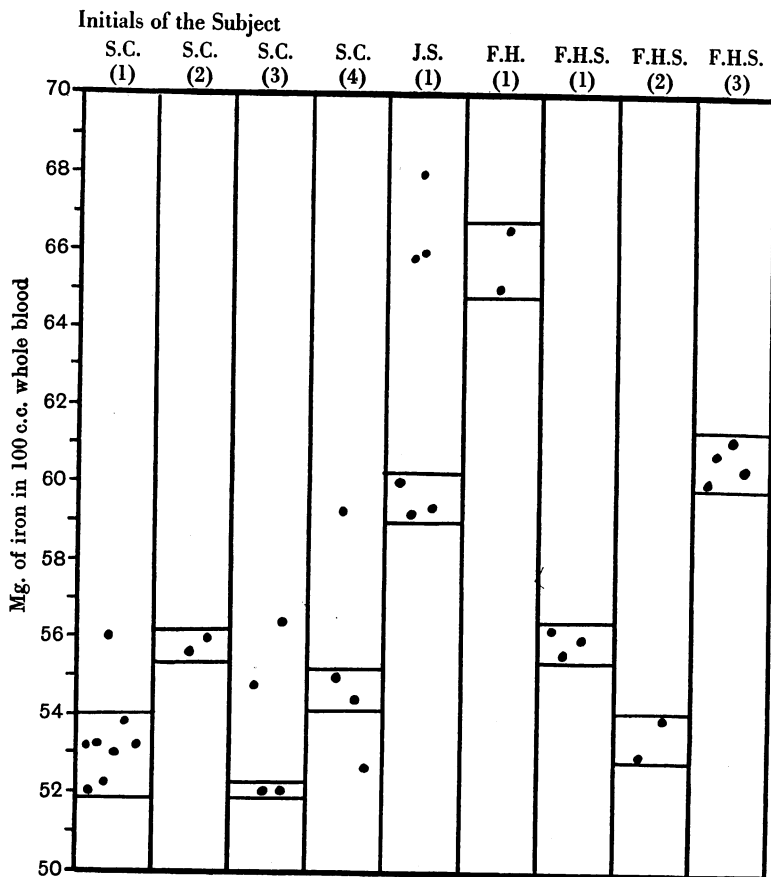


Fig. 1. The normal variability in the composition of capillary blood samples taken from finger pricks.

(b) *The procedure of sampling venous blood.*

*Errors resulting from the congesting or heating of a limb from which a venous blood sample is taken.* A separate investigation of this subject was made by Smirk [1928].

Congestion increased the concentration of hæmoglobin and caused a transference of chlorine ions from plasma to corpuscles.

Heat produced a venous blood which was bright red and the hæmoglobin content of the samples was sometimes raised.

It was concluded that venous samples taken with momentary congestion were satisfactory if subsequently they were shaken with air to remove  $\text{CO}_2$ . If they were not shaken with air the  $\text{CO}_2$  content of the blood would vary with the circulation rate through the limb, and so also would the distribution of chlorine ions between plasma and corpuscles.

(c) *An effect of meals on blood composition.*

It was thought advisable to discover whether meals might affect the blood composition in a way which would lead to misinterpretation of results.

In five out of six experiments an increase in the whole blood chloride was observed during the hour following a meal, in which no fluid or salt as such were taken. The whole blood chloride usually returned to normal in 2 or 3 hours.

Dodds and Smith [1923] reported a slight fall in the serum chloride after test meals, but for this they used a pint of liquid. It is possible that my results are due to a dilution of blood with tissue fluid. The increased proportion of plasma would cause an increase in the whole blood chloride.

It is clearly important to wait at least 3 hours after a meal before administering water, as a recent meal might obscure blood dilution from other causes.

(2) *The results of water-drinking experiments.*

The changes in blood concentration after water administration (Figs. 2, 3, 4, 5) have been expressed as a percentage of the blood concentration before giving water. 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 as a 4 p.c. dilution. In Figs. 2, 3, 4, 5 the horizontal lines represent the initial blood concentration. The distances of the points below this line represent the degrees of dilution of individual blood samples expressed as a percentage of the average blood concentration before water was given. The vertical line represents the beginning of water administration. The amounts of water given were either 1000 or 1500 c.c. and the time taken to drink this was usually 5 min. The distances of the points to the right of the vertical line indicate the times after giving water at which the blood samples were taken. Figs. 2, 3, 4 and 5 summarize the results obtained

in fourteen experiments on nine subjects. Figs. 6, 7 and 8 give the results of three individual tests.

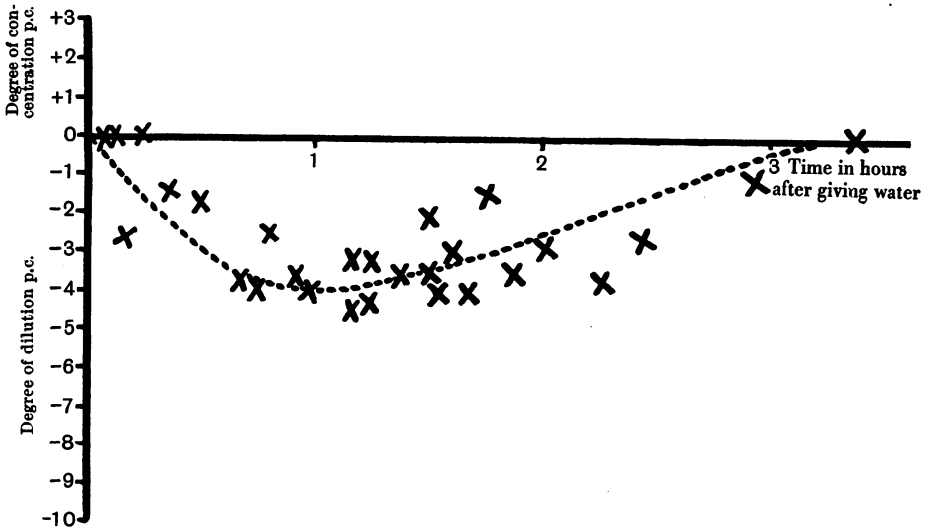


Fig. 2. The effect of water drinking on the whole blood chloride of capillary samples.

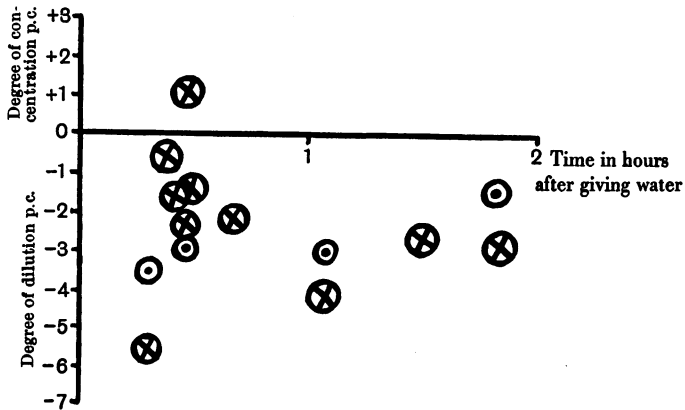


Fig. 3. The effect of water drinking on the p.c. of chloride in the plasma and whole blood of venous samples.

⊗ P.c. dilution of chlorine in plasma.    ⊙ P.c. dilution of chlorine in whole blood.

(a) *The effect of the administration of 1500 c.c. of water upon the whole blood chloride of capillary samples.*

Three experiments were made on the same subject. In each experiment (Figs. 6, 7) many samples were taken. The results of the three



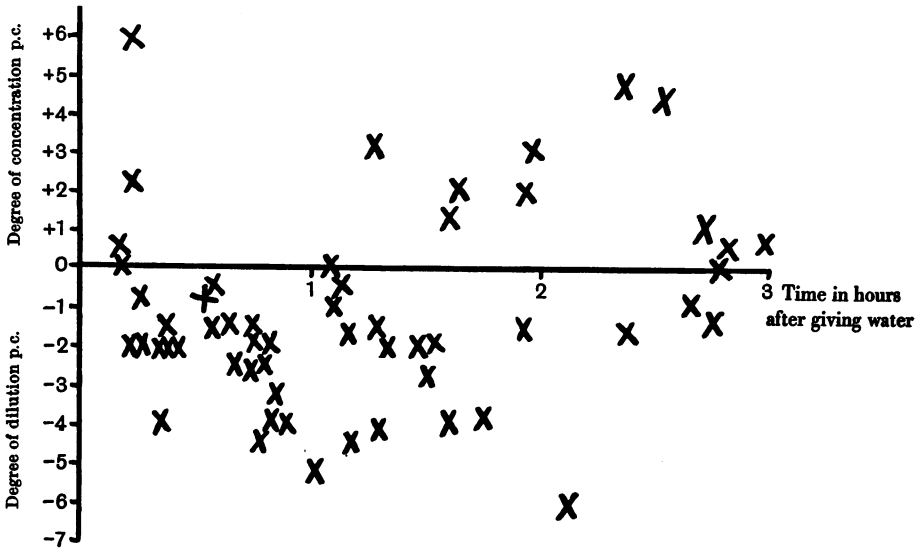


Fig. 4. The effect of water drinking on the p.c. of iron and hæmoglobin in samples of capillary blood.

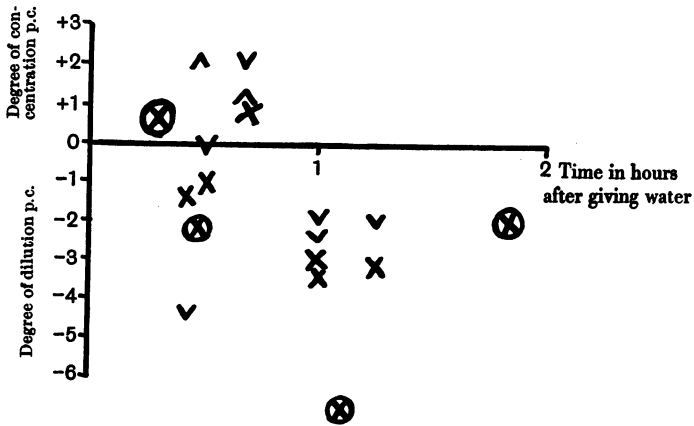


Fig. 5. The effect of water drinking on the p.c. of iron and hæmoglobin in whole venous blood and on the p.c. of protein in the plasma of venous blood.

- ⊗ P.c. dilution of iron in whole venous blood.
- × P.c. dilution of hæmoglobin in whole venous blood.
- v P.c. dilution of plasma protein.
- ^ P.c. dilution of the total solids in whole blood.

experiments are combined in Fig. 2. The chlorine content of whole blood is reduced, and judged by the distribution of the points on Fig. 2, as also by Figs. 6 and 7, the maximum degree of dilution appears at about 40 to 60 min. It will be seen that the maximum diuresis in these experiments was reached about  $1\frac{1}{2}$  hours after giving water. Therefore the maximum dilution of whole blood chloride may be attained before the maximum rate of urine formation and, indeed, before the rate of urine formation has been greatly increased.

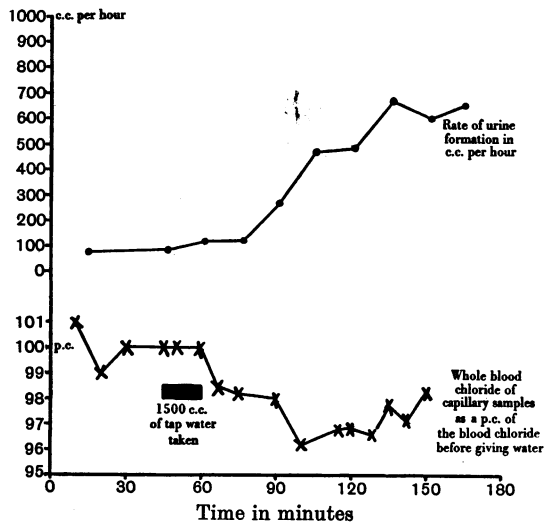


Fig. 6. The effect of water drinking on the whole blood chloride of capillary samples and on the rate of urine formation.

It will be observed also (Figs. 6, 7) that as many as four or five consecutive chlorine determinations are constant within chemical error at the time when the greatest change in the rate of urine formation takes place.

Capillary samples were also taken in a fourth experiment, in which 1000 c.c. of water were given to another subject. Some of the samples in this test showed slight concentration.

The degree of blood chloride dilution is greater than would be expected from an equal partition of additional water among the water-bearing tissues of the body.

(b) *The effect of the administration of 1000 to 1500 c.c. of water upon the plasma chloride and whole blood chloride of venous samples.*

Each point on Fig. 3 is the average of two or more chlorine determinations. It appears that there is a clear dilution of the whole blood and plasma chlorides of venous samples.

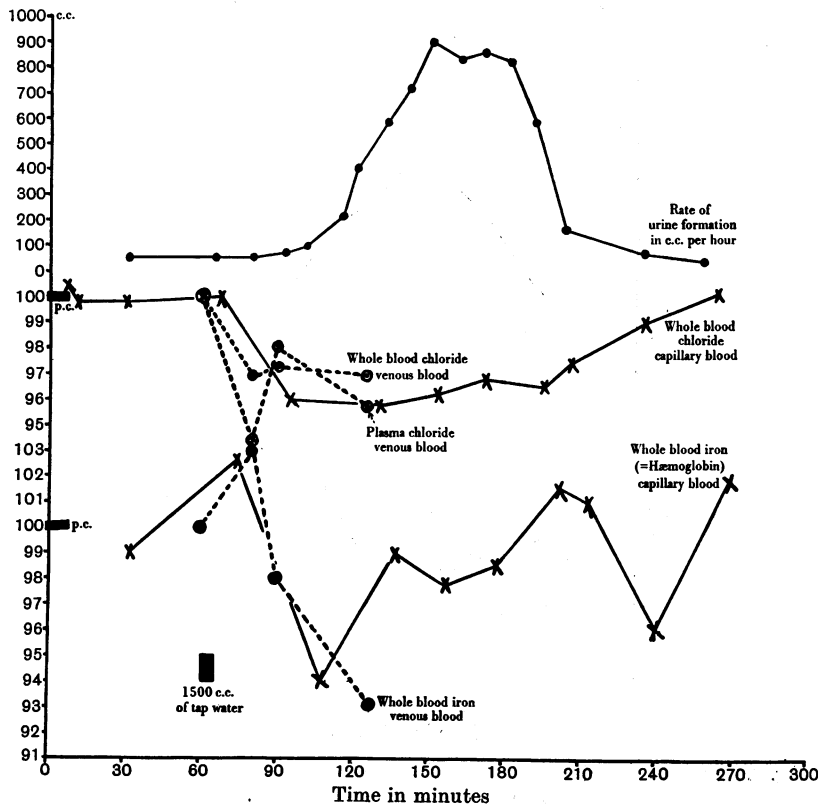


Fig. 7. The effect of water drinking on the whole blood and plasma chloride and on the iron percentage of whole blood, in relation to the rate of urine formation.

The degree of dilution is usually a little greater and sometimes definitely in excess of what would be expected from an equal partition of the additional water among the water-bearing tissues of the body.

Thus, if a 75 kg. man is composed of 60 p.c. water, the uniform distribution of a dose of 1500 c.c. among his water-bearing tissues would produce a blood dilution of about 2.7 p.c., and a dose of 1000 c.c. would produce a 1.8 p.c. dilution (assuming 80 p.c. water in blood).

(c) *The effect of the administration of 1000 to 1500 c.c. of water upon the hæmoglobin percentage of whole blood estimated in capillary samples.*

In Fig. 4 it will be observed that of fifty-five capillary samples removed for hæmoglobin or iron determinations at various times after giving water forty samples show dilution up to 6 c.c. and twelve samples show concentration, but between 20 and 70 min. after giving water twenty-four of the twenty-five samples then taken show dilution. Where

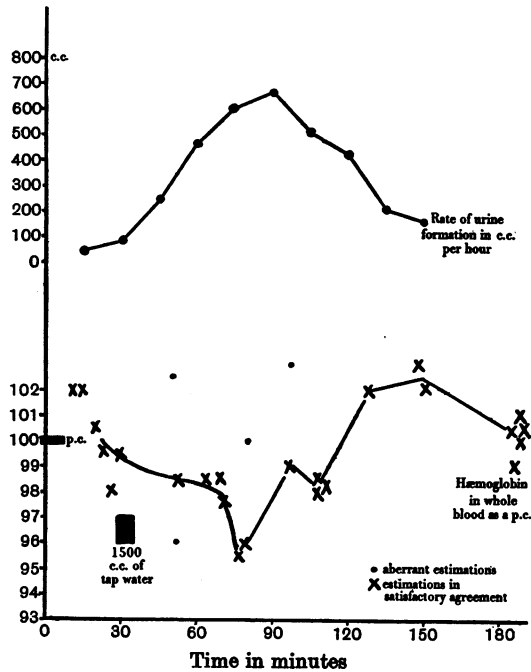


Fig. 8. The effect of water drinking on the hæmoglobin concentration in whole blood and on the rate of urine formation.

several capillary samples have been taken at short intervals of time the range of values obtained has been such as would be expected from the preliminary study of variability in capillary samples (Section 1 (a)); *i.e.* of four or five capillary samples we may expect close agreement in three or four. Applying this to Exp. 14, where a total of twenty-eight capillary samples was taken, four of the capillary samples (see Fig. 8) were clearly aberrant values. It is evident from the agreement of samples taken in triplicate that actual concentration of the hæmoglobin percentage has occurred towards the end of diuresis in Exp. 14 (Fig. 8).

The degree of blood dilution averages about 2 p.c., but may be as great as 6 p.c. It is seen from Fig. 4 and is also evident in the results of individual experiments that the degree of hæmoglobin dilution at any given time bears no proportional relationship to the rate of urine formation at that time.

In Exps. 3, 13 and 14 (Figs. 7, 8), performed on the subject F. H. S., the maximum dilution of hæmoglobin is met about 40 min. after giving water, and this is followed by a reconcentration during the period of rapid urine formation and is succeeded by a slight re-dilution. This is somewhat similar to the changes described by Marx and Mohr in man and in the dog [1927].

(d) *The effect of the administration of 1000 to 1500 p.c. of water upon the hæmoglobin percentage of whole blood, the dried weight of whole blood and the protein nitrogen of plasma estimated in venous samples.*

Fig. 5 shows that where venous samples are used an increased concentration in hæmoglobin and in the dried weight of whole blood (which depends mainly on hæmoglobin) is met in the first half hour after water administration. One hour after giving water a blood dilution of 2 to 3.5 p.c. appears to be the normal rule. Only two dilutions out of a total of eighteen observations are more than 4 p.c.

The initial increase in concentration of the non-diffusible blood constituents is probably not of methodical origin, the subsequent slight general blood dilution is, I think, unquestionably present in most experiments after water drinking.

#### DISCUSSION.

In the rat, guinea-pig and rabbit the relationship between water absorption, storage and diuresis has been considered by Heller and Smirk [1932 *a*, 1932 *b*], and between blood composition and diuresis in the rabbit [Smirk, 1932]. In the rabbit diuresis seemed unrelated to the degree of general blood dilution as estimated by the hæmoglobin percentage, the dried weight of whole blood and the plasma protein percentage. In the rat and also, though less certainly demonstrated, in the guinea-pig, water absorption is well in advance of diuresis, so that the maximum load of absorbed but as yet unexcreted water precedes the maximum diuresis by as much as 20–30 min. Also in the preceding paper it has been shown that there is a similar relationship between the water load and diuresis in man. It appears that, at any given moment, water diuresis is not proportional either to the degree of general blood dilution or to the excess of water then present in body tissues.

*The partition of water between blood and tissues.*

It is beyond question [Priestley, 1921 and others] that the changes which occur in blood composition after water drinking are small compared with the enormously increased output of urine. My results provide examples of the slight degrees of dilution obtained by Haldane and Priestley [1916] and, though less frequently, of the relatively great dilutions recorded by Marx and Mohr. It has been shown in the control observations that the reliability of any single capillary sample must be questioned, but where a consistent change has been observed in many capillary samples there is little chance of methodical error explaining the results. In venous samples methodical error is minimized when two or more blood constituents are used as indices of dilution, and simple chemical errors are avoided by duplicate or triplicate analysis.

I consider that in most of my experiments the variable blood dilution as determined by the analysis of capillary and venous samples represents an actual variability in the degree of dilution in circulating blood. The variations depend upon the subject of the experiment, the time after drinking water at which samples of blood are taken and upon the rates of absorption and diuresis. A similar conclusion was reached in rabbits.

Using capillary samples and hæmoglobin or iron as an index of blood dilution, a fall in hæmoglobin percentage is not invariable and concentration may be observed, as also in venous samples using the hæmoglobin percentage and the plasma protein. In both capillary and venous samples an increase in the hæmoglobin percentage is not infrequently met a few minutes after swallowing water. This rise in hæmoglobin was reported by Marx and Mohr. It has also been observed in the experiments where pituitrin was given subcutaneously in addition to the water. The plasma chloride was regularly diluted to between 1 and 6 p.c. The whole blood chloride was diluted in twenty-eight out of thirty-two samples, taken after water administration and four out of the four samples which failed to show dilution occurred in the same experiment. A slight increase of the chlorine content of whole blood is not inconsistent with chlorine dilution in plasma. (See Introduction.)

The degree of dilution measured both by diffusible and non-diffusible constituents is frequently greater than would be expected if all water given was absorbed and distributed uniformly throughout the water-bearing tissues of the body. This is still more convincingly illustrated in some of the experiments in the subsequent paper where diuresis has been prevented by pituitary hormone. One may quote here the results of

Margaria [1930] who observed a fall in the vapour pressure of blood after water drinking. The change in vapour pressure (expressed in terms of a sodium chloride p.c.) indicates a degree of dilution which is greater than would be expected if the water had been equally distributed among the water-bearing tissues of the body. Is this increased degree of dilution the result of delay in attaining equilibrium, or is the treatment of a new supply of absorbed water different from that of water already present and well established in body tissues? In other words, is only a part of the body water in a "labile" or "free" state so that the addition of a fresh supply of water produces a greater effect than would be expected from its proportion to the total water.

When the excretion of water is prevented by pituitrin hormone the excess of water in the blood is even greater, and a period of usually 40 or 50 min. passes before the blood has reconcentrated to something about what would be expected from an equal partition of water. If we assume that this degree of blood dilution is merely the expression of a time lag in the disposal of water, then one must comment upon the slowness with which equilibrium is established under certain circumstances.

In this respect Hill [1930 b] has shown that if we define "free" water as the weight of water in 1 g. of fluid or tissue which can dissolve substances added to it with a normal depression of vapour pressure, then practically all of the water in blood (98 or 100 p.c.) and 77 p.c. of the total 80 or 81 p.c. of water in frog's muscle is free. The preliminary experiments of Margaria have shown that at least part of a fresh supply of water absorbed from the alimentary canal also remains "free" to depress the vapour pressure. It would appear that any distinction there might be between a new supply of absorbed water and the water already present in tissues is not likely to be the "free" or "bound" state as defined by the power to dissolve substances with the normal depression of vapour pressure.

*The relationship between water absorption and blood dilution.*

As the distribution of extra water between blood and tissue varies, quantitative deductions as to the amount of water absorbed by any individual subject could not be made from changes in blood composition after water drinking. Yet it is evident in Exps. 1, 2, 3 and 4 where several blood samples have been taken that the maximum degree of whole blood and plasma chloride dilution is met as frequently in the second half hour after giving water as at any subsequent period. It has been shown in the previous paper that a litre of warm water taken by mouth probably will be

absorbed in 30–50 min. There is also some evidence that the water is more rapidly absorbed when the subject is fasting and in a sitting posture.

The maximum or almost the maximum chlorine dilution is usually met before or about the onset of diuresis. This has also been recorded by Rioch [1927, 1930] on dogs and on man. The electrical conductivity was used as an index of dilution and the changes observed may be fairly taken to indicate a diminished concentration of the chief electrolyte sodium chloride.

But the dilution of the diffusible chlorides may be produced as easily by chlorine leaving as by water entering the blood. The absorption of water and its storage in tissues may cause salt to be withdrawn from the blood in order to maintain this stored water in a more nearly isotonic condition. The storage of water in tissues after water administration has been demonstrated by Baer [1927], Tashiro [1926], Heller and Smirk [1932*a*]. If this alone caused chloride dilution the attainment of maximum dilution before diuresis is fully established would agree with the conclusion already drawn, that a large part of the administered water is absorbed before the urinary output is greatly increased. There remains, however, an alternative explanation for part of the chloride dilution; chlorides may enter the water in the alimentary canal prior to its absorption. In some experiments on rats in which 5 p.c. body weight of warm water was administered by stomach tube the chloride percentages in the residual water at a time when absorption was nearly complete were as follows: in the stomach 0.1 p.c. NaCl and in the intestine 0.55 p.c. NaCl. The total amounts of chlorine involved were calculated approximately. Translated into human terms they would be sufficient to alter measurably the chlorine content of blood. We are not aware of the relative surfaces of alimentary mucous membrane per kg. body weight in the rat and man, and it is probable that passage of chlorine to the water in the alimentary lumen plays a smaller part in man. Controls showed that about half the chloride found might be accounted for by chloride already present in the alimentary tract. Nevertheless, this process may explain part of the chloride dilution. The hæmoglobin and plasma protein percentages are uninfluenced however by migration of chlorine into the intestinal contents. In Fig. 7 it will be observed that degrees of dilution recorded are as great in the second half-hour period after water administration as at any subsequent period. This observation is consistent with the conclusion that a considerable absorption of water has taken place before diuresis is established (see the preceding paper).



*The relationship between blood composition and urinary output.*

It will be clear from Figs. 3 to 8, and Exps. 1 to 12 also show that the maximum degree of blood dilution is usually attained between 30 and 60 min. after giving water as would be expected from the duration of water absorption (see the previous paper). At this time the rate of urine formation has increased but slightly, the maximum rate being attained 20 or 30 min. later. If then the maximum dilution both of diffusible and non-diffusible blood constituents occurs usually some 20 min. before the maximum diuresis, then at any given moment the rate of urine formation will not be proportional to the degree of blood dilution then present. This is the equivalent of the results of Rioch who observed that the plasma chloride is roughly in inverse proportion to the diuresis if we make allowances for a lag of some 20–30 min. between the changes in blood and urine. He concludes from this that the most probable cause of water diuresis is the electrolyte dilution.

While supporting the observations of Rioch for many reasons I do regard this parallelism with a lag as evidence that the electrolyte dilution and water diuresis are causally related. For, once a dilution of blood has been established as a result of water absorption, the degree of dilution may be expected to diminish as the excess of water is excreted. In other words, the mere fact that absorption is in advance of diuresis explains the dilution of various blood constituents and the presence of a lag between blood dilution and diuresis.

Nevertheless, for reasons outlined in the Introduction, it seems that any change in blood composition which is to influence the kidney cells is probably a change in the diffusible constituents of blood. It has been shown, however [Heller and Smirk, 1932 b], that if rats are depleted of 5 p.c. body weight of water or rabbits of 4 p.c. body weight of water and then this water is given back to them by stomach tube, a diuresis ensues which is not much less than that of control animals receiving 5 or 4 p.c. body weight of water without previous depletion. What is it that determines the typical water diuresis in these animals? There is no excess of water in the body as a whole, and so far as we can tell there has been no excretion of salt. Salt excretion would account for electrolyte dilution if the salt and water lost were replaced by water alone. It is said that rabbits do not sweat, and it certainly appeared in our experiments that the water was excreted through the respiratory system. It may be said that, although in these experiments there is no excess of water in the body as a whole, an excess is present in the blood and this determines

diuresis. Clearly the initial electrolyte dilution could start a diuresis; the difficulty is to explain why it should continue when this temporary excess of water in the blood has been excreted by the kidneys. At this stage there is no excess of water either in the blood or in the tissues, and so far as one can say there is likewise no electrolyte dilution either in the plasma or in the tissues.

I do not consider that this experiment invalidates the theory that electrolyte dilution or an increase in the diffusion pressure of water is the cause of diuresis. It might well be that it is the change in electrolyte percentage rather than the absolute concentration which determines diuresis. As an analogy may be quoted the action of muscarine upon the heart in which it is the passage of the drug into and not the presence within the cell substance which determines its activity.

In man the loss of water by sweating is accompanied by a marked reduction in the urinary output. But in sweating both water and salt are lost. The conditions, therefore, are not the same as in the water-depletion experiments on rats and rabbits. It is highly probable, however, that the processes leading to water diuresis are essentially similar in mammals. This difficulty in accepting the absolute level of the diffusion pressure of water as the factor controlling water diuresis applies equally whether we consider that it operates upon the kidney directly or by some indirect mechanism such as the removal of the inhibitory influence of the pituitary hormone [Verney, 1929]. Verney's hypothesis does not assume, of course, that changes in the absolute level of the water-diffusion pressure control the activity of the pituitary gland.

But unless these experiments are explained by some factor which we have overlooked, it would appear that any satisfactory theory concerning the mechanism of water diuresis must ultimately show how in these animals, with no excess of water, the body is able to distinguish between water that has been well established in the body and a fresh supply of water. Why is it that in rats and rabbits a fresh supply of water is excreted although it is manifestly needed to replace water already lost, and it is eliminated at a time when there is no excess of water in the body?

Perhaps when water is first absorbed it is not in the same "state" as when it has been in the body for some time. For example, there may be a relatively slow penetration to within the tissue cells, or a time interval before the new water is taken up by colloids, so that this new water may be more available or perhaps is more in the tissue spaces and less in the cells. For a period of time it can perhaps exert some function which well-established water can perform less readily. Thus its presence might

influence the tissue of the pituitary gland and check the secretion of its anti-diuretic principle. Alternately a fresh supply of water may pick up some hormone-like body which is directly or indirectly the cause of the renal response and would explain why, in this particular case, the response is independent of the immediate needs of the organism.

These speculative suggestions are yet, and may remain, without foundation. They perhaps serve the purpose of indicating more clearly the nature and difficulties of the problem: why is much dilute urine passed after drinking water?

#### SUMMARY.

1. In man the drinking of 1 to 1.5 litres of water is usually associated with dilution of the blood as estimated by the plasma chloride, whole blood chloride, whole blood iron, hæmoglobin and plasma protein.

2. The maximum degrees of dilution are found as early as 30–60 min. after giving water. This agrees with the previous conclusions as to the time expended in water absorption.

3. The fall in concentration of various blood constituents is often greater than would be expected from an equal partition of water among the water-bearing tissues of the body.

4. When blood dilution is determined by estimating the chloride or hæmoglobin percentage in whole blood or by the protein in plasma, there appears to be no relationship between the rate of urine formation and the degree of blood dilution present at the time when the urine was formed.

5. Together with previous work on animals these observations throw light upon, and are discussed in relation to, the cause of water diuresis.

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