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THE EFFECT OF WATER DRINKING, MINERAL STARVATION AND SALT ADMINISTRATION ON THE TOTAL OSMOTIC PRESSURE OF THE BLOOD IN MAN, CHIEFLY IN RELATION TO THE PROBLEMS OF WATER ABSORPTION AND WATER DIURESIS

By EDWARD J. BALDES[1] and F. H. SMIRK[2].

(*From the Department of Physiology and Biochemistry, University College, London, and the Medical Unit, University College Hospital.*)

(*Received April* 27, 1934.)

Introduction.

The object of this paper is firstly to study the relationship between the total osmotic pressure of the blood and water diuresis; and secondly, in the light of previous papers, to present an account of water diuresis in man and lower animals.

The relationship between T.O.P.[3] and diuresis after water drinking has been studied in some detail, and we describe experiments in which changes in the T.O.P. are produced by mineral starvation and by giving salt.

Experimental procedure. With the human subject, blood samples were taken from the ante-cubital veins by a strictly uniform technique. The skin was cleaned and sterilized with absolute alcohol. The veins were tapped smartly to make them prominent and, after a period of about 5 sec. of venous congestion, induced by pressure of the subject's free hand upon the upper arm, a dry sterile needle connected to a dry record syringe was inserted into the vein and a sample of 4–10 c.c. of blood was removed without delay. The sample was defibrinated by shaking with glass beads for 12 min. In order to remove small particles of fibrin the de-

[1] Mayo Foundation and Mayo Clinic. [2] Beit Memorial Fellow.

[3] Total osmotic pressure.

fibrinated blood was filtered through a filter paper perforated with small holes into a small glass bottle with a ground-glass stopper. All blood samples were kept in the refrigerator for at least 6 hours before the osmotic pressure was measured. The reason for this was that small changes in the T.O.P. of the whole blood took place during the first 3 or 4 hours after it was removed. In the absence of bacterial contamination the osmotic pressure of the blood remained steady after the fifth hour. The measurements of osmotic pressure were made by Hill's [1930*a*] vapour-pressure method and are given in terms of an isotonic NaCl solution. Adopting the precautions of Baldes [1934] the accuracy is about a tenth to a fifth of 1 p.c. As our measurements were made on whole blood and at 5 p.c. CO_2 tension no special care was taken to prevent loss of blood gases.

When the subjects consumed water, it was given to them at 37° C., and on these occasions, except for emptying the bladder, they were kept at rest in the sitting posture for some hours before and during the experiment. To obtain further uniformity the subjects were given about 700 c.c. of water 3 or 4 hours before the test diuresis, and it was arranged that the test dose of water was given at a time when the rate of urine flow had just subsided to normal. In this way we tried to ensure comparable degrees of tissue hydration at the outset of each experiment. The importance of this has been demonstrated by Klisiecki, Pickford, Rothschild and Verney [1933].

Subjects were placed on the mineral-free diet for 8 or more days. This diet was prepared by Miss Marshall, the lady dietitian at University College Hospital, to whom our thanks are due. Its main constituents were:

Sugars.

Salt-free butter, shortbread and biscuits.

Vegetables boiled in distilled water (including tomatoes and onions in small quantity).

Jelly prepared by a method described by Aitkin [1929].

The total calorific value of this diet is 1885. There are 316 g. carbohydrate, 72 g. protein, and 37 g. fat.

The mineral content of a day's food is probably well within the limit set out in the following table:

	g.		g.
Calcium	0·10	Phosphorus	0·32
Magnesium	0·10	Chlorine	0·23
Potassium	0·89	Sulphur	0·48
Sodium	0·17	Iron	0·003

When salt was administered it was usually given as a 5 p.c. solution. The urinary chlorides were determined by the method of Volhard.

A few experiments were performed on rats. The method of handling them has been described previously by Heller and Smirk [1932].

Results.

The effect of water drinking on the total osmotic pressure of the blood. In man, after drinking a litre of water, there is a fall in the T.O.P. of the defibrinated blood (Fig. 1). This fall continues to take place rapidly for 25–45 (average 35) min. after drinking water, and may continue more slowly until 35–60 (average 48) min. after drinking water.

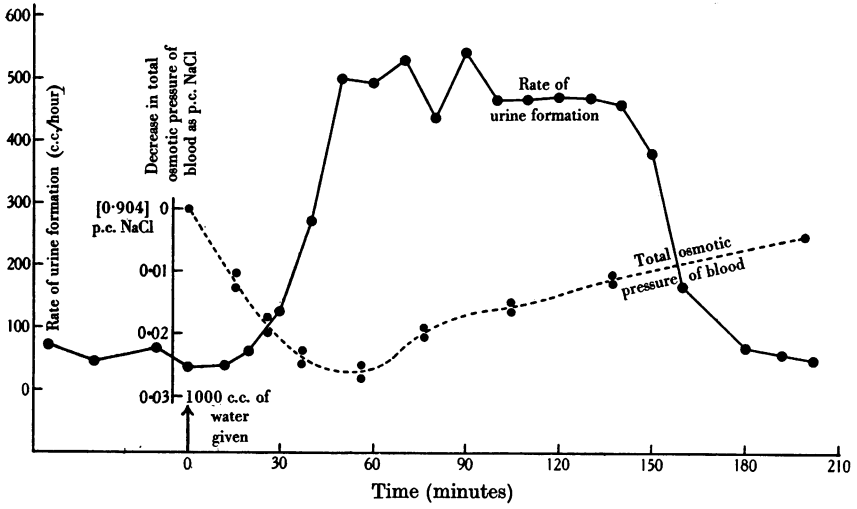

Fig. 1. The relationship between the T.O.P. of the blood and the rate of urine formation after giving water.

These changes in the T.O.P. resemble changes already described in the chlorine percentages of plasma and whole blood [Smirk, 1933*b*]. The fall in the T.O.P. of the blood would appear to reflect the absorption of water from the gut (Fig. 2), for the period of time in which the T.O.P. of the blood is falling corresponds with the absorption time for one litre of water measured in man by the abdomen- and leg-weighing and other methods [Smirk, 1933*a*, *b*, *c*].

In subjects weighing 64–75 kg. the average fall in the T.O.P. lies between 1·5 and 2·75 p.c. of the T.O.P. Thus the maximum degree of dilution may be less and in one instance has been slightly greater than would be expected if all the water was absorbed, retained and distributed

equally among the water bearing tissues of the body. If we assume that 60 p.c. of the body weight is water, then it is probable [Hill, 1930*b*; Hetherington, 1931] that 59 p.c. of the water in the body is in a free state. On this basis, with even distribution, 1 litre of water when ab-

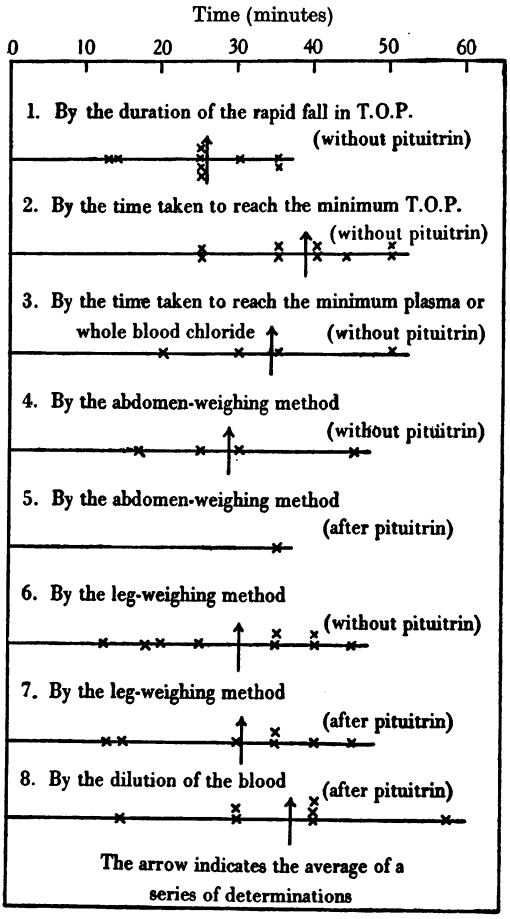

Fig. 2. The time for absorption of 1 litre of water in man as determined by different methods.

sorbed by a 65 kg. man should cause a fall of about 2·63 p.c. in the T.O.P. of the blood. In two subjects weighing 65 kg. the observed changes are between 2·08 and 2·77 p.c. in five experiments.

The relationship between total osmotic pressure and diuresis. In all our experiments it is clear that a considerable fall in the T.O.P. of the blood takes

place before there is an appreciable increase in the rate of urine flow. Usually the maximum dilution of blood precedes the maximum urine flow (Fig. 1) and corresponds with the water absorption time in man determined in other ways (Fig. 2). It is also evident that a constant rate of urine flow may be maintained while the T.O.P. of the blood is changing considerably (Fig. 1). These observations indicate clearly that the changes in urine flow in water diuresis do not depend on the momentary changes in the T.O.P.

The effect of a mineral-poor diet on the total osmotic pressure of the blood and on the renal excretion of water. If the subject is placed on a mineral-

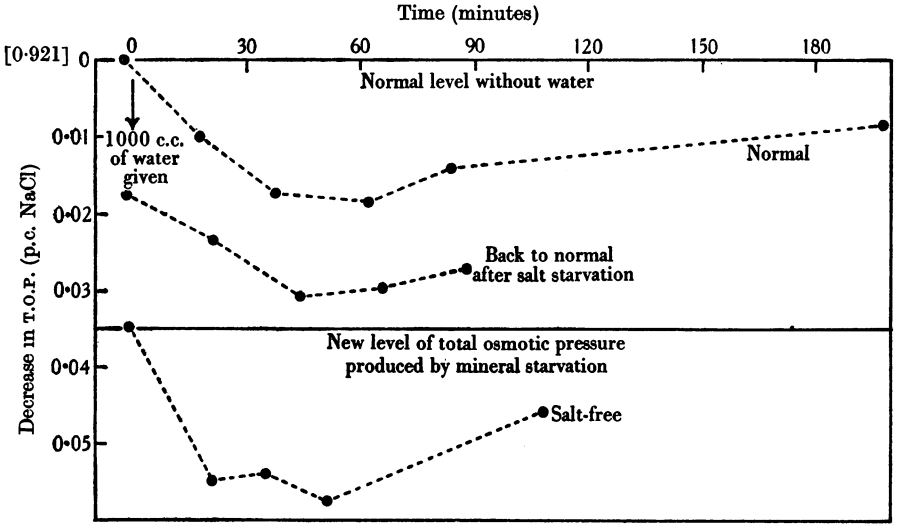

Fig. 3. The effect of water administration and of a mineral-free diet on the T.O.P. of blood.

poor diet for a period of 7 or 8 days and is made to lose salt by sweating, there is a fall in the osmotic pressure of the blood to a new level (Fig. 3). In two subjects the fall in osmotic pressure amounted to 0·0375 and 0·0265 g. NaCl per 100 g. H_2O which is greater than the falls in osmotic pressure produced acutely in the same subjects by drinking 1 litre of water. In a third subject the fall in the T.O.P. was not so great as this (0·017) but was greater than the fall in the T.O.P. produced by drinking a half-litre of water (0·010).

These gradual falls in the T.O.P. of blood produced by mineral starvation do not give rise to a state of diuresis, whereas the acute fall in the T.O.P. produced by drinking $\frac{1}{2}$–1 litre of water caused a considerable increase in the rate of urine flow.

If, after a period of salt starvation when the T.O.P. of the blood is already low, a dose of water is given there is a further acute fall in the T.O.P. of the blood and then, after the usual delay, a diuresis results. It is evident, therefore, that the kidneys are able to respond by diuresis to the administration of water, and the absence of diuresis when a low T.O.P. is produced by mineral starvation is not to be explained by total loss of the capacity to have a water diuresis. Yet it is clear from our results that the organism has not retained fully its capacity for diuresis, because in all subjects on a mineral-poor diet the rate of urine flow after drinking 1 litre of water was much less than in the same subjects when on a normal diet (Fig. 4).

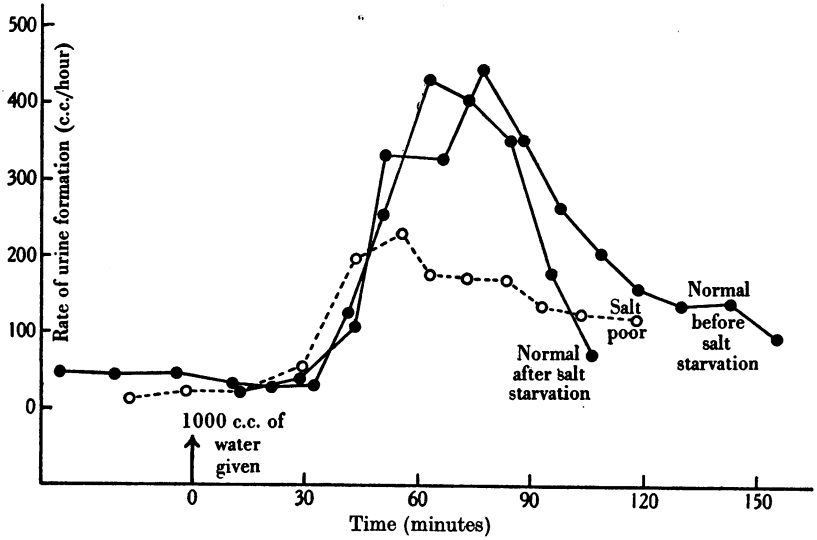

Fig. 4. The effect of salt starvation on the rate of urine formation.

In one subject, for example, three successive litres of water were given. Despite this great excess of water in the body the urine flow reached only a third of the value attained as the result of one litre either before deprivation or after restoration of salt. The urine flows after the second and third doses of water were actually less than after the first dose.

The effect of salt administration on the total osmotic pressure of the blood and on the renal excretion of water. Normal subjects were given 500 c.c. of 5 p.c. salt solution which they consumed in a period of 2 hours, starting 20 min. before breakfast or lunch. Three hours later the bladder was emptied and after this several urine samples and one blood sample were collected. The subjects then consumed 1 or 2 litres of water and urine

samples were collected at 10 min. intervals and blood samples at approximately 20 min. intervals.

The changes in urine flow which we encountered resemble those described by Baird and Haldane [1922] in that a diuresis sometimes but not always followed the administration of water. In the one subject where a diuresis was obtained the administration of water was seen to have produced the typical fall in the T.O.P. of the blood, but as the T.O.P. had been raised previously by giving salt, the giving of water did not cause it to fall below the normal range (Fig. 5).

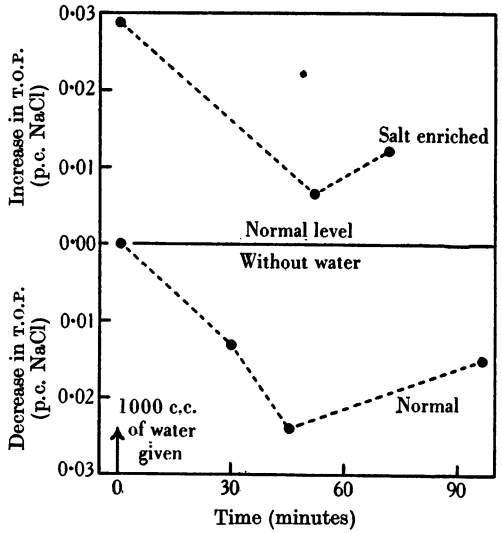

Fig. 5. The effect of water administration on the T.O.P. of blood in the salt-enriched subject.

It appears from this and the previous section that it is not the actual level of the T.O.P. of the blood that determines the onset of diuresis, since a diuresis was induced when the T.O.P. of the blood after water drinking was not sub-normal and also a low T.O.P. of the blood due to salt starvation did not induce diuresis.

The effect of salt administration on the total osmotic pressure of the blood and on the renal excretion of water in rats. This section was undertaken partly to obtain additional evidence on the matter referred to in the previous section, and partly to explain some previous experiments made on rats. A series of 24 white rats were placed on a uniform diet of dry oats with 0·2 p.c. salt solution to drink. Twenty-four hours before making an experiment they were given distilled water to drink instead of 0·2 p.c. salt solution.

A series of 6 rats were placed in small cages and the rates of urine flow were observed. Their necks were well cleaned with absolute alcohol and, when the urine flow was normal, they were stunned by a blow on the head and about 4 c.c. of blood were obtained by severing the carotid arteries with a razor. Coagulation of the blood was prevented by heparin, and loss of CO_2 was prevented by closing the collecting tube with a rubber stopper. The T.O.P. of the plasma of these blood samples was then determined.

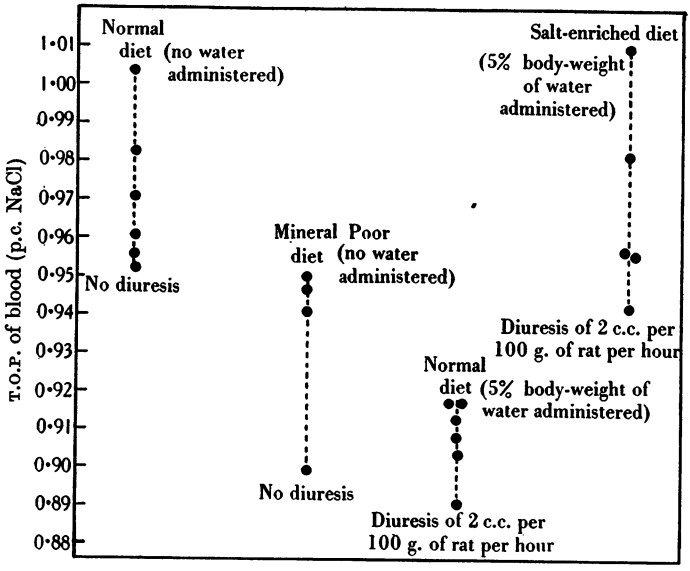

Fig. 6. The effect of water administration on the T.O.P. of plasma in normal and salt-enriched rats.

Another set of 6 rats were prepared as before but, when the rate of urine flow was basal (about 0·1 c.c. of urine per 100 g. of rat per hour), each animal was given 5 p.c. of its body weight of warm water by stomach tube. The rates of urine flow were observed, and as each rat attained a rate of 2 c.c. of urine per 100 g. of rat per hour it was killed and a sample of blood was collected as before.

A third set of 6 rats were given 5 p.c. of their body weight of 5 p.c. salt solution. The urine flow was increased by this excess of salt, but when it subsided a dose of 5 p.c. body weight of water was given.

Despite the great excess of salt which remained in the body this dose of water produced a typical water diuresis, and as the rate of urine flow

attained 2 c.c. per 100 g. of rat per hour, the animals were killed and a blood sample collected.

The results are summarized in Fig. 6. It will be seen that there is evidence of a considerable blood dilution after water drinking, the average dilution being probably 7 p.c. This finding is supported by a few micro-determinations of chlorine and iron in blood samples taken with regional anæsthesia of the tail before and after giving water.

One of the 6 rats which received a dose of 5 p.c. salt solution excreted a great part of the salt administered before the salt diuresis ceased. The remaining 5 rats retained much of their salt, and when the dose of water was given subsequently it did not suffice to bring the T.O.P.'s below the normal range of osmotic pressure as judged by the 6 normal rats. Nevertheless, these salt-enriched rats had a copious water diuresis. It is of interest to note that despite the excess of salt in the blood the urine excreted after giving water contained a low percentage of salt, in all cases well below the percentage of salt in the plasma.

In the remaining rats an attempt was made to reduce the T.O.P. of the blood by mineral starvation. There appeared to be a slight fall in the T.O.P., but the experiments were not as satisfactory as were the corresponding experiments in man.

Discussion.

The composition of the urine depends upon what the kidney does with the blood supplied to it. It is evident (Fig. 1) that the characteristic delay in the onset of diuresis does not depend on delay in the absorption of water, for it is in the latent period that most of the change in blood composition takes place. During this latent period the excess of water in the blood has not caused diuresis, but later when the degree of dilution in the blood is actually less there is a rapid excretion of much watery urine. In other words the excretion of watery urine is not caused by the excess of water then present in the blood but by a change in the kidneys; until this change in kidney activity has taken place, despite blood dilution, there is no diuresis.

In water diuresis the excretion of water in the urine is increased out of proportion to any other urine constituent so that the T.O.P. of the urine falls well below the T.O.P. of the blood. The greater the urine flow in water diuresis the greater is the separation between the T.O.P.'s of the blood and urine, and the more work performed in its formation.

We may deduce then that this change in the kidney which is responsible for water diuresis involves the per-

formance of secretory work and the exercise of selective activity: namely, the selective excretion of water. It appears more reasonable to consider secretion and selection as attributes of the tubule cells rather than of the glomeruli which appear to be filters. Therefore we are inclined to think that this functional change responsible for water diuresis is located in the renal tubule cells. There seems no escape from the conclusion that water diuresis is not the result of an automatic excretion of whatever excess of water is then present in the blood. It is due to change in the activity of the kidney. This change involves the selective excretion of water and is not instantaneous but takes time to develop.

We must now consider the nature of the stimulus that induces the change in kidney activity responsible for water diuresis. The central problem is whether the excess of water in the blood acts directly upon the kidney or through some intermediate mechanism. While the direct hypothesis has the advantage of simplicity it has appeared to many that some of the indirect hypotheses which assume the intervention of an intermediary offer a more complete explanation of the phenomena of water diuresis. Various aspects of this problem are discussed in papers by Klisiecki, Pickford, Rothschild and Verney [1933], Heller and Smirk [1932] and Smirk [1933*b*]. It has been shown by Newton and Smirk [1933, 1934] that the process of water diuresis takes place in a normal manner after the removal of the alimentary canal and the liver (water given intravenously), the pituitary gland and all the brain tissue lying above the tentorium cerebelli which includes the hypothalamus. These experiments seem to be irreconcilable with the views that

1. Water passing through the portal system extracts a diuretic hormone which is the primary cause of water diuresis [Cow, 1914].

2. That water diuresis is controlled by the pituitary gland, the changes in the rate of urine flow being an expression of changes in the amount of pituitary hormone present in the circulating blood [Motzfeldt, 1917; Starling and Verney, 1925; Verney, 1926; Klisiecki, Pickford, Rothschild and Verney, 1933].

3. That it is the expression of changes in the hypothalamus and that this is an essential part of the mechanism for controlling water diuresis.

It is not suggested or thought that these experiments exclude participation of any or all of the above-mentioned mechanisms in the process of water diuresis, when it takes place in the whole animal. What we would

emphasize is that, after removal of these organs, the diuresis after giving water has not differed notably from the normal.

It is quite possible that some other entirely unsuspected intermediate mechanism is responsible for water diuresis, but so far we have been unable to discover it. Therefore, while remembering that there may be an unsuspected indirect mechanism, we will turn to the direct hypothesis of Haldane and Priestley, that the rate of urine formation is controlled by the diffusion pressure of water in blood, in other words by the T.O.P. of the blood. Priestley [1921] showed, and his results have been confirmed many times, that after water drinking the electrical conductivity of plasma is reduced and there is a fall in the plasma chloride. There was reason to believe that there was also, at the same time, a fall in the T.O.P. of plasma: that is a fall in the crystalloidal plus the colloidal osmotic pressure. Margaria [1930], using Hill's vapour-pressure method, verified this deduction and we have confirmed his findings in this paper.

There appears to be a constant association between water diuresis and dilution of the blood as judged by the electrical conductivity, plasma chloride and the T.O.P. of the plasma. While the rate of urine formation is not proportional to the degree of blood dilution then present we agree with Rioch [1930] that usually diuresis is proportional to the electrolyte dilution if we make allowance for a lag of some 20–30 min. between the changes in blood and urine.

But the constant association between dilution of the diffusible constituents of the blood and water diuresis is not sufficient evidence that they are causally related, for such dilution is the logical consequence of the absorption of water, provided always that excretion does not take place immediately. Likewise this parallelism with a lag is no evidence of a causal relationship, 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. The fact that absorption is in advance of diuresis explains the dilution of various blood constituents as also the lag between blood dilution and diuresis. Also we have shown (Fig. 1) that this parallelism with a lag is not always present.

When we reduced the T.O.P. of the blood by mineral starvation, we were able to maintain for some days a lower level of osmotic pressure than we produced by giving 1 litre of water. This gradual change in the T.O.P. of the blood when produced by mineral starvation did not cause diuresis. It must be emphasized that the fall in T.O.P. from mineral starvation indicates an increase in the proportion of water molecules to total molecules and that, as after water drinking, there is a fall in the

plasma chloride. It appears therefore that it is not the actual level of the T.O.P. of the blood that determines the onset of diuresis.

The converse of this experiment was performed once in man and several times in rats. The T.O.P. of the blood was first raised above the normal by the administration of salt. It was found that when the T.O.P. was reduced again but not below the normal level, by drinking water, a diuresis often resulted without there being an excess of water molecules in the blood. Again this indicates that water diuresis is not initiated or controlled only by the level of the osmotic pressure of blood. This last experiment appears to explain the observation of Heller and Smirk [1932] 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.

It seems not unlikely that the change in osmotic pressure is of greater importance than the absolute value in determining diuresis. It is evident that given time the kidney is able to adjust itself to a limited change in its environment, and it may be that it resembles the thermal receptors in the skin which indicate a rise or fall in skin temperature more accurately than the actual temperature.

It is of interest that the diuresis after water is much less in mineral-starved and in salt-enriched subjects than in subjects with a normal salt intake, but we are unable to offer any explanation of this at present. It should be noted, however, that equal falls in the T.O.P. of the blood produced by giving equal doses of water caused a smaller diuresis in salt-depleted subjects. Must we regard this as evidence that water diuresis does not depend upon a sudden change in the T.O.P. of the blood, or must we say that mineral starvation so alters the response of the kidney to its natural stimulus (an excess of water molecules) that the diuresis is greatly reduced?

At present the phenomena of water diuresis seem best interpreted by the hypothesis that a mechanism which resides in the kidney is stimulated directly as a result of a sudden increase in the proportion of water in the blood; there is however a delay before the kidney responds by diuresis to this stimulus.

SUMMARY.

1. Water diuresis is not the result of an automatic excretion of whatever excess of water is then present in the blood. It is due to change in the activity of the kidney, and if the glomeruli are filters this change in activity must be located in the renal tubules. The change in the activity of the kidney involves the selective excretion of water and requires time for its development.

2. In man a fall in the level of the T.O.P. of the blood if produced gradually by mineral starvation does not induce a state of diuresis. If the osmotic pressure of the blood is first raised artificially, a dose of water then given may cause diuresis although the T.O.P. of the blood has not fallen below the normal limits. The significance of these findings is discussed.

3. The changes in the T.O.P. of the blood after water drinking enables an estimate to be made of the absorption time for water in man. Such estimates agree with those previously obtained by the abdomen- and leg-weighing methods.

We wish to thank Prof. A. V. Hill for his advice during the course of the present investigation and the facilities which enabled us to make the measurements of vapour pressure in his laboratory. We are also indebted to Mr J. L. Parkinson for technical assistance.

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