LXI. STUDIES ON KIDNEY FUNCTION.

II. THE EXCRETION OF UREA AND CHLORINE ANALYSED ACCORDING TO A MODIFIED FILTRATION-REABSORPTION THEORY.

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In the preceding paper the filtration-reabsorption theory was discussed and it was concluded that the present state of the theory was such that a modification was needed.

The main cause for the present crisis in the filtration-reabsorption theory is, I think, the way in which the threshold substances are defined. According to the theory as usually accepted all substances which are present in the reabsorbed fluid in more than insignificant amounts are termed threshold substances. The consequence of this definition has been that now practically all substances must be regarded as threshold substances, a view which makes the whole theory seem absurd. The reason for this is that in the present form of the theory no distinction has been made between active reabsorption and mere back-diffusion. In the modified form put forward in this paper the substances supposed to be present in the reabsorbed fluid are divided into two classes: (1) substances present because of active reabsorption by the tubule cells, or threshold substances proper, and (2) substances present because of mere back-diffusion, which are not threshold substances at all. To make clear the working hypothesis on which the experiments, dealt with in this paper, were started I shall ask the reader to make in his mind an experiment illustrating the process in the tubules according to my views.

Suppose we have a tube through which we send fluid containing in solution a mixture of substances all present in the same concentration. We suppose water to be absorbed by the tubule walls during the passage. If now the walls of the tube are supposed to be impermeable to all the substances, all of them will evidently be concentrated to the same extent. But if we suppose the wall to be extremely permeable to some substances, slightly permeable to others and impermeable to some, then these last will evidently be concentrated as before—the first will practically not be concentrated at all and the rest will be concentrated differently according to the ease with which they diffuse through the walls. Let us send through the tube 1000 cc. of

fluid containing in solution 1 mg. % of each of the five substances: *A*, *B*, *C*, *D* and *E*.

The tube walls are supposed to be impermeable to *A*, increasingly permeable to *B*, *C* and *D* and extremely permeable to *E*.

Let us now suppose 900 cc. of water to be absorbed during the passage through the tube. The 100 cc. of fluid left will contain all of the substance *A* which will amount to a 10 mg. % solution and only one-tenth of *E* or a 1 mg. % solution as before. The three other substances will be more or less concentrated, and between one-tenth and the whole amount of the substance will be present in the fluid. Suppose that we absorb 990 cc. instead of 900. *A* will be concentrated a hundred times, *E* still practically not at all and the three others between one and a hundred times. The concentration in which one of these three substances is present will be higher than in the preceding example but the total amount will be less because more has diffused back, as the concentration rose. According to a process like the one assumed, the fluid issuing from the tube will therefore contain the different substances in concentrations which are equal to or higher than in the original fluid, whilst the fluid which has been reabsorbed contains the substances in concentrations varying from zero to one compared with the original fluid.

Let us now suppose part of the tubule walls to possess the ability to absorb actively one of the substances, *D*. In this case the concentration of *D* may become much less in the fluid issuing from the tube than in the original fluid, whilst in the reabsorbed fluid the concentration is higher. It is therefore possible to distinguish, by means of the concentrations in the resulting fluid, between active reabsorption and loss by diffusion.

How would such a process apply to the formation of urine? We should filter out fluid from the glomeruli; in the tubules there must be cells for reabsorption of water, sugar, sodium and perhaps other substances. These substances, which are supposed to be absorbed by specialised cells and which may almost disappear from the urine and may be present in the reabsorbed fluid in a concentration higher than that of the blood, would be the threshold substances. The rest, the no-threshold substances, should always be present in the urine if present in the blood, but in varying concentrations according to the ease with which they diffuse back through the tubule walls; the concentration ratios however must in each case be related to one another according to certain laws. The main difference between this formulation of the theory and that of Cushny is that according to Cushny a substance is a threshold substance if it is present in the reabsorbed fluid—according to the formulation here given a substance may be present in the reabsorbed fluid in large amounts as a result of mere diffusion—it is only a threshold substance if the percentage in the reabsorbed fluid can under certain circumstances be higher than in the blood. It is in reality not a mere question of the definition of the term threshold substance but at the same time an accentuation of the difference between active reabsorption and diffusion. This way of distinguishing

between threshold substances and no-threshold substances brings back again among the latter all substances which, since Cushny, have been transferred to the threshold group. Perhaps it will even be possible to reduce Cushny's original threshold group considerably, so that, compared with the present state of the theory, we attain a real simplification. The theory, moreover, has the same advantage as Cushny's that it is possible to test it quantitatively. It should be possible—as demonstrated in the preceding paper—to estimate the amounts of filtrate and the amounts of the different substances filtered. If we compare these with the amounts excreted we should be able to decide if there is a difference in the reabsorption of threshold substances and no-threshold substances, as we should expect if, in the first case, we have to do with active reabsorption and in the other with mere back-diffusion. We should, moreover, be able to decide whether the threshold substances as thus defined are reabsorbed, as Cushny suggested, in a constant concentration.

Is it possible that diffusion can occur to such an extent? I think that good reasons can be given in support of such an assumption. If the organism were able to make a wall which was impermeable for the substances, the elimination of which constitutes the work of the kidney, we should expect the wall of the bladder to be equipped with this ability too. But the bladder has been clearly demonstrated to be permeable to at least some of the substances concerned to a not insignificant extent [Vickers and Marshall, 1924].

Moreover, if we compare the concentration of alcohol and acetone in the blood and in the urine we find that the concentration is practically the same [Widmark, 1915, 1920]. These substances must therefore, if filtration has occurred, have been present in the reabsorbed fluid in the same percentages as in the blood. They resemble the substance E of our example. According to the old definition they should be threshold substances which were reabsorbed; according to the new definition they are no-threshold bodies to which the tubule walls are extremely permeable. It must be admitted that these substances are in a class by themselves; but, when diffusion takes place with these substances to such an extent, why should it not play a part in the excretion of other substances too? Here the back-diffusion is so considerable that it lowers the concentration in the urine to practically the same percentage as in the blood; should the tubules then really be impermeable for all other no-threshold substances? Should we not expect the tubule walls to be permeable for these substances in the same way as the bladder? If they are permeable even to a very slight extent the immense surface (more than 50,000 cm.^{2} if we assume 2,000,000 kidney units) will make diffusion a factor of importance.

In order to calculate the concentration of a substance in the fluid reabsorbed from the glomerular filtrate during the passage through the tubules, parallel estimations of at least two substances are required. One of these substances must be a no-threshold substance to which the tubule walls may be taken as impermeable. For reasons given in the preceding paper creatinine

has been chosen as basis for the calculations, which are carried out as follows.

Suppose F to be the amount of filtrate, U the amount of urine, $Cr_P\,\%$ the percentage of creatinine in the plasma and $Cr_U\,\%$ the percentage of creatinine in urine then

$$(1)\quad \frac{F \times Cr_P\,\%}{100} = \frac{U \times Cr_U\,\%}{100}.$$

This gives (2) $F = \frac{Cr_U\,\%}{Cr_P\,\%} \times U = C \times U$ when $\frac{Cr_U\,\%}{Cr_P\,\%} = C$ (index of concentration); or, in words: by means of a substance creatinine, which is supposed not to diffuse back, we calculate how much the urine has been concentrated during its passage through the tubules (our index of concentration). This multiplied by the amount of urine gives the amount of filtrate formed. And for the substance B, which we want to study, we get

$$(3)\quad \frac{F \times B_P\,\%}{100} = \frac{R \times B_R\,\%}{100} + \frac{U \times B_U\,\%}{100},$$

where R is the amount of fluid reabsorbed and $B_R\,\%$ is the percentage of the substance B in the reabsorbed fluid.

Now we have

$$R = F - U = C \times U - U = (C - 1)\,U$$

and (3) therefore gives

$$B_R\,\% = \frac{F \times B_P\,\% - U \times B_U\,\%}{R} = \frac{C \times U \times B_P\,\% - U \times B_U\,\%}{(C-1)\,U},$$

or

$$(4)\quad B_R\,\% = \frac{C \times B_P\,\% - B_U\,\%}{C-1}.$$

The basis for this formula is the assumption given above that all of the creatinine present in the glomerular filtrate is also present in the urine. But this is only an assumption and is perhaps not true for any substance present in urine. How will it affect our formulae if the assumption is erroneous? Evidently the urine has then been more concentrated than our index of concentration shows, more filtrate has been formed, more of the substance B has filtered out and more has diffused back. The formula given for $B_R\,\%$ (4) may be written

$$(5)\quad B_R\,\% = \frac{C}{C-1}\,B_P\,\% - \frac{1}{C-1} \times B_U\,\%.$$

If in this formula C increases, the first term $\frac{C}{C-1} \times B_P\,\%$ will approach the value of $B_P\,\%$ and the second term $\frac{1}{C-1}\,B_U\,\%$ will approach zero, that is, with increasing index of concentration and constant $B_P\,\%$ and $B_U\,\%$ the value of $B_R\,\%$ will tend towards the value of $B_P\,\%$: or if part of the creatinine used in our calculations has diffused back our calculated value for $B_R\,\%$ will differ more from $B_P\,\%$ than the true value.

With this in mind we are able to use the formula even if the creatinine on which the calculations are based partly diffuses back into the blood during the passage through the tubules.

In the present paper two series of experiments are given; one dealing with a substance regarded as a no-threshold substance, urea; the other with a substance universally agreed to be a threshold substance, chlorine.

The excretion of urea.

The aim of this series of experiments was to study the excretion and especially the "reabsorption" of urea in varying conditions; diuresis, high blood urea, etc.

The variations in the excretion were induced by different means. In some experiments only the creatinine needed for the index determination was taken, in others the ingestion of creatinine was followed by large doses of urea in order to drive the urea-content of the blood up above the normal. Sometimes the ingestion of urea was followed by water drinking in order to get dilute urines with high blood urea values, or the water was drunk without previous urea ingestion to get a water diuresis with normal blood urea.

The arrangement of the experiments was as stated in the paragraph on creatinine excretion. The determination of the urea in the plasma was carried out by means of the micro-urease method described in an earlier paper [Rehberg, 1925, 1].

The determination of the urea in the urine was made by the ordinary Van Slyke method with parallel determination of the ammonia.

As examples of the experiments I shall give the following protocols with the resulting calculations.

Time		Time	
9.30	5 g. creatinine + 200 cc. of water taken	1.37	4th sample of urine, 68 cc.
10.10	20 g. urea + 200 cc. of water	2.05	5th sample of urine, 189 cc.
11.02	1st sample of blood	2.15	4th sample of blood, 400 cc. of water
11.06	bladder emptied	2.27	6th sample of urine, 335 cc.
11.42	1st sample of urine, 73 cc.	2.54	7th sample of urine, 520 cc.
12.05	2nd sample of blood	3.18	5th sample of blood
12.10	2nd sample of urine, 43 cc.	3.33	8th sample of urine, 279 cc.
12.15–40	lunch + 400 cc. of fluid	3.57	9th sample of urine, 49 cc.
12.58	3rd sample of urine, 53·5 cc.	4.15	6th sample of blood
1.09	3rd sample of blood, 200 cc. of water		

Result of analysis.

Sample	Creatinine mg. per 100 cc. plasma	Urea mg. per 100 cc. plasma	
1	8·14	1	$\left\{\begin{matrix}57·6\\57·3\end{matrix}\right\}$ 57·5
2	6·93	2	$\left\{\begin{matrix}49·3\\49·3\end{matrix}\right\}$ 49·3
3	6·58	3	$\left\{\begin{matrix}47·3\\41·7\end{matrix}\right\}$ 44·5
4	4·82	4	41·8
5	4·33	5	$\left\{\begin{matrix}39·2\\40·5\end{matrix}\right\}$ 39·9
6	3·46	6	$\left\{\begin{matrix}38·8\\40·0\end{matrix}\right\}$ 39·4

Sample	Creatinine mg. per 100 cc. urine	Urea-N + NH_3-N mg. per 100 cc. urine	NH_3-N mg. per 100 cc. urine	Urea mg. per 100 cc. urine
1	515	1000	17	2103
2	582	1178	28	2460
3	861	1470	47	3025
4	426	1112	36	2300
5	97	314	12	646
6	40	141	3·2	295
7	35	123	3·1	256
8	68	233	10·9	475
9	177	622	28	1250

By means of the values from the blood analyses curves of plasma creatinine and plasma urea are drawn. On these curves are marked the intervals corresponding to the different urine samples and the values of plasma creatinine and plasma urea corresponding to these time intervals are read off from the curves (Fig. 1).

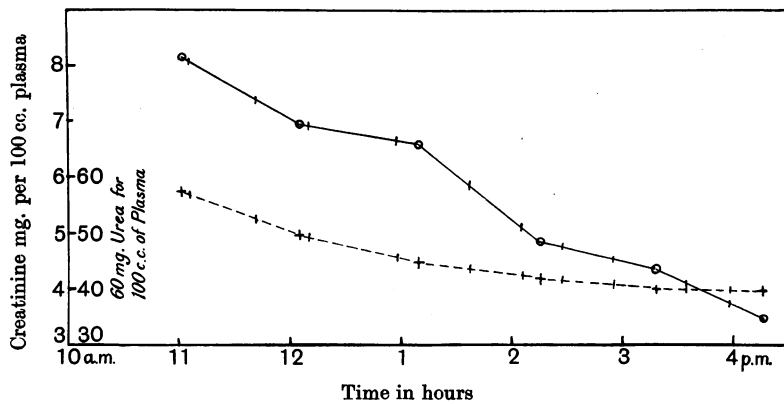

Fig. 1. Curves of plasma creatinine (○) and plasma urea (+).

The calculations are now carried out according to the formulae given on p. 464.

(1) The mg.-percentage of creatinine in the urine divided by the corresponding mg.-percentage in the blood gives the concentration ratio of creatinine or the concentration index of the urine $\left(C = \frac{Cr_U\,\%}{Cr_P\,\%}\right)$.

(2) The concentration index multiplied by the amount of urine per min. gives the amount of filtrate per min. ($C \times U = F$).

(3) The amount of filtrate per min. less the amount of urine per min. gives the amount of fluid reabsorbed per min. ($R = F - U$).

(4) The amount of filtrate multiplied by the percentage of urea in plasma gives the filtered amount of urea $\left(\frac{F}{100} \times U_P\,\% = F_U\right)$.

(5) The filtered amount of urea less the amount excreted gives the amount reabsorbed ($F_U - U_U = R_U$).

(6) The reabsorbed amount of urea divided by the amount of reabsorbed fluid gives the percentage of urea in the reabsorbed fluid

$$\left(\frac{F_U}{F} \times 100 = U_R\,\%\right).$$

(7) The amount of urea excreted divided by the amount filtered gives the percentage of the filtered amount of urea which is excreted

$$\left(\frac{U_U}{F_U} \times 100 = E_U\,\%, \text{ excretion percentage}\right).$$

Table I.

Urine sample	Creatinine mg. per 100 cc. urine	Creatinine mg. per 100 cc. plasma	Concentration index $\frac{Cr_U\,\%}{Cr_P\,\%}$	Amount of urine cc. per min.	Amount of filtrate cc. per min. $C \times U$	Amount of fluid reabsorbed cc. per min. $F - U$	Urea mg. per 100 cc. plasma
	$Cr_U\,\%$	$Cr_P\,\%$	C	U	F	R	$U_P\,\%$
1	515	7·75	66·5	2·03	135	133	54·60
2	582	7·10	82	1·54	126	124·5	50·50
3	861	6·70	129	1·11	143	141·9	47·25
4	426	6·30	67·6	1·74	118	116·3	44·30
5	97	5·50	17·6	6·75	119	112·3	42·80
6	40	4·85	8·25	15·22	126	110·80	41·60
7	35	4·60	7·6	19·25	146	126·8	41·00
8	68	4·35	15·6	7·16	112	104·80	40·00
9	177	3·90	45·3	1·26	57	55·8	39·60

Urine sample	Urea mg. per 100 cc. urine	Urea filtered mg. per min. $F \times U_P\,\%$	Urea excreted mg. per min. $U \times U_U\,\%$	Urea reabsorbed mg. per min. $F_U - U_U$	Urea % in the reabsorbed fluid $R_U \times 100$	Filtered urea % which is excreted $\frac{U_U}{F_U} \times 100$	Concentration ratio of urea $\frac{U_U\,\%}{U_P\,\%}$
	$U_U\,\%$	F_U	U_U	R_U	$U_R\,\%$	$E_U\,\%$	C_U
1	2103	73·6	42·75	30·85	23·2	58	38·6
2	2460	63·6	37·9	25·7	20·6	59	48·7
3	3025	67·5	33·6	33·9	23·9	50	64
4	2300	52·1	40·0	12·1	10·4	77	52
5	646	51·0	43·6	7·4	6·6	85	15·1
6	295	52·20	45·0	7·2	6·5	86	7·1
7	256	60·00	49·3	10·7	8·45	82	6·25
8	475	44·80	34·0	10·8	10·3	76	11·9
9	1250	22·60	15·75	6·85	12·3	70	31·6

If we now look at the details of this experiment we see that the plasma creatinine falls rapidly—that the water drinking induces a violent diuresis reaching the highest value observed in these experiments: 19·25 cc. per min. When we compare the amount of urine with the calculated amount of filtrate we see that, though the diuresis is accompanied by a rise in the amount of filtrate, the amounts formed are by no means especially high in this case (maximum = 146 cc.). The diuresis has mainly been of tubular origin and is brought about by a relative decrease in the reabsorption. In the last period recorded the filtration (and reabsorption) falls to the lowest value observed in these experiments (57 cc. per min.). Perhaps this means only that the kidneys are returning to a normal resting state, but it is possible that it is a depression similar to that observed by Barcroft and Straub [1910] in the

oxygen consumption of the kidney of the cat after injection of 0·5 g. urea; a depression which these authors ascribed to a toxic action of the urea, but which is perhaps only due to fatigue.

The most interesting columns of the table are the last three, (U_R %) the percentage of urea in the reabsorbed fluid, (E_U %) the percentage of the filtered urea which is really excreted, and (C_U) the concentration ratio for urea.

From column U_R % we see that urea is present in the reabsorbed fluid in a rather high percentage just as in Mayrs' experiments on rabbits. The percentage varies considerably, rising to a maximum of 51 % of the corresponding percentage in the plasma, when the urine is highly concentrated (period 3), and falling to a minimum of 15 % of the corresponding percentage in the plasma, when the urine is highly diluted (periods 5 and 6).

Because of this back-diffusion only part of the filtered amounts of urea is really excreted. In this experiment the excreted amount (E_U %) is seen to vary from 50 % to 86 %, the highest excretion percentage occurring when the urine is only slightly concentrated. The kidney appears to be less effective in excreting urea than in excreting creatinine, and for this reason the curve for blood urea does not show a fall as rapid as the curve for blood creatinine.

The last column (C_U) gives the concentration ratio for urea, which varies, of course, with the concentration index though it is invariably lower.

In another experiment in which no urea was taken the progress of the experiment was as follows.

Time		Creatinine mg. per 100 cc. plasma	Urea mg. per 100 cc. plasma
10.19	5 g. creatinine 200 cc. water		
10.57	1st blood sample	8·40	18·20
11.48	2nd blood sample	8·80	16·30
12.10–30	lunch, 300 cc. fluid		
12.40	3rd blood sample	7·25	17·40
1.45	4th blood sample	5·55	18·3
2.37	5th blood sample	5·10	17·0
3.27	6th blood sample	4·25	16·6
4.17	7th blood sample	3·70	17·85

By means of these values curves were drawn and the values corresponding to the time intervals of the urine samples were read off. These values together with the result of the urine analysis are given in the table. The calculations are carried out in the same way as in the preceding example. The result is presented in Table II.

In this experiment where no water or urea was ingested the concentration index is very high, increasing steadily throughout the experiment and reaching the maximum value observed; namely 293. The diuresis is very low and the filtration shows only small variations; there is especially no decrease with the decreasing diuresis. The small diuresis is caused by a large reabsorption. The blood urea is low, with but small variations during the experiment.

The percentage of urea in the reabsorbed fluid is considerable, rising from

Table II.

Urine sample	Time	Creatinine mg. per 100 cc. urine Cr_U %	Creatinine mg. per 100 cc. plasma Cr_P %	Concentration index $\frac{Cr_U\ \%}{Cr_P\ \%}$ C	Urine cc. per min. U	Filtrate cc. per min. $C \times U$ F	Fluid reabsorbed cc. per min. $F - U$ R
1	11.07–11.54	1258	8·65	145	0·67	97	96·3
2	11.54–12.50	1261	7·70	164	0·70	115	114·3
3	12.50–1.57	1111	6·20	179	0·78	140	139·2
4	1.57–2.49	1223	5·20	236	0·54	127	126·4
5	2.49–3.36	1096	4·50	244	0·51	124	123·5
6	3.36–4.26	1129	3·85	293	0·44	129	128·6

Urine sample	Urea mg. per 100 cc. plasma U_P %	Urea mg. per 100 cc. urine U_U %	Urea filtered mg. per min. $F \times U_P$ % F_U	Urea excreted mg. per min. $\frac{U \times U_U\ \%}{100}$ U_U	Urea reabsorbed mg. per min. $F_U - U_U$ R_U	Urea % in reabsorbed fluid $\frac{R_U}{R} \times 100$ U_R %	Filtered urea % which is excreted $\frac{U_U}{F_U} \times 100$ E_U %	Concentration ratio of urea $\frac{U_U\ \%}{U_P\ \%}$ C_U
1	16·90	1392	16·4	9·33	7·07	7·35	57	82
2	17·10	1436	19·70	10·06	9·64	8·44	51	84
3	18·0	1522	25·2	11·9	13·3	9·55	47	85
4	17·35	1840	22·05	9·95	12·10	9·55	45	106
5	16·70	1890	20·65	9·65	11·0	8·9	46·5	113
6	17·35	2080	22·4	9·16	13·24	10·3	41	120

a value of 44 % to 59 % of the corresponding percentage in the blood as the concentration index rises.

The excretion percentage is small owing to the high concentration of the urine; only about half of the amount of filtered urea is excreted, in the last period only 41 %. The concentration ratio for urea varies as before with the concentration index but to a smaller extent.

The results of these and a series of similar experiments are shown in the Figs. 2, 3, 4 and 5.

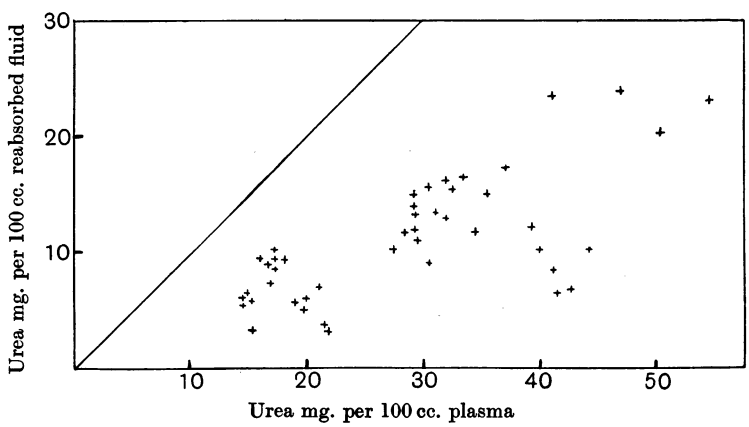

Fig. 2. The percentage of urea in the reabsorbed fluid.

On Fig. 2 is shown how the percentage of urea in the reabsorbed fluid varies with the percentage in the blood. The percentage in the reabsorbed

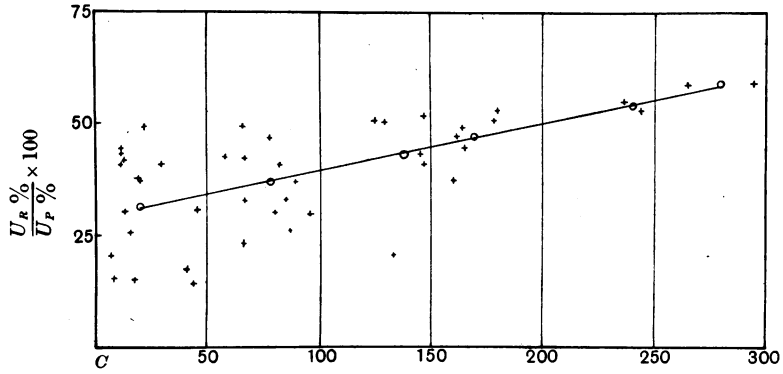

Fig. 3. $\frac{U_R\,\%}{U_P\,\%} \times 100$ with varying concentration index. The curve has been drawn through the mean values calculated graphically as the centres of gravity for the groups between the lines.

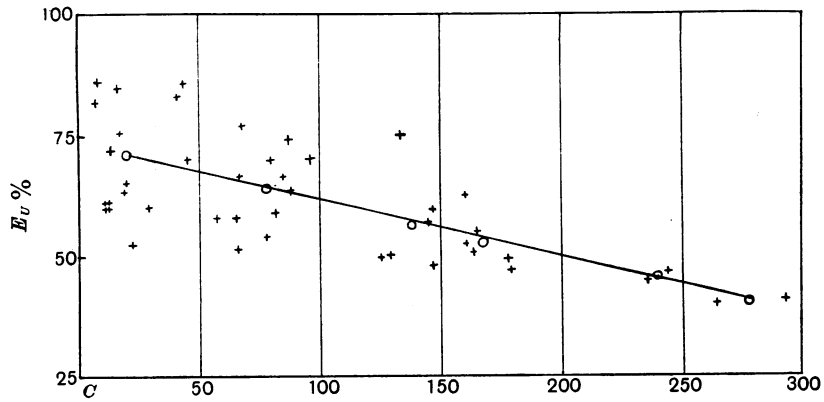

Fig. 4. The decrease in the excretion percentage with increasing concentration index. The curve drawn as in the preceding figure.

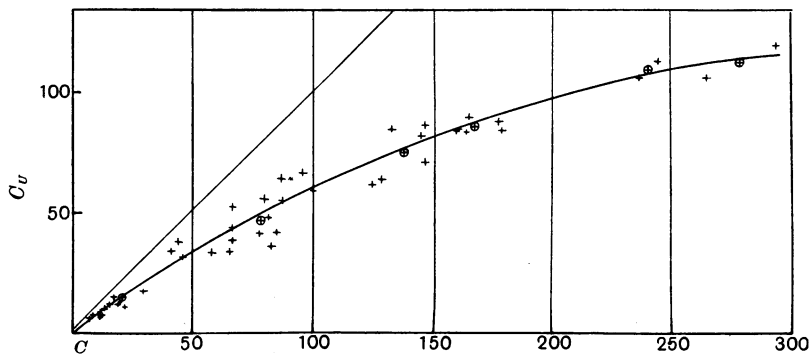

Fig. 5. The variation in the concentration ratio of urea with increasing concentration index.

fluid is always the lowest. In no single case is it necessary to suppose any active reabsorption. The result is in accordance with the result of Mayrs, previously mentioned, on rabbits, but the conclusion is different. Mayrs concluded, using Cushny's definition of threshold substances, that urea was a threshold substance reabsorbed actively because it was of value for the organism. According to the modified definition urea is a no-threshold substance present in the reabsorbed fluid because of back-diffusion.

In Fig. 2 it is impossible to see the influence of the concentration index on the reabsorption, but in Fig. 3 is shown how the percentage in the reabsorbed fluid approaches the percentage in the blood when the concentration index rises, though it never gets higher than about 60 % of the percentage in the blood.

In the next figure (4) are shown the variations in the excretion percentage with varying concentration index; that is, how large a percentage of the filtered amount of urea is excreted. The excretion percentage is on the whole much larger with low values for the concentration index than with high. The points in this and in the preceding figure are, however, very scattered. There are several reasons for this. One is that two of the estimations upon which the calculations are based do not give very accurate results. The urea, and especially the creatinine, determinations in plasma may have errors which are not insignificant. An error in the plasma creatinine determination of 5 % will give the same error in the calculated amount of filtrate, and if the error in the urea determination goes in the same direction the calculated E_U % may differ considerably from the true value.

For example, the determination of creatinine in the third blood sample in the experiment reported on p. 465 is probably erroneous. From the curve (Fig. 1) it seems that 5·90 mg. per 100 cc. instead of 6·58 mg. is more likely. If we use 5·90 mg. this will influence the values calculated for the periods 3, 4 and 5. Period 4, for instance, will give a concentration index of 74 instead of 68 and an excretion percentage of 70 instead of 77. In other cases the change in the excretion percentage may be even greater. Another possible reason is the error due to the amount of urine to be expelled from the "dead space." This error is, as demonstrated in the preceding paper, impossible to overcome. Moreover, some of the points which fall farthest away from the curves arise in periods where the rate of diuresis was rapidly changing. For example, the points corresponding to C 133 originate from a period with a diuresis of 1·18 cc. per min. whilst the preceding and subsequent periods gave 0·47 cc. and 12·12 cc. respectively. The high E_U % for the intermediate period may perhaps be explained as a washing out of concentrated urine from the tubules by the increasing filtration. Lastly, a large part of the variation is probably real and due to variations in the surface of the tubules.

In the next figure (5) is shown how the concentration ratio for urea $\left(C_U = \frac{U_U\,\%}{U_P\,\%}\right)$ follows the rise in the concentration index C without reaching

the same high values, so that when the urine has been concentrated 280 times the urea has only been concentrated about 120 times.

The result is in agreement with the working hypothesis, while I think it will be difficult to explain by the secretion theory that the extent to which urea is concentrated is dependent in this way on the extent to which creatinine is concentrated.

To summarise the results:

(1) the calculated percentage of urea in the reabsorbed fluid is always lower than the corresponding percentage in the plasma;

(2) it approaches the percentage in the plasma the more concentrated the urine is;

(3) that percentage of the calculated filtered urea which is excreted is the higher the less the urine has been concentrated;

(4) the urea is the more concentrated the more the creatinine is concentrated.

These four results are all in accordance with what could be expected if the excretion of urea is mainly a question of how much diffuses back through the tubule walls during the concentration process.

The excretion of chlorine.

The next series of experiments deals with the excretion of chlorine. The arrangement of the experiments was similar to that used in the experiments on urea. In some experiments only creatinine has been taken, in others large doses of NaCl were ingested in order to increase the Cl-content of the blood—sometimes the NaCl intake was supplemented by water drinking in order to get dilute urines corresponding to a high blood chlorine. In other experiments large amounts of water were drunk in order to obtain low values for the blood chlorine.

The value for blood chlorine to be used in the calculations is of course only the part present in the blood in a filterable form; that is, the amount present in the plasma of arterial blood. As the distribution of the total chlorine between corpuscles and plasma varies with the carbon dioxide tension, it was necessary to separate the plasma from the corpuscles with such precautions that this tension was not changed. This was done by collecting and centrifuging the blood under paraffin oil. The determination of the chlorine in the plasma was made in duplicate by the method described in another paper [Rehberg, 1926]. The difference between duplicate estimations was usually about 2–3 in the third decimal place and very seldom more than 5 mg. %.

The determination of chlorine in the urine was made in a similar way. In a 20 cc. flask were measured out 2 cc. of 0·15 N $AgNO_3$, 1 cc. of conc. HNO_3 and 5 cc. of a strong solution of ferric alum. To this was added an amount of the urine to be analysed varying from 0·5 cc. to 10 cc. according to the dilution of the urine and the expected concentration of chlorine. The

flasks were next filled to the mark with distilled water. The contents were shaken and left until the precipitate had settled. 1 cc. of the clear fluid was pipetted off in one of the small test tubes used in the blood analyses and titrated by means of 0·1 *N* thiocyanate with the micro-burette [Rehberg, 1925, 2]. Two or more samples from the same flask were titrated and the mean taken. The difference between two titrations does not exceed 1 % and is usually less.

As an example of the experiments the following two may serve .

Time		Creatinine mg. per 100 cc. plasma	Chlorine mg. per 100 cc. plasma
9.27	5 g. creatinine		
10.38	1st blood sample	8·30	371 371 } 371
11.30	2nd blood sample	7·78	371·6 373·0 } 372
12.30–50	lunch		
12.50	water drinking started		
1.03	3rd blood sample	5·13	366 369 } 368
2.03	4th blood sample	6·08	360 363 } 361
2.17	water drinking stopped (in all 3000 cc.)		
3.33	5th blood sample	3·83	365 369 } 367
4.33	6th blood sample	3·83	369 370 } 369

By means of these values curves of plasma creatinine and plasma chlorine were drawn. On the curves the time intervals corresponding to the urine samples were marked, and the values for plasma creatinine and plasma chlorine corresponding to these intervals were read off from the curves. These values together with the results of the urine analyses are given in Table III.

The object of this experiment was to get low plasma chlorine values. It was not very successful as the chlorine went down only from 372 to 361 mg. per 100 cc. The lowest value which I have been able to obtain in this way for the chlorine content of the plasma is 356 mg. per 100 cc.

The result of the water drinking was a very low concentration index while

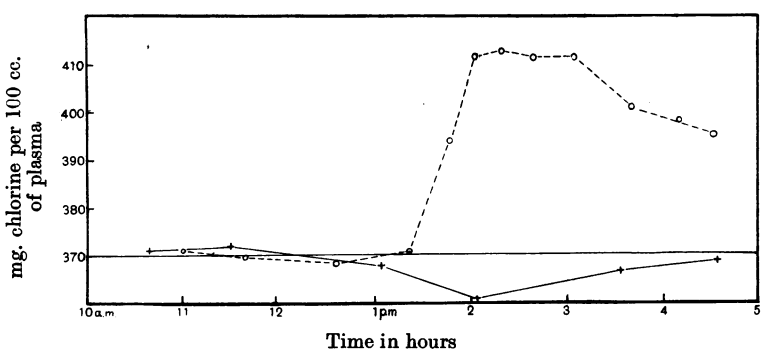

Fig. 6. The percentage of chlorine in the reabsorbed fluid (○) during an experiment with low plasma chlorine (+).

Table III.

Urine sample	Time	Creatinine mg. per 100 cc. urine	Creatinine mg. per 100 cc. plasma	Concentration index $\frac{Cr_U\ \%}{Cr_P\ \%}$	Urine cc. per min.	Filtrate cc. per min. $C \times U$	Reabsorbed fluid cc. per min. $F - U$
		$Cr_U\ \%$	$Cr_P\ \%$	C	U	F	R
1	10.44–11.15	907·0	8·05	113·0	1·77	200	198·2
2	11.15–12.01	992·0	7·40	134·0	0·96	128	127·0
3	12.01–1.09	1161·0	5·90	197·0	0·71	139	138·3
4	1.09–1.36	225·0	5·45	41·0	2·90	119	116·1
5	1.36–1.54	68·5	5·80	11·8	12·20	144	131·8
6	1.54–2.10	44·8	6·00	7·5	17·60	132	114·4
7	2.10–2.27	39·4	5·70	7·6	17·30	131	113·7
8	2.27–2.49	42·1	5·25	8·0	14·95	120	105·0
9	2.49–3.22	38·4	4·55	8·4	14·7	124	109·0
10	3.22–3.58	44·4	3·95	11·2	9·95	112	102·0
11	3.58–4.21	48·0	3·83	12·5	10·5	132	121·5
12	4.21–4.42	54·4	3·83	14·1	8·7	123	114·3

Urine sample	Chlorine mg. per 100 cc. plasma	Chlorine mg. per 100 cc. urine	Chlorine filtered mg. per min. $\frac{F \times Cl_P\ \%}{100}$	Chlorine excreted mg. per min. $\frac{U \times Cl_U\ \%}{100}$	Chlorine reabsorbed mg. per min. $F_{Cl} - U_{Cl}$	Chlorine % in the reabsorbed fluid $\frac{R_{Cl}}{R} \times 100$	Filtered chlorine % which is excreted $\frac{U_{Cl}}{F_{Cl}} \times 100$
	$Cl_P\ \%$	$Cl_U\ \%$	F_{Cl}	U_{Cl}	R_{Cl}	$Cl_R\ \%$	$E_{Cl}\ \%$
1	371·5	428·0	741	7·58	733·4	371·0	1·0
2	372	594·0	499	6·68	492·3	369·9	1·3
3	370	668·0	514·5	4·72	509·8	368·5	0·9
4	366·5	185·6	438	5·36	432·6	371·0	1·2
5	364·5	44·4	526·5	5·43	520·1	394·1	1·0
6	362	34·8	476·5	6·14	470·4	411·7	1·3
7	362·5	32·1	475	5·56	469·4	412·9	1·2
8	363·5	27·5	435	4·12	430·9	411·5	0·9
9	365·5	23·6	455	3·48	451·5	411·5	0·8
10	367	22·5	411	2·24	408·8	401·0	0·5
11	368·7	22·1	486	2·32	483·7	398·7	0·5
12	369·3	27·2	464	2·37	461·6	395·3	0·5

the amount of filtrate kept within the normal limits. Although the change in plasma chlorine was so slight the kidney reacted very distinctly towards it. The Cl percentage in the reabsorbed fluid, which at first was lower than in the blood, rose high above it when the Cl in the blood fell below 370 mg. % (cf. Fig. 6).

In other experiments sodium chloride was ingested in order to increase the Cl percentage in the plasma.

Time		Creatinine mg. per 100 cc. plasma	Chlorine mg. per 100 cc. plasma
9.19	5 g. creatinine		
9.53	10 g. NaCl		
10.55	1st blood sample	7·58	380
11.13	10 g. NaCl		
11.54	2nd blood sample	5·87	395
12.50	3rd blood sample	5·07	399
12.55–1.10	lunch		
1.10	600 cc. water		
2.25	4th blood sample	4·44	397·5
3.40	5th blood sample	3·81	382·5
4.35	6th blood sample	3·74	381·5

Table IV.

Urine sample	Time	Creatinine mg. per 100 cc. urine Cr_U %	Creatinine mg. per 100 cc. plasma Cr_P %	Concentration index $\frac{Cr_U\ \%}{Cr_P\ \%}$ C	Urine cc. per min. U	Filtrate cc. per min. $C \times U$ F	Reabsorbed fluid in cc. per min. $F - U$ R
1	11.01–11.49	933	6·65	140	0·92	129	128·1
2	11.49–12.32	546	5·60	98	1·29	126	124·7
3	12.32–1.16	302	5·05	60	2·18	130	127·8
4	1.16–1.53	144	4·75	30	4·58	139	134·4
5	1.53–2.41	244	4·45	55	2·23	122	119·8
6	2.41–3.33	267	4·05	66	1·65	109	107·35
7	3.33–4.00	252	3·85	65·5	1·70	111	109·3
8	4.00–4.41	264	3·77	70	1·59	111	109·4

Urine sample	Chlorine mg. per 100 cc. plasma Cl_P %	Chlorine mg. per 100 cc. urine Cl_U %	Chlorine filtered mg. per min. $\frac{F \times Cl_P\ \%}{100}$ F_{Cl}	Chlorine excreted mg. per min. $\frac{U \times Cl_U\ \%}{100}$ U_{Cl}	Chlorine reabsorbed mg. per min. $F_{Cl} - U_{Cl}$ R_{Cl}	Chlorine % in reabsorbed fluid $\frac{R_{Cl}}{R} \times 100$ Cl_R %	Filtered chlorine % which is excreted $\frac{U_{Cl}}{F_{Cl}} \times 100$ E_{Cl} %
1	388	920	498	8·43	489·6	384·5	1·7
2	396	1028	499	13·3	485·7	389·5	2·7
3	398·5	1061	520	23·2	496·8	387·3	4·5
4	398	878	553	40·2	512·8	381·65	7·3
5	396	1003	484	22·4	461·6	384·8	4·6
6	388·5	1007	423	16·6	406·4	379·5	3·9
7	383	1034	427	17·6	409·4	372·9	4·1
8	381·75	1028	423	16·3	406·7	372·4	3·9

In this experiment in which sodium chloride was taken first and later 600 cc. water were drunk the plasma chlorine went up as high as 399 mg. per 100 cc. The kidney reacted towards this by excreting chlorine in high concentration. The calculation of the percentage of chlorine in the reabsorbed fluid shows that this also is high (Fig. 7).

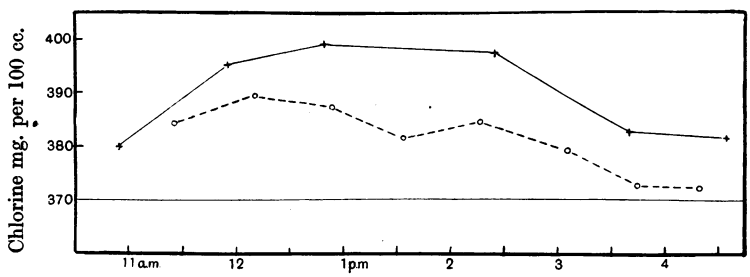

Fig. 7. The percentage of chlorine in the reabsorbed fluid (○) during an experiment with high plasma chlorine (+).

The results of a series of experiments of this kind are shown in the following figures. In Fig. 8 is shown how the percentage of chlorine in the reabsorbed fluid varies with the percentage of chlorine in the blood. The observations evidently fall into three groups. In one group the percentage of chlorine in the plasma varies but little from 370 mg. %. As long as the plasma chlorine keeps within limits of about 365–375 mg. % the amount of chlorine in the

reabsorbed fluid is practically the same. As long as the chlorine varies within these limits, which are the limits for the "optimal percentage," the kidneys

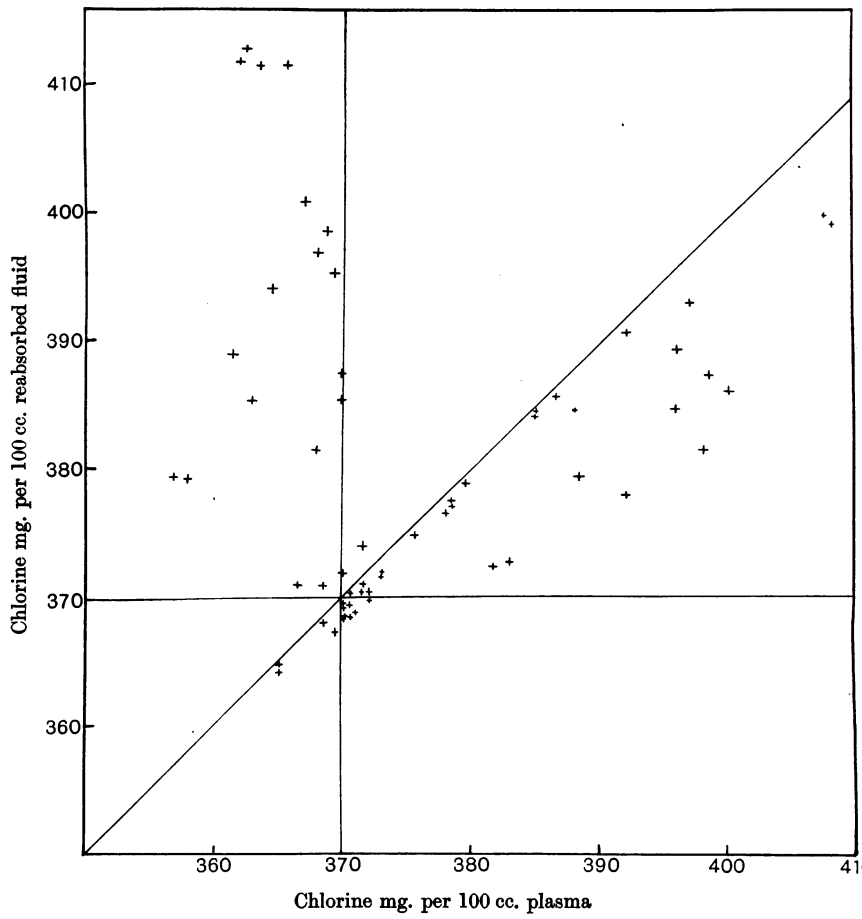

Fig. 8. The percentage of chlorine in the reabsorbed fluid.

keep up the percentage by reabsorbing chlorine in such amounts that the reabsorbed fluid gets the same concentration of chlorine. This is true, however, only when the chlorine of plasma is practically constant, which for normal persons is perhaps always except when they are living on a salt-rich diet or come under experimental conditions. The figure shows that $Cl_R\%$ varies considerably as soon as the chlorine content of the plasma differs appreciably from 370 mg. %. It might be a cause for surprise that whether the chlorine content of the plasma falls or rises the result is that the percentage in the reabsorbed fluid is higher than normally, but there is the very significant difference that when $Cl_P\%$ is low $Cl_R\%$ is higher than $Cl_P\%$ whilst the opposite is the case when the percentage in the plasma is high.

Fig. 9 illustrates the relation between the concentration index and that part of the filtered chlorine which becomes eliminated in the excreted urine

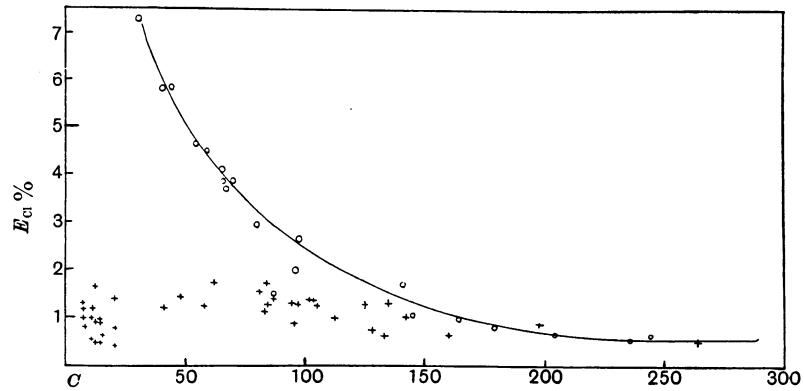

Fig. 9. The excretion percentage of chlorine: +, when plasma chlorine is below 375 mg. %. O, when plasma chlorine is above 375 mg. %.

(the excretion percentage). Here the observations fall into two groups. One group of points belonging to experiments in which the chlorine content of the plasma was below 375 mg. % lies in a narrow band along the abscissa axis. Irrespectively of the concentration index the amount of chlorine excreted is very nearly 1 % of the filtered quantity. With highly concentrated urines only the figure is perhaps a little lower. The other group belonging to experiments with a plasma chlorine above 375 mg. % is, however, distinctly dependent on the concentration index, that amount of the filtered chlorine which is finally eliminated rising as the concentration index falls.

Discussion.

How are these facts to be explained? If we compare Figs. 8 and 9 with Figs. 2 and 4 respectively we find that the data obtained on chlorine when the content of the plasma is above 375 mg. % resemble closely those obtained on urea.

As the percentage of chlorine in the plasma rises the percentage in the reabsorbed fluid rises too, as was the case with urea, though it never reaches the value of the plasma chlorine just as the value of U_R % did not reach the value of U_P %. Further, when the plasma chlorine is above 375 mg. %, the excretion percentage of chlorine is dependent on the concentration index just as was the excretion percentage of urea (Figs. 9 and 4). The only difference between the two substances is that the percentage of chlorine in the reabsorbed fluid is always nearer to the corresponding percentage of chlorine in the blood than the U_R % was to U_P %. The easiest explanation is that when the plasma chlorine is above 375 mg. % the chlorine is treated as a no-threshold substance, the excretion of which is simply determined by the amount of chlorine filtered

and the concentration index of the urine. The main difference between the two substances is that chlorine diffuses back through the tubule walls with much greater ease than does urea, so that the value of $Cl_R\,\%$ approaches the value of $Cl_P\,\%$ much more than the corresponding values for urea.

In Fig. 10 is shown how the value of $Cl_R\,\%$ approaches the value of $Cl_P\,\%$

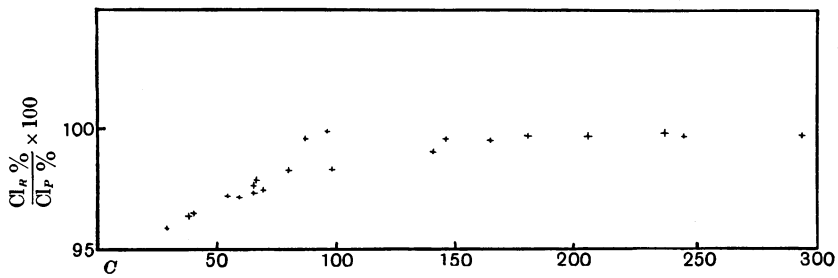

Fig. 10. $\frac{Cl_R\,\%}{Cl_P\,\%} \times 100$, when the plasma chlorine is above 375 mg. %.

when the concentration index rises. With a concentration index of 30 the percentage of chlorine in the reabsorbed fluid is about 96 % of that in the plasma, whilst at a concentration index of 150 the value of $Cl_R\,\%$ is about 99 % of the value of $Cl_P\,\%$. For urea the values at the same concentration indices are about 32 % and 45 %.

Because of this very rapid back-diffusion the percentage of the filtered chlorine which is excreted at a certain concentration index is much smaller than the amount of the filtered urea excreted at the same concentration index (*e.g.* concentration index 50, $E_{Cl}\,\% = 5\,\%$ and $E_U\,\%$ about 65–70 %). On the other hand, the data obtained show that when the chlorine content of the plasma falls below 375 mg. % the chlorine is treated in quite another way by the kidney. As long as the content of the plasma does not fall below 370 mg. % the percentage in the reabsorbed fluid is practically the same, but when the content falls lower the percentage in the reabsorbed fluid rises high above the percentage in the plasma. If we suppose active reabsorption to begin when the plasma chlorine falls below 375 mg. % these facts are in striking agreement with the hypothesis. But how is the unexpected result which has emerged to be explained; namely, that the excretion percentage is practically independent of the concentration index of the urine when the plasma chlorine is below 375 mg. %? Whatever the concentration index is, about 1 % of the filtered chlorine is excreted, whence it appears that the reabsorption of the chlorine is not helped by the rising concentration of the urine. An explanation of this is needed and that which offers itself is that the main concentration of the urine takes place at a lower place in the tubules than the reabsorption of the chlorine[1].

[1] Starling and Verney [1924] come to the same conclusion from their experiments on the isolated kidney. Without discussing these experiments, which from the standpoint of the

In the previous paper it was stated that if the pressure in the cavity of the capsule were able to drive out the urine it was necessary to postulate that the main reduction in volume should take place in the proximal convoluted tubules. Is this in contradiction with the postulate here, that the main concentration of the urine must take place at a lower level than the reabsorption of the chlorine? It is not, as the two terms reduction in volume and concentration, as I have used them, do not mean the same thing. If from 100 cc. filtrate we reabsorb 90 cc. water it is a great reduction in volume though the concentration index will be only 10; if however we reabsorb 9 cc. more this will raise the concentration index from 10 to 100. In reality we may expect the reabsorption of water to be very rapid in the beginning when the osmotic resistance to be overcome is relatively small. If, therefore, we picture to ourselves the process in the following way we have both postulates realised.

A certain amount, say 100 cc. of filtrate containing 360 mg. chlorine, passes through the tubules. As the chlorine content is below 375 mg. % the excretion percentage is about 1 % so that 99 % or about 357 mg. chlorine are reabsorbed. This chlorine we will suppose to be reabsorbed along with 80 cc. of fluid ($= R_1$) in passing the proximal convoluted tubules. If no further reabsorption occurred we should get 20 cc. of urine containing 3 mg. chlorine or a urine with a concentration index of 5, a chlorine concentration of 15 mg. % and an excretion percentage for chlorine of 0·83 %, whilst the corresponding reabsorbed fluid would have a chlorine concentration of $\frac{357 \times 100}{80}$ or 447 mg. %.

But suppose 15 cc. of fluid ($= R_2$) to be absorbed farther down the tubules, then in all 95 cc. of fluid ($R_1 + R_2 = R$) would have been reabsorbed together with 357 mg. chlorine. The 5 cc. of urine would have a concentration index of 20 and a chlorine content of 60, so that the total amount of chlorine and the E_U % are the same.

If we suppose a process like this the result will be in accordance with the facts observed and will explain that the fraction of the filtered chlorine which is excreted is practically independent of the concentration index. Moreover we see that, though the fluid assumed to be reabsorbed in the proximal convoluted tubules (R_1) is of a constant composition, the percentage of chlorine in the total reabsorbed fluid R varies and is dependent on the concentration index. If we compare the values for Cl_P %, Cl_R % and C met in the real experiments we find exactly the same interdependence as Fig. 11 shows.

"modern theory" appear to furnish strong arguments in favour of the mixed filtration-reabsorption-secretion theory, the results are in agreement with what would be expected according to the modified theory here presented. If the influence of hydrocyanic acid is to stop reabsorption and to make the tubule walls permeable for all substances, we should expect a kidney, after treatment with cyanide, to excrete urine of a composition like the deproteinised plasma in a volume which is determined by the pressure in the glomeruli and the diameter of the tubules during the experiments.

The data obtained on the excretion of chlorine may be summarised as follows:

(1) The calculated percentage of chlorine in the reabsorbed fluid is above 370 mg. % but below the percentage in the plasma when this percentage is above 375 mg. %.

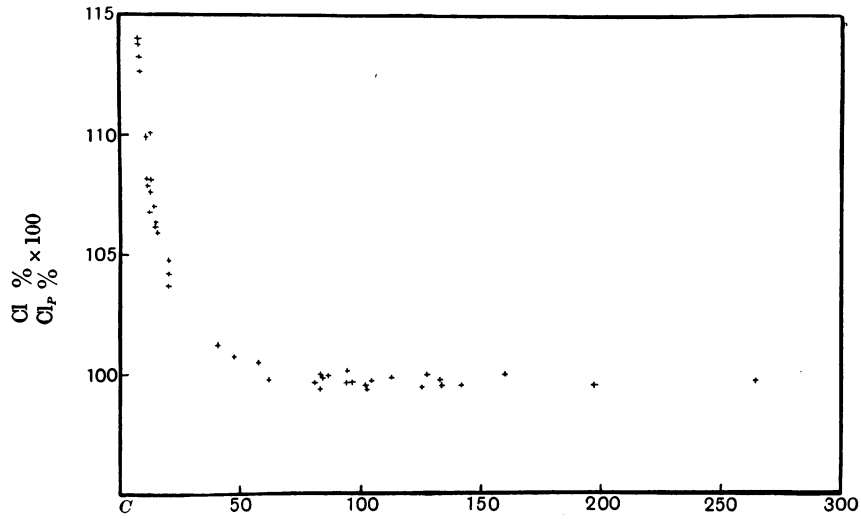

Fig. 11. $\frac{Cl_R\ \%}{Cl_P\ \%} \times 100$, when the plasma chlorine is below 375 mg. %.

(2) The calculated percentage of chlorine in the reabsorbed fluid is the same as in the plasma when the concentration of chlorine in the plasma is near 370 mg. %.

(3) The calculated percentage of chlorine in the reabsorbed fluid is higher than 370 mg. % when the concentration in the plasma is below 370 mg. %.

(4) The calculated percentage of chlorine in the reabsorbed fluid approaches the percentage of chlorine in the plasma when the concentration index increases.

(5) When the chlorine content of the plasma is above 375 mg. % the percentage of the filtered chlorine which is excreted is dependent on the concentration index. Most is excreted with a low concentration index.

(6) When the chlorine content of the plasma is below 375 mg. % the percentage of the filtered chlorine which is excreted is practically independent of the concentration index.

Conclusion.

These results, as well as those obtained on the excretion of urea, may perhaps be rejected by the supporters of the secretion theory by saying that the only observed fact is the interdependence between the concentration ratios of the different substances, whilst the rest is the result of mere theoretical

assumptions made on the basis of the unproved postulate that the creatinine is filtered out through the glomeruli. I think however that the results obtained are in such good agreement with what would be expected from the working hypothesis that it is not unwarranted to put forth the following theory for the work of the kidney.

Large volumes of filtrate are formed in the capsules of Bowman. This filtrate contains in solution all diffusible substances from the plasma. In the tubules we have cells for the active reabsorption of the threshold substances. These substances are—drawing conclusions from the behaviour of the chlorine —only actively reabsorbed when the percentage in the plasma falls below a certain percentage; the optimal percentage. When the concentration in the plasma is above this percentage the threshold substances are treated by the kidney as no-threshold substances. The reabsorption of chlorine—and probably of the other threshold substances—takes place along with the greater part of the water in the proximal convoluted tubules. The real concentration of the urine takes place farther down the tubules by the reabsorption of rather small volumes of water. The percentages and amounts in which the no-threshold substances—and the threshold substances when their concentration in the plasma is above their optimal concentration—are excreted are determined by the amount of filtrate, the concentration index of the urine and the ease with which the different substances diffuse back through the tubule walls. As the concentration index rises the concentration ratios of the different substances rise too, but at different rates. The group of no-threshold substances thus includes substances which may be concentrated to very different extents, from alcohol, which is not concentrated at all, to creatinine which may be concentrated several hundred times.

It is a further problem to decide which substances are threshold substances and which no-threshold substances. Sugar is of course a threshold substance and urea has been proved to be a no-threshold substance. It has here been demonstrated moreover that chlorine behaves as a threshold substance. In reality I do not think that chlorine is the true threshold substance but only acts as an indicator of the real threshold substance, sodium. Only as long as the chlorine really may be taken as an indicator of the sodium can we expect to get results like those obtained in this paper. If the normal relation between sodium and chlorine in plasma is disturbed, as in acidosis, the excretion of chlorine follows other laws, which perhaps could be studied by a simultaneous analysis of the excretion of sodium, bicarbonate and chlorine according to the method used in this paper.

Summary.

(1) The filtration-reabsorption theory and especially the way in which it distinguishes between threshold and no-threshold substances is discussed.

(2) The difference between active reabsorption and mere back-diffusion is emphasised and a working hypothesis is put forth.

(3) The excretion of urea and chlorine is analysed in two series of experiments according to this hypothesis.

(4) The conclusions drawn from the experiments are:

(*a*) urea is a no-threshold substance which is never actively reabsorbed. Its presence in the reabsorbed fluid is supposed to be due to mere diffusion;

(*b*) chlorine is a threshold substance which is actively reabsorbed when its percentage in the plasma is below 375 mg. %;

(*c*) when the percentage of chlorine in the plasma is above 375 mg. % chlorine is treated as a no-threshold substance;

(*d*) chlorine (and the other threshold substances) are supposed to be reabsorbed together with most of the water in the proximal convoluted tubules;

(*e*) the rest of the water is reabsorbed farther down the tubules.

(5) Chlorine is held to act only as an indicator for the real threshold substance, sodium.

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