THE EXCRETION OF CYANOL, AZOFUCHSIN I AND WATER BY THE KIDNEYS OF RABBITS*

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In renal disorders function and morphology have not yet been correlated satisfactorily. It is true that the fundamentals have been clarified by the work of Volhard and Fahr; but when we come to details, our knowledge is still incomplete. As recent advances, both in the understanding of renal function and in the experimental production of renal diseases, justified a new attempt in this direction, certain renal disorders have been reproduced, and a comparison of the morphological changes with the changes in function has been made.

It may be regarded as definitely established that certain substances are eliminated by the kidneys solely or mainly by filtration through the glomeruli, while others are mainly secreted by the tubules. Therefore, in the present experiments we studied the elimination by the kidneys: (*a*) of water; (*b*) of a dye generally regarded as eliminated by glomerular filtration; and (*c*) of a dye believed to be secreted by the tubular epithelium. While it has often been said that dye tests are of no great value, more recent studies have shown that a properly applied phenol red test, for example, is at least as useful as the urea clearance test (Shaw, 1925; Ockerblad, 1928; Chisholm, 1930; Chapman and Halsted, 1933). Watanabe, Oliver and Addis (1918) appear to have been right when they pointed out that these tests in reality give more than either rate of excretion or blood concentration alone. If the same amount of dye always is injected, the

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plasma concentration tends to be constant, and thus the ratio between the amount of the dye in the blood and in the urine, or in other words, the clearance, can be obtained.

For a "glomerular" dye, cyanol was chosen; and for a "tubular" dye we at first used phenol red. As the latter is an indicator, we found it difficult, however, to determine its concentration when the urine was rich in phosphates, or when the concentration of the dye was low: therefore azofuchsin I[1] was finally selected. As little was known about the excretion of these dyes in rabbits, we first had to study their normal elimination. The results of this study are the subject of this paper.

LITERATURE

Cyanol was first studied by Hoeber and his pupils. According to Yoshida (1924) and Orzechowski (1930), it is a highly diffusible, lipoid insoluble, acid dyestuff which easily diffuses through parchment cylinders, but does not enter erythrocytes or the lipoid mixture of Nirenstein. They found that in double perfusion experiments with a modified Ringer solution, in frogs, the dye either did not appear at all, or scarcely appeared, in the urine, when offered to the tubules alone. However, if it was offered to the glomeruli only, or to both glomeruli and tubules, it was concentrated one to two times (Yoshida, 1924; Schulten, 1925; Hoeber, 1927; Scheminzky, 1929; Robbins and Wilhelm, 1933).

Studying the kidney of aglomerular toadfish, Hoeber (1930) was unable to detect any dye in the urine. Marshall and Grafflin (1932) were also unable to find any dye after injections of 10 to 15 mg. per kilo. However, if they injected such tremendous doses as 125 to 300 mg. per kilo, they found a small amount in the urine. This latter finding was later confirmed by Grafflin (1936).

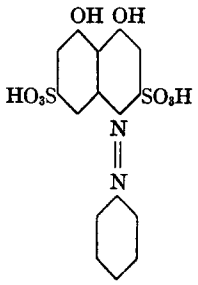
In glomerular sculpins which had been rendered functionally aglomerular by two or more doses of 200 to 300 mg. of phlorizin per kilo, Marshall and Grafflin (1932) found no excretion of cyanol, whereas normal sculpins excreted the dye and concentrated it 1.3 to 2.4 times (Grafflin, 1936). In two phlorizinized sculpins Grafflin found the cyanol clearance to be but 67 to 77 per cent of the simultaneous glucose clearance. However, both these fish had received tremendous doses of cyanol.

In rabbit urine Steffanutti (1930) found phenol red nine times as concentrated as cyanol, both dyes having been given simultaneously. In a rabbit which had been poisoned with uranium the phenol red concentration amounted to but a third of the cyanol concentration. Cope (1934) who studied the cyanol clearance in

[1] For the choice and the supply of both the dyes we are indebted to Dr. R. Hoeber.

rabbits as compared with the xylose and sucrose clearances, found the sucrose clearance to be about 25 per cent higher than the cyanol and xylose clearances. In phlorizinized rabbits the glucose clearance rose to equal the xylose clearance, whereas the cyanol clearance fell to about 42 per cent of the two clearances. As from our experience, which conforms with that of Smith,[2] the cyanol figures of Cope appear to be much too high, and as the possibility cannot be excluded that the drop observed by this author may have been due to factors other than change in the renal elimination of the dye, we do not attach weight to this observation at present.

Azofuchsin I has been used by Hoeber and his pupils only (1930, 1932). Like cyanol it is a highly diffusible lipoid insoluble, acid dyestuff which does not permeate into erythrocytes, and which is practically insoluble in the lipoid mixture of Nirenstein. Its formula has been given as



In double perfusion experiments with a modified Ringer solution, in frogs, Orzechowski (1930) found it to be concentrated 11 to 65 times, if it was offered to the tubules only. In excised frog kidneys which were placed in oxygenated salt solution containing azofuchsin, the dye behaved exactly like phenol red (Hoeber and Meirowsky, 1932).

Method

Male Chinchilla rabbits weighing 1500 to 2200 gm. were used. Animals with evidence of spontaneous renal disease were discarded. At the beginning of an experiment each animal received 100 cc. of tap water by stomach tube. When the resulting diuresis was in progress, usually 1 hour after the introduction of the water, the dyes were injected intravenously. In all experiments 2 cc. of a 0.1 per cent solution were given. At first both dyes were given simultaneously, as done by Steffanutti (1930). But since the natural color of the urine changed a great deal during the experiments, we actually had to measure three colors in the same specimen. Since this could not be done with accuracy, we later injected but one dye at a time.

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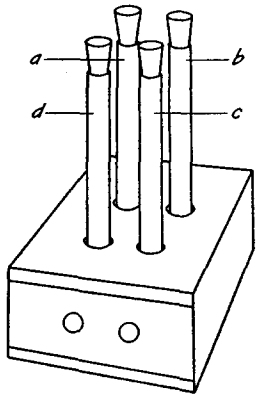
[2] Smith, Homer, oral communication.

The urine was collected by fine catheters which were inserted into the bladder immediately after the introduction of the water. The catheters held about 1.5 to 2.0 cc. of fluid. The volume of the urine was measured every 15 or 30 minutes, and after the injection of the dye, every 10 minutes. As a rule, urine passed freely when diuresis was in progress. If there seemed to be a retention of urine, the bladder was gently massaged each time urine was to be collected. A few experiments in which blood appeared in the urine were discarded.

The concentration of the dyes was tested with standard solutions which were prepared from the same solutions as those which were injected. To compensate for the varying color of the urine, it was found necessary to introduce into the comparator block a tube with urine of the same concentration as that of the specimen to be tested (Text-fig. 1). If the dye was concentrated very highly, it



TEXT-FIG. 1. Sketch of the comparator used. Tube (*a*) contained the urine specimen to be tested; (*b*) the standard dye solution in water; (*c*) the urine control which was diluted to the color of the urine to be tested, and (*d*) water. The front of the comparator was covered by milk glass.

was found to be more accurate to dilute the specimen with 4 or 9 parts of water before the determination. If the urine contained precipitated phosphate, this was dissolved by dilution with water, or if the dye concentration was low, by adding a trace of acid.

RESULTS

1. The excretion of *water* has been studied in 83 experiments (in 63 rabbits; 35 with cyanol, 38 with azofuchsin, 10 without dye). In 14 experiments in which 100 cc. of tap water was given by stomach tube on the day of the experiment ("dry" animals), the average rate of urine formation was 0.023 cc. per minute during the first 30 min-

utes, 0.063 cc. during the second, 0.197 cc. during the third, 0.223 cc. during the fourth and 0.273 cc. during the fifth period of 30 minutes. In 69 experiments in which the animals received 100 cc. of water the day before the experiment as well ("wet" animals), the corresponding figures were 0.018, 0.400, 0.447, 0.587 and 0.537 cc. per minute. The highest rate obtained amounted to 1.5 cc. per minute during a 10 minute period, or to 1.31 cc. during a period of 1 hour.

TABLE I

The Rate of Urine Excretion during the First 2½ Hours after the Ingestion of 100 Cc. of Water

	Rate of urine excretion per min.						
	0.02–0.07 cc.	0.08–0.2 cc.	0.2–0.4 cc.	0.4–0.6 cc.	0.6–0.8 cc.	0.8–1.0 cc.	1.0–1.15 cc.
"Dry" animals	7*	2	3	2	0	0	0
"Wet" animals	0	13	24	12	13	5	2

* Number of experiments.

TABLE II

The Peak of Urine Excretion after the Ingestion of 100 Cc. of Water

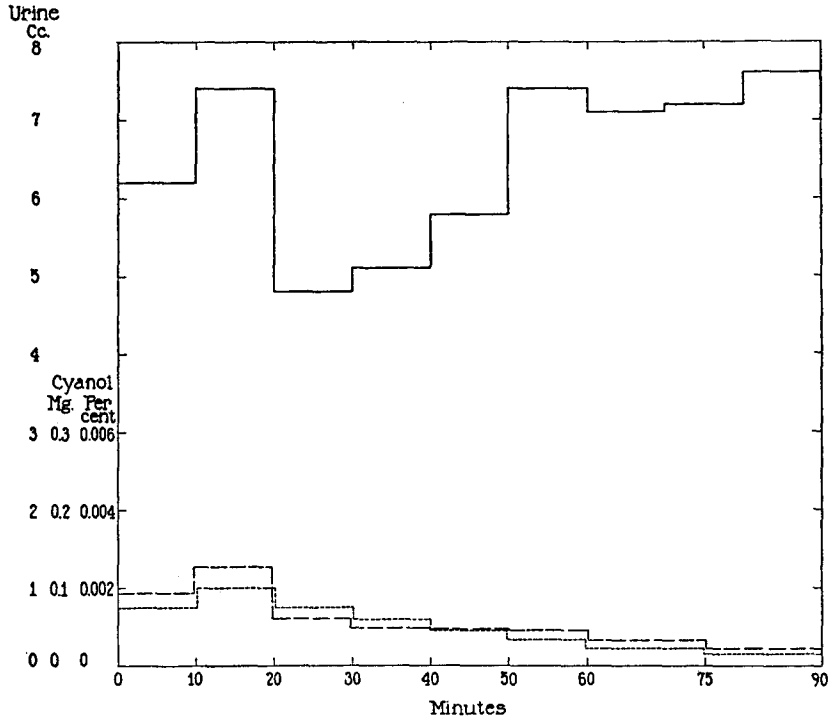
	Periods of 30 min.					
	1	2	3	4	5	6
"Dry" animals	0*	0	0	2	3	9
"Wet" animals	0	8	5	25	11–21†	10–20†

* Number of experiments.

† In 10 of these the experiment was stopped after the fifth period.

The individual rates varied a great deal (Table I). But whereas in most dry animals the rates were between 0.02 and 0.07 cc. per minute, in most wet animals they were between 0.08 and 0.8 cc. A similar variation was found in respect to the time the diuresis was at its peak (Table II). But whereas in the dry animals this was not reached before the fourth period and in the majority of the experiments at the sixth period only or later, in the wet animals it was reached as early as in the second period and in the majority of the experiments in the fourth or fifth period, *i.e.*, from 1½ to 2½ hours

after the ingestion of water. As these results are in close conformity with those of Rees (1918) and Heller and Smirk (1932), as far as the peak of the diuresis is concerned, and with those of Oehme (1921) and Walker, Schmidt, Elsom and Johnston (1937), as far as the difference between dry and wet animals is concerned, it may be taken for certain that under ordinary laboratory conditions a better water

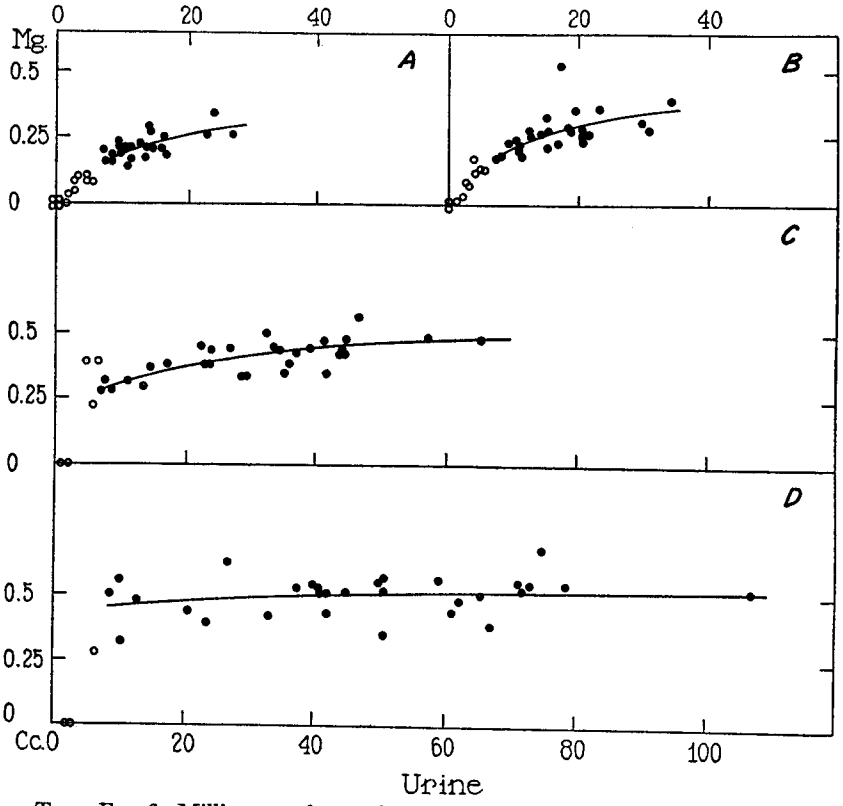


TEXT-FIG. 2. Average quantities of urine (————) and cyanol (mg. – – – –, concentration per cent .....) excreted in 10 minute periods after the intravenous injection of 2 mg. of the dye.

diuresis is obtained from rabbits, if they are given 100 cc. of water the day before the experiment as well as on the day of the experiment, and that under these conditions the diuresis is well in progress in the 2nd hour after the ingestion of water.

2. The excretion of *cyanol* has been tested in 35 experiments (in 30 rabbits). The highest rate of excretion, measured in mg., was

found to be in the first 10 minute period after the injection of the dye in 5 experiments, and in the second 10 minute period in 18 experiments. In all these experiments the rate of urine formation amounted to 0.35 cc. per minute or more during the first 20 minutes. In the remaining 12 experiments, in which the highest rate of dye



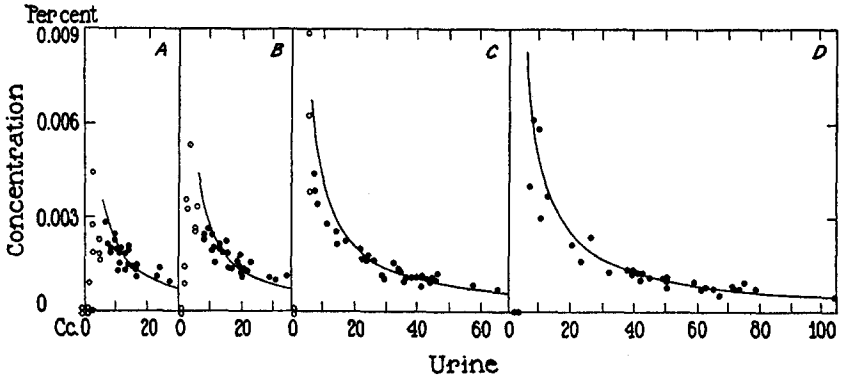
TEXT-FIG. 3. Milligrams of cyanol excreted during the first 20 (*a*), 30 (*b*), 60 (*c*) and 90 (*d*) minutes of the experiments as compared with the cc. of urine produced during these periods.

excretion was observed later than in the second 10 minute period, the rate of urine formation was 0.25 cc. per minute or less. The highest amount of dye excreted in a 10 minute period was 0.226 mg.; the highest concentration 0.017 per cent.

If we only take into account those experiments in which the urine

rate amounted to more than 0.25 cc. per minute during the first 20 minutes, the average rates of cyanol excretion during the first 9 periods follow the curve shown in Text-fig. 2. It should be noted that the urine rate drops considerably after 20 minutes, and that the concentration curve rises above the absolute excretion curve at this time, to drop below again after 50 minutes, when the urine rate has regained its original speed.

When we plot the milligrams of dye excreted during 20, 30, 60 and 90 minutes against the volumes of urine excreted during this time (in all our experiments), we find a definite increase in the dye excretion with increasing water diuresis (Text-fig. 3). This increase is most

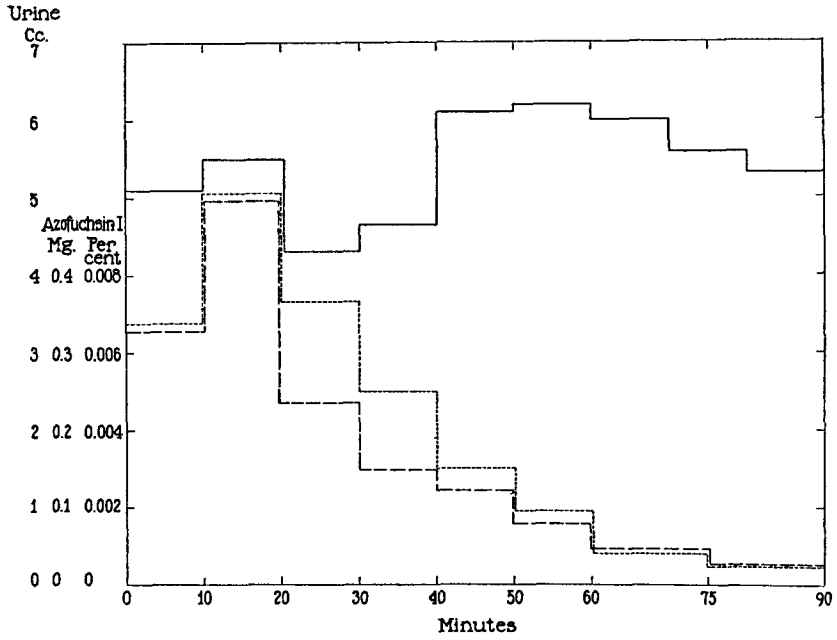


TEXT-FIG. 4. Concentration percentages of cyanol as compared with the urine volumes during the first 20 (*a*), 30 (*b*), 60 (*c*) and 90 (*d*) minutes of the experiments.

marked after 20 and 30 minutes and least so after 90 minutes. If we plot the concentration of the dye against the urine volume, we get similar results (Text-fig. 4). We then get curves which at low urine volume, are considerably below, and at high volume, above hyperbolas which have been constructed with the formula $x = \frac{k}{y}$, where x is the average concentration of the dye, and y the average urine volume, k being equal to the average mg. excreted times 100.[3]

[3] For the dead space in our experiments which we estimated as approximately 3 cc., the hyperbolas have been calculated only from urine volumes that amounted to at least 7 cc. Though it is obvious that there remains an error which becomes smaller with increasing urine volume, it can be seen from Text-figs. 4 and 7 that for any urine volume above 7 cc. this error is rather constant.

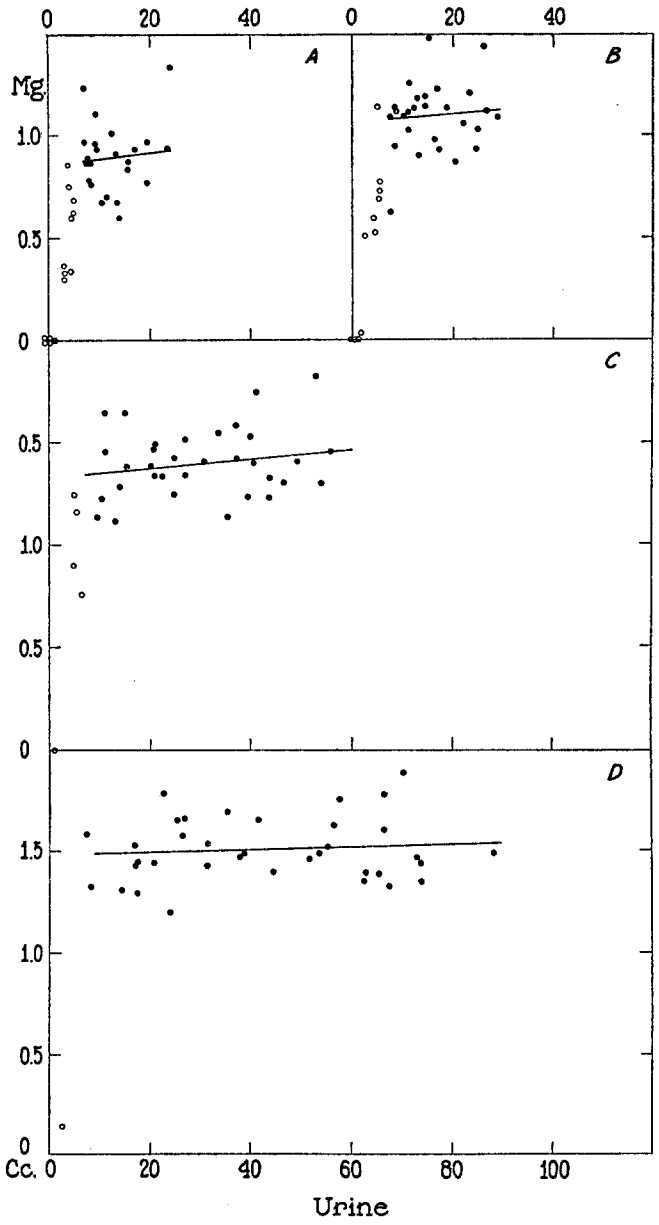
3. The excretion of *azofuchsin I* has been studied in 38 experiments (in 33 animals). In 10 experiments the peak of the excretion was observed in the first 10 minutes after the injection of the dye; in 21 experiments in the second 10 minute period. In all these experiments the urine rate amounted to at least 0.15 cc. per minute during the first 20 minutes. In the remaining 7 experiments in which the peak was reached later, the urine rate was 0.15 cc. or less. The larg-



TEXT-FIG. 5. Average quantities of urine (————) and azofuchsin I (mg. – – – –, concentration per cent .....) excreted in 10 minute periods after the intravenous injection of 2 mg. of the dye.

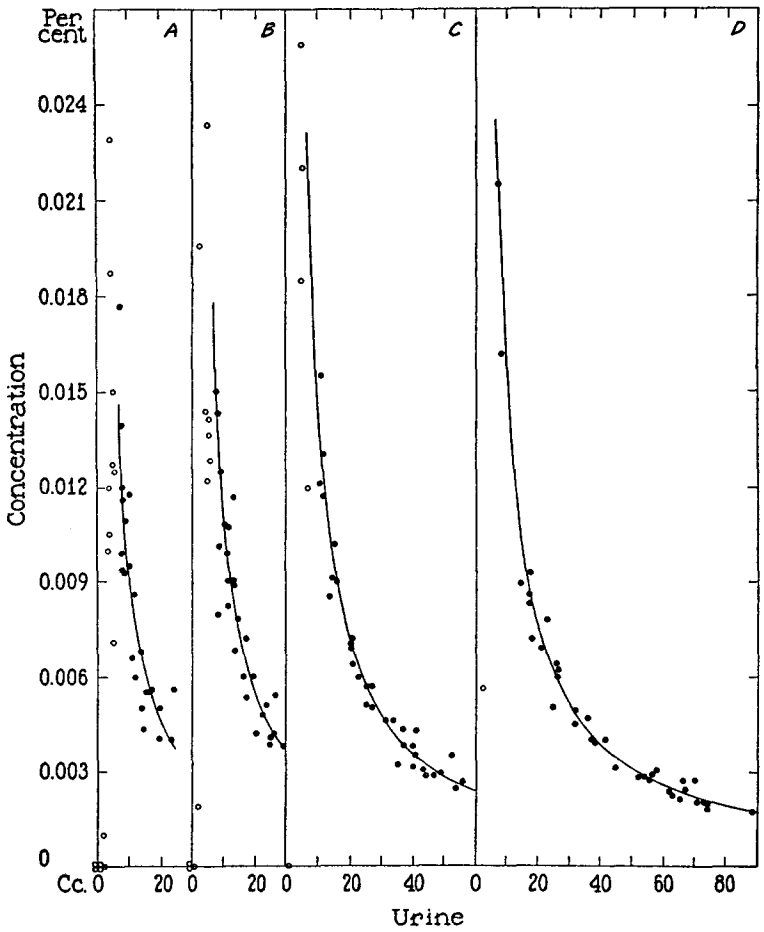
est amount of dye excreted during a 10 minute period was 1.05 mg., the highest concentration 0.04 per cent.

If, as in the case of cyanol, those experiments are taken into account in which the urine rate amounted to more than 0.15 cc. per minute, we find an average excretion curve as outlined in Text-fig. 5. With this dye also there is but a slight depression in the urine volume after 20 minutes. This, as in the case of cyanol, leads to a discrepancy between the concentration and absolute excretion curves.



TEXT-FIG. 6. Milligrams of azofuchsin I excreted during the first 20 (*a*), 30 (*b*), 60 (*c*) and 90 (*d*) minutes of the experiments as compared with the cc. of urin produced during these periods.

When we plot the mg. of dye excreted during 20, 30, 60 and 90 minutes against the volumes of urine excreted during this time, we find, unlike the case of cyanol, only a slight rise in dye excretion with



TEXT-FIG. 7. Concentration percentages of azofuchsin I as compared with the urine volumes during the first 20 (*a*), 30 (*b*), 60 (*c*) and 90 (*d*) minutes of the experiments.

urine volumes above 7 cc. (Text-fig. 6). When we plot the concentration of the dye against the urine volume, we have similar results; namely, curves which closely follow hyperbolas constructed as above (Text-fig. 7).

Variations in body weights, 86 per cent of which varied from 1600 to 1980 gm., appeared to exert no distinct differences, either in the cyanol or in the azofuchsin experiments.

DISCUSSION

From the foregoing results it is obvious that cyanol and azofuchsin I are excreted in a very different manner. Whereas the cyanol excretion amounted to no more than an average of 0.218 mg. in 20 minutes and 0.502 mg. in 90 minutes, *i.e.*, 10.9 per cent and 25.1 per cent of the injected dye, the corresponding figures for azofuchsin were 0.889 and 1.504 mg., *i.e.*, 44.4 per cent and 75.2 per cent. Whereas the cyanol excretion was slow (flat curve), azofuchsin was excreted very rapidly (steep curve; compare Text-figs. 2 and 5). Whereas the cyanol excretion depended on the urine volume, the azofuchsin excretion was essentially independent of this factor.

To evaluate these differences, we may compare our findings with available published data in the recent literature. If *cyanol* is a glomerular dye, its excretion should compare with a clearance which is regarded as a true measure of glomerular filtration, such as the inulin clearance, the curve of which in rabbits has been given by Kaplan and Smith (1935). As in our experiments there was a dead space which has been estimated as approximating 3 cc. of urine, we first corrected this error by using the formula m_2 (corrected mg.) $= \frac{v \cdot m}{v-3}$, v being the urine volume in cc., and m the actual amount of dye excreted in mg. The clearance (cc. of plasma cleared per minute) was then calculated by dividing the corrected mg. (m_2) by the number of minutes in which they were excreted, and by dividing this rate by the average mg. of dye contained in 1 cc. of plasma during this period, *i.e.*, by $\left(2.0 \text{ (mg. of dye injected)} - \frac{m_2 \text{ (mg. of dye excreted)}}{2}\right) : 100$ (100 being the approximate number of cc. of plasma contained in our rabbits). A further correction was necessary because we frequently found a considerable amount of dye in the small intestines, when the animals were killed 2 hours after the injection of the dye; we believe that this fraction, which was probably excreted by the liver,

may amount up to 50 per cent of the total dye; therefore, another clearance (2) was calculated by dividing the rate by $\left(2.0 - \frac{2m_2}{2}\right)$:100. Since the data of Kaplan and Smith were given on the basis of square meters of body surface, and since their animals were larger than ours, we finally divided our clearances by the average body surface of our animals which according to the table of Taylor, Drury and Addis (1923) amounted to about 0.1415 sq. m.

If now we compare our clearances thus derived from various points of Text-fig. 3 *a* and *b* with the inulin clearances of Kaplan and Smith, we find, considering the values after 20 minutes only (Text-fig. 3 *a*), that at urine rates of 2.47 to 9.89 cc. per minute per sq.m. the cyanol clearances amount to an average of from 8.1 to 8.9 per cent of the inulin clearances (Table III, 3 *a*), and if we consider the values after 30 minutes only (Text-fig. 3 *b*), that at urine rates from 3.06 to 8.25 cc. they amount to an average of from 6.8 to 7.5 per cent (Table III, 3 *b*). In both instances the deviations from the average are remarkably small. However, if we consider the values at urine rates below 2.47 cc. per minute per sq. m., we find considerably higher cyanol clearances as compared with the corresponding inulin clearances (Table III, 3 *a*,*b*,*c*). The explanation for this may be found in the "dead space" of our experiments, for it is obvious that the dye which is excreted diffuses in the urine contained in the dead space, and that at a very slow urine flow the diffusion introduces an error which considerably elevates the corrected excretion rate.[4] It may be noted that this error is of importance when the urine rate is below 1.65 to 2.47 cc. per minute per sq. m., which corresponds closely to the point below which urine volumes have been omitted in the calculation of the hyperbolas in Text-figs. 4 and 7.[3]

Comparison of our clearances with the xylose clearances of Kaplan and Smith shows no correlation (Table III). We find, for example, that after 20 minutes (Table III, 3 *a*) with an increase in diuresis from 2.47 to 9.89 cc. per minute per sq. m., the cyanol clearance in-

[4] That this actually occurs is illustrated by the observation of dye in as little as 1.2 cc. of urine 10 minutes after the injection of the dye, the catheter holding 1.5 to 2.0 cc. of urine.

creases 24 to 30 per cent, and the inulin clearance 26 per cent,[5] whereas the xylose clearance increases as much as 74.6 per cent. From all these figures it is evident that the cyanol excretion, as far as its curve is concerned, closely resembles the inulin clearance, whereas the xylose clearance follows a very different curve. We regard this as evidence that cyanol is handled by the kidney of the rabbit as are inulin and

TABLE III

Cyanol Clearance as Compared with Corresponding Inulin and Xylose Clearances as Obtained by Kaplan and Smith

Fig. from which data were derived	Urine	Dye	Period	Urine per min. per sq. m.	Dye excretion (corrected)								
					Amount	Clearance							
						Per min.		Per. min. per sq. m.		Amount as compared with inulin clearance		Amount as compared with xylose clearance	
						1	2	1	2	1	2	1	2
	cc.	*mg.*	*min.*	*cc.*	*mg.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*per cent*	*per cent*
3*a*	4	0.075	20	1.41	0.300	0.818	0.882	5.8	6.2	11.2	11.9	24.2	25.8
"	7	0.150	20	2.47	0.262	0.701	0.754	5.0	5.3	8.4	8.9	14.9	15.8
"	12	0.200	20	4.24	0.267	0.717	0.770	5.1	5.7	7.7	8.6	11.6	13.0
"	20	0.260	20	7.07	0.306	0.828	0.903	5.9	6.4	8.1	8.8	11.0	12.0
"	28	0.290	20	9.89	0.325	0.884	0.970	6.2	6.9	8.3	9.2	10.6	11.8
3*b*	4	0.100	30	0.92	0.400	0.739	0.831	5.2	5.9	11.1	12.6	28.1	31.9
"	7	0.180	30	1.65	0.315	0.570	0.623	4.0	4.4	7.4	8.1	15.4	16.9
"	13	0.250	30	3.06	0.325	0.589	0.647	4.2	4.6	6.7	7.4	11.2	12.3
"	20	0.300	30	4.71	0.353	0.645	0.715	4.6	5.1	6.8	7.5	10.0	11.1
"	35	0.350	30	8.25	0.383	0.706	0.790	5.0	5.6	6.8	7.6	8.9	10.0
3*d*	7	0.450	90	0.55	0.787	0.544	0.721	3.8	5.1	9.3	12.4	27.1	36.4
3*c*	7	0.275	60	0.83	0.481	0.456	0.228	3.2	3.7	7.0	8.0	18.3	21.1

creatinine, the clearances of which appear to be true measures of filtration (Kaplan and Smith, 1935). If this is correct, then we may estimate that in rabbits at plasma levels of about 2 mg. per cent no more than 10 per cent of the dye is available for filtration.

If we turn now to *azofuchsin I*, we see at once that there is no correlation between its rate of excretion and that of inulin, creatinine

[5] These figures have been calculated from the data of Kaplan and Smith.

or xylose. It is true that the concentration of this dye also rises with increasing urine volume (Text-fig. 6), but this rise does not exceed that which is caused by the dead space in our experiments. If we calculate the clearance as above, we find that 20 minutes after the injection of the dye the clearance amounts to about 21 to 24 cc. per minute per sq. m., *i.e.*, about 34 to 39 per cent of the inulin clearance at a urine flow of 2.47 cc. per minute per sq. m., or 28 to 32 per cent at a flow of 8.48 cc.; after 30 minutes it amounts to 18 to 20 cc. per minute per sq. m., *i.e.*, 33 to 37 per cent of the inulin clearance at a urine flow of 1.65 cc. per minute per sq. m. or 25 to 28 per cent at a flow of 7.07 cc. It is self evident that the differences in these figures at different urine flows cannot be explained by reabsorption. In this case the reverse should be expected, namely a smaller clearance with low urine flow and *vice versa*. It appears to be evident, therefore, that the excretion of azofuchsin I cannot be explained by filtration alone.

However, if we compare the excretion of azofuchsin with that of phenol red which is known today to be mainly secreted, we find a close correlation in at least three respects.

1. Whereas in man after intravenous injection Rowntree and Geraghty (1909–10, 1912) recovered 68 per cent of the injected phenol red in the urine 1 hour after the injection; Shaw (1925) 40 per cent after 15 minutes, 57 per cent after 30 minutes, 69.5 per cent after 1 hour and 74 per cent after $1\frac{1}{2}$ hours; and Chapman and Halsted (1933) 36 per cent after 15 minutes, 54 per cent after 30 minutes, and 66 per cent after 1 hour; we recovered 44 per cent of the injected azofuchsin after 20 minutes, 54 per cent after 30 minutes, 70 per cent after 1 hour, and 75 per cent after $1\frac{1}{2}$ hours. Furthermore, as after subcutaneous or intramuscular injection the amount of phenol red excreted in 1 hour was 50 to 51 per cent in man, dogs and rabbits (Rowntree and Geraghty, 1909–10, 1912; Eisenbrey, 1911; Frothingham, Fitz, Folin and Denis, 1913), it appears that the values in man and in rabbits are comparable, which would mean that phenol red and azofuchsin I both have the same excretion curve.

2. Like the azofuchsin excretion the elimination of phenol red is essentially independent of the urine volume. For phenol red this has been demonstrated in toadfish, frogs, rabbits, dogs and man

(Rowntree and Geraghty, 1909–10; Marshall and Kolls, 1919; Cushny, 1926; Scheminzky, 1929; Marshall and Grafflin, 1932; Chapman and Halsted, 1933; Shannon, 1935).[6]

3. If we assume that azofuchsin like phenol red (Chambers and Kempton, 1933; Richards, 1935) and cyanol (see below) is not reabsorbed, and that, as in the case of phenol red (Grollman, 1925, 1925–26), in rabbits at plasma concentrations below 2 mg. per cent, 95 per cent of the dye is bound by the plasma proteins, we arrive at azofuchsin clearances which are considerably higher than those mentioned above. 20 minutes after the injection of the dye they would amount to 680 to 780 per cent of the inulin clearance, at a urine flow of 2.47 cc. per minute per sq. m., or to 560 to 640 per cent, at a flow of 8.48 cc. If the inulin clearance represents the filtration rate, this would mean that at the lower urine flow 85 to 90 per cent of the excreted dye was secreted, and at rapid flow 82 to 84 per cent. If we compare these figures with the phenol red figures available in the literature we find a close correlation. In dogs, Shannon (1935) found the secreted proportion to approximate 83 per cent, at plasma levels of 0.5 to 1.5 mg. per cent. Similar figures can be calculated from Marshall's data (1932), namely 82 to 90 per cent, at plasma levels of 0.21 to 0.54 mg. per cent, or from Sheehan's data (1936), namely 70 to 90 per cent, at plasma levels of 0.37 to 3.00 mg. per cent, both in dogs. In man, Goldring, Clarke and Smith (1936) found the secreted proportion to amount to 95 per cent, at plasma levels below 1 mg. per cent. For the rabbit, comparable data appear to be lacking, excepting the studies of Elsom, Bott and Walker (1937) who investigated the renal blood flow and excretion of phenol red; however, it is difficult to compare these results with ours because of very different experimental conditions employed.

We believe that these figures present evidence that azofuchsin is mainly secreted. If this is true, the drop in the calculated secretion

[6] If MacKay and Oliver (1930) found the phenol red excretion in rabbits dependent on the urine volume, this is explained by the tremendous amount of dye they injected. Since it has been generally found that the secretory capacity of the tubules is limited (in dogs apparently at 0.4 mg. per 100 cc. of plasma (Sheehan, 1936)), it can be calculated from Sheehan's figures that at a plasma concentration of 40 mg. per cent the filtration exceeds the secretion 10 times.

ratio from 85 to 90 per cent at slow urine flow to 82 to 84 per cent at rapid flow would indicate either that the diffusibility of azofuchsin is higher than that of cyanol, or, if the drop actually occurs, that in rabbits the secretion also depends on the rate of urine formation. The latter possibility could be easily understood, for at high urine flow there is a considerable dilatation of the tubules which cannot be without influence on the function of the epithelial cells.

In attempting to answer the question whether our deductions are mere hypotheses, or whether they are supported by convincing or conclusive evidence, we have to consider that the kidney has three major functions; namely, filtration, secretion and reabsorption. If the assumption is correct that the inulin clearance is a true measure of glomerular filtration, if the inulin clearance curve of Kaplan and Smith represents the actual filtration curve at different urine rates, and if our determinations are accurate enough and sufficient in number, it appears that in the case of cyanol, reabsorption can be ruled out, for it would depress the clearance values at low urine flow. In our experiments, however, the reverse was observed, that is an actual increase when the urine flow sank below 2.47 cc. per minute per sq. m. As to a possible secretion of cyanol, the evidence is not so striking. But if we consider the literature (page 750) and if we recall that with an increase in the urine rate from 2.47 to 9.89 cc. per minute per sq. m. the clearance curve of the dye closely followed the curve of the inulin clearance, whereas the clearance of azofuchsin varied from 34 to 39 per cent to less than 28 to 32 per cent at the same rates, we feel that the evidence that cyanol is only or mainly filtered is at least strongly suggestive.

Concerning azofuchsin I, it has been shown that its excretion is fundamentally different from that of cyanol, inulin or xylose. Conclusive evidence has been presented that this difference cannot be explained by reabsorption of the dye (page 763). Logically there remains, then, only the possibility that azofuchsin is secreted in part, a conclusion which is corroborated by the close resemblance of its excretion to that of phenol red. Concerning the relative amount secreted, no definite information is available at present. However, from the data presented it appears that this percentage is not much different from that of phenol red.

SUMMARY

1. The excretion of water has been studied in a large number of experiments on rabbits. After the ingestion of 100 cc. of water, the day before the experiment and as part of the experiment, the average diuresis amounted to 0.6 cc. per minute during a half hour period. The highest individual rate was 1.5 cc. per minute.

2. The excretion of cyanol and of azofuchsin I has also been studied. It has been shown that the cyanol excretion curve closely parallels the inulin excretion curve of Kaplan and Smith. Evidence is presented that cyanol is disposed of entirely or mainly by filtration.

3. The excretion of azofuchsin I is not only very different from that of cyanol or inulin, but almost identical with that of phenol red. Evidence is presented that at low plasma concentration azofuchsin is, in the main, secreted.

BIBLIOGRAPHY

Chambers, R., and Kempton, R. T., *J. Cell. and Comp. Physiol.*, 1933, **3**, 131.
Chapman, E. M., and Halsted, J. A., *Am. J. Med. Sc.*, 1933, **186**, 223.
Chisholm, C. A., *Canad. Med. Assn. J.*, 1930, **22**, N.S., 788.
Cope, C. L., *J. Physiol.*, 1934, **80**, 253.
Cushny, A. R., The secretion of urine, London, Longmans, Green & Co., 1926.
Eisenbrey, A. B., *J. Exp. Med.*, 1911, **14**, 462.
Elsom, K. A., Bott, P. A., and Walker, A. M., *Am. J. Physiol.*, 1937, **118**, 739.
Frothingham, C., Fitz, R., Folin, O., and Denis, W., *Arch. Int. Med.*, 1913, **12**, 245.
Goldring, W., Clarke, R. W., and Smith, H. W., *J. Clin. Inv.*, 1936, **15**, 221.
Grafflin, A. L., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 178.
Grollman, A., *J. Biol. Chem.*, 1925, **64**, 141.
Grollman, A., *Am. J. Physiol.*, 1925–26, **75**, 287.
Heller, H., and Smirk, F. H., *J. Physiol.*, 1932, **76**, 1.
Hoeber, R., *Klin. Woch.*, 1927, **6**, 673.
Hoeber, R., *Arch. ges. Physiol.*, 1930, **224**, 72.
Hoeber, R., and Meirowsky, A., *Arch. ges. Physiol.*, 1932, **230**, 331.
Kaplan, B. J., and Smith, H. W., *Am. J. Physiol.*, 1935, **113**, 354.
MacKay, E., and Oliver, J., *J. Exp. Med.*, 1930, **51**, 161.
Marshall, E. K., *Am. J. Physiol.*, 1932, **99**, 77.
Marshall, E. K., and Grafflin, A. L., *J. Cell. and Comp. Physiol.*, 1932, **1**, 161.
Marshall, E. K., and Kolls, A. C., *Am. J. Physiol.*, 1919, **49**, 302.
Ockerblad, N. F., *J. Am. Med. Assn.*, 1928, **91**, 635.
Oehme, C., *Arch. exp. Path. u. Therap.*, 1921, **89**, 301.
Orzechowski, G., *Arch. ges. Physiol.*, 1930, **225**, 104.
Rees, M. H., *Am. J. Physiol.*, 1918, **45**, 471.

Richards, A. N., in Berglund, H., *et al.*, The kidney in health and disease, Philadelphia, Lea and Febiger, 1935.
Robbins, S., and Wilhelm, M. L., *Arch. ges. Physiol.*, 1933, **232,** 66.
Rowntree, L. G., and Geraghty, J. T., *Arch. Int. Med.*, 1909–10, **1,** 578.
Rowntree, L. G., and Geraghty, J. T., *Arch. Int. Med.*, 1912, **9,** 284.
Scheminzky, F., *Arch. ges. Physiol.*, 1929, **221,** 641.
Schulten, H., *Arch. ges. Physiol.*, 1925, **208,** 1.
Shannon, J. A., *Am. J. Physiol.*, 1935, **113,** 602.
Shaw, E. C., *J. Urol.*, 1925, **13,** 575.
Sheehan, H. L., *J. Physiol.*, 1936, **87,** 237.
Steffanutti, P., *Arch. ges. Physiol.*, 1930, **226,** 148.
Taylor, F. B., Drury, D. R., and Addis, T., *Am. J. Physiol.*, 1923, **65,** 55.
Walker, A. M., Schmidt, C. F., Elsom, K. A., and Johnston, C. G., *Am. J. Physiol.*, 1937, **118,** 95.
Watanabe, C. K., Oliver, J., and Addis, T., *J. Exp. Med.*, 1918, **28,** 359.
Yoshida, H., *Arch. ges. Physiol.*, 1924, **206,** 274.