FILTRATION AND REABSORPTION OF PROTEIN BY THE KIDNEY*

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Plates 1 and 2

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The findings of Walker and Oliver (1) indicate that the glomerular filtrate in mammals may not be entirely protein-free. A large body of evidence, recently reviewed by Rather (2), makes it clear that the cells lining the proximal convoluted tubule are able to reabsorb protein from the glomerular filtrate, and, in some instances, at least partially to degrade it. Dock (3) and Gilson (4) have labelled certain of the plasma proteins *in vivo* by injecting the dye T-1824 intravenously and have found the dye concentrated in the cells of the proximal convoluted tubule. Both workers have interpreted this as evidence of glomerular filtration and tubular reabsorption of plasma protein. It has recently been demonstrated (5) that the not inconsiderable normal urinary protein of the rat has the electrophoretic and solubility characteristics of serum alpha and beta globulin. If the glomerular membrane of the rat allows the passage of large serum globulin molecules, it would seem that the much smaller serum albumin molecules would enter the glomerular filtrate even more readily. This has been construed as strong evidence that in this species, serum albumin is filtered through the glomerular capillary and is constantly reabsorbed by the cells of the renal tubule. A small but constant proteinuria exists in man, and it has been shown that the A/G ratio of the urinary proteins is the reverse of that in the plasma (6).

The concentration of protein in glomerular filtrate is not known with certainty. The only direct measurements are those of Walker, Bott, Oliver, and MacDowell (7). Using methods that could not distinguish protein concentrations of less than 30 mg. per cent with certainty, they observed values ranging from less than 30 mg. per cent to less than 200 mg. per cent. Dock (3), using Bickford and Winton's technique of paralyzing tubular function by perfusion of the rabbit kidney with ice-cold serum (8), obtained protein concentrations of 15 to 22 mg. per 100 cc. of "glomerular filtrate." The present study

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is an attempt to measure quantitatively the amount of protein reabsorbed daily by the renal tubules of the rat kidney.

The dye T-1824 forms a stable blue complex with the plasma proteins. When the dye is injected into the blood stream in low concentrations, it attaches preferentially to plasma albumin. As the concentration of dye is increased, plasma albumin becomes saturated, and the dye begins to attach to the plasma globulins, selecting first alpha globulin. Rawson (9) has shown that the concentration of free dye in the equilibrium system between dye-protein complex, plasma protein, and free dye is infinitesimally small. Allen and Orahovats (10) have described the forces binding T-1824 to plasma protein and consider the renal clearance of T-1824 a measure of the clearance of plasma protein. We have previously demonstrated that when 25 mg. of T-1824 is injected intravenously into 200 gm. rats, no measurable amount of free T-1824 exists in the circulating blood, and when proteinuria is produced, all of the T-1824 found in the urine is similarly protein-bound (11). It was reasoned therefore that T-1824 present in the lumen of the nephron is protein-bound and that the complex T-1824–protein is reabsorbed by the cells of the proximal convoluted tubule. Since T-1824 is carried into the tubule cell attached to protein, and since it is seen to accumulate in the renal cells in the form of intensely blue droplets, by measuring the total T-1824 content of the tubule cells, and the mean ratio T-1824/protein per unit time in the plasma, one can calculate the milligrams of protein reabsorbed by the kidney during any desired time interval. This has been done in the present work.

Methods

Animals.—Female rats of the Slonaker-Addis strain weighing 200 to 210 gm. were used in all the experiments.

Technique of Injection.—All animals received 20 mg. of T-1824 in 1.0 ml. of 0.89 per cent sodium chloride solution intravenously. Animals were bled while under ether anesthesia, by severing the abdominal aorta with a sharp blade. Blood was collected directly into a centrifuge tube held just below the severed vessel.

Chemical Procedures.—Total serum proteins were determined by the micro Kjeldahl method. T-1824 in serum, and kidney extracts were determined with the Coleman spectrophotometer at a wave length of 620 mμ.

Procedure for Determining T-1824 Content of Kidney.—Animals were injected at time zero with 20 mg. of T-1824. At the end of the desired time interval the animals were anesthetized with ether, the abdomen was opened and the aorta and inferior vena cava exposed. Ligatures were placed around the aorta just cephalad to the renal arteries, and approximately ½ inch caudad to the renal arteries. The mesenteric vessels were ligated. A 20 gauge needle was inserted into the aorta between the caudad aortic tie and the renal arteries. An incision was made in the inferior vena cava, and the kidneys were perfused through the needle with 500 ml. of 0.89 per cent sodium chloride solution, at a pressure of 125 cm. of water. At the end of this time, the perfusate from the inferior vena cava was free of visible dye, and the bladder contained several milliliters of colorless fluid. Unstained frozen sections of these kidneys show that dye had been completely removed from the renal blood vessels

and tubular lumen system. The kidneys were removed, and their capsules stripped. Each kidney was homogenized in a mortar with 5 gm. of sea sand and 6 ml. of distilled water. Twenty-five ml. of 1.0 per cent aerosol OT was added, and the mixture allowed to stand with frequent agitation for 30 minutes. The T-1824 passed from the tissue into the liquid phase. Fifty ml. of acetone was added to precipitate the tissue proteins and the mixture was shaken frequently for 15 minutes before centrifuging. After centrifugation, the blue supernatant solution was decanted and placed into a 250 ml. volumetric flask. The remaining precipitate which had lost its blue color was resuspended in 50 ml. of a mixture of 6 parts water, 25 parts aerosol OT, and 50 parts acetone, and recentrifuged. The supernatant, which usually had a faint blue tinge, was added to the previous supernatant in the 250 ml. volumetric flask. The precipitate was then free of blue dye, and additional washing of this material revealed no additional T-1824 on spectrophotometric determination. The material in the 250 ml. volumetric flask was made to volume with the mixture of aerosol, acetone and water. An aliquot was taken for the spectrophotometric determination of T-1824.

Determination of Ratio T-1824/Protein in Serum.—Animals were injected with 20 mg. of T-1824 at time zero. At frequent time intervals ranging from 30 minutes to 48 hours after injection, groups of rats were bled from the abdominal aorta. The concentration of T-1824, and total proteins in the serum were determined by the methods outlined above.

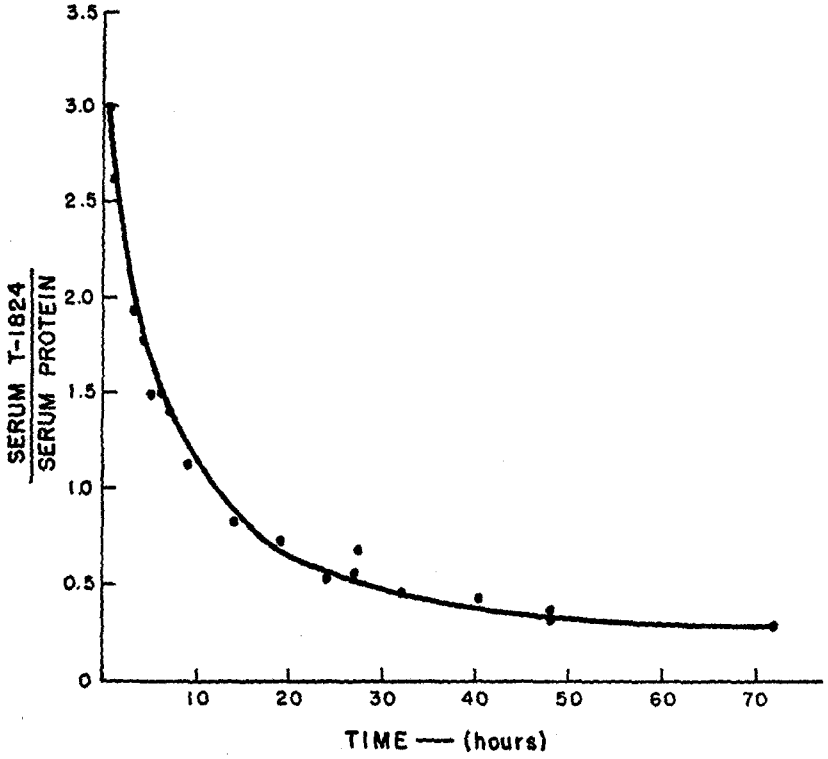
RESULTS

The T-1824/protein ratio is plotted against time in Text-fig. 1. Each point on the curve represents the mean of determinations made on three animals. In order to determine the mean T-1824/protein ratio in the serum for any desired time interval, planimetric integration was performed by measuring the area under the curve during this time interval with a compensating polar planimeter.

Unstained frozen sections of the kidney of rats taken 15 minutes after the intravenous administration of 25 mg. of T-1824 revealed blue dye in the peritubular capillaries, and to some extent in the capillary forming the glomerular tuft. Sections taken 30 and 60 minutes after injection were colorless. At 90 minutes, a few small blue droplets were seen in cells lining the proximal convoluted tubules, and were found close to the luminal border of the cell (11). Sections taken 3, 7, 19, and 24 hours after injection showed progressively more dye in these cells, and more of the cells were seen to contain the dye. The droplets appeared to become larger, and to occupy the entire cell from luminal to capillary border. Dye was not seen elsewhere in the kidney, except for occasional staining of the elastic layer of the larger arterioles (Figs. 1 to 3). There was usually less dye in the cells of the proximal tubule in sections taken 48 hours after injection (Fig. 4), while 72 hours later the amount of dye was always reduced. From these sections, it is clear that when T-1824 is extracted from the perfused kidney by the methods described above, it can be said to come entirely from the cells of the proximal convoluted tubule.

The total T-1824 content of the two kidneys of the rat is plotted against time after injection in Text-fig. 2. Each point is the mean of determinations made on the kidneys from three rats.

From the data below (Table I), we have calculated the milligrams of protein reabsorbed by the cells of the proximal tubule (Text-fig. 3). For the first 4 hours after dye injection, protein is reabsorbed at a rate of 4 to 6 mg. per hour per rat. As time goes on, this rate appears to fall and level off at a value of about 2 mg. per hour per rat.

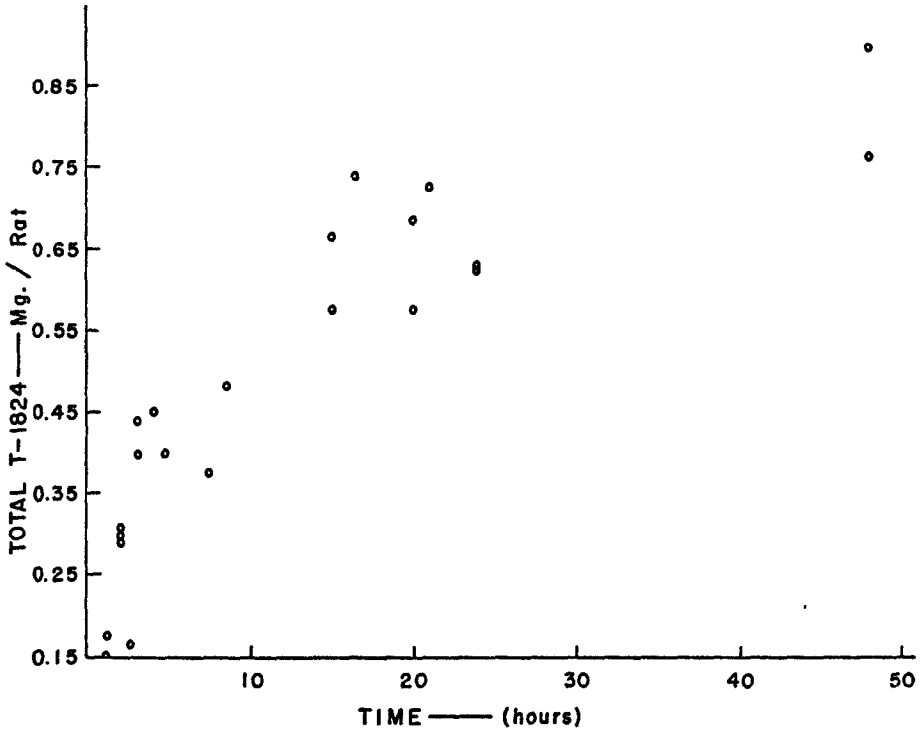


TEXT-FIG. 1. The rate of fall of the T-1824/protein ratio in rat serum following the intravenous injection of 20 mg. T-1824.

DISCUSSION

In studying the rate of protein reabsorption by the kidney, one aims in so far as possible to study the reabsorption of native, unaltered protein. We have found it necessary to tag native rat plasma protein with dye T-1824. While it does not seem unreasonable that certain properties of the serum proteins under investigation could be altered by this procedure, all available evidence indicates that tagged and unaltered plasma protein behaves in an identical manner. Rawson (9) has shown that T-1824 albumin has the same electric mobility as untagged albumin. It has been demonstrated (12) that tagging plasma albumin with T-1824 does not alter the highly specific immunochemical char-

acteristics of this substance. Others have shown that curves describing the disappearance of T-1824–albumin from the circulation are not significantly different from those of albumin tagged with I^{131} (13, 14). On the basis of these observations, we have assumed that T-1824–protein undergoes glomerular filtration and tubular reabsorption in a manner identical with that of untagged plasma protein.



TEXT-FIG. 2. The rate of uptake of T-1824 in the total renal tissue following a single intravenous injection of 20 mg. of the dye.

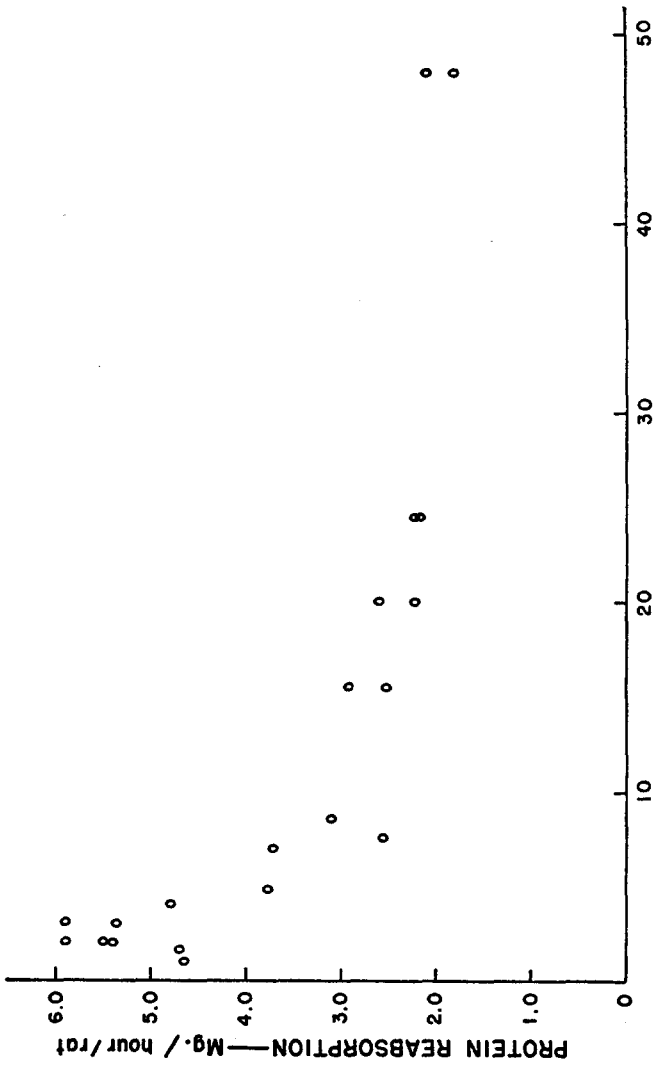
Once T-1824–protein is reabsorbed into the cells of the proximal convoluted tubule it appears to be concentrated into droplets. Here, it is most likely that T-1824 is split off and remains in the droplet, while the protein moiety of the complex is more or less degraded, and the degradation products are discharged into the peritubular venous capillary. The droplets containing T-1824 accumulate in the cells for at least 24 hours, and after 48 to 72 hours sections of the kidney reveal less dye than was observed previously. Rather (15) has shown that reabsorbed hemoglobin disappears from the cells of the convoluted tubules in a matter of hours, while Oliver (16) states that it takes 2 to 3 days for re-

absorbed egg white droplets to nearly completely disappear, and Smetana (17) finds that diazotized serum proteins persist indefinitely within the renal epithelium. We have assumed that all of the T-1824 reabsorbed by the tubules over a given time period remains in the cell and that we recover this by our extraction procedure. It is entirely possible that much of the T-1824 accumulates into droplets, but that some traverses the cell in a highly dispersed form and passes directly into the renal vein blood. Oliver and Lund (18) have described this sort of dual transport of dye during the tubular secretion of neutral

TABLE I

Time after injection	Mean ratio $\frac{\text{Mg. T-1824}}{\text{Mg. protein}} \times 10^{-2}$	Mean total T-1824 content of kidneys	Protein reabsorbed
hrs.		*mg.*	*mg./rat hr.*
1.0	2.90	0.138	4.76
2.0	2.63	0.288	5.48
2.0	2.63	0.310	5.89
2.0	2.63	0.290	5.50
2.0	2.63	0.252	4.80
3.0	2.48	0.440	5.91
3.0	2.48	0.400	5.38
4.0	2.36	0.450	4.80
4.75	2.25	0.400	3.74
7.0	1.98	0.513	3.70
7.5	1.94	0.375	2.58
8.5	1.86	0.483	3.10
15.5	1.48	0.575	2.50
15.5	1.48	0.665	2.90
20.0	1.31	0.685	2.61
20.0	1.31	0.575	2.20
24.0	1.20	0.725	2.17
24.0	1.20	0.625	2.17
48.0	0.89	0.900	2.11
48.0	0.89	0.763	1.80

red. In so far as dye is reabsorbed and leaves the cell before the experimental time has expired, our figures will be in error and it must be realized that the figure obtained represents a minimal figure for protein reabsorption. It is possible that the fall in protein reabsorption rate described in Text-fig. 3 can be explained on this basis. During the first several hours of the experiments, the rate of protein reabsorption ranges from 4 to 6 mg. per hour per rat. After this, the rate falls and appears to level off at approximately 2 mg. per hour per rat. If T-1824 does leave the cell, once it has been reabsorbed, this must be a process that takes time, else the dye would not accumulate in the cells as it is shown to do in Fig. 1 to 4. Hence, less T-1824 will have left the cells during the first hours



TEXT-FIG. 3. Calculated rate of protein reabsorption by the total renal tissue as measured over the indicated time intervals.

of the experiment, and the fall in rate of protein reabsorption seen in later hours may well be an error due to loss of T-1824 into renal venous blood. For these reasons, we consider the figures obtained during the first 3 to 4 hours as the most reliable index of the rate of protein reabsorption, and will take 5 mg./rat hour as being the mean rate of protein reabsorption for this period.

The best available figure for the rate of glomerular filtration in the rat is 0.6 ml./100 gm. body weight/min. (19) or for animals weighing 200 gm., 1.2 ml./min. This is equivalent to 72 ml./hour. Female rats weighing 200 gm. excrete protein in their urine at a rate of about 0.5 mg. per hour. Hence, if protein is being reabsorbed at a rate of 5 mg. per hour, the glomerular filtrate would contain 5.5 mg. of protein in 72 ml. of fluid or would contain protein to the extent of 7.6 mg. per cent. This concentration is well below the limits discernible by the methods employed by Walker *et al.* and is somewhat lower than the values obtained by Dock in the perfused rabbit kidney.

The circulating blood volume of the 200 gm. rat is about 15 ml. and the hematocrit value about 60 per cent (20). If the concentration of plasma protein is 6 gm. per cent, there is a total of 360 mg. of circulating plasma protein. Consequently, the rat filters and reabsorbs 120 mg. of protein per day, or approximately 33 per cent of its circulating plasma protein. If the reabsorbed protein is at least partially degraded in its passage through the renal tubule cell, the kidney can be said to metabolize 33 per cent of the circulating protein per day and may be considered an important organ in regulating the rate of protein synthesis.

It is not held that the calculated rates of protein reabsorption represent precisely what takes place in the kidney. We do believe, however, that it is a first approach to obtaining a minimal value for protein reabsorption in the intact animal. As such, it is permissible to report these data, inexact though they may be, in the hope that from here, more precise methods may be developed and the figure may constantly be revised until something approaching actual values will be obtained.

SUMMARY AND CONCLUSIONS

Plasma proteins of the rat have been labelled by the *in vivo* injection of the dye T-1824.

From a study of the rate of disappearance of T-1824 from the circulating blood, and the total T-1824 content of the perfused kidney the rate of protein reabsorption from the glomerular fluid by the cells of the renal tubule has been calculated.

It is concluded that protein reabsorption by the cells lining the proximal convoluted tubule of the rat kidney proceeds at a rate of at least 5 mg. per hour, equivalent to a daily filtration and reabsorption of 33 per cent of the circulating plasma protein.

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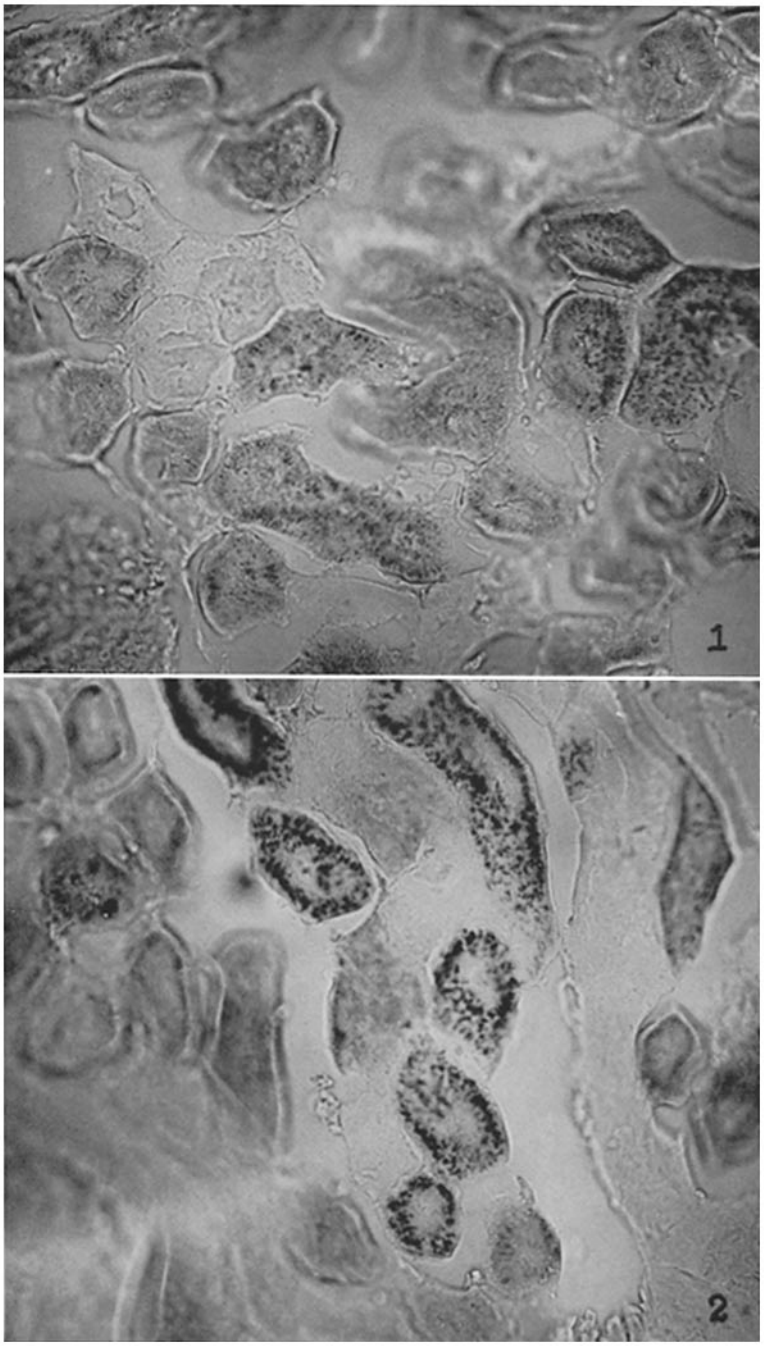
EXPLANATION OF PLATES

PLATE 1

FIG. 1. Unstained frozen section of rat kidney removed 3 hours after the intravenous injection of 20 mg. T-1824. Note a few scattered dark droplets containing T-1824 in some of the proximal convoluted tubule cells. These droplets are actually bright blue in color. × 535.

FIG. 2. Seven hours after the intravenous injection of 20 mg. T-1824. There is a sharp increase in the number of droplets over that seen in Fig. 1. × 535.

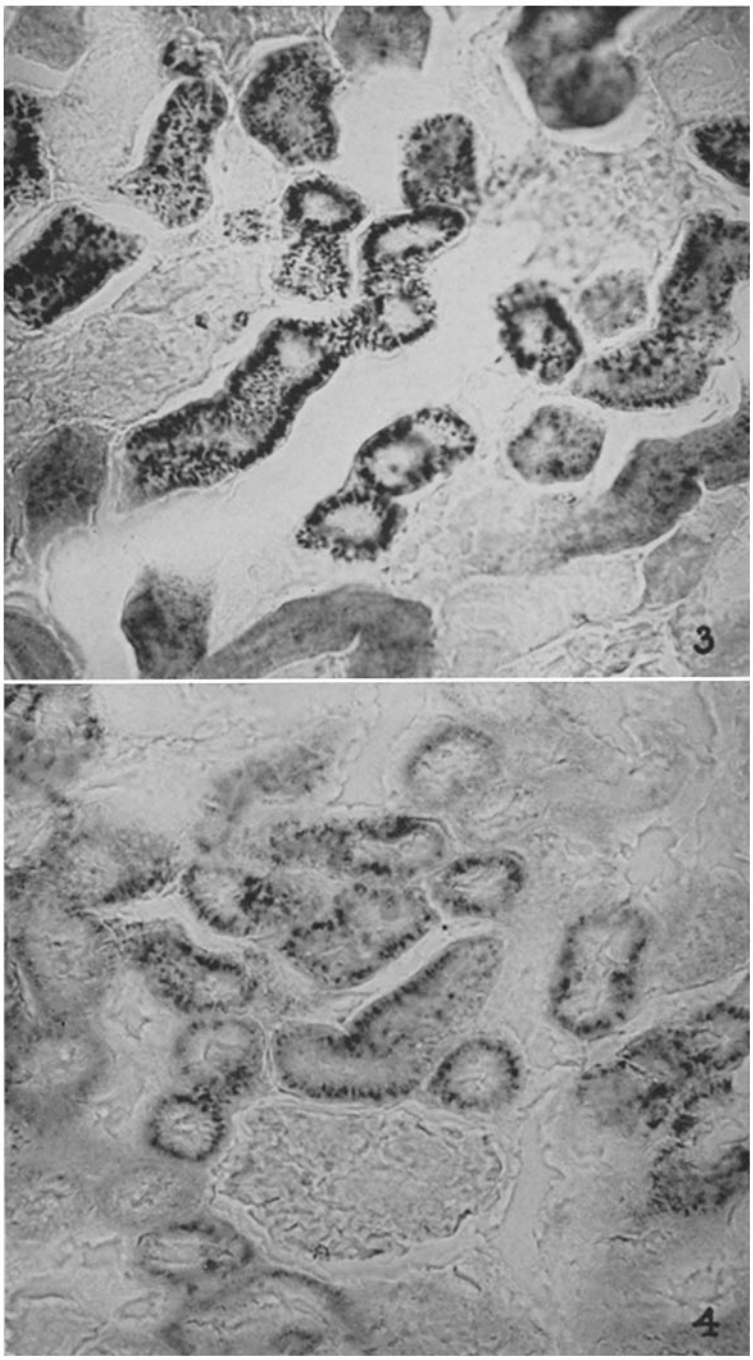


(Sellers *et al.*: Filtration and reabsorption of protein by the kidney)

PLATE 2

FIG. 3. Twenty-four hours after T-1824 injection. The number of droplets and the number of tubule cells containing droplets are maximal at this time. × 535.

FIG. 4. Forty-eight hours after T-1824 injection. There is a marked decrease in the number of T-1824 droplets since the 24 hour section. × 535.



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