MECHANISM OF PROTEINURIA

III. A Comparison of the Functional and Structural Aspects of the Effects of Certain Intraperitoneally Administered Proteins on Hemoglobin Excretion in the Rat*

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Plates 23 to 27

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From an *a priori* consideration, it would seem that the rate of urinary protein excretion is dependent upon four major factors: the rate of glomerular filtration, the permeability of the glomerular membrane, the rate of tubular absorption, and the rate at which the tubular epithelium can dispose of absorbed protein. In order to understand the mechanism of any particular proteinuria, it is necessary to consider each of these factors and the extent to which it determines the rate of urinary protein excretion.

In the rat, an indication of the glomerular filtration rate can be obtained with reasonable accuracy from the exogenous creatinine clearance (1). The influence of this factor upon the rate of protein excretion can thus be determined.

The glomerular permeability to protein can be determined by the use of hemoglobin as an indicator. When glomerular permeability is increased, as by the administration of bovine albumin, a sharp rise in the hemoglobin excretion rate occurs (2).

The tubular absorption of protein can be indicated by the threshold serum concentration at which hemoglobin appears in the urine, provided that the glomerular filtration rate is known. A more direct indication of tubular protein absorption can be obtained by the direct histologic observation of absorbed protein, in the case of hemoglobin by a modified benzidine stain, as described in a later portion of this study.

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For the purposes of investigation over short time periods, the tubular disposal rate of hemoglobin can be neglected, since it is of a very low order of magnitude when compared to the other rate-determining factors (3).

At the start of this investigation, it was known that glomerular permeability to hemoglobin in the rat is sharply increased by the administration of another protein, bovine albumin, which is similar in molecular size but of somewhat dissimilar molecular shape (2). It was known also that the urinary excretion rate of rat serum protein is increased in a like manner by the administration of bovine albumin, but not by hen egg white, a mixture which contains protein constituents that are very dissimilar from rat serum proteins in all respects (4). The work reported here compares by methods of functional examination the effect of bovine albumin and the dissimilar proteins of egg white upon the glomerular permeability and tubular absorption of hemoglobin. In addition, two of the constituent proteins of hen egg white, ovalbumin and ovomucoid, of similar molecular size but different chemical constitution, were examined to see whether they affected the excretion of hemoglobin similarly or differently. The functional findings thus obtained are compared and correlated with the structural aspects of tubular and glomerular activity as they can be seen in the kidney tissue derived from similar experiments.

To those who are accustomed to think of protein absorption as it occurs in the intestinal tract, where the proteins are digested and absorbed principally as amino acid fragments, the absorption of protein in the kidney may seem physiologically unorthodox. It is probable that the tubular epithelium absorbs protein molecules intact. Within a few minutes after the intravenous administration of hemoglobin to the normal animal, droplets of hemoglobin appear in the tubular epithelium cells and can be stained with relative specificity by the benzidine reaction. Other proteins of medium molecular size also appear promptly as droplets in the cells of the proximal convoluted tubules (5). It is difficult to conceive of an absorption mechanism which would require the degradation of protein molecules on one side of the cell membrane, with their immediate reconstitution after crossing the cell boundary.

From this viewpoint, it seemed useful to learn whether and in what manner the preliminary saturation of the tubule cells with one protein might affect the subsequent absorption of another protein. The following experiments were undertaken for this purpose.

A. FUNCTIONAL ASPECTS

Methods and Materials.—In the functional portion of the investigation approximately 200 rats of the Slonaker strain[1] were used, each weighing about 150 gm. The general experimental

[1] Our colony is directly descended from the Addis colony, which was started at the Stanford University School of Medicine in 1924 by Dr. Thomas Addis, with rats obtained from Professor J. R. Slonaker of Palo Alto. The Slonaker colony was started in 1903 with rats from a Chicago colony, believed to be the progenitor of the Wistar Institute colony (see *J. Gen. Psychol.* 1930, **3**, 324).

plan previously detailed (2) was followed closely, with minor modifications as indicated. Control groups received three intraperitoneal injections, at 9.30 a.m., 4.30 p.m., and 9.30 a.m. the following morning. Each injection consisted of 16 ml. of 0.85 per cent sodium chloride solution.

Groups studied for the effect of hen egg proteins received three intraperitoneal injections of 0.85 per cent sodium chloride solution, on the same schedule as the control groups. The substance being tested, 1 gm. crude egg white strained through gauze, 1 gm. ovalbumin, or 0.6 gm. ovomucoid, was dissolved in the fluid of the second injection. Groups studied for the effect of bovine albumin received three intraperitoneal injections on the same schedule as the control groups but each injection contained 1 gm. bovine albumin dissolved in the sodium chloride solution.

It was necessary to vary the dose of protein administered by intraperitoneal injection because of differences in absorption and excretion rate, in solubility, and in toxicity. The objective in each case was to fill the tubule cells with the respective proteins, as a preliminary to hemoglobin injection. That we succeeded in this endeavor was shown by the morphologic appearance of the absorbed protein in the tubule cells that will be described later.

Hemoglobin was administered intravenously 5½ hours after the third intraperitoneal injection. The dose was varied by diluting 7 per cent hemoglobin solution with 7 per cent human albumin, so that the total volume injected was always 1.6 ml. and the tonicity of the solution remained reasonably constant. The creatinine clearance was determined simultaneously. For this purpose 0.10 ml. of 5 per cent creatinine solution was added to the hemoglobin solution. Groups killed at 2 and 15 minute intervals after the intravenous injection were utilized for the determination of hemoglobin and creatinine serum concentrations and urinary excretion rates. A short interval was used in order to minimize the difference between initial and final concentrations in calculating the clearances. The urine volume collected was usually from 2 to 3 ml. The procedure for termination of the urine collection was modified from the previous method (6). At the end, each rat was anesthetized with ether while held in place over the collection funnel. The abdomen was then opened widely and the bladder was examined. Any urine remaining in the bladder was evacuated by syringe and added to the urine collection.

For reasons previously discussed, we have reported rates of excretion in terms of the predicted kidney weight in gm. (GKWP) (2, 7). Since a 150 gm. rat of our colony has a total predicted kidney weight of about 1100 mg., our rates can be multiplied by the factor 0.73 to obtain a rough equivalent to the rate per 100 gm. body weight.

Total hemoglobin concentration was measured by the method of Evelyn and Malloy (8). Creatinine clearances were determined by the "undisturbed" method (6), as modified above, and creatinine determinations were performed by the method of Bonsnes and Taussky (9). Mean hemoglobin and creatinine concentrations were calculated by assuming an exponential rate of fall in concentration during the experimental period (2).

The bovine serum albumin used corresponded to fraction V of Cohn.[2] The human albumin was diluted from concentrated, salt-poor albumin produced by The American National Red Cross. The human hemoglobin solution was prepared by Pennell (10) and was subjected to osmotic pressure determinations after a long period of storage (11). There was no significant change from the expected mean molecular weight of 68,000. The same lot of hemoglobin has been used in all of these studies. Crystalline ovalbumin was obtained commercially from Eli Lilly and Company. Ovomucoid was prepared by the method given by Plimmer (12).

Results.—As in experiments previously reported, all the animals had a pronounced diuresis after the hemoglobin injection, with hemoglobin appearing in the first voided urine, often as soon as 2 minutes after injection.

[2] Provided by the kindness of Dr. J. D. Porsche, Armour Laboratories, Chicago.

TABLE I

Simultaneous Determination of Creatinine Clearance, Hemoglobin Excretion, and Serum Concentration

KWP	Hemoglobin				Creatinine					*FS* column (1) × column (2)
	Initial concentration	Final concentration	(1) Mean concentration	Excretion	Initial concentration	Final concentration	Mean concentration	Excretion	(2) Clearance	
mg.	*mg./ml.*	*mg./ml.*	*mg./ml.*	*mg./min./GKWP*	*mg./ml.*	*mg./ml.*	*mg./ml.*	*mg./min./GKWP*	*ml./min./GKWP*	*mg./min./GKWP*
Controls										
1285	2.22	1.84	2.05	0.0742	0.0731	0.0412	0.0557	0.0796	1.44 ± 0.30*	2.95
1277	6.65	5.18	5.99	0.334	0.0750	0.0379	0.0561	0.0766	1.37 ± 0.11	8.21
1306	10.06	7.90	9.09	0.515	0.0869	0.0393	0.0621	0.0771	1.26 ± 0.21	11.45
								Mean...	1.36 ± 0.22	
Bovine albumin injections										
1073	1.60	1.38	1.50	0.110	0.0864	0.0387	0.0577	0.0960	1.69 ± 0.28	2.54
1170	7.03	4.61	5.88	0.632	0.0927	0.0370	0.0629	0.0852	1.36 ± 0.21	7.99
1112	10.43	7.44	9.03	1.037	0.0880	0.0457	0.0667	0.0944	1.43 ± 0.21	12.91
								Mean...	1.49 ± 0.25	
									P = 0.25‡	
Egg white injections										
1170	2.27	1.80	2.06	0.0624	0.0983	0.0482	0.0729	0.0960	1.32 ± 0.22	2.72
1182	6.35	4.97	5.74	0.285	0.0867	0.0442	0.0649	0.0758	1.17 ± 0.09	6.72
1195	9.02	7.61	8.40	0.412	0.0834	0.0426	0.0628	0.0696	1.12 ± 0.31	9.41
								Mean...	1.20 ± 0.22	
									P = 0.15	
Ovalbumin injections										
1085	2.60	.16	2.40	0.124	0.0975	0.0348	0.0632	0.0831	1.32 ± 0.14	3.17
1050	7.77	6.00	6.98	0.507	0.0877	0.0321	0.0574	0.0899	1.58 ± 0.21	11.03
1066	12.03	10.05	11.14	0.688	0.0856	0.0350	0.0586	0.0827	1.41 ± 0.13	15.71
								Mean...	1.44 ± 0.18	
									P = 0.4	
Ovomucoid injections										
1122	2.33	1.97	2.15	0.0877	0.0751	0.0405	0.0640	0.0877	1.40 ± 0.25	3.01
1141	6.76	5.16	5.99	0.403	0.0877	0.0432	0.0646	0.0976	1.52 ± 0.22	9.10
1129	11.31	9.62	10.60	0.619	0.0870	0.0465	0.0638	0.0997	1.58 ± 0.21	16.75
								Mean...	1.50 ± 0.19	
									P = 0.2	

* Standard deviation of the mean (6 determinations in each group).

‡ P calculated by method of Fisher (14) to indicate significance of difference from mean value of controls.

The administration of egg white is associated with circulatory disturbances obvious in the gross. Immediately following intraperitoneal egg white injection, there is a period of oliguria lasting several hours. The animals become edematous, with swollen faces, paws, and subcutaneous tissue. After 4 to 6 hours, there is a diuresis and the edema rapidly disappears, although the proteinuria continues. It is noteworthy that Clark and MacKay (13) prevented this sequence of events by the use of vasoconstrictive agents. Extended investigations over the several years that egg white has been used in these laboratories for various purposes have shown that, in the doses here described, it produces no structural damage to the kidney of the rat. The time for our excretion studies was chosen so as to follow the period of oliguria and diuresis, but also to remain within the period of maximal proteinuria.

Following the administration of ovomucoid, there was moderate subcutaneous edema, but much less and of shorter duration than the edema that followed egg white administration. The edema was least following the administration of ovalbumin. The latter protein was incompletely absorbed, a small portion remaining as a coagulum in the peritoneal cavity at the time of autopsy.

Kidneys of the animals injected with bovine albumin showed a 22 per cent increase in weight over that predicted for normal rats of the same size. Those injected with egg white showed a 16 per cent increase in weight, while the control animals showed an increase of only 6 per cent. After ovomucoid injection the kidney weight increase was 9 per cent, after ovalbumin injection the kidney weight increase was 6 per cent, and neither increase was considered to be significantly different from the controls. The significance of such differences in kidney weight was discussed in connection with the earlier work (2). The gain in kidney weight during the experiment was attributed to accumulation of absorbed protein in tubule cells and, in the case of bovine albumin, to the accumulation of fluid in the dilated tubules. This interpretation is supported by the histologic changes that have been described as accompanying bovine albumin and egg white proteinuria in the rat (5), and our new results, both functional and structural, support these conclusions. Since we consider these weight changes to be non-functional, we have continued to express excretion rates in terms of the predicted kidney weight for a normal rat of the same size.

In the control animals, the mean creatinine clearance was 1.36 ml./min./GKWP. The various protein injections produced slight changes in the creatinine clearance which were not statistically significant by the criteria of Fisher (14). (Table I.) The creatinine clearance did not change significantly or consistently with a rise in the hemoglobin dosage.

The following formula expresses the relationship of hemoglobin excretion rate to the various factors affecting it:

$$E = -Tm + GFS$$

where

E = hemoglobin excretion rate,
Tm = maximum tubular rate of hemoglobin absorption,
G = relative glomerular permeability to hemoglobin,
F = glomerular filtration rate (creatinine clearance),
S = serum hemoglobin concentration.

F can be measured, is constant, and independent of the serum hemoglobin concentration under conditions that are otherwise constant. If it is assumed that, under such conditions, Tm and G are likewise constant, then a plot of E against the product FS should result in a straight line of which the negative y axis intercept is in direct proportion to Tm and the slope is equal to G.

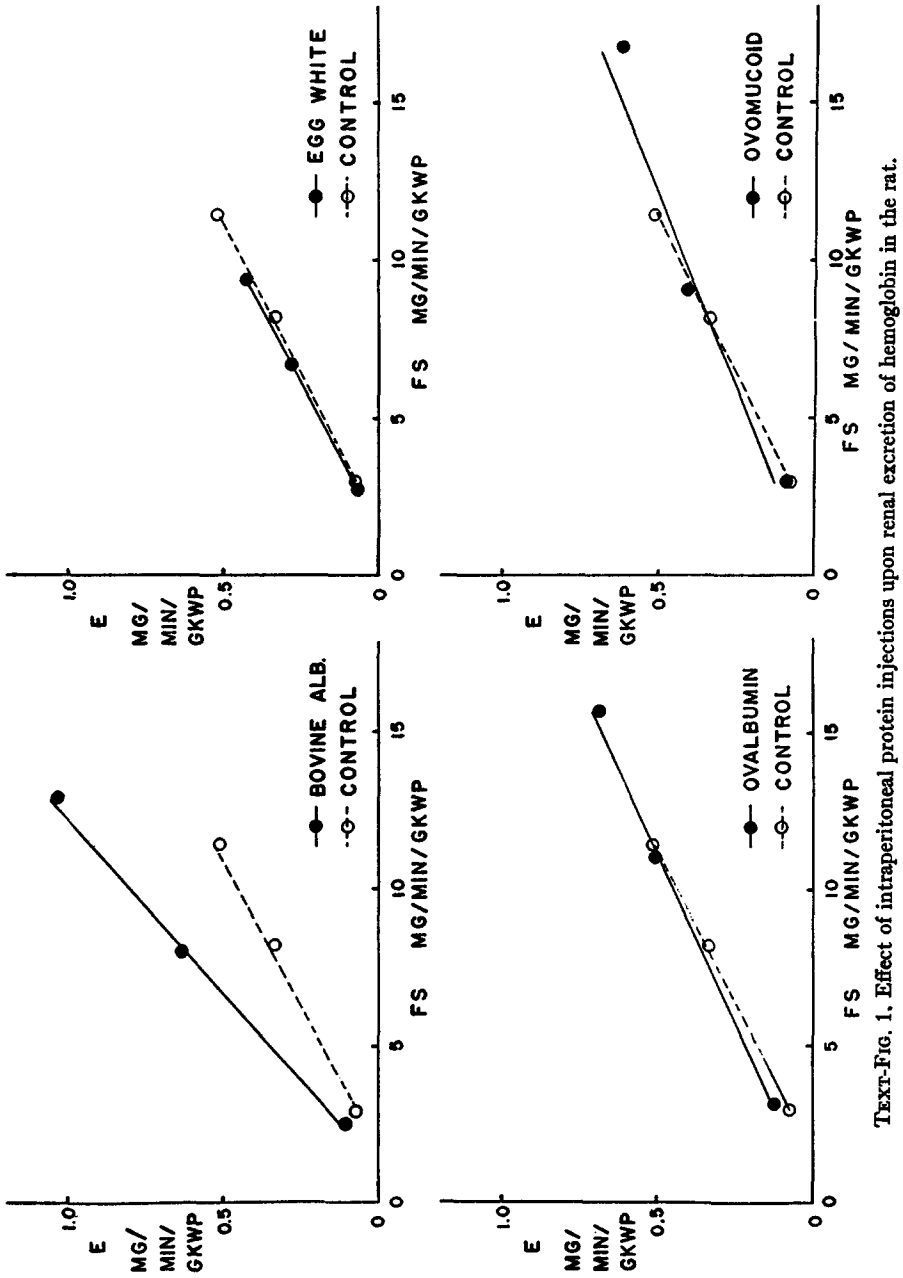
When the data are plotted in such a way (Text-fig. 1) it is seen that bovine albumin injections sharply increase the linear slope, while the other proteins injected produce no significant effects. The bovine albumin injections also appreciably reduce the negative y axis intercept as compared to the control curve, while the other proteins again produce no significant effects.

From their functional aspects, therefore, the experiments would seem to indicate that bovine albumin increases glomerular permeability to hemoglobin and diminishes the tubular absorption of hemoglobin. In contrast, the various proteins derived from hen egg white, which are more unlike hemoglobin than bovine albumin, have no significant effect upon glomerular permeability to hemoglobin or its tubular absorption.

B. STRUCTURAL ASPECTS

From the morphologic aspects of the process it is known that the absorption of various proteins, as they pass down the renal tubule, is performed by a specific portion of the proximal convolution. Certain cytologic changes accompany this absorption and the renal cells in the end are more or less filled with droplets of the absorbed material (5). It might therefore be supposed that if these functioning cells were to be filled with droplets of one sort of protein there would be less taken up of another. Physical space, indeed, appears lacking in some instances for the accommodation of additional droplets. Moreover, the exhaustion of mitochondrial and other cytoplasmic materials which participate in the formation of the droplets (5) would also seem to be a factor limiting the amount of protein that might thus be absorbed.

The fact that the observed droplets are not composed purely of absorbed protein modifies considerably the cogency of such *a priori* reasoning. If the drop is a complex "coacervate," composed of many substances, could it not take up a second protein that reached it from the tubule lumen? The problem is obviously less simple than the classic concept of "protein drops" and "blockage" would suggest. This is indeed suggested by the apparently paradoxical results of the functional experiments just described which showed that bovine



TEXT-FIG. 1. Effect of intraperitoneal protein injections upon renal excretion of hemoglobin in the rat.

albumin interferes with the subsequent tubular absorption of hemoglobin while egg white proteins, either in native mixture or in prepared fractions, do not.

As has been stated, the rate at which a protein is absorbed from the tubule lumen as it passes down the proximal convolution is only one of the factors that determine the rate of protein elimination. Another is the rate of its filtration through the glomerular membrane. Here, too, a striking contrast has been manifested in our functional examinations of the effect of two different proteins on the filterability of a third, hemoglobin. Bovine albumin markedly increases the hemoglobin filtration rate while egg white proteins have little or no effect upon it.

All these proteins can be seen in the cells as droplets and the hemoglobin can be stained specifically. The amount of hemoglobin that has passed the glomerular filter may be estimated visually by its appearance within the nephron lumen. Therefore, it would appear that herein lies an opportunity not only for a cooperative correlation of the structural and functional aspects of proteinuria, but also for a direct observation of the mechanisms by which preliminary injections of two proteins produce different effects upon the excretion of a third.

Methods.—The experiments were similar in general design to those used in the functional study. 5½ hours after the last injection of each of the preliminary "blocking" proteins, bovine albumin, egg white, ovalbumin, and ovomucoid, each animal received an intravenous injection of 2.0 ml. 7 per cent hemoglobin. After 1 hour the animal received a second injection of hemoglobin and 1 hour later was killed. Control experiments of two sorts were run. In one set the preceding injections of proteins were replaced by injections of saline. In the other the final injections of hemoglobin were replaced by injections of saline. In all 60 rats were used. The kidneys were sliced sagitally. One portion was fixed in 10 per cent formalin and another portion in Helly's solution. Paraffin sections of the formalin-fixed material were stained by the benzidine method of Ralph (15). Sections from the Helly-fixed material were stained with iron-hemotoxylin, the Gram method, and pyronine-methyl green. Visual examination of the sections was made both by conventional and by phase microscopy. In order to observe the faint yellow color of the benzidine reaction, a bright phase objective was used. With the former, both in visual and photographic examination, a blue Wratten C5 filter was used.

Cytologic Appearance of Various Protein Droplets after Individual Administration

The manner of formation and evolution of various protein droplets in the cells of the proximal convolution has been previously described in detail (5). The present description is limited to that which was seen in control experiments of the present series in which only one protein was being excreted in the urine and absorbed by the renal cells.

Bovine Albumin.—Sections of the kidney excreting bovine albumin show the renal cells in a majority of the cross-sections of the cortical proximal convolutions filled with droplets approximately 2 to 3μ in diameter. In regions where the droplets predominate, the mitochondrial rodlets have disappeared. In those

cells in which there are few or no droplets the original rodlets are well preserved. The droplets are Gram- and pyronine-positive, an indication that they are not pure bovine albumin but that they contain considerable amounts of a cytoplasmic element, ribonucleic acid. Other histochemical tests show them to contain phospholipids. (Fig. 1.)

Egg White.—Almost all the cells of the cortical proximal convolutions are crowded with droplets measuring from 3 to 6μ in diameter. Except for their size and great number, they are similar to those seen in the bovine albumin experiments. They are also pyronine- and Gram-positive. (Fig. 2.)

Ovalbumin.—The sections present an appearance intermediate between those seen after the bovine albumin and egg white. The droplets are larger than the former, more of them are within the cells and a larger part of the convolution is filled. (Fig. 3.)

Ovomucoid.—When living cells of the proximal convolutions are observed by phase microscopy suspended in physiologic media, they are seen to be filled with droplets that are similar in appearance to those of the other absorbed proteins. In fixed and stained material a striking difference is observed. Coagulation of this highly hydrated substance by the fixative results in separation of its excess water so that an irregular, empty vesicle surrounded by a thin skin of coagulated protein is formed. The result of this transformation is to produce the appearance of a foamy, vacuolated cytoplasm in which no solid droplets are seen. (Fig. 4.)[3]

Hemoglobin.—In sections stained with iron-hematoxylin, the appearance of the proximal convolution cells is not dissimilar from that seen when bovine albumin or egg white droplets fill the cells. (Fig. 5.) When the specific benzidine stain is used all the cortical cross-sections of proximal convolutions are seen to be filled with hemoglobin, while the medullary, terminal portions contain only traces. (Fig. 7.) The hemoglobin appears in two forms: as bright yellow-orange droplets of small size that fill the renal cells, and as a diffuse, yellowish staining of the cytoplasm between the droplets. (Fig. 10.) In none of the animals that had received only hemoglobin and saline were accumulations of the pigment visible either in the glomerular spaces or in tubular lumens. Again the pyronine and Gram stains disclose the presence of ribonucleic acid.[4]

Cytologic Appearance of Various Protein Droplets after Combined Administration

In all the experiments, with the exception of those with ovomucoid, in which two proteins were being excreted in the urine, droplets were seen in sections stained with iron-hemotoxylin or by the Gram method, filling the cells of the

[3] Absorbed gelatin and globin present the same vacuolar appearance in histologic sections.

[4] For preliminary statements on this finding see Oliver, J., *J. Mt. Sinai Hosp.*, 1948, **15**, 175; *Tr. Conf. Renal Function*, October 20–21, 1949, Josiah Macy, Jr., Foundation, New York, 1950.

cortical proximal convolutions. Since both protein droplets and hemoglobin droplets are similarly stained, the sections from the different experiments looked remarkably alike. The benzidine-stained sections, however, showed striking differences in the various combinations. Low power fields, such as those of Figs. 7 to 9, show renal cortex and medulla of typical examples from the saline-hemoglobin, egg white-hemoglobin, and bovine albumin-hemoglobin experiments, photographed with a blue filter to bring out the yellowish color of hemoglobin as a dark tone. At this magnification, the discrete droplets can hardly be made out so that the general intensity of the dark tone represents the total hemoglobin, droplet and free, in the cells of the cortical proximal convolutions. It will be seen that there is no great difference between the intracellular accumulation (absorption) of hemoglobin in the cortex of the control saline-hemoglobin (Fig. 7) and egg white-hemoglobin experiments (Fig. 8), whereas there is a definite cortical pallor in the bovine albumin-hemoglobin experiment. (Fig. 9.)

Another difference is the appearance in the kidney of the bovine albumin hemoglobin experiment of large accumulations of hemoglobin which appear in the photomicrographs as black cast-like deposits in the glomerular space and in segments of the tubule. (Figs. 6 and 9.) Such accumulations were not seen in any of the animals that had received saline-hemoglobin or egg white-hemoglobin.

The detail of the intracellular accumulations of hemoglobin in the various experiments is best seen with a "bright phase" oil immersion objective. By its use the advantage of increased contrast outlines the droplets sharply and yet leaves the yellow color of the benzidine reaction unchanged. In Fig. 10 are seen the small, somewhat irregular, intensely yellow droplets in a proximal convolution from a saline-hemoglobin experiment and also the considerable diffuse yellow of "free" cytoplasmic hemoglobin. In Fig. 11 the droplets of egg white are easily distinguished from the hemoglobin droplets by their greater size and number, but they also are deeply tinged with the yellow reaction of hemoglobin. There is also a considerable diffuse yellow cloud of free cytoplasmic hemoglobin. Fig. 12 shows proximal convolutions from the bovine albumin-hemoglobin experiments. The droplets can be recognized by their size and round contours as the bovine albumin administered previously, but there is only a faint yellowish coloring imparted by hemoglobin.

The intracapsular and tubular accumulations of hemoglobin are also an intense orange-yellow under bright phase contrast examination but stain black with iron-hemotoxylin. In the tubule wall, limited to the immediate proximity of the high concentrations of hemoglobin, are seen "droplets" in the epithelial cells which are also stained an intense yellow or deep black. (Fig. 6.) Besides in the intensity of their color, they also differ from the smaller hemoglobin droplets that are diffusely and evenly distributed throughout the entire cortical portion of the convolution in the great variation of their size. Some are small

but the majority are very large, measuring up to 8μ in diameter. It seems obvious from the limitation of these large droplets to the localized accumulations of hemoglobin filling short sections of tubule, that these "absorption" droplets have a different functional significance from the diffusely scattered small droplets that are evenly distributed through the length of the proximal convolution. The small droplets are the morphologic expression of the "normal" physiologic absorption of hemoglobin that accompanies the excretion of this protein; the large droplets represent an exaggerated or "abnormal" absorption related to the excessive and localized accumulation of high intratubular concentrations of hemoglobin in the tubule lumen.

In the experiments in which the two major components of egg white, ovalbumin and ovomucoid, were injected prior to the administration of hemoglobin, structural changes were found in the benzidine-stained sections similar in their significance to those described as following the injection of native egg white. After ovalbumin the cortical portions of the proximal convolutions were evenly filled with droplets, larger than those seen following administration of hemoglobin alone and equally deeply tinged with the yellow color of the benzidine reaction. After ovomucoid injection the histologic picture was similar to that of ovomucoid control experiments. The cytoplasm of cortical cross-sections of the proximal convolutions were transformed into what appeared like a vacuolar emulsion. The hemoglobin lay in the interstices between the vacuoles, in concentrated, irregular, linear or crystal-like deposits, with no suggestion of droplet form. The general appearance of these preparations was, therefore, very different from that of the other proteins, a difference readily understandable as an artifact produced as a result of the coagulation by the fixative of the highly hydrated droplets of ovomucoid-hemoglobin.

C. DISCUSSION

A correlation of the functional and morphologic aspects of the proteinurias that develop after the intraperitoneal injection of certain proteins indicates that the preliminary administration of bovine albumin sharply increases the glomerular permeability to hemoglobin in the rat kidney. In addition, by both functional and morphologic criteria, bovine albumin inhibits the tubular absorption of this substance and in contrast, native hen egg white and its fractions ovalbumin and ovomucoid do not produce significant changes in glomerular permeability to hemoglobin or in its tubular absorption.

In regard to absorption phenomena, the morphologic findings show that in both sets of circumstances the absorbing renal cells are "saturated" or, better, filled with the droplets of bovine albumin or the egg white proteins. If there is any difference in the degree of this "saturation" it would appear to be greater with egg white proteins. Yet the "blocking" effect of the latter is insignificant.

The explanation of this apparent paradox is found in the fact that the intracellular concentrations are complex mixtures of various substances that com-

bine in droplet form. The absorbed proteins form only a fraction of their total content (5, 16). Droplets of egg white proteins have the property, for reasons not yet clear, of combining with hemoglobin under the conditions of intracellular activity. They can therefore "take up" hemoglobin from the tubule lumen. The droplets of bovine albumin do not have this property, and absorption of hemoglobin is therefore "blocked."

SUMMARY

Intraperitoneal administration of bovine albumin sharply increases glomerular permeability to hemoglobin in the rat kidney. In addition, bovine albumin "saturates" the tubular absorption mechanism and inhibits the tubular absorption of subsequently administered hemoglobin. Egg white, ovalbumin, and ovomucoid, in contrast, do not produce significant changes in glomerular permeability to hemoglobin or in the tubular absorption of hemoglobin. These effects have been demonstrated both by physiologic measurements and by the morphologic demonstration of the absorbed proteins.

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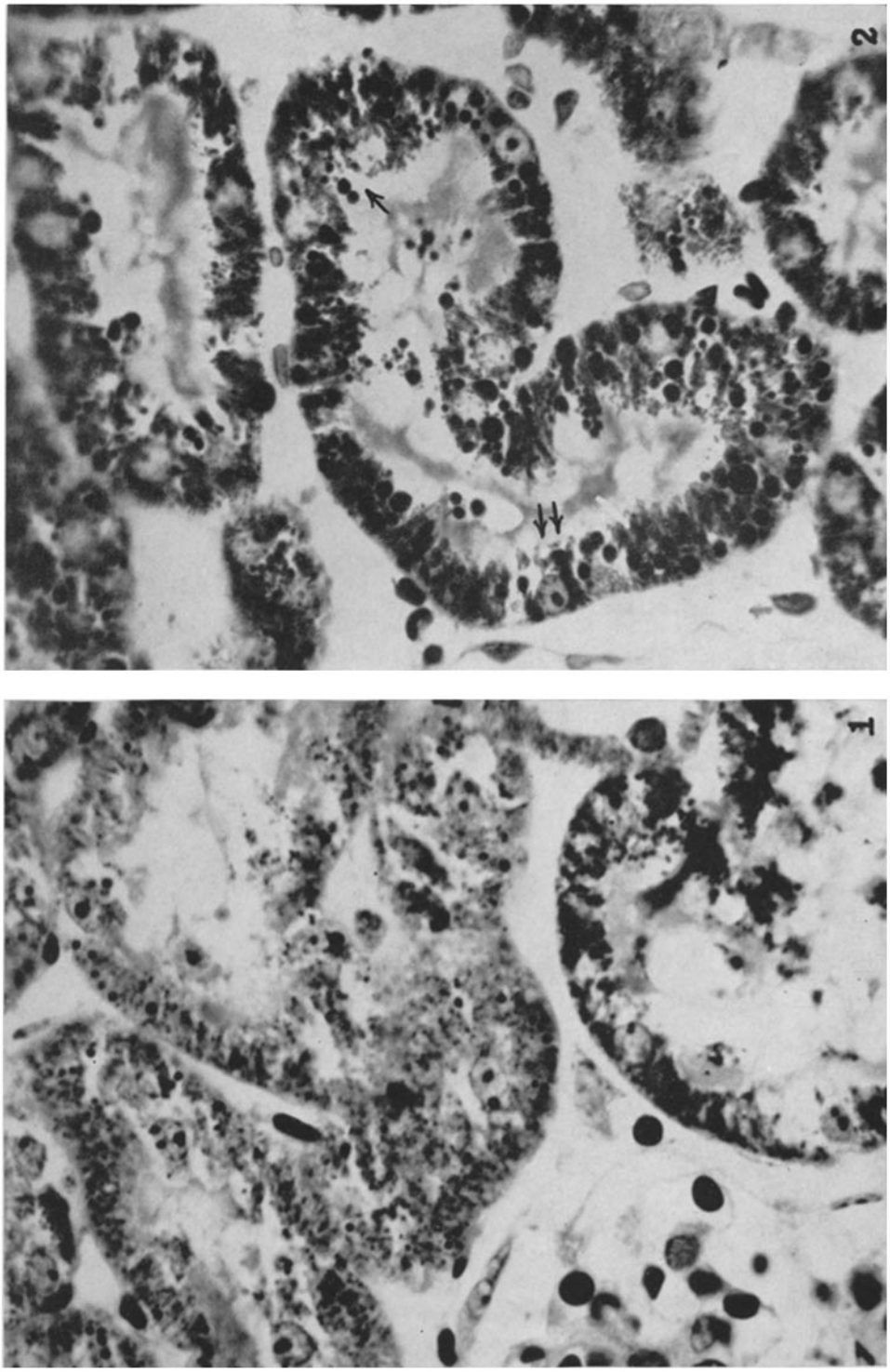
EXPLANATION OF PLATES

PLATE 23

FIG. 1. Cross-sections of cortical proximal convolutions filled with droplets after intraperitoneal injection of bovine albumin. The droplets appear densely black. Helly's fixation, iron-hemotoxylin stain. × 1000.

FIG. 2. Cross-section of a proximal convolution showing large droplets in the renal cells after intraperitoneal injection of hen egg white. In some cells the original mitochondrial rodlets persist (arrows). Same fixation and stain × 1000.



(Lippman, Ureen, and Oliver: Mechanism of proteinuria. III)

PLATE 24

FIG. 3. A similar section showing droplets in the renal cells of the proximal convolution after intraperitoneal injection of crystalline ovalbumin. Same stain and fixation. × 1000.

FIG. 4. A similar preparation from the kidney of a rat after intraperitoneal injection of ovomucoid. There are no droplets; instead the coagulation of the intracellular ovomucoid has produced a vacuolar appearance. × 1000.

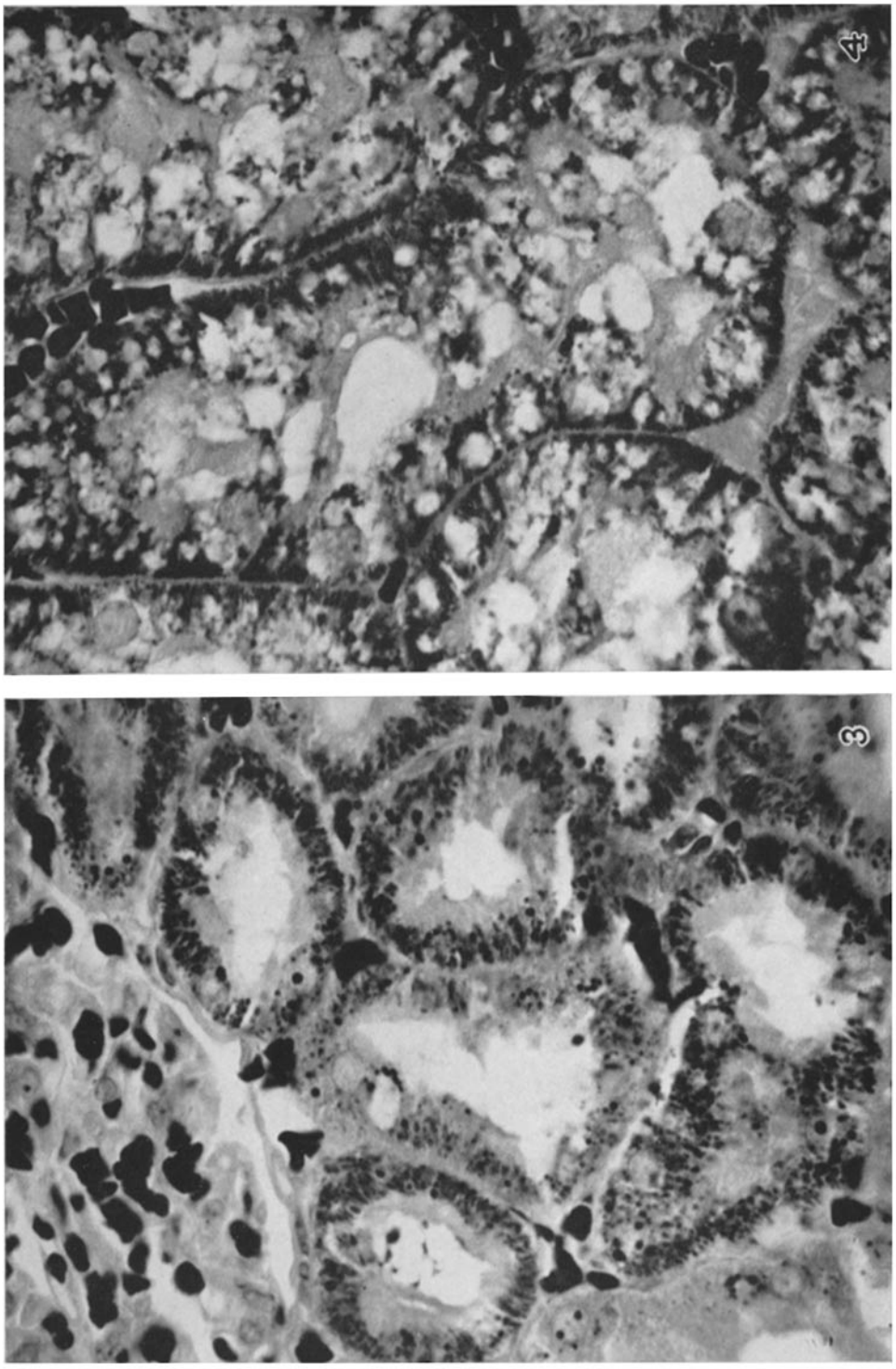


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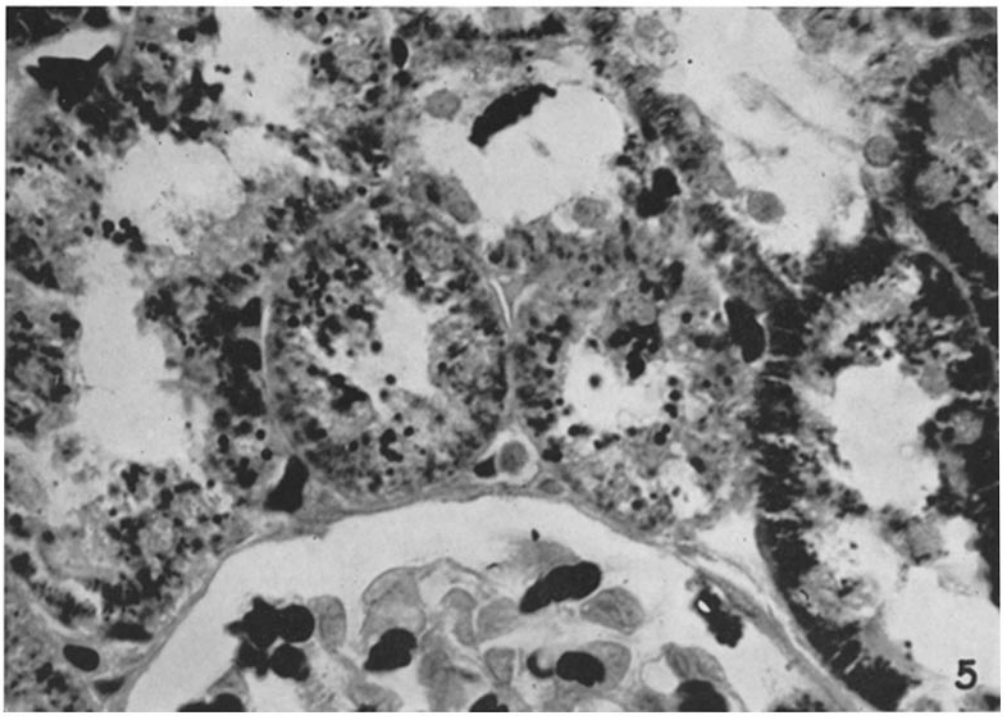
PLATE 25

FIG. 5. A similar preparation from the kidney of a rat after intravenous injection of hemoglobin. × 1000.

FIG. 6. High power view of a glomerulus (left) and cross-sections of its proximal convolution from the kidney of a rat which had received a preliminary injection of bovine albumin and subsequent intravenous injections of hemoglobin. The capsular space is distended with fluid rich in hemoglobin as is the lumen of the connecting proximal convolution. Benzidine stain photographed with a blue filter. The epithelial cells show varying degrees of localized hemoglobin absorption. Above center, note that only the brush border is stained. Other cells are completely filled with dense, black, coarse droplets of hemoglobin. In the other proximal convolutions, which do not contain the localized accumulation of hemoglobin, the very faint droplets of bovine albumin can be just distinguished. × 350.



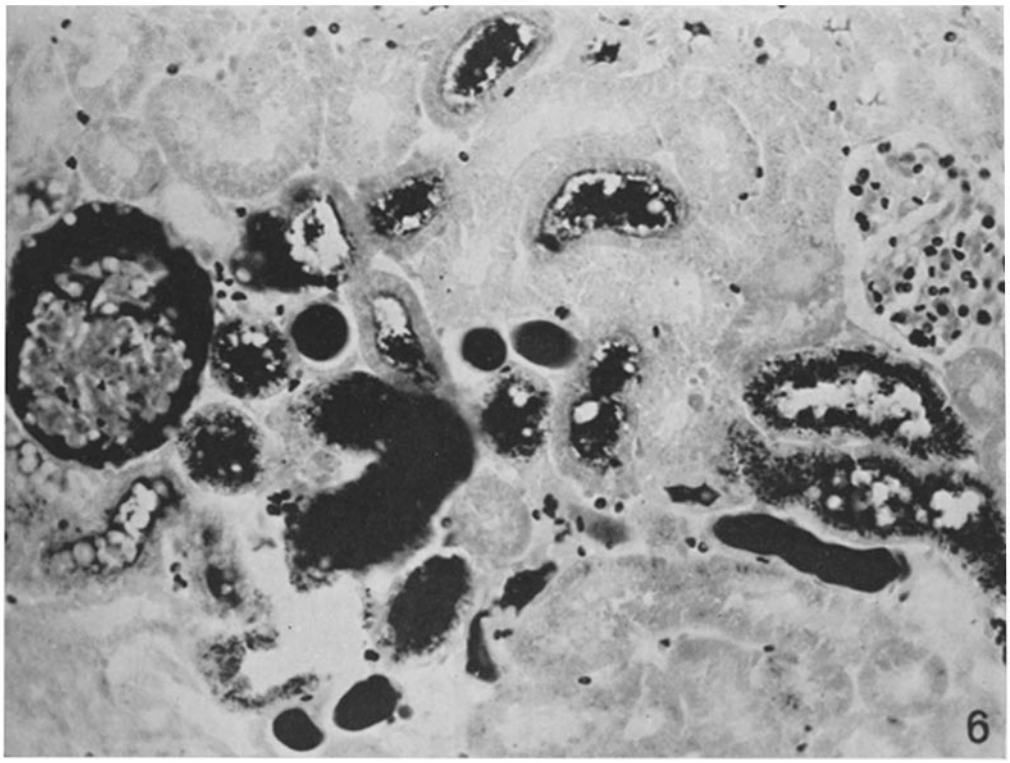


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PLATE 26

FIG. 7. A low power field from the kidney of a rat which had received intravenous injections of saline and hemoglobin. The section was stained by the benzidine method and on visual observation at this magnification only the faintest yellow coloration of the tubules could be seen. The photograph was taken with a blue filter so that the faint yellow appears as a dark tone. In the cross-sections of the cortical proximal convolutions the tubule cells are filled with hemoglobin; there are only traces in the medullary portions. Note the dense black of red blood cells in glomerular and intertubular capillaries. There are no collections of hemoglobin to be seen in the glomerular spaces or tubular lumens. × 60.

FIG. 8. A similar preparation from the kidney of a rat that had received a preliminary injection of egg white and then intravenous hemoglobin. The appearance is not significantly different from that of the preceding slide: the cells of the cortical tubules are filled with hemoglobin and there are no intratubular collections of it. × 60.

FIG. 9. A similar preparation from the kidney of a rat that had received a preliminary injection of bovine albumin and then hemoglobin intravenously. In marked contrast to Figs. 7 and 8, there is so little hemoglobin in the cells of the cortical proximal convolutions that the demarcation of the two zones is indistinct. The capsular space of some glomeruli and the lumen of many tubules are filled with heavy concentrations of hemoglobin. × 60.

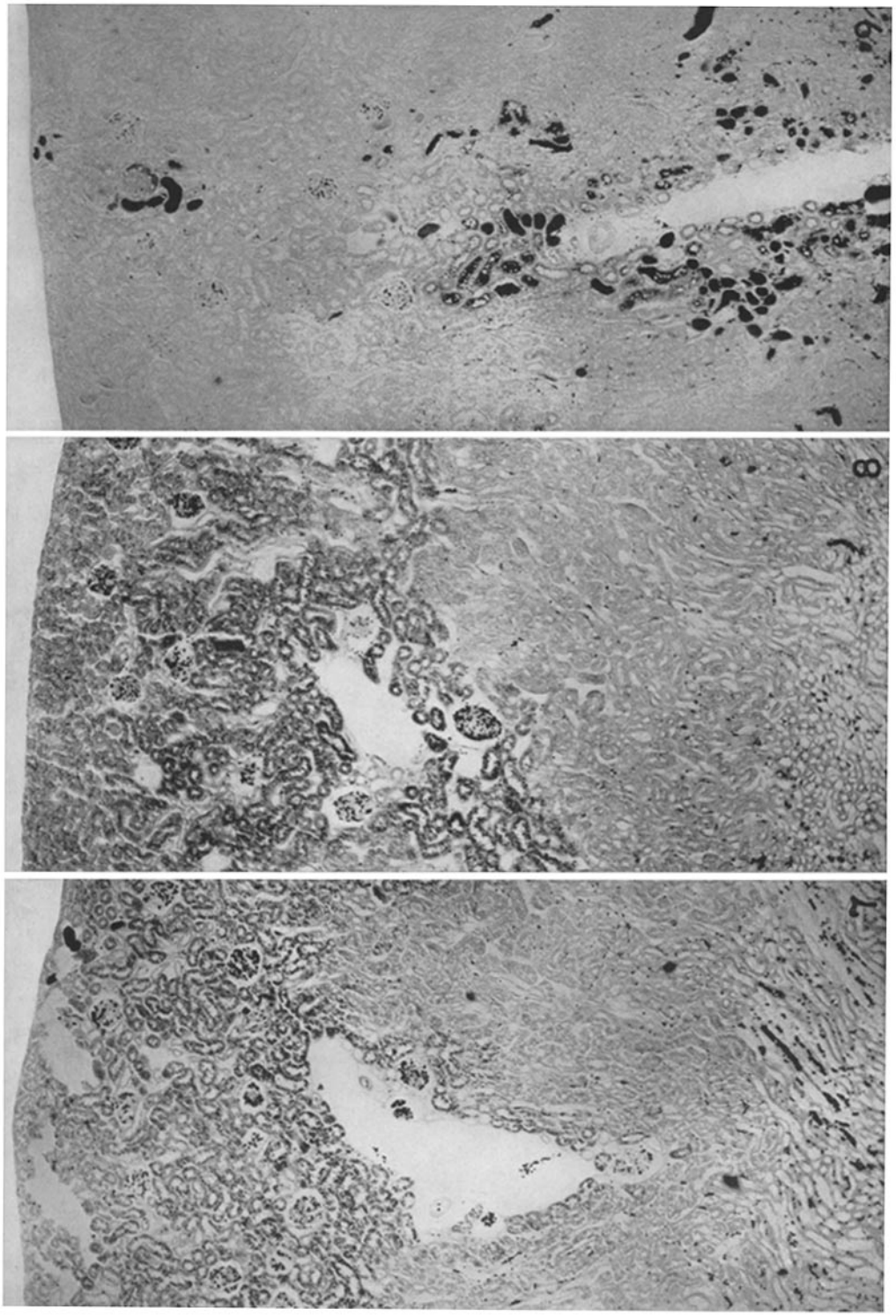

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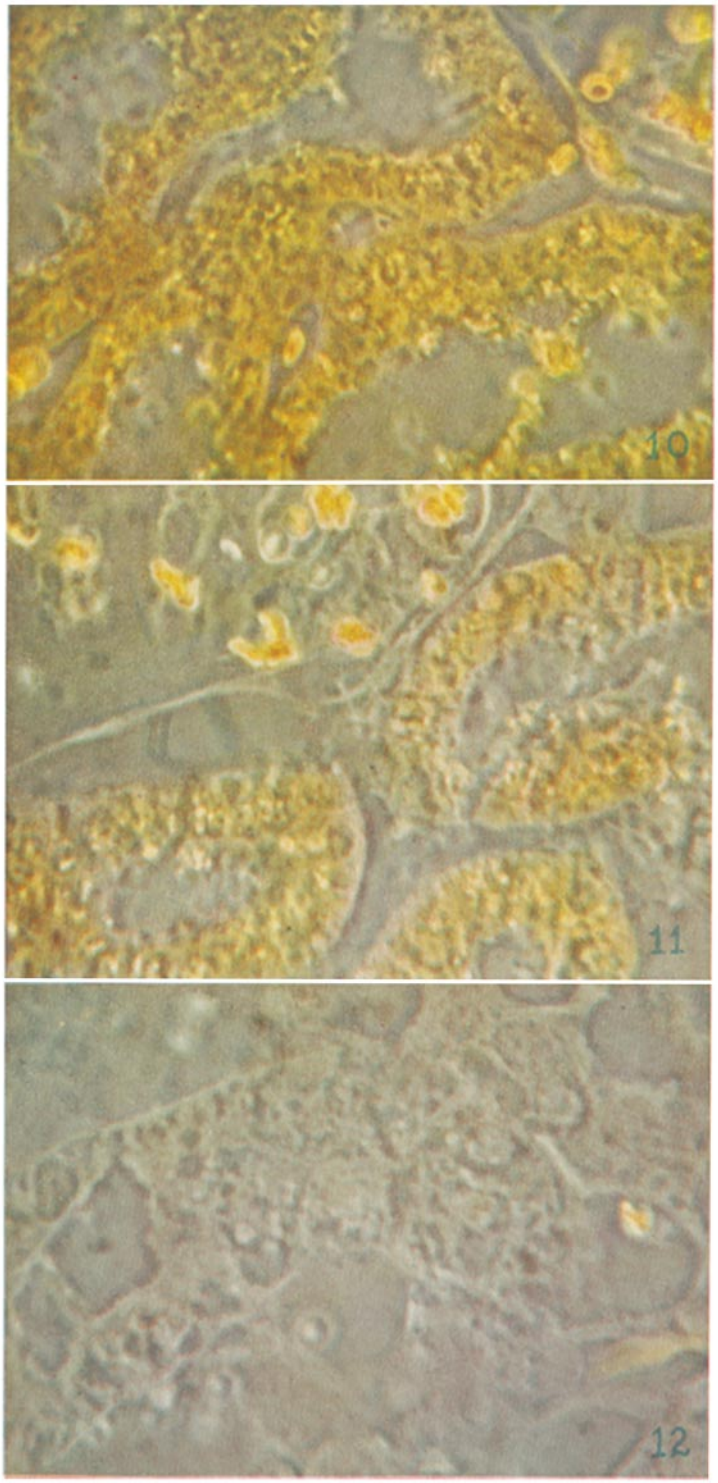
PLATE 27

FIG. 10. Kodachrome of a section of cortical proximal convolutions from the kidney of a rat that had received intravenous injections of saline and hemoglobin, stained with benzidine and photographed with American Optical Co. bright phase oil immersion 0.25 0.14A+. At the upper right a glomerulus in which the bright yellow hemoglobin of red blood cells can be seen. In the cross-sections of cortical proximal convolutions are many fine bright yellow droplets. There is also a considerable diffuse yellow coloration of the cytoplasm. × 1000.

FIG. 11. A similar preparation from a rat that had received preliminary intraperitoneal injections of egg white and subsequent intravenous injections of hemoglobin. A glomerulus with red blood cells above. The proximal convolutions are filled with droplets of egg white of various sizes, some quite large. They are deeply tinged with hemoglobin and there is also a diffusion of it throughout the cytoplasm. × 1000.

FIG. 12. A similar preparation from a rat that received preliminary injection of bovine albumin and subsequent intravenous injections of hemoglobin. The droplets of bovine albumin, much larger than those of hemoglobin (*cf.* Fig. 10), are but faintly tinged, if at all, with the yellow hue indicative of hemoglobin. × 1000.



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