EFFECTS OF PROTEINURIA ON THE KIDNEY*

PROTEINURIA, RENAL ENLARGEMENT, AND RENAL INJURY CONSEQUENT ON PROTRACTED PARENTERAL ADMINISTRATION OF PROTEIN SOLUTIONS IN RATS

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PLATES 32 TO 34

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Renal damage due to cast formation and tubular plugging by protein material, is well known in diseases associated with hemoglobinuria (1), Bence-Jones proteinuria (2), and "albuminuria" (3). The magnitude of this type of damage in Bright's disease is greatly influenced by the globulin content of the urine (4). Whether proteinuria results in renal damage other than that caused by the blockage of tubules, has not been fully determined. Cast formation may sometimes be a result of damage, as well as a cause of damage (5), and tubular lesions which did not appear to be due to obstruction have been observed with hemoglobinuria (6). However, as emphasized in Lucké's review (7), hemoglobinuria as observed clinically is often associated with shock, dehydration, and other factors, which may themselves contribute to the production of renal injury, and in diseases characterized by "albuminuria" or Bence-Jones proteinuria, one cannot always distinguish between possible effects of protein on the kidney, and other manifestations of the disease in question. The use of hemoglobin in investigations designed to determine the effects of protein itself on the kidney, is complicated by the presence of the prosthetic group.

With the advent of the treatment of nephrosis by the parenteral administration of large amounts of protein, usually with an exaggeration of the degree of proteinuria during the period of therapy, the question of the possible harmful effects of proteinuria on the kidney assumed greater importance. The same question also enters into the choice of diet in renal diseases, since the urinary protein often is increased by raising the dietary protein level.

In order to study the effects of proteinuria on the kidney, and particularly to determine whether the passage of protein through the kidney might result in renal damage other than that attributable to tubular obstruction, it was decided to attempt the production in rats of continuous proteinuria of abnormal degree, for extended as well as brief periods of time, and to follow the changes which occurred in renal morphology and composition during and following cessation of treatment, together with the changes in blood, urine, and other organs.

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Preliminary experiments indicated that fairly continuous additions of various proteins to the blood stream, and increases in the amount of protein in the urine could be induced by twice daily intraperitoneal injections of solutions of the proteins. Efforts were made to adjust fluid and electrolyte intake, and the amount of protein administered, in such a manner that tubular obstruction would not occur in the majority of animals, and that the results would not be influenced by differences in urine volume, dehydration, electrolyte imbalance, or shock.

In addition to observing the effects of the protein injections on the kidney, some information was obtained concerning the degree and duration of retention of the various proteins in the plasma, and it was possible to make observations of a preliminary nature bearing on the questions of the mechanism of proteinuria, the passage of protein through the glomerular membranes, and the reabsorption of protein by the tubules.

EXPERIMENTAL

Materials and Methods

Animals.—Two hundred and eighty-five male albino rats of a Sherman strain were given injections as described below. The animals were approximately 24 days old when the injections were started. The maximum variation in initial weight of animals used in any series was less than 10 gm. Approximately 100 large adult rats were used as donors for rat serum which was administered to some of the experimental rats, and about 50 additional rats were employed in auxiliary experiments.

Protein Solutions.—Three per cent solutions of the following proteins, each dissolved in 0.45 per cent sodium chloride solution, were employed for injections in some of the treated groups of animals: gelatin,[1] human albumin,[2] and bovine gamma globulin.[3] Phenyl mercuric borate 0.002 per cent was present in the gelatin solution and was added to the other solutions. In addition to the experiments employing 3 per cent protein solutions, a few rats received a solution containing approximately 6 per cent gelatin in a physiological solution of sodium chloride, with 0.004 per cent phenyl mercuric borate.[1] The solutions were prepared and handled aseptically, and refrigerated when not in use.

Rat Serum.—Rat serum diluted with an equal volume of distilled water (giving approximately a 3 per cent protein solution in half-physiological salt solution), and with 0.002 per cent phenyl mercuric borate added, was used for injections in two groups of animals. The serum administered to the first group was obtained from adult rats decapitated after ether anesthesia of sufficient depth to cause respiratory depression or paralysis. When it appeared that certain undesirable effects of the serum might be due to ether retained by the serum, the serum used for

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[1] Purified gelatin in isotonic solution of sodium chloride, oncotic pressure 70 mm. Hg, supplied by the Upjohn Company, Kalamazoo, Michigan, diluted with an equal volume of water. The stock solution containing approximately 6 per cent gelatin was administered without dilution to a few animals of series 9.

[2] Normal serum albumin (human) concentrated, salt-poor, E. R. Squibb and Sons, supplied by the American Red Cross.

[3] Fraction II from bovine plasma, supplied by The Armour Laboratories, Armour and Company, Chicago.

TABLE I

Summary of Experiments

	Series								
	1	2	3	4	5	6	7	8	9
No. of animals receiving following solutions*									
Saline	8	6	6	21		18	8	4	6
Sucrose	12								
Urea			6				8	4	
Casein hydrolysate			6	22	3		8		
Gelatin	12		6	20	3	18			12‡
Human albumin		8	6		3	12			
Bovine globulin		8	6		3	12			
Rat serum							6	4	
Total animals receiving injections (285)	32	22	36	63	12	60	30	12	18
Length of injection period, *days*	42	35	14	2	7	9	9	4	6
Time of autopsy of animals									
During course of injection period (animals)	12	6			4			4	
6 to 18 hrs. after end of injection period	6	4	30	18	8	16	30	8	6
2 wks. " " " " "						28			
2 mos. " " " " "	12	8							
At intervals of a few days after end of injection period				45		16			12
Urine collections for protein determinations									
Near end of injection period	x§	x			x	x	x	x	
1 and 2 days after end of injection period						x			
2 mos. after end of injection period	x	x				x			
Determinations of specific proteins in urine‖			x			x			
Animals and kidneys weighed at autopsy	x	x	x	x	x	x	x	x	
Determinations on blood at autopsy									
Urea	x	x	x			x		x	
Total plasma protein ($CuSO_4$ method)	x	x	x			x	x		
Plasma albumin and total nitrogen (Kjeldahl)‖						x	x		
Hemoglobin ($CuSO_4$ method)	x	x	x			x	x		
Determinations on renal cortical tissue									
Moisture content			x			x	x		
Total nitrogen (Kjeldahl)‖			x			x	x		
Tests for specific proteins‖		x	x			x			x
Kidneys examined microscopically	x	x		x	x		x	x	x

* Solutions previously described in text.

‡ Six of these animals received the more concentrated gelatin solution (see footnote 1).

§ x indicates that the examination indicated was done.

‖ These determinations were done in a limited number of cases.

the second group was obtained after decapitation, from large rats which had been placed in a nitrogen chamber until respiration stopped. These animals frequently remained in an atmosphere with little oxygen for 5 minutes or longer, and convulsions during this period were not uncommon. In both cases, the blood from the donor animals was collected with minimal contamination into tubes by means of paraffin-covered funnels and allowed to clot. The serum was separated promptly by centrifugation, placed in tubes, and immediately frozen. Portions of serum sufficient to last 24 to 36 hours were thawed and diluted as needed for injections, with constant refrigeration of the diluted serum.

Control Solutions.—One per cent urea, and 3 per cent casein hydrolysate,[4] each dissolved in 0.45 per cent sodium chloride solution, and the 0.45 per cent sodium chloride solution alone, were administered individually to control groups of animals. Phenyl mercuric borate 0.002 per cent was added as a preservative. The solutions were aseptically prepared and handled, and refrigerated between injections. In some of the later experiments, distilled water rather than half-physiological salt solution was used in the preparation of the urea and protein hydrolysate solutions in order to decrease peritoneal irritation.

General Plan of Experiments.—Comparable groups of the young rats were given, for various periods of time, twice daily intraperitoneal injections, 1 cc. per 10 gm. of body weight at each injection, of the protein, serum, and control solutions. The different series of injections, and the determinations which were done on the animals of each series are summarized in Table I. The injections were made without anesthesia, through a 21 gauge needle, care being taken to maintain aseptic conditions and to avoid injury of the liver and intestines. In order to insure an equal fluid intake and the elimination of urine of comparable volume and concentration by the animals of the different groups, *no fluid* except that administered in the injections already described was allowed. A stock diet[5] was offered *ad libitum*. For several days prior to and during the urine collections, a low residue synthetic diet[6] was substituted for the stock diet in order to decrease the bulk of the feces. Because vitamin-free injected materials were to some extent utilized in place of food by animals of some of the groups, complete vitamin supplements[7] were offered *ad libitum* to all animals. A complete salt mixture[8] was also supplied separately so that the animals could adjust their own salt intake to some extent. The animals were weighed at intervals of a few days and the amounts of injected materials were increased as indicated. Urine was collected from time to time for protein determinations. Animals from various groups were autopsied at intervals during, at the end of, and at periods following terminations of injection periods. Blood was collected for study, weight of kidneys was determined, and kidney tissue was preserved for chemical and histological study. Animals not autopsied during, or at the end of, injection periods were given water *ad libitum* beginning 24 hours after the final injection. The occasional animals which became seriously ill or died were not included in the determinations of kidney size.

Urine Collections.—Urine was collected by means of metabolic cages placed above large paraffin-coated funnels which drained into flasks containing a little toluene. Because of the

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[4] Amino acids—I. C., lyophilized (acid hydrolysate of casein with added tryptophane) supplied by Biochemical Division, Interchemical Corporation, Union, New Jersey.

[5] Purina fox chow.

[6] The diet employed was the same as that described elsewhere (8), with 0.3 per cent choline chloride added. The changes in the diet did not appear to influence appreciably the kidney weight–body weight ratios or the amount of protein in the urine. The dietary treatment of protein-treated and control groups was the same in each experiment.

[7] The vitamin mixture was the same as that used in the synthetic diet (8), with added choline chloride.

[8] Osborne-Mendel salt mixture No. 2, Eimer and Amend, New York.

small size of the animals, from 3 to 6 rats were placed together in each cage. Collections were made for periods of from 12 to 24 hours. When injections were continued during the periods of urine collections the animals were not returned to the metabolic cages until leakage from peritoneal cavity, if present, had ceased. Since fairly uniform results were obtained, it was thought that subsequent leakage did not occur. Because of technical difficulties, the collections were only approximately quantitative. As indicated in Table I, urine collections were made at various times after termination of injections, as well as near the end and sometimes at earlier stages of the injections.

Urine Examinations.—The urine specimens were cleared by centrifugation, and filtered if necessary. Volume was recorded, and protein concentration determined by the Shevky-Stafford (9) or the biuret (10) method. Gelatin was precipitated by addition of tungstate or Tsuchiya's reagent after removal and estimation of other proteins by addition of an equal volume of 10 per cent trichloracetic acid. Human albumin was estimated in a few cases by determination of the turbidity produced by addition of antiserum prepared from rabbits.[9] Bovine globulin was tested for qualitatively by serological precipitation, and the relative globulin content of urines from various groups was estimated by comparing the precipitates formed by adding equal volumes of 44 per cent sodium sulfate, after adjusting pH of the urine specimens to 7.4 (11).

Urine sediment was examined microscopically in a few instances.

Blood Examinations.—At the time of autopsy, during, or sometime after termination of the injections, blood was collected into heparin-containing flasks after decapitation of the animal. Total plasma protein and hemoglobin levels were estimated from specific gravity determinations by the copper sulfate method (12). Urea was determined by the hypobromite method (13). Total plasma nitrogen and plasma albumin determinations were done in a few instances by a micro Kjeldahl method (14), using pooled plasma from the various groups.

Examination of Organs.—After comparable animals of different groups had been weighed, killed by decapitation, and thoroughly drained of blood, the peritoneal cavities were inspected for free fluid, and for evidence of peritoneal irritation, infection, or injury to organs. When fluid was present in the peritoneal cavity, the weight of the animal was corrected by deducting the weight of the free fluid. Tissues were observed for evidence of dehydration or overhydration. The size, color, and other gross features of kidneys, liver, and spleen were specifically noted. Differences in size and shade of kidneys were most impressive when the organs from comparable control and protein-injected animals were compared directly by placing the kidneys side by side. The kidneys and sometimes the liver and spleen were removed for weighing, and tissue was preserved for histological study of gross and microscopic sections, and for total nitrogen and moisture determinations.

Determination of Relative Weight of Kidneys.—The animals of the different groups were of approximately the same weight at the beginning of the injection periods, and the growth rate of all the animals was fairly uniform, at least for several weeks. In experiments which were continued for a number of weeks, there was sometimes considerable variation in weight of the animals at the time of autopsy. In the early experiments, the kidneys were removed without attached tissue, but with capsules in place, and were weighed accurately. After it was observed that some of the injected solutions produced significant thickening and increase in weight of the renal capsules, the capsules of all kidneys were removed carefully before weighing. In order that the kidneys of various animals might be compared more accurately, the ratio of kidney weight to corrected body weight was determined in each case. In each series of experiments, animals of various groups were treated similarly. In most cases, autopsies were

[9] We are indebted to Dr. Henry G. Kunkel for the determinations of human albumin in urine and renal cortical tissue.

performed about 18 hours after the last injection, with no oral fluid during the intervening period. In some of the early experiments, however, the time interval between the last injection and autopsy was less than 18 hours.

Histological Examinations of Kidneys and Other Organs.—After the kidneys were weighed, they were sectioned along the median sagittal plane, and direct comparisons made of kidneys of animals of various groups. Kidney tissue was then fixed in alcohol-formol-acetic acid,[10] or sometimes in 10 per cent neutral formalin or in absolute alcohol. Paraffin sections were prepared as routine, and stained for microscopic examination with hematoxylin and eosin or with Giemsa's stain, and frequently with Mallory's connective tissue stain. A few sections from kidneys of gelatin-treated animals were fixed in absolute alcohol and stained with eosin and with several other stains in absolute alcohol, without passage through aqueous solutions. Frozen sections of formalin-fixed tissue were stained for fat in a few instances. Liver sections from some animals and sections of other organs from occasional animals also were studied microscopically.

Determinations of Water and Other Constituents of Cortical Tissue.—Cortical tissue was prepared by bisecting decapsulated kidneys and removing the medullary portion. The cortical tissue was then pressed against blotting paper to remove free fluid present in tubules and blood vessels.

Moisture determinations were made on kidneys from most of the animals. This was done by placing the cortical tissue from one-fourth to one-half of a kidney in a weighing bottle, accurately determining the wet weight of the tissue, drying in an oven at 104°C. for 6 hours, and again weighing. The percentage loss of weight by the wet tissue on drying was then calculated.

After tissue had been taken for sections and for moisture determinations, the remaining cortical tissue was placed in tubes, weighed, and frozen. Total nitrogen determinations were performed on this tissue in a few cases by a Kjeldahl method (14). In some other cases, the tissue was ground in water or saline with sand or by means of a Potter homogenizer. The solutions were cleared by high speed centrifugation, and gelatin, human albumin, and bovine globulin were precipitated as already described for the proteins in urine. Results on kidney tissue from control and protein-treated animals were compared in each instance. In a few cases, the protein injections were discontinued and the animals given water for 2 to 7 days before autopsy in order to eliminate most of the injected protein from blood and urine, small amounts of which were retained by the renal tissue. Although a few quantitative estimations of the specific protein were made, the results were of only qualitative significance because certain factors were not well controlled.

RESULTS

Most of the animals which received injections of the various substances remained well and grew fairly normally. The animals which received injections for the longer periods grew from weanlings to rats weighing approximately 200 gm. without receiving any fluids by mouth. Disturbances in health and growth of animals due to intraperitoneal infections or injuries produced at the time of injections, were rarely encountered. There was no evidence of sensitization of the protein-injected animals to the proteins, though temperatures of the injected animals were not determined following injections and sera were not examined for antibodies against the proteins. The impression was obtained that the animals which were given gelatin were less excitable and struggled less

[10] Eighty per cent alcohol 900 cc., 40 per cent formalin 50 cc., and glacial acetic acid 50 cc.

during injections than those of other groups. In the early experiments, the animals receiving protein hydrolysate appeared to be less able than animals of other groups to tolerate periods of more than 12 hours without an injection of fluid. This difference was partially removed by administering the protein hydrolysate in water rather than in sodium chloride solution, and by offering a complete salt mixture separately.

Data obtained from groups of animals injected with rat serum are included in tables and charts along with data from other groups. However, since the experiments with the homologous serum were not considered entirely satisfactory, they will be discussed separately.

Absorption of Injected Materials from Peritoneal Cavity.—The control (non-protein) solutions were absorbed somewhat more rapidly from the peritoneal cavity than were the protein solutions. In most experiments, even the protein solutions were fairly completely absorbed during the period from one injection to the next, and only in the case of one group receiving the usual 3 per cent solution of gelatin (series 4), the group receiving 6 per cent gelatin (series 9), and particularly the groups receiving rat serum did intraperitoneal accumulations of injected material present a serious problem. The animals receiving urea became dehydrated and the peritoneal surfaces did not have the normal moist appearance, though the hemoglobin levels did not appear to rise appreciably.

Changes in Plasma Proteins and Hemoglobin Levels Resulting from Injections.—Plasma protein and hemoglobin levels, which were determined in animals of the various groups at the time of autopsy of the animals, during the course of, and at intervals following the termination of injection periods, varied somewhat from group to group, and with the length of the injection period, and with the period of time elapsing between the last injection and the autopsy of the animal, but, qualitatively, the results were similar. Data obtained from animals of series 3, 6, and 7, are presented respectively in Tables II, III, and IV. Little or no change in serum protein or hemoglobin levels was observed with gelatin injections. Unfortunately studies were not done on animals of series 4 receiving the usual gelatin solution, or on those of series 9 receiving the more concentrated gelatin solution, some of which retained fluid in the peritoneal cavity, and some of which developed severe tubular damage. Albumin produced significant elevations in total plasma protein, plasma albumin, and in the ratio of albumin to globulin, with a coincident decrease in the hemoglobin level. Globulin induced an even greater rise in total plasma protein and plasma globulin, but the decrease in hemoglobin concentration was of about the same magnitude as with albumin. After termination of injections, the plasma protein and hemoglobin values returned fairly promptly toward normal.

Effect of Injections on Blood Urea Levels.—The animals receiving injections of control and protein solutions, including those of the long term experiments were found at the time of autopsy to have blood urea nitrogen values ranging between 12 and 28 mg. per cent, with the majority in the lower two-thirds of the

range, and there was no consistent difference between control and protein-treated animals. Some of the higher values were found in animals which appeared ill, but neither illness nor high urea nitrogen values were more common in protein-treated than in control animals, and neither could be attributed with any degree of certainty to specific effects of the injected materials on the kidneys. In animals given continued injections for prolonged periods and then observed for several weeks or months on a normal regime of food and water by mouth, the blood urea nitrogen values were still found to be normal. Blood urea levels were not determined in series 4 and 9 where retention of fluid in the peritoneal cavity, and renal damage, were observed in some of the animals receiving gelatin.

TABLE II

Total Plasma Protein and Hemoglobin Levels of Rats of Series 3

Injected solutions*	Total plasma protein	Hemoglobin
	gm. per cent	*gm. per cent*
Saline	6.5	10.6
Urea	6.2	9.9
Casein hydrolysate	6.5	11.0
Gelatin	6.4	10.1
Albumin	8.5	8.4
Globulin	9.5	8.3

The animals were autopsied about 6 hours after the last injection, following an injection period of 2 weeks. Each figure represents an average from specific gravity determinations on 3 rats by the $CuSO_4$ method.

* Solutions previously described in text.

Influence of Injections on Proteinuria and Urine Output.—Rapidly occurring diuresis after each injection was noted in animals receiving injections of urea, and the volume of urine excreted in 24 hour periods by these animals was usually somewhat greater than that excreted by comparable animals of other groups, despite the fact that the fluid intakes of comparable animals of all groups were equal. The rate of urine excretion was more uniform throughout the 24 hour periods in the protein-treated animals than in those receiving saline and amino acids, but the total urine volumes of animals of all these groups were approximately equal.

Urine from saline-injected animals always contained small amounts of protein, nearly always less than 1 gm. per liter, and often in the range of 0.5 gm. per liter or less. The total protein excreted per day varied with the size and age of the rat, but after an injection period of 2 weeks, starting with weanling rats, the amount was usually found to be between 1 and 2 mg. per rat per 24 hours. The amounts of protein excreted by the animals receiving amino acids

TABLE III

Plasma Protein and Hemoglobin Levels of Rats of Series 6

Injected solutions*	18 hrs. after final injection			2 days after final injection		5 days after final injection		10 days after final injection		
	Plasma protein‡	(Plasma protein) A/G§	Hb‡	Plasma protein‡	Hb‡	Plasma protein‡	Hb‡	Plasma protein‡	(Plasma protein) A/G§	Hb‡
	gm. per cent		*gm. per cent*	*gm. per cent*	*gm. per cent*	*gm. per cent*	*gm. per cent*	*gm. per cent*		*gm. per cent*
		(6.0)							(5.7)	
Saline	5.0	1.8	12.2	5.5	12.7	5.7	12.6	5.7	1.6	11.4
Casein hydrolysate	5.2	—	13.3	—	—	—	—	—	—	—
		(4.8)							(5.5)	
Gelatin	5.3	1.7	12.1	—	—	5.7	12.7	5.4	1.6	10.8
		(6.7)							(5.3)	
Albumin	6.5	3.0	10.2	5.8	11.7	—	—	5.4	1.6	10.9
		(9.5)							(5.9)	
Globulin	8.6	0.33	10.5	8.0	13.0	6.5	14.0	6.0	1.3	11.2

After an injection period of 9 days, the animals were autopsied at intervals as indicated in the table. Animals were given water *ad libitum* beginning 24 hours after the final injection.

* Solutions previously described in text.

‡ Average values calculated from specific gravity determinations on a total of 40 rats by the $CuSO_4$ method.

§ Single determinations on pooled plasma, using Howe separation and micro Kjeldahl.

TABLE IV

Total Plasma Protein and Hemoglobin Levels in Rats of Series 7

Injected solution*	Total plasma protein	Hemoglobin
	gm. per cent	*gm. per cent*
Saline	5.7	12.5
Urea	5.6	12.8
Casein hydrolysate	6.0	11.9
Rat serum	7.8‡	11.4

Animals were autopsied about 18 hours after the last injection, following an injection period of 9 days. Each figure represents an average value calculated from specific gravity determinations on 6 rats by the $CuSO_4$ method.

* Solutions previously described in text.

‡ A single determination on pooled plasma indicated that the albumin fraction was only slightly elevated.

and urea were in the same range as that of the saline animals, though the values for the urea-injected animals appeared to be consistently slightly higher than those of the other control groups, perhaps due to the diuretic effect. Additions

of sodium sulfate according to the method for globulin precipitation, to urines from the control groups, produced not more than a faint cloud, and most of the protein was presumably albumin. Urine specimens collected 1 to 2 weeks after starting injections, from the protein-treated animals, almost always contained more than 1 gm. of protein per liter of urine. The animals receiving albumin sometimes excreted as much as 5 gm. per liter or more over the 24 hour period. Globulin induced less marked elevations in urinary protein levels, while gelatin injections were usually accompanied by greater degrees of proteinuria than those observed with albumin.

The increase in urinary protein in animals injected with gelatin was due principally to the presence in the urine of gelatin; the other urinary proteins (precipitated by an equal volume of 10 per cent trichloracetic acid) did not appear to be regularly or markedly altered. Gelatin appeared in the urine and was occasionally seen in the glomerular capsules before there were appreciable changes in kidney size or visible alterations in the tubular cells. In animals injected with human albumin, preliminary determinations of the human albumin in the urine by the serological precipitin reaction suggested that only a part, probably less than one-half of the urinary protein increment was composed of the injected human albumin. The remainder was presumably rat protein, probably chiefly albumin. As in the case of the albumin, it appeared unlikely that the injected globulin was present in the urine in sufficient quantities to account for all the increase in urinary protein which accompanied injections of that protein.

In animals receiving injections of albumin and globulin, the urinary protein levels, like the plasma protein levels already discussed, remained definitely elevated for at least several days after termination of the injections. However, when urine specimens from the various groups receiving proteins were examined after periods of 2 weeks to 2 months, the urinary protein levels were found to be within the same range as in the specimens from comparable control animals.

Examination of urinary sediment from protein-treated animals revealed no abnormalities.

Gross Changes in Organs Resulting from Injections.—Examinations of the peritoneal cavities sometimes revealed adhesions between liver or intestines and omentum or adjacent organs, due to puncture wounds produced at the time of injections. Significant peritoneal infections were never recognized, if present. Prolonged injections of urea solution, and particularly of protein hydrolysate solution, appeared to produce peritoneal irritation, with thickening and opacity of peritoneal surfaces including hepatic and renal capsules. In the case of urea, the peritoneal surfaces were often less moist than with the other solutions. Saline and the 3 per cent protein solutions produced no peritoneal changes. However, the more concentrated gelatin solution used in series 9 produced definite peritoneal thickening, with rounding of the liver edges.

Occasional animals, without respect to treatment, were observed to have large dark spleens.

It was noted during the early periods of the study that the kidneys of animals which had received repeated injections of gelatin appeared enlarged and pale. The correctness of this observation was confirmed by side by side comparisons of the kidneys of control and gelatin-treated animals (Figs. 1 and 3). When sagittal sections of kidneys from gelatin-injected animals were compared with similar sections from comparable control kidneys, it was further evident that the enlargement of the kidneys from animals receiving gelatin was principally in the cortical portion. The cortical tissue appeared pale particularly through the outer two-thirds of its depth, and was separated from the pale medullary tissue by a narrow dark band at the corticomedullary junction.

In experiments employing albumin and globulin, similar but usually somewhat less marked renal enlargement was noted (Figs. 2 and 3). Paleness of some degree was seen frequently with injections of these proteins, particularly when enlargement of considerable degree occurred, but it was usually much less than with gelatin.

No gross alterations, other than the differences in size and depth of color, were noted in any of the kidneys, and the pelves and ureters always appeared normal.

There appeared to be no changes in the liver or spleen attributable to injections of protein, though these organs were accurately weighed in only a few cases. No changes were noted in the adrenal glands, which were not studied extensively.

Change in Relative Size of Kidneys Due to Injected Substances.—Since there was always some variation in size of the experimental animals at the time of autopsy, the feasibility of expressing the relative kidney size as the ratio of the weight of both kidneys divided by the weight of the animal, was investigated. It was found that this ratio was quite constant over the range of variation in animal size encountered in the individual experiments, particularly in those of not more than a few weeks' duration. This ratio, multiplied by 100, decreased steadily with growth of the animals, from approximately 1.25 in the youngest rats autopsied to about 0.67 in the rats of series 1 which were autopsied after being observed for 2 months at the end of the 6 weeks injection period.

In the early experiments, the kidneys were weighed without decapsulation. However, after the introduction of urea, amino acid, and rat serum solutions, it was observed that these substances caused sufficient thickening and increase in weight of the renal capsules to alter appreciably the kidney weight–body weight ratios, and in subsequent studies the kidneys were carefully decapsulated before weighing.

The animals receiving urea and protein hydrolysate solutions, like those receiving saline alone, exhibited little change in relative kidney size. The kidney weight–body weight ratios of the animals receiving urea were usually slightly

elevated compared with those of the other control groups, perhaps because of a decrease in body weight due to dehydration.

Protein solutions uniformly caused an increase in relative kidney size and weight. The most rapid enlargement appeared to occur in the early part of

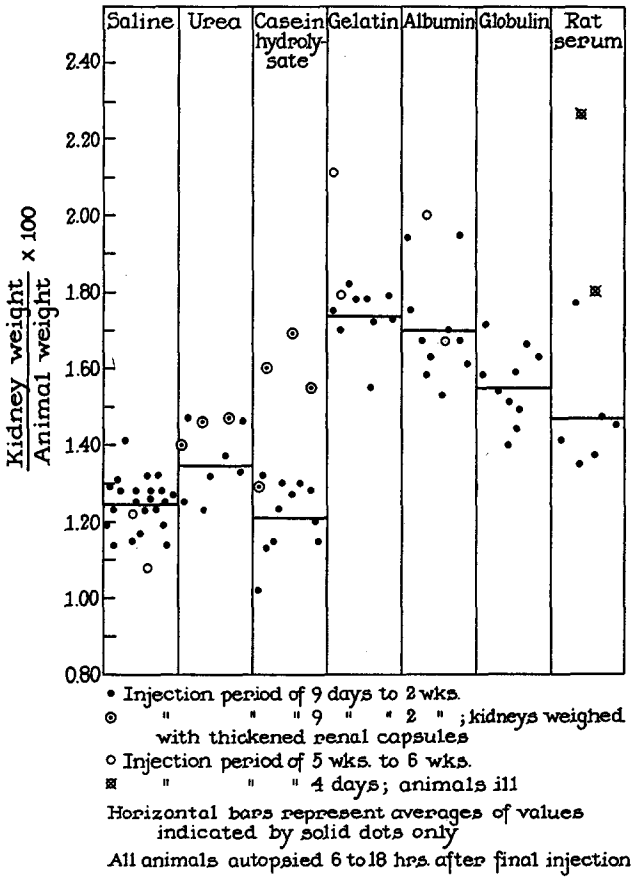

CHART 1. Relative renal size of comparable rats injected with various protein and control solutions.

the injection periods, but the enlargement was maintained as long as the injections were continued.

Gelatin caused the most rapid and usually the most marked increase in kidney size. Definite enlargement occurred within a few days, and perhaps within 1 day. Albumin ultimately caused almost as great enlargement as gelatin but the enlargement appeared to develop more slowly. Globulin produced less enlargement than gelatin or albumin.

Chart 1 shows the results of determinations of relative renal size which were made in the animals receiving injections for 9 days or longer and autopsied within 1 day after the final injection. The animals were from series 1, 2, 3, 6, and 7, with the two ill animals from series 8. Chart 2 contains further data from series 4 on the enlargement produced by short term injections of gelatin.

Reversibility of Renal Enlargement after Termination of Injections.—Chart 2 shows the changes in relative kidney size over a period of 8 days in rats of series 4, given 3 single injections of gelatin solution, compared with those of rats given injections of saline and casein hydrolysate. For some unknown reason,

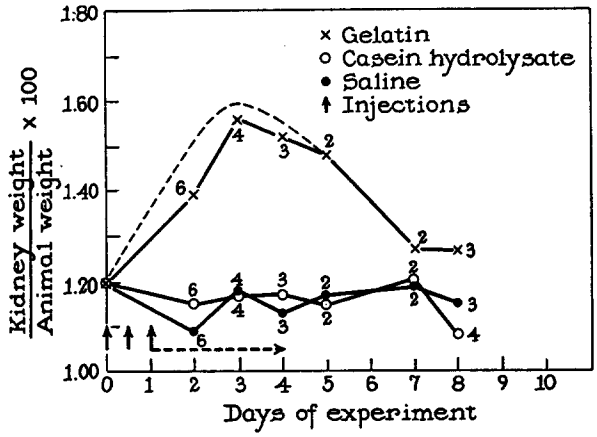

CHART 2. Showing changes in relative renal size on successive days produced by three injections of gelatin, compared with control injections. Absorption of gelatin was unusually slow, as indicated by horizontal arrow, and weight of animals was not corrected for weight of peritoneal fluid. Broken line indicates approximate correction. Numerals indicate number of observations. Average weight of gelatin-treated animals autopsied on various days was: 2nd day, 46 gm., 3rd day, 48 gm., 7th and 8th days, 58 gm. Data from series 4.

possibly because the animals were autopsied at an earlier age than most, it was found that the gelatin solution had not been as completely absorbed as usual, and fluid was present in the peritoneal cavities of many of the rats for 2 or 3 days after the final injection. This continued absorption probably accounted for the further increase in relative kidney size after injections were discontinued. The chart demonstrates that a prompt increase in the relative size of the kidneys of the gelatin-treated animals occurred, followed by a prompt return toward normal as soon as the injections were discontinued and the gelatin was all absorbed from the peritoneal cavity. Evidence of some degree of renal damage was observed in the kidneys of several rats of this group, as will be discussed in more detail later.

Chart 3 shows the changes in the kidney weight–body weight ratios, during

the 10 day period following termination of a 9 day injection period, in animals receiving various protein solutions. The final injection is indicated by vertical arrow. Protein solutions had been almost completely absorbed when the first animals were autopsied on the 10th day.

The decrease in kidney weight–animal weight ratio after termination of protein injections, particularly when observations were made over a considerable period, was due in part to a dilution of the kidney weight increment which resulted from the injections, due to normal growth of the kidneys. In addition to this effect, however, there was a prompt decrease in the absolute difference

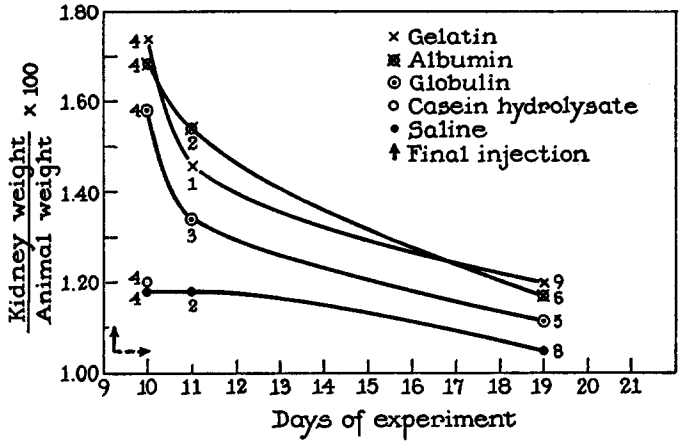

CHART 3. Showing return of kidneys of protein-treated animals toward normal size, following termination of a 9 day injection period. Data are from series 6. Numerals on chart indicate number of observations at each point. Horizontal arrow indicates duration of presence of protein solutions in peritoneal cavities of animals. Average weights of various groups of animals autopsied at different points on chart were: 10th day, 51 to 52 gm., 11th day, 52 to 56 gm., 19th day, 75 to 86 gm.

in size between the kidneys of protein-treated and control animals. Charts 2 and 3 indicate that some residual enlargements of the kidneys of the protein-treated groups probably remained at the end of the periods of observation. In series 1, there appeared to be slight enlargement of the kidneys of the gelatin-treated animals 2 months after the injections were discontinued. However, since any residual enlargement becomes a progressively smaller portion of the total renal tissue as the animals grow older, and greater variation in animal size occurs, the long term observations on small groups of animals were of doubtful significance. It can only be concluded that there was a prompt return of the kidneys of the protein-injected animals in the direction of normal size, after protein injections were discontinued.

Composition of Kidneys of Animals Receiving Various Solutions.—After it was apparent that injections of protein solutions resulted in renal enlargement,

it seemed of importance to determine whether the enlargement was due simply to an increase in the water content of the renal tissue, or whether it was due to an increase in material of approximately the same solid content as normal renal tissue. Water determinations were used to answer this question. Since the enlargement appeared to involve chiefly the cortical portion of the kidney, cortical tissue was used for the determinations. It is evident from data in Table V, representing water determinations on kidney tissue from a total of 70 animals, that only a slight part, if any, of the enlargement was due to an increase in the proportion of water to solids in the renal tissue of the protein-treated animals. The increase in weight was due to addition of water and solids in almost the same proportion present in normal kidney tissue. There was a slight increase in average relative water content of the tissue from animals

TABLE V

Moisture Content of Renal Cortical Tissue of Rats from Series 3, 6, and 7

Injected solutions*	Moisture	
	18 hrs. after final injection	10 days after final injection
	per cent	*per cent*
Saline	77.2	77.3
Urea	76.8	—
Casein hydrolysate	76.8	—
Gelatin	78.0	77.2
Albumin	78.8	78.3
Globulin	76.9	77.5
Serum	77.5	—

Injections were continued for 9 days to 2 weeks.

* Solutions previously described in text.

receiving albumin, and possibly in that of those receiving gelatin, 18 hours after termination of the injections. The 10 day values are less reliable because of the smaller number of determinations and greater spread of individual values.

Total nitrogen determinations on a few kidneys of various groups gave approximately similar results in most of the control and protein-treated animals and indicated that the protein concentration was probably normal in the protein-treated animals. The increase in kidney weight was apparently due in most instances to addition of water and protein in nearly the same ratio existing in normal kidneys.

Injected Protein in Renal Tissue.—Homogenates of renal cortical tissue from gelatin-injected animals were usually found to contain definitely more material resembling gelatin, in that it was precipitated by tungstate but not by a final concentration of 5 per cent trichloracetic acid, than was found in the renal tissue from other groups. This was true even when the determinations were made several days after gelatin injections were discontinued. However, efforts

were not made to insure quantitative extraction of gelatin or other specific proteins from the renal tissue and quantitative determinations of gelatin were not made in most cases. The desirability of further studies was evident when, during the course of the experiments, it was found that kidney tissue from *control* animals sometimes contained more than the minimal amounts of "gelatin" observed in the early experiments.

In extracts of kidneys from rats that received human albumin and bovine globulin, serological precipitin tests showed the presence of these injected proteins. The amounts of extracted human albumin were much smaller than necessary to account for the total enlargement of the kidneys associated with injections of that protein, and it was not established beyond question that the results could not be accounted for on the basis of human albumin in the blood and urine retained by the renal tissue. Quantitative determinations of the globulin were not attempted.

These results were regarded as suggestive but not conclusive evidence that the renal parenchymal cells contained quantities of the injected proteins.

Microscopic Observations.—No abnormalities or differences were noted on microscopic examination in the kidneys of animals receiving saline, (Fig. 4), urea, or protein hydrolysate.

Kidneys from animals receiving gelatin showed, in addition to the enlargement and paleness apparent on gross examination, rather marked alterations in the cells of the convoluted tubules, particularly in the outer two-thirds of the cortex (Figs. 5 and 6). The proximal tubules were probably more markedly involved than the distal segments. The cells of the convoluted tubules were enlarged and contained what appeared to be clear spaces in the cytoplasm. These clear spaces were observed in sections of tissue that had not been exposed to aqueous solutions, as well as in those subjected to the routine staining procedures. There was no increase in fat content of the cells as shown by fat stains. The lumina of the convoluted tubules appeared to be decreased in width and in some cases they were almost closed, due to encroachment by enlarged cells.

Although the kidneys of the animals receiving solutions of albumin and globulin were definitely enlarged in the gross, on microscopic examination no definite and consistent changes were noted which might have been responsible for the enlargement. There appeared to be slight cytoplasmic alterations in the cells of the convoluted tubules in some cases. Increases in size of the cells of the convoluted or other tubules, or changes in caliber of the tubular lumina, if present, were not of sufficient magnitude to be recognized, and there was no other apparent explanation for the increase in bulk of the cortical tissue. In most of the animals of the protein-treated groups, no changes suggestive of an increased production of new cells in the cortex, were observed; mitotic figures were noted in the tubules of some animals autopsied early in the course of gelatin injections.

In the kidneys of some animals receiving proteins, protein material was visible in occasional glomerular capsules and tubular lumina. Rare isolated tubules in the region of the straight segments appeared to be plugged with protein material but similar tubules also were seen from time to time, but perhaps less frequently, in control kidneys. No abnormalities were noted in glomeruli, blood vessels, or interstitial tissue, even in animals treated and observed for prolonged periods. No changes suggestive of sensitization of the animals to the injected proteins were observed.

Three or four gelatin-injected animals of series 4—the series in which absorption of the injected 3 per cent gelatin solution was unusually slow—differed further from most of the protein-treated animals in that definite evidence of renal damage was observed on microscopic examination of the kidneys. These animals in which definite damage was recognized were among those which were autopsied from 2 to 5 days after gelatin injections were discontinued. The morphological alterations were much more extensive in the kidneys of one of the animals (Figs. 7 and 8) than in those of the others. These alterations were localized principally in the inner cortical and outer medullary zones where glomerular capsules and tubules appeared dilated and contained some protein material, and the tubules were lined by flattened epithelium containing mitotic figures, large hyperchromatic nuclei, and basophilic cytoplasm. Similar dilation of glomerular capsules and tubules, not accompanied by evidences of tubular necrosis or regeneration, was present at the time of autopsy in the kidneys of some of the gelatin-treated animals of series 9, particularly those receiving the more concentrated solution. In the kidneys of the majority of animals receiving each of the 3 per cent protein solutions, no changes were observed which could be considered evidence of renal damage, unless the cytoplasmic alterations in the tubular cells induced by gelatin are interpreted as evidence of damage.

In the kidneys of animals receiving gelatin, the appearance of the cells of the convoluted tubules gradually returned toward normal after injections were discontinued, with the clear spaces decreasing in size and finally disappearing. While there was considerable individual variation, the morphological alterations largely disappeared in most cases within about a week, during which time the renal enlargement and the "gelatin" in the renal tissue also decreased.

During the periods following the injections no impressive renal cytologic changes were observed in the animals receiving the control solutions, or those receiving albumin or globulin.

No consistent microscopic changes were observed in the livers of any of the groups of protein-treated animals.

Observations on Animals Treated with Rat Serum.—Since renal enlargement and an increase in urinary protein excretion were induced by injections of heterologous proteins, it was desirable to determine whether similar effects would result from injections of homologous proteins. The experiments with

the proteins already discussed, particularly the rather preliminary studies on the specific proteins in the urine, suggested that the increased proteinuria was not dependent on the heterologous nature of the injected proteins but would be produced by homologous proteins as well. Because purified rat proteins were not available, it was decided to investigate the effects of injecting homologous serum. Some of the data from these studies have been included in the tables and charts already presented, in order to facilitate comparisons with data from other groups. However, for several reasons, separate discussion of the experiments with rat serum seemed desirable.

In the first experiment with rat serum (series 7 of Table I), the animals with the possible exception of one, appeared quite well throughout the experimental period. Some enlargement of the kidneys occurred (Chart 1), but this was of less magnitude than with the other protein solutions. At the time of autopsy, more than 12 hours after the final injection, large amounts of unabsorbed fluid were found in the peritoneal cavities of some of the animals. In experiments where solutions of gelatin, albumin, or globulin had been injected for similar periods of time, absorption had nearly always been more complete. In addition, the serum-injected rats showed evidence of peritoneal irritation with definitely thickened renal capsules, not observed in the animals receiving the 3 per cent solutions of single refined proteins. Furthermore, urine collections made on the last 2 days of the experiment, which were the only specimens collected, showed no definite increase in protein content as compared with control specimens.

It was thought that perhaps ether retained in the rat serum (the donor animals were deeply anesthetized with ether) caused progressive peritoneal irritation with a corresponding decrease in the rate of absorption of injected serum, and that this explained the fluid retention in the peritoneal cavities, the low levels of urinary protein excretion, and the absence of greater renal enlargement. A second experiment (series 8) was then performed, using rat serum which was collected from donor animals after they were rendered unconscious by anoxia in a nitrogen chamber. In this experiment, the serum-treated animals retained much of the injected serum solution in the peritoneal cavities from the beginning, excreted little urine, and became definitely ill with elevated blood urea levels. Two of the four animals died on the 4th day after becoming quite pale and cold. The other two animals were autopsied and found to have greatly enlarged kidneys. Urine collected on the 3rd and 4th days from animals of this group contained considerably increased protein levels.

Microscopic Observations on Serum-Treated Animals.—Sections from the kidneys of all but one of the animals of the first serum-treated group appeared entirely normal, with the exception perhaps that the tubular lumina contained more protein material than normal. Changes similar to those observed in the gelatin-treated animals of series 4 with the severer forms of injury, were seen in milder degree in sections of the kidneys of one rat of this first group.

When the greatly enlarged kidneys of the second serum-treated group were sectioned, a discrete band occupying the region about the corticomedullary junction, which obviously contained large amounts of calcium (Fig. 9), could be seen with the unaided eye. Microscopic examination of sections confirmed the presence of extensive tubular damage with necrosis and calcification in the zone including the deepest portion of the cortex and adjacent medullary tissue (Figs. 10 and 11). Many glomerular capsules contained protein precipitates, and the tubules in the area beneath the zone of calcification were dilated, lined by flattened epithelium, and contained large amounts of deeply staining protein material.

DISCUSSION

Only the inconclusive data of Bordley and Richards (15) and Walker *et al.* (16) are available concerning the protein content of the normal glomerular filtrate, though the constant occurrence under uniform conditions of fairly uniform levels of protein in the urine of the normal rat (17) appears to be presumptive evidence that the glomerular filtrate of that animal contains some protein. Accumulated evidence (18–21) indicates that the glomerulus is at least the chief source of the protein which appears in the urine under the various abnormal conditions which have been studied; it does not eliminate the possibility, however, that all or a part of the protein in the urine under certain circumstances may be present as a result of defective tubular reabsorption of protein normally or abnormally filtered from the glomeruli (22). It was evident from examination of the sections in the present study that the increased proteinuria which occurred with the protein injections was associated with an increased amount of protein in the glomerular filtrate. Furthermore, gelatin appeared in the urine prior to the development of the characteristic changes in the tubular cells which were presumably associated with the presence of gelatin in the cells.

Because of the chronic nature of the experiments there may have been a compensatory increase in hemoglobin levels of the protein-injected animals, and the hemoglobin levels at the end of the injection periods may not have adequately reflected the magnitude of the blood volume changes.

Proteinuria has been observed in dogs following injections of homologous plasma (23), and in human patients without renal disease following injections of homologous albumin (24). In the present experiments, an increase in urinary protein excretion was observed in some, but not in all the rats receiving homologous serum; interpretation of the results was complicated by the occurrence in some of the animals of incomplete absorption of fluid, oliguria, and renal damage.

The rather cursory study of the specific proteins in the urine of the rats seemed to indicate that increased amounts of homologous protein, as well as quantities of the heterologous protein, appeared in the urine following injec-

tions of human albumin and bovine globulin, which accumulated in the plasma, but not following gelatin which was passed into the tubular lumina to at least as great extent, but which caused less hemodilution than the other proteins. These observations might be interpreted as suggesting that the filtration of a protein present in the plasma may be increased, with no increase in the concentration of the protein, by changes in dynamics of the glomerular circulation associated with an increase in blood volume, though other explanations of the observations are conceivable.

Reversible renal enlargement, comparable in degree and rapidity of development to that observed in the present experiments following the injections of proteins, has been shown to occur in rats on diets containing high levels of various proteins, including casein (25–27). This renal hypertrophy under conditions of high protein diets has usually been considered a result of an increase in renal work associated with the metabolism and excretion of products of protein digestion. Some degree of renal enlargement due to feeding of urea itself has been observed by some investigators (28), but others have obtained essentially negative results (25). Renal injury also has occurred under certain circumstances with high protein diets (29, 30).

In the present study, renal enlargement such as occurred with the protein injections, was not observed following injections of urea or casein hydrolysate. It perhaps is possible, in spite of careful planning of the experiments, that differences in food intake, nutritional state, or hydration of tissues, might have been responsible for the difference in relative renal size which developed in the animals receiving protein hydrolysate, as contrasted with those receiving protein itself. However, there was no evidence from examination of the animals that such differences were present to an important degree.

Another explanation of the occurrence of renal enlargement with injections of proteins but not with injections of protein hydrolysate or urea, which must be considered, is that the renal enlargement might have been caused at least in part by effects on the kidney of protein molecules *per se*, perhaps more specifically by effects on the tubular cells of the increased amount of protein filtered through the glomeruli, rather than entirely by effects of products of protein digestion and metabolism reaching the kidney through the blood stream. It might be pointed out that an elevation in urinary protein excretion has been observed incidentally accompanying an increase in the dietary protein level in rats (30, 31); however, it is not intended to suggest that the increase in proteinuria was the cause of the renal hypertrophy which occurred with the high protein diets. It is difficult to understand why the protein hydrolysate injections in the present experiments did not induce some increase in proteinuria and renal enlargement through the same mechanisms as were involved with increasing the protein content of the diet.

Morphological observations by a number of European investigators, and

more recent studies by Oliver (21), Smetana and Johnson (32), Smetana (33), and Rather (34), have provided fairly conclusive evidence that at least certain proteins present in the fluid passing through the tubules may be reabsorbed by the tubular cells and accumulate to some extent within these cells. However, the results of Bott and Richards (20) indicated that not more than a small portion of the filtered protein was reabsorbed under the conditions of their experiments. The reabsorbed protein under normal conditions presumably is digested by the tubular cells (34). The observations of others on protein reabsorption, together with our own morphological observations, particularly with gelatin which was the only protein seeming to produce recognizable enlargement of the tubular cells, suggested that the renal enlargement might have been caused in some part by enlargement of individual tubular cells as a result of reabsorption and temporary accumulation within the cells of protein passing through the glomeruli, together with water associated with it. The cytoplasmic changes produced by gelatin were similar to those described by Popper (35) and Skinsnes (36) in human kidneys following gelatin administration. Furthermore, an increase in metabolic activity of the renal cells associated with an increased reabsorption and degradation of protein might have been responsible for some degree of enlargement of the kidneys.

An increase in volume of intraluminal material within the renal tubules very probably was present in a few animals in which severe renal injury occurred, and in some other gelatin-treated animals particularly during the early periods of the injections, but it appeared unlikely that changes of this nature were responsible for the renal enlargement which regularly followed the protein injections.

In the majority of animals receiving each of the 3 per cent protein solutions, there was no evidence of gross disturbances of renal function. Renal clearance studies have not been done, though such studies might yield interesting results. The changes in renal size and the alterations in the appearance of the convoluted tubules were regarded for the greatest part as reversible morphological expressions of exaggerated physiological processes, and no inflammatory, necrotic, or sclerotic changes were observed in the kidneys, either during or following the injections. Urinary protein excretion promptly returned to normal levels after injections were discontinued. It was concluded that under the conditions of the experiments, prolonged continuous proteinuria of the degree obtained with these injections in most cases did not lead to a persistent increase in glomerular permeability or to any other form of chronic or progressive renal injury.

Definite evidences of tubular injury of a severe degree were observed, however, in a few animals receiving 3 per cent gelatin, and dilatation of glomerular capsules and tubules was present in some of the animals of series 9 receiving gelatin. Whereas most of the gelatin deposition presumably occurred in the tubular cells in the outer two-thirds of the cortex, the zone of injury was about

the corticomedullary junction. Whether this injury was a result of plugging of the tubules, or was due to some other action of gelatin, or was unrelated to any effects of gelatin on the kidney, was not determined with certainty. While the destruction of cells and proliferative changes in the dilated tubules might be considered evidence against mechanical plugging alone as the cause of the tubular lesions, at least the damaged tubules in most cases contained more protein within the lumina than usually was observed in undamaged tubules. Urine from the animals in which severe injury occurred with gelatin, unfortunately was not collected or examined. The possibility that these animals became dehydrated, due to failure to absorb part of the injected fluid, or due to failure for some reason to drink water after the injections were discontinued, in the case of the animals of series 4 which were not autopsied for several days after injection, and that dehydration contributed to the production of the injury,—as has been observed with hemoglobin (37),—must be considered. It should be noted that the concentration of protein in the urine of most of the animals in the present experiments, particularly those receiving albumin and globulin, and those of the first group receiving serum, was not as great as is often observed clinically. Furthermore, in animals with already diseased kidneys, the effects of protein injections and increased proteinuria might have been quite different from the effects observed in the present experiments employing animals with normal kidneys.

Before examining the kidneys of the second series of rats receiving homologous serum, it was considered likely that the erratic absorption of serum, and the oliguria or anuria which occurred, and perhaps also the large amounts of protein in the urine, were due to shock rather than to primary renal damage. However, the remarkable lesions which were observed were not similar to any lesions which have been attributed to disturbances of the renal circulation accompanying shock. Tubular plugging may have played a part in the production of the lesions; both the tubules and the glomerular capsules often contained large amounts of protein. The lesions were similar in certain respects to those which occurred in some of the rats receiving gelatin. Because of the great difference in the results obtained in the first and second experiments with rat serum, the profound anoxia to which the donor animals in the second experiment were subjected warrants further investigation as the possible cause of the noxious effects of the serum. It may be that the injury was due to bacterial or chemical contamination of the serum or changes in the serum subsequent to collection, and, until an attempt has been made to repeat the results, no conclusions concerning the etiology of the lesions are justified.

Finally, it might be noted that the kidneys of the protein-treated animals of the present experiments, particularly those receiving gelatin, bore certain morphological resemblances to "nephrotic" kidneys. These similarities in appearance suggested the *possibility* that, as has been concluded by a number

of investigators, certain of the changes which are seen in kidneys in diseases characterized by high levels of protein in the urine may be secondary alterations due to excessive amounts of protein passed through the glomerular membranes. Obviously no conclusions regarding the nature or the site of the fundamental disturbances in nephrosis can be drawn from these observations.

SUMMARY

Repeated intraperitoneal injections twice daily of various proteins into young rats were regularly accompanied by an increase in the protein content of the urine, significant renal enlargement, and often some degree of renal pallor. The most marked changes were induced by gelatin, followed in order by human albumin and bovine globulin. Rat serum produced similar but less conclusive changes. Similar changes were not produced by equivalent amounts of urea or casein hydrolysate.

In sections from the kidneys of animals receiving gelatin, the cells of the convoluted tubules appeared enlarged, and they contained clear "spaces" throughout the cytoplasm. The tubular cells of the animals receiving the other solutions were not obviously altered in size or shape, and the cytoplasmic changes were slight or absent. There was little evidence of increased multiplication of cells or of tubular dilatation in the kidneys of any of the groups.

Changes in concentrations of plasma proteins and hemoglobin, and the results of preliminary studies of the injected proteins in urine and renal tissue following the injections, are described and their possible significance discussed.

It appears that the renal enlargement, as well as the increase in proteinuria and the tubular alterations which followed the protein injections, might have been caused in part by effects on the kidney of protein molecules *per se*, perhaps most likely by the effects on the tubular cells of an increased amount of protein filtered through the glomerular membranes, rather than entirely by effects of products of protein digestion and metabolism reaching the kidney through the blood stream.

In the majority of animals there was no evidence from the morphological or functional studies, that the prolonged and continuous proteinuria induced by the protein injections resulted in renal damage, unless the renal enlargement, and the cytoplasmic changes which occurred regularly with gelatin, are considered evidence of damage. Renal enlargement and proteinuria promptly regressed after injections were discontinued.

Lesions characterized by severe degrees of tubular damage, possibly as a result of tubular plugging, were observed in some of the animals of one group receiving gelatin solution of the usual concentration, and dilatation of renal tubules and glomerular capsules was present in some other gelatin-treated animals autopsied after relatively brief injection periods. A description is also presented of lesions

of remarkable character which developed in the kidneys of all the animals of one small group receiving homologous serum obtained from severely anoxic donors.

The possible relationship between the renal changes in the protein-injected animals and certain alterations of the kidneys observed in diseases characterized by large amounts of protein in the urine, is considered.

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EXPLANATION OF PLATES

PLATE 32

The photographs were made by Mr. R. F. Carter.

FIG. 1. Comparing left kidney from gelatin-treated rat (above) with homolateral kidney from comparable saline-injected control animal (below). The organs had not been fixed. The animals were from series 1 and received injections for 6 weeks. Note enlargement, particularly in the cortical portion, and paleness of the kidney from the gelatin-injected animal. Weights are as follows:—

Control rat: weight of kidneys, 1.85 gm.; weight of rat, 146 gm.; $\frac{\text{Weight of kidneys}}{\text{Weight of rat}} \times 100 = 1.27$

Gelatin rat: weight of kidneys, 2.50 gm.; weight of rat, 140 gm.; $\frac{\text{Weight of kidneys}}{\text{Weight of rat}} \times 100 = 1.79$

Magnification × 2.

FIG. 2. Comparing left kidney from albumin-treated rat (above) with control kidney (below). The animals were from series 2, and received injections for 5 weeks. Note enlargement and cortical thickening in kidney of albumin-treated animal. Weights are as follows:—

Control rat: weight of kidneys, 1.54 gm.; weight of rat, 126 gm.; $\frac{\text{Weight of kidneys}}{\text{Weight of rat}} \times 100 = 1.22$

Albumin rat: weight of kidneys, 2.10 gm.; weight of rat, 126 gm.; $\frac{\text{Weight of kidneys}}{\text{Weight of rat}} \times 100 = 1.67$

Magnification × 1.5.

FIG. 3. Comparing right kidneys from comparable rats of series 6 receiving various solutions. Animals received injections for 9 days. Top row, left to right:

Gelatin: weight of kidneys, 1.10 gm.; weight of rat, 64 gm.; $\frac{\text{Weight of kidneys}}{\text{Weight of rat}} \times 100 = 1.72$

Albumin: weight of kidneys, 1.03 gm.; weight of rat, 66 gm.; $\frac{\text{Weight of kidneys}}{\text{Weight of rat}} \times 100 = 1.56$

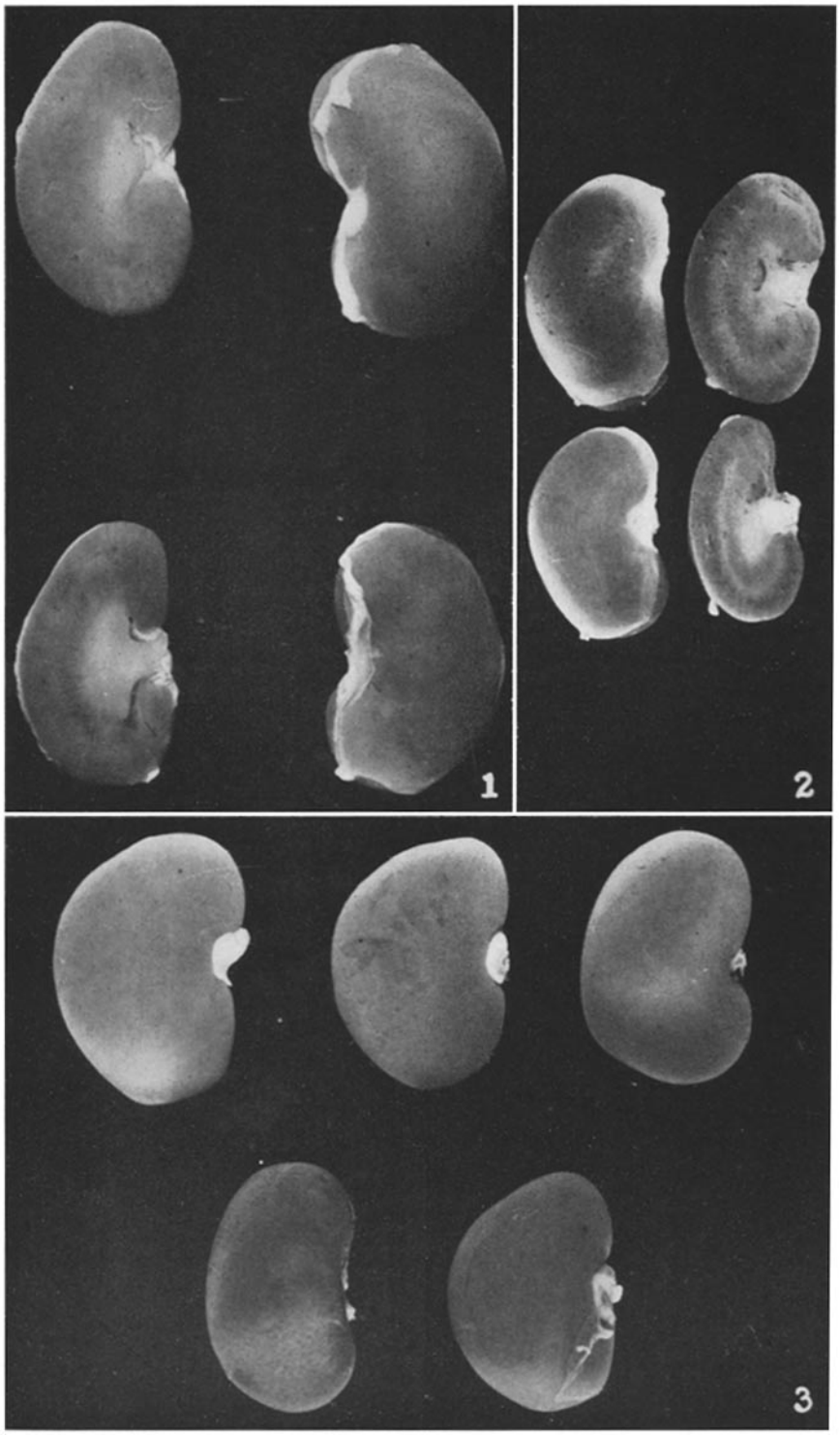
Globulin: weight of kidneys, 1.03 gm.; weight of rat, 66 gm.; $\frac{\text{Weight of kidneys}}{\text{Weight of rat}} \times 100 = 1.56$

Bottom row, left to right:

Casein hydrolysate: weight of kidneys, 0.78 gm.; weight of rat, 64 gm.; $\frac{\text{Weight of kidneys}}{\text{Weight of rat}} \times 100 = 1.22$

Saline: weight of kidneys, 0.78 gm.; weight of rat, 64 gm.; $\frac{\text{Weight of kidneys}}{\text{Weight of rat}} \times 100 = 1.22$

Magnification × 4.



(Baxter and Cotzias: Parenteral protein and the kidney)

PLATE 33

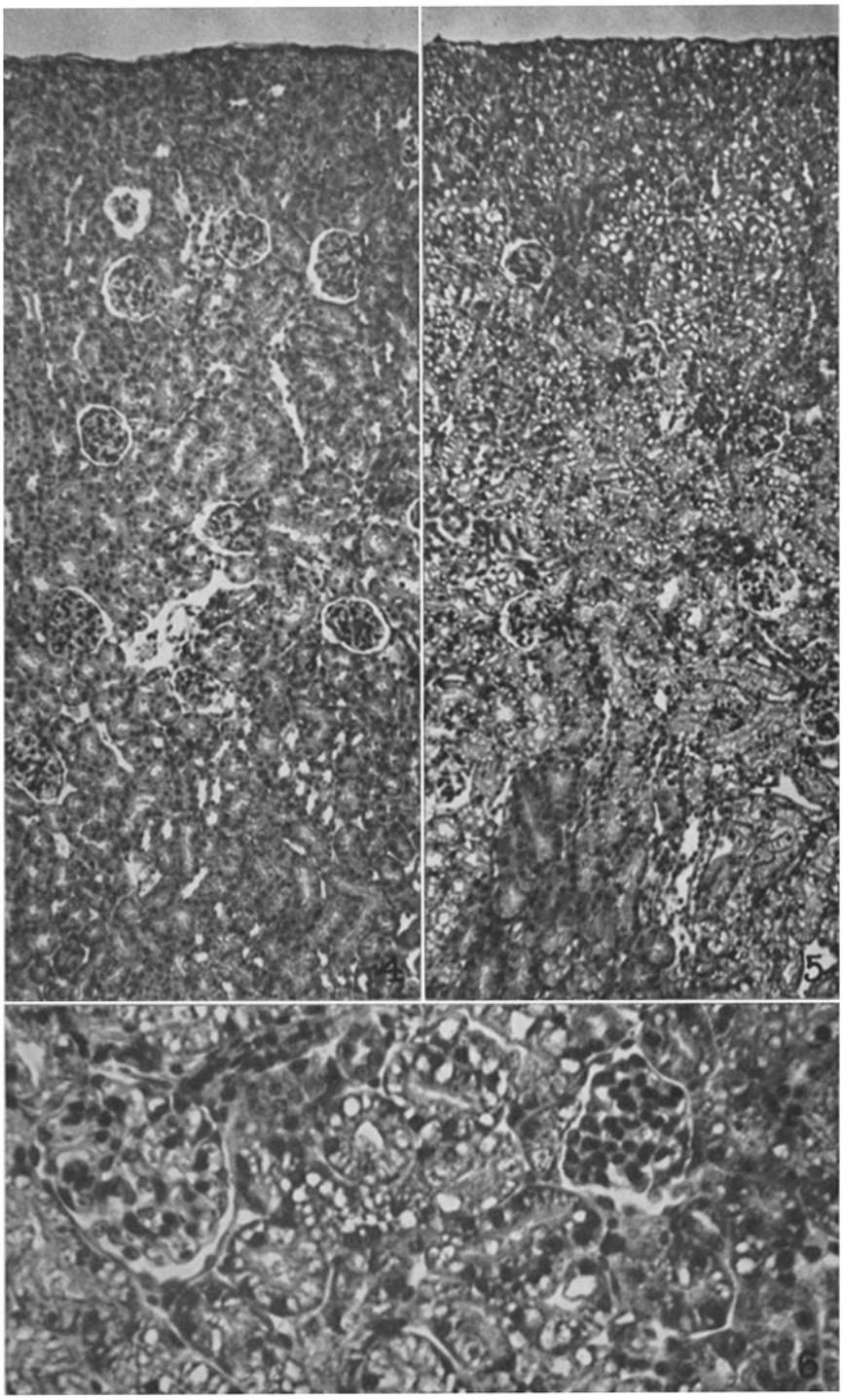
All the sections were stained with hematoxylin and eosin.

The photographs were made by Mr. J. A. Carlile.

FIG. 4. Sagittal section through a control kidney of same group as one shown in Fig. 1. × 120.

FIG. 5. Similar section through kidney of comparable gelatin-treated animal of series 1. Note enlargement of cells of convoluted tubules, and what appear to be clear spaces within the cytoplasm of the cells. × 120.

FIG. 6. Higher magnification of section shown in Fig. 5, showing cytoplasmic alterations in greater detail. × 350.



(Baxter and Cotzias: Parenteral protein and the kidney)

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All the sections were stained with hematoxylin and eosin.

The photographs were made by Mr. C. F. Reather, Johns Hopkins School of Medicine.

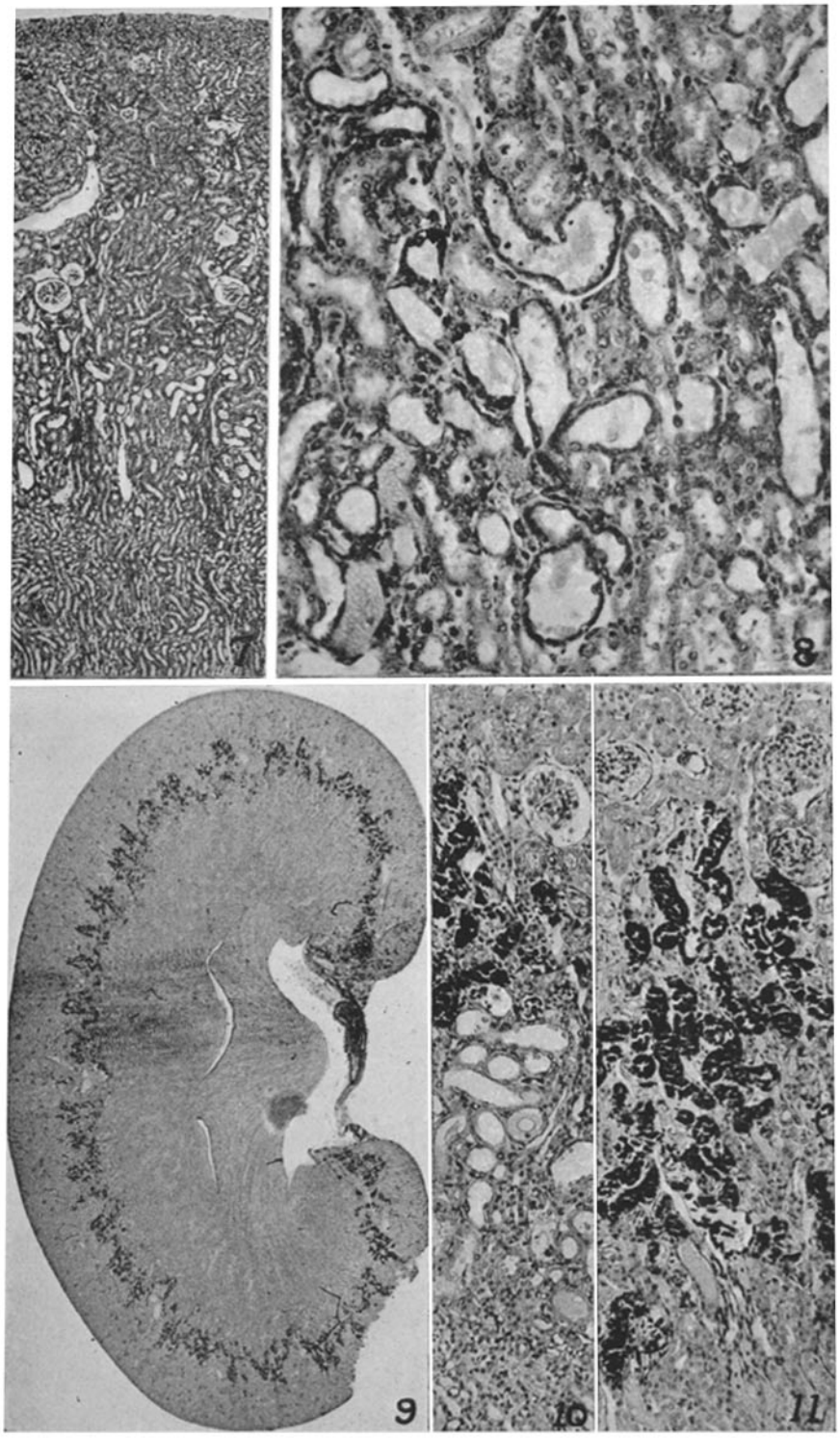
FIG. 7. Sagittal section through kidney of gelatin-treated animal of series 4. This animal received only three injections of gelatin at 12 hour intervals, and was autopsied 54 hours after the last injection. At a higher magnification clear spaces could still be seen in the cells of the convoluted tubules in the outer two-thirds of the cortex. Deeper in the cortex and in the outer portion of the medulla, many glomerular capsules and tubules are dilated. This section shows the most extensive damage observed in any of the kidneys of the animals receiving the 3 per cent solution of gelatin. × 40.

FIG. 8. Higher magnification of the section shown in Fig. 7, showing details of the tubular alterations in the zone of the most extensive injury. Many of the tubules are dilated, lined by flattened and markedly basophilic epithelium, and contain pink-staining protein material. Mitotic figures and other nuclear changes indicative of active cellular proliferation were also present. × 200.

FIG. 9. Sagittal section from kidney of a serum-treated animal of series 8, which became obviously ill and was autopsied on the 5th day. This animal was anuric through a considerable part of the injection period but excreted a moderate amount of urine containing large amounts of protein during the 24 hours before autopsy. The blood urea level was approximately 100 mg. per cent at the time of autopsy. The zone of tubular necrosis and calcification, and the zone of tubular dilatation, are shown well. × 8.

FIG. 10. Higher magnification of section shown in Fig. 9. The zone of calcification can be seen about the region of the corticomedullary junction. Adjacent to and toward the pelvis from this calcified zone, there is the zone of dilated tubules, many of which contain protein. Many tubules far down toward pelvis, which were not dilated, also were full of protein material. × 100.

FIG. 11. A similar section from kidney of the other serum-treated animal of series 8 which was autopsied on the 5th day. In this kidney most of the glomerular capsules contained protein precipitates. × 100.



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