GLOMERULAR LESIONS AND THE NEPHROTIC SYNDROME IN RABBITS GIVEN SACCHARATED IRON OXIDE INTRAVENOUSLY*

With Special Reference to the Part Played by Intracapillary Precipitates in the Pathogenesis of the Lesions

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Plate 5

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In an experiment done recently for other purposes, glomerular lesions and generalized edema were observed in several rabbits given repeated injections of saccharated iron oxide intravenously. Further study has shown that an iron-containing precipitate regularly forms in the blood within a few minutes following injection of the iron preparation into rabbits. This precipitate occludes many of the glomerular capillaries and gives rise to glomerular lesions characterized initially by signs of acute injury to the cells of the glomerular tufts, and later by proliferation of the epithelial cells, fibrosis, and siderosis. Proteinuria regularly develops some 5 to 7 days following injection of the iron, and this becomes marked and sustained when the injections are repeated, with hypoproteinemia developing later, and hypercholesterolemia, generalized edema, and death following in some instances. The findings provide an example of correlated structural and functional changes occurring in the kidney under controlled conditions, and they bear upon the pathogenesis of the nephrotic syndrome as it occurs naturally in human beings.

Methods and Materials

The general plan of the experiments was as follows: Rabbits were given saccharated iron oxide (hereinafter SIO) intravenously, and the character and distribution of the intravascular precipitates were studied histologically at appropriate times thereafter. Observations were also made of the precipitates formed *in vitro* when SIO was added to whole blood, plasma, or serum. In further studies with animals given single or repeated injections of SIO, the excretion of protein in the urine and the concentration of the serum proteins, cholesterol, and blood urea nitrogen were determined at intervals; these observations were correlated with structural changes found in the kidneys upon biopsy or autopsy.

Saccharated Iron Oxide.—The saccharated iron oxide solution (proferrin) was generously

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provided by the Sharp and Dohme Division of Merck and Co., Inc., West Point, Pennsylvania, through the courtesy of Doctor W. P. Boger. It was a dark brown liquid which contained 20 mg. of iron per cc. and had a pH of 10.8. The solution was devoid of precipitate or sediment.

Rabbits.—Market-bought animals, males and females, of mixed breeds, weighing between 500 and 3500 gm., were used. The differing weights of the animals when considered significant are noted in the text. The animals were housed individually in either metabolism or regular cages. All animals were maintained on a diet of rabbit pellets liberally supplemented with feedings of mixed green vegetables and water *ad libitum*. The pellets (Rockland rabbit ration) contained protein 17 per cent, fat 1.5 per cent, and fiber 16 per cent. The animals were weighed at regular intervals throughout the periods of observation.

Urine Determinations.—The 24 hour urine specimen was collected in a beaker placed beneath a metabolism cage with a metal grid and a funnel bottom. The feces were separated from the urine by the metal grid and by fine cotton gauze stretched over the top of the beaker. The rate of protein excretion was determined by Addis' method (1) using Tsuchiya's reagent. To provide optimum protein precipitate, the method was modified so that 7 cc. of urine was added to 7 cc. of Tsuchiya's reagent instead of the usual volume of $\frac{1}{10}$ the hourly output. As a rule the specimens of urine collected over a week-end were pooled for the 48 hour period and analyzed as a single specimen. In selected instances the centrifuged sediment of the urine was examined microscopically and tested for occult blood with benzidine.

Preparation and Studies of Sera.—5 to 10 cc. of blood was obtained from an ear vein, though occasionally blood was obtained by cardiac puncture. The amounts of blood removed were approximately the same for all the animals, and control rabbits were bled at the same time. The blood was allowed to clot, the sera removed, centrifuged, and stored at −20°C. A few serum specimens showing hemolysis were discarded. Determination of serum proteins was carried out by steam distillation using a Pregl-Parnas-Wagner micro-Kjeldahl apparatus (2). The blood urea nitrogen was determined by the method of Van Slyke and Cullen, as described by Hawk, Oser, and Summerson (3). Electrophoretic analysis of the sera was carried out by the method of Flynn and De Mayo (4). The strips were scanned with a photoelectric densitometer (elphor-Bender and Hobein model, Munich), and the concentrations of the various components were calculated from nitrogen determinations of the materials tested and planimetric measurements of the electrophoretic patterns. Serum cholesterol was determined by the method of Bloor, Pelkan, and Allen (5).

Values for 40 normal rabbits were obtained as follows:—

	Average	Range
Total protein	6.4 gm. per cent	5.0 – 8.5 gm. per cent
Albumin	4.2 " " "	3.1 – 5.8 " " "
Alpha globulin	0.78 " " "	0.38– 1.08 " " "
Beta globulin	0.76 " " "	0.42– 1.08 " " "
Gamma globulin	0.69 " " "	0.29– 1.40 " " "
Serum cholesterol	82. mg. " "	26. –148. mg. " "
Urea nitrogen	18. " " "	10. – 22. " " "

Anatomical Studies.—Postmortem examinations were performed on all animals. Sections of the kidneys, liver, spleen, heart, lung, adrenals, and various other selected organs were fixed in Zenker-formol and 10 per cent formalin solution. The blocks of tissue were embedded in paraffin, and sections were stained according to Mallory's or Masson's trichrome method, the periodic acid-Schiff reaction, and Gomori's ferrocyanide method for iron pigment (6).

Intravascular Precipitates and Other Changes Resulting from the Intravenous Injection of Saccharated Iron Oxide in Rabbits

When 2 or 3 drops of concentrated sulfuric acid were added to a solution containing 0.5 cc. of the saccharated iron oxide preparation in 9.5 cc. of distilled water, a copious brown precipitate formed almost immediately; this was comprised of minute brown granules, as the microscope disclosed. Identical precipitates also formed, though somewhat more slowly, when 0.2 cc. of the SIO preparation was added to 3 cc. of serum or plasma procured from normal rabbits. Furthermore, a similar precipitate was seen microscopically in three wet preparations of heart's blood procured from as many adult rabbits some 15 minutes after each animal had been given 10 to 20 cc. of the saccharated iron oxide intravenously. Brown precipitates similar to those just described were regularly found in the capillaries of the lungs, kidneys, and other viscera of rabbits that died or were sacrificed 15 minutes to 7 days after 10 to 20 cc. of the SIO preparation had been injected intravenously. The character of the precipitates seen in the glomerular capillaries within a few minutes following injection of the SIO is illustrated in Fig. 1. Such precipitates were comprised at least in part of iron, for they all stained intensely blue with ferrocyanide, as many histological observations showed. The precise conditions under which the intravascular precipitates arose were not defined. It seems probable, however, that the reduction in pH of the SIO preparation, which presumably took place when this was admixed with blood, had something to do with the formation of the precipitate; for previous workers have noted that saccharated iron is stabilized by the presence of alkali and sugar, and that it undergoes a sol to gel change when the pH is lowered (7, 8).

Table I provides details of the distribution of the intravascular precipitates in 12 of 20 rabbits given a single injection of the saccharated iron oxide preparation and examined after intervals of a few minutes to several days; the recorded findings are representative of those in the entire group.

Ten to 20 cc. of SIO preparation was injected into the marginal ear vein of each of the 20 rabbits during about 5 minutes. After 5 to 10 cc. of the SIO solution had been injected, the animals became relaxed, and their respirations became rapid and shallow. Most of the rabbits urinated within 10 minutes after the injections had been completed, the urine being amber; the next urine passed, usually within 1 hour, was always quite brown, however, and resembled the color of the injected SIO; it regularly gave a highly positive reaction for iron when mixed with potassium ferrocyanide and hydrochloric acid. 11 of the 20 rabbits continued to have dyspnea and died after intervals of 1 to 54 hours. Intravascular precipitates were conspicuous in the alveolar capillaries in all these animals, and pulmonary edema was marked as well, as the histological studies showed (for examples, see rabbits 4, 5, and 6 of Table I).

The remaining rabbits were sacrificed or died at intervals varying from 15 minutes to 7 days after injection. Large amounts of the intravascular precipitate were present in the capillaries of the lungs, liver, spleen, and kidneys. In general, the visceral distribution of the precipitates was similar to that reported by Polson, who used histological and chemical

TABLE 1

Distribution of Intravascular Precipitates and Other Changes in Rabbits Given a Single Injection of Saccharated Iron Oxide

Rabbit No.	Weight	SIO-injected intravenously	Manner of death	Interval between injection of SIO and death or sacrifice	Distribution of intravascular precipitates and other changes
	gm.	*cc.*			
1	1600	15	Sacrificed	¼ hr.	Intracapillary precipitates forming casts in almost 100 per cent of glomeruli; very many in capillaries of alveolar septa also. Large amount of precipitate in sinusoids of liver and spleen with conspicuous phagocytosis by sinusoidal lining cells. Small number of capillary casts in adrenal, pancreas, and heart.
2	2600	20	Sacrificed	¼ hr.	Findings similar to those in animal 1. In lungs the precipitate often lined or coated the larger branches of pulmonary artery. Moderate numbers of small hemorrhages in alveoli.
3	1425	20	Sacrificed	1 hr.	Over 90 per cent of glomeruli contain precipitates which form casts in most of the capillaries. Other organs not examined histologically.
4	2975	20	Died	4 hrs.	Intracapillary casts in almost 100 per cent of glomeruli and alveolar septa. Marked edema and hyperemia of lungs. Moderate hydropic swelling of cells of proximal convoluted tubules. Massive amount of precipitate in splenic sinusoids; slightly less in hepatic sinusoids. Small number of capillary casts in adrenal.
5	890	20	Died	18 to 24 hrs.	Intracapillary precipitates in almost 100 per cent of capillaries of alveolar septa and glomeruli; massive precipitates in splenic and hepatic sinusoids; occasional casts in adrenal and pancreas. Marked edema, hyperemia, and focal hemorrhages of lungs. Moderate hydropic swelling of the cytoplasm of the cells lining the proximal convoluted tubules.
6	2650	20	Died	24 hrs.	Identical with those of rabbit 5.
7	1650	20	Died	48 hrs.	Changes similar to those in rabbit 5.

TABLE 1—*Continued*

Rabbit No.	Weight	SIO injected intravenously	Manner of death	Interval between injection of SIO and death or sacrifice	Distribution of intravascular precipitates and other changes
	gm.	*cc.*			
8	2775	20	Sacrificed	54 hrs.	The capillaries of one or more lobules of nearly every glomerulus are filled with precipitate. Other organs not examined histologically.
9	2650	20	Sacrificed	4 days	Identical with those of rabbit 8.
10	2650	20	Sacrificed	5 days	Identical with those of rabbit 8.
11	2825	20	Sacrificed	6 days	Less than half of the glomeruli have capillaries occluded by intracapillary precipitates. Marked phagocytosis of brown precipitate by endothelial cells. Slight hydropic swelling of cytoplasm of the cells of the proximal convoluted tubules.
12	2825	20	Sacrificed	7 days	Intracapillary precipitates forming casts are less numerous than in rabbit 11, phagocytosis and hydropic change similar. A few glomerular capillaries contain marginated leukocytes.

methods in studying the distribution of intravenously injected colloidal iron in rabbits (9). Relatively little precipitate was found in the capillaries of the adrenals, pancreas, and myocardium.

As is shown in Table I, the glomeruli of the rabbits that were sacrificed or died during the first 5 days following injection of the iron contained huge amounts of the brown precipitate, which regularly filled one or more lobules of nearly every glomerulus and many of the peritubular capillaries of the cortex and medulla as well (Fig. 1). The precipitate was uniformly distributed, virtually every glomerulus being vividly blue in sections stained according to Gomori's ferrocyanide method for iron. The intravascular precipitates were found as early as 15 minutes after the injections, and they persisted with remarkably little variation in size or number for several days thereafter, as the findings of Table I show. On the 6th and 7th day after the injections, however, less than half the glomeruli contained precipitates (rabbits 11 and 12, Table I). The renal changes occurring later than 5 days after injection of the SIO will be described further on. Meanwhile a brief description will be given of another change noted in the kidneys of rabbits given a single injection of the iron preparation.

Transitory hydropic changes in the renal tubular epithelium resembling closely those seen following the administration of hypertonic sucrose were

regularly observed, in addition to the widespread intravascular precipitates in the kidneys of rabbits given the SIO (10). Within 4 hours after the intravenous injection of the SIO, the cytoplasm of the cells lining the proximal convoluted tubules became pale, swollen, and diffusely vacuolated (Fig. 1), so that the tubular lumina were partially narrowed. These changes remained constant for approximately 5 days, after which time the epithelial cells took on again their normal appearance. The following observations indicate that the hydropic change was related to the sucrose fraction of the injected solution and not to the iron. 9 rabbits were given intravenous injections of similar quantities of a control solution that was devoid of iron but contained the same concentration of sucrose (33 per cent) as the SIO solution. These animals were sacrificed from 15 minutes to 7 days after injection; in each case the hydropic swelling of the cells lining the proximal convoluted tubules was identical, as judged histologically, with that found in the animals given the SIO solution.

Proteinuria, Hypoproteinemia, and Hypercholesterolemia Following a Single Injection of SIO Intravenously

During the course of the work, 8 young adult male rabbits survived for some time following the injection of a relatively large amount (20 cc.) of the SIO intravenously, and repeated studies were made of their blood and urine. The findings in one of these animals are given in Chart 1; they will now be considered in detail.

The rabbit, a 2000 gm. hybrid male, was held under observation for 10 days before the iron was injected. Its urine was devoid of protein during this period, and its serum proteins were normal in amount, as electrophoretic determinations showed (Chart 1). The animal remained lively following the careful and slow injection of 20 cc. of SIO intravenously on the 11th day of the experiment. Its urinary output diminished during the next 48 hours, however, from an average of 13.4 cc./hour during the 7 days prior to the injection to 3.5 cc./hour during the 48 hours following the injection; thereafter for the next 7 days the urinary volume varied from 7.7 to 12.1 cc./hour in each of the 24 hour specimens, the average for the week being 9.4 cc./hour. On the 16th day of the experiment (and 5 days following injection of the iron), the rabbit excreted 940 mg. of protein, and it continued to excrete great quantities of protein each day thereafter for many days (Chart 1). Marked hypoproteinemia, with depletion of the serum albumins to 1.9 gm. per cent, was manifest in the specimen of blood taken on the 19th day (8 days post injection), the values for the serum globulins being normal. By the 26th day, the marked proteinuria having continued, the serum albumins had fallen to a level of 0.59 gm. per cent, while the alpha globulin levels had increased to 1.16 gm. per cent. By this time the serum cholesterol had also risen to an abnormally high level (230 mg. per cent, as compared with 96 and 139 mg. on the 10th and 19th days respectively). It is noteworthy that by the 36th day the serum albumins had risen to a level of 2.32 gm. per cent, even though the massive proteinuria had persisted; and on this day it was found that the serum cholesterol level had fallen to 156 mg. per cent, while the alpha globulins remained elevated. The proteinuria was less marked after the 36th day—200 to 600 mg. of protein being excreted each day instead of 800 to 1000 mg. or more as before; by the 55th day the level of serum albumins had reached 4.15 gm. per cent, while the serum cholesterol level had dropped to 54 mg. per cent.

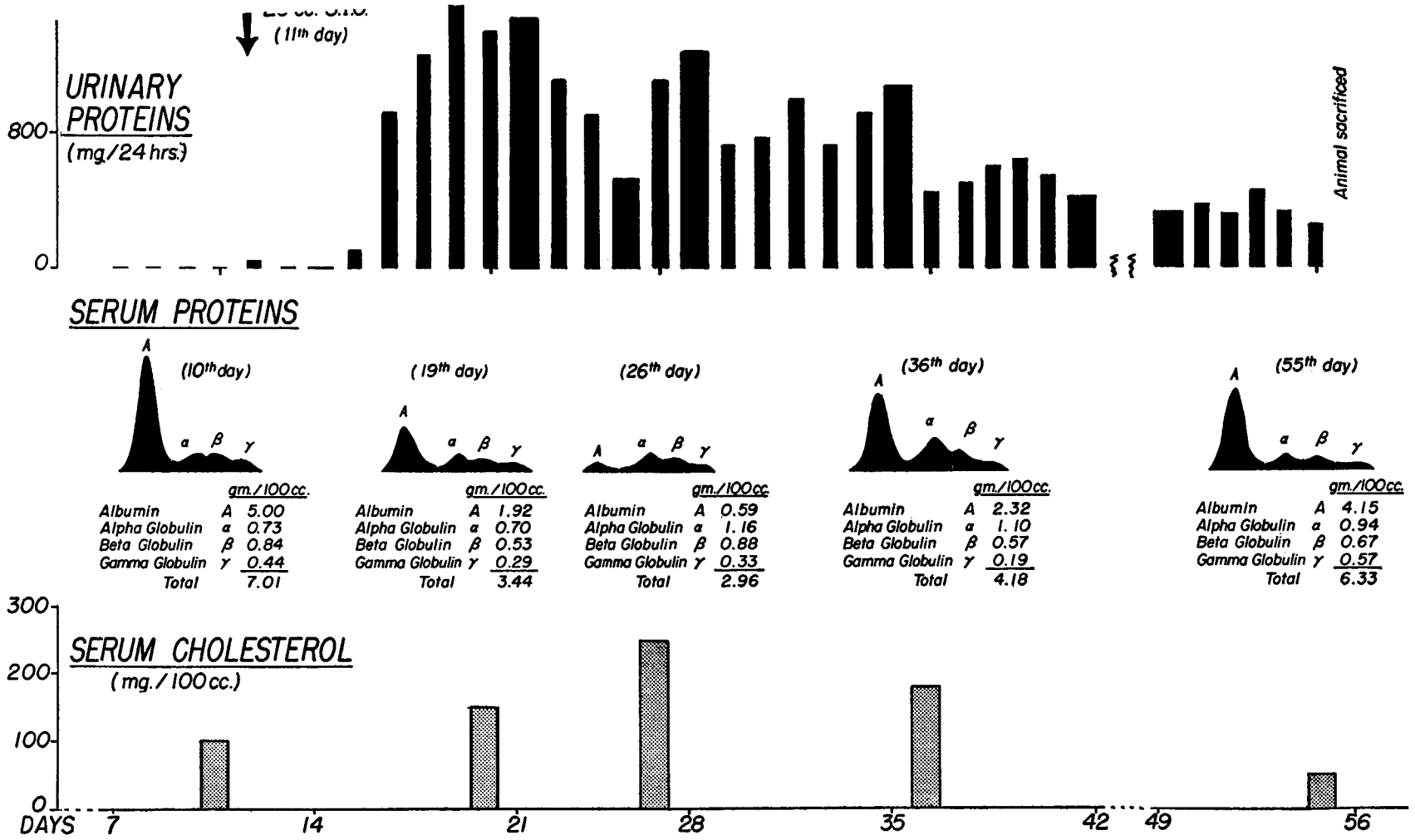

CHART 1. Proteinuria, hypoproteinemia, and hypercholesterolemia following a single injection of saccharated iron oxide intravenously in a rabbit.

The blood urea nitrogen values remained normal throughout the experiment. Several specimens of urine taken during the period of marked proteinuria were examined with benzidine for blood; these were regularly negative. In addition, microscopic examinations of the sediment from centrifuged specimens failed to disclose erythrocytes or casts.

From the findings given in Chart 1, it is plain that a marked and prolonged proteinuria and hypoproteinemia, together with moderate hypercholesterolemia, followed the intravenous injection of a relatively large amount of SIO into the rabbit cited. A control observation was made to learn whether the hypoproteinemia could have resulted from the binding of serum proteins by the injected iron.

Seven young adult rabbits were first bled from the ear, then given 20 cc. of SIO intravenously. The serum protein levels in specimens of blood procured 4 and 24 hours after the injections were approximately the same as those in the specimens obtained beforehand. The cholesterol values, too, were unchanged in the specimens procured after the injections.

In 4 additional rabbits functional changes similar to those just described followed a single intravenous injection of the SIO, while in three comparable animals that were given the same amount of the iron preparation, there was neither proteinuria nor hypoproteinemia. The findings in rabbits given repeated injections of the SIO were more uniform, as will now be shown.

The Nephrotic Syndrome in Rabbits Given Repeated Injections of SIO Intravenously

Thirteen rabbits were given repeated injections of the SIO preparation and observed over long periods of time. The findings from a typical animal are given in Chart 2; they will now be discussed in detail.

The rabbit, a 625 gm. hybrid male, was given 5 cc. of SIO intravenously on days 1, 12, 20, 58, 65, 77, and 90. The rabbit remained lively and continued to grow, so that by the 147th day its weight had increased to 3050 gm. The observations in Chart 2 begin at this point and show an initial proteinuria of 200 to 400 mg./day and a hypoproteinemia and hypoalbuminemia of modest proportions which were probably caused by previous injections. Additional injections, indicated on the chart by arrows, were given on the 151st, 162nd, and 171st days; these were followed by massive proteinuria ranging from 800 to 3000 mg./day for approximately 35 days, as is indicated on the chart. A final injection of SIO was given on the 193rd day; thereafter the proteinuria gradually diminished to levels comparable to those initially charted.

Hypoproteinemia was progressive during the period of maximal proteinuria, as the electrophoretic determinations recorded in Chart 2 show. This was due primarily to a depletion of the serum albumin—the alpha, beta, and gamma globulins being either normal or, as in the case of alpha globulin, actually elevated, as will be discussed later on. 4 days after the onset of marked proteinuria, the serum albumin, previously 2.7 gm. per cent, had diminished to 1.9 gm. per cent (day 172) and 12 days later was further reduced to 0.40 gm. per cent (day 184). As the proteinuria subsided, the serum albumin rose from 0.40 gm. per cent to 2.9 gm. per cent between the 184th and 246th day. Simultaneously, alterations were observed in the alpha globulin and cholesterol levels. Elevation of the alpha globulins occurred only after the albumin had reached low levels; for, as the chart shows, the alpha globulin was normal on the 172nd day when the albumin value was 1.88 gm. per cent; on the 184th and 193rd days, however, when the albumin level was further depressed (below 1.0 gm. per cent), the alpha

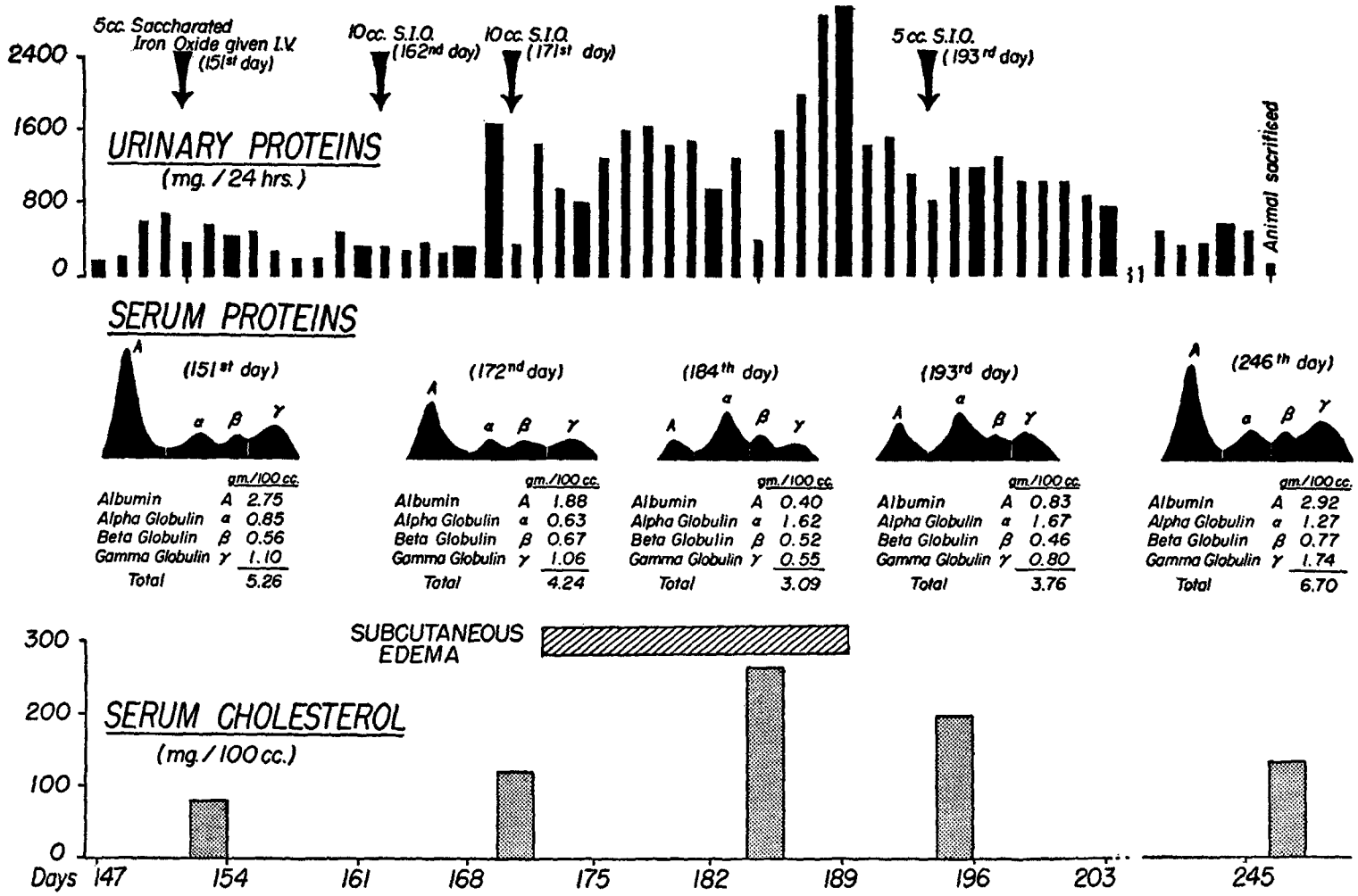

CHART 2. Proteinuria, hypoproteinemia, edema, and hypercholesterolemia following multiple injections of saccharated iron oxide intravenously in a rabbit. The rabbit was given injections of saccharated iron oxide (5 cc.) intravenously on days 1, 12, 20, 58, 65, 77, and 90.

globulin was elevated to 1.6 gm. per cent. As is indicated by the determinations of the 246th day, the elevated serum alpha globulin values diminished but did not return to normal, despite subsiding proteinuria and an increase in the level of albumin. Hypercholesterolemia of 260 and 180 mg. per cent was observed on the 184th and 193rd day, respectively, and was associated with the period of marked proteinuria, hypoalbuminemia, and elevated serum alpha globulins. Thereafter the serum cholesterol levels returned to a more normal level. It should be noted that the increase in the amount of gamma globulin, first noted on the 246th day, did not occur during the episode of proteinuria, hypoalbuminemia, and edema, but only after the urine and serum changes had begun to return to normal. The significance of the delayed elevation of gamma globulin and its relation, if any, to the renal lesions remains unknown.

Several specimens of urine taken during the period of marked proteinuria were examined with benzidine for blood; these tests were regularly negative. In addition, microscopic examinations of the sediment from centrifuged specimens failed to disclose erythrocytes or casts.

Edema developed on the 172nd day and continued during the period of maximal proteinuria for 17 days. It was manifested by a soft pitting of the subcutaneous tissues which was especially marked in the dependent portions of the axillary and inguinal folds and the vaginal tissues, and by a 20 per cent gain in weight. Ascites was also present, as was shown by abdominal protuberance and a fluid wave when the side of the abdomen was tapped gently. After the animal had been edematous for 17 days, spontaneous diuresis occurred and the excessive fluid was lost in a few days. It seems noteworthy that although edema occurred during the period of maximal hypoalbuminemia, it disappeared after 17 days although the albumin level during this time remained at relatively low levels (0.40 to 0.83 gm. per cent).

A moderate but nevertheless progressive azotemia developed during the period of observation before the animal was sacrificed, as is shown by the following findings. The blood urea nitrogen was 17 mg. per cent on the 135th day, 25 mg. per cent on the 151st day, 29 mg. per cent on the 184th and 193rd day, and 56 mg. per cent on the 246th day. During the entire period the rabbit remained lively, maintained its body weight between 3200 and 3400 gm., and had a normal urinary volume.

Twelve additional rabbits were given multiple injections of SIO and held under observation for prolonged periods of time, the majority being studied from 6 to 11 months. In general the findings were similar to those in the animal of Chart 2; they will now be briefly summarized.

Proteinuria and hypoproteinemia were regularly found in all 12 rabbits. Electrophoretic analysis of the urine of 2 animals with marked proteinuria showed that the urine from both contained protein fractions—corresponding with those found in rabbit serum. The electrophoretic samples were prepared by concentrating 10 to 15 cc. of freshly voided urine to a volume of approximately 1 cc. by dialysis for 6 to 8 hours in a cellophane bag against 20 per cent dextran. The electrophoretic analysis of one of the concentrated specimens of urine together with that of serum procured on the same day from the same animal gave the following values:—

	Urine	Serum
	per cent	*per cent*
Albumin	71.7	28.9
Alpha globulin	17.1	43.3
Beta globulin	9.9	22.0
Gamma globulin	1.3	6.7

The proteinuria lasted weeks or months in all the animals of this group, and often 600 to 3000 mg. of protein was excreted in each 24 hour specimen for long periods of time. The lowest values for the serum albumin in each of the various animals ranged from 0.6 to 1.7 gm. per cent in six instances, from 2.0 to 2.2 gm. per cent in five instances, and was 3.1 gm. per cent in one animal. In the latter the proteinuria was of relatively short duration and modest proportions. During the period of maximal hypoalbuminemia, the alpha globulin levels were elevated from 1 to 2 times their normal values in 10 of the 12 test animals, the exceptions having the mildest degrees of hypoalbuminemia of the entire group. In each instance, the serum alpha globulin levels approached or exceeded the albumin concentrations. It seems significant that the albumin and alpha globulin concentrations returned to normal levels in three instances in which the animal lived for several weeks after the cessation of proteinuria.

Transient elevations of serum cholesterol (ranging from 150 to 508 mg. per cent and similar to those shown in Charts 1 and 2) were present in 10 of the 12 rabbits. In 1 of the animals in which elevated cholesterol was not demonstrated, sufficient serum was not available to perform the necessary tests. The reason for the discrepancy in the 2nd animal remains obscure; the proteinuria and hypoproteinemia being equally as marked in this animal as in those rabbits with hypercholesterolemia. In 10 rabbits hypercholesterolemia, was always manifest during the period of maximal proteinuria and hypoalbuminemia. Inanition may give rise to elevated cholesterol values in rabbits, as a number of observations made in this laboratory have shown. For example in a control experiment, 6 adult hybrid rabbits of both sexes were placed on a restricted intake of rabbit pellets (Rockland) and lost 22 to 28 per cent of their body weight within 2 to 3 weeks. In 5 of the 6 animals, the serum cholesterol values had by this time reached levels of 2 to 3 times the prefasting control values. The weight records of the rabbits given SIO showed that in 5 animals the weight remained constant during the period of hypercholesterolemia, while in 2 others there was moderate weight loss, and in 3 the weight was elevated because of edema. 2 of the rabbits developed a second elevation of the serum cholesterol but at this time had only minimal proteinuria and hypoalbuminemia. These observations indicated that the elevated cholesterol values are not obviously related to any of the other changes noted.

Azotemia ranging from 64 to 510 mg. per cent was present terminally in 7 of the 12 rabbits. All of these animals had regularly lost weight for several weeks or months, and the majority were sacrificed because of their cachectic state. The urinary output remained essentially normal in all instances, except for 2 rabbits that developed marked oliguria 3 days prior to death. The reasons are not entirely clear why the other 5 rabbits of the group failed to develop azotemia. 2 of the animals were under observation for a relatively short interval of time (105 and 160 days respectively), and in 2 others the duration and degree of the proproteinuria and hypoalbuminemia were modest. The 5th animal that did not develop azotemia likewise failed to show elevated cholesterol values. 4 of the animals that did not develop azotemia were all well nourished at the time they were sacrificed, whereas the other became cachectic and was sacrificed.

Considered together the findings just given make it clear that prolonged proteinuria and hypoalbuminemia regularly followed multiple injections of the SIO intravenously in rabbits, and that transitory elevations of the alpha globulins and serum cholesterol usually followed as well, with some of the animals also exhibiting a transitory subcutaneous edema. The hypoproteinemia and hypoalbuminemia were always found in rabbits with marked proteinuria of some days duration. But the massive proteinuria continued only so long as the injections of SIO were given, and the lowered protein levels usually returned

to normal when the proteinuria diminished. A number of the rabbits developed progressive azotemia and weight loss and either died or were sacrificed because of their poor general condition. The morphologic alterations noted in the kidneys of these animals will be discussed in the next section.

Glomerular Lesions in Rabbits Given SIO Intravenously

The formation of the intravascular precipitates in rabbits given SIO intravenously, their accumulation and persistence in the glomerular capillaries for several days, and their disappearance, have been taken up in a previous section. The signs of acute injury to the glomeruli that became manifest soon after the onset of proteinuria will be described next. Some of these are illustrated in Fig. 2.

Two rabbits weighing 2600 and 3000 gm. were given a single injection of 20 cc. of SIO intravenously. 1 of them developed proteinuria of 3 gm./day 7 days later, while the other excreted 1 gm. of protein during the 8th day after the injection. The animals were sacrificed. In both rabbits there was a marked accumulation of protein material in Bowman's space in about 50 per cent of the glomeruli (Fig. 2). This pink-staining material extended as homogeneous casts through many of the proximal convoluted tubules, and into the loops of Henle, and the distal convoluted tubules as well. In a few of the glomerular capillaries moderate numbers of polymorphonuclear leukocytes were seen, together with karyorrhexis and pyknosis of some of the endothelial cells. The epithelial cells increased in size and number, and in places they separated the capillary loops to an abnormal degree. Despite these evidences of acute injury to the glomerular capillaries, abnormalities of the basement membranes were not observed in sections treated with the periodic acid–Schiff reaction. A small amount of brown granular pigment was distributed diffusely within the endothelial cells of the glomerular capillaries. In approximately 25 per cent of the glomeruli larger masses of brownish iron precipitate occluded one or more capillaries, the remainder being patent. It was impossible to decide whether this precipitate was phagocytized or free within the capillary lumen. The epithelial cells of the proximal convoluted tubules were markedly vacuolated and, in addition, contained many small hyaline deposits. The hyaline droplets were faintly pink in sections treated with Mallory's trichrome stain.

Proliferation of the epithelial cells was more advanced in a 3rd rabbit that was sacrificed 3 days after the onset of massive proteinuria (1.2, 1.6, and 1.0 gm. of protein per each 24 hour period). The rabbit received 3 injections of SIO, 10, 20, and 20 cc. respectively, during the 11 days before the onset of proteinuria. Microscopic sections showed that the glomerular and capsular epithelial cells were increased in size and number. In addition, the capsular and visceral epithelial cells were often fused—thereby forming bridges within, or partially obliterating, Bowman's space. In sections treated with periodic acid–Schiff reaction, and in others stained by Mallory's trichrome method, the glomerular and capsular basement membranes appeared distinct and unaltered.

The proliferative changes in the glomerular epithelial cells were even more prominent in renal biopsies procured from 3 animals 17, 20, and 26 days after the initial onset of proteinuria. A typical glomerulus from one of these biopsied animals is shown in Fig. 3.

The proliferated epithelial cells of many of the glomeruli in the kidneys of these 3 rabbits appeared continuous and formed a thin membrane which enveloped the capillary loops.

Many of the proliferated epithelial cells were greatly swollen and vacuolated, and some contained hyaline droplets (Fig. 3). The pale vacuolated cytoplasm of these cells was faintly pink in the section stained with periodic acid Schiff reaction but failed to stain with oil red O or Sudan IV. The hyaline droplets were round, of various sizes, and intensely red in preparations stained by Mallory's trichrome method. The majority of the capillaries of the glomeruli appeared patent, and contained red blood cells, but in some glomeruli, one or more lobules appeared denser than normal, and in these the capillary lumina did not appear patent and did not contain red cells. Brown, finely or coarsely granular pigment that gave a positive ferrocyanide test for iron was quite conspicuous in the endothelial cells, but intracapillary casts were absent. In general, the glomerular basement membranes appeared normal, though in a few of the glomeruli, in areas where there was conspicuous fusion of the capillary tufts and the glomerular capsule, the capsular and capillary basement membranes were thickened, reduplicated, or fragmented. Occasionally the proliferated epithelial and capsular cells were spindle-shaped, accumulated in layered masses, and crescent-like. Collagen was not present in these areas, however, as sections stained with Mallory's trichrome method showed.

The tubules of the kidneys of the rabbits given SIO showed three distinct changes. One of these—the transitory hydropic swelling referable to the sucrose contained in the injected material—has already been described (Fig. 1). A second change was regularly found in rabbits that had manifested a marked and sustained proteinuria. It consisted of the presence of numerous "hyaline droplets," mainly in the cells of the proximal convoluted tubules, quite like those generally attributed to the reabsorption of protein from tubular urine (11, 12); see, for examples, Figs. 2, 3. The third change was a manifestation of generalized siderosis and consisted in the presence of many small, brown, iron-positive granules in the cells of the proximal convoluted tubules (Fig. 4).

Chronic renal lesions characterized by marked proliferation of the epithelial cells of the glomeruli, together with fibrosis and siderosis, were found in each of 13 animals given repeated injections of SIO over long intervals of time. 8 of the animals had had azotemia for varying periods of time when they died or were sacrificed, and the glomerular changes in these were particularly marked and constant; but similar, though less marked changes were found in the kidneys of the 5 rabbits that had not manifested azotemia. Fig. 4 illustrates the chronic renal lesions seen in the rabbit of Chart 2.

Many of the glomeruli of these 13 rabbits were small because of partial or complete obliteration and fibrosis. Crescents of proliferated epithelial cells around the margins of Bowman's space were frequently seen. These structures showed early collagenization in sections stained by the Mallory trichrome method. In other small, partially fibrotic glomeruli the remains of Bowman's space was, in many instances, lined with a cuboidal epithelium forming tubules which partially encircled the glomerulus. Many of the remaining glomeruli were enlarged (Fig. 4) and showed a moderate to marked increase in the number and size of the epithelial cells lining Bowman's space (Fig. 4). A moderate degree of interstitial fibrosis was quite diffuse in the cortex, and many of the cortical tubules were atrophic. These alterations resulted in a decrease in the over-all width of the renal cortex. The cells lining many proximal tubules contained hyaline droplets, and moderate numbers of homogeneous casts were seen in the proximal and distal tubules.

All of the glomeruli of animals given repeated injections of SIO showed varying degrees

of siderosis (Fig. 4). In most instances the brown, granular pigment was clearly within the cytoplasm of cells lining either patent or obliterated capillaries, and therefore presumably of endothelial origin. In some instances, however, the masses of pigment were so large that it was impossible to decide whether the pigment was within or outside the capillary lumina. The cytoplasm of the cells lining the proximal and distal convoluted tubules likewise contained many small granules of iron pigment as did many of the atrophic tubules.

In summary, the findings show that the essential renal changes involved principally the glomeruli. During the first several days following injection of the SIO, the capillaries of the glomeruli were filled with iron-containing precipitates which largely disappeared after 5 to 7 days (Fig. 1 and Table I). At about this time, signs of acute injury to the glomerular capillaries—namely a sparse accumulation of neutrophils within them and pyknosis of some of their endothelial cells and early proliferation of the epithelial elements—became manifest, along with morphological signs that protein in great quantities was leaking through the capillary tufts (Fig. 2). The basement membranes of the capillary walls at this time appeared wholly normal, however, and there were no red blood cells in the spaces of Bowman or in the tubules. Later changes following after repeated injections of the SIO, consisted of marked proliferation of the epithelial cells, with partial fibrosis and atrophy of many of the glomeruli, and siderosis (Fig. 3 and 4). An attempt will be made in the discussion to correlate these structural changes with functional alterations described in the previous sections.

DISCUSSION

The essential observation here reported is that renal lesions involving principally the glomeruli and functional alterations identical with those of the nephrotic syndrome regularly followed repeated intravenous injections of large amounts of saccharated iron oxide into rabbits.

Proteinuria was of prime importance amongst the functional alterations, as the findings made clear. It was always the first such abnormality to become manifest, and under certain conditions it remained the only one. But when the injections of iron were continued, and in consequence the proteinuria became marked and sustained, depletion of the serum albumin regularly followed, and often generalized edema as well, with progressive azotemia and death in some instances. Conversely, when the injections of iron were curtailed the proteinuria diminished, and the serum albumins, if they had become depleted, were usually promptly reconstituted. The cholesterol and alpha globulins of the blood were frequently increased in amount when proteinuria and hypoalbuminemia had been marked for some weeks; but these were always late manifestations, and often they were transitory.

Visible alterations in the glomerular capillaries had an interesting relationship to the onset of proteinuria. For within a few minutes following an injection of the iron preparation intravenously, iron-containing precipitates formed intra-

vascularly, and many of these lodged in the capillary lumina of the glomeruli and remained there for several days, as microscopic observations showed; yet during this period the urine, although occasionally somewhat diminished in volume, remained devoid of protein. The precipitates within the glomerular capillaries as a rule diminished markedly in size and number between the 5th and 7th day following the injection, perhaps by resorption into the blood stream as well as by phagocytosis; at this time proteinuria and signs of damage to the capillary wall, as manifested by pyknosis of endothelial nuclei and margination of leukocytes were initially observed (Fig. 2). These observations suggest that, as the intracapillary precipitates dwindled and disappeared, the blood began again to flow through capillary loops that had previously been occluded, and protein then began to leak in large quantities through their damaged walls. It is plain that the capillaries were not ruptured, however; for there was no sign of hemorrhage into Bowman's space, and the urine, though loaded with protein, was devoid of blood. Furthermore, at the onset of proteinuria, Mallory's and Masson's trichrome stains and the periodic acid–Schiff preparations all failed to disclose visible alterations in the capillary basement membranes. Whether the iron-containing precipitates produced injury to the glomerular capillaries through the agency of anoxia or by some other mechanism cannot now be decided. The iron itself was not wholly responsible for the renal injury, however. For in many instances protein in large amounts passed through glomeruli that contained but little iron pigment (Fig. 2); while, conversely, in some animals given only one injection of the iron preparation, phagocytosed pigment giving a positive histochemical reaction for iron was often found in abundance in the glomeruli long after the proteinuria had subsided. It is clear also that epithelial proliferation and glomerular fibrosis were secondary and later consequences of the injury, for they occurred after the onset of proteinuria and at times persisted after it had disappeared.

Viewed in the large, the glomerular changes in the experiments here reported resemble quite closely those sometimes found in naturally occurring glomerulonephritis with the nephrotic syndrome (13, 14), and also those seen in experimental nephritis induced with nephrotoxic immune serums (15–17). Partial or complete obstruction of many of the glomerular capillaries by "hyaline" or "fibrinoid" material has been reported in each of the conditions enumerated above, and in others as well (18, 19). The present observations emphasize the importance of intracapillary precipitates as a cause of glomerular injury with proteinuria. But they do not disclose the essential nature of the anatomical change in the glomeruli which is responsible for the proteinuria, nor the precise means whereby this is brought about.

SUMMARY

Intravascular precipitates, comprised at least in part of iron, formed regularly in rabbits given one or more injections of a saccharated iron oxide preparation

intravenously, and these lodged in numerous capillaries throughout the body, particularly those of the lungs and kidneys. Large numbers of the brownish precipitates remained in the capillaries of the renal glomeruli during the first few days following injection of the iron, but most of them disappeared after 5 to 7 days, with only moderate amounts of brown pigment remaining in the endothelial cells of the renal glomeruli. Signs of acute injury of the glomerular tufts—namely, pyknosis of some of the endothelial cells, margination of leukocytes within the glomerular capillaries, and slight proliferation of the epithelial cells—also developed some 5 to 7 days following injection of the iron, along with marked proteinuria, which proved transitory if no further injections were given. When the iron preparation was given repeatedly over prolonged intervals, however, the proteinuria persisted and became extreme, and hypoproteinemia developed, often with hypercholesterolemia and transitory edema as well. Histological studies of the kidneys of rabbits manifesting the nephrotic syndrome, as just described, disclosed that virtually all the renal glomeruli were greatly altered, mainly owing to proliferation of the epithelial cells, together with some fibrosis and atrophy. Some of the rabbits having marked proteinuria and other functional changes eventually developed azotemia following repeated injections of the iron, and several of them lost weight and died; the renal glomeruli of these animals showed changes like those just described, but the alterations were more extensive.

Considered together, the findings provide evidence that the intravascular precipitates first occluded the glomerular capillaries for a period of several days following injection of the iron and then largely disappeared from them just prior to the development of morphologic signs of glomerular injury and proteinuria. Hence the possibility was considered that the intracapillary precipitates might have produced acute injury to the walls of the glomerular capillaries through the agency of anoxia. But it is plain that the findings of the present study do not disclose the essential nature of the anatomical change responsible for the proteinuria, or the means whereby this was produced.

The findings as a whole were briefly considered in relation to the pathogenesis of the nephrotic syndrome as it occurs naturally in human beings.

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EXPLANATION OF PLATE 5

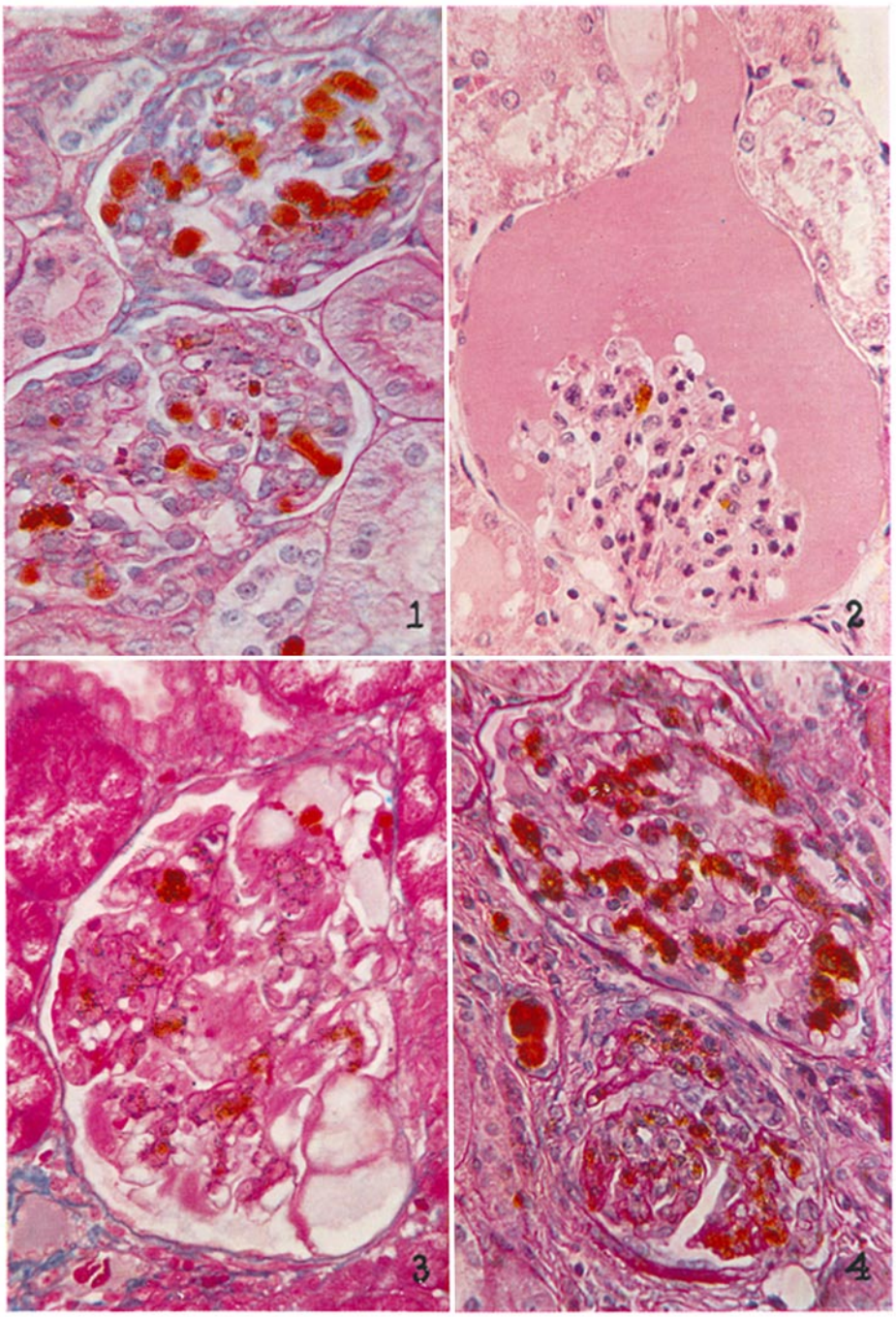
The photographs were made by Mr. Julius Mesiar.

FIG. 1. To show the brown precipitate in the glomerular capillaries of a rabbit given 20 cc. of the saccharated iron oxide preparation intravenously and killed 48 hours later. The precipitate forms casts in many of the glomerular capillaries and is seen in an intertubular capillary as well. Approximately 50 per cent of the glomeruli of this animal contained precipitates such as those shown; these were intensely blue in sections stained with Gomori's ferrocyanide method for iron pigment. The cytoplasm of the cells of the proximal convoluted tubules show hydropic swelling, presumably owing to the sucrose contained in the injected SIO (see text). Periodic acid–Schiff reaction. Hematoxylin counter stain. ×600.

FIG. 2. To show changes in the glomeruli 48 hours after the initial onset of proteinuria. A rabbit was given 20 cc. of SIO intravenously. The urine was devoid of protein for 6 days but on the 7th day it contained 3 gm. of protein, and the rabbit was sacrificed. Bowman's space is dilated and filled with a protein-rich fluid. There is pyknosis of the nuclei of some of the endothelial cells and margination of leukocytes in capillaries. There were no intracapillary precipitates such as are regularly found during the first few days following injection of the SIO (see Fig. 1), but a small amount of brown phagocytosed granular material may be seen. Of the remainder of the glomeruli approximately 25 per cent contained larger masses of the brownish iron precipitate that occluded one or more capillaries. Periodic acid–Schiff reaction. Hematoxylin counter stain. ×450.

FIG. 3. Biopsy of kidney of rabbit with marked proteinuria of 26 days duration. Proliferated epithelial cells envelop the capillaries and many contain hyaline droplets. There is obliteration of Bowman's space adjacent to the vascular pole and collapse of some of the capillary lumina, and marked phagocytosis of brown pigment by endothelial cells. Mallory's trichrome stain. ×450.

FIG. 4. To illustrate the chronic glomerular changes seen in the animal of Chart 2. One glomerulus is small and fibrotic; the other is hypertrophied and shows epithelial proliferation with partial obliteration of Bowman's space. The basement membrane of the capsule is irregularly thickened, while the capillary basement membrane appears essentially unchanged. There is marked endothelial siderosis. Periodic acid–Schiff reaction. Hematoxylin counter stain. ×450.



(Ellis: Iron oxide and the nephrotic syndrome)