Microembolic Renal Disease in Rats Induced with Sephadex

Hypertension, Lesions and Serum Urea Nitrogen

K. Solez, BA and G. W. Richter, MD

Sephadex particles (20–80 μ in size) were injected into the abdominal aorta of 134 male Sprague-Dawley rats near the renal arteries. In 31 rats, the right kidney was then removed. The Sephadex particles lodged in glomerular capillaries, afferent glomerular arterioles and interlobular arteries, creating renal infarcts, some of which were grossly visible. Shortly after injection, arterial blood pressure rose significantly in most animals. The hypertension in uninephrectomized rats was not demonstrably different from that in rats with two kidneys. Severity and duration of hypertension (up to 8 months) were positively correlated with the number of Sephadex particles in renal vessels, and there was also a positive correlation between the degree of hypertension and serum urea nitrogen levels, and between degree of hypertension and degree of cardiac hypertrophy. The vascular permeability in acutely hypertensive rats was abnormal, as judged from penetration of iron-dextran into vessel walls. This experimental model resembles atheromatous microembolic renovascular disease, which may play a significant role in the pathogenesis of unexplained hypertension in patients with advanced aortic atherosclerosis. (Am J Pathol 66:163–188, 1972)

MOORE AND MERSEREAU [1] HAVE SUGGESTED that many cases of so-called essential hypertension may be caused by the lodging in small renal vessels of microemboli that originate from thrombi on atherosclerotic plaques in the aorta or arteries elsewhere. These investigators devised an experimental model in which platelet emboli from a wire placed in the aorta of rabbits produced a hypertensive state and structural changes in the kidneys that resembled essential hypertension and benign nephrosclerosis in man. Alexander, Heptinstall and Pickering [2] produced hypertension in rabbits by injecting small seeds, shrimp eggs and other particles into the left renal artery. Koletsky and Rivera-Velez [3] produced sustained microembolic renal hypertension in rats by means of plastic microspheres. Jorgensen *et al* [4] brought about sustained elevation of blood pressure in rabbits by inducing aggregation of platelets with adenosine

From the Department of Pathology, The University of Rochester Medical Center, Rochester, NY 14642.

Supported in part by grant GM-00133 from the National Institute of General Medical Sciences, National Institutes of Health, Bethesda, Maryland.

Accepted for publication August 3, 1971.

Address reprint requests to Dr. Richter.

diphosphate in the aorta proximal to the renal arteries. It has remained uncertain to what extent the number of embolic particles in renal vessels or the degree of infarction determines the severity of the subsequent hypertension.

The experiments now to be reported were done to obtain more detailed information about the relationship of microembolic renal damage to the development and course of hypertension and its sequelae.

The first paper of this study deals with the production of hypertension and renal disease by injection of Sephadex microspheres into the aorta of rats at the level of the ostia of the renal arteries above a clamp. Special attention is given to the relationship between the number of Sephadex particles found in renal vessels and the time course and severity of the hypertension produced. Since Sephadex microspheres stain very heavily with periodic acid–Schiff (PAS) reagent, they are easily located, counted and measured in histologic sections.

Materials and Methods

Animals

Male rats of the CFE-Sprague Dawley strain (Carworth) weighing 250–350 g were used in these experiments. The animals were housed in separate cages and given Purina rat chow and water *ad libitum*.

Introduction of Sephadex Microemboli

After the animals were anesthetized with ether, a midline abdominal incision was made and the left kidney was freed from surrounding fat and reflected ventrally towards the midline. The abdominal aorta was uncovered by blunt dissection, using sterile cotton-tipped applicator sticks. A small clamp was placed on the aorta a few millimeters caudad to the ostium of the left renal artery. An autoclaved suspension of 6 mg of Sephadex G-25 (particle size 20-80 μ) (obtained from Pharmacia, Ltd, Uppsala, Sweden) in 1.0 ml of sterile 0.9% NaCl solution was injected slowly into the aorta at the level of the ostia of the renal arteries just cephalad to the clamp, retrograde against the flow of blood, using a syringe with a No. 27 needle. Immediately after injection, the clamp was removed and the needle withdrawn. Bleeding was contained by using Gelfoam (The Upjohn Company, Kalamazoo, Mich). The left kidney was returned to its original position, and the midline incision was closed using catgut and clips.

Owing to hemodynamic differences, to variations in the sedimentation of Sephadex in the syringe and to occasional loss of particles outside the aortic lumen, the injections resulted in variable degrees of infarction and ischemic atrophy of both kidneys, usually more severe in the left than in the right kidney. Brownish-yellow areas of ischemic kidney parenchyma were apparent shortly after injection of Sephadex. Occasionally, small incipient infarcts were noted in the adrenals. Infarction of other organs was not observed either at the time of operation or at necropsy.

The operation was performed without modification on 103 rats. In an additional 31 rats, right-sided uninephrectomy was carried out immediately after injection of

Sephadex by transecting the right renal artery and vein between ligatures, dissecting the right kidney free from fat and fascia, and removing it. Sixty-two rats of the former group survived the operation and were sacrificed either 10 days (SI-acute rats) or 4–8 months (SI-chronic rats) later. Twenty-two uninephrectomized rats (USI rats) survived the operation and were sacrificed 2½–6 months later. The time of sacrifice was determined to some extent by the health of the animal, and rats that appeared gravely ill were sacrificed immediately. Operative mortality among the SI groups was in some cases due to anesthesia, whereas all the operative deaths in the USI group occurred several hours to days after the operation, apparently as a result of hemorrhage or acute renal failure.

An additional 30 rats did not receive intrarenal injections of Sephadex. In 12 of these rats (TL rats), the left renal artery and vein were transected between ligatures, thus depriving the left kidney of its main blood supply, but this kidney was left *in situ* in the animal. In 6 animals (U rats), a simple right-sided uninephrectomy was performed. Another 6 rats received extrarenal injections of Sephadex as follows: 2 received 25 mg Sephadex in the left gastrocnemius muscle (SG rats), 2 received 25 mg Sephadex subcutaneously in the back (SS rats) and 2 received 6 mg Sephadex in the right iliac artery (SL rats). In 6 rats (C rats), no operation of any type was done. Table 1 summarizes the various treatments.

Measurement of Blood Pressure

Systolic blood pressure was measured with an inflatable tail cuff, pneumatic pulse transducer and sphygmomanometer after the rats had been placed in a thermostatically controlled warming unit. This equipment is manufactured by Narco Bio-Systems, Inc, Houston, Texas. Each rat was warmed at 38 C to induce vasodilation until tail pulse tracings of suitable magnitude appeared on a polygraph used for recording (usually after 2–5 minutes); then the systolic pressure was determined five times at intervals of 1 minute. Five determinations for each rat

Table 1—The Experimental and Control Groups

Group	No. of rats	Time of sacrifice after operation	Treatment
SI-acute	14	10 days	Intrarenal injection of Sephadex via the aorta
SI-chronic	48	4–8 months	Intrarenal injection of Sephadex via the aorta
USI	22	2½–6 months	Intrarenal injection of Sephadex via the aorta and right uninephrectomy
TL	12	4 months	Left renal artery and vein transected between ligatures
U	6	4 months	Right uninephrectomy
SG	2	5 months	25 mg Sephadex into left gastrocnemius
SS	2	5 months	25 mg Sephadex subcutaneous into back muscle
SL	2	5 months	6 mg Sephadex into leg through iliac artery
C	6	4–8 months	Normal controls
Total No. = 114			

were averaged, and this average was regarded as the blood pressure value for the day. Blood pressures were measured once before the operation (on a total of 164 normal rats), twice a week during the first 2 post-operative weeks, once a week for the following 2 weeks and once every 2 weeks thereafter.

Sacrifice and Preparation of Tissues for Gross and Microscopic Examination

Prior to sacrifice, each rat was anesthetized with diethyl ether. A midline abdominal incision was made. Three milliliters of venous blood were then collected from the inferior vena cava for blood chemical determinations. In order to demonstrate the patency of blood vessels in sections prepared for microscopic examination, 3 ml of iron-dextran (Imferon, Lakeside Laboratories, Milwaukee, Wis) was injected with a syringe into the inferior vena cava. The rats frequently developed tonic-clonic convulsions shortly after injection.

After allowing time for the iron-dextran to course through the renal circulation, the kidneys were excised and the animal was allowed to die by exsanguination. The renal capsules were stripped off, and the kidneys were examined for scarring and then split from pole to pole into anterior and posterior halves with a razor blade. Blocks of renal cortex 1 cu mm in size were fixed in 2% glutaraldehyde in Millonig's buffer[5] at pH 7.3 and 4 C for 3 hours, washed six times in Millonig's buffer during 1 hour, postfixed in 1% OsO_4 in Millonig's buffer and processed for electron microscopy by routine methods, using Luft's Epon embedding (Ladd Research Industries, Inc, Burlington, Vt). The bulk of the kidney tissue, as well as portions of heart, spleen, pancreas, small intestine, adrenal and lung were fixed in Bouin's fluid or in buffered 10% formalin. Sections embedded in paraffin were cut at 4 μ and stained with H&E, PAS, Perls' stain for ferric iron and Weigert–Van Gieson elastic tissue stain.

Determination of Serum Urea Nitrogen

Serum urea nitrogen was determined by the urease rapid colorimetric method of Chaney and Marbach as described by Kaplan,[6] using samples of 0.010 ml of serum. Determinations were done in triplicate.

Observations

Distribution of Sephadex Microspheres

Paraffin sections of each kidney stained with PAS were scanned at 100× magnification for Sephadex particles, and the number and location of such particles in each cross section of kidney were noted. The microspheres stained a very deep red (almost black) with the PAS stain, and were easily located even at low power (Fig 1). In sections stained with Perls' stain and lightly counterstained with basic fuchsin, Sephadex particles appeared as prominent, unstained, highly refractile, regularly shaped, circular bodies. The particles were less prominent in sections stained with H&E or by the Weigert–Van Gieson method, in which they appeared as almost transparent but slightly basophilic bodies that were not easily distinguishable from surrounding structures.

Sephadex particles were most numerous in arterial vessels of the outer renal cortex and were only very rarely seen in those of the medulla or

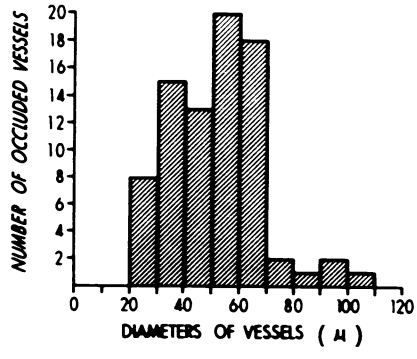
hilar fat. They were occasionally observed in small numbers in the adrenal gland, small intestine and spleen.

Sephadex particles found in two 4-μ sections cut through the middle of each kidney along its long axis were counted, and the average number per section was determined for each rat. For uninephrectomized rats, two sections from the remaining kidney were used. The inside diameter of occluded vessels and the apparent size of the occluding particles were determined with a calibrated occular micrometer. No corrections for planes of sectioning or for possible shrinkage of particles during processing for microscopy were made.

The size distributions of Sephadex particles and of the cross-sectional diameters of occluded vessels did not differ significantly in individual rats. Text-fig 1 shows a histogram of sizes of vessels obstructed in a typical rat. In general, the smallest particles, 20–25 μ in diameter, were situated in glomerular capillaries; slightly larger particles, 25–45 μ in diameter, in the afferent arterioles; and the largest particles, 45–80 μ in diameter, in interlobular and arcuate arteries. Occasionally, several particles obstructed a single vessel (Fig 2). In some instances, Sephadex emboli were observed that appeared considerably smaller than the lumens of the vessels that contained them. This was especially true at points of branching of larger arterioles, where a sudden change in vessel diameter occurred and prevented further progress of the embolus (Fig 3). More frequently, however, vessels appeared markedly distended by the particles within them, which suggested that there was little or no shrinkage of the Sephadex particles during fixation.

Systolic Blood Pressures

The average preoperative blood pressure in 164 normal rats was 120 mmHg with a standard deviation of 8.9 mmHg. The mean of average



TEXT-FIG 1—Histogram of inside diameters of vessels occluded by Sephadex microspheres in a typical rat. Mean diameter is 55.4 μ ±17.9 (SD).

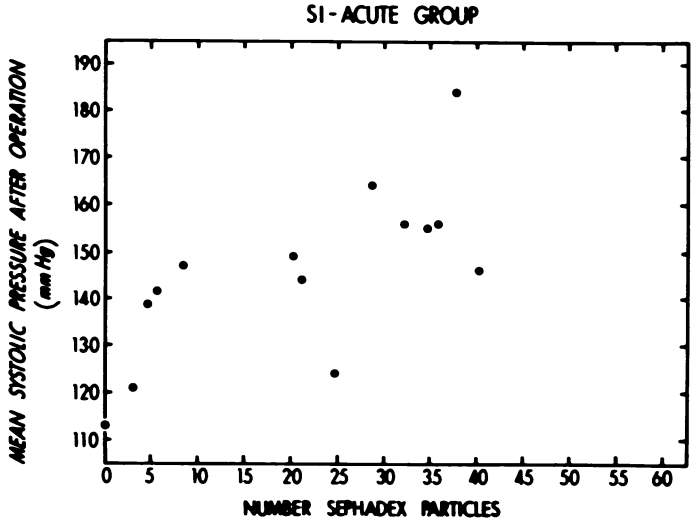
blood pressures of the 18 control rats in groups U, C, SG, SS and SL over a 4–6 month period was 117 mmHg with a standard deviation of 4.5 mmHg. There were no significant differences in systolic pressure among these five groups. Rats with an average systolic pressure of greater than 130 mmHg after operation (>3 SDs above the mean for controls) were regarded as being hypertensive. In computing the duration of hypertension, rats were considered to be hypertensive for that period of time during which blood pressures of greater than 147 mmHg were recorded (>3 SDs above average preoperative blood pressures).

Table 2 shows the distribution of hypertensive and normotensive rats in various groups, on the basis of these criteria. It is of interest that 2 rats in the group in which the left renal artery and vein were doubly ligated and cut (TL rats) became hypertensive, 1 markedly so (peak systolic pressure 178, average postoperative pressure 145).

Text-fig 2 to 4 depict the relationship between the average postoperative systolic blood pressure and the average number of Sephadex microspheres in longitudinal sections of kidneys stained with PAS, from the SI-acute, SI-chronic and USI groups. Although the points in these graphs are widely scattered, they indicate that the presence of more than a few Sephadex particles in the vessels was, as a rule, associated with definite systolic hypertension and that, in the SI-acute and SI-chronic groups, a significant positive correlation existed between blood pressure and number of embolic particles. The correlation coefficient (r) for a linear regression of blood pressure (y) on the number of Sephadex particles per kidney section (x) was +0.5307 for the SI-chronic group ($P < 0.001$) and

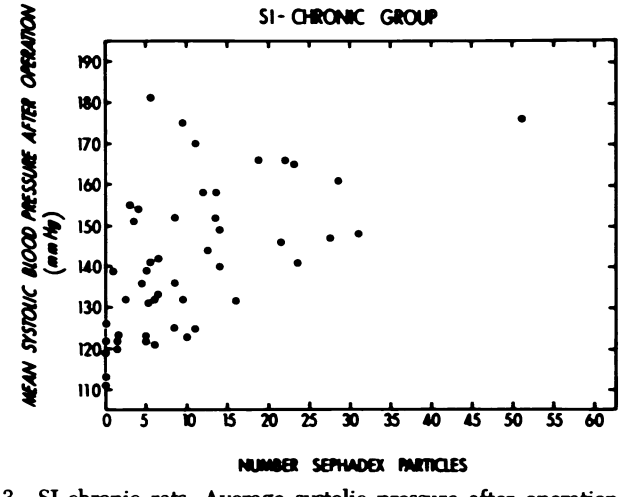
Table 2—Distribution of Hypertensive and Normotensive Rats in Experimental and Control Groups According to the Number of Sephadex Particles per Kidney Section

Group	Sephadex particles/ kidney section	Hypertensive		Normotensive		Total
		No.	%	No.	%	No.
SI-acute and SI-chronic	0–5	8	43	11	57	19
	5.5–10	13	81	3	19	16
	10.5–20	11	100	0	0	11
	20.5–51	15	94	1	6	16
USI	0–5	5	42	7	58	12
	5.5–10	2	50	2	50	4
	10.5–20	1	33	2	66	3
	20.5–51	3	100	0	0	3
TL	(0)	2	17	10	83	12
U, SG, SS, SL and C	(0)	0	0	18	100	18
						Total No. = 114

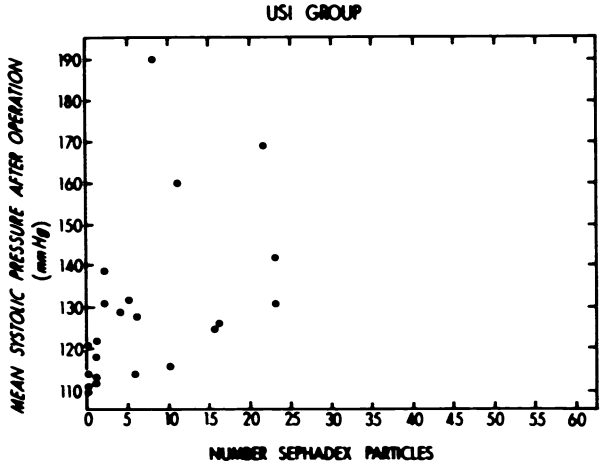


TEXT-FIG 2—SI-acute rats. Average systolic pressure after operation versus number of Sephadex particles per kidney section.

+0.6189 for the SI-acute group ($P < 0.02$). The correlation in the USI group ($r = +0.2798$) was not significant ($P > 0.1$), but the failure of animals in this group with more than 25 Sephadex particles per section to survive the operation resulted in an inadequate sample. The scattering of points in the graphs is unrelated to the different times of sacrifice and presumably represents a combination of sampling error and real variation in hypertensive response to the same number of Sephadex parti-



TEXT-FIG 3—SI-chronic rats. Average systolic pressure after operation versus number of Sephadex particles per kidney section.



TEXT-FIG 4—USI rats. Average postoperative systolic pressure versus number of Sephadex particles. Mortality of uninephrectomized rats with >25 Sephadex particles per kidney section was 100%.

cles. No definite differences in hypertensive response in the three groups of hypertensive rats are evident in the graphs, but it is of interest that the presence of more than 25 Sephadex particles per kidney section is apparently incompatible with life in uninephrectomized rats, whereas up to 50 Sephadex particles (2×25) are tolerated by rats with two kidneys. Rats with more than these numbers of Sephadex particles per section (25 or 50, respectively) survived no longer than a day or two after operation and are therefore not included in the graphs.

The time course of the hypertension was quite variable. Eighteen rats of the SI-chronic and USI groups developed stable hypertension which persisted until the time of sacrifice. While most rats in which sections of kidneys revealed large numbers of Sephadex particles had sustained hypertension, 3 such rats developed heart failure with irregular pulse and wheezing respiration and became normotensive or hypotensive. In cases in which sections showed only a moderate number of Sephadex particles, the rats often had had only transient hypertension after operation or had had wide fluctuations in blood pressure. The time course of Sephadex-induced hypertension will be discussed in the second paper of this study in relation to changes in excretion of urine.

Table 3 shows the relationship between persistence of hypertension (number of nonconsecutive or consecutive weeks in which systolic pressure had been above 147 mmHg) and the average number of Sephadex spheres observed in PAS-stained sections of kidneys in *hypertensive* animals of the SI-chronic and USI groups sacrificed 4–6½ months after oper-

Table 3—Relation Between Number of Sephadex Particles and Duration of Hypertension in Hypertensive Animals Sacrificed 4–6 Months after Operation

Sephadex particles/ kidney section	Average duration of hypertension (weeks ± SD)	No. of animals‡
2.0–5	5.3 ± 4.0	7
5.5–10	7.2 ± 7.8	13
10.5–20	8.2 ± 4.4	9
20.5–51	10.1 ± 3.6	9

* In this table, the blood pressure determined for a given rat on one occasion is regarded as that rat's blood pressure for the period between that measurement and the next, and so on.

‡ Includes only hypertensive rats of the SI-chronic and USI groups.

ation. The hypertension in the group with 20.5–50 Sephadex particles per section persisted significantly longer ($P < 0.02$) than that in the group with two to five Sephadex particles per section. Overall, there appears to be a positive correlation between the number of microspheres per section and duration of hypertension, although this is not statistically significant ($P > 0.1$).

Renal Function: Serum Urea Nitrogen

Table 4 shows the relationship between the average systolic blood pressure in the experimental rats after operation and serum urea nitrogen (SUN) values at time of sacrifice.

As can be seen from Table 4, the impairment of renal function in the hypertensive rats as reflected by increased SUN is related to blood pressure and tends to be greater in the chronic groups (SI-chronic and USI) than in the acute group (SI-acute), and greater in the uninephrectomized group injected with Sephadex (USI) than in the corresponding group of rats with both kidneys intact (SI-chronic). The association of hypertension with elevated SUN was not an obligatory one, since a few hypertensive rats had normal SUNs and some normotensive rats (especially those in the USI group) had moderately elevated SUNs (20–30 mg%). The positive correlation between mean systolic blood pressure after operation and serum urea nitrogen at the time of sacrifice was significant in all three groups ($P < 0.01$ for the SI-chronic group, $P < 0.02$ for the SI-acute group and $P < 0.001$ for the USI group).

Cardiac Hypertrophy

At the time of sacrifice, the heart of each rat was removed and weighed intact before blocks were taken for sections. Ratios of this weight to the weight of the whole animal before sacrifice were calculated. Table 5

Table 4—Serum Urea Nitrogen in Control and Experimental Groups, Subdivided by Mean Systolic Blood Pressure after Operation

Group	Mean systolic BP (mmHg)	Mean SUN* (mg/100 ml ± SD)	Range	No. of rats
S, SG, SL, U, SS and TL†		16.0±2.1	12–24	30
SI-chronic	110–130	18.0±4.3	12–25	13
SI-chronic	131–150	21.9±6.3	12–32	21
SI-chronic	151–190	38.0±38.2	14–108	14
SI-acute	110–130	14.2±1.4	13–15	3
SI-acute	131–150	21.9±8.0	16–39	6
SI-acute	151–190	29.1±8.1	20–42	5
USI	110–130	20.0±4.2	15–29	14
USI	131–150	25.6±7.4	17–37	4
USI	151–190	114.6±71.9	24–205	3

* SUN = serum urea nitrogen at time of sacrifice.

† There were no significant differences between the SUN values in these groups.

relates cardiac hypertrophy (heart weight and heart weight/body weight) to the severity of hypertension in each group of rats. Except in the SI-acute group (sacrificed 10 days after operation), the severity of hypertension is positively correlated with cardiac hypertrophy (heart weight/body weight) ($P < 0.001$ for the SI chronic group; $P < 0.04$ for USI group).

Gross Pathologic Findings

At the time of sacrifice 10 days after operation, the kidneys of many rats in the SI-acute group showed gray, wedge-shaped subcapsular areas of infarction involving as much as 12 mm of continuous cortical surface on cross section (Fig 4). In the SI-chronic and USI groups sacrificed 4–8 months after operation, depressed gray-white scars with dystrophic calcification of renal parenchyma were frequently observed, and often the renal capsule adhered firmly to the cortex. No such lesions were noted in control animals. In animals of the SI-acute and SI-chronic groups, infarcts in the left kidney tended to be more extensive than in the right.

A curious finding was the frequent absence of obvious infarcts or other lesions when the kidneys of hypertensive rats of the USI, SI-acute and SI-chronic groups were grossly inspected at necropsy. The kidneys of 17 rats in the SI-chronic group with moderate hypertension (average systolic pressure after operation: 150–170 mmHg) and with up to 12 Sephadex emboli per histologic section, appeared grossly normal at necropsy with a nonadherent capsule and without noticeable scars. Eight of these animals had sustained hypertension until the time of sacrifice.

All uninephrectomized rats showed hypertrophy of the remaining kid-

Table 5—Cardiac Hypertrophy and Blood Pressure

Group	Mean systolic BP after operation (mmHg)	Mean heart wt / Mean body wt (±SD) × 10^{+5}	Mean heart wt* (g ± SD)	No. of rats
U, SG, SS, SL and TL†	110–130	309±39	1.340±.151	28
SI-chronic	110–130	328±41	1.333±.167	13
SI-chronic	131–150	342±42	1.403±.154	21
SI-chronic	151–190	495±135	1.713±.270	14
SI-acute	110–130	344±55	0.920±.136	3
SI-acute	131–150	375±62	1.096±.168	6
SI-acute	151–150	361±63	1.023±.093	5
USI	110–130	306±48	1.149±.243	14
USI	131–150	319±38	1.120±.159	4
USI	151–190	482±92	1.790±.206	3

* Lower heart weights in SI-acute group reflect the younger age of these rats at time of sacrifice.

† The 2 hypertensive TL rats are not included in this table.

ney. In all TL rats, the left kidney, which had had its main blood supply interrupted, was a shrunken, calcified mass at necropsy and defied ordinary microtomy. With a magnifying glass, vascularity of the superficial renal cortex could be discerned in some cases, probably the result of collateral capsular circulation.

In 10 rats of the USI and SI-chronic groups, the presence of propagating arterial thrombi provided evidence of embolic occlusion of part of the mesenteric arterial circulation. However, this situation did not result in grossly visible intestinal infarcts or in intestinal perforation.

Small calcified infarcts were occasionally observed in one adrenal, usually the left. Bilateral infarction or complete necrosis of either adrenal was not observed.

Microscopic Observations

In the SI-acute group sacrificed 10 days after operation and in those rats that died a few days after operation, numerous well-demarcated wedge-shaped areas of atrophic parenchyma with incomplete or complete infarction were frequently observed in the superficial renal cortex (Fig 4). These areas often consisted of a central dead zone with necrotic glomeruli and tubules which exhibited pale-staining cytoplasm and loss of nuclei, and a marginal zone, similar to that described by Alexander *et al*,[2] where the cytoplasm of tubule cells was more eosinophilic and where there was a greater number of persisting nuclei (Fig 5). In the USI and SI-chronic groups sacrificed 3–8 months after operation,

these atrophic areas, which had developed extensive interstitial fibrosis, were less sharply set off from intervening areas of more normal parenchyma, except in cases where dystrophic calcification of old infarcts was found (Fig 6).

The persistence of Sephadex in the tissues and the evident resistance of these particles to resorption *in situ* in the animal is remarkable. In both the acute and chronic groups of rats, there was a striking lack of cellular infiltrative inflammatory response around the Sephadex beads lodged in the renal arterioles. The Sephadex particles were usually round and uniformly stained, with smooth, sharply demarcated edges, even in animals sacrificed 8 months after operation (Fig 7). Glomeruli with Sephadex particles lodged in afferent arterioles or in glomerular capillaries were still identifiable after 8 months (Fig 8). Complete fibrous obliteration of such glomeruli was seldom seen, although epithelial crescents and thickening and reduplication of the capsular basement membrane were frequently observed. In some cases in which Sephadex particles occluded afferent arterioles, there was focal necrosis of glomerular tufts, yet glomeruli containing a Sephadex embolus within a glomerular capillary often showed only minimal changes, with good perfusion of other portions of these glomeruli, as evidenced by the presence of red blood cells and/or iron-dextran tracer in capillary loops (Fig 9). The presence of the iron-dextran tracer in glomerular capillary loops was demonstrated with the Prussian blue reaction and by electron microscopy. The electron-dense, iron-dextran particles, 20–100 Å in greatest diameter, were obvious in perfused capillaries (Fig 10).

Interlobular and arcuate arterioles occluded by Sephadex beads developed striking medial and intimal hypertrophy and periadventitial fibrosis which greatly increased the apparent size of the vessels and, together with ischemic atrophy of the intervening parenchyma, gave the appearance of an unusual concentration of large arterioles in a small area (Fig 11).

In the SI-chronic and USI rats with sustained hypertension, there was marked thickening of the walls of afferent renal arterioles, and discontinuity and reduplication of the inner elastic lamina of larger arterioles together with medial and intimal thickening and marked narrowing of the arteriolar lumina (Fig 12). Tubular atrophy and interstitial fibrosis were prominent. Some tubules became markedly dilated (Fig 1 and 11) and many contained "colloid" casts. Glomerular basement membranes were irregularly thickened, and there was diffuse glomerular fibrosis, which in some areas completely obliterated glomerular tufts. Interstitial lymphocytic infiltration of renal parenchyma similar

to that seen in chronic pyelonephritis was observed in some rats (Fig 7 and 12).

Medial and intimal thickening of arterioles in organs other than the kidneys, often with associated reduplication and discontinuity of the inner elastic lamina, was observed in the heart, pancreas and periadrenal fat of 12 hypertensive rats (Fig 13 and 14). Evidence of myocardial necrosis and pulmonary edema was noted in a few hypertensive rats, with necrosis of cardiac muscle fibers, scarring in the myocardium and edema fluid and "heart-failure" cells in pulmonary alveoli.

In sections stained with Perls' stain for ferric iron and lightly counterstained with basic fuchsin, iron-dextran injected intravenously at the time of sacrifice could be seen in many kinds of vessels, including many glomerular tufts. A mixture of apparently unperfused and perfused cortical glomeruli was found in control animals as well as in animals of the SI-acute, SI-chronic, and USI groups. However, "unperfused" glomeruli, which did not contain iron-positive material, were more numerous in animals of the latter three groups, and in these animals obstructing Sephadex emboli were frequently observed in unperfused areas.

In hypertensive SI-acute animals, iron-dextran was sometimes diffusely spread throughout extensively infarcted areas. Elsewhere in the kidneys of these animals, iron-dextran could be seen in the intima and media of small arterioles and venules, suggesting abnormal permeability of these vessels. Penetration of iron-dextran into intima and media of arterioles and venules also occurred in the pancreas and in other organs of the SI-acute rats, but was most striking in the kidneys. The musculoelastic branch pads seen jutting into the lumen at sites of branching of interlobular and arcuate arterioles in SI-acute rats contained a great deal of iron-positive material, suggesting that these pads were more permeable than other areas of the vessel wall (Fig 4). Attention has recently been drawn to enlargement of these branch pads in experimental hypertension produced in rats by chronic ingestion of salt.[7] Iron-positive material was not demonstrated in the walls of vessels of rats in other groups, which may indicate that abnormal vascular permeability in hypertensive rats is more marked at 10 days than it is several months after operation.

Discussion

The findings demonstrate that both severity and duration of microembolic renovascular hypertension are positively correlated with the number of embolic particles in the renal circulation. A plot of average

systolic pressure versus average number of embolic particles encountered in cross sections of kidneys in this experiment bears a marked resemblance to a plot of blood pressure versus "renal contraction" secondary to narrowing of one renal artery, published by Swales and Blake.[8] There is similar scatter and nonlinearity in both plots. This suggests that "renal contraction" and "average number of embolic particles observed" are different indices of the same basic parameter, perhaps renal ischemia.

While sustained hypertension after embolic renal infarction in experimental animals has been described by other authors,[1,3,4] Alexander *et al*[2] observed only transient elevations in blood pressure in a careful study of embolic renal disease in rabbits. Most cases of embolic renal infarction in man are recognized only at autopsy,[9] which makes it difficult to relate the embolic event to the clinical course of hypertension. In those few reports in which the embolic event was recognized clinically, and in which accurate blood pressure records were kept, hypertension was usually transient,[9–11] although several patients have been described who were markedly hypertensive up to the time of death 1–3½ months after the embolic episode.[10,12,13]

Arnold *et al*[14] have attributed the variable hypertensive response in patients with renal infarction in part to inherent individual differences in vascular response, but have pointed out that this in itself is not a sufficient explanation. The present study, which demonstrates that the severity and duration of the hypertensive state is a function of the number of embolic particles, suggests that embolic renovascular hypertension may be either transient or sustained, depending on the magnitude of the embolic shower.

The fact that only 2 of the 12 TL rats became hypertensive supports the thesis that sudden complete ischemic necrosis of one kidney does not cause hypertension.[15] The hypertension observed in 2 TL rats was possibly due to the presence of collateral circulation from the renal capsule which kept the superficial cortex viable, though ischemic, and allowed for revascularization.

The positive correlation between elevated serum urea nitrogen and elevated blood pressure—with some exceptions—suggests that impaired renal function and hypertension are related, but that the former is not the direct cause of the latter.

No significant difference between the range of hypertensive response of uninephrectomized and non-uninephrectomized rats was found in these experiments. If a difference did exist, it must have been obscured by the variable responses within each group or by the high mortality in the USI group. Dahl and co-workers[16] have developed inbred lines of

Sprague-Dawley rats with markedly different susceptibilities to Goldblatt clamp or salt-induced hypertension. It is possible that similar genetic variation in susceptibility to renovascular microembolic hypertension exists. While the CFE-SD rats used in the present study are similar to each other in many ways, possible differences in the responses of uninephrectomized and non-uninephrectomized rats could be elucidated more clearly with littermates or with very highly inbred groups of rats.

It is noteworthy that in the present study little cellular inflammatory reponse to the Sephadex particles was observed in histologic sections. Dixon *et al* [17] have described pulmonary "Sephadex granulomas" with multinucleate giant cells, epithelial cells, histiocytes, lymphocytes and plasma cells after intravenous injections of Sephadex G-25.

The fact that most of the Sephadex particles were observed in vessels of the outer renal cortex and that almost none were found in the medulla is consistent with the heterogeneity of renal blood flow demonstrated by Thorburn *et al* [18] and recently confirmed by McNay and Abe.[19,20] In the experiments of the latter two investigators, the renal medulla of dogs received less than 1.3% of total renal blood flow. These investigators and others [21] have demonstrated that the distribution in the kidney of radioactive plastic microspheres, up to 35 μ in size, is a good measure of relative blood flow in different zones of the renal cortex and medulla.

The observation that uninephrectomized rats, with half the normal functioning renal mass could withstand only half as many Sephadex particles as normal rats suggests that immediate postinjection mortality is related both to the number of emboli injected into the renal circulation and to the amount of functioning renal tissue originally present. Koletsky, Pavlicko and Rivera-Velez [22] found that in rats one kidney could be subjected to severe renal ischemia by reducing renal artery pressure to 20–30 mmHg as long as the other kidney was kept *in situ*. Uninephrectomized rats, in contrast, could tolerate only a moderate reduction in renal arterial pressure, to levels of not less than 60 mmHg, without becoming uremic.

The presence of well-perfused and poorly-perfused glomeruli existing side by side in the renal cortex of normal rats, as demonstrated by the presence or absence of iron-dextran tracer within the glomerular capillaries may simply reflect a normal variation in blood flow in neighboring arterial territories in the kidney, of the sort recently postulated by Bradley.[23] Alternatively, it may be a consequence of vascular spasm with resulting patchy ischemia produced by the injection of the hyperosmolar, acidic (pH 5.2–6.0) iron-dextran solution into the circulation.

The observation that arterial and arteriolar walls in acutely hypertensive rats sacrificed 10 days after operation were permeable to iron-dextran particles confirms the abnormal vascular permeability in acute hypertension found by others [24-28] who used different vascular tracers. Abnormal permeability of arteriolar branch pads, observed in this study, has not been described previously.

The fact that chronic microembolic renovascular hypertension can exist in rats in the absence of grossly evident renal scars raises the possibility that in man atherosclerotic embolic renal disease with secondary hypertension may, at times, be confused with benign nephrosclerosis secondary to essential hypertension. In one large autopsy series, the incidence of cholesterol-atheromatous emboli observed in the kidney (presumably from atherosclerotic plaques in the aorta) in males over 50 years of age was 4.7%.[29] Although the ultimate fate of such emboli is uncertain, there is evidence that, unlike Sephadex emboli which remain intact in vessels, cholesterol emboli may be extruded through vessel walls,[29] or disappear altogether.[30] The traces of such emboli could easily be overlooked in routine autopsy or biopsy sections [31,32] or be confused with polyarteritis,[33] and it is possible, therefore, that cholesterol embolization to the kidney is a more common event than is generally appreciated.

Moore *et al* [1,34-36] feel that cholesterol emboli are a relatively late complication of aortic atherosclerosis, but that renal platelet emboli discharged from the surface of atherosclerotic plaques in the aorta occur earlier and much more frequently. These platelet emboli, analogous to the "white plugs" sometimes seen in the fundi of patients with transient retinal ischemia,[37-39] are seldom seen in autopsy or kidney biopsy sections, but the previous presence of such emboli in the renal circulation could account for both the hypertension and the fine cortical scarring of the kidneys frequently observed in patients with advanced aortic atherosclerosis.[1,34]

It is difficult to ascribe most cases of "essential hypertension" to microembolic phenomona, but it seems probable that microembolic renovascular disease plays a significant role in the pathogenesis of unexplained hypertension in some patients with severe aortic atherosclerosis. In the experiments here reported, a single large shower of emboli resulted in a rapid rise in blood pressure over a period of a few days. Recurrent embolic showers of smaller magnitude might result in the more gradual rise in blood pressure characteristic of essential hypertension in man.

References

1. Moore S, Mersereau WA: Microembolic renal ischemia, hypertension, and nephrosclerosis. Arch Pathol 85:623–630, 1968
2. Alexander N, Heptinstall RH, Pickering GW: The effects of embolic obstruction of intrarenal arteries in the rabbit. J Pathol Bacteriol 81:225–237, 1961
3. Koletsky S, Rivera-Velez JAM: Renin-angiotensin system in microembolic renal hypertension. Arch Pathol 85:1–9, 1968
4. Jorgensen L, Glynn MF, Hovig T, Morphy EA, Buchanan MR, Mustard JF: Renal lesions and rise in blood pressure caused by adenosine diphosphate-induced platelet aggregation in rabbits. Lab Invest 23:347–357, 1970
5. Millonig G: Further observations on a phosphate buffer for osmium solutions in fixation, Electron Microscopy. Vol 2. Edited by SS Bruse. New York, Academic Press Inc, 1962, p 8
6. Kaplan A: Urea nitrogen and urinary ammonia, Standard Methods of Clinical Chemistry. Vol 5. Edited by S Meites. New York, Academic Press Inc, 1965, pp 245–256
7. Jaffe D, Sutherland LE, Barker DM, Dahl LK: Effects of chronic salt ingestion: morphologic findings in kidneys of rats with differing genetic susceptibilities to hypertension. Arch Pathol 90:1–16, 1970
8. Swales JD, Blake J: The relation between blood pressure level and ischaemic renal contraction in the rat. J Pathol 100:149–154, 1970
9. Heptinstall RH: Pathology of the Kidney. Boston, Little, Brown and Company 1966, pp 193–233
10. Fishberg AM: Hypertension due to renal embolism. JAMA 119:551–553, 1942
11. Ben-Asher S: Hypertension caused by renal infarction. Ann Intern Med 23: 431–436, 1945
12. Hallan JA Jr, Radigan LR, Morrow AG: Hypertension due to segmental infarction of the kidney. Am J Med 22:303–305, 1957
13. Prinzmetal M, Hiatt N. Tragerman LJ: Hypertension in a patient with bilateral renal infarction: Clinical confirmation of experiments in animals. JAMA 118:44–46, 1942
14. Arnold MW, Goodwin WE, Colston JAC: Renal infarction and its relation to hypertension. Urol Survey 1:191–219, 1951
15. Loomis D: Hypertension and necrotizing arteritis in the rat following renal infarction. Arch Pathol 41:231–268, 1946
16. Dahl LK, Knudsen KD, Heine M, Leitl G: Effects of chronic excess salt ingestion: genetic influence on the development of salt hypertension in parabiotic rats: evidence for a humoral factor. J Exp Med 126:687–699, 1967
17. Dixon AC, Glen GC, Burham WA, Owen TL, Goldstein JD: The pathologic changes associated with the intravenous use of Sephadex. Am J Clin Pathol 52:587–592, 1969
18. Thorburn GD, Kopald HH, Herd JA, Hollenberg M, O'Morchoe CCC, Barger AC: Intrarenal distribution of nutrient blood flow determined with Krypton in the anesthetized dog. Circ Res 13:209–307, 1963
19. McNay, JL, Abe Y: Pressure-dependent heterogeneity of renal cortical blood flow in dogs. Circ Res 27:571–587, 1970

20. McNay JL, Abe Y: Redistribution of cortical blood flow during renal vasodilatation in dogs. Circ Res 27:1023–1032, 1970
21. Slothoff LM, Logan A, Jose P, D'Avella J, Eisner GM: Microsphere measurement of intrarenal circulation of the dog. Circ Res 28:158–166, 1971
22. Koletsky S, Pavlicko KM, Rivera-Velez J: Renin-angiotensin activity in hypertensive rats with a single ischemic kidney. Lab Invest 24:41–44, 1971
23. Bradley SE: Renal microvasculatures and microenvironments. N Engl J Med 284:498–499, 1971
24. Esterly JA, Glagov S: Altered permeability of the renal artery of the hypertensive rat: an electron microscope study. Am J Pathol 43:619–638, 1963
25. Giese J: Acute hypertensive vascular disease. 2. Studies on vascular reaction patterns and permeability changes by means of vital microscopy and colloidal tracer technique. Acta Pathol Microbiol Scand 62:497–515, 1964
26. Jelinek H, Hagy Z, Hüttner I, Balint A, Kocze A, Kerenyi T: Investigations of the permeability changes of the vascular wall in experimental malignant hypertension by means of a colloidal iron preparation. Br J Exp Pathol 50: 13–16, 1969
27. Wiener J, Lattes RG, Meltzer BG, Spiro D: The cellular pathology of experimental hypertension. IV. Evidence for increased vascular permeability. Am J Pathol 54:187–207, 1969
28. Giacomelli F, Weiner J, Spiro D: The cellular pathology of experimental hypertension. V. Increased permeability of cerebral arterial vessels. Am J Pathol 59:133–160, 1970
29. Gore I, McCombs HL, Lindquest RL: Observations on the fate of cholesterol emboli. J Atheroscler Res 4:527–535, 1964
30. Otken LB: Experimental production of atheromatous embolization. Arch Pathol 68:685–689, 1959
31. Kassirer JP: Atheroembolic renal disease. N Engl J Med 280:812–818, 1969
32. Zac FG, Elias K: Embolization with material from atheromata. Am J Med Sci 218:510–515, 1949
33. Richards AM, Eliot RS, Hanjuh VI, Bloemendaal RD, Edwards JE: Cholesterol embolism: A multiple system disease masquerading as polyarteritis nodosa. Am J Cardiol 15:696–707, 1965
34. Moore S: The relation of superficial cortical scars of the kidney to aortic atherosclerosis. J Pathol Bacteriol 88:471–478, 1964
35. Moore S, Mersereau WA: Platelet emboli and renal ischemia. J Pathol Bacteriol 90:579–588, 1965
36. Sieniewicz DJ, Moore S, Moir FD, McDade DF: Atheromatous emboli to the kidneys. Radiology 92:1231–1240, 1969
37. Retan JW, Miller RE: Microembolic complications of atherosclerosis. Arch Intern Med 118:534–545, 1966
38. Fisher CM: Observations of the fundus oculi in transient monocular blindness. Neurology 9:333–347, 1959
39. Ross Russell RW: The source of retinal emboli. Lancet 2:789–792, 1968

Presented in part at the Fifty-Fifth Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, Ill, April 12–17, 1971.

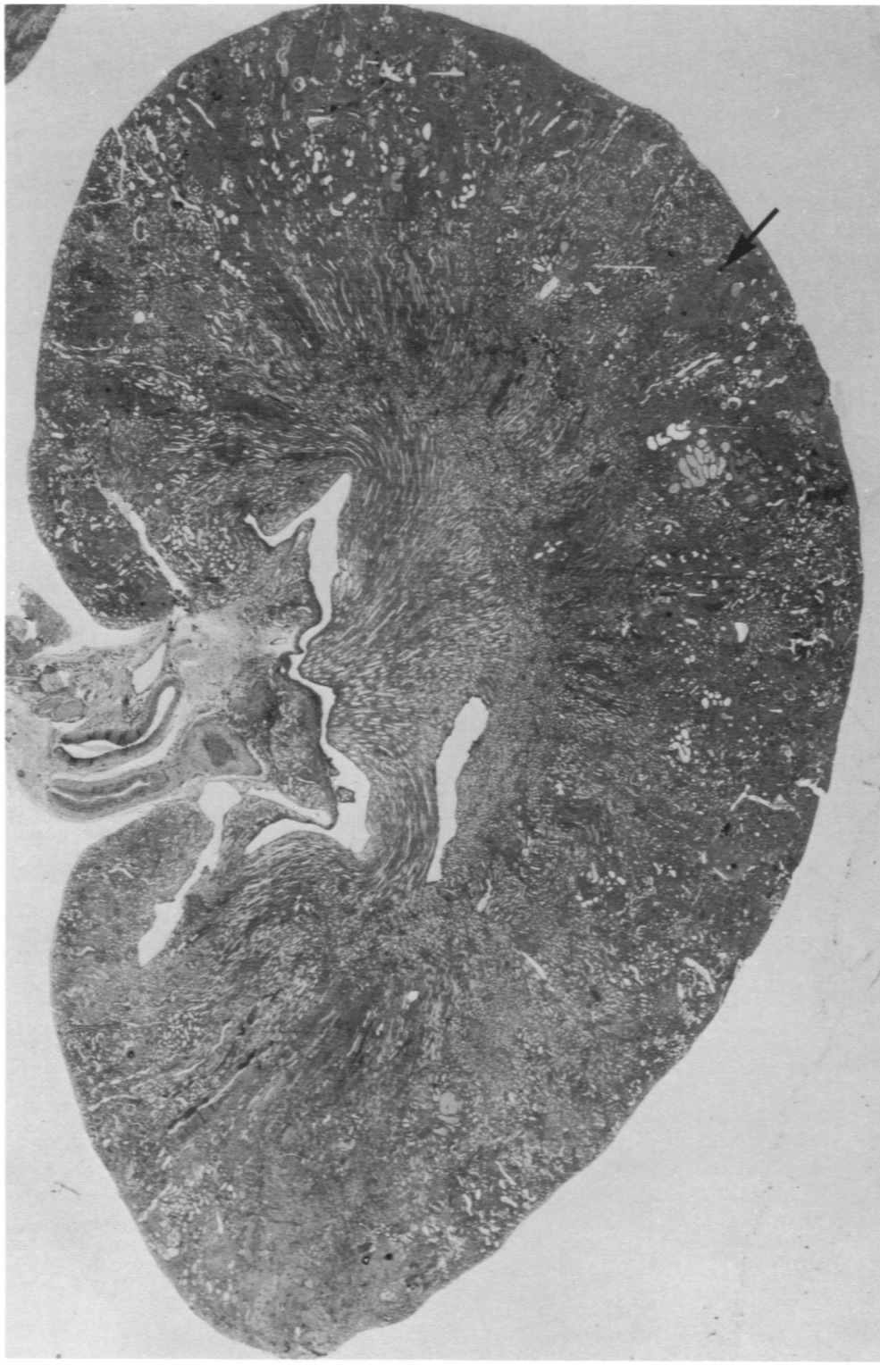


Fig 1—A midsagittal section of a kidney from an animal sacrificed 10 days after Sephadex was injected. Even at this low magnification, the darkly staining Sephadex practicles (*arrow*), dilated tubules, and subcapsular infarcts may be readily seen (PAS, × 10).

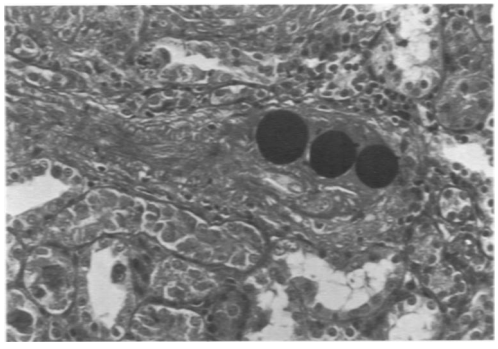


Fig 2—Three spheres lodged in an interlobular artery in an animal sacrificed 10 days after operation (PAS, × 180).

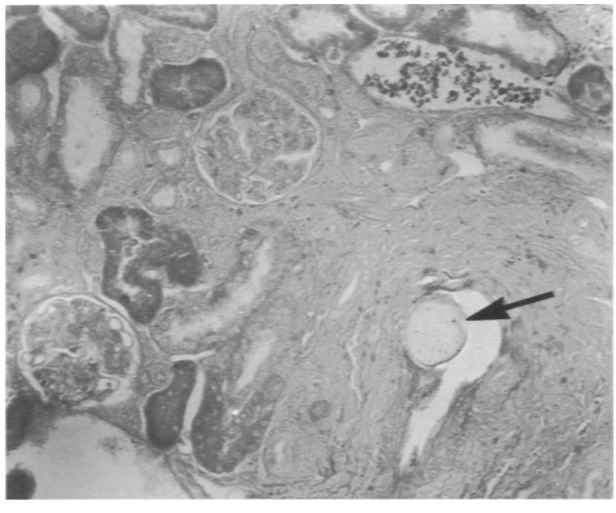


Fig 3—A Sephadex particle (*arrow*) caught in an arcuate artery where the latter branches into interlobular arteries. On the original slide, it could be seen that the hypertrophic musculoelastic branch pad and the intima of the interlobular artery stained heavily for iron, indicating permeability to iron-dextran. The glomerulus in the upper left-hand corner is totally unperfused. The rat from which this section was taken had an average systolic pressure of 164 mmHg after operation, and was sacrificed on the tenth postoperative day. (Perls' stain for ferric iron lightly counterstained with basic fuchsin, × 180).

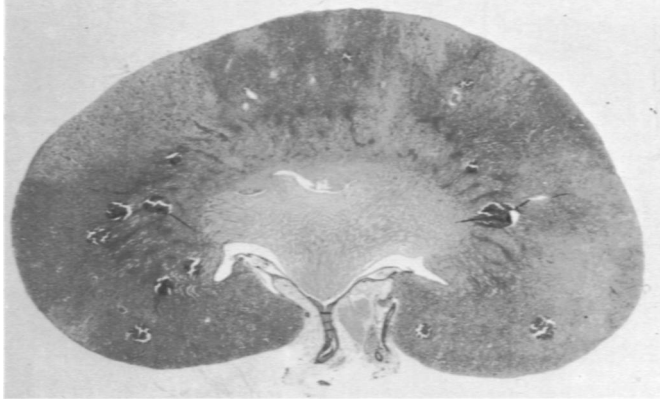
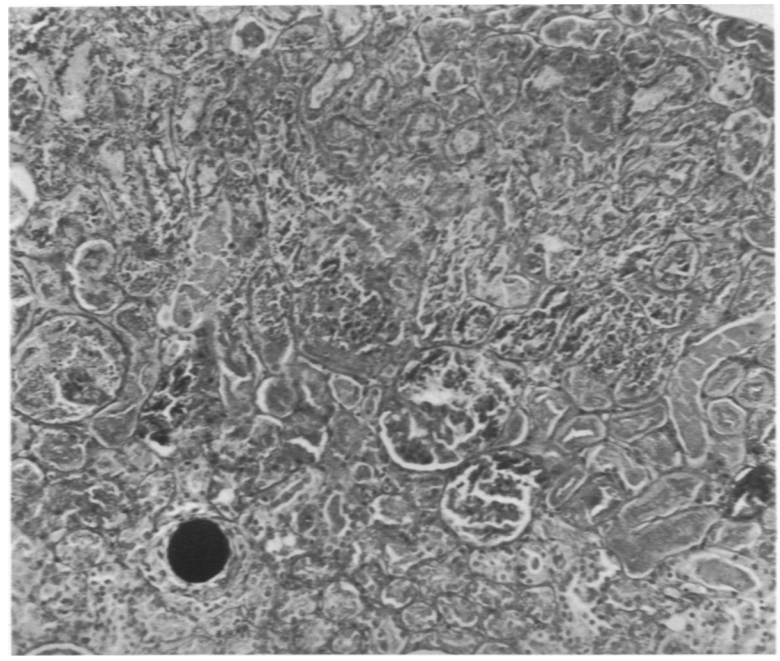
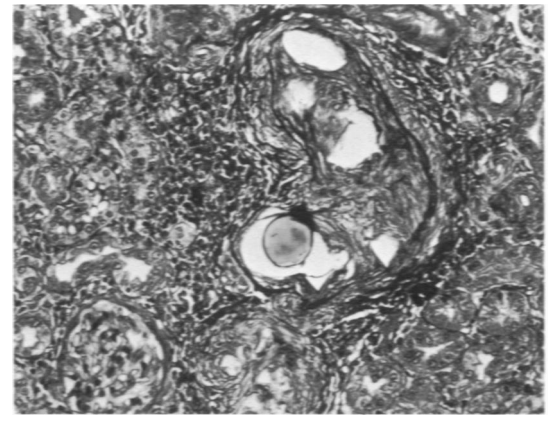


Fig 4—Acute infarcts in an animal that died 4 days after Sephadex was injected (H&E, × 4).



5

6



7

Fig 5—Microscopic detail of an acute infarct in an animal that died 6 days after Sephadex was injected, showing the central dead zone, the marginal zone, and the zone of relatively intact parenchyma in which a Sephadex sphere is seen. (PAS, × 100). **Fig 6**—Extensive old infarcts with dystrophic calcification in a hypertensive rat (average systolic pressure, 180 mmHg) sacrificed 3 months after injection (H&E, × 30). **Fig 7**—Intact Sephadex particle in a renal arteriole 8 months after injection. There is a moderate amount of chronic inflammatory infiltration around the arteriole and within it is an organized, recanalized thrombus (Weigert–Van Gieson stain, × 180).

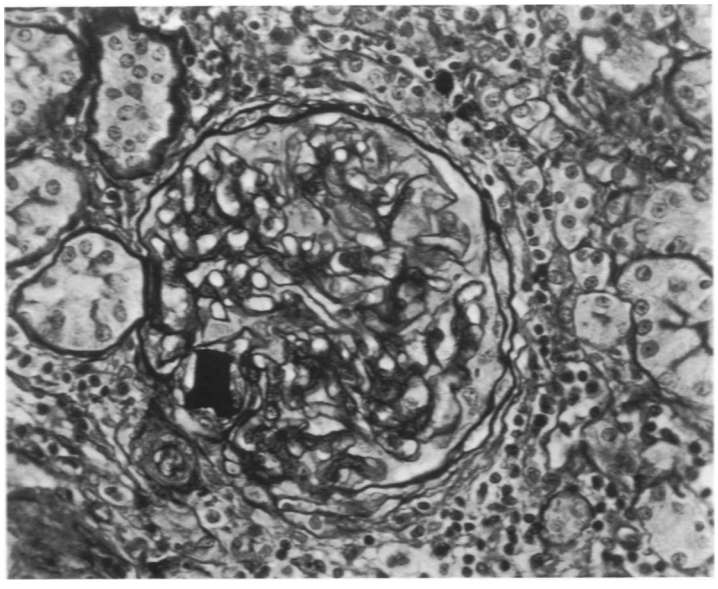


Fig 8—Glomerulus with its afferent arteriole partially occluded by an unusual polyhedral Sephadex particle. Epithelial crescent and reduplication of the capsular basement membrane are seen here 8 months after operation (PAS, × 380).

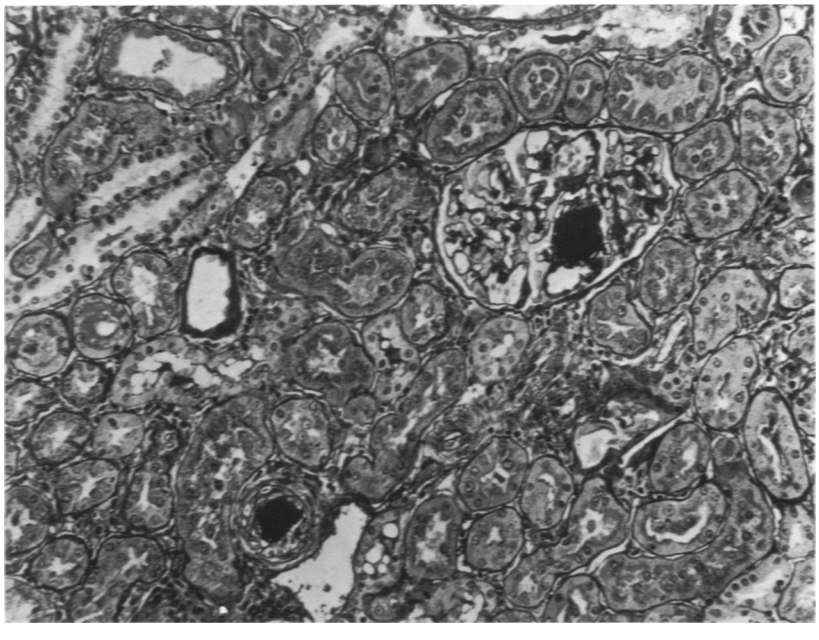


Fig 9—Glomerulus shows well-preserved architecture and good perfusion of capillary loops with red cells despite the presence of a Sephadex sphere within a capillary in a hypertensive rat (average systolic pressure, 148 mmHg) sacrificed 5 months after injection. Perfusion of such glomeruli was also demonstrated with iron-dextran. An interlobular arteriole, obstructed by a Sephadex microembolus, is seen at the lower left (PAS, × 180).

Fig 10—Dense particles (micellar ferric hydroxide) of iron-dextran within a perfused loop of a glomerular capillary from a moderately hypertensive rat (average systolic pressure, 148 mmHg) sacrificed 4 months after injection. Most of the very dense particles measure between 20 and 100 Å in greatest diameter (stained with uranyl acetate and lead citrate, × 8000).

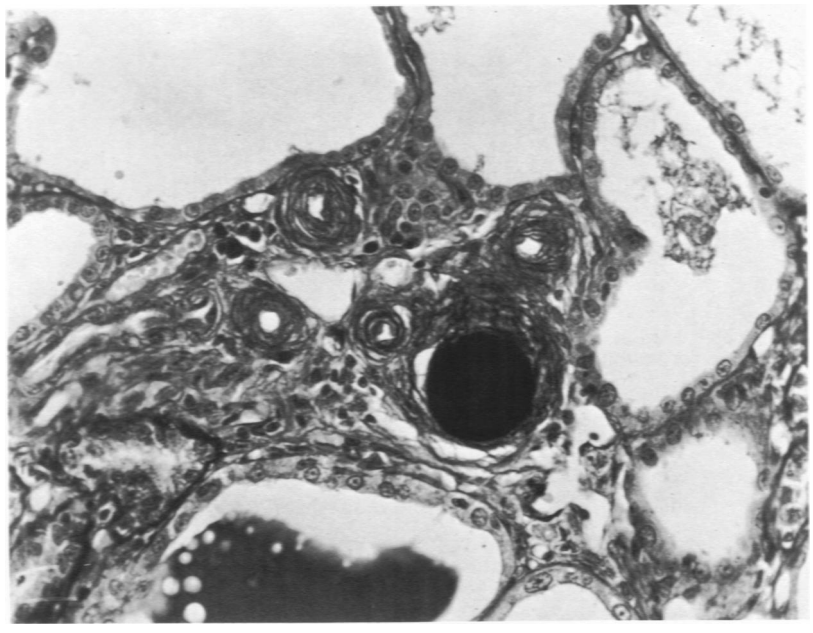
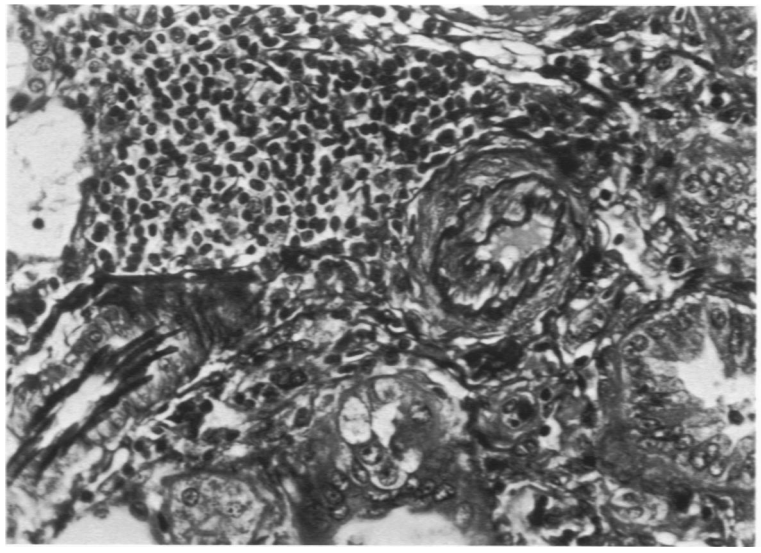


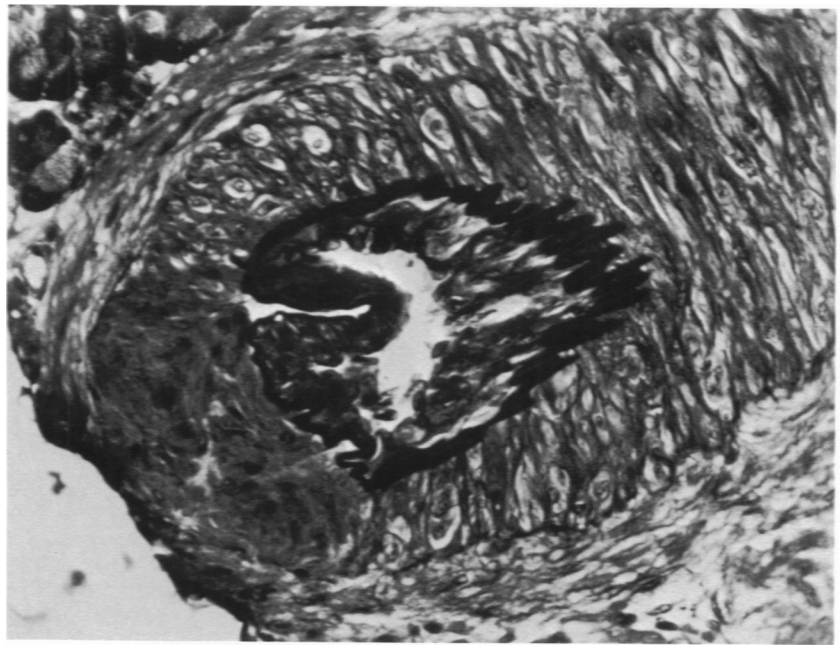
Fig 11—Dilated atrophic tubules (one with cast) in a markedly hypertensive rat (average systolic pressure, 190 mmHg) sacrificed 4 months after Sephadex was injected. Note four patent arterioles with thickened walls and a larger arteriole occluded by a Sephadex bead (PAS, × 380).

Fig 12—Medial hypertrophy and reduplication of the inner elastic lamina in renal arteriole of a hypertensive rat of the SI-chronic group (average systolic pressure, 164 mmHg) sacrificed 8 months after operation. There is a prominent round-cell infiltrate at the upper left (Weigert–Van Gieson, × 180).

Fig 13—Medial and intimal proliferation of a large pancreatic arteriole. Same hypertensive rat as that in Fig 12 (Weigert–Van Gieson, × 380).



12



13

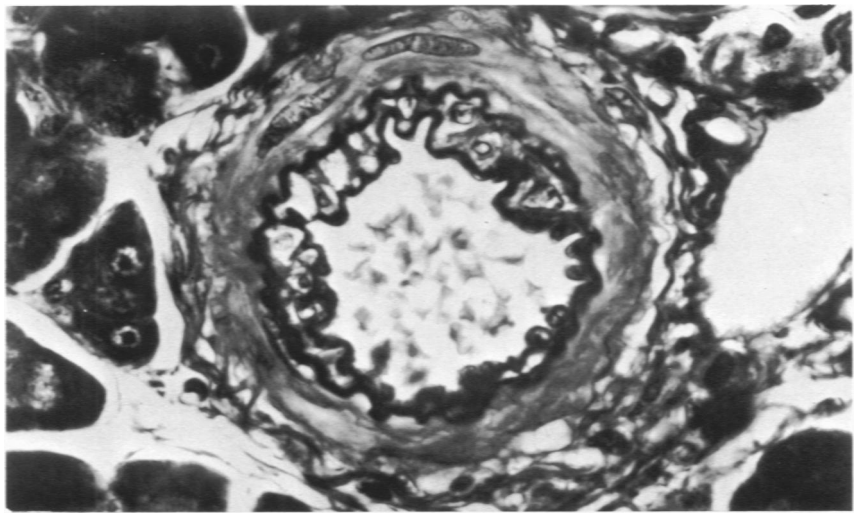


Fig 14—Intimal and medial thickening with reduplication of the inner elastic membrane of a small arteriole in the pancreas of the same hypertensive rat as that in Fig 11 (Weigert–Van Gieson, × 750).