STUDIES OF THE MECHANISM OF EXPERIMENTAL NEPHRITIS WITH FLUORESCEIN-LABELED ANTIBODY

II. Localization and Persistence of Injected Rabbit or Duck Anti-Rat-Kidney Serum During the Course of Nephritis in Rats

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Glomerular nephritis in rats follows injection of specific anti-kidney serum, anti-placenta serum or anti-lung serum. The present communication is the second in a series [1] describing the distribution of such nephrotoxic antiserums in the tissues of the host. The purpose of these experiments has been to determine the localization, relative concentration and persistence in the rat of selected rabbit or duck antiserums to rat kidney. The course and histologic features of the nephritis produced by these antiserums have been followed over a period of 1 to 291 days. Fluorescein-labeled antibodies have been used to identify the injected nephrotoxic globulins in the rats' organs and to determine the relative concentration of this globulin in acute and chronic nephritis of varying severity.

Material and Methods

Preparation of Kidney Antigen: Production of Antibodies

Anesthetized Sprague-Dawley or Long-Evans rats were perfused through the aorta, under sterile conditions, with 0.85 per cent NaCl which then escaped through an incision in the right auricle. When the kidneys appeared free of blood, they were excised, with precautions to maintain sterility, and stored in a CO_2 refrigerator at approximately −65° C. Five or 10 per cent suspensions of the renal tissue in physiologic saline were prepared with the Waring blender for intraperitoneal injection into rabbits according to a schedule previously described.[2] The rabbits were exsanguinated 10 to 14 days following the last injection, the serum separated aseptically and stored at 4 to 8° C. without preservative.

The duck anti-rat-kidney serums were those used in previous studies.[3] All antiserums were tested for agglutinins to rat erythrocytes and, if the titer exceeded 1 to 8, were absorbed with rat red blood cells. Serums from normal rabbits and ducks were obtained for injection into control rats.

Injection of Rats: Tests for Renal Damage

Five anti-rat-kidney serums from rabbits and 4 anti-rat-kidney serums from ducks, differing in titer of nephrotoxic activity, were injected intravenously into 51 Sprague-Dawley or Long-Evans rats in volumes varying from 0.2 to 1.5 ml. When 0.4 to 0.8

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ml. of serum was given, 2 injections were used; with larger volumes, 3 injections were given on successive days. Small repeated injections, in contrast to a single large injection, minimized the nonspecific toxic effects of many of the antiserums which, in large volumes, sometimes caused capillary damage and pulmonary edema. Sixteen rats were given injections of a total of 1.5 ml. of normal rabbit or duck serum. All serums were heated to 56° C. for 30 minutes prior to injection because such treatment reduced the incidence of toxic reactions.

The animals were inspected, weighed, and urine specimens were collected and tested for protein as described previously.[1] Determinations of urea nitrogen and total cholesterol were made on serum obtained at the time of necropsy.

Necropsy

The nephritic rats were killed 1 to 291 days following injection of the anti-kidney serums, whereas the control animals, injected with normal rabbit or duck serum, were examined within 1 month. Necropsy procedures and methods for histologic evaluation of the nephritis have been described.[1]

Fluorescent Antibody Studies

Nephrotoxic globulin in kidneys and other organs was identified by "staining" sections of frozen tissues with fluorescein-labeled antibodies to duck or rabbit globulin. The relative amounts of nephrotoxic globulin in the tissues were estimated by determining the minimum amount of unlabeled globulin required to block subsequent staining with fluorescein-labeled globulin.[1]

RESULTS

Course of Nephritis

Fifty-one rats received injections of rabbit or duck antiserum to rat kidney. Thirty-three of these animals were killed in the ensuing month, during the acute stage of nephritis; the other animals remained under observation for longer periods until subacute or chronic nephritis had developed.

Observations on 21 rats with acute nephritis after injection with 1 of 5 different rabbit antiserums are presented in Table I. The 5 rats which received 0.4 ml. of serum 800 or 0.9 ml. of serum 465–466 (groups 1 and 2) developed a severe disease that was characterized, in 4 animals, by a nephrotic syndrome with generalized edema and massive proteinuria. Serum urea nitrogen and total cholesterol were elevated terminally in 4 rats tested except for 1 animal (SDC280) in which the urea nitrogen was 24 mg. per cent. Severe renal lesions were found in all 5 of these rats. Text-figure 1 presents the course of nephritis in rat SDC207 (group 1) which was killed 6 days after injection. The animal was grossly edematous, having gained 50 per cent of its original body weight; proteinuria reached a maximum value of 6.5 gm. per cent. At the time of death the serum urea nitrogen was 42 mg. per cent and the serum cholesterol was 270 mg. per cent.

Disease of less severity followed the injection of the other 3 rabbit anti-rat-kidney serums. The 4 rats that received 0.9 ml. of rabbit serum

TABLE I

COURSE OF ACUTE NEPHRITIS IN RATS FOLLOWING INJECTION WITH RABBIT ANTI-RAT-KIDNEY SERUM

Group and serum	Rat number	Amt. (ml.)	Abnormal proteinuria		Killed post injection (days)	Terminal serum		Severity of renal lesion	Rabbit globulin in glomeruli (%)
			Day of onset*	Maximum (gm. %)		U.N.† (mg. %)	Chol.‡ (mg. %)		
1	SDC207	0.4	—1	6.5	6	42	270	Marked §	100
800	SDC217	0.4	—1	6.9	6	—	—	Marked §	100
	SDC280	0.4	—	2.9	16	24	206	Marked	90
2	SDB707	0.9	—2	4.3	4	52	350	Marked §	100
465–466	SDB708	0.9	—2	5.7	26	44	163	Marked §	100
3	SDC186	0.9	—1	1.9	1	—	94	Moderate	50
209–210	SDC15	0.9	—	—	5	22	189	Moderate to marked	50
	SDB737	0.9	—1	2.6	6	—	—	Moderate	50
	SDB734	0.9	—1	3.7	20	40	72	Moderate	50
4	SDC205	1.5	1	2.1	6	—	—	Moderate	50
799	SDC219	1.5	—1	5.0	6	20	212	Moderate to marked	50
5	SDC187	0.7	—1	6.3	5	—	143	Marked	100
797–798	SDB963	0.6	1	2.6	3	22	—	Marked	90
	SDC190	0.5	—1	5.0	5	23	132	Marked	90
	SDC61	0.47	1	3.8	5	—	—	Moderate	25
	SDC56	0.45	—1	1.7	2	—	265	Moderate	50
	SDC192	0.4	—1	2.2	5	32	68	Moderate	50
	SDC353	0.4	1	4.0	5	—	—	Moderate	50
	SDC194	0.3	1	2.3	5	34	63	Moderate	50
	SDC80	0.3	—1	6.9	13	37	166	Moderate	50
	SDC352	0.2	1	3.2	1	—	—	Mild	25

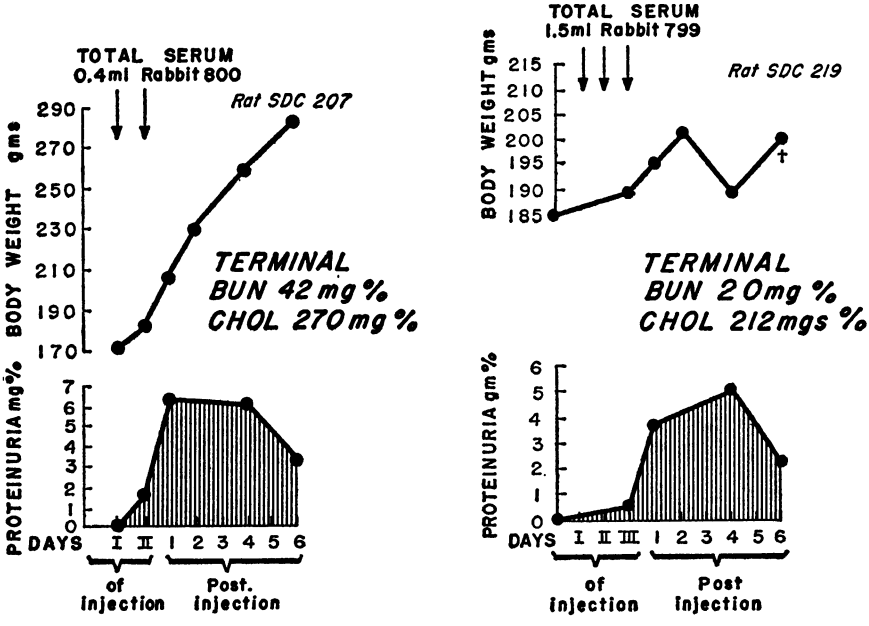
* —2 = 1 day before last injection; —1 = day of last injection (prior to injection); 1 = day following last injection.

† Urea nitrogen.

‡ Cholesterol.

§ Gross edema and ascites.

209–210 (group 3) did not show abnormal gain in weight or edema, although proteinuria was marked. Serum urea nitrogen was elevated in 1 of 2 animals tested, and serum cholesterol exceeded 100 mg. per cent in only 1 of 3 examined. The renal lesions were moderately severe. Two rats (group 4) received 1.5 ml. of serum 799, which was 66 per cent greater than the amount injected into the rats of groups 2 and 3, and 270



TEXT-FIGURE 1. Rat SDC207 given injections of 0.4 ml. anti-rat-kidney serum from rabbit 800. Note the increase in proteinuria beginning 24 hours after the first injection and the rapid rise in body weight apparent 1 day after injection. Fifteen ml. of fluid was recovered from the peritoneal cavity at necropsy, and there was a small amount of fluid in the thoracic cavities. Serum urea nitrogen and serum cholesterol were elevated.

TEXT-FIGURE 2. Rat SDC219 given 1.5 ml. of rabbit serum 799. Abnormal proteinuria had its onset on the last day of injection. The gain in weight was within normal limits. No gross edema was apparent at necropsy. Serum cholesterol was elevated.

per cent greater than that given to the rats in group 1. The nephritis produced by this serum was similar to that seen in the group 3 rats. Text-figure 2 presents the course of the disease in rat SDC219 killed 6 days after the injection of serum 799. There was no gross edema and no abnormal gain in weight. Proteinuria reached 5 gm. per cent; the terminal serum urea nitrogen was 20 mg. per cent and the serum cholesterol was 212 mg. per cent.

The 10 rats in group 5 were given injections of rabbit serum 797–798 in amounts varying from 0.7 ml. to 0.2 ml. Neither gross edema nor abnormal gain in weight were observed in any animal; the maximum

amount of protein found in the urine ranged from 1.7 to 6.9 gm. per cent, and, terminally, urea nitrogen was elevated in 3 of 5 and cholesterol in 4 of 6 serums tested. Rats receiving injections with larger volumes of antiserum had renal lesions graded as markedly severe; animals given smaller amounts had less severe renal lesions.

The time of onset of abnormal proteinuria was determined in 19 of the 21 rats (Table I). In 13 of these, abnormal proteinuria was present before the final injection of serum, and in the remaining 6 it was demonstrable on the day following the last injection.

Data concerning acute nephritis in 12 rats given injections of 1 of 4 pools of duck antibody to rat kidney are given in Table II. Three of 4 animals in group 6, given 0.6 to 0.75 ml. of Pool IV, developed marked proteinuria but showed no edema or abnormal gain in weight. Serum urea nitrogen was 20 and 26 mg. per cent, respectively, in the 2 animals tested, and the serum cholesterol was 213 mg. per cent in 1 of them. The nephritis was graded as moderately severe in 2 rats and markedly severe in the other 2.

The 3 duck antiserums given to the rats in groups 7, 8 and 9, in amounts of 1.3 to 1.5 ml., caused no edema or excessive gain in weight. Abnormal proteinuria was present in 5 of the 8 animals. Serum urea nitrogen exceeded 25 mg. per cent in 4 of the 6 rats examined, but the serum cholesterol was normal in all 4 instances in which it was determined. Histologic examination of renal tissue from these rats showed only mild lesions of acute nephritis.

The abnormal proteinuria seen in 8 of the rats (Table II) occurred before the last injection of serum in 1 animal, in 2 on the day following the last injection, and in 5 rats from 3 to 7 days later.

Sixteen control rats, receiving injections of 1.5 ml. of normal rabbit serum or normal duck serum, failed to develop edema, abnormal weight gain or proteinuria. These animals were killed 21 to 24 days following injection, at which time the serum urea nitrogen varied from 13 to 19 mg. per cent and the serum cholesterol from 30 to 70 mg. per cent. Renal lesions were not found on histologic examination of the kidneys.

Table III presents the results obtained in 18 rats under observation for longer periods of time. Ten rats were given injections of one of the rabbit antiserums used for groups 1, 3, 4 and 5 (Table I) and were killed at intervals from 60 to 291 days. After injection these animals excreted abnormally large amounts of protein in the urine for the rest of their lives, but the maximum amount varied from 1.2 to 7.7 gm. per cent. The terminal serum urea nitrogen was more than 25 mg. per cent in 6 of 8 rats, and serum cholesterol exceeded 100 mg. per cent in 4 of 6 animals studied. Lesions characteristic of chronic nephritis were found in 7 of

TABLE II

COURSE OF ACUTE NEPHRITIS IN RATS FOLLOWING INJECTION WITH DUCK ANTI-RAT-KIDNEY SERUM

Group and serum	Rat number	Amt. (ml.)	Abnormal proteinuria		Killed post injection (days)	Terminal serum		Severity of renal lesion	Duck globulin in glomeruli (%)
			Day of onset*	Maximum (gm. %)		U.N.† (mg. %)	Chol.‡ (mg. %)		
6 Pool IV	SDB998	0.6	—	None	1	—	—	Moderate	100
	SDC100	0.75	1	4.2	1	—	—	Moderate	100
	SDB997	0.6	7	4.3	15	26	213	Marked	100
	SDC101	0.72	1	2.9	19	20	—	Marked	50
7 Pool I	SDC354	1.5	3	0.7	4	—	—	Mild	50
	SDC355	1.5	5	0.6	11	—	80	Mild	90
8 Pool II	SDB991	1.3	—1	1.6	1	34	80	Mild	10
	SDB996	1.5	—	None	14	17	—	Mild	10
	SDB992	1.3	—	None	18	32	—	Mild	10
9 Pool III	SDB990	1.3	—	None	1	24	71	Mild	10
	SDB994	1.3	4	1.8	18	26	74	Mild	25
	SDC103	1.4	4	1.7	18	28	—	Mild	25

* —1 = day of last injection (prior to injection); 1, etc. = day(s) following last injection.
† Urea nitrogen.
‡ Cholesterol.

TABLE III

COURSE OF SUBACUTE AND CHRONIC NEPHRITIS IN RATS FOLLOWING INJECTION OF RABBIT OR DUCK ANTI-RAT-KIDNEY SERUM

Group and serum	Rat number	Amt. (ml.)	Abnormal proteinuria		Killed post injection (days)	Terminal serum		Type and severity of renal lesion	Rabbit or duck globulin in glomeruli (%)
			Day of onset*	Maximum (gm. %)		U.N.† (mg. %)	Chol.‡ (mg. %)		
1A 800	SDB691	0.7	—2	5.8	212	—	—	Chronic, marked	25
3A 209–210	SDB805	0.9	1	4.3	151	40	137	Chronic, marked	25
	SDB806	0.9	1	3.6	197	42	176	Chronic, marked	10
4A 799	SDC206	1.5	—1	3.1	60	42	—	Subacute/chronic, moderate	50
	SDC218	1.5	1	7.7	60	28	—	Chronic, moderate to marked	25
	H914	1.5	1	1.2	203	—	—	Subacute, mild	10
	H912	1.5	3	4.3	291	24	60	Subacute/chronic, mild	5
5A 797–798	SDC60	0.4	—1	3.2	150	44	108	Chronic, moderate to marked	10
	SDC59	0.35	1	3.3	286	32	108	Chronic, moderate to marked	10
	SDC58	0.3	1	1.7	286	20	64	Chronic, moderate	5
6A Pool IV	J268	1.0	1	3.5	42	24	246	Subacute, moderate	100
	SDC198	1.0	1	8.6	85	32	—	Chronic, marked	90
	SDC184	0.6	3	4.7	97	23	155	Chronic, marked	50
7A Pool I	SDC345	1.5	—	None	109	—	51	Subacute, mild	25
	SDC343	1.5	9	5.8	242	24	107	Chronic, marked	50
8A Pool II	SDC197	2.0	6	0.6	94	23	71	Acute/subacute, mild	10
	SDC185	1.5	5	1.4	97	20	74	Acute/subacute, mild	10
	SDB995	1.5	—	None	174	34	63	None	10

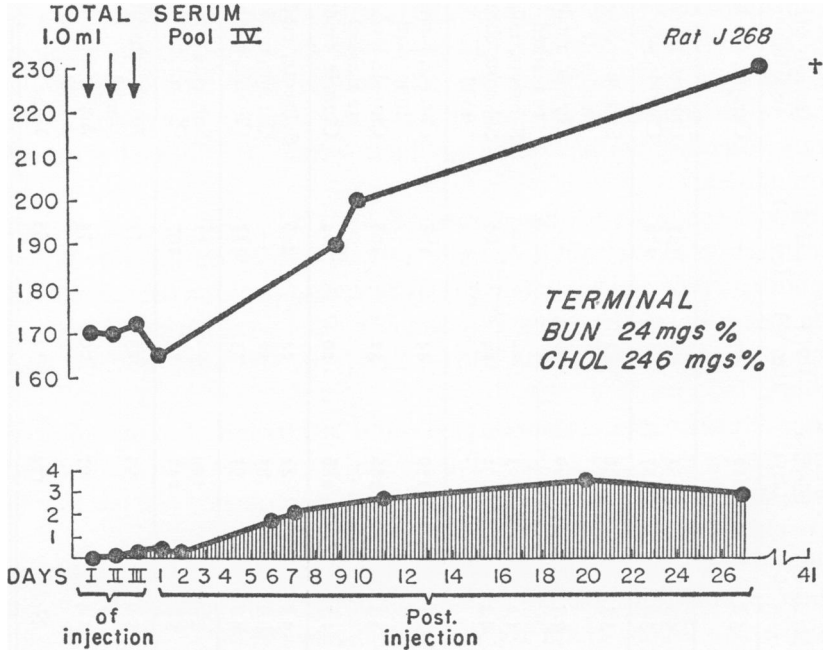
* —2 = 1 day before last injection; —1 = day of last injection (prior to injection); 1, etc. = day(s) following last injection.

† Urea nitrogen.

‡ Cholesterol.

the 10 rats, 2 showed renal lesions varying between those characteristic of the subacute and chronic stages, and in 1 animal the renal lesions were classified as subacute. Markedly severe renal lesions were seen only in the 3 rats given serums 800 or 209–210.

Eight rats injected with 1 of 3 duck anti-rat-kidney serums were killed 42 to 242 days later. The 3 rats given Pool IV serum had abnormal pro-



TEXT-FIGURE 3. Rat J268 given 1.0 ml. of anti-rat-kidney duck serum, Pool IV. Abnormal proteinuria was not recorded for 6 days after injection but reached the high level of 3 gm. per cent where it remained for the 41 days of observation. At necropsy the peritoneal surfaces were moist. Serum cholesterol was elevated.

teinuria within 1 to 3 days; this persisted throughout the course of the nephritis. Terminally, 42 to 97 days after injection, nitrogen retention was demonstrable in the serum of 1 of 3 animals tested, and the cholesterol was elevated in both of 2 serums examined. Text-figure 3 illustrates the course of the nephritis in rat J268, which was killed 42 days after injection of 1.0 ml. of Pool IV. Weight gain proceeded at a normal rate and no edema was found. Proteinuria reached a maximum value of 3.5 gm. per cent. Terminal serum urea nitrogen was 24 mg. per cent, and serum cholesterol was elevated to 246 mg. per cent.

Abnormal and persistent proteinuria occurred in 3 of 5 rats receiving Pool I or Pool II duck anti-renal serum, after a latent period of 5 to 9 days. Serum urea nitrogen was in excess of 25 mg. per cent in 1 of 4 rats tested, and cholesterol was elevated in 1 of 5 rats tested. Renal lesions

varying from mild acute to subacute nephritis to marked chronic nephritis were found in 4 of the 5 rats.

Pathologic Features Found at Necropsy

Thirty-three rats given either rabbit or duck antibody to rat kidney (Tables I and II) were necropsied 1 to 26 days later. Marked edema of the subcutaneous tissue and mesentery, with ascites, was found in 4 of the 5 rats in groups 1 and 2 (Table I). In these animals the kidneys were moist on section. No gross abnormalities were recognized in the tissues of the remaining rats, killed in the early stages of nephritis. The 16 rats receiving normal rabbit or duck serum also showed no gross abnormalities.

The kidneys in 13 of the 18 rats (Table III) killed 42 to 291 days after injection of the rabbit or duck anti-rat-kidney serum were larger than normal, pale and finely or coarsely pitted. On section they displayed prominent tubular striations. No abnormalities were observed grossly in the renal tissues of 5 rats, H914, SDC345, SDC197, SDC185 and SDB995.

There were no gross lesions which could be attributed to the injected antiserums in the other organs. Pneumonitis, the most common disease of rats, was found in the older animals with a frequency comparable to that in old untreated rats.

Microscopic examination of the renal tissues from rats killed within the first month after injection with rabbit or duck antibody to rat kidney showed acute lesions characterized by increased cellularity of the glomerular tufts, thickening of the glomerular basement membranes, some cellular infiltration of the interstitial tissue and the presence of tubular casts with variable amounts of damage to tubular epithelium. The extent of these lesions varied considerably from rat to rat and were graded as of mild, moderate or marked severity, depending upon the relative degree of damage seen.[1] Figures 1 and 2 illustrate the renal lesions in rats SDC207 and SDC219 (Text-figs. 1 and 2). The former animal was considered to have maximally severe acute nephritis. The lesions in the kidney of SDC219 were somewhat less marked.

Lesions of acute nephritis were usually seen only within the first 4 weeks after injection of nephrotoxic serum, but 2 rats, SDC197 and SDC185, given the weakly nephritogenic serum Pool II and killed 3 months later, had many glomeruli showing lesions considered to be characteristic of the acute disease. Lesions of subacute nephritis also were present.

Subacute nephritis was characterized by more advanced thickening of the glomerular basement membranes, adhesions between the tufts and

the capsule, and early crescent formation. The tubular epithelium appeared normal or was flattened in some areas where casts were numerous. There was increased interstitial cellular infiltration. This type of renal damage usually was seen in rats killed in the second to fourth month after injection, but it was found to occur earlier in some animals injected with nephrotoxic serum of high titer. Rat SDB708 (Table I), killed 26 days after injection of the highly nephrotoxic serum 465–466, exhibited lesions of both acute and subacute nephritis. In this limited series there were only 2 animals in which the renal lesions were characteristic primarily of the subacute disease. Rat J268, given a high-titered duck antiserum (Text-fig. 3), showed lesions of subacute nephritis after 42 days (Fig. 3). Rat H914, given a relatively weak rabbit anti-rat-kidney serum, still exhibited similar lesions after 203 days.

Chronic nephritis was characterized by fibrosis of glomeruli, leading eventually to complete occlusion of the capillaries. Associated with this fibrosis there was flattening of the tubular epithelium, blocking of the lumen of tubules by large casts in many cases, and marked interstitial cellular infiltration (rat SDB805, Fig. 4). The lesions, as seen in rats of this series given either rabbit or duck anti-rat-kidney serum, did not differ from those previously reported.[3,4]

The rapidity with which severe chronic nephritis developed was related to the severity of the acute disease and therefore to the nephrotoxic antibody titer of the anti-renal serum. This may be seen from an inspection of Table III, which shows that more severe and chronic disease followed the injection of those rabbit or duck antiserums which produced the more severe acute lesions, namely the serums given to rats in groups 1A, 3A and 6A.

Localization of Rabbit and Duck Anti-Rat-Kidney Serum

Blocks of kidney tissue were frozen in a CO_2-alcohol bath and stored in the CO_2 refrigerator. Fragments of lung, heart, liver, spleen, adrenal, lymph node and ovary from selected animals were similarly treated. Sections from these tissues were cut in the cryostat and examined for the presence of rabbit globulin or duck globulin by "staining" with fluorescein-labeled antibody to the respective globulin. In all cases the injected nephrotoxic globulin was found in the glomeruli. Figures 5 and 6 illustrate the appearance of the bright, rather sharp lines of fluorescence in the glomeruli in sections of the kidneys from SDC207 and SDC219 (Text-figs. 1 and 2), where the nephrotoxic rabbit globulin bound the fluorescent duck antibody to rabbit globulin.

Nephrotoxic globulin persisted in the glomeruli during progression of the nephritis. Figure 7 illustrates the presence of duck nephrotoxic

globulin in the glomeruli of J268 (Text-fig. 3) 42 days after injection, at a time when this rat had subacute nephritis. Figure 8 shows the continuing presence of rabbit nephrotoxic globulin in the glomeruli of rat SDB805, killed at a time when the rat had chronic nephritis, 151 days after the injection of rabbit nephrotoxic serum.

The spleen, adrenal and ovary were the only other tissues in which rabbit or duck nephrotoxic serums were encountered with any consistency. The injected globulins were demonstrable in the cells of the red pulp of the spleen in 18 of 24 rats, in endothelial cells of the capillary sinuses in the cortex of the adrenals in 23 of 25 animals, and in what appeared to be reticular cells of the ovary in 5 of 8 rats tested. After 5 months the globulins were not demonstrable in the spleen, but they were present in 2 instances at a later period in the adrenals and the ovaries. The hearts of 6 rats appeared to be free of nephrotoxic globulins. The livers of 6 rats also were tested for the presence of these foreign proteins. A few cells in the livers of 2 animals in group 5 (Table I), apparently Kupffer cells, contained rabbit globulin. The lungs of 11 rats and lymph nodes of 3 failed to show evidence of the nephrotoxic globulins.

Three types of control experiments were performed in order to verify the immunologic specificity of the "staining" with the fluorescein-labeled antibody to rabbit or duck globulin.[1] In each instance the control experiments demonstrated the immunologic specificity of the "staining."

Rabbit globulin was not specifically localized in the glomeruli of the 8 rats given normal rabbit serum. Small amounts of the foreign globulin were demonstrable in some of the endothelial cells lining capillaries between tubules and in an occasional cell, probably an endothelial cell, in the glomerular tufts as well as in a few scattered cells in the spleens and ovaries. Rabbit globulin could not be demonstrated in adrenals of these rats. Duck globulin was not found in the kidneys, adrenals or ovaries in rats given normal duck serum. This antigen, however, was found in a few cells of the spleen.

Relative Amounts of Nephrotoxic Serums Localized in Tissues

The relative amount of nephrotoxic globulin bound in the tissues in each animal was estimated, as in the case of the anti-lung serum,[1] by determining the minimum concentration of unlabeled antibody (duck antibody to rabbit globulin or rabbit antibody to duck globulin) required to block specific "staining" of the injected nephrotoxic globulin by the corresponding labeled antibody. In order to compare results obtained with the duck and rabbit antiserums, the maximum amount of unlabeled antibody necessary to block "staining" was assigned the value 100. Lesser amounts of unlabeled serum found necessary to block renal "stain-

ing" in other animals was expressed as a per cent of 100. These values have been recorded in the last column of Tables I to III.

It may be seen (Table I) that the frozen kidney sections from 4 rats in groups 1 and 2 with the nephrotic syndrome required the maximum amount of unlabeled duck anti-rabbit globulin to block "staining" with the fluorescein-labeled antibody. The fifth animal required slightly less. Blocking of the "staining" of all the renal tissues in groups 3 and 4 was accomplished by only half as much unlabeled antibody globulin. The amount of unlabeled antibody required to block the "staining" of the renal tissue in the 10 rats receiving decreasing amounts of serum 797–798 varied from 100 per cent to 25 per cent.

The renal tissue from rats receiving Pool IV required the largest amounts of unlabeled rabbit antibody to duck globulin to block "staining" with the fluorescein-labeled antibody (Table II). Comparable amounts were required for the 2 animals in group 7. Only 10 to 25 per cent of unlabeled antibody blocked the "staining" of the kidneys from the rats in groups 8 and 9.

In those animals which had lived sufficiently long to develop subacute or chronic nephritis following the injection of rabbit nephrotoxic serum, 5 to 50 per cent of unlabeled antibody was required to prevent "staining" with the fluorescein-labeled antibody to rabbit globulin (Table III). The amount of unlabeled rabbit antibody to duck globulin required to prevent "staining" of the renal sections from the rats with subacute or chronic nephritis produced by duck nephrotoxic serum varied from 10 to 100 per cent. The renal tissue from rat SDB995, which was without evidence of glomerulonephritis, nevertheless contained some of the nephrotoxic globulin in the glomeruli.

The last two columns of Table I and II show that when the amount of unlabeled antibody to rabbit or to duck globulin required to block the "staining" of renal tissue from rats with acute nephritis was compared with the severity of the renal lesion, the more severe nephritis occurred in animals which had the larger amounts of nephrotoxic globulin in the glomeruli.

The 17 animals listed in Table III with lesions of subacute or chronic nephritis had survived a variable period of time (42 to 291 days), and the relative amount of nephrotoxic globulin found in the glomeruli covered a wide range from 5 to 100 per cent. Two thirds of these animals, however, had less than 50 per cent of the amount presumably present immediately after injection. The nephrotoxic globulin was slowly lost from the renal tissue.

Those animals in which the spleen, the adrenal or the ovary contained fluorescent cells were also examined to determine the minimum amount

of untagged antibody required to block "staining" with the specific fluorescein-labeled antibody. About 20 per cent as much unlabeled antibody was required to block "staining" of these tissues as was necessary to block the "staining" of the glomeruli from the same animal.

Discussion

Nephritis produced in the rat by antiserums to renal tissue has been repeatedly described.[3,4] The purpose of the present investigation was to produce nephritis in a series of rats with antiserums of varying potency obtained from either rabbits or ducks in order to study possible causes for the variation in the severity and rate of progression of the ensuing disorder. It was evident that although the schedule of immunization in all the rabbits was essentially identical, the resulting antiserums differed considerably in nephrotoxic antibody titer. The rabbit serums used in these experiments were titered individually, by injection into rats; those that produced marked nephritis when identical volumes were injected were pooled. In order to avoid the possibility of serum disease, no serum was given in excess of 1.5 ml. The relatively strong serum pool from rabbits 465–466 was prepared in 1950; the somewhat weaker serum from rabbits 209–210 was produced in the following year; rabbits 797, 798, 799 and 800 were immunized in the summer and fall of 1956. All animals were under immunization at least 5 months. The data show that serum 800 was the most effective in producing severe acute nephritis, whereas serum 799 had the lowest nephrotoxic antibody titer. There was thus no way of predicting when rabbits would yield optimal antiserums.

The duck anti-rat-kidney serums[3] represented pools of serums obtained from many ducks. Individual variations in nephrotoxic antibody titer may have been equalized in most instances. Nevertheless, Pool IV produced a distinctly more severe disease than did the other duck serums.

It was apparent that the differences in antibody titer of both rabbit and duck antiserums represented actual differences in potency of the antiserums, rather than variation in susceptibility among rats, since a given serum produced nephritis of rather constant severity.

Rats receiving injections of a serum causing severe acute nephritis were found to develop severe subacute or chronic nephritis relatively rapidly. On the other hand, animals given antiserums that caused a mild acute nephritis showed slow progression of the disease. Months after injection the renal lesions might be those of mild subacute or chronic nephritis. Similar findings have been reported for rats receiving duck anti-rat-lung serums with varying titer.[1]

The fluorescent antibody technique was used to investigate the relationship of the severity of the renal lesions to the relative amount of nephrotoxic serum bound in the glomeruli of the injected rats. Preliminary experiments indicated that binding of the foreign nephrotoxic globulin in the glomeruli was accomplished within 20 minutes after injection of both rabbit and duck anti-rat-kidney serum. This is illustrated in Figure 9 where a glomerulus of a rat killed only 20 minutes after the injection of 0.16 ml. of serum 800 is shown to be "stained" with fluorescein-labeled antibody to rabbit globulin. The amount of unlabeled anti-rabbit globulin required to block this "staining" was about 50 per cent of that necessary in the case of rat SDC207, given 0.4 ml. of the same serum. It thus seems probable that most of the available nephrotoxic globulin had been localized in the specific renal tissue in this short period of time. Similar results have been obtained when duck serum Pool IV was injected. The nephrotoxic globulin was found to remain in the glomeruli in large amounts for long periods of time. During the period of acute nephritis there was no demonstrable loss, while in the subsequent months the amount of globulin decreased very slowly and could be demonstrated during the entire period of observation. It was found that the antiserums which produced the most severe acute disease were present in the glomeruli in relatively greatest amount. Conversely the animals with the least amount of foreign nephrotoxic globulin bound in the affected areas developed the mildest acute nephritis.

Summary

1. The course of acute, subacute and chronic nephritis produced in the rat by the injection of rabbit or duck anti-rat-kidney serum was followed for periods up to 291 days.

2. Both injected rabbit and duck globulin were demonstrated, during all periods of observation, in the glomeruli of nephritic animals.

3. The rats which had the most severe acute nephritis showed the greatest amount of nephrotoxic globulin localized in the glomeruli.

4. Progression of renal lesions to chronic glomerulonephritis occurred most rapidly in those animals which suffered the most severe acute disease.

5. Cells in the spleen and adrenal contained small amounts of rabbit or duck globulin up to 5 months after injection. This was the case also in animals given injections of duck anti-rat-lung serums.

6. Rabbit or duck anti-rat-kidney serums, localized in rat glomeruli, as in the case of anti-rat-lung serums, initiated nephritis; progression of this might be attributable to persistence of the foreign globulin in the glomeruli.

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[Illustrations follow]

Legends for Figures

FIG. 1. Kidney, rat SDC207, given injections 6 days previously of 0.4 ml. of rabbit anti-rat-kidney serum 800. There is some edema of the tissues, thickening of glomerular basement membranes and damage to tubular epithelial cells. Periodic acid-Schiff (PAS) stain. × 500.

FIG. 2. Kidney, rat SDC219, given injections 6 days previously of 1.5 ml. of rabbit anti-rat-kidney serum 799. Changes similar to those seen in the kidney of SDC207 are present, namely, thickening of the basement membranes and damage to tubular epithelium. PAS stain. × 500.

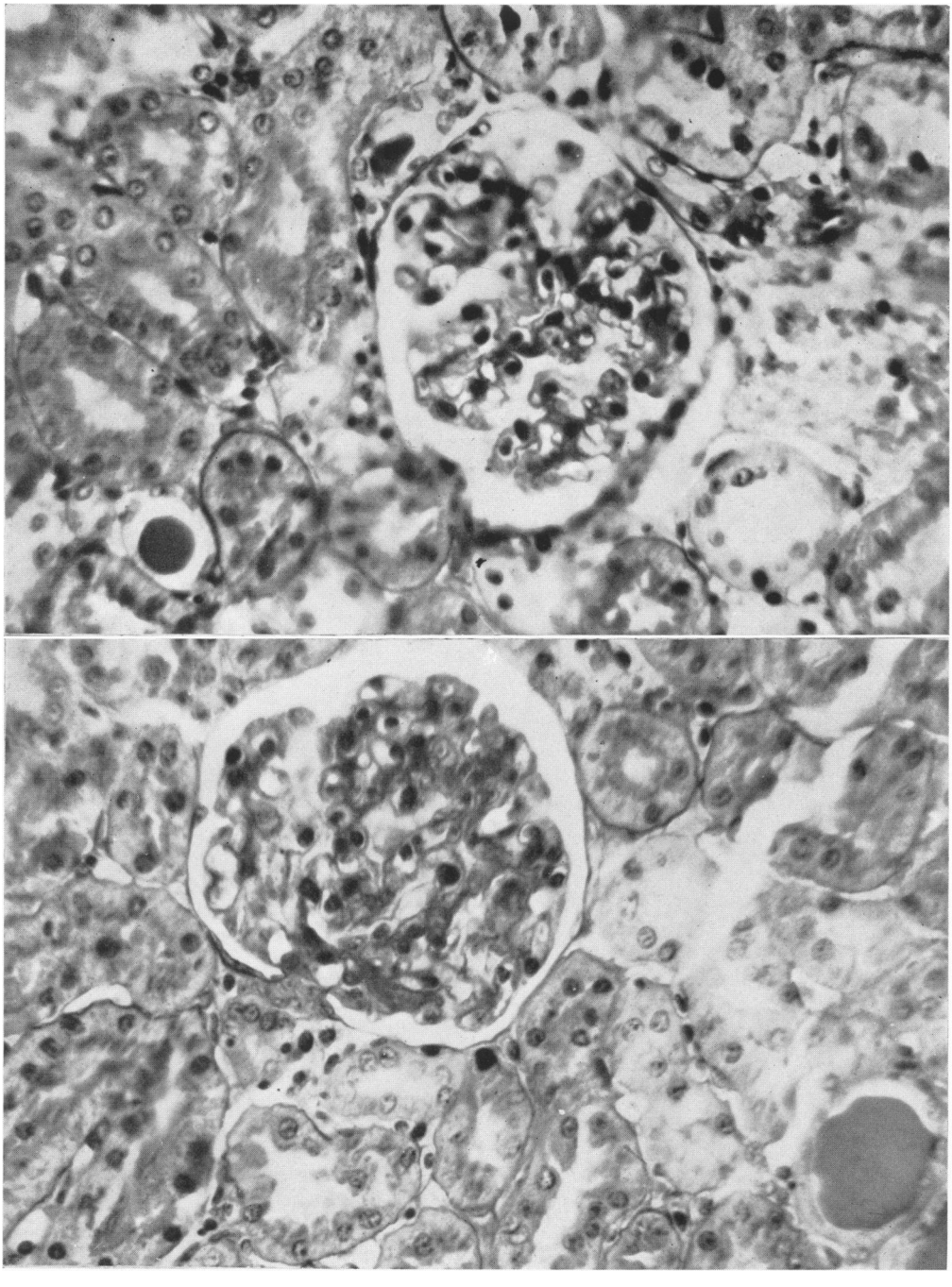

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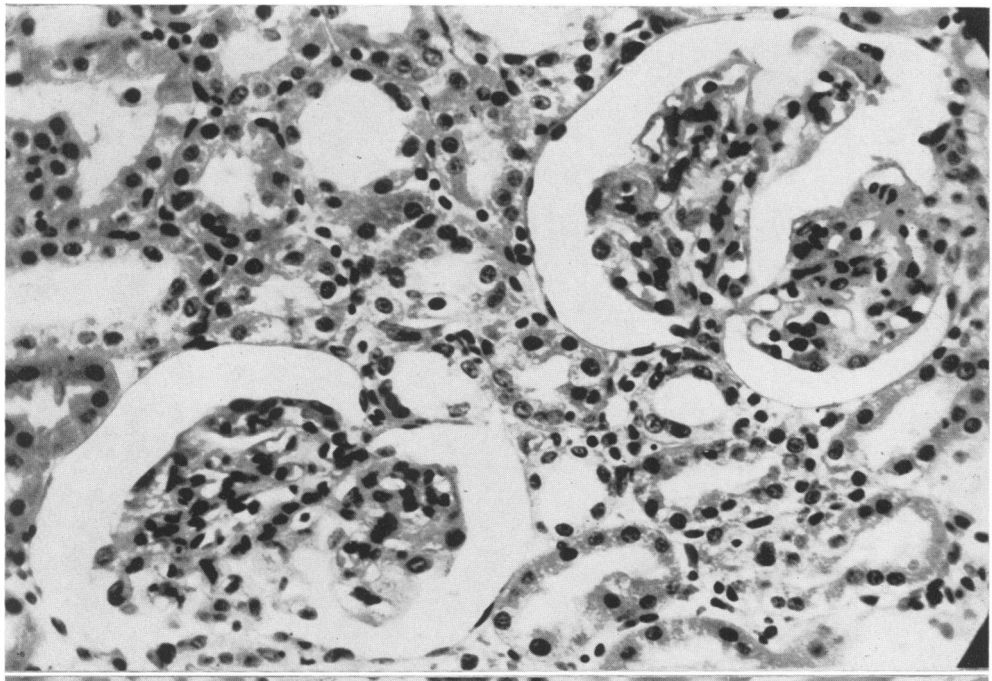
FIG. 3. Kidney, rat J268, 42 days after injection of 1.0 ml. of duck anti-rat-kidney serum, Pool II. The glomeruli show swelling of the tufts with occlusion of the capillary spaces, some cellular infiltration, and early crescent formation. Hematoxylin and eosin stain. × 400.

FIG. 4. Kidney, rat SDB805, 151 days after injection of 0.9 ml. of serum of 209-210. Occlusion of Bowman's space and of capillaries of the glomerular tufts by connective tissue proliferation is seen. The presence of casts, the flattening of tubular epithelium and scarring of the interstitium are evident. Hematoxylin and eosin stain. × 540.



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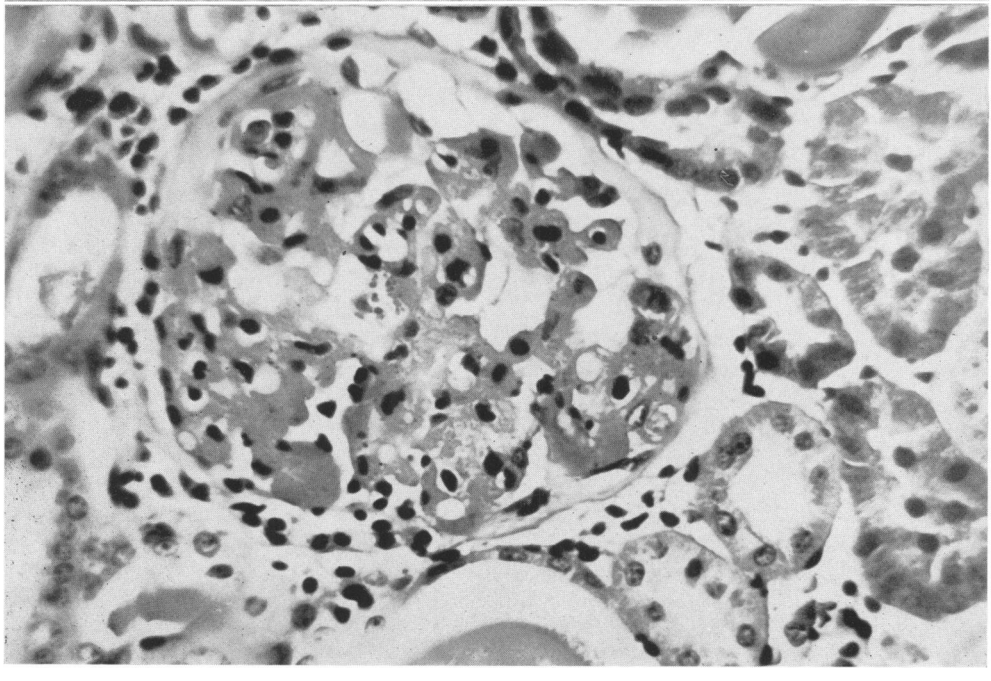

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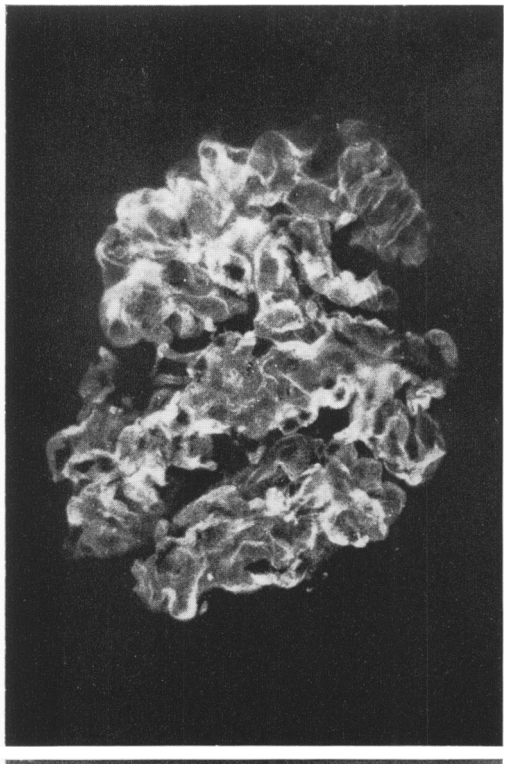
FIG. 5. Kidney, rat SDC207 (Text-fig. 1), "stained" with fluorescein-labeled duck antibody to rabbit globulin. The brightly fluorescent lines and less brightly fluorescent cytoplasm indicate where nephrotoxic rabbit globulin has localized. × 370.

FIG. 6. Kidney, rat SDC219 (Text-fig. 2) "stained" as above. Localization of nephrotoxic globulin is evident in a glomerulus. × 380.

FIG. 7. Kidney, rat J268 (Text-fig. 3) "stained" with fluorescein-labeled rabbit antibody to duck globulin. Distribution of the duck nephrotoxic serum is similar to that seen for rabbit nephrotoxic serum (Figs. 5 and 6). × 665.

FIG. 8. Kidney, rat SDB805, killed 151 days after the injection of 0.9 ml. of rabbit anti-rat-kidney serum 209-210, "stained" with fluorescein-labeled duck antibody to rabbit globulin. The rabbit nephrotoxic globulin is still clearly present in the glomeruli. × 330.

FIG. 9. Kidney, rat J239, dead 20 minutes following injection of 0.16 ml. of rabbit anti-rat-kidney serum 800. The nephrotoxic serum has already been bound specifically in the glomeruli. This is demonstrated by "staining" with fluorescein-labeled duck antibody to rabbit globulin. × 460.



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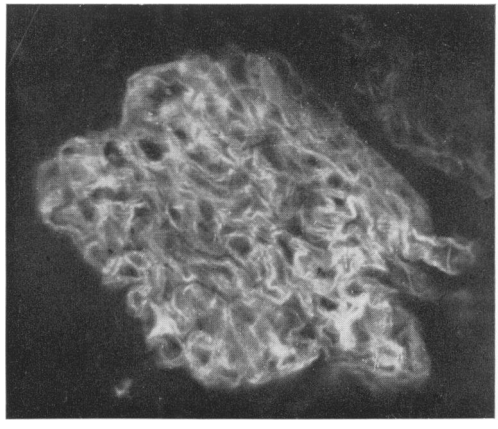

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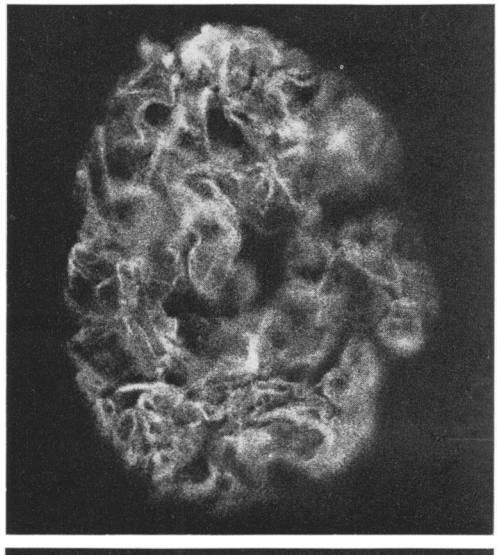

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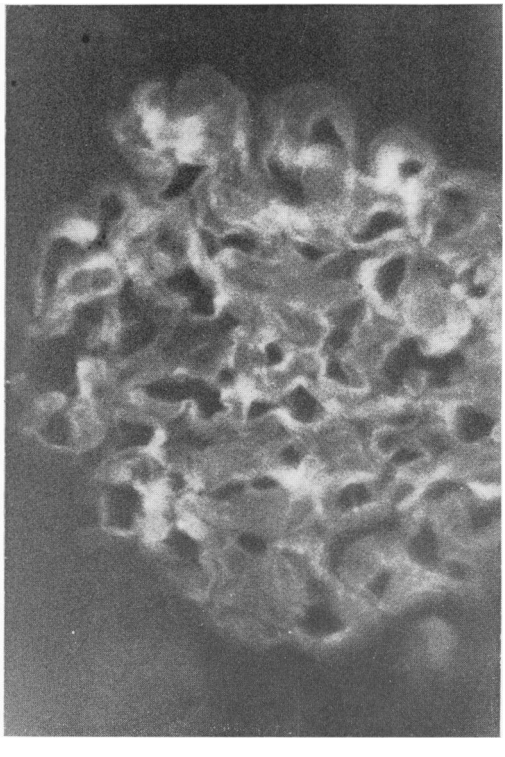

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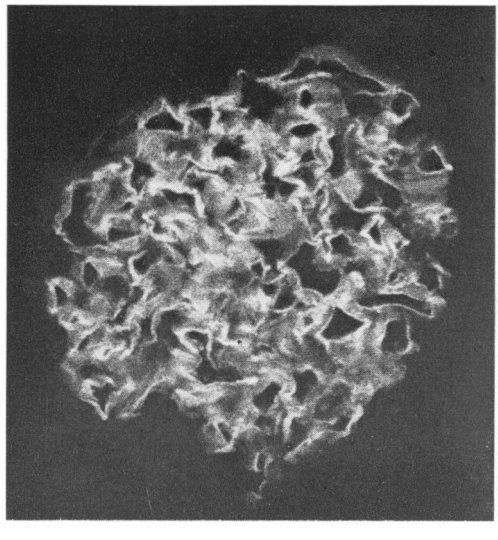

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