room temperature. Autoradiograms were then prepared by the stripping film technic of Pelc(2), and were left to expose in a light tight box at 4°C. Exposure time varied from a few days to 8 weeks according to isotope used and its concentrations in the kidney. After suitable exposure autoradiograms were developed, fixed, dried and permanently mounted. As staining technics were not altogether satisfactory, the preparations were photographed using both transmitted light and phase contrast microscopy.

Results. By this method the site of action of certain labelled substances has been localized to specific portions of the nephron. For example, a rat treated with 2 mg of C_{14} Vit. D_2 (spec. activity 1.15 μc mg)‡ showed that in the kidney the site of activity was confined to proximal convoluted tubule (Fig. 1a and b) and not in the remaining portion of the nephron (Fig. 2a and b), whereas in the dog 200 mg of Mersalyl§ (sodium salt of 2-(3-hydroxymercuri-2-methoxy-propylcarbamoyl) phenoxyacetic acid) labelled with 1 mc of Hg_{203} (spec. activity 5 μc/mg) acted in the lower end of proximal convolution, and not in the remaining portion of the nephron. On the other hand, rats conditioned by increasing doses of iodinated Pitressin, when injected intravenously with 8-16 units of Pitressin‖ labelled with I_{131} (spec. activity 1 μc/unit) showed that the activity was to be found in the distal convoluted and collecting tubules (Fig. 3a and b).

The potential value of this method for investigation of the site of action of substances within the kidney offers a useful adjunct to present technics and may be particularly effective for physiological substances which cannot be detected by staining procedures.

Summary. A method is described in which the combination of microdissection and a stripping film technic has provided autoradiograms of the isolated nephron. Accurate localization of site of action of suitable tagged materials was obtained. For example, after injection of C_{14} Vit. D_2, activity was found only in the proximal convoluted tubule, while with I_{131} Pitressin it was localized to the distal convoluted and collecting tubules.

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‖ We wish to thank Messrs. Parke, Davis Co., England for this material.

1. Darmady, E. M., Stranack, F., *Brit. Med. Bull.*, 1957, v13, 21.

2. Pelc, S. R., *Nature*, 1947, v160, 749.

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Production of Nephrotic Syndrome in Rats by Freund's Adjuvants and Rat Kidney Suspensions.* (24736)

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It has been shown that renal disease produced in rats by intravenous injection of anti-rat kidney serum obtained from rabbits simulates the nephrotic syndrome as observed in infants and children(1). This observation has stimulated the view that the disease in man may be due to an antigen-antibody reaction. The following suggestive evidence supports this hypothesis: 1) It has been noted that in nephrotic patients complement activity in blood serum is decreased(2,3), and 2) Increased deposition of globulins has been noted in the glomerular structures of nephrotic kidneys(4). Even though heteronephrotoxic antibodies have been shown to induce the ne-

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phrotic syndrome in rats(1) it has thus far not been possible to produce nephrotic renal disease experimentally by iso- or autosensitization to kidney proteins. The following studies suggest this immunochemical process as the underlying pathogenetic mechanism.

Methods and procedures. Rats of Sprague-Dawley strain were used. They were fed Friskies† and weighed 150-180 g at onset. Methods for chemical determinations in urine and blood sera have recently been described (5). Freund's adjuvants consisted of 8.5 ml Bayol F, 1.5 ml Arlacel A and 20 mg killed tubercle bacilli. One ml of this suspension was thoroughly mixed before each injection with 0.4 cc of low speed supernate (3000-4000 rpm for 1 to 1½ hr) of a 1:1 blood-free rat kidney, liver, lung or muscle suspension in saline. Because of the smaller amounts of kidney material available, a 25% kidney suspension was used in rats injected with their own kidney obtained by previous unilateral nephrectomy. 0.25-0.5 ml of the adjuvant-tissue suspension mixture was given every second week by intraperitoneal injection for a maximum of 6 times. Whenever proteinuria in excess of 150 mg/24 hr was noted no further injections were given. Determinations of protein nitrogen in various tissue suspension supernates revealed that the 0.066 ml used with each injection contained for kidney 2.45 mg, liver 1.3 mg, lung 1.0 mg and muscle 0.48 mg of proteins. Paper electrophoresis indicated these to be chiefly globulins. Blood pressure was measured in unanesthetized, unheated animals with the foot method described by Kersten, *et al.*(6).

Results. After long-term injections of blood-free rat kidney extracts failed to produce renal disease by subcutaneous and intraperitoneal injections, rats were also injected subcutaneously and into footpads with rat kidney extracts and Freund's adjuvants. No renal disease was noted. When 5 rats were treated by intraperitoneal injections every other week with 0.25 cc of a suspension containing 1 cc of Freund's adjuvants and 0.4 cc of a low speed supernate of a 1:1 blood-free rat kidney suspension, proteinuria developed after third to sixth injection. This was noted only once after the second injection. Fig. 1 shows that proteinuria, without gross or microscopic hematuria, developed in all 5 rats so treated. It was of considerable degree with values over 200 mg/24 hr in 4 of the 5 animals. After injections had been discontinued, renal disease continued without remission for over 3 months at which time the animals were sacrificed. Within that period azotemia did not develop, and hypoproteinemia and hyperlipemia were marked, reaching maximal values for total lipids in serum of 3450 mg % and for cholesterol of 577 mg %. Blood pressure remained within normal limits (6). When the supernate of rat liver suspensions was used instead of kidney material, only one of 6 rats developed a mild nephrotic disease. When Freund's adjuvants were injected without additional rat tissue suspension none of the 5 rats so treated developed proteinuria. Whether or not edema and ascites developed is difficult to state, because in some animals, and independent of ensuing renal disease, considerable degrees of ascites were observed after second or third injection. This subsided spontaneously within one to 2 weeks and did not recur even when subsequent intraperitoneal injections were given. The ascites was probably due to a sterile granulomatous inflammation of the peritoneum seen at post mortem examination in all animals treated with adjuvants. Adhesions between loops of small intestines were noted in all animals. At time of death the peritoneal surface was smooth and glossy. Cultures obtained at time of death from the peritoneal cavity were negative. Cultures for tubercle bacilli and inoculations of guinea pigs also all remained negative.

A second series of rats were treated in the same fashion except that the dose was doubled (Fig. 2). Ten rats of the same strain (weighing 170-180 g) were injected intraperitoneally every other week with 0.5 cc of the suspension containing adjuvants and rat kidney supernate. Every rat developed severe nephrotic disease after the third or subsequent injection. Again proteinuria (without hematuria), hypoproteinemia and hyperlipemia were marked,

† Ralston Purina Co., St. Louis, Mo.

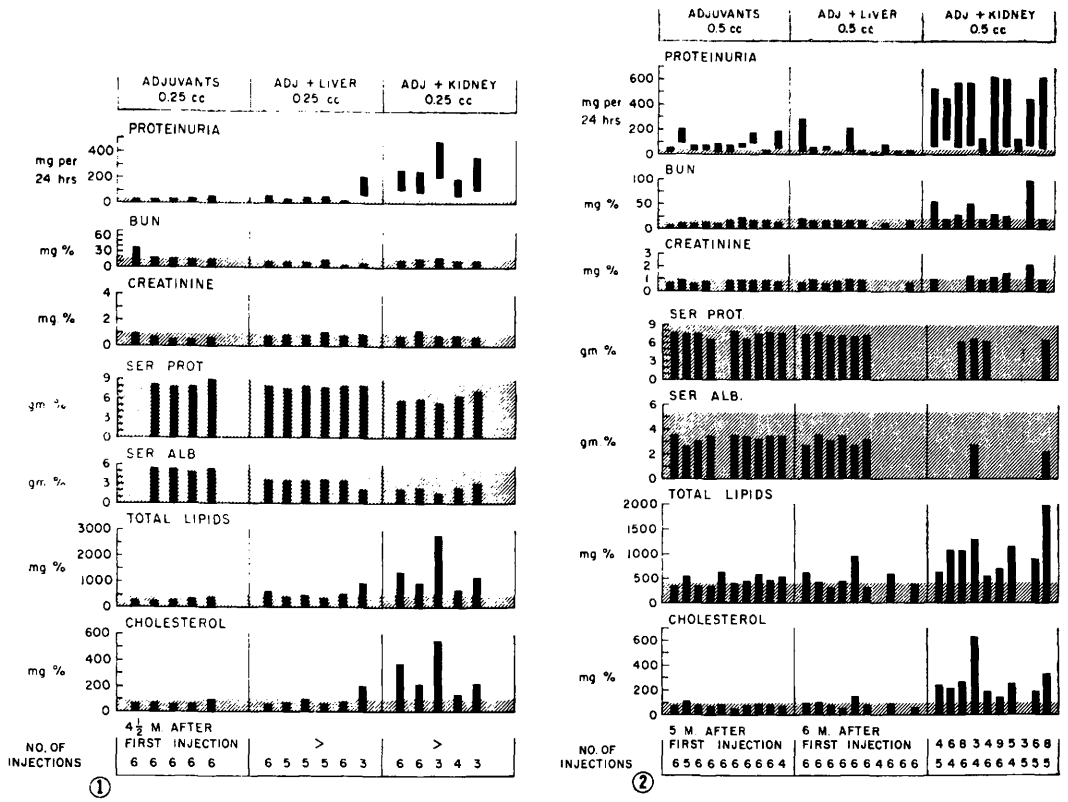

FIG. 1. Blood chemistry and urine findings in rats given 0.25 cc of Freund's adjuvants, with or without added liver or kidney suspensions. Shaded areas indicate range of normal values.
FIG. 2. Same as Fig. 1, except for use of 0.5 cc of Freund's adjuvants.

and some azotemia was noted in 3 rats and increased blood creatinine concentration was noted in 3 rats. Two of the 10 rats injected with 0.5 cc of adjuvants developed a mild nephrotic disease.

Twenty-five additional rats were divided into 5 groups of 5 animals each. These were injected according to the described schedules with 0.25 ml of adjuvant-kidney mixture, adjuvant and liver, adjuvant and muscle, adjuvant and lung, and adjuvants without added tissue suspension supernate. No proteinuria, hypoproteinemia or hyperlipemia was noted in any of these rats, with the exception of the 5 rats treated with the adjuvant-kidney mixture. Marked proteinuria, hypoalbuminemia and hyperlipemia developed in each of the 5 animals after 3, 5 and 6 injections.

Fifteen animals were then injected every second week into the abdominal cavity for a total of 6 injections. One to 2 injections were with 0.5 cc of adjuvants and rat-kidney-extract supernate, and 4-5 injections were with rat-kidney-extract supernate without adjuvants. Only 4 of these 15 rats developed proteinuria, hypoproteinemia and hyperlipemia.

Seven male rats weighing 430-500 g were subjected to unilateral nephrectomy. Four to 13 weeks later they were treated every 2 weeks by intraperitoneal injection with 0.25 cc of adjuvants plus 25% rat-kidney-extract supernate. Each rat received the kidney-extract supernate prepared from his previously removed kidney. Five of these animals developed a sever nephrotic syndrome, while none of the control rats injected with the same amount of adjuvants developed proteinuria.

Microscopic examination of kidneys of all rats given adjuvants and rat-kidney suspension supernate revealed a similar picture (Fig. 3). There was moderate to marked thickening of the basement membrane regions of the

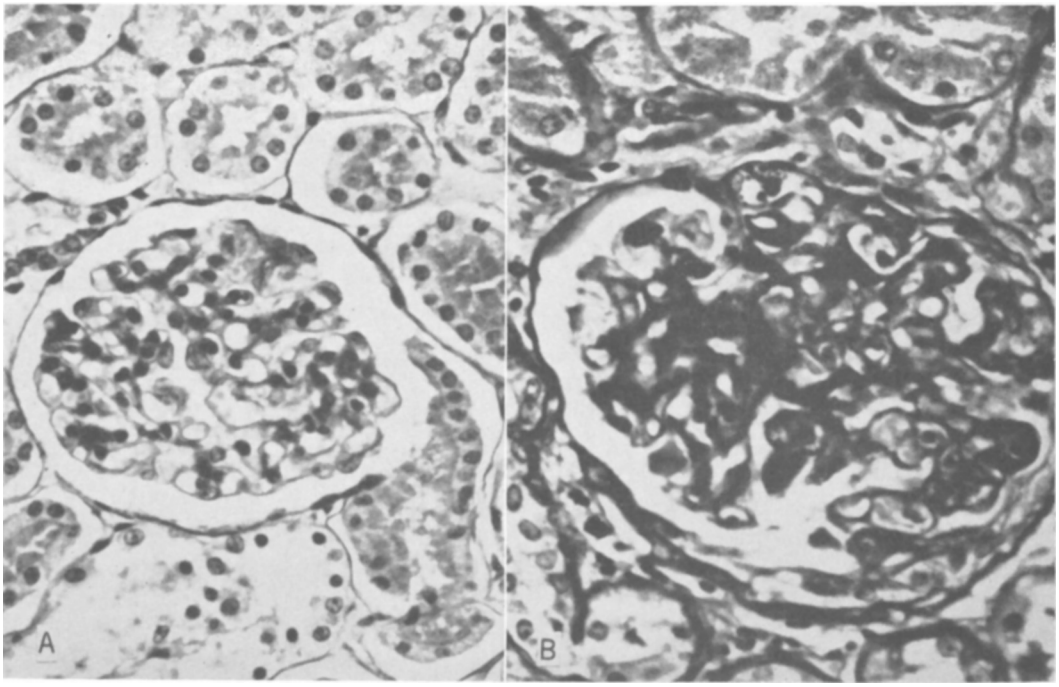

FIG. 3 (A). Normal kidney. Note patent glomerular capillaries, thin basement membrane. Periodic-acid Schiff stain. 480 ×.

FIG. 3 (B). Kidney of rat inj. with adjuvants plus rat kidney suspension. Note marked thickening of basement membrane regions, synechiae, and slight cellular proliferation of capsular epithelium. Periodic-acid Schiff stain. 480 ×.

glomerular capillaries, as seen in periodic acid-Schiff (PAS) stained sections, and slight swelling of endothelial cell cytoplasm. There was only slight encroachment on the patency of the capillary lumina, however, in most instances. Rare glomeruli showed epithelial crescent formation. Numerous eosinophilic, PAS-positive casts were present in the tubular lumina which were often slightly dilated. There were fat droplets (Sudan IV stain) in slight to moderate amounts in the basal portion of the cells, and small focal collections of lymphocytes and plasma cells were present in the interstitial tissue. No evidence of amyloid deposition was seen in sections of kidney, liver or spleen which were stained with crystal violet(7). Lesions of a comparable character but less in degree were seen in the rats who developed proteinuria after injection of adjuvants alone, or adjuvants plus liver. Most of the control rats treated with adjuvants alone, or adjuvants plus liver, that had not developed proteinuria, also had histological changes of similar character, but lesser in severity, in their kidneys.

Discussion. While it has been noted that repeated intraperitoneal injection of Freund's adjuvants alone in large doses did produce a mild nephrotic disease in 3 of 10 rats, and even though it has been shown that smaller amounts of adjuvants resulted frequently in slight histological alterations of the glomerular structure even when no proteinuria was noted, we have shown that addition of rat kidney supernate enhances this effect markedly. The observation that rat liver supernate did this in a milder form only 3 out of 21 times, while muscle and lung suspensions remained without effect, and the fact that the amounts of kidney proteins injected in 2-week intervals were furthermore impressively small (2-2.5 mg), supports the interpretation that an iso- and autosensitization mechanism is at play. In view of the fact that 5 of 7 rats treated with adjuvants and the supernate of 25% kidney suspension developed the ne-

phrotic syndrome makes it unlikely that the failure of liver, lung and muscle to produce the same effect was due to lower concentration of tissue proteins in these suspensions. Further immunological studies to evaluate this view are in progress.

Summary. 1) A severe nephrotic syndrome was noted in 20 rats given Freund's adjuvants and a supernate of rat kidney suspension by intraperitoneal injection. When kidney tissue was replaced by liver, only 3 of 21 animals developed a much milder renal disease. Lung or muscle suspensions failed to induce proteinuria in 10 rats. 2) In large doses (0.5 ml) Freund's adjuvants alone produced a mild nephrotic disease in 3 of 10 rats. Even though the smaller amounts of the adjuvants usually used (0.25 ml) never produced proteinuria, slight histological alterations of the glomerular structure were frequently noted. 3) In contradistinction to other tissue suspensions addition of rat kidney supernate enhanced the effect of adjuvants markedly and regularly. 4) The small amounts of rat kidney proteins used, support the interpretation that an iso- and autosensitization mechanism is at play.

1. Heymann, W., Lund, H. Z., *Pediatrics,* 1951, v7, 691.

2. Lange, K., Slobody, L., Craig, F., Ojur, G., Oberman, J., LoCasto, F., *ibid.,* 1951, v8, 814.

3. Wedgwood, R. J. P., Janeway, C. A., *ibid.,* 1953, v11, 569.

4. Mellors, R. C., Ortega, L. G., *Am. J. Path.,* 1956, v3, 455.

5. Heymann, W., Nash, G., Gilkey, C., Lewis, M., *J. Clin. Invest.,* 1958, v37, 808.

6. Kersten, H., Bresene, W. G., Jr., Ablondi, F., Subbarow, Y., *J. Lab. and Clin. Med.,* 1947, v32, 1090.

7. Lieb, E., *Am. J. Clin. Path.,* 1947, v17, 413.

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Precocious Sexual Development in Female Rats with Hypothalamic Lesions.* (24737)

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Appropriate hypothalamic lesions may impair selectively the secretion of several anterior pituitary hormones(1,2). This strongly suggests that the nervous system can exert discrete stimulating, or at least permissive, influences upon anterior lobe function. The converse possibility, that it may also act to suppress trophic hormone secretion, has been raised by several recent findings(3-6). This report, on effects of hypothalamic lesions in weanling male and female rats, deals further with this possibility.

Methods. Holtzman rats were weaned and subjected to bilateral electrolytic hypothalamic lesion placement at 18-19 days of age. Littermates of the same sex and approximate weight served as sham- and unoperated controls. Each litter was kept in a small cage and fed Big Red dog pellets and tap water, *ad lib.* During the first 3-4 post-operative days they were also given evaporated milk. Litters were sacrificed 10-15 days after surgery *i.e.,* at 28-33 days of age. Brains were fixed in 10% neutral formalin, trimmed to blocks of diencephalon, Tissuemat-embedded and sectioned serially at 10 μ. Every 9th and 10th section was stained with thionine for lesion localization studies. Other tissues were fixed, after rapid trimming and weighing, in Susa-picric acid or Helley's fluid. Routine 6 μ sections were stained with Gomori's trichrome-hematoxylin. Ovaries, sectioned serially at 10 μ, were also trichrome-stained. Hypophyses of females were examined by means of both periodic acid - Schiff reagent - hematoxylin (PAS - 6 μ) and aldehyde-fuchsin - trichrome - hematoxylin (AF - 4 μ) stained

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