TABLE I. Average Daily Urinary Excretion of Free Glycine and Free Amino Nitrogen by Control and Vit. E Deficient Rabbits. (Results expressed as mg/kg body wt ± stand. error.)

	Deficient animals			Control animals		
Period of exp. (wk)	Glycine	Amino N	Glycine N as % of amino N	Glycine	Amino N	Glycine N as % of amino N
1st	19.3 ± 1.5	8.4 ± 3.1	43	26.4 ± 3.4	10.9 ± 1.4	45
2nd	23.5 ± 4.3	8.9 ± .3	49	25.8 ± 2.1	9.5 ± 2.0	51
3rd	19.5 ± 2.1	8.6 ± 2.4	42	25.8 ± 3.8	11.8 ± 1.0	41
4th	16.9 ± 3.2	15.5 ± 3.7	20	17.7 ± .8	7.4 ± 1.0	45

Results of quantitative determinations of glycine and aminonitrogen are summarized in Table I. The slight decrease of urinary glycine in the deficient group is inconclusive in view of a decrease of glycine in the control group. But the increase of amino-nitrogen in the deficient group is certainly not paralleled by an increase of glycine. This becomes clearer from columns 4 and 7 of Table I, where glycine-nitrogen (calculated from the glycine content) is expressed as percent of total free amino-nitrogen content. In the control group glycine accounts for 41 to 51% of amino-nitrogen, glycine content in the deficient drops to 20% of amino-nitrogen.

The present results seem of interest in view of findings of other authors that glycine is in some way implicated in the dystrophic state. Tallan(8) reported increased concentration of most free amino acids in muscle extracts of dystrophic rabbits except for glycine, which was significantly reduced. This was confirmed by Smith and Nelson(9). Smith and Nehorayan(10) showed recently that reduction of tissue phosphatase observed in dystrophic rabbits is prevented by addition of 1% glycine to the diet.

Summary. 1. That excretion of free amino nitrogen in urine is elevated in dystrophic state of rabbits on Vit. E-free diet has been confirmed. 2. Glycine shows an exceptional behavior insofar as increased amino nitrogen excretion is not paralleled by increased glycine excretion.

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Autoradiography of the Isolated Nephron.* (24735)

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Detection of radioactive substances in tissues by autoradiography has been widely used. Difficulty has sometimes been experienced in interpreting the exact site of activity within the kidney owing to its complex anatomy and that histological sections do not give

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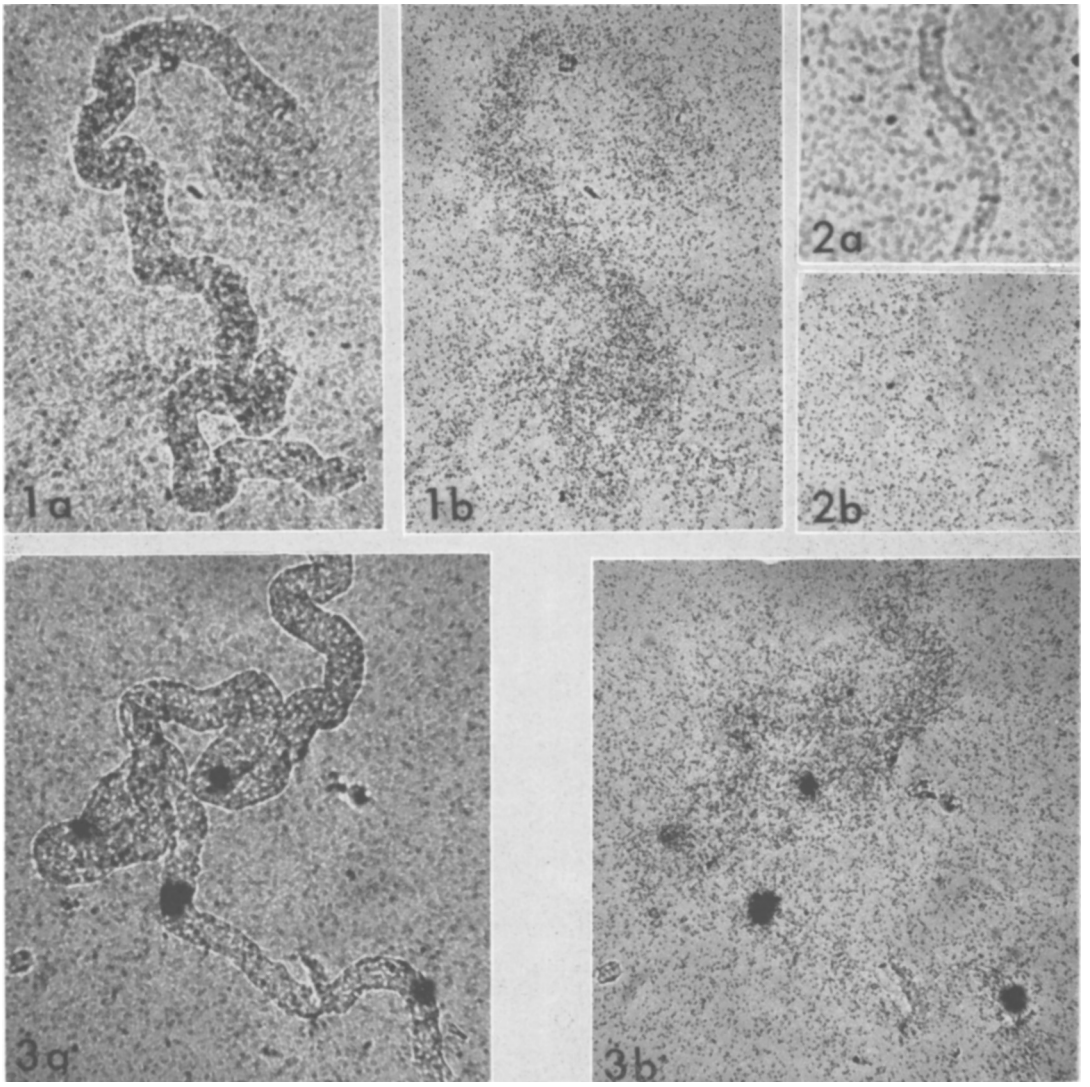

FIG. 1. Autoradiogram of proximal convoluted tubule from a rat receiving 2 mg of C_{14} Vit. D_2 (spec. activity 1.15 μc/mg). (a) Focused on tubule (left); (b) Focused on stripping film overlying the same area and showing marked activity in proximal convoluted tubule (right) × 95.

FIG. 2. Autoradiogram of ascending limb from same kidney as in Fig. 1. (a) Focused on tubule (top right); (b) Focused on stripping film over the same. Note absence of activity (bottom right) × 95.

FIG. 3. Autoradiogram of ascending limb and distal convoluted tubule from a rat inj. intrav. with 16 units of I_{131} Pitressin (spec. activity 1 μc/unit). (a) Focused on tubules (left); (b) Focused on stripping film overlying same area, showing a lack of activity over ascending limb, *i.e.*, between the 2 artifacts, but with marked activity over distal convoluted tubules (right) × 95.

continuity in the picture of the nephron. The technic described below overcomes these difficulties and enables more precise localization to be made.

Methods. After injection of radioactive material into an animal, it was killed and the kidneys removed and fixed quickly in 10% Formal saline. The nephrons were then prepared by microdissection by the method previously described(1), and mounted on microscope slides. Adherence of the nephrons was insured by allowing the preparation to dry at

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.

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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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