Summary. The ether, neutral, and phenolic fractions of the urine of normal individuals and of patients with carcinoma of the breast, cervix, gastrointestinal tract, and with various leukemias were analyzed polarographically. Reducible compounds were found in all the fractions. In the urine phenolic fraction the ratio (of the number of polarographically reducible compounds with an $E_{1/2}$ more negative than —0.5 V vs SCE to that more positive than—0.5 V) was significantly different for male and female patients with gastrointestinal cancer as compared to normal persons of both sexes. There was no difference in this ratio between the neutral urine fraction of normal persons and cancer patients. The ether fraction of the urine of males with gastrointestinal cancer had a much higher ratio (number of compounds with an $E_{1/2}$ more negative than —0.5 V vs SCE/number of compounds with $E_{1/2}$ more positive than —0.5 V) than that of the same fraction of normal males. Finally a significant difference was found in the ratio (of the number of reducible compounds with an $E_{1/2}$ more negative than —0.76 V vs SCE to that more positive than —0.76 V) of the ether fraction of normal male urine as compared with that of men with gastrointestinal cancer. Patients with various types of cancer did not excrete reducible compounds characteristic of any form of this disease.

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An *in Vitro* Demonstration of Cellular Sensitivity in Experimental Autoimmune Nephrosis in Rats* (32914)

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The precise mechanism by which injections of Freund's adjuvant and kidney produce the nephrotic syndrome in rats is not completely clear. Recent studies in our laboratory (1) would indicate that antigens from the apical, periluminal portion of the proximal tubular cell, complexed to the antibody they initiate, deposit in the glomeruli of the sensitized animal, inducing glomerular damage. The role, if any, that might be played by a delayed hypersensitivity phenomenon has not been fully elucidated, though this mechanism has been suggested (2). Independent reports by Heymann (3) and by Hess (2) demonstrate that a nephrotic disease can be produced in normal, immune tolerant Sprague-Dawley rats by the transfer of splenic or lymph node cells from donors with autoimmune nephrosis. The fact that this transfer has not been repeatable in an isogeneic strain (4) does not negate or explain that something was induced in the recipients of sensitized cells that was not induced in recipients of normal or control cells. It seemed reasonable, therefore, to examine the cells involved from both allogeneic and isogeneic rats for evidence of a cellular sensitivity similar to that seen in delayed hypersensitivity, namely the specific inhibition of cell migration from capillary tubes in tissue culture by the sensitizing antigen.

Materials and Methods. Autoimmune nephrosis was produced in ten Sprague-Dawley and ten Lewis rats by intraperitoneal injections of a suspension of complete

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Freund's adjuvant and homologous rat kidney by methods described by Heymann *et al.* (5). Five other Sprague-Dawley rats were made nephrotic by the single intravenous injection of 1.0 ml/100 gm of a 2% solution of the aminonucleoside of puromycin (6) as a disease control group. Five normal rats, five rats injected with kidney alone and five rats injected with complete Freund's adjuvant alone were also included as controls.

Test antigens were prepared from the saline homogenates of rat kidney cortex, liver, lung, and intestine by centrifugation at 100,000*g* for 1 hour. The supernatant of this was dialyzed against normal saline, lyophilized and frozen at —20°C until used.

The method of placing cells in culture is based on that of George and Vaughan (7). Nephrotic rats with well-established disease of 5–12 weeks duration were given a booster injection of adjuvant-kidney suspension. The animals were sacrificed 7–10 days later and their spleens removed, which were minced and teased in tissue culture medium-199 (TC-199).[1] The resulting cell suspension was separated by pipette from the larger tissue pieces and centrifuged at 315*g* for 10 min. The red cells in the mixture were lysed by two successive exposures to 0.35% saline after the method of Janowsky *et al.* (8). The cells were again washed with TC-199. This fourth and final wash was free of rat globulin by immunoelectrophoresis and capillary precipitation against rabbit antirat gamma-globulin serum. After resuspension in TC-199, the cells were drawn into capillary tubes,[2] and centrifuged at 315*g* for 10 min. The tubes were broken at the cell–fluid interface and the segments containing the packed cells were placed in 35 mm tissue culture petri dishes.[3] These cell preparations were 90–95% mononuclear cells of which 80–90% were lymphocytes. Cell viability as measured by trypan blue or eosin exclusion ranged from 85–95%. Spleens from aminonucleoside and control rats were handled in the same fashion.

Each experiment included a minimum of two tubes per chamber. One chamber served as a control containing 2.2 ml of tissue culture medium and 10% normal rat serum. An additional chamber was prepared for each antigen tested, the lyophilized antigen was dissolved in 2.2 ml of the tissue culture–normal serum mixture to obtain a final concentration of 50 mg/100 ml of antigen protein. The chambers were sealed with sterile silicone grease and incubated at 37°C for 24 hours. During this time, cells migrated from the open end of the capillary tube onto the floor of the chamber. This area of migration was photographed at a standard magnification, traced, then measured with a planimeter. The degree to which migration was inhibited in each experiment is expressed as the cytotoxic index (CI), which is the ratio of the average area of migration in the antigen chamber to the average area of migration in the control.

The presence of precipitating antibodies to whole kidney homogenate was determined by the method of Ouchterlony (9). The severity of the renal disease was determined by the degree of proteinuria according to the following scale: 1+ = proteinuria of 0.3–0.8 gm/100 ml or 0.05–0.1 gm/24 hours; 2+ = proteinuria of 0.9–1.5 gm/100 ml or 0.1–0.2 gm/24 hours; 3+ = proteinuria of 1.5–2.0 gm/100 ml or 0.2–0.4 gm/24 hours; 4+ = proteinuria of >2.0 gm/100 ml or >0.4 gm/24 hours. The nephrotic syndrome was confirmed by the presence of proteinuria, hypoproteinemia, and hyperlipemia. Anasarca or ascites was seen only in the aminonucleoside nephrotic rats.

Results. The migration of splenic cells from Sprague-Dawley animals with autoimmune nephrosis was inhibited by kidney antigens as shown by the example in Fig. 1. The specificity of the reaction is demonstrated by the moderate inhibition by liver but not by lung antigens.

Table I presents the results on all Sprague-Dawley autoimmune nephrotic rats related to disease severity and the presence or absence of precipitating antibodies to kidney. All but one animal demonstrated marked inhibition with kidney with a cytotoxic index ranging from 0.06 to 0.51. Most showed moderate

[1] Obtained from Difco Laboratories.

[2] A. H. Thomas, Company.

[3] Kimax-Kimble Glass Company.

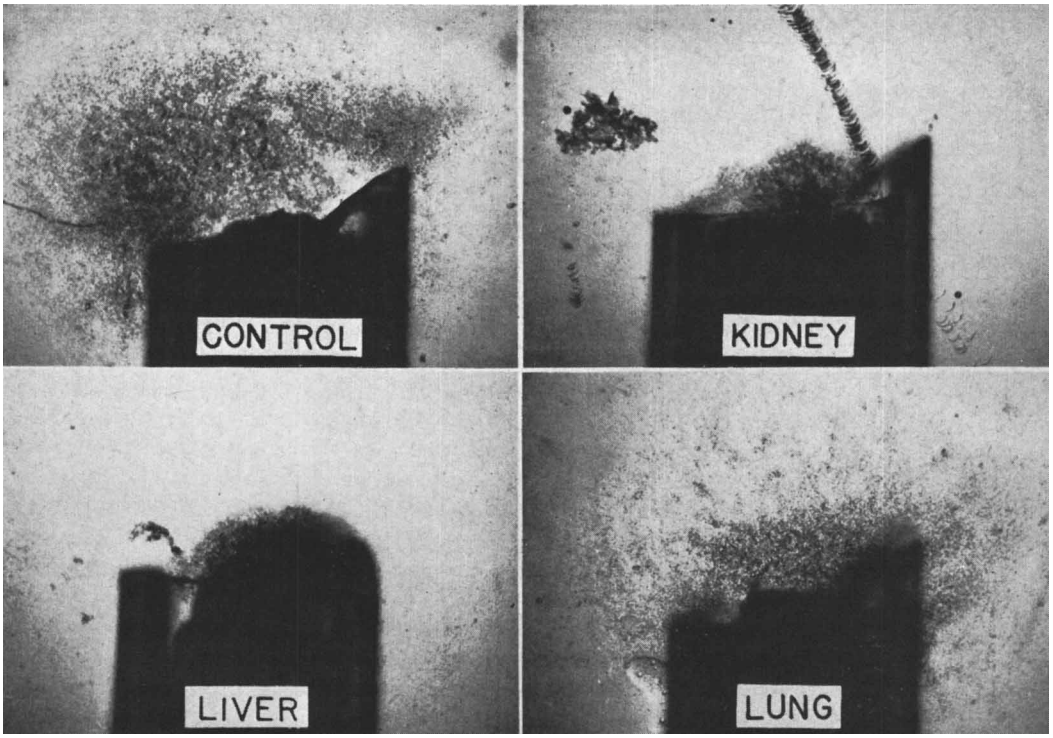

FIG. 1. Effect of tissue extracts on the migration of splenic monocytes from a rat with autoimmune nephrosis. Upper Left: Cells in control media; Upper Right: Cells inhibited by media containing kidney antigens (C.I. 0.38); Lower Left: Cells inhibited by media containing liver antigens (C.I. 0.40); Lower Right: Cells in media containing lung antigens.

TABLE I. Results of Migration in Sprague-Dawley Rats with Autoimmune Nephrosis.

Rat no.	Cytotoxic index				Precipitating antibodies	Severity of disease
	Kidney	Liver	Lung	Intestine		
1	0.06	0.24	1.10	1.45	—	2+
2	0.12	0.46	0.92	—	—	2+
3	0.42	0.67	1.16	—	1+	1+
4	0.51	0.73	—	1.00	0	1+
5	0.28	0.37	0.35	—	2+	2+
6	0.29	1.13	0.71	1.53	0	2+
7	0.13	1.36	1.20	0.92	0	1+
8	1.10	0.97	1.25	—	3+	2+
9	0.43	0.72	1.44	0.82	1+	0
10	0.21	0.36	0.78	—	0	0

inhibition with liver, but not with lung or intestine.

The results in Lewis rats with autoimmune nephrosis related to disease severity is presented in Table II. Again migration was markedly inhibited by kidney antigens (CI 0.14–0.57) and moderately inhibited by liver (CI 0.36–0.91).

Cells collected from animals with nephrosis produced by aminonucleoside were not inhibited *in vitro* by the same antigens as shown in Fig. 2. Nor was inhibition evident in any

TABLE II. Results of Migration in Lewis Rats with Autoimmune Nephrosis.

Lewis AK	Cytotoxic index			Disease severity
	Kidney	Liver	Lung	
1	0.33	0.46	0.87	2+
2	0.36	0.36	0.60	2+
3	0.57	0.45	0.80	3+
4	0.17	0.57	—	2+
5	0.14	0.91	0.89	3+
6	0.29	0.65	—	2+
7	0.38	0.40	0.92	2+
8	0.25	0.71	0.82	3+
9	0.21	0.98	0.84	3+
10	0.31	0.62	0.87	3+

normal rat or in control rats injected with either Freund's adjuvant alone or kidney homogenate alone.

The average cytotoxic index for each antigen in each group of animals is summarized in Table III. Since there was no inhibition in any of the three control groups, the data for these were pooled in the statistical analysis. The average migration of splenic cells from both the allogeneic strain (Sprague-Dawley) and the isogeneic strain (Lewis) was markedly inhibited by exposure to kidney antigen when compared with the control animals ($p<.001$). The degree of inhibition was somewhat less ($p = .01–.02$) with liver in

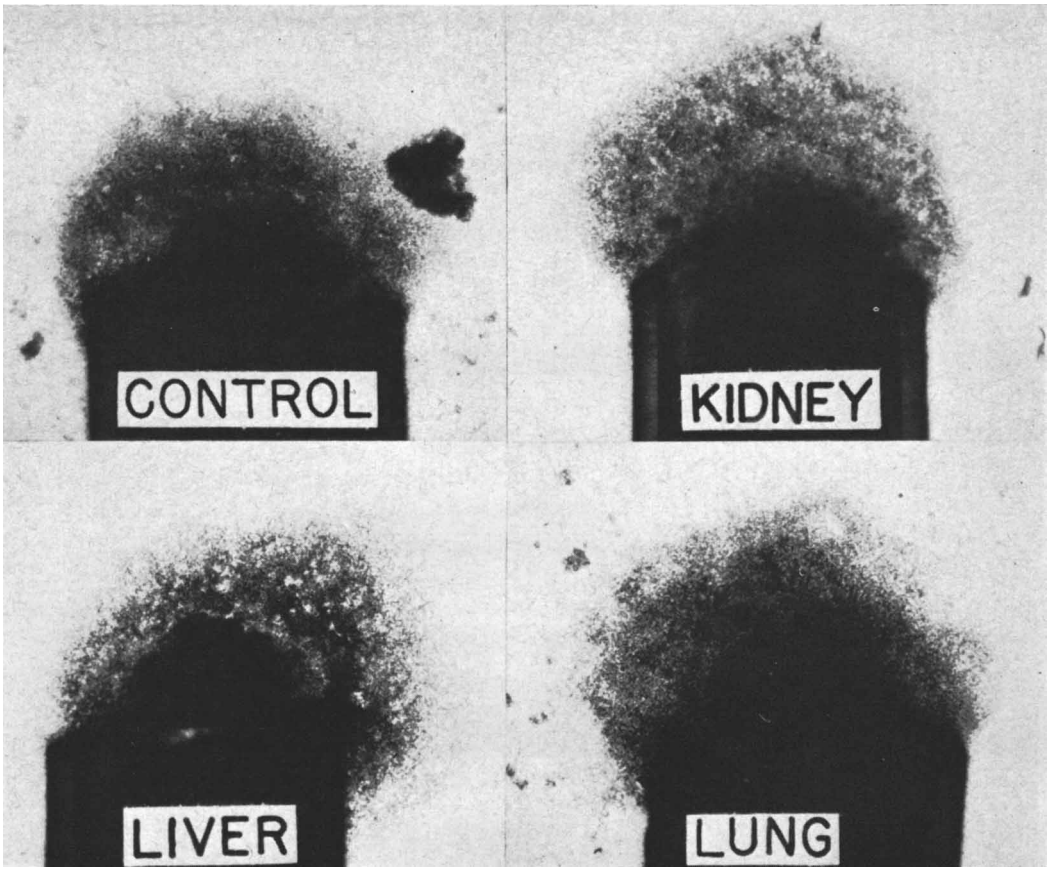

FIG. 2. Effect of tissue extracts on the migration of splenic monocytes from a rat with aminonucleoside nephrosis. Upper left: cells in control media; upper right: Cells in media containing kidney antigens; lower left: cells in media containing liver antigens; lower right: cells in media containing lung antigens. No significant inhibition is present. Results are similar to that seen with normal and control animals.

TABLE III. Summary of Migration Results.

Rats	No.	Av cytotoxic index			
		Kidney	Liver	Lung	Intestine
SDAK[a]	10	0.35 ($p<0.001$)	0.70 ($p=0.02$)	0.98	1.14
Lew AK[b]	10	0.31 ($p<0.001$)	0.61 ($p=0.01$)	0.83	—
IVAN[c]	5	0.82	1.01	—	—
Control[d]	15	0.89	0.81	0.80	0.86

[a] Sprague-Dawley rats immunized with Adjuvant-Kidney.

[b] Lewis rats immunized with Adjuvant-Kidney.

[c] Nephrotic syndrome produced by intravenous aminonucleoside of puromycin.

[d] Includes 5 normal rats, 5 rats injected with Freund's adjuvant alone, and 5 rats injected with kidney homogenate alone.

both strains compared to the control rats. These sensitized cells were not inhibited by extracts of either lung or intestine; if anything, in the Sprague-Dawley animals, lung and intestine stimulated migration, presumably by supplementing the nutrient media.

Conclusions. The migration of splenic cells from animals with autoimmune nephrosis is clearly inhibited *in vitro* by exposure to extracts of rat kidney but not inhibited by lung or intestinal extracts. An interpretation, however, depends on the specificity of the test used for the detection of the delayed hypersensitivity state. Previous studies (7,10,11) have clearly demonstrated that peritoneal exudate cells from guinea pigs exhibiting delayed hypersensitivity skin reactions are inhibited by the specific antigen whether or not these animals are also producing circulating antibody. In addition, cells from animals producing circulating antibody, but not displaying delayed hypersensitivity reactions, are not inhibited *in vitro*. Likewise, in this study, it was shown that the inhibition of cell migration was independent of the demonstration of circulating antibody. Animals 4, 6, 7, and 10 (Table I) never demonstrated antibodies to kidney antigens during the course of their immunization though all demonstrated clear inhibition with kidney antigen. Animal 8, on the other hand, had the most pronounced and persistent antibodies in the group but failed to demonstrate inhibition with any antigen.

It would appear, therefore, that this *in vitro* test is specific for the detection of delayed hypersensitivity and that in autoimmune nephrosis such cellular sensitivity is specifically directed to kidney related antigen. Further interest is added by the deomonstration of Holm (12) that lymphoid cells obtained from autoimmune nephrotic rats are cytotoxic to rat kidney cells *in vitro*.

The moderate sensitivity demonstrated to liver is interesting in that this organ is known to share many antigens with kidney (13,14). This cellular sensitivity is specific for the disease autoimmune nephrosis; this is revealed by the fact that the same syndrome produced by aminonucleoside, a disease with no known immunologic basis (6,15), fails to demonstrate any significant inhibition of migration.

There was no apparent correlation between the degree of inhibition and the disease severity (Tables I and II). Even more significant, perhaps, is that animals 9 and 10 (Table I) never developed the nephrotic syndrome yet both demonstrated significant inhibition by kidney antigen while animal 8 (Table I) was capable of developing a moderately severe disease without demonstrable cellular sensitivity.

The finding that splenic cells from animals with autoimmune nephrosis display such cellular sensitivity might suggest that the transfer disease in previous reports (2,3) could be mediated by these cells. However, the same degree of sensitivity was demonstrated in an isogeneic strain where such a transfer has thus far not been possible (4). Nevertheless, this demonstrates yet another immunologic response in these animals that would allow the further use of this experimental model in the study of the delayed hypersensitivity phenomenon and the evaluation of renal antigens.

Summary. The migration of mononuclear cells isolated from the spleens of rats with experimental autoimmune nephrosis was inhibited by extracts of rat kidney in both allogeneic and isogeneic strains. The migration was partially inhibited by the presence in the

medium of rat liver but not by other tissue extracts. This effect appeared whether or not circulating precipitating antibody against kidney antigens could be deomonstrated. The specificity of this reaction was further demonstrated in aminonucleoside nephrosis which involves no known immunologic mechanism; splenic monocytes from these animals, as well as from control animals, failed to show any inhibition of migration in the presence of these same antigens. There was no correlation between the degree of inhibition and the severity of disease. The role, if any, played by this specific cellular sensitivity in the pathogenesis of autoimmune nephrosis remains to be elucidated.

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Ovulation in the Rabbit Related to Dosage of Human Chorionic Gonadotropin and Pregnant Mare's Serum* (32915)

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Since the rabbit is an induced ovulator, it is very useful for the study of chemical stimuli for ovulation. Hill (1) reported that ovulation in the rabbit is due to the release of a neurosecretory substance, the chemical nature of which was not elucidated. Saxton and Greene (2) provided evidence that the female rabbit hypophysis releases follicle-stimulating hormone (FSH), luteinizing hormone (LH), and probably adrenal stimulating hormones as a result of mating. White and Leonard (3) demonstrated a rise in threshold of ovulatory response to anterior pituitary hormone and prolan (extract of pregnancy urine) gonadotropin in a rabbit hypophysectomized while in heat. A wide range of dosages of LH and human chorionic gonadotropin (HCG) used as a single stimulus (i.v.) for the induction of ovulation has produced ovulatory responses similar in type and timing to those obtained with natural mating (4–6). Unlike LH and HCG preparations, pregnant mare's serum (PMS) has been used primarily as an ovulatory priming substance as a consequence of

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