compared with Morris hepatoma 5123 are in progress.

In the search for drugs effective against hepatic tumors one must take into account the fact that a drug may be active against some types of liver hepatomas but inactive against others. Thus, a screening program for drugs effective against hepatomas should include both the malignant slow growing "minimal-deviation" tumors and the rapidly growing "multiple deviation"(8) tumors.

Summary. Several types of transplantable hepatomas were assayed for their ability to metabolize Cl^{36}-DCM *in vitro*. The Novikoff tumor lacked the ability to metabolize DCM. The tumor 3683 had a slight amount of enzyme activity. Other tumors assayed, *i.e.*, 5123, 7800, 5123 TC and Reuber hepatomas had activity similar to normal liver. Possible therapeutic implications of these findings were discussed.

The authors wish to thank Miss Maggie J. Earle for technical help in enzyme assays.

1. Oliverio, V. T., Davidson, J. D., *J. Pharmacol. Exp. Therap.*, 1962, v137, 76.

2. Loo, T. L., Adamson, R. H., *Biochem. Pharmacol.*, 1962, v11, 170.

3. Misra, D. K., personal communication.

4. Adamson, R. H., Fouts, J. R., *Cancer Research*, 1961, v21, 667.

5. Novikoff, A. B., *ibid.*, 1957, v17, 1010.

6. Morris, H. P., Sidransky, H., Wagner, B. P., Dyer, H. M., *ibid.*, 1960, v20, 1252.

7. Reuber M. D., *J. Nat. Cancer Inst.*, 1961, v26, 891.

8. Potter, V. R., *Cancer Research*, 1961, v21, 1331.

9. Oliverio, V. T., Davidson, J. D., *J. Pharmaceut. Sci.*, 1961, v50, 353.

10. Weber, G., Morris, H. P., *Proc. Am. Assn. Cancer Res.*, 1962, v3, 370.

Received October 4, 1962. P.S.E.B.M., 1962, v111.

Transfer of Experimental Auto-Immune Nephrosis in Rats.* (27857)

WALTER HEYMANN, JANET L. P. HUNTER, DONALD B. HACKEL,† AND FRANCIS CUPPAGE

Departments of Pediatrics and Pathology, Western Reserve School of Medicine, Cleveland, Ohio

It has been reported that repeated intraperitoneal (i.p.) injection of rat kidney suspensions incorporated in Freund's complete adjuvants resulted in severe nephrotic renal disease in 87% of 92 rats(1,2). The following observations suggested an autosensitization to rat kidney proteins as the underlying pathogenetic mechanism: (a) The injection of muscle, lung or intestinal mucosa obtained from rats failed to produce renal disease(2); (b) renal cortex proved to be more antigenic than renal medulla; (c) ACTH and cortisone prevented and nitrogen mustard (H_2N) significantly reduced disease incidence(2); (d) complement activity in serum decreased simultaneously with development of proteinuria(3); (e) precipitating antibodies to rat kidney proteins were detected in sera and ascitic fluid, using double diffusion agar plates according to Ouchterlony, and antibodies in sera were also noted, using hemagglutination technics(3).

It is reported here that renal disease thus produced can be transferred to healthy rats by 2 different methods: (a) by parabiosis, and (b) by i.v. injection of emulsions of spleens of rats with incipient renal disease into litter mates treated at birth with emulsions of spleen obtained from other rats of the same litter.

Methods and procedures. Unless otherwise stated, methods and procedures were the same as previously described(1,2,3). The only change in procedures pertaining to disease production was that tissue suspensions

* Supported by grant from Nat. Inst. of Health, P.H.S. and from Am. Heart Assn., Inc.

† Now at Duke University, School of Med., Dept. of Pathology, Durham, N. C.

were no longer centrifuged for 1 hour at 3000 r, but were stirred overnight in a magnetic mixer in the cold (20°C) in order to remove the coarse particles that tended to plug the 20-gauge needles used for i.p. injections.

Transfer by parabiosis. Inbred female litter mates of the Sprague-Dawley strain weighing 180-200 g were parabiosed (skin, muscles and scapulae were joined; abdominal cavities remained closed). They remained undisturbed thereafter for 6-8 weeks. One of the paired animals was then injected every 2 weeks i.p. with the antigenic material (0.25 ml of 0.4-ml rat kidney suspension incorporated in 1.0 ml of Freund's complete adjuvants) up to 10 times, or until proteinuria of >0.3 g % had developed. Thereafter, no further injections were given. The separate collection of urine of parabiosed rats was accomplished by cutting a 1-liter polyethylene bottle lengthwise along one side, permitting the bottle to be unfolded into 2 halves. The insides of the bottles were reinforced by well fitted plastic mesh. For urine collection, parabiosed animals were kept for 4-6 hours, one to each half of the bottle. The neck end of the bottle was tilted downward and an inch-square window cut in the lower parts of the bottle wall permitted the outflowing urine to be collected in a small beaker placed underneath. A fine metal mesh kept feces from slipping through. Urine was collected weekly after parabiosis had been established successfully for 3-4 weeks. Urinary proteins were determined quantitatively and recorded in g % values. At time of death, blood was obtained by cardiac puncture and analyzed for blood urea nitrogen (BUN), creatinine, serum protein and albumin, cholesterol and total lipids. In a control study, 12 single, non-parabiosed rats weighing 130-400 g were injected every second week, 3-9 times, with 0.2 ml of the same antigenic material, intravenously. This was injected slowly within 10 minutes into a tail vein. In these rats, urine was examined twice a week.

Transfer by i.v. injection of spleen emulsions. Inbred litters of rats were divided into 3 groups: a, b and c. Within a few hours after birth and under sterile conditions, 2 spleens of Group a rats were cut into fine pieces and, by stirring with a glass rod, were suspended in 0.4 ml of equal parts Ringer's and 5% dextrose solution. 0.1 ml was given once daily, for 3 consecutive days by i.p. injection to rats of Group b. These were then left alone and permitted to grow, with the exception of 2 rats in which unilateral nephrectomy was performed when they were 3 and 5½ months old respectively. Group c rats, when 150-200 g in weight, were given i.p. injections of rat kidney suspensions incorporated in Freund's adjuvants every 2 weeks until proteinuria of more than 100 mg/24 hr was noted. One more injection was then given, and the animals were killed 5-6 days thereafter. The spleen was minced, suspended in 5 ml of ½ Ringer's, ½ 5% glucose solution and immediately given intravenously to rats of Group b, in 2 injections spaced 8 hours apart. The total amount of lymphocytes injected varied between 100 and 150 million. Urine was examined 2-3 times weekly thereafter until they were sacrificed. A group of 6 control rats was treated in the same fashion. Group b rats were, however, injected with spleen emulsions obtained from Group c animals that had not been given i.p. injections of the antigenic material. All rats of this control group had been subjected to unilateral nephrectomy when 3-5 months old.

Results. Parabiosis transfer. Results of these experiments are summarized in Table I. Nine parabiosed pairs of rats were successfully carried through the stages of parabiosis and production of renal disease by i.p. injection of antigenic material started 6-8 weeks after operation. Parabiosis was established when the animals were 2½ to 3 months old; the treatment of the one rat to be injected was not started before 6-8 weeks thereafter; disease production in the injected rat could take as long as 4 months and these pairs were kept under observation for an additional 1-4 months. Thus, these rats were 10-13 months of age at time of death. A group of 5 parabiosed animals not listed in Table I was observed for a similar period. In these animals renal disease had not been produced in the injected rat in spite of 2-10 i.p. injections of the same antigenic material. Proteinuria was not observed, blood chemistry data were nor-

mal at time of death, and no significant histological alterations were noted in their kidneys. Table I shows that renal disease was produced in the injected animal of all 9 pairs, and that evidence of renal disease in the non-injected parabiont was unquestionably obtained in 8 of these. Even the non-injected rat #6 (Table I) had on 2 consecutive urine examinations some slight proteinuria and azotemia at time of death. Proteinuria had, however, been noted only for one week prior to sacrifice, and hypoalbuminemia and hyper-

TABLE I. Disease Transfer by Parabiosis.

No.	Rats	No. of inj	Maximum proteinuria, g%	Disease duration, mo	Serum chemistries					Severity of glomerular histologic abnormality
					BUN, mg%	Creatinine, mg%	Cholesterol, mg%	Total lipids, mg%	Serum protein, g%	Albumin, g%	
19	Inj	8	2.3	4	39	2.4	285	1610	5.8	2.9	++
20	Not inj		1.42	3¼	36	2.6	336	1320	6.2	3.9	++
44	Inj	5	2.1	4½			220	1060			—
45	Not inj		2.3	3	51	1.3	210	1100	7.3	3.1	+
3	Inj	8	1.8	1	30	1.0	194	740	7.3	3.0	++
4	Not inj		2.1	½	24	1.0	160	860	6.5	2.8	++
5	Inj	8	2.0	1½	13	.7	128	580	7.5	3.4	+
6	Not inj		.35	¼	55	.8	125	460	7.3	3.7	—
13	Inj	7	1.38	1½	24	.8	195	930	7.9	3.9	—
14	Not inj		1.7	1½	20	.8	209	750	6.5	2.7	—
42	Inj	5	1.9	3							
43	Not inj		2.4	2½							
46	Inj	6	2.0	3							
47	Not inj		3.62	3							
9	Inj	6	.648	1							
10	Not inj		.576	¼							
48	Inj	6	.94	2	30	1.0	100	610	8.6	3.8	+
49	Not inj		.72	1¼	20	.98	130	580	8.4	4.5	—
	Normal values (avg)		.288		16	.9	108	388	6.5	4.2	

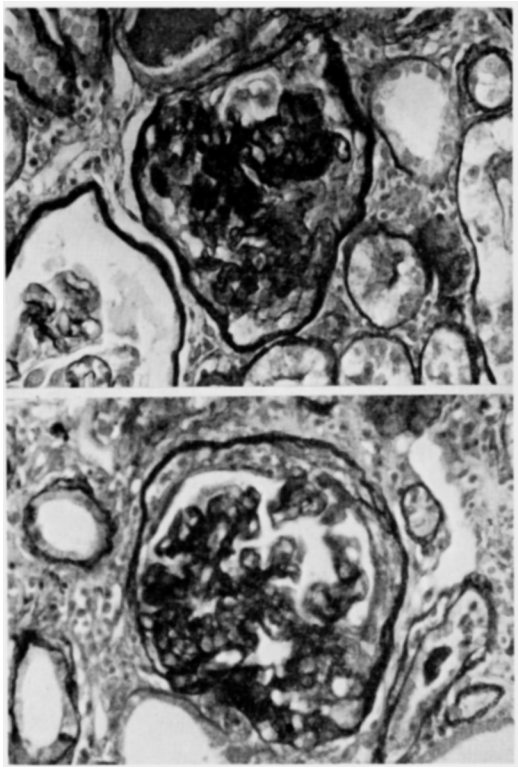

FIG. 1. 6 μ sections of kidneys of parabiosed rats 19 and 20 (Table I). Periodic acid Schiff stain. Magnification 245 ×. Lower glomerulus from the injected rat 19, upper glomerulus from non-injected parabiont 20. Both show moderate focal thickening of basement membrane of the glomerular tuft with distortion of many capillaries. Some capillary lumens are nearly completely occluded. Cytoplasm of both visceral and parietal epithelial cells is swollen. Multiple adhesions extend between capillary tufts and thickened Bowman's capsule.

lipemia were not with certainty beyond the range of normal variations. Onset of proteinuria in the non-injected rats occurred twice simultaneously with the proteinuria in the injected animal; in the remaining 6 pairs, it developed 2-5 weeks later. In the sera of 2 of these pairs, precipitating antibodies (Ouchterlony technic) to rat kidney proteins were observed at time of death in the sera of both injected and non-injected rats. The alterations of glomerular structure noted in parabiosed rats 19 and 20 of Table I are seen in Fig. 1. The severity of the changes is similar in both animals. This, however, was not always observed. In rats 44 and 45 (Table I), the histological alterations were more marked in the non-injected rats; in rats 5, 6, 42, and 43, they were more marked in the injected animal. In 5 rats, sections of the kidneys stained with HE and PAS did not reveal abnormal findings in spite of marked proteinuria, azotemia, hypoalbuminemia, hyperlipemia with hypercholesterolemia.

Effect of repeated i.v. injections of rat kidney suspensions incorporated in Freund's adjuvants was studied in 12 rats. No proteinuria was noted at any time in 7. Slight and inconsistent increases of excreted urinary proteins were observed in the remaining 5 animals. When sacrificed, blood chemistry values were obtained in sera of 8 animals. Slightly increased values for BUN, creatinine, cholesterol and total lipids were noted in 2 or 3 rats respectively. All other values were within normal range in the remaining 5-6 animals. Aside from regularly noted enlargement of the spleens, the autopsies did not reveal gross abnormalities. Histological examinations of the kidney did not show any abnormalities in 10 rats. In 2 rats, the glomerular basement membranes showed slight to moderate focal thickening. Endothelial cells appeared unaltered in one animal, but there was a focal increase in endothelial cell nuclei within glomerular tufts in the other. Whereas no adhesions or crescents were noted in the latter, few glomeruli of the former rat had adhesions between the tufts and capsule and rare crescentlike formations were identified in the more adherent glomeruli.

Transfer by i.v. injection of spleen emulsions. Nine rats, treated at birth with spleen emulsions obtained from litter mates, were given 5-8 months thereafter spleen suspensions by i.v. injections, which were obtained from litter mates in whom nephrotic renal disease had just been produced by repeated intraperitoneal injections of rat kidney suspensions incorporated in Freund's adjuvants. Their urine was tested thereafter 2-3 times weekly, and they were observed for up to 2½ months. No proteinuria was noted in 7 of these and when sacrificed, blood chemistry data and the histology of the kidneys did not reveal any abnormality. Two rats that had been subjected to unilateral nephrectomy 2½ and 4 months, respectively, prior to the i.v.

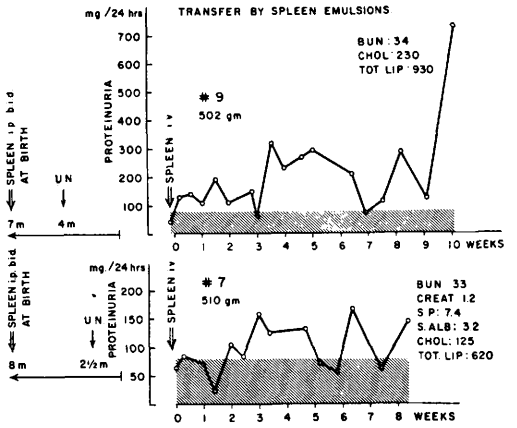

FIG. 2. Ordinate = Proteinuria (mg/24 hr) after i.v. injection of spleen emulsion. Abscissa = Time in wk after i.v. injection of spleen emulsion. Shaded area = Range of proteinuria in control rats 2-4 mo after unilateral nephrectomy. To left of ordinate: double arrow indicates i.p. injection of spleens from litter mate at birth, 7-8 mo, respectively, prior to injection of spleen emulsions obtained from litter mates with incipient autoimmune renal disease. Single arrow at left of ordinate indicates time of unilateral nephrectomy (U.N.) prior to transfer of spleen emulsions. Chemistries obtained at time of death: Chol = Serum cholesterol, mg %; Total Lip = Total lipids, mg %; Serum protein (S.P.) and serum albumin (S. Alb.) in g % and Creatinine (Creat) in serum, mg %.

injection of spleen emulsions, developed increasing proteinuria after 2 days and 2 weeks, respectively (Fig. 2). At time of death, azotemia and hyperlipemia were noted in both (Fig. 2). The histological lesions in the kidneys of both rats were similar and showed moderate focal thickening of the glomerular basement membrane with many adhesions between the glomerular tuft and Bowman's capsule. Early crescents were also noted in both. As controls, 6 rats treated at birth with spleen suspensions obtained from litter mates, were subjected to unilateral nephrectomy 3-5½ months thereafter, and were given suspensions of the spleen of untreated healthy litter mates by i.v. injections 2½-4 months thereafter. Proteinuria was not noted in any of these animals. At time of death, blood chemistry values and the histology of the kidneys were within normal limits.

Discussion. Proteinuria, hypoproteinemia and hyperlipemia developed in 9 non-injected rats that had been living in parabiosis with litter mates that developed nephrotic renal disease after repeated i.p. injections of isologous kidney material incorporated in Freund's complete adjuvants. The lack of definite histological abnormalities in the kidneys of 5 of these parabiosed rats is in keeping with our experience that early stages of this experimental disorder have frequently been characterized by a paucity of histological alterations visible by light microscopy. Changes in the basement membrane are usually noted in diseases of longer duration. The question could be raised, however, whether renal disease observed in the non-injected parabiont could be explained by transfer of the antigenic material rather than by transfer of antibodies or antibody producing cells. It is well to remember that the subcutaneous, intramuscular, or foot pad injections of the same material have thus far failed to produce renal disease in rats. Moreover, in the control studies the same antigenic material given repeatedly by i.v. injection, did not produce nephrotic renal disease in 12 rats, though 2 of these animals showed some histological evidence of glomerular injury, and some slight and inconsistent proteinuria was noted in 5 of these rats. We thus believe that renal disease in the non-injected parabiont occurred by immunochemical transfer, rather than by transfer of antigenic substances into this parabiont.

The transfer of the disease by i.v. injection of suspensions of lymphocytes obtained from the spleens of animals with incipient renal disease into rats made immune tolerant after birth, is in agreement with the above statement. In our studies only 2 of the 9 so treated rats developed severe membranous glomerulonephritis while Hess, Ashworth and Ziff(4) noted this in 13 of 14 animals, perhaps because these authors used the same animal as donor for lymphocytes to induce tolerance and to transfer antibody producing cells later, whereas we used 2-3 different litter mates for this purpose. That our 2 positive transfers were only observed in the 2 animals that had been subjected to unilateral nephrectomy prior to the spleen transfer may be explained by the diminished amount of renal tissue available for a limited antibody exposure.

The possibility of transferring this experi-

mental renal disorder by parabiosis as well as by a technic first described by Paterson (5), supports the contention that this experimental membranous glomerulonephritis is due to an autoimmune mechanism. Whether the described circulating antibodies(3) produce renal disease, or whether a delayed hypersensitivity mechanism is at play, is not clear.

Summary. 1. Fourteen pairs of parabiosed litter mates were used. In 9 of these autoimmune nephrosis was produced in one of the paired rats by repeated i.p. injection of rat kidney suspensions incorporated in Freund's complete adjuvants. Nephrotic renal disease was noted in all 9 non-injected parabionts, simultaneously with or shortly after onset of proteinuria in the injected rat. In the 5 parabiosed pairs in which the injected rat failed to develop renal disease, the parabiont that had not been treated did not develop nephrotic renal injury. 2. The same antigenic material was given 3-9 times at 2-week intervals by i.v. injection to 12 rats. Slight and inconsistent proteinuria was noted in 5 and histological alterations of glomerular structures were observed in 2. A nephrotic syndrome of comparable severity was not noted in any of these animals. 3. Suspensions of lymphocytes obtained from the spleens of rats with incipient autoimmune renal disease were given by i.v. injection to 9 litter mates in which immune tolerance had been established at birth. Severe membranous glomerulonephritis was produced in 2 of these animals that had been subjected previously to unilateral nephrectomy. None of the 6 identically treated control rats developed renal disease.

1. Heymann, W., Hackel, D. B., Horwood, S., Wilson, S. G. F., Hunter, J. L. P., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 660.

2. Heymann, W., Hunter, J. L. P., Hackel, D. B., *J. Immunol.*, 1962, v88, 135.

3. Hunter, J. L. P., Hackel, D. B., Heymann, W., *ibid.*, 1960, v85, 319.

4. Hess, E. V., Ashworth, C. T., Ziff, M., *J. Exp. Med.*, 1962, v115, 421.

5. Paterson, P. Y., *ibid.*, 1960, v111, 119.

Received July 17, 1962. P.S.E.B.M., 1962, v111.

Responses to Gonadotrophin in Intact and Hypophysectomized Immature Female Rats as Related to Age.* (27858)

EDWARD H. SOPER AND AARON J. LADMAN†
Department of Anatomy, University of Tennessee Medical Units, Memphis

The development of maximal responsiveness to gonadotrophin of the ovary of the immature rat is closely associated with the chronological age of the animal's endocrine system(1). Current interest in the relationship between ovarian reactivity and age has been aroused by recent studies of induction of ovulation in immature mice and rats(2-6), and the possible use of this response as a means of bioassay for ovulatory gonadotrophin(s)(7-9). The use of intact immature animals for assay of gonadotrophins in place of hypophysectomized ones implies that endogenous gonadotrophins are either absent or that their presence has little or no influence upon the organ responses to administered exogenous gonadotrophins. Indirect evidence that the immature animal secretes gonadotrophin has been adduced from bioassays of pituitary content at varying ages prior to puberty and from parabiosis experiments with normal and gonadectomized animals(10-11). The former experiments assume that changes in pituitary content of gonadotrophin can indicate secretion of the hormone (an assumption which many question), whereas the latter experiments apparently do not consider that gonadectomy may effectively alter the normal pattern of development and secretion of the pitui-

* Supported in part by grant from Nat. Inst. for Arthritis and Metab. Dis., U.S.P.H.S. and from the Division of General Medical Sciences, Public Health Service.

† Research Career Development Award recipient.