It is generally held that the action potential which triggers the contractile process depends on a transmembrane ionic exchange characterized first by an inward movement of sodium followed rapidly by an efflux of potassium (13). Adrenergic blocking agents, in relatively high doses, have been shown to interfere with the transmembrane exchange of ions in resting muscle incubated *in vitro* and this is accompanied by an impaired contractility measured *in vivo, in situ* or *in vitro*(2). Pitressin, which can shift sodium and potassium in the continuing presence of such an adrenergic blockade, restores contractility to the rat gastrocnemius during the limited period of its effectiveness. Perhaps the observation of Ingle and Li rests on the same ability of Pitressin to transfer sodium and potassium (with water) across the cell membrane.

Summary. Intracellular sodium concentration of stimulated rat gastrocnemius muscle increased 3-fold while potassium decreased by almost one-third after 20 minutes of stimulation (20/sec.). Dibenzylene abolished these changes and strikingly reduced the amplitude of muscular contraction. Pitressin (80 mU intravenously) in Dibenzylene treated rats restored both the cationic shifts and muscular contractions during its period of effectiveness. We suggest that the ability of Pitressin to restore work performance is brought about by a direct action on cationic exchanges across the muscle membrane.

1. Sréter, F. A., Friedman, S. M., *Am. J. Physiol.,* 1959, v197, 478.

2. ———, *J. Pharmacol. and Exp. Therap.,* in press.

3. Friedman, S. M., Friedman, C. L., Nakashima, M., *Nature,* 1957, v180, 194.

4. Daniel, E. E., Dodd, A., Hunt, J., *Arch. Internat. Pharmacodyn.,* 1959, v119, 43.

5. Ingle, D. J., Li, C. H., *Endocrinology,* 1955, v57, 383.

6. Ledingham, J. M., *Clin. Sci.,* 1954, v13, 535.

7. Ross, G., Mokotoff, R., *J. Biol. Chem.,* 1951, v190, 659.

8. Hastings, A. B., Eichelberger, L., *ibid.,* 1937, v117, 73.

9. Nelson, N., *ibid.,* 1944, v153, 375.

10. Somogyi, M., *ibid.,* 1945, v160, 61.

11. Barker, S. B., Summerson, W. H., *ibid.,* 1941, v138, 535.

12. Higashi, A., Peters, L., *J. Lab. and Clin. Med.,* 1950, v35, 475.

13. Hodgkin, A. L., *Biol. Rev.,* 1951, v26, 339.

Received July 29, 1960. P.S.E.B.M., 1961, v106.

Immunologic Mechanism of Transmission of Experimental Glomerulonephritis in Parabiotic Rats.* (26221)

KURT LANGE, MAX WACHSTEIN AND STEPHEN E. MCPHERSON

Departments of Medicine and Pediatrics, New York Medical College, New York, N. Y., and Dept. of Pathology, St. Catherine's Hospital, Brooklyn, N. Y.

We reported(1) that in patients with glomerulonephritis antibodies against human kidney could be detected by means of the very sensitive but also very delicate collodion technic. These findings were confirmed by Pfeiffer *et al.*(2) and by Brod(3) with the same technic and by Vorlaender(4) with a sensitive complement fixation technic. More recently Liu and McCrory(5) using the tanned red cell technic and the Ouchterlony gel-precipitation method have come to identical conclusions. On the other hand, Heyman(6) and Goodman(7) could not find such antibodies. All investigators who demonstrated such antibodies agreed with us that antibody titers were lowest or absent in the acute stage of glomerulonephritis and became higher and more consistent later in the disease. We assumed at first that in the acute stage the antibody was completely absorbed by the kidney which was supposed to act like

* This work was supported by grants from Inst. of Arthritis and Metabol. Dis., U. S. P. H. S. and from Kidney Disease Foundation of New York.

TABLE I. Parabiosis of Nephritic Rats.

No. of animals	Primary member: Duration of disease on day of parabiosis (days)	Secondary member: Duration of parabiosis on day of sacrifice (days)
7	8	14
3	16	10
3	16	14
5	45	14
2	55	8

a sponge leaving no or very little free antibody in the circulation. This assumption, however, is not tenable. For we could show (8) that in the experimental rabbit disease produced by antirabbit kidney duck serum, the serum of an animal with acute glomerulonephritis on the second or third day of the disease can produce immediate nephritis when transfused to a sensitized but normal rabbit. This corresponds to a passive transfer of antibody. Therefore, at least in this experimental disease antibody is present in excess practically from onset and is not completely absorbed by the kidney.

One may thus be forced to assume that the antibody against healthy kidney tissue found in human glomerulonephritis and occurring rather late in the disease is possibly not the original antibody causing the disease. It may rather be the result of destruction or alteration of kidney tissue, which then acts as an antigen giving rise to this secondary antibody of unknown significance. To clarify the role of such an antibody which appears late in the disease, we followed the suggestion of Pfeiffer(9) and performed the following experiments.

Methods. Female rats of the Sprague-Dawley strain, weighing approximately 150 g, were made nephritic by intravenous injection of 0.8 ml of antiratkidney rabbit serum. They showed a continuous 4+ proteinuria which began within 24 hours. A series of them was sacrificed at daily intervals during the first 10 days of disease. Others, as shown in Table I were then parabiosed to normal rats according to the method of Bunster and Meyer, by lateral attachment of the peritoneum, but without creating a common peritoneal cavity. Twenty parabioses of such pairs were successfully performed. By intravenous injection of fluorescein into the tail vein of one partner and appearance of a fluorescent urine in the other partner, one can find that it takes approximately 5 days before a good vascular connection between the two partners is established. Following parabiosis, attempts were made to collect daily urines of primary and secondary partners. This was not always possible, since such urines had to be expressed manually from the bladder to avoid mixture with the urine of the other partner. Thus only percentage of protein in the urine could be measured but no 24 hour outputs. In general, after the 5th day of parabiosis, the proteinuria in the secondary partner increased from the value, maximally 100 mg %, found in normal rats to 200 to 800 mg %.

Results. Table I shows duration of disease of the primary nephritic partner in the different groups when he was parabiosed to a healthy partner, and duration of parabiosis before sacrifice. Table II shows a typical example of the proteinuria in a parabiosed pair.

When 6 healthy pairs of rats were parabiosed, no abnormal proteinuria was observed except in some on the day of operation.

Both groups of parabiosed animals and the single animals of the first to the 10th day of disease were then sacrificed and their kidneys studied by routine histology and by Coon's technic of fluorescein labelled antibodies, but using fluorescein isothiocyanate as conjugating agent rather than fluorescein isocyanate. All kidneys of the single animals and of the primary partners showed an intense staining

TABLE II. Proteinuria in Parabiotic Rats (mg %).

Day	Primary partner	Secondary partner
−4	560	90
−2	980	70
−1	540	105
	Parabiosis	
+ 2	1290	116
+ 3	560	anuric
+ 6	1500	380
+ 7	1000	400
+ 8	700	anuric
+ 9	1200	800
+12	1100	760

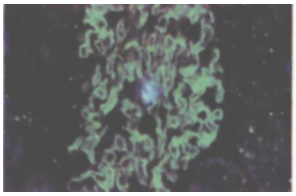

FIG. 1. Cryostat section (3 μ) of kidney of rat 1 day after 0.8 ml antiratkidney rabbit serum i.v. Stained with antirabbit gamma globulin horse serum conjugated with fluorescein isothiocyanate.

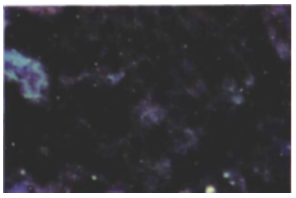

FIG. 2. Cryostat section (3 μ) of kidney of rat 1 day after 0.8 ml antiratkidney rabbit serum i.v. Stained with antirat gamma globulin rabbit serum conjugated with fluorescein isothiocyanate.

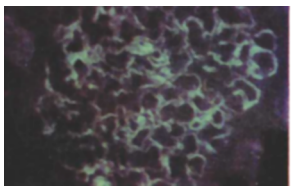

FIG. 3. Same as Fig. 2, but 3 days after 0.8 ml antiratkidney rabbit serum i.v.

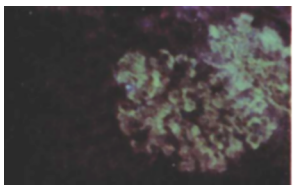

FIG. 4. Same as Fig. 2 and 3, but 7 days after 0.8 ml antiratkidney rabbit serum i.v.

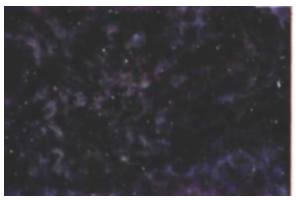

FIG. 5. Same as Fig. 2, 3 and 4, but 30 days after 0.8 ml antiratkidney rabbit serum i.v.

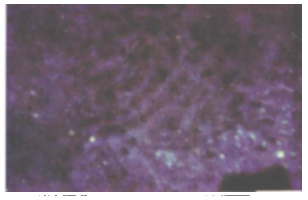

FIG. 6. Cryostat section (3 μ) of kidney of healthy rat parabiosed to nephritic rat for 14 days prior to sacrifice. Stained with antirabbit gamma globulin horse serum conjugated with fluorescein isothiocyanate.

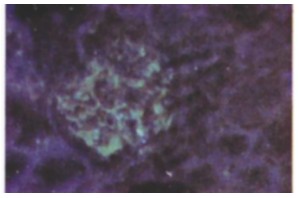

FIG. 7. Same as Fig. 6, but stained with antirat gamma globulin rabbit serum conjugated with fluorescein isothyocyanate.

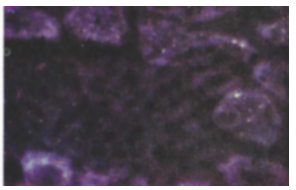

FIG. 8. Same as Fig. 7, but 32 days after parabiosis.

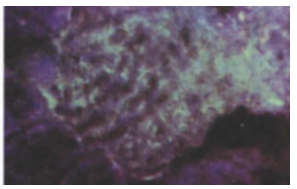

FIG. 9. Same as Fig. 7, but obtained from the parabiosis of two healthy rats.

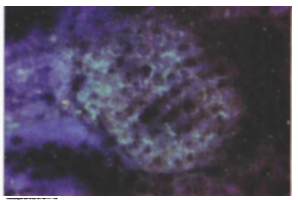

FIG. 10. Same as Fig. 7, but obtained from a rat which 5 days prior to parabiosis was unilaterally nephrectomized and then parabiosed to a nephritic rat.

with antibodies for rabbit gamma globulin from the first day (Fig. 1). The basement membrane of all glomeruli showed this intense fluorescence while all other parts of the kidney remained unstained. When such primary nephritic animals were sacrificed at daily intervals after injection of the nephrotoxic serum no staining for rat gamma globulin which would possibly indicate an auto-antibody could be obtained until the 5th or 6th day (Fig. 2, 3). Then however, the rats began to show increasing evidence for the presence of rat gamma globulin, presumably antibody, on their glomeruli. This rat gamma globulin could be demonstrated on the basement membrane for as long as 80 days after onset of the disease (Fig. 4, 5). The rat gamma globulin was indeed immunologically precipitated on the basement membrane and not encountered there in passage during the filtration process, since rat gamma globulin could not be demonstrated before the 5th to 6th day in spite of the severe proteinuria which existed from the first day. In addition, there was no staining of the glomeruli with labelled sera for rat albumin in this or any other stage, indicating that proteins which are just filtered do not stick to the glomerular membrane. Positive staining for albumin could be seen, however, in the protein droplets in the tubuli and in casts in the tubular lumina.

The secondary partners showed no staining for rabbit gamma globulin at any time (Fig. 6) indicating that at time of parabiosis no rabbit serum in demonstrable quantities could be left in the circulation of the primary partner, which could then attack the kidneys of the secondary partner. Similarly, Pfeiffer, *et al.*(9a) have reported that they too have been unable to discover any rabbit globulin on the glomeruli of the secondary partners using fluorescein isothiocyanate-labelled sera against rabbit globulin. All these kidneys showed, however, varying intensities of staining for rat gamma globulin presumably indicating the presence of an antirat kidney antibody. Degree of staining varied and not always did all glomerular loops seem to be involved. The reaction was weaker when primary partners had the disease for a long time or only to a mild degree. No other component of the kidney stained (Fig. 7 and 8). There was no staining with labelled antisera for rat albumin or for human gamma globulin. These antisera were used as controls to demonstrate the specificity of the staining for rat gamma globulin. No partner of a parabiosis of 2 healthy rats showed any staining of the glomeruli with antisera for rat gamma globulin, or for that matter, for any other precipitation of proteins in the kidney (Fig. 9).

To confirm the specificity of the staining for rat gamma globulin further, secondary partners of 3 pairs of rats were unilaterally nephrectomized 5 days prior to parabiosis. In these animals, staining of the remaining kidney for rat gamma globulin after parabiosis was considerably more intense than in the animals with 2 kidneys (Fig. 10). Apparently, all available antikidney antibody was now concentrated on one kidney instead of 2, thus leading to an increased deposition of antibody and increased renal damage.

Microscopic examination of the kidneys of the primary partners showed the typical changes described by many investigators in experimental rat glomerulonephritis. In the secondary partner there were seen alterations in both glomeruli and tubules. The changes were more marked in the unilaterally nephrectomized animals. The glomeruli showed a varying, moderate degree of hypercellularity, and thickening and reduplication of basement membranes. No thrombi were seen in capillary lumina. Proximal convoluted tubules showed PAS positive droplets varying in size from 1 to 3 μ. These are apparently protein absorption droplets. Only few protein casts were noted. There was focal atrophy of proximal convoluted tubules.

Discussion. One may thus assume that in the experimental glomerulo-nephritis of the rat a secondary auto-antibody is formed by the glomerulo-nephritic rat which is able to attack and alter healthy glomeruli. This appears to be another instance where diseased tissue can act as an antigen which produces an antibody which in turn can attack normal tissue. One should also consider the possibility that in the disease of the primary part-

ner, an antigen-antibody complex is formed which circulates in the parabiotic pair and becomes a new antigen to which antibodies are formed. This secondary antigen-antibody complex could then cause the disease in the secondary partner similarly to the glomerulitis caused by injected beef gamma globulin(10).

It is possible that a similar mechanism plays a role in causation of subacute or chronic glomerulonephritis. Hume and Merrill(11) have reported several instances in which acute glomerulonephritis occurred in a healthy kidney transplanted from an identical twin to a patient with far advanced glomerulonephritis. Much more study will be needed to evaluate the significance of the secondary antibody in the genesis of chronic glomerulonephritis.

Summary. The glomerular basement membranes in kidneys of rats made nephritic by antirat-kidney rabbit serum show immediately the presence of rabbit gamma globulin when examined with a fluorescein labelled antirabbit gamma globulin serum. Rat gamma globulin, presumably rat antibody, appears only after 6 days on these membranes. The parabiosis of such nephritic rats to healthy rats leads to a mild but clear cut nephritis in the healthy partner with proteinuria and typical histologic changes. Rat gamma globulin, presumably antibody, but not rabbit gamma globulin can be shown on the glomeruli of such secondarily diseased animals. The parabiosis of 2 normal rats does not lead to such a deposition of antibody or to clinical disease. Thus the primary nephritic partner seems to form an auto-antibody against its own altered kidney tissue. This antibody is then in turn able to attack the healthy kidney tissue of the secondary partner.

1. Lange, K., Gold, M. A., Weiner, D., Simon, V., *J. Clin. Invest.*, 1949, v28, 50.

2. Pfeiffer, E. F., Bruch, H., *Verhdlg. Dtsch. Ges. Inn. Med. Kong.*, 1950, v56, 189.

3. Brod, J., Pavkova, L., Fencl, V., Hejl, Z., Kratkova, E., *Lancet*, 1958, v1, 760.

4. Vorlaender, K. O., *Z. f. d. Ges. Exp. Med.*, 1952, v118, 352.

5. Liu, C. T., McCrory, W. W., *J. Immunol.*, 1958, v81, 492.

6. Heyman, W., Proc. 8th Nephrosis Conf., Washington, D. C., 1957.

7. Goodman, H., *ibid.*

8. Lange, K., Wenk, E. J., Noble, J., Wachstein, M., *Am. J. Med. Sc.*, 1958, v236, 767.

9. Pfeiffer, E. F., *et al., A. f. d. Ges. Exp. Med.*, 1954, v124, 471.

9a. Pfeiffer, E. F., Ditschuneit, H., Oho, I., Menk, W., Sandritter, W., Schulenburg, O., *Verhandl. Dtsch. Ges. Inn. Med. Kongr.*, 65, p450, 1959.

10. Weigle, W. O., Dixon, F., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 231.

11. Merrill, J. P., *Proc. 1st Int. Cong of Nephrology*, 1960, Evian, France.

Received July 8, 1960. P.S.E.B.M., 1961, v106.

Hepatic Alterations in Rats Fed 1,2-dihydro-2,2,4-trimethyl-quinoline, *Flectol H.* (26222)

B. J. PANNER AND J. T. PACKER (Introduced by J. L. Orbison)

Departments of Pathology and Pharmacology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

A variety of agents is capable of causing hepatic injury with attendant fatty metamorphosis, loss of cytoplasmic basophilia, and hepatic necrosis. Severe acute or chronic injury may result in fibrosis, cirrhosis, parenchymal and biliary-duct hyperplasias, and tumor formation. Relationships among these phenomena are complex and variable. Thus, signs of injury may be reversible or progressive, while tumors may or may not be preceded by fibrosis and cirrhosis. Exploration of apparently unrelated pathways by which diverse agents produce similar ends may disclose common basic mechanisms. This report calls attention to another means for pro-