liferation of injected cells was necessary for expression of its lethal effect.

Although growth and proliferation of heterologous (rat) myeloid cells have been successfully demonstrated in lethally irradiated mice injected with rat bone marrow, colonization by immunologically active lymphoid cells has not been conclusively shown. Gengozian *et al.*(10) and Zaalberg *et al.*(11) have reported the repopulation of lymphoid tissue of rat-mouse chimeras by donor cells. However, cell types and ability of these cells to function immunologically was not tested. Conflicting reports have also appeared regarding presence (12) or absence(13) of circulating rat type gamma globulin in rat-mouse chimeras. In view of the lack of conclusive evidence bearing on proliferation of immunologically active heterologous (rat) lymphoid cells, serious reservation must be placed on a graft *vs.* host reaction as an explanation for "late deaths" *in rat-mouse chimeras.*

Summary. A comparison was made of time and incidence of death between lethally irradiated mice injected with non-immune rat bone marrow and those injected with marrow from rats pre-immunized against recipient strain of mice. Statistical analysis of data showed no significant differences between the 2 groups of animals. Similarly, a lack of significant difference was observed with marrow-treated mice given a supplementary injection of moderate amounts of either non-immune or pre-immunized rat spleen cells. The failure of sufficient numbers of immunologically active cells to colonize and proliferate is discussed as the most probable interpretation of the results.

1. Nowell, P. C., Cole, L. J., Habermeyer, J. G., Roan, P. L., *Cancer Res.*, 1956, v16, 258.

2. Makinodan, T., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 174.

3. Congdon, C. C., Lorenz, E., *Am. J. Physiol.*, 1954, v176, 297.

4. Cole, L. J., Habermeyer, J. G., Bond, V. P., *J. Nat. Cancer Inst.*, 1955, v16, 1.

5. Fishler, M. C., Cole, L. J., Bond, V. P., Milne, W. L., *Am. J. Physiol.*, 1954, v177, 236.

6. Cosgrove, G. E., Jr., Upton, A. C., Schwartz, E. E., Congdon, C. C., Makinodan, T., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 417.

7. Shekarchi, I. C., Makinodan, T., *ibid.*, 1959, v100, 414.

8. Chin, P. H., Silverman, M. S., *J. Immunol.*, in press.

9. Boyse, E. A., *Immunology*, 1959, v2, 170.

10. Gengozian, N., Urso, I. S., Congdon, C. C., Conger, A. D., Makinodan, T., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 714.

11. Zaalberg, B. O., van Bekkum, D. W., *Transplantation Bull.*, 1959, v6, 91.

12. Grabar, P., Courcon, J., Illbery, P. L. T., Loutit, J. F., Merrill, J. P., *Compt. rend. Acad. Sci.*, 1957, v245, 950.

13. Gengozian, N., *J. Immunol.*, 1959, v83, 173.

Received May 24, 1960. P.S.E.B.M., 1960, v104.

Histochemical Changes in Kidneys and in Salivary Glands of Rats with Experimental Hypertension. (25892)

F. GROSS AND R. HESS (Introduced by F. F. Yonkman)

Pharmaceutical Dept., CIBA Ltd., Basle, Switzerland and Inst. of Pathology, University of Basle

Experimental hypertension produced in the rat by overdosage with cortexone acetate (DCA) and salt leads to disappearance of renin from the kidneys and to diminution of glucose-6-phosphate dehydrogenase activity in the macula densa(4). Identical changes may be observed in the unclamped kidney of rats in which hypertension is provoked by clamping one renal artery(5). These observations led us to conclude that the unclamped kidney in animals with renal hypertension is subject to similar pathogenic influences as are the kidneys of animals overdosed with DCA and salt. The fact that glucose-6-phosphate dehydrogenase (G6PD) activity is demonstrable in other structures in addition to the macula densa, *e.g.*, the small and medium ducts of salivary glands, induced us to deter-

mine whether the activity of this enzyme in the salivary gland shows changes comparable with those seen in the kidney under identical experimental conditions.

Methods. Inbred male albino rats, initial weight 120-145 g, were used in groups of 3 or 4. They were fed commercial ("Nafag") pellets and had free access either to tap water or, in the DCA overdosage experiments, to 1% saline. Hypertension was produced either by subcutaneous injection of DCA at a dose of 50 mg/kg of a microcrystalline suspension given at weekly intervals, or by unilateral clamping of the renal artery with a silver ribbon clip. Systolic blood pressure was measured under light ether anesthesia using a tail plethysmograph(1). The effect of adrenal insufficiency was studied in bilaterally adrenalectomized rats maintained on a low sodium diet and which were sacrificed 7 days after operation. All the animals were killed by cervical dislocation and were bled. Blocks of tissue from the middle part of both kidneys and whole submaxillary glands were removed quickly and immediately frozen in CO_2 and stored in a cryostat at −20°. G6PD, di- and triphosphopyridine nucleotide diaphorase (DPN- TPN-diaphorase) activity was demonstrated histochemically in 8 μ tissue sections by a cobalt-formazan technic(2,3). Untreated and sham-operated animals maintained on tap water or saline served as controls.

Results. In control animals, moderately strong G6PD activity could be demonstrated histochemically in the macula densa cells of the renal cortex and in the excretory duct epithelium of both serous and mucous parts of the salivary gland(3) (Fig. 1,4). Variations in enzyme activity in these structures occurred in animals with experimental hypertension or adrenal deficiency.

Overdosage with DCA and salt. Concomitant with development of hypertension, a gradual decrease of G6PD activity occurs in the macula densa cells of the kidneys (Fig. 5) and the activity was greatly diminished after 4 weeks' treatment(4). When G6PD activity in macular cells was compared with activity in salivary duct epithelium, a simultaneous decrease could be demonstrated in both structures. In the salivary glands, decreased G6PD activity was accompanied by flattening of the epithelium and distension of the excretory ducts (Fig. 2). The latter was similar to the widening of the lumen of the cortical tubular system in the kidney which can be observed to follow DCA-saline overdosage of more than 2 weeks' duration. Decrease of G6PD activity was confined to the salivary duct system. The high activity of this enzyme in elements of the autonomic ner-

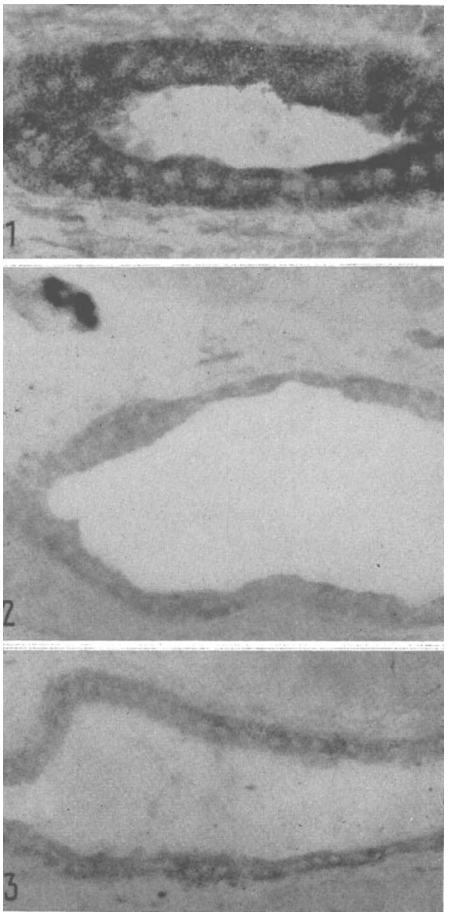

FIG. 1. Normal (high) activity of G6PD in excretory duct epithelium of rat submaxillary gland. 380 ×.

FIG. 2. DCA-NaCl treatment for 4 wk. Markedly decreased G6PD activity in ductal epithelium, in contrast to that in autonomic ganglion cells (upper left). 200 ×.

FIG. 3. Widened salivary duct, with epithelial cells displaying weak G6PD activity, from a rat bearing a Goldblatt clamp. 200 ×.

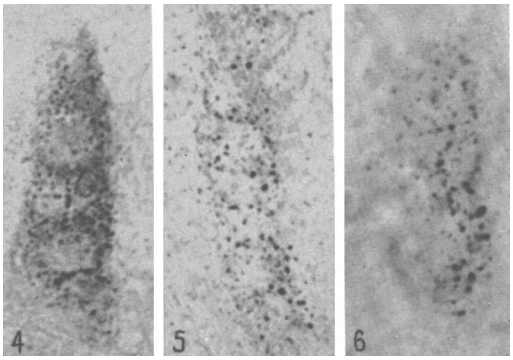

FIG. 4. Normal activity of G6PD in macula densa cells (untreated control animal). 1000 ×.

FIG. 5. Decreased G6PD activity in macula densa of a rat treated with DCA-NaCl for 2 wk. 1000 ×.

FIG. 6. Same animal as Fig. 3. Weak G6PD activity in macula densa cells of unclamped kidney. 1000 ×.

vous system within the gland was preserved (Fig. 2). TPN diaphorase activity in the excretory ducts was slightly diminished as compared with control animals. No change was noted in activity of DPN diaphorase.

Renal hypertension. Four to 5 weeks after unilateral clamping of the renal artery, G6PD activity in the salivary duct epithelium was depleted, the rats at this time having a raised blood pressure of 185-205 mm Hg (Fig. 3). Decreased enzymatic activity was accompanied by dilatation of the excretory ducts and closely resembled changes noted after DCA-NaCl overdosage for 3 weeks. As estimated by visual comparison, the decrease in G6PD activity in the salivary ducts was accompanied by a similar fall of activity in the macula densa which regularly occurred in the non-clamped kidney (Fig. 6). The clamped kidney, in contrast, showed increased G6PD activity in macular cells(5). No marked changes in diaphorase activities were observed.

Adrenal insufficiency. In rats showing signs of adrenal insufficiency, G6PD activity in the salivary duct epithelium was approximately equal to that seen in control animals. Confirming a previous observation(4), G6PD activity in the macula densa was shown to rise following adrenalectomy.

Discussion. The fact that in 2 different forms of experimental hypertension G6PD activity shows parallel changes in 2 organs with completely different functions demonstrates that variations hitherto observed only in the kidney also occur under similar conditions in other tissues. Although it is tempting to speculate that a humoral factor might be responsible for these parallel changes, no proof for such a hypothesis is available and the alternative explanation that a common underlying pathogenic mechanism is responsible for the alterations in various organs without interference of a humoral factor is equally probable. In the 2 forms of experimental hypertension, changes in sodium balance also occur and it is known that after a period of hypertension comparable to that after which these histochemical analyses were done, accumulation of sodium may be demonstrated in various tissues(6,7). On the other hand, the kidney as well as the salivary glands are secretory organs, eliminating salt and water, and similar changes in enzymatic activity in their duct systems may be the expression of attempts to adjust the function of these organs to special conditions. This interpretation agrees with the opinion expressed previously that in animals with unilateral clamping of the kidney, decrease in renin content and in G6PD activity in the untouched kidney represent efforts to counteract retention of sodium(8). The clamped kidney, however, cannot adapt its enzyme activity in the same way as the unclamped kidney. It is not possible to decide whether these changes in enzyme activity are directly involved in these functions or whether they are only an expression of diminution of activity in a primary energy yielding process on which some as yet unknown or unidentified mechanism for sodium transport depends.

Summary. In rats with experimental hypertension due either to overdosage with DCA and salt or to unilateral clamping of renal artery, activity of glucose-6-phosphate dehydrogenase (G6PD) was determined histochemically in the macula densa and simultaneously in ducts of submaxillary gland. In both kidneys of animals with DCA hypertension and in unclamped kidney of animals with renal hypertension, G6PD is diminished to a comparable degree, both in macula densa cells and

in the duct epithelium of submaxillary gland, whereas activity in the clamped kidney is higher than normal. Simultaneously there is a widening of ducts in the salivary gland comparable to that seen in cortical tubular system in the kidney. Adrenalectomy is followed by increased G6PD activity in the macula densa and a high normal activity in the submaxillary gland.

1. Byrom, F. B., Wilson, C., *J. Physiol.*, 1938, v93, 301.

2. Hess, R., Scarpelli, D. G., Pearse, A. G. E., *J. Biophys. Biochem. Cytol.*, 1958, v4, 753.

3. Scarpelli, D. G., Hess, R., Pearse, A. G. E., *J. Biophys. Biochem. Cytol.*, 1958, v4, 747.

4. Hess, R., Gross, F., *Am. J. Physiol.*, 1959, v197, 869.

5. Hess, R., Pearse, A. G. E., *J. Exp. Path.*, 1959, v40, 243.

6. Tobian, L., Binion, J. T., *J. Clin. Invest.*, 1954, v33, 1407.

7. Gross, F., Schmidt, H., *Arch. exp. Path. u. Pharmakol.*, 1958, v233, 311.

8. Gross, F., *Klin. Wschr.*, 1958, v36, 693.

Received May 24, 1960. P.S.E.B.M., 1960, v104.

Spontaneous Hydronephrosis in the Rat.* (25893)

ALVIN L. SELLERS, SHELDON ROSENFELD AND NATHAN B. FRIEDMAN
Inst. for Medical Research, and Division of Laboratories, Cedars of Lebanon Hospital, Los Angeles

Investigators using the Slonaker-Addis albino rat have long been aware of the frequent occurrence of spontaneous hydronephrosis in this strain. Recent interest in experimental production of pyelonephritis in the rat stimulated us to study this phenomenon in more detail.

Animals used represent a widespread strain of albino rats. Slonaker brought originators of the colony to California in 1903 from the Neurological Dept., University of Chicago. From the same colony, at a later date, the Wistar Institute Colony was derived. Thomas Addis used these rats to start the Stanford University Colony in 1924. In 1948 Addis moved a portion of the colony to Cedars of Lebanon Hospital, where it has been referred to as the Slonaker, and more recently, the Slonaker-Addis strain. Since 1924, the colony has been inbred, and great care taken to prevent cross-breeding with other strains.

Methods and results. Presence of hydronephrosis was determined by simple inspection of sliced kidney. Hydronephrosis of all degrees was seen, but in the majority of instances resembled that shown in Fig. 1. Groups of male and female rats weighing 90-120 g, and 200-350 g were sacrificed to determine incidence of hydronephrosis in the colony as a function of age and sex. The results are shown in Table I. In all instances, hydronephrosis occurred in the right kidney. Absence of hydronephrosis in adult female rats was striking, and it was suggested that pregnancy might be responsible. A group of 50 pre-puberty female rats were allowed to mature. Twenty-five females were mated and survived a normal pregnancy. The other 25 were allowed to mature without becoming pregnant. There were no deaths in either group. All animals were sacrificed after achieving a weight in excess of 200 g. Hydronephrosis was not found. Thus, right hydronephrosis, found in approximately 45% of immature female rats, disappears as animals mature regardless of whether or not they have borne young. Hooded rats of Long-Evans strain, as well as albino rats of Wistar strain, were inspected for hydronephrosis, and none was found (Table I). *X-ray Technic.* Presence of hydronephrosis can be determined without sacrificing the animal by intravenous urography. The x-ray technic most suitable is as follows: 42 kilovolts, 1/60 sec., 300 milli-

* Aided by grants from Nat. Heart Inst., U.S.P.H.S. and Southern Calif. Chapter, Nat. Kidney Disease Fn.