11. Davis, B., Ann. N. Y. Acad. Sci. **121**, 404 (1964).

12. Kabat, E. and Mayer, M., "Experimental Immunochemistry," 2nd ed., Thomas, Springfield, Illinois (1961).

13. Nelson, R., Jr., Jensen, J., Gigli, I., and Tamura, N., Immunochemistry **3**, 11 (1966).

14. Snyderman, R., Gewurz, H., and Mergenhagen, S., J. Exptl. Med. **128**, 261 (1968).

15. Shin, H., Snyderman, R., Friedman, E., Mellors, A., and Mayer, M., Science **162**, 361 (1968).

16. Gewurz, H., Shin, H., and Mergenhagen, S., J. Exptl. Med. **128**, 1049 (1968).

17. Ward, P., Cochrane, C., and Müller-Eberhard, H., J. Immunol. **11**, 141 (1966).

Received Jan. 6, 1969. P.S.E.B.M., 1969, Vol. 131.

Measurement of Peripheral Renin Activity in the Rat* (33841)

J. M. HAYES, J. M. B. O'CONNELL, L. SIEGEL, AND G. E. SCHREINER

Renal and Electrolyte Division, Department of Medicine, Georgetown University School of Medicine, Washington, D.C. 20005

Measurement of circulating renin activity in the rat is complicated by the need to obtain amounts of blood which are large in comparison with its blood volume. Removal of blood samples of the necessary magnitude required for standard methods (1–3) induces physiological responses which lead to an alteration of the level of peripheral renin.

This difficulty has been approached in several ways. Gross and associates (4) have utilized an isovolemic cross circulation technique to demonstrate an alteration in renin concentration in the rats circulation. This approach lacks specificity and is expensive in its requirement of assay rats. Boucher and co-workers (5) have devised a micromethod, employing exogenous rat renin substrate, which enables assay of renin activity in the plasma of the unanesthetized rat. The small size of the sample taken does not cause any disturbance of the circulating levels of peripheral renin activity. They have established in a quantitative fashion the levels found in a variety of states (6).

We have utilized dialysis of the serum sample against disodium EDTA to inactivate angiotensinase. This dialysis is accomplished without volume alterations when 1 ml of serum is used. Employing bilateral nephrectomy immediately before collection of a large blood sample we clearly separated the renin activity results occurring in several differing states.

Methods. Experimental animals. Sprague-Dawley rats, weighing 250–350 g, of both sexes were used. Blood samples were collected from the right carotid artery after bilateral nephrectomy had been performed under sodium pentobarbital anesthesia, 40 mg/kg ip. The interval between nephrectomy and sacrifice by exsanguination was 15 min. The serum was stored frozen until dialyzed.

Groups of rats were subjected to the following treatments: bilateral adrenalectomy without replacement therapy for 7 days; hemorrhage with the kidneys *in situ*; deoxycorticosterone (DOCA) in peanut oil im, 5 mg administered on alternate days for a total dose of 95 mg with 0.9% saline as drinking water; and 5% dextrose, 10 ml/kg im on one occasion.

Peripheral renin activity determination (PRA). One-ml aliquots of sera were dialyzed in 8/32-in. cellophane tubing sacs against 0.003 *M* disodium EDTA in 0.9% sodium chloride solution for 16 hr in the cold. Two-tenths-ml of serum was added to a siliconized tube containing 0.2 ml of rat renin substrate; 0.1 ml of 0.001 *M* phenylmercuric acetate; 0.1 ml of 0.5 *M* sodium phosphate buffer, pH 5.5; 0.01 ml of 5% diisopropylfluorophosphate in isopropyl alcohol; and 0.4 ml of 0.9% sodium chloride solution. Incu-

* Supported by PHS Grant FR-5360-06, the John A. Hartford Foundation Inc., and the Georgetown Kidney Research Fund. J. M. H. was a Fellow of the National Kidney Foundation during the period in which this work was performed.

bation was performed for 16 hr at 38° at pH 5.5 The reaction was terminated by heating in a boiling water bath for 10 min and a clear supernatant was obtained by centrigufation at 2500 rpm for 60 min in the cold. More angiotensin can be generated at pH 6.5 (5), but heat precipitation of the proteins in the mixture, at this pH, produces a gel. The supernatant was assayed in the 24-hr nephrectomized rat, ganglion blocked with pentolinium tartrate 3 μg per g sc. Mean carotid pressure was recorded by a Sanborn pressure transducer (267 B) via a Sanborn preamplifier (350-1100 C) on a 2-channel Sanborn recorder (296). Responses from unknowns were compared with asparaginyl[1] valyl[5] angiotensin II (7). Angiotensin recovery was determined by adding rat angiotensin, generated from rat renin substrate, to serum samples which had been previously assayed. The recovery was 82 ± 11% ($n = 9$).

Rat renin substrate was prepared from the serum of male Long-Evans rats (8) which had been nephrectomized 24 hr previously to increase the yield and to remove circulating renin. Substrate assay carried out at pH 5.5 using an excess of angiotensinase-free dog renin[1] showed 9.9 ± 7 μg of substrate/ml ($n = 6$).

Student's t test was used to determine the significance of differences between means. All results are expressed as means ± SEM.

Results. The data are illustrated in Fig. 1. A twentyfold elevation in PRA is shown in sera obtained from adrenalectomized rats, $p<0.001$. Acute hemorrhage was a less potent stimulus but the fivefold rise was still highly significant, $p<0.001$. The level found in untreated rats was 16 ± 1 ng/0.2 ml ($n = 26$) and depression to half this value was seen in the animals treated with DOCA, $p<0.01$. The administration of a placebo dextrose injection did not cause any change.

Discussion. Boucher and associates (6) demonstrated increased PRA in the rat in response to volume and sodium depletion and decreased PRA following the administration of DOCA. Considering the difference in pH and the amount of serum employed our control data are in agreement with the value they obtained, 15 ng/0.1 ml per 12-hr incubation at 37°.

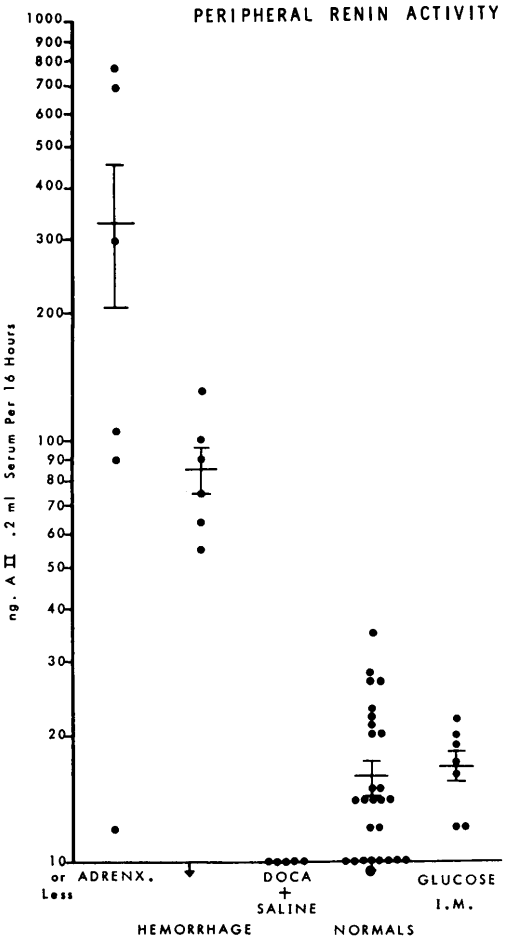

FIG. 1. PRA of rat sera; a significant increase occurred after bilateral adrenalectomy or severe hemorrhage; a significant reduction occurred after administration of DOCA. The mean ± SEM is shown on a double-cycle logarithmic scale.

The values found in our study represent the PRA in a situation where a number of opposing stimuli were in effect. They denote the sum of the influences of anesthesia and surgery, both of which would promote an increase, minus the effect of disappearance of renin from the circulation following bilateral nephrectomy (9). Despite these manipulations, elevation of PRA can be shown in sera from adrenalectomized rats and rats subject-

[1] Kindly supplied by Dr. E. Haas, Mount Sinai Hospital, Cleveland, Ohio.

ed to severe hemorrhage and suppression in sera from rats treated with DOCA.

Summary. Bilateral nephrectomy followed by immediate exsanguination allows the collection of blood from rats for the estimation of PRA in a manner that preserves the expected alterations to varying stimuli. This procedure can be used where relatively large samples of blood are required for treatment to inactivate angiotensinase and for the generation of angiotensin.

We wish to express our appreciation to Mr. F. H. Pryce for his technical assistance.

1. Helmer, O. M. and Judson, W. E. Circulation **27**, 1050 (1963).

2. Boucher, R., Veyrat, R., De Champlain, J., and Genest, J., Can. Med. Assoc. J. **90**, 194 (1964).

3. Pickens, P. T., Bumpus, F. M., Lloyd, A. M., Smeby, R. R., and Page, I. H., Circulation Res. **17**, 438 (1965).

4. Schaechtelin, G., Regoli, D., and Gross, F. Am. J. Physiol. **205**, 303 (1963).

5. Boucher, R., Menard, J., and Genest, J., Can. J. Physiol. Pharmacol. **45**, 881 (1967).

6. Boucher, R., Menard, J., and Genest, J., Clin. Res. **15**, 352 (1967).

7. Boonshaft, B., O'Connell, J. M. B., Hayes, J. M., and Schreiner, G. E., J. Clin. Endocrinol. **28**, 1641 (1968).

8. Haas, E., Goldblatt, H., Gipson, C., and Lewis, L., Circulation Res. **19**, 739 (1966).

9. Schaechtelin, G., Regoli, D., and Gross, F., Am. J. Physiol. **206**, 1361 (1964).

Received Dec. 22, 1968. P.S.E.B.M., 1969, Vol. 131.

Response of Chromosomal Puffs to Crystalline Hormones *in Vivo** (33842)

WALTER J. BURDETTE AND MASATOSHI KOBAYASHI

The University of Texas M. D. Anderson Hospital, and Tumor Institute at Houston, Texas 77025 and The Sericultural Experimental Station, Tokyo, Japan

Since the isolation and identification of alpha-ecdysone and ecdysterone by Butenandt, Karlson, Huber, Hoffmeister, and their collaborators (10, 11, 13), additional compounds differing in hydroxylation have been isolated not only from *Bombyx* but also from crustaceans and even from plant sources such as bracken and fir (9, 15, 16, 18, 20–22). In previous studies, Burdette and Bullock (5) extracted five active fractions from *Bombyx,* and Burdette and Anderson (3) found that the puffing pattern in Drosophila responded to ecdysones just as Clever and Karlson reported for Chironomus (13), although the chromosomes seemed refractory at times. Since the present pattern of sensitivity for puffing is activated by ecdysones and more than one ecdysone probably occurs naturally, it seemed appropriate to expose Drosophila larvae to several crystalline ecdysones and to determine the visible response of chromosomes to these hormones. In addition, proteinic brain hormone derived from *Bombyx* was also inoculated into larvae and the size of puffs was determined subsequently.

Procedures. A single test consisted of 20 late fifth-instar Drosophila larvae inoculated with 10 μl of hormone as a 10% solution in Ringer's saline. Stock proteinic brain hormone was used in two concentrations, one *Bombyx* unit in 100 μg (designated A) and one *Bombyx* unit in 50μg (designated B). Injections were made with a microsyringe and needle into anterior segments which were undisturbed until the time designated for the particular test had elapsed. [The method has been described previously (1).] In respective tests observations were made 20, 40, 60, and 90 min after injection.

Salivary glands were removed from the an-

* Aided by grants CA08910, CA10037, and CA10043 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

[1] The technical assistance of Miss Nancy Henkel, Mrs. Elaine Thornell, and Mr. Michael Gallagher is gratefully acknowledged.