ficiency affect intracellular metabolism similarly but independently.

Summary. The intracellular potassium concentration was found to be normal in diabetic rat liver.

1. Hastings, A. B., Teng, C. T., Nesbett, F. B., and Sinex, F. M., *J. Biol. Chem.*, 1952, v194, 69.

2. Renold, A. E., Teng, C. T., Nesbett, F. B., and Hastings, A. B., *ibid.*, 1953, v204, 533.

3. Hastings, A. B., Renold, A. E., and Teng, C. T., *Trans. Assn. Am. Phys.*, 1953, v66, 129.

4. Gardner, L. I., Talbot, N. B., Cook, C. D., Bermenand, H., and Uribe, R., *J. Lab. and Clin. Med.*, 1950, v35, 592.

5. Eliel, L. P., Pearson, O. H., and White, F. C., *J. Clin. Invest.*, 1952, v31, 419.

6. Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, v100, 485.

7. Lowry, O. H., and Hastings, A. B., *ibid.*, 1942, v143, 257.

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

Received August 10, 1955. P.S.E.B.M., 1955, v90.

Renin and Antirenin in Treatment of Long Term Experimental Renal Hypertension in the Dog.* (21951)

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A number of reports have suggested that the pathogenesis of experimental renal hypertension changes with time. Thus it has been suggested that in acute experimental renal hypertension the pathogenesis is renal and in chronic experimental renal hypertension the pathogenesis is extrarenal or (according to one group of investigators) neurogenic. There have been no reported studies on the pathogenesis of long term experimental renal hypertension (*i.e.*, hypertension of 4 or more years duration) in the dog. Thus Ogden and colleagues(1,2) showed that certain sympatholytic and general anesthetic drugs decreased the blood pressure of chronic renal hypertensive rats but not of acute renal hypertensive rats. They also showed that the removal of a unilaterally constricted kidney in the rat caused a decrease in blood pressure in acute but not in chronic renal hypertensive rats. On the basis of these findings, Ogden suggested that the pathogenesis of experimental renal hypertension changes with time from a renal pressor mechanism to a neurogenic mechanism. Likewise, several groups of British investigators have reported that complete nephrectomy in the acute renal hypertensive rat produces a decrease in blood pressure, whereas there is no decrease during survival in the chronic hypertensive rat(3,4). These results are similar to those of earlier workers who reported that total nephrectomy in renal hypertension of one week's duration in the rabbit results in normotension but after 2 to 4 months of hypertension, total nephrectomy results in persistence of the hypertension during survival(5,6). Floyer and others have interpreted these findings as demonstrating an extrarenal mechanism for the pathogenesis of chronic experimental renal hypertension. Moss and Wakerlin(7) and Page(8) however, found no significant difference in the effects of sympatholytic and general anesthetic drugs in acute and chronic experimental renal hypertension in dogs. Moss and Wakerlin considered acute experimental renal hypertension in dogs to last 6 months following renal artery constriction and most of their acute hypertensive dogs were 3 to 6 months hypertensive. Since then, Haynes and Dexter(9) showed that an increase in plasma renin following renal artery constriction in the dog persists for some weeks and Shorr and colleagues(10) showed that increased plasma VEM is balanced by VDM 8 to 12 weeks following renal

* Aided by grants from the National Heart Institute, the Chicago Heart Assn.; and the American Heart Assn.

artery constriction in dogs. These observations suggest that the dividing line between acute and chronic experimental renal hypertension in dogs may be 3 rather than 6 months. In keeping with this, Ohler and Wakerlin(11) found that dibenzyline produced no decrease in the blood pressure of 7 renal hypertensive dogs within 3 months following renal artery constriction whereas a decrease occurred in dogs which were hypertensive for 6 months or more. However, the decrease was not to the normotensive level and certainly not to the hypotensive level produced by dibenzyline in normotensive dogs. Recently, Ogden and colleagues(12) reported that pentobarbital decreased the blood pressure of dogs with renal hypertensions of more than one year's duration but not to normotension. These findings suggest that vasomotor tonus is in abeyance during acute renal hypertension but returns to at least a normal level during chronic renal hypertension in the dog.

To study the pathogenesis of long term experimental renal hypertension in dogs, we observed the effect of semipurified hog renin therapy and of passive immunization with antirenin to hog renin in dogs with renal hypertensions of 50 to 121 months duration. Two years ago, our research group showed that a course of semipurified hog renin from renal cortex protected against the development of experimental renal hypertension in dogs and was effective in the treatment of chronic experimental renal hypertension of 8 to 37 months duration in dogs, whereas numerous control tissue extracts were without effect(13). Since the effectiveness of prophylaxis and treatment was well-correlated with the serum antirenin titre, the findings were considered good evidence for the hypothesis that renin (or a closely related renal protein) plays the principal pathogenetic role in experimental renal hypertension in the dog for at least the first 3 years. A pathogenetic role for renin is also suggested by the antihypertensive effect of homologous antirenin to hog renin passively transferred to renal hypertensive dogs(14).

Methods. Ten dogs with renal hypertensions of 50 to 121 months duration were treated for 6 to 12 months with one or two courses of daily intramuscular injections of semipurified hog renin from cortex in doses varying from 2.5 to 20.0 D. U. (Goldblatt) per kg. The semipurified hog renin was prepared by a modification of the method of Marshall and Wakerlin(15) or the method of Haas and Goldblatt(16). The purity of the renin varied from 16-175 D. U. per mg N, but most of it was 75-175 D. U. per mg N. Antirenin titres on the serum were determined monthly by a method already described(13). The time between courses of hog renin therapy in the 6 dogs receiving a second course of hog renin was at least 6 months, during which the blood pressure reached its pretreatment hypertensive level and the antirenin titre fell to 0. Male and female dogs were made hypertensive by a standardized modification of the Goldblatt technique of renal artery constriction already reported(13). Mean blood pressure readings were obtained by femoral artery puncture one or 2 times per week, with occasional comparison by the strain gauge technic. Studies of blood urea nitrogen, urinalyses, and determinations of body weight and clinical condition were made at monthly intervals or more often when indicated.

For the passive transfer study, 3 of the dogs with renal hypertensions of 93 to 108 months duration were given high titre homologous antiserum containing antirenin to hog renin. Each of these dogs had previously been treated with a course of semipurified hog renin with a sufficient lapse of time (6 months or more) to permit a decrease in the antirenin titre to 0 and a return of the blood pressure to the pretreatment hypertensive level for 2 to 3 months. Blood pressure was measured every other day for one week prior to antirenin administration and the antirenin titre was determined to be 0 A. U. per cc of serum. Antirenin in total amounts varying from 947 A. U. to 3,560 A. U. per kg was administered subcutaneously in 4 divided doses over a 2-day period. Blood pressures and serum antirenin titres were determined on the 3rd, 5th, and 7th days following the first day of antirenin administration and subsequently at weekly or semi-weekly intervals. Urinalyses were made prior and subsequent to the administration of antirenin and the clinical condition of the dogs

TABLE I. Antihypertensive Effect of Semipurified Hog Renin from Cortex in Experimental Renal Hypertension in 10 Dogs with Hypertensions of 50 to 121 Months Duration.

Dog No.	Sex	Max antirenin titre (A.U./cc)	Duration of hypertension	Blood pressure in mm Hg: Normotension	Hypertension	Max response to treatment	Effect on hypertension, %
1	♀	37	50	125 ± 7.0*	183 ± 3.7*	151 ± 5.8*	−55
2	♀	29	51	122 ± 7.8	162 ± 5.4	153 ± 7.2	−23
3	♂	32	51	123 ± 5.0	167 ± 7.5	129 ± 8.0	−86
4	♂	0	51	115 ± 5.4	201 ± 6.9	219 ±10.5	+21†
5	♀	50	56	127 ± 6.8	158 ± 3.8	142 ± 4.8	−50
6	♂	24	60	120 ± 6.8	185 ± 4.0	140 ±10.5	−69
7	♀	18	66	117 ± 5.2	185 ±10.5	138 ± 8.4	−68
8	♂	20	67	127 ± 9.1	172 ± 5.5	147 ± 6.6	−55
2	♀	68	69	122 ± 7.8	172 ± 4.8	148 ± 4.0	−48
9	♂	22	71	117 ± 4.6	164 ± 9.4	145 ± 7.6	−47
7	♀	21	81	117 ± 5.2	166 ± 6.7	140 ± 3.9	−53
9	♂	85	82	117 ± 4.6	170 ±11.0	142 ± 6.6	−53
8	♂	16	87	127 ± 9.1	166 ± 5.8	150 ± 6.6	−41
3	♂	40	98	123 ± 5.0	171 ± 7.0	147 ± 9.1	−50
10	♂	0	99	129 ± 9.0	160 ± 2.7	183 ± 7.2	+74†
10	♂	11	121	129 ± 9.0	166 ± 3.2	146 ± 5.9	−54

* Avg ± stand. dev.
† Maintained chronic pressor effect in the absence of antirenin.
The P value (t test) was <.01 in all experiments.

was observed daily. Control experiments with normal serum were conducted.

Results. Table I summarizes results with hog renin injections. The normotensive and hypertensive blood pressure levels are each based upon a minimum of 12 to 18 readings over a period of 2 to 3 months. The maximal response to treatment level is based on a minimum of 6 to 12 readings over a period of one to 2 months. All of the dogs showed a significant antihypertensive effect ranging from 23 to 86% reduction in their hypertensions, except the 2 animals which did not develop antirenin. None of the dogs showed adverse effects from treatment. Appetite, body weight, and general clinical condition were well maintained. Urinalyses and blood urea nitrogen determinations consistently gave normal findings.

The maximal antihypertensive effect for the first course of injections occurred during the third to the sixth months of treatment as the antirenin titre approached its maximum and was maintained until treatment was discontinued. The maximal antihypertensive effect for the second course was observed during the second to the fourth months of treatment, correlating with the more rapid anamnestic development of antirenin. Following the end of treatment, the blood pressure gradually returned to its pretreatment level over a period of 2 to 6 months as the antirenin titre fell toward 0. Fig. 1 shows a typical antihypertensive effect from a course of semipurified hog renin in a long term renal hypertensive dog. The 2 dogs which did not develop anti-

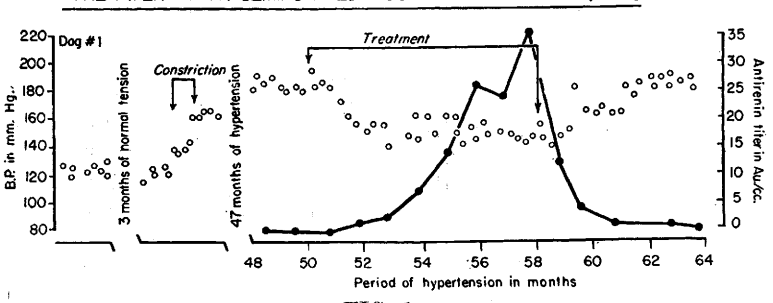

FIG. 1.

TABLE II. Antihypertensive Effect of Homologous Antirenin to Hog Renin Passively Transferred to Dogs with Long Term Experimental Renal Hypertension.

Dog No. (see Table I)	Sex	Total antirenin dose (A.U./kg)	Max antirenin titre	Duration of hypertension	Blood pressure in mm Hg: Normotension	Blood pressure in mm Hg: Hypertension	Max response to antirenin	Effect on hypertension, %
7	♀	3560	9	93	117 ± 5.2*	169 ±11.6*	128	− 79
7	♀	947	5	97	117 ± 5.2	177 ± 9.0	102	−100
7	♀	0	0	99	117 ± 5.2	166 ± 8.7	164 ± 7.0	− 4*
9	♂	1353	4	96	117 ± 4.6	164 ± 4.0	133	− 66
10	♂	1700	15	108	129 ± 9.0	164 ± 2.5	114	−100
10	♂	0	0	114	129 ± 9.0	166 ± 5.2	166 ± 3.0	0*

* Normal serum controls.

renin not only did not show an antihypertensive effect but showed a maintained chronic pressor effect which has been discussed elsewhere(17).

Table II summarizes results with the passive transfer of antirenin. The 4 passive transfer experiments with antirenin produced 66 to 100% reductions in the hypertensions of the three long term renal hypertensive dogs compared with antihypertensive effects in the same dogs of 54 to 68% as a result of 5 courses of hog renin. The maximal antihypertensive effect was obtained on the third or fifth day of the passive transfer experiment, usually simultaneously with the maximum antirenin titre. The blood pressure then gradually returned to the previous hypertensive level over a period of two weeks or more as the antirenin titre fell toward 0. Control passive transfer experiments with normal serum in 2 of the 3 dogs were without antihypertensive effect. None of the animals showed adverse changes in clinical condition or abnormalities in urinalyses following antiserum or serum administrations. Fig. 2 illustrates a passive transfer of antirenin and a control experiment in one of the dogs.

Discussion. The results with hog renin and with the passive transfer of antirenin support the hypothesis that renin (or a closely related renal protein) plays a significant role in experimental renal hypertension of many years duration in the dog. Although our research group has already shown that numerous control tissue extracts are without antihypertensive effect(13), the lack of response in the 2 long term hypertensive dogs failing to develop antirenin on hog renin therapy, further rules out a non-specific foreign protein effect as the cause of the antihypertensive response. The failure of most of the long term hypertensive dogs to show a decrease in blood pressure to normotension whereas most of the dogs with chronic hypertensions of 8 to 37 months showed such a decrease on semipurified hog renin therapy(13), suggests at least three possible explanations: (1) the pathogenesis of experimental renal hypertension in the dog may undergo a gradual change with the passage of years whereby renin plays a decreased role; (2) progressive arteriolosclerosis may develop over a period of years; and (3) compensatory mechanisms opposing the antihypertensive effect of actively produced antirenin may be enhanced in long term as contrasted with chronic experimental renal hypertension in the dog. The decrease in blood pressure to normotension or near normotension produced by the passive transfer of antirenin is opposed to (1) and (2). Likewise an extensive study of the passive transfer of antirenin in renal hypertensive dogs to be published later(18) indicates that the duration of chronic hypertension is without effect on the antihypertensive response to passive immunization with antirenin. Moreover, histological examination of the arterioles of long term hy-

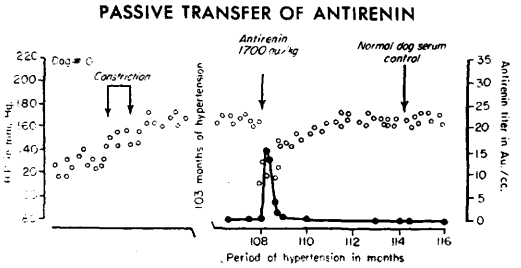

FIG. 2.

pertensive dogs by us confirmed the previous observation of Goldblatt that experimental renal hypertensive dogs show a minimal amount of arteriolosclerosis. This leaves the suggestion that compensatory hypertensive mechanisms come more prominently into play when serum antirenin is gradually increased by semipurified hog renin than when the serum antirenin titre is quickly developed by passive immunization with antirenin. Our observations shed no light on what these compensatory mechanisms may be although increased vasomotor tonus may be involved. Recently our research group reported an increase in renal renin concentration proportional to the antirenin titre in normotensive and renal hypertensive dogs on hog renin therapy but found no difference in this respect among acute, chronic, and long term renal hypertensive dogs(19).

Floyer(20) has suggested that possibly renin may exercise its pathogenetic role in chronic experimental renal hypertension by acting on the kidney perhaps to decrease the production of the blood pressure regulatory or depressor renal hormone (loss of which by complete nephrectomy results in renoprival hypertension)(21). Conceivably this action might evolve gradually during acute experimental renal hypertension and become significant only in chronic experimental renal hypertension. This hypothesis would reconcile the conflicting findings reviewed in the introduction of this report and would be in agreement with our finding that renin still plays a dominant role in the pathogenesis of long term experimental renal hypertension. The decreased antihypertensive response of long term experimental renal hypertension to hog renin injections might be due to failure of the blood pressure regulatory renal hormone to increase to the normal level as the serum antirenin titre is gradually increased by active immunization with hog renin. Certainly this intriguing hypothesis should be investigated.

Summary and conclusions. 1. Courses of intramuscular injections of semipurified hog renin from cortex administered to dogs with experimental renal hypertensions of 4 or more years duration produced a significant reduction in blood pressure in those dogs which developed antirenin but not to normotension as previously found in dogs with chronic renal hypertension of 6 to 37 months duration. The 2 dogs which did not develop antirenin showed a maintained chronic pressor effect. 2. Passive immunization with homologous antirenin to hog renin produced a reduction in blood pressure to normotension or near normotension in dogs with experimental renal hypertensions of more than 7 years duration. 3. The mechanism of the decreased response to hog renin therapy in long term experimental renal hypertension in the dog is unknown. The results with passive immunization suggest that a lessened pathogenetic role for renin is not involved nor is progressive arteriolosclerosis. Possibly compensatory hypertensive mechanisms are more effective in the face of the gradually increasing antirenin titre of hog renin therapy than of the abruptly produced antirenin titre of passive immunization. 4. The results indicate that renin (or a closely related renal protein) is the principal pathogenetic factor even in experimental renal hypertension of many years duration in the dog.

1. Reed, R. K., Sapirstein, L. A., Southard, F. D., and Ogden, E., *Am. J. Physiol.*, 1944, v41, 707.

2. Ogden, E., Collings, W. D., and Sapirstein, L. A, *Spec. Pub. N. Y. Acad. Sc.*, 1946, v3, 153.

3. Floyer, M. A., *Clin. Sc.*, 1951, v10, 405.

4. ———, Ciba Foundation Symposium on Hypertension, Boston, 1954, p. 155, Little, Brown & Co.

5. Pickering, G. W., *Clin. Sc.*, 1945, v5, 239.

6. Daniel, P. M., Prichard, M. M. L., and Ward-McQuaid, J. N., *ibid.*, 1954, v13, 247.

7. Moss, W. G., and Wakerlin, G. E., *Am. J. Physiol.*, 1950, v161, 435.

8. Page, I. H., *Factors Regulating Blood Pressure,* Trans. First Conf., New York, Josiah Macy, Jr. Found., 1947, p18.

9. Haynes, F. W., and Dexter, L., *Am. J. Physiol*, 1947, v150, 190.

10. Shorr, E., *Hypertension,* A Symposium, Univ. of Minn. Press, 1950, p86.

11. Ohler, E. A., and Wakerlin, G. E., *Factors Regulating Blood Pressure,* Trans. 5th Conf., New York, Josiah Macy, Jr., Found., 1951, p111.

12. Mandel, M. J., Greene, R. W., Sapirstein, L. A., and Ogden, E., *Am. J. Physiol.*, 1954, v176, 352.

13. Wakerlin, G. E., Bird, R. B., Brennan, V. B., Frank, M. H., Kremen, S., Kuperman, I., and

Skom, J. H., *J. Lab. and Clin. Med.*, 1953, v41, 708.

14. Kuperman, I., and Wakerlin, G. E., *Fed. Proc.*, 1953, v12, 80.

15. Marshall, J., and Wakerlin, G. E., *ibid.*, 1949, v8, 106.

16. Haas, E., Lamfrom, H., and Goldblatt, H., *Arch. Biochem. and Biophysics*, 1953, v42, 368.

17. Wakerlin, G. E., Kremen, S., Frank, M. H., Schmid, H. E., and Graham, L., *Circulation Res.*, 1954, v2, 201.

18. Wakerlin, G. E., Kuperman, I., Schmid, H. E., and Graham, L., to be published.

19. Schmid, H. E., and Wakerlin, G. E., *Fed. Proc*, 1955, v14, 132.

20. Flover, M. A., personal communication, 1955.

21. Grollman, A., Muirhead, E. E., and Vanatta, J., *Am. J. Physiol.*, 1949, v157, 21.

Received August 11, 1955. P.S.E.B.M., 1955, v90.

Technic for Preparation of Glycerol-extracted Whole Frog Hearts for Study of Myocardial Fiber Responses.* (21952)

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Since the demonstration by Szent-Györgyi that glycerinated skeletal muscle fibers contract on exposure to adenosine triphosphate, interest has developed in this preparation as a model for the study of muscle contraction (1). Attention has been directed largely to glycerinated psoas muscle fibers because of their length and relative absence of connective tissue(2-4). Glycerol-treated cardiac muscle fibers are more difficult to handle and have not been extensively studied. However, Taeschler and Bing(5) were able to prepare and observe glycerol-extracted trabecular fibers of dog hearts. The purpose of this communication is to describe a glycerinated frog-heart preparation which is suitable for the study of contraction-relaxation cycles.

Method. The frog heart has no coronary circulation, so that tissue nutrition depends upon the diffusion of solutes through the endocardium. The quantity of diffusion is enhanced by numerous fissures in the ventricular walls(6). Therefore, thorough glycerine extraction of the whole heart should be achieved by perfusion and immersion in a glycerol solution. After extraction, ventricular perfusion with suitable solutes should produce contraction of the muscle fibers. Large bullfrogs (*Rana catesbiana*) are employed. For the preparation of glycerinated hearts, the central nervous system is destroyed and the thorax opened ventrally. The pericardium is excised and the heart is dissected from the mediastinum, care being taken to avoid injury to the ventricle. The fresh heart is placed in Ringer's fluid at room temperature and allowed to beat for several minutes until largely cleared of blood. The heart is then tied to an applicator stick by a ligature tightly drawn around the aortae and upper portion of the atria. A small wire hook is inserted through the ventricular muscle near the apex; the free end of the hook is tied to the stick so as to apply a slight stretch to the ventricle. A needle introduced through the atrium permits the infusion of 50% glycerol into the heart until it is moderately distended. This procedure removes all blood, and the tissue becomes translucent. The mounted specimen is then placed in a capped test tube containing 50% glycerol and is stored at −30 to −10°C for 48 to 72 hours. A thin coat of ice may form at the glycerol-air interface; the tissue should be kept below this level.

Results. After extraction for 2 to 3 days, contraction and relaxation of the glycerinated hearts may be produced by using the perfu-

* This work was performed under a grant-in-aid (H-519) from the National Heart Institute of the National Institutes of Health.

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