In summary, it has been demonstrated that increasing the fat level in a riboflavin-low ration has a corresponding deleterious effect on the growth of young rats, and that the administration of adequate amounts of riboflavin completely corrects this deficiency.

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Reductions in Blood Pressures of Renal Hypertensive Dogs by Hog Renin.*†

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A number of attempts to reduce the blood pressures of renal ischemic hypertensive dogs have been reported recently. Most of these have been unsuccessful, although a few investigators have obtained results showing some promise. Thus Davis and Barker[1] reported that potassium sulphocyanate reduced the blood pressure of renal hypertensive dogs. Mansfield, *et al.*,[2] found that nephro-splenopexy but not nephro-omentopexy caused a more or less permanent decrease in the blood pressure of dogs rendered hypertensive by the Goldblatt technic. Similar results were later reported by Cerqua and Samaan.[3] Obviously methods involving the production of an increase in the collateral circulation to the ischemic kidney have at best a limited applicability to the therapy of essential and malignant hypertension in the human since the ischemia in the clinical condition is ordinarily due to arteriosclerosis rather than narrowing of the renal arteries. Katz and coworkers[4] reported that transplanted autolyzing kidney tissue reduced the blood pressure of renal hypertensive dogs but Goldblatt[5] was unable to confirm this

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† We appreciate the technical assistance of Messrs. B. Gomberg and M. L. Goldberg.

1 Davis, L., and Barker, M. H., *Ann. Surg.*, 1939, **110**, 1016.

2 Mansfield, J. S., Weeks, D. M., Steiner, A., and Victor, J., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 708.

3 Cerqua, S., and Samaan, A., *Clin. Sc.*, 1939, **4**, 113.

4 Rodbard, S., Katz, L. N., and Sokolow, M., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 360.

5 Goldblatt, H., personal communication.

finding. Harrison and collaborators[6] reported reductions in the blood pressure of renal hypertensive dogs and of humans with essential and malignant hypertension by means of a kidney extract containing the antipressor substance believed to be present in normal renal tissue. Because of untoward effects observed in a number of their dogs, as well as for other reasons, these authors reserved judgment relative to the ultimate specificity and value of this form of therapy. Page, *et al.*,[7] using a similar antipressor renal extract, reported favorable effects on the blood pressures of hypertensive dogs and humans. Both of these groups considered this antipressor substance to be different from any of the known depressor agents. However, more work is necessary to determine the comparative effectiveness of this type of renal extract and other antihypertensive agents, as well as its specificity for experimental renal hypertension and clinical essential or malignant hypertension.

Several months ago we reported the successful production of an antiserum for dog renin in the rabbit and for hog renin in the dog.[8] The active principle of the antiserums, which we designated "antirenin", was shown to neutralize the acute pressor effect of the antigenic renins. We have since produced an antiserum for rabbit renin in the guinea pig and for hog renin in the rabbit. We also noted that rabbit antiserum for dog renin was somewhat less effective in neutralizing rabbit renin. Since then we have found that the dog antiserum for hog renin is likewise slightly less effective in neutralizing dog renin.

In view of the increasing possibility that renin is the pathogenetic pressor agent in experimental renal ischemic hypertension, we stated[8] that, among other things, we purposed to determine the value of antirenin, actively produced, in the therapy of experimental renal hypertension. At this time, therefore, we present preliminary results with the treatment of renal ischemic hypertension in dogs by injections of hog renin.

Methods. Four dogs were rendered hypertensive by the Goldblatt technic and the resulting hypertension was allowed to stabilize over a period of 3 to 23 months before hog renin injections were begun. Mean blood pressure readings were obtained by femoral

[6] Grollman, A., Williams, J. R., Jr., and Harrison, T. R., *J. Am. Med. Assn.*, 1940, **115**, 1169.

[7] Page, I. H., Helmer, O. M., Kohlstaedt, K. G., Fouts, P. J., and Kempf, G. F., *Proc. Cent. Soc. Clin. Res.*, 1940, p. 8.

[8] Johnson, C. A., and Wakerlin, G. E., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 277.

artery puncture 2 or 3 times per week. Blood urea nitrogen studies, urinalyses, and body weight determinations were made at biweekly intervals. The 4 hypertensive dogs were treated with daily intramuscular injections of hog renin for 4 months each. The renin solution was equivalent to 1 g of fresh kidney cortex per cc and was administered in a dosage of 1 cc per kg of body weight. The method used for the preparation of renin was essentially that described by Grossman,[9] except that acetone was used as a dehydrating agent and much of the associated protein was removed by isoelectric precipitation.

Blood serums were examined for antirenin before treatment with hog renin and at 2-week intervals subsequently. The technic previously described,[8] consisted essentially in mixing 2 volumes of serum with one volume of renin solution, allowing the mixture to stand at least overnight at 4°C, and assaying the acute pressor effect of the mixture intravenously on the etherized, nephrectomized dog or the brain pithed, nephrectomized cat. The dosage of renin solution employed was 0.5 cc per kg of assay animal. In all instances the serum tested for antirenin was suitably controlled with normotensive dog serum and frequently with serum from untreated hypertensive dogs. Antirenin titers for both hog and dog renins were determined.

At the present writing, one of the dogs has been observed for 7 months and the other 3 for 2 months since hog renin injections were discontinued.

Results. The reductions in the blood pressures of these 4 hypertensive dogs resulting from intramuscular hog renin are illustrated by Figs. 1-4.

The first hypertensive dog showed an average femoral blood pressure of 164 mm of Hg with a maximum of 184 mm and a minimum of 146 mm, during the 3 months preceding treatment. As shown by Fig. 1, the blood pressure of the animal fell more or less steadily throughout the period of hog renin injections until the normal or prehypertensive range was reached in the fourth month of treatment. During the 2 months following renin therapy, the blood pressure dropped somewhat below the original normotensive level. In the succeeding 5 months the pressure slowly rose, so that it has now reached the pretreatment hypertensive range.

The second dog was hypertensive for 9 months prior to renin injections, although only the last 6 months of this period are recorded in Fig. 2. As illustrated, the blood pressure decreased during

[9] Grossman, E. B., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 40.

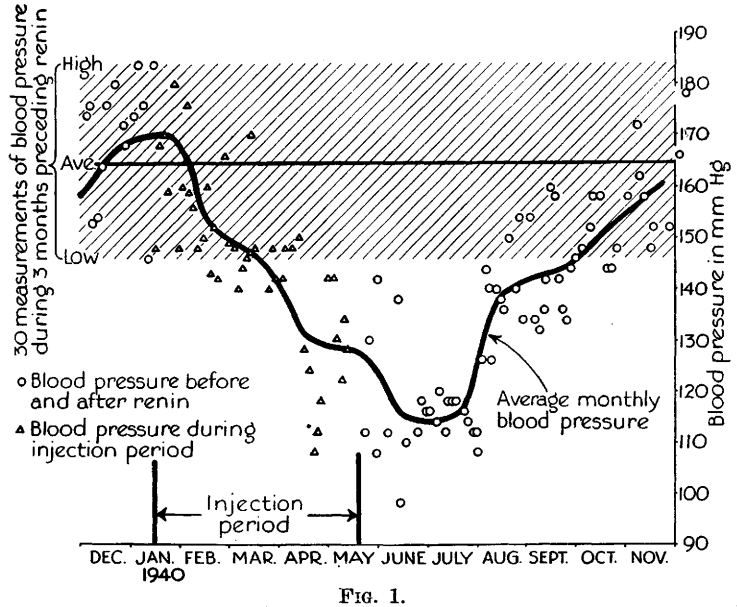

FIG. 1.

The effect of daily injections of hog renin on the blood pressure of renal hypertensive dog No. 1.

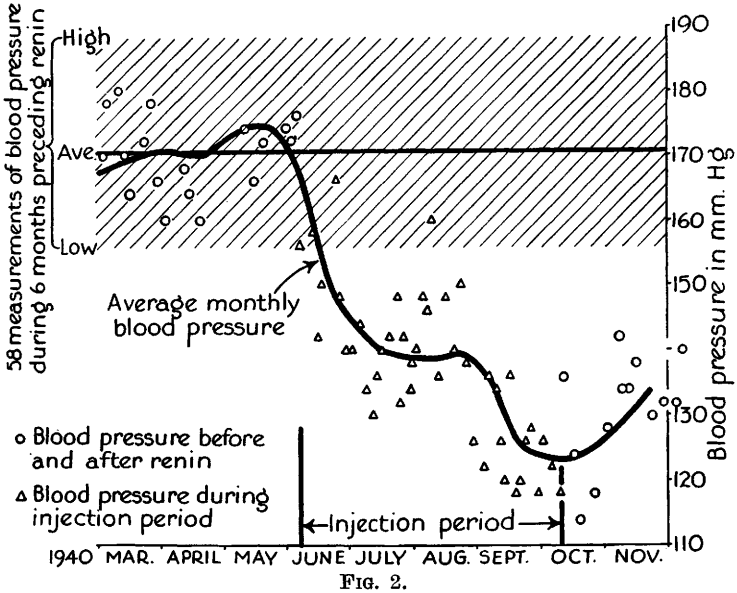

FIG. 2.

The effect of daily injections of hog renin on the blood pressure of renal hypertensive dog No. 2.

the 4 months of treatment until the prehypertensive level of the animal was reached by the end of the third month and a range

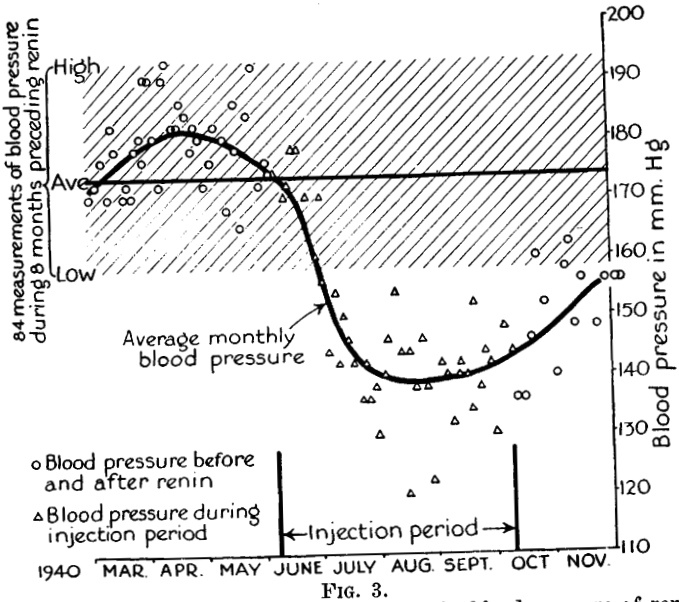

FIG. 3.

The effect of daily injections of hog renin on the blood pressure of renal hypertensive dog No. 3.

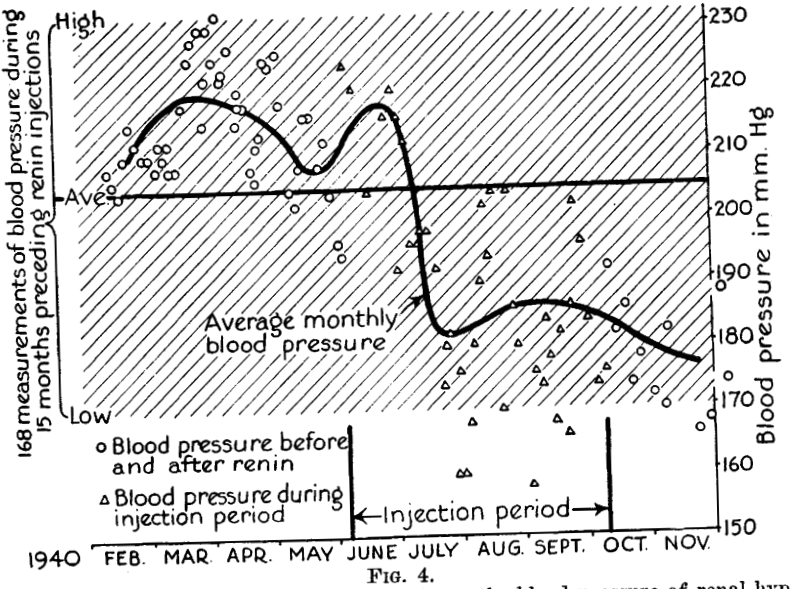

FIG. 4.

The effect of daily injections of hog renin on the blood pressure of renal hypertensive dog No. 4.

slightly below the original normotensive level was attained in the fourth month. During the 2 months following treatment, the

pressures have risen to the original normotensive range.

The third dog (Fig. 3) was hypertensive for 17 months prior to renin therapy, although only the last 8 months of this period are recorded. As indicated, the blood pressure fell to the prehypertensive level by the end of the second month of treatment and remained in this range throughout the remainder of the treatment period. During the months which have elapsed since the last renin injection, the blood pressure has shown a gradual rise toward the hypertensive range.

The fourth dog (Fig. 4) was hypertensive for 23 months before hog renin injections were begun, the last 15 months of this period being recorded. The blood pressure of this dog fell to the lower limits of the hypertensive range during the first 2 months of treatment and remained there for the rest of the treatment period. During the 2 months following renin injections the pressures have remained essentially unchanged.

At no time during treatment or subsequently was there any clinical evidence of untoward effects. The appetites of the 4 dogs remained excellent, their weights constant, and their blood urea nitrogens and urinalyses normal throughout the period of observation.

Before hog renin injections, the serums of the dogs showed no neutralizing effect for hog or dog renin. Repeated examinations of the serums of the 20 normotensive and 10 untreated hypertensive dogs used as controls likewise never showed any neutralizing effect for these renins. Antirenin to hog renin became demonstrable by the end of the first month of treatment and with variations in the 4 dogs reached a sufficient titer by the end of the third month to neutralize completely the acute pressor effect of hog renin by the particular assay technic employed. With fluctuations, the antirenin titers for hog renin of the serums of the 4 dogs remained essentially unaltered during the observation periods following hog renin injections (7 months for the first and 2 months each for the other 3 dogs). During hog renin injections and subsequently the neutralizing effects of the serums for dog renin more or less paralleled the antirenin titers for hog renin.

Discussion. This report is obviously preliminary in character. However, the blood pressure reductions obtained in these 4 hypertensive dogs in the absence of any overt evidence of toxic effects are so striking that we have every reason to believe they will be duplicated on a larger series of animals. In our opinion no comparable therapeutic results have thus far been reported in this form of experimental hypertension. In general, there was some corre-

spondence between the magnitude of the fall in blood pressure and the length of the period of hypertension preceding treatment with hog renin. Thus, as indicated in Figs. 1-4, the decrease in blood pressure was greatest in the first dog which had been hypertensive for only 3 months and least in the fourth animal which had been hypertensive for nearly 2 years preceding hog renin injections. Most probably these differences are related principally to the degree of arteriosclerotic change present.

A number of control experiments are obviously necessary: 1. The effect of a similar course of injections of hog renin inactivated by heat must be studied. To date we have treated 2 renal ischemic hypertensive dogs with heat-inactivated hog renin for 4 months without any blood pressure effect, toxic manifestations, or the appearance of antirenin in the serums. These 2 dogs would almost certainly have shown some reduction in blood pressure during the treatment period if active hog renin had been employed. 2. The effect of dog renin and other species of renin must be studied. In this connection, one of us[10] reported that the daily injection of similar doses of dog renin into 2 hypertensive dogs for one and 2½ months respectively had no effect except to elevate slightly the blood pressure of the animals. No antirenin studies were made on these dogs. The second of these animals was the fourth dog (Fig. 4) which received the dog renin injections 18 months prior to hog renin. Consequently, we do not believe that homologous renin will prove effective in reducing the blood pressure of renal hypertensive dogs, although other heterologous renins most probably will.† 3. The effect of highly purified renin preparations, as well as the optimal frequency, dosage, and mode of administration must also be studied. 4. The action of extracts of various tissues from the hog and other species prepared after the manner of renin must be determined.

We have already emphasized the lack of harmful effects in the 4 hypertensive dogs treated with hog renin. The absence of albuminuria is cogent evidence against significant renal damage.[11] However, in view of the deleterious effects of renal extracts in nephrectomized dogs reported by Winternitz, *et al.*,[12] we purpose

[10] Wakerlin, G. E., and Gaines, W., *Am. J. Physiol.*, 1940, **130**, 568.

† Since the preparation of this report we have injected one hypertensive dog with dog renin for 2½ months without the appearance of antirenin or any effect except slightly to increase the blood pressure.

[11] Brucer, M., and Robinson, S. C., *Am. J. Clin. Path.*, 1940, **10**, 800.

[12] Winternitz, M. C., Mylon, E., Waters, L. L., and Katzenstein, R., *Yale J. Biol. Med.*, 1940, **12**, 623.

to examine our treated hypertensive dogs with additional tests of renal, hepatic, and other functions, as well as grossly and microscopically at necropsy. Worthy of note is the fact that, contrary to the suggestion of others,[13] the observed reductions in blood pressure *per se* did not cause toxic manifestations.

Certainly much work remains to be done before the mechanism of this striking reduction in the blood pressure of renal hypertensive dogs by hog renin is clarified. The fact that the hog and dog antirenin titers of the serums of the 4 animals rose simultaneously with the reductions in blood pressure suggests the possibility that antirenin may be responsible. However, the failure of the antirenin titers (especially to dog renin) to fall as the blood pressures of the dogs increased during the months following renin, argues against this. Thus the blood pressure of the first hypertensive dog is now within the original hypertensive range (Fig. 1) and yet the antirenin titer to both hog and dog renin is still such as to give almost complete neutralization of renin pressor effect. Parallel results were obtained when the serums of the 4 treated hypertensive dogs were tested with a crude renin prepared from dog kidney by extraction with physiological salt solution. While the available evidence, therefore, does not favor antirenin as the mechanism responsible for the reductions in blood pressure, this possibility is by no means ruled out. The fact that a heterologous (hog) renin and not the homologous (dog) renin was effective speaks for the probability that the reductions in blood pressure of the 4 hypertensive dogs resulted from some type of immune or antihormone response to renin or less probably to some other heat labile constituent of the renal cortex of the hog. Another, though less likely, possibility is that of a significantly greater concentration of some directly antihypertensive agent in the hog renin solution as compared with the dog renin solution.

Without much question the antihypertensive action of the hog renin injections was not due to the coincidental presence of the antipressor substance under investigation by Harrison and coworkers[6] and by Page and associates.[7] Thus the amounts of kidney equivalent used by them were much larger than those employed by us, the blood pressure increases following cessation of therapy were more prompt in their animals than in ours, and frequently signs of toxicity accompanied their reductions in blood pressure. Moreover,

[13] Williams, J. R., Jr., Grollman, A., and Harrison, T. R., *Am. J. Physiol.*, 1940, **130**, 496.

Harrison, *et al.*,[14] have shown that their principle inhibits the acute pressor effect of renin and that it is extractable from dog kidney and presumably when so obtained effective in the hypertensive dog.

If the promise of our preliminary findings is substantiated by further work this type of treatment will be studied in essential hypertension in man.

Conclusions. 1. Daily intramuscular injections of hog renin for 4 months produced striking reductions in the blood pressures of renal ischemic hypertensive dogs, whereas heat inactivated hog renin and active dog renin were without effect. 2. No toxic manifestations resulted from renin treatment or from the reductions in blood pressures. 3. The serums of the dogs treated with active hog renin neutralized the acute pressor effect of renin in assay animals. 4. The mechanism of these reductions in blood pressure is not clear. Most probably an immune (antihormone?) response to heterologous hog renin is involved.

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Effect of Angiotonin on the Gall Bladder and the Duodenum.

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Angiotonin was first described by Page and Helmer[1] who prepared it by incubating a renin solution with a pseudo-globulin ("activator") fraction of blood serum. Substituting our renin[2] into their process, we too were able to prepare angiotonin. During the period of testing and comparing our product with the original angiotonin some additional pharmacologic actions were observed.

Gall Bladder. Two different series of preparations were used to demonstrate a contraction-stimulating action on the gall bladder. The first group of experiments was performed upon the guinea pig gall bladder (15 tests) according to the *in vitro* technic of Doubilet and Ivy.[3] Strong contractions were observed when rela-

[14] Grollman, A., Williams, J. R., Jr., and Harrison, T. R., *J. Biol. Chem.*, 1940, **134**, 115.

* Florsheim Fellow.

[1] Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 29.

[2] McEwen, E. G., Harrison, S. P., and Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 254.

[3] Doubilet, H., and Ivy, A. C., *Am. J. Physiol.*, 1938, **124**, 379.