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Antiserum for Renin.*

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In some earlier experiments on renin tachyphylaxis in the dog,[1] it was occasionally noticed that when an animal had become tolerant to dog renin is would still give a pressor response to the injection of rabbit renin. This observation, in the light of increasing evidence to support the protein-like character of renin, suggested the possibility of a species difference in this pressor substance as extracted from the renal cortex of these animals. Consequently, the idea occurred to us that a study of the immune responses to renin may furnish a new experimental approach to elucidate further its nature and properties Even if renin is a hormone, as has been suggested, it may still produce recognizable immune or antihormone responses equally valuable for our immediate purpose.[2]

This report is concerned with the preparation and properties of an antiserum produced in rabbits injected with pressor active extracts of the renal cortex of the dog. A preliminary observation on the pressor negating effect of serum from a dog receiving injections of hog renin is also recorded.

The method used for the extraction of renin was essentially that described by Grossman.[3] Dry, powdered, cortical residue, left after alcohol or acetone extraction, was used as crude material from which extracts of the pressor active principle were made. These (extracts) were further purified to remove much of the associated protein. About 30 cc of this partially purified extract was mixed with an equal volume of colloidal aluminum hydroxide. The colloidal aluminum hydroxide with the proteins, including renin, adsorbed on it was concentrated to half the volume by centrifuging and this gelatinous mixture injected intramuscularly into one rabbit.[4] At the end of 2 or 3 weeks the rabbits were bled to ascertain the presence of antibodies in the serum. If antibodies in appreciable titer were present

* This work was aided by a grant from the Graduate School Research Fund of the University of Illinois.

1 Wakerlin, G. E., and Johnson, C. A., *Proc. Am. Phys. Soc.*, 1940, p. 192.

2 Collip, J. B., *Ann. Int. Med.*, 1934, **8**, 10.

3 Grossman, E. B., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 40.

4 Hektoen, L., and Welker, W. H., *J. Infect. Dis.*, 1933, **53**, 309.

the animal was bled to death and the antiserum preserved in the refrigerator. If a low titer serum was found several intravenous injections of the original extract were given to augment its titer. Immunization may also be accomplished by the more common method of injecting the soluble antigen (renin) intravenously in increasing amounts at 3-day intervals.

Since the antigen used was a mixture of renin, kidney protein(s), and serum proteins (especially pseudoglobulin), precipitins for the last were demonstrable in the antiserum. Precipitins for dog serum pseudoglobulin in the antiserum could be removed by *in vitro* adsorption without affecting the renin antibody. To demonstrate the presence of an antibody for renin we had to depend on the bioassay of mixtures of the antiserum and renin. Such assays were always controlled in the same animal by a similar dose of renin mixed with normal rabbit serum. After a number of attempts to determine the optimum quantity relationship between antiserum and renin we routinely mixed 2 volumes of antiserum with one volume of renin and allowed these mixtures to remain at 4°C at least overnight.

Dogs were used for all assays. The femoral artery was cannulated under light ether or local (procaine) anesthesia and the blood pressure recorded with a mercury manometer. Some of the dogs were also subjected to bilateral nephrectomy. After recovery from these operative procedures, the animal was given an initial dose of renin intravenously, usually equivalent to one-half gram of

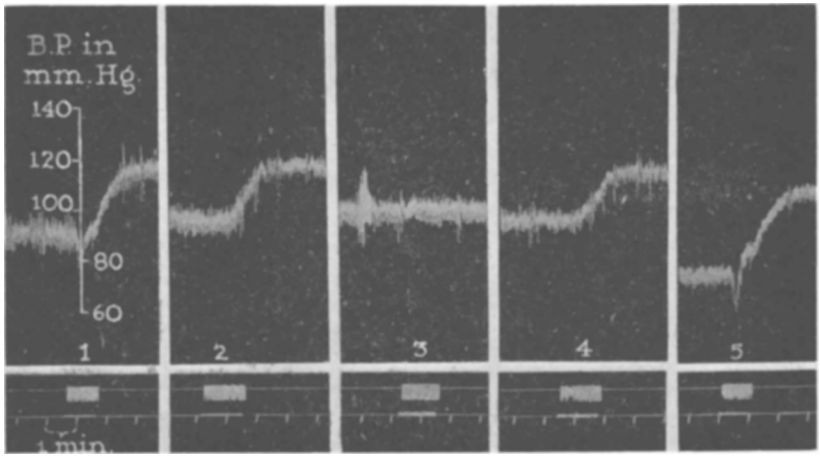

FIG. 1.

Dog, 7 kg, male, bilaterally nephrectomized. 1 Initial dose (2 cc) of dog renin injected, 2 two cc dog renin mixed with four cc normal rabbit serum, 3 two cc dog renin mixed with four cc antiserum for dog renin, 4 repeat of 2, 5 repeat of 1.

kidney per kilo of body weight, to establish the degree of pressor response. When the blood pressure had again assumed its original level a control dose of renin mixed with normal rabbit serum was injected. Figs. 1 and 2 illustrate results which are typical of those obtained by the assay of antiserum from 4 rabbits on a total of 15 dogs.

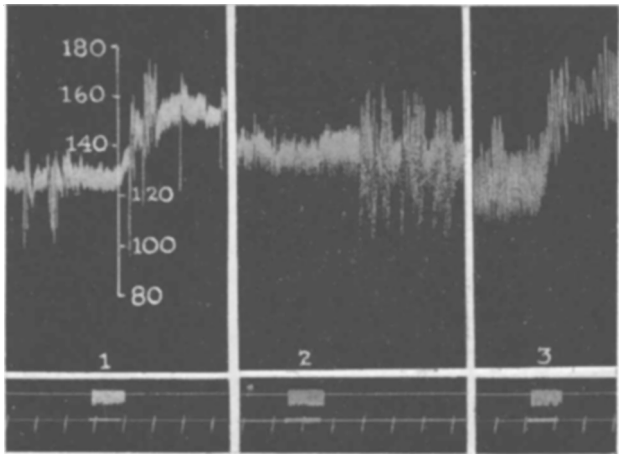

FIG. 2.

Dog, 8 kg, female, not nephrectomized. 1 One part (2 cc) dog renin mixed with two parts normal rabbit serum, 2 one part dog renin mixed with two parts antiserum for dog renin, 3 repeat of 1.

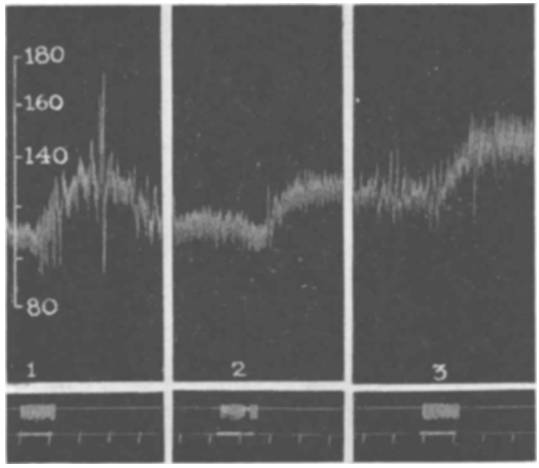

FIG. 3.

Dog, 20 kg, male, not nephrectomized. 1 One part (5 cc) rabbit renin mixed with two parts normal rabbit serum, 2 one part rabbit renin mixed with two parts antiserum for dog renin, 3 repeat of 1.

In 4 dogs we observed the effect of the injection of mixtures of rabbit renin and the antiserums for dog renin just described. The neutralizing effect of these antiserums on the pressor response to rabbit renin was distinctly less than it was for dog renin, as illustrated by Fig. 3. These results suggest a partial antigenic similarity between dog and rabbit renins although further work is necessary to prove this point conclusively.

Since pituitrin has a pressor effect similar to renin on the peripheral vascular bed it was of interest to determine whether the antiserum had any effect on the pressor response to pituitrin. Comparing injections 4 and 5 in Fig. 4, it is apparent that the mixing of pituitrin and antiserum did not change the character or degree of response to this agent as determined on 2 dogs.

In connection with another phase of our study of renin we had occasion to examine the serum of a dog which had received daily intramuscular injections of hog renin in a dosage of one gram of kidney equivalent per kilo for a period of 12 weeks. When serum from this dog was mixed with hog renin and injected into 3 assay dogs, a decidedly diminished response was observed (Fig. 5). No precipitins for hog serum proteins were demonstrable in this antiserum.

The neutralizing substance for renin demonstrated by these results may prove useful in throwing light on the question of whether renin is the pathogenetic agent in experimental renal ischemic hyper-

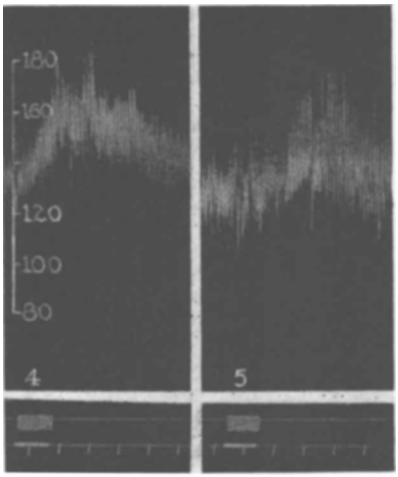

FIG. 4.

Dog, (same as in Fig. III.). 4 Three units pituitrin mixed with four cc antiserum for dog renin, 5 three units pituitrin mixed with four cc normal rabbit serum.

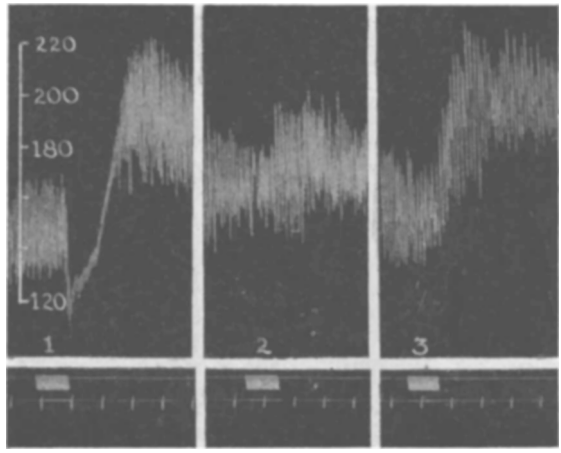

FIG. 5.

Dog, 7 kg, male, not nephrectomized. 1 One part (2 cc) hog renin mixed with two parts normal dog serum, 2 one part hog renin mixed with two parts serum from dog which had received injections of hog renin, 3 repeat 1.

tension. Speculating further, such an "antirenin" may conceivably have therapeutic value, when passively administered or actively produced in experimental renal ischemic hypertension. Experiments aimed at elucidating these possibilities, as well as a study of the immune responses to renins of other species, are now in progress.

Conclusions. 1. The rabbit is able to produce an antiserum which counteracts the pressor effect of dog renin. 2. The antiserum for dog renin appears to diminish the pressor response to rabbit renin. 3. This antiserum has no effect on the pressor action of pituitrin. 4. The dog appears able to produce an antiserum to hog renin. 5. The active principle of the antiserums (antirenin) is most likely a non-precipitating antibody or possibly an antihormone. 6. Studies are now in progress to determine the value of antirenin in the therapy of experimental renal hypertension and also in the elucidation of the possible pathogenetic rôle of renin in this form of hypertension.