REDUCTION IN THE BLOOD PRESSURES OF RENAL HYPERTENSIVE DOGS WITH HOG RENIN[1]

A NUMBER of attempts to reduce the blood pressure 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. Several months ago we reported the production of an antiserum for renin and stated that we purposed to determine the value of "antirenin" actively produced in the therapy of experimental renal hypertension.[2] At this time therefore preliminary results obtained by the treatment of hypertensive dogs with hog renin are presented.

Four dogs rendered hypertensive by the Goldblatt technique were treated for four months with daily intramuscular injections of hog renin representing 1 gram of kidney equivalent per Kg of body weight. Blood serums were examined for antirenin before treatment and subsequently at two-week intervals by a technique previously described.[2]

The history of the first dog is typical of the striking reductions in blood pressure observed in the four animals.

This 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 three months preceding treatment. 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 two months following renin therapy, the blood pressure dropped to an average of 114 mm of Hg or somewhat below the original normotensive level. In the succeeding five months the pressure slowly increased, so that it has now reached the pretreatment hypertensive range.

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

Typical of the four dogs, antirenin to hog and dog renin was demonstrable in the serum of the dog cited above by the end of the first month of treatment and in the third month reached a maximum which was maintained with fluctuations during the observation period of seven months following therapy.

Probably the mechanism of these reductions in blood pressure involves an immune (antihormone?) response to the heterologous hog renin, inasmuch as dog renin and heat-inactivated hog renin were shown to be without effect on the blood pressure of other renal hypertensive dogs. However, the failure of the antirenin titres (especially to dog renin) to fall as the blood pressures of the four dogs increased during the months following renin treatment is difficult to explain on this basis. Conceivably the immune response may be due to some other heat labile constituent of the renal cortex present in the hog 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[3] and by Page and associates.[4] 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, Harrison *et al.*[5] 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 now in progress this type of treatment will be studied in essential hypertension in man.

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

[2] C. A. Johnson and G. E. Wakerlin, *Proc. Soc. Exp. Biol. Med.*, 44: 277, 1940.

[3] A. Grollman, J. R. Williams, Jr., and T. R. Harrison, *Jour. Am. Med. Asn.*, 115: 1169, 1940.

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

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

THE CYANIDE STABLE RESPIRATION OF THE SEA URCHIN EGG

BARRON and Hamburger[1] claim complete inhibition of the respiration of the fertilized sea urchin egg by cyanide. This can not be confirmed by others.[2,3] The observation,[4] that the inhibition is complete just after the addition of cyanide, but soon decreases until about 90 minutes after the admixture, when a constant value is reached, explains this discrepancy. Later stages of development behave similarly, though here the inhibition decreases much faster.[5] Further the cyanide resistant respiration of the sea urchin egg, as in some strains of yeast,[6] turned out to be dependent on the oxygen pressure. Owing to these facts Lindahl con-

[1] *Jour. Biol. Chem.*, 96: 299, 1932.

[2] J. Runnström, *Acta Zool.*, 9: 445, 1928; *Protoplasma*, 10: 106, 1930; *Biol. Bull.*, 68: 327, 1935.

[3] I. Korr, *Jour. Cell. Comp. Physiol.*, 10: 461, 1937.

[4] *Zeitschr. vergl. Physiol.*, 27: 136, 1939.

[5] All experiments performed according to Krebs (*Biol. Jour.*, 29: 1920, 1935) to avoid escape of HCN from the medium.

[6] Tamiya and Kubo, *Acta phytochim.*, 10: 317, 1938.

cludes, that the CN resistant respiration is not a residual part of the normal respiration, but arises under the influence of cyanide. This view is supported by the lower temperature coefficient of the cyanide stable respiration.[7]

RQ of the developing sea urchin egg is known to be about 0.90 (for literature see[8]). Using a modification of the method of Dickens and Simer[9] my co-worker Öhman[10] recently determined RQ of the 1–2 hour stage as 0.73 ± 0.01 and of the 7–8 hour stage as 0.85 ± 0.01. With the 3 manometer method of Warburg I find an RQ of the cyanide resistant respiration of 1.22 ± 0.01 and an almost equal value in both the above mentioned stages, which means a considerably higher value than for the normal respiration. This excludes that an oxidation of cyanide to cyanate could be an important factor of the cyanide stable respiration as suggested for yeast by Pett.[11]

Using a strain of bakers yeast from Rotebro without substrate I have found HCN (0.001 m) to cause an increase of RQ. With rising oxygen pressure the O_2 consumption as well as the CO_2 production rises, the latter more than the former. This proves that CO_2 is not formed by fermentation. An analysis of HCN and NH_3 reveals that a complete oxidation of HCN does not form any considerable part of the cyanide stable respiration. This will probably be the case for sea urchin eggs also. The high RQ of the cyanide stable respiration of the sea urchin egg suggests either a complete oxidation of some substrate with this RQ or an oxidative decarboxilation taking place. The first substrates of this kind to be considered are glyceric acid and pyruvic acid (RQ = 1.20). Both these compounds, the latter in the form of cyanhydrin, increase the cyanide resistant respiration of the sea urchin egg as long as it has not reached its maximal value. Once this rate is reached there is no effect at all.

The inhibition of the respiration in the unfertilized sea urchin egg by cyanide is known to be rather small.[12, 13] In view of the above reported facts, it seems possible that a larger inhibition in the unfertilized egg may be masked by a cyanide resistant respiration. For further investigation the cyanide resistant respiration of the fertilized and unfertilized egg (0.001 Mol KCN) is measured at different oxygen pressures, ranging from 3 to 100 per cent. The function of the dependence on the oxygen pressure is the same in both cases. The CN resistant respiration does not increase at the same rate as the normal respiration at the fertilization and is in the fertilized egg proportionally much smaller than in the unfertilized. It is thus impossible to determine the real inhibition caused by cyanide in the unfertilized sea urchin egg. In 3 per cent. O_2 the inhibition was at least 70 per cent. Under the same O_2 pressure the normal respiration decreased by 3 per cent. A full description of the experiments will appear in *Archiv för Kemi och Mineralogi,* Stockholm.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

SPIRIT BLUE AGAR: A MEDIUM FOR THE DETECTION OF LIPOLYTIC MICROORGANISMS

THE detection of lipolytic microorganisms is important in the study of certain types of food spoilage, in taxonomic bacteriology and in other phases of microbiology. In a recent comparative study,[1] it was noted that each of the media which is used conventionally for the detection of lipolytic organisms falls short in one respect or another. Those media in which a dye is employed as the indicator of lipolysis are often quite toxic for certain types of microorganisms; *e.g.,* Turner's Nile blue sulfate technique inhibits the growth of micrococci.[1, 2] A sensitive differential medium which could be prepared without difficulty, which would not harm appreciably the growth of the organisms and which would permit indisputable detection of fat-splitting microorganisms would be of value in the study of lipolysis.

A medium of the following composition meets these requirements:

Agar	30.0 gm
Tryptone	10.0 "
Yeast extract	5.0 "
*20 per cent. cottonseed oil emulsion	25.0 ml
0.3 per cent. alcoholic solution of Spirit Blue (National Aniline)	50.0 "
Distilled water to make	1000.0 "

* Prepared by grinding thoroughly in a mortar or colloidal mill: 100.0 ml of *fresh* cottonseed oil, 10.0 gm finely powdered gum arabic and 400.0 ml of warm distilled water. Thorough grinding will result in a smooth, permanent emulsion in which most of the fat globules are less than 10 micra in diameter. Certain samples of cottonseed oil, because of high acidity, are unsatisfactory for use in spirit blue agar; Wesson oil is entirely satisfactory for this purpose.

[7] I. Korr, *op. cit.*
[8] *Archiv f. Zool.,* 32 A, N:o 15.
[9] *Biol. Jour.,* 905, 1930.
[10] *Archiv f. Zool., op. cit.*
[11] *Biol. Jour.,* 30: 1438, 1936.
[1] H. F. Long and B. W. Hammer, *Iowa State College Journal of Science,* 11: 343–351, 1937.
[2] R. H. Turner, *Jour. Inf. Dis.,* 44: 126–133, 1929.
[12] J. Runnström, *op. cit.*
[13] I. Korr, *op. cit.*