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A Pressor Substance Produced by Anaerobic Autolysis of Renal Cortex.

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Many attempts have been made to identify the postulated pressor substance that causes high arterial pressure in animals with partially constricted renal arteries. Most of the attention by investigators of this problem has been focused on renin.[1, 2] Recently a heat-stable pressor substance that passes through a collodion membrane has been found in autolyzed renal cortex.[8] In this communication we wish to present information about a pressor substance that is produced by the renal cortex under anaerobic but not under aerobic conditions.

There is suggestive evidence pointing to oxygen or the lack of oxygen as the important factor controlling the production of a pressor substance in the kidney.[3-6] To test this factor the following experiment was performed.

Methods. Under aseptic conditions the renal cortex of the normal dog was separated from the medulla. The tissues were sliced by hand with a razor into sections less than .5 mm thick. These were then incubated in 2 parts, by weight, of dog plasma or Krebs[7] bicarbonate buffer solution in an atmosphere of 95% N_2-5% CO_2 for from 24 to 46 hours. Cultures of these 5 preparations revealed contamination by *B. subtilis* in all portions of one. The fluid portion was then separated from the tissue by centrifuging and then filtering through 6 layers of cheese cloth. The filtrate was injected intravenously into normal, completely nephrectomized, and hypertensive dogs. The standard dose was 1 cc (equivalent to 0.3 g of tissue) per 5 lb of body weight. Systolic blood pressure was measured by

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2 Helmer and Page, *J. Biol. Chem.*, 1939, **127**, 273.

3 Goldblatt, Lynch, Hanzal, and Summerville, *J. Exp. Med.*, 1934, **59**, 347.

4 Harrison, Blaloch, Mason, and Williams, *Arch. Int. Med.*, 1937, **60**, 1058.

5 Houssay and Taquini, *Compt. rend. Soc. de biol.*, 1938, **129**, 860.

6 Taquini, *Compt. rend. Soc. de biol.*, 1939, **130**, 459.

7 Krebs and Henseleit, *Hoppe-Seyless Z. f. Physiol. Chem.*, 1932, **210**, 33.

8 Williams, Harrison, and Mason, *Am. J. Med. Sci.*, 1938, **195**, 339.

palpation of a Van Leersum carotid loop or the dorsalic pedis artery peripheral to a Riva Roca pressure cuff.

Results. In 18 of 20 instances the fluid obtained from anaerobic autolysis of renal cortex caused a rise in systolic blood pressure from 30 to over 300 (estimated) mm of Hg. Eight of these tests were made with normal dogs. The systolic blood pressure rose in 6 instances from 30 to 155 mm of Hg and remained elevated from 2 to 21 minutes. In the other 2 instances the systolic blood pressure fell 5 and 46 mm respectively. Nine injections in dogs with experimental renal hypertension raised the systolic blood pressure immediately. The range of increase varied from 68 to 300 (estimated) mm of Hg and the pressure remained above the base line from 30 to over 100 minutes.

Four bilaterally nephrectomized dogs (2 days postoperative) showed an increase in systolic blood pressure in each of the animals varying from 40 to 105 mm of Hg. The elevation of blood pressure lasted from 10 to 123 minutes. One animal in which a relatively poor response was obtained had had a severe hemorrhage during the operation 2 days previously.

The intravenous injection of material obtained by anaerobic autolysis of *renal medulla* produced no significant change in the blood pressure of 2 normal, 3 nephrectomized dogs, and 1 hypertensive dog. In one nephrectomized animal a rise of 105 mm Hg for 2 minutes followed the injection. However, in this animal, the injection of the cortical substance caused a rise of 105 mm Hg for over 50 minutes.

In contrast, when oxygen was substituted for nitrogen in the preparation of the kidney autolysates no pressor substance was manifest. On the contrary a strong depressor effect was produced by injection of the cortical substance. A fall in the systolic blood pressure of 40 to 186 mm Hg occurred in 8 of 10 cases. The injection of material derived from oxygenated medulla tissue was followed by no change in the blood pressure.

Other properties of the pressor substance may be mentioned. The amount of pressor substance increases with prolongation of anaerobiasis. The injection of 2 preparations that had been incubated for 24 hours raised the blood pressure of 3 normal dogs—5, 40 and 80 mm respectively while the injection of 3 preparations incubated for 42 hours raised the systolic blood pressure of 4 normal dogs 50, and more than 150, 150 and 155 mm Hg respectively. The pressor substance passes through a collodion membrane (6% parlodion in alcohol; ether, 60:40 and 4% glacial acetic acid). The ultrafiltrate so

obtained is active after 20 minutes of boiling and after standing for 4 days exposed to air in an icebox at 5°C. When injected into a hypertensive dog this ultrafiltrate raised the systolic blood pressure over 300 mm Hg. The pressor effect occurs immediately after intravenous injection. Whether this substance is a pressor amine has not been ascertained.

That neither cells nor bacteria are involved in the production of this pressor substance is shown by the following observations. Two preparations, one from fresh dog, the other from fresh beef kidney, were made by grinding the cortex and medulla separately. These were suspended in 2 parts by weight of distilled water for 1 hour, made isotonic with NaCl, centrifuged, filtered through filter paper and then through Seitz filters. The cell-free sterile (cultured) filtrates were set up, under the same condition as described above for the tissues, for 3 days. Control portions of the filtrates were kept on ice and tested at the same time as the incubated filtrates. With both beef and dog renal medullary and cortical filtrates the pressor effects were the same as were obtained with autolysed tissue under the same conditions. The untreated cortical filtrate produced a renin-like response while the anaerobically incubated cortical filtrate produced a pressor effect similar to that described for the anaerobically autolysed cortex. The aerobically incubated cortical filtrate produced a strong depressor substance as did the aerobically autolysed cortex. No comparable effects were observed with the filtrates of the medulla.

Conclusion. The above experiments show that anaerobic autolysis of renal cortex or cell-free cortical extract of the dog produces a powerful heat stable pressor substance that passes through a collodion membrane. This substance is not obtained from kidney medulla that has been subjected to the same conditions. Oxygen inhibits the formation of the pressor substance. The pressor action is manifest immediately after intravenous administration and it is greater and more sustained in hypertensive than in normal dogs. Increased quantities of pressor substance are produced as the anaerobic period is prolonged. The pressor substance is formed just as well in a substrate-free medium as in plasma.