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Epinephrine Sensitivity of Blood Vessel Strips from Salt-Fed and Castrated Rats.* (22810)

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The observation that high sodium chloride intake increased blood pressure in rats has been made by several investigators, including Tobian, Fregly, Meneely and associates, Sapirstein, and others(1-4). The salt diet apparently induced hypertension by increasing cardiovascular reactivity, since Raab(5) found that salt and DCA potentiated the pressor effects of epinephrine in rats. That blood vessels were involved was also suggested by findings of Tobian and Binion(6) that the aorta of rats on high salt diet contained more sodium than those on low salt.

In vitro studies of reactivity of blood vessels have been made by Franklin(7) and Furchgott and Bhadrakom(8), who showed that vessel strips constricted a measurable degree, when immersed in solutions of dilute epinephrine. Since high salt diet apparently increased sensitivity of the entire cardiovascular system to epinephrine, it appeared likely that vasoconstrictor functions of blood vessels might be implicated in the response. The object of this study was to determine if blood vessels from salt-fed rats differed from those of control animals with regard to epinephrine sensitivity.

Materials and methods. In the first experiments with 50 albino rats of Sprague-Dawley strain, about same age and size (80-100 g, and 60 days old), were used. The rats were separated at random into 2 groups, one fed a normal laboratory rat diet and tap water, while the other was given the same diet except that 2% sodium chloride by dry weight had been added, and drinking water changed to 0.9% saline. At the end of 10 weeks, control and salt-fed rats were removed in pairs at daily intervals and killed by blow on the head. The aortae were removed immediately from the level of the aortic arch to the origin of renal arteries. A segment 42 mm long was cut out and split longitudinally as shown in Fig. 1A. Incomplete transverse cuts were then made alternately from each side of flattened strip as shown in Fig. 1B. Control and salt-fed preparations were suspended in aerated Evans' Ringer's solution in 37° bath. Strips were attached to long heart levers, counterbalanced so that tension supplied by weight of levers was approximately 4 g. This weight acted to elongate the cut strips in such a way that circular and spiral

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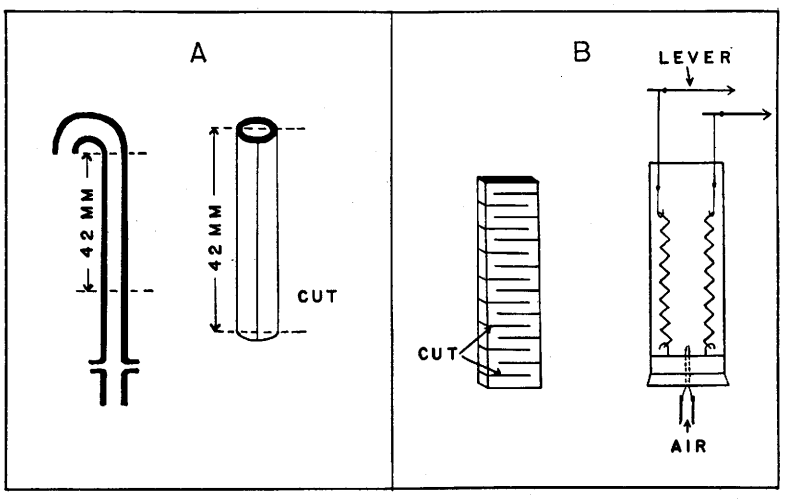

FIG. 1. Method of preparing aortic strips. A. Section cut from rat aorta and split lengthwise. B. Left, incomplete transverse cuts made across flattened strip of aorta. Right, aortic strips attached to levers and suspended in Evans' Ringer's solution.

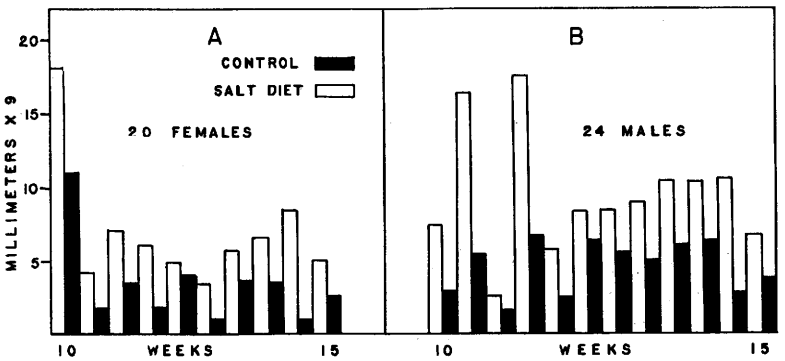

FIG. 2. Contractions produced by epinephrine in aortic strips. A. Response to epinephrine of aortic strips from control and salt-fed female rats. B. Same procedure using aortic strips from control and salt-fed male rats. Ordinate, amount of shortening of strip multiplied by magnification factor of lever system. Abscissa, number of weeks rats were kept on salt diet.

muscle layers of the aorta were oriented in a vertical direction (Fig. 1B). In preliminary experiments it was found that smooth muscle was usually contracted by the cutting procedures, and that best results were secured if the strip was allowed to relax for about 1 hour in aerated Ringer's solution previous to the testing procedures. Experimental and control strips were placed together in Ringer's solution to which l-epinephrine‡ was added, to make a concentration of 0.2 μg/ml. Usually 2 or 3 trials could be made for each pair of vessels provided relaxation of the muscle was induced by replacing the epinephrine solution with fresh Ringer's between tests. In a second series a similar group of 50 rats was separated as to sex, and half of the males and females castrated under ether anesthesia. The animals were allowed to recover for 3 weeks before being placed on the salt diet described above. After 10 weeks or longer the aortae of salt-fed castrated animals were compared with those of control diet castrates for sensitivity to epinephrine. As a further check on results, a group of intact rats from the same source was given either

‡ Supplied as Suprarenin, courtesy of Winthrop-Stearns, Inc.

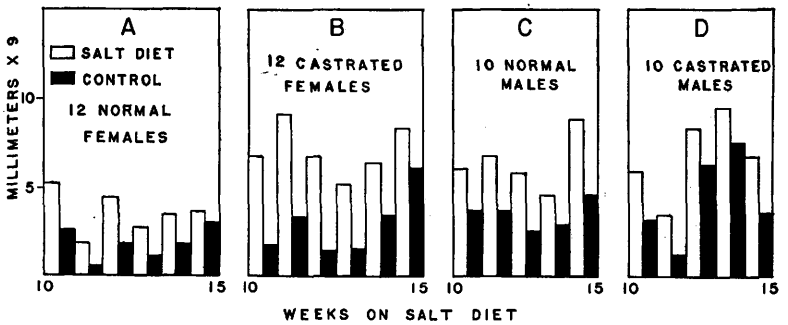

FIG. 3. Effects of castration on epinephrine contraction of aortic strips. A. Comparison of contractile response of aortic strips from control and salt-fed female rats. B. Procedures as in A, except all rats were ovariectomized previous to being placed on salt or control diets. C. Procedures as in A, except all animals were intact male rats. D. Procedures as in A, except all rats were castrated previous to being placed on salt or control diets. Ordinate and abscissa as in Fig. 2.

salt or control diets and their aortae tested as described above.

Results. Effects of salt diet on aortic epinephrine sensitivity for intact rats are shown in Fig. 2. These data indicate that responsiveness to epinephrine of aortic strips from salt-fed rats of both sexes was about double that of control strips. Statistical treatment of the data by Fisher's "t" method (9) gave a P value of 0.001, indicating a highly significant difference in sensitivity between salt-fed and control rats of both sexes. The results shown in Fig. 2 also disclosed a sex difference in the response of the aortae from salt-fed and control groups, the male aortic strips being more responsive to the same epinephrine solution than female strips. Statistical treatment gave $P = 0.001$, indicating a significant difference between the two groups.

By comparing Fig. 3A and B it can be seen that castrated females on control diet yielded aortae that were slightly but not significantly more sensitive to epinephrine than did normal females ($P = 0.2$). However, when castrated females on salt diet are compared with normal salt-fed females, (Figs. 3A and B) it is clearly evident that ovariectomy greatly increased the sensitivity of aortae to the salt diet. Statistical treatment of data gave a P value of 0.01, indicating that the difference was not likely due to chance.

In contrast to results found in operated females, the castration of males did not cause any similar increase in sensitivity of the aortae to epinephrine. When the salt diet was imposed on castrated males there was also no appreciable increase in reactivity of the aortae over those of normal males on the salt diet, as can be seen by comparing Fig. 3C and 3D. The normal rats shown in Fig. 3A and 3C confirm the finding that high salt diet increased epinephrine sensitivity of the aorta in both sexes.

Discussion. The finding that a high salt diet resulted in deposition of increased sodium in tissues, and that this was associated with development of hypertension, has been established by several investigators cited previously. From our observations on isolated blood vessel strips, it is apparent that one result of sodium deposition is an increased sensitivity of circular smooth muscle to the stimulating effects of epinephrine.

Sex differences found in the aortic response to epinephrine are in accord with the findings of Stamler(10) that salt-induced hypertension could be produced readily in male dogs and cockerels, while at most only a slight response could be produced in female dogs and laying hens. Estrogens were reported by Stamler to have anti-hypertensive effects, in that this hormone lowered blood pressure, or prevented the expected rise when DCA plus salt diets were imposed on cockerels.

Removal of ovaries and a high salt diet in our rats caused increases in aortic epinephrine response to the extent that castrated females

developed about the same aortic sensitivity as males. In comparison to females, castration did not have much effect on males, since little difference was noted when the aortae of operated animals were compared to normal ones. Likewise, the salt diet did not increase aortic responsiveness to epinephrine in castrated males beyond that found in normal males. These findings indicate that the presence of female sex hormones is required to maintain a low reactivity to epinephrine in the aorta. Removal of the male hormone, on the other hand, did not change the reactivity of the aorta, nor sensitize the animal to the salt diet. The mechanism whereby the salt diet increases the reactivity of vessel strips to epinephrine may be explainable by the theory of Raab(5) that increased intracellular sodium in smooth muscle of blood vessels potentiates the constrictor effects of epinephrine, possibly by raising the membrane electrical potentials of muscle cells upon which catecholamines act.

Summary. Strips were prepared from aortae of rats and their reactivity to epinephrine tested by means of a lever system that recorded shortening of the smooth muscle. A 2% salt diet increased the sensitivity of the vessel to l-epinephrine in both sexes. Male aortae in all instances had greater sensitivity to epinephrine than those of females under the same conditions. Castration markedly increased the sensitivity to epinephrine in females, especially when the castrates were placed on the salt diet. In contrast, male aortae were not particularly changed by castration.

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Insulin Secretion Following Carbutamide Injections in Normal Dogs.* (22811)

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The hypoglycemic action of orally active sulfonylureas may be due to one or more of the following reasons: a) destruction of the pancreatic A cells with decreased glucagon production(1), b) inhibition of one or more of the enzyme systems involved in liver glycogenolysis(2-6), c) decreased intestinal absorption of glucose(7), d) enhancement of insulin action(8), e) inhibition of the enzymes responsible for insulin degradation (9), and f) stimulation of insulin secretion (10-12). The possibility that these drugs may act through pituitary, adrenals, thyroid, parathyroid, testicles or the central nervous system appears unlikely(13) while the possibility that they may stimulate peripheral glucose utilization is uncertain(14,15). Purpose of this work was to investigate the hypothesis that carbutamide stimulates release of insulin into pancreatic blood.

Materials and methods. The problem was investigated by means of 7 pancreatic-femoral and 5 mesenteric-femoral cross-circulation ex-

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