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Received May 19, 1952. P.S.E.B.M., 1952, v80.

Adenosine Triphosphatase Activity of the Vascular System.* (19611)

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The contractile mechanism which specializes skeletal muscle for quick movement is presumably the same as that which enables smooth muscle to maintain its tonus state. It has been shown that the energy-rich phosphate bonds of adenosine triphosphate are the sole immediate source of energy for skeletal muscle contraction. In addition, evidence points to the fact that ATP plays a central role in the maintenance of smooth muscle tone and in the initiation of its contraction. Dubois and Potter(1) devised a method to determine the amount of adenosine triphosphatase (ATPase), the enzyme which is responsible for ATP hydrolysis, in various tissues. Krantz, Carr and Bryant(2) showed that the acutely-acting vasodilators of the nitrite-nitrate series inactivated ATPases present in the aorta of rabbits. The interference with enzyme activity occurred with therapeutic levels of the vasodilators. Thus it appears possible that these vasodilators may exert their action through the ATP-ATPase mechanism as the target enzyme system in arterial tissue.

Owing to this observation we considered it of interest to study the ATPase activity of various segments of the vascular bed. Furthermore, the normal variations within a single species of animal appeared important. In addition, the variation of the vascular ATPase activity of different species was determined, and the effect of age upon the enzyme concentration in the vascular wall was estimated.

* The expense of this investigation was defrayed in part by a grant from the National Institutes of Health.

Method. The principal studies were carried out on male mongrel dogs approximately in middle life. The animals were maintained on a balanced laboratory ration for a period of several months until a good nutritional state was established. The animals were anesthetized with an intravenous dose of pentobarbital sodium, and the vessels removed immediately after anesthesia ensued. It was previously determined that this barbiturate did not affect the ATPase activity of the vessels. All determinations were carried out within one-half hour after tissue removal. It was determined that during this period the ATPase activity did not undergo diminution. This same procedure with suitable modifications, to adapt it to the various species, was employed in all animals studied, omitting barbiturate anesthesia. Rats were used for the studies concerning the influence of age on the enzyme owing to the ease of obtaining animals of definite age. The ATPase activity of the tissues was determined by the method of Bell, Carr and Krantz(3). This method employs the tissue incubation of DuBois and Potter, the precipitation of the phosphate as magnesium ammonium phosphate recommended by Griswold(4), and the use of ammonium sulfate as suggested by Lowry and Lopez(5). The recovery of phosphate from tissue by the method is approximately 90%. The reproducibility of the method on a single specimen of tissue is shown by a standard deviation of 0.18 unit and a coefficient of variability of 3%. The values are expressed in ATPase units as adopted by DuBois and Potter, *i.e.*, the number of gam-

TABLE I. ATPase Activity of Vascular System of the Dog.

	No. of animals	High	Low	Mean	S.E.
Arterial tissue					
Aorta	21	16.5	7	11.6	±.7
Carotid	21	8.5	1.5	5.1	±.5
Coronary	32	8.1	1	4.6	±.4
Renal	19	7.7	2	4.8	±.3
Femoral	20	7.3	1	3.3	±.4
Venous tissue					
Jugular	19	No activity			
Renal	1				
Brachial	1				
Pulmonary	1				
Femoral	1				
Vena cava	1				

TABLE II. Aortic ATPase of Young and Old Male Rats.

Group No.	Wt, g	No. of rats	ATP units
	Young rats		
1	50–60	3	26
2	"	3	26
3	45–55	4	49
4	"	4	32
5	"	4	24
	Young adult rats		
1	150–180	1	34
2	"	2	36
3	150–200	3	29
	Old rats		
1	450–500	1	41
2	"	1	34
3	"	1	26
4	430–500	2	28
5	"	2	31

TABLE III. Aortic ATPase of Various Species.

	Animal No.	ATP units
Chelonia mydes—turtle	1	0
	2	0
Rana virescens—frog	1	7.8
	2	8.5
	3	4
Gallus domesticus—chicken	1	1.5
	2	>1
	3	>1
Epemys norvegicus—wild rat	1	22
	2	22
	3	21
Cavia cobaya—guinea pig	1	7
	2	10.5
	3	8.7
Lepus cuniculus—rabbit	1	5
	2	5.8
	3	6.3

ma of inorganic phosphorus liberated by 1 mg of tissue in 15 minutes at 37°. The value expressed as ATPase activity may be due to one or more enzymes present in the tissue as pointed out by Swanson(6). For example, liver has been shown to contain several kinds of enzymes measured as ATPase. In our incubation system we have maintained a pH value of 7.4 and Ca^{++} was used as the activator. The enzyme or enzymes acting under these conditions have been considered a measure of the ATPase tissue activity.

Results. (See Tables 1, 2, 3)

Discussion. An examination of Table I reveals that the distribution of ATPase varies greatly in the arterial system. From our studies in the dog, the venous system is apparently devoid of the enzyme. Kováts(7) found the actomyosin content of different parts of the heart proportional to their mechanical activity. Csapó(8) observed that the uterus was relatively poor in actomyosin until pregnancy occurred. During pregnancy it was observed that the actomyosin activity increased and its distribution bore a direct relation to the functional activity of the organ during gestation and at term. Similarly, the vascular system of the dog enjoys a broad variation in ATPase activity. Nevertheless the data clearly show that the enzyme is present in greatest quantity in the aorta. The coronary, carotid and renal arteries appear to share about equally in the enzyme distribution. It has been shown by Baló, Banga and Josepovits(9) that the human aorta contains from 20 to 31% of water-soluble protein, and it is this portion of aortic tissue which contains ATPase.

The rat's aorta (Table II) contains a very high concentration of ATPase. It appears from our studies that this value is not affected by age or captivity in this species.

Our principal concern in these studies is the relationship of the concentration of ATPase in arteries to the phenomena of vasoconstriction and the action of vasodilators. Indeed it appears that vasodilators which inhibit ATPase activity might have ATP-ATPase as their target enzyme system and through this inhibition elicit their response. One may postulate that the inactivation of ATPase pre-

vents ATP from hydrolyzing and thus deprives the arterial tissue of the energy which is released from the high energy phosphate bond. If through the decomposition of ATP by its enzyme ATPase, tonus and constriction of the artery are maintained, it is possible that a drug which acutely relaxes the artery may do so by interfering with the ATPase activity of the tissue. Studies aimed at clarifying this postulate are in progress.

Summary. The ATPase activity of the vascular system of the dog has been studied. It has been shown that the enzyme content of the different arteries enjoys wide variation. The venous system does not contain the enzyme. ATPase activity of the aorta of the rat was not influenced by age or captivity. The aortic ATPase activity of various species of animals is presented.

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Received May 26, 1952. P.S.E.B.M., 1952, v80.

Gastric Secretion in Heidenhain Pouches Following Section of Vagus Nerves to Main Stomach. (19612)

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The cephalic phase of gastric secretion is nervous in origin and is mediated via the vagus nerves to the stomach. Apparently the gastric phase is humoral in origin and is mediated by "gastrin" which is elaborated by the mucosa of the gastric antrum. A Heidenhain pouch is a separated, vagally denervated, greater curvature, gastric pouch. Thus, the exocrine secretion from this pouch is stimulated, probably exclusively, by the humoral phases of secretion, *i.e.*, antral and intestinal phases. Any observed change in Heidenhain pouch secretion produced by sectioning the vagi to the main stomach may be presumed to reflect a proportional change in the circulating chemical substances (hormone(s) and secretagogues) which are responsible for the chemical phases of secretion.

Although it is well known that the cephalic and gastric secretory mechanisms can function independently of the other, it is not yet established whether the normal function of either is impaired by the absence of the physiological activity of the other. Grossman(1) recently stated ". . . that vagal stimuli and gastrin can act together in stimulating gastric secretion is certainly in accord with observations, in the sense that any two different stimuli can act together additively. However, the view that either of these two mechanisms for secretion regularly participates in producing a response to the other is open to serious question." Evidence is accumulating(2-4) that the gastric secretory response to vagal stimulation is decreased when the antral mucosa is defunctionalized by cocainization, vascular impairment, or excision. The conclusion has been advanced that the cephalic phase is actually neurohumoral and not solely neural as formerly held. There is much less evidence of participation of the vagi in the gastrin mechanism. To our knowledge, no quantita-