the latter was between 8 and 8·5, this enzyme system differed from a microsomal aromatic hydroxylation enzyme system[8] in that it was found primarily in the soluble (72,000 g-supernatant) fraction. After continuous dialysis for 7 hr, the soluble fraction remained active, thus yielding no information as to cofactor requirements. The oxidation rate was greatly diminished when the reaction was conducted anaerobically. The enzyme system was also inhibited by β-diethylamino-ethyl diphenylpropylacetate (SKF 525-A) at a final concentration of 10^{-3}M.

The presence of the enzyme system has been demonstrated in the liver of the rhesus monkey, rat, mouse, rabbit and guinea pig. Its absence in the liver of the dog is consistent with the finding *in vivo* that no metabolite of DCM was excreted in the bile or urine by this species.[6] Of all the animals thus far studied, the rabbit is the only one that showed enzyme activity in the small intestine. Further work is in progress to characterize the enzyme system, and to elucidate its cofactor requirements and substrate specificity.

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Enzymic conversion of valine^5-angiotensin-I into an oxytoxic principle by impure preparations of alpha-amylase

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RECENTLY, Bumpus *et al.*[1] have shown that isoleucine^5-angiotensin-II and the heptapeptide, arginyl-valyl-tyrosyl-isoleucyl-histidyl-prolyl-phenylalanine, are the only peptides which could arise from isoleucine^5-angiotensin-I that show significant oxytocic activity. The measure of oxytocic activity, then, is a direct index of the conversion. Previous work by Huggins and Walaszek[2, 3, 4] on a vasopressor polypeptide with oxytocic activity, formed by incubating fraction IV-4 of human plasma protein with a crude preparation of alpha-amylase, suggested that such preparations may contain enzymes which convert angiotensin-I to an oxytocic principle.

Alpha-amylase was obtained from Nutritional Biochemicals Corporation and was labelled batch #7942. Synthetic valine^5-angiotensin I and II were generous gifts of Professor Rolfe Meier, CIBA, Ltd., Basle, Switzerland. Samples of human saliva were obtained from our laboratory associates, separated into two groups, smokers and non-smokers. All assays were carried out on the isolated rat uterus, as described by Carlini, Picarelli and Prado.[5] Uteri were taken from virgin rats weighing approximately 200 g, injected on the previous day with 10 μg of diethylstilbesterol per 100 g of body weight. The uteri were suspended in 10 ml of de Jalon's solution at 30 °C and activity was recorded with a frontal lever on a smoked drum. The contact time of the agonist with the tissue was 1–½ min and 5-min intervals elapsed between applications. Incubations of enzymes with substrates were carried out at 30 °C; for studies of heat denaturation, the enzymes were placed in a boiling water-

min.

The results in Fig. 1 show the time-course formation of an oxytocic principle in the incubation mixture of valine^5-angiotensin-I and impure alpha-amylase. The incubation mixture of de Jalon's solution contained 0·25 per cent of the crude alpha-amylase and 0·25 μg of valine^5-angiotensin-I per ml. Maximum formation of the oxytocic activity was found at approximately 10 minutes. "Angiotensinase" enzymes are present in the impure preparation of alpha-amylase, since incubation for periods longer than 30 min resulted in complete loss of oxytocic activity. When 0·005 μg of valine^5-

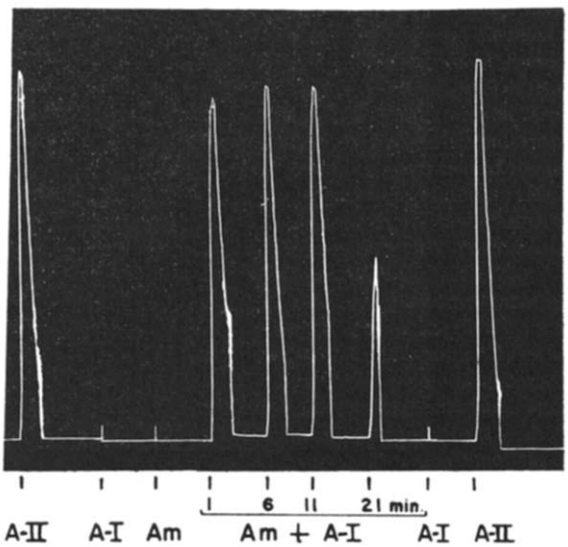

FIG. 1. Isolated rat uterus, 10-ml bath, 30°C; A-II and A-I are 0·005 and 0·05 μg of Valine^5-angiotensin-II and I, respectively; Am represents 0·2 ml of a 0·25% solution of a preparation of crude alpha-amylase; and Am + A-I, 0·2 ml of an incubation mixture of the same solution (0·25%) of crude alpha-amylase and valine^5-angiotensin-I, 0·05 μg/0·2 ml. A dose of angiotensin-I (0·05 μg) which produced no contraction prior to exposure to the preparation of alpha-amylase, elicited an uterine response after incubation with the crude enzyme.

TABLE 1. THE ENZYMIC CONVERSION OF VALINE^5-ANGIOTENSIN-I INTO AN OXYTOCIC PRINCIPLE BY PREPARATIONS CONTAINING ALPHA-AMYLASE

Val.^{5}-angiotensin-I incubated with—	Number of experiments	Conversion	Inactivation of oxytocic principle
Alpha-amylase, NBC #7942, non-crystalline	5		Within 30 min
Alpha-amylase, crystalline	1	—	
Boiled alpha-amylase, NBC #7942, non-crystalline	2	—	
Saliva*	12	+	No destruction after 60 min
Boiled saliva	4	+	No destruction after 60 min

* Saliva obtained from 8 smokers and 4 non-smokers.

angiotensin-II was added to the muscle bath a strong contraction occurred (Fig. 1), whereas 10 times this amount of valine[5]-angiotensin-I had no effect until it was incubated with the crude enzyme.

The data presented in Table 1 show the results of several experiments using different sources of alpha-amylase. It can be seen that saliva possesses the necessary enzymatic components for converting angiotensin-I into an oxytocic principle. Initially, we separated the saliva into two groups, smokers and non-smokers; however, since both groups produced an oxytocic principle on incubation and neither could be distinguished in our assay system, we have combined them into one group in Table 1. Two interesting observations are recorded; first, saliva does not possess "angiotensinase" activity, since we could not show destruction by extended incubation periods and, second, the enzyme in saliva which is responsible for formation of oxytocic activity was stable to heating in a boiling water bath for 10 min. This latter may be contrasted with the enzyme in the crude preparation of alpha-amylase which is destroyed by a similar period of heating. Crystalline alpha-amylase was not effective in forming an oxytocic principle.

It has been reported by Skeggs *et al.*[6] that angiotensin exists in two forms. The first, angiotensin-I, a decapeptide, was the initial product of the action of the renal enzyme, renin, upon its plasma substrate. The second, angiotensin-II, an octapeptide, was produced by the action of a "converting enzyme" in the plasma. Since this earlier report, numerous studies have appeared which are relevant to the presence of "converting enzyme" in tissues other than plasma.[1, 5, 7–10]

It should be noted that the data presented in this communication do not afford sufficient evidence to conclude that the enzyme in the impure alpha-amylase preparations which forms the polypeptide, substance A from fraction IV-4 of human plasma protein, is the same one that converts angiotensin-I to an oxytocic principle. These aspects of this problem are under investigation.

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