Werte, die 2–3mal höher sind, als bei Erwachsenen. Die Befunde werden im Bezug auf die niedrige Lipaseaktivität des gastrointestinalen Traktes sowie auf die hohe Fettaufnahme während der Säuglingsperiode diskutiert.

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Distribution of a Renin-Like Enzyme in the Bovine Adrenal Gland

The adrenal gland of the rabbit contains an enzyme that reacts in vitro with native renin substrate to form a vasopressor polypeptide, probably angiotensin I[1]. Recently, Hayduck, et al.[2], found a similar enzyme in the adrenal gland of the dog. Whether the renin-like enzyme reacts with renin substrate in vivo is not known. However, in view of the effects of angiotensin on the secretion of aldosterone and medullary catecholamines[3,4], the possibility exists that the enzyme influences specific adrenal gland functions. In the present investigation, a renin-like enzyme was found in bovine adrenal gland, and a study was made of its distribution.

Adrenal glands were dissected (at 4°C) as soon as possible (1–2 h) after slaughter. The glands were cut with a razor blade into cross-sections (2–3 mm thick). Each cross-section was cut to yield the following pieces: 1. cortex, 2. corticomedullary junction, 3. medulla and 4. central vein. Adherent capsular tissue was not removed from the cortical pieces. Similarly, it was not possible to separate the central vein from a small amount of medullary tissue.

The tissue pieces were weighed, homogenized, and then distilled water was added, 2 ml/g wet weight of tissue. The homogenates were frozen and thawed twice using a solid CO_2-ethanol slurry. The homogenates were centrifuged at $1{,}690 \times g$, at 4°C, for 30 min to remove tissue debris. The supernatants were dialyzed against Na_2HPO_4-citric acid buffer, pH 2.6, at 4°C, for 24 h, then against water for 6 h, and finally against 3 m*M* Na_2 · EDTA for 24 h. If, at the end of this time, the extracts were not free of catecholamines (estimated in terms of effects on mean arterial blood pressure[5]), dialysis was continued using 1 m*M* sodium phosphate buffer, pH 7.0, for another 24 h. Precipitates formed during dialysis were removed by centrifugation at $1{,}690 \times g$, at 4°C, for 30 min. Protein concentrations were measured by the method of Warburg and Christian[6].

One ml of dialyzed extract was added to 2 ml of rabbit renin substrate (2,000 ng of angiotensin content/ml) buffered at pH 6.0, the pH optimum of the reaction[1,7,8]. Soybean trypsin inhibitor, Na_2 · EDTA and dimercaprol were added to inhibit kallikrein and angiotensinase enzymes[7–9]. The reaction mixtures were free of angiotensinase activity. The incubation was carried out at 37°C. Samples were taken for assay after 1, 2 and 3 h of incubation. Renin-like activity was measured in terms of ng of angiotensin formed/ml/h. Angiotensin was assayed using the mean arterial blood pressure response of the pentolinium-treated, anesthetized rat[5]. Asn[1], Val[5]-angiotensin II was used as the standard reference compound.

Results are shown in the Table. Per g wet weight, the medulla contained 11-times more renin-like activity than did the cortex. Possibly because of the higher lipid content of cortex, the enzyme activities of cortex and medulla per mg of protein were much closer. The cortical pieces of tissue were not freed of adherent capsule, raising the possibility that the cortex itself has little or no renin-like activity. On this point, it may be relevant that Goormaghtigh and Handovsky[10] have described modified smooth muscle cells, similar to the juxtaglomerular cells of the renal afferent arterioles, in the capsule of the adrenal gland. Further support for the possibility that cortex contains little or no renin-like activity arises from the observation that the enzymic activity of the corticomedullary junction, per g wet weight, is not the mean of the cortical and medullary activities. In fact, the specific activity (activity/mg of protein) of the renin-like enzyme of the corticomedullary junction is less than that of either cortex plus capsule or medulla.

The central vein was of interest because of its large apical crescent of smooth muscle, which is penetrated by a variety of small veins draining the cortex and medulla. It was thought that a large concentration of renin-like enzyme in or around the apical crescent could, by releasing angiotensin, affect venous drainage and thereby either reduce flow or affect a redistribution of flow within the gland.

As shown in the Table, the renin-like activity of the central vein is about 65% that of medulla and thus is in great excess of that accounted for by trapped blood (average renin activity of 1 ng/ml/h). However, it was not possible to obtain central vein tissue without some

Distribution of the renin-like enzyme in bovine adrenal gland

Tissue	ng of angiotensin released per h	
	per g wet weight	per mg of protein
Cortex	18	15
Corticomedullary junction	37	10.5
Medulla	200	27
Central vein	128	14.5

Tissue segments were separated, extracted and assayed as described in the text. These values are the means of 4 experiments. The variation among experiments was less than 10%.

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adherent medullary tissue. By inspection, less than 10% of the weight of the central vein pieces was made up of medullary tissue. Therefore, unless there is a gradient of renin-like enzyme in the medulla, such that most of the medullary enzyme is adjacent to the central vein, it would appear that central vein itself contains relatively large quantities of renin-like activity.

These data show that the bovine adrenal gland contains an enzyme that is similar to renin in all respects tested. The enzyme does not pass through dialysis membranes and does not have an absolute requirement for those metals chelated by EDTA and dimercaprol[7-9]. The enzyme is stable for several hours at pH 2.6, 4°C. It reacts with rabbit renin substrate to form a product capable of causing a transient rise of mean arterial blood pressure. No product is formed in the absence of substrate, and no product is formed if either the enzyme or substrate is heated in a boiling water bath before preparing the reaction mixture. The reaction product is stable in boiling water but is inactivated by trypsin and chymotrypsin. The adrenal renin-like enzyme is distinguished from pseudorenin in that the adrenal enzyme is reactive with native renin substrate in the presence of other plasma components[11].

The apparent wide distribution in bovine adrenal gland of an enzyme capable of releasing angiotensin is consistent with the concept that the intraglandular release of angiotensin could regulate some adrenal secretions. As shown by LARAGH, et al.[3] and FELDBERG and LEWIS[4], angiotensin II, in low concentrations, stimulates the release of medullary catecholamines and aldosterone. Higher concentrations stimulate the secretion of corticosterone[12]. Although renin-like enzymes occur in several other tissues, in no other organ or gland are there more clearly demonstrated effects of angiotensin. However, there is, as yet, no evidence that the adrenal renin-like enzyme has access to and reacts with renin substrate in vivo.

Résumé. La glande surrénale bovine contient un enzyme qui réagit avec l'angiotensinogène pour former de l'angiotensine. L'enzyme est distribué dans toute la glande surrénale, en plus grande quantité dans la médullosurrénale et dans la veine centrale. Vu les effects bien connus de l'angiotensine sur la sécrétion de l'aldostérone et sur les catécholamines de la médullosurrénale, il est possible que l'enzyme «rénine-semblable» influence les fonctions spécifiques de la glande surrénale.

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The Reaction of Ca^{++} with the Inner and Outer Membrane of Mitochondria

In addition to the energy-linked transport of Ca^{++}[1], and to the metabolism independent Ca^{++} binding[2], another type of Ca^{++} binding has been described in isolated mitochondria[3,4]. In this reaction, small amounts of Ca^{++} are bound with very high affinity to specific sites in the mitochondrial membranes. Scatchard plots of this type of binding have led REYNAFARJE and LEHNINGER[3] to postulate carrier molecules specific for Ca^{++}, a conclusion also reached by MELA and CHANCE[5,6]. The role of the high affinity binding in the active translocation of Ca^{++} has been discussed[3,7-9], and the two processes go parallel in some mitochondrial and submitochondrial preparations[3]. It was thought that information on this problem could be obtained from a study of the distribution of the Ca^{++} binding sites between the inner (IM) and the outer (OM) mitochondrial membrane. Indeed, the latter does not participate in the energy-linked transport of Ca^{++}; if the high affinity sites play any role in the active translocation, they should be absent from the OM.

Materials and methods. Mitochondria were prepared from rat livers by the standard sucrose procedure of SCHNEIDER[10]. The 2 membranes were separated according to SCHNAITMAN and GREENAWALT[11]. Malic dehydrogenase and monoamine oxidase were determined as described by SCHNAITMAN et al.[12], but the temperature of the monoamine oxidase medium was 21°C instead of 37°C. Cytochrome oxidase was determined polarographically with a Clark electrode, in a medium containing 0.04 *M* phosphate buffer, pH 7.4, 0.0004 *M* $AlCl_3$, 0.015 *M* Na-ascorbate, 0.0001 *M* cytochrome C, 1 mg lubrol, and 0.05–2 mg of enzyme protein. Volume, 2 ml, temperature, 25°C. Respiratory control by ADP or by

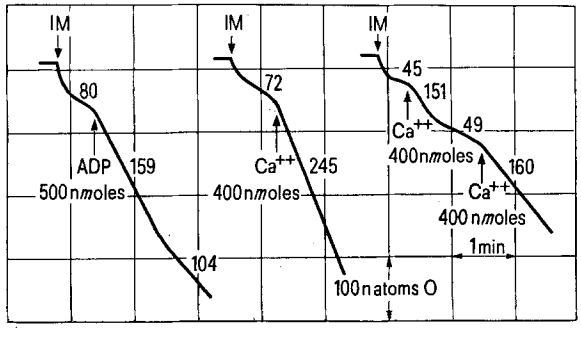

Fig. 1. Stimulation of the respiration of the inner membrane plus matrix fraction by ADP and by Ca^{++}. Technical details are described in Methods. Temperature 25°C. Final volume 2 ml. 1st and 2nd trace from the left: inorganic phosphate present. 3rd trace from the left: inorganic phosphate absent.

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