type. The results of the crosses given in Table 1 strongly indicate that the *rex* gene lies between the *N* and C_I genes.

It should be noted that the identification of the *rex* locus as a separate gene demonstrates that the C_I gene is not the only λ gene to be expressed in the lysogenic state.

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Renin-Like Enzyme in the Adrenal Gland

Abstract. *The rabbit adrenal gland contains an enzyme which reacts with renin substrate to form a vasopressor polypeptide, probably angiotensin I. In view of the strong effects of angiotensin on secretion of aldosterone and catecholamine, this finding suggests that there may be an intra-adrenal mechanism for the control of adrenal secretions.*

The finding (*1, 2*) that tissues other than the kidney contain renin or a renin-like enzyme suggests that the renin-angiotensin system is possibly a local hormone system (*2, 3*). Although the adrenal gland has not been surveyed in this regard, there is evidence that it may contain renin. Goormaghtigh and Handovsky (*4*) found modified smooth muscle cells, similar to the juxtaglomerular cells of the renal afferent arterioles, in the capsule of the adrenal gland. Granzer (*5*) found an enzyme in adrenal homogenates which degraded renin substrate. However, evidence that angiotensin was one of the degradation products was not presented. These observations, along with the demonstrations that angiotensin causes the release of medullary catecholamines (*6*) and aldosterone (*7*), led to a study of adrenal tissue for an enzyme capable of releasing angiotensin.

Four experiments, each requiring five normal adult rabbits, were performed as follows. Immediately after killing an animal with an intravenous overdose of pentobarbital (60 mg/kg), the adrenal glands were removed aseptically, weighed, and then homogenized with mortar and pestle. Distilled water was added (10 ml per gram of wet tissue); this suspension was frozen and thawed twice in a slurry of dry ice and acetone, and then tissue debris was removed by centrifugation at 2000 rev/min at 4°C for 30 minutes. The supernatant was dialyzed at 4°C against 5 liters of Na_2HPO_4-citric acid buffer, *p*H 2.6 (*8*) for 24 hours, 5 liters of distilled water for 6 hours, and then 5 liters of 3 m*M* disodium ethylenediaminetetraacetate (EDTA) for 24 hours. At the end of this time, if the contents of the dialysis sac were not free of catecholamines, dialysis was continued against 5 liters of 1 m*M* sodium phosphate buffer, *p*H 7, for 24 hours. The precipitate that formed during dialysis was removed by centrifugation at 2000 rev/min at 4°C for 30 minutes.

The supernatant (5 ml) was mixed with 5 ml of soybean trypsin inhibitor (*9*) (1 mg/ml), 5 ml of 40 m*M* EDTA, 0.5 ml of 0.4*M* dimercaprol (BAL) (*10*), and 10 ml of renin substrate that was prepared as described (*11*). Soybean trypsin inhibitor and EDTA were in 0.1*M* sodium phosphate buffer, *p*H 6.0, with chlorhexidine gluconate (*12*) (0.01 percent weight to volume). The final *p*H of the reaction mixture was 6.0. When prepared in this manner, the reaction mixture was free of kallikrein, angiotensinase, and converting-enzyme activities (*13*).

The mixture was incubated at 37°C for 20 hours. The reaction was stopped by the addition of 5 ml of trichloroacetic acid (20 percent weight to volume). After 60 minutes at 4°C, precipitated protein was removed by centrifugation. The clear, colorless supernatant was saved, and the precipitate was washed with 5 ml of 3-percent trichloroacetic acid and then again centrifuged. The combined supernatants were extracted three times with two volumes of diethyl ether. The aqueous layer was neutralized to *p*H 7.0 with 2*N* NaOH and then dried in a rotary evaporator to about 4 ml. This solution was extracted twice with 4 ml of *n*-butanol, previously equilibrated with 0.05*M* sodium phosphate buffer, *p*H 7.0. The butanol extracts were combined, and a portion (6 ml) was applied as the upper phase in the first tube of a ten-transfer countercurrent train. The countercurrent system developed was *n*-butanol for the upper phase and 0.05*M* sodium phosphate buffer (*p*H 7.0) for the lower phase (*14*). The upper phase was two volumes (6 ml per tube) in respect to the lower phase.

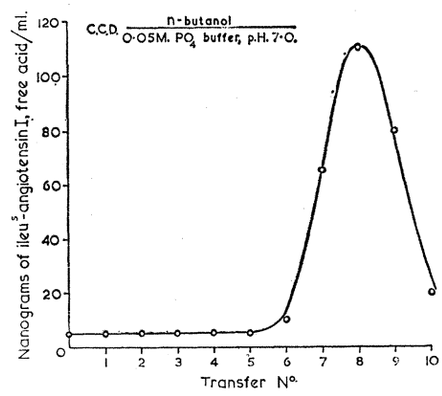

Fig. 1. Distribution of the vasopressor product of the reaction of adrenal enzyme with renin substrate.

At each step, angiotensin was assayed; the criterion was the change in the mean arterial blood pressure of the anesthetized rat treated with pentolinium (*15*). 5-Isoleucine-angiotensin I (200 ng/ml) or 5-valine-angiotensin II-β-amide (*16*) (100 ng/ml) was used as the assay standard.

Results of a countercurrent distribution are shown in Fig. 1. The apparent distribution coefficient of the activity peak was 2, and it corresponded to that of 5-isoleucine-angiotensin I. In this system, 5-isoleucine-angiotensin II, 5-valine-angiotensin II, epinephrine, norepinephrine, and vasopressin have distribution coefficients of 0.2 or less. Recovery of angiotensin in transfer tubes 7, 8, and 9 was 53 percent of that applied to the countercurrent-distribution train and 38 percent of that in the original incubation mixture.

The vasopressor material in the aqueous phase of transfer tubes 7, 8, and 9 was incubated at 37°C, *p*H 7.8 (0.01*M* tris HCl buffer) with trypsin or chymotrypsin (*17*). The ratio of enzyme to substrate was 1 : 1 by weight. Reactions were stopped by heating samples in a boiling water bath

for 5 minutes. With either enzyme, all vasopressor activity was lost during a 2-hour incubation. Samples incubated without trypsin or chymotrypsin retained full activity.

Formation of the polypeptide similar to angiotensin I occurred only in incubated reaction mixtures of adrenal extract and renin substrate. No vasopressor product was found in incubated mixtures that omitted either adrenal extract or renin substrate. Similarly, unincubated mixtures containing adrenal extract and renin substrate did not contain a vasopressor compound.

In eight other experiments, the renin-like activity of paired adrenal glands was compared with that of kidneys and whole blood. After a rabbit was killed, the thorax and abdomen were opened, and blood (3 to 4 ml) was taken by puncture of the left ventricle. The syringe contained heparin to give a final concentration no greater than 20 units per milliliter of blood. Then the adrenal glands and kidneys were removed. The dialysis and extraction procedures were as described, except that whole blood was not homogenized or diluted with water. The erythrocytes were hemolyzed by freezing and thawing. The incubation mixtures were scaled down proportionately, so that 0.5-ml samples of tissue extracts could be used. Incubation reactions were stopped after 15 minutes (kidney) to 60 hours (whole blood) by heating in a boiling water bath for 5 minutes. The clear supernatants collected after centrifugation were assayed for angiotensin. With an initial renin substrate concentration of 1000 ng of angiotensin content per milliliter, adrenal extracts released angiotensin at rates of 13 to 54 ng per hour per gram (wet weight) of original tissue, that is, at rates about 10 to 40 times greater than that of whole blood. Kidney extracts were 250 to 800 times more active than adrenal extracts.

These data show that the rabbit adrenal gland contains an enzyme that is like renin in the following respects. The enzyme is nondialyzable, is stable for several hours at *p*H 2.6, 4°C, and does not have an absolute requirement for those metals chelated by EDTA and BAL (*13, 18*). It reacts with renin substrate to form a product capable of causing a quick, transient rise of mean arterial blood pressure. This product is soluble in dilute salt solutions, trichloroacetic acid, and *n*-butanol, but not in diethyl ether. It is not inactivated in a boiling water bath but is inactivated by trypsin and chymotrypsin. On countercurrent distribution, the product behaves like angiotensin I and very unlike either known form of angiotensin II. The activity of the renin-like enzyme in adrenal glands was 10 to 40 times that of whole blood and could not be accounted for by trapped blood or plasma.

Whether this enzyme has access to and reacts with renin substrate in vivo remains to be determined. However, in view of the profound effects of angiotensin on aldosterone and catecholamine secretion, it is possible that the adrenal enzyme, by releasing angiotensin, influences adrenal secretions.

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Efficiency of the First Component of Complement (C'1) in the Hemolytic Reaction

Abstract. *With the use of the first component of guinea pig complement (C'1) labeled in vivo with ^{14}C-amino acids, we have obtained evidence that, under the conditions required for the assay of C'1, each molecule of C'1 capable of interaction with cell surface antigen-antibody complexes is capable of initiating the reaction sequence that leads to lysis of the cell.*

The lysis of cells by antibody and complement (C') results from the sequential interaction of cell-surface antigen, corresponding antibody, and the nine recognized components of C' (*1*). Analysis, on a molecular basis, of the interaction of the complement components has been possible since the development of modern tools of molecular biology and the introduction of the single-site (one-hit) theory of immune hemolysis.

The single-site theory of immune hemolysis states that the completion of the complement reaction-sequence at a discrete site on a cell surface is necessary and sufficient for lysis of the cell (*2*). This theory, however, does not exclude the possibilities that hemolytically inactive C' components may participate in the reaction sequence at a site that does not lead to lysis nor that more than one molecule of any of the C' components may act at a single site. Molecular analyses of the hemolytic dose-response curves for C'1a (the activated form of the first component of C') of guinea pig and human complement indicated that a single effective molecule of this component is sufficient to initiate the reaction-sequence leading to lysis of the cell (*3*). Because these measurements were based solely on hemolysis, however, it was not possible to determine whether every molecule of C'1a was hemolytically active.

Becker (*4*) has shown C'1a to be an enzyme whose substrates are C'4 and C'2 (*4, 5*). Reutilization of C'1a due to transfer from site to site at the cell surface may lead to an overestimation of hemolytic C'1a. Hemolytic C'1a is assayed under conditions where transfer of C'1a from site to site at the cell surface is about 1 percent (*3*), and therefore it cannot be reutilized to any significant degree.

In the assay of C'1a one could underestimate C'1a content owing to in-