emphasized that recent work with muscle preparations indicate that the relationship between ion flux and REP may require a more complex explanation than provided by the Nernst equation(5).

Loss of K^+ from adipose tissue is significantly reduced by insulin, with no glucose in the medium(6). There is no effect of insulin on the net inward movement of Na^+ or water. The relative decrease of K^+ accumulating in the medium, in response to insulin, might be related directly to the increase of REP associated with insulin(1,2). The results given here, decrease of fat pad REP as K^+ in the medium is increased, are consistent with these findings. Alternatively, it may be proposed that as insulin causes transport of K^+ from an extracellular to intracellular locus an increase of REP would result. However, studies in muscle by Zierler(7) indicated that increase of intracellular K^+ was too small and too late to account for the rise in REP, in response to insulin. Zierler (7) proposed that the REP associated with insulin caused the K^+ shift. Similarly, it is possible that the primary effect of insulin or other hormones is upon fat cell REP which, in turn, alters transport rate of ions, such as K^+, and of substrate, such as glucose.

Summary. Adipose tissue transmembrane or resting electrical potential (REP) of young rats is decreased by elevation of K^+. Substitution of medium high in Na^+ for one high in K^+ restored the REP to its control level or slightly higher. Adipose tissue from aged rats did not demonstrate any significant change of REP in response to alteration of K^+ or Na^+.

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Purification of Thyroid Hormone-Transaminase from Rat Kidney Mitochondria.* (28817)

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We have previously shown that extracts of rat kidney mitochondria contain oxidase and transaminase systems which deaminate L-thyroxine(1). Recently we found that a partially purified preparation with transaminase activity obtained by stepwise elution of the crude transaminase on DEAE cellulose column catalyzes the deamination of thyroxine, triiodothyronine, and diiodotyrosine(2). The work herein reported was undertaken to determine whether the transamination of these iodinated amino acids is effected by a single transaminase.

Material and method. All preparative procedures were carried out at 0° to 4°C. The dialyzed ammonium sulfate fraction, labeled crude transaminase, prepared from extracts of rat kidney mitochondria(2) was purified by gradient elution from DEAE cellulose. The crude transaminase, which contained 60

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mg of protein and had 876 units of thyroxine transaminase activity† with but little thyroxine oxidase activity, was transferred on 1.2 × 18 cm column of DEAE cellulose pre-equilibrated with 0.005 M phosphate buffer‡ at pH of 7.4. There were 150 ml of 0.005 M phosphate buffer in the mixing chamber, and the upper chamber contained 300 ml of 0.1 M phosphate buffer, both at pH 7.4. The eluate was collected in 3 ml volumes. Transaminase activities in each tube were measured by use of thyroxine, triiodothyronine and diiodotyrosine as substrates in the presence of added pyridoxal phosphate and α-ketoglutarate(2). The incubation medium contained 0.3 ml of Na-phosphate buffer at pH of 6.4, 1 ml of each eluate, 1.6 μmols of α-ketoglutarate, 6 μg of pyridoxal phosphate and 10 mμmols of thyroxine or triiodothyronine containing the corresponding radioactive compound for a total volume of 1.6 ml. In the experiment with diiodotyrosine, 0.8 ml of each eluate and 10 mμmols of radioactive diiodotyrosine were used instead of 1 ml of eluate and thyroid hormone in the above medium. The enzyme activity in 1 ml of each eluate was calculated by multiplying the value obtained with 0.8 ml of eluate by 1.25. The pH of the incubation media at which transaminase activities were manifest ranged from 7.0 to 7.1. One unit of transaminase was defined as that amount which catalyzes the degradation of 1 mμmol of substrate during one hour of incubation at 37° under the conditions described above. Protein was measured according to Kalckar's method(3). Specific activity was expressed in units per mg protein.

Results and discussion. A typical elution pattern is shown in Fig. 1. The eluate from DEAE cellulose column that contained thyroxine-transaminase activity also catalyzed the transamination of triiodothyronine and that of diiodotyrosine. Fractions in the 16th to 25th tube showed especially high specific

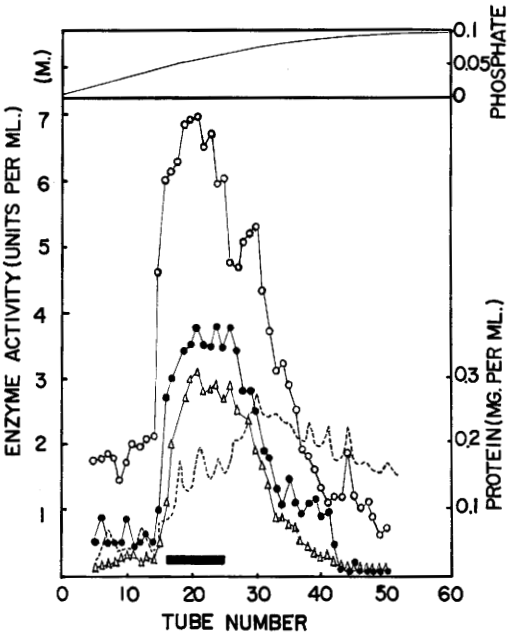

FIG. 1. Gradient elution of transaminase from DEAE cellulose. Contents of each tube were assayed for protein (-----) and transaminase activities with thyroxine (○——○), triiodothyronine (●——●), and diiodotyrosine (△——△) as substrates. Shaded area at bottom of figure indicates the samples which were identified as purified enzyme with high specific activities.

activities in transaminating the 3 iodinated compounds used. The specific activity of the purified enzyme in tubes 16 to 25 was calculated to be 44 units per mg protein, *i.e.*, some 3 times greater than the specific activity of the crude transaminase which was 14.6 units per mg protein, with thyroxine as the substrate. About the same increase in specific activity after column treatment was observed when triiodothyronine or diiodotyrosine was the substrate. These findings were duplicated in 2 other experiments. The inability to separate these activities by purification suggests that transamination of these 3 iodinated amino acids is effected by a single enzyme.

Summary. Crude transaminase from extracts of rat kidney mitochondria which catalyze the deamination of thyroxine, triiodothyronine and diiodotyrosine was purified by means of a DEAE cellulose column. The elution pattern suggests that the deamination of these three iodinated compounds is effected by one transaminase. Column treat-

† Transaminase and oxidase activities were measured at pH of 7.0, with or without added pyridoxal phosphate and α-ketoglutarate.

‡ Phosphate buffer consisted of Na_2HPO_4 and HCl to obtain pH of 7.4.

ment produced a 3-fold increase in the enzyme.

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Effects of Cortexone and Salt on Renin Content, Juxtaglomerular Index and Renal Hypertension in Rabbits. (28818)

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Juxtaglomerular cells have been considered as the source of renin, an enzyme which, acting on plasma angiotensinogen as a substrate, is responsible for the formation of the vasopressor angiotensin. Hypersecretion of renin by ischemic kidneys has been held to be the direct cause of nephrogenic hypertension. More recently, Hartroft and associates have shown that the degree of granulation of the juxtaglomerular apparatus, taken as an indicator of the rate of renin production, decreases after cortexone and salt administration(1) and is directly proportional to the thickness of the zona glomerulosa of the adrenal cortex(2). This finding was corroborated by Gross and associates, who have independently demonstrated that in the rat (3) as well as in the rabbit(4), renal renin content is decreased by cortexone and salt treatment. The significance of the juxtaglomerular apparatus as one part of the homeostatic mechanism governing sodium balance has been confirmed and extended by the demonstration that angiotensin increases the output of aldosterone from the adrenal cortex(5,6).

In view of the dual action attributed to the renin-angiotensin system, it seemed indicated to study the effects of cortexone administration, supplemented by salt on the one hand, and combined with renal ischemia on the other hand. It was reasoned that, were angiotensin the effective mediator of renal hypertension, degranulation of the juxtaglomerular apparatus following cortexone and salt administration, either should be reversed after clamping of the renal artery, or should prevent the rise in blood pressure, which normally follows the latter procedure. The following experiment was performed to test these possibilities. The rabbit was selected as the experimental animal because in this species cortexone is known to have no hypertensive action(7).

Materials and methods. As an initial step, the right kidney was removed in adult male and female rabbits weighing 2-3 kg, who were next randomly divided into 2 groups. The removed kidney was used to determine the normal values for renin content and juxtaglomerular granulation index (JGI). On the day of the nephrectomy the rabbits in one group were given one dose of 0.4 ml per kg body weight of a cortexone trimethylacetate suspension, a commercial preparation supplied by Ciba* under the trade name "Percortène" and containing 25 mg of the active microcrystalline substance per 1 ml of buffered saline. The rabbits of the other group, which served as controls, were injected with 0.4 ml per kg body weight of the same medium but without Percortène, which was also supplied by Ciba. In addition, the test animals were given as a drinking fluid a 0.5% solution of sodium chloride in distilled water. A second similar dose of either cortexone or drug vehicle was given on the 11th day of the experiment. Fourteen days after unilateral nephrectomy, during which interval blood pressure measured at the ear by a sphigmomanometric method(8) was re-

* Ciba, Basle, Switzerland.