a decreased fluoride inhibition as compared to homologous controls. Malonate inhibition of tumor tissue also tended to be minimal. The findings were discussed in terms of the metabolism of tumor tissue.

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Adrenal Cortex and Peptidase Activity of Rat Tissue.* (18865)

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There is general agreement that overactivity of the adrenal cortex results in a net increase in protein catabolism. This may result from a decreased rate of protein anabolism, an increased rate of protein catabolism, or both mechanisms may be operative(1). If the rate of protein breakdown were appreciably increased by adrenal cortical stimulation, a concomitant enhancement of proteolytic enzyme activity might not be unexpected. Therefore, an investigation of the effect of ACTH administration on the activity of several peptidases of various rat tissue homogenates and extracts was undertaken.

Materials and methods. Tissue extracts were prepared essentially as described by Smith(2). After a 48-hour fast, adult male rats of the Vanderbilt strain were anesthetized with nembutal, exsanguinated through the aorta, and one or more of various tissues were removed, rapidly weighed and transferred to a Waring blendor containing crushed ice and water. After mincing for 2 minutes, an aliquot of the homogenate was diluted with an equal amount of 0.02 M barbital buffer at pH 7.8 and refrigerated. The remaining homogenate was spun at 12,000 RPM for 30 minutes in a Servall centrifuge in a cold room at 4°C. An aliquot of the clear supernatant solution was similarly buffered at pH 7.8. Peptidase activity of these buffered mixtures was determined within a few hours after they were prepared. A modification of the procedures of Beloff and Peters(3) and Fruton(4) was used in preparing crude extracts of skin. About 5 g of skin was removed from the abdominal wall of an anesthetized rat. After subcutaneous tissue was scraped off, the skin was cut into strips about 2 mm wide. A coiled strip of skin was placed on a freezing microtome and the 50 μ sections taken were placed in 10 ml of 2% NaCl. The suspended sections were stirred for 3 hours at room temperature and then centrifuged at moderate speed for 30 minutes. Peptidase activity was determined on an aliquot of the clear supernatant solution. This entire procedure was carried out on skin obtained from 2 separate sites from each animal. The nitrogen content of unbuffered homogenate, supernate, and weighed portions of oven-dried whole tissue were determined by a semi-micro-Kjeldahl technic. Peptide hydrolysis proceeded in 10 ml Erlenmeyer flasks continuously shaken in a water bath maintained at 38°C. The hydrolysis mixture consisted of 0.5 ml of tissue preparation, 0.5 ml of 0.1 M substrate, 0.5 ml of 0.02 M barbital buffer (pH 7.8), and 0.5 ml of 0.85% N Cl. Hydrolysis mixtures containing glycyl-glycine as substrate were made 0.001 M with respect to cobalt. At 1, 2, and 18 hours, 0.2 ml samples were taken from the

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TABLE I. Rat Tissue Extract Peptidase Activity.

Tissue	Treatment	LGGase Homogenate C*	LGGase Homogenate 18 hr†	LGGase Supernate C	LGGase Supernate 18 hr	GGGase Homogenate C	GGGase Homogenate 18 hr	GGGase Supernate C	GGGase Supernate 18 hr	GGase + Co++ Homogenate C	GGase + Co++ Homogenate 18 hr	GGase + Co++ Supernate C	GGase + Co++ Supernate 18 hr
Liver	N‡	2.3	27.5	3.6	25.1	.7	26.6	.8	24.2	1.4	24	1.7	25.1
		(5)	(5)	(5)	(5)	(3)	(3)	(3)	(2)	(3)	(2)	(3)	(2)
	ACTH	2.1	26	3	23.1	.5	22.8	.4	22.4	1.7	25.5	1.4	22.6
		(3)	(3)	(3)	(3)	(2)	(1)	(2)	(1)	(2)	(3)	(2)	(2)
Muscle	N	.3	24.3	.8	24	.1	24.6	.3	30.3	.2	24.5	.5	24.6
		(3)	(4)	(5)	(4)	(2)	(2)	(3)	(1)	(2)	(1)	(2)	(1)
	ACTH	.2	21.7	.8	23.7	.1		.3		.2	21.4	.4	24.1
		(1)	(1)	(3)	(3)	(1)		(2)		(1)	(1)	(3)	(2)
Lung	N	3	35.5	4	31.5	1.5	41.5	1.8	31.7	3.9	24.1	4.9	25.4
		(3)	(3)	(4)	(3)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
	ACTH			3.1	34.6			.7	32.8			6.7	25.5
				(4)	(1)			(3)	(2)			(3)	(3)
Spleen	N	1.6	26	2.6	25.5	.7	28.6	.7	27.1	.9	25.3	1.1	25
		(2)	(2)	(3)	(2)	(2)	(2)	(4)	(2)	(2)	(2)	(2)	(2)
	ACTH			1.9	23.5			.8	21.5			1	23.5
				(2)	(2)			(2)	(2)			(2)	(2)
Kidney	N	2.3	34	3.1	35	1.8	42.3	1.5	34.8		25.5	6.3	23.7
		(2)	(2)	(2)	(2)	(2)	(2)	(4)	(2)		(1)	(3)	(3)
	ACTH			2.1	25.1			2.6	22			8.5	24.9
				(2)	(1)			(2)	(1)			(2)	(2)
Skin	N			.5	32							1.6	25.5
				(4)	(4)							(1)	(1)
	ACTH			.6	33.8							1.1	24.9
				(4)	(8)							(4)	(8)

* C = the number of micromoles of substrate hydrolyzed per min per mg of nitrogen contained in 1 ml of hydrolysis mixture, calculated on the assumption that cleavage of only 1 peptide bond occurs. Each value shown is an average of the number of animals shown in parentheses.

† 18 hr represents, for 1 ml of hydrolysis mixture the number of micromoles of substrate hydrolyzed in 18 hr assuming splitting of only 1 peptide bond. Each ml of hydrolysis mixture initially contained 25 M of substrate per ml. The numbers in parentheses represent the number of animals used for each average determination shown.

‡ N = normal.

hydrolysis mixture. The amount of substrate hydrolyzed was determined, in each sample, by a ninhydrin photometric procedure(5). From analysis of the 1 and 2 hour samples obtained and, assuming the cleavage of only 1 peptide bond, *the number of micromoles hydrolyzed per minute per mg of N contained in 1 ml of hydrolysis mixture* was calculated. This value, C, remained fairly constant over a 2 hour period, the first sample generally being somewhat higher than the second. In the interests of simplicity, only the average of the C values of the 1 and 2 hour samples is shown in Table I. A preparation of ACTH (Armour)‡ was injected intraperitoneally 4-6 mg per day in 3 divided doses over a 7 day period. Rats so treated were fasted during the last 48 hours on ACTH administration and then sacrificed.

Results. As indicated in Table I, no striking changes in peptidase activity toward l-leucylglycylglycine (LGG), glycylglycylglycine (GGG) or cobalt-activated glycylglycine (GG) appeared in extracts or homogenates of tissues from animals receiving ACTH. Nor is there a definite trend discernible in the minor changes which occur. The range of values in control and ACTH-treated animals indicated that further investigation would not be fruitful. Moreover, in those instances where enough observations were made to make statistical analysis meaningful, P values ranged from >0.1 to >0.5. In addition, available data, which are not presented, clearly indicate that this lack of effect of ACTH administration on tissue extract peptidase activity re-

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‡ Kindly provided by Dr. John R. Mote of Armour Laboratories.

mains apparent irrespective of whether peptidase activity is calculated on the basis of nitrogen content of tissue, wet or dry weight of tissue or weight of the animals. It is of interest that, judging from the amount of hydrolysis occurring in 18 hours when the liberation of 25 μM/ml presumably indicates complete splitting of 1 peptide bond, lung and kidney extracts and homogenates were capable of hydrolyzing considerable quantities of dipeptide formed from the initial cleavage of LGG and GGG. This finding is supported by the fact that of all the tissues studied, *cobalt-activated* GGase activity is highest in lung and kidney.

Discussion. The hypothesis that the protein catabolic action of ACTH may be reflected, at least in part, in increased catheptic activity is not supported by this study. However, it may be that such changes in proteolytic enzyme activity, if they do exist, may be discernible only in viable tissue. This possibility is being explored using a procedure(6) which permits quantitative measurement of GGase activity in the surviving rat diaphragm.

Summary. The administration of ACTH to fasted rats has no appreciable effect on LGGase, GGGase and cobalt-activated GGase activity of crude extracts of liver, muscle, lung, spleen, kidney or skin.

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Comparative Utilization of Carotene Administered Orally and Parenterally. (18866)

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The problem of utilization of carotene when administered parenterally to rats was shown by Tomarelli *et al.*(1) to be primarily a matter of the solvent used and the state of dispersion of the carotene. When carotene was solubilized in water by the use of a surface active agent (Tween 80), these workers found that one intramuscular injection of 0.5 mg carotene produced as good growth response in vit. A-deficient rats as did the same dosage given orally. In contrast, when carotene was dissolved in sesame oil and injected, the growth response and survival time were markedly reduced. We have confirmed these findings and have carried out experiments to determine the relative utilization of aqueous carotene when given orally and parenterally.

Methods. Male, weanling rats were caged individually and fed a purified vit. A-free diet composed of purified casein* 22%, salts(2) 4%, cottonseed oil (Wesson) 2%, vitamin-mix in casein 2%, and sucrose 70%. The vitamin-mix contained per 100 g: Thiamine hydrochloride 25 mg, riboflavin 50 mg, pyridoxine hydrochloride 25 mg, calcium pantothenate 250 mg, niacin 100 mg, folic acid 10 mg, biotin 10 mg, 2-methyl-1,4-naphthoquinone 25 mg, inositol 5 mg, p-aminobenzoic acid 50 mg, crystalline vit. B_{12} 0.005 mg, and choline chloride 5 g. In addition, 2 mg dl-α-tocopherol and 150 U.S.P. units of vit. D were added per 100 g ration. The food intake was restricted during the depletion period in an attempt to produce uniform weight gains. Purified, all-trans B-carotene(3) and crystalline vit. A acetate† were dissolved in water, using 20% Tween 40, or in cottonseed oil, by

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* General Biochemicals, Inc.

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