TABLE I.
Lethal Effect of Digitoxin Assayed by Method of U.S.P. XII.

	Control animals			DCA-treated animals		
	No.	Lethal dose of 0.0025% digitoxin, cc/kg	Total assay time, min	No.	Lethal dose of 0.0025% digitoxin, cc/kg	Total assay time, min
Normal diet	1	10	45	A	12	50
	2	12	55	B	12	55
	3	15	70	C		
	4	13	66	D	13	62
Avg		12.5	59		12.3	55.7
High salt	5	15	72	E	11	54
	6	11	53	F		
	7	13	62	G	15	73
	8	13	63	H	12	55
Avg		13.0	62.5		12.7	60.7
Avg of combined groups		12.75	60.75		12.5	58.2

significant difference between average adrenal weights if these were expressed as percentages of the body weights ($P = <0.2$ but >0.1).

Conclusion. These observations failed to support the hypothesis that DCA potentiates the toxic effects of digitoxin.

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Relation of Adrenal Cortex to Serum Peptidase Activity.*

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Attention has been drawn by several investigators[1-3] to the rapid hydrolysis of *l*-leucylglycylglycine (LGG) by the sera of rabbits, swine, horses, rats and humans. The splitting of LGG was attributed by Grassmann and Heyde[1] to the action of a serum "aminopolypeptidase," but a recent study[4] has presented evidence for the participation of at least 2 enzymes in the hydrolysis of this substrate by rabbit serum, and presumably by other sera as well. One of these enzymes has been identified as a manganese-activatable leucine aminopeptidase, for which *l*-leucinamide acetate (LA) is a satisfactory substrate. The other serum enzyme which splits LGG is a peptidase with a different, but hitherto undefined, specificity. Both of these enzymes are closely related in several properties to LGG-splitting enzymes found in extracts of skin and lung,[4] intestinal mucosa,[5] muscle,[6] and in leucocytes and lymphocytes.[7] In view of the widespread distribution, in

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† James Hudson Brown Junior Fellow 1946-1947.

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2 Maschmann, E., *Biochem. Z.*, 1941, **308**, 359.

3 Weil, L., and Russell, M. A., *J. Biol. Chem.*, 1938, **126**, 245.

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TABLE I.
Hydrolysis of Leucylglycylglycine (LGG) and Leucinamide (LA) by Mouse Serum

Substrate	cc of mouse serum per cc test solution	pH	Time in min	Hydrolysis: No activator added %*	Hydrolysis: No activator added K°	Hydrolysis: 0.001 M $MnSO_4$ present %*	Hydrolysis: 0.001 M $MnSO_4$ present K°
LGG	0.2	8.0	15	13	0.87		
			30	26	0.87		
			45	35	0.78		
			60	45	0.75		
	0.1	8.0	15	6	0.40		
			30	13	0.43	36	1.20
			45	20	0.45		
			60	27	0.45	66	1.10
LA	0.16	8.2	30	24	0.80	56	1.87
			60	47	0.78		
			90	69	0.77		
	0.12	8.3	30	16	0.53	45	1.50
			60	36	0.60	82	1.37
			90	54	0.60		
	0.08	8.0	30	12	0.40	31	1.03
			60	23	0.38	61	1.02
			90	38	0.42		

* % of the hydrolysis expected on the complete splitting of one peptide linkage.

tissues, of similar proteolytic enzymes which act on LGG, the possibility has been considered[4] that these enzymes have a common origin in ubiquitous cells such as lymphocytes. Furthermore, the suggestion has been made that the presence of the LGG-splitting peptidases in the sera of all animals tested might be due to the liberation of these enzymes into the circulation in the course of the disintegration of lymphoid tissue. In previous studies,[8] it was shown that the rate of dissolution of lymphoid tissue is controlled by the hormonal secretion of the adrenal cortex. The experiments reported in the present communication support the view that the serum peptidases which act on LGG arise, to a significant degree, from the turnover of lymphoid tissue, since the administration, to mice, of adrenal cortical extract or of pituitary adrenotrophic hormone, results in a marked increase in the peptidase activity of the sera of these animals.

Methods. Male mice (7-14 weeks old) of the CBA strain (Strong) were used. Water and Purina Fox Chow were available to the animals at all times. The diet was supplemented by a mixture consisting of 91% calf meal and 3% each of cod liver oil, dried yeast powder and wheat germ. Blood was obtained by heart puncture and pooled sera were used for the determination of enzyme activity. In the enzyme experiments, the substrate concentration was 0.05 mM per cc of the test solution, the pH was maintained near pH 8 with 0.02 M veronal buffer, and the temperature was kept at 38°. The rate of proteolytic action was followed by the titration method of Grassmann and Heyde.[9] The synthesis of the substrates LGG and LA is described elsewhere.[10,11] The hormone preparations employed in this study were an aqueous extract of adrenal cortex (Wilson), adrenal cortical steroids in oil (Upjohn), and hog

[8] Dougherty, T. F., and White, A., *Am. J. Anat.*, 1945, **77**, 81.

[9] Grassmann, W., and Heyde, W., *Z. physiol. Chem.*, 1929, **183**, 32.

[10] Behrens, O. K., and Bergmann, M., *J. Biol. Chem.*, 1939, **129**, 587.

[11] Bergmann, M., Zervas, L., and Fruton, J. S., *J. Biol. Chem.*, 1935, **111**, 225.

TABLE II.
Effect of Hormones on Serum Peptidase Level of Mice.

Treatment of mice	Substrate	No. of mice	No. of serum pools	K°*	% increase	"P"†
None	LGG	24	12	0.42 ± 0.09		
	LA	22	10	0.38 ± 0.22		
				0.86 ± 0.28‡		
Aqueous adrenal cortical extr. (1 cc) inj. 3-3.5 hr before bleeding	LGG	6	4	0.93 ± 0.07	120	<0.01
Cortical steroids in oil (0.1 cc) inj. 5-5.5 hr before bleeding	LGG	8	4	1.42 ± 0.42	235	<0.01
Cortical steroids in oil (0.1 cc) inj. 9-9.5 hr before bleeding	LGG	8	7	1.61 ± 0.56	272	<0.01
	LA	12	12	0.83 ± 0.56	126	0.038
				1.93 ± 0.87‡	136	0.018
Adrenotrophic hormone (1 mg) inj. 9-10 hr before bleeding	LGG	8	8	1.24 ± 0.17	188	<0.01
	LA	6	6	0.61 ± 0.43	64	0.3
				1.45 ± 0.84‡	69	0.1

* Mean value and standard deviation for 0.1 cc of serum per cc of test solution.
† Calculated according to "Student's" t method of Fisher.[13]
‡ Determined in the presence of 0.001 M $MnSO_4$.

pituitary adrenotrophic hormone[12] (2 mg per cc of aqueous solution). These preparations were administered by subcutaneous injection.

Hydrolysis of LGG and LA by Mouse Serum. The data in Table I show that LGG is hydrolyzed extremely rapidly by mouse serum. As was noted previously for rabbit serum,[4] the initial rate of hydrolysis accords with the kinetics of a zero order reaction. The rate may be expressed therefore by the constant $K°_{LGG}$ which is defined as per cent hydrolysis per minute. Within the limits of enzyme concentration given in Table I, the value of the constant is proportional to the amount of serum per cc of the test solution.

For a series of 24 mice, from which there were obtained 12 samples of pooled sera, the value of $K°_{LGG}$ was found to be 0.42 ± 0.09 for 0.1 cc of mouse serum per cc of test solution (*cf.* Table II). This value is much greater than that observed for rabbit serum (0.18) or for human serum (0.035).[4]

It will be noted from Table I that the addition of 0.001 M $MnSO_4$ greatly accelerates the hydrolysis of LGG by mouse serum, thus indicating the presence of appreciable amounts of leucine aminopeptidase. This observation is similar to the result obtained with guinea pig serum,[2,4] but is in contrast to the behavior of rabbit and horse sera which do not appear to be rich in aminopeptidase activity.

The conclusion that a large part of the hydrolysis of LGG by mouse serum is due to a manganese-activatable leucine aminopeptidase is confirmed by the finding that LA is split rapidly by this serum, and that the rate of hydrolysis is greatly increased by the addition of $MnSO_4$ (Table I). As in the case of the hydrolysis of LGG, the initial rate of enzymatic action follows the kinetics of a zero order reaction, and the rate may be expressed, therefore, by the constant $K°_{LA}$. It will be noted that the variation in the capacity of various mouse sera to split LA is much greater than in the case of LGG (*cf.* Table II).

Effect of Administration of Hormones on the Serum Peptidase Level. The data in Table II show that, following the subcutaneous injection of adrenal cortical extracts or of adrenotrophic hormone, there occurs an appreciable rise in the value for $K°_{LGG}$ per unit volume of serum. It is of interest that the most marked effect is observed about 9 hours after the administration of adrenal cortical steroids in oil. Previous studies have shown that, in mice, the degree of lymphocyte dis-

[12] Sayers, G., White, A., and Long, C. N. H., *J. Biol. Chem.*, 1943, **149**, 425.

[13] Fisher, R. A., *Statistical Methods for Research Workers*, London, 7th edition, 1938.

[14] Dougherty, T. F., and White, A., *Endocrinology*, 1944, **35**, 1.

solution,[8] and resultant blood lymphopenia,[14] are maximal 6 to 9 hours after the injection of this hormone preparation. During this period, there is also an increased rate of release of protein from lymphoid tissue to the circulation.[15]

Following injection of adrenal cortical steroids in oil, the increase in activity toward LGG is accompanied by an increase in the rate of hydrolysis of LA. The latter result was not found to be as reproducible as was the effect on the hydrolysis of LGG, but the difference in aminopeptidase activity for the sera of treated and untreated mice appears to be statistically significant. On the other hand, the injection of adrenotrophic hormone did not cause a significant rise in activity toward LA. This result may be due to the small number of serum samples tested.

It is of interest that the addition of 0.001 M $MnSO_4$ results in an activation of the hydrolysis of LA by sera from injected animals. This suggests that the increase in the activity toward LA, as well as towards LGG, following the administration of hormone, is due to a mechanism other than an increase in a serum constituent with a mode of action similar to that of $MnSO_4$. Although the possibility cannot as yet be excluded that the injection of hormone releases an enzyme activator of unknown nature, or removes a substance which is an inhibitor, the conclusion seems justified that there occurs an actual increase in the amount of circulating peptidases. That this increase cannot be accounted for by hemoconcentration resulting from hormone administration is shown by the data for the protein content of the sera of normal and treated mice. Determinations of the total nitrogen content of a series of untreated mice gave a value of 10.5 ± 1.5 mg per cc, while a comparable group of sera from mice treated with aqueous adrenal cortical extract showed a nitrogen content of 9.9 ± 1.2 mg per cc. In the case of mice which had received adrenal cortical steroids in oil, the nitrogen content of the sera was 11.8 ± 1.8 mg per cc, and for mice treated with adrenotrophic hormone, the serum nitrogen was found to be 10.3 ± 1.2 mg per cc.

It may be added that preliminary studies on rabbits have shown that the injection of cortical steroids in oil or of adrenotrophic hormone also produces a significant increase in the capacity of the sera of these animals to hydrolyze LGG. The effect observed in rabbits, however, does not appear to be as marked as that noted for mice.

Discussion. The control of the serum peptidase level by the hormonal secretion of the adrenal cortex may aid in explaining increases in serum proteolytic activity observed following a nonspecific stimulus known to augment pituitary-adrenal cortical secretion. Thus, an abrupt rise in the serum peptidases of the dog, cat, rat and calf was reported[6] to result from the burning of a superficial skin area. This type of injury is a powerful activator of the pituitary-adrenal cortical mechanism.[16] It may be noted also that sera from patients with fever have been found[1] to exhibit a greater activity toward LGG than that observed for sera from normal humans.

In addition, the data in the present investigation focus attention on the proteolytic enzymes of lymphoid tissue. It has been found[17] that extracts of calf thymus are unusually rich in enzymatic activity toward LGG and that the enzyme specific for this substrate may readily be separated from the accompanying manganese-activatable leucine aminopeptidase which hydrolyzes LA. Calf thymus, therefore, is a suitable starting material for the purification of the LGG-splitting peptidase preparatory to a study of the specificity and physiological effects of this enzyme.

Summary. A single, subcutaneous injection, in mice, of adrenal cortical extracts or of pituitary adrenotrophic hormone causes an appreciable rise in the serum peptidase level of these animals. It has been suggested that this increase in enzyme activity is a result of the acceleration, by the hormones of the adrenal cortex, of the rate of turnover of lymphoid tissue.

[15] White, A., and Dougherty, T. F., *Ann. N. Y. Acad. Sc.*, 1946, **46**, 859.

[16] Harkins, H. N., and Long, C. N. H., *Am. J. Physiol.*, 1945, **144**, 661.

[17] Fruton, J. S., unpublished experiments.