TABLE I. Mean Weights of Adrenal Glands and Microscopic Evaluation of Regeneration and Lipid Deposition in Adrenal Glands One Month after Transplantation.

Group	Procedure	No. of rats	Adrenal wt* (mg)	Adrenal wt* (mg/100 g body wt)	Adrenal cortex† Width (mm)	Adrenal cortex† Regeneration	Adrenal cortex† Lipid deposition
A	No hypophysectomy; no ACTH given	14	29.6 ± 1.8	10.3 ± .6	1.15	3.0 ± .4	3.0 ± .3
B	Hypophysectomy; 5 and 10 units ACTH given twice daily	17	14.8 ± 1.9	7.3 ± .7	.77	2.3 ± .8	2.7 ± .3
C	Hypophysectomy; no ACTH given	10	6.8 ± .9	3.5 ± .5	.18	1.8 ± .7	.6 ± .8

* Mean ± S.E.

† Regeneration and lipid deposition are rated on scale of 0 to 5, a value of 2.5 being comparable to appearance in unoperated normal rats.

lipid than autografts in non-hypophysectomized rats. The cells were vacuolated and composed of light cytoplasm, suggesting possible overstimulation. Autografts in hypophysectomized rats without ACTH showed some viable lipid-laden cortical tissue, but most of each graft was necrotic and regeneration remained minimal (Fig. 3, Table I).

Discussion. The results show that regeneration of an apparently well organized adrenal cortex can be stimulated by ACTH alone in the hypophysectomized rat. Extent of regeneration is less than occurs in presence of the anterior pituitary. Amounts of long-acting ACTH used in these experiments were very large. It is possible that the response of the adrenal graft to exogenous ACTH could be normalized if the ACTH were given continuously. It is also possible that some hypophyseal hormone other than ACTH plays a role in regeneration of adrenal cortical grafts.

Summary. Large doses of ACTH stimulated autografts of adrenal glands in hypophysectomized rats to regenerate cortical tissue. Extent of regeneration was less than occurs in the non-hypophysectomized rat.

1. Ingle, D. J., Griffith, J. Q., Jr., *The Rat in Laboratory Investigation,* Lippincott, Philadelphia, 1942, Chapt. 16.

2. Ingle, D. J., Higgins, G. M., *Endocrinol.,* 1938, v22, 458.

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Colorimetric Determination of Amino Acids in Presence of a Peptide. (25522)

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In investigating the splitting of peptides by peptidases, there has been need for a simple, quick photometric method to determine quantitatively the amino acids present after hydrolysis. The familiar Moore and Stein ninhydrin method(1) for determination of amino acids is not applicable as color intensity from peptides, on a molar basis, is nearly the same as that from amino acids. Most investigators use a titration method(2) for estimation of liberated carboxyl groups. In the present investigation, it was observed that when pH of the buffer in the ninhydrin reagent was 4.0 instead of 5.5, and development of ninhydrin color was at 60°C instead of 100°C, as used by Moore and Stein, amino acids gave the usual amount of color while peptides remained almost colorless. The glycyl peptides, which gave about 50% as much color as amino acids, were an exception to this. The hydrolysis of peptides can be readily followed, when these modifications of the Moore and Stein procedure are used to determine ninhydrin-reacting substances, since the color developed is

due almost exclusively to the amino acids liberated during the hydrolysis.

Materials and methods. Ninhydrin reagent. 0.5 g of ninhydrin and 25 mg of hydrindantin were dissolved in 18.75 ml of methyl cellosolve (peroxide free). This solution was diluted to 25 ml with 0.05 *M* potassium acid phthalate, pH 4.0, and was made up fresh for immediate use because this reagent was not stable for more than 1-2 hours. *Standard Solutions of Amino Acids and Peptides.* Amino acid solutions were made up at 3.0 *mM* in 0.025 *M* tris (hydroxymethyl) aminomethane buffer at pH 8.0. Peptide solutions were made up at 1.5 *mM* in the same buffer. *Determination of standard curves.* A standard curve showing change of optical density with concentration was prepared for a dipeptide and its hydrolysis products as follows: A mixture of a dipeptide and its constituent amino acids were prepared to simulate a range of products present after enzymatic hydrolysis of the dipeptide. To a series of 5 test tubes were added 0, 0.17, 0.33, 0.67, and 1.0 ml of a solution prepared by mixing equal volumes of 2 standard 3.0 *mM* amino acid solutions, each amino acid then being present at a 1.5 *mM* concentration. To the same series of test tubes were added 1.0, 0.83, 0.67, 0.33, and 0 ml of a standard 1.5 *mM* solution of the dipeptide, making a total volume of 1 ml per tube. One-tenth ml aliquots were removed from each tube and placed in another series of 5 tubes, which contained 0, 0.025, 0.05, 0.10, and 0.15 μmole of each amino acid plus 0.15, 0.125, 0.10, 0.05, and 0 μmole respectively of dipeptide. The blank contained 0.1 ml of 0.025 *M* tris buffer, at pH 8.0. One ml of ninhydrin reagent was added to each tube, which were then immersed 30 minutes in a 60°C water bath to obtain maximum color development. The tubes were removed, cooled, and diluted to 10 ml with 50% ethanol. After standing 5 minutes, the ninhydrin color was read at 575 mμ in a Lumetron photometer.

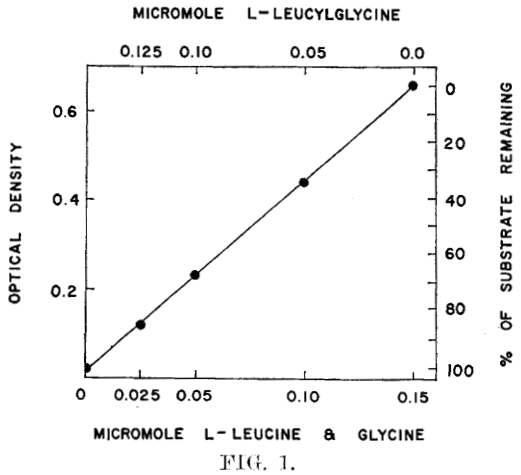

FIG. 1.

TABLE I. Color Yields on a Molar Basis from Amino Acids and Peptides Relative to Glycine.

Compound	Color yield	Compound	Color yield
Glycine	1.00	Leucylglycine	.03
Leucine	.78	Leucylalanine	.09
Alanine	.97	Leucylphenylalanine	.03
Valine	.97	Alanylglycine	.04
Lysine	.98	Phenylalanylglycine	.04
Arginine	.90	Valylglycine	.00
Histidine	.75	Lysylglycine	.03
Phenylalanine	.17	Arginylglycine	.09
Glycylglycine	.57	Leucylglycylglycine	.12
Glycylalanine	.54	Leucinamide	.12
Glycylleucine	.40		

Results. Fig. 1 was used as a reference standard for determining enzymatic hydrolysis products of L-leucylglycine. Similar reference curves (not shown) were prepared for analysis of reaction products from other peptides used as substrates. Other buffers, such as citrate and acetate, were employed in the ninhydrin reagent. Potassium acid phthalate, although having the least buffering capacity, was chosen because it gave the most linear curve over a range of zero to 0.30 μmole of amino acids plotted against optical density.

Table I demonstrates color yields on a molar basis of amino acids and peptides tested, relative to glycine value of unity. These were determined by adding 0.1 ml of a 1.5 *mM* solution of an amino acid or peptide in 0.025 *M* tris buffer to one ml of ninhydrin reagent. Procedure for development of color was carried out as described above. Color yields as listed in Table I are ratios of optical densities of amino acids or peptides to optical density of glycine. Color yields from peptides seem to be related to their structures. Glycyl peptides give considerably more color than those peptides containing a free amino group

attached to a carbon atom with a side chain. For example, glycylalanine yields more than 10 times as much color on a molar basis as alanylglycine.

Summary. A photometric method for determination of free amino acids in presence of a peptide has been described. It is applicable to estimation of free amino acids present after hydrolysis of peptides by peptidases.

1. Moore, S., Stein, W. H., *J. Biol. Chem.*, 1954, v211, 907.

2. Grossman, W., Heyde, W., *Z. Physiol. Chem.*, 1928, v183, 32.

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Effect of Subconvulsive Audiogenic Stress in Mice on Turpentine Induced Inflammation.* (25523)

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In previous studies we explored certain mechanisms involved in mobilization of organism's defenses against injury. Various procedures were employed, including effect of cortisone(1,2), of antireticulocytotoxic serum (ACS)(3), of somatotrophic hormone (STH) (4), and audiogenic stress(5) upon wound healing. Results in each instance suggested that delay or inhibition of wound healing probably relates to a number of common underlying mechanisms. Such a concept would be forced to recognize that stress may well be a major factor capable of affecting such basic physiologic adaptive processes. It was shown that mice subjected to audiogenic stress on 10 consecutive days have definitely impaired capacity for producing good, healthy granulation tissue after subjection to a single surgical wound. The deficit in such tissue repair does not appear to be mediated entirely through the pituitary-adrenal axis(5). There are differences in degree of response in different strains of inbred mice, and sound stimulation is more effective in producing audiogenic seizures when applied at night, rather than during the day. Also, there is a fair degree of parallelism between wound healing effect and nocturnal *vs.* diurnal timing of sound exposure.

Procedures. Because of well known stress effect upon animals of handling or confinement, preliminary studies were carried out to minimize all extraneous factors that might influence the animal other than auditory stimulus. As a result of these tests a small 6′ x 9′ room was constructed with facilities for 70 standard mouse cages, but placed upon specially wired racks with automatic electronic controls to set the interval, time and duration of each bell ringing exposure. This room was tested to determine effective delivery of stress by such bell ringing audiogenic stimulation as to evoke subconvulsive seizures in susceptible strains of mice. Time duration of each exposure ranged from 10 seconds initially to 60 seconds with 5 second increments. Control animals were housed in a comparable room and were handled in identical fashion, except that they were not exposed to bell ringing. Groups of C_{57}BL/6 (seizure resistant) and DBA/1 (seizure susceptible) mice were treated as follows after one week exposure to stress: 1. 20 C_{57}BL/6 and 20 DBA/1 mice given surgically induced wounds. 2. 40 C_{57}BL/6 and 40 DBA/1 mice injected subcutaneously with 0.025 cc turpentine. 3. Control groups of both strains, *not* exposed to stress, but otherwise treated identically. Results revealed a marked delay in wound healing and repression in extent and severity of the inflammatory reaction to turpentine as compared with that observed in the controls. These findings prompted the following to determine the morphologic characteristics of these altered responses and their underlying mechanisms. Groups of 20 mice each of

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