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.

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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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DBA/1 (seizure susceptible) and A/Jax (seizure resistant) strains, 12 to 14 weeks old and uniformly distributed in respect to sex and weight, were treated as follows: *Group I.* 20 A/Jax and 20 DBA/1 strain mice were exposed to audiogenic stress as described, for 7 consecutive nights, then injected immediately beneath surface epithelium (intracutaneously) with 0.025 cc of turpentine. Stress was applied nightly for 14 nights in accordance with predetermined schedule of sacrifices indicated below. *Group II.* 20 A/Jax and 20 DBA/1 strain mice were exposed for same 7 day pretreatment stress, then injected similarly with 0.025 cc of turpentine, but no further stress was applied. *Group III.* Controls: 20 A/Jax and 20 DBA/1 strain mice were injected with 0.025 cc of turpentine without exposure to stress stimulation. Five animals from each of the 6 groups were sacrificed as follows: 1) 24 hours after turpentine injection, 2) 4 days, 3) 7 days, and 4) 14 days after turpentine injection. Animals were all autopsied and tissues taken from local inflammatory area, from adrenals, liver, spleen and kidneys routinely, and randomly from other organs including lungs, heart and thyroid. Tissues were fixed in 10% formalin, sectioned and stained with hematoxylin and eosin. Slides were numbered by code and studied histologically by one of us (LWS) without previous knowledge of their identity, or treatment given.

Results. Local lesions (*e.g.* site of turpentine injection along with surrounding subcutaneous tissue and overlying epithelium) were graded on a 1-4 scale as follows: *Grade 1,* a relatively small localized cystic lesion containing very few cells and showing only a very narrow limiting zone of inflammatory cellular infiltration; *Grade 2,* the cystic area tends to be somewhat larger, containing small amount of leucocytic exudate and fibrin, limiting inflammatory zone is considerably wider, but there is little or no extension into surrounding tissues; *Grade 3,* the lesion still tends to be localized and cystic but is practically filled with pus, and inflammatory reaction extends widely into surrounding subcutaneous tissues, possibly very early fibroblastic development; *Grade 4,* evidence of fibrous tissue proliferation and epithelization (in the few cases where ulceration was noted), but with marked residual inflammatory cellular infiltration of tissues. Complete healing was not found in any instance.

Any changes observed in various viscera were also graded on a 1-4 scale. In liver and kidney such minor changes as were noted were of a transient, mild, irritative or toxic nature relating principally to various degrees of granular changes of the cytoplasm. In the spleen the gradation was expressed on the basis of follicular hyperplasia and by congestion. There was little or no consistency in these essentially insignificant variations except perhaps that they were most numerous in 24 hour and 4 day animals. For double check, random white blood cell and differential counts were taken from animals in each group. No significant rise in numbers nor alteration in percentage differential count was found, further evidence of the very minor or totally negligible systemic injury produced by audiogenic stress.

Discussion. As preliminary analysis of the statistical data revealed no demonstrable difference in the animals in Groups I and II, they have been grouped together in the final analysis. Nor was there statistically valid difference in the lesions in the 2 strains of mice, although the A/Jax mice appear to show rather less suppression of the inflammatory response during the first 24 hours or even by the fourth day than do the DBA/1 mice. However, these minor differences tend to disappear with the time factor, by the seventh day.

Critical review of the statistical data fails to give a clear-cut answer to the problem of total effect of audiogenic (subconvulsive) stress upon physiologic response of the body to inflammation. On the other hand, the data seem to indicate that stress exerts a repressive effect upon normal inflammatory response, and upon subsequent repair process, much as it was shown to do previously, in causing delay in wound healing experiments. The mechanism of this physiologic response to stress is not clear. The evidence is not adequate to explain it on the basis of adrenal cor-

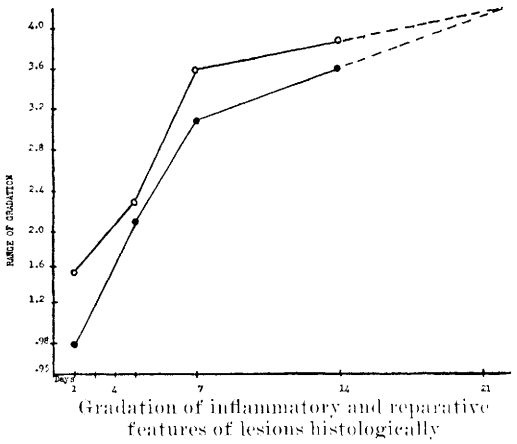

FIG. 1. ●——● experimental; ○——○ control.

tical stimulation, though the effect is comparable to that obtained by the use of cortisone. In this connection, Dr. Robert Henkin, Univ. of California, Los Angeles, has reported complex hormonal stimulation in animals subjected to unpleasant sounds, whereas no such effect is obtained in the presence of pleasant sounds such as music.

Two points have emerged from these experiments: 1) stress of this type does delay and repress to a considerable degree the usual inflammatory reaction to an injurious agent and 2) it likewise represses to a very considerable degree the repair process during the first 10 days to 2 weeks. Ultimately, however, the healing catches up, but in general leaving less residual scarring.

To ascertain whether the age of the mice might have modified the results, the experiments were repeated with 36 young (20 g) DBA/1 strain mice (seizure susceptible). Two or 3 animals from each group were sacrificed on the same 24 hours, 4 day, 7 day and 14 day schedule, their tissues taken and examined microscopically. These supplemental data were somewhat more sharply defined and give added weight to our earlier results. These statistics have been added to the other data and a composite graph (Fig. 1) emphasizes the major differences of experimental *vs.* control response to injection subcutaneously of turpentine following application of subconvulsive audiogenic stress.

Summary and conclusions. 1) A/Jax and C_{57}BL/6 (both seizure resistant) and DBA/1 (seizure susceptible) strain mice were exposed to subconvulsive audiogenic stress for 7 days before, and in some instances up to 14 days after intracutaneous injection with 0.025 cc of turpentine. 2) The effect of pretreatment audiogenic stress resulted in moderate repression of ordinary inflammatory response observed in control animals. This was characterized by a sharp localization of the lesion with only a very narrow limiting zone of inflammatory cells. 3) In addition to relative repression of inflammatory response, delayed repair of injury was observed, with a minimal amount of fibroblastic proliferation during first 10 days or more in the majority of animals. 4) No differences were observed in these reactions in mice subjected to post-treatment stress supplementing the initial pre-treatment application of stress. This suggests that the mechanism initiated by such audiogenic stress persists for a considerable time. 5) The data were inadequate to establish the way in which the reaction operates, but a certain parallel may be drawn between inhibition of inflammatory response clinically by use of adrenal cortical steroids to suggest that adrenal cortical stimulation may be involved.

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