Failure of Adrenalectomy Immediately Following Stress to Prevent Eosinopenia in Rats.* (18290)

WILLIAM D. LOVE. (Introduced by Carl V. Moore.)

From the Department of Internal Medicine, Washington University School of Medicine, St. Louis, Mo.

It is an old clinical observation that eosinopenia frequently accompanies severe illness. Dalton and Selye described this as a constant part of the general alarm reaction(1). Subsequent work(2,3) has demonstrated that a fall in eosinophiles may be produced by administration of 17-hydroxycorticosterone and 11-dehydro-17-hydroxycorticosterone in man. Since epinephrine, in the presence of intact pituitary and adrenal function is also capable of initiating this phenomenon, the change in circulating eosinophiles induced by epinephrine has become the basis for clinical tests of adrenal cortical responsiveness(4).

After epinephrine is injected into normal animals, the number of eosinophiles in the peripheral blood decreases gradually during a 4 hour period. It has not been determined whether the progressive decrease is due to the action of adrenal steroids secreted within a few minutes after the epinephrine stimulation, or indicates that there must necessarily be a continued elaboration of adrenal hormones during the time required for development of the maximum eosinopenia. The experiments described in this report were designed to answer this question. Bilateral adrenalectomies were performed rapidly on a series of rats so that both adrenal pedicles were clamped within 10 or 15 minutes after epinephrine administration. Eosinopenia was as great in these as in normal or in sham-operated animals. It is evident, therefore, that the quantity of adrenal steroids secreted within 10 to 15 minutes after epinephrine had been given was sufficient to induce eosinopenia. In addition, the fall in circulating eosinophiles was gradual and progressive over 4 hours in each group of animals, indicating that a time interval is necessary before the effect of the steroids on eosinophiles is apparent.

Method. Untrained, albino rats of the Fairfield strain, males and females, weighing 125 to 250 g were used. The test consisted of picking up an animal and injecting it subcutaneously with 0.2 mg/kg of epinephrine,† after which it was restrained in a tail cage for the 4 hours of the procedure. Counts of the total eosinophiles were made approximately 10 minutes after the injection, at 2 hours, and at 4 hours after the initial alarm of handling. Eosinophile counts were done by a modification of the Randolph technic(5), counting a total of 11.4 cu mm of a 1/20 dilution of tail blood (0.57 cu mm of blood) in aqueous 50% propylene glycol with 0.1% eosin. The standard deviation measured by 18 counts with a mean value of 131 eosinophiles per cu mm was 9 eosinophiles per cu mm. Single stage adrenalectomies were performed rapidly under ether anesthesia through a single mid-line dorsal skin incision, under direct visualization, with minimal traction on the adrenal glands themselves. Sham-adrenalectomy was done in the same manner except that after opening the abdomen through an identical incision the only procedure was displacement of the intestines and gentle traction on the perirenal fat. The time required to complete an adrenalectomy was measured from the time an animal was first picked up until the pedicle of the second gland was clamped. With practice, it became possible

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1. Dalton, A. J., and Selye, H., *Folia haematol.*, 1939, v62, 397.

2. Hills, A. G., Forsham, P. H., and Finch, C. A., *Blood*, 1948, v3, 755.

3. Perara, G. A., Pines, K. L., Hamilton, H. B., and Vislocky, K., *Am. J. Med.*, 1949, v7, 56.

4. Recant, L., Hume, D. M., Forsham, P. H., and Thorn, G. W., *J. C. Endo.*, 1950, v10, 187.

† Parke-Davis Adrenalin, diluted 1/5 with distilled water and prepared immediately before use.

5. Randolph, T. G., *J. Allergy*, 1944, v15, 89.

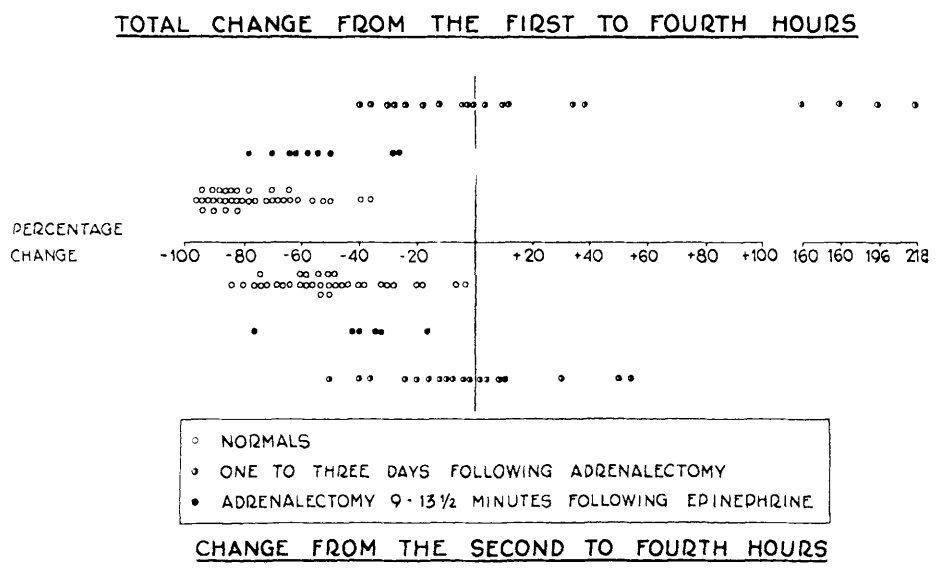

FIG. 1.
Changes in eosinophile counts in rats following injection of 0.2 mg/kg epinephrine subcutaneously.

to remove a rat from its cage, inject the epinephrine and complete the adrenalectomy rapidly enough so that the first count could be taken from ten to fifteen minutes after the animal had first been handled.

Results. The decrease in eosinophiles which had occurred by the end of 4 hours in response to epinephrine in 35 normal rats had a median value of 80% (range 38% to 95%). Three tests during sham-adrenalectomy gave results similar to the normals. The latter animals again responded normally 24 hours after the operation. Nineteen tests were done from one to 3 days after adrenalectomy as a test of the completeness of the operation and in order to make sure that epinephrine stimulation would no longer cause an eosinopenia. The median value for percentage change in eosinophiles showed no fall (range 40% fall to 220% rise).

In the 9 rats given epinephrine and adrenalectomized within approximately 10 minutes (13½, 11, 10, 10, 10, 10, 10, 9 and 9 minutes), the median value of the fall in eosinophiles was 58% (range 78% to 26%).

In order to compare the groups for differences in the progressive and gradual nature of the fall in eosinophiles, the changes in circulating eosinophiles during the second half of the 4 hour period were determined. The observed values for the final eosinophile levels relative to the level at the second hour were: normals, a median fall of 50% (range 0% to 80%); adrenalectomized animals, a median fall of 5% (range 60% rise to 60% fall); and in rats with adrenalectomy 9 to 13½ minutes following epinephrine injection, a median fall of 38% (range 15% to 78%). The results of all tests are summarized in Fig. 1.

Discussion. These results confirm the observation of Recant *et al.*(4) that eosinopenia follows injection of epinephrine in intact, but not in adrenalectomized rats. A significant decrease in circulating eosinophiles occurs even if the adrenals are removed ten minutes after epinephrine injection. Therefore, an appreciable quantity of adrenal steroids must be secreted within 10 minutes of the initial alarm, under the conditions of these experiments. This conclusion is in agreement with the observation of Long(6) that there is a significant fall in adrenal ascorbic acid within 20 minutes after stress in rats. In addition, Vogt(7) has shown that

6. Long, C. N. H., *Bull. N. Y. Acad. Med.*, 1947, v23, 260.

the cortical hormone content of the blood of the adrenal veins of dogs rises within 15 minutes after an infusion of physiological amounts of epinephrine. Despite the prevention of further cortical hormone production by adrenalectomy approximately 10 minutes after epinephrine injection, the continued normal 4 hour fall in circulating eosinophiles takes place. This indicates that the progressive eosinopenia occurring in normal rats need not depend on persistent or increasing elaboration and secretion of adrenal hormones.

Conclusions. 1. In rats, an eosinopenia follows stress even though adrenalectomy is completed within 10 minutes. 2. The progressive fall in circulating eosinophiles over a 4 hour period occurs even without the continued secretion of the adrenal cortex.

7. Vogt, M., *J. Physiol.*, 1944, v103, 317.

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Effect of Sinusoidal Stimulation upon Circulation Time.* (18291)

HARRY T. ZANKEL.
From the Department of Physical Medicine Rehabilitation, Crile VA Hospital, Cleveland, Ohio.

The primary motivation for this work was the hope of Dr. V. L. Tichy, attending in Surgery, Crile V. A. Hospital, to cut down on the incidence of thrombosis and embolism, post-operatively. With this end in view, Dr. Tichy approached the author late in 1946 with the desire to stimulate electrically, with periodic stimulation, the calf muscles of post-operative and other patients confined to bed. Sinusoidal stimulation was suggested by the author as a likely possibility. A routine technic was then established for post-operative sinusoidal stimulation of the calves of both legs. A preliminary report of this work was given by Dr. Tichy and the author before the Cleveland Chapter of the American Society of Experimental Biology and Medicine in November, 1947. Since then two clinical reports have been given(1,2).

The present report deals with the investigative results on the effect of sinusoidal stimulation (such as in being used post-operatively) on circulation time. The effects of sinusoidal stimulation upon circulation time have not previously been reported. However, Gaskell(3) and Bulbring and Burn(4) more recently, studied the blood flow during muscular contraction produced by faradic stimulation. As regards the effect of exercise, Smith, Allen, and Craig(5) have shown that lifting and dropping a leg, 40 times per minute, while wiggling the foot vigorously at the same time, reduced the circulation time in 4 patients by 24% to 40%.

Blood chemical changes. In order to ascertain the effect of sinusoidal stimulation upon circulation time, two types of examinations have been made. The first was suggested by Dr. J. R. Kahn, Director of Laboratories, Crile V. A. Hospital, and was performed by Leonard Skaggs, chemist at this hospital. Venous blood was drawn from the foot of stimulated as well as non-stimulated patients 24 hours post-operatively, and studies were made of the O_2 and CO_2 tensions, and CO_2 combining power of the specimens. These studies showed that sinusoidal stimulation has no definite effect on blood O_2, plasma CO_2, or the plasma CO_2 combining power.

Sodium cyanide test. In searching for a simple, accurate, yet comparatively safe method, the use of the sodium cyanide test as developed by Smith, Allen, and Craig(5) seemed the most feasible. This method is

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2. Tichy, V. L., and Zankel, H. T., *Arch. Phys. Med.,* 1949, vXXX, 711.

3. Gaskell, W. H., *Art a.d. Physiol. Inst., Leipzig,* 1877, v12, 45.

4. Bulbring, Edith, and Burn, J. H., *J. Physiol.,* 1939, v95, 203.

5. Smith, Lucian A., Allen, Edgar V., and Craig, W. McK., *Arch. Surg.,* 1940, v41, 1366.