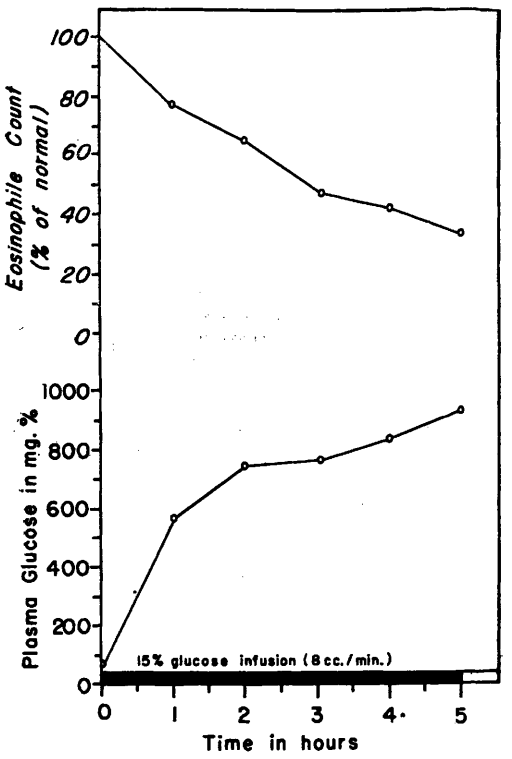

FIG. 2.

Mean eosinopenic responses in dogs during intravenous infusion of 15% glucose solution.

keeping with the response following the administration of epinephrine, ACTH, or C_{11} steroids.

These data do not elucidate the mechanism of action of hyperglycemia in effecting adrenal cortical discharge. It is conceivable as suggested by Vogt(10) and Goldlowski(11) that hyperglycemia may initiate the release of insulin which may directly stimulate the release of adrenocorticotrophic hormone from the anterior pituitary.

Summary. 1. The absolute eosinophile count in the dog varies markedly from animal to animal. The variability of the eosinophile count in the dog is reflected by a mean adjusted coefficient of variation of ± 11.1%.

2. Hyperglycemia produced by the oral or intravenous administration of glucose in the unanesthetized dog causes an eosinopenic response presumably mediated through activation of the pituitary-adrenal mechanism. In this respect hyperglycemia may be construed as a non-specific stress factor.

10. Vogt, M., *J. Physiol.*, 1947, v106, 394.
11. Goldlowski, Z. Z., *Brit. Med. J.*, 1948, v1, 46.

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Susceptibility of Newborn Mice of an Otherwise Apparently "Resistant" Strain to Inoculation with Leukemia.* (17642)

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A strain of transplantable lymphatic leukemia that originated spontaneously(1) in an 11-month-old female of our colony of Ak mice† has been maintained in our laboratory by successive intraperitoneal inoculations of Ak mice; this strain, at the present time in its 52nd transfer, has been designated "Ak Leukemia." Small pieces of liver, spleen, and lymph glands, removed aseptically from Ak mice that had developed leukemia as a result of inoculation, were weighed, then cut and ground in a sterile mortar, 0.85% solution of sodium chloride being added to obtain cell suspensions of 20% concentration. Only such cell suspensions were used for inoculations in experiments reported in this study; the potency of these cell suspensions, at least for mice of the Ak line, was such, however, that dilutions exceeding 1:10,000 reproduced

* Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the author are the result of his own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

1. Cole, R. K., and Furth, J., *Cancer Research*, 1941, v1, 957.

† Our colony of Ak mice has been raised from a litter of Ak-n mice obtained in 1945 from Dr. J. Furth, Cornell University, New York City.

regularly acute leukemia when inoculated into the peritoneum, or under the skin of mice, of either sex and any age, of the Ak line. The pathogenic potency of cell suspensions of Ak leukemia appeared, at first, to be limited only to mice of the Ak line. In this respect this strain of leukemia did not seem to differ from many other tumors that develop in mice of pure inbred lines. A few months ago, however, as a part of an experimental project(2) only indirectly related to the present study, a group of mice of a C3H subline‡, consisting of both adult parents and their 5 infant mice 24 hours of age, was inoculated subcutaneously with Ak leukemia: the infants received 0.05 cc, the parents 0.25 cc, each, of the leukemic cell suspension. As a result, all 5 infants developed acute leukemia within 12 days after the inoculation; their parents, however, remained in perfect health. This accidental observation prompted a systematic study, the results of which can be summarized as follows:

Eighty-five suckling mice, 1 to 7 days old, of either the C3H line§ or of the An (C3H) subline‡ were inoculated subcutaneously (0.05 cc each) with Ak leukemic cell suspensions; in 82 of these 85 baby mice, subcutaneous tumors developed within 12 days at the site of inoculation, followed by a generalized and fatal leukemia. This striking susceptibility of the suckling infant mice to inoculation with leukemia, was found, however, to diminish rapidly after the seventh day of life. Thus, when 57 suckling mice, 8 to 15 days old, of either the C3H or An line, were inoculated subcutaneously (0.05 to 0.2 cc) with AK leukemia, only 27 of them developed tumors at the site of inoculation; three of these tumors regressed spontaneously; the others were followed by a generalized leukemia.

‡ A colony of foster nursed mice of a C3H subline, free from the mammary carcinoma agent, has been raised in our laboratory (and designated by the symbol "An") from a litter obtained in 1945 from Dr. H. B. Andervont, National Cancer Institute, Bethesda, Md.(3)

2. Gross, L., experiments to be published.

3. Gross, L., *J. Immunol.*, 1947, v55, 297.

§ Our colony of C3H mice has been raised from a litter obtained in 1944 from Dr. J. J. Bittner, University of Minnesota.(3)

Leukemia resulted less frequently when older infants were used for the tests: 60 infant mice, 16 to 30 days old, of the same inbred lines, were injected subcutaneously or intraperitoneally with doses varying from 0.05 to 0.3 cc of Ak leukemia; only in 15 mice local tumors resulted from these inoculations; of these, 5 regressed spontaneously; the others, however, progressed into a generalized leukemia.

Adult mice, 2 to 6 months old, of both the C3H and An lines, were found to resist either subcutaneous or intraperitoneal inoculations of substantial doses of Ak leukemia. Forty-nine mice were inoculated subcutaneously, and 9 intraperitoneally, with doses varying from 0.2 cc to 1 cc of the leukemic cell suspensions; none of the 58 inoculated animals developed local tumors or leukemia.

Further experiments demonstrated, however, that the resistance of the adult mice of either the C3H or An line to inoculation with Ak leukemia could be broken, provided that overwhelming doses were injected, in particular by the intraperitoneal route of inoculation. Thus, 5 adult (2 month old) females of the C3H line were inoculated with massive doses subcutaneously, each mouse receiving 3 cc of the leukemic cell suspension. In 3 animals small subcutaneous tumors developed within 10 days at the site of inoculation, but 2 of them regressed spontaneously; only 1 of these progressed eventually into a generalized leukemia. When, however, 6 adult (2 month old) females of the C3H line were inoculated intraperitoneally with similar, massive doses (3 cc each) of the leukemic cell suspensions, all 6 animals died from leukemia within 12 days following the inoculation.

Those mice of either the C3H line, or of the An (C3H) subline that did not apparently react to the inoculation of Ak leukemia, have been under observation for periods of time not yet exceeding 4 months; they now appear to be in perfect health. It remains to be seen, nevertheless, whether all of them will remain free from leukemia for the balance of their lives.

Summary. 1. A strain of transplantable lymphatic leukemia which originated in a mouse of the Ak line was found to be highly

pathogenic for suckling infants of the C3H inbred line. Cell suspensions (20%) prepared from liver, spleen and lymph glands of leukemic mice of the Ak line, reproduced acute leukemia within 12 days when inoculated under the skin (0.05 cc) of newly born suckling mice of the C3H line.

2. This striking susceptibility of suckling C3H infants to inoculation with Ak leukemia appears to be limited to their first few days of life. Practically all infants of the C3H line (82 out of 85) developed leukemia when inoculated within the first 7 days of life; only 27 out of 57, however, were found to be susceptible when inoculated at ages varying from 8 to 15 days. Of 60 infants, 16 to 30 days old, only 15 reacted to inoculation.

3. Forty-nine adult mice of the C3H line were inoculated subcutaneously and 9 intraperitoneally with large doses (0.2 to 1 cc) of Ak leukemia and none reacted. When, however, overwhelming doses (3 cc each) of the Ak leukemic cell suspension were injected into adult mice of the C3H line, 1 out of 5 inoculated subcutaneously, and all 6 inoculated intraperitoneally died from leukemia.

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Inhibition of Hyaluronidase *in vivo* by Adrenal Cortical Activation. (17643)

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Among the actions of the adrenal steroids is the inhibition of the spreading effect of hyaluronidase injected into the skin(1). We have attempted to measure the decrease in hyaluronidase activity following stimulation of the adrenal cortex in the human. Of the various methods employed to measure hyaluronidase activity, the skin diffusion technic seemed the most practical for wide clinical application. In this study, a dilute preparation of fluorescein was used as an indicator dye for intracutaneous injection with the enzyme. Fluorescein was shown by Bukantz and Dammin(2) to be satisfactory for evaluating the effect of antihistaminic drugs upon the action of histamine. Activation of the adrenal cortex following the administration of epinephrine was established by Vogt(3) and Long(4); the possible magnitude of these responses being studied also by Vogt. The mode of stimulation of the adrenal cortex through the anterior pituitary(4) and, perhaps hypothalamus(5) has been shown. In the present study, epinephrine has been used to stimulate the pituitary-adrenal system in control patients in order to demonstrate inhibition of hyaluronidase injected intracutaneously when the adrenal cortex is activated.

Method. A test for skin diffusion was performed using fluorescein (1:25,000) and hyaluronidase* (150 TRU/cc) drawn into a tuberculin syringe for intracutaneous injection. From 0.01 to 0.03 cc of the enzyme are added to the dye to produce a volume of 0.1 cc which is injected into the volar surface of the forearm. The endpoint for the test is noted when the presence of the dye in the skin cannot be appreciated. It is essential to have either daylight or ultraviolet light in the form of a G.E. Purple X bulb or a Wood's lamp to observe the dye disappearance. Gentle stroking with an alcohol sponge enhances the color and the wheal produced by the injection. The test has been done in the fasting state before and 4 hours after a subcutaneous injection of 0.3 cc of 1:1000 epinephrine. Other dyes have been used in an effort to improve on

1. Opsahl, J. C., *Yale J. Biol. and Med.*, 1949, v21, 255.
2. Bukantz, S. C., and Dammin, G. J., *Science*, 1948, v107, 224.
3. Vogt, M., *J. Physiol.*, 1944, v103, 317.
4. Long, C. N. H., *Fed. Proc.*, 1947, v6, 461.
5. Hume, D. M., *J. Clin. Invest.*, 1949, v28, 797.

* Wyeth, Inc., Hydase, supplied by Dr. George E. Farrar.