Summary. The immunizing value of a living vaccine prepared from the first hamster-egg passage was of a high order against challenge by injection. Although a slightly lower degree of protection was obtained, the vaccine is considerably less pathogenic when given by the stick or wing-web method of vaccination.

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Eosinopenia Due to Glucose Administration.* (17641)

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In recent years the physiology of the adrenal cortex has received enormous consideration because of its importance in maintaining homeostatic equilibrium under normal conditions as well as during periods of stress. It is generally considered that the activity of the adrenal cortex is under the influence of adrenocorticotrophic hormone which is liberated by the anterior pituitary. Forsham *et al.*(1,2) believe that the depression of the circulating eosinophiles of the blood is a sensitive physiological criterion of increased adrenal cortical activity. They have shown that 11-oxygenated corticosteroids, ACTH, and epinephrine(3) will decrease the eosinophile count in subjects with normally active adrenal cortical tissue, but that the response is absent when this tissue is functionally deficient. The eosinopenia, which is maximum about 4 hours after adrenal cortical stimulation, is accompanied by an increased renal excretion of sodium, chloride, potassium, uric acid, 17-ketosteroids, and 11-oxysteroids, which is further indication of increased adrenal cortical activity. Pincus *et al.*(4) have shown that oral administration of glucose in the rat produced a lymphopenia which was synchronous with the rise in the blood sugar. This was interpreted as evidence of increased adrenal cortical discharge. A significant correlation was noted between the magnitude of the hyperglycemia and the lymphopenic response. Jailer(5), using comparable doses of glucose in normal man, found that there was no such correlation and that the changes in the lymphocyte levels were within the limits of experimental error. Forsham *et al.*(1) have also cast doubt upon the validity of lymphopenia as an index of adrenal cortical discharge, since they demonstrated an overlap in this response in Addisonian and non-Addisonian patients following ACTH administration. The recent observations of Steeples(6) that adrenal cholesterol increased within 30 minutes following the oral administration of glucose in the rat, was interpreted as indicative of adrenal cortical inhibition. The variance in these results motivated us to evaluate the action of hyperglycemia in terms

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TABLE I.
Variability of the Eosinophile Count in Normal Dogs.

Dog	Eosinophil counts (cells/mm³)						
		Hr after tapwater stomach tubing					
	Control	1	2	3	4	5	Adjusted c.v.,* %
B	1078	1078	1133	1122	1056	1034	± 6.2
Q	2266	2431	2068	2178	2244	2354	± 11.4
C	1298	1298	1474	1188	1155	1144	± 20.0
H	1771	1870	1738	1815	1859	—	± 6.6
G	1991	2167	2233	2233	2244	—	± 9.6
Qi	2079	2090	1683	1837	2145	1958	± 15.2
S	1177	1133	1188	1177	1133	1056	± 8.0
T	2178	2332	2475	2343	2387	2101	± 12.0

* Coefficient of variation.
Mean of 8 adjusted c.v. 11.1%.

of another index of increased adrenal cortical activity, namely the circulating blood eosinophiles.

Methods: Hyperglycemia was produced in two ways. Glucose was administered orally to trained unanesthetized dogs by stomach tube in doses of 2, 4, and 8 g per kilo body weight. Each dose was dissolved in 200 cc of water and given in 2 portions 30 minutes apart. Hyperglycemia was also produced by the intravenous infusion of a 15% glucose solution at the rate of 8 cc/min. for periods up to 6 hours. Plasma glucose determinations were made by the method of Nelson(7) using a Somogyi filtrate.(8) Eosinophile counts were made using a modification of the acetone-eosine method of Dunger.(9) Four Spencer Bright line chambers were counted in each determination. The maximum variability allowed between chambers in control counts was 10%. The average of four chambers was multiplied by a factor of 11.1 to give the number of cells/mm³ of blood. In order to evaluate the variability of our techniques, control counts were done on 8 dogs before and after stomach tubing with tap water. Counts were then repeated at hourly intervals on each dog for 5 hours. A coefficient of variation (c.v.) for the 6 counts in each dog was calculated. Each c.v. was multiplied by 2 to yield an adjusted c.v. so that the average of the 6 counts ± this value would include 95% of a given dog's counts. The mean of the eight adjusted coefficients of variation was found to be ± 11.1%.

Results. The control stomach tubing procedure did not appreciably alter the number of circulating eosinophiles in this series of trained dogs (Table I). These data were of value in estimating the variability of the eosinophile count as observed in our laboratory. Since the absolute eosinophile counts varied markedly from dog to dog, eosinopenic reactions to stress were made in terms of per cent deviations from an average control count for each dog. Alterations in the circulating blood eosinophiles and changes in plasma glucose levels in six unanesthetized dogs following glucose tolerance tests at three dosage levels are presented in Fig. 1. These data indicate that oral glucose in doses of 2, 4, and 8 g/kg will produce a significant eosinopenia occurring 2.5 to 4.5 hours after the administration of glucose. A given dose of glucose did not evoke a comparable eosinopenic response in every dog, however progressive increases in hyperglycemia with increasing doses of glucose produced progressive decreases in the eosinophile counts. Analysis of the 4 hourly eosinopenic responses following glucose administration revealed statistically significant differences between both the 2 and 8 g doses and the 4 and 8 g doses. The differences in eosinopenia effected by 2 and 4 g of sugar were not statistically valid. The eosinophile responses to the 3 levels of oral glucose

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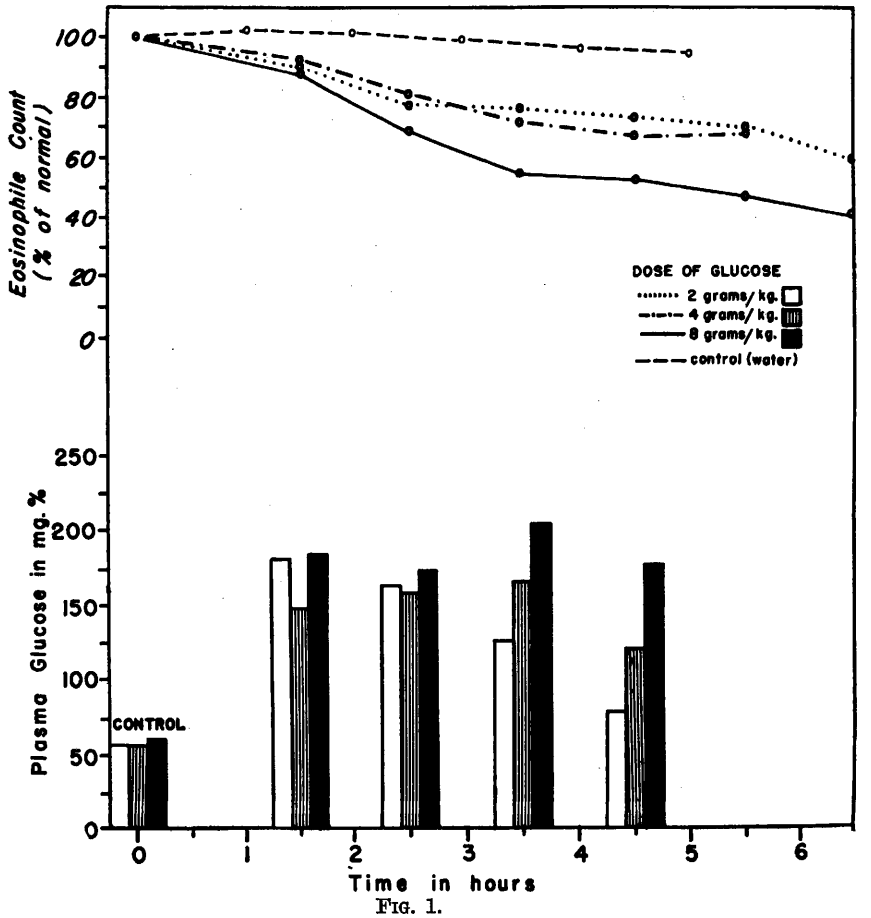

FIG. 1.
Mean eosinophile responses following the oral administration of glucose in 6 dogs.

yielded a parabolic type of dose-response curve.

The mean eosinophile and plasma glucose changes in 7 dogs following the infusion of 15% glucose at 8 cc/min. for 6 hours are illustrated in Fig. 2. The plasma glucose concentration reached a plateau after the first two hours of infusion, while the eosinophile counts continued to fall throughout the infusion. The constancy of hematocrit values after the first hour of glucose infusion obviated the possibility that the decrease in circulating eosinophiles was due to progressive hemodilution.

Discussion. There is evidence to the effect that exposure to stress results in the release of adrenocorticotrophic hormone from the anterior pituitary which in turn stimulates the secretion of the adrenal cortex. That the eosinopenic response is a manifestation of this reaction is indicated by its failure to appear in animals with adrenal cortical insufficiency. The data presented here suggest that hyperglycemia leads to an activation of the adrenals. The hyperglycemia response to oral glucose rose to a maximum within the first 1½ hours and gradually subsided over a 5 hour period. The eosinophile response, however, was maximum after 2.5 to 4.5 hours. Temporally speaking, the hyperglycemic and eosinopenic reactions in the dog following the oral administration of glucose were asynchronous. This is in contrast to the work of Pincus in the rat and man where the height of the blood sugar response coincided with the maximum lymphopenic reaction at the end of one half hour. The maximum eosinopenic response reported here, occurring at about 4 hours, is in

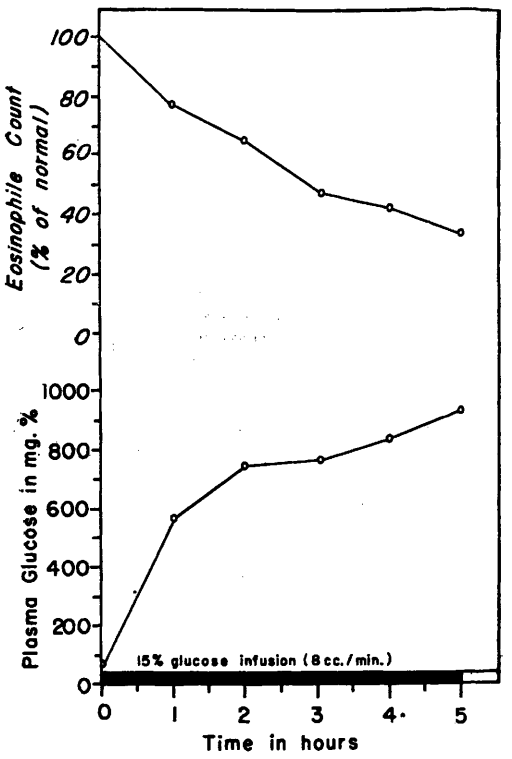

FIG. 2.

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.

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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.

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† 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.