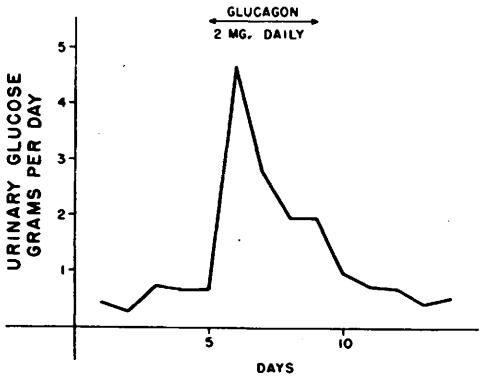

FIG. 2. Effect of continuous intravenous injection of glucagon upon glycosuria of a partially depancreatized rat.

glucose which reached a peak during the 2nd day and then fell to preinjection levels or lower by the 4th day. The data are in Fig. 1. Similar individual rats were given glucagon in doses of 0.1 to 2 mg/rat/day for 5 days. Each rat showed exacerbation of the glycosuria which reached a peak on the 2nd day and then abated. The response of one animal is shown in Fig. 2.

Discussion. Glucagon has a positive effect upon the glycosuria of the partially depancreatized rat. Our data indicate that the exacerbation of diabetes is temporary but definite conclusions may not be warranted. Some veins were found to be infected at autopsy although it was demonstrated that the infusion fluid still entered the blood in those animals which are reported here. There is evidence that the pancreas secretes a hyperglycemic agent(4) which may or may not be identical with glucagon. At this time the origin of glucagon, its hormonal nature and its role in body economy have not been clearly established.

Summary. When a highly purified preparation of glucagon was administered to partially depancreatized, force-fed rats by continuous intravenous injection there was temporary exacerbation of the diabetes. The continuous subcutaneous injections of glucagon to normal and to partially depancreatized rats were ineffective in the doses (0.05 to 1 mg/rat/day) tested.

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1. Ingle, D. J., Beary, D. F., and Purmalis, A., *Endocrinology*, 1953, v52, 403.

2. Staub, A., Sinn, L., and Behrens, O. K., *Science*, 1953, v117, 628.

3. Shaffer, P. A., and Williams, R. D., *J. Biol. Chem.*, 1935, v111, 707.

4. Foa, P. P., Magid, E. B., Glassman, M. D., and Weinstein, H. R., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 758.

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Eosinophils of Blood During Prolonged Exposure to Cold and Chronic Administration of Cortisone Acetate.*† (20908)

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In accordance with the concept of the General-Adaptation-Syndrome, it has been postulated that an increased secretion of adreno-cortical steroids occurred when an animal was placed under prolonged exposure to stress(1). This increased release of adrenal corticoids has been demonstrated indirectly by changes in the concentration of different metabolites in the blood during the period of exposure(1). The use of the adrenal ascorbic acid or adrenal cholesterol depletion tests as indices of adrenal activity have also revealed an immediate and marked release of adreno-corticoids(2). However, both substances returned to normal or above normal during prolonged exposure to cold(3). Similarly, prolonged exposure to a low atmospheric

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pressure(4) caused an initial depletion of adrenal ascorbic acid which returned to normal levels after adaptation to the environmental stress had occurred. It has been suggested that the recovery of the adrenal ascorbic acid and cholesterol were indicative of a new level of homeostasis and the gland was still in a hyperactive state.

Recently Ingle(5,6) has indicated that the hormones of the adrenal cortex play a permissive rather than a regulatory role in many adjustments to stress. Since the requirements for the cortical hormones are greatly increased during severe stress, he goes on to say . . . "that increased amounts of hormone would be required to support a state of eucorticalism and, consequently, permissive action, during severe as compared to a mild stress." The present study was undertaken to determine the level of adreno-cortical activity in rats during prolonged exposure to cold. Changes in the level of circulating eosinophils were used as an index of adrenal activity and comparisons made between normal rats, rats exposed to cold and rats treated with cortisone acetate.

Materials and methods. Mature female rats of the Harvard-Wistar strain, weighing between 150 and 250 g were used in the following experiments. The animals were divided into groups of 8 to 12 rats and 2 groups were exposed to a low environmental temperature (2 ± 2°C) for a period of 90 days. Four other groups were treated daily with various doses of cortisone acetate for a period of 21 days. The hormone was injected subcutaneously in a volume of 0.1 ml at dosages of 20, 50, 100, and 200 μg. A seventh group of animals served as controls and received 0.1 ml of saline daily. The animals were bled at regular intervals and the number of eosinophils/mm^3 of blood was determined. Peripheral blood was obtained from the tail and used for all the counts. The eosinophils were stained by the method of Pilot(7) and the number of cells was determined by counting the total number of eosinophils present in the entire field on both sides of a Neubauer Haemocytometer. Another group of rats received a single injection of 100 μg of cortisone acetate at the termination of a 90-day exposure to cold. This was done in order to determine whether the eosinophils were still sensitive to cortisone. A final group of rats was used for diurnal rhythm studies. The control animals and those treated with cortisone acetate were kept at a constant temperature of 22-24°C and supplied with food and water *ad libitum*. The animals exposed to the cold (2 ± 2°C) were kept in individual cages and given free access to food and water. All rats were fasted for 24 hours prior to each bleeding, but water was available at all times. Three eosinophil counts were made on all animals prior to treatment or exposure to cold and the subsequent counts were expressed as a percentage of the control values.

Results. A marked decrease of 86% in the circulating eosinophils occurred within 6 hours after the rats were exposed to cold (Fig. 1). However, the number of eosinophils returned to normal (—9%) within 24 hours in spite of the continued exposure. For the remainder of the period of cold exposure the level of eosinophils remained similar to, or greater than the pre-exposure value, with a significant drop observed on the 15th day. From the 29th day of exposure until the termination of the experiment on the 90th day the eosinophil levels remained consistently above normal.

The sensitivity of the eosinophils to exogenous cortisone acetate was tested in a second group of animals which had been in the cold for 90 days. An average eosinopenia of

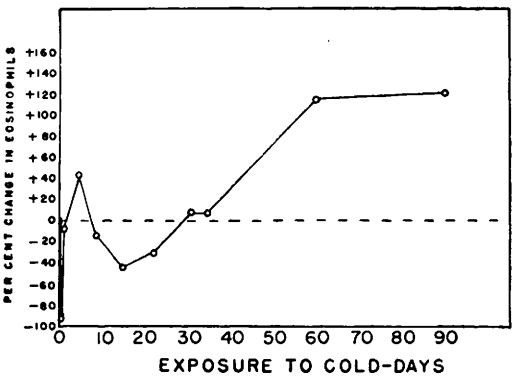

FIG. 1. Changes in level of circulating eosinophils following exposure to cold (2 ± 2°C). Note an initial drop followed by a rise with a secondary drop and a final rise which was maintained.

TABLE I. Diurnal Variation in Eosinophils of Rats Maintained at Room Temperature and during Exposure to Low Temperature (2 ± 2°C) for a Prolonged Period.

Treatment	% decrease in eosinophils at midnight, compared to noon level	"P" value
0 (room temp.)	−45	—
Cold (7 days)	−35	.70*
" (60 ")	−74	.10*

* As compared against room temperature decrease.

minus 76% was obtained within 6 hours after a single injection of 100 μg of cortisone acetate.

The diurnal rhythm of eosinophils was determined at room temperature and during cold exposure in another group of rats. The eosinophils were counted at noon on every third day for a 9-day period and at midnight on every third day for a second 9-day period. The same procedure was repeated after the animals had been exposed to the cold for one week. A 45% decrease in eosinophils was noted at midnight as compared with the noon values for the rats maintained at room temperature and 35% drop in eosinophils in the rats exposed to cold (Table I). The levels of eosinophils at noon and at midnight were again determined after the animals had been in the cold for 60 days. At this time a 74% decrease in the level of eosinophils was noted at midnight.

The level of circulating eosinophils was followed for 21 days in 4 groups of rats receiving different daily doses of cortisone acetate. In this way it was possible to demonstrate that a continuous eosinopenia could be maintained with a constant daily injection of an adequate amount of cortisone acetate. The number of circulating eosinophils was determined at 3-day intervals for a 21-day period of treatment and this was compared to normal values which had been obtained prior to the beginning of the hormone administration.

The administration of 20 μg of cortisone acetate daily had no effect on the level of circulating eosinophils. The number varied from —19 to +20% of the pre-injection level throughout the period of treatment (Fig. 2). The animals in the second group received 50 μg of the hormone and again no significant change in the number of eosinophils was noted for the duration of the experimental period. The level of eosinophils ranged from —25 to +19% during this period. The administration of 100 μg of cortisone acetate to the third group of rats resulted in a significant depletion in the number of eosinophils. The eosinopenia varied from —62 to —91% of the pre-injection level and was maintained for the entire period of treatment. Within 6 days after the cessation of treatment the number of eosinophils returned to normal. An average eosinophil depletion of —87 to —91% was observed in the rats that received 200 μg of cortisone acetate daily. With this dose the eosinophil level did not return to the pre-injection value until 9 days after the treatment had been stopped (Fig. 2).

Discussion. The conclusions that may be derived from these experiments depend on the validity of the eosinophil depletion test as an index of adreno-cortical activity. Dalton and Selye(8) were the first to indicate the presence of an eosinopenia in the alarm reaction. Since then a great deal of work has been published on the eosinophil response and its possible use as an index of pituitary adreno-cortical activity(9-14). In addition the eosinopenic reaction has been suggested as an assay for cortisone(15,16) and urinary corticoids(17). Recently the specificity of this response has been questioned since it was shown that epinephrine caused an eosinopenia in the adrenalectomized mouse(18) and in the Addisonian patient(11). Furthermore the eosinophil reaction could not be correlated with blood levels of compound F(19) and treatment with cortisone at a dosage that failed to produce eosinopenia in the adrenalectomized animals, would do so if combined with epinephrine(20). Spiers(21), however, claims that in adrenalectomized mice the eosinopenia response to epinephrine is due to the presence of adrenal rests. In general it can be concluded that the eosinopenia may be due not only to a release of adrenal corticoids but also to some other reaction. However, since the presence of adequate amounts of the 11-oxy-

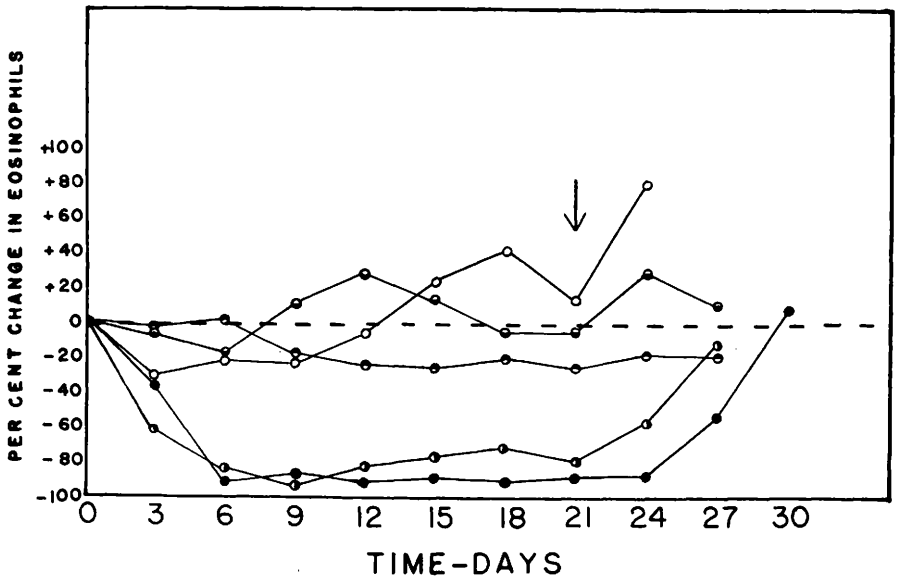

FIG. 2. % changes in circulating eosinophil of rat following treatment with 20, 50, 100 and 200 μg of cortisone acetate daily for 21 days. A control group was injected with 0.1 ml saline for a similar period. A significant drop in eosinophils was observed only in the rats receiving 100 or 200 μg of cortisone acetate, and in both groups the eosinopenia was maintained throughout the period of treatment. The arrow indicates cessation of treatment. Circle—0.1 ml saline; circle (bottom shaded)—20 μg cortisone acetate; circle (top shaded)—50 μg cortisone acetate; circle (side shaded)—100 μg cortisone acetate; solid circle—200 μg cortisone acetate.

corticoids always produces an eosinopenia, this reaction may be used to determine the absence of these hormones. Nevertheless, it must be admitted that our knowledge of the factors involved in determining the number of circulating eosinophils is inadequate to make any definite statement, and all conclusions reached here regarding adreno-cortical activity are tentative and subject to new interpretation.

The present results indicate that daily treatment with cortisone acetate will maintain a continuous eosinopenia (Fig. 2). Although the 50 μg dose did produce an eosinopenia, the average decrease was not significant except for day 12, due to the high degree of variation (Table II). However, the eosinopenia produced by the 100 and 200 μg dose was statistically significant throughout the period of treatment at the 0.10 and 0.05 level.

The initial eosinopenia which occurred shortly after the rats were exposed to the cold is presumably due to increased adreno-cortical activity. Within 24 hours, however, the number of eosinophils had returned to the pre-exposure level and by 48 hours a slight eosinophilia was present. In view of the eosinopenia which results from daily administration of cortisone acetate it appears that either the adrenal gland is not secreting excessive amounts of cortical steroids after initial exposure to cold or that adreno-cortical activity is increased only to the extent necessary to maintain a eucorticoid state in the new environment. The latter concept would be in agreement with the theory of the permissive action of the adrenal gland(6).

The diurnal variation in the number of circulating eosinophils was first demonstrated by Halberg and Visscher(22) who found a decrease in the number of eosinophils in mice during the night. This diurnal rhythm has been correlated with periods of generalized activity and increased adrenal secretion. Present results confirm previous findings that the diurnal rhythm present in the rat at room temperature is maintained after exposure to cold(23). This maintenance of a diurnal variation during the period of cold exposure is probably indicative of a diurnal rhythm in the adrenal cortex after the animal has been adapted to cold.

TABLE II. Statistical Significance of Results on the Level of Eosinophils in Rats Treated with Cortisone Acetate or Exposed to Cold.

Treatment	"P" values for No. of circulating eosinophils at* 6	12	18 days
Cold	>.1	>.1	>.1
50 μg cortisone acetate	>.1	.05	>.1
100 *idem*	.10	.10	.05
200 "	.05	.05	.10

* Eosinophil values of above groups were compared with eosinophil values obtained in normal, saline-treated, control rats.

The possibility that the eosinophils were refractive to adrenal corticoids is ruled out by their ability to respond to exogenous cortisone acetate after exposure to cold for 90 days. A single injection of 100 μg of cortisone acetate resulted in the characteristic eosinopenia that is normally found within 6 hours. This can be interpreted as indicating that the eosinophils are still responsive to cortisone and from this one may assume that there would be a continuous eosinopenia if the adrenal gland was secreting large amounts of cortical hormone during the period of adaptation.

A possible adaptation by the organism to hypercorticalism has been suggested from data indicating a failure of cortisone to maintain high liver glycogen values(24) in spite of continued treatment. Since the cortisone-treated rats showed no change in the eosinopenia after prolonged treatment it is apparent that the eosinophil reaction does not fall into this category. Hence it is difficult to assume that the failure to maintain an eosinopenia in the cold-exposed rats is due to adaptation. The present data seem to indicate that the increased amounts of adrenal corticoids present during the initial phase of exposure to cold are counteracted by the increasing needs of the organism and that, consequently, after the animal has become adapted a eucorticoid state exists.

Conclusion. Evidence has been presented to show that the circulating eosinophils do not remain depressed in rats exposed to cold for a period of 90 days. The level of eosinophils dropped 6 hours after the animals were exposed to the cold, but returned to above the normal values within 48 hours and in general remained high for the duration of the exposure. Different doses of cortisone acetate were administered to intact rats and it was determined that a daily dose of 100 μg was necessary to maintain a depressed level of eosinophils during the period of treatment. The evidence suggests that a eucorticoid state may develop during adaptation to cold. The permissive action of the adrenal gland and its relation to the adaptation of rats to a low environmental temperature is discussed.

1. Selye, H., *Stress,* p. 405, Acta Inc., Montreal, Canada, 1950.

2. Sayers, G., Sayers, M. A., Fry, E. G., White, A., and Long, C. N. H., *Endocrinol.,* 1944, v16, 361.

3. Sayers, G., and Sayers, M. A., *Recent Progress in Hormone Research,* 1948, v2, 81.

4. Darrow, D. E., and Sarason, E. L., *Endocrinol.,* 1944, v23, 1.

5. Ingle, D. J., *J. Endocrinol.,* 1952, v8, 23.

6. ———, Trans. 4th Conference on Adrenal Cortex, Josiah Macy, Jr. Foundation, 1952, p12.

7. Pilot, M., *Am. J. Clin. Path.,* 1950, v20, 850.

8. Dalton, A. J., and Selye, H., *Folia Haematologica,* 1939, v62, 397.

9. Hills, A. G., Forsham, P. H., and Finch, C. A., *J. Hemat.,* 1948, v3, 755.

10. Laragh, J. H., and Almy, T. P., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 499.

11. Recant, L. D., Hume, D. M., Forsham, P. H., and Thorn, G. W., *J. Clin. Endocrinol.,* 1950, v10, 187.

12. Gabrilove, J. L., *J. Clin. Endocrinol.,* 1950, v10, 637.

13. Bonner, C. D., *J.A.M.A.,* 1952, v148, 634.

14. Fisher, B., and Fisher, E. R., *Am. J. M. Sci.,* 1951, v221, 121.

15. Spiers, R. S., and Meyer, R. K., *Endocrinol.,* 1949, v45, 403.

16. ———, *ibid.,* 1951, v48, 316.

17. Rosemberg, E., and Lewis, R. A., *J. Applied Physiol.,* 1950, v3, 164.

18. Stein, K. F., Martin, C., and Falk, D., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 110.

19. Kelley, V. C., Ely, R. S., Raile, R. B., and Bray, P. F., *ibid.,* 1952, v81, 611.

20. Henry, W. L., Jr., Oliver, L., and Ramey, E. R., *Am. J. Physiol.,* 1953, v174, 455.

21. Speirs, R., *Am. J. Physiol.,* 1953, v172, 520.

22. Halberg, F., and Visscher, M. B., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 846.

23. Louch, C., Meyer, R. K., and Emlen, J. T., *ibid.,* 1953, v82, 668.

24. Ingle, D. J., Meeks, R. C., and Beary, D. F., *ibid.,* 1953, v84, 239.

Received February 23, 1954. P.S.E.B.M., 1954, v85.