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Effect of Epinephrine in Decreasing Number of Circulating Mononuclear Leucocytes in the Rat.*

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Recent evidence suggests that the number of circulating mononuclear leucocytes is under the control of adrenal cortical hormones[1,2] since these cells are decreased when an excess of cortical hormone is present and increased after adrenalectomy. Epinephrine has been reported to stimulate adrenal cortical secretion.[3-5] Epinephrine administration should, therefore, cause a decrease in the mononuclear count of the blood. Lymphopenia following epinephrine administration has recently been reported to occur in intact but not adrenalectomized dogs.[6]

The problem remains whether epinephrine changes the blood picture by direct action on the adrenal cortex or whether the mechanism is mediated through the pituitary gland. In an effort to add evidence to this point, the effect of epinephrine on the mononuclears of rat blood was determined in the presence or absence of the endocrine glands in question (pituitary and adrenal).

The results indicate that epinephrine administration is followed by a decrease in the number of circulating mononuclear leucocytes in normal, adreno-demedullated, and hypophysectomized rats but not in adrenalectomized rats.

Methods. Healthy growing male rats of the Long-Evans strain, 60 to 70 days of age and kept under similar environmental conditions were employed. They received the regular stock diet (XIV)† *ad libitum* supplemented twice weekly with fresh lettuce.

Hypophysectomy was performed under ether anesthesia. Adreno-demedullation and adrenalectomy were performed under sodium pentobarbital anesthesia. Hypophysectomized and adrenalectomized rats were used on the tenth postoperative day. Adreno-demedullated rats were used 20 or 30 days after the operation. Adrenalectomized rats were maintained on drinking water containing 1% sodium chloride.

Blood counts were made from tail vein blood. The smears were stained with Wright's stain and between 100 and 200 leucocytes were counted. White blood cells were classified as mononuclear or neutrophilic leucocytes.

Epinephrine chloride, 1:1000 (Parke Davis and Co.), was injected subcutaneously in the amount of 0.07 cc per 100 g body weight. For the purpose of control, saline solution (0.9%) was injected subcutaneously in the amount of 0.07 cc per 100 g body weight.

Results. Fifteen to 30 minutes after administering epinephrine to normal rats (Table I) a slight but consistent elevation in the number of mononuclear leucocytes occurred. After one hour, the number of mononuclears began to decrease, reaching the lowest level in 2 hours. Thereafter, the number of mononuclears began to increase, reaching the normal level after 6 to 8 hours. Neutrophilic leucocytes increased rapidly throughout the

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1 Dougherty, T. F., and White, A., *Endocrinology*, 1944, **35**, 1.

2 Reinhardt, W. O., Aron, H., and Li, C. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 19.

3 Vogt, M., *J. Physiol.*, 1944, **103**, 317.

4 Long, C. N. H., *Fed. Proc.*, 1947, **6**, 461.

5 Sayers, G., and Sayers, M., *Endocrinology*, 1947, **40**, 265.

6 Malmejac, J., Chardon, G., and Gros, A., *Bull. l'Acad. Med.*, 1946, **130**, 492.

† Diet XIV (stock diet) consisted of ground whole wheat 67.5%, casein 15.0%, whole milk powder 10.0%, NaCl 0.75%, $CaCO_3$ 1.5%, hydrogenated vegetable oil (Crisco or primex) 5.25%. To each kg of diet were added 3.5 g of sardilene (fish oil concentrate containing 3000 USP units of vitamin A and 400 chick units of vitamin D per g).

TABLE I. Number of Circulating Leucocytes in the Rat After Administration of Epinephrine or Saline Solution.

Treatment	Experimental animals: Type	No.	B.W. (g)	Age (days)	Mononuclear counts, Hr after injection: 0	½	2	4	Neutrophil counts, Hr after injection: 0	½	2	4
Epinephrine chloride	Normal	10	233	71	11600 (1300)	11800 (1150)	5500* (750)	9000 (1600)	2900 (500)	3750 (1050)	7850* (1200)	15200* (1650)
	Adrenodemedullated	8	245	68	10400 (550)	9400 (1100)	6200* (300)	9400 (900)	2700 (350)	3300 (450)	8600* (850)	16000* (1900)
	Adrenalectomized	10	247	66	13600 (2100)	17600 (2000)	15400 (1350)	19400 (2200)	4250 (850)	4800 (550)	11300* (1250)	17100* (2200)
	Hypophysectomized	8	205	70	22000 (1400)	19200 (750)	14000* (1400)	18000 (2100)	3000 (350)	4000 (700)	5000† (650)	10900* (1250)
0.9% NaCl	Normal	10	262	69	10750 (900)	7550† (650)	7300* (600)	10650 (900)	2600 (400)	2600 (450)	3900† (400)	5250† (850)
	Adrenodemedullated	8	218	60	10150 (600)	9300 (750)	12000 (1000)	13000† (1100)	2700 (300)	3850† (300)	5050* (350)	5500* (500)
	Hypophysectomized	8	180	65	15200 (950)	15300 (800)	17700 (1000)	23000* (1400)	3200 (400)	4000 (450)	5200 (800)	9600* (950)

Leucocyte counts are expressed as the number per mm³ (mean value with standard error in parenthesis).
* Denotes highly significant values ($p = <.01$) when compared with the 0 hour count for that group.
† Denotes probably significant values ($p = <.05$) when compared with 0 hour count for that group. Other values presented are not significant when compared in this way.

entire period. Twenty-four hours later the number of both types of leucocytes was slightly lower than the pretreatment number. Essentially the same response was obtained by injecting epinephrine into adreno-demedullated rats. Having established that the minimum level in the number of circulating mononuclear leucocytes was reached 2 hours after administering epinephrine, blood counts were restricted to a 4-hour period and were made at the intervals shown in the table.

Two hours after administering epinephrine to hypophysectomized rats, the number of mononuclear leucocytes was significantly decreased, although the percentage decrease was not as great as in the epinephrine treated normal rats. The number of neutrophils increased throughout the 4-hour period.

In adrenalectomized rats the number of mononuclear leucocytes did not fall but was slightly increased after administering epinephrine. The number of neutrophils increased as in the other groups.

In order to determine the error which might be incurred by repeated blood counts taken from the tail, and from handling the rats, 0.9% saline solution was injected into normal, adreno-demedullated, and hypophysectomized rats and blood counts were taken at the same intervals. Although the number of mononuclears was decreased in normal rats injected with saline solution, this change was not of the same magnitude as that following epinephrine injection. (It probably can be attributed to stimulation of the adrenal cortex by discharge of epinephrine resulting from excitement.) This decreased count was not observed in adreno-demedullated or hypophysectomized rats after injection of saline solution.

Statistical evaluation. The pretreatment value (0 hour) was considered as normal for the group in question and subsequent values for that group were compared with this initial count in calculating P values.[7] A change in the leucocyte count was considered highly significant if the P value was 0.01 or less and

[7] Fisher, R. A., and Yates, F., *Statistical Tables*, Oliver and Boyd, London, 1938.

probably significant if P was 0.05 or less (Table I).

It was not surprising that the initial mononuclear counts in different groups were not the same as it is well known that mononuclear leucocytes are increased after adrenalectomy or hypophysectomy. Comparison between groups, therefore, was not possible without converting all values to a percentage figure of the pretreatment count which would thus give an initial common basis for comparison. Percentage figures are not presented since this procedure did not alter the conclusions or significance of the data.

Discussion. Harvey[8] reported an increase in the mononuclear count 15 minutes after injection of epinephrine into rabbits. Bjure and Svensson[9] reported an increase after 30 minutes but a decrease after 2 hours in normal human beings. Fegler[10] reported a leucopenic phase 2 hours following administration of epinephrine to dogs. Martin[11] reported a lymphocytosis in human patients but did not make blood determinations longer than 40 minutes after injection of epinephrine. Camp[12] reported a lymphopenia but did not specify the time interval elapsing after injecting epinephrine. Harlow and Selye[13] reported a relative lymphopenia after administering epinephrine twice daily for 2 days. The effect of epinephrine in elevating the blood sugar may be related to the changes in the mononuclear counts. Following glucose administration, Elmadjian, Freeman, and Pincus[14] reported a lymphopenia which was lowest when the blood sugar was highest. This response could not be obtained in adrenalectomized rats.

Two hours after intravenous injections of epinephrine into intact dogs, Malmejac, Chardon, and Gros[6] reported a lymphopenia. The number of lymphocytes returned to the normal level after 4 to 6 hours. A slight lymphocytosis developed after injecting epinephrine into adrenalectomized dogs. These are the same time relationships which are reported in this paper following subcutaneous injection of epinephrine into rats.

The dose of epinephrine employed in the experiments reported here is admittedly too high to be within physiological limits. It is to be pointed out that epinephrine gave the responses indicated only in the presence of the adrenal gland. No attempt was made to determine the minimum effective dose to produce these effects.

Conclusions. The administration of epinephrine to normal or adreno-demedullated rats produced within 2 hours a definite fall in the number of circulating mononuclear leucocytes. The number of mononuclears did not decrease after administering epinephrine to adrenalectomized rats. This indicates that the presence of the adrenal cortex is necessary for epinephrine to cause these effects. After administering epinephrine to hypophysectomized rats, the number of mononuclears decreased as in the epinephrine treated normal rats although the percentage decrease was less. This would seem to indicate that at least one effect of epinephrine is to act directly on the adrenal cortex, releasing substances which lower the level of circulating mononuclear leucocytes.

Since this paper was submitted for publication, Gellhorn and Frank[15] have reported that epinephrine causes a reduction in the number of circulating lymphocytes in normal but not in adrenalectomized rats.

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9 Bjure, A., and Svensson, J., *Studia Biologica Medica*, Sect. VI, Uppsala, 1921.

10 Fegler, G., *Compt. Rend. Soc. Biol.*, 1927, **97**, 966.

11 Martin, H. E., *J. Physiol.*, 1932, **75**, 113.

12 Camp, W. J. R., *J. Lab. Clin. Med.*, 1927, **13**, 206.

13 Harlow, C. M., and Selye, H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 141.

14 Elmadjian, F., Freeman, H., and Pincus, G., *Endocrinology*, 1946, **39**, 293.

15 Gellhorn, E., and Frank, S., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 426.