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Sensitivity of the Lymphopenic Reaction to Adrenalin.*

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Injection of adrenotrophic hormone causes a decrease in the ascorbic acid and cholesterol content of the adrenal cortex (Long and collaborators[1,2]) and induces a lymphopenia (Dougherty and White[3]). Since the latter effect is absent in adrenalectomized rats it was interpreted as being due to the increased secretion of adrenocortical hormone resulting from excitation of the adrenal cortex through the adrenotrophic hormone. It was also reported that increased activity of the adrenal cortex accompanied conditions of stress and led to a decrease in ascorbic acid and cholesterol content of the adrenal gland (Long and collaborators), increased excretion of 17-ketosteroids (Pincus,[4] Hoagland[5]) and lymphopenia (Long and *alii,* Elmadjian and Pincus[6]). Since Vogt[7] has shown that injection of adrenalin causes an increase in the concentration of adrenocortical hormones in the adrenal vein and since various conditions of stress activate the sympathetico-adrenal system (cf. Gellhorn[8] for literature) it seemed probable that the alteration in the chemistry of the adrenal cortex as well as the lymphopenia resulted from the secretion of adrenalin which activated the hormones of the adrenal cortex. Such an interpretation presupposes a great sensitivity of the pituitary-adrenal cortex mechanism to adrenalin. The examples cited by Long indicate that the ascorbic acid content of the adrenal cortex may be lowered significantly by infusion of adrenalin in a dosage of 2 γ 100 g/hr. As far as the effect of the injection of adrenalin on the white blood corpuscles is concerned the data published in the literature suggest that its effect is mainly to produce leucocytosis (Walterhofer,[9] Naegeli[10]). An increase in the number of lymphocytes has been reported by Garrey and Bryan[11] whereas a biphasic reaction (leucocytosis followed by leucopenia) was found by Fegler.[12] Our own earlier work showed that conditions such as anoxic and anemic anoxia (Cress, Clare and Gellhorn[13]) and chemically or electrically induced convulsions (Clare, Cress and Gellhorn[14]) induce leucocytosis through secretion of adrenalin since this effect was absent in adreno-demedullated animals. In view of the fact that the experiment showing leucocytosis as a result of injection or secretion of adrenalin involved temporal relations quite different from those in which lymphopenia occurred as a result of conditions of stress a reinvestigation of this problem appeared desirable. The experiments reported in this paper show that lymphopenia may result from the injection of minute quantities of adrena-

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TABLE I.
Effect of Injection of Adrenalin on White Blood Cells of the Rat. Ten Rats Each Test.

Dose/100 g, γ	Leucocytes in %				Lymphocytes in %				t	p	Area in mg
	0′	20′	40′	60′	0′	20′	40′	60′			
A. Normal Rats.											
1	100	70	59	60	100	64	54	54	11.6	<.001	267.2
0.2	100	71	62	60	100	64	56	49	8.38	<.001	271.6
.1	100	76	58	56	100	62	52	49	8.96	<.001	230.9
.05	100	74	63	57	100	72	62	54	5.7	<.001	199.5
.03	100	94	89	87	100	93	88	85	0.79	<.4	47.5
B. Adrenalectomized Rats.											
1	100	94	92	94	100	95	92	93			36

lin. Moreover, an attempt is made to analyze the mechanism involved.

Method. The experiments were performed on Sprague-Dawley rats (mostly adults, 150-200 g, but also some of lower weight as stated below) after a 16 hour fast. The counting of red and white blood cells including differentials followed standard procedures. It was based on duplicate or triplicate determinations. Blood samples were taken from the tail with a minimum of excitation by proper selection of tame rats and careful handling. Adrenalin was injected intraperitoneally while eserine sulphate was administered subcutaneously. The blood samples were taken at 20 minute intervals for 1 hour after the injection of adrenalin.

Results. The effect of intraperitoneal injection of adrenalin on the white blood count of rats was studied on 5 groups of animals which received this substance in quantities varying from 0.03 γ to 1 γ/100 g weight. Table I shows that the number of leucocytes and lymphocytes decreases markedly with doses from 0.05 to 1 γ/100 g whereas the effect of 0.03 γ/100 g is only slight. The individual data showing the changes in the number of lymphocytes were graphed and the area representing the fall in the number of lymphocytes was weighed. The mean value in milligrams is given under "area" in the table and the statistical significance of these changes was calculated by determination of t and P values when each of these series of experiments was compared with that performed on adrenalectomized rats (Table I, B). The latter showed only an insignificant fall in the number of leucocytes and lymphocytes.

The table shows that the injection of adrenalin in doses varying from 0.05 to 1 γ/100 g causes a significant fall in leucocytes and lymphocytes whereas 0.03 γ/100 g adrenalin is ineffective. There seems to be little variation in the degree of leuco- and lymphopenia when the doses of adrenalin varied 20 fold.

A further group of experiments was performed on young rats averaging 40 g in weight. These animals reacted likewise with a marked leuco- and lymphopenia to the injection of 0.05 γ adrenalin per 100 g. This shows that 0.02 γ adrenalin can be readily detected by this method. Since the adrenalin content of blood is about 0.1 γ/cc (West)[15] this method appears to be suitable for such studies.

Fig. 1 shows the effect of adrenalin on neutrophils as compared to lymphocytes. It is evident that increase in neutrophils and lymphopenia characterizes the effect of adrenalin on the white blood count of the rat. In spite of some differences in the time relations the experiments of Dougherty and White on the effect of the injection of the adrenotrophic hormone on the leucocytes and our own on the action of adrenalin show qualitatively and quantitatively similar results.

Two further groups of experiments were performed. In the first saline was injected in volumes the same as those of adrenalin and failed to alter the white blood count. This proved that the injection *per se* did not excite the animals sufficiently to cause an alteration

[15] West, G. B., *J. Physiol.*, 1947, **106**, 426.

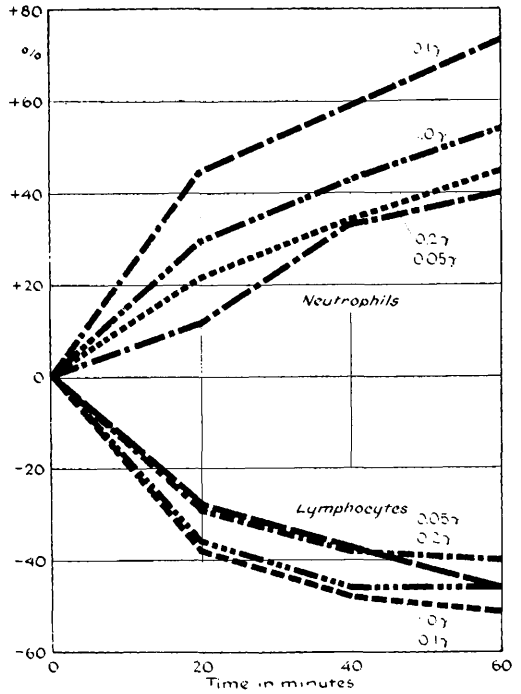

FIG. 1.

The effect of the injection of adrenalin on the percentage changes of neutrophils and lymphocytes. Each graph represents the mean of 10 experiments. Doses of adrenalin of 0.05, 0.1, 0.2, 1 γ/100 g respectively were injected at 0′ minutes.

in the white cell count. Also, rats were injected with doses totalling 3 cc of Upjohn's adrenocortical extract† in 24 hours, and 2 hours after the last injection (1 cc) the effect of adrenalin was studied. This experiment was performed since Sayers and Sayers[16] had noted that the effect of adrenalin and of various forms of stress on the concentration of ascorbic acid in the adrenal cortex was abolished by pretreatment with adrenocortical hormones. In a control test for this experiment normal rats injected with this extract showed no significant changes in the white blood count between the second and third hour after the last injection of adrenocortical extract. Therefore any alteration in the white count which would occur during this period after the injection of adrenalin could be causally related to the latter. However, as Fig. 2 shows, adrenalin fails to alter the white cell count of adrenalectomized rats in spite of ample supply of adrenocortical hormones.

This result suggests that the failure of adrenalin to alter the white count in adrenalectomized rats is not due to general metabolic disturbances resulting from the loss of the adrenocortical hormones unless one assumes that the particular adrenal hormone necessary for this response is not present in the extract. However, such an interpretation is incompatible with the following experiment in which the action of adrenalin on the white cell count of normal rats with and without previous treatment with large quantities of adrenocortical extract was studied. Table II shows that the pretreatment of normal rats with adrenocortical extracts greatly reduces the effectiveness of adrenalin on the lympho-

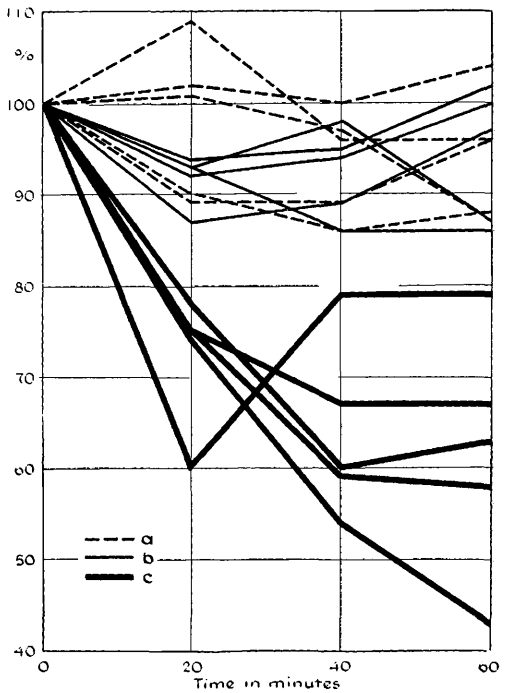

FIG. 2.

The effect of the injection of adrenalin (at 0′ min.) on the leucocyte count; a, adrenalectomized rats injected with 3 cc of adrenocortical extract (Upjohn) in 24 hours. Adrenalin (0.2 γ/100 g) administered 2 hours after the last injection of the Upjohn extract; b, adrenalectomized rats injected with 1 γ/100 g of adrenalin; c, normal rats, injected with 0.2 γ/100 g of adrenalin.

† Kindly supplied by Drs. Ingle and Hailman.

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TABLE II.
Effect of Adrenocortical Extract on Action of Adrenalin (0.2 γ/100 g) on Lymphocyte Count of Normal Rats.

No. of rats	Adrenocortical extract	Lymphocytes in % 0′	30′	60′	t	p	Area in mg
12	0	100	60	49	8.64	<0.001	172.7
14	3 cc	100	83	81			36.4

cyte count. This confirms in principle the work of Sayers and Sayers and suggests strongly that adrenalin acts primarily on the adrenocortical hormones in the tissues. If their concentration is sufficiently lowered the anterior pituitary is stimulated, more adrenotrophic hormone is secreted which in turn causes an increased secretion of adrenocortical hormones and thereby a replenishment of these hormones in the tissues.

Discussion. Intraperitoneal injection of adrenalin in doses varying from 0.05 to 1 γ/100 g causes in rats a marked lymphopenia while the number of neutrophils increases. However the effect on the lymphocytes is so great that the decrease in the total number of white blood cells is a reliable indicator of the action of adrenalin. This reaction is similar to that observed by Dougherty and White on injection of adrenotrophic hormone. The absence of this effect in adrenalectomized rats is not due to a general deficiency in adrenocortical hormones since the injection of adrenalin fails to alter the white blood count even in the adrenalectomized rats maintained with ample doses of adrenocortical extracts. The data suggest that adrenalin alters the white blood cell count through increased secretion of the adrenocortical hormones. The method is recommended for the assay of adrenalin since even 0.02 γ of adrenalin can be detected in small rats.

The primary factor involved in the action of adrenalin on the lymphocytes seems to be the lowering of the concentration of adrenocortical hormones in the tissues. This interpretation, advanced by Sayers and Sayers[16] on the basis of experiments on the ascorbic acid content of the adrenal cortex, was confirmed by our studies on lymphopenia since in both instances pretreatment with adrenocortical extracts prevented or greatly diminished the action of stress and of injection of adrenalin.

The lowered concentration of these hormones in blood and tissues furnishes the effective stimulus for the anterior pituitary which through the secretion of adrenotrophic hormone makes an increased secretion of adrenocortical hormones possible. This interpretation makes it understandable that adrenalin produces lymphopenia in normal and adrenodemullated but not in adrenalectomized animals even after treatment of the latter with adrenocortical extracts.

The observation of Long[1] that adrenalin does not alter the ascorbic acid concentration of the adrenal cortex in hypophysectomized rats even after injection of anterior pituitary extracts or implantation of the hypophysis does not invalidate this interpretation. It rather suggests that the increased secretion of the adrenotrophic hormone which follows depletion of adrenocortical hormones in the tissues is initiated by impulses in the hypothalamus. This interpretation will be tested in the near future.

Summary. Adrenalin injected intraperitoneally causes neutrophilia and a marked lymphopenia in rats in doses varying from 0.5 to 1 γ/100 g. This effect is absent in adrenalectomized animals and it is greatly diminished in normal rats injected with large quantities of adrenocortical extracts. It is suggested in agreement with Sayers and Sayers that adrenalin increases the consumption of adrenocortical hormones in the tissues and that the lowering of their concentration in the blood leads to increased secretion of the adrenotrophic hormone which in turn increases secretion of adrenocortical hormones. The latter induce lymphopenia.