17100. Lymphopenia and the Secretion of Adrenalin. *

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It was shown in a preceding paper[1] that the injection of adrenalin in doses of from 0.05 to 1 γ/100 g significantly lowers the lymphocyte count of the rat while the number of neutrophils is reduced to a lesser extent. This result (lymphopenia and a relative neutrophilia†) is not seen in adrenalectomized animals. The great sensitivity of this reaction makes it probable that it may occur under conditions involving not the injection but the secretion of adrenalin. Since the question of increased adrenalin secretion in physiological conditions is still controversial (See Rogoff,[2] for the older literature see Gellhorn[3]) it was decided to investigate the action of hemorrhage, emotional excitement and insulin hypoglycemia on the lymphocyte count. Elmadjian and Pincus[4] showed already that struggle (excitement) and exposure to cold resulted in lymphopenia and that this effect was absent in adrenalectomized rats. However the experiments of these authors did not deal with the question whether the secretion of adrenalin formed a necessary link in the chain of events leading to lymphopenia under conditions of struggle, etc. The present work was undertaken to fill this gap.

Method. The experiments were performed on adult Sprague-Dawley rats (weight 150 to 200 g) after a 16 hour fast unless otherwise stated. Blood samples were taken from the tail blood and the lymphocytes were determined before and 20, 40 and 60 minutes after the experimental procedure. Three procedures were used. The first consisted in hemorrhage (1 cc blood/100 g weight removed from the tail veins); the second involved emotional excitement induced by a slight electric shock which elicited a "rage" reaction but did not produce a convulsion. As a third procedure insulin‡ was injected intraperitoneally and the effect of varying degrees of hypoglycemia was studied. The blood sugar was determined as in previous papers.[5] The experiments were performed on normal and adrenodemedullated rats. The latter were used not earlier than 10 to 14 days after operation and only after a test with adrenalin 0.1 γ/100 g i.p. had resulted in a marked lymphopenia. If this effect was not clear cut the animal was either discarded or used after a second adrenalin test taken one or two weeks later had proved the responsiveness of the adrenal cortex to injected adrenalin.

Results. I. *The effect of hemorrhage on the lymphocyte count.* Table I shows the percentage changes in lymphocytes and polynuclear leucocytes following hemorrhage. In 60 minutes the former fell to 66.9% of the control value in normal rats whereas adrenodemedullated animals showed only an insignificant fall (to 91.5%). This difference is statistically significant ($P < 0.01$). The polynuclear cells fell only slightly. As the red cell count indicates the difference existing between the normal and adrenodemedullated rats is not accounted for by differences in the dilution of the blood and must therefore be attributed to a reaction dependent on the secretion of adrenalin. The fact that the reaction observed in normal rats has the same characteristics (lymphopenia and relative neutrophilia) as seen after injection of adrenalin supports this conclusion.

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

† Through an oversight this effect was described in the preceding paper as lymphopenia and neutrophilia instead of lymphopenia and *relative* neutrophilia.

2 Rogoff, J. M., *J. Genetic Psychol.*, 1945, **32**, 249.

3 Gellhorn, E., *Autonomic Regulations*, New York, 1943.

4 Elmadjian, F., and Pincus, G., *Endocrinology*, 1945, **37**, 47.

‡ Kindly supplied by the Eli Lilly and Co.

5 Safford, H., and Gellhorn, E., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 98.

TABLE I.

	Lymphocytes in %				Neutrophils in %				Neutrophils / Total leucocytes				P†	% of red blood cells at 60′
	0′	20′	40′	60′	0′	20′	40′	60′	0′	20′	40′	60′		
A. Effect of Hemorrhage on the Blood Count of the Rat.*														
Normal	100	85	78	67	100	99	84	80	100	110	114	121		94
Adrenodemedullated	100	91	95	92	100	83	92	86	100	95	100	96	$<.01$	96
B. Effect of the Rage Reaction on the Blood Count.														
Normal	100	76	55	46	100	88	84	72	100	114	127	131		101
Adrenodemedullated	100	91	84	82	100	101	100	103	100	110	116	116	$<.001$	98

* Each group consisted of 10 rats which were fasted 16 hours prior to the experiment.
† Probability coefficient applied to the lymphocytes in the 2 groups at 60 minutes.

II. *The effect of "rage" on the lymphocyte count.* Normal and adrenodemedullated rats were given a slight electroshock applied to both ears for 0.4 second. The rats reacted strongly to the stimulus with struggling and vocalization but were quiet a few minutes later. The effect on the lymphocytes was similar to that described in the first group but the difference in the reaction between normal and adrenodemedullated rats was even greater ($P < 0.001$. The change in the neutrophil count was again much less than in that of the lymphocytes, leading to a lymphopenia and a relative neutrophilia as on injection of adrenalin. No changes occurred in the neutrophils of the demedullated group and the fall in the lymphocytes was very slight. The red cell count before and 60 minutes after the "rage" reaction showed clearly that the changes in the lymphocytes of normal rats in conditions of "rage" are not due to a dilution of the blood.

III. *The action of hypoglycemia on the lymphocyte count.* A series of experiments listed in Table II was performed to determine the action of insulin hypoglycemia on the lymphocyte count of normal and adrenodemedullated rats. Since the latter are more sensitive to insulin than normal rats the experimental conditions had to be slightly modified for the two groups in order to obtain similar degrees of hypoglycemia. Table II shows that this was accomplished by varying the time interval of insulin injections and the duration of fasting. If both groups (normal and demedullated rats) were given 2 successive injections of 0.5 unit/kilo insulin i.p. at an interval of 20 minutes the demedullated rats were in coma at about 30 to 40 minutes but the normal rats at 60 minutes. By delaying the second injection by 10 minutes the demedullated group went into coma at about the same time as the unoperated group. As the Table shows the effect of insulin on blood sugar was very similar in groups A (normal) and D (demedullated); likewise the mild hypoglycemic effects in groups C and E were about the same. In the groups A and D in which each animal was in coma at 60 minutes the average lymphocyte count was 60 and 70.2% respectively of the preexperimental level in the normal and the

TABLE II.

A. Effect of Insulin Hypoglycemia on the Blood Count of the Rat. Dose 0.5 μ/kg.

Group	Hrs fasted	Dose	Blood sugar at 60′	% of lymphocytes				% of neutrophils				Neutrophils / Total leucocytes			
				0′	20′	40′	60′	0′	20′	40′	60′	0′	20′	40′	60′
A. Normal*	36	at 0′ and 20′	28.3 ± 8.48	100	83	73	60	100	91	83	76	100	112	122	136
B. " †	16	at 0′ and 20′	43.3 ± 6.39	100	83	77	70	100	89	84	83	100	107	115	123
C. "	16	at 0′	50.2 ± 4.96	100	89	83	75	100	93	83	80	100	106	106	112
D. Adrenodemedullated	24	at 0′ and 30′	31.8 ± 9.6	100	82	75	72	100	94	84	87	100	101	102	107
E. "	16	at 0′ and 20′	46.4 ± 14.97	100	92	85	79	100	92	85	88	100	98	99	113

* Group A and D consisted of 11 animals.
† Group B, C, and E consisted of 10 animals.

B. Significance of the Changes in Lymphocytes.

Groups compared	P*
A, C	<.01
A, D	<.05
D, E	<.2
A, B	<.05
C, D	<.9
C, E	.5

* Probability coefficient applied to lymphocytes in the two groups at 60′.

C. Effect of Insulin Hypoglycemia on the Blood Count of the Adrenalectomized Rat.*

Blood sugar at 60′ (mg%)	% of lymphocytes				% of neutrophils				Neutrophils / Total leukocytes				% red blood cells at 60′
	0′	20′	40′	60′	0′	20′	40′	60′	0′	20′	40′	60′	
31 ± 2.9	100	101	98	90	100	101	95	107	100	99	98	111	101%

* Insulin 0.2 μ/kg was injected in 10 rats fasted for 16 hours and resulted in deep coma.

demedullated group. The statistical evaluation shows that P is < 0.05 suggesting probable significance. As the hypoglycemic effect diminishes from A to C in the normal group (the final, 60 minutes—blood sugar being 28, 43 and 50 mg % respectively) the lymphopenia becomes progressively less (60% in group A, 70% in B and 75% in C). Comparison of A to C on the basis of the lymphocyte count at 60 minutes gives $P < 0.01$ indicating that the difference is statistically significant. On the other hand similar differences in hypoglycemia in the demedullated group (D and E) do not lead to statistically significant differences in lymphocyte count. These data suggest that in hypoglycemia the increased secretion of adrenalin contributes to lymphopenia. However further control experiments appeared in order. Therefore a final group of insulin tests was performed on adrenalectomized rats in which the count of red cells and lymphocytes, and the blood sugar were determined. As Table II shows adrenalectomized rats show only a very slight fall in lymphocytes and no characteristic changes in neutrophils. In view of the fact that the lymphocytes fell more in demedullated than in adrenalectomized animals subjected to insulin coma it is suggested that in addition to the lymphopenia initiated by the increased secretion of adrenalin some other effect of low blood sugar on the adrenal cortex occurs which leads to lymphopenia without the intervention of functional changes in the secretion of adrenalin. Here again the red blood count remained unchanged.

Our investigations show that hemorrhage, general ("emotional") excitement and, to a lesser extent, insulin hypoglycemia lead to a lymphopenia and a relative neutrophilia in normal but not in adrenodemedullated rats. As was shown previously similar changes may be induced by the injection of adrenalin. Since hemorrhage, general excitation and injection of adrenalin no longer cause these effects in adrenalectomized rats it must be concluded that emotional excitement and hemorrhage lead to an increased secretion of adrenalin which indirectly, probably through a decrease in the level of cortical hormones in blood and tissues (Sayers and Sayers,[6] Gellhorn and Frank), leads to a discharge of adrenocortical hormones resulting in lymphopenia. Insulin hypoglycemia causes a similar reaction but our experiment suggests an additional action of hypoglycemia on the hypophyseal-adrenocortical system since hypoglycemia acts much more on the lymphocyte count of adrenodemedullated than on that of adrenalectomized rats. This action appears to be the cause of the lesser difference between adrenodemedullated and normal rats under conditions of hypoglycemic coma than was found between these groups of animals subjected to hemorrhage and excitement. Observations by Parsons and collaborators[7] on the action of insulin hypoglycemia and injection of adrenalin on the blood count of schizophrenics agree with our present and previous experiments except for the fact that in these experiments lymphopenia is associated with an absolute neutrophilia whereas in our work on rats injection or secretion of adrenlin leads to lymphopenia with relative neutrophilia. However, this difference is probably accounted for by the fact that adrenalin in large doses causes lymphopenia and neutrophilia (Hungerford).[8]

Summary. Hemorrhage (1 cc/100 g body weight) and general excitement (induced by the action of a subconvulsive electrical shock) cause lymphopenia and relative neutrophilia in normal but not in adrenodemedullated rats. Insulin hypoglycemia leading to coma induces a greater lymphopenia in normal than in adrenodemedullated animals. It is concluded that hemorrhage, general excitement and insulin coma cause an increased secretion of adrenalin followed by an increased discharge of adrenocortical hormones. Hypoglycemia seems to induce some increased adrenocortical secretion even in the absence of the adrenal medulla.

[6] Sayers, G., and Sayers, M. A., *Endocrinology*, 1947, **40**, 265.

[7] Parsons, E. H., Gildea, E. F., Ronzoni, E., and Hulbert, S. Z., *Am. J. Psychiat.*, 1949, **105**, 573.

[8] Hungerford, G. F., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 356.

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