THE INFLUENCE OF PROTEIN-FREE LIVER AND SPLEEN EXTRACTS ON THE BLOOD REGENERATION AND RESPIRATORY EXCHANGE OF ANEMIC RABBITS.

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PLATE 30.

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I. INTRODUCTION.

This series of experiments is a continuation of the experimental work upon blood regeneration in rabbits. The purpose of the series was to gain further evidence of the nature of the action of the liver and spleen extracts upon blood regeneration in highly anemic animals as measured by means of heat production.

The respiratory exchange of anemic animals has been investigated by several authors as is shown in the reviews given by Meyer and Du Bois (1), and Tompkins, Brittingham and Drinker (2). With the exception of Bauer (3), most of the authors (4–6, 13) found the respiratory exchange within normal limits. Kraus, Chvostek and Bohland have reported metabolism values above or on the upper limit of normal. According to Grafe (11) and Eberstadt (12) in posthemorrhagic conditions there is a difference in the respiratory exchange depending on the state of the bone marrow. Anemic animals with normal bone marrow have shown normal metabolism and anemic animals with exhausted bone marrow have shown diminished metabolism. From these experiments Grafe concluded that active blood formation increases metabolism. Distinctly increased metabolism was found by Lukjanow (7) and Hári (8). Hári reported a 12 per cent increase after large hemorrhages in dogs. Two objections have been raised against these and similar experimental results: First that the animals were investigated too shortly after bleeding, and second, as pointed out by Plesh and Mohr (9), that the increased metabolism besides indicating a possible effect on the activity of blood-forming organs, might also arise from a more rapid respiration and heart rate.

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The results given by Hári, Lukjanow and Grafe have been partially confirmed by the experiments of Tompkins, Brittingham and Drinker. The latter authors found that blood transfusion in anemic states caused a diminution of metabolism, diminution of pulse and respiratory activity, a drop in temperature if it had previously been elevated, a rise in the red count and hemoglobin content. The effects were not due to a decrease in muscular activity, because the lowering of metabolism began some days after the transfusion. Therefore, they conclude that in anemic animals in addition to the increase in metabolism due to increased respiratory movement and heart activity there is evidently some type of stimulation of the body cells in general which increases metabolism.

The rôle of this stimulating factor was a primary concern in our experiments. We have sought further evidence of the existence of some kind of a stimulating factor for blood cell formation in protein-free extracts of liver substance by making simultaneous respiratory exchange determinations in addition to the blood investigations during the recovery of anemic animals.

In a second series of experiments the influence of a protein-free spleen extract (spleen Extract III, *b* prepared as well as the liver extract by Dr. Jobling of Columbia University) upon blood regeneration and respiratory exchange was studied.

II. Method.

The lightly ether anesthetised animals were bled either by heart puncture or from the carotid artery. Six-tenths of the whole blood was taken when possible. The total volume of blood was computed as 5 per cent of the body weight. This computation, however, does not give the same value for the blood volume in lean and in fat animals.

The extracts were freshly made by Dr. Jobling immediately before starting the experiments. A fresh 1 per cent solution was prepared each week from the dried extract. This was made up with sterile salt solution and kept in the ice box. 2 cc. of the solution (20 mg. dried substance) were given intraperitoneally to each animal on each Monday, Wednesday and Saturday. Measurements of the respiratory exchange were made each Tuesday and Friday.

Blood examinations (blood counts Hb. determination, reticulated cells, smears) were made before and after bleeding and once each week on the same day until complete recovery or death. A Leitz counting chamber and Sahli's hemoglobinometer were used.

At the end of the experiments the animals were sacrificed and the tissues examined histologically.

The respiratory exchange apparatus used was a modified Haldane. Each respiratory exchange measurement covered a 2 hour period. The animals were

TABLE I.

	Rabbit No.	Before bleeding. Percentages of						1 wk. after bleeding. Percentages of						2 wks. after bleeding. Percentages of						3 wks. after bleeding. Percentages of					
		Total white cells	Neutro.	Eosino.	Baso.	Mono.	Lympho.	Total white cells	Neutro.	Eosino.	Baso.	Mono.	Lympho.	Total white cells	Neutro.	Eosino.	Baso.	Mono.	Lympho.	Total white cells	Neutro.	Eosino.	Baso.	Mono.	Lympho.
Control animals (anemic)	1	10,000	20	1.6	0.4	15	63	6,400	23	0.5	1	4.5	71	8,400	20	2	—	7	71	10,200	47	—	3	9	41
	2	8,200	32.4	3	2	6	56.6	6,800	29	3	2	8	58	8,000	38	3	4	10	51	9,600	24	0.5	2	8.5	65
	3	12,200	24	2	—	14	60	10,200						12,400						14,200	34	1	3	12	52
Animals treated with liver extract (anemic)	4	7,900	10	4	—	7	70	8,800	60	2	—	8	30	9,600	36	1	2	11	50	12,600	29	—	2	6	63
	5	10,200	22.4	1	1	5.6	70	10,200	45	1	2	10	42	9,000	41	2	3	15	39	8,600	52	—	1	5	42
	6	11,600	29	1	4	16	50	20,000						10,400						18,000	38	2	3	10	47
Animals treated with spleen extract (Anemic)	7	10,600	28	1	2	20	49	9,800	16	8	2	12	62	11,200	5	4	1	7	83	11,600	6	8	—	14	72
	8	14,660	21	2	1	6	70	7,800	6	3	1	4	86	14,200	12	22	—	8	58	10,800	17	5	4	4	70
	9	5,800	39	1	2	15	52	12,600						8,000						10,400	19	6	2	12	61
(Normal)	10	12,200	32	2	3	14	49													11,800	25	4	6	15	50
	11	14,200	28	4	3	17	48													12,200	10	6	—	8	76

TABLE II.

Reticulated Cells.

	Rabbit No.	Before bleeding	1 wk. after bleeding	2 wks. after bleeding	3 wks. after bleeding
		per cent	*per cent*	*per cent*	*per cent*
Control animals (anemic)	1	1.0	7.2	9.6	6.9
	2	3.0	3.2	9.8	3.9
	3	1.4	4.0	16.0	3.0
Animals treated with liver extract (anemic)	4	1.4	15.0	10.0	3.7
	5	1.7	9.0	3.0	0.6
	6	1.8	11.2	9.2	0.4
Animals treated with spleen extract (Anemic)	7	0.9	3.7	2.6	3.5
	8	0.8	3.5	1.0	4.0
	9	1.1	3.0	3.0	4.0
(Normal)	10	1.7	—	—	1.2
	11	0.9	—	—	1.1

TABLE III.

Summary of the Respiratory Exchange Measurements.

	Average metabolism before bleeding		Average metabolism during 1st wk. after bleeding		Average metabolism during 2nd wk. after bleeding		Average metabolism during 3rd wk. after bleeding	
	Total calories in 2 hrs.	Calories per kg. per hr.	Total calories in 2 hrs.	Calories per kg. per hr.	Total calories in 2 hrs.	Calories per kg. per hr.	Total calories in 2 hrs.	Calories per kg. per hr.
Group I								
Control anemic animals untreated (3 cases)	15.27	3.06	12.88	2.64	13.53	2.71	13.84	2.71
Group II								
Anemic animals treated with liver extract (3 cases)	13.80	2.54	13.80	2.68	14.70	2.99	14.03	2.85
Group III								
Anemic animals treated with spleen extract (3 cases)	12.73	2.73	12.27	2.67	12.93	2.73	13.10	2.68
Group IV								
Normal animals treated with liver extract (2 cases)	13.10	2.25	15.03*	2.56	15.67	2.60	14.89	2.51
Group V								
Normal animals treated with spleen extract (2 cases)	12.41	2.91	12.39	2.80	13.13	2.97	11.53	2.62

* One animal pregnant.

fed for the last time 18 hours before the experiment. The standard diet (alfalfa hay, oats, with carrots or greens once a week) used in the laboratories of Montefiore Hospital was offered to the animals in equal amounts.

III. Presentation of Data.

Respiratory exchange measurements have been made on 9 anemic and 4 normal animals. For convenience of description the experi-

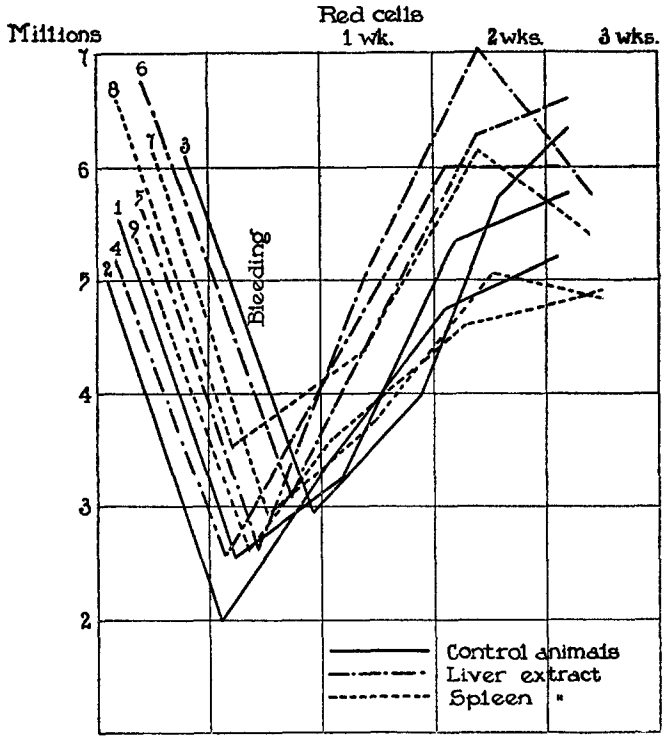

CHART 1.

ments have been grouped as follows: Group I comprises 3 anemic animals untreated and serving as controls; Group II includes 3 anemic animals treated with liver extract; Group III includes 3 anemic animals treated with spleen extract; Group IV includes 2 normal animals treated with liver extract; and Group V includes 2 normal animals treated with spleen extract.

A general summary of all the animals is given in Table III, in which the figures on heat production before bleeding represent the averages of 2 weeks readings while those after the bleedings represent the average weekly readings. The respiratory exchange measurements are also given in Charts 3 and 4. Chart 3 gives the data of the anemic

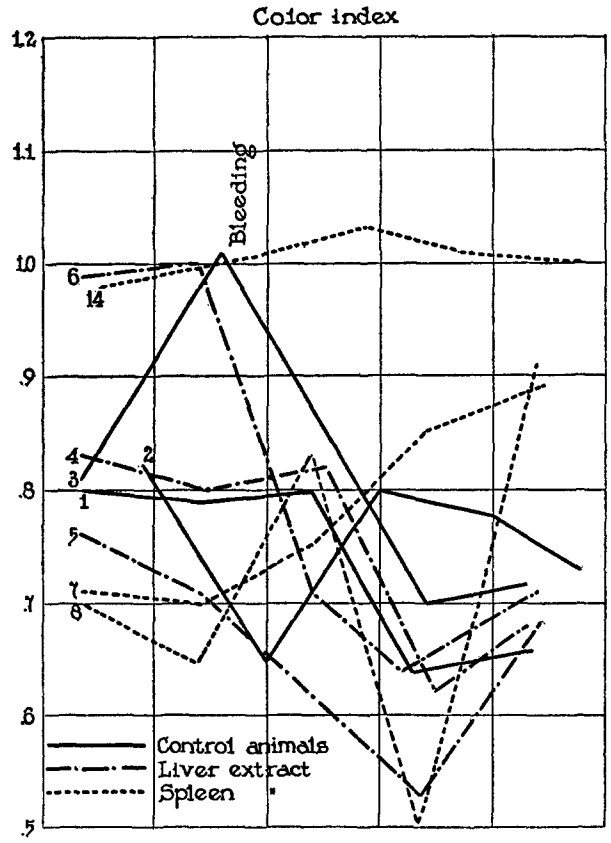

CHART 2.

animals and Chart 4 those of the normal animals. The number of red cells and color index of the anemic animals are given in Charts 1 and 2. The quantitative and qualitative changes in the white blood elements are shown in Table I. The figures of the reticulated cells of the anemic animals are contained in Table II.

IV. DISCUSSION.

A study of the respiratory exchange measurements of the five groups of experiments (see Table III) shows only slight differences in the total metabolism. There is a slight increase in the group of anemic animals treated with liver extract, while in those treated with spleen extract no notable change occurred.

The blood pictures, hematological and histological findings in all groups were approximately the same as those observed on a larger series of anemic rabbits, when we studied the question of blood regeneration under different conditions.

The anemic animals treated with liver extract showed a more rapid numerical restoration of the red blood cells, more reticulated cells, a lower color index and more prominent lymphoid tissue throughout the body than did the controls or those treated with spleen extract.

The slight increase in heat production observed during the period of blood regeneration in animals treated with liver extract coincides with and may be correlated with the hematological findings and suggests that liver extract exerts a stimulating effect on blood cell formation (Fig. 1). As to the nature or mode of action of this liver extract nothing definite can be said. In this connection we may recall that Joannovics and Pick (14) in 1909 obtained an alcohol and acetone-soluble hemolytic substance from the liver of animals treated with toluylenediamine and we know from experiments of others as well as from our own that the destruction products of the red cells do accelerate red blood cell formation. It may be possible too that in the liver extract a specific substance is present which stimulates blood cell regeneration.

Anemic animals treated with spleen extract showed that the numerical restoration of the red blood cells was not complete, while the heat production measurements indicated a possible slight decrease. Perhaps the spleen produces a substance inhibiting blood regeneration (see Chart 1 and Fig. 2), and in the meantime lowering the general metabolism (see Charts 3 and 4).

Richet (18) published in 1912 a paper in which he stated that splenectomized dogs need more food to keep their body weight in balance than normal animals. It is said that after splenectomy there

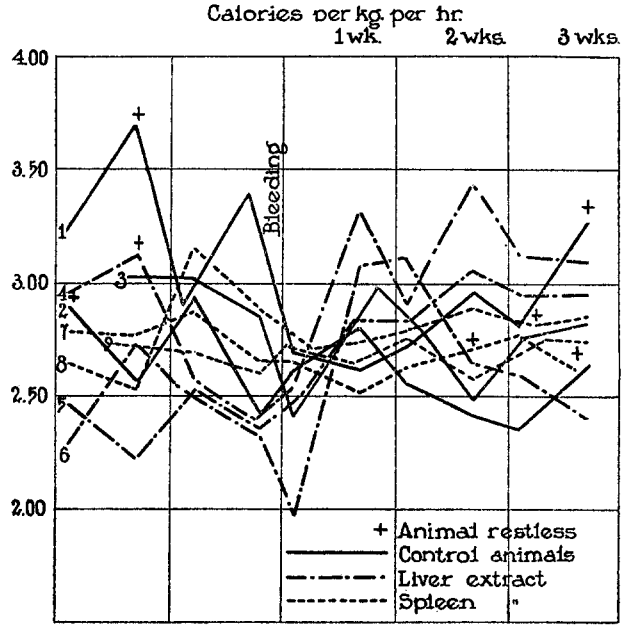

CHART 3.

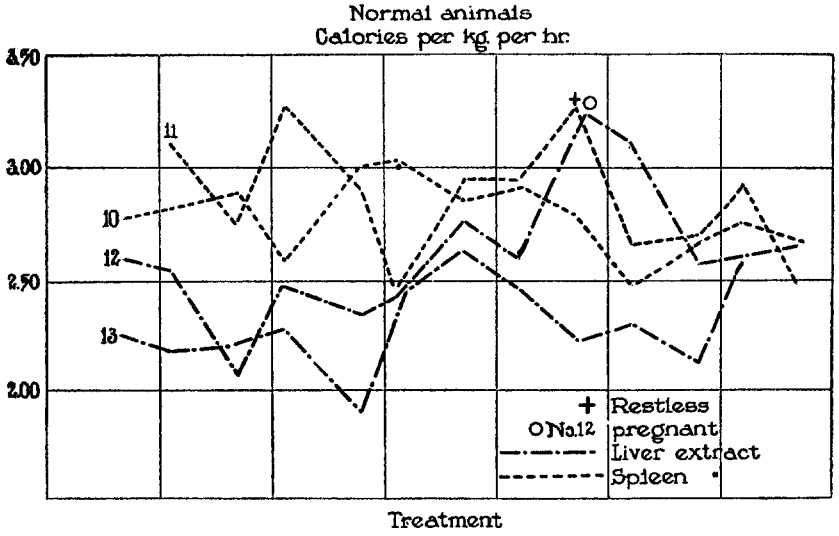

CHART 4.

is a hyperplasia of the thyroid gland. In cases of goiter, enlargement and fibrosis of the spleen is frequently seen. The possible antagonistic relation between the spleen and thyroid gland was first investigated by Asher and Streuli (19). They reported that after splenectomy, rats with intact thyroids are less resistant toward diminished partial pressure of oxygen than normal rats. After thyroidectomy, on the contrary, the rats became more resistant against asphyxia. After splenectomy in rats the basal metabolism of animals was found to be increased by Danoff (20), Asher and Hauri (21). Marine and Baumann (22) found no significant change in rabbits. We produced some sort of a "hypersplenia" with a protein-free spleen extract. We observed a fall in the basal metabolism both in normal and anemic animals treated with spleen extract. The blood cell regeneration was retarded also in these cases. 1 year after I finished my experiments M. and A. Leffkowitz (23) published their results, obtained with extracts from the spleen of anemic animals. This extract inhibited moderately red cell formation. The temperature curves of the several groups of experiments remain normal throughout the period of observation. The thyroid glands were more vascular and histological examination showed them to be slightly hypertrophic. This was undoubtedly true in those animals treated with liver extract. Such evidence of increased functional activity of the thyroid has long been noted in anemias, both in man and animals, and was formerly used in support of the view that the thyroid was directly concerned with blood formation (Fig. 3).

VI. SUMMARY AND CONCLUSION.

1. Respiratory exchange measurements may be used for gaining further evidence concerning the body changes during anemia, and are complementary to the hematological and histological data.

2. The heat production during anemia was slightly decreased. At the 3rd week it began to rise.

3. A protein-free liver extract has accelerated blood regeneration and at the same time increased the respiratory exchange of anemic animals.

4. A protein-free spleen extract has distinctly lowered the respiratory exchange of normal animals. The metabolism of anemic animals thus treated was practically the same as before bleeding and treatment.

The recovery of the spleen extract-treated animals was not complete during the period of observation. This extract may have been somewhat toxic. In spite of this disturbing factor we are not inclined to accept the view of C. D. and E. W. Leake (15) and Thalhimer (16) that the spleen takes part in the stimulating effect upon blood regeneration when given in combination with red bone marrow by mouth.

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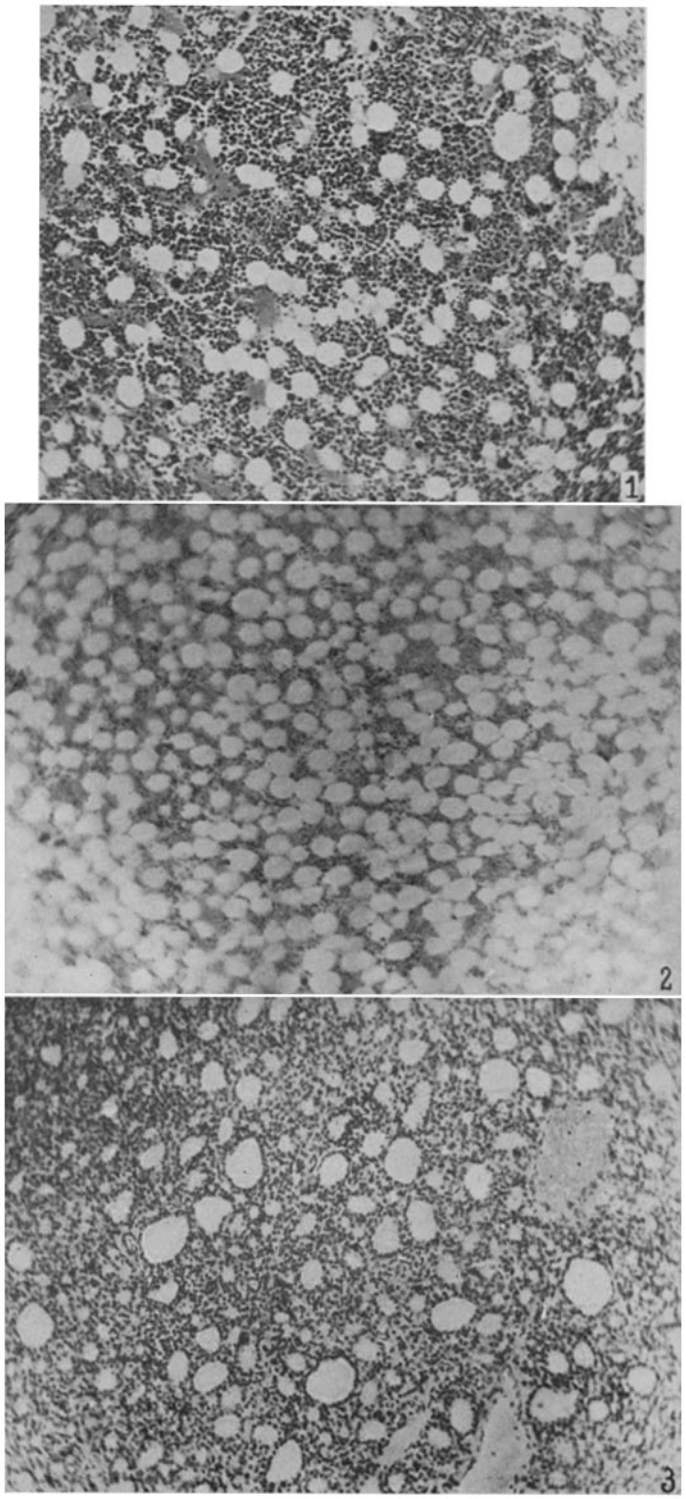
EXPLANATION OF PLATE 30.

FIG. 1. Bone marrow of Rabbit 5. Anemic animal treated with liver extract. 4 weeks after bleeding.

FIG. 2. Bone marrow of Rabbit 7. Anemic animal treated with spleen extract. 4 weeks after bleeding.

FIG. 3. Thyroid gland of Rabbit 5. Anemic animal treated with liver extract. 4 weeks after bleeding.



(Jeney: Blood regeneration and respiration.)