THE EFFECT OF PENTOXYL ON THE BONE MARROW LIPIDS OF RABBITS WITH EXPERIMENTAL LEUCOPENIA

E. N. Morozova

Department of Biological Chemistry (Head – Prof. S. N. Nedzvetskii), Leningrad Sanitary-Hygiene Medical Institute
(Presented by Active Member AMN SSSR S. V. Anichkov)
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The object of the present investigation was to study the changes in content of total lipids, phospholipids and cholesterol in bone marrow of rabbits with experimental leucopenia induced by Embichin, as affected by an increase in myeloblastic function of the bone marrow by pentoxyl – a stimulator of myelopoiesis [1].

EXPERIMENTAL METHOD

The experiments were carried out on rabbits 3-4 months old.

In the first series of experiments the injection of Embichin inhibited myelopoiesis in animals [2]. Embichin was injected intravenously daily in physiological solution at a dose of 0.3 mg/kg body weight. With the onset of distinct leucopenia accompanied by granulocytopenia, the injection of Embichin was stopped and pentoxyl was injected at a daily subcutaneous dose of 15 mg/kg body weight. When total leucocytes and granulocytes in peripheral blood attained a normal value, the animals were sacrificed. Twenty experiments in all were carried out with pentoxyl. Rabbits killed when acute leucopenia and granulocytopenia developed served as controls.

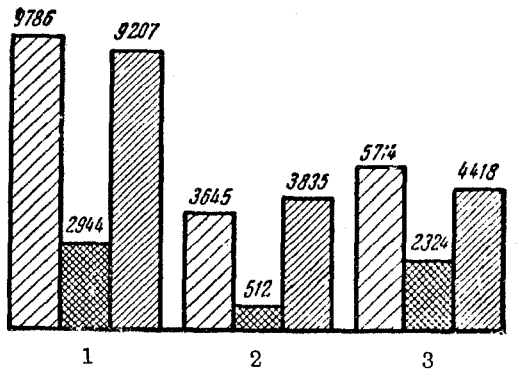
The bone marrow of normal rabbits (28 animals) was studied simultaneously.

The total bone marrow of the epiphysis and diaphysis of the femoral bones was removed for the investigation.

Extraction and determination of the lipids was carried out in the following manner. A weighed portion of ground bone marrow (from 2 to 3 g) was extracted at room temperature with ethyl alcohol, followed by ether, each for 1 hour; finally the marrow was extracted with a boiling mixture of chloroform and methanol (1: 1) for 4 hours and returned to the refrigerator. The solvent was boiled off from the combined alcohol-ether-methanol-chloroform extract on a water bath at reduced pressure and in a current of CO_2. The lipids were extracted from the half-dry residue obtained with petroleum ether. The solvent was boiled off from the petroleum ether extract in a current of CO_2 with reduced pressure. The residue obtained was dried in a vacuum desiccator over $CaCl_2$, after which the total lipids were determined by weighing. The total lipids were dissolved in a mixture of alcohol, ether, methanol and chloroform and the phospholipid and cholesterol content determined in specific parts of this extract. The phospholipids were precipitated and fractionated into a readily hydrolyzable fraction and a fraction hydrolyzable with difficulty according to the method of Hack [7]. The phosphorus of the total and readily-hydrolyzable phospholipids was determined after combustion with H_2SO_4 and $HClO_4$ according to the Fiske-Subbarow method. The phosphorus of the phospholipids hydrolyzable with difficulty was calculated by the difference between the content of phosphorus in the total and easily-hydrolyzable phospholipids. Cholesterol was determined according to the Schoenheimer and Sperry method.

EXPERIMENTAL RESULTS

Leucopenia developed in all rabbits (see figure) with injection of Embichin. Significant change in the lipid content of bone marrow was also noted.



Content of leucocytes, pseudoeosinophils and lymphocytes in blood of intact rabbits (average of 16 experiments), rabbits receiving Embichin (average 12 experiments) and rabbits receiving pentoxyl (average of 19 experiments). Light columns, intact rabbits; cross-hatched columns, rabbits receiving Embichin; densely striped columns, rabbits receiving pentoxyl. 1) Total number of leucocytes; 2) absolute content of pseudoeosinophils; 3) absolute content of lymphocytes.

As seen from the data presented in Table 1, total lipids decreased on the average to one-half the normal level, and furthermore in certain cases the level was extremely low (experiment No. 2). This decrease always accompanied an increase in water content. The dry residue in the bone marrow free from lipids changed only slightly, and decreased only in those cases where the number of leucocytes decreased to 1000 and less in 1 ml blood (experiment No. 4).

When pentoxyl was injected (Table 2) an increase in the bone marrow myelopoiesis was observed in all cases, with the exception of one (experiment 20), after only 3-4 days (see figure). The total leucocytes in peripheral blood increased 3.1 times on the average in comparison with leucocytes in Embichin leucopenia; at the same time the absolute number of granulocytes (pseudoeosinophils) increased 7.5 times, attaining a normal value.

A predominance of pseudoeosinophils over lymphocytes was observed in a number of cases. The condition of the animals during the whole period remained good.

The content of bone marrow lipids changed at the same time. The results obtained were very similar, so that in Table 2, data from only some of the experiments are presented.

As seen in Table 2, total lipids in the bone marrow of rabbits receiving pentoxyl varied from 23.9 to 48% and averaged 35.8%. The dry residue free from lipids varied within narrow limits, equalling 13.8% on the average. The phosphorus in the phospholipids averaged 32.7 mg %, while the phosphorous of phospholipids hydrolyzed with difficulty varied from 3.5 to 15 mg %, averaging 11 mg %.

Comparing the chemical compounds of the bone marrow of rabbits receiving Embichin and pentoxyl, it can be noted that under the influence of pentoxyl increase in the number of granulocytes (pseudoeosinophils) in the peripheral blood was accompanied by a 1.5-fold increase in total lipids, which almost reached the normal value. Such a significant increase in the amount of total lipids can originate only at the expense of neutral fat, since neutral fat comprises a large part of the bone marrow lipids.

When the development of leucopenia progressed (experiment No. 20) in spite of pentoxyl injection and the number of leucocytes in the peripheral blood decreased to 850,000 in 1 mm^3, the lipid content in bone marrow decreased. This change in fat content in bone marrow and concomitant disturbances of its erythroblastic function has been noted by a number of authors [3, 6, 8, 9].

These data indicate that the content of neutral fat in bone marrow is dependent on the condition of its hematopoietic function. It is possible that active metabolism of fat occurs in the bone marrow, since it has been established that synthesis of fatty acids from labeled acetate occurs in bone marrow homogenates [4, 5].

The phospholipid content of bone marrow varies with respect also to the state of myelopoiesis. In rabbits receiving pentoxyl, the phosphorus content of phospholipids in bone marrow increased 1.5-fold in comparison with the phosphorus content of this fraction during Embichin leucopenia. Furthermore, its value is higher than normal. It is known that phospholipds enter into the composition of formed elements. Consequently, it is possible that during restoration of myelopoiesis in the bone marrow, an accumulation of phospholipids takes place – phospholipids being a substrate necessary for the formation of formed elements.

The water content in the bone marrow of rabbits receiving pentoxyl varied widely. In normal animals, in Embichin-leucopenia animals and in animals injected with pentoxyl the water content was found to be in reverse proportion to the lipid content.

TABLE 1

Composition of Bone Marrow of Femoral Bones of Rabbits with Embichin Leucopenia, Not Receiving Pentoxyl (calculated on wet weight of tissue)

Expt. No.	Time of determination	Hemoglobin (in %)	Erythrocytes in blood (10^6)	Leucocytes in blood (10^3)	Wt. of animal (in g)	Weight of bone marrow (in g)	Water (in %)	Lipids (in %)	Dry residue, free from lipids (in %)	Phosphorus of phospholipids (in mg %)			Cholesterol, total (in mg %)
										total	easily hydrolyzed	hydrolyzed with difficulty	
1	Before expt.	68	4 540	7 300	1 720	3.24	68.3	19.7	12.0	22.7	16.2	6.5	113.6
	6 days after Embichin injection	58	3 720	2 900	1 650								
2	Before expt.	70	6 430	10 900	2 150	3.43	84.8	5.0	10.2	23.9	17.6	6.3	121.3
	6 days after Embichin injection	75	5 870	3 100	1 950								
3	Before expt.	70	5 460	9 720	1 850	2.71	55.1	33.7	11.2	28.6	22.8	5.8	97.0
	7 days after Embichin injection	72	5 290	2 700	1 670								
4	Before expt.	72	4 910	8 550	1 870	3.05	52.4	39.3	8.3	13.4	9.3	4.1	84.9
	7 days after Embichin injection	65	4 550	550	1 870								
Av. of 15 expt.	Before expt.	67	5 006	9 789	1 861	3,10 (2.34—4.07)	64.9 (51.3—84.8)	23,7 (5.0—39.3)	11.3 (8.1—14.6)	22.1 (13.4—29.9)	16.0 (9.3—24.4)	6.1 (2.1—8.3)	111.5 (84.9—133.2)
	After Embichin injection	67	4 640	2 944	1 748								
Control rabbits (average values of data obtained from 28 animals)		70	5 142	9 174	1 900	3.70 (2.10—5.68)	46.0 (38.9—68.8)	42.0 (17.0—50.7)	12.1 (9.2—14.2)	27.3 (20.6—33.2)	21.4 (15.5—26.5)	5.9 (2.22—8.68)	101.6 (45.0—137.8)

TABLE 2

Composition of Bone Marrow of Femoral Bones of Rabbits with Embichin Leucopenia, Receiving Pentoxyl (calculated on wet weight of tissue)

Expt. No.	Time of determination	Hemoglobin (in %)	Erythrocytes in blood (10^6)	Leucocytes in blood (10^3)	Weight of animal (in g)	Wt. of bone marrow (in g)	Water (in %)	Lipids (in %)	Dry residue, free from lipids (%)	Phosphorus of phospholipids (in mg%)			Cholesterol (in mg %)
										total	easily hydrolyzable	hydrolyzable with difficulty	
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Before the experiment	65	3 840	7 350									
	6 days after Embichin injection	65	3 740	1 425									
	3 days after pentoxyl injection	60	3 550	8 450	1 900	4.44	42.2	48.0	9.8	28.2	20.7	7.5	123.2
2	Before the experiment	55	4 240	6 750	1 650								
	6 days after Embichin injection	57	4 220	2 150	—								
	3 days after pentoxyl injection	55	4 045	6 575	—	2.49	51.0	33.5	15.5	45.9	38.7	7.2	—
3	Before the experiment	70	5 430	9 930	1 650								
	6 days after Embichin injection	58	4 560	4 725	—								
	3 days after pentoxyl injection	70	5 040	8 150	1 580	3.71	45.2	42.8	12.0	30.8	20.3	10.5	109.1
4	Before the experiment	69	5 080	7 650	1 750								
	6 days after Embichin injection	69	4 570	3 200	1 620								
	3 days after pentoxyl injection	61	4 645	8 125	—	3.35	63.6	23.9	12.5	36.45	—	—	112.8
5	Before the experiment	73	5 315	10 475	1 620								
	6 days after Embichin injection	67	4 660	2 575	1 570								
	3 days after pentoxyl injection	66	5 220	14 450	1 550	2.16	47.3	39.1	13.6	36.1	21.4	14.7	76.3
6	Before the experiment	71	5 350	7 400	1 900								
	6 days after Embichin injection	67	5 240	2 925									
	3 days after pentoxyl injection	84	5 115	10 850	1 930	2.41	52.6	31.8	15.6	37.4	33.9	3.5	62.1

(concluded on next page)

TABLE 2 (concluded)

1	2	3	4	5	6	7	8	9	10	11	12	13	14
7	Before the expt.	67	4 870	9 625	1 920								
	6 days after Embichin injection	66	4 775	2 225	1 900								
	3 days after pentoxyl injection	69	5 210	6 900	1 950	3,28	59,1	25,4	15,5	36,0	20,9	15,1	105,2
8	Before the expt.	79	5 080	9 325	2 300								
	6 days after Embichin injection	74	4 810	1 550	—								
	3 days after pentoxyl injection	70	4 675	9 275	2 320	4,67	40,1	46,3	13,6	24,9	14,2	10,7	102,8
	Av. value of 19 expts.	70	4 910	9 207	1 878	3,07	50,4 (40,1—63,6)	35,8 (23,9—48,0)	13,8 (9,8—15,7)	32,7 (24,9—45,9)	21,7 (13,9—38,7)	11,0 (3,5—15,0)	99,5 (62,1—125,1)
20	Before the expt.	76	5 110	13 850	2 180								
	6 days after Embichin injection	65	5 100	3 250									
	3 days after pentoxyl injection	65	5 005	850	1 800		71,9	19,6	8,5	13,4	8,8	4,6	108,1

Note: The minimum and maximum values are presented in parentheses.

On the basis of data obtained, it can be concluded that when pentoxyl is injected into rabbits with Embichin leucopenia, an increase of granulocytes in the peripheral blood is accompanied by an increase in the total lipids in the bone marrow mainly at the expense of the neutral fat fraction and the phospholipids. It can be assumed that these changes are related to the myeloblastic function of bone marrow.

SUMMARY

A rise of the total lipid content was noted in the bone marrow of rabbits with Embichin leucopenia during pentoxyl administration with the rise of the pseudoeosinophil count (granulocytosis) in the peripheral blood. The rise of the lipid content occurs mainly at the expense of the increase of the neutral fat and phospholipid fraction. It may be suggested that these changes occur in connection with the bone marrow myelopoiesis.

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