INFLUENCE OF ACETYLCHOLINE AND ADRENALINE ON THE HEMATOPIETIC CELLS OF THE BONE MARROW IN A TISSUE CULTURE

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Previous papers [1, 2, 3,] have indicated the influence of the nervous system on the course of the blood-forming process in the bone marrow. The presence of nerve receptors [4] in bone marrow tissue confirms the possibility of direct influence by the nervous system on bone marrow blood formation. In this connection it is without question necessary to take into account the influence of the products of excitation of the vegetative nervous system on the blood-forming process.

The theory of the chemical nature of nervous excitation is an integral part of the general theory on the tropic influence of the nervous system, a theory based on the works of L. M. Sechenov, S. P. Botkin, and I. P. Pavlov. It is necessary to consider that the regulatory influence of the nervous system on blood formation is also effected by means of liberated humoral executants of nervous influences. From the literature it is known that denervation of the organs produces in them considerable sensitization to the mediators. A similar manifestation of hypersensitization of the blood-forming organs in relation to the mediators after denervation of the bone marrow may also occur.

In the present work the object was to study the biological properties of the bone marrow cells in tissue cultures upon subjecting them to the action of acetylcholine and adrenaline. In a special series of investigations the influence exerted on the isolated cells of the previously denervated bone marrow by sympathicotropic and parasympathicotropic substances obtained by chemical means was studied.

The work was performed on the cat bone marrow. Denervation was carried out according to the method described by us in the previous papers [1, 2].

Cultures of the bone marrow were placed as a hanging drop on a glass cover and also in Carrel dishes. In the first case the substances under study (acetylcholine and adrenaline) were added to the nutrient plasma, in the second to the liquid phase used to wash the specimens of the bone marrow.

The effect of various attenuations of acetylcholine and adrenaline was studied. However, taking into account the diphasic action of adrenaline it was used only at large attenuations (1:1,000,000). Acetylcholine and adrenaline were diluted in Ringer solution.

The cultures were then studied in a living and fixed state. At various times of cultivation, smears from the central portions of the cultures were prepared according to the method of A. D. Timofeevsky. Before setting up the cultures the cellular elements of the bone marrow were calculated, and the cells in the smears prepared from the cultures at various times of explantation were calculated.

The material was fixed by methyl alcohol and stained with azure II eosin.

Study of the culture of the normal bone marrow on addition of acetylcholine showed that it creates favorable conditions for the development of bone marrow cells in the tissue culture. At various times of cultivation, cells were constantly found at all stages of differentiation. In the granulopoietic series a certain stimulation of the processes of differentiation was noted. This was expressed in intensified formation of myelocytes and particularly rod-nuclear and segment-nuclear granulocytes.

TABLE 1

Influence of Acetylcholine and Adrenaline on the Blood Forming Cells of the Bone Marrow in Tissue Cultures Adrenaline*

Designation of cells	Bone marrow in %	Bone marrow cultures								
		control	5 hours after introduction in %		control	18 hours after introduction in %		control	48 hours after introduction in %	
			acetyl-choline	adrenaline		acetyl-choline	adrenaline		acetyl-choline	adrenaline
Reticular	0.25	–	–	–	–	–	–	–	–	–
Hemocytoblasts	0.5	0.25	–	–	–	10.0	–	–	1.0	1.0
Myeloblasts	2.75	4.0	1.5	2.25	0.5	2.5	1.0	2.5	3.0	1.5
Myelocytes neutrophils	7.5	6.5	12.75	9.25	10.0	12.0	8.0	17.0	13.0	13.5
Myelocytes eosinophils	1.5	1.25	2.0	0.5	3.0	3.0	1.5	2.5	1.5	–
Young neutrophils	17.5	29.25	29.25	18.0	17.0	23.0	20.5	22.0	20.0	17.0
Young eosiniphils	1.5	0.5	0.5	0.25	4.0	1.0	0.5	14.5	2.0	1.0
Rod nuclear neutrophils	6.0	27.75	39.5	19.0	29.5	32.5	20.5	20.0	25.0	24.0
Rod nuclear eosiniphils	3.75	1.5	2.5	4.0	4.5	2.0	2.5	2.5	2.5	1.0
Segment neutrophils	0.25	1.25	1.5	1.0	3.0	8.0	3.5	0.5	9.5	5.0
Segment eosiniphils	0.5	0.25	–	–	3.0	–	–	–	1.0	1.5
Lymphocytes	12.5	7.75	0.5	3.75	3.0	1.0	0.5	2.0	2.5	4.5
Megacariocytes	1.25	0.75	–	–	1.5	–	–	–	0.5	0.5
Plasmatic	1.25	1.25	1.25	3.25	2.5	2.0	6.0	2.5	1.5	3.5
Polyblasts-macrophage	2.5	8.5	3.25	34.0	9.0	4.0	30.5	3.75	7.5	22.5
Proerythroblasts	1.5	2.75	1.25	0.5	1.0	2.0	–	–	2.5	–
Basophil erythroblasts	1.0	2.25	2.0	1.0	1.5	3.0	1.0	0.5	–	0.5
Polychromatophil erythroblasts	2.5	1.5	1.0	3.0	0.5	0.5	0.5	–	1.5	0.5
Normoblasts	5.5	1.75	1.25	0.25	1.5	2.0	3.5	4.5	1.0	2.5
Total white cells	83.75	80.75	90.0	58.0	77.5	85.0	58.5	89.5	81.5	69.0
Total red cells	10.5	8.25	5.5	4.75	4.5	7.5	5.0	5.0	9.0	3.5

* Acetylcholine and adrenaline at an attenuation of 1:5,000,000

In the erythropoietic series a considerable increase in the number of cellular elements was observed. This increase may be due to both an intensification of the proliferating capacity of the cells under the influence of acetylcholine and to a certain retardation in their maturation which was noted by us in comparison with the control and the cultures with added adrenaline. With the purpose of illustrating these points, the results of calculations of the cellular elements in one experiment are presented in Table 1.

Upon addition of acetylcholine a reduction of the degenerative processes in the cultures was also constantly observed in the bone marrow culture. The dystrophic changes in the cellular elements in this case were always less than in the cultures with added adrenaline, and in certain experiments even less pronounced than in the control cultures (see Table 2).

TABLE 2

Experiment No. 11

Correlation of Red and White Cells and Number of Degenerating Cells In Bone Marrow Cultures

Time of cultivation	Object of investigation	Correlation of number of red and white cells		Number of degenerating cells per 100 normal ones
		% of red cells	% of white cells	
5 hours	Control culture	32.0	68.0	9.0
5 hours	Addition of acetylcholine	33.5	66.5	6.0
5 hours	Addition of adrenaline	29.5	70.5	14.0
1 day	Control culture	29.5	70.5	40.0
1 day	Addition of acetylcholine	21.0	79.0	38.5
1 day	Addition of adrenaline	17.0	83.0	61.0
2 days	Control culture	12.5	81.5	50.0
2 days	Addition of acetylcholine	23.5	76.5	59.0
2 days	Addition of adrenaline	9.0	91.0	79.0

Table 2 shows that addition of adrenaline to the bone marrow cultures produced a considerable intensification of the degenerative processes in the blood-forming cells in comparison with the cultures with added acetylcholine and especially in comparison with the control cultures. A striking feature in these cultures was the considerable activation of formation of polyblasts-macrophagocytes, the number of which in bone marrow cultures with addition of adrenaline greatly exceeded the number of macrophagocytes in the cultures with addition of acetylcholine and in the control cultures (see Table 1). In the cultures to which adrenaline was added there was a certain acceleration of maturation of cellular elements of the erythropoietic series, manifest in an increased number of normoblasts in such cultures and also in the presence in them of a large number of normoblasts with ejected nuclei.

In the experiments on the effect of acetylcholine and adrenaline on the blood-forming cells of the previously denervated bone marrow, analogous but more clear-cut findings were obtained.

Study of the cultures of the denervated bone marrow showed that denervation makes the blood-forming elements more sensitive to the action of acetylcholine and adrenaline (Table 3).

In the denervated bone marrow under the influence of adrenaline, degenerative processes in the cells were even more sharply marked. At the same time with the effect of acetylcholine these processes were less intensive. In the denervated bone marrow tissue cultures, to which adrenaline was added, a greater increase in the number of erythropoietic cellular elements than in the normal bone marrow cultures was observed. Maturation of the cells of the granulopoietic series proceeded here at a faster rate as a result of which an increased number of rod-nuclear and segment-nuclear granulocytes was observed. The cultures of the denervated bone marrow upon addition of acetylcholine constantly showed themselves more viable.

Study of the denervated bone marrow cultures with addition of adrenaline showed, apart from strongly marked degeneration of the bone marrow cells, greater development of polyblasts-macrophagocytes.

TABLE 3

Experiment No. 12

Influence of Acetylcholine and Adrenaline on Cells of Denervated and Normal Bone Marrow in Tissue Cultures

	bone marrow		Bone marrow culture											
			After 3 hours						After 1 day					
	Normal	Denervated	normal	adding acetyl-choline	adding adrena-line	de-nerva-ted	adding acetyl-choline	adding adrena-line	normal	adding acetyl-choline	adding adrena-line	de-nerva-ted	adding acetyl-choline	adding adrena-line
Total white cells in %	32,5	78,5	85,0	86,0	79,0	81,0	79,0	72,0	86,0	72,0	78,0	91,0	70,5	74,5
Total red cells in %	14,0	14,5	5,0	12,0	9,0	6,0	17,0	6,0	10,0	16,0	9,0	6,0	23,5	8,0
Number of degenerated cells per 100	18,0	20,5	22,0	9,0	23,0	37,0	13,0	30,0	26,0	13,0	32,0	34,0	30,0	53,0

The experiments presented thus show the important influence of acetylcholine and adrenaline on the course of development of the blood-forming cells of the bone marrow.

Acetylcholine at attenuations from $1:5\times10^{-6}$ to $1:5\times10^{-7}$ exerted a stimulating influence on the viability of the bone marrow cells in the tissue culture. Besides a favorable effect on the development of young cells this was also manifest in a considerable reduction in the degenerative changes in the cellular elements.

Adrenaline at attenuations from $1:5\times10^{-7}$ to $1:10^{-8}$ in general impairs the state of the bone marrow cells, expressed in an intensification of the degeneration processes in the blood-forming cells of such cultures.

At the same time in the adrenaline cultures a considerable activation of formation of polyblasts-macrophagocytes was observed.

Our experiments enable one to conclude that acetylcholine and adrenaline have a direct influence on the biological properties of the blood-forming cells of the bone marrow (survival capacity in the tissue culture, and growth and maturation ability).

Our findings may also be used in the study of the problem of chemical mediators in the transmission of nervous impulses.

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