EFFECT OF ADRENALIN AND ACETYLCHOLINE ON THE PHAGOCYTIC FUNCTION OF THE LEUKOCYTES IN ONTOGENESIS

F. F. Shchastnyi

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Most authors recognize that the nervous system can regulate the activity of the ingestive stage of phagocytosis and adjust its intensity in accordance with changes in the conditions of the external and internal milieu of the organism [1,5,9].

Investigations carried out in recent years have demonstrated the influence of mediators and autonomotropic substances on the digestive stage of phagocytosis also in the leukocytes of the adult organism [10,12].

The influence of the nervous system on phagocytic activity is effected through the sensitivity of the leukocytes to adrenergic agents stimulating phagocytic function, and to cholinergic agents with an inhibitory action on phagocytic function [6,8,10-12].

No information can be found in the literature regarding whether the sensitivity of the leukocytes to autonomotropic substances is present at all periods of life or whether it is absent in the early stages of ontogenesis and appears in the process of development and age differentiation of the systems and functions of the organism. In face of ideas concerning the evolution of the nervous system and certain findings in relation to age changes in the influence of the nervous system of other functions of the body, the second of these views may be regarded as most likely [2,4,7].

The object of this investigation was to determine the times of appearance of sensitivity of the leukocytes to adrenalin and acetylcholine.

EXPERIMENTAL METHOD

Most of the investigations were conducted on the leukocytes of rats which are born relatively less mature than other laboratory animals. Altogether 81 young rats were investigated at the age of 5-10 h and 3 and 6 days. All the young rats were investigated at once. Each litter was divided into three groups so that the age changes in the adrenergic and cholinergic reactivity of the leukocytes could be studied in animals from the same mother.

To determine to what extent the principles discovered in the rats may be extended to animals born in a more mature state, a series of investigations was carried out on the leukocytes of puppies aged 5-10 h and 5 days. Some of the puppies were investigated twice, the rest once. Altogether 42 puppies were studied. The method of V. N. Berman and E. M. Slavskaya [2] was used. A suspension of leukocytes in Ringer's solution (pH 7.3-7.4) was prepared from 0.3 ml of the peripheral blood of the investigated animal. The suspension was poured into experimental and control tubes. Adrenalin or acetylcholine was added to the experimental tube and Ringer's solution to the control tube. The final concentrations of adrenalin and acetylcholine were $1 \cdot 10^{-9}$ and $1 \cdot 10^{-8}$ g/ml, respectively. After addition of a suspension of a nonpathogenic serotype of Escherichia coli in a concentration of 180 million microorganisms/ml, the tubes were incubated for 40 min at 37°, after which their contents were centrifuged and the residue of leukocytes was used for seeding Petri dishes with 1.5% agar. The microorganisms were grown on agar for 2 h.

During the first minutes of growth and after this period, impressions were taken from the agar on glass slides, and these were fixed with Nikiforov's mixture and stained by the Romanovsky-Giemsa method. The impressions taken during the first minutes of growth were used to study the intensity of phagocytosis in the ingestive stage. The number of microorganisms ingested by 200 neutrophils was counted. The intensity of phagocytosis in the final stage was determined from the impressions made after subculture. By carrying out subculture on agar for 2 h, it was possible to distinguish with certainty between the leukocytes in the cytoplasm remaining capable of metabolism and

Laboratory of Pathophysiology, Institute of Pediatrics, Academy of Medical Sciences of the USSR, Moscow. Director of Research N. V. Puchkov (Presented by Active Member of the Academy of Medical Sciences of the USSR V. V. Parin). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 62, No. 9, pp. 76-79, September, 1966. Original article submitted May 15, 1965.

TABLE 1. Effect of Adrenalin and Acetylcholine on Ingestive and Digestive Stages of Phagocytosis in Neutrophils of Newborn Rats

Preparations	Index	Bacterial cells ingested by 200 neutrophils						Leukocytes completing phagocytosis (in %)					
		1 day		3 days		6 days		1 day		3 days		6 days	
		Control	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control	Experiment
Adrenalin	Number of investigations	25	25	11	11	10	10	18	18	10	10	10	10
	M ± m	53,48±4,93	62,52±5,75	53,09±2,76	74,63±5,40	57,00±3,55	81,30±4,79	28,13±1,57	31,84±1,78	41,35±3,05	44,33±2,71	48,83±1,73	59,85±1,91
	Difference between experimental and control series	+9,04		+21,54		+24,30		+3,7		+2,98		+11,02	
	Difference (in %)	+16,4		+40,59		+42,56		+13,5		+7,23		+22,56	
	t	1,18		3,57		4,27		1,55		0,73		4,30	
	P	≈0,25,		<0,01		0,002		0,25		>0,25		0,002	
Acetylcholine	Number of investigations	11	11	15	15	10	10	11	11	15	15	10	10
	M ± m	49,54±8,44	38,63±7,87	68,60±4,18	50,53±4,10	68,80±3,31	46,56±3,08	28,05±1,94	24,84±1,82	27,42±1,78	21,69±1,80	50,33±1,64	42,18±1,25
	Difference between experimental and control series	−10,91		−18,07		−22,3		−3,21		−5,73		−8,15	
	Difference (in %)	−21,9		−26,55		−32,7		−11,8		−20,8		−16,19	
	t	0,97		3,17		4,93		1,22		2,3		3,43	
	P	>0,25		<0,01		<0,002		≈0,25		0,05>P>0,02		<0,002	

TABLE 2. Effect of Adrenalin and Acetylcholine on Ingestive and Digestive Stages of Phagocytosis in Neutrophils of Newborn and Adult Rats

Preparations	Index	Bacterial cells ingested by 200 neutrophils						Leukocytes completing phagocytosis (in %)					
		1 day		5 days		adults		1 day		5 days		adults	
		Control	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control	Experiment	Control	Experiment
Adrenalin	Number of investigations	10	10	11	11	7	7	10	10	11	11	7	7
	M ± m	30,40±3,82	49,10±3,63	47,91±3,94	74,00±4,57	88,77±5,40	122,8±7,10	29,48±1,57	32,25±1,78	28,49±2,2	36,43±2,13	58,88±2,02	67,18±2,00
	Difference between experimental and control series	+18,7		+26,9		+34,15		2,77		+7,94		+8,20	
	Difference (in %)	±61,51		+54,35		±38,7		±10,6		+27,8		+16,8	
	t	3,57		4,35		3,85		2,08		2,74		2,86	
	P	<0,01		<0,002		<0,01		>0,5		0,02>P>0,01		0,05>P>0,02	
Acetylcholine	Number of investigations	12	12	9	9	7	7	10	10	11	11	7	7
	M ± m	50,50±4,77	32,50±3,20	48,60±3,64	30,20±3,27	88,77±3,40	59,0±5,84	11,58±1,18	13,05±0,84	26,73±1,90	18,89±1,80	58,88±2,02	52,21±1,78
	Difference between experimental and control series	−18,0		−17,9		−29,71		+1,47		−7,84		−6,57	
	Difference (in %)	−32,7		−36,8		−33,5		+12,4		−28,7		−11,15	
	t	3,16		4,76		3,74		1,20		2,93		2,49	
	P	<0,01		<0,002		<0,01		>0,25		<0,01		0,05>P>0,02	

phagocytosis of microorganisms and dead leukocytes. The result was expressed as the number of neutrophils completing phagocytosis in 100 phagocytic cells. Altogether 200 neutrophils were investigated in the impression.

EXPERIMENTAL RESULTS

The results of the investigations conducted on the leukocytes of the rats in the neonatal period of life are given in Table 1. This table shows that, in the rats during the first 24 h after birth, despite some difference in the mean results in the experimental and control series, no significant sensitivity of the leukocytes exists to the concentrations of the autonomotropic substances used.

The effect of adrenalin and acetylcholine on the ingestive activity of the neutrophils of the rats appeared between 1 and 3 days, and on the final stage of phagocytosis, between 3 and 6 days after birth.

In the puppies the effect of the autonomotropic substances on the ingestive stage of phagocytosis was discovered after the first day of life (Table 2). In the newborn puppies, the autonomotropic substances had no effect on the final stage of phagocytosis of the neutrophils. It appeared about the fifth day after birth.

The absence, followed by the appearance and the increase in intensity of this influence of adrenalin and acetylcholine on the phagocytic function of the leukocytes in mammals in the neonatal period of life demonstrate the immaturity of the final links in the chain of nervous reflex influence of the body on phagocytic protection in the first days after birth.

The facts discovered demonstrate the profound functional modification of the leukocytes in the early period after birth and the considerable approximation of their physiological properties at this time to the properties observed in the adult.

These changes reflect important age changes in the regulatory, reactive, and protective powers of the developing organism in the neonatal period of life. The times of appearance of the influence of autonomotropic substances on the phagocytic function of the leukocytes suggest that this phenomenon is closely linked with the processes of adaptation of the body to the conditions of postnatal life.

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