CHANGES IN RESISTANCE OF SKELETAL MUSCLE TO CHLORAL HYDRATE DURING DEVELOPMENT OF IMMUNITY TO TUBERCULOSIS IN ALBINO MICE AFTER VACCINATION

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Experiments on albino mice vaccinated with BCG showed that vaccination modifies the response to nonspecific chemical (chloral hydrate) action of skeletal muscle resistance to tuberculosis. The changes discovered take place not only in cell systems responsible for excitability, but also in the contractile protein of the muscles.

Investigations conducted in the department of experimental pathology of the Leningrad Research Institute of Tuberculosis have shown that in response to BCG vaccination changes develop in the functional state of the systems of the body and of its organs and tissues [2-5]. Initially these changes have the character of a chaotic, and sometimes uncoordinated, yet reversible reaction of injury or stimulation. The response gradually subsides, and the corresponding indices return to their initial level [4].

Determinations of the affinity of tissues for the vital dye neutral red at various times after vaccination of mice [8-10] and guinea pigs [11] have revealed marked changes in the sorption properties of the cells of both immunologically competent and immunologically intact organs.

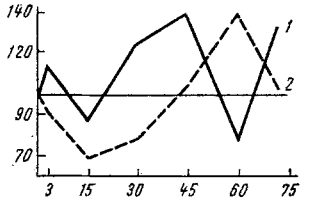

Fig. 1

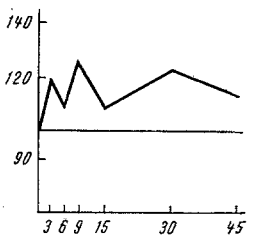

Fig. 2

Fig. 1. Period of contraction of muscles of albino mice (1) and of glycerol models of the muscles (2) in 1% chloral hydrate solution at various times after vaccination. Here and in Fig. 2: Abscissa, days after vaccination; ordinate, period of survival (in percentages of control).

Fig. 2. Duration of survival of muscles of albino mice in 0.5% chloral hydrate solution after vaccination.

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TABLE 1. Changes in Sorption Properties of Skeletal Muscles of Vaccinated Mice after a Stay of 25 min in 0.5% Chloral Hydrate Solution (in % of initial level; n = 8)

Time after exposure (in min)	Statistical index	Day after vaccination							
		3rd	6th	9th	15th	30th	45th	60th	75th
15	M± m	119,4± 10	70,3± 6,4	89,4± 8,4	130,9±7,2	110,4±5,2	105,2±6,1	82,6±9,7	99,8±6
	P	>0,05	>0,05	>0,1	< 0,05	>0,1	>0,05	>0,1	—
30	M± m	85,1± 5	128,2± 5,5	82± 3,8	100	80,0±4,5	88,2±7,2	66,9±4	112,6±7,4
	P	<0,05	<0,05	>0,05	—	>0,05	>0,05	<0,05	>0,05
60	M± m	87± 4,6	126,4± 7,2	81,4± 4,9	111,9± 6,1	97± 3,6	79± 13	103,3± 3,9	110,3± 8
	P	>0,05	<0,05	>0,1	>0,05	—	>0,1	>0,5	>0,1
120	M± m	101± 14,5	97,2± 7,7	100	109,1± 13	104,5± 3,8	100	104,7± 9,5	113± 4,9
	P	—	>0,5	—	>0.5	>0.5	—	>0,5	>0,1

The object of the present investigation was to determine the resistance of immunologically intact skeletal muscles of albino mice to chemical action nonspecific in nature as regards tuberculosis, and to examine its changes during the development of immunity to tuberculosis after vaccination.

EXPERIMENTAL METHOD AND RESULTS

The effect of a 1% solution of chloral hydrate (a cell narcotic with a well studied mechanism of action) was investigated 3, 6, 9, 15, 30, 45, 60, and 75 days after subcutaneous injection of BCG vaccine into mice weighing 16-18 g in a dose of 1 mg. The duration of survival of the muscles was determined from their contraction in response to electrical stimulation. In each experiment 8 experimental mice and 8 intact mice were investigated.

The resistance of the isolated anterior tibial muscles to the action of chloral hydrate showed phasic changes, with an increase on the 30th, 45th, and 75th days (by 24, 39, and 31%, respectively) and a decrease on the 60th day after immunization by 22.5% (Fig. 1).

To determine the degree of participation of the contractile system of the muscles in the reaction, the effect of chloral hydrate was studied on glycerinized muscle "models," prepared by Szent-Gyorgyi's method by treating muscles with 45% glycerol solution in the cold [1]. Preservation of contractile function of the glycerinized fibers was determined from their contraction in the presence of ATP.

Control experiments with the muscles of intact animals showed that the contractile function persists longer than the function of excitability: the muscles ceased to respond to electrical stimulation after 15-20 min in 1% chloral hydrate solution, while the "models" ceased to contract in the presence of ATP after 32-86 min in the solution. This phenomenon was also observed in the vaccinated mice. After exposure to chloral hydrate the contractile power of the muscles persisted longer than their electrical excitability. Ushakov [6, 7], who studied the thermostability of whole muscle fibers and the thermostability of the contractile system of the muscles separately, found that the decisive role in the termination of the response to electrical excitation is played not by the contractile protein, but by the more thermolabile soluble proteins of the muscle cell cytoplasm.

Apparently the main factor terminating the response to electrical stimulation of the muscle when acted upon by chloral hydrate likewise is not a contractile protein. In confirmation of this statement, all the muscles still remained capable of forming "models" in the present experiments after they had ceased to respond to electrical stimulation.

Phasic changes in resistance to chloral hydrate (Fig. 1) were found in the contractile system of the skeletal mus-

cles of the vaccinated mice, just as in the muscles themselves: weakening of resistance on the 15th day by 32%, and an increase in the resistance of 39% on the 60th day of immunization. As these results show, the changes in resistance of the contractile system of the muscles to chloral hydrate did not always correspond to the changes in resistance of the whole muscles.

Because of the short period of survival of the muscles when treated with 1% chloral hydrate solution, the action of a weaker stimulus had to be determined. For this purpose, experiments were carried out with 0.5% chloral hydrate solution. The period of survival of the muscles of the intact mice in 0.5% chloral hydrate solution varied from 33.3 to 40.8 min. The muscles of the vaccinated mice survived longer than those of the intact mice: on the 3rd day of immunization by 19%, on the 9th day by 25%, and on the 30th day by 23% (Fig. 2).

To study reactivity, the changes in vital staining (0.2% neutral red solution for 5 min) 15, 30, 60, and 120 min after the end of the reversible effect of 0.5% chloral hydrate solution (25 min) were determined. The sorption of neutral red by the muscles of the intact mice showed no statistically significant change after treatment with chloral hydrate. On the 6th and 15th days of immunization, an increase in sorption was found (Table 1), indicating a response of excitation or injury to the stimulus in accordance with Nasonov's denaturation theory. This reaction is readily reversible: no changes in staining properties were observed after 1-2 h. Characteristically, in the period of increased resistance of the muscles to chloral hydrate, when the survival period was increased, fixation of the dye was unchanged or was actually weakened (3rd day). Weakening of ability to fix the dye was also found on the 60th day after vaccination.

The results described above demonstrate changes in the functional state of the cells of a skeletal muscle rendered resistant to tuberculosis as the result of vaccination, and these changes lead to changes in nonspecific reactivity.

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