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EFFECT OF IMMUNOSTIMULATORS ON MOUSE PERITONEAL EXUDATE MACROPHAGES ANALYZED BY MATHEMATICAL MODELING AND PLANNING METHODS

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Biologically active substances of varied chemical nature, isolated from plants, animals, and bacteria, and also artificially synthesized, possess immunostimulating properties [2]. Immunostimulators (IS) induce profound and lasting transformations in the plasma membrane of cells of the mononuclear phagocytic system. This is shown, in particular, by changes in the phospholipid layer of the membrane [6], and also in the activity of 5'-nucleotidase (EC 3.1.3.5) one of the principal enzymes of purine catabolism, which is a marker of the cytoplasmic membrane [4]. It is accordingly interesting to study IS by assessing their effect on 5'-nucleotidase activity.

The aim of this investigation was to analyze changes in 5'-nucleotidase activity in mouse peritoneal exudate macrophages (PEM) after intraperitoneal injection of IS, using methods of mathematical modeling and planning.

EXPERIMENTAL METHOD

In the experimental part of the work 5'-nucleotidase activity was measured in PEM of CBA mice aged 3 months, at selected time intervals after intraperitoneal injection of IS. The following IS were used: salmosan (a polysaccharide of bacterial origin), tuftsin and rigin (tetrapeptides), and a polysaccharide of animal origin (PAO).

The preparations were injected in a single dose of 100 μg per mouse. Enzyme activity was determined by a modified method of Dixon and Purdom [4]. PEM were obtained by the method in [1]. The experimental data were subjected to statistical analysis by methods of regression analysis [3].

EXPERIMENTAL RESULTS

It will be clear from Table 1 that all the IS used induced a long-term decrease in activity of this enzyme. Regression analysis of the data showed that the change in 5'-

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TABLE 1. 5'-Nucleotidase Activity of PEM of CBA Mice after Intraperitoneal Injection of IS (in % of control)

	Salmosan		PAO		Tuftsin		Rigin	
	x	y	x	y	x	y	x	y
1	8	6,67	7	23,09	7	54,55	10	10,1
2	8	10,01	7	15,04	7	50,2	10	6,13
3	8	8,39	7	12,02	7	70,15	10	7,8
4	8	5,02	20	47,25	14	83,16	10	6,5
5	13	12,35	20	53,34	14	60,06	10	8,2
6	13	8,52	20	45,7	14	64,6	10	9,14
7	13	15,43	40	91,25	14	86,7	20	54,8
8	20	20,76	40	80,94	21	71,4	20	46,4
9	20	14,7	40	73,75	21	76,5	20	47,1
10	20	22,5	40	73,72	21	81,6	20	53,2
11	24	25,28	—	—	21	87,78	20	55,9
12	24	26,48	—	—	30	92,4	20	50,85
13	24	29,4	—	—	30	111,65	—	—
14	—	—	—	—	30	100,1	—	—

Legend. x) Time (in days), y) 5'-nucleotidase activity.

TABLE 2. Results of Regression Analysis of Data in Table 1

Preparation	$\hat{\theta}_0$	$\hat{\theta}_1$	$\Delta\hat{\theta}_1$	S
Salmosan	10,91	0,41	0,038	8,44
PAO	6,56	1,87	0,026	7,16
Tuftsin	45,9	1,79	0,026	9,55
Rigin	—39,15	4,36	0,018	2,18

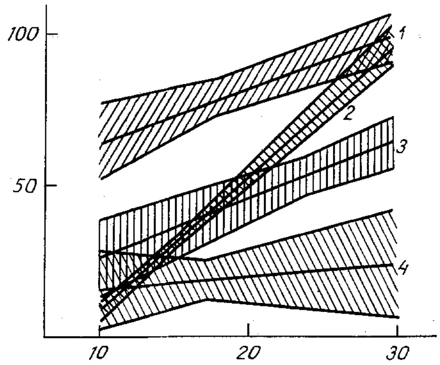

Fig. 1. Regression lines with Graybill—Boyden confidence bands for IS chosen for study. Abscissa, time (in days); IS: 1) tuftsin, 2) rigin; 3) PAO; 4) salmosan.

nucleotidase activity with time at the selected times of investigation, under the influence of each IS, in adequately described by a unidimensional linear regression model:

$$y=\theta_0+\theta_1 x+e, \tag{1}$$

where y is the 5'-nucleotidase activity at time x after injection of the immunostimulator; e denotes random interference; θ_0 and θ_1 are unknown parameters, exact and interval assessments of which are given in Table 2. If the estimates are substituted for the coefficient θ_0 and θ_1, equation (1) assumes the following form: for salmosan $\hat{y}$ = 10.91 + 0.41 x; for tuftsin $\hat{y}$ = —39.15 + 4.356 x; for PAO $\hat{y}$ = 6.56 + 1.869 x.

These relationships, together with the 95% confidence bands of Grabill and Bowden, are shown in Fig. 1. They enable enzyme activity to be predicted at arbitrary moments of time (within the duration of the investigation) and the accuracy of the prediction to be judged.

The results suggest that under the influence of certain IS changes in 5'-nucleotidase activity with time will also be linear in character. Accordingly, optimal planning of the regression experiment can be considered, aimed at determining the best estimates of the coefficients θ_0 and θ_1 in model (1) for newly tested IS.

TABLE 3. Measured Values of y and Predicted Values $\hat{y}$ Predicted by the Model for 5'-Nucleotidase Activity after Intraperitoneal Injection of Rigin

x, days	y, % of control	$\hat{y}$, % of control
14	27,7 24,4 22,5	21,83±5,7
30	97,8 100,1 109,95	96,5±8,3

We know from the theory of optimal planning [5] that to achieve the best quality when evaluating unknown parameters of regression model (1) an equal number of measurements of enzyme activity y must be made at two extreme periods of the investigation x_- and x_+, bounding the region of planning, within which the model (1) is true. The times x_- and x_+ must be chosen on the basis of a priori information about the IS to be studied, and also taking account of the results of investigations carried out with other preparations. The value of x_- must be knowingly exceed by a certain amount the time x_{min} during which enzyme activity remains at the maximally reduced value after injection of the preparation. The upper limit x_+ is determined by the time x_{max} at which enzyme activity is close to the control values. As the results of these investigations show (Fig. 1), time x_{min} varies for the IS used between 3 and 7 days. The value of x_{max} changes within a much wider range than x_{min}. Thus for the most powerful of the IS used, namely salmosan, this time is about 80 days, compared with only 27 days for tuftsin. If there is no a priori information about an IS newly chosen for study, when x_+ is chosen, the value of x_{max} of the preparation with the weakest immunostimulating action must be taken into consideration. It was shown that all the IS studied, when injected intraperitoneally, lower 5'-nucleotidase activity for at least 21 days. It can accordingly be recommended that 5'-nucleotidase activity be investigated at the times: $x_- = 10$, $x_+ = 20$.

The arguments given above were used to study changes in 5'-nucleotidase activity of mouse PEM under the influence of the intraperitoneally injected IS, rigin (Tables 1 and 2).

It will be clear from Table 2 that the most accurate model was constructed for rigin. This is shown by the lowest value of the confidence interval for the coefficient θ_1 and the least value of the estimate of dispersion of interference S.

To test the predicting and interpolation properties of the model for rigin, additional measurements of enzyme activity were made on the 14th and 30th days after its injection. The results of these measurements and also predicted values of enzyme activity based on the model constructed, are given in Table 3. Comparison of these data shows the good prognostic properties of the model as constructed.

The results thus showed that changes in 5'-nucleotidase activity after intraperitoneal injection of IS of different chemical and biological nature takes place in accordance with the same linear law at the chosen times of investigation. By making use of the idea of experimental planning, the most accurate characteristics of the change in 5'-nucleotidase activity can be obtained for the IS chosen for study, and with the minimal number of investigations.

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