in experimental thiamine deficiency. During analysis of the mechanism of disturbance of an energy-dependent process such as phagocytosis, the very important role of vitamin B_1 in the supply of energy to the cell must be recalled in the first place. Thiamine is known to participate through pyruvate dehydrogenase in the oxidative decarboxylation of pyruvic acid and through α-ketoglutarase dehydrogenase in the oxidative decarboxylation of α-ketoglutaric acid [4]. Inhibition of the activity of the above enzymes leads to disturbance of the basis of the energy economy of the cell.

It is difficult at present to specify the causes of the increase in the serum lysozyme concentration which was found. Administration of HT may perhaps lead to increased permeability of the subcellular and plasma membranes of the leukocytes, with the liberation of lysozyme, a lysosomal enzyme, into the blood stream.

LITERATURE CITED

1. N. B. Lutsyuk and A. K. Morozova, Vopr. Pitan., No. 5, 56 (1975).
2. Yu. M. Ostrovskii, Active Centers and Groups in the Thiamine Molecule [in Russian], Minsk (1975).
3. A. N. Razumovich, "Energy metabolism during aging in tissues with different functional specialization," Author's Abstract of Doctoral Dissertation, Vilnius (1971).
4. V. B. Spirichev, in: The Molecular Basis of Pathology [in Russian], Moscow (1966), p. 244.
5. E. F. Rogers, Methods Enzymol., 18A, 245 (1970).
6. N. K. Sarkar, Int. J. Biochem., 6, 423 (1975).

CYTOPHILIC IMMUNOGLOBULINS ON THE SURFACE OF POLYMORPHS IN MICE IMMUNIZED PERORALLY WITH LIVE VACCINE FROM SUPPRESSOR REVERTANT *Salmonella typhimurium* Rev 8

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The spleen cell migration inhibition test in the presence of monospecific antisera against mouse immunoglobulins of the G, A, and M classes was used to detect cytophilic antibodies on the surface of mouse granulocytes. Peroral administration of live vaccine from suppressor reverant *Salmonella typhimurium* Rev 8 to AKR mice protected the animals against infection with a virulent strain of *S. typhimurium*. An increase in the number of cytophilic IgG on the surface of the polymorphs was observed in the immunized mice.

KEY WORDS: cytophilic antibodies; neutrophil granulocytes (polymorphs); mouse typhus; antisera against mouse immunoglobulins; enteral immunization.

The nature of the resistance developing after immunization with live *Salmonella* vaccines, especially those prepared from *Salmonella typhimurium*, has been inadequately explained [5]. Definite importance is attached to antibodies which are cytophilic for phagocytic cells [4], [8-10]. The object of the present investigation was to study immunoglobulins on the surface of neutrophilic granulocytes (polymorphs) of mice immunized with *S. typhimurium* vaccine and at the same time, to evaluate postvaccinal resistance, circulating antibodies, and cellular immunity.

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TABLE 1. Resistance of Intact and Vaccinated Mice to Infection with *S. typhimurium* Strain No. 415

Group of mice	Cycle of immunization	
	1-st	2-nd
Nonimmunized ($^1/_9$)		
Immunized:		
with live vaccine	7/9	8/9
with killed vaccine	3/9	2/9

Legend. Number of surviving mice in numerator, total number of mice in group in denominator.

TABLE 2. Levels of Humoral and Cellular Immunity in Mice Immunized with Living and Killed *S. typhimurium* Rev 8 Vaccine at Moment of Infection

Group of mice	Cycle of immun.	Serum immunoglobulin concentration, mg/ml*			Antibody titer against O antigen in PHT†	Inhibition of spleen cells migration with a bact. conc. of‡
		IgM	IgG	IgA		
Nonimmunized	1-st	0,45±0,01	1,8±0,01	0,16±0,03	1:10	—$25 \cdot 10^7$
Immunized:						
with killed vaccine	1-st	0,5±0,03	1,9±0,02	0,18±0,02	1:20	+$25 \cdot 10^6$
	2-nd	0,48±0,01	2,1±0,03	0,25±0,02	1:80	+$25 \cdot 10^7$
with live vaccine	1-st	0,51±0,02	1,85±0,01	0,24±0,01	1:160	—$25 \cdot 10^7$
	2-nd	0,53±0,04	2,1±0,01	0,22±0,03	1:320	—$25 \cdot 10^7$

*M±m for group of 5-8 mice.
†Mixture of sera from five mice of the same group.
‡Test carried out with cell suspensions from 5 mice of the same group and considered to be positive (+) if index of inhibition exceeded 25%.

TABLE 3. Titers of 50% Inhibition of Spleen Cell Migration by Monospecific Antisera

Group of mice	Cycle of immun.	Antisera		
		anti-IgM	anti-IgG	anti-IgA
Nonimmunized		1:45	1:85	1:15
Immunized:				
with killed vaccine	1-st	1:18	1:90	1:10
	2-nd	1:18	1:100	1:10
with live vaccine	1-st	1:45	1:250	1:10
	2-nd	1:45	1:600	1:10

Legend. Test carried out on day of infection with cell suspension from 5 mice of the same group. Each of the 4-5 dilutions of antisera was added simultaneously to four identical cultures of spleen cells and mean indices of inhibition of migration were calculated; from these values the 50% inhibition titers were themselves calculated later [2, 10].

EXPERIMENTAL METHOD

Mice of line AKR/Rap were immunized perorally with live vaccine from suppressor revertant *S. typhimurium* Rev 8 [3] or with a heat-killed suspension of *S. typhimurium* strain 415. In

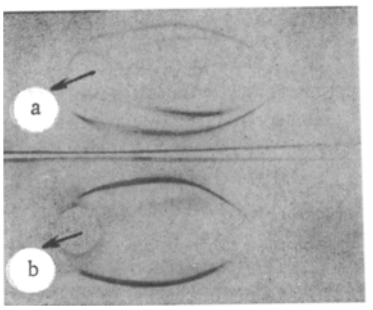

Fig. 1

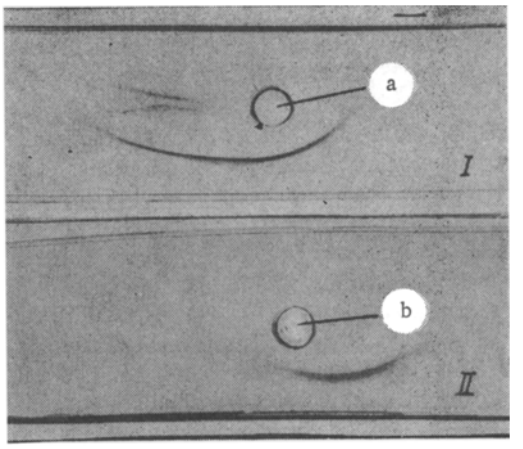

Fig. 2

Fig. 1. Immunoelectrophoresis of sera of mice with MORS-21 and RPC-5 plasmacytoma. Wells contain: serum of mice with plasmacytoma MORS-21 (a) and serum of mice with plasmacytoma RPS-5 (b). Trough contains rabbit antiserum against mouse IgG. Two precipitation bands in both cases indicate that the anti-G reagent reveals both IgG-1 and IgG-2.

Fig. 2. Immunoelectrophoresis of serum of BALB/c mice. Well (a) contains serum of BALB/c mice. Trough contains rabbit monospecific antiserum against mouse IgA (I) and IgM (II).

the first immunization cycle the animals received 10^9 bacterial cells daily for 3 days; the second cycle was carried out 10 days after the end of the first. Each mouse received $5 \cdot 10^9$ bacterial cells daily for 3 days. Three weeks after the first and second immunization cycles, individual groups of mice were infected perorally with 10^5 living cells of *S. typhimurium* strain 415, after which they were observed for 1 month and the number of survivors counted. Serum antibodies were titrated in the passive hemagglutination test (PHT) with erythrocytic *Salmonella* O diagnostic serum 1, 4, 12, produced by the Moscow Scientific-Research Institute of Experimental Medicine. To detect delayed hypersensitivity (HDT) to *Salmonella* antigens, the mouse spleen cell migration inhibition test was carried out in the presence of a suspension of *S. typhimurium* treated with formaldehyde and then washed. *Salmonellas* were added to the culture of splenic leukocytes in a concentration of $25 \cdot 10^5$, $25 \cdot 10^6$, and $25 \cdot 10^7$ bacterial cells/ml.

To detect cytophilic immunoglobulins on the surface of the polymorphs, spleen cells of immunized mice were cultured in capillary tubes [2], with the addition of monospecific antisera against mouse immunoglobulins of classes G, A, and M to the culture medium. As shown previously, the migration front of the spleen cells from the capillary tube is formed by polymorphs, and antibodies against the surface determinants of these cells, including against immunoglobulins located on the surface, caused migration inhibition [2, 4]. A titer of 50% migration inhibition by this antiserum served as the measure of the presence of cytophilic immunoglobulins on the surface of the polymorphs and was calculated as in [9].

Antisera were obtained by immunizing rabbits with purified myeloma immunoglobulins of classes M, G, and A as described in [1], and were standardized by radial immunodiffusion [6].

EXPERIMENTAL RESULTS AND DISCUSSION

The number of animals which survived after infection with the virulent strain of *S. typhimurium* is given in Table 1. Postvaccinal resistance was found only in mice immunized with live vaccine [the difference from the group of unimmunized mice is already significant ($P<0.01$), after the first immunization cycle]. Injection of killed vaccine did not produce immunity.

Some indices of humoral and cellular immunity of the vaccinated mice are given in Table 2. Immunization caused no significant change in the concentrations of immunoglobulins of the M, G, and A classes. Antibodies against O-antigen, the titers of which were highest after

immunization with live vaccine, were found in the sera of the vaccinated mice. Meanwhile, inhibition of spleen cell migration in the presence of formalinized *Salmonellas* (the HDT index) was observed only in mice receiving the killed vaccine.

The results of determination of titers of 50% inhibition of migration of spleen cells by monospecific antiglobulin reagents are given in Table 3. Antisera against mouse IgM and IgA had low 50% inhibition titers, which were the same in the control and the immunized animals. Antiserum against IgG was more active than the other two antiglobulin reagents and it inhibited migration of polymorphs from unimmunized mice. However, the highest inhibition titers were observed in experiments with spleen cells of animals receiving live *Salmonella* vaccine. In this case the inhibition titer rose in the course of immunization. The increase in the sensitivity of the migrating spleen cells to the inhibitory action of antisera was evidence of an increase in the dose of the corresponding antigenic determinants on the surface of the most mobile cells. Consequently, the increase in the inhibitory activity of anti-IgG antiserum can be regarded as proof of an increase in the quantity of fixed (cytophilic) IgG on the surface of the polymorphs of mice immunized with live *Salmonella* vaccine.

The results of immunoelectrophoresis given in Figs. 1 and 2 demonstrate the specificity of the antiserum used.

If the immunologic indices in mice immunized with killed and live *Salmonella* vaccine are compared it will be evident that the appearance of circulating anti-O antibodies and of HDT to *Salmonella* antigens was not accompanied by the development of postvaccinal resistance. Meanwhile, in animals protected against infection, an increased quantity of cytophilic IgG was found on the polymorphs. This suggests that, during immunization with live vaccine, antibodies cytophilic toward granulocytes may play a role in specific resistance to mouse typhys.

LITERATURE CITED

1. V. A. Aleshkin and V. V. Lebedev, Proceedings of the Second Moscow Medical Institute, Vol. 92, Series: Pharmacology, No. 3 [in Russian], Moscow (1977), pp. 76-80.
2. O. V. Protasova, N. P. Perepechkina, and A. N. Mats, Zh. Mikrobiol., No. 11, 132 (1971).
3. Yu. Yu. Shuster and V. G. Likhoded, Zh. Mikrobiol., No. 9, 39 (1976).
4. V. I. Levenson and N. I. Braude, Dokl. Akad. Nauk SSSR, 174, 468 (1967).
5. F. M. Collins, Bact. Rev., 38, 372 (1974).
6. J. L. Fahey and E. M. McKelvey, J. Immunol., 94, 84 (1965).
7. P. M. Henson, Immunology, 16, 107 (1969).
8. C. R. Jenkin and D. Rowley, Bact. Rev., 27, 391 (1963).
9. V. P. Juhasz, Ann. Immunol. Hung., 11, 279 (1968).
10. D. Rowley, I. Auzins, and C. R. Jenkin, Aust. J. Exp. Biol. Med. Sci., 46, 447 (1968).