INTENSITY OF THE SYNTHESIS OF NONSPECIFIC PROTEINS OF THE ORGANISM UNDER THE INFLUENCE OF THE LIPOPOLYSACCHARIDE COMPLEX FROM TYPHOID BACILLUS

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In our previous works, we showed [2, 3] that a single introduction of typhoid vaccine produces an increase in the intensity of synthesis of nonspecific proteins in the organism even during the first hours.

The intensity of the incorporation of a labelled amino acid into the protein or the organism is substantially activated immediately after the introduction of this vaccine, reaching a maximum in 4 h, and returning to the values observed in the control animals after 72 h.

In a study of the intensity of protein synthesis in these experiments, we were forced to administer large numbers of microbial cells to the animals in order to guarantee a good production of antibodies after a single introduction of the vaccine. The hypothesis arose that the activation of protein synthesis is accomplished by the decomposition products of the microbial bodies introduced into the abdominal cavity, in particular, by the soluble lipopolysaccharide complex, which, as is well known, gives rise to the formation of highly specific antibodies, and, at the same time, is a nonspecific stimulator of antibody formation.

In this work we investigated the intensity of protein synthesis in a number of organs in the animals after their administration of various doses of the lipopolysaccharide of typhoid bacteria.

EXPERIMENTAL PROCEDURE

All the experiments were conducted on rabbits of the chinchilla breed, from 2 to 2.5 kg in weight. The lipopolysaccharide complex was produced from B. typhi abdominalis (strain No. 4446) according to a somewhat modified Boivin procedure [4].

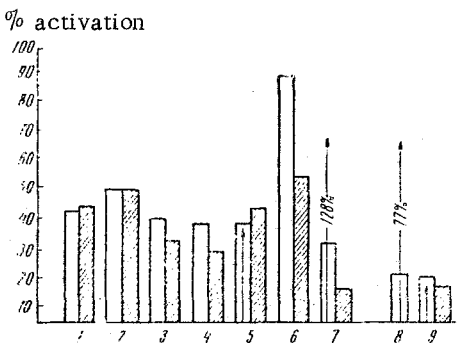
The dose of the antigen producing sufficient antibody formation after a single administration was titrated beforehand. At the same time, the pyrogenic effect of the titrated dose was determined. For our preparations, such a dose was 3.5-5 μg of the preparation per g of weight of the animal. In a single introduction, this dose produced the formation of antibodies in a titer of 1:3200. The injection of 15 μg of the same lipopolysaccharide complex simultaneously with an immunizing dose of typhoid vaccine considerably stimulated the antibody production induced by it. We also used this dose in our investigations. Both doses gave a pyrogenic effect.

Methionine-S^{35} was used as the radioactive amino acid in all the experiments; it was injected intravenously in an amount of 20 thousand counts per g of weight of the animal.

The dry powder of the lipopolysaccharide, dissolved in physiological solution, was administered to 1 group of experimental rabbits in a dose of 3.5 μg/g, intraperitoneally (in a volume of 1.5-2 ml), while the other group received a dose of 15 μg per rabbit intravenously.

TABLE 1. Intensity of the Incorporation of Methionine-S^{35} into the Proteins of the Organism Four Hours after the Introduction of Doses of the Complete Antigen According to Boivin (in counts/min in 2 mg of protein)

Dose of endotoxin	Group of rabbits	Group of animals	Radioactivity									
			Blood serum			Liver	Spleen	Adrenals	Lymph nodes			
			Total proteins	Albumins	Globulins				Retroperitoneal	Mesenteric	Mediastinal	Popliteal
3.5-5 μg/g	Experimental	14	212	132	198	142	195	278	213	180	160	139
	Control	12	148	88	140	102	139	148	161	148	131	158
Percent activation			43	50	41	39	40	90	32	22	22	–
15 μg per rabbit	Experimental	2	159	104	162	135	197	288	112	–	123	–
	Control	4	110	69	121	104	137	153	98	–	104	–
Percent activation			44	50	33	30	43	55	14	–	17	–



Intensity of the synthesis of nonspecific proteins of the organism (columns) and plasmocytary reaction (arrows) after the injection of the lipopolysaccharide complex from S. typhi abdominalis (in counts/min in 2 mg of protein). White columns – introduction of endotoxin in a dose of 3.5 μg/g, intraperitoneally; dark columns – in a dose of 15 μg per rabbit intravenously; 1) total proteins; 2) albumins; 3) globulins; 4) liver; 5) spleen; 6) adrenals; 7) retroperitoneal lymph nodes; 8) mesenteric lymph nodes; 9) mediastinal lymph nodes.

The labelled amino acid was adminstered to the animals 4 h after injection of the antigen, since in the preceeding experiments the maximum increase in the intensity of protein synthesis was observed at this time. The group of control rabbits received injections only of methionine-S^{35} in the same amounts.

The animals were decapitated 18 h after the injection of the labelled amino acid, and the intensity of its incorporation into the serum proteins, serum albumin, and serum globulin, into the proteins of the liver, spleen, adrenals, and lymph nodes (regional and distant) was determined.

In addition to the intensity of protein synthesis, the reaction of the plasma cells to the introduction of antibodies during the 1st h was investigated in the lymph nodes.

The production of the proteins and their treatment for a count of the radioactivity were described earlier [1, 2]. The radioactivity was counted in 2 mg of dry protein with an end-window counter.

EXPERIMENTAL RESULTS

From the data cited in Table 1, it is evident that after the animals were administered various doses of the total antibody according to Boivin, activation of the synthetic intensity in the summary proteins of the blood serum, serum albumin and globulin, proteins of the liver, spleen, and adrenals reaches a high level.

In our previous works, we showed that if the experimental results exceed the results obtained in the control by more than 27%, then in a statistical treatment of the material they prove reliable. On the basis of this, we performed a statistical treatment of the results pertaining only to the lymph nodes. For the retroperitoneal lymph nodes, $t > 3$, i.e., the changes are statistically quite reliable; for the mediastinal and mesenteric nodes, $t \leq 2$, i.e., the changes are statistically unreliable.

Thus, the lipopolysaccharide complex from typhoid bacteria produces an increase in the intensity of protein synthesis in the serum and its fractions, the proteins of the liver, spleen, and regional lymph nodes (retroperitoneal) 4 h after intraperitoneal injection. The intensity of protein synthesis in the adrenals is especially sharply activated. In the distant lymph nodes (under the conditions of our experiment, in the popliteal lymph nodes), no activation of the synthetic intensity was observed.

TABLE 2. Plasmocytary Reaction in the Regional and Distant Lymph Nodes During the First Hours After Injection of the Antigen (in 50 visual fields)

Group of animals	Number of animals	Spleen	Lymph nodes			
			Retroperitoneal	Mesenteric	Mediastinal	Popliteal
Experimental	7	94	164	110	173	60
Control	8	68	72	62	155	60
Percent Activation	—	38	128	77	11	—

Since antibodies represent gamma globulins adapted to an antigen, while globulin synthesis is closely related to plasma cells, in addition to our study of the intensity of protein synthesis of the organism, we conducted a parallel investigation of the plasmocytary reaction in the regional and distant lymph nodes during the 1st hours after introduction of an immunizing dose of the lipopolysaccharide complex (Table 2).

As can be seen from the figure, the intensity of protein synthesis of the organism and the plasmocytary reaction after the injection of the antigen are correlated. The closest regional lymph nodes—retroperitoneal, then the mesenteric and spleen—respond most actively to the introduction of the antigen. In the mediastinal lymph nodes, activation of the synthetic intensity and the plasmocytary reaction are weakly pronounced. In the popliteal lymph nodes, no plasmocytary reaction developed. It is interesting that after the injection of a stimulating dose of the lipopolysaccharide complex (15 μg), we registered a no less pronounced activation of the intensity of protein synthesis in analogous organs (see Table 1 and the figure).

We believe that the general stimulation of the intensity of protein synthesis, noted during the inductive phase of antibody formation, is a necessary link in the mechanism of antibody production. After this link has been accomplished, probably the adjuvant action of small doses of lipopolysaccharides is also effected; in other words, the adjuvant action of the endotoxin is evidently explained by their ability to stimulate the intensity of protein synthesis in the organism.

Since small doses of lipopolysaccharide (15 μg) increase the intensity of the incorporation of the labelled amino acid to approximately the same degree as doses capable of producing antibody formation, we might assume that the action of the vaccine in stimulating protein synthesis, which we detected earlier, is explained by the properties of the endotoxin that it contains.

Probably the stimulation of protein synthesis alone is insufficient for the formation of the antibody molecule—an antigen is needed, but without it, the antigen is relatively or entirely ineffective.

Our hypotheses find indirect confirmation in studies in which it was shown that factors inhibiting cell metabolism during the inductive phase of antibody formation prevent its accomplishment. Especially interesting from this standpoint are the data of Kind and Johnson [5], who established that the administration of endotoxin to irradiated animals partially restores their lost ability to manufacture antibodies.

SUMMARY

Lipopolysaccharide complex of typhoid bacilli was injected intravenously to chinchilla rabbits. The dose of the antigen was titrated according to the pyrogenic effect and the capacity for inducing the antibody production. Each rabbit received a stimulating antibody-producing dose of 15 γ and an immunizing dose of 3.5-5 γ of the antigen per kg of the animal's body weight.

Methionine S^{35} was used as a radioaminoacid. It was administered to experimental animals 4 h after the antigen had been introduced. Control rabbits received only methionine S^{35}.

It was experimentally shown that administration of lipopolysaccharide complex (in various doses) stimulated, during the first hours, the synthesis intensity in proteins of blood serum, in its albumin and globulin fractions, and in proteins of the liver, spleen, adrenal glands, as well as in proteins of the regional lymph nodes (retroperitoneal). No synthesis activation was observed in distal lymph nodes.

Simultaneously with the activation of the intensity of the body protein synthesis there occurred a marked plasmocytic reaction in the regional lymph nodes and the spleen.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
