# INDUCTION OF SPECIFIC IMMUNOLOGIC AREACTIVITY TO AN ENTERALLY ADMINISTERED ANTIGEN

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After enteral immunization of guinea pigs with diphtheria toxoid, the synthesis of specific antibodies begins in the lymphoid organs. If enteral immunization is carried out daily for a long time (3 months), the guinea pigs develop specific immunologic paralysis toward this antigen, but most of the animals under these circumstances develop partial areactivity, affecting predominantly the regional lymph glands relative to the site of injection of antigen.

Recent years have seen the intensive study of the mechanism of formation of the human automicroflora. The dominant role of specific immune systems of the body in this process has been demonstrated [3, 4, 6, 7]. However, the complete picture has not yet been obtained. Experimental evidence is required to show whether a powerful and specific immunologic reaction, or tolerance, to an antigen can be induced in an animal in response to the enteral administration of living microorganisms or their antigens.

The object of the present investigation was to study the immune response to an antigen when administered enterally for different periods of time to an immunologically mature organism.

## EXPERIMENTAL METHOD

Guinea pigs were fed with native concentrated diphtheria toxoid. The daily dose was 1 ml of toxoid (1300 Lf). Guinea pigs of groups 1 and 2, starting from the 8th day after birth, received diphtheria toxoid enterally daily (except Saturdays and Sundays) for 3 months. Guinea pigs of groups 3 and 4 received toxoid starting from the 8th day after birth (four 3-day cycles at intervals of 4 days, then one 3-day cycle 2 months later, and a 7-day cycle 3 months after birth). The animals of groups 2 and 4 were immunized with toxoid at 3 points 4 days after the end of enteral immunization: subcutaneously into the heel of one hind limb, into the submandibular region, and intraperitoneally, in a total dose of 160 Lf. Some animals of group 2 were immunized, in addition, with two injections of crystalline bovine serum albumin (BSA) at 3 points: subcutaneously into the heel of a hind limb, into the submandibular region, and intraperitoneally. The total single dose was 20 mg. Animals of groups 5 and 6 were immunized with diphtheria toxoid subcutaneously into the heel of the hind limb once (group 6) and twice (group 5). Group 7 consisted of control unimmunized animals. The guinea pigs were sacrificed on the 4th and 12th days after the last immunization. Lymphoid tissue of the pharyngeal ring, the submandibular, cervical, popliteal, and mesenteric lymph glands, the spleen and the Peyer's patches were studied. The number of antibody-containing cells was studied in serial sections through the organs. The luminescence-serological method of staining was used to detect diphtheria antibodies and nonspecific immunoglobulins or diphtheria and protein antibodies simultaneously (for details on the method of staining and counting the cells, see [1] and [2]).

## EXPERIMENTAL RESULTS

In the guinea pigs receiving toxoid enterally in 3-day cycles (group 3), antibody-containing cells were found on the 4th day after the last immunization in all lymphoid organs studied, although in small quantities

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TABLE 1. Number of Cells Containing Diphtheria Antibodies in Lymphoid Organs of Enterally and Subcutaneously Immunized Guinea Pigs

| Lymphoid organs | Group 1 | | | 2 | | 3 | 4 | 5 | | 6 | | 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4/6 | 12/2 | 4/7 | 4/6 | 12/3 | 4/6 | 4/8 | 4/7 | 12/5 | 4/4 | 12/4 | -/10 |
| Lymphatic tissue of pharyngeal ring | 0 | 0 | 0 | 0 | 0 | Single | Single | Single | Occasional | 0 | 0 | 0 |
| Submandibular and cervical lymph glands | 0 | 0 | 0 | 0 | 0 | Tens | Hundreds | " | " | 0 | 0 | 0 |
| Peyer's patches | 0 | 0 | 0 | Single | Occasional | Occasional | Single | " | " | 0 | 0 | 0 |
| Large intestine | 0 | 0 | 0 | 0 | 0 | " | " | Occasional | 0 | 0 | 0 | 0 |
| Mesenteric lymph glands | 0[1] | 0 | 0 | Occasional | Occasional | " | Tens | Single | Occasional | 0 | 0 | 0 |
| The same | 0 | 0 | 0 | " | 0 | " | Single | " | " | 0 | 0 | 0 |
| Spleen | 0 | 0 | 0 | Tens | Occasional | " | Hundreds | Tens | Single | 0 | Occasional | 0 |
| Popliteal lymph glands | 0 | 0 | 0 | Hundreds | Single | " | Thousands | Thousands | Tens | 0 | Tens | 0 |

[1]Three luminescent cells were found in 2 animals.

Note. Numerator gives days after immunization, denominator number of animals.

(Table 1). Their number was greatest in the submandibular and cervical lymph glands. Antibody-containing cells were found constantly in the lymphoid collections of the pharyngeal ring. They were fewer in number in the Peyer's patches, mesenteric and popliteal lymph glands, and spleen. However, substantially fewer luminescent cells were observed in all the organs than after double subcutaneous immunization (groups 5 and 6).

In animals receiving diphtheria toxoid enterally in 3-day cycles, followed by subcutaneous and intraperitoneal injections of the toxoid (group 4), many antibody-containing cells were found in all studied organs on the 4th day after immunization. They were no fewer than after double subcutaneous immunization (group 5).

These results indicate the immunological effectiveness of enteral vaccination with toxoid. The chief portals of entry for antigen were evidently the pharyngeal lymphoid structures, or Pirogov's ring. The intensity of the immune response in enteral immunization was lower than that observed when the antigen was injected subcutaneously. Despite weak antibody formation after enteral vaccination, numerous memory cells relative to the antigen accumulated in the body, indicating an intensive immune response to a single subcutaneous injection of toxoid into animals previously vaccinated enterally. In most guinea pigs receiving toxoid by mouth daily for 3 months (group 1), no specific antibody-containing cells could be found in any lymphoid organs. Only 1-3 such cells were found in all sections examined from the mesenteric lymph glands of only 2 of the 8 animals. In animals immunized enterally with toxoid by the group 1 scheme, and receiving toxoid subcutaneously and intraperitoneally for 4 and 12 days before sacrifice (group 2), the following picture was found. No antibody-containing cells could be found in any lymphoid organ of 7 animals sacrificed on the 4th day. In the remaining 9 animals, sacrificed on the 4th and 12th days after immunization, no antibody-containing cells were found in the lymphoid collections of the pharyngeal ring, the submandibular or cervical lymph glands. Occasional antibody-containing cells were seen in the Peyer's patches and mesenteric lymph glands, but in the spleen and popliteal lymph glands these cells were presented in large numbers. Some animals of this group received BSA subcutaneously and intraperitoneally as well as the toxoid. In all these animals, numerous cells producing antibodies against BSA were observed in all their lymphoid organs.

These last observations indicate that during prolonged enteral administration of an antigen, specific immunologic areactivity arises to it, although in most cases it is incomplete and affects mainly the regional lymphoid organs relative to the site of entry of the antigen. This areactivity may arise in an immunologically mature organism, and it is evidently similar in its mechanism to Felton's paralysis [5]. The possibility

that partial areactivity resembling Felton's paralysis may arise to antigens of bacterial origin, entering through the wall of the digestive tract, removes many of the difficulties preventing our understanding of the regulatory action of specific immune mechanisms during the formation of the autoflora of the digestive tract. It can be postulated on the basis of these findings and of others published in the literature that a true, complete tolerance or a tolerance resembling Felton's paralysis is created against only a few species of microorganisms. These microorganisms evidently form the true, permanent group of the autoflora. Partial areactivity is formed against many species in the body. Microorganisms of this group proliferate relatively well in the body, but not so freely as those of the preceding group. Species of microorganisms found in the human external environment enter the digestive tract inconstantly and infrequently, and they cannot induce immunologic paralysis but, on the contrary, the body develops an immune response to them inhibiting their proliferation. These last microorganisms constitute the group of the autoflora, variable and few in their total number.

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