

THE COMPARATIVE ANATOMY OF IMMUNITY

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In this paper we describe the results of a study of the reactivity of the lymphatic tissue of birds. Antigenic stimulation was used to detect the transformation of the lymphatic tissue cells.

EXPERIMENTAL METHOD

Experiments were conducted on 50 animals. In the first series of experiments 30 adult chickens of local breeds were used. Paratyphoid vaccine was injected subcutaneously into the medial surface of the thigh. The immunization program is shown in the graphs. The birds were sacrificed by bleeding from the jugular vein. The blood was investigated for the presence of agglutinins.

After post-mortem examination of the animals, the spleen was excised together with the small intestine and appendix. Pieces of the organs were fixed in neutral formalin. The spleen was weighed before fixation. Paraffin-wax sections were stained by the periodic acid - Schiff method and with methyl green - pyronine. The reticulum cells, lymphoblasts, and plasma cells in the sections were counted. Separate counts were made in the follicles and pulp of the spleen, in the follicles, interfollicular tissue, and mucous membrane proper of the appendix, and in the intestinal mucous membrane. In the follicles two diameters were measured and their mean obtained, from which the area of the follicles was deduced. In a similar fashion the area of the fields of vision was converted into square meters. For our comparative studies we worked in terms of an area of 0.1 mm^2 of follicle or section. These numerical results were treated statistically.

In the second series of experiments we used 10 chickens and 10 ducks, which were immunized by the same program. The material was treated in the same manner as in the first series of experiments. Observations were made for the purpose of comparing the reactivity of the lymphatic tissue of birds, some of which possess lymph glands (chickens), others not (ducks). Also, in the chickens, we studied the lymphatic tissue of the esophagus and the conjunctival membrane of the eye. Since the cellular changes observed in smears and sections of the lymphatic organs in the course of chicken immunization have already been described [7], information of this character is not given in this paper.

EXPERIMENTAL RESULTS

Graphs showing the changes in the weight of the spleen and in the numbers of PAS-positive plasma cells in its follicles and pulp in the course of immunization are given in Fig. 1. These results confirm reports in the literature [6, 7] that during immunization of chickens the spleen enlarges and the increased agglutinin titer is preceded by an increase in the number of plasma cells.

In Fig. 2 the graphs demonstrate the changes in the number of plasma cells in the tissues of the alimentary tract. It is particularly noteworthy that PAS-positive plasma cells appeared first of all in the mucous membrane of the appendix, and subsequently in the small intestine and the interfollicular tissue. Very interesting results were obtained by comparing the numbers of these cells in an area of section of 0.1 mm^2 during the period of observation. In the splenic follicles the number was 8.5, in the interfollicular tissue of the spleen 58.5, in the follicles of the appendix 11.8, in the interfollicular tissue of the appendix 129.1, in the mucous membrane of the appendix 636.6, and in the small intestine 130.4.

The results of the second series of experiments are given in the table. The numbers of mitotic figures and lymphoblasts in the follicles of the appendix of the ducks and chickens showed relatively slight fluctuations. Meanwhile, a considerable difference was observed in the numbers of reticulum cells. In the ducks, not until the

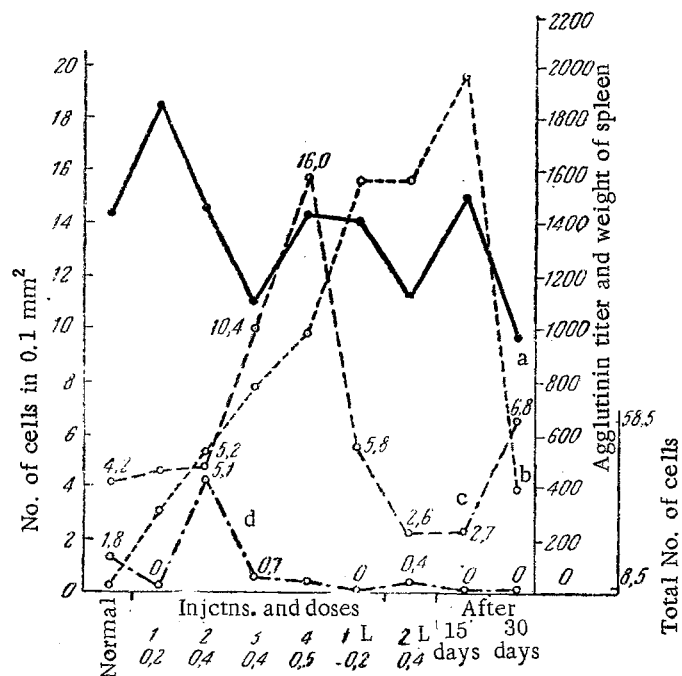


Fig. 1. Relative weight and number of plasma cells in the splenic follicles and pulp, and agglutinin titer in blood of chickens. a) Weight of the spleen; b) agglutinin titer; c) number of cells in the splenic pulp; d) number of cells in the splenic follicles; L) living culture.

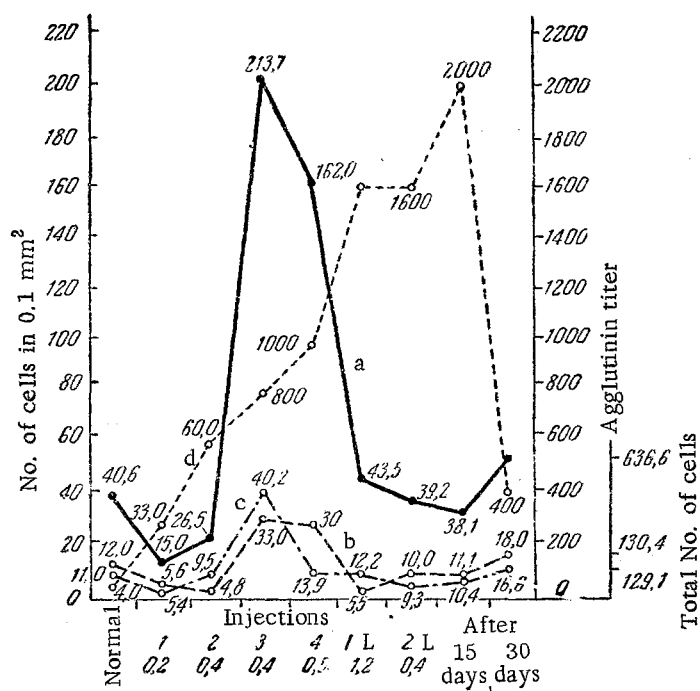


Fig. 2. Changes in the number of plasma cells in the mucous membranes proper of the appendix and ileum and in the interfollicular tissue of the appendix during immunization. a) Mucous membrane of the appendix; b) intestinal mucosa; c) interfollicular tissue; d) agglutinin titer; L) living culture.

Pyroninophilic Cells in the Follicles of the Appendix in Ducks and Chickens

Animal No.	Ducks										Chickens									
	No. of mitotic figures					lymphoblasts					No. of chan- ges					mitotic figures				
	M	σ	m	M	m	M	σ	m	M	m	M	σ	m	M	σ	m	M	σ	m	M
1	22	2,3	0,51	0,1	0,09	1,6	0,44	0,09	21	4,3	0,9	0,22	0,04	3,8	0,92	0,2	2,5	1,04	0,22	2,5
2	80	0,9	0,08	0,01	0,03	0,6	0,26	0,03	5	0,8	0,09	0,14	0,03	0,4	Her	0,07	1,1	0,2	0,15	1,1
3	36	2,3	1,3	0,2	0,08	1,3	0,48	0,08	14	8,1	1,3	0,16	0,005	4,2	0,6	0,1	0,8	0,46	0,1	0,8
4	20	6,3	1,26	0,28	0,05	0,6	0,2	0,05	4	0,15	0,03	0,49	0,08	3,1	1,46	0,4	4,7	1,22	0,2	4,7
5	26	3,9	1,73	0,34	0,2	2,7	1,2	0,2	19	5,0	1,0	1,08	0,3	1,1	0,4	0,1	7,2	3,05	0,8	7,2
6	23	1,7	0,3	0,07	0,12	1,8	0,58	0,12	19	3,8	0,8	0,17	0,05	1,1	0,4	0,1	4,1	0,7	0,2	4,1
7	—	No. of follicles found in the sections										19	4,4	4,8	4,2	0,9	19,8	8,5	1,9	19,8
8	27	2,3	0,88	0,17	0,3	3,1	1,3	0,3	9	6,0	1,1	0,33	0,1	0,3	0,48	0,16	14,5	5,2	1,7	14,5
9	18	2,7	1,14	0,27	0,08	0,4	0,3	0,08	24	9,4	2,3	2,02	0,4	2,5	0,5	0,1	23,6	6,5	1,4	23,6
10	22	1,3	1,0	0,2	0,13	0,9	0,6	0,13	15	7,4	1,6	0,91	0,3	—	—	—	—	—	—	—

30th day after immunization was any increase observed in the number of pyroninophilic reticulum cells. In contrast to this, in chickens under the same experimental conditions, the pyroninophilic reticulum cells began to increase in number after the third injection, and a more than nine-fold increase was observed on the 30th day after the end of immunization. Was this accidental? Evidently not. Counts of the pyroninophilic cells in the interfollicular tissue and in the mucous membrane proper of the intestinal rugae of these chickens showed that, of the 1,139 pyroninophilic cells of the interfollicular tissue counted in 50 fields of vision, 933 (or 81.6%) were reticulum cells, and 46.3% of these cells were fixed. Of the 2,449 pyroninophilic cells found in a total count of 50 intestinal rugae, 1,544 (or 61.9%) were reticulum cells, of which 19.4% were fixed cells. The fixed reticulum cells began to show pyroninophilia after the fourth injection, and it was particularly obvious after injection of the living culture, when these cells accounted for 88% of the total number of pyroninophilic cells in the interfollicular tissue.

Antigenic stimulation caused definite changes in the cell composition of the esophageal follicles of the chickens. In the course of immunization the number of PAS-positive cells in these follicles was doubled or trebled. By the end of immunization it had fallen almost to half the normal value. Fifteen days after immunization the number of these cells increased four-fold. One month later, the number of PAS-positive cells in an area of 0.1 mm² reached 28 ± 1.1 , of which 17.8 ± 0.9 were plasma cells.

The lymphatic tissue of the conjunctiva of the eyelids, which we studied by the method described by Hellman [5], was also affected by antigenic stimulation. The results illustrated in Fig. 3 indicate that the conjunctival lymphatic tissue increased in size by almost $2\frac{1}{2}$ times in the course of immunization.

During analysis of results relating to the comparative morphology of immunity, phylogenetic considerations must be taken into account. In multicellular animals, the body of which has become divided into kino- and phagocytoblasts, at a certain stage of development differentiation of the phagocytoblast into internal and external has taken place [1]. Subsequent specialization has led to the result that the latter has become the source of the tissues of the internal medium, one of the properties of which is protection, expressed initially in the form of phagocytosis and lysis. In warm-blooded animals the power to form antibodies has appeared.

In the embryonic state, the primogenital cell of the tissues of the internal medium in mammals and birds is mesenchymal; in the adult the reticulum cell is mesenchymal. The immunological processes must therefore be related to



Fig. 3. Lymphatic tissue of the conjunctiva of the lids and its changes in the course of immunization in chickens. In all cases part of the preparation measuring 1.25 mm in length was taken, and its outlines were traced on paper by means of a drawing apparatus. The outlined areas were cut out and weighed. Hen No. 1 - normal; hens Nos. 2-5 - after 1-4 injections of a killed culture; Nos. 6 and 7 - after 1st and 2nd injections of living culture; No. 8 - 15 days; No. 9 - 30 days; and No. 10 - 60 days after the end of vaccination.

the different forms of development of the reticulum cells. Lymphatic tissue, being a derivative of the mesenchyme, cannot remain indifferent to antigenic stimulation.

In our investigations, especially in the first series, we used the method of counting cells giving a positive PAS-reaction. It has been claimed [8, 9, 12] that the PAS-positive component of the plasma cells is a glycoprotein, concerned in antibody formation. If this is so, it must be considered that the site of antibody formation in chickens during hyperimmunization with a corpuscular antigen may be not only the spleen, but also the lymphatic tissue of the organs bordering the external environment.

Most immunomorphologists accept that during antibody formation the reticulum cells undergo transformation in accordance with a definite cycle [2, 3, 4, 10, 11]. The results described in this paper are in agreement with their findings and confirm their theoretical conclusions.

SUMMARY

A study was made of the antigenic reactivity of the spleen, appendix, intestine and esophagus in hens by estimating the PAS - positive and pyroninophilic cells. The amount of lymphatic tissue was determined in the hen conjunctiva. The greatest number of PAS - positive cells was revealed in the membrane proper of appendices and intestine, the least - in the splenic follicles. The lymphatic tissue of conjunctiva increased almost 2.5-fold during the observation period.

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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.