THE STUDY OF THE IMMUNOLOGY OF EMBRYOGENESIS

COMMUNICATION II. PHAGOCYTOSIS OF THE RED CELLS OF CHICK EMBRYOS BY LEUKOCYTES OF ADULT FOWLS

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At the end of the last centruy I. I. Mechnikov [6, 7] and A. O. Kovalevskii [5] demonstrated the important role of the phagocytes in processes of metamorphosis of the Echinodermata, Amphibia, and Insecta. These findings were later confirmed by the researches of other authors [1, 2, 10, 11, 12]. As a result of this research, Mechnikov [8] expressed his views on the morphogenic importance of the reaction of phagocytosis.

On the basis of these findings and of certain theoretical considerations, N. N. Zhukov-Verezhnikov [4] put forward his theory of primary immunological reactivity. One of the premises of this theory states that in the course of individual development certain definite immunological relationships arise between the protein systems of the tissues of different levels of development.

During the experimental investigation of this aspect of the theory, one of us, [3] was able to show that the tissue fluids of chick embryos contain substances which behave as antibodies in the complement fixation reaction with tissue extracts of chick embryos at earlier stages of development. These findings suggested that the immunological relationships between the tissues at different levels of development manifest themselves as humoral reactions of the antigen – antibody type.

We considered it of interest to ascertain by direct experiments whether these relationships were also manifested in the form of cellular reactions, and in particular of the reaction of phagocytosis. In order to solve this problem we carried out the experiments described below, in which we studied the phagocytosis of the red cells of chick embryos by the leukocytes of adult fowls.

EXPERIMENTAL METHODS

In order to obtain leukocytes from fowls one of the carotid arteries was opened (in some cases blood was taken from the comb). Clotting of the blood was prevented by the addition of a 2% solution of sodium citrate. The citrated blood thus obtained was centrifuged slowly (on a manual centrifuge) for an hour. As a result the red cells were deposited at the bottom of the tube and the leukocytes remained in the supernatant fluid (in the plasma), which was carefully aspirated with a Pasteur pipette*. In later experiments we used Sorokin's method [9], as follows. A ring of filter paper was placed on the surface of the yolk of a hen's egg after incubation for 52 hours**.

* To check completeness of deposition of the red cells, the supernatant fluid was examined under the microscope.

** The blood of 52-hour embryos is known not to contain its own leukocytes.

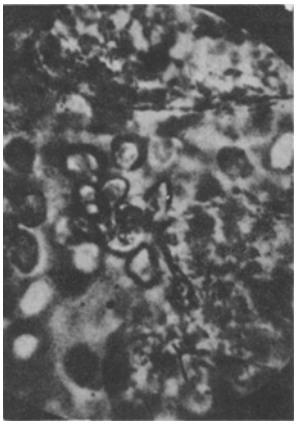

Fig. Macrophage with attached red cells, Magnification 900X.

The yolk sac was carefully incised with ophthalmic scissors around the outer margin of the ring. As a result, when the ring was removed with forceps, the embryo was inside the ring. One drop of fowl plasma containing leukocytes was then placed on the ventral aspect of the embryo. The ring was then transferred to a chamber constructed by V. I. Sorokin, enabling in vivo observations to be made on the processes of development of chick embryos for periods of more than 24 hours. Under a microscope with a stage heated to 38-39°, observations were then made on the cells moving in one of the vessels of the embryonic disk. Studies were also made of the phagocytosis of the red cells of chick embryos incubated 3-4 days in vitro.

For this purpose a drop of blood from the embryo was mixed with a drop of fowl plasma containing leukocytes. This mixture was then placed in a watch glass on the heated stage and studied under the microscope.

EXPERIMENTAL RESULTS

The study of phagocytosis in vivo. Altogether 40 embryos were studied. To the ventral surface of 20 of these ambryos was applied a drop of fowl plasma containing leukocytes. The remaining 20 embryos acted as controls and were cultivated without the additionof plasma.

In all 20 experimental embryos, roughly 1-1½ hours after application of the plasma, active fowl's leukocytes penetrated through the vessel walls into the blood stream. They usually moved against the flow of blood, attaching themselves by their pseudopodia to the endothelial wall of the vessels.

When a phagocyte came into contact with any red cells moving towards it, the red cell was seized by the leukocyte and stayed near it. The red cell was so firmly held by the small protoplasmic processes of the leukocyte that it could not detach itself in spite of the strength of the current of blood and collisions with other red cells. In time the phagocyte was usually attached also to the wall of a vessel by a readily visible, large pseudopodium. Other red cells, colliding at this time with the phagocyte, were not attached to it. After the leukocyte, tightly held to the vessel wall by its pseudopodium, had once again resumed its round shape, the next red cell to come into contact with it was attached, and so on.

Having seized 3-4 red cells, the phagoctye detached itself from the vessel and passed into the general blood stream, where it evidently trapped new red cells on its way. In this manner (usually 2½-3 hours after the beginning of the experiment) were formed characteristic accumulations of cells which were often seen in the blood stream of the embryo. On closer examination (usually at the bifurcation of vessels, where these agglomerations of cells were usually held up) two types of cell could always be seen. In the center was the phagocyte, and around it were arranged the red cells attached to it (see figure). In other words the typical picture of the initial stages of phagocytosis was observed.

The process of phagocytosis did not always terminate in this way. The red cell seized by the protoplasmic processes was gradually drawn inside the phagocyte. From 6 to 8 hours after the beginning of the experiment macrophages could sometimes be seen with several ingested red cells. It must be pointed out, however, that it was not always easy to determine whether in fact the red cells were inside the macrophage, for the same appearance was presented by red cells situated beneath the phagocyte but not actually within its substance.

Besides macrophages, microphages were also concerned in the phagocytosis of red cells. In these cases, however, a smaller number of red cells was attached — usually 2-3.

The study of phagocytosis in vitro. Red cells from the blood of 10 chick embryos of 72-80 hours incubation were used in the experiment. It was found that the active leukocytes of the adult fowls exhibited positive taxis in relation to the red cells of the embryos immediately after their entry into the blood stream. Some 30-40

minutes after the beginning of the experiment the formation of characteristic agglomerations of cells could be observed, consisting of two types of cells: In the center was usually to be found an adult chick leukocyte, and around it were embryonic red cells, attached by small protoplasmic processes to the phagocyte. The number of these accumulations of cells gradually increased but no further development of the picture of early stages of phagocytosis took place in vitro.

From the results described it can thus be concluded that the primary immunological reactivity, i.e., the immunological relationship between the tissues when at different stages of individual development, also manifested itself in the form of the reaction of phagocytosis. It is interesting to observe that the process of phagocytosis of the red cells of chick embryos by the leukocytes of adult fowls followed a course which was absolutely analogous to the process of phagocytosis of foreign goose red cells, injected into the peritoneal cavity of a guinea pig, by the leukocytes of the recipient animal, as observed by I. I. Mechnikov.

The experimental findings suggest that the reaction of phagocytosis plays just as active a role in the process of embryonic development of birds as in the processes of metamorphosis of the Echinodermata [6], the Insecta [2, 5, 10, 11, 12],and the Amphibia [1, 7].

SUMMARY

The author studied the phagocytosis of red cells in chick embryos after 52 hours of incubation by the phagocytes of adult chickens. Experiments were conducted in vivo on the developing chick embryo to which fowl leukocytes were added, as well as in vitro, by mixing the red cells obtained from the embryo with the fowl leukocytes. The experiments demonstrated that fowl phagocytes behave with chick embryo red cells as with foreign cells, by effecting the initial stages of phagocytosis.

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