SPLEEN REGENERATION AFTER CHANGES RESULTING FROM ADMINISTRATION OF LIVING ANTIPLAGUE VACCINE

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Regeneration of organs in mammals can have two origins. The first of these is operative intervention - removal of part of the organ. The second origin, which can be termed a regenerative process, is injury of the organ by some agent - radiant energy, chemical substances, or microbes.

Regeneration after resection of part of the organ has received diverse and detailed study, whereas regeneration occurring after pathological changes has drawn the attention of investigators only to a smaller extent. The majority of works deal with regeneration after radiation. There are not many experimental investigations devoted to study of regeneration of organs after diseases caused by microorganisms [3].

Moreover, these data have not been correlated with the results of the study of reparative regeneration and have not been analyzed from the general point of view.

METHODS

Experiments were conducted on white mice weighing 16-18 g. Cultures of the vaccine strain of P. pestis were introduced into the mice intravenously, as a suspension containing 2000 microbial organisms in 0.1 ml of normal saline. Spleens of all the mice were examined twice: 12 and 40 days after the introduction of the vaccine.

Material examined on the 12th day was obtained after spleen biopsy. The spleen was brought to the exterior through an incision through the abdominal wall on the left side. Examination during the operation revealed that in 40-50% of the mice a considerable portion of the spleen was replaced by numerous polymorphic whitish nodules. Spleens were biopsied and subsequently studied only in those cases where numerous nodules were found.

Approximately half of the spleen was removed at biopsy. The remaining portion of the spleen was re-inserted into the peritoneal cavity and the wound was sutured layer after layer. The removed portion of the spleen was cut into three parts. The first part was fixed in Bouin's fluid, the second in 10% neutral formalin and the third was used to make touch (or impression) preparations. Mice were chloroformed 28 days after the operation. The spleens were removed, weighed and processed in the same way as the portion removed at biopsy. Fragments of spleens fixed after biopsy or after killing were embedded in paraffin and sectioned at 3 microns. Sections from fragments fixed in Bouin's fluid were stained with haemalum-eosin, with van Gieson's stain or according to Dominici-Kedrovsky. Reticulin fibers in sections from fragments fixed in formalin were impregnated according to Gomori. Touch preparations were fixed with methyl alcohol and stained with azur-eosin according to Noht-Maximow. The reconstruction of the pathologically altered spleen was studied by means of comparison of sections and touch preparations from the portion of the spleen removed at biopsy with those from the same spleen obtained 28 days later, when the regeneration of the organ had been taking place.

The possible effect of the operation on the course of the regeneration process is a separate problem and was not studied in the present work.

RESULTS

As has been indicated previously, investigation was undertaken only of those mice in which at the time of the operation, i.e., 12 days after vaccination, the spleen had markedly altered. Its size exceeded that of the spleen of the control animals 2-2$^1/_2$ times. The spleen was without exception studded with whitish dense polymorphic nodules.

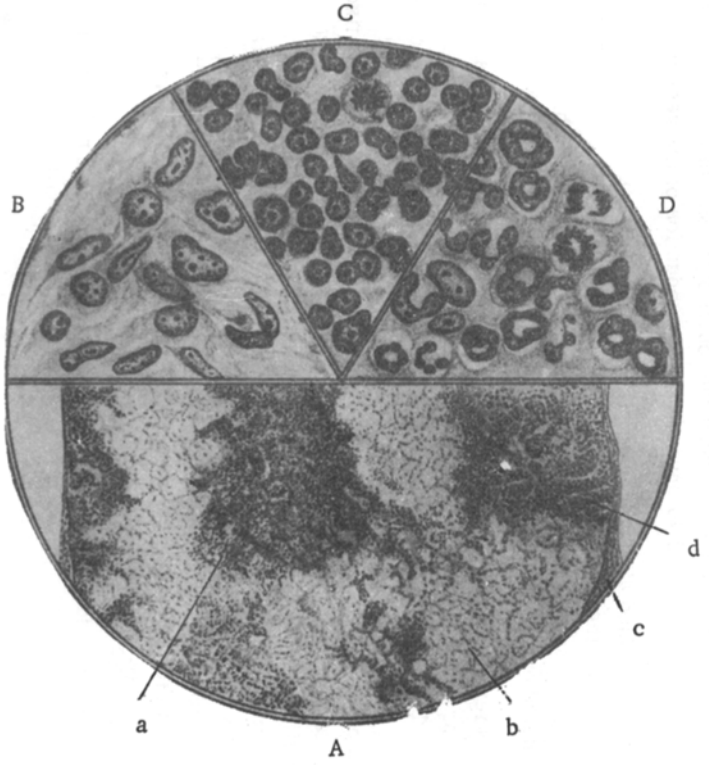

Fig. 1. Spleen of mouse No. 1 12 days after internal introduction of 2000 microbic bodies of vaccine strain EV. A) General view of portion of a cross section through the spleen: a) remains of Malpighian corpuscle; b) reticulocellular nodules; c) thickening of spleen capsule over reticulocellular nodule; d) myeloid hemopoisis focus. Magnification: objective 6 ×, ocular 6 ×; B) section of reticulocellular nodule. Magnification: objective 90 ×, ocular 6 ×; c) lymphocytes in remains of Malipighian corpuscle. Magnification: objective 90 ×, ocular 6 ×; D) remains of myeloid hemopoiesis focus. Mitosis. Magnification; objective 90 ×, ocular 6 ×.

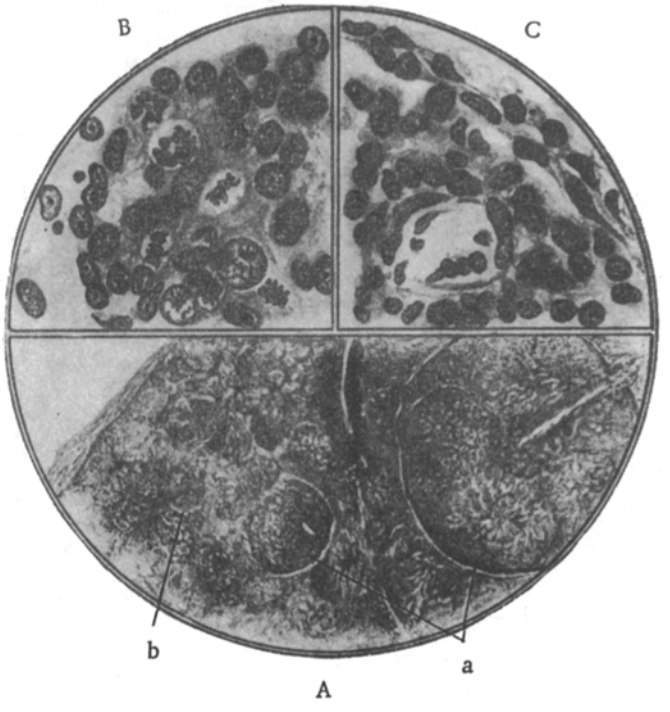

Fig. 2. Spleen of mouse No. 1 40 days after introduction of vaccine (28 days after operation). A) General view of portion of cross section through spleen; a) Malpighian corpuscle; b) red pulp. Magnification: objective 6 ×, ocular 6 ×; B) remains of embryonic center. Mitosis, Magnification: objective 90 ×, ocular 6 ×; C) remains of Malpighian corpuscle, central artery. Magnification: objective 90 ×, ocular 6 ×.

These nodules were united and replaced the spleen tissue, which was retained as red filaments and constituted no more than 15-20% of the whole of the organ tissue.

Study of sections prepared from spleen fragments removed at the time of the operation showed that the microscopic structure of the spleen at this period differed markedly from the usual. Red or white pulp could not be distinguished in the sections (Fig. 1,A). Typical Malpighian corpuscles were absent. Groups of cells of the lymphoid series were encountered only rarely near the central arteries (see Fig. 1, A; Fig. 1,B). About 40-60% of the total area of the section was occupied by nodules consisting of large, uniform cells with oval or elongated chromatin-deficient nuclei. The nodules had a circular or oval shape, sometimes being united and acquiring an apparent outline (see Fig. 1, A). Distribution of cells in such nodules was more or less regular (Fig. 1, B). In the mass of the nodule nuclei were distributed radially and became more elongated the nearer they were to the center. Nearer the periphery of the nodules its cells assumed a polygonal shape, but in the surface layer itself again became elongated, here being distributed concentrically. In the center of the nodule a caryopicnosis or caryopexis picture was as a rule observed, and also single, often disintegrated, leucocytes with polymorphic nuclei. If the nodule was located directly under the spleen capsule a reactive peritoneal node was formed over it (see Fig. 1,A). Most of the pulp unoccupied by nodes had regions of myeloid series cells at various stages of development (see Fig. 1,A; Fig. 1, D). In the preparations (especially clearly in the prints) impoverishment of spleen tissue by cells of the lymphoid and erythroid series was observed and increase in number of myeloid series cells. Large reticular cells were encountered more frequently than in the control.

The reticular spleen cells had been altered not only quantitatively, but also qualitatively. Besides normal reticular cells differing in the oval, poor nuclear chromatin and the pale cytoplasm having no clear boundary, altered forms were often encountered. They were characterized by a somewhat rounded nucleus and a pronounced basophilia of the cytoplasm. Cell forms were also often observed which in their structure were intermediate between the reticular cells and the lymphoblasts. They were characterized by a very large rounded nucleus with nucleoli, similar to a lymphoblast nucleus, and by a markedly basophilic, clearly delimited cytoplasm. These cells differed from the lymphoblasts in that they did not possess the normal round shape.

Thus, at the time of the operation the spleen of the mice had changed markedly. The changes were manifested in the formation of numerous reticulocellular nodules and areas of myeloid hemopoietic foci, replacing both the red and the white pulp.

By the time of the autopsy of the mice which had been operated upon (40 days after vaccination) the dimensions of the remaining spleen fragment had not changed or they had decreased in size only slightly. The spleen had a nearly normal color. Its surface was uneven, often with poorly defined grayish spots, as if repeating the marking of the reticulocellular nodules piercing the spleen at the time of the operation. The original shape of the organ was not restored.

A study of the sections showed that by this time spleen structure was quite close to normal. Most apparent was a definite separation into a red and white pulp. Malpighian bodies were clearly delimited (Fig. 2, A), in them being visible the central arteries and embryonic centres with dividing cells (Fig. 2, B and C). The structure of the red pulp differed somewhat from normal, in it being observed numerous small lymphoid hemopoietic foci (see Fig. 2, A), and a network of argyro fibers denser than usual. In the majority of the animals investigated the reticulocellular nodules penetrating the entire spleen at the time of the operation were entirely absent in the sections. Only in one case were small groups of cells found, in their structure reminiscent of the cells of which the nodules were formed. Cells of the lymphoid, erythroid and myeloid series were found in the red pulp as small islands. In these islets numerous mitoses were visible. The spleen capsule was thickened in places. The impression was created that these swelling occurred in locations where at the time of the operation there were reticulocellular nodules.

A similar scene was observed in the print-preparations, where lympoid series cells predominated. Compared with normal, the number of lymphoblasts had increased, and they had often divided by the mitotic method. Groups of erythroid and especially of myeloid cells were encountered much less frequently.

Thus, it can be considered established that in 28 days (the observation period in this experiment) restoration of spleen structure can occur, in appearance very close to normal. A similar process has been observed by A. Ya. Friedenstein [4] in guinea-pigs in the case of reversible development of the granules arising after introduction of vaccine B TsZh.

It has already been indicated above that in our experiments spleen weight in the mice after introduction of vaccine EV had increased 2-2½ times after 12 days. 28 days after the operation the weight of the remainder of the

organ had not changed substantially; consequently, restoration of normal structure of the organ had been effected by substitution of pathologically altered tissue by normal spleen tissue. Only in one instance was formation of a small scar observed, evidently at the location of the reticulocellular nodule. Degree of restoration of the organ varied in the various animals and depended on the extent of the injury.

The data obtained shows that spleen regeneration can take place not only after its partial removal but also after replacement of the major part of the tissue of this organ by reactive structures formed as a result of introduction of antiplague vaccine. The similarity of these processes is shown by the fact that in both cases regeneration proceeds within the organ throughout the whole of its tissue. In the spleen, as in most internal organs, regeneration takes place as a result of a type of regenerative hypertrophy [2]. Together with this, well-known differences between the two processes are observed. Regeneration of an altered spleen is based on the stroma contained within it to a greater or lesser degree. The regeneration process amounts to substitution of altered tissue structure by a structure approximating the usual. In this regard, structure normalization can be accompanied by some decrease in the weight of the organ, which is possibly connected with the reduction in volume of myeloid hemopoiesis in the spleen. In contrast to this, in reparative regeneration formation of a considerable amount of new spleen tissue occurs, as a result of which the weight of the remainder of the organ increases.

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