AN EXPERIMENTAL STUDY OF THE PHAGOCYTIC APPARATUS OF THE WALL OF THE RABBIT'S APPENDIX

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny,
Vol. 53, No. 6, pp. 91-95, June, 1962
Original article submitted March 18, 1961

The morphology and physiology of the vermiform appendix of the rabbit have now been the subject of considerable research [2, 3, 13, 17]. Its results indicate that this part of the rabbit's intestine plays a definite part in a group of important physiological processes and, in particular, in digestion in the cecum.

Special interest has been shown in the unique phagocytic apparatus found in the lymphoid follicles in the wall of the rabbit's appendix, first mentioned in the works of Bizzozero [12], Ribbert [18], and Ruffer [19]. During subsequent years many articles were published on the description of the phagocytic processes in the wall of the rabbit's appendix and on their relationship to the morphology of the organ [11, 14]. It is only recently, however, that as a result of the use of new histochemical staining methods, new details were discovered of this phenomenon that had previously escaped the attention of researchers [6, 7].

More recent work has shown that vast numbers of microorganisms, comprising the natural flora of the cecum and giving a well defined histochemical reaction for polysaccharides, are continually penetrating through the epithelium of the appendix of normal rabbits into the submucosa. These microorganisms are ingested by the reticulum cells of the lymphoid follicles and gradually destroyed (Fig. 1). Meanwhile, in the cytoplasm of the reticulum cells of the germinal centers of the lymphoid follicles of polymorphic PAS-positive material is found, which, as special investigations have shown, differs histochemically from the substances sometimes found in macrophages and giving a positive reaction for polysaccharides [4].

The distinctive topography of the appendix in the adult rabbit, and also the presence of a continuously functioning macrophage apparatus in its wall, have enabled this organ to be used on more than one occasion as a very convenient model for the study of both general and local problems in experimental pathology [5, 6, 9, 20].

We undertook an experimental study of the phagocytic apparatus of the vermiform appendix of the adult rabbit in the following conditions: 1) a change in the structure of the wall of the organ; 2) changes in the topographic relations between the appendix and the large intestine (isolation of the appendix from the cecum).

EXPERIMENTAL METHOD

As an agent to produce changes in the mucous membrane of the organ in situ we used a 5% aqueous solution of $AgNO_3$ possessing a weak astringent action on the tissue [17]. This solution, in a volume of 2-3 ml, was introduced by means of a Pasteur pipet into the lumen of the appendix through a puncture in its apex. Test material in the form of pieces of the wall of the organ were taken on the 2nd, 5th, 7th, 10th, 15th, and 25th days, fixed by Shabadash's method and embedded in paraffin wax. Sections were stained for polysaccharides (PAS reaction) [8, 15, 16] and counterstained with Ehrlich's alum hematoxylin.

The appendix was isolated from the cecum by means of an operation of our own conception. The principle of this operation was to create an "internal fistula" of the isolated distal segment of the appendix by anastomosing it with the proximal portion of the small intesting (Fig. 2). As a result of this operation the required isolation of part of the appendix was unaccompanied by any disturbance of the mechanism of drainage of secretion from its lumen. It should also be pointed out that the presence of a nonisolated proximal part of the appendix enabled the dynamics of the morphological and functional changes in the wall of the isolated part of the organ to be studied and compared with their normal course.

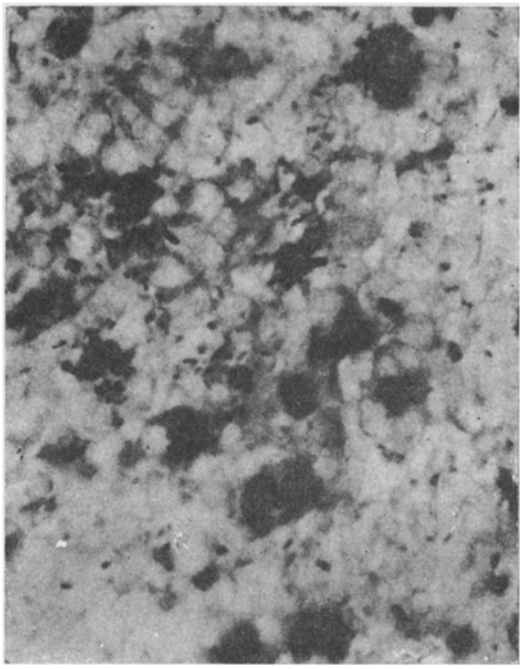

Fig. 1. The appendix of an adult rabbit. Microorganisms in a lymphoid follicle. Phagocytosis of microorganisms by macrophages of the lymphoid follicle. PAS reaction. Objective 45 ×, eyepiece 10 ×.

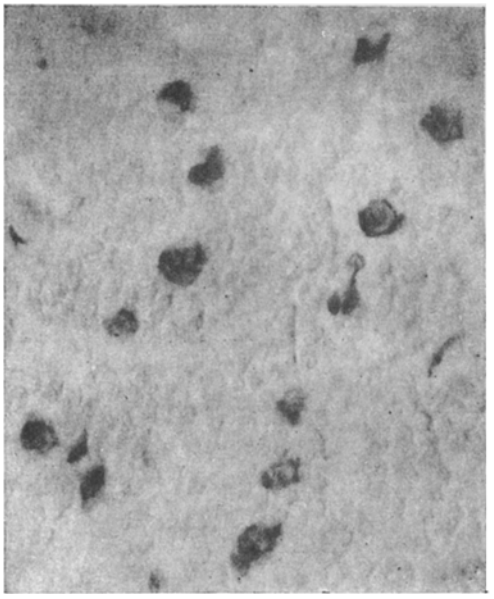

Fig. 3. Isolated appendix of a rabbit. Time after operation $1^1/_2$ months. Absence of free and phagocytosed microorganisms in the lymphoid follicle. No masses of PAS-positive material are present in the cytoplasm of the macrophages. Objective 24 ×, eye-piece 10.

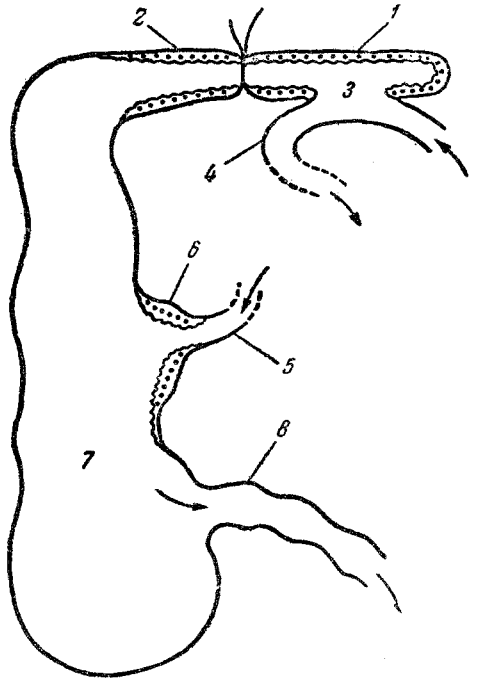

Fig. 2. Isolation of the vermiform appendix of a rabbit from the cecum (scheme). 1) Isolated part of the appendix; 2) nonisolated part of the appendix; 3) anastomosis between the appendix and the jejunum; 4) jejunum; 5) ileum; 6) lymphoid diverticulum of the ileum; 7) cecum; 8) colon.

The operation was performed under ether anesthesia. The appendix was mobilized. Its contents were expressed into the cecum, after which it was ligated roughly in the middle with a silk thread in such a way that the blood supply to the isolated part was preserved. A side-to-side anastomosis was formed between the isolated part and the proximal portion of the jejunum. The intestinal wound was closed by a single continuous Mateshuk's suture [1] as modified by E. Shtro [10]. The material was taken at intervals of 2 weeks, and $1^1/_2$, 2, and $4^1/_2$ months after the operation. Both the isolated and nonisolated parts of the appendix of each animal were treated simultaneously.

EXPERIMENTAL RESULTS

Introduction of a 5% aqueous solution of $AgNO_3$ into the lumen of the appendix. The stage of the wall of the appendix during the first 48 hours after introduction of the $AgNO_3$ solution was characterized by severe destructive changes in the mucous membrane of the organ. The epithelium of the orifices of the crypts was wrinkled and disintegrating, with ulceration here and there. Consequently, in some cases at this point large and small epithelial cysts were

being formed in the mucous membrane of the appendix. Particularly noteworthy were the cysts formed as a result of obliteration of the crateriform crypts. These cysts consisted of spherical, sterile spaces, lined with a single layer of flattened epithelial cells among which were goblet cells. The continuous epithelial lining of the cyst was surrounded by a layer of loose connective tissue. As a result of this process of cyst formation the part of the lymphoid follicles corresponding to these cysts became isolated from the lumen of the intestine, and their interior became favorable to the development of sterility. No microorganisms were observed inside such follicles, but many macrophages could still be seen there, containing droplets and granules of a PAS-positive material in their cytoplasm.

The greater part of the crypts, however, retained their typical structure. The epithelial lining in the depth of the crypts remained unchanged as a rule. Necrobiotic changes were not found in the epithelium lining the heads of the lymphoid follicles forming the base of the crateriform crypts. The structure of the lymphoid follicles was unchanged. Their phagocytic apparatus, however, showed definite changes, consisting essentially of the complete disappearance of microorganisms from the apical portions of the follicles and a decrease in the number of macrophages in these areas. Well defined pictures of the initial phases of phagocytosis in the apical portions of the lymphoid follicles, characteristic of the normal appendix, would not be found in these circumstances. The cytoplasm of the macrophages of the subepithelial zone was pale pink and contained fine granules but, as a rule, no formed inclusions of PAS-positive material. Only in the basal portions of the lymphoid follicles was the number of macrophages greatly increased. They characteristically contained microorganisms which now showed considerable destructive changes. A highly polymorphic bacterial detritus and large drops of PAS-positive material were seen in the cytoplasm of these macrophages.

The decrease in the infiltration of microorganisms into the apices of the lymphoid follicles is evidently explained by the partial sterilizing action of the colloidal silver formed after introduction of $AgNO_3$ solution into the lumen of the appendix. Naturally this action cannot last long. In fact, starting on the 5th day, solitary microorganisms could be seen in the subepithelial layer of individual follicles. Meanwhile an increase in the number of macrophages was observed in the heads of the follicles, some of them actively engaged in phagocytosis. The intermediate phases of ingestion of microorganisms in the macrophages of the middle zone of the follicles were not well defined. In the basal areas, however, the macrophages contained much bacterial detritus and loosely packed collections of PAS-positive material. Meanwhile the reticulum cells of the germinal centers were highly vacuolated, and their content of PAS-positive material was less than normal.

Between the 7th and 25th days the phagocytic apparatus gradually returned to normal. This was not observed, however, in the follicles separated from the lumen of the intestine by epithelial cysts. In this case sterile conditions were maintained in the follicle, and the macrophage apparatus continued to regress. The cytoplasm of the macrophages "emptied" and became pale pink in color. The germinal centers, consisting of large, polygonal, reticulum cells, rich in cytoplasm and with large nuclei, lost all their PAS-positive material except for pigment granules staining black with Sudan.

Isolation of the appendix from the cecum. During the whole period of observation no significant morphological or functional changes could be detected in the wall of the nonisolated part of the appendix. Many microorganisms could always be found in the lymphoid follicles of this part, and the picture of their completed phagocytosis were identical with those characteristic of the normal organ.

Two weeks after the operation no severe destructive changes could be seen in the wall of the isolated part of the appendix and the natural relations were maintained between its layers. Only a slight decrease in the size of the submucosal layer could be seen, as a result of flattening of the lymphoid follicles. The muscular coat, on the other hand, showed a tendency to hypertrophy. Hardly any microorganisms were seen in the lymphoid follicles. Solitary phagocytosed bacterial cells were found only in the macrophages of the apical parts of the follicles, and phagocytosis was completely absent in the middle and basal parts of the sections. The very few macrophages scattered throughout the follicle had a homogeneous, pale pink cytoplasm. The germinal centers were poorly supplied with PAS-positive material, and disintegration of compact masses of this material into small particles could be clearly seen. Some reticulum cells in the germinal centers were completely emptied, and contained only polymorphic pigment granules in their cytoplasm.

After $1\frac{1}{2}$ months the layer of lymphoid follicles in the wall of the isolated part was reduced to approximately half its normal size. They contained no microorganisms and very few macrophages, which stood out among the lymphocytes because of their homogeneous, pale pink cytoplasm. The germinal centers were atrophic and poorly supplied with PAS-positive material.

After 2 months the lymphoid follicles had become round in shape. No microorganisms were seen in them. As a rule few macrophages were present. The reticulum cells in the germinal centers contained mainly pigment granules.

More striking changes in the structure of the wall of the isolated part developed $4\frac{1}{2}$ months after the operation. The lymphoid follicles were reduced still further and sclerosed. The epithelial crypts pitted deeply into the submucosa, sometimes reaching the muscular coat. The latter was greatly hypertrophied. The large reticulum cells of the lymphoid follicles contained pigment granules, and only a few showed traces of PAS-positive material. The germinal centers were empty. Phagocytosis of microorganisms was absent, although in the depth of some follicles solitary bacterial cells could be seen.

These details show that the phagocytic apparatus constantly functioning in the wall of the appendix of the adult rabbit possesses a complex mechanism and can utilize all its resources only when certain conditions are satisfied. A change in the normal morphological relations in the wall of the appendix, sterilization of its lumen, or disturbance of the normal functional and anatomical relationships with the large intestine (the cecum) may be reflected in the state of the phagocytic apparatus, including inhibition of macrophage activity and a modification of the histochemical characteristics of the process as a whole.

The results obtained after experimental isolation of the appendix from the large intestine also indicate the close relationship between its phagocytic apparatus and the enzymic processes taking place in the cecum of adult rabbits. A special role in these processes is played by symbiotic microorganisms, capable of synthesizing intracellular polysaccharides.

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