STRUCTURAL AND IMMUNOMORPHOLOGICAL CHARACTERISTICS OF THE HUMAN FETAL APPENDIX

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The lymphatic system of the human fetus has not yet been adequately studied despite the importance of this problem to the understanding of the mother–fetus system. One reason for this may be difficulty in obtaining fresh material with which to study this system and its wide dispersion throughout the body. This last factor makes investigations on fetuses particularly difficult, because organs of the immune system are not laid down at the same time, and each one completes its development independently. After the discovery of the central organ for B lymphocyte differentiation in birds, the bursa of Fabricius, attempts were made to discover the analog of this organ in mammals. This accounts for the increased interest in the study of the appendix as a possible analog of the bursa of Fabricius.

Nearly all research in this direction has been undertaken in experiments on rabbits [8, 9, 12] and very few on the human appendix [6, 11]. Some workers [9, 12] state that this organ can be regarded as the analog of the bursa of Fabricius, whereas others [5, 8] reject this view. In one or two publications [1, 6] brief information is given on the embryogenesis of the human appendix. T and B lymphocytes are known to be present in the appendix of children and adults [11]. Workers in the writers' laboratory [3, 4] previously determined immunomorphological parameters of some of the most important lymphopoietic organs of the human fetus, and these are used in the present study for comparison with the corresponding parameters of the developing appendix.

The aim of the present study was to discover the time of appearance of lymphoid follicles (LF) in the wall of the human fetal appendix, to identify the character of the reticular stroma of LF and the character of lymphocyte differentiation, and to determine the direction of lymphocyte migration from LF.

EXPERIMENTAL METHOD

Ninety appendices were used for histological investigations: they were obtained from fetuses at between 12 and 31 weeks of intrauterine development, obtained from clinically healthy mothers. The age of the fetus was determined from the last maternal ovulation and the length of the fetus. The organ was fixed whole in a 10% neutral formalin solution and in Carnoy's fluid. Sections were stained with hematoxylin and eosin, by Brachet's method, and with Schiff's reagent, accompanied by the corresponding controls; reticulin fibers were determined by Gordon's method [2]. For the immunomorphologic investigations a lymphocyte suspension was obtained from the appendix by means of a glass homogenizer, and this was followed by isolation of the mononuclear fraction on a Ficoll–Verografin density gradient. The cell suspensions thus obtained were washed three times with medium 199 and the concentration was adjusted to 1-2 million cells/ml. The number of dead cells in the test with 0.1% trypan blue solution did not exceed 5%. T lymphocytes (E-RFC) were determined by the methods of spontaneous rosette formation of human lymphocytes with sheep's erythrocytes by the method in [10], and B lymphocytes (EAC-RFC) were determined by the spontaneous rosette formation test of lymphocytes with bovine erythrocytes, loaded with antibodies of the primary response and with complement, obtained from fresh noninbred mouse serum diluted in the ratio of 1:10. The E-RFC and EAC-RFC were counted in a Goryaev's chamber per 200 lymphocytes and expressed in percentages. The numerical results were subjected to statistical analysis on the Nairi K computer.

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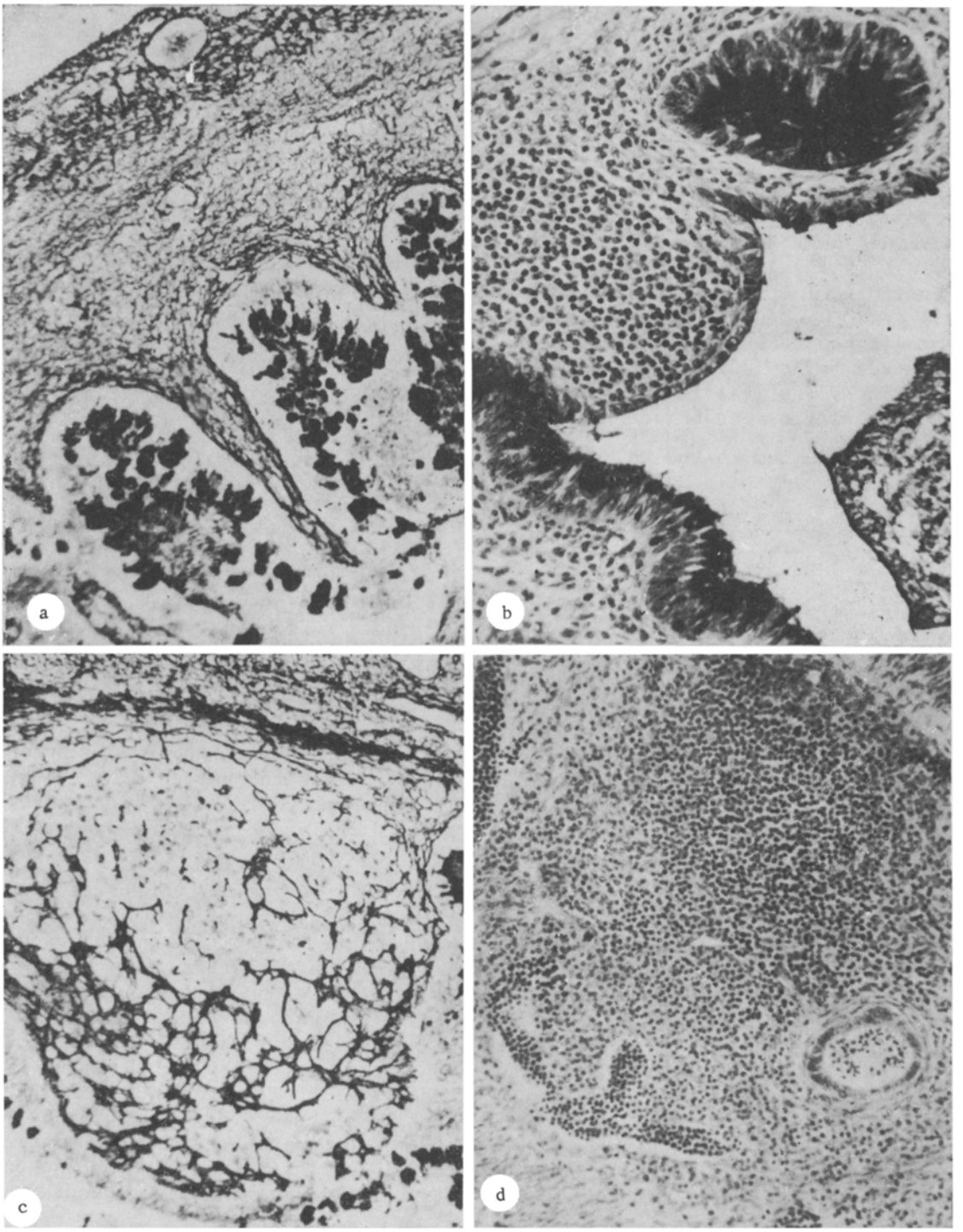

Fig. 1. Wall of human appendix at 15-31 weeks of development: a) 15-week fetus, impregnation with silver nitrate by Gordon's method, 260×; b) 24-week fetus, stained with Schiff's reagent, 260×; c) 30-week fetus, impregnation with silver nitrate by Gordon's method, 260×; d) 23-week fetus, stained by Brachet's method, 160×.

EXPERIMENTAL RESULTS

Complex processes of formation of the mucous membrane take place in the human fetal appendix before the 15th week of development and they call for special study. The inner surface of the organ was covered by simple cylindrical epithelium, secreting mucus, beneath which there was a dense network of thin reticulin fibers (Fig. 1a). By the 15th week of development only solitary lymphocytes or small groups of them could be identified in this stroma and they

could not be removed from the organ into a suspension. The first small LF could be detected in the appendix in a 17-week fetus. From this time a lymphocyte suspension could be obtained and its cellular composition determined. Meanwhile the network of reticulin fibers in the intestinal wall was altered. In the fetal appendix at 17-19 weeks both E-RFC and EAC-RFC could be identified (Table 1). Their relative percentages were almost identical. A very small decrease in the percentage of EAC-RFC was observed. In fetuses from 20 to 31 weeks of development the number and size of the LF in the appendix increased. They were always located between high lateral folds of mucous membrane, covered by cylindrical epithelium producing a mucous secretion, just as over the whole surface of the organ. In places of LF formation (Fig. 1b) there was a broad round swelling, clearly demonstrable in rabbits [12] and called the cupola. In this area of the intestine changes in the surface epithelium took place. From simple cylindrical it was converted into cubical or even squamous. In the cytoplasm of the epithelial cells no neutral glycoproteins could be detected, evidence that secretion of mucus by the cells had ceased in the region of the cupola and that their intercellular connections were weakened. The latter enabled better penetration of the lymphocytes through the epithelium into the intestinal lumen. At this time lymphocytes were present in the lumen of the appendix and could be seen between the epithelial cells.

Beneath the epithelium in the region of the cupola the network of reticulin fibers became sparse (Fig. 1c), all the spaces in it were filled with lymphocytes, and two zones of reticulin fibers could be distinguished in it. Previously regions corresponding to zones of T and B lymphocytes were identified in the reticular stroma of the human fetal spleen [4]. Comparison of the reticular stroma of the splenic LF with the network of reticulin fibers in the appendix suggests that here also there were LF of mixed type (Fig. 1c), in which, in the 30-week human fetus, zones of T and B lymphocytes were present. The first zone was characterized by the presence of thick, more compactly arranged reticulin fibers. This network was located at the top and sides of the cupola and corresponded to its description in rabbits [12]. The zones of B lymphocytes contained thin fibers, sparsely distributed. This zone lay at the lowest levels of LF. No reactive center was yet present in it in the fetus. During growth of the organ and an increase in the number and size of the follicles, the number of T and B lymphocytes in the appendix increased. The latter became rather more numerous (Table 1) and their percentage increased from 16 to 26, whereas the percentage of E-RFC changed very little, while not differing significantly from the former. After 20 weeks of development the lymphatics surrounding LF became filled with lymphocytes, evidence of an outflow of the latter from LF and from the organ (Fig. 1d).

LF could be identified in the appendix wall of the 17-week fetus. Composite tissue structures appeared in the intestinal mucosa at this time, including epithelium of a special structure, reticular tissue, and lymphocytes and they could be identified as independent formations, to which the name epitheliolymphons was given. These latter were cupola-shaped and the epithelium covering them had modified histophysiological properties, facilitating passage of the lymphocytes to the surface of the mucosa, whereas the reticular tissue created the particular microenvironment necessary for lymphocyte differentiation. Lymphocytes penetrated from the fetal epitheliolymphon into the lumen of the intestine and departed from the appendix along the lymphatics. The presence of T and B lymphocytes, the latter slightly more numerous, was demonstrated for the first time in a cell suspension from the human fetal appendix, as identified by surface markers. The appearance of B lymphocytes in the appendix of the 17-week fetus, when according to published data [7], B cells are already present in its bone marrow at the 14th week of development, does not permit the human appendix to be

TABLE 1. Number and Kinetics of E-RFC and EAC-RFC (in %) in Human Fetal Appendix

Age of fetus, weeks	Number of fetuses	E-RFC	EAC-RFC
17—19	3	18,7±2,33	16±7,02
20—23	10	12,7±1,6	18,2±2,27
24—27	5	19,2±1,9	22,2±1,5
27—31	5	18,2±5,03	26±6,8

regarded as the analog of the bursa of Fabricius, but justifies the conclusion that it is a peripheral organ participating in local immune reactions and forming part of the general system of immunity.

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A NEW HEMATOLOGIC METHOD OF DETECTING LATE SEQUELAE OF TRANSIENT MYOCARDIAL ISCHEMIA

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Changes in the cell composition of the peripheral blood in experiments on animals have been studied only during the first few hours after "subcritical" [11], transient myocardial ischemia [10]. Routine hematologic tests in the subsequent period can no longer reliably reveal evidence of previous transient disturbance of the blood supply to the heart [1]. Moreover, as has been pointed out, even in the case of irreversible disorders of the coronary circulation, involving the development of a myocardial infarct, the cell composition of the peripheral blood showed changes during the next few days in only a proportion of cases [9].

Accordingly the writers suggest a new technique for hematologic monitoring of the sequelae of reversible disturbances of the coronary blood flow, during the use of which repeated determinations of the cell composition of the blood are combined with a function test that stimulates an increase in the number of circulating leukocytes. The object of the present investigation was to test the effectiveness of this method of discovering latent postischemic leukocytosis by the use of a model of reversible local disturbances of the coronary blood flow in chronic experiments on dogs.

EXPERIMENTAL METHOD

To prepare the animal for the experiment a device for controlling the blood flow in the circumflex branch of the left coronary artery was implanted [5]. Some 2 to 3 weeks later the animal was used in the experiment, for which either the animal received no medication or it was preceded by a single intramuscular injection of 0.1 mg fentanyl and 5.0 mg droperidol. These drugs were given only when the dog strapped to the frame exhibited increased motor activity. Altogether 21 chronic experiments were performed on five animals, in which the leu-

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