It can be concluded from the results that in the stages of initial organogenesis, when the placenta, liver, and other structures capable of metabolizing acetaldehyde were not present [12], this product of ethanol metabolism may represent a greater risk for embryonic development than ethanol itself, and moreover, in concentrations which may actually arise in human blood after taking alcohol [5, 12]. Incidentally, the spectrum of anomalies induced by acetaldehyde is wider than that induced by ethanol; the embryotoxic effect of acetaldehyde, moreover, is realized within a short time (a few hours). However, despite the much greater embryotoxicity of acetaldehyde than of ethanol, which the experiments in vitro revealed, it is probably more correct when speaking of embryos developing *in utero* of their combined action of the fetus, for each of these substances possesses embryotoxic properties to some degree or other. The degree of the harmful action of each compound (ethanol or acetaldehyde) on the fetus probably depends on the character (quantity and frequency) of alcohol consumption and on individual features of the metabolism of these substances in the mother.

LITERATURE CITED

1. A. P. Dyban, V. F. Puchkov, V. S. Baranov, et al., in: Objects in Developmental Biology [in Russian], Moscow (1975), p. 510.
2. V. B. Popov, B. L. Vaisman, and V. F. Puchkov, Byull. Éksp. Biol. Med., No. 5, 613 (1981).
3. N. A. Brown, E. H. Goulding, and S. Fabro, Science, 206, 573 (1979).
4. A. G. Fantel, J. C. Greenway, et al., Life Sci., 25, 67 (1979).
5. M. A. Karsten, S. Matsuzaki, L. Feinmen, et al., New Engl. J. Med., 292, 386 (1975).
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
7. A. P. Streissguth, S. Langesman-Dwyer, J. C. Martin, et al., Science, 209, 353 (1980).
8. D. A. T. New, in: Methods in Mammalian Embryology, San Francisco (1971), p. 305.
9. K. S. O'Shea and M. H. Kaufman, J. Anat., 132, 107 (1981).
10. R. Rognstad and N. Grunnet, in: Biochemistry and Pharmacology of Ethanol, Vol. 1, New York (1979), p. 65.
11. P. V. Veghelyi and M. Osztovics, Experientia, 34, 195 (1978).
12. H. Weiner, in: Biochemistry and Pharmacology of Ethanol, Vol. 1, New York (1979), p. 125.

STRUCTURE OF THE HUMAN SPLEEN AND IMMUNOMORPHOLOGICAL PARAMETERS OF ITS LYMPHOCYTES DURING EMBRYOGENESIS

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UDC 616.411-091-07:616.411-008.939.624-097

KEY WORDS: spleen; human fetus; morphogenesis; lymphocytes.

The spleen is a complex multifunctional organ. It produces more immunoglobulins (IgM and IgG) than other organs [9, 11]. After splenectomy their content in the body falls and the risk of development of septicemia and of the patient's death is increased [13]. Recent investigations have shown that most of the T suppressors [4] affecting the immunologic reactivity of the body are concentrated in the spleen. Finally, the spleen performs the function of blood depot, and many metabolic processes take place in it. The spleen thus plays an active part in various reactions aimed at maintaining normal homeostasis. However, there have been few studies on the structure and function of this organ in man, especially in the embryonic period of development, when the main components of the spleen are formed. The authors of several publications [1-3, 6, 8, 10, 12] have emphasized the need for the study of the spleen during this period of development.

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 12, pp. 728-731, December, 1981. Original article submitted May 5, 1981.

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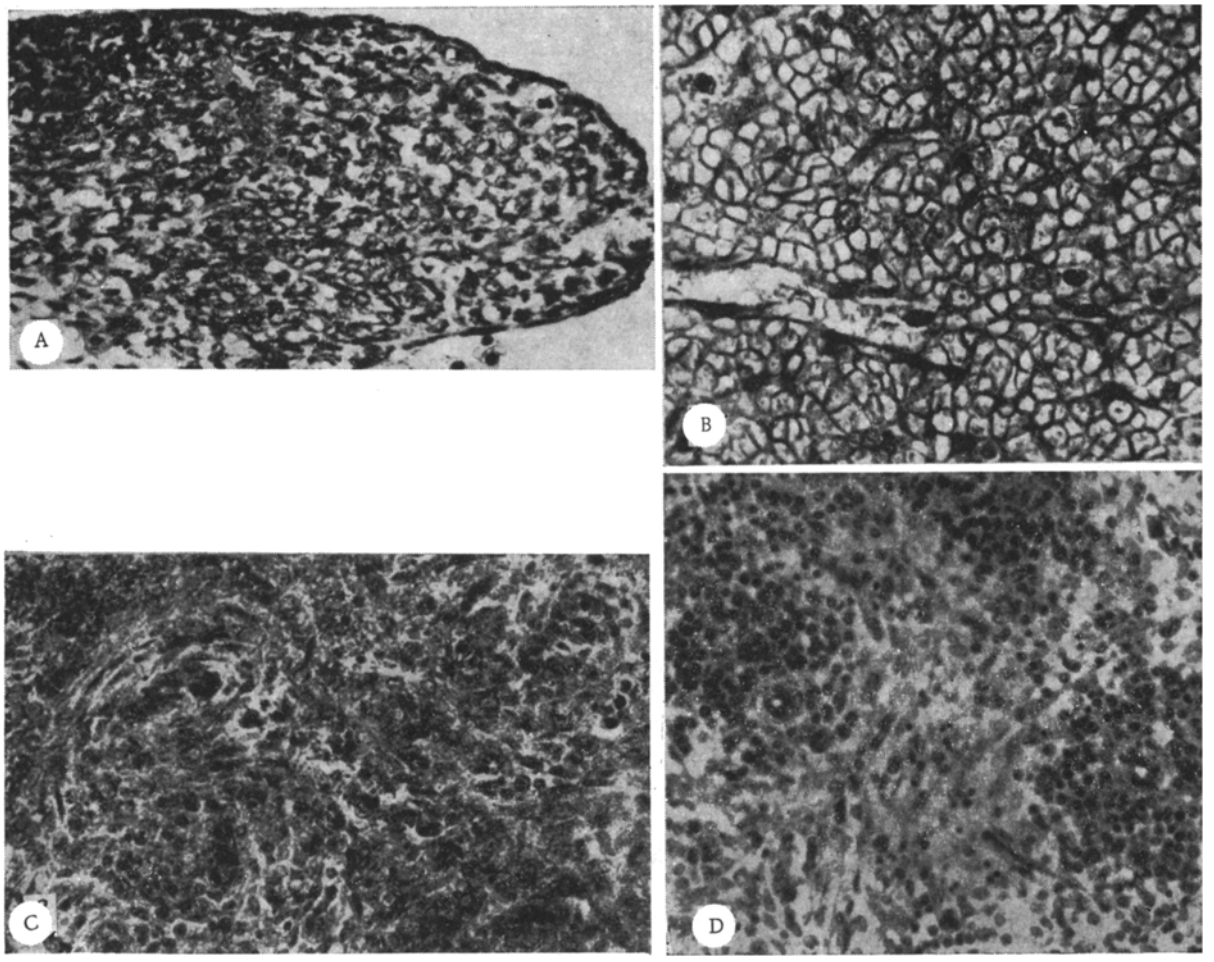

Fig. 1. Human fetal spleen at different times of development: A) 8 weeks, b) 9–10 weeks, C) 14 weeks, D) 17 weeks. Fixation with Carnoy's fluid. Staining: A, B, C) hematoxylin and eosin, D) Brachet. Magnification: A, C, D) 250 ×, 500 ×.

The development and differentiation of the cellular and tissue structures of the human fetal spleen were studied in the investigation described below. There have been no previous studies devoted simultaneously to tissue formation and lymphocyte differentiation in this organ in man.

EXPERIMENTAL METHOD

Spleens of 100 fetuses aged from 5 to 34 weeks, obtained from clinically healthy mothers in various hospitals, were used. The age of the fetus was determined from its length and the time of the mother's last ovulation. Sections were stained with hematoxylin and eosin and by Brachet's and McManus' methods [5]. The number of lymphocytes in the whole organ and in 1 mg tissue was determined in a suspension of spleen tissue. The monocyte fraction was isolated from the cell suspension in a Ficoll-Verografin gradient. The cell suspension, washed 3 times with medium No. 199, were adjusted to a concentration of 2–4 million cells/ml. The number of dead cells in the test with 0.1% trypan blue solution did not exceed 5%. The number of T lymphocytes was determined by the method of spontaneous rosette formation of lymphocytes with sheep's red blood cells (E-RFC) according to Jondal et al.

B lymphocytes with surface Ig were determined by the indirect immunofluorescence method using monospecific rabbit serum against human IgG and commercial donkey serum against rabbit Ig labeled with fluorescein isothiocyanate. Determination of T and B lymphocytes was accompanied by the usual control. Rosettes and luminescent cells were counted per 200 lymphocytes in a Goryaev's chamber and the results expressed in per cent. The data were subjected to statistical analysis on the Nairi-K computer.

TABLE 1. Gravimetric Indices and Percentages for the Human Fetal Spleen in Embryogenesis

Age of fetus, weeks	Weight of organ, mg	Number of lymphocytes		T lymphocytes (E-RFC)	IgG^+- cells
		in whole organ	in 1 mg of organ	%, $M \pm m$	
9—10	—	—	—	0 (n=2)	0 (n=4)
12—14	9 (n=2)	$175 \cdot 10^3$ (n=2)	$24{,}9 \cdot 10^3$ (n=2)	2,5±2,5 (n=3)	13,0±2,0 (n=3)
14—17	65,8 (n=2)	$7{,}2 \cdot 10^6$ (n=2)	$96 \cdot 10^3$ (n=2)	—	—
18—22	379,8 (n=8)	$50{,}8 \cdot 10^6$ (n=8)	$253{,}3 \cdot 10^3$ (n=7)	15,8±4,0 (n=13)	26,3±2,4 (n=8)
27—34	2340,4 (n=5)	$817{,}6 \cdot 10^6$ (n=6)	$368{,}6 \cdot 10^3$ (n=5)	11,0±1,5 (n=44)	31,0±2,9 (n=6)

Legend. n) Number of fetuses studied; IgG^+-cells) lymphocytes with class G immunoglobulin receptors on their surface.

EXPERIMENTAL RESULTS

In the earliest stages of development (5-6 weeks) the spleen tissue was very compact and consisted of densely arrange mesenchymal cells, in which the lumen of the blood vessels was hardly distinguishable. At the 8th week the structure of the spleen showed very little change. The compactness of the cells was reduced, the lumen of the blood vessels was more clearly defined, and the vessels contained large nucleated erythroid cells (megaloblasts) (Fig. 1A).

In the 9-10-week fetus the spleen acquired its characteristic appearance. At this stage the picture was that of an "empty spleen" being virtually filled with an enormous number of anuclear erythrocytes (Fig. 1B). This period evidently coincides with the following morphological phenomenon: invasion of the hilus of the spleen by large vascular trunks, which begin to divide and to form numerous thin-walled vessels; loss of their nuclei by the splenic erythrocytes; granular disintegration of hemoglobin and its discharge from the erythrocytes; commencing disintegration of the erythrocytes themselves. These processes were more intensive still at the 11th week, when rapid development of trabeculae with muscle cells and a simultaneous increase in size of the invading blood vessels occurred in the spleen. At this time a few blood islets consisting of normoblasts and erythrocytes, the latter with the features of disintegration, were found in the spleens. Thus the function of blood depot predominated over all other functions of the spleen at this period. What appeared to be physiological hemorrhage into the tissues of the organ was observed. It can be postulated that these processes in the spleen evidently take place in connection with the beginning of the bile-excreting function of the fetal liver, for breakdown products of erythrocytes necessary for the formation of bile pigments were found at the biliary pole of the hepatocytes. At this time at the periphery of the spleen transversely cut trabeculae were found beneath the capsule, and these could be mistaken for lymphoid follicles. However, our investigations showed that no true colonization of the organ by lymphocytes had yet occurred at this stage.

The stromal skeleton of the first lymphoid follicles was detected in the spleen at the 13th-14th week of fetal development (Fig. 1C). It contained a central artery with reticular cells and blood sinuses, containing a few lymphocytes, arranged concentrically around it. Tissue filled with anuclear erythrocytes appeared to be lying between the developing lymphoid follicles in the form of bands forming the red pulp of the organ. The spleen could now be weighed for the first time, and the result was 9 mg (Table 1), the lymphocyte count was 175×10^3, or 24×10^3 lymphocytes in 1 mg tissue, reflecting the density of the lymphocytes in the spleen. T and B lymphocytes could be clearly identified, with the latter more numerous. The period of 13-14 weeks of development of the spleen was thus characterized by the combination of several functions in the organ: lymphopoietic, depot, hemolytic, and, to only a weak degree, erythropoietic. As Table 1 shows, at 15-17 weeks there was a sharp increase in the weight of the spleen and in the number of lymphocytes contained in it, whether expressed per whole organ or per milligram tissue. These processes were accompanied by an increase in size of the lymphoid follicles (Fig. 1D) and in the density of the lymphocytes colonizing them.

The period of development of 18-22 weeks was distinguished as the critical period in the lymphopoietic function of the spleen. The weight of the organ and the number of lymphocytes in it continued to increase later (Table 1), but there was virtually no change in the density of the lymphocytes per milligram tissue after the 22nd week of development. The percentage of T lymphocytes (T-RFC) rose sharply toward this time, but thereafter remained unchanged. Ryabchikov, working in our laboratory, recently distinguished for the first time among T lymphocytes of the fetal spleen cells carrying receptors for the Fc-fragment of IgG on their surface (Tγ-cells), which other worker described in adults as T suppressors. Their percentage increased with the age of the fetus, but did not exceed 5.6 by the 28th week of development. The number of T lymphocytes also doubled by the 22nd week, but thereafter was virtually unchanged until birth. However, although the number of B lymphocytes remained constant, their qualitative differentiation still continued [7].

The spleen was thus shown to have a variety of functions even in the fetal period of development. In the 9-11-week fetus it has depot, hemolytic, and weak hematopoietic functions. At 13-14 weeks lymphoid follicles and T and IgG^+-lymphocytes can be detected in the spleen. By 18-22 weeks lymphocytes are present in the organ with a certain density, and the percentages of both lymphocyte populations are constant. These parameters remained virtually unchanged until the end of embryonic development. Hence it can be concluded that the human spleen is one of the chief organs for Ig formation and for the production of immunologic reactivity even in the embryonic period. If the view is accepted that Tγ cells belong to the T suppressor class, the fetal spleen is the site of their accumulation in man as early as in the embryonic period.

LITERATURE CITED

1. T. S. Adzhimamudova, Tr. 2-go Mosk. Med. Inst. Ser. Immunol., 61, No. 1, 3 (1978).
2. O. V. Volkova and M. I. Pekarskii, Embryogenesis and Age Histology of Human Internal Organs [in Russian], Moscow (1976).
3. N. A. Zharikova, Peripheral Organs of the Immunity System [in Russian], Minsk (1979).
4. R. V. Petrov and A. A. Mikhailova, Immunogenesis and Cell Differentiation [in Russian], Moscow (1978), p. 176.
5. A. G. E. Pearse, Histochemistry, Theoretical and Applied, Little, Brown, and Company, Boston (1960).
6. L. I. Falin, Human Embryology. Atlas [in Russian], Moscow (1976).
7. N. A. Chuich, Byull. Éksp. Biol. Med., No. 1, 38 (1980).
8. G. E. M. Asma, W. Pichler, H. R. Schult, et al., Clin. Exp. Immunol., 29, 278 (1977).
9. I. Barta, The Spleen [in Russian], Budapest (1976).
10. R. V. Furth, M. D. Henrica, R. E. Schuit, et al., J. Exp. Med., 122, 1173 (1965).
11. B. D. Icinkovic, K. Isakovic, L. Rakic, et al., Folia Biol. (Prague), 25, 307 (1979).
12. A. R. Hayward and G. Erer, Cell. Exp. Immunol., 17, 169 (1979).
13. M. E. Trigg, South Afr. Med. J., 72, 593 (1979).