

MORPHOLOGY AND FUNCTION OF THE FETAL AND NEONATAL RAT THYMUS AFTER VENOUS OCCLUSION

**B. B. Annanepesova, E. D. Ustyugov,
and N. M. Pasman**

UDC 611.423+611.161+611.428:618.214+618.3

KEY WORDS: thymus; venous occlusion; pregnancy.

Much clinical evidence has now been obtained to show that embarrassment of the blood flow and changes in the coagulation properties of the blood in the microcirculatory basins of the fetoplacental system take place in the majority (up to 97%) of pregnant women [2]. For that reason the study of the effect of vascular disturbances in the fetoplacental zone on the development of components of the immunocompetent system of the progeny has become an urgent problem.

The aim of this investigation was to analyze changes in the thymus of fetal and neonatal rats after antenatal venous occlusion.

EXPERIMENTAL METHOD

Experiments were carried out on 49 fetal and 60 neonatal Wistar rats. The caudal vena cava was ligated on the 15th day of pregnancy in 21 female rats. Open ether anesthesia was used. Material was taken for histologic investigation on the 21st day of pregnancy and again on the 1st and 14th days of postnatal development. The anesthetized animals were removed from the experiment by decapitation. Material was fixed in Tellyesniczky's fluid [5]. Serial and semiserial sections were cut on a rotary microtome. The relative area of the structural components of the thymus (cortex, medulla, capsule, interlobular septa) was determined in sections stained with Mayer's hematoxylin and eosin, using the dot counting method [3]. The relative number of parenchymatous cells in the thymus was determined for a constant surface area of section [4], stained with azure-2-eosin. The blood clotting system of the pregnant rats was studied by the microcoagulation test [1].

The numerical results were subjected to statistical analysis on the "Neiron" personal computer (the program was written in the laboratory for problems in ontogeny, Research Laboratory of Clinical and Experimental Lymphology, Siberian Branch, Russian Academy of Medical Sciences, by A. V. Zalavin.)

EXPERIMENTAL RESULTS

The creation of experimental venous occlusion led to changes in both mother and fetus. This was proved by a significant decrease in the time of onset of maximal blood coagulability of the pregnant animals of the experimental group (Table 1). Measurement of the body weight of the fetuses showed that the creation of venous occlusion caused a significant decrease in weight of fetuses of the experimental group by 29% (Table 2). The mass of the lymphoid organs in fetuses of the experimental group was significantly reduced: the thymus by 38%, the spleen by 46% (Table 2). On the 1st day after birth the mass of the young rats in the experimental group was reduced, the reduction being significant in the case of young females by 29%, whereas the body weight of the young males had only a tendency

Research Laboratory of Clinical and Experimental Lymphology, Siberian Branch, Russian Academy of Medical Sciences, Novosibirsk. (Presented by Academician of the Russian Academy of Medical Sciences Yu. I. Borodin.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 114, No. 10, pp. 422-424, October, 1992. Original article submitted February 7, 1992.

TABLE 1. Parameters of Microcoagulation Test in Rats during Intact Pregnancy and after Creation of Venous Occlusion ($\bar{X} \pm S_x$)

Parameters of test	21 days of pregnancy, control	21 days of pregnancy, venous occlusion
Coagulability at 2nd minute, A_2 , %	35.5±2.7	41.1±3.8
Maximal coagulability, M_S , %	110.5±7.6	114.8±5.6
Time of occurrence of maximal coagulability, T, min	5.5±0.5	4.0±0.5*

Legend. Here and in Tables 2-5: *) differences significant compared with control at $p < 0.05$ level.

TABLE 2. Results of Measurements of Body Weight and Weight of Thymus and Spleen in Rat Fetuses with Intact Pregnancy and after Creation of Venous Occlusion ($\bar{X} \pm S_x$)

Parameter	21 days of pregnancy, control	21 days of pregnancy, control, venous occlusion
Mass of fetus, g	3.8±0.1	3.0±0.1*
Mass of thymus, mg	5.5±0.4	3.4±0.1*
Mass of spleen, mg	4.4±0.2	2.4±0.1*

to decrease (Table 3). Parallel with the decrease in body weight, the mass of the thymus also was reduced in offspring of the experimental group: in young males by 39% and in young females by 43% (Table 3). The mass of the spleen in young rats in the experimental group, on the other hand, increased: in young males by 45%, in young females by 70% (Table 3). On the 14th day of postnatal development, in the experimental group the body weight of the young males was significantly reduced by 18% and that of the young females by 15% (Table 3). In the experimental group there was even greater retardation in development of the organs: the mass of the thymus in young males was significantly reduced by 78% and in young females by 79%. The mass of the spleen also was reduced: in young males by 47% and in young females by 37% (Table 3).

Analysis of cellular composition was carried out in the subcapsular, cortical, and medullary zones of the fetal thymus. Venous occlusion led to a significant increase in the number of reticuloepithelial cells and of cells in a state of mitosis in the subcapsular zone (Table 4). In the cortical zone the number of lymphoblasts in fetuses of the experimental group was significantly reduced, but the number of macrophages and mast cells was significantly higher than the control level. The number of medium-sized lymphocytes in the medullary zone was significantly less than in the control (Table 4).

The morphometric data are evidence that on the 1st day after birth the relative area occupied by the connective-tissue structures of the thymus had a tendency to increase in the progeny of the experimental group (Table 5). In the young males and in young females in the experimental group the relative area of the cortex of the thymus was significantly reduced by 29 and 44% respectively, whereas the relative area of the medulla had a tendency to decrease. On the 14th day of postnatal development in the experimental group the relative area of the cortex was significantly reduced in the young male rats by 41% and in the young females by 31% compared with the control.

TABLE 3. Results of Measurement of Body Weight and Weight of Thymus and Spleen in Offspring of Rats with Antenatal Venous Occlusion ($\bar{X} \pm S_{\bar{x}}$)

Parameter	Experimental conditions	1 day		14 days	
		males	females	males	females
Body mass, g	Control	6,0±0,1	5,9±0,1	20,4±0,3	17,2±0,5
	Experiment	5,8±0,07	4,2±0,1*	16,7±0,4*	14,7±0,1*
Mass of thymus, mg	Control	10,6±0,6	9,6±0,7	101±7,3	110,2±8,6
	Experiment	6,5±1,2*	5,5±1,6*	22,3±2,0*	24,0±2,5*
Mass of spleen, mg	Control	11,5±1,2	9,8±0,8	115,2±6,7	110,2±4,1
	Experiment	16,3±2,0*	15,3±2,0*	61,7±4,4*	69,6±6,6*

TABLE 4. Cellular Composition of Subcapsular, Cortical, and Medullary Zones of Rat Fetal Thymus with Intact Pregnancy and after Creation of Venous Occlusion ($\bar{X} \pm S_{\bar{x}}$)

Parameter	21 days of pregnancy, control	21 days of pregnancy, venous occlusion
Subcapsular zone		
Lymphoblasts	0,2±0,03	0,2±0,04
Medium-sized lymphocytes	0,1±0,02	0,07±0,005
Small lymphocytes	0,4±0,02	0,4±0,03
Macrophages	0,03±0,003	0,01±0,002
Reticuloepithelial cells	0,05±0,01	0,1±0,02*
Mast cells	0,003±0,001	0,007±0,003
Mitosis	0,004±0,003	0,02±0,004*
Cortical zone		
Lymphoblasts	0,05±0,008	0,03±0,004*
Medium-sized lymphocytes	0,07±0,009	0,02±0,03
small lymphocytes	0,7±0,02	0,8±0,08
Macrophages	0,01±0,002	0,08±0,07*
Reticuloepithelial cells	0,04±0,007	0,03±0,08
Mast cells	0,001±0,001	0,005±0,001*
Mitosis	0,006±0,003	0,009±0,02
Medullary zone		
Lymphoblasts	0,05±0,01	0,07±0,01
Medium-sized lymphocytes	0,08±0,008	0,4±0,01*
Small lymphocytes	0,5±0,01	0,6±0,2
Macrophages	0,03±0,006	0,03±0,089
Reticuloepithelial cells	0,2±0,02	0,2±0,2
Mast cells	0,004±0,003	0,01±0,009
Mitosis	0,008±0,003	0,01±0,02

TABLE 5. Relative Area of Cortex, Medulla, Capsule, and Interlobular Septa of Young Rats after Antenatal Creation of Venous Occlusion ($\bar{X} \pm S_{\bar{x}}$)

Parameter, %	Experimental conditions	1 day		14 days	
		males	females	males	females
Cortex	Control	45,1±3,7	43,8±2,1	58,7±3,6	54,0±4,9
	Experiment	32,2±4,6*	24,6±3,4*	34,6±2,8	37,4±3,7*
Medulla	Control	31,1±4,7	30,4±3,8	26,1±5,8	27,2±7,9
	Experiment	21,0±6,6	22,6±5,3	32,2±4,7	24,7±5,2
Capsule	Control	14,9±5,1	17,3±4,7	5,8±5,8	10,1±9,0
	Experiment	24,4±7,4	26,3±7,4	17,1±5,2	18,4±8,5
Interlobular septa	Control	8,7±5,2	8,3±4,8	9,2±6,1	9,1±8,5
	Experiment	22,1±8,1	26,2±9,1	15,6±5,8	19,3±6,9

In the experimental group the relative area of the medulla in young males had a tendency to increase, whereas in young females it had a tendency to decrease. The area of section occupied by the capsule and interlobular septa showed a stable tendency to increase in the experimental group in offspring of both sexes (Table 5).

The morphology and functional state of the fetal and postnatal rat thymus thus undergoes considerable changes after antenatal creation of venous occlusion. Besides a decrease in mass of the thymus, all the zones of the thymus and their cellular composition undergo reorganization. To sum up the findings of this investigation it has to be emphasized that the onset of circulatory disturbances in the utero-placental zone is reflected in the formation of the central organ of immunogenesis, namely the thymus, in fetuses and its development in the postnatal period.

REFERENCES

1. Z. S. Barkagan, Hemorrhagic Diseases and Syndromes [in Russian], Moscow (1988).
2. E. M. Vikhlyaeva, Vestn. Ross. Akad. Med. Nauk, No. 4, 1 (1987).
3. A. A. Glagolev, Geometric Methods of Quantitative Analysis of Aggregates Under the Microscope [in Russian], Moscow–Leningrad (1941).
4. M. R. Sapin, V. Sh. Belkin, S. B. Stefanov, and M. V. Kuinova, Arkh. Anat., No. 8, 85 (1988).
5. R. Lillie, Histopathological Techniques and Practical Histochemistry [Russian translation], Moscow (1969), p. 40.