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CHANGES IN COMPOSITION OF HEART PROTEINS IN EARLY ONTOGENY UNDER NORMAL CONDITIONS AND WHEN THE UTERO-PLACENTAL BLOOD FLOW IS REDUCED

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UDC 618.32:547.963:618.36

The heart of rabbit embryos on the 11th, 12th, 14th, 16th, and 18th days of development was studied under normal conditions and when the utero-placental blood flow was reduced. Normally the ratio weight of heart–weight of embryo is particularly high in the period of rapid development of the hemodynamic functional system embryo–placenta–uterus (12th and 14th days), but later the ratio falls. The increase in total nitrogen in the heart and changes in the relative proportions of its fractions as a result of an increase in the content of contractile proteins and proteins of the stroma, was particularly great toward the 18th day. Under pathological conditions the weight of the embryo and the weight of the heart were reduced on all days of the experiments. The ratio weight of heart–weight of embryo toward the 18th day indicated the onset of spontaneous rehabilitation as a result of a gradual improvement in the utero-placental circulation. However, the total nitrogen content in the heart in all groups investigated remained the same as in the control or it increased, possible evidence of dehydration of the heart. Changes in the fractional composition of the heart proteins pointed to profound biochemical disturbances in the organ which could be one cause of the disturbance of its functional state.

KEY WORDS: embryogenesis; heart; contractile proteins; utero-placental circulation.

The development of the heart and, in particular, the formation of its contractile function have attracted and continue to attract the attention of biochemists and physiologists. In the accessible literature there is practically no information on the fractional composition of the heart proteins from the time when the organ begins to function in the embryos of placental animals under normal and pathological conditions.

The circulation in the utero-placental region is considered to be more than sufficient to supply the embryo with nutrients and oxygen [5]. However, as the writers' investigations have shown, there is a connection between the decrease in intensity of the utero-placental circulation and the development of the embryo, the mechanism of which is a disturbance of the coupled development of the hemodynamic functional system of embryo–placenta–uterus [1]. The data in this respect are of considerable interest not only for age and comparative biochemistry, but also for perinatal medicine, for an important problem at the present time is that of delayed development of the fetus [3, 6].

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 0007-4888/78/8612-1610$07.50

TABLE 1. Characteristics of Development of Heart of Rabbit Embryos from 11th to 18th Days Under Normal Conditions and with Reduced Utero-Placental Blood Flow After 8th Day of Pregnancy (M ± m)

Index	Control embryos					Experimental embryos			
	11th	12th	14th	16th	18th	12th	14th	16th	18th
	days of intrauterine life								
Embryo: wet weight, mg	23,0±0,68 (73)	72,2±3,01* (15)	327±9,21* (7)	750±20,3* (63)	1573±19,68* (41)	47,0±3,23* (19)	174,5±8,59* (9)	634±14,5* (30)	1400±28,08* (38)
decrease in weight relative to weight of control groups, %	100	100	100	100	100	35	46	15	11
Heart: weight, mg	0,52±0,03* (171)	1,08±0,07* (217)	2,67±0,20* (65)	4,67±0,13* (119)	9,99±0,46* (69)	0,79±0,04* (82)	2,23±0,12 (9)	4,23±0,09 (47)	8,27±0,29* (31)
decrease in weight relative to weight of control groups, %	100	100	100	100	100	27	16	9	17
ratio to weight of embryo, %	2,26	1,44	0,8	0,6	0,6	1,68	1,3	0,7	0,6
total nitrogen, mg/g wet weight of tissue	—	10,96±1,16 (51)	—	10,17±0,41 (43)	12,54±0,64* (17)	10,38±2,47 (12)	12,82±0,68 (12)	11,14±0,55 (11)	14,32±1,55* (28)
content of cytoplasmic proteins, mg/g wet weight of tissue	9,69±1,23 (95)	12,48±0,98* (166)	13,6±2,26 (56)	13,4±0,87 (71)	12,0±1,39 (29)	10,6±0,81 (70)	—	14,3±0,28 (22)	14,8±1,56 (20)
content of contractile proteins, mg/g wet weight of tissue	—	5,60±0,77 (166)	4,4±1,3 (58)	5,3±0,61 (64)	9,0±0,89* (47)	4,2±0,50 (70)	—	5,3±1,44 (29)	10,0±0,9 (20)
nitrogen of residue, mg/g wet weight of tissue	—	3,0±0,26 (37)	3,23±0,85 (36)	3,82±0,51 (30)	4,48±0,16* (44)	2,87±1,01 (61)	4,67 (10)	4,02 (7)	3,72 (3)

Legend. 1. Numbers in parentheses denote number of embryos or number of hearts in experiments.
2. Asterisk indicates $P < 0.05$ compared with corresponding control.

In the investigation described below the fractional composition of the heart proteins was studied in rabbit embryos on the 11th, 12th, 14th, 16th, and 18th days of intrauterine life developing either under normal conditions or when the utero-placental blood flow was reduced.

EXPERIMENTAL METHOD

Experiments were carried out on 902 embryos from the 11th to the 18th days of development inclusive, obtained from 130 chinchilla rabbits weighing from 2800 to 3500 g. All the animals were pregnant for the first time. The first day of pregnancy was taken to be the day after copulation, which took place at the same time of day. Embryos of the experimental group developed when the utero-placental blood flow was reduced by ligation of about half the branches of the preplacental vessels directly adjacent to the chorionic sac of each second embryo, under aseptic conditions, on the 8th day of pregnancy. The conditions for removal of the embryonic heart were described previously [2]. The total nitrogen in the heart was determined by the micro-Kjeldahl method. Cytoplasmic proteins were extracted from a homogenate of the exsanguinated heart in the cold with 10 volumes of 0.03 M phosphate buffer, pH 7.4. After extraction of the cytoplasmic proteins, contractile proteins were extracted from the residual tissues. The first extraction was carried out with 10 volumes of Weber's solution for 18-20 h, the second with 5 volumes of 0.6 M KCl for 15 h. The extracts were separated from the stroma by centrifugation and pooled, after which the nitrogen of the stroma was determined. Protein in the fractions was determined quantitatively by Lowry's method, allowing for the fact that some reagents in high concentrations distort the results [4].

EXPERIMENTAL RESULTS

The experimental results are given in Table 1. The weight of the embryonic heart in the control experiments (from intact rabbits), on comparison of each group with the next, showed an almost twofold increase from the 11th day until the 18th day, the last day of observation; the weight of the embryo increased threefold from the 11th to the 12th day, fourfold from the 12th to the 14th day, and twofold from the 14th to the 16th and again from the 16th to the 18th days. The ratio of the weight of the heart to the weight of the embryo fell from 2.26% on the 18th day. This shows that the increase in the weight of the heart obeys the functional demands of the circulation, which develop particularly rapidly in the embryo–placenta hemodynamic system at the beginning of its formation.

The composition of the heart proteins in early ontogeny also undergoes appreciable changes. The total nitrogen content per gram wet weight of heart tissue increased until the 18th day of development. The concentration of contractile proteins also increased to this time. The concentration of cytoplasmic proteins, expressed per gram wet weight of heart tissue, increased only a little from the 11th to the 12th day, and on all subsequent days it remained unchanged. The concentration of proteins in the residue, as reflected in the nitrogen level (stromal proteins), increased after extraction of the cytoplasmic and contractile proteins in the period from the 12th to the 18th days. The results are in agreement with the more than 18-fold increase in the weight of the heart during the period of observation. Consequently, the protein composition of the rabbit embryonic heart in the early stages of development and in the process of maturation undergoes quantitative and qualitative changes even under normal conditions.

Significant changes were observed in the heart of embryos which developed when the utero-placental blood flow was reduced. Compared with the control embryos, the weight of the experimental embryos was reduced at all times of investigation. The weight of the heart by the 12th day (on the 4th day after the operation) was reduced by approximately one-third, and by the 18th day by approximately one-fifth. However, the total nitrogen content, calculated per gram wet weight of heart tissue, by the 12th day remained the same as in the control group, whereas by the 18th day it was increased significantly ($P < 0.01$). By the same time there was a significant increase in the content of cytoplasmic proteins ($P < 0.05$). The content of contractile proteins per gram wet weight of heart tissue in the experimental embryos on all days of the experiment was practically the same as in the controls. The protein nitrogen content of the stroma also corresponded to the control. These data indicate that changes in the fractional composition of the proteins of the embryonic heart may be one cause of the disturbance of its function.

The data on the ratio between the weight of the heart and the weight of the embryo on the 12th and 14th days of development show that under pathological conditions synthesis of the heart proteins was delayed by a lesser degree than synthesis of total body proteins. Later (16th and 18th days) signs of recovery of the embryo were clearly defined. By the 14th day of development its weight was 46% behind, but on the 18th day only 11% behind the control. This process may evidently develop on account of a gradual and spontaneous improvement of the utero-placental circulation.

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PORPHYROBILINOGEN BIOSYNTHESIS FROM δ-AMINOLEVULINIC ACID BY THE VISCERA OF ALBINO RATS

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UDC 612.015.36:[547.979.733:547.484.451

Ability to synthesize porphyrobilinogen (PBG) from δ-aminolevulinic acid (ALA) was determined in homogenates of tissues of the lungs, heart, liver, kidneys, spleen, pancreas, and small intestine of 77 albino rats. All these organs were found to be able to synthesize PBG. Highest ALA dehydratase activity was found in the liver tissue, followed in descending order by the kidneys, lungs, pancreas, small intestine, heart, and spleen. On the addition of a lead solution to the synthesizing system a significant decrease in enzyme activity was observed in the liver tissue, but in kidney tissue its activity was unchanged. On the addition of lead and D-penicillamine simultaneously no changes were found in the toxic effect of lead.

KEY WORDS: porphyrins – synthesis, localization in organs and tissues of rats; lead poisoning.

The biological role of heme and heme-containing compounds is exceptionally great. Functions such as participation in oxygen and electron transport require the presence of heme-containing compounds in every cell of the body. Whether porphyrins and heme also are formed in every cell or whether they are transported to the cells from the organs which synthesize porphyrins has not yet been finally settled.

Barta [1] considers that porphyrin is synthesized in the erythrocytes and liver. Idel'son [2] considers that porphyrin biosynthesis takes place in all cells of the living organism. There are few reports in the literature on the comparative study of the ability of different tissues to synthesize porphyrins. In particular, the synthesis of porphyrobilinogen (PBG) from δ-aminolevulinic acid (ALA) by homogenates of various rabbit organs and also by the liver, kidneys, and Harder's gland of rats [5] has been studied. The results have shown that the liver, kidneys, and bone marrow have the highest activity as regards PBG formation from ALA in both species of animals. According to the same workers, ALA dehydratase, an enzyme converting ALA into PBG, is widely distributed in nature and is evidently present in all cells with aerobic metabolism.

ALA dehydratase is known to be inactivated by lead; moreover, this action in vivo is abolished by various complexones, including D-penicillamine (D-PAM) [6-10]. The mechanism of the therapeutic action of D-PAM in lead poisoning is linked with the "exposure" of SH groups necessary for restoring the activity of the enzymes, and the chelating reaction of the compound with lead and its subsequent elimination. The study of the more precise mechanism of action of D-PAM could be assisted by an investigation of whether D-PAM can interact with lead in vitro, but this problem has received virtually no attention in the literature.

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(Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 12, pp. 687-689, December, 1978. Original article submitted October 24, 1977.