UPTAKE OF ^{14}C-LABELED PHOSPHOLIPIDS INJECTED INTO RATS IN THE LAST STAGE OF PREGNANCY BY FETAL TISSUES

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An important role in the pathogenesis of fetal hypotrophy and retarded development in late toxemias of pregnancy [3, 9] is unquestionably played by disturbances of lipid metabolism. This is confirmed by the fatty degeneration of the placenta associated with a low phospholipid (PL) content in it in toxemia of pregnancy [8], the low content of triglycerides and high PL content in maternal and umbilical blood, and the therapeutic effect of exogenous essential PL — in the form of the preparation "Essentsiale" which largely normalizes these disturbances of lipid metabolism in mother and fetus and prevents retarded development of the fetus [1]. The effectiveness of the therapeutic action of "Essentsiale" may perhaps be linked with its normalizing effect on the trophic functions of the placenta, on account of the lipotropic properties of PL, and also with the fact that it is an additional source of "essential" PL required for construction of the cellular structures of the fetus.

Considering that in experiments with doubly labeled [^{3}H,^{14}C]-PL intact PL molecules were shown to penetrate into the liver tissue without preliminary degradation or structural change in the course of 3 h and also, in small quantities, even into the animal's brain [10], it was decided in the present investigation to study the possibility of transplacental transfer and utilization of [^{14}C]-PL, injected into the mother, by the fetal tissues.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar albino rats weighing 240-270 g pregnant for the first time. The experiments were conducted in two stages. In the first stage, to obtain a preparation of [^{14}C]-PL, with carbon-labeled fatty acids, sodium [2-^{14}C]acetate was injected subcutaneously into four rats in a dose of 1 mCi/kg body weight. The rats were killed 1 h later and total lipids were extracted from their liver tissue with a mixture of chloroform and methanol by Folch's method, as described previously [2]. By thin-layer chromatography on silica-gel [4], the total PL fraction free from contamination with other lipids and nonlipid components was isolated from this extract. PL were eluted from the silica-gel and pooled. In the second stage of the experiment a phospholipid suspension was made of this [^{14}C]-PL preparation in physiological saline and injected into rats on the 20th day of pregnancy subcutaneously or intramuscularly in a dose of 23 mg [^{14}C]-PL per rat. After 1 h the rats were killed, the lung and brain tissue of all the embryos was removed, and total lipids were extracted from it; the total PL fraction was then isolated by thin-layer chromatography on silica-gel, having previously taken aliquots to determine the PL content by the method in [5]. The radioactivity of total PL of the fetal tissues was determined with an "Isocap-300" scintillation counter without elution from the silica-gel and expressed in counts per minute per total quantity of PL of all the investigated lung or brain tissue. The specific radioactivity (SR) of PL was expressed in cpm/mg PL.

EXPERIMENTAL RESULTS

In the first stage of the experiment a preparation of labeled [^{14}C]-PL was obtained from the liver of four rats in a quantity of 350 mg with SR of 2750 cpm/mg. This preparation was injected subcutaneously or intramuscularly into 10 rats on the 20th day of pregnancy. The re-

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TABLE 1. Radioactivity of PL of Lung and Brain Tissue of 20-Day Rat Embryos 1 h after Injection of [^{14}C]-PL into Mother

Rat	Number of embryos	Lungs		Brain	
		total radioactivity of tissue PL, cpm	SR, cpm/mg PL	total radioactivity of tissue PL, cpm	SR, cpm/mg PL
Subcutaneously					
1	7	300	32	259	25
2	12	190	18	150	9,0
3	12	336	24	172	11
4	7	57	20	56	10
5	8	120	25	41	7
Intramuscularly					
6	10	24	4	37	4
7	7	58	5	72	9
8	9	39	7	25	2
9	4	39	7	16	2
10	6	85	9	46	6

sults of determination of radioactivity of PL in embryonic lung and brain tissue are summarized in Table 1. The aim of this experiment was simply to determine whether it is possible, in principle, for exogenous labeled [^{14}C]-PL to pass from the maternal blood stream into the body of the fetus, and the table contains primary data before statistical analysis, since the number of embryos varied considerably from one experiment to another (from 4 to 12), and the experimental conditions could not therefore be standardized (the quantity of embryonic tissue from each rat and, consequently, the intensity of its uptake of [^{14}C]-PL). The results are evidence that 1 h after injection of [^{14}C]-PL into the mother, it was found in the fetal lung and brain tissue. However, after subcutaneous injection of the preparation, both total radioactivity of PL of the fetal lung and brain tissue and SR (calculated per milligram of PL) were considerably (2-5 times) higher than after intramuscular injection. This difference was evidently due to the different rate of entry of the [^{14}C]-PL preparation into the blood stream from the site of injection. It will be noted that by both methods of injection [^{14}C]-PL the specific radioactivity of PL in the fetal lungs of all animals studied was twice to three times higher than that of PL in the brain of the same embryos. It can accordingly be concluded that uptake of PL by the fetal lung tissue took place more intensively than by brain tissue, and this correlates well with data in the literature indicating that uptake of other lipid components (cholesterol [6] and fatty acids [7]) from the blood stream by the fetal brain is delayed in the last stage of embryonic development on account of the formation of the fetal blood–brain barrier and intensification of endogenous synthesis of lipids in the embryonic brain tissue.

Nevertheless, the fact that radioactivity was found in PL of the embryonic tissue is itself evidence that exogenous PL, having entered the maternal blood stream, pass through the placental barrier into the fetus and are utilized to form cellular membrane structures in the lung tissue. The fact that [^{14}C]-PL penetrates into the fetal brain is evidence that the PL also can surmount a second barrier, namely the blood–brain barrier of mature embryos, and are utilized to form the cellular structures of nerve tissue.

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