PROTEINS AND ENZYMES OF THE FETAL BLOOD SERUM AND LIVER AFTER ADMINISTRATION OF THE TERATOGENIC COMPOUND PYRIMETHAMINE TO PREGNANT RATS

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Administration of pyrimethamine to pregnant rats gave rise to various external malformations and developmental disturbances of the liver in fetuses at the 19th-20th day of development. Changes in the serum and liver proteins, disturbance of the isoenzyme spectra of lactate, malate, and sorbitol dehydrogenases, and an increase in the specific activity of serum lactate and malate dehydrogenases were observed in the abnormal fetuses.

The possible teratogenic action of therapeutic agents has now been studied for several years, but so far only the frequency of appearance and the morphological characteristics of the developmental defects have been studied [6, 13]. Another important aspect is the analysis of the biochemical mechanisms at the basis of teratogenesis [1, 7]. Experimental reproduction of models of developmental defects in animals by administration of teratogenic substances can also be regarded as a promising method of studying the pathogenesis of congenital anomalies in infants. The liver is an interesting object in which to study the characteristics of protein and enzyme biosynthesis in the course of fetal development [9].

The object of the investigation described below was to study the protein composition of the liver and blood serum and to investigate the specific activity and distribution of isoenzymes of lactate, malate, and sorbitol dehydrogenases (LDH, MDH, and SDH, respectively) in the serum of normal rat fetuses at the 19th-20th day of intrauterine development and in fetuses with developmental anomalies (including those of the liver) induced by administration of the teratogenic compound pyrimethamine to pregnant rats.

EXPERIMENTAL METHOD

Altogether 1250 fetuses, at the 19th-20th day of development, from 150 pregnant Wistar rats weighing 180-220 g were studied. Pregnancy was counted from the day when spermatozoa were first found in vaginal smears. The experimental animals were given pyrimethamine on the 13th day of pregnancy in a dose of 7 mg by mouth through a special tube. The animals were killed on the 19th-20th day of pregnancy. To obtain serum the fetuses were taken from the uterus and blood collected from the heart or umbilical vein by means of a capillary tube. After formation of the serum it was separated by spinning the blood for 1 min in a microcentrifuge by Shklyar's method. Soluble fetal liver proteins were extracted by Kuzovleva's method [4]. Blood serum and liver taken from several fetuses of the same female were used in each experiment.

Electrophoresis of the blood serum and liver proteins was carried out in agar gel.

Total (specific) activity of the serum LDH, MDH, and SDH was determined by a modification [8] of Warburg's optical test. Serum isoenzymes were detected by enzyme-electrophoresis in agar gel [3] followed by van der Helm's histochemical reaction [12]. The relative percentages of the individual fractions

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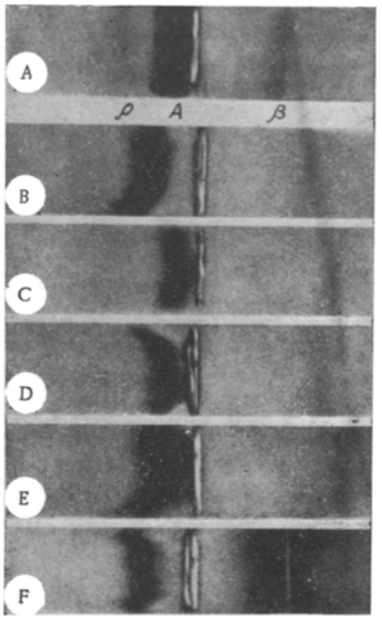

Fig. 1. Electrophoresis of serum proteins of normal and abnormal rat fetuses on the 19th day of development. A) Serum protein of normal fetuses; B-E) unusual albumin fractions in serum of abnormal fetuses; F) additional globulin components in serum of abnormal fetuses.

were calculated densitometrically. Statistical analysis of the numerical results was carried out with the aid of a BÉSM-2 computer.

EXPERIMENTAL RESULTS

Administration of pyrimethamine to the rats was followed by the appearance of developmental defects in the fetuses, affecting mainly the limbs, the facial skeleton, and the brain, in accordance with published observations [2]. Pyrimethamine also induced developmental defects of the liver. Changes in the lobular structure and in the size and shape of the organ and the appearance of accessory lobules were observed. The weight of the liver relative to the total weight of the fetus was greater in the abnormal fetuses.

Three protein fractions were found in the blood serum of rat fetuses on the 19th day of development (Fig. 1A), two of them (albumin and prealbumin) migrating toward the anode and one (β-globulins) toward the cathode. The content of albumin in the fetal serum at this stage was 75.2%.

A statistically significant ($P = 0.02$) decrease in the protein content (2.0 g% in normal fetuses, 1.67 g% in abnormal) was found in the serum of fetuses with developmental anomalies. Electrophoresis of the serum of these fetuses also revealed three fractions, but the albumin fraction was heterogeneous. In some cases, it contained components migrating faster than the main mass of the albumin, as a result of which the albumin stain appeared sickle-shaped (Fig. 1B-E). In individual cases additional globulin components, absent in normal fetuses, appeared in the blood serum of the abnormal fetuses (Fig. 1F).

Electrophoresis in agar gel showed that the liver proteins of normal 19-day fetuses divided into nine fractions. These were numbered in accordance with the corresponding protein fractions of adult animals (Fig. 2A). Four fractions (35%) migrated toward the anode and five fractions (65%) toward the cathode. The protein content in liver extracts from fetuses at this period was 1.5 g%. After administration of the teratogenic compound the liver proteins of 19-day rat fetuses contained only five fractions, of which four migrated toward the anode and only one toward the cathode. Most liver globulins thus migrated toward the cathode without separating into individual fractions (Fig. 2B). There was also a decrease in the relative percentage of anodic fractions in the abnormal fetuses: the area of the peaks of fractions 2, 5, and 6 was increased by a statistically significant degree. The impression was obtained that after administration of the teratogenic compound the maturation of proteins was disturbed in the liver of the abnormal fetuses, as reflected in the abnormal protein composition of the organ.

Enzyme electrophoresis of the blood serum of normal 20-day rat fetuses revealed five LDH isoenzymes: two fractions, LDH-1 and LDH-2, migrated toward the anode and three isoenzymes toward the cathode (Fig. 3A). The total activity was distributed as follows: LDH-1 1.2%, LDH-2 4.4%, LDH-3 15.7%, LDH-4 35.1%, and LDH-5 43.6%. The predominance of LDH isoenzymes of M-type in the fetal serum at this period is noteworthy. Five LDH isoenzymes, indistinguishable in mobility from isoenzymes in the serum of normal fetuses (Fig. 3B), also were found in the blood serum of the abnormal fetuses. Substantial differences were observed in the distribution of total activity. In fetuses with developmental anomalies all five isoenzymes showed statistically significant changes: LDH-1 4.2%, LDH-2 13.2, LDH-3 21.5%, LDH-4 28.2%, and LDH-5 32.9%. The existence of such considerable changes suggests distortion of the LDH isoenzyme spectrum in the blood serum of the abnormal fetuses. Spectrophotometric investigations showed that the specific activity of serum LDH in normal 20-day fetuses was 31.4 i.u.; activity of the enzyme in the serum of the abnormal fetuses was more than doubled (71.6 i.u.) and came close to the characteristic value observed in the adult animal.

Two MDH isoenzymes were found in the blood serum of normal 20-day rat fetuses (Fig. 3C). Both fractions, containing serum prealbumin and albumin, migrated toward the anode. The distribution of the total activity was as follows: MDH-1 36.6% and MDH-2 64.4%. The serum of the abnormal fetuses also

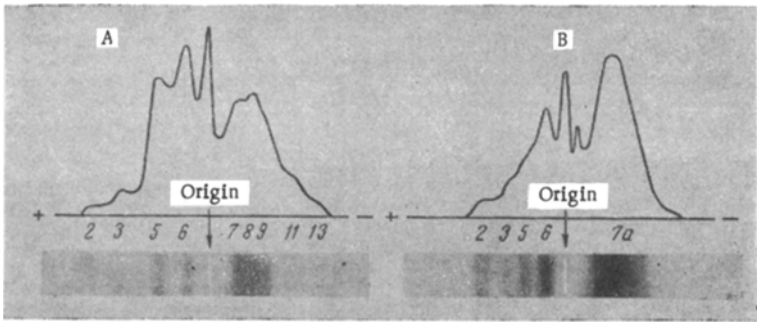

Fig. 2. Electrophoresis of soluble liver proteins of 19-day fetuses: A) normal fetuses; B) abnormal fetuses.

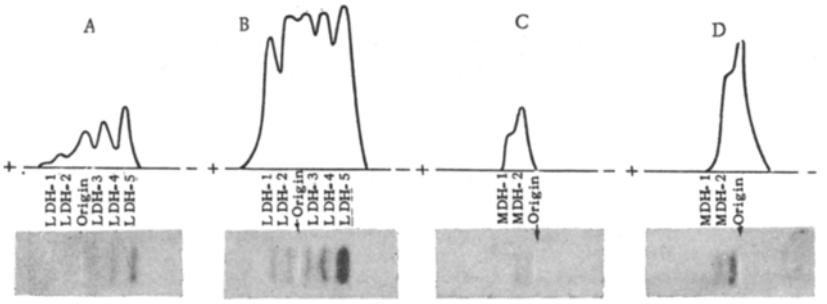

Fig. 3. LDH and MDH isoenzymes in the blood serum of rat fetuses: A) LDH isoenzymes in serum of normal 19-day fetuses; B) LDH isoenzymes in serum of abnormal fetuses of the same age; C) MDH isoenzymes in serum of normal 20-day fetuses; D) MDH isoenzymes in serum of abnormal fetuses of the same age.

contained two MDH isoenzymes (Fig. 3D), but a statistically significant difference was found in the distribution of the total activity: MDH-1 25.3% and MDH-2 74.7%. However, the most significant results were obtained on determining total MDH activity in the blood serum of the abnormal fetuses. Its value was 7.4 times higher (219.3 i.u.) than that in normal fetuses of the same age (29.6 i.u.).

By contrast with the two previous enzymes, only one fraction of SDH (SDH-1) was found in the serum of the 20-day normal rat fetuses, although on the day before birth two SDH isoenzymes were detected.

One SDH isoenzyme also was present in the blood serum of fetuses with developmental anomalies, but the results of densitometric investigations showed that the peak was 1.5 times higher. Incidentally, the serum protein concentration of the abnormal fetuses was lower than that of the normal fetuses. Total SDH activity could not be detected in the serum of the normal and abnormal fetuses by means of Warburg's optical test.

These results show that pyrimethamine, if given to the pregnant animal, disturbs the protein composition of the blood serum and liver and distorts the isoenzyme spectrum of the three serum enzymes studied in abnormal fetuses. This is evidently because pyrimethamine is an antagonist of folic acid, a coenzyme catalyzing reactions of protein and nucleic acid synthesis in the body [5, 14]. The results agree with data in the literature [10, 11] on the distortion of the isoenzyme spectra of acid and alkaline phosphatases and of LDH in the tissues of rat fetuses after administration of certain teratogenic agents to the pregnant female.

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