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# The Effect of Ethyl Alcohol on Fetal Fibroblasts *in vitro*

Wpływ alkoholu etylowego na fibroblasty płodowe *in vitro*

Negative effect of excessive alcohol consumption on human organism is well known. Alcohol causes serious changes, especially in parenchymatous organs (1, 3—5, 9, 14). It is also known that alcohol evokes teratogenic effect. With children's age there grows the number of showing alcohol syndrome traits in the case of both alcohol dependent mothers and fathers (12). Lesions were found in hepatocytes' ultrastructure (11) and also in the cells of the proximal canaliculi of the kidney (10) in the internal organs of rat foetuses of alcohol intoxicated rats. The effect of alcohol on the nervous system structures was also shown (5, 13). In the available literature there are few works concerning the effect of ethanol on mesenchymatous structures.

The paper aimed at morphohistochemical assessment of fibroblasts *in vitro*, collected for examinations from rat's foetuses of alcoholized parents.

## MATERIAL AND METHODS

Research was made on fibroblasts coming from the skin of rat foetuses. For 2—3 months white rats of Wistar strain, of both sexes, were given only 20% alcohol (the solution was made of 96% spirit produced by "Polmos"). Pregnant females were still given alcoholic solution. At the same time, water was given to a group of control rats. All the rats stayed in the same conditions and were administered standard granulated feeding stuff.

Rat foetuses were decapitated up to two days before birth, and the skin sections from backs and sides of their bodies were excised for samples. The collected sections of the skin served to obtain fibroblasts' cultures. The cells were cultured on the Parker's medium (199) with an addition of 10% calf serum produced by "Hungarpol". The medium 199 and other fluids applied in the culture came from Sera and Vaccines Manufacturers in Lublin.

After the first passage the cells were placed in vessels with cover-glasses, to which fibroblasts stuck. The passage was carried out after applying 0.25% solution of trypsin. On the third and fourth day since the moment of passage the cover-glasses with stuck cells were washed with PBS fluid, and then histochemical reactions for the activity of the following dehydrogenase were performed:

succinate dehydrogenase (SDH) E.C.1.3.99.1 by the Nachlas method, lactate dehydrogenase (LDH) E.C.1.1.1.27 and isocitrate dehydrogenase (ICDH) E.C.1.1.1.42 by the Pearse method, as well as for the activity of glucose-6-phosphatase ($G_6$ P-ase) E.C.3.1.3.9, by the Wachstein-Meisel method. Then the cells were fixed in the Baker's fluid for 10 min. The reaction for the activity of acid phosphatase (AcP), E.C.3.1.3.2, was performed on cells fixed in Baker's fluid. The review staining with the Giemsa's reagent was carried out on cells fixed in methyl alcohol. The preparations were assessed and microphotographs were taken by means of light microscope with a microphotographic countershaft produced by "Jenamed".

## RESULTS

Fetal fibroblasts from the alcoholized animals, in comparison with the culture of controls (Fig. 1), often contained numerous tiny vacuoles in the cytoplasm (Fig. 2), due to which the cytoplasm was foam-like. Sometimes fregmented cellular nuclei were found (Fig. 3). The cells lay multidirectionally.

The examination of succinate dehydrogenase (SDH) activity showed a delicate granular reaction in the whole cytoplasm, as well as more numerous granules of the reaction product in the perinuclear region. In the cells coming from the tissues of alcoholized animals the grains of the reaction product showed a slightly higher staining.

A distinctly heightened activity of lactate dehydrogenase (LDH) was noted in the cultures led out of the skin of alcoholized animals' foetuses in comparison with the control group (Figs 4 and 5). Numerous, highly stained grains of formasan were mostly localized in the paranuclear region of the cytoplasm.

No differences were found in the activity of isocitrate dehydrogenase (ICDH) in the cultures coming from both groups.

Much less intensive reaction for the activity of glucose-6-phosphatase ($G_6$ P-ase) was also shown by fetal fibroblasts from the alcoholized group. The brown grains of the reaction product were more numerous in comparison with the control cultures (Figs 6 and 7).

The product of reaction for the activity of acid phosphatase (AcP) in the control cultures was localized in higher amounts in the perinuclear region, and in lower amounts — in the peripheral cytoplasm and in the processes (Fig. 8). The fibroblast from the alcoholized group contained distinctly smaller amount of grains of the reaction product (Fig. 9), the localization being similar to the one in the control cultures.

## DISCUSSION

Ethyl alcohol is metabolized in the organism, mainly in the liver, in three ways (2, 4, 6). Hepatocytes metabolize ethanol with the participation of alcohol dehydrogenase (ADH) located in the cytosol (2, 4, 6). The second way, defined as MEOS, microsomal system of alcohol oxidation, is localized in the smooth

Fig. 1. Control group. Fetal fibroblast from the skin — passage I. Staining after Giemsa. Magn. ca 1600 ×

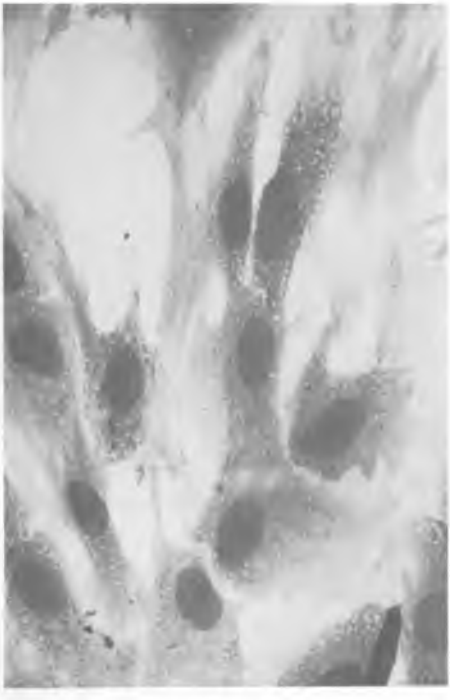

Fig. 2. Experimental group. Fetal fibroblasts from the skin — passage I. Staining after Giemsa. Magn. ca 1600 ×

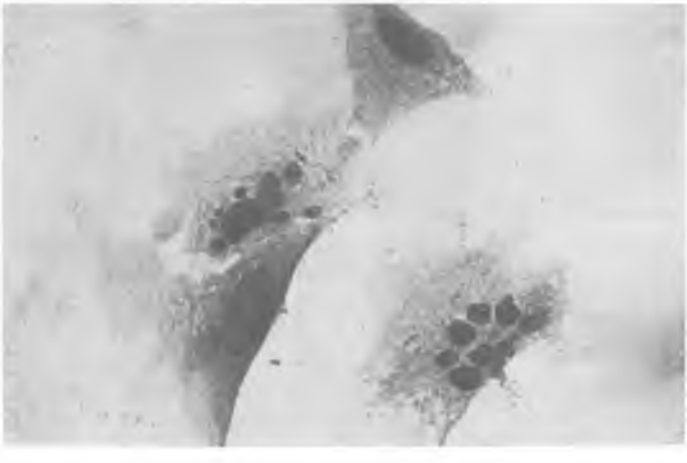

Fig. 3. Experimental group. Fetal fibroblasts from the skin — passage I. Staining after Giemsa. Magn. ca 1600 ×

Fig. 4. Control group. Fetal fibroblasts from the skin — passage I. Reaction for lactate dehydrogenase activity (LDH). Magn. ca 1600 ×

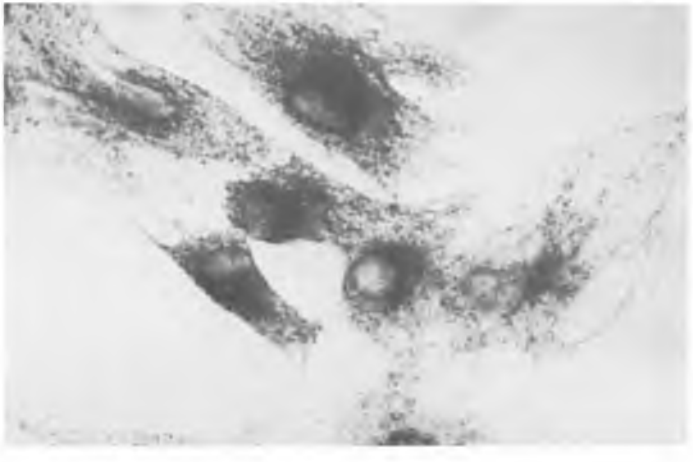

Fig. 5. Experimental group. Fetal fibroblasts from the skin — passage I. Reaction for lactate dehydrogenase activity (LDH). Magn. ca 1600 ×

Fig. 6. Control group. Fetal fibroblasts from the skin — passage I. Reaction for glucose-6--phosphatase ($G_6$ P-ase) activity. Magn. ca 1600 ×

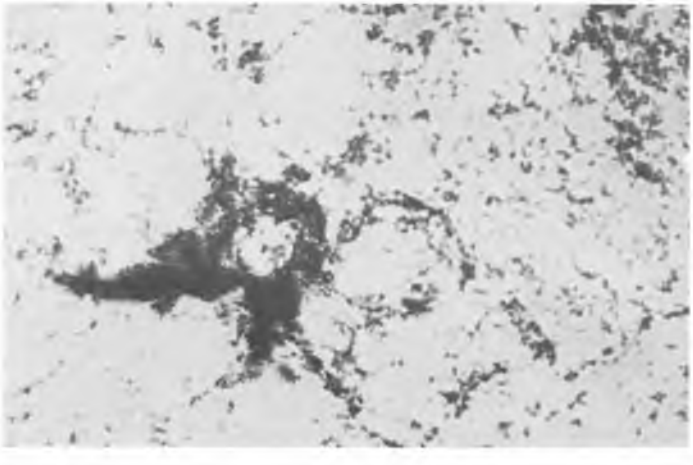

Fig. 7. Experimental group. Fetal fibroblasts from the skin — passage I. Reaction for glucose-6-phosphatase ($G_6$ P-ase) activity. Magn. ca 1600 ×

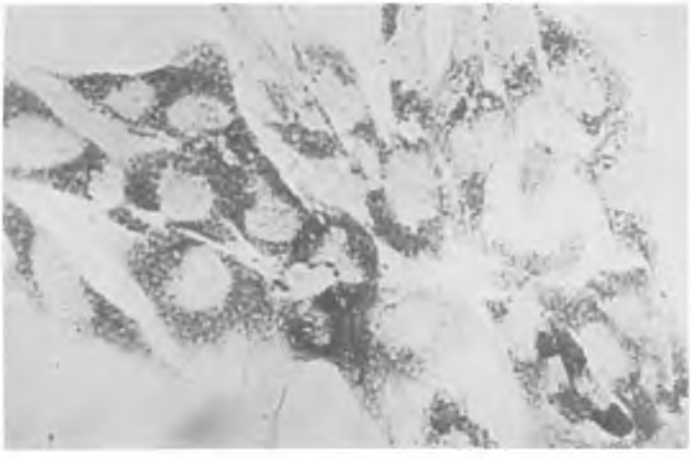

Fig. 8. Control group. Fetal fibroblasts from the skin — passage I. Reaction for acid phosphatase (AcP) activity. Magn. ca 1600 ×

Fig. 9. Experimental group. Fetal fibroblasts from the skin — passage I. Reaction for acid phosphatase (AcP) activity. Magn. ca 1600 ×

endoplasmic reticulum. In this system ethanol becomes an energy-absorbent substrate (2, 6, 8). The third way consists in the participation of peroxysomal catalase and of microsomes (2, 5).

Alcohol goes through the placental barrier leading to developmental disturbances and to a typical alcohol syndrome (12). Consuming of alcohol by pregnant females affects fetal liver to a certain extent. In accordance with the above rat foetuses of alcoholized parents showed an increased quantity of glycogen in the liver, lesions in mitochondrial structure (11). Other authors noted lesions in fetal kidneys after prolonged intoxication with ethanol, such as widened calans of intraplasmatic reticulum, the swelling of mitochondria (10).

Since alcohol is also excreted to a small extent through the skin, in the present paper we observed fetal fibroblasts *in vitro* coming from the skin. In accordance with the assumptions considerable changes occurred in them.

Frothy cytoplasm of fibroblasts, observed with the help of a microscope, is frequent in toxic damage of cells and is regarded as a degenerative change (7). Multidirectional system of cells also indicates anomalies in the proliferation of fibroblasts' cultures (7).

As was shown by histochemical examinations long-term intoxication of pregnant females with ethanol causes disturbances in the activity of many enzymes in fetal fibroblasts. An increase of the reaction for the activity of lactate dehydrogenase (LDH) and a slight rise in the activity of succinate dehydrogenase (SDH) point to an increased energy requirement of the cell. Alcohol metabolism connected with expenditure of energy takes place mostly in microsomal system (10, 14).

A considerable decrease in the activity of acid phosphatase (AcP) as the lysosome marker can be indicative of the damage of the digestive system of fibroblasts.

An increased activity of glucose-6-phosphatase ($G_6$ P-ase) as the marker of smooth intraplasmatic reticulum as well as the noticed changes in the activity of the remaining enzymes examined and the changes in morphology of fetal fibroblasts evidence permanent character of disturbances in cell metabolism, since the latter keep up in the conditions of cell culture after excluding the toxic effect of ethanol.

## Conclusions

1. Changes in fibroblasts displayed in the experiment indicate the negative effect of ethyl alcohol consumed by pregnant females on the connective tissue of foetuses.

2. The observed phenomena may induce us to undertake research on the effect of ethyl alcohol on the systemic disesases of connective tissue, many of which are causally related to the conditions of fetal life.

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## STRESZCZENIE

Szczury białe obojga płci otrzymywały wyłącznie do picia w okresie 2—3 mies. 20% roztwór alkoholu etylowego. Ciężarne samice nadal otrzymywały do picia tylko roztwór alkoholu. Przed rozwiązaniem 1—2 dni ze skóry płodów wyprowadzono hodowle fibroblastów. Po pierwszym pasażu fibroblasty barwiono odczynnikiem Giemsy oraz przeprowadzono reakcje na aktywność dehydrogenaz: bursztynianowej (SDH), mleczanowej (LDH) i izocytrynianowej (ICDH), a także wykrywano aktywność fosfatazy kwaśnej (AcP) i glukozo-6-fosfatazy ($G_6$P-azy). Stwierdzono wzmożenie odczynu histochemicznego LDH, SDH, $G_6$P-azy i osłabienie AcP. Obserwowane zmiany w morfologii komórek pozwalają wnosić, że alkohol spożywany przez samice miał szkodliwy wpływ na komórki tkanki łącznej płodu.

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