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Changes in the Thyroid Gland of Animals Chronically Treated with Alcohol

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Abstract: The study presents an experimental model of alcoholism. Female and male rats of Wistar albino were given 15% ethanol as the only drinking solution daily one month before mating, and the said amount was further administered to the females during both pregnancy and lactation. Control rats (n=22) received tap water. It was generally accepted that long-term administration of ethanol affected the thyroid gland morphology as well as the thyroid hormone level.

The results suggest that the effect of long-term alcohol administration induced changes in thyroid gland structure. Its secretion hormones are responsible for the regulation of growth and metabolism in general. Excessive thyroid activity induces general restlessness, more rapid heart rates and other undesired effects. Cythologic and morphometric procedures were used to describe the changes induced by ethanol in the tissues of baby rats. The baby rats were given 15% ethanol as the only drinking solution before and after birth. Point counting was used to determine the volume density of epithelium (Vve), colloid (Vvc), interfollicular spaces (Vvi), capillary (Vvs) and the activation index of the thyroid gland (Ia, Ia =Vve/Vvc). The most prominent cytological changes in the thyroid gland of the alcoholized baby rats were changes in follicular epithelia, C-cells and thyroid activation. Stereological analysis showed a statistically significant increase in the Vve, Ia, and significant reduction in Vvk, T3 and T4 in alcoholized animals.

Key words: follicles, follicular cells, C- cells, interfolicular space, colloid, follicular epithelia.bv.

Introduction

A group of anomalies occurring with the progeny whose mothers consumed alcohol during pregnancy were jointly termed fetal alcohol syndrome (FAS). These

anomalies include: retarded growth and development, heart and kidney damages, microcephaly and damage of the neural tube (Joneset and Smith 1973). It is important to underline that the progeny exposed to the action of alcohol *in utero* may experience serious biochemical, hormonal and neural anomalies even when the morphologically recognizable teratogenous effects are not expressed, since FAS may be expressed also in changes on cellular and subcellular level (Guerri *et al*. 1984, Weinberg 1994).

Since the normal development and maturing of the nervous system requires the presence of thyroid hormones – their deficiency during the critical periods in development being likely to induce permanent neurological and behavioral damages - it may be assumed that the changes in some of the levels of the hypothalamic-pituitary-thyroid axis of both mother and fetus could be, at least partially, responsible for the development of the anomalies recorded in FAS.

So far a number of researchers have studied the impact of alcohol-treated mothers on their progeny. The results of these investigations indicate that the effect of alcohol consumption of mothers on their progeny is mostly dependent on the severity and duration of alcoholization (Mennella and Beauchamp 1991).

In the light of the fact that the deficiency of thyroid hormones is induced by alcoholization of mothers also resulting in anomalies characteristic of FAS such as the retarded development of the central nervous system and retarded growth, the alcohol impact on thyroid gland has been increasingly studied. Former investigations have been restricted mainly to the determination of the status of thyroid hormones of the young of alcoholized mothers. Furthermore, data are lacking on the effect of ethanol on the cytophysiology of the thyroid gland itself. Therefore, the objective of the present study has been to examine the changes in the structure and function of the thyroid gland during the first 24 hours of postnatal life of baby rats, whose mothers were treated with 15% ethanol solution before and during the pregnancy as well as during the lactation. Due to the fact that alcoholism is the most widely distributed type of drug addiction both worldwide and in our country, focus should be given to the observation of all of its harmful effects especially those exerted by the alcoholization of parents on the progeny.

Material and Methods

The trial was performed at the Laboratory of Comparative Histology and Embriology of the Institute of Histology in Novi Sad, where long-term experiments on the impact of alcohol on the thyroid gland were conducted.

The investigation of the impact of ethyl-alcohol (ethanol) on the juvenile thyroid gland involved 10-day-old white laboratory baby rats of the Wistar strain.

At the beginning of the experiment, the sexually mature females, used for mating, were divided into two groups. The first group of animals were treated with 15% ethanol solution as the only liquid both during day and night one month before and during mating and pregnancy as well as throughout the course of lactation. The other group of animals, used as a control, were given water in the same period. A month later, the female rats were mated with the males. The males mated with alcoholized females were also treated with the 15% solution of ethanol for one

month. Upon delivery, the baby rats of both the alcoholized and the control females were sacrificed 10 days after birth (20 alcoholized and 22 control baby rats).

The animals were sacrificed by decapitation, and the thyroid glands were removed within five minutes of sacrificing. The thyroids were fixed in Bouin's liquid for twenty-four hours and molded in paraffin afterwards. Paraffin molds were cut on Reichert's microtone in serial 4 μm thick sections. The sections were dyed by the Haematoxylin edzin method; Florentin's method of dying with haematoxylin, eosin and lichtgrine and Dominici's method of dying with eosin, orange 6 and toluidine blue.

Immediately following the thyreoidectomy, the glands used in electron microscopic studies, were cut to small pieces under the control magnifying glass.

Polymerization at 55^0C lasted 24 hours. The fitted tissue was cut on an LKB Ultramicrotome III. Semithin toluidine blue stained sections were mounted on subject glasses. The preparations were used for lightmicroscopic analysis.

Stereological analysis of the thyroid glands of the baby rats of both alcoholozed and control mothers were performed using a Wild binocular microscope with the objective lens and eyepiece magnifications of 60 and 10 times, respectively. Weibl's multifunctional test system with 42 points (M42) was built in the eyepiece (Weibel et al. 1966).

Determination was made of the voluminous density of epithelia (Vve), colloid (Vvk) interstitium and blood vessels (Vvi, s) mastocytes (Vvm), numeric density of mastocytes (Nvm) and thyroid gland activation index (Ia=Vve/Vvk) (Kališnik 1971).

Upon decapitation of the animals, the animal blood was collected in test tubes and left to coagulate at room temperature for ten minutes; thereafter it was centrifuged for ten minutes in a centrifuge at 1500 revolutions per minute. The drained blood serum was immediately frozen and kept at -20^oC until the analysis was made. The concentration of total thyroxine (T4) and triodothyronine (T3) in the blood serum was determined by radioimmunological analysis (RIA).

The concentration of T4 and T3 was determined following the instructions of the diagnostic sets for the quantitative determination of thyroid hormones of the Nuclear Energy Institute, Zemun – i.e. RIA T4 (PEG) and RIA T3 (PEG), respectively. The principle of the test was based on the competitive binding of serum T4 and radioactive T4 (125-J-T4) for antigen (125J) determinants on the specific antibodies for T4, where marked and non-marked immunocomplexes were created. Upon reaction, both complexes were settled by polyethyleneglicole (PEG).

The significance of differences in specific stereological parameters obtained with the control and experimental animals was checked by the Student's t-test.

Results

The thyroid glands of the young of the alcoholized 10-day-old mothers were characterized by the decreased follicle volume, higher level of follicular epithelia and increased interfollicular spaces as compared to the control animals (Fig. 1). The C-cells were observed to increase the volume in the glands of the treated baby rats as compared to the control animals (Fig.1).

The semithin sections enabled a more detailed insight into the structure and localization of these cells.

The C- cells in the glands of the treated baby rats were mainly localized in the interfollicular space (Figs. 2a and 2b), but they were also observed in the follicle composition (Fig. 2c). The C-cells located in the follicles increased in volume.

The occurrence of a larger number of mastocytes of higher volume than in the control animals was observed in the interfollicular binder (Figs. 3 and 4). These cells contained numerous granulations.

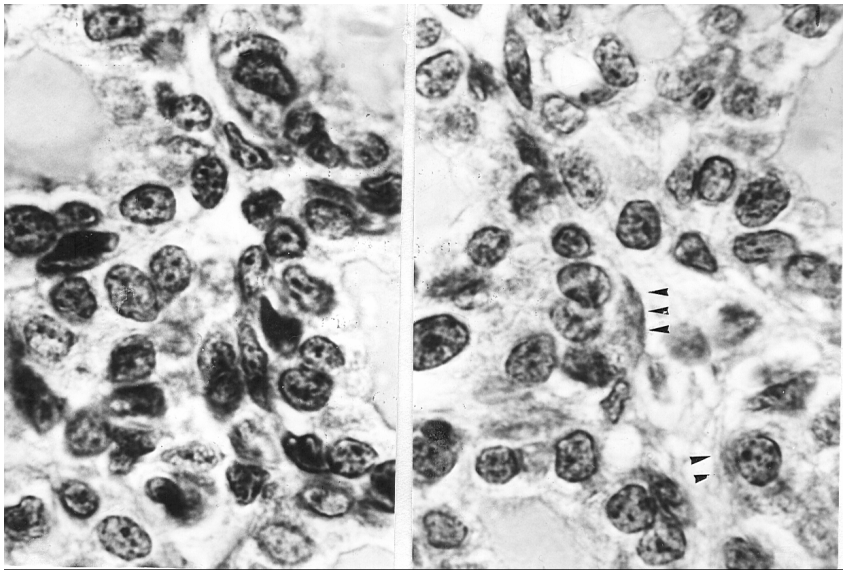

Fig. 1. Thyroid gland of 10-day-old rats: a) control group; b) alcoholized animals, higher follicular cell volume; hypertrophy of C-cells in the interfollicular spaces. Bouin, HE. oc10. Obj. IMM.

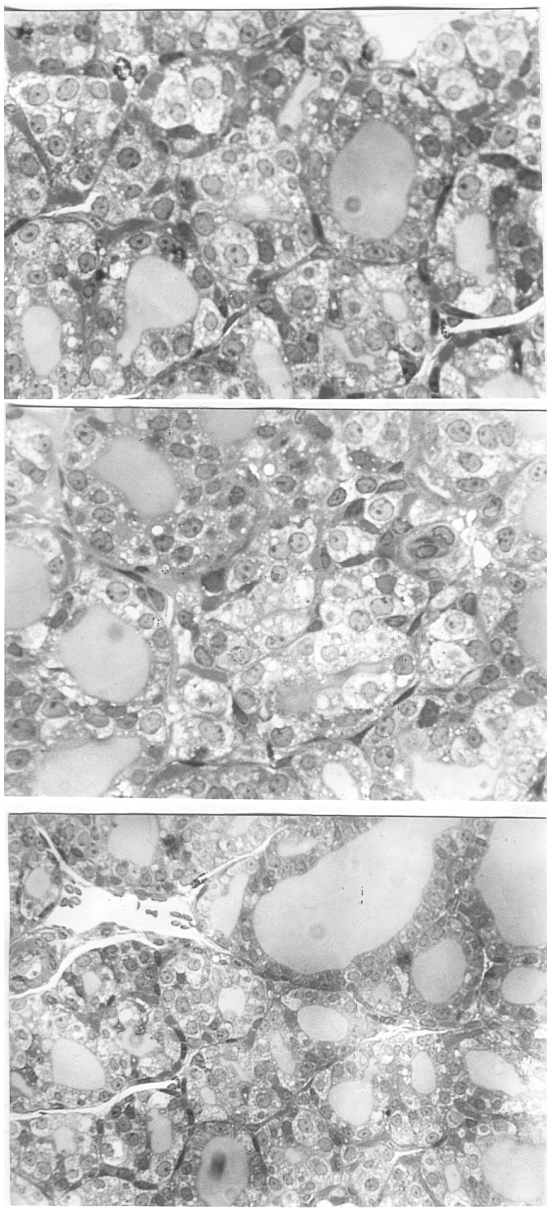

Fig. 2. Thyroid gland of 10-day-old alcoholized animals, C-cell hypertrophy
a) oc 10 obj.40; b) and c) oc.10 obj.63

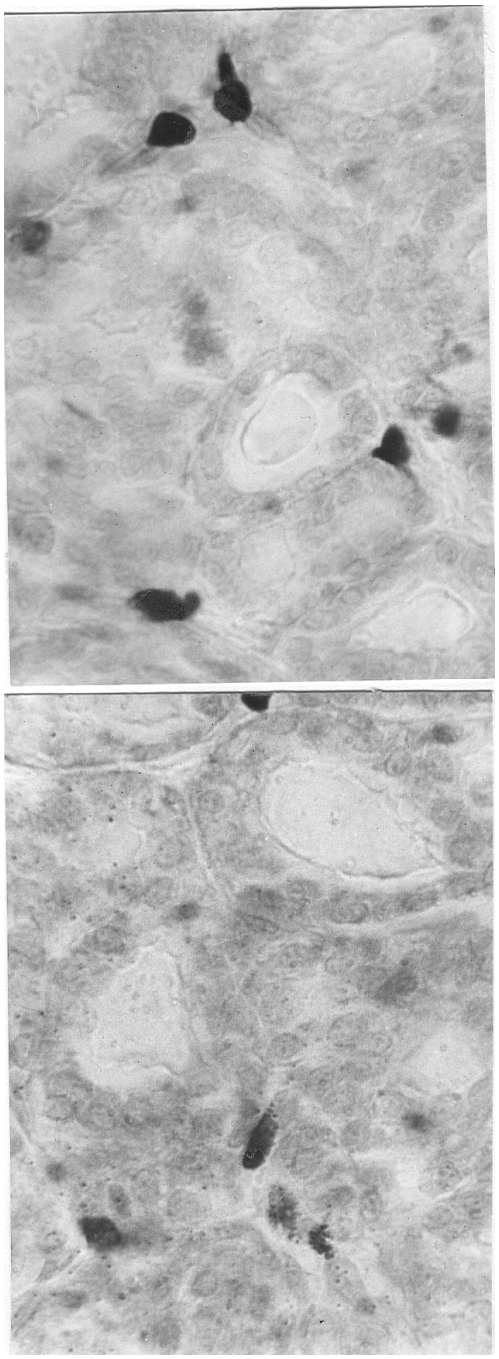

Fig. 3. Thyroid glands of 10-day-old rats: a) control animal; b) alcoholized animal, higher volume of mast cells in interfollicular spacies. Bouin, Dominici.oc10.0bj.63

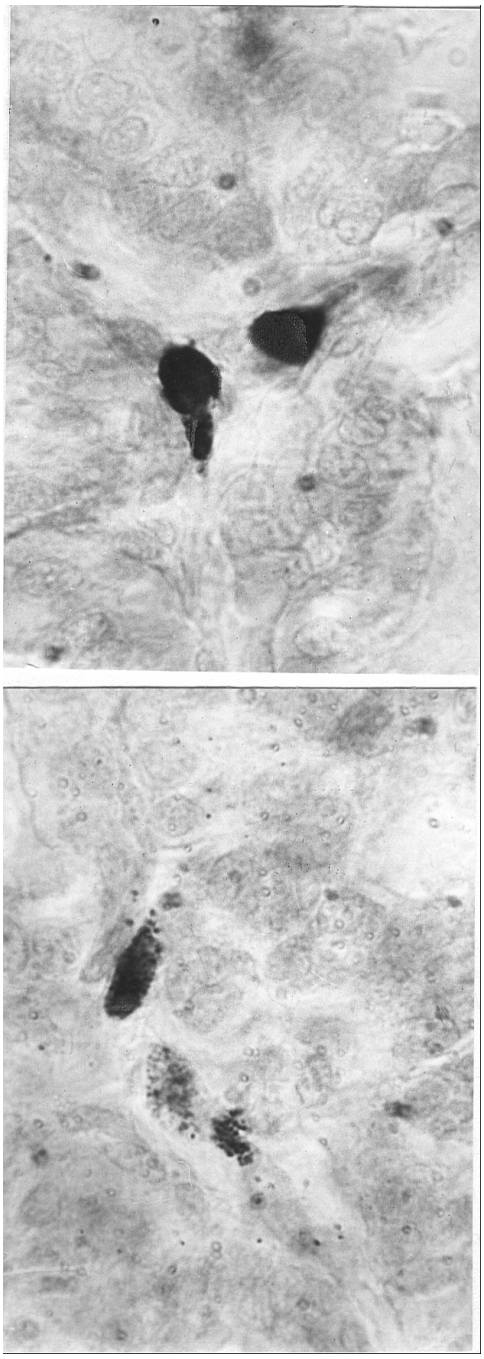

Fig. 4. Thyroid glands of 10-day-old rats: a) control animal; b) alcoholized animal, higher volume of mast cells in interfollicular spaces. Bouin, Dominici. Oc.10. obj. IMM

The stereological analysis demonstrated that the thyroids of the 10-day-old animals, the mothers of which were alcoholized, revealed significantly increased values, voluminous density of epithelia (Vve), the average value with the control animals being 63.38± 1.51%, and that with the baby rats of the alcohol-treated mothers – 71.68± 2.30% (Fig. 5).

The volume density of colloid (Vvk) in the control animals was 11.32± 0.88%, the value in the alcoholized ones being significantly decreased -7.97± 0.26% (Fig 5).

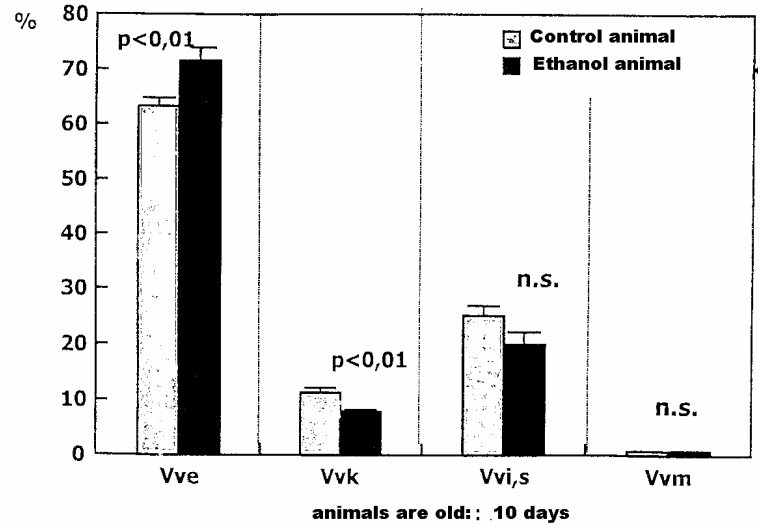

Fig. 5. Volume density of epithelium (Vve), colloid (Vvc), interfollicular spaces (Vvi), capillary (Vvs) and mast cell (Vvm) of the thyroid gland of 10-day-old rats

The thyroid gland activation index (Ia) (Ia=Vve/Vvk) in the baby rats of the alcohol-treated mothers was significantly increased, its mean in the control and experimental animals being 6.66± 0.45 and 9.74± 0.24, respectively (Fig 6).

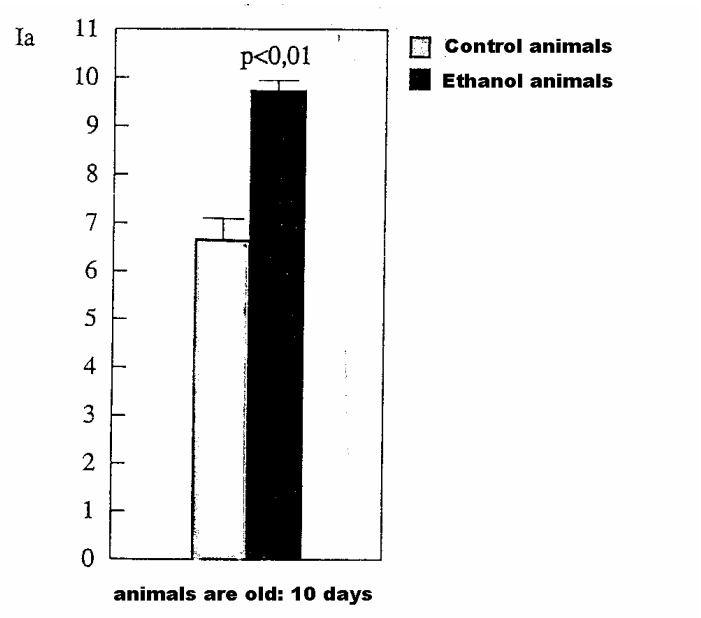

Fig. 6. The activation index of the thyroid gland of 10-day-old rats

The volume density of the interfollicular binder including the blood vessels (Vvi,s) was insignificantly decreased in the alcoholized animals, being 20.05± 2.23%, whereas its value in the control animals was 25.20±0.72% (Fig 5). The volume density of the interfollicular mastocytes (Vvm) remained unchanged. In the alcoholized animals it was 0.79± 0.09%, and in the control ones – 0.77± 0.06% (Fig 5), whereas the numerical density of mastocytes (Nvm) in the interfollicular binder of the experimental animals was 28,898.10± 2104mm^{-3} It was insignificantly lower than in the control animals - 30,805.10 ± 1,590.69 mm^{-3} (Fig. 7).

In the 10-day-old baby rats of the alcoholized mothers, there was a significant decrease in the level of thyroid hormone, triiodthyronines (T3).

The value of T3 in these animals was 0.22± 0.02 nmol/l, being significantly decreased as compared to that in the control animals – 0.34± 0.03 nmolol/l (Fig. 8). The T4 (thyroxin) level in the baby rats of the alcoholized mothers was 46.75± 2.29 nmol/l and it insignificantly decreased as compared to the control animals, where it was 54.50± 2.96 nmol/l. (Fig. 9).

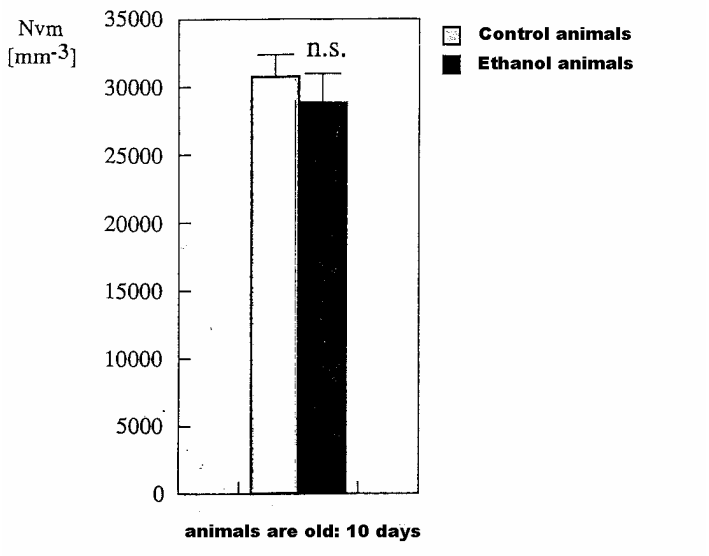

Fig. 7. The numerical density (Nvm) of thyroid gland mast cells in 10-day-old rats

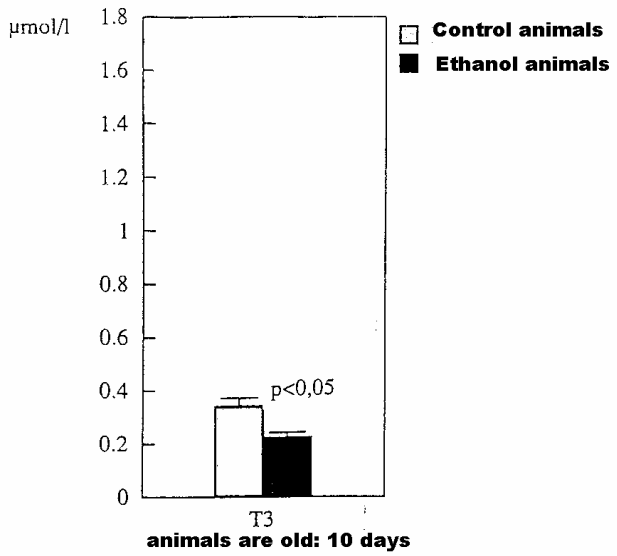

Fig. 8. Serum triiodothyronine (T3) levels in the thyroid gland of the 10-day-old rats of the control and ethanol groups

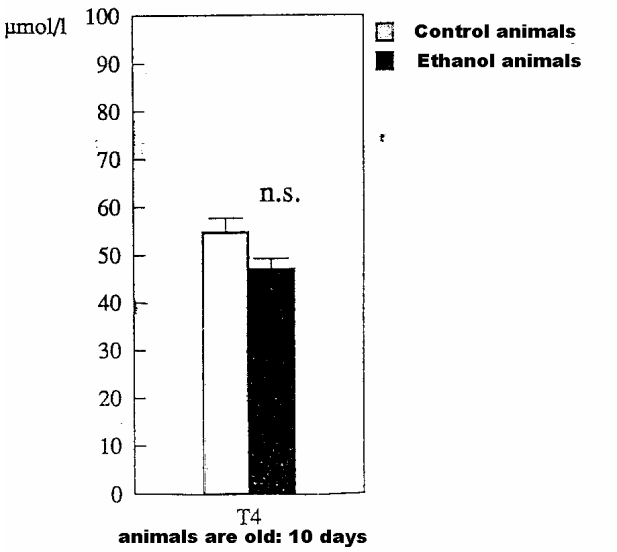

Fig. 9. Serum thyroxine (T4) levels in the thyroid gland of the 10-day-old rats of the control and ethanol groups

Discussion

The histological and stereological analyses suggested that the alcoholization of rat females, prior to and during pregnancy, as well as throughout the period of lactation induced the activation of the thyroid gland of the baby rats, which was followed by an initial decrease in the thyroid hormones T3 and T4 levels, as compared to the control animals. The significantly increased Ia was correlated with the plasma TSH level (Kalisnik 1971, 1981). The T3 and T4 levels in the 10-day-old rats of the alcohol-treated mothers decreased. The volume density of the interfollicular binder including the blood vessels insignificantly decreased in the alcoholized animals. The Ia (index of activation) of the baby rats of the alcoholized mothers was significantly increased. The volume density of colloid in the alcoholized animals was significantly reduced.

The stereological analysis revealed that the thyroids of the progeny of the alcohol-treated mother showed decreased Tiroxine (T4) levels in one-day-old rats (Kojima and Hershman 1974). The T3 level in initial postnatal days decreased. (Gayo *et al.* 1985). The index of activation (Ia) showed the activity of the thyroid gland, as reported by a number of experimental studies (Uotila and Kanas 1952, Palkovits 1963, Grant and Cunningham 1965). A hypothesis presented here implies that ethanol facilitates the adoption of T3 and T4 by the cells in various tissues inducing "intracellular hyperthyroidism" and, most likely, a decrease in the thyroid hormone levels of plasma, and affecting the intracellular metabolism of the thyroid hormones, activating the deiodinase

isoenzyme (Baumgartner et *al.* 1994). Importantly, T3 induces various changes in intracellular molecules and processes in peripheral tissues that are also affected by ethanol, such as the G-protein (Shank *et al.* 1984). Increased TSH in blood plasma is also evidenced by the presence of a large number of mastocytes(Melander *et.al.* 1971).

Conclusions

- The increased activity of the thyroid glands of the 10-day-old baby rats of the mothers alcoholized with 15% ethanol was also evidenced by the presence of a large number of microfollicles, increased follicular epithelia and decreased colloid.
- The increased activity of the glands in the initial 10 days of postnatal life was also evidenced by the presence of a large number of thyreocytes in mitosis.
- The stereological analysis demonstrated that the thyroids of the young of the alcohol-treated mothers were characterized by increased voluminous density of follicular epithelia.
- The volume density of the intrafollicular colloid amounts in the gland of the control animal increased, whereas its value in the alcoholized animals decreased.
- The thyroid gland activation index (Ia=Vve/Vvk) increased in the alcoholized animals as compared to the control ones.
- The thyroid gland activation in the baby rats of the alcoholized mothers induced hypertrophy and hyperplasion of C-cells as compared to the control animals.
- The volume density of the interfollicular binder including the blood vessels insignificantly decreased in the baby rats of the alcoholized mothers as compared to the control ones.
- The volume density of the interfollicular mastocytes (Vvm) increased in the alcoholized animals as compared to the control ones. The numerical density of mastocytes (Nvm) in the interfollicular binder of the experimental animals decreased as compared to the control animals.
- The T3 level in the alcohol-treated animals significantly increased as compared to the control animals. The T4 (thyroxin) level in the baby rats of alcoholized mothers insignificantly decreased as compared to the control animals.

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PROMENE TIREOIDNE ZLEZDE KOD ŽIVOTINJA HRONICNO TRETIRANIH ALKOHOLOM

- originalni naučni rad -

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Rezime

Na eksperimentalnom modelu alkoholizma u kome su muzjaci ženke pacova soja Wistar, mesec dana pre sparivanja, pile 15% etanol kao jedinu ponuđenu tečnost a ženke nastavile da piju alkohol i tokom graviditeta i laktacije, odabranim citološkim kvalitativnim i kvantitativnim metodama kao i merenjem nivoa tireoidnih hormona (T3 i T4) ustanovili smo da je kod njihovih mladunaca postnatalne starosti od deset dana došlo do aktivacije tireoidne zlezde.

Gledajuci sumarno, rezultati pokazuju da dugotrajna alkoholizacija majki izaziva poremećaj tireoidne funkcije njenih mladunaca, pa su prvih 10 dana bili izrazito hipotireoidni. Uočen je raskorak između, s jedne strane, strukturnih znakova stimulacije žlezde, a s druge strane sniženog nivoa T3 i T4.

Pošto je održanje određenog homeostatskog nivoa tireoidnih hormona neophodno za rast organizma i posebno za sazrevanje centralnog nervnog sistema u kritičnim fazama razvoja tokom fetalnog i ranog postnatalnog života može se konstatovati značajan udeo tireoidne disfunkcije u razvoju nekih od anomalija u FAS-u.