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Influence of zinc on ethanol-induced placental changes in the rat

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Summary. For normal fetal growth and developmet, an ample sumpply of nutrients and oxygen is essential. The placenta is the conduit for nutrient transfer and thus any factor that alters normal placental structure and function may adversely affect the nutritional status of the fetus. The effect of ethanol ingestion and zinc supplementation on placental structure was investigated by the simultaneous administration of ethanol and zinc to pregnant Sprague-Dawley rats from gestational day 6 through 12. One group of animals was given an ethanol liquid diet, a second group received the ethanol liquid diet plus zinc, and another group was pair-fed a control liquid diet. Placentas were recovered on day 20 of gestation. The mean placental weight in the ethanol group was significantly higher than that in either the pair-fed control or the ethanol plus zinc group. The ethanol-treated group revealed more stagnated blood in the basal-decidual and in the basal-labyrinthine junctions. Intervillous spaces in the labyrinthine zones were markedly dilated and filled with more blood corpuscles compared to the pair-fed control group. The giant cells of the basal zone were also larger in size in the ethanol-treated group. The frequency of occurrence of stagnated blood in either the labyrinthine zone and in the basal-labyrinthine junction was less in the ethanol plus zinc group compared to the ethanol group.

Key words: Alcohol embryopathy, Prevention, Zinc, Rat, Early development

Introduction

Excess ethanol consumption during pregnancy is recognized as the most prevalent cause of developmental anomalies in humans (Abel and Sokol, 1987). Offspring of chronic alcoholic mothers are characterized by a pattern of congenital anomalies that is known as the fetal alcohol syndrome (FAS). Microcephaly, mental retardation, prenatal and postnatal growth retardation, and facial anomalies are developmental characteristics associated with FAS (Jones et al., 1973). Retarded fetal growth, congenital anomalies, increased resorptions and skeletal abnormalities have been experimentally induced with ethanol in rat and mice fetuses (Weinberg, 1985; Sanchis et al., 1987). Growth and developmental retardation also have been detected in cultured whole rat embryos exposed to ethanol during the period of organogenesis (Brown et al., 1979; Fadel and Persaud, 1992).

The mechanism underlying the embryopathic effects of ethanol is not clear. For normal fetal growth and development, an adequate supply of essential nutrients such as amino acids, glucose, trace elements, vitamins, and oxygen is necessary. The placenta is responsible for the transfer of these nutrients to the fetus. Thus, any factor that disrupts placental structure and function could adversely affect the nutritional status of the fetus.

Impaired placental transport of zinc, as a result of maternal ethanol intake, has been reported (Ghishan et al., 1982). Both long-term and short-term ethanol feeding resulted in a significant reduction in placental and fetal uptake of zinc. In addition, long-term ethanol consumption significantly decreased fetal, placental, and serum zinc concentrations.

Administration of ethanol in experimental animals has been reported to interfere with zinc metabolism (Antonson and Vanderhoof, 1983; Assadi and Ziai, 1986), and zinc deficiency impairs ethanol metabolism (Das et al., 1984). Following ethanol exposure, decrease rate of absorption (Antonson and Vanderhoof, 1983) and increased urinary loss of zinc have been documented (Sullivan and Lankford, 1965). Chronic alcoholism decreased serum/plasma zinc levels in animals (Ghishan et al., 1982; Das et al., 1984) and humans (Sullivan and Lankford, 1965; Flynn et al., 1981). In addition, lower levels of hepatic zinc and decreased hepatic level/activity of alcohol dehydrogenase, the major enzyme responsible for metabolism of ethanol, have been reported (Sullivan, 1962; Prasad et al., 1967). Inverse relationships between maternal alcohol dehydrogenase activity and fetal abnormalities/maternal

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blood alcohol levels (Chernoff, 1980) and between maternal plasma zinc concentrations and incidence of fetal anomalies similar to FAS have been reported (Flynn et al., 1981).

Zinc deficiency acts as a coteratogen with ethanol in FAS. This was supported by lower fetal weight, a higher incidence of external anomalies, and a higher frequency of resorptions in mice fed a low zinc diet and ethanol compared to mice given an ethanol diet with adequate levels of zinc (Keppen et al., 1985).

The present study is designed to investigate whether or not zinc supplementation has any influence on ethanol teratogenicity as a result of changes in the placenta.

Materials and methods

Nulliparous albino Sprague-Dawley rats (220-240 g) and sexually mature males of proven fertility were used. All animals were kept in an environmentally controlled room. After adaptation for a period of 3-5 days the animals were mated and the day of finding spermatozoa in the vaginal smear was considered the first day of gestation.

Once pregnancy was confirmed, animals were randomly assigned to pair-fed control, ethanol, and ethanol + zinc groups. Each group consisted of 10 pregnant animals.

The ethanol group received the liquid diet containing ethanol ad libitum, and the pair-fed control group received an isocaloric control liquid diet, with maltose-dextrin substituted for ethanol. Each rat in the pair-fed control group was paired to a rat fed the liquid ethanol diet and also was fed the amount consumed by the respective pair-mate the previous day. The inclusion of such a pair-fed (restricted-fed) group was important because ethanol feeding during pregnancy has been reported to result in reduced food intake (Lieber and DeCarli, 1982). Pure 100% ethanol (Canadian Industrial Alcohol and Chemicals Limited), diluted to 95%, was used to make the ethanol liquid diet. The liquid ethanol diet contained 6.4% ethanol and the animals obtained about 36% of their calories from ethanol. The control diet was isocalorically balanced with maltoxe-dextrin.

The ethanol + zinc group received the liquid ethanol diet which was supplemented with zinc sulfate. Zinc sulfate ($ZnSO_4$, molecular weight 161.44, Sigma Chemical Co.) was administered intraperitoneally (i.p.) at a dose of 15 mg/kg body weight. Granular zinc-sulfate, 750 mg, was dissolved in 100 ml of physiological saline and a volume of 0.5 ml was injected i.p. every morning throughout the treatment period.

During the entire treatment period the liquid diet was the only source of nutrients and fluid for the animals. The daily food intake of each animal was recorded every morning and animals were weighed and weight gain was recorded on days 1, 6, 12 and 20 of gestation.

On gestational day 20, gravid females were killed by an overdose of ether and the placentae were recovered and weighed. Six to seven placentae from each of the three groups were randomly selected. Following routine processing for light microscopy, the placentae were embedded in paraffin and cut into sections of 4 μm thickness using a Sorval-JB-4 microtome. The sections were then mounted on glass slides, stained with haematoxylin and eosin (H&E) and cover-slipped for microscopic examination.

The sections were examined for evidence of structural and vascular alterations using a Nikon binocular light microscope. The following structures were examined and were used as indices of functional as well as structural changes in the placenta: basal zone of the placenta, labyrinthine zone, intervillous spaces, giant cells, and trophoblasts.

Results

The results revealed no statistically significant differences in food intake between the ethanol-fed and its pair-fed control or the ethanol + zinc groups (Table 1). Therefore, the dietary nutrient calories and zinc intakes of the various groups of animals were similar.

Ethanol consumption in the ethanol and ethanol + zinc groups were also found to be similar, 3.70±0.01 and 3.70±0.01 g/day, respectively (Table 1). This amounts to about 36% of the total calories obtained from the diet daily throughout the duration of the treatment.

The mean placental weight of fetuses of ethanol-fed animals (5.1±0.1 g) was significantly increased ($p<0.01$), compared to the pair-fed control (4.2±0.1 g) and the ethanol + zinc groups (4.4±0.1 g).

Photomicrographs of placental sections, prepared from pair-fed control, ethanol and ethanol + zinc treated rats, stained with haematoxylin and eosin (H&E), are shown in Figures 1-6. Placental sections in the ethanol

Table 1. Treatment schedule and dietary intakes of animals.

TREATMENT GROUPS	NUMBER OF ANIMALS	TREATMENT	ENERGY INTAKE (Kcal/day)	ZINC INTAKE (mg/day)	ETHANOL INTAKE (g/day)
Pair-fed control	10	Maltose-dextrin control liquid diet (restricted)	71.7±1.5	0.54±001	-
Ethanol	10	Ethanol liquid diet	71.4±1.7	0.54±0.01	3.70±0.01
Ethanol + zinc	10	Ethanol liquid diet and zinc	71.5±2.1	0.54±0.01	3.70±0.01

Pure ethanol was incorporated in the liquid ethanol diet (6.4%) and maltose-dextrin substituted the calories derived from ethanol in the control (maltose-dextrin) liquid diet. Zinc, in the form of $ZnSO_4$, was administered i.p. (15 mg/kg). Results are expressed as mean±SDM (ANOVA).

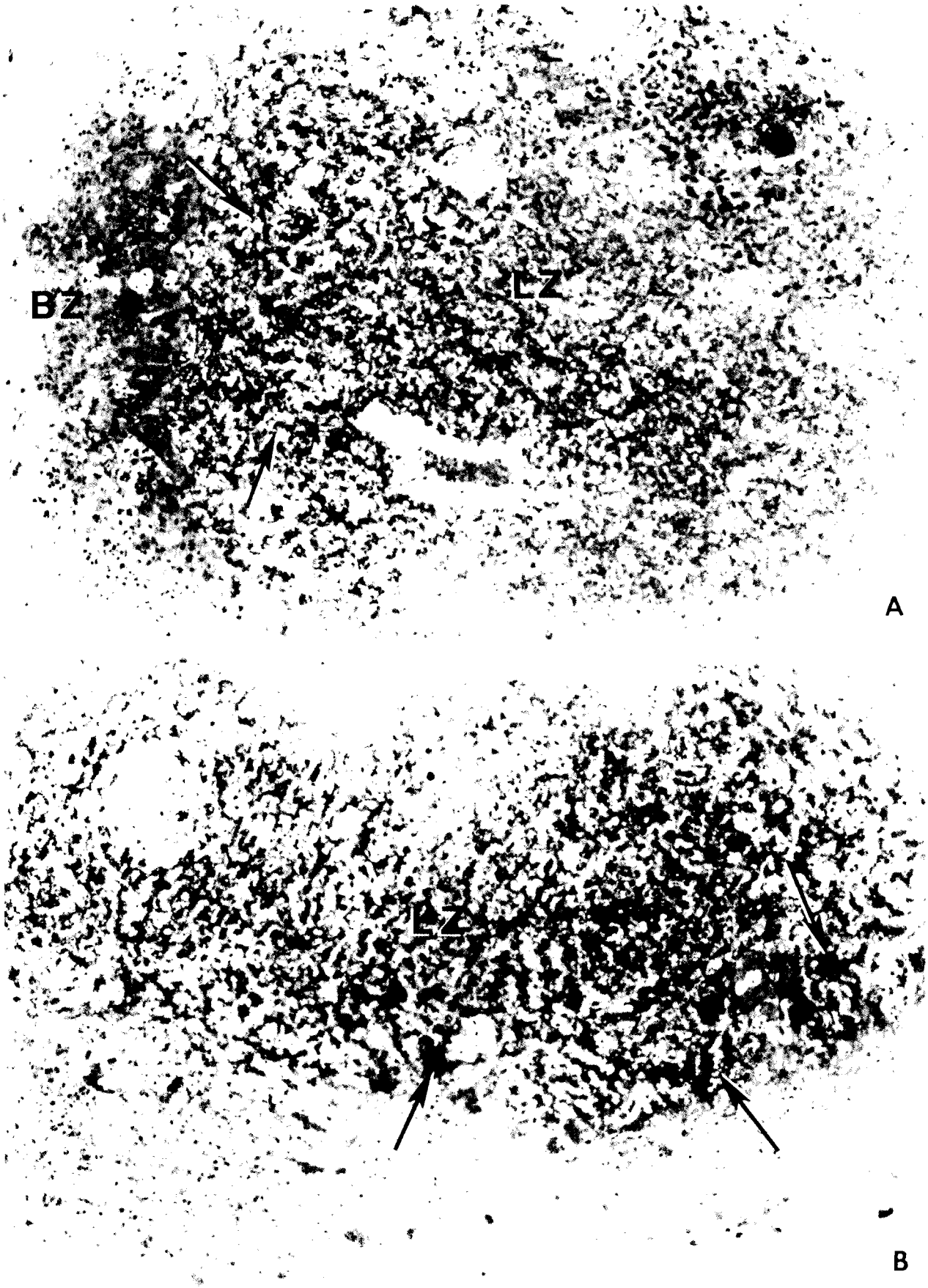

Fig. 1. Placental sections showing the basal zone (BZ), the labyrinthine zone (LZ) and the basal-labyrinthine junction (arrow). **A.** Placenta of a pair-fed control animal. **B.** Placenta of an ethanol treated animal showing intervillous spaces in the labyrinthine zone (LZ) and in the basal-labyrinthine junction (arrows) more filled with maternal blood. x 168

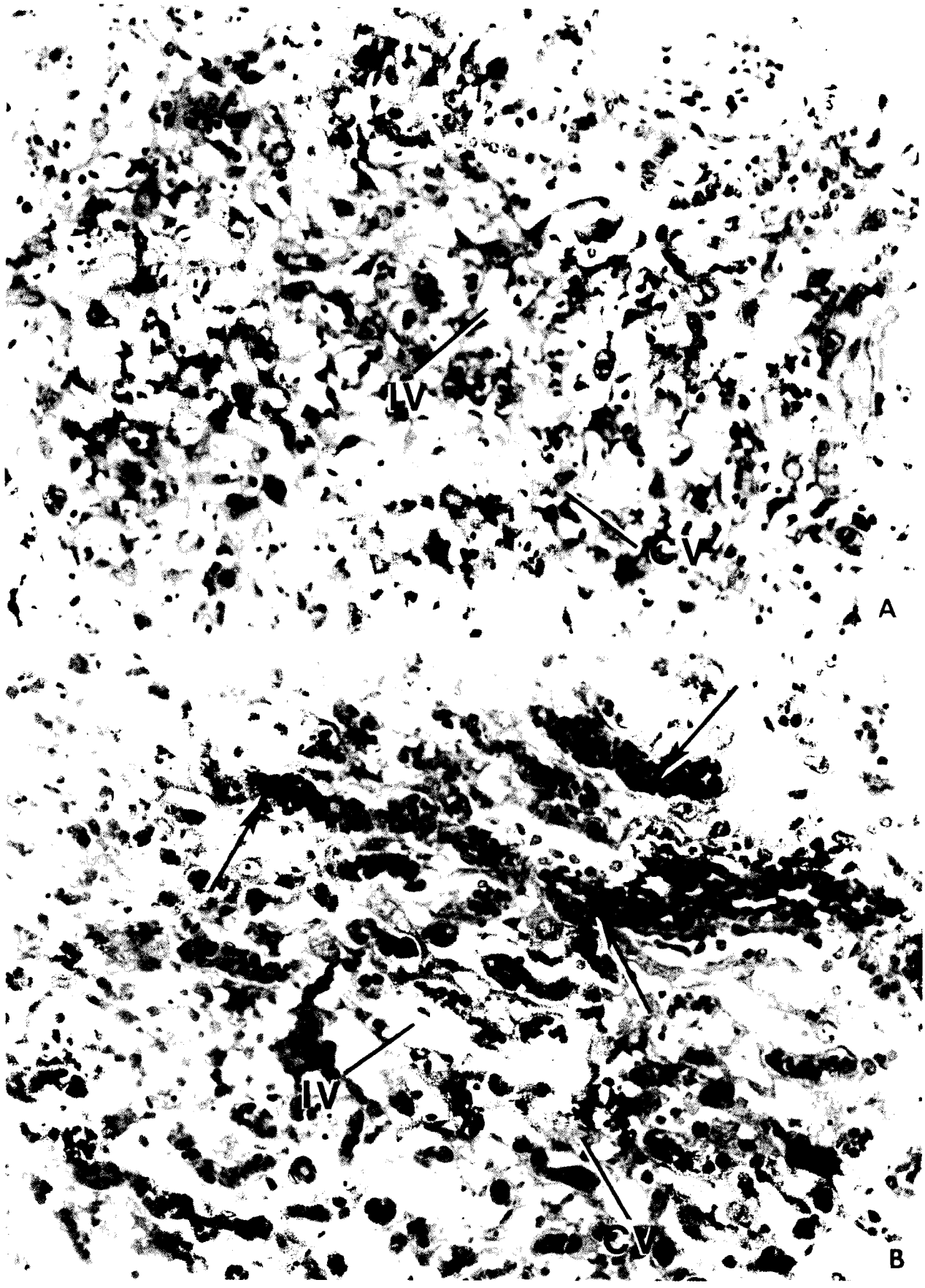

Fig. 2. Placental sections showing the labyrinthine zone. **A.** Placenta of a pair-fed control animal showing chorionic villi (CV) and intervillous spaces (IV). **B.** Placenta of an ethanol treated animal showing increased stagnating blood and blood corpuscles in the intervillous spaces (arrow). Intervillous spaces (IV) also appear dilated. x 420

treated group appeared to have more stagnated blood in the basal-decidual and in basal-labyrinthine junctions. Intervillous spaces in the labyrinthine zone were more dilated, and filled with maternal blood more frequently in the ethanol than in the pair-fed control group. In addition, the giant cells, located in the basal zone of the placenta, also appeared larger in the ethanol group compared to the pair-fed control. The frequency of occurrence of stagnated blood in either the labyrinthine zone or in the basal-labyrinthine junction appeared to be lower in the ethanol + zinc group compared to the ethanol group.

Discussion

In the present study, ethanol feeding of pregnant rats resulted in changes in the microscopic structure of the placenta. Increased stagnation of maternal blood in the labyrinth and in the basal-labyrinthine junction, and enlarged giant cells in the basal zone of the placenta in excess of those in the corresponding pair-fed control group, were observed and this could have accounted for the enlarged placenta in the ethanol treated rats. The fact that these alterations in the placental structure were apparent on day-20 of gestation, long after treatment of the animals was discontinued, appear to suggest that the effect of ethanol on the placenta is long lasting.

The stagnated maternal blood (in the maternal blood channels) could impair the placental transfer of nutrients and oxygen to the fetus. As a result, fetal malnutrition and hypoxia will occur and enhance the embryopathic effects of ethanol. Similar vascular and cellular changes in the placenta have also been reported in pregnant rats treated with ethanol (Eguchi et al., 1989). Maternal blood channels in the labyrinth were more dilated and filled with blood and the giant cells were larger in size and more in number in the ethanol treated group. These structural and cellular changes were suggested to be responsible for the significantly increased placental weight observed in the ethanol treated animals compared to that in the pair-fed controls (Eguchi et al., 1989). The placental weight in the ethanol treated animals was also significantly higher in the present study.

Histological study of the placenta showed that the increased appearance of stagnated maternal blood in the labyrinth or in the basal-labyrinthine junction, in ethanol treated animals, appeared to have been reduced in the animals treated with ethanol plus zinc. Chronic ethanol

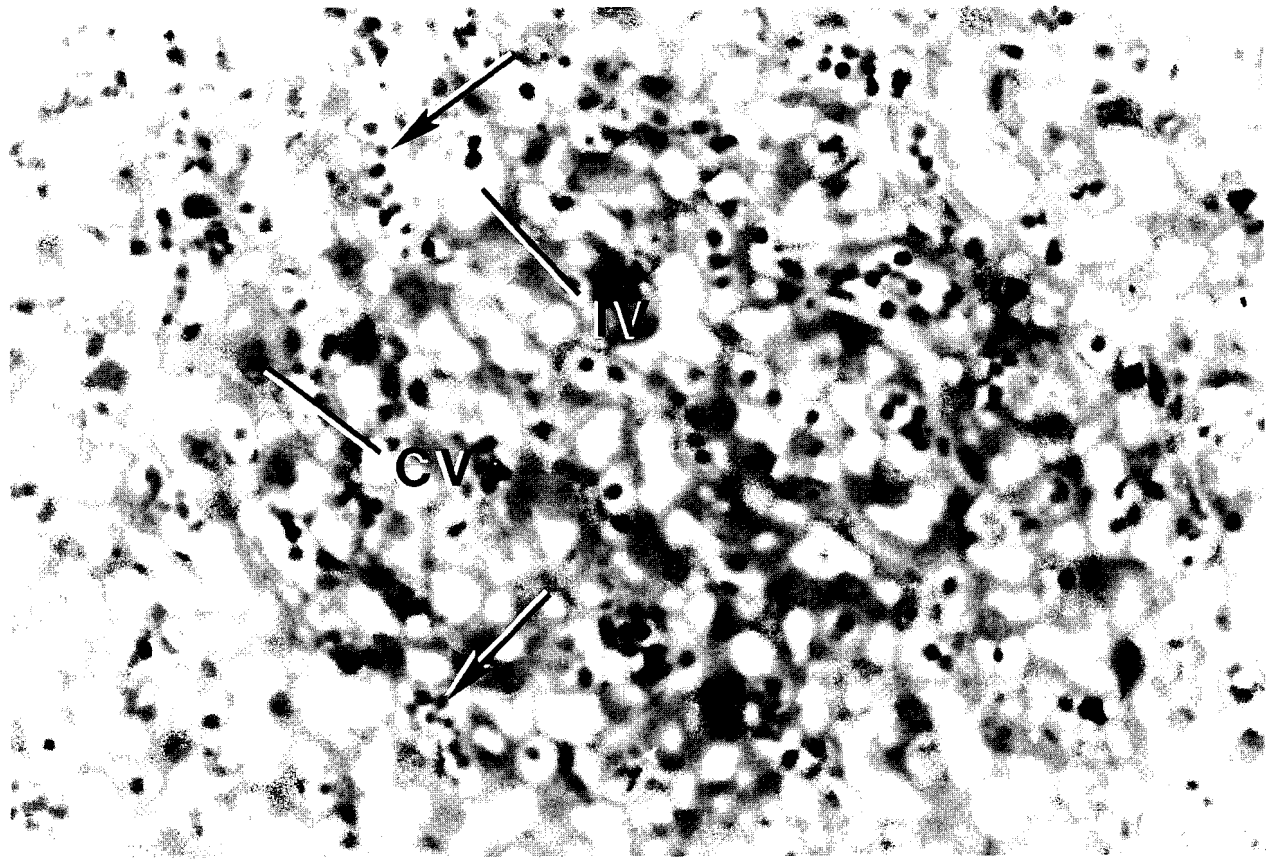

Fig. 3. Placenta of an ethanol plus zinc treated animal showing the labyrinthine zone with abundant chorionic villi (CV). The size of the intervillous space (IV) and the maternal blood (arrow) in it appeared to be reduced, compared to only ethanol treatment. x 420

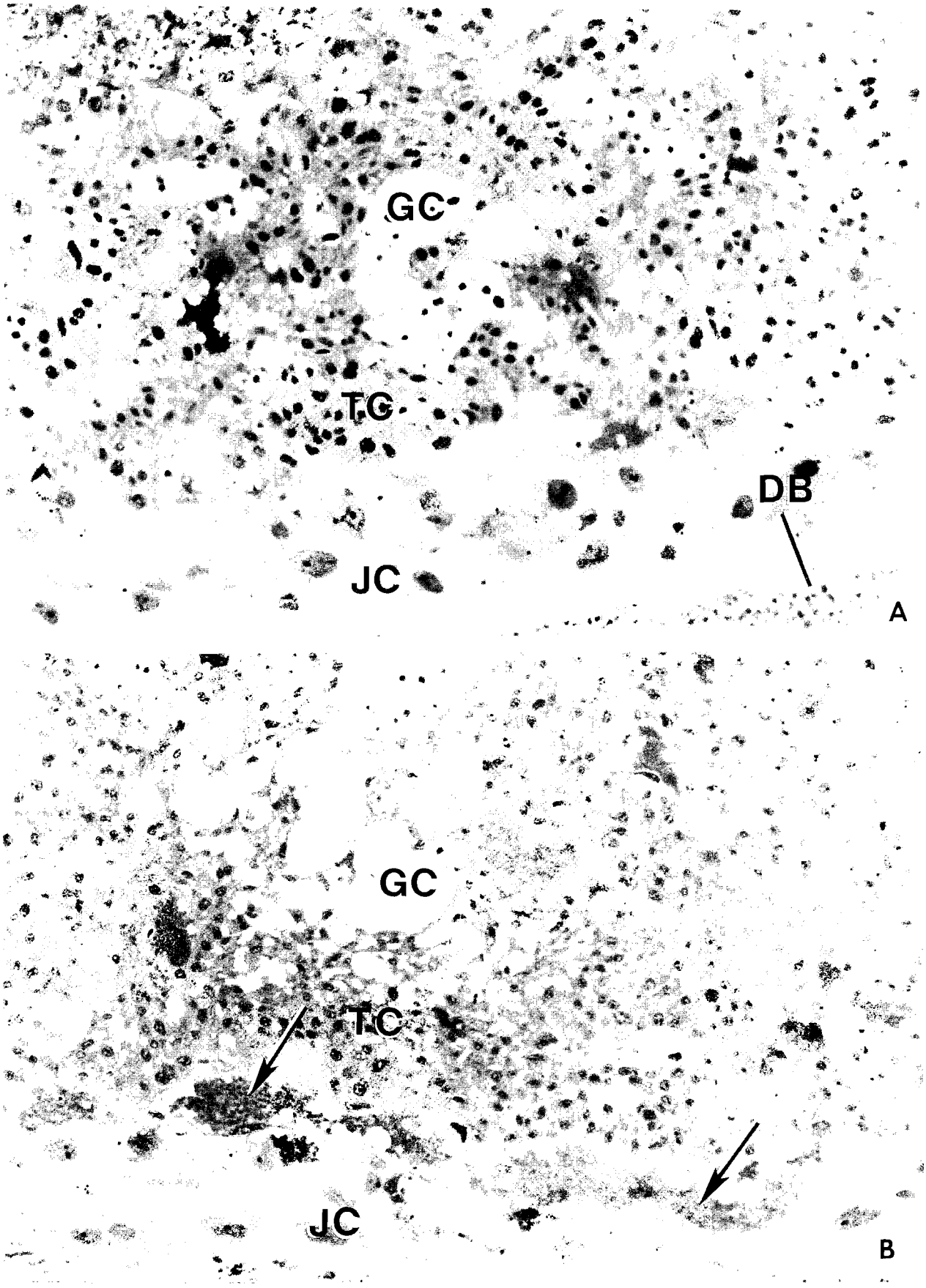

Fig. 4. Placental sections showing the basal zone with decidua basalis (DB), giant cells (JC), trophoblast (TC) and glycogen cells. **A.** Placenta of a pair-fed control animal. **B.** Placenta of an ethanol treated animal: the basal-labyrinthine junction is more filled with maternal blood (arrows). x 420

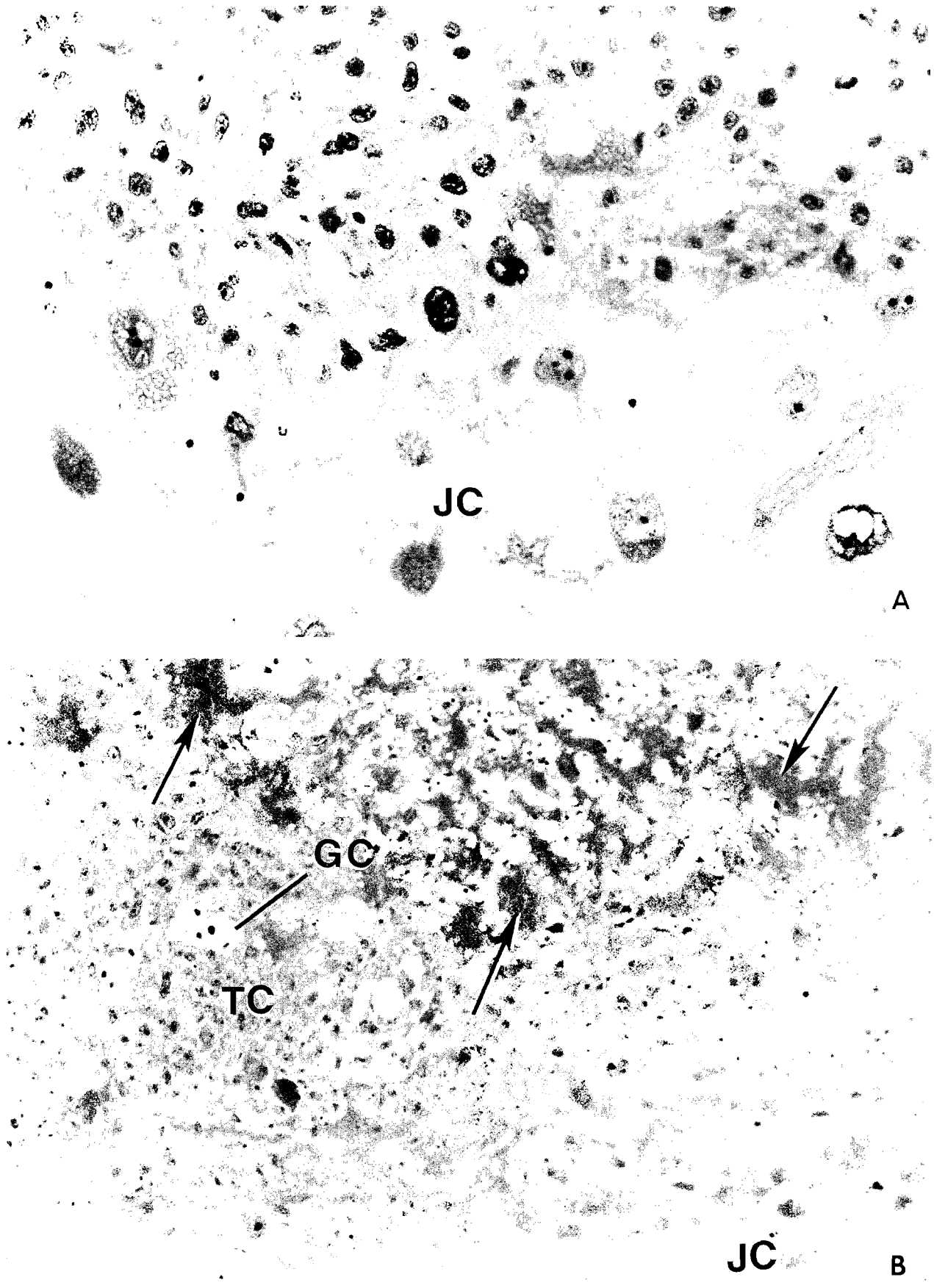

Fig. 5. Placental sections showing the basal zone. **A.** Placenta of a pair-fed control animal. **B.** Placenta of an ethanol treated animal showing decidua basalis (DB), trophoblast, and larger giant cells (JC). x 840

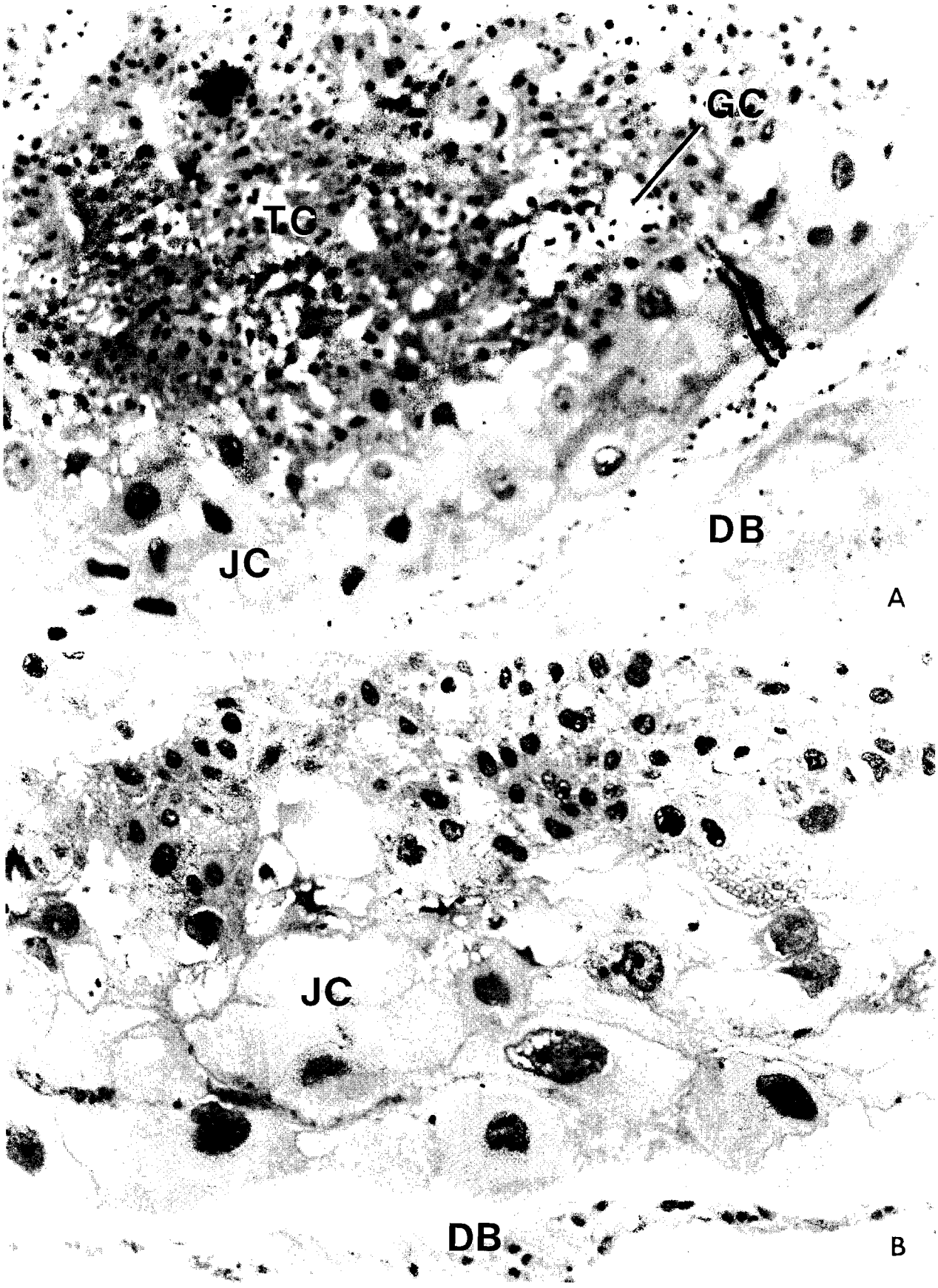

Fig. 6. Placental sections showing the basal-zone with decidua basalis (DB), giant cells (JC), trophoblast (TC) and glycogen cells (GC). **A.** Placenta of a pair-fed control animal. **B.** Placenta of an ethanol treated animal showing maternal blood and blood corpuscles at the junction of the giant cell (JC) and trophoblast cell layers (arrows). Note also abundant blood corpuscles, probably degenerating erythrocytes, in the cytoplasm of the giant cells. x 420

feeding during gestation has been reported to decrease placental blood flow in the rat (Jones et al., 1981). As a result, nutrient availability and transfer to the fetus could be affected. These ethanol-induced impairment may be ameliorated by substances that lower circulating ethanol levels. Reduced serum ethanol concentrations were observed following supplementation of ethanol treated pregnant animals with zinc (Seyoum et al., 1994).

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