Effect of Ethionine on Fetal Pancreatic Development in the Rat* (32889)

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Ethionine, a metabolic antagonist of the naturally occurring amino acid, methionine, has been shown to have marked pathological and growth-inhibitory effects on a wide variety of organisms. Young rats fed an ethionine diet show a significant reduction in bone growth (1). A marked decrease in hepatic ATP levels, followed by inhibition of both protein and RNA synthesis, is reported after ethionine administration to adult rats (2,3). Perhaps the most marked morphological effect of ethionine is its rather selective degeneration of the acinar epithelium of the pancreas in rats. Up to 95% of the acinar architecture can be destroyed by daily ethionine injections over a 10-day period (4). Since 1955, considerable work has been done on the effects of ethionine on the developing rat fetus, the most frequently reported results being an increased rate of fetal resorption and a decrease in the size of the young (5,6). Some investigators have specifically studied the effects of ethionine on the pancreas of the developing fetus (7,8), but in all cases, the fetal pancreas was reported to be completely normal, despite considerable damage to maternal pancreatic tissue. It was our opinion that an effect on fetal pancreatic tissue might be observed if sufficiently large doses of ethionine were injected during the final weeks of pregnancy, when acini are being formed and zymogen synthesis in progress.

Materials and Methods. Twenty-three pregnant rats of the Sprague-Dawley CD strain, consisting of 8 experimental and 15 control rats, were placed in individual cages and fed rat chow and water *ad libitum*. The day on which sperm was found in a vaginal smear was designated as the first day of pregnancy.

A dosage schedule was worked out which would cause maximum damage to the maternal pancreas, yet permit survival of both mother and fetuses to the end of gestation. The daily dosage used was 0.5 gm of ethionine per kg maternal body weight. A solution of *dl*-ethionine in physiological saline (3 gm of ethionine per 100 ml of saline) was prepared, adjusted to pH 7.4 with 3.0 *N* NaOH, and injected intraperitoneally each day from days 14 through 20 of pregnancy. Control rats were injected with equivalent volumes of physiological saline. Both controls and experimentals were sacrificed on day 21 of the 22-day gestation period. The young were removed by laparotomy while the mother was under ether anesthesia. Immediately following removal from the uterus, the fetuses were weighed, decapitated, and the fetal pancreas and attached organs (spleen, stomach, and duodenum) were removed and fixed in FAA (85 parts 70% ethanol, 10 parts formalin, 5 parts glacial acetic acid). The following day, the fetal pancreas was dissected from the surrounding organs and weighed. After removal of all fetuses from the uteri, the mothers were decapitated and their pancreases were removed, weighed, and fixed. The maternal uteri were then slit open lengthwise and examined visually for resorbed fetuses. All tissues were embedded in paraffin and histological sections were prepared at 6 μ and stained with hematoxylin and eosin.

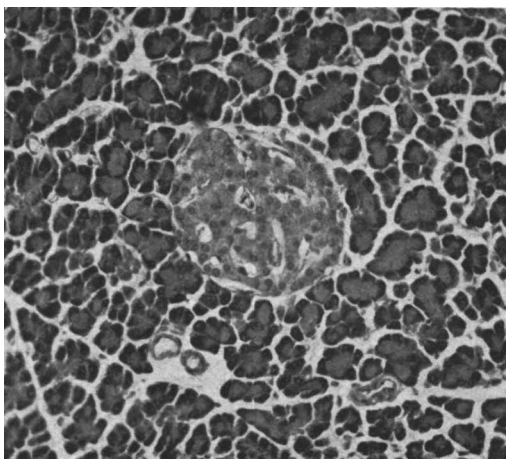

FIG. 1. Normal pancreas of female rat on day 21 of pregnancy. × 215.

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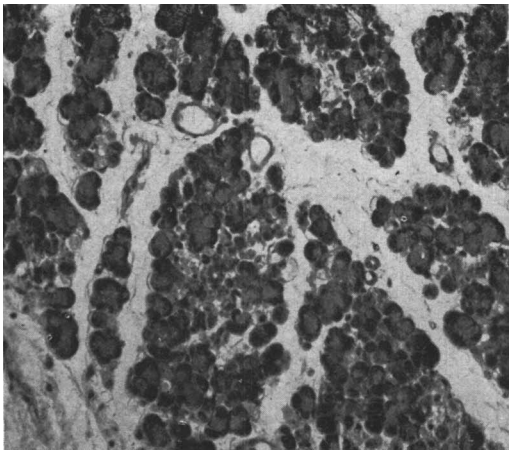

FIG. 2. Maternal pancreas on day 21 of gestation following daily ethionine injections from days 14 through 20 of pregnancy. × 215.

Results. Maternal effects. Histological examination of the maternal pancreas revealed marked degeneration in all mothers injected with ethionine (Figs. 1 and 2). All pancreatic tissue from this group showed prominent interstitial edema, disruption of acinar architecture, vacuolization of acinar cells, loss of cytoplasmic basophilia, and a general decrease in zymogen content. These degenerative changes are the typical ethionine effects noted in numerous other studies of both normal (4) and pregnant rats (8).

Fetal effects. Litter size. As in previous studies on the effects of ethionine on pregnancy (5,6), a decrease in litter size was observed in the experimental group, relative to the saline-injected control group. As of day 21, the average litter size in the eight ethionine-treated mothers was 9.75 ± 1.33 fetuses per litter, while that in the eight controls was 13.38 ± 0.71 (see Table I). Since conditions at conception were identical in both groups, the significant ($p<0.01$) differences in litter size can be attributed to ethionine-induced resorption of fetuses during pregnancy. This conclusion was confirmed by the discovery that an average of 19% of the experimental fetuses had undergone resorption, while no fetal deaths were observed among the controls. The possible causes of ethionine's toxic effect on fetuses can be divided into two groups: (a) those resulting from the direct effect of injected ethionine of fetal metabolism, and (b) those caused by damage to maternal systems (8).

The evidence for at least some direct action seems quite strong. Ethionine has been shown to be rapidly transported across the placenta to the fetus, where it becomes a part of the free amino acid pool of this developing organism (9,10). The mechanism by which ethionine exerts a direct resorptive effect remains unclear. One possibility might involve the competitive incorporation of ethionine for methionine into fetal proteins. A second possibility might be related to the reduced levels of ATP which this analogue produces in fetal tissues (11). Insufficient levels of ATP should have the same inhibitory effects on protein and ribonucleic acid synthesis that are observed in the adult liver following ethionine administration (2,3). Both of these possible mechanisms might lead to insufficient levels of essential proteins at critical periods of development, resulting ultimately in death and resorption of the fetus.

Fetal body weight. One of the most significant differences in development between experimental and control groups was the marked reduction in the body weight of the 21-day fetuses exposed to ethionine for a week. The average weight of control fetuses was 4.40 ± 0.03 gm, while that of experimental fetuses was 2.24 ± 0.04 gm, which is a significant difference ($p<0.01$) (see Table

TABLE I. Effect of Ethionine (0.5 gm/kg of body wt.) on Fetal Rats During the Last Week of Pregnancy.

Group	Total litter size	Av litter size	Av body wt. (gm)	Av relative wt. of pancreas (%)
21-day controls	107	13.38 ± 0.71	4.40 ± 0.03	0.732 ± 0.007
21-day ethionine group	78	9.75 ± 1.33	2.24 ± 0.04	0.549 ± 0.009
20-day controls	84	12.00 ± 0.44	2.26 ± 0.03	0.635 ± 0.007

I). This reduction in body weight was to be expected since the greatest incremental growth of the fetus occurs during the final week of gestation and this was the period during which ethionine was administered. The cause of this marked reduction is probably a direct toxic effect on the fetus by injected ethionine. Thus, there is probably some inhibition of protein synthesis in the developing fetus, with the result that growth is markedly impaired. It is also possible that the severe disturbances in maternal metabolism caused by ethionine are a contributing factor to the small size of the fetus exposed to ethionine. For example, the depressed function of the maternal pancreas might result in nutritional deficits in the mother, deficiencies which would be expected to be reflected in her fetuses as well.

In an effort to determine whether or not ethionine was affecting the development of all fetal tissues equally, seven control rats were sacrificed on day 20, instead of day 21, in order to obtain normal animals whose body weights were approximately equal to those of the ethionine-treated fetuses. In this way it could be determined whether ethionine was retarding the growth of the entire fetus, or whether certain tissues, specifically the pancreas, were being selectively retarded in their growth and differentiation. The average fetal body weight in the 20-day controls was 2.26 $\pm$ 0.02 gm; the difference between this group and the ethionine group (2.24 $\pm$ 0.04 gm) was negligible (see Table I).

Effects on the fetal pancreas. Perhaps the most important parameter being studied here is the difference in pancreatic relative weights in experimental and control fetuses. The relative weight of the fetal pancreas in the ethionine-treated group was 0.549 $\pm$ 0.009%, which was significantly ($p<0.01$) less than either the 21-day controls (0.732 $\pm$ 0.007%) or the 20-day controls (0.635 $\pm$ 0.007%) (see Table I).

Histological examination of fetal pancreatic tissue revealed marked differences between ethionine and control groups at the histological and cellular level. Pancreatic development in both control groups was apparently quite normal (Fig. 3). Microscopic examination revealed well-formed acini filled with zymogen granules in the apical regions of the cells.

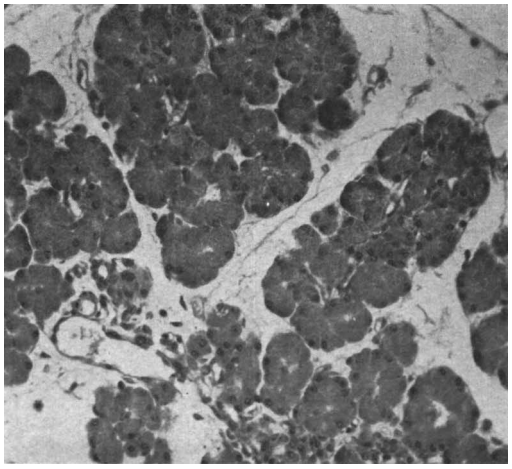

FIG. 3. Normal fetal pancreas on day 21 of gestation. × 215.

Nuclei were located in the basal regions and exhibited prominent nucleoli. The pancreas of the 20-day fetus was similar to that of a 21-day fetus except for a decreased level of cytoplasmic zymogen.

The pancreas of ethionine-treated fetuses, however, showed marked deviations from the normal structure described above (Fig. 4). There was a slight increase in interstitial edema, similar to the more extensive edema seen in adult pancreatic tissue exposed to ethionine. Secondly, there was a disruption of the normal acinar architecture; acinar epithelial cells were not found in the orderly circular arrangements seen in control groups.

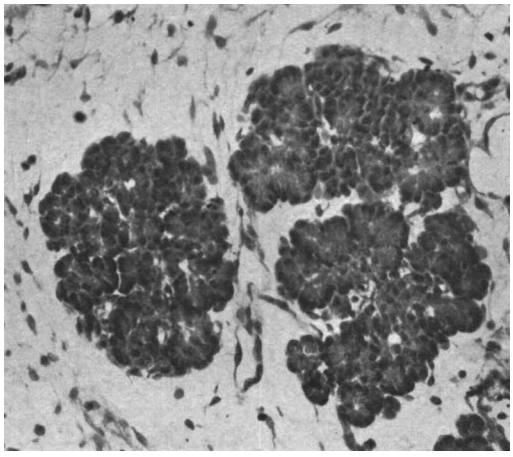

FIG. 4. Fetal pancreatic tissue removed on day 21 of gestation following ethionine injections to mother from days 14 through 20 of pregnancy. × 215.

Cellular integrity was maintained in all cases, and nuclei continued to display prominent nucleoli. Those acini still remaining intact were usually much smaller than the ones in control glands. Finally, there was a decrease in the zymogen content of the acinar cells. Thus, fetal pancreatic tissue from ethionine-injected mothers is quite different from that of either control group, but is not affected as much as adult glands.

Discussion. The most important finding in this study—the disruptive effect of ethionine on the fetal pancreas—has not been reported elsewhere. In fact, two studies (7,8) failed to demonstrate that ethionine had any observable effect on the development of the fetal pancreas. However, neither of these studies utilized a sufficiently high dose of ethionine during the final week of pregnancy. The highest dose given by Lee *et al.* (7) was less than half that given in the present experiments, while in the study by Proffit and Edwards (8) the doses were adequate but no injections were administered during the last week of gestation.

This period is of special importance because zymogen granules and secretory acini first become discernible on day 16 or 17 of gestation in the rat. Thus, it is during this time that the fetal pancreas assumes those morphological attributes which characterize the adult pancreas, an organ mature enough to be susceptible to the deleterious effects of ethionine. It is to be expected that ethionine should exert its disruptive effect on the fetal pancreas when this organ most resembles the adult organ, both morphologically and metabolically. The last week is important morphologically because acini are being formed, and metabolically, because zymogen synthesis begins.

High doses of ethionine are essential for the simple reason that the biochemical pathology of this analogue depends on its favorable competition with methionine for certain enzymes (12). Competitive inhibition almost always operates on the principle of "the more analogue, the more complete the inhibition." For this reason, a maximum dosage was determined which would still allow successful completion of pregnancy.

Disruption of fetal pancreatic structure was observed at the acinar and cellular levels. There was a definite decrease in zymogen content and a general disruption of the acinar architecture, similar to that observed in the maternal pancreas. The measures of relative weight also indicate a selective effect on the fetal pancreas. The difference between the experimental and 21-day controls (0.549 ± 0.009 vs 0.732 ± 0.007, respectively) is highly significant ($p<0.01$). As previously noted, a second control group was established during the course of the experiment in an attempt to see whether ethionine was slowing down fetal development as a whole, or whether a selective effect was really operating on the pancreas. If the relative weights of the pancreas in this 20-day control group and the 21-day ethionine-treated group were the same, it could be presumed that ethionine had simply slowed down the development of the entire fetus by about 24 hours. This was not the case, however; the relative weight of the pancreas in the ethionine-treated group was still significantly less ($p<0.01$) than in the 20-day controls (see Table I) despite their similar absolute body weights. Marked morphological differences also existed between these two groups, as noted above. Thus, ethionine apparently did exert a specific effect of fetal pancreatic development, much as it does on the adult pancreas.

The exact mechanism of ethionine's effect on fetal pancreatic tissue remains unclear, although two possible explanations suggest themselves. The first is that ethionine actually interferes with the mechanism by which pancreatic epithelial cells organize into acini during days 17 through 19, and that normal acini are never formed in these fetuses. The second possibility is that ethionine exerts its degenerative effects after the pancreatic acini have already formed to some extent, the mechanism of degeneration being similar to that acting on the adult pancreas. At this time it is impossible to choose between these two possibilities or rule out any other explanations of the observed effect. A more extensive study of the day-to-day changes in the fetal pancreas during ethionine injections is necessary before a definite mechanism can be offered.

Summary. The effects of *dl*-ethionine on fetal development in the rat were studied, with

particular reference to the formation of the exocrine pancreas. Pregnant rats were injected from day 14 through day 20 of pregnancy, and sacrificed on day 21 of the 22-day gestation period. Controls were injected with equivalent volumes of saline. Ethionine caused significant decreases in litter size and fetal body weight, and had a selectively disruptive effect on the formation of the fetal pancreas as indicated by measures of pancreatic relative weight and histological examination of fetal pancreatic tissue.

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Effect of Calcium Infusion on Gastric Function in Rats (32890)

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A number of clinical observations and experimental data seem to indicate the existence of a relationship between parathyroid glands, vitamin D, calcium, and gastric function. Whether morphological and secretory changes of glandular mucosa of stomach represent a direct effect of parathormone or vitamin D, or are due to hypercalcemia, is unknown (1, 2). Many conflicting observations have been reported in man and in animals treated with parathormone, vitamin D, and calcium. The results of these studies appear to depend on the species studied, on the state of gastric innervation, on the rapidity of blood calcium changes, on the methods of stimulation gastric function and on the duration of the experiment (2, 3).

The present short report is concerned with the effect of prolonged infusion of calcium on gastric function in rats bearing permanent gastric fistula.

Gastric secretion was collected in these animals for 24 hours during the infusion. At the end of the experiment, blood and gastric tissue pepsinogen was also determined.

Materials and Methods. Under ether anesthesia, a gastric fistula was produced in adult male Sprague-Dawley rats and a miniature stainless steel cannula of Pavlov's type was inserted (4). Collection experiments started after a postoperative period of 10 days. Prior to infusion studies, rats were conditioned to the procedure by keeping them in Bollman restraining cages (5) for periods of several hours. Rats were fasted for 24 hours prior to the infusion study but water was allowed as desired. On the morning of the experiment, each animal was placed in a Bollman cage and the stopper screw of the cannula was removed. After the initial drainage for 30 min the infusion and collection began. Normal saline or calcium gluconate, 300 mg/100 ml in saline were infused into a femoral vein at the rate of 100 ml/kg of body wt. per 24 hours. Gastric content was collected during the infusion for 24 hours (6,7). Very uniform infusion was obtained using the "Infusion Withdrawal Pump" (Harvard Apparatus Co., U.S.A.). Volume, pH, free and total HCl, and pepsin (8) were determined in the 24-hour gastric secretion.