10-day embryo.

The observations presented here warrant the conclusion that the pattern of response to irradiation in embryonic rats undergoes a qualitative change when cellular differentiation begins within the embryo. A similar change in the pattern of response appears to have occurred in a series of irradiated mouse embryos studied by Russell(3), if due allowance is made for the relatively faster developmental schedule of the mouse. The data presented by Job, Leibold, and Fitzmaurice(4), on the irradiation of rat embryos over a wide range of ages, lack sufficient detail to support a positive conclusion, although the possibility of a change in the response to irradiation between the 8th and 9th days of gestation is not contradicted by their results.

Summary. X-irradiation of rat embryos on the 8th day of gestation had a more limited effect on subsequent development than did similar treatment on the 9th or 10th days. The only residual effect after doses of 12½ to 100r on the 8th day was retardation of growth. Exposure to comparable doses on the 9th and 10th days has been shown previously to result in the development of malformations and in an increase in mortality, as well as in retardation of growth. This difference in reactivity appears to be dependent upon whether cellular differentiation within the embryos has begun at the time of irradiation.

1. Wilson, J. G., Karr, J. W., *Am. J. Anat.*, 1951, v88, 1.
2. Wilson, J. G., Jordan, H. C., Brent, R. L., *Am. J. Anat.*, 1953, v92, in press.
3. Russell, L. B., *J. Exp. Zool.*, 1950, v114, 545.
4. Job, T. T., Leibold, G. J., Fitzmaurice, H. A., *Am. J. Anat.*, 1935, v56, 97.

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Equal Effectiveness of L and D-Ethionine in Producing Tissue Damage in Rats and Mice. (20027)

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L-ethionine has been found to be as effective in producing fatty changes in the liver of female rats as the racemic dl-ethionine(1). Dl-ethionine produces renal changes in the rat similar to that produced by dl-serine(2). The nephrotoxic action of serine, however, is only caused by the d-isomer and not by the naturally occurring l-serine(3). It seemed therefore of interest to test both the l- and d-isomer of ethionine as to their possible nephrotoxic action as well as to their effect on the pancreas. In addition the influence of both isomers upon the liver and pancreas of male albino mice was tested. Changes induced by ethionine in the albino mouse will be briefly described since this species is apparently well suited for studies of this kind.

Material and method. The L- and D-ethionine were obtained from the California Foundation for Biochemical Research in Los Angeles. The L-ethionine had a specific rotation of $[\alpha]_D^{25} = +23.5°$ as a .85% solution in 0.2 normal HCl. The D-ethionine had a specific rotation of $[\alpha]_D^{25} = -23.6°$ as a .8% solution in .2 normal HCl. Dl-ethionine was obtained from the Nutritional Biochemical Corp., Cleveland, Ohio. Young male albino rats of the Wistar strain were given a protein free diet as previously described(4). After having been on this diet for 8 days, they received 1 mg ethionine per g body weight in 3 divided doses via the intraperitoneal route. The animals were killed 48 hours later. Male albino mice of the Swiss strain were fed a synthetic diet containing 16% casein. This diet was supplemented with either .5% of L- or D-ethionine. The animals were killed after 5 days. Total liver lipids of some of these mice as well as of controls were measured gravimetrically after extraction with chloroform. In order to study the influence of ethionine on the mouse, young albino mice of

TABLE I. Results of Intraperitoneal Inj. of 1 mg/g Body Wt of L- or D-Ethionine on Pancreas and Kidney of Male Rats on Protein Depletion Diet, Sacrificed after 48 Hr.

No. of animals	Administered	Renal necrosis	Pancreas		
			Loss of basophilia	Swelling of zymogen granules	Necrosis
8	l-ethionine	+++	+	++	+
7	d-ethionine	+++	+	++	+

TABLE II. Influence of Supplementation of a Synthetic Diet Containing 16% Casein with .5% L- and D-Ethionine 5 Days on Pancreas and Liver Including Total Liver Lipids in Male Mice.*

No. of animals	Ad-ministered	Renal necrosis	Pancreas			Liver		Total liver lipids,* % (wet wt) ± stand. dev.
			Loss of basophilia	Swelling of zymogen granules	Necrosis	Liver damage	Histologic fat	
6	—	0	0	0	0	0	0	6.85 ± .23
12	l-ethionine	0	+	++	+	+	+++	30.33 ± 2.32
11	d-ethionine	0	+	++	+	+	+++	34.15 ± 1.58

* 4 animals in each group.

the Swiss strain weighing 20 to 30 g were given the casein diet supplemented with .5% dl-ethionine. Animals were sacrificed after 5 and after 28 days. In order to study the maximum effect of chronic ethionine feeding a group of 60 male and 60 female mice were allowed to live for longer periods of time. Of these animals only 8 survived between 50 and 61 days.

Results. As can be seen from Table I there was no difference in the nephrotoxic action of L- and D-ethionine in the rat. The changes observed were essentially similar to those previously described with dl-ethionine(2). There was also no appreciable difference in the effect of both isomers on the pancreas. A comparable degree of pancreatic damage was also seen in mice after they had been given a casein diet supplemented with either L- or D-ethionine for 5 days. The changes in the liver were likewise identical in both groups, and comparable to those found when the racemic dl-ethionine was fed. As can be seen from Table II there was a highly significant increase of total liver lipids in the mice fed either L- or D-ethionine.

The pancreatic changes in the mouse are similar to those previously described in the rat(4-6). There was no significant difference between male and female animals. In the pancreas of mice killed after 5 days there was loss of characteristic basophilia at the base of cells composing the acini and marked swelling of the cytoplasm as well as focal vacuolization and liquefaction necrosis. There was also focal infiltration of inflammatory cells. In the pancreas of some animals after 28 days and in all animals after 50 to 61 days almost complete disappearance of the normal acinar cells containing zymogen granules was found. There were, however, many collapsed acini composed of cells whose nuclei were surrounded by a narrow layer of undifferentiated cytoplasm. There was in addition some fibroblastic proliferation and infiltration by lymphocytes and plasma cells. The islet tissue appeared normal.

The livers of both male and female animals sacrificed after 5 days were markedly enlarged and yellow in color. Microscopically the liver cells were distended by innumerable small droplets which stained intensely with Sudan IV. Focal areas of degenerating liver cells often surrounded by areas of hemorrhage were found irregularly distributed within the liver lobules. After 28 days and even more distinctly in the animals surviving 50 to 61 days the liver architecture was found to be conspicuously distorted on microscopic examination. Single liver cells as well as groups of liver cells were separated from each other by bands of proliferating fibroblasts. In their vicinity a network of reticulum fibers were clearly identified with appropriate staining.

Many of the liver cells showed vacuolization due to infiltration by fat. Some liver cells contained giant nuclei with irregular chromatin particles and from one to several nucleoli.

Fatty infiltration of the proximal convoluted tubules was observed in the kidneys being more pronounced in animals killed after 5 days than in those examined after longer periods. Necrosis of the distal portion of the proximal convoluted tubules was not seen. Since this change has been previously noticed predominantly in rats on a protein deficient diet following the intraperitoneal injection of 1 mg ethionine per g of body weight a similar experiment was carried out with mice. However, renal necrosis was not observed in either male or female animals.

Discussion. The results of these experiments as well as those of Jensen and his coworkers(1) demonstrate clearly that both isomers of ethionine are equally effective in their tissue damaging action. Ethionine was found to have an inhibitory effect on the uptake of methionine into tissue proteins of rats and mice(7). Ethionine is apparently incorporated into tissue proteins with the subsequent formation of abnormal proteins(8). Both optical isomers of methionine can be utilized in various species including the rat and mouse(9). It is now demonstrated that both optical isomers of the methionine antagonist ethionine, are equally effective at least under the conditions tested.

The identical effect of both isomers on the rat kidney is in distinct contrast to that of serine which is limited to the d-isomer. Another difference has been previously noted, namely that the nephrotoxic effect of serine but not that of ethionine can be prevented by the simultaneous administration of several amino-acids for example alanine(10). It should, however, be noted that the nephrotoxic effect of ethionine has so far only been observed in the rat but not in the mouse, dog, cat and monkey(11).

Hepatic and pancreatic lesions can be produced in the mouse by ethionine and thus the mouse may prove to be another suitable species for experiments of this type. It should be pointed out that in the mouse, fatty changes in the liver are considerably more pronounced than in the rat. Severe fatty infiltration is found in both male and female mice after having been on an ethionine supplemented diet for 5 days. Following chronic ethionine feeding, fatty infiltration is almost absent in the liver of rats with intralobular fibrosis(12) while considerable amounts of stainable fat are found in liver cells of mice with ethionine induced intralobular fibrosis. In additional experiments these fatty changes although somewhat less marked were also found in mice fed an ethionine supplemented complete Rockland mouse diet containing 21% protein, instead of the synthetic casein diet. There exists, therefore, in the mouse, a distinct similarity between liver changes produced by ethionine and those induced by diets deficient in lipotropic factors.

Summary. The L- and D-isomers of ethionine are equally effective in producing pancreatic and renal changes in the rat as well as fatty infiltration of the liver and pancreatic damage in the albino mouse. Chronic ethionine administration produces intralobular hepatic fibrosis which is preceded by severe fatty infiltration in both male and female mice.

1. Jensen, D., Chaikoff, I. L., and Tarver, H., *J. Biol. Chem.*, 1951, v192, 395.

2. Wachstein, M., and Meisel, E., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 648.

3. Artom, C., Fishman, W. H., and Morehead, R. P., PROC. SOC. EXP. BIOL. AND MED., 1945, v60, 284.

4. Wachstein, M., and Meisel, E., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 569.

5. Farber, E., and Popper, H., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 838.

6. Goldberg, R. C., and Chaikoff, I. L., *Arch. Path.*, 1951, v52, 230.

7. Simpson, M. V., Farber, E., and Tarver, H., *J. Biol. Chem.*, 1950, v182, 81.

8. Levine, M., and Tarver, H., *J. Biol. Chem.*, 1951, v192, 835.

9. Albanese, A. A., in *Advances in Protein Chemistry*, 1947, v43, 253.

10. Wachstein, M., and Meisel, E., *J. Mt. Sinai Hospital*, 1952, v19, 221.

11. DeAlmeida, A. L., and Grossman, M. I., *Gastroenterology*, 1952, v20, 554.

12. Koch-Weser, D., and Popper, H., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 34.

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