The sex difference observed in this study is much less striking than that found previously in studies on fatty liver and interference with protein metabolism. It is most clearly apparent in the experiments in Series III. Since there also appears to be a sex difference in the inhibition of glucose absorption by methionine, the differences between the sexes with ethionine might be predominantly due to an interference with alimentary tract function rather than with liver function.

Previous studies have shown that the fatty liver induced by ethionine may be prevented or cured by the administration of glucose or sucrose by stomach tube(1,10). From this study, it is clear that ethionine-treated female rats given glucose by stomach tube do not store much glycogen in the liver. The protective effect of glucose therefore is probably not mediated through increased hepatic glycogen formation. Possibly the metabolism of the excess neutral fat(2) in the livers of rats treated with ethionine is facilitated by glucose oxidation.

Summary. The intraperitoneal injection of DL-ethionine has been found to have the following effects on carbohydrate metabolism: 1) decrease in liver glycogen in both male and female rats given glucose by stomach tube 2) decrease in liver glycogen in male and female rats given glucose subcutaneously 3) increase in glucose unabsorbed from the alimentary tract in male and female rats given glucose by stomach tube and 4) decrease in liver glycogen in female rats given glucose by stomach tube or subcutaneously 3 hours prior to the administration of ethionine. Female rats generally showed changes of greater magnitude than males under the same conditions. Methionine prevented in part some of the effects on liver glycogen but had an effect similar to ethionine on glucose absorption from the alimentary tract.

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Nitrogen Mustard as a Teratogenic Agent in the Mouse.* (21207)

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While studying certain effects of nitrogen mustard on mice it was found that this substance is a powerful teratogenic agent when administered during the second week of pregnancy. Many of the anomalies produced are clear-cut and, within limits, predictable. In certain respects some of them are similar to those observed by Ingalls *et al.*(1) in the progeny of mice subjected to severe hypoxia and by Russell(2) in young following x-ray treatment. They also resemble anomalies in rat fetuses found by Gillman *et al.*(3) after treatment with trypan blue and by Wilson(4) after azo blue. In gross manifestations the present anomalies include a wide spectrum of forms ranging from squat, edematous, exophthalmic embryos with practically no legs or tails, to those with defects so minor as to be detected only on the dissection of half grown young. Localized manifestations appear as hydrocephalus, cerebral hernia, abdominal hernia,

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eye and ear defects, hare lip, deficient lower jaw, curly tails and abnormal feet with either an excess or deficiency in digits. Especially interesting are very small, translucent embryos with conspicuous vascular trees and strong heart action. It appears that the exact time of treatment is an important factor in determining the kind of anomaly produced, and it seems probable that an analysis of any of these aberrant responses would throw a good deal of light not only on teratogenesis as such, but on factors involved in normal morphogenesis as well. Only effects registered in the feet are reported at this time.

Procedure. Hydrochloride of the "HN_2" form of nitrogen mustard, methyl bis (β chloroethyl) amine hydrochloride, was used throughout. In ordinary dosages this substance is very toxic to pregnant mice, usually causing rather prompt abortion, and even in amounts too small to produce noticeable clinical effects on the mother it may do permanent damage to the young *in utero*. The dosage that we have found most effective in the production of fetal anomalies is from one to 2 μg of nitrogen mustard to a gram of pregnant mouse. This is administered on the 10th to 12th day of gestation as a single intraperitoneal injection of about .05 cc of a freshly prepared solution made by dissolving 10 mg of the dry crystals in 20 cc of .75% saline. The fetuses are then examined on the 14th or some subsequent day, or are allowed to be born and observed during the postnatal period.

Results. Following this procedure, some or all members of most litters are found to show aberrant development. The potential number of anomalies, however, is probably less than recorded because many embryos die immediately after the injection, and defective fetuses which go to term are less likely to survive the hazards of birth and maternal cannibalism. Nevertheless, there are a few litters that show no indication of loss and no abnormal young. While such litters must be counted in the totals, it is probable that some of them represent instances in which the injection (.05 cc) was not successfully administered or fell below an effective threshold. Individual and racial differences in response to treatment have become evident as the work progressed. In all groups

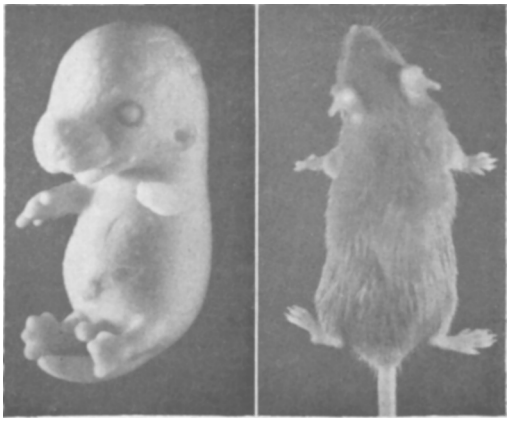

Fig. 1 Fig. 2

FIG. 1. A 14-day embryo obtained 3 days after treatment of the mother (an F_2 animal from a C3H $\times$ J cross) with about 50 μg of HN_2. Left front foot is a "peg" with no distinct toes; left hind foot has three toes (apparently II, III and IV); right hind foot, 4 toes, V being absent; right front foot has 5 toes, but I and II are somewhat fused with the former obscured in the photograph. Individuals in the litter from which the specimen came ranged from very defective to apparently normal.

FIG. 2. A somewhat less aberrant specimen at 9 weeks of age. Treatment was similar, except that only about 25 μg of HN_2 was administered to the mother (S-br strain). In this case left front foot lacks digits I, IV and V, and left hind foot digit V. Both right feet are normal. The only litter mate recovered was a normal female.

the pattern of observed anomalies seems to indicate definite and consistent responses to an altered humoral environment rather than the haphazard effects of localized injuries.

Of 475 available young from 78 treated females, there were 178 abnormal specimens of which 150 (about 31% of the total) showed deficiencies in the feet. It appears that when reduction occurs, it usually involves one or more fifth digit, which may be slightly reduced, greatly reduced, or completely suppressed (Fig. 1). Next most susceptible to reduction or disappearance is digit I, and so on down to a single third digit or partially fused aggregate which give the end of the monodactyl limb a peg-like appearance. Deficiency in the digits is reflected in the morphology of the more proximal skeletal elements.

Of the 150 animals with deficiency in foot development the defects were limited to, or greater on, the left side in 64 cases and on the

right side in 2 cases, indicating that where there is a difference in the response of the 2 sides it is greater on the left perhaps 30 times as often as on the right. Fig. 1 and 2 show typical responses of this sort. A related point of interest is found in the evidence for a certain degree of autonomy for each of the limbs individually. In extreme cases all 4 limbs may be reduced to mere pegs, but if only one foot is reduced it is almost invariably the left front one. Next most often affected is the left hind foot, followed by the right hind foot, with the right front foot being lowest in both the grade and frequency of anomalies.

Discussion. Whether the nitrogen mustard can traverse the placenta and act directly on a growing embryo at a critical moment in its development, or can produce its effect only through some indirect chain of reactions, is not clear. In light of other recent findings, the latter possibility might seem the more probable, for it has become apparent that somewhat comparable results can be produced in a variety of ways. Hogan(5,6), Evans(7), and their coworkers have found that the expression of hydrocephaly and other anomalies in the rat can be regulated in some measure by diet and by an anti-folic acid agent; Gillman *et al.*(3), and Wilson(4) have produced fetal anomalies in rats by use of 2 out of a considerable number of tested dyes which are taken up by the reticuloendothelial system; Russell(2) produced them by x-ray and Ingalls *et al.*(1) by hypoxia. In their structural formulas the 2 effective dyes have points in common, but the similarities among agents thus far found to be effective seems to end at this point unless, as seems not unlikely, they all produce some degree of toxicity or anoxia which, catching the embryo at a critical moment, can permanently alter its course of development. Of special interest is the fact that an amount of nitrogen mustard too small to produce detectable results in the mother can profoundly influence the young and that, if needed for morphological studies, stages in the development of certain specific anomalies could be produced almost at will.

Summary. A single intra-abdominal injection of nitrogen mustard in an amount equivalent to about 1 μg per g of body weight and administered on the 10th to 12th day of gestation has no apparent effect on a pregnant mouse, but may have a pronounced effect on the young *in utero*. Some of the latter are killed outright and others show a wide range of resultant anomalies. Only anomalies involving the feet are reported here. The grades and distribution of deficiencies in the feet reveal a significant difference in responses on the 2 sides of the body and a considerable developmental autonomy on the part of each individual foot.

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