Only preliminary data may be presented for the hybrids obtained from the other crosses, since many of them are still under observation, with the youngest animals being approximately 20 months of age.

Confirming the observations of Andervont and Dunn(1-4,6,7), in hybrids obtained by mating C females with the C_3H/An males more mammary tumors are being found among mice born in early litters than was seen in any other cross. Of those which have died, 40% have had mammary cancer at an average age of 580 days. When Za males were tested, the hybrids cast by C females after they became infected have shown an incidence of 86% at an average age of 275 days, with 6 of the 49 mice still living. No evidence that the D_8 males infected any of the C females was obtained. Of their 126 CD_8F_1 progeny which have died, 4% have had tumors at an average of 538 days. This compares with an incidence of 3% at 572 days in 102 $CZbF_1$ hybrids, all of which are now dead.

Summary. Females of the agent-free, but susceptible, C (Bagg albino) stock remained free of mammary cancer after they were bred with males of the C strain and with males of another stock lacking the mammary tumor agent (MTA). However, when they were mated with males of high cancerous strains, differences were noted in the ability of the males of these strains to transfer the agent and infect the C females. After C females were mated with males of the cancerous $Z(C_3H)$ strain, over 50% of the females developed mammary cancer, and the same incidence was observed in females with amputated uterine horns as in intact litter-mate controls. The source of the agent may be of some significance in male transmission experiments. In some crosses, only preliminary data could be reported in the hybrids.

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Effects of Ethionine upon Hepatic Glycogen Formation from Glucose in Intact Rats.* (21206)

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Ethionine (S-ethyl homocysteine), an analogue of methionine, has been found to induce a fatty liver in fasting female rats(1,2). Male rats and females pretreated with testosterone fail to develop this fatty change in the liver (3). Ethionine also inhibits, in female rats, the incorporation of labeled DL-methionine and glycine into liver protein *in vivo*(4) as well as the incorporation of L-cystine, DL-leucine, L-valine, and L-lysine into liver protein and plasma protein(5). This inhibition

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occurs at least several hours before there is a detectable increase in liver lipids. Male rats fail to show this inhibitory effect on amino acid incorporation(5,6) but do share with females other inhibitory effects of ethionine on liver metabolism, such as inhibition of transmethylation from methionine to choline and inhibition of the conversion of the methionine methyl group to formate(6,7). Thus, a correlation has been found between the inhibition of amino acid incorporation into liver protein and the subsequent development of fatty liver in rats given ethionine.

In an effort to investigate further the pathogenesis of the fatty liver and to establish whether the correspondence between disturbances in protein and lipid metabolism is significant, other metabolic activities in the liver are being studied in animals which are either susceptible or resistant to the fatty change in the liver. This report is concerned with the influence of ethionine upon the conversion of glucose into hepatic glycogen.

Experimental. White Wistar rats (Carworth), weighing 150 to 250 g were fasted for 24 hours. Three series of experiments were performed: Series I—groups of male and female rats received by stomach tube 2.5 g of glucose, dissolved in 3 ml of water, at the end of the initial 24-hour fast period. These animals also received ethionine (experimental), an equal volume of saline (control), methionine (control), or a mixture of ethionine and methionine (experimental) by intraperitoneal injection. The ethionine group was given 0.75 mg DL-ethionine per g of body weight in 2 divided doses: one-half initially at the end of the 24-hour fast period and one-half 2 hours later. Methionine (DL) when used was administered in amounts equimolar to ethionine. Methionine and ethionine were dissolved in the same solution when given together. The rats were fasted until sacrificed 6 hours after the administration of glucose. Series II—groups of male and female rats received subcutaneously in 4 divided doses 1.3 g of glucose dissolved in 13 ml of water: 4 ml at the end of the initial 24-hour fast period, and 3 ml every hour thereafter for 3 hours. These animals also were injected with ethionine, methionine, ethionine plus methionine or saline in the same manner as in Series I. The rats were fasted until sacrificed 6 hours after the first injection of glucose. Series III—groups of male and female rats received either glucose by stomach tube or subcutaneously as did the animals in Series I or II. However, they received no ethionine or saline until 3 hours after the initial dose of glucose when 3 animals were sacrificed and the remainder received either ethionine or saline in 2 divided doses as in Series I. These remaining rats were sacrificed 7 hours after the first dose of glucose. Three rats were used for each experimental and control group in Series I and II, and 9 animals were used in each experiment in Series III. The ethionine was dissolved in water to a concentration of 25 mg per ml. The glucose solution for subcutaneous injection contained 75 TR units of hyaluronidase per 100 ml to facilitate absorption. The animals were sacrificed by a blow on the head and the liver rapidly removed and weighed. Duplicate samples of the liver weighing approximately 1 g each were placed in tared 40 ml centrifuge tubes containing 3 ml of 30% KOH and the tubes were reweighed. Liver glycogen was isolated and hydrolyzed by the method of Good, Kramer, and Somogyi(8). Glucose was determined by Nelson's method(9). In some animals, residual glucose in the alimentary tract was determined at the time of sacrifice. The upper end of the esophagus and lower end of the rectum were tied with string and the alimentary tract between the ligatures was removed and placed in boiling distilled water. The tract was then opened and repeatedly washed with hot water. After cooling, the washings were diluted to an appropriate volume (500 or 1000 ml). To 10 ml of this suspension were added 5 ml of 0.3 N $Ba(OH)_2$, 5 ml of 5% $ZnSO_4$, dilute Na_2SO_4 solution to remove excess Ba^{++} and water to 50 ml. After filtration, 1 ml aliquots were removed for glucose determination by Nelson's method(9).

Results. In Series I, female rats injected with ethionine had virtually no liver glycogen 6 hours after the administration of 2.5 g of glucose by stomach tube (Table I). Male rats showed more glycogen than females but still much less than the control animals. It

TABLE I. Influence of Ethionine upon Hepatic Glycogen and Residual Alimentary Glucose following Glucose by Stomach Tube (Series I).

Treatment	Sex	Liver glycogen 1: No. of animals	Liver glycogen 1: Per 100 g wet liver, g	Liver glycogen 2: Per liver/100 g body wt, mg	% diff. from control*	Glucose in alimentary tract: No. of animals	Glucose in alimentary tract: Total, mg	Glucose in alimentary tract: % unabsorbed
S†	♀	8	2.74 ± .16‡	82.6 ± 4.9‡		5	108 ± 28‡	4.3
E	♀	6	<.05	1.3 ± .1	−98	3	669 ± 64	26.8
M	♀	5	2.71 ± .25	81.1 ± 5.3		3	1082 ± 29	43
E-M	♀	9	1.90 ± .5	60.9 ± 6.8	−25	6	1163 ±153	47
S	♂	8	1.84 ± .21	52.5 ± 5.1		6	54 ± 17	2.2
E	♂	9	.75 ± .17	20.8 ± 5.2	−60	6	358 ± 34	14.3
M	♂	6	2.67 ± .19	80.8 ± 6.0		3	475 ±264	19
E-M	♂	6	1.80 ± .19	56.7 ±11.5	−30	3	1475 ±118	58

* Figured on column 2. Saline group is the control for the ethionine group and the methionine is control for the ethionine-methionine.
† S = Saline; E = Ethionine; M = Methionine.
‡ Stand. error of mean.

was noted that, at the time of sacrifice, the stomachs of the ethionine-treated females were filled with fluid while those of the controls were empty. The experimental males likewise showed fluid in their stomachs but less than the females. It was therefore thought possible that ethionine might be preventing glucose from reaching the liver by interfering with the passage or absorption of glucose in the alimentary tract. To test this, the glucose remaining in the alimentary tract at the end of the 6-hour experimental period was determined in many subsequent experiments. It was found that approximately 25% of the administered glucose remained unabsorbed in females given ethionine while only about 4% remained in the control animals (Table I). Male experimental rats showed about 14% unabsorbed glucose as compared to about 2% in the controls. The simultaneous occurrence of inhibition of liver glycogen and decrease in the glucose absorption suggested that the interference with glucose absorption might be an important factor in the decreased hepatic glycogen formation in animals injected with ethionine.

TABLE II. Influence of Ethionine upon Hepatic Glycogen following Subcutaneous Administration of Glucose (Series II).

Treatment	Sex	Liver glycogen 1: No. of animals	Liver glycogen 1: Per 100 g wet liver, g	Liver glycogen 2: Per liver/100 g body wt, mg	% diff.* from control
S†	♀	9	2.65 ± .35‡	76.1 ±10.4‡	
E	♀	6	.74 ± .20	21.3 ± 6.2	−72
M	♀	6	1.81 ± .22	54.4 ± 5.5	
M-E	♀	3	1.34 ± .4	39.5 ± 5.1	−27
S	♂	5	2.34 ± .41	68.0 ±15.3	
E	♂	6	1.03 ± .23	27.0 ± 5.8	−60

* See footnote (*), Table I.
† S = Saline; E = Ethionine; M = Methionine.
‡ Stand. error of mean.

These results demonstrated that ethionine has a more direct effect on liver glycogen content in addition to its effect on the alimentary tract. However, the nature of this effect on the liver was in doubt. Conceivably, ethionine could inhibit synthesis of glycogen or could initiate a breakdown of preformed glycogen. A third series of experiments was therefore performed in order to help decide between these possibilities. Animals were given glucose either by stomach tube or subcutaneously and were allowed to synthesize liver glycogen in the absence of ethionine or administered saline. At the end of a 3-hour period one group was sacrificed and the remaining animals received ethionine or saline. The animals receiving glucose by stomach tube had at 3 hours approximately one-half the glycogen content of the controls sacrificed at 7 hours

The experiments in Series II were conducted to test this possibility. To overcome the possible effect of diminished glucose absorption on liver glycogen formation, the glucose was administered subcutaneously instead of by stomach tube. In these experiments, ethionine-treated females still showed much less liver glycogen than control females (Table II). The difference between the sexes was less than in the first series.

TABLE III. Effect of Ethionine on Preformed Hepatic Glycogen (Series III).

Treatment	Sex	Liver glycogen, mg/liver/100 g body wt 3 hr*	7 hr*
S—st†	♀	36.8 ± 2.9‡(5)§	72.4 ± 7.1‡(6)§
E—st	♀		31.4 ± 9.6 (6)
S—st	♂	37.3 ± 2.8 (3)	75.4 ± .17(3)
E—st	♂		71.7 ± 5.1 (3)
S—sc	♀	58.3 ± 4.7 (6)	59.9 ± 7.8 (6)
E—sc	♀		30.6 ± 8.1 (6)
S—sc	♂	51.9 ± 8.5 (3)	48.7 ±11.2 (3)
E—sc	♂		38.2 ± 8.4 (3)

* Fasted animals given glucose as indicated and liver glycogen determined on representative animals at end of 3 hr. At this time the remaining animals were treated with saline or ethionine and sacrificed 4 hr later or 7 hr after glucose admin.

† S = Saline; E = Ethionine; st = glucose by stomach tube; sc = glucose subcutaneously.

‡ Stand. error of mean.

§ No. of animals.

(Table III). At 7 hours, ethionine-treated females showed no mean rise over the value at 3 hours. However, the liver glycogen values were extremely variable in the experimental rats, some animals showing virtually no glycogen while others had amounts equal to the control animals. The control animals, on the other hand, had values close to each other. This suggested a breakdown of preformed liver glycogen in some animals treated with ethionine. This was confirmed in other experiments in which the glucose was administered subcutaneously. In this group, the liver glycogen during the early period increased more rapidly than in those given glucose by stomach tube but the level at the end of 7 hours was lower (Table III). The ethionine-treated females had liver glycogen values much below the control females. The males showed almost no effect of ethionine on liver glycogen. When glucose was administered to males by stomach tube, ethionine had no detectable influence on liver glycogen levels. With subcutaneous glucose, the male rats demonstrated a questionable decrease in liver glycogen.

Since ethionine is believed to owe some of its biological effects to an interference with methionine metabolism, the ability of methionine to reverse the effects on liver glycogen was tested. Methionine partially prevented the deleterious influence of ethionine on liver glycogen, both when glucose was administered by stomach tube (Table I) and subcutaneously (Table II). However, the reversal was never complete with the dosage of methionine used (equimolar). Like ethionine, methionine when administered alone increased significantly the per cent glucose unabsorbed from the alimentary tract. Yet, despite the interference with glucose absorption, the liver glycogen values were not consistently less than those of the controls. Again, methionine plus ethionine caused the greatest interference with glucose absorption (ca 50%) but the liver glycogen values were higher than found with ethionine alone.

Discussion. It is evident from this study that ethionine has effects both upon glucose absorption from the alimentary tract and upon the glycogen content of the liver.

The interference with the function of the gastrointestinal tract is apparently not a unique property of ethionine, since methionine has the same effect and since the greatest inhibition was found when both amino acids were administered together. It is not clear from this study whether the action is primarily on motility of the gastrointestinal tract or on absorption or whether this effect is characteristic of this type of S-containing amino acid or is shown by other amino acids as well.

Ethionine may inhibit synthesis of liver glycogen from glucose, accelerate its breakdown or have both effects. The results of experiments in Series III suggest an effect primarily upon breakdown, since some female animals showed virtually no liver glycogen. However, since the glycogen content at any time is the resultant of the relative rates of the 2 opposing reactions, synthesis and breakdown, any inhibition of synthesis coupled with a normal rate of breakdown might account for the observed decreases in hepatic glycogen.

It appears probable that the observed decrease in liver glycogen with ethionine is at least in part the result of interference with some metabolic function of methionine, since methionine administration gave partial protection against the ethionine effect on liver glycogen. There is, however, no known metabolic function of methionine which can explain this action of methionine.

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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