The considerable protective action of methionine described in this communication confirms and enlarges observations previously made by Véghelyi and his co-workers(9). The weight loss of animals on a methionine supplemented diet was of almost the same magnitude as that on the basic protein free diet with or without supplementation with 2% casein.

The changes on the basic protein free diet differ markedly from those induced by ethionine. The atrophic lesions induced by protein deficiency developed much slower. There is complete absence of any necrosis of acinar cells. Moreover the acinar cells do not show loss of perinuclear basophilia and de-differentiation which make them resemble fibroblasts (3). There is also a considerable difference in the effect on the liver since in protein deficiency necrosis of liver cells with subsequent diffuse fibrosis which follows the ingestion of methionine(13,14), is not observed. These considerable differences suggest that the effect of ethionine is probably not due to a conditioned methionine deficiency only, but is rather of a considerably more complex nature.

Summary. The development of typical atrophic lesions of the pancreas in the rat on a completely protein free diet is described. Supplementation of such a diet with 2% casein is without influence on the pancreas, while either 4% casein or 1% methionine have a marked beneficial effect. On a synthetic diet containing 7.5% casein the microscopic appearance of the pancreas is almost normal.

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Conversion of C^{14} Carotene to a Non-Saponifiable Substance or Substances in the Rat.* (20866)

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The conversion of carotene to vitamin A is a well established process; however, it remains to be demonstrated if this compound is a precursor for other body constituents. The object of this investigation was to determine if a part of the absorbed carotene was converted to some substance or substances in a carotene-free, non-saponifiable fraction in different tissues of the rat.

Method. A sample of uniformly labelled C^{14} carotene (90% beta)† having a specific activity of 0.4 microcurie per milligram, a total activity of 1.5 microcuries, and 4900 counts per minute was suspended in 3 ml of

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† The carotene was purchased from Nuclear Instrument and Chemical Corp., Chicago, Ill.

water using Tween 80 as the emulsifier and fed by a stomach tube to a male, white rat weighing about 200 g. The rat was sacrificed by decapitation 24 hours later. A second rat weighing approximately the same was given a similarly prepared dose of carotene having 2100 counts per minute. This animal was sacrificed at the end of 6 hours. The non-saponifiable substances were extracted from feces, liver, small intestine, and extrahepato-intestinal tissue according to the method of Hjarde(1). Liver, small intestine, and extra-hepato-intestinal tissue extracts were freed from carotene by the chromatographic procedures of Glover *et al.*(2). No carotene was demonstrated in these extracts when they were analyzed in a Beckman Spectrophotometer at 450 mμ. Throughout this paper, the carotene-free, non-saponifiable fraction will be referred to as the CFNS fraction. The carotene content of the non-saponifiable fraction from feces was measured spectrophotometrically without separating it from the extract. The radioactivity of the dry extracts was measured under an end window Geiger-Muller tube. The vit. A of the CFNS fractions was determined colorimetrically using $SbCl_3$ according to the technic of Glover *et al.*(2). The extinction of the petroleum ether solutions was measured at 450 mμ and accepting the $E^{1\%}_{1\,cm}$ for beta-carotene as 2500, the amount of carotene present was calculated.

Results. Table I summarizes the results of this investigation. The 24-hour animal absorbed approximately 30% of the total C^{14} fed. The remaining 70% of the administered dose remained in the feces. The CFNS extracts of liver, small intestine, and extra-hepato-intestinal tissue were found to contain respectively 4.4, 6.1, and 20.0% of the C^{14} fed. The 6-hour animal absorbed approximately 54% of the isotope fed. In the liver, intestine, and extrahepato-intestinal tissues there was found 6.7, 14.1, and 33.3%, respectively, of the dose administered. If the amounts of C^{14} found in the extracts of the liver, intestine, and extrahepato-intestinal tissues were calculated in terms of the per cent of dose absorbed, then the tissues of the twenty-four-hour animal contained 14.4, 20.1, and 65.5% and those of the 6-hr animal had 12.4, 26.0, and 61.5% respectively.

TABLE I. Isotope Concentration in Carotene-Free Non-Saponifiable Fractions of Rats Fed C^{14} Labelled Carotene.

The 24-hr animal was fed 3.75 mg carotene containing 4900 c.p.m. of uniformly labelled C^{14}. The 6-hr animal was fed 1.56 mg carotene containing 2100 c.p.m. of uniformly labelled C^{14}.

Fraction isolated	C^{14} found, c.p.m.	% of total dose fed*
24-hr animal		
Carotene-free non-saponifiable		
Liver	216	4.4
Small intestine	301	6.1
Extrahepatointestinal tissue	980	20.0
Total non-saponifiable		
Feces	3500	71.0
6-hr animal		
Carotene-free non-saponifiable		
Liver	141	6.7
Small intestine	297	14.1
Extrahepatointestinal tissue	700	33.0
Total non-saponifiable		
Feces	900	42.7

* $\dfrac{\text{C.p.m. in fraction isolated}}{\text{C.p.m. in carotene fed}} \times 100.$

The content of vit. A found in liver, intestine, and extrahepato-intestinal tissue after feeding the carotene was essentially similar for both the 24- and 6-hr rats, namely, approximately 1000 μg, 30 μg, and 90 μg, respectively. The carotene content of the non-saponifiable fraction from the feces of the 24-hr rat was 2.64 μg and 0.69 μg for the 6-hr animal.

Discussion. Since the combined CFNS tissue extracts from each animal accounted for approximately 100% of the absorbed C^{14}, one may infer that carotene is converted to some substance or substances found in the non-saponifiable fraction and is not changed into any water soluble or saponifiable material.

With the procedure used in this experiment it was not possible to demonstrate that vit. A was the only active substance found in this fraction. It is interesting to note that as soon as 6 hours after feeding the C^{14} labelled carotene, the highest percentage of C^{14} was found in tissues outside the liver and small intestine, thus indicating that the conversion product or products of carotene are rapidly

distributed throughout the body. Evidence has been presented to indicate that the intestine is a site where carotene is converted to vit. A(2). The finding that the uptake of C^{14} in the CFNS fraction was higher at the end of 6 hours than after 24 hours may be considered as presumptive evidence that the small intestine is one of the sites for this conversion process.

Summary. The feeding of C^{14} labelled carotene resulted in the deposition of all of the absorbed C^{14} in the carotene-free, non-saponifiable fraction from the total animal. The highest concentration of C^{14} was found in the extrahepato-intestinal tissues, and the least amount was recovered in the liver.

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Congenital Malformations Produced by Injecting Azo Blue into Pregnant Rats.* (20867)

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Gillman and his coworkers(1) have shown that the injection of trypan blue into female rats before and during pregnancy causes maldevelopment in the offspring. A single dose of 1 cc of 1% aqueous solution during the first 9 days of pregnancy caused anomalies in 8.6% of the young, but when treatment was given before as well as during pregnancy, 32.4% of the young were congenitally defective.

As an extension of this significant observation the present author has undertaken to study the teratogenic activity of other azo compounds more or less closely related to trypan blue. The original experimental procedure of Gillman and collaborators has been simplified and standardized to facilitate a comparison of the results of trypan blue with those of other compounds tested. One cc of 1% aqueous solution was injected subcutaneously into pregnant rats on the 7th, 8th and 9th days of gestation. (Pregnancy was considered to be of 1 day's duration 24 hours after sperm were found in the vaginal smear.) On the first day of treatment (7th day of gestation) the females were laparotomized to verify pregnancy and to determine the number of implantation sites. Control females were similarly laparotomized and injected with distilled water during pregnancy. No further treatment was given after the last injection on the 9th day. The animals were killed on the 20th day, one day prior to expected delivery, in order that living fetuses as well as resorption sites might be observed *in situ*. The living offspring were removed, weighed and examined externally for malformations. They were then preserved in Bouin's fluid for later dissection or histologic sectioning. Dissection consisted of making serial transverse slices with a razor blade at 1-2 mm intervals through the head and trunk. The slices were examined and further dismembered, if necessary, under a dissecting microscope. This made possible the recognition of a wide range of visceral and other internal malformations without the tedium of preparing and studying serial histologic sections.

Results. Of the compounds tested to date, azo blue has proven the most effective in producing developmental abnormalities. Its effects on both pregnancy and on the offspring are summarized and compared in Table I with the effects of trypan blue tested under similar conditions. The data suggest that azo blue is less likely than trypan blue to cause early termination of pregnancy as a result of ma-

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