son of histometric and radiometric methods for TSH detection in the stasis tadpole will be considered elsewhere.

Summary and conclusions. 1) The uptake of I^{131} and P^{32} by the developing thyroid of Rana tadpoles has been determined in normal and accelerated metamorphosis. Radioactive measurements of the isolated gland (estimated weight, 50-200 μg) disclose a progressive increase in radioiodine accumulation throughout normal development. The effect is not paralleled with radiophosphorus although significantly increased P^{32} thyroidal uptakes eventually occur in advanced development. Exogenous thyrotrophin augments I^{131} and P^{32} accumulation in the thyroids of young tadpoles. The applicability of the thyroidal response to the bioassay of thyrotrophin is discussed. 2) The enhancement of iodine and phosphorus uptake by the larval thyroid is considered to reflect increased tempo of thyroid function in amphibian development. The fundamental rôle of pituitary-thyroid interaction in anuran metamorphosis is reemphasized.

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Lack of Lipotropic Action by Thiomethyl Derivatives of Glucose.* (22584)

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The fatty infiltration of the livers of rats on low protein diets is generally ascribed to a deficient synthesis of choline as a result of the inadequate supply of biologically transferable ("labile") methyl groups. In methionine, which is the major dietary source of such groups, the methyl is attached to a sulfur atom; and the same type of attachment is present in methionine sulfoxide and in various thetins (dimethylpropio-, dimethyl-, methylethyl thetin, and methionine ethylsulfonium), which have been shown to be efficient methyl donors in the rat(1). It seemed possible that other compounds possessing terminal thiomethyl groups may also be active in this respect. In the present investigation, the lipotropic action of 2 such compounds, methyl-thioglucoside (MTG) ($CH_3 \cdot S \cdot CH_2 \cdot (CHOH)_4 \cdot CH_2OH$) and glucose dimethylmercaptal (GDM) ($(CH_3 \cdot S)_2 \cdot CH \cdot (CHOH)_4 \cdot CH_2OH$), was tested.†

* Technical assistance was given by C. Downs and M. Holton. This investigation was done under Contract No. AT-(40-1)-1638 with the U. S. Atomic Energy Commission. Stearate-1-C^{14} was obtained from a commercial source on allocation of the U. S. Atomic Energy Commission.

† The thiomethyl derivatives of glucose were synthesized by Herstein Laboratories, New York City. The author is indebted to the Sugar Research Foundation for the generous gift of these compounds and for their interest in the project.

TABLE I. Average Phospholipid and "Fat" Contents of Liver of Rats on a Low-Protein Diet with or without Supplements.*

Dietary supplement†	Gain in body wt, g	Liver wt for 100 g rat, g	Per g of liver: Phospholipids, mg	Per g of liver: "Fat," mg	Per liver of 100 g rat: Phospholipids, mg	Per liver of 100 g rat: "Fat," mg
None (23)	5.99 ± 1.3	5.86 ± .4	24.9 ± 2.0	112.0 ± 15.3	150.3 ± 11.9	675.7 ± 107
Choline (21)	6.77 ± 1.4	4.82§ ± .3	26.0 ± 2.1	41.7‡ ± 5.2	121.0§ ± 8.0	202.1‡ ± 23
MTG (15)	7.62 ± 2.86	5.73 ± .6	27.6 ± 4.0	132.7 ± 23.5	154.4 ± 22.2	775.8 ± 148
GDM (23)	9.13 ± 1.34	5.58 ± .4	25.0 ± 1.4	108.4 ± 13.2	135.4 ± 6.5	609.1 ± 84

* Values preceded by ± are stand. errors of means.

† No. of rats in parentheses.

‡ Significantly different ($P < 0.01$) from corresponding value of group on the unsupplemented diet.

§ Significantly different ($P < 0.05$) from corresponding value of group on the unsupplemented diet.

Moreover, since choline-deficient livers show a decreased ability to oxidize isotopic fatty acid *in vitro*(2), the possibility that such an effect could be prevented by supplementation of the diet with MTG or GDM was also investigated.

Methods. Male weanling rats (Rockland Farms, initial weight 35-45 g) were kept on an experimental diet (Diet 46, containing casein 8%, sucrose 28%, dextrin 28%, Crisco 28%, Ruffex 2%, cod liver oil 2%, salt mixture (USP XII) 4%). A B vitamin mixture (2) was incorporated in the diet. The rats were housed not more than 6 per cage and were given food and water *ad libitum*. In each experiment one group was maintained on the unsupplemented diet. Other groups received 3 mM of choline chloride, MTG, or GDM, per 100 g of diet, except in one experiment in which the methyl glucose compounds were fed at levels calculated to contain the same number of methyl groups as choline (9 mM of MTG, or 4.5 mM of GDM). After 21 days on the diets, the rats were killed by decapitation. The liver of each animal was weighed and its content of total lipids (weight of the chloroform extract(3)) and lipid phosphorus(4) was determined. The differences between total lipids and total phospholipids (lipid P x 25) are indicated as "fat". Such values, in addition to triglycerides, include cholesterol and other unsaponifiable substances. In the experiments on fatty acid oxidation, homogenates prepared from livers of rats on the supplemented, or unsupplemented, diets were incubated simultaneously in the presence of stearate-1-C^{14}. After 3 hours in air at 37°C, the $C^{14}O_2$ produced was determined. For further details on the procedures, see Artom(2).

Results. For the sake of brevity, only the averages and standard errors of the results obtained on the individual rats are recorded in Table I. Phospholipid and "fat" values have been calculated both per g of tissue and for the whole liver of a 100 g rat: the latter method of expressing the results allows a direct comparison of the values obtained in the various groups, in spite of considerable variations in the size and fat content of the liver. The differences between the averages of the groups on the supplemented diets and those of the control rats on the unsupplemented diet were considered significant, when the ratios of these differences to their standard errors were greater than 2 ($P<0.05$), or 3 ($P<0.01$).

The results, expressed by either method, indicate that choline supplementation of the diet lowered significantly the fat content of the liver. Total phospholipids were also lower, but only when the results were referred to the whole liver of a 100 g rat. On the other hand, it is apparent that the fatty infiltration in the livers of rats receiving MTG or GDM is of the same degree as that seen in the livers of the controls on the unsupplemented diet.

In Table II the results of one of our experiments on the *in vitro* oxidation of stearate-1-C^{14} are recorded. Data on the $C^{14}O_2$ produced during the incubation (expressed as counts per minute per mg N of tissue, as well as per liver of a 100 g rat) indicate that the livers of the rats on the choline-supplemented

TABLE II. $C^{14}O_2$ Production from Stearate-1-C^{14} by Liver Homogenates.*

Rat	Dietary supplement	$C^{14}O_2$ (counts/min.) Per mg N	Per liver of 100 g rat
a	None	121	14,750
b	"	212	25,800
c	Choline	835	70,000
d	"	1000	116,000
e	GDM	256	32,500
f	"	163	17,000

* Each value is avg of data obtained on duplicate flasks containing the same homogenate. Each flask contained, in a total volume of 6 ml of Ringer-phosphate without Ca and Mg (pH 7.4), 0.375 g of homogenized liver, 12 μmoles of adenosine triphosphate, 3 mg each of penicillin and dihydrostreptomycin and 1 μmole (= 1 μC) of stearate-1-C^{14}.

diet oxidized isotopic stearate at a much higher rate than the livers of the control rats. On the other hand, $C^{14}O_2$ produced by the livers of the animals receiving MTG or GDM was about the same as that produced by livers of rats on the unsupplemented diet.

It is now established that the transfer of the methyl group of methionine must be preceded by the conversion of the free amino acid into an active form, S-adenosylmethionine(5). It seems probable that transmethylation from the thiomethyl groups present in other compounds should require a similar activation of the sulfur to the "onium" level. The lack of specific enzymes for the conversion of the glucose derivatives which we have tested, into active forms might perhaps explain their inability to act as methyl donors in the organism.

Summary. Supplementation of a high fat, low-protein diet with two thiomethyl derivatives of glucose (glucose dimethylmercaptal and methyl-thioglucoside) did not prevent the fatty infiltration of the liver, and did not enhance the ability of the tissue to oxidize fatty acid added *in vitro*.

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Free Electrophoresis of Lipoproteins. (22585)

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There are comparatively few reports on serum lipoprotein investigations by moving boundary electrophoresis(1,2), because of the difficulty inherent in the interpretation of the patterns due to the low density of the lipoproteins(3) and their possible transformations during dialysis. Paper electrophoresis (4,5) has yielded some information on these substances but this method too, has its shortcomings because of the adsorption of lipid or lipoproteins and their stains on the paper(6). In addition, differences have been found between moving boundary and paper electrophoresis for α_2-, β-, and γ-globulins(7).

In the present investigation, advantage is taken of the ultracentrifugal technic for isolation of the low density lipoproteins(8). The characteristics of the latter as revealed by moving boundary electrophoresis were studied and it is believed that this procedure can be used profitably in comparative lipoprotein investigations.

Methods. Blood (80 ml) was collected from each of 5 healthy male medical students,