Effects of prolonged ingestion of glucose or ethanol on tissue lipid composition and lipid biosynthesis in rat

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ABSTRACT The effects on lipid metabolism of long-term feeding of large amounts of ethanol or glucose differed from those that have been reported in short-term experiments. Three groups of male rats were investigated. The first was fed lab chow and 15% (v/v) ethanol ad lib.; the second was pair-fed with the first and given isocaloric amounts of glucose in lieu of ethanol; the third was fed lab chow and water ad lib. All three groups consumed nearly the same number of calories, and about 30% of the calories in the first group were derived from ethanol.

Neither glucose nor ethanol added to a nutritionally adequate diet promoted the development of a fatty liver, although both stimulated acetate-^{14}C utilization for hepatic lipid synthesis. In all three groups more than 80% of the label in hepatic lipid was found in fatty acids, and the distribution of label amongst the fatty acids of different chain lengths was virtually the same.

Ethanol decreased while glucose increased the quantity of lipid in fat depots, and each altered the fatty acid composition of the lipids in adipose tissue, kidney, liver, and hepatic subcellular fractions in a different manner. The most striking of these changes was the relative increase in monounsaturated fatty acids and the decrease in essential fatty acids produced by glucose.

KEY WORDS ethanol · glucose · prolonged feeding · rat · liver · adipose tissue · fatty acids · composition · incorporation · acetate-^{14}C

SINGLE OR MULTIPLE large doses of ethanol, in contrast to carbohydrate, consistently produce a fatty liver in experimental animals (3) and in man (4). This apears to be due primarily to enhanced hepatic triglyceride synthesis from fatty acids derived from adipose tissue (5, 6), although other factors, including decreased fatty acid oxidation (7), enhanced hepatic fatty acid synthesis (8), and diminished triglyceride release from the liver (9), may play a contributory role.

Since little information is available on the effects of chronic ethanol ingestion on lipid metabolism, we have investigated the composition of hepatic and other tissue lipids and the utilization of acetate for lipid synthesis in rats fed 15% (v/v) ethanol in lieu of drinking water for prolonged periods. The results have been compared with those obtained in pair-fed animals given an isocaloric amount of glucose instead of ethanol and in animals fed the same stock diet and water ad lib. Our findings, to be described in this report, indicate that the short-term and long-term effects of ethanol and glucose feeding on lipid metabolism differ, and that there are marked differences as well as certain similarities between the effects of ethanol and of glucose on tissue lipid composition and utilization of acetate in vivo.

MATERIALS

Sodium acetate-1-^{14}C (50 mc/mmole) was obtained from the New England Nuclear Corp., Boston, Mass. Ethanol (95%) was purchased from the U.S. Industrial Chemicals Co., New York, and diluted with water to obtain a 15% (v/v) solution which contained 0.119 g of ethanol per ml. Materials for GLC were obtained from Analabs Inc., Hamden, Conn., and for TLC from Brinkmann Instruments Inc., Great Neck, New York.

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

Preliminary abstracts of this work have been published (1, 2).

Standards from the Hormel Foundation, Austin, Minn., were used to identify lipids. All other chemicals were reagent grade materials from commercial suppliers.

METHODS

Animal Feeding

Male weanling rats of the Sprague-Dawley strain (Charles River Rat Farm, Brookline, Mass.) were housed in individual cages and fed ground Purina Lab Chow[1] and water ad lib. for 1 week, at which time their average weight was 60 g. They were then randomly separated into three groups: (*a*) a control group, maintained on ground Purina Lab Chow and water ad lib.; (*b*) an ethanol group, fed ground Purina Lab Chow and 15% (v/v) ethanol in water ad lib. in lieu of drinking water [the ethanol was administered in graduated Richter tubes (10) to prevent evaporation]; and (*c*) a glucose group, which was group pair-fed with the ethanol group but given isocaloric amounts of glucose instead of ethanol (crystalline glucose was added to the diet, and free access to water was permitted). One milliliter of 15% ethanol is equivalent to 0.22 g of glucose if the caloric value of glucose is taken to be 3.74 kcal/g and of ethanol 7.11 kcal/g (11). Food, which was offered in tared glass cups, and ethanol consumption were measured daily. Usually the glucose group ate all food offered but if they did not, the remainder was mixed with the next day's diet. All animals were kept in a constant temperature room at 25°C with a relative humidity of 50–55%.

Tissue Fractionation and Lipid and Protein Analyses

One rat from each group was killed by decapitation *without prior fasting* on the days indicated in the text. The livers were removed, weighed, minced with scissors, and homogenized with 9 volumes of 0.25 M sucrose, using a Potter-Elvehjem glass homogenizer with a Teflon pestle. Lipids were extracted from aliquots of the sucrose homogenates by the method of Folch, Lees, and Sloane Stanley (12).The phase containing lipid was evaporated to dryness under a stream of nitrogen at 40°C or in a rotary evaporator, and the lipid residue was redissolved in chloroform. Aliquots of this solution were used for further analyses. Total carbon was determined (13) after first removing all the chloroform under a stream of nitrogen. Other aliquots were used for the determination of triglyceride (14) and of fatty acid ester content (15), and for the preparation of the methyl esters of the total fatty acids (16) to be used for the subsequent analyses by GLC. Glycerol was used as the standard in the triglyceride assay.

The remainder of the sucrose homogenate was centrifuged in a Servall refrigerated angle centrifuge with an SS-34 head for 10 min at 700 × *g*, after which the supernatant fraction was centrifuged at 10,000 × *g* for 20 min in order to sediment the mitochondria. The supernatant fluid was collected by decantation and centrifuged at 41,000 rpm for 1 hr in a No. 50 rotor in a Beckman L 2 centrifuge. The sedimented microsomes so obtained were resuspended in a volume of 0.25 M sucrose so that 1 ml contained microsomes from 1 g of liver. The supernatant fluid remaining after sedimentation of the microsomes contained the cell sap fraction. The mitochondria were suspended in 20 ml of 0.25 M sucrose, resedimented by centrifugation at 10,000 × *g* for 20 min, and then suspended in the same manner as the microsomes. All operations prior to lipid extraction were carried out at 4°C. Lipid solvents contained 0.01% (w/v) hydroquinone to prevent oxidation of the lipids.

Kidney and brain were homogenized with 3 volumes of 0.25 M sucrose and lipids were extracted from the homogenates by the method previously described for liver. Epididymal and perirenal fat pads were removed completely and together constituted the adipose tissue investigated. The tissue was thoroughly minced with scissors, and lipid from 1.00 g was extracted with 100 volumes of chloroform–methanol 2:1. Protein analyses were carried out by the method of Lowry, Rosebrough, Farr, and Randall (17).

Injection of Acetate-^{14}C

Eight animals in each group were injected intraperitoneally with 14 or 75 μmoles of sodium acetate-1-^{14}C (50–150 μc) dissolved in 1 ml of isotonic saline, and were sacrificed 1 hr later. Aliquots of the lipid extracts from the whole homogenate and subcellular fractions of liver were transferred to glass counting-vials and evaporated to dryness under a stream of nitrogen, and the residue was redissolved in Liquifluor[2] (Pilot Chemicals, Inc., Watertown, Mass.). Radioactivity was estimated in a Nuclear-Chicago liquid scintillation spectrometer, and the degree of quenching determined by a channels ratio method (18). TLC was used to separate the lipids into classes (19), and the radioactivity in each class was determined as described previously (6).

GLC and Assay of ^{14}C

Fatty acid composition was analyzed by GLC using

[1] According to the supplier, the diet contained 3.78 calories per g, 23.4% protein (9.5% in the form of a mixture of 12 amino acids including all the essential ones), 50.6% carbohydrate, 3.8% lipid, 4.9% fiber, and 4.3% salts. The remainder included vitamins, 0.16% choline, and ash.

[2] The Liquifluor concentrate was diluted with toluene so that the final solution contained 4 g/liter of 2,5-diphenyloxazole and 0.05 g/liter of 1,4-bis[2-(5-phenyloxazolyl)]-benzene.

Barber-Colman Series 5000 gas chromatographs with both flame ionization and argon "electron drift" (20) (tritium foil, 100 mc) detectors. U-shaped glass columns, 6 ft × 3 mm I.D., were packed with Anakrom ABS, 80–90 mesh, coated with 15% (w/w) ethylene glycol succinate polyester and operated at 170°C. Quantitative results with National Heart Institute Fatty Acid Standards agreed with the stated composition, the relative error being less than 5% for major components and less than 12% for minor components. In determining fatty acid isotope content, components were also separated on a column in which the liquid phase was 5% (w/w) SE-30 (silicone rubber gum, General Electric) plus 1% (w/w) ethylene glycol succinate polyester. This combination of liquid phases prevented adsorption of fatty acids by the support and gave separations that were as good as on SE-30 alone. A calibrated stream splitter was introduced between the column and the detector (21) in order to collect 70% of the column effluent by one of two methods: either condensation in Teflon tubing (9 inches × $^1/_{16}$ inch I.D.) and rinsing into a counting vial with 12 ml of Liquifluor containing 0.1% carrier stearic acid (isotope recovery was greater than 95%), or collection in cartridges packed with toluene-wetted Chromosorb W (60–100 mesh) and placed in the effluent stream by means of a Packard Model 830 gas chromatography fraction collector. The entire contents of the cartridges were transferred to counting vials, thoroughly mixed with 12 ml of Liquifluor, and counted. Chromosorb sediments in Liquifluor, but it was established that more than 95% of methyl palmitate-^{14}C collected in this fashion was consistently recovered.

All analyses were performed in duplicate. The data for each group are expressed as the mean ± SEM. The statistical significance of differences between groups was calculated by the paired "*t*" test (22).

RESULTS

Diet and Growth Rates

Fig. 1 shows that the total caloric intake of the three groups of rats was almost identical and increased with age. It ranged from 75 to 107 kcal/day for the animals of the control group and from 68 to 99 kcal/day for the glucose and ethanol groups. About 30% of the calories consumed by the ethanol group was derived from the alcohol. Although protein and essential amino and fatty acid intake was less in rats receiving glucose and ethanol, the growth rates of these animals were only slightly less than those of the controls (Fig. 2). All animals consumed at least six times the daily requirement of essential fatty acids in the form of linoleic acid

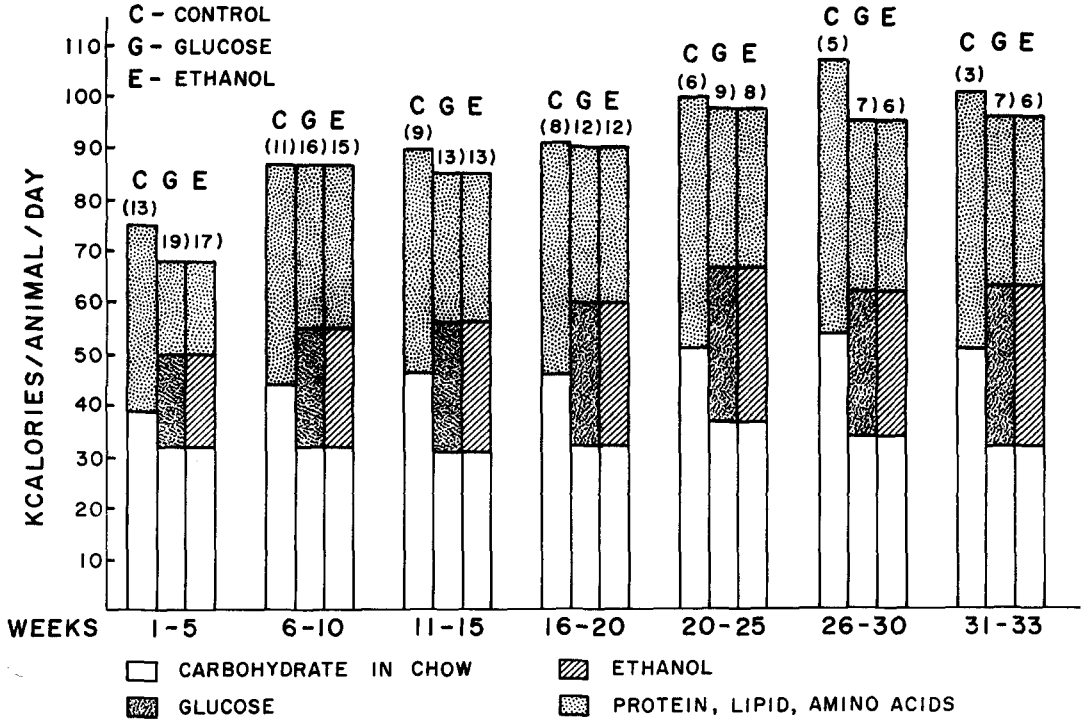

FIG. 1. Caloric intakes of male rats on the three dietary regimens. The animals were fed as indicated in the experimental sections. Numbers in parentheses refer to the number of rats in each group. The ordinate is the mean number of kcal consumed per day by each animal in the various groups during the period indicated on the abscissa.

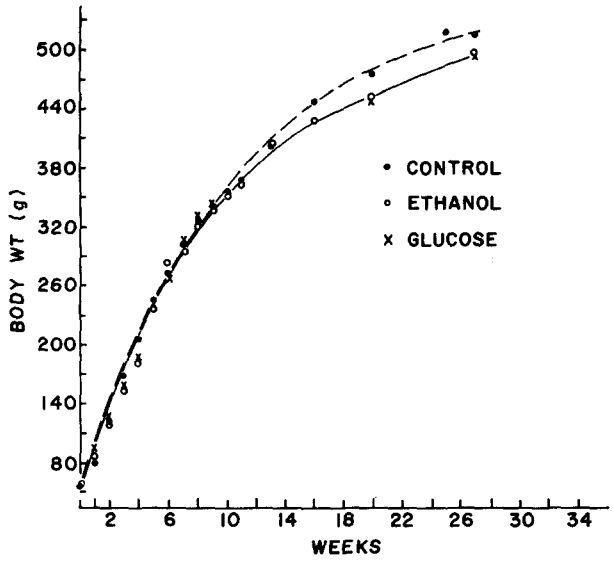

FIG. 2. Growth rate of male rats on three dietary regimens. The ordinate represents the mean body weight in grams of the animals in each group whose dietary intake is shown in Fig. 1.

(23) and nine times the normal daily choline requirement (24). The percentage distribution of fatty acids in the diet was identical for the three groups and consisted of 1.8% 14:0, 22.7% 16:0, 8.0% 18:0, 3.9% 16:1, 30.3% 18:1, 30.3% 18:2, and 3.0% 18:3, as determined by GLC.

Alterations in Liver and Adipose Tissue Weight and Hepatic Triglyceride Content

One animal from each group was sacrificed 29–260 days after the dietary regimens had been established. Liver weights, adipose tissue weights, and hepatic triglyceride levels were determined. In Table 1 the results are divided into three successive time periods of 29–101, 101–199, and 199–260 days.

It is seen that neither glucose nor ethanol feeding had any significant effect on the liver weights during the three time periods. However, as compared to the controls, the combined weight of epididymal and perirenal adipose tissue was increased in the glucose-fed group and decreased in the ethanol-fed group. These differences in adipose tissue mass appeared in the time period 29–101 days and became increasingly larger as the experiment progressed: the adipose tissue weight in the glucose group during the time periods of 101–199 and 199–260 days was nearly twice that of the ethanol group. In addition, data not presented in Table 1 showed that the esterified fatty acid concentration increased from 2.3 to 3.2 meq/g of tissue with glucose feeding and decreased to 1.3 meq/g of tissue with ethanol feeding at 136 days.

Although the quantity of adipose tissue differed in the three groups, it is seen in Table 1 that the triglyceride content of the liver was not significantly altered by prolonged ingestion of glucose or ethanol. It appeared that glucose feeding might have led to a slight increase after 101 days, but histological examination throughout the whole experiment revealed that all the livers were normal and confirmed the absence of lipid accumulation in any of the animals. Moreover, other data not in Table 1 showed that the total carbon content in lipid extracts and the protein concentration in homogenates of the liver were the same in the three groups.

Changes in Fatty Acid Composition of Tissue Lipids Produced by Ethanol and Glucose

Figs. 3 and 4 show the fatty acid composition of liver and adipose tissue in rats that had been on their respective diets for 29–199 days. In liver, glucose caused an increase in 16:0, 16:1, and 18:1 but a decrease in 18:0 and 18:2, while ethanol caused an increase only in 18:1. These changes apparently had occurred during the first 29 days, since the fatty acid composition from 29 to 199 days was relatively stable, as reflected by the small standard deviations.

From Figs. 3 and 4 it is apparent that the liver was rich in the saturated fatty acids, palmitic and stearic,

TABLE 1 WEIGHTS OF LIVER AND ADIPOSE TISSUE AND CONCENTRATION OF HEPATIC TRIGLYCERIDES

Days on Diet	Liver Weight			Adipose Tissue Weight			Hepatic Triglyceride		
	Control	Glucose-fed	Ethanol-fed	Control	Glucose-fed	Ethanol-fed	Control	Glucose-fed	Ethanol-fed
	mg/g body weight			*mg/g body weight*			*μmoles/g liver*		
29–101	34 ± 3.3	33 ± 2.1	36 ± 3.1	17 ± 4.4	21 ± 3.6	12 ± 2.2	10 ± 1.7	8 ± 1.7	8 ± 1.3
101–199	28 ± 2.3	28 ± 2.7	31 ± 3.0	31 ± 3.1	44 ± 3.1	22 ± 3.0	11 ± 1.6	13 ± 2.9	10 ± 1.1
199–260	26 ± 2.5	29 ± 2.3	33 ± 3.1	35 ± 3.0	46 ± 3.0	24 ± 3.0	9 ± 1.3	14 ± 2.0	11 ± 2.1
Over-all mean	29 ± 1.9	29 ± 1.5 (NS)*	33 ± 1.3 (NS)*	28 ± 3.7	36 ± 4.5 ($P < 0.01$)*	20 ± 2.2 ($P < 0.05$)*	10 ± 1.5	12 ± 1.3 (NS)*	10 ± 1.6 (NS)*

One animal in each dietary group had been on the stated regimen for 29, 38, 70, 84, 101, 136, 155, 175, 199, 224, 227, 239, or 260 days. The values are expressed as the means ± SEM.

* Glucose- and ethanol-fed animals compared with controls.

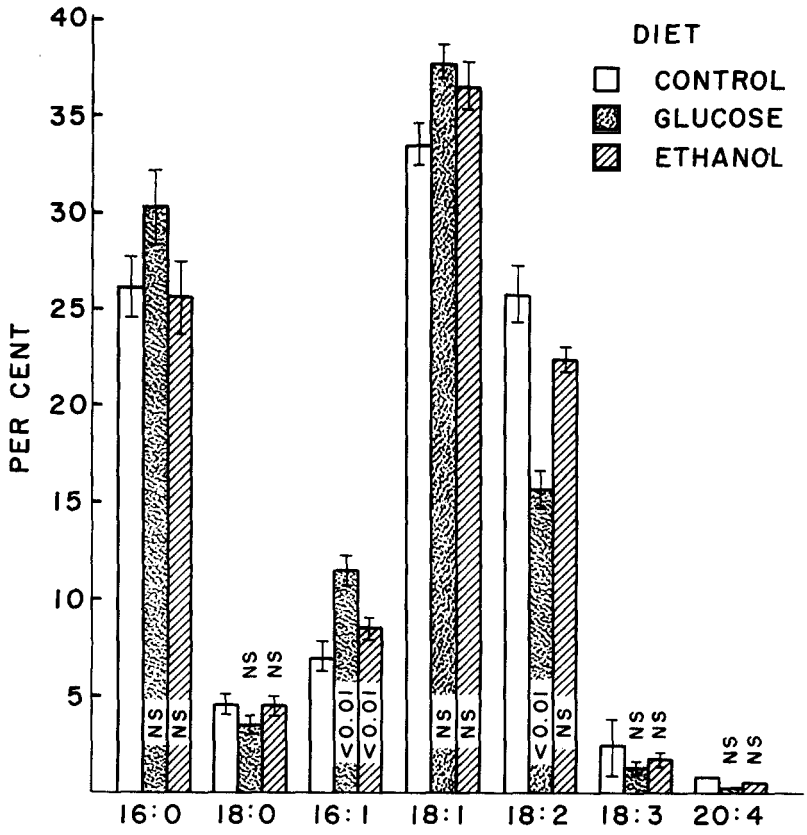

FIG. 3. Fatty acid composition of rat adipose tissue. One rat from each diet group was killed 29, 38, 70, 84, 101, 136, 155, 175, and 199 days after the experiment was begun and the fatty acid composition of the combined perirenal and epididymal fat pads was determined by GLC. The bars represent the means and the vertical lines the SEM.

whereas adipose tissue contained a greater proportion of the monounsaturated fatty acids, palmitoleic and oleic. Neither glucose nor ethanol supplements altered the proportion of saturated fatty acids present in liver or adipose tissue. Glucose, and to a lesser extent ethanol, increased the proportion of monounsaturated fatty acids and reciprocally decreased the proportion of the essential fatty acids, linoleic and arachidonic, in both tissues. However, only with glucose feeding was the reduction in essential fatty acids statistically significant.

The fatty acid composition of *kidneys* was determined in five sets of rats that had been on the diets for 29, 38, 155, 179, and 199 days. Glucose supplements produced changes similar to those seen in liver and adipose tissue, but ethanol had virtually no effect. In the control animals and those fed ethanol the saturated fatty acids comprised 37.8 and 38.8% of the total, monounsaturated fatty acids 28.2 and 27.8%, and essential fatty acids 34.0 and 33.4%, respectively. However, in animals fed glucose there was an increase in monounsaturated fatty acids to 36.3% and a concomitant decrease in essential fatty acids to 28.7%. The total lipid content of kidney was the same in the three groups.

Analyses of *brain tissue* in four animals in each of the three groups failed to demonstrate any changes in fatty acid composition following glucose or ethanol administration.

The fatty acid composition of the various *subcellular fractions* of hepatic tissue in the three groups was also determined. The cell sap in all groups was rich in palmitate (33–35%) and oleate (28–38%) and low in stearate (4–5%) and arachidonate (1–4%), whereas mitochondria and microsomes had much higher concentrations of stearate (17–23%) and arachidonate (19–24%). Ethanol and glucose produced changes in fatty acid composition of the individual cell fractions that were similar to those observed in whole homogenates.

In Vivo Incorporation of Acetate-1-^{14}C into Total Liver Lipids

When 75 μmoles of acetate-^{14}C was injected, the fraction of isotope incorporated into total hepatic lipid was 1.7 times greater in both the glucose- and ethanol-fed animals than in the controls (Table 2). Because the weight and lipid content of the liver were virtually the same in all three groups (*see* above), these differences

TABLE 2 INCORPORATION OF SODIUM ACETATE-1-^{14}C INTO TOTAL HEPATIC LIPIDS

Dietary Regimen	Acetate-^{14}C Injected	Amount Incorporated into Liver Lipid	Amount Incorporated into Liver Lipid
	μmoles	*%*	*mμmoles/g liver*
Control	75	0.97 ± 0.31	52.5 ± 6.0
Glucose	75	1.68 ± 0.41	84.8 ± 6.1 ($P < 0.05$)
Ethanol	75	1.74 ± 0.28	85.6 ± 4.4 ($P < 0.05$)
Control	14	0.63 ± 0.21	7.4 ± 1.5
Glucose	14	1.88 ± 0.22	20.3 ± 1.0 ($P < 0.05$)
Ethanol	14	1.75 ± 0.03	16.7 ± 1.3 ($P < 0.05$)

Each of the groups given 75 μmoles of acetate-1-^{14}C intraperitoneally contained five animals. One animal in each group had been on the stated dietary regimen for 155, 199, 224, 227, or 239 days.

Each of the groups given 14 μmoles of acetate-1-^{14}C intraperitoneally contained three animals. One animal in each group had been on the stated dietary regimen for 84, 175, or 260 days.

are in the same relation whether incorporation is expressed as the absolute amount of isotope recovered in lipid of the whole liver or per gram of liver. When 14 μmoles of acetate-^{14}C was administered, a significantly lower amount of acetate was incorporated into hepatic lipids than after the administration of 75 μmoles in all three groups. Both 14 and 75 μmoles of acetate were administered to establish that increased utilization of the label was not a reflection of the amount of metabolite injected. The glucose- and ethanol-fed animals still fixed two to three times more acetate into total liver lipids than did the controls. In all groups, irrespective of the amount of acetate injected, more than 80% of the label present in the lipids was found in the fatty acid fraction.

The total liver lipids from four sets of animals were separated into classes by TLC. Forty-five per cent of the isotope was found in the triglyceride fraction in both the ethanol and control groups, whereas 56% was found in the glucose group. Phospholipid in the control, glucose, and ethanol groups contained about 38, 31, and 41% of the label, respectively. The distribution of isotope in the various liver lipid classes was the same when the animals were given either 75 or 14 μmoles of acetate-1-^{14}C.

The percentage distribution of label in the various fatty acids found in the lipid extracts of liver microsomes, mitochondria, and cell sap of these animals is shown in Table 3. Neither glucose nor ethanol altered the pattern of labeling of the fatty acids found in these cell fractions. Saturated fatty acids, especially palmitic, contained the bulk of the isotope in all cell fractions. Between 10 and 20% of the label appeared in the monounsaturated fatty acids, while most of the remainder was found in the unsaturated fatty acids containing 20 and 22 carbon atoms. Since no differences in the pattern of ^{14}C labeling were observed in animals given either 14 or 75 μmoles of acetate, the results in the two groups were pooled. Though not shown in Table 3, the subcellular distribution of ^{14}C-lipid was found to be the same in all three groups and was 35% in

FIG. 4. Fatty acid composition of rat liver. The livers from the rats referred to in Fig. 3 were analyzed for fatty acid composition.

TABLE 3 PERCENTAGE DISTRIBUTION OF ^{14}C IN FATTY ACIDS OF HEPATIC SUBCELLULAR FRACTIONS AFTER INJECTION OF ACETATE-1-^{14}C

Fatty Acids	Microsomes			Mitochondria			Cell Sap		
	Control	Glucose-fed	Ethanol-fed	Control	Glucose-fed	Ethanol-fed	Control	Glucose-fed	Ethanol-fed
16:0	62	55	60	56	50	55	60	54	66
16:1	5	5	3	4	4	4	11	12	9
18:0	12	11	10	11	13	12	3	3	3
18:1	8	15	15	11	12	11	13	19	12
Others	13	14	12	18	21	18	13	12	10

The values presented are the means for five animals in each group. The animals were the same as those used for the analyses recorded in Table 2 and included three in each group given 75 μmoles and two given 14 μmoles of acetate-1-^{14}C.

cell debris, 17% in mitochondria, 33% in microsomes, and 15% in cell sap.

DISCUSSION

Many alterations in lipid metabolism are observed after the administration of a single dose of ethanol to the intact animal. These include fatty infiltration of the liver with concomitant increases in the concentrations of oleate and linoleate and a decrease in stearate (5, 6, 25). The fatty acid pattern of adipose tissue is unchanged after an acute dose of ethanol. It is clear from our data (Figs. 3 and 4) that the effects of prolonged feeding of ethanol differ from those produced by a single large dose in that oleate of liver and palmitoleate of adipose tissue increase. In addition, no decrease of any fatty acid in either tissue is observed after prolonged ingestion of ethanol. Further, even though a single dose of 7.5 g of ethanol per kg causes fatty infiltration of the liver in the rat, still greater amounts of ethanol (Fig. 1) consumed on a daily ad lib. basis failed to do so in our experiments (Table 1). During the first five weeks of the experiment our animals consumed an average of 20 g of ethanol per kg of body weight daily and, though subsequently this decreased, the daily intake never fell below 9 g/kg. Failure to observe hepatic triglyceride accumulation may in part be related to the fact that male rats are more resistant to toxic injury than females (26). Preliminary experiments indicated that female rats accumulate more hepatic triglycerides than do males following acute ethanol intoxication.[3]

In contrast, Mallov (27) found that chronic ethanol administration increased hepatic fat about 36% above controls given isocaloric amounts of glucose. However, his male rats were fed twice daily by stomach tube and were given a ration that contained about 4 g of ethanol per kg of body weight in each feeding. It is possible that the diet contained limiting amounts of choline (25 mg/100 g of diet) or its precursors, and was responsible for the minor degree of fatty infiltration (28) and the depressed growth rate observed in his ethanol-fed animals, since the choline requirement increases to 0.08% of the diet when alcohol is consumed in these quantities (29). As in previous studies (30), in the present experiments the animals which received adequate amounts of lipotropic materials did not develop a fatty liver when ethanol or glucose supplemented the basal diet. It has also been reported (4) that more pronounced fatty infiltration of the liver develops in rats given 36% of their daily caloric intake in the form of ethanol rather than sucrose. However, the interpretation of this observation is complicated by the fact that the liquid formula diet used in this study contained about 40% neutral fat. It would be of greater significance if similar results could be obtained with more modest amounts of dietary fat, since, in the absence of ethanol, the inclusion of this much fat without added carbohydrate gives rise to a fatty liver (31) and decreases the rate of alcohol oxidation (32).

Prolonged administration of either ethanol or glucose stimulated acetate incorporation into lipids of rat liver (Table 3) as has been reported following single large doses (33) of these materials or high carbohydrate diets (34). The increased utilization of acetate by the glucose- and ethanol-fed animals may be in part due to the slightly decreased amount of dietary fat they consumed, since dietary lipid is known to depress hepatic fatty acid synthesis (35). The increased incorporation of acetate-^{14}C in the ethanol-fed animals is even more striking since these animals would be expected to have increased blood acetate levels (36), which would tend to dilute the label and thus decrease ^{14}C incorporation into lipid. In any event, fatty livers did not develop in either the glucose- or the ethanol-fed animals despite their increased utilization of acetate for fatty acid synthesis. Moreover, since the pattern of labeling of individual fatty acids was the same in livers of each of the three groups (Table 3), it is unlikely that hepatic fatty acid synthesis accounted for the changes in fatty acid composition observed either in liver or in adipose tissue.

[3] N. M. Alexander and R. Scheig, unpublished observations.

Single large doses of ethanol do not affect the fat content of adipose tissue (37). In contrast, prolonged ethanol feeding in the present experiments led to a decrease while prolonged glucose feeding produced an increase in the amount as well as concentration of lipid in the fat depots (Table 1). Moreover, the increase in depot fat induced by glucose feeding was accompanied by an alteration in its fatty acid composition, characterized by a decrease in linoleate and an increase in palmitate (Fig. 3), a pattern reminiscent of that seen in experimental obesity (38). These changes plus the increase in monounsaturated fatty acids and the decrease in stearate observed following prolonged glucose administration (Table 2) are the very opposite of those seen in the diabetic rat (39) and, hence, suggest that they may be dependent on the increase in insulin output known to follow glucose feeding. Since insulin promotes triglyceride synthesis and inhibits lipolysis in adipose tissue (40), it could very well account for the increase in depot fat observed in our glucose-fed animals. In addition, it is conceivable, in the light of our observations, that insulin plays a role in determining the fatty acid composition of adipose tissue.

Since the number of potential acetyl CoA units consumed was virtually identical in our glucose- and ethanol-fed animals (2.1 moles of ethanol were consumed for every mole of glucose), the fact that the weight of the perirenal and epididymal fat pads increased in the glucose-fed animals and decreased in those fed ethanol suggests that glucose is more lipogenic than ethanol. An alternative possibility is that ethanol and glucose ingestion each lead to a different redistribution of fat. Similarity in the body weights of the three groups does indicate that alterations in body water or weights of tissues other than liver may have occurred.

The mechanism underlying the changes induced in adipose tissue by ethanol is unknown. The possibility that the adrenal hormones are involved, as has been postulated in the case of the acute effects of ethanol (41), merits consideration, since these hormones mobilize fatty acids from adipose tissue and produce fatty livers (41, 42). Horning, Wakabayashi, and Maling (41) have proposed that ethanol affects the hormonal control of fat transport because adrenalectomy prevents the development of fatty liver following short-term administration of ethanol (43). Consistent with this observation are the in vitro experiments that have shown that ethanol is less readily metabolized by adipose tissue than is glucose (44). Since adipose tissue contains little, if any, alcohol dehydrogenase (45), ethanol probably cannot be utilized for lipid synthesis in the fat depots until it has been converted to another lipid precursor by other tissues.

Ethanol and glucose also differ in their effects on polyunsaturated fatty acid metabolism (Figs. 3 and 4). Glucose feeding depresses the proportion of linoleate in adipose tissue, but because of the increase in tissue mass the total amount remains the same as in the control. On the other hand, ethanol depletes the amount of linoleate in adipose tissue, but, unlike glucose, does not significantly modify the amounts of essential fatty acids in liver and kidney. The significance of these alterations in polyunsaturated essential fatty acids is unknown.

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