trast, the transition temperature was 22.5 ± 1.2° for cells from animals fed the coconut oil diet. This 3.8° difference, which was associated with a lipid alteration, suggests that the transition temperature was due to a lipid phase change. The values of the transition temperature for melphalan transport of cells from the sunflower fed animals (18.7°) were similar to the lower transition temperature of the electron spin resonance studies (19.5° for 5-nitroxide stearic acid and 18.5° for 12-nitroxide stearic acid) [4]. Likewise, the cells from animals fed the coconut oil diets had transition temperatures for melphalan transport (22.5°) which were similar to the transition temperatures of the electron spin resonance study (22.0° for both probes). This is further evidence that the break in the Arrhenius plot for melphalan transport by the L1210 cell probably was due to a lipid phase change. It has been reported that fatty acid alteration of Ehrlich cells resulted in changes in transition temperatures for phenylalanine transport without affecting the kinetic parameters [11]. This amino acid is transported by system L which is the low-affinity system responsible for transport of a majority of melphalan at higher concentrations.

We have shown previously that alteration of the fatty acid composition and fluidity of plasma membranes has a marked effect on the transport of methotrexate by intact L1210 cells [4]. The K'_m for transport of methotrexate by L1210 cells from animals fed a polyunsaturated rich diet was 2.9 ± 0.4 μM compared to 4.1 ± 0.1 μM for cells from animals fed a more saturated-fat rich diet ($P < 0.02$). Since the transport of melphalan, like that of methotrexate, is an active carrier-mediated process, we examined the effect of fatty acid composition on the kinetic parameters of melphalan transport. There were no differences in K'_m or V'_{max} of either component of melphalan transport. This lack of effect of membrane lipid composition and fluidity on melphalan transport as compared to methotrexate transport cannot be explained by data currently available.

In summary, the transport of melphalan by L1210 lymphoblastic leukemia cells was markedly temperature dependent, and the Arrhenius plot demonstrated a biphasic pattern. Modification of the lipid composition of the cell phospholipids had a significant effect on the transition temperature for melphalan transport even though the K'_m and V'_{max} were the same in both types of cells. This shift in transition temperature demonstrates that the discontinuity in the Arrhenius plot was the result of a lipid phase transition and not of an interaction of the two carrier-mediated transport processes. These data indicating differential membrane lipid effects on the transport mechanism provide further evidence of a relationship between lipids and membrane drug transport.

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Effects of clofibrate and ethanol on the pathways of initial fatty acid oxidation

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Clofibrate administration to rats increases peroxisomal oxidation many-fold and perhaps mitochondrial oxidation [1–4]. Clofibrate also stimulates a variety of hepatic microsomal enzymatic activities [5].

A microsomal fraction of liver catalyzes the hydroxylation of the terminal carbon of fatty acids. The resulting omega-hydroxylated fatty acids are then oxidized to their corresponding dicarboxylic acids [6–8]. We have developed a method for estimating the contribution of omega-oxidation relative to beta-oxidation in the initial oxidation of fatty acids [9]. The changes produced by clofibrate on fatty acid oxidation by the different hepatic subcellular fractions prompted us to examine whether or not clofibrate changes the proportion of fatty acids undergoing omega-oxidation in the rat. Ethanol has profound effects upon lipid metabolism including the inhibition of fatty acid oxidation [10]. We therefore elected also to assess the effect of ethanol administration on the relative contribution of omega-oxidation to fatty acid oxidation.

Our estimations depend upon the fate of the omega-carbon of a fatty acid [9, 11]. Thus, if [16-^{14}C]palmitic acid undergoes beta-oxidation, its terminal two carbons will yield [2-^{14}C]acetyl CoA. The hexadecanedioic acid formed from it by omega-oxidation will have one of its carboxyls

Table 1. Effects of clofibrate and ethanol feeding on the distribution of ^{14}C in acetate formed by rats from [16-^{14}C]palmitate

		Control					Drug-fed				
		Initial rat	Acetate	% ^{14}C in		%	Initial rat	Acetate	% ^{14}C in		%
Drug	Expt.	wt (g)	(dpm × 10^3)	Carbon 1	Carbon 2	recovery	wt (g)	(dpm × 10^3)	Carbon 1	Carbon 2	recovery
Clofibrate	1	174	2.9	20.6	79.4	95	184	2.6	14.1	85.9	105
Clofibrate	1	170	2.8	21.0	79.0	100	175	2.8	13.9	86.1	97
Clofibrate	1	177	9.2	21.5	78.5	98	170	13.2	14.2	85.8	100
Clofibrate	2	200	9.2	40.2	59.8	100	223	9.0	22.0	78.0	100
Clofibrate	3	195	5.0	42.2	57.8	102	160	9.4	21.4	78.6	97
Ethanol	1	254	9.7	29.5	70.5	97	249	4.2	29.1	70.9	105
Ethanol	2	250	3.4	12.8	87.2	98	192	6.3	17.2	82.8	100

labeled with ^{14}C, and on subsequent beta-oxidation the diacid will yield [1-^{14}C]acetyl CoA. The distribution of ^{14}C in acetyl CoA then is the measure of extent of initial omega-oxidation compared to beta-oxidation of the palmitate.

Three experiments were performed to study the effect of clofibrate. Rats were male, of the Sprague–Dawley strain, and were housed in metabolic cages. Weights of the rats at the beginning of the study are recorded in Table 1. In the first experiment, three rats (control rats) were fed powdered stock chow (Purina Rat Chow, Purina Co., St. Louis, MO) for 7 days. Three other rats (clofibrate-fed rats) were fed the chow mixed with the sodium salt of clofibric acid (5 g/kg of chow) for the 7 days [12]. Beginning on day 8, all six rats had mixed in their diet phenylaminobutyric acid (3.5 g/kg of chow) and on days 8, 9, and 10, each of the rats was injected intraperitoneally with 5 μCi of [16-^{14}C]palmitic acid (46 μCi/mmole).

Phenylaminobutyric acid was administered since following its ingestion it is acetylated and the acetylated phenylaminobutyric acid is excreted in urine from which it can be readily isolated. The phenylaminobutyric acid thus allows sampling of the pool of acetyl CoA utilized in its acetylation [13]. Urines from each rat were collected from days 8 through 11 and then the rats were killed. The acetylated phenylaminobutyric acid that each rat excreted was isolated and purified. It was hydrolyzed and the acetate that formed was degraded to yield each of the two carbons of the acetate as CO_2. The CO_2 was assayed for ^{14}C [11]. During the 11-day period of the experiment, the increase in the weights of the control rats ranged from 26 to 32% of their initial weights and of the clofibrate-fed rats, somewhat less, from 12 to 20%. The liver weights at killing of the control rats ranged from 9.1 to 10.0 g and of the clofibrate-fed rats from 12.9 to 13.6 g, in accord with the reported effect of clofibrate on liver weight [12, 14].

The second experiment was identical to the first experiment except that there was only one control and one clofibrate-fed rat, and phenylaminobutyric acid addition to the diet and [16-^{14}C]palmitic acid injections were begun on the tenth day after the rats were begun on chow. When the rats were killed on day 13, the weight of the control rat had increased by 56% and that of the clofibrate-fed rat by 46%. The third experiment was identical to the second experiment except that the phenylaminobutyric acid addition and [16-^{14}C]palmitic acid injection were begun on the eleventh day after placing the rats on the chow diet. The weight of the control rat at killing had increased by 69% and that of the clofibrate-fed rat by 70%.

In each of two experiments, one rat was fed a liquid ethanol-rich diet [15] for 7 weeks and a control rat was fed the identical diet except that a maltose–dextrin mixture was substituted isocalorically for the ethanol. The rats were female, of the Sprague–Dawley strain, weighed 192–257 g when begun on the diet, and were housed in metabolic cages. The control rat ingested on the average about 65 ml of the liquid diet daily in the first experiment and 95 ml in the second. The ethanol-fed rat ingested an average of 70 ml daily in the first experiment and 75 ml in the second. The weights of the control rats increased by 38 and 118% during the 7-week period and those of the ethanol-fed rats 23 and 82%.

For the last 4 days of week 7, phenylaminobutyric acid was added to the liquid diet of each rat (100 mg/80 ml of diet the first two days and 200 mg/80 ml of diet the last two days). The intake of diet by the rats was not altered by the addition of the amino acid. On each of the first 3 days of the 4-day period, each rat was injected with 5 μCi of [16-^{14}C]palmitate intraperitoneally. Urine was collected, and acetate was isolated from the excreted acetylated amino acid and was degraded as described for the study of the effect of clofibrate.

The distributions of ^{14}C in the carbons of acetate from

each experiment are recorded in Table 1 along with the initial weights of the rats and the disintegrations per minute in the acetates. The ^{14}C specific activity in each carbon is expressed as the percentage of the sum of the specific activity in both carbons and hence the percentages in the two carbons sum to 100%. Before degrading each acetate, an aliquot was combusted to CO_2 and the specific activity of the CO_2 was determined. Percent recovery is 100 times the specific activity of the ^{14}C in carbon 1 and the ^{14}C in carbon 2 divided by the specific activity of the ^{14}C in the carbons in the aliquot combusted. These recoveries range from 97 to 105%, giving good evidence for the purity of the acetates and the adequacy of the degradations.

In each of the three experiments, clofibrate decreased the quantity of [1-^{14}C]acetate relative to [2-^{14}C]acetate formed from [16-^{14}C]palmitic acid. Therefore, clofibrate decreased the fraction of fatty acid oxidized initially via omega-oxidation. From the distributions in acetates from the control rats one can estimate that 28–56% of the initial metabolism of the fatty acid was via omega-oxidation and that in the clofibrate-fed rats 16–31% was via omega-oxidation [11]. These estimates are for contributions of omega-oxidation in the cellular environment in which the acetylation of phenylaminobutyric acid occurs. The percentage in carbon one varied in the control rats from 21 to 42%. However, in the experiment done in triplicate, the distributions were essentially identical among the three rats. The reason for variations between experiments is uncertain, but we have observed such variations in the past [11, 16]. The variations do not detract from the clear effect of clofibrate on the distributions. The distributions in acetate were not different between the control and ethanol-fed rats. Thus, ethanol administration did not alter the fraction of fatty acid initially oxidized via omega-oxidation relative to beta-oxidation.

In summary, clofibrate, but not ethanol, administration to the rat decreased the proportion of palmitic acid oxidized initially via omega-oxidation as compared to beta-oxidation.

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New method to increase the serotonin level in brain by carotid injection of desoxyfructo-serotonin in mice

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Serotonin (5-HT) has multiple functions in blood and brain [1]. If the level of serotonin can be increased by suitable means, it could be valuable in the treatment of diseases associated with a decrease in serotonin.

Desoxyfructo-serotonin (1-desoxy-1-[5-hydroxytryptamino]-D-fructose or DF-5HT) (Fig. 1) is a sugar derivative of serotonin [2] which seems to cross the blood–brain barrier, since central effects were observed with this compound after intravenous as well as intraventricular administration [3]. It was, therefore, considered necessary to obtain data on the penetration of desoxyfructo-serotonin from the blood to the brain and study its metabolism in this compartment.

Materials and methods

The experiments were performed on male Swiss albino mice weighing between 24 and 26 g. [2-^{14}C]-5-Hydroxytryptamine binoxalate (54 mCi/mmole) obtained from New England Nuclear (Boston, MA) was used for the preparation of desoxyfructo-[2-^{14}C]-5-hydroxytryptamine oxalate [2]. Purity of the radioactive material was controlled by TLC. Solution in 0.9% NaCl of 2.3 mM [2-^{14}C]-DF-5HT, having 0.5 mCi/mmole activity, was freshly prepared and 0.125 ml of the solution was injected into the carotid artery [4]. Following injection, at various intervals from 2 min to 60 min, the radioactivity was assessed in the brain.