$CuSO_4$, 10 µmol of sodium fumarate, 10 µmol of ascorbic acid, 1 µmol of pargyline, 1500 units (50 µg) of catalase, and 20 µmol of tyramine hydrochloride. Incubation was carried out at 37 °C for 45 min. A sample of diluted plasma boiled at 95 °C for 5 min was used for blank incubation.

Changes in serum DBH activity during development of Japanese monkeys (*Macaca fuscata fuscata*) are shown in the Figure. Plasma DBH activity in young Japanese monkeys at 3 months after birth was very low, but increased gradually during development to the adult level (0.59 nmol/min/ml plasma), and the developmental changes were parallel to those of body weight. These developmental changes in monkey plasma DBH activity are similar to those in human beings.

Plasma DBH activity in various species of adult monkeys, i.e., chimpanzee (*Pan troglodytes*), hamadriad baboon (*Papio hamadryas*), and thick-tailed galago (*Galago crassicaudatus*) were: 0.05, 0.05 and 0.08 nmol/min/ml plasma, respectively. Therefore, the activities are different among various monkey species regardless of the difference in their posture, and distinctly lower than those of human beings (mean value, 43 nmol/min/ml serum or plasma[4]).

It is concluded that although developmental changes in plasma DBH activity of monkeys are similar to those of human beings, the enzyme activity in plasma of various species of adult monkeys is much lower than that of human beings.

Cholesterol Esterification Activities in the Intestines and Pancreas of the Albino Rat

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Summary. Cholesterol esterification activities in intestines and pancreas are much greater with unsaturated fatty acids than with the saturated ones; the maximum activity is with arachidonic acid in intestines and with oleic acid in pancreas. The pancreatic cholesterol esterification activity is higher than the intestinal one.

In biological systems, there are at least three different mechanisms for the cholesterol esterfication activities. The esterification reaction taking place in incubated serum is due to a transesterase which does not require any cofactor for its activity[1–3]. This enzyme has a specificity for fatty acids characteristic of the normal pattern of cholesterol esters. The liver is suggested as the source of the plasma enzymes. Contrary to the above mechanism, cholesterol esterification in the liver involves the presence of CoA, ATP, etc. as cofactors[4,5]. This enzyme has been shown to be a fatty acyl-CoA-cholesterol acyl transferase. In a third mechanism, the apparent direct reaction of free cholesterol with free fatty acid seems to be responsible for cholesterol ester synthesis in both the pancreas and the intestines[6,7]. This reaction has been studied with highly purified enzyme systems and certainly does not proceed through the formation of a fatty acyl CoA intermediate. In the present paper, the effects of two dietary fats on cholesterol esterification activities in the intestines and pancreas are discussed.

Table 1. Cholesterol esterification activity in rat intestines

Group	Palmitic	Stearic	Oleic	Linoleic	Arachidonic
10% Safflower oil	6.8	6.6	48.5	61.6	81.2
20% Safflower oil	7.8	7.8	41.4	69.3	85.1
30% Safflower oil	9.8	10.4	46.0	72.0	86.8
50% Safflower oil	8.0	9.0	50.4	70.4	84.8
10% Lard	10.0	9.4	53.3	67.4	86.0
20% Lard	10.0	9.4	50.0	66.0	86.4
30% Lard	12.4	12.4	52.0	70.8	88.0
50% Lard	10.1	10.7	49.6	68.5	83.5

The values are expressed as nmoles of cholesterol esterified/mg protein/h. The enzyme assay system contained in a total volume of 5 ml: 25 mg of intestinal powder corresponding to 8 mg protein, potassium phosphate buffer 0.1 *M*, pH 6.1, 3 ml, sodium taurocholate 25 mg, cholesterol 5 µmoles and fatty acid 10 µmoles.

Materials and methods. 8 groups of 5 weanling male albino rats were fed safflower seed oil (highly unsaturated vegetable oil) or lard (animal fat) at 10, 20, 30 and 50% of their total calorie intake. The total calorie intake was kept constant. The rats were sacrificed at the end of an 8-week experimental period and cholesterol esterification activities in the intestines and pancreas determined by the method of Murthy et al.[7]. Intestines and pancreas were cut and washed with ice-cold saline to remove food particles. They were pressed on filter paper and placed in −15° acetone. Acetone-dried powder was prepared by grinding the tissues in 10 volumes of acetone precooled to −15 °C. The powder was suspended in distilled water (1 g/10 ml) for 1 h and centrifuged at 10,000 *g* for 10 min (0–5 °C) and the clear supernatant was used as the enzyme source. Intestinal and pancreatic enzyme assays were carried out at 37 °C in a metabolic shaker for 1 h and 30 min respectively. Reactions were terminated by adding 5 ml ethanol to the system and total and free cholesterol determined before and after the incubation period[8]. Protein was determined by the method of Gornall et al.[9].

Results and discussion. It is clear (Table I) that the process of esterification in intestines is much greater in the presence of unsaturated fatty acids than of saturated ones. The enzyme activity is the same when incubated

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Table II. Cholesterol esterification activity in rat pancreas

Group	Palmitic	Stearic	Oleic	Linoleic	Arachidonic
10% Safflower oil	35.2	38.0	980.0	773.0	575.0
20% Safflower oil	41.2	45.0	1156.0	820.0	597.0
30% Safflower oil	44.6	46.4	1158.0	924.0	758.0
50% Safflower oil	46.0	46.4	1094.0	864.0	652.0
10% Lard	48.2	52.0	1223.0	806.0	581.0
20% Lard	48.6	48.6	1318.0	924.0	724.0
30% Lard	50.0	50.0	1313.0	912.0	768.0
50% Lard	48.1	49.3	1106.0	877.0	697.0

The values are expressed as nmoles of cholesterol esterified/mg protein/h. The enzyme assay system is the same as in Table I, except that 10 mg of pancreatic powder corresponding to 3 mg protein was used as the enzyme source.

with palmitic or stearic acid. A progressive increase is noticed for unsaturated fatty acids; the more the double bonds, the greater the enzyme activity. There is a slight increase in the cholesterol esterification activities as the amount of dietary fat is increased from 10% to 30% in both oil groups. Exceptions are seen in the 20% fat-fed groups with respect to $C_{18:1}$. The activity found is slightly greater for lard groups than for the safflower oil groups when incubated with C_{16}, $C_{18:1}$, C_{18} and $C_{20:4}$. With $C_{18:2}$ a slight decrease is noticed in the lard groups compared to the safflower seed oil groups, except when the rats are on 10% fat. Since the esterification of cholesterol is higher with unsaturated fatty acids, and since, as is well known, the majority of the cholesterol is esterified during absorption, the present work supports the view that unsaturated fatty acids enhance cholesterol absorption. On the other hand, the results show little esterification activity when the intestinal enzymes are incubated with saturated fatty acids. The dietary fats do not seem to change the esterifying capacity of the intestines. This is in agreement with the findings of MURTHY et al.[10] who observed no change in the esterifying activity of the intestines as a result of change in dietary fat given at a level of 10%.

The pancreatic enzymes, like the intestinal ones, esterify the cholesterol preferentially with unsaturated fatty acids (Table II). The esterification is slightly greater with C_{18} than with C_{16} in all the groups of rats. Maximum activity is reached when the enzymes are incubated with $C_{18:1}$. This is in contrast to the enzyme specificity seen in the intestines. This specificity for fatty acids confirms the concept that the pancreatic cholesterol esterifying enzymes are different from the intestinal ones. In pancreas also, a slight increase in the esterification activities is observed as the amount of the dietary fat increases from 10% to 30%. The cholesterol esterification activities of all fatty acids are greater in rats fed lard diets than safflower seed oil diets. This activity is significantly higher in the pancrease than in the intestines – 3.5 to 5 times with respect to C_{16} and C_{18}, 20 times with $C_{18:1}$ and 12–14 times with $C_{20:4}$.

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Lipolytic Response of 'Diabetic' Mice (*db/db*) to Isoproterenol and Propranolol in vivo

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Summary. The genetically diabetic and obese *db/db* mice responded lipolytically to isoproterenol and propranolol similarly to normal mice in vivo. However, considering the large amount of triglyceride in a *db/db* mouse, we conclude that the in vivo response of *db/db* adipose tissue is deficient in magnitude.

Many in vitro studies have shown that the adipose tissue of genetic obese mice (*ob/ob*, *db/db*, A^{vy}/a, A^{y}/a) does not respond to lipolytic agents, especially catecholamines, so well as that of normal mice[1–3]. The major implication of these observations is that the obesity of these mice is caused by a defective lipolytic mechanism. However, ABRAHAM et al.[4] found that *ob/ob* mice responded to catecholamines in vivo. In that study, the *ob/ob* mice were of a strain background[5] different from that of C57BL/6J on which most of the in vitro studies were based. The phenotypic expressions of the *obese* gene and the *diabetes* gene are affected by the strain genome[6–8]. It is therefore difficult to compare the results of ABRAHAM et al.[4] with the results obtained in vitro because of the difference in the strain background. In this study, we investigated the in vivo response of *db/db* mice to isoproterenol. The mice we used were of the same strain background as those we used in previous in vitro studies[1] so that unequivocal comparisons could be made between the in vivo and the in vitro data.

Materials and methods. Genetic diabetic and corpulent *db/db* mice and lean mice (db^{+}/db^{+} normals) of the C57BL/KsJ strain were obtained from the Jackson Laboratory, Bar Harbor, Maine. The animals used were 2 to 4 months of age. They were housed in groups of 3 in filter-capped, transparent plastic breeding cages maintained at about 25 °C with lights on from 06.00 h to 18.00 h. The mice were allowed at least 1 week to become acclimated to our animal room. Purina Laboratory Chow and water were available ad libitum.

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