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Studies on Carbohydrate Metabolism in Hypercholesteremic Rhesus Monkeys.* (27457)

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We have reported(1), increased plasma non-esterified fatty acids (NEFA) levels in rhesus monkeys in which hypercholesteremia was produced by feeding cholesterol along with different edible oils. Dole *et al.*(2) suggested that changes in blood NEFA concentration might provide a useful index of alterations in carbohydrate metabolism, as suppression of glucose utilization by adipose tissue was followed by a brisk release of NEFA into the blood and diminution of NEFA when glucose utilization was stimulated. Waddell *et al.*(3) observed diminished glucose tolerance in patients with hypercholesteremia although the fasting blood sugar was normal. A fall in plasma NEFA concentration in normal subjects after an injection of insulin(4) and a progressive rise in NEFA values in diabetic patients(5) suggested a possible relationship between plasma insulin and NEFA concentration. The mobilization of NEFA from adipose tissue by epinephrine was found by Shafrir and Steinberg(6) to be dependent on intact adrenals. It was, therefore, of interest to study the adrenal cortical and plasma insulin-like activities in hypercholesteremic rhesus monkeys and the relation between blood glucose and plasma NEFA of these animals after an oral dose of glucose. The results are presented here.

Experimental. Ten rhesus monkeys were fed a basal diet(1) for a week. They were fasted overnight and a fasting blood sample was collected next morning. Plasma insulin-like activity was determined in these samples using rat diaphragm as described by Willebrands *et al.*(7). 0.5 ml of plasma was used for the determination. The results have been expressed in terms of glucose uptake in mg by rat diaphragm per g of dry tissue per 90 min. incubation per ml of plasma and not in absolute insulin units.

After the above studies, the animals were divided into 5 groups and fed basal diet only; basal diet with 3 g cholesterol mixed with the diet; 3 g cholesterol and 30 g mustard oil mixed with the basal diet; 3 g cholesterol and 30 g coconut oil mixed with the diet and 3 g cholesterol and 30 g sesame oil mixed with the diet as described previously(1). The diets were fed for 8 months. The animals were fasted over night and after a fasting venous blood was collected, each animal was fed 50% glucose, 4 ml/kg. Samples of blood were withdrawn at intervals of 30 minutes for 2 hours. Blood glucose(8) and plasma NEFA were estimated(9) in the different blood samples. Plasma total cholesterol(10) and plasma insulin-like activity(7) were determined in the fasting blood samples.

The animals were then placed in metabolism cages for collection of urine. Twenty-four hour urine samples were collected for consecutive 3 days without addition of any preservative. An aliquot of urine was hydrolyzed with hydrochloric acid, extracted with carbon tetrachloride and 17-ketosteroid estimated by the Zimmermann reaction as modified by Callow *et al.*(11). The average of 3 days' excretion is given in Table I.

Results. The results are given in Fig. 1 and 2 and in Table I. *Glucose tolerance test.* All the animals had normal fasting blood sugar values. Blood sugar of monkeys fed

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TABLE I. Plasma Cholesterol, Plasma Insulin and Urinary 17-Ketosteroids of Rhesus Monkeys on Different Diets, Fed for 8 Months.

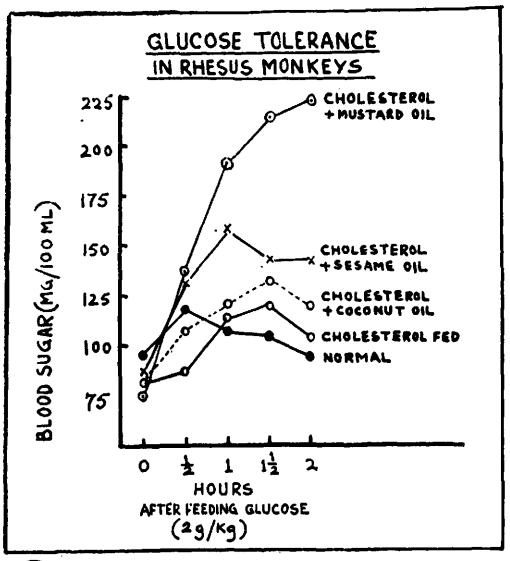
Monkey No.	Treatment	Total plasma cholesterol (mg/100 ml)	Insulin-like activity* Initial	Insulin-like activity* After 8 mo	24-hr urinary 17-ketosteroids (mg) †
20	Basal diet only	138	—	12.6 (2)	.770
21	*Idem*	105	—	13.3 (2)	.709
3	Basal + cholesterol, 3 g/day	114	4.6	8.6	1.907
5	*Idem*	118	8.1	12.3	2.613
6	Basal + cholesterol + mustard oil	210	12.0	7.6	1.322
7	*Idem*	248	13.2	5.1	1.322
1	Basal + cholesterol + coconut oil	242	5.8	no uptake (10)	1.593
4	*Idem*	198	4.3	*idem*	1.206
13	Basal + cholesterol + sesame oil	636	11.8	"	1.064
14	*Idem*	554	6.2	"	1.370

* Insulin-like activity expressed as mg glucose uptake by rat diaphragm incubated with 1 ml of plasma/g dry wt/90 min. incubation. Figures in parentheses indicate No. of determinations carried out.

† Avg of 3 days' excretion.

cholesterol along with the different oils did not come down to the fasting level at 2 hours after the feeding of glucose and maximum rise was in the monkeys fed mustard oil. Only a slight rise in blood sugar at this time could be seen in animals fed cholesterol without the supplement of oils (Fig. 1). *Plasma NEFA values.* The mean fasting plasma NEFA levels of monkeys fed cholesterol along with the oils were slightly higher than the normally fed animals. The monkeys fed only cholesterol had fasting NEFA values similar to the normal controls. Despite some fluctuations, mean plasma NEFA concentrations at 2 hours after feeding of glucose were within the normal range in the monkeys fed the basal diet only or the basal diet and cholesterol without any oil supplement. Plasma NEFA values after 2 hours of feeding glucose were well above the fasting levels in animals receiving mustard oil and coconut oil along with cholesterol. Plasma NEFA values could not be estimated in the animals fed sesame oil after glucose (Fig. 2). *Plasma insulin-like activities.* The plasma insulin-like activity in monkeys fed cholesterol without any of the oils for 8 months did not change from the initial activity. There was no glucose uptake by rat diaphragm incubated with plasma from monkeys fed sesame oil and coconut oil along with cholesterol for 8 months. Monkeys fed mustard oil with cholesterol showed diminished plasma insulin-like activity as compared to the initial activity. *Plasma cholesterol.* Hypercholesteremia was marked in monkeys fed cholesterol with the different oils. The animals fed basal diet and cholesterol, without any oil supplement, did not show any change in plasma cholesterol. *Urinary 17-ketosteroids.* Twenty-four hour urinary excretion of 17-ketosteroids by the experimental monkeys fed cholesterol, with or without the different oils, increased considerably as compared to the excretion by animals fed only the basal diet (Table I).

Discussion. It is evident that monkeys fed cholesterol along with the different oils showed diminished utilization of glucose. These experimental monkeys also showed hypercholesteremia. The increased plasma NEFA values after glucose administration in these animals, therefore, might be due to defective utilization of glucose, or impaired response of adipose tissue to glucose loads. The monkeys fed cholesterol only did not show hypercholesteremia. They also showed normal glucose tolerance and had normal plasma NEFA values. Hypercholesteremia *per se,* therefore, interferes with normal carbohydrate utilization. All the hypercholesteremic monkeys showed diminished plasma insulin-like activity. It might be possible that this insufficiency was responsible for the defective utilization of glucose which consequently resulted in increased production of plasma NEFA.



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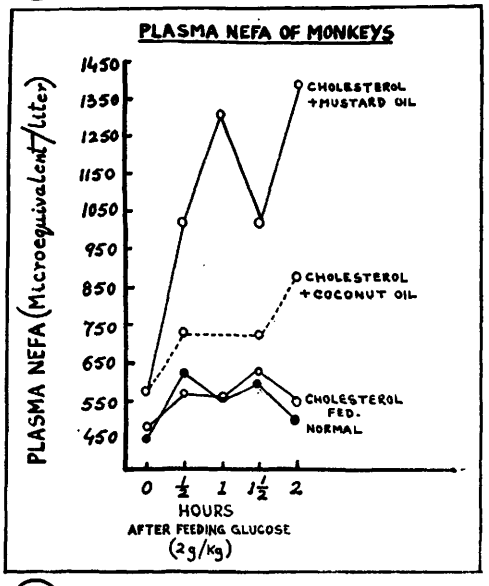

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FIG. 1. Oral glucose tolerance test in rhesus monkeys on different diets. Dose of glucose: 2 g/kg body wt.

FIG. 2. Plasma nonesterified fatty acids (NEFA) of monkeys after glucose feeding, 2 g/kg body wt.

All the monkeys fed cholesterol, with or without the different oils, had hyperactive adrenal cortex as evidenced by increased urinary excretion of 17-ketosteroids. Cholesterol of the diet might have been utilized for the increased synthesis of steroids by the adrenal cortex. Utilization of glucose is defective in hyperactivity of the adrenal cortex. It seems probable that the diminished glucose utilization in hypercholesteremia is the combined effect of insulin lack and hyperfunction of the adrenal cortex. Although the monkeys fed cholesterol without the oils showed hyperfunction of the adrenal cortex, they could utilize glucose like normal animals. This is possibly due to normal plasma insulin in these animals which antagonize the adrenal cortical steroid action on carbohydrate metabolism. Glucose utilization in these animals being normal, the NEFA values of plasma did not increase.

Summary. Hypercholesteremia in rhesus monkeys was associated with defective utilization of glucose as evidenced by diminished glucose tolerance and increased plasma NEFA values. Hypercholesteremic rhesus monkeys excreted increased amounts of 17-ketosteroids and had diminished plasma insulin-like activity. The impaired carbohydrate metabolism of these animals was possibly due to combined effects of insulin insufficiency and hyperfunction of the adrenal cortex.

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