Summary. Rabbits fed *ad lib.* on a high cholesterol diet were exercised 8 hours daily for 2 months. This regimen did not inhibit atherogenesis as measured by serum and aortic tissue cholesterol levels.

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Hormonal Effects on C-14 Acetate Metabolism in the Human.† (24587)

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An important and intriguing segment of intermediary metabolism is concerned with reactions, both synthetic and degradative, involving the common 2 carbon fragment of metabolism, "acetate." Many of these reactions can be studied in a general way in relatively undisturbed human subjects with the aid of carbon-14 labeled compounds. We studied the effect of glucocorticoid administration and diabetes on certain of the fates of 1 and 2-C^{14} labeled acetate in the human. Though some results are preliminary in nature, we believe them of sufficient interest, to report at this time.

Procedure. Well controlled diabetics served as subjects to determine the effect of 3 types of diabetes on synthesis of various lipid fractions from 1- and 2-C^{14} acetate. In an attempt to prevent a compensatory secretion of insulin which might obscure the glucocorticoid effect, the same diabetics were also used as subjects for studying the effect of glucocorticoids on utilization of C^{14} acetate. These subjects were hospitalized and were on a constant intake of carbohydrate, protein and fat. Their weights were stable for several weeks prior to each experiment. Their usual dose of long acting insulin was given 24 hours prior to the experiment and (except for the stable adult diabetic (D.M.) following steroid administration), each subject had a normal fasting blood sugar and was non-ketotic at the start of each experimental procedure. Thirty mg of prednisone (delta-1-cortisone) was given orally 9 and 3 hours prior to several experiments, to study glucocorticoid effect on utilization of acetate. No food was allowed on day of experiment until 8 hours from start of procedure, at which time the usual evening meal was given. Interval between experiments in the same subject was 1-3 months. Breath samples for respiratory $C^{14}O_2$ excretion, blood samples for pyruvic and alpha ketoglutaric acids, lipids, ketones, and glucose were collected at various intervals after administration of 40-100 microcuries of 1 or 2-C^{14} acetate intravenously. Respiratory CO_2 was trapped by the method of Baker, Shreeve *et al.*(1). Specific activity of respiratory CO_2 was determined by the method of Van Slyke *et al.*(2). Specific activity of pyruvic and alpha ketoglutaric acids was determined as follows: Whole blood keto acids were determined by paper chromatographic method of Seligson and Shapiro(3). Pyruvic and alpha keto glutaric acids were determined quantitatively. A portion of the eluate from the paper strip was transferred to Van Slyke combustion

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tube and dried. Radioactivity was then determined by wet carbon combustion and determination of radioactivity of the evolved CO_2 by the method of Van Slyke *et al.*(2). Lipid samples were chromatographed on silicic acid columns by a modification of the method of Fillerup and Mead(4). Triglycerides were determined colorimetrically on an eluate from the column, by the method of Stern and Shapiro(5). Cholesterol was determined quantitatively on an eluate from the column by the method of Carr and Drekter(6). After saponification, extraction, and washing, another portion of the eluate from each major lipid fraction studied was mixed with 15 ml of 2-phenyl-5 (4 biphenyl) 1,3,4,-oxiadiazole 0.6% (PBD) in toluene and radioactivity was determined in a Packard Tri-Carb liquid Scintillation counter. All results have been corrected to the same amount of administered radioactivity/kg body weight.

Results. In each of 4 studies, administration of prednisone has been associated with a 10-15% decrease in 24 hour cumulative excretion of radioactivity in respiratory CO_2 as compared to the control experiment in the same subject(Fig. 1). The most striking changes are seen in early breath samples which show as much as 20-30% decrease in specific activity as compared to the control experiment. Administration of prednisone has been associated consistently in these experiments with decrease in peak specific activity of CO_2, a late peak in activity and a decrease in cumulative excretion of C-14 in respiratory CO_2. Logarithmic decline of specific activity is essentially parallel in the 2 series of experiments. These findings suggest that the effect of adrenal steroids in decreasing respiratory $C^{14}O_2$ following administration of C^{14} acetate is not due to enlargement of body CO_2 pool, since the straight line logarithmic fall off in specific activity is thought to be a measure of this pool. Rather, these findings suggest that glucocorticoids may exert some effect on initial disposition of acetate which decreases its early oxidation to CO_2.

We also compared respiratory $C^{14}O_2$ following administration of carboxyl and methyl labeled acetate to the same subject. Previous *in vitro* experiments with plant seeds and animal tissues and *in vivo* experiments with lactating cows have shown that carboxyl carbon of acetate is more rapidly oxidized to CO_2 than is methyl carbon and total CO_2 production from carboxyl carbon is much greater. This difference can be readily demonstrated in the human (Fig. 2). As pointed out by Weinman, Strisower, and Chaikoff(7), this phenomenon can be partially explained on the basis of known reactions of the Krebs Cycle. The carboxyl carbon of acetate is oxidized in one turn of the cycle while methyl carbon is not. However, since oxidation of acetate occurs almost explosively and radioactivity is rapidly randomized in the Krebs cycle acids, oxidation of methyl carbon should speedily approach that of the carboxyl carbon in an isolated Krebs cycle. Since production of respiratory $C^{14}O_2$ from methyl labeled acetate does not approach that from carboxyl labeled acetate during the 24 hour period of study, it can probably be assumed that Krebs cycle acids readily and rapidly enter other compounds which are less rapidly oxidized. Perhaps due to its longer stay in the Krebs cycle acids, the methyl carbon of acetate enters these compounds more extensively than does the carboxyl carbon. Regulation of amounts of Krebs cycle substrates going to such pools may be an important means of regulating energy production in the cell.

As perhaps another measure of cellular oxidative processes, we determined the specific activity of whole blood pyruvic and alpha ketoglutaric acids following administration of C^{14} acetate. In almost all of approximately 60 samples analyzed specific activity of alpha ketoglutarate has been higher than that of pyruvate, undoubtedly reflecting the dilution of radioactive pyruvate, derived probably from previously intra-mitochondrial oxaloacetate, with non-radioactive pyruvate resulting from glycolysis.

Incorporation of 2-C^{14} acetate into free cholesterol, ester cholesterol and triglyceride fatty acid was studied in a young unstable and in an adult stable diabetic before and after administration of prednisone. Though specific activity of the earliest triglyceride samples was increased in both subjects following steroid administration, there was an asso-

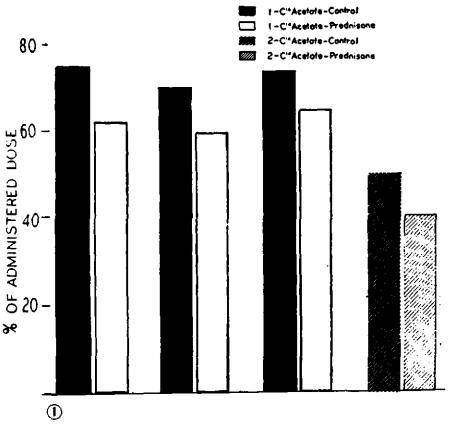
ciated decrease in absolute levels of plasma triglycerides, so that total incorporation of radioactivity into plasma triglycerides was actually diminished by 25-50%. These preliminary findings suggest that adrenal 17-hydroxycorticoids in high dosage may decrease mobilization of fat from the depots.

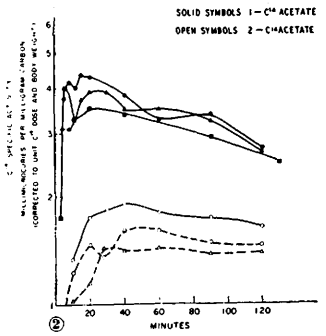
No striking differences in specific activity of early samples of plasma free cholesterol were noted after acute administration of prednisone. There is a suggestion of a later decrease in specific activity following prednisone. These studies do not show the striking increase in cholesterol specific activity noted by Gould *et al.*(8), following prolonged administration of cortisone to chronically ill patients. It seems likely, therefore, that an increased synthesis of cholesterol under the influence of adrenal steroids depends on secondary metabolic changes and is not a primary effect of administration of adrenal 17 hydroxycorticoids.

A comparison was also made between specific activity of these lipid fractions following administration of 1- and 2-C^{14} acetate to the same subject in a comparable state of diabetic control. Since much more C^{14} was present in expired CO_2 following administration of carboxyl labeled than methyl labeled acetate, we expected to find greater radioactivity in lipids synthesized from methyl labeled than from the carboxyl labeled compound. This was not the case. Actually, the specific activity of triglycerides and cholesterol was approximately twice as great following administration of carboxyl labeled acetate, despite the fact that much less radioactive acetate should have been available for synthetic purposes as indicated by respiratory C^{14} O_2. Feller has found in *in vitro* studies that specific activity of the fatty acids of rat adipose tissue is approximately 2 times as great following administration of carboxyl labeled acetate as it is following administration of the methyl labeled compound(9).

TABLE I. Peak Specific Activity of Plasma Lipids following I. V. 2-C^{14} Acetate.

	DPM/mg	
	Triglyceride fatty acids	Free cholesterol
Lipoatrophic diabetic	271	440
Young unstable "	235	242
Middle aged control	110	111
Stable adult diabetic	44	78





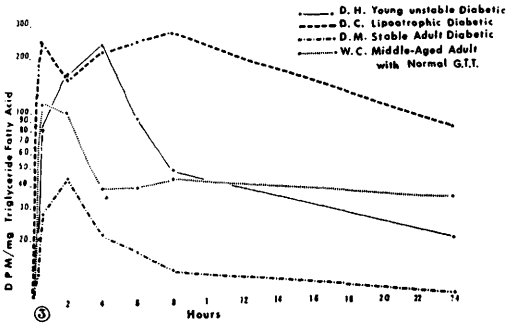


FIG. 1. Effect of prednisone on 24-hr cumulative excretion of $C^{14}O_2$ from C^{14} acetate.

FIG. 2. Comparison of specific activity of respiratory CO_2 following administration of 1- and 2-C^{14} acetate to same subject.

FIG. 3. Specific activity of plasma triglyceride fatty acids following administration of I.V. 2-C^{14} acetate.

We studied lipid synthesis from 2-C^{14} acetate in each of Lawrence's 3 types of diabetics (10) (Table I). Because 2-C^{14} acetate was administered intravenously in these studies,

whereas previous studies of triglyceride synthesis in non-diabetics by Lipsky *et al.*(11) have been done following oral administration of 1-C^{14} acetate, only a very general comparison of these studies with those of Lipsky can be made.

In a well controlled young unstable diabetic (D. H., age 27) triglyceride synthesis is quite rapid with a peak approximately 1/6-1/2 that found by Lipsky. The general shape of the curve of rise and decline in specific activity is similar to that found by Lipsky (Fig. 3). In a 14-year-old girl with classical lipoatrophic diabetes† (12) peak specific activity is reached more rapidly, but is about the same level as found in the young unstable diabetic. However, a high level of activity in triglycerides persists longer than normal. Thus, the striking loss of adipose tissue in this patient does not seem to be due to an increased utilization of fat, since a more rapid disappearance of radioactivity than normal should have been seen if this were the case. The persistence of high specific activity in plasma triglycerides may be due both to less dilution with unlabeled fat from the adipose tissue and to less deposition of labeled fat in adipose tissue. It seems likely that individuals with this condition are unable to store and perhaps to synthesize fat in the adipose tissue. The findings in this patient seem quite compatible with Lawrence's suggestion that the metabolic defect in this syndrome is primarily in the adipose tissue and that "diabetes" in these patients may be a symptom of inability of the adipose tissue to use carbohydrate for synthesizing fat. Oxidation of 2-C^{14} acetate was not grossly different in pattern from that in other diabetics.

The stable adult diabetic shows a surprisingly low level of radioactivity in plasma triglyceride fatty acids. Peak specific activity is much lower than in either of the 2 young diabetics and is very much lower than has been noted in individuals without diabetes(11).

Because it seemed possible that the striking decrease in specific activity of plasma triglycerides found in the stable adult diabetic might be an effect of age, rather than of diabetes, a similar study was done in an adult of same age and body build, but with normal glucose tolerance. This subject showed much less active triglyceride synthesis than did the young diabetics. However, specific activity of plasma triglycerides was approximately 2½ times greater than in the stable adult diabetic. It will be of extreme importance in understanding diabetes to determine whether a decrease in ability to synthesize fatty acids from acetate is a consistent defect in the stable adult diabetic with normal fasting blood sugar as compared to younger individuals and to subjects in the same age group. It is interesting that Shreeve *et al.*(13) have found no decrease in the portion of C^{14} glucose oxidized to CO_2 in the stable adult diabetic. It is tempting to speculate, as Lawrence already has, that the defect in glucose utilization in the obese adult diabetic may be one in conversion of carbohydrate to fat. However, much work will be required before this postulate can be confirmed or denied.

The synthesis of plasma free cholesterol was also studied in these same patients (Table I). The young unstable diabetic under good control attains a peak specific activity quite comparable to the average found by Gould *et al.* with 1-C^{14} acetate(8). Peak specific activity in the young girl with lipoatrophic diabetes is approximately twice as great. The middle aged adult with normal glucose tolerance attains a peak specific activity which is just at the lower limits of Gould's range. The stable adult diabetic shows an even lower peak specific activity, being greater than 2 S.D. from Gould's average value. However, because of limitation of dose of C^{14} in normal individuals of all ages, and particularly in young individuals, it may be many years before normal ranges of lipid synthesis from 1- and 2-C^{14} acetate in various age groups can be established with certainty.

Incorporation of radioactivity into ester cholesterol lags behind that of free cholesterol since it is formed from it. The differences in radioactivity in ester cholesterol between these patients merely reflect and confirm the previously noted differences in plasma free cholesterol.

† Studied with help of Robert W. Schwartz, Boston Children's Hosp.

Summary. These data suggest the following tentative conclusions: 1. Adrenal 17-hydroxycorticoids appear to exert an effect on utilization of acetate and to decrease oxidation of this 2 carbon fragment to CO_2. 2. In the human as in tissues of lower animals and in plant seeds, carboxyl carbon of acetate is more readily oxidized than is methyl carbon. The finding of a decrease in lipid synthesis form methyl labeled acetate in association with a decrease in oxidation of this compound as compared to carboxyl labeled acetate suggests that the carboxyl carbon may also be used preferentially for lipid synthesis in the human. 3. The syndrome of lipoatrophic diabetes appears to be associated with a decreased ability to store and perhaps to synthesize lipids in the adipose tissue rather than an increase in utilization of lipids of adipose tissue. 4. The stable adult diabetic may have a defect in conversion of 2 carbon fragments to fatty acids, even when the fasting blood sugar is normal.

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Effect of Dietary Sucrose and Glucose on Plasma Cholesterol in Chicks and Rabbits.* (24588)

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The influence of certain components of diet on level of cholesterol in blood has been the subject of much investigation and speculation. That type of protein used may alter cholesterol level was indicated as early as 1940(1), and the association of different types of fat with various serum cholesterol values has also been described(2). In many such studies the effect of carbohydrate has not been considered. However, it has recently been reported (3) that cholesterol-fed rats developed significantly higher serum cholesterol levels when sucrose was substituted for starch in the diet. A similar but less pronounced effect was noticed when sucrose replaced glucose. The purpose of this study was to investigate the difference between glucose and sucrose in regulation of plasma cholesterol in chicks and rabbits.

Methods. Day-old single-comb White Legborn male chicks‡ were given water and purified basal diets (Table I) *ad lib.* with appropriate supplementation for 28 days. At end of this period blood was removed by heart

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