Serum Lipoid Level in Diabetic Children

By

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The view according to which transportation is the only function of plasma lipoids has considerably been modified in recent years [3, 9]. It is with proteins, their special vehicles, that lipoids are transported; these proteins have well defined structures, as shown by the sequence of their amino acids, among others. The lipoid part is composed of free cholesterol, cholesterol ester, phospholipids, triglyceride and non-esterified fatty acid. Ultracentrifugation yields fractions of high (S_{0-4}, S_{4-20}), and of low ($S_{f\,0-12}$, $S_{f\,12-24}$, $S_{f\,20-400}$) density, further chylomicra [2]. A decrease in specific weight goes parallel with an increase in the proportion of triglyceride and a decrease in that of proteins and phospholipids. The fact the turnover rate of the various lipoprotein fractions is different in the course of metabolism shows that it is justified to regard lipoproteins as both functional and transport substances.

It follows that the quantitative estimation of a single lipoid component such as cholesterol or triglyceride does not yield information about changes in lipoid metabolism, a fact that considerably affects the diagnostic value of quantitative serum lipoid determinations in pathologic conditions.

The most important biochemical defect of diabetes mellitus is known to be connected with the transport and utilization of lipoids and glucose. Reports on the changes of the serum lipoid components in diabetics are contradictory. While JOSLIN *et al.* [7] registered high triglyceride concentrations in 34 cases of grave diabetes, DAVIDSON and KAYE [1] found in juvenile diabetics normal total lipoid, total esterified fatty acid and triglyceride concentrations with a high cholesterol level. Such contradictions will be better understood if analysed against the background of changes occurring in the lipoprotein fractions. $S_{f\,12-400}$, the lipoprotein fraction with the lowest specific density increases significantly in diabetes, whereas the fraction $S_{f\,0-12}$ undergoes no change; administration of insulin normalizes the low specific density fraction.

The present study had the purpose to observe correlations between the quantitative changes of the various serum lipoids in diabetic children,

TABLE I

Total, esterified, and free cholesterol as well as lipid phosphorus and triglyceride levels in the plasma of fasting normal and diabetic children

	Cholesterol, mg/100 ml			Lipid phosphorus, mg/100 ml	Triglyceride, mEq/100 ml
	Total	Ester	Free		
Diabetic					
mean	245.3	189.3	65.4	215.7	0.61
S. E.	±3.6	±5.6	±2.1	±8.8	±0.15
n	(81)	(78)	(69)	(78)	(69)
Control					
mean	180.3	125.9	55.2	194.7	0.59
S. E.	±1.3	±2.5	±5.6	±7.8	±0.06
n	(47)	(47)	(47)	(36)	(35)
Ratio Diabetic / Control	1.3	1.3	1.2	1.1	1.0

further, to establish whether the plasma triglyceride, cholesterol ester and phospholipids could be regarded as indicators of chylomicra and of the lipoprotein fractions of low and high density.

MATERIAL AND METHOD

The material of this study consisted of 20 diabetic children aged between 4 and 15 years; they were partly in and partly outpatients and were clinically well controlled. Each patient was repeatedly examined. In two cases the diabetes had developed shortly before admission. Fasting blood samples were taken before insulin administration. JEZERNICZKY's method [6] was used for the determination of total esterified cholesterol, a modified version of McDONALD and HALL's procedure [9] for that of phospholipids, and that of VERHEYDEN and NYS [12] for plasma total esterified fatty acid. Although we determined practically the total lipoid-ester bonds by the last named method, it is possible to compute gravimetrically that amount of fatty acid which is bound to phospholipids and cholesterol. By deducting this value from that of the total esterified fatty acid we obtain the amount of triglyceride, provided the amount of mono- and diglyceride is negligible. The following formula was used for this computation. Triglyceride (mEq/l) = TEFA

$$(mEq/l) - \frac{P}{39.65} + \frac{CH}{38.6}$$

where TEFA = total esterified fatty acid

P = inorganic phosphorus in phospholipid (mg/litre)

CH = cholesterol (mg/litre)

$\dfrac{P}{39.65}$ = fatty acid contained in phospholipid (mEq/litre)

$\dfrac{CH}{38.6}$ = fatty acid contained in cholesterol ester (mEq/litre).

Blood sugar was determined from capillary blood by the Hagedorn—Jensen method. Statistical significance was computed by Student's t method.

RESULTS

Values for lipoid in the blood of controlled diabetics and healthy controls of the corresponding age groups are listed in Table I. It was the cholesterol level (and, within it, that of cholesterol ester) which showed the most striking increase in diabetic children. Triglyceride concentration was practically unchanged, although the scattering was wide.

Depending on the insulin dose and the condition of the patients, the fasting blood sugar varied from 150 to 450 mg per 100 ml. Since the variations covered a wide range, and a large number of determinations was performed, it was possible to subject to statistical analysis the correlation between the fasting blood sugar level and that of the serum total lipoids. Increase in the blood sugar level and in that of the total lipoids showed a parallel course (Fig. 1). This correlation was, however, not significant, as shown by the wide scattering of the regression coefficient. There was, on the other hand, a statistically signif-
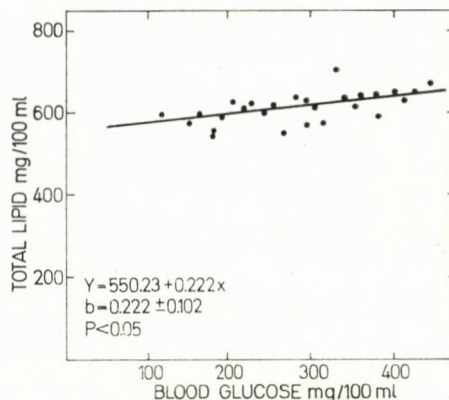

Fig. 1. Serum total lipoid level in diabetic children, in the function of the fasting blood sugar level

icant correlation between the changes in the fasting blood sugar level and those in the concentration of free cholesterol (Fig. 2). This was in contradiction to the assumption that an increase in total lipid concentration is chiefly due to the increased level of cholesterol ester. There seemed, on the other hand, no regular correlation to exist between the blood sugar and cholesterol ester levels or between those of blood sugar and phospholipids (Figs. 3, 4). It may be that the
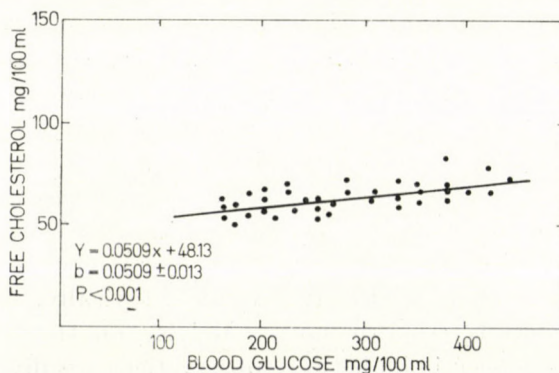

FIG. 2. Serum free cholesterol level in diabetic children, in relation to the blood sugar level
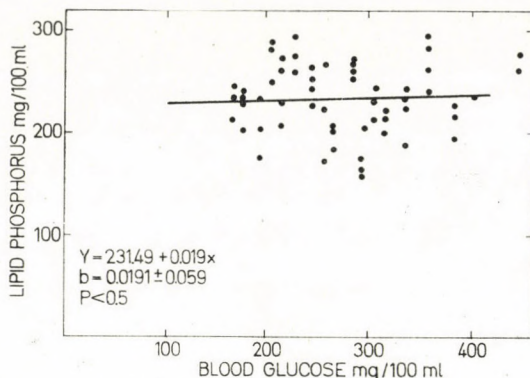
FIG. 3. Serum phospholipid and fasting blood sugar levels in diabetic children
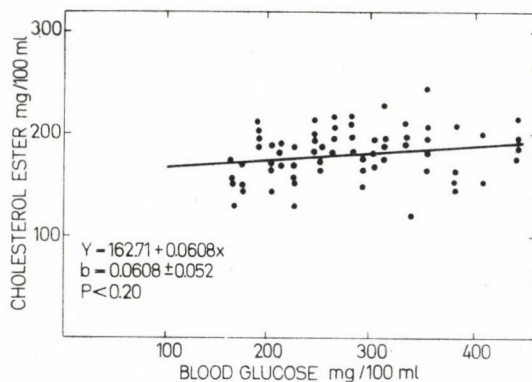

FIG. 4. Serum cholesterol ester and fasting blood sugar levels in diabetic children

increased cholesterol production of insulin-treated children is accompanied by a change in the interproportion of cholesterol ester and free cholesterol. This possibility seems to be supported by Fig. 5a—b where the cholesterol ester and free cholesterol values are shown as functions of the total cholesterol concentration. The regression line of cholesterol ester and that of free cholesterol ran practically parallel in the controls, whereas no such parallelism was seen in diabetics in whom the increase in the total cholesterol level was accompanied by a lesser elevation of the free cholesterol level than of that of cholesterol ester. Comparison of the regression coefficients showed that, as regards cholesterol ester, there was no significant difference between controls and diabetics, whereas the difference between the regression coefficients of total and free cholesterol was significant statistically.

Relying on the evidence of these observations we divided the diabetic children into three groups according
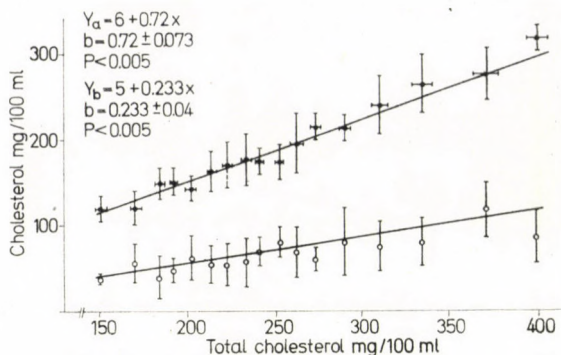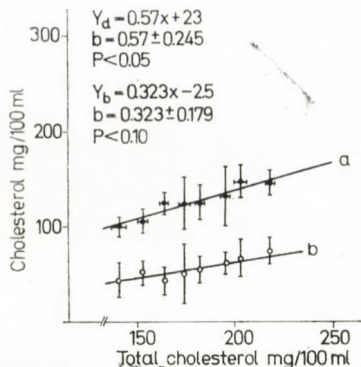
The first graph (left) contains:

$Y_d = 0.57x + 23$
$b = 0.57 \pm 0.245$
$P < 0.05$

$Y_b = 0.323x - 2.5$
$b = 0.323 \pm 0.179$
$P < 0.10$

Cholesterol mg/100 ml (y-axis), values 100, 200, 300; curves labelled a and b.

Total cholesterol mg/100 ml (x-axis), values 150, 200, 250.

The second graph (right) contains:

$Y_a = 6 + 0.72x$
$b = 0.72 \pm 0.073$
$P < 0.005$

$Y_b = 5 + 0.233x$
$b = 0.233 \pm 0.04$
$P < 0.005$

Cholesterol mg/100 ml (y-axis), values 100, 200, 300.

Total cholesterol mg/100 ml (x-axis), values 150, 200, 250, 300, 350, 400.

FIG. 5a. Serum total cholesterol and cholesterol ester (black circles) and free cholesterol (empty circles) levels in control children. Y_a = cholesterol ester, Y_b = free cholesterol

FIG. 5b. Serum total cholesterol and cholesterol ester (black circles) and free cholester[o] (empty circles) levels in diabetic children. Y_a = cholesterol ester, Y_b = free cholesterol

to their total cholesterol level. The first group consisted of patients with a total cholesterol concentration of 150 to 200 mg per 100 ml, the second of those with one of 200 to 250, the third of those with one of more than 250 mg per 100 ml. The controls were not divided into separate groups. This distribution of the patients left the possibility open for any given individual to belong to more than one group on the evidence of periodic examinations.

Next, we examined the controls and the three diabetic groups for the correlation between the respective serum triglyceride and cholesterol ester levels (Fig. 6). The serum triglyceride level was found to vary directly with the increase in the concentration of esterified cholesterol, a phenomenon observed in the controls and the diabetic groups alike. It is evident from Table II that any increase in the total cholesterol level went hand in hand with an increase in the choles

TABLE II

Fasting plasma cholesterol ester and triglyceride levels in control and in the three different groups of diabetic children

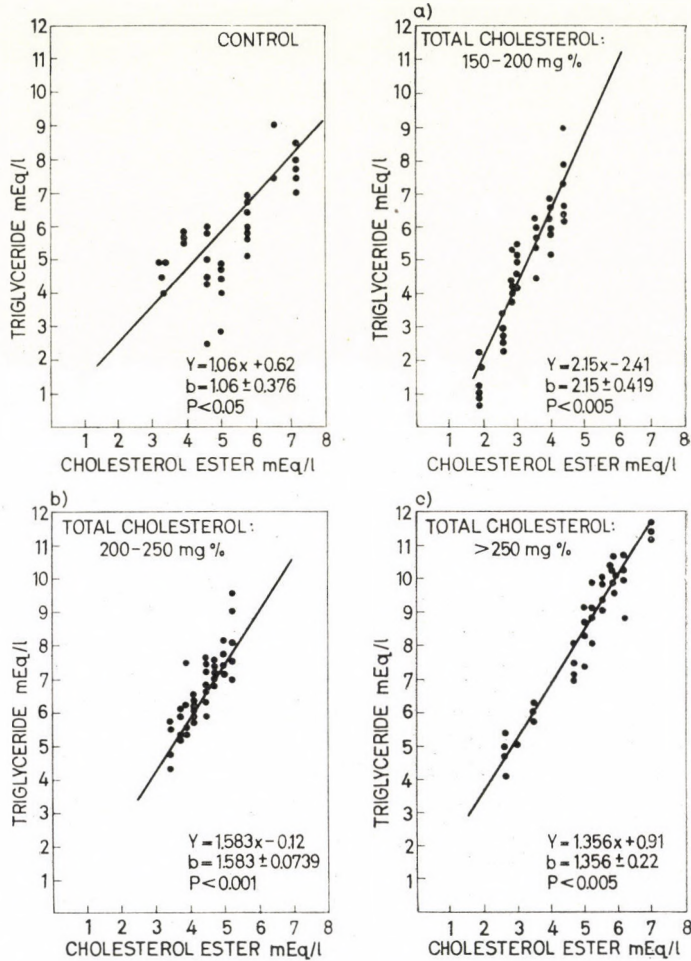|	Control	Group A	Group B	Group C
		total cholesterol, mg per 100 ml		
		150—200	200—250	250
Cholesterol ester mEq/l	4.55	4.65	5.29	6.29
Triglyceride mEq/l	5.88	4.52	6.67	8.08

FIG. 6. Serum triglyceride level in the function of the cholesterol ester level

terol ester and triglyceride levels. It is, therefore, clear that, as compared with the healthy controls, the correlation between triglyceride and cholesterol remains unchanged in the serum of well-controlled diabetic children.

The situation was different as regards the serum concentrations of cholesterol ester and phospholipids. Fig. 7 shows that these were inversely

related in the controls: an increase in the cholesterol ester level was associated with a decrease in the phospholipid level. The interproportion of these compounds showed a notable change in diabetics inasmuch as the increase of the cholesterol ester level was accompanied by an increase of the phospholipid level in the first diabetic group. This correlation was, however, not significant statistically,
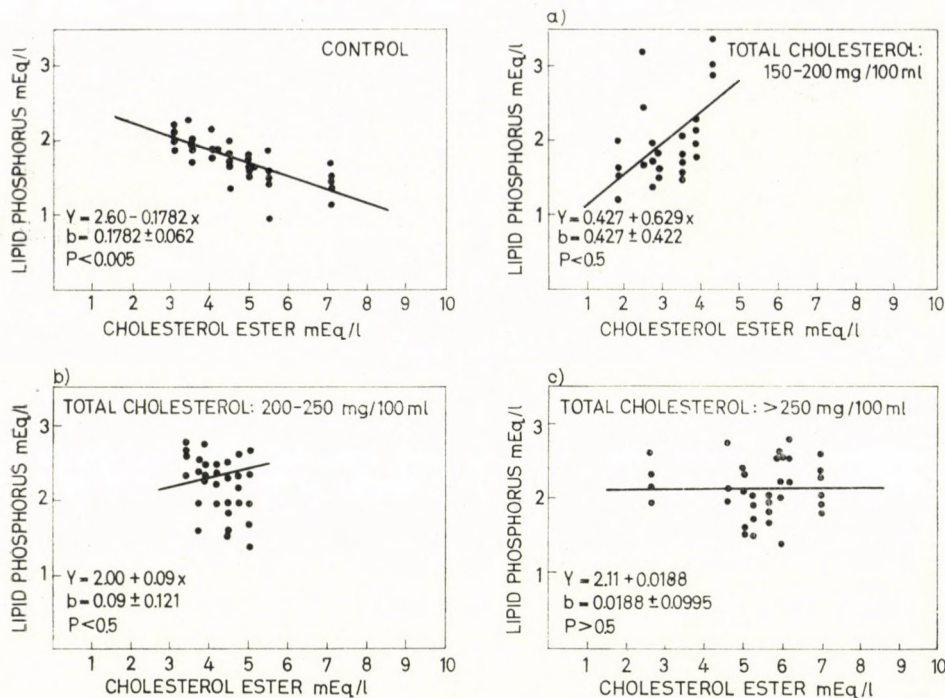
FIG. 7. Serum phospholipid and cholesterol ester levels in control and diabetic children

since the correlation became less pronounced with the elevation of the cholesterol ester level.

DISCUSSION

Earlier literary data have made it clear that the lipoprotein fraction $S_{f\ 20-40}$ increases in the blood of diabetics [11]. In human blood, the lipoproteins of low density constitute an intensively dynamic system whose principal function consists in the transportation of triglyceride between the liver and the fat stores. It is in the liver that triglyceride is linked with the lipoprotein fraction $S_{f\ 0-12}$ transforming it into lipoprotein $S_{f\ 20-400}$, i.e. a fraction rich in triglyceride is split off from the compound by lipoprotein lipase in the fat stores so that it changes back into $S_{f\ 0-12}$ [3].

Liberation and outflow of non-esterified fatty acid is considerably enhanced in diabetes [13, 14, 15]. The rate of liberation is a function of accelerated triglyceride lipolysis [3, 5]. This rate is considerably diminished by insulin and glucose; insulin promotes the utilization of glucose and impedes the liberation of non-esterified fatty acid. It is obvious that, in these circumstances, lipoids do not or do hardly pass into the adipose tissue of diabetics from the lipoprotein fraction $S_{f\ 20-400}$, a fact that in itself

suffices to explain the increased $S_{f\ 20-400}$ lipoprotein concentration in diabetic patients.

It is commonly known that lipoids are accumulating in the diabetic liver, a phenomenon due to the increased mobilization of non-esterified fatty acids in the fat depots. In the liver of fasting subjects and of diabetics, the major part of active acetate is provided by the breakdown of fatty acids. The access of active acetate to the citrate cycle is considerably diminished in the diabetic liver [4], while 50% more than the normal amount of hydroxymethyl-glutaryl coenzyme A is formed [16]. The significance of this coenzyme was pointed out by LYNEN et al. [8] who demonstrated that the acid arising from this metabolite under the effect of a specific reductase represents the precursor of cholesterol synthesis.

These data confirm our observations that in diabetic children the increased cholesterol concentration is a sign of an increased activity of the hydroxymethyl-glutaryl-CoA cycle. Since an increase of the esterified cholesterol level is accompanied by a relative decrease of the free cholesterol level (Fig. 5b), increase in the concentration of non-esterified fatty acids might enhance the esterification of cholesterol in the liver.

An increase in the concentration of serum cholesterol does not, in itself yield information about the manner in which the plasma lipoprotein fractions undergo changes in either controlled or non-controlled diabetes. In these fractions phospholipids are the main component of high-density lipoproteins, while triglyceride is the main component of the low-density fraction $S_{f\ 20-400}$. Cholesterol ester and free cholesterol occur in all lipoprotein fractions; they account for approximately 30% of the total lipoids in the high-density fractions as also in the fractions $S_{f\ 0-12}$ and $S_{f\ 12-20}$, while only 15% in the fraction $S_{f\ 20-400}$. Comparison between the serum cholesterol ester and triglyceride levels as well as between those of cholesterol ester and phospholipids in normal children are in harmony with this observation, since higher concentrations of cholesterol ester were always accompanied by higher triglyceride levels. The regression coefficient is but slightly significant in this connection; this might be due to that the proportion of triglyceride increases and that of cholesterol ester decreases with the diminution of specific weight. A high cholesterol ester level is accompanied by a low phospholipid level in normal children. This correlation is significant statistically and points to the fact that an increase in the low-density lipoprotein fraction is mainly responsible for the elevation of the cholesterol ester level and the simultaneous decrease in the proportion of the high-density phospholipid-rich lipoprotein fraction. There is a similar correlation between the serum cholesterol ester and triglyceride levels in diabetic children; the correlation is always strongly significant. There is, on the other hand, no correlation between the respective concentration of cholesterol ester and phospholipids.

Our observations are in harmony with the findings of other authors in that it is the lipoprotein fraction $S_{f\ 20-400}$ which increases in insulin-treated diabetic children. Our results have moreover shown that the composition of this fraction may change. Enhanced cholesterol synthesis is associated with an increase in the cholesterol contents of the lipoprotein fraction of the lowest density, a phenomenon confirmed by the figures in Table II.

It seems justified to suppose that a change occurs also in the composition of the lipoproteins of high density. The present investigations have moreover proved that the fasting blood sugar value alone affords no reliable information concerning the fat metabolism of children treated with insulin.

Summary

(1) The serum total lipid level is slightly increased in fasting insulin-treated diabetic children, a phenomenon for which cholesterol is responsible.

(2) There is a significant correlation between the free cholesterol and the fasting blood sugar levels, while the correlation between the fasting blood sugar, total lipoid, cholesterol ester and phospholipid levels is not significant statistically.

(3) An increase in the concentration of total cholesterol is asssociated with a parallel increase of the cholesterol ester level in both diabetic and healthy children, whereas the concentration of free cholesterol increases considerably less in diabetics. Enhanced esterification modifies the interproportion between esterified and non-esterified cholesterol in diabetic patients.

(4) The same linear correlation between the serum cholesterol ester and triglyceride levels exists in both healthy and diabetic children.

(5) The serum cholesterol ester and phospholipid levels showed no correlation in insulin-treated diabetic children, whereas the said levels were inversely related in the controls.

(6) It is suggested that, while the lipoprotein fraction of the lowest specific weight increases in the serum of diabetic children treated with insulin, the lipoid composition of the fraction is also changed. It is moreover supposed that the composition of high-density lipoproteins, too, undergoes changes.

References

1. Davidson, M. H., Kaye, R.: A study of blood glucose and ketone bodies and plasma-free fatty acids, cholesterol, P-lipids and total lipids in juvenile diabetes. J. Pediat. **66**, 313 (1965).
2. DeLalla, O., Elliot, H. A., Gofman, J. W.: Ultracentrifugal analysis of serum lipoproteins. Amer. J. Physiol. **179** 333 (1968).
3. Fredrickson, D. S., Gordon, R. S. Jr.: Transport of fatty acids. J. clin. Invest. **37**, 1504 (1958).
4. Frohman, C. E., Orten, J. M.: Tracer studies of the acids of the tricarboxylic

acid cycle. I. The fate of labeled acetate in the livers of normal and diabetic rats. J. biol. Chem. **216,** 795 (1955).

5. JEANRENAULD, B.: Dynamic aspects of adipose tissue metabolism. A review. Metabolism **10,** 535 (1961).

6. JEZERNICZKY, J.: Cholesterin és cholesterinester meghatározása kismennyiségű szérumban. Kísérl. Orvostud. **19,** 326 (1967).

7. JOSLIN, E. P., ROOT, H. F., WHITE, P., MARBLE, A.: The Treatment of Diabetes Mellitus. Lea and Febiger, Philadelphia 1958.

8. LYNEN, F., HENNING, U., BUBLITZ, C., SÖRBO, B., KROPLIN-RUEFF, L.: Der chemische Mechanismus der Acetessigsäurebildung in der Leber. Biochem. **330,** 269. (1958)

9. McDONALD, J. W., HALL, R. J.: The conversion of casein into microbial proteins in the rumen. Biochem. J. **67,** 400 (1957).

10. OLSON, R. E., VESTEY, J. W.: Nutrition endocrine interrelationships in the control of fat transport in man. Physiol. Rev. **40,** 677 (1960).

11. TULLER, E. F., MANN, G. V., SCHERTENLEIB, F., ROEHRIG, C. B., ROOT, H. F.: The effect of diabetic acidosis and coma upon the serum lipoproteins and cholesterol. Diabetes **3,** 279 (1954).

12. VERHEYDEN, J., NYS, J.: Estimation of fatty acid esters in serum. Clin. chim. Acta **7,** 262 (1962).

13. WENKOVA, J., PAV, J.: Release of non-esterified fatty acids from adipose tissue in normal and diabetic rats. Nature (Lond.) **184,** 1147 (1959).

14. WERTHEIMER, E., HAMOSH, M., SHAFRIR, E.: Factors affecting fat mobilization from adipose tissue. Amer. J. clin. Nutrit. **8,** 705 (1960).

15. WERTHEIMER, E., SHAFRIR, E.: Influence of hormones on adipose tissue as a center of fat metabolism. Recent Progr. Hormone Res. **16,** 196 (1961).

16. WIELAND, O., LÖFFLER, G., WEISS, L., NEUFELD, T.: Zur Acetessigsäure und Cholesterinbildung bei experimenteller Ketose. Biochem. Z. **333,** 10 (1960).

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