ORIGINAL CONTRIBUTIONS

Dietary Fats, the Diurnal Blood Lipids and Ketones in Juvenile Diabetes

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Summary. The fasting levels and diurnal variation in blood lipids were studied in about 150 children and adolescents of varying diabetic duration. The diabetics had cholesterol, phospholipid and triglyceride levels within the normal range but elevated free fatty acids, glycerol and ketone bodies. Postprandial determination does not seem to be any major advantage. The duration of diabetes was without significant influence. Diabetics receiving insulin twice daily almost normalize their FFA values and decrease their ketones in comparison with those taking insulin only once a day. An isocaloric, socially acceptable diet rich in polyunsaturated fat lowered blood lipid levels in a controlled trial over a period of eighteen months.

Graisses de régime. Les lipides et les corps cétoniques du sang au cours de la journée dans le diabète juvénile.

Résumé. Les taux à jeun des lipides sanguins et leurs variations diurnes ont été étudiés chez environ 150 enfants et adolescents ayant un diabète de durée variable. Les diabétiques présentaient des taux de cholestérol, de phospholipides et de triglycérides dans des limites normales mais une élévation des acides gras libres et des corps cétoniques. La détermination postprandiale ne semble pas être de quelque avantage majeur. Des sujets diabétiques recevant de l'insuline deux fois par jour normalisèrent presque les valeurs de leurs acides gras libres et présentèrent une

diminution des corps cétoniques par comparaison avec les sujets ne recevant de l'insuline qu'une fois par jour. La durée du diabète était sans influence significative. Un régime isocalorique, socialement acceptable, riche en graisse polyinsaturée a abaissé les taux de lipides sanguins au cours d'un essai de contrôle d'une durée de 18 mois.

Diätetische Fette. Die Tagesschwankungen der Lipide und Ketonkörper beim jugendlichen Diabetes.

Zusammenfassung. Bei ungefähr 150 Kindern und Jugendlichen mit verschieden lang bestehendem Diabetes mellitus wurden die Nüchternwerte und Tagesschwankungen der Blutlipide untersucht. Die Cholesterin-, Phospholipid- und Triglyceridespiegel der Diabetiker lagen im Normalbereich, doch waren die freien Fettsäuren und Ketonkörper erhöht. Die postprandiale Bestimmung scheint keinen größeren Vorteil zu bieten. Im Vergleich mit Diabetikern, die nur einmal täglich Insulin spritzen, normalisieren sich bei Diabetikern, die zweimal täglich Insulin bekommen, die Werte der freien Fettsäuren, und auch die Ketonkörper fallen ab. Die Dauer des Diabetes war ohne signifikanten Einfluß. Eine isokalorische, sozial annehmbare, an hochungesättigten Fetten reiche Diät führte bei einem Kontrollversuch über eine Periode von 18 Monaten zu einer Abnahme des Blutfettspiegels.

If dietary polyunsaturated fats are of value in lowering serum lipids, thereby preventing or retarding atherosclerosis, the diabetic patient might benefit from such a diet. The chief aim of this investigation was to evaluate the effects of a socially acceptable diet rich in polyunsaturated fat on blood lipids in juvenile diabetics.

A marked individual variability in cholesterol, phospholipids and triglycerides in non-diabetic adult subjects has been reported [4, 7]. In a previous study from our department [12] we did not find any striking differences in the fasting levels of these lipid fractions between diabetic and non-diabetic children.

Information on postprandial lipid values as well as variation of free fatty acids (FFA), glycerol and ketone bodies might allow better discrimination of diabetics from non-diabetics.

The present investigation is designed to study the influence of the following factors on various blood lipid levels in diabetic children and adolescents:

- a) diurnal variation
- b) duration of diabetes
- c) a practical diet rich in polyunsaturated fat

Material and clinical methods

All diabetic patients included in the study regularly attended the clinic and were seen by the authors. The organization and function of our clinic has previously been reported on [8, 9]. Most patients were on Lente and Semilente insulin in various combinations. All the diabetics had been given detailed dietary instructions using exchange lists according to the system recommended by the American Diabetes Association as applied for Swedish use. During the period 1960-61, 27 patients of varying duration of diabetes were instructed about an isocaloric diet rich in polyunsaturated fat (about 22% of total fat). The following recommendations were given:

a) Margo margarine, containing 45% unsaturated fat (linoleic acid from cotton oil), to be substituted for all ordinary margarine and butter;

b) skimmed milk to be substituted for ordinary milk;

c) special cheese (prepared from Margo margarine¹) with 45% of the fat unsaturated to replace all other cheeses;

¹ Supplied by the courtesy of Svenska Margarinbolaget.

d) ham, pork and other kinds of animal meat rich in fat to be shunned; and

e) a high consumption of fish, especially fat fish, advocated.

Twenty-nine diabetic patients on ordinary diet served as the control group. The composition of the prescribed diet for both groups is given in Table 1.

The dietary checks made at the regular visits to the clinic were tabulated at the end of the trial and were the basis for deciding which patient could be regarded as having kept to the unsaturated fat diet. Only 18 patients (in the following called Du) fulfilled our criteria, 5 had totally failed and 4 partially. Among the controls, 3 had to be excluded because of their partly unsaturated diet, which they had unintentionally begun during the trial. Clinical data at the follow-up for the remaining 26 controls (Dc) are given in Table 3 and for the trial group in Table 2.

All patients were questioned by experienced dietitians at the end of the study about their food consumption during the previous 24 hours. Blood samples were taken several times before, during and at the end of the trial. Two young boys (Du) were withdrawn because blood samples could not be taken. The venous blood was usually taken after an overnight fast,

and the insulin withheld that morning. At the beginning and end of the study the sampling took place at 7 a.m., 3 p.m. and 8 p.m. During these special days the children kept to their ordinary diet and physical activity. The patients' meal times were at approximately 7 a.m., 11.30 a.m., 3.30 p.m., 6 p.m. and 8.30 p.m.

Similar diurnal blood sampling was also performed on about 100 diabetic patients on an ordinary diet (cf. Table 1, Dc) with a duration of diabetes of 0 to 20 years. These samples were usually taken on the first day after admittance to hospital for a routine check-up. The material was collected over a period of 3 years. The patients were tested after 2, 5, 10, 15 and 20 years of diabetes respectively. Twentyfour non-diabetic, non-hospitalized children (13 boys, 11 girls), aged 13 to 14 years served as a control group (called C). Angiopathy was estimated according to criteria previously reported from this department [9]. The over-all frequency of cases with retinopathy in the present "duration" material was 23%, and with permanent proteinuria 7%. When relating the frequency of retinopathy to the duration of diabetes in periods of 2, 5, 10, 15, 20 years, the following figures were arrived at: 0, 5, 47, 75 and 85%.

Table 1. Composition of the prescribed and consumed diet. Mean values in g and per cent of total calories

Calories	Prescribed diet				Consumed diet			
	Du 1975		Dc 1790		Du 1980		Dc 1820	
	g	%	g	%	g	%	g	%
protein	85	18	78	18	83	17	82	18
carbohydrate	207	42	190	42	207	42	188	41
fat	86	40	78	40	90	41	83	41
saturated	67	31	72	37	69	31	77	38
polyun- saturated	19	9	6	3	21	10	6	3

Table 2. Clinical data on 16 diabetics (Du) treated with unsaturated diet

Case No.	Sex	Age yrs.	Height cm	Weight kg	Insulin dose units (times/day)	Duration of diabetes at dietary prescription yrs.	Time on diet (days)
1	F	26	160	50	38 (2)	14.0	568
2	F	16	163	54	32 (1)	0.1	542
3	M	11	151	39	32 (1)	0.1	530
4	F	10	152	42	52 (1)	1.5	1124
6	M	9	131	26	20 (1)	1.7	471
9	M	18	180	63	54 (2)	10.5	426
10	F	10	144	33	26 (1)	1.8	426
12	M	14	157	43	20 (2)	0.1	407
14	M	14	146	36	18 (1)	4.6	373
15	M	14	180	60	16 (1)	0.9	358
17	F	10	150	41	36 (2)	3.2	957
19	F	10	143	37	8 (1)	0.1	266
20	F	11	136	32	32 (1)	0.1	266
23	M	11	148	38	24 (1)	0.1	233
24	M	15	167	54	24 (1)	0.1	228
26	F	10	145	29	14 (1)	0.1	157
Mean	—	13.1	153.3	42.3	27.9	2.4	458.3

Table 3. *Clinical data on 26 diabetic controls (Dc) at the time of interview*

Case No.	Sex	Age yrs.	Height cm	Weight kg	Insulin dose units (times/day)	Duration of diabetes
1	M	12	148	33	32 (1)	4
2	F	10	141	30	24 (1)	3
3	M	15	165	44	44 (1)	11
4	F	17	157	68	36 (2)	6
5	F	7	118	22	40 (1)	5
6	F	15	162	44	84 (2)	9
7	F	18	159	59	60 (2)	8
8	M	13	152	49	52 (1)	8
9	M	13	132	28	8 (1)	4
10	M	15	169	53	64 (2)	6
11	M	7	125	24	16 (1)	4
12	F	12	147	36	18 (1)	2
13	M	25	170	74	52 (2)	20
15	M	18	180	73	72 (2)	8
16	M	26	179	64	84 (1)	12
17	M	15	164	50	64 (2)	3
18	F	17	169	49	60 (1)	2
19	F	15	170	61	84 (2)	6
20	M	8	129	26	18 (1)	1
22	M	8	126	23	26 (1)	2
23	M	8	131	28	18 (1)	2
24	M	10	139	33	24 (1)	2
25	F	13	154	39	48 (1)	1
28	F	8	129	25	22 (1)	5
29	F	14	169	52	8 (1)	1
30	M	15	176	56	8 (1)	0.3
Mean	—	13.6	152.3	43.9	41.0	5.2

Table 4. *Diurnal blood lipids (mean values) in 89 diabetic patients with different duration of diabetes*

Duration of diabetes (yrs.)		0	2	5	10	15	20
Age (yrs.)		11	13	12	17	23	25
No. of cases		13	13	21	19	15	8
Cholesterol mg/100 ml	7 a.m.	214	192	206	202	213	214
	3 p.m.	202	192	200	189	208	209
	8 p.m.	200	190	204	196	225	220
	7 a.m.	206	185	209	185	208	211
Phospholipids mg/100 ml	7 a.m.	221	203	217	210	220	217
	3 p.m.	237	226	238	219	235	231
	8 p.m.	231	222	239	224	256	245
	7 a.m.	214	198	217	197	223	220
Glyceride-glycerol mg/100 ml	7 a.m.	1.55	0.82	1.08	1.04	1.01	1.05
	3 p.m.	1.76	1.06	1.71	1.50	1.57	1.60
	8 p.m.	1.36	0.84	1.44	1.27	1.27	1.71
	7 a.m.	1.02	0.76	1.19	0.94	0.97	1.09

Biochemical methods

At each outpatient visit the urine was examined for sugar, acetone and protein, and the capillary blood sugar was determined according to EK and HULTMAN [5]. All the venous blood samples were analysed for cholesterol [3], phospholipids [13] and glyceride-glycerol [2]. In some cases, FFA [15], ketone bodies (as total acetone by GREENBERG and LESTER as modified by WERK et al.) [16] and glycerol, by an enzymatic method according to WIELAND [18], were also determined. All samples were run in duplicate. The analytical errors for the different procedures, *expressé* *en* percentages, were as follows: cholesterol 1.1, phos-

pholipids 2.9, glyceride-glycerol 2.8, glycerol, 4.4, ketone bodies 6.4 and FFA 4.3%.

Results

Juvenile diabetics ($n = 15$) at the onset of diabetes and before receiving insulin therapy had high fasting blood lipids: cholesterol 277 mg%, phospholipids 225 mg% and glyceride-glycerol 2.3 mmol/l. After 3 weeks on insulin, before discharge from hospital the levels had fallen (Table 4, group 0). Diurnal blood lipids in the various groups of diabetic duration are given in Table 4. No obvious influence of duration upon the diurnal mean blood sugar could be seen. The above-

mentioned lipid values were not influenced by the number of insulin injections per day.

The results of lipid analysis for the non-diabetic controls are given in Table 5.

The composition of the diets actually consumed is given in Table 1. The authors' classification of the Du group agreed well with the results of the interviews with the children and the separate questioning of their mothers. Thus, the four who were judged as partially keeping to the diet consumed 5% of their total calories as unsaturated fat and the five who had totally failed 3%.

The mean number of outpatient visits during the trial was the same for the Du as for the Dc group. The mean of the individual means for outpatient values of fasting blood sugar and glycosuria per 24 hours were lower in the Du than in the Dc group. However, the differences were not statistically significant.

The mean fasting levels of cholesterol, phospholipids and glyceride-glycerol before the trial were somewhat higher for the Du group than for the Dc. Each patient is represented by a mean of two to five values sampled on different days over a period of several weeks. The influence of duration of diabetes on these pre-test values was examined in each group. Those of the Du group (Table 2) with a duration of one or two months (A), had higher values than the others (B), whose lipid values were comparable with the Dc group, in which an influence of duration could be detected (cf. Table 4).

After beginning on the unsaturated diet, the blood lipid values fell within a few weeks in both Du groups (A and B). The lowest mean value was found after two months; during the following months no systematic difference could be detected within the Du group. Fasting blood lipid values (about 5 samples in each patient) among the Dc group did not show any clear variation over a period of a year.

Fig. 1. Diurnal lipid variation (mean values) at follow-up
 ——— Diabetics on unsaturated diet (Du)
 - - - - - Diabetics on ordinary diet (Dc)
 Shaded area represents mean values $\pm$ error for the mean in non-diabetic controls

Table 5. Diurnal blood lipids and ketone bodies (mean $\pm$ standard error of the mean) among 24 non-diabetic controls (C)

	No. of cases	7 a.m.	3 p.m.	8 p.m.	7 a.m.
Cholesterol mg/100 ml	20	177 $\pm$ 6.2	175 $\pm$ 5.7	174 $\pm$ 5.0	175 $\pm$ 4.7
Phospholipids mg/100 ml	20	196 $\pm$ 5.1	210 $\pm$ 5.9	212 $\pm$ 4.9	196 $\pm$ 3.9
Glyceride-glycerol mMol/l	20	0.82 $\pm$ 0.06	1.23 $\pm$ 0.11	1.19 $\pm$ 0.10	0.67 $\pm$ 0.04
FFA mMol/l	24	0.55 $\pm$ 0.03	0.57 $\pm$ 0.04	0.48 $\pm$ 0.04	0.58 $\pm$ 0.03
Ketone bodies mMol/l	24	0.14 $\pm$ 0.007	0.13 $\pm$ 0.006	0.11 $\pm$ 0.006	0.12 $\pm$ 0.01

Table 6. Diurnal blood lipid and sugar values (mean $\pm$ standard error of the mean) at the end of the trial in 16 diabetic subjects on unsaturated (Du) and among 26 on ordinary diet (Dc)

		8 a.m.	3 p.m.	8 p.m.
Cholesterol mg/100 ml	Du	169 $\pm$ 9.3	166 $\pm$ 6.2	168 $\pm$ 7.1
	Dc	178 $\pm$ 5.1	178 $\pm$ 6.1	179 $\pm$ 5.9
Phospholipids mg/100 ml	Du	196 $\pm$ 5.9	207 $\pm$ 5.5	211 $\pm$ 9.1
	Dc	197 $\pm$ 5.2	222 $\pm$ 7.1	225 $\pm$ 6.8
Glyceride-glycerol mMol/l	Du	0.58 $\pm$ 0.06	0.91 $\pm$ 0.07	0.84 $\pm$ 0.08
	Dc	0.74 $\pm$ 0.08	1.36 $\pm$ 0.18	1.14 $\pm$ 0.13
Blood sugar mg/100 ml	Du	170 $\pm$ 21.2	164 $\pm$ 19.9	196 $\pm$ 25.1
	Dc	202 $\pm$ 21.1	198 $\pm$ 21.6	224 $\pm$ 24.0

The results at the end of the trial for diurnal lipid and blood sugar variation are given in Table 6 and illustrated in Fig. 1. At this follow-up, the mean duration of diabetes was 3.7 years among the Du group and 5.2 among the Dc. The Du group had lower values at all times of sampling. However, *t*-tests between mean values showed no significant differences except for the logarithmic glyceride-glycerol values (skew distribution) at 8 p.m. ($p < 0.01$). An analysis of variance, taking into account the individual diurnal lipid variation — that seen among individuals within each group — was also not significant.

Fig. 2. Diurnal ketone and FFA variation (mean values)

— Diabetics taking insulin once daily (A)
 - - - Diabetics taking insulin twice daily (B)

Shaded area represents mean values $\pm$ error of the mean in non-diabetic controls. Mean blood sugar in group A was 158, 179, 217 and 186 mg/100 ml and in group B 150, 145, 167 and 160 mg/100 ml at the times indicated

The mean fasting values of FFA and ketone bodies were higher among diabetics (Fig. 2) than among non-diabetics (Table 5). The influence of the number of insulin injections per day is illustrated in Fig. 2. The difference between fasting levels of FFA is significant ($p < 0.01$). Fasting values for FFA and ketones showed a significant correlation ($p < 0.01$) in the total diabetic material. There did not appear to be any variation with regard to sex or age. No correlation between blood sugar and FFA was found, but the fasting ketone concentrations increased with those of glucose.

A limited number of fasting glycerol values were available for diabetics. In the age group 1–8 years ($n = 6$) a mean of 0.19 and in the group 9–17 years ($n = 18$) a mean of 0.16 mmole/l was found. These values could be compared with our unpublished results among nondiabetics: in the younger group ($n = 15$) 0.18 ± 0.014 mmole/l, and in the older ($n = 51$) 0.093 ± 0.010 mmole/l.

Discussion

The fasting levels of cholesterol, phospholipids and triglycerides in diabetic children and adolescents on an ordinary diet fall within the normal limits for non-diabetics. The mean values after five years' duration of diabetes are slightly higher than in non-diabetics.

Neither the non-diabetics nor the diabetics show any diurnal variation of cholesterol. That of phospholipids and glyceride-glycerol is marked in both groups, being more pronounced among diabetics. The variability between repeated fasting levels is greatest in both materials for triglycerides. This finding is consistent with that of CROMIE et al. [4] for non-diabetic adults. The controls did not show any such variability in the FFA levels in contrast to findings in adults [6].

As in our previous study [12], the main difference in blood lipids between diabetics and non-diabetics is to be found in the FFA values. However, this is true only for the fasting levels, postprandial levels being even lower among the diabetics. A similar result is found for the blood ketone bodies, the values being significantly correlated with those of the FFA and increasing with increasing blood sugar. Our findings are very similar to those of WERK et al. [17] on adult diabetic subjects. No lipid value was correlated with that of blood glucose. Fasting free glycerol was likewise higher among diabetics than among non-diabetics. These results could be interpreted as an increased lipolysis in fasting diabetics, the most likely cause being a relative lack of insulin. This is born out by the fact that diabetics taking insulin twice daily show a normalization of the FFA level, a lowering of ketone bodies, and steadier diurnal blood sugar. No such influence of insulin could be shown on the levels of cholesterol, phospholipids or triglycerides. If an elevation of ketone bodies or FFA levels is prognostically unfavourable, our results indicate that juvenile diabetics ought to be given insulin twice daily. This seems to be the situation in spite of the availability of long-acting insulin preparations.

As in other studies [10], untreated juvenile diabetics at onset had high fasting blood lipid levels which could be lowered by insulin therapy. On ordinary diet the levels were lowest after a duration of two years, probably only reflecting the "postinitial stabilization phase" of juvenile diabetes. They then increased somewhat but did not show any clear changes between 5 and 20 years of duration. Nor did duration of diabetes or age seem to affect the diurnal variation of blood lipids. The frequency of diabetic retinopathy and nephropathy increased with duration, as has been found in many studies (for ref. see 9). Some cases with more than 15 years of diabetic duration had vascular calcifications of the lower extremities as signs of premature advanced atherosclerosis, but no elevation of serum lipids was yet found.

The unsaturated diet in the trial was chosen so as to be as socially acceptable as possible, the patients were well instructed, and great efforts were made to supervise them. But even so, $\frac{1}{3}$ of the patients could not keep to the diet over a period of about eighteen months. On the other hand, the remaining $\frac{2}{3}$ of the patients did consume the prescribed quality and quantity of calories as judged by various means. After taking into consideration the influence of a short

diabetic duration (weeks) on the blood lipids, a lowering of all lipid components was found, and, in most studies with dietary unsaturated fat, this effect was noticeable within a few weeks. The favourable effect of such a diet in juvenile diabetics, as indicated by SALT et al. [11] and TRAISMAN and NEWCOMB [14], was obtained after such short periods. However, as will be seen in Table 6 and Fig. 1, at the end of the trial the treated group (Du) not only had lower mean cholesterol, phospholipid and glyceride-glycerol values than the control group of diabetics (De) but also lower values than non-diabetic controls (C). Few of the differences are statistically significant, but they could hardly be so, for pre-test levels were well within the normal range. LLOYD et al. [10] on the other hand claim to have shown a significant lowering of the β -lipoprotein lipids in a group of juvenile diabetics given corn-oil.

The treated group had somewhat lower blood sugar levels. We did not find any correlation between individual blood sugar and cholesterol, phospholipids and triglycerides; we regard the supervision of both groups as the same; and we found no obvious differences between the groups except for diet. Thus it seems possible, but not proved, that the unsaturated diet contributes to greater stabilization of the blood sugar level, and its introduction might well be recommended for this reason also.

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References

- [1] ALBRINK, M.J.: Diet, Diabetes and serum lipids. *Diabetes* **13**, 425—29 (1964).
- [2] CARLSSON, L.A., and L. WADSTRÖM: Determination of glycerides in blood serum. *Clin. chim. Acta.* **4**, 197—205 (1958).
- [3] CRAMÉR, K., and B. ISAKSSON: An evaluation of the Theorell method for the determination of total serum cholesterol. *Scand. J. clin. Lab. Invest.* **11**, 213—16 (1959).
- [4] CROMIE, J.B., K.J. THOMSON, O.S. CULLIMORE and E.F. BEACH: Studies in serum lipids. With special reference to spontaneous variations and the effect of short-term dietary changes. *Circulation* **27**, 360—65, (1963).
- [5] EK, J., and E. HULTMAN: Determination of glucose and laevulose in body fluids. *Nature* **181**, 780—1 (1958).
- [6] HOLLISTER, L.E., and J.E. OVERALL: Sources of variation in repeated measurement of plasma free fatty acids in rested fasting subjects. *Amer. J. clin. Nutr.* **15**, 149—57 (1964).
- [7] KUO, P.T., and J.C. CARSON: Dietary fats and the diurnal serum triglycerides levels in man. *J. clin. Invest.* **38**, 1384—93 (1959).
- [8] LARSSON, Y.: The organization and functions of a clinic for juvenile diabetes. *Acta. paediat. (Scand.)*, Suppl. **135**, 137—43 (1962).
- [9] —, and G. STERKY: Long-term prognosis in juvenile diabetes mellitus. *Acta. paediat. Scand.* **51**, Suppl. 130 (1962).
- [10] LLOYD, J.K., A.S. FOSBROOKE, O.H. WOLFF and H.B. SALT: Effect of dietary corn oil on lipaemia in diabetic children. *Lancet* **1962 I**, 1329—30.
- [11] SALT, H.B., O.H. WOLFF, A. NESTADT and J.K. LLOYD: Control of lipaemia in children with diabetes mellitus. The role of insulin and the effects of a diet rich in unsaturated fatty acids. *Lancet* **1960 I**, 71—75.
- [12] STERKY, G., Y. LARSSON and B. PERSSON: Blood lipids in diabetic and non-diabetic schoolchildren. *Acta. paediat. Scand.* **52**, 11—21 (1963).
- [13] SVANBORG, A., and L. SVENNERHOLM: Plasma total lipid, cholesterol, triglycerides, phospholipids and free fatty acids in a healthy Scandinavian population. *Acta med. scand.* **169**, 43—49 (1961).
- [14] TRAISMAN, H.S., and A.L. NEWCOMB: The effect of safflower oil upon serum lipids and proteins in juvenile diabetes mellitus. *Quart. Bull. Northw. Univ. med. Sch.* **35**, 143—46 (1961).
- [15] TROUT, D., E.H. ESTES and S.J. FRIEDBERG: Titration of free fatty acids of plasma. A study of current methods and a new modification. *J. Lipid Res.* **1**, 199—202 (1960).
- [16] WERK, E.E., Jr., H.T. MCPHERSON, L.W. HAMRICK, Jr., J.D. MYERS and F.L. ENGEL: Studies on ketone metabolism in man. I A method for the quantitative estimation of splanchnic ketone production. *J. clin. Invest.* **34**, 1256—67 (1955).
- [17] —, and H.C. KNOWLES, Jr.: The blood ketone and plasma free fatty acid concentration in diabetic and normal subjects. *Diabetes* **10**, 22—32 (1961).
- [18] WIELAND, O.: Eine enzymatische Methode zur Bestimmung von Glycerin. *Biochem. Z.* **329**, 313—20 (1957).

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