Biochemical Pharmacology, Vol. 19, pp. 2963–2971. Pergamon Press, 1970. Printed in Great Britain

ON THE EFFECT OF THE HYPOLIPIDAEMIC PHENYL ETHER CH 13 437 ON THE LIVER METABOLISM OF THE RAT

U. SCHACHT and E. GRANZER

Farbwerke Hoechst AG, vorm. Meister Lucius & Brüning, Frankfurt (M)-Höchst, Germany

(*Received* 4 *December* 1969; *accepted* 26 *May* 1970)

Abstract—The new tetrahydronaphthalene derivative 2-methyl-2-*p*-(1,2,3,4-tetrahydro-1-naphthyl)-phenoxy-propionic acid (CH 13 437) is a compound with a strong hypolipidaemic effect with a direct influence on the liver metabolism. *In vitro* inhibition occured at the stage of the acetyl-CoA carboxylase. *In vivo* experiments in the rat were performed in which liver enzymes of the carbohydrate and lipid metabolism and the concentrations of metabolic intermediates in the liver were determined. The compound CH 13 437, *in vivo*, inhibits key enzymes of gluconeogenesis (phosphoenolpyruvate carboxykinase) and those of glycolysis (pyruvate kinase, phosphofructokinase); citrate synthase shows a moderate increase in activity and malic enzyme a considerable increase paralleled by a high activity increase of the microsomal NADPH-oxidase. The activity of the citrate cleavage enzyme was reduced in refeeding experiments. The concentrations of acetyl-CoA, acetoacetate, citrate and pyruvate—due to the enzyme activity changes found—have undergone a dose-dependent increase. According to the results discussed, the findings indicate inhibition of lipogenesis and retarded glycolysis in the liver as the cause of the hypolipidaemic effect of the compound CH 13 437.

THE TETRAHYDRONAPHTHALENE derivative 2-methyl-2-*p*(1,2,3,4-tetrahydro-1-naphthyl)-phenoxy-propionic acid (CH 13 437)[1] is a hypolipidaemic agent which can lower both the serum cholesterol level and the serum triglyceride level in rats and dogs as well as alimentary hypertriglyceridaemias induced by fructose loading in the rat. Hypertrophy of the liver, which occurs in the male rat after the administration of higher dosages of CH 13 437, the simultaneous increase in the smooth endoplasmatic reticulum and the microbodies (peroxysomes), and the increased activity of oxidizing and hydroxylizing enzymes as well, which are independent of sex and thyroid function, indicate a direct site of action in the liver as causing the hypolipidaemic effect.[1,2] Lowered concentrations of cholesterol and triglycerides[1] as well as an increased uptake of extrahepatic fatty acids into the triglyceride fraction of the liver[2] might be based on restricted lipid synthesis, especially reduced fatty acid formation.

Our investigations in the field of the liver metabolism of rats also indicate that lipogenesis is inhibited by compound CH 13 437, and are in accord with the assumption of a blocking of the rate-limiting step of fatty acid synthesis on the acetyl-CoA carboxylase reaction; acetyl-CoA carboxylase obtained from chicken liver is competitively inhibited by CH 13 437 and clofibrate.[3]

In the present study liver enzymes of the carbohydrate and lipid metabolism, enzymes of the respiratory chain and the liver concentrations of a few metabolites were examined. The enzymes investigated include phosphoenolpyruvate carboxykinase (EC 4.1.1.32), fructose-1,6-diphosphatase (EC 3.1.3.11) and glucose-6-phosphatase

(EC 3.1.3.9) as key enzymes of gluconeogenesis, glucokinase (EC 2.7.1.2), hexokinase (EC 2.7.1.1), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), phosphofructokinase (EC 2.7.1.11) and pyruvate kinase (EC 2.7.1.40) as key enzymes of glucose decomposition, citrate cleavage enzyme (EC 4.1.3.8), malic enzyme (EC 1.1.1.40) and citrate synthase (EC 4.1.3.7) as auxiliary enzymes of lipid metabolism, as well as NADH-cytochrome c-reductase (EC 1.6.99.3) as enzyme complex of the respiratory chain. The concentrations of the following metabolites were measured: ATP, ADP, AMP, pyruvate, lactate, acetoacetate, citrate and acetyl-CoA.

METHODS

1. *Treatment of the animals*

Male Wistar strain rats weighing about 200 g were treated with the compound CH 13 437, dissolved in polyethyleneglycol (molecular weight 400), for periods of 14 days on an average. Administration of the drug was carried out in dosages of 10 mg/kg respectively once a day by gavage (0·2 ml/100 g body wt.).

The animals under inspection were then divided into three groups: (a) animals which were kept on a normal balanced chow diet; (b) animals which had fasted for the last 2 days of the experimental period; (c) animals which were refed with a carbohydrate-rich diet in the form of pure oats, following 2 days of starvation at the end of the period. In all cases groups of rats serving as the controls were kept under identical conditions respectively and were given the solvent polyethyleneglycol. On the last day of the period, 3 hr after administration, the rats were killed by a blow in the neck and then exsanguinated.

2. *Preparation of the extracts*

For determination of the enzyme activities, the livers were immediately removed and homogenized with ice cooling; five different extracts had to be prepared.

Extract 1. One portion of liver was homogenized in the Potter–Elvehjem Teflon homogenizer in 4 vol. of buffer containing 100 mM triethanolamine (pH 7·6) and 10 mM EDTA. After centrifuging at 14,000 *g* for 2 min glucose-6-phosphate dehydrogenase, phosphofructokinase, fructose-1,6-diphosphatase, pyruvate kinase and malic enzyme were determined in the supernatant.

Extract 2. In the Teflon–Potter, one portion of liver was homogenized in 4 vol. of 100 mM Tris buffer (pH 7·4), 1 mM EDTA and 1 mM monothioethyleneglycol, and the homogenate centrifuged in the Beckman L2-50 centrifuge at 105,000 *g* and 0° for 30 min. The supernatant was used for measuring the activity of glucokinase and hexokinase.

Extract 3. One portion of liver was homogenized in 9 vol. of 250 mM saccharose, the homogenate centrifuged in the Beckman L2-50 centrifuge at 105,000 *g* and 0° for 30 min, and phosphoenolpyruvate carboxykinase and citrate cleavage enzyme were determined in the supernatant.

Extract 4. To determine the activity of the citrate synthase, one portion of liver was homogenized twice for 30 sec with the Ultra-Turrax (Janke & Kunkel, Staufen i. Br.) in 9 vol. of 100 mM phosphate buffer (pH 7·3) and 2 mM EDTA; the total homogenate was inserted in the test mixture.

Extract 5. One portion of liver was homogenized in the glass–Potter in 9 vol. of 100 mM citrate buffer (pH 6·5), the extract filtered through glass wool, and glucose-6-phosphatase measured in the filtrate.

Mitochondrial fraction. The mitochondrial fraction for determination of the NADH-cytochrome c reductase was obtained by homogenization of one portion of liver in 4 vol. of 250 mM saccharose, 20 mM triethanolamine (pH 7·4) and 1 mM EDTA followed by centrifugation at 600 *g* and 0° for 10 min, and by centrifugation of the supernatant at 8000 *g* and 0° for 10 min. The precipitate was washed, and the mitochondrial fraction obtained at 8000 *g* taken up in ice-cooled 66 mM potassium–sodium-phosphate buffer, pH 7·0 (2·5 ml/g of liver).

Extract for substrate determinations. About 2 g of liver were taken by freeze-stop, pulverized in liquid nitrogen in a mortar and then homogenized in the Teflon–Potter with ice cooling in 8 ml 6 per cent (w/v) perchloric acid. The homogenate was centrifuged at 2500 *g* and 0° for 15 min, and the supernatant adjusted to approximately pH 6·0 with 2 N KOH.

3. *Measurement of the enzyme activities*

Enzymes, coenzymes and substrates had been purchased from Boehringer Mannheim GmbH.

With the exception of phosphoenolpyruvate carboxykinase, glucose-6-phosphatase and the mitochondrial NADH-cytochrome c reductase, it was possible to determine all enzymes by applying Warburg's optical test. Hexokinase, glucose-6-phosphate dehydrogenase, phosphofructokinase, fructose-1,6-diphosphatase and pyruvate kinase were measured in the test compositions given by Bücher, Luh and Pette.[4] Glucokinase was determined in 50 mM Tris buffer (pH 7·4) by the method of DiPietro and Weinhouse.[5] Malic enzyme was determined in 50 mM Tris buffer (pH 7·4) according to Ochoa,[6] citrate cleavage enzyme in 100 mM triethanolamine buffer (pH 7·3) according to Srere,[7] and citrate synthase in 100 mM Tris buffer (pH 8·0) according to Ochoa[8] in the modification of Brdiczka *et al.*[9]

Measurements were carried out at 334 and 366 nm respectively, and at 25° using an Eppendorf filter photometer (Netheler & Hinz GmbH, Hamburg). The enzyme activities are given in international units (1 IU = 1 μmole substrate exchange per minute at 25°), related to 1 g of liver weight.

The activity of the phosphoenolpyruvate carboxykinase was measured at 30° on the basis of Nordlie's and Lardy's method,[10] with the modifications given by Seubert and Huth.[11]

Glucose-6-phosphatase was determined by the method of Swanson[12] using 100 mM citrate buffer (pH 6·5), and the liberated inorganic phosphate estimated by using Fiske's and Subbarow's method.[13]

NADH-cytochrome c reductase was measured by Mackler's method,[14] the rate of cytochrome c-reduction being recorded at 546 nm and 25°. The activity of NADH-cytochrome c reductase is related to 1 mg protein, determined by the biuret-method.[15]

4. *Measurement of the substrate concentrations*

The substrates were measured in the supernatant of the $KClO_4$-precipitate spectrophotometrically by enzymatic analysis:[16] ATP by Lamprecht's and Trautschold's method, ADP and AMP according to Adam, pyruvate according to Bücher *et al.*,

lactate according to Hohorst, and acetoacetate according to Mellanby and Williamson. Citrate was determined according to Moellering and Gruber.[17]

The acetyl-CoA was assayed by a combined optical test with the aid of citrate synthase and malate dehydrogenase.[8] The reaction mixture, in a total volume of 2·24 ml, contained 320 μmoles Tris-buffer (pH 8·0), 5·0 μmoles EDTA, 10 μmoles NAD, 120 μmoles L(−)-malate (neutralized), 36 IU malate dehydrogenase and 1·0 ml of extract; the reaction was started by the addition of citrate synthase (2·8 IU), and measured at 334 nm (d = 2 cm).

RESULTS

1. *Influence on the enzyme activities*

In Tables 1 and 2 those results are summarized, which were obtained with the groups of rats kept on the normal balanced chow diet.

TABLE 1. ENZYME ACTIVITIES IN RAT LIVER AFTER ORAL ADMINISTRATION OF 10 mg/kg CH 13 437 DAILY*

Enzyme	CH 13 437 (IU/g liver)	n†	Control (IU/g liver)	n†	P‡
Phosphoenol-pyruvate carboxykinase	0·85 ± 0·35	18	0·90 ± 0·44	16	not significant
Fructose-1,6-diphosphatase	7·76 ± 1·40	17	7·91 ± 1·42	18	not significant
Glucose-6-phosphatase	12·8 ± 1·8	18	12·2 ± 2·1	18	not significant
Glucokinase	3·96 ± 0·60	18	3·36 ± 0·81	17	P < 0·05
Hexokinase	0·59 ± 0·17	18	0·69 ± 0·22	17	not significant
Glucose-6-phosphate dehydrogenase	2·21 ± 0·57	15	2·47 ± 0·65	14	not significant
Fructose-6-phosphate kinase	1·79 ± 0·43	6	1·77 ± 0·42	6	not significant
Pyruvate kinase	12·5 ± 3·7	18	24·2 ± 5·4	18	P < 0·001
Malic enzyme	4·01 ± 1·36	18	1·30 ± 0·60	18	P < 0·001
Citrate cleavage enzyme	0·68 ± 0·26	15	0·86 ± 0·33	15	not significant
NADH-cytochrome c reductase	15·3 ± 3·8§	5	20·8 ± 1·6§	5	P < 0·05

* The drug was solved in polyethylene glycol 400 and administered orally on an average of 14 days; the rats of the control group obtained polyethylene glycol 400 only. Food and water in both animal groups *ad lib.*

Enzyme activities are expressed in international units (IU = μmoles substrate/min). The results are given as mean values ± S.D.

† *n* = number of animals.

‡ Calculation of significance by Student's *t*-test.

§ The activity of the NADH-cytochrome c reductase is expressed in IU × 10^{-2}/mg protein.

In the group of gluconeogenetic enzymes, both fructose-1,6-diphosphatase and glucose-6-phosphatase remain unchanged in the two dosages of 10 mg/kg CH 13 437 daily and 100 mg/kg CH 13 437 respectively, whilst the activity of phosphoenol-pyruvate carboxykinase in the higher dosage has become reduced by 53 per cent as compared with the controls; despite the considerable standard deviation, the lowering of activity is still significant (P < 0·05).

Among the enzymes of glucose decomposition there is a pronounced influence on pyruvate kinase: in a dosage of 10 mg/kg CH 13 437 daily, the enzyme activity has

TABLE 2. ENZYME ACTIVITIES IN RAT LIVER AFTER ORAL ADMINISTRATION OF 100 mg/kg CH 13 437 DAILY*

Enzyme	CH 13 437 (IU/g liver)	n†	Control (IU/g liver)	n†	P‡
Phosphoenol-pyruvate carboxykinase	0·72 ± 0·60	9	1·53 ± 0·84	9	P < 0·05
Fructose-1,6-diphosphatase	5·00 ± 1·82	9	6·01 ± 1·43	9	not significant
Glucose-6-phosphatase	10·6 ± 1·9	9	11·1 ± 2·5	9	not significant
Glucose-6-phosphate dehydrogenase	2·94 ± 1·34	9	2·94 ± 0·91	9	not significant
Fructose-6-phosphate kinase	1·62 ± 0·30	6	2·02 ± 0·36	6	P < 0·1
Pyruvate kinase	9·2 ± 2·1	15	30·5 ± 7·0	15	P < 0·001
Malic enzyme	12·72 ± 4·96	15	1·54 ± 0·49	15	P < 0·001
Citrate cleavage enzyme	0·90 ± 0·25	14	0·96 ± 0·54	14	not significant
Condensing enzyme	6·02 ± 0·42	8	5·10 ± 0·39	8	P < 0·001
NADH-cytochrome c reductase	12·1 ± 2·5§	9	18·7 ± 2·8	10	P < 0·001

* The drug was solved in polyethylene glycol 400 and administered orally on an average of 14 days; the rats of the control group obtained polyethylene glycol 400 only. Food and water in both animal groups *ad lib.*

Enzyme activities are expressed in international units (IU = μmoles substrate/min). The results are given as mean values ± S.D.

† n = number of animals.

‡ Calculation of significance by Student's t-test.

§ The activity of the NADH-cytochrome c reductase is expressed in IU × 10^{-2}/mg protein.

become reduced significantly (P < 0·001) by about 48 per cent on an average, after administration of the daily dose of 100 mg/kg of CH 13 437 enzyme inhibition amounts to 70 per cent. After a daily administration of 100 mg/kg of the drug phosphofructokinase shows an insignificant decline of about 20 per cent, and its activity in the low dosage of 10 mg/kg remains unchanged, whilst glucokinase activity after a daily administration of 10 mg/kg of CH 13 437 has become increased by 18 per cent (P < 0·05) on an average; the behaviour of this enzyme in a higher dose of the compound was not studied in detail. The other enzymes of this group, hexokinase and glucose-6-phosphate dehydrogenase, do not exhibit any change in activity.

The activity of malic enzyme has become considerably increased under the influence of compound CH 13 437: after a daily administration of 10 mg/kg its average activity has become more than three times as much; after administration of a daily dose of 100 mg/kg its activity has increased to eight times of the one found in the controls. CH 13 437 lowers the activity of the citrate cleavage enzyme slightly but not significantly in normally fed rats.

The citrate synthase shows a significant increase in activity of 18 per cent after administration of 100 mg/kg of CH 13 437.

After a daily treatment with 100 mg/kg of CH 13 437, in the mitochondrial fractions isolated from the livers, the activity of NADH-cytochrome c reductase related to mg of protein of the mitochondrial fraction has become reduced by 35 per cent (P < 0·001) on an average. After treatment of the rats with 10 mg/kg of CH 13 437 daily, the inhibition of NADH-cytochrome c reductase in the liver mitochondrias amounts to 26 per cent.

In vitro, in mitochondrial preparations from rat heart, the compound CH 13 437 causes a dose-dependent inhibition of NADH-cytochrome c reductase (complex I + III

of the respiratory chain according to Green).[18] CH 13 437 in a concentration of 1×10^{-3}M inhibits this enzyme complex by about 70–80 per cent, in a concentration of 5×10^{-4}M by about 40–45 per cent, and in a concentration of 1×10^{-4}M by about 10–20 per cent. The inhibition is abolished, when albumin in a concentration of 5 per cent (w/v) is added to the test mixture.

The results of the effect of the drug (10 mg/kg) on enzyme suppression during starvation and on enzyme induction after refeeding animals which fasted before are shown in Tables 3 and 4.

TABLE 3. ENZYME ACTIVITIES IN RAT LIVER AFTER ORAL ADMINISTRATION OF 10 mg/kg CH 13 437 DAILY AND AFTER 2 days OF STARVATION*

Enzyme	CH 13 437 (IU/g liver)	n†	Control (IU/g liver)	n†	P‡
Pyruvate kinase	16·4 ± 3·6	5	29·9 ± 2·7	5	$P < 0.001$
Malic enzyme	1·65 ± 0·59	5	1·12 ± 0·56	5	not significant
Citrate cleavage enzyme	0·29 ± 0·06	5	0·43 ± 0·06	5	$P < 0.01$

* The drug was solved in polyethylene glycol 400 and administered orally over a period of 14 days; the rats of the control group obtained polyethylene glycol 400 only.
48 hr before killing the food was withdrawn, water was given *ad lib.*
Enzyme activities are expressed in international units (IU = μmoles substrate/min).
The results are given as mean values ± S.D.
† n = number of animals.
‡ Calculation of significance by Student's t-test.

TABLE 4. ENZYME ACTIVITIES IN RAT LIVER AFTER ORAL ADMINISTRATION OF 10 mg/kg CH 13 437 DAILY AND AFTER 2 days OF REFEEDING*

Enzyme	CH 13 437 (IU/g liver)	n†	Control (IU/g liver)	n†	P‡
Pyruvate kinase	22·4 ± 3·2	5	52·6 ± 9·3	4	$P < 0.001$
Malic enzyme	7·78 ± 3·37	5	3·66 ± 0·46	4	$P < 0.05$
Citrate cleavage enzyme	0·63 ± 0·13	5	1·30 ± 0·13	4	$P < 0.001$

* The drug was solved in polyethylene glycol 400 and administered orally over a period of 14 days; the rats of the control group obtained polyethylene glycol 400 only.
96 hr before killing the food was withdrawn, water was given *ad lib.*
48 hr before killing the animals were refed with a carbohydrate-rich diet.
Enzyme activities are expressed in international units (IU = μmoles substrate/min).
The results are given as mean values ± S.D.
† n = number of animals.
‡ Calculation of significance by Student's t-test.

Citrate cleavage enzyme activity has become significantly lowered by 32 per cent ($P < 0.01$) in starved animals and lowered by 52 per cent ($P < 0.001$) on refeeding with a carbohydrate-rich diet. Pyruvate kinase has become inhibited by 45 per cent in the fasted state and by 57 per cent in the refed animals. Malic enzyme, on the contrary, shows an insignificant increase in activity by 47 per cent in starved rats and a significant increase in activity by 112 per cent after refeeding.

In the case of pyruvate kinase and citrate cleavage enzyme, the enzyme induction

caused by refeeding is largely suppressed by compound CH 13 437, whereas it is enhanced with malic enzyme.

2. *Influence on the substrate concentrations*

The concentrations of some metabolic intermediates in the liver, measured after administration of the drug to normally fed rats, are given in Tables 5 and 6.

TABLE 5. CONCENTRATIONS OF METABOLIC INTERMEDIATES IN RAT LIVER AFTER ORAL ADMINISTRATION OF 10 mg/kg CH 13 437 DAILY*

Metabolite	CH 13 437 (nmoles/g liver)	n†	Control (nmoles/g liver)	n†	P‡
ATP	3990 ± 1230	6	4170 ± 560	6	not significant
ADP	1370 ± 100	6	1250 ± 270	6	not significant
AMP	420 ± 140	6	330 ± 160	6	not significant
Pyruvate	68 ± 32	6	49 ± 22	6	not significant
Lactate	3470 ± 1600	5	2575 ± 540	6	not significant
Citrate	248 ± 96	6	200 ± 54	6	not significant
Acetoacetate	74 ± 17	3	48 ± 5	3	not significant
Acetyl-CoA	47 ± 10	6	27 ± 7	6	P < 0·01

* The drug was solved in polyethylene glycol 400 and administered orally on an average of 14 days; the rats of the control group obtained polyethylene glycol 400 only
To both animal group food and water were given *ad lib.*
The results are expressed as mean values ± S.D.
† *n* = number of animals.
‡ Calculation of significance by Student's *t*-test.

TABLE 6. CONCENTRATIONS OF METABOLIC INTERMEDIATES IN RAT LIVER AFTER ORAL ADMINISTRATION OF 100 mg/kg CH 13 437 DAILY*

Metabolite	CH 13 437 (nmoles/g liver)	n†	Control (nmoles/g liver)	n†	P‡
ATP	3800 ± 630	6	3860 ± 700	6	not significant
ADP	1200 ± 480	6	1060 ± 410	6	not significant
AMP	370 ± 250	6	340 ± 200	6	not significant
Pyruvate	100 ± 35	6	42 ± 6	6	P < 0·01
Lactate	2180 ± 810	6	2220 ± 380	6	not significant
Citrate	276 ± 105	6	186 ± 36	6	not significant
Acetoacetate	142 ± 29	6	49 ± 15	6	P < 0·001
Acetyl-CoA	55 ± 12	6	29 ± 8	6	P < 0·01

* The drug was solved in polyethylene glycol 400 and administered orally on an average of 14 days; the rats of the control group obtained polyethylene glycol 400 only.
To both animal group food and water were given *ad lib.*
The results are expressed as mean values ± S.D.
† *n* = number of animals.
‡ Calculation of significance by Student's *t*-test.

Despite the inhibition of the respiratory chain observed in liver mitochondria, both the adenosine phosphate concentrations and the lactate concentration remain unchanged. The liver concentrations of all other metabolites investigated have become increased depending on dosages, although not always significantly. The pyruvate

content increased by about 39 per cent after having administered a daily dosage of 10 mg/kg of CH 13 437; after administration of the higher dosage of 100 mg/kg daily a significant increase ($P < 0{\cdot}01$) by 138 per cent on an average was measured. Non-significant though dose-dependent increases of 24 or 48 per cent on an average were found on determining the citrate.

After administration of 10 mg/kg of CH 13 437, acetoacetate concentration was assayed in three animals only and found to have increased by 54 per cent. Under the higher dosage of the drug the acetoacetate content of the liver rose to the 3-fold value ($P < 0{\cdot}001$). Finally, CH 13 437 causes a significant increase ($P < 0{\cdot}01$) in the acetyl-CoA concentration, i.e. 74 per cent under the lower, and 90 per cent under the higher dose.

DISCUSSION

The important role of malic enzyme and citrate cleavage enzyme in lipogenesis seems now to be well established. In animal experiments both liver enzymes show a pronounced adaptive behaviour towards hormonal influences and varied feeding conditions, the activities of both enzymes changing in parallel with the extent of fatty acid synthesis.[19-23]

The great increase in activity of the malic enzyme measured under normal feeding conditions thus at first sight appears to be an incomprehensible biochemical effect of a drug which lowers the lipid content in the blood and liver. In the examination of microsomal liver enzymes after feeding CH 13 437 to rats, an approximately equal activity increase of the NADPH-cytochrome c reductase is found;* this microsomal enzyme complex oxidizes the hydrogen of the NADPH with atmospheric oxygen to water, no ATP being formed. Through an ATP-dependent reaction cycle which runs between pyruvate, oxaloacetate and malate, hydrogen may be transferred from NADH to NADPH[11,22,24] and its oxidation finally achieved by the microsomal NADPH-cytochrome c reductase. In this reaction cycle malic enzyme catalyses the third and last reaction, the NADP-dependent oxidative decarboxylation of malate to pyruvate, NADPH being formed. It is conceivable that inhibition of the mitochondrial respiratory chain observed under CH 13 437 medication is closely connected with an acceleration of this transfer reaction of hydrogen. Not only the increased activity of the malic enzyme indicates an accelerated reaction in this cycle but also the increase in concentration of acetyl-CoA which represents an essential activator for pyruvate carboxylase.[25] The pyruvate carboxylase reaction is at the same time the first step in gluconeogenesis starting from pyruvate.[25,26] However, as indicated by the reduced activity of phosphoenolpyruvate carboxykinase, the gluconeogenetic pathway at this step is impaired by the drug.

The elevated concentrations of acetyl-CoA and acetoacetate must be considered as the consequence of acetyl-CoA carboxylase-inhibition;[3] on the other hand they may originate from an increased fatty acid oxidation. Yet the distinct inhibition of the citrate cleavage enzyme in the refeeding experiment at the same time indicates inhibition of lipogenesis.

The observed increase in citrate concentration results from the reduced activity of the citrate cleavage enzyme. The increased concentrations of acetyl-CoA and citrate

* F. Beyhl and H. Kief, personal communication.

on their part might cause the elevated pyruvate level; for both metabolites are inhibitors of the mitochondrial pyruvate dehydrogenase complex.[27-29]

For the intermediate metabolism, the diminished activity of the two glycolytic pacemaker enzymes, pyruvate kinase and phosphofructokinase, means a slowing down of glucose decomposition. The reduced requirement of acetyl-CoA which might be referred to the inhibition of fatty acid synthesis, is reflected in this.

It will depend on the results of further investigations if, besides inhibition of lipogenesis, also accelerated fatty acid decomposition is responsible for the lipid lowering effect caused by compound CH 13 437; the two mechanisms possibly are acting in a synergistic manner.

REFERENCES

1. R. Hess and W. L. Bencze, *Experientia* **24**, 418 (1968).
2. R. Hess, R. Maier and W. Stäubli, in *Drugs Affecting Lipid Metabolism* (Eds. W. L. Holmes, L. A. Carlson and R. Paoletti), Advances Exp. Med. Biol., Vol. 4 (1969).
3. M. E. Maragoudakis, *J. biol. Chem.* **244**, 5005 (1969).
4. T. Bücher, W. Luh and D. Pette, in Hoppe-Seyler/Thierfelder: *Handbuch der Physiologisch- und Pathologisch-Chemischen Analyse* Vol. 6 A, 10 ed., Springer-Verlag, Berlin-Heidelberg-New York p. 292 (1964).
5. D. L. DiPietro and S. Weinhouse, *J. biol. Chem.* **235**, 2542 (1960).
6. S. Ochoa, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan), Vol. 1, p. 739, Academic Press, New York (1955).
7. P. A. Srere, *J. biol. Chem.* **234**, 2544 (1959).
8. S. Ochoa, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan), Vol. 1, p. 658. Academic Press, New York (1955).
9. D. Brdiczka, D. Pette, G. Brunner and F. Miller, *Europ. J. Biochem.* **5**, 294 (1968).
10. R. C. Nordlie and H. A. Lardy, *J. biol. Chem.* **238**, 2259 (1963).
11. W. Seubert and W. Huth, *Biochem. Z.* **343**, 176 (1965).
12. M. A. Swanson, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan), Vol. 2, p. 541. Academic Press, New York (1955).
13. C. H. Fiske and Y. Subbarow, *J. biol. Chem.* **66**, 375 (1925).
14. B. Mackler, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan), Vol. 10, p. 551. Academic Press, New York (1967).
15. G. Beisenherz, H. J. Boltze, T. Bücher, R. Czok, K. H. Garbade, E. Meyer-Arendt and G. Pfleiderer, *Z. Naturforsch.* **8b**, 555 (1953).
16. H. U. Bergmeyer (Ed.), *Methoden der enzymatischen Analyse*, Verlag Chemie, Weinheim (1962).
17. H. Moellering and W. Gruber, *Analyt. Biochem.* **17**, 369 (1966).
18. D. E. Green and D. H. McLennan, in *Metabolic Pathways*, (Ed. D. M. Greenberg). 3rd edn., Vol. 1, p. 47. Academic Press, New York (1967).
19. J. A. Olsen, in P. D. Boyer (Ed.) *Ann. Rev. Biochem.* **35**/II, 559 (1966).
20. P. A. Srere, *Nature, Lond.* **205**, 766 (1965).
21. F. J. Ballard and R. W. Hanson, *J. Lipid Res.* **8**, 73 (1967).
22. M. S. Kornacker and J. M. Lowenstein, *Biochem. J.* **94**, 209 (1965).
23. D. E. Atkinson, in *Citric Acid Cycle* (Ed. J. M. Lowenstein), p. 137. Marcel Dekker, New York (1969).
24. J. M. Lowenstein, *J. biol. Chem.* **236**, 1213 (1961).
25. M. F. Utter, D. B. Keech and M. C. Scrutton, in *Advances in Enzyme Regulation* (Ed. G. Weber), Vol. 2, p. 49. Pergamon Press, New York (1964).
26. M. C. Scrutton and M. F. Utter, in P. D. Boyer (Ed.), *Ann. Rev. Biochem.* **37**, 249 (1968).
27. P. B. Garland and P. J. Randle, *Biochem. J.* **91**, 6 C (1964).
28. C. K. Silbert and D. B. Martin, *Biochem. biophys. Res. Commun.* **31**, 818 (1968).
29. O. Wieland, B. v. Jagow-Westermann and B. Stukowski, *Hoppe-Seyler's Z. Physiol. Chem.* **350**, 329 (1969).