decrease. The percent inhibition of oxygen consumption by IAA also increased up to 12 weeks and then decreased up to 33 weeks. There was a significant increase in inhibition in the older rats.

The increase in inhibition of oxygen uptake of the liver by IAA during developmental period indicates an increase in the activity of gly-3P-DH and of the Krebs cycle during this period. A decrease in the inhibition after the attainment of maturity at 12 weeks shows a decrease in the activity of Krebs cycle and possibly a relative increase in the activity of hexose monophosphate pathway (HMP) for glucose oxidation. A 33-week-old rat is a fully grown adult. It is seen that even though there is no difference in the oxygen consumption of the liver of 33- and 70-week old rats, the inhibition by IAA is significantly higher in the older rat. This shows that in the old rats, the activity of gly-3P-DH and of Krebs cycle is increased again. Such shifts to HMP from Krebs cycle occurs also in the liver of goldfish adapted to cold temperature[7,8].

Oxygen consumption and percentage of inhibition with IAA by the liver homogenates of rats of various ages with D-glucose as substrate

Age (weeks)	ml O_2/g wet wt./h	P (t'-test)	% Inhibition	P (t'-test)
6	0.50	0.001	61.0	0.001
12	0.65	0.001	80.0	0.001
33	0.45	0.20	47.0	0.001
70	0.47		73.0	

Each value represents the mean of 8–9 animals.

Zusammenfassung. Die Aktivität des Krebszyklus scheint während der Wachstumsperiode bis zur 12. Woche stetig anzusteigen. Während des Adultstadiums hingegen tritt ein Wechsel nach dem Hexosemonophosphatweg ein, um im Seneszenzstadium eine Aktivierung des Krebszyklus aufzuweisen.

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Influence of 2-Methyl-2-(*p*-1,2,3,4-Tetrahydro-1-Naphthylphenoxy)Propionic Acid on the Oxidation of Cholesterol by Rat Liver Mitochondria

HESS and his co-workers[1,2] have reported that one of a series of aryloxy derivatives of short-chain fatty acids, 2-methyl-2-(*p*-1, 2, 3, 4-tetrahydro-1-naphthylphenoxy) propionic acid (Su-13,437), has potent lipopenic properties in normal as well as hyperlipemic rats. This compound exerts a number of metabolic effects similar to those exhibited by ethyl *p*-chlorophenoxyisobutyrate (CPIB). In view of these observations we thought it would be of interest to determine if this new compound would also affect mitochondrial oxidation of cholesterol in a manner similar to that seen with CPIB[3]. This report describes the effects of a diet containing 0.3% Su-13,437 upon serum and liver lipids of rats as well as its effect upon cholesterol oxidation by rat-liver mitochondrial preparations.

Materials and methods. In both experiments, male Wistar rats were fed a diet consisting of 70% mixed cereal; 7% wheat germ; 21% skim milk powder; and 2% vitamin mix[4]. The test groups were fed the same diet in which 0.3% of the cereal was replaced by Su-13,437.

After 3 weeks the rats were decapitated and the livers quickly excised and placed in cold 10% aqueous (w/v) sucrose. Aliquots of liver were taken for lipid determination, the remainder homogenized in sucrose, and the mitochondria prepared by the method of WHITEHOUSE et al.[5]. The oxidation of 26-^{14}C-cholesterol was carried out using previously detailed methods[5,6]. When boiled supernatant fraction (cytosol) was omitted it was replaced by an equal volume of 10% sucrose.

Liver aliquots were homogenized in chloroform-methanol, 2:1 and the extract used for lipid determination. In serum and liver, cholesterol was determined by the method of MANN[7], triglycerides by the method of VAN HANDEL and ZILVERSMIT[8] and phospholipid by the method of FISKE and SUBBAROW[9].

Results and discussion. In the first experiment (Table I), it was observed that after 3 weeks on diet, the weight gain of rats fed Su-13,437 was lower than that of the controls, but their liver weight was significantly higher. There were no differences in serum cholesterol levels, but serum triglycerides were lower and phospholipids higher in the test group. Only cholesterol levels of the liver were determined in this experiment and lower levels were observed in the test group ($p < 0.01$).

In the second experiment (Table I) the changes in serum and liver lipid levels were similar to those observed in experiment 1. In the serum, the cholesterol levels were the same but triglycerides were lower ($p < 0.02$) and phospholipids higher in the group fed Su-13,437. In the liver, cholesterol levels were lower in the test group, but no differences were observed in triglyceride or phospholipid levels. The total serum-liver lipid pools were elevated

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Table I. Influence of 2-methyl-2-(*p*-1,2,3,4-tetrahydro-1-naphthylphenoxy) propionic acid (Su-13,437) on serum and liver lipids in rats[a]

	Experiment 1 Control	Su-13,437	Experiment 2 Control	Su-13,437
No. of animals	6	6	6	6
Starting wt. (g)	172	172	148	148
Wt. gain (g)	109	43	68	3
Liver wt. (g)	10.6 ± 0.6[b]	14.5 ± 0.9[c]	8.6 ± 0.2	11.9 ± 0.3[d]
Liver as percentage body wt.	3.77	6.74	3.98	7.88
Serum cholesterol, mg/100 ml	27.2 ± 4.9	35.4 ± 2.8	24.0 ± 3.1	32.3 ± 4.5
Serum triglyceride, mg/100 ml	81.6 ± 11.1	60.0 ± 8.2	73.0 ± 6.9	41.2 ± 6.5[e]
Serum phospholipid, mg/100 ml	59.8 ± 2.9	74.3 ± 2.5[c]	68.7 ± 6.6	78.0 ± 3.0
Liver cholesterol, mg/100 g	159 ± 8.2	117 ± 6.2[c]	168 ± 8.3	139 ± 11.2
Liver triglyceride, mg/100 g	–	–	313 ± 76	300 ± 51
Liver phospholipid, mg/100 g	–	–	94 ± 6.8	92 ± 6.8
Serum plus liver cholesterol pool (mg)	19.14	19.25	16.01	18.00
Serum plus liver triglyceride pool (mg)	–	–	31.65	37.57
Serum plus liver phospholipid pool (mg)	–	–	12.52	15.16

[a] Drug fed at 0.3% of diet for 21 days. [b] Standard error. [c] $p < 0.01$. [d] $p < 0.001$. [e] $p < 0.02$.

in the Su-13,437 fed rats by 12.4% for cholesterol, 18.7% for triglyceride and 21.1% for phospholipid.

HESS administered Su-13,437 to rats at a level of 10 mg/kg in some experiments, and, in an acute study of the influence of this compound upon liver lipid levels[1], the daily dose was 100 mg/kg. Our diet contained 0.3% of the test material, which amounts to 15 mg/day (75 mg/kg) if we assume that the rats ingested as much as 5 g of food daily. Under these conditions the reductions in liver cholesterol were 26% and 17% in experiments 1 and 2, respectively. Experiment 2 showed practically no change in liver triglyceride or phospholipid. This is of interest since at this dose level, CPIB shows both hepatomegaly and elevation of liver triglyceride[3,10].

In the first experiment we observed that the gross oxidation of 26-^{14}C-cholesterol to $^{14}CO_2$ by liver mitochondrial preparations from rats fed Su-13,437 was higher than that by normal liver preparations, but when percent oxidation was corrected for mg mitochondrial *N* it was lower. Neither difference was statistically significant. Working with CPIB-fed rats[3], we found that when the cytosol was omitted, the usual reduction in percent oxidation seen with normal mitochondrial preparations[5], did not occur. Using liver mitochondria from rats fed Su-13,437 we observed a similar effect. In this experiment the gross oxidation by the complete system was significantly higher with preparations from the test group ($p < 0.001$), but the difference was not statistically significant after correction for mg/*N*. When cytosol was omitted, the extent of oxidation was significantly higher when calculated on a gross ($p < 0.001$) or corrected ($p < 0.02$) basis.

HESS et al.[2,11] have shown that both Su-13,437 and CPIB increase the level of certain types of oxidative enzyme activity (catalase, cytochrome oxidase) in rat liver. Our findings with CPIB[3] show no significant increases over normal of liver acid phosphatase or β-glucuronidase activity, suggesting that the increased oxidation of cholesterol is not due to lysosomal activity. The possibility that the high level of oxidation of cholesterol by liver mitochondrial preparations from rats fed CPIB or Su-13,437 in the absence of cytosol may be a consequence of the large increase in liver protein is under investigation.

Table II. Influence of 2-methyl-2-(*p*-1,2,3,4-tetrahydro-1-naphthylphenoxy) propionic acid feeding (0.3% for 3 weeks) on oxidation of 26-^{14}C-cholesterol to $^{14}CO_2$ by rat liver mitochondria

Group[a]	Cytosol	% Oxidation Gross	Correction for mg/*N*
Experiment 1			
S	+	10.2 ± 1.3	5.8 ± 1.1
C	+	9.2 ± 1.2	
Experiment 2			
S	+	14.4 ± 1.2[b]	8.2 ± 0.7[c]
C	+	6.4 ± 0.8	
S	–	10.6 ± 1.0[b]	6.0 ± 0.5[d]
C	–	4.0 ± 0.5	

Average of 6 incubations.
[a] S, experimental group; C, control; [b] $p < 0.001$; [c] not significant; [d] $p < 0.02$.

Zusammenfassung. Es wird gezeigt, dass bei männlichen Wistar-Ratten im 3-Wochen-Diätexperiment mit Zusatz von 0,3% 2-Methyl-2-(*p*-1, 2, 3, 4-Tetrahydro-1-naphthylphenoxy)-Propionsäure (Su-13437) die Oxydation von 26-^{14}C-Cholesterin zu $^{14}CO_2$ durch Lebermitochondrien (bezogen auf mg/*N*) ähnlich wie bei den Kontrollen war. Bei den Versuchstieren war jedoch bei Abwesenheit von Cytosol im System die Oxydation durch Lebermitochondrien erhöht.

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