Table II. Metabolism of glucose and glucose-6-phosphate

Substrate concentration (mM)	Glucose 5.5	55.0	Glucose-6-phosphate 6.6	66.0
Glycogen and oligosaccharides	331 ± 32	2440 ± 160	15 ± 5	208 ± 7
Glucose and Hexose phosphate esters	135 ± 12	2628 ± 163	29 ± 5	1798 ± 126
Lactate	764 ± 58	1852 ± 93	334 ± 96	2520 ± 46
Phosphoglycerate	96 ± 16	556 ± 63	166 ± 13	2522 ± 63

Results are expressed as nmoles of converted and unchanged substrate/100 mg of tissue wet weight. Mean values of 5 experiments ± S.E. mean are reported.

66.0 mM level. The amount of radioactive carbon from glucose increases in the tissue proportionally to the external concentration, while in the case of the hexose ester an appreciable amount is found only at the higher concentration.

The differences in ratios of incorporation of ^{14}C into CO_2 and lactate with respect to glycogen, shows that glucose-6-phosphate was utilized as such, rather than rapidly hydrolyzed to glucose.

Assuming that 2 compartments coexist, it seems likely that glucose-6-phosphate would not enter each compartment with equal facility. In this case, the cellular system should be more affected by factors able to modify the equilibrium constants of the enzymes than by concentration factors. The results obtained, on the other hand, clearly show that mass effects are more important (Tables I and II) than possible regulatory actions of glucose-6-phosphate. If this action was to be limited by glucose-6-phosphate concentration in the cell, the ratios must remain unaffected by changes in glucose-6-phosphate concentration in the medium. The concentration effects could be due to a permeability factor. Glucose-6-phosphate, in fact, requires a threshold concentration[8–10] before it can be utilized. If the cells discriminate to a greater or lesser extent against the entry of substances from the external medium, this phenomenon would not require different effects, experimentally not evidenced, on the 2 compartments. At the higher concentration, instead, the 2 pools, if they exist independently of the experimental conditions, are completely mixed.

Riassunto. È stato studiato l'effetto della concentrazione sul metabolismo del glucosio e del glucosio-6-fosfato nel diaframma isolato di ratto. Si discute brevemente della esistenza di due pools di glucosio-6-fosfato in relazione alle diverse condizioni sperimentali.

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Alterations in the Activity of Glyceraldehyde-3P-Dehydrogenase of the Rat Liver as a Function of Age

Alteration in the activities of several enzymes are known to occur in the tissues of aging animals[1–4]. Our earlier reports[5,6] on the lactate and malate dehydrogenases of the heart, brain, skeletal muscle and liver of rats have shown that the tissues become more aerobic during old age. The present study on glyceraldehyde-3P- dehydrogenase (gly-3P-DH) shows that the activity of Krebs cycle is increased in old age of the rat and supports the above conclusion.

The liver of 6-, 12-, 33- and 70-week-old male albino rats of Wistar strain was taken out and a 20% (w/v) homogenate of the tissue was prepared in 0.25M sucrose at 0–2 °C using a Potter-Elvehjem homogenizer and teflon pestle. The oxygen consumption and the percent inhibition of oxygen consumption by iodoacetic acid (IAA; 0.125M) of the liver homogenate were measured manometrically using an Aminco Warburg Respirometer. The total volume of the incubation medium was 3.0 ml which included 0.9 ml of sucrose (0.25M), 0.4 ml of phosphate buffer (0.066M; pH 7.4), 0.1 ml of $MgCl_2$ (0.1M), 1.0 ml of homogenate, 0.5 ml of glucose (0.1M) and 0.1 ml of IAA (0.125M final concentration). The centre well contained 20% KOH. The temperature of measurement was 37 °C and the gas phase was air. Readings were linear for 60 min after which the oxygen consumption was measured as ml O_2/g wet wt./h. The effect of IAA was expressed as percent inhibition of oxygen consumption.

The Table shows that the rate of oxygen consumption of the liver increases significantly with development of the rat up to 12 weeks after which it decreases significantly up to 33 weeks. Thereafter, there was no significant

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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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