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DIFFERENTIATION OF LIVER METABOLISM ON THE MOLECULAR LEVEL DURING CHRONIC APPLICATION OF HALOTHANE

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Abstract—Mice were treated with Halothane (0·5% in O_2) 1 hr daily for 4 weeks. During this period, enzyme activities and substrate contents were measured in the liver and compared with the livers of untreated mice. During Halothane treatment, the relative liver weight increased rapidly (30–50 per cent in the first week) while protein and glycogen content of the liver and the general enzyme pattern remained constant (related per g liver wt.); that means that a coordinate growth occurs.

Only few enzymes changed in their activity; there was a decrease in the enzyme activity of pyruvate kinase and an increase of malic enzyme and glycerol-1-phosphate oxidase activity. The substrate content in the trioses of the Embden Meyerhof chain was diminished, whereas substrates of the citric acid cycle were increased. The effects observed are discussed with respect to the observations made by other authors and compared with the effects after barbiturate treatment.

A YEAR ago, Kunz and Siess reported that the livers of mice were greatly enlarged after the chronic application of small doses of Halothane (bromochlorotrifluoroethane).[1] The enlargement of the whole liver amounted to more than 50 per cent in 2 weeks. In these experiments, the concentration of Halothane remained fixed at 0·5% Halothane in O_2 in a subnarcotic range and the exposure of the animals was

Abbreviations used in this paper are as follows:

Enzymes	
G6PDH	Glucose-6-phosphate dehydrogenase
GAPDH	Glyceraldehyde phosphate dehydrogenase
PGK	Phosphoglycerate kinase
GDH	Glycerol-1-phosphate dehydrogenase
GPT	Glutamate-pyruvate transaminase
GOT	Glutamate-oxaloacetate transaminase
GPox	(mitochondrial) Glycerol-1-phosphate oxidase
T-IDH	NADP (TPN)-depending isocitrico dehydrogenase
LDH	Lactate dehydrogenase
ME	Malic enzyme
M-MDH	(mitochondrial) Malate dehydrogenase
Enzymes	
C-MDH	(cytoplasmic) Malate dehydrogenase
PK	Pyruvate kinase
PGK	Phosphoglycerate kinase
Substrates	
G6P	Glucose-6-phosphate
F-1,6-P	Fructose-1,6-phosphate
GAP	Glyceraldehyde phosphate
DAP	Dihydroxyacetone phosphate
α-GP	Glycerol-1-phosphate
3-PG	3-Phosphoglycerate
2-PG	2-Phosphoglycerate
PEP	Phosphoenolpyruvate
α-KG	Ketoglutarate
Oxalac.	Oxaloacetate
L/P	ratio Lactate/pyruvate
G/D	ratio Glycerol-1-phosphate/ dihydroxyacetonephosphate

only 1 hr daily. According to the histological examination, the authors described the development of fatty livers, but only a small amount of cell-destruction or necrosis could be observed (compare also refs. 2, 3 and for clinical aspects 4–8).

The analysis of the livers of Halothane-treated animals by the electron microscope showed differences in the structure of mitochondria (Vogell[9]). These mitochondria had a more spherical form and the matrix space was enlarged.

Independently of the question of the possibly toxic nature of this narcoticum, it is of common interest to study the induction of liver growth by Halothane and to compare this inductive step with the well known liver enlargement after, e.g., the application of barbiturates. That was the motive for our repeating the experiments with Halothane under the same experimental conditions as described earlier.[1]

We measured, in livers of Halothane-treated mice, enzyme activities and substrate contents which are linked with important steps in the metabolic chains (Fig. 1).

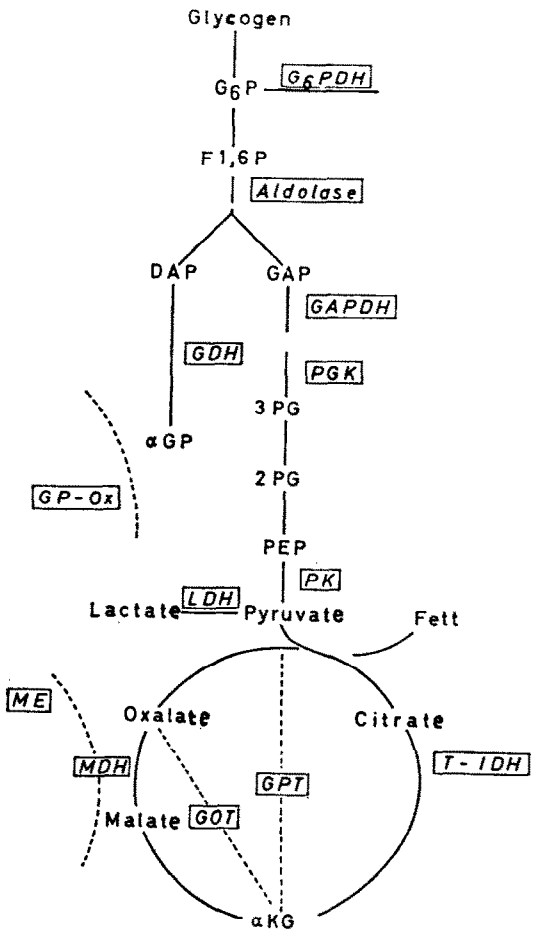

FIG. 1.

MATERIALS AND METHODS

Mice, strain NMRI—Tübingen, Kissleg (Germany), weighing 20–25 g at the beginning of the experiments, were exposed to Halothane (0·5% in O_2) for 60 min daily. The mice showed no signs of narcosis or abnormal behaviour during Halothane exposure. The animals were fed with a standard mouse diet (Fa. Laats) and water *ad libitum*. Every second day, two mice were removed from the group for analysis. Under slight ether anaesthesia, a part of the liver was taken off for substrate measurements with Wollenberger clamps.[10] The rest of the liver was subjected to enzyme analysis and the determination of liver weight. The determination of substrates was carried out as described by others.[11] The enzyme activities in the supernatant of an Ultraturrax homogenate were tested after centrifugation at 80,000 *g* for 10 min (0°, Spinco).

The preparation of glycerol-1-phosphate oxidase (GPox) used for measurements was prepared as follows: fresh samples of mouse livers were carefully homogenized with a glass Teflon Potter homogenizer in 0·1 M phosphate buffer, pH 7·2 (volume: sample = 10:1). The homogenate was centrifuged, the supernatant was discarded and the sediment re-homogenized in the phosphate buffer with the Ultraturrax homogenizer. The activity of the GPox was tested in the remaining whole homogenate as described elsewhere.[12] Neutral fat was determined by enzymatic determination of glycerol after alkaline hydrolysis, adapting the method of Kreutz[13] for tissue samples. The soluble protein was measured according to the Biuret method,[14] calculating with a factor $F = 16{\cdot}5$.

RESULTS

The changes described here, in liver weight, substrate contents and enzyme activities, develop progressively during Halothane treatment. In order to distinguish the effects and establish a time sequence for the observed changes we have summarized the results weekly and compared average weekly values in tables.

Liver weight and substrate content

We can confirm the observations of Kunz and Siess that livers of mice greatly enlarge within a few days of Halothane treatment (Table 1). After only 1 week the increase in the relative liver weight amounts to 27 per cent, rising to 62 per cent in the second week. This value seems to be limit of induced enlargement attainable under these experimental conditions, because the further increase in liver weight during the next two weeks is small (see Table 1).

The content of neutral fat, glycogen and soluble protein does not change in the same manner. The content of neutral fat rises continually during Halothane treatment. In livers of untreated mice, we find a mean value of 25 μmoles neutral fat/g wet wt. After an experimental period of 4 weeks' Halothane treatment, the content of neutral fat increases by 290 per cent In spite of the increase in fat and of the fast liver growth, the glycogen level and the content of soluble protein do not change in that period (differences in the glycogen content, given in the tables, are statistically not significant).

Table 2 shows data of the content of special intermediates, most of them belonging to the Embden–Meyerhof chain and the citric acid cycle. In general, we find a decrease

in the content of trioses of the glycolytic pathway and an increase of substrates of the citric acid cycle. Maximum values of the changes described are reached in the third week. After that time, hydrogenated substrates of the glycolytic pathway, such as glycerol-1-phosphate and lactate, begin to rise.

TABLE 1. COMPARISON OF SOME GENERAL PARAMETERS OF THE LIVERS OF HALOTHANE-TREATED AND UNTREATED ANIMALS

Conditions	Liver wt. (in % of body wt.)	Neutral fat (μmoles/g wet wt.)	Content of soluble protein (mg/g wet wt.)	Glycogen content (μmoles/g wet wt.)
Untreated animals	4·5	25	92	197
Halothane				
1 week	5·7 (+ 27%)	45 (+ 80%)	90	220
2 weeks	7·3 (+ 62%)	66 (+ 164%)	88	190
3 weeks	7·2 (+ 60%)	62 (+ 250%)	86	240
4 weeks	7·7 (+ 71%)	97 (+ 290%)	87	250

TABLE 2. CONTENT OF SUBSTRATES IN LIVERS OF MICE DURING CHRONIC EXPOSURE OF HALOTHANE, COMPARED WITH UNTREATED ANIMALS

Substrate (mμmoles/g wet wt.)	Untreated mice	Halothane-treated animals				Substrates, which do not change markedly during Halothane treatment: control values (mμmoles/g wet wt.)	
		1 week	2 weeks	3 weeks	4 weeks		
Glycerol-1-phosphate	250	207	184	152	340	Dihydroxyacetone-phosphate	35
3-Phosphoglycerate	180	110	130	53	115	Glyceraldehyde-phosphate	47
Phosphoenolpyruvate	86	60	56	38	51	Glucose-6-phosphate	295
Pyruvate	140	115	70	45	56	Fruktose-1,6-phosphate	13
Lactate	2200	2100	1600	1200	2300	ATP	2460
Malate	730	1450	1350	1400	1100	ADP	1080
Citrate	320				560	AMP	830
Ketoglutarate	55	70	80	100	60	Adenine nucleotides	4370
L/P	15	18	23	27	41		
G/D	7	6·5	4·9	5·5	8		
ATP/ADP	2·3	2·7	3·1	3·3	2·2		

The ratio lactate/pyruvate (L/P) increases continuously during Halothane treatment, independently of the changes in lactate levels. The ratio of the redox system glycerol-1-phosphate/dihydroxyacetone phosphate (G/D) decreases at first (as a consequence of the decreased GP-levels) and begins to rise at the end of the experimental period.

The ratio ATP/ADP, used as an approximative term for the energy pool of the liver metabolism, shows a tendency to rise during Halothane treatment. The controlling value of the ratio ATP/ADP of 2·3 is probably too low owing to an experimental failure (Table 2). Mice seem to be more sensitive to ether anaesthesia than rats.

We can assume from our data that in the livers of untreated mice the ratio ATP/ADP is about the same as that in the livers of rats.[15] That means that the ATP/ADP ratios, shown in Table 2, are generally shifted to lower values under our experimental conditions.

Enzyme activities

In Table 3, the activities of the enzymes tested are summarized. From all the enzymes measured, only three show a definite change in their activity, namely the enzymes pyruvate kinase, the malic enzyme and glycerol-1-phosphate oxidase. The activities of pyruvate kinase and the malic enzyme change very rapidly but in opposite directions. In the first week, the activity of pyruvate kinase decreases by about 35 per cent whereas the activity of the malic enzyme increases by 58 per cent (mean values). The activity of the glycerol-1-phosphate oxidase (GPox) begins to rise in the second week and increases up to 140 per cent by the end of the experimental period. In contrast to this enzyme, which is located in mitochondria, the activity of the cytoplasmic glycerol-1-phosphate dehydrogenase does not change to the same extent (increasing by only 30 per cent).

DISCUSSION

One of the most remarkable effects of the chronic application of Halothane is the rapid enlargement of the liver. While the liver weight is nearly doubled in a period of 3 weeks, the content of soluble protein and the whole pattern of enzyme activities remain practically constant (related to g wet wt. and mg protein). This means that a very balanced and coordinated growth of the organ takes place.

The second important point is that we find only very few and distinctive changes, attested by enzyme activities and substrate contents, in the metabolism of the liver. These distinct effects make it possible to describe the changes observed as a special differentiation in the liver metabolism during chronic Halothane treatment.

The tables show some parallelism, in time sequence and direction, between changes in enzyme activities and those observed in the substrate contents. This consideration includes questions of cellular coordination and compartmentation of metabolic chains and therefore the interpretation of the observed effects seems to be difficult.

We note for instance from our tables that the considerable increase in the content of malate is accompanied by changes in the activity of the malic enzyme (ME) only, the activity of the malate dehydrogenase (MDH) is not affected (i.e. the sum of M-MDH and C-MDH; a shift in the distribution of the activity within the compartments would, theoretically, be possible). We can assume that a doubled or tripled subtrate content should not influence the capacity of the very active MDH. The activity of the ME amounts only to 1/100 that of the MDH. But our data give no further information between a direct relationship of the high malate level (and other substrates of the citric acid cycle) and the increase of the activity of the cytoplasmic ME.

The same uncertainty occurs for the decrease of triose phosphate levels in relation to the decrease of the activity of pyruvate kinase (PK). It is probable that there exists a relationship of these partners of the same metabolic chain. At present we don't know the first step of this chain reaction. It is surprising that only one of the kinases,

TABLE 3. ENZYME ACTIVITIES (I.U. $\times 10^2$) IN LIVERS OF UNTREATED MICE AND CHANGES IN ENZYME ACTIVITIES DURING CHRONIC APPLICATION OF HALOTHANE

	Enzyme activity					
	Changing during Halothane treatment				Unchanged	
	Pyruvate kinase	Malic enzyme	Glycerol-1-phosphate oxidase	Glycerol-1-phosphate dehydrogenase	Glucose-6-P dehydrogenase Aldolase Glyceraldehyde-P-dehydrogenase	130 232 11,000
Untreated Halothane	2310	266	100	3460	3-P-Glycerate kinase Lactate dehydrogenase	13,000 14,500
1 week	1500 (−35%)	420 (+58%)	95 (−5%)	3900 (+13%)	Isocitricodehydrogenase (NADP-dependent)	2020
2 weeks	900 (−61%)	950 (+260%)	162 (+62%)	4700 (+36%)	Malate dehydrogenase	35,500
3 weeks	1100 (−53%)	860 (+220%)	214 (+114%)	4500 (+30%)	Glutamate-oxaloacetate transaminase	9180
4 weeks	840 (−63%)	100 (+275%)	240 (+140%)	4600 (+33%)	Glutamate-pyruvate transaminase	521

the PK, is affected and not also the phosphoglycerate kinase (PGK). This difference could be a question of different turnover of the enzymes.

It seems that most of the observed effects during Halothane treatment concern the mitochondrial compartment or reactions linked with it. That applies to the increase of substrates in the citric acid cycle, increased content of cytochrome *c* in liver mitochondria during Halothane treatment,[16] a decrease of the content of NADPH in liver mitochondria,[17] the electron microscopic findings mentioned earlier,[9] the increase of the activity of the malic enzyme as a connecting link between cytoplasmic and mitochondrial compartments[18] and the increase of the activity of the glycerol-1-phosphate oxidase (GPox).

The rise of GPox activity is of particular interest. This enzyme, hormonally controlled by the thyroid gland,[19, 20] operates also as an essential part of the glycerol-1-phosphate cycle between the cytoplasmic and mitochondrial compartment. Changes in the activity of this enzyme have been reported only rarely and mainly occurred under hormonal influence. If the increase of substrates in the citric acid cycle indicate a reduction of hydrogen transport from this cycle to the respiratory chain, the increased activity of the GPox could be regarded as a compensation for direct hydrogen transport from the glycolytic pathway to the respiratory chain. In general the liver enlargement and the special differentiation of liver metabolism can be regarded as an adaptive and compensatory process caused by a partial poisoning of the liver by Halothane (probably through products originating from decomposition).

We have no indication so far, that under Halothane, the endoplasmic reticulum is included in the differentiation. The predominant participation of mitochondria in the changes of metabolism during Halothane treatment distinguishes, to a certain degree, this type of liver enlargement from that caused by barbiturates or insecticides.[21–24] Liver growth under the application of barbiturates is also more than a net increase of the endoplasmic reticulum.[25] But changes in structure and enzyme systems of microsomes are predominant. That means there are two general types of drug induced, compensatory liver growth, one more or less affecting the oxidative metabolism—the mitochondria, the other the biological transformation—the microsomes.

The fact that only few and distinctive changes occur in metabolism during chronic treatment of Halothane make this model of interest in respect to the (unknown) first action inducing liver growth and to study the coordination of metabolic pathways observing the phenomenons of compensation.

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