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PRELIMINARY COMMUNICATIONS

ALKANE FORMATION DURING LIVER MICROSOMAL LIPID PEROXIDATION

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During treatment of animals with the hepatotoxin carbon tetrachloride (CCl_4) short-chain alkanes, e.g. ethane and n-pentane, are formed (1 - 6). It is now generally accepted that these alkanes originate from the decomposition of lipid hydroperoxides (7). However, studies with model compounds demonstrated that metal ions, e.g. iron, are involved in the breakdown of lipid hydroperoxides to alkanes (8, 9). Because we were interested whether metal ions are also involved in CCl_4-induced lipid peroxidation we studied the effect of ferrous ions on CCl_4-induced alkane formation in rat liver microsomes. This could be important, because lipid peroxides are fairly stable in absence of metal ions (10). First of all we had to develop an in vitro system which gives reproducible measurements of alkanes during microsomal incubation. Furthermore, we had to examine whether these alkanes are indeed formed in hepatic microsomes due to CCl_4. On the other hand, we had to find out what the optimal iron concentration in this system would be.

METHODS

Rat liver microsomes were prepared according to standard procedures (11). The 5 ml microsomal incubation mixture (1 mg protein/ml) was essentially the same as previously described (12) and contained a NADPH-regenerating system. Incubations were carried out in special incubation flasks at 37° (Figure 1). Before the incubation, we allowed synthetic air (hydrocarbon-free) to stream through the incubation flask containing Tris-KCl-buffer and microsomes by moderate evacuation (Figure 1). This removed all contaminants and replaced the laboratory air. Then the vacuum pump was disconnected by removal of needle 12 (Figure 1). After 3 min pre-incubation all reagents were added to the microsomal mixture using syringes and needles stuck through the septum. Before each gas sampling the atmosphere above the incubation mixture (19 ml) was diluted with 8 ml synthetic air. This was attained by removing 8 ml of the head space gas via the sampling syringe, as 8 ml synthetic air bubbled through the water barrier 5 (Figure 1). The design allowed a homogenous distribution of the atmosphere by pumping the 8 ml gas present in the syringe backward and forward for several times. Afterwards, from the 8 ml sample 5 ml were analysed for ethane, propane and n-pentane by gas chromatography as already described (3) using a temperature program of 90° - 200° with a heating rate of 12°/min. The amounts of alkanes formed per mg microsomal protein were calculated using calibration gases (3) taking into account the dilutions and the removals.

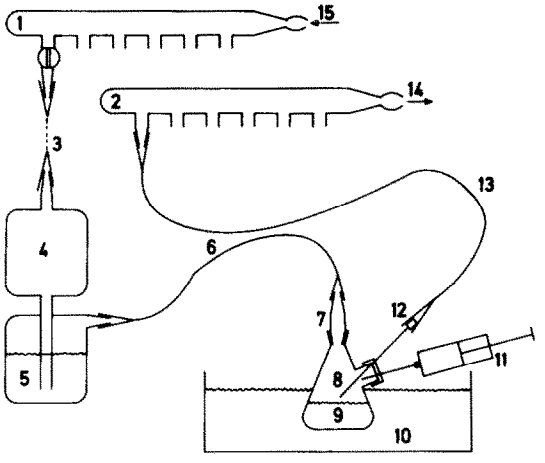

Figure 1: Scheme of the system used for microsomal incubations and for sampling of alkanes. The all-glass part (1) was connected to a synthetic air supply (15) and via teflon tubes (3) to six special glass flasks of 33 ml (4) containing 15 ml H_2O (5). Each was connected to an 22 ml Erlenmeyer flask (8) via teflon tube (6) and ground glass (7). The Erlenmeyer flask with side arm, screw and teflon-coated silicon septum contained the microsomal mixture (9) which was shaken in a water bath of 37° (10). A needle (12) connected to another all-glass part (2) and to a vacuum pump (14) via teflon tube (13) was stuck through the septum. Another needle connected to the gas sampling syringe (11) was also stuck through the septum.

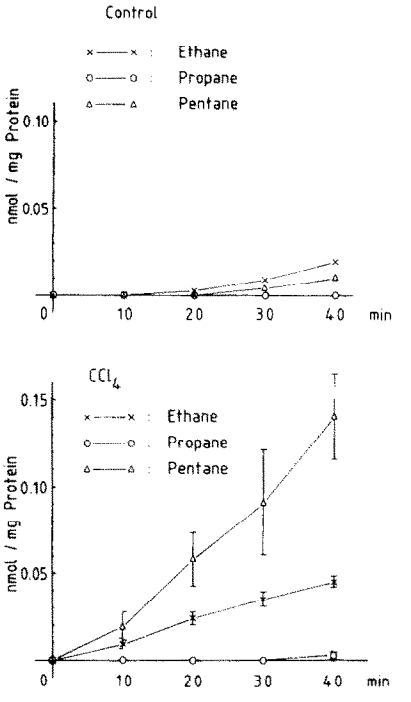

Figure 2: Ethane, propane and n-pentane formation in hepatic microsomes incubated in presence of a NADPH-regenerating system without (upper part) and with (lower part) addition of CCl_4 (10 mM). In general, mean values ± S.D. of experiments with microsomes from 4 different rats are shown.

RESULTS AND DISCUSSION

Our results demonstrate that the system used for microsomal incubations and alkane sampling (Figure 1) gives reproducible measurements of ethane, propane and n-pentane. In contrast to other systems used during in vitro incubations and alkane measurements (1, 13, 14) the system allows to remove several gas samples from the same incubation flask.

We demonstrate for the first time that CCl_4 induces ethane, propane and n-pentane formation in hepatic microsomes and that this alkane formation depends on NADPH supply (Figure 2). Additionally, we could show that microsomal alkane formation due to ferrous ions is also NADPH-dependent (Figure 3). Furthermore, we found that the iron concentration dependence curve leads to a plateau of alkane formation at similar ferrous ion concentrations as already described for malondialdehyde (15). On the other hand, from Table 1 it is obvious that Fe^{2+} increases the CCl_4-induced alkane formation in microsomes, the effect not being solely attributable to Fe^{2+}-initiated lipid peroxidation. The same was true for the small "endogenous" alkane formation (without CCl_4). This indicates that besides the involvement of Fe^{2+} in the initiation of alkane formation Fe^{2+} catalyses the breakdown of already pre-formed lipid peroxides to alkanes. This effect of iron ions has already been described for malondialdehyde formation (10).

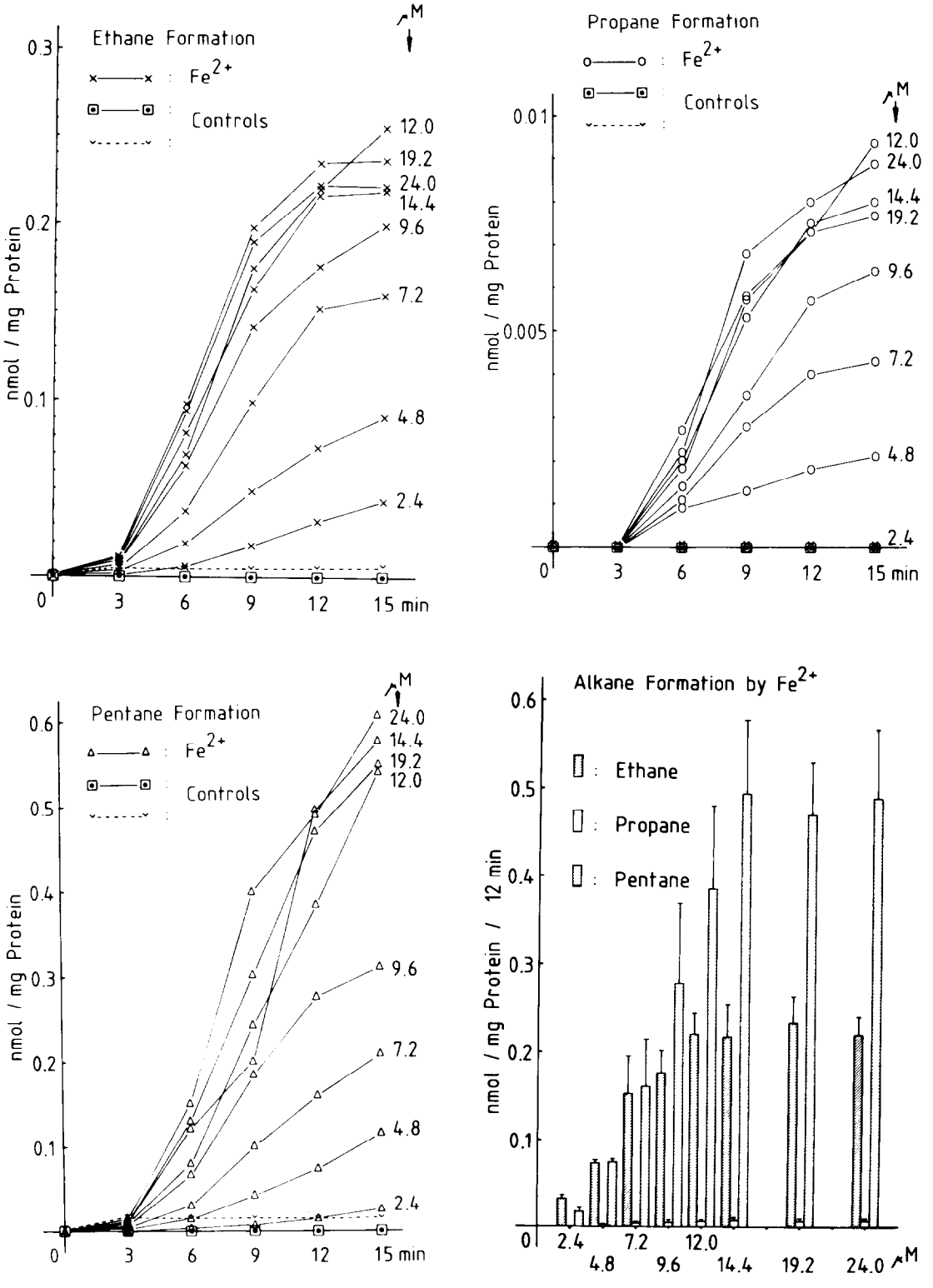

Figure 3: Ethane, propane and n-pentane formation in hepatic microsomes incubated in presence or absence of a NADPH-regenerating system with different concentrations of $FeCl_2 \cdot 4H_2O$. Control incubations were carried out either with $FeCl_2$ (24 uM), but without NADPH, or with NADPH, but without $FeCl_2$. The bottom right panel shows mean values ± S.D. of experiments with microsomes from 4 different rats, whereas all other panels represent typical experiments.

Table 1: Liver Microsomal Alkane Formation

Additions at zero time	after 30 min incubation	pmol/mg microsomal protein/33 min ethane	propane	n-pentane
NADPH	-	4 ± 1	n.m.	6 ± 2
10 mM CCl_4	-	9 ± 2	n.m.	4 ± 2
NADPH	24 µM $FeCl_2$	66 ± 9	2 ± 1	86 ± 9
NADPH + 10 mM CCl_4	-	28 ± 3	n.m.	58 ± 6
NADPH + 10 mM CCl_4	24 µM $FeCl_2$	112 ± 22	5 ± 1	236 ± 38

Mean values ± S.D. of experiments with microsomes from 4 different rats are shown. n.m. = not measurable. When $FeCl_2$ and the NADPH-regenerating system were added at zero time 9 ± 1 pmol ethane, no propane and 11 ± 2 pmol n-pentane were formed per mg microsomal protein within 3 min (see also Figure 3).

We suggest that iron ions play an important role in the propagation step of microsomal lipid peroxidation, also when induced by CCl_4. This might be relevant in vivo when the formation of various toxic products formed during breakdown of lipid peroxides is concerned.

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