Acute Effects of Carbon Monoxide on the Metabolism of Perfused Rat Liver

By DAVID L. TOPPING*

Department of Cardiovascular Pharmacology, Tobacco Research Council Laboratories,
Otley Road, Harrogate, North Yorkshire HG3 1PY, U.K.

(Received 3 September 1975)

1. Perfusion of livers with whole blood containing carboxyhaemoglobin decreased hepatic O₂ consumption and triglyceride secretion and raised free fatty acid oxidation.
2. Perfusion with [¹⁴C]carboxyhaemoglobin indicated that there was negligible hepatic uptake of ¹⁴CO.
3. The observations appear to be due to a decrease in O₂ consumption rather than to specific effects of carboxyhaemoglobin.

Correlations have been made between both smoking (Jenkins *et al.*, 1968) and hypertriglyceridaemia (Tzagournis *et al.*, 1968), and coronary heart disease. The liver is the major source of plasma triglycerides present in VLD lipoproteins† arising from the esterification of free fatty acids (Stein & Shapiro, 1959; Havel *et al.*, 1962; Mayes & Felts, 1967). Nicotine and tobacco smoke raise the concentrations of plasma free fatty acids (Kershbaum, 1968). The nicotine-induced rise in free fatty acids is accompanied by increased secretion of plasma triglycerides, probably by the liver (Topping & Turner, 1975). Exposure to CO, a tobacco smoke constituent, increases diet-induced atherogenesis and it has been proposed that CO is the causative link between smoking and coronary heart disease (Astrup *et al.*, 1970).

It was therefore decided to investigate the effects of carboxyhaemoglobin on hepatic metabolism and VLD-lipoprotein secretion. Rat livers were perfused at raised concentrations of carboxyhaemoglobin (Astrup *et al.*, 1970) and free fatty acids encountered in human smokers. To determine whether any metabolic effects of carboxyhaemoglobin were due to a transfer of CO to the liver, a separate series of experiments was performed with [¹⁴C]carboxyhaemoglobin and the uptake of ¹⁴CO measured.

Materials and methods

Livers from fed male Wistar albino rats (340–360 g) were perfused *in situ* for an experimental period of 90 min with 100 ml of defibrinated, dialysed whole rat blood by a modification of the method of Mayes & Felts (1966) for perfusion of the isolated organ. The rate of perfusion was 12 ml/min and the inflow pO₂ was maintained at 90–110 mmHg (12–13 kPa). Where the perfusate contained carboxyhaemoglobin,

10 ml of blood saturated with CO+CO₂ (95:5) was added at the start of the experiment which gave a concentration of approx. 12% of total haemoglobin. Less than 2% of the CO present was shown to be in solution in serum. To maintain blood concentrations of carboxyhaemoglobin, 3 ml of blood saturated with CO+CO₂ was added to the perfusate at 30 min intervals. In control perfusions blood was equilibrated with air+CO₂ (95:5) and added to the perfusate as described. Livers were continuously infused with [1-¹⁴C]oleate as a complex with bovine serum albumin (Topping & Mayes, 1972). The concentration of haemoglobin was measured spectrophotometrically in 10 µl of blood. Perfusate CO was determined by g.l.c. (Klimisch & Meissner, 1972) and blood carboxyhaemoglobin content calculated assuming a binding of 1.39 ml of CO/g of haemoglobin (Gregory, 1974). Blood O₂ content was determined polarographically and hepatic O₂ consumption calculated from blood samples taken from the hepatic portal vein and hepatic vein at the end of the experiment.

When livers were perfused with [¹⁴C]carboxyhaemoglobin, blood was equilibrated with 300 ml of CO+CO₂ (95:5) containing 500 µCi of ¹⁴CO (The Radiochemical Centre, Amersham, Bucks., U.K.) and added to the perfusate as described above. Livers were washed free of blood with approx. 25 ml of 0.9% NaCl and combusted in a stream of O₂ at 750°C. ¹⁴CO₂ so formed was trapped in ethanolamine and counted for radioactivity. Perfusate ¹⁴CO was released and similarly converted into ¹⁴CO₂ for calculation of specific radioactivity.

All other analytical techniques and relevant details have been described previously (Topping & Mayes, 1972).

Results and discussion

Addition of CO-saturated blood did not alter the rate of perfusion or the gross appearance of the liver.

* Present address: Isotope Unit, Royal Veterinary College, Royal College Street, London NW1 0TU, U.K.

† Abbreviation: VLD lipoproteins, very-low-density lipoproteins (*d* < 1.006).

Table 1. *Effects of carboxyhaemoglobin on O₂ consumption and carbohydrate metabolism of perfused rat liver*

Livers from fed rats were perfused *in situ* with whole blood for an experimental period of 90 min. Blood saturated with CO was added as indicated to give carboxyhaemoglobin concentrations of 12% of total haemoglobin. Free fatty acid (oleate) was infused as a complex with albumin to maintain concentrations of approx. 0.8 $\mu\text{mol/ml}$ of serum. Values are shown as the means $\pm$ S.E.M. of determinations in the steady state (30–90 min), except O₂ consumption and liver glycogen, which are terminal values; the numbers of observations are in parentheses.

Additions to perfusate	Perfusate haemoglobin (g/100ml of blood)	Perfusate carboxy-haemoglobin (% of total)	O ₂ consumption (ml/h per g of liver)	Perfusate glucose ($\mu\text{mol/ml}$)	Perfusate lactate ($\mu\text{mol/ml}$)	Liver glycogen (% of wet wt.)
None (controls)	13.3 $\pm$ 0.4 (4)	<0.5 (4)	7.22 $\pm$ 0.28 (3)	9.60 $\pm$ 0.03 (4)	3.38 $\pm$ 0.14 (3)	1.9 $\pm$ 0.3 (4)
Carboxyhaemoglobin	13.7 $\pm$ 0.4 (4)	12.2 $\pm$ 0.9 (4)	5.91 $\pm$ 0.25 (4)	9.60 $\pm$ 0.07 (4)	3.00 $\pm$ 0.24 (4)	1.4 $\pm$ 0.1 (4)

($P < 0.05$)

The weight of the liver at the end of perfusion (average 11.7 g) and the mean rate of bile production (1.3 ml/h per liver) did not differ between the groups.

Blood gases. (a) *Perfusate carboxyhaemoglobin.* Mean total haemoglobin concentrations declined slightly during the experiment owing to the diluting effect of the infusion, and so although blood CO content stayed constant, carboxyhaemoglobin concentrations rose slightly. The mean concentration was calculated to be 12.1% in the carboxyhaemoglobin group. Less than 0.5% of the perfusate haemoglobin was combined with CO in control perfusions (Table 1).

(b) *Hepatic O₂ consumption and perfusion with [¹⁴C]carboxyhaemoglobin.* In all perfusions the $p\text{O}_2$ of the perfusate was 90–110 mmHg and total perfusate haemoglobin (including that combined with CO) did not differ between the groups. Hepatic O₂ consumption was significantly decreased from 7.2 ml/h per g of liver in controls to 5.9 ml/h per g of liver in carboxyhaemoglobin-perfused livers (Table 1). This fall in O₂ consumption is that which may be expected from the combination of CO with haemoglobin and the resulting shift in the O₂ dissociation curve of rat blood (Gray & Steadman, 1964) calculated according to Roughton & Darling (1944). Although Haldane (1895) concluded that CO toxicity at high concentrations of carboxyhaemoglobin was due to a fall in the O₂-carrying capacity of the blood, it was possible that the observed effects were due to a transfer of CO to liver haemoproteins. CO is an inhibitor of the mitochondrial respiratory chain (Chance *et al.*, 1970), and Tobias *et al.* (1945) reported that in human volunteers significant amounts of [¹⁴C]carboxyhaemoglobin were located in the extravascular compartment of the liver. When three livers were perfused with [¹⁴C]carboxyhaemoglobin at 10.1 $\pm$ 0.2% of total haemoglobin, 3.7 $\pm$ 1.0 μl (or 0.30 $\mu\text{l/g}$ of liver) remained in the liver. Hepatic cytochromes *P*-450 and *a*₃, of which the former has the greater affinity, may complex with CO (Estabrook *et al.*, 1970), but its distribution between them in the hepatocyte, with resulting effects on O₂ consumption, are unknown. Although the liver was perfused free

of blood, some contamination due to traces of blood, may have occurred, and it may be inferred that uptake of perfusate CO was less than that required to cause the observed decrease in hepatic O₂ consumption.

(c) *Carbohydrate metabolism.* Blood glucose and lactate remained constant throughout the experiment and perfusion with carboxyhaemoglobin did not alter their concentrations in this steady state or alter the liver glycogen content at the end of perfusion (Table 1).

Lipid metabolism. (a) *Free fatty acids.* Steady-state concentrations of serum free fatty acids did not alter between the groups (Table 2), thus hepatic uptake was unchanged by carboxyhaemoglobin.

(b) *Oxidation and esterification of free fatty acids.* Control livers oxidized some 5% of the total infused [¹⁴C]oleate to ¹⁴CO₂ and incorporated 73% into esterified products (Table 2). Perfusion with carboxyhaemoglobin significantly decreased esterification to 60% of the total label infused and increased ¹⁴CO₂ production. Calculation of the ratio (d.p.m. oxidized)/(d.p.m. esterified) showed that this was significantly ($P < 0.01$) raised from 0.068 $\pm$ 0.011 in controls to 0.122 $\pm$ 0.008 in carboxyhaemoglobin-perfused livers, emphasizing the shift towards fatty acid oxidation in the latter group. Although oxidation was raised by carboxyhaemoglobin, there was no change in the rate of ketogenesis or in the ratio [β -hydroxybutyrate]/[acetoacetate], which averaged 8.1 $\mu\text{mol/h}$ per g of liver and 1.04:1.00 respectively.

(c) *VLD-lipoprotein secretion and incorporation of [¹⁴C]oleate into liver and perfusate lipids.* Control livers secreted 7.4 μmol of triglyceride fatty acid/h per g of liver. This rate was decreased significantly to 5.9 $\mu\text{mol/h}$ per g of liver by carboxyhaemoglobin. There was a similar decrease in the incorporation of ¹⁴C-labelled free fatty acids into VLD-lipoprotein lipids from 28 to 21% of total [¹⁴C]oleate infused. In both groups 95% of the label secreted in VLD lipoproteins was in triglycerides, with only traces of radioactivity in other lipids. Carboxyhaemoglobin also decreased the incorporation of ¹⁴C-labelled free

Table 2. Effect of carboxyhaemoglobin on VLD-lipoprotein secretion and metabolism of serum ^{14}C -labelled free fatty acids by perfused rat liver

Livers from fed rats were perfused as described in Table 1 and infused continuously with $5\text{ }\mu\text{Ci}$ of $[1\text{-}^{14}\text{C}]$ oleate. The incorporation of ^{14}C -labelled free fatty acid into the various metabolic products and the secretion of VLD-lipoprotein triglycerides were measured over 90 min. Concentrations of perfusate free fatty acids were determined in the steady state (30–90 min). Values are shown as the means $\pm$ s.e.m. with the numbers of observations in parentheses.

Additions to perfusate	Perfusate free fatty acid ($\mu\text{mol/ml}$ of serum)	VLD-lipoprotein production (μmol of triglyceride fatty acid/h per g of liver)	Incorporation (%) of $[1\text{-}^{14}\text{C}]$ oleate into			
			$^{14}\text{CO}_2$	Esterified products	VLD-lipoprotein lipids	Liver lipids
None (controls)	0.94 ± 0.06 (3)	7.4 ± 0.7 (4)	4.9 ± 0.7 (4)	72.7 ± 2.1 (4)	27.5 ± 1.9 (3)	47.3 ± 2.6 (4)
Carboxyhaemoglobin	0.83 ± 0.06 (4)	5.9 ± 0.3 (3) ($P < 0.05$)	7.3 ± 0.4 (4) ($P < 0.05$)	59.9 ± 1.3 (4) ($P < 0.01$)	20.5 ± 1.5 (3) ($P < 0.05$)	38.1 ± 1.6 (4) ($P < 0.05$)

fatty acids into liver lipids from 47.3 to 38.1%. The distribution of radioactivity in chromatographically separated lipids was unchanged except that incorporation of label into triglycerides was significantly ($P < 0.05$) decreased from 28.0 to 18.7% of the total. Of the remaining $[1\text{-}^{14}\text{C}]$ oleate incorporated, more than two-thirds was in phospholipids, with only trace quantities in other lipids.

General discussion and conclusions. These data clearly demonstrate that perfusion with carboxyhaemoglobin significantly decreases hepatic triglyceride secretion and increases free fatty acid oxidation. This was probably due to a decrease in O_2 consumption, as similar changes have been shown to result from simple hypoxia (P. A. Mayes & J. M. Felts, personal communication). The mechanisms of the effect of carboxyhaemoglobin-induced hypoxia on free fatty acid metabolism remains unclear. It is unlikely that ATP became restricted because at an O_2 consumption less than that in the carboxyhaemoglobin group, its supply was not limiting to hepatic function (Hems *et al.*, 1966). Alternatively, it is suggested that the hepatic supply of *sn*-glycerol 3-phosphate was changed. Availability of this compound has been suggested to determine the balance between the hepatic oxidation and esterification of free fatty acids (Fritz, 1961; Tzur *et al.*, 1964). However, in adipose tissue there is a very poor correlation between *sn*-glycerol 3-phosphate concentration and net esterification (Denton & Randle, 1967).

Whatever the precise mechanism, the presence of carboxyhaemoglobin limited the hepatic production of VLD lipoproteins. In view of the attention focused on CO as a possible causative agent in the aetiology of coronary heart disease (Astrup *et al.*, 1970), it is suggested that CO in inspired tobacco smoke may in fact counteract the effect of nicotine-induced raised free fatty acid concentration on the hepatic secretion of triglycerides.

Excellent assistance was given by Miss J. O'Brien and Mr. M. E. Targ. CO was determined by Mr. K. Phillips and ^{14}CO by Mr. J. B. Hopper.

- Astrup, P., Kjeldsen, K. & Wanstrup, J. (1970) *Ann. N.Y. Acad. Sci.* **174**, 294–300
- Chance, B., Erecinska, M. & Wagner, M. (1970) *Ann. N.Y. Acad. Sci.* **174**, 193–204
- Denton, R. M. & Randle, P. J. (1967) *Biochem. J.* **104**, 423–434
- Estabrook, R. W., Franklin, M. R. & Hildebrandt, A. G. (1970) *Ann. N.Y. Acad. Sci.* **174**, 218–232
- Fritz, I. B. (1961) *Physiol. Rev.* **41**, 52–129
- Gregory, I. C. (1974) *J. Physiol. (London)* **236**, 625–634
- Gray, L. H. & Steadman, J. M. (1964) *J. Physiol. (London)* **175**, 161–171
- Haldane, J. S. (1895) *J. Physiol. (London)* **18**, 201–207
- Havel, R. J., Felts, J. M. & Van Duyne, C. M. (1962) *J. Lipid Res.* **3**, 297–308
- Hems, R., Ross, B. D., Berry, M. N. & Krebs, H. A. (1966) *Biochem. J.* **101**, 284–292
- Jenkins, C. D., Roseman, R. H. & Zyzanski, S. J. (1968) *Circulation* **38**, 1140–1155
- Kershbaum, A. (1968) *Acta Cardiol.* **23**, 317–329
- Klimisch, H. J. & Meissner, K. (1972) *Beitr. Tabakforsch.* **6**, 216–219
- Mayes, P. A. & Felts, J. M. (1966) *Proc. Eur. Soc. Study Drug Toxic.* **7**, 16–29
- Mayes, P. A. & Felts, J. M. (1967) *Nature (London)* **215**, 716–718
- Roughton, F. J. W. & Darling, R. C. (1944) *Am. J. Physiol.* **141**, 17–31
- Stein, Y. & Shapiro, B. (1959) *Am. J. Physiol.* **196**, 1238–1241
- Tobias, C. A., Lawrence, J. H., Roughton, F. J. W., Root, W. S. & Gregersen, M. I. (1945) *Am. J. Physiol.* **145**, 253–263
- Topping, D. L. & Mayes, P. A. (1972) *Biochem. J.* **126**, 295–311
- Topping, D. L. & Turner, D. M. (1975) *Nutr. Metab.* in the press
- Tzagournis, M., Chiles, R., Ryan, J. M. & Skillman, T. G. (1968) *Circulation* **38**, 1156–1163
- Tzur, R., Tal, E. & Shapiro, B. (1964) *Biochim. Biophys. Acta* **84**, 18–23