Short Communications

SC 2251

Inhibition of the autoxidation of unsaturated fatty acids by haematin proteins

Many investigators have demonstrated that haemoglobin and other haematin proteins have a powerful catalytic effect on the rate of oxidation of unsaturated fats and fatty acids[1,2]. Recently, however, BANKS *et al.*[3], showed that cytochrome *c* in relatively high concentrations ($3.5 \cdot 10^{-5}$ M) inhibited oxidation of unsaturated fatty acids but that $1.75 \cdot 10^{-5}$ M cytochrome *c* was an active catalyst. High concentrations of cytochrome *c* were considered to destroy peroxide.

These findings were reported whilst extensive studies on fatty acid peroxide formation were in progress in this laboratory. Our results are in agreement with those of BANKS *et al.*[3] and we have found that similar inhibitory effects can be observed with haemoglobin, haemin and tissue homogenates in addition to cytochrome *c*.

The oxidation of freshly prepared emulsions of linoleic acid (0.017 M) in phosphate buffer (pH 7.4) was followed manometrically at 37° by measuring the oxygen uptake and by determination of the peroxide formed colorimetrically[4]. Dilute ($4 \cdot 10^{-6}$ M) solutions of haemoglobin catalysed a rapid rate of oxidation but strong ($4 \cdot 10^{-5}$ M) solutions inhibited oxidation (Fig. 1). The peroxide concentration in a typical control experiment with no added haemoglobin rose in 1 h to $2 \cdot 10^{-3}$ M, whereas in presence of $4 \cdot 10^{-5}$ M haemoglobin it had fallen to 10^{-4} M. Similar results were obtained using

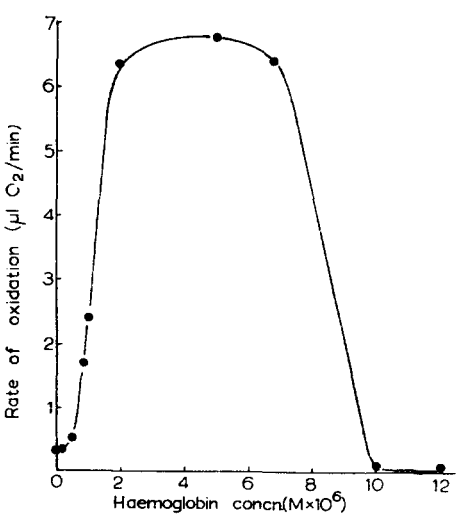

Fig. 1. Effect of haemoglobin in various concentrations on the rate of oxidation of linoleic acid emulsion. 0.017 M linoleic acid.

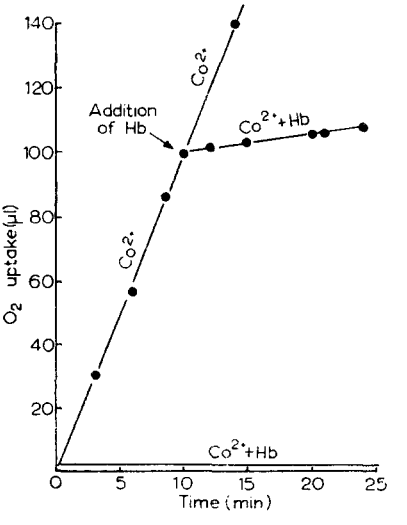

Fig. 2. Effect of haemoglobin (Hb) on Co^{2+}-catalysed linoleic acid oxidation. 0.017 M linoleic acid, $5 \cdot 10^{-5}$ M haemoglobin, $2 \cdot 10^{-4}$ M Co^{2+}.

cytochrome *c* or haemin, but stronger solutions of haemin (*e.g.* 10^{-4} M) were necessary to produce inhibition. Experiments were normally continued for a minimum period of 1 h, but if this period was extended it was found that when the concentration of haemoglobin was minimum for production of inhibition (10^{-5} M), a rapid oxidation began in some experiments after a long induction period. More concentrated solutions of haemoglobin did not initiate any oxidation of linoleic acid for at least 3 h.

The effect of haemoglobin on the rate of oxidation of the emulsion depended on the ratio of the haemoglobin concentration to the emulsion concentration, the more concentrated emulsions requiring more concentrated haemoglobin solutions for a maximum rate of oxidation. Thus, although $4 \cdot 10^{-6}$ M haemoglobin was optimal for catalysis of the oxidation of 0.017 M linoleic acid emulsion, it inhibited the oxidation of $5 \cdot 10^{-3}$ M linoleic acid and the oxidation of this dilute emulsion was effectively catalysed by $5 \cdot 10^{-7}$ M haemoglobin.

The inhibitory effect of haemoglobin could be shown most clearly by adding strong solutions ($5 \cdot 10^{-5}$ M) to emulsions of linoleic acid rapidly oxidising in presence of Co^{2+} as a catalyst. Immediate cessation of oxidation occurred (Fig. 2).

Addition of bovine serum albumin (0.1 %) or $5 \cdot 10^{-3}$ M solutions of alanine, histidine, or glutamic acid had no effect on the catalysis of linoleic acid oxidation by haemoglobin ($5 \cdot 10^{-6}$ M). Lysine, cystine, cysteine, methionine and tyrosine did not affect the final rate of oxidation but caused a delay in the period before oxidation began. Oxidation normally began immediately if no amino acid was added. For most amino acids this delay period varied between 10 and 35 min. Cysteine, however, caused a prolonged induction period which varied with the concentration of cysteine and oxidation of linoleic acid did not begin until all the cysteine had been destroyed (Fig. 3). This phenomenon has recently been discussed in detail by LEWIS AND WILLS[5].

Homogenates of rat liver, heart and spleen behaved in a similar manner to haemoglobin solutions, being effective catalysts of linoleic acid oxidation in dilute (0.5 %, w/v) suspensions and inhibitory in high concentrations (5 %, w/v). BERNHEIM AND BERNHEIM[6] showed that catalysis of the oxidation of unsaturated fatty acids of

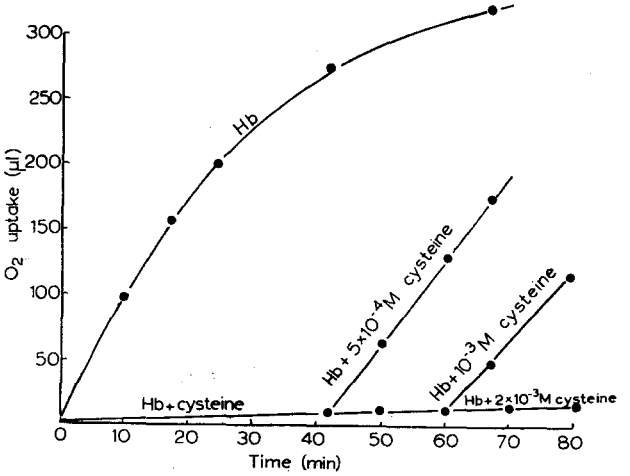

Fig. 3. Effect of cysteine on haemoglobin (Hb) catalysis of linoleic acid oxidation. $5 \cdot 10^{-6}$ M haemoglobin, 0.017 M linoleic acid.

phospholipids by liver homogenates was stimulated by vanadium. On re-examination of these results we found that vanadium (10^{-3} M vanadate) had little effect when weak homogenates (0.5 %, w/v) were used, but that the main action was to overcome the inhibitory action of concentrated homogenates (Table I). Haemoglobin inhibition cannot normally be overcome by addition of vanadium.

TABLE I

EFFECT OF VANADIUM (10^{-3} M) AND LIVER HOMOGENATES ON THE RATE OF OXIDATION OF LINOLEIC ACID EMULSION

Liver homogenate concentration (%)	*Rate of oxidation of linoleic acid (µl O_2/min)*	
	No addition	*Vanadium added*
0.25	2.7	2.7
0.5	4.5	4.5
1.0	5.15	6.6
2.5	0.25	5.7
4.0	0.20	5.15

These findings may have important biological significance. Peroxides of unsaturated fatty acids are known to be toxic to animals[7], to enzymes[8] and to oxidise –SH-containing amino acids and proteins[5]. It is clear that wherever haematin proteins are present *in vivo* in relatively high concentration no peroxide will be formed, but at lower concentrations within a certain critical range rapid catalysis of the oxidation of unsaturated fatty acids and peroxide formation can occur.

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The effect of citrate on the metabolism of acetate by sheep tissues *in vitro*

Many workers have shown that low concentrations of [^{12}C]citrate stimulate the incorporation of [^{14}C]acetate into long-chain fatty acids *in vitro*[1,2] and that higher concentrations of citrate inhibit this incorporation[3–5]. Evidence has been produced