While such an immune-mediated metabolic impairment is therefore unlikely to be directly responsible for the development of the pathophysiology of Reye's syndrome, this does not preclude the possibility that aspirin itself or a metabolic product may play a more indirect role in the aetiology of this disease.

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Comparison of the effects of Fe^{2+} and Cu^{2+} on prostaglandin synthesis in rabbit kidney medulla slices

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The effects of Fe^{2+} and Cu^{2+} on the generation of medullary prostaglandins E_2 and $F_{2\alpha}$ have been compared. Fe^{2+} markedly promoted the lipid peroxidation of rabbit kidney medulla slices. The lipid peroxidation induced by Fe^{2+} inhibited both prostaglandin E_2 and prostaglandin $F_{2\alpha}$ formation to a similar extent. While Cu^{2+} produced only a small increase in lipid peroxidation, it had a powerful inhibitory effect on prostaglandin E_2 formation. Simultaneously, prostaglandin $F_{2\alpha}$ production was increased. In the presence of Cu^{2+} the net increased amount of prostaglandin $F_{2\alpha}$ was much smaller than the net decreased amount of prostaglandin E_2 (15–20%). These results suggest that Cu^{2+} has the potential to modulate prostaglandins E_2 and $F_{2\alpha}$ synthesis by affecting endoperoxide E_2 isomerase or endoperoxide reductase independent of its peroxidative action.

The ions of a number of transition metals are effective catalysts for the rapid peroxidation of unsaturated lipids. It has been reported that cupric copper (Cu^{2+}) is an active catalyst of lipid peroxidation, and acts as a strong pro-oxidant by catalysing the decomposition of hydroperoxides (Haase & Dunkley 1969). We have reported that Cu^{2+} stimulates the lipid peroxidation in rabbit renal cortical mitochondria (Fujimoto et al 1984). We have also shown that lipid peroxidation induced by ascorbic acid and Fe^{2+} inhibits medullary generation of prostaglandin E_2 (Fujimoto & Fujita 1982; Fujimoto et al 1983). The present study was undertaken to investigate the effect of Cu^{2+} on the biosynthesis of prostaglandins E_2 and $F_{2\alpha}$ in kidney medulla slices in comparison with that of Fe^{2+}.

* Correspondence.

Materials and methods

Kidney medulla slices were prepared from male rabbits (2–2·5 kg) as described by Fujimoto & Fujita (1982). In all experiments the slices (0·4 g) were preincubated in 4·0 mL of 0·15 M KCl/0·02 M Tris-HCl buffer, pH 7·4, at 4 °C for 5 min. Following preincubation, the medium was discarded, the slices rinsed twice with the Tris-HCl buffer and incubated with the indicated concentrations of $FeSO_4$ or $CuSO_4$ at 37 °C for 30 min.

At the end of the incubation period, the slices were quickly removed from the medium, blotted lightly on filter paper, reweighed and homogenized in 5 mL of the Tris-HCl buffer. Aliquots, 5 mL, were mixed with 1·25 mL of 40% trichloroacetic acid and assayed for malondialdehyde by the thiobarbituric acid method (Tappel & Zalkin 1959), and it was expressed as thiobarbituric acid values (absorbance at 532 nm g^{-1} tissue).

We reported previously that the major prostaglandins produced in our incubation of medulla slices and recovered in the medium were prostaglandins E_2 and $F_{2\alpha}$ (Fujimoto et al 1983). Prostaglandins E_2 and $F_{2\alpha}$ in

the incubation medium were simultaneously determined by a high pressure liquid chromatographic (HPLC) method as described by Fujita et al (1986). Briefly, prostaglandins E_2 and $F_{2\alpha}$ extracted with ethyl ether (approximately pH 3) were measured after esterification of prostaglandins with 9-anthryldiazomethane (ADAM) (Nimura & Kinoshita 1980). Since ADAM contains many impurities which interfere with the HPLC determination, the purification of prostaglandins esterified with ADAM (PGs-ADAM) was attempted using a normal-phase silica cartridge (Sep-pak, Waters Associates). The cartridge was prepared by rinsing it with 5 mL of methanol followed by 10 mL of benzene–ethyl acetate (60 : 40). The sample was passed through the cartridge. The cartridge was washed with benzene–ethyl acetate (60 : 40, 7 mL) and the PGs-ADAM was then quantitatively eluted with benzene–ethyl acetate–methanol (60 : 40 : 5, 7 mL). Peak heights were measured for the quantification of the PGs-ADAM relative to the standard derivatives prepared from authentic prostaglandins E_2 and $F_{2\alpha}$.

The values are the mean ± standard error. Statistical significance was calculated using Student's paired *t*-test.

Results

Fig. 1 shows the effects of Fe^{2+} and Cu^{2+} on the lipid peroxidation of rabbit kidney medulla slices. Fe^{2+} was able to promote the lipid peroxidation in medulla slices markedly. The effect of Fe^{2+} appeared to be concentration-dependent. Increasing the concentration from 0·2 to 1·6 mM produced a progressive increase in the values of thiobarbituric acids of the slices (4·1 to 10·6-fold). Although Cu^{2+} slightly stimulated the lipid peroxidation, the stimulatory effect was kept low compared with Fe^{2+}. These results imply that Fe^{2+} has a more powerful stimulatory effect on the lipid peroxidation of medulla slices than Cu^{2+}.

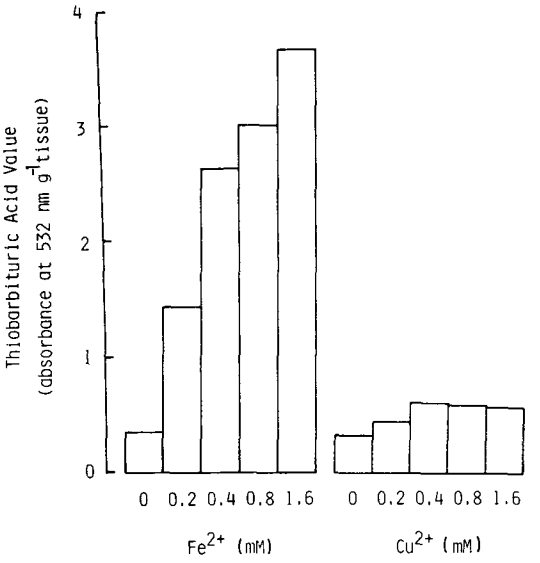

FIG. 1. The influence of Fe^{2+} and Cu^{2+} on the lipid peroxidation of rabbit kidney medulla slices. Slices were incubated with different concentrations of Fe^{2+} or Cu^{2+} for 30 min at 37 °C in 0·15 M KCl/0·02 M Tris-HCl buffer. Values are the means of five experiments.

Previous studies from our laboratory (Fujimoto et al 1983) and by others (Erman & Raz 1979) have shown that the major prostaglandins produced in the incubation of medulla slices and recovered in the medium were

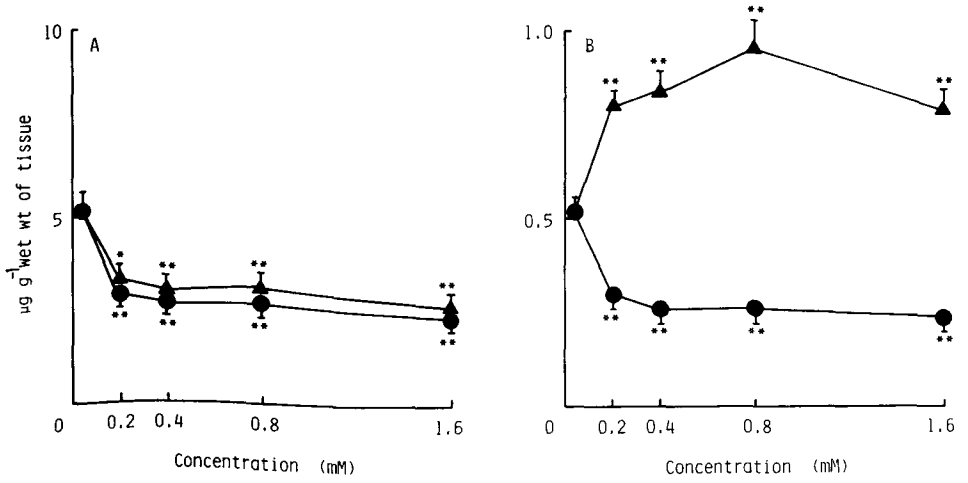

FIG. 2. Effects of Fe^{2+} and Cu^{2+} on prostaglandin E_2 (A) and prostaglandin $F_{2\alpha}$ (B) synthesis in rabbit kidney medulla slices. Slices were incubated for 30 min at 37 °C in 0·15 M KCl/0·02 M Tris-HCl buffer in the presence of different concentrations of Fe^{2+} (●) or Cu^{2+} (▲). Each point indicates the mean of seven experiments; vertical lines show s.e. $^*P < 0{\cdot}02$ compared with the corresponding value in the absence of Fe^{2+} or Cu^{2+}. $^{**}P < 0{\cdot}01$ compared with the corresponding value in the absence of Fe^{2+} or Cu^{2+}.

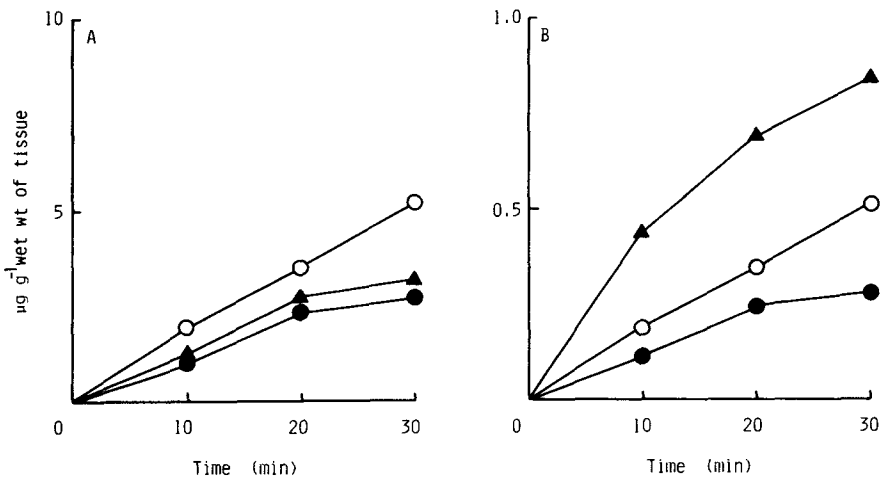

FIG. 3. Time course of prostaglandin E_2 (A) and prostaglandin $F_{2\alpha}$ (B) release from rabbit kidney medulla slices. Incubations were for 30 min at 37 °C in 0·15 M KCl/0·02 M Tris-HCl buffer in the absence (○) and the presence of 0·4 mM Fe^{2+} (●) or 0·4 mM Cu^{2+} (▲). Each point indicates the mean of five experiments (s.e. values were less than 5%).

prostaglandins E_2 and $F_{2\alpha}$. As shown in Fig. 2, medulla slice preparations under basal conditions, without the addition of Fe^{2+} or Cu^{2+}, produce prostaglandin $F_{2\alpha}/E_2$ in a ratio of 0·10. The rate of prostaglandin E_2 synthesis appears to be significantly higher than prostaglandin $F_{2\alpha}$. A similar tendency has been described for rat kidney medulla slices (Speziale et al 1984).

Fe^{2+} and Cu^{2+} reduced the production of basal prostaglandin E_2 (Fig. 2A). Cu^{2+} inhibited medullary generation of prostaglandin E_2 to a level comparable with that obtained with Fe^{2+} at concentrations ranging from 0·2 to 1·6 mM (Cu^{2+}, 36–50% inhibition; Fe^{2+}, 44–54% inhibition). Fe^{2+} at four concentrations inhibited the generation of prostaglandin $F_{2\alpha}$ by 42–53% (Fig. 2B). On the other hand, Cu^{2+} stimulated the production of prostaglandin $F_{2\alpha}$ and maximal stimulatory effect was observed at the concentration of 0·8 mM. Thus, Fe^{2+} did not alter the prostaglandin $F_{2\alpha}/E_2$ ratio (0·10), whereas Cu^{2+} increased it (0·24–0·31).

The effects of Fe^{2+} (0·4 mM) and Cu^{2+} (0·4 mM) on prostaglandins E_2 and $F_{2\alpha}$ generation were apparent within 10 min after addition to the incubation mixture, and they persisted for 30 min (Fig. 3).

Discussion

The conversion of archidonate to prostaglandin E_2 or $F_{2\alpha}$ may be separated essentially into two components. Firstly, prostaglandin endoperoxide synthetase (cyclo-oxygenase) catalyses the oxygenation of arachidonate to prostaglandin G_2 and the subsequent reduction of prostaglandin G_2 to prostaglandin H_2 (Nugteren & Hazelhof 1973; Hamberg et al 1974; Miyamoto et al 1976; Van der Ouderaa et al 1977; Ogino et al 1978). Secondly, an endoperoxide E_2 isomerase catalyses rearrangement of prostaglandin H_2 into prostaglandin E_2, or an endoperoxide reductase catalyses reduction of prostaglandin H_2 into prostaglandin $F_{2\alpha}$ (Hamberg & Samuelsson 1973).

We have reported that lipid peroxidation induced by ascorbic acid and Fe^{2+} inhibits the formation of prostaglandin E_2 and that this inhibitory effect may be mediated by lipid peroxides via the inhibition of cyclo-oxygenase (Fujimoto et al 1983). In the present study, Fe^{2+} stimulated the lipid peroxidation of medulla slices and inhibited both prostaglandin E_2 and prostaglandin $F_{2\alpha}$ formation to the same degree. These results support the idea that the inhibition of prostaglandins E_2 and $F_{2\alpha}$ generation by Fe^{2+} occurs at the cyclo-oxygenase reaction. Egan et al (1976) also demonstrated that prostaglandin cyclo-oxygenase was irreversibly deactivated by radicals, possibly hydroxyl radicals. The polyunsaturated fatty acids form free-radical intermediates during lipid peroxidation. These free radicals react with protein, and with thiol and non-thiol enzymes (Lewis & Wills 1962; Chio & Tappel 1969; Tappel 1973). Thus free radicals produced by Fe^{2+} during lipid peroxidation may de-activate prostaglandin cyclo-oxygenase. Although the lipid peroxidation observed with Cu^{2+} was much less than that observed with Fe^{2+}, nevertheless Cu^{2+} had a potent inhibitory effect on prostaglandin E_2 formation. Simultaneously, Cu^{2+} was capable of stimulating prostaglandin $F_{2\alpha}$ generation. Thus, lipid peroxidation is unlikely to be responsible for the modulation of prostaglandin synthesis by Cu^{2+}.

Lands et al (1971), Lee & Lands (1972) and Maddox (1973) reported a selective enhancement of the biosynthesis of prostaglandin F at the expense of other prostaglandin-like materials when cupric ions and copper–dithiol complexes were added to sheep vesicular preparations. They did not know for sure whether these agents which favour the production of prostaglandin F

during biosynthesis act by chemical or enzymic means. Chan et al (1975), after experimenting with bovine seminal vesicle microsomes, concluded that the increase in prostaglandin F formation caused by copper–dithiols was not due to their inherent abilities to serve as cofactors (enzymic means) but rather to non-enzymatic reduction of prostaglandin G or prostaglandin H (chemical means). In these studies, the net increased amount of prostaglandin F was larger than the net decreased amount of prostaglandin E. In contrast, our present results with rabbit kidney medulla slices (Fig. 2) showed that in the presence of Cu^{2+} the net increased amount of prostaglandin $F_{2\alpha}$ was much smaller than the net decreased amount of prostaglandin E_2 (15–20%). If copper favouring prostaglandin $F_{2\alpha}$ formation during biosynthesis acts by chemical means, it is difficult to understand why the ratio of net increased prostaglandin $F_{2\alpha}$ formation to net decreased prostaglandin E_2 formation is not 1:1 or greater. It is possible that Cu^{2+} participates in a process which led to inactivation of endoperoxide E_2 isomerase with a concomitant activation of endoperoxide $F_{2\alpha}$ reductase in rabbit kidney medulla slices. The reason for the difference between the present and previous work (Lands et al 1971; Lee & Lands 1972; Maddox 1973; Chan et al 1975) is not known; it may relate to preparation of tissues or a species difference.

Further studies are needed to clarify the mechanism by which Cu^{2+} modulate prostaglandins E_2 and $F_{2\alpha}$ generation; however, the present study serves to emphasize that Cu^{2+} plays an important role in the control of prostaglandin synthesis by enzymic means.

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