

This effect could not be explained by a direct action at receptors in smooth muscle or by action at the nerves or ganglia, as contractions of guinea-pig ileum in response to acetylcholine, histamine, 5-hydroxytryptamine, PGE<sub>2</sub>, bradykinin or nicotine were unchanged (see Fig. 1). Inhibition of intestinal motility was also observed when the drug was administered intravenously or into the peritoneal cavity of guinea-pigs anaesthetized with nembutal (40 mg/kg, I.P.).

The effect of TYA on synthesis of prostaglandins in guinea-pig ileum was then examined using suspensions of guinea-pig ileum tissue powdered after rapid freezing in liquid nitrogen (Willis, Davison, Ramwell, Smith & Brocklehurst, 1972). PGE<sub>2</sub> and PGF<sub>2α</sub>, formed from arachidonic acid (Sigma) were isolated by selective solvent extraction (Shaw & Ramwell, 1969), and two stage thin layer chromatography in Green & Samuelsson's (1964) AI and AII solvent systems (Willis, 1970). TYA added five min prior to addition of arachidonic acid markedly inhibited PG synthesis in a graded dose-dependent fashion. The dose for 50% reduction being 1.5 µg/ml.

These results support and extend the findings of Bennett & Posner (1971) who used prostaglandin antagonists, and of Ferreira, Herman & Vane (1972) who used anti-inflammatory drugs.

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#### **The effect of caffeine on the antipyretic action of aspirin administered during endotoxin induced fever**

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Since several 'over the counter' preparations contain mixtures of antipyretics and caffeine, it seemed important to assess the effect of caffeine on the antipyretic action of aspirin. Fever was produced by an i.v. injection of 0.05 µg 'Pyrogen E' into an ear vein, control animals received 0.9% sodium chloride solution. The fever was assessed by computing the area of the fever curve [°C × time (h)] during the

5 h following endotoxin, and expressed as the thermal response index (TRI). The effect of aspirin (100 mg/kg) and caffeine (13.3 mg/kg) separately and together on the fever response was measured. The drugs were dissolved in 6% potassium citrate and administered intraperitoneally 15 min before the endotoxin, control animals received 6% citrate solution. It will be seen from Table 1 that, in the absence of endotoxin and fever, the drugs were without significant effect, whereas the animals receiving endotoxin plus caffeine developed a significantly greater fever than those receiving endotoxin alone. Further, though aspirin markedly reduced the endotoxin fever, the aspirin/caffeine mixture had no antipyretic action.

TABLE 1. *Temperature response (mean values) in the conscious, restrained rabbit at ambient temperature of 21–23° C*

No. of animals	Dose of pyrogen (μg)	Drug (i.p.)	Latency of temp. response (min)	Max. temp. increase °C±S.D.	Thermal response index (TRI)±S.D.
6	0.05	Saline	19.6	1.45±0.10	4.82±0.60
6	0.05	Aspirin	26.3	0.83±0.39	2.93±0.93
6	0.05	Caffeine	19.0	1.70±0.16	5.93±0.54
6	0.05	Caffeine/ aspirin	23.4	1.52±0.28	6.00±1.76
4	Saline	Saline	—	0.63±0.45	1.37±0.85
6	Saline	Aspirin	—	0.60±0.33	1.44±1.07
6	Saline	Caffeine	—	0.77±0.41	1.80±1.26
6	Saline	Caffeine/ aspirin	—	0.85±0.45	1.95±0.89

These results showed, therefore, that in the rabbit caffeine significantly potentiated endotoxin fever and also prevented the antipyresis produced by aspirin. The mechanism by which caffeine exerts these effects on body temperature cannot be determined from these experiments. It is possible that caffeine raises levels of 3'5' cyclic AMP, a substance reported to raise body temperature (Robison, Butcher & Sutherland, 1971) by inhibiting 3'5' cyclic AMP phosphodiesterase. Prostaglandins are known to increase 3'5' cyclic AMP levels (Butcher & Baird, 1968), and since Milton & Wendlandt (1970; 1971) reported the hyperthermic effects of prostaglandins, and suggested that the antipyretics might act by preventing the release of prostaglandins, it is possible that, in the experiments described here, there is some interaction between caffeine, prostaglandins and 3'5' cyclic AMP. It is important to repeat these experiments in man since if caffeine has similar effects in this species, then caffeine and mixtures containing caffeine would be inadvisable during fever.

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