obtained with an EFA-deficient diet, supplemented with hydrogenated coconut oil (6% of calories as saturated fat).

By contrast, however, levels of prostaglandins E_1, E_2 and $F_{2\alpha}$ were markedly decreased in all tissues examined (Table 1b).

The results suggest that in the rabbit basal turnover of these prostaglandins is more directly related to dietary status rather than phosphoglyceride stores. The question raised by these results is: are there different metabolic pools for basal and stimulated prostaglandin synthesis?

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Release of slow-reacting substance from guinea-pig and human lung by calcium ionophore A23187

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Slow-reacting substance of anaphylaxis (SRS-A) was first described in the effluent from guinea-pig sensitized lungs *in vitro* following antigenic challenge. Consequently, many subsequent studies have been directed towards the immunological mechanisms involved. However, it has been reported that a slow-reacting substance(s) (SRS) with the characteristics of SRS-A may be released by a non-immunological stimulus, calcium ionophore A23187 from human leucocytes (Conroy, Orange & Lichtenstein, 1976) and rat peritoneal cells (Bach & Brashler, 1974). The experiments described were performed to determine whether A23187 would release SRS from guinea-pig and human lung.

Unsensitized or actively sensitized male guinea-pigs (350–600 g) were killed, the lungs removed and perfused with Tyrode via the pulmonary artery until free of blood. The lung tissue was cut into pieces, approximately 2 mm^3, weighed into 1.0–1.7 g portions and incubated in 4.5 ml of A23187 (5 μg/ml) in Tyrode for 45 min at 37°C or challenged with antigen (15 min at 37°C). Macroscopically normal human lung, obtained from operative specimens resected for bronchogenic carcinoma was washed in Tyrode, cut into small pieces, divided into 450 mg replicates and incubated with A23187 as above. The SRS activity in the supernatant was assayed on smooth muscle stripped from guinea-pig ileum (Rang, 1964) and blocked with mepyramine and hyoscine. Histamine was assayed fluorimetrically.

During incubation with A23187 unsensitized guinea-pig lung released SRS (560 ± 183 mu ml^{-1}, $n = 7$, for units see Engineer, Niederhauser, Piper & Sirois, 1978). After pretreatment with indomethacin (1 μg/ml) for 1 h and subsequent incubation with A23187 and indomethacin (1 μg/ml), the release of SRS was significantly greater than with A23187 alone (930 ± 234 mu/ml, $P < 0.005$). When sensitized guinea-pigs were used, the chopped lung released SRS with A23187 and SRS-A following antigenic challenge. Both these substances were antagonized by FPL 55712 (1 μg/ml) (Augstein, Farmer, Lee, Sheard & Tattersall, 1973), destroyed by arylsulphatase and by low pH but were stable to base hydrolysis. In unsensitized and sensitized lung cysteine (10^{-3} M) caused an increase (mean 92%) in ionophore-induced SRS release. In experiments with human lung SRS was also released by A23187 (5 μg/ml) and the release potentiated by cysteine (10^{-3} M). Human SRS was found to be indistinguishable from guinea-pig SRS. A23187 also caused a dose-dependent release of histamine from human and guinea-pig lung.

These findings extend earlier observations (Bach & Brashler, 1974; Conroy *et al.*, 1976) and support the possibility that a SRS, which is similar or identical to SRS-A associated with IgE and IgG-mediated allergic reactions, may be involved in non-immunological inflammatory processes. Since indomethacin increased the ionophore-induced release of SRS, some product(s) of arachidonic acid metabolism by cyclooxygenase may modulate its release.

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On the preparation of highly purified slow reacting substance of anaphylaxis (SRS-A) from biological extracts

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Ever since its discovery by Kellaway & Trethewie in 1940, Slow Reacting Substance of Anaphylaxis (SRS-A) has defied all attempts to identify it. This is partly due to the lack of suitable techniques for purification and preparation and thus an investigation of the pharmacology of this most interesting mediator has been hampered. We now present a method for the production of SRS-A of very high purity and specific activity, which is eminently suited for pharmacological investigations. Crude rat SRS-A, generated either by antigen challenge (Orange, Valentine & Austen, 1968) or by the calcium ionophore technique (Bach & Brashler, 1974) was diluted with ice-cold ethanol (1:4), the precipitated protein was removed by filtration and the ethanolic solution of SRS-A was evaporated to dryness and resuspended in distilled water. After base hydrolysis (1 N NaOH for 1 h) the aqueous mixture was pumped through a column of Amberlite XAD-8, and washed through with distilled water. The SRS-A activity was then eluted with ethanol/water (80:20, v/v). After concentration under vacuum the SRS-A was applied to a column of Sephadex G-10 equilibrated with 1% ammonium bicarbonate. Serial fractions were collected and those containing SRS-A-like activity on the guinea-pig ileum were pooled. Water and buffer salts were subsequently removed by lyophilization, and the residue was applied to a silicic acid column equilibrated in hexane. Successive elutions were made with hexane, ether and ethyl acetate and continued with increasing concentrations of methanol in chloroform. SRS-A always eluted between 40–60% methanol. Subsequent high pressure liquid chromatography (HPLC) was performed with a Waters 6060 liquid chromatograph equipped with a μBondapak-C_{18} reversed phase column equilibrated with distilled water and eluted with a linear gradient of ethanol. When first prepared SRS-A eluted as a single peak of biological activity (at an ethanol concentration of about 50%), but subsequently chromatographed as two or more active compounds of similar polarity perhaps indicating the presence of tautomers. Active fractions were pooled and further purified using the HPLC in an isocratic mode with 60% methanol–40% water as the eluting solvent. SRS-A prepared in this way has a specific activity of over 100,000 u/mg (1u $\triangleq$ 0.2 ng histamine) and is free from all other mediators.

When treated with diazomethane, SRS-A lost all biological activity but this could be (partially) restored by hydrolysis with 0.2 N NaOH at 80% for 15 minutes. This technique allows the purification of SRS-A as the less polar methyl ester and if labelled diazomethane is used, labelled methyl SRS-A may be prepared. Treatment with sodium borohydride did not inactivate SRS-A or change its chromatographic mobility. This data implies the presence of a free carboxyl, but not a ketone group on the molecule. SRS-A generated by antigen challenge or calcium ionophore was chromatographically indistinguishable.

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