Table 1. Effects of capsaicin treatment (50 mg kg^{-1} s.c. on day 2 of life) on nociceptive threshold in rats with normal and yeast-inflamed paws in the paw-pressure test 3 months later. Number of animals in parentheses.

	Nociceptive threshold (*g* force) Normal paw	Inflamed paw
Capsaicin treated	570 ± 104 (7)*	443 ± 26 (6)**
Vehicle treated	352 ± 42 (7)	122 ± 24 (7)

* Significantly different to vehicle $P < 0.05$.
** Significantly different to vehicle $P < 0.001$ using Student's unpaired *t*-test.

with the findings of Jansco et al (1977). The reasons for this difference could be that the previous workers failed to accurately quantify the response to a mechanical stimulus and that the stimulus might have been of a different quality, i.e. a pinch rather than a gradual increase in pressure. Moreover, in the rat paw-pressure test it could be that as pressure to the paw is gradually increased, release of a chemical pain mediator may occur, leading to activation of chemoreceptors in addition to the already activated mechanoreceptors. In fact, it has been demonstrated that capsaicin treatment reduces the size of antidromically evoked action potentials in C_2-type fibres, having no effect on C_1, A-α-β and A-δ-type fibres (Szolcsanyi 1977). Our results confirm that capsaicin treatment is more effective in a situation where the main influence on the pain response is chemical and possibly via C-type fibres, i.e. when the paw is inflamed or a noxious substance is injected intravenously. In the case of the normal paw it could be that the mechanical stimulation leads to activation of the A-δ-type fibres and that these are subserved by the C-type fibres that are affected by capsaicin treatment.

In interpreting the result obtained in the yeast-inflamed paw, the possibility that capsaicin may also have reduced the severity of the yeast-induced inflammatory response must be considered. Indeed, it has been demonstrated that rats treated with capsaicin on day 2 of life and used 4 months later, show significantly reduced hind-paw vasodilation following antidromic stimulation (Lembeck & Holzer 1979). Further, Gamse et al (1980) were able to inhibit neurogenic plasma extravasation by more than 80% in rats treated in an identical fashion. Thus, it is highly probable that capsaicin could have reduced the inflammatory response in our experiments, and therefore contributed to the more marked analgesic effect observed in these animals.

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The use of Sephadex LH-20 to improve yield in the initial stages of the purification of slow reacting substance of anaphylaxis

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In any investigation of the properties of slow reacting substance of anaphylaxis (SRS-A) it is necessary to purify SRS-A from biological fluids which usually contain inorganic salts, histamine and other pharmacologically active compounds. Many multistep techniques have been reported in which an early step is to separate SRS-A from histamine and physiological salts using either charcoal (Morris et al 1978) or the Amberlite XAD-2, 7 and 8 ion-exchange resins originally described by Orange et al (1973). However, other workers in this field (Takahashi et al 1976; Bach et al 1979; Whelan 1980) failed to obtain high, reproducible yields of SRS-A using such methods. Furthermore in a recent study using Amberlite XAD-8, Lee et al (1979) could only separate SRS-A from histamine by introducing an additional elution into the procedure. Recently, SRS-A was separated from histamine and physiological salts by solvent extraction (Whelan 1980); this method gave a mean recovery of SRS-A of 55 ± 11% which was greater than that obtained using Amberlite XAD-2 chromatography. Therefore, it is desirable that any extraction procedure should be simple and give a reproducible and high percentage yield of SRS-A.

Orange et al (1973) used a column of Sephadex LH-20 to determine the molecular weight of SRS-A and recently Yecies et al (1979) have used Sephadex LH-20 chromatography in the isolation of a slow reacting substance released by the ionophore A 23187. In the experiments described below a calibrated column of Sephadex LH-20 has been used to separate SRS-A from histamine and physiological salts such that over 90% recovery of SRS-A is effected.

Sephadex LH-20 (Pharmacia) was slurried in methanol–water (4 : 1 v/v) and packed into a borosilicate glass

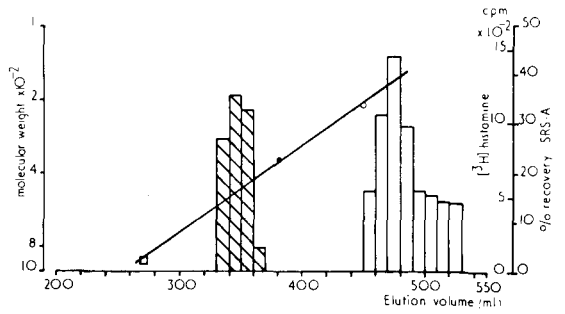

FIG. 1. The separation of SRS-A (hatched columns) and histamine (open columns) by Sephadex LH-20 gel filtration chromatography. The column (32 mm × 720 mm) was calibrated with [^{3}H]noradrenaline hydrochloride (○), [^{14}C]prostaglandin E_2 (●) and 2-[^{3}H]-arachidonyl phosphatidylcholine (□) and eluted with methanol–water (4:1 *v/v*) at a rate of 1·5 ml min^{-1}.

column (bed size 32 × 720 mm). The column was eluted with methanol–water (4:1 v/v) at a rate of 1·5 ml min^{-1} and 10 ml fractions were collected. The column was calibrated with [1-^{14}C] prostaglandin E_2 (mol. wt 352) 1-[7,8 ^{3}H]noradrenaline hydrochloride (mol. wt 205) (Radiochemical Centre, Amersham) and 2-[^{3}H]-arachidonyl phosphatidylcholine (mol. wt 890) prepared in this laboratory. As shown in Fig. 1 a linear plot of molecular weight against elution volume was obtained for these substances.

SRS-A (5000 U), prepared as described by Whelan (1980), was dissolved in a small volume of methanol–water (4:1 v/v) and applied to the calibrated Sephadex LH-20 column. The column was eluted and fractions collected as described above. Fractions were evaporated to dryness, reconstituted in a small volume of 0·15 M NaCl and assayed for biological activity on the guinea-pig isolated ileum preparation as described by Coleman et al (1979). SRS-A was found to elute between 350 and 380 ml corresponding to a molecular weight of 450 (Fig. 1) confirming the value obtained in this chromatographic system by Orange et al (1973).

[^{3}H]Histamine dihydrochloride (1·0 μCi) (Radiochemical Centre, Amersham) dissolved in a small volume of methanol–water (4:1 v/v), was applied to the column and eluted as described above. Aliquots (1 ml) of each fraction were taken and the tritium content measured by liquid scintillation counting. Histamine dihydrochloride was eluted from the column between 470 and 530 ml, an elution volume which is consistent with its molecular weight of 184.

From the results described above one would expect to be able to separate histamine from SRS-A. To verify this extrapolation rat peritoneal anaphylactic fluid was collected and treated with ethanol to remove any protein as described by Whelan (1980). The residue obtained by evaporation of the resulting supernatant was dissolved in 10 ml methanol–water (4:1 v/v), filtered, applied to the column and eluted as described above. The eluate was collected between 330 and 450 ml evaporated and the residue assayed on the guinea-pig isolated ileum for SRS-A as described by Coleman et al (1979). Histamine was assayed on the guinea-pig isolated ileum in the presence of 10^{-6} M FPL 55712, a specific antagonist of SRS-A (Augstein et al 1973).

Table 1. The recovery of SRS-A following Sephadex LH-20 gel filtration chromatography.

	Units SRS-A applied	Units SRS-A recovered	% recovery
Expt. No. 1	22560	31200	138
2	128092	101432	79
3	43700	36800	84
4	75060	81540	108
5	55575	49875	89
mean	64997	60169	99
s.e.	17924	13510	11

The percentage recovery of SRS-A following Sephadex LH-20 chromatography in five experiments is shown in Table 1, the mean recovery of SRS-A being 99 ± 11%. In all experiments the histamine content of the 330–450 ml fraction was less than 10 ng/1000 U SRS-A. 100 units of SRS-A being defined as the amount of SRS-A required to produce a contraction of the guinea-pig isolated superfused ileum equivalent to that produced by 100 ng prostaglandin E_2. Thus in a typical experiment a partial purification, measured in terms of ng histamine per 1000 U SRS-A, of 160 fold is obtained.

The material recovered from the column, using the elution volumes described above, is a mixture of substances with molecular weights in the range 280–550 which includes SRS-A. SRS-A is then purified further using established techniques (Orange et al 1973; Morris et al 1978; Yecies et al 1979; Whelan 1980).

This technique is used routinely in my laboratory to separate SRS-A from histamine and physiological salts and had advantages over established techniques in that it is simple, reproducible and the column can be used many times.

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