response at any of the doses employed. 'Safety Factors' of about 78 and 104 were obtained in the two rabbits tested separately. The remaining 5 substances are tabulated in decreasing order of 'Safety Factor', the worst being (+)-tubocurarine with which some vagolytic action was seen even with doses that did not fully paralyse.

Comparison of the present results from the rabbit with data for the cat (Riker & Wescoe 1951; Bonta et al 1968; Hughes & Chapple 1976) suggest that the rabbit is relatively more resistant to the vagolytic effects of the non-depolarizing relaxants. The limited data for decamethonium (Abrahams & Hilton 1962) also suggests that the rabbit is more resistant than the cat to the vagolytic effects of this depolarizing relaxant.

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An improved technique for the partial purification of SRS-A

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Slow reacting substance of anaphylaxis (SRS-A) is released immunologically from several animal tissues and is believed to be a major mediator in the pathogenesis of human asthma. A simple technique for the purification of SRS-A would greatly help the study of this substance. Several multistep techniques have been reported, many based upon the use of Amberlite XAD-2, -7 and -8 resins as originally described by Orange et al (1973). However, only the latter authors quantify the separation of SRS-A from histamine at the Amberlite chromatography stage. Furthermore, workers in this field (Orange 1978; Bach et al 1979) report that Amberlite chromatography gives extremely variable recoveries of SRS-A.

For our investigations we required SRS-A of reasonable potency, free from histamine and physiological salts. After considering the methods available in the literature we elected to use Amberlite XAD-8 in a single step process. An anaphylactic perfusate containing SRS-A was produced from perfused isolated lungs of sensitized guinea-pigs by the method of Engineer et al (1978). The perfusate was collected between 2 and 20 min after antigen injection. In all experiments indomethacin (1 μg ml^{-1}) was present in the perfusion fluid to increase the yield of SRS-A and prevent the formation of prostaglandins (Engineer et al 1978). The perfusates were pooled and centrifuged at 1000 *g* for 20 min at 4°C to remove contaminating cells. All bioassays of SRS-A were performed on guinea-pig isolated ileum in the presence of atropine (10^{-6} M) and mepyramine (10^{-6} M). Histamine was assayed fluorimetrically. Recoveries of SRS-A were determined by comparison of the activity of the purified sample with an aliquot of the original perfusate which had been stored at −20°C until the time of assay on the following day. Previous experiments using freeze-dried SRS-A as a standard had shown that storage of crude perfusate for this length of time did not lead to loss of activity.

After some preliminary investigative work an experiment was performed using conditions as described by Orange et al (1973). In this experiment guinea-pig lung perfusate (50 ml) was chromatographed on a glass column (2·5 cm diameter) containing pre-washed Amberlite XAD-8 (65 g). The SRS-A recovery in the ethanolic fraction was 63%, but this fraction also contained 76% of the original histamine. This was contrary to the findings of Orange et al (1973) who reported a complete separation of SRS-A and histamine.

Because it has been suggested that SRS-A may bind more strongly to Amberlite at a pH below 7 (Jakschik, personal communication), we investigated the effect of pH on the separation of SRS-A and histamine. Table 1 summarizes some of the results obtained using 10 ml perfusate and 10 g Amberlite XAD-8 in columns 1 cm diameter, in which the Amberlite had been equilibrated with phosphate buffers of different pH. The anaphylactic perfusate was adjusted with buffer to the same pH before addition to the column. The column was eluted with buffer (40 ml) and then 80% ethanol. In these experiments the elution of SRS-A and histamine was carried out at the same pH.

* Correspondence.

Table 1. Effect of pH on recovery of SRS-A and histamine after chromatography of anaphylactic guinea-pig lung perfusate on Amberlite XAD-8. Results are from individual experiments.

pH (perfusate & column)	% Recovery in 80% ethanol SRS-A	Histamine
unadjusted*	55	37
7·0	52	1·2
6·5	42	0·7
6·0	36	0·7

* The perfusate was usually at a pH of approximately 9.

It is clear from these results that a slightly acidic pH is required for optimal removal of histamine, whereas an alkaline pH leads to better recovery of SRS-A. This pH dependence of the elution characteristics exhibited by histamine (base) and SRS-A (acidic lipid) is explicable in terms of the weaker adsorption affinity of Amberlite XAD resins for molecules in their ionized form (BDH 1977).

As a consequence of these observations we developed the following dual pH procedure for the routine partial purification of SRS-A.

A silicon coated column (4·5 cm diameter) containing 200 g (based on approximate dry weight) of pre-washed Amberlite XAD-8 is adjusted to pH 6·5 using 0·01 M NaH_2PO_4 buffer of pH 6·5 (approximately 1 litre). Freshly prepared guinea-pig lung perfusate (200 ml) is adjusted to pH 6·5 by the addition of 0·5 M NaH_2PO_4 buffer of pH 6·5 (2–3 ml). After the perfusate has been run onto the column the histamine is eluted at approximately 25 ml min^{-1} using 1700 ml of 0·01 M NaH_2PO_4 buffer of pH 6·5. The column is then adjusted to pH 8·0 using 0·01 M NaH_2PO_4 buffer of pH 8·0 (approximately 450 ml), washed with a further 400 ml of distilled water to remove buffer salts and then the SRS-A is eluted in 80% ethanol (800 ml). The ethanolic solution is evaporated to dryness at 40°C under vacuum and the residue stored at −20°C until the following day, when it is reconstituted in isotonic saline immediately before assay.

Using these conditions high recoveries of SRS-A and almost complete removal of histamine are achieved as shown in Table 2.

Comparison by Students *t*-test of recoveries of SRS-A from fresh and 24 h old perfusate (stored at −20°C) reveals a significant difference ($P < 0·01$), whereas the removal of histamine is the same in each group. At present we are unable to explain the reason for this difference, but preliminary results do not suggest that storage of perfusate for periods longer than 24 h necessarily leads to even lower recoveries of SRS-A.

Table 2. Recovery of SRS-A and histamine after chromatography of anaphylactic guinea-pig lung perfusate on Amberlite XAD-8 using dual pH procedure. Experiments 1–6 used freshly prepared perfusates. Experiments 7–9 used perfusates which had been stored overnight at −20°C. Each experiment used perfusate from a different group of animals.

Experiment	% Recovery in 80% ethanol SRS-A	Histamine
1	100	0
2	100	0·4
3	100	0·3
4	91	0·8
5	97	0·7
6	109	0·5
Mean ± s.e.m.	99·5 ± 2·4	0·4 ± 0·1
7	82	0·9
8	85	0·3
9	80	0·3
Mean ± s.e.m.	82·3 ± 1·5	0·5 ± 0·2

Although we routinely use this technique to purify guinea-pig SRS-A, similar results have been obtained by us using SRS-A obtained from rat peritoneal cavity according to the method of Orange et al (1968).

We believe this technique is a significant advance over other published methods of partial purification of SRS-A. Separation of SRS-A from histamine is almost absolute, and SRS-A is recovered in high yield with predictable consistency.

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