assessed by extraction and colorimetric assay. Samples of SPF contain no appreciable amounts of low molecular permeability factors and produce no contraction or relaxation of isolated smooth muscle.

The permeability effect of SPF is exerted indirectly through the release of histamine and 5-hydroxytryptamine (5-HT) from mast cells as indicated by the following findings (1) the blueing effect of SPF is almost completely abolished by the previous systemic application of a combination of mepyramine and methysergide in doses which specifically antagonize histamine and 5-HT respectively; (2) both histamine and 5-HT are released by SPF from rat peritoneal mast cells *in vitro*; (3) after depleting rat skin of histamine and 5-HT with compound 48/80 in doses which do not impair the permeability effect of histamine, SPF loses its permeability effect. The histamine releasing effect of SPF in isolated mast cells is abolished by 0·1 mM iodoacetate, but in contrast to anaphylactic histamine release it is unaffected by Ca-lack and by raising temperature to 45° C for 5 min.

Chemical modification of spleen permeability factor

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The permeability factor obtained from the spleen (SPF), referred to in the preceding communication, is a mixture of priteins extracted from the spleens of pig or rat. Attempts have been made to purify those components responsible for the permeability activity. Gel filtration, molecular membrane filtration, ion-exchange chromatography, acrylamide electrophoresis, and isoelectric focussing yield a variety of protein fractions from SPF, electrophoretically and chromatographically distinct, which possess biological activity comparable to that of the original material. This activity may be due to structural features shared by many proteins, likely to be of a simple type depending on distribution of amino acid side chains rather than complex features of tertiary structure.

The use of reagents which modify the proteins of SPF has helped to define those features essential for permeability activity. Two categories of reagents have been used; those which unwind the tertiary structure of proteins, and those which react with particular amino acid side chains to effect a chemical modification. In practice these two effects are not clearly separable, since extensive chemical modification will produce changes in tertiary structure, whilst maximum disruption of tertiary structure is effected only by reagents which cause some chemical modification.

The treatment of SPF with modifying agents has led to the following findings:

(1) The unfolding of tertiary structure followed by re-folding, as caused by prolonged exposure to 6M urea and its removal prior to bioassay, does not diminish biological activity.

(2) Further disruption of tertiary structure by similar treatment with urea plus mercaptoethanol reduces activity threefold. Blockage of native and formed sulphydryl groups causes no further reduction in activity.

(3) Blockage of ϵ-amino groups of lysine and α-amino terminal groups with three different reagents causes a reduction in activity to 1/20th of the original level.

(4) Blockage of aspartic and glutamic carboxylate (and C-terminal) groups by conversion to an ester or an amide increases the activity of SPF by factors ranging from 4 to 24-fold.

Our interpretation of these results is that unique features of tertiary structure are not responsible for SPF activity. Intact primary amino groups are largely responsible for activity. The enhanced activity seen after the conversion of carboxylate groups to electroneutral derivatives may be caused by the destruction of charge interactions and/or hydrogen bonding between carboxylate and primary amino groups, allowing the latter to exist in a free state at the surface of the protein molecules.

The effects of β_2-adrenoceptor stimulants, salbutamol and terbutaline on gastric acid secretion and mucosal blood flow in conscious dogs with Heidenhain pouches

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Isoprenaline is a potent inhibitor of pentagastrin-induced gastric acid secretion in dogs; this inhibition is not secondary to decreased mucosal blood flow, nor antagonized by propranolol (Curwain & Holton, 1972). In order to investigate whether these properties are shared by other drugs which stimulate β_2 receptors, we have examined the effect of salbutamol and terbutaline.

Gastric acid secretion was stimulated by pentagastrin [1·0–4·0 (μg/kg)/h] and gastric mucosal blood flow measured by radioactive aniline clearance (Curwain & Holton, 1971; Curwain, 1972). Both salbutamol sulphate [0·1–0·5 (μg/kg)/min] and terbutaline sulphate [0·1–0·5 (μg/kg)/min], infused intravenously for 30 min, decreased gastric acid secretion, and the effect was dose related. In 5 experiments in 3 dogs salbutamol [0·1 (μg/kg)/min] reduced acid secretion to a mean of 54% ±S.E. of mean 12% and the effect was antagonized by propranolol (1 mg/kg, I.V.) given 25 min earlier (3 experiments in 3 dogs).

In 6 experiments in 3 dogs terbutaline [0·2 (μg/kg)/min] reduced acid secretion to 48% ±4% and in each case the effect was abolished by propranolol. Heart rate, measured by palpation, rose to 163% of pre-dose level during salbutamol infusion and 127% during terbutaline. Propranolol abolished the tachycardia.

The effects of salbutamol and terbutaline on mucosal blood flow were studied in 3 dogs. Salbutamol 0·1 (μg/kg)/min for 30 min in each of 2 experiments in 2 dogs decreased acid secretion and mucosal blood flow, but the ratio of blood flow to secretion (G/P) increased markedly. Terbutaline 0·2 (μg/kg)/min for 30 min gave similar results (2 experiments in 2 dogs).

In a dose of 1 (μg/kg)/min both salbutamol and terbutaline increased gastric mucosal blood flow without affecting secretion when given on a plateau of histamine-induced secretion.

These results are similar to those previously reported for isoprenaline (Curwain, Endersby & Holton, 1971; Curwain & Holton, 1972) except that isoprenaline inhibition of gastric secretion is not sensitive to blockade by propranolol. The inhibi-