In contrast to the effect on the utilization of TPNH in crot-CoA-reduction, U-9189 had little or on effect on glutathione reductase,* another system in which the reduced nucleotide is required. Concentrations between 5×10^{-4} M and 2×10^{-3} M exhibited variable and inconsistent inhibitions of from 10 to 25 per cent. The order of introduction of U-9189 was of no significance.

Thus, 1:1:3-tricyano-2-amino-1-propene not only is an inhibitor of an enzymic step important in the synthesis of fatty acids in liver, but also it may possess a specificity of activity which could facilitate elucidation of the mechanistic details of the reactions involved. Black and Hudson[5] have presented evidence for a flavin prosthetic group in yeast glutathione reductase, and Lynen recently reported[6] that flavin mononucleotide is an intermediate in the reduction by TPNH of α, β-unsaturated acyl CoA-compounds during fatty acid synthesis by yeast. Wakil has suggested[7] that precedence favours the flavin intermediate in TPNH-double bond reductions. If this generalization is valid, in the microsomal system it seems most probable that U-9189 is actually interfering with hydrogen-transfer from a flavin to the model substrate.

* A preparation from yeast obtained from Sigma Chemical Co.

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SRS–S concentrates from normal swine lung without anaphylaxis

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THE release of a smooth muscle-stimulating substance, in addition to histamine, from the lung tissue of guinea pig during anaphylaxis has been reported,[1-5] and is of interest in research in allergy. This "substance" has not been proven by organic chemical data to be a single substance, but is characterized primarily by biological effects and the relation of biological response to chemical fractionation. This substance causes a prolonged contraction of isolated smooth muscle, in contrast to the rapid contraction caused by histamine; consequently, it has been designated "slow-reacting substance" or SRS[1, 2] or SRS-A[5] (A for anaphylaxis).

Previous methods[1-5] for extraction of SRS or SRS-A from guinea pigs required sensitization of the animals with antigen, usually egg albumin. The lungs were then removed, and anaphylaxis was induced by perfusion or incubation of the sensitized lungs with antigen solution. The aqueous effluents contained SRS and histamine only after anaphylaxis. The existence of SRS in these effluents was based on observations of the characteristic contraction of the guinea pig ileum after suppression of the action of histamine with antihistaminic agents.[2] In our study, this procedure served as the basis of an assay[5, 6] for activity of samples.

The preparation of concentrates of SRS for biological and chemical studies is greatly hampered by the small quantities available from the lungs of guinea pigs and by the sensitization requirement. Although the background research seemed to preclude the presence of SRS in normal lung tissue, lungs from swine have been investigated as a source for SRS, and very active concentrates have been prepared. In the absence of definitive characterizing data on SRS obtained from different sources and

by isolation steps which do and do not include anaphylaxis, we are tentatively designating our active material SRS-S (S for swine).

It was found that Tyrode solution extracted much activity from fresh swine lungs that are relatively free of blood. Background information for our isolation procedure included work by Chakravarty[3] and Brocklehurst.[5, 7] For example, advantage was taken of the fatty acid-like properties of SRS; i.e., water-soluble in weakly basic solution and organic solvent-soluble in acidic solution.

Tissue residue was removed by centrifugation from the Tyrode extract, and salts and proteins were removed by precipitation with alcohol. The alcohol was removed *in vacuo*, and the aqueous solution was acidified. SRS-S was then separated from histamine and other water-soluble components by extraction with chloroform. The chloroform-soluble lipids were fractionated by extraction with sodium bicarbonate solution. The aqueous extract was acidified and extracted with chloroform. Evaporation of the solvent yielded about 50 mg of a histamine-free lipid concentrate from 1 kg of wet tissue; this material contracted the guinea-pig ileum at a level of 0·025–0·125 μg/ml of bathing fluid.

Certain other smooth muscle-stimulating substances have been isolated from blood. In particular, G-acid[8] a fatty acid, has properties similar to SRS. The possibility that SRS-S was derived from residual blood in the lung tissue seems to be unlikely; no activity of SRS-S was found when swine blood was similarly processed.

A basic physiological significance for "slow-reacting substance(s)" acting in possible relationship with histamine in allergic syndromes is not fully elucidated, and could hardly be expected at this time when active concentrates have been available only on a micro scale. The new findings reported herein may facilitate the isolation of a pure substance(s), the elucidation of structure, and comparisons with other substances active in the rather nonspecific assay.

Acknowledgement—In May, 1960, we discussed with Dr. Brocklehurst the possibility of obtaining SRS from lung tissue without sensitization and anaphylaxis; he has since written that during incubation of fragments of swine lung, SRS activity is freed into the bathing fluid. Professor Börje Uvnas has kindly tested a sample of SRS-S and found it to be active.[10] We are grateful to Dr. Brocklehurst for exchange of assay samples and particularly for helpful discussions in his laboratory.

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