CHAPTER FIFTEEN

PHARMACOLOGICAL MEDIATORS OF HYPERSENSITIVITY REACTIONS

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RECENT TRENDS OF STUDY

A few years ago this chapter might well have been headed 'Histamine', and the clinician might have been justified in paying little attention to it. At present it is known that at least two other substances contribute to the symptoms resulting from hypersensitivity reactions, and several more substances are suspected. Research is still in progress over much of this field and the work is far from complete, but in some respects the results are already convincing, and tomorrow's therapeutic measures must be related to this new knowledge. It is to be expected that animal experiments will frequently not correspond to the situation in man, but occasionally it has been possible to use human material instead of the usual animal tissue

in the experimental procedure, and then a surprising degree of similarity has been found. In an attempt to follow the reaction in allergic tissue step by step, pharmacologists have recently used some of the methods of the biochemist to trace the events in the immediate type reaction. These studies have shown that one of the first events resulting from antigen-antibody combination is the activation of enzymes, or a change in balance between existing active enzymes and their inhibitors. This in turn leads to the release or formation in the tissue of substances having pharmacological activity.

Studies have been directed almost exclusively to reactions of immediate type, and the biochemical and pharmacological events of the delayed reaction remain practically unknown. The delayed type reaction is essentially *in vivo* and consequently cannot be studied by any of the new techniques by which we have gained more understanding of the immediate reaction. Furthermore, the experiments are necessarily cumbersome because of the large numbers needed to overcome natural variation in response, and the rather small increments in effect produced by multiples of the challenging dose.

EVENTS PRECEDING THE APPEARANCE OF PHARMACOLOGICALLY ACTIVE SUBSTANCES

The technique which has contributed very greatly to knowledge of these events is the use of chopped tissue in the study of anaphylaxis *in vitro*. Tissue, usually guinea-pig lung taken from a sensitized animal, is chopped into slices or fragments, and thoroughly washed in Ringer solution (usually Tyrode's solution). The material can be pooled and then subdivided to give a large number of replicate samples. The undamaged tissue is living, and retains its histamine stores well. If this tissue is incubated at 37° C in Ringer solution at pH 7.5-7.9 in tubes gently agitated to ensure diffusion and moderate oxygenation, the addition of the antigen causes a sudden release of some of the stored histamine. The released histamine is easily and accurately assayed biologically on the guinea-pig ileum. The percentage released from the tissue varies in different experiments between 10 and 30 per cent, depending on the level of sensitization of the tissue. In any one experiment the level of histamine release is surprisingly constant in replicate samples, but is readily altered by modifying the treatment of the tissue. Reduction of the histamine yield can therefore be used as an indication of interference with the normal chain of events set off by challenge of the tissue.

Indications that the Reaction Involves Enzymes

Mongar & Schild (1956, 1957, 1958, 1962) devised and applied this method. They found that the reaction was dependent on the presence of calcium, and that it had a pH optimum at 7.8. It was very dependent upon temperature, so that at 30° C the release of histamine was very slight, at 38-40° C it was maximal, and after the tissue had been warmed to 44° C for 15 minutes no reaction occurred. They also observed that many antipyretic substances, e.g. phenol, phenylbutazone, cinnamic acid, amidopyrine, phenazone, salicylate, if present at the time of challenge, prevented histamine release, whilst not preventing desensitization of the tissue. In the course of this work it was shown that iodoacetate, N-ethylmaleimide, azide and dinitrophenol, and also complete deprivation of oxygen could prevent histamine release. They also found that oxygen utilization increased after challenge. Mongar and Schild therefore concluded that the tissue must be capable of oxidative metabolism for the anaphylactic reaction to occur. This has since been interpreted by others as 'anaphylaxis is an energy-requiring reaction', but this statement is not fully justified, as will be discussed later. Several groups of workers using modifications of the technique, have confirmed and extended the findings of Mongar and Schild (Högberg & Uvnäs 1960; Chakravarty *et al* 1959, 1960; Yamasaki *et al* 1961; Hayashi *et al* 1960). Moussatché & Prouvost Danon (1956, 1960) also used intermediates and inhibitors of the tricarboxylic acid cycle to show that this source of energy influenced anaphylaxis in chopped tissue.

Mast Cells

Mota (1957, 1959, 1960) and also Boreus & Chakravarty (1960) used the mast cell count as a means of evaluating the severity of anaphylaxis. Nearly all the histamine in practically every tissue is found in the granules held within the mast cells (Riley 1959). These granules contain heparin, and can therefore be stained with basic dyes such as toluidine blue. In anaphylaxis and in many forms of tissue damage these granules are lost from the mast cells which often totally disintegrate. The numbers of identifiable mast cells present in a tissue before and after challenge will thus bear some relation to the severity of the antigen-antibody reaction. Mota studied this in fragments of various tissues, and experimented particularly on fragments of the very thin mesentery attaching the small intestine of the guinea-pig (and rat) and has obtained results in agreement with those of Mongar and Schild. He has also shown that the anaphylactic

damage to mast cells can be reduced if nicotinamide or other inhibitors of diphosphopyridine nucleotidase are present.

Attempts to Identify Specific Enzymes

Studies on anaphylaxis *in vitro* have contributed much to our knowledge, but the interpretation of results relies upon the specificity of the antagonist or the enzyme inhibitor, and in many instances the degree of specificity is poor or unknown. This criticism applied particularly to experiments in which high concentrations of inhibitor have to be added, because an increase in the ionic strength of Tyrode solution $\geq$ 20 mM NaCl, will itself reduce the amount of histamine released in anaphylaxis. High concentrations also invite the danger of inhibition by a subsidiary property of the inhibitor, which will thus lead to incorrect conclusions regarding the mechanism involved.

Since the results of Mongar and Schild clearly indicated an enzymic step or steps as intermediates in the release of histamine, the obvious development was the identification of the enzymes. Austen & Brocklehurst (1961) approached this problem by offering alternative substrates (synthetic esters and peptides), to act in competition with the normal substrate in the tissue and thus reduce the effectiveness of the events leading to histamine release. This technique relies upon the readiness of enzyme and synthetic substrate to combine and is not concerned with the ability of the enzyme to split the substrate subsequently. Thus the results in respect of any given enzyme might differ quantitatively from those of the biochemist who is concerned with the splitting of substrate. The method nevertheless provides much more useful information than study of the fission products as evidence of enzyme activity, because these may relate to enzymes in no way connected with the release or formation of pharmacologically active products.

By building up a pattern of experimental data showing the relative effectiveness (or failure) of a large number of substrates, Austen and Brocklehurst concluded that an enzyme having the characteristics of chymotrypsin was essential to the reaction, and were able to exclude all the enzymes known to be activated from the various components of complement, with the exception of C′3. They obtained suggestive but inadequate evidence that the esterase from C′3 was involved in the reaction. These results do not mean that other enzymes may not be activated, but indicate that these are not essential factors in the release of histamine and formation of SRS-A during anaphylaxis.

TABLE 15.1

Procedures which Modify the Anaphylactic Release of Histamine

pH: optimal range about 7.8 minimal activity at 6.2	
temperature: optimal range 40° C to 41° C; total inhibition at 15° C and 45° C 5 minutes at 45° C irreversibly prevents the response of tissue to events started by the Ag-Ab union	
ionic strength: limited enhancement if moderately hypotonic, inhibition if hypertonic	
Ca^{++}: total lack prevents reaction; levels below about 1 mEq/litre depress it	
Reaction reduced or abolished by: reduction in S–H groups (1) by very low O_2 tension (2) by iodoacetate, N-ethyl maleimide, oxidized glutathione, and many other thiol inhibitors including sulphite	
inhibitors of chymotrypsin (1) antipyretics and compounds of indole, phenol, nicotinic acid, etc. (2) ester and peptide substrates (3) di-isopropylfluophosphonate	*Note.* In corresponding tests, inhibitors of trypsin, carboxypeptidase and leucine aminopeptidase do not suppress the reaction
salicylaldoxime and phlorizin presumed to be acting against the esterase from C'3	
saturated fatty acids pentanoic to dodecanoic (also ε-aminocaproic)	Mode of action not known. Must be influenced by metabolic processes in the tissue, but presumed to be related to fat metabolism and only incidentally to the production of energy
Reaction enhanced by: succinic acid and other substances in the Krebs' cycle which are capable of raising the concentration of succinic acid	
maleic acid	
No effect with cytochrome poisons CO, 2 heptyl 4 hydroxyquinoline N-oxide (dinitrophenol and CN effective only in large doses, and in anomalous fashion)	
fumaric acid	Component of the citric acid cycle, related to succinic acid, and also to maleic acid which is not part of the cycle

Anoxia and Metabolic Activity

Very recently Mongar has investigated the actual levels of oxygen lack needed to prevent the antigen-antibody reaction and found that 50 per cent inhibition is produced by 0.5 per cent oxygen in nitrogen. This inhibition is greatly potentiated if small amounts of cysteine are present during anoxia. From this, and studies of substances able to disrupt or inactivate S-H and S-S groups in tissue, he has concluded that the way in which O_2-lack reversibly influences the tissue, is probably by altering these active groups or bonds on the enzymes or their precursors or possibly on the antibody itself. The need for O_2 would then not necessarily be related to metabolic processes, and indeed it is difficult to see how oxidative metabolism could survive the combination of oxygen-lack and CO treatment which failed to prevent the anaphylactic release of histamine in the experiments of Austen and Brocklehurst. Many workers have shown that the rate of oxygen utilization rises after an antigen-antibody reaction in a Warburg apparatus. However, the time between contact with antigen and the end of the active phase of histamine release, is probably much less than 2 minutes, whereas Warburg measurements refer to periods of at least 15 minutes and thus cover not only histamine release, but all the subsequent and concurrent events.

Nevertheless some of the products of tissue metabolism can influence antigen-antibody reactions since small increments of succinic acid can enhance these; however, maleic acid is at least as potent in this respect whereas fumaric acid is inactive, and the effect therefore seems to be at best only indirectly related to the level of available metabolic energy. Table 15.1 is an attempt to present in an accessible form the more important observations and their probable meaning. Hypothetical schemes for the actual course of events in the reaction are of little merit because the total number of steps is unknown, and the arrangement of those considered to be identified is largely a matter of speculation.

Ions

It has been stated that magnesium is not essential to the reaction, but this is questionable. The fact is that calcium ion is essential, since complete removal of Ca^{++} by the chelating agent EDTA* will prevent the reaction. EDTA binds Mg^{++} a little less strongly than Ca^{++}, so that restoration of the Ca^{++} by adding excess will also restore Mg^{++} by displacing it from the EDTA. On the other hand, the addition of Mg^{++} will not displace Ca^{++} from chelation. Thus we can show that Ca^{++} is essential, but cannot be

* EDTA = Ethylene diamine tetra-acetic acid, disodium salt.

sure that Mg^{++} is not also necessary, or at least normally involved in hypersensitivity reactions.

The Site of the Reaction

The intact mast cell provides visible evidence of anaphylactic damage and histamine release both *in vitro* and *in vivo*, in many species. When small pieces of mesentery are used in *in vitro* tests, very few other types of cell are present, and it has been concluded that the mast cell contains all the requirements for anaphylactic release of its contained histamine. It is tempting to consider that the mast cell is not only the source of histamine, but the specific tissue type where fixed antibody is localized and from which all the processes of the anaphylactic reaction originate. If this were the case it would make the concept and the study of the reaction much more simple. Unfortunately it cannot be accepted for several reasons. Firstly passive anaphylactic reactions can be produced in the skin of rats which have been intensively treated with compound 48/80 to destroy the mast cells. Secondly, visible anaphylactic damage occurs to other cell types. Thirdly, the amount of antibody necessary to sensitize any tissue is so small that localization of uptake at this level cannot be demonstrated.

Workers on isolated mast cells and on destruction of mast cells by relatively specific agents such as compound 48/80, have used the rat because such studies are not satisfactory in the guinea-pig, nor as far as we know, practicable in other species. Recent work has shown that the isolated mast cells of the rat contain an active enzyme resembling chymotrypsin, and that they lose their histamine rather easily. Other species examined have mast cells which probably contain a precursor but do not contain the active enzyme, and are less ready to lose their histamine. Rat tissues may thus prove to be atypical in respect of reactions involving histamine release or the interaction of proteins.

Factors Influencing the Reactions of Different Tissues

We know that antibody is easily and rapidly taken up on tissue, and now think that this is a rather non-specific surface phenomenon which will vary somewhat for different cell-membranes. If this is true we must expect that the union of antibody and antigen will occur on the membrane of many if not all types of cell, but that the subsequent events will be dependent on the type of cell and its immediate surroundings. It is evident that cell membranes must differ because of the wide range of reactivity shown by different tissues in contact with the same biologically active agent – e.g. histamine or 5-hydroxytryptamine. Table 15.2 shows

that this is true even in tissues as closely related as the smooth muscle of the trachea and the bronchioles of the same animal. Histamine does not contract all smooth muscle, neither does 5-hydroxytryptamine.

We should therefore expect some variation in the intensity of the initial trigger (antigen-antibody union) and a considerable variation in the effect this has on the cell membrane. This first set of variables might thus be called 'membrane susceptibility'. The next variable might be headed

TABLE 15.2

Comparison of the Minimum Effective Doses of 5-hydroxytryptamine, Histamine and Acetylcholine on the Isolated Circular Muscle of the Trachea and Rings of Bronchioles from the Same Animal

	Bronchioles			Trachea		
	5-HT	Hist.	ACh	5-HT	Hist.	ACh
Cat	0.01	2.0	0.1	0.02	>20	0.05
Rat	0.01	>5	0.04	0.1	>5	0.04
Dog	0.05	0.3	0.1	As bronchioles		
Guinea-pig	0.4	0.4	0.4	As bronchioles		
Rabbit	>8	0.5	0.2	>8	>5 R	0.4
Rh. monkey	>20R	0.5	0.1	0.02	0.5	0.01
Man	>20R	0.2	0.1	—	—	—

R denotes relaxation to large doses.
Doses given as μg base per ml bath fluid.

'intermediate reactions', and this would include the activation of enzymes. This activation could result from changes in ionic environment or the effects of co-enzymes or unmasking of active groups. Whilst there must be a general scheme of enzymes in all living cells, the special functions of each cell type make it safe to assume that the pattern of enzyme activation will vary with each tissue. The next phase concerns pharmacologically active mediators, and the number and quantity of these will obviously depend both on the stores or precursors of such mediator substances and on the effectiveness of the enzymes able to free or form them. The range and persistence of the active enzymes will thus determine in very large measure the severity and the characteristics of the whole reaction. Finally, the mediators (possibly with some contribution by the intermediate reaction and also some from the primary membrane reaction) produce the clinical picture. Here the difference between tissues will largely be one of physiology, that is, the range of possible effects which a mediator

can produce in one particular tissue, for example skin or gut or bronchiole. In addition there will be modifying factors such as the proximity between susceptible cells and the active mediator, and the rate of destruction or dilution of the mediator in the tissue spaces. In the case where mediation is by polypeptides, the active level may rise or fall very sharply, since it depends on the balance of rapid rates of formation and destruction. In most other instances, release or formation appears to be fairly rapid, and destruction thereafter reduces the level progressively. A less obvious factor which may limit the range of the effect of a mediator, but incidentally prolong its local action, is the adsorption of the substance close to its site of formation. In sum, there would be great differences between the observed reactions in different tissues even if the same amounts of the same mediators were freed in each. With the increasing number of known mediator substances, the range of effects becomes correspondingly multiplied and the overall picture infinitely more complex.

FINAL MEDIATORS OF TISSUE REACTIONS IN ANAPHYLAXIS

We may reasonably assume that a substance is mediator of an effect observed in a sensitized tissue challenged with antigen, if the following points can be demonstrated:

1. That addition of the substance can mimic the effect.
2. That the substance is present at an effective level in the tissue at the time of the reaction.

Additional evidence, which might be acceptable instead of (2), would be:
(*a*) abolition of the effect by a moderate concentration of an antagonist possessing a high degree of specificity,
(*b*) reduction of the effect by depletion of the substance or its precursor in the tissue (or if blood-borne, in the whole animal).

The exclusion of any particular substance from a reaction is much more difficult.

Histamine

There are several useful reviews of the early work connecting histamine with anaphylaxis (e.g. Dragstedt 1941; Dale 1950, 1952, and an up-to-date account by Feldberg (1961)). The first intimation of such a connection was by Dale and Laidlaw (1910), yet the final clear connection with clinical practice did not come until the work of Schild *et al* in 1951. This work was an application of the classic methods of pharmacology to prove

that histamine was set free when living bronchioles and lung tissue from human asthmatic subjects were challenged *in vitro* by the various specific allergens. This work showed clearly that histamine was released from human tissue *in vitro* in the same experimental procedure as had been previously used for guinea-pig tissue, but the failure of these workers to find any additional substance led them to attribute an important role to 'intrinsic histamine', which we would not now accept (see later).

The well-known pharmacological actions of histamine are as follows:

1. Contracts many smooth muscle organs including human bronchioles.
2. Given locally (e.g. by iontophoresis or intradermal injection) causes an increase of vascular permeability leading to oedema.
3. Given intravenously in cat causes a brief increase in pulmonary vascular resistance and a fall of carotid blood pressure. Large doses cause capillary spasm and vascular collapse.
4. By intravenous infusion in man, causes headache (with a sensation of pressure) and flushing of the face, with some fall of carotid blood pressure and some tightness of the chest. There is a considerable increase of acid gastric secretion. The release of endogenous histamine by chemical releaser substances causes similar effects (Lacomte 1957). The rise in haematocrit reading indicates loss of fluid from the circulation into the extracellular compartment.
5. Increases nasal and lacrymal secretions and is thought to increase secretion of bronchial glands.

Thus the local release of histamine could easily be the cause of urticarial conditions, hay fever and angioneurotic oedema, and also the bronchospasm seen in acute anaphylactic shock.

Antihistamine Drugs

In these conditions, the effectiveness of potent modern antihistamine drugs in very many cases, not only supports this explanation but suggests that histamine is the major causative agent. This rationale may not, however, be as conclusive as it seems, because the antihistamine drugs are not entirely specific, and are usually used near the highest tolerable level, so that their other actions may be responsible for some part of the clinical improvement. This likelihood is strengthened by the facts that experimentally the anti-allergic potencies of drugs do not follow the order of their antihistaminic potency, and that many drugs successful clinically are not the most potent antihistaminics but have numerous other actions. Amongst these unspecific actions are local anaesthesia, atropine-like activity, antagonism of 5-hydroxytryptamine and reduction of tonus in

certain smooth muscle preparations. The most general of these is the local anaesthetic potency, which is often as great as that of xylocaine. This may be the cause of sedation experienced as a side-effect, and could also help to account for the reduction of pruritis and the axon reflex flare, in skin reactions. The other properties, such as antagonism of acetylcholine, vary enormously between different drugs, but it is clearly probable that one or another may be particularly useful in treatment of the symptoms of allergic reactions, depending on the site or the tissue involved.

The Physiological Role of Histamine

It has been thought in the past that in spite of its ubiquity histamine had no physiological role except in gastric secretion. Recent work has suggested that the release of histamine locally in trauma or other forms of tissue damage assists in the mobilization of phagocytes (Goszy & Kato 1957) and stimulates or facilitates tissue repair. The latter effect may be indirect, the important factor being the release of ground-substance material from the mast cells; however this may be, the depletion of histamine stores, or the local application of antihistaminics, retards healing and gives a weaker repair after incisions (Boyd & Smith 1959; Kahlson 1960, 1962). These observations may indeed offer a teleological explanation of how histamine comes to be involved in anaphylaxis. The mechanism begins as a line of defence, with products giving physiological advantages, but when grossly magnified the same process can cause grave distress to the body by an excess of those same products. One may therefore question the desirability of depleting the body of histamine, or of saturating it with antihistamine drugs continuously over long periods, but in all acute allergic manifestations *excess* histamine is released, and it is logical to counteract its effects by specific drugs. Antihistamine drugs alone may not be enough to suppress all symptoms because histamine may be only one of several active agents, as will be shown later.

Histamine Metabolism

The importance of histamine has also to be considered in the light of recent evidence regarding the dynamics of histamine release, destruction and resynthesis. These findings are mainly to the credit of Schayer (1959, 1961), who has developed an isotope labelling technique to follow these physiological events. For instance, intravenous ^{14}C histidine is decarboxylated *in vivo* to give ^{14}C histamine, which can then be estimated quantitatively after repeated recrystallization with carrier histamine. The advantages of the technique are that physiological amounts are used, and the

animal is not subjected to stress, restraint or trauma, quite apart from the usual advantages that labelled material can be followed with certainty and considerable quantitative precision. Furthermore the final products of metabolism can be separated by chromatography and identified by parallel comparison with known materials, they can then be quantitatively estimated by the ^{14}C label, and this can also show the presence of unknown end-products. Schayer's important results include the following:

Excretion. Histamine ingested in food or formed in the gut by bacteria, is not stored but excreted, the excretion products varying with species. In man the urine contains mainly free imidazole-acetic-acid, and 1-methyl imidazole-acetic acid, showing that deamination by mono-amine oxidase, before or after conjugation, is the main metabolic pathway: very little is excreted unchanged.

Storage. Histamine which is stored in mast cells, and which constitutes nearly all of the content of the tissue, is formed from histidine, and is presumed to be formed in the cell. The turnover of the stored histamine is slow because this seems to be an emergency supply, but once freed, this histamine is treated as extrinsic and is not returned to storage but detoxicated and excreted.

Utilization. Many tissues (and possibly all) have a fairly high turnover of histamine formed in or near the vascular endothelium by histidine decarboxylase. This is not stored, and the rate of destruction is high, therefore it contributes little to the amount of histamine extracted from tissue. The activity of histidine decarboxylase is increased as a result of stress (including cold) and any traumatic or chemical or other histamine-releasing process to which the animal is subjected. Kahlson has recently shown that it is present in high concentration where growth is rapid, e.g. regeneration of tissue, wound healing and foetal development. Schayer considers that it is probably a local regulator of circulation at the level of the capillaries, thus resurrecting the views of Dale and Lewis in the light of new evidence.

Histidine Decarboxylase. When tissue is repeatedly subjected to histamine release, as in repeated trauma or frequent treatment with chemical releasers, the histidine decarboxylase rises during the resynthesis and restoration of histamine stores, and continues higher than normal afterwards (Schayer 1960). Since repeated histamine release is inevitable in all allergic states it seems likely that allergic tissue has abnormally high histamine synthesis and a correspondingly raised level of active histamine continually present. Such tissue would be expected to have a lowered threshold of response to histamine from mast cells or other extrinsic

sources, and might also be expected to inactivate any extra histamine at a comparatively slow rate. This would offer an explanation of the many reports that asthmatics respond to lower doses of histamine and other substances than do non-asthmatics, or correspondingly, give a response where normal subjects do not.

Possible Action of Glucocorticoids. The striking anti-allergic action of the glucocorticoids is probably due to many separate effects which include the increased stability of many cell membranes such as those of capillaries and smooth-muscle cells, as well as the depression of enzyme activity, particularly in connection with injury and repair. It is known that the levels of tissue histamine fall, that repair is slowed and that antibody synthesis is reduced under the influence of the anti-inflammatory corticoids. All these changes may be explained as depression of enzyme activity, an effect which would produce particularly striking changes where the symptoms were enhanced because an enzyme was abnormally active, as postulated regarding histidine decarboxylase in chronic allergic states. This is of course only one of several possible ways in which the corticoids might act. Two obvious ones are, firstly the suppression of the enzymic events triggered by the union of antigen with antibody and leading to the release of active substances, and secondly the decreased reactivity of all tissues to the active products produced.

Slow-reacting Substance

Occurrence and Role. A detailed review of SRS-A (slow-reacting substance of anaphylaxis) has recently been published (Brocklehurst 1962). The chemical structure of this substance is not yet known, and until it can be obtained in moderate amounts and comparative purity, the full pharmacology and clinical significance will remain uncertain. Nevertheless there is a strong case for believing that in asthma this substance may be of overriding importance in the production of prolonged bronchospasm.

There are many 'slow-reacting substances' – the term simply implies a material of unknown composition which can cause a relatively slow contraction of an isolated smooth muscle preparation. The one we are concerned with was first noted by Kellaway & Trethewie (1940). They found that when the lungs of a sensitized guinea-pig were perfused through the vascular tree with Tyrode solution, anaphylactic bronchospasm was caused by challenge with antigen, and at this time biologically active substances were detectable in the effluent perfusate. When tested on the guinea-pig ileum, the perfusate caused a contraction which began

like that produced by histamine, but the subsequent relaxation was much slower than that after pure histamine. The main contraction was attributed to histamine, and the persistent late effect to a subsidiary contraction caused by a 'slow-reacting substance'. Brocklehurst (1956) unmasked the SRS by the use of antihistamine drugs, and by parallel quantitative assay against bradykinin, substance P, and 5-hydroxytryptamine showed that it differed from them. As the pattern of pharmacological activity in tests with many different tissues did not fit with that of any substance previously described, the suffix -A was added to indicate that this was a particular slow-reacting substance associated with anaphylaxis. In the doses available, it fails to cause a response in many pharmacological tests, but a positive result of great interest is its ability to cause bronchoconstriction in isolated human bronchioles. This contraction is not inhibited by mepyramine or clinically tolerable levels of atropine. Thus the occurrence of SRS-A can explain the failure of antihistaminic drugs in allergic asthma. It can also account for the mepyramine-resistant component of the anaphylactic reaction of the isolated bronchioles from an asthmatic patient, which was observed by Schild *et al*, but attributed to 'intrinsic histamine'. SRS-A is formed in many tissues, notably in lung and blood vessels, as a result of an antigen-antibody reaction in the tissue itself. The greatest amount is formed in the first 5 minutes after sudden brief exposure of sensitized tissue to antigen, but it is still detectable 1 hour later. When excess antigen is left in contact with the tissue the release of SRS-A is more prolonged. Little or none can be extracted from unchallenged sensitized tissue.

SRS-A obtained by perfusion of human asthmatic lung taken fresh after pneumonectomy for carcinoma of the bronchus (and in other cases from tissue fragments of asthmatic lung), has been compared with the SRS-A from guinea-pig, which is the usual source. In purification and pharmacological tests the two are indistinguishable. The relative importance of histamine and SRS-A in asthmatic bronchospasm can be calculated from the actual amounts of each coming from the tissue, and the ratio of the equi-active doses on isolated human bronchioles. For instance, if during one particular minute 4 μg of histamine and 400 units of SRS-A are found in the effluent perfusate, and we take 0.2 μg and 4 units respectively, as concentrations producing equal contractions of the chain of bronchioles, it follows that we have twenty doses of histamine and 100 doses of SRS-A set free during the same minute. The results of such a calculation show that only during the first 3 or 4 minutes after antigen, is histamine present at more effective levels than is SRS-A, and that

5 minutes later, the response to SRS-A is twice that to histamine, and by 30 minutes is six times as great. There are other considerations, such as the very persistent effect of SRS-A, and suggestive evidence that it is adsorbed in tissue near the site of its formation, which make it likely that the relative effectiveness of SRS-A is even greater than these values suggest. There is no adequate evidence yet for or against the participation of SRS-A in any reaction other than asthma, but it would seem to be a likely factor in food allergy at least, because the gut (of guinea-pig) will both form and respond to SRS-A.

Chemistry. SRS-A is an acidic substance, very soluble in water. It readily associates with lipids, and the complex then acquires some degree of solubility in organic solvents. After purification it shows a marked tendency to adsorb on glass or agar or any solid medium, and for this reason can best be separated by electrophoresis using a density gradient. It is resistant to trypsin, chymotrypsin, papaine, pepsin and alkaline phosphatase, and is only slowly destroyed at 20° C and pH 2 or pH 13, or by boiling at pH 7.5. It is quite quickly destroyed by organic peroxides and by bacterial action.

Inhibition. There are as yet no reasonably potent and *specific* inhibitors of SRS-A, but all the bronchodilators in common use will overcome it. It is notable, however, that as soon as short-lived bronchodilators (e.g. adrenaline) are metabolized or washed away, the SRS-A contraction returns. Calcium exerts a definite but limited antagonism. Many drug-screening schemes now include tests for SRS-A antagonism, so the development of effective drugs may be expected. Homochlorcyclizine is only a weak antagonist, and has many other properties, but its usefulness as an anti-asthmatic drug may be due to its action against SRS-A.

The purification and characterization of this substance presents special difficulties, but once these are overcome the development of antagonists will become rational, and full investigation of the pharmacology of SRS-A will be possible.

5-Hydroxytryptamine (5-HT: Serotonin: Enteramine)

There is no direct evidence connecting 5-HT with anaphylactic conditions, with the exception of certain reactions in rabbits and possibly in rats and mice (Waalkes *et al* 1957; West 1961). The interpretation of results is difficult because the most widely used antagonist, bromolysergic acid diethylamide, has unspecific actions in the rather high concentration needed to suppress anaphylaxis. These include reduction of blood pressure (and therefore skin blood flow) in the intact rat, and reduction of the response of isolated tissue (e.g. rat uterus to bradykinin).

For some time it was thought that 5-HT might be the cause of asthmatic bronchospasm in man because 5-HT given as an aerosol was bronchoconstrictor in guinea-pig, dog and cat. Herxheimer (1953, 1955) was unable to produce this effect in normal man, and the effect in asthmatics was unconvincing when the solution was at physiological pH (see also Table 15.2). The results of recent trials of new drugs having strong anti-5-HT action show that this property does not suppress asthma.

Bradykinin

Very recently it has been shown that bradykinin is a basic nonapeptide, and its synthesis has been achieved. It has been known for some time that when the pseudoglobulin fraction of plasma is digested with pure trypsin, this substance (or others of similar chemistry and pharmacological properties) is formed, and that chymotrypsin will degrade the peptide further and destroy its biological activity (R. e Silva 1951). The substrate (bradykininogen) has been located in the α_2 globulin fraction. Plasma, which contains both the substrate and the precursors of suitable enzymes, can be activated by glass so that bradykinin is formed and later destroyed (Margolis 1960). Bradykinin is the best known and most fully characterized of a group of similar substances referred to as plasma kinins (Lewis 1960). This substance is formed *in vitro* from plasma following contact with glass or processes associated with clotting, or by treatment with trypsin or certain proteolytic venoms. It causes contraction of many, but not all, types of smooth muscle, is a vasodepressor, causes increased vascular permeability and leucotaxis, and is associated with increased secretion of sweat and salivary glands (Schachter 1959).

Participation in Allergic Reactions. Considerable fluctuations in the protease-antiprotease balance of blood during anaphylaxis have been recognized for many years, and it seemed highly probable that bradykinin would reach effective concentrations at least in some organs. Detection presents some difficulty because of the ease with which bradykinin may be formed inadvertently by the experimenter. Beraldo (1950) found that bradykinin was sometimes present in the blood of dogs during anaphylaxis. Brocklehurst & Lahiri (1961) found that bradykinin is present (for a brief period very shortly after challenge) in the blood of rat, guinea-pig and rabbit. If the antigen is freed only slowly from a depot dose, the blood contains bradykinin over a period of hours, and the amount of precursor in the plasma is greatly reduced, showing that the actual turnover (i.e. formation and destruction) of bradykinin, is quite high. The enzyme or enzyme-activator which forms bradykinin, is derived

from tissue such as lung and skin but not from the blood, and whilst the antigen-antibody reaction frees this activity, histamine does not. It is unlikely that activation of plasmin is involved in this reaction, because according to Lewis plasmin forms plasma kinins rather slowly, whereas the substance present in perfusion fluid from shocked, blood-free lung, acts on heated dog plasma to give bradykinin in under 2 minutes. Since the precursor of bradykinin is present in the plasma as a small globulin, some at least must be present in lymph and this amount will increase with raised vascular permeability. Release of suitable enzymes from cells damaged by an allergic reaction or resulting from activation of complement is therefore virtually certain to form bradykinin, but the production of symptoms will depend very much on the rate of destruction. We know that destroying enzymes must normally be present, because bradykinin produces only short-lived reductions in blood pressure or increased vascular permeability, but the distribution of this activity in different tissues, and the effect of allergic reactions on relative rates of formation and destruction, are unknown. In human skin, bradykinin causes redness, oedema and itching, it also stimulates pain receptors (Armstrong *et al* 1957). When given by aerosol it produces bronchoconstriction in the guinea-pig and in man (Collier *et al* 1960; Herxheimer & Stresemann 1961). During asthma the increase of vascular permeability and the possible stimulation of the bronchial mucous glands may be equally important.

Acetylcholine (Ach)

The inclusion of this substance among the active agents is prompted only by the need to explain the usefulness of atropine, because Ach has never been implicated directly in any allergic reaction in tissue. For instance it is not found in the effluent from isolated lung during anaphylaxis.

Parasympathetic nerves supply the secretory tissue of the respiratory tract and stimulation of the vagus causes bronchoconstriction. It must be assumed that some secretion and smooth muscle tone is maintained by normal parasympathetic activity. There is no doubt that stress whether physical or psychological can unbalance the autonomic nervous system, and it has been assumed that vagal activity increases during an asthma attack. The use of atropine will suppress both bronchial tone and secretion to subnormal levels and will also abolish any effects which the postulated parasympathetic over-activity would otherwise cause. This is rational therapy, but does not mean that Ach is a product of the hypersensitivity reaction.

It thus seems to be unwise to study the effects of Ach as a model of

asthma, because even if the pattern of response is nearer to the natural picture than that produced by any other substance, all the available evidence shows that the underlying pharmacology is quite different, and the effectiveness of various treatments will differ correspondingly.

Other Possible Chemical Mediators

The substances in this group are potentially contributors to the general pattern of hypersensitivity syndromes but it must be clearly stated that as yet this role is entirely speculative.

G_2 α-*Globulin*

This electrophoretically purified globulin was shown by Miles and Wilhelm to increase vascular permeability, and later found to act by an enzymic mechanism (see Wilhelm 1962). This substance, being normally present as an inactive precursor in plasma, is activated by separation from other plasma constituents and then has very considerable potency. It must therefore be considered as likely to prolong and possibly intensify the reaction set off by some other process such as direct capillary damage, or tissue response to histamine, bradykinin, or in some species to 5-HT.

Leucotaxin

One of the characteristics of allergic reactions is the accumulation of leucocytes. There is an influx of eosinophils to the site in chronic reactions of immediate type, and a very great influx of monocytes which roughly parallels the development of the tuberculin response. These effects can only be due to chemotaxic material produced as part of the reaction. The nature of these materials is unknown, and studies on chemotaxis are notoriously difficult (Harris 1961) but since polypeptides have been shown to possess this property, we have some justification for supposing that the enzymes active during antigen-antibody reactions form leucotaxic products from the proteins of the tissue and lymph which will vary with the site and the enzymes involved and thus give the various observed patterns of leucocyte infiltration.

Delayed Reactions

Nothing definite is known about the mediators of reactions of delayed type, histamine has not been shown to play any major role, although there are great changes in the histamine content of the tissue as the lesion develops and the leucocytes migrate in (Inderbitzen 1956). This reflects the slow time course of anti-antibody combination and may be the key to the

difference in pharmacology between the immediate and delayed types of reaction. Even if the reactions gave rise to the same mediators, the effects would differ in at least the same way as an intravenous injection does from a drip infusion. In fact, the kind of damage suffered by the cells involved is liable to differ greatly, and lead to a change in the relative importance of the mediators formed or freed, even if the actual substances involved are quite similar. The testing of such speculations regarding reactions of delayed type must await the development of antagonists or inactivators of such potential mediator substances as G_2 α-globulin, SRS-A and bradykinin.

Therapeutic Measures

Much new knowledge has been acquired in recent years concerning the essential details of hypersensitivity reactions, but so far none of this has provided any significant advance in therapy. In fact there has been a tendency to produce drugs having a wider range of action – e.g. antagonism to histamine, 5-hydroxytryptamine, acetylcholine, etc., and often being bronchodilators also – rather than having few and more specific actions as would seem to be the ideal. Drugs providing such an effective blanket must inevitably bring a heavy load of side-effects. This trend probably results from the swing away from the unitarian ideas centred on histamine, towards the acceptance of a multiplicity of agents in the allergic state, only a few of which are at present recognized. Proof that this is an unduly pessimistic view must await effective antagonists against the agents at present known, but in the meantime the more rational aim should be to provide such tailor-made antagonists, if possible without too many other actions. The three major agents encountered are the base histamine, the acidic substance SRS-A, and the nonapeptide bradykinin. These are all so different that a single antagonist to all three is most unlikely and thus three separate treatments will probably be needed. This is important, for it may quite well be that each of these agents separately contributes so much to the total reaction, that antagonism of any one alone will provide only unconvincing relief, and even antagonism of two simultaneously might still leave an embarrassingly large intractable reaction. For this reason the pharmacologist will have no satisfaction until the effects of each known substance can be annulled, and the clinician must be aware that each of several active agents may require individual treatment by drugs, and so not be too ready to dismiss as useless any single drug because of its limited ability to control the whole range of symptoms.

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