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Many facts, but insufficient knowledge: the story of asthma

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In the last decade, great strides have been made towards our understanding of asthma, and it seems that soon we will have sufficient knowledge to devise treatment of the asthma attack at least. I believe that this view is justified, but am mindful of similar optimism repeatedly expressed by highly experienced researchers during the last 40 or 50 years. Indeed it is a salutary and humbling experience to look back over the various theories, emphasis and outlook which have been current at different times, and to realize that these must occasionally have hindered real advance. The complexity of nearly all biological functions has provoked the remark 'The Creator did not shave with Ockham's razor', yet this principle of opting for the simplest theory compatible with the data, is a cornerstone of scientific logic. The principle fails when the data are biased because certain aspects have received particular attention whereas others have been ignored or may have been undiscovered at the time. It becomes even less reliable when individual workers give preference to discoveries in their own speciality or become committed to an interpretation which has gained a high award for them.

Nowadays, when it is common knowledge that asthma is an allergy, and the causative agent is inhaled as a dust, it is not easy to envisage the time when immunology was limited to the production of protective antibodies. There must have been many untoward reactions to second or third doses of antigen when animals were receiving injections to boost the concentration of antibodies in their blood so that the serum could be used to give passive protection against infection in man. However, the crude broth cultures of micro-organisms, or tissue extracts used, must themselves have caused quite drastic cardiovascular reactions, and any changes during the course of injections did not attract attention. The animals most used were the rabbit and the horse, both of which are relatively insensitive to allergic reactions, and exhibit them as cardiovascular rather than respiratory manifestations. In 1902, Portier & Richet caused a fatal reaction in a dog by one of the usual boost injections intended to make the animal immune to jelly-fish toxin. Since the reaction was excessively violent, with cardiovascular, respiratory and gastrointestinal symptoms, whereas earlier doses of the toxin had been well tolerated, the observers concluded that the protective process had miscarried and that the animal had become more susceptible to the toxin, rather than less. Hence the description 'anaphylactic shock'.

It was not until 1911 that Dale & Laidlaw tentatively suggested that histamine could be the single agent responsible for the symptoms of anaphylactic shock. The reasoning was sound because there was close analogy in several species with the pharmacology of histamine, which Dale & Laidlaw were reporting. The caution was mandatory, since histamine had never been detected in mammals, except as a product of putrefaction, and by 1910 anaphylactic reactions had been provoked by minute amounts of non-toxic proteins such as white of egg. Between 1902 and 1910 the gross symptomatology of anaphylactic shock in response to harmless substances such as the serum of another species had been described for most of the laboratory and domestic animals. Amongst these data was the description of the syndrome in the guinea-pig, which died with lungs grossly inflated and airways totally constricted. This work in 1910 by Auer & Lewis must have provided a powerful clue to Dale, and was the first clear illustration of the probable mechanism of asthma.

Before 1910, anaphylactic shock had been ascribed to changes in the blood, and the evidence was good. If blood or serum containing antibody was incubated with antigen it became toxic whereas blood without antibody or without antigen did not. When the toxic serum was given intravenously to a normal animal, it produced an acute syndrome superficially similar to anaphylactic shock in that species. The acquired toxicity was ascribed to the production of 'anaphylatoxin', which was assumed to be a product of the marked proteolytic activity also discovered in toxic sera. This was a total theory, with no conceptual gaps such as the origin of histamine, and it had strong adherents for the next 20 years. During this time the

'Histamine theory' collected data but had to wait until 1927 before histamine was isolated from fresh tissue. Then, in 1932, Bartosch, Feldberg & Nagel found histamine in the effluent solution used to perfuse a guinea-pig isolated lung during an anaphylactic reaction, and in 1936 Dragstedt & Mead showed that it was present in the blood of a dog during anaphylactic shock. Thus the Humoral theory, and anaphylatoxin were overshadowed. By now, Lewis had produced a detailed picture of the human pharmacology of histamine, and the association between histamine, allergy and asthma was firmly established. Were not all the criteria satisfied? . . . the immune reaction did produce the substance and it could account for the observed effects . . . successful therapy simply awaited the discovery of specific histamine antagonists to replace the transient and cardio-active adrenaline and sympathomimetics which had already proved successful as unspecific pharmacological antagonists. The first clinically useful antihistamine drug was synthesized as a potential anti-adrenaline compound but was found by an alert pharmacologist to inhibit the histamine-induced contraction of the guinea-pig uterus, and led to 'Antergan'. Soon there were several antihistamines of proven efficacy in histamine-induced reactions in man, including the classic wheal, flare in skin, but to everyone's dismay they were only marginally effective in asthma. For a time it was thought that more powerful drugs would prevail, but this was not so, and then Dale put forward the concept of 'intrinsic' histamine. This theory envisaged the site of action of histamine to be so spatially close to the site of release or so inaccessible, that adequate amounts of antagonist could not reach it. Two apparently parallel examples were cited; the failure of the drugs to block histamine evoked gastric secretion, and the inability of atropine to block parotid secretion resulting from chorda stimulation. However, the histamine theory had taken a body blow, and the new modification was neither particularly plausible nor readily amenable to study. Thus the field was wide open for alternatives notably the possibility of direct action on susceptible tissue by blood-produced agents, such as anaphylatoxin, or bradykinin or other polypeptides, or perhaps such substances as 'darmstoff' or a 'slow reacting substance' produced by slight damage to cells within the shock organ.

By now the year was 1950, a time when methods for purifying peptides and identifying them were being developed, so the humoral theory could be investigated in some depth but not yet with great precision. Immunology was profiting from these advances, with studies on complement and the separation and quantitation of antibodies. Upon this scene in the early 1950s there suddenly burst another candidate—5-hydroxytryptamine (5-HT, serotonin, enteramine). Interest in 5-HT was greatly stimulated by studies in the rat, which responds relatively weakly to histamine, but strongly to 5-HT. The rat was also popular because it responded well to two 'anaphylactoid' agents, 'compound 48/80', which releases histamine and 5-HT from rat mast cells, and dextran of a suitable polymer size which also evokes amine release from mast cells. Anaphylactoid agents produce their effects on first administration and so differ from allergens which must first stimulate the production of antibodies, and will not set off an allergic reaction until the animal is re-exposed to exactly the same antigen several weeks later. The 'releaser' 48/80 was widely used because it gave reproducible and dose-related responses in the skin or the whole animal. It was regarded as a valid model of the antigen-antibody reaction, which at that time was very variable and not easily produced in rats. The interest in the rat was closely linked with the recognition that the mast cell was the site of histamine stores and the source of the histamine released in allergic reactions. The mast cells of the rat are large, numerous and easily recognized whereas those of the guinea-pig are difficult to study and react poorly with compound 48/80. A major outcome of this phase of research was recognition that the tissue mast cell was a central feature of allergic reactions, including asthma, and that the blood basophil could be regarded as its circulating equivalent. 5-HT did not find a star role in asthma because in man it is not very active on the musculature of the small airways, and is not very plentiful in the mast cells. Many of the findings were of indirect relevance to the understanding of asthma. These include the discovery that histamine is bound in mast cell granules to an acidic heparin-like molecule from which it can be displaced by other bases or cations, notably Na^+. The introductory work on mast cells by Riley & West stimulated activity in the whole matter of the physiological role and biochemistry of histamine. It was established that histamine was not absorbed, but was always produced as needed from histidine, by a very adaptable enzyme system. Histamine synthesis occurred in rapidly growing and relatively undifferentiated cells in sites such as the foetus and wounds undergoing repair as well as in mast cells where it was both produced and stored. Thus, histamine became a

respectable member of the group of local agents controlling tissue nutrition and acting to minimize the extent of damage. So what goes wrong in asthma? . . . the best guess is that the challenge by antigen evokes a widespread synchronized response which grossly overloads the system; the lung of any species is at special risk due to the large area of wet mucosa which soluble protein can readily penetrate, but man has the added disadvantage that his lung tissues are highly susceptible to the agents released from mast cells by an allergic reaction.

Although mast cells, rats and 5-HT were in the limelight in the 50s, attention was also being paid to other agents which might contribute to the pharmacology of asthma. The most important were kinins and the slow-reacting substance of anaphylaxis (SRS-A), the former being a product of proteolytic activity on an α2-globulin present in plasma and lymph, and the latter being formed in tissue including blood vessels and lung parenchyma, as a consequence of the reaction between the specific antigen and antibody located on cell membranes. Both substances had a good claim to be considered, and both were difficult to study. The kinins caused contraction of many types of smooth muscle, stimulated secretion of glands, and caused oedema by increasing the leakiness of capillaries—but study on a quantitative basis was almost impossible because they were very quickly destroyed. For this reason, a significant effect would only be apparent in asthma if there was continuous production of kinin as a consequence of long activity of the arginine esterase 'kallikrein'. This enzyme is released from lung by an allergic reaction, but agents such as aprotinin which inhibit the enzyme, and the fenamates which oppose the actions of the kinins have not found a place in the treatment of asthma. The role of the kinins is therefore thought to be a minor one. In 1940, SRS (slow reacting substance) had been found together with histamine in the perfusate leaving a guinea-pig lung during an anaphylactic reaction. The name simply described its one known pharmacological effect, viz. to cause a slower but more long-lasting contraction of the guinea-pig ileum than that of histamine. In the 1950s antihistamine drugs were used to unmask the SRS activity, and to work out its pharmacological profile. The more specific name SRS-A (to denote its relationship to anaphylaxis) helped to identify it as separate from other 'lipid soluble acids' or totally uncharacterized gut-contracting agents. SRS-A was shown to be a very active constrictor of human bronchioles *in vitro* and to be released in significant amounts from human asthmatic lung, but it proved to be extremely difficult to purify, and attempts to characterize it by enzymic destruction or chemical reactions were inconclusive.

The biochemistry of the anaphylactic release of mediators from sensitized lung was also studied in the 1950s. It was found that enzymes were involved, because the reactions were greatly temperature-dependent. Energy was required because metabolic blockade prevented release. Esterases were involved because selective inhibitors or specific substrates could interfere with release. Ionic calcium was essential. Intact cells were necessary so presumably the process was highly organized and active rather than the consequence of irreparable change such as destruction of the plasma membrane.

By 1960 the simplistic view of asthma had been replaced by a morass of uncertainty. Many questions were posed. Histamine is involved but does it really do much? SRS-A seems to be important, but is it formed *in vivo*? . . . and what progress can be made by studying an unidentified factor? 5-HT causes wheeziness so why does it not also constrict small airways? Aerosols of acetylcholine cause all the symptoms of asthma—are you sure that acetylcholine is not released? Does the reaction require any mediator?—could not the stimulation of union between antibody and antigen be directly on the responding cells? How do you account for 'intrinsic' asthma (i.e. where no allergen has been identified) where there may not even be an allergen? Why are the glucocorticosteroids so effective in asthma?—they don't seem to inhibit any of the mediators very much! Man is unique, what can be learned from animals? What about the psychological aspects? Fortunately, within a few years new techniques for handling and characterizing proteins had permitted immunology to burst forth in a torrent of new discoveries, enthusiasm, and theories, which spilled over into related disciplines and caused much cross-fertilization and exciting activity in the 'no man's land' between the clinic and the various branches of biological science. Among the findings were many clues to the nature of asthma, and some answers to the questions.

Ishizaka and his colleagues separated IgE from the serum of an asthmatic and showed that this was the 'reaginic antibody' which would passively sensitize normal human tissue, simply by becoming firmly adsorbed to cells *in vitro* or *in vivo*. The phenomenon was already well known but not the type of antibody involved. The structural characteristics of IgE were established and similar antibodies were found in

several other species, thus supporting the validity of experiments in these. Passive sensitization as an experimental technique made it possible to do crucial experiments *in vitro* on human tissue and to avoid mistakes due to species differences, as well as permitting more reproducible experiments. The mystique surrounding reagins was thus swept away.

Basing his theory on studies with the endotoxins of *Bordetella pertussis*, which stimulate rats to make large amounts of IgE and also make them unusually responsive to histamine by reducing their counterbalancing response to adrenaline, Szentivanyi proposed that asthma was the consequence of some deficiency in the β-adrenergic mechanisms of sufferers. It was already known that adrenal damage or the use of β-adrenergic blockers made asthmatics worse, as would be expected since adrenaline is a bronchodilator. The new theory inferred some failure of response to adrenaline in the cells involved in the allergic reaction, or the production of IgE, or both. It was persuasive and plausible, but early attempts to verify it in man produced inconsistent data, irrespective of whether the study was biochemical or based on airway resistance or cardiovascular changes. These ambiguous findings did not kill the theory, but rather widened the scope of study to take in the whole matter of autonomic control and of stress. Human leucocytes *in vitro* will release histamine when subjected to a reagin-mediated allergic-reaction, and are thus one of the few human tissues in which biochemical comparison is possible between asthmatics and others. It has recently been shown that the resting concentration of cAMP in leucocytes of asthmatics presently or recently under treatment, is of the order of 50% lower than that of normal subjects or those with other respiratory infections. The response to isoprenaline is also about 50% lower. The difference is greatest in the B lymphocytes, and in spite of wide scatter in the increase of cAMP induced by adrenaline, the active asthmatics stand out as a group of poor responders although asthmatics in remission are not distinguishable. Glucocorticoids and steroid contraceptives improve the response. B lymphocytes are stimulated to multiply and produce antibody when exposed to appropriate antigens, and this response is preceded by DNA synthesis. The response is reduced by high concentrations of cAMP, and is therefore likely to be greater in asthmatics. Adjuvants increase the production of antibody in response to concurrent antigen, and a well-known adjuvant causing increases in IgE is *B. pertussis* which also reduces the response (of rats) to β-adrenergic agents. The inference is that low concentrations of cAMP in B lymphocytes will result in increased response to antigen, and a high production of IgE. The reason for the poor response to adrenaline remains unknown, but it is not confined to leucocytes and respiratory tissues, and is said to be transferred by serum. If this is so an antibody or some other inhibitor of the β-receptor would be suspected. The increase in cAMP caused by prostaglandin E is normal in asthmatics, showing that the 'weak link' can be bypassed.

Improved methods of measuring respiratory function and vagal activity led to the discovery of an important reflex arc in which afferent impulses were carried in the vagus from sensory receptors for tension or chemical irritants to the autonomic centres in the brainstem, and after processing returned in the vagus to the lung. The efferent limb of the arc was thus cholinergic and caused bronchoconstriction and the secretion of viscid mucus. The ancient asthma remedies employing stramonium or belladonna had always suggested a role for acetylcholine but direct methods failed to detect it. These failures are now seen as methodological since isolated lungs or animals with total block of respiratory reflexes were used until recently.

There was also a mild resurgence of the old interest in active substances from blood plasma. The broad outline of the activation of complement was established long before 1950. Complement is a group of proenzymes in plasma which can be activated in an ordered sequence by adsorption upon a complex of IgG and antigen. The outcome is damage to the plasma membranes of cells in contact with the complex. During the 1960s the detailed chemistry of the many steps of the complement 'cascade' was substantially unravelled and two fragments were found to have the biological characteristics of anaphylatoxin. More complication was to follow, for the activation of the later part of the cascade, which included the anaphylatoxins did not necessarily involve IgG, but could be achieved through the much less specific 'properdin' or alternative pathway. The anaphylatoxins can thus be produced after complement activation by antigen-antibody complexes, release of cell enzymes, changed cell-membranes and some adsorptive surfaces. The main actions of the anaphylatoxins are to release the stored mediators from mast cells, to cause increases in vascular permeability, and to attract polymorphonuclear neutrophils. The relevance of complement to asthma only became of interest when it was discovered that the

Arthus reaction (which is the result of cell damage by complement) becomes more severe when an IgE reaction also occurs, and when it was appreciated that long-established asthmatics often have high concentrations of IgG as well as IgE to the specific antigen.

In recent years there has been much speculation concerning the part which prostaglandins may play in asthma. Prostaglandins are formed whenever tissue is damaged, and they are found in isolated lung subjected to mild trauma or anoxia in greater quantities than are produced by anaphylactic reactions. Furthermore, aspirin and indomethacin prevent the synthesis of PGs, yet few asthmatics have claimed benefit from these drugs. However, it would be premature to conclude that the PGs and their close relatives the thromboxanes have no significant role in asthma, since the pharmacology of this large family of substances and their metabolites is incomplete. $PGF_{2\alpha}$ and its 15-keto metabolite both contract human bronchioles, PGE and PGA dilate them. Further complications arise since the lung is the organ in which prostaglandins in the circulation are quickly destroyed, and since the redox potential of a tissue determines whether the precursor is changed to the E or F series of PGs, with hypoxia favouring F,—which seems rather unlikely *in vivo*. However, metabolites of $PGF_{2\alpha}$ have been found in the urine after attacks of asthma, so the question remains open. Asthmatics show increased airway resistance when they inhale an aerosol of $PGF_{2\alpha}$, in quantities thousands of times smaller than those producing a comparable effect in normal subjects. The reasons are not known. The effect is unlikely to be attributable to previous exposure to PGs, but rather to interaction with another agent, which is either potentiated or released. Mutual potentiation between $PGF_{2\alpha}$ and SRS–A has been observed, and PGs decrease the pain threshold, so may increase the effects of kinins or enhance afferent activity in the vagal reflex.

Two other factors released by reagin-antigen reactions are the eosinophil chemotactic factor (ECF–A), and the platelet activating factor (PAF). The chemotaxic agent accounts for the long-recognized eosinophilia in asthmatics, and the accumulation of eosinophils in the site of antigen challenge. Eosinophils are rich in a wide range of enzymes, so they may well have many functions; one of special interest is their ability to destroy SRS–A, albeit rather slowly. PAF causes aggregation of platelets and release of their contents. Platelets contain ATP, 5-HT, thromboplastin, thromboxanes, PGE_1, as well as lysosomal enzymes, but the reaction will take place in the bloodstream and may be of greater significance for the venous side of the pulmonary circulation than for the airways.

On present evidence, SRS–A must be regarded as the most important mediator in asthma except during the first few minutes of a sudden challenge, when a flood of histamine would be predominant. It has a considerable duration of action on the muscle of human airways, and there is good reason to believe that more SRS–A is synthesized during the course of the attack of asthma. *In vivo* it binds to plasma, and it has been recovered from blood during asthma. Until recently, available antagonists were unspecific but recently a compound very active *in vitro* has been reported. Unfortunately it has a short life *in vivo*, but may be a way of testing the belief that antagonists of SRS–A would be effective along with other drugs in the treatment of asthma. The rational design of such inhibitors awaits discovery of the chemical structure of SRS–A.

SRS–A has a pK of about 4·6 and a molecular weight of 400 to 500 daltons. No characteristic physical properties or colour reactions have been found and it is difficult to purify. The recent report that the biological activity was lost after incubation with a purified arylsulphatase is the strongest clue to date: it has previously resisted destruction by enzymes including proteases, phosphatases, phospholipases, neuraminidase, nucleotidases and 15 hydroxy-PG-dehydrogenase. However, attempts to introduce a radioactive S into SRS–A by giving ^{35}S to a guinea-pig before challenge, or during prolonged perfusion of a lung before anaphylactic shock, have not been successful, although ^{35}S has appeared in other lung constituents.

Summary of the current view of an attack of asthma:

1. *Immune trigger*

Antibody molecules attached to the membrane of mast cells can be bridged by polyvalent antigen molecules, and some kind of distortion of the membrane is caused.

(i) The nature of the changes is not known but glucocorticosteroids are thought to minimize them.

(ii) The magnitude of the effect is dependent upon the number of molecules of IgE reacting in unit time, so displacement of bound reagin by another IgE molecule of different antigen-specificity will reduce the effect.

(iii) The ratio of cyclic AMP (inhibitory) to cyclic GMP (enhancing) modulates the activation of cell-processes (refer to sections 2 and 3 below).

(iv) Cyclic AMP concentrations are raised by increased synthesis from ATP. β_2-Adrenergic agents stimulate adenylate cyclase, so do PGE and PGA, and perhaps histamine working through H_2 receptors.

(v) Cyclic GMP concentrations are raised when the cell is depolarized, e.g. by cholinergic activity or entry of K^+: Ca^{++} is needed.

2. *Biochemical events*

Proesterases in the cytoplasm are activated. One type is involved in the formation of SRS–A, another in the release of both stored and newly-formed mediators viz. histamine, SRS–A, ECF–A and PAF.

(i) Ionic calcium is involved and seems to be an important factor in the activation process. It may enter the cell or be released from binding within the cell.

(ii) Phosphonate esters which inactivate serine esterases block both esterases after activation. The proesterase concerned with production of SRS–A is also blocked, but not the one involved in active secretion.

3. *Active secretion of mediators*

Factors known to be required are:

Calcium
Energy: glycolysis and ATP/cAMP
Microtubules

The granules are moved into contact with the plasma membrane and the granule membrane fuses with it to open a 'pore' into the granule through which the contents may be released or Na^+ enter and displace histamine from its bound state. Other granules may fuse with the membrane of the discharged granule and thus have access to the extracellular environment.

The process is dependent on temperature and pH. The cell quickly synthesizes more histamine, but little if any of the released histamine is reabsorbed.

4. *Other agents active in lung*

Prostaglandins, thromboxanes and kallikrein. The unsaturated fatty acid precursors for the two types of lipid are thought to be generated from the plasma membrane of the mast cell (or others) by activation of a phospholipase (see section 1). The origin of the arginine-esterase kallikrein has not been studied.

5. *Activity of mediators*

(i) Histamine. Bronchoconstriction; increased permeability of blood vessels; increased secretion of glands. Inactivated fairly quickly.

(ii) SRS-A. Bronchoconstriction; potentiates activity of other agents; long acting. No other significant activity known.

(iii) ECF-A. Attracts eosinophils, (? scavenging): these contain enzymes able to inactivate SRS-A and histamine.

(iv) PAF causes accumulation and aggregation of platelets and so has potential to involve 5-HT, kinins, thromboxanes and PGE_1. Importance not yet assessed.

(v) Prostaglandins. $F_{2\alpha}$ is bronchoconstrictor, so is the major metobolite (15 keto $F_{2\alpha}$). E_2 is bronchodilator. They are vaso-active, mostly increasing resistance in the pulmonary circulation. Half life in lung is brief and amounts found are small. The thromboxanes, which have the same precursors, have bronchoconstrictor and vasomotor activity, but studies are incomplete.

(vi) Kinins. Bronchoconstriction not certain, but increased bronchial secretions and vascular permeability likely. Very short half-life.

6. *Feedback*

Stress will cause the physiological release of suprarenal catecholamines and steroids which will act together to damp down the reactivity of mast cells and dilate airways. Release may fall off in prolonged stress.

Vagal reflexes will have the potential to set up a vicious circle. The cholinergic activity will cause bronchoconstriction and secretion of thick mucus, and may increase the reactivity of the mast cells. It is a system which does not readily fatigue. The mediators released will increase the mechanical stresses within the lung, and probably stimulate the 'irritation receptors' as well as reducing their threshold for firing.

I feel that I must now justify the title of this discourse. Asthma has been on the "frontier" of scientific interest for a long time, and in most periods it must have seemed that confirmation of ideas or the big break-through was imminent. These expectations were unfortunately based on knowledge in limited fields and total oblivion of factors since recognized as important. There may be blind

spots even now, but the current problem is to give the correct weight to those factors which have been identified. This is where knowledge is still in short supply.

BIBLIOGRAPHY

Suggested reading includes mainly recent reviews or reports of symposia and collected accounts of older work. Single papers have been selected only as examples. The following codes are given at the end of each reference, to identify its content; **B** Historical; **A** asthma; **H** histamine; **M** the mast cells; **K** kinins; **E** 5-hydroxytryptamine; **S** SRS-A; **L** PG; **I** biochemistry and immunology; **P** physiology.

REFERENCES

AUGSTEIN, J., FARMER, J. B., LEE, T. B., SHEARD, P. & TATTERSALL, M. L. (1973). *Nature New Biology*, **245** 215–217. (S).

AUSTEN, K. F. (1973). *Biochemical characteristics and pharmacologic modulation of the antigen-induced release of the chemical mediators of immediate hypersensitivity.* Excerpta Medica. in Internat. Congress Series 323—Tokyo: North Holland. (I) (M).

AUSTEN, K. F. & LICHTENSTEIN, L. M., Eds. (1973). *Asthma: physiology, pharmacology and treatment.* Chapters by: Austen, Gold, Ishizaka, Lichtenstein, Nadel, Parker. New York: Academic Press. (A) (I) (P).

BARTOSCH, R., FELDBERG, W. & NAGEL, E. (1932). *Pflugers Arch. ges Physiol.*, **230**, 129–153. (B).

BINAGHI, R. A., BENACERRAF, B., BLOCH, K. J. & KOURITSKY, F. (1964). *J. Immun.* **92**, 927. (I).

BROCKLEHURST, W. E. (1960). *J. Physiol., Lond.*, **151**, 416–435 (S).

BROCKLEHURST, W. E. (1962). In: *Progr. Allergy*, **6**, 540–560. Editor: Kallos, P., Basle: Karger (S).

BROCKLEHURST, W. E. (1975). In: *Clinical Aspects of Immunology*, pp. 821–846. Editors: Gell, P. G. H Coombs, R.R.A. & Lachmann, P. J. Oxford: Blackwell (A).

BRONFENBRENNER, J. (1948). *J. Allergy*, **19**, 71–82. (B).

COLLIER, H. O. J. & SHORLEY, P. G. (1963). *Br. J. Pharmac. Chemother.*, **20**, 345–351. (K).

COMROE, J. H. (1974). *Physiology of Respiration*—2nd Edn. pp. 72–82., pp. 142–153. Chicago: Year Book Medical Publ. (P).

DALE, H. H. (1920). *Proc. Roy. Soc. B.*, **91**, 126–146. (B).

DALE, H. H. (1950). *Ann. N.Y. Acad. Sci.*, **50**, 1017. (M).

DAWSON, W., LEWIS, R. L. & TOMLINSON, R. (1975). *J. Physiol, Lond.*, **247**, 37P–38P. (S).

ERDOS, E. G. (Ed) (1963). *Ann. N.Y. Acad. Sci.*, **104**, 1–464 (K).

FELDBERG, W. (1941). *Ann. Rev. Physiol.*, **3**, 671. (B).

FOREMAN, J. C., MONGAR, J. L. & GOMPERTS, B. D. (1973). *Nature*, **245**, 249–251. (M).

GOETZL, E. J. & AUSTEN, K. F. (1975). *Proc. Nat. Acad. Sci. U.S.A.*, **72**, 4123–4127. (I).

HAMBERG, M., SVENSSON, J. & SAMUELSSON, B. (1974). *Ibid.*, **71**, 3824–3825. (L).

HAMBERG, M. & SAMUELSSON, B. (1974). *Ibid.*, **71**, 340–4. (L).

HORTON, E. W. (1972). *Proc. Roy. Soc.* (B). **182**, 411–461. (L).

ISHIZAKA, K. (1971). In *Immunological Diseases.*, pp. 202–219. Editor: Lamter. Boston: Little Brown Co. (I).

JONASSON, O. & BECKER, E. L. (1965). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **24**, 250. (K).

KAHLSON, G. & ROSENGREN, E. (1972). *Experientia*, **28**, 993–1128. (H).

KALINER, M. & AUSTEN, K. F. (1973). *J. exp. Med.*, **138**, 1077–1094. (I) (M).

KELLAWAY, C. H. & TRETHEWIE, E. R. (1940). *Q.J. exp. Physiol.*, **30**, 121–145. (S).

KOLATA, G. B. (1975). *Science*, **190**, 770, 771, 812. (L).

LICHTENSTEIN, L. M. & HENNEY, C. S. (1974). In: *Progress in Immunology*, II, **2**, 73. Editors: Brent & Holborow. Amsterdam: North Holland, Elsevier (I).

LICHTENSTEIN, L. M., LEVY, D. A. & ISHIZAKA, K. (1970). *Immunology*, **19**, 831–842. (I).

MATHE, A. A., STRANDBERG, K. & ÅSTROM, A. (1971). *Nature (New Biol.)*, **230**, 215–216. (L).

MILLS, J. E. & WIDDICOMBE, J. G. (1970). *Br. J. Pharmac.*, **39**, 724–731. (P).

MONGAR, J. L. & SCHILD, H. O. (1962). *Physiol. Rev.*, **42**, 226–270. (H) (M) (I).

MULLER-EBERHARD, H. J. & VALLOTA, E. H. (1971). In: *Biochemistry of the acute allergic reactions*, pp. 217–228. Editors: Austen, K. F. & Becker, E. L. Oxford: Blackwell. (I).

ORANGE, R. P. & AUSTEN, K. F. (1971). *Progress of Immunol.*, pp. 173–186. Editor: Amos, B. New York: Academic Press. (I) (M).

ORANGE, R. P., MURPHY, R. C., KARNOVSKY, M. L. & AUSTEN, K. F. (1973). *J. Immunol.*, **110**, 760–770. (S).

OSLER, A. G. (1971). *Int. Arch. Allergy.*, **41**, 49–55. (I).
PADAWAR, J. (Ed). (1963). *Ann. N.Y. Acad. Sci.*, **103**, 1–492. (H) (M) (E).
PIPER, P. J. & VANE, J. R. (1971). *Ibid.*, **180**, 363–385 (L).
RAMWELL, D. W. & SHAW, J. E. (1970). *Recent Progr. Hormone Res.*, **26**, New York: Academic Press. (L).
RILEY, J. F. (1959). *The mast cells.*, Edinburgh: Livingstone. (M).
RUDDY, S., GIGLI, I. & AUSTEN, K. F. (1972). *New. Engl. J. Med.*, **287**, 489–495, 545–549. (I).
SCHACHTER, M. (1969). *Physiol. Rev.*, **49**, 509–547. (K).
SAMUELSSON, B. (1972). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **31**, 1442–1450. (L).
SCHAYER, R. W. (1963). *Prog. in Allergy*, **7**, 189–212, Basle: Karger. (I).
SCHILD, H. O. (1956). *Ciba Foundation Symposium on Histamine*, pp. 139–149. London: Churchill. (B).
SMITH, W. G. (1964). *Allergy and tissue metabolism.* (Includes historical review with good cover of older classic refs.) London: Heinemann. (B).
STRANDBERG, K. & UNVAS, B. (1971). *Acta physiol. scand*, **82**, 358–375. (S).
SZENTIVANYI, A. (1968). *J. Allergy*, **42**, 203–232. (A) (I).
TAYLOR, W. A. FRANCIS, D. H., SHELDON, D. & ROITT, I. M. (1974). *Int. Arch. Allergy*, **46**, 104–120. (A) (I).
UDENFRIEND, S. & WAALKES, T. P. (1959). *International Symposium on Mechanisms of Hypersensitivity*, pp. 219 226, Editors: Shaffer, J. H., Lo Grippo, G. A. & Chase, M. W. London: Churchill, (E).
UNVÄS, B. (1974). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **33**, 2172–76. (M).
VOGT, W. (1974). *Pharmac. Rev.*, **26**, 126–169. (I).
WASSERMAK, S. I., GOETZL, E. J. & AUSTEN, K. F. (1975). *J. Immunol.*, **114**, 645–649. (S).
YU, D. Y. C., GALANT, S. P. & GOLD, W. M. (1972). *J. appl. Physiol.*, **32**, 823–828. (A) (P).