REVIEW

Elements of the history of our present concepts of anaphylaxis, hay fever and asthma

E. L. BECKER

Department of Pathology, University of Connecticut Health Center Farmington, CT, USA

Introduction

What follows is an attempt to sketch, in an admittedly very incomplete and imperfect fashion, how many of our present ideas of the mechanisms underlying anaphylaxis and other human immediate ('anaphylactic') type syndromes arose and developed. The human anaphylactic-type syndromes I shall be discussing are hay fever and allergic asthma. To include the latter two as anaphylactic-type syndromes is in accord with most of present day thinking, but is itself the result of an historical process. I have included 'most' in the above sentence because it is now realized that although allergic asthma has an immediate type component there is also another pathway using CD4$^+$ T-helper cells [1].

I have tried to avoid history both as a string of scientific 'begats' and Butterfield's, 'Whig Interpretation of History', i.e. 'to produce a story that is a ratification if not the glorification of the present' [2]. I hope I shall illustrate that our ideas concerning several of these topics did not arise as direct and ineluctable inferences from a smooth series of results but rather out of the clash of opposing interpretations of contradictory observations. In what follows, I wish to try to answer the questions — Why did our forefathers make the observations they did? What questions were they designed to answer and why were these questions considered important? Of course, in a number of instances crucial observations were stumbled on while searching for something else: one example is the discovery of anaphylaxis by Richet and Portier (Fig. 1). But what was that 'something else' and why did the individual investigator and those that came after him undertake to follow the 'accidental' observations and attribute significance to them? In other words, not only, what were the problems our forebearers set themselves but what were the presuppositions and the set of beliefs and assumptions which they brought to bear in choosing the problems and trying to solve them.

Correspondence: E. L. Becker, Department of Pathology, University of Connecticut Health Center Farmington, Connecticut 06030–3105, USA

In the beginning

The work of Pasteur and Koch, their co-workers and their followers had by the 1880s established the germ theory of disease. Part of the difficulties that these pioneers initially faced in convincing the majority of the reality of the microbial causation of infectious disease is aptly illustrated by the following quotation from a pioneering hygienist appealing to direct experience.

I was brought up by scientific men and ignorant women distinctly to believe that smallpox was a thing of which there once was a specimen in the world, which went on propagating itself in a perpetual chain of descent, just as much as there was a first dog [or first pair of dogs] and that smallpox would not begin by itself any more than a new dog would without there having been a parent dog. Since then, I have seen with my eyes and smelled with my nose smallpox growing up in first specimens, either in close rooms or in overcrowded wards, where it could not by any possibility have been 'caught' but must have begun. Nay more, I have seen diseases begin, grow up and pass into one another. Now dogs do not pass into cats. I have seen, for instance, with a little overcrowding, continued fever grow up, and with a little more, typhus, and all in the same ward or hut. For diseases, as all experiences show, are adjectives not noun substances.

The specific disease doctrine is the grand refuge of weak, uncultured, unstable minds, such as now rule in the medical profession. There are no specific diseases: there are specific disease conditions (quoted in [3]). Florence Nightingale is the author of this quotation; an individual as forceful as her prose style, with, as she points out and as we all know, a direct and compelling experience of infectious disease and its control.

Within the amazingly short period, 1879–1884, Pasteur and his colleagues discovered immunization against chicken cholera, anthrax, swine erysipelas and rabies and the general techniques which Pasteur called 'vaccination' in homage to Jenner and his inoculation against small pox [4]. Out of this interest in and practical concern with the use of immunological

Fig. 1. Commemorative postage stamp. Charles R. Richet (1850–1935) and Paul J. Portier (1866–1962). Discovered anaphylaxis.

agents for the prevention and treatment of infections, the end of the nineteenth and early part of the twentieth century became a time of bubbling activity and excitement in immunology. *Bliss was it in that dawn to be alive.*

The recognition and early exploration of anaphylaxis was part of this activity; the discovery of anaphylaxis in 1902 by Richet and Portier (see below) was the unexpected result of their study of the toxin of the sea anemone. The latter, in turn, was part of the concern of many investigators with toxins of various kinds. Knowledge of toxins started with Roux and Yersin's discovery of diphtheria toxin in 1888 and Kitasato's (a student of Koch) studies of tetanus toxin in 1891. These findings led Kitasato and von Behring (an assistant to Koch) to the discovery of tetanus antitoxin in 1890 and almost immediately afterward to von Behring's production of diphtheria antitoxin. The first case of diphtheria was treated with antitoxin in 1891 and by 1894 it was in regular use in clinical medicine [4]. The problems incident to the standardization of diphtheria antitoxin in experimental animals so it could be employed therapeutically in man and the reaction in humans resulting from its clinical use were the immediate stimuli for the continuing study of anaphylaxis.

In addition to the knowledge of toxins and antitoxins, investigators were also accumulating information on the other properties of antigen and antibodies and this served as the growing framework within which anaphylactic phenomena were investigated and interpreted. A bit of potted history [4] will describe this framework. In 1889, Charring and Roger showed that serum from animals injected with *Pseudomonas pyocyaneaus* would agglutinate these organisms and in 1896 Durham and Grubler demonstrated the specificity of the agglutination reaction. The specific bacteriolytic properties of antisera were reported by Pfeiffer in 1894, and the bactericidal properties of antibodies by Buchner in the same year. A year later Bordet showed that this depended on two factors in serum, a heat-stable specific factor, antibody and a heat-labile nonspecific substance that he termed 'alexin' but since Ehrlich

has been called 'complement'. In 1897, Krause discovered the precipitin reaction. Thus, by 1903, von Dungern [5] could devote almost an entire monograph, called simply 'Die Antikörper', to a review of the current knowledge of antibodies.

All discoveries have forerunners; if one has sufficient incentive to look hard and long, one can always find that no one is as original as first thought. Anaphylaxis is no stranger to this dictum. Magendie in 1839 [6], described the sudden death of dogs following repeated injections with egg white. Von Behring in 1893, noted that guinea pigs frequently were more sensitive to the second injection of toxin occurring some time after the first; he called it the 'paradoxical toxin reaction' (see below). Flexner in 1894 in rabbits, and Richet and Herricault in dogs found that a second injection of a foreign serum led to a more or less violent reaction not seen with the first injection (reviewed in [4]). However, it remained for Portier and Richet in 1902 [7] to realize that these reactions were not only interesting curiosities but represented a phenomenon worth investigating and, not unimportantly, naming.

Because of the general interest in toxins, referred to above, Portier and Richet studied the toxicity of extracts of the Physalia, the Portuguese man-of-war. While on the Prince of Monaco's yacht they discovered that dogs which survived an initial dose of the extract, instead of being protected as they expected, could actually be killed with a second injection of the same or even a lesser amount of the toxin [7]. The development of an increased sensitivity to the toxin Richet termed 'anaphylaxis' (against protection) in contrast with prophylaxis, the protection they anticipated. Richet emphasized that to obtain an anaphylactic reaction, an incubation period of at least 10 days had to elapse between the first and second injection and once induced the anaphylactic state lasted many weeks. In their conclusions, the authors remarked upon the analogy between the 'decreased immunity' in the anaphylactic reaction and the 'decreased immunity to tuberculin' (the latter, a reaction shown by Koch in 1891 [4]).

With Richet's encouragement, in 1903 Arthus demonstrated the inflammatory reaction which developed at the site after repeated injection of serum into the skin of rabbits [8]. This response, which we now call the 'Arthus reaction', he termed 'local anaphylaxis', a misnomer which still lingers. At the same time, Arthus also reported that rabbits sensitized to horse serum by local or intraperitoneal injection of serum would give a severe and even lethal systemic response to the injection of serum into the ear veins, thus demonstrating that anaphylaxis did not require that the sensitizing substance be toxic as Richet had first believed.

Soon after the introduction of antitoxin therapy in 1891, numerous reports were published describing reactions in humans resulting from the injection of antitoxic horse serum. In 1903, von Pirquet and Schick observed that a child who had received a second injection of antitoxin had clinical symptoms the same day though on the first injection

some time previously, her clinical symptoms appeared only after the tenth day. They hypothesized that '... the time of incubation is the time necessary for the formation of these antibodies' [9]. From there, Pirquet went on with Schick to a comprehensive study of serum reactions, their results being published in 1905 in a monumental monograph, 'Serum Sickness' [10] This study is a model of what a clinical investigation should be and can still be read today with enjoyment and profit. Fortunately, for those who do not read German, a charming English translation prepared by Schick himself, is available. In this monograph, von Pirquet and Schick not only described the variety of clinical manifestations following upon the injection of a foreign serum but gave the name to the syndrome. They concluded that the disease was a manifestation of the reaction of newly formed antibody with still-circulating horse serum antigen; an explanation still accepted today. As von Pirquet and Schick point out, their '... conception of antibody did not coincide with the usual term of antitoxin but [they] used the term in a quite general way as the sum of the specific products of reaction of the organism created by the introduced antigen' [10]. They also pointed out the close relationship of their observations in humans to those of Richet and Arthus in lower animals.

A year later in 1906, von Pirquet published the paper in which he coined the terms 'allergy', 'allergic' and 'allergen' [11] (Allergy from the Greek 'allos' other and 'erogos' work). Allergy he defined in the following words: 'The vaccinated person behaves toward vaccine lymph, the syphilitic toward the virus of syphilis, the tuberculous patient toward tuberculin, the person injected with serum towards this serum in a different manner from him who has not previously been in contact with such an agent. Yet, he is not insensitive to it. We can only say of him that his power to react has undergone a change.' [12]

This conception of allergy included 'hypersensitivity' and 'immunity' as well as those responses which seemingly were included under neither. The use and meaning of the term underwent many vicissitudes. Although not explicitly stating so in his original definition, in later work von Pirquet made it clear that he intended the term allergy to be applied only to immunological reactions [12]. Nevertheless, Doerr in his review of 1914 offered a classification of allergy which did not accept this limitation. He divided allergy into hypersusceptibility to antigenic substances as well as altered reactivity to nonantigens, e.g. morphine addiction [13]. However, in later versions Doerr went back to a more limited view of its meaning [14]. In a review of 1926 Coca, exemplifying the feelings of a number of investigators, pointed out the numerous conflicting meanings ascribed to the term allergy by various authorities and urged its abandonment [15]. In that same paper, Coca applied the term 'anaphylaxis' to those phenomena in which the antigen–antibody reaction was established as the initiator and classified under 'allergy' all those conditions where he considered an antibody mechanism had not been demonstrated. Under the latter rubric, he included drug 'idiosyncrasies', serum sickness in man and hay fever. This point of view was long lasting, for example, Boyd in the 2nd edition (1947) of his well-known and standard textbook of immunology defined allergy as all forms of hypersensitivity except anaphylaxis [16].

The lack of agreement as to the meaning of the term allergy, as well as to the classification of allergic disease was inevitable at the time, given the multiple forms and patterns of allergic disease and the inability of clinicians and experimentalists alike to have any firm and well-founded concept of the theoretical basis for their varied manifestation. We have certainly gained in understanding and have much clearer theoretical concepts but it is still not possible to obtain complete agreement on the meaning of 'allergy'. Not too many years ago, an attempt was made by an international committee sponsored by the International Union of Immunological Societies to decide upon a nomenclature for hypersensitivity. The attempt failed and one rock upon which it foundered was our inability to agree on the meaning to be attached to the word 'allergy'. However, clinicians diagnosing and treating hay fever, asthma, serum sickness, drug reactions, etc., desired a brief, convenient, not too limiting group of terms describing what their specialty was and what it was about. As a consequence, they turned more and more to the use of 'allergy', 'allergic', etc. so that these terms became embedded in clinical usage. This was recognized, for example, by the editors of the *Journal of Allergy* when in the first issue of the Journal they stated, '... We believe that it [allergy] does not possess an established meaning in scientific usage.' (They then quoted Karsner and Ecker [17] as the source for this belief). 'However, the term is very generally employed by clinicians who apply it to conditions of specific hypersensitiveness exclusive of anaphylaxis in lower animals ... it seems the title of this journal corresponds to current medical usage.' The editors also could have invoked the authority of Horace's Ars Poetica. 'Many a word long disused will revive and many now high in esteem will fade if custom wills it, in whose power lie the arbitrament, the rule and the standard of language' (Translation of E. H. Blakeney [18]). However, Coombs and Gell in the book they edited in 1963 [19] used the term in von Pirquet's original sense, employing it as rational basis for the classification of allergic conditions; type I being anaphylactic, type II cytolytic or cytotoxic; type III being the Arthus reaction or toxic complex syndrome and type IV, the delayed-type syndrome. This classification brought great clarity to the field in the United Kingdom and Europe and served as a focus for the 'Cambridge School' which flourished in the 1960s.

Theobald Smith noted in 1903 that guinea pigs used to

standardize diphtheria toxin and injected with mixtures of toxin and horse serum-antitoxin often died when injected several weeks later with normal horse serum. He described these results to Ehrlich in 1904, who put R. Otto, then in his laboratory, to work on what he termed the 'Theobald Smith Phenomenon' [20,21]. Rosenau and Anderson in an attempt to understand cause of a patient given diphtheria toxin also studied the effect of injecting horse serum into guinea pigs [22]. As early as 1903, von Pirquet and Schick had termed similar accidents, 'anaphylactic' [9], also studied the effects of injecting horse serum into guinea pigs. The two groups of workers independently arrived at many of the same conclusions and reported them almost simultaneously in 1905 and 1906. They showed that normal horse serum could serve as both the sensitizing and challenge dose; there had to be an interval of ≈ 10 days between the sensitizing and challenge dose of horse serum and that the reaction was specific with a specificity the same as that of antigen–antibody reactions in general. Rosenau and Anderson found that guinea pigs given a daily injection of horse serum could not be shocked after the usual incubation period [23]. Otto demonstrated that guinea pigs who survived acute shock, did not show any symptoms if a short time later they were reinjected with horse serum. The decreased reactivity obtained by both procedures was termed 'antianaphylaxis' by Nicolle and investigated in detail by Besredka and Steinhardt in 1907 [24]. The latter workers claimed that the desensitization was long lasting but this was quickly refuted by Otto, Gay and Southard, and others. It was not until his Croonian lectures of 1920 that Dale distinguished between desensitization and what was presumably tolerance developing from repeated injections of large quantities of protein [25].

Both Rosenau and Anderson, and Otto maintained that the same substance could both sensitize for and elicit anaphylaxis. This was denied by Besredka, Gay and Southard, and Richet. The quantitative experiments by Doerr and Russ in 1909 showing that distinctly less amounts of antigen were required to sensitize than to shock [26] removed the most cogent experimental objection to the acceptance of the first view.

In 1907, Gay and Southard, and Otto in guinea pigs, Nicolle in rabbits and Richet in dogs (all cited in [25]) independently demonstrated that normal animals could be passively sensitized for anaphylaxis by prior injection of the serum from sensitized animals. Only Otto correctly attributed the sensitizing capacity of the serum to its content of antibody [20,21]. Otto emphasized the necessity for the antiserum to be given at least 24 h prior to challenge of the guinea pig with antigen for an anaphylactic reaction to be elicitable. He attributed this 'latent period' to the necessity for the antibody to be distributed throughout the body and 'anchored' to certain cells. The requirement for a latent period in guinea pigs was confirmed by a number of observers and served as the prime experimental basis for the 'cellular' or 'histogenic' 'theory of anaphylaxis'. However, Richet in 1913 reported no requirement for a latent period in passive transfer in dogs [27] and Friedman in 1909 [28] and Scott in 1911 [29] reported that no latent period was necessary in the passive transfer of anaphylaxis in rabbits.

Otto in guinea pig anaphylaxis [20] and von Pirquet and Schick [10] in serum sickness refused to equate the anaphylactic antibody with the precipitin mainly because they frequently failed to find precipitating antibodies in the serum of guinea pigs undergoing anaphylaxis and humans undergoing serum sickness. However, Besredka in 1907 (cited in [24]) and also Friedberger [30] in his earlier theorizing in 1910, considered the two antibodies to be identical and that essentially, anaphylaxis was due to *in vivo* precipitation on cells. Doerr and Russ had reported that in guinea pigs the anaphylactic potency of rabbit antiserum paralleled its precipitin content [26]. Weil in 1916 demonstrated that guinea pigs injected with well-washed antigen–antibody precipitates could be shocked by the subsequent injection of specific antigen [31]. These and other findings not only provided powerful support for the concept of the identity of the precipitating and anaphylactic antibodies (however, see below) but also for Besredka's and Friedberger's notion of anaphylaxis as an *in vivo* precipitation reaction.

The site of the anaphylactic reaction

Essentially there were two theories as to the site of the anaphylactic reaction. The earlier theory was that the reaction was due to antigen reacting with antibody bound to cells. This 'cellular', 'histogenic' or 'sessile antibody' hypothesis was first suggested by Wasserman to explain the paradoxical increase of the toxicity of diphtheria toxin on its second injection into guinea pigs and, as stated above, was advocated by Besredka and temporarily by Friedberger. The early experimental backing for the theory was the finding of Otto [20] that a latent period was required in the passive transfer of anaphylactic sensitivity and he, in fact, so interpreted his finding. Hamburger's idea (cited in Friedman [28]) that anaphylaxis was due to mechanical plugging of capillaries by antigen–antibody precipitates was an exception to this. However, the inability of von Pirquet to detect the precipitates in sera from serum sickness patients or Otto to find them in the sera of sensitive guinea pigs disposed of this concept as a general explanation.

In some versions of the histogenic theory, an enzyme in the sensitized cell acted upon the protein antigen to split off a poison (this was before the antigenicity of carbohydrates was known). Dale considered that the union of antigen with antibody upset the colloidal state of the protein of the muscle fibre [32]. Doerr in 1922 modified this hypothesis considering that the antigen–antibody precipitate formed on the surface of the cell disturbed its permeability [14].

The cellular theory, however, was submerged for a considerable time by the humoral theory; the idea that the antigen–antibody reaction took place in the blood stream to cause the release of one or another form of poison. The idea that the anaphylactic reactions was due to a poison liberated extracellulary from the interior as a consequence of the union of antigen and antibody was generally accepted. This agreement arose partly because the idea of toxins was in the air and partly because of the nature of the anaphylactic reaction itself, the abrupt onset and the dramatic and often lethal systemic effects. However, there was no agreement but rather shifting waves of opinion as to the source of the poison. One assumption was based on the then current idea that antibodies were designed to assimilate and eliminate foreign antigens by digesting them. Erlich's side chain theory, Gengou's original concept in 1903 that complement fixation was due to an 'albuminolysin', and Pfeiffer's concept of immune bacteriolysis as cell dissolution leading to the liberation of 'endotoxins' were all exemplifications of this kind of thinking. The assumption that the putative digestion of the antigen led to the splitting off of toxic by-products seemed to obtain experimental confirmation by Vaughan and Wheeler's demonstration [33] that toxic products were obtained by the alkaline, alcoholic hydrolysis of all proteins tested. It was also from this point of view that Biedl and Krause in 1909 [34] tested the effects of peptone in dogs and guinea pigs and pointed out the similarity to anaphylaxis of the reactions obtained from those protein split products.

In attempting to demonstrate such a 'poison' *in vivo*, Friedmann [28] incubated rabbit antibody with beef red cells in the presence of fresh, normal rabbit serum, centrifuged off the cells and injected the supernatant into normal rabbits. It gave acute reactions which were identified with anaphylaxis and Friedman concluded that he had produced *in vitro* the 'anaphylactic poison'. Since this did not occur with heated rabbit serum he further concluded that the 'poison' was produced from the erythrocytes by the action of complement. He could not, however, obtain his poison if he used beef serum as an antigen. Friedberger [30] repeating Friedmann's work, incubated a washed antigen–rabbit antibody precipitate or sensitized bacteria with guinea pig serum as a source of complement, removed the suspended material and injected the supernatant into guinea pigs. This produced symptoms typical of anaphylactic shock. Friedberger initially assumed that complement acted as a proteolytic enzyme digesting the antigen in the precipitate to split off a toxic product which he called 'anaphylatoxin'. However, Keyser and Wasserman in 1911 showed that kaolin, and Bordet in 1913 that agar, starch or inulin gave the same sort of toxic activity when reacted with guinea pig serum (cited in [34]). These substances, containing no protein, indicated that 'anaphylatoxin' could not be derived from the protein antigen. The anaphylatoxin theory was then amended to suppose that the guinea pig serum proteins were the substrate for the enzymatic action of complement activated by the antibody antigen complex. Alternatively, Jobling and Peterson in 1914 advocated that the kaolin, agar or the specific precipitate absorbed 'antitrypsin' from the serum, allowing the 'serum trypsin' to hydrolyse the toxic moiety from the serum proteins (cited in [35]). Dale and Laidlaw gave a completely new turn to the idea of an anaphylactic poison in 1910 when in their pioneering studies on the physiological actions of histamine they recorded ... 'as a point of interest and possible significance that the immediate symptoms with which an animal responds to an injection of a normally inert protein to which it has been sensitized are to a large extent those of poisoning by β-iminazolyethylamine [histamine]' [36]. Although Dale bravely refused to go beyond this until further evidence developed, others from this time on kept mentioning the possibility that histamine was the anaphylactic poison (see below).

In 1910 Schultz demonstrated that washed intestinal strips and other smooth muscle preparations from sensitized guinea pigs would specifically contract when in contact with antigen [37]. Dale in 1913 confirmed and extended these findings employing the uterus from virgin guinea pigs [38]. Thus, both authors, but especially Dale considered these results as powerful arguments for the validity of the cellular theory of anaphylaxis. This viewpoint was powerfully supported by Manwaring [39], and Pearce and Eisenbrey in 1910 [40] and others later, who showed that the blood of a sensitized dog could be removed and replaced with that of a normal dog without reducing the ability of the former to undergo anaphylaxis. These and other studies on similar lines —Dale's forceful advocacy of the cellular theory [32] and equally forceful denigration of the humoral theory in his Croonian Lecture of 1920 [25], followed by the study of Dale and Kellaway in 1922 [41] — strongly argued against the significance of anaphylatoxin in anaphylaxis. These resulted in the submergence of the humoral theory until its revival in a modified form by Hahn and Rocha e Silva in 1950 (see below).

Early studies on the pathophysiology of anaphylaxis

Concluding from the generalized nature of the signs of anaphylaxis, Richet, Besredka, Gay, and Southard, Rosenau and Anderson, originally thought that the train of pathophysiological changes seen in anaphylaxis was due to a toxic attack on one or another part of the brain. Biedl and Krause in 1909 [34] confirmed Richet's original observation of the immediate and profound fall in blood pressure associated with anaphylaxis in the dog but suggested that it was due to a peripheral vasomotor paralysis. They also drew attention

to the profound leukopenia in this condition and, as had Arthus, also pointed out the incoagulability of the dog's blood. Pearce, and Eisenbrey in 1910 [40] demonstrated the large accumulation of blood in the liver of dogs undergoing anaphylactic shock. However, it was Manwaring, also in 1910, who by removal of various viscera and removing the liver from circulation demonstrated that the liver was necessary for the development of full blown anaphylactic symptoms in this species [40]. By means of cross-circulation experiments, he showed the appearance of a circulating vaso-depressor substance during anaphylaxis and here and in later studies [42] concluded that '... the anaphylactic reaction in dogs is essentially an explosive hepatic intoxication, the formation or liberation of hepatic products having a histamine-like reaction on the extra hepatic blood vessels'.

Emphysema of the lungs in guinea pig anaphylaxis was first mentioned as a prominent sign by Gay and Southard in 1908; they attributed it to paralysis of the respiratory centre (cited in [42]). Auer and Lewis [43], in a superb physiological study published in l910, were the first to describe the emphysema in detail and showed it was due to the contraction of the bronchiolar smooth muscles. They considered this stenosis of the bronchioles the major cause of symptoms and death in the animals. They also demonstrated, by destroying the brain and spinal cord and by vagotomy, that neither the peripheral nor the central nervous system was necessary for the development of the emphysema. This confirmed for the guinea pig the essentially similar findings in dogs of Manwaring [39], and Pearce and Eisenbray published slightly earlier [40]. It is not without interest that the well-founded conclusion that anaphylactic reactions could occur *in vivo* without the intervention of the nervous system was transformed by many to the implicit or explicit feeling that the nervous system, peripheral or systemic, played no role in anaphylaxis or allergic reactions in general.

Arthus in 1909, emphasized the circulatory collapse in anaphylaxis in the rabbit [44] and Auer in 1911 demonstrated that bronchospasm was not a prominent part of anaphylaxis in this species [45]. He noted the pronounced dilation of the right heart and he attributed this to cardiac failure. Coca in 1919 [46], and Drinker and Bronfenbrenner in 1924, observed an increase in pulmonary arterial pressure in the rabbit beginning within 30 seconds of antigenic challenge. Coca observed a greatly increased resistance to perfusion of saline through the pulmonary artery during anaphylaxis in rabbits and inferred that the right heart dilatation was secondary to the '... contraction of the muscular of the (pulmonary) arteries comparable with that of the bronchial musculature in anaphylactic shock in the guinea pig'.

Simmonds in 1919 attributed the liver engorgement in anaphylaxis of the dog to spasm of the hepatic veins which he related to the relatively enormous amount of smooth muscle present in the walls [47]. Referring to the large amount of smooth muscle in the bronchial tree of the guinea pig he suggested that 'the basis for these differences in the reaction of different [species of] animals in [anaphylactic] shock ... is an anatomic difference in the distribution of smooth muscle in the body'. Wells in 1921, pointed out that the high degree of development of the muscular coat of the large and small pulmonary arteries of the rabbit was in accord with this conclusion [48]. Thus, Doerr [14] in his review of 1922 wrote of 'shock organs', meaning those organs which seemingly bore the brunt of the anaphylactic reaction and whose reaction was responsible for the major part of the signs and symptoms. He also accepted the notion of shock tissues and, like Dale, included among them not only smooth muscle but endothelium especially of the capillaries.

Thus, by around 1920 the anaphylactic syndrome had been demonstrated in most mammalian species, including man and even in some avian species. Its immunological nature had been defined, i.e. its specificity, the requirement for antibody, passive sensitization, and the requirement for antigen. The gross features of the pathophysiology, the definition of shock tissues and shock organs and their relation to the different manifestations in various species had also been worked out. These experimental findings were held in a theoretical framework which by the beginning of the 1920s was the cellular theory, primarily as described by Dale. Lastly, there was the idea of some that histamine might be important, largely on the basis that it could reproduce most, if not all, of the manifestations of anaphylaxis in various species.

The beginning of our concepts of hay fever and asthma as allergic diseases

The word 'asthma', meaning panting, was employed by Greek physicians of antiquity. Aretaeus in the second century AD [49] gave what was probably the first clinical description of asthma. The early suggestions of Willis and Laennec and the observations of numerous workers through the nineteenth century slowly led to the conclusion that the underlying cause of the attack of bronchial asthma was a spasm of the bronchial musculature (reviewed in [50]). However, Berkert in his 1878 book on asthma asserted that the dominant theory was that asthma was a nervous disorder (cited in [51]). Melzer in 1910, pointing to the just published work of Auer and Lewis showing that anaphylactic shock in the guinea pig was due to spasm of the bronchioles (see above), suggested that asthmatics are individuals sensitive to a specific substance, that is 'asthma is an anaphylactic phenomenon' [52].

The association between asthma and hay fever had been

pointed out by numerous observers in the nineteenth century. Human sensitivity to roses and other flowers had been reported as early as 1565, but Bostock in 1819, in a case report in which he was the subject, was the first to describe hay fever as a disease with a definite symptomatology. In a later paper in 1828 he described the use of the popular phrase 'hay fever' but preferred to call it 'summer catarrh' [53]. This was the first time the term 'hay fever' appeared in the medical literature. (Finn gives a short description of Bostock's life and work [54].) Elliotson in 1831 also questioned the validity of the term 'hay fever' considering it was probably due to pollen (cited in [51]). This supposition was supported by the observations of Morrill Wyman but was definitely established by Blackley in 1873 in a series of superbly planned clinical experiments executed over a period of 14 years [55]. The experiments were largely carried out by Blackley on himself, a hay fever sufferer. He applied fresh and dried pollen, in and out of season, directly to the nose, to the tongue, lips and face and he inhaled it; in each instance he obtained a reaction. He also applied pollen extracts to the conjunctiva and performed skin tests by placing pollen on abraded skin and obtained an erythema and weal. He was thus the first to use conjunctival and skin tests. He also exposed glass slides coated with adhesive to the atmosphere and showed a relationship between the number of pollen grains collected in 24 hours and the intensity of symptoms.

Blackley's work was published shortly before the time when the germ theory of disease was being continually validated by fresh discoveries and held the reigning attention; in addition, it led to no advance in therapy. Thus, his work did not make the impact that its completeness, thoughtfulness and ingenuity might lead one, looking backwards, to expect. Therefore, various theories, including that of a bacteria aetiology, were considered until the end of the nineteenth century. In 1903, Dunbar [56] confirmed Blackley's work on the aetiological role of pollen and others soon followed. Dunbar considered that pollen induced the symptoms of hay fever by virtue of the putative toxin it contained. He isolated the supposed toxin from pollen and injected it into horses to produce an 'antitoxin' he called 'Pollatin'. This was widely used therapeutically. These various findings led Wolff-Eisner in 1906 to suggest the allergic nature of hay fever [57] a suggestion that was readily accepted by many but not all.

In 1911, Noon introduced specific injection therapy for hay fever [58]. He also was impressed with the idea that pollen contained a toxin, and thus gave repeated subcutaneous injections of pollen extracts to hay fever patients so they would produce their own antitoxin. He reported they obtained substantial therapeutic relief. After his premature death due to tuberculosis, his devoted colleague, John Freeman [59] and soon numerous other workers used pollen extract therapy and by 1920 it was a standard method of treatment among allergists. (Note, that Freeman considered the injections to be a vaccination.) The scratch test, although used by Blackely [55], was first employed diagnostically as a test for food allergy by Schloss in 1912 [60]; the intracutaneous test was used diagnostically by Cooke and Vander Veer in 1916 [61]. In this report based on 621 cases, Cooke and Vander Veer emphasized the presence of an hereditary component '... not only in hay fever but also in those other clinical manifestations of sensitization–bronchial asthma, urticaria, angioneurotic oedema and acute gastroenteritis following the ingestion of certain foods ...'. They concluded that the offspring of a sensitive parent are not born sensitive; a parent may transmit a tendency to sensitization without being sensitized and where both parents are sensitive the sensitivity tends to develop earlier and be more severe. They suggested sensitization was inherited as a dominant characteristic.

In the same paper, Cooke and Vander Veer argued against the idea of a toxin being present in pollen extracts and by extension that the results of specific injection therapy were due to the development of an antitoxin. Rather they considered the human clinical manifestations to be anaphylactic in nature. This latter opinion is striking, in view of Cooke's later agreement with Coca in sharply separating these forms of human allergy from anaphylaxis. Coca in 1920 [62], considered that in human hypersensitivity, heredity was the sole determining factor. In 1923, Coca and Cooke [63] proposed that asthma, hay fever, urticaria, etc. were manifestations of 'atopy' which they categorically separated from anaphylaxis. In their view atopy was peculiar to humans, whereas anaphylaxis was characteristic of lower animals; in atopy, heredity was the determining factor, whereas, it was not operative in anaphylaxis. With Coca and Grove's confirmation of the existence of human reaginic antibodies ([64] see below), atopy was then defined as a genetically determined type of hypersensitivity peculiar to man giving a wealing type of skin reaction and characterized by circulating reagins.

The sharp separation of anaphylaxis and human allergy was hotly contested by Doerr, Zinsser and others and was too radical for many clinical allergists. Even Coca and especially Cooke modified their views over the years.

Early concepts of the nature of the antibodies of anaphylaxis and human anaphylactic type allergies

Passive transfer experiments by Otto and others and the demonstration that the specificity of anaphylactic reactions was similar to that of acknowledged antigen–antibody reactions eventually convinced all that antibodies mediated anaphylaxis. Yet, acceptance of the notion was not immediate. As already pointed out, initially neither Richet, nor Gay

and Southard, for example, considered that antibodies were responsible for the ability of serum from actively sensitized animals to passively transfer.

Even with later general acceptance that antibody was responsible for sensitizing an animal for anaphylaxis there was controversy about the nature of the antibody. Various workers expressed doubt that the anaphylactic antibody was the same as precipitins. The basis of the uncertainty was in part experimental, precipitating activity was not always found in anaphylactically sensitive animals and not all workers could repeat Russ and Doerr's finding that the precipitating activity and sensitizing activity of an antiserum paralleled each other. In part, the uncertainty also stemmed from conceptual biases. Those who considered anaphylaxis to be an *in vivo* precipitation on cells inevitably were impressed by the evidence suggesting that the anaphylactic antibody was precipitating. Others, without this preconception, found the evidence less convincing.

The question of the nature of the anaphylactic antibody partly arose from Ehrlich's original concept that a different kind of antibody was responsible for each different antibody function. The terms 'precipitin', 'agglutinin' and 'antitoxin' originally were considered to designate not only different functions but different kinds of antibodies. Almost from the beginning, however, the concept that different antibody functions were due to different antibodies was not accepted by all. In the early years of the twentieth century, Paltauf had suggested that agglutinins and precipitins represented the same antibody (quoted in [65]). Bail and Hoke as early as 1909, (quoted in [65]) suggested that there were no separate bacteriolytic, precipitating or agglutinating antibodies in sera as such, but rather, the different reactions were the result of different conditions under which they were carried out. This and other work led Zinsser in 1915 [66], and Dean independently in 1917 (cited in [65]), to what Zinsser later termed [67] the 'Unitarian Concept of Antibodies'. This was the idea that a single pure antigen produced only a single variety of antibody. This antibody when reacted with antigen could carry out all of the various antibody functions including 'sensitizing effects in the anaphylactic sense' [67]; which one became manifest depended upon the physical state of the antigen, the presence and the nature of the co-factors, e.g. complement, leucocytes, etc. and the environmental or experimental condition under which the antigen–antibody reaction occurred. Initially, even Zinsser advocated the separation of antitoxins from other antibodies due to the fact that antitoxins possessed the power of neutralization in multiple proportions and did not seem to precipitate or fix complement. Nevertheless, after Ramon introduced the toxin–antitoxin flocculation test in 1923 that showed antitoxin precipitated, Zinsser included antitoxin in the Unitarian concept.

Fig. 2. (a) Otto Carl W. Prausnitz (Giles) (1876–1963). (b) Heinz Küstner (1897–1963). The passive transfer of immediate skin reactivity by interdermal injection of serum from an allergic patient ('reagin') is called the Prausnitz–Küstner (or P–K) test. (Reproduced with permission from Excerpts from Classics in Allergy, second edition; eds Cohen SG, Samter M. Symposia Foundation, 1992 (Oceanside Publications, Providence, Rhode Island).

Reagin or skin-sensitizing antibodies

Following the proposal by Wolf-Eisner in 1906 and by Melzer in 1910 that hay fever and asthma, respectively, were anaphylactic in nature, numerous attempts were made to transfer anaphylactic sensitivity to experimental animals using the serum from hay fever or asthmatics. The results were variable, some investigators reporting success others complete failure. In 1919, Ramirez reported that a normal recipient two weeks after receiving a blood transfusion from a donor sensitive to horse serum developed asthma upon being exposed to horses [68]. The first clear-cut demonstration of the presence of a sensitizing antibody in humans was the report by Prausnitz and Küstner in 1921 (Fig. 2) [69] that Prausnitz gave a positive reaction to fish extract when this was injected into a site which previously had been injected intradermally with serum from Küstner, a fish-sensitive individual. (A translation of this paper appears in [70].) They termed the sensitizing agent 'reagin' because they were not sure it was an antibody. Their finding was confirmed in 1923, when de Besche transferred serum from five asthmatic individuals sensitive to horses to the skin of normal individuals and 24 hour later injected the sites with horse serum. The sites reacted specifically to the injection [71]. It is of interest that although de Besche made reference to the study of Prausnitz and Küstner, he implied that the impetus for his experiments came from the report of Ramirez.

Coca and Grove, in 1925 in the work already mentioned [64], showed that the skin-sensitizing activity in the serum was present in the blood of all asthmatic and hay fever patients they examined. The activity was decreased by heating to 56°C, it remained in the skin of normal recipients

for at least four weeks and they agreed with Prausnitz and Küstner in being unable to transfer the activity to guinea pigs. Moreover, they demonstrated that the transfer required a latent period but could find no precipitating or complement-fixing activity associated with it. In addition, although the skin-sensitizing activity could be neutralized by the specific allergen *in vitro* or in the skin, the activity of the allergen did not seem decreased. They found that the skin of about 11% of normal individuals would not accept passive transfer. They considered it desirable to avoid the use of the word 'antibody' in describe the serum activity. This was because they had no evidence that the activity in serum appeared as a result of immunological stimulation and, as Coca later indicated, he wished to underline the apparent qualitative difference between the skin-sensitizing activity and anaphylactic antibody. They therefore called it the 'atopic reagin'. However, on finding that the reagin content of the blood increased two to four times after therapeutic injection of pollen extracts, Coca very shortly after agreed that reagin was an 'immune body'. Pepys *et al.* 1962 [72], showed that reagins were not the only antibodies involved in anaphylactic-type reactions such as asthma in man. These authors demonstrated by immunoelectrophoresis that in a minimum of 162 sera from humans, precipitins to thermophilic actinomycetes played a major role in Farmer's lung. (For further detail, see [1].)

The results of Coca and Grove were repeated by numerous investigators who also showed that reagins were not exclusively found in atopic individuals, e.g. they could be demonstrated in normal individuals injected with extracts of *Ascaris* spp. Bell and Eriksson in 1931 [73], recognized that reagin was unable to pass the placenta; another property by which this antibody seemed to differ from all antibodies known in lower animals and was apparently peculiar to humans.

Coca and many allergists stressed that reagin was qualitatively different from other antibodies, particularly anaphylactic antibodies. This opinion was not held universally. Zinsser conceded the possibility of a qualitative difference between reagins and other antibodies, but was skeptical of its reality. Others, such as Topley, considered that there might be '... minor differences in the antibodies ... but there seemed not adequate reasons for regarding reagins and anaphylactic antibodies as belonging to essentially different types,' [74]. The difference in viewpoint as to the nature of the antibodies involved in 'atopy' and experimental anaphylaxis was bound up in and paralleled the dispute as to the nature of the differences between atopy and anaphylaxis. In regard to the latter, the group exemplified by Coca, Cooke and many clinical allergists insisted that although similarities were present, the differences they noted between the clinical atopies or allergies and anaphylaxis of experimental animals were sufficiently great as to indicate a basic qualitative difference. Others, particularly experimental immunologists such as Zinsser, or Topley, and in addition, Doerr, considered that the differences, although present, were either quantitative or merely species' differences. In any event they were not large enough to obscure the overriding general similarities in mechanism.

The development of the quantitative precipitin technique by Heidelberger and Marrack and its systematic application from 1929 primarily by Heidelberger and his students led to the demonstration that rabbit antibody to Type I or Type II pneumococcal polysaccharide was quantitatively the same whether measured as complement fixing antibody, as agglutinating antibody or as precipitating antibody. This was taken as 'the first conclusive evidence for the so-called Unitarian theory of antibodies ...' [65]. Final confirmation of the Unitarian theory seemed to be afforded when Chow and Wu, in 1937 showed that rabbit antipneumaccocal Type I polysaccharide isolated in a pure state from specific precipitates would not only precipitate Type I pneumococcal polysaccharide, agglutinate Type I pneumococcal organisms, fix complement, and protect mice from a lethal infection with Type I pneumococcus but also sensitize guinea pigs for anaphylaxis [75]

Development of the concept of immunoglobulins

Despite this seeming triumph of the Unitarian Hypothesis, there was very early evidence of physicochemical heterogeneity of antibodies. Wells in his monograph on the chemical aspects of immunity published in 1929, pointed out that one of the objections to the Unitarian Hypothesis was that different antibodies had different solubilities, some being found in the euglobulin of serum, others in the pseudo-globulin fraction [35]. Moreover, the evidence of physicochemical heterogeneity increased with the development of further techniques of fractionation and analysis. Ethanol fractionation of serum and similar techniques were developed during World War II and cellulose ion exchange chromatography by Sober and Peterson during the early 1950s [76]. As these and other techniques of fractionation and analysis were introduced (see below) they were applied to the 'reagin problem' during the 1940s and 1950s. Yet, in terms of answering the basic questions as to the nature of reagin and its relation to other antibodies, little progress was apparent. This is evidenced by the statement of Kabat and Mayer in the second edition of their *Experimental Immunochemistry*, published in 1960 [76]. After critically reviewing the subject of the reported differences between human skin-sensitizing antibodies and other antibodies they could only conclude '... that whether this [difference] is due to the unusually high sensitivity of the Prausnitz-Küstner reaction ... or whether this type of antibody differs intrinsically from the usual antibodies is not known'. In the same vein,

Stanworth opened his extensive review on reaginic antibodies, published as late as 1963, with the confession 'Reagin still represents a nebulous concept to many immunologists' [77]. How the well-founded doubt and uncertainty expressed by these quotations were finally dispelled is worth reviewing in some detail both as a matter of history and as one of the triumphs of modern immunology and allergy.

Methodological and conceptual inadequacies initially prevented the solution of the reagin problem. However, the methodology was at hand by the early 1950s with the introduction of the immunochemical methods of analysis by immunoelectrophoresis, the Ouchterlony technique and the biochemical separation technique of cellulose ion exchange chromatography. After that time, it was a matter of developing a clear and correct concept of the nature of antibody macroheterogeneity. The understanding of the basis of the heterogeneity of antibodies awaited the development and application of newer physicochemical and immunochemical techniques of analysis. In the late twenties Svedberg developed the ultracentrifuge and in the early thirties Tiselius perfected moving boundary electrophoresis. Heidelberger in 1937, in Svedberg's laboratory showed that human antibody had a sedimentation coefficient of approximately 19S, indicating a molecular weight of $\approx 1\,000\,000$, whereas that rabbit antibody was $\approx 7S$, indicating a molecular weight of around 150 000. This sort of molecular weight heterogeneity was later shown for antibodies existing within the same species, including humans. In 1939, Kabat, in the laboratory of Tiselius, demonstrated that antibodies were in the group of proteins with the slowest electrophoretic mobilities, i.e. they were what Tiselius had previously defined as the γ-globulin fraction, although, as later brought out, some apparently had faster mobilities (reviewed in [76]). The relationship between electrophoretic and molecular weight heterogeneity of antibodies was, however, not clarified until the employment of immunoelectrophoresis developed by Grabar and Williams in 1953 and the technique described by Ouchterlony in 1948. Both of these techniques were adaptations of the gel precipitin procedure first introduced by Oudin in 1945. Employment of these and other methods, allowed the demonstration by 1957 that the electrophoretically slow γ-globulin, called variously $\gamma2$ or γ-globulin, was 7S (what we now call IgG) and the protein with somewhat greater electrophoretic mobility called $\alpha_1 m$ or $\beta_2 m$, etc., (what is now termed IgM) had a high molecular weight of 19S and these two globulins had similar antigenic determinants (reviewed in [76]).

Concomitant with these developments was the recognition that the paraproteins produced in patients with multiple myeloma were related to the immunoglobulins. The relationship of myeloma proteins in the blood to immunoglobulins was not thought of until the moving boundary electrophoresis technique of Tiselius showed that myeloma paraproteins moved in the γ-globulin peak. On the basis of quantitative precipitin analysis, Kunkel, Slater and Good concluded that multiple myeloma paraproteins were modified γ-globulins [78]. The application of immunoelectrophoresis and Ouchterlony analysis allowed the realization that in addition to myeloma proteins being related to [$\beta_2 M$], others belonged to another group, called by Williams and Grabar in 1955, $\beta_2 A$, (IgA). In 1959, Heremans isolated $\beta_2 A$ from normal serum and showed its antigenic similarity to γ-globulins [79].

In the same year, in a landmark paper, Heremans enunciated what he termed the 'immunoglobulin concept' [80]. He pointed out that 'all the data seems to point to the existence of a system of closely related, though not identical proteins which are capable of acting as antibodies. These are (a) γ-globulins, (b) $\beta_2 A$-globulin …; (c) $\beta_2 M$-globulin. The outlined similarities in nature and function clearly call for the adoption of a common name for all these substances. A word such as ''immunoglobulins'' would seem suitable'. He also showed that this term also included the myeloma proteins.

Starting in the late 1950s, Porter and Edelman, followed by a host of others, worked out the structure of immunoglobulins and the basis for their differentiation into classes. The completion of the initial phases of this work was signalized by the 1964 report of the WHO group on immunoglobulin nomenclature [81]. Nevertheless, even before this, as the paper of Hereman's indicated, workers interested in the nature of reagins were able to define the direction of their studies in terms of the implicit question; to what immunoglobulin class do reagins belong?

Anaphylactic-type antibodies in experimental animals

The conceptual climate expressed by this question was undoubtedly strengthened by the work proceeding at the same time on the nature of anaphylactic-type antibodies in experimental animals. As early as 1941, Wittich described spontaneous asthma in a dog due to ragweed that was associated with an antibody capable of sensitizing the skin of dogs [82]. In 1943, Weil and Reddin reported that cattle with skin sensitivity to ragweed possessed a heat-labile antibody that could sensitize the skin of cattle (cited in [82]). As pointed out by Kabat and Mayer in the first edition of their well-known textbook published in 1948, these findings suggested '… that there is no essential difference in immunological mechanisms between sensitivity in humans and animals' [83]. More specifically, these observations seemed to throw doubt upon the idea that possession of reagin was a purely human attribute. Clinical allergists were less impressed by this viewpoint and the observations were not mentioned in most of the textbooks of clinical

allergy of that time. One argument for this among many workers, was that one of the attributes of the reagin antibody was considered to be its ability to sensitize human skin and these animal skin-sensitizing antibodies could not do this.

In 1963, Mota reported the existence of a heat-labile 'reaginic' antibody in the rat [84]. Ovary, Benacerraf and Bloch [85] and independently, White and co-workers [86] in 1963 demonstrated the ability of heat stable $\gamma1$ antibody in the guinea pig to sensitize guinea pigs for passive cutaneous anaphylaxis (PCA). Zvaifler and Becker [87] in 1966 reported a heat-labile antibody in the rabbit sensitized rabbits for PCA. The finding that guinea pig $\gamma1$ did not sensitize the rat for PCA whereas the corresponding $\gamma2$ antibody did, by analogy, provided insight into the older observations that human reagin would not sensitize guinea pigs for PCA but 'blocking antibody' would (see above). In addition, the observations on guinea pigs and the related observation in rats and rabbits provided the basis for the definition in 1966 of 'homocytotropic antibodies', as those antibodies capable of sensitizing the species in which they were produced or closely related species but not unrelated species and 'heterocytotropic antibodies' as those capable of sensitizing unrelated species but not the species in which they were engendered [88].

### Identification of human reagin as IgE antibody

Several authors immediately following Heremans' and co-workers isolation of β_2A (IgA) [79], suggested that β_2A might be the reaginic antibody. Heremans and Vaerman pointing out that reagins had never been clearly identified with either γ (IgG) or β_2M (IgM) immunoglobulin, presented evidence that skin-sensitizing activity was associated with a fraction of serum containing a very high proportion of IgA and only small amount of IgM and IgG [89]. Fireman, Vannier and Goodman in 1963 [90], reported that skin-sensitizing activity was precipitated by an antiserum apparently specific for β_2A. In 1963 Ishizaka (Fig. 3) and his colleagues also showed that β_2A preparations from normal individuals blocked sensitization of human skin with reagin but isolated IgM or IgG did not [91]. They considered it unlikely that an impurity in the β_2A preparation was responsible for the blocking effect. They indicated, with all due caution and circumspection, that '. . . present data suggest that reagins are β_2A globulin.' This suggestion was strengthened the next year when Vaerman *et al.* demonstrated skin-sensitizing activity in fractions from serum that contained only IgA as demonstrated by gel diffusion [92]. Thus, for a time there was general, if not complete, conviction that reagin was IgA.

This belief, however, was short lived. Loveless in 1964 reported the presence of reagin in an individual who formed no detectable IgA [93]. Furthermore, in the study by Vaerman

Fig. 3. Teruko (1926–) and Kimshige (1925–) Ishizaka. Characterized reagins as IgE.

et al., just quoted, it was noted that in one sample of serum the skin-sensitizing activity was present in both IgG and IgA fractions [92]. The latter observation suggested to Ishizaka the possibility that '. . . reaginic antibody is not necessarily β_2A globulins'. Acting on this idea, he and his colleagues in 1966 reported that, in fact, skin-sensitizing activity was not associated with the IgA present in various serum fractions [94,95]; a conclusion Perelmutter, Rose and Goodfriend reached at the same time [96]. In 1966, Ishizaka, Ishizaka and Hornbrook [97] developed an antiserum which after absorption with IgG, and its various subclasses, IgA, IgM, and IgD (a new immunoglobulin whose discovery was reported by Rowe and Fahey in 1965 [98]) still precipitated protein in immunoglobulin fractions and also precipitated skin-sensitizing activity. As they stated 'the results suggest the presence of a unique immunoglobulin as a carrier of reaginic activity'. This protein they tentatively designated IgE-globulin and over the two following years, in a brilliant series of investigations, they laboriously checked and cross checked this conclusion and left no doubt of its validity (reviewed in [99]).
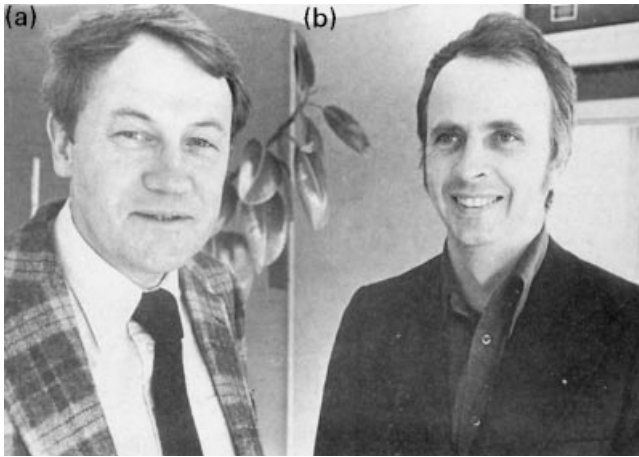
Fig. 4. (a) Hans Bennich (1930–). (b) Gunnar Johansson (1938–). Identification of IgND as IgE immunoglobulin and with L. Wide developed the radioallergosorbent technique (RAST). (Courtesy of Pharmacia, Uppsala, Sweden).

The conclusion was confirmed by a completely different approach when Johansson and Bennich (Fig. 4) discovered in 1965 (but reported in 1967) a myeloma protein which they demonstrated did not belong to any of the four known immunoglobulin classes [100]. The myeloma protein was shown to block skin-sensitizing activity and an antiserum prepared against it had the same specificity as the anti IgE-globulin prepared by Ishizaka. In an international conference held in 1968 and attended, among others, by K. Ishizaka, Bennich and Johansson, it was agreed to call the new class of proteins to which reagins belonged 'immunoglobulin E' (IgE) [101].

Blocking antibody

The other kind of antibody found in human immediate type allergic disease is the so-called 'blocking antibody.' This was discovered by Cooke *et al*. in attempting to find out why the injection treatment for hay fever was successful [102]. On the theory that it might be possible to demonstrate an antibody that conferred immunity to hay fever, he and his co-workers transfused untreated hay fever patients with large amounts of blood from ragweed patients who had undergone injection treatment and noted clinical improvement in the recipients. They then compared the serum of patients before and after injection treatment and found that mixtures of ragweed extract and post-treatment serum did not give an immediate reaction when injected into the skin of normal recipients, whereas, pretreatment serum, containing as much or even less skin-sensitizing activity, did. Correspondingly, skin sites injected with the mixture of pretreatment serum and allergen did not respond 48 hours

later to an injection of the allergen, whereas, sites injected with a mixture of post-treatment serum and allergen did. They interpreted these findings as '... the development under treatment of a peculiar blocking or inhibiting type of immune antibody that prevented the action of the allergen on the sensitizing antibody (reagin) ...'. Harley confirmed these results and showed that the blocking activity disappeared from the skin within 24 hours, unlike the skin-sensitizing activity itself which previously had been shown to persist for weeks [103]. Cooke, Loveless and Stull demonstrated that injection of ragweed into nonragweed-sensitive individuals gave rise to blocking but not skin-sensitizing activity [104]. This seemingly disposed of the idea which both they and Harley previously had considered, that the blocking activity acted upon the tissue. However, the concept that the blocking activity was due to a binding of the antibody with the allergen was first explicitly stated by Loveless when she found that blocking activity, unlike the skin-sensitizing activity was heat stabile, withstanding heating at 56°C [105]. She also confirmed that blocking activity disappeared rapidly from the skin. At about the same time, Sherman, Hampton and Cooke reported that blocking antibody readily passed the placenta [106], further differentiating blocking antibody from reagin. Subsequent work showed that blocking antibody was a typical 7S IgG globulin (reviewed in [107]).

Blocking antibody was important for a number of reasons. The differences between it and reagin reinforced the belief of allergists and others that reagin was different from other antibodies. In addition, it seemed to offer a respectable, 'scientific' reason for the efficacy of the injection treatment of hay fever, despite the uncertainty that arose as to whether the development of blocking antibody was responsible for the therapeutic relief claimed for the treatment.

Mediator cells

Paul Ehrlich, while still a medical student at the University of Freiburg, tested a new basic synthetic dye, 'dahlia' and discovered that some connective tissue cells contained large granules which avidly took up the dye and changed its colour to a reddish purple (metachromasia). He reported this in 1877 [108]. He named these cells 'mast' cells, i.e. well fed cells, giving them this name because he believed the cell granules were products of cell overfeeding. Following the discovery in 1937 by Lison that heparin stained metachromatically, Jorpes, Holmgren and Wilander [109], and Holmgren and Wilander [110], showed there was a good correlation between the mast cells and heparin content of various tissues. From this they concluded that mast cells were the source of heparin. In 1938, Wilander reported that

the dogs during anaphylactic shock discharged their mast cell granules [111]. This led to the demonstration by Waters, Markowitz and Jaques [112] of a marked increase in the heparin titre of the blood of dogs during anaphylactic shock, and three years later in 1941, to the actual isolation of heparin from their blood by Jaques and Waters [113]. Thus, the two groups of workers succeeded in explaining the marked incoaguability of the blood occurring during the course of peptone or anaphylactic shock in dogs.

Alam and his co-workers reported in 1939 that curare released histamine from dog muscle *in vivo* [114]. During the late 1940s and subsequently, a number of workers showed that numerous, simple organic compounds, particularly basic substances, were histamine releasers *in vivo*. Among other uses, the compounds were important in the demonstration that tissue mast cells contained histamine. Riley and his co-worker West were the first to provide convincing evidence of this [115,116]. Two considerations led Riley to suspect that histamine arose from mast cells. The first was that peptone shock in the dog led to heparin release and, as was shown later, also to histamine liberation, heparin, as just related, was recognized to come from mast cells. The second was the demonstration that mild trauma to the skin lesions of patients with urticaria pigmentosa resulted in a triple response. Lewis had shown the latter to be given by histamine and the lesions of urticaria pigmentosa were known to be focal collections of mast cells [117].

Riley first showed that an histamine liberator, stilbamidine was concentrated in the granules of the mast cells of the peritoneum of the rat and he and West in a series of publications starting in 1952, demonstrated the close correlation between mast cell number and histamine content of a number of tissues of various species including man (see above). In this initial work, Riley and West consciously adapted the approaches used by Jorpes, Holmgren and Wilander [109] to demonstrate mast cells as a source of heparin. Fawcett [118] in 1954, used microscopic and pharmacological approaches to study the histamine release from mast cells. He concluded the release was an enhancement of normal secretory processes. His inference received indirect confirmation in 1963 when Thiery, also by means of the electron microscope, showed these cells were still alive after extrusion of their granules [119]. Bloom and Haegermark, in 1967 [120] also using the electron microscope, reached the same conclusion as Fawcett.

Following the study of Jaques and Waters on mast cell degranulation in anaphylaxis already referred to, Stuart in an abstract in 1952, reported that anaphylaxis in the mouse, rabbit and guinea pig was associated with degranulation of mast cells [121]. Mota correlated the presence of mast cell degranulation in the tissues of sensitized rats and guinea pigs with signs of anaphylaxis on challenge with antigen [122]. Subsequently, he and Vugman demonstrated not only degranulation of mast cells in guinea pig anaphylaxis but a clear-cut correlation between the decrease in the number of mast cells and of histamine in the lungs of sensitized guinea pigs and challenged with antigen [123,124].

In 1955, Benditt *et al.* were the first to show the presence of serotonin in rat mast cells [125] and Humphrey and Jacques (cited in [126]) at the same time demonstrated its presence in rabbit platelets. Later work demonstrated that only the mast cells of rodents contained serotonin and that it could be released from these cells by appropriate immunological and other stimuli (reviewed in [126]). Thus, by approximately the mid 1950s, evidence was available that mast cells contained the mediators, histamine, serotonin and heparin, and that mast degranulation was related to the release of these mediators during anaphylaxis in several species. By that time, moreover, it was also evident that the tissue mast cell was not the only mediator cell.

Two years after describing the mast cell, Ehrlich noted the presence of cells with metachromatic granules in blood [127]. Although he termed them 'blood mast cells', he proposed that unlike the tissue mast cells the blood cells were derived from bone marrow and were essentially equivalent to the neutrophil and eosinophil; cells he had also described. This view with some few dissenting opinions, was substantiated by later workers who, emphasizing the similarity to the other granulocytes, renamed the blood mast cell, the basophil. Ehrlich and Lazarus in 1898 observed that the basophil count usually increased in myeloid leukaemia (cited in [128]). In 1952, Graham and her associates related the basophilia of chronic myeloid leukaemia to the high blood histamine levels found in this condition. Based on this clue, she and her associates demonstrated that the basophils of human blood contained at least half and possibly all of the blood histamines [128]. This immediately implied that the histamine came from the basophil [129]. Katz reported that specific antigen added to the blood of actively-sensitized rabbits induced histamine release into the plasma (cited in [129]), Minard as early as 1937 had discovered that at least 97% of the histamine content of rabbit blood was contained in the platelets [130]. He pointed out that the platelets of dog blood did not contain histamine and subsequent workers verified that the rabbit platelet was unique in this respect. In 1955, Humphrey and Jaques [131] verified directly that the platelet was the source of the histamine when they demonstrated that antigen added to isolated platelets in plasma from sensitized rabbits induced histamine and serotonin release.

Mediators

Histamine

Histamine was the first substance to be considered an anaphylactic mediator (see above). A number of workers, Barger and Dale in 1911, Mellanby and Twort in 1912 and Abel and Kub in 1919, demonstrated the presence of histamine in various tissues of the body, but in each instance it was argued that this could have resulted from the bacterial decarboxylation of histidine. Not until 1927 did Dale, for reasons he admitted were irrelevant to the histamine theory of anaphylaxis, organize the workers of his laboratory to demonstrate the presence of histamine in various tissues under conditions where this objection was not present [132].

Lewis previously demonstrated that a histamine-like agent ('H substance') was liberated from the skin during the local anaphylactic reaction. However, he did not consider histamine and H substance to be identical [117].

Dale in his Croonian Lectures in 1929, cited the experiments demonstrating the presence of histamine in tissues, the ideas and the observations of Lewis and the ability of intravenously given histamine to mimic the actions of anaphylactic shock on the organs of various species and went on to say:

'We … may picture the anaphylactic shock therefore as a result of cellular injury, due to the intracellular reaction of antigen with an aggregating antibody. Whether this is generalized or localized in a particular organ, histamine will be released and its effects will be prominent in the resulting reaction imposing a general resemblance to the syndrome produced by histamine itself on the symptoms seen in each species. The cell injury, however, is not limited to the degree required to produce a release of histamine, and involves other and more direct results. Such a conception is in accordance with all the facts as yet available, and it has the advantage of rendering intelligible, not only the striking resemblance between symptoms of the anaphylactic reaction and those produced by injecting histamine but also the various and equally significant points of difference between the two syndromes' [25].

Like all good theories, that of Dale's anticipated the evidence, for despite numerous previous attempts, no one had succeeded in demonstrating the release of histamine during and as a consequence of the anaphylactic response. It was not until 1932, that Gebauer Fuelnegg in Dragstedt's laboratory [133], Bartosch working with Feldberg [134] and Spinelli [135] actually succeeded in demonstrating the release of histamine during *in vitro* and *in vivo* anaphylaxis. These experiments were not the first attempts to demonstrate histamine release, rather they were the first successful ones. The experimental and conceptual atmosphere in which

they were carried out is strikingly conveyed by Dragstedt in a later reminiscence [136]. (When the late Gebauer-Fuelnegg and I began our work in 1932, we were aware that we would be called upon to traverse a cemetery of departed hopes. The circumstantial evidence indicating that the anaphylactic reaction was mediated by some toxin resembling histamine was so convincing, however, that we were persuaded to look for incriminating evidence in spite of the many negative results in the literature.) Part of the reason for the success of these workers was that they looked for the rise in histamine sufficiently early during the course of the reaction, whereas the preceding investigators did not. Following these successes, numerous workers in numerous ways confirmed their results.

The theory and the consequent studies stressing the great significance of histamine in anaphylaxis and by assumption, in human allergic disorders, were important both in the discovery of the antihistamines and in the intensity of their later development. In 1937, Staub and Bovet [137] demonstrated that thymoxydiethylamine antagonized the pharmacological actions of histamine and prevented or ameliorated anaphylactic shock of the guinea pig. In 1942, the first clinically useable antihistamine 'Antergan' was introduced and this was the forerunner of many more [138–140].

The histamine theory was welcomed by the generality of workers as providing an intellectually satisfying explanation of many of the aspects of anaphylaxis but almost immediately doubts were raised as to its general validity. Histamine, under some circumstances, could not reproduce the qualitative manifestations of anaphylaxis. In 1940, Kellaway [141], showed that antigen contracted the sensitized rat uterus and pointed out that as far back as 1912 histamine was known to relax the same organ. He concluded that 'Taking all the evidence together it appears almost certain that histamine plays no significant part in the production of anaphylactic phenomena in the rat'. Following Kellaway, Schild showed that the sensitized guinea pig uterus desensitized by large doses of histamine would still contract on the addition of antigen (cited in [142]). In addition to these and other qualitative difficulties, workers also brought out quantitative discrepancies between the histamine released and the associated symptoms. Schild [143], for example, pointed out that a hundred times more histamine had to be administered to the guinea pig lung than was released from shocked lungs to have the same effect in contracting the bronchi. Anaphylatoxin was found to liberate much more histamine from perfused guinea pig lung than did antigen from sensitized lung, yet the degree of bronchoconstriction produced by these two agents was the reverse [144]. The action of the antihistamines in preventing anaphylaxis — although taken as a prediction from the theory and, as its strong support also, when studied more closely — seemingly cast doubts concerning the general validity of the histamine

theory [142,145]. As a result of these discrepancies, Goodman and Gilman [146] in the 1956 edition of their influential textbook of pharmacology, were willing only to conclude that '... few would deny to histamine an important, if not the major role, in the characteristic syndrome of anaphylaxis in certain animal species' and they carefully left undefined how important was 'important'.

Two explanations were offered for these difficulties. In the first, Schild considered that in the guinea pig, the amounts of histamine released were consistent with the histamine theory if it were assumed that relatively small amounts were effective [142] due to its high concentration at the site of release. In 1950, Dale [147] conceded the likelihood that other active constituents are released with the histamine but that in most cases the discrepancies were explicable by the assumed difference between 'extrinsic' and 'intrinsic' histamine. Intrinsic histamine was the histamine released from the cells and tissues that reacted to the mediator, e.g. smooth muscle cells, whereas extrinsic histamine was histamine whose source was outside the reactive tissue. In the same paper, Dale proposed not only to explain the various discrepancies in the histamine theory by means of this putative difference between intrinsic and extrinsic histamine, but suggested it as a possible explanation for the lack of efficacy of the antihistamines in asthma. In 1966 [139], Dale went back to his original viewpoint of 1929, and considered that in the guinea pig or allergic human being there were broadly speaking two components in the overall allergic reaction, one of which consisted of those responses or portions of response due to the release of histamine and the other consisted of those that were not. The second type of explanation was to invoke the action of other mediators.

Recognition of other mediators

As is evident from the above discussion, the experiments of Kellaway in the rat, Schild in the guinea pig and others not mentioned, pointed to the probable existence of other mediators of anaphylaxis. This expectation was borne out by the discovery of SRS-A, serotonin and then other agents.

Slow reacting substance of anaphylaxis SRS-A
Kellaway and Trethwie in 1940, reported that the lungs or jejunum of sensitized guinea pigs perfused with antigen released a substance which gave a slow, sustained contraction of guinea pig ileum [148]. This was unlike the sharp, short contraction given by histamine and, accordingly, they named the substance, slow reacting substance, SRS (see [149] for further background). No further attention was paid the material until Brocklehurst, in 1953 confirmed this work demonstrating the principle in perfusates of sensitized guinea pig lungs challenged with antigen which gave a slow contraction of guinea pig ileum even with an antihistamine in the bath [150,151]. He renamed the agent SRS-A, the slow reacting substance of anaphylaxis.

Bradykinin

Rocha e Silva, Beraldo and Rosenfield added trypsin or snake venom to serum globulin and obtained a peptide which was hypotensive, simulated smooth muscle and also a vasodilator [152]. They termed the peptide 'bradykinin' because it gave a contraction of smooth muscle that was somewhat slower than histamine. In 1950, Beraldo, demonstrated the liberation of bradykinin into the blood of dogs undergoing anaphylaxis [153]. These results over the succeeding 10 years were confirmed and extended to other species.

Biochemical mechanisms of mediator release

Investigators who began our knowledge of anaphylaxis inevitably speculated as to its basic initiating mechanisms. As already described, some of the earliest ideas concerned the intervention of proteolytic enzymes in the anaphylactic reaction. Dale in the course of Croonian Lectures of 1929, signalled the demise of these early theories remarking that 'There was, and there is still, no convincing evidence either of an immediate protein digestion resulting from the union of antigen and antibody, or of the liberation of histamine itself in the enzymatic cleavage of proteins.'

Many hypotheses, however, like old soldiers, never die but simply fade away. Nonetheless, they sometimes can be revived, usually in a modified form. The late 1940s and 1950s seemed to be a time for such resuscitation. Among those revived was that of the involvement of proteases in anaphylaxis. This came about from a number of investigators. Rocha e Silva was impressed with the experiments of Feldberg *et al.* in 1937 and 1938 showing that snake venom liberated histamine from guinea pig lung. Knowing that many snake venoms contain proteolytic enzymes, he showed as early as 1939 that crystalline trypsin could contract smooth muscle and liberate histamine from guinea pig lung [154] and suggested that this came about through a proteolysis of a putative histamine–amide bond in tissues. He further suggested that this proteolytic liberation of histamine occurred in anaphylaxis. In 1947, and subsequently, Ungar presented evidence supporting the notion that serum fibrinolytic enzyme and/or tissue proteases upon activation by antigen–antibody complexes played the causal role in anaphylaxis [155,156]. The hypothesis broke down in a welter of conflicting evidence but did play a role as part of the background in the revival of ideas concerning the activation of enzymes as a basic biochemical mechanism of anaphylactic reactions (see below).

The idea of anaphylatoxin being involved in anaphylaxis

was also refurbished. In 1950, 1954, Hahn and Oberdorf showed that antihistamines prevented anaphylatoxin shock in guinea pigs [157,158] earlier, Rocha e Silva, Bier and Aronson [144] had demonstrated that anaphylatoxin liberated histamine from isolated guinea pig lungs. This and other evidence prompted Hahn, Rocha e Silva and several others to champion the idea that anaphylatoxin was an intermediate in anaphylaxis. The humoral theory reborn! However, the revival of anaphylatoxin as an obligatory or important intermediary in anaphylaxis was relatively short lived. This was partly because of the evidence by Friedberger, Engelhardt and Meineke [159] that desensitizing animals to the effects of anaphylatoxin neither abolished nor modified their anaphylactic response. Mainly, however, the evidence revealing that mediators were responsible for the major manifestations of anaphylaxis and the growing interest in the mechanism of the release of mediators from isolated tissues and cells made anaphylatoxin, like Laplace's God, an hypothesis that was not needed. This short history of the lives and deaths of anaphylatoxin would, however, be incomplete without at least mentioning its resurrection in the late 1960s and later as fragments of complement components active not only in increasing vascular permeability, and contracting smooth muscle but as chemotactic agents and secretogogues for white cells [160].

The major focus of attention started to shift in the 1950s to the direct study of mechanisms of mediator release from tissues or cells. However, interest in this approach goes back earlier. Dragstedt in 1941 stated that the 'The mechanisms by which the antigen–antibody reaction leads to the rather special type of cell injury resulting in the discharge of the agents characteristic of the anaphylactic reaction presents the next challenge in the study of this interesting subject . . .' [161]. The challenge was not taken up immediately, although Parrot in 1942 showed that anoxia inhibited histamine release from guinea pigs. Intensive work on the problem began in the 1950s by a number of investigators, mainly pharmacologists. They included, among others, Schild and his students using whole lung and lung slices and Uvnäs and Moustache using rat mesentery, and then isolated mast cells and Mota also using the last. In all systems studied, the process of immunological mediator release was shown to be an active one requiring metabolic energy, Ca^{2+} and to be temperature dependent (reviewed in [142,149]).

Ideas concerning the underlying mechanism came from several sources. Mongar and Schild proposed that the complex of antigen- and cell-fixed antibody reacts with an heat-labile enzyme precursor which in the presence of calcium is tranformed into a short-lived active enzyme. The active enzyme is concerned with the further intracellular events leading to histamine release (reviewed in [142]). The hypothesis was based, *inter alia*, on their studies

showing a requirement for calcium, and the inhibitory effects of phenol and cooling.

In 1967 Thon and Uvnäs [162], using pharmacological and morphological approaches, concluded that the release of histamine was a two-step process. The first was a primary energy-requiring transport of histamine-containing granules to the exterior of the mast cells with a secondary, energy-dependent exchange of the histamine of the granules with extra cellular sodium.

Brocklehurst, prompted by Levine's [163] finding that di-isopropyl fluorophosphate (DFP) inhibited immune haemolysis (see below), showed that DFF also inhibited histamine release and SRS-A from sensitized guinea pig lung; he than suggested that the antigen–antibody reaction activated a cell-bound protease required for histamine release (quoted in [149]).

Feldberg, Holden and Kellaway [164] found that snake venom stimulated smooth muscle and concomitantly released lysolecithin, suggesting that the antigen–antibody complex might activate a phospholipase.

Later Hogeberg and Uvnäs observed that preparations of phospholipase caused release of histamine from mast cells; other enzymes did not. They also suggested that a phospholipase present in an inactive form on the mast cell was activated by the antigen–antibody reaction and destroyed the permeability of the cell membrane releasing histamine [165].

As is evident, the concept that activation of one or another enzyme was involved in mediator release was a popular notion. Partly this arose from the demands of the studies themselves but there also was the conceptual atmosphere which stimulated the notion. A portion of this 'atmosphere' was the work on the enzymatic mechanism of complement occurring at approximately the same time. From 1954 to 1956, the studies and co-workers of Lepow [166,167], Levine [163] and Becker [168,169] established that the first component of complement was a precursor enzyme which was activated by the antigen–antibody reaction, acted upon the fourth and second components. The possible analogy of complement activation to the mechanisms of histamine release in anaphylaxis was stressed [170].

Most of the earlier workers on the mechanism of histamine release implicitly or more usually explicitly assumed that the release was due to tissue or cellular damage or disruption. However, as early as 1954, Fawcett, as mentioned, studying release of histamine from the mast cells of the rat peritoneal cavity suggested that the action of the histamine releaser, 48/80 was to enhance the normal secretory processes of the mast cell [118]. This view was echoed by Smith [171] who in 1958, studying the release of histamine by protamine or polymyxin, concluded that the action of these compounds did not require cell disruption and death and that the mast cell is '. . . an endocrine cell

which elaborates histamine and other products and secretes them when appropriately stimulated'. As mentioned, ultra-structural studies of the morphology of mast cells induced to degranulate by 48/80, first by Thiery in 1963 [119] and then by Bloom and Haegermark in 1967 [120], supported this view. It was further buttressed by the functional studies of Thon and Uvnäs [161] and of Diamant [172]. Nevertheless, immunologists in general in discussing or describing ana-phylactically induced histamine release, continued to talk of mast cell or basophil 'damage' or 'disruption', [e.g. 122,168]. This was partly a holdover from the views of the original workers of anaphylaxis, who impressed by florid symptoms and frequently rapidly lethal nature of anaphylaxis, considered that the basis had to be severe cellular and tissue damage. It was also partly due to the Arthus reaction where tissue damage was clear and promi-nent being considered a form of 'local anaphylaxis' (see above), to the morphological appearance by light micro-scopy of the mast cell reaction and possibly as much, to the happy innocence of the bulk of immunologists of the work of morphologists and pharmacologists. Thus, it was only in 1968, at the same symposium where other immunologists were using the terms mast cell 'damage', that Becker [173] and Lichtenstein [174], also ignorant of the work of Fawcett [118] and others, independently suggested that immunolo-gically induced mediator release from mast cells and baso-phils was noncytotoxic and involved secretory mechanisms of secretion. This suggestion received independent experimen-tal verification a year later by Johnson and Moran [175].

But certainly not the end

The story I have attempted to tell stops in all cases before 1970, in some cases distinctly before then. By then the major mechanisms of anaphylaxis and immediate (anaphy-lactic) allergies had been identified. Moreover, I feel that to go beyond this date would intrude into today and tomor-row's history. I hope, however, that it shows the intricate turnings which have led to our present understanding of some aspects of anaphylaxis, hay fever and asthma. I also hope it transmits my admiration for those upon whose work this understanding is based.

References

1 Kay AB. (Ed.) Blackwell Science Oxford 1, 1997:29–32.
2 Butterfield H. The Whig Interpretation of History (Preface) W. W. Norton Inc., New York 1965 v. 171.
3 Dubos RJ. Louis Pasteur Lance of Science. Little Brown and Co., Boston, 1950:249.
4 Bulloch W. The History of Bacteriology Oxford University Press, London 1937.
5 von Dungern E. Die Antikorper, Jena, G. In: Jena G, eds. Fisher 1903.
6 Magendie F. Lectures on the blood. Harrington, Barington and Hasswell, Philadelphia 1839.
7 Portier P, Richet C. Action anaphylactique des quelques venims. C R Soc Biol 1902; 54:170–2.
8 Arthus M. Injections rpts de serum de cheval chez le lapin. C R Soc Biol 1903; 22:817–20.
9 von Pirquet C, Schick B. Zur Theorie der Inkubationzeit. Vorlaufige Miteilung Wien Klin Wochenschrift 1903; 16:758.
10 von Pirquet C, Schick B. Die Serum Krankheit. Serum Sickness (English Translation, 1951 William and Wilkins Co., Baltimore 1905.
11 von Pirquet C. Allergie. Mnch Med Wochenschr 1906; 30:1457–61.
12 von Pirquet C. Allergy. Arch Int Med 1911; 7:259–88; and 382–8.
13 Doerr R. Neuere ergebnisse der anaphylaxieforschung. Ergebnisse Immunittsforsch Hyg Bakteriol un Exp Therap 1914; 1:257–76.
14 Doerr R. Die Anaphylaxieforschung in Zeitraume von 1914–21. Hyg Bakt 1922; 5:71–84.
15 Coca AF. Relation of atopic hypersensitiveness (hay fever, asthma) to anaphylaxis. Arch Path 1926; 1:116–8.
16 Boyd WC. Fundamentals of Immunology. Interscience Pub-lishers, New York, 1947 :326.
17 Karsner H, Ecker E. Principles of Immunology Lippincott Co. Philadelphia, 1921 :308.
18 Blakeney EH. The complete works of Horace. In: Kraemer J, eds. Modern Library. Random House, New York 1936:399.
19 Coombs RRA, Gell PGH. The classification of allergic reac-tions responsible for allergic reactions underlying diseases. In: Gell PGH, Coombs RRA, eds. Clinical Aspects of Immu-nology, Blackwell Scientific Publications, Oxford, 1963: 217–37.
20 Otto R. Das Theobald Smitsche Phnomen der Serum-Ueberempfindlichkeit. Gedenkschrift F Rudolph Von Leut-hold 1905; 1:155–72.
21 Otto R. Zur Frage der Serum-Ueberempfindlichkeit. Mnch Med Wochenschrift 1907; 34:1665–70.
22 Rosenau MJ, Anderson JF. A study of the cause of sudden death following upon the injection of horse serum. Hygienic Lab Bull 1906; 29:7–95.
23 Rosenau MJ, Anderson JF. Studies upon hypersusceptibility and immunity. Hygienic Lab Bull 1907; 36:7–67.
24 Besredka A. Anaphylaxis and anti-anaphylaxis and their experimental foundation. Glyone, St Louis, 1919 :5.
25 Dale HH. Some chemical factors in the control of the circulation. Lancet Croonian Lectures 1929; 1:1233–7 and 85–90.
26 Doerr R, Russ VK. Studien uber Anaphylaxie III. Der Anaphylaktische Immunkrper und Seine Beziehungen Zum Eisureissartigen Zeitschrift F Immunittsforsch 1909; 3:181–08.
27 Richet CR. Anaphylaxis. Translated by J. M. Blich. Constable and Co., London 1913.

28 Friedemann U. Weitere Untersuchungen ber den Mechanismus der Anaphylaxie. Z Immunittforsch T11 1909; 2:591–43.

29 Scott WM. Anaphylaxis in the rabbit: the mechanism of the symptoms. J Path Bact 1910–11; 15:31–49.

30 Friedberger E. Weitere untersuchungen ber Eiwessenanaphylaxie. IV Mitteilung Zeit Immunittsforschung 1910; 4:636–42.

31 Weil R. Studies in anaphylaxis. XIV. On the relation between precipition and sensitizion. J Immunol 1916; 1:1–18.

32 Dale HH. The biological significance of anaphylaxis. Proc Roy Soc B 1920; 91:126–46.

33 Vaughn VC, Wheeler SM. The effects of egg-white and its split products on animals: a study of susceptibility and immunity. J Infect Dis 1907; 4:476–508.

34 Biedl A, Kraus R. Experimentelle Studien ber Anaphylaxie. Wien Klin Wochenschrift 1909; 22:363–70.

35 Wells HG. The chemical aspects of immunity. New York, Chemical Catalogue Co., 1929 :115.

36 Dale HH, Laidlaw PP. The physiological action of beta-iminozolethylanine. J Physiol 1910; 41:318–44.

37 Schultz W. Physiological studies in anaphylaxis. I. The reaction of the smooth muscle of the guinea pig sensitized with horse serum. J Pharmacol Exp Therapy 1910; 1:549–67.

38 Dale HH. The anaphylactic reaction of plain muscle in the guinea pig. J Pharmacol Exp Therapy 1913; 4:167–23.

39 Manwaring WH. Serophysiologische untersuchungen. I. Der physiologische mechanismus des anaphylaktischen schocke. Zeit F Immunittforsch 1910; 8:1–23.

40 Pearce RM, Eisenbrey AB. The physiology of anaphylactic ''shock'' in the dog. J Infect Dis 1910; 7:565–76.

41 Dale HH, Kellaway CH. Anaphylaxis and anaphylatoxins. Philosophical Trans 1922; 211:273–15.

42 Manwaring WH, Chilcote RC, Brill S. The hepatic mechanical factor in canine anaphylactic shock. Trans Soc Exp Biol Med 1922; 20:184–96.

43 Auer J, Lewis P. The physiology of the immediate reaction of anaphylaxis in the guinea pig. J Exp Med 1910; 12:151–73.

44 Arthus M. Sur la sera-anaphylaxie. Press Med, Paris 1909; 17:305–7.

45 Auer J. Lethal cardiac anaphylaxis in the rabbit. J Exp Med 1911; 14:476–96.

46 Coca AF. The mechanism of the anaphylaxis reaction in the rabbit. J Immunol 1919; 4:219–30.

47 Simonds JP. The fundamental physiologic reaction in anaphylactic and peptone shock. Preliminary report. J Am Med Assoc 1919; 73:1437–45.

48 Wells H. The present status of the problems of anaphylaxis. Physiol Rev 1921; 1:44–68.

49 Major RH. Aretaeus on asthma. Classic Descriptions of Disease. C C Thomas, Springfield, IL, 1939 :631–2.

50 Major RH. The history of asthma. In: Underwood EA, ed. Science Medicine and History, Oxford University Press, London 1910: 518.

51 Emanuel MD, Howarth PH. Asthma and anaphylais: a relevant model for chronic disease? An historical analysis of directions in asthma research. Clin Exp Allergy 1995; 25:15–26.

52 Melzer SJ. Bronchial asthma as a phenomenon of anaphylaxis. J Am Med Soc 1910; 55:1021–4.52.

53 Bostock J. On Catarrhus aestivus or summer catarrh. Medico-Chirurgical Transactions 1828; 12:437–46.

54 Finn R, JB. Hay fever and the mechanism of allergy. Lancet 1992; 340:453–5.

55 Blackley CH. Experimental Researches on the Causes and Nature of Catarrhus Aestivus (Hayfever or Hay-Asthma). Reprinted by Dawson's of Pall Mall. London 1959 Originally published by Balliere, Tindall & Cox 1873, London.

56 Dunbar CP. Zur Frage betreffend die aetiologic und specifische therapie des Heufiebers. Ber Klin Wochschr 1903; 40:537–69.

57 Wolff-Eisner A. Das Heufieber: Sein Wesen und Seine Behandlung. J. F. Lehmann, Munich 1906.

58 Noon L. Prophylactic inoculation against hay fever. Lancet 1911; 1:1572–3.

59 Freeman J. Vaccination against hay fever; a report of results during the last three years. Lancet 1914:1178–80.

60 Schloss O. A case of allergy to common foods. Am J Dis Child 1912; 3:341–62.

61 Cooke RA, Vanderveer A. Human sensitization. J Immunol 1916; 1:201–305.

62 Coca A. Tice practice of medicine. Hypersensitiveness WF Prior Co NY 1920; i:107–98.

63 Coca AF, Cooke RA. On the classification of the phenomena of hypersensitiveness. J Immunol 1923; 8:163–82.

64 Coca AF, Grove EF. Studies in hypersensitiveness. XIII. A study of atopic reagins. J Immunol 1925; 10:445–64.

65 Zinsser H, Enders FF, Fothergill D. Immunity, Principles and Applications in Medicine and Public Health. McMillian Co., New York 1939.

66 Zinsser H. The more recent developments in the study of anaphylactic phenomena. New York Med J 1915; 16:223.

67 Zinsser H. On the essential identity of the antibodies. J Immunol 1921; 6:289–99.

68 Ramirez MA. Horse asthma following blood transfusion. J Am Med Assoc 1919; 73:984–5.

69 Prausnitz C, Kstner J. Studien uber die Ueberempfindlichkeit. Centralbe F Bakt 1 Abt Orig 1921; 86:160–8.

70 Coombs RRA, Gell PGH. Classification of allergic reactions responsible for clinical hypersensitivity and disease. In: Coombs RRA, ed. Clinical Aspects of Immunology, Blackwell Science Publishers, London, 1968:575–96.

71 de Besche A. Studies on the reactions of asthmatics and on passive transference of hypersusceptibility. Am J Med Sci 1923; 21:117–28.

72 Pepys J, Jenkins PA, Festenstein GN, Gregory PH, Lacy M, Skinner FA. Farmers lung; chemophilic actenomyels as a source of farmers lung hay antigen. Lancet 1936; 2:607–11.

73 Bell SD, Erickson Z. Studies in the transmission of sensitization from mother to child in human beings. J Immunol 1931; 20:347–57.

74 Topley WC. An Outline of Immunity. William Wood and Co., Baltimore, 1931 :211.

75 Chow BF, Wu H. The unitarian hypothesis of antibodies. Chinese J Physiol 1937; 11:183–92.

76 Kabat EA, Mayer M. Experimental Immunochemistry. C. C. Thomas, Springfield, IL 1960.

77 Stanworth DR. Reaginic antibodies. Adv Immunol 1963; 3:181–260.

78 Kunkel HG, Slater RJ, Good RA. Relation between certain myeloma proteins and normal gamma globulins. Proc Soc Exp Biol Med 1951; 76:190–3.

79 Heremans JF. Isolation and description of a few properties of the β_2A globulin of human serum. Clin Chem Acta 1959; 41:96–102.

80 Heremans JF. Immunohistochemical studies on protein pathology. The immunoglobulin concept. Clin Chim Acta 1959; 4:639–46.

81 Ceppelini R, Dray S, Edelman J et al. Nomenclature for human immunoglobulins. Bull WHO, 1964:447–50.

82 Wittich FW. Allergic diseases in animals. Prog Allergy 1949; 2:58–67.

83 Kabat EA, Mayer M. Experimental Immunochemistry. CC Thomas, Springfield, IL, 1948 :157.

84 Mota I. Biological characterization of 'mast cell sensitizing' antibodies. Life Sci 1963; 1:465–74.

85 Ovary Z, Benacerraf B, Bloch KJ. Properties of guinea pig 75 antibodies. II. Identification of antibodies involved in passive cutaneous and systemic anaphylaxis. J Exp Med 1963; 117:951–64.

86 White RG, Jenkins GC, Wilkinson PC. The production of skin sensitizing antibody in the guinea pig. Int Arch Allergy 1963; 22:156–65.

87 Zvaifler NJ, Becker EL. Rabbit anaphylactic antibody. J Exp Med 1966; 123:935–50.

88 Becker EL, Austen KF. Mechanisms of immunologic injury of rat peritoneal mast cells. I. The effect of phosphate inhibitors on the homocytotropic antibody-mediated histamine release and the first component of rat complement. J Exp Med 1966; 124:379–95.

89 Heremans JF, Vaerman JP. β_2A globulin as a possible carrier of allergic reaginic activity. Nature (Lond) 1962; 193:1091–3.

90 Fireman P, Vannier W, Goodman H. The association of skin-sensitizing activity with the β_2A globulins in sera from ragweed sensitive patients. J Exp Med, 1963:603–17.

91 Ishizaka K, Ishizaka T, Hornbrook MM. Blocking of Prausnitz-Kstner sensitization with reagin by normal human b2A globulin. J Allergy 1963; 34:395–403.

92 Vaerman JP, Epstein W, Fudenberg H, Ishizaka K. Direct demonstration of reagin activity in purified a1A. Nature 1964; 203:1046–8.

93 Loveless MH. Reagin production in a healthy male who forms no detectable b2A immunoglobulins. Fed Proc 1964; 23:403.

94 Ishizaka K, Ishizaka T. Physicochemical properties of human reaginic antibody. I. Association of reaginic antibody with an immunoglobulin other than gA or gG globulin. J Allergy 1966; 37:169–85.

95 Ishizaka K, Ishizaka T, Hornbrook MM. Physicochemical properties of reaginic antibody. III. Further studies on the reaginic antibody in βA-globulin presss. J Allergy 1966; 38:108–19.

96 Perelmutter L, Rose B, Goodfriend L. The relationship between skin-sensitizing antibodies and γA protein in the sera of ragweed and allergic individuals. J Allergy 1966; 37:236–45.

97 Ishizaka K, Ishizaka T, Hornbrook MM. Physicochemical properties of reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. J Immunol 1966; 97:75–85.

98 Rowe DS, Fahey JL. A new class of human immunoglobulins. I. A unique myeloma protein. J Exp Med 1965; 121:171–99.

99 Ishizaka K, Ishizaka T. Human reaginic antibodies and immunoglobulin E. J Allergy 1968; 42:330–63.

100 Johansson SGO, Bennich H. Immunological studies of an atypical (myeloma) immunoglobulin. J Immunology 1967; 98:381–94.

101 Bennich H, Ishizaka K, Johansson SGO, Rowe DS, Stanworth DR, Terry WD. Immunoglobulin E, a new class of human immunoglobulin. Bull WHO 1968; 38:151–2.

102 Cooke RA, Barnard SH, Hebald AT, Stull A. Serological evidence of immunity with co-existing sensitization in a type_of human allergy, hay fever. J Exp Med 1935; 62:733–50.

103 Harley D. The effect of pollen therapy on the skin reactions. II. A reaction of inhibiting substance in the serum of treated patients. J Path Bact 1937; 44:589–60.

104 Cooke RA, Loveless MH, Stull A. Studies on immunity in a type of human allergy (hayfever): serological response of non-sensitive individuals to pollen injection. J Exp Med 1937; 66:689–96.

105 Loveless MH. Immunological studies of pollenosis. I. The presence of two antibodies related to the same pollen-antigen in the serum of treated hay fever patients. J Immunol 1940; 38:25–58.

106 Sherman WB, Hampton SF, Cooke RA. The placental transmission of antibodies in the skin-sensitive type of human allergy. J Exp Med 1940; 72:611–21.

107 Sehon AH, Geynes L. Antibodies in atopic patients and antibodies developed during treatment. In: Samter M, ed. Immunological Diseases. Little Brown and Co., Boston 1971: 785–91.

108 Ehrlich P. Beitrage zur Kenntnis der Anilifarbungen und ihrer Verwedung in der Mikroskopischen Technik. Arch Mikr Anat 1877; 13:263–77.

109 Jorpes JE, Holmgren H, Wilander O. Ueber das vorkommen von heparin in den gefasswandes und den augen. Ein Beitrag zur physiologie der ehrlischen mastzellen. Zeit Zellenforsch Mikroskop Anal 1937; 42:279–301.

110 Holmgren H, Wilander O. Beitrag zur Kenntnis der Chemie und Funktion der Ehrlichschen Mastzellen. Zeit Zellenforsch Mikroscop Anat 1937; 42:242.

111 Wilander O. Studien ber heparin. Skand Arch Physiol Suppl., 1938 :15–23.

112 Waters ET, Markowitz J, Jaques R. Anaphylaxis in a liverless dog and observations on the anticoagulant of anaphylactic shock. Science 1938; 87:582–3.

113 Jacques LB, Waters ET. The identity and origin of the anticoagulant of anaphylactic shock in the dog. J Physiol (London) 1941; 99:454–66.

114 Alam M, Anrep GV, Barsoum GS, Talaat M, Weiniger E.

Liberation of histamine from skeletal muscle by curare. J Physiol (Lond) 1939; 95:148–56.

115 Riley JF, West GB. Histamine in tissue mast cells. J Physiol (London) 1952; 172:72–3.

116 Riley JF. The Mast Cells. E. D. Livingston Ltd., Edinburgh 1959.

117 Lewis T. The blood vessels of the human skin and their responses. Shaw and Sons Ltd, London 1927.

118 Fawcett DW. Cytological and pharmacological observations on the release of histamine by mast cells. J Exp Med 1954; 94:217–24.

119 Thiery JP. Etude au microscope electronique de la maturation et de l'excretion des granules des mastocyte. J Microscopie 1963; 2:549–56.

120 Bloom GD, Haegermarke Ö. Studies on morphological changes and of histamine release induced by bee venom, N-decylamine and hypotonic solutions in rat mast cells. Acta Physiol Scand 1967; 71:257–69.

121 Stuart EG. Mast cell responses to anaphylaxis. Anat Record 1952; 112:344.

122 Mota I. Mast Cell Damage by Anaphylaxis, Peptone Shock and Compound 48–80 Thesis, Universidade de Sao Paulo, Brasil, Sao Paulo 1953.

123 Mota I, Vugman I. Effects of anaphylactic shock and compound 48/80 on the mast cells of the guinea pig lung. Nature 1956; 177:427–9.

124 Mota I. Effect of antigen and octylamine and mast cells and histamine content of sensitized guinea pig tissues. J Physiol (London) 1959; 147:425–36.

125 Benditt EP, Wong RL, Arase M, Roeper E. 5-hydroxytryptamine in mast cells. Proc Soc Exp Biol Med 1956; 90:303–4.

126 Udenfriend S, Waalkes TP. On the role of serotonin in anaphylaxis. Mechanisms of Hypersensitivity. On the role of serotonin in anaphylaxis. Little Brown and Co., Boston 1959.

127 Ehrlich P. Beitrage zur Kentnis der granulierten Blutgewebzellen und der eosinophilen leukocyten. Verk. Physiol. Gesellsch. zur Berlin. Arch Anat und Physiol Ab 1879: 166–73.

128 Graham HT, Wheelwright F, Parrish HH, Marks AR. Distribution of histamine among blood elements. Fed Proceed 1952; 11:350.

129 Katz G, Cohen S. Experimental evidence for histamine release in allergy. J Am Med Assoc 1782; 1941:117:?–3.

130 0Minard D. The presence and distribution of histamine in blood. Am J Physiol 1941; 132:327–35.

131 Humphrey JA, Jaques R. The release of histamine and serotonin by antigen-antibody reactions (in vitro). J Physiol (London) 1955; 128:9–27.

132 Best CH, Dale HH, Dudley HW, Thorpe WV. The nature of the vasodilator constituents of certain tissue extracts. J Physiol (London) 1927; 62:397–17.

133 Gebauer-Fuelnegg E, Dragstedt CA, Mullenix RB. Observations on a physiologically active substance appearing during anaphylactic shock. Proc Soc Exp Biol Med 1932; 29:1084–6.

134 Bartosch R, Feldberg W, Nagel E. Das Freiwarden eines

histaminhnlichen. Stoffes bei der Anaphylaxie des Meerschweinchens. Pfgers Arch Ger Physiol 1932; 230:129–53.

135 Spinelli A. Demonstration de la mise en libert de substance type histamine du pumon isole du cobaye en choc anaphylactique. Bull Sez Ital Soc Int Microbiol 1932; 14:257–64.

136 Dragstedt CA. The physiological significance of histamine. Quart Bull NW Univ Med Sch 1940; 14:283–9.

137 Staub A, Bovet D. Action de la Thymoxyethyldiethylamine (929F) et des ethers sur le choc anaphylactique. C R Soc Biol (Paris) 1937; 125:818–21.

138 Dale HH. Antihistamine substances. Brit Med J 1948; 2:281–3.

139 Dale HH. Foreward to histamine and anti-histamines. Part 1. In: Rocha E Silva M, ed. Handbook of Experimental Pharmacology. Springer-Verlag, Berlin, 1966: 26–35.

140 Goodman L, Gilman A. The Pharmacological Basis of Therapeutics The MacMillan Co, New York 1970.

141 Kellaway CH. The anaphylactic reaction of the isolated uterus of the rat. Brit J Exp Path 1940; 11:72–80.

142 Schild HO. Mechanism of the anaphylactic reaction as studied by means of inhibitors. In: Shaffer JH, Logrippo GA and Chase MW, eds. Mechanisms of hypersensitivity. Little, Brown and Co., Boston 1959.

143 Schild HO. Histamine release and anaphylactic shock in isolated lungs of guinea pigs. Quart J Exp Physiol 1936; 26:165–77.

144 Rocha e Silva M, Bier O, Aronson M. Histamine release by anaphylatoxin. Nature 1951; 168:465–6.

145 Hawkins DF, Rosa L. Some discrepancies in the histamine theory of anaphylaxis in smooth muscle. In: Wolstenhome GEW, O'Connor CM, eds. Ciba Foundation Symposium on Histamine Little, Brown, Boston 1956 180–2.

146 Goodman LA, Gilman A. The Pharmacological Basis of Therapeutics, The Macmillan Co., New York, 1956 :629.

147 Dale HH. The pharmocology of histamine: with a brief survey of the evidence for the occurence, liberation and participation in natural reactions. Ann NY Acad Sci 1950; 50:1017–21.

148 Kellaway CH, Trethwie ER. The liberation of a slow-reacting smooth muscle stimulating substance in anaphylaxis. Quart J Exp Physiol 1940; 30:121–45.

149 Austen KF, Humphrey JH. In vitro studies of the mechanisms of anaphylaxis. Adv Immunol 1963; 3:3–96.

150 Brocklehurst WE. Occurrence of an unidentified substance during anaphylactic shock in cavy lung. J Physiol (Lond) 1953; 120:16P.

151 Brocklehurst WE. Slow reacting substance and related compounds. Prog Allergy 1962; 6:539–88.

152 Rocha e Silva M, Beraldo W, Rosenfeld G. Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma globulin by snake venoms and trypsin. Am J Physiol 1949; 156:261–73.

153 Beraldo WT. Formation of bradykinin in anaphylactic and peptone shock. Amer J Physiol 1950; 163:283–9.

154 Rocha e Silva M. Liberation de l'shistamine par la perfusion du poumon du cobaye au moyen de la trypsine. Soc Biol 1939; 130:186–8.

155 Ungar G, Mist SH. Observations on the release of serum

fibrinolysin by specific antigen, peptone and certain polysaccharides. J Exp Med 1949; 90:39–51.

156 Ungar G, Hayashi H. Enzymatic mechanisms in allergy. Ann Allergy 1958; 126:542–81.

157 Hahn F, Oberdorf A. Antihistaminica and anaphylaktoide Reaktionen. Z Immuntt Forsch 1950; 107:538–3.

158 Hahn F. Zur anaphylatoxinfrage. Naturwissenschaften 1954; 41:465–70.

159 Friedberger KD, Engelhardt G, Meineke F. Untersuchungen ber die anaphylatoxin-tachyphylaxie und ber ihre Bedetung fr den Ablauf echter anaphylactischen Reaktionen. Int Arch Allergy 1964; 25:154–81.

160 0Lepow IH. Progress in Immunology. In: Amos B, eds. Biologically Active Fragments of Complement Academic Press, New York 1971.

161 Dragstedt CA. Anaphylaxis. Physiol Rev 1941; 21:563–9.

162 Thon L, Uvns B. Degranulation and histamine release, two consectivutive steps in the response of mast cells to 48/8. Acta Physiol Scand 1967; 71:303–15.

163 Levine L. Inhbition of immune hemolysis by di-isopropyl fluorophosphate. Biochim Biophys Acta 1955; 18:283–4.

164 Feldberg W, Holden HF, Kellaway CH. The formation of lysolecithin of a smooth muscle-stimulating substance by snake venoms. J Physiol Lond 1938; 94:232–40.

165 Hogberg B, Uvns B. Further observations on the disruption of rat mesentery caused by compound 48/80 antigen-antibody reaction, and decylamine. Acta Physiol Scand 1960; 48:133–45.

166 Lepow IH, Pillemer L. Studies on the mechanism of inactivation of human complement by plasmin and by antigen-antibody aggregates. II. Demonstration of two reaction stages in complement fixation. J Immunol 1955; 75:63–70.

167 Lepow IH, Ratnoff OD, Rosen FS, Pillemer L. Observations on a proesterase associated with partially purified first component of human complement (Cl). Proc Soc Exp Biol Med 1956; 92:32.

168 Becker EL. Concerning the mechanism of complement action. I. Inhibition of complement activity by di-isopropyl-fluorophosphate. J Immunol 1955; 77:462–8.

169 Becker EL. Concerning the mechanism of complement action. II. The nature of the first component of guinea pig complement. J Immunol 1956; 77:469–78.

170 Becker EL. Hypersensitivity. J Cell Comp Physiol Suppl 1957; 50:303–25.

171 Smith DE. Nature of the secretory activity of the mast cell. Am J Physiol 1958; 193:573–5.

172 Diamant B. Effects of compound 48/80 and distilled water on the adenosine triphosphate content of isolated rat mast cells. Acta Physiol Scand 1967; 71:283–90.

173 Becker EL. Nature and significance of antigen–antibody activated esterases. Biochemistry of the Acute Allergic Reactions. F. A. Davis, Philadelphia, 1968 :199–213.

174 Lichtenstein LM. Biochemistry of the Acute Allergic Reaction. Mechanism of allergic histamine release from human leukocytes. F. A. Davis, Philadelphia, 1968 :153–74.

175 Johnson AR, Moran NC. Selective release of histamine from rat mast cells by compound 48/80 and antigen. Am J Physiol 1969; 216:453–9.