ADVANCES IN
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PREFACE

The third volume of *Advances in Immunology* appears under the editorship of Frank J. Dixon and J. H. Humphrey, the senior editor, William F. Taliaferro, having retired last year. On behalf of ourselves and of the many readers who may have found the first two volumes useful and stimulating we wish to record our gratitude to Dr. Taliaferro for the wisdom, care, and energy that he devoted to initiating the series and to producing the first volumes. Immunology continues to develop rapidly and to have an increasing impact on biology and medicine. Whatever doubts may have been expressed three years ago about the need for *Advances in Immunology* must have been dispelled by the reception it has received and by the readiness with which authors have been willing to take the time and trouble required to survey their special fields. With the continued cooperation of our scientific colleagues and the expert assistance of Academic Press, we foresee that the serial publication will remain of a high standard for many years to come.

The present volume contains four chapters concerned with biological effects of antigen–antibody interactions. The first, by Frank J. Austen and J. H. Humphrey, reviews *in vitro* studies of anaphylaxis in the several model systems which have been investigated during recent years, and attempts to draw these together so as to make clear the extent to which the underlying mechanisms follow common or divergent patterns. The fourth chapter, in which D. R. Stanworth summarizes current knowledge of reaginic antibodies in Man and puts forward his ideas about how their peculiar properties might be explained, is to a considerable extent complementary to the first chapter. In the second chapter Chandler A. Stetson takes up the continuing controversy about whether or not homograft rejection is mediated by humoral antibodies, and marshals a powerful set of arguments and of experimental evidence in favor of the thesis that antibodies play an essential part. David S. Nelson, in the third chapter, reviews the history and current concepts of immune adherence, a phenomenon that not only provides an exceedingly sensitive method for detecting antibodies but that may have a hitherto largely unrecognized importance for the destruction of sensitized cells.

The remaining three chapters concern three different aspects of immunology. Dan H. Campbell and Justine S. Garvey from the wealth of

their own experience discuss the very topical problem of the fate and intracellular persistence of antigen in relation to its capacity to provide a continuing antigenic stimulus. Irving S. Stone and Malcolm R. Irwin review the knowledge of blood groups in species other than Man, and some of its genetic implications. Readers who are unfamiliar with this field may be surprised to learn how much is known, for example, about the blood groups of cattle. Finally, C. S. Jenkin outlines a new approach to parasitism based on the idea that when a parasite and its host share important antigenic determinants, the capacity of the host to give an immunological response to the parasite may be seriously impaired.

In conclusion we wish once again to thank the authors for their cooperation and Academic Press for smoothing the path of the editors and for arriving at the technically excellent production for which they are reputed.

September, 1963

F. J. Dixon, Jr.
J. H. Humphrey

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In Vitro Studies of the Mechanism of Anaphylaxis

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The symptom complex exhibited during systemic anaphylaxis varies from species to species. During systemic or local anaphylaxis direct damage to a variety of cells in the body may occur, but an increasing volume of evidence suggests that the main part of the symptom complex is caused by the release of pharmacologically active agents or their activators. These materials are released from a rather small number of cell types, and when other cells or tissues are involved they become so, secondarily. Some of the factors contributing to species variation include: the *relative proportion* of the four, presently recognized, pharmacologically active products of the anaphylactic reaction, namely, histamine, slow reacting substance, plasma kinins, and serotonin; the particular host organ subjected to the highest concentration of these materials, the so-called *"shock" organ*; the susceptibility or *reactivity* of host smooth muscle and vascular tissue to each of these substances; the rate at which each species *degrades* them; and the extent of direct participation by antigen–antibody *complexes*. Despite the variation in the anaphylactic symptom complex, it is a tenable thesis that the mechanism whereby the chemical mediators are released is very similar even in different species.

Quantitative experiments of the type needed to study the possible steps in the anaphylactic release of the chemical mediators of anaphylaxis *cannot* be performed in the whole animal. However, their release *in vitro* from replicate samples of sensitized tissue can be quantitated and has been used by numerous workers to investigate the mechanism of anaphylaxis. *In vitro* experiments in which the tissue is thoroughly washed permit a study of the reaction between antigen and antibody fixed to tissue without the complicating presence of free serum factors. It is even more convenient to investigate *in vitro* anaphylaxis in a homogeneous suspension of sensitized cells capable of giving detectable evidence of their response, since this eliminates non-participating cell types. On the other hand, with each refinement of the *in vitro* test system one must exert greater caution in applying the findings to systemic anaphylaxis. The mere observation that cytotoxic antibody and serum or that preformed antigen–antibody complexes can damage or release some pharmacological material from cells *in vitro* is not evidence that a similar mechanism is important for anaphylaxis *in vivo*. Furthermore, as systemic anaphylaxis in a given species may involve several chemical mediators arising from different cell types, an *in vitro* system may eliminate one or more important materials. With these reservations, *in vitro* anaphylaxis or, more correctly, the antigen-induced release of chemical mediators from tissue or cells, may be a useful means of

investigating the mechanism whereby antigen–antibody interaction produces systemic anaphylaxis.

The *in vitro* tissue or cell systems to be considered in detail include the chopped guinea pig lung, the rat or guinea pig mesentery, the rat peritoneal mast cell, the rabbit platelet, and the human white blood cell. Data obtained with similar systems in other species will also be included when available. Studies with other *in vitro* systems, such as guinea pig ileum, rat or guinea pig uterus, dog or rabbit liver, guinea pig, dog, or rabbit skin, and guinea pig heart, will be considered only to the extent that the available data contribute to our understanding of the mechanism of anaphylaxis. Cytotoxic systems—immune hemolysis or the lysis of ascites tumor cells by antibody against a cell surface constituent and complement—will be considered only for purposes of comparison, since the details of the complement system have recently been reviewed by M. M. Mayer (1956, 1961a) and by Osler (1961). There is no convincing evidence one way or the other that cytotoxic reactions are really suitable models for anaphylaxis. Complement will again be considered in the section on serum factors in anaphylaxis; this section will also include anaphylotoxin and antigen–antibody activated serum enzyme activities. Immunological reactions in tissue culture will not be reviewed. Studies with chemical histamine or serotonin releasors will not be included since there are data with the guinea pig lung (Mongar and Schild, 1957a; Yamasaki *et al.*, 1960) and rabbit platelet (Humphrey and Jaques, 1955) that point out important differences between histamine release by the *p*-methoxyphenyl-ethylmethylamine and formaldehyde condensation product (48/80) and by antigen. In systems involving the rat mast cell the similarity between histamine release by 48/80 and by antigen is considerable (Hogberg and Uvnas, 1960). The limits set for this review result from our wish to evaluate data strictly pertinent to the mechanism of anaphylaxis *in vitro*. Accordingly, studies on the variety of para-anaphylactic (anaphylactoid) phenomena will be largely neglected.

Before proceeding to a detailed consideration of anaphylaxis *in vitro*, it will be necessary to review briefly the characteristics of the pharmacological materials used as indicators of this reaction. We shall also consider the *in vivo* evidence for the release of these materials during anaphylaxis.

I. Pharmacology

A. Histamine

Histamine has been of great interest to workers in the field of anaphylaxis since Dale and Laidlaw (1910) and Dale (1929) pointed

out the many resemblances, and some differences, between its effects and those of anaphylaxis in the guinea pig. In 1932, direct evidence was presented that it could be released from tissue *in vitro* or *in vivo* by an anaphylactic reaction (Bartosch *et al.*, 1932; Dragstedt and Gebauer-Fuelnegg, 1932). Histamine is widely distributed in mammalian tissue (Feldberg, 1956) but the concentration in a given organ shows great species variation. Much of the tissue histamine is contained in mast cells (Riley and West, 1953, 1955). The histamine content per mast cell shows surprising constancy in normal tissue as illustrated in Table

TABLE I

THE HISTAMINE CONTENT PER MAST CELL IN NORMAL TISSUE FROM DIFFERENT SPECIES

Species	Histamine content per mast cell (μμg.)
Dog tissue	7 – 16
Beef liver capsule	32
Guinea pig tissue	21 – 34
Rat (isolated cells)	10 – 40

I which summarizes the data from several groups of investigators. Graham and her associates (1955), based on mast cells counts and total tissue histamine analyses, concluded that the histamine content of the dog mast cell ranged from 7 to 16 μμg., whereas that of the beef liver capsule mast cell was 32 μμg. Boreus and Chakravarty (1960a), using a similar method, reported that the histamine content of guinea pig mast cells ranged from 21 to 34 μμg. The histamine content of isolated, rat, peritoneal mast cells ranged from 10 to 20 μμg. in the experiments of Humphrey *et al.* (1963), whereas Archer (1960) reported a value of 40 μμg.

The mast cells, which are located mainly in connective tissue in relation to blood vessels (Riley, 1953), are *not* the only cells containing histamine. Histamine has been found in platelets (Humphrey and Jaques, 1955) and in basophilic leucocytes (VanArsdel *et al.*, 1958); histamine is present in high concentration in fetal liver (Kahlson, 1960) and in the parietal cell region of the stomach (Feldberg, 1956) even though mast cells are virtually absent from these latter sites. It is of interest that in the rat, at least, the histamine that resides in mast cells is released by 48/80, whereas that in non-mast cell areas such as the fundus and duodenum is not (Mota *et al.*, 1956). Regardless of whether or not tissue histamine is contained in mast cells, it is formed from L-histidine (Schayer, 1959). Schayer (1960) has recently presented evidence that histidine decarboxylase in tissues not necessarily

rich in mast cells increases in activity in response to a variety of stimuli. Schayer refers to the product of this "adaptive form" of enzyme activity as induced histamine to distinguish it from the stored histamine of the mast cells and suggests that this induced histamine is formed by vascular endothelium and may be an important intrinsic regulator of the microcirculatory system.

Some of the established pharmacological effects of histamine include capillary vasodilatation with increased permeability, bronchiolar and other smooth muscle constriction, and stimulation of the glands of exocrine secretion. Despite this diverse activity and the widespread distribution of histamine, the physiological significance of this material is still obscure. The evidence that parietal cell stimulation is a physiological function of histamine has been summarized by Code (1956); Whelan (1956) has concluded that it is doubtful whether histamine plays a role in the regulation of blood flow during reactive or post-exercise hyperemia; and Miles (1959) is not convinced that histamine is primarily responsible for the increased capillary permeability of inflammation. Recently, Kahlson (1960) has suggested that histamine is connected with anabolic events such as growth, development, and repair.

B. Slow Reacting Substance

The term "slow reacting substance" (SRS) refers to material or materials that contract smooth muscle, usually guinea pig ileum, more slowly than histamine or acetylcholine. Feldberg and Kellaway (1938) introduced this term to describe a substance obtained from lung during perfusion with cobra venom, and 2 years later, in 1940, Kellaway and Trethewie (1940) reported the presence of a similar material in the effluent collected during anaphylactic shock of the perfused guinea pig lung. The evidence for the presence of a slow reacting material was indirect and based on the observation that the effluent caused a more prolonged contraction of the guinea pig ileum than did histamine alone. In 1953, Brocklehurst (1953, 1960), using antihistamines to abolish the response of the ileum to histamine, presented direct and conclusive evidence for the anaphylactic release of slow reacting material from guinea pig lung. This material was termed SRS-A to indicate that it was obtained as a consequence of antigen–antibody interaction.

The chemical properties of the slow reacting material produced in guinea pig lung by the lecithinase A in cobra venom are consistent with those of an unsaturated fatty acid (Vogt, 1956). The material released from guinea pig lung by anaphylaxis has not been characterized chemically,

but the properties of the partially purified preparation suggest that it is acidic and lipid. It is always obtained rather firmly associated with some protein (Brocklehurst, 1962).

Slow reacting substance contracts only a limited number of isolated smooth muscle preparations. These include the guinea pig ileum, the rabbit jejunum, the fowl rectal cecum, and the human bronchiole; it does not contract tissues such as rat colon, rat uterus, or the bronchiole of the guinea pig, dog, rabbit, or cat (Brocklehurst, 1962). On intravenous injection it did not depress the blood pressure of the cat or rabbit. It is easily differentiated from bradykinin or serotonin, as shown in Table II. Bradykinin contracts the rat uterus, is inactive on the human

TABLE II

THE DIFFERENTIAL BIOLOGICAL ACTIVITY OF SRS-A, BRADYKININ, AND SEROTONIN

Parameter	SRS-A	Bradykinin	Serotonin
Contraction of guinea pig ileum	+	+	+
Contraction of rat uterus	—	+	+
Contraction of human bronchiole	+	—	—
Reduction of arterial pressure (cat)	—	+	+
Tachyphylaxis	—	—	+
Destruction by chymotrypsin	—	+	—

bronchiole, depresses arterial pressure, and being a polypeptide is destroyed by chymotrypsin. Serotonin contracts the rat uterus, has no effect on the isolated human bronchiole except in high doses which cause relaxation rather than constriction, depresses arterial pressure, and produces tachyphylaxis of the guinea pig ileum.

Whereas the antigen–antibody reaction releases *preformed* tissue histamine, SRS is both *formed* and *released* as a result of antigen–antibody interaction. This was demonstrated by Brocklehurst (1960) who found appreciable amounts of SRS-A in shocked tissue and the effluent therefrom, but only trace amounts in unshocked tissue and, of course, none in the effluent.

The predominant source of SRS as well as histamine in the guinea pig is lung tissue. As will be discussed shortly, SRS has also been obtained *in vitro* from rabbit, monkey, and human lung but not from horse or goat lung (Brocklehurst, 1960). The *in vivo* release of SRS has recently been demonstrated in the rat (Rapp, 1961). Although Uvnas and Thon (1959) and Boreus and Chakravarty (1960b) have presented evidence that SRS originates in the mast cell, there are data to the contrary which will be considered later.

C. Physiologically Active Polypeptides

In the early 1930's, Frey and his associates (1930, 1933) demonstrated that the intravenous injection of pancreatic juice into dogs produced hypotension; the active principle in the pancreatic extract was termed "kallikrein." *In vitro* experiments several years later (Werle *et al.*, 1937) revealed that kallikrein reacted with blood to form a smooth muscle stimulating principle, but the realization that kallikrein was an enzyme which acted on a protein substrate in blood to produce the muscle stimulating principle was delayed for more than 10 years. In 1949, Rocha e Silva *et al.*, showed that a muscle stimulating and vasodilator polypeptide, which they termed bradykinin, was produced by the enzymatic action of trypsin or snake venom on the plasma proteins.

Apart from trypsin, there are now several enzymes of possible physiological significance capable of elaborating a plasma kinin in varying degrees of proficiency. Two of these, plasmin and salivary gland kallikrein, are known to arise from an inactive precursor state, termed, respectively, plasminogen and kallikreinogen (Hilton and Lewis, 1957). Although both these enzymes act on a substrate in the plasma proteins, split the synthetic ester *p*-toluenesulfonyl-L-arginine methyl ester (TAME), and are inhibited by diisopropylfluorophosphate (DFP) (Webster and Pierce, 1961), some important differences in their action have been demonstrated, and these must be kept in mind because of their pertinence to the subsequent discussion of antigen–antibody activated serum enzymes. Salivary gland kallikrein, in contrast to plasmin, forms a kinin from the plasma proteins with great rapidity, is resistant to inhibition by the soybean trypsin inhibitor, and does not split casein (Lewis, 1959, 1960).

A kinin-forming enzyme which arises from a precursor in plasma has now been delineated (Elliott, 1963). It is differentiated from plasmin by virtue of its marked kinin-forming and poor fibrinolytic capacity (Schachter, 1963). These two plasma enzymes may also be distinguished by differential activation (Margolis, 1963). Indeed, the studies of Margolis (1958, 1963) indicate that the initial step in the elaboration of plasma kallikrein involves activation of the Hageman factor, and it has even been suggested that the permeability factor of Miles is synonymous with plasma kallikrein.

Purification of bradykinin obtained from the action of crystalline trypsin on pseudoglobulin from ox blood was achieved by Elliott *et al.* (1960a, 1960b) who found that it was a nonapeptide. Synthesis was accomplished by Boissonnas and associates (1960, 1963). Recent studies

indicate that human plasma kallikrein, like trypsin, produces a nonapeptide (kallidin I), whereas salivary kallikrein and an analogous enzyme in urine produces a decapeptide (kallidin II) which differs from the nonapeptide only in the addition of an *N*-terminal lysine (Webster and Pierce, 1963).

Using the pure nonapeptide (bradykinin or kallidin I), Elliott *et al.* (1960c) demonstrated five pharmacological activities: smooth muscle stimulation, vasodilatation, increase in capillary permeability, migration of leucocytes, and stimulation of pain fibers. On a molar basis (Fox *et al.*, 1961) bradykinin is an extremely active vasodilator, and this could prove to be a significant consequence of its activation during anaphylaxis. The decapeptide (Webster and Pierce, 1963) has similar pharmacological activity which differs in degree depending on the assay. There is some evidence that these kinins produce bronchoconstriction when given intravenously or intraperitoneally to the guinea pig, but this action is absent on inhalation (Collier, 1963).

The possibility that a plasma kinin might play a role in systemic anaphylaxis was first suggested by Beraldo's finding of bradykinin-like activity in dog blood during anaphylactic shock (Beraldo, 1950). Recently Brocklehurst and Lahiri (1962) have shown that significant amounts of plasma kinin appear in the blood of sensitized guinea pigs, rats, and rabbits within 2 to 5 minutes of injecting antigen intravenously. These workers also found that the effluent from perfused, shocked, guinea pig lung contained no detectable bradykinin, but would produce bradykinin when incubated with plasma pseudoglobulin which had been heated to destroy kinin-inactivating enzymes. Since the effluent contained the kinin-forming capacity but not kinin, it is reasonable to assume that the kinin-forming enzyme or some precursor thereof was derived from the lung tissue. Diniz and Carvalho (1963) have reported that the plasma substrate for bradykinin formation is depleted during systemic anaphylaxis in the rabbit.

D. Serotonin

In 1955 Humphrey and Jaques showed that serotonin was released from rabbit platelets *in vitro* by antigen–antibody interaction, and subsequently Waalkes and his associates (1957) demonstrated serotonin release *in vivo* during rabbit anaphylaxis. These observations naturally stimulated further investigation into the possible role of serotonin in the anaphylactic reaction.

In mammalian tissue, serotonin is localized primarily in the mucosal layer of the gastrointestinal tract, and to a lesser extent in brain tissue