Volume Four

PROGRESS IN MEDICINAL CHEMISTRY

Edited by
G. P. ELLIS, B.Sc., Ph.D., F.R.I.C.
and
G. B. WEST, B.Pharm., D.Sc., Ph.D.

BUTTERWORTHS

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G. P. ELLIS, B.Sc., Ph.D., F.R.I.C.

Department of Chemistry and Biology, Welsh College of Advanced Technology, Cardiff

and

G. B. WEST, B.Pharm., D.Sc., Ph.D.

School of Pharmacy, University of London

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PREFACE

THE present volume of five reviews provides up-to-date information on a wide variety of topics. The first two chapters are short and contain material about the mechanism of action of drugs in general; the third attempts to provide the latest ideas on the combination of drugs with cells at the subcellular level; and each of the last two chapters is a more detailed account of a specific type of drug.

The first chapter is devoted to the study of experimental hypersensitivity reactions in an attempt to determine some of the more important factors involved in human allergy. The severity of the reaction appears to be controlled in part by the glucocorticoid hormones of the adrenal cortex and hence results from changes in the metabolism of carbohydrates. The review on the mechanisms of toxic action, however, shows how little understood are these effects, how important is the species of animal used, and how results in animals cannot be translated in many cases to man.

At the present state of knowledge, any discussion on drug-receptor interactions must be highly speculative. We know much about the structure of drugs but very little about the receptor material in the cell with which the drug combines. The future isolation of the receptor substance is now so important. The interaction between these materials is discussed in the third chapter of the present volume.

We have allocated one chapter of nearly 100 pages to the synthesis, assay and clinical importance of various polypeptides because it appears that major advances in this field of medicinal chemistry are not far away. The importance of insulin, oxytocin and vasopressin is well known but not so much stress has been laid on the polypeptides, angiotensin and bradykinin.

The final chapter on the chemical aspects of analgesic drugs is complementary to that on the testing and development of these compounds which appeared in Volume 2. Major advances in the metabolism of analgesic drugs and in the pharmacology of analgesic antagonists have provided some insight into their mechanism of action, and the structure-action relationships are discussed in the present volume.

Lastly, we are grateful to reviewers and others for their encouragement and suggestions. Reviews take many months to compile and further months to edit and print so that delay between completion and publication is inevitable. Our thanks are due to the staff of Butterworths and to the authors, societies, and publishers for permission to use illustrations and tables in this and previous volumes.

G. P. ELLIS
G. B. WEST

February, 1965

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EXPERIMENTAL HYPERSENSITIVITY REACTIONS

P. S. J. Spencer and G. B. West

INTRODUCTION

A substantial proportion of the human race each year suffers to a varying degree from asthma, hay fever, food allergies, drug allergies, contact dermatitis, and other unpleasant kinds of allergic manifestations. The undesirable exogenous substance (termed the antigen) reacts with some form of endogenous antagonistic substance (termed the antibody), and a train of events suffered by these unfortunate individuals is started. Very little is known as to how damage is then caused to the cells of the body, and in fact there are still no concise definitions as to the general chemical nature of either antigen or antibody.

In the earlier studies in this field, the specific changes in the response of the individual concerned immunity, or the immune state which remains after recovery from a bacterial or viral disease. A peculiar sort of protein belonging to the globulin fraction of blood and combining with the undesirable substance was generally found in the plasma of the immune organism. These so-called antibodies were then shown to possess protective and curative properties. Later, antibodies were found to be formed not only against pathogenic micro-organisms and their products of metabolism but also against a great variety of both proteins and polysaccharides. Simple chemical substances are now known to be able to form complex antigens and induce the production of specific antibodies, and hence it is impossible to state that such antibodies have a protective or any other useful function.

After primary contact with the antigen, the response of the individual to renewed contact is so altered that peculiar local and sometimes general reactions may result. It is this state of specifically altered reactivity which is generally termed allergy. The immediate allergic reaction involves antibodies in the circulation and may have a mechanism similar to that involved in the anaphylactic reaction in animals. In this reaction, the animal has received a small dose of the antigen two or three weeks before a fairly large dose of the same antigen is given intravenously. The second dose of antigen combines with the antibodies located mostly in the tissues and the animal may die from anaphylactic shock. Many patients who react badly to aspirin, for example, seem to have had a history of an allergic disease such as asthma, and so may well be placed in this category. On the other hand, the delayed allergic reaction does not appear to be the initial reaction of antigen and antibody, as antibodies cannot be found in the free state in the plasma or tissues of such individuals. This type of response may have a mechanism similar to that involved in the anaphylactoid reaction in animals, where the first dose of antigen or antagonistic substance produces a delayed

severe allergic-like response. Many individuals who have not had a history of an allergic disease but possess some unexplained hypersensitivity to drugs such as the barbiturates, sulphonamides or salicylates may be included in this category of delayed reactivity.

Although allergy is usually assigned to the subject of immunology, its reactions are distinctly pharmacological in nature. The responses are complex and the symptoms include eczema, oedema, gastro-intestinal upsets, urticaria and asthma. The human skin is highly sensitive to capillary poisons and therefore furnishes a useful index of allergic hypersensitivity. A number of drug idiosyncrasies show such a striking likeness to most of the characteristics of protein idiosyncrasies (for example, food poisoning) and to allergic reactions generally that an intimate connection between these suggests itself. All involve only small doses of the irritant and all may involve similar tissues of the host. Whatever else the allergic reaction may do to cells, histamine is released from them and this release is one of the more important consequences of cell stimulation or injury. Many but not all of the profound local and systemic signs and symptoms of allergic reactions can be traced to the actions of the released histamine. Although histamine produces vasodilatation and increases the capillary permeability, two vascular effects often occurring in allergy, this is not the sole chemical agent released. Other agents to be considered are 5-hydroxytryptamine (5-HT), the slow-reacting substances (S.R.S.), and various kinins (for example, bradykinin).

STUDIES ON HISTAMINE

Histamine (I) is a natural constituent of many plant and animal tissues. It was first synthesized in 1907 by Windaus and Vogt[1] who prepared it by a Curtius degradation of histidine (II). The discoverers were unaware of the powerful biological properties of their compound and histamine aroused little immediate interest. Three years later, Barger and Dale[2] showed that it was a constituent of ergot, and Ackermann[3] found that putrefactive bacteria form histamine when incubated with the amino acid. Dale and Laidlaw[4] studied its pharmacological actions and confirmed its presence in the intestinal mucosa of mammals. These authors were impressed by the similarity between the immediate symptoms of anaphylactic shock and those evoked by large doses of histamine, and even in 1911 suggested that histamine plays a role in anaphylaxis.

During the 1920's, histamine was shown to be released in sensitization reactions and then found to play a major role in the 'triple response' of the skin (Lewis[5]). Some years later, mainly during the World War II, came the discovery and development of the antihistamine drugs. The diverse pharmacological actions of histamine, coupled with its unknown physiological significance, had stimulated the search for effective blocking agents and it was abundantly clear that such antagonists would be valuable research tools and might even be useful in therapeutics. Today, the range of clinically-effective antihistamines is probably wider than that of any other group of drugs.

Another major advance came with the discovery of the well-known histamine liberators. These are substances capable of freeing histamine from

the tissues without causing gross tissue damage. In 1939, it was shown[6] that crude curare releases histamine from muscles of the dog, and ten years later MacIntosh and Paton[7] published their classical paper on chemical histamine liberators. More recent work has shown that the property of releasing histamine is common to many simple compounds. The knowledge gained by the use of histamine liberators in animals has been considerable, and this has been surveyed by Paton[8] in a comprehensive review of the mechanism of histamine release.

The most recent contribution of outstanding significance to the study of histamine has been the finding of Riley and West[9] that the bulk of the histamine in a number of tissues is located in mast cells. Earlier work[10] established that there is a correlation between the heparin content of a tissue and its mast cell population, and later work by Asboe-Hansen[11] showed that the hyaluronic acid content of many normal and pathological tissues also parallels their mast cell content. Thus three substances—histamine, heparin and hyaluronic acid—are closely linked with the physiology of these cells. They may contain other highly-active agents (for example, slow-reacting substances), and their function remains a matter for speculation.

When tissues are injured, mast cells disrupt and release their contents into the tissue fluids. It is remarkable that the highest concentrations of histamine and mast cells generally occur at surfaces where the organism is in contact with the outside world (that is, the skin, the lungs and the alimentary tract). Paton[12] suggested that perhaps histamine is in these locations to produce vasodilatation and so reduce the pathogenicity of invading bacteria. It is significant that many mast cells are found at perivascular sites and the histamine they release acts by increasing capillary permeability. This in turn floods the tissues with a protein-rich oedema fluid and so assists in the mobilization of the fixed mesenchymal tissue and in the removal of foreign matter[13].

The wide distribution of histamine in animal tissues led to speculation about its origin. The presence of an enzyme in mammalian tissues capable of decarboxylating histidine to form histamine was first shown by Werle[14] in 1936, and later studies, particularly by Waton[15], illustrate that the distribution of this enzyme varies widely from species to species. It now appears that most of the tissue histamine is not in equilibrium with the main metabolic stream, as it reaches the body from various sources. Animals which do not make their own histamine probably absorb it from the gut[16]. Later studies by Waton[17] have supported this hypothesis.

The fact that animal tissues inactivate histamine was presented for the first time by Dale and Laidlaw[4] in 1911, and later it was found that there are marked species differences in the distribution of the enzyme termed histaminase[18]. This enzyme catalyses the oxidative deamination of histamine to 4-imidazolylacetic acid (III) which then appears in the urine as 1-ribosyl-4-imidazolylacetic acid[19]. It has been known for some time that the urine of many mammalian species also contains some free histamine and some in a conjugated form having properties similar to those of acetylhistamine[20,21] (IV). Since estimates of conjugated histamine in the urine depend largely on the diet, Gaddum[16] suggested that estimates of free histamine in the

urine are most likely to be of value as an index of the amount of histamine released in the whole body. Another major metabolic pathway in some species is methylation of the imidazole ring nitrogen[22]. Dogs for example use methylation as the principal means of inactivating injected histamine, and cats and man methylate both oral and injected histamine. The product formed is 1-methylhistamine (V) which is then in some species deaminated to form 1-methyl-4-imidazolylacetic acid (VI).[23,24] It is of interest that female rats and mice excrete much more free histamine than do the males of these species.

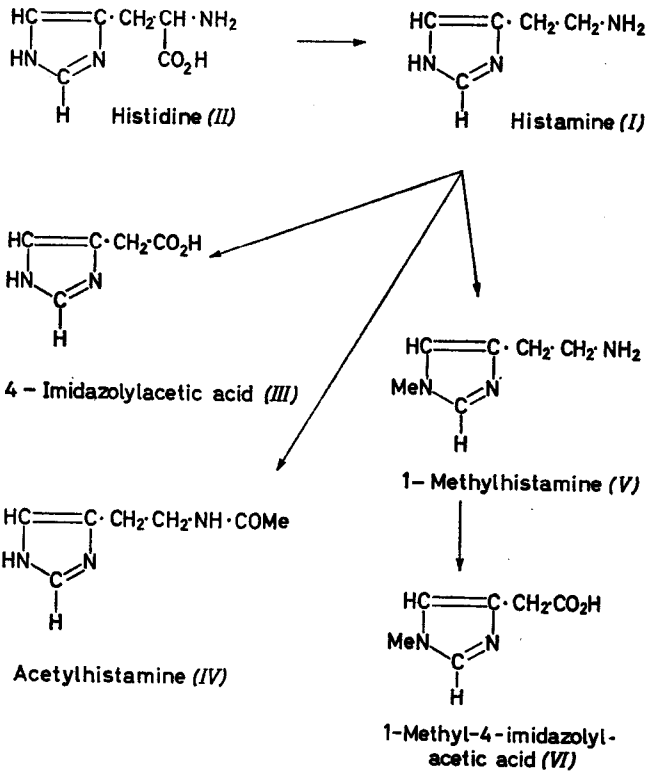


Figure 1.1. Principal pathways in the metabolism of histamine

STUDIES ON 5-HYDROXYTRYPTAMINE

The history of 5-HT covers no more than 15 years and the compound has been referred to under many names. These include vasoconstrictine, vasotonin, spatgift, thrombocytin, thrombotonin, enteramine and serotonin. The largest quantities of 5-HT are found in the gastro-intestinal tract where it is held chiefly in enterochromaffin cells in the mucosa. In the blood, 5-HT is contained mostly in platelets where it appears to be bound to adenosine triphosphate. The amounts of 5-HT in platelets of different species vary enormously, there being for example 15 times more in those of the rabbit than in human platelets. Considerable amounts are also to be found in the

spleen, and in the amygdala, hypothalamus and mid-brain areas of the central nervous system. The only other considerable source of 5-HT in mammals is in the mast cells of the rat and mouse. It is the mast cells which give the skin of these two species their high 5-HT content. Venoms and sting fluids such as those of the wasp, scorpion, toad, octopus, stinging nettle and cowhage also contain much 5-HT.

The biological synthesis of 5-HT was largely worked out[25] in 1957 and its course is indicated in *Figure 1.2.* Synthesis starts from tryptophan (VII)

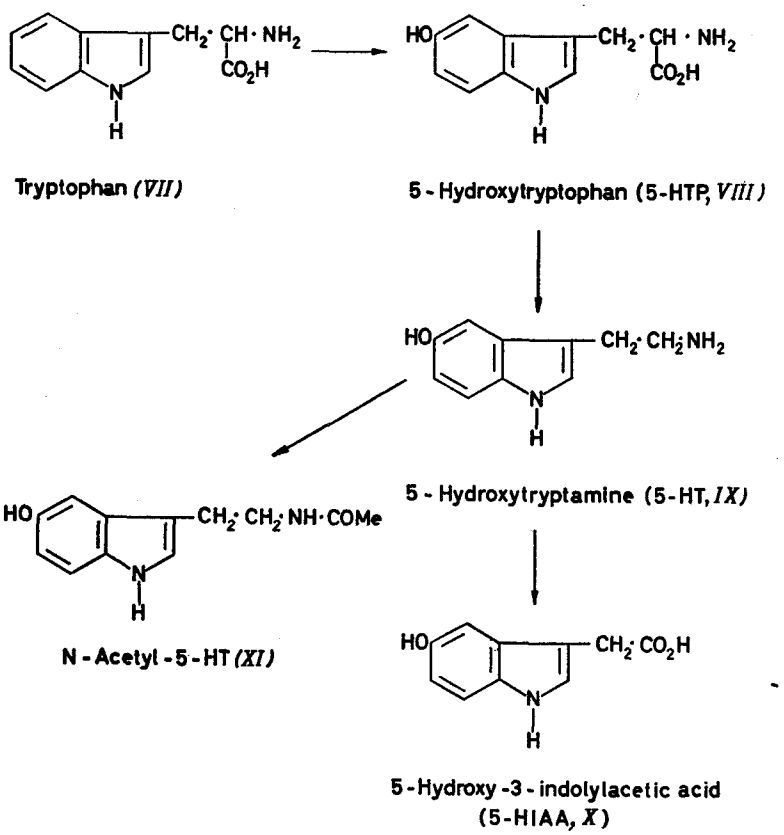


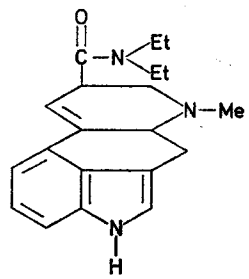
Figure 1.2. Principal pathways in the metabolism of 5-hydroxytryptamine

and this is hydroxylated into 5-hydroxytryptophan (VIII) although little is known of the sites in the body at which this stage occurs. The decarboxylating enzyme responsible for the formation of 5-HT (IX) from 5-HTP (VIII) is widely distributed and its activity in many tissues is considerable. Pyridoxal-5-phosphate is its co-enzyme since 5-HT synthesis is markedly depressed in pyridoxal deficiency. 5-HT is easily oxidized in the body to 5-hydroxy-3-indolylacetic acid (X) which is excreted in the urine. The other principal metabolite is N-acetyl-5-HT (XI) which may account for up to 25 per cent of the total.

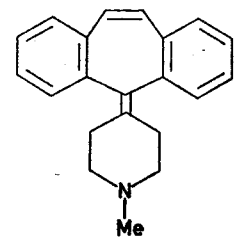
The changes in the circulation after the intravenous injection of 5-HT

into experimental animals are complex and have not been completely analysed. On the respiratory system, hyperpnoeic and apnoeic responses may both be seen. Generally, smooth muscle of mammalian origin is contracted by 5-HT. Evidence that 5-HT plays a physiological role in peristalsis was obtained when Bülbring and Crema[26] found that 5-HT is released from the intestinal wall into the lumen of the gut and that this release was related to the intra-luminal pressure both in the isolated preparation and in the living animal. When 5-HTP is injected into mammals, there is a rise in the 5-HT content of the brain, liver, heart and blood, and this is accompanied by tremors, ataxia, lachrymation and diarrhoea. Changes in hydroxyindole metabolism or in the concentration of 5-HT in the blood have been found in the malignant carcinoid syndrome, in some forms of mental deficiency, and in some blood diseases. Carcinoid tumours may develop anywhere along the gastro-intestinal tract and in such cases there is an increased excretion of both 5-HT and 5-HIAA in the urine. Phenylketonuria is an inherited condition due to a recessive gene and is associated with mental deficiency usually of a gross degree. There is a failure to oxidize phenylalanine to tyrosine which leads to a high blood phenylalanine concentration and to the formation and excretion in the urine of a number of products of phenylalanine metabolism normally not formed or only formed in small amounts. Of these, the ones found in largest amounts are phenylacetic, phenylpyruvic and phenyllactic acids.

Much work has been done using drugs with actions antagonistic to those of 5-HT and the subject has been reviewed[27]. At first it was considered that all antagonists must contain the indole structure (for example, lysergic acid diethylamide, XII) but recently compounds without such a structure have been shown to be potent antagonists (for example, cyproheptadine, XIII).



Lysergic acid diethylamide *(XII)*



Cyproheptadine *(XIII)*

The injection of reserpine into an animal is followed by a large increase in the excretion of 5-HIAA (X) in the urine and a loss of 5-HT (IX) from sites where it is normally found. This type of treatment has been extensively used in studying the role of 5-HT in allergic and hypersensitivity reactions. Humphrey and Jaques[28] were the first to observe in 1954 that 5-HT was released with histamine from rabbit platelets during the antigen-antibody reaction, and other workers have found that the urinary excretion of 5-HIAA

is increased after anaphylactic shock in rabbits[29]. In rats, however, anaphylaxis occurs even after the animals have been treated with reserpine to remove 5-HT from the brain, gastro-intestinal tract, platelets, spleen and skin, so there may be considerable species variation in the importance of 5-HT.

The functions of histamine and mast cells are closely related and the location of some 5-HT in the skin mast cells of the rat and mouse suggest that there may also be a function of 5-HT linked with that of mast cells and histamine. In these two species, 5-HT is many times more potent than histamine in increasing capillary permeability[30] and it is possible that at least in these two species 5-HT takes over the postulated defence role of histamine in other species[31].

STUDIES ON BRADYKININ

In 1949, Rocha e Silva, Beraldo and Rosenfeld[32] described the release of an active peptide from serum globulin by trypsin or snake venoms. They named the peptide bradykinin, because it caused a relatively slow contraction of the isolated guinea-pig ileum. These workers distinguished this substance from acetylcholine, histamine, adenosine and other active substances by a series of biological tests. For example, bradykinin contracts most isolated smooth muscle preparations such as the intestine of the guinea-pig, rabbit, cat and dog but relaxes the duodenum of the rat. It is also a powerful vasodilator and markedly increases capillary permeability. The isolated rat uterus, though one of the most sensitive test preparations *in vitro*, is practically unaffected by bradykinin *in vivo*[33]. Bradykinin produces bronchoconstriction in the guinea-pig but fails to contract isolated bronchial muscle of dog or man. It produces pain when applied to a blister base on human skin[34]. It is of interest that the bronchoconstrictor action of bradykinin in the guinea-pig is specifically suppressed by relatively small doses of acetylsalicylic acid, phenylbutazone and amidopyrine[35].

Bradykinin is a nonapeptide[36], although the early studies indicated that it was an octapeptide[37]. There are many other kinins which develop in plasma under a variety of conditions, but all seem to be derived from α_2-globulins. They are of considerable interest from the pharmacological point of view since their biological potencies are often as great as those of acetylcholine or adrenaline. The recent progress in the isolation and synthesis of these peptides however will undoubtedly accelerate the discovery of their significance in physiology and pathology. It is possible that bradykinin-like material is formed during the antigen-antibody reaction in some species and antagonists will also help in determining its role in pathology.

THE ANAPHYLACTOID REACTION

In the course of their experiments with egg-white, Parker and Parker[38] reported difficulty in separating the symptoms due to anaphylaxis from those due to the immediate toxicity of the protein. The rats reacted in a similar way after the initial dose and after the challenging injection of antigen, showing mild congestion of the lungs and intestines. Later, Selye[39] noticed a peculiar reaction in rats after the first intraperitoneal injection of fresh egg-white. This was characterized by oedema and hyperaemia especially of the face and extremities. A more thorough investigation of the

reaction[40] showed that the rat is naturally hypersensitive to egg-white and the symptoms attributed to anaphylaxis by previous workers might well have been due to this hypersensitivity state. However, even after large doses of egg-white, no fatalities were reported and the response was termed an anaphylactoid reaction.

Egg-white is not the only substance to elicit this reaction in the rat. A similar response is given with dextran, a high molecular weight polymer of glucose[41], and other examples are glycogen, globin, kaolin, yeast, hyaluronidase and bradykinin. The anaphylactoid reaction is readily elicited on intravenous, intraperitoneal or intrapleural injection of the agents. Small amounts of dextran injected subcutaneously also produce a marked local reaction in the paws and in distant shock organs. Selye[39] suggested that a combination occurred between dextran and the tissues of the shock organs and this was responsible for the anaphylactoid reaction in regions remote from the local injection site. After a single intraperitoneal injection of dextran or egg-white, the rat develops violent scratching especially of the face, and the pruritus soon becomes generalized. Vasodilatation and oedema of the snout and paws follow to reach a maximum about 90 minutes after injection. The symptoms then regress and by 6 hours nothing remains but patches of hyperaemia. The anaphylactoid reaction is now accepted as an acute inflammatory reaction elicited in the rat.

The role of histamine in the anaphylactoid reaction has been indicated by many workers[42–44], yet the symptoms observed are not reproduced in full by injections of histamine, and relatively large doses are necessary to elicit local reactions in the rat. In most areas of skin of the rat, Parratt and West[45] found that there is a relationship between the histamine content and mast cell count on the one hand and the 5-HT content on the other, and 5-HT was considered as another amine involved in the anaphylactoid reaction. Previously, Rowley and Benditt[46] reported that egg-white and dextran each released both histamine and 5-HT from rat skin. Furthermore, the intraperitoneal injection of egg-white or dextran was shown to produce the full anaphylactoid reaction even after the histamine content had been lowered to minute amounts. Depletion of tissue 5-HT, on the other hand, prevented the onset of the full reaction to these substances. Parratt and West[45] also detected small amounts of 5-HT in the oedema fluid but no histamine. Treatment with antagonists of 5-HT prevented the reaction whereas treatment with specific antihistamines such as mepyramine had no effect. Lastly, 5-HT is more than 100 times as effective as histamine in increasing capillary permeability in the rat[47]. However, whilst 5-HT may be more important than histamine in the anaphylactoid reaction, neither amine when injected alone fully reproduces the reaction and it is possible that other substances such as heparin[10], hyaluronic acid[11], bradykinin[37] and slow-reacting substances[48] are involved.

The anaphylactoid reaction in rats has recently been re-investigated by Harris and West[49], since these authors found that more than one-fifth of the rats secured from one colony failed to react to dextran and egg-white, no matter by what route or in what dose they were given. The skin of rats resistant to dextran and egg-white (the non-reactors) was not deficient in either histamine or 5-HT, and these rats were not less sensitive to injected

histamine and 5-HT than were the reactor rats. Although dextran released no histamine from the perfused hind quarters of the non-reactors, more powerful histamine liberators such as polymyxin B were effective. Procedures which inhibited the reaction in reactor rats included the production of alloxan diabetes and the pretreatment with glucose[50] or 2-deoxyglucose[51] but non-reactors were not diabetic and they had no glycosuria. Procedures which enhanced the reaction in reactor rats did not change the resistance of non-reactor rats. It was also noted that anaphylaxis was induced both in guinea-pigs using non-reactor serum as antigen and in non-reactor rats using horse serum as antigen. This result illustrates that rats resistant to egg-white and dextran produce antibodies to foreign protein and also that their serum acts as an antigen in a heterologous species. Consequently anaphylaxis may occur in a rat which does not show the anaphylactoid response. Guinea-pigs and rabbits are similar in this respect as the anaphylactoid reaction has never been found in these two species.

The combination of dextran or egg-white with a blood or tissue component necessary to effect the release of histamine and 5-HT may not occur in non-reactor rats. This component may be an enzyme, a metabolic product or an antibody. Since dextran reactivity is closely linked with carbohydrate transport as exemplified by the action of glucose and insulin and by the work of Beraldo, Dias da Silva and Lemos Fernandes[52] with sugars other than glucose, an abnormal metabolic intermediate may be formed. Alternatively, if an antigen-antibody reaction is involved in the production of the anaphylactoid response[53], then it is likely that this antibody to dextran is lacking in non-reactor rats. This non-reactivity has been found only in the Wistar strain of rat but it can be outbred into other strains. Recent work shows that this character is controlled by an autosomal recessive gene[54]. This important finding may have a counterpart in human allergy which is usually of an hereditary character. The mechanism by which the non-reactivity property in rats is brought about has not so far been established.

INFLUENCE OF THE ADRENAL GLAND

Dale and Richards[55] were the first workers to draw attention to the possibility of an antagonism between adrenaline and histamine and to suggest that a function of adrenaline is to maintain capillary tone against the depressant action of histamine and other products of cellular injury and metabolism. Later, Dale[56] showed that adrenalectomy in the cat renders it several times more sensitive to the effects of histamine. In the rat, adrenalectomy lowers the resistance to both histamine[57] and anaphylaxis[58]. Some years later, Perla and Gottesman[59] showed that this hypersensitivity in adrenalectomized rats is reversed by adrenaline or by adrenal cortical extracts. These results have been repeatedly confirmed and the protection has been found to be due to the gluco-corticoids and not to the mineralo-corticoids.

In a similar manner, adrenalectomy profoundly increases the intensity of the anaphylactoid reaction. This was first shown by Selye[39] and confirmed later by other workers[60–62]. Egg-white or dextran when injected into such rats elicits within minutes the severe symptoms of shock and vascular collapse, followed by death. Repeated large doses of cortisone protect adrenalectomized rats against the lethal effects of dextran[63], and often acute

pre-treatment with adrenaline is effective[61]. Treatment with ACTH for several days prior to challenge also inhibits the reaction in intact animals but is ineffective in adrenalectomized rats. Both the adrenal cortex and the adrenal medulla therefore secrete hormones which increase the resistance of the intact rat to dextran, egg-white, histamine and anaphylaxis.

In 1938, Rose and Browne[64] showed that injected histamine was less readily inactivated by rats after adrenalectomy, an effect which was counteracted by adrenal cortical extracts. The ability of the rat to inactivate exogenous histamine therefore appears to be influenced by changes in the level of adrenal cortical secretion. Endogenous histamine is similarly affected and after adrenalectomy the histamine content of various rat tissues is markedly raised[65–69]. Tissue levels of 5-HT are also raised after adrenalectomy[67], effects which are overcome by giving cortisone (but not deoxycorticosterone, a mineralo-corticoid). In 1961[70], a marked reduction in the skin histamine and 5-HT levels in the rat was reported after the administration of some new synthetic gluco-corticoids. Those compounds possessing the greatest gluco-corticoid activity were the most active whilst the mineralo-corticoids were inactive.

The formation and binding of new histamine in rat abdominal skin was shown by Schayer, Davis and Smiley[71] to be controlled by the adrenal cortex. Using radioactive histidine and the tracer techniques, these workers found that the rate of binding of new histamine is strongly inhibited by cortisone and increased after adrenalectomy. Telford and West[72] investigated the histidine decarboxylase activity of rat tissues and found that the liver has a far greater histamine-forming capacity than any other tissue, suggesting that this tissue in the rat supplies the histamine requirements of most of the body. Gluco-corticoids depressed the liver histidine decarboxylase activity in amounts sufficient to account for the depletion of histamine from the skin and lungs. Cass and Marshall[73] later confirmed the effects on the tissue histamine and 5-HT levels but attributed these changes to an action on the uptake and storage of these amines and not to a depression of synthesis. It is clear, however, that the adrenal cortical secretion, which is mainly corticosterone in the rat and thus gluco-corticoid in nature, exerts a functional control over the general metabolism of histamine and 5-HT, and this property may explain the therapeutic effect of gluco-corticoid hormones in allergic and inflammatory reactions in other species including man.

INFLUENCE OF THE THYROID GLAND

It is well known that thyroxine potentiates anaphylaxis in most animal species and thyroidectomy reduces the severity of the shock[74]. There is also considerable evidence to show that the thyroid hormones influence histamine metabolism in the rat. Gotzl and Dragstedt[75], for example, found that the removal of the thyroid gland decreased the histamine content of rat tissues whereas injections of thyroid extract increased them. A similar trend in skin histamine levels has been noticed by Feldberg and Loeser[76]. On the other hand, Arvy[77] showed that rats made hypothyroid by feeding with thiouracil showed a marked increase in the numbers of tissue mast cells and thus by implication the histamine content was raised. The literature is thus confusing on this point.

On the anaphylactoid reaction in rats, Leger and Masson[60] showed that the mild oedematous response after egg-white changed to a severe one resulting in death when the animals had received thyroxine. Thyroidectomy produced the opposite effects. More recently, Parratt and West[80] showed that, besides egg-white, doses of dextran, polymyxin B and compound 48/80 produce severe shock and death in thyroxine-treated rats. A pattern of events similar to that found in adrenalectomized animals is recorded, and microscopical examination of the submucosa of the jejunum revealed extensive haemorrhagic lesions. Oedema extended up to the villi where the epithelial cells showed desquamation, just as is seen in anaphylaxis in this species[78]. Parratt and West also found that rats under thyroxine treatment became much more sensitive to histamine and 5-HT, amines liberated in both anaphylaxis and the anaphylactoid reaction. The rats excreted more free histamine and the histaminase activity of the intestine was markedly reduced. Thus the greatly enhanced anaphylactoid reaction in thyroxine-treated rats is probably due to the marked increase in sensitivity to histamine and 5-HT (about 30-fold) and an impaired ability to inactivate these amines.

Later work by Spencer and West[79] showed that daily subcutaneous injections of either thyroxine or tri-iodothyronine greatly increased the severity of the anaphylactoid reaction in both male and female rats. Males proved to be more sensitive than females, whilst tri-iodothyronine was more potent in this respect than was thyroxine. The degree of oedema, as measured by a plethysmographic apparatus[80], did not increase although the speed of oedema formation was markedly accelerated in thyroxine-treated rats. A peak time of sensitivity occurred at 14 days in males and 17 days in females when treated with thyroxine sodium. Despite further treatment, the severity of the anaphylactoid reaction declined towards control levels, suggesting that some internal compensatory mechanism had become effective. The adrenal cortex and its secretions were thought to be involved.

Various aspects of histamine and 5-HT metabolism were therefore examined after making the rats hyperthyroid by injecting tri-iodothyronine. Marked increases in the sensitivity of the tissues to histamine and to 5-HT were found, as with thyroxine treatment, and the rate of removal of dextran-liberated histamine was partially reduced. Intestinal histaminase levels were somewhat lowered and tissue histamine contents were temporarily raised. The increase in the severity of the anaphylactoid reaction after tri-iodothyronine treatment thus appears to be mainly the result of the increased sensitivity of the tissues to the amines released by the anaphylactoid agents.

When hypothyroidism was induced in rats by thyroidectomy or by feeding antithyroid drugs (for example, methylthiouracil), the severity of the anaphylactoid reaction was reduced. This also was only temporary and within 14 days the sensitivity to dextran had returned to control levels. Again, as with hyperthyroidism, an internal compensatory mechanism was considered to be operating.

The mouse, like the rat, is resistant to the systemic effects of histamine and 5-HT, but this resistance is lowered by pre-treatment with *Haemophilus pertussis* vaccine[81,82] or by adrenalectomy[83]. Spencer and West[84] found that daily doses of thyroxine increased the sensitivity of mice to both histamine and 5-HT, coupled with increases in tissue amine levels and decreases in the