STUDIES ON THE MECHANISM OF ANAPHYLAXIS IN CATTLE

A Thesis

submitted for

The Degree of Doctor of Philosophy

in

The Faculty of Veterinary Medicine

of

The University of Glasgow

by

Maureen MacKenzie Aitken, B.V.M.S., M.R.C.V.S.

Department of Veterinary Pharmacology

University of Glasgow

November 1970

ACKNOWLEDGEMENTS

I wish to express my thanks for the help I received from the following people:

Dr. J. Sanford for supervising this research project, instructing me in pharmacological techniques and providing invaluable help and advice throughout.

Professor Sir William L. Weipers, who also supervised this work, for his continued interest and encouragement and the reassurance of his advice on particular difficulties.

The technicians of the Department of Veterinary Pharmacology, particularly Miss Janet Warnock and also Miss Ann McConnell for their competent technical assistance.

Mr. Alan MacKenzie, head stockman, for looking after the experimental cattle and for his help in their handling and restraint.

Miss Rita Wilson for carrying out the haematological estimations.

The staff of the Department of Veterinary Pathology for carrying out post mortem examination of experimental animals, preparing sections of tissues for histopathological examination and advising me on the interpretation of histopathological features.

Messrs. A. Finnie and A. May for taking and preparing the photographs.

Miss Winifred Boyle for typing many of the tables and Mrs. Isobel Gerrard for typing the manuscript.

I would also like to express my appreciation to Dr. H.O.J. Collier, Dr. W.E. Brocklehurst, Mr. G.E. Davies, Mr. G.G. Beadle and Dr. A. Green for extremely helpful discussions and advice. References to findings of Dr. Brocklehurst, Mr. Davies and Mr. Beadle, using material from our experimental animals, are made in the discussion (Section III).

I am also grateful to the Agricultural Research Council who provided a grant to finance this work for 3 years and the

Horserace Betting Levy Board from whom I received a research training scholarship for one year.

The following drug firms are thanked for their generosity and cooperation in donating drugs used in this investigation: Fisons Pharmaceuticals Ltd. (disodium cromoglycate), I.C.I. Ltd. (propranolol), Merck, Sharp and Dohme (cyproheptadine), Parke, Davis Ltd. (meclofenamate), Wellcome Research Laboratories (B.W. 501C67).

TABLE OF CONTENTS

INTRODUCTION

The word "anaphylaxis" is derived from the Greek meaning guarding (phylaxis) reversed (ana). It suggests the reverse of a beneficial phenomenon, namely immunity, and is applied to reactions, damaging to cells of the animal body, which occur as a result of immunological responses involving interactions of antibody and antigen. According to von Pirquet (1906) the term "immunity" must be restricted to those processes in which the introduction into the organism of the foreign substance to which the organism is immune causes no evident adverse reaction, where, therefore, complete insensitivity exists. "Anaphylaxis" usually refers to experimentally or artificially produced reactions whereas those occurring naturally are termed "hypersensitivity" or "allergy" (Coombs and Gell, 1963).

Little is known of the mechanism of anaphylaxis in ruminants. Hypersensitivity may underlie many diseases of cattle, such as laminitis, the syndrome of acute respiratory distress known as "fog fever," and adverse reactions to warble fly larvae, antibiotics, foot and mouth disease vaccine and dietary constituents. Diseases of hypersensitivity in humans have received a great deal of attention and the mechanism of anaphylaxis has been extensively investigated in the guinea-pig, dog, rabbit, rat and mouse. The degree of species variation, however, has greatly limited the application of findings in these species to other species such as the bovine.

The objects of the investigation described in this thesis were:-

1) To determine the nature of the acute, systemic, anaphylactic reaction in cattle and the conditions necessary for their sensitisation in such a way that reactions of

predictable severity could be consistently induced (Section II. 1. and 2).

2) To assess the roles of histamine, 5-hydroxy-tryptamine, bradykinin and slow reacting substance of anaphylaxis (SRS-A) in mediating the reaction. This was done by examining the effects of some of these substances on cattle and on isolated bovine tissue (Section II. 3.) and by testing the effect of specific or selective antagonists of these substances on the anaphylactic reaction (Section II.4). The effects of antigen on isolated tissue of sensitised animals were examined, with the aim of comparing such effects to those of possible mediators (Section II. 5). Estimations of the concentrations, in tissues and fluids, of histamine, in relation to anaphylaxis in vivo and in vitro, were also carried out, in order to detect evidence of release of this substance from tissues (Section II. 6).

3) To induce active and passive cutaneous anaphylaxis and to assess the value of such reactions in the detection of hypersensitivity (Section II. 7).

SECTION I

REVIEW OF LITERATURE

CLASSIFICATION OF ALLERGIC REACTIONS

Coombs and Gell (1963) classified allergic reactions as being of 4 main types on the basis of initiating mechanisms rather than ultimate manifestations. Anaphylaxis they designated a Type I response, being initiated by antigen reacting with tissue cells which had been sensitised by antibody produced elsewhere. This resulted in the release of pharmacologically active substances, producing local or general manifestations in the body. They distinguished this from a Type II reaction, termed cytolytic or cytotoxic, which was initiated by antibody reacting with either an antigenic component of tissue cells or an antigen or hapten in intimate association with tissue cells. Haemolytic disease of the newborn exemplifies a Type II reaction.

A Type III or Arthus reaction they described as occurring when antigen reacted in tissue spaces with precipitating antibody and produced either microprecipitates in and around

the small vessels causing cellular damage, or soluble complexes of potentially precipitating antibody and antigen, when the latter was present in excess. These complexes, deposited in blood vessel walls, produced local inflammation. This type included the local Arthus reaction and serum sickness.

As Type IV, or delayed hypersensitivity, they described the reaction of specifically modified mononuclear cells containing a factor able to respond to antigen deposited at a local site. This response was characterised by infiltration at the local site, of cells of reticulo-endothelial origin, as seen in reactions of sensitised individuals to tubercle bacilli or to material derived from these organisms injected intradermally.

As generalised by Coombs and Gell (1963) the manifestations of anaphylaxis are due to contraction of smooth muscle and increased capillary permeability.

ANAPHYLACTOID REACTIONS

It is important to distinguish the above allergic reactions from "anaphylactoid" reactions in which antibodies are not involved and hence no initial stimulus for their formation is necessary. Reactions of this sort occur on the first occasion of administration of a substance which directly stimulates release of pharmacologically active substances, principally histamine but also slow reacting substance (SRS) (Paton, 1956) and, in the rat, 5-hydroxytryptamine (Parratt & West, 1957). Ankier and Starr (1967) investigated the possible importance of kinins and concluded that they could be excluded from the mediation of the anaphylactoid reaction in rats. Selye, in 1937, first described anaphylactoid reactions produced in Albino rats by fresh egg white injected intraperitoneally. The rats showed severe oedema of the paws and nose. A similar effect was found by Morrison, Bloom and Richardson in 1951 to be produced in rats by dextran.

Anaphylactoid reactions in other species to certain substances, which appeared species specific in this respect, were recorded. All these substances were, like dextran, of large molecular size. Dextran of molecular weight of 100,000 was found by Halpern (1956) to provide optimum anaphylactoid activity in rats. Feldberg and Schachter, in 1952, produced the effect in cats with horse serum and Halpern and Briot, in 1953, in dogs with polyvinylpyrrolidone (PVP). The exact mechanism of release of the mediators in anaphylactoid reactions is not known. Paton (1956) in reviewing the mechanism of histamine release classified substances with the ability to cause histamine release as follows:-

1) Sensitising compounds, i.e. antigens and haptens.

2) Compounds damaging tissues, e.g. venoms, toxins.

3) Proteolytic enzymes, e.g. trypsin.

4) Surface-active agents, e.g. Tween 20.

5) Large molecules, e.g. egg white, dextran.

6) Histamine liberators, e.g. compound 48/80, dibasic and polybasic compounds.

7) Monobasic compounds, e.g. Alkylamines, octylamine.

The "histamine liberators," such as compound 48/80, were distinguished from the monobasic compounds such as octylamine by Paton as they appeared to differ in that the former caused a short explosive release whereas the latter caused a more prolonged release as demonstrated by Wilson's experiments on urinary histamine excretion in rats in 1954.

a) EXPERIMENTALLY INDUCED ANAPHYLAXIS

The methods used to induce anaphylaxis, the nature of the reaction and the mediators involved in the guinea-pig will be outlined. This will be followed by a similar review of the extensive literature dealing with anaphylaxis in dogs, rabbits, rats and mice. Less information is available regarding anaphylaxis in cats, pigs, sheep, cattle and horses. Relevant experimental work which has been carried out in these species will be reviewed. This will be followed by an account of naturally occurring diseases in which there is reason to believe that hypersensitivity is involved. Those diseases affecting cattle will be discussed in detail. Those affecting horses, sheep, pigs, cats and dogs will also be described and the vast literature on hypersensitivity diseases of humans will be summarised.

1) ANAPHYLAXIS IN THE GUINEA-PIG

INDUCTION

Otto, in 1906, recorded anaphylaxis produced in guinea-pigs by foreign serum. He showed that an interval of 10 days elapsed after the sensitising dose before sensitivity was established, and that reactions did not occur when large injections of serum were given at shorter intervals. Rosenau and Anderson (1906, 1907) established that guinea-pigs, sensitised to horse serum, reacted specifically to that antigen. They confirmed that a latent period of at least 10 days was necessary and that hypersensitivity, once established, lasted for several months. Rosenau and Anderson found that a sensitising dose of as little as 1.0 µl. of serum was effective but larger challenge doses of the order of 0.01-0.1 ml., were necessary to induce anaphylaxis. However, it was later established (Coulson and Stevens, 1949) that the challenge dose need not be larger than the minimal sensitising dose.

A challenge dose of 0.5 µg. ovalbumin produced reactions in guinea-pigs sensitised with 1.0 ug. ovalbumin. Coulson et.al. (1949) found that 5½ month old guinea-pigs required sensitising doses which were 20 times smaller than those required by animals of one year old. Thus the dose required varied with age.

It was established (Otto, 1907; Doerr and Russ, 1909) that sensitivity could be transferred passively by injecting serum from a hypersensitive animal into a normal animal. The species of donor animal proved important. Serum from guinea-pigs, rabbits and humans was effective in guinea-pigs. That from horses, pigs, sheep and goats was ineffective. Doerr and Russ (1909) showed that the degree of sensitivity produced was related to the precipitating antibody content of the serum. Benacerraf and Kabat (1949) showed that there was a quantitative relationship between the dose of antibody transferred and the latent period necessary between injection of serum

and challenge with antigen. This period, usually from 4 to 24 hours, was believed to be necessary for fixation of antibody to tissue cells (Weil, 1912; 1914). With large doses of antibody Benacerraf and Kabat showed that immediate reactions could be obtained but this may have involved different mechanisms. Kabat and Landow (1942) showed that the amount of antigen required for the challenge dose was 50 times that giving optimal precipitation with the amount of antibody in the blood. This implied that antigen was required in excess of that neutralised by circulating antibody and supported the early finding by Weil (1912) that high levels of circulating antibody protected guinea-pigs against anaphylaxis

DESCRIPTION

Within minutes of intravenous injection of serum sensitised guinea-pigs showed severe respiratory distress, dying in up to 10 minutes (Otto, 1906). Auer and Lewis in 1910 diagnosed

as did Biedl and Kraus (1910), that this was due to bronchoconstriction, leaving the lungs over inflated and unable to collapse when the thorax was opened after death. Auer and Lewis (1910) demonstrated anaphylactic bronchoconstriction after destruction of the central nervous system and after cutting of the vagi (Auer, 1910). This showed it to be independent of central and reflex vagal mediation. Dale (1920) reproduced it in isolated lung perfused clear of blood. The abundance of smooth muscle in guinea-pig lungs was pointed out by Miller (1921) as contributing to the relative severity of the bronchoconstrictor response in this species. Using labelled antigens to challenge sensitised guinea-pigs Dixon and Warren (1950) observed specific uptake of antigen by the lung.

Intra-peritoneal administration of antigen to sensitised guinea-pigs could produce a different result, in that respiratory embarrassment was not marked. Hypotension, coma and

death after several hours occurred. Congestion of the liver and intestines, with only patchy oedema and haemorrhage in the lungs, was described (Martin and Croizat, (1927).

Protracted anaphylactic shock was produced by subcutaneous injection of antigen in actively sensitised guinea-pigs (Stone, 1958a). These showed pruritis, dyspnoea, bristling of fur and hypothermia, with death occurring after several hours. Stasis and haemorrhage of the alimentary tract was described at necropsy. From experiments on passively sensitised guinea-pigs Stone (1958b) postulated that high levels of circulating antibody prolonged the reaction.

The relationship between the dose of intravenously administered antigen and the severity of anaphylactic bronchoconstriction was investigated at intervals after sensitisation to a constant dose of antigen by Hicks and Okpako (1968). Anaphylactic hypersensitivity was found to increase with corresponding shifts in antigen dose-response curves, reaching

a maximal level after 6 weeks. It was found that with smaller sensitising doses hypersensitivity developed and declined more rapidly than when larger doses were used.

In 1963 Parish, Hall and Coombs investigated the effect of anaesthesia on anaphylaxis in guinea-pigs. They found that death due to asphyxia following bronchoconstriction occurred when sensitised animals anaesthetised with halothane, diethyl ether, nitrous oxide and trichloroethylene or sodium pentobarbitone were challenged by intravenous injection of antigen. When the antigen was administered by inhalation, only anaesthesia by diethylether conferred some protection. This work showed that, in guinea-pigs at least, observations on anaesthetised animals, with the opportunity to measure blood pressure and other changes in such a situation, would be applicable to anaphylaxis in conscious animals. It also showed that sudden death of hypersensitive individuals could occur as a result of inhalation of regurgitated antigen or

administration of antigen in the form of drugs or foreign serum during anaesthesia or deep sleep.

Schultz (1910) and Dale (1913) demonstrated specific responses to antigen of isolated intestine and uterus from sensitised guinea-pigs. Following an anaphylactic contraction responses to successive doses of antigen were absent or greatly diminished in size. They believed this to be due to exhaustion of tissue-bound antibody as sensitivity was restored by exposure of the tissues for several hours to serum from sensitised guinea-pigs (Dale, 1920).

MEDIATORS

i) HISTAMINE

Dale and others postulated the release of some substance from the cells of the sensitised tissue as a consequence of antibody combining with antigen (Dale and Laidlaw, 1911; Dale, 1920; Dale and Kellaway, 1922; Dale, 1929). They suggested that histamine might be the substance in question and in 1932

Bartosch, Feldberg and Nagel demonstrated liberation of histamine from isolated sensitised guinea-pig lung by perfusion with antigen. Bartosch (1935) showed that there was a difference between the histamine content of perfused and unperfused lung from the same animal, confirming that release of histamine had occurred. Schild (1939) demonstrated histamine release by antigen from aorta, intestine and stomach. Similar release from skin was demonstrated by Emmelin, Kahlson and Lindstrom in 1941.

Riley and West focused interest on mast cells as a source of histamine when, in 1953, they pointed out a correlation of histamine content with density of mast cells in many tissues. Mota and Vugman in 1956 and Boreus and Chakravarty in 1960 produced evidence that the tissue mast cell was the source of the histamine released in anaphylaxis. Mota and Vugman showed a depletion of about 70% of the mast cell content of guinea-pig lung following anaphylaxis.

Boreus and Chakravarty (1960) carried out in vitro experiments incubating lung, aorta, trachea, uterus, skin, heart, liver and striated muscle with antigen and found a good correlation between disappearance of mast cells and release of histamine. Release of histamine and mast cell disappearance were increased by increasing the concentration of antigen and inhibited by enzyme inhibitors such as iodo-acetate. The aerobic nature of the mechanism of histamine release from sensitised tissue by antigen and the importance of SH and S-S groups was demonstrated in incubation experiments using chopped lung by Edman, Mongar and Schild (1964). Although the mast cell source of histamine was not disputed, it became clear that histamine could be released from mast cell granules without degranulation being apparent on histological examination (West, 1956, 1962). Boreus (1960, 1961a) found depletion of mast cells of the nasal mucosa of anaesthetised guinea-pigs to occur after anaphylaxis in vivo. Depletion of mast cells was proportional to the challenging dose of antigen and the

severity of response. Anaphylactic (Schultz - Dale) contractions of uterus and ileum could occur with only small reductions in the numbers of mast cells (Boreus, 1961b). Changes occurring in mast cells during anaphylaxis have been reviewed by Mota (1963).

Code (1939), Giertz, Hahn, Hahn and Schmutzer (1962) and Logan (1967) found a 5 to 10 fold increase in blood histamine levels during anaphylaxis.

Histamine alone could not be held responsible for all the manifestations of anaphylaxis. The development of tools for investigation in the form of antihistamines, drugs specifically antagonistic to histamine, strongly suggested that other substances were involved. The situation was reviewed by Mongar and Schild in 1962. Antihistamines were found to reduce the bronchoconstrictor response to antigen given by injection (Staub and Bovet, 1937) or by inhalation (Armitage, Herxheimer and Rosa, 1952). The failure of

antihistamines to completely abolish the response could be explained either on the grounds that released histamine was less readily antagonised than injected histamine or that other mediators were also involved. Dale's view (1948) that even very high concentrations of antihistamines would be unlikely to successfully compete with histamine released at its site of action and, therefore, present at receptor sites in very high concentrations, seemed reasonable. However, other mediators were also identified, namely 5-hydroxytryptamine, slow reacting substance of anaphylaxis (SRS-A) and kinins. Adenosine triphosphate (ATP) and prostaglandins have since come under suspicion but their role has not been confirmed.

ii) 5-HYDROXYTRYPTAMINE

5-Hydroxytryptamine stimulated bronchial constriction but was shown not to play a major role in anaphylaxis in the guinea-pig (Herxheimer, 1955). Its release from sensitised lung and spleen in vitro was demonstrated, however, by Sanyal

and West (1958b). Collier and James (1967), using the 5-hydroxytryptamine antagonist, methysergide, agreed with Hexheimer and others that 5-hydroxytryptamine did not participate significantly in the bronchoconstriction observed in anaphylaxis in the guinea-pig. 5-Hydroxytryptamine was shown to be released from intestine and spleen during anaphylaxis, by Engelhardt in 1960. A combination of lysergic acid diethylamide (LSD), antihistamine and atropine was found necessary by Geiger and Alpers in 1959 to suppress completely the Schultz-Dale reaction of the guinea-pig uterus.

iii) SLOW REACTING SUBSTANCE OF ANAPHYLAXIS (SRS-A)

Slow reacting substance (SRS) was so called because of the slow contraction which it evoked in guinea-pig ileum, in contrast to the more rapid contraction evoked by histamine. Its appearance in perfusate from isolated, sensitised guinea-pig lung during perfusion with antigen was first detected by Kellaway and Trethewie in 1940. Feldberg and Kellaway (1937b) had

observed release of a similar substance from lung perfused by snake venom 3 years previously. Brocklehurst (1953, 1955) observed release of this substance on perfusion of sensitised lung with antigen. He showed it to differ in its pharmacological properties from 5-hydroxytryptamine, bradykinin, substance P and histamine. As its activity on guinea-pig ileum was unaffected by mepyramine, SRS could be assayed on this preparation in the presence of this antihistamine. Brocklehurst called his substance SRS-A (slow reacting substance of anaphylaxis) to distinguish it from other slow reacting substances (Brocklehurst, 1960). Continued investigation of this substance revealed that the output of SRS-A from perfused lungs of sensitised guinea-pigs was slower in onset and more prolonged than that of histamine. SRS-A was formed as a result of the antibody-antigen reaction in the lung, unlike histamine which was released from pre-existing stores in the lung. Antigen perfusion diminished the histamine

content of lung whereas unshocked lung had a very low content of SRS-A compared to shocked lung. Brocklehurst (1960) eliminated blood platelets as a source of SRS-A. He obtained SRS-A from the perfused lungs of guinea-pigs which had been depleted of their platelets by treatment with anti-platelet serum. He also showed that tissue from organs other than lung could form SRS-A. Vascular tissue and, to a lesser extent, salivary gland, spleen and uterus had this property. After incubation of minced tissue with antigen, using the technique of Mongar and Schild (1956), Brocklehurst was able to show the presence of SRS-A in the bathing fluid but this technique proved less satisfactory for detection of SRS-A than was perfusion. Brocklehurst (1960) found that SRS-A was present in the effluent after antigen had been added to the perfused lungs of sensitised rabbits, monkeys and asthmatic humans. No SRS-A or histamine was detected in lungs of sensitised rats, horses or goats. SRS-A has never been detected in blood.

The exact chemical structure of SRS-A is unknown. Berry and Collier (1964) found that, in their hands, the properties of SRS-A were slightly different from those described by Brocklehurst and suggested that SRS-A contained more than one component. Berry and Collier (1964) found that SRS-A prepared by them differed from that of Brocklehurst particularly in that it had a constrictor effect on the isolated tracheal muscle of the guinea-pig. Marquis (1966) ascribed some of the constrictor effects described by these workers to the presence of cysteine which was used as an anti-oxidant in the perfusion fluid. Collier and James (1967) reported extensive investigations of the role of SRS-A in relation to other humoral factors in acute anaphylactic bronchoconstriction in the guinea-pig. They measured bronchoconstriction by the method of Konzett and Rössler (1940) as modified by Collier, Holgate, Schachter and Shorley (1960) and used specific and selective antagonists

to suppress the effects of individual substances. They found that acetylsalicylic acid (aspirin) and meclofenamate, which antagonise SRS-A, kinins and ATP, suppressed part of the response to intravenously administered antigen. By inducing tachyphylaxis separately to bradykinin and to SRS-A, by repeated administration of sub-lethal doses, they were able to show that both these substances participated. Meclofenamate, which is the sodium salt of N-(2,6-dichlorom-tolyl) anthranilic acid (Winder, Wax and Welford, 1965) resembles acetylsalicylate in its pharmacological properties but is more potent. Collier and James (1967) showed that the antihistamine, mepyramine and meclofenamate together suppressed most, but not all, of the anaphylactic response. They also proved that release of catecholamines during the reaction moderated the bronchoconstriction. Piper, Collier and Vane in 1967 demonstrated that histamine, bradykinin, SRS-A and anaphylaxis all liberated catecholamines, mainly adrenaline, into the circulation of the guinea-pig.

The main difficulty in studying SRS-A is, as was pointed out by Marquis (1966), the uncertainty as to the degree of purity of samples being investigated. Marquis (1966) described a method of obtaining SRS-A, from perfusate of guinea-pig lung, by ethanol extraction of freeze dried lung perfusate followed by adsorption on specially prepared activated alumina and elution by passage of a solvent containing decreasing concentrations of ethanol in water. He re-investigated the pharmacology of this alumina purified material, distinguishing it from SRS-A of other workers and relating differences in activity to differences in degrees of purity and methods of lung perfusion. Smith (1962) and Anderson, Goadby and Smith (1963) presented evidence for the release of sialic acids during anaphylaxis in guinea-pig lungs in vitro and concluded that SRS-A was a mixture of neuraminic acid glycosides. This possibility was re-investigated by Cirstea, Niculescu, Rusovici and Suhaciu (1967) who found

that the threshold concentrations of all of the sialic acid derivatives with gut stimulating activity were over 20 times higher than that of sialic acid in anaphylactic fluid contracting guinea pig ileum in the presence of mepyramine. Also SRS-A proved to be less resistant to increased temperature, low pH and storage than free sialic acids. This disproved the hypothesis that SRS-A was a mixture of neuraminic acid glycosides. These workers found that SRS-A was dialysable and considered that it was not truly bound to proteins as stated by Brocklehurst (1962).

iv) KININS

Collier and James (1967) concluded that kinins participated in anaphylactic bronchoconstriction, their antagonism contributing to the protective effect of meclofenamate in anaphylaxis. Kinins are polypeptides, consisting of 9 or 10 amino acids. They include bradykinin, kallidin and other related compounds, largely indistinguishable by their

pharmacological properties. Brocklehurst and Lahiri (1963) demonstrated kallikrein, an enzyme which acts on kininogens to form kinins, in the effluent from perfused, sensitised guinea-pig lung. No kinin was present but kininase which destroys kinins was found. Jonasson and Becker (1966) also demonstrated kallikrein release from sensitised guinea-pig lung following antigen perfusion. They suggested that this lung kallikrein was identical with plasma kallikrein and produced evidence suggesting that activation of the enzyme by the antigen-antibody reaction proceeded through the Hageman factor involved in blood clotting. Although it has been proved that kinins are very rapidly destroyed, particularly in the lungs (Ferreira and Vane, 1967; Vane, 1968), Brocklehurst and Lahiri in 1962 showed a rise in blood kinin content 2.5 minutes after intravenous challenge of sensitised guinea-pigs. Other workers showed a corresponding fall in the

kininogen level of plasma (Greeff, Scharnagel, Luhr and Strobach, 1966). The action of bradykinin in inducing bronchoconstriction in the guinea-pig was first described by Collier, Holgate, Schachter and Shorley (1959, 1960). Suppression of this bronchoconstrictor response to kinin, as to SRS-A, by aspirin and related antipyretic drugs, acting as local antagonists, was demonstrated by Collier, James and Piper (1965); Collier, James and Schneider (1966), Collier and James (1967).

v) ADENOSINE TRIPHOSPHATE (ATP)

Adenosine-5-triphosphate (ATP) was shown to induce bronchoconstriction in guinea-pig lungs (Collier, James and Schneider, 1966). Its potency was lower than that of histamine or bradykinin in this respect but it was antagonised, like bradykinin and SRS-A, by meclofenamate. Kitamura (1965 was unable to demonstrate release of ATP from lung during anaphylaxis in the guinea-pig and in 1967 Collier and James

accounted for the protective effect of meclofenamate in anaphylaxis as being entirely due to antagonism of SRS-A and bradykinin.

vi) PROSTAGLANDINS

The prostaglandins are a group of substances, most of which are ketones, with activity on smooth muscle. The first was detected in human semen but they have also been detected in most other tissues including lung (Editorial, 1968). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been found in the lung and $PGF_{2\alpha}$ was shown to be among those substances increasing air overflow volume in the Konzett-Rossler preparation of guinea-pig lungs (Berry and Collier, 1964). This prostaglandin contracted isolated human bronchial muscle (Sweatman and Collier, 1968). They distinguished it from SRS-A by producing tachyphylaxis to either substance independently. Prostaglandins E_1 and E_2 relaxed human bronchial muscle. Thus, it is possible that certain prostaglandins participate

in the bronchoconstrictor response of anaphylaxis either by intensifying the effect or, like the catecholamines, by modifying it.

vii) ACETYLCHOLINE

Acetylcholine contracts bronchial muscle (Dale, 1914) but Collier and James (1967) found that atropine failed to reduce anaphylactic bronchoconstriction and Brocklehurst (1958) detected no acetylcholine in the perfusate of the isolated lungs of sensitised guinea-pigs. Auer and Lewis (1910) and Alberty (1959) found that atropine reduced the bronchoconstrictor response to intravenous antigens, although cutting and degeneration of the vagi (Auer, 1910) did not reduce the severity of the response. Armitage, Herxheimer and Rosa (1952) found that atropine had a protective effect against inhaled antigen. Collier and James (1967) ascribed these discrepancies in the results of different workers to

differences in the sensitisation procedure, depth of anaesthesia and, in the case of his animals, pre-treatment with a β-adrenergic receptor antagonist. High doses of atropine may also be acting non-specifically.

Mills and Widdicombe (1970) found that vagotomy reduced both the reduction in total lung conductance and the reduction in lung compliance occurring during anaphylaxis in guinea-pigs. Animals used in their experiments were anaesthetised, treated with a muscle relaxant and artificially ventilated. Mills and Widdicombe also suggested that discrepancies in the findings of different workers in relation to the role of the vagus nerve in anaphylaxis might be related to the depth of anaesthesia and to the use of pithed animals (Collier and James, 1967; Collier, Holgate, Schachter and Shorley, 1960).

CORTICOSTEROIDS

Collier (1968), reviewing the role of humoral factors in bronchoconstriction, excluded corticoids as major participants on the grounds that injection of corticoids had little effect on bronchoconstriction and adrenalectomy was less effective than β-adrenergic blockade in intensifying the response.

SUMMARY

Collier (1968) demanded 3 types of evidence for the participation of a humoral factor in anaphylactic bronchoconstriction:-

1) It should appear or increase in a body fluid at the time and site of action and disappear or decrease as the reaction wanes.

2) The factor should on injection ellicit the reaction it is claimed to be responsible for, at blood levels achieved during the natural reaction.

3) Its specific antagonists should wholly or partly inhibit the natural reaction.

On these grounds histamine, SRS-A and kinins are mediators and catecholamines modifiers of the reaction in the guinea pig. However, Collier (1968) pointed out that even after antagonism of the 3 major mediators there was a residual degree of bronchoconstriction which was intensified by adrenergic blockade. Even in the guinea-pig, which has been extensively studied, the mediation of anaphylaxis is not yet fully understood.

2) ANAPHYLAXIS IN THE DOG

INDUCTION

The phenomenon of anaphylaxis was first observed in the dog. Portier and Richet in 1902, while investigating the toxicity of extracts of sea anemones in dogs, found that where animals tolerated an initial small dose of extract, showing no ill-effects, a second administration of the same dose 3 to 4 weeks later, resulted in a severe reaction, fatal within one to 2 hours. The dogs collapsed showing symptoms associated with the alimentary tract, namely vomiting, blood and diarrhoea. Arthus in 1909 showed that this phenomenon could be developed to non-toxic proteins, such as egg albumin, to which antibodies were formed.

DESCRIPTION

Biedl and Kraus in 1910 detected marked hypotension during anaphylaxis in the dog. Manwaring (1910) experimentally

excluded various abdominal organs from the circulation by means of ligatures and examined the subsequent effect on anaphylaxis. Total exclusion of the abdominal organs prevented shock but exclusion of stomach, intestines, kidneys, adrenals and spleen was ineffective. The reaction was later shown to be prevented by exclusion of the liver from the circulation (Voegtlin and Bernheim, 1911). Weil (1917) observed that, after anaphylaxis in the dog, the liver was congested and suggested that stagnation of blood in this organ could account for the severe fall in systemic blood pressure. Manwaring, Hosepian, O'Neil and Moy (1925) provided more conclusive evidence of the importance of the liver in anaphylaxis in the dog by transfusing the liver of a sensitised dog from the circulation of another normal unsensitised dog or an eviscerated dog. They showed that injection of antigen produced a fall in systemic blood pressure and a rise in intracystic pressure. The occurrence

of leucopenia and thrombocytopenia in anaphylaxis was recorded by Dean and Webb in 1924. Webb (1924) showed this to be associated with accumulation of leucocytes, particularly polymorphonuclear neutrophil leucocytes, in the capillaries of the lungs.

MEDIATORS

i) HISTAMINE

Manwaring et.al. (1925) found that injection of antigen resulted in release, from the liver of a sensitised dog, of a chemical substance with hypotensive and smooth muscle stimulating properties. Such a substance was detected in thoracic duct lymph of a dog showing anaphylaxis by Dragstedt and Gebauer-Fuelnegg in 1932 and in 1936 Dragstedt and Mead recovered the same substance from the circulation immediately after injection of antigen. Dragstedt et.al. (1932, 1936) deduced from its chemical and pharmacological

properties that this substance was histamine. Code in 1939 confirmed their findings and Ojers, Holmes and Dragstedt (1941) showed that the histamine content of the liver was reduced after anaphylaxis by as much as 90%.

ii) HEPARIN

Arthus (1909) and Biedl and Kraus (1910) observed that during anaphylaxis in the dog the blood clotting time was increased. Jaques and Waters in 1941 demonstrated that this was associated with heparin release into the circulation when they isolated heparin from the blood of dogs during anaphylaxis. They found that they were unable to isolate heparin from the blood of unsensitised control dogs or of sensitised dogs challenged after removal of the liver. They deduced that heparin was liberated with histamine from the liver of the dog during anaphylaxis.

MAST CELLS. In 1946 heparin was recognised by Jorpes as a constituent of mast cell granules and in 1953 Riley and West demonstrated the association between the histamine content of tissues and the density of their population of mast cells. Akcasu and West (1960) carried out investigations into changes occurring in mast cells during anaphylaxis in the dog and compared the histamine and 5-hydroxytryptamine contents of different tissues of the dog with their mast cell content. They found that liver contained many mast cells and large amounts of 5-hydroxytryptamine as well as histamine. Sanyal and West (1958b), however, had found that, while histamine was released from both the liver and the spleen as a result of anaphylaxis in the dog, they could detect no loss of 5-hydroxytryptamine from either the liver or the spleen. Akcasu and West (1960) observed extensive mast cell damage in the liver after induction of anaphylaxis. Unlike histamine

release and mast cell damage observed after administration of histamine liberators such as compound 48/80, the histamine release and mast cell damage observed after antigen administration appeared to be confined largely to the liver. They explained this selective effect as being due to the presence of antibody in liver mast cells but not in mast cells at other sites. They found that dogs sensitised to two antigens were simultaneously desensitised to both by a challenge dose of one of them.

iii) KININS

In 1950 Beraldo reported finding a smooth-muscle-stimulating substance other than histamine in the blood collected from dogs for several minutes after anaphylactic shock. This substance, which was active on guinea-pig ileum in the presence of an antihistamine, was also found in the blood after shock produced by administration of peptone.

It was believed to be bradykinin as it was a polypeptide inactivated by incubation with trypsin. Beraldo's results showed no correlation between the severity of the reaction exhibited by the animal and the amount of bradykinin present, nor was the amount of bradykinin related to the amount of histamine detected. The technical difficulties involved in investigating bradykinin formation in certain situations were stressed by Lewis (1962). It is very rapidly destroyed by kininase (Erdos and Sloane, 1962) and also liable to be formed inadvertently during the handling of samples by contact with glass (Armstrong, Jepson, Keele and Stewart, 1957).

SUMMARY

In the dog, anaphylaxis is believed to involve an antigen-antibody reaction leading to release of histamine and heparin from the liver and formation of bradykinin. The histamine constricts the hepatic vein with consequent stagnation of blood in the liver. This, together with the effects of

histamine and bradykinin on vascular permeability and smooth muscle, leads to systemic hypotension and alimentary symptoms.

3) ANAPHYLAXIS IN THE RABBIT

INDUCTION

The rabbit was found to be less readily sensitised to foreign proteins than the guinea-pig or the dog (Grove, 1932). Sanyal and West (1958b) sensitised rabbits to horse serum by daily intraperitoneal injections of 1 ml. for 6 days while in the same experiment guinea-pigs were sensitised by a single intraperitoneal injection of 0.5 ml. and dogs by 2 injections, one subcutaneous of 2 ml. and one intravenous, 2 days later, of 3 ml.

DESCRIPTION

Systemic hypotension was detected as an outstanding feature of anaphylaxis in the rabbit as in the dog (Arthus, 1909). Increased pulmonary arterial pressure was shown to be a primary effect (Airilia, 1914; Drinker and Bronfenbrenner, 1924). By means of arterio-radiographs generalised constriction

of the arterial system was demonstrated (Pasteur Vallery-Radot, 1949). Constriction of the pulmonary arterial branches led to dilation with blood of the right side of the heart.

MEDIATORS

i) HISTAMINE

Rabbit blood platelets were shown to be rich in histamine (Minard, 1937; Humphrey and Jaques, 1954). Leucocytes also contained histamine (Rose and Weil, 1939; Rose, 1941; Humphrey and Jaques, 1954). Antigen, in vitro, was shown to cause transfer of histamine from cells to plasma in the blood of sensitised rabbits (Katz, 1940). Dragstedt, Arellano and Lawton, in 1940, and Rose and Weil, in 1939, found that the histamine content of the blood was lowered following antibody-antigen reactions.

ii) 5-HYDROXYTRYPTAMINE

Humphrey and Jaques (1955) showed that antigen released 5-hydroxytryptamine together with histamine from blood platelets of sensitised rabbits in vitro. Evidence that release of 5-hydroxytryptamine from platelets was responsible for the rise in plasma 5-hydroxytryptamine during anaphylaxis in vivo was provided by Waalkes, Weissbach, Bozicevich and Udenfriend in 1957. They showed that this rise was prevented by pretreating rabbits with reserpine which lowered the 5-hydroxytryptamine content of platelets. An increase in urinary 5-hydroxytryptamine metabolites during anaphylaxis was shown to occur by Fischer and Lecomte (1956). Waalkes and Coburn (1959a) described trapping of platelets in the pulmonary blood vessels with a corresponding fall in the 5-hydroxytryptamine content of whole blood and a rise in the lung content of 5-hydroxy-tryptamine. Heparin administration was found to inhibit

these effects. Sanyal and West (1958b) removed similar pieces of lung from anaesthetised animals before and 20 to 30 minutes after challenge and showed an increase in lung content of histamine and 5-hydroxytryptamine after anaphylaxis. Waalkes and Coburn (1959b) reproduced the change in distribution of 5-hydroxytryptamine between whole blood and lungs, with trapping of platelets in the lungs, by intravenous injection of glycogen.

Cohen and Sapp (1960) found, however, that depletion of the 5-hydroxytryptamine content of rabbit tissues by reserpine administration did not prevent subsequent development of anaphylaxis. Lecomte and Fischer (1957; 1958) found that 5-hydroxytryptamine antagonists did not reduce the severity of anaphylaxis in the rabbit. These findings suggested that the role of 5-hydroxytryptamine was less important than that of histamine in this species.

MAST CELLS. Although blood clotting time was increased approximately 10 fold in anaphylaxis, Adams (1953) found that this could not be attributed to heparin release as the presence of protamine sulphate, which inactivates heparin, did not affect the clotting time of blood from shocked rabbits. Tissue mast cells in the rabbit are few but the basophils or blood mast cells, are numerous and have been shown to contain histamine. Injection of compound 48/80 decreased the number of circulating basophils (Boseila, 1958). There is, however, no evidence for their participation in anaphylaxis (Graham, Lenz, Lowry, Parish and Wheelwright, 1955). The blood platelets were concluded to be the main source of histamine (Code, 1952) and these contain no heparin.

SUMMARY

Schachter (1953) demonstrated release of histamine during anaphylaxis from perfused rabbit skin, liver and, to a lesser extent, small intestine but generally it is accepted that, in

the rabbit, anaphylaxis is mediated mainly by histamine released from blood platelets. This histamine constricts the pulmonary arterial system resulting in death due to right-sided heart failure.

4) ANAPHYLAXIS IN THE RAT

INDUCTION

Hochwald and Rackemann in 1946 clarified many of the factors involved in the induction of anaphylaxis in the rat. Earlier work (Longcope, 1922) suggested that the rat was particularly insusceptible to anaphylactic shock. Longcope showed that the titre of precipitating antibodies in the rat reached a peak 10 days after administration of the last sensitising dose of antigen, but he allowed an interval of at least 3 weeks between the last sensitising dose and the challenge dose and failed to induce anaphylactic shock. Parker and Parker (1924) succeeded using an interval of 10 days between sensitisation and challenge. Hochwald and Rackemann (1946) confirmed that the rat was susceptible to anaphylaxis only between the tenth and fifteenth day after sensitisation. They also showed that the number of sensitising injections was more important than the total quantity of protein injected.

Schultz-Dale reactions as described in guinea-pig tissues (p. 17) were demonstrated in sensitised rat tissues by Parker and Parker (1924). Kellaway (1930) found that responses were obtained more regularly from isolated tissues passively sensitised by incubation with heterologous antibody. This might be expected, as tissues obtained from actively sensitised rats might vary in degree of sensitivity, depending on the stage at which the animal was killed and the course of sensitising injections which it had received.

DESCRIPTION

Parker and Parker (1924) described dyspnoea, abdominal cramps, hypothermia and collapse as the symptoms of anaphylactic shock in the rat. At necropsy the small intestine showed most marked changes, namely oedema and haemorrhage of the submucosa with desquamation of the mucous membrane.

ADJUVANTS

FREUND'S ADJUVANT. As the symptoms of anaphylaxis were generally found to be mild, (Parker and Parker, 1924; Hochwald and Rackemann, 1946) attempts were made to produce more severe reactions. By using adjuvants, Lipton, Stone and Freund (1956) produced lethal anaphylactic shock in rats. They sensitised animals with bovine serum albumin (BSA) combined with adjuvants and injected intracutaneously at several sites. They then challenged the rats by intravenous injection of BSA. "Complete" adjuvant contained killed mycobacterium tuberculosis, Bayol F and the emulsifying agent, Arlacel A, in a water-in-oil emulsion (Freund, Thomson, Hough, Sommer and Pisani, 1948). "Incomplete" adjuvant differed in that it did not contain M. tuberculosis. Lipton et.al. (1956) found the incidence of fatal reactions to be higher where complete adjuvant was used. These workers also elicited skin reactions in rats sensitised using paraffin

oil emulsions and found that the reactions in animals receiving tubercle bacilli in the emulsion were more extensive and of longer duration than in rats sensitised without mycobacteria. In the absence of adjuvants Longcope (1922) and Opie (1924) failed to demonstrate skin sensitisation in the rat by repeated injections of horse serum. Lipton et.al. (1956) attributed their high incidence of fatal systemic anaphylactic reactions and intense skin reactions to the high levels of precipitating antibody achieved by combining antigens with paraffin oil and killed mycobacteria. These workers also demonstrated the importance of not exceeding the optimal antigen concentration in the proceedure of skin testing sensitised animals. Ovary (1952) failed to elicit passive cutaneous reactions in rats using rat antisera, but Lipton et.al. (1956) referred to unpublished experiments where passive cutaneous anaphylaxis was demonstrated using rat serum with a sufficiently high titre of precipitating antibodies.

HAEMOPHILUS (BORDETELLA) PERTUSSIS VACCINE. Malkiel and Hargis (1952b) produced fatal anaphylaxis in rats sensitised with a mixture of horse serum and Haemophilus pertussis vaccine and challenged with horse serum. Haemophilus pertussis vaccine was believed to act by temporarily increasing susceptibility to histamine. Sanyal and West (1958a) confirmed that anaphylaxis in the rat was aggravated by pretreatment with this vaccine. The vaccine increased the rat's sensitivity to histamine and 5-hydroxytryptamine. Injection of H.pertussis vaccine alone gave no reactions on subsequent administration of either the vaccine or foreign protein. Tissue content, urinary excretion and release, during anaphylaxis, of histamine and 5-hydroxy-tryptamine were unaltered by H.pertussis vaccine and it did not increase precipitin formation.

Where they used egg white as antigen Sanyal and West (1958a) took into account the anaphylactoid reaction produced and manifested in normal rats as peripheral oedema. As this

oedema was prevented by hypotension occurring during anaphylaxis, they accepted the presence of oedema as a negative sign and the absence of peripheral oedema as a positive sign of anaphylaxis when using egg white as antigen.

HYPOGLYCAEMIA

Hypophysectomy (Molomut, 1939), adrenalectomy (Weiser, Golub and Hamre, 1941) and injection of insulin (Sanyal, Spencer and West, 1959), immediately before the challenge dose of antigen, increased the susceptibility of rats and mice to anaphylactic shock. Sanyal in 1960 showed that all these procedures as well as the injection of H.pertussis vaccine at the time of sensitisation, resulted in hypoglycaemia. In 1967 Dhar, Sanyal and West investigated the relationship of blood sugar level to the severity of anaphylaxis. Animals sensitised by injection of horse serum and H.pertussis vaccine intraperitoneally showed hypoglycaemia 12 days later, at which time they were challenged. Injection of insulin 30 minutes

before challenge produced hypoglycaemia and it was found that the severity of anaphylaxis was increased. Hyperglycaemia, produced by injection of alloxan or glucose, resulted in reduced severity of anaphylaxis and death, where it occurred, was delayed.

Dhar et.al. (1967) found that anaphylaxis in mice was affected by changes in glucose levels in the blood in the same way as was anaphylaxis in rats. In guinea-pigs hypoglycaemia aggravated anaphylaxis but it was not modified by hyperglycaemia. The converse was true in the rabbit where hyperglycaemia conferred protection but hypoglycaemia did not increase the severity of anaphylaxis.

MEDIATORS

i) HISTAMINE AND 5-HYDROXYTRYPTAMINE

The findings of Sanyal and West (1958a) relating the increased susceptibility to anaphylaxis of H.pertussis treated

rats, to increased susceptibility to histamine and 5-hydroxytryptamine, suggested that these amines might be important mediators in the rat. In 1955 Benditt, Wong, Arase and Roeper showed that rat peritoneal mast cells contained both histamine and 5-hydroxytryptamine. They liberated the amines into the suspending fluid by alternately freezing and thawing the cells. Mota (1957, 1958) investigated the role of the mast cell and histamine in anaphylaxis in the rat. He described mast cell degranulation and a rise in plasma histamine levels during anaphylaxis, and later reported (1958), in contrast to the findings of Sanyal and West (1958a) that these effects were increased when H.pertussis vaccine was used in sensitisation. Mota (1957) differed from Sanyal and West (1958b) also in his findings that rats were protected against anaphylactic shock by antihistamine pretreatment. He found that when rats were given 8 daily intraperitoneal injections of

increasing doses of compound 48/80 and challenged 24 hours after the last dose, both anaphylactic shock and the increase in plasma histamine were prevented. He deduced that nearly all the histamine liberated in anaphylaxis came from mast cells.

Sanyal and West (1958b) found that rats whose skin and intestine had been depleted of histamine and 5-hydroxytryptamine showed severe, unmodified anaphylaxis. They criticised Mota's results on the grounds that the degranulation he described was frequently seen in tissue spreads from control rats and that he did not report plasma histamine levels after administration of antigen to control rats. Also the method of sensitisation which he used, namely, 3 daily injections of alum-precipitated horse serum, produced only mild reactions, making assessment of protection questionable. They suggested that, as histamine and 5-hydroxytryptamine could not be held predominantly responsible for anaphylaxis in the rat, H.pertussis might also increase the sensitivity of rats to other mediators.

In 1966 Chayen, Darracott and Kirby postulated that mast cells might detoxicate rather than eject histamine. Pieces of lung from rats were maintained for 24 hours in a synthetic, non-proliferative culture system, as described by Trowell (1959). Some pieces were then removed and chilled at - 70°C in hexane and the others were maintained in a similar culture solution, which contained histamine. Pieces were then removed from this solution at intervals of 5, 15 and 30 minutes and sections cut and stained. It was found that culturing for 24 hours increased the number of mast cells. Numbers were more markedly increased by exposure to histamine for 5 or 15 minutes. Exposure to histamine in culture for 30 minutes resulted in a decline in the number of mast cells. Chayen et. al. postulated that histamine was taken up by mast cells, but that on prolonged exposure the granules were disrupted. These findings were obtained in vitro in conditions rather remote from those existing in vivo.

ii) KININS

Brocklehurst and Lahiri (1962) found that, as in the rabbit and guinea-pig, plasma levels of bradykinin were raised after anaphylaxis in the rat. Dawson and West (1965) found that the times when sensitised rats were most sensitive to antigen coincided with the times when they showed increased susceptibility to administered bradykinin. Dawson, Starr and West (1966) found that anaphylaxis and bradykinin administration resulted in similar types of damage to the right ventricle of the heart. When the antigen used was horse serum, cardiac lesions were more severe than when it was egg albumin. The latter resulted in more marked congestion and haemorrhage in the lungs and small intestine. Dawson, et.al. found that the levels of bradykinin, bradykininogen and kinin-forming enzymes reached a peak in plasma 10 days after sensitisation. Levels were measured in control rats and in sensitised rats 5 minutes after challenge.

They found that administration of ascorbic acid and mepyramine protected rats against anaphylactic shock at 10 days but not at 20 days after sensitisation. This protective effect was accompanied by reduced levels of bradykinin and bradykininogen after challenge. Ascorbic acid alone conferred a less marked protective effect. Dawson et.al. proposed that there were two phases in anaphylaxis in the rat, namely, that occurring 10 days after sensitisation, in which bradykinin was a mediator, and a later phase not involving bradykinin. As the mixture of mepyramine and ascorbic acid did not reduce the toxicity of bradykinin in sensitised animals, the protection appeared to be due to reduced activity of the kinin-forming enzyme.

Csaba and West (1968) found that, whereas antagonists of histamine and 5-hydroxytryptamine conferred little or no protection against anaphylaxis, trasylol (an inhibitor of kinin formation) and a bradykinin antagonist, reduced the

mortality rate in anaphylaxis in the rat. The bradykinin antagonist used by Csaba and West was Rheopyrin, a mixture of amidopyrine and phenylbutazone, and they found its protective effect to be enhanced by simultaneous administration of an antagonist of either histamine or 5-hydroxytryptamine. Csaba and West used H.pertussis vaccine in sensitising their rats to ovalbumin. Three daily sensitising injections were given and the animals challenged 12 days after their first injection. They deduced that kinins played an important part in anaphylaxis in the rat but that histamine and 5-hydroxytryptamine might also be involved.

SRS-A

Rapp, in 1961, first demonstrated SRS-A in the peritoneal cavity of rats passively sensitised by intraperitoneal injection of hyperimmune rabbit antiserum and challenged 4 hours later by the same route. This SRS-A was indistinguishable from that recovered from perfused guinea-pig

lungs. Mota, in 1962, showed that rat mast cells were not altered by exposure to rabbit antiserum followed by antigen. Orange, Valentine and Austen (1967) found that SRS-A formation in vivo in the rat, under the circumstances described by Rapp, was inhibited if the rats were previously depleted of polymorphonuclear leucocytes. Formation was not affected by depletion of mast cells or of thymic lymphocytes. Removal of complement from the rats reduced SRS-A production. These findings, indicating the importance of the polymorphonuclear leucocyte, were later confirmed by the same workers (Orange, Valentine and Austen, 1968a) using homologous rat antiserum in place of heterologous rabbit antiserum.

ANTIBODIES (IMMUNOGLOBULINS)

Serum proteins have been separated by electrophoresis into albumin and alpha (α), beta (β) and gamma (γ) globulins in order of decreasing electrophoretic mobility. Antibody

activity has been shown to reside in the gamma fraction. These gamma globulins were further subdivided, on the basis of differences in antigenic determinant sites, into immunoglobulin (Ig), G,M,A and E in the human species. Bellanti (1968) reviewed the structural and functional properties of immunoglobulins and their classification. The immunoglobulins of class G (IgG) have been shown to include most of the antiviral and antibacterial antibody and those of class E (IgE) are believed to include homocytotropic, mast cell-sensitising, reaginic or skin-sensitising antibodies in allergic humans (Ishizaka, Ishizaka and Hornbrook, 1966a, b).

In the rat, Stechschulte, Austen and Bloch (1967) showed that the peritoneal cavity could be prepared for antigen-induced release of different chemical mediators of anaphylaxis by two different homologous immunoglobulins. One of these had been found to sensitise

rat mast cells in vivo and in vitro (Becker and Austen, 1966; Mota, 1963) for the release of histamine and 5-hydroxytryptamine. This was termed the "mast cell-sensitising" (Mota, 1964) or homocytotropic (Becker and Austen, 1966) antibody. The second antibody (Stechschulte et.al., 1967) was associated with the IgG fraction of rat antisera and prepared the rat for selective release of SRS-A. Orange, Valentine and Austen (1968a) showed that mast cells were necessary for histamine release while polymorphonuclear leucocytes were necessary for release of SRS-A by antigen in the peritoneal cavity of rats treated with the appropriate hyperimmune antisera. These workers also showed that such release of SRS-A, but not of histamine, was inhibited by diethylcarbamazine. Orange, Valentine and Austen (1968b) showed that diethylcarbamazine did not interfere with the antibody-antigen reaction in vitro or with the bioassay of SRS-A. Another compound, disodium cromoglycate, (sodium

salt of 1, 3-bis-(2-carboxychromon-5-yloxyl)-2 hydroxypropane) was reported by Cox in 1967 to inhibit the anaphylactic process initiated by reaginic antibody-antigen interactions. It suppressed the release of histamine and 5-hydroxytryptamine initiated by such interactions. Orange and Austen (1968) demonstrated that release of histamine and SRS-A in the rat could be selectively blocked by these drugs.

Morse, Bloch and Austen, in 1968, showed that the antibodies of the IgG fraction responsible for SRS-A release appeared in the serum of actively sensitised rats one week after injection of antigen in complete Freund's adjuvant. They reached a peak concentration at 2 to 3 weeks and had declined by 10 weeks after sensitisation. They were found to be stable to heating for 4 hours at 56^{o}C and were identified as belonging to a subclass IgGa of IgG. The homocytotropic antibodies, responsible for histamine release, were heat-labile. However, in 1969, Morse, Austen and Bloch

showed that intraperitoneal release of histamine by antigen could also be mediated by heat-stable IgGa antibodies. The heat-labile and heat-stable antibodies responsible for histamine release were distinguishable by chromatography and by their different latent periods for passive sensitisation. The optimal latent period for the heat-labile homocytotropic antibody was 48 hours, whereas it was 4 hours for the heat-stable IgG antibody. This difference was demonstrated by their ability to sensitise skin for passive cutaneous anaphylaxis (PCA) after different latent periods. Morse et.al (1969) also showed that intraperitoneal release of histamine, but not of SRS-A, by IgGa, was suppressed by disodium cromoglycate. The fact that disodium cromoglycate suppressed histamine release associated with either homocytotropic or IgGa antibodies supported the view that this agent acted subsequent to antibody-antigen interaction, although it did not affect the pharmacological actions of

histamine. Morse et.al. (1969) in drawing conclusions from their findings, recognised that separate heat-stable antibodies with the characteristics of IgGa might be responsible for release of histamine and SRS-A respectively. Rats which received sensitising injections which included complete Freund's adjuvant produced only heat-stable antibodies. Those which did not receive Freund's adjuvant, but did receive killed Bordetella (Haemophillus) pertussis organisms with their sensitising injections, produced both heat-stable and heat-labile antibodies.

SUMMARY

Rats have been used by many workers to investigate the immunological aspects of anaphylaxis. It has been clearly shown in the rat that the type of antibody produced can be determined by the method of sensitisation and, particularly, by the nature of the adjuvants used.

It has also been shown that the chemical mediators of subsequent anaphylactic reactions can vary depending on the nature of the antibody involved. The complexity of the situation in this species was revealed as a result of the failure of early attempts to sensitise rats and induce anaphylaxis, as in other species, by simple injections of foreign proteins. Histamine, 5-hydroxytryptamine, kinins and SRS-A have all been shown to be involved as mediators under certain circumstances. It is doubtful that any of the findings obtained in the rat could be applied directly to other species but it is likely that in all species adjuvants may influence the antibodies and mediators involved in hypersensitity reactions.

5) ANAPHYLAXIS IN THE MOUSE

INDUCTION AND DESCRIPTION

Weiser, Golub and Hamre in 1941 described the symptoms of anaphylaxis in mice as depression, lacrimation, partial paralysis of the limbs, cyanosis, convulsions, prostration and death within 30 minutes of challenge. Malkiel and Hargis (1952a) showed that administration of Haemophillus pertussis vaccine at the same time as the sensitising injection of antigen increased the susceptibility of mice to anaphylaxis. This vaccine was shown by Kallos and Kallos-Deffner (1957) and Kind (1957) to increase the sensitivity of mice to both histamine and 5-hydroxytryptamine. Fink and Quinn (1953) found genetic variation in the ability of mice to produce demonstrable circulating antibody. Antibody titres were measured 10 and 12 days after sensitisation. Mice aged 4 to 5 months produced more antibody

than did those aged 2 months. Fink and Quinn (1953) also found that mice produced antibody more readily to egg white than to pneumococcus polysaccharide. The route of administration of antigen had an effect in that the antibody response to intra-abdominally administered egg white was greater than when it was administered intra-muscularly. Unlike the findings of Hochwald and Rackemann (1946) in rats, division of the total dose into 5 doses, injected on alternate days did not alter the response in mice. Anaphylaxis in mice was shown to be accompanied by haemoconcentration and changes in the electrolyte content of the tissues (Fox, Nelson and Freeman, 1951; Nelson, Fox and Freeman, 1952). These workers wished to determine whether electrolyte changes observed in shock due to burns or trauma also occurred in anaphylactic shock. Kind (1955) found hypothermia to be a readily measureable feature. Body temperature fell gradually until death and the fall

was greatest in mice in which death was delayed. Haemoconcentration was shown by Fulton, Harris and Craft (1957) to occur regularly in anaphylaxis. Harris and Fulton (1958) found that the increase in haematocrit was a sigmoidal function of the log dose of bovine albumin in the challenge antigen preparation. The relationship was linear over a given range and provided a method for drawing quantitative comparisons in studies of mouse anaphylaxis.

MEDIATORS

HISTAMINE AND 5-HYDROXYTRYPTAMINE

Fox, Einbinder and Nelson (1958) investigated the effect on the incidence of fatal anaphylaxis in mice of pretreatment with a large number of different drugs. Lysergic acid diethylamide (LSD), reserpine, phenothiazine tranquillisers and glucocorticoids reduced the rate of

mortality. Antihistamines, sodium salicylate, phenylbutazone, mineralocorticoids and adrenaline were without effect. This suggested that 5-hydroxytryptamine might be an important mediator of anaphylaxis in the mouse and supported earlier findings.

Gershon and Ross, in 1962, showed that administration of reserpine for 2 days preceding challenge protected mice against anaphylaxis. Release of 5-hydroxytryptamine by reserpine was associated with depletion of the entero-chromaffin cells of the duodenum. Similar depletion was produced by anaphylaxis or administration of 5-hydroxy-tryptamine itself. Fink (1956) found that LSD and reserpine inhibited the Schultz-Dale reaction of uterine muscle and that this tissue was more sensitive to 5-hydroxytryptamine than to histamine. Fink and Rothlauf (1955) found uterine mast cells to be unaltered by in vitro anaphylaxis. Einbinder,

Nelson and Fox (1954) showed the protective effect of glucocorticoids to be related to their effects on electrolyte levels in tissues of mice.

ANTIBODIES

Mota and Peixoto (1966) detected thermolabile antibody in the sera of actively sensitised mice. This antibody sensitised mouse skin for PCA reactions. Mast cell disruption was not observed after such reactions. However, in 1967, Prouvost-Danon, Peixoto and Queiroz Javierre concluded from their experiments that homologous thermolabile, reagin-like antibody passively sensitised mouse peritoneal mast cells for histamine release by antigen. These workers believed that active and passive sensitisation of mouse peritoneal cells involved different antibodies. Active sensitisation resulted in thermostable precipitating antibody which did not fix to mast cells (Prouvost-Danon, Queiroz Javierre and

Silva Lima, 1966). Passive reactions could be induced by antibody which did fix to mast cells (Prouvost-Danon, Silva Lima and Queiroz Javierre, 1966). In the mouse, as in the rat, mast cells are believed to contain 5-hydroxytryptamine as well as histamine (Parrat and West, 1957).

SUMMARY

In the mouse, as in the rat, there is evidence for the involvement of more than one type of antibody in the development of hypersensitivity. 5-Hydroxytryptamine appears to be an important mediator of anaphylaxis. Mast cells have not been shown to be involved.

6) ANAPHYLAXIS IN THE CAT

INDUCTION

In contrast to the large amount of literature on anaphylaxis in rodents and in dogs there is little published information on anaphylaxis in the cat. Wilson and Miles (1964) in Topley and Wilson's textbook "Principles of Bacteriology and Immunity" stated that the cat was peculiarly insusceptible to anaphylaxis. Akcasu, in 1963, carried out experiments to determine why cats could not be sensitised to foreign proteins. Following intravenous infusion of 100 ml. of foreign serum he detected an increase in the γ globulin content of the recipient's serum. He believed this to be due to transformation, in cat tissue, of heterologous albumin. Following injection of egg albumin, or cow's milk, albumin was found to be excreted so rapidly by the kidney that antibodies were not detected and sensit-

ivity was not achieved. The ability of cats to transform foreign protein and excrete egg albumin was not shared by guinea-pigs, dogs or rabbits. Akcasu deduced that cats were not readily sensitised because of their ability either to change the antigenic specificity of protein or to excrete it before antibody formation was stimulated.

McCusker and Aitken, however, in 1966, accidentally induced anaphylaxis in 2 cats, which they were using to investigate the serological and dermal reactions of the cat to parenterally administered foreign protein. One cat received 4 subcutaneous injections of bovine serum at intervals of several days, followed by an intravenous injection 19 days later. This injection resulted within one minute in scratching, dyspnoea, salivation, vomiting, incoordination and collapse. Recovery began after 15 minutes and was complete after 12 hours. The second cat

was injected at several sites, subcutaneously, intradermally and intramuscularly with bovine gamma globulin (BGG) emulsified in complete Freund's adjuvant. These injections were repeated on 4 occasions at intervals of several days. After 7 weeks labelled gamma globulin was given intravenously to investigate antigen elimination. This produced no reaction. The cat was not used again for approximately 9 months when the injections of BGG in Freund's adjuvant were repeated to stimulate precipitating antibody production. Four months later an intravenous injection of BGG resulted in collapse, dyspnoea with blood-stained frothy fluid exuding from the nostrils and mouth and death within 3 minutes. Post mortem examination revealed enlarged lymph glands, pulmonary emphysema, oedema and haemorrhage and congestion of the liver. Leucopenia with accumulation of leucocytes in the pulmonary arteries and arterioles was described. McCusker and Aitken judged the bronchioles to be

less patent than normal. Intravenous injection of BGG had no adverse effects on 7 control cats tested.

In 1969 Aitken and McCusker reported the occurrence of anaphylactic reactions in a further 9 cats. These were 9 out of a total of 15 cats which were being used to investigate antibody production. The protein antigen, emulsified with Freund's adjuvant was injected into the footpad, subcutaneously or intramuscularly. Intravenous challenge was carried out in 8 cats after 17 days. Two of these developed symptoms of anaphylaxis. The remaining 7 cats were challenged after 25 and 27 days and all showed anaphylaxis. No conclusions could be drawn as to the importance of the time interval between sensitising and challenge injections, however, as these 7 cats had received larger sensitising and challenge doses of protein than had those challenged after 17 days. Also, the same antigen was not used in every animal. The cats developed precipitating

antibodies to egg albumin, bovine fibrinogen and pig gamma globulin but not to bovine serum albumin (BSA). Anaphylaxis was not produced in the cats which received BSA and Aitken and McCusker suggested that cats might have a tolerance to this protein as a result of early dietary exposure to milk and meat.

Unlike the 2 cases previously encountered (Aitken and McCusker, 1966), the symptoms of anaphylaxis in cats described by Aitken and McCusker in 1969 were skin irritation and hyperactivity of the alimentary tract. Symptoms began within seconds of the intravenous injections and lasted for periods ranging from 10 minutes to 24 hours. Two cats died and post mortem examination revealed intense congestion of the small intestine with no macroscopic pulmonary lesions. There was considerable variation in the severity of the symptoms but none of thecats showed the respiratory distress described previously (McCusker and Aitken, 1966). This difference was

attributed to the difference in the degree of sensitisation in the two situations. The cats described in 1966 received a larger number of sensitising injections and longer time intervals elapsed before challenge. The possibility of individual variation in the organs affected was suggested.

Brodie (1900-01) described respiratory and cardiac arrest and vasodilation produced in cats by intravenous injection of serum from any source. The effects were abolished after vagotomy and were attributed to excitation of the pulmonary branches of the vagus. Gilding and Nutt (1943-44) also investigated serum toxicity in cats. They observed that another feature of the reaction was leucopenia, that storage of serum was associated with toxicity and that atropine or vagotomy abolished the reaction. The "Brodie phenomenon" was unlikely to be involved in the reactions observed by Aitken and McCusker as they used freshly prepared solutions

of antigen and as they never observed reactions in unsensitised cats.

Aitken and McCusker did not attempt to detect possible mediators in the affected cats but Austen and Humphrey (1963) recorded cat lung to be 200 times more sensitive to 5-hydroxytryptamine than to histamine. Although cat pulmonary tissue was described by Riley and West (1953) as being rich in mast cells, such cells were found to be totally absent in the lung of a cat dying during anaphylaxis (McCusker and Aitken, 1966).

SUMMARY

The experiments carried out by Aitken and McCusker were not devised to investigate systemic anaphylaxis in the cat. Their findings were incidental to their investigation of allergic skin reactions in cats, however, they did show that systemic anaphylaxis could occur in the cat and that the nature of the reaction could vary with different sensitisation procedures.

7) ANAPHYLAXIS IN THE PIG

SENSITISATION

Thomlinson and Buxton (1963) produced active anaphylaxis with egg albumin, and reversed passive anaphylaxis with Escherichia coli (E. coli) in pigs in order to compare the symptoms and lesions produced to those of gastro-enteritis and oedema disease associated with E. coli infection. To sensitise pigs to egg albumin Thomlinson and Buxton used the same dose per unit body weight as used in preliminary experiments in guinea-pigs (Thomlinson and Buxton, 1962). A dose of approximately 100 mg/Kg subcutaneously or 50 mg/Kg intravenously was given to pigs weighing about 20 Kg. The animals were challenged intravenously with a dose of 50 mg/Kg or 25 mg/Kg after intervals of 6 to 12 days. Thirty minutes after intravenous challenge they repeated the dose subcutaneously with the aim of prolonging the reaction.

All 7 pigs sensitised in this way developed symptoms on challenge. The onset in 4 cases was one minute after injection, in one 5 minutes and, in 2, slowly over one hour. Circling, incoordination, staggering, coughing, congestion of skin and conjunctivae, convulsions, collapse and respiratory distress, with periods of apnoea were described. Defaecation, straining and vomiting followed with muscular tremors developing after 20 to 40 minutes. Swelling of the eyelids with cyanosis and prolonged respiratory distress were described. The duration of reactions ranged from 2 to 6 hours. In less severely affected animals vomiting did not occur and respiratory symptoms were less severe. One animal was given a further dose of egg albumin after 2 hours when it had recovered from the challenge doses. This caused symptoms to recur and continue for a further 2 hours. Three unsensitised pigs, given the same dose of egg albumin intravenously, showed no symptoms. Reactions were encountered

in pigs, which were receiving repeated intravenous injections of egg albumin at intervals of 2 days in order to produce antiserum. These reactions occurred after injection on the fourth day and on each subsequent occasion. When injected again after an interval of 24 days no reactions occurred. This appeared not to have been a Type I anaphylactic reaction and may have been a manifestation of Type III, serum sickness or Arthus reaction.

Reversed passive anaphylaxis was induced by intravenous injection of E. coli antigen, followed 30 minutes later by intravenous injection of homologous E. coli antiserum. The symptoms shown by these pigs were similar to those of active anaphylaxis but were more prolonged, showing no signs of recovery after 4 hours when they were killed.

Post mortem examination of animals which had shown moderately severe, active anaphylactic shock revealed characteristic lesions of oedema disease. Mildly affected

animals showed a catarrhal enteritis while severe protracted symptoms, resulting from reversed passive anaphylaxis to E. coli were associated with lesions of haemorrhagic gastro-enteritis. In the lungs emphysema, congestion, bronchoconstriction and peribronchial oedema were described and, in the heart, subendocardial haemorrhages with oedema and haemorrhage of the interstitial tissue of the myocardium. Thomlinson and Buxton considered oedema disease, haemorrhagic gastro-enteritis and catarrhal enteritis to be hypersensitive reactions of an anaphylactic type to E. coli organisms. The ultimate manifestations they judged to be determined by the severity and duration of the reaction, which, in turn, depended on the immune status of the individual pig and the rate of multiplication and absorption of E. coli. Their results suggested that hypersensitivity reactions of Type III as well as Type I were involved. Thomlinson and

Buxton (1963) did not measure the physiological changes occurring in their pigs nor did they speculate on the possible mediators of the reactions.

MEDIATORS

i) HISTAMINE

The actions of histamine on the cardiovascular and respiratory systems of pigs were described by Smith and Alpert (1955), Smith and Coxe (1951) and Heaton (1962). Smith and Alpert (1955) found 1.35 mg/Kg to be the LD_{50} of histamine base when injected intravenously in a group of 26 young pigs anaesthetised with sodium pentobarbitone. Respiratory distress was described, with respiratory failure preceeding cardiac arrest by 10 to 15 minutes. The pathological features were enlargement of the right side of the heart and distension of the coronary veins. The lungs were distended showing alveolar rupture and areas of atalectasis.

The authors stated death to be due to bronchoconstriction and pulmonary vasoconstriction leading to heart failure. No lesions were described in other organs.

Heaton (1962) found the intravenous LD_{50} in 15 un-anaesthetised pigs to be slightly higher, namely, 2.0 mg/Kg. He also diagnosed that right-heart failure was preceeded by bronchoconstriction and respiratory arrest. Pigs showed vomiting, salivation, dyspnoea and convulsions. Heaton observed an increase in femoral venous pressure and a fall, followed by a rise, in carotid arterial pressure. Post mortem examination revealed dilatation of the right side of the heart and pulmonary oedema. Heaton concluded that pulmonary venous constriction occurred and eliminated pulmonary arterial constriction on the grounds that the latter would not allow development of pulmonary oedema. Heaton did not measure pulmonary blood pressures but his theory was supported by

the finding of Smith and Coxe (1951) that isolated pig pulmonary artery was unresponsive to histamine whereas pulmonary vein constricted.

8) ANAPHYLAXIS IN THE SHEEP

SENSITISATION AND DESCRIPTION

Code and Hester (1939) attempted to extend the investigation of histamine release in anaphylaxis from the dog and guinea-pig (Code, 1938) to include observations on larger domestic animals, namely the horse, calf, sheep and goat. However, they failed to induce anaphylaxis in sheep by the technique of sensitisation used. Two sheep were given 30 to 40 ml. egg white subcutaneously daily for 10 consecutive days and 2 were given dog serum, alternating the subcutaneous and intravenous routes of injection for 9 consecutive days. They were challenged by intravenous injection of 50 ml. of the appropriate antigen after an interval of 2 weeks. The only effect of this injection was a slight increase in rate and depth of respiration. Blood histamine concentration, measured by the modified Barsoum-Gaddum method (Code, 1937), was unchanged. Similar attempts to sensitise two goats were equally unsuccessful.

Anaphylaxis was induced in sheep by Alexander, Eyre, Head and Sanford in 1970. They injected 1.0gm. crude hen egg albumen as a 10% solution in saline on 2 or 3 occasions at intervals of 2 days. After intervals of 2 to 4 weeks these animals were challenged by intravenous injection of 1.0 gm. alum-precipitated ovalbumin. This dose was equivalent to approximately 50 mg/Kg. Within 15 to 30 seconds the sheep showed dyspnoea, nose-licking, defaecation and urination. Coughing, with small amounts of mucus in the mouth and nares was described. Recovery began after 10 to 15 minutes and was apparently complete after 40 to 60 minutes. Further injections of antigen given one to 4 hours after recovery produced no manifestations of shock. Packed cell volume (PCV) was found to be increased by 10% to 40% within 5 minutes of injection of antigen and this was followed by haemodilution. Similar reactions were produced in animals sensitised by a different method. These were injected

intramuscularly with 0.25 mg. egg albumen mixed with 5 ml. complete Freund's adjuvant. This was repeated after 7 days. After 4 to 6 weeks graded doses of alum-precipitated ovalbumin (10, 10, 20, 50 and 100 ug) were injected intravenously at 2 day intervals and the animals used for experiments after a latent period of at least 2 weeks.

Challenge of sensitised animals anaesthetised with pentobarbitone allowed detection of a fall in carotid blood pressure and a rise in the pressure in the pulmonary artery, vena cava and hepatic vein. Tracheo-bronchial resistance, measured by the method of Konzett and Rössler (1940), increased.

MEDIATORS

Alexander et.al. (1970) found that specific antagonists of histamine and 5-hydroxytryptamine did not influence the anaphylactic response to egg albumen but sodium meclofenamate, an antagonist of bradykinin and SRS, did have an inhibitary

effect. The dose of sodium meclofenamate used (0.25 mg/Kg) antagonised the fall in blood pressure and slight increase in bronchial resistance produced by 0.001 mg/Kg bradykinin but also appeared to partly inhibit responses to histamine and 5-hydroxytryptamine. Mepyramine (5 mg/Kg) and methysergide (0.25 mg/Kg), antagonised administered histamine (1 ug/Kg) and 5-hydroxytryptamine (10 ug/Kg) respectively.

Alexander et.al. (1970) also administered the histamine releasers, compound 48/80, octylamine and polymyxin to sheep. These produced similar effects to those seen during anaphylaxis, both in conscious and in anaesthetised sheep. The effects of polymyxin were partially antagonised by the antihistamine mepyramine but the effects of 48/80 and octylamine were not antagonised by mepyramine or the 5-hydroxytryptamine antagonist methysergide. Repeated doses of histamine releasers, given intraperitoneally on 3 to 5 consecutive days, failed to deplete the tissues of histamine. These results suggested that

bradykinin and SRS might play a more important role than histamine or 5-hydroxytryptamine in anaphylaxis in sheep. They also indicated that the effects of histamine releasers in sheep were not entirely due to histamine release. No significant changes in mast cells were described.

The effects of histamine and 5-hydroxytryptamine were described in some detail by Alexander et.al. in 1967. In unanaesthetised sheep histamine caused haemoconcentration, respiratory embarrassment and collapse. 5-Hydroxytryptamine produced similar effects with the exception of haemoconcentration. In anaesthetised sheep histamine caused a fall in systemic blood pressure, a rise in pulmonary arterial blood pressure, bronchoconstriction and increased intrapleural pressure. The outflow from the pulmonary vein of an isolated lung was reduced by histamine. 5-Hydroxytryptamine produced a fall then a subsequent rise in systemic blood pressure but other effects

were similar to those of histamine. Collapse, oedema and haemorrhage was described as being present in the lungs after administration of either substance.

Halmagyi, Starzecki and Horner in 1963 administered histamine and 5-hydroxytryptamine to sheep in the course of investigating endotoxin shock. They found that the anti-histamine promethazine and the 5-hydroxytryptamine antagonist, 1-methyl-d-lysergic acid butanolamide, protected the animals against the effects of histamine and 5-hydroxytryptamine respectively. Endotoxin administration, after pretreatment with these antagonists, however, still produced a rise in pulmonary arterial and arterial wedge pressure, a fall in cardiac output and systemic arterial pressure and a fall in lung compliance due to terminal airway closure. Isoproterenol (isoprenaline) reversed these effects. Endotoxin-resistant sheep were also resistant to pulmonary embolism and incompatible blood transfusion, suggesting that a common mediator was involved in these three conditions.

Eyre (1969) examined the effects of various substances on sheep tracheobronchial muscle in vitro. He found that histamine contracted the tracheal and major bronchial muscles and relaxed the muscles of the lesser bronchi and bronchioles. The stimulant, but not the relaxant action, was antagonised by mepyramine. 5-Hydroxytryptamine contracted the musculature from all parts of the respiratory tract. This effect was antagonised by both methysergide and atropine. Bradykinin also had a stimulant action on tracheobronchial muscle but the high doses required indicated that the tissue was relatively insensitive to this substance. The most interesting feature of Eyre's report was the finding of a relaxant effect of histamine. This could not be antagonised by adrenergic blocking drugs or local anaesthetic but was antagonised by barbiturates. This was significant as experiments involving measurement of resistance of the lungs to inflation were carried out under barbiturate anaesthesia (Alexander et.al., 1967).

CUTANEOUS REACTIONS

Eyre (1970a)investigated passive cutaneous anaphylactic reactions in sheep and found that 5-hydroxytryptamine and kinin and/or SRS-A appeared to be more important as mediators than histamine. He found that the effects of injecting 48/80 intradermally could be antagonised either by the antihistamine mepyramine or by the 5-hydroxytryptamine antagonist, methysergide, injected intravenously 15 minutes before challenge. This suggested that 48/80 might release 5-hydroxytryptamine in addition to histamine in sheep. Passive cutaneous anaphylactic reactions were inhibited by methysergide and sodium meclofenamate to a greater extent than by the antihistamines, mepyramine and promethazine.

9) ANAPHYLAXIS IN CATTLE

INDUCTION AND DESCRIPTION

The first report of the experimental production of hypersensitivity reactions in cattle was by Gerlach in 1922. He observed the effects of single and repeated subcutaneous and intravenous injections of various sera in cows to be itching, urticaria and oedema of the skin with congestion and swelling of the mucous membranes as well as salivation, ruminal tympany, pulmonary emphysema and sub-pleural haemorrhage. The stimulus for Gerlach's work was the occurrence of adverse reactions in cattle vaccinated against anthrax over the years 1919 and 1920. Equine immune serum was administered together with the anthrax vaccine (sero-vaccination). The symptoms produced by subcutaneous and intravenous administration differed only in their rate of onset. This ranged from 15 minutes to 2 hours. Gerlach found that reactions to

equine sera occurred in animals with no previous known exposure to equine sera and described these as serum sickness as distinct from anaphylaxis. Anthrax culture had no influence on the reactions which were attributed wholly to the heterologous sera. He attempted to determine the duration of the stage following a reaction when an animal was resistant to further injections of serum. Subcutaneous injections of serum were given at 2 to 3 week intervals and he first observed recurrence of a reaction after $3\frac{1}{2}$ months. He attempted unsuccessfully to transfer hypersensitivity by injection of sera from sensitive cattle into guinea-pigs and rabbits.

Code and Hester in 1939 produced anaphylaxis in 4 Guernsey calves, under 6 months old, in order to determine whether liberation of histamine occurred. Two calves were daily given 6 alternating intravenous and subcutaneous injections of 5 mls. of a 20% solution of fresh egg white in saline while another two were similarly injected with undiluted horse

serum. Having determined, before beginning the course of sensitisation, that an intravenous injection of 30 mls. of the antigen solution produced no reaction in the calves, they allowed an interval of 5 to 7 weeks after the last sensitising injection then gave the challenging dose of 30 mls. antigen solution intravenously. The interval between the control intravenous injection and the beginning of the sensitisation course was not stated. Two calves were challenged in the conscious state, one sensitised to each of the two antigens, and two under anaesthesia. The conscious calves subsided immediately to their knees showing hyperpnoea which lasted 3 to 10 minutes. Abdominal distension then developed over 20 to 30 minutes leading to respiratory impairment. Tympany persisted for 6 hours. In the anaesthetised calves stomach movements, respiratory movements and carotid blood pressure (B.P.) were recorded. Within 3 minutes of injection of antigen the stomach contractions

ceased for approximately 45 minutes and tympany was the most outstanding feature observed. There was only a gradual, slight decline in B.P. (20 m.m. Hg.). These animals were destroyed after 2 hours. At post mortem, organs other than the distended stomach were apparently normal. It was presumed that "stomach" referred to rumen.

Blood samples taken from sensitised animals before and 10 minutes after challenge by intravenous injection of antigen showed a reduction in histamine content in all cases. Before sensitisation such an intravenous injection produced no change. Clotting time was measured in a clean glass test tube and found to be unchanged after anaphylactic shock.

Dungworth (1965) sensitised 6 month old Holstein-Friesian and Hereford cattle to egg proteins in an attempt to produce an allergic pneumonitis to compare with the naturally occurring syndrome of acute pulmonary emphysema, or fog fever, of cattle. His animals were given 8 weekly intramuscular injections of

350 mg. dried egg white, or 250 mg. purified ovalbumin, in complete Freund's adjuvant. Booster injections were given at unspecified intervals until challenge was carried out by exposure of the animals to antigen administered by aerosol. Serum precipitin levels were measured by Dungworth using the method of Kabat and Mayer (1961) and by Ouchterlony's method of double diffusion in gel (Ouchterlony, 1964). Passive skin tests were carried out in unsensitised calves to demonstrate skin sensitising, heat-labile, reaginic antibodies, by injection of antigen intravenously or intradermally 48 hours after intradermal injection of dilutions of sera from sensitised calves. Challenge with aerosolised ovalbumen as particles 0.7 to 1.2 μ in diameter was carried out at a time when precipitins and reagins were maximally developed. This appeared to be delayed until 4 to 15 months after completion of the sensitising course. Exposure to a low concentration of the vapour was achieved by placing the animals in

a room 10' x 20' x 10' with minimum ventilation and to a higher concentration by placing their heads in a 4' cube plastic chamber.

Of 10 animals challenged by aerosol administration of ovalbumen 3 showed no response after 3 to 5 hours exposure to the antigen in high concentration. One animal reacted acutely within 15 minutes, showing loud wheezing, open-mouthed breathing and coughing. These symptoms rapidly regressed on cessation of exposure to the antigen. Generally responses occurred within 90 minutes of exposure to high concentrations and within 4 hours of exposure to low concentrations. Two animals died, one after exposure for one hour to high concentration and one after 31 hours exposure to low concentration. A severe response occurred in a further case exposed to low concentration for 9 days. The 3 remaining animals showed moderate to mild reactions. The severity of the reactions produced was apparently unpredictable and there

was no correlation between the degree of severity and precipitin or reagin levels.

Increased respiratory rate, fluid sounds on auscultation of the thorax, coughing and dyspnoea with expiratory grunting were the clinical features shown. Post mortem examination revealed interstitial pulmonary oedema, emphysema, consolidation and haemorrhage. Histologically, Dungworth considered that the bronchioles appeared constricted with sloughing of the epithelium. Mediastinal and bronchial lymph nodes were enlarged, emphysematous and oedematous. Hyaline membranes were described in some alveoli and deposition of acidophilic homogenous material with accumulations of polymorphonuclear leucocytes in the lumina and on the walls of capillaries in intra-alveolar septa. The latter, Dungworth identified as a manifestation of a local Arthus reaction, involving precipitins. The bronchial constriction he believed to be related to reaginic antibodies. After several days of exposure, proliferation

of plasma cells in pulmonary connective tissue and lymph nodes was marked and taken to indicate a secondary immune response.

Dungworth found that a leucocytosis occurred, consisting mainly of neutrophilia with reduced numbers of lymphocytes and eosinophils. This effect was maximal 6 hours after an exposure of 15 minutes duration. Where exposure was continuous, return to normal numbers of white cells occurred on the second day. Dungworth interpreted these haematological changes to be due to increased adrenocorticosteroid activity in response to stress.

Wray and Thomlinson in 1969 sensitised calves to egg albumin in order to determine the conditions under which anaphylaxis might give rise to gastro-intestinal lesions. They were investigating the extent to which anaphylaxis was involved in the pathogenesis of colibacillosis in calves. Wray and Thomlinson used calves of various breeds, all of which were

under 2 weeks old when the experiments were begun. They produced active, passive and protracted anaphylactic shock by adopting the following proceedures:-

1) Active sensitisation was carried out in some animals by injecting intravenously 1 gm. of hen egg albumin as a 10% solution in saline. This was equivalent to a dose of 22.2 to 40 mg/Kg. In others the intravenous injection was accompanied by an equal dose of egg albumin given subcutaneously and a third group received 3 intravenous injections at 48 hour intervals. Challenge was carried out by intravenous injection of 1 gm. egg albumin after intervals ranging from 6 to 20 days. No reaction was recorded in the single animal challenged after 6 days but a positive reaction was obtained where the interval was of 9 days or more. Where sensitisation had involved 3 intravenous injections serum antibody titres to egg albumin were generally higher both before and after challenge. Such titres were determined by the tanned red blood cell haemoglutination technique described by Boyden (1951).

2) Passive sensitisation was carried out by intravenous injection of whole antisera, or purified gamma globulin. Such animals were challenged in the same way as those actively sensitised, after fixation periods of 48 or 60 hours. Antisera were prepared in calves by injecting 5 doses of 0.2 gm. intravenously at 48 hour intervals and repeating this course of injections 4 times at intervals of 10 days.

3) Protracted anaphylaxis was produced by giving a further injection of antiserum intravenously to passively sensitised calves immediately before they were challenged. It was suggested by Stone (1959) and by Thomlinson and Buxton (1962) that protracted anaphylaxis in guinea pigs required the presence of circulating antibody at the time of challenge.

Wray and Thomlinson encountered no fatal reactions in their calves and found that multiple sensitising doses were necessary to ensure that severe reactions could be produced

on challenge. The symptoms of acute anaphylactic shock were similar both in actively and passively sensitised animals. Hyperpnoea, salivation, muscular tremors and signs of abdominal discomfort were described. Severely affected animals showed incoordination, nystagmus, opisthotonus, dyspnoea, cyanosis and lacrimation. Symptoms usually appeared 2 to 3 minutes after injection and persisted for one hour. Animals passively sensitised showed reactions related in severity to the sensitising dose of antiserum. Post mortem examination of animals after acute anaphylaxis revealed petechiae in the lungs with areas of emphysema and of collapse and also oedema of the interlobular and subpleural connective tissue. Oedema and congestion of the mucosa of the abomasum and hyperaemia of the small intestine as well as oedema of the renal pelvis was described.

Antisera given immediately before challenge in some cases resulted in the onset of symptoms being delayed for 5 to 6 minutes with hyperpnoea persisting for 2 to 3 hours. During this time the animals were reluctant to move, showing tension of the abdominal muscles and trembling intermittently. Post mortem examination after protracted anaphylaxis revealed mainly mild pulmonary lesions limited to a few petechiae but severe gastro-intestinal lesions. These consisted of hyperaemia of the mucous membrane and oedema of the submucosa of the abomasum and small intestine with hyperaemia and haemorrhage in the Peyer's patches. The mesenteric lymph nodes were enlarged and oedematous. Leucopenia was developed by all animals showing anaphylaxis whether acute or protracted.

Severity of anaphylaxis was not found by these workers to be dependent on titre of precipitating antibodies. The nature of the pulmonary lesions, they believed, suggested that constriction of the bronchioles was responsible for the

respiratory symptoms. Wray and Thomlinson concluded that anaphylaxis might be involved in colibacillosis of calves as the lesions they produced resembled those described by Osborne (1967a; 1967b) after oral and intravenous administration of E. coli cultures and cell-free culture supernatants. Where antiserum was injected before challenge to produce protracted reactions they considered the possible involvement also of antigen-antibody complexes in the circulation causing an Arthus-type reaction.

The degree of immunological competence of the calves used by Wray and Thomlinson was in some doubt. Two of their animals were only one day old and all were under 2 weeks of age. On the basis of the indirect haemaglutination test they found considerable individual variation in immune response. Kerr and Robertson (1954), Pierce (1955) and Kerr (1956) using trichomonas, brucella and salmonella antigens were unable to detect an immune response in calves less than

30 days old. Roberts, Worden and Evans (1954) did detect antibodies to Salmonella dublin in calves 6 to 7 days old after repeated doses of vaccine. Smith and Ingram (1965) investigated antibody production in young animals. They tested the ability of calves whose ages ranged from one day to 2 months to produce antibodies against antigens of different chemical and physical properties. To bacterial and polysaccharide antigens detectable antibodies were not produced in animals under one month of age. When a soluble protein antigen, namely human serum albumin, was injected in one day old calves, antibodies were demonstrated after 7 to 8 days. This response, however, was dependent on human serum albumin being incorporated in Freund's incomplete adjuvant. Without adjuvant it failed to stimulate antibody production even in calves which were 6 months old. Smith and Ingram concluded that the nature of the antigen used affected the immune response of young calves.

Jain, Lasmanis and Schalm in 1967 sensitised an adult cow to egg albumin by giving 6 intramuscular injections of an homogenate of 250 mg. ovalbumin dissolved in 2.5 ml. of saline, 2.5 ml. Freund's adjuvant, 100,000 units of penicillin G and 125 mg. of streptomycin sulphate. The injections were given over a period of 35 days at weekly intervals. The reason for inclusion of antibiotics was not explained but may have been simply a prophylactic measure against infection. Precipitating antibodies to ovalbumin were detected, after 3 such injections had been given, by the precipitation ring test and agar gel-diffusion method of Ouchterlony (1964). One week after the sixth sensitising injection, sensitivity was tested by intravenous injection of 20 mg. ovalbumin. Within 30 seconds symptoms developed. Collapse with the neck extended, respiratory difficulty and shivering were recorded. Recovery began within 5 minutes and was complete in 30 minutes. The primary aim of Jain and Lasmanis was to

investigate the effect of leucopenia on experimental and natural mastitis. They used anaphylaxis as a means of producing leucopenia. By exposing the animal to the antigen, by intravenous drip, over 6 hours and subsequently over 10 hours, they caused the leucopenia, which appeared within 10 minutes of the start of administration, to be prolonged for 48 hours. Clinical signs also became apparent within minutes of starting the drip. These were restlessness, sneezing, salivation, defaecation, lacrimation, nasal discharge, head shaking, coughing, tachypnoea, tachycardia and congestion of mucous membranes. Symptoms lasted for 4 hours being reduced in severity after one hour although administration of ovalbumin was continued for 6 hours. Precipitating antibody was undetectable 2 hours after the drip started and for at least 2 days subsequently. In samples taken on the fifteenth day precipitating antibody was detected. Re-exposure to the drip 62 days after the first exposure resulted in more

marked clinical signs including mucus and blood in the faeces, cyanosis and sweating. These symptoms lasted for about 24 hours. Exposure on the second occasion was for 10 hours. Post mortem examination of the animal was not described. Leucopenia resulted in prolonged multiplication and delayed inflammatory response to certain mastitis producing organisms. The development of leucopenia has been explained in other species as being due to clumping, trapping and destruction of leucocytes in the capillary beds, especially in the lungs (Webb, 1924).

10) ANAPHYLAXIS IN THE HORSE

INDUCTION AND DESCRIPTION

Gerlach (1922) extended his investigations of reactions to foreign sera in cattle to other species including horses. Forty-two horses were given bovine serum subcutaneously. Fourteen reacted to their first injection. Symptoms appeared after a few hours and lasted for several hours. These were similar, but less severe than the reactions produced by equine sera in cattle and were characterised by urticaria, itching and oedema of mucous membranes. Human and rabbit, but not pig sera, produced similar reactions. The severity of the reaction was independent of the dose. Gerlach tried to establish the maximum safe interval between doses of serum and found this to be highly specific for the particular serum involved and to be shorter than that in cattle.

Code and Hester (1939) included 2 horses in their investigation of blood histamine changes in anaphylaxis. One horse was sensitised to dog serum and the other to egg white. The initial intravenous administration of 100 ml. dog serum produced no effects but the horse which received 500 ml. egg white showed a mild reaction lasting about 30 minutes and a slight fall in blood histamine. Daily intravenous administration of 45 ml. dog serum was carried out for 12 days and a challenge injection of 100 ml. dog serum given intravenously, after an interval of 3 weeks, to the first horse. Symptoms, which consisted mainly of dyspnoea, increased peristalsis and sweating, lasted for about one hour. Challenge repeated with 500 ml. dog serum 3 weeks later produced a more severe reaction. The second horse received alternating daily subcutaneous and intravenous injections of 40 to 50 ml. egg white for 12 days and was likewise challenged 3 weeks later by intravenous injection

of egg white. This resulted in death within 8 minutes, attributed to respiratory dysfunction.

Blood histamine was found to fall during the reaction to below detectable levels (0.01 ug/ml). White blood cells were reduced in numbers as judged by the volume of the white cell layer in centrifuged blood.

SUMMARY

In the large domestic animals anaphylaxis has been encountered accidentally and produced experimentally by a variety of methods. The symptoms have been described as affecting mainly the alimentary system in pigs and young calves, in the latter particularly where anaphylaxis was protracted, and the respiratory system in sheep, adult cattle and horses. The role of histamine has been examined in these species, but no definite evidence of its release provided. In sheep indirect evidence has suggested a lesser role for histamine and 5-hydroxytryptamine in relation to other mediators such as SRS and kinins.

b) DISEASES OF HYPERSENSITIVE ORIGIN

INTRODUCTION

Guinea-pigs, rabbits, rats, mice and dogs have been used to investigate the basic mechanisms involved in anaphylactic reactions. The value of information obtained in these species rested, with the exception of the dog, which itself was of clinical interest, in applying such findings to diseases of hypersensitive origin in domestic animals and in man. Where anaphylaxis was induced in cats, pigs, sheep, cattle and horses the object was generally to compare such reactions to specific diseases or to accidentally encountered adverse reactions to medication.

1) CATTLE

i) ATYPICAL INTERSTITIAL PNEUMONIA

DESCRIPTION

Atypical interstitial pneumonia of cattle, described by Jubb and Kennedy (1963) as a clinical and pathological syndrome, rather than a specific disease, has been given a variety of names including fog fever, pulmonary emphysema, pulmonary adenomatosis, lunger disease and rape poisoning (Jubb and Kennedy, 1963). Gibbons (1962) recorded bovine asthma, panting disease, emphysema-adenomatosis complex, bronchiolitis fibrosa obliterans and the new pneumonia complex as other terms used. The syndrome was reviewed by Gibbons (1962) and Mackenzie (1965, 1966). The main difficulty in studying this disease has been the lack of a clear definition on which to base diagnosis. The characteristic features have been described as severe dyspnoea of sudden onset and usually of short duration. Death or recovery

was usually found to occur within one hour to 4 days but death has been reported after the disease had persisted for 6 months (Jubb and Kennedy, 1963). The condition was found not to be contagious or associated with pyrexia. Mackenzie (1966) described the characteristic appearance of animals in respiratory distress. Cattle stood with the neck extended, mouth open and tongue protruding. They showed gasping respiration with expiratory grunting, coughing producing frothy fluid and the development of cyanosis. Where death or recovery had not occurred, subcutaneous emphysema was seen after 24 hours. On post mortem examination oedema and emphysema involving all lobes of the lungs was found. Alveolar epithelialisation and proteinacious exudate in the alveoli were the main features on histological examination.

Many different aetiological agents have been shown to be capable of producing these symptoms and pathological changes (Gibbons, 1962) and the syndrome has arisen in different parts

of the world under different circumstances. In Britain these circumstances, as listed by Mackenzie (1965) were:- 1) transfer to grazing on lush pastures or on the aftermath of hay called "foggage", hence the term "fog fever", 2) infection or reinfection with lungworms, 3) exposure of housed cattle to atmospheric dust, spores or mouldy feed, 4) exposure of cattle to smog. It was believed to occur only in cattle over one year old but a similar syndrome was reported in 1966 in calves of 2 to 6 months old, which were being fed barley (Kinch and Omar, 1966; MacLeod, 1966). Mackenzie considered that the pathology of fog fever as it occurred in Britain was similar to bovine emphysema occurring in cattle in North America. The nature of the pulmonary lesions was related to the duration of the illness. Where this exceeded 24 hours hyaline membranes were found in the alveoli. The lesions were often complicated by those of parasitic bronchitis.

Pulmonary adenomatosis was first reported in Texas in 1953 (Monlux, Fitte, Kendrick and Dubuisson). The condition was investigated by Seaton in 1958. It was called adenomatosis because of the tumour-like proliferation of cells lining the alveoli. These cells were phagocytes and septal cells. Hypertrophy and hyperplasia of the respiratory epithelium were outstanding features, although pulmonary oedema and emphysema were also present. Another feature of adenomatosis was a general hyperaemia of all organs of the body. It was not altogether clear whether pulmonary adenomatosis was a separate disease from pulmonary emphysema (Gibbons, 1962).

AETIOLOGY

In considering the aetiology of this syndrome there are two major schools of thought. The first is that it may be the result of direct injury to the lungs caused by inhalation or ingestion of some irritant or toxic substance. The second

is that it is due to a hypersensitivity reaction provoked by inhaled or ingested allergens.

DIRECT

NITROGEN DIOXIDE. Seaton (1958) reproduced the clinical signs and pulmonary lesions of adenomatosis in one cow by subjecting her, on two occasions, to nitrogen dioxide inhalation. Initial exposure to the gas for 30 minutes had no effect but symptoms appeared 24 hours after exposure to the fumes for a second time 96 hours later. She died after showing respiratory distress for 94 hours. Attempts to repeat this experiment in another animal, which was given nitrogen dioxide gas by stomach tube, failed. Lesions similar to those of pulmonary adenomatosis in cattle were produced in mice and guinea-pigs by exposure for 2 minutes to nitrogen dioxide.

Seaton experimented with nitrogen dioxide as a result of observing similarities between the lesions of pulmonary adenomatosis in cattle and "silo-filler's disease" in man. The latter had been proved to be due to inhalation of nitrogen dioxide and other oxides of nitrogen derived from fresh silage (Lowry and Schuman, 1956). Hypertrophy and hyperplasia of alveolar and bronchiolar epithelium were the outstanding features of this disease in man. In spite of the limited experimental evidence of the effects of oxides of nitrogen in cattle, the clinical recoveries obtained as a result of changing the diet of affected animals led Seaton (1958) to suspect that the cause was related to the chemical constituents of food plants possibly including silage. Dougherty, Stewart, Nold, Lindahl, Mullenax and Leek (1962) showed that, in cattle and sheep, eructated gasses from the rumen were presented to the lungs in significant quantities. Moulton, Cornelius and Osburn (1963) examined rumen gasses

in cattle suffering from acute bovine pulmonary emphysema and in animals which had recovered from the disease, but found no difference between the two groups. There is therefore little direct evidence in support of the theory that the syndrome results from inhalation of gasses such as nitrogen dioxide produced in the rumen when animals are being fed a certain diet.

AMMONIA. Of some relevance are the findings, in sheep, of Annison, Lewis and Lindsay (1959) that when sheep were transferred from stalls to pasture there was a four-fold increase in rumen ammonia. Garan in 1938 showed that ammonia could liberate histamine from guinea-pig lungs so that it might in this way produce pulmonary oedema. Methylamine also has a histamine releasing action and was found by Hill and Mangan in 1964 to be present in the rumen.

TRYPTOPHAN. Dickinson, Spencer and Gorham in 1967 induced in cattle a syndrome similar in onset, course, clinical signs and pulmonary lesions to fog fever by oral administration of the amino acid DL-tryptophan. They suggested that a sudden increase in a naturally occurring dietary constituent, possibly this amino acid, was either directly responsible for the syndrome or, by conversion to some other toxic substance, was indirectly responsible. They found that there was no significant change in total white cell or differential counts throughout the course of the syndrome.

INDIRECT(HYPERSENSITIVITY)

The second school of thought on the aetiology of bovine pulmonary emphysema or atypical interstitial pneumonia is that it is the result of a hypersensitivity reaction provoked by inhaled or ingested allergens including migratory parasites (Mackenzie, 1965). Jenkins and Pepys (1965) noticed a

similarity between this disease in cattle and a respiratory disease known as "farmer's lung" in man. The latter had been shown to be the result of a hypersensitivity reaction to inhaled thermophilic actinomycetes, particularly Thermopolyspora polyspora, present in mouldy hay. Affected individuals were found to have precipitating antibodies of the Ig G type in their sera against these farmer's lung hay (FLH) antigens. Jenkins and Pepys therefore tested the sera of affected and normal cattle for the presence of precipitins against FLH antigens. Positive reactions were obtained only in cattle which had been exposed to mouldy hay, and the highest percentage, 71%, of reactors was in the group of 28 animals suffering from fog fever. Of apparently normal cattle which had been exposed to mouldy hay 21% of a total of 68 animals had precipitins to FLH antigens in their sera. These results suggested that at least some of the cases of fog fever were of the same aetiology as farmer's lung in man. Just as many other

inhaled organic antigens have been shown to produce allergic respiratory disease in man (Pepys, 1966) many different inhaled substances may result in pulmonary manifestations of hypersensitivity in cattle.

Walton in 1968 recorded hypersensitivity reactions to ingested allergens in cattle. Although reactions were usually manifested as subcutaneous oedema mainly affecting the eyelids, face and vulva, one animal showed acute respiratory embarrassment.

Dungworth (1965), as described previously (p.101), produced a reaction similar to pulmonary emphysema by exposure of sensitised cattle to ovalbumin by inhalation. Maki and Tucker (1965) investigated acute bovine pulmonary emphysema by deliberately subjecting cattle to a sudden change from dry pasture to a lush type of pasture previously associated with high incidence of the disease. They carried out haematological examination of 17 cows before and 6 days after they had been grazing such pasture. They found leucopenia with

a neutropenia, a reduced haematocrit, but a rise in the number of erythrocytes. These changes occurred even when clinical pulmonary emphysema was not produced. Maki and Tucker (1965) measured blood histamine and 5-hydroxytryptamine levels in some of their animals but found no apparent correlation between those levels and pulmonary emphysema. They also failed to reproduce the syndrome by transferring rumen contents from affected animals on lush pasture to a fistulated normal animal kept in dry lot.

Michel, in 1954, described a syndrome similar to fog fever occurring at certain stages of infection with the lungworm of cattle, Dictyocaulus viviparus. He related these to the stages of development of the lungworm, particularly to self-cure and the elimination of worms from the host. In 1960 Jarrett, Jennings, McIntyre, Mulligan, Sharp and Urquhart described reinfection phenomena manifested as severe coughing of sudden onset in immune animals. Reactions to larvae killed in the

small bronchioles were believed to be responsible. Michel and Mackenzie (1965) demonstrated eosinophilia to be a regular feature on reinfection or challenge of vaccinated animals with Dictyocaulus spp. larvae. The eosinophilic responses did not occur in animals which had not been previously exposed to the parasite and were considered to indicate hypersensitivity reactions.

THE ROLE OF HISTAMINE IN ATYPICAL INTERSTITIAL PNEUMONIA

The role of histamine in this syndrome was discussed by Hull in 1965. He administered histamine hydrochloride intravenously at a rate of 2.0 mg/min. to 2 Hereford cows and found that salivation, dyspnoea and an increase in heart rate was produced. There was a fall in the mean pulmonary arterial blood pressure and the animals became restless and cyanotic. Hull did not state for how long the histamine had to be infused to produce these effects, but increasing the rate of administration to 5.0 mg/min. produced a further increase in

heart rate, dyspnoea and salivation and death occurred within 15 minutes. Post mortem examination revealed that the lungs only partially collapsed when the thorax was opened and only a small amount of interstitial emphysema was observed. The lungs were stated to be "relatively dry." Endocardial haemorrhage and petechiation of the trachea and epicardium were described with blanched areas in the abomasum. Histologically, a small amount of oedema and some alveolar emphysema were described. Hull stated that histamine produced a definite bronchial constriction in the bovine although he produced no direct evidence in support of this, apparently making the deduction from his clinical and pathological observations. The total dose which these animals received was about 200 mg. of histamine hydrochloride. Hull did no more than speculate that histamine might be involved in the aetiology of pulmonary emphysema.

Hull did suggest that there were differences between the effects of histamine and those of apparent anaphylaxis. In the latter, which he considered to be exemplified by a fatal reaction to antibiotics in a Holstein heifer, the lungs were extremely oedematous with blood stained foam in the trachea and bronchi.

Desliens, in 1958, found the lethal dose of histamine given intravenously to cattle was 0.06 mg/Kg. This was 10 times less than the lethal dose for horses and guinea-pigs and 160 times less than for dogs. He recorded symptoms of dyspnoea, cyanosis, urination, defaecation, tachycardia haemoconcentration and a fall in aortic pressure. Desliens differed in his findings from those of Hull in that he found histamine to produce a rise in pulmonary arterial pressure. He did not describe in detail the pathological changes produced but he indicated that pulmonary oedema was a feature.

Nilsson (1963), in the course of an investigation into laminitis, administered up to 30 mg. histamine subcutaneously. He observed increased respiratory rates and reductions in systemic blood pressure among other changes which will be discussed in relation to laminitis (p.134).

In conclusion, it seemed possible that histamine might be involved in the pathogenesis of atypical interstitial pneumonia, either being directly released by ingested or inhaled substances or indirectly released as a result of a hypersensitivity reaction. Antihistamines have been used in treating affected animals (Gibbons, 1962) but their value is doubtful. Reports of successful therapy have generally involved only mildly affected cattle and spontaneous recovery can occur.

ii) LAMINITIS

Nilsson (1963) published the results of his investigations and observations on the clinical, morphological

and experimental aspects of laminitis in cattle. In some animals, particularly those of the Friesian breed, laminitis only affected the hind hooves. Cows appeared to be predisposed to laminitis during the month following parturition and the disease often occurred in conjunction with acetonaemia, retained foetal membranes, metritis, gastroenteritis, mastitis and internal abscesses. Nilsson measured blood pressure by inserting a needle through the wall of the rectum into the aorta and connecting the needle to a mercury manometer. He found that the blood pressure was usually low in affected animals, especially in acute and sub-acute cases.

Nilsson produced laminitis by subcutaneous injections of histamine. The degree of response to histamine varied in different animals. Some showed obvious reactions after receiving 10 mg. while others were unaffected by as much as 30 to 50 mg. Of 24 treated animals 10 reacted with very obvious clinical signs of laminitis. Three animals showed

no changes and the remaining 11 showed less distinct signs of laminitis. The clinical signs characteristic of laminitis and reproduced by subcutaneous injection of histamine were distension of the digital arteries and veins, with pulsation in the arteries, stiffness, crossing of the fore feet and a reaction suggesting pain on compressing the hoof with pincers. These symptoms appeared 30 minutes to 2 hours after injection. Hyperaemia, oedema, cell infiltration and haemorrhages in the corium with infiltration of cells and morphological changes in cells in the epidermis were observed in animals killed after receiving histamine. Nilsson found fewer mast cells than normal in the corium of the foot of cattle suffering from acute laminitis. Where the disease was subacute or chronic they were more numerous. Blood histamine levels in acute and exacerbated cases were found to be lower and in subacute and chronic cases higher than in normal cattle. Administration of histamine

regularly caused a reduction in blood pressure and increased pulse and respiratory rates. After injection of the drug a decrease in blood histamine level was found and not an increase as might have been expected. Most animals showed an increase in total white blood cells after injection of histamine whereas leucopenia was frequently encountered in animals suffering from laminitis.

Nilsson found antihistamines to be an effective form of treatment in laminitis particularly in its acute form. Corticosteroid therapy and phlebotomy were also effective. He considered that histamine was involved in the pathogenesis of the disease and that, in some cases at least, it was the result of an allergic reaction.

The disease often occurred in association with changes in the diet such as feeding mouldy hay, concentrates or clover. Nilsson produced an exacerbation of laminitis in 2 cows by feeding them mouldy hay to which they had been exposed

previously when first showing the condition. He injected possible allergens, including milk, extracts from fungi, cottonseed oilcake and red clover, in various dilutions intracutaneously and found increases in skin thickness to be greatest between 2 and 6 hours after injection. Positive reactions could be correlated with incidence of laminitis and access to the particular allergen involved. However, positive skin reactions sometimes occurred in animals which had no history of laminitis. Nilsson suggested that laminitis might not always be an independent disease, but often a symptom of other simultaneously occurring diseases and that the aetiology differed in different cases, being in some related to hypersensitivity to a dietary constituent.

Maclean (1965) described 61 cases of laminitis seen over 2 years and his findings were similar to those of Nilsson. The incidence was highest in cows during the month following calving and laminitis was often associated with mastitis,

metritis, acetonaemia or digestive disturbances. He measured blood histamine levels in 18 animals, 9 of which were suffering from laminitis, and found that the affected animals had lower histamine levels than his controls. An elevated blood histamine level was encountered in a sample taken from one animal one hour before the onset of symptoms of laminitis. A second sample taken about 1½ hours later and a third taken about 5½ hours later showed that the level had fallen. This observation was not confirmed in the case of the only other animal sampled before and during an attack of laminitis. Blood histamine levels in this second animal were consistently low. Maclean found that administration of antihistamines was a less successful form of therapy than was administration of corticostercids or ruminal contents where digestive disturbance was also present, or phlebotomy. He found no haematological deviations from normal other than those sometimes associated with concurrent diseases. Maclean's

findings regarding blood histamine levels would have been of greater value had they all been based on repeated rather than single blood samples.

iii) ANAPHYLACTIC REACTIONS TO ANTIBIOTICS

In 1963 Brisbane reported the results of a survey carried out in Alberta, Canada to determine the significance of reported reactions in cattle following administration of antibiotics. Over a period of 3 years, 1,200 animals were reported to show such reactions of which 20 were fatal. Reactions were described as anaphylaxis characterised by dyspnoea, staggering, collapse and salivation, or urticaria characterised by oedema of the eyelids, vulva and rectum. Urticaria was a milder type of reaction. Sixty-eight animals showed only local reactions in the form of discomfort at the site of injection after oxytetracycline and chloramphenicol administration. These might simply have been due to a direct irritant action of the drugs. Penicillin was responsible for

720 cases of anaphylaxis with approximately half as many reacting to mixtures of penicillin and streptomycin. Oxytetracycline was responsible in 27 cases and neomycin in 3. Penicillin and mixtures of penicillin and streptomycin were responsible for the urticartal forms of reaction. Brisbane pointed out that in many cases there was no history of the animal having been previously exposed to the antibiotics eliciting the reactions. Adrenaline was stated to be the drug of choice in the treatment of acute anaphylaxis, but antihistamines were reported to be effective in urticarial reactions. The frequency of incrimination of individual antibiotics in anaphylaxis and urticaria was generally a reflection of the frequency of their use.

Leemann, De Weck and Schneider (1969) reported that carboxymethyl-cellulose (CMC) could be responsible for anaphylactic reactions to drugs in cattle. They embarked on an investigation of anaphylactic reactions to antibiotics

following numerous reports of such reactions in Switzerland. Penicillin was the antibiotic most frequently involved and the incidence greatly increased following a campaign of vaccination against foot-and-mouth disease. On skin testing animals, which had reacted to penicillin previously, these workers found that positive reactions were only obtained with commercial preparations of penicillin which contained CMC added as a stabilising substance. Positive urticarial reactions were produced on intradermal injection of CMC in allergic cows, but not in non-allergic cows. Leeman et.al. failed in attempts to transfer the skin hypersensitivity to guinea-pigs, goats, or non-allergic calves using sera from allergic cattle. Fatal systemic anaphylaxis was produced in some vaccinated cows by intravenous injection of 10 mg. of CMC. These showed pulmonary oedema and eosinophilia. No precipitating antibodies to CMC were detected in the sera of allergic cattle. This was an illustration of allergic

reactions developing to a carbohydrate substance previously believed to be unable to act in this way. This substance is used in many drug formulations and foods. The exact role of foot-and-mouth disease vaccine in sensitisation to CMC was not established.

iv) ANAPHYLACTIC REACTIONS TO VACCINES

Anaphylactic reactions were reported by De Quiroz, Sutmöller and Barroeta in 1964 as occurring in cattle in Venezuela after vaccination against foot-and-mouth disease and rabies. In 1962 an outbreak of foot and moutn disease occurred, which was due to a different subtype of virus from that used routinely in the inactivated foot-and-mouth disease vaccine. A live-virus vaccine of the subtype responsible passaged in chicken embryos was therefore used. On initial vaccination there were some reports of anaphylaxis and on revaccination the following year such reactions became cause for alarm. Reactions were also found at this time to occur

after rabies vaccination. The symptoms were salivation, lacrimation, urticaria, increased respiratory rate, coughing and mucosal oedema. Symptoms usually appeared within a few minutes of intramuscular injection of the vaccine, but occasionally were delayed in onset for 1½ hours. In non-fatal reactions recovery was complete in one to 3 hours. Post mortem examination revealed pulmonary oedema, poor coagulation of the blood, congestion of all organs, mucosae and muscles and haemorrhagic infarcts in the spleen.

The live virus foot-and-mouth disease vaccine was prepared from the hearts of 14 day chick embryos or from the carcases of 8-day-old mice. Streptomycin and penicillin were added to the final dilution. The Flury vaccine used against rabies contained total chicken embryo juice and streptomycin and penicillin. The inactivated type of foot-and-mouth disease vaccine also contained streptomycin and penicillin. It was prepared from cattle tongue epithelium.

The virus was adsorbed on aluminium hydroxide and inactivated by heat and formol.

De Quiroz et.al. carried out experiments on cattle in order to determine which vaccine components or other factors were responsible for the anaphylactic reactions. No anaphylactic reactions were observed when the live virus vaccine, prepared without antibiotics, was given intramuscularly whereas 25% of the cattle innoculated with this vaccine containing antibiotics showed marked reactions. Intradermal innoculation of the inactivated vaccine which contained antibiotics, although in smaller quantities than in the live-virus vaccine, did not cause reactions unless given simultaneously with live-virus vaccine containing antibiotics. In the case of the Flury rabies vaccine, however, reactions were obtained to whole chicken embryo juice alone. In this case antibiotics could not be held responsible. Animals, which had been found to be sensitive to this embryo juice, did not react to the embryo heart tissue

of live virus foot-and-mouth disease vaccine. This was thought to be due to a difference between the proteins in the heart tissue and in the whole embryo juice. Although inactivated foot-and-mouth vaccine did not produce reactions, the incidence of reactions to the live-virus vaccine containing antibiotics was greater where animals had also been receiving inactivated vaccine. De Quiroz et.al. pointed out the danger of sensitising cattle populations on a large scale to agents incorporated in vaccines. They considered addition of antibiotics to vaccines to be contraindicated. Rossi (1963) had already shown that reactions of cattle in Argentina to subcutaneous innoculations of inactivated foot-and-mouth disease vaccine were due to the presence in the vaccine of dihydrostreptomycin. In his report cows which had been vaccinated on 4 or 5 occasions were mainly involved. Anaphylactic reactions to certain types of foot-and-mouth disease vaccine occurred recently in Europe (Beadle, 1969, personal

communication), where sensitivity to antibiotics did not seem to be responsible. Results of PCA reactions suggested reaginic antibodies to be involved.

v) REACTIONS ASSOCIATED WITH INFESTATION BY LARVAE OF HYPODERMA SPP. (WARBLE FLY)

Hadwen and Bruce in 1917 reported the occurrence of adverse reactions in cattle following the destruction of larvae of Hypoderma bovis and Hypoderma lineatum within the tissues of the host. They described the reactions as anaphylaxis on the basis of their clinical and post mortem observations. Salivation, tears, defaecation, oedema and irritation of the skin were described. Death in fatal cases was ascribed to asphyxia and blood after death failed to clot. Hadwen and Bruce postulated that animals were sensitised by the excretions of warble larvae and that rupture of the larvae under the skin of the animals' back produced reactions. Such reactions were also produced by intravenous

injection of the protein material contained in the larvae. Thereafter animals did not react to further injections for a period of at least 30 days. Warble extracts administered to rabbits and guinea-pigs had no direct toxic effects but anaphylaxis occurred following a second injection. Instillation of warble extract into the eye produced lacrimation, swelling of the conjunctiva and irritation in sensitised cattle and rabbits. Similar instillation produced no effects in unsensitised rabbits, horses, pigs and lambs. Hadwen and Bruce unfortunately did not record administration of warble extract to cattle never previously exposed to it. MacDougall (1930) repeated the work of Hadwen and Bruce. He also believed the reactions to be manifestations of anaphylaxis.

The introduction of organophosphorus insecticides for systemic use in the control of warble fly confused the picture as direct toxic effects of the insecticides could be responsible for symptoms observed. Inflammatory responses around dead

larvae, leading to oesophageal occlusion and respiratory failure or paralysis of the hindquarters due to pressure on the spinal cord (Gebauer, 1965) could also produce dramatic effects. Beesley (1965) showed that extracts from the mid-gut of the first-instar larvae had direct toxic effects similar to anaphylaxis and he produced anaphylactoid shock in unsensitised rabbits and calves. This reaction was similar to that produced in rabbits and calves sensitised, either by a previous injection of larval extract, or by implanting larvae and killing them by dermal application of an organo-phosphorus insecticide. True anaphylaxis was elicited by successive injections of haemolymph from first-, second-or third instar larvae. Beesley found that, after treatment of rabbits and calves, in which larvae had been implanted, with systemic insecticides, the first dead larvae were encountered at post mortem examination only after several days. He therefore suggested that reactions occurring within 48 hours

of treatment were likely to be related to the insecticide rather than to release of material from the larvae. Reactions occurring 4 to 5 days after treatment could be due to the direct toxic action of material liberated from killed larvae. Beesley noted that in practice no larvae were recovered from most of the cattle subjected to post mortem examination after reactions.

Anderson and Kirkwood (1968) described experiments demonstrating the direct toxic actions of extracts of larvae of H. bovis in 4 calves which had never been exposed to warble fly. Extracts consisted of haemolymph and gut contents drained from first stage larvae of H. bovis obtained from the epidural fat of slaughterhouse animals. Anderson and Kirkwood hoped to distinguish anaphylactic reactions from direct toxic effects of the larvae and direct organophosphorus toxicity by means of blood histamine levels, white blood cell differential counts and erythrocyte cholinesterase activity.

Intravenous administration of extracts from 13, 30 and 45 larvae respectively to 3 of the calves produced reactions characterised by coughing, dyspnoea, lacrimation, mucosal erythema, ataxia, defaecation and urination. The severity of the reaction was related to the number of larvae represented in the extract. The fourth animal received extract from $1\frac{1}{2}$ larvae and showed only a very mild reaction in the form of erythema of the nasal and vulval mucosae and ears. After an interval of 2 weeks 12 larvae were given. This failed to produce a reaction but administration of 13 larvae 2 weeks later did produce a typical reaction. This attempt to sensitise an animal by giving an initial small dose appeared to be unsuccessful. Blood histamine levels were found to be slightly reduced after reactions but the changes were not thought to be significant, nor were they related to the severity of the reactions. The cholinesterase activity of erythrocytes was unchanged and haematological

changes were small and inconsistent. In all cases the lymphocyte/neutrophil ratio was reduced and the packed cell volume and red blood cell count was increased in the animal which received the largest dose of larvae. From findings in sheep (Alexander et.al. 1967) this haemoconcentration might have been related to histamine release. There was no direct evidence of histamine release or of cholinesterase inhibition in the host. Anderson and Kirkwood believed that Beesley's (1965) findings regarding the delayed effect of organophosphorus insecticides on larvae did not apply in cases of natural infestation and that sufficient larvae would die within the period of 24 hours following insecticide application to produce a concentration of toxins sufficient to account for the clinical symptoms. Whether or not this is the case it was conclusively shown that reactions unrelated to hypersensitivity to Hypoderma bovis larvae could by produced in cattle. The chemical nature of the toxic substance in the first-stage larvae is unknown.

vi) RESPONSES TO BACTERIAL TOXINS

The haemodynamic effects of gram-negative endotoxin have been found to show species variation and to be similar to those of anaphylaxis (Kuida, Hinshaw, Gilbert and Visscher, 1958; Tikoff, Kuida and Chiga, 1966; Kuida, Gilbert, Hinshaw, Brunson and Visscher, 1961). Tikoff et.al. (1966) considered the effects of Escherichia coli endotoxin to be indirect involving a blood and/or tissue reaction with vasoactive mediators but did not suggest that hypersensitivity was involved. However, in the considerable literature on the effects of Vibrio foetus endotoxin authors have used the terms "hypersensitive," "allergic" and "anaphylactoid" loosely, basing their diagnosis on the nature of the clinical signs and overcoming the lack of history of previous exposure by postulating passive sensitisation during pre-natal development (Osborne and Smibert, 1962). Osborne and Smibert in 1964 postulated that the abortifacient action of Vibrio foetus in cattle, as well as in goats, sheep, pigs and

rabbits, was allergic in nature. Abortion occurred in cows following intravenous innoculation of V. foetus whole cells although bacteraemia was not demonstrable. Pregnancy apparently increased the susceptibility of rabbits to V.foetus toxin. Reactions to this toxin were produced also in newborn calves and pigs which had not received colostrum.

2) HORSES

i) PULMONARY EMPHYSEMA

Chronic alveolar emphysema or "heaves" in horses was first suggested by Larsson in 1936 to be the manifestation of a complex of allergic phenomena. The condition is gradual in onset and usually encountered in housed horses fed poor quality roughage (Blood and Henderson, 1963). It is frequently associated with a dusty atmosphere and dusty food and Alegren and Carlstrom (1940) stated the cause of heaves to be constriction of the bronchioles caused directly by irritation of the mucous membrane by cold and dusty air. On skin testing affected horses Larsson (1936) found that they reacted positively to extracts of mould obtained from mouldy hay. Andberg, Boyd and Code (1941) showed that histamine injected intravenously into normal horses caused a complex of symptoms similar to that of heaves and Obel and Schmiterlow (1948) found that horses suffering from heaves were more

sensitive than normal horses to the bronchoconstricting action of histamine. This was true even when the symptoms of the disease were absent as a result either of spontaneous regression or of atropine pretreatment. This was of interest in relation to a similar finding in humans that asthmatic individuals were more than normally sensitive to histamine (Weiss, Robb and Blumgart, 1929). Obel and Schmiterlow also showed that in horses suffering from heaves the intra-thoracic pressure during expiration was positive due to the abdominal muscular effort required to expel air from the lungs.

The work of Obel and Schmiterlow supported the theory that histamine might be involved in the pathogenesis of alveolar emphysema but did not prove it to be an allergic reaction. More recently Jenkins and Pepys (1965) carried out a limited investigation in horses suffering from this disease, testing their sera for the presence of precipitins against the FLH antigens of mouldy hay and against the

source of these antigens, the thermophilic actinomycete, Thermopolyspora polyspora. No positive reactions were obtained but, as they pointed out, allergens other than those of FLH might be responsible.

ii) LAMINITIS

Åkerblom in 1934 carried out an investigation of the aetiology and pathogenesis of laminitis in horses. On the basis of aetiology he classified laminitis, or founder as it is often called, as parturition, feeding, toxic, metastatic, rheumatic, overloading or over exertion founder. Of those types he found that associated with feeding was the most common, having an incidence of 67.4% of all cases of laminitis he recorded. A wide range of food stuffs have been incriminated by different authors. Eberlein (1908) and Thum (1912) maintained that it could be induced by all types of grain and hay. Eberlein gave the name rheumatic founder to cases

associated with chilling and obscure causes in which allergy was possibly involved.

Åkerblom (1939) found that in horses suffering from acute laminitis blood histamine levels were elevated but Amman (1949) found no significant difference between the values obtained during laminitis and normal values. Åkerblom showed that histamine-forming coli bacteria were present in the intestine of horses suffering from laminitis associated with feeding. However, he differentiated between histamine involvement or what ne termed "histaminosis" and allergy.

Åkerblom (1934) produced laminitis experimentally in the horse partly by feeding rye and partly by intravenous and subcutaneous injections of histamine. The pathological changes produced in this way were similar to those of spontaneous laminitis. Mintschew (1938) produced laminitis

and periodic opthalmia (moon blindness) by intra-arterial injection of histamine but Dozza and Rampichini in 1959 were not able to produce laminitis by injecting the histamine releasing agent, compound 48/80, into the digital arteries although this did increase the histamine content of circulating blood.

Augustinus in 1945 and Chavance in 1946 reported that antihistamine treatment was successful in laminitis in horses. This was supported by Åkerblom (1952). Corticosteroid therapy was reported also to be successful (Lannek, Nordström and Asheim (1959). Åkerblom (1934) also advocated phlebotomy.

There appears to be evidence of histamine being involved in equine laminitis but no real evidence that it is an allergic reaction.

iii) ANAPHYLACTIC REACTIONS TO VACCINES

Graham (1940) discussed the possible causes of adverse reactions in horses to chick-embryo equine encephalomyelitis vaccine. Hypersensitivity was suspected to be involved but attempts to reproduce the reactions experimentally, by injecting double doses of vaccine by various routes, failed. Schoening (1940) reported anaphylactic reactions to this vaccine occurring mainly in animals receiving a second dose but stressed that many animals were vaccinated twice without showing any adverse symptoms.

Jackson (1969) commenting on untoward reactions of the horse to injection of antigenic substances observed that the reported incidence of anaphylactic reactions was lower than might have been expected as the horse is frequently injected with immunising materials. Purpura haemorrhagica has been reported to occur as a result of strangles

vaccination (Bryans, 1966). The extensive use of antisera in infectious disease has been associated with cases of serum sickness (Kral and Schwartzman, 1964).

iv) SKIN DISEASES

Skin manifestations in the horse of allergic response to ingested allergens were recorded by Walton (1968). The dietary constituents involved included wheat, oats, barley, horse cubes, horse tonic and certain plants. Horses showed either a generalised papular response with or without pruritis or generalised oedema of the skin particularly over the face and eyelids. Skin reactions resulting from administration of antibiotics, tetanus toxoid, antisera and phenothiazine were described by Kral and Schwartzman (1964) and contact dermatitis resulting from contact with allergens in substances used for cleaning tack were confirmed (Walton, 1968). Insect bites have also been incriminated in allergic skin reactions of the horse. Riek (1954, 1955) investigated the

condition known as "sweet itch" or "Queensland Itch" and found it to be related to the bites of the sand fly, Culicoides robertsi. He found that in summer the plasma histamine level of susceptible horses was elevated at the time of day of maximum activity of C. robertsi. Also the histamine content of the skin at the site of intradermal injection of C. robertsi was increased. Intradermal tests, positive in susceptible horses, were negative in non-susceptible horses and the latter showed no marked fluctuations in plasma histamine levels. Thermolabile skin sensitising antibody to C. robertsi was detected in susceptible animals. Greatorex (1969) reviewed current information on conditions of the horse, involving skin lesions, of possible allergic origin, namely urticaria, blue nose and purpura haemorrhagica. Blue nose is characterised by sudden onset of oedema of the nostrils, face and eyelids. The skin around the nostrils becomes blue in colour and desquamation follows. Urticarial

lesions may also be present on the body. There may be evidence of jaundice and behaviour changes and this disease may be fatal within several days. Clovers have been suspected as the sensitising agents responsible.

Purpura haemorrhagica is a condition of obscure aetiology involving oedema of the head and limbs with widespread petechial haemorrhages often accompanied by urticarial lesions. As it was often found to accompany a systemic infection such as influenza or strangles, appearing when the acute symptoms were subsiding, it was thought to be an allergic reaction to streptococcal protein, however, not all cases have been associated with streptococcal infection.

In all the above conditions antihistamine therapy has been advocated but has generally proved disappointing.

3) SHEEP

Diseases associated with allergic reactions in sheep are not common. As in cattle it was claimed by Osborne and Smibert in 1964 that enzootic abortion associated with Vibrio foetus organisms in sheep was related to hypersensitivity. Endotoxins of Brucella abortus and B. melitensis increased sensitivity of smooth muscle to oxytocin and histamine. (Urbaschek, 1964 and Urbaschek and Versteyl, 1965).

Halmagyi, Starzecki and Horner (1963) described the effects of injections of Coli-lipopolysaccharide and staphylococcus toxins in sheep but they did not consider hypersensitivity to be involved in the response to endotoxin. They deduced that endotoxin caused constriction initially of pulmonary blood vessels. This led to right ventricular failure and respiratory arrest due to changes in lung mechanics.

Anaphylaxis in sheep was claimed by Hadwen and Bruce (1917) to be produced by intravenous injection of extracts of the larval form of Oestrus ovis. They described a natural case of what they termed anaphylaxis in a lamb where no larval infestation was found, suggesting that sensitivity had been inherited. Following reactions there was a period of about one month when the animals did not react on administration of the larval extracts. The reactions shown by the sheep consisted of defaecation, salivation, respiratory distress, cyanosis, nasal discharge and staggering. At necropsy it was found that blood failed to clot. The lungs and mucous membranes of the trachea and bronchi were congested as were the thoracic lymph glands. Petechiae were found in the lungs. The main difficulty in assessing the findings of these early workers is their assumption that animals were in a sensitised state without determining how they became sensitised or trying to sensitise them experimentally.

Atypical interstitial pneumonia in sheep was described by Pascoe and McGavin in 1969. Four outbreaks of this disease, not previously reported as occurring in sheep, occurred in Queensland, Australia during summer in sheep-grazing stubble of wheat, barley and canary grass. Morbidity ranged from 2 to 25%. The clinical signs and pathological changes including the presence of alveolar hyaline membranes were similar to those described in cases of atypical interstitial pneumonia of cattle. Symptoms appeared within 6 days of sheep being introduced to new grazing and Pascoe and McGavin considered this disease to be the same as that described in cattle, assuming in the latter species that it was a hypersensitivity reaction. Antihistamines were administered intravenously to some of their cases but they were considered to have been of value only when used within 24 hours of onset of the symptoms.

4) PIGS

Oedema disease and gastro-enteritis in pigs were shown to be associated with multiplication of certain Escherichia coli serotypes in the stomach and intestines (Sojka, Erskine and Lloyd, 1957; Roberts and Vallely, 1959; Thomlinson and Buxton, 1962). Gregory (1955) and Erskine, Sojka and Lloyd (1957) reproduced oedema disease in pigs by innoculating culture filtrates and extracts of these organisms. Buxton and Thomlinson in 1961 showed that pigs were hypersensitive to the common E. coli serotypes associated with oedema disease and haemorrhagic gastro-enteritis and postulated that an anaphylactic reaction to E. coli, rather than a direct toxic effect following the absorption of E. coli polysaccharide from the intestines, might be responsible for these diseases. When Thomlinson and Buxton in 1963 experimentally produced active anaphylaxis and reversed passive anaphylaxis in pigs they found that the

reactions and the lesions produced resembled those of naturally occurring oedema disease and haemorrhagic gastro-enteritis. This work has already been discussed in detail (see page 83).

5) CATS AND DOGS

a) SYSTEMIC REACTIONS

REACTIONS TO RABIES VACCINE IN DOGS

Anaphylactic shock in dogs resulting from vaccination against rabies, was reported by Baer, Goodrich and Dean in 1962. The incidence of such reactions was not high. Baer et. al. recorded 9 cases out of 500,000 dogs vaccinated (approximately 0.002%).

ANAPHYLACTIC REACTIONS IN CATS

As previously described (p. 76) McCusker and Aitken encountered acute anaphylaxis in cats. Two showed respiratory distress with pulmonary lesions revealed on necropsy (McCusker and Aitken, 1966) and 9 showed involvement of the alimentary tract and skin (Aitken and McCusker, 1969). Klaus (1965) described spontaneous idiopathic pulmonary oedema in a Siamese cat which showed a sudden onset of very severe respiratory

distress. Klaus considered this to be a manifestation of anaphylaxis, comparing the reaction to fog fever in cattle. Antihistamine therapy had little effect. Miller (1960) and Peckenpaugh (1961) described bronchial asthma occurring in cats. The outstanding features of their cases were sudden onset of dyspnoea and rapid response to adrenaline or corticosteroid therapy. Siamese cats appeared to predominate in the incidence of asthmatic reactions.

REACTIONS TO INGESTED ALLERGENS

Walton, Parish and Coombs (1968) described a case of naturally occurring allergy to cow's milk where a cat developed enteritis and dermatitis. When milk was injected intradermally a weal developed and serum from the affected cat was shown to have high agglutinating antibody titres to whole milk and to the α-lactalbumin fraction. Serum from the cat failed to sensitise the skin of guinea-pigs for PCA and passive skin sensitisation was achieved only in one of 8 normal cats.

REACTIONS TO INHALED ALLERGENS

An account of allergy to ragweed in a dog was given by Patterson in 1959. The symptoms, namely conjunctivitis, rhinitis and respiratory distress were reproduced by allowing the animal to inhale ragweed pollen or ragweed extract. On skin testing, positive reactions were obtained to ragweed antigen. Other plant pollens, poultry and horse danders have been incriminated in similar allergic reactions in dogs ranging from hay fever to more severe respiratory distress (Walton, 1968).

b) CUTANEOUS REACTIONS

The manifestation of allergic response most commonly encountered in cats and dogs has been dermatitis. Walton (1967) gave an account of 100 confirmed cases of skin responses to ingested allergens, 82 in dogs and 18 in cats. Diagnosis was carried out most successfully by test meal

administration. The skin responses were, in 16 cases, accompanied by enteric involvement and, in 2, by respiratory involvement. Walton pointed out that of the many causes of skin disease in small animals allergy was responsible in only about 1% of all animals showing skin disease.

Walton (1966) described allergic skin changes occurring in dogs and cats in association with roundworm, tapeworm and ectoparasite infestations. Hypersensitivity to bacteria and fungi was also suspected in some dogs and cats. Contact with carpets or polishes was often responsible for contact dermatitis (Walton, 1968). These lesions sometimes appeared suddenly after many years of exposure to the agent responsible. Eisen in 1959 showed that such sensitising agents combined strongly with animal protein. Frey and Wenk in 1956 found regional lymph nodes to play an essential role in initiation of contact dermatitis in the guinea-pig. The skin response to contact with the appropriate allergen could be delayed

for as long as 8 days, making identification of the allergen difficult (Walton, 1968).

McCusker and Aitken (1967) produced lesions experimentally with the object of clarifying the aetiology and pathogenesis of feline eczema. McCusker (1965) showed that the mast cell and histamine content of the skin was increased in this condition. They produced cutaneous hypersensitivity to bovine normal serum and serum albumin in cats by injections of the antigens in Freund's adjuvant (McCusker and Aitken, 1967). Intradermal challenge after an interval of 10 or 11 days resulted, within several minutes to 4 hours, in local cutaneous anaphylaxis and, after approximately 4 to 24 hours, in local Arthus reactions. No relationship was apparent between the severity of reactions and circulating levels of precipitating antibody. Mast cell degranulation was described in cutaneous anaphylactic but not in Arthus reactions.

6) HUMANS

Allergic disorders in man have been extensively investigated. This subject was reviewed by Gladstone (1964).

General anaphylaxis has not been described frequently in man. Sutliffe in 1930 observed anaphylaxis in 6.1% of 371 patients who were injected with serum in the treatment of pneumonia. The time interval between sensitising and shocking doses was at least 12 days but reactions could occur on exposure after an interval of as long as 10 years. The reaction could be produced by as little as 0.05 ml. serum injected subcutaneously. Reactions in humans affecting the lungs primarily, as in the guinea-pig, or the pulmonary arterial system, as in the rabbit, or showing hypotension as the predominant symptom, as in the dog, have been described. Thus the nature of the reaction appeared more variable in humans than in other species.

The Arthus Phenomenon (Arthus, 1903), involving damage to capillary endothelium, extravasation of blood and necrosis, was produced in man as a result of injecting horse serum subcutaneously repeatedly at intervals of several days. Although the skin is the site most frequently affected, any organ may be involved. Pepys (1966) believed this form of reaction to be involved in pulmonary hypersensitivity diseases.

The term "atopy" was applied to allergic diseases of the immediate type which occurred only in certain individuals and was believed to be associated with hereditary factors. The symptoms of such atopic reactions varied, ranging from urticarial skin lesions to gastro-intestinal disturbances and asthma. The nature of the symptoms was influenced by the route of exposure to the allergen. The antibodies involved in atopy were given the name "reagins." These could not be detected in serum by complement fixation, agglutination

or precipitation tests, but were detected by means of the Prausnitz-Kustner reaction (P-K reaction)(Prausnitz and Kustner, 1921). This reaction was produced by injecting serum from an atopic individual into the skin of a normal individual. When the allergen to which the atopic individual was sensitive was injected after an interval of 24 hours, a positive reaction was manifest at the site of the serum injection within 1½ hours, as a wheal and flare. The ability of the reagins to prepare skin for such a reaction was destroyed by heating the serum for 4 hours at 56°C (Kuhns and Pappenheimer, 1952). Reaginic antibody was identified by Ishizaka, Ishizaka and Hornbrook (1966a,b) as belonging to the immunoglobulin class E designated IgE. Wide, Bennich and Johansson in 1967 showed that allergic patients had 6 times as much IgE in their serum as had control individuals and that the capacity of individuals to produce reagins or IgE was related in cases of multiple allergic sensitivity to the

number of allergens involved. The hereditary factor involved in atopy was believed to be a predisposition to produce reaginic antibodies (Smith, 1964). Although reagins did not readily sensitise tissue passively for Schultz-Dale reactions, Schild, Hawkins, Mongar and Herxheimer (1951) showed that a Schultz-Dale response could be obtained from the tissues of the allergic individual in which the reagins were produced. They demonstrated this using bronchial rings from a child sensitive to pollen.

DRUG ALLERGY

Among the allergens of a non protein nature and low molecular weight, incriminated in producing hypersensitivity reactions, are many drugs such as penicillin and sulphonamides. Landsteiner (1945) showed that such haptens combined in vivo with protein and that this was necessary for sensitisation to occur. Eissen, Orris and Belman (1952) showed that the formation of covalent irreversible bonds with skin protein

was necessary also for elicitation of delayed allergic skin reactions to haptens. Drug Allergy could be manifested in a variety of ways including immediate anaphylaxis, atopy, delayed hypersensitivity and, in the case of drugs such as sulphanamides which persisted in the blood for several days, serum sickness.

REACTIONS TO INHALED ORGANIC ANTIGENS

ASPERGILLUS FUMIGATUS. Pepys (1966) reviewed the subject of pulmonary hypersensitivity disease due to inhaled organic antigens. He described how inhaled spores of Aspergillus fumigatus could act as allergens and on subsequent exposure produce asthma in some individuals. In 10% to 38% of such patients immediate skin reactions, mediated by reaginic antibodies, were obtained and on inhalation of antigen the bronchial mucosa became oedematous and infiltrated with eosinophils. Some cases of asthma were complicated by eosinophilia with transitory pulmonary infiltration of eosinophils. These patients were found to possess precipitating as well as reaginic, antibodies to aspergillus allergen.

On skin testing, as well as an immediate reaction, a second, more delayed, Arthus type of reaction was obtained 3 to 4 hours after intradermal injection of the allergen. Aspergillus, being a common saprophyte or pathogen of the respiratory tract, was present in many humans who were not hypersensitive to it. Such individuals showed neither the immediate nor the more delayed skin reaction, although their sera contained precipitins.

Siqueira and Bier (1961) and Bier, Passos and Siqueira (1968) investigated a dual type of skin reaction which appeared to require both reagins and precipitins in guinea-pigs. They concluded that the PCA reaction in some way other than by increasing permeability enhanced uptake of antigen-antibody aggregates by vascular endothelium. Dual reactions to inhalation tests were also produced using Aspergillus by Pepys, Riddell, Citron, Clayton and Short in 1959. These took the form of an immediate asthmatic reaction followed several hours

later by a more severe and prolonged reaction accompanied by fever. The initial reaction was believed to be mediated by reaginic antibodies and the more delayed reaction by precipitins.

FARMERS' LUNG AND SIMILAR DISEASES. This occurred commonly in farmers as a result of repeated inhalation of dust from mouldy hay and other vegetable matter. "Pigeon fanciers' lung" or "bird breeders' lung" occurred as a result of inhalation of dust of pigeon or budgerigar excreta. The therapeutic inhalation of bovine or porcine pituitary snuff, in patients suffering diabetes insipidus, was also found to produce the syndrome. Farmers' lung affected the peripheral part of the respiratory system, interfering with gaseous exchange. The symptoms of coughing, dyspnoea and fever developed at varying intervals after exposure to the antigen involved. Pulmonary ventilation perfusion and compliance was reduced.

Dual skin reactions mediated by reagins and precipitins were obtained as in pulmonary aspergillosis. In the case of Farmer's lung precipitins against thermophilic actinomycetes, particularly Thermopolyspora polyspora extracted from mouldy hay, were detected in the sera of 87% of affected individuals. These antigens have been termed "farmer's lung hay antigens" (FLH antigens). Skin tests in farmers' lung cases were described as unsatisfactory but inhalation of extracts of mouldy hay or Thermopolyspora polyspora produced symptoms after 5 or 6 hours. Precipitins were found in the sera of 18% of individuals exposed to FLH antigens but not suffering from the disease.

MEDIATORS OF ALLERGIC REACTIONS IN HUMANS

Brocklehurst (1956) found that histamine and SRS-A were liberated, on exposure to pollen allergen, in vitro from perfused lung obtained from an individual who was sensitive to pollen. In vivo exposure to pollen

resulted in an attack of asthma in this individual. Brocklehurst showed that human bronchioles were more sensitive to SRS-A than were those of any other species. Also SRS-A increased the sensitivity of tissues to histamine. Rose (1947) showed that individuals showing the atopic form of hypersensitivity were more than normally sensitive to histamine. These findings suggested that histamine and SRS-A might be involved in the mediation of atopic reactions in humans.

Antihistamines have been extensively used in the treatment of asthma but their efficacy has been poor (leading article, Lancet, 1968). Disodium cromoglycate was shown to inhibit release of histamine and SRS-A from sensitised human lung, in vitro (Sheard, Killingback and Blair, 1967) and to have a specific inhibitary effect on anaphylactic processes initiated by reaginic antibody-antigen interactions (Cox, 1967). This drug was developed for use in the treatment of asthma. Altounyan (1967) found that inhalation of disodium cromoglycate

inhibited immediate asthmatic reactions to inhaled allergen and Howell and Altounyan (1967) reported significant clinical improvement in asthmatic patients treated by inhalation of disodium cromoglycate with isoprenaline. Corticosteroids were popular for many years in the treatment of asthma. Schayer (1963) postulated that a physiological function of the glucocorticoids was to moderate the effects of induced histamine. Weissman and Thomas (1962) found that corticosteroids had a protective effect on lysosomes. This explained their ability to inhibit Arthus type (Type III) reactions. Lysosomes were known to be liberated from polymorphonuclear leucocytes which, in the early stages of the Arthus reaction, ingested and were destroyed by antigen-antibody aggregates combined with an activated component of complement.

SUMMARY

Even in humans, in which the importance of hypersensitivity has been a subject of investigation for many years, therapy of allergic reactions is not regularly effective. There persists a tendency to assume that diseases of animals are hypersensitive in origin if associated changes in blood and tissue concentrations of histamine have been demonstrated and, conversely, that the symptoms of such diseases should be modified by administration of antihistamines. It is unlikely that identification of the mediators of experimentally induced anaphylaxis in cattle would lead to greater therapeutic success in veterinary than has been achieved in human medicine. However, in investigating a reaction where species variation is considerable, advantage is to be gained from carrying out experimental procedures on the same species in which ultimately diagnosis and therapy of diseases of hypersensitivity is intended.

SECTION II

MATERIALS, METHODS AND RESULTS OF EXPERIMENTAL INVESTIGATIONS

1. THE NATURE OF ANAPHYLAXIS IN CATTLE AND THE CONDITIONS NECESSARY FOR ITS INDUCTION

MATERIALS AND METHODS

ANIMALS. Ayrshire, Friesian, Ayrshire-cross and Friesian-cross calves aged 4 to 6 months and weighing 100 Kg. to 200 Kg. were purchased from farms in the West of Scotland. These were housed and fed a diet of hay and concentrates. Ninety percent of the calves were castrated males, 4% entire males and 6% females. Sex differences were not found to affect anaphylaxis.

ANTIGENS. Proteins foreign to the bovine species were used as antigens. These were albumen(egg)powder (The British Drug Houses Ltd.), purified egg albumin or ovalbumin (Grade III, electrophoretic purity approx. 90%) (Sigma Chemical Co.), horse albumin (Cohn fraction V) (Koch-Light Laboratories Ltd.) and horse serum. The egg-white powder, ovalbumin and horse albumin were administered as 5% solutions in sterile saline. Horse

serum was obtained from blood taken under sterile conditions from healthy horses. The blood was incubated for 4 hr. at 37°C and for a further 20 hr. at room temperature (approximately 20°C) to allow clotting and retraction of the clot (Dacie and Lewis, 1963). The serum extruded from the clotted blood was removed by aspiration and used within the next hour. Horse serum contains 7.1 (± 0.4) gm. protein per 100 ml. (Jennings and Mulligan, 1953). Unless otherwise stated solutions of antigens were injected intravenously and animals were kept under observation for at least one hour following the injection. When adjuvant was used this was Freund's complete adjuvant (Difco Laboratories) which was emulsified with solutions of ovalbumin and injected intramuscularly.

The animals and antigens used in all experiments described in section II (1 to 7) of this thesis were as described above, unless otherwise stated.

SENSITISING DOSE

In a preliminary experiment involving 3 animals, albumen egg powder was administered at a dose rate of 25 mg/Kg. This was half the dose used by Thomlinson and Buxton (1963) to sensitise pigs. Subsequently a dose of 15 mg/Kg. was found to be equally effective and this dose of purified ovalbumin was used in the 36 animals employed in the main investigation of the conditions necessary for induction of anaphylaxis in cattle. Sensitisation was not detected following doses of 3 mg/Kg. but determination of the minimum dose necessary for sensitisation was not carried out.

In 5 of a total of 15 animals, which received crude albumen egg powder, tachypnoea and coughing developed one to 2 min. after administration of the sensitising injection. No such effects were produced by purified ovalbumin, horse albumin or horse serum.

DETERMINATION OF LATENT PERIOD

a) OVALBUMIN

Twenty calves received a sensitising dose of 15 mg/Kg. of ovalbumin by intravenous injection. After an interval of 24 hr., 2 of these were challenged by administration of a second intravenous injection of ovalbumin at the same dose rate. A second pair of calves was challenged in the same way on the second day following the sensitising injection and a third pair on the third day. This procedure was continued until the seventh day when both calves showed respiratory distress manifested as predominance of abdominal respiratory movements, extension of the neck with the head held low and the mouth open and grunting on expiration. As indicated in Table 1. 1, increased respiratory rates were observed on challenge as early as 2 days after the sensitising injection but an interval of 7 days was necessary to produce a reaction involving definite dyspnoea as well as coughing,

NOTE: Assessment of Severity of Symptoms as Shown in Tables 1. 1, 1. 2, 1. 4 and 1.6.

Tachypnoea: respiratory rate increased x > 2

Coughing: + → +++ = occasional → frequent

Dyspnoea: + → +++ = mild → very severe

Collapse + = recumbent for < 30 sec.

++ = recumbent for 30 to 60 sec

+++ = recumbent for > 60 sec.

Nasal Discharge,)

Lacrimation,): + → +++ = slightly increased →

Salivation) very profuse

TABLE 1.1 EFFECTS OF CHALLENGE WITH OVALBUMIN AT DIFFERENT TIME INTERVALS AFTER INITIAL INJECTION

Interval days	Calf No.	Symptoms						
		Tachypnoea	Coughing	Dyspnoea	Collapse	Nasal Discharge	Lacrimation	Salivation
1	1	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-
2	3	+	+	-	-	-	-	-
	4	+	+	-	-	-	-	-
3	5	-	-	-	-	-	-	-
	6	+	-	-	-	-	-	-
4	7	+	-	-	-	-	-	-
	8	-	-	-	-	-	-	-
5	9	+	-	-	-	-	-	-
	10	+	+	+	-	-	-	-
6	11	+	++	+++	+++	-	-	-
	12	+	++	-	-	-	-	-
7	13	+	+++	+++	++	+	+++	+
	14	+	+++	++	+	-	++	++
	15*	+	+	+++	+++	++	+	+++
	16	+	++	+++	+++	++	+++	+++
14	17	+	++	++	+	+	++	+
	18	+	+++	+	-	+	++	+
	19	+	+++	++	-	-	++	++
21	20	+	+	++	+	++	++	+

* Died

salivation and lacrimation. This was confirmed on challenging a further pair of calves 7 days after sensitisation. Increasing the time interval between sensitising and challenging injections beyond 7 days did not increase the severity of the response, the outcome of which was fatal in one animal challenged on the seventh day. In all subsequent experiments, intervals of 7 or 14 days between sensitisation and challenge, and of at least 7 days between successive challenges were used.

b) HORSE SERUM

When horse serum was used, 2 sensitising injections followed by an interval of not less than 7 days were found to be necessary. Eight calves received intravenous injections of horse serum at a dose rate of 15 mg. protein per Kg. body weight. These animals did not show anaphylactic reactions when they received second injections of horse serum, at the same dose rate, 4, 7 or 14 days later. Third injections of this antigen given to one animal 3 days after the second

injection and to another 4 days after the second injection produced no effects. However, reactions were produced in all 6 animals which received the third injection of horse serum 7 days after the second injections. These findings are summarised in Table 1. 2.

c) HORSE ALBUMIN

Two animals received horse albumin intravenously at a dose rate of 15 mg/Kg. weekly for 5 weeks. Anaphylaxis was not induced on any occasion. Each animal subsequently showed a typical anaphylactic reaction to 3.0 mg/Kg. of ovalbumin administered one wk. after a sensitising injection of 15 mg/Kg. of this protein.

One animal received horse albumin intravenously at a dose rate of 15 mg/Kg. followed by 3.0 mg/Kg. weekly for the next 2 wk. and 7.5 mg/Kg. for 2 further wks. A positive reaction occurred only on the fourth occasion of challenge.

TABLE 1.2 EFFECTS OF CHALLENGE WITH HORSE SERUM AFTER DIFFERENT TIME INTERVALS

Interval (Days) after 1st injection	Calf No.	Tachypnoea	Coughing	Symptoms				
				Dyspnoea	Collapse	Nasal Discharge	Lacrimation	Salivation
1st Challenge								
4	1	-	-	-	-	-	-	-
7	2	-	-	-	-	-	-	-
	3	+	-	-	-	-	-	-
	4	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-
14	6	+	+	-	-	-	-	-
	7	-	-	-	-	-	-	-
	8	-	-	-	-	-	-	-
2nd Challenge								
4 + 3	1	-	-	-	-	-	-	-
7 + 4	2	-	-	-	-	-	-	-
7 + 7	3	+	+	+	-	-	+	-
	4	+	++	+++	-	+++	+	+++
	5	+	-	-	-	-	-	-
14 + 7	6	+	+	+++	-	++	+	+
	7	+	+	++	-	-	++	-
	8	+	+	+++	+++	-	+++	+++

SYMPTOMS OF ANAPHYLACTIC SHOCK

When a challenge dose of 15 mg/Kg. ovalbumin was given intravenously 7 days after a sensitising dose of 15 mg/Kg. ovalbumin, a severe systemic reaction was produced. Within 2 min. of completion of the injection the animals collapsed on to their sides. The neck was extended and the legs made kicking movements. Nystagmus was shown and cyanosis of visible mucous membranes developed following periods of apnoea lasting 10 to 30 sec. The animals made violent respiratory efforts with the mouth open and coughing often produced cream frothy fluid mixed with blood, or mouthfuls of bright red blood. In 2 cases respiration ceased 7 and 10 min. respectively after injection of the antigen. Survivors showed less respiratory distress after 5 to 10 min., raised their heads and remained in sternal recumbency for a further 10 to 15 min. then stood up. Salivary, nasal and lacrimal secretions were profuse and the skin of the muzzle and mucous

membranes showed congestion. One animal collapsed for a second time after 15 min., with blood-stained cream frothy fluid streaming from the nostrils. This animal showed increasingly severe dyspnoea and died after 25 min. Depression and dyspnoea was evident for 30 to 40 min. following the injection in most animals. They stood with the neck extended, head low, mouth open and tongue protruding. Abdominal respiratory movements were marked and grunting accompanied each expiration. Rectal temperature did not rise by more than $1^{o}C$. Contractions of the rumen were suppressed during the phase of dyspnoea and moderate tympany of the rumen was sometimes seen. Four animals on different occasions showed evidence of pruritis, attempting to scratch their ears, licking their muzzles and rubbing themselves against the sides of their pens. In 3 of these animals this symptom was shown only once, although each was challenged on at least 6 occasions. The fourth animal showed pruritis every time it was challenged. One third

of all the animals challenged showed a variable degree of trembling. With 5 exceptions animals appeared to have recovered completely within 2 hr. One animal died after showing dyspnoea for 30 hr. after challenge, 3 showed slight dyspnoea for 48 hr. after challenge but recovered and one was killed with a humane killer when still severely depressed and showing no reduction in respiratory distress after 48 hr.

The severity of the reaction varied in relation to the challenge dose. Animals reacting less severely showed less respiratory distress and usually did not collapse. Staggering and incoordination were frequently seen. The minimal respiratory changes recorded as a positive reaction were abdominal respiratory movements and increased respiratory rate.

The symptoms of anaphylaxis are summarised in Table 1. 3. under the heading "regular","frequent" and "occasional". Assessment of the severity of the reaction was based on those symptoms which occurred regularly, with the exception of

TABLE 1. 3

SYMPTOMS OF ANAPHYLAXIS IN CATTLE

Regular	Frequent	Occasional
Tachypnoea	Collapse	Trembling
Dyspnoea	Nasal discharge	Pruritis
Coughing	Lacrimation	Ruminal tympany
Depression	Conjunctival congestion	Sneezing
	Salivation	Defaecation
		Urination

depression which was a subjective observation, and those which occurred frequently. Tachypnoea was recorded if the respiratory rate doubled. Dyspnoea ranged in severity from obvious abdominal respiratory movements with harsher bronchial respiratory sounds than normal (+), through expiratory grunting (++) to open-mouthed gasping (+++). In mild reactions coughing was non-productive while in severe reactions coughing produced fluid and blood. High pitched ronchi were heard on auscultation of the chest of mildly affected animals. In addition fluid râles were marked in severely affected cases.

The time of onset of the reaction was measured as the time from completion of the injection until the appearance of the first symptoms. This time interval ranged from 15 to 120 sec. The duration of the reaction was measured as the time from the onset of the symptoms until symptoms were no longer present. Tachypnoea was judged to be absent when the respiratory rate had returned to the preinjection value and adventitious

respiratory sounds were no longer detectable. The duration of reactions ranged from 10 to 120 min. with the exception of the 5 instances described above.

A symptom of more delayed onset was diarrhoea. This developed 48 to 72 hr. after challenge and persisted for 48 to 72 hr.

DETERMINATION OF A SUITABLE CHALLENGE DOSE

Challenge by intravenous administration of 15 mg/Kg. of ovalbumin, which was the dose used for sensitisation, resulted in severe respiratory distress in all 36 animals and in death in 3 of the animals which received this dose. In order to determine the minimum effective challenge dose 6 animals received doses of ovalbumin ranging from 0.5 to 15 mg/Kg. As summarised in Table 1. 4, the 3 animals challenged with a dose of 0.5 mg/Kg. showed only coughing and tachypnoea. One of the 3 collapsed but got to his feet again in less than 30 sec. Doses of 1.0 mg/Kg. and 1.25 mg/Kg. produced

TABLE 1.4 DETERMINATION OF MINIMUM EFFECTIVE CHALLENGE DOSE OF OVALBUMIN

Dose mg/Kg.	Calf No.	Onset sec	Duration min	SYMPTOMS						
				Tachypnoea	Coughing	Dyspnoea	Collapse	Nasal Discharge	Lacrimation	Salivation
0.5	E2	30	15	+	+	-	-	-	-	-
	E4	15	15	+	+	-	+	-	-	-
	E1	300	15	+	+	-	-	-	-	-
1.0	E3	30	20	+	+	+	+	+	-	-
1.25	E7	2	15	+	+	+	+	+	-	-
1.5	412	2	30	+	++	++	+	+++	+++	++
	412	2	25	+	++	+	-	+	++	+
	E2	15	15	+	++	-	++	+	+	-
	E4	10	10	+	++	+	-	-	-	-
	E1	0	15	+	+	+	-	-	+	-
	E3	15	30	+	++	+	++	+	-	+
	E7	30	15	+	+	+	-	-	+	+

slight dyspnoea, tachypnoea, occasional coughing, collapse for less than 30 sec. and slight nasal discharge in the animal receiving each dose. All 6 animals showed more marked symptoms when a dose of 1.5 mg/Kg. was used. This dose was taken as being the minimum effective challenge dose and in subsequent experiments doses ranging from 1.5 mg/Kg. to 15 mg/Kg. were used.

GROUPING OF ANIMALS

The numbers of animals used in the above experiments are summarised in Table 1. 5. Twenty animals were used to determine the latent period for hypersensitivity to ovalbumin to develop. Eight of these were subsequently used to determine the latent period for hypersensitivity to horse serum to develop. Three animals received horse albumin and a group of 6 was used to determine the minimum challenge dose. A total of 36 animals were sensitised by intravenous injections as described above.

TABLE 1.5 NUMBERS OF CALVES USED IN EXPERIMENTS

Experiment	No. of calves
Sensitisation	36
Determination of latent period (ovalbumin)	20
Determination of latent period (horse serum)	8
Determination of latent period (horse albumin)	3
Determination of minimum challenge dose	6
Total number of calves used	36

CHALLENGE OF ANIMALS SENSITISED TO TWO ANTIGENS

Two animals, sensitised to both ovalbumin and horse serum, were challenged by intravenous injections of horse serum to which they reacted typically. When challenged 24 hr. later with ovalbumin, they reacted minimally, showing only an increase in respiratory rate with abdominal respiratory movements. In one case, 20 min. after injection of ovalbumin, cutaneous lesions became visible on the head and neck. These were circular, firm, raised, freely-moveable swellings 5 to 35 mm. in diameter. After 30 min. similar lesions appeared on the body and the legs. They did not seem to cause the animal any discomfort. They were still palpable but not visible after 15 hr. and were no longer palpable after 18 hr. When the above injection procedure was repeated, using the same animals the results were confirmed, the skin reactions being shown only by the same animal as before. Injection of ovalbumin alone 7 days later did not produce these cutaneous reactions but severe respiratory distress and other regular features of anaphylaxis occurred.

CHALLENGE BY ROUTES OTHER THAN INTRAVENOUS

Administration of ovalbumin subcutaneously to 2 and intramuscularly to 4 sensitised calves, at the same dose rate as that producing reactions when given intravenously, produced no effects.

SENSITISATION USING FREUND'S ADJUVANT

Six calves were sensitised using the technique described by Dungworth (1965). These were given 8 serial intramuscular injections at weekly intervals, of 250 mg. ovalbumin as a 5% solution in saline emulsified with 5 ml. complete Freund's adjuvant. These animals were challenged by intravenous injection of ovalbumin at intervals after completion of the sensitisation course. As shown in Table 1. 6, all 6 reacted severely to relatively small challenge doses. Reactions were produced by as little as 0.03 mg/Kg. Animals sensitised by an intravenous injection of antigen required challenge doses of at least 1.5 mg/Kg. Two animals showed respiratory distress

TABLE 1.6 EFFECTS OF CHALLENGE AFTER SENSITISATION USING FREUND'S ADJUVANT

Calf No.	Dose mg/Kg	Interval days	Symptoms							
			Onset sec	Duration min	Collapse	Dyspnoea	Cough	Nasal Discharge	Lacrimation	Salivation
68	0.03	330	15	35	-	++	+	+	+++	+++
68	0.03	345	60	40	-	+	+++	-	++	-
50	0.3	14	15	120	+++	+++	-	-	+++	+
69*	0.3	75	60	120	+++	+++	++	+	+++	++
51	1.5	7	60	60	+++	+++	+	+	++	++
35•	1.5	54	15	60	+++	+++	+	-	+	-
34•	3.0	54	10	75	+++	+++	++	++	++	++

• Respiratory distress shown after 7th intramuscular injection.

* Challenge dose of ovalbumin replaced by horse albumin

Interval = Number of days after last intramuscular sensitising injection.

and signs of irritation of the skin after the seventh intramuscular injection of antigen and Freund's adjuvant. These symptoms lasted for approximately 15 min. One animal (No. 69) sensitised, like the others, by injections of ovalbumin and Freund's adjuvant was injected intravenously with 0.3 mg/Kg. horse albumin. This, as shown in Table 16, produced a severe reaction. Animals sensitised by intravenous injection of ovalbumin without Freund's adjuvant showed no response to the intravenous injection of 1.5 mg/Kg. horse albumin. It appeared that the use of adjuvant resulted in a less specific sensitisation. Firm, apparently painless, swellings developed at the sites of intramuscular injections of antigen and adjuvant emulsion. These persisted for 3 to 4 wk. regressing slowly.

2. THE PHYSIOLOGICAL, HAEMATOLOGICAL AND PATHOLOGICAL CHANGES OCCURRING DURING ANAPHYLAXIS

a) PHYSIOLOGICAL

MATERIALS AND METHODS

Physiological parameters were measured in anaesthetised animals. Food was witheld from these animals for 48 hr. prior to anaesthesia in order to reduce the risk of ruminal tympany, regurgitation and inhalation of ruminal contents. Induction of anaesthesia was achieved by intravenous injection of sodium pentobarbitone (Abbott Ltd.) as a 6% solution at a dose rate of 14 mg/Kg. Animals were maintained, for the 3 to 4 hr. for which the experiments lasted, in the second plane of the third stage of anaesthesia. This medium depth of anaesthesia was characterised by central fixation of the eyeball, regular thoraco-abdominal respiration and absence of the pedal, palpebral, conjunctival and corneal reflexes. Sodium pentobarbitone was administered to effect

during the experiments when lightening of anaesthesia was detected on testing ocular reflexes. The average total dose of sodium pentobarbitane administered to 16 animals was 30 mg/Kg. At the conclusion of each experiment which did not involve a fatal reaction the animal was killed by an additional dose of 20 mg/Kg. sodium pentobarbitone.

Tracheotomy was carried out to allow insertion of an endotracheal tube which was attached, via a Fleisch pneumotachograph (Metabo Epalinges Lusanne), to a differential pressure transducer (the Infra Red Development Co., Ltd.) to measure air flow. This was recorded, as were all other parameters measured, on the heat sensitive paper of a multichannel physiological pen recorder (Type M8 Devices, Ltd.). From the record obtained respiratory rate could be read. Respiratory minute volume was measured over alternate 30 sec. periods by means of a Wright's respirometer (British Oxygen Co., Ltd.) attached to the Fleisch tube (Fig. 2. I)

RESPIRATORY RECORDING APPARATUS

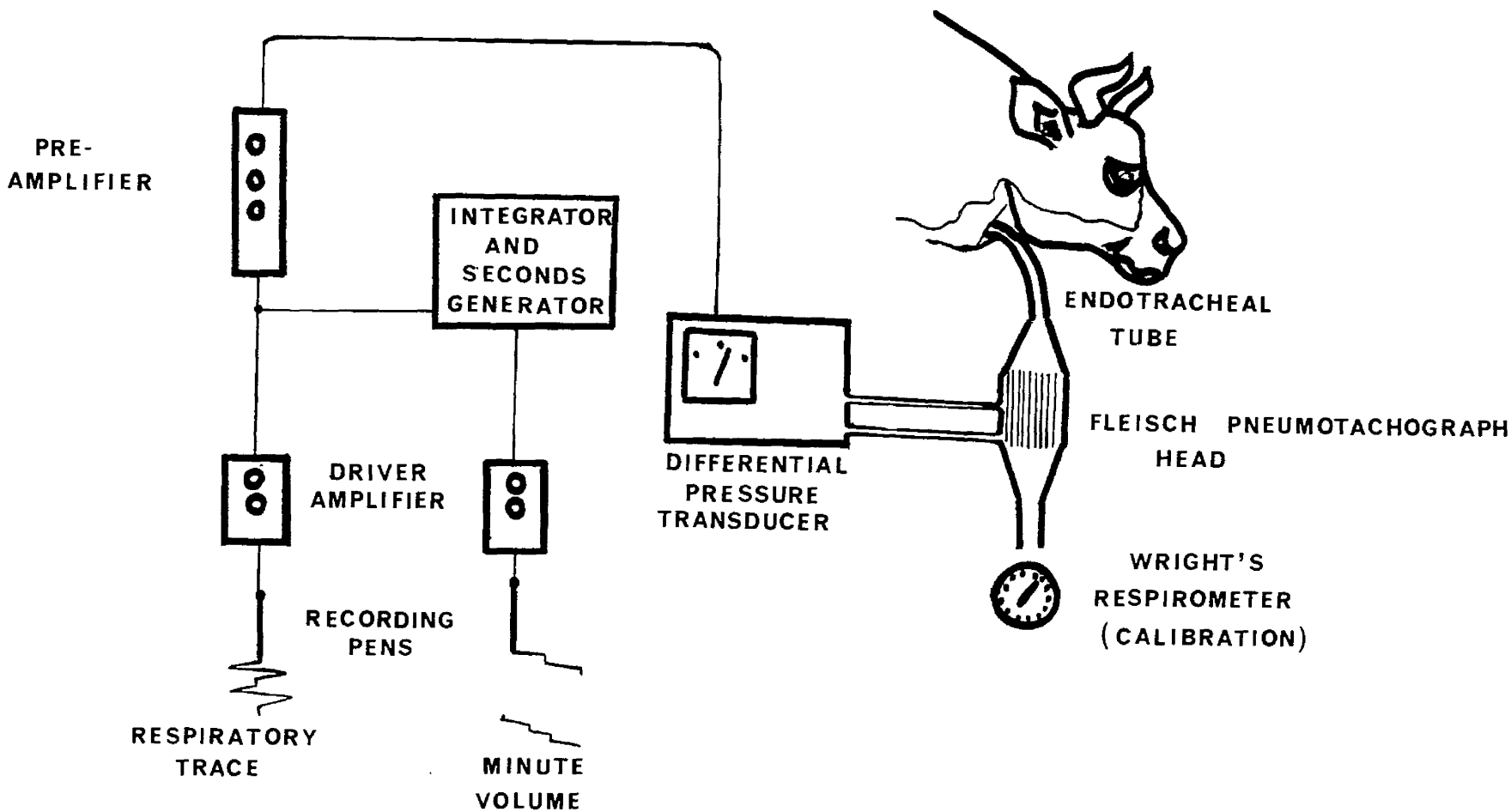


Fig. 2.1 - Diagramatic representation of measurement of air flow and minute volume in anaesthetised calves.

or measured continuously by means of an integrator (Devices Ltd.). The input to the pen recorder from the differential pressure transducer was passed to the integrator as shown in Fig. 2. 1. Volumes were integrated over 10 sec. either on inspiration or on expiration. This in turn was recorded by means of the physiological pen recorder. The integrator was calibrated prior to each experiment by means of the Wright's respirometer.

Heart rate was measured by a Neilson instantaneous heart rate meter (Devices Ltd.) driven by a signal from an electrocardiograph (Devices Ltd.) using standard limb lead II.

Systemic blood pressure was measured from a cannula introduced into a carotid artery and connected to a Statham strain guage pressure transducer, calibrated prior to each experiment by means of a mercury manometer. Pulmonary blood pressure was measured in the same way after catheterisation of the pulmonary artery. To reach this artery a catheter

was inserted in a jugular vein and insertion continued until a length of catheter approximately equal to the distance between the point of insertion in the neck and the third rib, had been passed into the vein. The catheter was then judged to have reached the right atrium or the right ventricle of the heart. Examination of the form and magnitude of the pressure being recorded allowed detection of passage of the catheter, on further insertion, from the right ventricle into the pulmonary artery. Fig. 2. 2 shows a recording obtained when the catheter was first in the right ventricle and then in the pulmonary artery. In every case the position of the catheter was checked by post mortem examination. Failure to catheterise the pulmonary artery was due, in those cases where this occurred, to coiling of the catheter in the right atrium. The catheters and cannulae used were of polythene or nylon (Portex Ltd.) of external diameter 2 to 3 mm. These were filled with anticoagulant heparin solution (Boots Pure Drug Co.) containing

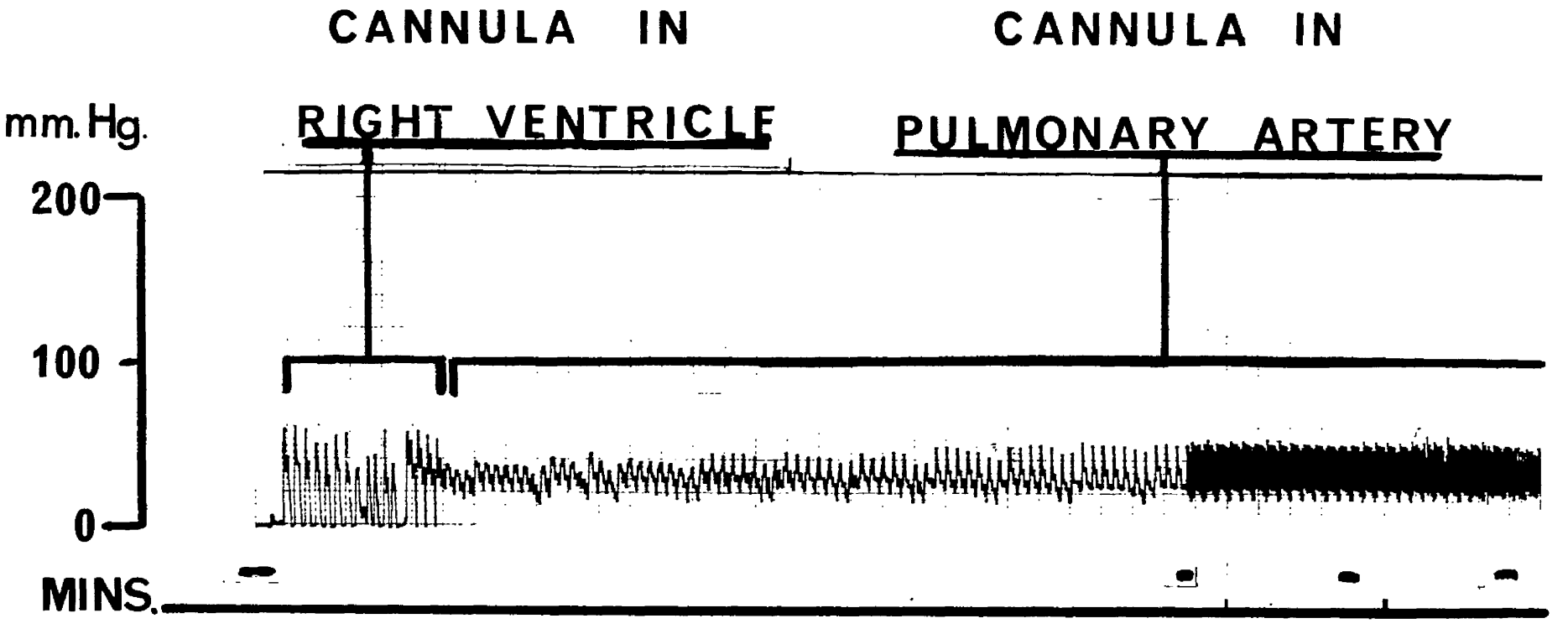


Fig. 2.2 - Record of pressure during passage of cannula through right ventricle into pulmonary artery.

1,000 units per ml. before insertion into the blood vessels. All drugs were administered through a cannula inserted into a recurrent tarsal vein and, immediately following administration of each drug, 5 ml. of saline containing 10 units of heparin per ml. was injected. Injections were carried out at a rate of approximately 0.5 ml. per sec. Antigens were administered in the form of 5% solutions with the exception of horse serum which was approximately equivalent to a 7% solution of protein. Animals therefore received injected antigen at rates of approximately 25 mg. or 35 mg. per sec.

RESULTS

Ovalbumin was administered to 5 anaesthetised animals in doses ranging from 7.5 mg/Kg. to 100 mg/Kg. Of these one animal was unsensitised, never having been exposed previously to ovalbumin, one had been sensitised using Freund's adjuvant and the remaining 3 had been sensitised

by intravenous injections of ovalbumin without Freund's adjuvant. Horse serum (12.5 mg/Kg.) was administered to one animal, sensitised, without Freund's adjuvant, to this antigen. The challenge dose of antigen used and its effects in each case are summarised in Table 2. 1. The time of onset of the reaction was measured as the time interval between commencement of administration of antigen and manifestation of the first changes in respiratory, blood pressure or heart rate records. The duration of the reaction was measured as the time from onset of these changes until the respiratory and cardiovascular parameters had returned to preinjection levels or, in fatal reactions, until death supervened. Apnoea, as recorded in Table 2. 1, was the longest period during which no respiration occurred. Changes in minute volume, systemic and pulmonary blood pressures and heart rate, as recorded in Table 2. 1, were the maximum changes measured, expressed as percentages of preinjection values.

Table 2. 1.

EFFECTS OF CHALLENGE ON ANAESTHETISED CALVES

NOTE. The recorded change in minute volume accompanying apnoea was determined by the method used to measure minute volume. Where this was measured, over alternate 30 sec. periods by means of a respirometer, the reductions were recorded as 100% only where apnoea lasted for at least 30 sec. while respiratory volume was being measured. Where the respiratory tidal volume was integrated over 10 sec. periods, reductions of 100% in minute volume were recorded when apnoea lasted for 10 sec. or longer.

TABLE 2.1

EFFECTS OF CHALLENGE ON ANAESTHETISED CALVES

Antigen	Sensitisation	No.	Challenge Dose	Onset	Duration	Apnoea	Minute Volume	Systemic B.P.	Pulmonary B.P.	Heart Rate
			mg/Kg	sec.	min.	sec.	% Fall	% Fall	% Rise	% Fall
Ovalbumin	U	39231	20	-	-	0	0	0	0	0
Ovalbumin	FS	39350	7.5	12	8*	90	100	100	233	19
Ovalbumin	S	29438	100	20	4	50	50	40	100	N.M.
Ovalbumin	S	36220	12.5	12	5	42	66	78	100	31
Ovalbumin	S	32053	12.5	10	6	32	46	83	133	NM.
			12.5●	10	15*	0	15	100	71	N.M.
Horse Serum	S	33812	12.5	100	3	30	38	100	50	N.M.

U = Unsensitised
FS = Freund's sensitised (sensitised using Freund's Adjuvant).
S = Sensitised (sensitised without using Freund's Adjuvant)
* = Died
● = Second dose
N.M. = Not measured

The unsensitised animal showed no change in any of the parameters measured when it was subjected to a dose of 20 mg/Kg. of ovalbumin. Sensitised animals exhibited changes 10 to 100 sec. after commencement of administration of the antigen. There was simultaneous onset of apnoea, systemic hypotension and pulmonary hypertension. Apnoea persisted for 30 to 90 sec. and was accompanied by falls of 15% to 100% in minute volume. This was followed by increased minute volume before return to the preinjection volume in non-fatal reactions. Animals which died showed recurrence of apnoea 1 to 2 min. after the initial period of apnoea, making irregular gasping respiratory efforts before death. Systemic blood pressures fell rapidly by 40% to 100% within 10 sec. returning to preinjection levels in non-fatal cases after 3 to 5 min. Pulmonary blood pressures rose within 10 sec. by 50% to 233%. This rise was not sustained. After 30 sec. pulmonary arterial pressures fell to preinjection levels.

After one to 3 min. a secondary rise in pulmonary pressure occurred in 4 of 6 animals, falling again more slowly over one to 2 min.

Heart rate was not recorded in all cases but fell by 19% and 31% respectively in the 2 cases in which it was measured, returning to normal in the non-fatal case after 30 sec.

Fatal reactions occurred in 2 of the 6 animals challenged. One of those which died was the one animal sensitised using Freund's adjuvant and the challenge dose of ovalbumin which resulted in death after 8 min. was 7.5 mg/Kg. This dose consistently produced non-fatal reactions in conscious animals sensitised without the adjuvant. Doses of 12.5 mg/Kg. and of 100 mg/Kg. in one animal, produced non-fatal reactions in anaesthetised animals sensitised without the adjuvant. The other animal which died did so after receiving 2 doses of 12.5 mg/Kg. of ovalbumin separated by an interval of 12 min.

Systemic blood pressure was falling when the second injection was given. Death occurred 15 min. after administration of the second injection.

When a second dose of antigen, equal to the first, was given to 3 animals when all parameters had returned to preinjection levels no changes were found to occur. These injections were given 15 to 30 min. after administration of the initial challenging injections.

The records obtained during a non-fatal reaction in animal No. 36220 are shown in Fig. 2. 3. Those obtained during the fatal reaction in animal No. 39350 are shown in Fig. 2. 4. The blood pressure recording was interrupted as shown in Fig. 2. 4 in order to sample blood from the cannula in the carotid artery.

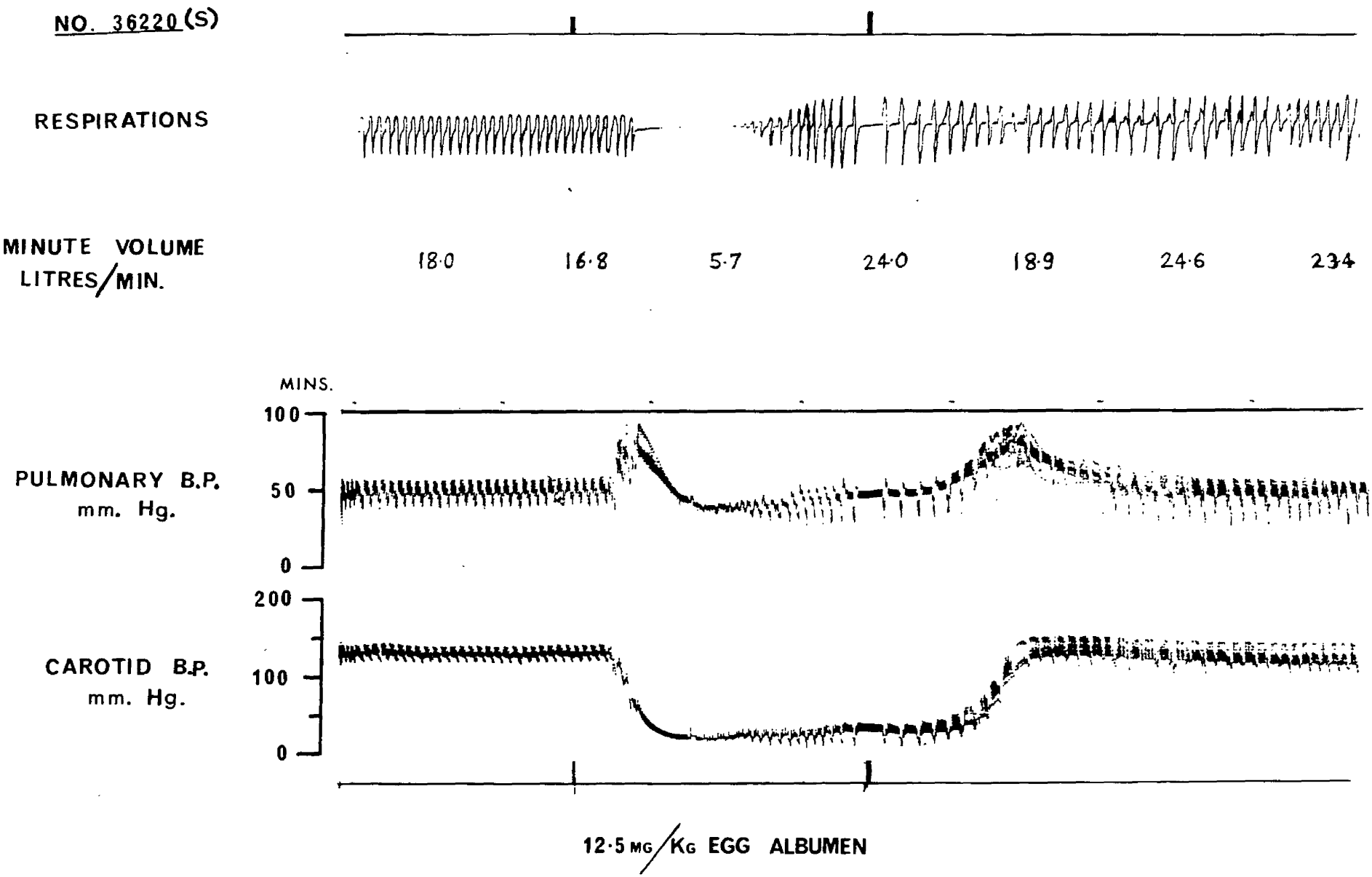


Fig. 2.3 - Effect of challenge on anaesthetised calf sensitised without adjuvant.
Antigen injected between the marks (I I).

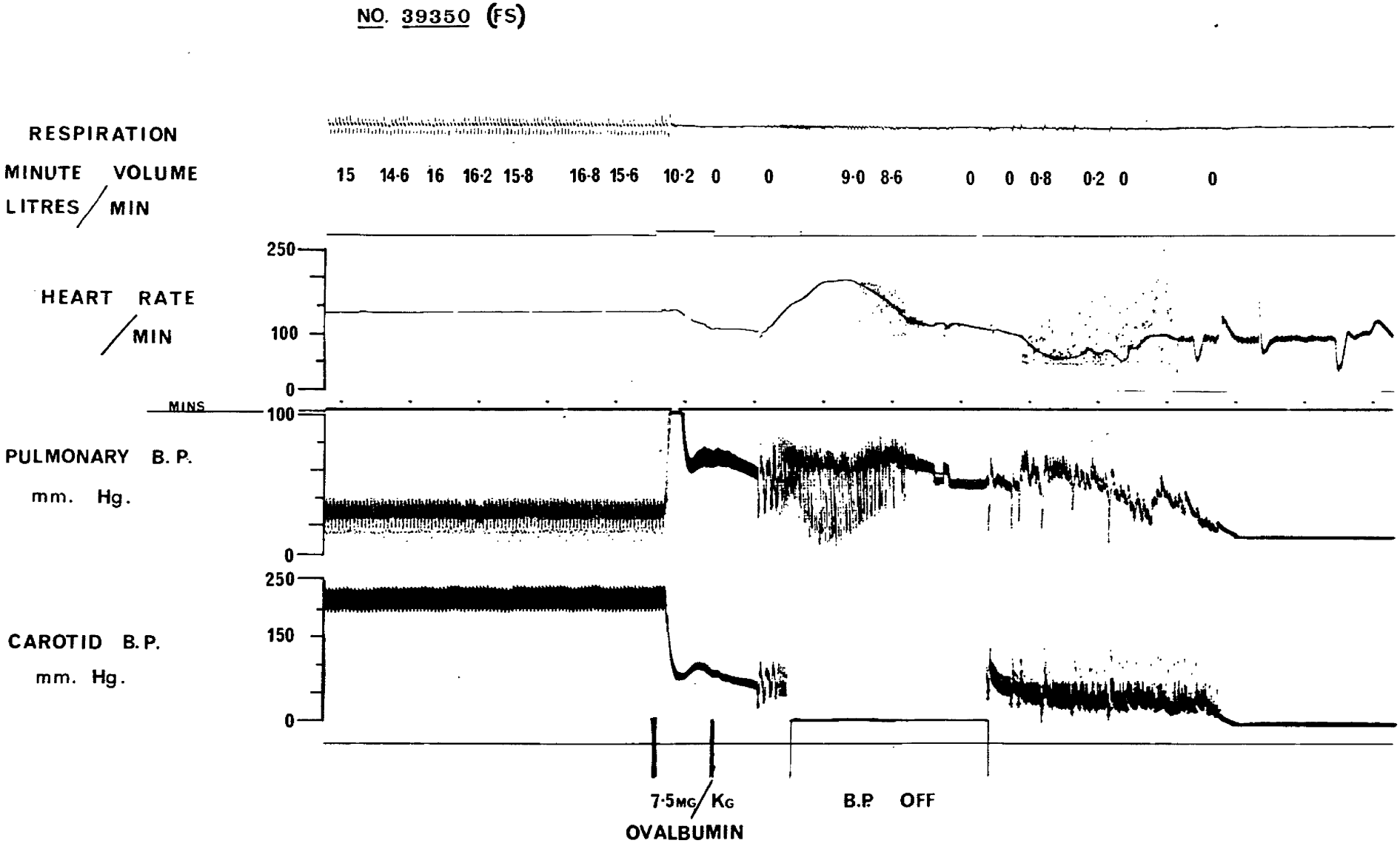


Fig. 2.4 - Effect of challenge on anaesthetised calf sensitised using Freund's adjuvant. Antigen injected between the marks (| |).

b) HAEMATOLOGICAL

MATERIALS AND METHODS

Samples of blood for haematological examination were obtained in conscious animals from the jugular and in anaesthetised animals from the recurrent tarsal vein. These were taken into bottles containing the anticoagulant ethylene diamine tetracetic acid (EDTA) (1 to 2 mg per ml blood). The packed cell volume (PCV) was measured by the technique of Fisher (1962) using the Hawksley haematocrit. The total erythrocytes (RBC) and total leucocytes (WBC) per cu. mm. were counted by an electronic particle counter (coulter counter, Model "B") (Crighton, 1965). A differential leucocyte count was carried out according to the method advocated by Dacie and Lewis (1963).

Samples of blood were taken before and 5 min. after injections of antigen solutions or saline. Samples were taken from some animals on more than one occasion of challenge. The numbers of such animals appear twice in Table 2. 2.

The severity of reaction was assessed by observation of respiratory distress and other symptoms as described in Section II. 1.

RESULTS

The results of total and differential WBC counts and PCV are shown in Table 2. 2 together with the mean and standard error (SE) for each of the 3 groups of cattle, namely controls, those which reacted severely ($>+$) and those which reacted mildly ($+,<+$). The changes associated with injection of antigen or saline, in total WBC, percentage of neutrophils, percentage of lymphocytes and PCV are expressed as percentages of those values before challenge. Changes in percentages of eosinophils are excluded as the small numbers of these cells counted give misleadingly high percent changes. The significance of the changes was assessed for each group by the t test for small samples (Bancroft, 1965). The mean difference and SE between haematological values before and after challenge are shown in Table 2.3 with the values of t and p.

After anaphylaxis WBC counts were found to fall, due largely to neutropenia, and PCV to rise. The severity of the reaction was

TABLE 2.2 HAEMATOLOGICAL CHANGES ASSOCIATED WITH I.V. INJECTION OF ANTIGEN OR SALINE IN SENSITISED CATTLE.

Severity of Reaction	Calf No.	Before Challenge					After Challenge					% Change			
		Total WBC	Neutrophiles %	Eosinophiles %	Lymphocytes %	PCV	Total WBC	Neutrophiles %	Eosinophiles %	Lymphocytes %	PCV	Total WBC (Fall)	Neutrophiles % (Fall)	Lymphocytes % (Rise)	PCV (Rise)
> + (Antigen)	41045	11,900	31	11.5	57.5	34.5	3,900	1.5	5.5	93	43.5	67.2	95.2	61.7	26.1
	39234	17,800	22.5	9.5	68	32.5	6,100	1.5	1.5	97	42.0	65.7	93.3	42.6	29.2
	41046	11,200	20	0.5	79.5	33.0	4,100	1	0	99	37.5	63.4	95	24.5	13.6
	40986	8,000	25	1.0	74	42.5	3,500	11	0.5	88.5	48.5	56.2	56	19.6	14.1
	37934	14,200	21	2.0	77	33	6,500	2	1	97	42.0	54.2	90.5	25.9	27.3
	40987	9,900	8	2.5	89.5	37	4,600	4.5	0	95.5	42.0	53.5	43.7	6.7	13.5
	41046	11,200	11.5	0.5	88	39.5	5,200	1.5	0	98.5	41.5	53.6	86.9	11.9	0.5
	41045	10,900	34.5	2.5	63	34.5	5,800	9.5	0.5	90	50	46.7	72.5	42.8	44.9
	37935	12,500	11.5	1.0	87.5	37	7,000	4.5	1.5	94	46.5	44	60.9	7.4	25.7
	41046	12,600	32	2.0	66	30.5	9,100	24.0	0.5	75.5	35.5	27.7	25.0	14.4	16.4
	Mean	12,020	21.7	3.3	75.0	35.4	5,580	6.1	1.1	92.8	42.9	53.2	71.9	25.7	21.1
	S.E. ±	830.3	2.9	1.23	3.56	1.14	538.0	2.27	0.5	2.2	1.42	3.7	7.8	5.7	3.8
+ and < + (Antigen)	41588	11,100	21.5	4.0	74.5	28.0	9,000	20.5	5.0	74.5	26.5	18.9	4.6	0	-5.3
	39351	19,950	26.0	1.0	73.0	36.0	17,100	22.0	4.0	74.0	34.5	14.3	15.4	1.3	-2.7
	39366	13,950	22.0	5.0	72.5	37.5	12,150	21.0	2.5	75.5	40.5	12.9	4.5	4.1	8.0
	33366	12,900	24.5	0.5	75	39.5	11,800	31.5	0.5	68.0	39.5	8.5	-28.6	-9.3	0
	Mean	14,475	23.5	2.6	73.75	35.25	12,512	23.75	3.0	73.0	35.25	13.6	- 1	-1	0
	S.E. ±	1,917	1.06	1.1	0.59	2.52	2,546	2.6	0.98	1.70	3.2	2.1	9.54	2.9	0
− Controls (Saline)	41806	8,300	13.5	0	96.5	32.5	8,800	6.5	2	91.5	32.5	-6	51.8	-5.1	0
	41649	10,300	13.0	0.5	86.5	30.5	10,700	17	0.5	82.5	33	-3.8	-30.7	-4.6	8.2
	43740	8,100	29.5	0.5	70.0	37.5	7,800	20.5	0	79.5	37.5	3.7	30.5	13.5	0
	43741	11,600	31.5	1.0	67.5	34.5	11,400	25	0	75	34.5	1.7	20.6	11.1	0
	Mean	9,575	21.9	0.5	80.1	33.7	9,675	17.2	0.62	82.1	34.4	-1.1	18.05	3.7	2.05
	S.E. ±	838	4.95	0.205	21.8	1.49	832	3.94	0.47	3.48	1.12	0.85	17.5	4.9	2.05

TABLE 2.3 SIGNIFICANCE OF HAEMATOLOGICAL CHANGES ASSOCIATED WITH I.V. INJECTION OF ANTIGEN OR SALINE IN SENSITISED CATTLE.

Severity of reaction	Difference between values before and after challenge	WBC	% Neutrophiles	% Lymphocytes	PCV
> + (Antigen)	Mean	6,440	15.6	17.8	7.5
	S.E.±	736.2	2.68	3.23	1.2
	t	8.74	5.82	5.5	6.25
	p	< 0.001	< 0.001	< 0.001	< 0.001
+ and < + (Antigen)	Mean	1,962.5	- 0.25	- 0.75	0
	S.E. ±	362.5	2.36	2.17	1.05
	t	5.4	0.11	0.34	0
	p	< 0.02	< 0.9	< 0.08	< 0.9
- controls (saline)	Mean	- 100	4.62	2.0	0.625
	S.E.±	204.1	2.92	3.8	0.62
	Changes not significant				

related, as described in Section II. 1, to the challenge dose of antigen and was reflected in the magnitude of the haematological changes. The fall in WBC count was less than 20% where reactions were mild and, with one exception (No. 41046), over 45% when very severe (+++). The change in total WBC was highly significant in the group showing severe reactions and significant in those reacting mildly. The changes in neutrophils and lymphocytes were also highly significant in the severely reacting group but neither was significant in those reacting mildly. This was partly due to the fact that in one case (No. 33366) the percentage of neutrophils was increased and lymphocytes reduced following challenge. The animal nevertheless showed a small fall in total WBC. The change in PCV was highly significant in the group which reacted severely but in those showing mild reactions it was not significant. Two animals in the latter group showed reduced PCV after challenge and in one animal PCV was unchanged. The control group showed no significant changes in any of the parameters measured, following injection of saline.

c) PATHOLOGICAL

MATERIALS AND METHODS

Animals, which suffered fatal reactions or which were killed, either by injection of pentobarbitone or by humane killer after non-fatal reactions, were necropsied within 60 min. of death. For histopathological examination, blocks of lung, thoracic lymph nodes, liver, spleen, abomasum, small intestine, large intestine, turbinate bone and skin were fixed in corrosive formol (Carleton and Drury, 1957). They were dehydrated and cleared in an alcohol-amyl acetate-chloroform series and embedded in paraffin wax. Paraffin sections were stained routinely with haemalum and eosin (H. & E.).

RESULTS

In all cases the pathological changes were most marked in the respiratory system. On gross examination pulmonary oedema with cream, blood stained, frothy fluid in the trachea

and bronchi and pulmonary emphysema was found. This is shown in Fig. 2. 5. There were areas of congestion and petechiation of the mucosal lining of the trachea and bronchi. The alveolar and interstitial oedema and emphysema can be seen in Fig. 2. 6. Lymph nodes, particularly those in the thoracic cavity were hyperplastic, congested and in some cases haemorrhagic. Petechial haemorrhages were often present on the endocardium.

Other organs did not regularly show lesions. In 2 cases the abomasum and small intestine showed mucosal oedema, congestion and haemorrhage.

Histological examination confirmed the presence of intra-alveolar and interstitial oedema and emphysema with associated areas of collapse (Figs. 2. 7 and 2. 8). In some cases fibrinous material present in the oedema fluid was lined along the alveolar walls, giving the appearance of hyaline membranes. Intra-alveolar haemorrhage frequently was present. In one case thrombi were present in branches of the pulmonary artery. There was congestion of the blood vessels of the nasal epithelium.

Fig. 25. Longitudinal section through bronchus of diaphragmatic lobe of lung of calf killed by anaphylaxis. Frothy fluid in the bronchus, pulmonary oedema and emphysema are shown.

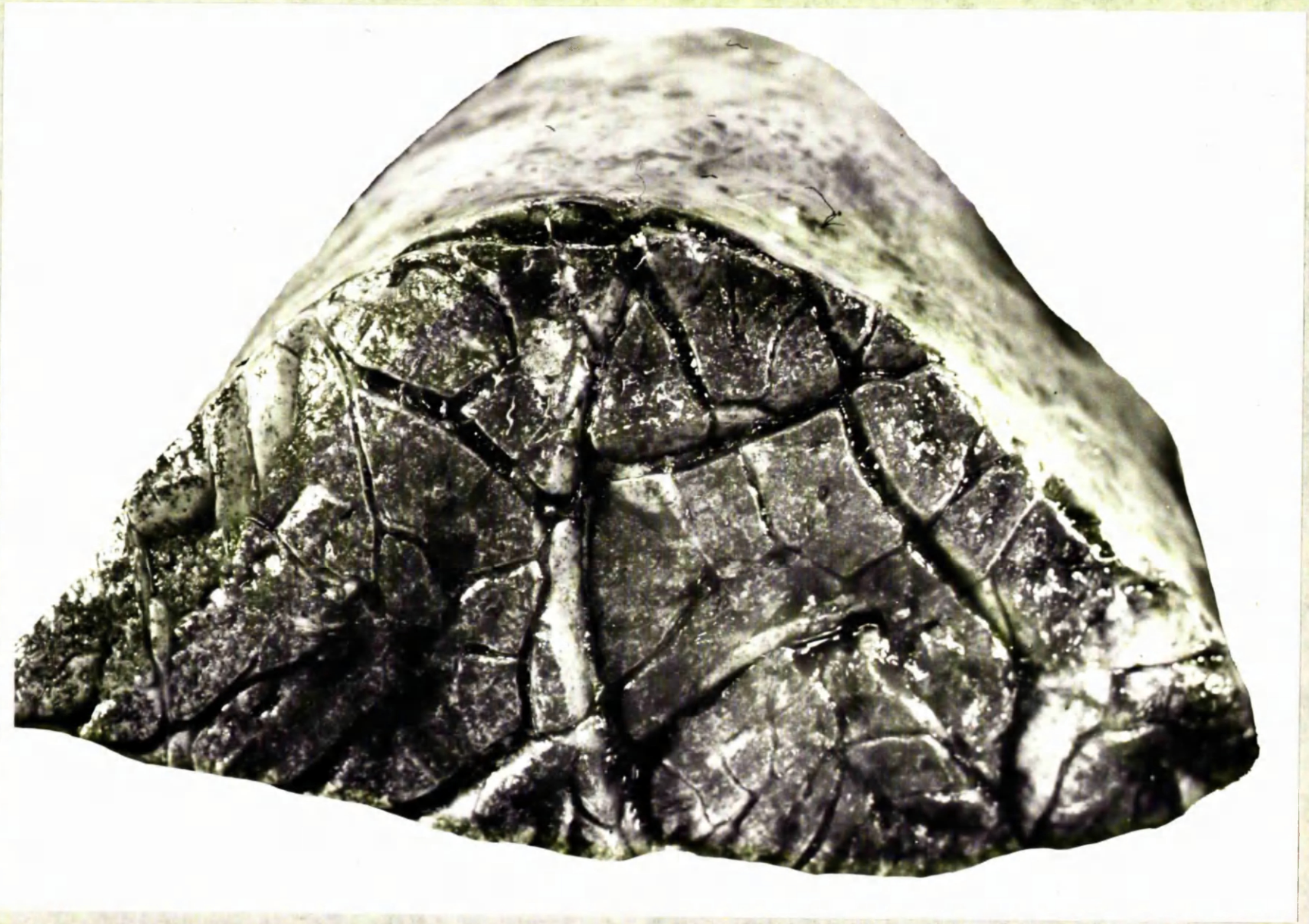


Fig.26. Transverse section of diaphragmatic lobe of lung of calf killed by anaphylaxis. Inter-lobular oedema and emphysema are shown.

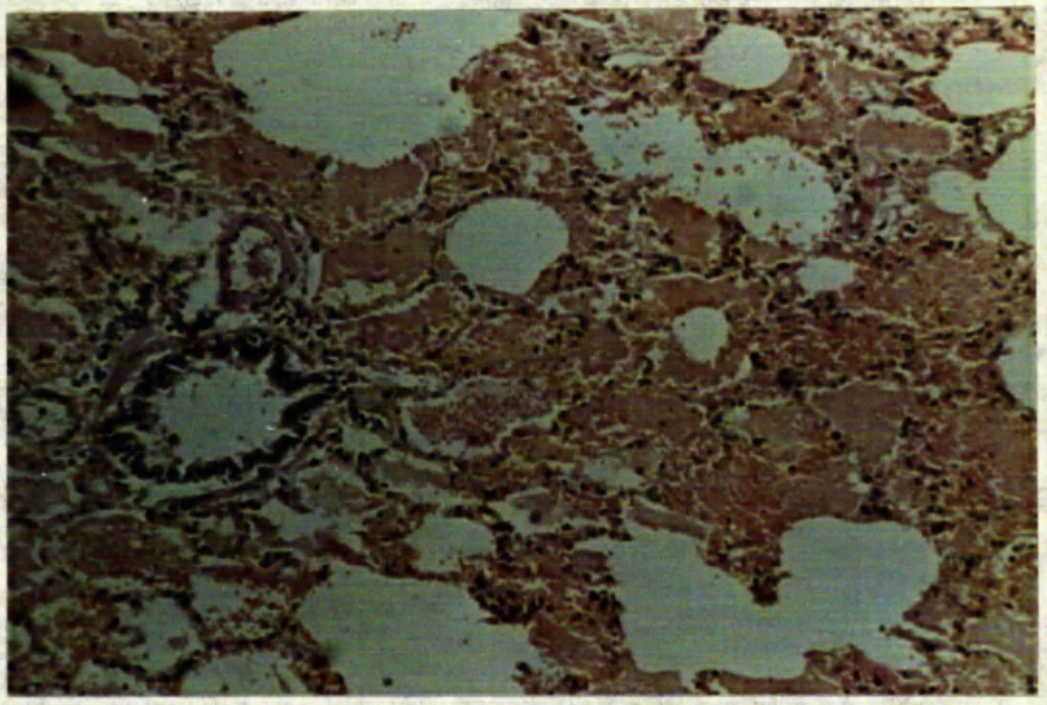


Fig. 2.7 - Photomicrograph of bovine lung after fatal anaphylaxis, showing intra-alveolar oedema and haemorrhage. (H & E x 110)

Fig. 2.8 - Photomicrograph of bovine lung after fatal anaphylaxis, showing alveolar emphysema. (H & E x 110)

3. THE EFFECTS OF HISTAMINE, 5-HYDROXYTRYPTAMINE (5-HT) AND BRADYKININ IN CATTLE AND MODIFICATION OF THESE EFFECTS BY ANTAGONISTS AND BY VAGOTOMY

INTRODUCTION

Histamine, 5-hydroxytryptamine and bradykinin are of major importance in the mediation of anaphylaxis in guinea-pigs, dogs, rabbits and rats (see Section I). These substances were administered to cattle in order to compare their effects with those of anaphylaxis. Findings obtained in vivo were examined further by means of isolated tissues and organs.

a) IN VIVO EXPERIMENTS

MATERIALS AND METHODS

ANIMALS

The animals used were as described in Section II. 1 with the exception that those which received bradykinin were 8 to 12 wk. old weighing 50 to 100 Kg.

DRUGS

Histamine dihydrochloride (Koch-Light Laboratories Ltd.)

Serotonin (5-hydroxytryptamine) creatinine sulphate (Sigma Chemical Co.)

Bradykinin triacetate (Sigma Chemical Co.)

Mepyramine maleate (May and Baker Ltd.)

Cyproheptadine hydrochloride (Merck, Sharp and Dohme Ltd.)

Methysergide bimaleate (Sandoz Ltd.)

B.W.501C67 (a-Anilino-N-2-m-chlorophenoxypropylacetamidine hydrochloride monohydrate) (Wellcome Research Laboratories)

Sodium meclofenamate (Parke, Davis Ltd.)

Propranolol hydrochloride (Imperial Chemical Industries Ltd.)

When administration of antagonists was carried out, this always preceded administration of agonists. The antagonist drugs and corresponding agonists are listed in Table 3. 1, with route of administration and dose of the antagonist and the time interval between administration of antagonist and agonist. In all cases agonists were administered intravenously. Weights of agonists are expressed as active base and of antagonists as the salts listed above.

TABLE 3.1 ANTAGONISTS

Agonist	Antagonist	Route	Dose mg/Kg	Interval mins
Histamine	Mepyramine	i.m.	5	20
		i.v.	2	5-10
5-Hydroxytryptamine	Cyproheptadine	i.v.	0.3	5
	Methysergide	i.v.	0.1	5
	501C67	i.v.	1-6	5
Bradykinin	Meclofenamate	i.v.	2	5-15

Cyproheptadine possesses activity as an antagonist of both histamine and 5-hydroxytryptamine and its activity is comparable to that of the most active known substances with such activity (Stone, Wenger, Ludden, Stavorski and Ross, 1961; Robinson, H.J., 1958).

B.W. 501C67 is an antagonist of 5-hydroxytryptamine, more active against the peripheral than the central actions of 5-hydroxytryptamine and more persistent in effect than methysergide or xylamidine (Mawson and Whittington, 1970). In other respects the pharmacological properties of 501C67 resemble xylamidine (Green, 1970, personal communication) which has been shown, at a dose rate of 0.15 mg/Kg, to abolish the bronchoconstrictor response to 5-hydroxytryptamine (20 ug/Kg) of anaesthetised guinea-pigs (Copp, Green, Hodson, Randall and Sim, 1967). Mepyramine, methysergide and meclofenamate were used successfully by Collier and James (1967) to antagonise bronchoconstriction induced by histamine, 5-hydroxytryptamine and bradykinin respectively in guinea-pigs. The doses of antagonists and times of administration of antagonists before agonists were based on those used by Collier

and James (1967). Propranolol, a β-adrenergic blocking drug, was used to enhance the response to bradykinin. Propranolol (5 mg/Kg) was injected intravenously 5 min. before bradykinin (Collier and James, 1967). The rate of injection was 1 mg/min.

Bilateral cervical vagotomy was carried out in 3 cases as some of the effects of histamine in the guinea-pig have been shown to be affected by vagotomy (Mills and Widdicombe, 1970).

Preparation of solutions for injection

Histamine, 5-hydroxytryptamine and bradykinin salts listed above were dissolved in sterile distilled water to form 0.2%, 0.1% and 0.01% solutions respectively. Mepyramine maleate was used as the 5.0% solution for injection.

Cyproheptadine hydrochloride was used as the 0.2% solution for injection.

Methysergide bimaleate was used as a 1.0% solution prepared by dissolving 100 mg of the salt in 1.0ml N.methanesulphonic acid and making the volume up to 10 ml with 5.0% glucose solution.

Sodium meclofenamate was very poorly soluble in water but dissolved in N or N/10 NaOH to form a 5.0% to 10% solution. N/10 Hcl was slowly added to reduce the pH to as close to 7.0 as possible without precipitation. The solution obtained was of pH 9.0 to 9.5. Precipitation tended to occur on storage for 24 hr. or more but solution was achieved again by heating.

The materials and methods used to investigate the physiological responses of anaesthetised animals and to examine pathological changes were as described in Section II. 2.

Mid-cervical vagotomy was carried out, in anaesthetised animals, after cannulae had been inserted in the trachea and blood vessels, 10 to 15 min. before administration of drugs. Identification of the nerves was confirmed by observing the effects of electrically stimulating each for approximately 5 sec. Stimuli of 15 volts for 4.0 milli-sec. at a rate of 20 per sec. were applied by means of a square wave stimulator (Palmer Ltd.).

Effects on conscious animals

Two groups of 4 animals received 0.03 mg/Kg. histamine and 0.07 mg/Kg. 5-hydroxytryptamine respectively. The dose of histamine was half as great as that found by Desliens (1958) to be lethal for cattle. Doses of 0.02 mg/Kg. and 0.04 mg/Kg. 5-hydroxytryptamine produced no apparent effects when tested in 2 animals. The symptoms produced by histamine and 5-hydroxytryptamine, their rate of onset and duration are listed in Table 3. 2. Both substances produced respiratory changes, congestion of the conjunctival mucosa and muzzle and lacrimation. Depression and increased salivary and nasal secretion was marked after histamine but not after 5-hydroxytryptamine, whereas staggering and sneezing were seen only after 5-hydroxytryptamine. The rate of onset of the symptoms was less rapid and they persisted for longer after histamine than after 5-hydroxytryptamine administration at these dose

TABLE 3. 2

EFFECTS OF HISTAMINE AND 5-HYDROXY-TRYPTAMINE IN CONSCIOUS CATTLE

Symptoms	Numbers of Animals Affected (Total = 4)	
	Histamine 0.03 mg/Kg	5-HT 0.07 mg/Kg
depression	4	0
tachypnoea	4	4
dyspnoea	4	3
coughing	3	3
lacrimation	1	4
congestion of conjunctiva & muzzle	4	1
salivation	2	0
nasal discharge	2	0
sneezing	0	1
staggering	0	2
Onset (sec)	90 (60-120)	15 (10-20)
Duration (min)	37.5 (30-40)	7.5 (5-10)

Note: Times of onset and of duration of symptoms expressed as mean of 4.
( ) = range of times.

rates. Neither substance caused the animals to collapse, tremble or show pruritis but otherwise the effects were similar to those of anaphylaxis.

Effects of antagonists

When animals were injected intramuscularly with mepyramine maleate (5.0mg/Kg.) 20 min. before receiving histamine, all the visible effects of histamine, with the exception of slight congestion of the muzzle, were prevented. Cyproheptadine hydrochloride, injected intravenously at a dose rate of 0.3 mg/Kg., 5 min. before 5-hydroxytryptamine, prevented all the visible effects of 5-hydroxytryptamine.

EFFECTS ON ANAESTHETISED ANIMALS

MATERIALS AND METHODS

Animals were anaesthetised with sodium pentobarbitone and treated as described in Section II. 2. Two animals received 0.03 mg/Kg. histamine. Two animals received 5-

hydroxytryptamine, one at a dose rate of 0.07 mg/Kg. and one at a dose rate of 0.05 mg/Kg. A further 2 animals received bradykinin at dose rates of 0.01 mg/Kg. and 0.1 mg/Kg. The animal receiving the smaller dose was pretreated with propranolol (0.1 mg/Kg.) injected intravenously 10 min. before bradykinin.

RESULTS

The effects of histamine, 5-hydroxytryptamine and bradykinin on respiration, systemic and pulmonary blood pressures and heart rate are summarised in Table 3. 3. These parameters, times of onset and duration of symptoms were measured as described in Section II. 2. Fig. 3. 1 shows the effects of histamine. Apnoea was accompanied by the accumulation, in the endotracheal tube, of frothy fluid. The respiratory recording apparatus was disconnected in order to clear the tube. Following apnoea respiratory flow and minute volume

TABLE 3.3 EFFECTS OF HISTAMINE, 5-HYDROXYTRYPTAMINE AND BRADYKININ IN ANAESTHETISED CATTLE

Drug	Dose mg/Kg	Onset sec	Duration min	Apnoea sec	Minute Volume % Fall	Systemic B.P. % Fall	Pulmonary B.P. % Rise	Heart Rate % Fall
Histamine	0.03	10	2.5	54	92	100	80	N.M.
	0.03	5	5.0	120	100	58	200	25
5-HT	0.07	24	7●	180	100	25	50	28
	0.05	24	5	20	N.M	57	100	24
Bradykinin	0.1	15	5	48	100	44	50	20
	0.01*	15	10	18	79	79	N.M	33

● = Died

* = After 0.1 mg/Kg propranalol

N.M = Not measured

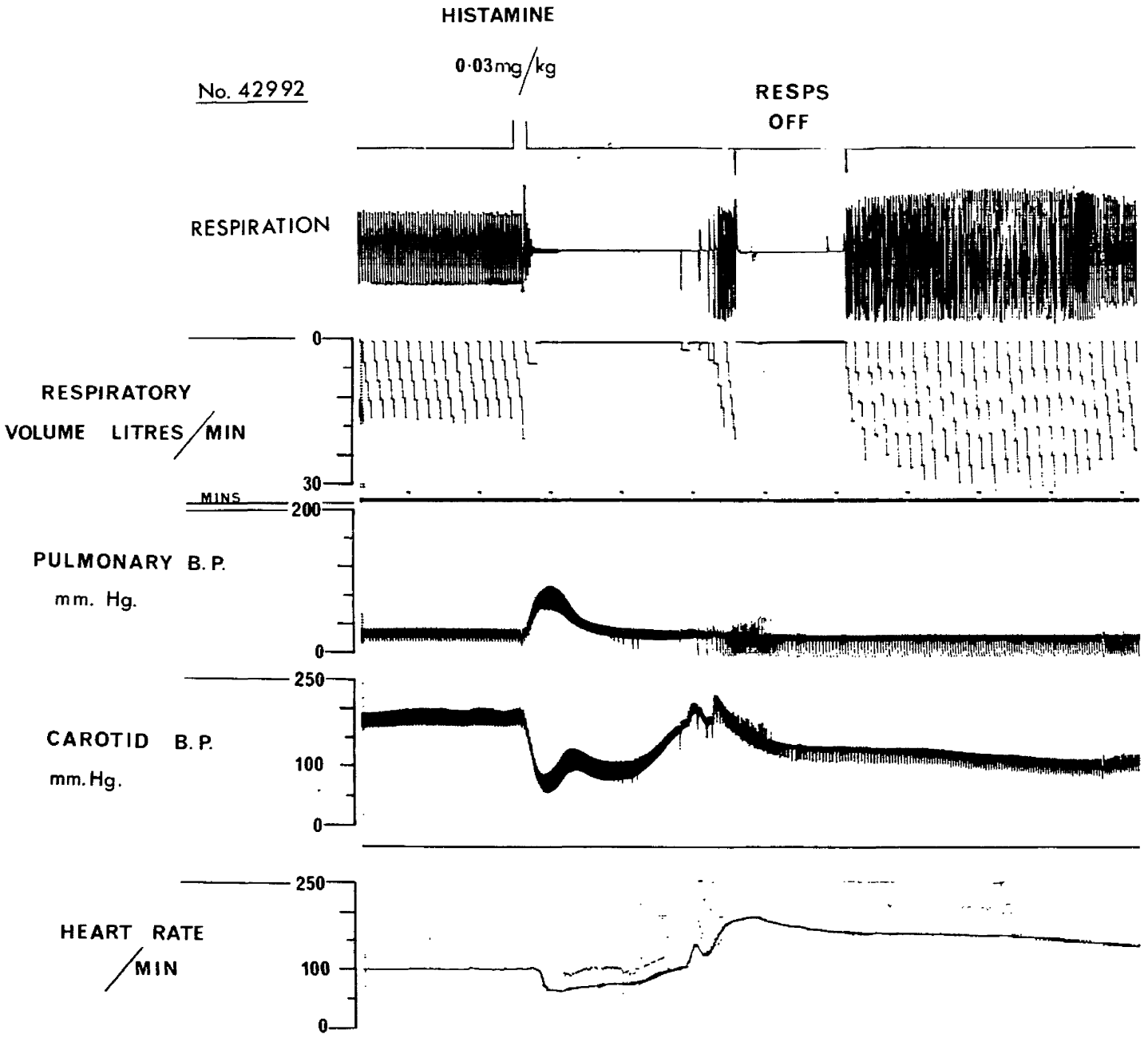


Fig. 3.1 - The effects of histamine on an anaesthetised calf.
Histamine injected between the marks (| |).

were increased. Simultaneously with the onset of apnoea pulmonary blood pressure rose, but returned after one min. to preinjection level. Systemic blood pressure fell rapidly but returned to preinjection level after 2½ min. It fell again gradually but was maintained at a level 42% lower than before histamine administration. The heart rate fell by 25% but this was followed after 2 min. by tachycardia which was maintained at a level 85% higher than the pre-injection rate.

Fig. 3. 2 shows the effects of a lethal dose of 5-hydroxytryptamine. This also produced apnoea. The animal breathed again after 3 min. but apnoea supervened for 90 sec. Respiration was irregular until death occurred after 7 min. The rise in pulmonary blood pressure and fall in systemic blood pressure was less marked than after histamine administration. Heart rate fell by 28% but was not followed by

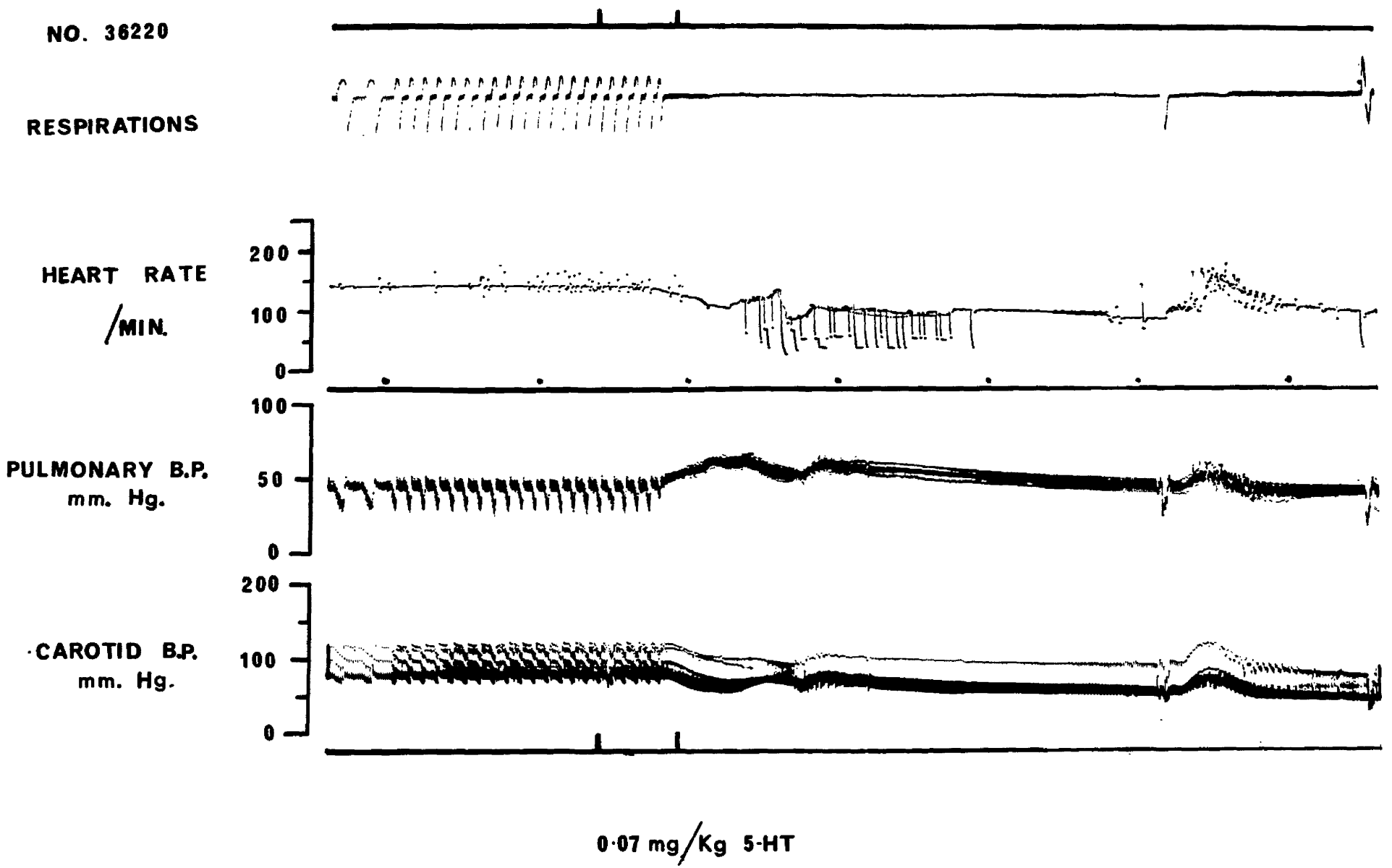


Fig. 3.2 - The effects of 5-hydroxytryptamine on an anaesthetised calf. 5-HT injected between the marks (! !).

tachycardia. The administration of a sub-lethal dose of 5-hydroxytryptamine (0.05 mg/Kg.) produced similar effects. Again tachycardia did not occur.

Fig. 3.3 shows the effects of 0.1 mg/Kg. bradykinin. These effects were similar to those of histamine and 5-hydroxy-tryptamine. Tachycardia occurred following the reduction in heart rate. In this case it did not prove possible to pass a catheter into the pulmonary artery and it was found, on post mortem examination, to lie in the vena cava. Pre-treatment with propranolol allowed comparable effects to be produced with a dose of 0.01 mg/Kg. bradykinin.

Effects after Vagotomy

The effects of electrical stimulation and section of the vagus are shown in Fig. 3. 4. Stimulation resulted in immediate apnoea lasting approximately 24 sec. and a 57% fall in systemic B.P. lasting for 12 sec. On sectioning the vagus respiratory flow and minute volume were reduced.

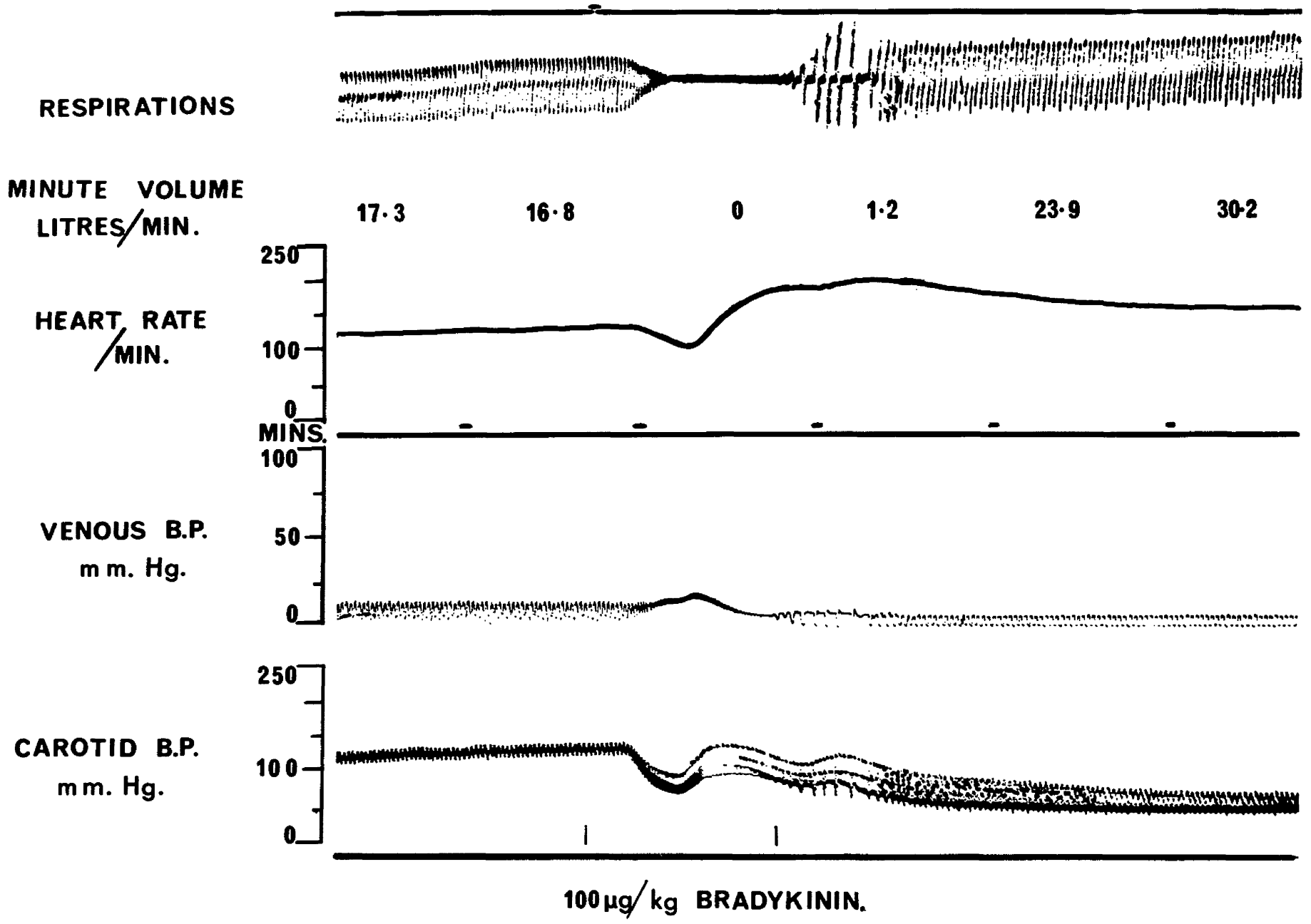


Fig. 3.3 - The effects of bradykinin on anaesthetised calf. Bradykinin injected between the marks (| |).

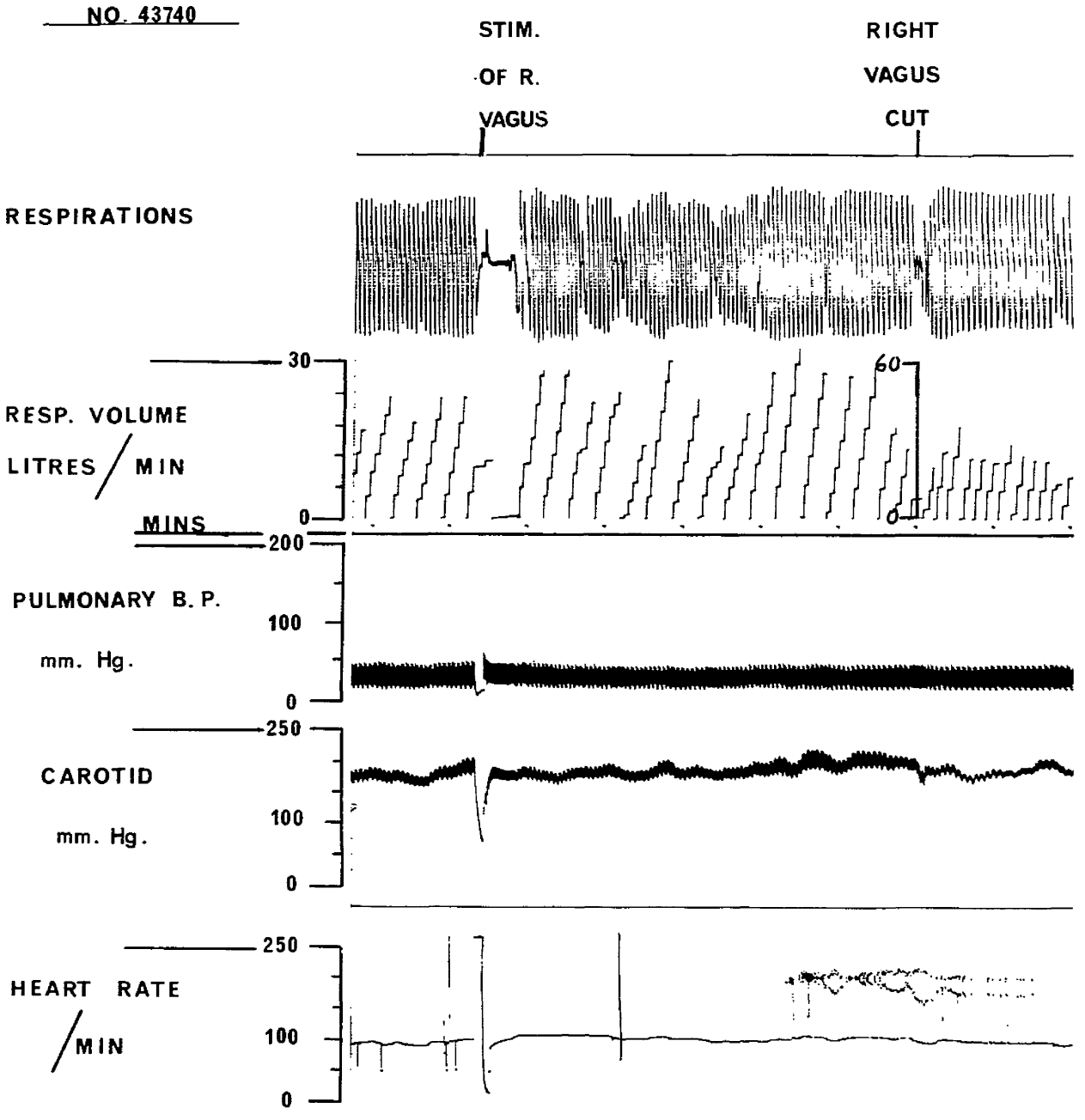


Fig. 3.4 - Effects of electrical stimulation and section of right vagus nerve of anaesthetised calf.

After vagotomy one animal was given 0.06 mg/Kg. of histamine and another 0.07 mg/Kg. of 5-hydroxytryptamine. The outcome in both cases was fatal within 15 min., as shown in Figs. 3. 5 and 3. 6. A third animal was given 0.03 mg/Kg. 5-hydroxytryptamine and survived although apnoeic for almost 4 min. The changes produced are summarised in Table 3. 4. The effects of histamine and 5-hydroxytryptamine appeared to be independent of the vagus nerve.

Effects after Antagonists

Mepyramine maleate was used to antagonise histamine, methysergide bimaleate or 501C67, to antagonise 5-hydroxytryptamine and sodium meclofenamate to antagonise bradykinin. The antagonists in all cases were administered intravenously. Table 3. 5 summarises the effects of histamine, 5-hydroxytryptamine and bradykinin administered after the animals

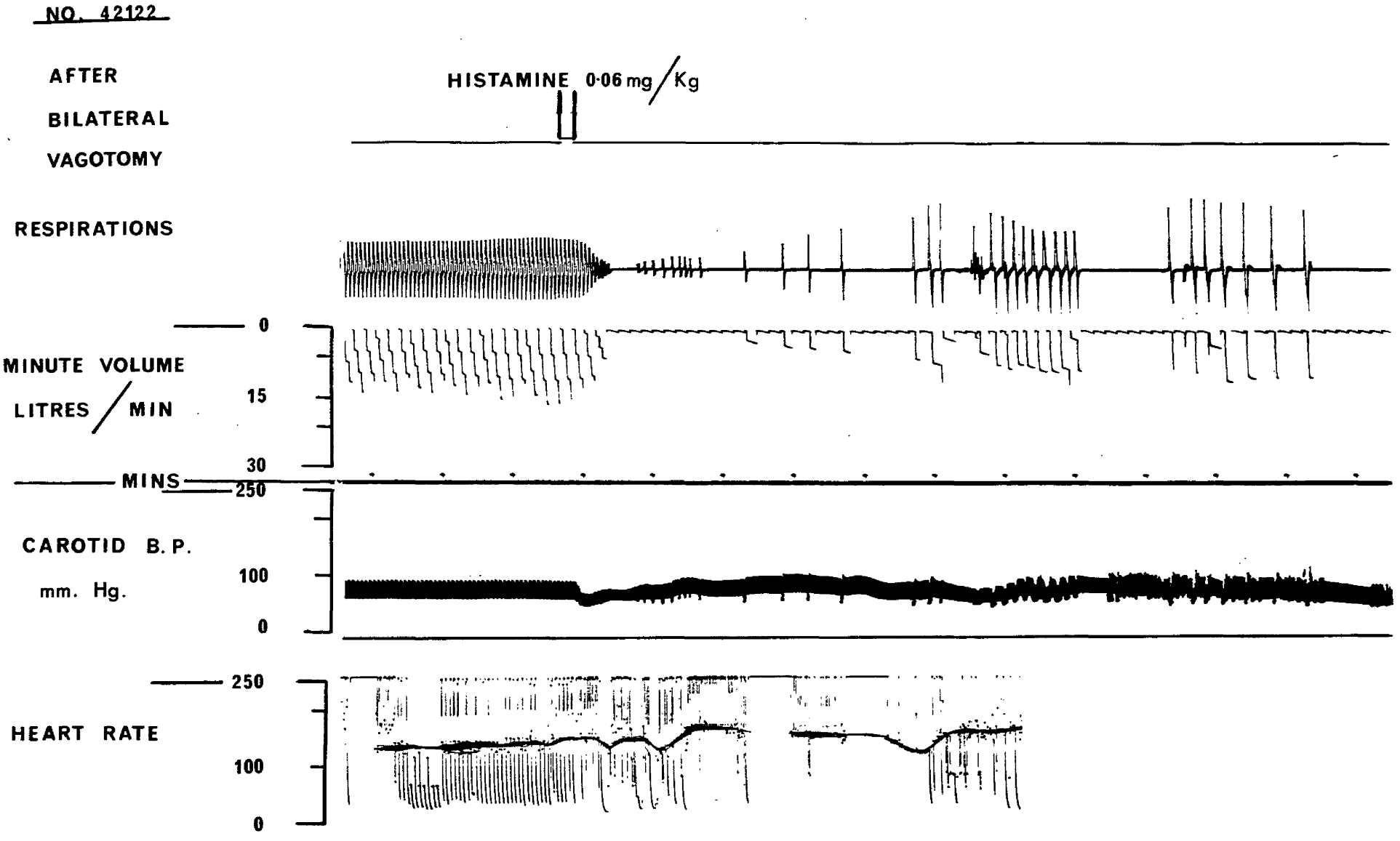


Fig. 3.5 - The effects of histamine on an anaesthetised, vagotomised calf.

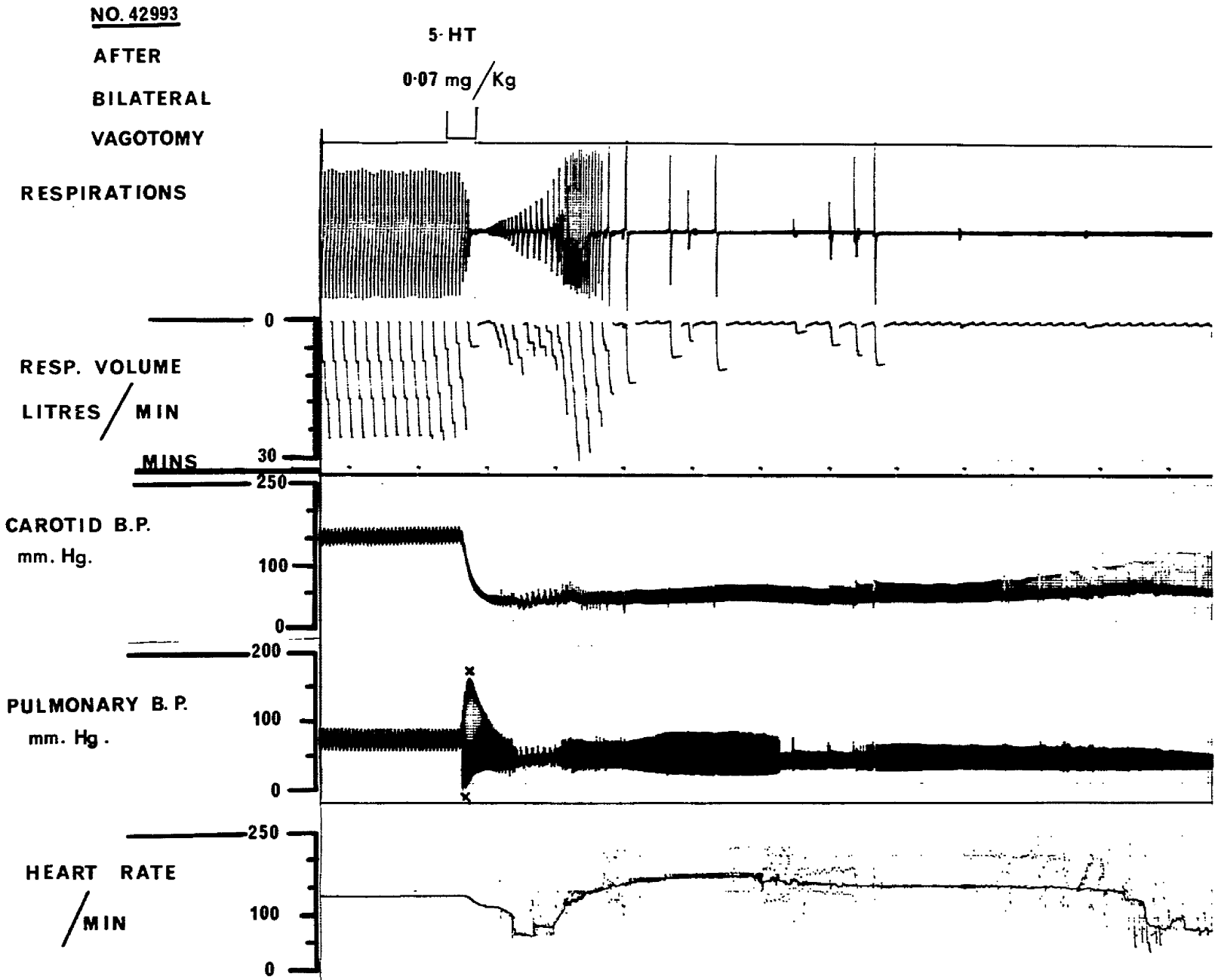


Fig. 3.6 - The effects of 5-hydroxytryptamine on an anaesthetised, vagotomised calf. (x x Cannula displaced into right ventricle)

TABLE 3.4 EFFECTS OF HISTAMINE AND 5-HYDROXYTRYPTAMINE AFTER VAGOTOMY

Drug	Dose mg/Kg	Onset sec	Duration min	Apnoea sec	Min.Vol. % Fall	Systemic B.P. % Fall	Pulmonary B.P. % Rise	Heart Rate % Fall
Histamine	0.06	5	12●	24	100	33	N.M.	17
5-Hydroxytryptamine	0.07	14	14●	16	100	80	140	46
	0.03	36	10	228	100	42	250	34

Note: ● = Died

TABLE 3.5 EFFECTS OF HISTAMINE, 5-HYDROXYTRYPTAMINE AND BRADYKININ AFTER ANTAGONISTS

Antagonist mg/Kg	Drug mg/Kg	Onset sec	Duration min	Apnoea sec	Min.Vol. % Fall	Systemic B.P. % Fall	Pulmonary B.P. % Rise	Heart Rate % Fall
Mepyramine 2.0	Histamine 0.03	15	2	0	0	28	0	N.M.
501C67 5.0	5-HT 0.05	12	7	12	91	33	0	16
501C67 1.0	5-HT 0.05	18	10	18	35	50	0	30
Methysergide 0.1 + 501C67 5.0	5-HT 0.05	12	5	12	54	23	0	25
Meclofenamate 2.0	Bradykinin 0.01	20	5	0	25	64	0	6
	Histamine 0.03	12	2.5	48	97	37.5	75	6
	5-HT 0.07	4	3.0	30	94	46	114	14

had been treated with the appropriate antagonist. Mepyramine pretreatment abolished all the effects of histamine with the exception of a slight fall in blood pressure. This is shown in Fig. 4. 16. Meclofenamate did not prevent the effects of histamine or 5-hydroxy-tryptamine. Bradykinin given after meclofenamate did not produce apnoea. Minute volume fell by 25% and systemic blood pressure fell by 64%. Pulmonary blood pressure was unchanged and heart rate reduced by only 6%. It was found that the production of apnoea and brady-cardia by 5-hydroxytryptamine was not prevented by a dose of 501C67, 5 times greater than that recommended, or by 501C67 followed by methysergide. The systemic hypotensive effect was reduced and pulmonary hypertension prevented (Fig. 3. 7).

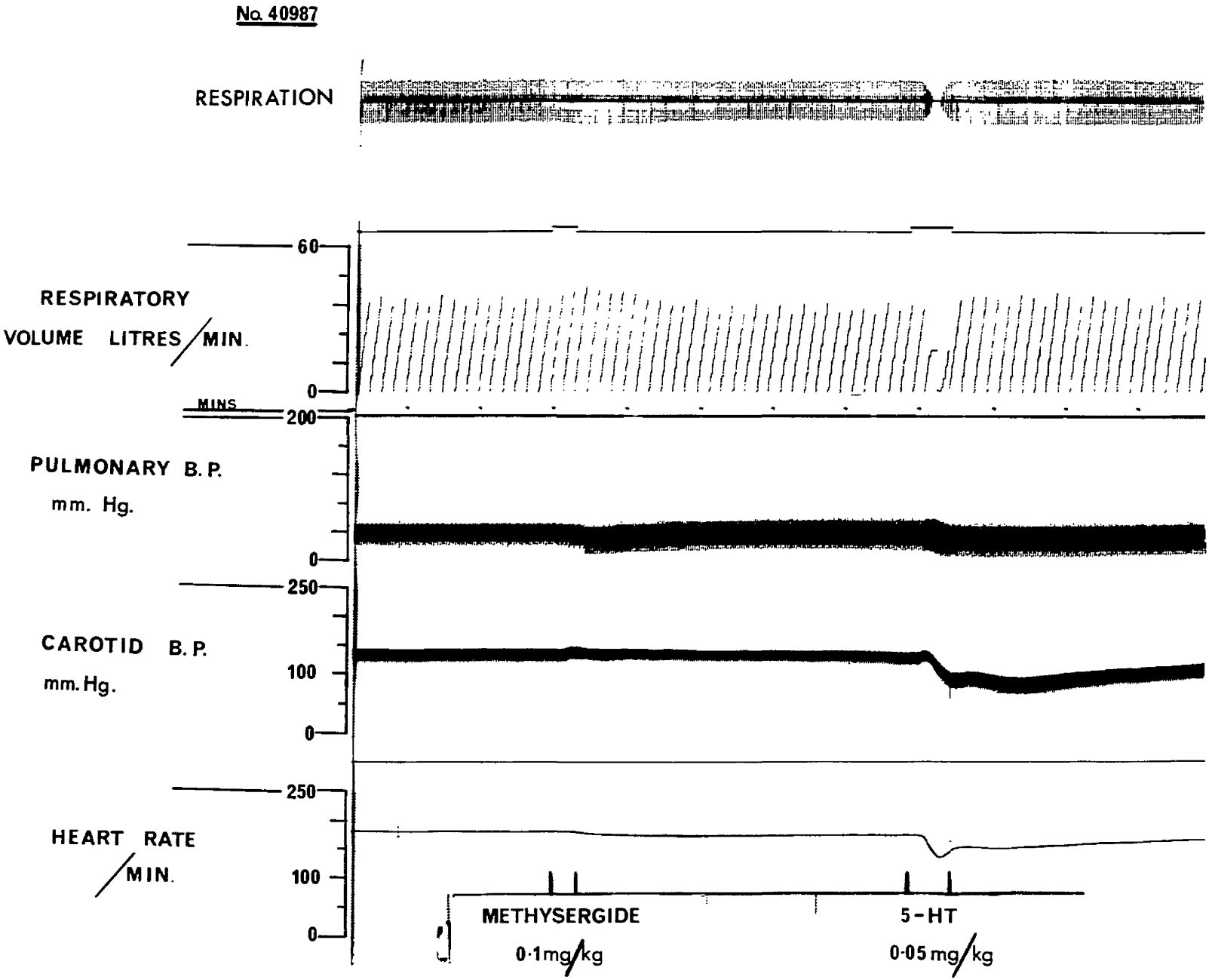


Fig. 3.7 - The effects of 5-hydroxytryptamine on an anaesthetised calf pretreated with B.W.501C67 and methysergide.(Methysergide and 5-HT injected between the marks (| |).

Pathological changes

Pulmonary oedema was always very severe after histamine administration and moderately severe after bradykinin but was minimal where animals received only 5-hydroxytryptamine. Even lethal doses of 5-hydroxy-tryptamine produced only patchy pulmonary congestion.

The number of animals in which pathological changes produced by histamine, 5-hydroxytryptamine or bradykinin and modification of these changes by antagonists could be assessed was limited by the fact that an anaesthetised animal frequently received more than one drug in the course of an experiment.

b) IN VITRO EXPERIMENTS

i) THE EFFECTS OF HISTAMINE, 5-HYDROXYTRYPTAMINE, BRADYKININ, ACETYLCHOLINE AND ADRENALINE ON BOVINE TRACHEABRONCHIAL AND PULMONARY VASCULAR TISSUE AND INHIBITION OF THESE EFFECTS BY SPECIFIC OR SELECTIVE ANTAGONISTS.

As histamine, 5-hydroxytryptamine and bradykinin all produced effects on the respiratory system of cattle their effects were examined on pulmonary smooth muscle in vitro.

MATERIALS AND METHODS

Specimens of lung were obtained from young cattle within 15 min. of slaughter and were placed in Krebs-Henseleit (1932) solution at 4°C. Under these conditions the tissues remained viable for up to 3 days. Strips of trachealis muscle, 2 cm. long x 5 mm., wide were dissected from the inside of a tracheal ring. Segments of bronchioles of less than 5 mm. diameter

were cut spirally to give a final length of approximately 4 cm. Segments of pulmonary artery and segments of pulmonary vein of about the same diameter were similarly cut spirally to give preparations of a similar length. Alternatively, segments of pulmonary artery and vein 5 mm. long and of approximately 2 cm. diameter were cut longitudinally to form strips approximately 4 cm. long. The tissues were suspended in 100 ml. organ baths containing Krebs-Henseleit solution at 37°C and aerated with a mixture of 95% oxygen and 5% carbon dioxide. Contractions were recorded isotonically using a linear motion transducer (Phipps and Bird) and potentiometric recorder (Citizen, Model TRE-5 or TOA Electronics Ltd., Model EPR-2TD) giving an overall magnification of x 200 to 400.

Drugs were allowed to remain in contact with trachealis muscle, pulmonary artery and pulmonary vein for 5 to 10 min. with 15 to 20 min. intervals between doses. Bronchiolar preparations responded more slowly and here contact periods

of 15 to 30 min. were used. Antagonists were administered one min. or 20 min. before agonists. In each case the recorder was switched off before washing the tissue and switched on again 5 min. before administration of the succeeding dose.

The drugs used were:

acetylcholine chloride (Wilcox, Jozeau & Co., Ltd.)

histamine dihydrochloride (Koch-Light Laboratories Ltd.)

serotonin areatinine sulphate (Sigma Chemical Co.)

bradykinin triacetate (Sigma Chemical Co.)

adrenaline tartrate (Evans Medical Ltd.)

atropine sulphate (Koch-Light Laboratories Ltd.)

methysergide bimaleate (Sandoz Ltd.)

mepyramine maleate (May and Baker Ltd.)

sodium meclofenamate (Parke, Davis Ltd.)

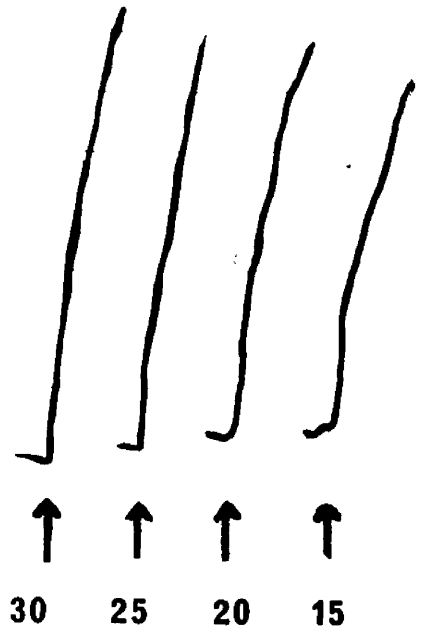
Meclofenamate and methysergide were dissolved as previously described (p.216) and diluted in Krebs-Henseleit solution. Other drugs were dissolved directly in this solution. Acetylcholine and adrenaline were diluted from 10% and 0.1% solutions respectively. Drugs were added to the organ bath by means of a 1.0 ml. syringe. Weights of agonists are expressed as active base. Weights of antagonists as salts.

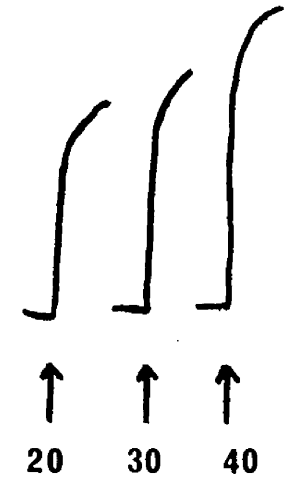
RESULTS

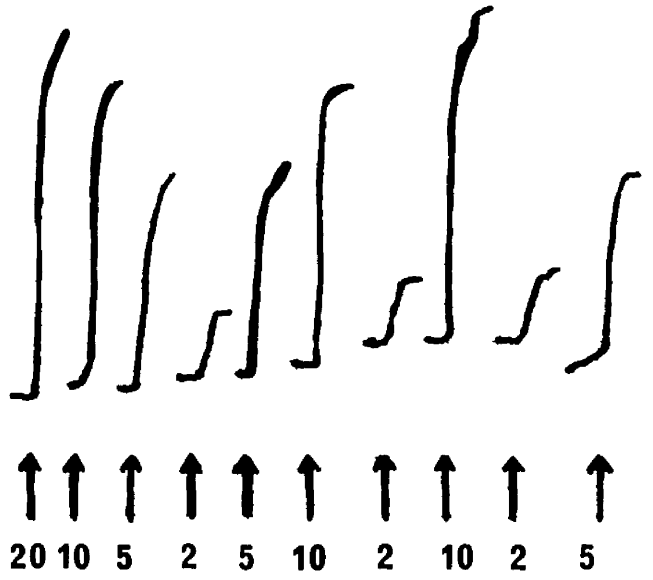
TRACHEALIS MUSCLE

Trachealis muscle contracted in response to histamine (0.01 to 5.0 ug/ml), 5-hydroxytryptamine (0.01 to 2.0 µg/ml), bradykinin (0.001 to 1.0 µg/ml) and acetylcholine (0.002 to 1.0 µg/ml). Contractions began 15 to 30 sec. after administration of the drugs and reached their maxima within 5 min. Responses of trachealis muscle are shown in Fig. 3. 8.

BOVINE TRACHEALIS MUSCLE.







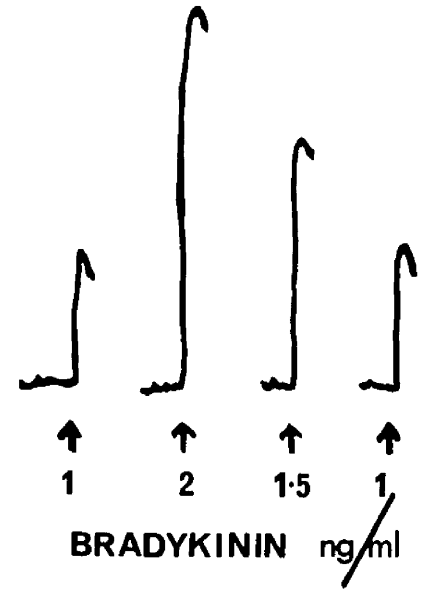


Fig. 3.8 (↑ = Injection of drug into organ bath)

Examples of the effects of antagonists are shown in

by mepyramine (0.005 µg/ml) and bradykinin (0.001 µg/ml) sed

by mepyramine (0.005 µg/ml) and bradykinin (0.001 µg/ml)

by meclofenamate (40 µg/ml) as shown in Fig. 3. 9. Acetylcholine (0.002 µg/ml) was antagonised by atropine (0.001 µg/ml) and 5-hydroxytryptamine (0.02 µg/ml) by methysergide (0.001 µg/ml) (Fig. 3. 10). Atropine added to the bath one min. or 20 min. before 0.1 µg/ml 5-hydroxytryptamine, in concentrations of up to 2.0 µg/ml., showed no antagonistic effect (Fig. 3. 10). A dose of 20 µg/ml atropine, which was 100 times greater than that antagonising an equi-effective dose of acetylcholine, almost abolished the response to 0.1 µg/ml 5-hydroxytryptamine. Antagonism of 5-hydroxytryptamine by methysergide was demonstrated in every preparation in which this was tested (9).

BRONCHIOLAR MUSCLE

Bronchiolar muscle contracted in response to histamine, 5-hydroxytryptamine, bradykinin and acetylcholine but required

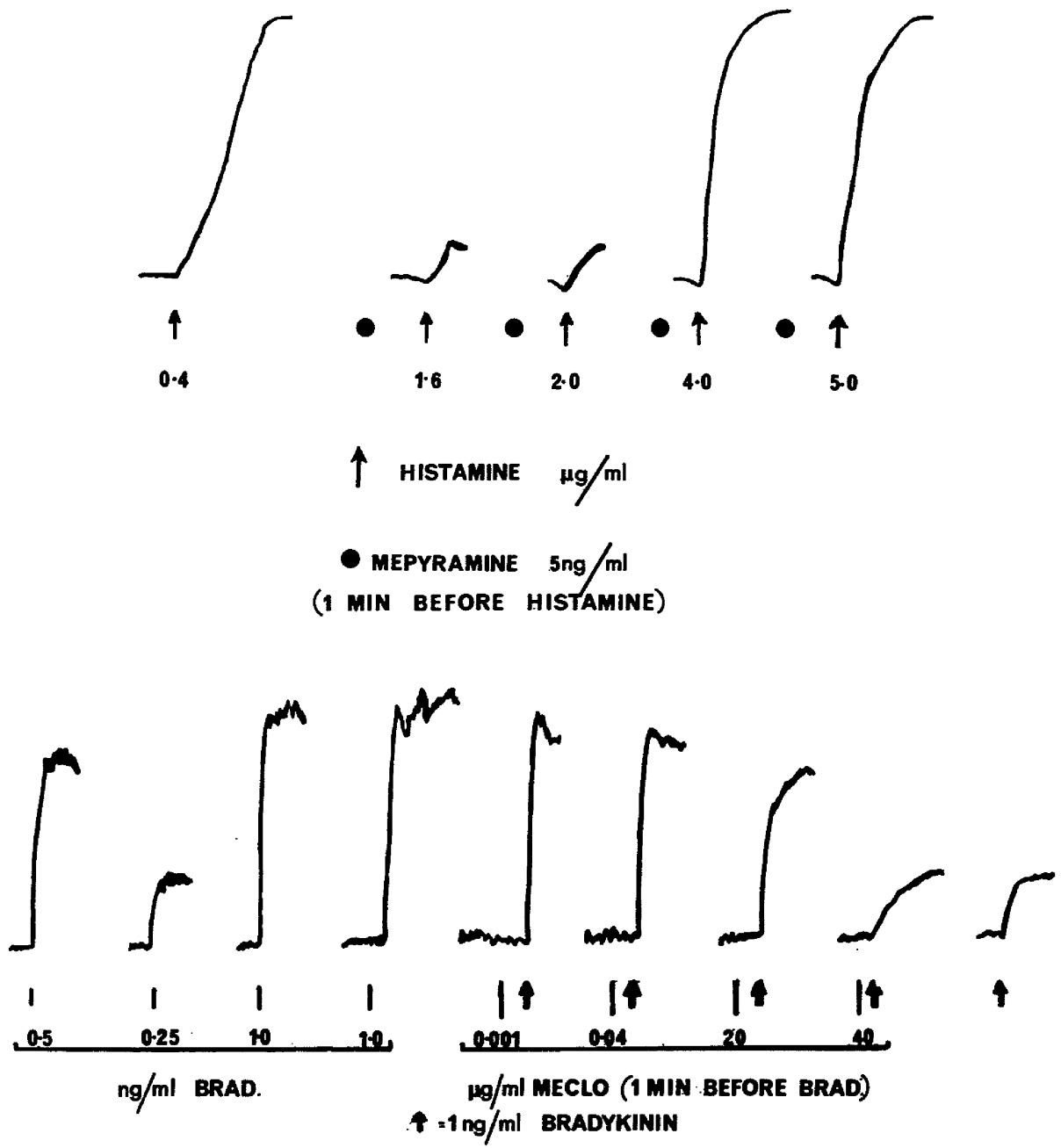

0·4
1·6
2·0
4·0
5·0
HISTAMINE µg/ml
MEPYRAMINE 5ng/ml
(1 MIN BEFORE HISTAMINE)
0·5
0·25
1·0
1·0
0·001
0·04
2·0
4·0
ng/ml BRAD.
µg/ml MECLO (1 MIN BEFORE BRAD)
= 1 ng/ml BRADYKININ

Fig. 3.9

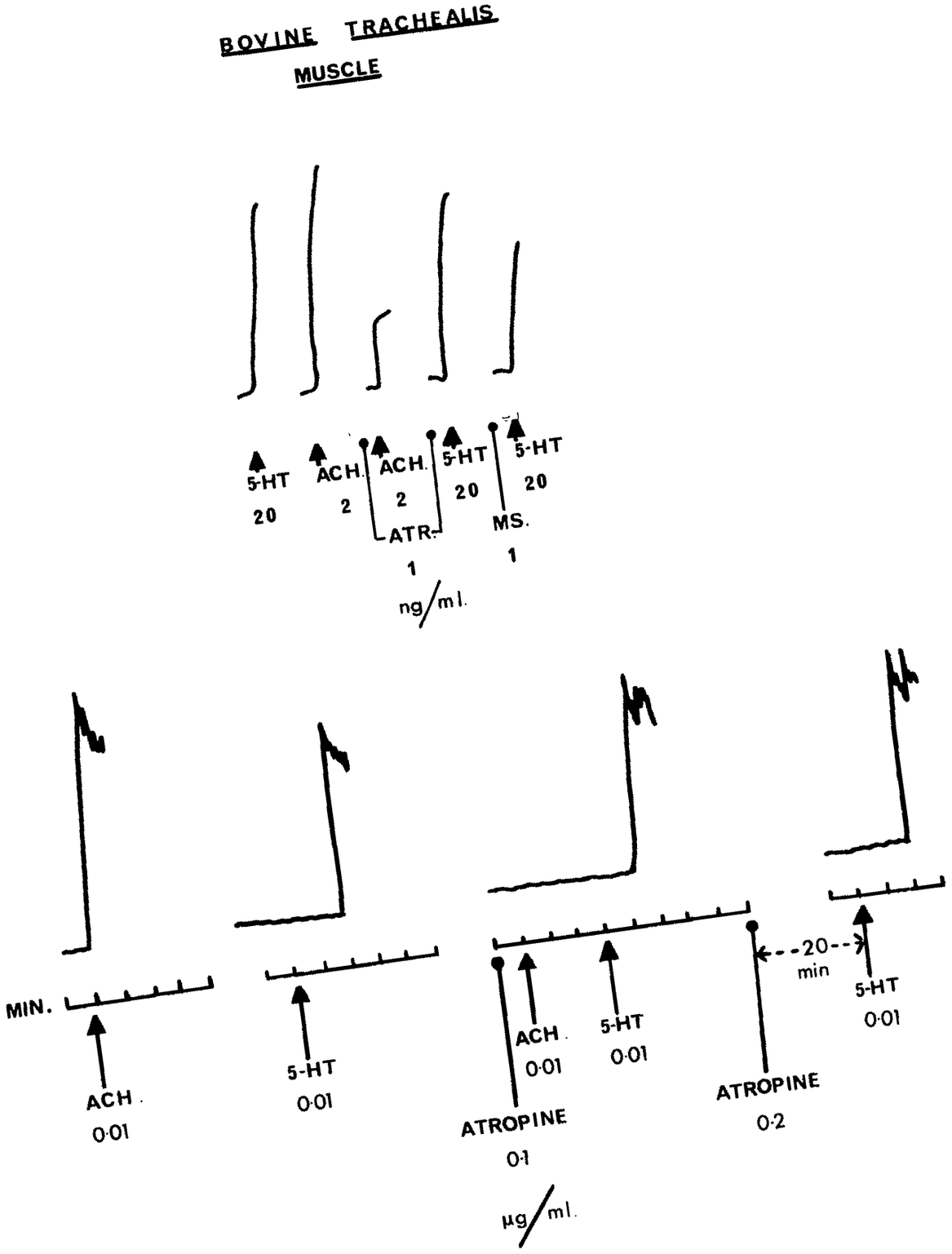
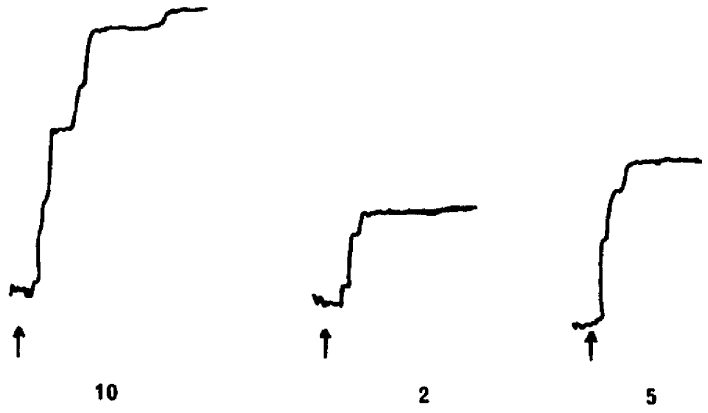

BOVINE TRACHEALIS
MUSCLE
5-HT
20
ACH.
2
ACH.
2
ATR.-
1
5-HT
20
5-HT
20
MS.
1
ng/ml.
MIN.
ACH
0·01
5-HT
0·01
ATROPINE
0·1
ACH
0·01
5-HT
0·01
ATROPINE
0·2
20
min
5-HT
0·01
µg/ml.

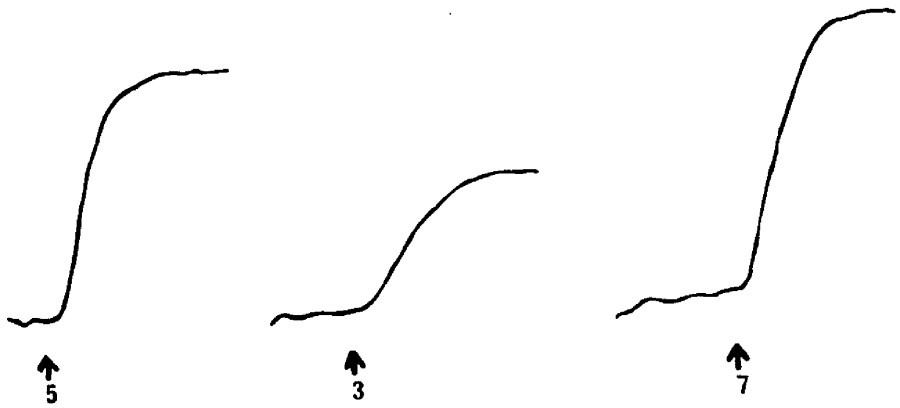
Fig. 3.10

doses 10 to 100 times greater than those which produced responses of trachealis muscle. Contractions in response to histamine, 5-hydroxytryptamine and acetylcholine began within 5 min. of administration of the drugs but responses to bradykinin were often delayed for up to 15 min. Rest periods of 30 to 60 min. were necessary to allow the tissue to relax fully between doses. Responses of bronchiolar muscle to acetylcholine, histamine and 5-hydroxytryptamine are shown in Fig. 3. 11 and to acetylcholine, 5-hydroxytryptamine and bradykinin in Fig. 3.12. Tachyphylaxis developed to 5-hydroxytryptamine (Fig. 3. 11) and to bradykinin. This prevented assessment of the activity of antagonists of these substances. Acetylcholine (0.5 to 2.0 μg/ml) was antagonised by atropine (0.1 to 0.2 μg/ml). Contractions of bronchiolar muscle in response to acetylcholine were maintained, while the tissue remained unwashed, for up to 30 min. Administration of adrenaline (1.0 to 10 μg/ml) produced relaxation within

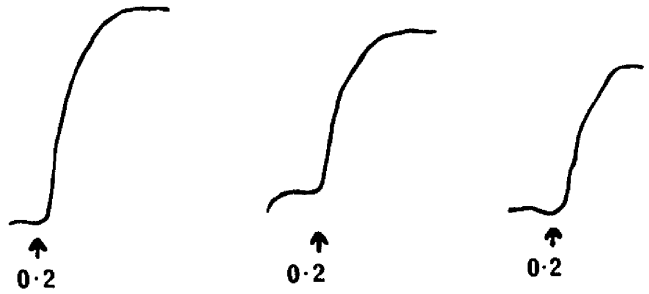
BOVINE BRONCHUS



ACETYLCHOLINE µg/ml



HISTAMINE µg/ml



5 HT µg/ml

Fig. 3.11

5 min. as shown in Fig. 3. 12. Administration of up to 20 µg/ml of adrenaline did not result in detectable relaxation when the bronchiolar muscle was not in a state of contraction.

PULMONARY ARTERY

Pulmonary artery showed no response to up to 15 µg/ml acetylcholine in 4 cases but 2 preparations contracted in response to 0.5 to 1.0 µg/ml. Contractions were produced by histamine (0.02 to 2.0 µg/ml), 5-hydroxytryptamine (0.005 to 0.2 µg/ml), bradykinin (0.1 to 20.0 µg/ml) and adrenaline (0.005 to 1.0 µg/ml) (Fig. 3. 13). This tissue showed tachyphylaxis to bradykinin (Fig. 3. 13). Histamine (0.1 to 1.0 µg/ml) was antagonised by mepyramine (0.01 to 0.02 µg/ml) and 5-hydroxytryptamine (0.01 to 1.0 µg/ml) by methysergide (0.02 to 0.3 µg/ml). The rate at which pulmonary arterial tissue contracted in response to drugs was similar to that described for trachealis muscle.

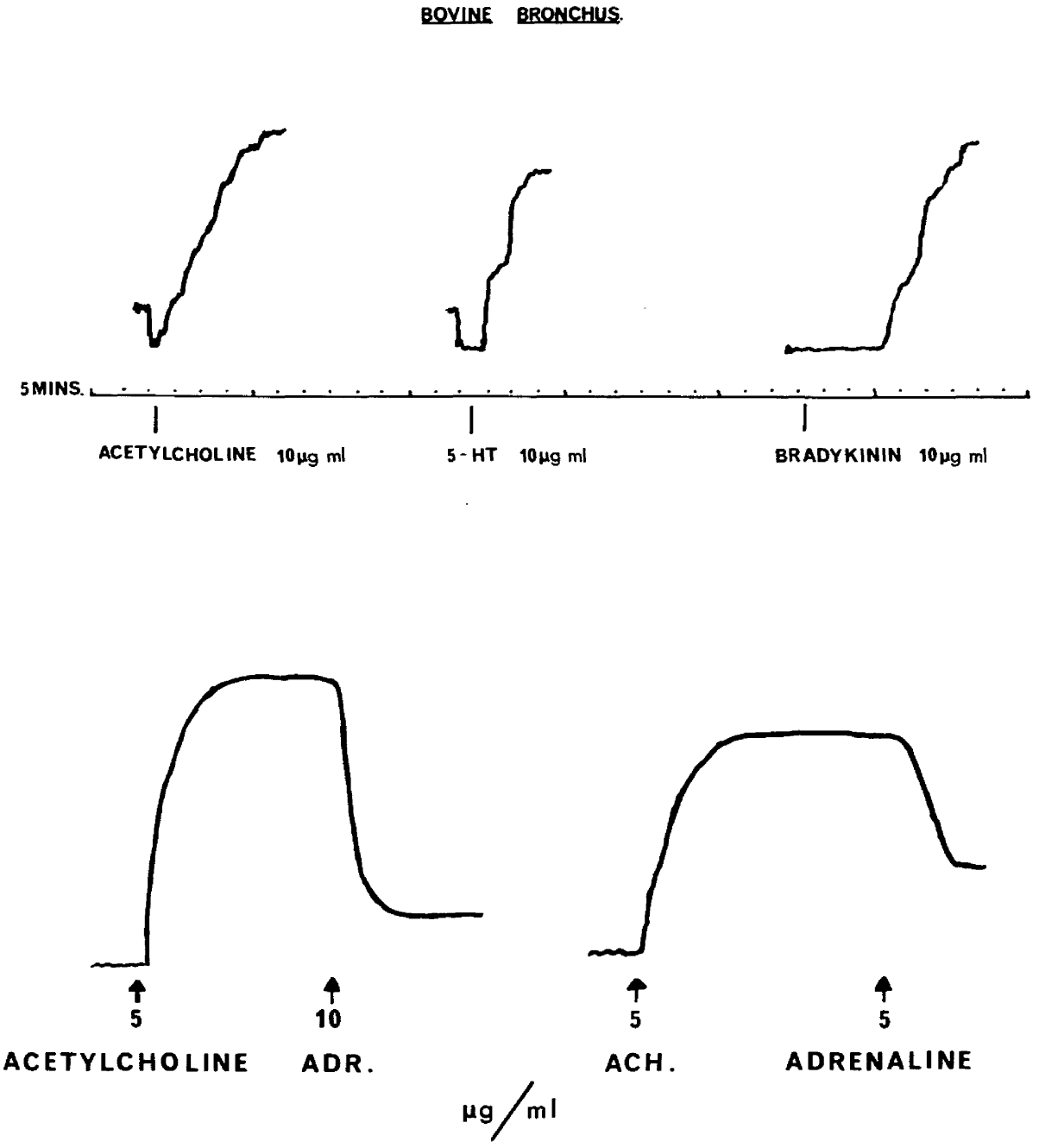
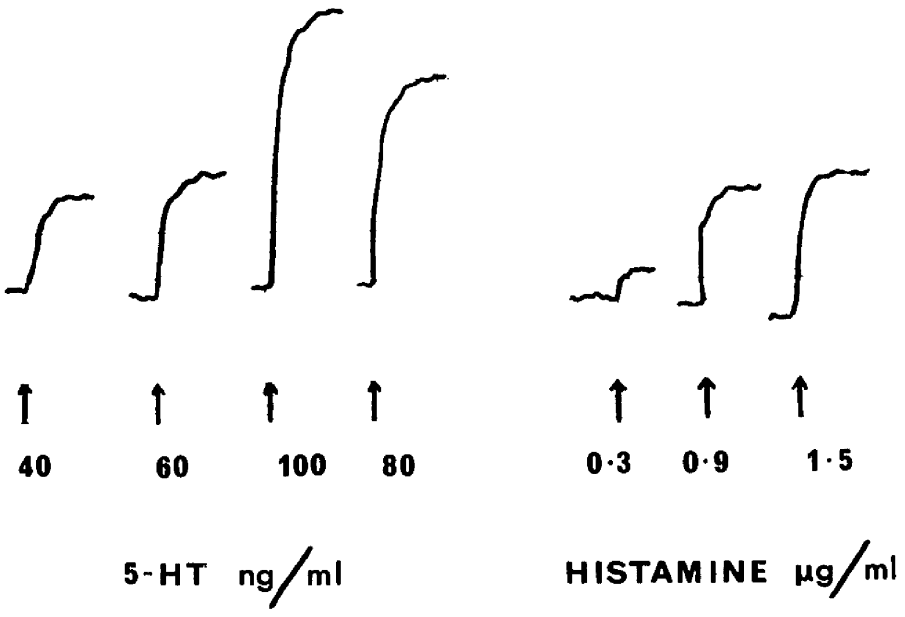
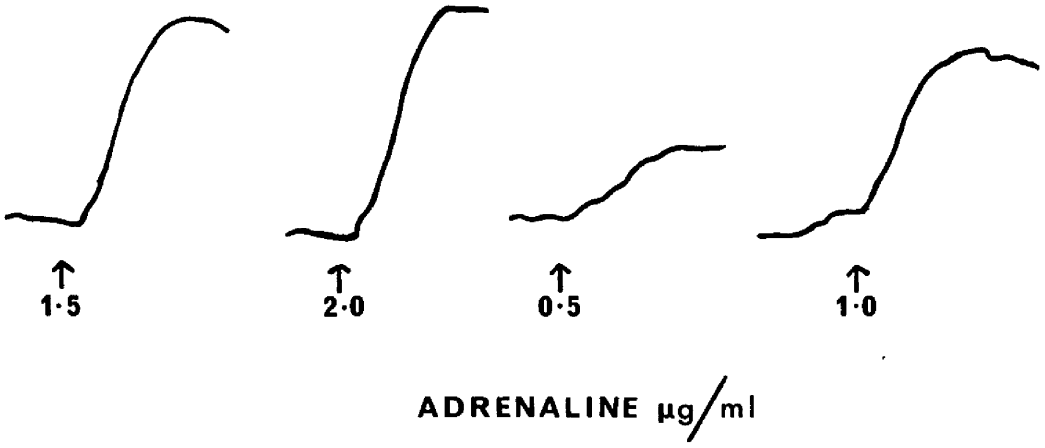

BOVINE BRONCHUS.
5 MINS.
ACETYLCHOLINE 10µg ml
5-HT 10µg ml
BRADYKININ 10µg ml
5
10
ACETYLCHOLINE
ADR.
5
5
ACH.
ADRENALINE
µg/ml

Fig. 3.12

BOVINE PULMONARY ARTERY





TACHYPHYLAXIS

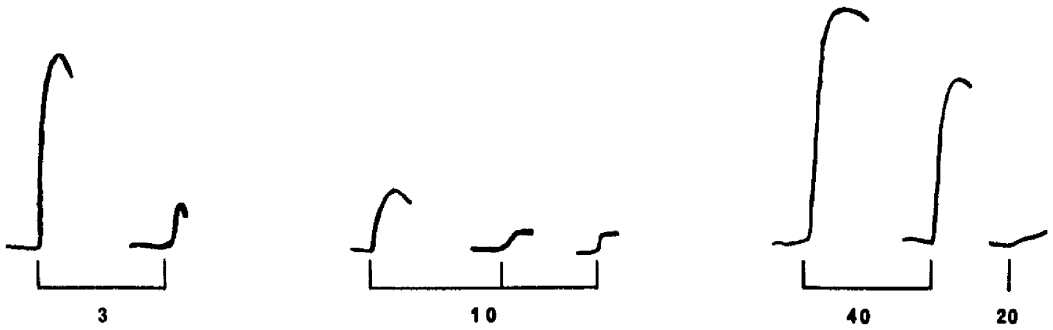


Fig. 3.13

PULMONARY VEIN

Pulmonary vein contracted in response to acetylcholine (0.005 to 30.0 µg/ml), histamine (10 µg/ml), 5-hydroxy-tryptamine (0.0002 to 0.1 µg/ml) and adrenaline (0.5 µg/ml).

The ranges of concentrations of acetylcholine, histamine, 5-hydroxytryptamine and bradykinin producing responses of at least 2 cm. in height at a magnification of x 200 are summarised in Table 3. 6, which includes the numbers of samples of trachealis, bronchiolar, pulmonary arterial and pulmonary venous tissue which responded to each dose range.

TABLE 3.6 RANGES OF CONCENTRATIONS OF DRUGS USED IN VITRO.

Drug (μg/ml)	Preparation							
	Trachea	No.	Bronchiole	No.	Pulmonary Artery	No.	Pulmonary Vein	No.
Histamine	< 1.0	4	< 1.0	4	< 0.1	1	1.0 - 10	2
	1.0 - 2.0	10	1.0 - 10	14	0.1 - 2.0	7		
	> 2.0	4	> 10	4	> 2.0	2		
5-Hydroxy-Tryptamine	< 0.1	9	< 1.0	3	< 0.01	3	< 0.01	4
	0.1 - 1.0	13	1.0 - 10	8	0.01 - 0.2	12	0.01 - 0.2	1
	> 1.0	2	> 10	1	> 0.2	5	> 0.2	1
Bradykinin	< 0.1	1	< 1.0	3	0.1 - 1.0	7		
	0.1 - 1.0	3	1.0 - 10	8	> 1.0	2		
Acetylcholine	< 0.01	4	< 1.0	7	0.5 - 1.0	2	0.005 -1.0	2
	0.01 -0.1	7	1.0 - 10	19			1.0 - 10	1
	0.1 - 1.0	13	> 10	9				
	> 1.0	3						

b) ii) EFFECTS OF DRUGS ON RESISTANCE TO AIRFLOW AND VASCULAR PERFUSION IN ISOLATED LUNGS

MATERIALS AND METHODS

Lobes of lungs of young adult cattle, obtained within 15 min. of slaughter were perfused using the technique described by Brocklehurst (1960), with minor modifications. One lobe of bovine lung was perfused by means of a cannula tied into the pulmonary artery whereas Brocklehurst perfused intact lungs of the smaller laboratory animals. The tissue was suspended by the bronchus, inside a polythene bag lightly closed at the top to prevent excessive cooling. The effluent solution ran from pulmonary veins and was allowed to escape through slits in the bottom of the polythene bag into a polythene filter funnel. Perfusion fluid was supplied by gravity flow from a 5 litre aspirator at a constant height of 80 cm. above the lungs, giving a perfusion pressure of approximately 60 mm. Hg. and passed through a warming coil to raise its

temperature to 37°C immediately before it entered the cannula. Drugs were injected into the perfusion fluid through a rubber connection between the warming coil and the cannula. In 3 cases the lung was simultaneously perfused with air through a cannula in the bronchus by means of an Ideal respiratory pump (C.F. Palmer Ltd.). The stroke volume used was 120 cc delivered at a rate of 13 strokes/min. Inflation was minimised by allowing air to escape from the alveoli through scarifications made on the surface of the inflated lung. The air perfusion pressure was measured in cm. water by means of a differential pressure transducer and recorded on a multi-channel physiological pen recorder (Devices Ltd.). Resistance to air perfusion was recorded as an increase in pressure. The rate of air perfusion was adjusted to maintain an initial pressure of 10 to 30 cm. water. The rate of perfusion was 1.0 to 0.5 ml/min/gm tissue. The rate of flow was adjusted by means

of a screw clip, distal to the pulmonary cannula. The perfusion fluid was oxygenated Tyrode solution. Before beginning recordings, attempts were made to flush the lungs free of blood. Perfusate was collected in glass or polythene bottles every consecutive or alternate min. or 30 sec. during the course of perfusion and the volume measured. The temperature of the lung was continuously monitored by means of a needle probe thermistor, inserted in the lung and attached to an electric thermometer (Light Laboratories). The efficiency of perfusion was checked by injecting Evan's Blue dye (Gurr Ltd.) (1.0 mg/ml) into the perfusion fluid at the conclusion of each experiment and examining the extent of blue colouration of the dissected lung (Fig. 3. 14). The apparatus is represented diagramatically in Fig. 3. 15.

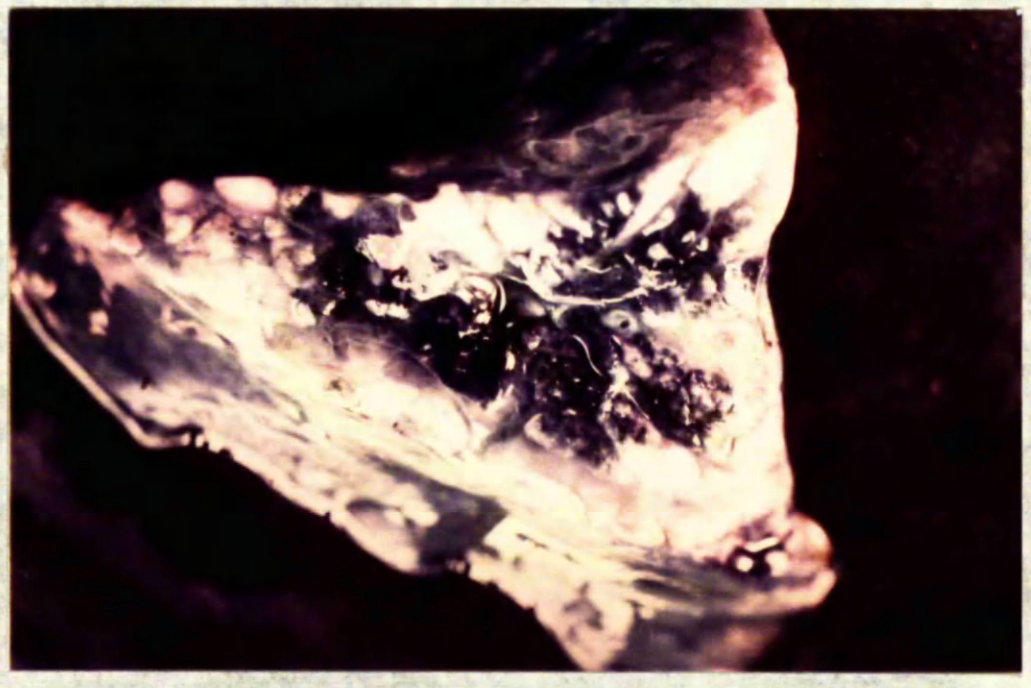


Fig. 3.14 - Transverse section of cardiac lobe of bovine lung after perfusion with Tyrode solution containing Evan's Blue dye.

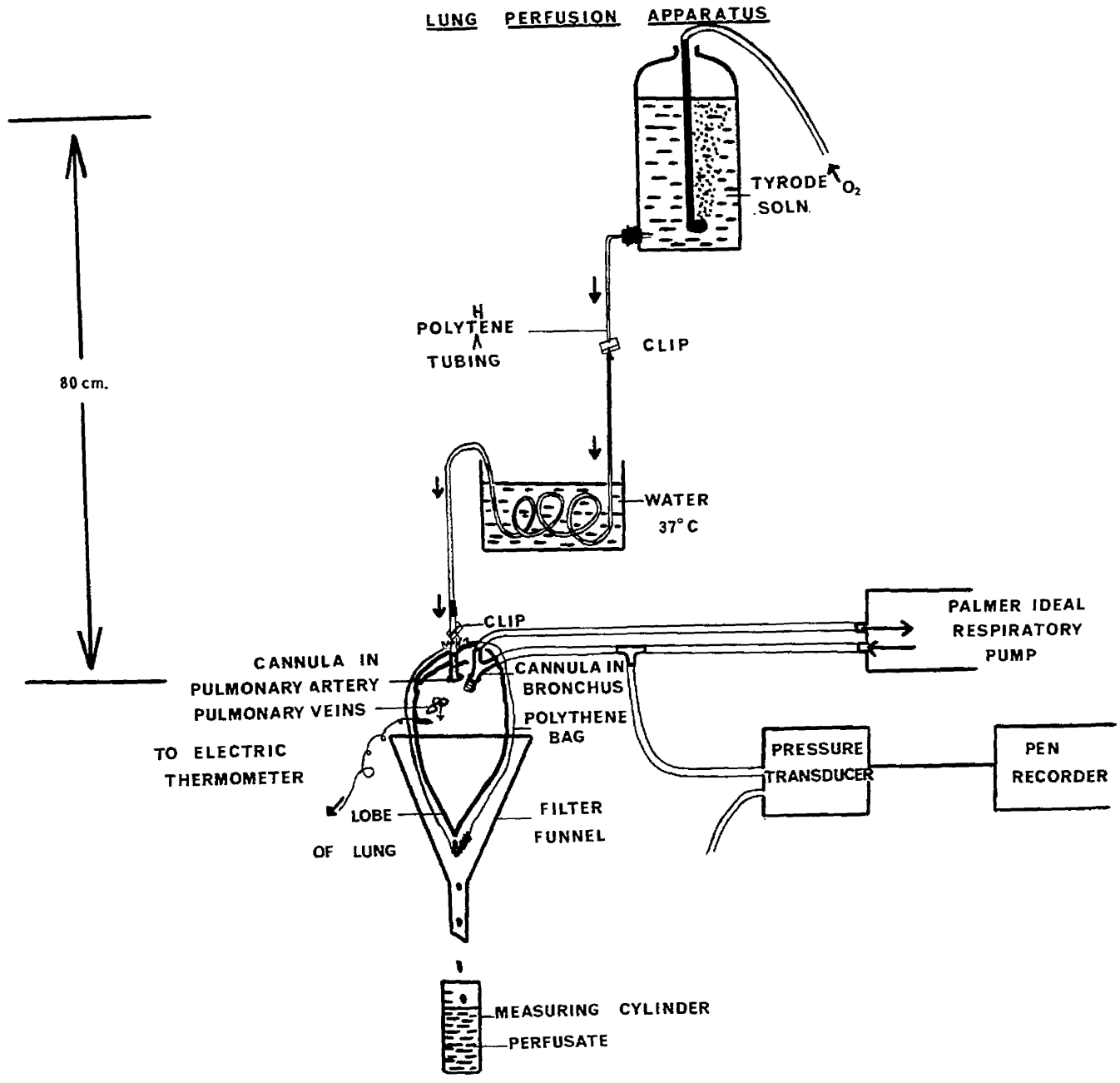


Fig. 3.15 - Diagramatic representation of apparatus used to perfuse bovine lung.

RESULTS

Air perfusion

Administration of acetylcholine, histamine, 5-hydroxytryptamine and bradykinin resulted in each case in an increase in air perfusion pressure. Adrenaline reduced the perfusion pressure. The results are summarised in Table 3. 7. Changes in pressure were apparent within 5 sec. of completing administration of the drugs. After responding once to 5-hydroxytryptamine the lung showed tachyphylaxis, a second dose given 63 min. later eliciting a reduced response and a third given 40 min. later having a negligible effect. Fig. 3. 16 shows the effects on air perfusion pressure of histamine, acetylcholine and adrenaline.

Results obtained using this preparation were considered to be of more qualitative than quantitative value as perfusion pressure was influenced by the ability of air to escape from the alveoli as well as by constrictor or relaxant effects

TABLE 3. 7

EFFECTS OF DRUGS ON AIR PERFUSION PRESSURE OF ISOLATED LUNGS

Drug	Dose (µg)	Lung No.	Pressure ($Cm.H_2O$)		
			Before	After	% Change
Acetylcholine	100	4	26	34	+ 31
	500	4	26	58	+123
	500	1	20	33	+ 65
	500	5	36	41	+ 14
	600	3	21	47	+123
	900	2	10	33	+230
	1,000	5	37	42	+ 13
Histamine	300	4	28	36	+ 28
	600	1	17	23	+ 35
	800	2	16	33	+106
	800	3	20	40	+100
	800	3	16	47	+194
	1,000	5	27	34	+ 25
5-Hydroxytrypt-amine	250	1	22	28	+ 27
	300	4	30	42	+ 40
	400	3	16	29	+ 81
	400	3	10	15	+ 50
	400	3	26	28	+ 7
	800	5	26	30	+ 15
Bradykinin	500	1	21	21	0
	800	2	16	28	+ 75
	2,500	5	37	38	+ 3
Adrenaline	600	2	33	10	- 70
	800	3	25	11	- 56

\+ = increased
\- = reduced

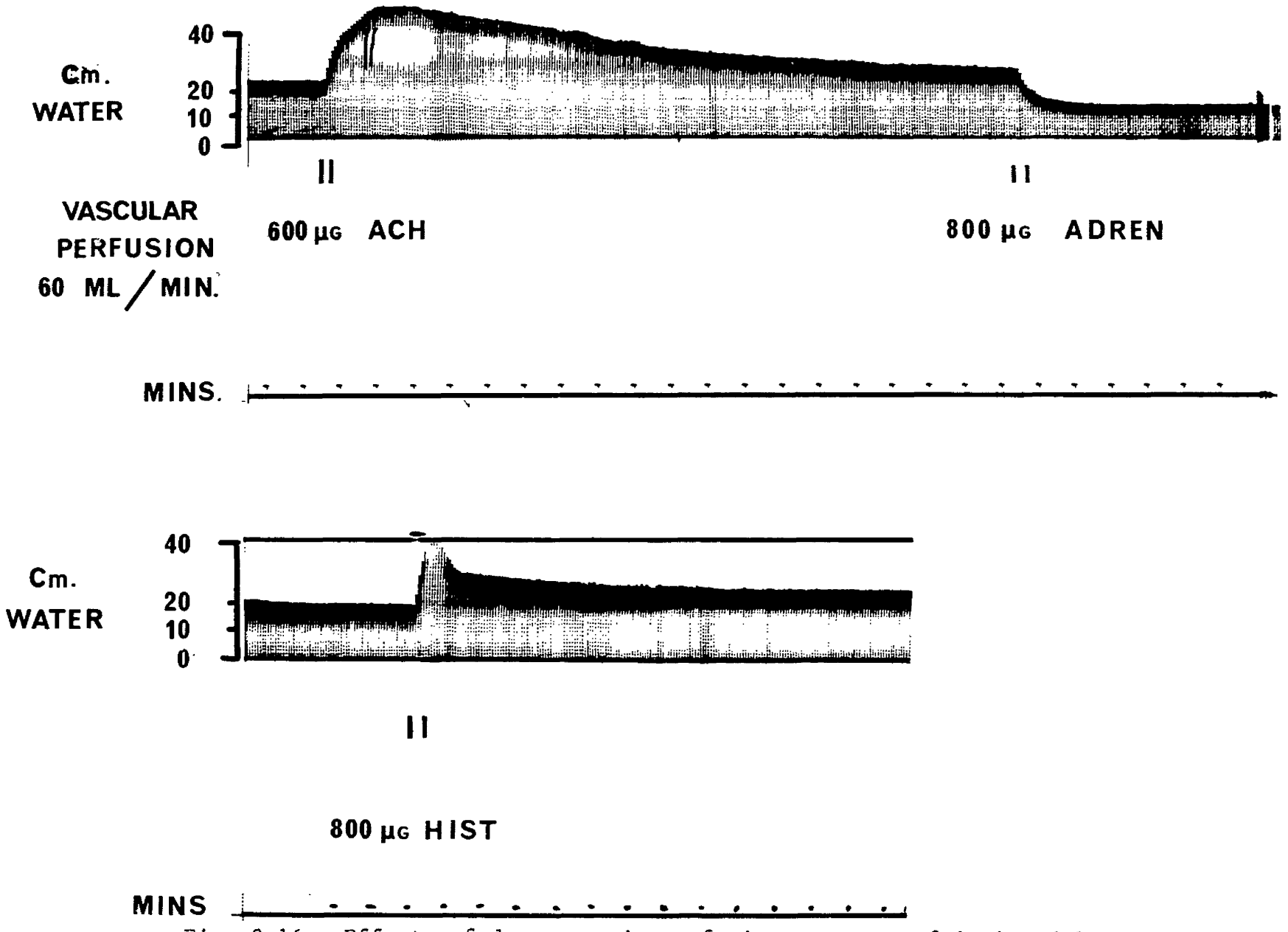


Fig. 3.16 - Effects of drugs on air perfusion pressure of isolated lungs. Drugs injected between marks (| |).

of drugs on bronchial smooth muscle. These drugs also had actions on vascular smooth muscle and on capillary permeability and the efficiency of vascular perfusion would affect the distribution of drugs throughout the lung. Intervals of 30 min. to one hr. between doses of drugs were often necessary to allow complete recovery.

VASCULAR PERFUSION

Perfusion rate, measured as the rate of outflow of fluid from the pulmonary vein, was reduced following injection of acetylcholine, histamine, 5-hydroxytryptamine and bradykinin. Adrenaline increased the rate of outflow. The results are summarised in Table 3. 8. The change in perfusion rate was taken as the difference between the average of at least 3 samples obtained consecutively before drug administration and the sample after drug administration, which showed the maximum increase or decrease in volume. There was poor correlation between dose and response, but the concentration

TABLE 3. 8

EFFECTS OF DRUGS ON VASCULAR PERFUSION RATE OF ISOLATED LUNGS

Drug	No. of Lungs Tested	Dose Range µg	% Change in Perfusion Rate Range
Acetylcholine	4	400 to 1,000	- 5 to - 43
Histamine	6	10 to 1,000	-11 to - 91
5-Hydroxytrypt-amine	6	50 to 500	-20 to - 69
Bradykinin	3	10 to 1,000	-21 to - 50
Adrenaline	1	1,000	+26

- = reduced

+ = increased

of drug reaching the tissue was difficult to estimate as the change in flow rate would affect the degree of dilution of the administered dose. The volume changes were seen at their maxima one min. after drug administration. Five to 30 min. were necessary to allow volumes to return to pre-injection values. Typical changes in vascular perfusion rate in relation to drug administration are shown in Fig. 3. 17.

Injection of ovalbumin (1 to 500 mg), as shown in Fig. 3. 17, did not appear to affect the vascular perfusion rate of unsensitised lung. As previously explained quantitative conclusions have not been drawn from these results. In Fig. 3. 17 a difference in rates of recovery between lung No. 1 and lung No. 2 can be seen.

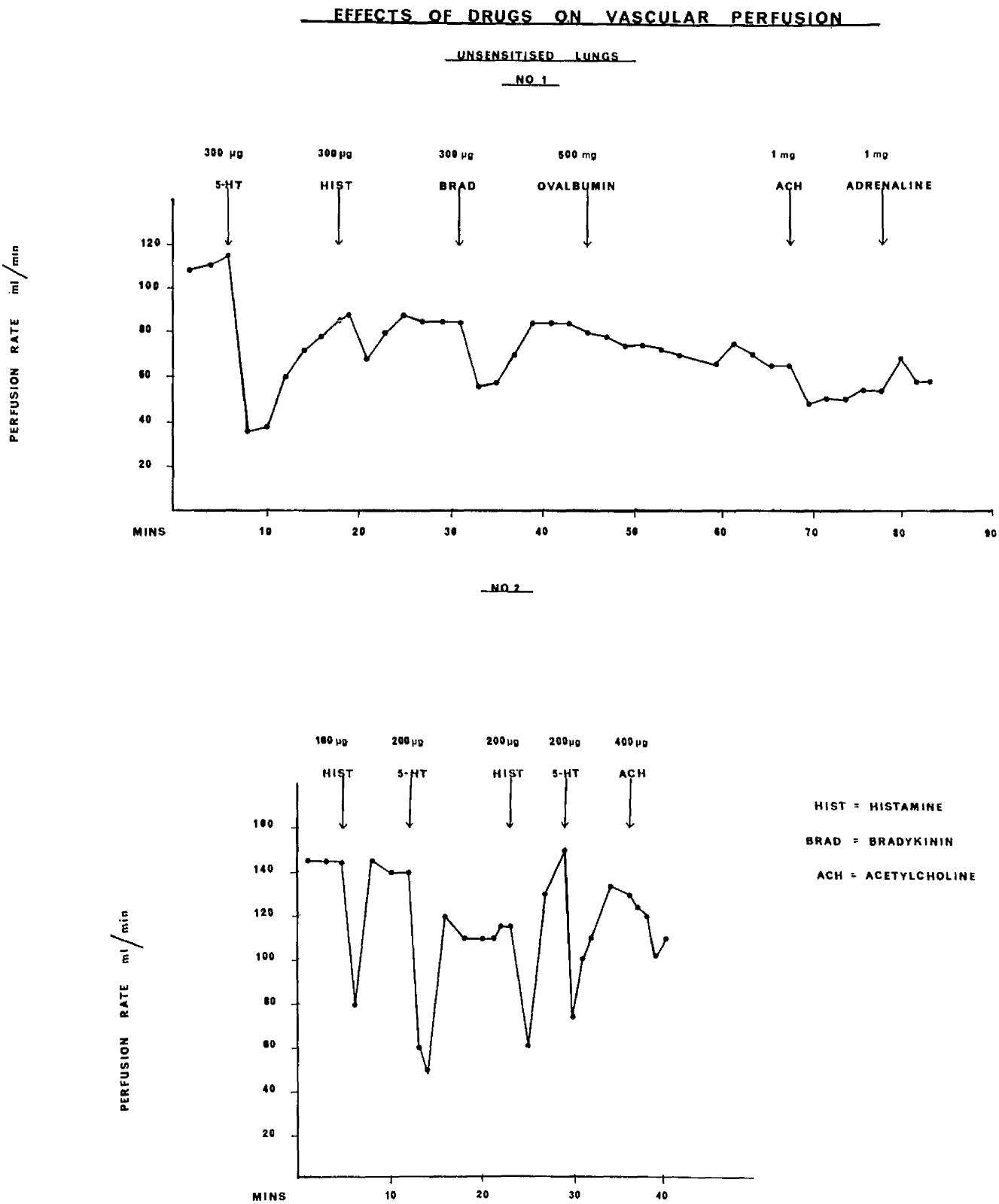

EFFECTS OF DRUGS ON VASCULAR PERFUSION
UNSENSITISED LUNGS
NO 1
300 µg
5-HT
300 µg
HIST
300 µg
BRAD
500 mg
OVALBUMIN
1 mg
ACH
1 mg
ADRENALINE
PERFUSION RATE ml/min
120
100
80
60
40
20
MINS
10
20
30
40
50
60
70
80
90
NO 2
160 µg
HIST
200 µg
5-HT
200 µg
HIST
200 µg
5-HT
400 µg
ACH
160
140
120
100
80
60
40
20
MINS
10
20
30
40
HIST = HISTAMINE
BRAD = BRADYKININ
ACH = ACETYLCHOLINE

Fig. 3.17

SUMMARY

1. Histamine and 5-hydroxytryptamine both produced effects similar to those of anaphylaxis in conscious animals.

2. Histamine, 5-hydroxytryptamine and bradykinin produced respiratory and circulatory changes similar to those seen during anaphylaxis.

3. Only histamine and bradykinin produced pulmonary oedema comparable to that produced by anaphylaxis.

4. The effects of all 3 substances appeared to be unchanged in vagotomised animals.

5. The respiratory effects of histamine and bradykinin were prevented by mepyramine and meclofenamate respectively but the respiratory effects of 5-hydroxytryptamine persisted in the presence of 501067 and methysergide.

6. All 3 substances caused contraction of isolated pulmonary smooth muscle and these effects were inhibited by specific or selective antagonists.

7. Histamine, 5-hydroxytryptamine and bradykinin reduced perfusion rate through both the airways and blood vessels of the isolated lung.

4. MODIFICATION OF ACUTE SYSTEMIC ANAPHYLAXIS BY ANTAGONISTS AND BY VAGOTOMY

ANIMALS

All animals used had been sensitised to ovalbumin or horse serum and had shown severe dyspnoea (+++) on challenge. All had been challenged on at least 2 previous occasions. Groups of 2 to 18 animals were used in cross-over tests, so that each animal served as its own control in the assessment of the effects of antagonists on the symptoms of anaphylaxis.

MATERIALS AND METHODS

Ovalbumin was prepared as a 5% solution in sterile saline as described in Section II. 1. Horse serum was also prepared, as described in Section II. 1 from clotted horse blood. A constant challenge dose of antigen (about 2.0 mg/Kg) was selected for each animal by preliminary tests, so that consistent, non-fatal reactions were produced.

The antagonist drugs used were:

atropine sulphate (0.1 mg/Kg)

mepyramine maleate (2.0 mg/Kg)

promethazine hydrochloride (May and Baker) (1.0 mg/Kg)

cyproheptadine hydrochloride (0.3 mg/Kg)

methysergide bimaleate (0.1 mg/Kg)

501C67 (1.0 mg/Kg)

sodium meclofenamate (1.0 to 2.0 mg/Kg)

phenylbutazone (Stevenson, Turner and Boyce Ltd.) (1.0 mg/Kg)

disodium cromoglycate (Fisons Pharmaceuticals Ltd.) (30 mg/Kg)

diethylcarbamazine citrate (Burroughs Wellcome & Co.) (20 mg/Kg)

Each of the above antagonists was administered singly and was injected intravenously 5 min. before challenge with antigen, with the exception of diethylcarbamazine and disodium cromoglycate which were injected intramuscularly 30 min. before challenge. Intravenous administration of diethylcarbamazine

was not repeated after this had resulted in signs of central nervous stimulation and collapse. Disodium cromoglycate was reported by the manufacturers to activate chemoreceptors in the pulmonary and coronary circulations of the dog when injected intravenously.

The effect of using mepyramine together with meclofenamate was subsequently investigated. Atropine was used with the object of blocking vagal reflex effects possibly produced by intravenous injection (Gilding and Nutt, 1943) and the dose was that recommended to counteract organophosphorus poisoning in cattle (Garner, 1961). This dose was found to inhibit the effects of 0.08 mg/Kg acetylcholine administered intravenously (See Fig.4. 11). The doses of mepyramine, cyproheptadine and methysergide were those found previously to inhibit the effects produced by administration of histamine (0.03 mg/Kg) and 5-hydroxytryptamine (0.07 mg/Kg) respectively in cattle. The dose of meclofenamate was as used by Collier

and James (1967) in guinea-pigs to antagonise bradykinin and SRS-A. The doses of phenylbutazone and promethazine were the therapeutic doses recommended by the manufacturers and the doses of disodium cromoglycate and diethylcarbamazine were those used by Orange and Austen (1968) to inhibit release of histamine and SRS-A respectively in rats. Each animal was observed for at least one hr. after challenge and the duration of collapse, degree of dyspnoea, frequency of coughing, increase in salivation, nasal discharge and lacrimation were scored in degrees of severity from + to +++.

Table 4. 1 lists the humoral factors possibly produced or released during anaphylaxis and the antagonists used to investigate their participation. The doses of antagonists, routes of administration and time intervals between administration of antagonist and challenge with antigen are summarised in Table 4. 1.

TABLE 4.1 ANTAGONISTS OF POSSIBLE MEDIATORS

Humoral Factor	Antagonist	Dose of Antagonist mg/Kg.	Route	Interval min.
Acetylcholine	Atropine	0.1	i.v.	5
Histamine	Mepyramine	2.0	i.v.	5
	Promethazine	1.0	i.v.	5
	Cyproheptadine	0.3	i.v.	5
	Disodium Cromoglycate	30.0	i.m.	30
5-Hydroxytryptamine	Cyproheptadine	0.3	i.v.	5
	Methysergide	0.1	i.v.	5
	501C67	1.0	i.v.	5
Kinins	Meclofenamate	1.0 - 2.0	i.v.	5
	Phenylbutazone	1.0	i.v.	5
SRS-A	Meclofenamate	1.0 - 2.0	i.v.	5
	Phenylbutazone	1.0	i.v.	5
	Diethylcarbamazine	20	i.m.	30

Subsequently, the effects of challenge on anaesthetised animals pretreated with antagonists were examined. The drugs used in anaesthetised animals were atropine, mepyramine, 501C67 and meclofenamate. Three anaesthetised animals were subjected to bilateral vagotomy, carried out as described in Section II. 3, before challenge. Respiratory flow, minute respiratory volume, systemic arterial blood pressure, pulmonary arterial blood pressure and heart rate were measured and recorded in these animals as described in Section II. 2.

RESULTS

a) CONSCIOUS ANIMALS

The effect of pretreatment with atropine on the severity of anaphylactic shock is shown in Fig. 4. 1, where histograms represent the average severity of each symptom in a group of 4 animals. Salivation, nasal discharge and lacrimation were abolished. Coughing was less marked but dyspnoea was unchanged and the duration of collapse slightly increased in one animal after atropine pretreatment.

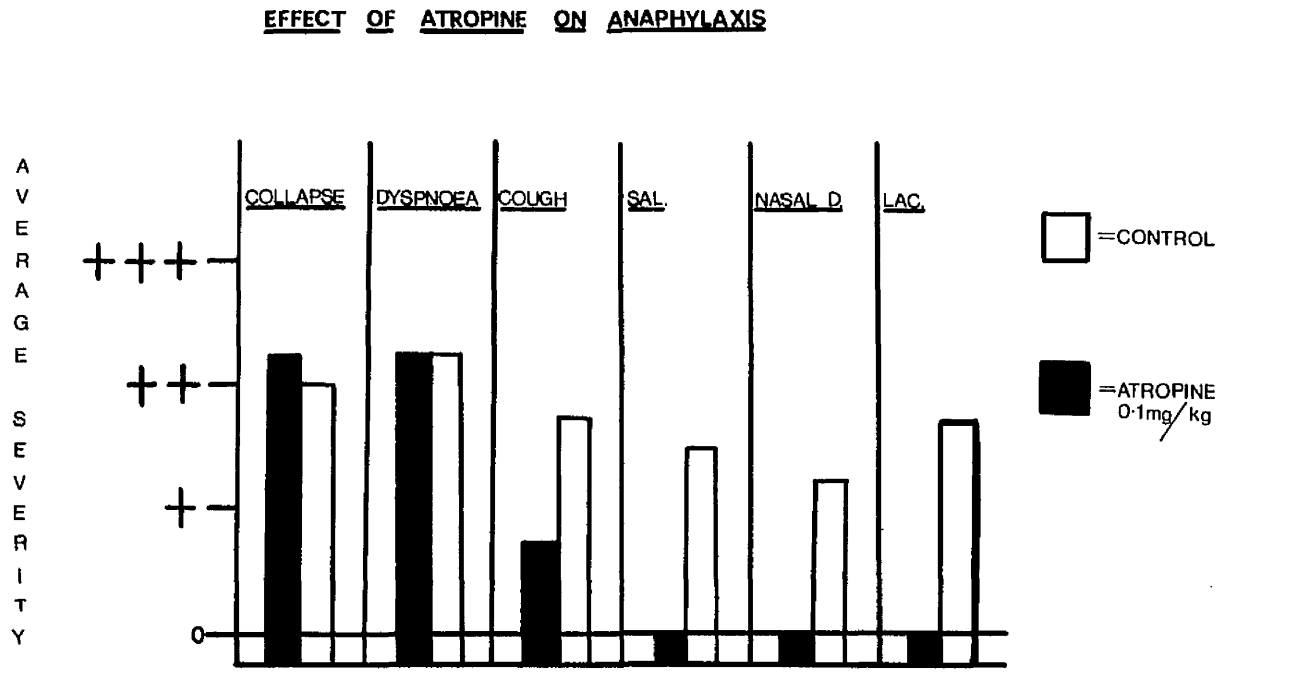

EFFECT OF ATROPINE ON ANAPHYLAXIS
AVERAGE SEVERITY
+++
++
+
0
COLLAPSE
DYSPNOEA
COUGH
SAL.
NASAL D.
LAC.
=CONTROL
=ATROPINE 0·1mg/kg
N=4

Fig. 4.1

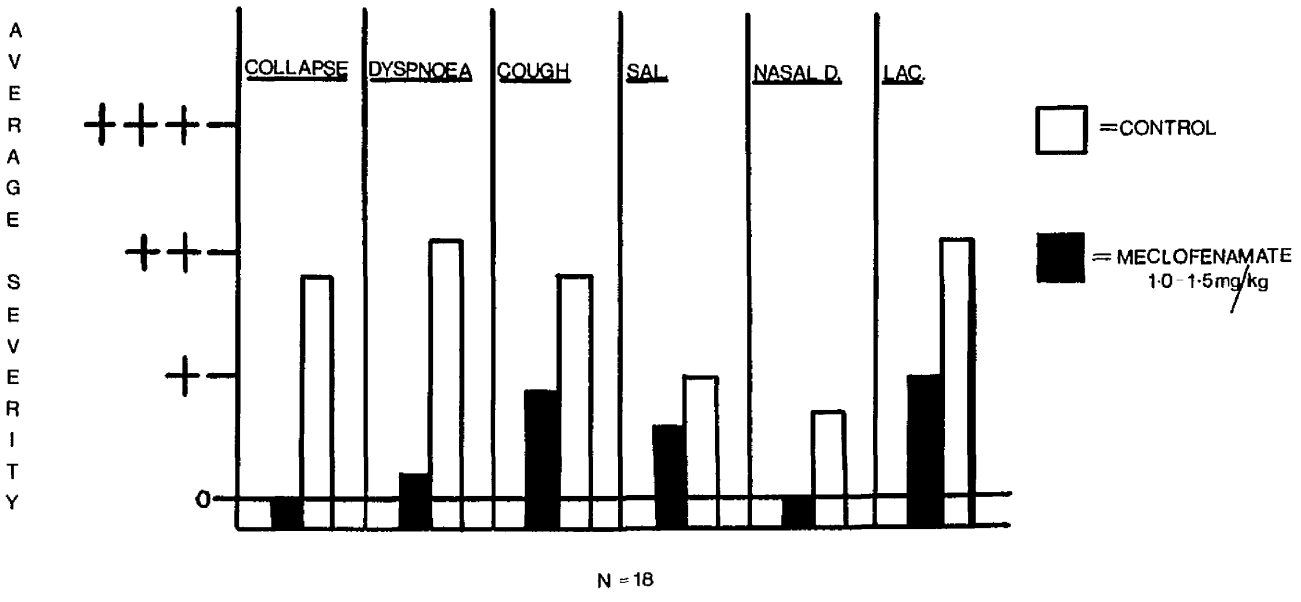
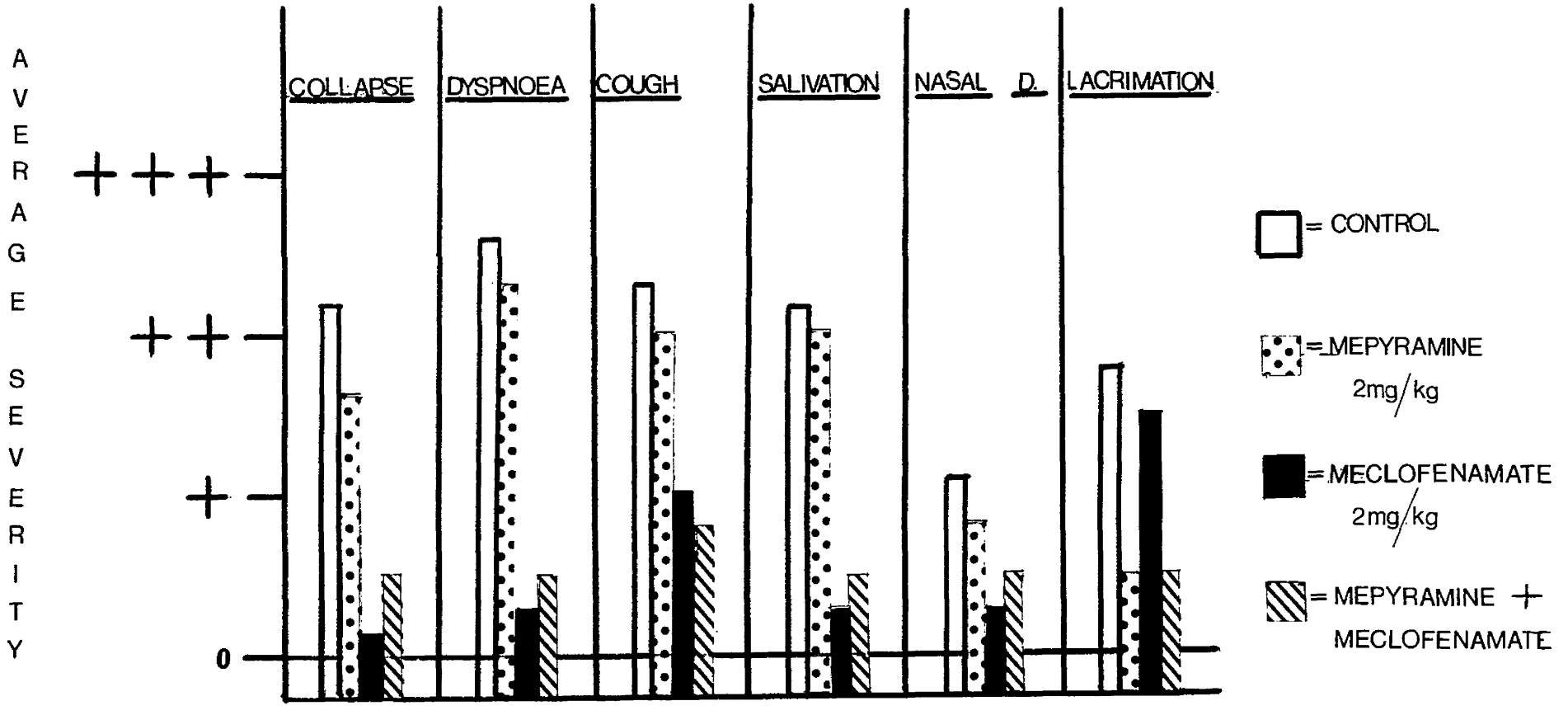

EFFECT OF MECLOFENAMATE ON ANAPHYLAXIS
AVERAGE SEVERITY
+++
++
+
0
COLLAPSE
DYSPNOEA
COUGH
SAL.
NASAL D.
LAC.
=CONTROL
= MECLOFENAMATE 1·0-1·5mg/kg
N = 18

Fig. 4.2

When a group of 6 animals were pretreated with meclofenamate the effects of subsequent challenge with antigen were largely suppressed. This protective effect of meclofenamate was tested in 2 further groups of 6 animals. At least 2 cross-over-tests were carried out on each animal. The findings obtained from a total of 18 animals are summarised in Fig. 4. 2. Collapse, respiratory distress and nasal discharge were regularly prevented. The reduction in coughing, salivation and lacrimation was more variable. Increasing the dose of meclofenamate from 1.5 mg/Kg to 2.0 mg/Kg did not increase the degree of protection. Fig. 4. 3 shows the effect, in a group of 6 animals, of pretreatment with meclofenamate alone, the antihistamine, mepyramine alone and meclofenamate together with mepyramine. Mepyramine alone had little effect on any of the symptoms of anaphylaxis with the exception of lacrimation which was reduced. Likewise mepyramine used together with meclofenamate improved on meclofenamate alone only in reducing lacrimation. Of 24 animals

EFFECT OF MEPYRAMINE AND MECLOFENAMATE ON ANAPHYLAXIS

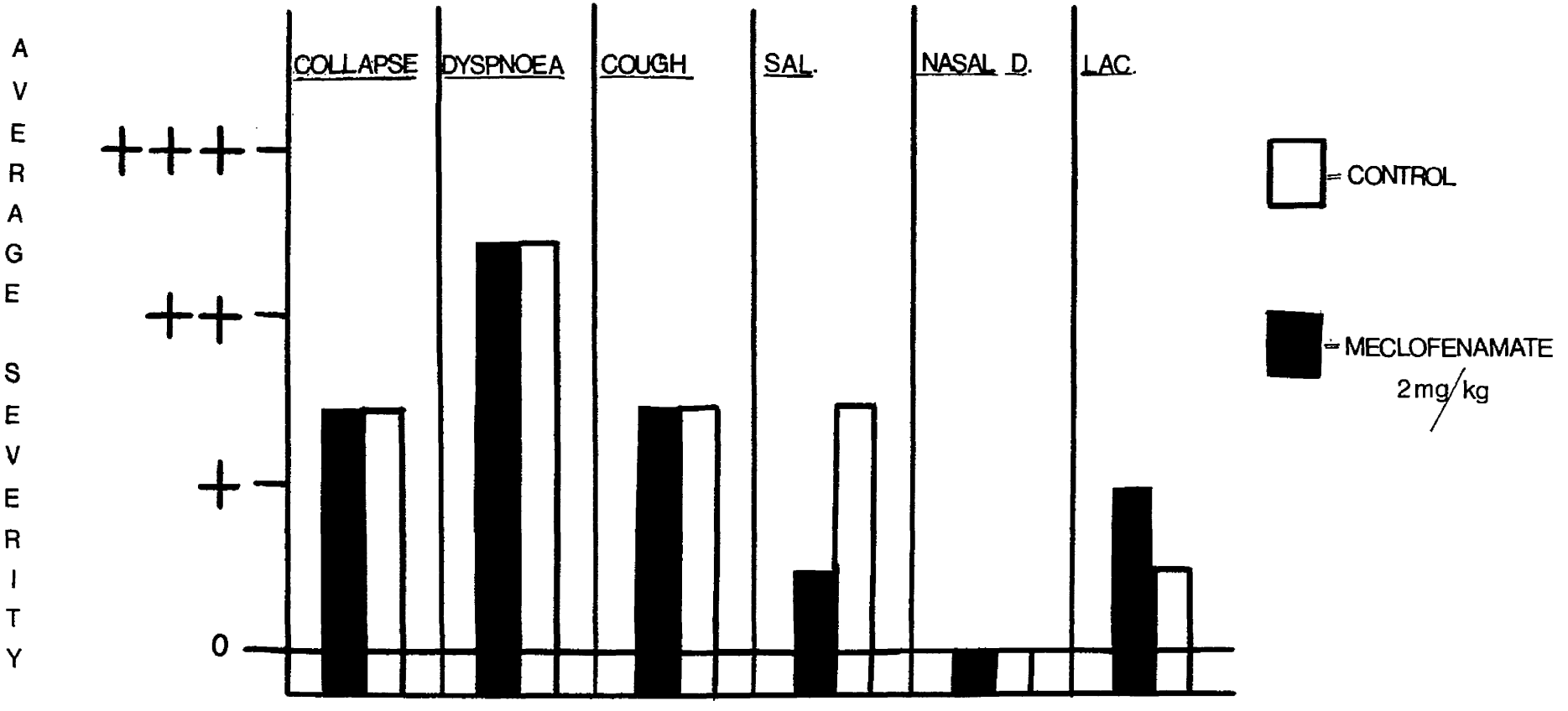


N=6

Fig. 4.3

pretreated with either meclofenamate alone or meclofenamate and mepyramine only one animal failed to be protected. Findings were similar whether the antigen involved was ovalbumin or horse serum. As shown in Fig. 4. 4,2 animals which had been sensitised by intramuscular injections of ovalbumin with Freund's adjuvant failed to be protected by pretreatment with 2 mg/Kg meclofenamate against challenge with a dose of 1.5 mg/Kg ovalbumin. As previously described in Section II. 1 animals sensitised in this way reacted more severely to equal doses of ovalbumin than did those sensitised without adjuvant. A challenge dose of 0.03 mg/Kg ovalbumin in an animal sensitised with Freund's adjuvant produced a reaction characterised by staggering but not collapse, dyspnoea (+++) with the mouth open, neck extended and expiratory grunting and other symptoms. Repeated tests on this animal, summarised in Table 4.2, revealed suppression by meclofenamate of an anaphylactic reaction of this degree of severity.

EFFECT OF MECLOFENAMATE ON ANAPHYLAXIS IN ANIMALS SENSITISED USING FREUND'S ADJUVANT



N = 2

Fig. 4.4

TABLE 4.2 EFFECT OF MECLOFENAMATE (2 mg/Kg) ON ANAPHYLAXIS IN AN ANIMAL SENSITISED TO OVALBUMIN USING FREUND'S ADJUVANT AND CHALLENGED REPEATEDLY BY I.V. INJECTION OF OVALBUMIN (0.03 mg/Kg).

Challenge (Day)	Pretreatment	Symptoms						Onset (sec)	Duration (min)
		Collapse	Dyspnoea	Cough	Lacrimation	Salivation	Nasal Discharge		
1	None	-	+	+++	++	-	-	60	40
8	Meclofenamate	-	-	-	+	+	-	30	30
15	None	+	++	+	+++	+++	-	15	35
80	None	-	+	++	+++	++	++	60	50
87	Meclofenamate	-	-	-	-	-	-	-	-

Fig. 4. 5 shows the failure of cyproheptadine in reducing the severity of anaphylaxis in a group of 4 animals. Every symptom was slightly more marked where cyproheptadine pretreatment had been carried out. Fig. 4.6 shows the effect of methysergide, like cyproheptadine, an antagonist of 5-hydroxytryptamine. This appeared to reduce coughing, salivation, nasal discharge and lacrimation but the incidence of collapse and degree of dyspnoea were unchanged.

Phenylbutazone is a compound similar to meclofenamate in its chemical properties and ability to antagonise broncho-constriction induced by SRS-A or kinins in the guinea-pig (Collier and Shorley, 1960, 1963; Berry and Collier, 1964). This reduced the severity of all the symptoms but less regularly and completely than did meclofenamate (Fig. 4. 7). One of the 4 animals tested suffered fatal anaphylaxis after phenylbutazone pretreatment. As meclofenamate acts as an antagonist of SRS-A (Collier and James, 1966) and diethyl-

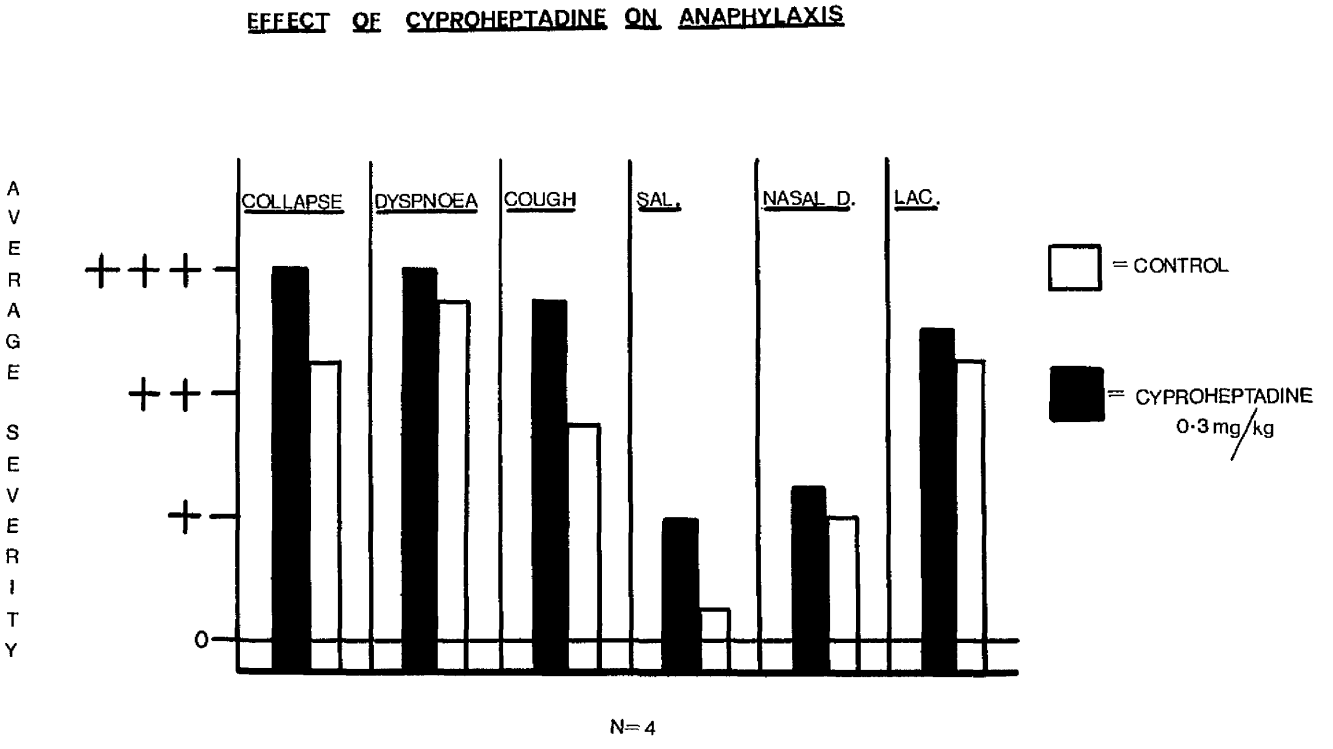

EFFECT OF CYPROHEPTADINE ON ANAPHYLAXIS
AVERAGE SEVERITY
COLLAPSE
DYSPNOEA
COUGH
SAL.
NASAL D.
LAC.
+++
++
+
0
= CONTROL
= CYPROHEPTADINE 0·3 mg/kg
N=4

Fig. 4.5

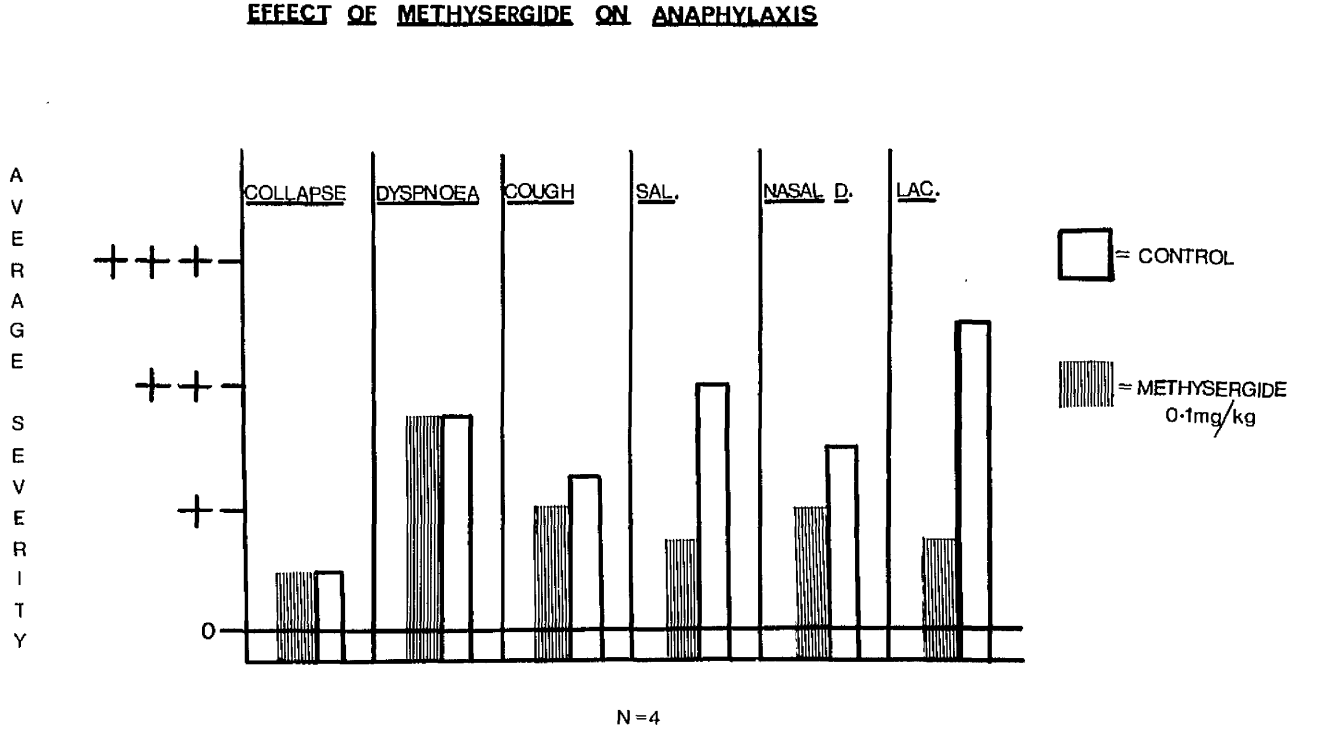

EFFECT OF METHYSERGIDE ON ANAPHYLAXIS
AVERAGE SEVERITY
COLLAPSE
DYSPNOEA
COUGH
SAL.
NASAL D.
LAC.
+++
++
+
0
= CONTROL
= METHYSERGIDE 0·1mg/kg
N=4

Fig. 4.6

carbamazine inhibits release of SRS-A in the rat (Orange and Austen, 1968), 4 animals were pretreated with diethylcarbamazine. The results are summarised in Fig. 4. 8. Two of the animals tested showed a marked reduction in dyspnoea and did not collapse when pretreated with diethylcarbamazine. The other 2 animals, however, showed unmodified reactions.

Promethazine, an antihistamine of less specific action than mepyramine, disodium cromoglycate, which inhibits histamine release in rats (Orange and Austen, 1968), and 501C67, an antagonist of 5-hydroxytryptamine (Mawson and Whittington, 1970) were each tested in one animal but as they showed no evidence of reducing the severity of any of the symptoms of anaphylaxis they were not investigated further.

b) ANAESTHETISED ANIMALS

The effects of challenging anaesthetised animals with antigen after bilateral vagotomy, administration of atropine, meclofenamate, mepyramine and 501C67 are summarised in Table 4. 3.

EFFECT OF DIETHYLCARBAMAZINE ON ANAPHYLAXIS

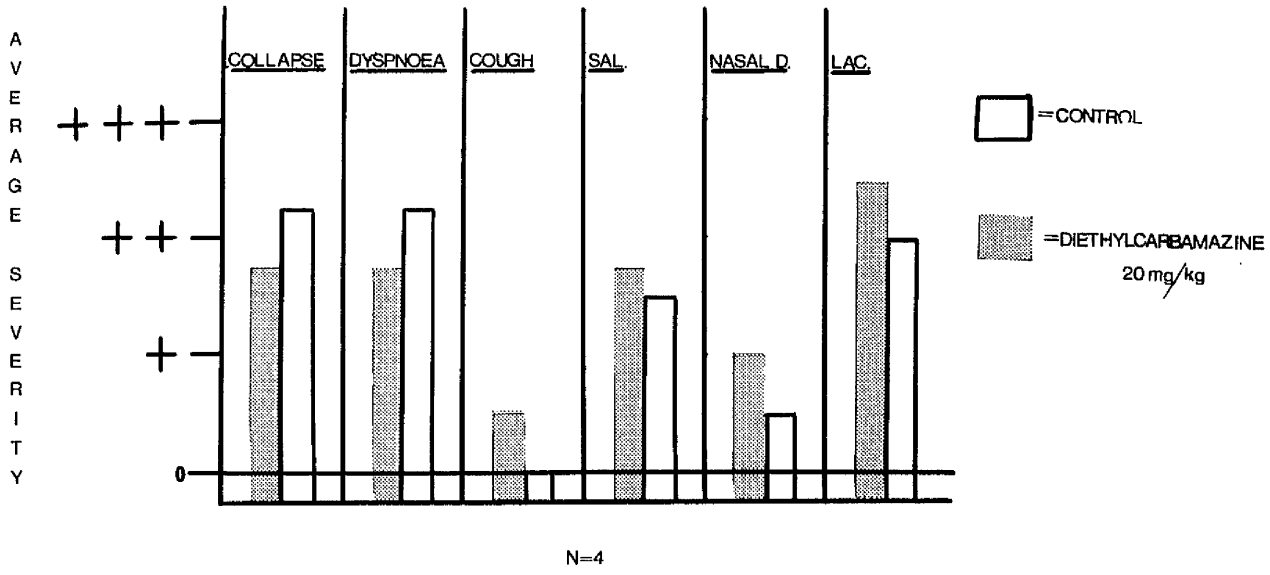


Fig. 4.8

EFFECT OF PHENYLBUTAZONE ON ANAPHYLAXIS

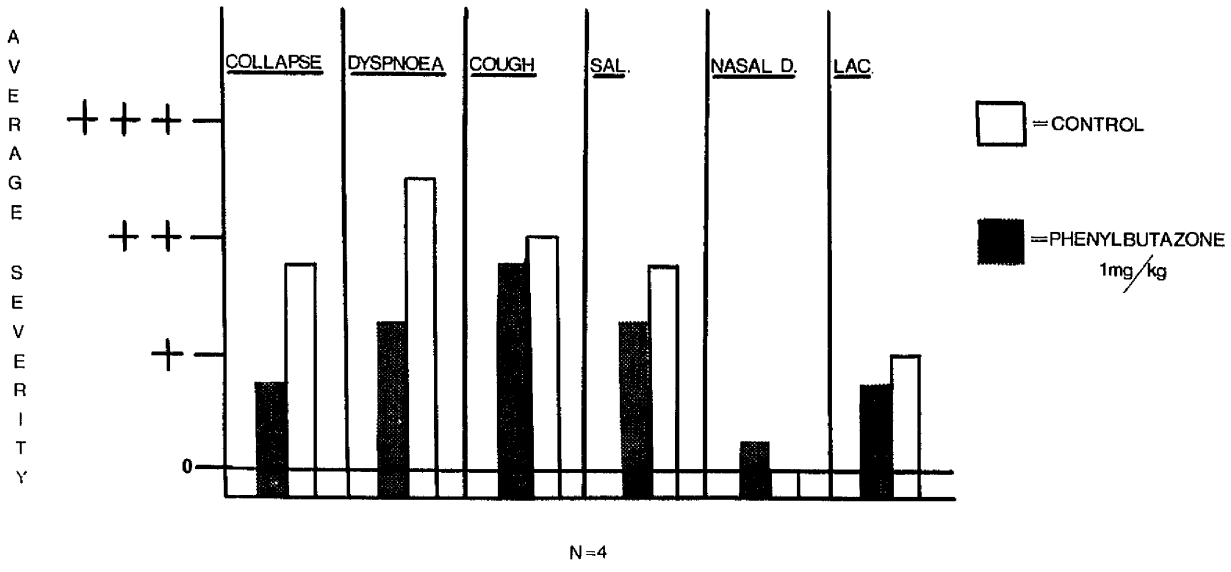


Fig. 4.7

TABLE 4.3 EFFECTS OF CHALLENGE AFTER VAGOTOMY AND AFTER PRETREATMENT WITH ANTAGONISTS

Pretreatment	Challenge Ovalbumin mg/Kg	No.	Onset sec	Duration min	Apnoea sec	% Change			
						Minute Vol % Fall	Systemic B.P % Fall	Pulmonary B.P % Rise	Heart Rate % Fall
Vagotomy	15	42122	25	24	0	18	66	N.M.	20
		42993	24	5	0	8	72	250	80
		43740	18	5	36	100	89	266	21
Atropine 0.1 mg/Kg	15	43741	12	5	18	100	65	400	11.5
Meclofenamate 2.0 mg/Kg	7.5	35790	24	4.5	0	+60	18.75	160	+3
		39234	84	16	0	0	14	100	+ 10
		40869*	30	7	30	50	51	80	14
Mepyramine 2.0 mg/Kg	7.5	37105	30	3.5	42	100	57	N.M.	N.M.
501C67 1.0 mg/Kg	7.5	40986	12	2.0	0	24	31	36	0

\+ = rise
N.M = not measured
* = sensitised using Freund's Adjuvant

In 2 of 3 vagotomised animals apnoea did not occur on challenge with ovalbumin. Both these animals showed a reduction in respiratory flow and minute volume (Fig. 4. 9). In the animal which did show apnoea this developed 2 min. after antigen administration while blood pressure changes occurred after 18 sec. (Fig. 4. 10). Animals challenged without pre-treatment consistently showed simultaneous onset of apnoea and blood pressure changes (Section II. 2). All 3 vagotomised animals suffered systemic hypotension and bradycardia following challenge with 15 mg/Kg ovalbumin. Pulmonary hypertension occurred in both animals in which this was measured.

Pretreatment with 0.1 mg/Kg atropine failed to prevent the typical effects of anaphylaxis, as shown in Fig. 4. 11. This dose of atropine prevented the hypotensive effects of acetylcholine (Fig. 4. 12).

Meclofenamate (2.0 mg/Kg) pretreatment resulted in suppression of the respiratory changes associated with anaphylaxis.

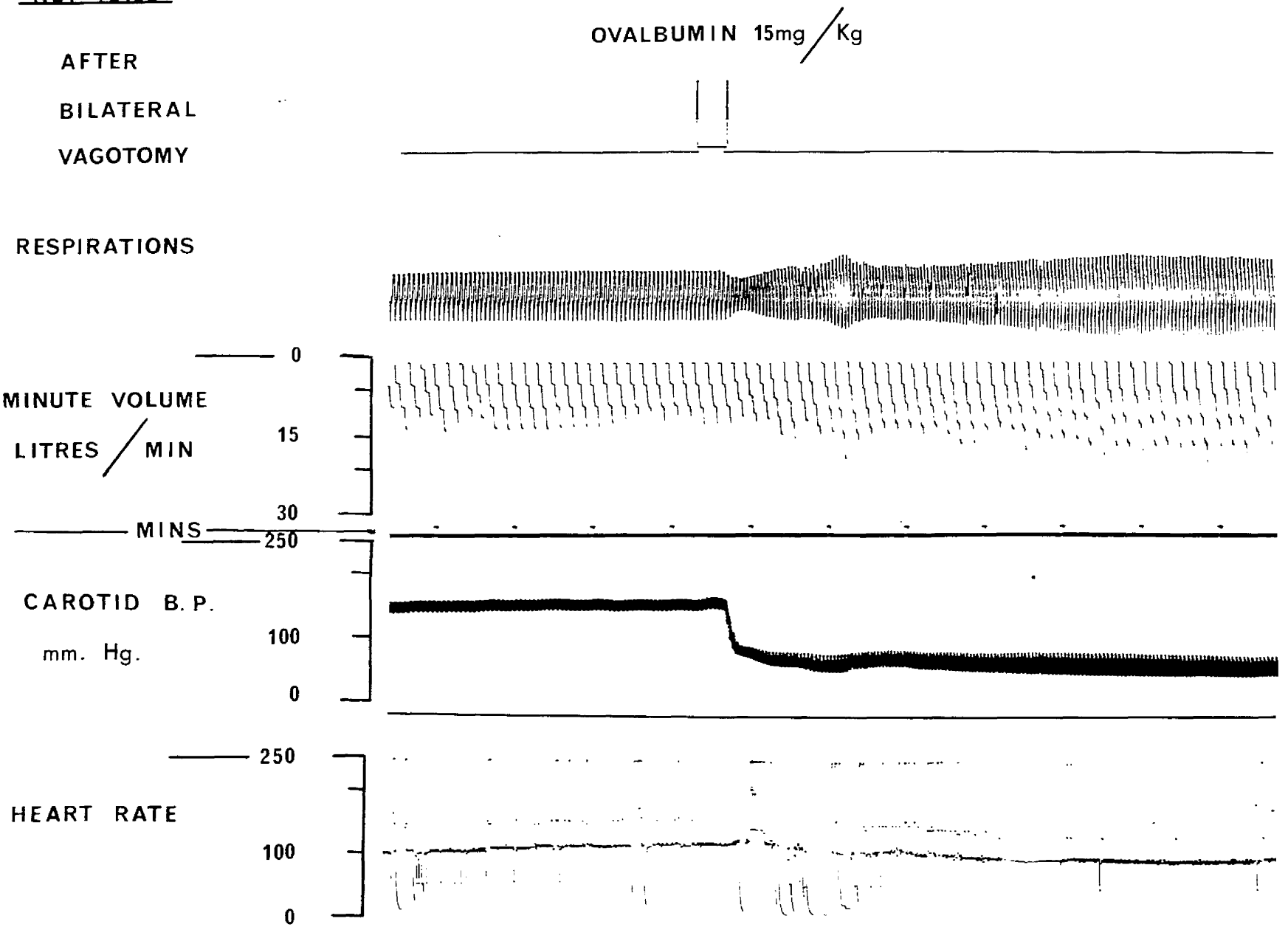


Fig. 4.9 - Effect of challenge on an anaesthetised, vagotomised calf sensitised to ovalbumin.

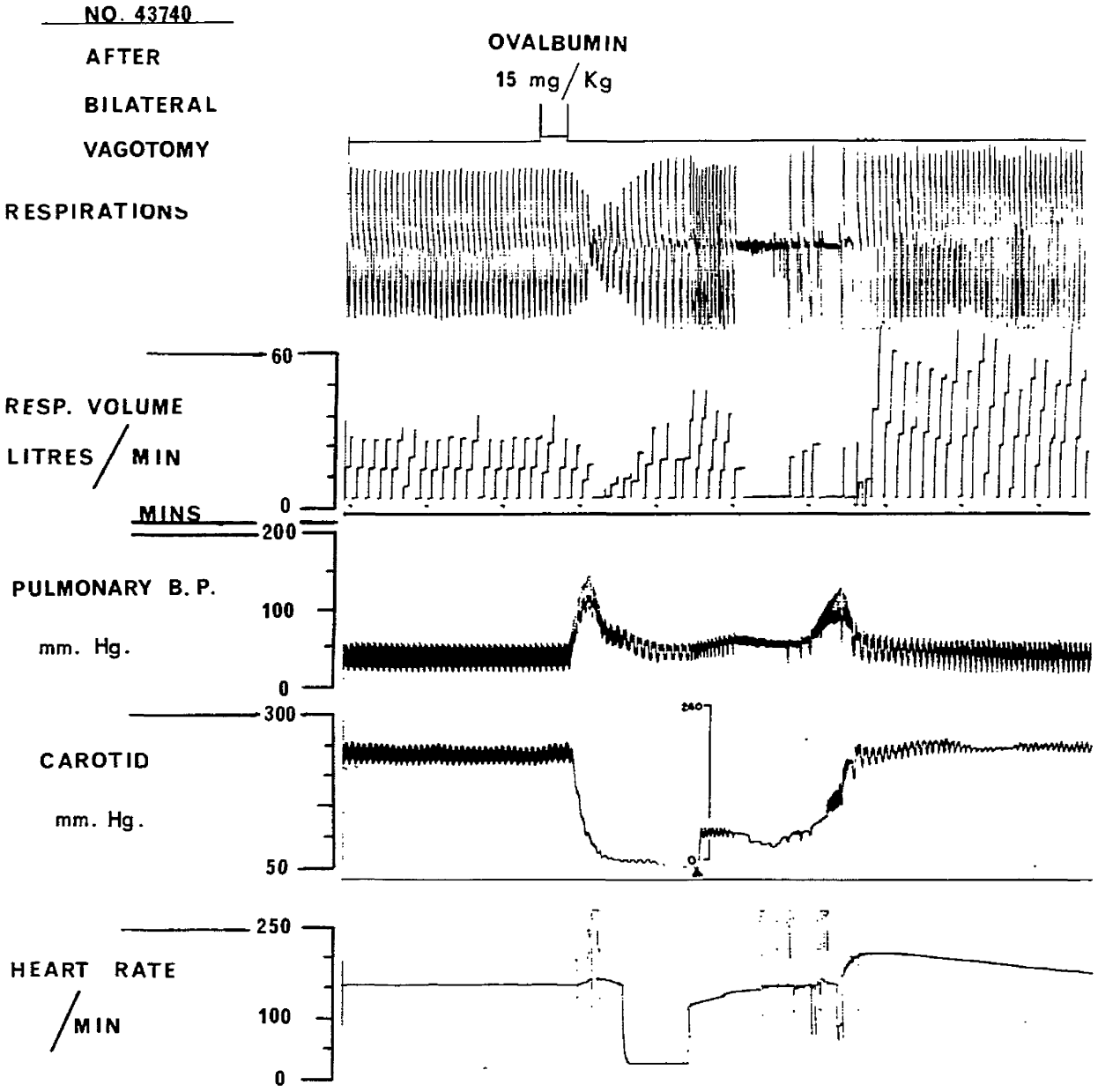


Fig. 4.10 - Effect of challenge on an anaesthetised, vagotomised calf sensitised to ovalbumin.

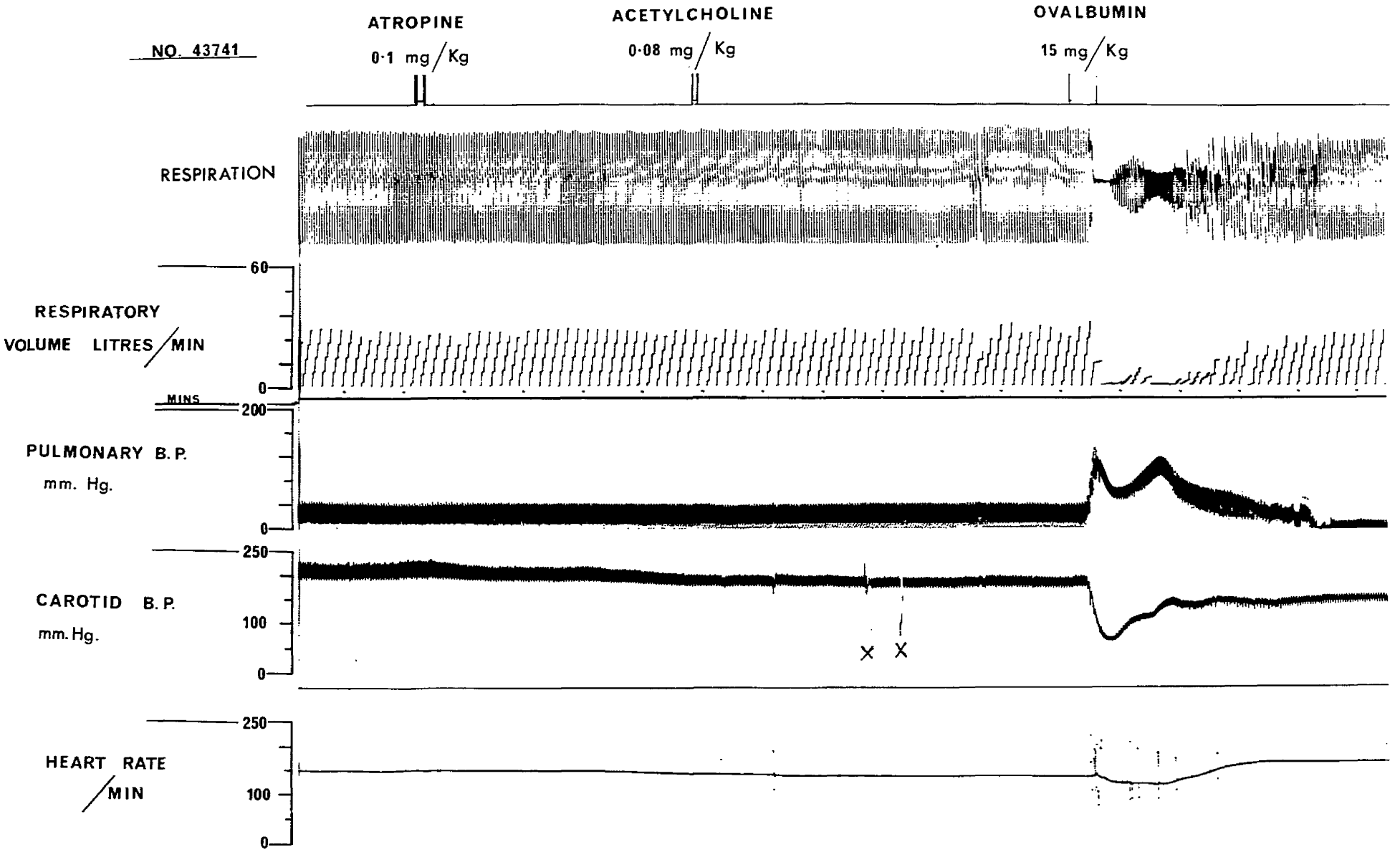


Fig. 4.11 - Effect of challenge on anaesthetised calf pretreated with atropine.
(X = artefact.)

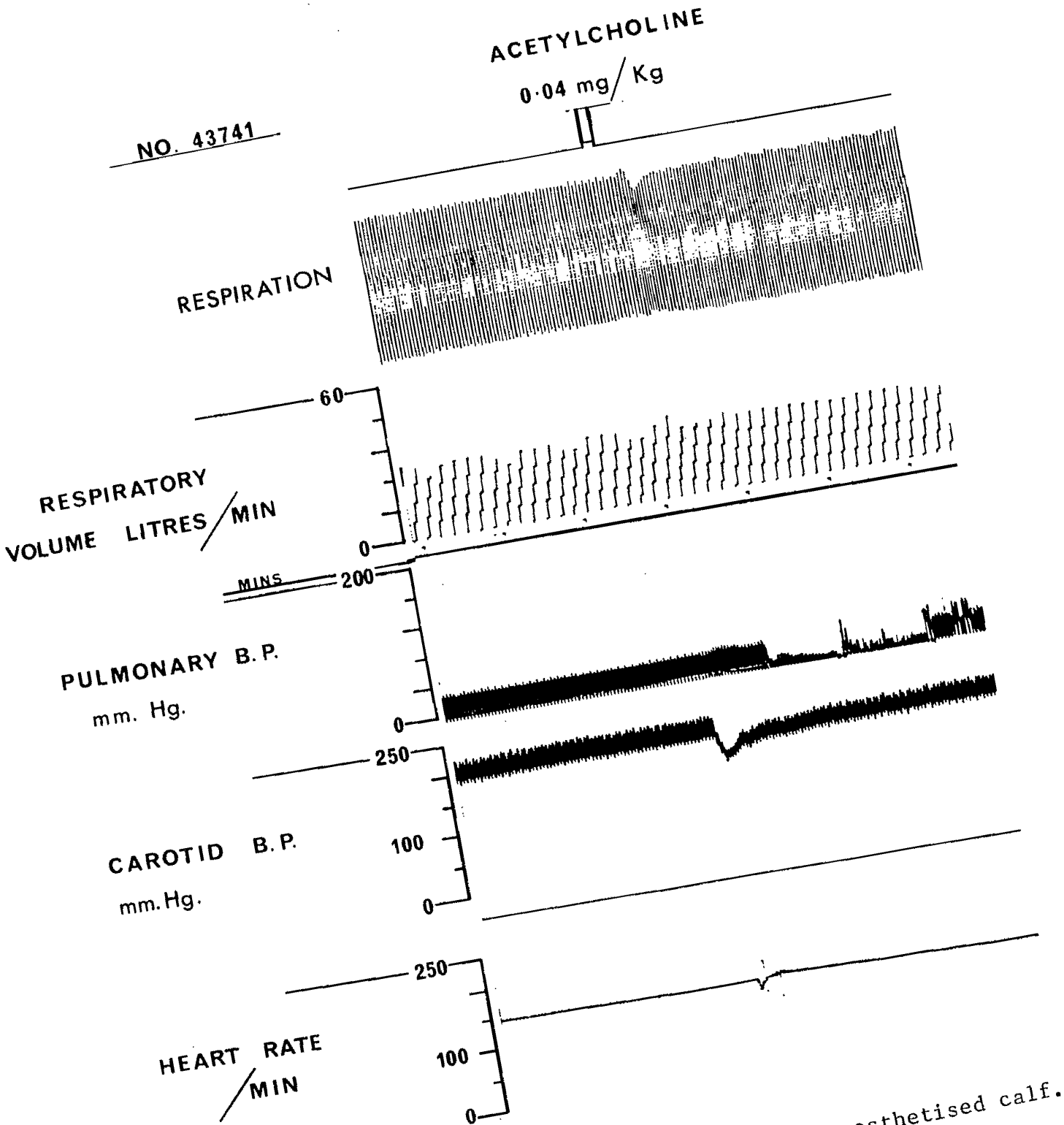


Fig. 4.12 - The effects of acetylcholine on an anaesthetised calf.

Bradycardia did not occur and the degree of systemic hypotension was reduced although pulmonary blood pressure still rose (Fig. 4. 13). As was found in conscious animals, this protective effect was not seen when an animal sensitised using Freund's adjuvant was challenged with 7.5 mg/Kg ovalbumin (Fig. 4. 14).

After pretreatment with 501067 apnoea did not occur although respiratory minute volume was reduced. Bradycardia and blood pressure changes occurred but were of relatively small severity (Fig. 4. 15). Pretreatment with mepyramine (2 mg/Kg) did not result in any modification of anaphylaxis (Fig. 4. 16).

c) HAEMATOLOGY

Blood samples obtained immediately before and 5 min. after injection of antigen were examined as described in Section II. 2 and changes in total white blood cells, differential white cell counts and packed cell volumes recorded.

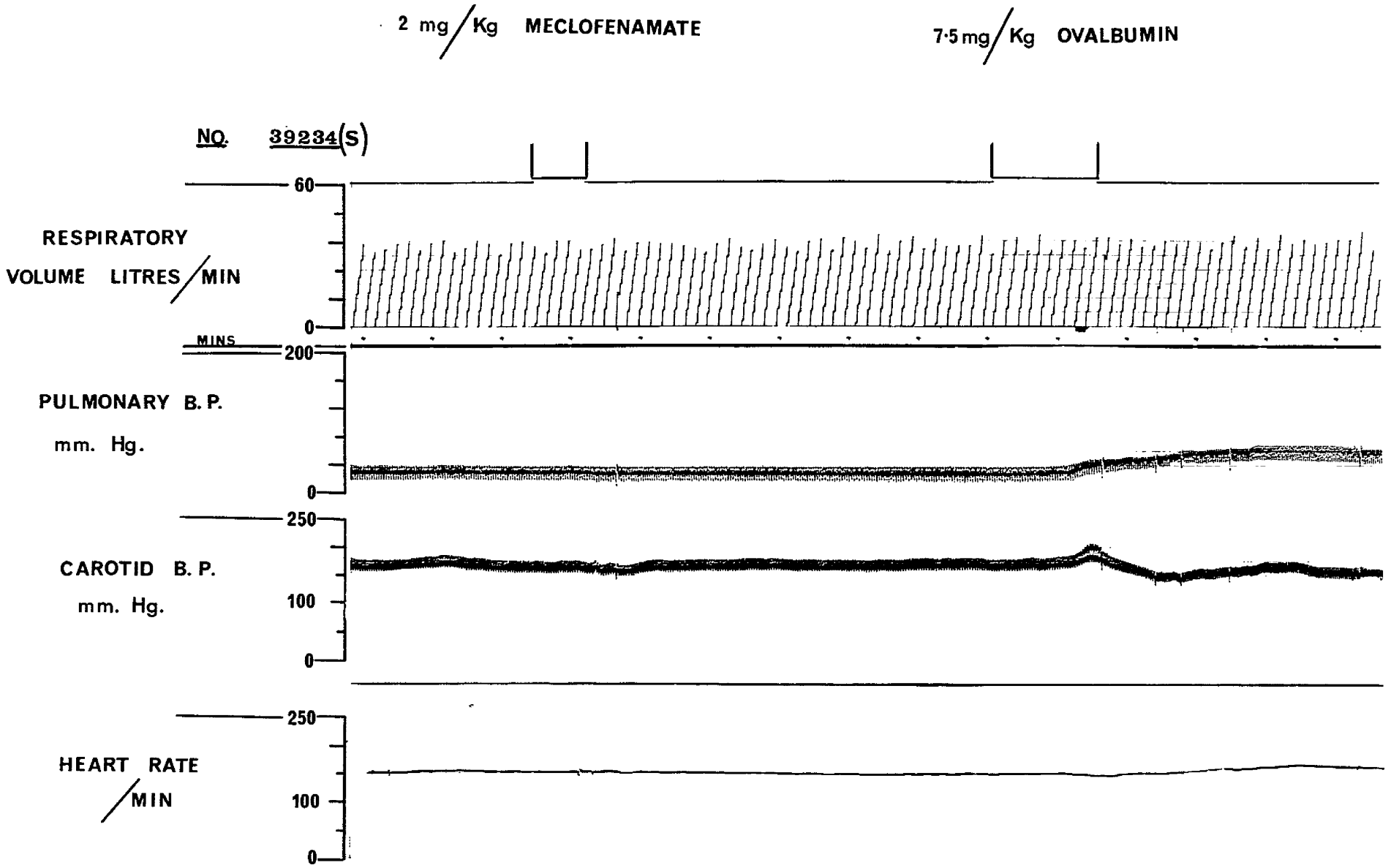


Fig. 4.13 - Effect of challenge, after pretreatment with meclofenamate, on an anaesthetised calf, sensitised without adjuvant.

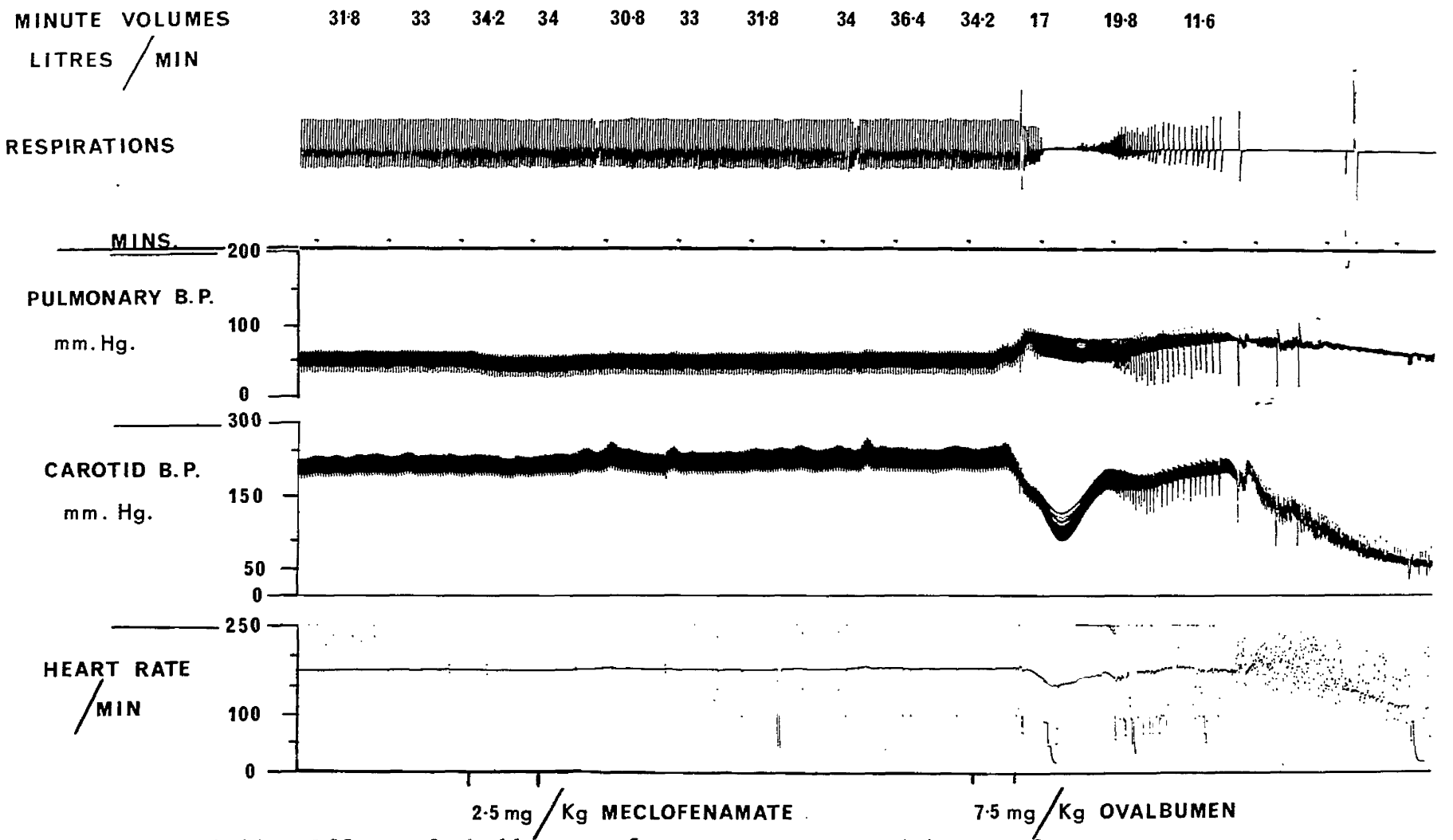


Fig. 4.14 - Effect of challenge, after pretreatment with meclofenamate, on an anaesthetised calf, sensitised using Freund's adjuvant.

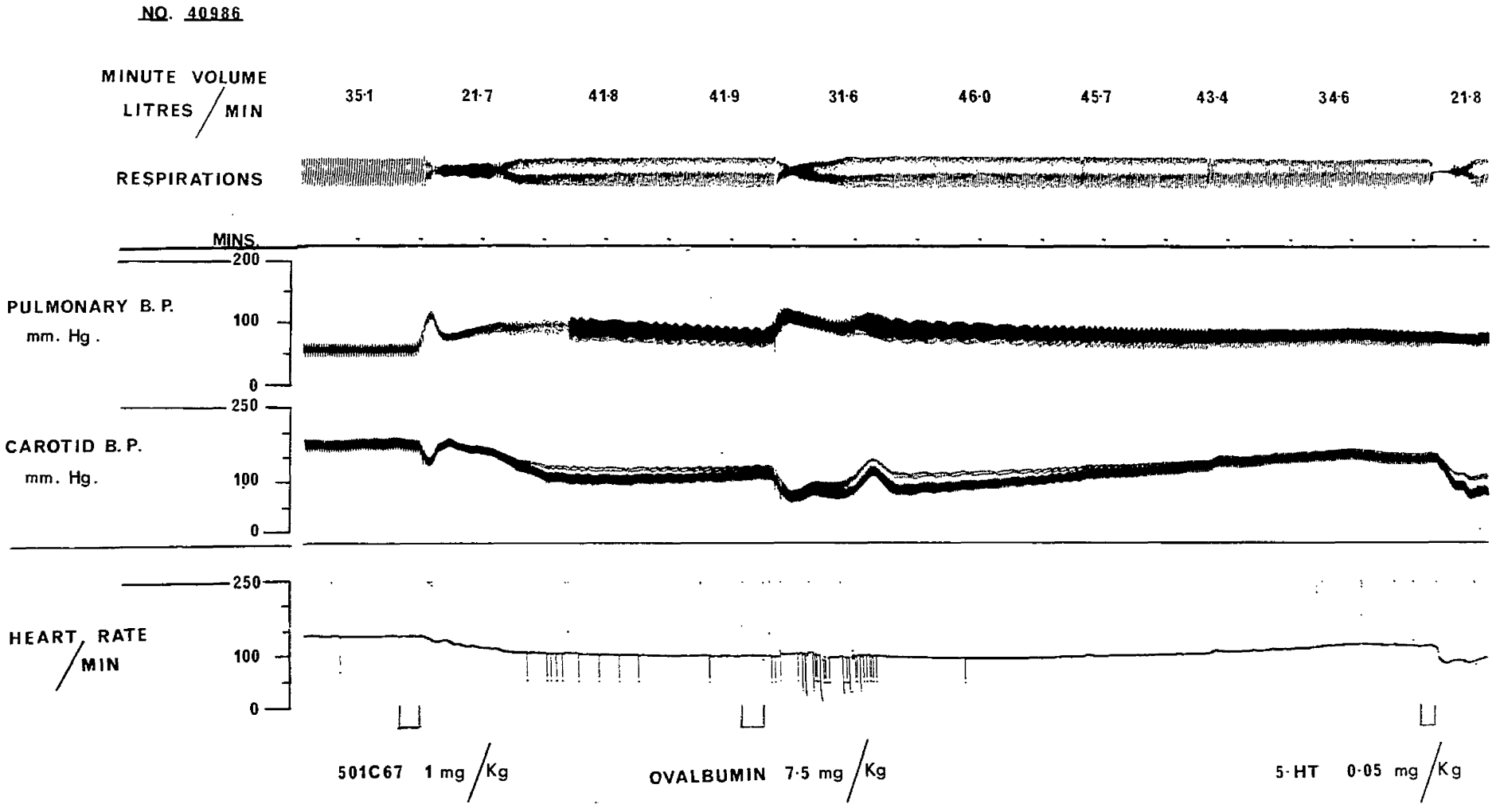


Fig. 4.15 - Effect of challenge and effect of 5-HT after pretreatment with B.W.501C67 on an anaesthetised calf, sensitised without adjuvant.

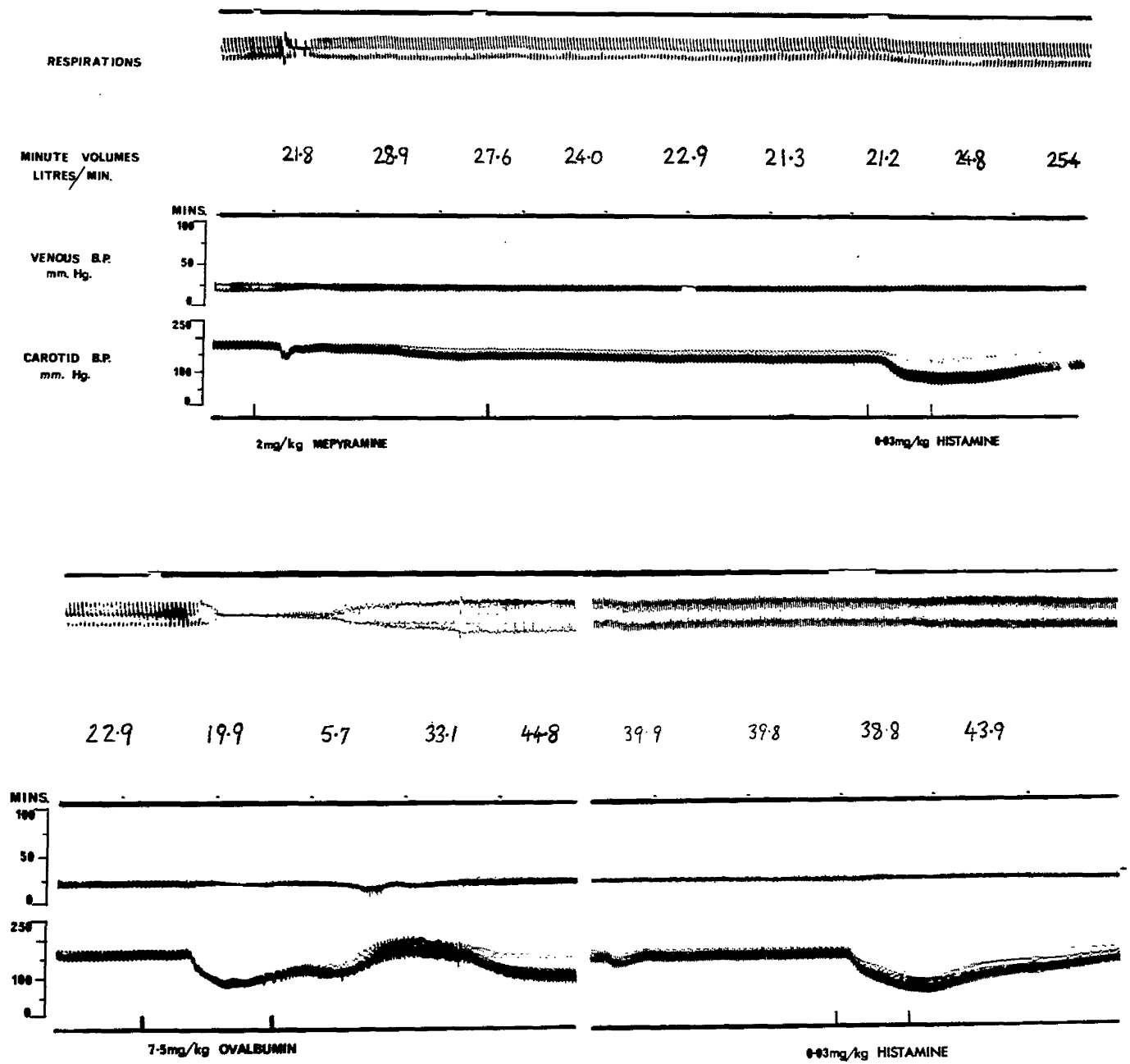


Fig. 4.16 - Effect of challenge and effect of histamine after pretreatment with mepyramine, on an anaesthetised calf sensitised without adjuvant.

Of 16 animals examined, only 2 failed to show a reduction in total WBC and % neutrophils after injection of antigen. One of these was a vagotomised animal, which had a low white blood cell count before challenge, the other was one pretreated with 501C67, the 5-hydroxytryptamine antagonist, in which the total WBC count increased after challenge. The haematological changes associated with injection of antigen following pretreatment with meclofenamate are summarised in Table 4. 4. All 5 animals pretreated with meclofenamate showed haematological changes typical of those occurring during anaphylaxis although the symptoms of anaphylaxis were largely suppressed. Suppression of symptoms by meclofenamate did not include suppression of haematological changes.

d) PATHOLOGY

Post mortem examination of animals challenged under anaesthesia after pretreatment with antagonists was carried

TABLE 4.4 HAEMATOLOGICAL CHANGES ASSOCIATED WITH CHALLENGE OF ANIMALS PRETREATED WITH MECLOFENAMATE (2.0 mg/Kg)

Calf No	Severity of Reaction	Before Challenge					After Challenge					% Change			
		Total WBC	% Neutrophiles	% Eosinophiles	% Lymphocytes	PCV	Total WBC	% Neutrophiles	% Eosinophiles	% Lymphocytes	PCV	Total WBC	% Neutrophiles	Lymphocytes	PCV
												Fall	Fall	Rise	Rise
39234	< +	28,600	46.5	9.0	44.5	39	5,500	8.5	3.5	88.0	43.0	80.7	81.7	97.7	10.2
39234	< +	18,600	24.5	9.5	66.0	31.5	6,100	1.5	1.0	97.5	36.5	67.2	93.8	47.7	15.8
35790	< +	19,900	37.0	4.0	59.0	N.M	5,800	12.0	11.0	77.0	N.M.	70.8	67.5	30.5	N.M.
41046	< +	12,600	23.5	0.5	76.0	30.5	5,600	7.0	0.5	92.5	36.5	55.5	70.2	21.7	19.6
41045	< +	10,300	26.5	2.0	71.5	32.5	7,000	5.0	0.5	94.5	33.5	32.0	81.1	32.2	3.07
Mean		18,000	31.6	5.0	63.2	33.4	6,000	6.8	3.3	89.8	37.4	61.2	78.9	45.9	12.2
S.D.		7,155	9.9	4.1	12.3	3.8	604	3.9	4.5	7.9	4.0	18.7	10.5	30.4	7.2
40869*	++++	14,400	50.5	0	49.5	40.5	3,500	35.0	0	65	47.0	75.6	30.6	15.5	16.0

* = Sensitised using Freund's adjuvant
N.M= Not measured

out as described in Section II. 2. After administration of ovalbumin the lungs of an intravenously sensitised animal pretreated with meclofenamate appeared normal on gross examination. A few small haemorrhages were present but the oedema and collapse typical of anaphylaxis were absent. The wall of the small intestine was haemorrhagic and the contents were mucoid and mixed with blood. Histological examination of the intestine revealed congestion and haemorrhage of mucosal blood vessels. After administration of ovalbumin to an animal sensitised by intramuscular injections of ovalbumin with Freund's adjuvant and similarly pretreated with meclofenamate the lungs showed severe pulmonary oedema and congestion of all lobes, endocardial haemorrhages and hyperaemia of the small intestinal wall. The effects of pretreatment with antagonists on the pathological changes associated with anaphylaxis, in all but the 2 cases described, could not be assessed as substances other than antigen namely histamine, 5-hydroxytryptamine or bradykinin were administered before the animals died or were killed.

SUMMARY

1. Only sodium meclofenamate, an antagonist of bradykinin and SRS-A, regularly reduced the severity of the respiratory and circulatory effects of anaphylaxis in both conscious and anaesthetised cattle.

2. The protective effect of meclofenamate was not apparent in animals sensitised using Freund's adjuvant but this seemed to be related to the greater severity of the reactions shown by such animals.

3. Challenge after pretreatment with meclofenamate resulted in haematological changes characteristic of anaphylaxis.

4. The respiratory effects of anaphylaxis were reduced in vagotomised animals.

5. B.W. 501C67 appeared to reduce the severity of the respiratory and circulatory changes in animals challenged under anaesthesia although conscious cattle were not protected by this drug or by other antagonists of 5-hydroxytryptamine.

5. EFFECTS OF ANTIGEN IN VITRO

a) ISOLATED SMOOTH MUSCLE PREPARATIONS (SCHULTZ-DALE REACTIONS)

MATERIALS AND METHODS

Lungs were removed from sensitised and unsensitised cattle within 15 min. of slaughter and strips of trachealis muscle, segments of bronchioles, segments of pulmonary artery and segments of pulmonary vein were suspended in 100 ml. organ baths containing Krebs-Henseleit solution as described in Section II. 3.

Segments of jejunum were also removed from animals within 15 min. of slaughter and placed in Tyrode solution at 4°C. Strips of longitudinal intestinal muscle, 4 cm. long x 5 m.m. wide, were dissected and suspended in 100 ml. organ baths containing Tyrode solution at 37°C and aerated with oxygen. Contractions of the intestinal muscle were recorded isotonically

in the same way as were those of pulmonary tissue. Drugs were allowed to remain in contact with jejunum for 3 min. with 20 to 30 min. intervals between doses to allow complete relaxation of the tissue.

Ovalbumin was used in these experiments as horse serum contained sufficient 5-hydroxytryptamine to stimulate contraction of all the smooth muscle preparations directly. Ovalbumin was administered in concentrations of 20 ug/ml to 50 mg/ml and allowed to remain in contact with the tissues for 5 to 30 min. The capacity of the tissues to respond to drugs was checked before and after administration of the antigen.

Passive sensitisation was attempted by incubating tissues from unsensitised cattle and from cattle sensitised without Freund's adjuvant in sera of cattle sensitised using Freund's adjuvant. Incubation at 37°C was carried out for periods of 1, 2 and 4 hrs. In 3 cases, where tissues failed to respond to ovalbumin 1 ml., serum obtained from animals sensitised

with Freund's adjuvant was injected into the 100 ml. organ bath 20 to 30 min. before subsequent injection of ovalbumin.

RESULTS

The ranges of sensitivity to drugs of pulmonary tracheobronchial and vascular tissue are summarised in Table 3. 6 (Section II. 3.) Jejunum contracted in response to acetylcholine (0.05 to 1.0 µg/ml), histamine (0.1 to 2.0 µg/ml) and 5-hydroxytryptamine (0.3 to 1.0 µg/ml). The numbers of animals from which specimens of each tissue were tested by being subjected to ovalbumin administration and the numbers of these which responded to the antigen are listed in Table 5. 1. These are divided into 3 groups; unsensitised animals, animals sensitised to ovalbumin by intravenous injection without Freund's adjuvant and animals sensitised by intramuscular injection of ovalbumin with Freund's adjuvant. No responses to ovalbumin were exhibited by tissues from unsensitised animals. Specimens of jejunum from 2 out of 4 animals

TABLE 5. 1

SCHULTZ-DALE REACTIONS

Tissue	Number of Animals					
	U		S		FS	
	T	R	T	R	T	R
Trachea	25	0	16	0	3	2
Bronchiole	25	0	16	0	3	2
Pulmonary artery	25	0	16	0	3	2
Pulmonary vein	2	0	2	0	1	1
Jejunum	2	0	4	2	3	2

U = Unsensitised

S = Sensitised without Freund's adjuvant

FS = Sensitised with Freund's adjuvant

T = Tested

R = Responded

sensitised without adjuvant were found to contract in response to ovalbumin. Trachealis muscle, bronchiole, pulmonary artery and pulmonary vein from these and from similarly sensitised animals failed to respond to antigen. All tissues (trachealis muscle, bronchiole, pulmonary artery and jejunum) from 2 of 3 animals sensitised using Freund's adjuvant responded to ovalbumin as did the one pulmonary vein tested. Responses to ovalbumin in all cases either failed to be elicited by a second dose of the antigen 20 min. to 3 hr. later or were reduced in magnitude. Fig. 5. 1 shows responses to ovalbumin of pulmonary artery, bronchus and jejunum obtained from an animal sensitised with Freund's adjuvant. Fig. 5. 2 shows responses to ovalbumin of jejunum from animals sensitised without Freund's adjuvant. Incubation of tissues from 3 other animals with serum from an animal, sensitised with Freund's adjuvant, did not result in their responding to ovalbumin. Similarly the presence of such serum in the organ bath did not cause a response to ovalbumin.

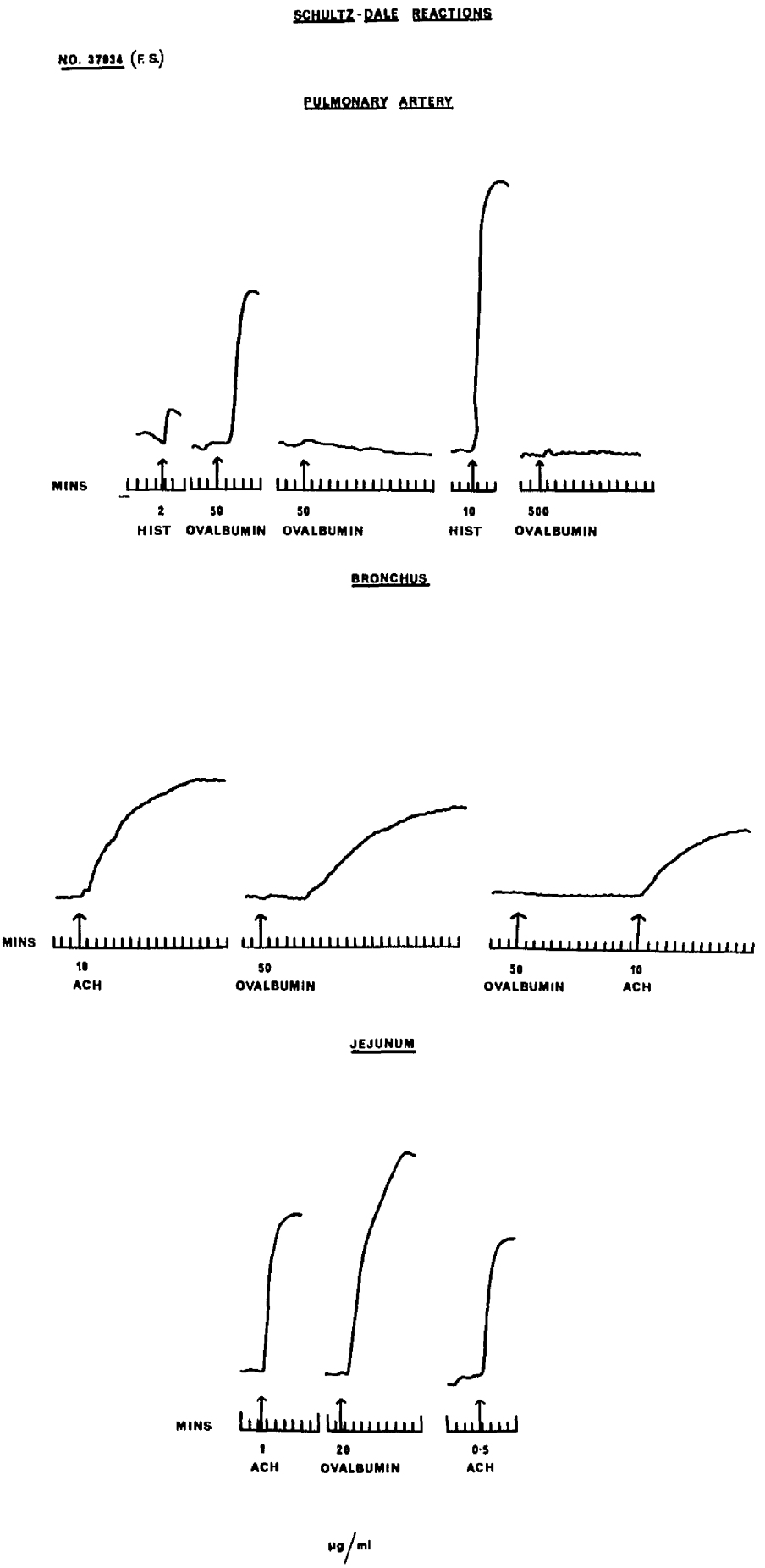


Fig. 5.1 - Effects of antigen on isolated tissues from an animal sensitised using Freund's adjuvant.

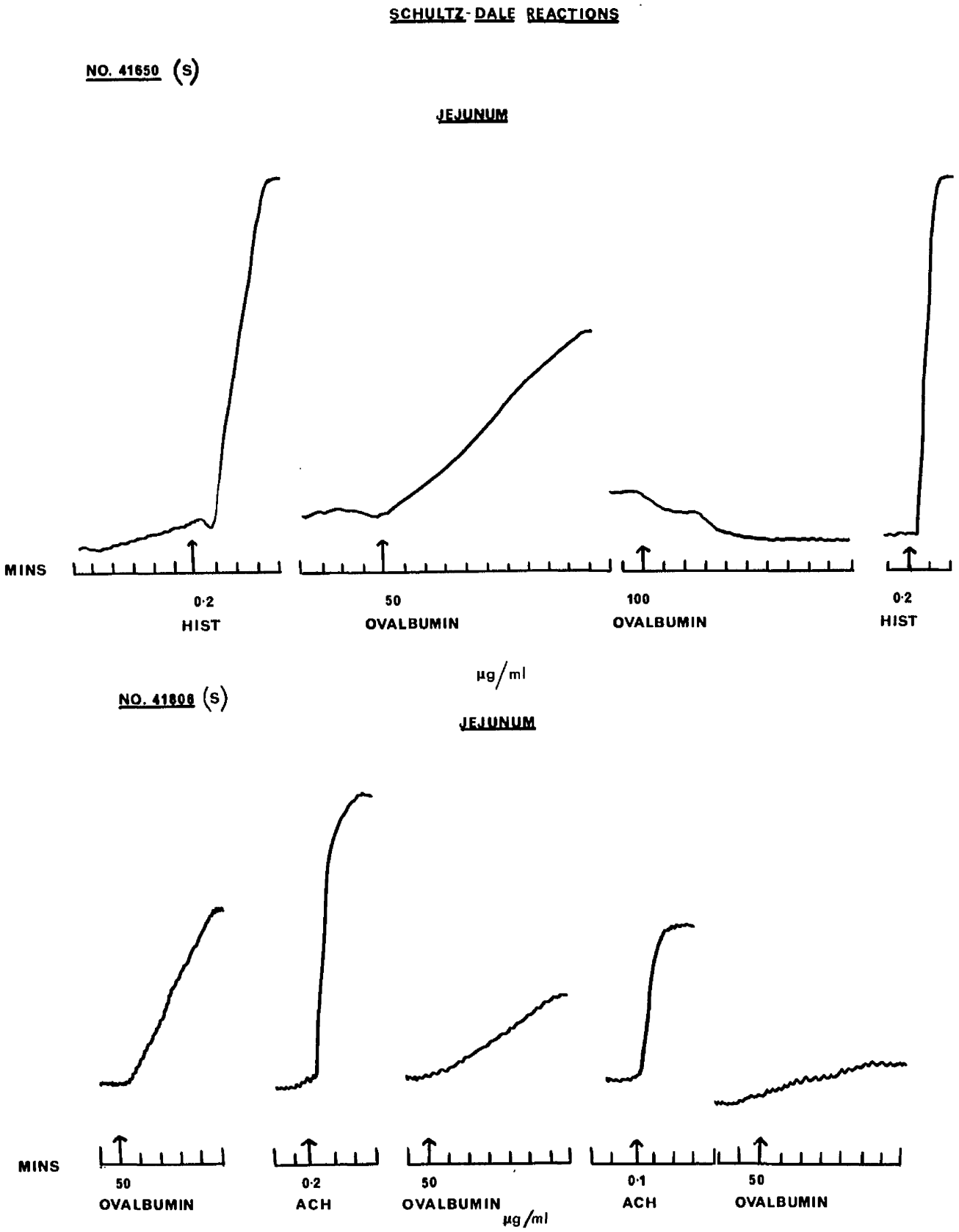


Fig. 5.2 - Effects of antigen on isolated jejunum from animals sensitised without adjuvant.

b) LUNG PERFUSION

EFFECT OF ANTIGEN ON VASCULAR PERFUSION RATE

MATERIALS AND METHODS

Isolated lobes of lung from sensitised and unsensitised animals were perfused with oxygenated tyrode solution as described in Section II. 3. Tyrode solution containing 1.0 mg/ml ovalbumin was supplied from a second aspirator by means of a polythene tube which, after passing through the water bath, communicated with the tube from the aspirator containing tyrode solution and with the cannula inserted in the pulmonary artery by means of a Y piece as shown in Fig. 5. 3. The volume of fluid flowing from the pulmonary vein was measured over alternate 30 sec. periods. The lung was perfused at a rate of 1 ml/gm/min. until blood was no longer grossly visible in the perfusate. The perfusion rate was then reduced to 0.25 to 0.5 ml/gm/min. and perfusion with Tyrode was carried out for 15 to 30 min. Clip A (Fig. 5. 3) was then closed and

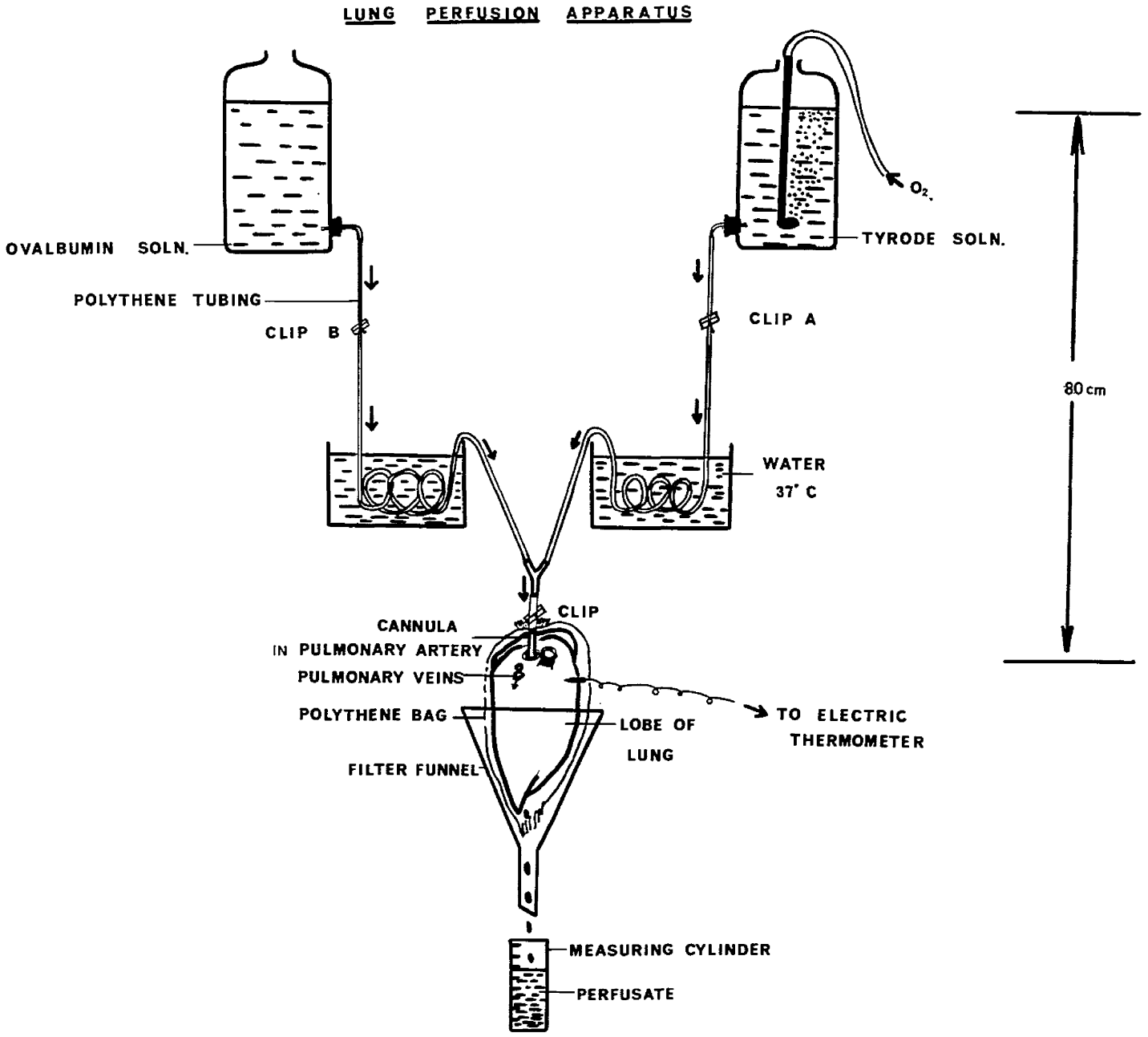


Fig. 5.3 - Diagramatic representation of apparatus used to perfuse bovine lungs with antigen.

simultaneously clip B opened to allow perfusion with ovalbumin solution (1 mg/ml) for the succeeding 15 to 30 min. Clip B was closed and A then re-opened, to allow perfusion with Tyrode to continue for the next 15 to 30 min. The efficiency of perfusion of the lung was checked at the conclusion of each experiment by injecting Evan's Blue dye as previously described (Section II. 3). Perfusion with Tyrode solution alone was carried out for one hr. in one case.

RESULTS

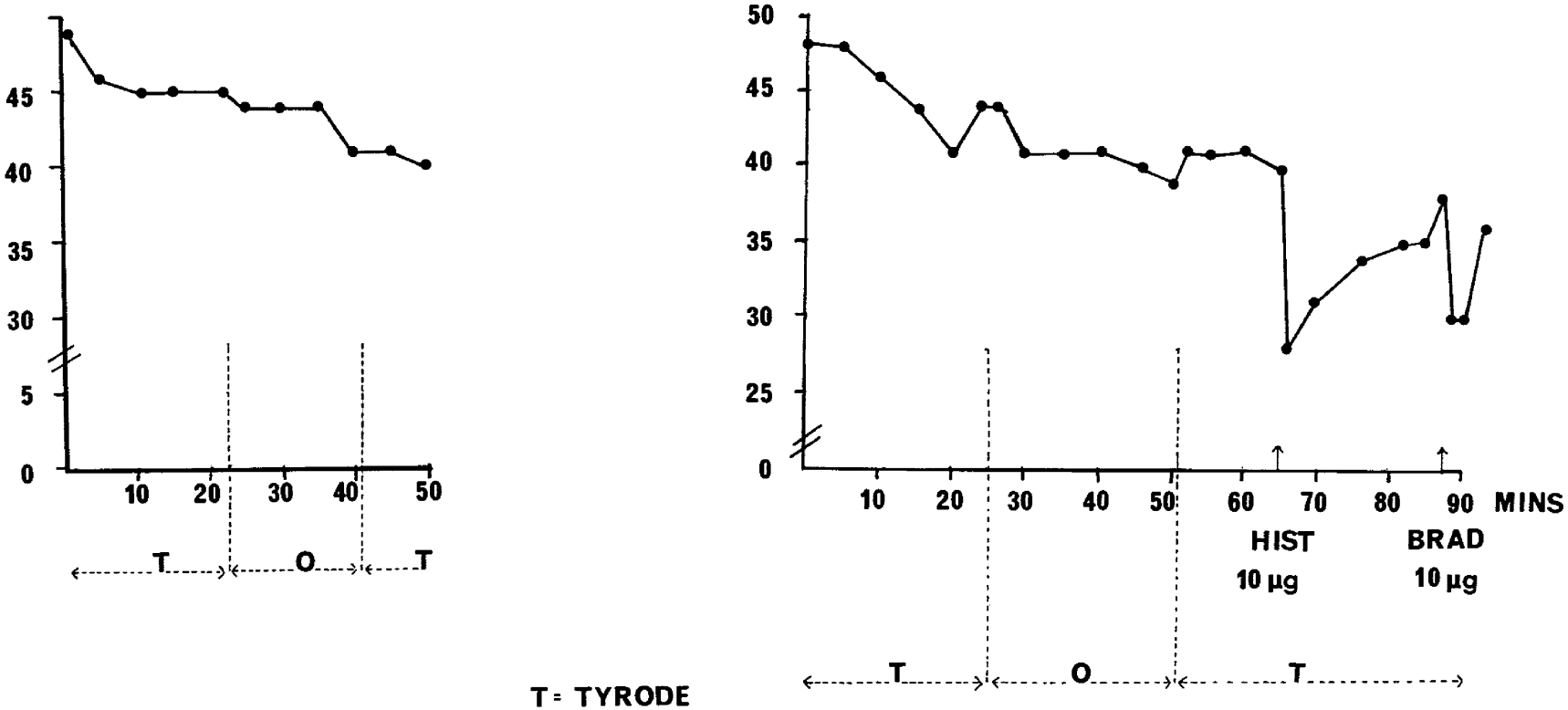
Changes in perfusion rate during perfusion with Tyrode solution before and after ovalbumin and the changes, which occurred during perfusion with ovalbumin, were calculated for the 3 groups of lungs namely, those lungs from a) animals sensitised without Freund's adjuvant, b) animals sensitised with Freund's adjuvant and c) unsensitised animals. Fig. 5. 4 and 5. 5 show the changes occurring during perfusion with Tyrode solution alone and with ovalbumin solution of

lung obtained from unsensitised and sensitised animals respectively. The rate of perfusion was found to decrease during the course of the experiments. However, the effects on the rate of perfusion of ovalbumin were not significant for any of the 3 groups of animals. The greatest changes occurred in lungs of animals sensitised using Freund's adjuvant (Fig. 5. 5). As shown in Fig. 5. 4 the ability of preparations to respond to drugs was checked at the conclusion of each experiment. The effect of raising the level of Tyrode in the reservoir was also checked and found to be negligible (Fig. 5. 5).

VASCULAR PERFUSION OF BOVINE LUNG

UNSENSITISED

SENSITISED (S)

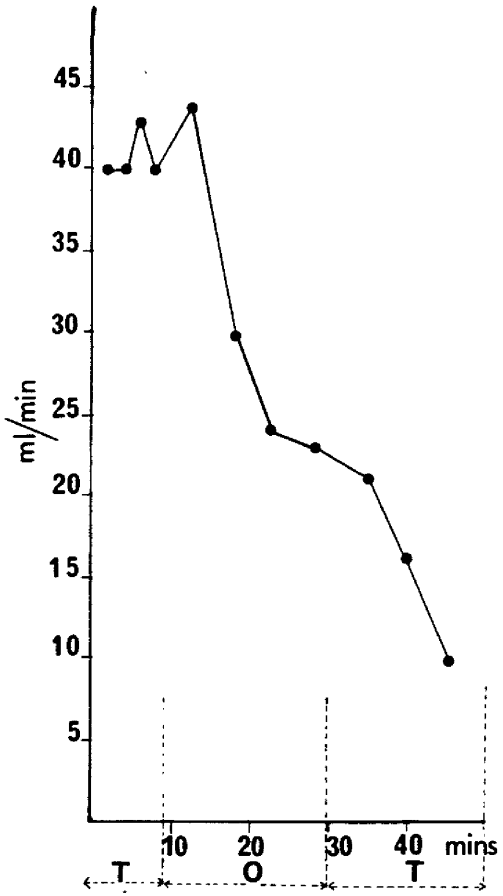


T = TYRODE
O = OVALBUMIN
HIST = HISTAMINE
BRAD = BRADYKININ

Fig. 5.4

VASCULAR PERFUSION OF BOVINE LUNG

SENSITISED (FS)



T = TYRODE
O = OVALBUMIN

SENSITISED (S)

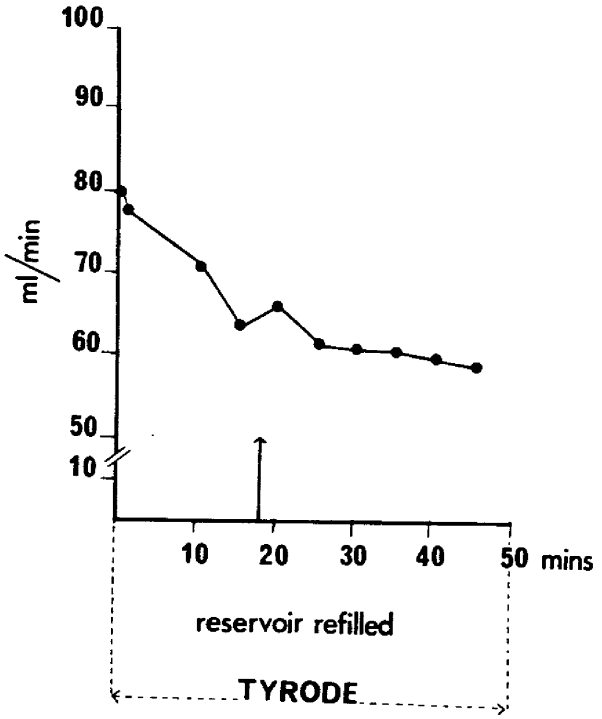


Fig. 5.5

SUMMARY

1. Schultz-Dale reactions were shown by pulmonary smooth muscle only from animals sensitised using Freund's adjuvant.

2. Schultz-Dale reactions were shown by jejunum from animals sensitised with or without Freund's adjuvant.

3. The effect of antigen in reducing the rate of vascular perfusion of isolated lung was variable. The reduction was greatest in an animal of the group sensitised using Freund's adjuvant but the change in perfusion rate was not significant for this group or for the group sensitised without adjuvant.

6. INVESTIGATION OF RELEASE OF PHARMACOLOGICALLY ACTIVE SUBSTANCES DURING ANAPHYLAXIS

a) CONCENTRATION OF HISTAMINE IN TISSUES

MATERIALS AND METHODS

Samples of lung, pleura, liver, liver capsule, abomasum, colon, ileum, small intestine, skin, omentum and subcutaneous tissue, weighing 200 to 500 mg., were removed within 15 min. after death from animals, which died of anaphylaxis, or which were killed within 30 min. after undergoing anaphylaxis and from control animals of the same breed and age killed by humane killer. Tissues from a group of 3 calves aged 4 days were also examined. Estimations of histamine content were carried out on 2 or 4 samples of each tissue and the histamine content expressed as the average in µg histamine base per gm. tissue. Histamine was extracted from the tissues by the method described by Feldberg and Harris (1953). Tissue was weighed on a single

pan analytical balance then transferred to a mortar containing 3 to 5 ml. N/3 hydrochloric acid and ground with sand. The tissue and sand together with washings, totalling approximately 5 ml., were then transferred to a flask and heated to boiling point on a hot plate. After boiling for 30 min. each flask was stoppered and stored at $-20^{\circ}C$ until assayed.

The neutralised extract was assayed for its histamine content on isolated guinea-pig ileum suspended in oxygenated Tyrode solution, containing atropine ($4 \times 10^{-7}M$) at $37^{\circ}C$. A standard solution of histamine was prepared from histamine dihydrochloride and a 3-point assay carried out by the conventional technique in which test and standard solutions of histamine were added at intervals of 2 min. to the organ bath. Each solution was allowed to act for 30 sec. Test solutions were added manually while an automatic assay apparatus (Casella Electronics) as described by Boura, Mongar and Schild (1954) administered standard solutions at regular intervals. All

dilutions of drugs and tissue extracts were made in Tyrode solution. Contractions of guinea-pig ileum were recorded by means of a linear motion transducer and a potentiometric recorder, giving a magnification of x 40 to 120. The minimum concentration of histamine detectable was 0.004 µg/ml. The nature of the contracting substance was confirmed by antagonism with mepyramine moleate (10^{-6}M).

RESULTS

The mean histamine content of each tissue obtained from control calves aged 3 to 6 months or 4 days and calves aged 3 to 6 months which had shown anaphylaxis within 30 min. before death is shown in Table 6. 1 a) b) c). The histamine content of the tissues varied widely between individual animals in all 3 groups. The calves aged 4 days tended to have lower histamine levels than did older calves. Tissues from animals which had shown anaphylaxis before death showed no evidence of a loss of histamine. Histamine levels in the

TABLE 6.1 HISTAMINE CONTENT OF TISSUES µg/gm

a) Control Calves aged 3-6 months

Tissue	Liver Capsule	Pleura	Lung	Ileum	Abomasum	Liver	Colon	Skin	Sub-Cut.	Omentum	Nasal Mucosa
No. of Animals	11	11	12	10	6	12	11	12	10	11	1
Range	196.4 / 1.14	78.41 / 21.57	44.3 / 9.2	81.78 / 8.26	41.68 / 7.81	26.87 / 3.51	22.24 / 6.42	18.96 / 3.88	11.78 / 1.03	11.19 / 0.94	
Mean	105.9	47.49	27.59	22.1	18.59	11.26	11.24	8.55	6.56	2.93	6.52
S.D. ±	69.6	17.33	10.86	21.5	12.2	7.39	4.22	6.23	4.0	2.9	

b) Control Calves aged 4 days.

Tissue	Liver Capsule	Pleura	Lung	Ileum	Liver	Colon	Skin	Sub-Cut.	Omentum
No. of Animals	3	2	2	3	3	3	3	3	3
Range	51.24 / 9.17	18.92 / 5.17	3.21 / 3.17	5.08 / 2.33	4.12 / 0.26	4.01 / 3.06	4.73 / 1.82	2.09 / 1.03	2.03 / 0.74
Mean	31.13	12.04	3.19	3.74	2.52	3.6	3.52	1.44	1.47

c) Shocked Calves

Tissue	Liver Capsule	Pleura	Lung	Ileum	Abomasum	Liver	Colon	Skin	Sub-Cut	Omentum	Nasal Mucosa
No. of Animals	2	2	3	3	2	3	2	2	2	2	1
Range	956.25 / 45.65	121.55 / 23.84	43.0 / 4.04	82.41 / 11.26	14.53 / 12.27	80.49 / 2.85	41.5 / 11.89	52.31 / 5.59	39.13 / 8.15	29.54 / 8.53	
Mean	500.95	72.69	18.87	35.17	13.4	32.38	26.69	28.95	23.64	19.03	4.04

tissues from such animals showed some tendency to be higher than those in tissues from control calves. The extreme variability of histamine levels made it very difficult to draw firm conclusion from the results. In each animal the relative histamine levels of different tissues were consistent in that liver capsule contained the highest concentration of histamine followed by pleura, lung and ileum.

b) BLOOD LEVELS OF HISTAMINE

MATERIALS AND METHODS

METHOD

The method of extraction of histamine from blood samples was that described by Sanford (1962). This was based on the method described by Adam, Hardwick and Spencer (1957). Blood was collected in nylon centrifuge tubes of 15 ml. capacity containing heparin (10 i.u[x]/ml. blood) to prevent clotting. After centrifuging at 5°C for 30 min. at 2.000 revs./min. the

[x] international units

plasma was removed and subjected to further centrifugation at 3,000 revs./min. for 30 min. to remove platelets. Duplicate samples of plasma (5 ml.) were then withdrawn and mixed with an equal volume of 6% trichloracetic acid to precipitate protein. After standing for one hour the samples were centrifuged for 60 min. at 3,000 revs./min. and the supernatant was removed. The volume of supernatant obtained was 7.0 to 7.4 ml.

Histamine was extracted from the supernatant by passage through columns containing a carboxylic ion-exchange resin (IRC 50) which had been previously prepared by treatment with sodium hydroxide. Samples were run through the columns at pH 7.92 using phosphate buffer to attain the correct degree of alkalinity.

At this pH free histamine is completely adsorbed by the resin. The adsorbed histamine was eluted with a fixed volume of 0.1N hydrochloric acid and the eluate was collected and subsequently neutralised and assayed biologically. The rate

of flow of solution through the columns was adjusted to 0.2 to 0.3 ml/min.

MATERIALS

Glass columns of 0.6 cm. internal diameter and 30 cm. long with the upper 10 cm. of the tube widened to 2 cm. int. dia. to form a cylindrical bulb, and a ground glass socket and cone fitted to the upper and lower ends respectively, were used.

Amberlite C.G.50 chromatography resin, Type I (100-200 mesh) B.D.H.). This was treated to remove small particles by suspending 30 gm. in 1.0 L of distilled water. This was poured off after 10 min. and the resin was re-suspended 6 times, after which it was dried at 50°C for 24 hrs.

Powdered cellulose (Whatman Chromatography Grade).

Phosphate buffer (pH 7.92). This was made from stock solutions and contained 53 ml. of 0.2M NaH_2PO_4 and 947 ml. of 0.2 M Na_2HPO_4 per litre. The mixture was adjusted to

pH 7.92 and stored in a refrigerator. Before use the buffer was diluted by making 438 ml. to up to 1.0 L in distilled water.

6% trichloracetic acid

0.1N hydrochloric acid

0.1N and 1.0N sodium hydroxide

0.01% neutral red solution

Heparin (Boots Ltd.) 1,000 units/ml.

METHODS

PREPARATION OF COLUMNS

Powdered cellulose (300 mg) and dry resin (50 mg) were mixed in a 25 ml. conical flask. Four ml. 0.1N NaOH was added and the flasks shaken to ensure thorough mixing. The mouth of each flask was then covered with aluminium foil and the flasks were allowed to stand overnight

Each glass column was set up in a rack and fitted with a glass wool plug. A 100 ml. tap funnel was fitted at the upper end and 4 ml. diluted buffer poured on to the column. Ten ml. diluted buffer was added to the activated resin and the contents of the conical flask were poured into the column forming a composite column of resin and cellulose about 4 cm. in length.

Further quantities of buffer were added slowly from the tap funnel until the fluid leaving the column had reached pH 7.92.

PREPARATION OF PLASMA SAMPLES, ADSORPTION AND ELUTION

Five ml. of centrifuged supernatant was transferred to a 10 ml., stoppered, graduated cylinder and one drop of neutral red indicator was added. The solution was neutralised with 1.0 NaOH added from a 5.0 ml. burette and the end-point was adjusted as necessary with small quantities of 0.1N NaOH and 0.1N HCl. One ml. of phosphate buffer was then added and

the volume made up to 10 ml. with distilled water.

The prepared sample was poured into the tap funnel and was allowed to run on to the column at a rate of 0.3 ml./min. The sample was followed by 3.5 ml. distilled water and the column was allowed to run dry.

Columns were eluted with 0.25 N HCl in the manner described by Sanford (1962) who found that histamine left the column when the pH of the eluate fell to 4.5. Four ml. of 0.25N HCl was added to the column and the rate of flow of fluid through the column adjusted to 0.2 ml./min. When the pH of the fluid leaving the column reached 4 the level was marked, a further 4 ml. 0.25 N HCl added to the column and the next 4 ml. of eluate collected. One drop of neutral red was added to each eluted sample which was stored at -20°C until neutralised and assayed biologically as described previously (p.265). A standard solution of histamine (0.2 ug/ml) was prepared with each group of samples. This was

treated in the same way as the neutralised samples and the calculated histamine content of the standard solution, on assay of the eluate, allowed the percentage recovery of histamine by the above method to be determined.

The plasma free histamine content of blood samples obtained from control animals and from sensitised animals 5 min. before and at intervals of one min. to 22 min. after injection of antigen was determined.

RESULTS

The recovery of histamine from standard solutions is shown in Table 6. 2. The number of the standard solution in each case corresponds to the number of the experiment involved. The percentage recovery for each experiment was used to correct the histamine estimations on blood samples obtained during that experiment. The mean recovery in 9 experiments was 72% ± 8.8% (S.D.).

TABLE 6.2 RECOVERY OF HISTAMINE FROM STANDARD SOLUTIONS CONTAINING 0.2 μg/ml

Number	Recovered Histamine μg/ml	% Recovery
1	0.154	77
2	0.126	63.25
3	0.140	70
4	0.158	79
5	0.130	65
6	0.178	89
7	0.152	76
8	0.133	66.5
9	0.125	62.5
Mean	0.144	72
S.D	± 0.01	± 8.8
S.E.	± 0.003	± 2.9

Table 6. 3 lists plasma free histamine levels of control animals including levels found in the blood of one animal from which samples were removed at daily intervals for 3 days. Table 6. 4 shows the changes in histamine levels in plasma following injection of antigen. On comparing histamine levels before and after challenge, using the t test for small samples (Bancroft, 1965), the changes were probably significant ($p = < 0.02$). However, repeated sampling of 2 animals at 5 min. intervals showed that the variations in histamine concentration before challenge could be greater than the changes which followed (Fig. 6. 1). Thus for animal E. 4 the sample taken after challenge could be regarded as either less or greater than normal, according to whether it was compared with the sample taken either 10 or 5 min. before.

TABLE 6. 3.

PLASMA HISTAMINE LEVELS OF CATTLE

Animal Number	Histamine (µg/ml)
1	0.021
2	0.008
3	0.012
4	0.007
5	0.007
6	0.042
7	< 0.004
8	< 0.004
9	0.023
10	< 0.004
11	0.013
12	0.035
13	0.014
14	0.013
15	0.007
16* (day 1)	0.012
16 (day 2)	0.028
16 (day 3)	0.036
Mean	0.016
S.D	± 0.012

* Sample taken at daily intervals for 3 days

< = less than

TABLE 6.4 PLASMA HISTAMINE LEVELS 5 MIN BEFORE AND 1 MIN AFTER CHALLENGE

Animal No.	Plasma Histamine μg/ml		
	Before	After	Change
E.1	0.049	0.047	- 0.002
E.4	0.011	0.033	+ 0.022
E.3	< 0.004	< 0.004	0
E.7	< 0.004	0.047	+ 0.043
412	0.013	0.100	+ 0.087
2	0.021	0.046	+ 0.025
T.1	0.016	0.036	+ 0.020
T.2	0.021	0.029	+ 0.008
39350*	0.037	0.113	+ 0.076
Mean	0.019	0.05	0.031
S.E.	0.005	± 0.01	± 0.010
t			3.1
p			< 0.02

* = Sensitised using Freund's adjuvant
\- = fall
\+ = rise

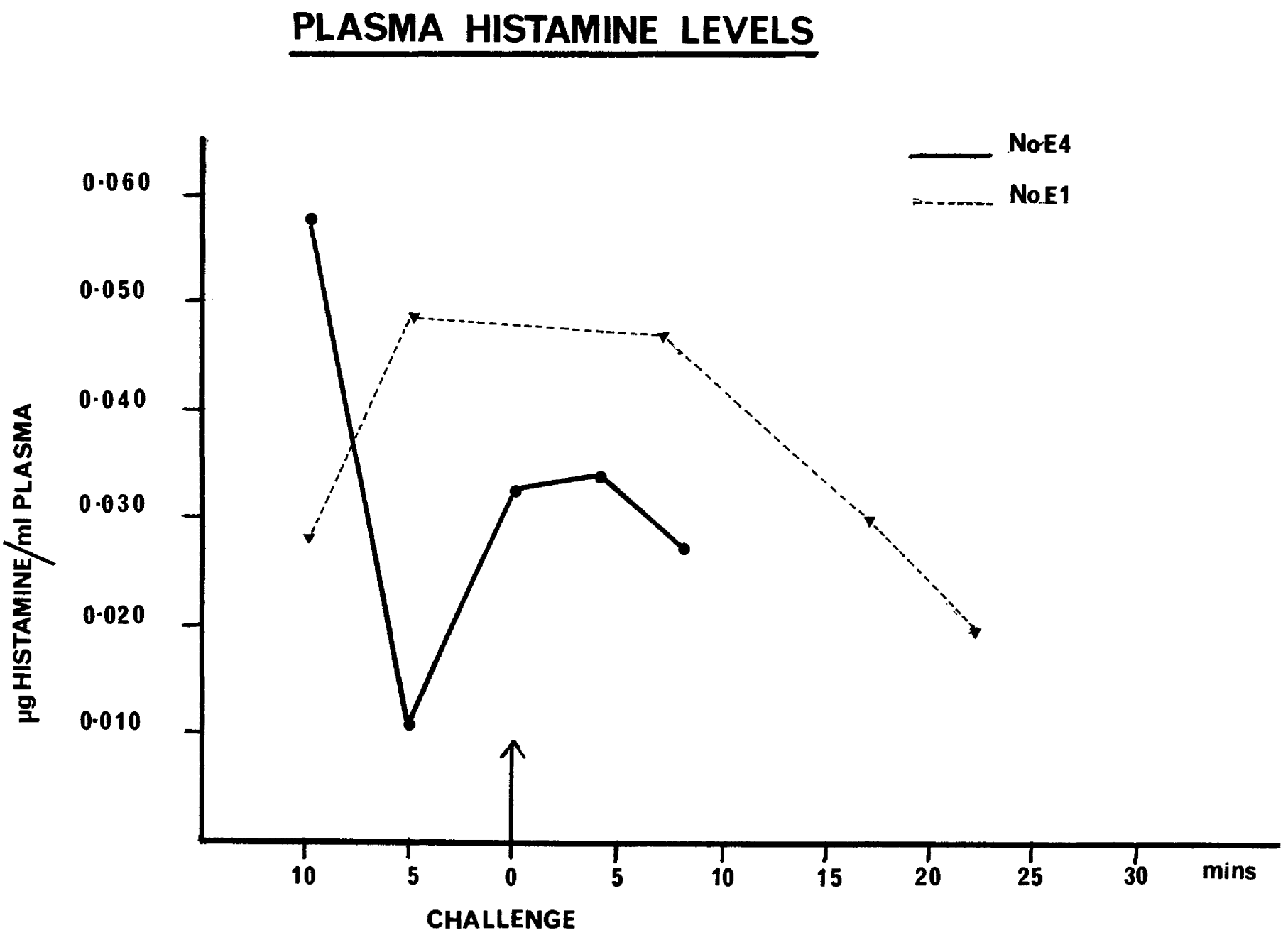

PLASMA HISTAMINE LEVELS
No E4
No E1
0·060
0·050
0·040
0·030
0·020
0·010
µg HISTAMINE/ml PLASMA
10
5
0
5
10
15
20
25
30
mins
CHALLENGE

Fig. 6.1

c) TISSUE INCUBATION EXPERIMENTS

HISTAMINE

TO INVESTIGATE HISTAMINE RELEASE

MATERIALS AND METHODS

The method of incubation of tissue was based on that described by Mongar and Schild (1956). Pieces of lung, pleura, liver capsule, nasal mucosa and skin were removed within 15 min. after death from animals sensitised to ovalbumin or horse serum and from unsensitised control animals of the same breed and age. Samples of skin of sensitised animals was taken only from those which had shown pruritis on challenge. All animals were killed by humane killer. The tissue samples were lightly blotted and chopped as finely as possible using a McIlwain mechanical chopper (The Mickle Laboratory Engineering Co.). They were then placed on filter paper in a Buchner filter funnel and washed. Washing was carried out by pouring Tyrode solution over the

chopped tissues in the filter funnel. The tissues were allowed to drain, then weighed. Duplicate samples of each tissue, weighing 200 to 400 mg., were placed in 25 ml. flasks containing 4 ml. Tyrode solution and shaken in an incubator (Gallenkamp) at 37°C for 20 min. This Tyrode solution was then removed, discarded and replaced by 4 ml. of Tyrode solution, Tyrode solution containing antigen or Tyrode solution containing histamine releaser. Incubation at 37°C with shaking was continued for 60 min. In one experiment one group of paired tissue samples was supplied, during incubation, with oxygen bubbled through the incubation fluid by means of a needle inserted through a rubber bung in the neck of each flask. A second needle allowed air to escape. The other group of paired samples was treated in an identical manner but did not receive oxygen. The supernatant fluid in each flask was removed, acidified by addition of 1 ml. N/3 HCl and stored at -20°C until assayed biologically

for its histamine content. The tissues were boiled in 5 ml. N/3 HCl for 30 min. to release the remaining histamine and stored at -20°C until assayed. Assay of histamine content was carried out, as described previously (p.265), on isolated guinea-pig ileum after neutralisation of the samples by addition of N/10 NaOH.

The antigens and the concentrations in which they were used were as follows:

ovalbumin : 5 mg/ml, 1.0 mg/ml, 0.1 mg/ml

horse serum : diluted to concentrations of protein of approximately 1.0 mg/ml and 0.1 mg/ml.

The histamine releasers and the concentrations in which they were used were as follows:

compound 48/80 : 1.0 mg/ml

octylamine : 1.0 mg/ml.

Any contractions of guinea-pig ileum, due directly to these concentrations of antigens or histamine releasers, were determined and subtracted from those attributed to the presence of histamine in the samples of supernatant fluid.

RESULTS

The results are summarised in Figs. 6. 2 and 6. 3. Incubation with histamine releasers resulted in the release of 50 to 99% histamine from all the tissues examined. Incubation in Tyrode solution alone resulted in release of 1.0 to 22% histamine from the tissues into the supernatant fluid. The amount of histamine released on incubating tissue from sensitised animals with antigen varied with the tissue. Less than 10% histamine content was released from liver capsule, less than 5% from lung and amounts ranging from 4% to 40% from pleura. From pleura of unsensitised animals incubation with antigen released up to 33% histamine (Fig. 6. 3). There was evidence of more specific histamine release by antigen from skin of ear (up to 56%) and muzzle (up to 79%) of sensitised animals (Fig. 6. 2).

The results obtained by incubation with and without oxygenation are shown in Table 6. 5. The presence of oxygen

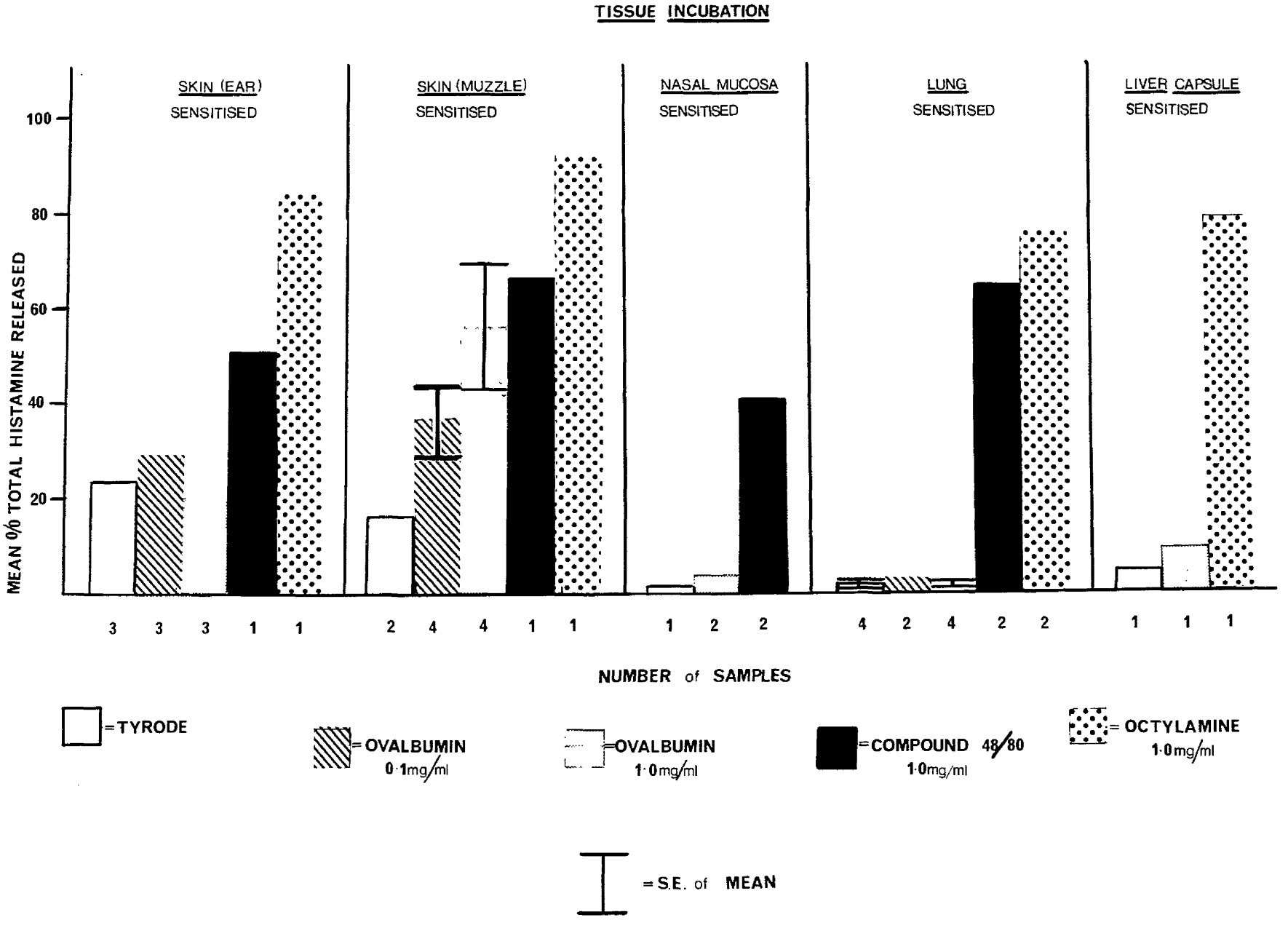


Fig. 6.2 - Histamine release during incubation of tissue from sensitised calves.

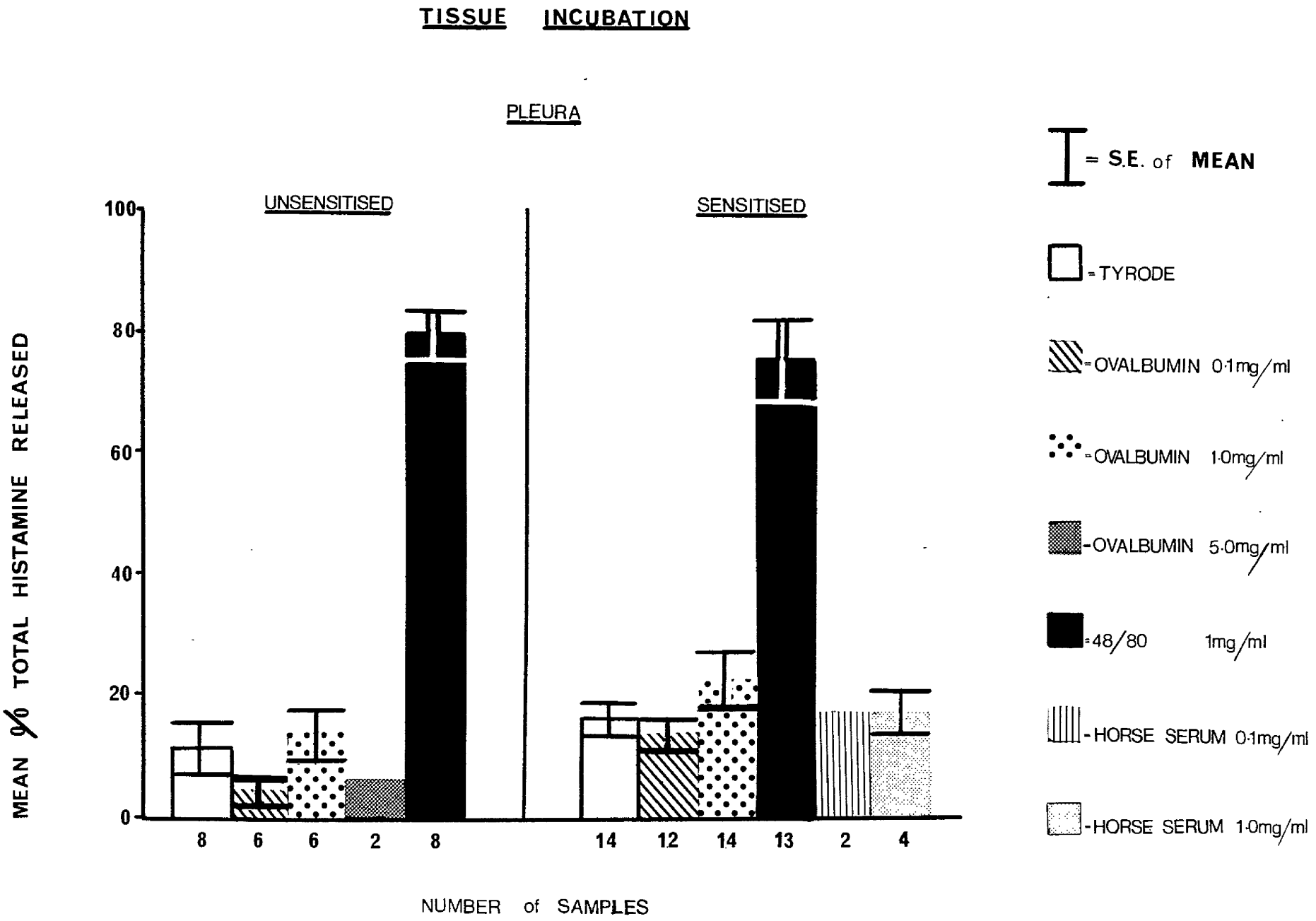


Fig. 6.3 - Histamine release during incubation of pleura from sensitised and unsensitised calves.

TABLE 6. 5.

EFFECT OF OXYGEN ON HISTAMINE

RELEASE FROM PLEURA IN VITRO

Incubation	Mean % Total Histamine Released			
	No.	With O_2	No.	Without O_2
Tyrode	2	4.9	1	21.9
Ovalbumin 1.0 mg/ml	2	11.6	2	8.5
CPD. 48/80 1.0 mg/ml	2	77.6	1	67.4

did not appear to have any effect on histamine release by antigen or histamine releaser.

d) LUNG PERFUSION EXPERIMENTS

MATERIALS AND METHODS

Lungs were removed from 16 cattle within 15 min. of death and isolated lobes perfused through the pulmonary artery as described in Section II. 5 using the apparatus shown in Fig. 5.3). Perfusion with 50 to 100 ml. per min. oxygenated Tyrode solution was carried out for 15 or 20 min. This was followed by perfusion for 20 min. with Tyrode solution containing 1.0 mg/ml ovalbumin. Perfusion with Tyrode solution alone was then reverted to and continued for a further 20 min. In 7 cases, following perfusion with ovalbumin, compound 48/80 was administered by injection at a concentration of 1.0 mg compound 48/80 per ml of Tyrode solution entering the lung per minute for 10 min. Throughout the experiment perfusate was collected over alternate

or consecutive periods of 30 sec. or 60 sec. The perfusate samples were centrifuged to remove any blood cells and stored at -20°C until they could be examined for biological activity. Such examination was carried out within 24 to 48 hr. of collection except in one case where the samples were stored for 7 days. Where, because of the need for rapid testing of a large number of samples, every sample was not examined, that collected every 5th min. was examined.

The activity of the samples in contracting isolated guinea-pig ileum was compared to that of histamine, in the presence of atropine (4×10^{-7}M) in the absence and then in the presence of mepyramine (10^{-8}M to 10^{-6}M). The percentage of activity of the perfusate samples persisting in the presence of concentrations of mepyramine, sufficient to suppress contractions of the ileum stimulated by previously equi-effective concentrations of histamine, was measured. The effect of meclofenamate on such activity was examined in one case. In 6

experiments the action of perfusate samples on isolated rat duodenum was tested and compared to that of bradykinin and of histamine. In using these tissues the activity of perfusate samples containing ovalbumin or compound 48/80 was estimated after subtraction of any activity due directly to 1.0 mg/ml ovalbumin or 1.0 mg/ml compound 48/80.

Guinea-pig ileum was bathed by Tyrode solution as previously described. Rat duodenum, which consisted of the proximal 3 cm. of duodenum, was suspended in a 10 ml. organ bath and bathed by oxygenated De Jalon solution at 37°C. A dose cycle of 3 or 4 min. with 30 sec. contact time was used (Horton, 1959). The minimum concentrations of histamine and bradykinin detectable by our methods of assay were 0.002 µg and 0.02 µg respectively per ml of sample.

Lungs were obtained from 3 unsensitised animals, 10 sensitised to ovalbumin without Freund's adjuvant and 3 sensitised to ovalbumin using Freund's adjuvant.

RESULTS

The number of samples examined and the activity of these samples of lung perfusate, expressed in terms of ranges of equivalent concentrations of histamine, on guinea-pig ileum is summarised in Table 6.6. The percentage of this activity persisting in the presence of mepyramine is summarised in Table 6. 7.

On perfusion of the lungs of unsensitised cattle samples obtained before exposure to ovalbumin showed less activity than that of 0.02 µg/ml histamine. During perfusion with ovalbumin samples of perfusate showed activity equivalent to less than 0.1 µg/ml histamine in 2 cases and between 0.1 and 1.0 µg/ml histamine in the third. Samples obtained during perfusion with Tyrode solution after ovalbumin showed similar activity. After injection of compound 48/80 most of the samples obtained produced contractions of guinea-pig ileum equivalent to 0.1 to 1.0 µg/ml histamine. In one case

TABLE 6.6

ACTIVITY OF LUNG PERFUSATE ON GUINEA PIG ILEUM

Animals		Histamine Equivalent µg/ml																			
		Before Ovalbumin					During Ovalbumin					After Ovalbumin					After Compound 48/80				
Group	No.	Samples	<0.02	<0.1	0.1-1.0	1.0-2.0	Samples	<0.02	<0.1	0.1-1.0	1.0-2.0	Samples	<0.02	<0.1	0.1-1.0	1.0-2.0	Samples	<0.02	<0.2	0.1-1.0	1.0-2.0
US	1	5	XM				10		X	M		3		XM			4			X	M
	2	5	XM				6		XM			4	X	M			9			XM	
	3	5	XM				6			XM		4			XM		4			XM	
S	41046	3	XM				4	XM				3	XM				0				
	41650	3	XM				6	XM				3	XM				0				
	36940	7	XM				8	XM				6	XM				5	XM			
	37238	4	XM				2			XM		2			XM		0				
	41649	3	XM				5		XM			4		X	M		0				
	33366	2	XM				10			XM		2			XM		7				XM
	37003	3	XM				6		XM			0					0				
	37005	4	X	M			6	X	M			5			XM		3			XM	
	37104	4	X	M			6		XM			5		XM			6				XM
	41806	4		XM			5		XM			4			XM		0				
FS	40868	4	XM				6			XM		4			XM						
	37934	3		XM			5			XM		3			XM		0				
	37935	3		XM			3			XM		3			XM		0				
	36851	3	XM				6			XM		0					4		XM		

Notes:

US = unsensitised
S = sensitised to ovalbumin without Freund's adjuvant
FS = sensitised to ovalbumin using Freund's adjuvant
X = largest number of samples
M = most active sample

TABLE 6.7

% ACTIVITY OF LUNG PERFUSATE NOT DUE TO HISTAMINE

Animals		% Activity persisting in presence of Mepyramine											
Group	No.	Before Ovalbumin			During Ovalbumin			After Ovalbumin			After Compound 48/80		
		<10%	10-50%	>50%	<10%	10-50%	>50%	<10%	10-50%	>50%	<10%	10-50%	> 50%
	1	XM			XM			XM					XM
US	2	XM			XM			XM			X		M
	3	XM				XM			XM		X	M	
	41046	XM			XM			XM					
	41650	XM			XM			XM					
	36940*			XM			XM			XM			XM
	37238	XM				XM		XM					
S	41649	XM			X		M	XM					
	33366	XM			XM			X		XM		XM	
	37003	XM			XM								
	37005	XM			XM				XM		XM		
	37104	XM					XM			XM		X	M
	41806■	XM					XM			XM			
	40868	XM			X	M		X	M				
	37934	X		M			XM			XM			
FS	37935	XM			XM			XM					
	36851	XM			XM						X		M

Notes: As for Table 6.6

* = stored for 7 days before being tested.

■ = activity in presence of mepyramine abolished by meclofenamate.

activity of up to 2.0 µg/ml histamine was present in one sample. The activity of all the samples of perfusate obtained from the lungs of 2 unsensitised animals was reduced by mepyramine to less than 10% with the exception of the samples obtained after injection of compound 48/80. These were reduced only slightly in one case, which suggested that compound 48/80 released from the lung active substances other than histamine. In the third case, samples obtained during and after perfusion with ovalbumin were reduced in activity to between 10% and 50% of that shown in the absence of mepyramine. These results showed that ovalbumin could release histamine and possibly other active substances from unsensitised lungs. Perfusate from lungs of unsensitised animals was not tested on rat duodenum.

On perfusion of the lungs of 10 animals sensitised to ovalbumin without Freund's adjuvant, it was found in 3 cases that no sample obtained at any stage of perfusion showed

activity equivalent to more than 0.02 µg/ml histamine, although examination of the distribution of Evan's blue dye in the lung, as described in Section II. 5, indicated efficient perfusion. In the remaining 7 cases, 6 showed an increase in activity from that equivalent to less than 0.02 µg/ml histamine or less than 0.1 µg/ml histamine before ovalbumin to up to 1.0 µg/ml histamine after ovalbumin. In the 7th case activity never exceeded that of 0.1 µg/ml. Injection of compound 48/80 was carried out in 3 of these cases and resulted in activity equivalent to up to 2.0 µg/ml histamine in the perfusate. Of the 7 cases where samples showed activity equivalent to more than 0.02 µg/ml histamine, in 2 this activity (in samples obtained during and after perfusion with ovalbumin) was not reduced in the presence of mepyramine but was abolished in one case by meclofenamate (10^{-4}M). In 2 further cases, although samples obtained during perfusion with ovalbumin were reduced to less than

10% by mepyramine, those after ovalbumin were reduced in one case by 50% and in the other not at all. In a fifth case mepyramine reduced activity of samples obtained during perfusion with ovalbumin to between 10% and 50% and after ovalbumin to less than 10%. Only in one of 3 cases where compound 48/80 was injected did mepyramine reduce the activity of samples subsequently obtained to less than 10%. In one case (36940), where samples which had shown activity equivalent to less than 0.02 µg/ml histamine were stored for 7 days, subsequent examination revealed activity in all samples. This was equivalent to up to 1.0 µg/ml histamine and was reduced by only 50% in the presence of mepyramine.

When tested on rat duodenum those samples showing activity in the presence of mepyramine caused relaxation equivalent to that produced by 0.02 to 0.2 µg/ml bradykinin. As the rat duodenum is stimulated to contract by acetylcholine, 5-hydroxytryptamine and histamine (Horton, 1959)

and as SRS-A was found by Marquis (1966) to produce no response when tested on this tissue, this relaxing effect was considered to indicate the presence of bradykinin in the samples.

Samples obtained by perfusion of the lungs of 4 animals sensitised using Freund's adjuvant showed greater activity and this was increased during and after ovalbumin perfusion. Activity increased from that equivalent to less than 0.1 µg/ml to that equivalent to up to 1.0 µg/ml histamine. In one case the activity of the samples obtained both during and after perfusion with ovalbumin was unaltered by mepyramine but in the other 3 cases it was reduced to less than 10%.

The activity of sequential samples of lung perfusate was inconsistent in that a sample of very low activity was often succeeded by several of high activity and vice versa. There was also variation in the results obtained from lungs of similarly sensitised animals. Histamine, bradykinin and

possibly other active substances were present in lung perfusate during and after perfusion with ovalbumin. The amount of bradykinin and of any other active substances excluding histamine, released or formed showed a tendency to be greater in sensitised than in unsensitised lungs.

SUMMARY

1. The concentration of histamine in tissues was extremely variable and did not appear to be reduced after anaphylaxis. Concentrations were higher in shocked than in control animals.

2. On comparison of 2 blood samples, one before and one after challenge, plasma free histamine levels showed a significant increase following anaphylaxis. The significance of this change became doubtful when great variability in histamine levels was revealed in serial samples removed at 5 min. intervals.

3. Histamine was not released by antigen in vitro during incubation from lung, pleura or liver capsule. It did appear to be released from the skin of some animals.

4. Perfusion of lungs of sensitised animals with antigen resulted in irregular release of histamine together with other active substances including bradykinin.

7. CUTANEOUS REACTIONS

MATERIALS AND METHODS

a) PASSIVE

ANIMALS

Twenty adult female English albino guinea-pigs, 12 white Wistar rats and 19 Ayrshire, Friesian, Ayrshire-cross and Friesian-cross calves aged 4 to 6 months were injected intracutaneously with serum obtained from calves which had been sensitised to ovalbumin and from unsensitised control calves. The calves from which the serum was obtained were of the same breeds and approximately the same ages as those to which the serum was administered.

SERUM

Sensitisation of the animals supplying the serum was carried out by the 2 procedures described in Section II. 1, namely intravenous injection of ovalbumin without adjuvant or 8 intramuscular injections of ovalbumin with Freund's

adjuvant. Animals were classed as sensitised when they reacted to ovalbumin administered intravenously at a dose rate of 1.5 mg/Kg by showing anaphylaxis with marked dyspnoea (+++).

Serum was obtained from blood collected into sterile glass bottles from a needle inserted in the jugular vein. The blood was allowed to clot at room temperature (approx. 20°C) and, after 24 hr., serum was aspirated and centrifuged for 10 min. at 2,000 revs./min. to remove any blood cells. In experiments involving investigation of heat labile reaginic antibody activity in the serum, half of the serum collected was incubated for 4 hr. at 56°C. The serum was then diluted with sterile saline x 5, x 10, x 25, x 50 and x 100.

TESTING PROCEDURE

Volumes of 0.1 ml of sterile saline and of undiluted and diluted unheated and, in 2 calves, heated sera were injected as described by Ovary (1958), into skin previously clipped

and cleansed. The skin of the back of guinea-pigs and rats and of the neck of calves was used. After intervals of 4 hr., 18 hr., 24 hr. or 48 hr. ovalbumin was injected intravenously. Calves received 300 mg to 2,500 mg of ovalbumin (6 ml to 50 ml of 5% solution). This was accompanied by intravenous injection of Evan's blue dye (50 mg) to all guinea pigs and rats and to 4 of the calves. Calves received 300 mg Evan's blue as a 2% solution.

The sites where serum had been injected were examined after 20 to 90 min. and the diameters of associated areas of swelling in the case of calves and blueing in the case of guinea-pigs and rats measured by means of calipers. Such cutaneous reactions in calves were recorded using the following scale:

+	=	5 - 10	m.m. diameter
++	=	11 - 20	" "
+++	=	21 - 30	" "
++++	=	31 - 40	" "
⊕	=	> 40	" "

Reactions of less than 5 m.m. were not recorded as positive reactions.

b) ACTIVE

Unsensitised calves and calves sensitised to ovalbumin and horse serum as described in Section II. 1 were injected intracutaneously with antigens. These animals were of the same breeds as described above. Their ages ranged from 3 months to 18 months. Volumes of 0.1 ml of saline, ovalbumin, horse albumin and horse serum were injected into the clipped and cleansed skin of the neck. The concentrations of ovalbumin and horse albumin used ranged from 0.1 mg/ml to 50 mg/ml. Horse serum was used undiluted and diluted x 10 and x 100. The sites of injection were examined after 20 min., 60 min., 90 min. and 48 hr. Reactions were measured and recorded as described above.

ANTAGONISTS

The ability of meclofenamate to inhibit passive cutaneous reactions was investigated by injecting 2.0 mg/Kg of this drug intravenously 5 min. before antigen was injected also intravenously.

In the case of active reactions mepyramine was injected intracutaneously together with antigen. The dose of mepyramine necessary to inhibit development of cutaneous lesions produced by histamine was first determined.

As intracutaneous injection of meclofenamate itself produced a reaction, this compound was given intravenously 5 min. before intracutaneous injection of antigen.

RESULTS

a) PASSIVE

Injections of bovine sera into the skin of guinea-pigs and rats failed in every case to sensitise the skin to subsequent

intravenous injection of antigen. Each guinea-pig and rat was injected at 4 sites with serum from unsensitised calves, serum from calves sensitised without adjuvant, serum from calves sensitised with adjuvant and saline. In 4 guinea-pigs and 2 rats an interval of 24 hr. between serum and antigen injections was allowed. In 8 guinea-pigs and 4 rats the interval was 18 hr. and in 8 guinea-pigs and 6 rats the interval was 4 hr. In the case of 2 guinea-pigs and 2 rats the dose of ovalbumin injected was 50 mg. All other guinea-pigs and rats received 1 mg of ovalbumin.

The results of injecting bovine sera into the skin of calves are summarised in Table 7. 1.

No passive cutaneous anaphylactic (PCA) reactions were induced as a result of injecting serum from control unsensitised calves into the skin of 4 similar calves. Each animal was injected at each of 2 sites with serum diluted x 1, x 10 and x 100. Each animal was injected at a total of 6 sites,

TABLE 7.1

PASSIVE CUTANEOUS REACTIONS

Serum	Interval between Serum & Antigen (hrs)	No. Tested	No. Reacting	Maximum Diameter of Reaction			
				+ No.	++ No.	+++ No.	++++ No.
US	48	4	0	-	-	-	-
S (Unheated)	4	5	1	1	-	-	-
	48	5	0	-	-	-	-
S (Heated)	4	2	0	-	-	-	-
FS (Unheated)	4	6	6	1	2	3	0
	48	8	5	0	0	2	3
FS (Heated)	4	2	0	-	-	-	-

Notes:
US = Serum from unsensitised animals
S = Serum from animals sensitised without Freund's adjuvant
FS = Serum from animals senitised using Freund's adjuvant.

Diameter of cutaneous reactions: + = 5-10 m.m.
++ = 11-20 m.m.
+++ = 21-30 m.m.
++++ = 31-40 m.m.

an interval of 48 hr. being allowed between the intracutaneous injection of serum and intravenous injection of ovalbumin.

Of 10 animals injected intracutaneously with serum from animals sensitised to ovalbumin without adjuvant, only one animal developed skin reactions. These appeared at sites of injection of serum diluted x 10, x 25 and x 50 but were weakly positive being 10 m.m. (+) in diameter.

Serum from animals sensitised to ovalbumin with Freund's adjuvant sensitised the skin of 11 of a total of 14 animals tested for PCA. This serum was obtained from 5 animals and was not uniformly effective in preparing skin for reactions to ovalbumin. Serum obtained from one animal (No. 51) failed to sensitise the skin of each of the 3 animals in which it was tested whereas that obtained from another (No. 34), which had received a similar course of injections of ovalbumin and Freund's adjuvant, induced PCA in all 11 animals in which it

was tested. This serum produced reactions at all sites at which it was injected in dilutions of x 1, x 5, x 10 and x 100. A typical record of PCA reactions in one calf is shown in Fig. 7. 1.

Reactions were produced allowing intervals of either 4 hr. or 48 hr. between injection of serum and antigen. Cutaneous reactions were not induced by serum after it had been heated. This was tested in 2 animals by administration of serum from animals sensitised with and without Freund's adjuvant.

The cutaneous lesions passively induced were discrete, circular, flattened, raised areas of firm consistency, freely moveable and apparently painless. They became visible approximately 15 min. after intravenous injection of antigen and reached their maximum size after 30 to 90 min. They regressed after 2 hr. and were not detectable after 24 hr. Intravenous injection of Evan's blue dye with the antigen resulted in discrete blue colouration of the raised areas of skin.

P.C.A.

No. 39350 (unsensitised)

Right side of neck

ES_1 x 1	Sal	FS_2 x 1	US x 1
30 m.m. +++	-	30 m.m. +++	-
FS_1 x 5	Sal	FS_2 x 10	US x 10
20 m.m. ++	-	20 m.m. ++	-

Left side of neck

US x 10	FS_2 x 10	Sal	FS_1 x 5
-	20 m.m. ++	-	20 m.m. ++
US x 1	FS_2 x 1	Sal	FS_1 x 1
-	15 m.m. ++	-	15 m.m. ++

Note:

FS_1 = Serum from Animal No. 37934 sensitised using Freund's adjuvant.

FS_2 = Serum from Animal No. 37935 sensitised using Freund's adjuvant.

US = Serum from unsensitised animal.

Sal = Saline.

Fig. 7.1 - Record of P.C.A. reactions in one calf. Serum or saline injected at 8 sites on each side of neck.

ANTAGONISTS

Four calves injected intracutaneously at 4 sites with serum from animals sensitised to ovalbumin using Freund's adjuvant were injected with meclofenamate 5 min. before ovalbumin and Evan's blue. In 2 calves this was after an interval of 4 hr. and in 2 after 48 hr. One of these developed no skin reactions but the other 3 developed typical reactions at the sites of injection of serum. Meclofenamate, the only antagonist tested on PCA, did not appear to prevent the reaction.

b) ACTIVE

Cutaneous reaction of sensitised animals to doses of ovalbumin ranging from 0.25 to 10 mg injected intracutaneously are shown in Table 7.2. Of 3 unsensitised animals tested, all showed reactions of approximately 5.0 mm diameter only to the highest concentration of ovalbumin and undiluted horse serum. All 24 animals sensitised to ovalbumin reacted positively on

TABLE 7.2 CUTANEOUS REACTIONS OF SENSITISED ANIMALS TO OVALBUMIN INJECTED INTRADERMALLY.

Ovalbumin Dose mg	No.of Animals	Diameter of Reactions Range mm
0.25	2	10 - 15
0.5	2	10 - 25
2.5	2	15 - 20
5.0	6	20 - 40
10.0	4	60 - 70

intracutaneous injection of ovalbumin (0.5 mg). Two of 5 animals tested, which had been sensitised intravenously to ovalbumin alone, reacted also to undiluted horse serum injected intracutaneously. All 3 animals sensitised intramuscularly by injections of ovalbumin with Freund's adjuvant reacted to horse serum (x 1 to x 100) and to horse albumin (0.5 mg) injected intracutaneously. These results are summarised in Table 7. 3. Typical cutaneous reactions are shown in Fig. 7. 2.

All animals sensitised to ovalbumin using Freund's adjuvant developed delayed skin reaction 24 to 48 hr. after injection of ovalbumin, horse serum and horse albumin. These were manifested as more diffuse swellings, harder in consistency than those of the immediate reactions. They ranged in size from 25 mm to 50 mm in diameter. These persisted for up to 7 days. Such delayed reactions did not occur in animals sensitised without Freund's adjuvant.

TABLE 7.3

ACTIVE CUTANEOUS REACTIONS

Sensitisation	Antigen (injected into skin)	No. Tested	No. showing immediate Reaction	Maximum diameter of immediate reaction					No. showing delayed Reaction
				+ No.	++ No.	+++ No.	++++ No.	⊕ No.	
S (Ovalbumin)	Ovalbumin	15	15	0	1	4	1	9	0
	Horse serum	5	2	2	0	0	0	0	0
	Horse albumin	5	0	0	0	0	0	0	0
S (Ovalbumin & horse serum)	Ovalbumin	2	2	0	0	0	2	0	0
	Horse serum	2	2	0	0	0	2	0	0
FS (Ovalbumin)	Ovalbumin	7	7	0	0	0	6	1	7
	Horse serum	3	3	0	0	0	2	1	3
	Horse albumin	3	3	0	0	0	2	1	3

Notes:

S = sensitised without Freund's adjuvant
FS = sensitised with Freund's adjuvant
Diameter of cutaneous reactions:

+ = 5-10 m.m.
++ = 11-20 m.m.
+++ = 21-30 m.m.
++++ = 31-40 m.m.
⊕ = > 40 m.m.

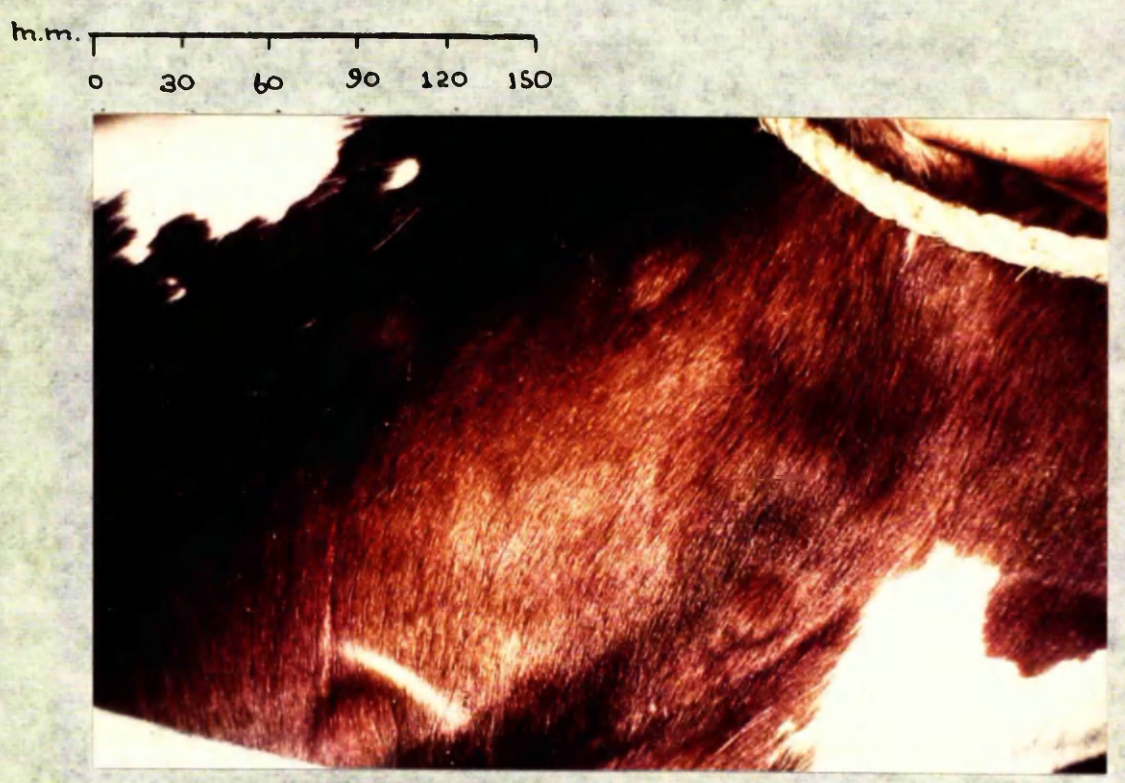


Fig. 7.2 - Skin of a sensitised Ayrshire calf showing active cutaneous anaphylactic reactions to intradermal injections of ovalbumin (5.0 mg and 2.5 mg).

ANTAGONISTS

i) MECLOFENAMATE

Intravenous injection of meclofenamate appeared to prevent immediate active cutaneous reactions in animals sensitised without adjuvant. Such pretreatment also reduced the magnitude of immediate reactions in animals sensitised with Freund's adjuvant but delayed skin reactions still developed in these animals 24 to 48 hr. after intravenous injection of meclofenamate and intracutaneous injections of ovalbumin.

ii) MEPYRAMINE

The dose of histamine producing a reaction of approximately the same magnitude as 5.0 mg ovalbumin in a sensitised animal was determined. Such a reaction to 2.0 µg histamine was found to be inhibited by simultaneous intracutaneous injection of 1.0 mg mepyramine (Table 7. 4). This dose of mepyramine had no effect in reducing active cutaneous reactions

TABLE 7.4 EFFECT OF MEPYRAMINE ON REACTIONS TO HISTAMINE INJECTED INTRADERMALLY

Histamine	Mepyramine Dose	No. of Animals	Diameter of Reactions Range
µg.	mg.		m.m.
0.4	0	4	20 - 25
2.0	0	4	25 - 35
4.0	0	4	60 - 65
2.0	0.001	4	20 - 25
2.0	0.1	4	12 - 20
2.0	1.0	4	5 - 8

to 5.0 mg ovalbumin (Fig. 7.3). Increasing the dose of mepyramine to 5.0 mg resulted in some reduction in immediate cutaneous reactions but not of delayed reactions, as shown in Table 7. 5.

SUMMARY

1. Only serum from animals sensitised using Freund's adjuvant sensitised the skin of calves, but not guinea-pigs or rats, for PCA reactions.

2. The skin sensitising property of such serum was lost on heating.

3. PCA was not prevented by pretreatment of animals with meclofenamate.

4. Immediate active cutaneous reactions were elicited in all sensitised animals. These were not specific in animals sensitised using Freund's adjuvant and such animals also showed non specific delayed skin reactions.

ANIMAL NO. 1S

Left side of neck

5 mg Ovalbumin	2 µg Histamine
(25 m.m.) +++	(28 m.m.) +++

Right side of neck

5 mg Ovalbumin and 1 mg mepyramine	2 µg Histamine and 1 mg mepyramine
(24 m.m.) +++	(6 m.m.) +

Fig. 7.3 - Record of cutaneous reactions in one calf produced by injections of ovalbumin and histamine, with and without mepyramine.

TABLE 7.5 EFFECT OF MEPYRAMINE ON CUTANEOUS REACTIONS OF SENSITISED ANIMALS TO OVALBUMIN INJECTED INTRADERMALLY

Dose of Mepyramine mg	Animals		Maximum Diameter of Reaction			
			Immediate		Delayed	
	No.	Sensitisation	Without Mepyramine	With Mepyramine	Without Mepyramine	With Mepyramine
1	4	S	+++	+++	-	-
5	2	S	++	+	-	-
	1	FS	+++	-	⊕	⊕

S = Sensitised without Freund's adjuvant
FS = Sensitised with Freund's adjuvant.

Diameter of cutaneous reactions :-
+ = 5-10 m.m.
++ = 11-20 m.m.
+++ = 21-30 m.m.
++++ = 31-40 m.m.
⊕ = > 40 m.m.

5. Immediate but not delayed reactions were reduced by intravenous injection of meclofenamate or by intracutaneous injection of mepyramine.

SECTION III

DISCUSSION

SUMMARY AND CONCLUSIONS

DISCUSSION

ANIMALS

It was essential to establish primarily, a method by which cattle might be sensitised to foreign protein so that acute, systemic anaphylactic reactions could be regularly produced. Calves aged 4 to 6 months were selected for this purpose as they could be relied upon at this age to be immunologically competent. As discussed in Section I (p.110) the immune response has been shown to be affected by the nature of the antigen and workers in this field have found that calves of under one month of age do not regularly respond to an immunological stimulus. All animals were free from disease at the commencement of experiments. Subsequently animals showing symptoms of disease in the course of the experiments were rejected. Among those rejected were 4 animals which developed mucosal disease, 4 suffering from severe ostertagiasis, 2 showing signs of interstitial pneumonia and one

which developed an abscess in the neck. The animals were not parasite-free but, to minimise their exposure to parasitic infection, were kept indoors in individual pens. At necropsy there was evidence of infestation by intestinal helminths and, in about 25% of the animals, dictyocaulus viviparus.

Calves of the Ayrshire and Friesian breeds were chosen as these are most easily obtainable in the West of Scotland. Previously no importance has been attached to breed in the incidence of diseases possibly associated with hypersensitivity and other workers who induced anaphylaxis in cattle used a variety of breeds (Wray and Thomlinson, 1969). Dungworth (1965) used Holstein-Friesians and Herefords and Code and Hester (1939) used Guernsey calves. In a preliminary test one Ayrshire and 2 Highland calves were used. The Highland calves reacted less severely than did the Ayrshire, particularly with respect to the respiratory symptoms. These animals,

although not included in the results described in Section II were retained and challenged at 2 to 3 week intervals for 5 months. The Highland calves on no occasion showed very severe respiratory distress and when they collapsed rose to their feet within 30 sec. Congestion of the conjunctivae and the skin of the muzzle was always seen. The scope of this investigation did not include an examination of breed differences, but it is possible that the nature of the anaphylactic reaction may be influenced by breed and some breeds of cattle may develop hypersensitivity more readily than others. This could be of importance in anticipating risks of anaphylaxis occurring in association with vaccination or drug administration.

ANTIGENS

As antigens, the proteins ovalbumin, horse albumin and horse serum were chosen. Bacterial endotoxin and drugs having direct effects unrelated to hypersensitivity have been used by other workers, but were excluded from this investigation

to avoid additional complication. Intravenous administration allowed the maximum control of the dose of antigen to which each animal was exposed. The sensitising dose of egg albumen used initially and based on that used by Thomlinson and Buxton (1963) in pigs was subsequently reduced but the minimum sensitising dose necessary was not determined. The latent period and challenge dose necessary were determined by experiment (Section II. 2). Differences were encountered depending on whether the antigen used was ovalbumin, horse serum, or horse albumin. The doses of horse serum were calculated from the total protein content of the serum. The protein in horse serum to which calves became sensitised was not identified. The failure to induce anaphylaxis using horse albumin suggested that this was not the protein in horse serum to which cattle became sensitised. Smith and Ingram (1965) found that the nature of the antigen used affected the immune response of calves under one month of age. As much of the literature on

the subject of anaphylaxis concerns experiments where ovalbumin was the antigen used, comparisons with the findings of other workers were simplified by using ovalbumin in most of our experiments.

The adverse effect of crude egg albumen on 5 unsensitised calves may have been anaphylactoid, like that observed in rats on injection of egg white (Selye, 1937; Paton, 1956; Parratt and West, 1957).

ADJUVANTS

As severe reactions were produced regularly by the very simple method of sensitisation by a single intravenous injection of ovalbumin, adjuvants were not deemed necessary. Failure to induce reactions on exposure of animals, sensitised in this way, to antigen by inhalation led to the adoption in 6 animals of the technique used by Dungworth (1965) of giving intramuscular injections of ovalbumin with complete

Freund's adjuvant. This resulted in the revelation of differences between sensitivity induced with and without adjuvant. Animals sensitised using Freund's adjuvant showed typical symptoms of anaphylaxis but reacted systemically to challenge doses smaller by 98% than did those sensitised without adjuvant. They also showed systemic and cutaneous cross-reactions to horse albumin and horse serum. Schultz-Dale reactions were demonstrated in bronchiole, pulmonary artery and jejunum from 2 out of 3 of such animals whereas although 2 of 4 animals sensitised without adjuvant provided jejunum which responded to antigen, pulmonary tissue from all of 16 animals sensitised without adjuvant failed to respond. The effects of antigen on perfusion of isolated lung were also more marked in the case of animals sensitised with Freund's adjuvant. The serum of the latter succeeded, where that of animals sensitised without adjuvant failed, in sensitising the skin of other calves for passive cutaneous

anaphylaxis. This was confirmed using goats in place of unsensitised calves (Beadle, 1970, personal communication). This skin sensitising activity of serum was lost on heating, suggesting that reaginic antibodies were responsible. It was found that the titres of precipitating and haemagglutinating antibodies in these animals were high (1:5,120 to 1:10,240) whereas such titres in animals sensitised without adjuvant were almost as low (1:40 to 1:320) as in control calves (1:40) Davies, 1968, personal communication).

Freund and McDermott (1942) described sensitisation of guinea-pigs to horse serum using an adjuvant containing killed tubercle bacilli, paraffin oil and a lanolin-like emulsifying agent. They found precipitin titres to be higher as a result of using adjuvant. They also encountered differences in the nature and duration of cutaneous reactions in animals sensitised in this way. Lewis and Loomis, as early as 1924 discovered that tuberculous guinea-pigs produced more

antibodies than non-tuberculous guinea pigs, when injected with various antigens not related to tubercle bacilli. Dienes, in 1928, showed killed tubercle bacilli to be as effective as living ones. The paraffin oil and emulsifying agent were believed by Freund and McDermott (1942) simply to protect the bacteria and retard separation of the antigen. The exact mode of action of immunological adjuvants, however, is still unclear. The active principle of Mycobacterium tuberculosis, a peptidoglycolipid, Wax D, was fractionated and characterised by Tanaka, Tanaka, Tsubone, Kuroda and Sugiyama (1965). This wax, the active principle of complete Freund's adjuvant, stimulated proliferation of plasma-cell elements and of reticuloendothelial cells (Rupp, Moore and Schoenberg, 1960). Koga, Ishibashi, Sugiyama and Tanaka (1969) provided evidence that the mycobacterial adjuvant exerted its effect by increasing the number of antibody-forming cells and also enhanced the antibody-producing capacity

of each cell. White (1968) pointed out the surface-active properties of the mycobacterial peptidoglycolipid and suggested the involvement of lysosomal rupture in adjuvant activity. White also pointed out the specific effect of mycobacterial adjuvants in increasing particular immunoglobulins and their association, in the guinea-pig, with increased delayed-type hypersensitivity. Koga et al (1969) suggested that the endotoxin of Gram-negative bacteria, such as Bordetella pertussis, acted as an adjuvant in a way similar to that of Mycobacterial adjuvants. If the effect of adjuvants were simply to quantitatively increase the immune response the situation would be simple. However, as stated by White (1968) and as outlined in Section I where the literature on anaphylaxis in the rat was reviewed, it was shown that, in the rat, sensitisation using different adjuvants, namely Freund's complete adjuvant or Bordetella pertussis, resulted in the production of different types of antibody. The two types were distinguishable on PCA

reactions on the basis of optimal latent periods and on their inhibition by different selective antagonists of histamine and SRS-A respectively (Stechschulte et al., 1967). Different types of antibody were produced also in mice using Freund's and Bordetella pertussis adjuvants (Clausen, Munoz and Bergman, 1969). Bordetella pertussis cells were shown to contain, in addition to endotoxin, a heat-labile substance which possessed an immunologic adjuvant activity (Pieroni and Levine, 1966; 1967). In addition, increased susceptibility to histamine and 5-hydroxytryptamine was shown by Sanyal and West (1958a) to be associated with pretreatment with Bordetella pertussis vaccine in rats and mice.

SYMPTOMS

Respiratory distress was shown in every case of anaphylaxis as was increased secretory activity. Ninety percent of all animals never showed symptoms of skin irritation

although severe respiratory changes occurred. Where pruritis was shown it was accompanied by mild, rather than severe, respiratory symptoms. As described in Section II (p.191) the occurrence of pruritis was unpredictable so that the influence of antagonist drugs on this symptom was not assessed.

CHALLENGE DOSES

The relationship between the challenge dose of antigen and the severity of the reaction allowed selection of doses which produced reactions of predictable severity. This was necessary for reasons of humanity,in order to avoid producing fatal reactions unintentionally and to allow repeated challenge to be carried out in assessment of the effects of antagonist drugs in modifying the severity of reactions.

ROUTES

Unlike Thomlinson and Buxton (1963) who claimed that,in pigs, additional subcutaneous injections of antigen prolonged

reactions on intravenous challenge, we did not observe this effect in calves. Intramuscular or subcutaneous challenging injections alone produced no symptoms. This might have been due to the slow rate of absorption from such sites, resulting in an insufficient concentration reaching the circulation to initiate a reaction.

Exposure to ovalbumin by inhalation of animals sensitised by an intravenous injection of ovalbumin or by a series of intramuscular injections of ovalbumin and Freund's adjuvant produced only tachypnoea and occasional coughing. Each animal was restrained in stocks and a polythene bag was secured round the head. A rubber tube led from the interior of the polythene bag to a Wright nebuliser (Aerosol Products, Colchester Ltd) containing a 5% solution of ovalbumin. Oxygen at 10L/min was allowed to flow through this solution. This allowed particles of approximately 1 μ diam. to form as vapour in the polythene bag. Animals were exposed to this vapour for

30 min. during which time approximately 30 ml of 5% ovalbumin was administered.

Attempts to induce anaphylaxis by inhalation suffered 2 major disadvantages in that the procedure was technically difficult to carry out without subjection of operators to ovalbumin vapour or impairing the supply of air to the animal. Also, inhalation of foreign material might result in bronchoconstriction due to an irritant effect unrelated to hypersensitivity (Banister, Fegler and Hebb, 1949). When Dungworth (1965) induced reactions on inhalation of ovalbumin he used periods of exposure of up to 9 days in a room 10' x 20' x 10'. Dungworth used an aerosol supplying particles 0.7 to 1.2 μ in diameter. Particles of ovalbumin supplied to guinea-pigs using the same nebuliser as used in calves were found to induce reactions in sensitised guinea-pigs. The size of particles formed by the nebuliser may have been unable to penetrate far enough in the respiratory tract of calves to initiate the

reaction which, in the guinea-pig, is largely due to contraction of bronchial muscle.

DURATION OF SENSITIVITY

None of the experimental animals were kept alive for longer than 18 months. The duration of sensitivity was not investigated specifically but 2 sensitised animals in which exposure to ovalbumin was witheld for 22 wk. reacted typically when challenged after this interval. Intervals of 11 wk. between successive exposures, allowed in 4 animals, did not alter the severity of subsequent reactions.

DESENSITISATION

Desensitisation was attempted in one animal by injecting 100 mg ovalbumin subcutaneously every second day for 32 days, followed by 150 mg ovalbumin given daily for a further 7 days. However, challenge by intravenous injection of ovalbumin 10 days after the completion of this course resulted in a severe

reaction. The feasibility of desensitising cattle was not investigated further but without using a large range of doses and of programmes of injection the possibility of desensitisation cannot be dismissed.

THERAPY

In experiments on unanaesthetised animals when the duration of the symptoms of anaphylaxis was not being measured, any animals which had not completely recovered by 30 min. after challenge were given 5.0 to 10 *mg* adrenaline subcutaneously. The value of this therapy was not a subject of investigation.

CHALLENGE OF ANAESTHETISED ANIMALS

PHYSIOLOGICAL CHANGES

Animals were challenged under anaesthesia for two reasons namely to facilitate measurement and recording of cardiovascular and respiratory parameters and to minimise the distress and discomfort of the animals during severe reactions. The anaesthetic, sodium pentobarbitone was shown by Parish, Hall and Coombs (1963) to confer no protection against anaphylaxis in guinea-pigs. Severe reactions were regularly produced in our anaesthetised animals, but as the possibility of modification by the anaesthetic could not be entirely excluded, quantitative results and assessments of the effects of antagonists were considered separately in conscious and in anaesthetised animals. Eyre (1969) found that barbiturate antagonised a histamine-induced relaxation of lesser bronchi and bronchioles in the sheep. This might intensify bronchoconstriction in sheep anaesthetised with

barbiturate. However, no relaxant effect was observed on isolated bovine tracheobronchial tissue except that induced by adrenaline (Section II. 3). The congestive effect on the lung of lateral recumbency of the animal and the abolition of the cough reflex would influence to some extent the respiratory and circulatory changes in anaesthetised animals.

On challenge, anaesthetised animals were found to undergo a rapid fall in systemic blood pressure. This, which could cause cerebral hypoxia, was considered to be responsible for the collapse observed in unanaesthetised animals. There was a simultaneous rise in pulmonary arterial pressure. Various factors, together or separately, might have been responsible for this phenomenon. Direct constriction of the pulmonary arterial system as described in the rabbit (Airilia, 1914), or of the pulmonary vein, by released or formed substances such as histamine, 5-hydroxytryptamine, kinins or SRS-A might have occurred. Pulmonary artery and pulmonary vein constricted

when exposed in isolation to 5-hydroxytryptamine, histamine and bradykinin. Both tissues, obtained from animals sensitised using Freund's adjuvant, constricted in response to ovalbumin. Eyre (1970b) found pulmonary vein to be at least 20 times more sensitive than artery to 5-hydroxytryptamine and histamine and obtained Schultz-Dale responses more readily from pulmonary vein than artery. The experiments described in Section II. 3 included 3 times more arteries than veins but, where both tissues were tested, both or neither responded to antigen. There was also a less marked difference between the tissues in their sensitivity to 5-hydroxytryptamine and histamine. Excluding any other factors, hypoxia has been shown to stimulate pulmonary arterial constriction in calves (Kuida, Brown, Thorne, Lange and Hecht, 1962) so that, even if not a primary effect, pulmonary hypertension would be expected to arise secondarily to respiratory embarrassment. Resistance to blood flow into the lung would also be increased

by an increase in the volume of air retained in the lungs due to bronchoconstriction and impairment of expiration. Pulmonary arterial and venous constriction could, in turn, result in decreased return of blood to the left side of the heart, leading to systemic hypotension. Vasodilation at other sites in the body would result in systemic hypotension also. There was no evidence on necropsy of pooling of blood in any other organ or tissue (Section II. 2).

Simultaneously with the onset of blood pressure changes, animals became apnoeic for 30 to 90 sec. Following apnoea respiratory flow and minute volumes were greater than before challenge. Accumulation of fluid in the tracheobronchial tree was also a feature. On examination post mortem, pulmonary oedema was always severe, with areas of congestion, emphysema and haemorrhage. The relative importance and sequence in time of oedema and bronchoconstriction was not clearly established. The technique of Konzett and Rossler (1940) for recording air

overflow volume used by Alexander et al. (1967) to measure bronchoconstriction in the sheep was not used in our experiments in calves. Although it is inferred that this method measures bronchoconstriction, what is actually being measured is resistance to the entry of air into the lungs. This resistance would clearly be increased also by the presence of material within the lumen of the bronchial tree and would not distinguish a constrictor from an obstructive effect. Bronchial muscle, removed from animals sensitised with Freund's adjuvant, contracted on exposure, in vitro, to antigen and in preliminary experiments on air perfusion of isolated lungs of such animals, resistance to air perfusion was slightly increased by antigen. The post mortem appearance of the lungs was unlike that of guinea-pigs dying of anaphylaxis in that the latter showed marked emphysema with no gross oedema. Sanyal and West (1958b) attributed the complete obstruction of the respiratory passages which is a characteristic feature of anaphylactic shock in the guinea-pig to the unusual arrangement in this species

of the smooth-muscle fibres in the trachea and bronchi. These are attached in the guinea-pig to the inner aspect of the cartilage.

The absence of any effect produced by injection of purified ovalbumin or horse serum in unsensitised animals confirmed the absence of anaphylactoid or reflex effects unrelated to hypersensitivity. Many chemically different substances, e.g. 5-hydroxytryptamine, egg white and horse serum in cats are known to produce apnoea, hypotension and bradycardia when injected intravenously due to stimulation of chemoreflexes from the heart and lungs (Dawes and Comroe, 1954).

SUCCESSIVE CHALLENGES

The absence of response to a second injection of the same antigen for a period of 6 to 7 days following a reaction might be due to depletion of mediating substances or of specific antibodies. Challenge with one antigen reduced the

severity of the subsequent response to a second antigen to which animals had also been sensitised. This finding favoured the importance of depletion of humoral factors or their precursors, responsible for mediating the reaction.

COMPARISONS WITH REACTIONS DESCRIBED BY OTHER WORKERS

Code and Hester (1939) described abdominal tympany as the outstanding feature of anaphylaxis in their calves. They considered the accompanying tachypnoea and hyperpnoea as secondary to gaseous distension of the rumen. Tympany of the rumen was observed occasionally in our animals, but was always preceded and accompanied by severe dyspnoea. Tympany never persisted for longer than 45 min. whereas Code and Hester found this symptom to persist for 6 hr. The exact age of Code and Hester's calves was not stated but they were under 6 months old. They did differ from our animals in that they were of the Guernsey breed and also in that they were sensitised by a series of daily injections. The

dose of antigen in relation to body weight was not stated, but was of the same order as that used in our experiments. Challenging animals with this amount by single intravenous injection would have been expected to result in a severe reaction. As the effects were relatively mild, the method of sensitisation used by Code and Hester appeared less effective than a single intravenous sensitising injection. Code and Hester measured carotid blood pressure by means of a mercury manometer and observed only a decline of approximately 15%. This occurred gradually over 3 min. and coincided with suppression of contractions of the rumen. The rapid fall in B.P. which we observed was not encountered. Gerlach (1922), by subcutaneous injections of horse serum, produced reactions similar to those of our animals. Urticaria and oedema of mucous membranes were more marked in Gerlach's cases and the rates of onset and duration of the reactions were more prolonged.

Our findings differed from those of Wray and Thomlinson (1969) in that these workers found marked gastro-intestinal as well as pulmonary lesions on post mortem examination following anaphylaxis. Lesions in the alimentary tract were more severe and pulmonary lesions less severe following protracted anaphylaxis, induced by Wray and Thomlinson (1969) by injecting antisera immediately before challenge. Gastro-intestinal lesions were present in only 2 of our animals when examined post mortem following anaphylaxis. One of these had been pretreated with sodium meclofenamate and had shown no respiratory changes, although challenged with a dose of ovalbumin lethal in unprotected calves. Although gastro-intestinal lesions were uncommon, animals frequently developed diarrhoea 48 to 72 hr. after challenge. This persisted for 48 to 72 hr. Wray and Thomlinson did not describe occurrence of diarrhoea but they did observe signs of abdominal discomfort with tension of abdominal muscles which were not detected in

our animals. It is possible that effects on the permeability of the alimentary tract and its vasculature are produced more slowly and in animals dying or killed within an hour of challenge have not had sufficient time to become apparent. The other symptoms described by Wray and Thomlinson agreed with those described in Section II. 1. Wray and Thomlinson used doses of ovalbumin larger than those which were found to be necessary for sensitisation (Section II. 1) yet they found multiple sensitising injections (3 at 48 hr. intervals) to be necessary to ensure that severe reactions could be produced on challenge by intravenous injection of the same dose. Their calves were of various unspecified breeds and were less than 2 wk. old. The difference in age might account for these animals proving less easily sensitised. Wray and Thomlinson suggested that their animals might have lesser quantities of pharmacodynamic agents available for release from the tissues. There was no evidence to suggest that this was the reason. A more likely

explanation was that such young animals were less immunologically competent than older calves. Although the severity of acute anaphylaxis was found to be independent of the titre of circulating antibody, the amounts of tissue-fixed antibodies were not known. Circulating antibody was confirmed by Wray and Thomlinson to be necessary for the production of protracted anaphylaxis which may involve antigen-antibody complexes producing an Arthus-type reaction.

Recently anaphylactic reactions to heat-inactivated Mycoplasma mycoides were described in 6 wk. old calves (Piercy, 1970). The lungs were primarily affected and reactions occurred in the absence of detectable circulating antibody and of cutaneous hypersensitivity. These animals reacted on their second occasion of exposure to the antigen 14 days after initial exposure. This illustrated the ease of sensitisation of calves as young as 6 wk., although circulating complement fixing antibody to Mycoplasma mycoides was formed only in animals over 8 wk. old.

The variations in the descriptions of acute anaphylaxis in the bovine species by different investigators suggest the possibility of the reactions being affected by age, breed, antigen and the method of sensitisation. The greatest problem in attempting to apply experimental findings to naturally occurring diseases is ignorance of the importance of the method of sensitisation.

PATHOLOGY

The diagnosis of bronchoconstriction, by examination of the histopathology of lung sections is to a large extent, subjective and was not attempted in our animals. Wray and Thomlinson (1969) judged bronchoconstriction to be present in their calves, as did Dungworth (1965). The degree of obliteration of the lumina of bronchioles described in guinea-pig lung by Dixon and Warren (1950) was not encountered in our calves. However, it is probable that a spasm of bronchial muscle would pass off after death. The deviations

from normality found in the lungs of our animals after acute anaphylaxis resembled closely those of fog fever or atypical interstitial pneumonia of cattle (Mackenzie, 1965). Bearing in mind that fog fever is a term applied to a range of respiratory diseases certain features of its pathology are regularly identified. Hyaline membranes in alveoli and alveolar ducts have been found in animals dying after suffering fog fever for at least 24 hr. Our animals, with 5 exceptions, died or had recovered within one to 2 hr. but those cases which were dyspnoeic for 48 hr. were found on post mortem examination to have hyaline membranes in their lungs.

HAEMATOLOGY

The leucopenia, due largely to neutropenia, and the haemoconcentration occurring during anaphylaxis was in agreement with the findings of Wray and Thomlinson (1969). Jain, Lasmanis and Schalm (1967) maintained a sensitised cow in a state of neutropenia for 24 hr. by infusion of ovalbumin over

6 hr. Leucopenia was described as a feature of anaphylaxis in the dog by Dean and Webb as early as 1924. This was explained as resulting from accumulation of leucocytes in the capillaries of the lungs and the liver (Webb, 1924). Polymorphonuclear neutrophile leucocytes were shown to be involved in the rat in SRS-A formation (Orange, Valentine and Austen, 1967). They are also known to contain histamine and 5-hydroxytryptamine in most species (Humphrey and Jaques, 1954). Migration of these cells to the lungs in anaphylaxis, as to sites of acute inflammation, is likely to be followed by subsequent participation in release and formation of mediators. Dungworth (1965) described a rise in total WBC with neutrophilia, eosinopenia and lymphopenia in cattle within 3 to 5 hr. of commencing inhalation of ovalbumin. He attributed these changes to increased adrenocorticosteroid activity in response to stress. However, he did not state the numbers of WBC before challenge and the numbers recorded immediately

after challenge began were lower (7,000 to 8,000/cu.mm.) than those of our animals before challenge (8,000 to 19,900/cu.mm.). It seems possible that Dungworth may have missed an early fall in WBC by his method of sampling.

Maki and Tucker (1965) found leucopenia, due to neutropenia, and a reduction in haemotocrit value in cattle following grazing on pasture known to be associated with the incidence of acute bovine pulmonary emphysema. They did not reproduce the disease and the implications of the haematological changes were not clear but suggested the presence of some factor in the pasture causing leucopenia with or without respiratory disease.

Haemoconcentration was also found by Alexander et al. (1970) to occur in sheep during anaphylaxis. Haemoconcentration accompanying oedema of the lungs or other organs can be explained on the basis of loss of fluid from the circulation into tissue spaces and airways of the lungs. The protein

content of exudate removed from the bronchi during anaphylaxis in vivo, estimated in 2 cases, was found to range from 4.0 to 5.0 gm per 100 ml. Pulmonary oedema indicates increased capillary permeability due possibly to direct effects of substances, such as histamine, on capillaries and post capillary venules, changes in hydrostatic pressure, due to pulmonary vascular constriction, impaired venous return and reduced cardiac output. The developing tissue hypoxia due to impaired respiratory function might be expected to aggravate the condition. The lack of pulmonary lesions in animals slaughtered one week to one month after having, on numerous occasions of challenge, shown severe respiratory distress was of interest and supported the observation that repeated challenge at intervals of 7 to 14 days did not result in the reactions increasing in severity.

MEDIATORS

CRITERIA AND LIMITATIONS

Collier (1968) reviewing the humoral factors in bronchoconstriction, distinguished a humoral factor from a mediator, the former acting positively or negatively while the latter is assumed to act only positively. The evidence demanded by Collier, to establish participation of a humoral factor in anaphylactic bronchoconstriction (see Section I, pp. 34, 35) might equally be applied to the establishment of participation of a mediator in anaphylaxis.

The ease with which such evidence may be obtained varies greatly with the substance being investigated. It is, for example, much more likely that changes in concentrations of histamine in a body fluid will be detected than will be changes in kinin or SRS-A levels. Histamine is an amine of known structure and chemical and pharmacological properties, for which an efficient method of extraction has been developed (Adam,

et al, 1957) whereas kinins are difficult to estimate accurately in body fluids (Lewis, 1962) and SRS-A is still chemically undefined and may be a group of substances rather than a single substance. Identification, by chemical characterisation, of any previously unknown substance was considered to be outwith the scope of this work. As well as technical difficulty in handling possible mediators, the degree of normal variation in the levels of such substances in body fluids must be appreciated. This difficulty was encountered in evaluating apparently significant changes in histamine concentrations.

The reproduction of the anaphylactic reaction by injection of the mediator in question, depends on that mediator being available in pure form in sufficient quantities for this purpose. Also, to administer such a substance at a dose rate related to blood levels achieved during natural reactions, these levels must be established. However, even with this

knowledge the concentration of a mediator released at its site of action may achieve much higher tissue concentrations locally than would be indicated from the levels in the blood. The route and rate of administration too will affect the effects produced. It is nevertheless, of some value to determine doses necessary to produce certain effects, and to compare these effects to those of anaphylaxis.

The ability of antagonists of possible mediators to inhibit the natural reaction is dependent on such drugs reaching the sites of action of the mediators in sufficient concentrations to block their effects. This will be more difficult where mediators are released to act locally in high concentrations. This was one of the reasons put forward for the much greater effect of antihistamines against added histamine than against antigen when tested on the bronchial chain of an asthmatic human, in vitro (Schild, Hawkins, Mongar and Herxheimer, 1951) or in sensitised guinea-

pigs, in vivo (Reuse, 1950). The conclusions which may be drawn from the effects of such antagonists on the natural reaction depend on the degree of specificity of their antagonistic action. For this reason, drugs of non-specific action such as corticosteroids, although of value in therapy of natural hypersensitivity reactions, were not included as antagonists for the purpose of this investigation.

In the investigation of the mediation of anaphylaxis in cattle more positive information was obtained indirectly by administration of possible mediators and their antagonists than directly by measurement of tissue and blood levels of such mediators.

As SRS-A was not available in pure form the substances administered to our animals were histamine, 5-hydroxytryptamine and bradykinin. Specific antagonists of histamine and 5-hydroxytryptamine were available but drugs antagonistic both to kinins and SRS-A did not distinguish between these substances.

EFFECTS OF POSSIBLE MEDIATORS

IN VIVO

No reference was found concerning the effects of 5-hydroxytryptamine or kinins in cattle. Several accounts have been given of the effects of histamine in this species. Our findings were in accordance with those of Desliens (1958) regarding the changes occurring following histamine administration and the lethal dose of histamine for anaesthetised cattle. Half the lethal dose administered to conscious calves produced reactions similar to moderately severe anaphylaxis and similar symptoms were produced by 5-hydroxytryptamine. In anaesthetised calves both histamine and 5-hydroxytryptamine caused apnoea, systemic hypotension, pulmonary hypertension and bradycardia. In the case of histamine, bradycardia was followed, within 2 min. by tachycardia, which was attributed to adrenaline release. This was observed in the case of 5-hydroxytryptamine only in vagotomised animals. The effects

of 5-hydroxytryptamine appeared to be influenced by anaesthesia in that a dose, producing only a moderate reaction in conscious animals, was lethal for an anaesthetised animal. Bradykinin produced effects similar to those of histamine, including tachycardia following bradycardia. The amount of bradykinin used was limited by its cost. For this reason it was administered only to anaesthetised animals and in one case, to allow reduction of the dose, pretreatment with propranolol was carried out to block the B effects of adrenaline. These effects include relaxation of bronchial muscle, which tends to reduce the severity of the broncho-constrictor effect of bradykinin. Thus B-adrenergic blockade intensifies the response to bradykinin as demonstrated by Collier, James and Piper (1965) in the guinea-pig. Piper, Collier and Vane (1967) showed that adrenaline release in the guinea-pig was stimulated by injection of bradykinin, histamine or SRS-A and adrenaline was released also during anaphylaxis.

Following administration of 5-hydroxytryptamine, pulmonary oedema did not occur. Severe pulmonary oedema with accumulation of fluid in the trachea and endotracheal tube resulted from histamine administration and was a regular feature following anaphylaxis. Bradykinin administration resulted in pulmonary oedema of moderate severity. The failure of even lethal doses of 5-hydroxytryptamine to produce the oedema characteristic of anaphylaxis, allowed the conclusion that 5-hydroxytryptamine could not alone be the mediator involved, although it might participate.

Administration of doses of 0·06 mg/Kg histamine and 0·07 mg/Kg 5-hydroxytryptamine to anaesthetised vagotomised animals resulted in death. This suggested that at least the major part of the actions of these substances was direct and precluded the possibility of 5-hydroxytryptamine acting in calves as in cats by stimulating coronary and pulmonary depressor chemoreflexes

(Dawes and Comroe, 1954). Detailed examination of a possible reduction in the severity of the changes produced was not carried out. However, the recent demonstration that vagotomy reduced the respiratory effects of histamine in spontaneously breathing and in artificially ventilated guinea-pigs (Mills and Widdicombe, 1970) suggested that the role of the vagus nerves in the manifestations of anaphylaxis and of the effects of drugs on the respiratory system, might be considerable. Mills and Widdicombe postulated the existence of afferent end-organs in the form of "lung irritant receptors." In rabbits (Karczewski and Widdicombe,1969a,b) and dogs (de Latona, Mata and Aviado, 1961: de Kock, Nadel, Zwi, Colebatch and Olsen, 1966) it has been shown that bronchoconstriction and rapid shallow breathing, resulting from injection of histamine, involve a vagal reflex. In man, atropine and hexamethonium reduced the respiratory and bronchomotor actions of histamine, suggesting a nervous

reflex mechanism (Bouhuys, Jonsson, Lichtneckert, Lindell, Lundgren, Lundin and Ringquist, 1960). As suggested by Mills and Widdicombe (1970) there are probably species differences in the relative importance of reflex and direct reactions to histamine and also to 5-hydroxytryptamine, ATP and other substances possibly involved in anaphylaxis.

Mepyramine and meclofenamate antagonised the effects of injected histamine and bradykinin in our animals. However, both methysergide and 501C67 failed, in normal and also in vagotomised animals, to prevent apnoea resulting from 5-hydroxytryptamine administration. This suggested that the respiratory effects of 5-hydroxytryptamine might be indirect involving release of some other substance not antagonised by methysergide or by 501C67.

IN VITRO

Histamine, 5-hydroxytryptamine and bradykinin were each capable of stimulating contraction of bovine trachealis muscle,

bronchiolar muscle, pulmonary artery and pulmonary vein and these effects on trachealis muscle were inhibited by mepyramine, methysergide and meclofenamate respectively. Offermeier and Ariens (1966) suggested that, in calf trachealis muscle, 5-hydroxytryptamine acted by releasing acetylcholine. These workers used the technique of adding consecutive doses of drug to the organ bath without washing out the bath between doses. They antagonised 5-hydroxytryptamine successfully with atropine but not with lysergic acid diethylamide (L.S.D.) on this tissue. We found that doses of atropine 100 times greater than those antagonising an equi-effective dose of acetylcholine were necessary to antagonise 5-hydroxytryptamine irrespective of whether the duration of exposure to atropine was one or 20 min. Methysergide regularly inhibited contraction of pulmonary artery induced by 5-hydroxytryptamine. Antagonism of 5-hydroxytryptamine on bronchiolar muscle and of bradykinin on pulmonary artery and on bronchiolar muscle was not tested

as these tissues showed tachyphylaxis to 5-hydroxytryptamine and bradykinin. Only a small number of pulmonary veins were tested but these tissues did not differ greatly in sensitivity from pulmonary arteries. The veins responded to smaller doses of acetylcholine than did the arteries. In order definitely to identify venous tissue the pulmonary vein was followed from the left atrium and the vessel wall cut transversely and opened to form a strip. Segments of what were believed to be small arterial branches were cut spirally. These could not be identified grossly with complete certainty as artery or vein. Eyre (1970b) found pulmonary vein to be 20 times more sensitive than artery to 5-hydroxytryptamine and histamine. He did not state the diameter of the vessels he used nor did we, during the experimental work, attach significance to this factor. It may, however, influence sensitivity to drugs. The number of observations which could be carried out on bronchiolar tissue was limited by the necessity for long rest

periods between doses. Intervals of at least 30 min. were always necessary, but up to 2 hr. were often required to allow recovery to be complete. Responses to adrenaline appeared to depend on the degree of contraction of the muscle

The reduction in vascular perfusion rate produced by histamine, 5-hydroxytryptamine and bradykinin in isolated lungs could result from constriction of the pulmonary artery or vein or both. It could also be attributed, in part, to leakage of fluid from capillaries. The increase in air perfusion pressure could be largely attributed to constriction of bronchial muscle but would also be affected by leakage of fluid into the airways. The main difficulty encountered in the experiments carried out using isolated lung was the permeability change affecting the lung vasculature and leading, even in the absence of drug administration, to the lung becoming progressively more oedematous during the course of perfusion.

The responses of isolated tissues showed that the respiratory dysfunction and pulmonary hypertension of anaphylaxis could possibly result from direct effects of histamine, 5-hydroxytryptamine or bradykinin on tracheo-bronchial and pulmonary vascular tissue. The logical extension of this work was to examine the effect of antigen on such tissues removed from sensitised animals and to identify the mediators released, following combination of antigen with fixed antibody, by means of specific antagonists. This identification was not achieved for two reasons. Firstly, as mentioned previously, pulmonary tissues from all animals sensitised without Freund's adjuvant failed to respond to antigen. Secondly, where a response to antigen was produced in longitudinal intestinal muscle or, in animals sensitised using Freund's adjuvant, in intestinal and in pulmonary tissues, a second dose of antigen produced either no response or a greatly reduced

response. This made assessment of inhibition by drugs, using the same tissue and testing with antigen before and after antagonist, impossible. If response to antigen had proved as predictable in isolated tissues as in the whole animal, some information might have been obtained by using different pieces of a tissue from the same animal and testing with and without antagonists. This, however, was not the case.

Eyre (1970b) found that positive Schultz-Dale reactions were produced more readily in pulmonary veins than arteries. However, we found that where such reactions were produced all pulmonary tissues gave positive reactions. Unfortunately, in many animals only pulmonary tissue was tested as the lung appeared to be most sensitive in vivo to the effects of antigen. In some animals intestinal tissue showed positive reactions when pulmonary tissues did not, namely where tissues were obtained from animals sensitised without Freund's adjuvant.

Eyre (1970b) did not give details of the relationship between the method of sensitising animals and the incidence of positive Schultz-Dale reactions. However, he also found that the use of Freund's adjuvant in sensitisation favoured the production of positive Schultz-Dale reactions (Eyre, 1970, personal communication).

The failure to sensitise tissues passively for the purpose of initiating Schultz-Dale responses by incubation with sera from animals sensitised using Freund's adjuvant was reminiscent of the finding that human reaginic antibody failed to induce passive sensitisation for this purpose, although Schultz-Dale reactions could be stimulated in bronchial rings removed from an allergic individual (Schild, Hawkins, Mongar and Herxheimer, 1951). Animals sensitised with and without Freund's adjuvant may differ only in the quantity of tissue fixed antibody available, or the latter may possess antibodies and/or their reactions involve mediators

located elsewhere than in the tissues tested. The conditions prevailing when a piece of muscle is suspended in physiological saline may not allow the reaction to occur. The possibility that blood might be essential was considered. Blood bathed organs were not used but the effect of adding autologous blood or plasma to the organ bath before or together with antigen was tested. Blood, serum or plasma alone unfortunately caused contraction of isolated smooth muscle. This was attributed to the presence of 5-hydroxytryptamine as such responses were reduced in the presence of methysergide.

The effects of antigen on vascular perfusion of sensitised lung were not significant. In isolated lung perfusion with antigen rather than injection of antigen was carried out to allow a longer period of contact between tissue and antigen.

EFFECT OF ANTAGONISTS ON ANAPHYLAXIS

The value of any results obtained regarding protection of animals against acute, systemic anaphylaxis depended on the regular production of reactions of predictable severity. In conscious animals, using cross-over tests, anaphylaxis could be produced as many times as was desired, in the presence and in the absence of pretreatment with an antagonist. Intervals of at least one week were allowed between successive challenges. Challenge under anaesthesia was carried out in each animal only once, so that the effects produced in an animal after a form of pretreatment could be compared only to the effects produced in a different animal without that pretreatment. Had reactions not proved predictable such experiments would not have been undertaken. No animal, reacting on challenge when conscious, failed to react on challenge under anaesthesia without pretreatment.

Protection against anaphylaxis in conscious animals was observed after pretreatment with meclofenamate. Although Collier and James (1967) found in the guinea-pig that meclofenamate together with mepyramine suppressed most of the anaphylactic bronchoconstrictor response, we found that the addition of mepyramine improved on the effect of meclofenamate alone only in reducing lacrimation. The protective effect of meclofenamate was confirmed in anaesthetised animals where the suppression of respiratory changes was complete. The fact that pulmonary arterial blood pressure still rose after meclofenamate pretreatment suggested that this was a primary effect independent of respiratory changes or changes in systemic blood pressure. Pathological changes were absent from the lungs of such protected animals but it was of interest that one of these animals showed congestion and haemorrhage in the alimentary tract. Slight inflammatory changes in the alimentary tract

were observed following administration of histamine. We found that responses to histamine and 5-hydroxytryptamine were not inhibited by meclofenamate while the response to bradykinin was prevented. Collier and Shorley in 1960 and 1963 and Berry and Collier in 1964 showed that in the guinea-pig the effect of meclofenamate, and other drugs similar to aspirin, in antagonising bronchoconstriction, was selective for kinins and SRS-A and that they did not antagonise histamine or 5-hydroxytryptamine. Alexander et al. (1970) reported meclofenamate to have some anti-histamine and anti-5-hydroxytryptamine activity in the sheep. The protective effect of meclofenamate suggested that bradykinin or SRS-A or both substances, possibly also including ATP might be of importance in the mediation of anaphylaxis in cattle. It was of interest that these findings in cattle were followed by reports of similar findings relating to the mediators involved in anaphylaxis

in sheep (Alexander et al., 1970). Ruminants may differ considerably from laboratory animals in the relative roles of endogenous substances such as histamine, 5-hydroxytryptamine, kinins and SRS-A.

When animals sensitised using Freund's adjuvant were challenged with the same dose of ovalbumin as used in animals sensitised without Freund's adjuvant no protective effect of meclofenamate was apparent. Pretreatment of such animals with either mepyramine (2.0 mg/Kg) or phenylbutazone (2.0 mg/Kg) was also without effect. However, when the challenge dose of ovalbumin was reduced to 0.03 mg/Kg the reaction produced was suppressed by meclofenamate. This suggested that animals sensitised using Freund's adjuvant differed quantitatively rather than qualitatively from those sensitised without adjuvant. It is possible that the former possessed greater amounts of the antibodies involved and that the quantities of mediators released was greater.

Alternatively the mechanism in animals sensitised with adjuvant might have involved a different type of antibody and different mediators.

Throughout experiments involving antagonists haematological changes were measured. These changes were not abolished even in the absence of all other effects of anaphylaxis. This proved a useful method of checking that an animal had been protected from the effects of mediators, rather than that an antibody antigen reaction had not occurred. It was shown by Mongar and Schild (1957) and Trethewie (1951) that acetylsalicylate did not inhibit interaction between antibody and antigen or release of humoral factors unless used in very much higher concentrations in vitro than those obtained in vivo, which inhibit the response to bradykinin and effects of anaphylaxis.

Although most of the work of Collier and his colleagues concerning meclofenamate has been in relation to broncho-

constriction, it was demonstrated that aspirin and meclofenamate also reduced the duration but not the depth of the hypotensive effect of intra-arterially injected bradykinin in guinea-pigs (Collier, Dinneen, Perkins, Piper, 1968). Meclofenamate resembles acetylsalicylate in pharmacological properties but is more potent (Winder et al., 1965; Collier, James and Schneider, 1966). A reduction in the hypotensive as well as the respiratory effects of bradykinin and of anaphylaxis was observed in our calves when pretreated with meclofenamate.

Two anaesthetised animals failed to show anaphylaxis on challenge after receiving B.W. 501C67 although the production of apnoea by injection of 5-hydroxytryptamine was not prevented. No protective effect of antagonists of 5-hydroxytryptamine was observed in conscious animals and the mechanism of this effect is unclear as it cannot be explained on the basis of antagonism of 5-hydroxytryptamine. B.W.501C67

is a new compound and investigation of an anti-anaphylactic effect might prove useful. In aiding detection of mediators it was less useful than meclofenamate the spectrum of activity of which is more clearly established.

EFFECTS OF VAGOTOMY ON ANAPHYLAXIS

Vagotomy was found to reduce the severity of anaphylaxis. In two cases apnoea was inhibited and in a third it was delayed. Atropine was ineffective in reducing changes in respiration and blood pressures. Only secretory activity was reduced. The role of the vagus nerve in anaphylaxis in other species is unclear and, as outlined in Section I, p. 31, there are conflicting findings. Mills and Widdicombe (1970) found that in guinea-pigs anaesthetised with sodium pentobarbitone, paralysed by intravenous injections of gallamine triethiodide and artificially ventilated, vagotomy reduced by 75% the decrease in total lung conductance and by 50% the decrease in lung compliance due to anaphylaxis. These parameters,

conductance and compliance, were obtained from measurements of transpulmonary pressure and tracheal airflow. It was put forward by Mills and Widdicombe (1970) that differences in the findings of workers on this subject might be related to some studies being carried out in pithed or very deeply anaesthetised animals (Collier and James, 1967; Collier, Holgate, Schachter and Shorley, 1960) in which nervously mediated changes in breathing and bronchomotor tone might be blocked. In lightly anaesthetised guinea-pigs anaphylaxis caused respiratory changes only if the vagi were intact (Auer and Lewis, 1910; Koller, 1967a, b; 1968). Koller (1967a, b; 1968) showed that vagal afferent fibres in the guinea-pig were stimulated during pulmonary anaphylaxis by inflation of the lungs. These may be identical to the lung irritant receptors of rabbits (Homberger, 1968; Mills, Sellick and Widdicombe, 1969) which are stimulated by chemical and mechanical irritants and by traction on the bronchial wall. Thus

vagal reflexes may be involved also in the pulmonary effects of anaphylaxis in cattle. It was of interest that in the vagotomised animals which failed to show apnoea on challenge there was also an absence of pulmonary oedema. It is therefore conceivable that therapy which blocks vagal conduction might reduce the reaction, although atropine, at the dose used, was without effect.

INVESTIGATION OF RELEASE OF POSSIBLE MEDIATORS

HISTAMINE

Tissue levels of histamine were measured in an attempt to detect differences between levels in control animals and animals dying during anaphylactic reactions. The wide individual variation in tissue histamine levels which was found to occur in calves was in agreement with the findings of Feldberg (1956) who stressed the degree of individual as well as species variation and variation in the histamine content of different organs. This reduced the possibility

of detecting real differences from normal in shocked animals. The fact that shocked animals, rather than showing evidence of release of histamine from tissues, had high levels of histamine might possibly have been related their having undergone anaphylactic reactions on several occasions before death, whereas control animals, obtained from the local abbatoir had presumably never suffered anaphylaxis. Animals of only 4 days old had lower levels of histamine than did 3 month old animals. This was in agreement with the findings of Riley and West (1953) that the histamine contentent of the lung of cow, man and cat, liver of cow and skin of cat increased with age. Feldberg and Kellaway (1937a) also found that the histamine content of lung was lower in kittens than in adult cats. Mast cells were recognisable by the presence of metachromatic granules, after staining of sections with Toluidine blue as described by Sanford (1962),

in all tissues, both in controls and in shocked animals. Mast cell counting was not carried out as it has been demonstrated that histamine may be released without disappearance of granules being apparent (West, 1956; 1962).

Levels of plasma free histamine were extremely variable on repeated sampling from individual animals and conclusions as to changes in plasma histamine occurring in association with anaphylaxis were approached with caution. Claims by other workers of detecting such changes must be assessed in relation to individual variation and variations encountered on repeated sampling over a period of time from a single animal. Changes in blood histamine levels were described by Code and Hester (1939) in association with anaphylactic shock and by Nilsson (1963) and by Maclean (1965) in association with laminitis. In comparing the results of different workers the differences in their methods of extraction of histamine must be appreciated. Code and Hester (1939)

used the method of Barsoum-Gaddum with Code's modification (Code, 1937) and found the level of histamine in whole blood of 4 calves to range from 0.017 to 0.064 µg/ml. Nilsson (1963) cited views that Code's method allowed transformation of histidine in haemoglobin to histamine and Nilsson used 2 different methods, namely that of Duner and Pernow (1958) and of Schmiterlöw (1949). Both methods measured the histamine content of whole blood. By the former method Nilsson found levels of 0.0858 $\pm$ 0.012 µg/ml in normal cattle. The method of extraction should not influence the nature of changes in histamine levels. Code and Hester found that blood histamine levels fell after anaphylaxis. Nilsson and Maclean both described reductions in histamine levels in association with acute laminitis but both encountered exceptions in which histamine levels were higher than normal. We found increases in histamine levels which were statistically significant

following anaphylaxis but the variation revealed on repeated sampling indicated the necessity of examining serial rather than paired blood samples. Examination of serial blood samples before and after exposure of an animal to a procedure or experience suspected of affecting blood histamine levels would appear, from our findings and those of other workers, to be essential.

Incubation of tissue, particularly lung, from sensitised guinea-pigs with antigen was used by many workers to demonstrate anaphylactic release of histamine in vitro (Boreus and Chakravarty, 1960). This was shown to be essentially an aerobic reaction, inhibited by exposing the tissues to an atmosphere of nitrogen (Edman, Mongar and Schild, 1964). In the experiments described in Section II. 6 oxygen was not supplied to the tissues but they were shaken continuously during incubation. An experiment carried out to assess the effect of oxygenation showed that this did

not alter the results of incubation in any way. Although incubation with histamine releasers, such as compound 48/80, resulted in histamine release, as described by Mongar and Schild (1953) using guinea-pig lung, no release resulted from exposure of lung, pleura or liver capsule to antigen. As pleura appeared in preliminary experiments to release higher percentages of histamine than did lung or liver capsule, most subsequent experiments were carried out using this tissue. Evidence was obtained of release by antigen of histamine from skin obtained from the muzzle of animals which had shown pruritis when challenged in vivo. The irregularity of occurrence of this symptom prevented investigation of its inhibition by antihistamines. It is possible that histamine is responsible, at least in some cases, for the symptoms of skin irritation, congestion of the muzzle, mucosae and conjunctivae occurring during anaphylaxis. The possibility of histamine release from tissues other than those investigated has not been excluded. As the lung is

the organ principally affected in anaphylaxis, release from pulmonary tissue seemed most likely. Liver capsule was also investigated because of its very high content of histamine. Kidney, spleen alimentary tract and white blood cells are other possible sites of histamine release. Incubation of WBC with antigen to investigate release of histamine, 5-hydroxytryptamine or SRS might prove very interesting. Suspension of WBC and platelets in a medium in which they were maintained intact and viable would be essential. Preliminary attempts to carry out such experiments were frustrated by failure to prevent spontaneous disruption of WBC and platelets. These were suspended in the medium prepared as described by Humphrey and Jaques (1955) who incubated WBC of rabbits with antigen and demonstrated release of histamine and 5-hydroxytryptamine.

5-HYDROXYTRYPTAMINE

The role of 5-hydroxytryptamine in anaphylaxis in cattle has not been clarified. After incubation of samples of lung for 60 min. with Tyrode solution or with ovalbumin there was found to be no detectable 5-hydroxytryptamine in the supernatant fluid and 5-hydroxytryptamine (10 µg/ml.) was found to be progressively reduced in quantity when assayed spectroflurimetrically after incubation with lung for periods of time ranging from 5 to 60 min. The finding that it was removed by lung from incubation fluid ($t\frac{1}{2}$ = approx. 10 min.) prevented investigation of release of 5-hydroxytryptamine by antigen in vitro. A similar effect has been demonstrated by incubation of 5-hydroxytryptamine with rat lung (Alabaster and Bakhle, 1970).

LUNG PERFUSION

The method of lung perfusion with a solution of antigen was basically that used by Brocklehurst (1960) to detect

SRS-A. Findings were inconsistent but there was some evidence of release or formation of histamine, kinin and possibly some other substance in sensitised lung on exposure to ovalbumin. Histamine was present in samples obtained during exposure to Tyrode solution alone and also in samples obtained from lungs of unsensitised animals. Samples which caused relaxation of rat duodenum were believed to contain bradykinin but samples which contracted rat duodenum in the presence of atropine were also encountered. These were found, when assayed on guinea-pig ileum, to contain 100 times less histamine than was necessary to stimulate duodenum to contract. Marquis (1966) obtained no response of rat duodenum to SRS-A so another unknown substance or substances may be present. In order to clarify the results obtained, extraction and purification of kinins and SRS-A from samples of perfusate should be carried out and assays performed as soon as possible after perfusion. Storing samples for 7 days increased activity attributable to bradykinin. Although collection of samples

was delayed at the beginning of each experiment until blood was absent from the perfusate traces of blood frequently appeared later in the course of the experiment. Samples were centrifuged to remove blood cells on collection but activity attributable to bradykinin was often found to be considerable in samples which had contained blood. Ideally the intact lungs of a sensitised calf might be perfused rather than isolated lobes. This would require very large volumes of fluid as each apical and cardiac lobe weighed approximately 200 to 300 gm. The entire lungs would weigh approximately 2 Kg and would require 2 L per min. of Tyrode solution for perfusion at the rate of 1 ml/gm/min. used by Brocklehurst (1960). Brocklehurst and Marquis detected SRS-A on perfusion of lungs from our sensitised animals (Brocklehurst, 1967, personal communication).

CUTANEOUS REACTIONS

PASSIVE

In eliciting passive cutaneous reactions, sera from animals sensitised using Freund's adjuvant was effective. Sera from animals sensitised without adjuvant was ineffective. Wells and Eyre (1970) found that sensitisation of calves using horse serum and Freund's adjuvant was the most effective method for the production of sera capable of transferring sensitivity to the skin of other calves. Heating at 56°C for 4 hr. removed the skin sensitising activity of the sera of our animals. This suggested that thermolabile antibodies, probably reagins, were responsible. Our findings concerning PCA were in agreement with those of Dungworth (1965). Positive reactions were elicited whether intervals of 4 hr. or 48 hr. between injections of sera and antigen were employed. In 2 cases reactions were obtained after 48 hr. but not after 4 hr. It is doubtful that a meaningful comparison can be made to the

findings of Stechschulte et al. in 1967 in rats. However, these workers distinguished 2 different antibodies on the basis of optimal intervals for induction of PCA. That for which 24 to 72 hr. was optimal was a heat labile, homocytotropic antibody responsible for release of histamine and 5-hydroxytryptamine from mast cells.

Investigation of the mediatiors of PCA in the calves was not undertaken but intravenous injection of meclofenamate 5 min. before the antigen did not inhibit the development of the reactions. Eyre (1970a) found PCA reactions in sheep to involve 5-hydroxytryptamine, kinins and/or SRS-A to a greater extent than histamine. These reactions in sheep were antagonised by methysergide and by meclofenamate while mepyramine had little effect.

The failure of serum from animals sensitised without adjuvant to induce PCA may be explained on the same basis as the other differences between animals sensitised with and

without adjuvant. It may simply be quantitative, in that only animals sensitised with adjuvant have sufficient heat labile antibodies in their sera to induce sensitisation or, alternatively, the antibodies may be qualitatively different. The destruction of skin sensitising ability by heating eliminated the possibility that heat stable precipitating antibodies were responsible. These were demonstrated in the sera of animals sensitised using adjuvant but not in those sensitised without adjuvant (Davies, 1968, personal communication). Our findings in calves were confirmed using the same sera in goats (Beadle, 1970, personal communication).

ACTIVE

Active cutaneous reactions in animals sensitised without adjuvant were specific for the antigens to which the animals had been sensitised. Non-specific reactions were encountered to undiluted horse serum but these may be attributed to the

amount of 5-hydroxytryptamine in such serum. Just as animals sensitised using Freund's adjuvant reacted non-specifically to intravenous injections of horse albumin and horse serum, they also showed non-specific skin reactions. Thus, depending on the method of sensitisation skin testing can be a useful and specific method of testing for sensitivity to certain antigens.

The large dose of mepyramine necessary to inhibit skin reactions and the finding that meclofenamate also appeared to have some inhibitary effect on immediate cutaneous reactions suggested that mediation did not depend entirely on histamine release.

SUMMARY AND CONCLUSIONS

1. Anaphylaxis may be regularly reproduced in cattle by intravenous injection of foreign protein 7 days or more after sensitisation by intravenous injection of the same protein.

2. The outstanding feature of anaphylaxis in cattle is respiratory dysfunction associated with severe pulmonary oedema, systemic hypotension, pulmonary hypertension and leucopenia.

3. The severity of the anaphylactic response is directly related to the challenge dose of antigen.

4. Hypersensitivity may be detected by intracutaneous injection of antigen resulting in localised swelling.

5. The severity of the symptoms of anaphylaxis may be reduced by pretreatment with a selective antagonist of bradykinin and SRS-A but not by pretreatment

with antihistamines, 5-hydroxytryptamine antagonists or parasympatholytic agents.

6. Plasma free histamine levels were raised after anaphylaxis but no significant amount of histamine was found to be released from tissues, with the exception of skin. In vitro, perfusion of sensitised lungs with antigen resulted in irregular release of histamine and another spasmogenic substance.

7. Sensitisation by intravenous injection of antigen may be replaced by 8 weekly intramuscular injections of antigen in Freund's adjuvant. Animals sensitised by the latter method show similar physiological, pathological and haematological changes during anaphylaxis but differ from those sensitised intravenously in several respects:

   a) They require 50 times smaller challenge doses of antigen to produce minimal or lethal effects.

b) They react non-specifically both systemically and cutaneously.

c) They show delayed cutaneous hypersensitivity reactions.

d) Their sera passively sensitise the skin of other calves for cutaneous anaphylaxis.

e) Smooth muscle from the lungs of such animals contracts in vitro in response to antigen.

In conclusion, a method has been described by which anaphylaxis may be produced in cattle for experimental investigation. This method does not involve the use of an adjuvant and the reactions produced in this way show more evidence of being mediated by bradykinin and/or SRS-A than by histamine or 5-hydroxytryptamine. The lung, is the organ primarily affected following intravenous challenge and death results from asphyxia. Examination of the effects

of histamine, 5-hydroxytryptamine and bradykinin on intact cattle and on isolated bovine lung, isolated bronchioles, trachealis muscle, pulmonary artery and vein revealed that none could be eliminated on the grounds of having effects incompatible with those seen during anaphylaxis. The failure of antagonists of 5-hydroxytryptamine to prevent apnoea produced by this agent and the difficulty of carrying out in vitro incubation experiments for 5-hydroxytryptamine estimation due to its rapid removal by lung tissue, reduced the grounds for elimination of 5-hydroxytryptamine. However, the minimal amount of pulmonary oedema produced by this substance suggested that it alone was unlikely to be involved.

The reasons for the differences between findings in animals sensitised with and without adjuvant are important. If the reason is simply a quantitative difference in the antibody involved, resulting in release or formation of mediators in such quantities that all antagonists fail to be effective,

this could be desirable in allowing in vitro reactions to be examined. This explanation was supported by the finding that reduction of the challenge dose in order to produce a mild reaction, allowed a protective effect of meclofenamate to become apparent. Only animals sensitised with Freund's adjuvant were found to have high circulating levels of precipitins.

That the reaction following sensitisation with Freund's adjuvant involves different antibodies and different mediators is a possibility. This would have an important bearing on the application of results, obtained in experimentally induced reactions, to natural diseases or reactions to drugs and vaccines.

Respiratory distress with pulmonary oedema may result from a variety of initiating mechanisms. Clearly, anaphylaxis is a reaction capable of producing such a syndrome. From our findings fog fever may, in some situations at least, result

from anaphylaxis, Other syndromes, namely reactions to vaccines, drugs and parasites may arise in a similar way. Such may possibly be identified by direct skin testing. Production of PCA and responses of tissues to antigen in vitro may be absent. Antihistamines and anti-5-hydroxy-tryptamine agents are less likely to be effective than are antagonists of bradykinin and SRS-A. Other as yet unidentified mediators may also play a part in anaphylaxis in cattle.

REFERENCES

ADAM, H.M., HARDWICK, D.C. and SPENCER, K.E.V. (1957) Br.J.Pharmac., 12, 397.

ADAMS, S.S. (1953) J.Pharm.Pharmac., 5, 580.

AIRILIA, Von.Y. (1914) Skand.Arch.Physiol., 31, 388.

AITKEN, I.D. and McCUSKER, H.B. (1969) Vet.Rec., 84, 58.

AKCASU, A. and WEST, G.B. (1960) Int.Archs.Allergy appl. Immun., 16, 326.

AKCASU, A. (1963) Int.Archs.Allergy appl.Immun., 22, 85.

ÅKERBLOM, E. (1934) "Über die Ätiologie und Pathogenese der Futterche bein Pferde." Diss.Stockholm. (Cited by Nilsson, 1963).

ÅKERBLOM, E. (1939) V.Nord.Vet.Congr.Copenhagen, Sect 4, 50. (Cited by Nilsson, 1963).

ÅKERBLOM, E. (1952) Medlemsbl.Sver.Vet Förb., 4, 278.

ALABASTER, V.A. and BAKHLE, Y.S. (1970) Br.J.Pharmac., 38, 440P.

ALBERTY, J. (1959) Int.Archs.Allergy appl.Immun., 14, 162.

ALEGREN, A. and CARLSTROM, B. (1940) Skand.Vet Tidskr., 30, 10.

ALEXANDER, F., EYRE, P., HEAD, K.W. and SANFORD, J. (1967) J.comp.Path., 77, 1.

ALEXANDER, F., EYRE, P., HEAD, K.W. and SANFORD, J. (1970) J.comp.Path., 80, 19.

ALTOUNYAN, R.E.C. (1967) Acta.Allerg., 22, 487.

AMMAN, K. (1949) Schweiz.Arch. Tierheilk., 91, 695. (Cited by Nilsson, 1963).

ANDBERG, W.G., BOYD, W.L. and CODE, C.F. (1941) J.Am.vet. med.Ass., 98, 285.

ANDERSON, D.M., GOADBY, P. and SMITH, W.G. (1963) Int.Archs. Allergy appl.Immun., 22, 131.

ANDERSON, P.H. and KIRKWOOD, A.C. (1968) Br.vet.J., 124, 569.

ANKIER, S.I. and STARR, M.S. (1967) Br.J.Pharmac., 31, 331.

ANNISON, E.F., LEWIS, D. and LINDSAY, D.B. (1959) J.agric. Sci., Camb. 53, 34.

ARMITAGE, P., HERXHEIMER, H. and ROSA, L. (1952) Br.J.Pharmac., 7, 625.

ARMSTRONG, D., JEPSON, J.B., KEELE, C.A. and STEWART, J.W. (1957) J.Physiol., Lond., 135, 350.

ARTHUS, M. (1903) C.r.Seanc.Soc.Biol., 55, 817.

ARTHUS, M. (1909) Archs.int.Physiol., 7, 471.

AUER, J. and LEWIS, P.A. (1910) J.exp.Med., 12, 151.

AUER, J. (1910) J.exp.Med., 12, 638.

AUGUSTINUS, R. (1945) "L'anaphylaxie et l'histamine en pathologie vétérinaire." Diss.Lyon.

AUSTEN, K.F. and HUMPHREY, J.H. (1963) Adv.Immunol., 3, 10.

BANCROFT, H. (1965) in "Introduction to Biostatistics," Hoeber Medical Division, Harper and Row, New York, Evanston and London, 172.

BAER, G.M., GOODRICH, W.O. and DEAN, J.D. (1962) J.Am.vet. med.Ass., 141, 1048.

BANISTER, J., FEGLER, G. and HEBB, C. (1949) Q.J.exp.Physiol., 35, 233.

BARTOSCH, R., FELDBERG, W. and NAGEL, E. (1932) Pflug.Arch. ges.Physiol., 230, 129.

BARTOSCH, R. (1935) Klin.Wschr., 1, 307.

BECKER, E.L. and AUSTEN, K.F. (1966) J.exp.Med., 124, 379.

BEESLEY, W.M. (1965) Parasitology, 55, 9P.

BELLANTI, J.A. (1968) Am.J.Dis.Child., 115, 239.

BENACERRAF, B. and KABAT, E.D. (1949) J.Immun., 62, 517.

BENDITT, E.P., WONG, R.L., ARASE, M. and ROEPER, E. (1955) Proc.Soc.exp.Biol.Med., 90, 303.

BERALDO, W.T. (1950) Am.J.Physiol., 161, 283.

BERRY, P.A. and COLLIER, H.O.J. (1964) Br.J.Pharmac., 23, 201.

BIEDL, A. and KRAUS, R. (1910) Wien.klin.Wschr., 22, 363.

BIER, O.G., PASSOS, H.C. and SIQUEIRA, M. (1968) Immunology, 14,291

BLOOD, D.C. and HENDERSON, J.A. (1963) in "Veterinary Medicine," 2nd Edition Baillière, Tindall and Cox, London, 218.

BOREUS, L.O. and CHAKRAVARTY, N. (1960) Acta Physiol.scand., 48,315

BOREUS, L.O. (1960) Acta physiol.scand., 48, 431.

BOREUS, L.O. (1961a) Acta physiol.scand., 52, 309.

BOREUS, L.O. (1961b) Acta physiol.scand., 52, 320.

BOSEILA, A.W.A. (1958) Proc.Soc.exp.Biol.Med., 98, 184.

BOUHUYS, A., JONSSON, R., LIGHTNECKERT, S., LINDELL, S.E., LUNDGREN, C., LUNDIN, G. and RINGQUIST, T.R. (1960) Clin.Sci., 19, 79.

BOURA, A., MONGAR, J.L. and SCHILD, H.O. (1954) Br.J.Pharmac., 9,24

BOYDEN, S.V. (1951) J.exp.Med., 93, 107.

BRISBANE, W.P. (1963) Can.vet.J., 4, 234.

BROCKLEHURST, W.E. (1953) J.Physiol.,Lond., 120, 16P.

BROCKLEHURST, W.E. (1955) J.Physiol.,Lond., 128, 1P.

BROCKLEHURST, W.E. (1956) Ciba Foundation Symposium on Histamine, Churchill, London, 175.

BROCKLEHURST, W.E. (1958) Proc.Third International Congress of Allergology, Pena, Ed.Halpern.B.N. and Holtzer, A., 361.

BROCKLEHURST, W.E. (1960) J.Physiol., Lond., 151, 416.

BROCKLEHURST, W.E. (1962) Prog.Allergy., 6, 539.

BROCKLEHURST, W.E. and LAHIRI, S.C. (1962) J.Physiol.,Lond.,160,15P

BROCKLEHURST, W.E. and LAHIRI, S.C. (1963) J.Physiol.,Lond.,165,39P

BRODIE, T.G. (1900-01) J.Physiol., Lond., 26, 48.

BRYANS, J.T. (1966) Cornell Vet., 56, 278.

BUXTON, A. and THOMLINSON, J.R. (1961) Res.vet.Sci., 2, 73.

CARLETON, H.M. and DRURY, R.A.B. (1957) Histological Techniques 3rd Ed. University Press: Oxford.

CHAVANCE, J. (1946) Vet.Med., 41, 199.

CHAYEN, J., DARRACOTT, S. and KIRBY, W.W. (1966) Nature, Lond., 209, 887.

CÎRSTEA, M., NICULESCU, V., RUSOVICI, L. and SUHACIU, G.H. (1967) Int.Archs.Allergy appl.Immun., 32, 105.

CLAUSEN, C.R., MUNOZ, J. and BERGMAN, R.K. (1969) J.Immun., 103,768.

CODE, C.F. (1937) J.Physiol., Lond., 89, 257.

CODE, C.F. (1938) Am.J.Physiol., 123, 40.

CODE, C.F. and HESTER, H.R. (1939) Am.J.Physiol., 127, 71.

CODE, C.F. (1939) Am.J.Physiol., 127, 78.

CODE, C.F. (1952) Physiol.Rev., 32, 47.

COHEN, S.G. and SAPP, T.M. (1960) J.Allergy., 31, 248.

COLLIER, H.O.J., HOLGATE, J.A., SCHACHTER, M. and SHORLEY, P.G. (1959) J.Physiol., Lond., 180, 13P.

COLLIER, H.O.J., HOLGATE, J.A., SCHACHTER, M. and SHORLEY, P.G. (1960) Br.J.Pharmac., 15, 290.

COLLIER, H.O.J. and SHORLEY, P.G. (1960) Br.J.Pharmac., 15, 601.

COLLIER, H.O.J. and SHORLEY, P.G. (1963) Br.J.Pharmac., 20, 345.

COLLIER, H.O.J., JAMES, G.W.L. and PIPER, P.G. (1965) J.Physiol., Lond., 180, 13P.

COLLIER, H.O.J. and JAMES, G.W.L. (1966) J.Physiol., Lond., 185, 71P.

COLLIER, H.O.J., JAMES, G.W.L. and SCHNEIDER, C. (1966) Nature, Lond., 212, 411.

COLLIER, H.O.J. and JAMES, G.W.L. (1967) Br.J.Pharmac., 30, 283.

COLLIER, H.O.J. (1968) The Scientific Basis of Medicine, Annual Reviews, 308.

COLLIER, H.O.J., DINEEN, L.C., PERKINS, A.C. and PIPER, P.J. (1968) "Naunyn-Schmiedebergs Arch.Pharmak.exp.Path.", Bd. 259, Heft 2. S159.

COOMBS, R.R.A. and GELL, P.G.H. (1963) in "Clinical Aspects of Immunology," Ed.Gell, P.G.H. and Coombs, R.R.A., Blackwell Scientific Publications, Oxford, 137.

COPP, F.C., GREEN, A.F., HODSON, H.F., RANDALL, A.W. and SIM, M.R. (1967) Nature, Lond., 214, 200.

COULSON, E.J. and STEVENS, H. (1949) J.Immun., 61, 1.

COX, J.S.G. (1967) Nature, Lond., 216, 1328.

CRIGHTON, G.W. (1965) PhD. Thesis, University of Glasgow.

CSABA, B. and WEST, G.B. (1968) Int.Archs.Allergy appl. Immun., 33, 99.

DACIE, J.V. and LEWIS, S.M. (1963) in "Practical Haematology," 3rd Edition, Churchill, London, 2.

DALE, H.H. and LAIDLAW, P.P. (1911) J.Physiol., Lond., 44, 318.

DALE, H.H. (1913) J.Pharmac.exp.Ther., 4, 516.

DALE, H.H. (1914) J.Pharmac.exp.Ther., 6, 147.

DALE, H.H. (1920) Proc.R.Soc.Edinb.B., 91, 126.

DALE, H.H. and KELLAWAY, C.H. (1922) Phil.Trans.R.Soc.B., 21, 273.

DALE, H.H. (1929) Lancet, 216, 1232 and 1285.

DALE, H.H. (1948) Br.med.J., 2, 281.

DAWES, G.S. and COMROE, J.H.Jr. (1954) Physiol.Rev., 34, 167.

DAWSON, W. and WEST, G.B. (1965) J.Pharm.Pharmac., 17, 246.

DAWSON, W., STARR, M.S. and WEST, G.B. (1966) Br.J.Pharmac., 27, 249.

DEAN, H.R. and WEBB, R.A. (1924) J.Path.Bact., 27, 65.

DE KOCK, M.A., NADELL, J.A., ZWI, S., COLEBATCH, H.S.H. and OLSEN, C.R. (1966) J.appl.Physiol., 21, 185.

DE LATONA, L.M.L., DE LaMata, R.C. and AVIADO, D.M. (1961) J.Pharmac.exp.Ther., 133, 195.

DE QUIROZ, R., SUTMÖLLER, P. and BARROETA, M.E. (1964) Am.J.vet.Res., 25, 1627.

DESLIENS, L. (1958) Presse méd., 66, 562.

DHAR, H.L., SANYAL, R.K. and WEST, G.B. (1967) Br.J.Pharmac., 31, 351.

DICKINSON, E.O., SPENCER, G.R. and GORHAM, J.R. (1967) Vet.Rec., 80, 487.

DIENES, L. (1928) J.Immun., 15, 153.

DIXON, F.J. and WARREN, S. (1950) Am.J.med.Sci., 219, 414.

DOERR, R. and RUSS, V.K. (1909) Z. immun.Forsch., 3, 181, 706.

DOUGHERTY, R.W., STEWART, W.E., NOLD, M.M., LINDAHL, M.S., MULLENAX, C.H. and LEEK, B.F. (1962) Am.J.vet.Res., 23, 205.

DOZZA, G. and RAMPICHINI, L. (1959) Atti.Soc.ital.Sci.vet., 13, 412.

DRAGSTEDT, C.A. and GEBAUER-FUELNEGG, E. (1932) Am.J.Physiol., 101, 512.

DRAGSTEDT, C.A. and MEAD, F.B. (1936) J.Pharmac.exp.Ther., 57, 419.

DRAGSTEDT, C.A., ARELLANO, MAX RAMIREZ DE. and LAWTON, A.H. (1940) Science, N.Y., 91, 617.

DRINKER, C.K. and BROWFENBRENNER, J. (1924) J.Immun., 9, 387.

DUNÉR, H. and PERNOW, B. (1958) Scand.J.clin.Lab.Invest., 10, 233.

DUNGWORTH, D.L. (1965) Proc.Symp.Acute Bovine Pulmonary Emphysema, University of Wyoming, Larami, Wyoming, N.l.

EBERLEIN, R. (1908) Die Hufkrankheiten des Pferdes Handbuck der tierärzte chirugie und Geburtshilfe, Frohner und Bayer, Tiel. 4.

EDITORIAL (1968) J.Am.med.Ass., 203, 592.

EDMAN, K.A.P., MONGAR, J.L. and SCHILD, H.O. (1964) J.Physiol., Lond., 170, 124.

EINBINDER, J.M., NELSON, C.T. and FOX, C.L.Jr. (1954) Am.J. Physiol., 179, 347.

EISEN, H.M., ORRIS, L. and BELMAN, S. (1952) J.exp.Med., 95, 473.

EISEN, H.N. (1959) in "Cellular and Humoral Aspects of Hypersensitive States," Ed. Lawrence, H.S., Cassell, London, p.90

EMMELIN, N., KAHLSON, G.S. and LINDSTROM, K. (1941) Acta physiol. scand., 2, 78.

ENGELHARDT, G. (1960) Arch.exp.Path.Pharmak., 238, 99.

ERDOS, E.G. and SLOAN, E.M. (1962) Biochem. Pharmac., 11, 585.

ERSKINE, R.G., SOJKA, W.J. and LLOYD, M.K. (1957) Vet.Rec., 69,301.

EYRE, P. (1969) Br.J.Pharmac., 36, 408.

EYRE, P. (1970a) J.Pharm.Pharmac., 22, 104.

EYRE, P. (1970b) Br.J.Pharmac., 40, 166P.

FELDBERG, W. and KELLAWAY, C.H. (1937a) Aust.J.exp.Biol.med.Sci., 15, 81.

FELDBERG, W. and KELLAWAY, C.H. (1937b) J.Physiol., Lond., 90, 257.

FELDBERG, W. and SCHACHTER, M. (1952) J.Physiol., Lond., 118, 124.

FELDBERG, W. and HARRIS, G.E. (1953) J.Physiol., Lond., 120, 352.

FELDBERG, W. (1956) Ciba Foundation Symposium on Histamine Churchill, London, 4.

FERREIRA, S.H. and VANE, J.R. (1967) Br.J.Pharmac., 29, 367.

FINK, M.A. and QUINN, V.A. (1953) J.Immun., 70, 61.

FINK, M.A. and ROTHLAUE, M.V. (1955) Proc.Soc.exp.Biol.Med., 90, 477.

FINK, M.A. (1956) Proc.Soc.exp.Biol.Med., 92, 673.

FISCHER, P. and LECOMTE, J. (1956) C.r.Seanc.Soc.Biol., 150, 1649.

FISHER, E.W. (1962) Br.vet.J., 118, 513.

FOX, C.L.Jr., NELSON, C.T. and FREEMA, E.B. (1951) Fedn.Proc. Fedn.am.Socs.exp.Biol., 10, 408.

FOX, C.L.Jr., EINBINDER, J.M. and NELSON, C.T. (1958) Am.J. Physiol., 192, 241.

FRANKLAND, A.W. and AUGUSTIN, R. (1954) Lancet, (i), 1055.

FREUND, J. and McDERMOTT, K. (1942) Proc.Soc.exp.Biol.Med., 49,548.

FREUND, J., THOMSON, K.J., HOUGH, H.B., SOMMER, H.E. and PISANI, T.M. (1948) J.Immun., 60, 383.

FREY, J.R. and WENK, P. (1956) Dermatologica, 112, 265.

FULTON, J.D., HARRIS, W.E. and CRAFT, C.E. (1957) Proc.Soc. exp.Biol.Med., 95, 625.

GARAN, R.S. (1938) Naunyn-Schmiedebergs Archs.exp.Path. Pharmak., 188, 250.

GARNER, R.J. (1961) in "Veterinary Toxicology," 2nd Edition Bailliere, Tindall and Cox, London. 259.

GEBAUER, O. (1965) Vet.- med.Nachr., 1, 39.

GEIGER, W.B. and ALPERS, H.S. (1959) J.Allergy, 30, 316.

GERLACH, F. (1922) Z.Immun.Forsch.exp.Ther., 34, 75.

GERSHON, M.D. and ROSS, L.L. (1962) J.exp.Med., 115, 367.

GIBBONS, W.J. (1962) Mod.vet.Pract., 43, 34.

GIERTZ, H., HAHN, F., HAHN, H. and SCHMUTZER, W. (1962) Klin.Wschr., 40, 598.

GILDING, H.P. and NUTT, M.E. (1943-44) J.Physiol.,Lond., 102, 446.

GLADSTONE, G.P. (1964) in "General Pathology," 3rd Edition, Ed. H. Florey, Lloyd-Luke (Medical Books) Ltd., London. p.932.

GRAHAM, R. (1940) J.Am.vet.med.Ass., 97, 38.

GRAHAM, H.T., LENZ, M.A., LOWRY, O.H., PARISH, H.H. and WHEELWRIGHT, F. (1955) Blood, 10, 467.

GREEF, K., SCHARNAGEL, K., LÜHR, R and STROBACH, H. (1966) Naunyn-Schmiedebergs Arch.exp.Path.Pharmak., 253, 235.

GREATOREX, J.C. (1969) Equine vet.J., 1, 157.

GREGORY, D.W. (1955) Vet.Med., 50, 609.

GROVE, E.F. (1932) J.Immun., 23, 101.

HADWEN, S. and BRUCE, E.A. (1917) J.Am.vet.med.Ass., 51, 15.

HALMAGYI, D.F.J., STARZECKI, B. and HORNER, G.J. (1963) J.appl.Physiol., 18, 544.

HALPERN, B.N. and BRIOT, M. (1953) C.r.Seanc.Soc.Biol., 147, 643.

HALPERN, B.N. (1956) Ciba Foundation Symposium on Histamine, Churchill, London, 92.

HARRIS, W.E. and FULTON, J.D. (1958) Proc.Soc.exp.Biol.Med., 97, 14.

HEATON, R.W. (1962) M.Sc.thesis, University of Vermont, U.S.A.

HERXHEIMER, H. (1955) J.Physiol., Lond., 128, 435.

HICKS, R. and OKPAKO, D.T. (1968) Int.Archs.Allergy appl. Immun., 33, 131.

HILL, K.J. and MANGAN, J.L. (1964) Biochem.J., 93, 39.

HOCHWALD, A. and RACKEMANN, F.M. (1946) J.Immun., 53, 191.

HOMBERGER, A.C. (1968) Helv.physiol.pharmac.Acta., 26, 97.

HORTON, E.W. (1959) Br.J.Pharmac., 14, 125.

HOWELL, J.B.L. and ALTOUNYAN, R.E.C. (1967) Lancet ii, 539.

HULL, M.W. (1965) Proc.Symp.Acute Bovine Pulmonary Emphysema, Laramie, Wyoming, Hl.

HUMPHREY, J.H. and JAQUES, R. (1954) J.Physiol., Lond., 124, 305.

HUMPHREY, J.H. and JAQUES, R. (1955) J.Physiol., Lond., 128, 9.

ISHIZAKA, K., ISHIZAKA, T. and HORNBROOK, M.M. (1966a) J.Immun., 97, 75.

ISHIZAKA, K., ISHIZAKA, T. and HORNBROOK, M.M. (1966b) J.Immun., 97, 840.

JACKSON, R.S. (1969) J.Am.vet.med.Ass., 155, 262.

JAIN, M.C., LASMANIS, J. and SCHALM, O.W. (1967) Am.J.vet.Res., 28, 1243.

JAQUES, L.B. and WATERS, E.T. (1941) J.Physiol., Lond., 99, 454.

JARRETT, W.F.H., JENNINGS, F.W., McINTYRE, W.I.M., MULLIGAN, W., SHARP, N.C.C. and URQUHART, G.M. (1960) Vet.Rec., 72, 1066.

JENKINS, P.A. and PEPYS, J. (1965) Vet.Rec., 77, 464.

JENNINGS, F.W. and MULLIGAN, W. (1953) J.comp.Path., 63, 286.

JONASSON, O. and BECKER, E.L. (1966) J.exp.Med., 123, 509.

JORPES, J.E. (1946) in "Heparin in the Treatment of Thrombosis," Oxford University Press, London.

JUBB, K.V.F. and KENNEDY, D.C. (1963) in "Pathology of Domestic Animals," Vol.1, Academic Press, New York and London, 208.

KABAT, E.A. and LANDOW, H. (1942) J.Immun., 44, 69.

KABAT, E.A. and MAYER, M.M. (1961) in "Experimental Immunochemistry" 2nd Edition, Charles C. Thomas Co., Springfield, Ill., 67.

KALLOS, P. and KALLOS-DEFFNER, L. (1957) Int.Archs.Allergy appl.Immun., 11, 327.

KARCZEWSKI, W. and WIDDICOMBE, J.G. (1969a) J.Physiol., Lond., 201, 271.

KARCZEWSKI, W. and WIDDICOMBE, J.G. (1969b) J.Physiol., Lond., 201, 293.

KATZ, G. (1940) Science, N.Y., 91, 221.

KELLAWAY, C.H. (1930) Br.J.exp.Path., 11, 72.

KELLAWAY, C.H. and TRETHEWIE, E.R. (1940) Q.Jl.exp.Physiol., 30,121.

KERR, W.R. and ROBERTSON, M. (1954) J.Hyg., Camb., 52, 253.

KERR, W.R. (1956) Vet.Rec., 68, 476.

KINCH, D.A. and OMAR, A.R. (1966) Vet.Rec., 78, 766.

KIND, L.S. (1955) J.Immun., 74, 387.

KIND, L.S. (1957) Proc.Soc.exp.Biol.Med., 95, 200.

KITAMURA, M. (1965) Jap.J.Pharmac., 15, 135.

KLAUS, G. (1965) Vet.Rec., 77, 1235.

KOGA, T., ISHIBASHI, T., SUGIYAMA, K. and TANAKA, A. (1969) Int.Archs.Allergy appl.Immun., 36, 233.

KOLLER, E.A. (1967a) Helv.physiol.pharmac.Acta., 25, 287.

KOLLER, E.A. (1967b) Helv.physiol.pharmac.Acta., 25, 353.

KOLLER, E.A. (1968) Helv.physiol.pharmac.Acta., 26, 153.

KONZETT, H. and RÖSSLER, R. (1940) Naunyn-Schmeidebergs. Arch.exp.Path.Pharmak., 195, 71.

KRAL, F. and SCHWARTZMAN, R.M. (1964) in "Veterinary and Comparative Dermatology," J.B. Lippincott Co., Philadelphia, Pena.

KREBS, H.A. and HENSELEIT, K. (1932) Hoppe-Seyler's Z. physiol.Chem., 210, 33.

KUHNS, W.J. and PAPPENHEIMER, A.M.Jr. (1952) J.expMed., 95, 375.

KUIDA, H., HINSHAW, L.B., GILBERT, R.P. and VISSCHER, M.B. (1958) Am.J.Physiol., 192, 335.

KUIDA, H., GILBERT, R.P., HINSHAW, L.B., BRUNSON, J.G. and VISSCHER, M.B. (1961) Am.J.Physiol., 200, 1197.

KUIDA, H., BROWN, A.M., THORNE, J.L., LANGE, R.L. and HECHT, H.H. (1962) Am.J.Physiol., 203, 391.

LANDSTEINER, K. (1945) in "The Specificity of Serological Reactions," Harvard University Press, Cambridge, Mass.

LANNEK, N., NORDSTROM, G. and ASHEIM, Å. (1959) Nord.vet.Med., 11, 11.

LARSSON, G. (1936) Svensk.Vet.Tidskr., 41, 408.

LEADING ARTICLE. (1968) Lancet,(ii), 160.

LECOMTE, J. and FISCHER, P. (1957) C.r.Seanc.Soc.Biol., 151, 1279.

LECOMTE, J. and FISCHER, P. (1958) Archs.int.Physiol.Biochim., 66, 50.

LEEMANN, W., DE WECK, A.L. and SCHNEIDER, C.H. (1969) Nature, Lond., 223, 621.

LEWIS, P.A. and LOOMIS, D. (1924) J.exp.Med., 40, 503.

LEWIS, G.P. (1962) The Scientific Basis of Medicine, Annual Reviews, 14, 242.

LIPTON, M.M., STONE, S.H. and FREUND, J. (1956) J.Immun., 77, 453.

LOGAN, G.B. (1967) J.Allergy, 40, 207.

LONGCOPE, W.T. (1922) J.exp.Med., 36, 627.

LOWRY, T. and SCHUMAN, L.M. (1956) J.Am.med.Ass., 162, 153.

McCUSKER, H.B. (1965) in "Comparative Physiology and Pathology of the Skin," Ed. A.J. Rook and G.S. Walton, Blackwell Scientific Publications, Oxford, 427.

McCUSKER, H.B. and AITKEN, I.D. (1966) J.Path.Bact., 91, 282.

McCUSKER, H.B. and AITKEN, I.D. (1967) Res.vet.Sci., 8, 265.

MACDOUGALL, R.S. (1930) Trans.R.Highld.agric.Soc.Scotl., 42, 75.

MACKENZIE, A. (1965) Proc.Symp.Acute Bovine Pulmonary Emphysema, University of Wyoming, Laramie, Wyoming, El.

MACKENZIE, A. (1966) Proc.R.Soc.Med., 59, 1008.

MACLEAN, C.N. (1965) Vet.Rec., 77, 662.

MACLEOD, N.S.M. (1966) Vet.Rec., 78, 856.

MAKI, L.R. and TUCKER, J.O. (1965) Proc.Symp.Acute Bovine Pulmonary Emphysema, University of Wyoming, Laramie, Wyoming, Al.

MALKIEL, S. and HARGIS, B.J. (1952a) Proc.Soc.exp.Biol.Med., 80,122.

MALKIEL, S. and HARGIS, B.J. (1952b) Proc.Soc.exp.Biol.Med., 81,689.

MANWARING, W.H. (1910) Z.immun.Forsch., 8, 1.

MANWARING, W.H., HOSEPIAN, V.M., O'NEILL, F. and MOY, H.B. (1925) J.Immun., 10, 575.

MARQUIS, V.O. (1966) "The Role of 'SRS-A' and Other Active Substances in Allergic and Inflammatory Conditions," Ph.D. thesis, Edinburgh University.

MARTIN, J.F. and CROIZAT, P. (1927) C.r.Seanc.Soc.Biol., 97, 95.

MAWSON, C. and WHITTINGTON, H. (1970) Br.J.Pharmacol., 39, 223P.

MICHEL, J.F. (1954) Vet.Rec., 66, 381.

MICHEL, J.F. and MACKENZIE, A. (1965) Res.vet.Sci., 6, 344.

MILLER, W.S. (1921) Am.Rev.Tuberc.pulm.Dis., 5, 689.

MILLER, R.M. (1960) Mod.vet.Pract., 41, 58.

MILLS, J., SELLICK, H. and WIDDICOMBE, J.G. (1969) J.Physiol., Lond., 203, 337.

MILLS, J.E. and WIDDICOMBE, J.G. (1970) Br.J.Pharmac., 39, 724.

MINARD, D. (1937) Am.J.Physiol., 119, 375.

MINTSCHEW, P. (1938) Tierärztl.Rundschau, 44, 748.

MOLOMUT, N. (1939) J.Immun., 37, 113.

MONGAR, J.L. and SCHILD, H.O. (1953) Br.J.Pharmac., 8, 103.

MONGAR, J.L. and SCHILD, H.O. (1956) J.Physiol., Lond., 131, 207.

MONGAR, J.L. and SCHILD, H.O. (1957) J.Physiol., Lond., 135, 301.

MONGAR, J.L. and SCHILD, H.O. (1962) Physiol.Rev., 42, 226.

MONLUX, W., FITTE, J., KENDRICK, G. and DUBUISSON, H. (1953) S.West Vet., 6, 267.

MORRISON, J.L., BLOOM, W.L. and RICHARDSON, A.P. (1951) J.Pharmac.exp.Ther., 101, 27.

MORSE, H.C., BLOCH, K.J. and AUSTEN, K.F. (1968) J.Immun., 101, 658.

MORSE, H.C., AUSTEN, K.F. and BLOCH, K.J. (1969) J.Immun., 102, 327.

MOTA, I. and VUGMAN, I. (1956) Nature, Lond., 177, 429.

MOTA, I. (1957) Br.J.Pharmac., 12, 453.

MOTA, I. (1958) Nature, Lond., 182, 1021.

MOTA, I. (1962) Immunology, 5, 11.

MOTA, I. (1963) Ann.N.Y.Acad.Sci., 103, 264.

MOTA, I. (1964) Immunology, 7, 681.

MOTA, I. and PEIXOTO, J.M. (1966) Life Sci., 5, 1723.

MOULTON, J.E., CORNELIUS, C.E. and OSBURN, B.I. (1963) J.Am.vet.med.Ass., 142, 133.

NELSON, C.T., FOX., C.L.Jr. and FREEMAN, E.B. (1952) J.invest.Dermat., 18, 113.

NILSSON, S.A. (1963) Acta.vet.scand., 4, Suppl.1.

OBEL, N.J. and SCHMITERLÖW, C.G. (1948) Acta.pharmac.tox., 4, 71.

OFFERMEIER, J. and ARIËNS, E.J. (1966) Archs.int.Pharmacodyn. Ther., 164, 192.

OJERS, G., HOLMES, A.C. and DRAGSTEDT, A.C. (1941) J.Pharmac.exp.Ther., 73, 33.

OPIE, E.L. (1924) J.Immun., 9, 231.

ORANGE, R.P., VALENTINE, M.D. and AUSTEN, K.F. (1967) Science, N.Y., 157, 318.

ORANGE, R.P., VALENTINE, M.D. and AUSTEN, K.F. (1968a) J.exp.Med., 127, 767.

ORANGE, R.P., VALENTINE, M.D. and AUSTEN, K.F. (1968b) Proc.Soc.exp.Biol.Med., 127, 127.

ORANGE, R.P. and AUSTEN, K.F. (1968) Proc.Soc.exp.Biol.Med., 129, 836.

OSBORNE, J.C. and SMIBERT, R.M. (1962) Nature, Lond., 195, 1106.

OSBORNE, J.C. and SMIBERT, R.M. (1964) Cornell Vet., 54, 561.

OSBORNE, J.C. (1967a) Cornell Vet., 57, 204.

OSBORNE, J.C. (1967b) Cornell Vet., 57, 227.

OTTO, R. (1906) Leuthold Gedenkschrift, 1, 153.

OTTO, R. (1907) Münch.med.Wschr., 55, 1665.

OUCHTERLONY, Ö. (1964) in "Immunological Methods, A Symposium." Ed. Ackroyd, J.F. Blackwell Scientific Publications, Oxford.

OVARY, Z. (1952) Int.Archs.Allergy appl.Immun., 3, 393.

OVARY, Z. (1958) Prog.Allergy, 5, 459.

PARISH, W.E., HALL, L.W. and COOMBS, R.R.A. (1963) Immunology, 6, 462.

PARKER, J.T. and PARKER, F. (1924) J.med.Res., 44, 263.

PARRATT, J.R. and WEST, G.B. (1957) J.Physiol., Lond., 139, 27.

PASCOE, R.R. and McGAVIN, M.D. (1969) Vet.Rec., 85, 376.

PASTEUR, VALLERY-RADOT, P. (1949) Précis des Maladies Allergiques, Paris: Flammarion et Cie, 18.

PATON, W.D.M. (1956) Ciba Foundation Symposium on Histamine, Churchill, London.

PATTERSON, R. (1959) J.Am.vet.med.Ass., 135, 178.

PECKENPAUGH, C.I. (1961) Mod.vet.Pract., 42, 61.

PEPYS, J., RIDDELL, R.W., CITRON, K.M., CLAYTON, Y.M. and SHORT, E.I. (1959) Am.Rev.resp.Dis., 80, 167.

PEPYS, J. (1966) Postgrad.med.J., 42, 698.

PIERCE, A.E. (1955) J.Hyg.Camb., 53, 261.

PIERCY, D.W. (1970) Res.vet.Sci., 11, 481.

PIERONI, R.E. and LEVINE, L. (1966) Nature, Lond., 211, 1419.

PIERONI, R.E. and LEVINE, L. (1967) Nature, Lond., 213, 1015.

PIPER, P.J., COLLIER, H.O.J. and VANE, J.R. (1967) Nature, Lond., 213, 838.

PIRQUET, C.von. (1906) Münch.med.Wschr., 30, 1457.

PORTIER, R. and RICHET, C. (1902) C.r.Seanc.Soc.Biol., 54, 170.

PRAUSNITZ, C. and KÜSTNER, H. (1921) Zentbl.Bakt. Parasit.Kde., Abt.I, orig., 86, 160.

PROUVOST-DANON, A., QUEIROZ JAVIERRE, M. and SILVA LIMA, M. (1966) Life Sci., 5, 1751.

PROUVOST-DANON, A., SILVA LIMA, M. and QUEIROZ JAVIERRE, M. (1966) Life Sci., 5, 289.

PROUVOST-DANON, A., PEIXOTO, J.M. and QUEIROZ JAVIERRE, M. (1967) Life Sci., 6, 1793.

RAPP, H.J. (1961) J.Physiol., Lond., 158, 35P.

REUSE, J.J. (1950) XVIIIth Int.Physiol.Cong.Abstracts of Communications, p.535.

RIEK, R.F. (1954) Aust.J.agric.Res., 5, 109.

RIEK, R.F. (1955) Aust.J.agric.Res., 6, 161.

RILEY, J.F. and WEST, G.B. (1953) J.Physiol., Lond., 120, 528.

ROBERTS, H.E., WORDEN, A.N. and EVANS, E.T.R. (1954) J.comp.Path., 64, 283.

ROBERTS, H.E. and VALLELY, T.F. (1959) Vet.Rec., 71, 846.

ROBINSON, H.J. (1958) Preclinical evaluation Cyproheptadine, Merck, Sharp and Dohme Research Laboratories.

ROSE, B. and WEIL, P. (1939) Proc.Soc.exp.Biol.Med., 42, 494.

ROSE, B. (1941) J.Allergy, 12, 441.

ROSE, B. (1947) Am.J.Med., 3, 345.

ROSENAU, M.J. and ANDERSON, J.F. (1906) Bull. U.S. Hyg.Lab., 29, 1.

ROSENAU, M.J. and ANDERSON, J.F. (1907) J.infect.Dis., 4, 552.

ROSSI, F. (1963) Gac.vet., 25, 299.

RUPP, J.C., MOORE, R.D. and SCHOENBERG, M.D. (1960) Archs.Path., 70, 43.

SANFORD, J. (1962) "Some Aspects of Histamine in the Sheep Stomach," Ph.D. thesis, Glasgow University.

SANYAL, R.K. and WEST, G.B. (1958a) J.Physiol., Lond., 142, 571.

SANYAL, R.K. and WEST, G.B. (1958b) J.Physiol., Lond., 144, 525.

SANYAL, R.K., SPENCER, P.S.J. and WEST, G.B. (1959) Nature, Lond., 184, 2020.

SANYAL, R.K. (1960) Allergie Asthma, 6, 317.

SCHACHTER, M. (1953) Br.J.Pharmac., 8, 412.

SCHAYER, R.W. (1963) Prog.Allergy, 7, 187.

SCHILD, H.O. (1939) J.Physiol., Lond., 98, 393.

SCHILD, H.O., HAWKINS, D.F., MONGAR, J.L. and HERXHEIMER, H. (1951) Lancet, (ii), 376.

SCHMITERLÖW, C.G. (1949) Acta.physiol.scand., 19, 260.

SCHOENING, H.W. (1940) J.Am.vet.med.Ass., 97, 39.

SCHULTZ, W.H. (1910) J.Pharmac.exp.Ther., 1, 549.

SEATON, V.A. (1958) Am.J.vet.Res., 19, 600.

SELYE, H. (1937) Endocrinology, 21, 169.

SHEARD, P., KILLINGBACK, P.G. and BLAIR, A.M.J.N. (1967) Nature, Lond., 216, 283.

SIQUEIRA, M. and BIER, O.G. (1961) Proc.Soc.exp.Biol.Med., 107, 779.

SMITH, A.N. and INGRAM, D.G. (1965) Can.vet.J., 6, 226.

SMITH, D.J. and COXE, J.W. (1951) Am.J.Physiol., 167, 732.

SMITH, D.J. and ALPERT, S. (1955) Fed.Proc., 14, 140.

SMITH, W.G. (1962) Life Sci., 1, 133.

SMITH, W.G. (1964) in "Allergy and Tissue Metabolism," Heinemann Medical Books Ltd., London.

SOJKA, W.J., ERSKINE, R.G. and LLOYD, M.K. (1957) Vet.Rec., 69, 293.

STAUB, A.M. and BOVET, D. (1937) C.r.Seanc.Soc.Biol., 125, 818.

STECHSCHULTE, D.J., AUSTEN, K.F. and BLOCH, K.J. (1967) J.exp.Med., 125, 127.

STONE, C.A., WENGER, H.C., LUDDEN, C.T., STAVORSKI, J.M. and ROSS, C.A. (1961) J.Pharmac.exp.Ther., 131, 73.

STONE, S.H. (1958a) Fed.Proc., 17, 536.

STONE, S.H. (1958b) Science, N.Y., 128, 1090.

STONE, S.H. (1959) J.Immun., 82, 138.

SUTLIFFE, W.D. (1930) J.Allergy, 1, 156.

SWEATMAN, W.J.F. and COLLIER, H.O.J. (1968) Nature, Lond., 217, 69.

TANAKA, A., TANAKA, K., TSUBONE, T., KURODA, Y. and SUGIYAMA, K. (1965) Int.Archs.Allergy appl.Immun., 28, 340.

THOMLINSON, J.R. and BUXTON, A. (1962) Res.vet.Sci., 3, 186.

THOMLINSON, J.R. and BUXTON, A. (1963) Immunology, 6, 126.

THUM, H. (1912) Z.Tiermed, 16, 428.

TIKOFF, G., KUIDA, H. and CHIGA, M. (1966) Am.J.Physiol., 210, 846.

TRETHEWIE, E.R. (1951) Aust.J.exp.Biol.med.Sci, 29, 443.

TROWELL, O.A. (1959) Expl.Cell Res., 16, 118.

URBASCHEK, B. (1964) Nature, Lond., 202, 883.

URBASCHEK, B. and VERSTEYL, R. (1965) Nature, Lond., 207, 763.

VANE, J.R. (1968) Scientific Basis of Medicine, Annual Reviews, 336

VOEGTLIN, C. and BERNHEIM, B.M. (1911) J.Pharmac.exp.Ther., 2, 507.

WAALKES, T.P., WEISSBACH, H., BOZICEVICH, J. and UDENFRIEND, S. (1957) Proc.Soc.exp.Biol.Med., 95, 479.

WAALKES, T.P. and COBURN, H. (1959a) J.Allergy, 30, 394.

WAALKES, T.P. and COBURN, H. (1959b) Proc.Soc.exp.Biol.Med., 101, 122.

WALTON, G.S. (1966) J.small Anim.Pract., 7, 749.

WALTON, G.S. (1967) Vet.Rec., 81, 709.

WALTON, G.S. (1968) Vet.Rec., 82, 204.

WALTON, G.S., PARISH, W.E. and COOMBS, R.R.A. (1968) Vet.Rec., 83, 35.

WEBB, R.A. (1924) J.Path.Bact., 27, 79.

WEIL, R. (1912) J.med.Res., 27, 497.

WEIL, R. (1914) J.med.Res., 30, 87.

WEIL, R. (1917) J.Immun., 2, 525.

WEISTER, R.S., GOLUB, O.J. and HAMRE, D.M. (1941) J.infect.Dis., 68, 97.

WEISS, S., ROBB, G.P. and BLUMGART, H.L. (1929) Am.Heart.J., 4, 664

WEISSMAN, C., and THOMAS, L. (1962) J.exp.Med., 116, 433.

WELLS, P.W. and EYRE, P. (1970) Vet.Rec., 87, 173.

WEST, G.B. (1956) Abst.20th Int.Physiol.Congr.Brussels, 964.

WEST, G.B. (1962) J.Pharm.Pharmac., 14, 618.

WHITE, R.G. (1968) Proc.R.Soc.Med., 61, 1.

WIDE,L., BENNICH,H. and JOHANSSON, S.G.O. (1967) Lancet, (ii), 1105.

WILSON, C.W.M. (1954) J.Physiol., Lond., 126, 141.

WILSON, G.S. and MILES, A.A. (1964) in ''Topley and Wilson's Principles of Bacteriology and Immunity,''5th Edition, Vol. 2, Arnold, London, 1397.

WINDER,C.V., WAX,J. and WELFORD,M. (1965) J.Pharmac.exp.Ther., 148, 422.

WRAY,C. and THOMLINSON,J.R. (1969) J.Path.Bact., 98, 61.

APPENDICES I to III

APPENDIX I

HISTAMINE CONTENT OF TISSUES

(Results summarised in Table 6, 1)

No.	Histamine µg/gm				
	Liver capsule	Pleura	Lung	Ileum	Abomasum
a) Control calves aged 3 to 6 months					
1	5.45	56.60	34.56	20.97	N.M.
2	24.49	78.41	44.30	20.80	N.M.
3	114.80	70.60	35.58	9.61	N.M.
4	78.55	36.19	25.99	N.M.	N.M.
5	196.40	40.44	13.99	15.45	N.M.
6	N.M.	N.M.	27.26	N.M.	7.81
7	139.47	21.57	41.89	8.26	13.93
8	132.85	43.75	9.20	81.78	15.51
9	184.32	48.65	23.54	16.58	19.56
10	161.53	27.77	16.20	15.90	10.65
11	131.55	58.51	25.82	10.44	N.M.

No.	Histamine µg/gm				
	Liver capsule	Pleura	Lung	Ileum	Abomasum
12	1.14	40.00	32.83	21.22	41.68
Mean	105.9	47.49	27.59	22.1	18.59
S.D.	69.6	17.33	10.86	21.5	12.2
b) Control calves aged 4 days					
1	51.24	18.92	N.M.	3.81	N.M.
2	9.17	N.M.	3.17	2.33	N.M.
3	2.98	5.17	3.21	5.08	N.M.
Mean	31.13	12.04	3.19	3.74	
c) Shocked calves					
1	956.25	121.55	43.0	82.41	N.M.
2	45.65	23.84	4.04	11.86	12.27
3	N.M.	N.M.	9.59	11.26	14.53
Mean	500.95	72.69	18.87	35.17	13.4

No.	Histamine µg/gm					
	Liver	Colon	Skin	Sub-Cut	Omentum	Nasal Mucosa
a) Control calves aged 3 to 6 months						
1	16.36	10.07	4.73	1.56	0.94	N.M.
2	8.57	11.10	10.30	2.10	2.41	N.M.
3	7.59	7.10	6.04	4.30	1.42	N.M.
4	9.86	11.34	3.88	N.M.	3.46	N.M.
5	4.68	9.90	4.61	1.69	1.47	N.M.
6	3.51	N.M.	4.79	N.M.	N.M.	6.52
7	9.31	6.42	7.31	8.39	1.56	N.M.
8	5.19	13.35	9.94	10.57	3.14	N.M.
9	13.19	22.24	9.13	10.97	1.29	N.M.
10	6.89	8.71	18.96	6.43	3.46	N.M.
11	23.15	12.87	17.54	11.78	11.19	N.M.
12	26.87	10.64	5.37	7.76	1.94	N.M.
Mean	11.26	11.24	8.55	6.56	2.93	
S.D.	7.39	4.22	6.23	4.0	2.9	

No.	Histamine µg/gm					
	Liver	Colon	Skin	Sub-cut	Omentum	Nasal Mucosa
b) Control calves aged 4 days						
1	4.12	3.79	4.00	1.19	0.74	N.M.
2	0.26	3.06	1.82	1.03	1.64	N.M.
3	3.20	4.01	4.73	2.09	2.03	N.M.
Mean	2.52	3.60	3.52	1.44	1.47	N.M.
c) Shocked calves						
1	80.49	41.50	52.31	39.13	29.54	N.M.
2	2.85	11.89	5.59	8.15	8.53	4.04
3	13.82	N.M.	N.M.	N.M.	N.M.	N.M.
Mean	32.38	26.69	28.95	23.64	19.03	

Note: Sub-cut = Subcutaneous tissue

N.M. = Not Measured

APPENDIX II

TISSUE INCUBATION

(Results summarised in Fig. 6. 2)

1. SKIN (EAR) (SENSITISED)

Incubation	No.	Histamine			
		Tissue		Supernatant	
		µg/gm	% total	µg/gm	% total
Tyrode	1	2.48	78.98	0.66	21.02
	2	4.40	77.75	1.26	22.25
	3	9.02	73.15	3.31	26.85
Mean		5.30	76.62	1.74	23.37
Ovalbumin (0.1 mg/ml)	1	2.60	82.02	0.57	17.98
	2	4.0	89.48	0.47	10.52
	3	0.81	39.66	1.24	60.34
Mean		2.47	70.38	0.76	29.61
Ovalbumin (1.0 mg/ml)	1	4.79	75.08	1.59	24.92
	2	2.85	83.33	0.57	16.67

Incubation	No.	Histamine Tissue µg/gm	Tissue % total	Supernatant ug/gm	Supernatant % total
	3	3.60	81.67	0.80	18.33
Mean		3.74	80.02	0.99	19.97
Compound 48/80 (1.0 mg/ml)	1	4.98	49.54	5.07	50.45
Octylamine (1.0 mg/ml)	1	1.36	15.44	7.45	84.56
2. SKIN (MUZZLE)		(SENSITISED)			
Tyrode	1	3.80	89.83	0.43	10.17
	2	1.59	78.29	0.44	21.71
Mean		2.69	84.06	0.43	15.94
Ovalbumin (0.1 mg/ml)	1	3.05	43.14	4.02	56.86
	2	4.06	78.68	1.11	21.32
	3	0.91	68.44	4.11	31.56
	4	0.77	64.08	0.43	35.91
Mean		2.19	63.58	2.42	36.41
S.E.±		0.80	7.47	0.96	7.47

Incubation	No.	Histamine Tissue µg/gm	Tissue % total	Supernatant µg/gm	Supernatant % total
Ovalbumin (1.0 mg/ml)	1	1.18	22.06	4.17	77.94
	2	0.82	24.48	2.53	75.52
	3	1.95	52.19	1.78	47.80
	4	3.05	78.95	0.81	21.05
Mean		1.75	44.42	2.32	55.57
S.E.±		0.49	13.38	1.42	13.38
Compound 48/80 (1.0 mg/ml)	1	2.411	33.97	4.68	66.02
Octylamine (1.0 mg/ml)	1	0.83	7.71	9.93	92.29
3. NASAL MUCOSA (SENSITISED)					
Tyrode	1	16.1	83.9	0.23	1.41
Ovalbumin (1.0 mg/ml)	1	7.63	98.41	1.45	1.59
	2	8.99	93.26	0.65	6.74
Mean		8.31	95.83	1.05	4.16

Incubation	No.	Histamine Tissue µg/gm	Tissue % total	Supernatant µg/gm	Supernatant % total
Compound 48/80 (1.0 mg/ml)	1	5.73	45.66	6.82	54.34
	2	12.55	73.10	4.62	26.90
Mean		9.14	59.38	5.72	40.62
4. LUNG (SENSITISED)					
Tyrode	1	44.74	99.48	0.23	0.52
	2	53.44	99.18	0.44	0.82
	3	16.99	96.72	0.57	3.22
	4	28.28	97.87	0.63	2.18
Mean		35.86	98.30	0.47	1.68
S.E.±		26.08	0.63	0.08	0.62
Ovalbumin (0.1 mg/ml)	1	29.38	97.91	0.62	2.09
	2	22.46	96.72	0.86	3.71
Mean		25.92	97.31	0.74	2.90
Ovalbumin (1.0 mg/ml)	1	43.70	99.68	0.14	0.32
	2	41.69	98.23	0.75	1.77

Incubation	No.	Histamine Tissue ug/gm	Tissue % total	Supernatant ug/gm	Supernatant % total
	3	34.67	98.60	0.49	1.40
	4	32.72	97.68	0.77	2.31
Mean		38.19	98.54	0.54	1.45
S.E.±		2.66	0.42	0.14	0.42
Compound 48/80 (1.0 mg/ml)	1	6.87	42.11	9.45	57.87
	2	8.38	28.38	21.16	71.63
Mean		7.63	35.24	15.30	64.75
Octylamine (1.0 mg/ml)	1	10.60	23.50	34.50	76.49
	2	12.49	34.65	38.16	75.34
Mean		11.54	24.07	36.33	75.91
5. LIVER CAPSULE (SENSITISED)					
Tyrode	1	78.25	95.22	3.92	4.78
Ovalbumin (1.0 mg/ml)	1	81.98	90.57	8.53	9.43
Octylamine (1.0 mg/ml)	1	17.25	20.72	66.01	79.28

TISSUE INCUBATION

(Results summarised in Fig. 6. 3)

1. PLEURA (UNSENSITISED)

Incubation	No.	Histamine			
		Tissue		Supernatant	
		µg/gm	% total	µg/gm	% total
Tyrode	1	31.73	91.53	2.94	8.47
	2	18.11	84.89	3.23	15.11
	3	80.25	98.85	0.94	1.15
	4	44.58	90.25	4.82	9.75
	5	39.02	93.64	2.65	6.36
	6	3.96	59.10	2.74	40.90
	7	21.63	96.87	0.70	3.13
	8	17.39	93.44	1.22	6.55
Mean		36.10	88.57	2.40	11.42
S.E.±		8.40	4.48	0.46	4.50
Ovalbumin (0.1 mg/ml)	1	20.75	84.84	3.71	15.16
	2	48.29	98.30	0.84	1.70

Incubation	No.	Histamine			
		Tissue		Supernatant	
		µg/gm	% total	µg/gm	% total
	3	66.49	99.51	0.33	0.49
	4	49.70	97.16	1.45	2.84
	5	33.39	97.12	0.99	2.88
	6	25.58	94.81	1.40	5.19
Mean		40.69	95.29	1.45	4.71
S.E.±		7.06	2.19	0.47	2.20
Ovalbumin (1.0 mg/ml)	1	24.10	86.37	3.80	13.63
	2	16.01	83.91	3.07	16.09
	3	70.40	97.21	2.02	8.79
	4	46.42	94.15	2.88	5.85
	5	14.01	67.26	6.82	32.74
	6	23.53	94.76	1.30	5.24
Mean		32.41	87.27	3.32	13.67
S.E.±		8.96	4.54	0.78	4.3
Ovalbumin (5.0 mg/ml)	1	40.16	94.05	2.54	5.95
	2	37.68	92.65	2.99	7.35

Incubation	No.	Histamine			
		Tissue		Supernatant	
		µg/gm	% total	µg/gm	% total
Mean		38.92	93.35	2.77	6.65
Compound 48/80 (1.0 mg/ml)	1	10.03	28.24	25.48	71.76
	2	11.17	32.10	23.64	67.90
	3	8.30	16.45	42.14	83.65
	4	8.37	15.76	44.75	84.24
	5	5.50	23.63	17.78	76.37
	6	7.18	23.36	23.56	76.64
	7	1.79	4.35	39.35	98.64
	8	10.62	20.17	42.01	79.83
Mean		7.87	20.5	32.34	79.86
S.E.±		1.09	3.0	11.24	3.3
2. PLEURA (SENSITISED)					
Tyrode	1	33.04	85.50	5.60	14.50
	2	18.17	73.74	6.47	26.26
	3	24.51	94.74	1.36	5.26

Incubation	No.	Histamine			
		Tissue		Supernatant	
		µg/gm	% total	µg/gm	% total
	4	25.76	94.34	1.25	4.66
	5	44.82	91.71	4.05	8.28
	6	40.14	86.88	6.06	13.11
	7	20.23	63.68	11.54	36.32
	8	34.65	75.49	11.25	24.51
	9	30.61	89.62	3.55	10.38
	10	44.93	66.38	22.75	33.62
	11	22.80	88.23	3.04	11.77
	12	12.45	87.60	1.88	12.49
	13	14.63	86.09	2.36	13.91
	14	21.36	85.07	2.76	14.92
Mean		27.72	83.5	5.99	16.42
S.E.		2.80	2.65	1.56	2.69
Ovalbumin (0.1 mg/Kg)	1	9.84	72.72	3.69	27.28
	2	8.90	69.46	3.91	30.51

Incubation	No.	Histamine			
		Tissue		Supernatant	
		µg/gm	% total	µg/gm	% total
	3	23.39	88.32	2.91	11.68
	4	15.77	88.67	2.04	11.33
	5	32.18	91.21	3.10	8.78
	6	38.28	92.19	3.24	7.80
	7	55.76	89.76	6.36	10.24
	8	17.93	75.82	5.72	24.18
	9	19.83	89.77	2.25	10.22
	10	23.15	92.68	1.82	7.32
	11	18.20	90.53	1.90	9.47
	12	26.05	93.13	1.92	6.86
Mean		24.11	86.19	3.24	13.8
S.E.±		3.72	2.3	0.43	2.39
Ovalbumin (1.0 mg/ml)	1	7.36	59.6	5.00	40.47
	2	24.58	48.71	25.89	51.30
	3	23.28	93.28	1.67	6.72

Incubation	No.	Histamine			
		Tissue		Supernatant	
		µg/gm	% total	µg/gm	% total
	4	30.19	95.88	1.27	4.12
	5	31.13	87.89	4.29	12.11
	6	28.31	91.56	3.53	8.43
	7	9.72	60.73	6.28	39.27
	8	15.07	67.72	7.18	32.28
	9	16.85	64.98	9.08	35.02
	10	10.45	58.27	7.48	41.73
	11	26.87	89.43	3.17	10.57
	12	21.58	87.05	3.21	12.95
	13	11.05	82.66	2.31	17.34
	14	19.86	87.43	2.87	12.57
Mean		20.45	76.79	5.94	23.2
S.E.±		2.50	4.3	1.68	4.3
Compound 48/80	1	2.626	17.45	12.417	82.56
(1.0 mg/ml)	2	3.961	12.68	27.265	87.30

Incubation	No.	Histamine			
		Tissue		Supernatant	
		ug/gm	% total	ug/gm	% total
	3	2.552	5.72	39.604	94.28
	4	3.68	5.46	63.61	94.54
	5	26.48	73.09	7.85	26.91
	6	4.758	8.99	48.182	91.01
	7	3.364	7.31	42.677	92.69
	8	<2.229	<3.92	54.688	90.08
	9	21.792	33.88	42.515	66.12
	10	9.961	28.89	24.515	71.11
	11	17.932	71.31	7.214	28.68
	12	7.794	20.46	30.30	79.54
	13	8.681	24.31	27.03	75.69
Mean		8.9	24.11	32.91	75.42
S.E.±		2.24	6.47	4.9	6.38
Horse serum (0.1 mg/ml)	1	35.509	89.65	4.099	10.35
	2	19.329	75.02	6.438	24.98
Mean		27.42	82.33	5.27	17.66

Incubation	No.	Histamine			
		Tissue		Supernatant	
		µg/gm	% total	µg/gm	% total
Horse serum (1.0 mg/ml)	1	42.422	90.98	4.204	9.02
	2	37.646	74.79	12.688	25.21
	3	26.581	79.79	6.735	20.21
	4	28.400	84.34	5.274	15.66
Mean		33.762	82.47	7.225	17.52
S.E.±		3.77	3.4	1.9	3.4

APPENDIX III

COMPOSITION OF PHYSIOLOGICAL SOLUTIONS

Solution	Salt	Concentration gm/l
Tyrode	Sodium chloride	8.0
	Potassium chloride	0.2
	Calcium chloride	0.2
	Magnesium chloride	0.01
	Sodium dihydrogen orthophosphate	0.065
	Dextrose	1.0
	Sodium hydrogen carbonate	1.0
Krebs-Henseleit	Sodium chloride	6.92
	Potassium chloride	0.354
	Calcium chloride	0.282
	Potassium dihydrogen orthophosphate	0.162
	Magnesium sulphate	0.294
	Sodium hydrogen carbonate	2.1
	Dextrose	2.0

De Jalon	Sodium chloride	9.0
	Potassium chloride	0.42
	Calcium chloride	0.06
	Dextrose	0.5
	Sodium hydrogen carbonate	0.5

STUDIES ON THE MECHANISM OF ANAPHYLAXIS IN CATTLE

Summary of a thesis submitted for the degree of Doctor of Philosophy of t

Faculty of Veterinary Medicine by Maureen M. Aitken, B.V.M.S., M.R.C.V.S

Although hypersensitivity may play a part in diseases of cattle the mechanisms of hypersensitivity reactions have been investigated in detail only in laboratory animals and the relative importance of endogenous pharmacologically active substances, namely histamine, 5-hydroxytryptamine, kinins and SRS-A in such reactions has been found to vary in different species.

Ayrshire and Friesian calves were sensitised to ovalbumin and horse serum. One intravenous injection of ovalbumin was adequate but two, separated by an interval of 7 days, were required of horse serum. Challenge by intravenous injection of the appropriate antigen after a latent period of at least 7 days resulted in the immediate onset of acute systemic anaphylaxis from which animals either died within 10 min. or recovered in most cases within 30 min. to 2 hr. Anaphylaxis was characterised by respiratory distress, coughing, apnoea, systemic hypotension, pulmonary hypertension, bradycardia, leucopenia, neutropenia and haemoconcentration. Necropsy revealed severe pulmonary oedema, emphysema and intra-alveolar haemorrhage. Diarrhoea occurred

48 to 72 hr. after non-fatal reactions. The severity of the symptoms depended on the challenge dose. A course of weekly intramuscular injections of ovalbumin with complete Freund's adjuvant also resulted in sensitisation to ovalbumin. Animals sensitised in this way showed similar symptoms on challenge but reacted to doses of ovalbumin 50 times smaller. These animals also reacted non-specificall on challenge with horse albumin or horse serum.

Administration of histamine, 5-hydroxytryptamine or bradykinin produced effects similar to those of anaphylaxis. 5-Hydroxytryptamine differed in that even lethal doses did not result in pulmonary oedema. All the effects of these substances were reduced in the presence of their specific or selective antagonists with the exception of the apnoeic effect of 5-hydroxytryptamine which persisted in the presence of methysergide and BW501C67. Vagotomy did not alter the effects of histamine or 5-hydroxytryptamine.

In vitro bovine tracheobronchial smooth muscle and pulmonary vascular tissue contracted in response to histamine, 5-hydroxytryptamine

and bradykinin. These responses were abolished by the appropriate antagonists. Vascular perfusion rate of isolated lobes of lung was reduced and air perfusion pressure increased by these substances.

Apart from a reduction in lacrimal secretion no modification of anaphylaxis was observed in animals pretreated with antihistamines or atropine. Meclofenamate, a selective antagonist of kinins, SRS-A and ATP prevented the respiratory changes and reduced the circulatory changes in animals sensitised without Freund's adjuvant. This suggeste that kinins and/or SRS-A and ATP might be important as mediators. The compound BW501C67, had a modifying effect on anaphylaxis. However, this appeared to be unrelated to its activity as an antagonist of 5-hydroxytryptamine. The occurrence of apnoea during anaphylaxis was prevented or delayed in vagotomised animals. Haematological changes were unmodified after pretreatment with antagonists. The protective effect of meclofenamate, in animals sensitised using Freund's adjuvant, was limited to inhibiting mild reactions only.

Responses of pulmonary tissues to antigen in vitro were shown only by tissues removed from animals sensitised using Freund's adjuvant. Schultz-Dale reactions were elicited from intestinal tissue of animals sensitised without adjuvant.

Antigen failed to release histamine in vitro from chopped lung, pleura or liver capsule of sensitised animals. Some release of histami from chopped skin did occur. Concentrations of histamine in the tissues of animals which died during or after anaphylaxis tended to be higher than in control animals. Plasma free histamine levels were elevated following anaphylaxis but serial blood samples showed great variation in plasma histamine levels. Perfusion of isolated lung with ovalbumin resulted in the presence of histamine, bradykinin and possibl other spasmogenic substances in the perfusate. Perfusate from the lungs of sensitised animals had a higher content of active substances than did that from the lungs of unsensitised animals.

Serum of animals sensitised using Freund's adjuvant had the property of sensitising the skin of other calves for PCA which could