

THE IN VIVO EFFECTS OF EXTRACTS OF THE ANTI-ASTHMATIC PLANT,
DESMODIUM ADSCENDENS ON ANAPHYLAXIS

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DEDICATED TO MY PARENTS



DECLARATION

The experimental work described in this Thesis was carried out by me in the Department of Biochemistry, University of Ghana, Legon, under the supervision of Dr. Marian E. Addy.

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A B S T R A C T

Desmodium adscendens is used as prophylaxis in asthma. Earlier work in this laboratory on the scientific basis for the therapeutic action of the plant indicated that both the aqueous and alcoholic extracts were anti-anaphylactic in vitro. Since extracts of the plant are taken orally by asthmatic patients, the present work was carried out to investigate the in vivo effects of both alcoholic and aqueous extracts of D. adscendens on anaphylaxis.

The methods used were the contraction of the isolated guinea-pig ileum, the histamine-induced spasm, the release of smooth muscle-stimulating substances from the guinea-pig lung and the histamine content of such lung tissue.

The results show that the extracts of D. adscendens caused a significant reduction in the anaphylactic contraction of the guinea-pig ileum and rendered it less sensitive to histamine when compared with controls. The quantities of mediators of smooth muscle contraction released from the lung tissue of the treated animals were significantly less than those in the untreated animals. Furthermore, the total histamine content of the lung tissue in animals treated with the extracts of D. adscendens was significantly less than that in the untreated animals.

The results have provided more evidence for the earlier indication of the anti-anaphylactic property of the plant extracts, a property which may underlie their usefulness as antiasthmatic agents in folklore medicine.

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INTRODUCTION AND LITERATURE REVIEW

A. GENERAL INTRODUCTION

Plant medicine is important in the primary health care requirements of a large number of Ghanaians, particularly the rural population, who have little or no access to hospital facilities. In traditional medical practice, use is made of local plant species in curing a number of ailments. Some of the plants used are fairly well-known to the public and these are usually sold in the ordinary market places. Information on the medicinal uses of some of these plants is in the local folklore and there is hardly any secrecy about them. Most of these plants are regarded as of proven efficacy. Knowledge of the alleged medicinal value of a larger number, however, is usually confined to professional traditional and psychic healers who keep this as a secret and who acquire and disseminate it in some peculiar ways (Dokosi, 1969). There is also documented evidence of a number of herbs which have been used for the treatment of diseases in this country. Examples of these are Desmodium adscendens (Papilionaceae), Thonningia sanguinea (Balanophoraceae) and Deinbollia pinnata (Sapendaceae) used for asthma; Bridelia ferruginea (Euphorbiaceae), Costus schlechteri (Zingiberaceae), Myrianthus arboreus (Moraceae) and Anthocleista kerstingii (Longaniaceae) for diabetes mellitus; Elaeophorbia drupifera (Euphorbiaceae) and Hillieria latifolia (Phytolacaceae) as filaricide in guinea-worm infestation;

Hoslundia opposita, Securidaca longipedunculata, Picralima nitida (Apocynaceae) and Combretum mucronatum (Combretaceae) for guinea-worm and herpes zoster (Ampofo, 1977). Also, Lippia multiflora (Verbenaceae) is used for treating hypertension (Noamesi, 1980). It is marketed under the trade name 'Healer Herb'.

In spite of the wide variety of medicinal herbs found in our forests, very little has been done to process these herbs into forms for general use in the treatment of tropical diseases that are prevalent here. Herbs are important in providing primary health care, especially to a nation with scarce foreign exchange which places a major constraint on the importation of drugs to meet the health needs of its people.

Conventional Western medicine is known to have its good and bad sides. Its areas of competence and shortcomings appear to be well defined and well documented. The vast number of drugs used are also well known and scientifically tested. However, in herbal medical practice, such scientific tests are lacking. The possible side effects of the use of any particular plant extract have not been properly investigated and documented, partly because the average herbalist is not well equipped for such studies, and partly because these effects may be of such a long term nature that they would not be immediately apparent to a practitioner. Other factors, such as the success stories of some herbal medicine could unfortunately prevent

one from realising the harmful and probably more dangerous side effects of herbal medicine. Very little work has been done on the toxicology and teratology of herbal medicines, but the possible dangers in its practice are gradually becoming apparent (Addae-Mensah, 1975).

There is the need for such studies in order to improve upon traditional medical practice. Such improvement will make it acceptable to sections of the population other than the rural dwellers, and therefore available to even greater numbers of the people. But such improvement or development for the proper use of herbs as medicine require a lot of scientific investigations. The complete knowledge about the plant specie, its medicinal property and how it affects the body are all necessary. Improper use of herbs in terms of incorrect identification or wrong dosage can be fatal. There is variety resemblance between herbs. They may be similar but that does not mean that they can be assumed to act in the same way and there is no written herbal pharmacopoeia to learn from.

In recent years, however, through the co-operation of many traditional healers in this country, it has been possible to document a number of these plants botanically and to record their medicinal uses (Dokosi, 1969, 1975).

There are many diseases and conditions which respond positively to herbal treatment and for which orthodox medicine has found no cure, e.g. asthma and herpes zoster (Ampofo, 1977). Traditional medicine appears also to be more effective in curing guinea worm infestation. Although

many plants have been credited with curative properties, the medicinal values of some are doubtful and herbalists are generally imprecise as regards the dosage of their drugs. Studies on dosage and proper classification are important for the development of herbal medical practice and so is the scientific basis for the therapeutic action of these plants found to be effective. Such studies will go a long way to help and improve our pharmacopoeia.

Co-operation between herbal practitioners and medical doctors is also necessary for the development of herbal medicine. In China and India work in this area has reached an advanced stage. Herbal medical practitioners are well organised into groups which are stationed in agricultural communes. There is an established working relationship between these groups and the doctors. In Ghana, this kind of co-operation is lacking and there is a wide range of disagreement between doctors and herbalists on the use of these herbs. There is therefore the need for studies to provide scientific basis for materials used by herbalists and how these effect their cure to enable the doctors realise the value in herbal medicine. The testing of pharmacological and physiological effects of various preparations from plant sources is very important as it will contribute to knowledge of possible side effects.

In Ghana the importation of drugs is limited by the country's foreign exchange earnings and the hospitals at present are ill-equipped.

Improvement of traditional medicine could help cut down on the dependence of primary health care on imported drugs and these standard hospitals. For the proper development of herbal medicine, the Ghana Government established the Centre for Scientific Research into Plant Medicine (CSRPM) at Mampong, Akwapim in November, 1973 to carry out scientific research into the medicinal properties of several plants used by herbalists. Apart from this government centre, there are also the Noamesi Laboratory, established by Dr. G.K. Noamesi at Hohoe in the Volta Region, Dr. Nartey's Institute of Herbal and Tropical Medicine at Nsawam, and the Traditional and Psychic Healers Association, all of which have some contact with the country's hospitals, and carry out some amount of research into medicinal plants. The CSRPM has been responsible for scientific testing of a number of herbs. Pre-clinical trials are carried out at the Centre and the plants found to be effective are given to the Universities and the Research Institutes which have the facilities for scientific investigations. Interest in this department in collaboration with the Centre is in several areas including asthma.

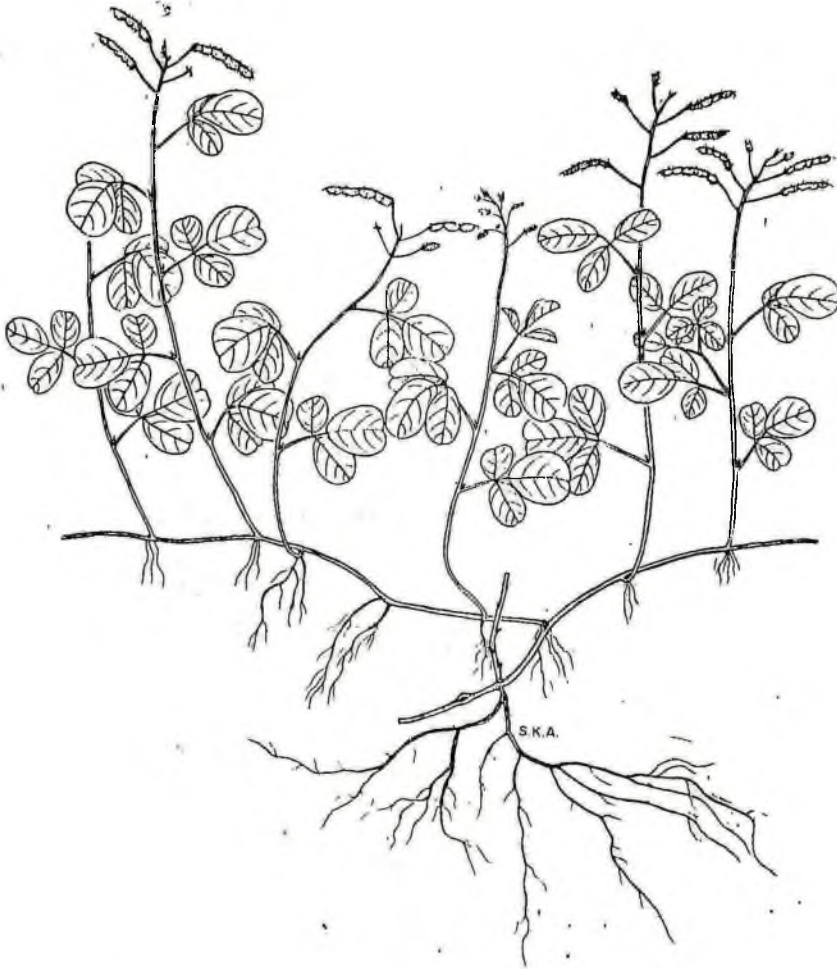
Asthma is known to be one disease for which orthodox medicine has no cure. Drugs used for bronchial asthma in modern medicine (e.g. adrenaline) are mainly applied during attacks whereas at the Centre medicinal plants can be used prophylactically until attacks are well reduced or completely eliminated (Ampofo, 1977). Of the many plants

available for bronchial asthma, D. adscendens, Thonningia sanguinea and Deinbollia pinnata have been most widely used at the CSRFM. The present work is a study of the scientific basis for the therapeutic action of D. adscendens, one of the plants used at the Centre for the treatment of asthma.

B. THE GENUS DESMÓDIUM

The genus *Desmodium* belongs to the family Leguminosae and sub-family Papilionaceae. It is a shrub with compound trifoliolate leaves, its fruits are jointed and deeply indented on one side, and breaks transversely into one-seeded portions. This description puts it in the tribe Hedysareae. About four dozen species have been recorded for this genus in India (Ghosal and Bhattacharya, 1972), sixteen species in Tropical Africa (Hutchison and Daiziel, 1954) and nine in Ghana (Irvine, 1961). Two varieties of D. adscendens exist. They are D. adscendens var. robustum and D. adscendens var. adscendens. The leaves of the former are often larger than those of the latter. D. adscendens var. adscendens which was used for the work reported here is an undershrub (Fig.1). Its stems are prostrate and has roots at the nodes with ascending branches on which flowers are borne. The leaflets are appressed and thinly pubescent above. The leaves are trifoliolate and often fold at night. The plant is often

Fig 1



Desmodium adscendens var *adscendens*

found in the Savanna forest or in closed forests where it reaches great heights. It grows along the forest paths in communities. The Akwapims call it 'akwanfanu' because it grows on both sides of the forest paths. The Ashantis call it 'Ananse akatee', literally meaning 'spider's groundnut' since its leaves are similar to that of groundnut.

The members of this genus are well known for their various medicinal uses (Ayensu, 1978; Irvine, 1961 and Chopra et al, 1956). The leaves of D. gangeticum are used for treating infections of the urinary system in the Ivory Coast and Upper Volta, whilst a root decoction with other plants is used in treating afflictions of the brain and chest in India. During an outbreak of chicken-pox, the Ewes of Ghana treat their patients with baths of macerated leaves of D. gangeticum. A paste of the ground leaves is also taken internally in water and some applied to the skin. The non-infected members of the community are also given the same treatment and they may either escape infection, or if they contract it, the eruption is normally insignificant. No chicken-pox scars are found on patients treated with this plant (Ampofo, 1976). Decoction of the bark of D. pulchellum is used in haemorrhage, diarrhoea and for eye diseases, whilst its flowers are used in biliousness in India. The leaves of D. triflorum are used as a galactagogue, for dysentery and for convulsion, whilst the roots are applied to wounds and abscesses. Stem-leaves of D. adscendens have

been reported to be galactogenic. Boiled leaf extracts of this plant are also used in treating constipation and ringworm infections (Ayensu, 1978).

At the CSRPM, D. adscendens is used in combination with Thonningia sanguinea and Deinbollia pinnata for the treatment of asthma. The leaves of D. adscendens may be given in the form of dry powder, one or two tea-spoonfuls, according to age, in warm water in three divided doses per day, or it can be made into an alcoholic extract. The powder may be soaked in alcohol (the local gin) and taken as above. The roots of Thonningia sanguinea and Deinbollia pinnata are used differently. The former is taken in honey or prepared in the form of alcoholic extract and the latter taken in palm soup or soda water. Each of these preparations is capable of reducing the incidence of bronchial asthma attacks or even stopping them, especially in children. But the best result obtained at the centre is by the administration of a combination of D. adscendens and either Thonningia sanguinea or Deinbollia pinnata. Clinical trials using placebo herbs and combinations of these plants showed continuous asthmatic attack in patients during placebo treatment and no attack during herbal therapy. Evidence points to the fact that D. adscendens and the other herbal preparations produced a satisfactory response in 75% of treated patients (Ampefo, 1977).

Few species of the genus *Desmodium* have been evaluated phytochemically. Chemical investigations have revealed the presence of several simple bases, mostly indole alkaloids in various plant parts of these species (Ghosal et al 1972).

Preliminary work on the chemical evaluation of D. adscendens indicates the presence of indole alkaloids, some of which were identified as tryptamine derivatives (Gbewonyo, 1980). The only record of pharmacological studies on D. adscendens var. adscendens is that mentioned above (Gbewonyo, 1980). Work on other species indicate, however that the medicinal properties ascribed to extracts from various parts of these plants reside essentially in the alkaloidal constituents (Ghosal et al., 1971, 1972 and Ghosal and Bhattacharya, 1972). The total alkaloids of D. triflorum have been shown not only to possess sympathomimetic activity which may be due to their catecholamine releasing effect, but also offer protection against acetylcholine and histamine-aerosol-induced bronchospasms (Ghosal et al., 1972). These pharmacological effects would account for the use of the plant extracts in the treatment of asthma and coughs. As mentioned earlier, D. adscendens is also used as an anti-asthmatic plant.

C. ASTHMA

The disease is defined as a condition of increased sensitivity of the trachea and bronchi to various stimuli. It is a complex disease in which allergic, psychological and bronchitic factors are inextricably bound up (Reed, 1968; Schild et al., 1975). Bronchial asthma is a multi-factorial disease for many patients, but the beta adrenergic blockade hypothesis,

proposed by Szentivanyi offers a unifying explanation for many of the diverse features characteristic of asthma (Szentivanyi, 1968).

The disease is brought about in genetically susceptible individuals by the sequential occurrence of sensitization, subsequent re-exposure to the same antigen, release of pharmacologically active substances by a series of incompletely understood biochemical events, and the action of these substances on blood vessels, mucous glands and smooth muscles. Individuals capable of developing atopic hypersensitivity as in asthma can be allergic to a great variety of antigens especially inhalants, pollens, mold, foods, drugs and injectables. Sensitization here gives rise to the characteristic reaginic or skin sensitizing antibody. This cytotropic antibody is operative in the immunological sequences leading to asthma. The initial event triggering off asthma is re-introduction of specific antigen and its interaction with antibody on the surface of sensitized cells. This reaction brings about certain fundamental changes in the cell resulting in the release of histamine and other pharmacologically active substances like serotonin, slow-reacting substance of anaphylaxis (SRS-A), prostaglandins, bradykinin and other ~~kinins~~ kinins (Barret, 1974).

The symptoms are due to a combination of spasm of the smooth muscles of bronchioles, oedema and swelling of mucous membranes. The trachea and bronchi lumen are filled with thick tenacious secretions. If these secretions are aspirated the patient is partly relieved (Schild et al, 1975).

Some of the pharmacological actions required in the treatment of bronchial asthma therefore include relaxation of bronchial muscle, decrease of oedema and swelling, sedation, expectoration and improved oxygen supply. No known drug possesses all these actions but the typical sympathemimetic drugs have the final effect of relaxing bronchial muscle and diminishing oedema. They are by far the most useful drugs in the treatment of either acute asthmatic attack or of chronic bronchial asthma. The more common drugs used are adrenaline, isoprenaline, ventolin and 'Intal' (disodium cromoglycate). With the exception of disodium cromoglycate which was found to reduce the immunological release of histamine and SRS-A from lung (Douglas, 1975), all the other drugs act when the attack had already occurred. The effect of these drugs is therefore temporal.

Experimental analogy to bronchial asthma can be created in mice sensitized with *Bordetella pertusis* or in guinea-pigs with egg albumen (EA) inoculation (Clausen et al., 1969). The sensitization causes a state of immunological imbalance very similar to the pharmacological and immunological abnormality found in bronchial asthma. It causes hypersensitivity to histamine, serotonin, acetylcholine and reduced sensitivity to catecholamines. The sensitization leads to an enhancement of antibody formation and production of homocytotropic antibodies in particular.

D. EXPERIMENTAL ALLERGY SYSTEM

1. Allergic or hypersensitivity reactions:

Hypersensitivity or allergy has been defined as a group of variably harmful immunological reactions leading to a heightened or accelerated response to a previously encountered antigen, or its haptens. Thus it is always an anamnestic reaction. Any allergic manifestation is the visible result of a long series of steps, each step frequently comprising one or more sequences of reactions (Gray, 1970; Becker, 1971). Hypersensitivity, an altered reactivity injurious to the host can also lead to immunity, the alteration beneficial to the host in resisting infection. However, the term allergy has come to refer only to those immunological reactions that are damaging to the host. Hypersensitivity and allergy have thus become synonymous. The re-exposure of hypersensitive individuals to the appropriate antigen triggers off allergic responses which may either be of the immediate or delayed type.

Immediate-type allergic reactions are antibody-dependent and specific since they depend on and can be transferred by cytotoxic antibody of the immunoglobulin E or G type (IgE or IgG). The reactions consist of local or general, occasionally fatal, inflammatory responses to antigens that are usually quite harmless to non-sensitized individuals. The medical importance of the hypersensitivity reactions is very considerable. The manifestations

include asthma, anaphylaxis, hay fever, gastroenteritis, urticaria and migraine. Sensitization occurs by contact, by inhalation, by ingestion or injection, or by invasion of bacteria. Immediate-type hypersensitivities can conveniently be divided into induced reactions, which seldom occur naturally and the relatively common spontaneous clinical allergies of the asthma-urticaria-hay fever group (Gray, 1970).

2. Anaphylaxis

Anaphylaxis means without protection and is the antonym of prophylaxis. It is a sequence of events initiated by the union of antigen with cell-bound antibody, formed from a previous exposure to a specific antigen or a structurally similar substance, followed by release of pharmacologically active substances which cause various pathophysiologic reactions to take place. Anaphylaxis seldom occurs spontaneously but can be readily produced experimentally by standard medical procedures including chemotherapy, antiserum therapy, sensitization with pollen extracts and active immunization with egg-based vaccines (Gray, 1970). Experimental anaphylaxis in the guinea-pig may provide the prototype for immediate-type hypersensitivities of which asthma is an example, the other forms differing only in the severity of the reaction and the shock organ.

The induction of the anaphylactic state varies with different animal species, as do the manifestations of anaphylactic shock. The special conditions required to induce anaphylaxis are illustrated by the guinea-pig

which is the best documented model: a single, very small sensitizing dose of antigen is needed, e.g. one-hundredth or even one-millionth of 1 ml. of horse serum or egg albumen; a delay of 14 to 21 days should then elapse in order to allow the hypersensitive state to develop; anaphylactic shock may be induced at this stage or later, on, by administering a large dose (2-3 ml) of the sensitizing antigen to tissues. Tissues of sensitized guinea-pigs can be brought into contact with antigen in vivo or in vitro (Humphrey and Mota, 1959). The observed signs and lesions in the guinea-pig reflect the pharmacology of histamine poisoning in this species viz: smooth muscle contracts all over the body and capillary endothelium is damaged.

The in vitro contraction of guinea-pig ileum was first reported in 1910 (Schultz, 1910). This tissue was then used to confirm the findings regarding desensitization in the guinea-pig uterus (Dale, 1912). It was noted that a pronounced anaphylactic contraction of either tissue left the smooth muscle refractory to any further additions of antigen. This reaction which is now referred to as the Schultz-Dale reaction uses isolated smooth uterine muscle or intestine from a guinea-pig sensitized with egg albumen, suspended in warm, oxygenated Tyrode solution. The isolated muscle contracts violently on the addition of antigen and is subsequently found to have become desensitized (Dale and Okpako, 1969; Austen and Humphrey, 1963).

3. The Chemical mediators of anaphylaxis

The term mediators is restricted to substances whose release or formation is triggered directly or indirectly by the initiating antigen-antibody reaction and whose action is responsible for one or more of the manifestations of a given allergic reaction. Not all types of allergic responses are due to the action of mediators. Nevertheless, a large number of different kinds of allergic reactions are, and in these the differences among the reactions are due in large part to the different kinds of mediators released. There are two groups of mediators, low molecular weight mediators comprising histamine, serotonin, kinins, SRS-A, and macromolecular mediators e.g. heparin, lysosomal enzymes, anaphylatoxins. The low molecular weight mediators are substances which have marked, direct pharmacological activity (Becker, 1971).

The in vitro anaphylactic release of histamine, SRS-A and kinin-forming enzymes was first appreciated in experiments with perfused, shocked, whole guinea-pig lung (Brocklehurst and Lahiri, 1962). However, neither the perfused whole tissue nor the isolated whole tissue technique is as suitable for studying anaphylaxis in vitro as the chopped lung method (Mongar and Schild, 1953). The technique was later modified (Austen and Brocklehurst, 1960). The lung tissue was perfused free

from blood prior to chopping so as to minimise any non-specific contribution from serum factors not firmly adsorbed onto the tissue. Another method for the study of the anaphylactic release of smooth muscle-stimulating substances, used portions of excised sensitized lung tissues (Nicoll and Campbell, 1940). Portions of lung tissues from sensitized animals were added to segments of non-sensitized guinea-pig ileum maintained in an organ-bath of oxygenated Tyrode-Ringer solution at 37°C. The addition of antigen to the bath fluid then produced a release of smooth muscle-stimulating material from the lung tissue which caused the contraction of the non-sensitized ileum.

The time course of the release of histamine and SRS-A after the addition of antigen to sensitized perfused, chopped guinea-pig lung is brisk (Austen and Humphrey, 1963). Histamine starts to appear within 10-15 seconds after adding antigen, and two-thirds of the total amount is released by the end of the first minute. SRS-A starts to appear 30-60 seconds after the addition of antigen and attains maximal release rate at the end of the first minute. That the time course of SRS-A release is slower than that of histamine has also been observed in non-perfused lung slices and perfused whole lung (Brocklehurst, 1960).

4. Anaphylactic contraction of smooth muscle

The indicator of anaphylaxis both in vivo and in vitro is a contraction of some organ or tissue. The pathway to anaphylactic contraction of smooth muscle is shown in Fig. 2. Smooth muscle contraction during anaphylaxis occurs when the mediators released act on the muscle (through receptors in some cases). The responses to chemical mediators could be ascribed to an action exerted on the cell membrane that results in increased permeability to calcium ions. This alters the membrane potential and the intracellular ionic movement leading to the contraction of the muscle. Relaxation may be assumed to involve a reduction in the intracellular concentration of calcium ions (Huxley, 1969).

The Schultz-Dale reaction in anaphylaxis is a consequence of strong stimulating action on smooth muscles by some chemical mediators. The sequence leading to the release of these active substances can be interrupted at various points using inhibitors and much information on the mechanism of anaphylaxis has arisen from studies of the action of such inhibitors (Mongar and Schild, 1962). However, certain pharmacological antagonists to known mediators fail to block the anaphylactic response. Another observation is that on abolishing or reducing the response to

some mediators as a result of their repeated administration, application of the antigen still elicits strong responses. On this basis, it was suggested that the mechanism of anaphylaxis in the smooth muscle is not confined to the participation of chemical mediators (Alonso-de Florida et al, 1968); it is also possible that antigens act directly on the membranes of sensitized muscle cells to cause depolarization and contraction.

A characteristic feature of the Schultz-Dale reaction in guinea-pig smooth muscle is the fact that exposure to antigen leads to a rapid desensitization (Hicks et al, 1968). It has, however, been observed that such desensitization reaction is not absolute, neither is it irreversible, since tissue sensitivity is known to become re-established within a few hours (Okpako, 1970). An explanation for this observation is based on the concept of drug-receptor interaction (Paton, 1961). It is assumed that the anaphylactic contractions elicited in smooth muscle is short-lived compared with the antigen-antibody complexes producing them, so that the contractions could be a measure of the rate of formation of these complexes and therefore of the concentration of the challenging antigen. The anaphylactic desensitization induced by moderate doses of antigen is therefore a dose-dependent phenomenon surmountable by increasing the dose of antigen,

and is reversible by simply washing in physiological solution. Antigen dose-response curves can therefore be obtained using moderate doses of antigen, and this could be used in the study of potentially anti-anaphylactic drugs in vitro.

5. Inhibition of anaphylactic contraction of smooth muscle

The study of inhibitors of the anaphylactic reaction can be expected to yield information about the underlying mechanism of anaphylaxis and also to provide methods for the discovery of potentially useful compounds for the treatment of allergic conditions (Mongar and Schild, 1956).

The anaphylactic contraction of plain muscle can be considered as a sequence of reactions initiated by the union of antigen with cell-bound antibody and leading to mediator release and muscular contraction. Various attempts have been made to inhibit one or other stage of the anaphylactic reaction and thus interrupt the whole sequence of events.

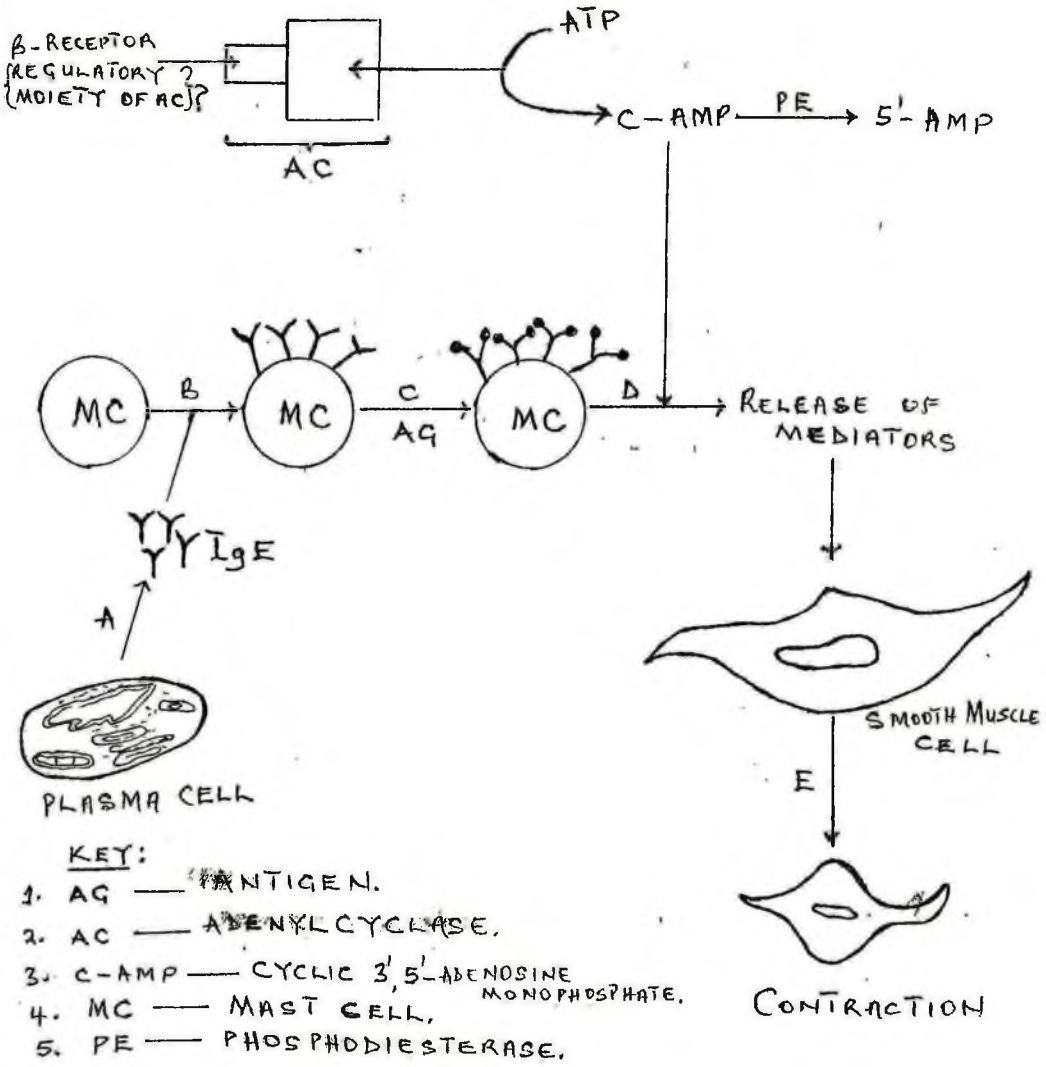
Initial attempts were with inhibitors such as haptens which interfere with antigen-antibody complex formation, and the antihistamines which interfere with the combination of released histamine with histamine receptors (Mongar and Schild, 1957). The most effective inhibitors are those which inhibit the last stage of the anaphylactic reaction, that of muscular contraction; for example, sympathomimetics and anaesthetics.

In view of the finding that the effectiveness of the sympathomimetics in allergic bronchial asthma depends on the ability of these compounds both to inhibit the anaphylactic mechanism and to relax bronchial smooth muscle (Assem and Schild, 1969), it was suggested that the sympathomimetics may have a dual effect of direct relaxation of bronchial smooth muscle and inhibition of the release of pharmacological mediators of immediate-type allergy.

Different inhibitors have different effects on the anaphylactic mechanisms. The explanation of these differences would seem to be fundamental to understanding the biochemical mechanism of the anaphylactic reaction and might be of practical importance in future approaches to the therapy of allergic bronchial asthma. The biochemical regulation of anaphylaxis is possible at several stages in the pathway of anaphylactic shock (see Fig. 2). Some of these include; regulation of immunoglobulin synthesis by use of immunosuppressants, prevention of antibody fixation to cells (hypothetical), chemical degranulation of mast cells prior to antigen administration, a treatment that would render the mast cell physiologically incapable of further histamine release, competitive inhibition of histamine and other mediators of anaphylaxis (Barret, 1974). Any control which will block the release of pharmacological mediators would be most effective since in this case the mediators do not get to the target organ.

FIG. 2

ASTHMA: IMMUNOPHARMACOLOGY.



Immediate-type allergic reactions in man, rat and guinea-pigs were found to be inhibited by disodium cromoglycate (DSCG) and beta adrenergic stimulant drugs like isoprenaline and salbutamol. DSCG is now well accepted in clinical practice for the management of perennial allergic asthma (Assem and Richter, 1971; Assem and Mongar, 1970; Mahajani and Kulkarni, 1977). Since its introduction (Cox, 1967), its inhibitory effect on type-1 hypersensitivity reactions mediated by heat-labile IgE-like antibodies (Goose and Blair, 1969; Sheard and Blair, 1970), its protective effect on the mast cell (Orr *et al.*, 1971; Marshall, 1972), its inhibitory effect on allergic reactions mediated by immunoglobulins other than IgE type (Pepys *et al.*, 1968), and its beneficial effects in exercise-induced asthma (Davies, 1968; Muittari and Kreuz, 1969) are well documented. The drug has been widely used in Europe in the prophylaxis of severe bronchial asthma especially the type that is clearly attributable to the inhalation of allergens. D. adscendens is also used prophylactically in the treatment of asthma (Ampofo, 1977). DSCG inhibits release of histamine and SRS-A from human lung and guinea-pig lung during allergic responses. The drug does not inhibit fixation of antibody, nor does it seem to interfere with the antigen-antibody reaction; rather it suppresses the response to this reaction.

It does

not also prevent mast cell-mediated hypersensitivity reactions in human

skin. There are clearly both tissue and species variations in the response to the drug (Assem and Mongar, 1970; Cox et al, 1970; Orange et al, 1971). However, there is general agreement on its inhibition of mediator release as a basis for its prophylactic use. Because D. adscendens is used in prophylaxis of asthma, methods used in its evaluation included the inhibition of release of mediators.

6. Histamine

The principal target cells of the hypersensitivity reactions of the immediate-type are the mast cells and basophils. Within the secretory granules, of these cells, histamine is stored along with a heparin-protein complex to which it is loosely bound by ionic forces, probably involving carboxyl groups. It is formed from L-histidine by enzymatic decarboxylation before being stored in the granules (Borje, 1974). Histamine has been of great interest to workers in the field of anaphylaxis since the many resemblances between its effects and those of anaphylaxis in the guinea-pig were pointed out (Dale and Laidlaw, 1910). Direct evidence was presented that it could be released from tissue in vitro and in vivo by anaphylactic reaction (Philips and Middleton, 1965). Histamine is widely distributed in mammalian tissue but the concentration in a given organ shows great species variation. The histamine content of the guinea-pig lung ranges between 5-25 µg/gm tissue; 21-24 pg per mast cell (Austen and Humphrey, 1963).

There is reported reduction in the histamine content of lung tissue from sensitized guinea-pigs treated with the anti-asthmatic plant preparation, Picrorhiza kurroa root powder (Mahajani and Kulkarni, 1977). It is therefore interesting to study the effect of D. adscendens on the lung histamine content. Apart from mast cells the cells containing histamine are platelets, basophilic leucocytes, foetal liver, parietal cell region of stomach, even though mast cells are virtually absent from these sites.

It was postulated that when a mast cell disintegrates during the anaphylactic reaction it creates a histamine gradient in its surroundings, and that the degree of muscular contraction will then depend on the number of smooth muscle cells reached by effective concentrations of histamine (Schild, 1956). Mast cells are in close association with the responsive structures in the so-called shock organ, as, for example, in bronchial muscle of the guinea-pig lung. The established pharmacological effects of histamine include capillary vasodilatation with increased permeability, bronchiolar and other smooth muscle constriction, and stimulation of the glands of exocrine secretion. The physiological significance of histamine is obscure despite diverse activity and widespread distribution. It had been observed that asthmatics undergoing treatment with DSCG show decreased responsiveness to histamine and increased responsiveness to atropine (Kerr et al., 1970). There is also a reported reduction in sensitivity to histamine in sensitized guinea-pigs undergoing treatment with the anti-asthmatic plant, Picrorhiza kurroa (Mahajani and Kulkarni, 1977). It is therefore necessary

to investigate the effect of this antiasthmatic plant, D. adscendens on sensitivity to histamine.

Since anaphylaxis underlies atopic asthma, studies were done in this laboratory to find out if the therapeutic effect of the plant D. adscendens used for the treatment of asthma, is due to the fact that it is anti-anaphylactic. These in vitro studies during which the plant extracts were added to the tissue bath showed that both alcoholic and aqueous extracts of the plant material inhibit the anaphylactic contraction of the guinea-pig ileum, and it appeared that the effect of the plant was to inhibit the release of pharmacological mediators (Gbewonyo, 1980).

In the present studies, the plant extracts were given to the animals while they were alive (in vivo), because extracts of the plant are taken orally by asthmatic patients. This was done to find out if the anti-anaphylactic properties observed in the in vitro experiments could also be observed in the circumstances under which treatment is given.

The effects of treating (oral administration) guinea-pigs with extracts (both alcoholic and aqueous) of D. adscendens during the sensitization period were investigated. The following parameters were used for the study:

1. Anaphylactic contraction of ileum isolated from guinea-pigs sensitized and treated with extracts of D. adscendens.
2. Histamine-induced spasms in such ileal pieces.
3. Anaphylactic release of mediators of smooth muscle contraction from lung tissue of guinea-pigs treated with the extracts.
4. Histamine content of such lung tissue.

CHAPTER II

MATERIALS AND METHODS

A. MATERIALS

(1) Chemicals:

Normal butyl alcohol, calcium chloride (anhydrous), citric acid monohydrate, egg albumen (powdered), glucose, magnesium sulphate, sodium hydroxide, sodium chloride, sodium dihydrogen phosphate, sodium hydrogen carbonate, and trisodium citrate dihydrate were purchased from B.D.H Chemical Ltd; ethanol (96%), hydrochloric acid, methanol (acetone free), perchloric acid and potassium chloride from May and Baker; n-heptane from Fluka Chemical; histamine, histamine diphosphate, and o-phthalaldehyde from Sigma Chemical Company.

(2) Animals:

Guinea-pigs were obtained from two sources: the animal house in the Biochemistry Department and from local breeders in the Achimota, Suhum and Koforidua areas. When purchased from the latter source, the animals were quarantined for at least two weeks in the departmental animal house before they were used. The animals were sensitized only when they weighed between 250 and 350 g. Both male and female animals were used. They were maintained on unrestricted supplies of water or plant extract and elephant grass supplemented with animal feed (composition: 40% wheat bran, 40% maize, 17% fish meal, 2% cod liver oil and 1% sodium chloride), purchased from the Agricultural Research Station, Nungua.

(3) Plant materials:

The plant material used, (stem-leaves of D. adscendens) was supplied by the Centre for Scientific Research into Plant Medicine. The plant was identified by the Silviculturist at the Centre.

B. METHODS

(1) Preparation of extracts of D. adscendens

The roots from the fresh plant material were removed and the stem and leaves washed, air-dried at room temperature for up to one week and then pulverized in a mill.

(a) Alcoholic extracts

About 600 g of the powdered material was extracted continuously for 48 hours, with 5 litres of ethanol, in a Soxhlet extractor. The resulting ethanolic extract was concentrated by evaporating the ethanol in a rotary evaporator until a thick slurry was obtained. This material weighing 200 g was then taken in 2 litres of distilled water to give a solution of final concentration 300 mg/ml (based on the weight of powdered material per volume). This was diluted 1 in 3 before it was given to the animals.

(b) Aqueous extract:

200 g of powdered material was extracted continuously for 48 hours with 2 litres of distilled water in a Soxhlet extractor. The concentration was

100 mg/ml (based on the weight of powdered material per volume). This was diluted 1 in 3 before it was given to the animals.

(2) Solutions:

The composition and preparation of the following solutions used are to be found in the Appendix:

- (a) Egg albumen (EA) (100 mg/ml, 45 mg/ml and 5 mg/ml).
- (b) Citrate buffer pH 4.0.
- (c) Histamine (100 µg/ml) and histamine diphosphate (30 mg/ml).
- (d) Tyrode Solution.
- (e) O-Phthalaldehyde (OPT) reagent.

(3) Bioassays:

(a) Sensitization

Young guinea-pigs, weighing between 250 and 350 g, were actively sensitized by injecting them first with 2 ml of freshly prepared solution of egg albumen as antigen in normal saline (100 mg/ml); 1 ml intraperitoneally and 1 ml subcutaneously. This was followed on the 8th day by another injection with the same antigen (5 mg/ml); 1 ml intraperitoneally and 1 ml subcutaneously. The sensitized animals were divided into three groups: one group received the alcoholic extract, another group received the aqueous extract of D. adscendens and the third group received water during the three-week sensitization period.

(b) Preparation of isolated tissues

Three weeks after the primary sensitization, the animals were starved for a day and then killed by a blow on the head. The abdomen was opened

and the caecum lifted forward to expose the ileum. A length of ileum was removed and placed in a dish containing aerated Tyrode solution. Great care was taken to avoid damaging the gut muscle. It was handled with the fingers rather than gripped with forceps. The mesentery was trimmed away and 2 to 3 cm pieces were cut from the length of the ileum, starting above the Peyer's patch. The starvation prior to the killing rendered the gut relatively clean enough to use directly. In cases where the ileum was not clean enough, it was necessary to wash out the contents by placing one end of the gut over the tip of a pipette containing Tyrode solution and applying a very small head of pressure by tilting the pipette to about $20-30^{\circ}$ to the horizontal. Ileal pieces were kept in the refrigerator (below 10°C) and a tissue obtained when required for assay. Unused ileal pieces from one animal were discarded after about five hours.

(c) Assay procedure

Anaphylactic responses to doses of antigen in isolated sensitized ileum preparations were recorded by a modification of the method of Schultz and Dale in an organ bath containing Tyrode solution thus: A piece of cotton thread was tied at each end of the piece of ileum, taking care to ensure that the ileum was left opened, i.e. the threads did not close the lumen. The thread at one end was made into a small loop by which the tissue was hooked to a platinum wire attached to a glass tube through which the organ bath was aerated. The piece of ileum was mounted in a 45-ml organ bath containing Tyrode solution which was kept at $37 \pm 0.5^{\circ}\text{C}$ and continuously

aerated. This was done by fastening the thread at the other end of the tissue to a lever attached to the transducer of an 8" Chart Mover (Model 450, Harvard Apparatus Co. Inc.). The transducer had a writing point which recorded the contractions on a chart. There was always a constant weight of 0.5 g on the lever to stretch the tissue. The speed of the Chart Mover was set at 10 mm/min., the minimum speed, and this was employed for all assays. Twenty to thirty minutes were allowed for the tissue to relax fully and the response to doses of histamine diphosphate (3 µg/ml) and antigen in varying concentrations were tested.

(d) Challenge with increasing doses of histamine

The effects of both the alcoholic and aqueous extracts on histamine-induced spasms in the isolated ileum were studied. This was done by assaying increasing doses of histamine on ileum from animals sensitized and receiving water (control), alcoholic extract or aqueous extract. Increasing doses of histamine were obtained by adding 0.1 ml, 0.2 ml, 0.3 ml and 0.4 ml of 10^{-5} M histamine diphosphate solutions to the bath in that order. These gave final bath concentrations of 2.22×10^{-8} M, 4.44×10^{-8} M, 6.67×10^{-8} M and 8.89×10^{-8} M histamine diphosphate respectively. After the addition of one concentration, the tissue was washed two to three times or until the writing point returned to the baseline before a higher dose was added. The time interval between doses was one to two

minutes. Maximum contraction to each dose applied was obtained within 30 seconds. The contractions were expressed as a percentage of maximum contraction to the highest dose of histamine diphosphate ($8.89 \times 10^{-8} \text{M}$).

(c) Challenge with 'cumulative' antigen doses

The challenging antigen (EA) solution, 45 mg/ml prepared fresh in Tyrode solution was diluted serially so as to obtain a final organ bath concentration range of 10^{-8} to 10^{-3} g/ml. A good size response of each mounted tissue was obtained by adding 0.3 ml of 10^{-5}M solution of histamine diphosphate to the bath. After a preliminary experiment in which the addition of the lower doses of 10^{-8} and 10^{-7} g/ml of the antigen to the tissue bath gave no contractions, the range used for subsequent studies was 10^{-6} to 10^{-3} g/ml. The anaphylactic contraction of each piece of ileum was obtained by the addition of these concentrations of egg albumen as antigen to the mounted tissue in increasing order. Tissues were washed 4 to 6 times with Tyrode solution before the next higher concentration of antigen was added. The washings were done by draining downwards. In cases where the writing point failed to return to the base line, the washing was continued until this was achieved. In most cases, the antigen challenge assays were done on the same tissue used for the histamine challenge assay. In this case, the antigen challenge assay was done after the addition of the highest dose of histamine. The anaphylactic contraction of each piece of tissue was expressed as the percentage of the

contraction of that piece to a large dose of histamine (0.3 ml of 10^{-5} M histamine diphosphate). The final bath concentration of histamine was 6.67×10^{-8} M. The expression of the anaphylactic contraction as a percentage of the contraction due to histamine provided a means by which anaphylactic contractions of ileal pieces from different segments of the ileum from the same animal or pieces from different animals could be assessed.

(f) Anaphylactic release of smooth muscle-stimulating substances from lung tissues

Segments of non-sensitized guinea-pig ileum were mounted in an organ bath of aerated Tyrode solution at 37°C . The maximum contraction of each mounted tissue was determined by adding 0.3 ml of 10^{-5} M histamine diphosphate solution. Excised lungs from sensitized animals were washed thoroughly in warm Tyrode solution and portions of the tissue weighing approximately 300 mg (wet weight) were incubated in Tyrode solution at 37°C for at least one hour. These portions of sensitized lung tissue were placed in the organ bath with the mounted segment of non-sensitized guinea-pig ileum. An amount of antigen, 1 ml of 45 mg/ml EA solution, giving a final bath concentration of 10^{-3} g/ml, was added to the bath fluid. A quick, sustained contraction of the ileal muscle similar to that caused by the addition of histamine diphosphate was recorded. The experiment was repeated with tissues from guinea-pigs sensitized and receiving the alcoholic or the

aqueous extracts. Two replicates were prepared from each lung tissue. The contractions of the ileal pieces due to anaphylactic release of smooth muscle-stimulating material from lung tissues were expressed as percentage of contraction to 0.5 ml of $10^{-5}M$ histamine diphosphate solution.

(4) Histamine content of lung tissues:

(a) Extraction of histamine from lung tissues

Approximately 1 g samples of lung tissues were weighed, cut into pieces and homogenized in 9 ml of 0.4 M perchloric acid in an all glass homogenizer. Each homogenate was allowed to stand at room temperature for about ten minutes and then centrifuged at a speed of 1250 g using the bench centrifuge. A 4-ml aliquot of the supernatant fluid was transferred to a boiling tube containing 10 ml n-butanol, 0.5 ml 5M sodium hydroxide and 1.5 g or more of solid sodium chloride. The tube was shaken for at least 5 minutes and centrifuged at 1250 g for 5 minutes. The organic phase was removed into another boiling tube and shaken with 5 ml sodium chloride-saturated 0.1M sodium hydroxide. After centrifugation, an aliquot of 8 ml of the organic phase was removed into another boiling tube containing 4 ml of 0.1M hydrochloric acid and 15 ml n-heptane. The tube was shaken for about 1 minute, centrifuged and the organic phase removed by

Fig. 3a

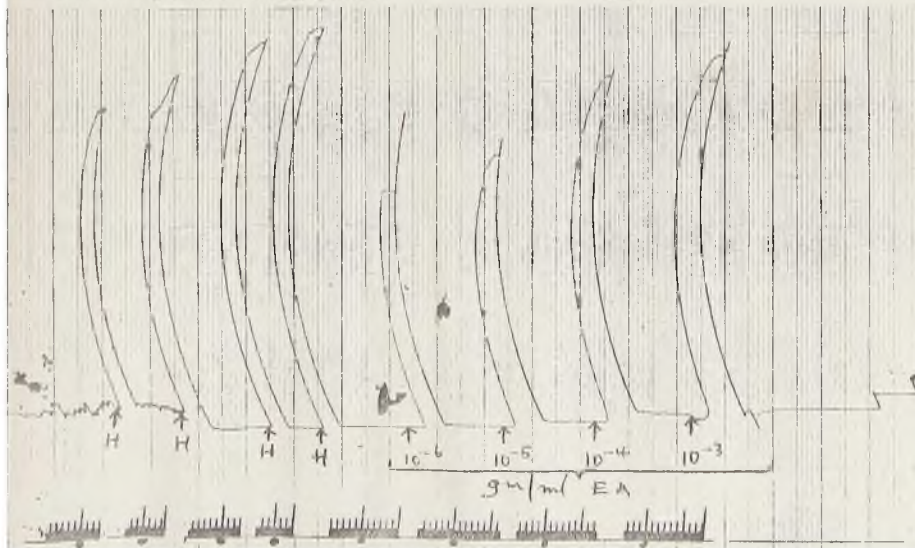
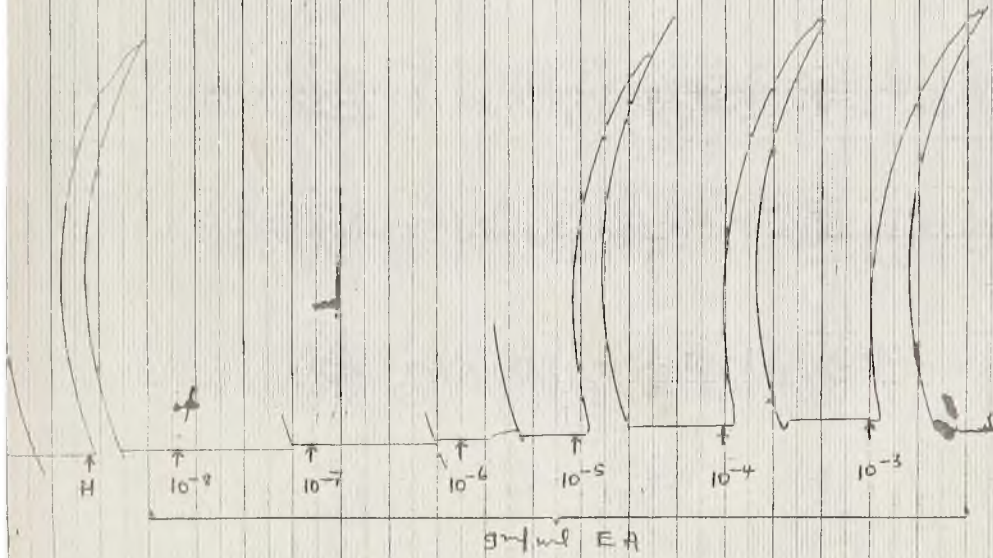
A typical set of recordings of anaphylactic contractions in isolated ileum from guinea-pigs sensitized to EA (antigen). The animals received water during the sensitization period.

The arrows represent points of application of increasing doses of histamine (H) and EA (10^{-8} to 10^{-3} g/ml). Time signal is shown at the base of each curve. Each small division is 10 seconds. The time interval between successive doses of antigen was approximately 10 minutes.

The upper graph shows contractions due to a single dose of histamine and increasing doses of antigen.

The lower graph shows contractions due to increasing doses of both histamine and antigen.

FIG. 3a: RECORDED CONTRACTIONS IN ILEUM FROM SENSITIZED ANIMALS RECEIVING WATER (CONTROL)



aqueous extracts. Two replicates were prepared from each lung tissue. The contractions of the ileal pieces due to anaphylactic release of smooth muscle-stimulating material from lung tissues were expressed as percentage of contraction to 0.3 ml of 10^{-5} M histamine diphosphate solution.

(4) Histamine content of lung tissues:

(a) Extraction of histamine from lung tissues

Approximately 1 g samples of lung tissues were weighed, cut into pieces and homogenized in 9 ml of 0.4 M perchloric acid in an all glass homogenizer. Each homogenate was allowed to stand at room temperature for about ten minutes and then centrifuged at a speed of 1250 g using the bench centrifuge. A 4-ml aliquot of the supernatant fluid was transferred to a boiling tube containing 10 ml n-butanol, 0.5 ml 5M sodium hydroxide and 1.5 g or more of solid sodium chloride. The tube was shaken for at least 5 minutes and centrifuged at 1250 g for 5 minutes. The organic phase was removed into another boiling tube and shaken with 5 ml sodium chloride-saturated 0.1M sodium hydroxide. After centrifugation, an aliquot of 8 ml of the organic phase was removed into another boiling tube containing 4 ml of 0.1M hydrochloric acid and 15 ml n-heptane. The tube was shaken for about 1 minute, centrifuged and the organic phase removed by

aspiration. 2 ml of the acid phase was then transferred to a small test tube for reaction with O-phthalaldehyde (OPT).

(b) Fluorometric assay

To a 2-ml aliquot of histamine solution in a small test tube (prepared as above) 0.4 ml 1M sodium hydroxide was added followed by 0.1 ml OPT reagent. After 4 minutes at room temperature, 0.2 ml 3M hydrochloric acid was added. The tube was shaken after each addition. The solution was then transferred to a fluorometer cuvet and fluorescence at 450 nm resulting from activation at 360 nm was measured in a Turner Spectrofluorometer Model 430. Histamine concentrations in the samples were estimated by using a standard curve for histamine prepared by diluting a histamine (free base) stock solution of 100 $\mu\text{g}/\text{ml}$ (in 0.1M hydrochloric acid) to working standards of 0.25, 0.50, 0.75, 1.00, 1.25, 1.50 and 1.75 $\mu\text{g}/\text{ml}$ in 0.1M hydrochloric acid. The fluorescence as measured in the spectrofluorometer was proportional to histamine concentration over the range indicated.

CHAPTER III

R E S U L T S

A. ANAPHYLACTIC CONTRACTION OF ILEUM

Typical recordings of anaphylactic contractions in the isolated guinea-pig ileum and histamine-induced spasms are shown in Fig. 3. The contractions (expressed as % of contraction to a large dose of histamine) are presented in Tables 1 and 2.

Fig. 3a

A typical set of recordings of anaphylactic contractions in isolated ileum from guinea-pigs sensitized to EA (antigen). The animals received water during the sensitization period.

Note (a): The arrows represent points of application of increasing doses of histamine (H) and EA (10^{-8} to 10^{-3} g/ml). Time signal is shown at the base of each curve. Each small division is 10 seconds. The time interval between successive doses of antigen was approximately 10 minutes.

The upper graph shows contractions due to a single dose of histamine and increasing doses of antigen.

The lower graph shows contractions due to increasing doses of both histamine and antigen.

FIG. 3a: RECORDED CONTRACTIONS IN ILEUM FROM SENSITIZED ANIMALS RECEIVING WATER (CONTROL)

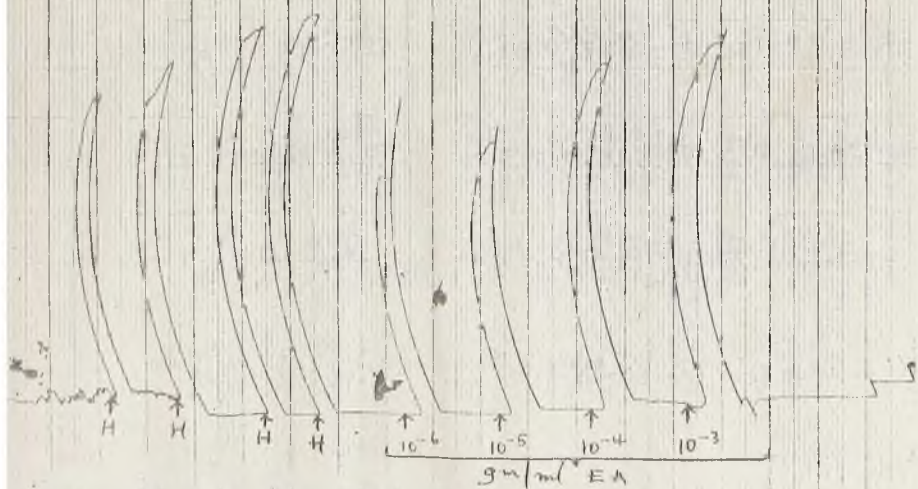
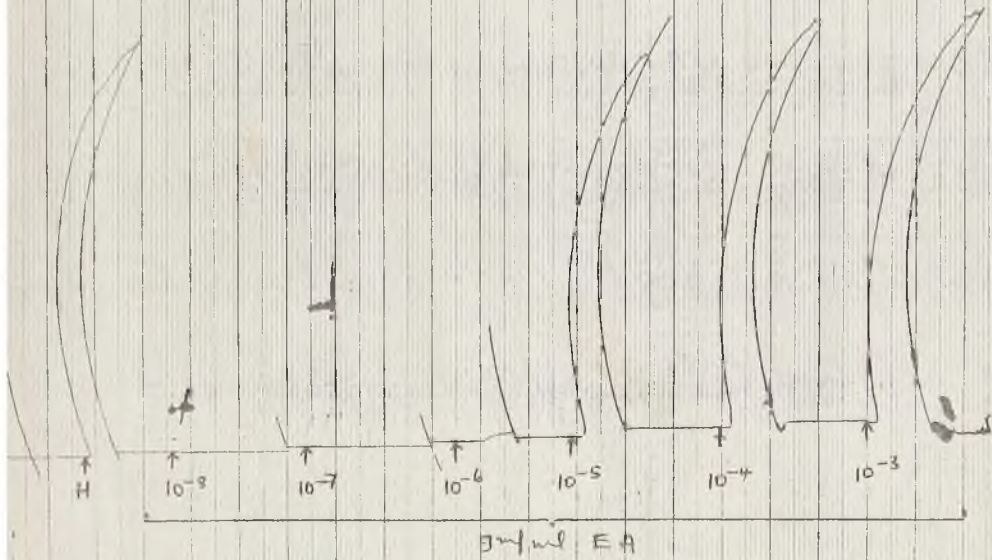


Fig. 3b

A typical set of recordings of anaphylactic contractions in isolated ileum from guinea-pigs sensitized to EA (antigen). The animals received alcoholic extract of D. adscendens during the sensitization period.

The arrows represent points of application of increasing doses of histamine (H) and EA (10^{-8} to 10^{-3} g/ml). Time signal is shown at the base of each curve. Each small division is 10 seconds. The time interval between successive doses of antigen was approximately 10 minutes.

The upper graph shows contractions due to a single dose of histamine and increasing doses of antigen.

The lower graph shows contractions due to increasing doses of both histamine and antigen.

FIG. 3b: RECORDED CONTRACTIONS IN ILEUM FROM SENSITIZED ANIMALS RECEIVING ALCOHOLIC EXTRACT OF DESMODIUM ADSCENDENS.

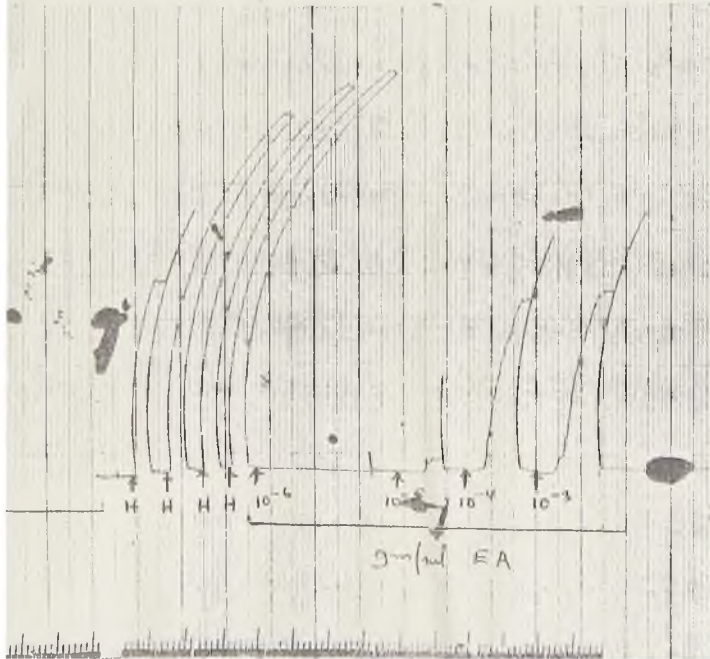
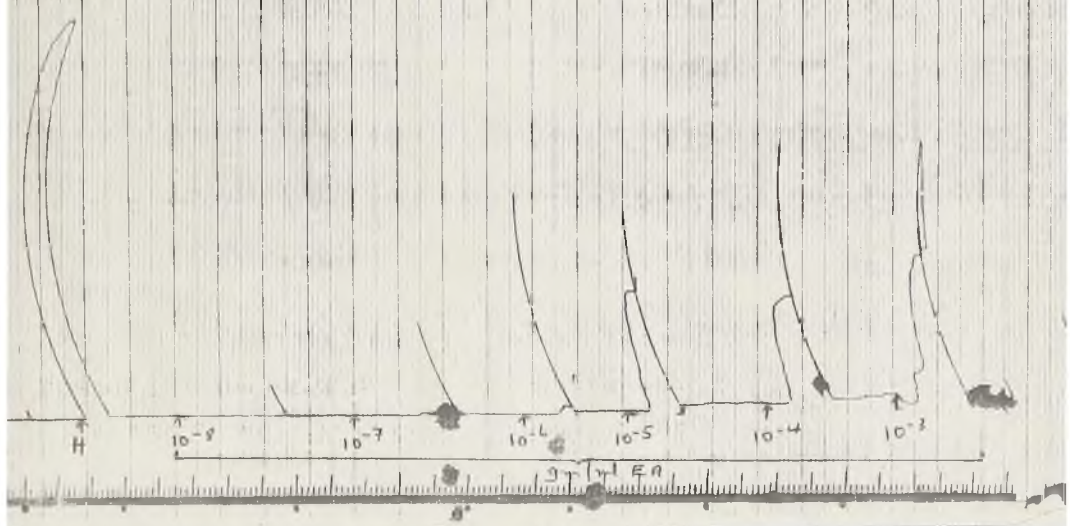


Fig. 3c

A typical set of recordings of anaphylactic contractions in isolated ileum from guinea-pigs sensitized to EA (antigen). The animals received aqueous extract of D. adscendens during the sensitization period.

The arrows represent points of application of increasing doses of histamine (H) and EA (10^{-8} to 10^{-3} g/ml). Time signal is shown at the base of each curve. Each small division is 10 seconds. The time interval between successive doses of antigen was approximately 10 minutes.

The upper graph shows contractions due to a single dose of histamine and increasing doses of antigen.

The lower graph shows contractions due to increasing doses of both histamine and antigen.

FIG. 3C: RECORDED CONTRACTIONS IN ULEUM FROM SENSITIZED ANIMALS
RECEIVING AQUEOUS EXTRACT OF DESMODIUM ASCENDENS.

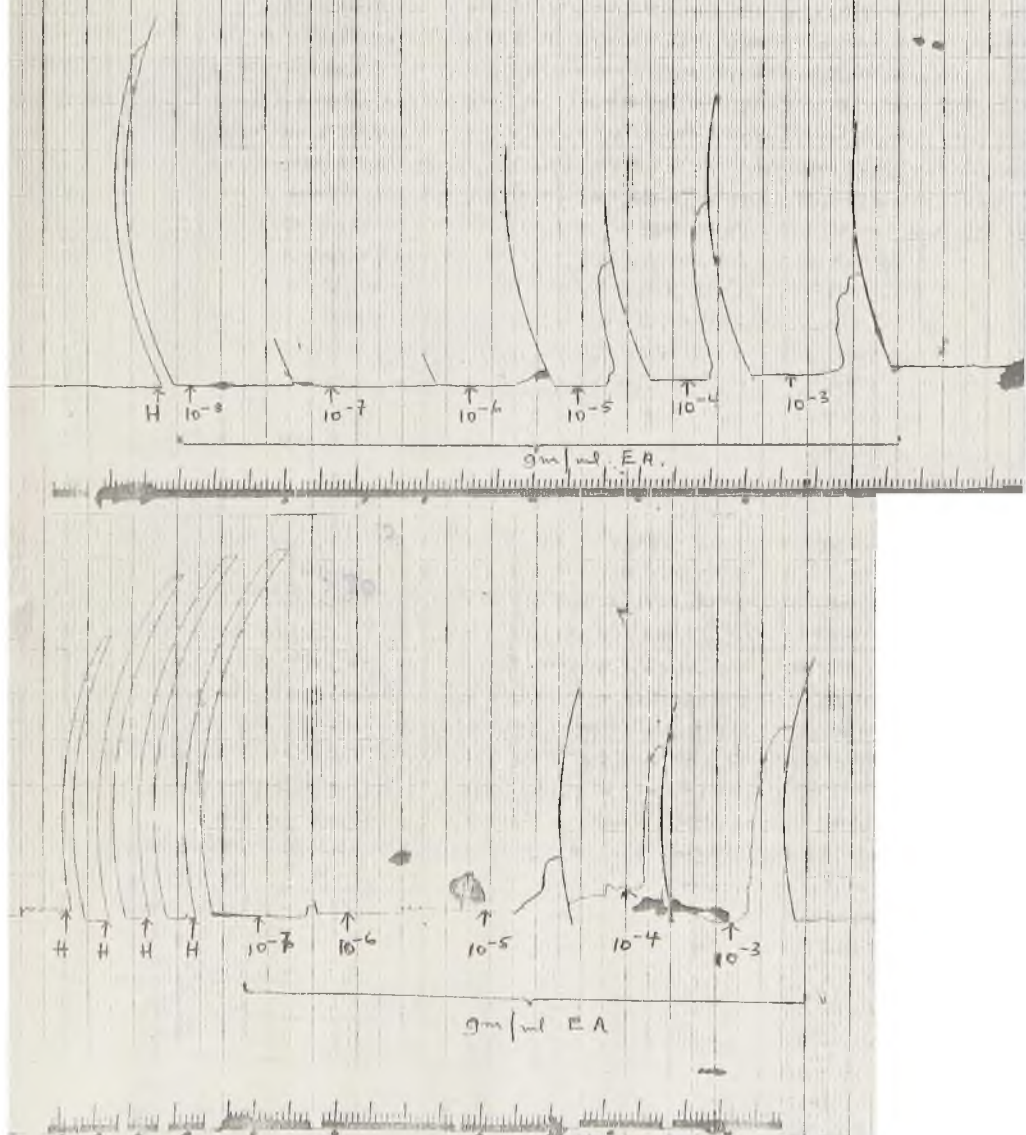


TABLE 1: ANAPHYLACTIC CONTRACTION OF GUINEA-PIG ILEUM. ANIMALS SENSITIZED AND CHALLENGED WITH EGG ALBUMEN AS ANTIGEN, VALUES ARE EXPRESSED AS PERCENTAGE OF CONTRACTION TO HISTAMINE DIPHOSPHATE ($6.67 \times 10^{-8}M$). NUMBERS I-V REFER TO LABEL ON DIFFERENT ANIMALS.

(a) SENSITIZED ANIMALS RECEIVING WATER (CONTROL):

CHALLENGING ANTIGEN DOSE (gm/ml)	I	II	III	IV	V
10^{-6}	27.61	45.51	45.19	1.89	9.52
10^{-5}	62.05	71.99	68.09	86.47	61.90
10^{-4}	67.70	92.41	87.35	100.84	88.89
10^{-3}	71.43	98.83	93.38	100.25	98.41

(b) SENSITIZED ANIMALS RECEIVING ALCOHOLIC EXTRACT OF DESMODIUM ADSCENDENS:

CHALLENGING ANTIGEN DOSE (gm/ml)	I	II	III	IV	V
10^{-6}	2.96	2.31	0	0	0
10^{-5}	38.88	30.66	1.25	3.49	12.11
10^{-4}	34.31	29.52	42.50	44.19	44.08
10^{-3}	32.18	38.77	53.75	46.51	48.00

(c) SENSITIZED ANIMALS RECEIVING AQUEOUS EXTRACT OF DESMODIUM ADSCENDENS:

CHALLENGING ANTIGEN DOSE (gm/ml)	I	II	III	IV	V
10^{-6}	3.39	2.07	4.41	2.47	1.25
10^{-5}	6.78	14.50	11.76	25.81	10.22
10^{-4}	10.17	15.19	26.47	44.05	29.44
10^{-3}	25.42	22.19	13.24	34.59	44.27

TABLE 2: MEAN VALUES OF ANAPHYLACTIC CONTRACTION OF THE GUINEA-PIG ILEUM FROM SENSITIZED ANIMALS RECEIVING WATER, ALCOHOLIC EXTRACT OR AQUEOUS EXTRACT OF *D. ADSCENDENS*: EACH VALUE IS A MEAN OF FIVE DETERMINATIONS, EXPRESSED AS PERCENTAGE OF CONTRACTION TO A HIGH DOSE OF HISTAMINE DIPHOSPHATE (SEE TABLE 1). THE STANDARD ERRORS ARE GIVEN.

CHALLENGING ANTIGEN DOSE (gm/ml)	ANIMALS RECEIVING WATER (CONTROL)	ANIMALS RECEIVING ALCOHOLIC EXTRACT	ANIMALS RECEIVING AQUEOUS EXTRACT
10^{-6}	25.94 ± 8.99	1.05 ± 0.65	2.72 ± 0.55
10^{-5}	70.10 ± 4.51	17.28 ± 7.49	13.81 ± 3.25
10^{-4}	87.44 ± 5.46	39.00 ± 2.97	25.06 ± 5.92
10^{-3}	92.46 ± 5.38	43.84 ± 3.77	27.94 ± 5.32

It can be seen from Table 1 that the dose of antigen (EA) that gave any contraction was 10^{-6} gm/ml. In Table 1b, however, this antigen concentration did not give any contractions in three of the animals. This may be attributed to biological variations. Generally, contractions increased with increasing doses of antigen. A comparison of Tables 1a, 1b, and 1c clearly shows that anaphylactic contraction of the guinea-pig ileum is greatly reduced in the sensitized animals receiving alcoholic and aqueous extracts of D. adscendens. Compared to the control, that is, sensitized animals receiving water, less than 50% of the maximum contraction was obtained in these animals at all antigen concentrations employed. This trend is more clearly seen in Table 2. There is a statistically significant reduction in the anaphylactic contraction in ileum from sensitized animals receiving extracts as compared with those receiving water ($p < 0.05$ for the lowest antigen concentration, 10^{-6} gm/ml and $p < 0.001$ for higher concentrations of 10^{-5} - 10^{-3} gm/ml for both alcoholic and aqueous extracts). Thus at the higher antigen concentrations, differences are highly significant.

Figs. 4, 5 and 6 show the antigen dose-response curves for the individual animals. Fig. 7 shows a plot of the mean contractions (% max) against log dose of antigen. There is a decrease in anaphylactic contraction by 62% and 65% for the aqueous extract and 48% and 49% for the alcoholic extract at higher doses (10^{-4} and 10^{-3} gm/ml respectively).

B. HISTAMINE-INDUCED SPASMS

Table 3 shows contractions (expressed as % of maximum) due to increasing doses of histamine diphosphate.

FIG. 4 DOSE-RESPONSE CURVES SHOWING ANAPHYLACTIC CONTRACTION OF ILEAL PIECES FROM SENSITIZED ANIMALS RECEIVING WATER (CONTROL); A FAMILY OF CURVES SHOWING RESPONSES FROM FIVE DIFFERENT ANIMALS.

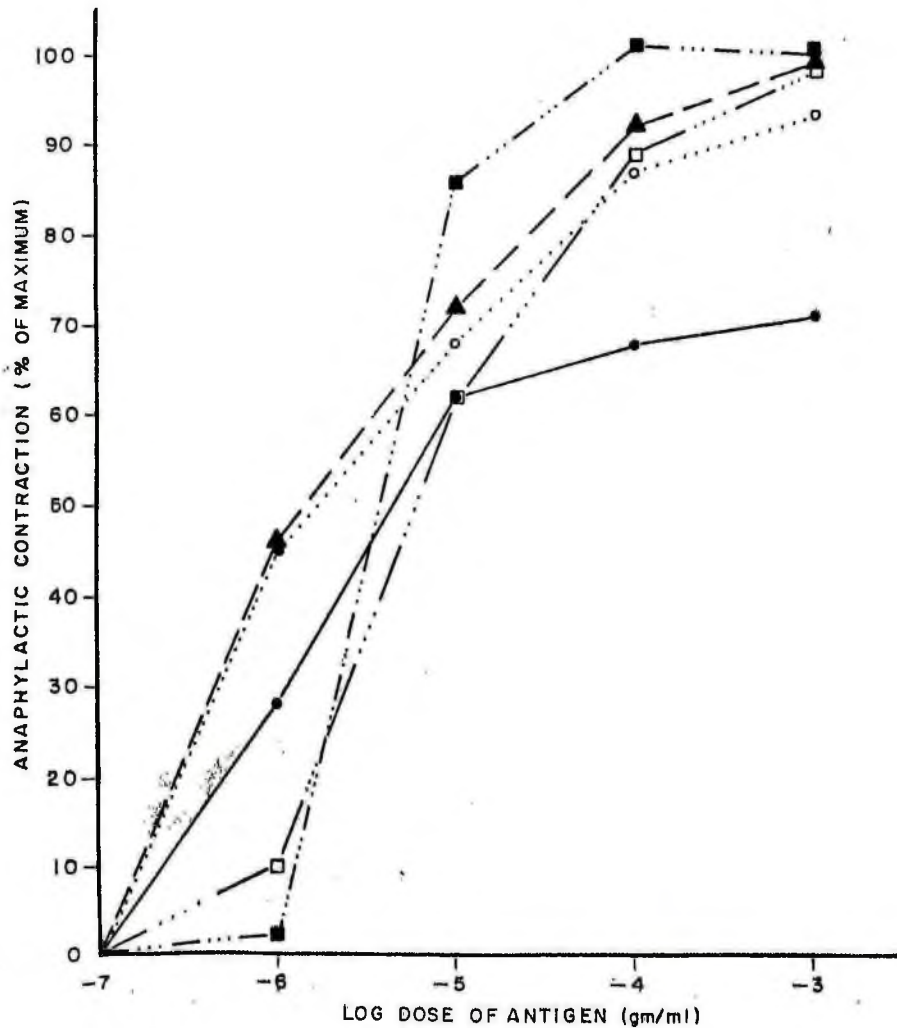


FIG.5
DOSE-RESPONSE CURVES SHOWING ANAPHYLACTIC
CONTRACTION OF ILEAL PIECES FROM SENSITIZED
ANIMALS RECEIVING ALCOHOLIC EXTRACT OF
DESMODIUM ADSCENDENS; A FAMILY OF CURVES
SHOWING RESPONSES FROM FIVE DIFFERENT ANIMALS

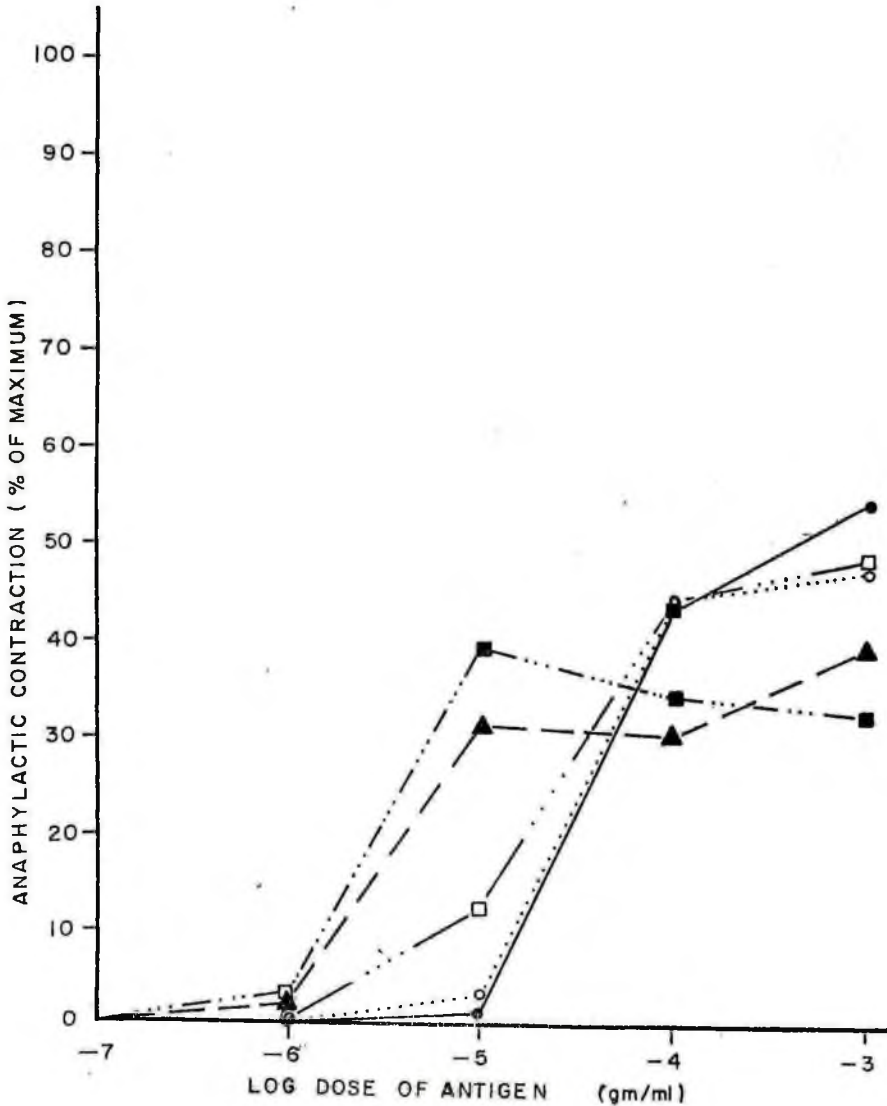


FIG. 6 DOSE - RESPONSE CURVES SHOWING ANAPHYLACTIC CONTRACTION OF ILEAL PIECES FROM SENSITIZED ANIMALS RECEIVING AQUEOUS EXTRACT OF DESMODIUM ADSCENDENS; A FAMILY OF CURVES SHOWING RESPONSES FROM FIVE DIFFERENT ANIMALS.

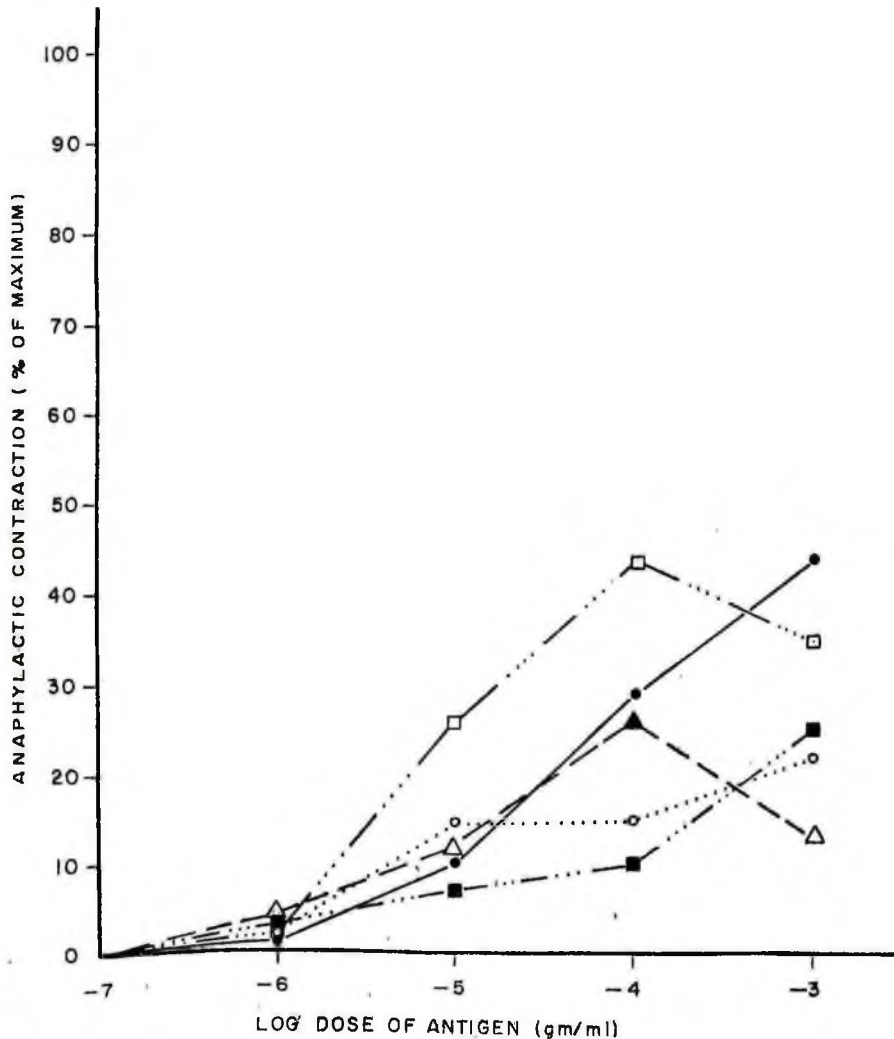


FIG.7 DOSE-RESPONSE CURVES SHOWING THE EFFECT OF EXTRACTS OF DESMODIUM ADSCENDENS ON ANAPHYLACTIC CONTRACTION OF ILEAL PIECES FROM SENSITIZED ANIMALS; VALUES ARE MEANS OF DETERMINATIONS IN FIVE SEPARATE ANIMALS. STANDARD ERRORS ARE INDICATED.

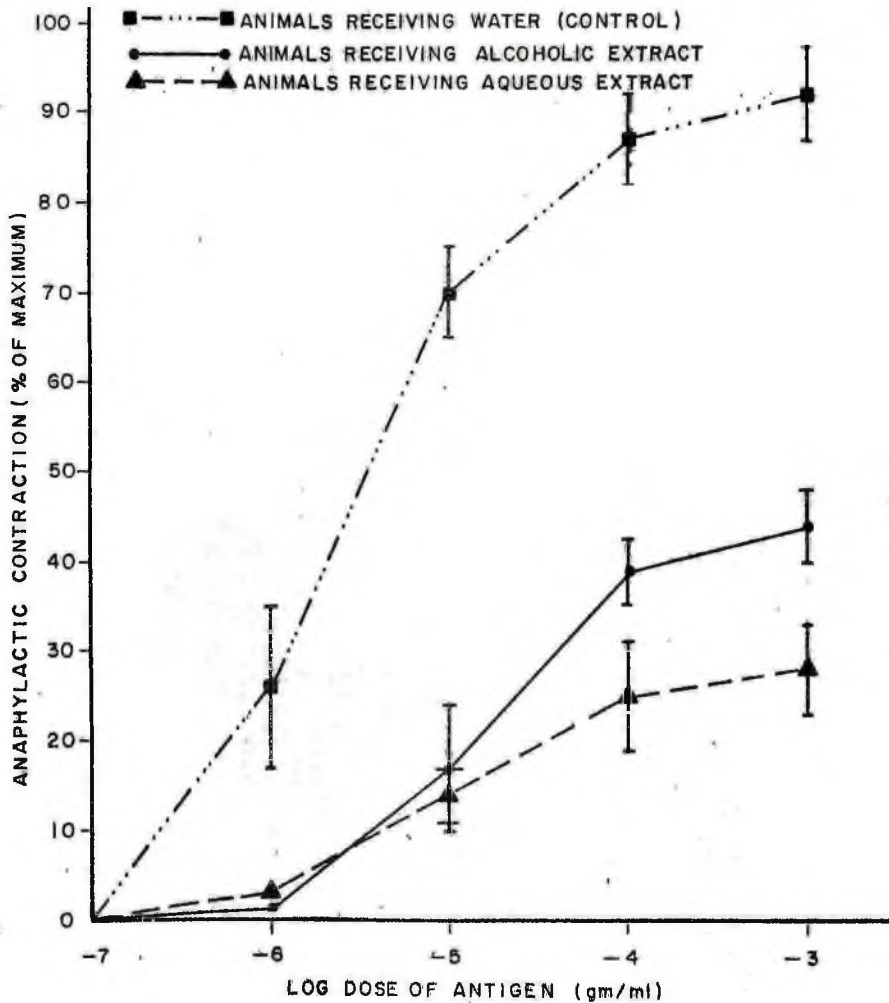


TABLE 3: MEAN VALUES OF HISTAMINE-INDUCED CONTRACTION OF THE GUINEA-PIG ILEUM FROM SENSITIZED ANIMALS RECEIVING WATER, ALCOHOLIC EXTRACT OR AQUEOUS EXTRACT OF *D. ADSCENDENS*: EACH VALUE, A MEAN OF FIVE DETERMINATIONS, IS EXPRESSED AS PERCENTAGE OF CONTRACTION TO THE HIGHEST DOSE OF HISTAMINE DIPHOSPHATE ($8.89 \times 10^{-8}M$). THE STANDARD ERRORS ARE GIVEN.

HISTAMINE DOSE ($\times 10^{-8}M$)	ANIMALS RECEIVING WATER(CONTROL)	ANIMALS RECEIVING ALCOHOLIC EXTRACT	ANIMALS RECEIVING AQUEOUS EXTRACT
2.22	75.57 \pm 3.23	62.50 \pm 5.74	64.23 \pm 2.04
4.44	92.27 \pm 1.32	87.05 \pm 3.86	85.77 \pm 2.39
6.67	97.75 \pm 0.67	96.17 \pm 1.40	94.50 \pm 0.86
8.89	100.00 \pm 0.00	100.00 \pm 0.00	100.00 \pm 0.00

The values in the table indicate that at low histamine diphosphate concentrations ($2.22 \times 10^{-8}M$ and $4.44 \times 10^{-8}M$) contractions elicited in ileal pieces from sensitized animals receiving extracts of D. adscendens are similar and lower than those from sensitized animals receiving water. There is therefore a reduction in histamine-induced spasms in ileum from the extract-treated animals at low histamine diphosphate concentrations. The differences are statistically significant ($p < 0.05$ and $p < 0.01$ for the alcoholic and aqueous extracts respectively) at the lower histamine diphosphate concentrations of $2.22 \times 10^{-8}M$. At the higher concentrations of $4.44 \times 10^{-8}M$ and $6.67 \times 10^{-8}M$, however, the differences are only significant for the aqueous extract ($p < 0.05$). Fig. 8 shows the histamine diphosphate dose-response curves for the three groups of animals. The curves show that both the alcoholic and aqueous extracts of D. adscendens reduced histamine-induced spasms in the guinea-pig ileum.

C. ANAPHYLACTIC RELEASE OF MEDIATORS OF SMOOTH MUSCLE CONTRACTION FROM LUNG TISSUE

Fig. 9 shows typical recordings of contractions of non-sensitized ileum due to the anaphylactic release of smooth muscle stimulating substances from the lungs of sensitized and treated animals. The contractions, expressed as percentages of contraction to $6.67 \times 10^{-8}M$, histamine diphosphate are presented in Table 4.

FIG. 8 DOSE-RESPONSE CURVES SHOWING THE EFFECTS OF EXTRACTS OF DESMODIUM ADSCENDENS ON HISTAMINE-INDUCED CONTRACTION OF ILEAL PIECES FROM SENSITIZED ANIMALS; VALUES ARE MEANS OF DETERMINATIONS IN FIVE SEPARATE ANIMALS. STANDARD ERRORS ARE INDICATED.

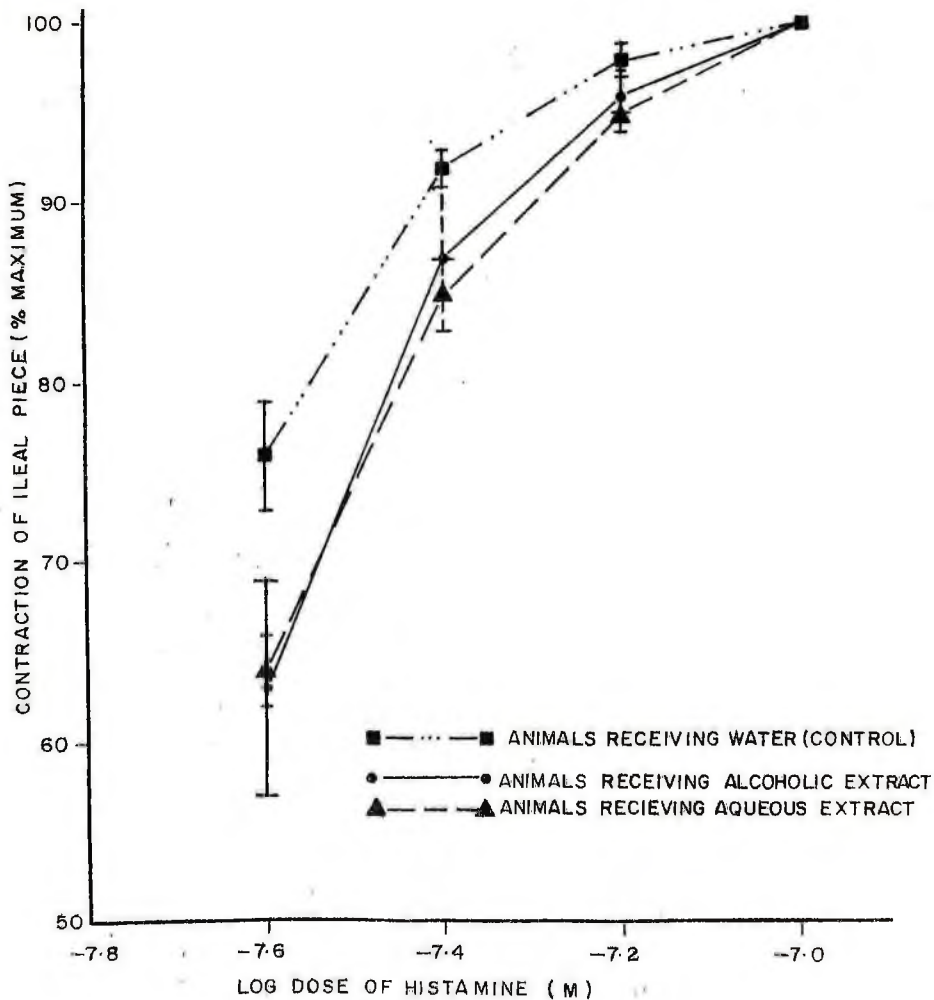


Fig. 9

A typical set of recordings of contraction of isolated non-sensitized guinea-pig ileum due to the anaphylactic release of smooth muscle-stimulating substances from the guinea-pig lung.

The arrows represent the points of application of antigen (10^{-3} g/ml EA). 'H' indicates the point of addition of histamine (0.3 of 10^{-5} M histamine diphosphate solution). 'A' represents contractions due to release of substances from lung tissue of sensitized animals receiving aqueous extract of D. adscendens; 'B' represents contractions due to release of substances from lung tissue from sensitized animals receiving water (control); 'C' represents contractions due to release of substances from lung tissue of sensitized animals receiving alcoholic extract of D. adscendens. The time signal is shown at the base of each curve. Each small division is 10 seconds. The time interval between additions of antigen was approximately 10 minutes.

CONTRACTIONS IN NON-SENSITIZED ILEUM DUE TO ANAPHYLACTIC RELEASE OF MEDIATORS FROM LUNGS OF SENSITIZED ANIMALS RECEIVING WATER, ALCOHOLIC EXTRACT OR AQUEOUS EXTRACT OF BERMUDIUM ABSCONDENS

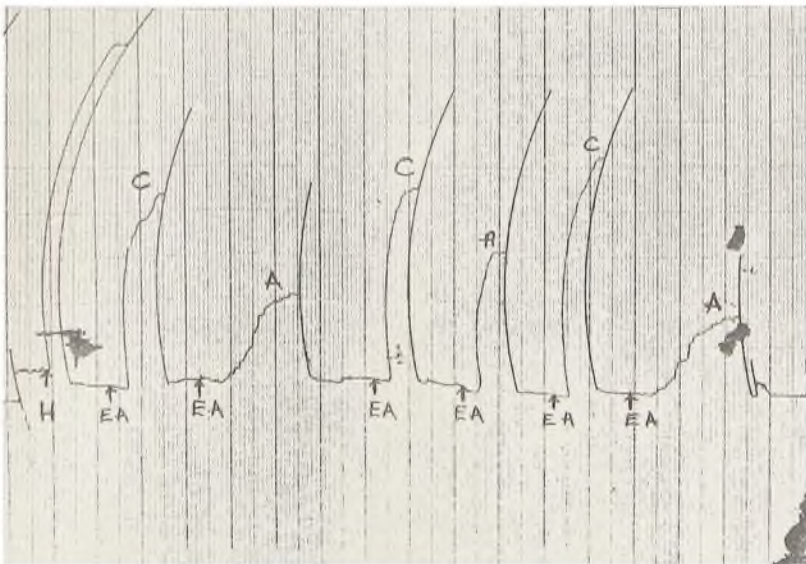


TABLE 4: CONTRACTION OF NON-SENSITIZED GUINEA-PIG ILEUM DUE TO ANAPHYLACTIC RELEASE OF SMOOTH MUSCLE-STIMULATING SUBSTANCES FROM LUNG TISSUE. THE SENSITIZED ANIMALS RECEIVED WATER, ALCOHOLIC EXTRACT OR AQUEOUS EXTRACT OF D. ADSCENDENS. VALUES, EXPRESSED AS PERCENTAGES OF CONTRACTION TO HISTAMINE DIPHOSPHATE ($6.67 \times 10^{-8}M$), ARE MEANS OF TWO DETERMINATIONS IN THE SAME ANIMAL.

SOURCE OF LUNG TISSUES		
FROM ANIMALS RECEIVING WATER (CONTROL)	FROM ANIMALS RECEIVING ALCOHOLIC EXTRACT	FROM ANIMALS RECEIVING AQUEOUS EXTRACT
77.78	64.81	31.48
89.66	59.65	46.88
73.66	55.17	31.48
79.47	22.22	22.26
69.23	48.72	28.46
77.96 \pm 3.42	50.11 \pm 7.46	32.11 \pm 4.06

It can be seen from the table that higher contractions were recorded for the sensitized animals receiving water than for the sensitized animals receiving the extracts of D. adscendens. It is also clear from the table that values for the sensitized animals receiving aqueous extract are lower than those for sensitized animals receiving alcoholic extract. The differences between the control values and the values for the alcoholic extract-treated, and aqueous extract-treated animals are statistically significant ($p < 0.05$ and $p < 0.001$ respectively). The differences between the values for the alcoholic extract-treated animals and the aqueous extract-treated animals are also statistically significant ($p < 0.05$). Fig. 10 is a histogram showing the effects of D. adscendens treatment on the anaphylactic release of pharmacologically active substances from the guinea-pig lung.

D. HISTAMINE CONTENT OF LUNG TISSUE

The histamine content of lung tissues from the three groups of animals, determined fluorometrically, are shown in Table 5. It can be seen from the table that the histamine content of lung tissues from sensitized animals receiving water are higher than those from sensitized animals receiving extracts of D. adscendens. A comparison of the mean values show that the differences between control and test values are

HISTOGRAM SHOWING THE
FIG. 10 EFFECT OF EXTRACTS OF DESMODIUM ASCENDENS ON ANAPHYLACTIC RELEASE OF SMOOTH MUSCLE-STIMULATING SUBSTANCES FROM THE GUINEA-PIG LUNG. THE VERTICAL LINES INDICATE STANDARD ERRORS.

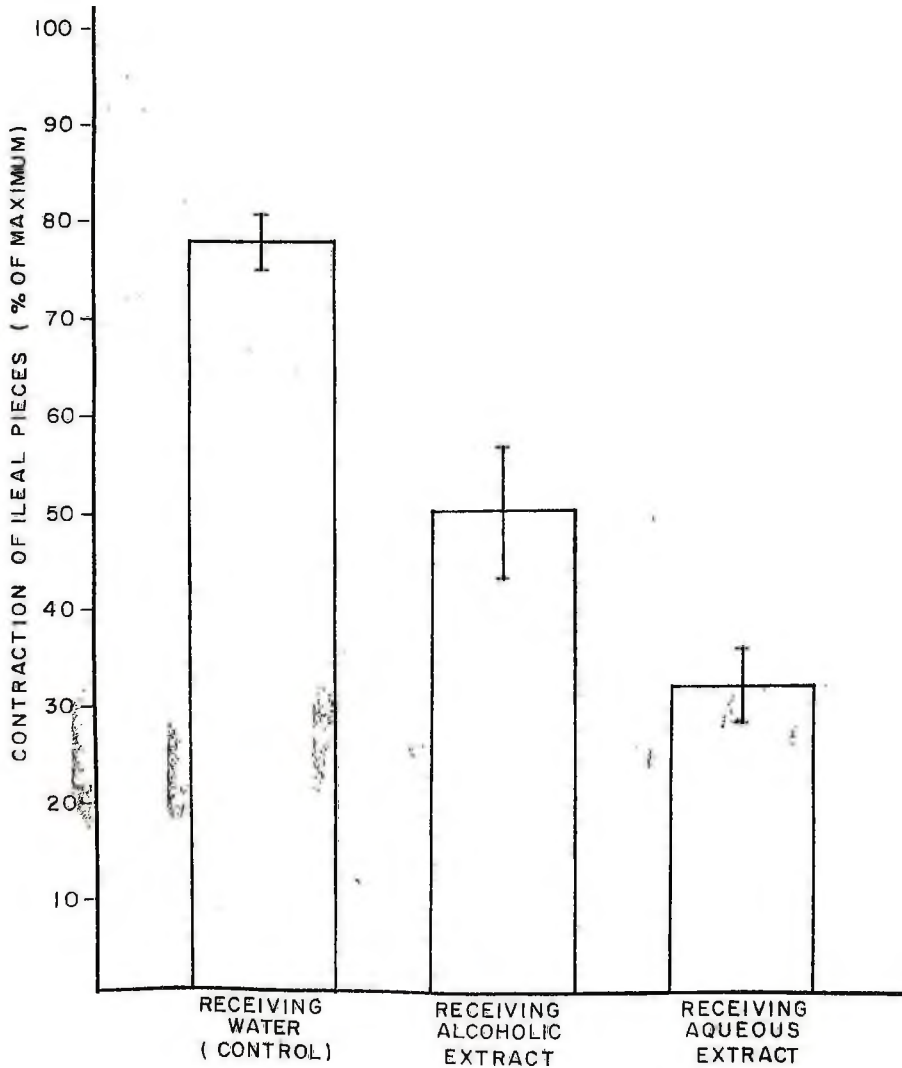
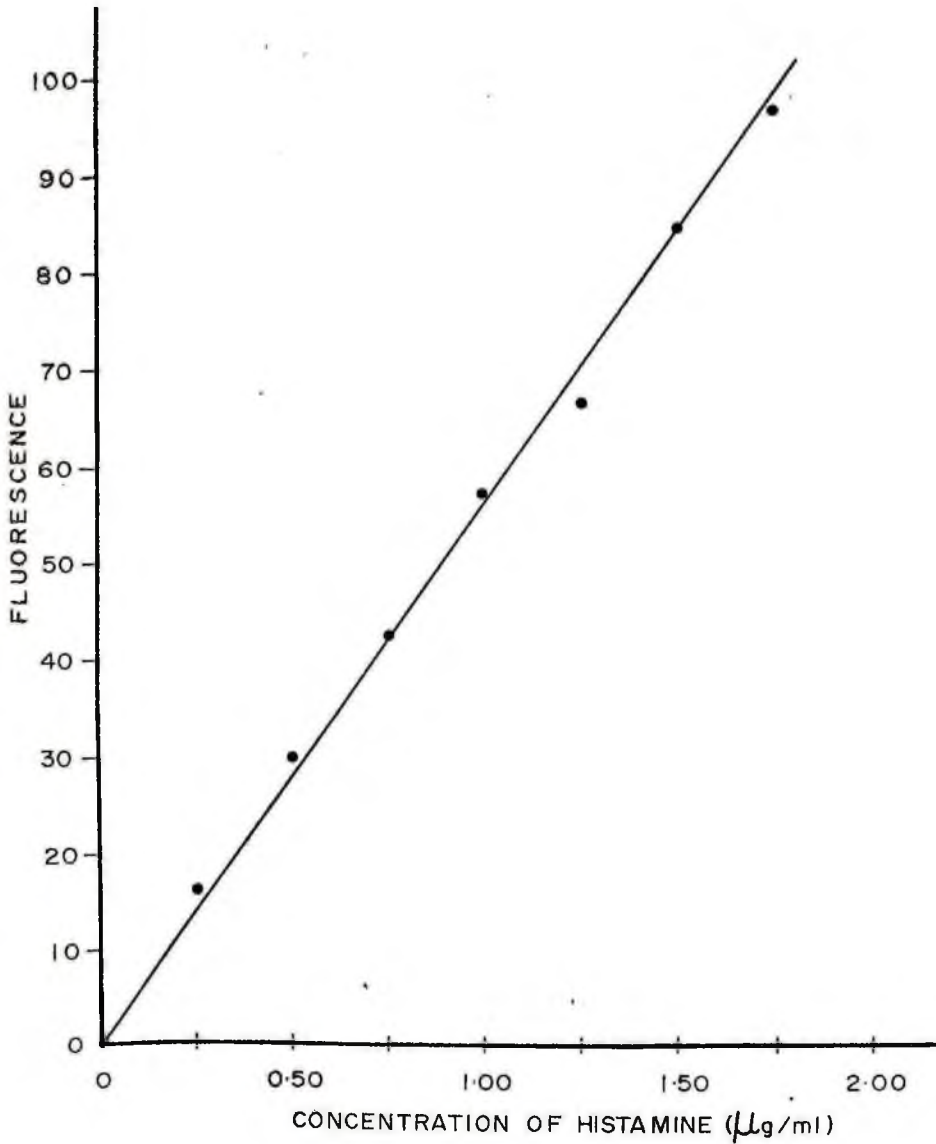


TABLE 5: FLUOROMETRIC DETERMINATION OF HISTAMINE CONTENT OF LUNG TISSUE FROM SENSITIZED ANIMALS RECEIVING WATER OR EXTRACTS OF D. ADSCENDENS; VALUES ARE EXPRESSED AS MICROGRAMMES PER GRAMME WET WEIGHT OF LUNG TISSUE. EACH VALUE REPRESENTS DETERMINATION FROM ONE ANIMAL.

ANIMALS RECEIVING WATER (CONTROL)	ANIMALS RECEIVING ALCOHOLIC EXTRACT	ANIMALS RECEIVING AQUEOUS EXTRACT
7.72	5.51	3.38
7.85	2.18	3.24
5.87	0.97	2.30
4.84	1.78	1.55
4.68	1.80	0.61
6.19 ± 0.68	2.45 ± 0.79	2.21 ± 0.52

statistically significant ($p < 0.005$ for alcoholic extract-treated animals and $p < 0.001$ for aqueous extract-treated animals). The values for the control compare favourably with those found in the literature (5 - 25 $\mu\text{g}/\text{gm}$ tissue). The standard curve for the estimation of histamine content of lung tissues is shown in Fig. 11. There is a linear relationship between fluorescence readings and concentration of histamine over the range indicated.

Fig. II STANDARD CURVE FOR HISTAMINE DETERMINATION;
FLUORESCENCE AGAINST CONCENTRATION OF HISTAMINE



CHAPTER IV

D I S C U S S I O N

The study of inhibitors of anaphylactic reaction and the investigation of the anti-anaphylactic effects of various drug preparations, including local plant extracts used for treating asthma and other allergic conditions can be expected to yield information about the underlying mechanisms of allergies. It can also provide methods for the discovery of potentially useful compounds for the treatment of allergic conditions (Mongar and Schild, 1956).

Studies have been done on the sensitivities of various smooth muscle preparations to pharmacological mediators, particularly histamine (Mahajani and Kulkarni, 1977). The effects of herbal preparations on these have also been studied. Inhibitory effects of sympathomimetic amines on histamine-induced bronchoconstriction, allergic bronchospasms and antigen-induced release of mediators from shock organs, particularly the lungs, provided useful methods for investigating the anti-allergic properties of various drugs and herbal preparations which have been acclaimed to be anti-asthmatic (Assem and Schild, 1969).

Studies employing separate pieces of ileum, which is the usual procedure for the study of anaphylactic reactions cannot provide very useful information on a potentially anti-anaphylactic agent. The finding that the anaphylactic desensitization produced by moderate doses of antigen

is a dose-dependent phenomenon surmountable by increase in the antigen dose, and reversible simply by washing in physiological solution, makes a new and quantitative approach to the study of the Schultz-Dale reaction possible (Okpako, 1970). The procedure described here, which involves the assay of increasing doses of antigen on the same ileal piece, provides a method for easy construction of dose-response curves. The approach ~~limits~~ the number of separate tissues needed to obtain a dose-response curve, and therefore, reduces the inherent error due to variations in sensitivity of pieces from different sections of the same intestine.

In spite of the finding that combinations of D. adscendens, Thonningia sanguinea and Deinbollia pinnata reduced attacks in asthmatics, research work on the scientific basis for the therapeutic action of these plants has not been done until recently. Previous work on D. adscendens in this laboratory indicated that the anaphylactic reactions, using isolated guinea-pig ileum, was inhibited by extracts of the plant added to the tissue bath (Addy and Gbewonyo, 1980). In that study extracts were added to the bath prior to the addition of antigen. However, at the CSRPM, the extracts are given orally to the asthmatics. Because of the oral administration, the animals were made to drink the extracts in this study.

Results from the present studies show that the extracts of D. adscendens inhibit the anaphylactic contraction of the guinea-pig ileum and have also provided more evidence for the earlier indication of the anti-anaphylactic property of the plant extracts.

The anaphylactic contraction of the guinea-pig ileum is significantly reduced when the sensitized animals received either the alcoholic or aqueous extracts of D. adscendens (Fig.7). At higher antigen concentrations there is enough challenging antigen to react with cell-fixed antibodies resulting in mast cell degranulation and mediator release. Alonso-de-Florida et al (1968), showed that antigens apart from reacting with cell-fixed antibodies also exert a direct action on the permeability of the muscle membrane. At the lower antigen concentration, it is probable that the antigen acts directly on the smooth muscle (ileum) membrane thus enhancing the permeability to inorganic ions and hence muscle contraction. The contraction at higher doses of challenging antigen is more the effect of mediator released as a consequence of antigen-antibody reaction. It is clearly seen that the extracts inhibit both the anaphylactic contractions as well as the contraction which may be due to the direct action of the antigen on the ileum.

An interesting finding of these experiments is the effect of extracts on histamine-induced spasms in the ileum. The extracts have the ability to reduce the sensitivity of the smooth muscles to histamine (Fig.8). There is a reduction in the histamine-induced spasms in ileum from animals receiving alcoholic and aqueous extracts of D. adscendens. Significant reductions are observed at lower histamine concentrations. Earlier

investigations in which the plant extract was added to the tissue bath indicated that the extracts were devoid of anti-histaminic activity with respect to the extent of the contraction (Gbewonyo, 1980). However, the time taken for the tissue to reach maximum contraction was longer when extract was present indicating some interaction between the added extract and the histamine receptors. One could infer from the present results that the extracts ^{probably} affect histamine receptors. The extracts may have the ability to combine with these receptors on smooth muscle. Thus, at low histamine concentrations there is little histamine-receptor interaction. Some of the histamine receptors are free to react with the extract and the effect of the latter is shown. At high histamine concentrations, however, all receptors would have been occupied and therefore no effect of the extract is observed. This indicates a competitive type of reaction.

Asthma is a disease characterised by an increased responsiveness of the trachea and bronchi to various stimuli. Drugs and herbal preparations like extracts of D. adscendens may be beneficial by virtue of their ability to reduce this increased responsiveness of the tracheo-bronchial smooth muscle. Since the manifestation of the allergic reactions occurs as a result of the anaphylactic release of the pharmacological mediators, any drug or herbal preparation which can reduce the amount of these mediators will be useful in the treatment of allergic conditions including asthma. D. adscendens extracts, if used prophylactically prevent or minimise asthmatic attacks (Ampofo, 1976). It has,

however, been observed in clinical trials that once there is an attack treatment with the extract is ineffective (Ampofo, 1977). This is an indication that the extract probably acts by interfering with a stage in the sequence of events leading to attack, other than the stage of bronchial smooth muscle contraction, when the mediators would have been released. The interference of the extracts with mediator release is of special significance as far as allergic hypersensitivity is concerned. For hypersensitive individuals it is the released mediators which cause the harm. A drug which is capable of interfering with the amount of mediator released is important.

As is evident from the results there is a marked reduction in the amount of mediators of smooth muscle activity released from the lungs of the animals receiving extracts, the aqueous extract being more effective than the alcoholic extract. This may suggest that the extracts interfere with some or all of the steps leading to the release of mediators. Considering anaphylaxis, the possible levels of action of the extracts in reducing the amount of these smooth muscle-stimulating substances released are as follows:

- (a) decrease in cell-bound antibody level, that is, the actual quantity of antibodies produced as a result of the sensitization.
- (b) the binding of these cytotoxic antibodies to mast cells.

- (c) interaction of challenging antigen and mast cell-bound antibody.
- (d) degranulation of mast cell after the binding of the antigen and hence the release of pharmacological mediators (Fig.2).

Further investigation into these possible interactions would contribute to a more detailed knowledge of the scientific basis for the therapeutic action of the plant material. On the other hand, the plant could work by reducing the actual amounts of these smooth muscle-stimulating substances in the mast cell.

Assem (1974) has shown that beta stimulants inhibit histamine-forming capacity of human leucocytes and suggested the role of cellular levels of c-AMP as the key factor. The results obtained for the histamine content in lungs show that there is a reduction in the amount of histamine in the lungs of the animals receiving the extracts. The differences between the control animals and the test animals were significant. The mean value for the control animals fall within the range given in the literature. The reduced histamine content in treated animals implies that the extracts inhibit the histamine-forming capacity of the cells. Cellular concentrations of c-AMP and enzymes may be implicated here. The action of adrenoceptor stimulants is thought to be related to their ability to increase the level of c-AMP in the smooth muscle cells (Tomiyama et al, 1973). Drugs

inhibiting c-AMP phosphodiesterase enzyme activity and thereby increasing cellular levels of c-AMP are known to potentiate the effect of beta stimulant drugs. It is therefore possible that the extracts may have an inhibitory effect on c-AMP phosphodiesterase enzyme. However this property of the extract needs further investigation. A combination of the inhibitory effect of the extracts on mediator release and a reduction in histamine content of lung tissue will make the plant more useful as an anti-asthmatic.

The results show that the aqueous extract was more potent in inhibiting the various parameters studied, than the alcoholic extract. The former might therefore contain more active principles than the latter. The water used as solvent might have extracted more active compounds than the alcohol (96% ethanol). The aqueous extract was found to grow mouldy after two days. However, the fact that the extracts were kept in the refrigerator and diluted daily before they were given to the animals would have eliminated any effect due to mouldiness.

An attempt was made to investigate the effect of extract on contractions due to substances other than histamine. The addition of mepyramine maleate (2.5×10^{-6} M, final bath concentration) and atropine sulphate (3×10^{-6} M, final concentration) to the assay

system used produced contractions which were too small to be used for comparison. A different assay system may be used to investigate this.

If one considers all the effects of oral administration of extracts of D. adscendens presented here, i.e. (i) reduced anaphylactic contraction of the ileum; (ii) apparent interaction with histamine receptors; (iii) reduction in the amount of mediators, of type-1 hypersensitivity released, and (iv) reduced content of histamine in the lung tissue, as modes of action of the plant, one cannot help but believe the extraordinary potential of D. adscendens as an anti-asthmatic plant.

SUGGESTIONS FOR FURTHER WORK

The present work has opened up more areas for further research.

The phytochemical evaluation of D. adscendens has not yet been done.

However, the medicinal value of the other species of *Desmodium* has been ascribed to the constituent alkaloids. The results from the present work have shown that both alcoholic and aqueous extracts of the plant inhibit anaphylaxis, the aqueous extract being more effective than the alcoholic extract.

There is therefore the need for further work to identify the fraction(s) in the extracts that are responsible for this inhibitory action. This may involve chromatographic and detailed phytochemical evaluation. This will show the difference, if any, between the alcoholic and aqueous extracts.

Since intracellular c-AMP levels are known to regulate the anaphylactic release of mediators of smooth muscle contraction, it may be necessary to investigate the effects of the extracts on cellular c-AMP levels to ascertain the mode of action of the plant material.

The present work also suggests the possibility of the extracts interfering with histamine receptors. This should be confirmed by further studies employing lower doses of histamine.

There is evidence from the present studies that the extracts of D. adscendens inhibit the anaphylactic release of mediators from the lungs of sensitized guinea-pigs. Lung perfusion studies may be carried out to

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give additional evidence for this all important effect. There are various steps leading to the release of mediators and it is possible that the plant may work at one or more points along the line. Investigations should therefore be carried out to find the point(s) or level(s) at which the plants works.

The extracts inhibited the anaphylactic contraction of the isolated ileum. Since the extracts are used in the treatment of asthma, it is suggested that similar studies be done using the tracheal chain, a tissue belonging to the respiratory tract and directly involved in the expression of the asthmatic symptoms. A study of the ability of the extracts to inhibit histamine-induced bronchoconstriction may also be done.

SRS-A is known to be important in allergic asthma. The effect of the extracts on the release of this particular mediator from guinea-pig lung subsequent to antigen challenge can be investigated. This may involve the use of substances like atropine, mepyramine and methysergide to block the effect of other mediators.

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A P E N D I X

Preparation of reagents, buffers and physiological solutions, Glass-distilled water was used in all cases.

(1) Egg albumen (EA)

Three concentrations of egg albumen were prepared as follows:

(a) 1 g of the protein was dissolved in 10 ml of sterile isotonic saline (prepared by dissolving 0.9 g of sodium chloride in 100 ml of distilled water to give a 0.9% solution; this was autoclaved at a pressure of 15 lb./sq. in. and a temperature of 121°C for 15 minutes). The resulting solution, 100 mg/ml EA was used in sensitizing the guinea-pigs.

(b) 0.05 g of the protein was dissolved in 10 ml of sterile saline. The resulting solution, 5 mg/ml, was used as a booster.

(c) 0.45 g of the protein was dissolved in 10 ml of Tyrode solution. This was diluted serially and served as challenging antigen solutions for the bioassay.

(2) Citrate buffer, pH 4.0

Solution A: 0.1M citric acid; 2.104 g of citric acid monohydrate was dissolved in a little amount of glass-distilled water and made up to 100 ml.

Solution B: 0.1M sodium citrate; 2.941 g of Trisodium citrate dihydrate was dissolved in a little amount of glass-distilled water and made up to 100 ml.

59 ml of solution A, prepared as above was mixed with 41 ml of solution B to make 100 ml of buffer solution. The pH was checked with a pH meter.

(3) Histamine solutions

(a) 30 mg/ml histamine stock; 0.3 gm of histamine diphosphate was dissolved in 10 ml of citrate buffer. This was diluted to a working standard of 3 $\mu\text{g/ml}$ (10^{-5}M) when required for ^{bio} assay.

(b) 100 $\mu\text{g/ml}$ histamine stock; 1 mg histamine (free base) was dissolved in 10 ml of 0.1M. This was diluted, when required, to working standards for the preparation of a standard curve for histamine determination, in lung tissues, fluorometrically.

(4) Tyrode solution

A stock Tyrode solution containing sodium chloride, potassium chloride, magnesium chloride, sodium dihydrogen phosphate and sodium hydrogen carbonate was prepared as follows:

Salt	NaCl	KCl	MgSO ₄ .7H ₂ O	NaH PO ₄ .2H ₂ O	NaHCO ₃
Weight (g)	160	4.0	2.0	1.0	20

The above weights of the various salts were dissolved in a minimum volume of glass-distilled water and made up to one litre.

Stock solutions of glucose and calcium chloride were prepared separately:

Glucose (10%): 50 g in 500 ml of distilled water.

Calcium Chloride (anhydrous, 1M): 55.5 g in 500 ml of distilled water. The stock solutions were appropriately diluted to give the complete Tyrode physiological solution. Fresh solution was prepared each time the experiment was performed. The following volumes of stock solutions, prepared as above, were added and diluted to 2 litres:

Solution	Stock Tyrode	10% Glucose	1M CaCl ₂
Volume(ml)	100	20	4.4

The CaCl₂ solution was added last, after most of the water had been added. This was done to avoid the risk of precipitating poorly soluble calcium salts.

(5) O-Phthalaldehyde (OPT) reagent

50 mg OPT was dissolved in 5 ml reagent grade, acetone-free methanol to give a solution of concentration 10 mg/ml. This reagent was used for fluorometric histamine determination. Fresh solutions were prepared as needed.

All stock solutions were stored in the refrigerator until required.

CURRICULUM VITAE

The author was born on the 18th day of May, 1952 at Gbi-Bla, near Hohoe in the Volta Region of Ghana. He attended the St. Francis' Demonstration Primary School, Hohoe from 1957 to 1963 and the Roman Catholic Boys' Middle School, Hohoe from 1963 to 1967, where he obtained the Middle School Leaving Certificate.

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