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Acute systemic anaphylaxis in the horse

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Summary

1. Histamine in small doses caused systemic depressor responses in horses, whereas greater doses caused biphasic effects. All doses of 5-hydroxytryptamine (5-HT) were pressor and all doses of bradykinin depressor. All three active substances raised pulmonary artery pressure and lowered central venous pressure. 5-HT reduced ventilation volume. Histamine caused brief apnoea followed by hyperpnoea only.

2. Acute anaphylaxis in the horse was accompanied by a severe systemic arterial depressor response, a pressor response in the pulmonary artery and vena cava, and alternating phases of apnoea and dyspnoea.

3. During anaphylaxis, profound haemoconcentration, leucopoenia, thrombocytopoenia and hyperkalaemia were in evidence. Early during anaphylactic shock (2 to 4 min) there were profound increases in plasma histamine (five to six-fold) and plasma kinin activity (four to five-fold). Plasma 5-HT concentrations were reduced initially but recovered. Later in anaphylaxis (10 to 20 min) whole blood histamine concentration fell significantly. This coincided with the most profound period of leucopoenia.

4. No significant differences were observed in histamine concentration in any of five tissues between six ponies subjected to anaphylaxis and six controls. Mast cell numbers were not reduced but mast cells were more metachromatic (pink) and there was spilling of mast cell granules.

5. Gross pathological changes were noted mainly in the lungs which were extensively oedematous and congested. Inflamed, congested and oedematous areas in the large colon and caecum were seen, and the kidneys, spleen and liver were engorged. Alveolar emphysema, peribroncheolar oedema (containing mononuclear cells and neutrophils) were recorded. Alveoli contained erythrocytes.

Introduction

Horses have made some classical but little-known contributions to early physiology and medicine. In 1732, arterial blood pressure was first recorded by the Rev. Hales in a mare using a goose quill. The first recorded cardiac catheterization was made in a horse by Bernard and Magendie in 1847, and the observation of Marley's Law was made by Chauveau and Marley in a horse in 1863 (Hamlin & Smith, 1970). In more recent times, horses have been used to produce antisera against important human diseases such as diphtheria and tetanus, among others. Furthermore, anti-lymphocytic sera for experimental use and for human therapy,

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have been produced in this species (Woodruff, 1969). It is thus surprising that detailed investigation of the horse as an experimental animal *per se* has been largely neglected.

It is of some interest that an obstructive respiratory disease of horses, variously known as 'emphysema', 'heaves' or 'broken-wind' closely resembles asthma of man. Stömmer in 1887 (cited by Cook & Rossdale, 1963) may have been the first to compare the asthma-like syndromes in the two species. Since that time, many clinical and pathological similarities have been noted. 'Asthmatic' horses show hyper-reactivity to histamine and muscarinic stimulants (Obel & Schmitterlöw, 1948) much like the bronchial hyperactivity described in human asthma subjects (Itkin, 1967). 'Allergic' horses also may have dermal sensitivity to specific antigens, especially moulds (Sertíc, 1968; Eyre, 1972a) and their sera may elicit Prausnitz–Küstner reactions (Eyre, 1972a). Pulmonary blood vessels taken from asthmatic horses contract *in vitro* to specific antigen: the Schultz-Dale phenomenon (Schultz, 1910; Dale, 1913; Eyre, 1972a).

Attempts to create and study experimental anaphylaxis in horses have been few (Gerlach, 1922; Ritzenthaler, 1924; Code & Hester, 1939). Recently new interest has centred on equine anaphylactic reactions (Eyre, 1972a, b; Lewis, Eyre & Downie, 1972; Mansmann, 1972; Mansmann, Wheat & Osburn, 1972; McGavin, Gronwall & Mia, 1972). In view of the incompleteness of information, it seemed of immediate importance to study primary cardiovascular and respiratory responses of horses to autonomic drugs, bioamines, kinins and acute systemic anaphylaclic shock: also to measure pathological and haematological parameters and investigate the contribution made by histamine, 5-HT, kinins and other putative mediators (transmitters) of acute anaphylaxis of horses.

Methods

Twenty adult ponies of mixed breed and either sex, weighing 120–200 kg, were used. Ten ponies were sensitized with 0·2 ml/kg whole bovine serum intravenously. A second injection of 4 ml bovine serum emulsified in an equal volume of Freund's complete adjuvant was injected subcutaneously three weeks later. After a further three to four weeks the ponies were anaesthetized with 25 mg/kg pentobarbitone sodium, given intravenously, and anaesthesia was maintained with supplementary pentobarbitone. With the animals in right lateral recumbency, blood pressures were recorded and measured in the left common carotid artery, the pulmonary artery and abdominal vena cava, as previously described in calves (Lewis & Eyre, 1972; Eyre, Lewis & Wells, 1973). Respiratory ventilation volume was also monitored as before. Drug administrations were made by means of a cannula in the tarsal vein.

After an equilibration period of 15–30 min, the effects of bradykinin acetate, histamine acid phosphate and 5-hydroxytryptamine creatinine sulphate, given intravenously were recorded in six serum-sensitized ponies and in a group of six unsensitized controls. Dose-response relationships to several randomly-administered doses of each agonist were established prior to challenge with bovine serum (0·2 ml/kg), given intravenously over a 2 min period, to induce anaphylaxis.

In a further group of four sensitized ponies, carotid arterial blood samples were obtained 15 min prior to challenge with antigen, at challenge and 0·5, 1, 2, 4, 6,

10 and 20 min thereafter. Plasma histamine concentration (Noah & Brand, 1961), plasma 5-HT concentration (Andén & Magnussen, 1967), whole blood histamine (Shore, Burkhalter & Cohn, 1959) and whole blood kinin equivalents (Boreham, 1968 ; Eyre & Lewis, 1972) were estimated.

Haematological measurements were made on arterial blood from the same ponies. Packed cell volumes (PCV) were determined by means of a Clay Adams Microhematocrit using blood collected with EDTA (the disodium salt of ethylenediamine tetraacetic acid). Haemoglobin concentration was estimated with a Coulter Hemoglobinometer. Red cell and leucocyte counts were undertaken with a Coulter Counter Model FN[3] and platelet counts made using Unopettes and Neubauer counting chambers. Two hundred leucocytes in stained blood smears from each sample were differentially counted.

A final group of four unsensitized (control) ponies were anaesthetized in the same way as described above and challenged with antigen. Serial carotid blood samples were assayed for amines, kinins and haematological changes as before. Plasma histamine levels were measured in only two controls, whereas all other parameters were taken in all four unsensitized ponies.

Every animal was killed with pentobarbitone at the end of each experiment and subjected to necropsy. Portions of lung parenchyma and pleura, liver parenchyma and capsule, small intestine and subcutaneous tissue were removed for determination of histamine concentration (Shore *et al.*, 1959). Tissue spreads of pleura, omentum, liver capsule and subcutaneous tissue were fixed in 80% ethanol, stained with Toluidine blue (Riley, 1953) and examined for changes in mast cell numbers and morphology.

Drugs

Histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate and bradykinin triacetate were obtained from Nutritional Biochemicals Co., Cleveland, Ohio, U.S.A. All drugs were dissolved in isotonic sodium chloride solution and all doses refer to the base. Bovine serum was obtained locally and was used undiluted either fresh or after being stored at −20° C for varying periods.

Results

The mean resting blood pressures measured in 10 ponies under pentobarbitone anaesthesia were: carotid 145 mmHg (120–160), pulmonary artery 13·5 mmHg (10–15), vena cava 11·0 mmHg (8–12).

Effects of histamine, 5-hydroxytryptamine and bradykinin in the anaesthetized pony

Drugs were tested in random order and dose-effect relationships were measured for each drug in both antigen/adjuvant-sensitized and in unsensitized animals.

A dose of histamine of 0·1 μg/kg or greater caused a distinct fall in systemic blood pressure (Figure 1). Within the dose range of 0·25–0·75 μg histamine/kg the hypotension was preceded by a sharp transient rise in pressure. Doses of histamine greater than 1·0 μg/kg caused a biphasic response in which an initial fall in pressure was followed by a transient rise which sometimes preceded further more prolonged hypotension. Ventilation was not markedly changed by doses <0·25 μg histamine/

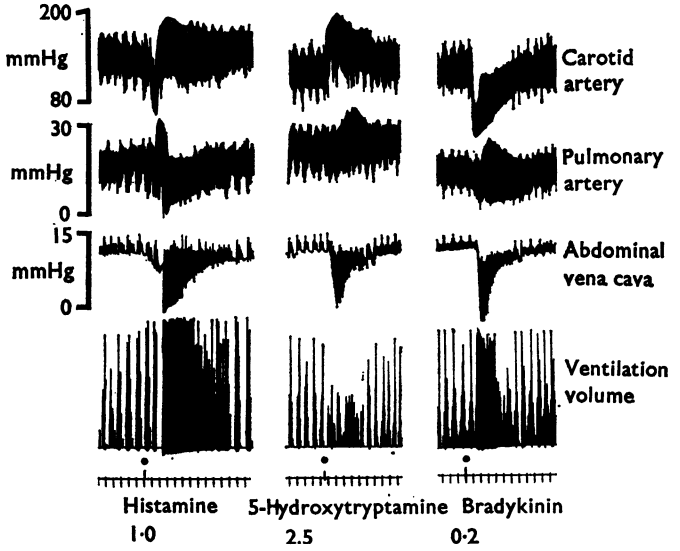

FIG. 1. Horse anaesthetized with pentobarbitone sodium (25 mg/kg). Recordings of blood pressures (mmHg) in the carotid artery, pulmonary artery and abdominal vena cava; and respiratory ventilation volume. Effects of histamine, 1·0 μg/kg; 5-hydroxytryptamine, 2·5 μg/kg and bradykinin, 0·2 μg/kg. Time marker indicates injection times and 30 seconds.

kg but larger doses of histamine caused a brief period of 'gasping' respiration followed by apnoea and a period of 2 to 3 min of increased respiratory frequency. There was a rise in pulmonary artery pressure accompanying doses of histamine sufficient to affect the system pressure. At doses of histamine $>1{\cdot}0$ μg/kg, a fall in pulmonary pressure was sometimes observed after the initial rise. Abdominal venous pressure was reduced by all doses of histamine tested.

All effective doses of 5-HT (i.e. $>1{\cdot}0$ μg/kg) were qualitatively similar. Increases in systemic and pulmonary artery pressures were accompanied by reduced venous pressure, increased frequency and reduced volume of respiration (Figure 1).

The horses were particularly sensitive to bradykinin which produced transient hypotension at doses as low as 10 ng/kg. Doses of bradykinin greater than 20 ng/kg induced more prolonged hypotension, increased pulmonary artery pressure and accelerated the respiratory rate. The venous pressure was reduced (Figure 1).

There appeared to be no differences in sensitivity to injected drugs between antigen-sensitized and non-sensitized animals.

Characteristics of acute anaphylaxis

Approximately 2 min after the beginning of antigen infusion there was a dramatic fall in carotid artery pressure, which coincided with an increased pulmonary artery pressure and a brief fall in vena cava pressure. Vena cava pressure increased suddenly and very markedly at about the third minute and this was coincidental with a brief period of apnoea (Figure 2). All blood pressure parameters remained steady until approximately the eighth to tenth minute when venous pressure fell sharply to normal. There was also a sudden systemic hypertension prior to the carotid pressure becoming normal at about 15 minutes. Pulmonary artery pressure remained elevated for a longer period but had become normal after 20–25 minutes.

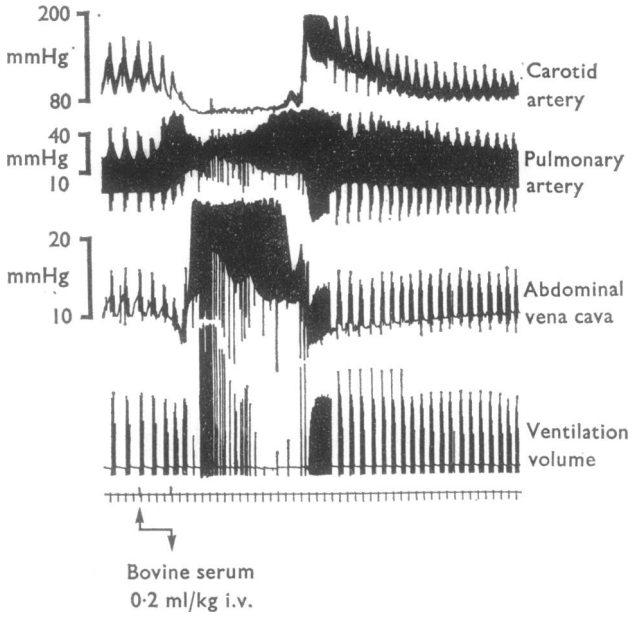

FIG. 2. Horse sensitized with bovin serum in complete Freund's adjuvant: anaesthetized with pentobarbitone sodium (25 mg/kg). Recordings of blood pressures (mmHg) in carotid artery, pulmonary artery and abdominal vena cava; and respiratory ventilation volume. Effects of bovine serum (0·2 ml/kg, i.v.), given over a 2 min period shown by the arrows. Time marker indicates injection period and 30 seconds.

Effects on respiration were divisible into four stages. The initial apnoea observed was followed by a period of 2 to 3 min of deep rapid breathing. A second longer period of apnoea, lasting 3 or 4 min, then preceded a short period of shallow rapid breathing before the normal respiratory pattern returned at approximately 12 minutes.

In all four sensitized ponies a second anaphylactic reaction could not be elicited by a similar dose of antigen given 30–45 min after recovery from the first 'shock'.

Unsensitized ponies which received identical doses of antigen under the same experimental conditions did not display any measurable haemodynamic changes.

Haematology

No differences were apparent in haematological parameters before and after induction of anaesthesia. During anaphylactic shock, as described above, one of the most profound changes was haemoconcentration in the order of 30–40% increase (Figure 3). This was accompanied by comparable increases in red cell count and haemoglobin concentration.

Plasma potassium ion concentration increased dramatically during anaphylaxis, commencing approximately 5 min after antigen injection. Plasma sodium concentration did not alter.

The total leucocyte count decreased markedly within 5 to 10 min of induction of anaphylaxis and had returned to normal within 30–40 minutes. The most dramatic change was in neutrophil numbers which in some horses fell to as few as two per cent of the total leucocytes. In addition there was distinct monocytopoenia while the lymphocyte counts remained normal.

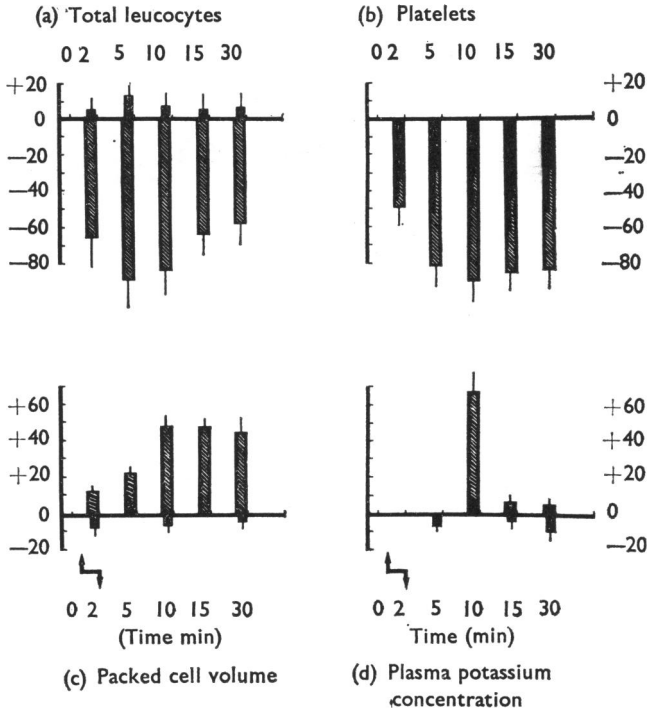

FIG. 3. Percentage changes in (a) total leucocytes, (b) platelets, (c) packed cell volume, (d) plasma potassium concentration in the blood of horses during anaphylactic shock induced by bovine serum (0·2 ml/kg, i.v.) given over a 2 min period shown by the arrows. Hatched columns represent four sensitized horses in anaphylaxis (±S.D.). Solid black columns represent four unsensitized (control) horses (±S.D.).

Concurrent with these changes in red and white cells there was a severe thrombocytopoenia—the platelet count being 25% of normal (or less) from 5 to 30 min following the start of anaphylaxis (Figure 3).

Blood histamine, 5-hydroxytryptamine and kinins

Figure 4 indicates that there was a marked five to six-fold increase in plasma histamine concentration approximately 2 to 4 min after the beginning of antigen infusion. The concentration fell by half at 10 min and remained so elevated at least until 20 minutes. At about 5 min there was a small increase in whole blood histamine concentration. The rising concentration of circulating histamine coincided with the most profound period of anaphylactic shock. Approximately coincident with histaminaemia, there was a four to five-fold increase in whole blood kinin activity, which reached a peak at 5 min, but had returned to normal at 10–12 min (Figure 5). Between approximately 10 and 20 min after the start of anaphylaxis the whole blood histamine concentration was reduced significantly (Figure 4). This period coincided with the time of profound leucopoenia and thrombocytopoenia in the same animals (Figure 3). At this time the concentration of histamine in the plasma remained elevated approximately four-fold.

Plasma concentrations of 5-HT were reduced significantly within 30–60 s of the beginning of antigen infusion (Figure 4). During anaphylaxis as shown by the depression of blood pressure, the plasma 5-HT concentrations increased to near

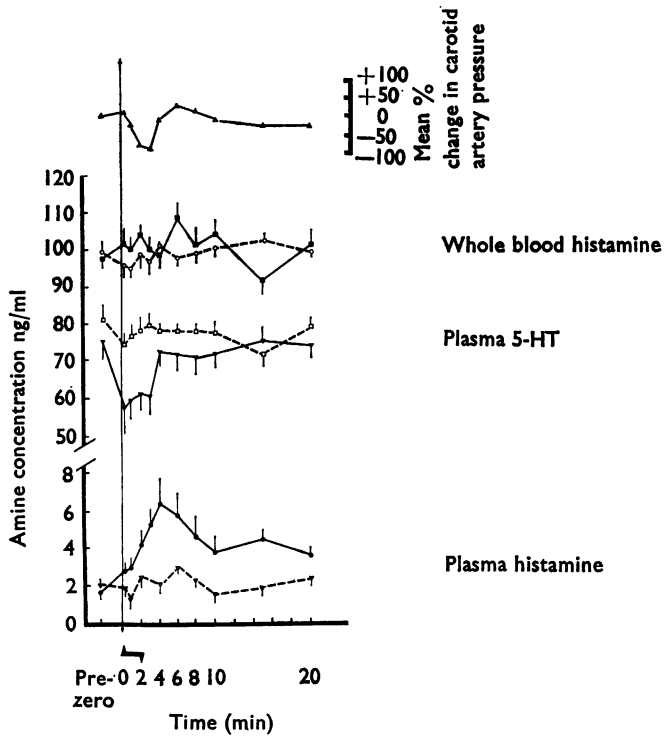

FIG. 4. Mean carotid artery blood pressure, whole blood histamine and plasma histamine and 5-hydroxytryptamine (5-HT) concentrations during anaphylaxis in horses sensitized to bovine serum. Seven graphs from above downwards: (i) mean % change in carotid pressure in six bovine serum-sensitized horses during anaphylaxis △——△; (ii) whole blood histamine concentration in four *unsensitized* controls ○- - -○; (iii) whole blood histamine concentration in four *sensitized* horses in anaphylaxis ■——■; (iv) plasma 5-HT concentration in four *unsensitized* controls □- - -□; (v) plasma 5-HT concentration in four sensitized horses in anaphylaxis ▼——▼; (vi) plasma histamine concentration in four *unsensitized* controls anaphylaxis ●——●; (vii) plasma histamine concentration in four *unsensitized* controls ▽- - -▽. All horses were anaesthetized with pentobarbitone and received bovine serum (0·2 ml/kg, i.v.) over the 2 min period indicated by the arrows. Pre-zero readings are measurements after anaesthetization and cannulation, approximately 5–15 min prior to antigen infusion. Amine concentrations were measured spectrophotofluorimetrically and are expressed in ng/ml. Time intervals are in minutes. Vertical bars represent standard deviations.

the pre-challenge level, but remained below normal for the whole 20 min period observed.

Tissue histamine concentrations

The data in Table 1 indicate that there was no significant difference in histamine concentration in any of five tissues examined from six sensitized ponies subjected to anaphylaxis compared with six controls injected with antigen.

Mast cells

Mast cells of the pleura, omentum, liver capsule and subcutaneous tissue of sensitized ponies displayed minor differences from the control tissues. For example, their mast cells had more metachromatic (pink) staining and there was some dispersal of granules into the tissue after anaphylaxis.

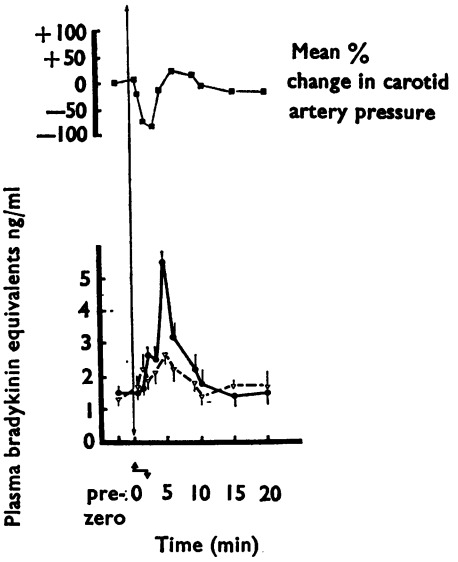

FIG. 5. Blood pressure and whole blood kinin activity during anaphylaxis in the horse. Top graph ■——■, represents mean % change in carotid pressure in six bovine serum-sensitized horses in anaphylaxis. Second graph ●——●, indicates whole blood bradykinin equivalents in four *sensitized* horses during anaphylaxis. Bottom graph ▽- - -▽ indicates whole blood bradykinin equivalents in four *unsensitized* controls. All animals were anaesthetized with pentobarbitone sodium and were injected with bovine serum (0·2 ml/kg, i.v.) over a 2 min period indicated by the arrows. Pre-zero readings are taken after anaesthetization and cannulation, approximately 5–15 min before challenge. Bradykinin equivalents were measured by bioassay on oestrous rat uterus and are expressed as ng bradykinin/ml. Time intervals are min and vertical bars represent standard deviations.

TABLE 1. *Concentrations of histamine in some tissues of six ponies subjected to acute anaphylactic shock* in vivo *and in six controls*

Tissue	Histamine concentration (μg/g) After anaphylaxis	Controls
Lung parenchyma	2·65±0·20	2·41±0·14
Lung pleura	1·94±0·14	2·12±0·13
Liver parenchyma	0·82±0·19	0·78±0·10
Ileum	3·61±0·21	2·91±0·13
Sub-cutaneous tissue	4·40±0·27	3·92±0·21

Each value is the mean of six animals±S.E.

Pathological changes

(a) *Macroscopic lesions* were mainly confined to the lungs, which in all cases of acute anaphylaxis were grossly congested and oedematous. In two animals there was inflammation in ' patchy ' areas of the large intestine and caecum, which were oedematous and haemorrhagic. The kidney, spleen and liver were consistently congested.

(b) *Histological changes.* Alveolar emphysema and early bronchiolar oedema were consistent findings in all anaphylactic ponies. Red cells could be seen in the alveoli. The peribronchiolar infiltrate contained some mononuclear cells and neutrophils.

Discussion

The systemic cardiovascular responses to histamine in the horse do not seem to differ markedly from those of other species. The secondary increase in systemic

blood pressure caused by histamine >0·25 μg/kg may have been due to catecholamine release. 5-Hydroxytryptamine induces a pressor response in horses as in most species, which is in contrast to the hypotensive action of 5-HT which we have reported in cattle (Lewis & Eyre, 1972). Bradykinin was the most potent of the three agonists tested and this compound induced profound hypotension. There were no measurable differences in reactivity to histamine, 5-HT or bradykinin between the protein-sensitized and unsensitized groups of animals.

Systemic anaphylaxis in the horse may be divided arbitrarily into four phases. The first phase was characterized by marked carotid hypotension and pulmonary arterial hypertension and a slight fall in vena cava pressure within 2 to 3 min of beginning antigen infusion. This period approximately coincided with appearance in the peripheral blood of histamine followed by kinins. At approximately 3 min there was a rapid elevation of vena cava pressure and a brief period of apnoea, while the peripheral arterial pressure remained depressed and the pulmonary arterial pressure remained elevated. At about this time, the levels of plasma histamine and kinins were approaching peak concentrations, and also the plasma 5-HT concentration increased sharply above the low level recorded immediately after challenge.

Blood pressure parameters stayed constant until approximately the twelfth minute during which time alternate phases of apnoea and rapid dyspnoea were observed. It is likely that the observations observed to this point were caused by the primary release of mediators (transmitters) such as histamine, 5-HT, kinins and possibly other substances (e.g. slow reacting substance, prostaglandins) not measured in these experiments.

The third phase of the anaphylactic response began at approximately 10–12 min and consisted of profound reduction in venous (caval) pressure, elevation of systemic arterial blood pressure to above normal, and an increased respiratory rate. It is possible that this phase may be associated with reflex activity and with secondary catecholamine release (Piper, Collier & Vane, 1967).

The final phase of anaphylaxis consisted of restoration of normal blood pressures and respiratory pattern, which was complete at about 20–25 minutes.

Some of the most striking changes in equine anaphylaxis occurred to the constituents and the consistency of the blood *per se*, and these alterations seemed to correlate with both the haemodynamic changes and the appearance in the blood of vasoactive amines and kinins. Increased packed cell volume may be due to loss of circulating fluid as a result of 'pooling' of blood and to extravasation of fluid due to increased vascular permeability in organs such as the intestine and lung where oedema was actually observed at post mortem. Similar observations have been made in sheep (Alexander, Eyre, Head & Sanford, 1967, 1970) and in calves (Eyre *et al.*, 1973 ; Wells, Eyre & Lumsden, 1973).

The source of the increased plasma potassium concentration in anaphylaxis can only be speculative in these experiments. One source may be efflux from smooth muscles occurring during contractile and permeability changes ; while another may be potassium release from target cells such as leucocytes and platelets during the liberation of vasoactive constituents. Extrusion of potassium is a well known event associated with cellular trauma.

Leucopoenia and thrombocytopoenia correlate closely with measured haemo-

dynamic changes and may result from the sequestration of these cells in vascular beds during anaphylaxis. The involvement of leucocytes and platelets in anaphylaxis (Rocha é Silva, 1950) is a prominent feature in rabbits (Lecomte, 1956), dog (Fidlar & Waters, 1946), monkeys (Kopeloff & Kopeloff, 1941) and cattle (Wells *et al.*, 1973) and it seems likely that leucocytes and platelets may play an integral part in the anaphylactic process of horses. It is possible that circulating leucocytes and platelets are more important target mediator cells than mast cells in this species because in the experiments described here there was no measurable reduction in tissue mast cell numbers and the only change in the sensitized equine mast cells during anaphylaxis was slight metachromasia. It was also interesting to note that in this same connection we also failed to show any significant alteration in tissue histamine.

However, it must be emphasized that several explanations are possible. It is feasible that the increased plasma levels of histamine and 5-HT originated from leucocytes and thrombocytes rather than mast cells. Furthermore, those tissues which we examined may not have been regions of active release, or alternatively lack of significant change may be caused by sample variation (there are regional differences in amine concentrations of amines in horse tissues and, owing to the large size of the organs, consistency of sampling is virtually impossible). It may also be that the histamine-forming capacity of the tissues had increased and compensated for small losses (Kahlson & Rosengren, 1968).

Although it is too early in the study to relate the present findings to clinical equine emphysema it is interesting that Brion, Pellerat & Castric (1948) reported elevated blood histamine concentrations in emphysematous horses. We have however been unable to confirm these findings (Eyre, 1972a), although we reported increased plasma 5-HT concentrations in 50% of emphysematous horses examined. None of these animals showed elevated blood kinin activity (unpublished observations).

Andberg, Boyd & Code (1941) reported that intravenously administered histamine caused an asthma-like condition in horses, not unlike the natural disease of ' heaves ', whereas Code & Hester (1939) reported that whole blood histamine concentration was reduced during experimental anaphylaxis in two unanaesthetized horses and four calves. Whole blood histamine concentrations were measured by Code & Hester at 10 min after injection of antigen and at no other time. We have shown that in both calves and horses the total leucocyte count is drastically reduced at approximately 10 min after the start of anaphylaxis. Since most of the circulating histamine is contained in leucocytes (Code & Mitchell, 1957 ; Greaves & Mongar, 1968) the reduced histamine concentration in whole blood during anaphylaxis might well be explained by the accompanying leucopoenia. Whole blood histamine is a notoriously bad indicator of pathological processes in any species (Porter & Mitchell, 1972). We have shown, however, that in both horses and cattle *plasma* histamine concentrations are significantly raised in anaphylaxis. Code & Hester's early observations were thus misleading and more significance should be attached to a possible role for histamine in the hypersensitivity reactions of ungulates than has previously been suggested. Our present results do not indicate an important role for 5-HT in equine anaphylaxis, whereas the highly significant elevation of plasma kinin activity suggests that these peptides might make a contribution to anaphylactic shock in this species. A study of specific drug anatagonists is now in progress, which should shed more light on the mechanism of anaphylaxis in the horse.

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