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STRESS IN THE HORSE AND ITS
MODIFICATION WITH DRUGS

BY

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Summary

The aim of this study was to estimate the effects of stress on horses by measuring alterations in adrenocortical and sympathetic nervous activity. The effects on the adrenocortical system were determined directly by measuring circulating levels of plasma 11 - hydroxycorticosteroids. The effects of stress on the sympathetic nervous system were assessed indirectly by recording changes in heart rate and circulating packed cell volume and in a few cases by direct measurement of circulating plasma catecholamine levels.

Plasma 11 - hydroxycorticosteroid levels were measured using a modified fluorometric technique. Standard reliability criteria were used to validate the method. The range of corticosteroid levels in horses and ponies under various natural and experimentally induced conditions was established. Changes in adrenocortical and sympathetic nervous activity were measured during physical stimulation (either exercise or surgical trauma) and following administration of certain drugs. The significance as a means of quantifying stress of alterations in plasma 11 - hydroxycorticosteroid levels and in sympathetic nervous activity was assessed.

A number of tranquillising drugs were compared both for their central nervous depressant and peripheral actions and the effects of these drugs on stress-induced changes in adrenocortical and sympathetic nervous activity were studied. Alterations in packed cell volume caused by tranquillising drugs was investigated.

Finally, changes in adrenocortical and sympathetic nervous

activity produced by the commonly used neuroleptanalgesic mixture of etorphine and acepromazine were studied and attempts were made to modify these changes by incorporating various tranquillising and adrenoreceptor blocking drugs in the etorphine/acepromazine combination.

It was concluded from this study that alterations in adrenocortical and sympathetic nervous activity can be used as means of assessing stress in the horse. As the tranquillising drugs used however modified adrenocortical and sympathetic activity, changes in these systems could not be employed in assessing alleviation of stress by these compounds. It was also concluded from these experiments that the marked effects of the etorphine/acepromazine mixture on sympathetic nervous activity could be modified by the central and peripheral actions of the various tranquillising drugs employed in this study.

GENERAL INTRODUCTION

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GENERAL INTRODUCTION

The term stress is widely used in both human and veterinary medicine and is employed in a great variety of contexts. While the definition of stress is vague, the factors which may produce stress are generally accepted. It is generally agreed that potentially harmful environmental stimuli can be resisted by a person or animal through a complex number of interacting local and general anatomical, biochemical, physiological or behavioural responses.

Cannon (1929) reported on the similarity of body responses to a number of different types of environmental disturbance and noted the activity of the sympathetic nervous system and the secretion of adrenaline in relation to these. He recorded that both these parameters increased during emotional disturbance and pain and that these elevations were responsible for a number of marked physiological changes. Selye (1956) on the other hand described stress as a general adaptation syndrome which was triggered by a number of stimuli such as trauma, cold and physical restraint.

In the veterinary field, stress has been used mainly where the physiological and biochemical alterations occurring have led to easily observable changes such as mortality, disease or reduced weight gain. For example, the 'acute stress syndrome' in pigs is responsible for a high mortality rate and is commonly associated with transportation and handling of these animals. Similarly neonatal scours in calves and pigs have been linked to social and

environmental disturbances during the first 2-3 days of life (Fraser, Ritchie and Fraser, 1975).

Ewbank (1973) suggested that the term stress should be restricted to the mechanisms by which an animal resists the effects of adverse stimuli. He also suggested that the stimuli provoking these stress responses should be referred to as stressors and can be further classified as heat, sound, emotional, physical etc.

Any individual stressor may produce a specific local response but will probably also result in a more generalised reaction. It should be possible to assess whether or not a state of stress has occurred by measuring the changes in any of a number of systems. In man the level of stress can be estimated to some extent by assessing his psychological state, but in animals objective criteria are required. Stress reactions are often considered to be deleterious to the animal (Bareham, 1973; Fraser et al, 1975) and indeed under prolonged or intense stimulation many detrimental effects can occur. It can also be argued however that stress reactions are beneficial in that they allow the animal to adapt and survive changes in its environment.

Drugs, such as tranquillisers and sedatives, are widely used in man to alleviate mental stress and they do this by means of their central nervous depressant actions (Greenblatt and Shader, 1973; Goodman and Gilman, 1975). Similar types of drugs are used on many occasions in animals usually to facilitate handling for minor operative procedures or for transportation. Measurement of certain parameters such as heart rate and plasma corticosteroid concentrations

are frequently used as means of assessing the efficacy of tranquillisers and sedatives in animals because these drugs are considered to lower the stress response to various stimuli (Smith, Maikel and Brodie, 1963; Aitken and Sanford, 1972; Jahti and Barsuhn, 1974).

The aim of this study was to measure the response in horses to a number of stressors of different types and to assess whether these responses constituted a reasonable method of estimating stress in the horse. The changes being measured were in the sympathetic nervous system and the central nervous system/pituitary adrenal system.

The same methods were also employed to assess the usefulness of a number of tranquilliser and sedative drugs in alleviating stress reactions produced in horses.

ANIMALS USED

ANIMALS USED

The animals used throughout this study are shown in Table 0.1.

The five Thoroughbreds and two heavy hunters were housed inside in individual loose boxes throughout the experimental period. They were allowed hay and water ad lib and were fed daily rations of oats, bran and horse nuts.

The three ponies were kept outside all year except when required for experimental work. Outside they were fed additional hay during the winter and inside were allowed hay and water ad lib.

Table 0.1 Animals used

No.	Type	Age (years)	Sex	Weight (lbs)
1	T.B.	9	Gelding	1100
2	T.B.	6	Gelding	960
3	T.B.	5	Gelding	980
4	T.B.	5	Mare	970
5	T.B.	21	Mare	940
6	H.H.	12	Gelding	1300
7	H.H.	13	Mare	1250
1	Pony	6	Gelding	320
2	Pony	7	Gelding	335
3	Pony	15	Gelding	440

T.B. = Thoroughbred

H.H. = Heavy Hunter

1. PHYSIOLOGICAL MEANS OF
ASSESSING STRESS

1.1 Introduction

Many of the physiological responses to stress, both physical and emotional, are mediated by sympathetic nerves of the autonomic nervous system. The sympathetic nervous system exerts widespread effects on body functions including heart rate, blood pressure, cellular metabolism and mental activity (Guyton, 1971). When stimulated it permits man and animals to perform physical activity and withstand certain disturbing stimuli to a much greater extent than normal.

Sympathetic activity is controlled by various centres in the brain, especially the hypothalamus, and stimulation of any part of the system generally results in stimulation of the entire sympathetic pathway (Guyton, 1971). Direct assessment of sympathetic activity can be carried out by measuring plasma levels of circulating catecholamines (adrenaline and nor-adrenaline) but the techniques involved are difficult and indirect methods are more commonly employed.

Heart rate is controlled almost entirely by the vasomotor centre in the brain which transmits excitatory impulses via sympathetic fibres and inhibitory impulses via the parasympathetic vagus nerve (Guyton, 1971; Rushmer, 1972). Generally, therefore, increased sympathetic activity will result in elevated heart rates but the mechanism is complicated by a number of reflexes involving parasympathetic nerves e.g. increased blood pressure causing a decrease in heart rate because of stimulation of stretch receptors

in the aortic and carotid bodies and vice versa (Rushmer, 1972).

Although changes in heart rate have been used as a means of assessing both physical effort and emotional stress in horses (Krzywanek, Wittke, Bayer and Borman, 1970; Witherington, 1971; Aitken and Sanford, 1972) they are not an ideal means of evaluation because of these parasympathetically mediated reflexes.

Sympathetic activity can also be assessed by measuring changes in circulating packed cell volumes. The spleen has been shown in the horse to be the main reservoir of red cells (Persson, 1967) and the splenic capsule is rich in adrenoceptors (Davies and Witherington, 1973). Increased-sympathetic tone resulting from many conditions including exercise, fright and adrenaline infusion leads to splenic contraction and expulsion of red cells into the circulation.

In these experiments, heart rates and packed cell volumes were measured and assessed as methods of estimating stress.

MEASUREMENT OF HEART RATE

Heart rate can be measured either directly by auscultation or indirectly by radiotelemetry.

The former involves direct recording of the heart sounds occurring during closing of the semilunar and atrial valves, and the latter, recording of the electrical impulses generated during contraction of the atria and ventricles.

Materials

ECG transmitter : TM 2T	M.I.B.	Tasman Vaccine Laboratory (U.K.) Ltd., Suffolk.
ECG receiver : TM 2R	M.I.B.	Tasman Vaccine Laboratory (U.K.) Ltd., Suffolk.
Devices, two channel pen-recorder		Devices Ltd., England.
Neilson heart rate meter		Devices Ltd., England.

Methods

1) Auscultation

Heart rates were recorded using a stethoscope and counting the number of beats over a 30 second period.

2) Radiotelemetry

Two metal discs approximately 9mm in diameter were applied to areas of shaved skin, one in front of the anterior angle of the scapula and one in midline over the xiphoid cartilage.

The sites of application were those advocated by Holmes, Alps and Darke (1966) and the electrodes were secured by means of elastoplast and collodion glue.

The electrodes were connected by two fine wires (which also acted as aerials) to the transmitter which was strapped to the withers of the horses by a surcingle. When heart rates were recorded during exercise the transmitter was strapped onto the left leg of the rider.

Signals from the transmitter were picked up on the radio-receiver, passed through a preamplifier and the Electrocardiogram (E.C.G.) recorded on a pen-recorder.

In initial experiments, carried out on resting animals in loose-boxes, records of heart rate were also made using a Neilson heart-rate meter.

This instrument gives a steady output between pulses representing the repetition frequency of the two previous pulses. If the rate becomes lower, the ratemeter anticipates this drop and the output falls. A continuous recording of heart rate is produced. However during and after exercise there is a considerable amount of spurious electrical activity due mainly to muscle movement and it was found that more reliable heart rates could be obtained by manually counting the number of ventricular beats over a fixed period (either 30 or 60 seconds) on the pen recording.

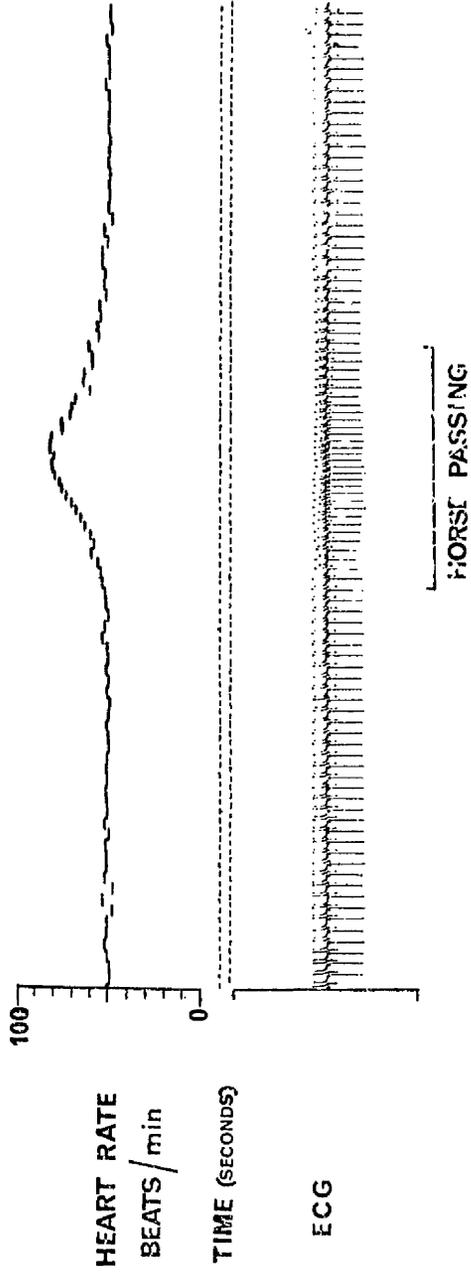
Results

Heart rates recorded by auscultation and radiotelemetry at various times in the seven horses included in this study are reported in Table 1.1.

Records obtained by radiotelemetry from one of the ponies (No. 1) unrestrained in a loose box and from horse No. 2 during and immediately after exercise are shown in Fig. 1.1.

HEART RATES RECORDED BY RADIOTELEMETRY

1. PONY UNRESTRAINED IN LOOSE BOX



2. HORSE DURING AND IMMEDIATELY AFTER EXERCISE

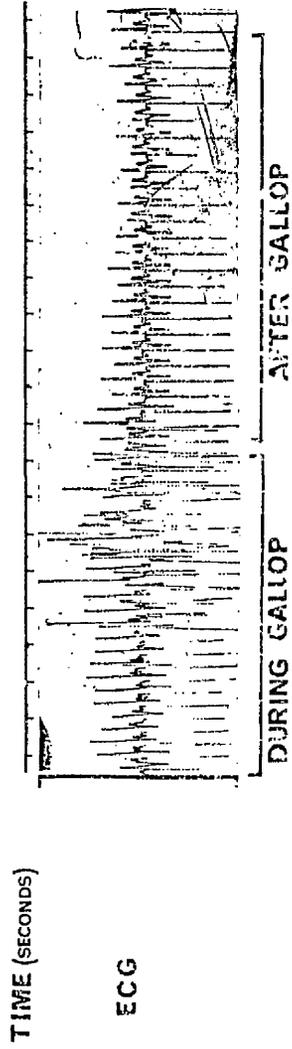


Table 1.1 Heart Rates (Mean \pm s.e.) recorded by Auscultation and Radiotelemetry from Horses at Rest and after Exercise

Means of Recording	Heart Rates recorded from Horses at Rest	Maximum Heart Rate recorded from Horses after submaximal Exercise
Auscultation	48 \pm 1.8 (36 - 52)	123 \pm 7.0 (81 - 144)
Radiotelemetry	42 \pm 3 (36 - 46)	157 \pm 12.0 (150 - 139)

Figures in brackets refer to ranges of levels recorded

1.3

MEASUREMENT OF PACKED CELL VOLUME

The packed cell volume (P.C.V.) represents the percentage of whole blood which consists of erythrocytes and leucocytes. By centrifuging whole blood at high speeds it separates into two distinct layers - the erythrocyte/leucocyte mass and the blood plasma. The former is expressed as a percentage of the total volume.

Materials

Heparinised tubes (for Hawksley microhaematocrit centrifuge)

Gelman - Hawksley Ltd., England.

Hawksley microhaematocrit centrifuge

Gelman - Hawksley Ltd., England.

Methods

The method of P.C.V. determination used in this study was the macrohaematocrit method.

Heparinised blood was drawn into capillary tubes and one end was sealed in a flame. The tubes were then spun at 4,000 r.p.m. for 4-5 min and read on a Hawksley Micro-haematocrit reader.

Samples were always measured in duplicate and the mean of the two values recorded.

Results

Ranges of packed cell volumes recorded during the course of

this study in horses and ponies under various conditions are expressed in Table 1.2.

Table 1.2 Packed Cell Volumes (%) in Horses and Ponies, recorded by the Microhaematocrit Method

Species	At Rest (Mean \pm s.e.)	Maximum Level recorded	Minimum Level recorded
Horse	38 \pm 2	63 *	30
Pony	31 \pm 0.4	35	25

* SAMPLES TAKEN FROM THOROUGHBRED HORSES AFTER MAXIMAL EXERCISE

1.4 Discussion

Heart rates in horses have been recorded by numerous authors using both auscultation and radiotelemetry (Littlewort and Hickman, 1969; Krzywanek et al, 1970; Witherington, 1971; Aitken and Sanford, 1972).

The latter method is considered to be the more accurate since Witherington (1971) pointed out the difficulties which may be encountered in distinguishing heart from respiratory sounds in some horses. This effect was noticed occasionally in the two heavy hunters included in these studies especially when resting heart rates were being recorded.

Another advantage of radiotelemetry is that there is no interference with the animal at the time when the recording is made. Aitken and Sanford (1972) have reported that auditory and visual stimuli could induce increases in heart rate of up to 200 per cent and thus it is possible that auscultation itself would produce elevations in heart rate especially when levels had fallen below certain values.

Heart rates recorded immediately after exercise by auscultation were lower than those recorded by radiotelemetry. This difference is due to two factors. Firstly, rates recorded by radiotelemetry are obtained immediately after exercise stops and the time constant using this method is much shorter than using auscultation. Secondly, heart rates have been shown to decrease very rapidly within the first 1-2 min after exercise (Aitken, Anderson, MacKenzie and

Sanford, 1975; Hall, Steel and Stewart, 1976) and any delay in recording will lead to falsely low results.

It proved difficult to record heart rates during exercise in these studies although several workers have reported such results (Marsland, 1968; Lindholm and Saltin, 1974; Hall et al., 1976).

Difficulties were encountered because at times during the exercise regime the horses and radiotransmitter were too far from the radioreceiver. During prolonged exercise, when the animals began to sweat, it was also noticed that the contact between the electrode and the skin was poor and in some instances contact was lost completely.

For several years the microhaematocrit method of packed cell volume determination has been used for routine blood samples in many laboratories (Allen and Archer, 1973). The method is considered to be more accurate and reproducible, less time consuming and to use less blood than is required for the Wintrobe (1933) haematocrit method (Schalm, Jain, and Carroll, 1975). The ranges of values recorded throughout these studies were within those reported in the same species by other workers and the various alterations noted are discussed in the appropriate chapters.

Summary

From these results it was decided to use radiotelemetry to record heart rates from the horses and ponies on all occasions when experimental work was being carried out indoors. During the

exercise programmes, radiotelemetry was used wherever possible to obtain recordings during and immediately after exercise. When this proved difficult however heart rates were measured by auscultation.

Packed cell volumes were measured on all occasions by the microhaematocrit method.

2. BIOCHEMICAL MEANS OF
ASSESSING STRESS

2.1 Introduction

The adrenal gland, which is part of the endocrine system, secretes hormones which play a large part in the regulation of body metabolism and are produced by this gland in response to stressful stimuli.

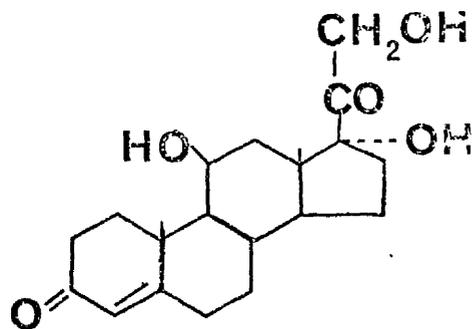
In the horse, as in man, all the corticosteroids are produced in the cortical region of the adrenal gland. By definition, the corticosteroids are those C - 21 steroids containing three or more oxygen atoms and which can be found in the adrenal glands, the blood and the urine. (Borth, 1956).

The basic structure of the corticosteroid molecule is shown in Fig. 2.1. All the steroids possess a $\Delta^4 - 3$ - ketone group which is believed to be essential for biological activity (Lorraine and Bell 1971).

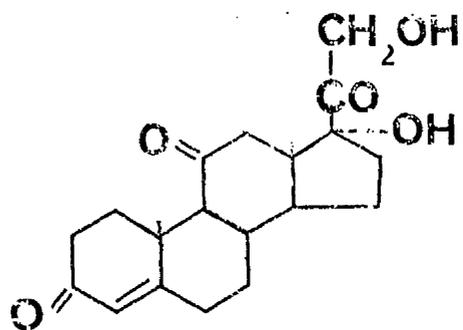
Generally, corticosteroids are defined as either glucocorticosteroids or mineralocorticosteroids depending on their relative effects on carbohydrate and electrolyte metabolism. The structural formulae of the three glucocorticosteroids present in greatest amounts in human plasma are shown in Fig. 2.1. These compounds are known collectively as the 11- hydroxycorticosteroids (11-OHCS) because they all have substituted groups on C - 11.

The glucocorticosteroids are synthesised in the two inner zones of the adrenal cortex namely the zona fasciculata and the zona reticularis from acetate via cholesterol Fig. 2.2.

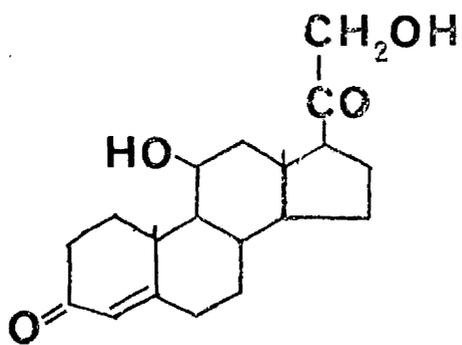
FIG. 2.1 STRUCTURAL FORMULAE OF
CORTISOL, CORTISONE AND CORTICOSTERONE



CORTISOL

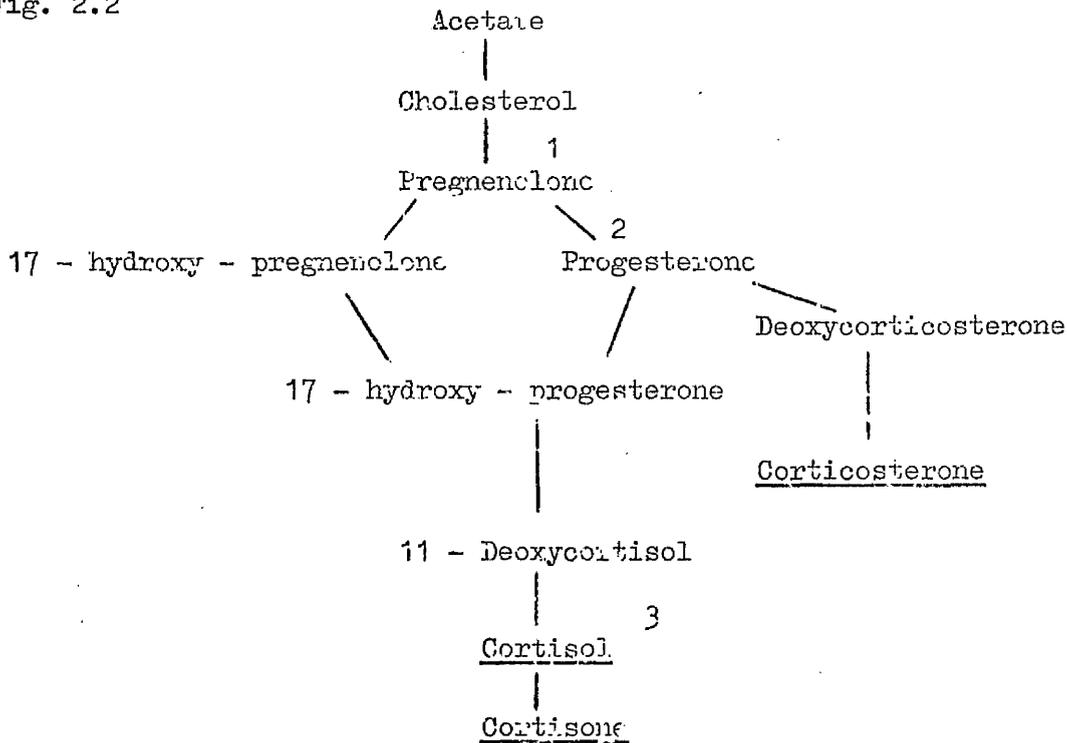


CORTISONE



CORTICOSTERONE

Fig. 2.2



The main enzyme systems involved at certain points in the metabolism are shown:-

1. Side chain cleavage enzyme
2. Reductase isomerase
3. 11 - hydroxylase

In the horse, it has been shown that the formation of 17 hydroxyprogesterone from both 17 hydroxypregnenolone and progesterone is rate-limiting and also that this reaction occurs more rapidly in the fascicular than in the reticular tissue (Cameron and Grant, 1967). However more recent studies in man have suggested that the rate-limiting step in corticosteroid biosynthesis is the conversion of cholesterol to pregnenolone. (Schulster, Burstein and Cooke, 1976).

The entire biosynthetic pathway takes place within the cortical

cells:-

- 1) cholesterol \rightarrow pregnenolone occurring within the cellular mitochondria.
- 2) pregnenolone \rightarrow 11 - deoxycortisol in the cytoplasm.
- 3) 11 - deoxycortisol \rightarrow cortisol in the cellular mitochondria.

The rate at which corticosteroids are synthesised is controlled by specific hormone adrenocorticotrophin (ACTH) which is secreted from the anterior pituitary gland in response to stimuli from higher centres in the central nervous system. The points at which ACTH acts in the biosynthetic pathway are thought to be at the level of cholesterol conversion to pregnenolone. The ACTH acts in the adrenal cell membranes, stimulating production of the enzyme adenylyl cyclase which in turn stimulates an increase in cyclic adenylylmonophosphate (AMP) concentrations. Cyclic AMP inside the cell binds to its specific receptor protein which exists as a regulatory complex with protein kinase and causes the release of the protein kinase from the receptor. The protein kinase in turn increases the production of free cholesterol in the lipid droplets (Garren, Gill, Masui and Walton, 1971). It is also possible however that ACTH may influence directly the enzyme system responsible for the side-chain cleavage of cholesterol (Schulster et al, 1976).

The major glucocorticoid circulating in the plasma of the horse is cortisol with corticosterone and cortisone as minor steroids (Zolovick, Upson and Eleftheriou, 1966; James, Horner, Moss and Rippon, 1970). Cortisol is also the major glucocorticoid present in the plasma of man (Dixon, Booth and Butler, 1967; Forsham, 1966).

ACTH is released from the anterior pituitary which is under the control of the hypothalamic area of the brain (Nelson 1972). The hypothalamus forms the floor of the 3rd ventricle of the brain and has many functions including the control of appetite, temperature and regulation of the pituitary gland (Martini and Ganong 1966; Sawin, 1969).

The anterior pituitary is formed from the oral region and has few direct neural links with the hypothalamus. Control of its secretions is exerted by means of chemical agents (peptides) secreted from the hypothalamus into the numerous portal-hypophyseal vessels carrying blood from the capillaries of the ventral hypothalamus directly to the anterior lobe (Green and Harris, 1949; Worthington, 1960 and 1963).

The chemical agent or releasing factor initiating the release of ACTH is called corticotrophin releasing factor (CRF) and the activity of the neurones producing CRF in the hypothalamus comes under the control of afferent impulses from higher centres in the brain (Hodges, 1976). The cerebral cortex midbrain, amygdala and hippocampus are all known to exert stimulatory or inhibitory effects on the mechanisms controlling CRF and hence ACTH release (Mangili, Motta and Martini, 1966).

Basal levels of circulating corticosteroids are controlled by a negative feedback system, with ACTH secretion being inhibited by increased plasma cortisol and cortisone levels and vice versa (Williams, 1968). Alterations in circulating levels of plasma 11-OHCS are thought to influence the rate of secretion of CRF from

the hypothalamus (Sawin, 1969; James and Landon, 1976) rather than to act directly on the pituitary since it has been shown (Sawin, 1969) that implants of corticosteroids in the hypothalamus will subsequently decrease ACTH secretion whereas similar implants in the pituitary do not produce this effect.

Apart from the negative feedback mechanism there is also a much more complicated control system responsible for the circadian rhythm of ACTH and 11-OHCS secretion. Circadian rhythm has been described in man, horses and other species (James and Landon, 1976; Krieger, Allen, Rizzo and Krieger, 1971; Campbell and Watts, 1973; Zolovick et al., 1966; Krieger, Silverberg, Rizzo and Krieger, 1968). Five to ten episodic bursts of ACTH secretion occur throughout the 24 hour period in man (Krieger et al., 1971; Ney, Shimizu, Nicholson, Island and Liddle, 1963) and are reflected in similar changes of plasma 11-OHCS levels. These alterations in both ACTH and plasma 11-OHCS levels have been related to a number of causes. Sleep/wake cycles, light/dark cycles, general activity cycles and feeding cycles have all been studied as causes of the fluctuations (Orth, Island and Liddle, 1967; Krieger, 1974; Natelson, Holaday, Meyerhoff and Stokes, 1975).

The circadian pattern of corticosteroid and ACTH secretion can be abolished by diffuse CNS disease or focal hypothalamic lesions (Krieger and Krieger, 1966; Slusher, 1964) and by the administration of certain drugs e.g. atropine (Krieger and Krieger, 1966) and betamethasone (Hodges and Mitchley, 1970).

The atropine effect would suggest that cholinergic mechanisms in the CNS are involved in the circadian release of ACTH over the

24 hour period. Since abolition of the circadian pattern did not result in an impaired response to either insulin-induced hypoglycaemia or lysine vasopressin (Krieger et al, 1968) it would also suggest that different CNS mechanisms and/or structures are involved in the regulation of circadian periodicity of 11-OHCS levels as opposed to stress initiated adreno-cortical responses.

A third controlling mechanism of the hypothalamic-pituitary-adrenal system is that which operates when external stimuli, such as exercise, trauma and surgical procedures, or any deviations from normal within the body, such as hypotension, haemorrhage and disease produce changes in corticosteroid levels independent of the feedback mechanism. It has been suggested therefore that there are two distinct areas in the hypothalamus mediating CRF secretion - one inhibiting it and the other stimulating it (Grizzle, Dallman, Schramm and Gann, 1974).

The effects of glucocorticoids are varied and widespread. They increase gluconeogenesis in the liver by increasing the conversion of amino acids to glucose (Cahill, 1971) and in turn decrease the amino acid uptake and incorporation into protein in muscle. They also increase lipolysis in adipose tissue resulting in release of glycerol and free fatty acids (Baxter and Forsham, 1972; Leung and Munck, 1975).

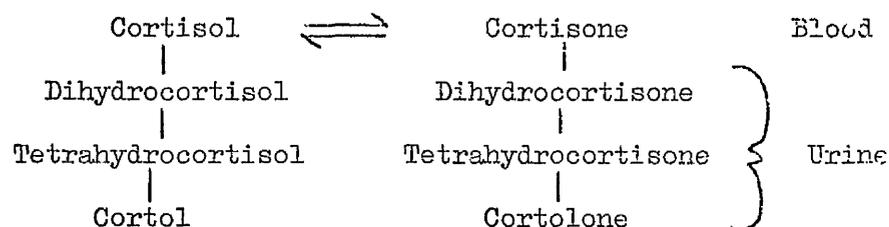
After secretion from the adrenal cortex, most of the corticosteroids are transported in the blood bound to protein. The greater proportion in man (about 80%) is bound to a specific binding globulin 'transcortin' and some of the remaining 20% to albumin.

(Sawin, 1969) Only a very small amount of the total corticosteroids produced (10%) is therefore 'free' in the plasma to bring about the various physiological actions on the body tissues (Sawin, 1969; James and Landon, 1976).

In man the plasma half-life of cortisol is between 60 and 90 min (Hellman, Nakada, Curti, Weitzman, Kream, Roffwarg, Ellman, Fukushima and Gallagher, 1970; Few, 1974; James and Landon, 1976) and a similar half-life has been recorded in the horse (James, Horner, Moss and Rippon, 1970).

A small amount of free cortisol is excreted unchanged by the kidneys, the remainder is metabolised mainly by the liver and excreted via the kidneys as inactive metabolites (50%) or as conjugated forms of these (30%) i.e. as cortols and cortolones (Fukushima, Bradlow, Hellman, Zumoff and Gallagher, 1960; Romanoff, Morris, Welch, Rodriguez and Pincus, 1961).

Fig. 2.3



The metabolic pathways of cortisol and cortisone in man are shown in Fig. 2.3, the metabolism of corticosterone occurring in a similar manner.

2.2

ESTIMATION OF CORTICOSTEROIDS2.2.1 Introduction

The metabolites of the corticosteroids found in urine can be measured by a number of methods. Unfortunately none of these are very specific since they estimate groups of metabolites rather than individual compounds and since most of the methods are based on a colour reaction nonsteroid substances can interfere and produce falsely high results. The small proportion of 'free' cortisol in urine can also be estimated using either a fluorometric technique similar to that used for plasma or a competitive binding method (Beardwell, Burke and Cope, 1968).

A method similar to that used for the assay of urinary corticosteroids has been devised for plasma utilising the Porter-Silber colourometric technique (Nelson and Samuels, 1952). Steroids measured by this method include cortisol, cortisone, 11-deoxycortisol, some metabolites of corticosteroids and other synthetic steroids such as prednisone and prednisolone. Corticosterone is not estimated by this technique. As in the colourometric reactions when applied to urine, lack of specificity of this method means that the materials assayed are referred to as 'Porter - Silber' chromogens, rather than corticosteroids.

Fluorometric methods of estimating plasma corticosteroid levels are also used, the most common one being that of Mattingly (1962). These techniques depend upon an ethanol-sulphuric acid induced

fluorescence of the 11-OHCS after their extraction from plasma with methylene chloride. Unlike the Porter - Silber reaction, this method does estimate corticosterone. While the fluorometric technique is simpler and requires less plasma than do the colourometric techniques, there is a disadvantage in that both methods are prone to interference from non-steroid materials.

The specificity of the fluorometric method has been assessed by a number of authors (De Moor, Steeno, Raskin, Hendriks, 1960; Mattingly, 1962) and it has been shown that synthetic steroids including prednisone, prednisolone and dexamethasone produce only minimal interference.

One of the more recent methods developed for corticosteroid estimation is the competitive protein binding assay - C.P.B. assay (Baum, Tudor and Landon, 1974). This technique uses selenium⁷⁵ labelled cortisol and rabbit serum and is a modified version of the competitive protein binding method of Murphy (1967) in which tritium labelled cortisol was used. The competitive protein binding assays are more specific than the fluorometric methods as they do not estimate any non-steroid material. However competition for binding sites on the binding protein between cortisol, corticosterone, cortisone, progesterone and 11-deoxycortisol can occur, (Murphy, 1967). Also, other steroids such as prednisolone have been found to cause high results with the competitive protein binding assay (Crawley, Garbien and Tuttlebee, 1975).

Another method recently developed for plasma cortisol estimation is that of radioimmunoassay - R.I.A. (Farmer and Pierce,

1974). This technique is more time consuming than any of the others but tends to be more specific because of the anti-sera used. Problems can arise due to cross-reactions of the anti-sera (Farmer and Pierce, 1974) or due to interference from the organic solvents and chromatographic materials used (Pizzaro and Kolanowski, 1972).

Although the R.I.A. method compares favourably with the C.P.E. Method (Farmer and Pearce, 1974) the former is generally considered to be too complicated a procedure for numerous routine applications.

All the methods outlined have been developed for the assay of corticosteroids in human plasma but have been used to estimate corticosteroid levels in animals. As can be seen later in this Chapter in Table 2.5, the plasma levels of corticosteroids in horses, as measured by the different methods, vary considerably.

Many comparative studies have been made of the various assay procedures. All of these however have been carried out using human plasma (Baum et al, 1974; Crowley et al, 1975; Gore and Lester, 1975). Bruton, Li and Smith (1973) recorded very poor correlation between the fluorometric method of Clark and Rubin (1969) and the Porter and Silber technique. They related this to the poor specificity of the colourimetric procedure.

Having considered all these facts and compared the costs of the various assays, it was decided to use the fluorometric technique of Mattingly (1962) for all corticosteroid estimations in this study.

The following study was therefore carried out to establish the fluorometric assay method and validate its application to the estimation of plasma 11-OHCS levels in the horse.

2.2.2 Materials

Glass extraction tubes (15 ml.). Quickfit.
 Automatic pipette. Eppendorf, West Germany.
 15 ml. glass extraction tubes, modified to form 2.5 ml. cuvette.
 J. J. McCulloch, Glasgow.
 Spectrofluorometer with modified lamp holder to contain extraction
 tube/cuvettes. Aminco-Bowman, Spectrophotofluorometer,
 American Instrument Co. Inc., Maryland, U.S.A.
 Liquid Scintillation Spectrometer, Packard Tri Carb, 3255.
 Scintillation vials - plastic (22 mm). Metal Box Limited,
 Plastics Group.

Chemicals

Dichloromethane. F.D.P.C. (B.D.H.)
 0.2 N NaOH. Volucon. (B.D.H.)
 Ethanol. Analar. Burroughs
 Concentrated sulphuric acid. Analar. (B.D.H.)
 Cortisol standard : hydrocortisone standardised vial (50 mg)
 Sigma Chemical Company.

The initial dilution to 28 nmol/litre was made in ethanol,
 subsequent dilutions to 1680, 1120, 560, 420, 280 and 140 n mol/
 litre were made in distilled water. Standards were stored in the
 dark at 4° C.

Quality Control Serum

A) Wellcontrol 1 (Bovine Serum) :

Estimated cortisol value 566 n mol/litre

B) Wellcontrol 2 (Horse Serum) :

Estimated cortisol value batch i) 311 n mol/litre

batch ii) 400 n mol/litre

The Wellcome Foundation Limited.

(1, 2, 6, 7 (n) ^{-3}H) Cortisol : supplied in a benzene/ethanol solution (9:1 V/V), 1 ml. containing 1m Ci activity.

Radiochemical Centre, Amersham

Liquid Scintillator ELS 93. Koch-Light Laboratories Ltd.

Decon 90 : cleaning fluid. B.D.H.

Corticosterone. Sigma Chemical Company.

Hydrocortisone. Sigma Chemical Company.

Heparin BP (mucoid.) 1000 units/ml. Evans Medical Limited.

Instagel. Koch-Light Laboratories Ltd.

2.2.3

METHODSCollection and preparation of blood for 11-hydroxycorticosteroid
(11-OHCS) analysis

Blood samples were obtained on all occasions from the jugular vein of the horses and ponies used during this study. When repeated sampling was necessary, a cannula was inserted into the jugular vein through a 16 gauge $1\frac{1}{2}$ " needle and held in place by surgical tape around the neck, once the needle had been withdrawn.

During these procedures the animals were restrained by means of a head collar and showed little or no signs of excitement or discomfort.

Blood was collected into heparinised plastic tubes and stored at 4° C until centrifugation (2,500 r.p.m.) which was generally within 30 minutes of collection. The plasma was removed and stored at -15° C until assay.

During sampling, the cannula was kept patent by flushing with 1-2 ml. of heparinised saline after each blood sample. The initial 3 ml. of each sample was discarded.

Collection and preparation of blood for ACTH estimation

Blood was collected from the jugular vein by venepuncture or through an indwelling cannula. It was placed immediately in plastic heparinised tubes on ice and then centrifuged at 4° C. The plasma was removed and stored in plastic tubes at -15° C until assay. Care

was taken to ensure that the samples did not come into contact with glass at any time, as ACTH adsorbs onto this substance (Landon, James, Wharton and Friedman, 1967).

Estimation of ACTH levels in plasma

A large number of bioassays have been developed to determine levels of circulating ACTH in plasma and in other body tissues but they are generally complicated, time consuming and not ideal for routine clinical purposes (Vernikas - Darnellis, Anderson and Trigg, 1966).

A radioimmunoassay has also been developed which is much more practical, sensitive and easy to use. The basic method is that of Landon and Greenwood (1968) using I¹²⁵ labelled antigen (Greenwood, Hunter and Glover, 1963) and anti-sera prepared in rabbits.

Preparation of the fluorescent reagent for plasma 11-OHCS estimation

Seven volumes of concentrated sulphuric acid were added slowly to three volumes of ethanol in a flask cooled to 0° C. The mixture was stirred continuously using a magnetic stirrer and stored in the dark at room temperature.

Statistical Analyses

All statistical analysis in this study were carried out using the paired 't' test and correlation coefficient.

Assay Method

The method was that of Mattingly (1962) with a few minor

modifications.

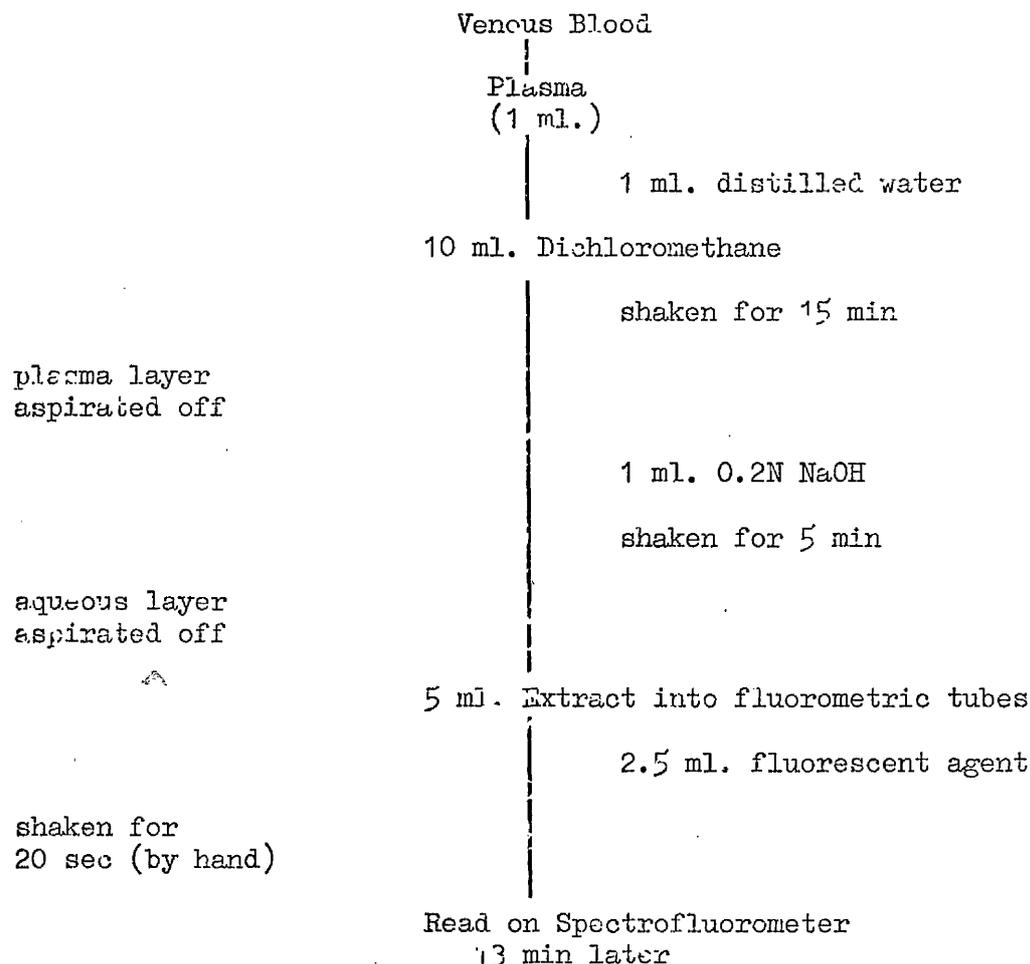
All glassware was soaked overnight in a commercial detergent solution (Decon 90) and rinsed thoroughly in distilled water before use.

1 ml. aliquots of thawed plasma were pipetted into 15 ml. extraction tubes containing 1 ml of distilled water.

1 ml. volumes of quality control serum and of each standard concentration (140, 280, 420, 560, 1120, and 1680 nmol cortisol/litre distilled water) were treated in the same manner. All five standards and the one quality control were assayed in duplicate and two 2 ml. distilled water blanks were also included.

A flow diagram representing the assay method is presented in Fig. 2.4

The modified spectrofluorometric tubes allowed them to be used for both the fluorometric step and for the reading of the fluorescence without transfer of the extract.

Fig. 2.4 Flow Diagram for 11-Hydroxycorticosteroid Assay

Because of the critical timing involved (Mattingly, (1962) described thirteen minutes as the time when interference from other fluorescing bodies was at a minimum) twenty-six tubes could be handled in this way per assay.

Readings of Relative Fluorescent Intensity (R.F.I.) were made (by multiplying the meter reading by the gain setting on the photomultiplier) at wavelengths of 472 μ excitation and 520 μ emission. These settings were ascertained by scanning a standard cortisol sample and setting the fluorometer to the peak excitation and emission wavelengths.

All readings were corrected by subtracting the distilled water blank. A standard curve of the fluorescence of the standard cortisol samples was plotted and the concentrations of the unknown samples read off from this scale.

Concentrations of the standard cortisol samples were found to remain stable for a period of at least two weeks when stored in the dark at 4° C.

2.2.4 Determination of the level of sensitivity of the assay

Borth (1957) had defined sensitivity as being the smallest single result which can be distinguished from zero.

Standard solutions of hydrocortisone (Sigma) were made up in ethanol and water concentrations ranging from 28 to 280 n mol/litre. Four samples of each concentration were assayed along with duplicate water blanks. After subtraction of the average blank R.F.I. values from the standard results the significance of the differences between consecutive concentrations was calculated.

Determination of the specificity of the assay

The specificity of the assay is defined as the ability of the method to determine solely the compound it purports to measure (Whitby, Mitchell and Moss, 1967).

In initial assays, the dichloromethane extract of the quality control serum samples exhibited a strong green colour, and it was noticed that on these occasions the recorded 11-OHCS levels were higher than those quoted for the quality control sera. Mattingly (1962) successfully reduced non-specific fluorescence in plasma by adding a washing step with 0.1N sodium hydroxide and this ^{was} step included in our assay. Estimated values of 11-OHCS in Wellcontrol 2 (Batch ii) were compared with quoted values before and after inclusion of a NaOH wash following dichloromethane extraction.

Determination of the accuracy of the assay

The accuracy of the method is defined as the concordance between

it and the true or most probable value of the quantity measured (Whitby et al, 1967).

Plasma was taken from a horse treated with dexamethasone twelve hours previously. This was assumed to be blank plasma since the levels of plasma 11-OHCS recorded in it were below the limit of detection of the assay.

A standard solution of cortisol (hydrocortisone) was made up in ethanol to a concentration of 1 mg/ml. Volumes of this standard equivalent to concentrations of 1680, 1120, 560 and 280 n mol/litre were then added to glass extraction tubes and blown dry under air at 55° C. 1 ml. of the blank plasma was added to each tube which was then mixed thoroughly and left at room temperature for 30 minutes.

The plasma samples, duplicate standards and distilled water blanks were then assayed as normal.

Determination of the precision of the assay

The precision of the assay is defined as the concordance of a series of measurements of the same quantity (Whitby et al, 1967).

Two quality control samples of the same value were included in every assay. Wellcontrol 1 is a control serum giving a high 11-OHCS reading and the Wellcontrol 2 (Batch ii) a low 11-OHCS reading.

Precision was calculated over a number of assays in which both types of Wellcontrol sera had been used. Standard deviations were estimated using the formula:- (Whitby et al, 1967)

$$SD = \frac{\sqrt{\sum (d)^2}}{2N}$$

where 'd' is the difference between duplicate readings and N the number of duplicate determinations performed.

The coefficient of variation was then calculated for each set of results using the formula:-

$$\text{C.V.} = \frac{\text{SD}}{\text{mean}} \times 100$$

Results

SENSITIVITY (TABLE 2.1)

Table 2.1 presents details of the significance of the differences amongst points of the hydrocortisone standard curve. It is apparent that the sensitivity of the standard curve lies at worst between 28 and 56 n mol/litre. It may be that a significant difference could also have been detected below the 28 n mol/litre level but for practical purposes in this thesis, the working range of the assay standard was considered to extend upwards from 56 n mol/litre.

SPECIFICITY (TABLE 2.2)

Initially, the assays carried out including Wellcontrol 2 (Batch ii) quality controls produced high readings of plasma 11-OHCS levels compared to those quoted by the manufacturer. These high readings were accompanied on each occasion by a strong green discolouration of the dichloromethane extract and of the fluorescent mixture. The inclusion of a sodium hydroxide wash in the assay after the extraction step, successfully removed this green colour and reduced the quality control readings to nearer the stated ones. NaOH

Table 2.1 The Significance of Differences amongst R.F.I. values on the Hydrocortisone Standard Curve

Concentration of Sample Assayed ($n = 4$)	Mean (\pm s.e.) R.F.I. reading	Mean (\pm s.e.) Corrected R.F.I. reading (Minus Blank)	Significance of Difference of corrected R.F.I.
Water Blank	20 (\pm 0.5)		
280 nmol/l	53.75 \pm 0.5	33.75 \pm 0.5	$p < .001$
210 nmol/l	46.75 \pm 0.5	26.75 \pm 0.5	$p < .001$
140 nmol/l	35.75 \pm 0.9	15.75 \pm 1.0	$p < .001$
56 nmol/l	27.75 \pm 0.5	7.75 \pm 0.5	$p < .001$
28 nmol/l	25.5 \pm 0.6	5.5 \pm 0.6	$p < .001$

Table 2.2 Plasma 11-OHCS levels found in Quality Control Serum

Quality Control Serum (Wellcontrol 2)		
Value Cortisol	Assayed Level 11-OHCS before NaOH wash	Assayed Level 11-OHCS after NaOH wash
311 n mol/l	439 n mol/l	304 n mol/l
	440 n mol/l	322 n mol/l
311 n mol/l	390 n mol/l	327 n mol/l
	395 n mol/l	322 n mol/l
311 n mol/l	363 n mol/l	318 n mol/l
	377 n mol/l	322 n mol/l
311 n mol/l	418 n mol/l	318 n mol/l
	427 n mol/l	318 n mol/l

washes were consequently included in all assays.

ACCURACY

The ability of the assay to determine accurately the amount of cortisol in horse plasma is shown in Fig. 2.5. The fluorescence values of the 'blank' plasma alone were subtracted from those of plasma plus cortisol in order to reduce any interference from endogenous cortisol.

The correlation coefficient was calculated and the equation of the line of the graph recorded.

$$r = 0.9973$$

$$y = -0.957 + 1.17 x$$

PRECISION

Standard deviations were calculated for each Wellcontrol value over approximately sixty assays. The overall results are shown in Table 2.3, the Coefficient of variation having been calculated separately for each of the two groups - Wellcontrols 1 and 2 (ii).

FIG. 2.5 THE ABILITY OF THE ASSAY TO
ACCURATELY DETERMINE THE AMOUNT OF
CORTISOL ADDED TO HORSE PLASMA

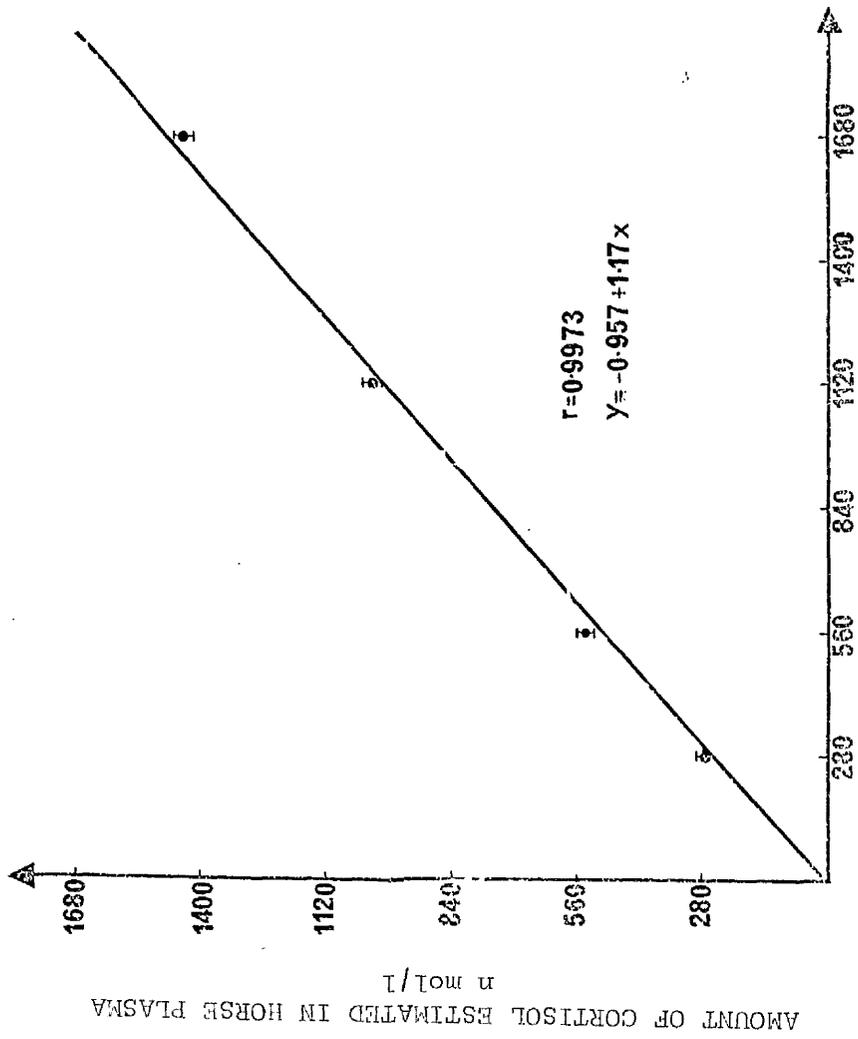


Table 2.3 Mean values and standard deviations of 11-OHCS in
Quality Control Serum samples

	Wellcontrol 1	Wellcontrol 2
Quoted levels cortisol present	566 n mol/l	400 n mol/l
Mean levels 11-OHCS found (No. samples analysed)	599.4 n mol/l (30)	398.9 n mol/l (37)
Standard Deviation	+ 12 -	+ 12.8 -
Coefficient of Variation	2.0%	3.2%

2.2.5 Recovery of (1, 2, 6, 7, (n) H^3) labelled cortisol from horse plasma

The fluorometric method of Mattingly (1962) has been evaluated previously using human plasma. It was important to assess the ability of this technique to extract 11-OHCS from horse plasma before applying the method.

Experimental procedure

The tritium labelled cortisol was supplied in a benzene/ethanol solution (9:1 V/V), 1 ml. containing 1m Ci activity.

0.1 ml. of this initial concentration was diluted in 100 ml. distilled water and 1 ml. of this solution diluted again in 100 ml. of distilled water. 100 μ l. of the final concentration was calculated to contain approx. 20,000 c.p.m. (\approx 500 pg cortisol).

100 μ l. of labelled cortisol was added to two plastic scintillation vials as controls and these were air dried in a water bath at 55^o C.

The same volume of H^3 cortisol was added to each of ten glass extraction tubes and was air dried at the same temperature. 1 ml. of horse plasma and 1 ml. distilled water was added to each tube. The tubes were mixed thoroughly for 30 seconds and left at room temperature for 30 minutes.

10 ml. of the extraction agent Dichloromethane were added to each tube and the tubes were shaken on a rotamix:-

- i) four tubes for 15 minutes

- ii) three tubes for 20 minutes
- iii) three tubes for 30 minutes

The plasma layer was aspirated off, 1 ml. of 0.1N sodium hydroxide was added to each tube and they were shaken on the rotamix for a further 5 minutes. The aqueous layer was again aspirated off and 2 ml. of the dichloromethane extract added to plastic scintillation vials. These were air dried at 55° C and 1 ml. distilled water and 10 ml. scintillation fluid added to each vial.

All the vials were counted on a liquid scintillation spectrometer and the recovered activity compared with that found in the vials to which labelled cortisol had been added directly.

Results

Recoveries obtained using different extraction times are shown in Table 2.4.

There appeared to be little benefit in continuing extraction for longer than 15 min.

Table 2.4 Recovery of H³ cortisol added to equine plasma and taken through the Mattingly (1962) extraction procedure using different extraction times, compared with an equal amount of H³ cortisol counted directly

	N*	% Recovery Mean values (Range)
15 min rotating	4	94 (92-97)
20 min rotating	3	90 (87-94)
30 min rotating	3	92 (87-94)

* number of tubes included

2.2.6 Practicability

Modification of the glass extraction tubes to incorporate a fluorometric cuvette reduced the time required for each individual sample and allowed twenty-six tubes to be assayed at one time. This, together with the fact that only 1 ml. of plasma was required for each estimation, meant that the assay could be carried out frequently and easily.

The fluorometric method was considerably cheaper than either the Porter - Silber colourimetric assay or the Competitive Protein Binding (C.P.B.) assay.

Application of the Method

Table 2.5 shows the results obtained by several authors for corticosteroid levels in horse plasma using different methods of corticosteroid assay.

Levels of 11-OHCS in resting animals recorded by the Mattingly (1962) method in this laboratory ranged from 70 - 426 n mol/litre and were slightly above the range of results recorded by other authors using similar methods.

Table 2.5 Plasma Cortisol Values (n mol/litre) recorded in Horses at Rest by Various Authors using Different Methods

Species	Assay Method	Resting values cortisol n mol/l	Author
Horse	Colourometric	499 - 720	Zolovick <u>et al</u> , 1966
Horse	C.P.B.	15 - 76	Bottoms <u>et al</u> , 1972
Horse	C.P.B.	142	Hoffsis <u>et al</u> , 1970
Horse	Fluorometric	299 - 352	Flisinska-Bojanowska <u>et al</u> , 1974
Horse	Fluorometric	83 - 360	James <u>et al</u> , 1970
Horse	Fluorometric	70 - 426	This Study

2.2.7 Discussion

Estimates of corticosteroid levels use methods involving either urine or plasma.

Using urine to estimate corticosteroid production however can result in numerous difficulties. There are problems associated with the variable dilution effects in urine and standardising these with reference to the amounts of creatinine present would not have been possible in some of our studies e.g. exercise tests. There is also a lag between the production of the urine and collection, such that the levels of corticosteroids measured would be the mean values over a period.

The various methods for measuring corticosteroids in plasma have been compared by numerous authors (Pirke and Stamm, 1972; Bruton et al, 1973; Gore and Lester, 1975; Crowley et al, 1975). The Porter - Silber procedure has difficulties in that a number of interfering chromogens in the extract may produce elevated results (Lorraine and Bell, 1971). It is however reproducible (Bruton et al, 1973) and is still used in some laboratories.

The fluorometric method of Mattingly (1962) has compared favourably with the more recent Competitive Protein Binding (C.P.B.) Assay described by Baum et al, (1974). Gore and Lester (1975) quoted a coefficient of correlation between the two of + 0.92, Crowley et al (1975) of + 0.94 and Bour et al (1974) of + 0.974.

Bruton et al (1973) on the other hand reported a poor correlation between the fluorometric technique of Clark and Rubin

(1969) and the Competitive Protein Binding Method of Murphy, Engelberg and Patti (1963). They attributed this difference to the fact that the former measured only the corticosteroids with an attached 11-hydroxyl group whereas the latter estimates all corticosteroids. The C.P.B. method of Murphy (1967) however was further modified by Baum et al (1974) and with this assay the correlation between the two techniques was better.

An important factor in determining which assay would be most practical and efficient for our purposes was the fact that many substances will interfere with both the fluorometric and C.P.B. assays producing falsely high results. De Moor et al (1960) and Mattingly (1962) added various substances including dexamethasone, prednisolone and betamethasone to plasma samples and assayed them using the fluorometric method. They concluded that whereas prednisolone produced a slight degree of fluorescence, dexamethasone did not. Crowley et al (1975) found similar abnormally high results following prednisolone treatment using both the fluorometric and the C.P.B. technique.

Gore et al (1974) and Crowley et al (1975) also reported that spironolactone, a drug used in the treatment of hypertension and structurally similar to aldosterone, caused fluorescence in plasma which lasted for several days following treatment.

It was important that drugs such as dexamethasone should not interfere with our assay specificity, since in the course of these studies a number of drugs of this type were to be administered to the horses.

Mattingly (1962) also reported that timing was critical in measuring the fluorescence after addition of the H_2SO_4 /ethanol mixture. Fluorescence due to non-specific agents increases with time, and the maximal point of 11-OHCS fluorescence occurs between ten and twenty minutes after addition of the fluorescent agent. Mattingly measured the fluorescence of his samples 13 minutes after adding H_2SO_4 /ethanol mixture and could assay only a limited number of samples because of the time involved in transferring them from glass tubes into cuvettes. Use of the combined glass tube/cuvette eliminated this step and enabled more samples to be read in the same time.

All three techniques, (colourometric, fluorometric and C.P.B.) were developed using human plasma but have been employed by different workers to estimate plasma corticosteroid levels in horses. As shown in Table 2.5 however the different methods do produce variable results in the horse. The high levels recorded by Zolovick et al (1966) using a colourometric technique may be explained by the fact that this assay is known to be subject to interference from many other substances.

The sensitivity as described for this technique was taken as the limit of detection of the assay. The smallest single result distinguishable from zero as reported by Mattingly (1962) was 64 n mol/litre and our result of 28 n mol/litre is considerably below this although levels of 15 n mol/litre have been recorded by Bottoms et al (1972). The limit of detection in this assay however was estimated using hydrocortisone standards made up not in plasma but in

ethanol and distilled water. It is possible that interference from other fluorescing substances in plasma would increase the limit of detection such that these results using distilled water might not truly reflect the sensitivity of normal assay conditions. For this reason we decided to take the limit of detection as 56 n mol/litre and in subsequent experiments, any plasma 11-OHCS concentration below this level was considered to be 'blank' plasma. Subsequent results suggest that the amount of interfering fluorescence in plasma cannot be high since in samples obtained when dexamethasone was employed to suppress adrenocortical function, many of the results obtained had levels below the limit of detection (56 n mol/litre).

De Moor et al (1960) and Mattingly (1962) have shown that the method is not completely specific for 11-OHCS with several other steroids producing fluorescence. However, the fluorescence produced by 11-OHCS (on a weight basis) with the sulphuric acid/ethanol mixture is considerably greater than with other steroids. Of the 11-OHCS present in human plasma, the greatest fluorescence is produced by corticosterone followed by cortisol (Mattingly, 1962). In the horse the latter is by far the more important (Zolovick et al, 1966; James et al, 1970) and therefore fluorescence due to corticosterone should be minimal.

The green discolouration present in the quality control samples appeared to be the cause of the over estimation of the results obtained since removal of the colour successfully reduced the recorded levels.

Silber and Porter (1954) and Silber and Busch (1956) used 0.1N sodium hydroxide to remove some steroidal and other phenolic and acidic compounds which interfered in their colourimetric assays. Mattingly (1962) also records the successful reduction in interfering fluorescence by certain synthetic steroids by incorporation a 0.1N sodium hydroxide wash in the extraction procedure. This step is often included in urinary assays as a further method of purification (Glenn and Nelson, 1953; Norymberski, Stubbs and West, 1953).

When a known amount of cortisol was added to blank plasma (plasma from an animal whose adrenal had been suppressed by administration of dexamethasone and whose basal plasma 11-OHCS level twelve hours later was below the limit of detection of the assay) and taken through the assay procedure, a linear relationship was found between the known amounts of added cortisol and the measured amounts (Fig. 2.5). These results of accuracy are similar to those reported by Mattingly (1962).

Precision was calculated over a large number of assays and can be taken to mean reproducibility as the assays were performed by the same person every time. The standard deviations recorded for both high and low Wellcontrol samples are lower than those reported by Mattingly (1962) for the original method and using a similar number of results. It is probable that the improvement in chemical reagents combined with our assay technique avoiding transfer of the extract from tubes to cuvettes would be the reason for this improvement in precision.

The recovery of tritium labelled cortisol added to equine plasma was within the range of 87 - 97% with a mean of 92% (Table 2.4) and

there was no significant difference in recovery with the length of time allowed for extraction. These results are similar to the recoveries reported in man by Mattingly (1962), but slightly lower than the ones reported by De Moor et al (1960). No corrections were made in subsequent assays for recovery.

3. DETERMINATION OF PLASMA CORTICOSTEROID

LEVELS IN THE HORSE

3.1 Introduction

A number of preliminary experiments were necessary prior to the use of corticosteroid estimations as a means of assessing stress in the horse. These studies were as follows:-

- i) estimation of the plasma half-life of cortisol in the horse since on a number of occasions repeated 11-OHCS measurements had to be taken over short periods of time.
- ii) assessment of the effect of venepuncture on plasma 11-OHCS levels. The majority of blood samples throughout this study were taken by this means and it was important to determine whether this sampling technique per se constituted a stress.
- iii) basal levels of plasma 11-OHCS levels were recorded in all the experimental animals. Blood samples were collected throughout this study at varying times of day and it was essential to establish whether a circadian rhythm of 11-OHCS and ACTH secretion as reported by other workers in horses, existed in our animals.
- iv) suppression of adrenocortical function by dexamethasone.
- v) assessment of the sensitivity of the adrenal cortex to small physiological doses of synthetic ACTH.

Both these latter tests are used in man to assess adrenocortical function and would possibly be a guide to the adrenal responses in our animals.

3.2

CALCULATION OF THE BIOLOGICAL HALF-LIFE OF
CORTISOL IN THE HORSE

This experiment was designed to calculate the half-life of tritium labelled cortisol in one pony.

Animals

Pony No. 3 was used in this study.

Materials

0.1 ml. of an aqueous solution of H^3 labelled cortisol containing 0.1 m Ci was calculated to give a sufficiently high count per ml. of plasma (i.e. 4.4×10^7 c.p.m.) and was diluted in 5 ml. of saline for injection.

Blood samples were centrifuged at $4^{\circ}C$ and $1\frac{1}{2}$ ml. plasma removed and added to plastic scintillation vials. 10 ml. Instagel was put into each vial which was shaken thoroughly before being counted in the scintillation counter.

Experimental Procedure

The left jugular vein of pony No. 3 was cannulated, as described previously, 2 hours before the start of the experiment.

The 5 ml. saline containing the 0.1m Ci of labelled cortisol was injected via the cannula. Blood samples (7 ml.) were taken at

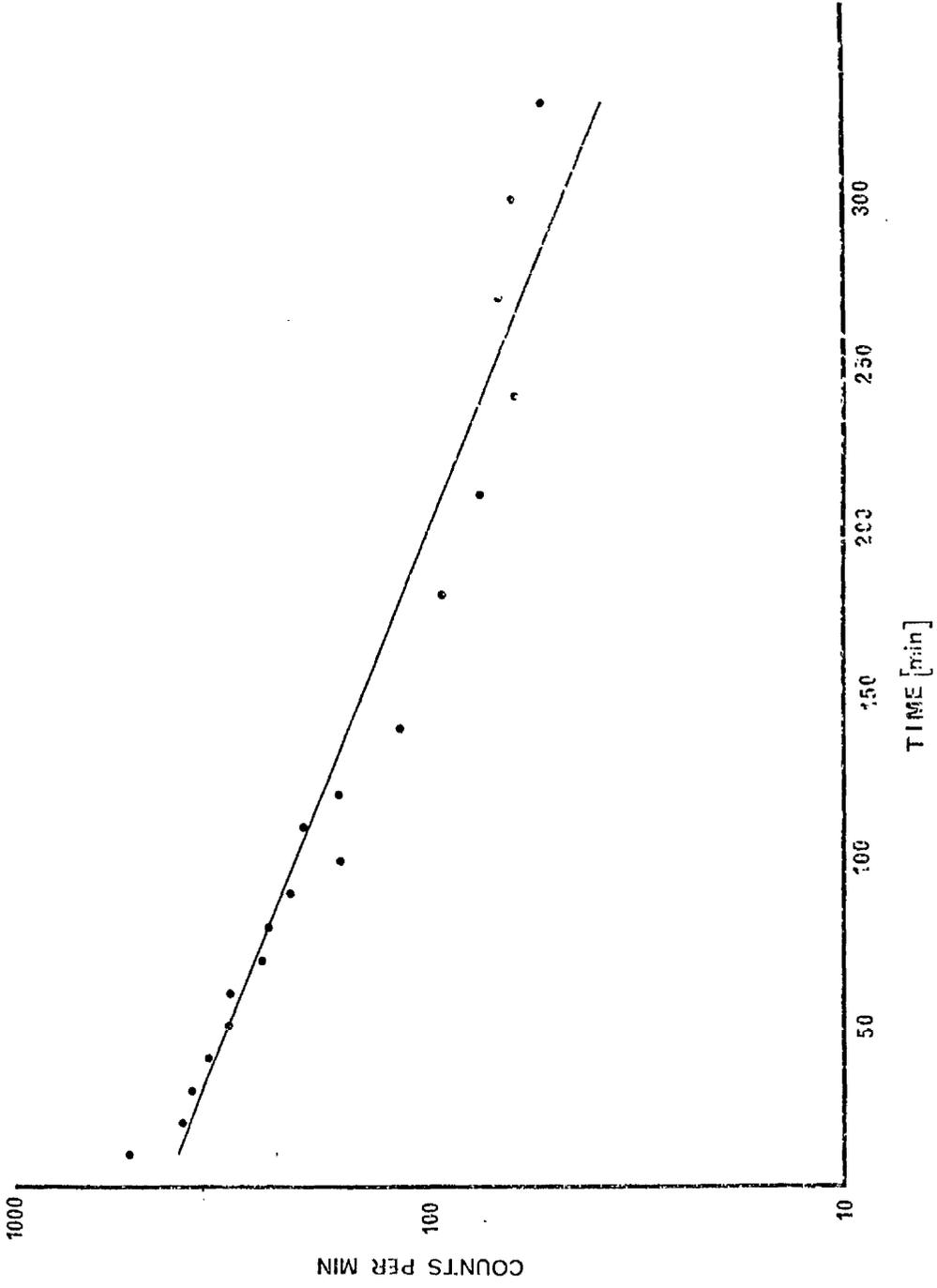
10 min intervals over a period of 2 hours and every 30 min for a further 4-5 hours.

Results (Fig. 3.1)

Counts per minute recorded were plotted against time on semi-log graph paper and since this revealed a mono-exponential pattern the equation of the line was calculated:-

$$y = 361.2 - 1.4 x$$

The half-life of cortisol calculated between 50 and 250 min was 103 min.



3.3 EFFECTS OF THE METHOD OF BLOOD SAMPLING ON THE LEVELS
OF 11-OHCS AND ACTH IN PLASMA

This experiment was designed to assess the effects of blood sampling by venepuncture on plasma 11-OHCS and ACTH levels.

Animals

Two ponies were used in this study (Nos. 1 and 3).

Materials

Cannula : intravenous nylon cannula set with 16G needle, I/D 1.0mm.

Length 30 cms. Portex Limited

Experimental Procedure

Cannulae (30 cms) were inserted through a 16 gauge needle into the left jugular veins of both ponies and the animals left to stand quietly for 2 hours. 25 ml. of blood was taken into plastic heparinised tubes - 15 ml. for ACTH and 10 ml for 11-OHCS estimation (C1). All blood was stored as previously described until and during centrifugation. Plasma was removed and stored at -15° C until assay for 11-OHCS and ACTH.

Five minutes later a blood sample was taken from the right jugular vein by venepuncture (V1) and 1 minute later a second sample was withdrawn via the cannula (C2). Finally, 2 minutes after C2

a third sample was withdrawn via the cannula (C3).

Results

The plasma levels of 11-OHCS and ACTH before and after venepuncture are shown in Table 3.1.

Plasma 11-OHCS values did not increase after venepuncture as compared to resting (C1) samples. Plasma ACTH levels increased at each successive sampling time reaching their highest levels at 3 min post venepuncture in both ponies (C3).

There was no significant correlation ($p > 0.05$) between plasma ACTH and 11-OHCS levels in either animal.

Table 3.1 Levels of plasma 11-OHCS and ACTH before and after Venepuncture in two ponies

Pony	Sample	Plasma 11-OHCS levels (n mol/l)	Plasma ACTH levels (ng/l)
1	C ₁	277	188
	V ₁	254	204
	C ₂	277	222
	C ₃	252	240
2	C ₁	247	120
	V ₁	168	162
	C ₂	250	173
	C ₃	250	200

3.4 LEVELS OF PLASMA 11-OHCS AND ACTH IN HORSES AND PONIES AT
REST AND VARIATION IN THESE DURING ONE HOUR AND
TWENTY-FOUR HOUR CYCLES

Before considering alterations in either plasma 11-OHCS or ACTH levels due to stressful situations, it was essential to estimate the range of these levels in clinically normal animals at rest and to determine any changes in these levels over short and long periods of time.

Animals

All seven horses and three ponies were used in this study.

Experimental Procedure

Blood samples for plasma 11-OHCS estimation were taken from the group of horses routinely over a period of 6 months. All samples were taken between 9 a.m. and 10 a.m. from animals which had been stabled overnight and had access to both hay and water.

A separate group of samples was taken from 20 thoroughbred race horses (2-7 years) before and during a training programme over a twelve month period. These samples were always taken from the animals at rest between 7 a.m. and 9 a.m.

In order to ascertain fluctuations in plasma 11-OHCS and ACTH levels over a short period of time, nylon cannulae were inserted into the left jugular veins of 2 ponies (Nos. 1 and 3) and blood samples

taken at 10 minute intervals over a period of 1 hour. As with all the experiments where cannulae were used, they were inserted more than 1 hour before blood sampling was due to start.

Diurnal variation in plasma 11-OHCS levels was studied by taking samples from alternate jugular veins in all 10 animals over a period of 48 hours. During this period, the animals were fed and watered as normal but were not exercised. Care was taken to ensure that samples, especially those taken during the night, were obtained with the minimum of interference and lighting. Samples were taken from 7 of the 10 animals at 8 a.m. and 10 p.m. for plasma ACTH estimation.

Results

The mean levels of plasma 11-OHCS in all of the 10 animals at rest were below 230 n mol/l, range 70 - 426 n mol/l (Table 3.2). There were no significant differences ($p > 0.1$) between mares and geldings, thoroughbreds and heavy hunters or ponies and horses and there was a large degree of variation within the levels of each animal.

The plasma concentrations of 11-OHCS in the group of thoroughbred horses in training are shown in Table 3.3. The levels fell within the range 155 - 465 n mol/litre for the group of 20 horses. This is a slightly higher range than was recorded in the previous experiment where heavy hunters and ponies were also included but the difference between the two groups was not significant ($p > 0.1$). Training also resulted in an increase in resting plasma 11-OHCS levels which were significantly above pre-training values ($p < 0.05$) after 3 and 9 months training.

Alterations in plasma 11-OHCS and ACTH levels over a 60 minute period in ponies No. 2 and 3 are shown in Table 3.4.

11-OHCS and ACTH values varied throughout the 60 minutes in both animals. The mean values of 163 and 220 n mol/litre of 11-OHCS are within the range of values found in the previous experiment for normal resting animals (Table 3.3). It is interesting to note that plasma ACTH levels in pony No. 3 were consistently higher than those in pony No. 2 although the 11-OHCS levels were not. Throughout the 60 minutes there was no significant correlation in either pony

Table 3.2 Levels of plasma 11-OHCS (mean \pm s.e.) in individual resting animals between 9 a.m. and 10 a.m.

	Type of Animal								
	Pony		Thoroughbreds		Heavy Hunter				
	Mares	Geldings	Mares	Geldings	Mares	Geldings	Mares	Geldings	
Plasma levels of									
11-OHCS		172 \pm 61.0 (27)	200 \pm 57.1 (35)	180 \pm 68.1 (40)	209 \pm 52.2 (40)	177 \pm 40.8 (30)	179 \pm 74.9 (25)		
(1 μ ol/l)		218 \pm 68 (23)	220 \pm 70.4 (40)	195 \pm 63.6 (40)					

Figures in brackets refer to number of occasions samples were taken for estimation in each animal

Table 3.3 Plasma 11-OHCS levels (n mol/l) in a group of twenty thoroughbreds before and during training

Horse No.	Sex	Occasions Sampled			
		Pre Training	3 months Training	6 months Training	9 months Training
1	S	211	327	272	338
2	F	222	338	155	393
3	S	211	379	216	299
4	S	278	355	449	
5	S	211	443	188	249
6	S	222	244	216	155
7	S	288	255	216	255
8	S	244	244	305	438
9	F	316	244	216	388
10	F	172	327		260
11	S		343	244	255
12	S	271	410	338	155
13	S		377	465	388
14	S	277	343	399	465
15	S	244	343	244	410
16	S	316	305	260	410
17	G	244	272	244	188
18	S	211	343	205	343
19	S	272	410	282	294
20	S	172	255	227	260
Mean \pm s.e		243 \pm 10.2	327 \pm 13.5	270 \pm 19.7	312 \pm 21.6

S - Stallion

G - Gelding

F - Filly

Table 3.4 Alterations in circulating levels of plasma 11-OHCS and ACTH in two ponies over a period of 60 minutes

Time (min)	Pony 2		Pony 3	
	Plasma 11-OHCS levels n mol/l	Plasma ACTH levels ng/litre	Plasma 11-OHCS levels n mol/l	Plasma ACTH levels ng/litre
0	218	149	268	323
10	186	162	309	330
20	186	199	227	294
30	197	125	191	302
40	145	111	172	350
50	134	244	154	186
Mean	163	165	220	297

between 11-OHCS and ACTH levels ($r = 0.2835$, $p > 0.1$; $r = 0.5078$, $p > 0.1$ respectively).

Fig. 3.2 shows the pattern of plasma 11-OHCS levels over 24 hours in all 7 horses sampled. The lowest mean 11-OHCS level occurred at 4 p.m. (147 n mol/litre) and the highest at 10 a.m. (266 n mol/litre). This difference was significant in all 7 horses combined ($p < 0.001$).

Fig. 3.3 shows the pattern of plasma 11-OHCS levels over a twenty-four hour period in the three ponies. In this case the lowest mean values occurred at 12 a.m. and the highest at 10 p.m.

There were no significant differences in 11-OHCS values at similar times between the mares and geldings or the horses and ponies. The differences between highest and lowest levels recorded are significant in each individual group (mares $p < 0.05$, geldings $p < 0.05$, ponies $p < 0.05$).

However there is great individual variation within the groups - standard deviation in some cases being as high as 108 n mol/litre.

There was also a large variation in the plasma ACTH levels between animals (range 26 - 441 ng/litre) and no significant correlation between these and the corresponding plasma 11-OHCS values ($r = 0.2516$, $p > 0.1$). In 3 of the 5 horses the lower ACTH levels occurred at 8 a.m. and a similar trend was noticed in all 3 ponies (Table 3.5). There was no significant difference between ACTH levels at 8 a.m. and 10 p.m. in the group of animals ($p > 0.1$).

FIG. 3.2 PLASMA 11-OHCS LEVELS IN SEVEN HORSES
(MEAN \pm s.e.) DURING A TWENTY-FOUR HOUR PERIOD

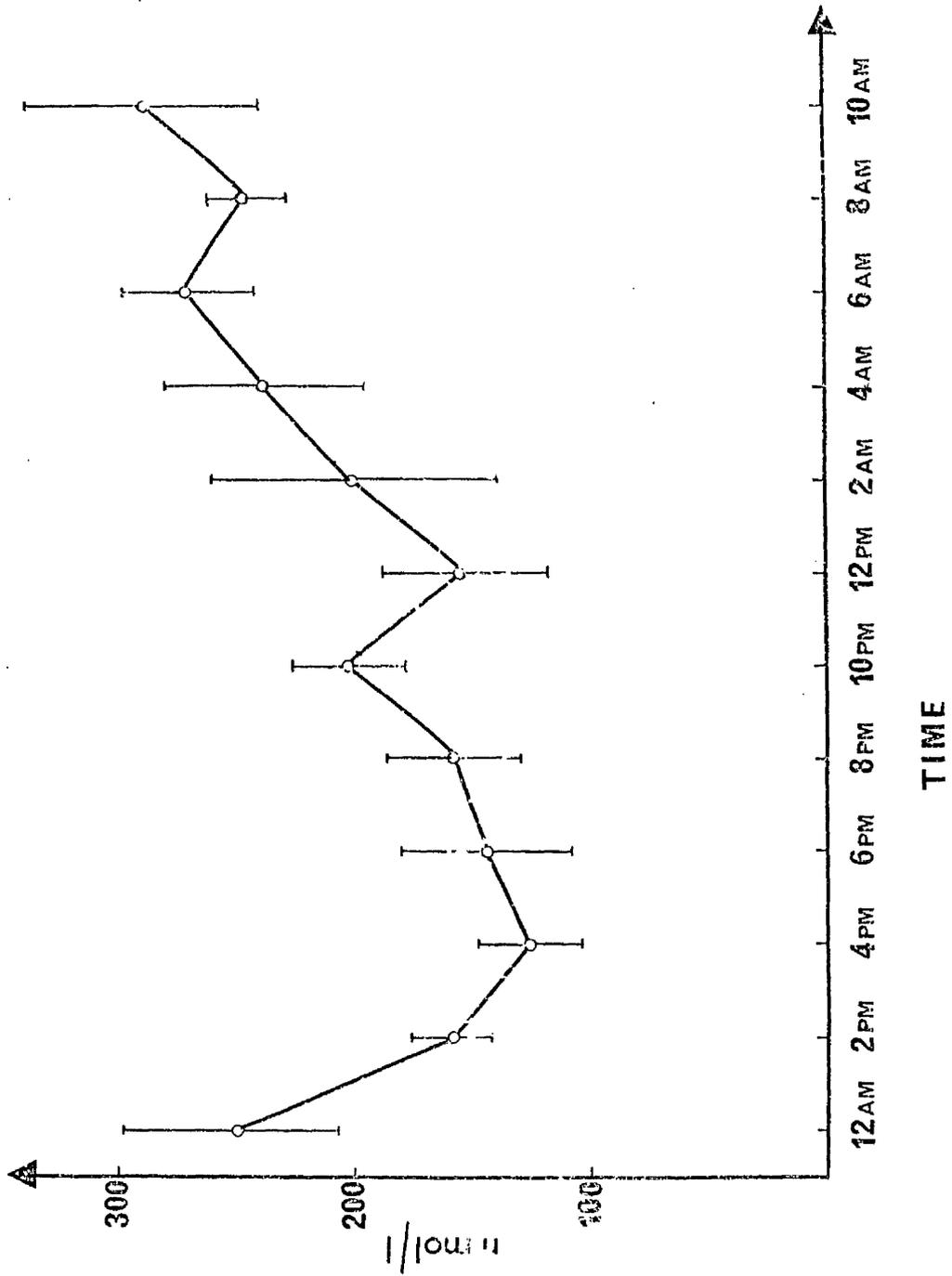


FIG. 3.3 PLASMA 11-OHCS LEVELS IN THREE PONIES
(MEAN \pm s.e.) DURING A TWENTY-FOUR HOUR PERIOD

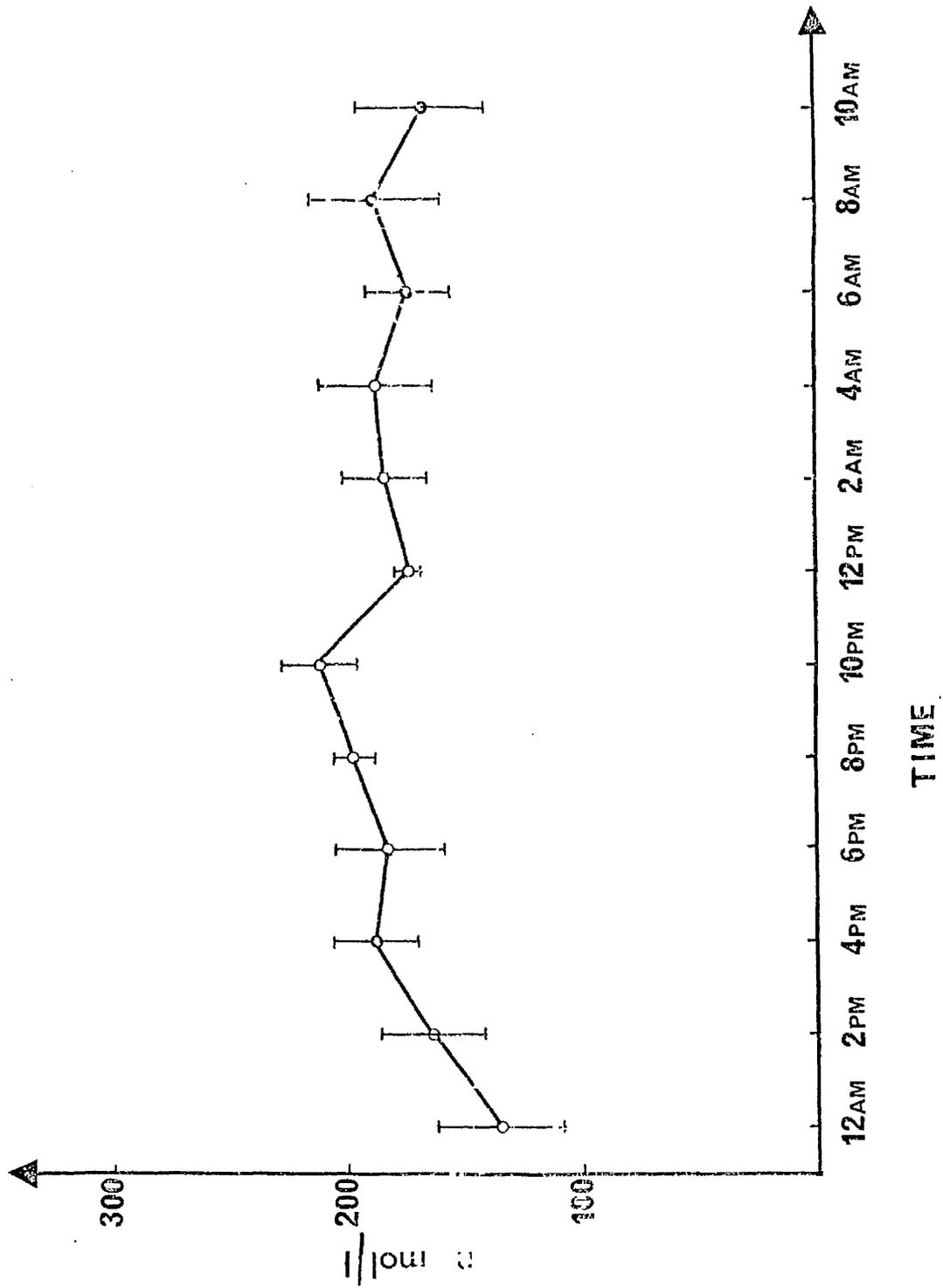


Table 3.5 Plasma 11-OHCS and ACTH concentrations at 8 a.m. and 10 p.m. Values in 5 horses and 3 ponies

	Time	Horses						Ponies		
		Sex	G	M	G	M	G	G	G	
Plasma ACTH levels (ng/l)	8 a.m.		196	213	53	146	54	86	413	234
	10 p.m.		84	-	195	238	-	300	441	348
Plasma 11-OHCS levels (n mol/l)	8 a.m.		218	209	218	145	200	170	168	120
	10 p.m.		104	134	191	182	184	186	175	154

'-' indicates samples not taken

3.5 EFFECT OF SUPPRESSION OF THE ADRENAL CORTEX BY DEXAMETHASONE
ON THE LEVELS OF 11-OHCS AND ACTH IN PLASMA

The initial object of this experiment was to ascertain whether or not dexamethasone would suppress adrenal function and thus produce a fall in circulating plasma 11-OHCS levels. If it could be shown that dexamethasone successfully suppress^{ed} adrenocortical function producing low levels of plasma 11-OHCS, it would then be possible to extend the investigation and measure the adrenal response to physiological doses of ACTH.

Animals

Three ponies (Nos. 1, 2 and 3) and one thoroughbred mare (No. 5) were used.

Drugs

Dexamethasone : Dexadreson (2 mg/ml). Intervet.

Experimental Procedure

Dexamethasone was administered intramuscularly at dose rates (on a weight basis) similar to those producing suppression of adrenocortical function in humans.

10 mg. dexamethasone (equivalent to approx. 0.06 mg/kg) was administered to each pony and 30mg. (equivalent to approx. 0.06

mg/kg) to the thoroughbred mare.

Plasma samples for 11-OHCS estimation were collected before injection and at 12, 24, 36 and 48 hours post-injection. In 2 of the ponies (Nos. 1 and 2) samples for 11-OHCS measurement were taken at 60, 72, 96, 120, 144 and 168 hours to ascertain the duration of adrenocortical suppression.

Results

In all the animals given dexamethasone there was a marked reduction in plasma 11-OHCS levels (Table 3.6).

Twelve hours after dexamethasone administration circulating plasma 11-OHCS values were below the limit of detection for the assay in all 4 animals and in ponies No. 1 and 2 they remained below the limit of detection (i.e. 56 n mol/l) for the first 48 hours. In horse No. 5 and pony No. 3, levels had almost returned to resting (pre-injection) values by 48 hours.

Duration of suppression achieved following this dose of dexamethasone was 96 hours, in ponies Nos. 1 and 2. By this time plasma 11-OHCS levels had returned to resting (pre-injection) values.

Table 3.6 Plasma 11-OHCS levels (n mol/litre) following dexamethasone injection (0.06 mg/kg)

Stage of Procedure	Animals				
	Horse No. 5	Pony No. 1	Pony No. 2	Pony No. 3	
Pre Injection	200	82	199	188	
Post 12 hrs.	-	-	-	-	
Post 24 hrs.	66	-	-	161	
Post 36 hrs.	102	-	-	161	
Post 48 hrs.	159	-	-	144	
Post 60 hrs.		100	116		
Post 72 hrs.		75	116		
Post 96 hrs.		122	199		
Post 120 hrs.		108	133		
Post 168 hrs.		180	188		

- refers to values below the limit of detection of the assay i.e. 56 n mol/litre

3.6 SENSITIVITY OF THE ADRENAL TO PHYSIOLOGICAL DOSES OF
SYNTHETIC ACTH

Having established in the previous experiment that administration of dexamethasone results in decreased levels of plasma 11-OHCS over a period of at least 24 hours, the response of the adrenal to physiological doses of ACTH was investigated in the 7 hour period beginning 12 hours after dexamethasone injection.

Animals

One pony (No. 2) and two Thoroughbreds (Nos. 1 and 3) were used in this study.

Drugs

Synacthen : synthetic Adrenocorticotrophic hormone (0.25 mg/ml)

Ciba

ACTH is a single chain peptide and the terminal 24 amino acids are identical in all species studied to date. Since this terminal portion of ACTH is the only part necessary for biological activity it has been possible to synthesise an identical peptide to the 24 amino acids of the natural hormone and this is Synacthen (James and Landon, 1976).

Experimental Procedure

Synthetic ACTH (Synacthen) was diluted to the required

concentrations in acidified saline (pH2) -- it has been shown (Landon, James, Wharton and Friedman, 1967) that this procedure reduces adsorption losses onto the glass bottles used.

Dexamethasone at the appropriate dose rate was administered at 10 p.m. after a blood sample had been taken for 11-OHCS estimation. At 8 a.m. the following morning a cannula was inserted into the left jugular vein and at 9.30 a.m. a blood sample taken.

At 10 a.m. (12 hours after dexamethasone administration) the following dosing and sampling regime was commenced. Injections were given through the cannula at 60 minute intervals and blood samples taken after each injection (10, 15, 20, 30 and 60 minutes). Immediately the 60 minutes sample had been taken, the next injection was administered and the procedure repeated.

A control injection of 1 ml. acidic saline was given to each animal at 9 a.m. before treatment with increasing doses of Synacthen from 25 to 1000 ng. was commenced at 10 a.m.

Blood samples, taken before and 11 hours after dexamethasone administration, were assayed rapidly for plasma 11-OHCS levels to confirm that a reduction in values had occurred.

Results

The responses of all 3 animals to this procedure are shown in Tables 3.7 and 3.8.

Plasma 11-OHCS levels decreased in all the animals to a level below that of the assay sensitivity 12 hours after dexamethasone administration. Injection of acidic saline appeared to produce no effect in any of the animals on circulating plasma 11-OHCS levels, neither did administration of 25 and 50 ng. Synacthen. In both horses Nos. 1 and 3 injection of 100 ng. Synacthen produced an increase in plasma 11-OHCS levels which at its maximum was 160% and 69% respectively above the levels immediately prior to that injection. Increasing doses of 250 and 500 ng. produced little change in horse No. 1 but after administration of 1000 ng. plasma 11-OHCS levels increased to almost resting (pre-dexamethasone) values.

Horse No. 3 on the other hand responded to increasing doses of Synacthen with gradually increasing levels of plasma 11-OHCS and following 1000 ng. these values were at 30 min post injection 39% higher than resting (pre-dexamethasone) levels.

Pony No. 2 did not respond to the increasing doses of Synacthen until the 250 ng. dose was administered. The maximum 11-OHCS level produced by that injection was 359% above the value immediately prior to it. Following the 500 and 1000 ng. doses the circulating plasma 11-OHCS levels increased by 44% and 93% respectively above resting (pre-dexamethasone) values.

Table 3.8 Alterations in plasma 11-OHCS levels (n mol/litre) after 250, 500 and 1000 ng. doses of Synacthen

Animal	Pre. Exp.	12 hr Post Dex.	Time (Minutes after injection)																							
			250 ng. Synacthen						500 ng. Synacthen						1000 ng. Synacthen											
			10	15	20	30	60	10	15	20	30	60	10	15	20	30	60	10	15	20	30	60				
Horse No. 1	212	-	122	128	106	138	122	106	160	160	240	128	144	198	288											
Horse No. 5	248	-	154	154	136	86	118	-	174	112	112	-	176	112	112	250										
Pony No. 2	268	-	80	96	116	224	164	332	332	386	332	276	304	380	456	516										

'-' indicates levels below limit of detection of the assay i.e. 56 n mol/litre

3.7 Discussion

Half-Life

The plasma half-life of cortisol recorded in this study (103 min) is slightly above the values of 60-90 min reported in man (Sawin, 1969; Hellman et al, 1970; Few, 1974; James and Landon, 1976) and also higher than the half-life of 80 min recorded by James et al (1970) in horses.

The half-life represents the rate at which cortisol is cleared from the plasma mainly due to its being metabolised in the liver and it has been shown in man that exercise will decrease and liver disease increase the half-life of cortisol (Few, 1974; James and Landon, 1976). The difference in our results compared to those of James et al (1970) could be due to a number of factors.

Firstly, although the pattern on the graph appeared to be mono-exponential there is a suggestion that there might be two phases of breakdown - the initial one being the more rapid of the two. Secondly there was a difference in the methodology employed in this experiment compared to that carried out by James et al (1970). These authors employed chromatographic separation before estimating plasma cortisol levels by a fluorometric method and they measured the half-life of cortisol by studying its rate of clearance from the blood after one intravenous injection. The method used in this study on the other hand does not differentiate between cortisol and its labelled metabolites and would therefore always tend to lead

to overestimation.

In this study there also appeared to be some variation in individual points around the line and this could be due in part to the low results obtained in these cases and the relatively high counting error (10%). Because of all these differences it would be of more interest to repeat this experiment in a greater number of animals in order to establish any patterns of individual variation.

Venepuncture

It was important to establish the effect of venepuncture on plasma ACTH and 11-OHCS concentrations because in many of the experiments carried out in this study it was not practicable to use a cannula.

The fact that no alterations in plasma 11-OHCS levels were found within 3 min of venepuncture agrees with the results of James et al (1970). In both our ponies however plasma ACTH levels had increased quite markedly by the time of the last sample suggesting that had blood sampling been continued over a longer period a similar rise in 11-OHCS values might have occurred.

During routine blood sampling from the animals at rest it was always possible to obtain blood within 3 min and therefore increases occurring after this time would not influence the results. On several occasions however sampling was continued at 10 or 15 min intervals and in these cases a cannula was inserted in order to minimise the effect of repeated stresses.

Plasma 11-OHCS and ACTH levels were never estimated immediately

following cannulation and having determined that $T_{\frac{1}{2}}$ for cortisol was in the region of 103 min in one of our ponies, a period of at least $1\frac{1}{2}$ hours was always allowed after cannulation before commencing each experiment. Resting (pre-experiment) blood samples were taken before cannulation of the vein and compared with a second resting sample taken immediately prior to the experiment for comparison.

Normal resting levels

The range of plasma 11-OHCS levels recorded in both groups of horses (Tables 3.3 and 3.4) are similar to those reported by other workers (James et al, 1970; Hoffsis, Murdick, Tharp and Ault, 1970; Flisinska-Bojanowska, Skwarlo, Lukaszewska, Bobilewicz, Wilk and Gill, 1974; Snow and Munro, 1975) but lower than those quoted by Zolovick et al (1966). This discrepancy could be due to the fact that latter authors used the colourimetric technique of Porter and Silber (1950) for cortisol estimations and as has been discussed previously this method can over estimate corticosteroid levels due to interference from other substances.

No significant differences in 11-OHCS levels were apparent between animals of different sex. This confirms the results of earlier authors (Hoffsis et al, 1970; Flisinska-Bojanowska et al, 1974). In man, the levels of plasma corticosteroids are thought to decrease with age (Bliss, Sandberg, Nelson and Eik-Ness, 1953) but no age related findings were obvious in this study nor in the work carried out by Hoffsis et al (1970).

The range of plasma 11-OHCS levels recorded in the thoroughbred horses throughout a training programme was slightly higher than that in our ten experimental animals. The increases in values of individual horses (Table 3.4) were spasmodic and apparently unrelated to specific animals or to the stage of training. There was however a significant difference between the mean resting levels of the untrained horses and the same animals after three and nine months of training. These results agree with those of Frenkl and Csabay (1970) and Buick and Tharp (1971) who reported an increase in resting plasma 11-OHCS levels in rats half way through a training period of six weeks.

Variations in plasma 11-OHCS and ACTH levels

Circadian variation in plasma 11-OHCS levels have been demonstrated in many species including man (Orth, Island and Liddle, 1967; Weitman, Fukushima, Nogeire, Roffwarg, Gallagher and Hellman, 1971) rats (Krieger, 1974), dogs (Halliwell, Schwartzman, Hopkins and McEvoy, 1971; Campbell and Watts, 1973), cattle (McAdam and Eberhast, 1972), pigs (Whipp, Wood and Lyon, 1970) and horses (Zolovick et al, 1966; Hoffsis et al, 1970; Bottoms, Roesl, Rausch and Akins 1972; Snow and Munro, 1975).

This pattern of 11-OHCS secretion, has been reported to coincide with similar changes in plasma ACTH levels (Krieger et al, 1971) and five to ten bursts of episodic secretion have been shown in man to occur throughout the 24 hour period (Hellman et al, 1970).

The initial experiment in this study was carried out to

recognise not only circadian rhythms of 11-OHCS secretion but also patterns of shorter periodicity i.e. 60 min. From these results it would appear that plasma 11-OHCS levels do alter over short periods of time and this would agree with the studies of Hellman et al (1970) who demonstrated secretory bursts of 11-OHCS production in man occurring every 20 min throughout a 24 hour cycle.

Plasma ACTH levels do not show a similar pattern, there being no significant correlation between the two sets of results in either pony. There was a coefficient of variation of 20% in the assay method used to estimate ACTH levels however (Gray, personal communication) which means that the values recorded may not truly reflect any rhythm of ACTH secretion. In man, Krieger et al (1971) have shown that at the great majority of times throughout the 24 hour period, a given ACTH peak preceded or occurred at the same time as an elevation in plasma 11-OHCS levels. As ACTH values have never been recorded previously in horses it is probable that a similar picture would emerge in this species if more work were carried out.

Combining the results from all ten experimental animals of 11-OHCS levels a definite pattern of circadian secretion emerges similar to that reported by other workers. Highest values were recorded in early morning and lowest in early evening. The exact mechanism controlling the periodicity of 11-OHCS secretion is unknown but a number of suggestions have been put forward. In man, the sleep-waking cycle is closely related to changes in plasma 11-OHCS levels and experimental alteration of this schedule over a

period of 7 days did result in changes in the pattern of 11-OHCS secretion (Orth et al, 1967; Perkoff, Eik-Nes, Fred, Nimer, Rush, Samuel's and Tyler, 1959; Hellman et al, 1970; Weitzman et al, 1971). Association with light-dark cycles has also been reported in rats (Fiske and Leeman, 1964; Cheifetz, Gaffud and Dingman, 1968) although in monkeys Natelson, Holaday, Meyerhoff and Stokes (1975) reported no correlation between illumination and cortisol secretion. In man, also Perkoff et al (1958) reported that total blindness had no effect on the circadian rhythm of 11-OHCS secretion. Krieger (1974) recorded that by restricting food and water intake in rats to a certain limited time a 12 hour shift in the time of peak plasma 11-OHCS levels occurred. This change was linked however to alterations in the animals sleep-wake pattern due to a need to obtain adequate food and drink.

The hypothalamus releases corticotrophin-releasing-factor to stimulate ACTH secretion and a circadian rhythm in this pituitary stimulatory activity of the hypothalamus has been described in mice (Ungar, 1964). Disease of the central nervous system has correspondingly been shown to alter the circadian pattern of patients especially those suffering from disease of the temporal lobe or hypothalamus (Krieger and Krieger, 1966) and experimental destruction of the anterior areas of the hypothalamus also inhibited the normal 5 p.m. rise in rats (Slusher, 1964). In both these cases however, normal corticosteroid responses to stressful stimuli such as noise were not abolished, suggesting that two distinct areas are involved in these aspects of ACTH release.

Circadian changes in plasma 11-OHCS levels can also be abolished by the actions of several drugs. Atropine, administered just prior to the time of expected elevations in 11-OHCS levels blocks this increase suggesting that cholinergic mechanisms are involved in ACTH release (Krieger and Krieger, 1966; Krieger *et al.* 1968). Atropine however did not block the adrenal response to administered ACTH, or insulin-induced hypoglycaemia suggesting that several CNS mechanisms or structures are responsible for the regulation of circadian periodicity as opposed to stress induced adrenal cortical responses.

Betamethasone on the other hand, when injected into rats suppressed both circadian and stress-induced changes in plasma corticosterone (Hodges and Mitchely, 1970). These observations agree with the suggestion that the mechanisms controlling circadian ACTH rhythm and stress-induced ACTH release are dissociated.

The circadian pattern recorded in our horses could be related to sleep-wake cycles although this is difficult to say since all the samples taken through the night involved wakening the animals. For this reason blood samples were taken at 2 hour intervals over a 48 hour period in the hope of reducing the interference caused, as much as possible. It was noticed that when considering the group of horses compared to the ponies, the latter did not show such an obvious circadian pattern. All the horses (Thoroughbreds and heavy hunters) were kept in loose boxes and had been over a number of years, the ponies were usually allowed to graze outside in a field and were only brought in for experiments. The peak plasma

11-OHCS levels occurred in the horses between 6 and 10 a.m. - stable routine involved work starting around 8.30 a.m. and feeding was always carried out around 9 - 9.30 a.m. By 6 p.m. when 11-OHCS levels were at their lowest, all the work was over for the day and the grooms had left. It is possible therefore that the pattern of corticosteroid secretion was, as well as being controlled by a sleep-wake cycle affected in some way by the day-to-day routine in the stables. A similar pattern has been demonstrated in dairy cattle (MacAdam and Eberhapt, 1972) subject to a strict daily schedule except that their peak levels occurred earlier at 4.30 a.m.

Dexamethasone suppression

Dexamethasone is the steroid most commonly used in man because at the doses required to produce suppression neither it, nor its metabolites influence plasma steroid levels as determined by the fluorometric method (James and Landon, 1976).

The effects of dexamethasone on circulating levels of plasma 11-OHCS have been recorded in horses by various workers. Hoffsis et al (1970) gave an intramuscular dose of dexamethasone and recorded maximal adrenal suppression occurring at between 12-24 hours and a gradual return to pre-treatment levels by 72 hours. James et al (1970) administered 0.04 mg/kg. dexamethasone intravenously and recorded depression of plasma 11-OHCS levels 24 hours later.

The results reported in these experiments therefore agree with those published elsewhere. The two ponies (Nos. 1 and 2) in which duration of suppression was recorded both showed complete depression

of 11-OHCS levels for 48 hours after dexamethasone treatment. This is slightly longer than was reported by Hoffsis et al (1970) but the latter gave no details for the dose of dexamethasone used.

There was a considerable degree of individual variation between the animals in that 2 of the 4 animals showed suppression lasting only between 12 and 24 hours. This variation could be due to different levels of threshold sensitivity i.e. certain animals might need higher levels of circulating corticosteroids before the negative feedback mechanism is triggered. It would be advisable therefore to confirm total suppression of the adrenal by estimating plasma 11-OHCS levels before commencing any further tests dependent on low basal corticosteroid values.

Adrenal sensitivity to physiological doses of ACTH

The low baseline levels which resulted from dexamethasone injection made the small changes in circulating corticosteroid levels in response to administered ACTH much more apparent. Also dexamethasone in doses sufficient to suppress endogenous 11-OHCS secretion did not appear to impair adrenocortical function. A procedure based on determining plasma 11-OHCS values immediately before and after nanogram amounts of synthetic ACTH has been used in man as a test of adrenal sensitivity (Landon et al, 1967).

In those studies both horses showed a greater degree of adrenal sensitivity to ACTH than did the pony. This may be a feature of fit Thoroughbred animals since training has been shown in rats to produce adrenal hyperplasia (Tharp, 1975).

It was surprising that in Horse No. 5 the peak level produced following the 100 ng. dose of Synacthen was higher than that recorded after either the 250 or 500 ng. dose although the maximum 11-OHCS levels were recorded following the final 1000 ng. dose of Synacthen.

Summary

From these studies it would appear that the modified fluorometric method of Mattingly (1962) is sufficiently sensitive to assay peripheral plasma 11-OHCS levels in horses.

The normal range of plasma 11-OHCS levels in horses at rest has been established and these correspond to those reported by other workers. It was also established that a circadian variation occurs in Thoroughbred horses but this could not be demonstrated as clearly in ponies. The response of the equine adrenal in a number of situations is similar to that of man and other animals.

4. THE EFFECTS OF VARIOUS STRESS SITUATIONS
ON THE ADRENAL CORTEX AND ON THE
SYMPATHETIC NERVOUS SYSTEM

4.1 Introduction

The adrenal cortex responds to a wide variety of stressful situations by increasing the rate of production and release of 11-OHCS. This response depends upon intact neural and hormonal pathways between the central nervous system, hypothalamus, pituitary and adrenal cortex.

It is possible to artificially stimulate either the entire hypothalamic-pituitary-adrenal (H.P.A.) axis or the adrenal cortex alone using a number of substances e.g. synthetic ACTH or insulin (James and Landon, 1976; Kehlet, Blichert-Toft, Lindholm and Rasmussen, 1976). By using such methods of artificial stimulation, the integrity of the H.P.A. axis can be assessed. These tests are used frequently in man and have also been carried out on horses (James et al, 1970).

Changes in plasma 11-OHCS levels are also associated with natural conditions such as physical exercise (Wasserman, Van Kessel and Burton, 1967; Wasserman and Whipp, 1975) and surgical trauma (Clarke, Johnston and Sheridan, 1970). These alterations have been extensively investigated in man but there is a paucity of similar results in the horse.

The following experiments were undertaken to compare the effects of various stressors, on heart rates, packed cell volumes, plasma 11-OHCS levels and a number of associated parameters.

4.2 EXPERIMENTAL STIMULATION OF THE H.P.A. AXIS BY INSULIN
AND INCREASING DOSES OF SYNTHETIC ACTH

4.2.1 Effects of insulin-induced hypoglycaemia on circulating
plasma 11-OHCS, ACTH and glucose levels

Insulin-induced hypoglycaemia is used in man to test the integrity of the hypothalamic-pituitary-adrenal system (Landon, Wyn and James, 1963; Greenwood, Landon and Stamp, 1966; Staub, Jenkins, Ratcliffe and Landon, 1973) and has been used in horses as a means of maximally stimulating glucocorticosteroid release (James *et al.*, 1970).

Insulin administration produces an increase in the uptake of glucose into muscle and an increase in the conversion of glucose to glycogen in the liver and in fat tissue. The net result is a fall in blood glucose levels (Goodman and Gilman, 1975). In order to increase the supply of glucose again many changes occur including increased gluconeogenesis and glycolysis. These changes are brought about by a number of hormones but mainly by the catecholamines, the glucocorticosteroids and glucagon. Glucagon and the catecholamines increase glycolysis in the liver and in muscle and the latter inhibit further insulin release. Together with the glucocorticosteroids, all three hormones stimulate gluconeogenesis in the liver and kidneys and also lipolysis in the adipose tissue (Williams, 1968).

The response recorded following insulin-induced hypoglycaemia should depend therefore on the fall in blood glucose produced (Landon *et al.*, 1963). The purpose of this experiment was to assess the

effects of varying doses of insulin on blood glucose levels in ponies and to compare the adreno-cortical responses to insulin-induced hypoglycaemia with those resulting from maximal stimulation by ACTH.

Materials

Insulin BP (40 units/ml).	Duncan, Flockhart and Co. Ltd., London.
Technicon Autoanalyser II.	Technicon Corp., Incorp., Tarrytown, New York.

Animals

Three ponies (Nos. 1, 2 and 3) were used. All were housed in loose boxes and allowed hay and water ad lib. Food was withdrawn throughout the experimental period.

Methods

Plasma 11-OHCS levels were measured as described previously.

Plasma ACTH levels were measured by radioimmunoassay.

Blood (approximately 3 ml) was added to tubes containing 20 mg. sodium fluoride/sodium oxalate mixture (1:3) and the plasma removed for blood glucose estimation. This was carried out on a Technicon Autoanalyser II using the method AA II - 2. (Neocuprøine Hydrochloride).

Heart rates were measured by auscultation in experiment 1.

Experimental Procedure

Cannulation of the jugular vein was carried out 2 hours before insulin injection (as described previously). A control blood sample for plasma 11-OHCS, ACTH and glucose estimation was taken 1 hour 45 min later.

Plasma 11-OHCS and glucose levels were measured 15, 30, 45, 60, 75, 90 min after insulin administration in both experiments.

Plasma ACTH levels were estimated in one pony in each experiment at 15, 30, 60 and 75 min after insulin.

EXPERIMENT 1

0.1 units/kg. of soluble insulin were administered intravenously to all three ponies.

EXPERIMENT 2

0.2 units/kg. of soluble insulin were administered intravenously to ponies No. 2 and 3.

All times quoted are times after insulin injection.

4.2.2 Results

EXPERIMENT 1 : 0.1 UNITS INSULIN/KG.

Pony No. 1

BEHAVIOURAL SIGNS

Between 30 and 60 min this pony showed mild sweating and restlessness.

HEART RATE (TABLE 4.1)

Heart rate altered slightly between 45 and 60 min.

BLOOD GLUCOSE (FIG. 4.1)

A marked hypoglycaemia was apparent, blood glucose levels falling to .92 mmol/litre within 60 min. By 2.5 hours the levels returned towards resting (pre-insulin) values.

PLASMA 11-OHCS AND ACTH (FIG. 4.1)

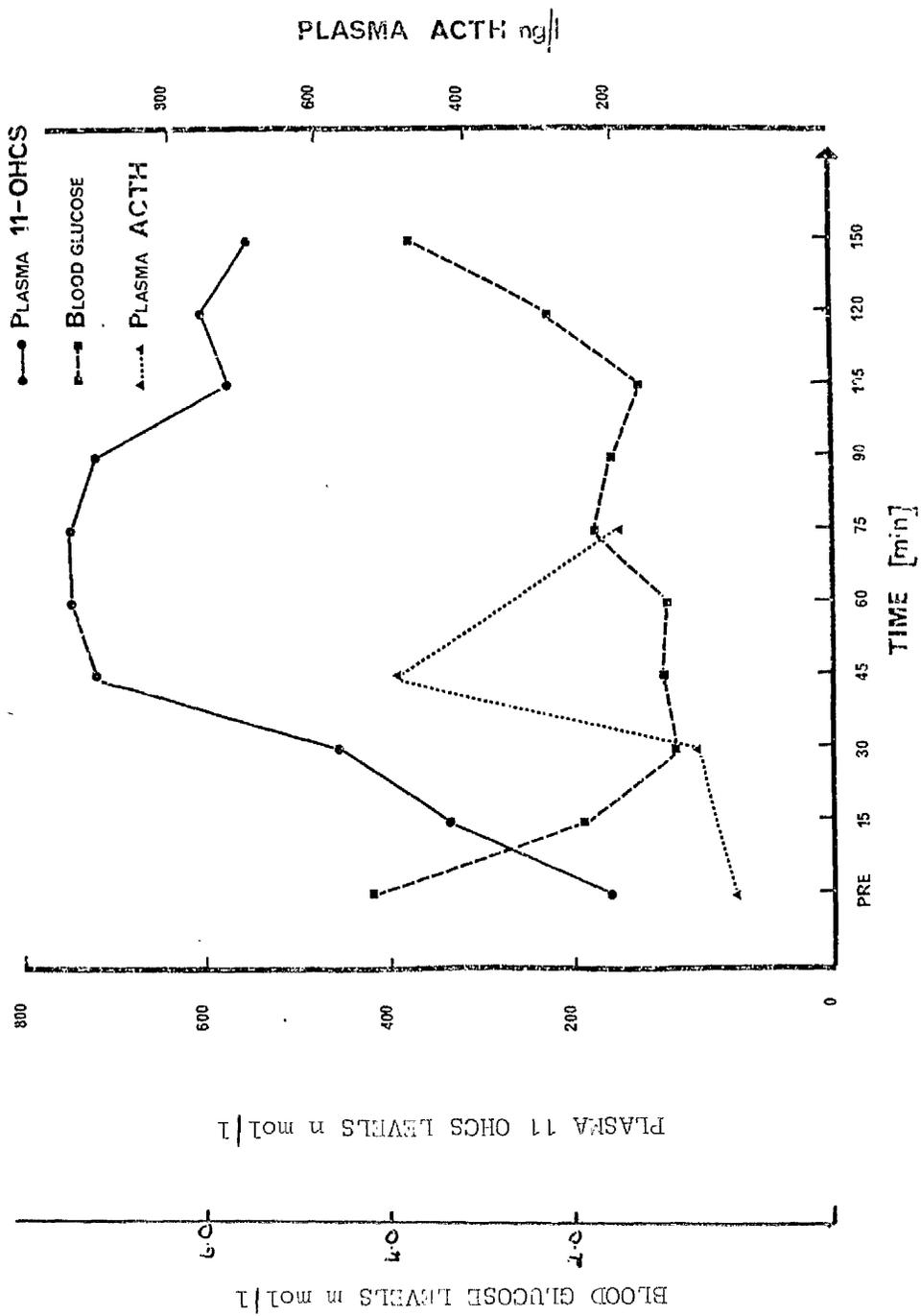
Alterations in plasma ACTH and 11-OHCS levels occurred at the same time as the fall in blood glucose values. Maximum ACTH levels (490 ng/litre) occurred at 45 min and maximum 11-OHCS levels (748 n mol/litre) at 60 min.

ACTH levels had fallen towards resting values by 75 min but plasma 11-OHCS values remained elevated 2.5 hours after insulin injection at which time blood glucose levels had returned almost to resting values.

Table 4.1 Heart rate (beats/min) in 3 ponies after 0.1 units/kg. Insulin i/v

Stage of Procedure	Animals		
	Pony No. 1	Pony No. 2	Pony No. 3
Pre Insulin	48	48	48
15 min Post Insulin	48	44	48
30 min Post Insulin	54	44	48
45 min Post Insulin	60	48	42
60 min Post Insulin	58	42	44
75 min Post Insulin	42	48	48
90 min Post Insulin	48	44	48
120 min Post Insulin	42	44	43

FIG. 4.1 ALTERATIONS IN PLASMA 11-OHCS, ACTH
AND GLUCOSE LEVELS IN PONY NO. 1 FOLLOWING
0.1 UNITS INSULIN/KG. ADMINISTERED INTRAVENOUSLY



Ponies No. 2 and 3

BEHAVIOURAL SIGNS

No abnormal behavioural signs were apparent.

HEART RATE (TABLE 4.1)

Heart rates remained unchanged throughout the 2.5 hour period.

BLOOD GLUCOSE (FIGS. 4.2 AND 4.3)

Blood glucose levels fell in both animals but not to values as low as those recorded in pony No. 1 (174 and 228 mmol/litre respectively). The response was also much slower, lowest levels being recorded between 60 and 75 min. By 2.5 hours the levels had returned almost to resting values.

PLASMA 11-OHCS (FIGS. 4.2 AND 4.3)

Changes in plasma 11-OHCS levels were less marked than those in pony No. 1. One of the ponies (No. 2) showed an increase in 11-OHCS values above pre-treatment levels - the peak occurring at 75 min. In pony No. 3 no definite pattern was apparent.

EXPERIMENT 2 : 0.2 UNITS INSULIN/KG.Ponies No. 2 and 3

BEHAVIOURAL SIGNS

Pony No. 2 began to sweat and become restless between 60 and 90 min. Pony No. 3 showed no behavioural changes.

BLOOD GLUCOSE (FIGS. 4.4 AND 4.5)

Blood glucose levels in these animals fell further than in the

FIG. 4.2 ALTERATIONS IN PLASMA 11-OHCS AND GLUCOSE
LEVELS IN PONY NO. 2 FOLLOWING 0.1 UNITS
INSULIN/KG. ADMINISTERED INTRAVENOUSLY

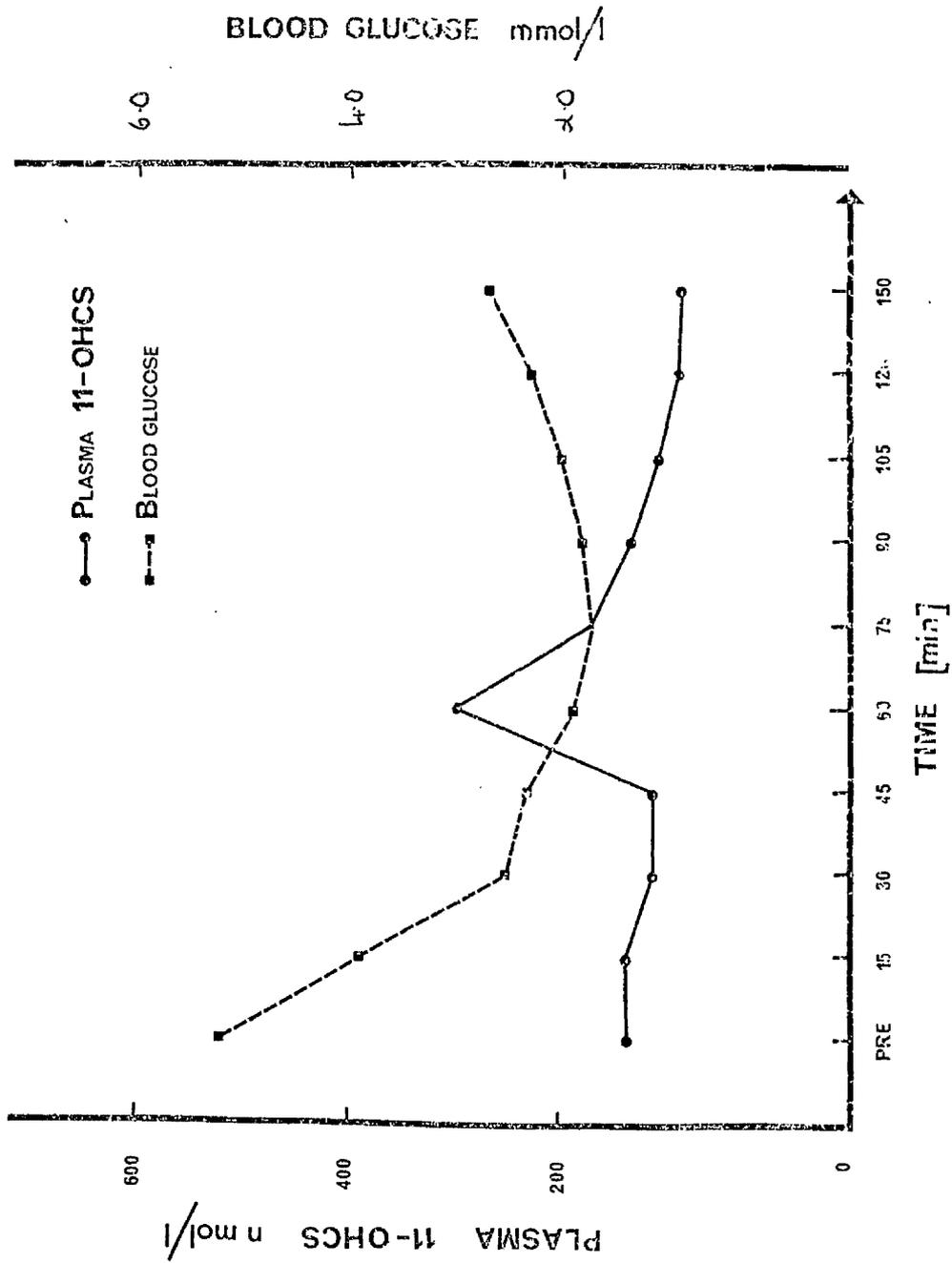
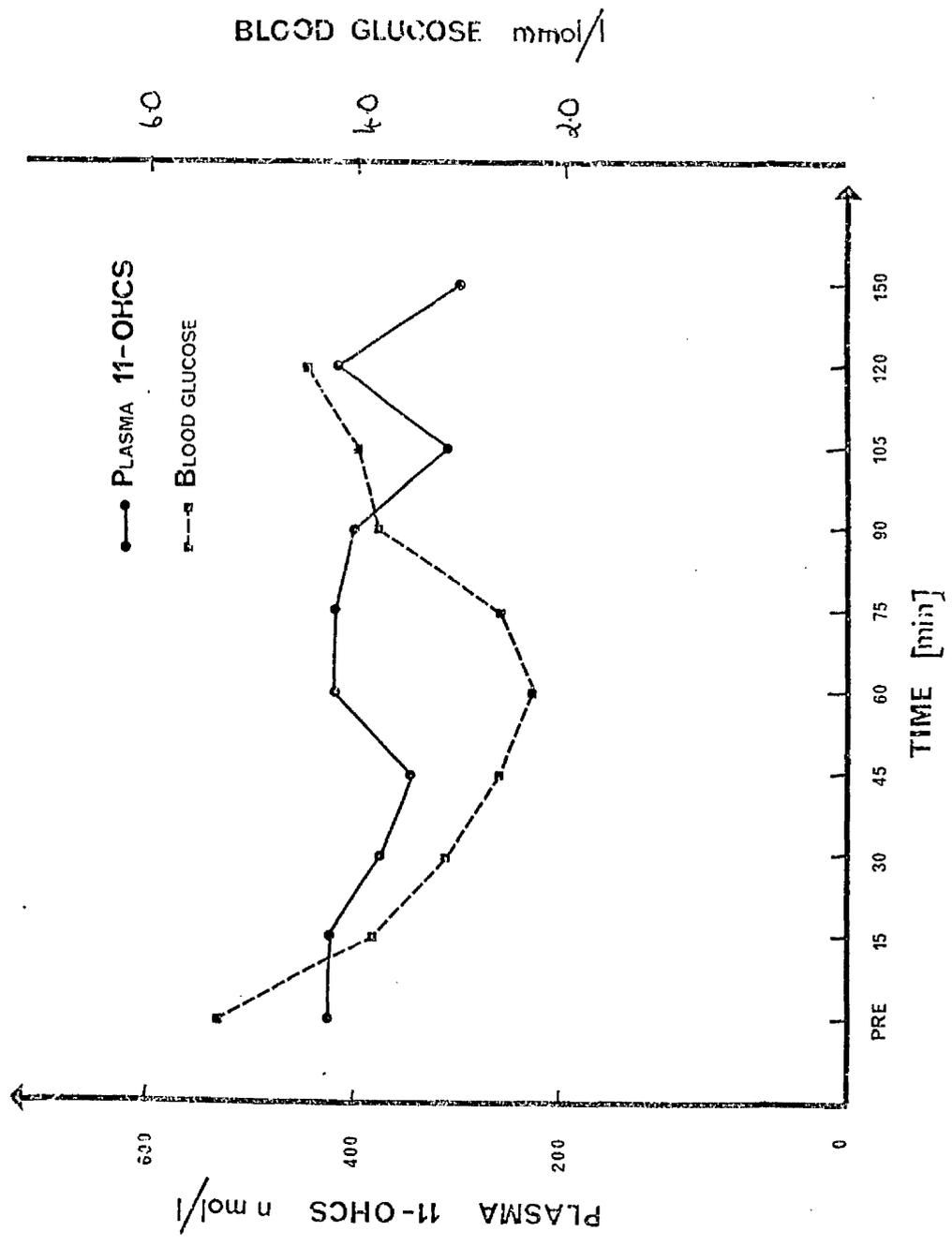


FIG. 4.3 ALTERATIONS IN PLASMA 11-OECS AND GLUCOSE
LEVELS IN PONY NO. 3 FOLLOWING 0.1 UNITS
INSULIN/KG. ADMINISTERED INTRAVENOUSLY



previous experiment reaching 100 and 128 mmol/litre respectively between 60 and 90 min. In pony No. 2 the levels were still below 200 mmol/litre, 2.5 hours after insulin but in pony No. 3 they had returned almost to pre-treatment values by this time.

PLASMA 11-OHCS AND ACTH (FIGS. 4.4 AND 4.5)

Plasma ACTH levels in pony No. 2 showed a similar pattern to those recorded in pony No. 1 following the lower dose of insulin although the response was much smaller. The peak level occurred at 45 min and was followed by the increase in 11-OHCS values.

Plasma 11-OHCS levels in these animals increased to values of 640 and 728 nmol/litre respectively between 60 and 75 min post-injection - times corresponding to the lowest levels of blood glucose. By 2.5 hours in both animals 11-OHCS levels had returned to resting (pre-treatment) values.

FIG. 4.4 ALTERATIONS IN PLASMA 11-OHCS, ACTH
AND GLUCOSE LEVELS IN PONY NO. 2 FOLLOWING
0.2 UNITS INSULIN/KG. ADMINISTERED INTRAVENOUSLY

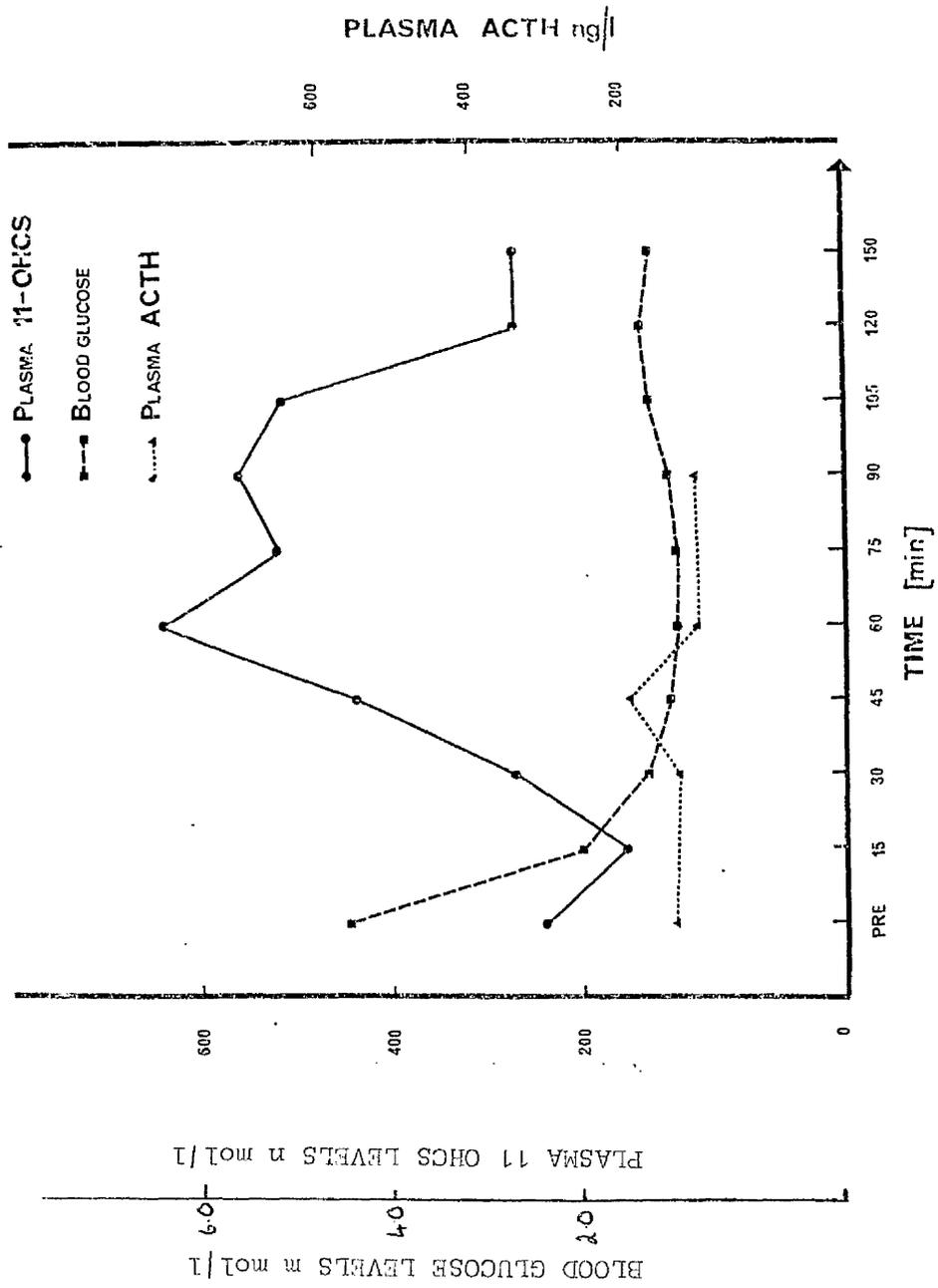
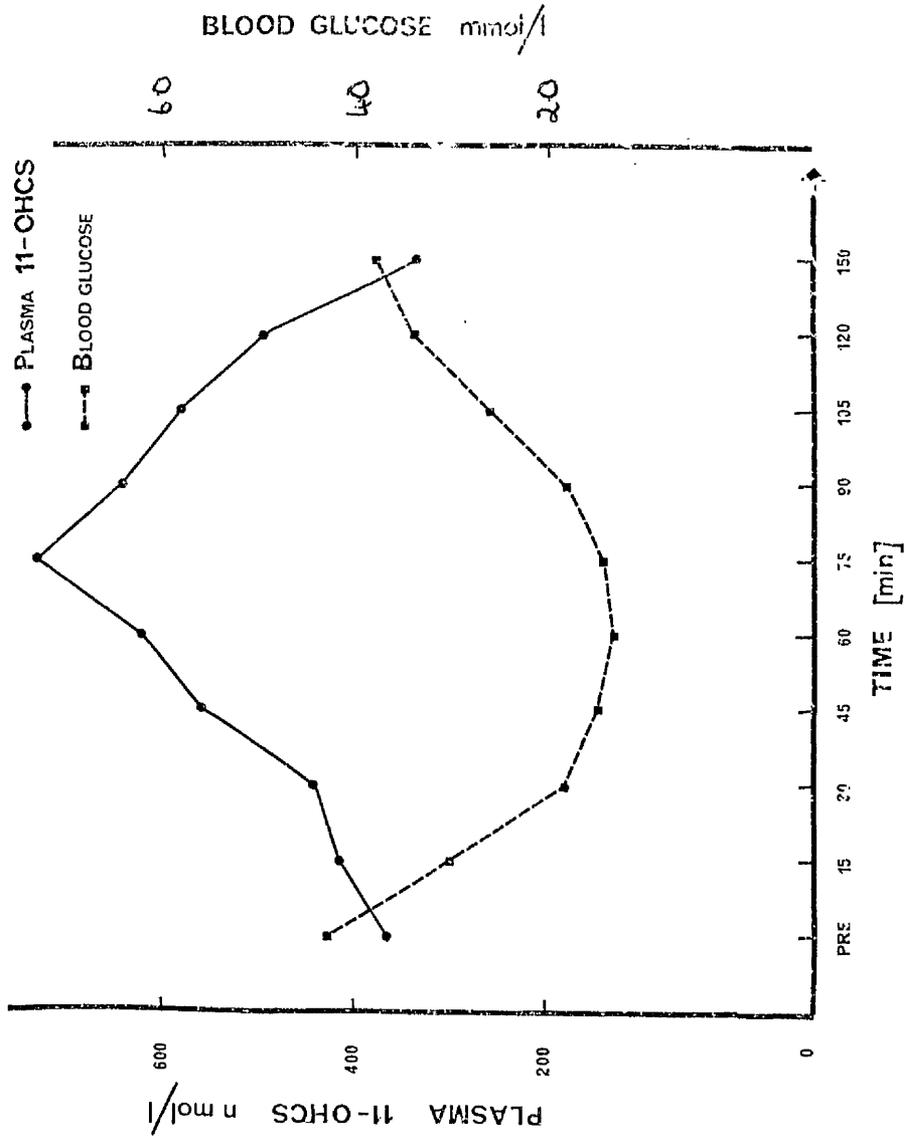


FIG. 4.5 ALTERATIONS IN PLASMA 11-OHCS AND GLUCOSE
LEVELS IN PONY NO. 3 FOLLOWING 0.2 UNITS
INSULIN/KG. ADMINISTERED INTRAVENOUSLY



4.3 EFFECTS OF STIMULATION OF THE EQUINE ADRENAL WITH INCREASING DOSES OF SYNTHETIC ACTH

The mode of action of ACTH and the control of its production and secretion have been discussed in detail in an earlier section.

The doses of ACTH used in this experiment were much greater than the normal physiological range and were used in order to find the dose of ACTH which would produce maximal adreno-cortical stimulation in these animals. A similar method has been employed in man (Landon and James, 1976) and has been used in horses (James *et al.*, 1970; Snow and Munro, 1975).

It was intended that the response of any stress in an animal might be quantitated by comparison of the plasma 11-OHCS levels produced with those produced maximally by this procedure.

Animals

Three ponies (Nos. 1, 2 and 3) were used.

Experimental Procedure

Three doses of synthetic ACTH (Synacthen) were administered - 50, 100 and 200 iu. Each dose was given intramuscularly to 2 of the 3 ponies on separate occasions. On each occasion, one pony acted as a control and was given an injection of sterile water. Blood samples for plasma 11-OHCS estimation were taken 30, 60, 120, 240, 360, 480 and in some cases 720 min after ACTH injection.

The ponies were restrained throughout the 8 hour period but were allowed access to hay and water. No pony was used more than once as a control animal and at least 10 days were allowed between experiments.

Results (Fig. 4.6)

50 IU SYNACTHEN - PONIES NO. 1 AND 2

The maximum response to this dose of ACTH was seen between 2 and 4 hours post injection when the plasma 11-OHCS levels rose by 136% and 222% respectively above pre-experiment values. A diphasic response was noted in both ponies. In both animals the levels had returned to pre-injection values by 480 min.

The plasma 11-OHCS levels of the control animal (No. 3) fluctuated over the 8 hour period but never exceeded 360 n mol/litre.

100 IU SYNACTHEN - PONIES NO. 1 AND 3

The maximum response was recorded at 4 hours post injection, when the plasma 11-OHCS levels increased by 74% and 42% respectively above pre-experiment values. Again the response appeared to be diphasic with two maxima at 60 and 240 min. Levels had returned to pre-injection values by 480 min post injection.

The plasma 11-OHCS levels in the control animal (No. 2) did not exceed 364 n mol/litre.

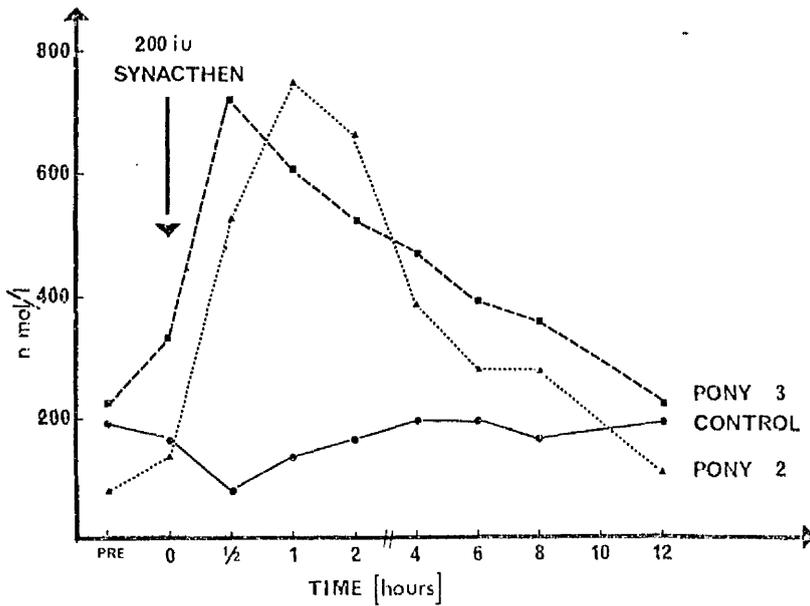
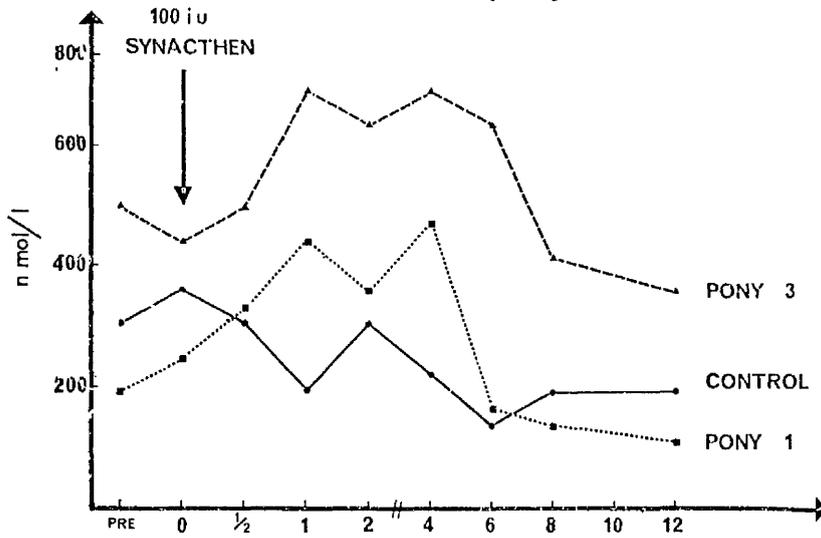
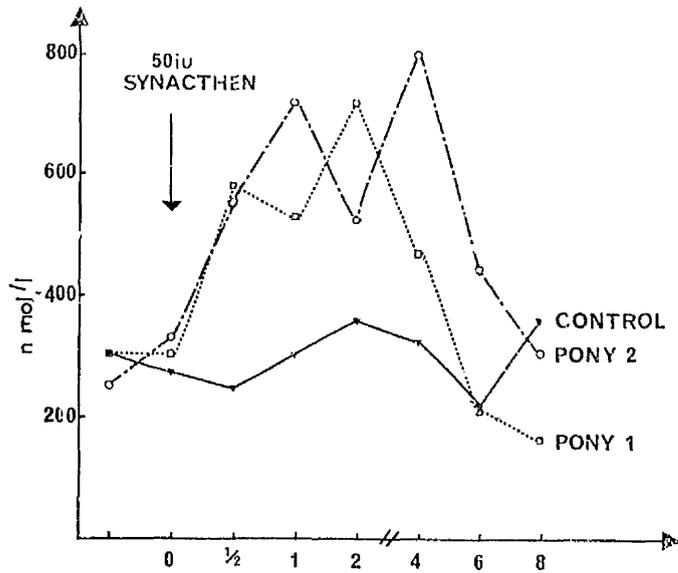
200 IU SYNACTHEN - PONIES NO. 2 AND 3

The maximum response following this dose of ACTH was seen much earlier. In pony No. 2 plasma 11-OHCS levels increased by 225% above control values at 30 min post injection and in pony No. 3 a 790% increase occurred at 60 min post injection.

There did not appear to be a diphasic response following this dose. Levels fell quickly however and had returned to pre-injection values by 720 min.

11-OHCS values in the control animal (No. 1) were always below 220 n mol/litre.

FIG. 4.6 ALTERATIONS IN PLASMA 11-OHCS LEVELS
IN THREE PONIES FOLLOWING 50, 100
AND 200 iu SYNACTHEN
ADMINISTERED INTRAMUSCULARLY



4.4 Discussion

Experimental stimulation of the adrenal and the H.P.A. axis resulted in increases in plasma 11-OHCS levels. The maximal increases in each pony produced by injection of synthetic ACTH (Synacthen) were taken to be the levels of maximal adrenal production.

The doses of Synacthen used in this study produced circulating plasma ACTH levels greater than those occurring naturally in any of our animals (Chapter 3.4) and should therefore ensure that maximal adrenal stimulation was occurring. Peak levels of plasma 11-OHCS were recorded in two of the three ponies (Nos. 1 and 2) following the lower doses of Synacthen suggesting that at this point the adrenals of these animals had reached maximum production. In pony No. 3 on the other hand the greatest increase in 11-OHCS levels was recorded after the 200 iu dose of Synacthen.

James et al (1970) and Hoffsis et al (1970) injected similar doses of Synacthen to those used in this study into horses and recorded little difference in the peak levels achieved following each dose. In these studies the range of maximum levels (Table 4.2) appeared to be within a narrow range, the only differences between ponies being in their sensitivity to the administered doses of ACTH.

This individual variation between animals was demonstrated in earlier experiments involving dexamethasone suppression and adrenal sensitivity to low doses of Synacthen. It would appear that this large individual variation is a constant feature of equine animals as it is of man and other species and suggests that experimental

Table 4.2 Maximum levels of 11-OHCS (n mol/l) recorded in each pony following various doses of Synacthen administered intramuscularly

Pony	Dose Synacthen		
	50 iu	100 iu	200 iu
1	720	471	220 *
2	803	364 *	609
3	360 *	692	748

* ANIMAL USED AS CONTROL

data should be collected from as large a group of animals as possible before drawing any conclusions on adreno-cortical function in horses.

The peak levels in our ponies were higher than any of those recorded by James et al (1970), Hoffsis et al (1970) or Snow and Munro (1975) at similar times post-injection but were within the range of levels reported in man following the 5 hour Synacthen test (James and Landon, 1976).

The rate of onset of the adrenal response in our animals was slowest following the 50 iu dose of Synacthen and most rapid after 200 iu. This is similar to the results of Hoffsis et al (1970) and Snow and Munro (1975). The increase in the plasma 11-OHCS levels in all our animals was as rapid as that reported by Hoffsis et al (1970) following intravenous administration of Synacthen and more rapid than that reported by Snow and Munro (1975) following a 100 iu dose administered intramuscularly.

This rapid increase could be due to variation in experimental animals - both previous workers were using horses of Thoroughbred and Standardbred types whereas ponies were used in this study. It also appears to be common practice in such procedures in man and animals that Synacthen doses are not calculated on a weight basis. If this were taken into account then the ponies used here would be receiving considerably higher doses than the horses in the other studies and this would explain the more elevated plasma 11-OHCS levels and the more rapid onset of action found in our animals.

The duration of corticosteroid responses in this study was

also similar to that reported by James et al (1970) and Hoffsis et al (1970). Both groups of workers noted that the length of time over which the plasma corticosteroid levels was increased was related to the dose of Synacthen administered and to the route of administration. Intramuscular injection of 100 iu (Hoffsis et al, 1970) produced elevated 11-OHCS levels during 8 hour period after injection and the same period of elevated 11-OHCS levels is visible in our results (especially pony No. 3).

The biphasic response recorded in these ponies following the 50 and 100 iu doses has not been reported previously in horses or man. It could be due to the normal circadian pattern of 11-corticosteroid secretion being superimposed on the increases produced by the injected Synacthen. If the circadian rhythm in 11-OHCS levels is mediated via ACTH it would be probable that the doses of Synacthen used would dominate any small changes in ACTH and therefore it seems unlikely that the biphasic pattern seen is due to this reason.

The mechanism by which hypoglycaemia produces an increase in plasma ACTH levels has been studied in man. It has been shown that the response is due entirely to the hypoglycaemia and not to any direct action of the insulin since administration of both insulin and glucose simultaneously produces no alteration in corticosteroid production (Landon et al, 1963).

The response to hypoglycaemia is mediated via the hypothalamus which is known to be an important regulatory centre for food uptake and to contain numerous receptors sensitive to glucose levels in the blood (Foster, 1953; Donovan, 1970). Stimulation of these centres leads to the release of catecholamines, glucagon and ACTH (which in

turn produces increases in glucocorticosteroid levels). All these hormones by their numerous actions increase blood glucose levels and tend to minimise the hypoglycaemic effects of the insulin.

Previous workers in man and in horses have used soluble insulin in dose rates ranging from 0.1 - 0.3 units/kg. in the human (Greenwood et al, 1966; Landon, Greenwood, Stamp and Wyn, 1966; Staub et al, 1973) to 0.4 and 0.8 units/kg. in horses (James et al, 1970). Argenzio and Hintz (1971) used zinc insulin in ponies which is more slowly absorbed at dose rates of 0.2 units/kg. but in this study the soluble type was more suitable as a rapid effect was required.

The dose of insulin used in experiment 1 was similar to that given to man and was selected in order that the sensitivity of the adrenal cortex to this degree of hypoglycaemia in the two species could be compared. The doses administered by James et al (1970) were higher but were chosen to produce maximal stimulation and their experiment was not intended as a means of assessing the adrenal sensitivity to varying degrees of hypoglycaemia.

Injection of the low dose of insulin (Chapter 4.2.1) produced a hypoglycaemia with blood glucose levels below 220 mmol/litre in two of the three ponies studied. In both these animals an accompanying rise in plasma 11-OHCS levels occurred but this elevation was most marked in pony No. 1 whose blood glucose levels fell to the greatest extent. Slight signs of hypoglycaemia (restlessness and sweating) were also visible in this animal and there appeared to be a small increase in heart rate - occurring at

the time of most marked hypoglycaemia.

The increased sensitivity of this pony to the low dose of insulin could perhaps have been due to its low resting blood glucose level as compared with those of the other two animals. It is probable that the response to insulin-induced hypoglycaemia depends to some extent upon the normal resting glucose values of individuals.

Landon et al (1963) concluded that in man the adrenal response to insulin-induced hypoglycaemia depended on the degree and duration of the hypoglycaemia and that there was no response in subjects whose blood glucose levels did not fall below 200 mmol/litre.

James et al (1970) however reported that in horses changes in plasma 11-OHCS levels were only achieved when blood glucose values fell below 100 mmol/litre.

From these results it would appear that the latter is the case in ponies but that the elevations in plasma 11-OHCS levels produced were not dependent on the hypoglycaemia below the 220 mmol glucose/litre level. This is especially apparent following administration of the larger doses of insulin in experiment 2. In pony No. 2, blood glucose levels fell as low as those recorded in pony No. 1 after 0.1 units/kg. and yet the plasma 11-OHCS levels remained lower than in the latter. Similarly with pony No. 3 - the peak plasma 11-OHCS values were higher than those recorded in pony No. 2 but the fall in blood glucose less.

The increases in plasma 11-OHCS levels recorded in all three ponies in these experiments were within the ranges reported in man during similar studies (Landon et al, 1963; Greenwood et al, 1966;

Staub et al., 1973) but higher than those quoted by James et al. (1970). This is interesting since the corresponding blood glucose levels reported by James et al. were considerably below 1.10 mmol/litre. The latter authors estimated corticosteroid levels however by a semi-automated fluorometric method after chromatographic separation and this could have resulted in some losses. In general, the majority of their results are well below those found in this study.

Plasma ACTH levels recorded in ponies No. 1 and 2 showed a pattern similar to that reported in man by Staub et al. (1973) following insulin administration. These authors found that although peak ACTH levels occurred consistently at 45 min post insulin injection, peak plasma 11-OHCS levels occurred at 60 min. They also reported that the plasma 11-OHCS levels achieved correlated very poorly with the ACTH values and a similar pattern is apparent in this study.

The maximum 11-OHCS levels in ponies No. 1 and 2 were above 600 nmol/litre but the corresponding maximum ACTH values differed by 300 ng/litre. This could be due to different levels of adrenal sensitivity between the two animals or that the suppressive effect of rising 11-OHCS levels on further ACTH release may be greater in one than in the other. The feedback effect may also explain why the ACTH levels reached their peak before the 11-OHCS and why they fell off rapidly while 11-OHCS levels were still increasing (Staub et al., 1973).

It has been suggested that insulin-induced hypoglycaemia will result in maximal stimulation of the adrenal (Landon et al., 1963)

and that injection of ACTH at the time of peak response will result in no further increase in 11-OHCS levels. Comparing peak 11-OHCS levels in our ponies following ACTH and insulin-induced hypoglycaemia however (Table 4.3) pony No. 1 appeared not to have been stimulated maximally by the large doses of Synacthen. The results further suggest that the degree of insulin-induced hypoglycaemia in ponies No. 2 and 3 was not sufficient to produce maximal 11- hydroxycorticosteroid secretion. Because the degree of hypoglycaemia recorded in all 3 ponies was similar this could mean that the H.P.A. axes of ponies No. 2 and 3 were less sensitive to this stress.

Summary

From these studies, it would appear that adrenal stimulation by (1) large, non-physiological doses of ACTH and (2) insulin-induced hypoglycaemia with blood glucose levels below 220 mmol/litre both resulted in elevated plasma 11-OHCS levels. If increases in plasma 11-OHCS concentrations are taken to indicate stress, it would also appear that the widely variable responses in adrenal function from animal to animal would make quantitative comparison of stress using this parameter very difficult.

The adrenal response in the horse to insulin-induced hypoglycaemia is similar to that produced by Synacthen stimulation and both are within the range of responses recorded in man under the same conditions. This would suggest that the same controlling mechanisms are present in both species and that the hypothalamus plays a similar role in respect of this aspect of body function

in both species.

Table 4.3 Maximum levels of plasma 11-OHCS (n mol/litre) recorded in each pony following Synacthen administration and Insulin-induced Hypoglycaemia

Pony No.	Maximum plasma 11-OHCS levels (n mol/litre) recorded	
	Following Synacthen administration (i/m)	Following Insulin-induced Hypoglycaemia
1	720	748
2	803	640
3	748	728

5. THE EFFECTS OF A MINOR SURGICAL
PROCEDURE ON HEART RATE,
PACKED CELL VOLUME AND
PLASMA 11-OHCS LEVELS IN HORSES

5.1 Introduction

Surgical procedures have been shown in man and horses to produce increases in adreno-cortical function which are dependent on the degree and duration of the surgery performed (Tyler, Schmidt, Eik-Nes, Brown and Samuels, 1954; Clarke, Johnston and Sheridan, 1970; James et al, 1970).

Disturbing stimuli have also been shown to produce increases in heart rate in horses (Aitken and Sanford, 1972) and as has been suggested in previous chapters, might also be expected to produce elevations in P.C.V. levels.

Alterations in heart rates and plasma 11-OHCS concentrations in response to stress have been employed in horses and some laboratory animals as quantitative means of assessing the effectiveness of tranquillising and antipsychotic drugs (Smith et al, 1963; Aitken and Sanford, 1972; Lahti and Barsuhn, 1974).

This study was designed to assess the effects of a minor surgical procedure on the above parameters.

If the procedure involved produced alterations in heart rate, P.C.V. and 11-OHCS levels indicative of stress it was intended to complete the experiment by using this technique to compare the effectiveness of three commonly used veterinary tranquillisers.

The surgical procedure employed was that of percutaneous muscle biopsy (Snow and Guy, 1976). These muscle samples were being taken in the course of a study into the biochemical and ultrastructural changes in muscle associated with training.

5.2 Animals

All seven horses were included in this study. Their temperaments are assessed in Table 5.1.

Materials

Lignocaine : 2% Xylocaine Astra

Methods

Heart rates were recorded by radiotelemetry as described previously.

Blood samples for P.C.V. and plasma 11-OHCS determinations were collected and stored and the estimations carried out as described previously.

Operative Procedure

The procedure involved the taking of small muscle biopsies from six limb muscles - the long head of the triceps, deltoid, lateral vastus, biceps femoris, middle gluteal and semitendinosus -- by percutaneous needle biopsy (Snow and Guy, 1976).

The main steps were

- i) shaving the area of skin over the biopsy site.
- ii) injecting 1-2 ml. of local anaesthetic (lignocaine) subcutaneously along the line of the proposed incision.

Table 5.1 Description of Horses used in study involving
Muscle Biopsies

Horse	Temperament *
1	+
2	+
3	+++
4	++
5	+
6	+
7	++

* = + quiet, easy to handle
 ++ moderately quiet
 +++ nervous, highly excitable

- iii) Making an incision about $\frac{1}{2}$ " in length through the skin and fascia.
- iv) inserting the biopsy needle through the incision and withdrawing a muscle sample.

The experiments were carried out between 9 a.m. and 12 noon, all resting samples and recordings being taken before 9.30 a.m. The entire procedure was generally completed within 45-60 min and the horses were restrained in stocks throughout.

Experimental Procedure

Heart rates were recorded continuously throughout the procedure and those quoted are the means of the heart rates monitored over a period of 1 min after commencement of each individual stage of the procedure.

Blood samples for P.C.V. and plasma 11-OHCS estimation were collected from the animals at rest before commencing the procedure, immediately after injection of the local anaesthetic and following the muscle biopsies.

Under ideal conditions, when the horses allowed the procedure to be completed, injection of the local anaesthetic took 2-3 min and the biopsies 5-10 min.

5.3 Results

BEHAVIOURAL SIGNS

In only three of the seven horses (Nos. 1, 5 and 6) was the entire biopsy procedure completed. All the animals showed some signs of discomfort viz. moving in the stocks especially after injection of the local anaesthetic and the four in which the biopsies were not taken became very violent at this stage. Failure to inject local anaesthetic was the reason for the procedure being abandoned on all four occasions.

HEART RATE (TABLE 5.2)

Increases in heart rate occurred throughout the procedure.

Maximum levels were recorded in association with injection of the local anaesthetic when rates were significantly elevated above resting values ($p < 0.05$). This increase was most marked in the horses in which the biopsy procedure was not completed. Incising the skin produced only a slight elevation in heart rates whereas following the muscle biopsies values were again significantly increased above resting levels ($p < 0.05$).

PACKED CELL VOLUME (TABLE 5.2)

P.C.V. levels increased after injection of the local anaesthetic and maximum values were recorded on completion of the muscle biopsies.

PLASMA 11-OHCS (TABLE 5.2)

Plasma 11-OHCS levels increased throughout the procedure and were significantly elevated above resting values ($p < 0.05$) following injection of the local anaesthetic. On completion of all the biopsies, values had almost returned to resting levels.

There was a significant correlation ($r = 0.7364$, $p < 0.05$) between the heart rates recorded and plasma 11-OHCS levels after injection of the local anaesthetic.

Table 5.2 Heart Rate, Packed Cell Volume and Plasma 11-OHCS Levels (mean \pm s.e.) Before and During Biopsy Procedure

Stage of Procedure	Heart Rate (beats/min)	Packed Cell Volume	Plasma 11-OHCS (n mol/l)
Pre (Control)	39 \pm 1.8 (7)	37.7 \pm 2.0 (7)	270 \pm 41 (7)
Before local anaesthetic	40 \pm 2.5 (6)	-	-
After local anaesthetic	91 \pm 11.0 * (7)	42.4 \pm 3.5 (7)	334 \pm 41 * (7)
Before muscle Biopsy	48 \pm 6.0 (3)	-	-
After muscle Biopsy	78 \pm 9.2 * (3)	44.0 \pm 3.7 (3)	291 \pm 55 (3)

figures in parenthesis indicate number of times each stage of the procedure successfully completed

* $p < 0.05$ (refers to difference from mean control levels)

'-' indicates samples not taken

5.4 Discussion

Aitken and Sanford (1972) described tachycardia in horses associated with various disturbing stimuli of either an auditory or a visual nature and concluded that these alterations could be used as an accurate means of assessing stress in the horse.

As discussed previously (chapter 1) heart rate is controlled by the vasomotor centre in the medulla (Rushmer, 1972; Guyton, 1971) and this in turn can be influenced by higher centres in the brain viz. the hypothalamus and cerebral cortex.

During this study elevations in heart rate occurred to the greatest extent following injection of the local anaesthetic but were also markedly increased after the biopsy procedure. Reaction to the injection of local anaesthetic was possibly due to the pain associated with this procedure.

Stimulation of the sensory nerve endings in the skin by a painful procedure results in reflex withdrawal of the part of the body concerned from the painful stimulus and also other psychological and physiological effects (Guyton, 1971). In the brain, impulses related to pain are believed to stimulate the cerebral cortex and the hypothalamus and in the case of the latter to result in increases in sympathetic nervous activity (Rushmer, 1972).

In man there is great variation in individual responses to pain and the same is apparently true of horses. In the four most nervous animals it was impossible to complete the biopsy procedure because of the violent reactions produced by attempts to inject the

local anaesthetic.

Marked elevations in heart rate were also recorded following the muscle biopsies suggesting that some degree of discomfort was present. Similar findings have been reported in man after muscle biopsies when feelings of dull discomfort have been described (Edwards, Lewis, Maunder and Pearce, 1973). Because stimulation of any individual part of the sympathetic system generally results in the entire system being stimulated (Guyton, 1971) the changes recorded in P.C.V. levels in this study were to be expected. As described in chapter 1 the splenic capsule is rich in adrenergic receptors (Davies and Withrington, 1973) and any increase in sympathetic activity results in contraction of the organ and expulsion of red cells into the circulation. The fact that the greatest elevation in P.C.V. levels did not occur at the same time as the peak heart rates could have been due to reflex responses involving vagal tone affecting the heart.

In the three quietest horses which allowed the entire procedure to be completed, P.C.V. levels were highest at the end of the biopsy procedure. As the biopsy techniques took the longest however (between 5 and 10 min) values would have had time to increase over this period more than during injection of the local anaesthetic which only took 1-2 min.

All the horses showed a significant increase in plasma 11-OHCS levels at the same time as maximum heart rates were recorded immediately after injection of the local. Following the muscle biopsies however 11-OHCS values had decreased whereas heart rate and P.C.V. levels were still elevated. These results would suggest

that increased sympathetic activity does not necessarily result in elevated plasma 11-OHCS levels or that it can affect different systems to varying degrees. Since the metabolic half-life of cortisol recorded in these studies was in the region of 103 min however it seems unlikely that 11-OHCS levels would have fallen as rapidly as this over such a short period of time. The low levels recorded following the biopsy procedure could therefore be due either to the small number of samples assayed or to methodological error.

Summary

From these results it would appear that muscle biopsy does constitute a stress as measured by elevations in heart rate, packed cell volume and plasma 11-OHCS levels. From the variations in the results recorded however, a constant stress would appear to affect different systems to varying degrees. These differences may be due to individual variations between animals. The timing of sampling may also be important in using 11-OHCS and P.C.V. levels as a means of assessing stress.

6. THE EFFECTS OF EXERCISE
AND TRAINING ON ADRENO-CORTICAL
AND SYMPATHETIC NERVOUS ACTIVITY

6.1 Introduction

Exercise in the horse is accompanied by changes in both physiological and biochemical parameters. These alterations are related to the intensity and duration of the exercise carried out and also to the state of fitness of the animal (Milne, 1974; Aitken, Anderson, MacKenzie and Sanford, 1975; Lindholm and Saltin, 1974; Milne, Skarda, Gabel, Smith and Ault, 1976).

Elevations in heart and respiratory rates and packed cell volumes and alterations in circulating plasma 11-OHCS levels, blood gas concentrations and in the acid-base status of the blood occur during and immediately following exercise. Many studies have been carried out in man and horses to monitor these effects and to determine the adaptive processes, if any, which occur in them following a period of training (Wasserman et al, 1967; Astrand and Rodahl, 1970; Milne et al, 1976).

In these experiments the effects of exercise of varying intensity and duration on heart rates, packed cell volumes and plasma 11-OHCS levels were recorded in order that the degree of stress produced by such exertion could be assessed. Alterations in blood gas concentrations and in the acid-base status of the blood were also measured.

In order to investigate the effect of training on cardiovascular, respiratory and adreno-cortical activity two of the exercise tests were carried out before and after a ten week training programme. The third test was carried out at a nearby racecourse using four

Thoroughbreds which after ten weeks training were considered to be fit.

All blood lactate levels included in this chapter were estimated by Dr. D. Snow in the process of his studies into the various changes occurring in blood biochemistry and muscle during exercise.

6.2 THE EFFECTS OF MAXIMAL EXERCISE ON HEART AND RESPIRATORY RATES,
P.C.V., PLASMA 11-OHCS LEVELS, BLOOD GAS CONCENTRATIONS AND
ACID-BASE BALANCE IN HORSES BEFORE AND AFTER A TEN WEEK
TRAINING PROGRAMME

Animals

Four Thoroughbreds (Nos. 1, 2, 3 and 4) and two heavy hunters (Nos. 5 and 6) were used. All were housed in loose-boxes and had been given only maintenance exercise consisting of limited walking and trotting every day except at weekends for the previous 3 months.

Diet consisted of bran, oats, horse nuts and hay fed twice daily in the initial stages of training. Later in the training programme the amount of oats given was increased, the bran decreased and a third mid-day meal included.

Methods

Heart rates were recorded by auscultation over 30 sec periods. Respiratory rates were recorded visually over 20 seconds.

Blood samples for P.C.V. and plasma 11-OHCS levels were collected and treated as described previously.

Blood samples (2 ml) for blood gas analysis were collected anaerobically into cold plastic heparinized tubes and stored on ice. Blood gas and pH measurements were made at 37° C on a direct leading electrode system (Eel Corning 165). Bicarbonate (HCO_3^-) and base

excess values were calculated automatically on the same machine.

Experimental Procedure

The training programme lasted ten weeks and consisted of submaximal work (trotting and cantering over a 10-15 m distance) four days per week and maximal work (galloping three times over 600 m) on a circular course two days per week. The amount of submaximal work was increased gradually from 10 to 15 Km. over the training period.

All the horses were tested before and after the training programmes. Each test was carried out in the morning after the animal was fasted overnight. The test exercise consisted of a maximal gallop over 600 m on a circular course. This was repeated three times (referred to as G_1 , G_2 , G_3) with a period of approximately 5 min between each gallop. Each horse was timed over the distance and the mean speeds before and after training calculated.

Heart and respiratory rates were recorded prior to the exercise, after each gallop and in some cases up to 30 min post-exercise.

Blood samples for P.C.V. and 11-CECS estimations were collected from the horses at rest in the stables prior to exercise, immediately after each gallop and at 15, 30, 60 and 90 min after completion of the 3rd gallop. Samples for blood gas determinations were collected at similar times up to 30 min post-exercise.

All blood tubes were stored on ice from the time of sampling until the plasma could be removed or the appropriate estimations made. When assays were not carried out on the same day as sampling,

the plasma was stored at -15° C until required. Plasma was never stored for periods of longer than 6-7 days before being assayed.

2.1 Results

The changes in the various parameters recorded were similar to both types of horses and therefore all the results have been considered together.

SPEED (TABLE 6.1)

All the horses were significantly faster after the training programme ($p < 0.01$).

HEART RATE (TABLE 6.2)

Heart rates before and after training increased significantly above resting values ($p < 0.05$) after each gallop and were still elevated 30 min post-exercise. There was no significant difference between the heart rates immediately after exercise in either the trained or the untrained horses.

RESPIRATORY RATE (TABLE 6.2)

Respiratory rates were increased significantly above resting values ($p < 0.05$) after each gallop in the trained and untrained animals. There was no difference in post-exercise rates between trained and untrained groups.

PACKED CELL VOLUME (TABLE 6.3)

P.C.V. increased progressively immediately after each gallop in both trained and untrained animals reaching maximum levels after G_3 and G_2 respectively. By 90 min post-exercise values had returned

Table 6.1 Horses included in maximal exercise test and time taken over 3 successive gallops before and after a period of training of ten weeks

Horse No.	Type	Time Taken for Three Gallops (mean \pm se)	
		Pre Training	Post Training
1	T.B.	43.7 \pm 0.9	41.9 \pm 1.2
2	T.B.	41.2 \pm 0.2	40.5 \pm 0.6
3	T.B.	44.1 \pm 1.5	40.8 \pm 0.5
4	T.B.	46.0 \pm 0	44.8 \pm 0.1
5	H.H.	54.3 \pm 0.7	53.3 \pm 2.4
6	H.H.	61.3 \pm 0.8	58.8 \pm 0.5

T.B. = Thoroughbred

H.H. = Heavy Hunter

Table 6.2 Heart and Respiratory Rates (Mean \pm s.e.) after 3 successive gallops before and after a training programme

	Heart Rate (Mean \pm s.e.)		Respiratory Rate (Mean \pm s.e.)	
	Pre Training	Post Training	Pre Training	Post Training
Pre Ex.	41 \pm 1.8 (6)	41 \pm 1.8 (6)	14 \pm 2.2 (6)	12 \pm 0 (6)
G ₁	182 \pm 5.9* (6)	150 \pm 6.8* (6)	53 \pm 5.0* (6)	61 \pm 5.2* (6)
G ₂	173 \pm 9.8* (6)	156 \pm 4.8* (6)	65 \pm 6.0* (6)	67 \pm 3.2* (6)
G ₃	164 \pm 18.0* (6)	158 \pm 5.3* (6)	66 \pm 7.0* (6)	71 \pm 3.6* (6)
Post 15 min.	90 \pm 8.1* (6)	94 \pm 5.5* (6)	52 \pm 10.0* (6)	40 \pm 10* (6)
Post 30 min.	77 \pm 6.7* (6)	82 \pm 5.5* (6)	38 \pm 9.0* (6)	38 \pm 11 (6)

Figures in parenthesis refer to number of horses from which recordings were taken

*=Values significantly ($p < 0.05$) altered from resting (pre-exercise) levels

TABLE 6.3 THE EFFECT OF MAXIMAL EXERCISE ON
A NUMBER OF PARAMETERS IN
6 HORSES (MEAN \pm s.e.)

Parameter	Stage of Training	Stage of Procedure							
		Pre Ex.	Post G1	Post G2	Post G3	Post 15min	Post 30min	Post 60min	Post 90min
Plasma 11-OHCS (nmol/l)	Pre	233 ±38	261 ±47	318 ±45	401* ±27	413* ±18	420* ±39	330* ±31	314 ±20
	Post	269 ±45	304 ±61	354 ±79	403* ±59	377* ±61	297 ±63	371* ±56	311 ±61
PCV (%)	Pre	33 ±1.8	50.5* ±2.6	52.7* ±3.7	54.9** ±2.7	45.7* ±3.5	42.9* ±2.6	37.7 ±1.8	35.2 ±1.5
	Post	33.8 ±2.4	51.3* ±4.8	54.5* ±4.4	54.3* ±4.0	46.8* ±3.5	40.5* ±1.6	39.7 ±2.2	36.4 ±2.4
Blood lactate (mmol/l)	Pre	0.8 ±0.2	11.0** ±1.1	16.2** ±1.9	18.8** ±1.9	16.8* ±2.1	14.5* ±2.5	7.4 ±2.1	4.1 ±0.5
	Post	0.7 ±0.2	14.0* ±2.1	20.7** ±1.8	22.8** ±2.7	22.1* ±3.4	14.8* ±2.6	6.0 ±1.2	3.6 ±0.6
Venous pH	Pre	7.386 ±0.006	7.159* ±0.013	7.122** ±0.044	7.101* ±0.078	7.185* ±0.057	7.229* ±0.064		
	Post	7.402 ±0.024	7.209 ±0.026	7.142* ±0.030	7.112* ±0.041	7.192 ±0.038	7.301 ±0.023		
Venous pCO ₂ (mmHg)	Pre	44.5 ±0.6	46.8 ±5.7	43.0 ±5.2	38.6 ±5.7	29.2* ±2.0	30.9* ±2.0		
	Post	44.6 ±2.0	42.5 ±1.9	38.0 ±2.1	29.6* ±2.7	30.3* ±2.0	34.2 ±1.7		
Venous pO ₂ (mmHg)	Pre	35.8 ±4.8	45.0 ±2.6	46.5 ±3.6	57.4 ±8.4	60.1* ±5.1	62.5* ±6.7		
	Post	40.6 ±3.8	46.7 ±2.6	54.3* ±3.9	66.5* ±3.9	69.7 ±12.7	56.9 ±14.7		
Venous bicarbonate (mEq/l)	Pre	26.3 ±1.0	21.4 ±5.3	13.2** ±0.7	11.7** ±1.2	11.5** ±2.1	14.0 ±2.7		
	Post	28.6 ±3.3	16.4 ±0.6	12.6* ±0.3	9.3* ±0.3	11.7* ±1.6	16.5 ±1.2		
Base Excess (mEq/l)	Pre	1.5 ±1.2	-11.2** ±2.2	-14.7** ±1.1	-17.2** ±2.7	-15.5* ±6.4	-6.4 ±4.6		
	Post	-2.4 ±3.8	-11.2 ±0.9	-15.7* ±1.1	-16.9* ±2.3	-10.5 ±5.5	-6.6 ±1.9		

** levels significantly ($p < .001$) altered from resting values

* levels significantly ($p < 0.05$) altered from resting values

almost to resting levels. P.C.V. recorded immediately after the individual gallops were higher in the Thoroughbreds than in the heavy hunters with one value of 65% being recorded (Horse No. 1).

VENOUS pH (TABLE 6.3)

Venous pH values decreased after each gallop, reaching the lowest levels on completion of G_3 both pre and post-training. In both cases the levels were still decreased 30 min after exercise. The lowest pH value recorded was 6.841 (Horse No. 1 pre-training, on completion of G_3). The drop in venous pH after each gallop was less marked in the trained animals although the corresponding changes in venous lactate levels were greater.

BLOOD LACTATE (TABLE 6.3)

Blood lactate levels markedly increased after each gallop and were still elevated 30 min post-exercise in both trained and untrained animals. In all the trained horses blood lactate levels immediately after each gallop were significantly higher than in the untrained ones ($p < 0.05$). There was a significant negative linear correlation ($r = -0.8516$ and -0.6842 $p < 0.05$) between blood lactate and pH levels in each group of horses before and after training.

VENOUS pO_2 (TABLE 6.3)

Venous pO_2 levels increased after each gallop and continued to rise until 30 min post exercise in the untrained animals. In the trained group maximum levels occurred at 15 min post exercise and by 30 min values were decreasing towards resting levels. All pO_2 values recorded were slightly higher in the trained animals.

VENOUS $p\text{CO}_2$ (TABLE 6.3)

Venous $p\text{CO}_2$ levels increased after G_1 and decreased after G_2 and G_3 and up to 15 min post exercise in the untrained animals. After training the lowest levels were recorded immediately after G_3 and in both groups values were increasing towards resting levels by 30 min post-exercise.

$p\text{CO}_2$ levels throughout the test were slightly lower in the trained horses.

VENOUS BICARBONATE (TABLE 6.3)

Venous HCO_3^- values fell progressively after each gallop reaching their lowest levels immediately after G_3 and 15 min post-exercise in the trained and untrained animals respectively. Values fell more markedly in the trained horses but also appeared to be returning towards control levels more rapidly in this group.

BASE EXCESS (TABLE 6.3)

Base excess values decreased progressively after each gallop. Lowest levels occurred immediately after G_3 in both trained and untrained animals.

PLASMA 11-OHCS (TABLE 6.3)

Plasma 11-OHCS levels increased steadily after each gallop both pre and post-training. In the former they continued to rise reaching maximum levels 30 min post-exercise whereas in the latter maximum levels occurred immediately after G_3 . By 90 min post-exercise in both groups values had returned almost to resting levels.

6.3 THE EFFECTS OF SUB-MAXIMAL EXERCISE ON HEART AND RESPIRATORY RATES, P.C.V., PLASMA 11-OHCS LEVELS, BLOOD GAS CONCENTRATIONS AND ACID-BASE BALANCE IN HORSES BEFORE AND AFTER A TEN WEEK TRAINING PROGRAMME

Animals

The same six horses (Table 6.1) were used in this study.

Feeding and exercise regimes were as described previously.

Methods

Heart rates were recorded by auscultation as described previously in all 6 horses before training. In four of the horses post training heart rates were recorded by radiotelemetry.

Respiratory rates, P.C.V., plasma 11-OHCS levels, blood gas and acid-base determinations were carried out as described earlier.

Rectal temperatures after exercise were recorded in several horses and increases from resting values of 38.0 ± 0.1 to 39.7 ± 0.2 after 11.2 km. and 39.3 ± 0.1 after 22.4 km. were obtained both before and after training. Blood pH, pCO_2 , pO_2 , HCO_3^- and base excess values were all corrected to post-exercise temperatures using a blood gas calculator (Radiometer)

Experimental Procedure

The training programme was the same as for the previous

experiment and the exercise tests were carried out before and after a ten week training period.

The tests were all carried out in the morning, the horses having been fasted overnight.

The horses were cantered at a steady pace around the perimeter of a large field. The 4 Thoroughbreds were exercised over a total distance of 22.4 km. and the 2 heavy hunters 11.2 km.

Heart and respiratory rates were recorded and blood samples for P.C.V., 11-OHCS and blood gas analysis collected from the animals at rest prior to the exercise (in the stables) after 5.6, 11.2, 16.8 and 22.4 km. and at 15 and 30 min post exercise.

It has been suggested that prolonged exercise results in exhaustion of the adrenals and a reduction in circulating plasma 11-OHCS levels (Chin and Evonuk, 1971). In order to investigate this possibility, 1 mg (100 iu) Synacthen was injected ^{30 min after exercise} intramuscularly into all six horses before training and five horses after training. Blood samples were then collected at 30 and 60 min post-injection.

6.3.1 Results

SPEED (TABLE 6.4)

Before and after training the Thoroughbreds were cantered at a speed of approximately 7 metres/s and the heavy hunters at 6 metres/s.

All six horses (except No. 1) maintained approximately the same speeds throughout the test on both occasions.

HEART RATE (TABLE 6.5)

Heart rates were increased at each intermediate distance, maximum levels in the trained and untrained animals occurring on completion of the longer distances (16.8 and 22.4 km. respectively). In both cases rates had returned towards resting values by 30 min post-exercise.

The rates recorded by radiotelemetry post-exercise in the trained animals were all higher than those recorded by auscultation in the same animals before training.

There was no significant correlation between the individual heart rates recorded immediately after each intermediate distance and the mean speed (metres/second) over the same distance either before or after training ($r = 0.2412$, $p > 0.1$).

RESPIRATORY RATE (TABLE 6.5)

Respiratory rates increased after each 5.6 km. distance in both trained and untrained animals. Maximum values being recorded on completion of 22.4 km. in the untrained horses and after 11.2 km. in the trained ones.

Table 6.4 Speeds of six horses during submaximal exercise before and after training, (individual values and means (\pm s.e.)).

Horse No.	Type	Stage Training	Speed (metres/s)				Mean (\pm s.e.)
			5.6 km.	11.2 km.	16.8 km.	22.4 km.	
1	TB	Pre	6.1	6.1	5.7	5.4	5.8 \pm 0.2
		Post	6.8	7.1	7.3	6.5	
2	TB	Pre	7.0	7.0	7.2	7.1	7.0 \pm 0.2
		Post	7.4	7.4	7.4	7.4	
3	TB	Pre	6.6	6.6	6.8	6.1	6.5 \pm 0.2
		Post	6.5	6.5	6.9	6.9	
4	TB	Pre	6.7	6.5	6.3	6.1	6.4 \pm 0.1
		Post	7.0	6.3	6.4	6.0	
5	HH	Pre	5.9	6.1	-	-	6.0 \pm 0.1
		Post	6.2	6.3	-	-	
6	HH	Pre	5.7	5.9	-	-	5.8 \pm 0.1
		Post	5.8	5.8	-	-	

TABLE 6.5 THE EFFECT OF SUBMAXIMAL EXERCISE
ON A NUMBER OF PARAMETERS IN
6 HORSES (MEAN \pm s.e.)

Parameter	Stage of Training	Stage of Procedure						
		Pre Ex.	5.6 Km.	11.2 Km.	16.8 Km.	22.4 Km.	Post 15min	Post 30min
Heart Rate (beats/min)	Pre	48 ±1.8	114* ±7.0	112* ±2.0	119* ±3.0	123* ±7.0	51 ±4.0	42 ±7.0
	Post	42 ±1.5	116* ±3.0	158* ±11.0	167* ±12.0	165* ±11.0	58 ±4.0	54 ±2.0
Respiratory Rate (respirations/min)	Pre	12 ±1.0	79* ±9.0	89* ±9.0	82* ±16.0	91* ±20.0		
	Post	10 ±1.0	77* ±11.0	80* ±9.0	79* ±10.0	70* ±9.0		
PCV (%)	Pre	34 ±2.0	43.8* ±6.5	41.5* ±2.0	41.3* ±1.7	41.8 ±5.9	36.2 ±1.0	37.5 ±1.6
	Post	30.7 ±1.1	45.7* ±2.1	42.1* ±2.5	46.8 ±4.6	46.8* ±3.1	42.8* ±1.7	40.2* ±1.7
Blood Lactate (mmol/l)	Pre	0.9 ±0.3	1.6 ±0.6	1.5 ±0.5	1.1 ±0.2	1.3 ±0.2	1.2 ±0.2	1.1 ±0.2
	Post	0.6 ±0.1	1.7 ±0.3	2.5 ±0.5	2.1 ±0.6	2.3 ±0.6	2.0 ±0.6	2.1 ±0.8
Venous pH	Pre	7.433 ±0.027	7.444 ±0.014	7.456 ±0.014	7.463 ±0.014	7.460* ±0.005	7.431 ±0.007	7.462 ±0.014
	Post	7.378 ±0.013	7.397 ±0.007	7.415* ±0.010	7.427* ±0.009	7.425 ±0.011	7.413* ±0.005	7.426 ±0.014
Venous pCO ₂ (mmHg)	Pre	46.2 ±2.7	42.1 ±1.4	39.4* ±1.1	38.9* ±0.6	40.3* ±0.6	43.2 ±0.8	43.4 ±1.0
	Post	54.2 ±2.0	47.6* ±1.5	42.7* ±1.9	44.3* ±2.9	47.0* ±1.9	46.9* ±1.8	47.2 ±2.0
Venous pO ₂ (mmHg)	Pre	40.8 ±1.5	49.7* ±1.9	50.0* ±2.0	50.2* ±2.5	50.0* ±2.1	44.1 ±2.3	39.9 ±2.5
	Post	47.7 ±9.2	59.6 ±2.2	50.0 ±2.0	57.9* ±2.2	50.0* ±2.1	53.9* ±9.7	42.9 ±1.5
Venous HCO ₃ ⁻ (mEq/l)	Pre	34.4 ±1.5	28.3 ±1.4	27.7 ±0.6	28.1 ±1.6	28.9 ±0.8	28.1 ±1.3	31.0 ±1.8
	Post	31.5 ±1.5	29.6 ±1.3	29.6 ±1.7	29.7 ±1.0	31.2 ±0.6	30.9 ±1.4	29.9 ±1.2
Base Excess (mEq/l)	Pre	5.6 ±1.7	3.7 ±1.2	3.9 ±0.	4.4 ±1.5	4.7 ±0.5	5.5 ±1.3	6.6 ±1.0
	Post	4.7 ±1.3	3.9 ±1.1	2.8 ±1.3	4.9 ±1.2	5.7 ±0.4	5.2 ±1.1	4.0 ±0.9

* = levels significantly ($p < 0.05$) altered from resting values

PACKED CELL VOLUME (TABLE 6.5)

P.C.V. values were elevated throughout the test in both trained and untrained animals. Maximum levels occurred pre-training on completion of the first 5.6 km. and post-training after 16.8 and 22.4 km. By 30 min post-exercise P.C.V. levels were still increased above resting values.

The magnitude of increase recorded appeared to be greater in the trained animals but this difference was not significant ($p > 0.1$).

VENOUS pH (TABLE 6.5)

Venous pH levels increased progressively after each 5.6 km. distance, maximum levels in both trained and untrained animals occurring after 16.8 km. The increases in venous pH were less marked in the trained horses although as in the previous study, the changes in venous lactate levels were greater in this group. By 30 min post-exercise pH levels were still above resting values.

BLOOD LACTATE (TABLE 6.5)

Blood lactate levels were only slightly elevated throughout the test before and after the training programme. These increases were greater in the trained than in the untrained animals and in both groups levels were still elevated 30 min post-exercise.

VENOUS pO_2 (TABLE 6.5)

Venous pO_2 levels were elevated by varying degrees after each 5.6 km. in both trained and untrained animals but to the greatest extent in the former.

By 30 min post-exercise in both groups levels had fallen to below resting (pre-experiment) values.

VENOUS $p\text{CO}_2$ (TABLE 6.5)

Venous $p\text{CO}_2$ levels fell during the test in both groups of animals. The magnitude of the decreases varied considerably but were greatest in the untrained group. Values were still below resting levels at 30 min post-exercise.

VENOUS BICARBONATE (TABLE 6.5)

HCO_3^- levels decreased during the test in both groups of animals but lowest levels were recorded pre-training after 11.2 km. 30 min post-exercise pre-training levels were still decreased when post-training values had almost returned to resting levels.

BASE EXCESS (TABLE 6.5)

Base excess values decreased during exercise in the untrained animals but increased half way through the test (16.8 km.) in the trained ones. 30 min post-exercise pre-training levels were slightly above and post-training levels slightly below resting values.

PLASMA 11-OHCS (FIG. 6.1)

Plasma 11-OHCS level increased throughout the exercise in both trained and untrained animals, reaching maximum levels on completion of the 22.4 km. The values appeared to be slightly higher in the trained animals. 30 min after exercise the levels in both groups were returning towards resting values, the decrease being more rapid in the trained horses.

30 min after injection of 100 iu Synacthen there was a marked elevation in 11-OHCS levels in both trained and untrained animals (the increase appearing greater in the trained horses). By 60 min post-injection the 11-OHCS values were returning towards resting levels.

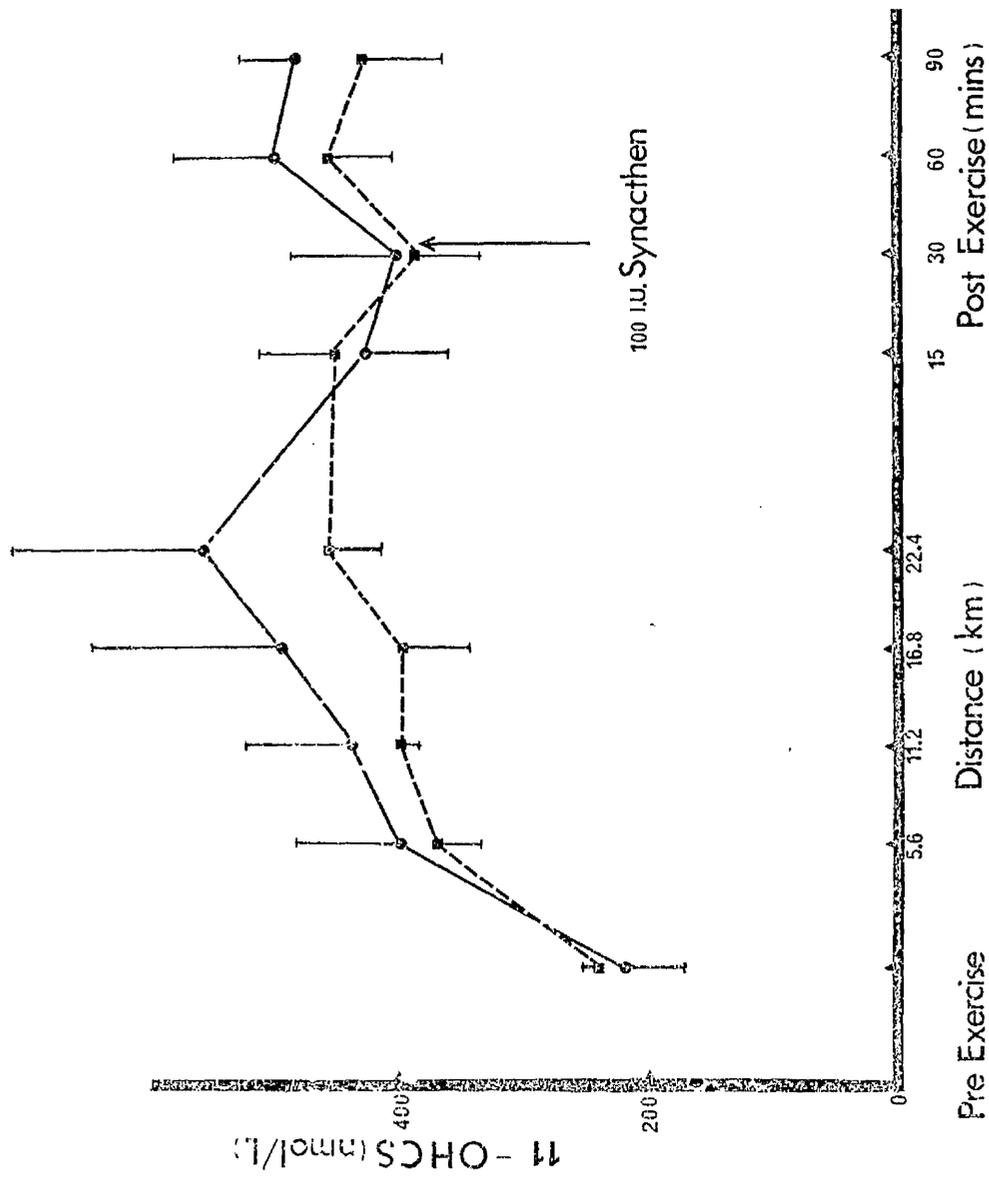
FIG. 6.1 EFFECTS OF SUBMAXIMAL EXERCISE ON PLASMA 11-OHCS LEVELS

IN SIX HORSES (n = 4 AT 16.8 AND 22.4 KM) AND

AFTER INTRAMUSCULAR INJECTION OF 100 IU SYNACTHEN

----- BEFORE TRAINING

_____ AFTER TRAINING



6.4 THE EFFECTS OF INCREASING DISTANCES OF MAXIMAL EXERCISE ON
P.C.V., PLASMA 11-CHECS, BLOOD GAS CONCENTRATIONS AND ACID-BASE
BALANCE IN HORSES AFTER A TEN WEEK TRAINING PROGRAMME

Animals

The four Thoroughbreds included in the two previous experiments were used.

This study was carried out after the 10 week training programme immediately following the maximal and sub-maximal exercise tests.

Exercise and feeding regimes have been described previously.

Methods

All determinations were carried out as described previously.

Experimental Procedure

The tests were carried out at a race course 30 miles from the stables. The horses were transported by horse-box on a number of separate occasions ranging over a 3 week period. The tests were sometimes carried out on consecutive days but never on Mondays. Each horse (except No. 3) was tested once over 2, 3, 5, 8, 12 and 16 furlongs (No. 3 was tested over 5, 8, 12 and 16 furlongs). When possible, the horses were galloped over each distance in groups of three but when riders were not available the horses were galloped singly or in groups of two.

All the tests were carried out between 10 a.m. and 12 noon. Each horse was timed separately over each distance.

Blood samples were collected for P.C.V., 11-OHCS and blood gas estimation from the horses before transportation, on arrival at the race-course, immediately after each gallop and at 15, 30, 60 and 90 min post exercise.

All blood tubes were stored on ice until such time as the appropriate assessments could be carried out (this was always within 3-4 hours of sampling).

6.4.1 Results

SPEED (TABLE 6.6)

The speeds of each horse varied considerably over the different distances. Horse No. 2 proved fastest over all except the 8 furlong distance and horse No. 1 although fast over the 2 and 3 furlong gallops was much slower over the longer distances.

PACKED CELL VOLUME (TABLE 6.7)

Packed cell volume increased significantly ($p < 0.01$) on all occasions by the time the horses arrived at the race course (Table 6.15). Following the gallops the levels became markedly elevated. All values falling within the range of 61-63%.

After exercise, the levels returned slowly towards resting values but at 90 min were still slightly increased.

BLOOD pH (TABLE 6.8)

A slight increase in pH values appeared to occur after transport

Table 6.6 Speeds (metres/s) of each horse over increasing distances

Horse	Distance Galloped (Furlongs)						
	2	3	5	8	12	18	
1	14.4	14.5	13.3	12.9	11.2	10.6	
2	15.2	16.4	16.1	14.5	14.0	13.0	
3	-	-	14.9	15.0	13.5	12.6	
4	13.0	14.0	14.3	13.9	12.7	10.8	

- indicates measurements not made

Table 6.7 Packed cell volumes in 4 horses (Mean \pm s.e.) after maximum exercise of varying distance

Stage of Procedure	Distance Furlongs							
	2	3	5	8	12	18		
Pre 1 (Before Transportation)	39.3 \pm 2.0	36.3 \pm 3.7	38.5 \pm 2.5	38.5 \pm 1.9	40.8 \pm 2.7	39.3 \pm 3.3		
Pre 2 (Racecourse)	42.3 \pm 3.5	42.7 \pm 1.8	39.3 \pm 3.8	45.0 \pm 1.0	41.5 \pm 0.9	43.3 \pm 2.0		
Post Gallop	62.3 ** \pm 0.9	62.7 ** \pm 0.9	61.0 ** \pm 1.3	62.3 ** \pm 1.9	63.0 ** \pm 0.5	62.8 ** \pm 2.1		
Post 15 min	55.3 ** \pm 0.3	54.5 ** \pm 6.0	53.0 ** \pm 3.0	55.7 ** \pm 3.3	58.5 ** \pm 3.2	56.3 ** \pm 3.4		
Post 30 min	46.0 \pm 3.5	46.7 ** \pm 3.8	47.0 ** \pm 2.5	50.8 ** \pm 2.6	49.8 \pm 2.9	49.5 \pm 1.0		
Post 60 min	42.7 \pm 2.0	46.0 \pm 1.5	43.3 \pm 1.9	46.3 ** \pm 1.1	44.0 \pm 2.3	40.5 \pm 3.5		
Post 90 min	-	41.7 \pm 0.9	42.5 \pm 1.0	44.0 \pm 1.0	42.3 \pm 1.9	40.3 \pm 3.7		

! indicates samples not taken; * results from 3 horses
 ** indicates results significantly ($p < 0.05$) altered from resting values

Table 6.8 Venous pH values (Mean \pm s.e.) in 4 horses after maximal exercise of varying distance

Stage of Procedure	Distance Furlongs						
	2	3	5	8	12	18	
Pre 1 (Before Transportation)	7.387 \pm .007	7.374 \pm .006	7.399 \pm 0.008	7.431 \pm .018	7.432 \pm .004	7.380 \pm .005	
Pre 2 (Racecourse)	7.398 \pm .008	7.407 \pm .009	7.430 \pm .010	7.424 \pm .013	7.432 \pm .013	7.361 \pm .014	
Post Gallop	7.162 \pm .066	7.089 ** \pm .067	7.104 ** \pm .053	7.097 \pm .076	7.089 ** \pm .052	7.013 \pm .168	
Post 15 min	7.326 \pm .041	7.222 \pm .088	7.215 ** \pm .063	7.20 \pm .082	7.216 ** \pm .067	7.195 \pm .109	
Post 30 min	7.374 \pm .010	7.330 \pm .044	7.334 ** \pm .028	7.316 \pm .069	7.362 \pm .038	7.254 \pm .056	

** results from 3 horses

** indicates results significantly ($p < 0.05$) altered from resting values

of the horses to the race course (Table 6.15). Immediately following each gallop pH values were markedly reduced, the lowest level (6.780) being recorded in horse No. 1 following the 1.8 furlong distance. There was a significant correlation between pH and blood lactate levels ($p < 0.05$) on all occasions. By 30 min post exercise pH levels were still below resting values.

BLOOD LACTATE (TABLE 6.9)

Blood lactate levels were markedly increased immediately after each gallop. Highest lactate levels were recorded after the 5, 8 and 18 furlong distances. By 30 min post exercise values were still above resting levels.

VENOUS pO_2 (TABLE 6.10)

Venous pO_2 levels increased after each gallop but most markedly following the 5 and 8 furlong distances. After 2, 3, 12 and 18 furlongs venous pO_2 values were at or below resting values.

15 min after exercise the highest values (post 5 and 8 furlongs) had decreased whereas those following the 2, 3, 12 and 18 furlongs had increased. By 30 min post exercise all except the 5 furlong levels were falling towards resting values.

VENOUS pCO_2 (TABLE 6.11)

Maximum venous pCO_2 levels occurred following the two shortest and two longest gallops. Values immediately after the 5 and 8 furlong distances were below resting levels.

15 and 30 min post exercise all levels were below resting values.

Table 6.9 Venous lactate levels (m mol/l) in 4 horses (Mean \pm s.e.) after maximal exercise of varying distance

Stage of Procedure	Distance Furlongs							
	2 *	3 *	5	8	12	18		
Pre 1 (Before Transportation)	1.1 \pm 0.2	-	-	-	1.3 \pm 0.4	0.8 \pm 0.3		
Pre 2 (Racecourse)	1.1 \pm 0.2	1.3 \pm 0.3	1.2 \pm 0.3	1.6 \pm 0.8	1.2 \pm 0.3	1.0 \pm 0.2		
Post Gallop	17.0 ** \pm 3.6	16.9 ** \pm 2.9	23.4 ** \pm 2.4	21.2 ** \pm 3.4	18.6 ** \pm 5.0	20.6 ** \pm 5.6		
Post 15 min	11.3 \pm 3.8	14.3 \pm 3.9	19.8 ** \pm 1.4	17.2 ** \pm 3.4	20.1 ** \pm 3.3	22.6 ** \pm 6.7		
Post 30 min	5.8 \pm 2.3	8.7 \pm 3.0	6.7 ** \pm 1.0	6.9 \pm 2.1	8.6 \pm 3.1	13.2 \pm 4.2		

'-' indicates samples not taken; * results from 3 horses

** indicates results significantly ($p < 0.05$) altered from resting (Pre 2) values

Table 6.10 Venous pO₂ levels (mm Hg) in 4 horses (Mean ± s.e.) after maximal exercise of varying distance

Stage of Procedure	Distance Furlongs					
	2*	3*	5	8	12	18
Pre 1 (Before Transportation)	33.4 ±0.9	32.9 ±0.7	34.2 ±0.8	32.1 ±2.0	40.8 ±7.5	34.0 ±1.5
Pre 2 (Racecourse)	34.3 ±3.8	29.8 ±1.9	33.7 ±0.9	32.8 ±0.7	33.1 ±0.2	36.7 ±2.0
Post Gallop	33.0 ±3.1	43.5 ±2.5	56.8** ±3.6	47.9** ±1.1	41.6 ±3.9	33.5 ±5.8
Post 15 min	38.3 ±1.9	46.9 ±3.5	44.5 ±4.0	43.2 ±7.9	52.4 ±6.5	66.5** ±11.2
Post 30 min	35.7 ±0.7	38.9 ±4.0	45.4 ±2.5	41.7 ±5.3	41.8 ±3.5	48.5 ±5.4

* results from 3 horses

** indicates results significantly ($p < 0.05$) altered from resting values

Table 6.11 Venous pCO₂ levels (mm Hg) in 4 horses (Mean ± s.e.) after maximal exercise of varying distance

Stage of Procedure	Distance Furlongs					
	2*	3*	5	8	12	18
Pre 1 (Before Transportation)	46.8 ±3.4	47.9 ±1.9	47.6 ±1.8	47.4 ±2.4	43.4 ±2.0	47.4 ±1.3
Pre 2 (Racecourse)	48.3 ±3.6	41.3 ±1.4	42.1 ±0.9	44.9 ±1.6	44.2 ±2.7	44.2 ±0.7
Post Gallop	69.3 ±12.6	56.6** ±2.9	45.8 ±6.7	44.3 ±7.2	55.6 ±11.0	58.2 ±15.0
Post 15 min	40.9 ±0.3	33.1 ±2.9	28.6*** ±2.5	36.4 ±1.3	33.9 ±3.3	33.4 ±4.7
Post 30 min	43.1 ±1.3	35.3 ±2.1	33.2 ±2.9	37.4 ±1.9	36.9 ±1.3	33.7 ±2.9

*** results from 3 horses

** indicates results significantly (p < 0.05) altered from resting values

VENOUS BICARBONATE (TABLE 6.12)

HCO_3^- levels fell markedly after each gallop the greatest differences being recorded after 2, 3, and 12 furlongs. By 30 min post exercise values were increasing slowly towards resting levels.

BASE EXCESS (TABLE 6.13)

Base excess was reduced after each gallop, lowest levels being recorded following 5, 8 and 18 furlongs. Following all but the 2 furlong gallop, levels were still markedly decreased 30 min post exercise.

PLASMA 11-OHCS (TABLE 6.14)

Plasma 11-OHCS levels were increased on all occasions by transportation of the horses to the race course (Table 6.15). Immediately after each gallop levels were further elevated but following all except the 2 and 12 furlong distances maximum levels occurred between 15 and 60 min post exercise.

Following the longer distances (8, 12 and 18 furlongs) the levels, although beginning to decrease by 90 min post exercise, were markedly higher than the corresponding resting values.

Table 6.12 Venous bicarbonate (HCO_3^-) levels (m Eq/litre) in 4 horses (Mean \pm s.e.) after maximal exercise of varying distance

Stage of Procedure	Distance Furlongs							
	2*	3*	5	8	12	18		
Pre 1 (Before Transportation)	27.9 ± 1.5	27.5 ± 0.4	28.2 ± 0.6	30.9 ± 0.1	26.4 ± 2.2	27.7 ± 0.4		
Pre 2 (Racecourse)	29.7 ± 1.9	25.8 ± 0.9	27.6 ± 0.3	28.7 ± 0.5	29.5 ± 2.5	24.6 ± 1.2		
Post Gallop	24.3** ± 0.7	17.2** ± 2.1	13.9** ± 1.1	12.9** ± 0.9	16.8 ± 1.8	13.5 ± 2.2		
Post 15 min	21.5 ± 2.1	14.5 ± 4.2	12.2** ± 2.4	15.0 ± 3.1	13.0 ± 3.6	15.0 ± 4.7		
Post 30 min	25.0 ± 0.1	19.0 ± 3.2	22.2 ± 5.0	19.7 ± 5.8	21.8 ± 2.9	14.9 ± 3.6		

* results from 2 horses
 ** indicates results significantly ($p < 0.05$) altered from resting values

Table 6.13 Base Excess Levels (m Eq/litre) in 4 horses (Mean \pm s.e.) after maximal exercise of varying distance

Stage of Procedure	Distance Furlongs							
	2	3	5	8	12	18		
Pre 1 (Before Transportation)	+4.0 \pm 1.1	+2.2 \pm 0.3	+3.4 \pm 0.2	+6.2 \pm 0.2	+4.0 \pm 1.1	+2.0 \pm 0.3		
Pre 2 (Racecourse)	+5.5 \pm 1.0	+1.5 \pm 0.9	+3.6 \pm 0.2	+3.8 \pm 0.5	+5.3 \pm 2.4	-0.8 \pm 1.3		
Post Gallop	-5.8** \pm 0.6	-12.8** \pm 3.2	-15.2** \pm 1.7	-18.6** \pm 1.1	-12.6** \pm 2.3	-19.8** \pm 6.1		
Post 15 min	-3.9 \pm 2.7	-12.0 \pm 5.5	-14.2** \pm 3.2	-15.9** \pm 2.7	-12.2** \pm 4.7	-13.1 \pm 6.7		
Post 30 min	0.2 \pm 0.3	-5.9 \pm 3.7	-6.9** \pm 2.6	-8.5** \pm 4.3	-3.9** \pm 2.9	-11.0 \pm 4.3		

** results from 3 horses

** indicates results significantly ($p < 0.05$) altered from resting values

Table 6.14 Plasma 11-OHCS levels (n mol/litre) in 4 horses (Mean \pm s.e.) after maximum exercise of varying distance

Stage of Procedure	Distance Furlongs							
	2	3	5	8	12	18		
Pre 1 (Before Transportation)	172 \pm 34	345 \pm 13	328 \pm 26	405 \pm 79	340 \pm 69	420 \pm 24		
Pre 2 (Racecourse)	288 \pm 9	417 \pm 24	404 \pm 54	488 \pm 56	442 \pm 100	537 \pm 27		
Post Gallop	460 ** \pm 25	552 ** \pm 17	495 \pm 52	595 \pm 72	699 \pm 43	641 \pm 66		
Post 15 min	416 \pm 67	424 \pm 92	541 \pm 60	624 \pm 46	624 \pm 96	642 \pm 106		
Post 30 min	430 \pm 108	706 \pm 8	434 \pm 46	624 \pm 81	697 \pm 113	685 \pm 96		
Post 60 min	365 \pm 96	363 \pm 24	432 \pm 35	593 \pm 42	511 \pm 128	689 \pm 99		
Post 90 min	-	367 \pm 56	357 \pm 7	-	511 \pm 160	575 \pm 85		

** indicates results significantly ($p < 0.05$) altered from resting values
 *** indicates samples not taken; * results from 3 horses

Table 6.15 Alterations in various parameters (Mean \pm s.e.)
before and after transportation to the race-course

Parameter	Before Transportation	After Transportation
P.C.V. (%)	38.8 ± 0.6	42.4* ± 0.8
pH	7.400 ± 0.010	7.408 ± 0.010
Venous lactate (m mol/l)	1.1 ± 0.1	1.2 ± 0.1
Venous pO ₂ (mm Hg)	34.6 ± 1.3	33.4 ± 0.9
Venous pCO ₂ (mm ² Hg)	45.8 ± 0.7	44.2 ± 1.0
Venous HCO ₃ ⁻ (m Eq/l)	28.1 ± 0.6	27.7 ± 0.8
Base Excess (m Eq/l)	3.6 ± 0.6	3.2 ± 1.0
11-OHCS (n mol/l)	335 ± 36	429* ± 35

* levels significantly ($p < 0.05$) above pre-
transportation values

6.5 Discussion

1. Physiological Changes

As expected, exercise resulted in marked increases in heart and respiratory rates in horses similar to those shown by other workers (Krzywanek et al., 1970; Aitken, Sanford and MacKenzie, 1973; Hall et al., 1975). These elevations are the result of increased anticipatory mental activity and also increased metabolic demands (Guyton, 1971; Wasserman and Whipp, 1975).

During this exercise study, it was intended to record heart rates using radiotelemetry but due to a number of factors (discussed in chapter 4 of this thesis) this was not always possible. Consequently the heart rates recorded by auscultation are much lower than those reported by other authors in similar types of horses and following similar types of exercise.

Rates reported using radiotelemetry range for 200-240 beats/min in Thoroughbreds immediately after racing (Krzywanek et al., 1970; Hall et al., 1976) and Standardbred horses trotting at maximal speed (Persson, 1967; Marsland, 1968; Asheim, Knudsen, Lindholm, Rulcker and Saltin, 1970; Lindholm and Saltin, 1974). Heart rates of 160-200 beats/min have also been recorded in Standardbred horses trotting at speeds of 4-7 metres/second (Persson, 1967; Asheim et al., 1970). On several occasions however, not connected directly with this study, heart rates have been successfully recorded by radiotelemetry in our experimental horses during and immediately after maximal exercise of short duration (Fig. 1.1). In these instances,

rates of over 200 beats/min have been measured which agree with the results published previously.

The difference in readings measured by radiotelemetry and auscultation is the result of two factors. Firstly, it usually takes a short time for the heart rates measured by auscultation to be recorded i.e. for the operator to approach the horse and locate the heart beat and secondly, the heart rate is a mean over a period of 30 seconds during a time when the heart rate is almost certainly decreasing rapidly (Aitken et al, 1973; Hall et al, 1976). Radiotelemetry on the other hand allows an instantaneous heart rate to be obtained.

Respiratory rates also increased after exercise but the levels recorded in these experiments were markedly lower than those reported by Aitken et al. (1973) who monitored rates of up to 118 respirations/min in Thoroughbreds and 125 respirations/min in heavy hunters after cantering a distance of 5.6 km. The length of time between the end of exercise and the time when the recordings were made will also influence the values reported however since respiratory rates fall more rapidly than heart rates post exercise (Aitken et al, 1973). This difference could also be due to the times at which recordings were taken post exercise from these horses. Heart rate recordings were always made immediately the animal stopped and, after these had been completed, respiratory rates were measured. Consequently it was always three or four minutes after exercise that respirations were recorded

2. Metabolic Changes

Increases in P.C.V. following exercise of varying intensity have

been previously reported in horses (Persson, 1967; Milne, 1974). In this species, as in others such as sheep, cattle and dogs, the spleen has been shown to be the main reservoir of erythrocytes and to be able to regulate the oxygen-carrying capacity of the blood whenever required (Persson, 1967; Torten and Schalm, 1964; Turner and Hodgetts, 1959; Gartner, Ryley and Beattie, 1965; Elenes, Ewald and Crosby, 1964). This ability to increase the oxygen-carrying capacity of the blood is very important in the horse especially during exercise.

The control of splenic contraction has been discussed previously (chapter 1) and increases in P.C.V. have been noted in horses following excitement and adrenaline infusion as well as exercise. (Irvine, 1958; Turner and Hodgetts, 1959; Persson, 1967; Sreter, 1959; Krzywanek, 1973).

Following maximal exercise Milne (1974) reported greater increases in P.C.V. than following submaximal work and in these studies a similar pattern is apparent. Highest levels occurred immediately after the exercise tests at the racecourse and all the values fell between definite limits suggesting that in these horses 60-63% was the maximum levels possible. Similar maximal levels have been reported by Persson (1967) following injection of adrenaline intravenously at dose rates of up to 1.0 mg/kg.

That P.C.V. values were highest after the maximum exercise tests is to be expected since the oxygen requirement during acute strenuous activity will be higher than for prolonged submaximal effort. The high P.C.V. levels after the gallops also reflect a high degree of splenic contraction which will be due to increased

sympathetic activity at maximal work loads. Bloom, Johnson, Park, Rennie and Sulaiman (1976) and Howley (1976) reported increased levels of circulating catecholamines in man following exercise of high intensity and similar alterations have been recorded in horses (Snow and Summers, 1977). Smaller increases in sympathetic activity during submaximal exercise (Snow, personal communication) would also explain why P.C.V. values were lower after this less strenuous effort.

During the tests carried out at the racecourse, an increase in P.C.V. was also apparent after transportation from the stables suggesting that the horses all became slightly excited or apprehensive during the journey.

Blood gas levels and the acid-base status of the blood also altered to varying degrees during the different exercise programmes. Throughout exercise, increased cardiovascular and respiratory function is required in order to increase the supply of oxygen to the exercising muscles and also the removal of carbon dioxide from the circulation. These processes are closely linked to the amount of work being performed and the energy requirements of the working muscle. At low levels of work intensity (i.e. below half of the maximum oxygen uptake) all the energy required can be supplied aerobically because adequate oxygen is available. Above this level however, at higher work intensities sufficient oxygen is not available and anaerobic pathways of metabolism have to be utilised. The end product of anaerobic metabolism is lactic acid and blood lactate levels begin to increase only after a threshold level

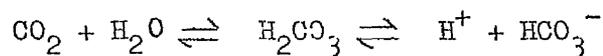
(approx. 50-60% of maximal oxygen uptake) has been reached (Wasserman and Whipp, 1975). The result of an increase in blood lactate values is a metabolic acidosis and fall in blood pH and bicarbonate levels, as was apparent in these studies following the maximal exercise tests.

Changes in the blood gas status and acid-base balance were monitored on all occasions on samples of venous blood. This is by no means the ideal situation but practical difficulties in obtaining arterial blood quickly made venous sampling the method of choice. Levels of pH, HCO_3^- and pCO_2 in arterial and venous blood have been compared at various stages of exercise and recovery, and it has been shown that venous values consistently mirrored the changes in arterial ones (Milne, 1974).

Metabolic acidosis as a result of maximal exercise in the horse has been reported previously (Krzywanek, 1974; Milne, 1974; Persson and Ullberg, 1974; Asheim et al., 1970; Krzywanek, Milne, Gabel and Smith, 1976) and extremely low levels of blood pH can be attained during this type of activity as is shown by the decrease in pH to 6.780 in Horse No. 1 following the 18 furlong gallop. At low levels of pH and consequently high blood lactate concentrations, exercise cannot be successfully continued for any length of time (Astrand and Rodahl, 1970). It is interesting to note that Horse No. 1 although proving fastest over the 2 furlong gallop became progressively slower over the longer distances as his blood pH level dropped lower than in any of the other horses. The other three horses were perhaps therefore more able to continue utilising aerobic pathways of metabolism before having to switch to anaerobic ones and were

capable of maintaining their speeds over the longer gallops.

The decreases in pH recorded can be attributed mainly to elevations in blood lactate levels as shown by the high inverse linear correlation between the two reported in experiment 6.2. However changes in venous $p\text{CO}_2$ levels will also affect pH values (Wasserman and Whipp, 1975). Excess CO_2 is produced from the buffering action of bicarbonate on the increased lactate levels and by 'blowing off' this excess CO_2 the animal may be able to partially compensate for the metabolic acidosis by shifting the equilibrium



to the left and thus raising the pH

This respiratory compensation might explain the elevation in blood pH 15 min after the 3 x 600 metre gallops when lactate levels in most of the horses were still increasing and also the smaller decreases in pH with given lactate levels following training in the same experiment.

It would also explain why the decreases in blood pH following the 5 and 8 furlong gallops were less than the others when in fact levels of blood lactate were highest after these two distances. In both cases venous $p\text{CO}_2$ values were only slightly above or (as in the 8 furlong test) below pre-exercise levels suggesting that during this exercise the horses were able to blow off CO_2 and compensate in part for the acidosis.

Hyperventilation will also occur in response to increased body temperature in the horse and it has been suggested (Milne, 1974)

that this temperature-controlling mechanism could be the cause of lowered $p\text{CO}_2$ and elevated pH values within a few minutes of exercise ceasing. Body temperature has been shown in man to be regulated at a higher level during work than at rest (Nielsen and Nielsen, 1962; Saltin and Hermanssen, 1966). As elevations in body temperature of 2-3° were recorded after the submaximal test it is most likely that similar or greater elevations would occur after maximal exercise and would be inducing a greater degree of hyperventilation increasing the effects on $p\text{CO}_2$ and pH already apparent.

Unlike the marked decreases in pH recorded after all the maximal exercises, submaximal effort produced a slight increase in blood pH values. It would appear therefore that under these conditions the animals were all able to maintain aerobic metabolism throughout the tests since only very slight elevations of blood lactate levels also occurred. The alterations in blood pH could have been due therefore to a respiratory alkalosis caused by hyperventilation and demonstrated by a reduction in venous $p\text{CO}_2$ and bicarbonate values (Milne, 1974).

Venous $p\text{O}_2$ and $p\text{CO}_2$ levels following the two types of exercise also differed. Measurement of venous $p\text{O}_2$ levels has been reported as not being a true estimation of the oxygen content of the blood (Milne, 1974). However Krzywanek *et al.*, (1976) found that although venous $p\text{O}_2$ values were lower than arterial ones, they did provide an overall picture of any alterations which might occur.

Venous $p\text{O}_2$ values increased to a greater extent after maximal

exercise and in some cases did not reach peak levels until 15 or 30 min after the exercise had ceased. This elevation had been attributed to the inability of the tissues to fully remove the elevated concentrations of arterial oxygen still being supplied to them (Milne, 1974) and also to the fact that the cardiovascular system does not respond immediately after exercise to the working muscles shutting down their high levels of oxidative metabolism (Krzywanek et al, 1976).

The more elevated levels of venous pO_2 recorded after the 5 and 8 furlong gallops at the racecourse occurred simultaneously with lower levels of pCO_2 and higher pH values than were recorded over any of the other distances. The combination of these three effects further suggests that by hyperventilating the animals in this case were able to partially compensate for the metabolic acidosis.

The more rapid return to resting values in both trained and untrained animals following submaximal exercise and in the trained group after maximal exercise suggests that in the former the oxygen-debt, which is due largely to lactate production (Knuttgen, 1962), is very small and that in the latter training might improve the capacity to overcome this oxygen-debt. This would also be borne out by the more rapid return to resting lactate levels which also occurred post-training in this group of animals.

Venous pCO_2 levels in horses after a single bout of strenuous exercise have been found to increase (Bergsten, 1974; Milne et al, 1976; Krzywanek et al, 1976) and then during recovery to decrease slightly below resting levels due to hyperventilation.

CO₂ during exercise is produced from two sources - the increased rate of metabolism in the working muscles and, as discussed previously, at high work loads from the buffering of lactic acid by bicarbonate (Wasserman and Whipp, 1975).

During and after the 3 x 600 metre gallop tests pCO₂ levels decreased. This reduction could have been partially due to the fact that no corrections on account of body temperature were made in this study. As has been discussed previously elevations in temperature of 2-3° can be expected after exercise and had all blood gas estimations been corrected accordingly the alterations in pCO₂ values would have been less marked although still apparent. A decrease in pCO₂ levels was also noted during the submaximal exercise test and in both tests this was probably a result of respiratory compensation for the decreased HCO₃⁻ levels and hyperventilation occurring in order to help control body temperature.

The lower pCO₂ levels recorded after the 10 week training programme in the 3 x 600 metre gallop test could be due to an increase in pulmonary ventilation (i.e. tidal volume) or in the alveolar diffusing capacity of the lungs (Wasserman and Whipp, 1975) since no apparent alterations in respiratory rates were noted before and after the training programme.

In the individual gallops pCO₂ values increased markedly after exercise except following the 5 and 8 furlong gallops. These elevations are similar to the results reported by Milne et al (1976) and Krzywanek et al (1976) and are attributed to increased metabolic rate during exercise.

The fact that $p\text{CO}_2$ values were lower after the 5 and 8 furlong gallops has been discussed previously and it has been suggested that this could be due to hyperventilation as a result of the decreased bicarbonate levels and possibly increases in body temperature.

The alterations in venous bicarbonate, as discussed previously, and in base excess levels recorded after maximal exercise are a further indication of the degree of metabolic acidosis produced (Wasserman *et al.*, 1967; Osnes and Hermanssen, 1972; Krzywanek, 1974; Milne, 1974). Base excess is a direct measurement of the excess or deficit of non-volatile acid in the circulation (Sigaard-Anderson, 1963) and changes in base excess reflect either an increase in non-volatile acids in the blood or a removal of base. It has been suggested that base excess measurements provide a more accurate estimation of metabolic acidosis than do blood pH estimations since the latter is affected by respiratory compensatory mechanisms (Osnes and Hermansen, 1972). These authors however also reported that decreases in base excess levels were higher than the corresponding increases in blood lactate concentrations and that this overestimation of acidosis could be due to the increase of other acids such as pyruvic acid in the blood after exercise. In these studies following maximal exercise, base excess values underestimated the degree of metabolic acidosis as indicated by blood lactate levels. This difference could possibly be due to the fact that no corrections were made in base excess values for increases in body temperature during these tests.

Following submaximal exercise both base excess and blood lactate levels altered only slightly indicating that a small degree of metabolic acidosis was present. The decrease in bicarbonate levels and venous $p\text{CO}_2$ however along with the fact that pH values increased slightly suggest that respiratory compensation had occurred and that in fact a slight respiratory alkalosis had ensued.

3. Hormonal Changes

Conflicting data exists on the effects of exercise of varying intensity on plasma 11-OHCS levels. It is generally considered (Thrap, 1975) that light to moderate exercise may produce increases, decreases or no change in circulating plasma 11-OHCS levels in man, rats and dogs (Cornil, De Coster, Copinschi and Franckson, 1965; Suzuki, Otsuka, Matsui, Ohukuzi, Sakai and Harada, 1967; Davies and Few, 1973; Few, 1974; Foss, Barnard and Tipton, 1971; Rose, Friedman, Beering and Cooper, 1970).

Moderate to exhaustive prolonged exercise on the other hand nearly always produces increases in 11-OHCS levels in man, rats, dogs and horses (Hartley, Mason, Hogan, Jones, Kotchen, Mougey, Wherry, Pennington, and Ricketts, 1972; Davies and Few, 1973; Sutton, Young, Lazarus, Hickie and Maksuytis, 1969; Chin and Evonuk, 1974; Foss et al., 1971; Snow and Munro, 1975; Flisinska-Bojanowska et al., 1974).

The diversity in all these results has been suggested to be due to variation in the duration and intensity of the exercise employed as well as differing methodology of 11-OHCS estimation (Foss et al., 1971).

The range of plasma 11-OHCS levels recorded from this group of four Thoroughbreds before transportation to the race course falls within that reported earlier (chapter 3) for a series of twenty Thoroughbreds before and during a training programme. There was however a significant increase in the corticosteroid levels after transportation in the horse box which could have been due to excitement apprehension or the physical effort of maintaining balance in the box while travelling.

The maximum plasma 11-OHCS levels recorded in this study following all three exercise tests viz. maximal, sub-maximal and maximal over varying distances are recorded in Table 6.16 and are also expressed in this table as percentage increases above the levels recorded immediately prior to exercise in each case (those samples collected at the race course i.e. Pre 2 were considered to be pre-exercise values for this test). Although the results in this study demonstrated that the greatest increase in 11-OHCS concentrations occurred up to 60 min after the gallops over distances varying from three to eighteen furlongs and immediately following the prolonged sub-maximal test when percentage increases above resting (pre-exercise) values are considered, this is not the case. The greatest percentage increase of plasma 11-OHCS levels occurred following prolonged sub-maximal exercise but when changes in corticosteroid values per unit time were considered the greater alterations were associated with maximal effort. These results therefore agree with those of Foss et al (1971) and Davies and Few (1973) who demonstrated a linear relationship between plasma 11-OHCS values and work

Table 6.16 Maximal plasma 11-OHCS levels recorded following maximal and sub-maximal exercise in 4 horses
 (mean \pm s.e.) expressed as a percentage increase above resting (pre-exercise) levels

Exercise Test	Stage of Training	Maximum Plasma 11-OHCS Values Recorded (n mol/litre)	Percentage Increase above Resting (Pre-Exercise) Levels
Maximal Exercise 3 x 600 metres	Pre	420	80
	Post	403	50
Sub-Maximal Exercise	Pre	665	127
	Post	556	155
Maximal Exercise (varying distance)		706	69

intensity in dogs and man.

The increase in plasma 11-OHCS levels for a length of time after the completion of exercise has been recorded by other workers in dogs (Foss et al, 1971) and could be due to a decrease in the rate of metabolic clearance of these steroids from the blood. Davies and Few (1973) reported that during exercise the rate of metabolic breakdown of cortisol is increased. Few (1974) also demonstrated that the rate of removal of labelled cortisol from the circulation immediately after exercise falls markedly whereas corticosteroid production continues for a short time.

Synacthen was administered to the horses after submaximal exercise because it has been suggested by several authors that 11-OHCS levels decline following prolonged exercise due to adrenal exhaustion (Frenkl and Csaly, 1970; Chin and Evonuk, 1971). Injection of 100 iu Synacthen produced elevations in plasma 11-OHCS values much lower than those reported in previous chapters in ponies, but similar to those recorded by Snow and Munro (1975). In neither group of animals were the elevations in 11-OHCS values as high as those immediately after exercise suggesting either that stores of corticosteroids had become slightly depleted or that because of the still high resting plasma 11-OHCS levels the negative feedback mechanism was functioning and that basal plasma ACTH values were low.

The duration of response was also much shorter in these horses compared to the results of Snow and Munro (1975) and to those recorded earlier in this study. This could be due to uptake of

Synacthen from the site of injection being more rapid in these horses as a result of increased blood flow to the muscles immediately following exercise.

There was no apparent change in pre-exercise 11-OHCS levels recorded in either of these exercise tests after the ten week training programme although such alterations have been previously reported in rats (Frenkl and Csabay, 1970; Buick and Tharp, 1971) and were noted in earlier studies (chapter 3.4) in a group of twenty Thoroughbreds during a twelve month training programme.

It would appear from these results however that training does cause an elevation in plasma 11-OHCS levels immediately after exercise and similar findings have been reported in both animals and man by other workers (Frenkl, Csabay, Csakvary and Zelles, 1968; Foss et al, 1971; White, Ismail and Bottoms, 1976). This increase could be due to adrenal hyperplasia caused by continued ACTH stimulation (Tharp, 1975) to some adaptive process in the CNS resulting in more ACTH being secreted, or to an increased sensitivity of the adrenal to the same amount of ACTH. In these studies, the results indicate that adrenal hyperplasia and/or increased adrenal sensitivity to the same degree of stimulation are responsible for the training effects since administration of Synacthen to the trained horses caused slightly greater elevations in 11-OHCS levels.

Summary

- i) Maximal and submaximal exercise in the horse result in elevations in heart and respiratory rates, P.C.V. and plasma 11-OHCS

values. Alterations in the acid-base status of the blood also occurred.

- ii) Maximal exercise produced the greatest increases in P.C.V. levels and also the greatest degree of metabolic acidosis. Changes in plasma 17-OHCS values on the other hand appeared to be influenced by the duration of the exercise as well as the speed.
- iii) Training produced less marked reductions in venous pH for given blood lactate levels and this effect could have been the result of more efficient respiratory compensation for the metabolic acidosis in the trained animals.
- iv) Training appeared to result in increased levels of plasma 17-OHCS levels after exercise and this together with the greater adrenal response to Synacthen post-training suggests that some degree of adrenal hyperplasia had occurred.

7. TILE USE OF CENTRAL DEPRESSANT

DRUGS TO ALLEVIATE STRESS

7.1 Introduction

A tranquilliser is a drug which produces central nervous depression without at the same time causing drowsiness, analgesia or anaesthesia. A sedative on the other hand is a drug which can produce sleep and which at high doses can be used as an anaesthetic (Soma, 1971).

The distinction between tranquilliser and sedative in veterinary medicine is not always made and the two terms are used by some authors synonymously. However, the distinction between tranquillisation and sedation can still be made in that with the former at the same degree of central depression there is much less motor involvement than with the latter.

Tranquillisers are widely used in all domestic animals as chemical restraining agents in order to facilitate handling for both routine work and for minor surgical procedures. They are also used widely as premedicants for general anaesthesia.

Prior to 1960 the sedatives chloral hydrate and pentobarbitone were widely used for animal restraint (Arthur, Vaughan and Yeats, 1953; Wright, 1958) but with the advent of tranquillisers, principally the phenothiazine group, these compounds have been largely superseded for this purpose although they are still used currently to a small extent.

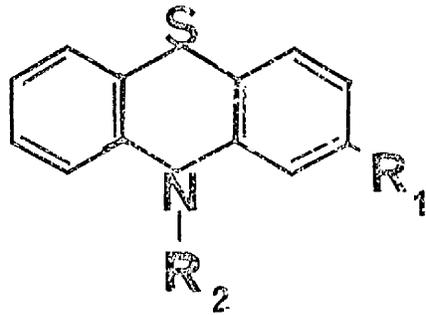
For the last decade the most commonly used tranquillisers in horses have been the phenothiazine derivatives promazine and acepromazine (Carey and Sanford, 1963; Pugh, 1964; Jones, 1972).

The general structure of the phenothiazine group is shown in Fig. 7.1 and the characteristic actions of these drugs are governed by the nature of the side-chain substituted at position R_2 on the nucleus. The development of useful derivatives has occurred in three separate groups 1) those compounds with a dimethylaminopropyl side-chain produce tranquillisation with marked sedation. 2) those compounds with a piperazinopropyl side-chain have very potent tranquillising properties but little sedative effect. They also possess a high incidence of extrapyramidal effects. 3) those compounds with a methylpiperidyl group have very low potency.

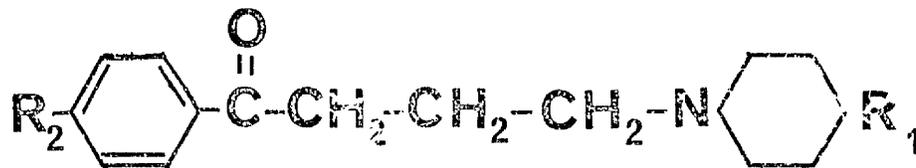
Further variations are possible within each group by substitution on the R_1 site and tranquillising ability has been shown to increase in order from unsubstituted to trifluoromethyl (Carey and Sanford, 1963). Promazine and acepromazine both belong to the first group of phenothiazines.

A newer group of tranquillisers introduced were the butyrophenones (Marsboom, 1971). Their general structure is also shown in Fig. 7.1 and highest neuroleptic potency is associated with the presence of a fluorine substituent in the R_2 position. Azaperone (4' fluoro-4 (4-(2-pyridyl)-1-piperazinyl)-butyrophenone) is one of this class of drugs which has been used extensively and successfully in pig practice (Mitchell, 1966; Marsboom and Symoens, 1968). It has been used in horses to produce tranquillisation (Roztocil, Nemecek and Pavlica, 1971; Aitken and Sanford, 1972; Lees and Serrano, 1976; Serrano and Lees, 1976) and in combination with metomidate, a short acting induction agent to produce

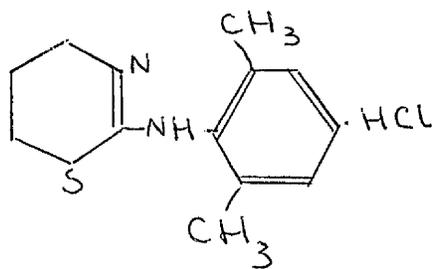
FIG. 7.1 THE GENERAL STRUCTURE OF THE PHENOTHAZINES,
THE BUTYROPHENONES AND XYLAZINE



1. PHENOTHIAZINES



2. BUTYROPHENONES



3. XYLAZINE

anaesthesia (Roztocil, Nemecek, Pavlica, Bouda and Misic, 1972; Hillidge, Lees and Serrano, 1975).

The third of the most commonly used drugs is the tranquilliser xylazine (2(2,6-dimethylphenylamino)-4H-5,6 dihydro-1,3-thiazine hydrochloride). Its general structure is shown in Fig. 7.1.

Xylazine has been shown to be extremely successful as a tranquilliser in horses (Clark and Hall, 1969; Fessel, 1970) and a number of workers (McCashen and Gabel, 1971; Kerr, Jones, Holbert and Huggins, 1972; Hoffman, 1974) have concluded that it is a superior tranquilliser to acepromazine and the other phenothiazines for minor surgical operations in terms of speed of onset, shortness of action and analgesic properties. A disadvantage of xylazine for routine use however is its high cost compared to phenothiazines.

Tranquillisers and sedatives lower the stress response to various stimuli and therefore measurement of this stress can be used as a quantitative means of assessing the efficacy of various tranquillisers (Greenblatt and Shader, 1973). As stress in this study is being measured by changes in the adrenal-cortical system and in sympathetic nervous activity, the ability of these drugs to reduce stress can be estimated from the changes, if any, which occur in these parameters during physical or pharmacological stimulation. Aitken and Sanford (1972) compared the effects of acepromazine, azaperone and xylazine on the changes in heart rate produced by various disturbing stimuli on horses and Smith *et al.* (1963) and Lahti and Barsuhn, (1974) evaluated the effectiveness of several tranquillising and antipsychotic drugs in rats by measuring

increases in plasma corticosteroid levels.

As well as reducing stress by their effects on the central nervous system however, these drugs can alter heart rate, blood pressure, respiratory rate and numerous biochemical parameters by direct pharmacological actions.

The narcotic analgesic etorphine is estimated to cause stress when this is assessed by both subjective and objective means and is normally administered in combination with a tranquilliser to reduce this stress. The two drugs are normally given simultaneously but it is probable that the maximal central depressant effect of the tranquilliser does not occur at the time of greatest stress produced by the etorphine. In addition the peripheral α - adrenergic blocking actions of the tranquillisers may play a major part in their value in this particular usage.

The following experiments were designed to clarify these points.

7.2 THE EFFECTS OF ACEPROMAZINE, AZAPERONE AND XYLAZINE ADMINISTERED INTRAVENOUSLY AND INTRAMUSCULARLY ON HEART AND RESPIRATORY RATES AND OF ACEPROMAZINE ADMINISTERED INTRAVENOUSLY ON HEART RATE AND ARTERIAL BLOOD PRESSURE IN PONIES

The initial experiments in this study involving the effects of intravenous and intramuscular administration of acepromazine, azaperone and xylazine on heart and respiratory rates in ponies were carried out concurrently with the studies on the actions of etorphine and 'Immobilon' in the same animals i.e. ponies Nos. 1 and 3 (see chapter 9).

The investigation into the effects of acepromazine on arterial blood pressure was undertaken at a later date. All three ponies were included in this and further studies on the actions of etorphine and 'Immobilon'.

Animals

All three ponies were used in these experiments.

Before monitoring arterial blood pressures all three animals were anaesthetised and the right carotid artery was transposed subcutaneously 1.5 inches dorsal of the jugular furrow. This raising of the carotid artery facilitated the measurement of arterial blood pressure in the animals. The surgical procedure used was that of Tavernor (1969).

A period of 12 weeks was allowed after surgery before the

animals were used experimentally.

Methods

Clinical observations were made following administration of each drug on onset of action, depth and duration of tranquillisation (assessed by the physical appearance of the animal, reluctance to move, reaction to handling). Other actions were also noted.

Heart rates were recorded by radiotelemetry as described previously and respiratory rates were measured visually over a period of 30 seconds. Blood pressure was monitored in the relocated right carotid artery. A 30 cm. (nylon) cannula was inserted into the artery through a 16 gauge 1.5 inch needle and was kept patent by injection of small volumes of heparinised saline. The cannula was attached to a Bell and Howell Type 4-422-0001 pressure transducer (previously calibrated to the height of the animal) and the blood pressure readings recorded on a Devices (M 19) pen-recorder.

Blood pressures quoted are mean blood pressures (diastolic blood pressure plus one third of pulse pressure).

Drugs

Acepromazine	: Acetylpromazine	10 mg/ml.	C-Vet Limited
Azaperone	: Suicalm	4% w/v.	Crown Chemical Company Limited
Xylazine	: Rompun	50 mg/ml.	Bayer UK Limited

The drugs were administered at the following dose rates.

- | | |
|------------------|--|
| i.) acepromazine | 0.2 mg/kg intramuscularly
0.1 mg/kg intravenously |
| ii) azaperone | 0.4 mg/kg intramuscularly
0.2 mg/kg intravenously |
| iii) xylazine | 3.0 mg/kg intramuscularly
0.5 mg/kg intravenously |

The doses of all three drugs were similar to those used by previous workers in horses (Aitken and Sanford, 1972; Pugh, 1964; Seriano and Lees, 1976; Clarke and Hall, 1969; Hoffman, 1974).

The dose of acepromazine used in the second experiment was also 0.1 mg/kg administered intravenously. This dose is equivalent to that included in the recommended dose of 'Immobilon' (0.5 ml/50 kg) which was employed in later experiments (Chapter 9) with these same ponies.

Experimental Procedure

EXPERIMENT 1

Heart and respiratory rates were recorded from the animals before tranquillisation with all 3 drugs and at 10, 20, 30, 40, 50 and 60 min after drug injection.

A period of at least 4 days was allowed between doses.

EXPERIMENT 2

Heart rate and arterial blood pressure were recorded from all three ponies prior to tranquillisation with acepromazine and at 1, 5, 10, 20, 30, 45 and 60 min after injection.

7.2.1 Results

EXPERIMENT 1

Intravenous administration of tranquillisers

BEHAVIOURAL SIGNS

All three drugs produced penile relaxation and drooping of the head, acepromazine and azaperone produced slight sweating in the groin and axillae.

Azaperone and xylazine produced signs of sedation within 5 min of injection which lasted between 40 and 50 min following each drug. Acepromazine produced signs of sedation within 10 min which lasted for 2.5-3 hours.

HEART RATE (TABLE 7.1)

Acepromazine and azaperone increased heart rate by 29% and 91% respectively during the first 20 min of sedation. Thereafter the rate fell gradually reaching pre-injection values 40 min later. Xylazine caused a 25% reduction in heart rate within 10 min of injection and the rate was still below resting values 50 min later. Dropped beats were seen during the first 3 min following xylazine administration.

RESPIRATORY RATE (TABLE 7.2)

Acepromazine appeared to produce a slight decrease in respiratory rate over the 60 min period whereas azaperone and xylazine produced no obvious effect.

Table 7.1 Heart Rates (Mean \pm s.e.) in 2 ponies after administration of acepromazine, azaperone and xylazine i/v and i/m

Stage of Procedure	Drugs							
	Acepromazine		Azaperone		Xylazine		Xylazine	
	i/v	i/m	i/v	i/m	i/v	i/m	i/v	i/m
Pre Inj.	48 \pm 2.0	44 \pm 2.0	44 \pm 2.9	45 \pm 1.7	48 \pm 1.8	47 \pm 1.2		
10 min Post Inj.	54 \pm 3.5	56 \pm 2.0	68 \pm 8.0	54 \pm 6.5	36 \pm 2.1	41 \pm 1.2		
20 min Post Inj.	62 \pm 5.3	64 \pm 2.0	84 \pm 2.1	57 \pm 5.2	39 \pm 3.0	40 \pm 2.4		
30 min Post Inj.	60 \pm 3.5	60 \pm 6.9	54 \pm 3.2	62 \pm 3.5	42 \pm 2.0	38 \pm 2.4		
40 min Post Inj.	54 \pm 2.1	48 \pm 2.3	42 \pm 1.6	48 \pm 1.3	-	-		
50 min Post Inj.	48 \pm 1.3	48 \pm 3.5	-	-	-	44 \pm 1.0		
60 min Post Inj.	48 \pm 4.0	46 \pm 4.0	42 \pm 2.3	-	46 \pm 2.0	46 \pm 1.0		

'-' indicates recordings not taken

Table 7.2 Respiratory Rates (Mean \pm s.e.) in 2 ponies after administration of acepromazine, azaperone and xylazine i/v and i/m

Stage of Procedure	Drugs							
	Acepromazine		Azaperone		Xylazine		Xylazine	
	i/v	i/m	i/v	i/m	i/v	i/m	i/v	i/m
Pre Injection	13 \pm 5	16 \pm 2	16 \pm 2	13 \pm 3	10 \pm 2	10 \pm 1	10 \pm 2	10 \pm 1
10 min Post Inj.	10 \pm 1	10 \pm 1	17 \pm 7	13 \pm 1	11 \pm 1	11 \pm 2	11 \pm 1	11 \pm 2
20 min Post Inj.	-	-	-	15 \pm 1	10 \pm 1	10 \pm 1	10 \pm 1	-
30 min Post Inj.	11 \pm 2	10 \pm 2	14 \pm 3	-	-	12 \pm 1	-	12 \pm 1
40 min Post Inj.	11 \pm 2	-	16 \pm 2	14 \pm 1	10 \pm 1	9 \pm 2	10 \pm 1	9 \pm 2
50 min Post Inj.	10 \pm 1	13 \pm 3	-	12 \pm 2	-	10 \pm 1	-	10 \pm 1
60 min Post Inj.	10 \pm 1	10 \pm 1	17 \pm 2	14 \pm 3	11 \pm 2	10 \pm 2	11 \pm 2	10 \pm 2

'..' indicates recordings not taken.

Intramuscular administration of tranquillisers

BEHAVIOURAL SIGNS

The clinical signs seen were similar to those recorded after intravenous administration. The onset of action of all the drugs was much slower following injection by this route. Azaperone produced signs of tranquillisation at 10-15 min, xylazine at 10-15 min and acepromazine at 50-60 min. The effects were still visible 1.5 hours after xylazine and 2.5 hours after acepromazine and azaperone.

HEART RATE (TABLE 7.1)

Acepromazine and azaperone produced increases in heart rate of 45% and 38% 20 and 30 min post-injection respectively. Xylazine on the other hand produced a 20% fall in heart rate 30 min post-injection. No dropped beats were recorded.

All heart rates had returned to pre-injection values by 60 min.

RESPIRATORY RATE (TABLE 7.2)

Acepromazine appeared to produce a slight decrease in respiratory rates over the 60 min period. Following azaperone and xylazine administration the rates appeared to increase but this effect was slight.

EXPERIMENT 2

BEHAVIOURAL SIGNS

The clinical signs observed were the same as those described

previously.

HEART RATE (TABLE 7.3)

Heart rate increased to a maximum 5 min post-injection (+50% above resting values) but had returned to pre-injection values within 60 min.

ARTERIAL BLOOD PRESSURE (TABLE 7.3)

Blood pressure decreased slowly over the first 30 min reaching its lowest level between 20 and 30 min post-injection. By the end of the 60 min period however it had returned almost to resting values.

Table 7.3 Heart rate and arterial blood pressure (Mean \pm s.e.) in 3 ponies after acepromazine injected intravenously (0.1 mg/kg)

Procedure	Heart Rates (beats/min)	Arterial Blood Pressure (mm Hg)
Pre Injection	42 \pm 3	73 \pm 9
1 min Post	-	70 \pm 20
5 min Post	66 \pm 3 *	70 \pm 6
10 min Post	58 \pm 2 *	52 \pm 3
20 min Post	58 \pm 3	42 \pm 6 *
30 min Post	-	43 \pm 4 *
45 min Post	48 \pm 2	50 \pm 6
60 min Post	42 \pm 3	68 \pm 3

* indicates recordings not taken

* indicates levels significantly altered from pre drug values (p<0.05)

7.3 THE EFFECTS OF ACEPROMAZINE, AZAPERONE AND XYLAZINE ON HEART RATE, PACKED CELL VOLUME AND PLASMA 11-OHCS LEVELS IN HORSES

This experiment was carried out concurrently with the work described previously on the effects of muscle biopsy. This surgical procedure was repeated in tranquillised animals, the doses of drugs used being higher than those employed in the preceding study.

However before administration of acepromazine, azaperone and xylazine prior to muscle biopsy, each drug was administered to three horses without carrying out the operative procedure. The effects of the drugs on heart rate, packed cell volume and plasma 11-OHCS levels were measured over a 60 min period as it had been shown in the previous study that the muscle biopsies could be completed within this time.

Animals

All seven horses were used in this study. Their temperaments are assessed in Table 5.1.

Methods

Clinical observations were made following administration of each drug as described in the preceding experiment.

Heart rates were recorded by radiotelemetry as described

previously.

Blood samples for P.C.V. and plasma 11-OHCS levels were collected and treated as described previously.

Drugs

All three drugs were administered intramuscularly at the following dose rates.

- | | |
|-----------------|-----------|
| i) acepromazine | 0.5 mg/kg |
| ii) azaperone | 0.7 mg/kg |
| iii) xylazine | 2.0 mg/kg |

The dose of acepromazine was five times that recommended by the manufacturers for horses and the maximum required to produce obvious signs of sedation in all horses (Hall, 1971) also Aitken and Sanford (1972) have shown that doses of 0.2 mg/kg acepromazine administered intramuscularly produced very little tranquillising effect on the responses to disturbing visual stimuli and for this reason also it was decided to use the 0.5 mg/kg dose.

The doses of azaperone and xylazine were within the ranges employed by other workers (Hillidge *et al*, 1975; Lees and Serrano, 1976; Aitken and Sanford, 1972; Clarke and Hall, 1969; Hoffman, 1974).

The schedule of drug administration is shown in Table 7.4.

Experimental Procedure

All the experiments were carried out between the hours of 9 a.m.

Table 7.4 Drugs administered in Control experiment

Horse	Drug *
1	ACP : X
2	AZ
3	X : ACP
4	AZ
5	X
6	ACP
7	AZ

*

ACP = Acepromazine

AZ = Azaperone

X = Xylazine

and 12 noon.

Heart rate recordings and blood samples for P.C.V. and 11-OHCS estimation were taken from the animals at rest prior to tranquillisation and at 30 and 60 min post-injection.

7.3.1 Results

BEHAVIOURAL SIGNS

Azaperone and xylazine produced visible signs of tranquillisation by 20 min post-injection compared with between 30 and 40 min following acepromazine. All three drugs produced penile relaxation and drooping of the head and ears and both acepromazine and azaperone produced varying degrees of sweating especially around the axillae and groin. Excess salivation was seen in the same three horses each time azaperone or xylazine was administered.

Twenty-four hours after acepromazine and azaperone injection the horses still showed slight signs of tranquillisation in that they were reluctant to exercise and when ridden showed slight incoordination of movement. Following xylazine the effects had worn off within 5 hours.

Horse No. 4 became hyperexcitable between 20 and 40 min following azaperone injection. This reaction was accompanied by severe sweating, trembling and restlessness.

HEART RATE (TABLE 7.5)

Acepromazine and azaperone both appeared to produce a slight elevation in heart rate 60 min after injection when levels were increased by 12% and 24% respectively above resting values.

Xylazine on the other hand produced little change in heart rate but caused occasional dropped beats especially within the initial 10-20 min.

Table 7.5 Effects of acepromazine, azaperone and xylazine on Heart Rate, P.C.V. and Plasma 11-OHCS levels in 3 Horses (mean \pm s.e.)

	Acepromazine 0.5 mg/kg				Azaperone 0.7 mg/kg				Xylazine 2.0 mg/kg			
	Stage of Procedure											
	Pre	30 min	60 min	Pre	30 min	60 min	Pre	30 min	60 min	Pre	30 min	60 min
Heart Rate /min	42 \pm 3	48 \pm 4	60 \pm 7	44 \pm 2	47* \pm 3	54* \pm 3	38 \pm 2	36 \pm 1	36 \pm 9	38 \pm 2	37 \pm 1	35 \pm 4
PCV (%)	37 \pm 3	34 \pm 3	31 \pm 2	38 \pm 2	35* \pm 1	30* \pm 2	38 \pm 2	37 \pm 1	35 \pm 4	38 \pm 2	37 \pm 1	35 \pm 4
11-OHCS (n mol/litre)	202 \pm 18	219 \pm 12	266 \pm 11	203 \pm 38	394* \pm 70	436* \pm 93	203 \pm 38	266 \pm 24	227 \pm 8	279 \pm 18	266 \pm 24	227 \pm 8

* = includes Horse No. 4 which showed signs of hyperexcitability following azaperone

PACKED CELL VOLUME (TABLE 7.5)

Both acepromazine and azaperone administration produced decreases in P.C.V. levels at 60 min post-injection which were 15% and 20% below resting values. Xylazine appeared to cause little alteration in P.C.V.

PLASMA 11-OHCS (TABLE 7.5)

Daily variations in plasma 11-OHCS levels occurred in all the horses but these values always fell within the ranges quoted in earlier studies (chapter 3). Acepromazine caused a slight increase in 11-OHCS values in all 3 horses. Azaperone also produced increases in 11-OHCS levels, the most marked occurring in horse No. 4 which exhibited a mild hyperexcitability reaction. Xylazine on the other hand produced little change in two of the horses and a decrease in 11-OHCS levels in the third.

7.4 REDUCTION OF THE STRESS OF SURGICAL PROCEDURES BY PREMEDICATION
WITH TRANQUILLISERS

This study was carried out at the same time as the experiment described previously on the effects of muscle biopsy on heart rate, P.C.V. and plasma 11-OHCS levels.

Premedication with acepromazine, azaperone and xylazine was employed to enable the biopsy procedure to be carried out in all including those the horses in which this had previously proved impossible.

Animals

All 7 horses used in previous studies were included in this experiment.

Methods

Heart rates were recorded by radiotelemetry as described previously.

Blood samples for P.C.V. and plasma 11-OHCS estimation were collected and treated as described previously.

Drugs

Acepromazine, azaperone and xylazine were administered intramuscularly at the dose rates used in the previous experiment

in section 7.3. The intramuscular route was chosen in preference to intravenous because the duration of tranquillisation following the former route was greater and was sufficient to allow completion of the biopsy procedure.

A higher dose of azaperone (0.9 mg/kg) was administered on one occasion to horse No. 3 which was the most nervous of all the animals.

Operative Procedure

The biopsy procedure was the same as described previously (chapter 5).

Experimental Procedure

All the experiments were carried out between 9 a.m. and 12 noon the resting samples and values always being recorded before 9.30 a.m. The entire procedure (drug injection to last biopsy) was always completed within 1.5 hours.

All the drugs were administered at least once to each of the 7 horses before the operative procedure and azaperone was used on 15 occasions. The order in which the drugs were given is shown in Table 7.6. There was an interval of at least 3 weeks between drug administrations to each horse except on the occasions when inadequate restraint resulted and on these occasions another drug was given 48 hours later.

Heart rates were monitored continuously throughout the procedure. Blood samples for P.C.V. and 11-OHCS estimation were

Table 7.6 Schedule of drugs administered prior to Muscle Biopsy

Horse	Drugs *
1	AZ : AZ : X : ACP
2	AZ : AZ : X : ACP
3	AZ : AZ : ACP : X : AZ
4	AZ : ACP : AZ : X : AZ
5	AZ : ACP : X
6	AZ : AZ : X : ACP
7	AZ : ACP : AZ : X : AZ

* = order in which drugs were given

ACP Acepromazine

AZ Azaperone

X Xylazine

taken after drug administration, injection of local anaesthetic and on completion of all the biopsies.

The results recorded when the procedure was attempted without tranquillisation are included in this study as a control group.

7.4.1 Results

BEHAVIOURAL SIGNS

All the drugs produced the same effects on behaviour and posture as described previously.

In one of the horses (No. 4) acepromazine produced a hyperexcitable reaction between 20 and 60 min after injection. Severe sweating, staggering and trembling occurred and the operative procedure was abandoned. On subsequent occasions when this animal was given azaperone (described in the previous section) a similar but milder reaction occurred.

Although all three drugs produced similar signs of central nervous depression in the horses, they did not produce similar degrees of tranquillisation. This was only regarded as adequate if the animals allowed completion of the operative procedure without showing signs of distress (indicated by excitability, apprehension and attempts to resist injection of local anaesthetic and biopsying).

As with the control studies when no tranquillisers were used, injection of the local anaesthetic produced most agitation and failure to inject the local anaesthetic into all the biopsy sites was the normal reason for the procedure not being completed.

Only azaperone was effective in all 7 horses on each occasion although in horse No. 3 a higher dose (0.9 mg/kg) was necessary. Acepromazine was effective in five horses (Nos. 1, 2, 3, 5 and 6) and xylazine in only four (Nos. 1, 2, 5 and 6). In three of the animals (Nos. 1, 5 and 6) however it was possible to complete the

procedure with no tranquillisation.

HEART RATE (TABLE 7.7)

Following premedication, as when no drugs were used, the biopsy procedure produced an increase in heart rate associated especially with injection of the local anaesthetic. This increase was most marked in those animals which did not allow completion of the procedure.

Premedication reduced the heart rate increases below those of the control group following injection of the local anaesthetic ($p < 0.05$) but not following the biopsies.

The dropped beats recorded following xylazine disappeared as the heart rate increased.

PACKED CELL VOLUME (TABLE 7.8)

No increases in P.C.V. were recorded following premedication at any time during the operative procedure. Contrary to the elevations in P.C.V. recorded after injection of local anaesthetic and after the biopsies when no tranquillisers were used, acepromazine and azaperone both decreased P.C.V. below resting values. Xylazine produced no effect on P.C.V.

PLASMA 11-OHCS (TABLE 7.9)

Following acepromazine premedication there appeared to be a slight increase in plasma 11-OHCS levels which was higher following the biopsies than the local anaesthetic. In the azaperone treated animals the 11-OHCS levels were markedly higher following the biopsy procedure than after injection of the local anaesthetic even though this group of horses showed least evidence of distress and

Table 7.7 Effects of Biopsy Procedure on Heart Rate

(beats/min Mean \pm s.e.)

Stage of Procedure	Control (No Tranquillisers)	Acepromazine	Azaperone	Xylazine
Pre Experiment	39 \pm 1.8 (7)+	40 \pm 1.3 (7)	42 \pm 1.0 (15)	38 \pm 1.5 (7)
Before local Anaesthetic	40 \pm 2.5 (7)	37 \pm 2.5 (6)	44 \pm 1.4 (14)	34 \pm 2.0 (7)
After local Anaesthetic	91 \pm 11.0* (7)	64 \pm 8.0 (6)	58 \pm 5.6* (15)	53 \pm 9.0 (7)
Before Biopsy	48 \pm 6.0 (3)	34 \pm 2.4 (5)	42 \pm 1.4 (15)	35 \pm 1.5 (4)
After Biopsy	78 \pm 9.2* (3)	66 \pm 14.0 (5)	61 \pm 6.0* (15)	62 \pm 7.2 (4)

figures in parenthesis indicates number of times each stage of procedure successfully completed

* = $p < 0.05$ (refers to difference from mean pre-tranquillising levels)

Table 7.8 Effects of Biopsy Procedure on Packed Cell Volume

(Mean \pm s.e.)

Stage of Procedure	Control (No Tranquilliser)	Acepromazine	Azaperone	Xylazine
Pre Experiment	37.7 \pm 2.0 (7)	35.2 \pm 1.0 (7)	38.5 \pm 0.8 (15)	37.6 \pm 1.3 (7)
After local Anaesthetic	42.4 \pm 3.5 (7)	28.0 \pm 1.6 (6)	29.1 \pm 1.0* (15)	36.0 \pm 2.8 (7)
After Biopsy	44.0 \pm 3.7 (3)	29.0 \pm 0.5* (5)	29.0 \pm 0.6* (15)	37.2 \pm 2.5 (4)

* = $p < 0.05$ (refers to difference from mean pre-tranquilliser levels)

Figures in parenthesis indicates number of times each stage of the procedure successfully completed

Table 7.9 Effects of Biopsy Procedure on Plasma 11-OHCS

Levels (n mol/l Mean \pm s.e.)

Stage of Procedure	Control (No Tranquilliser)	Acepromazine	Azaperone	Xylazine
Pre Experiment	270 \pm 41 (7)	269 \pm 25 (7)	208 \pm 17 (15)	196 \pm 26 (7)
After Local Anaesthetic	334 \pm 41* (7)	380 \pm 59 (6)	304 \pm 33* (15)	271 \pm 42* (7)
After Biopsy	291 \pm 55 (3)	395 \pm 24 (5)	401 \pm 46* (15)	174 \pm 30 (4)

* = p < 0.05 (refers to difference from mean pre-tranquilliser levels)

Figures in parenthesis indicate number of times each stage of the procedure successfully completed

agitation. Xylazine premedication, in accordance with the observations made on behaviour and heart rate, produced more elevated 11-OHCS levels after injection of the local anaesthetic than after the biopsies.

11-OHCS levels at the end of the operative procedure were higher following acepromazine and azaperone premedication than in the control studies when no tranquillisers were used.

7.5 Discussion

The phenothiazines and the butyrophenones share many pharmacological properties amongst them depression of the brain stem reticular system and vasomotor reflexes and an ability to block the dopamine receptors and thus the transmission of nervous impulses in various areas of the brain. These areas include the hypothalamus, basal ganglia, limbic system and chemoreceptor trigger zone and this action of the two groups of drugs explains many of their psychological and anti-emetic effects (Marsboom, 1971; Goodman and Gilman, 1975).

CLINICAL SIGNS

The clinical signs, onset and duration of tranquillisation recorded in this study following acepromazine and xylazine administration have also been reported previously by workers using similar doses of both drugs (Carey and Sanford, 1963; Aitken and Sanford, 1972; McCashin and Gabel, 1971; Burns and McMullan, 1972; Hoffman, 1974; Tronicke and Voke, 1970). Lees and Serrano (1976) reported similar clinical signs and onset of tranquillisation in ponies following azaperone (0.4 and 0.8 mg/kg intramuscularly) as have been described in this study but they recorded a duration of action of between 4 and 6 hours whereas signs of sedation were still apparent in our horses 24 hours post-injection. This difference could be due to the fact that all the horses concerned were riding animals and the effects noted after 24 hours were visible only as reluctance to exercise and slight hesitation of gait. This

prolonged duration of action of azaperone was not recorded in any of our ponies which would support the suggestion that the effect might not be apparent in restrained animals.

The increased doses of acepromazine and azaperone employed prior to the biopsy procedure (Sections 7.3 and 7.4) did not increase the degree of tranquillisation as assessed by visual signs but lengthened the period of tranquillisation. The fact that azaperone proved to be a more successful tranquilliser than acepromazine in reducing the stress of muscle biopsy (even when acepromazine was administered at five times the recommended equine dose) is similar to the findings of Marsboom and Symoens (1968), and Aitken and Sanford (1972). The former authors reported that azaperone was a more effective tranquilliser than propionylpromazine in the pig and the latter authors that it was more effective than acepromazine in the horse. The low success rate following xylazine premedication is surprising considering the reports of previous workers (Tronicke and Vocke, 1970; Hoffman, 1974; Kerr *et al*, 1972; McCashin and Gabel, 1971; Clarke and Hall, 1969). The lack of adequate sedative effect in our horses could have been due to inadequate dosage via the intramuscular route but this seems unlikely since the dose rates used were those recommended by the manufacturers and found by other workers to produce excellent tranquillisation (Aitken and Sanford, 1972; Burns and McMullan, 1972).

Xylazine has been reported, however, as being more effective in docile animals (Hoffman, 1974) and also to exert greater analgesic properties when administered intravenously than

intramuscularly. Both these effects could explain the lack of success with this drug during the muscle biopsy procedure. The animals in which it was impossible to complete the biopsy technique under xylazine were the more nervous of the seven horses and the use of xylazine intramuscularly might have produced insufficient analgesia for the injection of the local anaesthetic.

The salivation recorded in three of our horses (7.3) has also been recorded previously in cattle by the manufacturers of xylazine and in pigs and horses following azaperone by Marsboom and Symoens, (1968) and Lees and Serrano (1976). The reason for this effect is not known.

The hyperexcitability reaction reported in Horse No. 4 following administration of acepromazine (7.3) has also been recorded by other workers using phenothiazines in horses (Carey and Sanford, 1963; Jones, 1963). The exact cause of this reaction is unknown. Hall (1960) attempted to discover whether this excitement might be due to the hypotension produced by chlorpromazine and Carey and Sanford (1963) also considered this. Neither, however, could demonstrate a reduction in blood pressure coinciding with any excitement phase. Owen and Neal (1957) suggested on the other hand that muscle weakness due to the tranquillising effect of the drug might cause a panic reaction but again there is no evidence of this.

Carey and Sanford (1963) concluded that the phenothiazines might exert some fundamentally different action in certain individual horses but could not suggest what this action might be. If, however, it is some central effect of the drug, since both

acepromazine and azaperone act in similar ways, it is possible that this could explain why the same horse reacted similarly to both agents.

Acepromazine and azaperone produced slight sweating in all the animals. Sweating in the horse is considered to be under the control of the sympathetic nervous system (McEwan-Jenkinson, 1973) and recent work by Anderson and Aitken (1977) suggests that β receptors rather than α receptors are concerned. More detailed investigations by Snow (1977) have also demonstrated that β_2 rather than β_1 receptors are involved.

Acepromazine has been reported by Aitken and Sanford (1972) to potentiate the β -receptor actions of adrenaline on the heart as described by Webster (1966) and Sutherland (1970).

Several α - adrenergic receptor blocking drugs have been shown to increase noradrenaline release from nerve endings (Ehoro, Langer, Rothlin and Stefano, 1972; Cubeddu, Langer and Weiner, 1974) and some phenothiazines have been reported to reduce the uptake of catecholamines into tissues (Hertting, Axelrod and Whitby, 1961). Azaperone also possesses α - adrenergic blocking activity (Marshoorn, 1971; Lees, Tavernor and Hillidge, 1971) and another of the butyrophenone group, droperidol, has been demonstrated to increase circulating noradrenaline levels in cats by stimulation of post-ganglionic sympathetic nerve endings and inhibition of noradrenaline re-uptake (Herbaczynska and Staszewska, 1971). By either of these two mechanisms the end result would be the same as prolonged sympathetic activity. However, because of the α -

adrenergic blocking activities of these two tranquillizers, the net result would be that greatest potentiation will occur on β -receptors thus producing increased sweating.

CARDIOVASCULAR EFFECTS

The mild tachycardia recorded in both sections 7.2 and 7.3 following administration of acepromazine and azaperone has also been reported previously (Gabel, Hamlin, and Smith, 1964; Kerr et al., 1972; Lees and Serrano, 1976) and is thought to be a reflex response to the decrease in blood pressure produced by these drugs. This increase in heart rate occurs in response to signals from the cardioregulatory centre in the brain via the sympathetic system (Rushmer, 1972). Stretch receptors in the aortic arch and carotid sinus are very sensitive to alterations in blood pressure and relay information accordingly to the brain which in turn regulates the heart rate via the parasympathetic (inhibitory) and sympathetic (stimulatory) systems.

In section 7.1 when heart rate and blood pressure were recorded following acepromazine administration tachycardia did occur during the period of hypotension, suggesting that reflex control of the heart was responsible for the changes recorded in these studies. During the muscle biopsy procedure it was interesting to note that tachycardia was not recorded between stages of the operative technique following acepromazine and azaperone premedication.

Xylazine produced bradycardia when administered either intravenously or intramuscularly, the effect occurring more rapidly

following administration by the intravenous route. This action of xylazine has been recorded previously (Hoffman, 1974; Kerr *et al*, 1972; Garner, Amend and Rosborough, 1971; Clarke and Hall, 1969) and is suggested by the manufacturers to be due to a direct action of the drug on the myocardium. Kerr *et al* (1972) however claim that the bradycardia may result from a reflex response to the hypertension they recorded immediately following xylazine administration. This hypertension was present for less than 10 min, the blood pressure thereafter decreasing slowly to levels below resting values, whereas the bradycardia lasted for 60 min. A similar pattern of alteration in heart rate was recorded in these studies suggesting that some direct action of xylazine on the heart or, as described by Schmitt, Fournadjiev and Schmitt (1970), some centrally produced reduction in sympathetic tone might be the cause of the bradycardia.

Pretreatment with all three drugs reduced the changes in heart rate produced by the different stages of the biopsy procedure as compared to the increases recorded in the control studies without premedication. This effect may be due to some central action of these drugs on the hypothalamus involving suppression or blockage of nerve impulses to the pain centres (Guyton, 1971) or as described previously in the case of xylazine to some direct central effect on the heart rate.

Arterial blood pressure was recorded only following administration of acepromazine intravenously (Section 7.2). A fall in blood pressure was recorded similar to that reported by

other workers in dogs (Tavernor, 1962; Popovic, Mullane and Yhap, 1972) and in horses (Gabel et al, 1964; Kerr et al, 1972; Glen, 1972).

The hypotensive effect of acepromazine is attributed to its α - adrenergic blocking action causing a reduction of sympathetic vasoconstriction. Most of the phenothiazines possess α - receptor blocking activity although their potency varies (Bhargava and Chandra, 1964) and acepromazine has been shown to actively antagonise the pressor effect of adrenaline (Serrano and Lees, 1975).

The effects of the three drugs on respiratory rates were monitored only in section 7.2. Acepromazine produced a slight decrease in respiratory rates over a 60 min period and similar results have been recorded in horses by Kerr et al, (1972) and Muir and Hamlin (1975). They attributed this effect to the depressant action of acepromazine, most central nervous depressants producing some degree of respiratory depression, and xylazine produced no changes in respiratory rates, agreeing with the results of Roztocil et al. (1971) and Garner et al, (1971). Lees and Serrano (1976) on the other hand reported significantly increased respiratory rates following 0.8 mg/kg azaperone in ponies and suggested that this could be due to the hypotensive effect of the drug producing stagnant hypoxia causing stimulation of chemoreceptors in the aortic and carotid bodies. Several authors (Clarke and Hall, 1969; McCaslin and Gabel, 1971; Hoffman, 1974) also have reported a decrease in respiratory rate following xylazine administration to horses. The reason for the difference between these results could

be that the animals used in this study were less disturbed during the experiment since Garner et al (1971) and Hoffman (1974) suggested that animals with higher than normal respiratory rates were affected to a greater degree by xylazine administration.

The effects of acepromazine and azaperone on packed cell volumes recorded in experiment 7.3 have also been reported previously and will be discussed in detail in the following chapter of this study. Briefly, however, both drugs produced a fall in packed cell volume. The effects of phenothiazine tranquillisers on packed cell volume in sheep have been recorded by Turner and Hodgetts (1960) and in dogs by Collette and Meriwether (1965). Both groups reported that chlorpromazine lowered venous haematocrit and the latter authors that splenic enlargement also occurred. Aronson, Magora and Landon (1970) reported that droperidol (one of the butyrophenone group) produced a small decrease in venous haematocrit in humans and Roztocil et al (1971) and Lees and Serrano (1976) demonstrated a similar reduction in horses following azaperone.

This fall in packed cell volume has been attributed to the central depressant effects of the drugs (Turner and Hodgetts, 1960), causing splenic relaxation and an accumulation of red cells in the spleen, or to the α - adrenergic blocking action causing relaxation of the splenic capsule and an uptake of red cells from the circulation (Lees and Serrano, 1976). Weil and Chidsey (1968) found that in man sympatholytic agents produce a haemodilution due to an alteration in pre/post capillary resistance resulting in

changes in plasma protein concentrations. Such changes were noted in these studies (see next chapter) but were not recorded by Lees and Serrano (1976) following azaperone administration to ponies. It would appear therefore that the fall in packed cell volume seen following acepromazine and azaperone may be due both to an accumulation of erythrocytes in the spleen and to a lesser extent to haemodilution.

ENDOCRINE EFFECTS

The presence of a circadian variation in plasma 11-OHCS levels as recorded in our horses and by others (Bottoms et al, 1972) makes evaluation of the results in sections 7.3 and 7.4 difficult. By carrying out the procedures at the same times each day and within as short a time as possible it was hoped that these effects would be minimised.

The results in section 7.3 indicate that in the horse, azaperone and possibly acepromazine per se produce an increase in plasma 11-OHCS levels. Reports on the effect of phenothiazines on the secretion of 11-OHCS are contradictory. Mahfouz and Ezz (1958) suggest that chlorpromazine decreases the output of ACTH in response to stressful stimuli Holzbaur and Vogt (1954), Smith et al (1963) and de Wied (1967), however, carried out studies which indicate that chlorpromazine increases 11-OHCS secretion and furthermore that this increase is due to ACTH hypersecretion. Smith et al (1963) also demonstrated that the increases in plasma 11-OHCS levels in rats was directly proportional to the degree of

central activity of the phenothiazines. If this is the case it might explain why azaperone caused higher increases in plasma 11-OHCS levels than acepromazine. The mode of action of both drugs is very similar (Goodman and Gilman, 1975) and they might both affect ACTH secretion in the same manner. This effect of azaperone on ACTH secretion would also help to explain why 11-OHCS levels were greatest when the biopsy in section 7.4 was carried out following azaperone premedication, even although the horses were most tractable during this time.

Xylazine appeared to have no effect on plasma 11 OHCS levels when administered alone (Table 7.5). During the biopsy procedure however it did successfully reduce 11-OHCS values, below those recorded following either acepromazine or azaperone premedication.

If corticosteroid secretion is in some way influenced by increased sympathetic activity then the central action of xylazine decreasing sympathetic tone as described by Schmitt et al, (1970) might result in a decrease in adrenal cortical activity. This central effect might also be most obvious under conditions of increased sympathetic stimulation such as was seen to occur after injection of the local anaesthetic and the muscle biopsies.

Summary

Acepromazine and azaperone reduced the stress of a minor surgical procedure (muscle biopsy) as assessed by changes in heart

rate and packed cell volume, compared to the control experiments when no tranquilliser was used. The effect on heart rate was probably due to the central depressant actions of the drugs reducing the anxiety, apprehension and possibly pain associated with the various procedures whereas the effect on P.C.V. was due to a direct pharmacological action of both drugs. This effect on P.C.V. is investigated in more detail in the following study.

Both these drugs apparently produced elevations in plasma 11-OHCS levels per se suggesting that in this instance measurement of 11-OHCS values is not a useful means of assessing the efficacy of certain tranquillisers.

Xylazine reduced heart rate, both in resting animals and during the biopsy procedure. This effect was possibly due to a central depressant effect of the drug on sympathetic tone. No changes in P.C.V. were recorded following xylazine but a reduction in plasma 11-OHCS levels was apparent during the biopsy.

Of the three drugs used, azaperone proved to be most effective in allowing completion of the surgical procedure in horses of all temperaments.

8. AN INVESTIGATION INTO THE ACTIONS OF CENTRAL,
DEPRESSANT DRUGS ON CIRCULATING PACKED CELL VOLUMES
IN HORSES AND DOGS

8.1 Introduction

The splenic capsule is rich in α - adrenoreceptors and contraction of the splenic capsule occurs in response to increased sympathetic stimulation (Davies and Withrington, 1973).

In the quiet resting animal the splenic capsule is always in a state of partial contraction and the organ functions as a reservoir for red blood cells. In certain species e.g. horses, dogs and sheep this reservoir capacity is large (Schalm et al, 1975). The fall in circulating packed cell volume as recorded following premedication with acepromazine and azaperone in the previous studies could have been due to either:-

- i) local blockade of normal sympathetic activity at the α - adrenoreceptors in the splenic capsule
- ii) a general decrease in sympathetic activity due to central nervous depressant effects of these drugs
- iii) an increase in plasma volume

Several phenothiazine tranquillisers possess α - adrenoreceptor blocking actions (Courvoisier, Fournel, Ducrot, Kolsky and Koetschet, 1953) and a number of the butyrophenones have also been shown to exert this effect (Marsboom, 1971; Lees and Serrano, 1976).

A fall in circulating packed cell volume levels in horses has been recorded following injection of chlorpromazine (Martin and Beck, 1956) and a propionylpromazine/promethazine mixture (De Moor and Van den Hende, 1968). Turner and Hodgetts (1960) reported similar findings in sheep following chlorpromazine administration and

suggested that the fall in circulating red blood cells was due not to the local α - adrenoreceptor blocking actions of this drug on the splenic capsule, but to its central depressant actions.

In this study the effects of acepromazine and azaperone on packed cell volume and total plasma protein concentrations in horses were monitored. Total plasma protein concentrations alter as a result of either increased or decreased plasma volume and the former has been reported to occur following administration of certain tranquillising drugs including acepromazine and chlorpromazine (Gartner, Ryley and Beattie, 1965; Dalton, 1972). This change in plasma volume has been attributed to the α - adrenoreceptor blocking actions of these drugs causing a fall in capillary pressure and a transfer of protein free filtrate from the interstitial spaces into the vascular system.

To try and elucidate the relative importance of the three factors in reducing packed cell volume the following studies were carried out. Acepromazine and azaperone were administered to dogs before and after splenectomy and on one occasion to rabbits (animals known to possess a very small spleen with little or no reservoir capacity (Schalm et al, 1975)).

Finally, a number of drugs known to possess central nervous depressant actions similar to acepromazine and azaperone and a specific α - adrenoreceptor blocking agent (phenoxybenzamine) were administered to dogs in order to establish whether or not packed cell volume was affected by the central rather than the local actions of these drugs.

8.2 THE EFFECTS OF VARYING DOSES OF ACEPROMAZINE AND AZAPERONE
ON THE PACKED CELL VOLUMES AND TOTAL PLASMA PROTEIN
CONCENTRATIONS IN PONIES

Animals

All three ponies were used (Nos. 1, 2 and 3).

Methods

Packed cell volumes were measured as described previously.

Blood (5 ml) was taken into a heparinised plastic tube and 1 ml. plasma removed for total plasma protein estimation. These measurements were made on a Technicon Autoanalyser using the standard AAI - 14 method, which is based on the Biuret technique.

Signs of tranquillisation were assessed as before.

The Coefficient of Variation of this assay was 5%.

Drugs

Acepromazine at dose rates of 0.5, 0.25, 0.12 and 0.06 mg/kg and azaperone at dose rates of 0.7, 0.35, 0.18 and 0.09 mg/kg were injected intramuscularly into the three ponies on separate occasions.

Experimental Procedure

Blood samples for P.C.V. and plasma total protein estimation

were taken before the experiment and at 15, 30, 60, 90 and 120 min after drug injection.

Onset and degree of central depression produced were assessed visually in each animal.

A period of at least 5 days was allowed between experiments.

Results

BEHAVIOURAL SIGNS

Both drugs produced similar clinical signs which included drooping of the head, closing of the eyes, relaxation of the penis and reluctance to move at all but the lowest dose rates (0.06 mg/kg acepromazine and 0.09 mg/kg azaperone). The signs of central depression were apparent within 40 min following azaperone injection and between 45 - 60 min following acepromazine injection and were still visible after both drugs at the end of the 2 hour period.

PACKED CELL VOLUME (TABLES 8.1 AND 8.2)

All four doses of each drug produced a decrease in P.C.V. within 15 min of injection. The greatest changes in all cases occurred between 60 and 120 min after administration of the tranquilliser when levels on one occasion decreased by 21% below control (pre-injection) values. Decreasing doses of both drugs did not alter the effects on P.C.V. levels.

TOTAL PLASMA PROTEIN (TABLE 8.1 AND 8.2)

The responses to both drugs were varied but a slight decrease in total plasma protein (T.P.P.) was recorded especially between 60

Table 8.1 Decreases in P.C.V. and T.P.P. levels expressed as a percentage of resting (pre-injection) values.

Mean values in three ponies following acepromazine administration

Dose of Acepromazine	0.5 mg/kg		0.25 mg/kg		0.12 mg/kg		0.05 mg/kg	
	P.C.V.	T.P.P.	P.C.V.	T.P.P.	P.C.V.	T.P.P.	P.C.V.	T.P.P.
15 min Post inj.	-7	+1	-12	0	-16	-1	-10	+1
30 min Post inj.	-10	-1	-12	+1	-13	-1	-17	+2
60 min Post inj.	-14	-5	-24	-3	-19	-1	-20	+1
90 min Post inj.	-14	-2	-18	-6	-19	-4	-20	0
120 min Post inj.	-20	-6	-24	-5	-19	-4	-20	-3

Table 8.2 Decreases in P.C.V. and T.P.P. levels expressed as a percentage of resting (pre-injection) values.

Mean values in three ponies following azaperone administration

Dose of Azaperone	0.7 mg/kg		0.35 mg/kg		0.18 mg/kg		0.09 mg/kg	
	P.C.V.	T.P.P.	P.C.V.	T.P.P.	P.C.V.	T.P.P.	P.C.V.	T.P.P.
15 min Post inj.	-12	*	-6	-1	-12	0	-8	-1
30 min Post inj.	-15	-5	-10	-4	-15	-1	-10	-2
60 min Post inj.	-15	-14	-19	-6	-18	-1	-12	-2
90 min Post inj.	*	*	-16	-8	-12	-1	-14	-1
120 min Post inj.	-13	-8	-19	-6	-12	-1	-12	-3

*** = levels not recorded

and 120 min after injection. There was no marked difference between the 2 drugs except following the highest dose of azaperone (0.7 mg/kg) when a 14% drop in T.P.P. was recorded. In all cases levels were still decreased at the end of the 2 hour period.

8.3 THE EFFECT OF ACEPROMAZINE AND AZAPERONE ON PACKED CELL
VOLUME AND TOTAL PLASMA PROTEIN LEVELS IN DOGS

Animals

Four male greyhounds were used in this experiment. All the dogs weighed between 20 and 30 kg.

Methods

Blood samples for packed cell volume and total plasma protein estimation were taken by venepuncture from right and left cephalic veins alternately into heparinised tubes.

P.C.V. and total plasma proteins were estimated as described previously.

Tranquillisation was assessed visually using a scoring system:-

- + animal on feet throughout 2 hour period
- ++ animal recumbent between blood samples but on feet when disturbed
- +++ animal recumbent throughout 2 hour period and undisturbed by blood sampling

Drugs

Both drugs were administered intravenously, acepromazine at

dose rates of 0.25 and 0.5 mg/kg and azaperone at 0.5 mg/kg.

Experimental Procedure

Before commencing the experiment, the dogs were allowed to rest quietly for 1-2 hours in order to ensure that P.C.V. levels recorded were those of nonexcited animals.

Acepromazine was administered to all four dogs on two separate occasions once at a dose rate of 0.25 mg/kg and once at 0.5 mg/kg. The effects of both dose rates on rate of onset of effect, degree of central nervous depression and P.C.V. were recorded.

Azaperone was administered to the same four dogs and similar recordings made. Blood samples were always taken from the dogs at rest prior to drug administration and 15, 30, 60, 90 and 120 min later.

A period of at least 3 days was allowed between experiments and longer if resting P.C.V. levels had not returned to within the range of original control values.

Results

1. Acepromazine administration (0.25 and 0.5 mg/kg intravenously)

BEHAVIOURAL SIGNS (TABLE 8.3)

The onset of action of both doses of acepromazine occurred 5-10 min after injection and the effects lasted throughout the 2 hour period.

Table 8.3 Degree of Central depression produced by administration of various drugs in dogs

Drug	Degree of Central Depression
Acepromazine	+++
Azaperone	+++
Xylazine	+++
Diazepam	+
Chloral Hydrate	+++
Phenoxybenzamine	++

+ animal on feet throughout 2 hour period

++ animal lying down between blood samples but on feet when disturbed

+++ animal lying down all the time and undisturbed by blood sampling

PACKED CELL VOLUME (TABLE 8.4)

There was no apparent difference between the effects of the two dose rates on P.C.V. levels. In both cases, values were significantly below pre-injection levels by 15 min ($p < 0.02$) and by 120 min were even more depressed ($p < 0.001$).

TOTAL PLASMA PROTEIN (TABLE 8.5)

Total plasma protein levels were significantly below resting (pre-injection) values by 30 min ($p < 0.05$) and remained low throughout the remainder of the 2 hour period.

2. Azaperone administration (0.5 mg/kg intravenously)

BEHAVIOURAL SIGNS

The onset of action following azaperone was similar to that recorded after acepromazine. By 10 min post-injection the dogs were recumbent and remained thus throughout the 2 hour period.

PACKED CELL VOLUME (TABLE 8.6)

P.C.V. levels were significantly decreased ($p < 0.05$) throughout the 2 hour period. Percentage decreases were slightly greater than following the same dose (0.5 mg/kg) of acepromazine (Table 8.7).

TOTAL PLASMA PROTEIN (TABLE 8.6)

Total plasma protein levels were also decreased throughout the 2 hour period and were significantly below pre-injection values between 60 and 120 min post drug administration. Percentage decreases were slightly greater in this case than those recorded

Table 8.4 Packed Cell Volumes (%) in 4 dogs (Mean \pm s.e.) after acepromazine injection at 2 dose rates (0.25 and 0.5 mg/kg intravenously)

Stage of Procedure	Acepromazine	
	0.5 mg/kg i/v	0.25 mg/kg i/v
Pre Injection	57 \pm 1.9	58 \pm 1.1
Post 15 min	51 \pm 1.2*	53 \pm 1.1*
Post 30 min	49 \pm 1.2*	48 \pm 1.4*
Post 60 min	48 \pm 1.9*	48 \pm 0.8*
Post 90 min	47 \pm 1.6*	46 \pm 1.0*
Post 120 min	47 \pm 1.3*	47 \pm 1.2*

* refers to levels significantly ($p < 0.01$) below resting (pre-drug) values

Table 8.5 Total Plasma protein levels (g/litre) on 4 dogs (mean \pm s.e.) after acepromazine injection at two dose rates (0.25 and 0.5 mg/kg)

Stage of Procedure	Acepromazine	
	0.5 mg/kg i/v	0.25 mg/kg i/v
Pre Injection	58 \pm 2.5	60 \pm 2.4
Post 15 min	55 \pm 1.9	57 \pm 1.3
Post 30 min	53 \pm 1.4*	57 \pm 1.4*
Post 60 min	54 \pm 1.5*	55 \pm 1.9*
Post 90 min	54 \pm 1.9*	56 \pm 1.6*
Post 120 min	53 \pm 1.6	55 \pm 2.0*

* refers to figures significantly ($p < 0.05$) below resting (pre-drug) values

Table 8.6 P.C.V. and Total Plasma Protein Levels in 4 dogs (Mean \pm s.e.) after intravenous injection of azaperone (0.5 mg/kg)

Procedure	Packed Cell Volume (%)	Total Plasma Protein (g/litre)
Pre injection	60 \pm 4.6	63 \pm 6.2
Post 15 min	53 \pm 1.2*	59 \pm 5.5
Post 30 min	50 \pm 1.3*	58 \pm 5.7
Post 60 min	48 \pm 2.1*	56 \pm 6.6*
Post 90 min	49 \pm 3.5*	57 \pm 6.2*
Post 120 min	49 \pm 0.9*	59 \pm 5.3*

* refers to figures significantly ($p < 0.05$) below resting (pre-drug) values

following acepromazine injection (Table 8.7).

Table 8.7

The Decrease in P.C.V. and T.P.P. in 4 dogs following acepromazine and azaperone administration intravenously expressed as a percentage of pre experiment values

Stage of Procedure	Acepromazine (0.5 mg/kg)		Azaperone (0.5 mg/kg)	
	PCV (%)	T.P.P. (g/litre)	PCV (%)	T.P.P. (g/litre)
15 min Post Injection	-10	-5	-12	-6
30 min Post Injection	-14	-9	-17	-8
60 min Post Injection	-16	-7	-20	-11
90 min Post Injection	-18	-7	-18	-10
120 min Post Injection	-18	-9	-18	-6

8.4. THE EFFECT OF SPLENECTOMY ON THE ACTION OF ACEPROMAZINE
ON PACKED CELL VOLUMES AND TOTAL PLASMA PROTEIN
LEVELS IN DOGS

Animals

The four greyhounds used in experiment 2 were included in this study.

Methods

Packed cell volumes and total plasma protein levels were estimated in all blood samples as described previously.

Drugs

Acepromazine was administered intravenously at dose rates of 0.25 mg/kg since previous work had shown no apparent difference between the effects on P.C.V. of this and 0.5 mg/kg dose.

Experimental Procedure

Blood samples for P.C.V. and T.P.P. estimation were collected from all the dogs at rest prior to acepromazine injection and at 15, 30, 45, 60, 90 and 120 min intervals following this.

The dogs were splenectomised under halothane anaesthesia and were allowed 3 weeks to recover before the same procedure was

repeated. Resting P.C.V. levels were slightly lower in all the splenectomised dogs.

Results

1. Pre Splenectomy

BEHAVIOURAL SIGNS

The onset and signs of tranquillisation were as described in the previous experiment.

PACKED CELL VOLUME (TABLE 8.8)

After tranquillisation levels fell significantly ($p < 0.02$) below resting values and remained decreased throughout the 120 min period.

TOTAL PLASMA PROTEIN (TABLE 8.8)

T.P.P. levels fell gradually after tranquillisation and by 45 min post-injection were significantly ($p < 0.05$) below resting values. They remained decreased throughout the remainder of the 2 hour period.

2. Post Splenectomy

BEHAVIOURAL SIGNS

The onset and signs of tranquillisation similar to those described before splenectomy.

Table 8.8 P.C.V. and T.P.P. values in 4 dogs (Mean \pm s.e.) before and after removal of the spleen and following acepromazine administered intravenously (0.25 mg/kg)

Stage of Procedure	P.C.V. (%)		T.P.P. (g/litre)	
	Pre Splenectomy	Post Splenectomy	Pre Splenectomy	Post Splenectomy
Pre	55 \pm 0.7	51 \pm 0.3	63 \pm 1.3	63 \pm 1.9
15 min Post inj.	49 \pm 1.1*	50 \pm 0.6	60 \pm 2.8	62 \pm 3.8
30 min Post inj.	48 \pm 0.5*	50 \pm 0.4	60 \pm 1.2	61 \pm 1.8
45 min Post inj.	47 \pm 0.6*	50 \pm 0.4	57 \pm 1.5*	61 \pm 0.8
60 min Post inj.	47 \pm 0.6*	50 \pm 0.2	58 \pm 1.6*	59 \pm 2.2
90 min Post inj.	46 \pm 2.2*	51 \pm 0	59 \pm 2.9	62 \pm 0.6
120 min Post inj.	45 \pm 0.5	50 \pm 0.5	57 \pm 0.6*	62 \pm 2.3

* refers to levels significantly below control values ($p < 0.05$)

PACKED CELL VOLUME (TABLE 8.8)

P.C.V. levels remained constant throughout the 120 min.

TOTAL PLASMA PROTEIN (TABLE 8.8)

Plasma protein levels had fallen by 8% 60 min after acepromazine injection but had returned to almost resting values by the end of the 2 hour period.

8.5 THE EFFECTS OF ACEPROMAZINE AND AZAPERONE ADMINISTRATION
ON PACKED CELL VOLUME AND TOTAL PLASMA PROTEIN
LEVELS IN RABBITS

Animals

A white male rabbit weighing 3.5 kg was used in this study.

Methods

P.C.V. and T.P.P. values were determined as described previously.

Drugs

Acepromazine and azaperone were administered intramuscularly at dose rates similar to those used previously in dogs i.e. 0.5 mg/kg.

Experimental Procedure

Blood samples for P.C.V. and T.P.P. estimations were collected from the ear vein prior to drug administration and 15, 30, 60, 90 and 120 min later.

Results

PACKED CELL VOLUME (TABLE 8.9)

Following both drugs there was a slight decrease in P.C.V. levels, lowest values being recorded between 30 and 90 min post-injection (-8% in both cases below resting values).

TOTAL PLASMA PROTEIN (TABLE 8.9)

T.P.P. levels fell markedly following both drugs throughout the 2 hour period. Lowest values were recorded between 60 and 120 min (-17% following acepromazine and -34% following azaperone below resting values).

Table 8.9 P.C.V. and T.P.P. levels in one rabbit following intramuscular premedication with acepromazine and azaperone

Stage of Procedure	Acepromazine (0.5 mg/kg)		Azaperone (0.5 mg/kg)	
	PCV (%)	T.P.P. (g/litre)	PCV (%)	T.P.P. (g/litre)
Pre Injection	39	57	37	63
15 min Post Injection	37	51	35	49
30 min Post Injection	36	49	34	45
60 min Post Injection	36	52	34	51
90 min Post Injection	36	50	34	53
120 min Post Injection	37	47	35	41

THE EFFECTS ON PACKED CELL VOLUMES IN DOGS OF SEVERAL

8.6 THE EFFECTS ON PACKED CELL VOLUMES IN DOGS OF SEVERAL

CENTRAL DEPRESSANT DRUGS AND OF A SPECIFIC

α - ADRENORECEPTOR BLOCKING DRUG.

Animals

A separate group of four male greyhounds, all weighing between 20 and 30 kg. were used in this study.

Methods

Packed cell volumes and total plasma protein concentrations were measured as described previously.

Degree of tranquillisation where relevant was assessed using the visual score as in sections 8.3 and 8.4.

Drugs

Xylazine (10%) Bayer Agrochemical Ltd.

Chloral Hydrate (110% : w/v) May and Baker Ltd.

Valium : diazepam (5 mg/ml) Roche

Phenoxybenzamine hydrochloride Smith, Kline and French Ltd.

The drug (300 mg) was dissolved in absolute alcohol (11.9 ml), propylene glycol (15.6 ml) and N/1 hydrochloric acid (0.5 ml) to give a solution of 10.7 mg/ml.

Chloral hydrate was administered at a dose rate of 80 mg/kg

by slow intravenous injection (over a period of 3 min) in a volume of 20 ml. sterile saline, diazepam at 2 mg/kg by intravenous injection, xylazine at 3 mg/kg by intramuscular injection and phenoxybenzamine at 2 mg/kg by slow intravenous injection (over 2 min) in 20 ml. sterile saline.

Experimental Procedure

Samples for P.C.V. and T.P.P. estimation were taken from the dogs at rest before commencing the experiment.

Each drug was administered to all four dogs on separate occasions and blood samples were collected at 15, 30, 45, 60, 90 and 120 min after drug injection.

A period of seven days was always allowed between drug administrations.

Results

BEHAVIOURAL SIGNS (TABLE 8.3)

The onset of action was slower following xylazine administration, 15-20 min as compared to less than 5 min following chloral hydrate. The behavioural signs produced by both drugs were however similar to those seen after acepromazine and azaperone. Diazepam on the other hand produced none of the behavioural signs seen following the other drugs. Three of the four dogs receiving diazepam became markedly ataxic within 20 min of injection and began to struggle. This behaviour lasted between 15-20 min but the animals continued to be

unsteady on their feet throughout the two hour period especially when disturbed for blood sampling.

Phenoxybenzamine produced slight signs of tranquillisation in all the dogs similar to those seen following xylazine, chloral hydrate, acepromazine and azaperone. These effects lasted throughout the two hour period.

PACKED CELL VOLUME (TABLE 8.10)

P.C.V. levels remained unchanged throughout the two hours following xylazine administration and appeared to decrease slightly between 60 and 120 min after diazepam injection. Following chloral hydrate the levels were significantly below resting (pre-injection) values between 30 and 60 min ($p < 0.05$) but had returned towards control levels by the end of the two hour period.

After phenoxybenzamine injection P.C.V. values fell significantly ($p < 0.001$) within 15 min and remained decreased throughout the experiment.

TOTAL PLASMA PROTEIN (TABLE 8.11)

T.P.P. levels decreased slightly following diazepam injection and significantly ($p < 0.05$) 60 min after chloral hydrate administration. Following phenoxybenzamine levels also dropped and were significantly below resting values between 30 and 90 min after injection ($p < 0.02$).

There were no alterations in T.P.P. levels following xylazine premedication.

Table 8.10 Changes in Packed Cell Volumes (%) in 4 dogs (mean \pm s.e.) following treatment with xylazine diazepam, chloral hydrate and phenoxybenzamine

Stage of Procedure	Xylazine 3 mg/kg i/m	Diazepam 2 mg/kg i/v	Chloral Hydrate 80 mg/kg i/v	Phenoxybenzamine 2 mg/kg i/v
Pre Inj.	59.8 \pm 3.3	60.7 \pm 3.1	59.6 \pm 2.3	59 \pm 1
15 min Post Inj.	59.8 \pm 3.0	60.7 \pm 3.5	58.8 \pm 2.4	50 \pm 1.3**
30 min Post Inj.	62.0 \pm 4.0	60.3 \pm 2.1	57.2 \pm 2.2*	47 \pm 2.1***
60 min Post Inj.	59.5 \pm 4.3	60.0 \pm 3.5	56.2 \pm 2.6*	42 \pm 1.3**
90 min Post Inj.	60.2 \pm 4.6	58.0 \pm 3.6	58.0 \pm 1.8	44 \pm 1.4***
120 min Post Inj.	61.0 \pm 3.3	58.7 \pm 4.9	58.9 \pm 2.4	42 \pm 2.7**

* refers to figures significantly (p < 0.05) below resting pre-drug values

** refers to figures significantly (p < 0.01) below resting pre-drug values

Table 8.11 Total plasma protein concentrations (g/litre) in 4 dogs (mean \pm s.e.) following treatment with xylazine, diazepam, chloral hydrate and phenoxybenzamine

Stage of Procedure	Xylazine 3 mg/kg i/m	Diazepam 2 mg/kg i/v	Chloral Hydrate 80 mg/kg i/v	Phenoxybenzamine 2 mg/kg i/v
Pre Inj.	60 \pm 3.6	58.7 \pm 3.5	60.0 \pm 2.0	58 \pm 2.4
15 min Post Inj.	61.2 \pm 3.9	59.3 \pm 1.5	61.3 \pm 2.3	56 \pm 1.3
30 min Post Inj.	61.5 \pm 2.6	58.7 \pm 1.2	59.0 \pm 1.0	53 \pm 1.2 ^{**}
60 min Post Inj.	61.5 \pm 5.5	58.0 \pm 2.6	56.3 \pm 1.5 [*]	51 \pm 1.0 ^{**}
90 min Post Inj.	60.3 \pm 3.2	57.0 \pm 2.0	56.0 \pm 1.0	50 \pm 1.2 ^{**}
120 min Post Inj.	61.3 \pm 3.8	57.3 \pm 2.5	57.0 \pm 2.6	49 \pm 1.6

* refers to figures significantly ($p < 0.05$) below resting pre-drug values

** refers to figures significantly ($p < 0.02$) below resting pre-drug values

8.7 Discussion

From these studies, it would appear that the fall in P.C.V. levels recorded following premedication with certain drugs is due either to uptake of red cells by the spleen or to a haemodilution effect since on most occasions a decrease in P.C.V. was accompanied by a fall in T.P.P. levels. Both effects could be accounted for by local α - adrenoreceptor blockade brought about by the drugs or to a centrally induced reduction in sympathetic activity.

The smooth muscle of the splenic capsule in the majority of species is rich in α - adrenoreceptors (Davies and Withrington, 1973) and the contractions induced by adrenaline are mediated through these receptors.

Lippay, Mitchell and Potter (1950) first described reversal of adrenaline-induced contractions of the splenic capsule in cats using yohimbine, dibenamine and the ergot alkaloids. More recently, Dooley, Hecker and Webster (1972) described the effects of excitement and adrenaline on the splenic capsule in sheep. They recorded decreases in the thickness of the spleen following both adrenaline and excitement and also that the increase in venous haemoglobin concentration which followed splenic contraction returned to control levels after the spleen thickness had been restored to resting values.

Torten and Schalm (1964) and Persson (1967) and Persson, Ekman, Lydin and Tufuesson (1973) demonstrated an increase in venous haematocrit in horses following exercise and adrenaline administration. The increase after the latter has been shown to be due to release of

red blood cells from the spleen since Persson et al (197?) showed that this was abolished by splenectomy of the animals. Turner and Hodgetts (1959) made similar recordings in sheep following exercise and adrenaline administration.

The effects of various phenothiazine tranquillisers in reversing the adrenaline-induced rise in P.C.V. have also been reported. Turner and Hodgetts (1960) demonstrated this effect following chlorpromazine (0.46 to 0.7 mg/kg) in sheep, and De Moor and Van den Hende (1968) noted a similar effect in horses and cattle following injection of a mixture of propionylpromazine and promethazine although in this case they reported that there was no change in plasma volume. In cattle Gartner et al (1965) recorded decreases in venous haematocrit after chlorpromazine injection and noted that these changes were less in splenectomised animals. Collette and Meriwether (1965) reported that chlorpromazine, propionylpromazine and promazine administered separately to dogs all reduced the venous haematocrit and caused splenic enlargement which was visible on X-ray.

Less work has been carried out using the butyrophenone tranquillisers but a fall in venous haematocrit has been reported following droperidol administration in man (Aronson, Magora and Landon, 1970). A fall in haematocrit has also been recorded in ponies following azaperone injection (Lees and Serrano, 1976; Serrano and Lees, 1976).

Results of the current study indicate that acepromazine and azaperone both produce decreases in P.C.V. in ponies and dogs and

that in the range of doses employed in this study these effects appear to be independent of dose rate. Decreasing doses of both drugs produced reductions in P.C.V. levels in ponies even when no obvious central depressant effects were visible (0.06 mg/kg acepromazine and 0.09 mg/kg azaperone) and similar findings were recorded following two doses of acepromazine in the dogs. This suggests that the mechanism producing the alteration in P.C.V. is not related to the central depressant activity of the drugs as has been previously reported, (Turner and Hodgetts, 1960) and this aspect of the study will be discussed in more detail later.

As well as blocking the α - adrenoreceptors locally in the splenic capsule, acepromazine and azaperone will block peripheral sympathetic vasoconstrictor receptors and produce a slight decrease in blood pressure. This effect in turn could lead to an alteration in plasma volume brought about by the fall in capillary pressure and a transfer of protein free filtrate from the interstitial spaces into the vascular system (Dalton, 1972).

Increases in plasma volume have been reported in man following treatment with guanethidine, and adrenergic neuron blocking agent and phenoxybenzamine a specific α - adrenergic receptor blocker (Weil and Chidssey, 1968). Alterations in plasma volume have also been previously reported following administration of certain tranquillisers. Turner and Hodgetts (1960) recorded that plasma dilution caused by a water shift across the capillary bed accounted for only a small part of the fall in haematocrit produced by chlorpromazine injection in sheep and De Moor and Van den Hende

(1968) reported no change in plasma volume in horses after administration of a mixture of propionylpromazine and promethazine. Gartner et al (1965) on the other hand recorded a fall in serum protein concentration following tranquillisation with chlorpromazine in cattle and Dalton (1972) reported decreases in plasma protein concentrations and venous haematocrit in horses after acepromazine injection.

In these studies, both acepromazine and azaperone produced decreases in T.P.P. concentrations, the greater of these reductions occurring following injection of azaperone (0.7 mg/kg to the ponies and 0.5 mg/kg to the dogs). This is interesting since Lees and Serrano (1976) reported no similar changes in ponies after dose rates of 0.4 mg/kg azaperone and only a small drop (1.3%) in T.P.P. concentrations after 0.8 mg/kg. There is however no apparent reason for the difference between the results of Lees and Serrano (1976) and those recorded in this study.

The decrease in T.P.P. levels recorded in this study suggest that haemodilution could be responsible to some extent for the fall in P.C.V. noted following both acepromazine and azaperone administration. However as the reduction in P.C.V. was in both cases much greater than the fall in T.P.P. this would suggest that some other mechanism such as relaxation of the splenic capsule must also be involved.

Azaperone produced slightly more marked effects on both P.C.V. and T.P.P. than acepromazine suggesting that of the two drugs the former possesses the greater α - adrenoceptor blocking activity.

Serrano and Lees (1976) however recorded that azaperone (0.8 mg/kg) was less effective than acepromazine (0.1 mg/kg) in suppressing the pressor effects of adrenaline although it was the more potent hypotensive agent of the two. They concluded therefore that azaperone might also exert a centrally mediated depression of vasomotor tone. This might explain the greater effects on P.C.V. and plasma protein concentrations recorded in our animals after the higher doses of azaperone (0.7 mg/kg). Both acepromazine and azaperone share many pharmacological actions (chapter 7) and it is possible therefore that both drugs will exert some degree of central depression of the vasomotor centre. The exact mode of action of this effect is unknown.

Further evidence that the spleen is involved in producing the reductions in P.C.V. levels found is provided by the experiment on splenectomised dogs which showed that removal of the spleen abolished the effects of acepromazine on P.C.V. In addition the experiment on the rabbit, known to have a very small splenic reserve, showed that acepromazine and azaperone produced only small decreases in P.C.V. The alterations which did occur in the latter experiment were probably due to a haemodilution effect since T.P.P. levels also decreased quite markedly probably due either to peripheral or central actions of the drugs on sympathetic tone.

Turner and Hodgetts (1960) suggested that the effect of tranquilliser drugs in producing a fall in P.C.V. was due not to their α - adrenergic receptor blocking activity but to their central depressant action. They maintained that an animal in the

normal resting state is always under a certain degree of sympathetic stimulation and that by administration of these drugs this adrenergic tone in the splenic capsule was removed and consequently that the packed cell volume would decrease.

The three drugs investigated in experiment 4 were chosen for their potential central depressant qualities and lack of adrenergic blocking actions. All have been used previously in animals to produce various stages of tranquillisation or narcosis at dose rates similar to or above those used in this study (Lumb and Jones, 1973).

In this study diazepam (2 mg/kg) failed to produce obvious signs of sedation in any of the dogs. This is interesting since Karol, Brown and Sletten (1968) reported behavioural changes in dogs following 1 mg/kg and Dodman (personal communication) also recorded sedative effects in dogs at this dose level. The benzodiazepines in general (including diazepam) have been found to produce some degree of muscle relaxation and ataxia in man and this has been attributed to a potent central effect (Greenblatt and Shader, 1973; Goodman and Gilman, 1975). This action would therefore explain the ataxia recorded in three of these dogs. It is possible that there could be a breed difference in susceptibility to these agents - previous workers have used mongrel dogs whereas in this study greyhounds were used.

The sedative effects of both xylazine and chloral hydrate on the other hand were similar to those reported by other workers in dogs (Lumb and Jones, 1973) and also to those of acepromazine and azaperone recorded in sections 8.3 and 8.4.

Apart from depressant actions similar to acepromazine and azaperone only chloral hydrate produced a fall in P.C.V. and plasma protein concentrations similar to those recorded in our earlier studies (sections 8.3 and 8.4). This was probably due to the central depressant action of this drug on the vasomotor centre resulting in a fall in blood pressure. The hypotensive effect of chloral hydrate is normally associated with anaesthetic doses of the drug (Goodman and Gilman, 1975; Jamb and Jones, 1973). The loss of vasomotor tone would also account for the fall in plasma protein concentrations recorded with this drug. These could be caused by a transfer of fluid into the vascular system across the capillary membranes (Dalton, 1972). The percentage decrease in P.C.V. compared to that in plasma protein concentrations at 60 min post injection was very similar (6% and 7% respectively) suggesting that increased plasma volume was responsible for the fall in haematocrit.

Diazepam produced a slight decrease in both P.C.V. and plasma protein concentrations but this alteration was very small. In man the benzodiazepines are generally considered to exhibit few haemodynamic properties (Greenblatt and Shader, 1973). They have however been reported to produce transient hypotension in conscious and anaesthetised dogs (Abel, Reis and Staroscik, 1970; Bloor, Leon and Walker, 1973) and these effects have been attributed to some direct action of the drugs (particularly diazepam) on post ganglionic adrenergic and cholinergic vasodilating mechanisms. If this is the case then a transfer of fluid into the vascular

system as a result of the lowered capillary pressure could have been the cause of the slight alterations in P.C.V. and plasma protein concentrations recorded in this study.

Xylazine was the only one of the three drugs which did not produce any effect on either of the parameters monitored. This is interesting since it is reported to have central inhibitory effects on the vasomotor centres of the brain stem in anaesthetised dogs and cats (Schmitt et al, 1970) and has been recorded to produce a fall in P.C.V. in cattle (De Moor and Desmet, 1971). The dose rates administered in this study were higher than any of those used by previous workers suggesting that if anything the response in our dogs should have been more marked. In any of our experimental work however involving the use of xylazine in horses we have not recorded any alterations in either P.C.V. or plasma protein concentrations and it would appear that further detailed studies into the hypotensive effects of this drug are required.

From the results in this experiment it has been shown that the fall in P.C.V. recorded following acepromazine and azaperone is not due to their central depressant effects but to an α - adrenergic receptor blocking action. In order to complete this investigation phenoxybenzamine (a specific α - adrenergic receptor blocking agent) was administered to the same four dogs. It was intended to demonstrate with this drug similar decreases in P.C.V. to those recorded following acepromazine and azaperone. Although phenoxybenzamine did produce highly significant decreases in both P.C.V. and plasma protein concentrations it also produced a degree

of central nervous depression only slightly less than that recorded following acepromazine, azaperone, xylazine and chloral hydrate. This central depressant action of phenoxybenzamine has been recorded previously in man (Lewis, 1971) and is considered to be due not to the α - adrenergic blocking properties of the drug but to some non-specific central effect. Because of this central depressant effect however it was impossible to directly compare the α - adrenoreceptor blocking properties of phenoxybenzamine with those of acepromazine and azaperone without the possibility of central actions being involved.

Summary

It would appear that the α - adrenoreceptor blocking actions of acepromazine and azaperone are responsible to a great extent for the reduction in P.C.V. produced by both drugs. These effects are probably mediated through relaxation of the splenic capsule and to a lesser degree by haemodilution due to peripheral reduction of vasomotor tone. Whether or not central reduction of sympathetic tone is also involved is not certain but the effects do not appear to be linked to the central depressant actions of the drugs as assessed by the behavioural changes in the animals concerned.

9. NEUROLEPTANALGESIA

9.1 Introduction

In the past fifteen years a number of new drug combinations have been introduced to produce a state of neuroleptanalgesia in various animal species. Neuroleptanalgesia is a state of profound central nervous depression and analgesia (Lumb and Jones, 1973). The drug mixtures which result in this effect combine a narcotic analgesic with a tranquilliser and a number of different narcotic analgesics and tranquillisers have been used in combination.

In the veterinary field the most commonly used narcotic analgesic in the United Kingdom is etorphine hydrochloride. This is one of a group of derivatives of 6.14 - endo-etheno-tetrahydro-oripavine reported to have analgesic properties 5,000 - 10,000 times greater than morphine on a weight basis (Lister, 1964; Blane, Boura, Fitzgerald and Lister, 1967). The oripavines have structural similarity to morphine and are all derivatives of thebaine, an opium alkaloid with no therapeutic activity (Lister, 1964). The oripavines however and especially etorphine have been shown to exert pharmacological effects on laboratory animals which are very similar to those of morphine (Blane et al, 1967). At low doses they produce analgesia, depression of the respiratory and cough centres and inhibition of gastrointestinal motility - all signs characteristic of morphine like drugs. At higher doses however they will eventually produce recumbency (Lister, 1964) probably due to increased central depressant effects although other actions such as muscle relaxation may also be involved (Lees and Hillidge, 1976).

Etorphine has been widely used for the capture and restraint of wild animals, its characteristics of very high potency and therefore small volume required making it ideal for this purpose (Harthoorn and Bligh, 1965; Pienaar, Van Niekerk, Young, Van Wyk and Fairall, 1966; Keep and Keep, 1967; Wallach, Frueh and Lentz, 1967). Etorphine has been combined with a number of butyrophenone and phenothiazine tranquillisers to produce neuroleptanalgesia² (Harthoorn, 1965; Keep and Keep, 1967).

A considerable advantage of neuroleptanalgesic combinations is that the actions of the narcotic analgesic can be reversed. Blane et al., (1967) have shown that nalorphine will antagonise the effects of etorphine as readily as those of morphine. However the N - cyclopropylmethyl - analogue of etorphine (diprenorphine) shows enhanced activity as an antagonist over nalorphine in similar fashion to etorphine over morphine with the result that the antagonist supplied with etorphine can be given in equally small doses.

It is interesting that the effects of etorphine vary considerably between species. Harthoorn (1965) recorded the effects of etorphine at various dose rates ranging from those just sufficient to abolish postural reflexes to those high enough to produce deep narcosis in domestic animals. He reported an increase in heart rate in donkeys and cattle, a decrease in respiration in donkeys and goats and a complete abolition of postural reflexes in donkeys and horses resulting in the animals falling to the ground. Blane et al. (1967) on the other hand working with laboratory species

described bradycardia and hypotension in rats, dogs, cats and monkeys and attributed these effects to a morphine - type-induced increase in vagal tone.

A combination of etorphine and acepromazine was introduced ('Immobilon' : Large Animal) about ten years ago. Preliminary investigations carried out by Hillidge and Lees (1974) and results reported by other workers (Daniel and Ling, 1972; Jenkins, Crooks, Charlesworth, Blane and Ling, 1972; Schlarmann, Gorlitz, Wintzer and Frey, 1973) showed that 'Immobilon' administered to horses produced marked increases in heart rate, blood pressure, muscle activity and muscle tone throughout the period of recumbency along with respiratory depression.

In this study the effects of etorphine alone on the cardiovascular, respiratory and endocrine systems were assessed, also the modifications if any which occurred in these actions following administration of various tranquillisers in combination with etorphine.

9.2 THE EFFECTS OF ETORPHINE AND 'IMMOBILON' ON MUSCLE ACTIVITY
AND TONE, HEART AND RESPIRATORY RATES, BLOOD PRESSURE, P.C.V.
PLASMA 11-OHCS AND NORADRENALINE LEVELS IN PONIES

Materials

Etorphine Base (22.5 mg/ml). Made up in sterile normal saline for injection.

Reckitt and Colman, Pharmaceutical Division, Hull.

'Immobilon' : large Animal - Each ml. containing 2.45 mg. (0.225% etorphine base), 10 mg. acepromazine maleate B.P.C., chlorocresol 0.1% saline.

Reckitt and Colman, Pharmaceutical Division, Hull.

'Revivon' : large Animal. Each ml. containing diprenorphine 3 mg. as hydrochloride, methylene blue 0.001%, chlorocresol 0.1%

Reckitt and Colman, Pharmaceutical Division, Hull.

Animals

All three ponies (Nos. 1, 2 and 3) were used in this study.

Methods

Electrocardiograms were recorded from each pony by radio-telemetry as described in Chapter 1. Average heart rates were then calculated at specific times by counting the number of beats

over a 30 second period.

Arterial blood pressures were recorded as described previously.

Blood samples for plasma 11-OHCS and P.C.V. determinations were collected as described in prior experiments. Samples for nor-adrenaline estimation were collected into ice-cold heparinised glass tubes and centrifuged at -4° C. The plasma was removed as quickly as possible and stored at -15° C.

Blood samples before immobilisation and after recovery were taken by venepuncture from the left jugular vein. Because of gross fasciculations during neuroleptanalgesia blood samples throughout this period were collected via the arterial cannula.

Respiratory rates were counted visually over 60 second periods.

Experimental Procedure

The animals were fasted throughout the experimental period.

Blood samples for plasma 11-OHCS and P.C.V. estimations were collected from all the ponies before the start of the experiment with as little disturbance as possible, at 5 and 20 min after etorphine injection and 5 min after administration of the antagonist. Samples for plasma nor-adrenaline measurement were taken at similar times from two ponies (Nos. 1 and 3).

Heart rates and arterial blood pressure were monitored continuously prior to and throughout the period of recumbency and for 10 min after injection of the antagonist.

Control (resting) blood samples and heart rates were all taken prior to cannulation of the artery and the ponies were allowed to stand for 30 min after this procedure before drug administration.

Etorphine was administered intravenously at a dose rate of 22.5 $\mu\text{g}/\text{kg}$, as rapidly as possible. This was equivalent to the amount present in the recommended dose of 'Immobilon' and also that used by other workers (Daniel and Ling, 1972).

Immobilon was injected intravenously at the recommended dose rate equivalent to 22.5 $\mu\text{g}/\text{kg}$ etorphine and 0.1 mg/kg acepromazine.

The effects of both drugs were terminated 30 min post-injection by intravenous administration of the antagonist diprenorphine at dose rates equivalent to 30 $\mu\text{g}/\text{kg}$.

Results

CLINICAL SIGNS

i) Etorphine

All three ponies became recumbent within 1-1 $\frac{1}{2}$ min of etorphine injection and showed a marked degree of muscle activity and increased muscle tone. These effects lasted throughout the 30 min period of recumbency but muscle activity became more violent during the last 5-10 min. Paddling of the fore and hind limbs also occurred during this time. One of the three ponies (No. 2) although in a gravely depressed state made continuous efforts to stand. Sweating occurred moderately around the head, neck, axillae and groin regions.

Injection of the antagonist produced visible increases in respiratory rate within 45 - 60 seconds and the animals were able to stand within 2-4 minutes.

ii) 'Immobilon'

All three ponies became recumbent within 1 min of 'Immobilon' injection and showed signs of increased muscle activity and tone throughout the initial 15 min period.

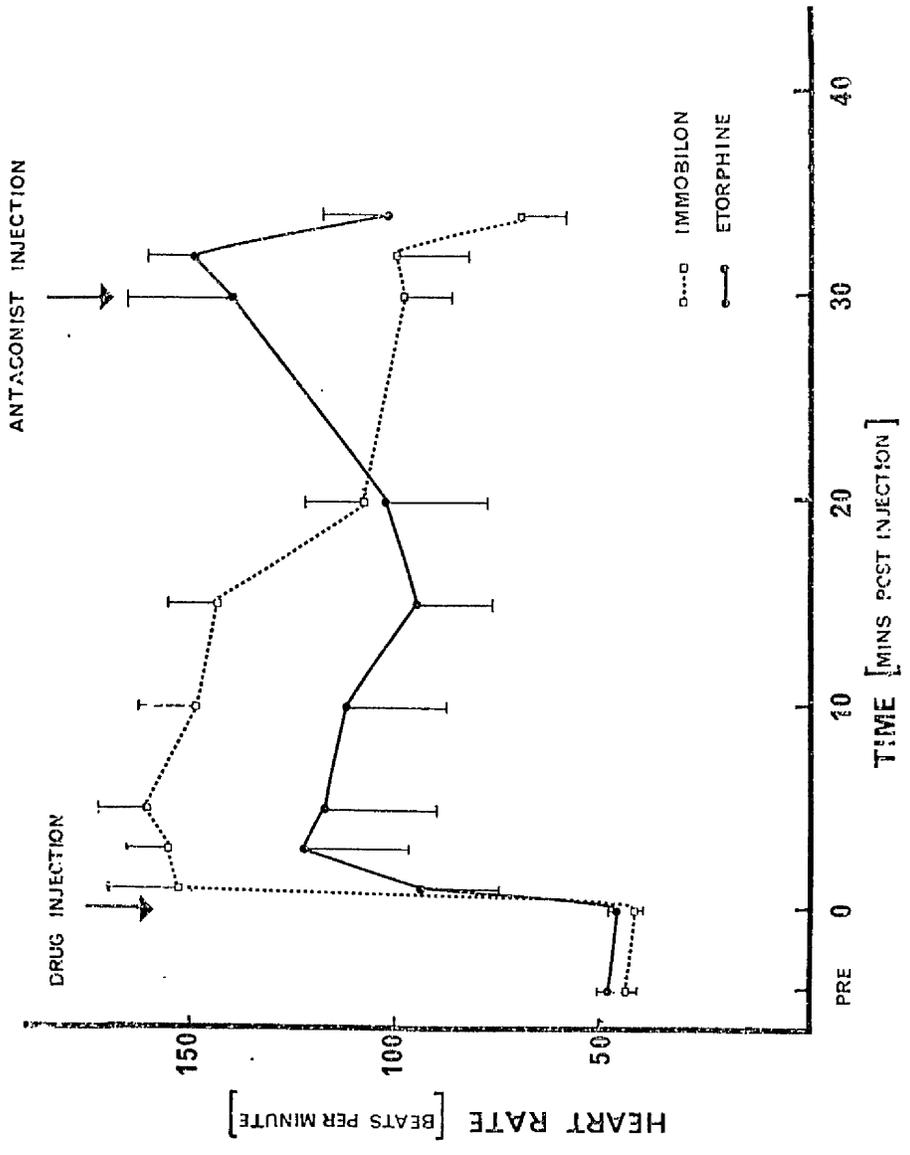
All three animals were observed to be sweating moderately around the head, neck, axillae and groin regions within 30 min. Muscle relaxation appeared to be better than with etorphine alone (experiment 1) and muscle tremors were occasional. Respiratory rates increased within 30 - 45 seconds of diprenorphine administration and within 3 min the animals were able to stand. Although the initial knock-down effect of the drug had been counteracted, all the ponies showed signs of tranquillisation viz. head down, ears ~~and~~ back and penile relaxation. These effects lasted for 2 - 3 hours.

HEART RATE (FIG. 9.1)

i) Etorphine

Etorphine produced heart rates significantly elevated above resting values ($p < 0.05$) within 3 min of injection. The rates remained high over the remainder of the 30 min period, increasing sharply between 20 and 30 min after etorphine injection and reaching maximum levels 2 min after diprenorphine administration. All these values were significantly above resting levels ($p < 0.001$). By 5 min post-diprenorphine the rate had begun to fall towards control values. Marked sinus arrhythmia occurred throughout the 30 min period (Fig. 9.4).

FIG. 9.1 INCREASES IN HEART RATE IN THREE PONIES (MEAN \pm s.e.)
FOLLOWING ETORPHINE AND 'IMMOBILON' ADMINISTRATION INTRAVENOUSLY



ii) 'Immobilon'

Heart rates were significantly elevated above resting (pre-injection) values throughout the 30 min period of recumbency ($p < 0.01$). The heart rates were highest during the first 10 min following Immobilon injection and decreased gradually until administration of the antagonist. Five minutes after diprenorphine injection they were still significantly elevated ($p < 0.02$). Marked sinus arrhythmia was present throughout the 30 min recumbency period (Fig. 9.4).

ARTERIAL BLOOD PRESSURE (FIG. 9.2)

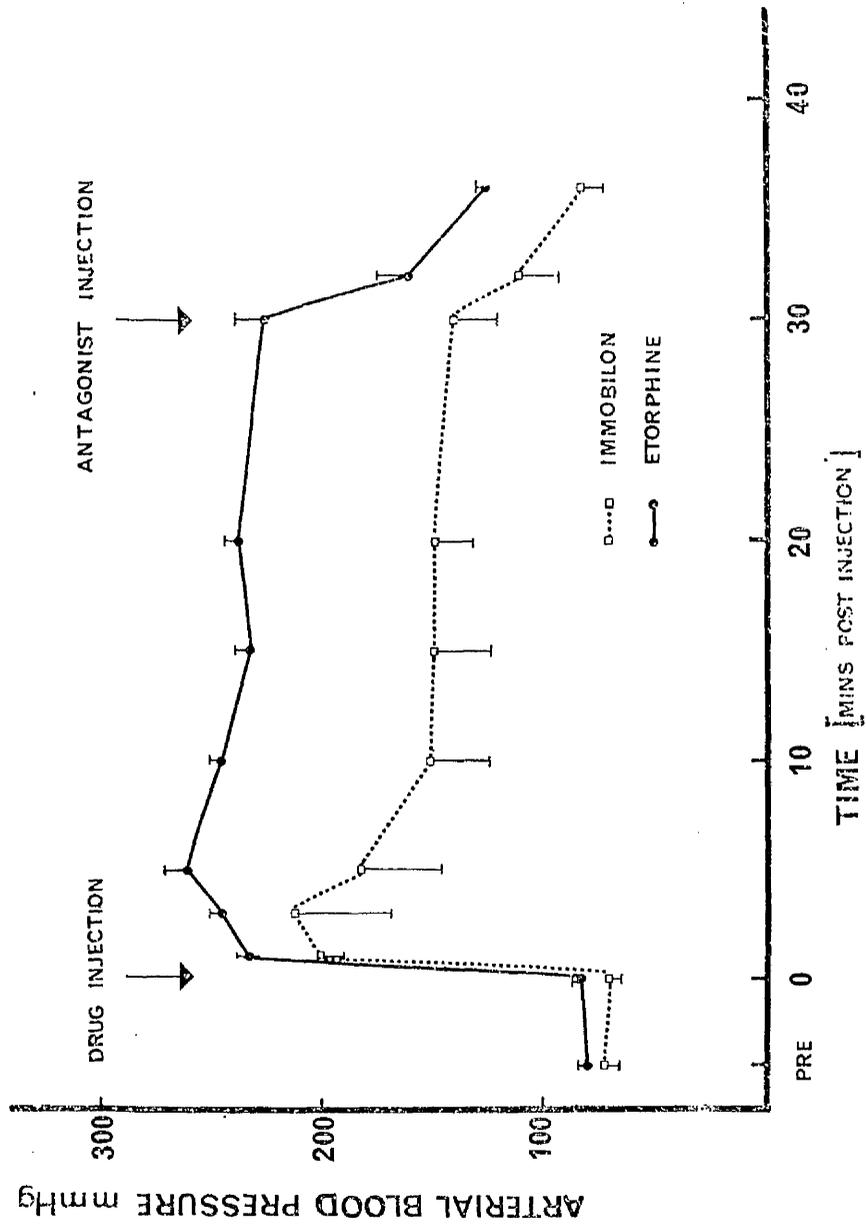
i) Etorphine

Blood pressure increased after etorphine injection - reaching a peak within 6 min and remaining elevated throughout the 30 min period. All these levels were significantly elevated above resting (pre-injection) values ($p < 0.01$). Five minutes after diprenorphine administration blood pressure had fallen markedly but was still significantly above resting values ($p < 0.02$).

ii) 'Immobilon'

Blood pressure increased after 'Immobilon' injection reaching its highest level within 3 min. This value was 150% above resting (pre-injection) levels. By 10 min post-injection the pressure began to decrease and continued to fall gradually although still 92% above resting values immediately prior to injection of the antagonist.

FIG. 9.2 INCREASES IN ARTERIAL BLOOD PRESSURE IN THREE PONIES
(MEAN [±] s.e.) FOLLOWING ETORPHINE AND IMMOBILON[®]
ADMINISTRATION INTRAVENOUSLY



PACKED CELL VOLUME (TABLE 9.1)

i) Etorphine

P.C.V. levels increased significantly above resting values throughout the 30 min following etorphine administration ($p < 0.001$) and were still significantly elevated 5 min after diprenorphine injection ($p < 0.001$).

ii) 'Immobilon'

P.C.V. increased to a significant level above resting values ($p < 0.01$) within 5 min of 'Immobilon' injection and were still elevated 15 min later. Five minutes after administration of the antagonist the P.C.V. had fallen to a level significantly below resting values ($p < 0.01$).

RESPIRATORY RATES (TABLE 9.2)

i) Etorphine

Respiratory rates fell significantly below resting values ($p < 0.02$) throughout the 30 min period of recumbency. Cyanosis was apparent in pony No. 3. Within 5 min of diprenorphine injection, respiratory rates had increased significantly above resting values ($p < 0.05$).

ii) 'Immobilon'

Marked respiratory depression was apparent in all ponies after 'Immobilon' injection and cyanosis was visible in one of the three (pony No. 3). Respiratory rates decreased significantly ($p < 0.05$) below resting values to levels of 1-2 respirations/min throughout the period of recumbency. After diprenorphine injection however the rates increased markedly to levels significantly above resting

Table 9.1 Packed Cell Volumes (%) in 3 ponies (mean \pm s.e.) during and after injection of etorphine and

Immobilon

	Drug	
Time of Sample	Etorphine	'Immobilon' (22.5 ug/kg. Etorphine 0.1 mg/kg. Acp)
Pre Injection	30.3 \pm 0.4	30.6 \pm 0.3
5 min Post Injection	39.2 \pm 1.1 **	36.3 \pm 0.8 **
20 min Post Injection	39.8 \pm 1.2 **	32.5 \pm 0.8
5 min Post Antagonist Injection	32.5 \pm 0.3 **	29.0 \pm 0.4 *

** refers to levels significantly ($p < 0.001$) above pre-drug values

* refers to levels significantly ($p < 0.01$) below pre-drug values

Table 9.2 Respiratory Rates (respirations/min) after etorphine and 'Immobilon' administration (Mean \pm s.e.)

Stage of Procedure	Drug	
	Etorphine	'Immobilon'
Pre Injection	13 \pm 0.6	18 \pm 3.5
5 min Post Etorphine	2 \pm 0 ⁺	2 \pm 1
25 min Post Etorphine	3 \pm 1.5	2 \pm 1
5 min Post Antagonist	74 \pm 11	49 \pm 4.1

values ($p < 0.05$).

PLASMA 11-OHCS (FIG. 9.3)

i) Etorphine

Plasma 11-OHCS levels increased to a maximum 20 min after etorphine injection. These values together with those recorded 5 min after diprenorphine administration were significantly above resting levels ($p < 0.01$).

ii) 'Immobilon'

Plasma 11-OHCS levels increased gradually throughout the period of recumbency being significantly elevated above resting values 20 min after 'Immobilon' injection ($p < 0.05$). Five minutes after diprenorphine administration the levels were at their highest.

PLASMA NORADRENALINE (TABLE 9.3)

i) Etorphine

Marked increases occurred in both ponies, the highest levels being recorded within 5 min of etorphine injection (151% and 184% above resting values respectively). Values were still increased 20 min later (42% and 404% respectively) but had fallen to almost resting levels within 5 min of diprenorphine injection.

ii) 'Immobilon'

Increase in plasma nor-adrenaline levels occurred in both ponies during the period of recumbency, maximum values being recorded at 5 min post Immobilon when levels were 140% and 484% respectively above pre-injection values. By 20 min post Immobilon

FIG. 9.3 INCREASES IN PLASMA 11-OHCS LEVELS IN THREE PONIES
(MEAN \pm s.e.) FOLLOWING ETOPHINE AND 'IMMOBILON'
ADMINISTRATION INTRAVENOUSLY

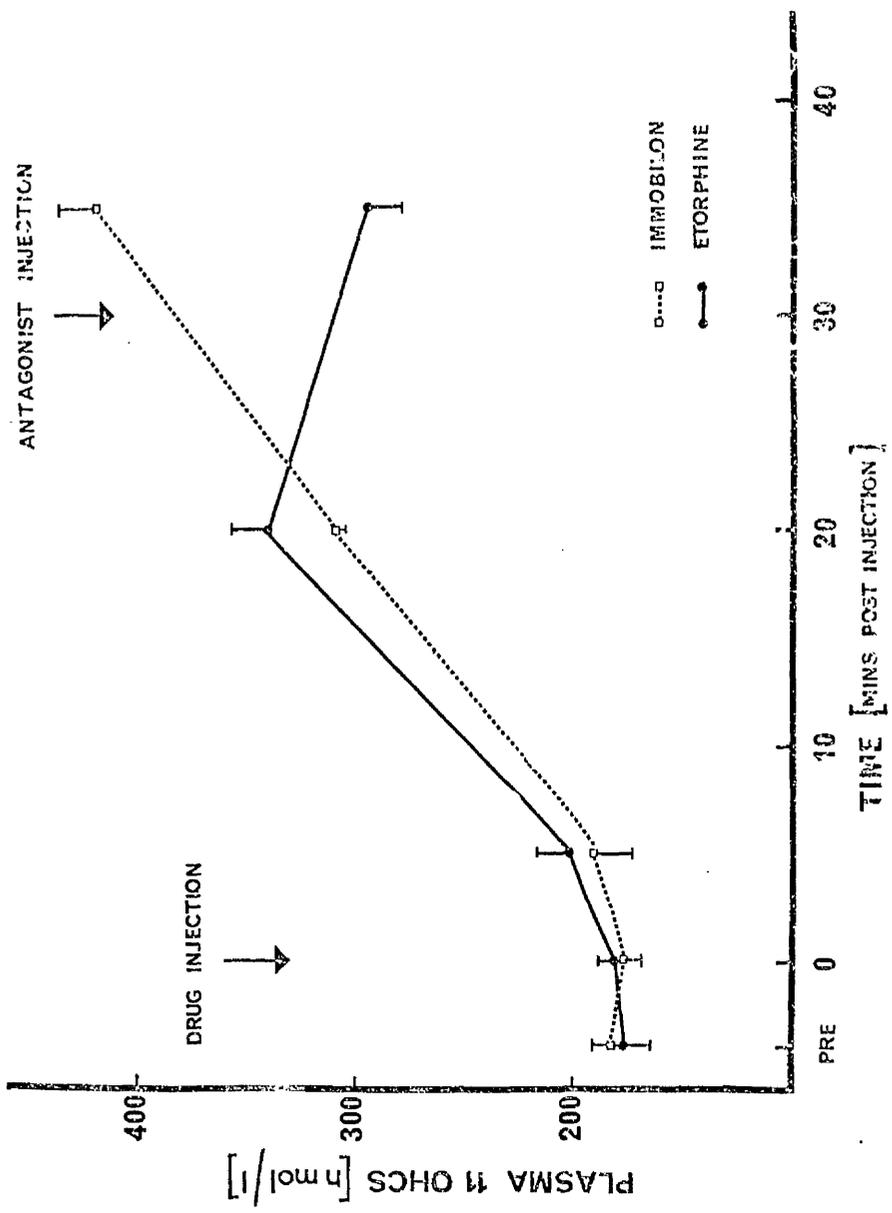


Table 9.3 Plasma nor-adrenaline levels (p mol/l) in 2 ponies after administration of etorphine alone and *Immobilon*

Stage of Procedure	Pony No. 1		Pony No. 3	
	Etorphine	Immobilon	Etorphine	Immobilon
Pre Injection	1.19	0.35	0.26	0.45
5 min Post Injection	2.99	0.84	5.05	2.63
25 min Post Injection	1.69	0.71	1.31	1.07
5 min Post Antagonist	0.98	0.30	0.24	0.62

the levels were considerably lower than at 5 min post Immobilon and after administration of the antagonist were at or below resting values.

9.3 EFFECTS OF PREMEDICATION WITH VARIOUS TRANQUILLISERS ON THE
ACTIONS OF ETORPHINE IN PONIES

In the previous experiments the actions of 'Immobilon' were compared with those of etorphine alone and it has been shown that acepromazine injected simultaneously with etorphine does modify a number of the effects of the latter.

It is known that the maximal central effects of acepromazine do not occur until between 10-15 min after intravenous injection since it is at this time that drooping of the head and penile relaxation occur. It appeared therefore that prior administration of the tranquilliser and injection of etorphine at the time corresponding to the peak central effect of the tranquilliser would prove a more successful means of administering the drug combination. There was also the possibility that acepromazine or any phenothiazine tranquilliser might not be the drugs of choice for inclusion in the mixture and that one of the butyrophenones, in particular azaperone, or the unrelated drug xylazine, might be more suitable.

This experiment was designed to compare the actions of etorphine (administered intravenously) with those of:-

- i) etorphine alone (i/v) after intravenous premedication with acepromazine, azaperone and xylazine.
- ii) etorphine alone (i/v) after intramuscular premedication with acepromazine, azaperone and xylazine.

Effects on heart rate, respiratory rate, muscle tone and activity and sweating were assessed.

Animals

Two ponies (Nos. 1 and 3) were used in this study.

Recordings

Heart rates were recorded by radiotelemetry as described previously.

Respiratory rates were measured by visual estimation over a period of 30 seconds.

Muscle tone and activity and sweating were assessed visually and graded according to a scale.

1. Muscle activity

+ legs and head shaking occasionally

++ legs and head shaking continuously

+++ legs and head shaking violently

2. Muscle tone

+ all relaxed

++ legs relaxed

+++ legs and neck tense

3. Sweat

+ damp around groin and axillae

++ damp all over

+++ drops of sweat on body

Drugs

The drugs used, their dose rates, routes of administration and time of administration before etorphine and diprenorphine are shown in Table 9.4. Etorphine and diprenorphine were given at the same dose rates as previously. Acepromazine, azaperone and xylazine were administered intravenously at dose rates either slightly below or similar to those used in other studies (Jones, 1972; Kerr *et al.*, 1972; Hoffman, 1974; Hillidge, Lees and Serrano, 1975) and intramuscularly at the dose rates recommended by Aitken and Sanford (1972) and Lees and Serrano (1976).

Experimental Procedure

This study was carried out on a separate occasion from the previous experiments and therefore for comparative procedures the results concerning administration of etorphine alone are reported again.

Heart and respiratory rates were recorded from the animals at rest before the experiment, throughout the period of recumbency and for 10 min after administration of the antagonist.

Etorphine was injected intravenously at the time of maximum effect of the tranquillisers and assessed in a previous experiment (chapter 8). Times of etorphine administration after intravenous and intramuscular premedication with the 3 drugs are shown in Table 9.5.

Etorphine alone was given to both colonies and the antagonist

Table 9.4 Drugs used as premedication prior to etorphine, their dosage rates and routes of administration

Tranquilliser	Dose/kg.	Route	Drug	Dose/kg.	Route			
Acepromazine	0.2 mg.	i.m.	Etorphine	22.5 µg.	i.v.			
	0.1 mg.	i.v.						
Azaperone	0.4 mg.	i.m.						
	0.2 mg.	i.v.						
Xylazine	3.0 mg.	i.m.				Diprenorphine	30 µg.	i.v.
	0.5 mg.	i.v.						

Table 9.5 Times of etorphine and diprenorphine injection following premedication with xylazine, azaperone and acepromazine

Premedication	Route of Administration	Dose /Kg.	Time to Etorphine (min)	Time to Diprenorphine (min after Etorphine)
Xylazine	i/v	0.5 mg	10	30
	i/m	3.0 mg	20	60
Azaperone	i/v	0.2 mg	10	30
	i/m	0.4 mg	40	30
Acepromazine	i/v	0.1 mg	15	60
	i/m	0.2 mg	60	60

was not administered until signs of recovery were seen. Following premedication with the tranquillisers prior to etorphine, the ponies were allowed to remain recumbent for 60 min unless showing signs of spontaneous movement before this.

Each tranquilliser was administered once intramuscularly and once intravenously to both ponies and at least 4 days was allowed between experiments.

Results

1. Etorphine alone

CLINICAL SIGNS (TABLE 9.6)

In both ponies etorphine produced recumbency lasting between 25 - 30 min after this time they began to paddle violently with fore and hind legs. Increased muscle activity and tone and sweating ^{were} apparent throughout the period of recumbency.

HEART RATE (TABLE 9.7)

Etorphine produced an increase in heart rate of 240% (equivalent to 164 beats/min) at 5 min post injection. The rate then fell gradually, but was still elevated by 150%, 20 min after etorphine. Between 25 and 30 min post-injection the rates increased to 144 beats/min (an elevation of 200% above resting values) and remained high until after diprenorphine injection.

Sinus arrhythmia was present throughout the period of recumbency (Fig. 9.4).

RESPIRATORY RATES (TABLE 9.8)

Etorphine depressed respiration during recumbency. Rates increased markedly however after injection of the antagonist being 480% above resting values within 10 min.

Table 9.6 Side effects seen during recumbency following 'Immobilon' and etorphine alone and with prior tranquilliser premedication

Drug	Route of Admin.	Side Effects		
		Muscle Activity	Muscle Tone	Sweating
Etorphine	i/v	+++	+++	+
	i/v	++	++	+
Xylazine	i/m	++	+	++
	i/v	+++	++	++
Azaperone	i/m	+++	++	+++
	i/v	++	++	+++
Acepromazine	i/v	++	+	++
	i/m	++	+	++

Key = (1) Muscle activity (2) Muscle tone (3) Sweat

+ leg and head
occasionally
++ legs and head
continuously
+++ legs and head
violently

+ all relaxed
++ legs relaxed
+++ legs and neck tense

+ damp around groin and axillae
++ damp all over
+++ drops of sweat on body

Table 9.7 Percentage increases in heart rate (above resting levels) in 2 ponies after intravenous premedication with acepromazine, azaperone and xylazine

Drug	Min Post Etorphine										Min Post Antagonist	
	1	3	5	10	20	30	40	50	60	2	5	
Etorphine	182	222	240	184	150	200						
Acepromazine i/v Premedication	235	235	173	160	128	78	50	42	34	26		78
Azaperone i/v Premedication	270	293	248	168	168	172				140		58
Xylazine i/v Premedication	83	96	88	76	50	28				187		105

Table 9.8 Respiratory Rates (Mean \pm s.e.) in 2 ponies following etorphine and after premedication with acepromazine, azaperone and xylazine before etorphine

Procedure	Drug									
	Etorphine		Acepromazine		Azaperone		Xylazine			
			i/v	i/m	i/v	i/m	i/v	i/m	i/v	i/m
Pre Inj.	10 \pm 2		13 \pm 5	16 \pm 2	16 \pm 2	13 \pm 3	10		9 \pm 1	
10 min Post Recumbency	3 \pm 1		4 \pm 1	5 \pm 1	3 \pm 1	4 \pm 1	4 \pm 1		1 \pm 0	
20 min Post Recumbency	3 \pm 1		4 \pm 1	3 \pm 1	3 \pm 1	4 \pm 1	-		1 \pm 0	
30 min Post Recumbency	4 \pm 0		3 \pm 1	2 \pm 0	4 \pm 3	2 \pm 0	3 \pm 1		2 \pm 1	
40 min Post Recumbency	2 \pm 0		2 \pm 0	2 \pm 0	-	-	-		1 \pm 0	
50 min Post Recumbency	3 \pm 0		1 \pm 0	2 \pm 0	-	-	-		-	
60 min Post Recumbency	2 \pm 1		2 \pm 0	3 \pm 1	-	-	-		1 \pm 0	
5 min Post Antagonist	44 \pm 4		43 \pm 1	55 \pm 11	51 \pm 3	40 \pm 8	48 \pm 6		48 \pm 5	
10 min Post Antagonist	58 \pm 6		47 \pm 13	54 \pm 6	47 \pm 5	51 \pm 15	63 \pm 3		56 \pm 5	

!-! readings not recorded

2. Intravenous premedication with acepromazine, azaperone and xylazine prior to etorphine

CLINICAL SIGNS (TABLE 9.6)

Azaperone and xylazine premedication produced satisfactory recumbency lasting for 30 min only whereas following acepromazine recumbency was satisfactory for up to 60 min when the experiment was terminated and the antagonist administered. All three drugs reduced the degree of muscle tone but only acepromazine and xylazine reduced muscle activity. Xylazine produced least sweating and acepromazine most in both ponies.

HEART RATE (TABLE 9.7)

The pre-etorphine heart rates were always between 42 - 48 beats/min. Azaperone premedication produced a maximum increase of 293% (173 beats/min) at 3 min post-etorphine, acepromazine of 235% (162 beats/min) at 5 min post etorphine and xylazine of 96% (94 beats/min) at 3 min post-etorphine. In all cases the heart rates decreased gradually over the period of recumbency.

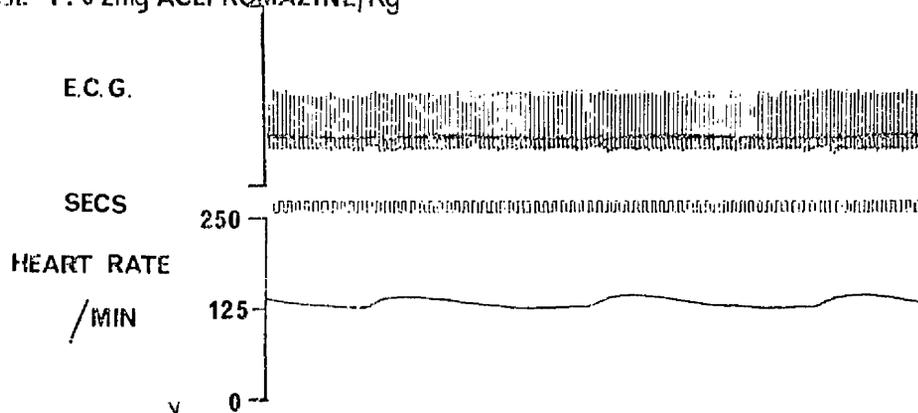
The greatest increase in heart rate following administration of the antagonist was seen in the ponies premedicated with xylazine where rates of 138 beats/min (an increase of 187% above resting values were recorded). Acepromazine premedication also produced an increase at this time of 78% (84 beats/min).

Sinus arrhythmia was present in both ponies after all three drugs (Fig. 9.4).

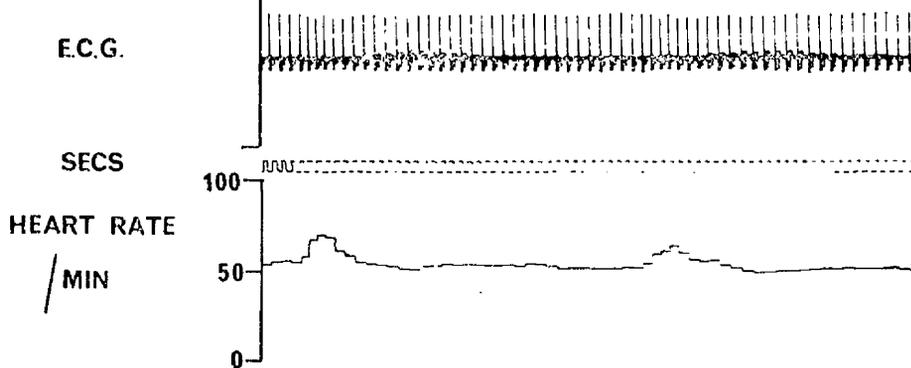
FIG. 9.4 . SINUS ARRHYTHMIA FOLLOWING ETORPHINE WITH TRANQUILLISER
PREMEDICATION

RECORDS OBTAINED BY RADIOTELEMETRY : SINUS ARRHYTHMIA
FOLLOWING ETORPHINE WITH TRANQUILLISER PREMEDIATION

HORSE 1: 0.2mg ACEPROMAZINE/Kg



HORSE 2: 3.0mg X_ALAZINE/Kg



RESPIRATORY RATES (TABLE 9.8)

Respiratory rates were depressed throughout recumbency with all three drugs. After administration of the antagonist the rates increased to levels similar to those recorded following etorphine alone.

3. Intramuscular premedication with acepromazine, azaperone and xylazine prior to etorphine

CLINICAL SIGNS (TABLE 9.6)

Azaperone premedication produced recumbency lasting for approx. about 30 min acepromazine and xylazine for approximately 60 min. Acepromazine and xylazine also produced complete relaxation in both ponies and reduced the degree of muscle activity. Xylazine and azaperone premedication resulted in less sweating than was seen following acepromazine.

HEART RATE (TABLE 9.9)

The greatest percentage increase in heart rate was seen following azaperone premedication when an elevation of 264% (164 beats/min) was recorded 10 min post-etorphine. After acepromazine the increase was 244% (144 beats/min) 1 min post etorphine and after xylazine 126% (106 beats/min) 1 min post-etorphine.

Sinus arrhythmia was noted in both ponies (Fig. 9.4) following all three drugs.

RESPIRATORY RATES (TABLE 9.8)

Respiratory rates were depressed following etorphine administration in all three instances but increased after injection of the antagonist.

Table 9.9 Percentage increases in heart rate (above resting levels) in 2 ponies after intramuscular premedication with acepromazine, azaperone and xylazine before etorphine administration

Drug	Min Post Etorphine										Min Post Antagonist	
	1	3	5	10	20	30	40	50	60	2	5	
Etorphine	182	222	240	184	150	200				220	150	
Acepromazine i/m Premedication	244	226	226	176	144	86	76	64	64	50	32	
Azaperone i/m Premedication	244	256	250	264	216	210				180	110	
Xylazine i/m Premedication	126	102	76	50	28	23	14	6	0	68	24	

9.4 EFFECTS OF PREMEDICATION WITH ACEPROMAZINE ON THE
HYPERTENSIVE EFFECTS OF ETORPHINE

Since the hypertension produced by the Immobilon mixture is probably the most dangerous side effect of the neuroleptanalgesic combination in that the few unexplained deaths which have occurred following 'Immobilon' could be a consequence of such increases in blood pressure, it was decided to investigate in more detail the timing of tranquilliser administration prior to etorphine with a view to reducing this effect.

It has already been established (chapter 7) that acepromazine exerted a hypotensive effect in these ponies. This experiment was designed to assess the changes in blood pressure produced by injecting etorphine at the time when these hypotensive actions of acepromazine were at their maximum.

Animals

Three ponies (Nos. 1, 2 and 3) were used in this study.

Methods

Arterial blood pressure were recorded as described previously.

Blood samples for plasma 11-OHCS levels and packed cell volumes were treated and the estimations carried out in the same manner as previously.

Drugs

Acepromazine and etorphine were administered at dose rates equivalent to those in the Immobilon mixture and diprenorphine was injected at the recommended dose rate (30 $\mu\text{g}/\text{kg}$).

Experimental Procedure

Blood pressures were recorded from the animals at rest and continuously throughout the study. Blood samples for plasma 11-OCS and P.C.V. estimations were taken before the experiment, at 5 and 25 min after etorphine and 5 min after diprenorphine.

Etorphine was injected at 25 min after acepromazine (the time of maximum hypotensive effect - chapter 7). The period of recumbency was terminated at 30 min after etorphine administration by injection of the antagonist.

A period of at least 10 days was allowed between experiments.

Results

CLINICAL SIGNS

As reported previously, acepromazine premedication decreased muscle activity and tone. The drug mixture produced moderate sweating in all three ponies.

BLOOD PRESSURE (TABLE 9.10 AND FIG. 9.5)

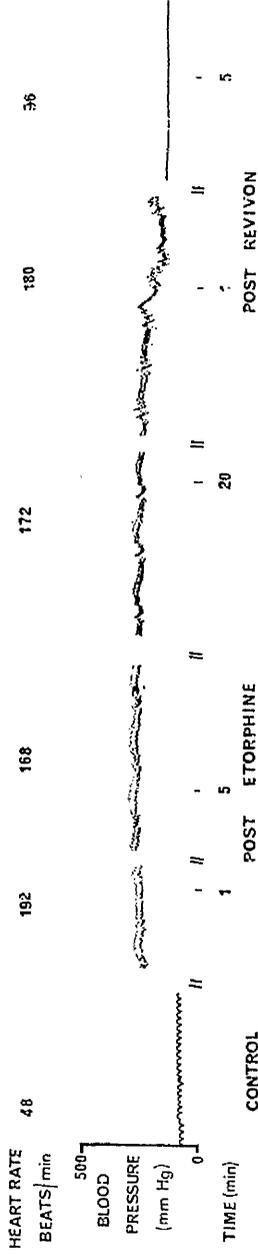
Arterial blood pressure was significantly lower 1 min post-etorphine than either following etorphine alone ($p < 0.01$) or

Table 9.10 Blood pressure readings (mm. Hg) in 3 ponies (mean \pm s.e.) after acepromazine, premedication and pretreatment with propranolol

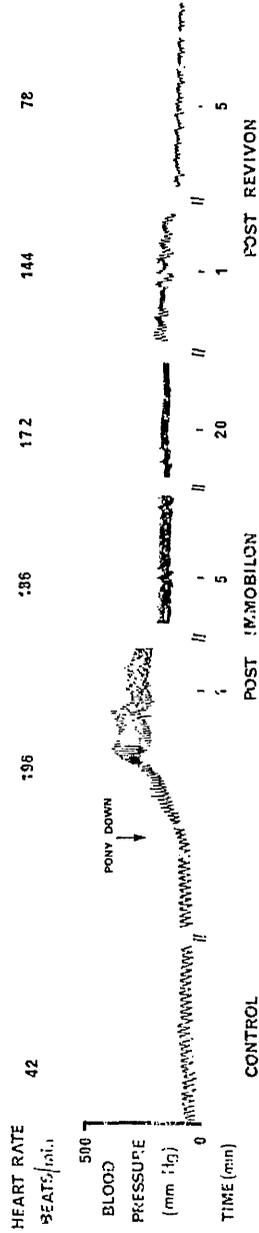
Drug	Pre.	Min Post Acp			Min Post Etorphine							Min Post Antagonist		
		15	20	25	1	3	5	10	15	20	25	30	2	5
Acepromazine Prior to Etorphine	70 ± 6	52 ± 8		53 ± 2	102 ± 6	130 ± 26	137 ± 23	138 ± 22	138 ± 20	138 ± 22	138 ± 23	137 ± 23	128 ± 25	95 ± 8
Acepromazine and Propranolol Prior to Etorphine	58 ± 6	47 ± 7	45 ± 5	45 ± 6	78 ± 12	105 ± 25	138 ± 24	130 ± 15	120 ± 5	127 ± 9	123 ± 9	117 ± 16	105 ± 3	88 ± 4
Etorphine	80 ± 8				233 ± 4	247 ± 6	260 ± 10	246 ± 3	233 ± 3	256 ± 4		225 ± 12	160 ± 14	125 ± 4
Immobilon	73 ± 10				200 ± 12	214 ± 14	183 ± 10	150 ± 24	148 ± 28	148 ± 18		140 ± 20	110 ± 18	84 ± 8

FIG 9.5 BLOOD PRESSURE RECORDINGS AND HEART RATES IN PONY NO. 1
FOLLOWING ETORPHINE, 'IMMOBILON' AND ACEPROMAZINE PREMEDICATION

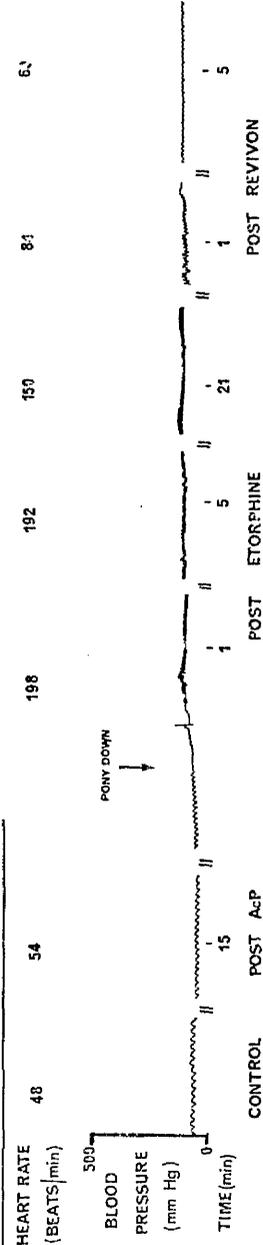
1. ETORPHINE ALONE



2. IMMOBILON



3. ACEPROMAZINE 25 min. BEFORE ETORPHINE



'Immobilon' ($p < 0.05$). The levels remained significantly lower than those recorded following etorphine ($p < 0.05$) and slightly below those recorded after 'Immobilon' throughout the 30 min period of recumbency. Within 5 min of injection of the antagonist the values had returned almost to resting (pre-injection) levels and were still significantly below ($p < 0.05$) those recorded following etorphine alone.

PLASMA 11-OHCS (TABLE 9.11)

Plasma 11-OHCS levels increased during the period of recumbency reaching maximum levels at 25 min post-etorphine injection. These levels were higher than any recorded following etorphine or 'Immobilon' administration (Fig. 9.2) and were still elevated 5 min after injection of the antagonist.

PACKED CELL VOLUME (TABLE 9.11)

Packed cell volumes increased to maximum levels within 5 min of etorphine injection. These values were significantly elevated above resting levels ($p < 0.01$) but were lower than those recorded following etorphine or 'Immobilon' administration at the same time (Table 9.1). By 25 min post etorphine the P.C.V. had fallen slightly and at 5 min after antagonist injection was significantly below resting (pre-experiment) levels ($p < 0.02$).

Table 9.11

Plasma 11-OHCS levels and packed cell volumes (mean \pm s.e.) in 3 ponies after acepromazine premedication and etorphine administration

Stage of Procedure	Plasma 11-OHCS levels (n mol/litre)	Packed Cell Volume (%)
Pre Acepromazine	224 \pm 56	31 \pm 1.0
5 min Post Etorphine	364 \pm 27	34 \pm 0.9*
25 min Post Etorphine	476 \pm 26	33 \pm 0.5
5 min Post Antagonist	448 \pm 36	29 \pm 0.6**

* refers to levels significantly ($p < 0.01$) above resting values

** refers to levels significantly ($p < 0.02$) below resting values

9.5 EFFECT OF PRETREATMENT WITH THE β -ADRENERGIC BLOCKING AGENT
PROPRANOLOL ON THE TACHYCARDIA PRODUCED BY ETORPHINE

Propranolol has been used by previous workers (Daniel and Ling, 1972; Schlarman *et al.*, 1973) to successfully reduce the tachycardia produced following 'Immobilon' administration. It was used in this study to compare its effect on the heart rate increase with those of the three tranquillisers and in particular xylazine.

Animals

Three ponies (Nos. 1, 2 and 3) were used in this study.

Methods

All heart rates, blood pressures and blood samples were recorded and collected as described previously.

Drugs

Propranolol Hydrochloride. Imperial Chemical Industries Limited.

Propranolol was dissolved in saline to a concentration of 20 mg/ml. The solution was then sterilised by passing through a 'millipore' filter.

Acepromazine, etorphine and diprenorphine were administered at the dose rates used in the previous study.

Propranolol was injected at a dose rate of 0.2 mg/kg. similar to that used by Daniel and Ling (1972). It was injected slowly into the vein over a period of 2-3 min, beginning 5 min before etorphine administration (Schlarmann et al., 1973).

Experimental Procedure

Heart rates and blood pressures were recorded from the animals at rest and blood samples were taken for 11-OHCS, nor-adrenaline and P.C.V. estimation.

Acepromazine was injected as in the previous study and heart rates and blood pressures continually recorded. Twenty minutes after acepromazine, the appropriate dose of propranolol was slowly injected into the vein and at 25 min post acepromazine etorphine was administered.

Blood samples for 11-OHCS, nor-adrenaline and P.C.V. were taken 5 and 25 min post etorphine and 5 min post diprenorphine.

The period of recumbency was terminated in all three ponies by injection of the antagonist 30 min after etorphine had been given.

Results

CLINICAL SIGNS

Injection of propranolol 5 min before etorphine produced no effect on muscle activity or tone nor on the degree of sweating recorded (as compared to the results of experiment 4).

HEART RATE (TABLE 9.12)

During the 5 min immediately following propranolol injection there was no alteration in heart rate recorded. Three minutes after etorphine administration the heart rate increased to its maximum level (123 beats/min). This was a mean increase of 207% above resting values and was 93% below the level recorded at the same time following 'Immobilon' injection (Fig. 9.1). Throughout the 30 min period the heart rate remained above resting values being very similar to the rates recorded following xylazine premedication (Table 9.9) but always below those found after etorphine alone and Immobilon. Injection of the antagonist did not produce further elevation only a more rapid return to resting (pre-injection) levels. Sinus arrhythmia was recorded in all 3 ponies throughout the 30 min period of recumbency.

BLOOD PRESSURE (TABLE 9.10)

Arterial blood pressure did not change in the 5 min following propranolol injection. After etorphine administration however it rose to maximum levels within 5 min (138 ± 24 mm Hg). This value was similar to that recorded in the previous experiment (experiment 4) but significantly ($p < 0.02$) lower than was reported following

Table 9.12 Heart Rate (beats/min) in 3 ponies (mean \pm s.e.) following propranolol administered intravenously 5 min before etorphine

Drugs	Pre.	Min Post Acp			Min Post Etorphine								Min Post Antagonist	
		15	20	25	1	3	5	10	15	20	25	30	2	5
Acepromazine /Propranolol /Etorphine	40 + -2	61 + -6	57 + -7	57 + -11	65 + -13	123 + -12	90 + -14	82 + -15	75 + -13	75 + -13	70 + -18	68 + -19	58 + -6	46 + -4
Etorphine	48 \pm 2				94 \pm 18	126 \pm 25	118 \pm 29	112 \pm 24	95 \pm 17	104 \pm 26	-	135 \pm 34	148 \pm 23	102 \pm 30
'Immobilon'	44 \pm 2				152 \pm 18	154 \pm 10	160 \pm 14	148 \pm 14	144 \pm 11	118 \pm 12	110 \pm 10	98 \pm 11	100 \pm 16	70 \pm 11
Xylazine i/v	40 \pm 4				90 \pm 12	94 \pm 17	90 \pm 16	84 \pm 16	76 \pm 16	72 \pm 12	-	60 \pm 6	124 +	92 +
Premed. i/m	41 \pm 2				113 \pm 17	92 \pm 8	89 \pm 11	74 \pm 9	63 \pm 9	65 \pm 6	-	58 \pm 5	45 \pm 2	63 \pm 11

:- indicated measurements not made

etorphine alone and 10% lower than that seen after 'Immobilon' injection. Over the 30 min period blood pressure steadily decreased at a faster rate than was recorded in any of the previous experiments and by 5 min post diprenorphine injection had returned almost to control values.

PLASMA 11-OHCS (TABLE 9.13)

Plasma 11-OHCS levels increased throughout the period of recumbency reaching maximum levels 25 min post-etorphine injection. These values were higher than those recorded following administration of etorphine alone (Fig. 9.3) but similar to those seen after 'Immobilon' and were still elevated 5 min after injection of the antagonist.

PACKED CELL VOLUME (TABLE 9.13)

Packed cell volumes were increased significantly above resting values 5 min after etorphine injection ($p < 0.01$) to levels similar to those recorded in experiment 4 (Table 9.11). Within 5 min of antagonist injection, they had fallen to values significantly below control levels ($p < 0.02$).

PLASMA NOR-ADRENALINE (TABLE 9.14)

Plasma nor-adrenaline concentrations increased in both animals reaching a maximum in pony No. 1 at 25 min post-etorphine (+1032%) and in pony No. 3 at 5 min post-etorphine (+1368%).

Within 5 min of injection of the antagonist, the levels had returned towards resting (pre-injection) values.

These nor-adrenaline levels are similar to those recorded in the same animals following etorphine administration but slightly

higher than those seen after 'Jimmobilon' (Table 9.3).

Table 9.13 Plasma 11-OHCS and P.C.V. levels (mean \pm s.e.) in 3 ponies following acepromazine premedication and treatment with propranolol

Stage of Procedure	Plasma 11-OHCS levels (n mol/litre)	Packed Cell Volume (%)
Pre Acepromazine	235 \pm 56	31 \pm 0.6
5 min Post Etorphine	266 \pm 73	34 \pm 0.7*
25 min Post Etorphine	440 \pm 22	33 \pm 0.7
5 min Post Diprenorphine	437 \pm 25	27 \pm 0.3**

* refers to levels significantly above (p < 0.01) resting values

** refers to levels significantly below (p < 0.02) resting values

Table 9.14 Plasma noradrenaline levels (p mol/litre) in 2 ponies following acepromazine and propranolol administration before etorphine

Stage of Procedure	Pony No. 1	Pony No. 3
Pre Experiment	0.25	0.34
5 min Post Etorphine	2.77	4.99
25 min Post Etorphine	2.33	1.80
5 min Post Diprenorphine	0.32	0.55

9.6 Discussion

In these studies administration of etorphine alone produced a greater degree of muscle activity and tone and larger increases in arterial blood pressure, packed cell volumes and plasma nor-adrenaline levels than 'Immobilon'.

The most interesting observation in these studies was that both etorphine and 'Immobilon' cause an increase in plasma nor-adrenaline levels. This suggests that etorphine either produces a centrally induced increase in sympathetic activity or that it causes release of adrenaline and nor-adrenaline directly from the adrenal medulla and post-ganglionic nerve endings. Increased sympathetic tone could also be due to the severe degree of hypoxia which was noted in these experiments and which has also been recorded by other workers in horses (Hillidge and Lees, 1971 and 1975; Schlarmann et al, 1973). The respiratory effects of etorphine will be discussed in detail in later pages but the presence of hypoxia must be considered as one of the causal agents of the increase in sympathetic activity which would account for a number of the effects recorded following both etorphine and 'Immobilon' viz. elevations in heart rate, blood pressure, packed cell volume, muscle activity and sweating (Hillidge and Lees, 1971 and 1975; Daniel and Ling, 1972; Schlarmann et al, 1973). It is unlikely however that hypoxia would have developed within 1-2 min of the animals becoming recumbent when the majority of these effects were already marked. Schlarmann et al (1973) suggested that ganglion stimulation was the

cause of the symptomatology of 'Immobilon' administration but such an effect would probably occur in all species and would not account for the different reactions recorded by this and other authors in dogs, monkeys and rats (Blane et al, 1967).

Although the assay employed measured only plasma nor-adrenaline levels it can be assumed that adrenaline levels would rise in parallel since either central or local actions on the sympathetic system would stimulate the release of both.

Under normal conditions of nor-adrenaline and adrenaline release both amines are rapidly removed from tissues either by reuptake into storage granules or by breakdown by a number of enzymes (principally monoamine oxidase (M.A.O.) and Catechol - O - methyl - transferase, (C.O.M.T.) found in the liver and elsewhere in the body (Goodman and Gilman, 1975). Under normal bodily conditions plasma levels of nor-adrenaline are low because breakdown and reuptake occur rapidly at the site of action in the tissues. It is only when marked increased sympathetic activity occurs that levels of nor-adrenaline in the circulation increase since too much is being produced to be removed quickly (Guyton, 1971).

Examination of the plasma nor-adrenaline levels recorded in this study following etorphine administration suggest that initially a large degree of stimulation occurred and that this decreased over the 30 min period. Consideration of the alterations in other parameters monitored shows that they too fit into this pattern.

Elevated levels of circulating catecholamines normally result in increased heart rate and blood pressure because of β - adrenergic

effects on the heart and α effects on systemic blood vessels (Goodman and Gilman, 1975). However these effects do not always parallel the levels of catecholamines in the intact animal because of reflex responses.

Stretch receptors in the carotid sinus and aortic arch exert a powerful influence on the cardio-regulatory centres in the brain - decreases in blood pressure producing increases in heart rate and vice versa (Rushmer, 1972). This regulatory effect on the heart is mediated through the sympathetic and parasympathetic nervous systems, the former supplying excitatory and the latter inhibitory effects. A balance between these two systems normally exists but can be influenced by higher centres e.g. hypothalamus or cerebral cortex (Bronk, Pitts and Larrabee, 1940; Guyton, 1971).

Reflex actions on the heart in response to hypertension may explain why the tachycardia following etorphine alone was less than that following 'Immobilon'. The heart slows reflexly in response to hypertension and therefore following etorphine administration when the blood pressure was very high heart rate was lower than that seen after 'Immobilon' injection. Because of the hypotensive actions of acepromazine, blood pressure after 'Immobilon' is lower and there is less reflex control of the heart thus leading to more elevated heart rates.

A second reason for the increased heart rates recorded following acepromazine and azaperone premedication prior to etorphine could be a direct effect of the tranquillisers. Aitken and Sanford (1972) reported changes in heart rate in horses after

intravenous injection of adrenaline (0.2 mg per animal) before and after tranquillisation with acepromazine (0.2 mg/kg). The mean increase in heart rate produced by adrenaline alone was 43% and following tranquillisation with acepromazine was 105%. They attributed this potentiating effect to an enhancement by acepromazine of the β - receptor actions of adrenaline on the heart as described by Sutherland (1970).

These effects of acepromazine have been discussed in some detail in chapter 7 with reference to the increased sweating noted following premedication with both acepromazine and azaperone. α -adrenergic receptor blocking drugs and members of both the phenothiazine and butyrophenone groups of tranquillisers have been shown to produce elevated circulating plasma levels of catecholamines either by increasing nor-adrenaline release from nerve endings (Cubeddu et al, 1974; Herbaczynska and Stabzewska, 1972) or by reducing the uptake of catecholamines into tissues (Hertting et al, 1961). Because of the α - adrenergic blocking properties of acepromazine and azaperone it is therefore possible that these elevated levels of catecholamines will exercise their greatest effect on the β - receptors.

Unfortunately in this study plasma nor-adrenaline levels were not estimated following acepromazine and azaperone premedication. Following 'Immobilon' injection however there was no apparent *further* elevation in circulating nor-adrenaline values suggesting that the higher heart rates were a result of the lower blood pressure levels recorded. Similar hypotensive effects of azaperone would also

explain the tachycardia noted following administration of this drug.

Towards the end of the 30 min period of recumbency following etorphine administration, heart rates increased markedly. This effect occurred at the same time as all three animals began to show signs of increased activity viz. paddling with all four legs and in the case of one pony trying to stand up. The tachycardia could be due to elevated sympathetic activity in association with the movement or more probably to the animals recovering from the effects of the analgesic and being aware of their surroundings with associated fear.

Xylazine premedication and pre-treatment with the specific β - adrenergic blocking drug propranolol both reduced the tachycardia following etorphine administration. The mode of action of xylazine is not known, although it has been reported to exert central and peripheral effects decreasing sympathetic tone (Schmidt et al, 1970). Such an action could bring about a reduction in heart rate and would also produce a decrease in blood pressure due to vasodilation. Although blood pressures were not recorded in these experiments previous workers have demonstrated hypotension in horses following administration of this drug (Kerr et al, 1972; Clarke and Hall, 1969).

Propranolol blocks both β_1 and β_2 receptors in the body (Lands, Arnold, McAuliff, Luduena and Brown, 1967; Goodman and Gilman, 1975) which mediate the positive chronotropic and inotropic effects of the sympathetic system. It has been employed previously to reduce 'Immobilon' induced tachycardia (Daniel and Ling, 1972;

Schlarmann *et al*, 1973) and in this study it proved as successful as xylazine in decreasing the elevation in heart rate produced by etorphine.

Plasma nor-adrenaline levels following propranolol administration remained as high as those recorded after etorphine alone because the former only blocks the β -receptor sites and does not interfere with the synthesis or release of catecholamines.

The sinus arrhythmia recorded in all three ponies following etorphine, 'Immobilon' and the various drug combinations was probably due to the influence of the medullary inspiratory centre on the cardioinhibitory centre (Swenson, 1970). Respiratory induced alterations in the activity of this cardioinhibitory centre result in rhythmic increases and decreases in its efferent outflow to the vagal nerves thus producing changes in heart rate. That the effect was not abolished by β -receptor blockade with propranolol further suggests that this action is centrally mediated and not due to local sympathetic activity on the heart.

The hypotensive effects of acepromazine have been discussed previously (chapter 7). It was interesting to note that administration of etorphine at the time corresponding to the peak of this hypotensive effect greatly reduced the increase in blood pressure produced by etorphine. The hypertensive effect was not abolished completely since acepromazine is a competitive α -adrenergic receptor antagonist and increased circulating catecholamine levels will still result in some degree of hypertension. In the practical usage of the acepromazine - etorphine mixture it would be of considerable benefit to use the acepromazine in advance of the

etorphine since it is probable that the majority of accidental deaths using the combination are a result of the excessive hypertension produced.

The most marked effects on blood pressure were seen when propranolol was administered along with acepromazine. This was to be expected because of the simultaneous α and β adrenergic blockade occurring.

An increase in sympathetic tone would also account for the changes in P.C.V. recorded after etorphine administration. As discussed previously (chapter 1) the splenic capsule is rich in α - adrenergic receptors under the control of the sympathetic system (Davies and Withrington, 1973). Following etorphine injection the P.C.V. remained elevated throughout the period of recumbency and after injection of the antagonist suggesting that an increase in sympathetic tone was present during all of this time.

The α - adrenergic blocking action of acepromazine has been demonstrated by numerous workers and in this study to block sympathetic activity in the spleen (Turner and Hodgetts, 1960; De Moor and Van den Hende, 1968; Gartner et al, 1965). With 'Immobilon', increases in P.C.V. still occurred probably as a result of the massive elevation in circulating nor-adrenaline levels not being completely blocked by the α - adrenergic blocking action of the tranquilliser.

The increased muscle activity and tone recorded following etorphine administration are possibly further indications of increased sympathetic tone although Lees and Hillidge (1976) suggested that

they more resembled a state of tonic convulsion brought about by the high doses of etorphine included in the 'Immobilon' mixture. This is further indicated by the fact that pretreatment with propranolol did not reduce the muscle activity or tone recorded following etorphine injection suggesting that peripheral sympathetic activity is not responsible for these alterations.

The reason for azaperone premedication producing no reduction in muscle activity throughout recumbency and only a slight decrease in muscle tone when acepromazine and xylazine reduced both is not known. It would appear however that modification of these effects is connected with the duration of recumbency produced by each drug combination. Both azaperone and xylazine administered intravenously produced satisfactory recumbency lasting for only 30 min reflecting their short duration of action when injected by this route compared to acepromazine.

Intramuscular premedication with azaperone however did not increase the duration of recumbency whereas xylazine administered by this route did. This difference could be due to the fact that the dose of xylazine intramuscularly was six times the intravenous dose whereas with azaperone there was only a two fold increase in dose rates by the different routes. In retrospect it would have been better to increase doses of each drug equally but at that time the dose rates chosen were according to dosage regimes used by other workers.

The degree of sweating recorded during recumbency was greatest following 'Immobilon' and acepromazine and azaperone premedication

prior to etorphine. A possible explanation for the effects of these tranquillisers has been discussed previously (chapter 7).

Severe respiratory depression was reported following etorphine administration on every occasion irrespective of pretreatment with any drug. This effect is well documented in both wild and domestic species (Pienarr et al, 1966; Blane et al, 1967; Schlarman et al, 1973; Hillidge and Lees, 1971 and 1975).

The degree of hypoxia produced has been suggested to be due to three factors - the position of lateral recumbency during immobilisation, the reduced respiratory rate due to the central depressant action of the drug and the laboured character of the respirations because of the generalised increase in muscle tone (Hillidge and Lees, 1975). These authors studied the respiratory effects of 'Immobilon' in detail and concluded that the degree of hypoxia produced must be regarded as a serious side-effect. They also suggested that some of the cardiovascular effects of 'Immobilon' such as hypertension and increased P.C.V. might be attributed either directly or indirectly to the effects of the hypoxia.

In this study respiratory depression was present throughout the period of recumbency whereas, as stated previously, plasma nor-adrenaline levels decreased towards the end of recumbency. This suggests that whereas hypoxia may be a contributory factor towards increased sympathetic tone it is not the only mechanism involved.

In their studies Hillidge and Lees (1975) recorded a significant fall in arterial oxygen tension and a small decrease in arterial pH with corresponding small increases in arterial carbon

dicxide tension. In these experiments a degree of cyanosis was noticed, particularly in pony No. 3. This effect has also been reported by other workers (Schlarman et al., 1973) and is probably due to a reduction in the oxygen uptake into the blood. (Nunn, 1969).

Premedication with all three tranquillisers produced little effect on the degree of respiratory depression recorded following etorphine. Since none of these drugs exhibited marked effects on respirations when administered alone this was not unexpected.

The sudden increase in respiratory rates after injection of the antagonist was probably due to the animals need to elevate arterial oxygen tensions.

Plasma 11-OHCS levels following etorphine administration were lower than those recorded after 'Immobilon' or acepromazine premedication. This could be due to the tranquilliser per se producing an increase in 11-OHCS values. It has been reported that chlorpromazine increases ACTH secretion from the pituitary and consequently produces an elevation in plasma 11-OHCS concentrations (Smith et al., 1963).

The fact that acepromazine premedication resulted in higher 11-OHCS values than 'Immobilon' could result from the time elapsed between tranquilliser and etorphine administration. In chapter 7 the effects of acepromazine per se on plasma 11-OHCS levels were recorded, maximum values occurring at 60 min post-injection. Peak levels following acepromazine premedication were noted at 50 min post-injection (25 min post-etorphine) suggesting that the increase produced by etorphine was further elevated by that produced by the

tranquilliser.

The increases in plasma 11-OHCS levels reported in this study occurred at the same time as the elevations in sympathetic tone as estimated by plasma nor-adrenaline levels. Although James *et al* (1970) recorded no increases in plasma cortisol levels following adrenaline infusion in the horse it would appear from these and other results in this study that the mechanisms of control of the H.P.A. system may be influenced by sympathetic stimulation. Certain other factors however such as the hypoxia associated with etorphine may also be involved.

Summary

From these results it would appear that etorphine administration results in an elevation in sympathetic nervous activity which may be due in part to direct central or local effects of the drug on the sympathetic system or to the degree of hypoxia produced. All the changes in heart rate, blood pressure and packed cell volume recorded can be attributed to this alteration in sympathetic tone and to reflex mechanisms controlled by higher centres in the brain.

The tachycardia produced by etorphine injection could be successfully reduced by premedication with xylazine or pretreatment with the β - adrenergic blocking drug propranolol. The hypertension produced by etorphine could also be successfully reduced by premedication with acepromazine.

Both acepromazine and xylazine premedication reduced the degree of muscle activity and tone recorded following etorphine

administration but acepromazine and azaperone produced a greater degree of sweating.

Premedication with the tranquilisers had no beneficial effects on the respiratory depression recorded following etorphine and as described previously acepromazine increased plasma 11-OHCS levels above those measured after administration of etorphine alone.

10. FINAL CONCLUSIONS

FINAL CONCLUSIONS

The aim of this study was to determine whether measurement of adrenocortical and sympathetic nervous activity could be successfully employed as a means of assessing stress in horses.

Estimation of adrenocortical activity was carried out using a modified Mattingly (1962) fluorometric technique which successfully measured plasma 11-OHCS values in horses. Sympathetic nervous activity was measured by indirect methods in all but one section of the study when plasma noradrenaline levels were estimated directly. This was due to the numerous difficulties associated with catecholamine assay in plasma and the convenience with which heart rate, packed cell volume and arterial blood pressure can be measured. Difficulties are involved in the interpretation of such indirect readings, including reflex responses such as when heart rate slows in response to increased blood pressure and vice versa (Rushmer, 1972).

The elevation in plasma nor-adrenaline levels following etorphine administration showed that increased sympathetic tone had occurred and this was probably responsible for all the alterations in the cardiovascular system noted at the same time. It is still not clear however whether this increased sympathetic activity is due to a direct action of the drug etorphine locally, at nerve endings or on the adrenal medulla, or whether it is due to central stimulation of the vasomotor and possibly other centres. Since propranolol, a specific β - adrenergic blocking drug, did not reduce the degree of

muscle activity and tone following etorphine administration suggests that this effect is not mediated through the autonomic system but could be due to a morphine like induced-convulsive state as reported by Hillidge and Lees (1976).

Etorphine produced elevations in heart rate, blood pressure, packed cell volume and plasma 11-OHCS levels as did the minor surgical procedure (muscle biopsy) and physical exercise. These three stimuli therefore all constitute stress if this is defined as any stimuli resulting in elevated adrenocortical and sympathetic nervous activity. Etorphine however produced these effects by mechanical actions and consequently it is difficult to use these parameters to assess whether or not any emotional or psychological disturbance also occurred in the animals. During muscle biopsy and the exercise tests the horses appeared disturbed or excited i.e. they moved around restlessly and often became agitated. From this point of view stress was apparent both visibly and in the changes recorded in adrenocortical and sympathetic nervous activity.

The number of central depressant drugs tested in this study all possessed varied pharmacological actions which interfered with their assessment as efficient tranquillising drugs (efficient being defined as drugs which would prevent or reduce any elevation in adrenocortical and sympathetic nervous activity produced by a stressor). Although azaperone successfully allowed muscle biopsies to be taken from all the horses viz. although it visibly appeared to reduce the stress the animals were undergoing, it and the other two drugs (acepromazine and xylazine) all produced per se effects on heart rate and/or packed cell volume and/or blood pressure and/or plasma

11-OHCS levels.

The effects of tranquillising drugs on the changes in heart rate and plasma 11-OHCS levels produced by disturbing stimuli have been employed previously in horses as means of assessing the efficacy of the drugs concerned (Aitken and Sanford, 1972). From the results in this study it would appear that this is not an ideal method. However, quantitative methods of assessing stress must be used in animals since no information can be obtained on the psychological or emotional state of the subjects. With horses it would appear that subjective assessment is also a useful guide to the degree of stress present.

The investigation into the α - adrenergic blocking actions of several commonly used tranquilliser drugs showed that the drop in packed cell volume, associated in particular with acepromazine and azaperone administration, is due mainly to relaxation of the splenic capsule and accumulation of red cells in the spleen and to a small extent to haemodilution brought about by increased plasma volume. There was no evidence that the central depressant actions of any of the drugs was the cause of the reduction in P.C.V. as previously suggested by Turner and Hodgetts (1960).

Premedication with acepromazine and propranolol (a specific β - adrenergic receptor blocker) also successfully reduced the cardiovascular effects of etorphine. Because 'Immobilon' is so widely used in the equine field and because of its potentially dangerous side-effects especially in animals which may suffer from cardiovascular or respiratory disease it was important to investigate

the possibility of modifying the pharmacological actions of etorphine by direct effects of other drugs. By administering these agents at their times of peak efficacy prior to etorphine, it is therefore possible to produce the same degree of neuroleptanalgesia with less physiological stress to the animal.

Stress must still be considered as being a difficult parameter, to define in the horse but measurement of adrenocortical and sympathetic nervous activity, if considered in conjunction with other physical signs can be employed usefully as an indicator of this condition.

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