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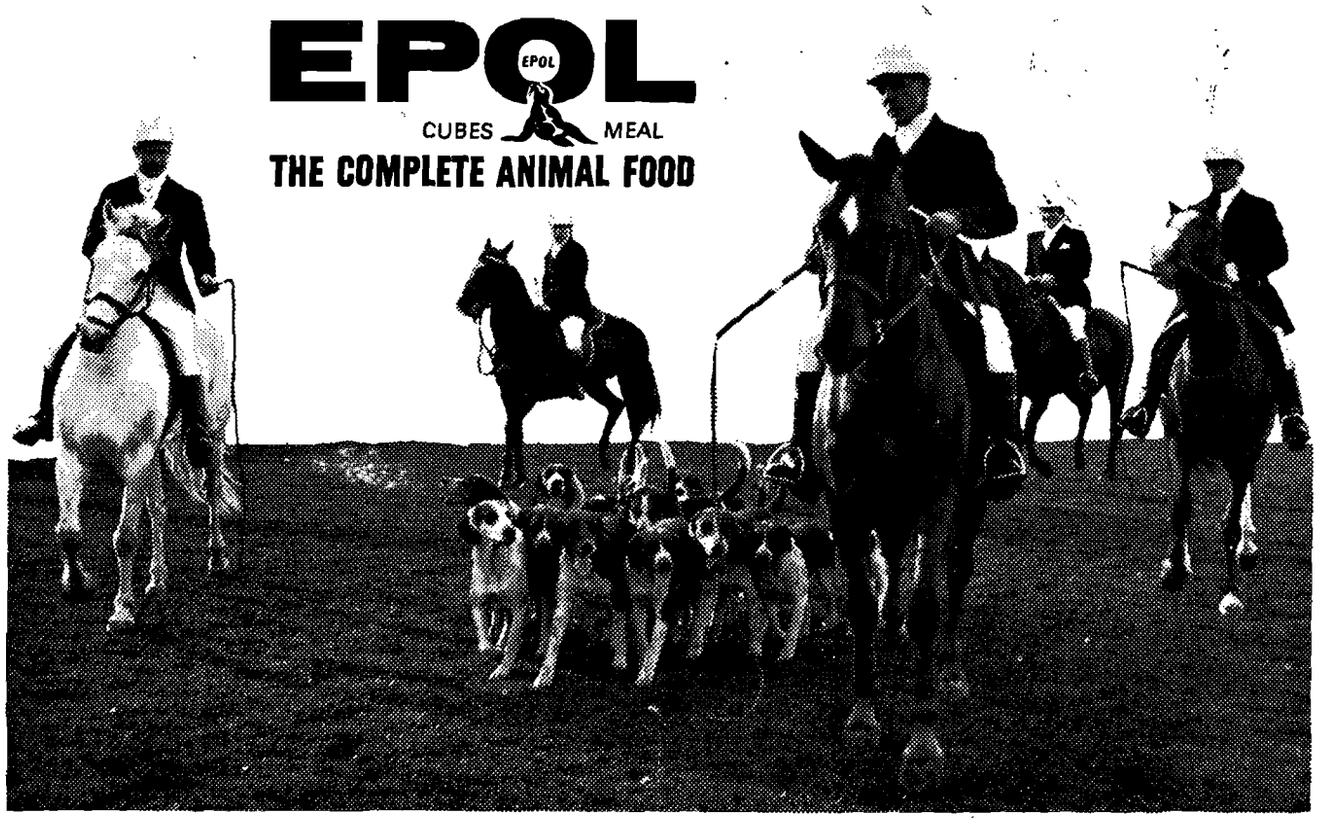

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EDITORIAL NOTE

PROCEEDINGS OF THE FIRST INTERNATIONAL EQUINE VETERINARY CONFERENCE, PRETORIUSKOP, KRUGER NATIONAL PARK, AUGUST 5-10, 1974

VERHANDELINGE VAN DIE EERSTE INTERNASIONALE HIPPIATRIESE KONGRES, PRETORIUSKOP, KRUGER NASIONALE WILDTUIN, AUGUSTUS 5-10, 1974

FIRST INTERNATIONAL EQUINE VETERINARY CONFERENCE 1974

In Western history for centuries the horse had been the primary object of veterinary attention, until the full impact of the advent of motor transport and the increasing demand for animal protein soon thereafter caused the veterinarian of necessity to turn his attention to farm livestock. Intensified urbanization, meanwhile, had elevated small animal practice.

Increasing affluence brought about indulgence in the sport of kings and of gentlemen; the horse, once regarded as on its way out, has become re-instated, in a nobler form than before. This swing of events is reflected in the holding of the First International Equine Veterinary Conference at Pretoriuskop in the Kruger National Park from August 5 till August 10, 1974, a month before the World Association of Buiatrics was due to hold its eighth international meeting in Milan.

The veterinary profession in South Africa, and particularly the Equine Practitioners Group of the South African Veterinary Association, is honoured and proud to have been able to take the lead in organizing a successful first international conference – hopefully the forerunner of many more – and to act as hosts to a gathering of distinguished visitors. Equally, the Editorial Committee of the Association is proud to be able to devote two issues of this Journal entirely to the Conference Proceedings.

It would be puerile to expect final answers in these papers but extremely helpful guidelines have been laid down.

Tribute is paid to Dr M.A.J. Azzie, founder of the Equine Practitioners Group of the South African Veterinary Association, for conceiving the idea of an international conference of this kind and for his dogged perseverence in bringing the idea to fruition. The major load of organization was willingly borne by him.

To Professor Frank J. Milne the Editorial Committee extend their deep indebtness for his meticulous, hard work as Conference Proceedings Editor. It was a major accomplishment on his part to be able to hand over the edited version of all the papers submitted (which means all the papers but two) to the Journal Editor before his departure from South Africa. A major part of the first session's discussions had also been under his care. It has been a pleasure and an educational experience to collaborate with him.

The infra-structure of the Conference, the social side and the 17 and 11 day tour organization were in the hands of a veritable Jeeves, in the person of the inimitable Bill Mounsey of Festivals and Conventions Trust (Pty) Ltd (FACT).

Except for the occasional group photograph, no report on the social activities, formal or informal, will appear in these pages. Nevertheless, it would be grossly unjust not to make mention in passing of the grand spirit exhibited and goodwill generated at the various get-togethers. Veterinarians the world over do indeed possess remarkable and unsuspected potentialities, as the feature pages in this and the next issue will show.

Readers and contributors should please note that the international metric system (SI) is followed as far as possible. Litres are indicated by *l*. – EDITOR, Jl S. Afr. vet. Ass.

FOREWORD

The December, 1974 and March, 1975 issues of the Journal of the South African Veterinary Association carry the Proceedings of the highly successful First International Equine Veterinary Conference held in the Kruger National Park during August, 1974. The part of the Conference which covered the parameters indicative of performance ability and state of fitness will be found in this, the December, 1974 issue, while that part of the Conference dealing with the alimentary tract will be featured in the March, 1975 Journal.

Because this typewriter can probably not spell in the Scottish dialect, at best, we can reproduce the philosophy of the great Robbie Burns by saying that the best laid plans of mice and men often go wrong, and it was unfortunate that the well-laid arrangements to have a steno-typist present to record the extemporaneously delivered contributions could, for reasons beyond control, not be fulfilled. This meant that at the last minute alternative arrangements had to be made to have the discussions taped, transcribed, typed and returned where possible for the confirmation of the speakers concerned. Unfortunately, distortion occurs in all forms of transmission, and this may be reflected in the discussion following the main presentations. No apologies are offered to those whose

extemporaneous remarks appear mutilated, for this could have been overcome, or at least avoided, if the persons concerned had accepted the invitation of the Programme Chairman to submit their questions and answers in writing!

To Professor H.P.A. de Boom and Miss Dianne Fulton, Dr. Azzie's efficient and hard-working secretary, my grateful thanks are due for all the work they did to publish these Proceedings within a reasonable time of the completion of the Conference.

It was an honour and a privilege for me to be invited to participate in this International meeting.

F.J. Milne,
Editor, Proceedings of the First International Equine Veterinary Conference.

INAUGURATION OF A WORLD EQUINE VETERINARY ASSOCIATION

INVITATION

Equine veterinary services have progressed so rapidly over the past fifty years that equine veterinarians have developed specialized practices all over the world.

The wave of enthusiasm, led by the AAEP, followed by the BEVA, has extended to other countries.

The South African Equine Practitioners Group held the first International Equine Veterinary Congress in August, 1974. The reproduction specialists held an International Symposium in the United Kingdom in July, 1974. Both of the above meetings were attended by approximately 200 delegates.

It is clear from the success of these two events and the continuing interest indicated by the attendance of domestic annual equine conferences in many countries of the world, that the time is now ripe to coordinate the scientific knowledge of the world.

The Convenor's introduction at the First International Equine Veterinary Congress indicates the needs and the advantages to be gained from a world body of this kind.

An informal meeting was held during this Congress to probe the individual feelings of the attending delegates. There were sixteen countries represented and the principle was favoured by all as individuals.

The Chairman of the Australian Equine Veterinary Association extended an invitation to hold the next International Equine Veterinary Conference at Fiji in 1976, where they would be hosts.

Attention was also drawn to the fact that the BEVA is holding its 1976 meeting in the Republic of Ireland. The importance of World Equine Veterinary Association to prevent overlaps, that might occur, is urgently required. I am sure many of us would like to attend both meetings.

The co-operation of Equine Veterinary Associations from most countries in the world could greatly assist the inauguration of a World Equine Veterinary Association and, I am sure, the knowledge of every Equine Veterinarian in the world and his country will benefit considerablv.

The Equine Practitioners Group of the South African Veterinary Association will work as Convenors until a Committee is formed.

The following objectives of the World Equine Veterinary Association have been drawn up provisionally:

1. To establish liaison between all Equine Veterinary Associations and Groups.
2. To provide an information service of all Equine Veterinary Meetings and new Equine veterinary literature.
3. To establish a register of all Equine Veterinary Associations and aim to have these national Associations as members of this World Group.
4. To advance and exchange veterinary knowledge,
   a. by regular International Conferences
   b. by action on the recommendations made by national associations that are of International importance, *i.e.*, the recognition of blood typing for parentage and registration of horses, standards of soundness examinations, clarification of destruction on humane grounds, international movement of horses and doping of race horses, etc.
   c. by liaison of post-graduate student exchange.

Further Recommendations for Discussion are Welcome

The above function will be carried out by a Committee formed by representatives from each Equine Veterinary Association or Group. It is hoped to have an inaugural meeting not later than June, 1976, when the representatives will meet to draft a constitution and make recommendations. It is not predicted to meet more than once a year, or more likely every second year. The expenses of travel and membership are envisaged to be the responsibility of the National Association and the venue will be selected as centrally for the expected attendance as possible.

An appeal is made to advise us of your interest and to make whatever suggestions you deem appropriate to:-

Dr. M.A.J. Azzie,
P.O. Box 4024,
Alrode 1451
Transvaal
South Africa

OPENING ADDRESS

A.B. LA GRANGE

PRESIDENT SAVA

It is indeed a very great honour and privilege to have been invited by the Chairman to this very select International Conference of the Equine Practitioners Group of the S A V A. As a prelude to this Conference I had the good fortune of winning my office pool's July fortune of R9 this year. It is the first clear profit I have ever shown on betting and the first money I have received while not having worked for it. My other ventures in this line have all been unsuccessful.

This First International Equine Veterinary Conference is a momentous occasion to which many private practitioners and members of our Faculty of Veterinary Science have been looking forward. It is not only important for those who are actively engaged in equine work but prides our entire profession, for what is being initiated here on an international level of exchange of knowledge can become a permanent institution.

We are proud to receive all our overseas' colleagues in our country under these special and unique conditions. We know that we will benefit greatly from your wide field of knowledge which you are willingly sharing with us and we trust to contribute meaningfully to the proceedings as well.

If one thinks of the equine species, be it as a student or as a veterinarian, or the average citizen, one automatically visualizes the Thoroughbred horse winning the July, or the Derby, or the Oaks, or other famous and traditional races all over the world. I do not think there is any other animal that grasps the imagination of the public to the extent that a fine and well-trained horse can do, be it as a July winner, or a Five-gaiter, or a Clydesdale pulling the beer wagons, or a jumper clearing his jumps, or seeing someone expertly mounted on the back of his best friend, trotting around the outskirts of the cities.

The horse has been one of mankind's biggest friends for many centuries. In fact, as an important part of man's activities, horses date back to well over 4 500 years. Tribes in places as far apart as Mesopotamia and China had domestic horses 4 000 years ago. Domestic horses were recorded in ancient Greece as early as 1 700 BC and in Egypt in 1 600 BC. There is reference to a mounted Olympiad about 640 BC. It was the Romans who standardized horses as a means of sport and recreation. Ironically, however, when Caesar invaded Britain in 55 BC he was astounded to find himself opposed by charioteers.

To the British must go the credit of organized horse racing and the developing of the noblest horse breed – the Thoroughbred. Over the centuries it has spread, as the Sport of Kings, to all countries, even to those without kings.

Thinking of countries like America, England, France, Japan, Australia, and many others, also this country, we realize that this is a massive industry and we are called upon to look after the health, soundness and continuation thereof. In the U.S.A. the earnings of top horses far outstrip their counterparts in the rest of the world, though Japan is showing remarkable progress. In the 1968 Japan Derby there were 161 000 spectators who betted 11 500 000 dollars on a single race.

Even in our small country the growth in meetings and gambling in horse races are quite astounding. It becomes impressive if we bring it in line with our definite, ultra-conservative approach to these matters. Our authorities firmly believe in the old saying:

If you win money on the races, that's Gambling.
If you win money at bridge, that's Social Amusement.
If you win money on the Stock Echange, that's a Miracle.

In 1968 there were 333 meetings with a total stake of 3,4 million and a total turnover of 54,2 million Rand. In 1973 these figures jumped to 437 meetings, with stakes of 6,2 million and the staggering total figure of 134 million Rand. It is estimated that the total gambling spree is in excess of 300 million Rand per annum. Over the corresponding period yearling sales have risen from 356 in 1968 to 702 in 1973. The value of these sales jumped from 1,2 million to well over 3 million Rand per annum.

It is to be expected that big money will always have a positive as well as a negative side, be it in horse racing, American saddler competitions and shows, or Arab shows, etc. In general, laws, rules and regulations of countries, societies and associations are daily being tested to the extreme by modern man. This is part of the life style of the modern era in which we live. Whether we like it or not, we are part of this society and part of this era, where the old traditions and the dignity and nobility of it is being opposed and undermined by conniving and all sorts of intrigues.

It gives me immense pleasure to be here today at the beginning of a conference of equine specialists who are geared towards the positive side of this enormous industry. On studying your congress programme one can only be impressed by the papers that will be read and the field that will be covered.

This does not only reflect the positive side of equine practice and research, but it also typifies the desire to learn. Continued education is an integral part of modern veterinary practice. It is the veterinarians' sincere desire and constant effort to keep abreast of science and technology, modern techniques and research.

It is unfortunately true that in many instances and occasions the extremely important contact and liaison between research institutions and faculties of veterinary science and private practice is not as close as it should be. Nothing can be more detrimental to progress than a lack of liaison and close collaboration between these three vital links.

I am sure that this international conference of equine specialists and practitioners which is now being opened will strengthen these links and that everybody present will leave here enriched and revitalized with knowledge, skills, techniques and new ideas.

On behalf of the Parent Body, I wish to congratulate and thank Dr Azzie and his co-workers for the inspired effort, the energy they have expended and work they have done to make this conference a success.

Mr. Chairman, it is indeed a very great honour and privilege to now declare your congress formally open and to wish you success, happiness and enjoyment for the days ahead.

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The First International Equine Veterinary Conference organisers would like to pay tribute to all the donors for their many generous contributions. This excellent support has enabled us to stage this Conference at a level in which we can all take pride.

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PROCEEDINGS OF THE FIRST INTERNATIONAL EQUINE VETERINARY CONFERENCE

INTRODUCTION

M.A.J. AZZIE
Convenor: First International Equine Veterinary Conference

The importance of a congress such as this to the equine community needs no emphasis.

As equine specialists, we will exchange scientific knowledge at an international level. We will meet our colleagues who are engaged in similar research or clinical investigations. This will provide an opportunity to exchange information on the progress of personal research. It will seed new thoughts of projects which require investigation. It will avoid over-lapping of research and the duplication of similar investigations without justification. This contact will provide guidance to new literature. This meeting provides an opportunity to discuss our projects with other experienced equine veterinary specialists, who may have different views which will be defended publicly.

The practitioner will be greatly rewarded by the consolidation of considerable research knowledge and by participating in its discussion.

The standard of veterinary science and clinical veterinary medicine can be perpetually elevated by regular meetings of this kind, and great professional pride will be accomplished by enhancing the progress of science. The economics of the entire equine industry will benefit.

It is hoped that the publication of the proceedings of this meeting will stimulate the desire of every country to support the inauguration of a World Equine Veterinary Association so that similar meetings will follow.

SECTION A: SOME PARAMETERS INDICATIVE OF PERFORMANCE ABILITY AND STATE OF FITNESS OF EQUINES

First Session: CARDIOLOGY, HAEMATOLOGY AND PERFORMANCE

Chairman: A. LITTLEJOHN

AN INVESTIGATION OF CARDIAC RHYTHM USING AN ON-LINE RADIOTELEMETRY/COMPUTER LINK

J.R. HOLMES*

SUMMARY

The paper describes the findings in various types of arrhythmia at rest and after exercise using an on-line radiotelemetry/computer link to measure and plot heart beat interval durations.

INTRODUCTION

From the haemodynamic point of view, the circulation can be regarded as a closed system with a pump at its centre. In an inanimate situation, to maintain regular flow, the pump should pulsate regularly; any irregularity will have repercussions on fluid flow in the system. In the living animal the analogy is not quite true, however, for a variety of other mechanisms besides cardiac contractions influence blood flow through the arterial, capillary and venous channels. Particularly in the resting horse, in which species the heart rate is comparatively slow, it is apparent that irregularities may occur in rhythm which are compatible with normality. Horses with a variety of arrhythmias at rest have been found to be capable of completing the requirements of Three Day Event Trials which represent a fairly severe test of cardiac efficiency[2]. It is likely that these irregularities, observed at rest, disappeared when heart rate accelerated and there is good evidence that in fact at high heart rates these horses have regular rhythm. The conclusion to be drawn from this kind of observation is that at slow resting heart rates (30-40 per minute), some apparently normal horses may have rhythm irregularities but that in many of them the irregularities disappear when heart rate accelerates and thus do not impair performance. The persistence of arrhythmia at high heart rates, however, during or immediately after exercise, would probably justify a guarded prognosis, for at these times circulatory efficiency is of major importance, particularly when a temporary oxygen debt may occur, the repayment of which is favoured by an efficient blood flow through lungs and periphery.

The resting heart rate of different horses varies and it may vary from time to time in an individual horse depending particularly on the activity of the animal and the influence of the environment. It is likely that cardiac rate is slowest when an animal is asleep,

* Department of Veterinary Medicine, University of Bristol, Bristol, England.

whereas movement and feeding may influence resting heart rate. In race-fit Throughbreds particularly, rate may readily be affected by stimuli from the environment. Thus, other horses moving about a stable yard or the approach of the feed bucket may result in short periods of excitement which are associated with transient tachycardia. This generally subsides as quickly as it appears.

The variations in resting heart rate may have a marked influence on the appearance of some arrhythmias (using the term arrhythmia in its broadest sense to include the dysrhythmias). Thus, resting heart rate at the time of examination is very important and in easily excitable horses it may be difficult to obtain an appreciation of rhythm at the real resting heart rate. The appearance of irregularities in rhythm at one examination and their absence at another may depend on the heart rate at the time of the examination. The character of the horse therefore may influence the kind of arrhythmia encountered at rest. The phlegmatic type or one undisturbed by its surroundings and with a slow resting heart rate may often show first and second degree partial AV block whereas the excitable horse with a relatively high "resting" heart rate may not show this dysrhythmia.

When heart rate accelerates, even the most bizarre rhythms tend to become less obvious, so that during exercise, when the heart rate may rise to 230 per minute, arrhythmia may be difficult to detect but it readily becomes apparent when heart rate begins to slow.

METHODS OF STUDYING CARDIAC RHYTHM

An appreciation of rhythm may be gained from pulse palpation or cardiac auscultation at rest and immediately after exercise. The use of electrocardiography has the advantage not only of providing a permanent record of the rhythm but of allowing diagnosis of the nature of any arrhythmia. At rest a conventional electrocardiograph may be used with leads attached directly between horse and machine but this method cannot be applied during exercise.

The first few minutes after the end of exercise is a critical period when heart rate decelerates very quickly, and potentially significant and yet transient irregularities in rhythm may occur. This period is generally missed if a horse has to be connected to a conventional electrocardiograph. The problems may be overcome by the use of radiotelemetry and the method we use has been described.[6] By radiotelemetry, ECG data may be viewed on an oscilloscope, written directly on an electrocardiograph and/or stored on magnetic tape. The introduction of computer methods has greatly increased the usefulness of the radiotelemetry technique. Using a PDP12 computer, the ECG signal received by radiotelemetry may be passed, after suitable amplification, into the computer's clock which may be set to measure time intervals between selected signal peaks such as RR- or SS-intervals and to store these values on sequential blocks of computer magnetic tape. Subsequently the results may be output to a teletype or displayed, 256 values at at time (=1 tapeblock) as a histogram or as 2 x 128 beat-by-beat plots. Thus, irregularities in rhythm may be easily recognized. At the same time, the incoming data are stored on a conventional tape recorder and can later be written on an electrocardiograph for confirmation or study of the nature or any arrhythmia demonstrated by the computer's display (Fig. 1).

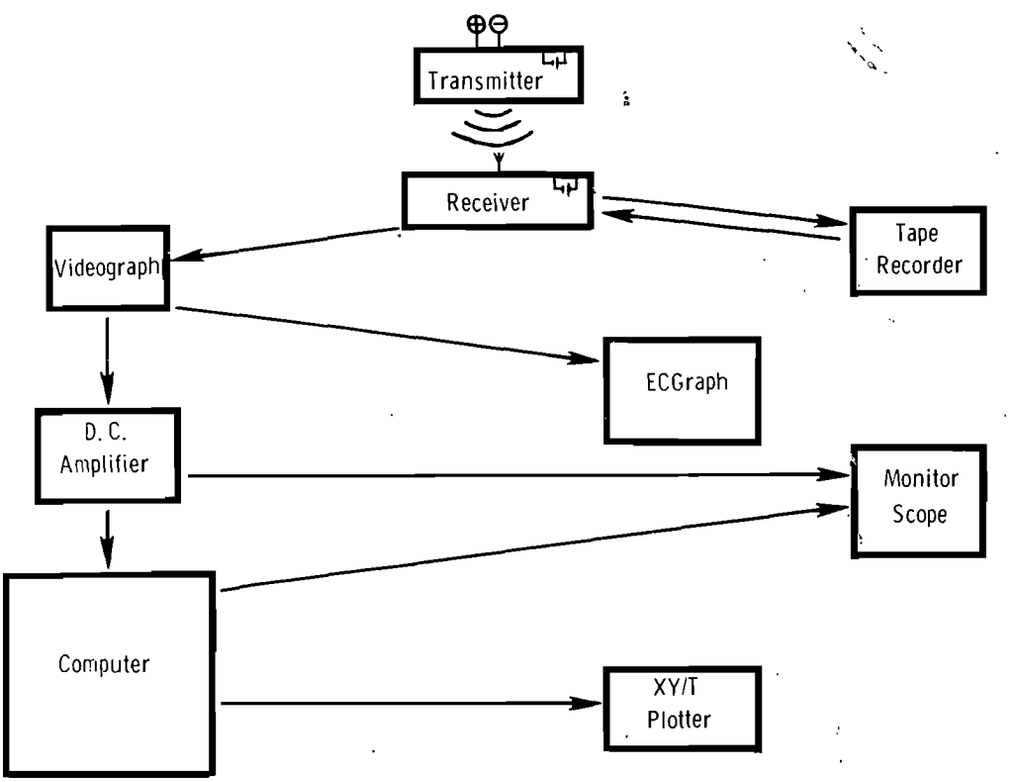

Fig. 1:Arrangement of the transmitter and receiving and recording equipment.

Any display on the computer's oscilloscope may be output to an XY/T plotter and the plots which are used for figures 3 to 16 were drawn directly under computer control. This is a very easy and rapid way of permanently illustrating various types of arrhythmia and any number of sequential beats may be measured and then plotted in blocks of 256 values. It is therefore ideal for long-continued monitoring at rest as well as for observations on the effect of exercise.[3]

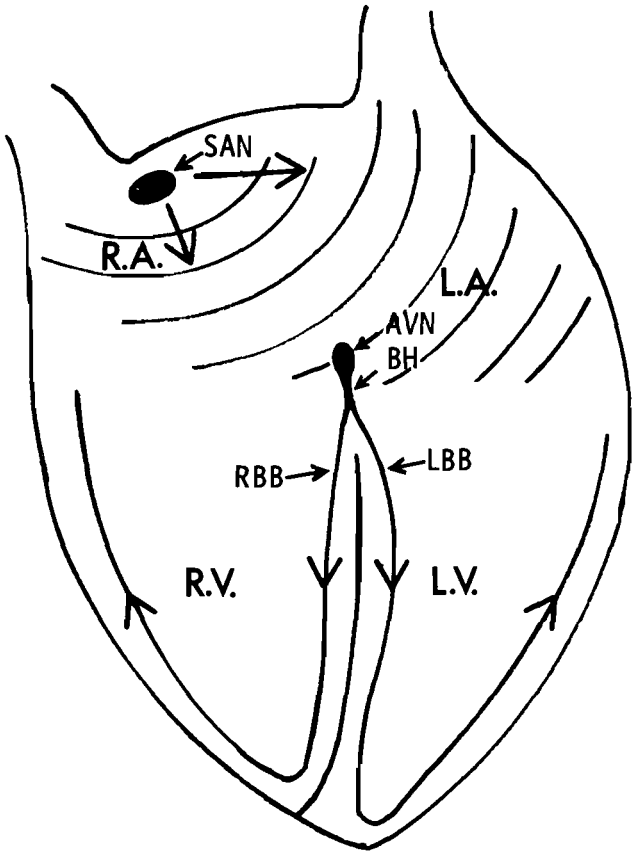

Fig. 2: Normal impulse conduction through the myocardium. The pacemaker is in the sinoatrial node (SAN) from where a ripple contraction mechnically crosses the right and left atria (RA and LA). From the atrioventricular node (AVN) the activation passes through the Bundle of His (BH) and thence *via* the Purkinje pathways in the right and left bundle branches (RBB and LBB) to activate the two ventricles virtually simultaneously.

CLASSIFICATION OF ARRHYTHMIAS

The nature of the more common arrhythmias may be appreciated by a consideration of the normal origin and pathway of impulse conduction in the myocardium (Fig. 2). The pacemaker in the sinus node initiates a relatively slow ripple contraction across the atria followed by stimulation of the AV-node and bundle of His, then rapid transmission through the Purkinje pathways to the right and left ventricles. Irregularities in rhythm may therefore arise as a result of disturbances in impulse formation and from interference in impulse conduction or a combination of the two.

Whilst there are certain clearly recognized arrhythmias which may often be diagnosed on auscultation, sometimes arrhythmias may be very complex with mixed irregularities. Some forms of arrhythmia, particularly atrial irregularities, are also open to different interpretations as to their nature.

Disturbances of impulse formation include effects in the sinus node (pacemaker) and ectopic foci in atria or ventricles which may initiate myocardial contractions. Disturbances of impulse conduction include various forms of heart block. Mixed disturbances of impulse formation and conduction include fibrillation, dissociation and parasystole.

Atrial Fibrillation

This is the commonest cause of the most bizarre rhythm. On auscultation, the profound irregularity, absence of atrial contraction sounds and variation in intensity of first and second heart sounds are indicative of atrial fibrillation. A histogram of 256 beats at rest will show typically a wide range of RR-intervals and the irregularity is very apparent on a beat-by-beat plot (Figs 3 and 4). During exercise, at high heart rates, the arrhythmia persists but is less obvious [4 7]. It quickly becomes very apparent as heart rate begins to slow immediately after exercise (Fig. 5).

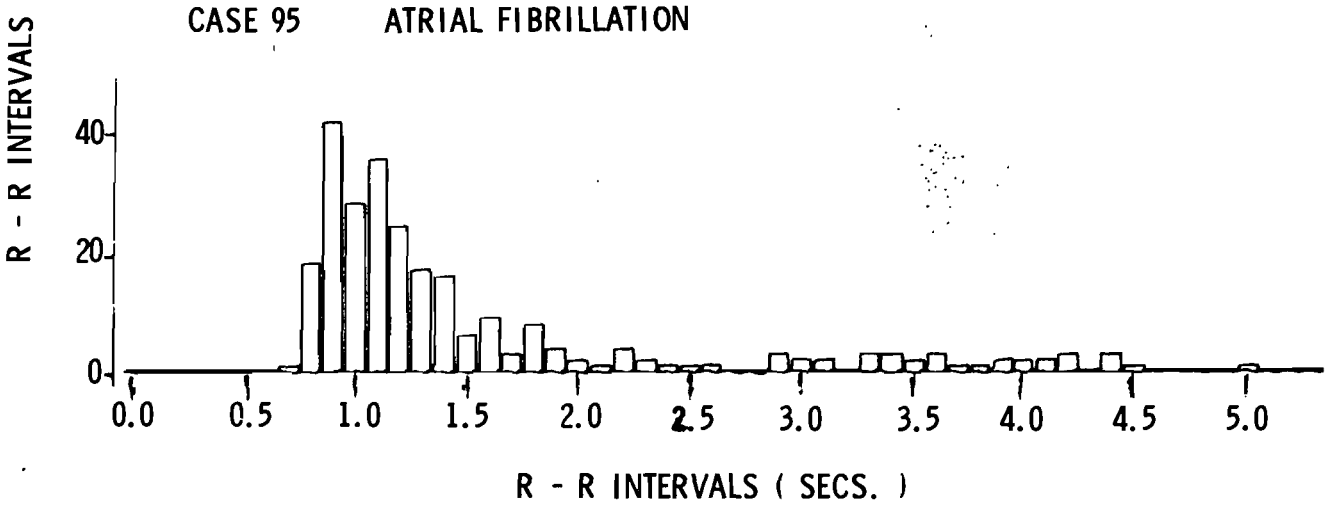

Fig. 3: Case 95. Twenty-years-old Hanoverian spayed mare. Atrial fibrillation. Histogram from 256 heart beat intervals at rest. This mare had had atrial fibrillation for at least 10 years and also had a marked pansystolic murmur. It had been regularly hunted during this period but had become progressively less efficient for sustained gallops and particularly when climbing hills. Note the wide range of RR-intervals typical of atrial fibrillation.

The minimum RR-interval was 0,7 s and the maximum was 5,0 s.

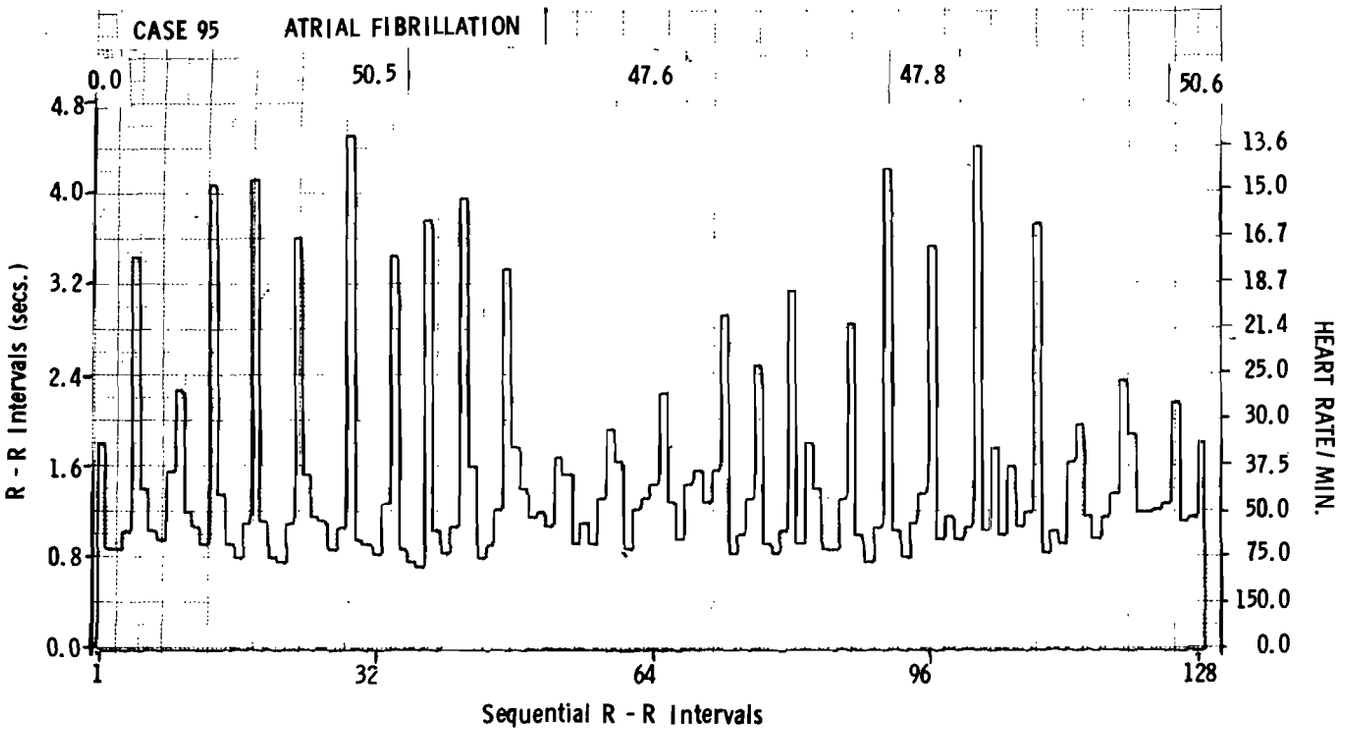

Fig. 4: Case 95. Plot of 128 sequential heart beats comprising part of the histogram of figure 3 to illustrate the bizarre rhythm. The longest interval was 4,53 s, representing an equivalent heart rate of 13/min and the shortest was 0,72 s representing a heart rate of 83/min. The total time for the 128 intervals was 196,5 s (3 min 16,5 s). Despite the marked irregularity, the times for 4 x 32 intervals were not very different (47,6 to 50,6 s).
In all beat-by-beat plots the values above the plots represent the time in seconds for each group of 32 intervals.

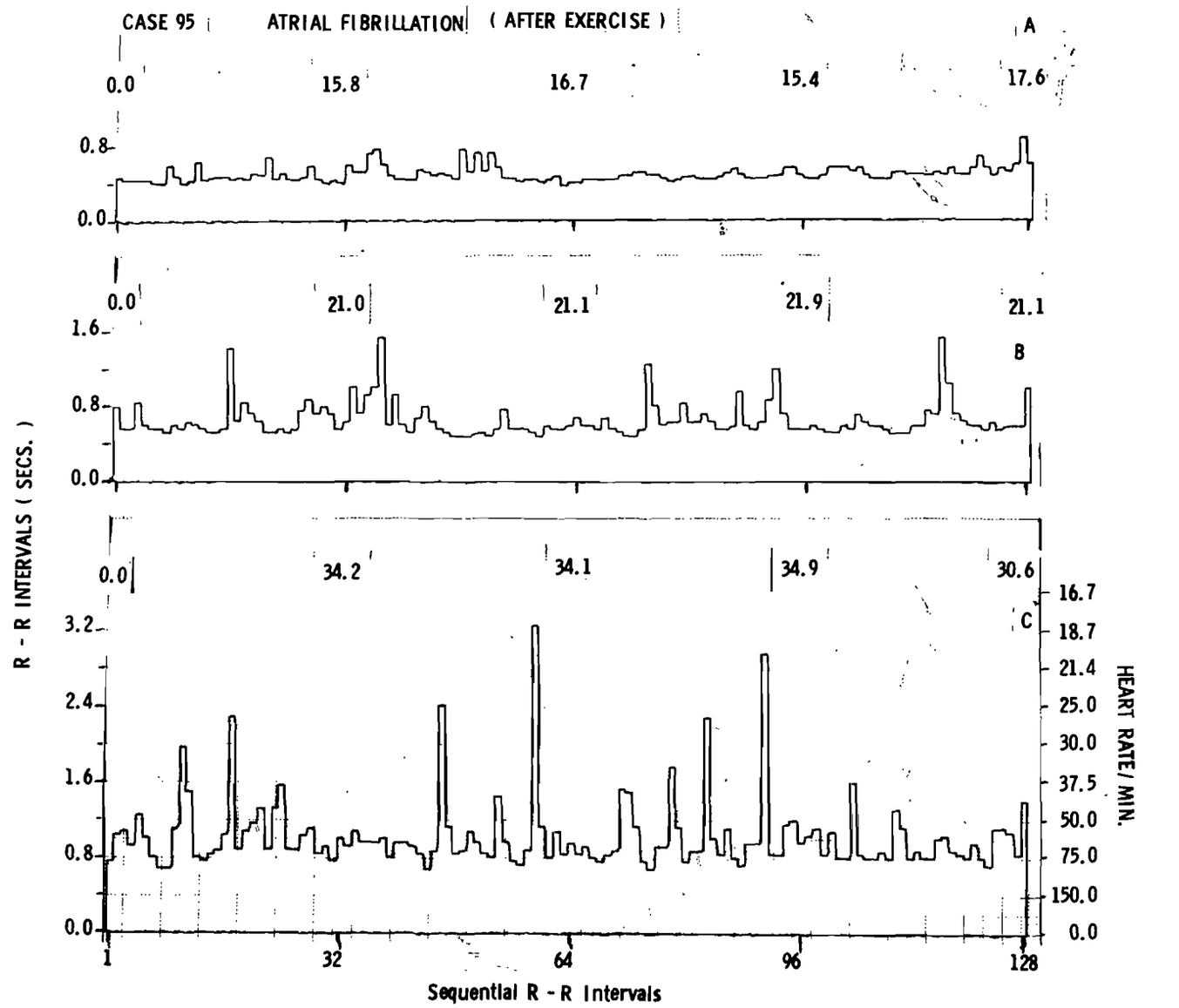

Fig. 5: Case 95. Plots of 128 sequential heart beats.
a) Immediately after 6,75 min lunging exercise.
b) 72,3 s after the send of a)
c) 555,6 s after the end of b)
Note the persistence of arrhythmia at high heart rates and the progressive development of a pattern resembling that observed at rest (Fig. 4).
Total times for the 128 intervals were:-

	Sequential beats	secs.
a)	1 – 128	65,5
b)	257 – 384	85,1
c)	1025 – 1152	133,8

Although there are undoubtedly horses with unsuspected atrial fibrillation which are racing, in our experience this is one cardiac abnormality most often associated with a history of poor performance and fading during racing, and, in the Hunter, dyspnoea on climbing hills. It is the most frequent cause of true jugular pulsation in the horse. Strangely, the condition seems to occur only in big horses and we have

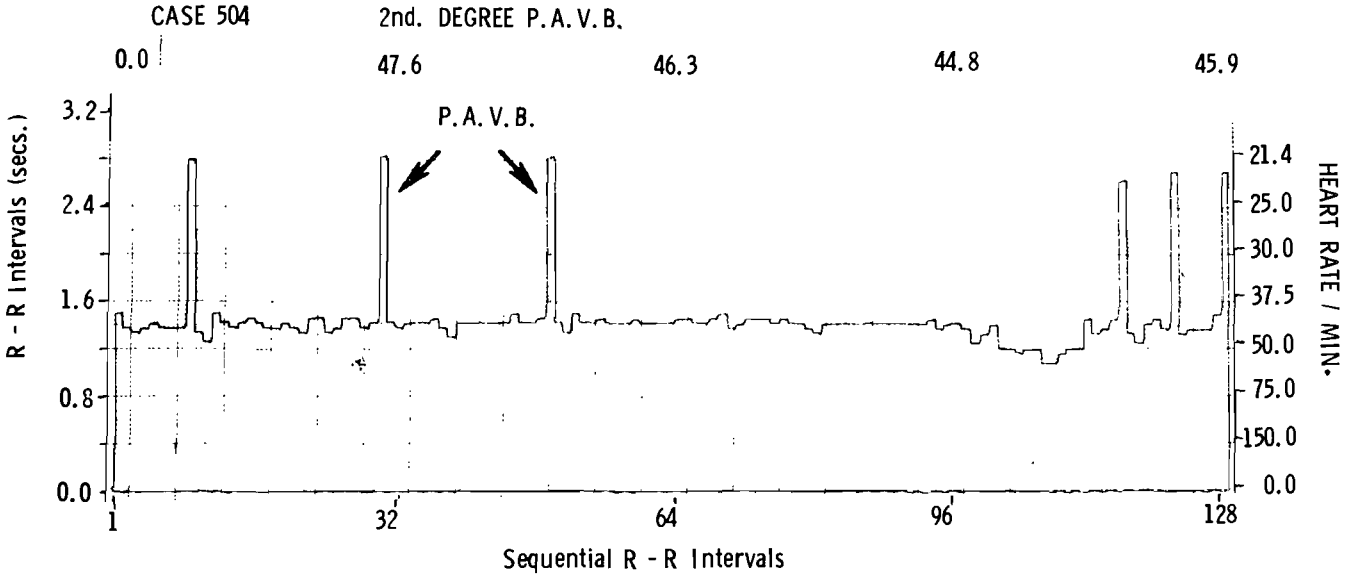

Fig. 6: Case 504. Six-years-old Thoroughbred mare. Plot of 128 sequential heart beat intervals at rest. Note the occasional intervals of approximately twice the normal length. These were due to second degree partial AV-block. The plot shows 6 missed beats in 128 ventricular contractions, occurring at a resting heart rate which averaged 43/min.

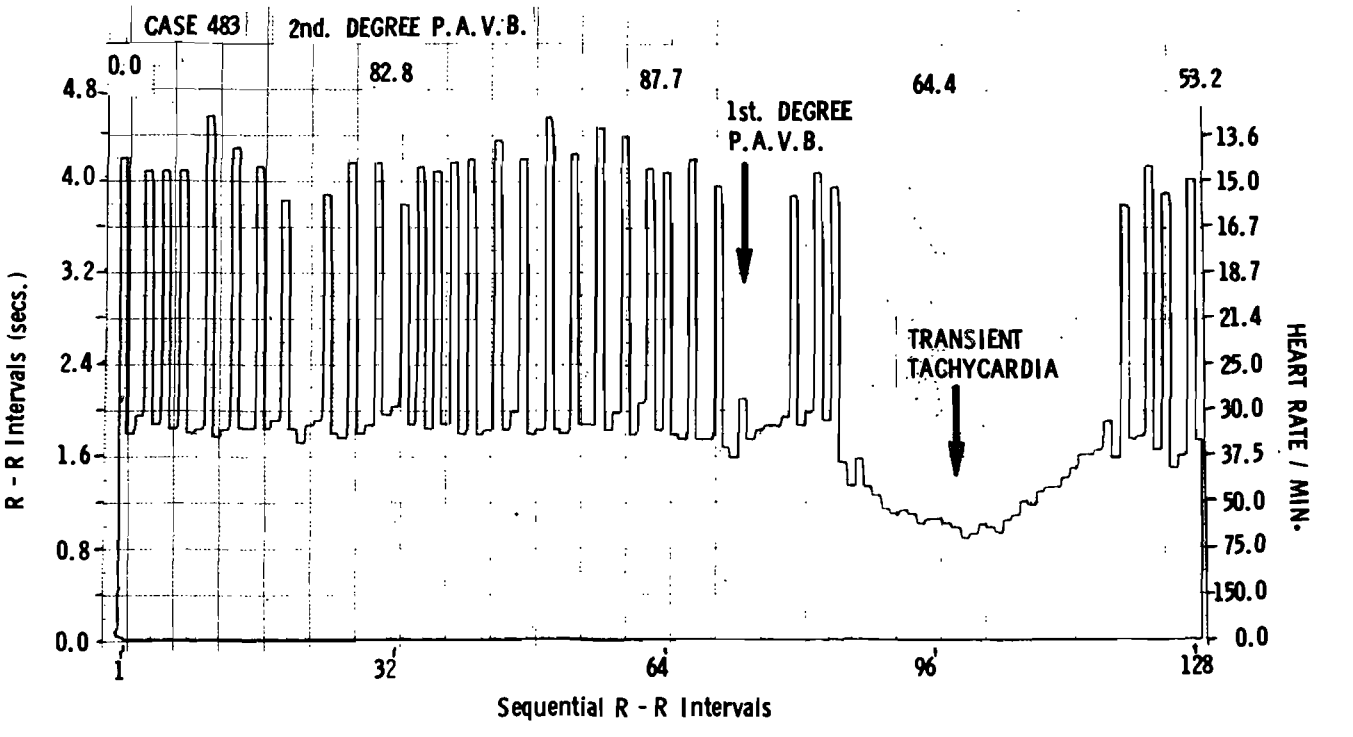

Fig. 7: Case 483. Five-years-old Thoroughbred gelding. Plot of 128 sequential heart beat intervals at rest. In this case the long intervals, which occurred very frequently, were due to second degree partial AV-block. There was also occasional first degree AV-block, the longer-than-normal PR-interval increasing the length of the associated RR-interval. Note that when heart rate accelerated, the AV-block temporarily disappeared. In this plot there were 33 missed beats during the period of 128 ventricular contractions. There were periods of both 2:1 and 3:1 block. They occurred when heart rate averaged 34/min and were absent when heart rate accelerated to over 45/min. Total time was 288,1 s (4 min 48,1 s).

never seen it in animals measuring under 14,0 hands. The heavy draught horse and the heavyweight Hunter seem to be more often affected. In our view it constitutes an unsoundness. In early cases free from murmurs, treatment with quinidine sulphate by mouth may re-establish sinus rhythm. Some cases show no significant gross lesions but others may have very severe atrial fibrosis [1]. It is difficult clinically to differentiate between the two. History of possible recent occurrence, absence of murmurs and ectopic beats and absence of jugular pulsation and/or oedema should be taken into consideration in deciding whether to advise treatment.

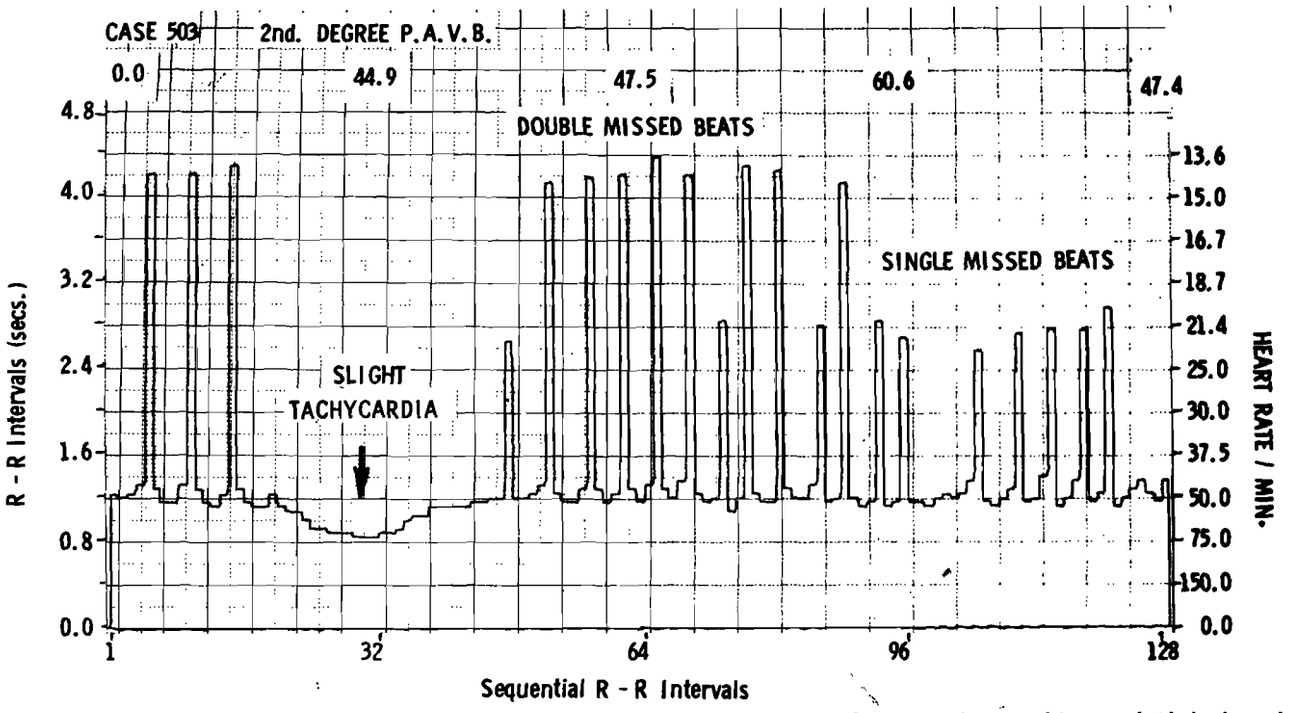

Fig. 8: Case 503. Seven-years-old pony mare. Histogram from 256 heart beat intervals at rest: At rest, this pony had single and double second degree partial AV-block.

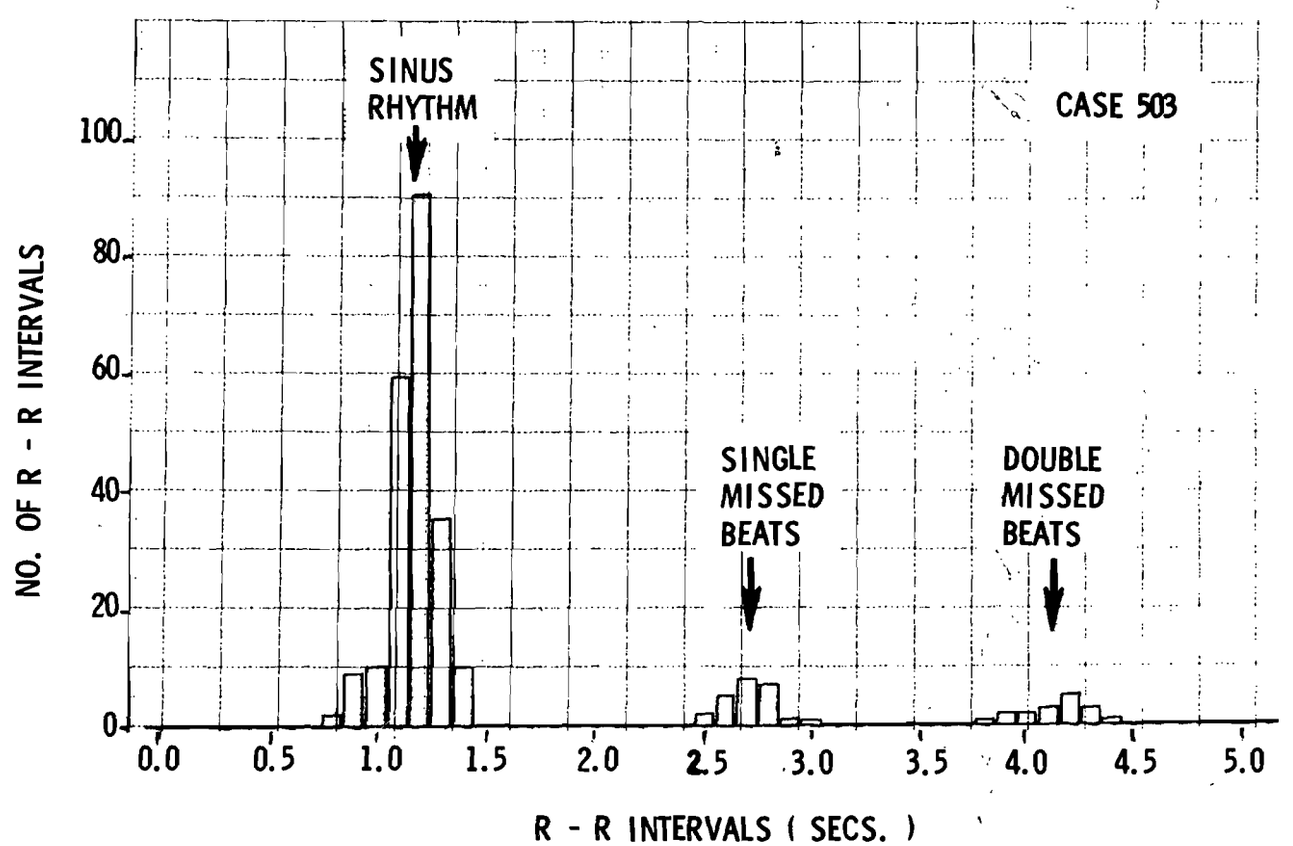

Fig. 9: Case 503. Plot of 128 sequential heart beats comprising part of the histogram of figure 8. In this plot there were 10 single and 11 double missed beats during 128 ventricular contractions when heart rate averaged 50/min. The single and double missed beats were distributed irregularly but both disappeared when heart rate accelerated slightly. After exercise in this pony, rhythm was regular.

Second Degree Partial AV-Block

This is the commonest form of rhythm irregularity observed in horses at rest, clinically recognizable by the appearance of missed beats during which, in the majority of cases (80 per cent), the atrial contraction sound can be heard. Thus, in most horses the condition can be diagnosed without difficulty on auscultation. It is often accompanied by first degree AV-block and is generally Wenckebach type I with variations in PR-interval which often progressively lengthen up to a missed beat [5]. Thus, on auscultation there is a corresponding increasing separation between the atrial contraction sound and the first heart sound. The missed beats may occur relatively infrequently and irregularly (Fig. 6) or appear very frequently, producing 2:1 or 3:1 block (Fig. 7). Often the appearance of missed beats is very rate-dependant and they may disappear if the heart accelerates slightly. Double missed beats are also not uncommon (Figs. 8 & 9).

It seems doubtful whether any clinical significance can be attached to the frequency or regularity. Heart rate at the time is probably an important controlling factor. The effect of pulse acceleration and particularly exercise on the missed beats is probably a much more important criterion of their significance.

Sino-atrial Block

Generally, the condition is recognizable by missed beats during which no atrial contraction sound can be heard. Much less common than partial AV-block, it may sometimes be confused with the latter. Theoretically the typical SA-block is represented by a pause of approximately twice the normal interval length, but in practice there may be considerable variation so that the picture may be rather reminiscent of atrial fibrillation and then would probably be better referred to as sinus node exit block of varying degree. The radio-computer method is particularly useful in depicting this type of irregularity (Fig. 10).

Ectopic Beats

Ectopic beats may arise from the atria or the ventricles. The AV-node may also act occasionally as a source of myocardial activation even when the sinus node is the dominant pacemaker. The majority arise from foci which stimulate the myocardium irregularly and unpredictably. Their presence becomes apparent only if they trigger an impulse outside the refractory period. Sinus rate and the timing of the ectopic beat relative to the previous sinus beat (coupling interval) determine whether they can be interpolated in the normal rhythm or whether they abolish the next sinus impulse resulting in a compensatory pause. Their prematurity, generally louder than a normal first heart sound and, when present, the ensuing compensatory pauses, are features of diagnostic significance on auscultation. Usually the sinus pacemaker is the dominant pacemaker because it is the fastest pacemaker but very occasionally the ectopic focus may possess rhythmicity. A condition of atrial or ventricular parasystole then exists, depending on the location of the ectopic focus.

Atrial ectopic beats may cause the pacemaker to "change step" or the sinus node may retain its rhythm in which case a compensatory pause follows the premature atrial beat (Fig. 11).

Ventricular ectopic beats may be interpolated in the normal rhythm although they are more often followed by a compensatory pause. Interpolation is very dependent on sinus rate and coupling interval. It is possible only at sinus rates which are sufficiently slow for the myocardium to recover from the premature ectopic contraction before the next sinus impulse arrives(Figs. 12 & 13).

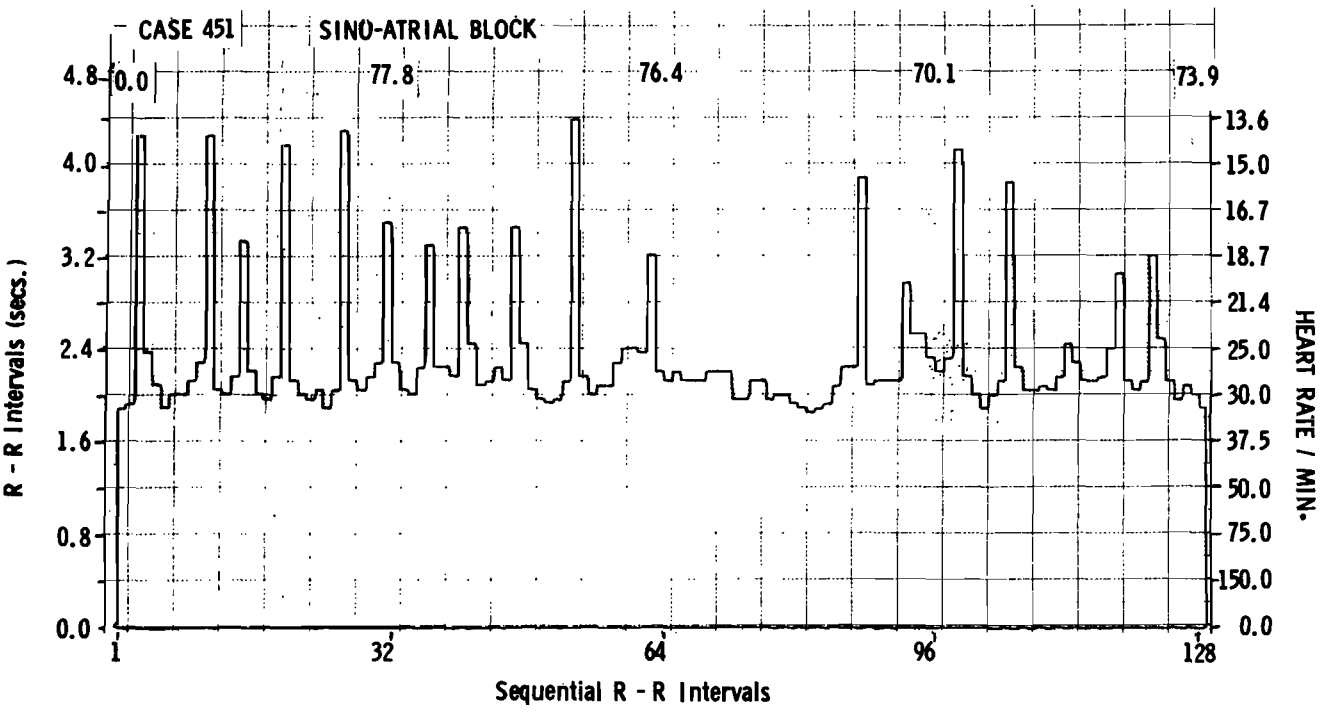

Fig. 10: Case 451. Aged Thoroughbred gelding. Plot of 128 sequential heart beats. The long RR-intervals were due to sinoatrial block and the intermediate intervals probably represent various degrees of sinus node exit block. This results in a markedly irregular rhythm, namely the basic cycle length and intermediate pauses of variable duration. Here there were 5 intervals of approximately twice the basic cycle length but there were 12 others of intermediate length.

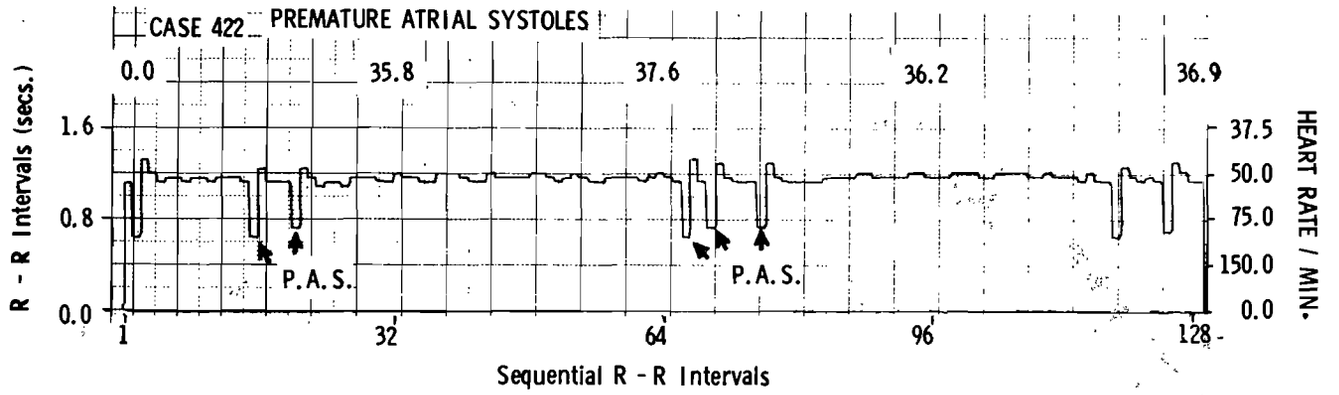

Fig. 11: Case 422. Six-months-old Thoroughbred colt foal. Found to have very marked arrhythmia when examined for insurance purposes at 5 weeks of age. Atrial ectopic beats occurred very frequently either singly or in groups. The foal was normally active and grew satisfactorily. The incidence of ectopic beats became progressively less and by 22 months of age were relatively infrequent. Plot of 128 sequential heart beats recorded when the foal was 6 months old. In this case the earlier-than-normal beats were not followed by a compensatory pause although the next RR-interval was slightly longer than the basic cycle length. Thus the sinus node had changed its rhythm. There were 8 premature beats (PAS) in the 128 intervals in this plot.

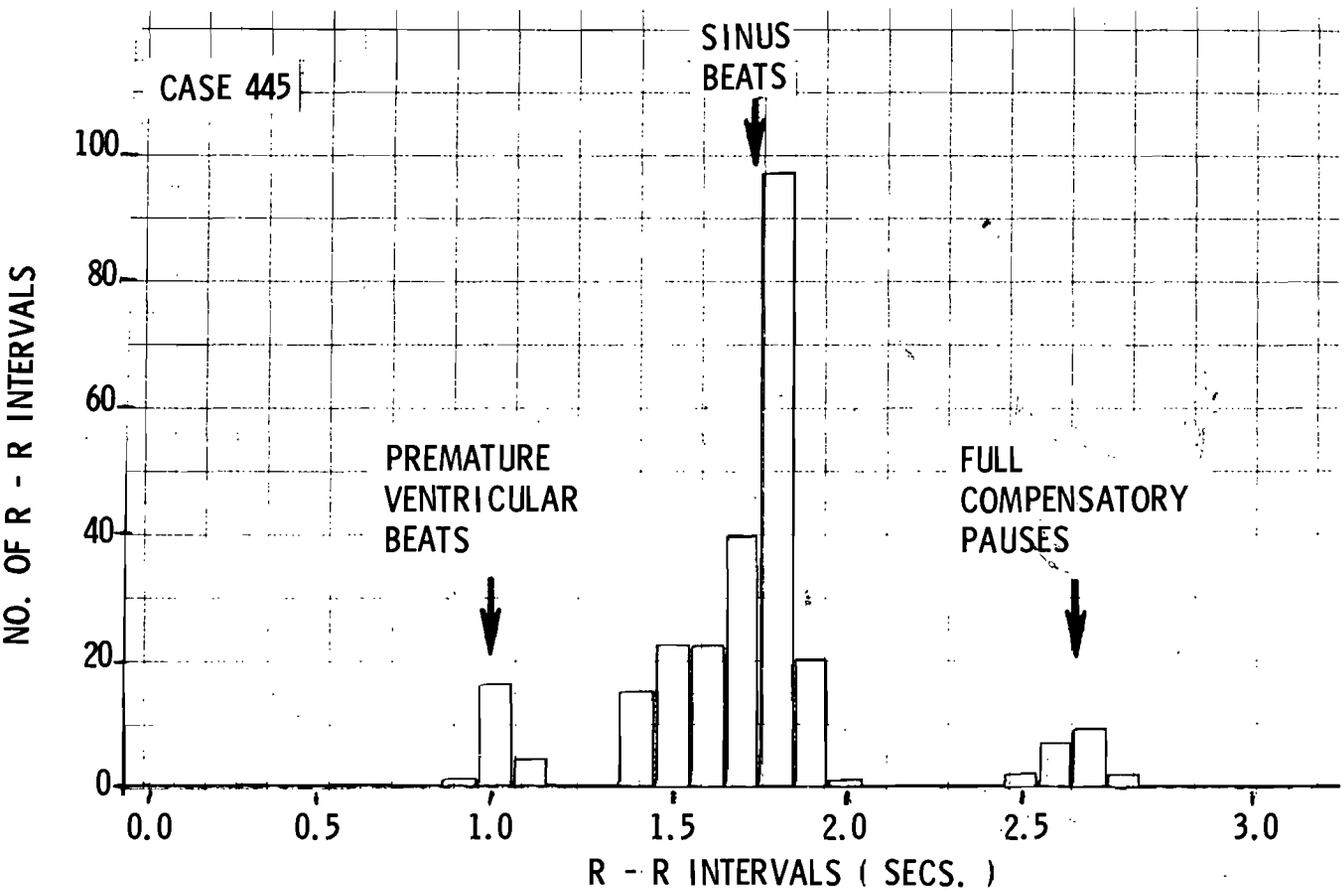

Fig. 12: Case 445. Sixteen-years-old draught mare. Histogram of 256 heart beat intervals at rest. This mare had very frequent ventricular ectopic beats.

Parasystole is relatively rare in the horse and few cases have been reported. By setting the computer's clock to trigger only on ectopic beats, ectopic intervals can be measured and plotted. Figure 14 provides an example of a histogram from a case of ventricular parasystole constructed from 1 024 sequential ectopic beats, illustrating how they occur in multiples of a basic cycle length because the focus possesses rhythmicity.

Mixed irregularities sometimes occur, such as second degree partial AV-block with premature atrial systoles and blocked premature atrial beats. Variations in sinus node exit block and second degree partial AV-block, not uncommonly accompanied by sinus arrhythmia, may also produce a very bizarre rhythm.

EFFECT OF EXERCISE ON CARDIAC RHYTHM

Our practice in assessing cardiac response to work is to gradually increase the amount of exercise. Thus, we generally first have the horse trotted a short distance in hand. This has the advantage that the induced tachycardia is of short duration and the effect on rhythm can be observed quickly. Later, the horse is ridden at the trot, then the canter and later at the gallop, the duration depending on degree of fitness and the findings in each case.

Oscilloscope monitoring of ECG signals by radiotelemetry indicates that at high heart rates, during exercise, rhythm is generally regular. The moment exercise stops, however, heart rate begins to slow, rapidly after light exercise and more slowly after more severe exertion. We do not adopt a standard exercise test partly because we are asked to examine a wide range of different types of horses and ponies and also because they are presented in varying states of fitness.

Thus, immediately after the end of exercise, rhythm is generally regular. After light exercise, however, a transient arrhythmia is not uncommonly observed in many apparently normal horses which have regular rhythm at rest. Such a pattern is shown in figure 15 and from ECG recordings we consider this to be sinus arrhythmia. It is generally very transient, indeed we would probably not have been able to record it if we had to connect the animal to an electrocardiograph, such is its short duration. It is probably associated with vagal activity related to the commencement of heart rate slowing. After more severe exertion it is usually absent.

Radiotelemetry has also shown interesting transient irregularities in some cases of second degree partial AV-block. Using a conventional electrocardiograph, it was observed that in most horses with partial AV-block at rest the missed beats disappeared after exer-

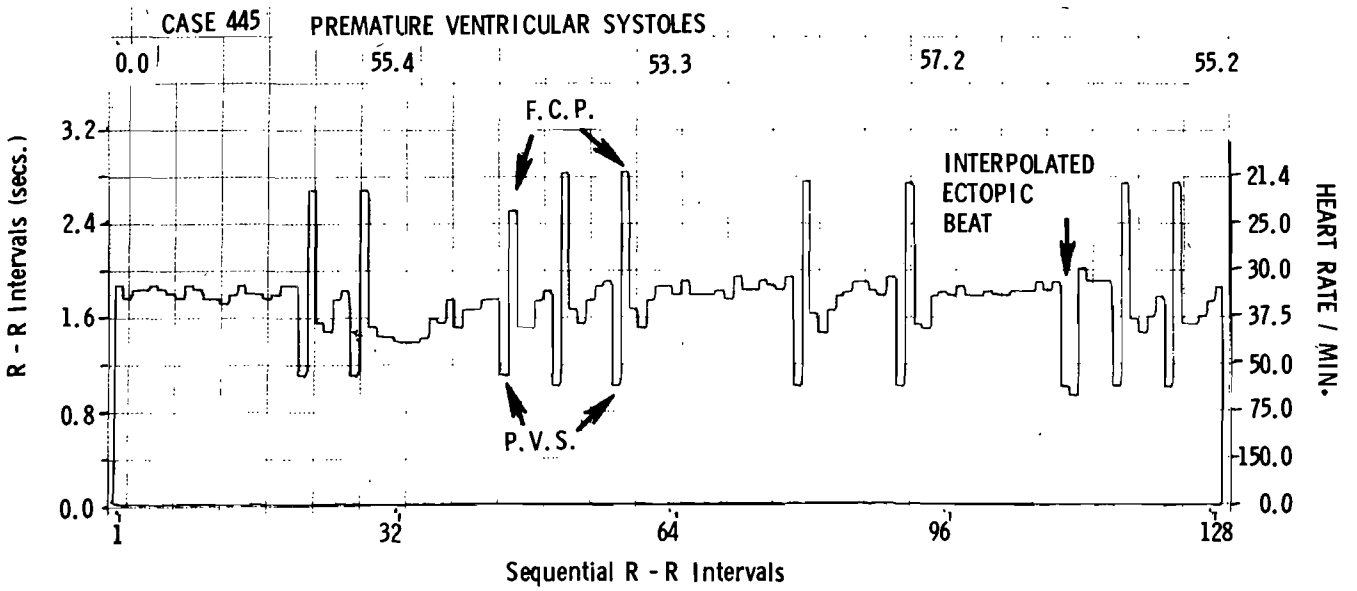

Fig. 13: Case 445. Plot of 128 sequential heart beats comprising part of the histogram of figure 12. In this plot there were 10 premature beats (P.V.S.). Nine of them were followed by full compensatory pauses (F.C.P.), whilst one was interpolated in the normal sinus rhythm.

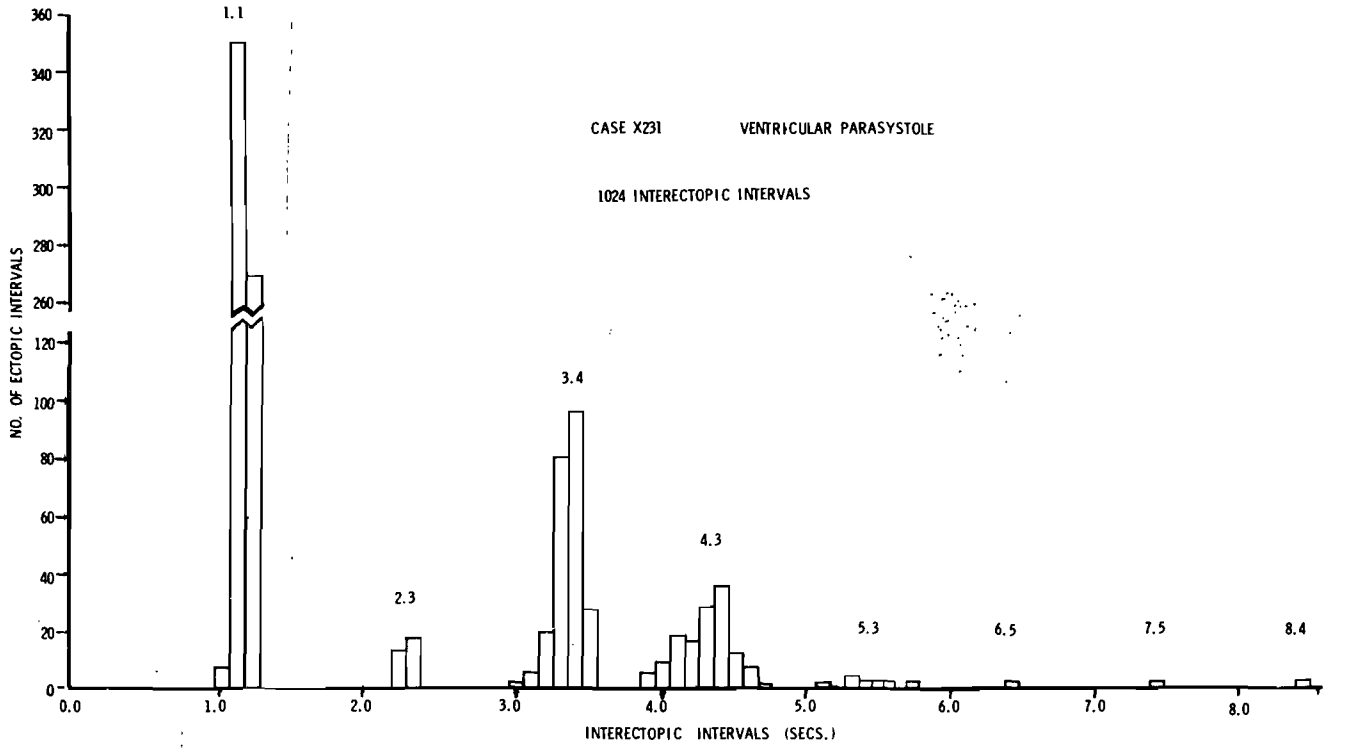

Fig. 14: Case X231. Three-years-old pony. Histogram of 1 024 sequential ventricular ectopic intervals. The ventricular focus possessed rhythmicity. Its basic cycle length was 1,1 s and the longer intervals were multiples of this basic cycle length. This is typical of ventricular parasystole. The numbers above the plots are the average values in each group.

cise and did not recur until heart rate approached resting rate [4]. On the other hand, using radiotelemetry, which permits immediate monitoring after the end of exercise, it has been found that a significant number of horses with partial AV-block at rest may also show missed beats, singles and sometimes doubles, for a short period immediately exercise is stopped. This transient period is then usually followed by regular rhythm until heart rate approaches resting rate, when partial AV-block reappears (Fig. 16). The transient nature of this arrhythmia suggests that it may be a manifestation of excessive vagal action associated with the onset of heart rate slowing, just as vagal influence may explain the frequency of partial AV-block in horses at slow resting heart rates. It may not therefore justify a grave prognosis.

Ectopic beats of atrial or ventricular origin may become more frequent after exercise [4]. Their appearance implies the presence of some irritant focus in the myocardium. Nevertheless, from our *post-mortem* studies, often no gross lesions are apparent. In man, Lewis observed that the presence of ectopic beats *per se* would not necessarily justify a grave prognosis but should emphasize the need for a close scrutiny of the heart. If no other signs of cardiac disease were detected, their significance became negligible [8]. They are, however, inefficient contractions and, if very frequent or associated with parasystole, may warrant a guarded prognosis. The majority of horses with ectopic beats seems to improve with rest and this is one of the few conditions in which a period of rest appears to be beneficial.

It is important not to get the problem of arrhythmia out of perspective. The majority of horses have regular rhythm at rest, as well as during and after exercise. Arrhythmia is obviously undesirable and yet certain forms of irregularity, notably second degree partial AV-block at slow heart rates, may be a way of resting the myocardium without impairing the circulation.

At high heart rates, as the heart slows after exer-

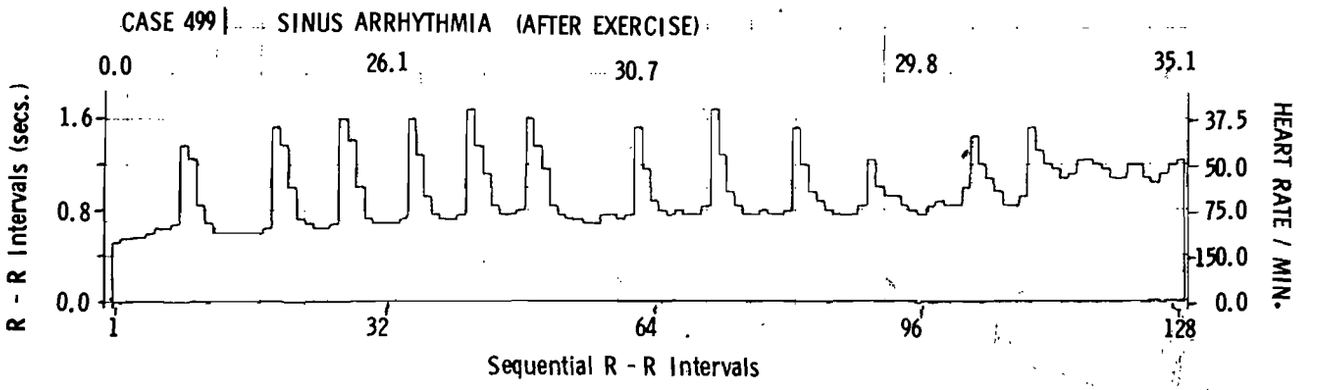

Fig. 15: Case 499. Twenty-one-years-old Hunter gelding. Plot of 128 sequential heart beats immediately after a short gallop. This horse had pulmonary emphysema. There were no cardiac murmurs on auscultation. Rhythm was regular at rest. The arrhythmia depicted was transient and rhythm later became regular as heart rate slowed. The RR-interval length at the beginning of the record was equivalent to a heart rate of 108/min. The total time for the 128 ventricular contractions was 121,7 s, during which there were 12 periods initiated by a long RR-interval followed by progressive shortening of the intervals back to the previous rapid rate. After 128 intervals the RR-interval length was equivalent to a heart rate of 47/min.

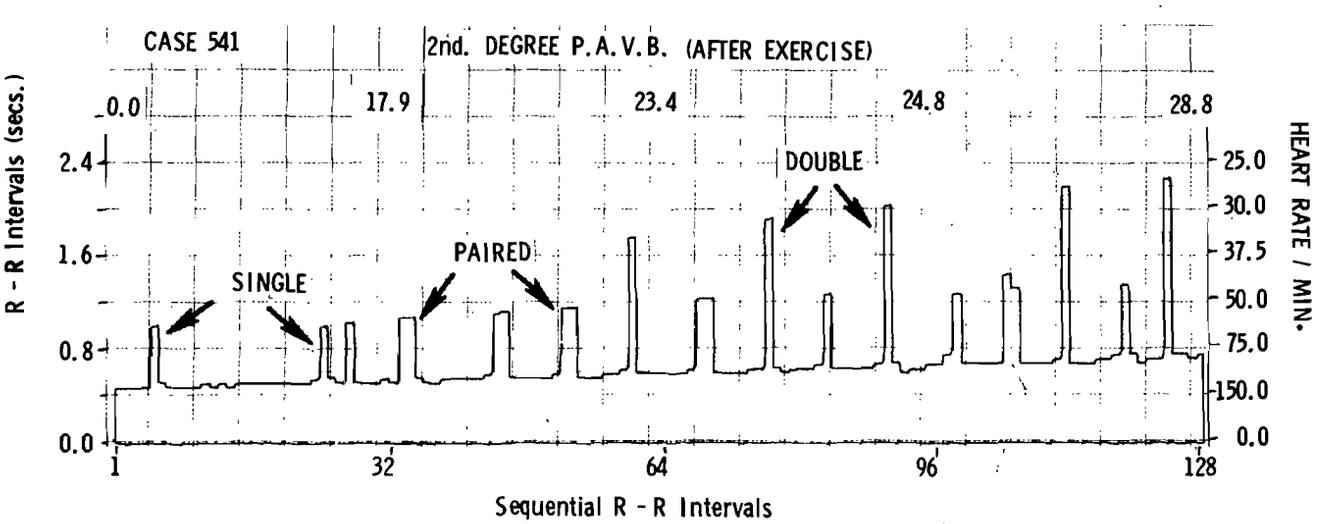

Fig. 16: Case 541. Six-years-old Thoroughbred gelding. Plot of 128 sequential heart beats immediately after 2,5 minutes' galloping. Note the presence of frequent single and later double missed beats which were due to second degree AV-block. This horse had frequent single and occasional double missed beats at rest. At the commencement of the plot, the RR-interval was equivalent to a heart rate of 117/min. Then during the following 94,9 s, the time for 128 ventricular contractions, there were 6 single, 5 paired and 5 double missed beats. In the next 21 intervals there was 1 double and 3 single missed beats after which rhythm was regular for the next 405 intervals measured. Missed beats did not occur after the first RR-interval equivalent to a heart rate of 60/min in this recording. Thus the post-exercise arrhythmia was transient and regular rhythm developed to be followed later by return of second degree partial AV-block when resting rate was approached.

cise, transient sinus arrhythmia is probably of no clinical significance. Even transient second degree partial AV-block, which may have a similar aetiology, may be benign, provided it appears only temporarily but perhaps a more serious view should be taken at present when it appears at high heart rates and persists.

Arrhythmia of mixed aetiology and atrial fibrillation with very bizarre rhythm must result in severe disturbance to blood flow and, over a period of time, impair circulatory efficiency.

Our knowledge of these matters is too insufficient to allow us to be dogmatic but evidence will gradually accumulate to permit a more rational prognosis in cases of arrhythmia.

ACKNOWLEDGEMENTS

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DISCUSSION

G. Frost: When examining horses such as hunters and jumpers for soundness, can arrhythmias which disappear upon exercise be ignored, or should they be mentioned? Are they to be regarded as a sympton of unsoundness?

J.R. Holmes: They should definitely be mentioned. For purposes of evaluation, all factors must be taken into account, such as the presence or absence of murmurs and the use to which the horse is being put. In the absence of other signs one would be justified in stating that they are of no significance.

J.D. Steel: If we exclude all horses required to act at speed, I would agree. This type of arrhythmia is less significant than when considering the racing horse. Because acceleration of heart rate leads to disappearance of discernible arrhythmia as recorded on the ECG it does not necessarily mean that it is not going to affect performance when speed is important. It has been shown that heart rate bears a linear relationship to speed of work. If we plot this ratio for horses moving between 400 to 800 m/min, a higher rate will be detected in those horses whose resting ECG's can be classified as abnormal. There is going to be a point in cardiac acceleration at which there is insufficient time for adequate ventricular filling and thus for sustained stroke volume. At higher heart rates performance must then be affected significantly.

G.F. Fregin: What would be considered an abnormally long PR-interval when considering first degree AV-block?

If the resting heart rate is 30, what would you consider a normal PR-interval to be in the thoroughbred?

I would agree with Dr Steel about the significance of incomplete AV-block with a short beat. It seems difficult to determine from the literature at what level the cut-off point should really be. Dr Steel in his 1963 monograph had taken 0,40 s as the cut-off point. It seems somewhat short from our experience. It seems Dr Steel had taken double the human criterion of 0,20 s. If we use that as basis, namely 0,40 to 0,44 s, as a maximum for incomplete AV-block with dropped beat, or first degree AV-block, there are categories of horses that will drop beats below this interval and also those that drop beats with intervals above this value. We have used this as a general guide line in trying to determine the actual significance. I think there are ways of looking at dropped beats in the horse. One that has not been mentioned is age. Below two years – say in a yearling – a dropped beat I would consider quite abnormal. In the majority of young animals with this symptom on which we have had the opportunity to perform an autopsy, myocarditis could be found, usually in the area of the AV node or in the septum. In young horses below two years of age dropped beats are probably indicative of heart disease. For those older than two years we have either taken performance, as Dr Steel has done, or we have taken PR-intervals of over 0,44 s. In those with multiple dropped beats usually there was very good evidence of decreased exercise performance. Single dropped beats with intervals of over 0,44 s, or multiple dropped beats – 2 to 3 in a row – in our experience proved to be relatively reliable evidence of cardiovascular disease. At autopsy each case had myocarditis in the vicinity of the conducting system. Many had histories of decreased exercise performance, or at the age of 4 to 5 years dropped considerably in their performance. Many of these horses can do a fair amount of work, as show animals or hunters, but when really put to the task of covering a long distance within a certain period of time they cannot cope.

J.R. Holmes: The PR-interval is rate dependent and we use a formula which works out the normal range of PR-intervals associated with particular heart rates. The formula is : $y = 5{,}682 \times {}^{1}/_{x} + 0{,}15$ with a Standard Deviation of $\pm$ 0,0564 s, where y = the PQ-interval in seconds and x = the heart rate per minute.

One could find a value lower then 0,40 s in a first degree AV-block.

WORLD VETERINARY ASSOCIATION

Announcement

The Secretariat of the World Veterinary Association intends to issue the following publications:

1. News Items (as until now 2 times a year)
2. Monthly News Letter
3. Report on the implementation of the resolutions of the 19th World Veterinary Congress, Mexico, 1971.
4. World catalogue of veterinary films and films of veterinary interest.

1. NEWS ITEMS OF THE WORLD VETERINARY ASSOCIATION

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(Continued on p. 271)

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ELECTROCARDIOGRAPHY OF THE HORSE AND POTENTIAL PERFORMANCE ABILITY

J.D. STEEL AND G.A. STEWART*

SUMMARY

The use of electrocardiography to determine heart size expressed as a heart score and the relation of heart score to cardiorespiratory function has been described. Attention was drawn to the relationship between heart score and racing performance expressed in terms of stakes won, and this was further examined by reference to the heart scores of horses that have won the A.J.C. Derby, the Victoria Derby, the Caulfield Cup and the Melbourne Cup. The winners of set-weight, Classic races like Derbies usually have heart scores of 120 or higher and while most major handicap winners have similar heart scores, these races may be won by horses with heart scores between 110 and 116. Preliminary analysis of data on the heritability of heart score indicates a high level of heritability and suggests an important rôle for the mare in the transmission of heart size.

Studies on the racehorse and the human athlete showing a relationship between the resting electrocardiogram and perfomance have been reported previously. [4 5 6 7 8 9 10 11]. The essential proposition in these reports is that if one carefully records a resting ECG and then, equally carefully, reads and interprets the tracing, two pieces of information become avialable. One is an assessment of heart size or heart weight, expressed as a heart score, and the other is knowledge of whether the ECG is normal or abnormal.

Because several aspects of the work going on in Sydney and Melbourne for over 20 years may not be fully understood, an attempt will be made to restate some old concepts and indicate some current avenues of interest. As about half the programme at this conference is concerned with "parameters indicative of performance ability and state of fitness", the contributions of Stewart and myself on two aspects of cardio-respiratory function should be seen in proper perspective. Figure 1 shows what might be called the oxygen transfer chain. It is seen as a functional chain for the transfer of oxygen from outside the body to metabolizing tissue. This transfer chain includes O_2 uptake (upper and lower respiratory tract function), O_2 carriage (red cells and haemoglobin), O_2 transport (the cardiac pump and circulation through the vascular system), O_2 delivery (dissociation, diffusion and 2,3-diphosphoglycerate) and O_2 utilization (myoglobin, mitochondria, oxidizable substrate and enzymes).

Although the papers by Stewart and myself are concerned with only two links in the chain – heart and haematology – we do have some appreciation of the wider horizon. Our view is that the oxygen transfer chain is only as strong as its weakest link. It is also

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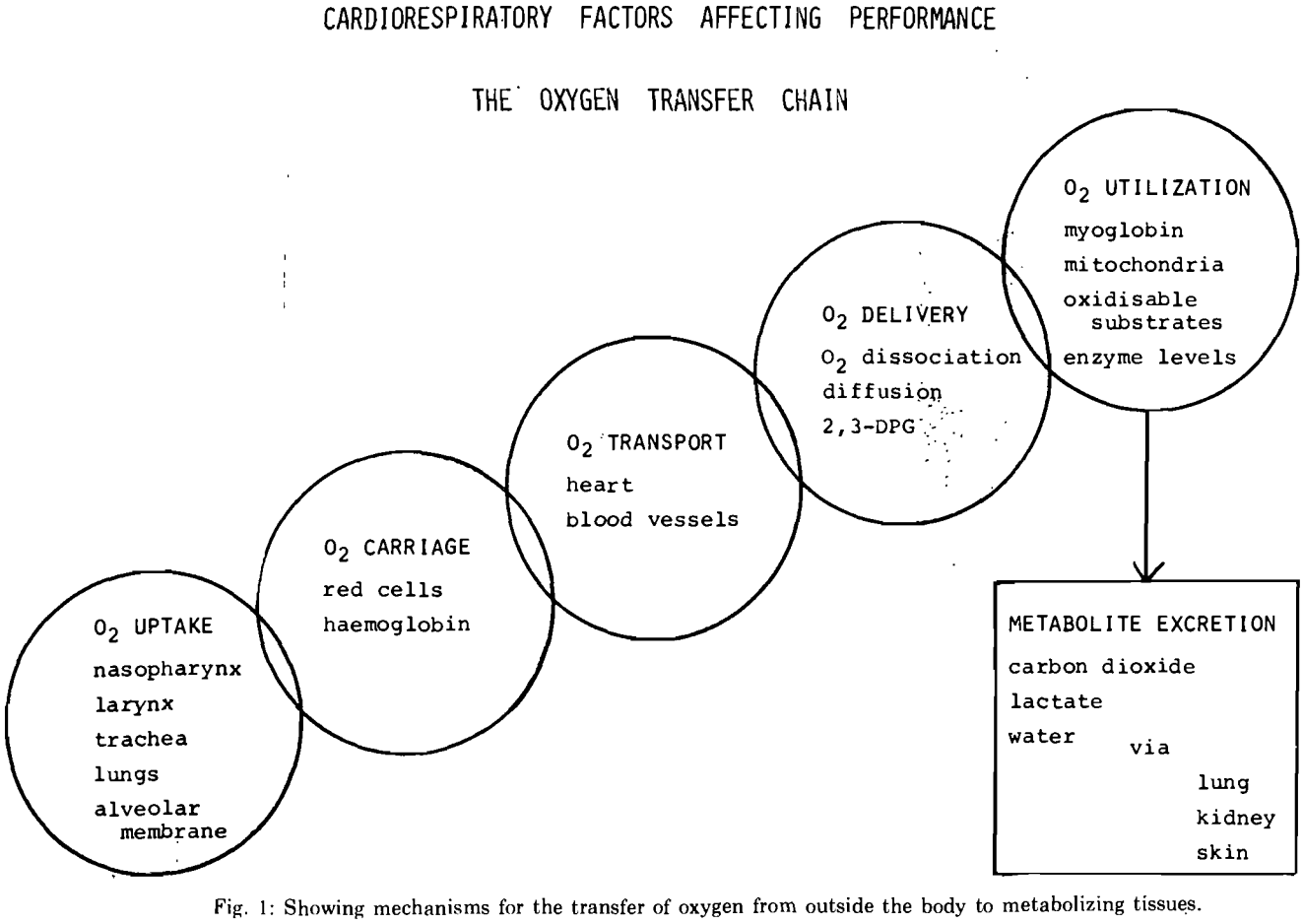

Fig. 1: Showing mechanisms for the transfer of oxygen from outside the body to metabolizing tissues.

suggested that the weakest link will vary from individual to individual, and from time to time in an individual.

The ECG fits into the concept of cardio-respiratory function because it is a non-invasive technique for obtaining some information about the cardiac pump. It is not a panacea for problems in the difficult business of horse racing, but it is often very useful and worthy of wider application. If the ECG is to be used effectively, the person concerned should not be under client pressure. Electrocardiography is a specialized field of knowledge, and the over-busy person will not do justice either to himself or his clients. The first requirement is a good, well-tuned, frequently serviced electrocardiograph. It should have a frequency response from 0,05 Hz to 80 Hz and a high input impedance. This should be a minimum of 10 mega-ohms so that proper balance within and at the current source can be maintained. In addition, because a high skin resistance or a difference in skin resistance at the electrodes of only 150 ohms can cause recording difficulty, there should be a high common mode rejection ratio. Although satisfactory recording is possible with instruments having a common mode rejection ratio of 10 000:1, existing technology permits the ratio to be raised to 100 000:1. Instruments meeting the higher specification have greater capacity to obviate errors due to electrode impedance imbalance. [2]

The patient cable should be about 4,5 m long and each part of the split section about 105 cm in length. The lead selector switch should permit the recording of leads I, II, III, aVR, aVL, aVF, and CV, CR, CF and CL leads from the left apex of the heart. The reason for recommending these lead combinations is that the first three are essential to determine the heart score and the remainder provide confirmatory and critical evidence as to whether the ECG is normal or abnormal. These recommendations are not as far from the standard 12 lead ECG which we use on human athletes as might be imagined. As we are not really interested in the anatomical localization of infarcts, the recording of V1 to V6 in athletes and the recording of unipolar and bipolar chest leads from one point on the equine thorax provide a procedure whereby the number of chest leads showing T-wave changes can be counted as an aid to the assessment of ventricular injury. In addition, because the equine T-wave is labile and subject to change of direction due to excitement, there is merit in scanning the heart by the use of bipolar chest leads rather than run the risk of causing excitement and tachycardia when electrode sites are changed.

When recording the equine ECG, it is essential to have a relaxed horse, with a slow heart rate and proper limb posture. In any quadruped, it is easy to change QRS-wave form and T-wave direction by changing the forelimb position at the time of recording. As an aside, it might also be noted that human wave forms are readily changed by recording at the full inspiratory or full expiratory chest positions.

Because of these factors, it is recommended that horse ECGs be recorded when the forefeet are squarely placed and level with one another. If this is impossible because of a restless horse, it is better to record when the left forefoot is on the ground some 10-15 cm in advance of the right forefoot. Do not record with the right forefoot in advance of the left because this affects the T-wave and makes the duration of the QRS-interval in lead I more difficult to determine. At any one time, all leads should be recorded with the feet in the one position.

If adequate records are to be obtained, the horse must be settled down so that its heart rate is less than 40 min^{-1}. The exceptions to this rule could be when a recording is made from a foal or weanling, or when an obvious abnormality is causing tachycardia. As a general rule, the recording of all the leads required takes 10-12 minutes, and when examining a group of horses the time allocation should be estimated on the basis of about three horses per hour. This amount of recording means the use of considerable paper. Do not economize on recording paper because unless one records at least 30-40 complexes from each of leads I, II and III, one may not get clean, artefact-free complexes with some showing the beginning or end points of QRS clearly associated with perpendicular time lines.

To obtain measurable tacings, it is best to record at a paper speed of 25 mm per second. When paper speeds of 50 mm per second are used, the turning points or elbows that mark the beginning and the end of the QRS complex become more rounded, flattened and less easy to read.

Determination of the heart score is carried out by the following procedures:

1. Measure the duration of the QRS-interval in each of the standard limb leads.
2. Calculate the arithmetic mean of the value observed in each of the three leads.

This will give the mean QRS-interval or heart score expressed in milliseconds.

All measurements of the QRS-interval are made with the aid of a 5 or 10 times magnifier. The QRS-interval in an individual lead is measured to the nearest 10 milliseconds or $\frac{1}{4}$ mm on tracing paper run at a speed of 25 mm per second. Because the resulting heart score is an arithmetic expression, the last figure of a heart score is the nearest one-third of 10 milliseconds.[3]

During the actual reading process, the following guidelines apply:

a. Select parts of the tracing from each limb lead that are free from artefact, in the centre of the tracing paper and without baseline wander.

b. Look at several complexes in each lead so that characteristic wave forms are selected.

c. Make actual readings from complexes in which the beginning and/or end of QRS can be accurately lined up against a perpendicular time line.

d. Make the readings so that the thickness of the tracing lines is not included in the QRS duration measured. The way in which this is done has been described previously.[9]

Difficulty may be encountered in identifying the S-T junction which marks the end of the QRS-interval. To overcome this problem, it is recommended that the reader look back along the S-T segment and regard a distinct turning point or elbow as indicating the end of QRS. When no such elbow can be identified the QRS duration should be read to the point that is isoelectric or level with the beginning of the Q or the R wave.

Figures 2-4 show examples of some of the QRS wave forms likely to be seen in leads I, II and III. They have been carefully selected so that the beginning and/or end points of QRS are lined up with perpendicular

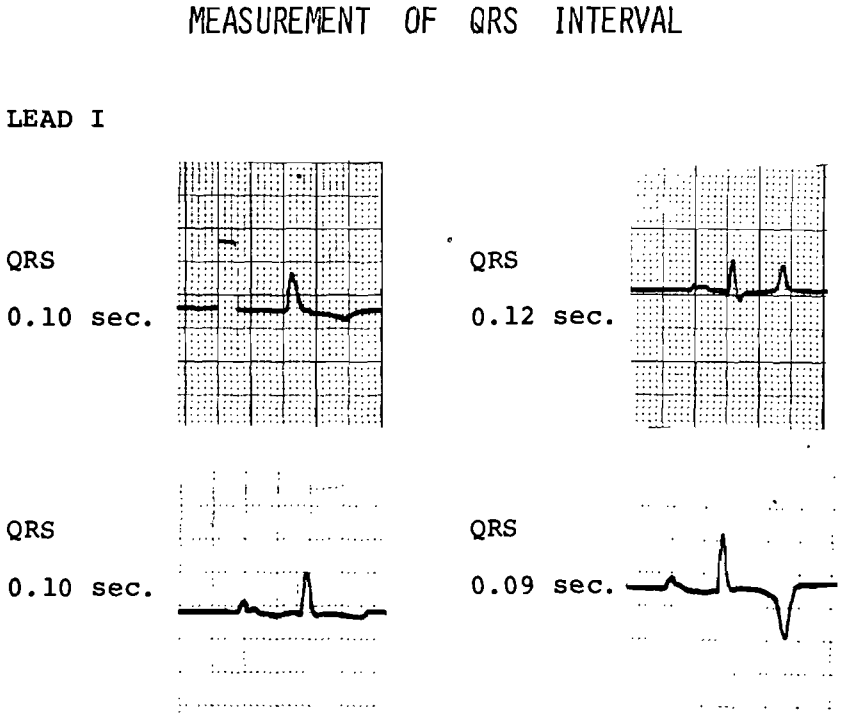

Fig. 2: Showing the QRS-interval in lead 1 from different horses.

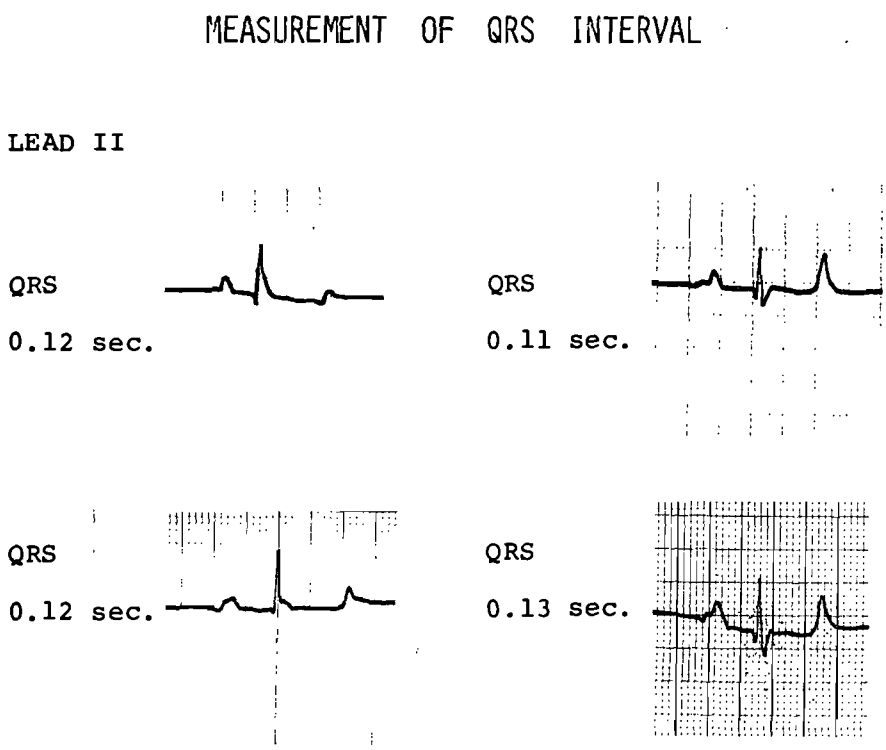

Fig. 3: Showing the QRS-interval in lead II from different horses.

time lines. If they are examined under a magnifier, the measuring points used to determine QRS durations are readily identifiable. With the exercise of patience and care during recording and reading, the determination of heart scores is a usable and practical procedure.

Observations showing that the heart mass of racehorses can vary from about 2 to 6 kg and that there is a positive correlation of 0,9 between the heart score and the heart mass have been reported previously [5 7 9]. These recorded observations indicate that heart mass will increase by about 0,8 kg for each 10 milliseconds rise in the heart score. They mean that if a horse has a heart score of 100, its heart mass will be approximately 3,0 kg; if the score is 110, the heart mass will be approximately 3,8 kg; if 120, it will be approximately 4,6 kg; and if 130, approximately 5,4 kg.

Although these generalizations over-simplify the true biological position, they are necessary if the question indicated by recent work by Kubo *et al.* [3] is to be answered. This work describes the determination of

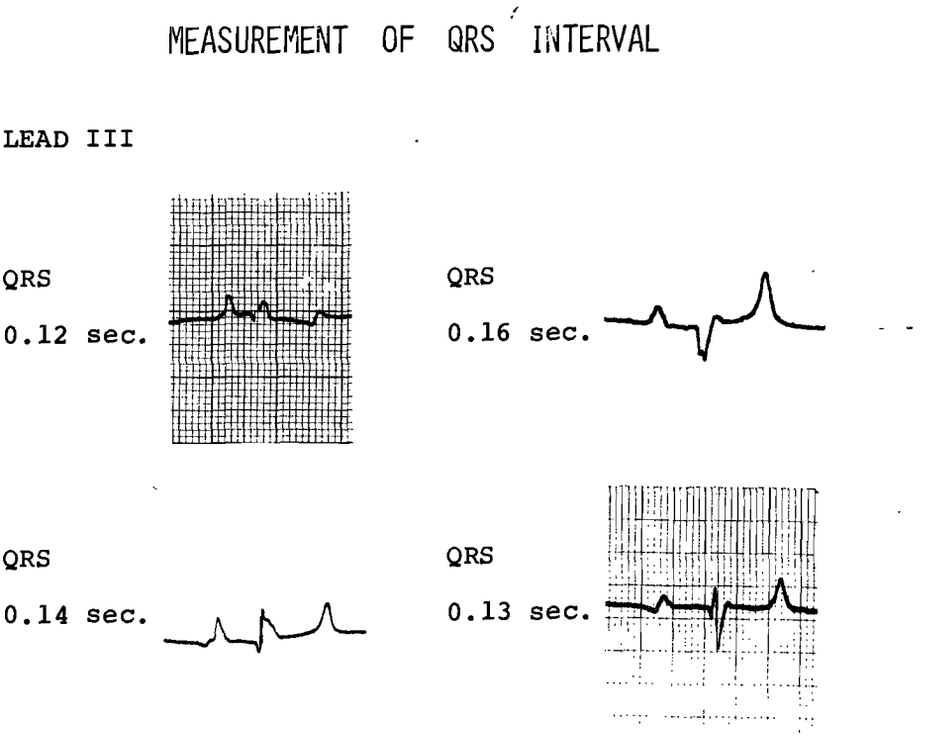

Fig. 4: Showing the QRS-interval in lead III from different horses.

cardiac output and stroke volume in standing, unanaesthetized Thoroughbred horses. The results obtained showed that cardiac output varied from 18 to 42 litres per minute and stroke volume from 400 to 1238 m*l*. Observations such as these immediately pose the question of the relationships, if any, between heart score, heart mass, cardiac output and stroke volume.

The relationship between heart score and racing performance expressed in terms of average total stakes won and average earnings per start in a race has been recorded [5 7 9]. Because prize money has risen substantially in recent years, another way of looking at the relationship between heart score and performance is the examination of class of race won. A starting point for such examination would be the three-year-old Classic races. In Australia, the two most important three-year-old Classic races are the A.J.C. Derby run in Sydney, and the Victoria Derby run in Melbourne. These races are run in early October and early November over a distance of 2400 metres (1½ miles). Since 1951, we have done ECGs on 18 horses that won these two races on 22 occasions. It means there are four dual Derby winners in the list. With one exception, all these horses had heart scores between 120 and 136. The exception was a horse with a heart score of 110, and in this Derby horses with heart scores of 106 and 113 ran second and third. There are no fillies in the list of Derby winners.

Unfortunately, during this period the ECGs of only six Oaks winners were recorded. The fact that all six winners had heart scores between 110 and 116 may help underline the earlier observation that, in general, fillies and mares have lower heart scores and smaller hearts than colts and geldings. This observation does not negate the idea that there are some mares with heart scores between 120 and 130 and these horses do at times beat colts under wieght-for-age or open handicap conditions.

The two richest handicap races in Australia are the Caulfield Cup of 2400 metres (1½ miles) and the Melbourne Cup of 3200 metres (2 miles).

Since 1953, ECGs have been recorded on 20 winners of these two races. Their heart scores have varied between 110 and 136, and all but five have had heart scores of 120 or more. The only mare to win one of these races had a heart score of 110. These observations on the winners of major handicaps indicate that while they are often won by horses that should be ranked in the top class, they are also won by horses with heart scores of 110 to 116. The ECGs and heart scores of some of these horses are shown in figures 5 and 6.

The observations now made on racehorses and human athletes permit the conclusion that in athletic events which place a considerable demand on the cardiovascular system, there is an advantage for those individuals that possess a high heart score and a large cardiac pump.

There is, however, a significant difference between the two species. Whereas human breeding is largely uncontrolled, the breeding of racehorses has been controlled by man for over 200 years. By using performance on the racetrack as a progeny test and then mating the best performers of both sexes, horse breeders have probably made unconscious selections for large heart size. If this is true, the following questions must be asked. Is heart score inherited? If so, how?

The slow breeding cycle in horses and the tendency for animals to scatter when they go to the stud, make the collection of data bearing on these questions formidable. The data we have on the heritability of heart score have not been fully analysed. A preliminary analysis of data from 140 progeny, 52 brood mares and a number of stallions used to produce these progeny indicates that heart score is highly heritable [1]. At present, the data available tend to support the well

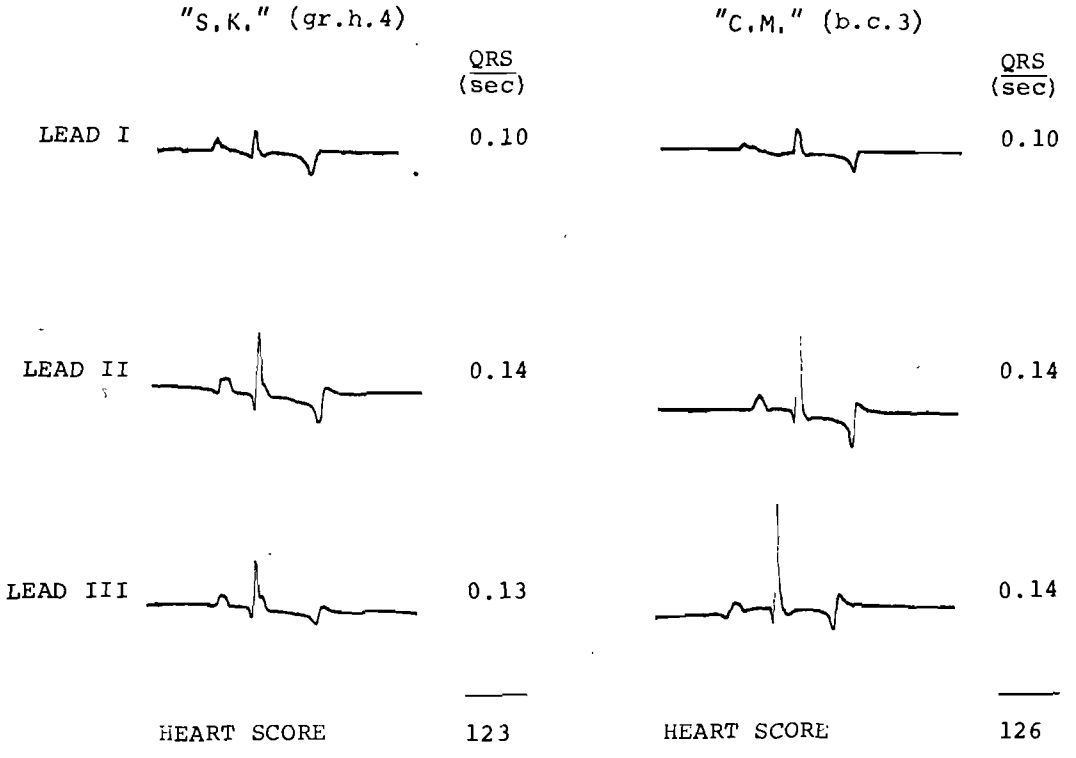

Fig. 5: Showing the heart scores of a Derby winner and a Derby and Caulfield Cup winner.

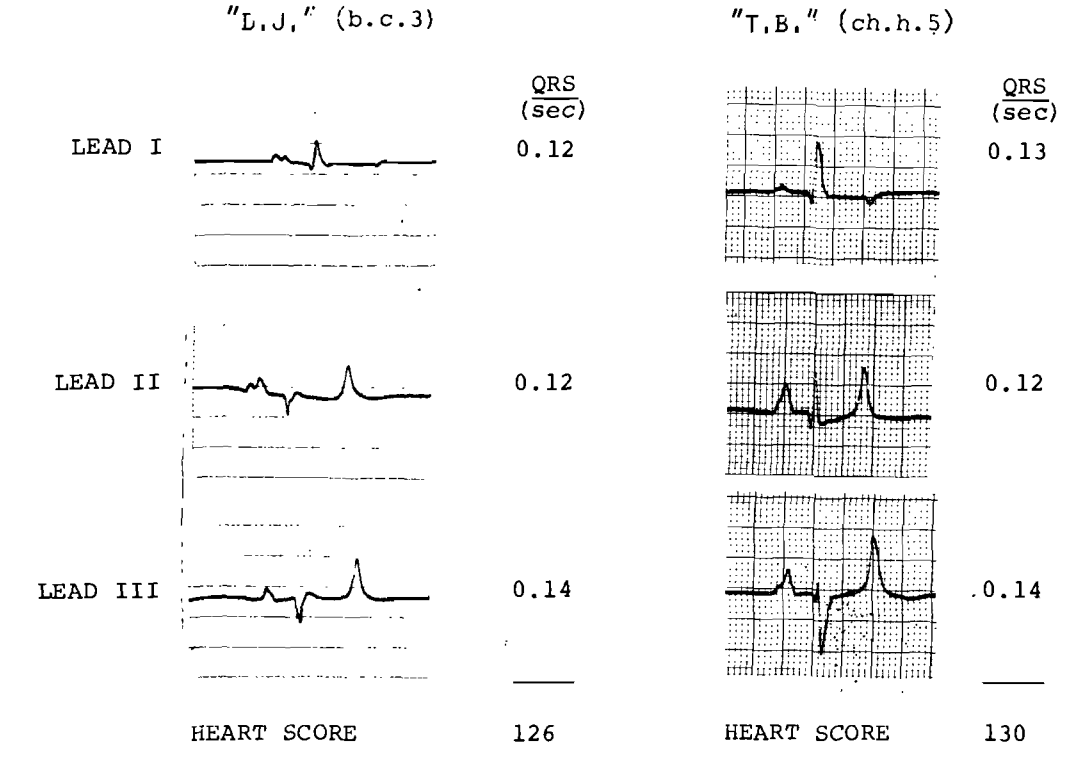

Fig. 6: Showing the heart scores of a Melbourne Cup winner and a dual Derby winner.

known American rule of horse breeding which says that 85% of winners come from the quality of the mare, but the 15% of top horses bred come from the quality of the stallions with which these mares are mated.

Finally, although the oxygen transfer chain indicates that assessment for performance in just one area is both difficult and complex, when ECGs are carefully recorded and read, the heart score, as an indicator of heart size, represents a useful clinical aid. At this point in time, individual judgement may vary on the question of whether any link in the chain is of special importance in relation to performance limitation. Despite this, the way ahead undoubtedly depends on further work aimed at devising tests or measures for:

1. Innate capacity or potentiality.
2. Responses to conditioning or training.

3. The identification of peaks of fitness using the word "fitness" to mean a readiness to win.

In the concept above, it should be noted that heart score is regarded as an indicator of innate capacity or potentiality.

ACKNOWLEDGEMENTS

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DISCUSSION

A. Littlejohn: Have you any data on the effect of age, for example, relating the heart scores of youngsters to those at the time when they are about to undertake their first race?
Secondly, have you had the opportunity of correlating heart scores with body mass of horses?

J.D. Steel: The first question revolves around the question of youngsters, whether they be school boys or horses. We have been going to one of the leading schools in Melbourne every year and doing ECGs on pupils who self-select themselves into the school athletic squad. The first ECG is done at about the age of 14 and then followed through annually until about the time of leaving school.
Heart score in man increases from birth until maturity, i.e. somewhere near the age of 20 years. Heart score in horses increases about thirty milliseconds from birth until three years of age, or approximately at the rate of ten milliseconds per annum. This means that if one takes a three-week old foal and it has a heart score of 73 one can anticipate that it will reach 103 at three years. If it is 96 at three weeks it will reach 126 at the age of three years. Regarding the second question, we have not had facilities for weighing our horses: of the 7 000 to 9 000 ECGs available, the body mass is known for only 3 or 4 horses. The answer thus is: No. In the case of the athletes observed by us, the body mass is known and, as shown on the slide, there is not necessarily a relationship between body size and heart size. In man, it is true, there is the general proposition that heart mass is related to body mass. Nevertheless, in observations on footballers and Olympic athletes and the like, one frequently observes small individuals with relatively high heart scores and, conversely, quite large individuals with relatively low heart scores.

M.A.J. Azzie: What is Prof. Steel's opinion on the value of heart scores in yearlings that have never been trained before, i.e. at presale level, particularly as he has indicated that the possible ideal stage to take an ECG is when animals are not in a state of fitness. Is there any possible link with hereditary factors?

J.D. Steel: A possible misconception has to be dispelled. When referring to the oxygen transfer chain, I did not intend to imply that the ECG has to be done when animals are fit and ready to race. The heart score is not modified by state of fitness. (Case in human athlete quoted as example.)
Regarding yearlings, one has to bear in mind that heart size is going to increase from birth to three years of age. The prognosis given at the yearling level depends on the actual age of the animal. When this is known at the time of recording it would be necessary to add 6, 10 or even 13 points to approximate the likely heart score at maturity.

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ACQUIRED CARDIOVASCULAR DISEASES AFFECTING EXERCISE PERFORMANCE – DIAGNOSIS, THERAPY, AND PROGNOSIS

G. F. FREGIN*

SUMMARY

Primary myocardial disease occurs more frequently following many of the common infectious, parasitic and toxic disease states than has generally been appreciated. Myocarditis may be followed by rapid recovery, sudden death, subacute illness with recovery and relapses, and by chronic heart disease. In general, management should be directed towards the systemic illness. Horses must be evaluated daily, however, since cardiac decompensation may develop rapidly. Satisfactory recovery following the onset of cardiac dilatation and signs of congestive heart failure is unlikely.

INTRODUCTION

Diseases of the heart of the horse occur more commonly than has generally been appreciated by the veterinary practitioner. Cardiac arrhythmias and murmurs are detected more frequently in this species than in any other domestic animal. Their significance, however, continues in many instances to be a subject of debate as well as of concern. Early decrescendo systolic heart murmurs in the aortic and pulmonic valve areas and cardiac arrhythmias such as incomplete atrioventricular block with dropped beats, for example, are detected in horses without other reliable evidence of cardiovascular disease.

In some horses, however, these same findings may be associated with decreased exercise performance and occasionally with *post-mortem* evidence of cardiac disease.

Although the only truly unassailable evidence of heart disease is the demonstration of histologic evidence of inflammation or the presence of other structural abnormalities, the following clinical findings have been used in our clinic as dependable signs of cardiovascular disease in the horse:

1. Grade 4 or greater systolic murmur at resting heart rate in the absence of anaemia. (Systolic ejection murmurs may appear with accelerated heart rates.)
2. Grade 3 or greater prolonged diastolic murmur at resting heart rate. (Pre-systolic and early diastolic flow murmurs may appear with elevated heart rates following excitement or exercise.)
3. Precordial thrill (in the absence of anaemia).
4. Generalized venous distension.
5. Pericardial friction rub.
6. Atrial fibrillation or flutter.
7. Complete heart block.
8. Intermittent claudication with loss of arterial pulse.
9. Atrial or ventricular premature systoles occurring frequently, when increased by exercise, or when arising from more than one ectopic focus.

Certainly, other clinical findings, although somewhat less reliable, are suggestive signs of heart disease:

1. Low intensity systolic or diastolic murmurs in animals in which they have not previously been heard, in older animals and in areas where flow murmurs are not usually audible.
2. Occasional atrial or ventricular premature beats.
3. Arrhythmias developing after exercise.
4. ST-segment and T-wave deviations.
5. Cough, dyspnoea, ascites or oedema, generalized weakness, cyanosis, fainting; tachycardia and fevers of unknown origins.

Since it is impossible to cover these findings in their entirety, it is the purpose of this paper to discuss only one of the more common types of cardiac disease that has been associated with these various clinical manifestations.

PRIMARY MYOCARDIAL DISEASE

Inflammatory or degenerative lesions of the myocardium may be the most frequent of all diseases of the heart, occurring in approximately 2 to 3 per cent of routine *post-mortem* examinations in the horse. Myocarditis has been noted following various infectious and parasitic diseases and as a result of extension from primary endocardial or pericardial lesions [2 5]. Sudden death, associated with inflammatory changes in the myocardial parenchyma or conduction system, has been reported in horses with chronic pharyngitis and in horses recently recovered from streptococcal infections and from influenza [2]. Toxic states also are encountered in metabolic disturbances (uraemia), nutritional deficiencies and drug intoxications which may produce acute myocarditis.

Electrocardiographic studies in man suggest a transient cardiac involvement of 5 to 15 per cent in most common infectious diseases with an even higher incidence in diphtheria, rickettsial diseases, poliomyelitis and Chagas' disease [4]. Similar studies, although uncommon on the horse, have indicated electrocardiographic abnormalities following *Streptococcus equi* and certain other infectious diseases. Significant changes, for example, were found in 8 (32%) of 25 Standardbred horses with fever, lumphadenitis and purulent rhinolaryngotracheitis from which *Streptococcus equi* had been isolated in 16 of the horses examined [1]. In four horses the electrocardiograms had returned to normal within two weeks; in two horses, several months were required and in one, abnormalities were present a year after the initial infection (one case was not followed up).

Diagnosis of myocarditis has often been made difficult because many horses apparently recover spontaneously without overt clinical signs. Myocardial injury also may be overlooked due to the preoccupation with the primary infection. The detection of acute myocarditis, however, would be more frequent if there were greater general awareness among clinicians of its association with many of the known bacterial, viral and parasitic diseases. The following clinical signs, although not pathognomonic, have been associated

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with myocarditis occurring usually 1 to 4 weeks after onset of the illness:

1. Decrease exercise performance, dyspnoea on exertion, fainting, or collapse and even sudden death.
2. Tachycardia disproportionate to fever.
3. Murmurs and gallop sounds.
4. Cardiac arrhythmias, *i.e.,* premature systoles, paroxysmal ventricular tachycardia, atrial fibrillation and incomplete atrioventricular block with dropped beats.
5. Electrocardiographic abonormalities such as ST-segment and T-wave deviations, slurring and prologation of QRS-complex, electrical alternans and prologation of PR- and QT-intervals.
6. Generalized congestive heart failure

Based on these criteria, 96 horses suspected of having primary myocardial disease were detected in a group of 298 horses referred for cardiovascular examination from July 1st, 1969 to June 30th, 1973. Seventy-one of the horses were seen at least once after the initial examination, while 20 have been followed up to the present time.

In 23 horses, atrial fibrillation was diagnosed, 11 had wandering atrial pacemakers and sinoatrial block, eight had atrial premature beats, nine had incomplete atrioventricular block with dropped beats, nine had ventricular ectopic beats, 28 had T-wave abnormalities and five had miscellaneous changes such as microvoltage and QT-interval prolongation.

Quinidine sulphate therapy was successful in 13 of the horses with atrial fibrillation, two horses, however, did revert, but have since been retreated and have remained normal up to the present time. One horse died during treatment, while a second horse did not respond to therapy. Eight horses were not treated: one reverted spontaneously, two were retired, two were kept as controls, while the remaining four developed signs of congestive heart failure.

Post-mortem examinations were done on 38 horses. Unfortunately histologic descriptions of the heart were available for only 21 of these animals. In nine horses, evidence of myocarditis was noted, eight had myocardial degeneration and fibrosis, while the remaining four had tumours involving the heart. Clinical signs of congestive heart failure were present in four horses, three of which had atrial fibrillation. Myocarditis, degeneration and fibrosis, or cardiac dilatation was present in all seven horses with atrial fibrillation which were available for *post-mortem* examination.

In those horses in which the cardiovascular manifestation began to dominate the clinical picture, the following diagnostic criteria, in order of severity, are suggested:

1. *Mild acute myocarditis* – minimal ST-segment and T-wave changes, wandering atrial pacemaker, sinoatrial block, incomplete atrioventricular block with dropped beats, occasional atrial or ventricular premature beats.
2. *Moderately severe acute myocarditis* – electrocardiographic findings as in (1), tachycardia disproportionate to fever, multiple atrial and ventricular premature beats, electrical and *pulsus alternans,* accentuated gallop rhythm and atrial fibrillation.
3. *Severe acute myocarditis* – clinical manifestation as in preceding, only more severe; functional murmurs, cardiac enlargement, signs of congestive heart failure, poor peripheral pulse and circulatory collapse.

exhibiting signs of mild acute myocarditis, with steady improvement usually occurring over a period of two weeks to several months. In a few horses, however, resumption of training may be associated with recurrence of minor electrocardiographic changes and an extended period of rest may be necessary.

In animals with moderately severe acute myocarditis, stall rest is absolutely essential with treatment again being directed primarily towards the systemic illness. These horses, however, must be evaluated daily to determine any change in cardiovascular status since signs of severe acute myocarditis with cardiac decompensation may develop rapidly. Electrocardiographic abnormalities (with the exception of atrial fibrillation) usually diminish within three to four months, although occasionally they may persist up to a year. Permanent damage obviously may be sustained in a small percentage, with racing performance usually being affected adversely.

In severe acute myocarditis, cardiac decompensation has usually occurred early in the course of the disease, with death from intractable heart failure intervening after several months, or, rarely, up to a year later, despite treatment.

THERAPY

The treatment of acute myocarditis is best divided into two categories, the first related to the systemic disease itself and the second related to the heart disease. In general, the management should be directed toward the systemic illness, since improvement of the myocarditis usually follows. Once myocardial injury is suspected, vigorous treatment with antibiotics and supportive therapy must be instituted to prevent permanent damage to the heart. The horse must be rested until the infection has subsided and the electrocardiogram has returned to a normal rate, rhythm and configuration.

Horses with atrial fibrillation, in the absence of any other reliable evidence of heart disease, are good candidates for treatment with quinidine sulphate [2 3]. Those with signs of congestive heart failure and atrial fibrillation, however, have responded poorly to digitalis, diuretics and anti-arrhythmic agents.

Frequent premature ventricular contractions in the absence of signs of congestive heart failure may be suppressed with agents such as lidocaine hydrochloride* or propranolol hydrochloride**, although experience with both drugs has been limited. Prolonged use, however, during training or racing is not deemed advisable. Resting these horses for four to six months, without further therapy, has usually resulted in disappearance of these ectopic contractions.

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* Xylocaine 2%: Astra Pharmaceutical Products, Inc. Worcester, Mass.
** Inderal: Ayerst laboratorise Inc. New York. N.Y.

DISCUSSION

J.R. Gillespie: Do you see myocarditis in the same locality without the ECG changes that you have described?

G.F. Fregin: Not in my experience.

J.R. Gillespie: What explanation do you have for the changes that you see with exertion or with excitation on the rotation of these vectors of the ECGs?

G.F. Fregin: These are due to changes in vago/sympathetic (autonomic) tone. Variations occuring from direct cardiac stimulation plus circulation catecholamines can be shown experimentally very nicely to cause alterations in repolarization of the mvocardium which can be seen on the electrocardiogram. It is a very complex type of change but one can expect to see it in all horses; that is why I always point them out.

J.R. Coffmann: Could you comment on the use of enzymology as an aid in the diagnosis of myocarditis, *e.g.*, CPK and LDH isozymes.

G.F. Fregin: Our experience has been relatively limited from the point of view of enzyme studies. Those having the most positive results have had tumours involving the heart. It is difficult to demonstrate enzyme changes unless one actually obtains serum during the acute phase. In many of the horses it is difficult to date the onset of the actual inflammatory process. This might be the reason why the enzymes have not always correlated well with the degree of damage that might be seen.

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(Continued from page 262)

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Jl S. Afr. vet. Ass. 45(4) 273-277
(Proc. 1st int. equine vet. Conf. Pretoriuskop 1974)

HAEMATOLOGY IN RELATION TO PERFORMANCE AND POTENTIAL

A GENERAL REVIEW

R.K. ARCHER*

SUMMARY

This paper consists of a review of existing knowledge on haematology as it is related to performance and potential in horses. Particular reference is made to the Thoroughbred in training.

It is to be noted that the circulation of blood occurs within vessels containing a substantial number of quite large lacunae, these lacunae being of such size, however, that erythrocytes will not normally pass through. It is for this reason that close interpretation of blood counts is impossible unless the animal is both at rest and has been repeatedly sampled so that its personal normal range is known.

INTRODUCTION

The ability of horses to achieve remarkable levels of exertion in a very short period of time is legendary. The limits of performance are of fundamental interest where the potential is to be considered. In the case of muscular performance, the limits are imposed by the amounts of oxygen and fuel that can be delivered to the musculature, the efficiency of the removal of the waste products from the muscle, including heat; and besides all this, the efficacy of heart, lungs, blood vessels and the other support mechanisms vital for effective muscular work. Blood is, in fact, the only parthway by which the musculature can be both supplied and relieved; it is this aspect of performance and potential which will be considered.

When an animal is exerted, the changes produced in circulating blood are remarkably swift and profound. In less time then blood takes to complete one cycle of circulation (say 90 seconds) there is a sharp increase in the level in unit volume of erythrocytes, leucocytes and platelets. There is an increase in concentration of some, but certainly not all, chemical constituents of blood plasma. This increase, however, is not precisely parallel to the increases in cell concentrations just mentioned, nor is the time sequence so swift. The response to exertion, then, is both complicated and exceptionally interesting.

VALUE OF HAEMATOLOGICAL OBSERVATIONS AT REST AND AFTER EXERTION

With a fluid so subject to natural variation as circulating blood, all practical steps to standardize that variation are of great value. Quantitation of partial exertion is probably impossible and bleeding horses during sleep is clearly impracticable. The most reproducible conditions occurs, therefore, when the horse is at rest, for preference in the morning after a night during which exertion has been minimal. Given these conditions, remarkably reproducible laboratory results can be obtained from horses, particularly Thoroughbreds, in any given locality. At Newmarket, England, the Equine Research Station has established accurate figures for the haematology of Thoroughbreds in training and at stud (Table).

It has to be accepted that some horses cannot be bled at rest, or perhaps it should be described as relaxed. In such instances 'normal' figures cannot be obtained, for even the presence of an attendant or the veterinary surgeon leads to sufficient stimulation that an increase in blood cells over the resting state is unavoidable.

After exertion there is a substantial change in curculating blood and this depends to some extent on the individual horse in addition to the degree and duration of the exercise. Horses which do not respond to exertion with an increased blood count are certainly abnormal.

To achieve meaningful laboratory figures with equine blood samples it is therefore essential to provide good clinical control when the specimens are taken; where this is not possible by reason of the temperament of the subject, some allowance in attempting interpretation is essential. Besides this, good laboratory quality control is most important and this can be very difficult to achieve, particularly in isolated areas. It is most important for the proper interpretation of equine haematology in trained racehorses to concentrate on the individual and not on the average of a group. The objective of the racehorse is to win: not to be an average horse. In fact, individual horses, as people, have particular characteristics which change very little; the mean cell volume (MCV) is an excellent example of this. The MCV of a particular horse seldom changes by as much as 0,5 per cent in a year, provided, of course, there is no intercurrent frank illness or disorder.

LABORATORY METHODS

In the last decade or so, haematological methods have been considerably changed. In the 1960's, probably the most reproducible single test in blood was the packed cell colume (PCV) or haematocrit. A little later, this was overtaken by haemoglobin estimation following the introduction of the cyanmethaemoglobin method and stable standards. Currently, with the advent of really accurate electronic equipment, erythrocyte counting is the most accurate and reproducible single haematological test, particularly since equipment is available which will give automatically a distribution curve, by size, of the cells being counted. The PCV is now regarded as one of the least accurate because of the difficulty of allowing for trapped plasma between the packed erythrocytes. Attempts to overcome this by using

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Table: NORMAL RED CELL VALUES IN THOROUGHBRED HORSES*

Age of Thoroughbred (number examined)		Hb g% ± S.D.	RBC $\times 10^6/mm^3$ ± S.D.	PCV % ± S.D.	MCV $\times 10^{-9}$ mm^3 ± S.D.	MCH pg ± S.D.	MCHC g% ± S.D.	P.V. mPa.s (Centipoises) ± S.D.
< 1 month	(78)	13,6 ± 1,90	9,85 ± 1,31	37,8 ± 5,00	38,5 ± 2,49	13,8 ± 1,07	36,1 ± 1,62	1,48 ± 0,10
1 – 9 months	(75)	12,7 ± 1,55	10,33 ± 1,24	35,3 ± 4,40	34,3 ± 2,92	12,3 ± 1,23	36,1 ± 2,25	1,56 ± 0,12
Yearling	(100)	13,7 ± 1,44	9,91 ± 1,11	37,5 ± 3,89	37,9 ± 1,90	13,8 ± 0,68	36,6 ± 1,36	1,59 ± 0,09
2 y.o.	(308)	14,6 ± 1,44	9,86 ± 0,98	39,9 ± 3,99	40,6 ± 2,53	14,7 ± 0,87	36,2 ± 1,02	1,50 ± 0,08
3 y.o.	(353)	15,1 ± 1,52	9,71 ± 1,06	41,4 ± 4,18	42,7 ± 2,54	15,6 ± 0,83	36,5 ± 1,07	1,51 ± 0,08
4 y.o.	(138)	15,0 ± 1,65	9,28 ± 0,98	40,8 ± 4,65	44,3 ± 2,43	16,7 ± 0,86	36,5 ± 1,30	1,53 ± 0,08
Over 4 y.o.	(302)	14,6 ± 1,61	8,80 ± 1,09	39,8 ± 4,70	45,5 ± 2,56	16,6 ± 0,96	36,5 ± 1,36	1,55 ± 0,10

* Normal red cell values in English Thoroughbred horses. In a series of more than a thousand blood samples from normal horses examined by the latest quality-controlled methods, means with S.D. are given for the red cell parameters and for plasma viscosity. Reprinted from: *The Equine Veterinary Journal* (1973) 5:136

other methods, such as the electrical resistance of blood, so far have not proved good enough to restore the measurement of PCV to its previous place. Nonethe-less, as a quick test for use in a practice laboratory, requiring a minimum of technical skill and apparatus, it retains a most important and probably supreme position.

Modern laboratory methods require careful and continuing quality control. Details of methods to achieve this are not appropriate here, but the principles may be of interest. These include the regular counting of a given sample from an individual in good health, the repetition on the next morning of the sample last done on the previous day; and, most important, wherever available, participation in a national quality control scheme which regularly issues blood samples of known constitution.

Modern methods are unlikely to include the use of

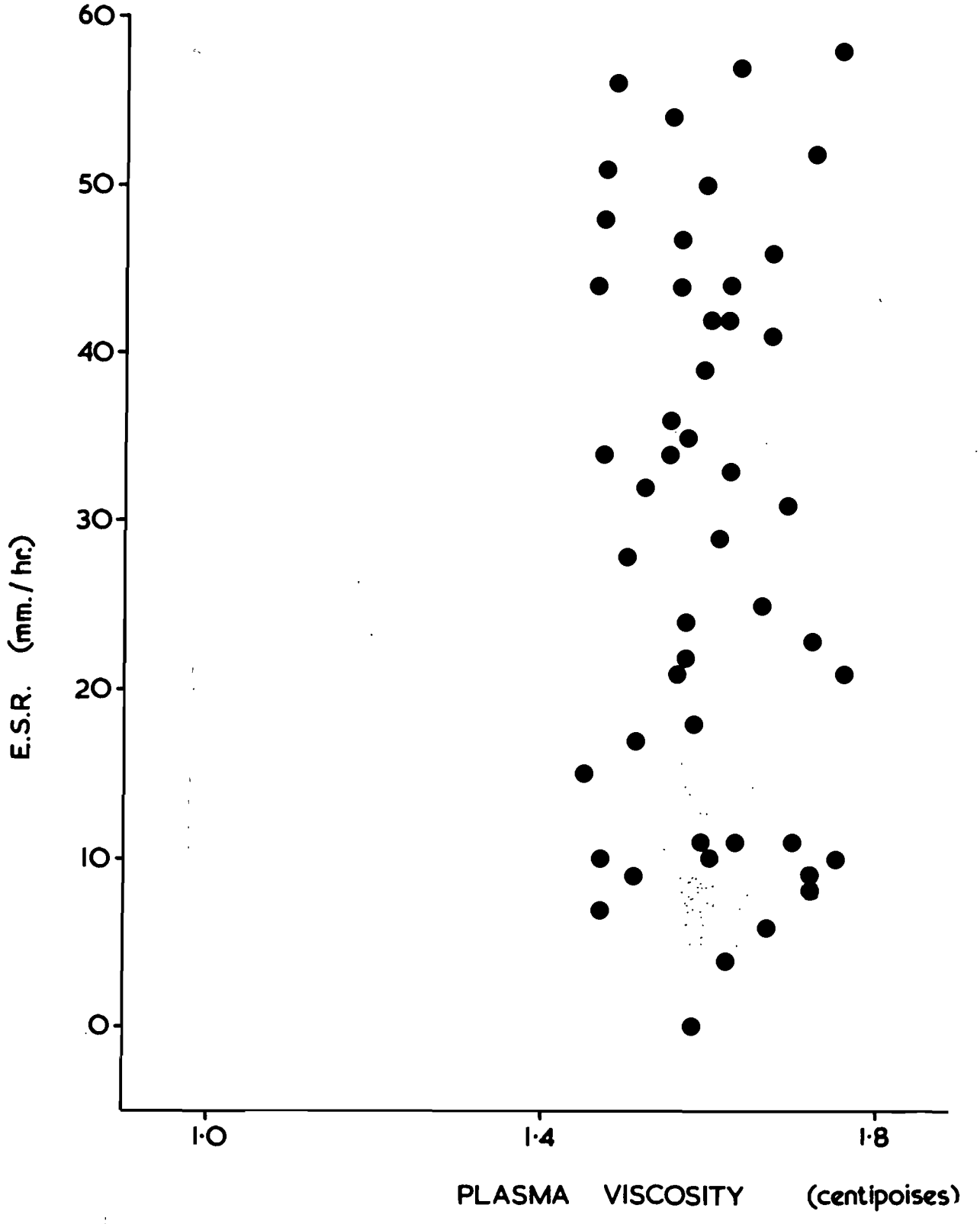


Fig. 1.: A series of 48 blood samples from normal Thoroughbred horses examined for ESR and plasma viscosity. Whilst the ESR varied from zero to 60 over the whole range, including pathological, each measurement of plasma viscosity fell between the normal limits of 1,4 to 1,8 mPa.S (centipoises). Redrawn from: *The Veterinary Record,* 86 (1970):362.

erythrocyte sedimentation rate (ESR). Whilst this is a valuable screening test, in the horse the sedimentation is always rapid since the erythocytes are small (MCV *ca* 45) and tend to form rouleaux. Increases in ESR cannot therefore be measured accurately; decreases are seldom of clinical importance, save in haemoconcentration, which is better revealed by other tests. The measurement of plasma viscosity is a valuable new technique, since both increases and decreases are easily measured (Fig.) and increases above about 1,8 mPa.s(centipoises) are certainly clinically significant.

EFFECTS OF EXERTION ON BLOOD

The effects of exertion upon blood are exceedingly difficult to quantify. Probably the shift of fluid into and out of the circulation is the most important single factor, but since the body is about two thirds water by mass, the problem is complex. Most of the body water is intracellular (about 60 per cent), with about a third in tissue space (35 per cent). Only 5 per cent of the body water is in plasma.

Capillary structure is very relevant to the status of circulating blood. Effectively, capillary wall is like a coarse net, with large holes, just smaller than the erythrocyte mean diameter (about 3 μ m). Presumably, electrostatic effects prevent platelets and large molecules from free movement through these holes, but one can, for example, visualize the cigar-shaped albumin molecules slipping in and out of a capillary quite freely.

The rôle of the spleen in the exerted horse has not yet been evaluated fully, but it is unlikely that any contraction of it could fully explain the apparent increase in circulating erythrocytes following exercise. A racehorse in training has a blood volume of about 50 litres (70 m*l*/kg; 700 kg) and the resting PCV is about 45. After exercise, the PCV may be about 60 if the work is fast, apparently representing some eight litres of extra red cells. The spleen of a Thoroughbred at necropsy weighs 1-3 kg, or rather more after destruction by barbiturate. It seems unlikely that anything like eight litres of packed cells could be released from this organ by contraction of a normal spleen. Of course, there are other organs in which erythrocytes could be sequestered (the lungs, for instance). Besides this, the effect may be relative, at least in part a reflection of fluid shift rather than an increase in circulating red cell mass.

We are actively engaged at this time on the study of peripheral blood taken from exerted horses. One of my post-graduate pupils, Sarah Catling, is working on exerted and trained ponies and will shortly be extending her investigations to cover the Thoroughbred. She has reconfirmed the extraordinary speed and profundity of the change in the circulating blood following the commencement of exertion. It has also been confirmed that at a given level of work, at the steady trot for example, there is a return towards the resting levels after about five or ten minutes, even though the exertion be continued. When exercise is stopped, the return to pre-exercise levels is rapid (say ten or fifteen minutes) in all the conditions investigated and, additionally, there is always a rebound for a variable time below the resting level.

By dye dilution experiments it has been shown that the circulating plasma volume decreases sharply, leading to haemoconcentration. This is accompanied by a modest increase in plasma protein concentration, indicating presumably that water is leaving the circulation at a higher rate than would be accounted for by plasma movements only. While there is also an increase in the circulating red cell mass during exertion, it is not to an equivalent extent in all cases and is unaccompanied by any change in MCV derived by calculation or by observation on 'Channelyser' plots. The leukocyte count is somewhat increased during exertion, along with the erythrocyte count.

These experiments are being repeated on ponies with isotope labels and will be extended to Thoroughbreds, including splenectomized individuals. In due course, Miss Catling's work should enlarge on the substantial contributions of Persson* and extend them to trained Thoroughbreds.

At my laboratory we now have a string of eight Thoroughbred two-year-olds in training. During training, these have been bled at rest every two weeks and Miss Catling has been examining the erythrocytes. From December to the following June, all these animals have shown an increase of erythrocyte count averaging 4 per cent per month: a substantial increase. During this period, the MCV has increased only very slightly and this has been confirmed by 'Channelyser" examinations. Both MCH and MCHC have also increased appreciably at the rate of about 1 per cent and 0,7 per cent per month, respectively. Perhaps the most striking finding, however, is that while all the Thoroughbreds in this string have haematological values within the normal limits, the limits of each individual are not only very much smaller than this, but also remain in a particular order relative to each other.

This current work and existing knowledge permit the tentative conclusion that changes in the concentration of erythrocytes and leukocytes tend to run in parallel in exerted horses and this forms a valuable piece of evidence to support the theory that much of the change in blood is attributable to fluid shift. The fact that there is evidence of a decrease in the albumin space, which is approximately the plasma volume, at a comparable time and to a comparable extent, supports the theory further. Bearing in mind the extraordinary structure of capillary wall, clearly adapted for rapid and repeated fluid shift substantially without cells, one may define a main mechanism of the haemoconcentration of exertion.

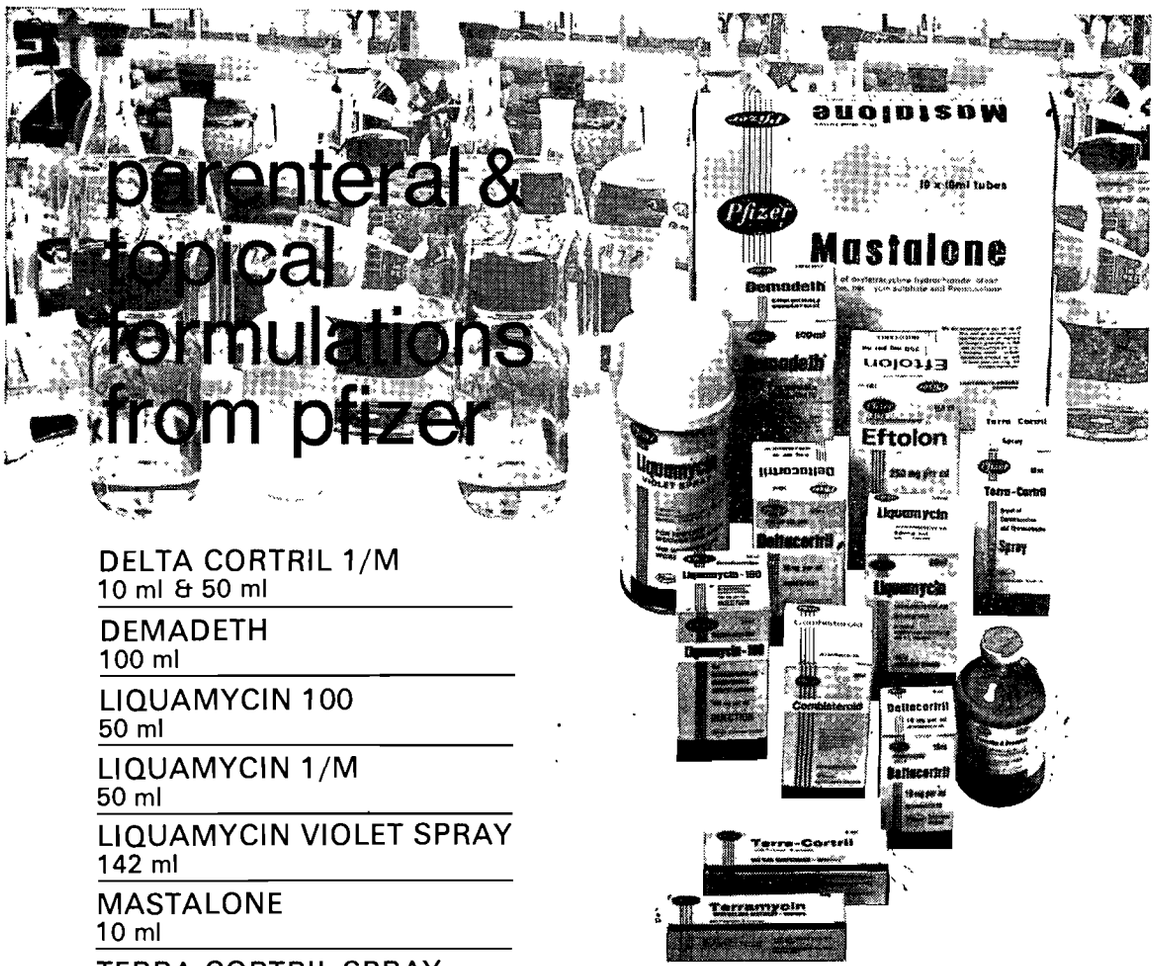
ASSESSMENT OF PERFORMANCE AND POTENTIAL BY THE HAEMATOLOGICAL EXAMINATION OF PERIPHERAL BLOOD

The conclusion from the evidence presented in this paper must be that from examinations of peripheral blood, subject as it is to both rapid and profound changes, cannot lead directly to the assessment of the potential performance of an individual. Much more can be said when an individual is well known from repeated laboratory examinations, for small deviations from the individual's norm can be noticed quickly and reliably. The limits of normality as defined by a particular laboratory, in a particular place and for a particular group of horses, can be specified fairly closely. Deviations from these limits are quickly seen, and such animals cannot, at the time concerned, be at the potential possible in normal health.

* Persson S.G.B. 1967 The blood volume and working capacity in horses. *Acta vet. scand.* Suppl. 19:1.

Two caveats are essential. The first is that all the foregoing depends upon the animals being sampled when truly at rest; as has been discussed earlier, with some horses this is simply not possible. Secondly, it is important to avoid the interpretation that it is an advantage for an individual to be at the upper end of the normal range. This is by no means true. Further, individuals above the upper limit of normality are at a definite disadvantage, although the mechanisms of this are not known. Probably they include increased blood viscosity, leading to poorer microcirculation, and the inability to respond to the challenge of exertion if, so to speak, the response is in part already made.

There is no doubt of the great, and increasing, use of haematology to assess or rather monitor the health status of horses. This assessment is powerfully improved by the use of clinical chemical tests and a consideration of these form the substance of the next paper.



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HAEMATOLOGY IN RELATION TO PERFORMANCE AND POTENTIAL

2. SOME SPECIFIC ASPECTS

L.B. JEFFCOTT*

SUMMARY

Some of the constituents of blood plasma are considered and their relationship to physical fitness and disease is discussed. The paper is divided into three main sections. Firstly, the properties of the major plasma proteins are described and values for normal Thoroughbreds from birth to adulthood presented. Some of the alterations that occur in the serum protein profile are reported and their clinical implications discussed. In the second part some aspects of clinical haemodynamics are touched upon with particular references to the horse's ability to rapidly alter the cell/plasma ratio in peripheral blood. Finally, the application of clinical biochemistry in relation to performance is considered. Serum enzymes C P K and G O T are particularly useful in judging the progress and status of the muscular system during training and could on occasion be used to assess performance potential. The evaluation of bone metabolism by serum biochemical estimations has not yet proved to be of much value clinically.

INTRODUCTION

The first part of the paper has dealt with the cellular elements of the blood; we will now consider some of the constituents of plasma and discuss their relationship to physical fitness and disease. The plasma not only acts as a suitable medium for suspending the cells of the blood but is largely responsible for the maintenance of homeostasis under the control of the nervous and endocrine systems.

Plasma is an exceedingly complex mixture of components. In the compass of this paper only a few aspects can receive consideration. About 93 per cent of plasma is water and the other components include proteins, enzymes, lipids, carbohydrates and salts. It is intended to consider first the plasma proteins and their main functions, followed by some aspects of clinical haemodynamics and finally to review some of the important biochemical features of muscle and bone metabolism in the horse.

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THE PLASMA PROTEINS

a) Properties

The plasma proteins form the major group of substances of the blood plasma. They are made up of some 40 odd proteins together with a large number of enzymes, hormones, clotting and complement factors [28]. For practical purposes they may be divided into two main groups, namely albumin and the globulins (Table 1). These perform a host of different functions including maintenance of blood pH, colloid osmotic pressure and viscosity. They are involved in the complex process of coagulation and by conjugation, in the transport of various hormones, vitamins, fats and essential ions. They also play a vital rôle in the body's defence mechanisms.

The albumin, which makes up about 50 per cent of the total serum protein, is a simple protein, soluble in water and has a molecular weight of 67 000. Albumin molecules have a characteristic corkscrew-shaped molecular conformation which apparently makes them particularly mobile in blood and tissue. By vir-

TABLE 1. MAJOR PROTEIN CONSTITUENTS OF PLASMA

PROTEIN		APPROX MOL. SIZE	SITE OF BIOSYNTHESIS	FUNCTION
	Albumin	67 000	Liver	Regulation of plasma volume by colloid osmotic pressure; storage form of protein; transport functions (e.g. Ca and P).
α_1 Globulins	Prealbumin	61 000	Liver	Binding of thyroxine
	α_1 Lipoprotein	195 000 – 435 000	Liver	Transport of fats & vitamins
α_2 Globulins	Ceruloplasmin	160 000	Liver	Carrier of copper
	Haptoglobulin	100 000	Liver	Binds Hb & transports it
	α_2 Lipoprotein	5 000 000 – 20 000 000	Liver	Transport of fats
β_1 Globulin	Transferrin	90 000	Liver	Transport of iron
	β Lipoprotein	3 200 000	Liver	Transport of fats, vitamins & hormones
β_2 Globulin	IgGT	152 000	R.E. system	Antibodies
γ Globulin	IgG	152 000	R.E. system	Antibodies
	IgM	1 000 000	R.E. system	Antibodies
	IgA	350 000	R.E. system	Antibodies
	Fibrinogen	341 000	Liver	Coagulation (Factor 1)

tue of this and of their relatively small size, they contribute much more to maintenance of fluid balance of the blood and to the colloid osmotic pressure (oncotic pressure) than do the globulins. Albumin also acts as a primary source of reserve amino acids for tissue protein when the need arises. These features are of particular importance in fit racehorses. Albumin also acts as a vehicle for a variety of smaller molecular substances by binding to them and preventing their excretion by the kidney.

The globulins listed in table 1 are only the major constituents and many more occur in trace amounts. As a group they do provide some colloid osmotic pressure, but are chiefly concerned with transport mechanisms and immunity. The last of the major plasma proteins is fibrinogen which is an essential factor of the blood-coagulation system. The site of synthesis of albumin, -α and β_1 globulin and fibrinogen is the liver. The immune proteins (β_2 and γ globulin) are produced by plasma cells and lymphocytes of the reticuloendothelial system.

b) Plasma Proteins in the Horse

There is comparatively little published work on plasma proteins in the horse compared with the literature on cellular haematology. The value of serum protein determinations by electrophoretic separation as an aid to clinical diagnosis has been referred to previously by Coffman [6] and Jeffcott [14]. There has been a number of references to serum protein estimations in horses, although a wide range in the number of protein fractions separated and in their respective concentrations have been reported [3 12 13 17 22 31]. Jeffcott [15] and Morgan [20] have reported serum protein levels in ponies and Standardbreds during the first year of life and the values for albumin have been determined in Thoroughbreds in training [21]. Coffman & Hibbs [7] and Jeffcott [15] have reported on the serum proteins of horses during immunization procedures.

c) Normal Values in Thoroughbreds

In order to show the alteration of serum proteins according to age, the results of a group of 19 normal Thoroughbred foals from one well-managed stud are given in table 2. A further 101 healthy horses from six months to six years of age were examined prior to going into training and during active training (Table 3). Total serum protein was estimated by the biuret method and the individual proteins (albumin, -α_1, α_2, β and γ globulin; see Fig. 1) differentiated on CAM strips [15].

In the foals, there was a very low total protein at birth but the level rapidly increased after colostrum ingestion. In the neonatal period there was a fall of passive γ globulin and to a less extent of the albumin. The low albumin levels during foalhood may have been associated with the great demands for tissue protein during the peak period of growth. There was a steady rise from two weeks of age to adulthood at four to six years of age and an accompanying increase in the total protein.

TABLE 2. SERUM PROTEIN LEVELS IN HEALTHY THOROUGHBREDS FROM ONE STUD (g/100 m/)

AGE	(No. of animals)	TOTAL PROTEIN	ALBUMIN	GLOBULINS			
				α_1	α_2	β	γ
0 hr.	(16)	4,25	2,87	0,19	0,59	0,63	0
24 hr.	(17)	5,24	2,95	0,18	0,36	0,98	0,76
7 dys.	(19)	5,12	2,65	0,26	0,56	1,04	0,63
14 dys.	(19)	4,97	2,44	0,26	0,54	1,21	0,54
28 dys.	(18)	5,03	2,46	0,29	0,64	1,25	0,43
2 mth.	(16)	5,34	2,45	0,29	0,81	1,34	0,45
3 mth.	(8)	5,81	2,74	0,22	0,91	1,40	0,52

TABLE 3: SERUM PROTEIN LEVELS IN HEALTHY THOROUGHBREDS (g/100 m/)

AGE	(No. of animals)	TOTAL PROTEIN	ALBUMIN	GLOBULINS			
				α_1	α_2	β	γ
6–9 mth.	(32)	5,88	2,81	0,27	1,03	1,25	0,53
1 yr.	(23)	5,82	2,96	0,21	0,96	1,25	0,42
2 yr.	(9)	5,98	2,99	0,31	0,88	1,16	0,53
3 yr.	(27)	6,07	3,45	0,29	0,71	1,03	0,61
4–6 yr.	(10)	6,46	3,74	0,27	0,66	1,11	0,70
6 mth.–6 yr.	(101)	5,99	3,13	0,26	0,88	1,18	0,54

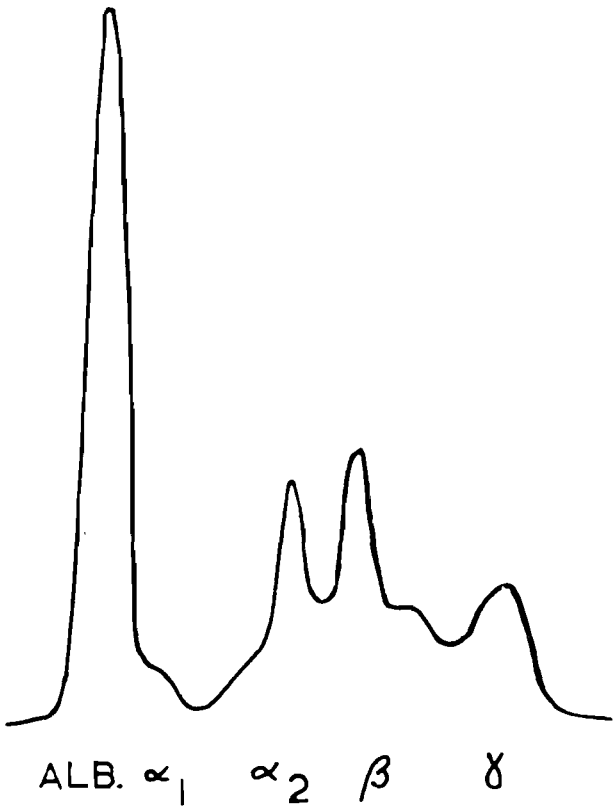

Fig. 1: Electrophoretic separation of normal serum proteins in the horse.

As immunological competence increased, there were rises in the α_2, β and γ globulins of the foals. Nevertheless, the pattern altered from the time the animals went into training as yearlings. The β globulin, which had risen steadily since birth, levelled off at one year and fell a little over the next two to three years. The α_2 globulin followed a similar pattern but showed a more pronounced drop with age. The γ globulin, which began with a steady rise from two months, declined temporarily at one year and then increased gradually. Levels remained below that recorded after birth, the latter resulting from colostrum ingestion. The α_1 globulin showed no significant alteration at any time from birth to adulthood. A very different picture was seen in ponies kept out at grass: these three fractions continued to rise in the second year, particularly the β_2 globulin.

The progressive reduction of values in globulin seen in one-, two- and three-years-olds may have been associated with the rather protected environment of a training establishment and the absence of significant parasitic infestation.

d) Alterations in the Serum Protein Profile

Some of the more important alterations in serum proteins are given in table 4. There will be an elevation of total serum protein in horses with dehydration and haemoconcentration. The changes in albumin concentration in Thoroughbreds usually prove to be the most informative. Sykes [29] reports that a fall in serum proteins, particularly albumin, occurs under conditions of increasing stress during training. Certainly, low albumin levels are seen in horses with intestinal damage. The albumin is the major protein to be lost because of its smaller molecular size. Where there is enteritis or ulceration of the mucosa in the large or small bowel, protein can leak out between the damaged cells into the intestinal lumen. There is usually some compensatory rise of the globulin fractions which tends to maintain colloid osmotic pressure and prevent oedema and ascites. As the liver is the site of synthesis of albumin, chronic or low grade hepatic damage may affect the serum albumin concentration. In cases of renal damage, there may be

TABLE 4: SOME FACTORS AND CONDITIONS AFFECTING THE SERUM PROTEIN PROFILE OF HORSES

PROTEIN	SERUM CONCENTRATION	CAUSES
Albumin	Low	– Intestinal damage – protein losing enteropathy – Chronic liver damage – Chronic parasitism
	High	– Dehydration & haemoconcentration
α_1 Globulin	High	– ?
α_2 Globulin	High	– Pyrexia & viral infections – Chronic suppurative conditions – Inflammatory reaction of bone or joints – Nephrosis & amyloidosis
β Globulin	High	– Systemic parasitism (e.g., *S. vulgaris*) – Chronic infections (e.g., Tuberculosis)
γ Globulin	Low	– Late pregnancy; poor passive immune status in foals
	High	– Chronic infections; lactation; cirrhosis; paraproteinaemia

considerable urinary loss of albumin although cases of nephritis in horses are rare. Hypoalbuminaemia is a common finding in helminth infections and in strongyle infections it can produce marked rises in the β globulin levels [25].

Hypergammaglobulinaemia and raised β globulin levels usually indicate some immune process, often the result of chronic infection or inflammatory focus. Nevertheless, other factors may be involved and it appears to be quite normal for lactating mares to have an increased γ globulin level until after weaning. Hypergammaglobulinaemia also occurs in cirrhosis of the liver and during immunization [7]. A number of paraproteinaemias also occasionally occur in Thoroughbreds and these are usually associated with lymphoid tumours such as lymphosarcoma.

Increased levels of α_2 globulin usually indicate some inflammatory reaction often involving bones and joints, although it may reflect damage to the kidney. Chronic purulent foci, osteomyelitis and suppurative arthritis (joint-ill) produce marked rises in the α_2 globulin. The first indication of these raised globulin values may be an elevation in the plasma viscosity [2] and it is always worth carrying out serum protein estimations in any horse which shows a viscosity greater than 1,80 mPa.s (centipoises).

Lowered γ globulin levels are seen in pregnant mares just prior to parturition. This is associated with the selective concentration of immunoglobulins by the mammary gland prior to foaling [16]. Hypogammaglobulinaemia is sometimes seen in young racehorses and presumably indicates some form of immune incompetence. Certainly, a careful clinical check on these animals should be kept to prevent untoward infections. A primary combined immunodeficiency has been reported in Arabian foals [19] but the condition has not yet been seen in Thoroughbreds.

CLINICAL HAEMODYNAMICS IN THE HORSE

The effects of exertion and excitement on the blood picture have already been mentioned. A marked alteration in the cell/plasma ratio of the peripheral venous blood takes place with an associated shift of intravascular to extravascular fluid. The source of the increased number of cells is considered to be wholly or partly from the spleen [23] although other organs of sequestration such as the gut and lungs may also play an important rôle.

The resting packed cell volume (PCV) of fit racehorses is about 40 per cent ± 4 per cent [1] while under exertion this will rise by 10 - 20 per cent, thereby greatly improving gaseous exchange and tissue oxygenation. There is, of course, a concomitant rise in blood viscosity at this time. While it is essential that levels be at least 35 - 45 per cent for PCV, some racehorses are haemoconcentrated at rest or show an apparent polycythaemia. The aetiology of this condition is not clearly understood but resting levels of PCV of over 50 - 60 per cent are seen in otherwise clinically normal-looking horses. These animals rarely perform well and often 'blow up' in a race, finishing well below their expected form. Probably what happens is that with the increasing PCV during exertion, the blood becomes so viscous that there is increased resistance to blood flow in small blood vessels and capillaries which leads to a rapid reduction in oxygenation of the tissues. Sykes [30] suggests administration of 500 m*l* of 5 per cent saline and sodium citrate to reduce PCV by temporary transfer of fluids in the blood stream. Restriction of fast work and DOCA (desoxycorticosterone acetate) implants have also produced a return to form in these horses.

The ability of horses to call on their reserves of erythrocytes by rapid haemoconcentration can easily be reversed and the situation of haemodilution obtained. A hypotensive drug such as acetyl promazine (ACP) can produce a significant drop in the blood cells (Table 5). This is accompanied by very little change in the plasma viscosity and serum proteins although there is probably some increase of intravascular fluid. Similar changes were recorded in horses given the drug by intravenous or intramuscular administration while the blood picture of control animals showed no significant alteration (Fig. 2). In this situation, which might prove a useful model for further study of haemodynamics, the cells presumably sequester or return to whatever reservoir of cells may be available. Persson, *et al.* have recently reported that after splenectomy there is very much reduced variation in the PCV and they conclude that the uneven distribution of erythrocytes in the vascular system is due largely to significant red cell accumulation in the spleen [24].

TABLE 5: ADMINISTRATION OF ACETYL PROMAZINE TO 55 HORSES BY INTRAMUSCULAR AND INTRAVENOUS INJECTION

(Dose rate 0,049 ± 0,0012 mg/kg)

ESTIMATION		EFFECT ONE HOUR AFTER INJECTION
(a)	BLOOD	
	TRC	20% Fall
	Hb	25% Fall
	PCV	15% Fall
	TWC	20%
	Platelets	No significant change
(b)	PLASMA	
	PV	3,5% Fall
	TP	2% Fall
	ALB	No significant change
	GOT	No significant change

SOME ASPECTS OF CLINICAL BIOCHEMISTRY IN RELATION TO PERFORMANCE

a) Serum Enzyme Activity in Muscle Metabolism

The skeletal musculature comprises some 50 per cent of the total body mass and is a particularly active tissue in such an athletic animal as the racehorse. It contains the following enzymes in fairly large concentrations GOT (glutamic oxaloacetic transaminase), GPT (glutamic pyruvic transaminase), CPK (creatine phosphokinase), LDH (lactic dehydrogenase), MDH (malic dehydrogenase), ALD (aldolase); there are smaller amounts of AP (alkaline phosphatase), SDH (sorbitol dehydrogenase) and GLDH (glutamic dehydrogenase) [10]. The two enzymes usually examined in the laboratory are GOT and CPK. The values for resting levels in normal healthy horses in our laboratory for GOT are 107 i.u./*l* (SD 20) and for CPK 37 i.u./*l* (SD 19).

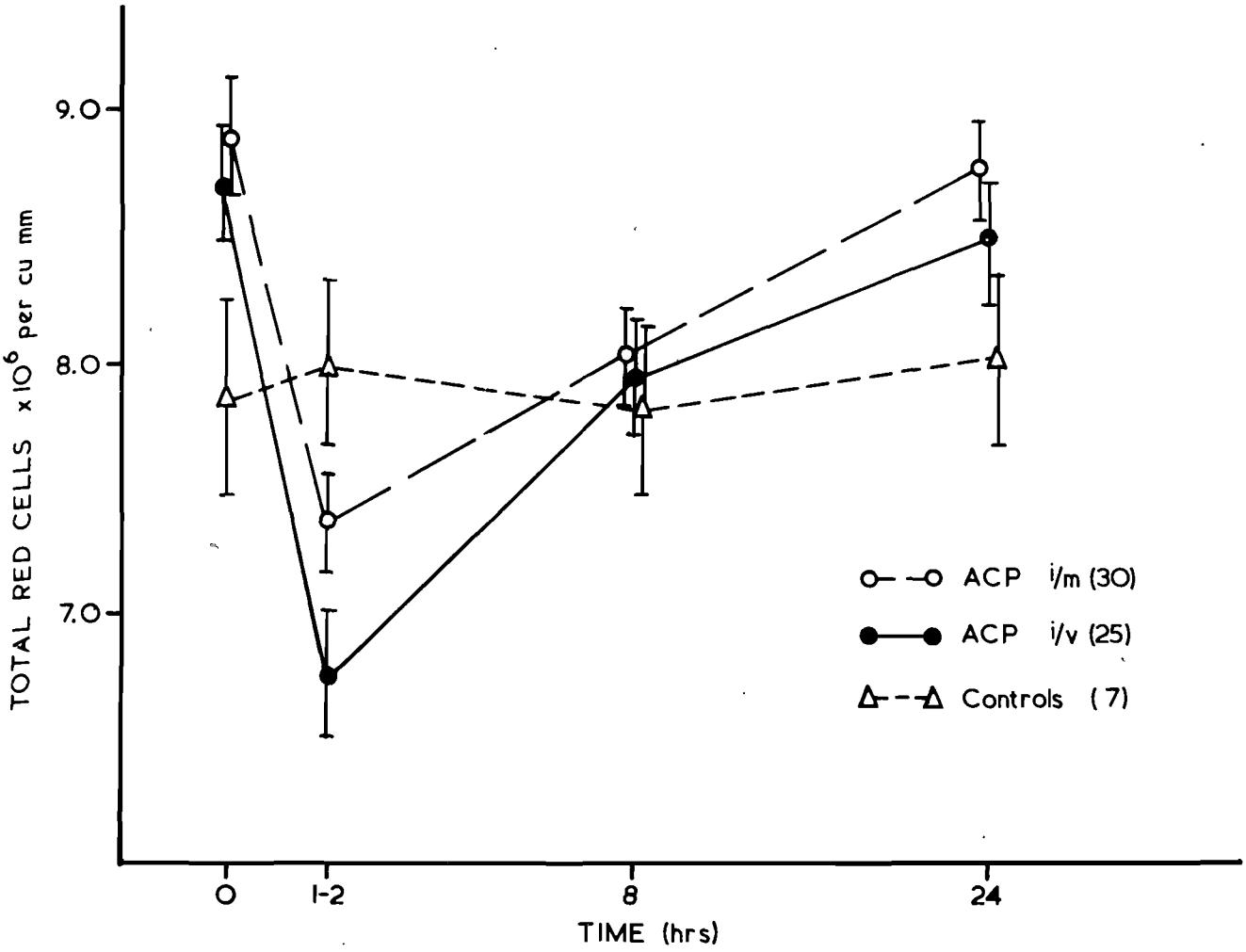

Fig. 2: Changes in total red cell values before and after administration of acetyl promazine.

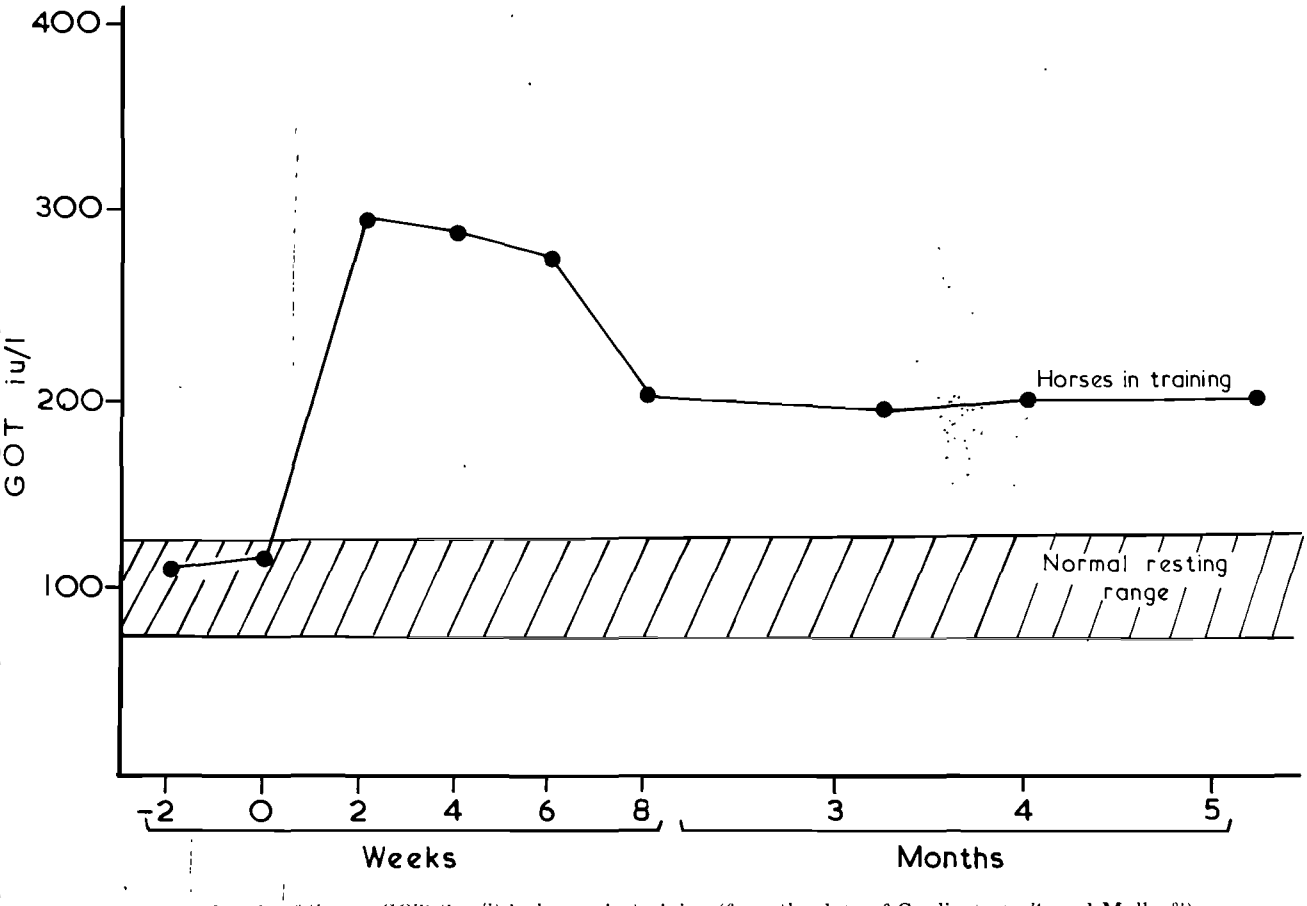

Fig. 3: Levels of Serum GOT (i.u./*l*) in horses in training (from the data of Cardinet *et al*[4], and Mullen[21])

It was first shown by Cornelius *et al* that horses in training have higher GOT levels than inactive horses [8]. This has since been confirmed by Cardinet *et al* [4] and Mullen [21], who determined the GOT activity throughout a training programme (Fig. 3). The levels rose rapidly by two to three times the resting value in the first two weeks of work. There followed a gradual fall over the ensuing two to three months to a 'fitness' value which was significantly higher than the normal resting levels. In the racehorses in Mullen's series, levels remained constant provided the horses were kept in regular work. Cardinet *et al.* [4] observed an increase in GOT activity from about the third to fourth month of the training programme but this was ascribed to some reduction in the exercise with the high feed intake maintained.

The enzyme changes in working animals are believed to be due to slight muscle damage, particularly at the onset of the training programme rather than to changes in the permeability of muscle cells. Where there is severe muscle damage, as in azoturia or setfast, there are marked increases in the muscle enzyme activity of the serum. This particular condition has been the subject of a considerable amount of investigation in recent years although the exact aetiology still remains obscure [4 5 8 10 11]. Attacks of azoturia result in an almost immediate release of muscular enzymes and this takes place even before the onset of clinical signs. CPK is the most useful indicator as it is a muscle-specific enzyme and shows the fastest reaction by reaching maximum activity often within 15 minutes but certainly not later than 24 hours of an attack. Gerber states that CPK and ALD are the most sensitive indicators of muscle damage followed by GOT, MDH, LDH, and GPT [11]. Liver damage will also produce somewhat elevated ALD levels; the other enzymes are found in considerable concentration in other organs. GOT is a non-specific enzyme and raised levels indicate more or less any kind of cellular damage but in horses it is most likely to be that of skeletal muscle.

A close correlation seems to exist between the extent of muscle damage and the serum enzyme activity. In addition, there is not much qualitative difference in the pattern of enzyme release between azoturia, tying-up, myositis, tetanus, muscular trauma or even the effects of physical exercise in untrained animals [10].

The determination of CPK and GOT levels in horses which do not perform particularly well can sometimes be a rewarding exercise. In a series of 14 fairly fit horses the muscle enzymes were determined before and after 20 minutes lunging or ridden exercise (Fig. 4). There was no obvious difference in values and no rise by 24 hours after exercise. In a series of five horses referred with a history of back trouble or obscure lameness, with no evidence of bone damage, the CPK rose somewhat immediately after exercise but showed a considerable elevation within 24 hours. There was also a doubling of the mean GOT values at this time. None of these animals showed obvious signs of azoturia or tying-up although a clinical diagnosis of muscle damage was made in each case.

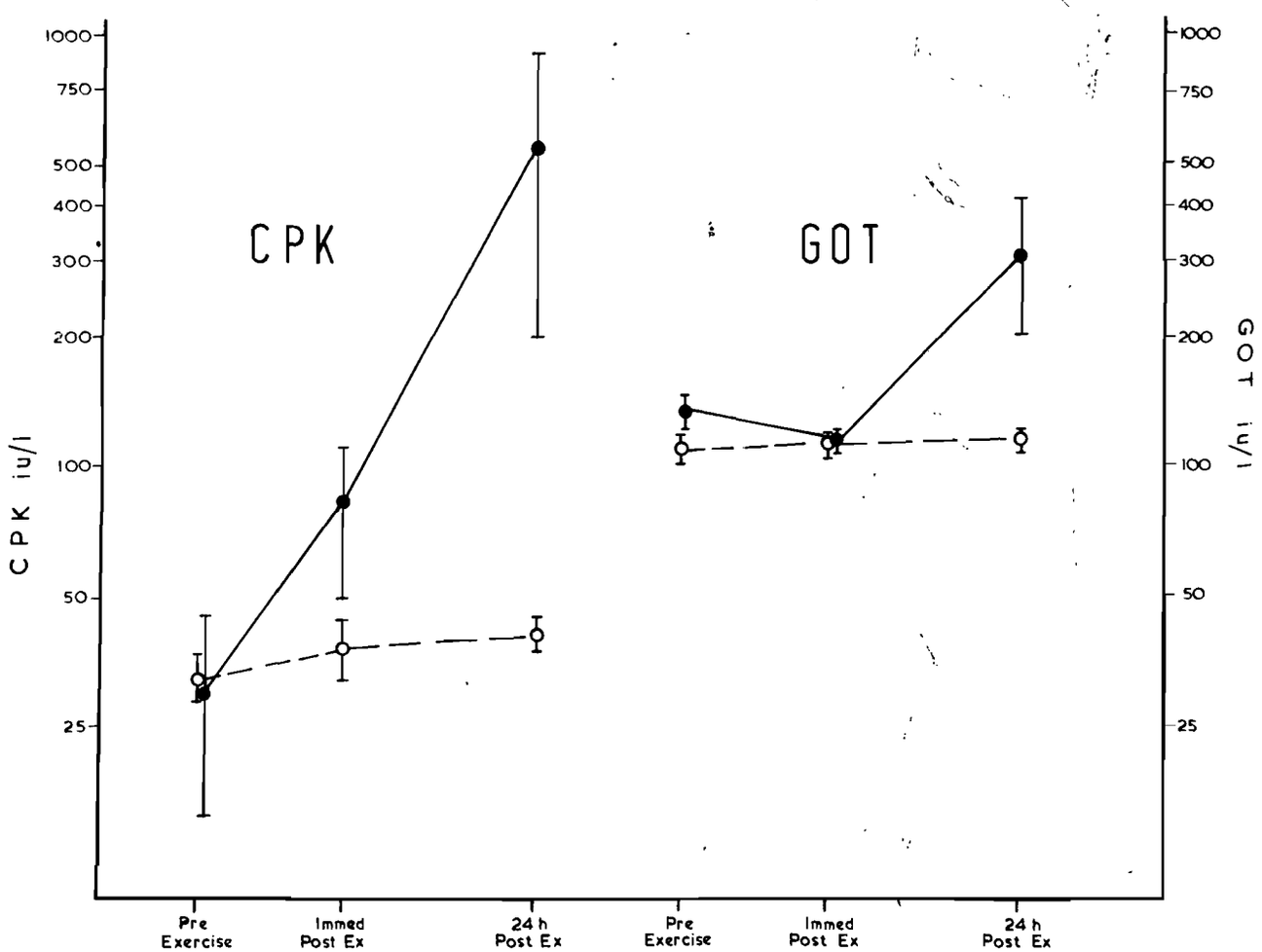

Fig. 4: Mean levels of muscle enzymes (CPK & GOT) before and after exercise in two groups of horses. (O–O horses showing no sign of mus damage; ●–● horses showing subclinical muscle damage).

b) Blood Levels of Ca/P and Alkaline Phosphatase (AP)

Serum analysis of Ca and P for an assessment of bone metabolism in relation to fitness and performance usually proves to be of little value. Serum Ca and P levels in horses (Blackmore, 1973, personal communication) from foalhood for four years of age are given in table 6. The physiological compensatory mechanisms that maintain normal serum levels are very efficient and the dietary intake must always be considered. Repeated determinations over a period of time may support a diagnosis of excess dietary phosphorus [18]. High and low Ca diets have little effect on serum Ca but a high P diet will produce a hypocalcaemia [27]. Hypercalcaemia occurs during hyperparathyroidism or from excessive administration of vitamin D. High P levels are sometimes seen in young animals showing clinical signs of epiphysitis.

Exercise may increase the dietary requirement for Ca and P [26] as substantial amounts of both ions are lost during sweating. The requirements of the mature working horse can probably be met by diets providing 0,4 per cent Ca and 0,3 per cent P.

Alkaline phosphatase is capable of hydrolysing monophosphate esters with the liberation of inorganic phosphate. There is a very high serum activity of this enzyme at birth. This is followed by a gradual and progressive fall with age [9 21]. AP will be raised in such conditions as rickets and osteomalacia.

TABLE 6: MEAN VALUES OF SERUM CALCIUM AND INORGANIC PHOSPHATE ACCORDING TO AGE IN 650 HORSES

Age	Calcium mg/100 ml (SD)	Inorganic Phosphate mg/100 m/ (SD)
Foals $\geq$ 1 y.o.	12,8 (0,4)	5,9 (0,4)
1 y.o.	12,6 (0,3)	4,9 (0,3)
2 y.o.	11,9 (0,3)	4,5 (0,6)
3 y.o.	11,9 (0,3)	4,2 (0,4)
4 y.o.	11,8 (0,3)	3,9 (0,6)

CONCLUSIONS

A number of biochemical tests can assist in assessing the fitness and health status of horses. In conjunction with the results of the blood cell picture, they can form part of a useful metabolic profile. Nevertheless, there is considerable scope for more work to further our understanding of the complicated physiological mechanisms associated with fitness.

Serum enzyme levels have a particular application in judging the progress and status of the muscular system during a training programme. It is possible to pick out horses showing clinical or subclinical muscular damage and therefore to assess to some extent their performance potential. Serum biochemical estimations for evaluation of bone metabolism are not meaningful because of the efficient compensatory mechanisms of homeostasis and the influence of dietary intake.

The horse has a reserve pool of erythrocytes that can be mobilized very rapidly during exertion or alternatively added when there is lowering of blood pressure. The resting PCV is a useful aid to assessing the health of a racehorse.

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DISCUSSION

Maureen Aitken: Did you measure CPK levels at hourly intervals in the period between cessation of exercise and 24 hours later or just immediately post-exercise and then 24 hours later?

L.W. Jeffcott: We examined only three blood samples in each of these horses, before exercise, immediately after exercise and after 24 hours. I have mentioned this regime simply as a clinical aid to diagnosis and was not trying to show the exact time of release of the enzyme. A significant rise takes place within 24 hours if a rise is going to take place at all.

Maureen Aitken: We have measured CPK levels in our horses and have shown a peak of release at five hours post-exercise.

M.A.J. Azzie: The CPK levels that you refer to at approximately 24 hours according to your graph reached a level of about 500 IU/litre. Is that correct?

L.W. Jeffcott: The 'normal' group of clinically fit and healthy animals did not show a significant rise at all. The levels were plotted on a log scale and in the group of horses with suspected muscular conditions involving either the back or hind limbs, there was a rise in CPK levels in the 24 hour post-exercise sample. The levels at this time rose from about 30 IU/litre to a mean of over 500IU/litre.

M.A.J. Azzie: The reason I asked this question was because in a survey we carried out here the highest levels reached in physically fit horses approximately five or six hours after a race were in the range of 85-95 IU/litre.

L.W. Jeffcott: Thank you, Dr Azzie that confirms my point. In fact we have also examined one or two horses that had been subjected to strenuous racing and have obtained a result similar to yours.

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HAEMATOLOGY OF THE FIT RACEHORSE*

G.A. STEWART AND J.D. STEEL**

SUMMARY

Studies relating the resting blood count to racing performance are reviewed, and the rôle of circulating red cells and haemoglobin as part of an oxygen transfer chain that affects cardio-respiratory function is indicated. As a result of changing from manual to automated laboratory techniques, some modification of the normal range of values for RBC, Hb and WBC has been described. The principal aim of studies on resting blood samples is to identify horses likely to perform below expectations. Low results are unlikely to have been significantly affected by excitement and splenic contraction, and can be associated with poor performance. It is considered that after laboratory quality control is taken into account, any blood count below one standard deviation from the mean is suboptimal from the point of view of successful racing in the Melbourne metropolitan area. Because of this, definition of optimal, sub-optimal and anaemic blood levels is useful when factors affecting racing performance are being considered.

INTRODUCTION

In the paper on electrocardiography at this conference [13], attention was drawn to a number of cardio-respiratory factors that affect racing performance. It was proposed that these factors form an oxygen transfer chain and that circulating red cells and haemoglobin constitute one of the links in that chain. It follows that a temporary or permanent deficiency of this or any other link could be a critical factor limiting capacity for performance.

In previous studies [3 11 12 14 16], an attempt was made to define optimal, sub-optimal and anaemic levels for the erythrocyte count (RBC), haemoglobin concentration (Hb) and packed cell volume (PCV).

The study by Clarkson [3] on 50 metropolitan winners sampled within three days of their win showed that 82 per cent had RBC, PCV and Hb levels which exceeded the breed means established from a study of 329 Thoroughbreds. Of the remainder, five, with values below the breed mean, were two-year-olds running over distances of about 1000 metres.

Following this, Stewart *et al.* [14] found that a group of 55 metropolitan winners and a group of 71 winners of lesser races on country racecourses had RBC, PCV and Hb levels that exceeded the population mean.

These observations led to the suggestion that levels within the normal range that were below the breed means should be regarded as sub-optimal for metropolitan racing especially when the horses were older than three years.

The mean values for RBC, PCV and Hb levels which permitted this division of the normal range were presented by Stewart and Steel [16].

CHANGES IN LABORATORY TECHNIQUE

Since publication of the data referred to above, there have been significant changes in some techniques used in our laboratory. These include the introduction of an automatic cell counter † and associated dual dilutor †† and haemoglobinometer††, which have been used routinely since December 7, 1971.

Previously, blood for RBC and total leukocyte counts (WBC) was diluted in standard diluting pipettes to concentrations of 3:1 000 and 1:20 respectively, according to the recommendations of Schalm [10]. The cells were counted in improved Neubauer counting chambers. Haemoglobin estimations were then done according to the cyanmethaemoglobin method of Evelyn and Malloy [4] using a commercial reagent and standard solution °, and pipettes calibrated to contain 0,02 m*l* blood.

There has been no change in the methods for determining PCV °° or ESR °°°.

When the new methods were adopted in the laboratory, comparative studies with samples estimated by both the old and new techniques indicated that the new methods gave slightly lower RBC, Hb and WBC estimates than the old methods.

Results obtained from control samples provided by the N.S.W. Blood Transfusion Service (Table 1) and others purchased from the manufacturer of the new apparatus, have led to the conclusion that the automatic cell counter and haemoglobinometer provide results which are closer to the true levels present in the samples. This necessitated re-definition of the normal range and the means used for defining optimal and sub-optimal levels for metropolitan racing. The old and new means are included in table 2. These effects of laboratory technique illustrate the need to take into account the methods and quality control of a laboratory when interpreting results received from it.

Quality Control

In the quality control programme employed in our laboratory, at least one duplicate sample is included in each batch of routine samples submitted to the laboratory for analysis. The duplicate is always collected from the same venepuncture immediately after the routine sample, and given a fictitious name and other details so as not to reveal its identity to the laboratory staff. From the duplicate estimations, a measure of the repeatability or precision of the laboratory method is obtained. The results of our latest analysis of 36 coded duplicates are shown in table 3. These indicate the precision of individual estimates of Hb, PCV, RBC and WBC as approximately ± 5%. This represents an improvement in precision of RBC and WBC over that reported by Stewart *et al.* [14] when cell counting was still being done manually.

*Presented by J.D. Steel.

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†Coulter Counter Model FN: Coulter Electronics Ltd., England.
††Coulter Electronics Ltd., England.

°–"Aculute" and "Acuglobin": Ortho Diagnostics, Sydney, N.S.W.
°° "Autocrit" Centrifuge: Clay Adams Inc., New York.
°°° "Sedirak": Becton Dickinson & Co. Ltd., Rutherford, N.J.

TABLE 1: QUALITY CONTROL PROGRAMME HAEMOGLOBIN – ABSOLUTE LEVELS*

Number of Control Tests:	17
Mean of Known Levels (A):	13,6 g/100 m/
Mean of Laboratory Results (B):	13,6 g/100 m/
Variance of Technique†:	0,106
Standard Deviation:	0,33 g/100 m/
Precision (95% probability):	± 0,69 g/100 m/ (i.e. ± 5.1%)

* Based on solutions of known concentration provided regularly by the N.S.W. Blood Transfusion Service.

† Based on differences (d) between individual estimates (B–A). $\hat{\sigma}^2 = \frac{\Sigma d^2}{17}$

TABLE 2: HAEMATOLOGICAL PARAMETERS* IN THOROUGHBREDS IN VICTORIA

TECHNIQUES		1966 – 1971 MANUAL CELL COUNTING ETC. †	1971 – 1973 AUTOMATIC CELL COUNTING ETC. †
No. of Observations:		2356	637
RBC	($\times 10^6/mm^3$)	10,1 ± 1,3	9,4 ± 1,3
Hb	(g/100 m/)	16,0 ± 1,7	15,0 ± 2,0
PCB	(%)†	40,9 ± 4,2	40,3 ± 5,7
MCV	($\times 10^{-9}$ mm^3)	40,7 ± 4,0	43,2 ± 3,0
MCHC	(g/100 m/)	39,3 ± 2,2	37,2 ± 1,8
MCH	(pg)	16,0 ± 1,6	16,1 ± 1,3
ESR	(mm/hr)†	39,4 ± 14,5	38,8 ± 16,6
WBC	($\times 10^3/mm^3$)	9,8 ± 2,1	8,9 ± 1,9

* Mean ± standard deviation.

† For details of methods see text.

TABLE 3: QUALITY CONTROL PROGRAMME – REPEATABILITY ESTIMATES*

	RBC ($\times 10^6/mm^3$)	Hb (g/100 m/)	PCV (%)	WBC ($\times 10^3/mm^3$)
Mean of Original Estimates (A)	9,0	14,3	38,8	8,52
Mean of Duplicate Estimates (B)	9,1	14,5	39,4	8,56
Variance of Technique†	0,044	0,090	0,802	0,056
Standard Deviation	0,21	0,30	0,90	0,24
Precision (95% probability) i.e.	± 0,42 (4,7%)	± 0,61 (4,2%)	± 1,8 (4,6%)	± 0,48 (5,6%)

* Based on 36 coded duplicates each collected after the original sample.

† Based on differences (d) between individual estimates (B–A): $\hat{\sigma}^2 = \frac{\Sigma d^2}{72}$

The Normal Range

Based on the conventional definition of the normal range as the mean ± 2 standard deviations, [1 17] the ranges obtained with the new laboratory techniques from Thoroughbreds in Victoria are shown in table 4. In our experience, the extremes of this range are too wide to be useful. They suggest that some anaemic and some polycythaemic animals have been included in the so called normal population. Accordingly, modification of the upper and lower limits of the normal range have been suggested in table 4.

working capacity of trotting horses and the THb, expressed as g/kg body mass. He also reported a highly significant correlation between the post-exercise haemoglobin level and racing performance expressed as kilometre time.

The degree of sympathetic stimulation associated with venepuncture and blood collection appears to vary considerably with the technique of the collector. A greater response is likely in excitable horses, particularly those which have had previous painful experiences associated with injections or venepuncture.

TABLE 4: NORMAL RANGE OF ERYTHROCYTIC PARAMETERS IN THOROUGHBREDS IN WORK IN VICTORIA

	Mean - 2 SD	Suggested Lower Limit *	Mean – Precision Estimate	MEAN	*Suggested Upper Limit	Mean + 2 SD
RBC ($x10^6/mm^3$)	7	7,5	9	9,5	11	12
Hb (g/100 ml)	11	12,5	14,5	15	17,5	19
PCV (%)	29	33	38	40	48	51
DIAGNOSIS	ANAEMIC	SUB-OPTIMAL / DOUBTFUL		OPTIMAL		POLY-CYTHAEMIC

* Suggested Limits ≃ Mean ± (Standard Deviation + Precision Estimate).

Bearing in mind the precision determined from quality control of the methods used, a doubtful area has been included at the lower end of the segment of the normal range regarded as optimal for metropolitan racing. Results below this, classed as sub-optimal or anaemic, are considered unsatisfactory for metropolitan racing, as it is unlikely that laboratory errors or disturbance during collection could have produced a false result.

Excitement during Venepuncture

Increases in RBC, Hb and PCV arising from disturbance or excitement of horses during collection of the blood samples are well recognized [2], and are presumably due to splenic contraction following sympathetic or adrenergic stimulation [9 18]. In an attempt to overcome this problem, which causes false high results, some workers have advocated procedures designed to produce more complete and constant splenic contraction and re-definition of the normal range on this basis. Irvine [5] suggested using a comparison of results obtained immediately after exercise with those obtained at rest, before finally assessing RBC, Hb and PCV for racing purposes. Likewise, Persson [6 7 8] placed greater reliance on Hb estimations from samples collected after exercise or adrenaline injection than from the resting subject. After calculating total body haemoglobin (THb) from the post-exercise Hb and the blood volume, Persson [7] reported a highly significant correlation between the

Ideally, the people associated with those experiences should not be present when a resting blood sample is being collected.

The technique of venepuncture and collection is extremely important. It should be done in a manner which causes minimum disturbance and discomfort to the animal. For venepuncture we routinely use 20-gauge $1\frac{1}{2}$ inch sterile disposable needles which can be inserted gently through the skin and into the jugular vein without the horse flinching. Aqueous antiseptic† is used in preference to alcoholic solutions to avoid the cooling sensation and odour which the horse may associate with unpleasant injections. The needle is then connected to an evacuated collection tube †† and 7 m*l* blood quickly withdrawn. Using this technique, it is usually possible to collect the sample from the horse standing unrestrained in its loose box. With some horses, it may be necessary for the animal's usual attendant to offer the minimum restraint of placing his hands on the horse's nose. Only in a few cases, when more restraint is thought necessary, are a head stall and lead placed on the animal. After a few routine collections, even in these, it is usually possible to collect the sample from the horse unrestrained.

If a horse does become disturbed, it is likely to be shown by the horse flinching when the jugular vein is

†"Hibitane": ICI Australia Ltd., Melborne.

††"Vacutainer", containing EDTA: Becton-Dickinson & Co. Ltd., Rutherford, N.J.

raised, by pulling back and resisting venepuncture, or simply by an increase in the force or rate of the cardiac impulse. If these signs occur, they are noted at the time of collection and less reliance is placed on the result.

Using the above technique, it is commonly possible for the sample to be obtained in less than 30 seconds after the collector first enters the loose box. In earlier studies on the effects of excitement during collection [15], it appeared that after the horse became disturbed, approximately 30 seconds elapsed before the RBC, Hb and PCV of the jugular blood became elevated.

When the duplicate sample is collected for quality control studies, it is always the second sample collected, and the total collection is achieved in approximately 45 seconds.

The 36 duplicate collections presented in table 3 were obtained in this way. In 15 of these samples, each of the RBC, Hb and PCV had increased slightly in the second sample, whereas in the other 21, there was no change or a decrease in at least one of these parameters. In the 15 which did show a consistent increase, it could be assumed that some excitement contributed to the result. In these, the mean increases in RBC, Hb and PCV were approximately 3% (Table 5). Before attempting to generalize, however, two aspects should be considered: (i) the effect occurred only in 15 of the 36 duplicate collections, and (ii) the time for the duplicate collection was longer after venepuncture than that taken for routine samples. Nevertheless, by using these duplicates as a basis for quality control, collection effects as well as laboratory precision have contribted to the over-all variability of approximately ± 5% (Table 3).

Since changing to the new techniques in December 1971, routine samples have been obtained from 46 winning horses, which won a total of 20 metropolitan and 40 country races within 28 days of the blood collection. All except one had haemoglobin levels above 13,0 g/100 m*l* (i.e. Mean – 1 SD). This horse with 12,8 g/100 m*l* was still above our suggested lower limit of 12,5 g/100 m*l*, which takes the precision estimate into account. Combining these observations with those of Clarkson [3], gives 96 individual winners, of which only one had a resting haemoglobin level more than one standard deviation below the breed mean. This horse had a heart score of 120, which may have helped to compensate for his haemoglobin deficiency.

In Melbourne metropolitan racing, there is little doubt that when the Hb level falls below 12,5 g/100 m*l*, the red cell count below 7,5 x 10^6 mm^3 and the packed cell volume below 33%, the chance of racing success is low.

It is this association between a low result and poor performance, or the identification of horses likely to perform below expectation, that is the principal aim of studies on the resting blood sample. The converse, namely, a correlation between a high blood count and good performance, presents greater difficulties because many factors contribute to winning or losing. While there is some concern that a high count may be due to inadequate technique, this is lessened by the fact that the object is not to say that certain horses will win, but rather to say that certain horses are unlikely to win.

At times, other authors seem to have interpreted the studies of Persson and ourselves as being contradictory, but this is probably not the case. In both points of view, there seems to be agreement that good racing performance requires adequate oxygen carriage in the blood and this means high circulating Hb levels. The differences between us appear to be in technique and approach, with Persson seeking to identify horses that will perform well, and we seeking to identify those horses which are likely to perform below expectations.

With this objective in mind, providing one takes care during collection and knows the precision of the laboratory, the resting blood sample can provide valid results. It is also simpler and more practical when the analysis of about 3 000 blood counts is being considered.

TABLE 5: PROBABLE EFFECT OF MINOR EXCITEMENT DURING COLLECTION OF 15 DUPLICATE SAMPLES

	RBC ($x10^6/mm^3$)	Hb (g/100 m*l*)	PCV (%)	WBC ($x10^3/mm^3$)
Mean of First Samples Collected	8,6	13,8	37,7	8,7
Mean Increase in Second Sample Collected*	0,3 ± 0,3 (3,7%)	0,4 ± 0,3 (3,2%)	1,3 ± 1,3 (3,4%)	0,1 ± 0,4 (1,5%)

* Mean ± standard deviation.

RELATIONSHIP OF ERYTHROCYTIC PARAMETERS TO RACING PERFORMANCE

It has been shown that the majority of metropolitan winners in Melbourne had higher resting blood counts than country winners and non-winners. This justifies the definition of an optimal range for RBC, Hb and PCV.

In the 50 metropolitan winners studied by Clarkson [3], no horse won with a Hb level more than one standard deviation below the mean. On this basis, reference to table 4 indicates that the suggested lower limit of 12,5 g/100 m*l* allows for a standard deviation down to 13 g/100 m*l* and a precision estimate of ± 0,5 g/100 m*l* down to 12,5 g/100 m*l*.

Acknowledgments

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DISCUSSION

D.H.G. Irwin: In our laboratory we find that some of our horses can run very adequately on what would be approximately 1½ standard deviations below our norm. Our figures from PCV – Hb are much higher than yours; I do not know if it is the altitude, because in Johannesburg we are at about 1700 m. Also we use a 2 500 rpm centrifuge, and not a microhaematocrit technique which may affect the PCV values. Do you consider it possible that these horses are capable of successful performance with haematological values about 1½ standard deviations below norm?

J.D. Steel: Well, fairly obviously there is going to be an occasional horse that will perform successfully at the figures you mention. Haematology is highly personalized in that you really have to establish normal standards for your own laboratory and, having done that, perhaps use other figures as a guide. It is not very commom in our environment for horses to run well one standard deviation below the mean. It also depends on the standard of competition. The other thing that can happen is that occasionally a horse will compensate for a low blood count by having a high heart score.

P.F.B. Williams: Did you test any other placed or unplaced horses in the races in which you tested the winners in comparison with the winners that have a PCV of 35 or thereabouts?

J.D. Steel: The figures that appeared on the board refer to Thoroughbreds. If you are going to bring in the Standardbreds you establish a new set of values related to that breed. A PCV of 35 in a Standardbred would not upset me because the population mean for Standardbreds may be as low as 38, so you are only three below breed mean.
Regarding your particular question, we have not yet analysed our taped data to obtain a critical comparison between blood values of winners and placed and unplaced horses in any specific race. A point I would like to make is that some people are overemphasizing the positive value of a haematological examination whereas it really ought to be a procedure where the emphasis is on the negative. The object is to identify animals not likely to come up to expectations. It is not a device for picking winners.

W.H.S. Bellinge: I refer to Dr Jeffcott's thesis relating to low blood counts following the use of acetyl promazine, which was mentioned this morning. In relation to this I would ask whether a horse with an exceptionally phlegmatic nature and which has a very low haemogram can fall outside your picture. We had an exceptionally good horse about four years ago, whose blood pictures were absolutely appalling but which won some very good races. When you walked into his box to take the samples he hardly woke: Could this extreme phlegmatism affect the blood picture?

J.D. Steel: Yes, it is biologically possible. All that we can really say is that there are exceptions to every biological situation and all we can really do, is try and play the numbers game: if we look at a hundred winners, we may have only one that is going to be below certain suggested limits. This means that if either a book-maker or I could have all this information, we could easily make some money by shelving the odds. It is no good to a punter, but it is very valuable to a book-maker.

A.M. Merritt: What do you consider the most common cause of anaemia in your horses and how would these findings influence your therapy?

J.D. Steel: That is difficult to answer and I really do not know. We have looked at the serum iron level; it is very difficult to determine and not a reliable indicator; even in man reliable criteria for diagnosis of iron deficiency have not been clearly or firmly established. We have looked at erythrocyte protoporphyrin levels; they might be slightly more useful, but the technique is cumbersome and time-consuming.
One must look upon iron deficiency in one form or another as being a cause, as well as the possibility of folic acid deficiency and perhaps vitamin B12 deficiency.

A.M. Merrit: Have you got bone marrow pictures?

J.D. Steel: No.

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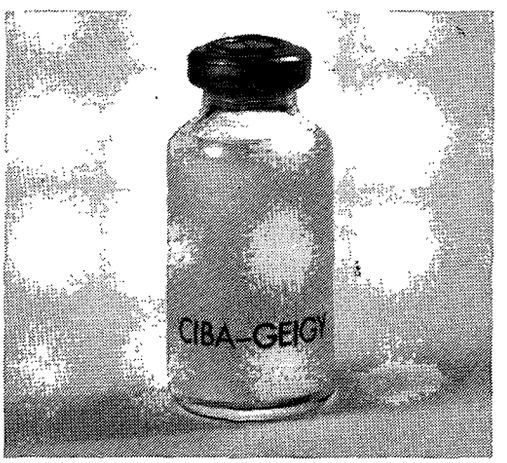

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BLOOD VOLUME IN RELATION TO EXERCISE TOLERANCE IN TROTTERS

S.G.B. PERSSON AND L.E. ULLBERG*

SUMMARY

Blood volume can be regarded as an expression of the dimensional capacity of the cardiovascular system; as such it should be regarded as a limiting factor of the 'oxygen conduction system' conveying oxygen from the air to the metabolically active tissues of the body. An unimpeded flow of oxygen to the tissues is also dependent on the functional capacities of the cardio-respiratory system. Thus, blood volume should be correlated to parameters describing functional capacities of the cardio-vascular system; both dimensional and functional capacities should determine the exercise performance in the horse as well as in other animal species.

In the present communication the relationship is demonstrated between blood volume and working capacity assessed from several types of exercise-tolerance tests, based on heart rate and blood lactate response to exercise. The dependency of exercise performance on these parameters is discussed, as well as the clinical applicability of these relationships.

Exercise tolerance is related to the aerobic capacity of the cardio-respiratory system. Thus, both dimensional and functional capacities of this organ system are limiting factors for maximal oxygen uptake and, consequently, for exercise performance, as aerobic energy production predominates during work lasting more than about one minute. During heavy exercise, however, both aerobic and anaerobic energy production contributes to the work output. Obviously, an unimpeded flow of oxygen to the working muscles requires that the different dimensional and functional capacities of the cardiovascular system be related to each other, which, in fact, has been demonstrated to be true in man[2]. Furthermore, an increasing demand on the energy production caused, for example, by continuous physical training, induces a corresponding dimensional and functional adaptation of the cardiovascular system [3 4]. It should be possible to predict the degree of adaptation to physical work, that is exercise tolerance, from parameters indicative of cardiovascular efficiency. In the horse, the easiest of such parameters to determine are total blood volume (TBV), total red cell volume (CV) and total haemoglobin (THb). Provided the splenic erythrocyte reservoir is mobilized by exercise or intravenous adrenalin injection, the dye-dilution method allows these determinations to be done with a precision of ± 3 per cent.[3]. The variation of this parameter between individuals is about ± 10 per cent if variations owing to body mass (Bm), sex, age and breed is taken into account. It is further reduced if the state of training is taken into consideration [3].

Several parameters are related to the functional capacity of the cardio-respiratory system. Many of those used in man, however, are not applicable to the horse. The obvious parameter describing the total work output in trotters is racing performance expressed, for instance, as kilometre time in seconds (kmt), *i.e.*, the total time taken divided by the distance in kilometres in a standard race. This parameter is a relevant expression for the aerobic work capacity as it is significantly correlated with TBV [3]. The racing performance, however, is affected by factors other than the aerobic capacity of the horse. Factors of general significance, such as anaerobic energy release, mechanical efficiency of the energy turnover, pace, body build and track conditions, tend to increase the variability of this parameter. Furthermore, racing performance in the trotter is greatly influenced by factors such as tactical driving and pulling up from the gallop.

For this reason, an exercise tolerance test based on the heart rate response to increasing, reproducible and measurable work loads on a treadmill was devised [3]. Work load is defined as treadmill velocity which can be measured exactly. The treadmill velocity causing a heart rate (HR) of 150 beats/min is extra- or intrapolated from 3 to 4 submaximal work loads on the treadmill and this parameter (V_{150} metres/sec) is considered to be an expression of the circulatory capacity of the horse and, thus, correlated with the stroke volume of the heart and also with the arterio-venous oxygen difference of the blood [3 4].

On the average, the blood lactate level does not increase significantly above the resting level until the heart rate exceeds 158 beats/min (Fig. 1). This indicates that a work load causing a heart rate of 150 beats/min should be performed almost entirely aerobically in most horses and, thus, V_{150} should be an expression for the aerobic capacity of the animal. It should be noted (Fig. 1) that a very steep increase of the blood lactate level occurs at an exercise heart rate of about 200 beats/min, indicating that above this level the anaerobic energy release starts to play a significant rôle in work output.

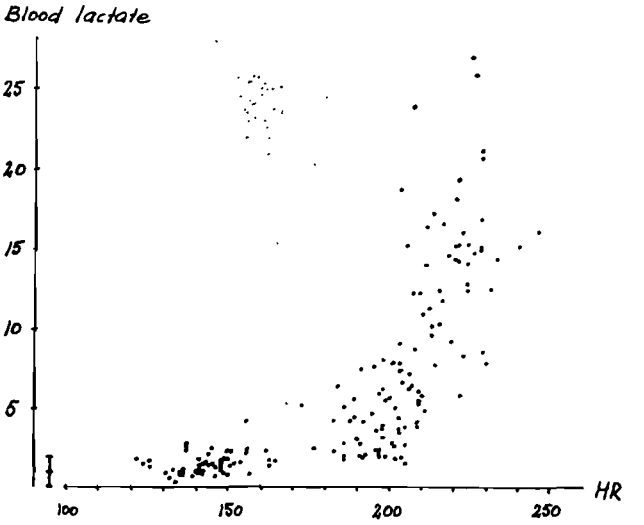

Fig. 1: Blood lactate (μmol/ml) in relation to heart rate (HR, beats/min) at rest (mean value + 2x S.D.) and during exercise in trotters.

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If V_{150} reflects the aerobic capacity of the cardiovascular system, a relation to the dimensional capacity, or, for instance, TBV should prevail. This is the case in normal trotters provided body mass is taken into consideration [3]. In adult mares and geldings this correlation is lacking, however. As the mean value for V_{150} is the same in trained adult mares and geldings as in adult stallions (7,0 m/s), in spite of lower TBV in mares and geldings, it seems that these have a hypokinetic circulation relative to the stallions, that is, they have a more efficient peripheral oxygen utilization and, thus, a wider arteriovenous oxygen difference during submaximal work. This might have a bearing on the lack of erythropoietic effect of testosterone on mares and geldings. Young horses, too, seem to be hypokinetic relative to adult stallions. This means that prediction of the aerobic working capacity from blood volume parameters in clinical work must take age and sex into consideration. The clinical application of this test has been described earlier [4].

A treadmill is an expensive piece of machinery. It is most useful for basic studies of cardio-respiratory function in the horse, but, for obvious reasons its use in clinical diagnostic work is restricted by economics. Therefore, the relevance of some exercise tolerance tests performed on the track was studied. These tests were based on the heart rate or the lood lactate response to work loads to which the horses were well accustomed.

The parameters were calculated from two types of experimental procedures. In one of these, the horse trotted at a constant speed of 10 m/s over 4 000 metres on a 1 000-metre track. The driver continuously checked the speed by timing the passing of poles 100m apart and the velocity was also checked by the investigator from the track tower. All the work tests were performed under standard track and weather conditions. Heart rates were calculated from electrocardiograms recorded by radiotelemetry towards the end of every 500-metre distance. As will be seen from figure 2, there was a gradual increase in heart rate throughout the work test. This is in accordance with earlier observations [1]. Two parameters with reference to the heart rate response to this test were calculated: the mean heart rate denoted as HR_{10}, that is, the mean HR at a speed of 10 m/s and the heart rate increase during the test denoted as HR_{Incr} expressed as the regression coefficient for the pulse/distance relationship. Immediately after the end of work, and 2,5 and 5 minutes later, blood samples were taken for determination of blood lactate concentrations. Two parameters with reference to blood lactate response to this submaximal exercise test were used, namely: the blood lactate level immediately after work, denoted as LA_{10}, that is, the lactic acid concentration at a work level of 10 m/s, and lactic acid clearance (LA_{Cl}) expressed as the regression coefficient for the blood lactate/time relationship during the 5-minute post-work period. Obviously, this parameter is negative for decreasing, and positive for increasing lactate levels.

The other exercise test was performed by trotting at four increasing submaximal speeds, each for 1 000 metres. The driver was instructed to maintain a constant speed at each level and the speed was calculated by the investigator. The work load at the highest submaximal level was chosen so that it would cause a

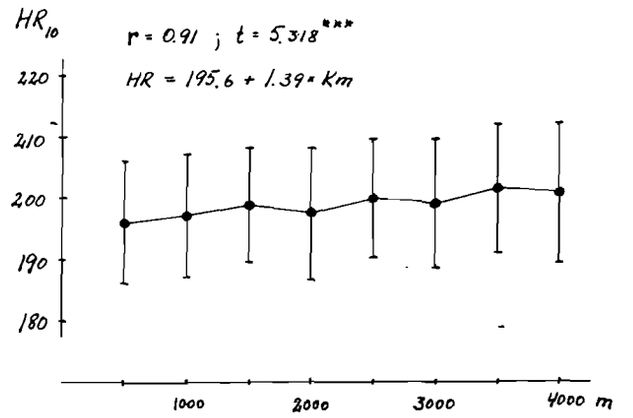

Fig. 2: Mean heart rate (HR_{10}) ± SD at 500 metres interval during trotting at 10 m/s over 4 000 metres in 76 horses. r = correlation coefficient for the mean heart rate/distance relationship. Km = kilometre.

heart rate slightly exceeding 200 beats/min. Immediately after the highest submaximal work level, the horse was made to trot one more round of 1 000 metres distance at its maximum speed. The heart rate at the end of each work level was assessed telemetrically as previously described and the blood lactate concentration was determined from samples taken in the same manner as after the constant-speed exercise test. The following variables with reference to heart rate and blood lactate response to this exercise test were used:- V_{200}, defined as the velocity attained at a heart rate of 200 beats/min, intrapolated from the linear pulse/speed relationship in metres/second; HR_{max}, which is the heart rate at the end of the round at maximum speed and considered to represent the maximal heart rate of the horse; LA_{max} which is the blood lactate concentration immediately after the trot at maximum speed; LA_{maxCl}, which is the regression coefficient for the blood lactate/time relationship during the 5-minute period following maximal work. This is very often positive, that is, the blood lactate concentration increases (Fig. 3). The maximal velocity attained (V_{max} in metres/second) was considered to represent the maximal work output ability, or, the sum of the maximal capacities for aerobic and anaerobic energy release of the horse.

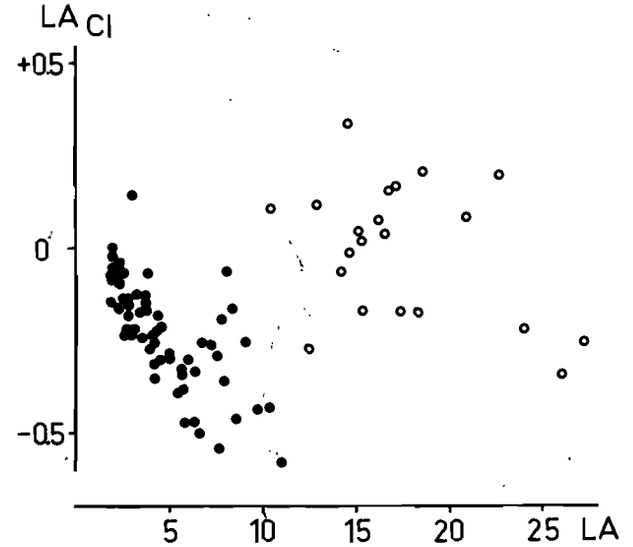

Fig. 3: Lactic acid clearance (LA_{Cl}; see the text) in relation to the blood lactate concentration (LA) at the end of submaximal (filled symbols) and maximal (open symbols) exercise in trotters.

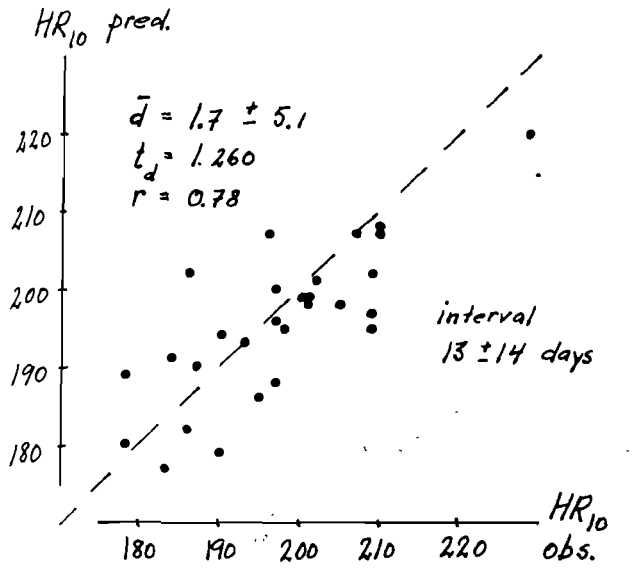

Fig. 4: Heart rate at a trotting speed of 10 m/s predicted from the pulse/submaximal work regression (HR_{10} pred.; see the text) in relation to that observed (HR_{10} obs.). Broken line = identity line; $\bar{d}$ mean difference between predicted and observed heart rates.

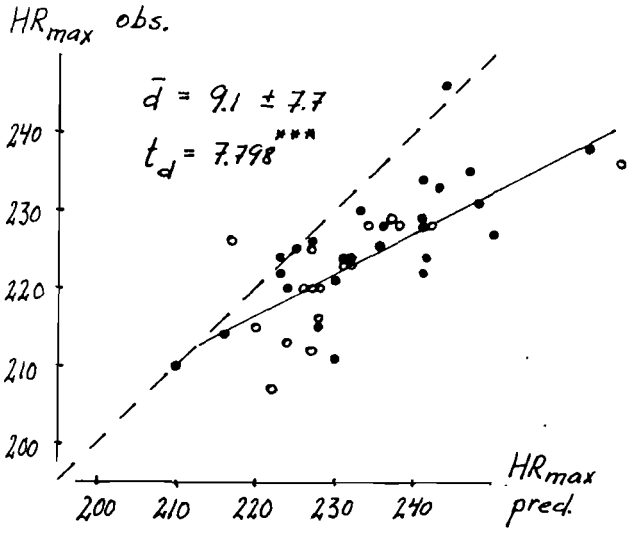

Fig. 5: Observed heart rate (HR_{max} obs.) in relation to heart rate extrapolated from the pulse/submaximal work regression (HR pred.) at maximal speed (see the text). Broken line = identity line; continuous line = regression line; $\bar{d}$ = mean difference between observed and predicted heart rates; open symbols = mares and geldings; closed symbols = stallions.

The reproducibility of the heart rate response to track work is indicated by the relationship between HR_{10} observed and predicted from the increasing work-load test in the same animal (Fig. 4). The presumption that the heart rate during the last work load is maximal is supported by the fact that in most cases it deviates from the pulse/submaximal work regression line (Fig. 5).

In the following discussions, these parameters, assumed to be related to the exercise tolerance of the horse, are usually considered to be dependent variables. Apart from the blood volume, or rather, the total red cell volume, age, sex, and body mass were used as independent variables affecting the variability of exercise tolerance. The linear correlation and multiple regression calculations were done using a computer and standard statistical programmes. The following degrees of probability (p values) for significance were used: $p \leq 0{,}001$*** highly significant; $0{,}001 < p \leq 0{,}01$** significant; and $0{,}01 < p \leq 0{,}05$* probably significant.

Y	X	Coefficient of correlation					
		All horses		♂		♀ + (♂)	
Age	CV/Bwt	0.55^{xxx}	(107)	0.65^{xxx}	(62)	0.46^{xx}	(45)
	LA_{10}	-0.31^{x}	(66)	-0.23	(41)	-0.45^{x}	(25)
	LA_{max}	0.35	(31)	0.47	(15)	0.28	(16)
	LA_{maxCl}	0.56^{xx}	(24)	0.69^{x}	(12)	0.22	(12)
	HR_{10}	-0.42^{xxx}	(76)	-0.45^{xx}	(49)	-0.38	(27)
	V_{200}	0.47^{xxx}	(61)	0.62^{xxx}	(37)	0.25	(24)
	HR_{max}	-0.26	(37)	-0.47^{x}	(22)	0.11	(15)
	V_{max}	0.55^{xxx}	(37)	0.53^{x}	(22)	0.54^{x}	(15)
CV/Bwt	LA_{10}	-0.38^{xx}	(63)	-0.23	(38)	-0.53^{xx}	(25)
	LA_{Cl}	0.39^{xx}	(57)	0.26	(36)	0.52^{x}	(21)
	HR_{10}	-0.36^{xx}	(72)	-0.38^{xx}	(45)	-0.32	(27)
	V_{200}	0.68^{xxx}	(57)	0.72^{xxx}	(33)	0.62^{xx}	(24)
	V_{max}	0.68^{xxx}	(35)	0.66^{xx}	(20)	0.62^{x}	(15)

Fig. 6: Correlation matrix (Abbreviations – see the text). Degrees of probability for correlations: $0.01 < p \leq 0.05$*; $0.001 < p \leq 0.01$**; $p \leq 0.001$*** Figures in parentheses indicate number of observations. Bwt (body weight) = Bm (body mass) in text.

Y	X	Coefficient of correlation					
		All horses		♂		♀ + (♂)	
LA_{10}	LA_{Cl}	-0.72^{xxx}	(60)	-0.68^{xxx}	(39)	-0.75^{xxx}	(21)
	LA_{max}	0.45	(19)	0.26	(10)	0.46	(9)
	HR_{10}	0.47^{xxx}	(65)	0.52^{xxx}	(41)	0.41^{x}	(24)
	HR_{Incr}	0.39^{xx}	(64)	0.40^{x}	(40)	0.49^{x}	(24)
	V_{200}	-0.37	(23)	-0.50	(13)	-0.37	(10)
	V_{max}	-0.66^{xx}	(20)	-0.55	(11)	-0.73^{x}	(9)
LA_{Cl}	HR_{10}	-0.28^{x}	(59)	-0.29	(39)	-0.32	(20)
	HR_{Incr}	-0.28^{x}	(58)	-0.27	(38)	-0.39	(20)
	V_{200}	0.39	(23)	0.59^{x}	(13)	0.25	(10)
	V_{max}	0.57^{xx}	(20)	0.49	(11)	0.56	(9)
HR_{10}	HR_{Incr}	0.32^{xx}	(74)	0.32^{x}	(48)	0.34	(26)
	V_{200}	-0.77^{xxx}	(29)	-0.74^{xxx}	(18)	-0.85^{xxx}	(11)
	HR_{max}	0.66^{xxx}	(22)	0.77^{xx}	(12)	0.54	(10)
	V_{max}	-0.74^{xxx}	(22)	-0.71^{xx}	(12)	-0.84^{xxx}	(10)
HR_{Incr}	V_{max}	-0.44^{x}	(22)	-0.57	(12)	-0.17	(10)
V_{200}	HR_{max}	-0.63^{xxx}	(37)	-0.73^{xxx}	(22)	-0.43	(15)
	V_{max}	0.60^{xxx}	(37)	0.57^{xx}	(22)	0.72^{xx}	(15)
V_{max}	LA_{maxCl}	0.48^{x}	(24)	0.57	(12)	0.19	(12)

Fig. 7: Symbols as in Fig. 6.

Some interrelationships found in the correlation matrix are shown in figure 6 and 7. As has been demonstrated earlier, there is a significant dependency of CV/Bm on age [3]. This is true in mares and geldings up to four years of age and in stallions to five years of age. This difference due to sex explains the higher correlation coefficient in stallions. After the age of four and five years, respectively, age alone has no influence on the cardiovascular dimension [3].

The influence of age on the blood lactate response to this submaximal work load also seems to end at maturity (Fig. 8). This might indicate that the lower dimensional and, consequently, the lower aerobic capacity is compensated for by a higher anaerobic energy release in young horses at this work level. The

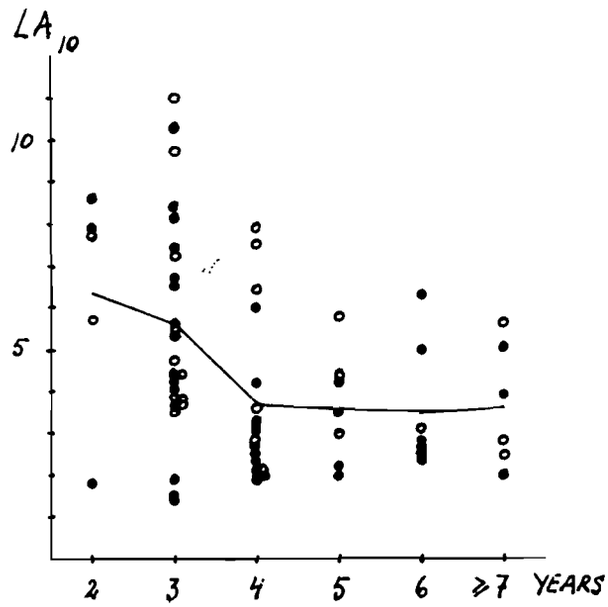

Fig. 8: Blood lactic acid at 10 m/s (LA_{10}; see the text) in relation to age. The line indicates mean values. Symbols as in Fig. 5.

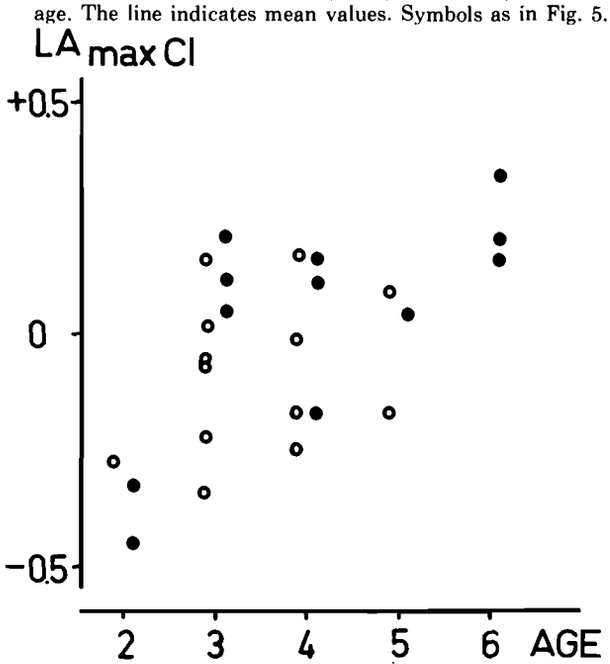

Fig. 9: Blood lactate clearance after maximal work (LA_{maxCl}; see the text) in relation to age in years. Symbols as in Fig. 5.

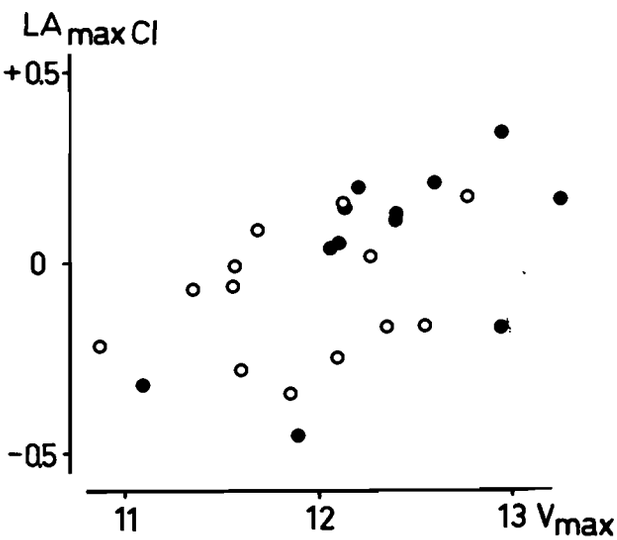

Fig. 10: Blood lactate clearance after maximal work (LA_{maxCl}) in relation to maximal trotting speed (V_{max}, m/s; see the text). Symbols as in Fig. 5.

higher correlation coefficient in mares and geldings could, in addition, have a bearing on their lower dimensional capacity. This will be further discussed later.

The positive dependency of LA_{maxCl} on age is somewhat confusing (Fig. 9), but this might be due to the reasonable, positive correlation between V_{max} and LA_{maxCl} (Fig. 10) as V_{max}, too, is positively correlated with age.

The heart rate response to exercise, expressed either as HR_{10} or as V_{200}, tends to be age-dependent as well only up to maturity (Fig. 11 and 12). The inverse correlation between HR_{max} and age in stallions is of interest as it is concordant with observations in man. The lack of correlation in mares and geldings, on the other hand, is not in accord with the situation in man. It should be kept in mind, however, that most horses in this study were relatively young, from a biological point of view, and the age range was limited.

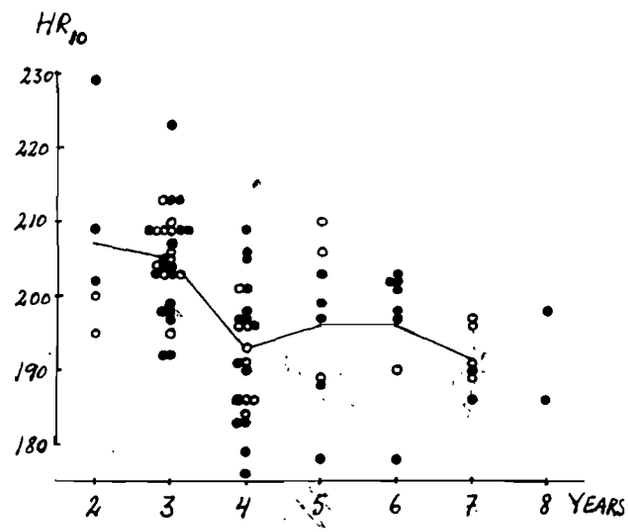

Fig. 11: Heart rate at 10 m/s (HR_{10}; see the text) in relation to age. Symbols as in Figs. 5 and 8.

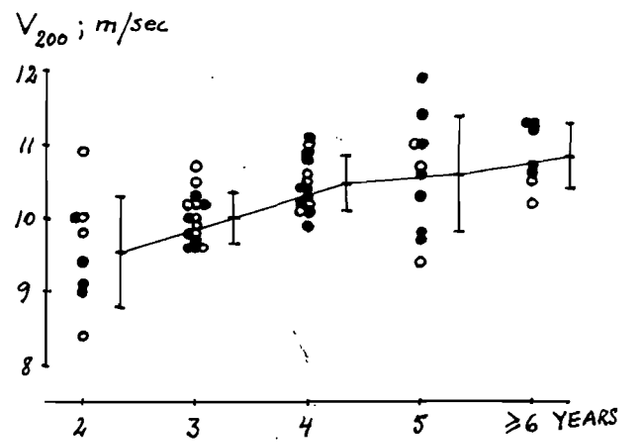

Fig. 12: Intrapolated trotting velocity with a heart rate of 200 beats/minute (V_{200}; see the text) in relation to age. Symbols as in Figs. 5 and 8.

Both parameters based on the lactic acid and the heart rate response to submaximal exercise are significantly correlated to the dimensional capacity of the cardiovascular system. The inverse dependency of LA_{10} on CV/Bm is significant for mares and geldings only (Fig. 13). This might be due to their sex-depen-

dent lower dimensional capacity with, therefore, a tendency to rely on anaerobic energy production even at this submaximal work load – a tendency significantly modified by increasing cardiovascular dimension. In almost half of the stallions this work load barely raised the lactate level.

The tendency towards a positive relationship between LA_{Cl} and CB/Bm, which means that the greater the CV/Bm the slower the LA_{Cl}, is somewhat confusing. One would have expected the reverse relationship. Probably the explanation is the strong inverse relation between LA_{Cl} and LA_{10}, which means that, up to a certain level, blood lactate is eliminated faster at increasing values for LA_{10}. This is also seen in figure 3.

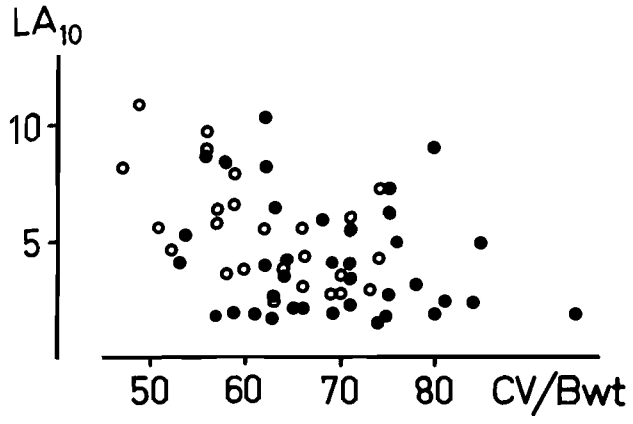

Fig. 13: Blood lactic acid at 10 m/s (LA_{10}) in relation to the total red cell volume divided by the body mass (CV/Bwt, m*l*). Symbols as in Fig. 5

The discrepancy in the dependency of the parameters based on heart rate response to exercise on CV/Bm is unexpected. The correlation between V_{200} and CV/Bm is fair and of the same degree as that between V_{150}, measured on the treadmill, and CV/Bm The sex difference found in the latter relationship is not to be foundin the V_{200}/CV-relation, at least not to the same extent. Obviously, V_{200} is as good an expression for the aerobic functional capacity as racing performance and V_{150}, judging by its dependency on the blood volume parameters. Further, the equally high correlation between the maximal work output, expressed as V_{max} and CV/Bm suggests that the former, to a large extent, is limited by the oxygen transport capacity of the horse. The predictability of V_{max} from CV/Bm is equally reliable in both sex groups as the regressions are statistically identical (Fig. 14).

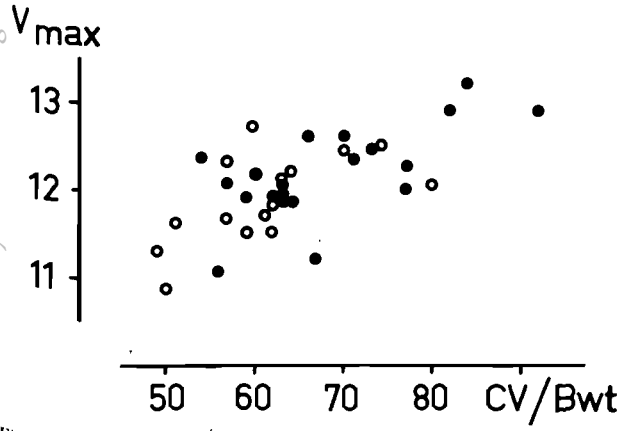

Fig. 14: Maximal trotting speed (V_{max}) in relation to the total red cell volume divided by the body mass (CV/Bwt). Symbols as in Fig. 5.

The correlation between LA_{10} and LA_{Cl} has been commented on previously. Obviously, the rate of elimination of lactate from the blood is dependent on the quantity of lactate to be removed. But, on the other hand, it is possible that the degree of accumulation of blood lactate is modified by the efficiency of removing lactate from the blood.

The dependency of LA_{10} on HR_{10} is probably an expression for an increasing demand on anaerobic energy production at increasing relative work loads (Fig. 15). As suggested by this relationship, there seems to be a gradually increasing blood lactate level with HR_{10} approaching HR_{max} that is, the correlation is curvilinear. At least in stallions, it seems that the anaerobic energy resource is not recruited until the heart rate exceeds 85 to 90 per cent of the maximal heart rate. Following this, it is not surprising to find a weak, but significant positive correlation between LA_{10} and HR_{Incr}, or, in other words, the higher the heart rate and the faster it approaches the maximal heart rate, the higher the lactate accumulation in the blood, or from another point of view, a gradual accumulation of lactate tends to increase the heart rate.

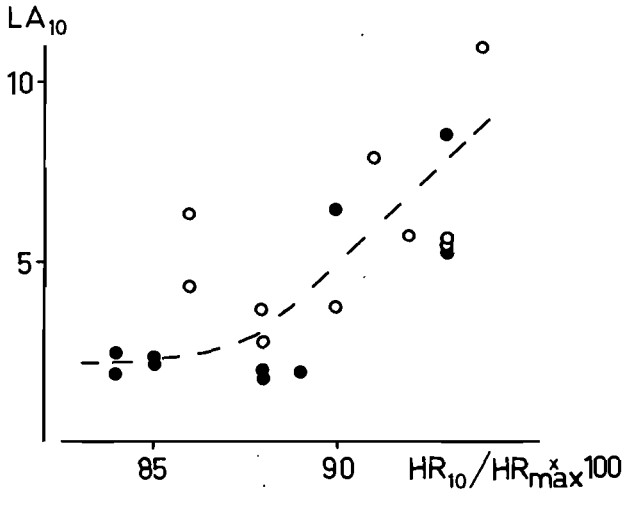

Fig. 15: Blood lactic acid (LA_{10}) in relation to heart rate at 10 m/s as a percentage of the maximal heart rate (HR_{10}/HR_{max} x 100). Symbols as in Fig. 5. The broken line indicates the probable regression line.

LA_{10} tends to be inversely dependent on the cardiovascular functional capacity, expressed as V_{200}. Although not statistically significant, this relationship seems logical, but, judgment on its relevancy is restricted by too few observations. The inverse relation between LA_{10} and $V_{\ldots}$ on the other hand, is fair and of considerable interest. It means that horses which can trot 10 m/s with a low lactate accumulation in the blood, have a high maximal performance and *vice versa*. This should mean that a low LA_{10} reflects a high aerobic capacity. The difference between the sex groups, with a higher correlation coefficient in mares and geldings, also seen in the LA_{10}/CV-relationship, is probably due to the fact that about 2/3 of the stallions exhibit almost no increase of blood lactate at 10 m/s (Fig. 16). The higher correlation between V_{max} and LA_{10} than between CV/Bm and LA_{10} should be ascribed to the addi-

tional information of the functional capacity included in V_{max} as is indicated by the dependency of this parameter on HR_{10}, which will be discussed later.

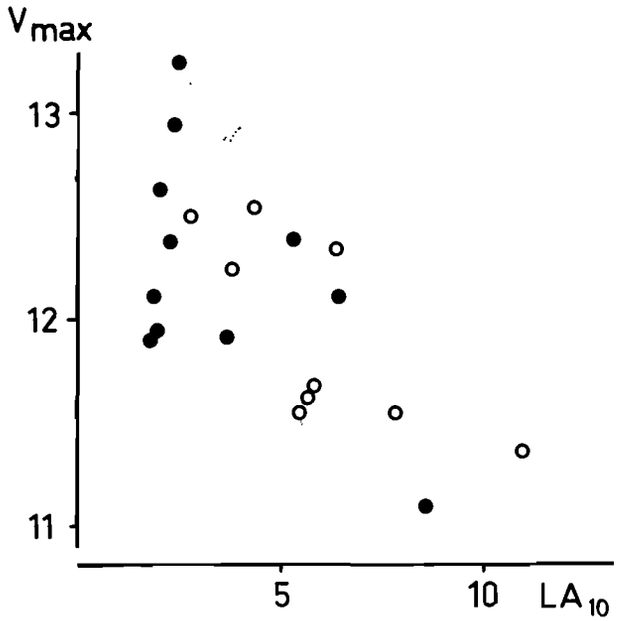

Fig. 16: Maximal trotting speed (V_{max}) in relation to blood lactic acid at 10 m/s (LA_{10}). Symbols as in Fig. 5.

The correlation between LA_{Cl} and V_{max} is probably due to the dependency of LA_{Cl} on LA_{10}. On the other hand, a high capability of eliminating excess lactate might be beneficial for maximal exercise performance.

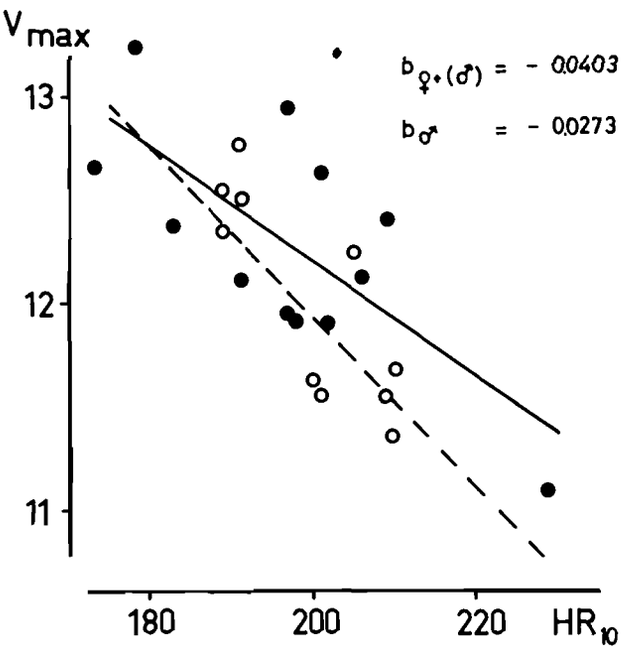

Fig. 17: Maximal trotting speed (V_{max}) in relation to heart rate at 10 m/s (HR_{10}). Broken regression line and open circles = mares and geldings; continuous regression line and closed circles = stallions; b = regression coefficient.

Apart from the relationships with V_{200} and HR_{max}, which are obvious by definition, HR_{10} is strongly correlated with V_{max}. Unexpectedly, this correlation is even better than that between V_{200} and V_{max}. In both correlations the highest correlation coefficients are found in mares and geldings. These correlations demonstrate the dependency of exercise tolerance on the cardiovascular functional capacity. This seems to indicate that mares and geldings, having a lower dimensional capacity, must rely on an increasing functional capacity in adaptation to continuous exercise stress. This is also indicated by the significantly higher regression coefficient in this sex group than in stallions in the V_{max}/HR_{10} relationship (Fig. 17).

So far, it might be concluded that mares and geldings, lacking the anabolic effect of androgens, seem to depend, to a higher degree, on increasing cardiovascular functional capacity and anaerobic capacity to improve their exercise tolerance than is the case with stallions, which primarily increase their cardiovascular dimensional capacity.

Dependent variable	Independent variables tested	significant	t	r	SD	%	n
V_{max}	Age, CV/Bwt, V_{200}, HR_{max}	CV/Bwt, V_{200}	3.328^{xx} 2.125^{x}	0.72	0.38	3.1	35
	Age, CV/Bwt, V_{200}, LA_{max-Cl}	CV/Bwt LA_{max-Cl}	4.017^{xxx} 2.343^{x}	0.76	0.37	3.0	23
	Age, CV/Bwt, LA_{10}, HR_{10} V_{200}	CV/Bwt HR_{10}	4.058^{xxx} 3.085^{xx}	0.86	0.29	2.4	20
	Age, CV/Bwt, LA_{10}, HR_{10} HR_{Incr}, LA_{Cl}	CV/Bwt HR_{10}	4.058^{xxx} 3.085^{xx}	0.86	0.29	2.4	20

Fig. 18: Stepwise multiple regression analysis of the dependency of maximal trotting velocity (V_{max}) on several variables. Abbreviations – see the text. t = test of hypothesis: b≠0, where b = regression coefficient. Degrees of significance as in Fig. 6. r = multiple correlation coefficient; SD = standard deviation; % = variation coefficient; n = number of observations. (Bwt (body weight) = Bm (body mass) in text.

Finally, in a limited number of horses, the dependency of the maximal exercise performance, V_{max} on several independent variables was tested, using a stepwise, multiple regression computer programme (Fig. 18). The results indicate that the main factors limiting maximal work performance in race horses are the dimensional and functional capacities, in that order, of the cardiovascular system, that is, an improved exercise tolerance is primarily achieved by an increased aerobic capacity.

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M.C. Morison: You said that higher lactic acid clearance was evident in mares and geldings than in stallions. If this is the case, could you explain why mares are more liable to suffer from azoturia than stallions? Does this mean that there is less lactic acid build-up in stallions than in mares that suffer from this condition? Do these mares have an inability to excrete higher levels of lactic acid?

S.G.B. Persson: I did mention that the blood lactate response to submaximal exercise (10 m/s) is more evident in mares and geldings, whereas most stallions barely increase their blood lactate at all at this work level. Merely for purposes of discussion I have postulated the possibility of higher lactic acid clearance in mares. If it is an accepted fact that azoturia is more common in mares than in stallions, this might have a bearing on the tendency of mares to rely more on their anaerobic energy release, as this would mean higher lactic acid concentration in the working muscles. There is no evidence of a slower elimination of lactate from the blood in mares.

J.R. Gillespie: I am concerned about the temporal effects on lactic acid build-up in the blood. There must be a different rate of build-up with a different intensity of exercise. What do you mean by maximum work?

S.G.B. Persson: As was pointed out in the paper, the blood lactate accumulation during exercise is dependent on the intensity of exercise, or, rather on the relative work load, expressed as the rate response relative to the maximal heart rate of the horse. It was also mentioned that in many horses this lactic acid accumulation in the blood continues for at least five minutes after work. This is especially the case at high relative work loads. That was one reason for studying the so called lactic acid clearance. The criteria for maximal work were that the horse was urged by the driver to trot as fast as it could; the heart rate then usually deviated significantly from the value extrapolated from the heart/rate submaximal work regression line.

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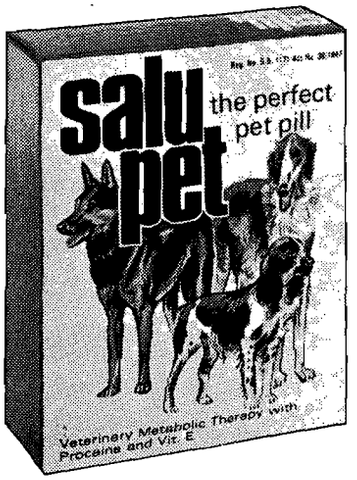

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OPEN DISCUSSION ON CARDIOLOGY, HAEMATOLOGY AND PERFORMANCE

Chairman: M.A.J. Azzie.

G. Buys: I found Dr Persson's clear delineation of lactic acid metabolism very interesting. The fact that anaerobic metabolism leads to the build-up of lactic acid has various implications. I would like Dr Persson's views on the fact that when one exercises a horse heavily, the lactic acid builds up because the horse has to depend on anaerobic metabolism. If one subjects this horse to heavy silage feed which contains lactic acid. I suppose that this lactic acid will increase the blood level and the blood level will remain high until the lactic acid is removed from the muscles. Could he elaborate on this aspect of lactic acid accumulation and destruction in the body, because I have an idea that the lactic acid is also a cause of stiffness in the horse. At what point do we get muscle dystrophy, where the horse will eventually peter out or even die. I have seen this happen when a hard-worked horse eats silage with high lactic acid content and is again worked the following day without having enough time to eliminate the lactic acid from the muscles.

S.G.B. Persson: I am afraid I am not able to comment on the nutritional aspect; there are people here who are much more competent to discuss that point. The accumulation of lactic acid to very high levels is interesting in relation to fading out or even death. In pigs there is the well known 'porcine stress syndrome'. It can be induced experimentally by strenulously exercising the untrained pig. In the trained pig the accumulation of lactic acid in the blood after standard work is much less than in the non-trained animal. In the non-trained animal it very often starts a vicious circle, the increasing lactic acid levels inducing a higher body temperature which triggers off more lactic acid accumulation until there is a complete or vast destruction of skeletal muscles; the pig often dies acutely from this condition or from simultaneous circulatory failure. I hope this answers your question.

A.M. Merritt: I just want to get clarification on the point regarding the usefulness of the packed cell volume as indicator of performance ability. I got the impression from both Drs Archer and Steel that it is not a good indicator but these gentlemen could possibly cast some light on the matter.

L.W. Jeffcott: I think the PVC is much more an indicator of poor performance. That is the point I was trying to get across, rather than saying the PVC is a good indicator of performance; I apologize for misleading you.

M.A.J. Azzie: First of all, is there any significant manner in which a blood sample can be identified as having been collected from a horse during excitement? After all, we do not know if the farrier was making a noise in the stable five minutes before we got there to collect our specimen. Secondly, Dr Persson in his work clearly indicated why he did not rely on haematocrits as an indicator of state fitness of the horse and that is why today he broached a completely different subject. Our own work in South Africa, involving about 10 000 blood tests, indicated that PVC is only a guide to a state of fitness but it is a highly individualized guide; a horse will have an optimum PVC for his own state of fitness and this will vary by as much as 2% either way within one week of performance. This is the fundamental reason why, when a horse's PVC can vary as much as 4% in Johannesburg, these tests are only a guide to a state of ill-health and not to a state of fitness. On this level I would like to ask our haematology men; what significance is attached to plasma volume changes having taken place as well?

J.D. Steel: We have not done any work on plasma volumes, therefore I cannot comment on whether changes in plasma volume, causing effects in haematological parameters, tend to interfere with either their interpretation or their usefulness. The reason we have not done blood volumes is that on account of the work involved we have not had the capacity to undertake these additional measurements. If you look upon the parameters of red cells, haemoglobin, and packed cell volume in an individual horse and you look at these regularly, there are going to be relatively small variations. I tried to point out that these variations in part are due to the animal and in part due to the precision of the laboratory techniques employed. One ends up in a situation where one can say that plus minus 5% in most of these parameters is not going to be really meaningful. One of the difficulties in haematology in relation to performance is that we have probably consistently tried to read too much into small changes. I personally believe that it is a valuable tool, providing you do not try to over-refine it by putting too much significance on small changes in the figures.

R.K. Archer: First of all, I agree entirely with what the previous speaker has said; no comments on that. The identification of blood samples taken during excitation, whether inadvertent or not is impossible from the haematologist's point of view; therefore it is essential to know whether excitation has occured, otherwise the blood sample is uninterpretable. There is no differential change associated with excitation sufficiently different from that which might arise from other causes. Concerning plasma volume, I would extend Prof. Steel insofar that the assessment of plasma volume is a fairly elaborate matter. It is not a question of putting a needle into a vein and taking a sample of blood, one has to do more than that. It is less of a routine matter and more an experimental procedure, rather than one to be used in practice. I did not mention quality control in my paper and if Prof. Steel did so, I missed it. It is quite essential if you are setting up quality control methods for use on horse blood, not to standardize on human material. All the commercially available equipment is designed for human material, particularly the Coulter 4C, which is probably the best known and certainly the best nationally available. It is useless, I repeat, useless for the horse. The reason is quite simple; the mean blood cell volume of man is around 85 and the mean cell volume of the horse is around 45. Coulter 4C and the commercial products are all made to measure a mean cell volume of around 70, designed to include human microcytic anaemia, but the apparatus used for counting human blood is designed to cut off with a lower threshold around 60 cubic microns. That is above the value for the horse's mean cell volume. Consequently, if one standardizes equipment designed for counting horse cells on human material, one will consistently get a higher MCV than true, because one's counter will be cutting off part of the sample. We even go so far as to say that in cases of samples having been tested in a human laboratory and then sent to us for an opinion, which happens too often for my personal comfort, the horse has 'Coulter S disease'.

Dr Steel, I think, mentioned the use of vacutainers. We never use vacutainers; in my experience vacutainers and haemolysis are inseparable. It just so happens that horse blood cells break more easily than do those of humans or cattle. In a vacutainer the 5 cm or so that the blood sprays from the back end of the needle to the bottom of the tube is enough to lyse about 1% of cells.

If you want reproducible work my advice is; abhor the vacutainer. The next point I would like to take up is anaemias. In England the commonest cause for anaemia in Thoroughbred horses is worms. The treatment is obvious, the use of haematinics is superfluous but harmless, that is my view. I should also perhaps add that it is well established that the horse manufactures its own vitamin B_{12} with the greatest ease, so, if one injects vitamin B_{12}, one is giving cobalt in a very expensive form. Cobalt chloride will work just as well. (The papers were published by Frank Alexander in Edinburgh about 5 years ago). There is certainly a dietary anaemia of Thoroughbreds attributable to deficiency of folic acid. Some of the Thoroughbreds fisnish racing in say October (beginning of our winter), go inside, hard tack, no grass. When they come out again, around next March, and are put to work, some of the animals fail to produce that work. Upon careful haematological examination, particularly if a channelizer is used, one sees that the cell size distribution has shifted to the left, *i.e.*, the mean cell volume is larger but it is not evenly distributed: it has a bi-modal course. That is a dietary deficiency of folic acid; it is very readily correctable by folic acid so long as that acid is injected. For some curious reason folic by mouth is almost inactive: almost 100 times the calculated amount has to be given by mouth to get

the same effect as by injection; I do not know why. We have some work running on that point.

The last thing I would like to take up is bone marrow biopsy. It is perfectly straightforward, we do it frequently. Its use in clinical haematology is limited. It is sometimes very handy, particularly in cases of horses which have an undiagnosed swelling of any kind, and the possibility of malignancy has to be considered. It is very handy indeed to look at the bone marrow and see if the accompanying anaemia is attributable to under-activity of the bone marrow or over-activity with destruction or arrest at any point. Leukaemia is incredibly rare; I only know of one case in the literature I would regard as proven – I have never seen one. Leukaemoid reactions, yes. Generalized lymphosarcoma can fox one a little.

J.D. Steel: Two brief comments concern firstly the standardization of the Coulter. It was human material from the blood bank which was used for the standardization of the automatic haemaglobinometer. Concerning standardizing the particle-counting part of it, we regularly purchase particles of known size and run those through to check that we are getting the counter to operate within ranges of red cell size one is likely to encounter in the horse.

S.G.B. Persson: May I just explain how I measure the total blood volume in the horse. Immediately after standard training work blood is drawn by venepuncture from the jugular vein for determination of the postwork haematocrit. Through the same needle I inject the measured amount of dye. After 15 minutes, a new blood sample is taken from the contralateral jugular vein for determination of dye concentration. The subsequent laboratory routine is very simple; determination of packed cell volume by haematocrit and determination of the dye concentration. This procedure is not very elaborate and can be done as a routine in the laboratory. The method gives quite reproducible and reliable results.

J.R. Holmes: If I may turn to Dr Fregin's paper, I agree with what he said in the first part of his contribution. Just for what it is worth, in my experience the pandiastolic murmur of all the murmurs in the horse is the one which we find associated with cardiac lesions. The majority by far concerns lesions on the aortic valve cusps. In my experience many practitioners fail to recognise the third heart sound or confuse the third heart sound with an early short diastolic murmur. The key to recognition is its relationship to the second heart sound and also the site of its maximum intensity.

I would just like to refer to the treatment of atrial fibrillation which Dr Fregin mentioned but had not time to deal with in detail. The literature describes the treatment of atrial fibrillation by very frequent and gradually increasing doses of quinidine sulphate. This involves a great deal of work for the practitioner, a stomach tube having to be passed 3, 4 or 5 times a day and that day after day. Frankly, I do not think this is practicable for the majority of practitioners. Some of the people we have advised to treat horses in this manner have, in fact, given big doses once a day by stomach tube. They have obtained just as good results, no better, nor worse, than with the method found in textbooks. We always give a test dose or advise a test dose of quinidine to make sure there is no individual idiosyncrasy. After that, in the Throughbred, there is an initial dose of 20 gm per day. This is increased by 5 or 10 gm per day until we get either sinus rhythm or signs of toxicity. Dosing does not go on longer than 10 days and if we get sinus rhythm the drug is gradually withdrawn. This is done, of course, after digitalizing the horse, using digitalis pulv. in the food. If I could summarize Prof. Steel's contribution, the larger the heart, the longer the time for ventricular depolarization, which we would all accept and expect. What does surprise me is that he advocates the use of paper speed of 25 mm/second. I would have thought the more rapid the paper speed the greater would be the accuracy. We use variable paper speeds up to 100 mm/second and experience no difficulty in detecting the position where wave forms pass the iso-electric line, which are the points of measurement for determining heart score. I must plead ignorance about the heart score, I can only think that many of you are going to make a lot of money in the future giving numbers to horses. I only hope you won't exclude potentially good horses in the process. I have not the experience that Prof. Steel has in evaluating this technique.

Dr Fregin, in discussing the effects of inflammatory reactions on the myocardium, spoke of ST-segment and T-wave changes. I am sure that he did not have the time to detail what he meant by this. I must just say something about T-wave changes. The two speakers have already indicated that the T-wave is an extremely labile wave form and that it is extremely rate dependent. It also varies according to the amount of exercise that a horse has been given. I myself am not so confident, not so sure, that we know enough yet about the significance of the T-wave to be able to attach clinical significance to its changes. Human medicine is in the same predicament; we do not know enough about repolarization of the myocardium. Repolarization is a process completely different from de-polarization with its specific conduction pathways. I emphasize this because it would be a mistake for you to go away from here believing, if you see a T-wave form change, this necessarily is of grave or any clinical significance. Heart rate is a factor which effects the T-wave but we do not know enough about the significance of the T-wave for it to be assessed clinically.

Dr Fregin deals with vectors in the latter part of his paper. I wonder if we ever think why we put electrodes on the limbs of horses. I suppose it is because we use the techniques that are used in man and the comparable parts of the body to wrists and ankles are the limbs of the horse. Yet it is doubtful whether any really accurate assessment has been made of the value of these limb leads in vectorial terms until fairly recently. I have no objection to Einthoven's limb leads if the technique is used for the recognition and diagnosis of arrhythmias in the horse. When this technique is used to calculate vectors, then I must disagree entirely. We found, and Dr Fregin's paper shows, that if one looks at Einthoven's limb lead II and III we have R-waves and a positive deflection in the lead forms. And yet, when one looks at body surface potentials, one finds that in a majority of horses activation at the time of the peak of ventricular depolarization during QRS is not backwards, but is upwards, forwards and leftwards. So here is a situation where Einthoven's limb leads do not give us a true representation of the cardiac electric field. The cardiac electric field does not matter at all if one is using the ECG for recognition of arrhythmia. If the ECG is used for an accurate portrayal of cardiac vectors in planes or in space, then a lead system is required which more accurately represents the true position. The procedure which we adopted was to place unipolar electrodes over the body surface, always with a standard site on the neck as a time base, and then to calculate from the deflection which we obtained by integration of the body surface potentials the resultant cardiac dipole moment at each instant in time, *i.e.*, the direction and magnitude of the spatial vector instant by instant through the cardiac cycle. It was quite a long procedure, which we did on 4 horses. The result was that at the peak of the R-wave (ventricular depolarization) the vector was upwards, forwards and leftwards. Yet in these horses Einthoven's leads put on the limbs in the conventional way showed a positive deflection in lead II and lead III, indicating the backwardly direction of the vector, thus clearly not representing the true nature of the cardiac electric field, there being a greater degree of positivity to the root of the hindlimb than to that of the forelimb and so causing an opposite deflection, which is not representative. The next procedure was to calculate the expected vector cardiograms in three planes and the expected scalar ECGs in three axes, the axis from left to right we call x, from behind we call y and the vertical we call z. From the data obtained, we calculated the expected ECG pattern. Electrodes were placed about the body of these four horses to find out where we would get the patterns we had predicted. From that we developed what I call a semiorthogonal system. We described what it is, and how we use it. We use it routinely. I am not suggesting for one second it is the perfect lead system, but if one wants to talk about vectors, then it is a great deal more accurate than Einthoven's lead system.

G.F. Fregin: Maybe it is best to start with atrial fibrillation, a problem that is commonly seen. At our university we have had about 50 cases over the last ten years. Thirty of these were selected for treatment. There appear to be two different populations of horses, maybe three, that are seen with atrial fibrillation. The cases that were reported by Sally-Ann Glendenning and others were usually older horses. Apparently, in the States, at least where I am, we see a number of younger horses, between the ages of two and three years that have atrial fibrillation which usually has quite an acute onset. Because they are engaged in racing or training at the time.

we usually get them fairly quickly after trotting. These animals are very good candidates for treatment if there are no other indications of cardiovascular disease. From the standpoint of therapy, quinidine sulphate is mixed into a suspension and given by stomach tube. I used to use a balling gun and put 10 gm of quinidine into a gelatine capsule and gave it that way, but often got into difficulties. Dosing the suspension by stomach tube yielded more satisfactory results. Most of the horses that we have treated have been able to convert to normal sinus rhythm on quinidine levels of about 40 gm/-day. Obviously there are different ways to achieve this. I give 10 gm every 2 hours; by the time I get up to 40 gm, or at the most 60 gm, the greater percentage of horses will convert at that level. A thousand pound horse can be given up to 60 gm of quinidine over a 12 hour period without getting into severe straits from toxicity. Once over 60 gm, one begins to run into difficulty; over 80 gm one is really asking for trouble. We have gone as high as 110 gm in a day's time. This was done on an experimental basis and these animals got really sick. In practice, if one wishes to be cautious, one could start the animal on 10 gm twice a day and work up to 10 gm three times a day until one gets to 40 to 50 gm. Once over the 60 gm level, one must do electrocardiographic monitoring. Other methods of treatment have been suggested, *e.g.*, intravenous quinidine administration. I think Dr Gerber published an article on three cases receiving intravenous quinidine, one of which converted to sinus rhythm. I tried that on a number of occasions but found it more difficult to control than oral dosing. When I gave the medication intravenously, no matter how small the dose, I ran into trouble with a ventricular type of tachycardia very quickly after the drug had been administered. Administration of the drug intravenously over a period of an hour was usually followed within 20 to 30 minutes by tachycardia, the heart rate going up to 160 to 180 beats per minute. Orally one does not run into that type of trouble until the 60 to 80 gm/day level is reached. I do not digitalize the animal. If an animal comes in with congestive heart failure, as far as I am concerned the last thing I would worry about is atrial fibrillation. I would digitalize that animal. But the majority of animals that come into our clinic with atrial fibrillation do not show clear signs of congestive heart failure; resting the heart rates are normal: in the thirties or so. Hence I have not used any digitalis preparations. Concerning continuation of therapy after the animal has converted to normal sinus rhythm, in the past I did give them quinidine until converted and then placed them on a maintenance dose of 10 gm twice a day for three to four days. On occasion the students would forget to give the following day's medication; after that had happened a number of times, we just decided not to give it; we have not had any unfavourable effects as far as I can tell. I give it until the animal converts or begins to show signs of toxity. Then I stop and do not give it any more quinidine after that. We request that the horse be rested for at least two or three months and then returned to the hospital for another check to see if there is normal sinus rhythm. I have them begin training the horse and I have a look half-way through its training period if the electrocardiogram is still normal. We have a number of horses that have gone back into racing. There are 22, I think, that have been treated for atrial fibrillation and that have gone back into racing. One of these horses relapsed and had to be retreated. We lost two or three. Most of them have gone back and raced as well as they did prior to the atrial fibrillation. We have had some fairly good horses that have had it. I do not know what Dr Holmes would say is a large dose.

R. Holmes: I go up to 40 gm a day but it is easy to attain conversion on doses as low as 10 gm. I cannot see any reason why, if you have taken the electrocardiogram and you are certain that the animal has atrial fibrillation that you could not try going up to 40 gm or so; I do not think you should get into trouble. We can talk later about the type of difficulties you can get into and what can be done to help circumvent them, but I do not know if there will be the time available.

F. Fregin: With regard to myocarditis, Dr Holmes commented on Dr Steel's and my opinions about ST-segments and T-wave changes. I thought I had shown on the slides that the T-wave certainly is extremely labile, just like the heart rate is in the horse. By exciting the horse one can get changes in both the T-wave and the P-wave. By exercising the horse there are obvious changes in the T-wave. We keep the limbs as parallel as we can to each other. We also try to keep the animal as quiet as we can by keeping him in his own box and using a long set of leads so that the recording machine is outside. We use personnel who are used to working with the animal. We have followed a fairly large group of horses, not in their thousands like these gentlemen have been talking about, but about 100 horses. These we have followed from the time they were two years old up to date, and we have done them from four to six times a year, just as a sort of experimental procedure. I can now look at the electrocardiogram and already know what horse it is on. The ECGs are very reproducible if they are done in the same way. Making a diagnosis of myocarditis on the basis of T-wave changes or ST-segment deviations, I would agree is taking a chance, but if one is using this in conjunction with other factors and clinical findings, I think it can be used as a diagnostic tool. We may even be able to use it in a prognostic sense. There are certainly non-specific T-wave changes that take place in the horse just as they do in man. These, however, I have not had in following the group of horses which we studied. The problem could be a liability if one were not very cautious.

I would concur that when a pandiastolic murmur is heard, it is mostly associated with cardiovascular disease, primarily disease of the aortic valve.

At a cardiology session last year we tried to talk about vectors. There were many arguments about the type of lead system that should be used: which lead system might be the best and what type of information we could use from the diagnostic point of view. As Dr Holmes has pointed out, just about any lead system can pick up sinus arrhythmia and premature beats. There is no question of that, but the use of vectors is a very difficult matter. I do not think now is the time to try to conclude whether Dr. Holmes' lead system is any better than the one we are using. When one analyses specific groups of horses like Standardbreds, Thoroughbreds or Quarter horses or race ponies it seems to me that in the more cold-blooded horses there is a greater percentage of animals that has QRS-vectors going dorsally and cranially according to our leads. When we look at the Thoroughbred, there are more animals in which these vectors are going dorsally, again, but by contrast also caudally and to the left. I do not know what this really means. I think there are probably different populations of horses. I am still not convinced that either lead system has any advantage over the other. Some forces may move over rapidly in one direction and may be of very short duration. One may have forces going in an opposite direction which may cancel these out. There are a number of factors that have to be taken into consideration and I believe it will be along time before we arrive at a completely reliable lead system. The question arises: what is one going to do once one has the QRS vector – of what diagnostic value is it going to be? One can make diagnoses, in humans at least, of right ventricular hypertrophy; it would be advantageous to apply that to the horse, irrespective of the lead system used.

The only time that I have seen and conducted a right ventricular hypertrophy was in horses with congenital heart disease: tetralogy of Fallot, interventricular septal defect and pulmonary stenosis. With our lead system such cases will have ventricular axes that have shifted clearly to the right. There is no question about a right axis deviation being present. In horses with interventricular septal defect in which pulmonary hypertension (elevated right heart pressure) has not developed, one does not usually see any electrocardiographic shift; at best a minimal shift may be present. The same situation holds for the chronically emphysematous horse. It is only when the condition has existed for a long time and the horse has developed very elevated right heart pressures that the vectors are shifted.

Left ventricular hypertrophy is very difficult to determine. We even have the difficulty in establishing criteria in dogs where we have colonies of dogs with congenital stenosis where we know left ventricular hypertrophy is going to develop. Even here it is difficult to pick out affected animals from the normal population of the same breed. I think perhaps the same goes for the horse. The QRS-complexes in the cases I have seen – and they are relatively few – had the same direction as the normal population.

A. Littlejohn: We know that atrial fibrillation is an unsoundness. But what is the judgment to be on a horse that has been successfully treated and in which sinus rhythm has been re-established? Is the animal now sound and must we regard atrial fibrillation in the same light as a nasty bout of influenza in such cases?

It is generally agreed that the Einthoven lead system certainly does give rather variable results because of differences in position of the limbs. To me this seems to be a recommendation for the orthogonal lead system as has been proposed by several workers in this field. It is certainly a system we propose to use in Pretoria.

C. Button: We find that about every horse on which we tried will stand crocodile clips on its skin. I would never have believed that this could be possible. It is much more convenient for holding electrodes on to animals. It does not worry the horse at all and there is no change in the herat rate.

Jl S. Afr. vet. Ass. 45(4) 305-309
(Proc. 1st int. equine vet. Conf., Pretoriuskop 1974)

SECOND SESSION: RESPIRATION, BIOCHEMISTRY, ELECTROLYTES AND PERFORMANCE

Chairman: A Littlejohn

THE ROLE OF THE RESPIRATORY SYSTEM DURING EXERTION

J.R. GILLESPIE*

SUMMARY

We measured maximal expiratory flow ($\dot{V}_{E\,max}$) in healthy horses to determine if dynamic events in the pulmonary airways might limit ventilation during maximal exercise. We found horses were capable of very high peak expiratory flow rates (540 kg horse, $\dot{V}_{E\,max}$ = 80 to 100 *l*/s). The general shape of the expiratory flow-volume curves from horses were similar to those of other mammals measured with the same technique.

Using prediction formula from the literature for oxygen uptake during maximal exercise, we determined that it is unlikely that healthy horses are limited by expiratory flow during maximal exercise. Horses with chronic obstructive lung disease have severe ventilatory limitations owing to dynamic narrowing and closure of intrathoracic airways during expiration.

INTRODUCTION

Grimby recently reviewed the subject of respiration during exercise in man. He discussed four factors which might limit strenuous exercise: 1) work of breathing; 2) the factors limiting flow; 3) capacity for transfer of oxygen and carbon dioxide; and 4) breathlessness or inhibition of exercise caused by neuromuscular responses initiated by the mechanoreceptors of the lung [2 3]. Strenuous exercise to the point of exhaustion within a few minutes, called 'maximal exercise,' was considered for three groups of individuals: 1) young sedentary persons, 2) athletes; and 3) elderly persons. He concluded that in young sedentary persons the respiratory function does not limit maximal exercise. In athletes with very high aerobic power maximal exercise may be limited by factors of respiratory gas flow. In elderly persons with aging processes in their lungs, their ventilation may be maximal during exercise, yet their blood gases are normal. He was unable to rule out curtailment of exercise in any of the groups as a result of the sensation of breathlessness caused by stimuli other than those of hypoxaemia or hypercapnoea.

Respiratory flows have been measured in horses so that we can begin to examine one of the four factors which might limit maximal exercise [4]. I will consider respiratory flow in horses which may limit their ventilation and thereby exercise.

MATERIALS AND METHOD

Six normal anaesthetized horses were suspended upright within an integrated-flow, pressure-volume plethysmograph after performance of tracheostomy and insertion of oesophageal balloons. We [4] measured quasi-static volume-pressure curves of lungs and of chest walls, and recorded flow-volume curves during passive and during forced deflation of the lungs.

RESULTS AND DISCUSSION

The following figures and data are based upon values from a healthy 540 kg Thoroughbred-type mare. Figure 1 represents a record of a slightly larger than normal resting tidal breath in which the lung volume is plotted (y-axis) against the gas flow rate (x-axis). Inspiratory events are to the left and expiratory events are to the right. The breath begins at functional residual capacity (FRC), and the volume increases at various rates of flow, to end-inspiration. Expiration follows in which flow rate increases to "peak" flow rate then decreases exponentially to zero at end-expiration with lung volume again at FRC. This represents one breath cycle and one can visualize a series of breaths during regular breathing retracing the loop in figure 1. Subsequent figures will show only expiratory events because it is during expiration that flow limitation is effort-independent and may occur during maximal breathing. Inspiratory flow limits exist too, but inspiration is effort-dependent.

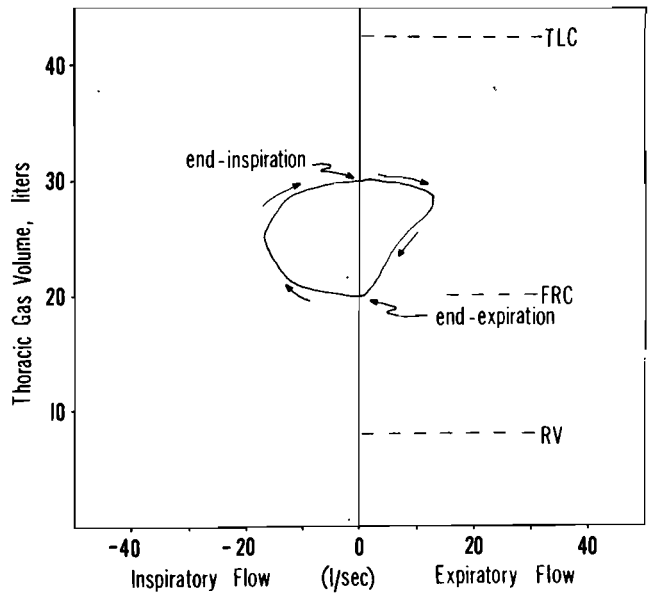

Fig. 1: Thoracic gas volume against inspiratory and expiratory flow rate during an entire breath in which the tidal volume is slightly larger than resting levels for a 540 kg horse. Inspiratory events to the left, expiratory events to the right.

Figure 2 shows a passive expiratory flow-volume curve from total lung capacity (TLC) to FRC. This figure shows the instantaneous flow rates out of the respiratory system at various lung volumes between TLC and FRC with no muscle force used to 'squeeze'

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the lung during expiration. Figure 3 shows a family of curves (*(a – e)* in which increasing expiratory force is employed to increase the flow rate and more completely empty the lungs. Curve *a* is the passive expiratory curve and is the same as the curve in figure 2. Curves *b, c, d* and *e* represent flow-volume curves in which increasing expiratory effort is employed with each subsequent expiration.

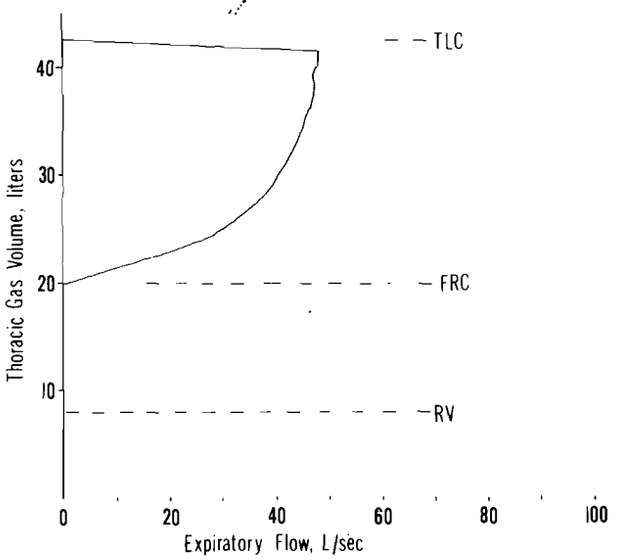

Fig. 2: Passive expiratory flow-volume curve for a 540 kg horse initiated at TLC and ending at FRC.

Curve *e* is unique. This curve represents the *maximal expiratory flow-volume curve* for this particular horse. We produced this curve by applying an expiratory force to the horse's respiratory system beginning at TLC. When we applied greater expiratory forces, the flow-volume curve did not change. At first sight this does not seem reasonable. One might expect greater expiratory force to always produce greater expiratory flow. I will discuss later the dynamic events that occur in the airways during forced expiration that cause flow limitation. Effort-independent expiratory flow limitation has been found in every mammalian species studied.

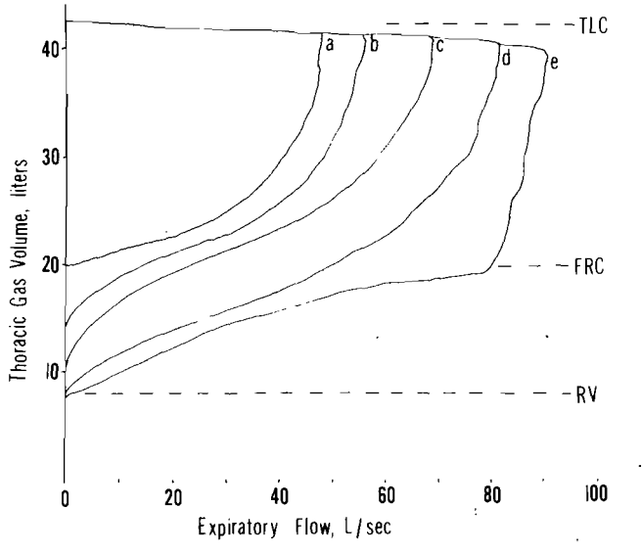

Fig. 3: A series of expiratory flow-volume curves for a 540 kg horse initiated from TLC. Curve *a* is a passive expiratory flow curve (see Fig. 2). Each curve (*a, b, c, d,* & *e*) is produced with greater expiratory force (a≺b≺c≺d≺e). Curve *e* is the maximal expiratory flow-volume curve between TLC and RV. Only curves *a* and *e* were traced from original data.

Figure 4 presents maximal expiratory flow-volume curves initiated at different lung volumes. Curve *e* is a complete maximal expiratory flow-volume curve and is the same as curve *e* in figure 3. Curve *a* is a maximal effort initiated at TLC and terminated after about seven litres are expired. Curve *b* is a maximal expiratory effort started seven litres below TLC and ends after expiration of seven litres. Curves are shown which are initiated at progressively smaller volumes (curve *c,* and *d*). At each new volume, maximal expiratory effort never produces a greater flow than can be produced by a complete maximal expiratory flow-volume effort at a comparable volume. From these curves it can be seen that maximal flow is volume-dependent, *i.e.,* peak flows decrease with decreasing lung volume (*a* ⟹ *b* ⟹ *c* ⟹ *d,* Fig. 4).

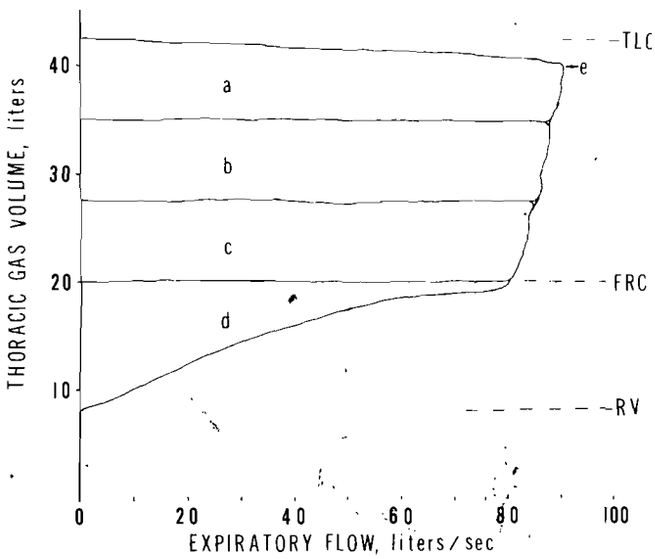

Fig. 4: A series of partial maximal expiratory flow-volume curves for a 540 kg horse with each subsequent curve initiated at a lower lung volume and each expiration equal to about 7 litres. Note the partial curves produce an outer shell which is the maximal flow-volume curve between TLC and RV (see Fig. 3). Only curve *e* was traced from original data.

Both tidal volume and breathing frequency can be increased from the flow-volume at rest (Fig. 1). Increases in frequency at any tidal volume will require increases in inspiratory and/or expiratory flow rate. Near rest (Fig. 1), a relatively small tidal volume and flow rate will meet the animal's ventilatory requirements. The healthy animal has a 'flow-reserve' which is the distance between the resting loop (Fig. 1) and the maximal loop (curve *e,* Figs. 3 and 4). With exercise, the flow-volume loop can enlarge until it reaches its limit, the maximal expiratory flow volume curve (curve *e* Figs. 3 and 4).

We now can consider the mechanisms which set the limit on expiratory flow. It has been shown in human beings that healthy individuals can develop expiratory muscle force far in excess of that required to achieve maximal expiratory flow. In horses, we found that we could demonstrate a maximal expiratory flow and that further increases in driving expiratory force did not change the expiratory flow. We lack direct evidence that healthy horses can develop sufficient expiratory muscle force to reach their maximal expiratory flow. We measured inspiratory pressures in excess of 1,5 x 10^4 Pa (150 cm H_2O) when horses breathed against an obstructed airway. For this reason, it seems likely to me that horses, like

man, have an excess expiratory muscle force. If they do not, then ventilation would be limited by muscular weakness and might be improved with specific respiratory muscular exercises.

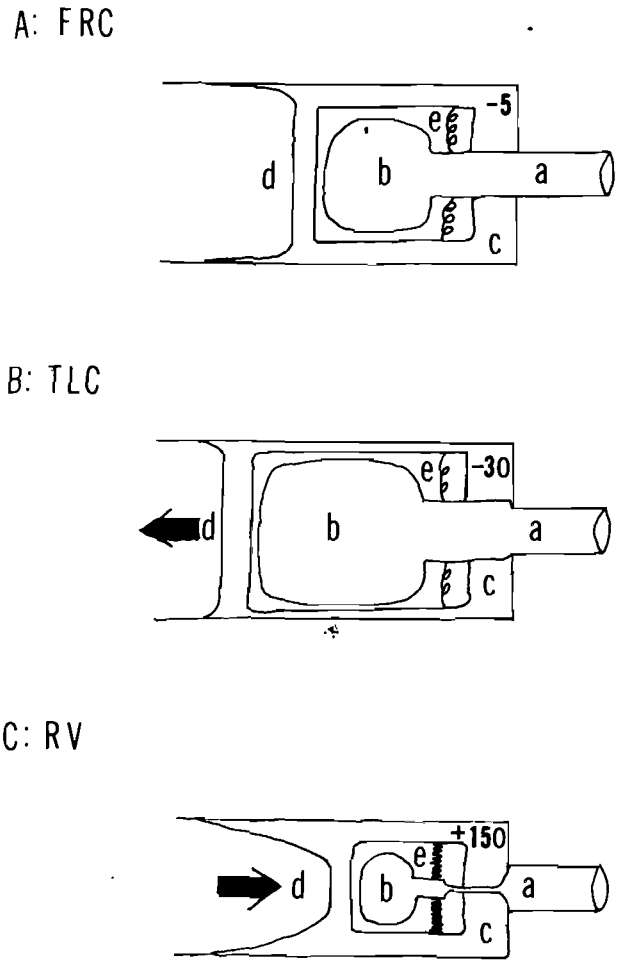

Fig. 5: Three models of the respiratory system with the parts labelled; a, airways; b, alveoli; c, pleural space, d, diaphragm; e, lung parenchyma with elastic element represented by spring. Model A; the system at rest, pleural pressure 5 x 10^2 Pa (5 cm H_2O) negative to atmospheric and lung volume at FRC. Model B; maximal (static) lung vol: pleural pressure 30 x 10^2 Pa (30 cm H_2O) negative to atmospheric and lung volume at TLC. Note the enlargement of the intrapulmonic airways as a result of increased parabronchial lateral tissue tension on the airways (stretched springs). Model C; maximal expiration pleural pressure 1,5 x 10^4 Pa (150 cm H_2O) positive to atmospheric and lung volume at RV. Note the narrowing of intrathoracic airways as a result of loss of parabronchial, parenchymal support (collapsed springs).

Figure 5 shows three models of the chest wall and lungs. In model A, the respiratory system is depicted at rest with pleural pressure 5 x 10^2 Pa (5 cm H_2O) less than atmospheric pressure. This is sufficient to resist the recoil of the lung and hold the lung volume at FRC and the airways open.

The cross-sectional area of airways is a critical structural feature which determines flow resistance. Airways in the lung depend in part upon surrounding lung tissue for their support. The conformation of the surrounding parenchyma depends upon the elastic elements in the lung tissue. The high tension on the elastic elements at high lung volumes increases the outward pull on the airway's walls and increases their cross-sectional area.

Model B, (Fig. 5) shows the respiratory system at a high volume. The diaphragm has contracted and increased the difference between the pleural pressure and the atmospheric pressure to 30 x 10^2 Pa (30 cm H_2O). The lung is stretched to a larger size and lung volume increased. The airways are pulled open and have a large cross-sectional area. The greater the volume inspired, the greater will be the cross-sectional area of the airways and the smaller the resistance to gas flow through them. Hence, resistance to gas flow decreases during inspiration and increases with decreasing volume during expiration.

Model C shows the respiratory system during a forced expiratory effort. In this instance, pleural pressure is positive to atmospheric pressure: the lung is 'squeezed'. The parabronchiolar tissue supports become ineffective and the airways are allowed to narrow and with additional squeeze they close. During expiration, resistance increases as airways narrow. Increasing expiratory force will increase expiratory flow rate until the expiratory force critically narrows and/or closes airways which increases resistance and limits flow. Additional force is wasted as it will yield no additional flow.

The maximal flows along the flow-volume curve are volume-dependent and effort-independent. In an individual, higher flows can be achieved near TLC than at lower lung volumes, and individuals with larger lungs can achieve higher flows than those with smaller ones.

CONCLUSIONS

Minute ventilation increases with exercise and can be changed by increases or decreases in tidal volume and/or frequency. There are mechanical restraints to large increases in tidal volume or frequency. At high lung volumes (near TLC) and at low volumes (near RV), the lung and chest wall become stiff (Fig. 6) and require greater changes in pressure for each unit volume breathed in and out. Large tidal volumes and high respiratory flows require large energy expenditures by the respiratory muscles. There are optimal (minimal) energy costs for each level of ventilation which are achieved by appropriately adjusting tidal volume and frequency. It is not known if breathing is finely controlled in the horse to achieve exercise ventilation with minimal energy costs.

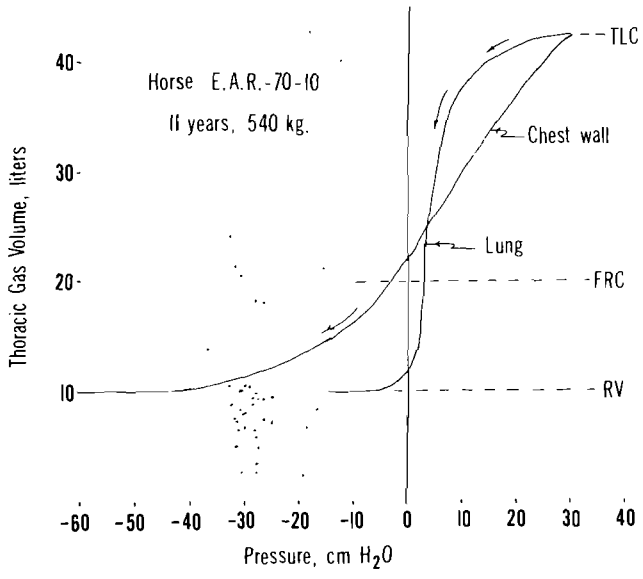

Fig. 6: Expiratory volume-pressure (compliance) curves of the lung and chest wall of a 540 kg horse.

With present data we can begin to define the limits of ventilation. If we assume horses have sufficient respiratory muscle strength to achieve their maximal expiratory flow-volume curve, we can approximate their maximal ventilation and make some prediction on the respiratory limitations to exercise.

Table 1 illustrates how respiratory flows set minute ventilation. In this example, I am estimating a mean respiratory flow rate (inspiratory and expiratory) of 75 *l*/s, based upon our records of maximal expiratory

Table 1: ESTIMATES OF MAXIMAL MINUTE VENTILATION FOR THE HORSE
Mean Inspiratory and Expiratory Flow = 75//s

Tidal Volume (/)	Breaths/min	Minute Ventilation (//min)
10	225	2 250
15	150	2 250
20	112	2 250
30	75	2 250

flows. If I asume equal inspiratory and expiratory periods, then the minute ventilation ($\dot{V}_{min}$) will be 2250 *l*/min. The vital capacity (TLC-RV) of a 540 kg horse is about 30 litres (see Fig. 6). For the example in table 1, I have selected tidal volumes ranging from 10 to 30 litres. Ten litres would likely be too small, as it would require a respiratory rate of 225 breaths/min, whereas 30 litres would be too large as it would have a high energy cost for the horse to breathe at the extremes of its vital capacity (see Fig. 6). Tidal volumes of 15-20 *l* seem more reasonable, and the respiratory rates of 122-150 breaths/min are in line with the counts of respiratory frequency ventilation made by Mead on racing horses [5]. This must be near maximum ventilation for a 540 kg horse and it is very large.

Table 2 shows the comparisons of minute ventilation, oxygen consumption ($\dot{V}o_2$) and cardiac output (CO) for human athletes during maximal exercise and estimated maximal values for a 540 kg horse assuming a $\dot{V}_{min}$ maximum of 2250 *l*/min, and a arteriovenous oxygen difference of 16 vol. per cent for both species. Athletes are able to achieve about 80 per cent of maximal ventilation during exercise. The values given in the upper line for the horse in table 2 are based upon estimate maximal $\dot{V}_{min}$ and the values in the lower line are 80 per cent of maximal values.

Taylor and co-workers [6] developed the expression for minimum cost of running (M (run) $ml\ O_2 g^{-1} km^{-1}$) for mammals: $M_{(run)} = 8{,}46\ W^{-0{,}40}$ where W is body mass in grams. I assumed a speed of 0,8 km/min and calculated a $\dot{V}o_2$ of 17,5 *l* O_2/min for a running 540 kg horse. If Taylor and co-worker's formula accurately predicts $\dot{V}o_2$ for the horse during maximal exercise, and if our measures represent maximal respiratory flows and volumes, then the healthy horse has a substantial ventilatory reserve with a flow limitation to exercise ventilation.

Table 2: MINUTE VENTILATION ($\dot{V}_{min}$), OXYGEN CONSUMPTION ($\dot{V}o_2$) AND CARDIAC OUTPUT (CO) IN HUMAN ATHLETES DURING MAXIMAL EXERCISE AND ESTIMATED MAXIMAL VALUES FOR A 540 KG HORSE.
Ventilatory Equivalent ($\dot{V}_{min}/\dot{V}o_2$) of 30 and arteriovenous difference of 16 vol. per cent were used for both species to calculate $\dot{V}o_2$ and CO.

	$\dot{V}_{min}$ (//min)	$\dot{V}_{min}$/kg (//min/kg)	$\dot{V}_{O_2}$ (//min)	$\dot{V}_{O_2}$/kg (//min/kg)	CO (//min)	CO/kg (//min/kg)
Man*	170	2,43	5,7	0,08	36	0,51
Horse	2 250	4,17	75	0,14	469	0,87
80% of values	1 800	3,33	60	0,11	375	0,69

*Data from Biological Handbook. 1970. *Respiration and Circulation*, P.L. Altman D.S. Dittmer (Eds). Bethesda, Maryland: Fed. Am. Soc. exp. Biol. p.80.

We must be cautious, however, in attempting to scale the horse to the human athlete. One would expect that the horse has evolved with far greater pressures than man to be able to run fast for long periods of time. Perhaps the excercising horse has a much greater $\dot{V}o_2$ than we are predicting. It is also possible that the horse has a very large ventilatory reserve for reasons other than gas exchange, *e.g.*, temperature regulation.

For whatever reason, it does appear that healthy horses have a very large ventilatory reserve. This is not always true for the horse with respiratory disease. We have recorded the passive and maximal expiratory flow-volume curves in a horse with bronchitis and emphysema[4]. This horse had essentially no flow reserve and its passive expiratory curve was essentially the same as its maximal expiratory curve. As in human beings, we expect this test in horses to be of value in measuring ventilatory impairment with disease.

I have considered only one of four possible respiratory factors which might limit exercise: respiratory flow limitation. Our data [4] described lung volume and respiratory flow limits for horses of particular sizes. My estimate based upon these data indicates that healthy horses have a large respiratory volume and flow reserve which would provide ventilations in excess of those currently thought necessary for exercise. We lack direct measures of ventilation and gas exchange during exercise.

We have collected some data on arterial oxygen and carbon dioxide tensions during exercise. These data may be of use in evaluating a second important factor of respiration, the gas-transfer function of the lung. We still lack data for the horse on work of breathing and on the sensation of breathlessness during exercise.

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D.H.G. Irwin: Would you consider answering a question concerning the horse which holds its breath. Sometimes trainers complain that horses hold their breath. On the other hand, as they jump from the starting gate, some horses take a breath with every stride. Is it perhaps the case that as the horse extends its stride it breathes in and as it collects itself it will breathe out. Some human athletes in the top echelon will run a 100 yards without taking a breath. Would you speak to this please.

J.R. Gillespie: Let me approach the breah-holding question first. The 100 yard dash for man compared to our usual sprint races for Thoroughbreds is shorter, and ventilatory demands for the two groups are quite different. Certainly horses could not hold their breath for the entire race. I would not be surprised if horses come out of the gate with a nearly full lung and take several strides before they take a breath. The large lung capacity of the horse very likely provides a substantial reserve for gas-change during the initial portion of the race. We have looked at movies of horses racing to ascertain whether or not they entrap, that is, breathe in some sort of multiple of their striding frequency. It appears to us that most horses do. They often inspire with body extension and expire with flexion.

Entrapment seems to be normal; it makes a lot of sense because of the mechanical advantage offered for breathing. A good way of examining breathing during racing is to watch movies of races in England, where they run the horses while it is fairly cold. One can count the breaths and correlate breathing phase with stride, viewing the condensation with each expiration.

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BIOCHEMICAL GENETICS AND PERFORMANCE ABILITY IN HORSES

D.R. OSTERHOFF*, M.A.J. AZZIE** AND J. OP'T HOF***

SUMMARY

Genetical markers identifying 18 pairs of chromosomes of the horse, including six different blood group systems, haemoglobin types, five serum protein types and six enzyme types, were used to find a relationship of the racing performance ability to these markers.

It was established that Thoroughbred horses in different parts of the world are very similar in their genetic make-up and that the variability of different phenotypes has diminished in the process of selection for performance ability.

Scores were alotted to the horses tested and suggestions were made for pre-selections according to the results obtained.

INTRODUCTION

During the thirty years which have passed since systematical studies of blood groups in farm animals were initiated by means of isoimmune antibodies, the subject of immunogenetics and the more recent field of biochemical genetics have developed greatly and a number of gene-controlled polymorphisms have been discovered and elucidated.

The term polymorphism refers to the occurrence in a population of two or more alleles of a given codon, in certain proportions. In general, according to Ford, the term polymorphism has been applied to those genetic systems where the less common gene has a frequency in excess of one per cent. There is in fact no clear dividing line between the frequency of what is usually termed a rare recessive gene and the frequency of a gene member of a polymorphic system.

Blood group research on horses is basically no less important than that done on other species but most of the work has been done by single persons in different countries and the results had not been compared until quite recently.

When a registered Thoroughbred mare in the United States or in the Republic of South Africa is bred to two registered Thoroughbred stallions, the resulting foal can be registered only as the offspring of both stallions. This method of double registry has obvious disadvantages when it comes to keeping records of pedigrees. The Jockey Clubs would like to keep the number of doubly registered horses at a minimum, hence the keen interest in this work of identification using genetical markers is understandable.

In South Africa, the first immunizations for the production of immune antisera were performed in 1967. Table 1 gives the summary of the South African production of horse blood group reagents.

Table 1: PRODUCTION OF HORSE BLOOD TYPING REAGENTS[5]

Year	No. of Immunizations	Reagents produced
1967	86	3
1968	552	21
1969	341	6
1970	117	0
1971	66	4
TOTAL	1 162	34

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All these reagents have been compared in four different International Horse Comparison tests.

In table 2, other genetic markets which have been compared with the mentioned blood typing reagents are included.

Table 2: INTERNATIONAL HORSE COMPARISON TESTS[5]

Year	Duty Laboratory	Participating Laboratories	South Africa's Contribution	
			Blood Group Reagent	Other Genetic Markers
1968	Davis, U.S.A.	5	11	4
1969	Paris, France	10	29	5
1971	Uppsala, Sweden	11	32	7
1973	Newmarket, Engl.	17	30	10

All factors belonging to these systems which have been compared and accepted internationally are compiled in table 3.

Table 3: BLOOD GROUP SYSTEMS IN HORSES

Genetic Systems	Antigenic Factors
A	A_1 A_2 H_1 H_2 A' Z_1 Z_2 F
C	C
D	D_1 D_2 E_1 E_2 E'G J_1 J_2
K	K
P	P_1 P_2 P'
Q	O R S_1 S_2 X
T	T_1 T_2
U	U_1 U_2

The most important aspect of the blood group factors to the practising veterinarian is that of *erythroblastosis foetalis*. Up to a year ago, no laboratory in the world was able to differentiate between the 'Rh positive' and the 'Rh negative' horse in order to predict the possible consequences. In the meantime it has been proved at Newmarket, England, that not a single Rh factor is responsible for haemolytic disease [10]. The factors which may cause isosensitization are A_1, Q, R, S and E_2. The usual cause is sensitization with A_1 or Q, less commonly with R and S and rarely with E_2. At a recent meeting in Davis, California, Watanabe [12] from the

Hokkaido University, Sapporo, Japan, reported on 39 cases of 41 mares that had bred and icteric foal. The following antibodies were noticed in the sera of the mares involved: A_1, C, P_1, Q and E_2. As to blood factor A_1, which is apt to produce antibody in the mare, the disease appeared from the first to the fifth birth; as to Q, from the third birth onward; as to C, P and E_2, from the sixth birth onward. The Japanese and British results are very similar and it seems that within a year or two the whole icterus situation will have been clarified.

Further investigations – also in our laboratory – led to biochemical polymorphism in different systems of haemoglobins, serum proteins and enzymes in horses.

The methods used in detecting genetic variation in these proteins are of much more recent nature, notably the techniques of starch gel electrophoresis. From many parentage tests performed in our laboratory two examples will be given, whereby both the serological and the starch gel electrophoresis techniques played their part. Table 4 represents a paternity test performed for a breeder of Arab horses in which four stallions were involved.

Only those haemolysins and agglutinins which actually reacted with the respective reagents, are given in the table; the others have been omitted. The case was solved with the final statement: the stallions Storik, Raktha and Masrik are excluded as possible sires and Azymdar qualifies as the sire of the foal with proof in the agglutinin, transferrin and carbonic anhydrase systems.

In the second case, the antigenic characters of the erythrocytes could not contribute to the solution. Table 5 represents a case in which the protein and enzyme systems settled the matter.

This case was solved with the final statement: the stallion Powder Rock was excluded as the possible sire and the stallion Veronese qualifies as the sire with proof in the pre-albumin-, 6-PGD-, esterase- and LDH-systems. The results demonstrated that these tests are efficient in solving problems of questionable parentage in horses and they confirm the validity of the methods.

MATERIAL AND METHODS

From different studs in Transvaal, 377 horse blood samples were obtained and the horses recorded as accurately as possible with regard to parentage, age and racing performance. The performance score was calculated as follows:
One race won: three points; one placing: one point. These are added up and the result is multiplied by a factor of 10. The classes were then divided as follows:

Class	Scores
1	Up to 30
2	31 to 60
3	61 to 90
4	91 to 120
5	121 and Higher

Table 6: GENETIC SYSTEMS INVESTIGATED

Blood group systems	6 different systems: A, C, D, K, P, Q
Haemoglobin	
Serum proteins	Transferrin Albumin Pre-albumin Post-albumin Pre-haemoglobin (Protein X)
Enzymes	Esterase Carbonic anhydrase 6-phosphogluconate dehydrogenase Acid phosphatase Phosphoglucomutase Phosphoglucose isomerase
TOTAL	Genetic markers of 18 chromosomes

Table 4: HORSE PARENTAGE CASE

Animal	Haemolysins									Agglutinins									
	A	C	5	7	8	14	17	22	25	J	K	1	2	4	5	Hb	Tf	Alb	Ca	Es
POSSIBLE SIRES:																				
STORIK	+	+	+	+	+		+	+	+			+			+	Aa	DH	AB	IS	FI
RAKTHA	+	+	+		+							+				Aa	DO	AB	IS	FF
MASRIK	+	+	+		+		+	+				+			+	Aa	DH	AB	IS	II
AZYMDAR	+	+	+		+					+		+		+		Aa	FF	BB	FS	II
DAM:																				
SALLY	+	+		+	+		+	+			+	+	+			Aa	HH	BB	SS	FF
FOAL:	+	+	+	+			+	+		+	+	+	+	+		Aa	FH	BB	FS	FI

Table 5: HORSE PARENTAGE CASE

	Hemolysins								Agglutinins										
	A	H	C	SA f	SA 14	SA 17	SA 19	SA 29	J2	NA 5	NA 7	Hb	Tf	Alb	Pre Alb	6– PDG	Est	LDH
Foal	+	+	+	+	+	+	+	+	–	+	+	Aa	DF	AB	FS	FS	FS	FS
Tasmin (Dam)	+	+	+	+	+	+	+	–	–	–	–	Aa	FF	AB	SS	FF	SS	FF
Powder Rock (Sire)	+	±	+	–	+	–	–	–	+	+	+	Aa	DO	AB	SS	FF	SS	FF
Veronese (Sire)	+	+	+	–	+	–	–	+	+	+	+	Aa	DD	BB	FS	SS	FS	FS

By using well-known serological, immunological and electrophoretic techniques, the following genetic systems were investigated (Table 6).

RESULTS AND DISCUSSION

Blood Groups

The results of blood typing of the 377 South African Thoroughbreds were very close to those of the American horses tested a few years ago. In our investigation, many new factors were also tested for, but not included in table 7.

Table 7: ESTIMATES OF THE FREQUENCIES OF ALLELES IN SIX BLOOD GROUP SYSTEMS[11]

Loci	Alleles	Frequencies in Thoroughbreds	
		American (276)	South African (377)
A	A_1	0,705	0,734
	A'	0,029	0,035
	H	0,004	0,000
	A'H	0,000	0,000
	a	0,262	0,231
C	C	0,732	0,684
D	D	0,000	0,000
	J	0,150	0,142
	d	0,850	0,858
K	K	0,064	0,059
P	P	0,205	0,310
	P'	0,091	0,087
	p	0,704	0,703
Q	Q	0,508	0,488
	R	0,000	0,004
	S	0,103	0,089
	q	0,389	0,419

No attempt at correlating these results with performance scores was made, because blood group typing thus far has not proven to be of any significant value in selective breeding programmes, although newer methods, like the electron microscope technique referred to during the above-mentioned meeting in California, could deliver promising results: the quantity and distribution of blood group antigens on the surface of red cells can now be studied by using indirect labelling methods and gene dosage effects, and differences between heterozygous and homozygous animals can be studied.

Table 8: PERCENTAGE OF HAEMOGLOBIN TYPES IN DIFFERENT HORSE BREEDS[6 7]

Breed	No. tested	Haemoglobin type			
		A_1	A_1A_2	A_1+A_2-	A_1-A_2+
Common horses	228	0	97,4	2,2	0,4
Arabs	46	0	98,1	1,9	0
Percheron	45	0	77,8	22,2	0
Thoroughbred	441	0	100,0	0	0
Welsh and Welsh cross	18	0	100,0	0	0
Experimental group	60	0	93,4	6,6	0

Haemoglobins

In Thoroughbreds, only one type of haemoglobin is found, not only in South Africa, but also in other countries where polymorphism in horses has been investigated. Table 8 gives a comparison of different breeds investigated in South Africa; it is obvious that this system cannot be used in any comparison of performance ability.

Serum Proteins

Of the proteins listed in table 6, the post-albumins and the pre-haemoglobins (protein X) were subject to very little variation and could not be used in further studies for correlations with performance ability.

Transferrin, on the other hand, proved to be the most variable and interesting protein type. In table 9, the frequency of transferrin alleles in Thoroughbreds of Belgium, Netherlands, Hungary, U.S.A. and South Africa is compared, proving the fact presented in table 7 of an excellent concordance of gene frequencies. It seems that strict selection for performance shaped the gene frequencies in these countries in a very similar way.

Table 9: FREQUENCY OF TRANSFERRIN ALLELES IN THOROUGHBREDS[2 3]

Country	No.	Tf^D	Tf^F	Tf^H	Tf^O	Tf^R
Belgium	333	0,314	0,488	0,066	0,090	0,042
Netherlands	35	0,300	0,442	0,043	0,129	0,086
U.S.A.	150	0,267	0,563	0,027	0,090	0,053
Hungary	208	0,313	0,471	0,019	0,101	0,096
South Africa	377	0,261	0,551	0,042	0,074	0,072

In table 10, the transferrin types are correlated with the performance of the horses. Unfortunately, only 206 horses could be included in these studies because not all the records were available at the time of writing; in many cases the horses had neither been raced nor had obtained any score. All these animals had to be eliminated in further investigation.

Table 10: TRANSFERRIN TYPES AND RACING PERFORMANCE

Transferrin Type	Horses (%) with score					Total (%)
	5	4	3	2	1	
FF	33	56	28	25	27	68 (33)
DF	33	37	37	27	25	61 (30)
FO		4	8	7	10	16 (8)
FR	33		8	9	6	14 (7)
DD			10	5	5	11 (5)
10 others		3	9	27	21	36 (17)
TOTAL	3	27	40	56	80	206 (100)

There is a significant surplus of FF horses in the score-class 4 which could indicate the superiority of these horses in their racing performance.

The determination of the frequencies of albumin types, when compared with those in other countries, showed a very similar pattern in all populations investigated. Table 11 presents the results of the comparison of racing performance with albumin types.

Table 11: SERUM ALBUMIN TYPES AND RACING PERFORMANCE

Albumin type	Horses (%) with score					Total (%)
	5	4	3	2	1	
SS	67	56	58	55	64	112 (59)
FS	33	26	30	18	24	49 (24)
FF		18	12	27	12	35 (17)
TOTAL	3	27	40	56	80	206 (100)

No clear-cut difference could be shown between the albumin types with reference to racing performance; in comparison with Basuto ponies, Arab horses and many other horse breeds in the world, it is clear that racing horses have a far greater frequency of the allele Alb^S, which indicates a clear preference of this allele in the breeding of such horses.

A new genetic system, only recently evaluated in family studies, is the pre-albumin system. Table 12 presents the results of this investigation with the respective racing performance of the horses.

Table 12: PRE-ALBUMIN TYPES AND RACING PERFORMANCE

Pre-albumin type	Horses (%) with score					Total (%)
	5	4	3	2	1	
LL	66	81	58	63	66	135 (65)
FS	33	4	5	4	1	7 (3)
FL		11	13	18	13	28 (14)
FF				4	3	4 (2)
IL			10	5	8	13 (6)
5 others			14	6	9	19 (10)
TOTAL	3	27	40	56	80	206 (100)

Further investigations seem to be necessary but it is apparent that the pre-albumin homozygous LL genotypes have a better racing performance, predominating in the score-class 4.

Enzymes

Of the six enzymes investigated, only two showed remarkable type variation, serum esterase and 6-phosphogluconate dehydrogenase. The variation in carbonic anhydrase, acid phosphatase, phosphoglucomutase and phosphoglucose isomerase (phosphohexose isomerase), through intensive selection for racing performance, has diminished to such an extent that there is really very little left. Thus, no correlation studies with these genetic systems could be performed.

Table 13: SERUM ESTERASE TYPES AND RACING PERFORMANCE

Esterase type	Horses (%) with score					Total (%)
	5	4	3	2	1	
II	100	84	90	92	88	161 (89)
IS		4	5	4	8	11 (5)
IF		4	5	4	4	8 (4)
FF						
FS		4				1 (1)
SS		4				1 (1)
TOTAL	3	27	40	56	80	206 (100)

Of the serum esterase polymorphism, the II type is the most frequent, and this is true for almost all breeds investigated. Table 13 correlates esterase types with racing performance.

Without any doubt, the breeder should concentrate on the esterase type II homozygous stallion in his selection programme and avoid all other combinations.

The 6-phosphogluconate dehydrogenase plays an important rôle in the hexose monophosphate shunt pathway. The oxidative decarboxylation of 6-phosphogluconate (6-PG) to ribulose 5 -phosphate (R 5P) is catalyzed by 6-phosphogluconate dehydrogenase (6-PGD), generating a second molecule of nicotinamide adenine dinucleotide phosphate (NADP). This enzyme has been found to vary both in electrophoretic pattern and catalytic activity. In table 14, horse breeds so far investigated for the different 6-PGD types are compiled, together with the present results.

Table 14: GENE FREQUENCIES OF 6–PGD IN DIFFERENT HORSE BREEDS (INCLUDING MULES & DONKEYS)[1 4 8]

Breed/ Type/ Species	D	F	S	Number	Author
North Swedish Horse	0,092	0,907	0,001	457	Sandberg and Bengtsson
Swedish Trotter	0,002	0,794	0,204	264	Sandberg and Bengtsson
Common German Horses	0,000	0,812	0,188	40	Bender et al.
Common S.A. Horses	0,000	0,837	0,163	135	Op't Hof & Osterhoff 1973
Mules	0,013	0,895	0,092	38	Op't Hof & Osterhoff 1973
Donkeys	0,100	0,871	0,029	35	Op't Hof & Osterhoff 1973
Thoroughbreds (all)	0,000	0,600	0,400	377	This report
Thoroughbreds (with score)	0,000	0,612	0,388	206	This report

There was no difference between horses with scores and those without, as yet, any performance results. This suggests that the young horses of the group investigated certainly stand a good chance to compete;

Table 15: 6-PHOSPHOGLUCONATE DEHYDROGENASE TYPES AND RACING PERFORMANCE

6-PGD Type	Horses (%) with score					Total (%)
	5	4	3	2	1	
FF		30	48	39	39	79 (38)
FS	60	55	35	47	46	94 (45)
SS	40	15	17	14	15	33 (17)
TOTAL	3	27	40	56	80	206 (100)

it also indicates that we should move in the direction of increasing the frequency of 6-PGD^S alleles by introducing homozygous SS stallions. Table 15 indicates that the heterozygous FS horses certainly performed well (see the significant deviation in the score-class 4).

In a report from Sweden presented at the California meeting in June, genetically controlled variants of NADH diaphorase were established in horse red cells [9]. The enzyme NADH diaphorase in red cells is considered to be a major agent in the reduction of methaemoglobin to haemoglobin. Deficiency of the enzyme has proved to be associated with congenital methaemoglobinaemia in man. Three types were established in horses and family data indicated that the variants were controlled by two co-dominant autosomal alleles, tentatively designed Dia ^{F} and Dia ^{S}.

This finding is mentioned as an example of the future direction of the present work. The typing of NADH diaphorase of the 377 samples collected during this investigation has already been started.

CONCLUSION

The results so far obtained are compiled in Table 16 and should serve as a guide for breeding race horses.

Table 16: PREFERRED COMBINATION FOR BREEDING HIGH PERFORMANCE RACE HORSES

Marker	Type
Transferrin	FF
Albumin	FS
Pre-albumin	LL
Post-albumin	SS
Esterase	II
6-PGD	SS
Acid Phosphatase	FS
PGM	FS
PGI	FS

The list of combinations will be lengthened when more results become available. This is the first study of its kind and should stimulate further investigation, not only in South Africa but elsewhere.

Future research should concentrate on those systems which are directly involved in the physiological and biochemical processes, and could provide new parameters indicative of performance ability in the equine.

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DISCUSSION

J.R. Gillespie: Because of its importance in the transport of oxygen, I was wondering if you could use 2-3DPG as a possible enzyme to be evaluated, as you have others, relative to performance and if that would get us anywhere.

D.R. Osterhoff: I could have added another enzyme that was presented just four weeks ago at the same meeting. The NADH diaphorase was established in a Swedish laboratory and also here, since this enzyme in red cells is considered to be a major agent in the reduction of methaemoglobin to haemoglobin and also it has been shown that this particular enxyme, if sufficient, has been proved to be associated with congenital methaemoglobinaemia in man. It is being studied at the moment. There is not only one, but a whole series, which should be followed up.

P. Boyazoglu: You had one chart which showed a comparison of about five different breeds of horses in which you indicated that 100% of Thoroughbreds had one haemoglobin type and 98,1% of Arabians had that type but also 1,9% had a type which the Thoroughbred did not have. If you look back into the development of the Thoroughbred, we see three Arabian stallions playing a prominent rôle in the development. How do you explain the vanishing of that type from the Thoroughbred?

D.R. Osterhoff: It is correct that the Arab played a tremendous rôle in the development of the Thoroughbred, but the Thoroughbred has been bred away from the Arab: probably these extra or odd types, and other types, have disappeared in this strong selection for speed. This is the only explanation I have. There were the Welsh crosses in the same table, consisting of 18 horses, none of which had this particular type. Of 441 Thoroughbreds all had only one type of haemoglobin. This is an indication, I think that the other types were lost in the evolution of the Breed.

If I may, this is the opportunity to mention the disparity of blood typing results and the urgent need for world-wide standardization and official recognition by breed societies. This may be the right group of people together here to consider these possibilities for the future, and in your respective countries to work out a policy with the different horse breed societies, which could then be accepted officially. In countries like Germany, Holland, Sweden, USA and England this is accepted and we are on the way to getting international standards accepted by our societies. If horses are transferred, or in the future semen is exported to or imported from different countries their blood group and type determinations should be acceptable to the countries involved. Already in the comparison tests 16 different countries are involved. This particular group of equine specialists should consider steps towards recognizing this type of test as a rule in horse breeding.

M.A.J. Azzie: I would like to compliment Dr Osterhoff on his investigations and also on the original work that he has done on typing of blood groups of horses. Two weeks ago in Cambridge there was much discussion about possible artificial insemination of horses and the attitude that should be adopted. The message that really comes home here is that there is no problem in relation to identification of progeny: the problem now is solved and it is up to us as a group to try to stimulate our various horse communities and horse industries to make use of tests of this kind in the advancement of breeding.

J.D. Steel: There is just one point which might not have been brought out strongly enough. Roumanet, who is the President of the Society for the Encouragement of Horse Racing in France, has been very anxious to make it possible for horses to move more freely from one country to another, so racing can become truly international. At the Third International Conference of Infectious Diseases of Horses which was held in Paris in 1972 this question of using blood groups as a means of identification was very thoroughly discussed. Roumanet is strongly pushing for the idea that all horses should have an identification passport and that part of the information to be included in that passport would be information about the blood groups. This is perhaps another way that this sophisticated and magnificent study might be put to practical use.

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GLUCOSE AND INSULIN BIORHYTHMS IN THE HORSE

J.W. EVANS*, P.G. THOMPSON* AND C.M. WINGET**

SUMMARY

The plasma concentration of insulin and glucose is the result of complex regulatory mechanisms. The data demonstrate circadian rhythms in plasma glucose and insulin. Glucose also has ultradian rhythms of 17 to 19 minutes and 70 to 80 minutes. The glucose data lend support to the concept that homeostasis is not the maintenance of a steady state but of a regulated state. The baseline of regulation varies over a 24-hour period. Because of the continuous changes in regulatory input and output of the system, there is a circadian rhythm. As a result of noise in the regulatory systems, a rapid oscillation or ultradian rhythm lasting a few minutes about the baseline also exists. No ultradian rhythms were observed for plasma insulin which lends support to the concept that the insulin system in the horse is a slow reacting system.

CIRCADIAN RHYTHMS

Introduction

Glucose metabolism and its control have been studied extensively in many species but has received little attention in the horse [1 2 3 5 9 13 14]. Argenzio & Hintz [2 3] suggested that changes in plasma glucose concentration in ponies are mediated by different mechanisms from those in ruminants, while the insulin response to glucose resembled that reported for man. Madigan & Evans [13] observed that, compared with man, the equine pancreas is slow to respond to a decline in plasma glucose concentration. Glucose is an important energy source in horses and Evans [5] has observed a two-fold increase of glucose in exercise-conditioned horses.

Many studies have demonstrated that changing periods of light and darkness affect the activities of many physiological processes in animals and man [4 12]. The horse is a complex organism which carries on a countless number of physiological processes in order to produce its most important commodity – the ability to run, trot or pace. The types and levels of performance are determined not only by the interactions of many physiological processes but also by the proper degree of each interaction. These interactions and degree of interactions of the various physiological processes are dependent upon rhythmical variations in certain processes. Changes in light-dark schedules alter the functions of many metabolic processes and thus the efficiency of energy utilization. Therefore, desynchronization of these processes will affect the ability of the horse to give a winning effort in a race. Gill, Skwarlo & Flisinka-Bojanowska [8] failed to observe a significant circadian rhythm in plasma glucose concentration, but the horses were not maintained under closely controlled environmental conditions. The purpose of our investigations was to determine the temporal fluctuations of plasma glucose and insulin and the effect of changes of the light-dark schedule on the temporal patterns.

Materials and Methods

To determine the circadian rhythm in plasma glucose and insulin, six Thoroughbred mares in anoestrus were maintained under a constant temperature of 24°C and a light-dark schedule of 12:12. The lights came on at 06h00 and went off at 18h00. The mares were allowed to eat a completely pelleted ration and drink water *ad libitum*, but fresh feed [5] and water were given at 08h30 each day. After the horses remained under the controlled conditions for a 45 day acclimatization period, blood samples were obtained at 4h intervals for 14 days. Plasma glucose was determined by the o-toluidine method on the Auto Analyzer [16] and insulin was determined by radioimmunoassay [13]. Owing to a laboratory accident, plasma samples from Horses 5 and 6 were not analysed for insulin. To investigate the effects of changes in the light-dark schedule, three of the above horses were subjected to the following light-dark schedule while blood samples continued to be obtained at 4h intervals. On experimental Day 15, the lights remained on at 06h00 until 18h00 hours so that the horses were subjected to an additional 12 hours of light and a subsequent reversal of the light-dark period. On Day 43, the lights remained off at 18h00 hours and came on at 06h00 hours of Day 44 so that the light-dark schedule was reversed and returned to the first 14-day schedule. On Day 65, the lights remained on at 18h00 and remained on until Day 98.

Results

Significant circadian rhythms in plasma glucose were observed in 5 of 6 horses during the first 14-day period (Fig. 1). In Horses 1, 2 and 6, a large and sharp peak was observed at 12h00, and minimal values were observed at 08h00. Mare 4 had a similar pattern except the peak was observed at 08h00. During the rest of the day, the plasma concentration remained constant. In Mares 3 and 5, minimal values were observed at 08h00, but the plasma concentration continued to increase until 16h00 to 20h00.

Horses 1, 2 and 6 had greater mean daily amplitudes and ranges of oscillation than Horses 3, 4 and 5 (Table 1). The amplitude, which is the greatest deviation from the daily mean, ranged from 2,2 to 9,6 mg per cent except for Horse 1 which had an amplitude of 21,6 mg per cent. It appears that the horse has a smaller daily amplitude than man and sheep, the amplitudes of which are about 20% and 11% of the daily mean, respectively [10 11].

The daily range of oscillation between the minimum and maximum values varied between 3,8 and 13,3 mg per cent except for Horse 1 which had a 28,9 mg per cent average. In comparison with other species, a range of oscillation of 30% of the daily mean has been observed for mice [6], 25% for rats [15], 40% for man [10] and 15% for sheep [11]. The horse appears to be similar to the ruminant in respect to the range of oscillation.

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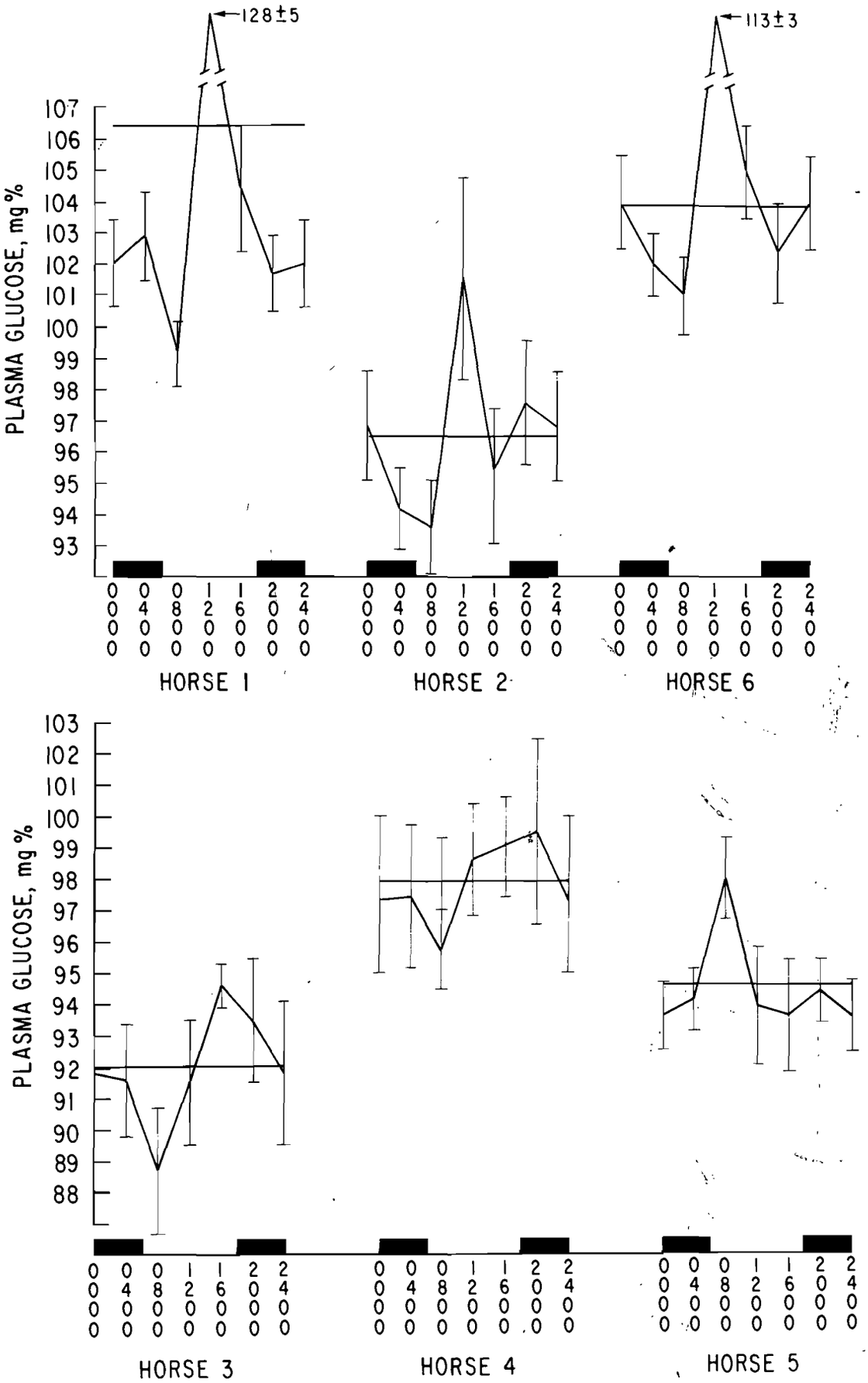

Fig. 1: Circadian rhythms in plasma glucose determined in six horses over a 14-day period. ■ – lights off, □ – lights on. Values are mean ± SE.

Table 1: GLUCOSE AND INSULIN CIRCADIAN RHYTHM VALUES

Day	Time of Lights on	Horse	Glucose			Insulin		
			Daily mean · mg %	Amplitude mg %	Range of Oscillation mg %	Daily Mean ng/ml	Amplitude ng	Range of Oscillation ng
1–14	0600	1	106,4	21,6	28,9	2,9	5,5	7,3
		2	96,5	5,1	8,0	1,1	0,9	1,5
		3	92,0	3,3	5,9	1,9	0,1	0,3
		4	94,6	3,4	4,4	1,9	0,2	0,3
		5	97,9	2,2	3,9	–	–	–
		6	103,9	9,6	13,3	–	–	–
30–43	1800	1	107,0	3,9	7,4	2,4	1,4	3,8
		2	95,0	2,1	2,9	1,2	0,2	0,4
		3	92,5	2,3	4,2	1,0	0,2	0,4
51–65	0600	1	105,1	9,6	12,9	3,0	2,1	3,3
		2	100,4	3,9	5,7	1,5	0,5	0,8
		3	91,3	2,6	4,6	0,7	0,2	0,4
85–98	Continuous	1	106,3	17,3	22,6	2,7	4,9	6,5
		2	101,4	11,5	15,4	1,2	1,7	2,2
		3	91,2	1,7	2,8	3,6	0,4	0,7

Reversal of the light-dark schedule by exposure to an additional 12 hours of light (experimental Days 15 to 43) depressed the mean daily amplitude and mean range of oscillation in Horse 1 during Days 30 through 43 (Fig. 2). The daily mean concentration was not changed (Table 1). The glucose rhythm did not undergo a phase shift and the rhythm had not re-synchronized by Days 30 to 43. In Horse 2, the glucose rhythm was abolished. In Horse 3, a phase shift occured so that the daily minimum value was observed 12 hours later at 20h00. The daily maximum value was phase-shifted to 12h00. The light-dark schedule did affect the glucose rhythms, but the time of adding fresh food to the manger may be a more important 'zeitgeber'. Unfortunately, the mares were not observed for determination of their eating habits. A second reversal of the light-dark schedule by an additional 12 hours of darkness, which returned the horses to the original light regime, caused a return of the glucose rhythm during Days 51 through 65 in Horse 1 resembling the rhythm of Days 1 to 14. The rhythm in Horse 2 did not re-synchronize. The amplitudes and ranges of oscillation of Horse 2 were decreased compared with Days 1 through 14 and the daily maximum was phase-shifted to 16h00. In Horse 3, the daily minimum value returned to 08h00 and the daily maximum values tended to be observed at 24h00. Therefore, Horse 3 did not re-synchronize within this time period. During the 85 to 98-day period after the horses had been under constant light for 2 weeks, Horses 1 and 2 had glucose rhythms almost identical to the period Day 1 to 14. In contrast, Horse 3 had no significant rhythm, but the daily minimum value tended to occur at 08h00.

The insulin circadian rhythms of Horses 1 and 2 were similar in their wave form during Days 1 through 14 (Fig. 1). Nevertheless, Horse 1 had a large amplitude of 5,5 ng compared with 0,9 ng for Horse 2 (Table 1). Horse 4 had a different type of temporal pattern in that a minimum value of 1,8 ng/ml was observed at 10h00. The daily minimum value was between two peak values observed at 12h00 and 20h00. The rhythm was not significant ($P \geq 0,05$). Horse 3 also had a daily minimum value at 08h00 but the maximum daily value was observed at 20h00. Reversal of the light-dark schedule resulted in changes in the characteristics of the rhythms. Horse 3 phase-shifted to re-synchronize its insulin circadian rhythm with the light-dark schedule. In Horse 3, the minimum value was phase-shifted from 08h00 to 16h00 and 20h00 and the daily maximum value was phase-shifted from 20h00 to 08h00. In Horses 1 and 2, the 12h00 peak was depressed but the general temporal pattern was unchanged. Horse 3 also re-synchronized its insulin rhythm after the next light-dark schedule change. The rhythms in Horses 1 and 2 during Days 51 to 65 did not change much compared with the rhythms for Days 30 through 43, except Horse 2 had a definite peak value at 16h00. Exposure to continuous light returned the insulin rhythms in Horses 1 and 2 to their original wave form observed during Days 1 to 14. Minimum values in Horse 3 were again observed at 04h00 to 08h00 hours, but the rest of the values tended to be rather constant.

ULTRADIAN RHYTHMS

Introduction

Within the last few years, the ultradian rhythms and episodic secretions of various metabolites and hormones have been investigated, particularly in man. These studies have demonstrated that there is considerable oscillation about the baseline of regulation for most blood constituents. Because of the day-to-day variations that were observed in glucose and insulin concentrations of plasma samples obtained at a given time each day, the purpose of our next investigation was to determine if ultradian rhythms in plasma glucose and insulin existed in horses.

Materials and Methods

Six Thoroughbred and Quarter Horse mares were kept under the same conditions as in Days 1 to 14 of the previous trial. The horses were trained to stand in a sampling cage connected to the environmentally

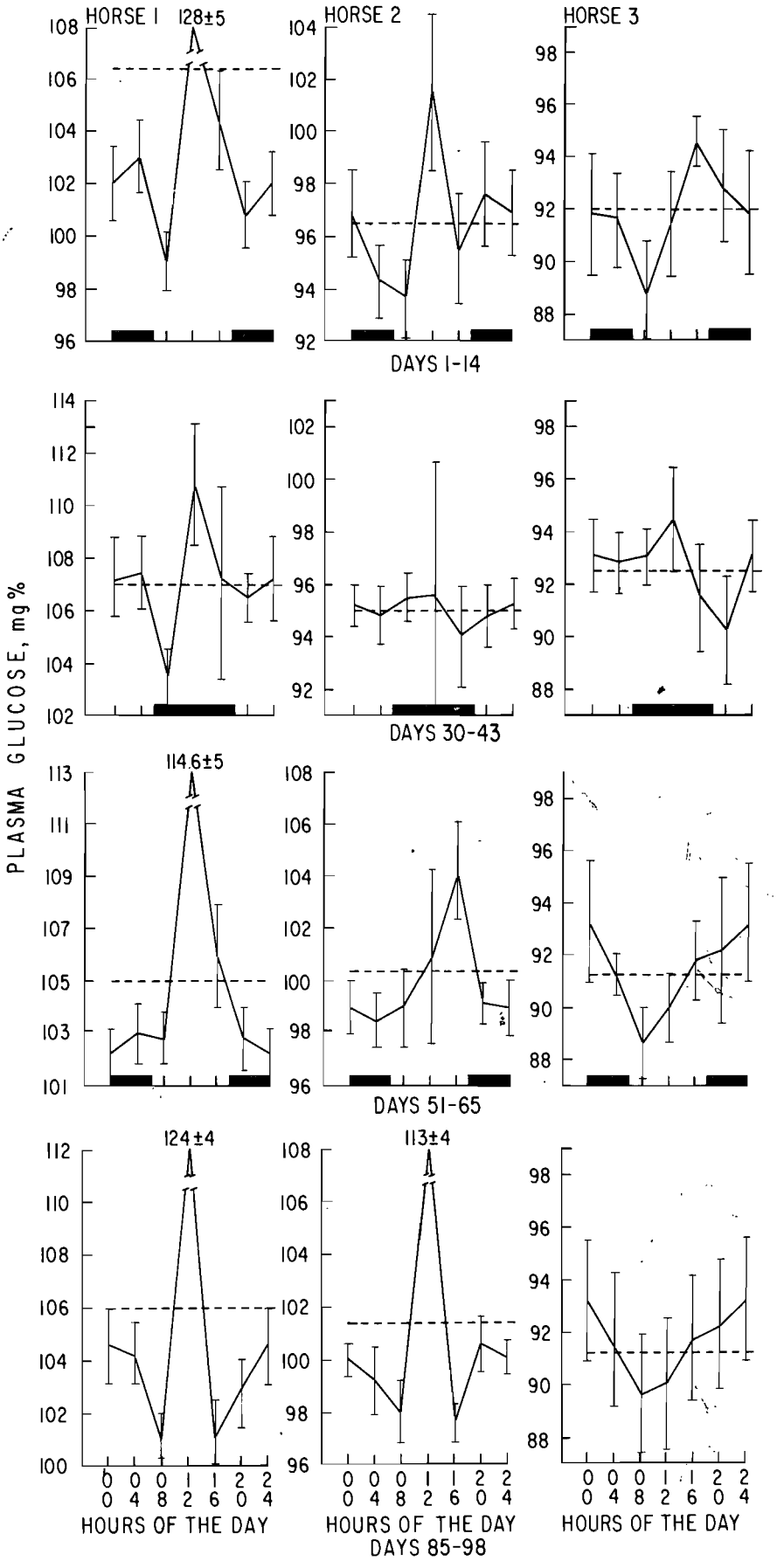

Fig. 2: Effect of changes in the light-dark cycle on the circadian rhythms in plasma glucose of three horses. ■ lights off. □ lights on. Each point is mean ± SE for 14 days.

controlled room. A jugular catheter was passed through a light-tight port in the cage so that the horse could be bled without being disturbed. A one-way mirror was installed so the horses could be observed if necessary. Blood samples were obtained at 20-minute intervals for 2 to 5 days and at 5-minute intervals from 15h00 to 19h00 for two consecutive days to determine the ultradian rhythms.

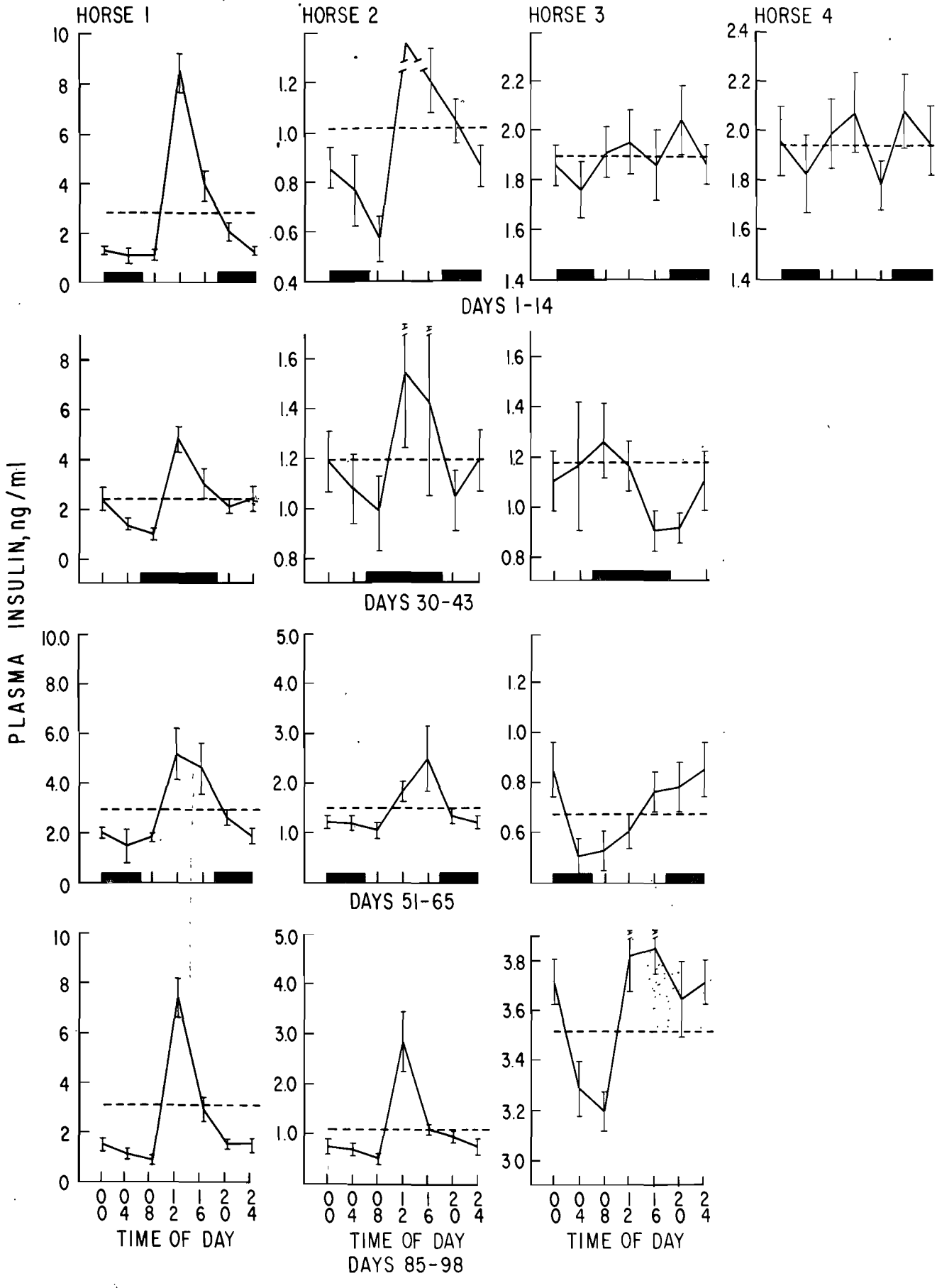

Fig. 3: Effect of changes in the light-dark cycle on the plasma insulin circadian rhythms. ■ – lights off, ▭ – lights on. Each point is mean ± SE for 14 days.

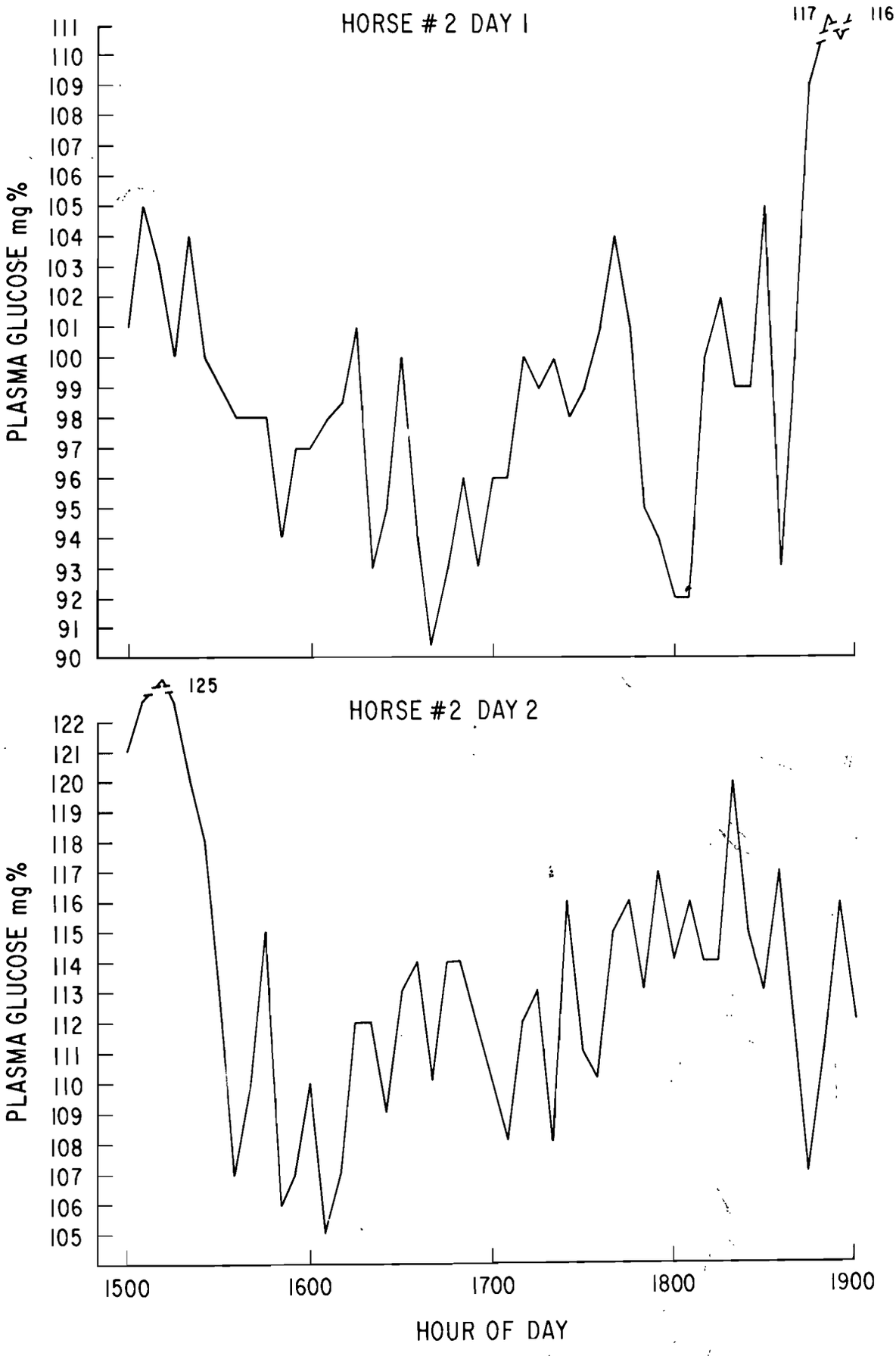

Fig. 4: Plasma glucose concentrations in blood samples obtained at 5-minute intervals for 4 hours fromHorse 2.

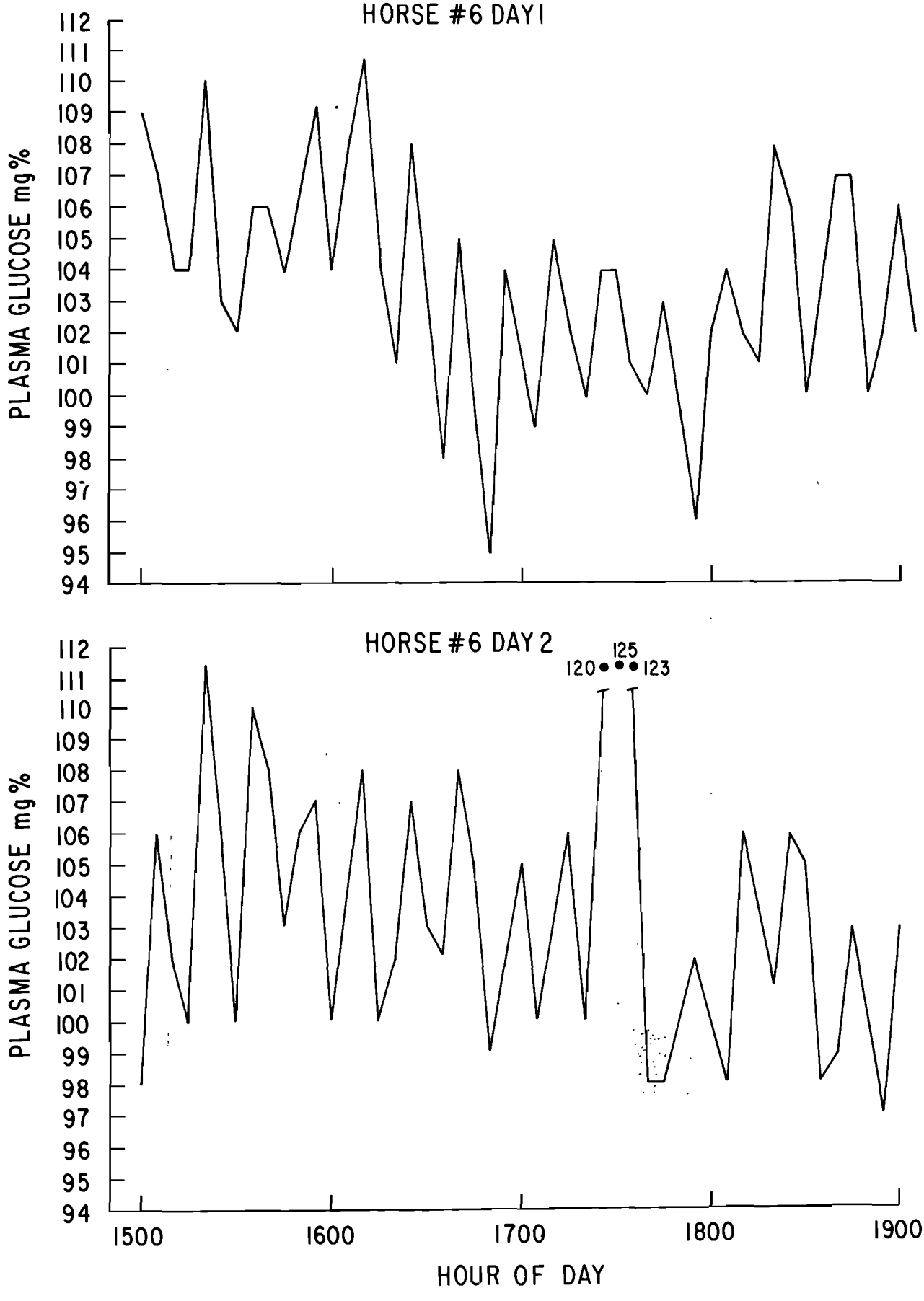

Fig. 5: Plasma glucose concentrations in blood samples obtained at 5-minute intervals for 4 hours from Horse 6.

Results

Typical short-term oscillations in plasma glucose are shown for Horses 2 and 6 in figures 4 and 5 respectively. Horse 6 had a smooth oscillating rhythm, whereas the oscillations in Horse 2 were not as consistent. Except for two other 4-hour periods, which resembled Days 1 and 2 for Horse 2, the oscillations resembled those for Horse 6. The periods of the oscillations were determined as shown in figure 5. Based upon this type of analysis, ultradian rhythms which averaged 16 to 19 minutes in length were observed for each of the horses (Table 2). The minimum period length observed during the twelve 4-hour periods was 10 minutes and the maximum 33 minutes.

Table 2: GLUCOSE AND INSULIN ULTRADIAN RHYTHMS

Horse	Glucose		Insulin
	min. ± SE	min. ± SE	min. ± SE
1	18,1 ± 0,7	77,8 ± 3,9	N. S. *
2	17,1 ± 1,1	69,5 ± 3,3	N. S.
3	16,2 ± 0,6	73,8 ± 5,5	N. S.
4	18,8 ± 1,4	71,4 ± 3,3	N. S.
5	18,1 ± 1,2	77,2 ± 5,7	N. S.
6	16,8 ± 0,4	67,9 ± 2,2	N. S.

* not significant: $P > 0.05$

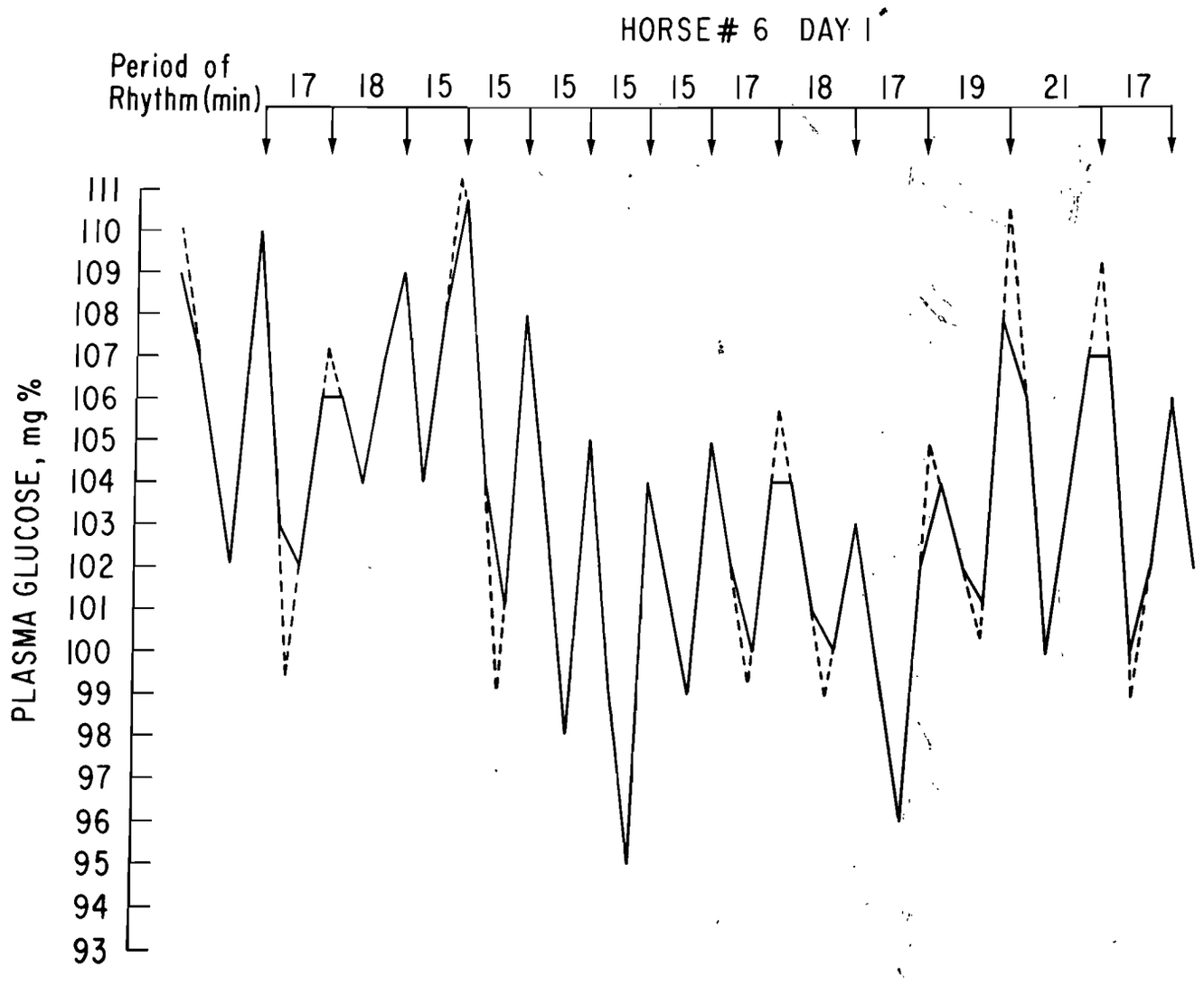

Fig. 6: Analysis of 5-minute interval glucose samples. Lines were drawn, based upon the fact that in many instances a straight line connected three or more points.

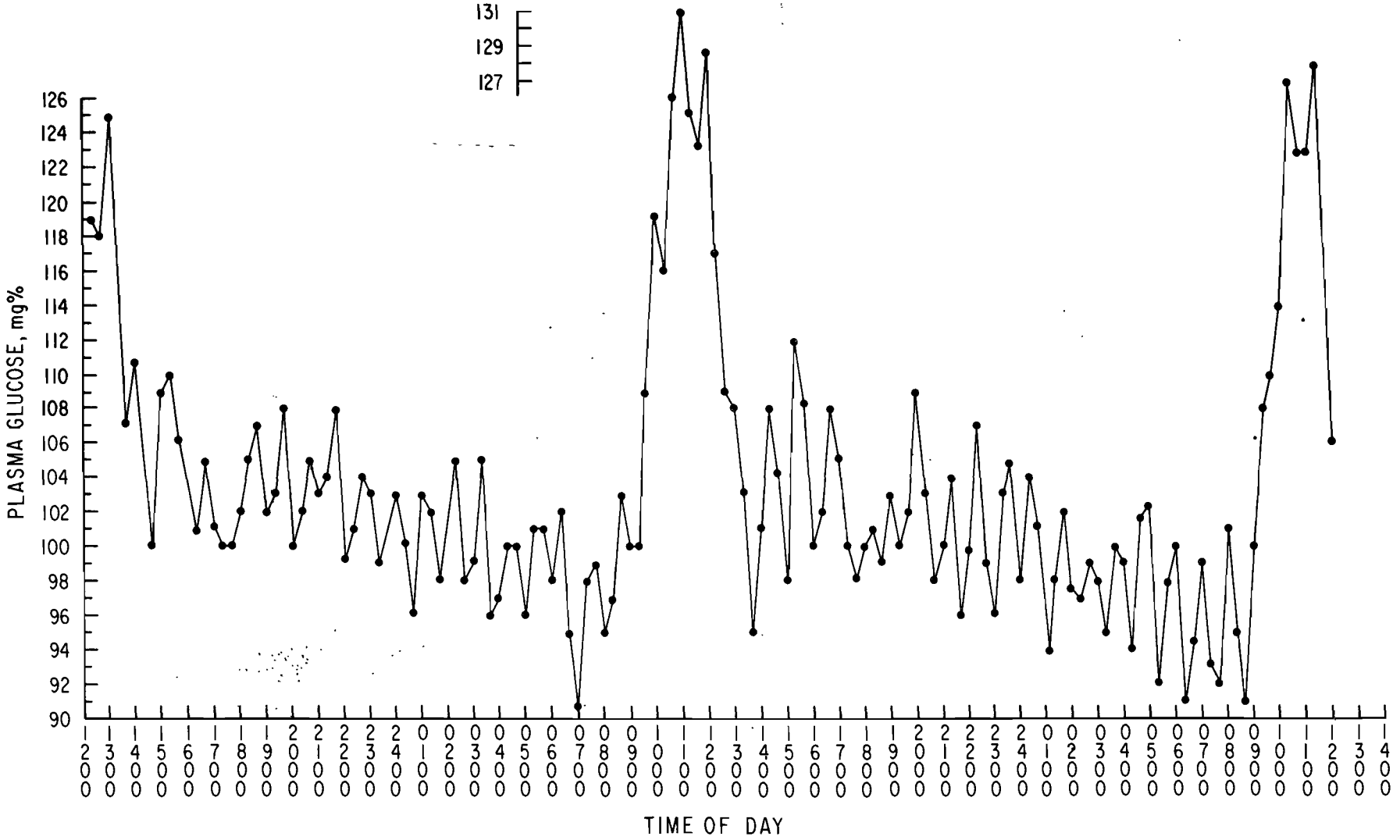

Fig. 7: Glucose concentration in plasma samples obtained at 20-minute intervals for 48 hours.

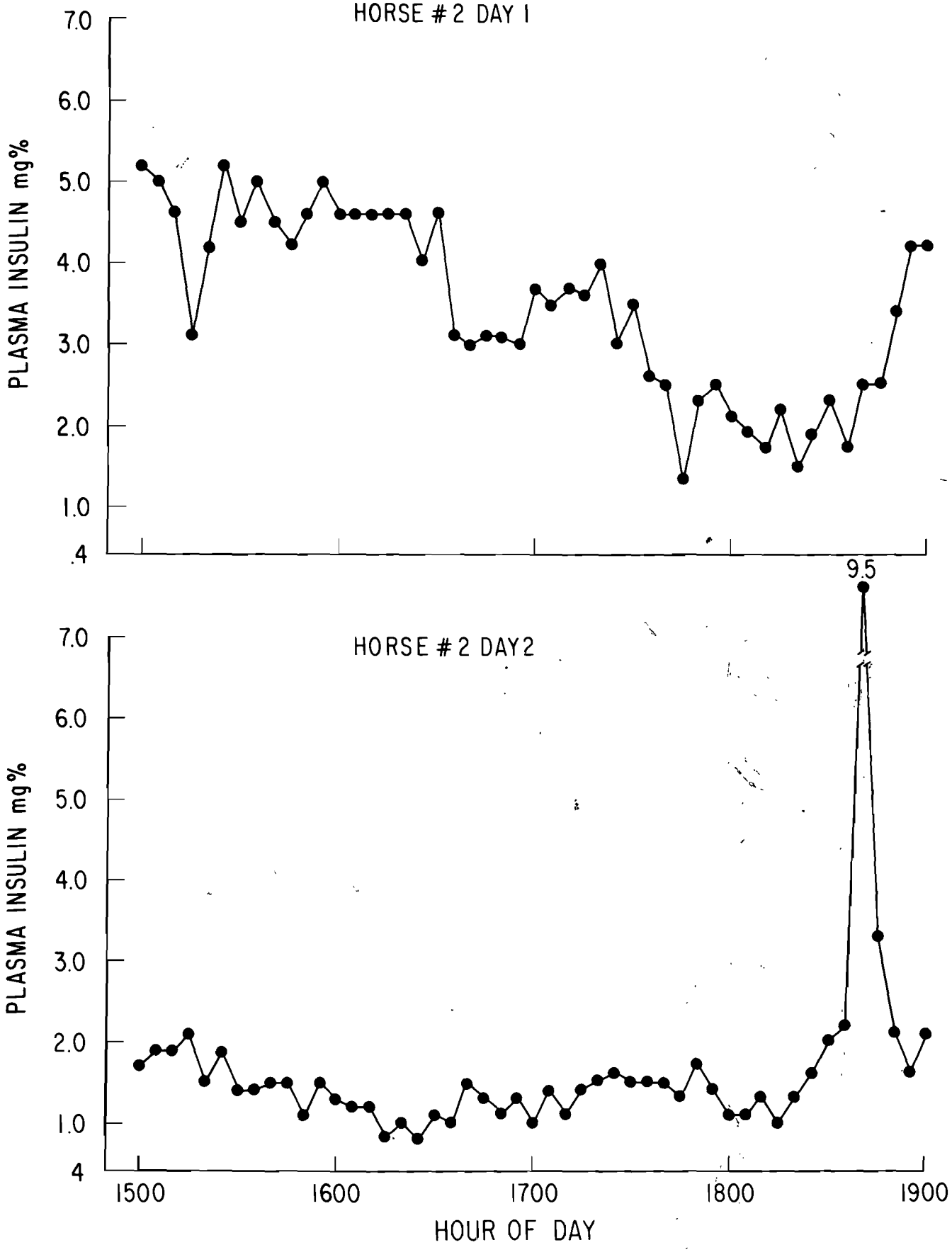

Fig. 8: Plasma insulin concentrations in blood samples obtained from Horse 2 at 5-minute intervals for 4 hours.

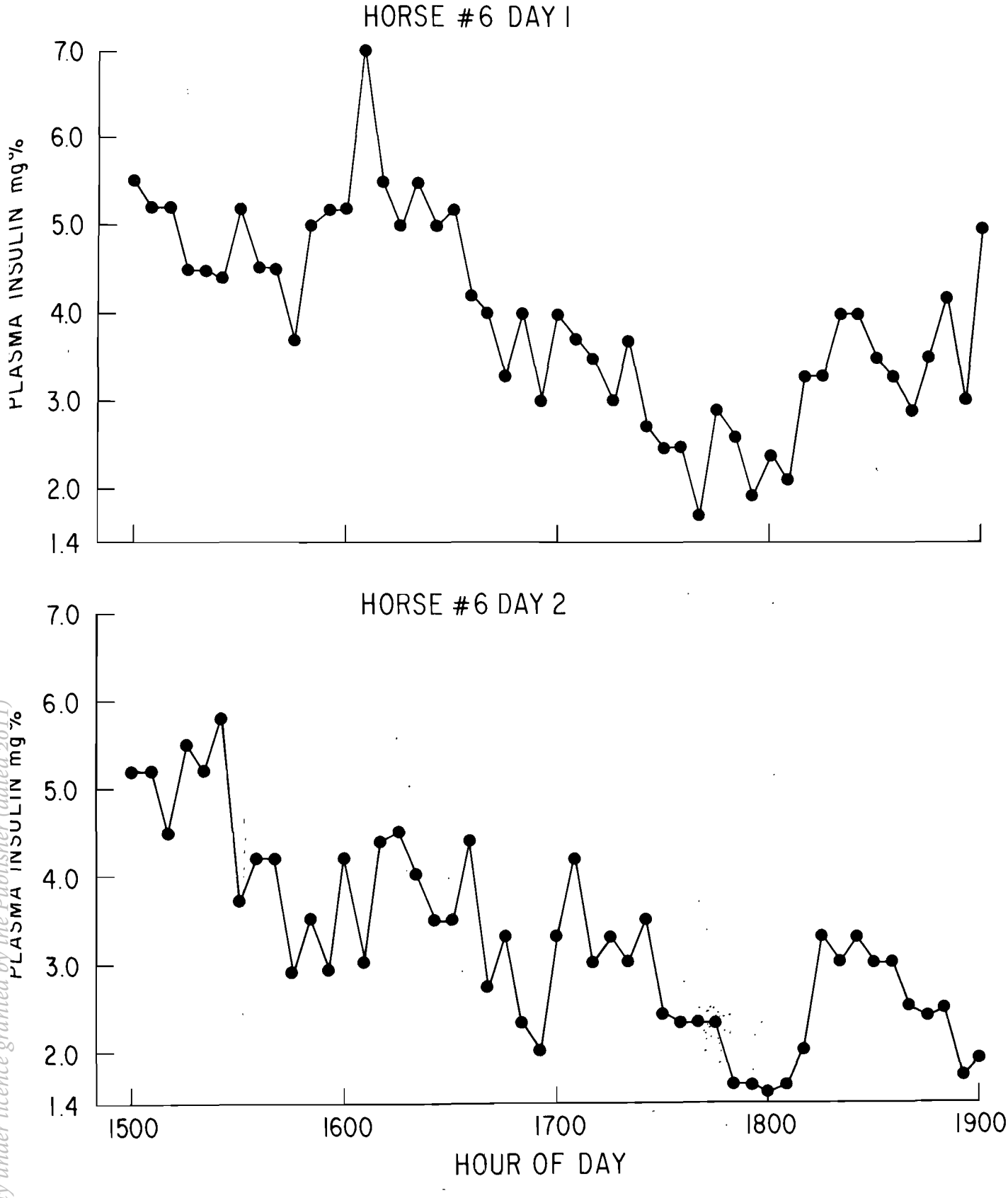

Fig. 9: Plasma insulin concentrations in blood samples obtained from Horse 6 at 5-minute intervals for 4 hours.

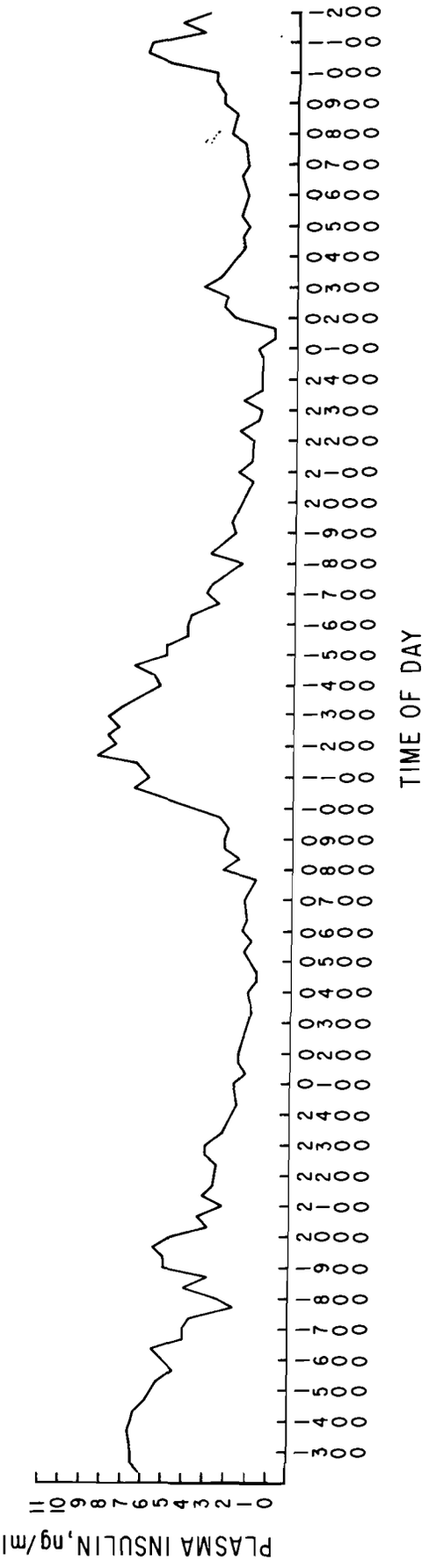

Fig. 10: Plasma insulin concentrations in blood samples obtained at 20-minute intervals for 48 hours.

In analysing the data obtained at 20-minute intervals, there were glucose rhythms with periods of 70 to 80 minutes in the six horses (Fig. 7 and Table 2).

The oscillations in plasma insulin were difficult to interpret. Typical oscillations that occurred in Horses 2 and 6 are shown in figures 8 and 9 respectively. During Day 1 in Horse 2, the insulin concentration had a three-fold change, but it is doubtful if a 15 to 20-minute rhythm was present. During the 4-hour period of Day 2, only minor changes were observed except for a four-fold increase and decrease that occurred during the last hour of observation. During Day 1 in Horse 6, the plasma concentration also went through a three-fold change along with other minor fluctuations. Day 2 was similar to Day 1, except the minor fluctuations were more pronounced. The other data were similar to the data in figures 8 and 9. Data for the 20-minute interval samples did not reveal a 70 to 80-minute rhythm as was observed for plasma glucose (Fig. 10 and Table 2). We do not feel that sufficient evidence exists to suggest an insulin ultradian rhythm with a period length of a few minutes. The important point, however, is that it is difficult to obtain a single plasma sample and use the value to indicate the physiological state of the animal in regard to insulin metabolism.

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J.R. Coffman: You mentioned that the horses were fed a pelleted ration at a standard time each day. Could you elucidate further the make-up of the ration, particularly the grain-to-hay ratio.

J.W. Evans: The completed ration consisted of alfalfa hay (66%), barley (20%), bran (9%), molasses (4%) and salt (1%).

J.R. Coffman: Have you looked at plasma cortisol?

J.W. Evans: Yes, we have. There appears to be an ultradian rhythm with a period length of 25 to 35 minutes. It is not uncommon to observe a 3 to 4-fold rise and fall of the cortisol concentration over a period of 6 to 8 hours. Our resting values are between 50 to 70 ng / ml plasma. They may stay within this range or they may fluctuate 2 or 3-fold with minimal values in this range.

J.R. Coffman: Could you correlate glucose, insulin and cortisol values – and could you demonstrate counter-regulatory patterns?

J.W. Evans: The data have not been analyzed in this respect, but will be analyzed very shortly. It was one of our objectives to observe the interrelationships between these three factors. Briefly, we had planned to use a computer, but its analysis depends upon a sine wave. The oscillations are not sine waves and thus require 'hand calculations.'

A.M. Merritt: I was curious to know if you observed any of these horses after they were fed?

J.W. Evans: No, we have not. I suspect that the horses that had a sharp peak in glucose at noon were meal-eaters and those that did not have peak values at noon were nibblers or continous eaters.

A.M. Merritt: Do you feel that light or diet is the more important?

J.W. Evans: I am not really sure. This is one of the areas we will be investigating. We are forming a group of horses to separate the effects of light *versus* meals, *i.e.* light *versus* one meal, two meals and four meals per day. The fasted condition must also be considered

Maureen Aitken: I have just one small question which is related to the last one. Did all the horses completely eat their entire ration at a fixed time every day. We have recorded some not very sophisticated, careful measurements of glucose. We found that if we feed the horses only on hay, then we do not see the normal rise which we do when they get their pelleted diet.

J.W. Evans: Yes, this is one of the parameters that has bothered us. It is one of the reasons we are going to separate the effects of light and diet. I suspect that the horses with definite rhythms ate all their feed at once, whereas, those horses that did not have much of a rhythm probably ate continuously over 24 hour period. We did not observe the animals because we did not want to influence them. The one-way mirror was installed for observation in the sampling cage for the ultradian rhythm experiment.

"... the safest procedure is to administer Sulphonamides in doses sufficient to establish an antibacterial effect until a day or so after the infection has cleared up"

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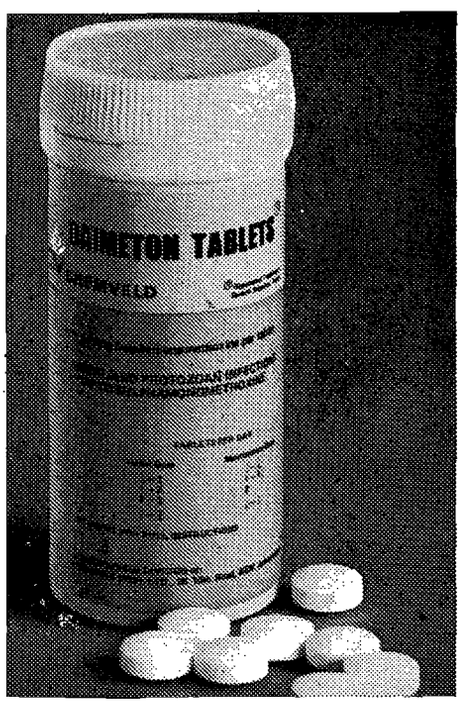

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SERUM ENZYME CHANGES AND HAEMATO-CHEMICAL LEVELS IN THOROUGHBREDS AFTER TRANSPORT AND EXERCISE

D. CODAZZA. G. MAFFEO AND G. REDAELLI*

SUMMARY

Serum enzyme changes and haemato-chemical levels in Thoroughbred horses after transport and exercise have been studied. The samples were collected at the commencement of travel (300 km), immediately after arrival, after resting periods of 24 and 48 hours, and finally after an extended canter of 1 500 m. The values concerning total proteins, blood glucose, glutamic-oxalacetic and pyruvic transaminase, creatine phosphokinase, lactic dehydrogenase, lactate, pyruvate and creatinine were measured and the serum levels of calcium, magnesium, sodium, potassium and phosphorus ions noted. A certain similarity of muscular function appears to occur both during transport and prolonged work.

INTRODUCTION

Race-horses are frequently moved from one centre to another to race. When the journey is reasonably short (up to 200 km), horses are usually moved a few hours before the event. Although nowadays modern means of transport are in use and transfer is carried out during the most suitable hours of the day, it is clear that these journeys by road could have an adverse effect on the physiological conditions and therefore the performance of the individual. Therefore, we investigated the serum-enzymatic changes and the haematochemical data involved during transport and muscular work of race-horses.

MATERIALS AND METHODS

The research was carried out on 40 clinically healthy Thoroughbred horses, three to five years old. Both sexes were included. Blood samples were taken: a) at the commencement of a 300 km journey, b) immediately on arrival; c) after resting for 24 and 48 hours, respectively, and d) following a 1,500 extended canter. Of these samples, taken from the jugular vein without anticoagulant, one was left to clot at room temperature and the other was deproteinated by adding different dilutions of $HClO_4$ (perchloric acid). The samples were frozen at collection and the evaluations carried out within six hours. The following investigations were carried out:-

1) Total serum-proteins [8]
2) Calcium and magnesium [3]
3) Sodium and potassium [19]
4) Inorganic phosphorus [4]
5) Creatinine [14]
6) Creatine phosphokinase [18]
7) Lactic dehydrogenase (LDH) [20]
8) Glumatic-oxalacetic transminase (GOT) [11] and pyruvic transaminase (GPT) [20]

The plasma obtained by centrifugation of the deproteinated blood was used to determine glucose, [12] lactate and pyruvate [9].

RESULTS

Since the investigation was carried out over a long period of time, under different experimental conditions, the results are 'graphically' recorded as percen-

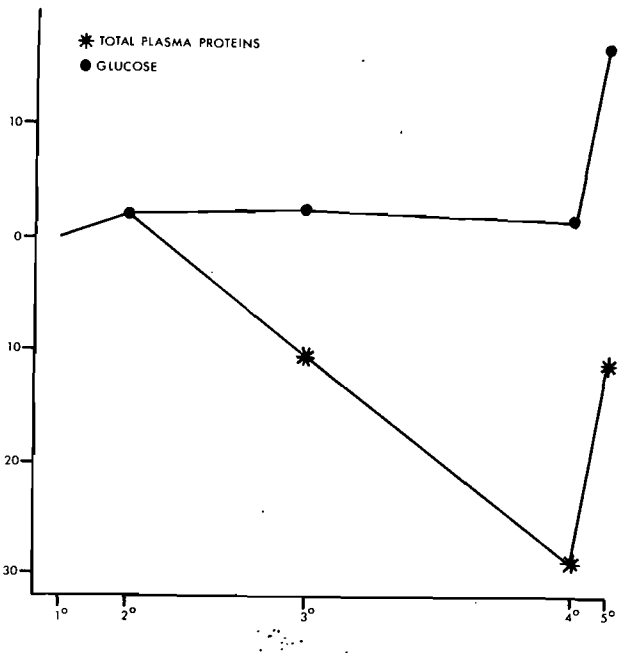

Fig. 1: The effect of transport, rest and exercise on total protein and glucose levels in the blood of Thoroughbreds. Time of sampling along x-axis. Percentage change along y-axis.

* Equine Research Station of Pathology and Prophylaxis of Infectious Diseases, University of Milan, 2013 Milano, via Celoria 10, Italy.

Table 1: THE EFFECT OF TRANSPORT, REST AND EXERCISE ON TOTAL PROTEIN, GLUCOSE, ENZYME, LACTATE, PYRUVATE AND CREATININE LEVELS IN THE BLOOD OF THROUGHBREDS

Activity	Sample No.	Time	Total Protein	Glucose	GOT	GPT	CPK	LDH	Lactate	Pyruvate	Creatinine
Transport	1^0	0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
	2^0	6h	102,1	101,9	84,2	105,4	160,1	130,1	110,3	118,1	106,7
Rest	3^0	24h	102,5	89,4	95,5	93,3	100,3	122,3	90,1	90,0	95,9
	4^0	48h	101,1	73,7	101,1	94,5	103,4	84,0	95,8	83,3	73,7
Exercise	5^0	51h	116,4	88,4	133,5	118,2	137,4	125,0	109,5	93,4	170,8

Values given as percentage change from pre-transportation levels taken as 100.

tage deviation from the values initially determined. Although the blood values of the horses examined appear to be within the normal range [10] it is interesting, however, to consider the following observations:-

a) There is no significant change in the total protein content of the blood serum when determined immediately after the journey and also shortly thereafter when the horses are at rest, but there is a definite increase following muscular exercise (+16,4% – Table 1 Fig. 1).

b) Blood glucose remains more or less constant during transport, decreases after rest (down to –26,3%), increases to some degree after exercise, but fails to return to the original values (–11,6% – Table 1, Fig. 1).

c) GOT values undergo a slight decrease following transport, but return to their original values at the third blood collection (after 24 hours rest), and then increase remarkably, during muscular work (+33,5% – Table 1, Fig. 2).

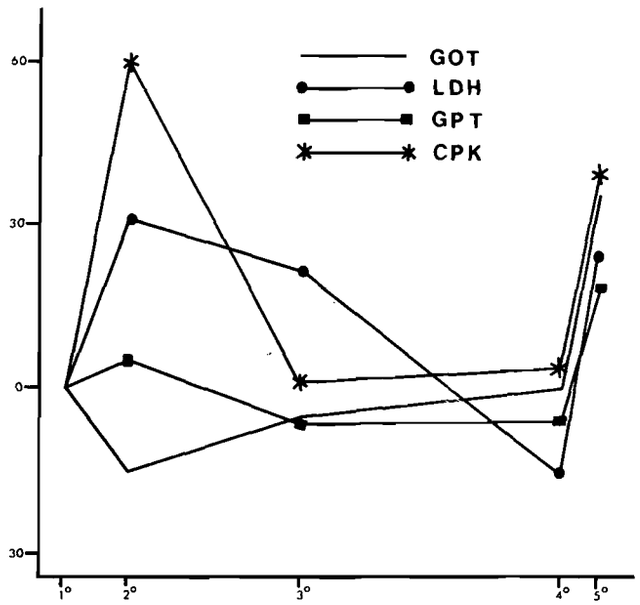

Fig. 2: The effect of transport, rest and exercise on serum enzymes of Thoroughbreds. Time of sampling along x-axis. Percentage change along y-axis.

d) GPT changes only slightly up to 48 hours, with an 18,2% increase after exercise (Table 1, Fig. 2).

e) CPK values are considerably increased after transport (+60,1%) but they return quickly to their normal values for 24 hours, before they rise again after exercise (+37,4% – Table 1, Fig. 2)

f) LDH undergoes an increase of 30,1% at the second collection and then decreases after rest to points lower than the original values (–16%) but increases again after exercise (+25% – Table 1, Fig. 2)

g) Lactate shows a curve similar to LDH, altough of lesser amplitude, with a final value of +9,5%) Table 1, Fig. 3).

h) After transport, the pyruvate curve reaches a value 18,1% higher than the one prior to the horses' departure. This decreases during rest to 16,7% below the original value and tends to increase during exercise to 6,6% below the original figure (Table 1. Fig. 3).

i) Serum creatinine increases slightly after transport (+6,7%) while decreasing at the third and fourth collection and then increasing again considerably after exercise to + 70,8% above the original level (Table 1, Fig. 3).

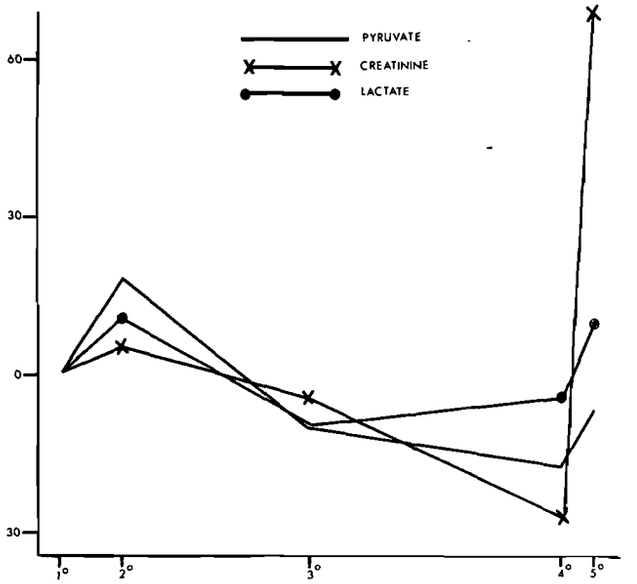

Fig. 3: The effect of transport, rest and exercise on blood pyruvate, lactate and creatinine levels in Thoroughbreds. Time of sampling along x-axis. Percentage change along y-axis.

j) Magnesium levels drop appreciably both after transport and exercise (–7,8% and –13,5% respectively while calcium values do not change significantly with a drop to –2,3% and ultimately to – 6,1% (Table 2, Fig. 4).

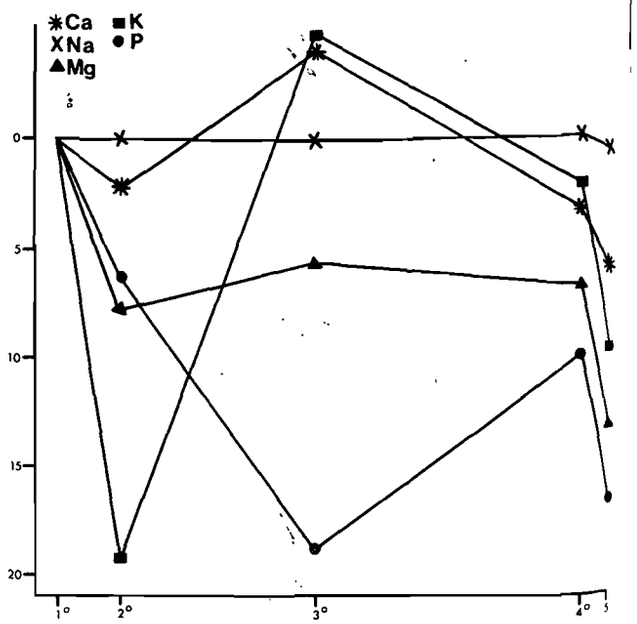

Fig. 4: The effect of transport, rest and exercise on blood electrolyte levels of Thoroughbreds. Time of sampling along x-axis. Percentage change along y.axis.

k) Phosphorus falls after transport (–5,4%) and undergoes a further decline during the first twenty-four hours of rest. Then it increases but does not revert completely to normal at the fourth blood

collection. Finally, muscular work causes a lowering of serum inorganic phosphate to a level 16,7% below the original value (Table 2, Fig. 4).

l) Serum potassium shows lower values both after transport and work (–19,4% and –9,7%); during the rest however, the level returns to normal. Sodium does not undergo any appreciable change (Table 2, Fig. 4).

Table 2: THE EFFECT OF TRANSPORT, REST AND EXERCISE ON BLOOD ELECTROLYTE LEVELS OF THOROUGHBREDS

Activity	Sample No.	Time	Ca	Mg	Na	K	P
Transport	1^0	0	100,0	100,0	100,0	100,0	100,0
	2^0	6h	97,7	92,2	99,7	80,6	94,6
Rest	3^0	24h	103,8	94,3	98,7	105,6	81,0
	4^0	48h	97,7	93,2	99,0	97,9	90,3
Exercise	5^0	51h	93,9	86,5	99,3	90,3	83,3

Values given as percentage change from pre-transportation levels taken as 100.

DISCUSSION AND CONCLUSIONS

As seen from the tables, transport appears to have influenced the blood enzyme levels, with increased LDH and CPK blood values. The CPK levels are even higher than those seen after muscular work (canter).

These changes could be explained by the degree of muscular work demanded by the standing and rocking position during transport.

In man, the CPK values increase under psychological stress, anxiety, depression, etc. [13] . Since the Thoroughbred horse is a very sensitive and excitable animal, assumption of the existence of similar conditions is warranted.

The changes of GOT and GPT transaminases after transport seem to exclude any damage to the skeletal musculature. In fact, even if we consider that serum values of these enzymes are expressions of the functions of different sections of the animal body, the variations observed are not sufficient to point out any specific functional damage.

After work, the changes proceed at the same constant degree, particularly as they affect the enzymatic activities and the creatinine, thus indicating some muscular work which, however, is different from that required during transportation. Moreover, the alteration of the lactate, pyruvate and creatinine in the two different stages of the experiment give an indication that different metabolic pathways are used to recover energy for the muscle fibres. In fact, the rapid increase of the creatinine blood values after exercise could also be explained as a recovery of the energy necessary to the muscle fibre contractions by the easiest route *i.e.* the creatine phosphate.

During transportation, on the other hand, when muscular work is prolonged, it seems possible to hypothesize that energy is recovered through a slower method, that is lactate and the Krebs cycle. The increase of blood protein during work is probably due to a measure of haemoconcentration [5]. This is not found during transportation and may be a further confirmation of a different kind of body response to different conditions causing fatigue.

Variations in blood glucose levels are more difficult to explain, although the increase (rather small during transportation, higher after the canter) could be grouped under the so called 'general adaptation syndrome' [16], which accounts for a sudden need for glucose because of the demands of muscular work.

The potassium changes could be explained likewise, although we must not forget the quantity of potassium eliminated through perspiration which is quite plentiful in this kind of animal [17]. Phosphorus changes also depend on carbohydrate metabolism; the lower value of glucose and phosphorus after strong muscular work and transportation might confirm this. We already know that inorganic phosphorus changes are associated with the metabolism of glucose [2]. As far as other mineral elements are concerned, only magnesium shows some noteworthy variations and our results agree with statements made by Refsum *et al,* [15] about athletes during performance.

The time of rest (48 hours) available to some subjects after transport, seems sufficient to permit a return to normal haematochemical values. These values are comparable to those recorded by others [16] and ourselves in previous research on race-horses subjected to similar sport activities without previous transportation [5].

From these observations and considerations we conclude that with regard to muscular function (*i.e.,* exercise and transport), the two different circumstances generally act in a similar way on the body of the animals.

It is interesting to note the different methods of metabolic response to the energy requirements necessitated by a moderate but prolonged demand during transport, compared with the sudden intense demand of athletic performance.

Finally it is concluded that it is not advisable to transport animals immediately before a race, even though the distances involved are not great (200-300 km).

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DISCUSSION

J.R. Gillespie: Each line represents samples for how many animals?

D. Codazza: It is the average value obtained from 40 animals; five or six animals were used at a time.

H.H. Krzywanek: What was the running distance and at what speed was it covered?

D. Codazza: It was 1500 metres at a canter (moderate exercise).

D.H.G. Irwin: a) From the practical point of view one would like to know when transporting a horse by road whether it is best for the horse to be standing with his long axis in the direction of travel or across the direction of travel.
b) Surely there will be some optimal size of floor surface with regard to proper support for the horse offered by the sides of the vehicle in which he is transported.

D. Codazza: With his long axis facing in the direction of travel and 1,20 m space allowed for a horse of normal size.

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Chairman: W.L. Jenkins

NORMAL AND ABNORMAL ELECTROLYTE LEVELS IN THE RACING HORSE AND THEIR EFFECT ON PERFORMANCE

H.M. WILLIAMSON*

SUMMARY

To elucidate poor race-track performance in clinically healthy race-horses with normal haematological parameters, normal serum enzyme levels and functionally normal hearts of adequate size (but some with moderate to severe T-wave changes) investigation of their serum electrolyte levels was undertaken. The values for Na^+, K^+, Cl^- and HCO_3^- of 200 winning performers, which fell within narrower limits than those recorded in the literature, served as standard.

The poor performers had various abnormalities of their serum electrolyte levels. Adrenal exhaustion with elevation of serum potassium and depression of sodium, chloride and ultimately of bicarbonate; hypochloraemic alkalosis with elevation of serum potassium; hypokalaemia; hyponatraemia; transient hypernatraemia; and acidosis with or without hyperchloraemia but not associated with adrenal exhaustion were identified. The clinical picture of these disorders is described briefly and the methods of therapy instituted on the basis of the underlying pathological physiology are outlined.

INTRODUCTION

The findings which this paper conveys are the result of observations made routinely upon animals under our care, in a desire to understand the pathology of a particular race-track syndrome. While the resulting observations have proven clinically valid in my practice, I do not claim to have completely elucidated the underlying physiology. This is complex and I have attempted to interpret it by extrapolation from conditions found in man: I know of no literature on the endocrinology of the horse as it applies to the humoral mechanisms controlling hormone production by the adrenal cortex. The findings which I intend to present on the electrolyte levels in the race-horse arose out of a desire to understand better a major problem faced by trainers: the so-called 'Morning Glory' which trains well, or well enough, but fails to produce form, or at least consistent form, under race conditions. This type of horse falls into one of two groups: those which frankly stop; *i.e.* actually get slower as the race progresses; and those which fail to run on; *i.e.*, fail to produce a sprint during the final part of the race, which, under New Zealand conditions, is the fastest part of the race. Haematology, lameness and electrocardiography all supply some of the answers to this problem but we found in our practice that we had a group of sound horses with normal haematological parameters and functionally normal hearts of adequate size but which failed to race well. We also found a group of horses with moderate to serve T-wave changes on electrocardiography, which were frank stoppers. This group had normal total white cell counts and serum enzyme levels; there was no clinical evidence of myocarditis in these cases. It was the investigation of this latter group which first led us to study serum potassium levels, then serum sodium levels and finally serum sodium, potassium, chloride and bicarbonate levels along with our routine haematological examinations. It is now three years since we commenced doing the complete range of tests during which time we have made 1 100 analyses on which our findings are based.

METHODS

Our methods are quite standard. Blood is collected from the jugular vein of the rested horse by means of a 21 gauge, 38 mm, double-ended collecting needle and evacuated glass tube**, a 5 m*l* vial with EDTA as anticoagulant for haematology, and a 10 m*l* vial with sodium heparin as anticoagulant for serology. Sodium and potassium estimations are done by flame photometer, chlorides by diphenyl carbozone titration with mercuric chloride, and the bicarbonate estimations by measurement of total carbon dioxide using the gasometric technique. The latter method was chosen for bicarbonate estimations as it dispensed with the necessity of drawing a further specimen under oil. Measurements were all made in mEq per litre.

As already stated, samples were always drawn from a rested donor to eliminate excitement as a source of error, and serum separation was carried out as soon as was practicable after collection to prevent leakage of electrolytes from within the cellular elements of the blood. Fortunately all the estimations were carried out by the same, highly competent technician, so minimizing the human error.

Samples were collected from both Thoroughbred and Standardbred horses, the latter outnumbering the former. No difference in parameters between breeds are evident in the results.

NORMAL SERUM ELECTROLYTE VALUES

A search of the literature for normal electrolyte values in the equine species left us somewhat bewildered. There was no real paucity of references – our search turned up 12 altogether – but the references to which we had access [3 5 14 16] gave ranges so wide that most of our measurements fell within the normal figure given! Since we are seeking not only the frankly ill horse but also the horse which deviated from its optimum state of health, we set out to ascertain a normal range of electrolyte values for ourselves; these, of necessity, had to fall within fairly narrow limits.

* 83 Evans Street, Timaru, New Zealand.

** Venoject. Jintan-Terumo Co. Ltd. Japan.

Normal levels of each of the electrolytes studied were established by taking the serum findings close to a winning run, *i.e.*, a few days before or after a winning performance, on the assumption that those horses providing the samples must be in good electrolyte balance to perform adequately. The results of samples from 200 winning performers are shown in table 1.

Table 1: NORMAL SERUM ELECTROLYTE LEVELS IN THE HORSE

Author	Na^+	K^+	Cl^-	HCO_3^-
Tasker[17]	132–146	2,4–4,7	99–109	–
Burch[3]	139–152	4,1–5,6	95–107	25–27
Spector[14]	146–152	2,7–3,5	98–106	28
Cosgrove[5]	135–150	4,1–5,6	98–110	
Williamson (winning runs)	139–143	3,7–4,0	100–103	26–28
Mean of Winners	141	3,8	101	27
Range from all analyses	125–149	2,3–7,0	92–108	22–35

When one compares those figures taken from winning horses with the range into which our over-all analyses fell, it is apparent that deviations from normal in the electrolyte levels of race-horses occur in both directions, that is, both excesses and deficiencies of sodium, potassium and chloride occur. The bicarbonate levels indicate race-horses also deviate into both alkalotic and acidotic states. The amounts of the deviation can be marked if the cause remains uncorrected; the effect on performance can be dramatic.

POTASSIUM LEVELS

As already stated, our initial electrolyte analyses were serum potassium estimations made while investigating the cause of T-wave changes in electrocardiographs of horses which were stopping in their races. It might be easiest to commence our discussion at this point. In the analyses which we reviewed for this paper, serum potassium levels ranged from 2,3 mEq/litre to 7,0 mEq/litre. Successful race-track performance was associated only with potassium levels ranging from 3,6 to 4,0 mEq/litre and T-wave changes were consistently seen in those horses whose serum potassium levels exceeded 4,8 mEq/litre. May I respectfully suggest to our cardiologists that here is one possible explanation for the T-wave changes which they see in horses which perform poorly but in which they find no lesions on subsequent investigation.

Adrenal Exhaustion

Horses with potassium levels of this magnitude initially do not display marked clinical signs except for poor race-track performance. When racing, these horses stop; this may occur as late in the race as the 200 metre pole or as early as the half-way mark, depending on the severity of the syndrome. In the early stages of the disease, these horses often baffle trainers by their apparent lack of distress as they do not show respiratory embarrassment and their general condition is good. For this reason these horses are often labelled 'cheats' or 'dogs', or are accused of being cunning and not trying. As the disease progresses, however, loss of condition becomes apparent; the horse becomes a poor sweater, the eye becomes sunken, and there is loss of skin turgor.

Haematological examination reveals haemoconcentration, with elevated haemoglobin, red cell count, and packed cell volume. The sedimentation rate is decreased – commonly to less than 10 millimetres per hour; the mean corpuscular volume is increased, and the eosinphil count is usually elevated. This is the typical hypotonic contraction described by Sykes[15].† Along with the elevated serum potassium level described, there is also a depression of both serum sodium and chloride levels. The sodium level may be depressed to a greater extent than the chloride level, owing to the development of a hyperchloraemic acidosis[9]. As this acidosis increases, the bicarbonate levels become more depressed.

In our work, 94 horses, or 8,5 per cent of cases, showed this clinical syndrome, which, I believe, is that of adrenal insufficiency, or adrenal exhaustion. As seen in the horse, adrenal exhaustion, as far as we can ascertain, is basically a loss of production of aldosterone by the adrenal cortex. The condition is highly responsive to therapy with mineralo-corticoids

†(I think this is also the haemoconcentration problem referred to by Dr Jeffcott.)

Table 2: CASE HISTORY: ADRENAL EXHAUSTION

Date	Record	Date	Record
18.10.71	Raced unplaced	7.2.72	Na 136 K 5,0 Cl 97 HCO_3 25
9.11.71	Raced unplaced	9.2.72	9 / Na salts, DOCA
12.11.71	Raced unplaced	12.2.72	Raced unplaced
17.11.71	Raced unplaced	14.2.72	Raced won
20.11.71	Na 134 K 6,2 Cl 96 HCO_3 25	16.2.72	Raced won
27.11.71	DOCA, 9 / NaCl	20.2.72	4,5 / Na salts
	NaCit, Na HCO_3	23.2.72	Raced unplaced
3.12.71	9 / Na salts	26.2.72	Raced unplaced
10.12.71	9 / Na salts	27.2.72	4,5 / Na salts
17.12.71	4,5 / Na salts	29.2.72	Raced 3rd
23.12.71	4,5 / Na salts DOCA	5.3.72	Na 137 K 4,8 Cl 98 HCO_3 26
	Na 140 K 3,8 Cl 102 HCO_3 27	7.3.72	DOCA, Na salts
1. 1.72	Raced won	13.3.72	Raced 2nd
3. 1.72	Raced won	15.3.72	Raced won
21. 1.72	Raced 4th	16.3.72	Na salts
23. 1.72	Na 137 K 4,4 Cl 99 HCO_3 26	18.3.72	Raced won
30. 1.72	9 / Na salts		

and the horses return to form without the necessity of supplying cortisol or hydrocortisone, which is the main requisite in the therapy of this condition in man[8].

Adrenal exhaustion arises in the horse which is not being allowed sufficient recovery time between stressful experiences. While the whole concept of training is to apply increasingly stressful stimuli to the race-horse, one must do this in a progressive manner, so that the animal passes into the stage of adaptation before facing the challenge of the next progressively stressful level of training. When a very quick progression through the preliminary period of training is made, a horse may develop the hypoaldosteronism which we have described. The onset of insufficiency may on other occasions be traced to a particularly meritorius race-day effort followed by a sudden, or occasionally progressive, loss of form. In these cases the race-day performance has been particularly exhausting and because the horse has not had enough time to recover, his subsequent workouts or races cause diminished aldosterone production and the horse becomes exhausted or 'trains off'.

Aldosterone is the controlling factor which maintains the water, sodium and potassium balances in the animal. In its absence the sodium pump mechanism breaks down, sodium levels increase intracellularly and potassium escapes from the cells into the extracellular fluids. In the absence of aldosterone the ability of the renal tubules to conserve water and sodium is also lost, and dehydration results. This is the cause of the decreased skin turgor and shrunken eye, and a major cause of the weight loss evident as the disease progresses.

Treatment consists of replacing the aldosterone. Aldosterone itself is available commercially but it is expensive and, having a short period of action, must be administered frequently. 9-ϕ fluorohydrocortisone can be given orally; it is the next most powerful mineralo-corticosteroid, but it must be given daily. Desoxycortisone may be given by injection and can be obtained if the form of salts which have a prolonged period of action. The dehydration, too, must be corrected and the hyponatraemia requires the provision of sodium ions, while alkaline radicals such as bicarbonate, lactate or citrate ions should be provided to correct the acidosis. I usually initiate treatment with intravenous saline containing added alkaline anions, and subsequently maintain water and electrolyte intake orally with four to nine litres of a compound drench providing sodium chloride, bicarbonate and citrate. Work is restricted for the first week of therapy.

Routine haematology and serology as an adjunct to training can prevent the occurrence of this syndrome by monitoring the adjustment which the race-horse in training is making.

Hypochloraemic Alkalosis

In our survey we also found another 214 serological evaluations – approximately 19 per cent – which also had serum potassium elevations – but had neither clinical or laboratory evidence of adrenal insufficiency. These cases had serum potassium levels in excess of 4,0 mEq/*l* but only rarely did they exceed 4,8 mEq/*l*. This clinical syndrome was elucidated when we began doing serum chlorides and bicarbonates.

Analysis had shown that the horses which raced best had a bicarbonate level of 26-28 mEq/*l* and a chloride level of 100-103 mEq/*l*. The bicarbonate levels, in horses suffering from this syndrome, however, were always elevated above 29 mEq/*l* and ranged as high as 35mEq/*l*. The horse from which this latter reading was taken was so severely affected that it could not even train without becoming severely distressed. These animals also had depressed sodium and chloride levels, with the chloride level being lowered to a greater degree than the serum sodium value.

These horses characteristically displayed increased depth and rate of respiration for a long time after exercise and are commonly described by trainers as being 'thick-winded' or 'fat inside' [11]. For this reason, they are usually allotted heavier training schedules which have a detrimental effect on the well-being of the horse. If this alkalotic condition presists, the affected horse becomes noticeably hyperexcitable, being easily startled, difficult to handle and wayward to ride or drive in the field. Some Standardbreds become rough-gaited, develop shadow jumping propensities and, under New Zealand's system of standing starts, often develop bad barrier manners and finish on the unruly list.

O'Connor [12] describes a fatigue condition of trail ride horses, which he calls thumps or spasm of the diaphragm and which he considers to be an alkalotic condition with potassium loss. I have seen Standardbreds exhibiting all the signs of the syndrome which O,Connor describes: the stiff attitude, a high pulse-respiration ratio(usually 1,0), and the thumping diaphragm. The severity of the attack and the urgency for treatment has not enabled us to wait for serum electrolyte analysis, but subsequent analysis of samples taken at the time of the attack have revealed serum potassium levels in the 4,1-4,6 mEq/*l* range, bicarbonate levels in excess of 30 mEq/*l* and depression of the serum chloride level to below 97 mEq/*l*. The syndrome described by O'Connor appears to be the final acute attack which these alkalotic horses suffer if continually insulted with the stress of exercise.

It must be borne in mind that the cause of metabolic alkalosis is almost always multi-factorial and that serum potassium levels are not a good indication of the body's total potassium status, since most of the body's potassium is intracellular. It is therefore possible to have elevated serum potassium levels in a horse which is actually potassium deficient, were it possible to measure the intracellular component of its total body potassium [9].

Adrenocorticotropin is secreted by the anterior pituitary gland and stimulates production of cortisol, corticosterone and perhaps desoxycorticosterone by the adrenal cortex. Circulating plasma levels of cortisol and cortisone reaching the anterior pituitary gland in turn regulate ACTH secretion by that endocrine gland. High plasma levels of these two corticosteroids will suppress ACTH production, while low plasma levels will result in enhanced secretion of the tropic hormone. This servo-mechanism maintains circulating plasma cortisol levels within fairly narrow limits except in the presence of stress, when this control is over-ridden. Stressful stimuli reaching the cerebral cortex cause the escape of the hypothalamic centres of the brain from the sustained inhibition of the limbic system under which they normally function. Within these centres large secretory neurons

produce cortiocotropin releasing factor which acts on the anterior pituitary gland to increase ACTH production irrespective of the level of plasma cortisol. Repeated stressful stimuli will therefore result in increased circulating plasma levels of cortisol and corticosterone. High levels of these hormones induce a hypochloraemic alkalosis. In such a state, chloride ions are excreted by the kidney with hydrogen ions as ammonium chloride, and with sodium ions as sodium chloride. Further hydrogen ions will also be transferred from extracellular fluids to intracellular locations which will displace potassium ions from within the cells, raising the levels found extracellularly. This relative hyperkalaemia is further contributed to by the dehydration induced by the loss of sodium and chloride ions, so that the developing potassium deficiency is masked still further [18].

stomach tube at the initial treatment. Depending on the severity of the syndrome, this therapy may have to be repeated several times. Milder cases need only be eased in their work but severe cases must be given a spell of rest at grass to effect recovery. A few days after initiating therapy 15 g potassium chloride is added daily to the feed and the dietary intake of salt is raised to 120 g per day.

Hypokalaemia

Low serum potassium levels were found to be an uncommon condition in this practice, occurring in only 21 instances, which is a mere 2 per cent of the survey. This is not suprising when it is realized that almost all New Zealand horses in training run at pasture for some part of every day. Their dietary intake of

Table 3: CASE HISTORY: HYPOCHLORAEMIC ALKALOSIS

Date	Record	Date	Record
1. 8.73	Na 139 K4,6 Cl 100 HCO_3 30	15. 9.73	Raced 2nd
3. 8.73	Saline and acid	29. 9.73	Raced unplaced
5. 8.73	Saline and acid	4.10.73	Na 141 K 3,9 Cl 102 HCO_3 27
8. 8.73	Saline and acid	26.10.73	Raced won
11. 8.73	Saline and acid	1.11.73	Na 139 K 4,6 Cl 98 HCO_3 29
18. 8.73	Acid saline drench	3.11.73	Saline and acid
25. 8.73	Acid saline drench	10.11.73	Saline and acid
30. 8.73	Na 142 K 3,9 Cl 102 HCO_3 28	13.11.73	Raced won
5. 9.73	Raced won	1.12.73	Raced third

Table 4: A CASE OF THUMPS

Date	Record	Date	Record
	Eleven unplaced performances prior to attack.		
4. 3.71	Na 129 K 4,5 Cl 97 HCO_3 34	26. 8.71	Raced unplaced
4. 3.71	5 / NaCl i/v	2. 9.71	Raced won
	1 + acid	16. 9.71	Raced fourth
6. 3.71	5 / NaCl i/v	9.12.71	Raced 2nd
	1 + acid	13.12.71	Raced 2nd
6. 4.71	Na 139 K 4,0 Cl 99 HCO_3 29	27.12.71	Raced won
10. 4.71	9 / acid mix		11 unplaced performances
17. 4.71	9 / acid mix	3. 3.72	Na 142 K 4,7 Cl 100 $HCO_3$30
10. 8.71	Na 141 K 3,6 Cl 101 HCO_3 28		

Treatment consists of providing adequate chloride ions to overcome the hypochloraemia initiating the syndrome, sodium replacement therapy and a change in training to relieve the stress causing the excessive corticosteroid production. After initiating this therapy, and only after initiation of therapy, it is desirable to increase the horse's potassium intake, to correct the intracellular deficiency of that ion which is produced in the syndrome. A serological analysis made during recovery will usually show a return towards normal levels of the sodium chloride and bicarbonate levels and a change from hyper- to hypokalaemia reflecting the basic potassium deficiency from which the animal is really suffering. This change is, of course, brought about by the return of the potassium to an intracellular location with the correction of the alkalosis. In treating these cases I have followed O'Connor [12] in giving 1 litre of normal saline intravenously to which has been added 1 m*l* of concentrated hydrochloric acid. In addition I always give a further 2 litres of normal saline without acid intravenously and 4 litres of a saline solution by

potassium normally, therefore, is quite high. Cases of hypokalaemia therefore, had to be due to malabsorption or excessive excretion of the cation concerned. As the kidney has no potassium conserving ability, the latter is the more likely situation. The majority of cases were found when doing check tests on horses during therapy for the alkalotic syndrome previously described. Cases of protein deficiency with an accompanying potassium deficiency were also seen and healing was successful only in these cases when concurrent potassium administration was effected along with the protein thearapy. The hypokalaemic state must always be borne in mind, however, as it is likely to arise at any time when large scale intravenous therapy with potassium-free fluids is attempted to correct dehydration. Two cases were also seen in horses mistakenly treated with mineralo-corticoids after wrongfully diagnosing adrenal exhaustion instead of hypochloraemic alkalosis. At the time, I believed serum potassium levels in excess of 4,0 m*Eq/l* to be due to hypoaldosteronism and, not having appreciated the significance of the elevated bicarbonate

level, had treated them accordingly. The initial response was good but form subsequently fell away and hypokalaemia was found when check tests were done. Replacement therapy with oral potassium chloride and ACTH to correct any suppression of the adrenal gland that may have occurred was effective and the horses returned to a satisfactory level of performance.

Therapy of hypokalaemia consists of oral supplementation using potassium chloride, 15 g daily in alkalotic states and potassium citrate when the animal is acidotic. Dextrose is usually administered concurrently to encourage passage of the potassium into cells, and small doses of testosterone or anabolic steroids are given to further enhance potassium uptake. Potassium had been administered intravenously by us only in conjunction with protein hydrolysate in those cases exhibiting hypoproteinaemia.

SODIUM LEVELS

Sodium is the major cation of the extracellular fluid and its concentration in the extracellular fluid determines water distribution between extra- and intracellular locations. In the racing horse hyponatraemia (Table 5) is the most commonly noted electrolyte disturbance. In this survey it was presented in 20 per cent of the estimations done. In 8,5 per cent of these horses it was associated with adrenal exhaustion which we have discussed but a further 11,5 per cent of the 115 cases were not associated with hypoaldosteronism. These horses were suffering from sodium deficiency owing to high sweat loss, insufficient water intake and inadequate amounts of sodium chloride in their diet, with associated dehydration. They showed a progressive weight loss, which, because it was gradual, often went unnoticed until well advanced. The affected horse was usually somewhat depressed and apathetic in demeanour, poorly muscled about the neck, with a shrunken eye, diminished skin turgor and lacking in stamina.

Table 5: CASE HISTORY: HYPONATRAEMIA

Date	Record	Date	Record
30. 9.72	Raced ninth	24.10.72	Na 138 K 3,8 Cl 101 HCO_3 24,5
5.10.72	Na 129 K 4,0 Cl 98 HCO_3 25	25.10.72	4,5 / NaCl Na HCO_3 orally
6.10.72	9 / NaCl $NaHCO_3$ orally	29.10.72	4,5 / NaCl $NaHCO_3$ orally
7.10.72	Raced ninth	30.10.72	Raced sixth
11.10.72	9 / NaCl $NaHCO_3$ orally	1.11.72	Na 140 K 3,8 Cl 101 HCO_3 26
14.10.72	Raced sixth	5.11.72	4,5 / NaCl Na HCO_3 orally
16.10.72	Na 134 K 3,8 Cl 95 HCO_3 22,7	7.11.72	Raced won near record
17.10.72	9 / NaCl Na HCO_3 orally	10.11.72	Raced 2nd near record
20.10.72	9 / NaCl $NaHCO_3$ orally		

Sodium loss induces a reduction in the concentration of this cation in the extracellular fluid. This suppresses the production of antidiuretic hormone so that water excretion by the kidney is increased until the sodium concentration is restored. This has been referred to as the sacrifice of volume in the interests of tonicity [9]. Once volume deficit assumes more significant proportions, however, disproportionate water loss no longer follows, and sodium loss and resultant hyponatraemia are established. Under these conditions there is also movement of water from the extracellular compartment to within the cells, which in turn impairs their function. Water intake is also diminished as the thirst reflex is not maintained in the presence of hyponatraemia, irrespective of severity of the dehydration.

Treatment consists of correcting the sodium and water deficits and any anion imbalance associated with them. By restoring the sodium balance in the extracellular fluids, the horse's willingness to drink will be restored, and the simplest method of administering fluids and electrolytes will once more be available. We have found the oral route to be very satisfactory in treatment of hyponatraemia. It allows the administration of large volumes of fluid quickly and simply. In severe cases we routinely give 9 *l* of solution by stomach tube and repeat the dose as frequently as we consider necessary. Intravenous therapy is also used at the initial treatment, usually in quantities of 4 *l* per administration. The solution used is dependent on the bicarbonate or chloride levels which we find on analysis. If acidosis is present, we administer lactate and citrate along with sodium chloride intravenously, and bicarbonate and citrate with the chloride orally. If alkalosis is present we administer only chlorides intravenously, but add bicarbonates and citrate to the sodium chloride given by stomach tube but in much reduced proportions, An effort is also made to persuade trainers to place large water containers in each stall or yard so that the horse has free access to a plentiful supply of water and the dietary intake of salts is raised to 120 g daily or 1 per cent of the ration.

Elevation of serum sodium levels was only rarely seen in these cases under review and was transient in nature as far as we could ascertain.

Of our survey, 45 cases or 4,1 per cent, had low bicarbonate levels not associated with adrenal exhaustion in which it is commonly seen; 35 cases had an accompanying serum chloride elevation or at least elevation of chlorides relative to sodium ions, indicating hyperchloraemic acidosis probably owing to reduced kidney function in the presence of dehydration. Of the horses under review 1 per cent did not show elevation of chlorides and these may have been cases of massive lactic acid production from excessive effort. Whatever the cause, the condition responds readily to therapy with bicarbonate, citrate or lactate given orally or intravenously.

CONCLUSION

I hope I have been able to show that there are at least three quite distinct clinical entities which result from, or produce imbalance of, electrolytes in the race-horse. From this I believe it is evident, too, that one cannot haphazardly administer electrolytes to the race-horse but must base therapy on precise laboratory estimation of the electrolyte status of the patient.

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DISCUSSION

A.M. Meritt: Have you seen the 'thumps' in some of the horses you described as alkalotic?

H.M. Williamson: I have seen cases in standard-bred horses which resemble the thumping which O'Connor described. We took serums from them because O'Connor's A.F.E.P. paper suggests that it might be a hypokalaemic condition but he did not actually present any figures to support this. When we saw these cases we took a serum sample before treating them but I cannot project the figures clearly enough here. See table 4.

A.M. Merritt: Did you also perhaps run calcium determinations? The only time we have seen 'thumps' was in cases with hypocalcaemia. We have seen numerous animals with a marked hypokalaemia and yet never a sign of 'thumping', yet we have had a couple of cases where 'thumping' and persistent hypocalcaemia were presented. When they become very hypocalcaemic, one of the outstanding signs was the 'thumping' of the diaphragm. When treated with calcium, the 'thumping' stopped. I wondered if you had also investigated calcium.

H.M. Williamson: I did not mention serum calcium but maybe the calcium ions are upset owing to the alkalosis. Horses with alkalosis not severe enough to go into 'thumps' become very bad race-horses. They shadow jump badly, they seem to be very nervous and are bad goers and performers. Such horses gallop away hopelesly from standing starts and are hard to handle. It may be that there is some tie-up of calcium ions or some conditions like that. We did not do calcium analyses. We certainly saw that condition but I would not say there were marked potassium elevations. I would say these were in the range of 4 mEq or more but under 4,8. I think once one gets into the high realms over 4,8 one runs into the adrenal exhaustion condition.

A.M. Merritt: You made a statement that the horse cannot conserve potassium. Maybe I misunderstood the statement, but if that is what you said, could you support it?

H.M. Williamson: I did not mention the horse as such; I said the physiology underlying this is extrapolated basically from hyman physiology and the kidney cannot conserve potassium.

P. Boyazoglu: Would you express any preference regarding the desirability of administering electrolytes in drinking water or in feed to correct deficiencies?

H.M. Williamson: I did not mention the horse as such; I said the imbalances and to maintain electrolyte levels one probably does better by feeding them. The racing economy of New Zealand is not as strong as the racing economy in other countries, so that many horses which we in New Zealand see as practice are not being looked after by having routine biochemistry done on them. Often we are only called in when the horse is presented as a case. Under these circumstances one has to administer electrolytes in such a way as to get reasonably large doses into the animals quickly. I think you can then maintain them by giving electrolytes in the feed or water.

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GLYCOGEN DEPLETION PATTERN AND THE BIOCHEMICAL RESPONSE TO VARYING EXERCISE INTENSITIES IN STANDARDBRED TROTTERS

A. LINDHOLM*

SUMMARY

In Standard-bred Trotters glycogen depletion was slight [0,3mmolx(kg x min)$^{-1}$] at slow trotting speed (3 min 20 s x km $^{-1}$)but rose to nearby 14mmol x (kg x min) $^{-1}$ at maximal trotting speed (1 min 20 x km $^{-1}$) for 6 x 400 m. At slow speeds predominantly the 'slow twitch' fibres became depleted and, once that had occurred, the 'fast twitch, high oxidative' fibres also became subject to depletion. During maximal speed, after 6 x 400 m, the 'fast twitch, low oxidative' fibres became strikingly depleted as well. There is thus a preferential fibre recruitment with increase in speed and duration of exercise.

Lactate accumulation in the blood did not increase strikingly until speeds exceeded 10m x s $^{-1}$.

Repeated 700 – 1 000 m exercise sessions at maximum to near-maximum speed, respectively, appears to be appropriate for training of fast twitch fibres, as well as for achieving a high heart rate and a high demand on anaerobic energy release.

INTRODUCTION

Both during racing and training, horses are subjected to severe stresses. Therefore, training methods are required which are both efficient and minimally stressful to the muscles, joints and skeleton of the horse. For the development of such training methods a detailed study of the adaptation of muscles to heavy work is required. This paper condenses previous publications by the author and co-workers [4 5 6].

MATERIAL AND METHODS

Sixty-eight clinically healthy Standardbred trotters (0,5-8 years old) were investigated. Horses older than two years were in professional training at Solvalla Race Track, Stockholm, Sweden.

Determinations were performed on muscle samples, taken mainly from the gluteus medius muscle and obtained using a needle biopsy technique [1].

Biochemical analyses were performed on muscle samples frozen in liquid nitrogen immediately after sampling. A modified Lowry technique [3] was used for biochemical investigations of glycogen and related metabolites.

Two different histochemical staining procedures were used for the identification of muscle fibres. Alkaline-stable myofibrillar adenosine triphosphatase (ATP-ase) and reduced nicotinamide dinucleotide diaphorase (NADH-diaphorase) were estimated as described by Padykula & Herman [8] and Novikoff *et al.* [7], respectively. The distribution of glycogen was estimated on the basis of PAS staining [9]. Blood from the jugular vein was analyzed for lactate, free fatty acids (FFA) and glucose according to Scholz *et al.* [10], Trout, Estes & Friedberg [11] and Hjelm & de Verdier [2], respectively.

Muscle temperature was determined using electrothermometry and the heart rate was telemetrically recorded.

RESULTS

Muscle Fibre Composition

Three major fibre types were identified. Fibres displaying high ATP-ase staining intensity were designated 'fast twitch', whereas fibres possessing a low level of staining intensity were designated 'slow twitch' (ST). Fast twitch fibres were stained either lightly or heavily for NADH-diaphorase. The latter was termed 'fast twitch, high oxidative' (FTH), whereas the former was termed 'fast twitch, low oxidative' (FT) fibre.

The 4 to 8-year-old horse displayed a fibre composition consisting of 22 per cent FT, 54 per cent FTH and 24 percent ST fibres. The size of all fibres increased with age and/or training and, in the mature horse, FT and FTH fibres comprised about 43 per cent of the total cross-sectional area, respectively.

Pattern of Glycogen Depletion in Muscle Fibres

Preferential fibre recruitment was according to speed and work duration. At a slow trotting speed (3 min 20 s x km $^{-1}$) horses became exhausted after 4 hours. Glycogen depletion at this speed was slight [0,3 mmol x (kg x min)$^{-1}$] and muscle lactate did not exceed 1,6 mmol x kg^{-1}.

At maximal trotting speed (1 min 20 s x km^{-1}) for 6 x 400 m, muscle lactate amounted to 10 mmol x kg^{-1} and horses tired after 3 min of exercise. Glycogen depletion amounted to nearly 14 mmol x (kg x min)$^{-1}$.

The present data indicate that predominantly ST fibres were depleted of glycogen at slow trotting speeds. Once some ST fibres were depleted for glycogen, FTH fibres also began to be depleted. During maximal speed, however, FT fibres were strikingly depleted of glycogen and after 6 x 400 m of maximal trotting, the reduction in the PAS staining intensity was as pronounced in FT as in FTH and ST fibres. The present results indicate that ST fibres were most active at slow trotting speeds, but FTH fibres became active when ST fibres were depleted or speed increased successively. During maximal trotting, the glycogen depletion indicated that FT fibres were the most active.

Metabolic Response to Exercise

Heart rate: It was found that at high exercise intensity, both the speed and duration of the trotting were of importance to the increase in heart rate. A maximal heart rate (± 240 beats x min^{-1}) was obtained in trotting at top speed (12,1-12,5 m x s^{-1}) for at least 700 m or in 1 000 m or more of trotting at close to maximal speed (+ 11,8 m x s^{-1}).

Muscle and rectal temperatures displayed a linear increase with increasing speed and work duration.

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Values as high as 43°C and 41°C, respectively, were recorded after repeated 700-1 000 m interval training sessions.

Lactate accumulation: When trotting was performed at a gradually increasing speed, lactate accumulation in the blood did not increase strikingly until speeds exceeded 10 x s^{-1}. During a normal training program (3 x 2 100 m) muscle and blood lactate concentrations comparable to those obtained during racing were achieved only during the third 2 100 m work-out. Trotting work amounting to 6 x 400 m at maximal speed (12,1 m x s^{-1}) was found to be too short a distance to elicit a good metabolic response and muscle lactate amounted to about 13 mmol x kg^{-1}. Repeated 700 m exercise sessions at maximal speed and repeated 1 000 m sessions at near-maximal speed appeared to constitute a suitable distance range for interval training for the horse. Muscle and blood lactate concentrations exceeded 20 mmol x kg^{-1} and l^{-1}, respectively, in both distances. Glycogen utilization was found to be directly related to work intensity. The greatest decrease in glycogen levels (20 mmol glucose units x kg^{-1} x min^{-1}) was observed in the first two heats when trotting at maximal speed – 6 x 700 m.

CONCLUSIONS

The importance of exercise intensity in the recruitment of FT fibres was clearly demonstrated in this study; it is obvious that maximal speed is required in order to bring most FT fibres into use.

The results also indicate that repeated 700-1 000 m work bouts (11,8-12,1 m x s^{-1}) is appropriate for training of the fast twitch fibres, as well as for achieving a high heart rate and a high demand on anaerobic energy release.

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DISCUSSION

S.G.B. Persson: Which pathway do you think is the most important for energy production in the horse during exercise and, further, do you think that glucose concentration is an indication of the pathway involved?

A. Lindholm: At these distances the horse is trotted for about 3 to 4 minutes. It is only carbohydrates which are utilized. During the four hour experiment they all produce free fatty acids in the metabolism. This is at a very low speed and as soon as the speed increases utilization of fat decreases and that of carbohydrates increases. In the resting condition the horse uses mostly fat. To transport a horse he uses mostly fat and not so much glycogen. As soon as the speed increases, there ia a higher demand on the carbohydrate. About the glucose concentration in the blood, if there is a high glycogen concentration delivered and a high concentration in the muscle, it does not matter what there is in the blood, because the fuel is in the muscles and if the muscles need more fuel, then they take it from the liver. The liver is the reserve for the muscle; so, during exercise, there is a build-up and break-down of glycogen all the time. In my four hour experiment the liver could supply the muscle glycogen for two hours, but after that time the liver was depleted and the glucose concentration also dropped. But just for work it is only the glycogen in the muscle which is important.

H.M. Williamson: You stated that in your examination of horses they were being trained by the executive training technique in your country, that is they were only trained twice a day. Under New Zealand and Australian conditions they are normally trained by fast work three times a day. I wonder if you have examined horses which had more frequent periods of training in fast paces than what is the normal situation in your country. If you have not, would you care to comment in any way on whether this could increase fitness as against the twice a week regimen.

A. Lindholm: If one waits two hours after working at maximum speed, the lactate is down to zero, but it takes about two hours. If one wants to train an animal twice within these two hours, one starts with a higher lactate level and one obtains a higher lactate value. If one trains them three times a day at longer than two hours intervals, one has the same situation as one would when training once a day. Because one can never deplete the muscles of glycogen, there is still a considerable amount of glycogen left in the muscle, even if the horse is exhausted.

H.M. Williamson: I think I have perhaps not expressed myself clearly, although I do appreciate the information that you are after. meaning a greater number of times per week rather than more frequently per day.

A. Lindholm: It is hard to say which is the best, because one cannot measure the oxygen uptake and there is nothing relevant to go by. In a few years' time we might be able to answer this question. Much more experimentation is required before we can say anything about it.

H.H. Krzywanek: I wonder whether there are always small differences between lactic acid concentration in muscle and in blood at the end of the competition. I think in humans measurements have been done and considerable differences have been found.

A. Lindholm: That is right. This is a problem, because in humans one can put a needle into the thigh of the experimental cases at once as soon as exercise stops, but a horse has to be taken to a quiet place which takes 30 seconds. That is why one gets somewhat lower values. They would be higher if one could sit on the horse and take the biopsy immediately.

G.F. Freign: I would like to compliment you on a most interesting paper. I am sure we all enjoyed it. Could you throw further light on the ratio between slow twitch and fast twitch muscle fibres. Does the ratio remain the same irrespective of age or is it modified in any way by training or use?

A. Lindholm: I showed you one slide in which a slight increase of the first twitch, high-oxidative fibres is apparent. The slow twitch fibres, however, remain the same from foals to adult horses, so the tendency is only a change from the fast twitch, low-oxidative to fast twitch, high-oxidative fibres. I do not believe that this is a transformation, because one may have some fast twitch, high-oxidative fibres in the untrained animal, but which upon training turn out to be more highly oxidative. That is why one sees little difference. The difference is significant but not highly so.

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BLOOD GASES, ACID-BASE BALANCE AND ELECTROLYTE AND ENZYME CHANGES IN EXERCISING HORSES*

D.W. MILNE**

SUMMARY

A study consisting of three separate experiments was carried out to evaluate blood gases, acid-base balance, electrolytes and enzyme changes in exercising horses. In the first experiment, comparisons were made between arterial and venous pH, PO_2, PCO_2 and HCO_3^- in unconditioned horses at rest, after two submaximal work loads and 15 minutes after completion of the heavier work load. The blood samples were obtained from the carotid artery and jugular vein by direct puncture within two minutes of the completion of each exercise period. There was no change in acid-base balance during moderate work but all horses developed a partially compensated metabolic acidosis during heavy work. The changes in acid-base parameters were still present 15 minutes after completion of the heavy work but were returning towards pre-exercise resting levels. The PaO_2 decreased during moderate work but was normal during heavy work. The O_2 carrying capacity and O_2 content of both arterial and venous blood increased during both submaximal work rates. There was a linear relation between the changes in arterial and venous pH, PCO_2 and HCO_3^-. Arterial pH, PCO_2 and HCO_3^- could be predicted accurately from a knowledge of venous blood during exercise in these horses. The PCO_2 and HCO_3^- of the venous blood were found to be consistently slightly higher and venous pH consistently lower than arterial blood. PaO_2 could not be accurately predicted from venous blood. Serum calcium increased significantly during heavy exercise but there was no significant change in serum potasium. Creatine phosphokinase (CPK) and serum glutamic oxalotransaminase (SGOT) increased significantly on completion of exercise.

In the second experiment, arterial, venous and mixed venous pH, PO_2, PCO_2 and HCO_3^- were measured in unconditioned horses at rest and after an anaerobic submaximal work load. The $(a-vO_2)$ of the horses after exercise at 700m/min increased two times over the resting levels.

In the third experiment, venous blood gases, ph, HCO_3^- and serum electrolytes were determined on sixteen horses before, during and after an 80 km endurance ride. The PCO_2 and bicarbonate were significantly decreased at the completion of the ride. The pH was significantly elevated at the middle of the ride. No significant change in venous PO_2, serum potasium or calcium was found during or after the event.

INTRODUCTION

Blood gas and acid-base status have been incompletely evaluated in the horse during exercise, although they have been studied extensively in man. Changes in acid-base balance and blood gases during exercise vary among different species. Respiratory alkalosis occurs in dogs performing exhaustive work [30 39]. In man, during strenuous work, metabolic acidosis occurs [1 3]. Engelhardt [8] and Krzywanek [19 20] found that arduous exercise will also cause metabolic acidosis in the horse. Most of these studies have utilized short exercise periods of submaximal or maximal work.

Numerous studies have evaluated changes in serum electrolytes in horses during endurance events. Serum potassium and calcium concentrations have been shown to decrease in endurance horses [23 24]. Few studies evaluating electrolyte changes in short duration exercise have been made in the horse. Sreter [36] found a significant increase in serum calcium and potassium in Thoroughbreds immediately following a one-to-three minute race (900–1 000m/min).

Changes in serum muscle enzymes have been studied in the horse. Cornelius [6] and Cardinet [5] found significant increases in serum glutamic oxalotransaminase (SGOT) after strenuous exercise in horses. Reithmuller [31] found an increase in resting serum SGOT, creatine phosphokinase (CPK) and lactic dehydrogenase (LDH) over a seven-month training period.

The purpose of this study was to evaluate the changes in acid-base balance and blood gases in exercising horses and to determine the accuracy of estimating arterial blood gases, pH and HCO_3 from venous blood during normal resting conditions, submaximal work and recovery from exercise. In addition, we wished to determine the arterial-venous O_2 difference $(a\text{-}vO_2)$ and the approximate area of anaerobic threshold in unconditioned horses, and to compare the changes in blood gases and acid-base balance in unconditioned horses and endurance horses.

This paper also presents the effects of different types of exercise on serum potassium and calcium and the effects of short-duration exercise on serum SGOT and CPK concentrations.

MATERIALS AND METHODS

The study was divided into three separate experiments.

1. In the first experiment, measurements were made on six mature healthy horses: one Thoroughbred and one Quarterhorse stallion; one Thoroughbred and one Arabian gelding; one Standardbred and one Quarterhorse mare, all of which were sedentary and had not been in training for at least six months. The mean mass and height of the six horses was 460 kg and 160 cm, respectively. Blood samples were drawn from the carotid artery and jugular vein by direct puncture. Heparinized samples were drawn anaerobically for determination of blood gases, pH and HCO_3^- by a blood gas analyzer.† Samples for venous lactic acid were drawn into oxalate tubes, placed in an ice bath and deproteinized with 0,6 mol perchloric acid. Lactic acid was determined by an anzymatic method [22 25]††. An ethylene diaminetetracetate-trea-

*In the unavoidable absence of the author, this paper was presented by Dr G.F. Fregin.

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†Corning No. 165 Blood Gas Analyser: Scientific Instruments, Mansfield, Mass.

††Eskalab: Smith Kline Inst., Palo Alto, California.

ted blood sample was drawn for the determination of packed cell volume (PCV) and total solids (TS). Serum total solids was determined by refractometry and the PVC by microhaematocrit technique. A clotted sample was obtained for determination of serum potassium and calcium, and serum SGOT and CPK. Serum potassium and calcium were measured with an atomic absorption spectrophotometer. The plasma activity of CPK and SGOT was determined spectrophotometrically by the Rosalki method [32] and Karmen method [17], respectively. Commercial reagents for blood determinations were utilized.†††

Resting blood samples were drawn while the horses were standing quietly in their stalls. Each horse was then worked under saddle at a rate of 350m/min for 7 km (20 min). The horses were allowed to rest for 30 minutes and then were worked at a rate of 700m/min over a distance of 3,5 km (5 min). The total mass of rider and saddle was 53 kg. All the samples were drawn within two minutes of completion of the exercise periods and 15 minutes after completion of the heavier exercise. Blood gases, pH, HCO_3^-, lactic acid, PCV and TS were determined immediately after sampling. The clotted samples were placed into a water bath for ten minutes at 37°C, the blood centrifuged and serum removed and frozen at -65°C for determination of electrolytes and serum muscle enzymes at a later date.

Blood gases, pH and HCO_3^- were analyzed by three-way analysis of variance for repeated measure, followed by a Student Newman Keul's Test when the "F" statistic was significant [35]. The level of significance for differences in sample values was determined for the location (location = either arterial or venous blood samples) and time (time = rest, 350m/min, 700m/min or 15 minutes post-exercise) (Figs 1-4). Lactic acid, PCV, TS, Ca++, K+, SGOT and CPK were analyzed by two-way analysis of variance for repeated measures followed by a Student Newman Keul's Test when the "F" statistic were significant (Figs 5-10).

2. In the second experiment, measurements were made on four mature healthy horses, 1 Thoroughbred stallion, 1 Thoroughbred gelding, 1 Standardbred mare, and 1 Quarterhorse mare, all of which were sedentary for two months. The mean mass and height of the four horses was 460 kg and 160 cm, respectively. Two of the horses had exteriorized carotid arteries to facilitate sampling for arterial blood.

   Prior to the exercise period, each horse was catheterized with a 110 cm Swan-Ganz flow directed balloon catheter°. The catheter was placed into the right jugular vein, the balloon inflated and the catheter passed into the right ventricle and jugular vein. Heparinized samples were position of the catheter tip in the right ventricle was monitored by pressure tracings using an oscilloscope°° and the pressure transducer°°°. The catheter was sutured securely to the horse. Resting blood samples were drawn while the horses were standing quietly. Samples were drawn simultaneously from the carotid artery, right ventricle and jugular vein. Heparinized samples were drawn anaeorobically for the determination of blood gases, pH and HCO_3^-. Each horse was then worked under saddle at a rate of 700 m/min for 3,5 km (5 min). Carotid, jugular and right ventricle samples were taken within two minutes of completion of the exercise. The mean values of the four horses were determined.

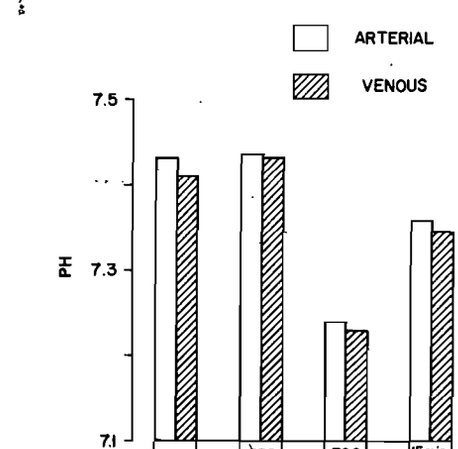
3. In the third experiment, measurements were made on 16 variably-conditioned endurance horses, which included Arabians, Quarter Horses and Thoroughbreds. The environmental conditions were moderate for the day of the ride. The temperature was 22°C and the relative humidity was 65 per cent. The evening before, in the middle and at the completion of an 80 km ride, venous blood samples were obtained. Heparinized samples were drawn for analysis of pH, PO_2, PCO_2 and HCO_3^- with a blood gas analyzer. An ethylene diaminetetracetate sample and a clotted sample were drawn for determination of PCV, serum TS and potassium and calcium, respectively. Serum calcium and potassium were measured by an atomic absorption spectrophotometer. Serum total solids was determined by refractometry and the PCV by microhaematocrit technique.

   The data were statistically analyzed by a two-way analysis of variance for repeated measures. When the "F" statistic was significant, the data were followed by a Student Newman Keul's Test.

RESULTS

Experiment 1

During rest and exercise, there was no significant difference between arterial and venous pH (Fig. 1). The venous pH values were consistently lower than arterial pH, the average difference being 0,01 units.



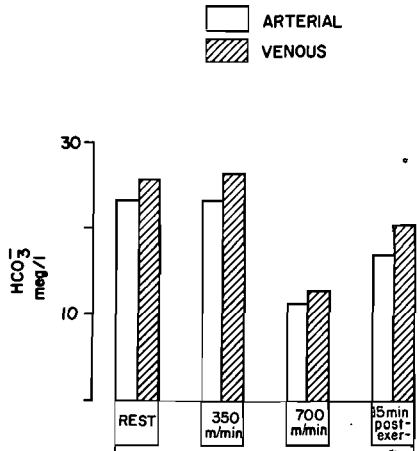
3 WAY ANALYSIS OF VARIANCE * (REPEATED MEASURES DESIGN)

SOURCE OF VARIATION	df	F	LEVEL OF SIGNIFICANCE
LOCATION	1	0.23	NOT SIG.
ANIMALS	5	–	–
TIME	3	26.85	99%
TIME VS. LOCATION	3	0.114	NOT SIG.
RESIDUAL ERROR	35		

Figure 1 - Arterial and venous pH from the carotid and jugular vein of unconditioned horses performing two submaximal work loads. Statistics* df = degrees of freedom.

†††Eskalab: Smith Kline Inst., Palo Alto, California.

°Edwards Laboratories, Santa Ana, California.
°°Datascope No. 860; Datascope Corporation, Paramus, N.J.
°°°Transducer P23Db; Datascope Corporation, Paramus, N.J.

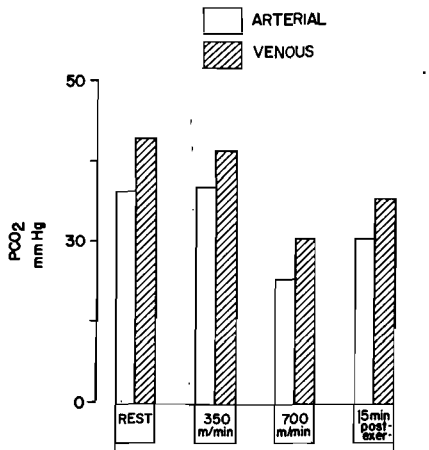


3 WAY ANALYSIS OF VARIANCE* (REPEATED MEASURES DESIGN)

SOURCE OF VARIATION	df	F	LEVEL OF SIGNIFICANCE
LOCATION	1	7.40	99%
ANIMALS	5	–	–
TIME	3	42.53	99%
TIME VS. LOCATION	3	0.081	NOT SIG.
RESIDUAL ERROR	35		

Figure 2 - Arterial and venous HCO_3^- from the carotid artery and jugular vein of unconditioned horses performing two submaximal work loads. Statistics* df = degrees of freedom.

There was a significant difference between arterial and venous HCO_3^- and between arterial and venous PCO_2 (Figs 2,3). The resting and working venous HCO_3^-, was consistently slightly higher than arterial HCO_3^-, the average difference being 2,47 mE/*l*. The rest and working venous PCO_2 were consistently slightly higher than arterial PCO_2, the average



3 WAY ANALYSIS OF VARIANCE* (REPEATED MEASURES DESIGN)

SOURCE OF VARIATION	df	F	LEVEL OF SIGNIFICANCE
LOCATION	1	25.87	99%
ANIMALS	5	–	–
TIME	3	29.20	99%
TIME VS. LOCATION	3	0.245	NOT SIG.
RESIDUAL ERROR	35		

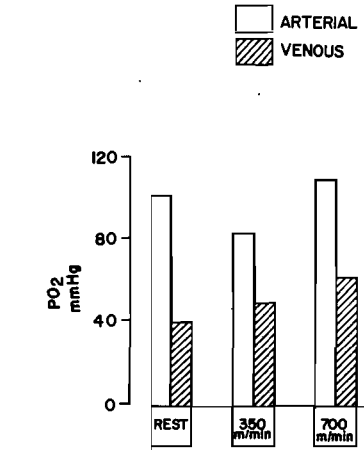
Figure 3 - Arterial and venous PCO_2 from the carotid artery and jugular vein of unconditioned horses performing two submaximal work loads. Statistics * df = degrees of freedom.

difference being 6,99 x 10^2Pa (5,24 mm Hg). There was no significant difference in pH, HCO_3^- or PCO_2 with respect to time *versus* location (Figs 1, 2, 3). The pH, HCO_3^- and PCO_2 of venous blood at each time (rest, 350m/min, 700m/min and 15 minutes post-exercise) consistently reflected the changes in arterial values.

The pH, HCO_3^- and PCO_2 were significantly different with respect to time (Figs 1, 2, 3). The pH, HCO_3^- and PCO_2 did not change significantly at moderate work (350m/min) but decreased significantly at heavy work (700m/min) and had begun a significant return towards normal by 15 minutes post-exercise.

Arterial oxygen (PaO_2) and venous oxygen (PvO_2) were significantly different (Fig. 4). The resting and working PvO_2 values were consistently lower than PaO_2. There was also a significant difference in PO_2 with respect to time *versus* location, *i.e.*, the PvO_2 did not reflect the changes in PaO_2.

The PaO_2 and PvO_2 were significantly different with respect to time (Fig. 4) The resting and working PvO_2 did not reflect the changes in PaO_2. The PaO_2 decreased significantly at a submaximal work load of 350m/min but the PvO_2 values did not change significantly. The PvO_2 increased significantly at 700 m/min and had begun to decrease by 15 minutes post-exercise, but was still significantly elevated over resting levels.



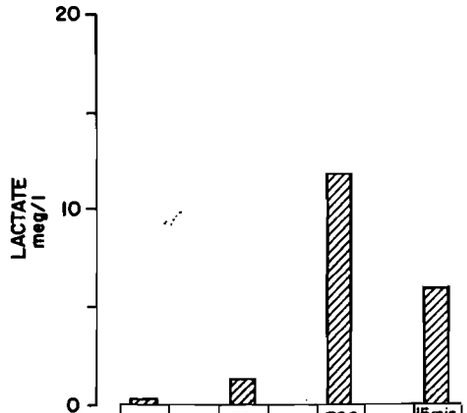
3 WAY ANALYSIS OF VARIANCE* (REPEATED MEASURES DESIGN)

SOURCE OF VARIATION	df	F	LEVEL OF SIGNIFICANCE
LOCATION	1	618.61	99%
ANIMALS	5	–	–
TIME	3	10.94	99%
TIME VS. LOCATION	3	3.63	95%
RESIDUAL ERROR	35		

Figure 4 - Arterial and venous PO_2 from the carotid artery and jugular vein of unconditioned horses performing two submaximal work loads. Statistics* df = degrees of freedom.

The lactate concentration in venous blood was significantly different with respect to time (Fig. 5). Venous lactic acid concentration did not change significantly during moderate work but had increased significantly at 700m/min and had begun to decrease by 15 minutes post-exercise but had not reached the normal resting values.

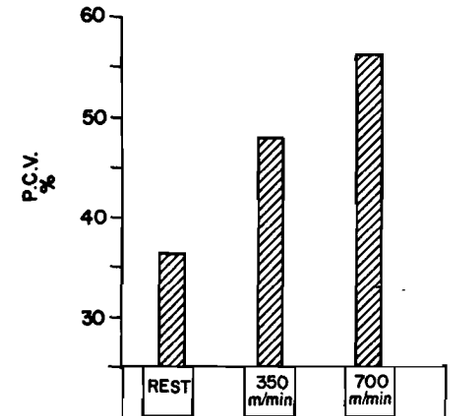
The PCV and TS were significantly elevated in venous blood at both moderate and heavy work (Figs 6,7). Compared to resting levels, the PCV rose 32 per cent at a work rate of 350m/min and 55 per cent at a work rate of 700m/min. The total solids rose 6 per cent during moderate work and 16 per cent during



2 WAY ANALYSIS OF VARIANCE * (REPEATED MEASURES DESIGN)

SOURCE OF VARIATION	df	F	LEVEL OF SIGNIFICANCE
TOTAL	23	–	–
ANIMALS	5	–	–
TIME	3	14.07	99%
ERROR	15		

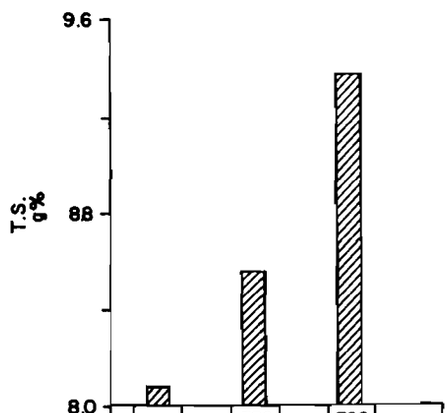
Figure 5 - Lactate from venous blood of unconditioned horses at two submaximal work loads. Statistics * df = degrees of freedom.



2 WAY ANALYSIS OF VARIANCE * (REPEATED MEASURES DESIGN)

SOURCE OF VARIATION	df	F	LEVEL OF SIGNIFICANCE
TOTAL	17	–	–
ANIMALS	5	–	–
TIME	2	18.34	99%
ERROR	10		

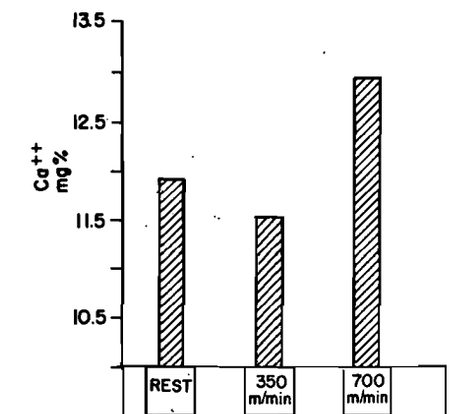
Figure 7 - P. C. V. from venous blood of unconditioned horses at two submaximal work loads. Statistics * df =degrees of freedom.



2 WAY ANALYSIS OF VARIANCE * (REPEATED MEASURES DESIGN)

SOURCE OF VARIATION	df	F	LEVEL OF SIGNIFICANCE
TOTAL	17	–	–
ANIMALS	5	–	–
TIME	2	25.43	99%
ERROR	10		

Figure 6 - Total solids from venous blood of horses at two submaximal work loads. Statistics * df = degrees of freedom.



2 WAY ANALYSIS OF VARIANCE * (REPEATED MEASURES DESIGN)

SOURCE OF VARIATION	df	F	LEVEL OF SIGNIFICANCE
TOTAL	17	–	–
ANIMALS	5	–	–
TIME	2	7.21	95%
ERROR	10		

Figure 8 - Calcium from venous blood of unconditioned horses at two submaximal work loads. Statistics * df = degrees of freedom.

Table 1: PHYSIOLOGIC DATA OBTAINED AT REST, AFTER 350m/min AND 700m/min IN UNCONDITIONED HORSES

($\bar{X}$ of Six Horses)

Condition	PCV %	Hb g %	O_2 Capacity Vol. %	pH	PO_2 mm Hg *	% Saturation	O_2 Content Vol. %
Rest $\bar{X}$	36,6	12,2	16,35	7,417	Arterial 102	97%	15,86
					Venous 40	75%	12,26
350 m/min $\bar{X}$	48,3	16,1	21,57	7,428	Arterial 93	95%	20,49
					Venous 49	81%	17,47
700 m/min $\bar{X}$	56,5	18,8	25,19	7,231	Arterial 109	95%	23,93
					Venous 62	85	21,41

* = 1,33 x 10^2 Pa

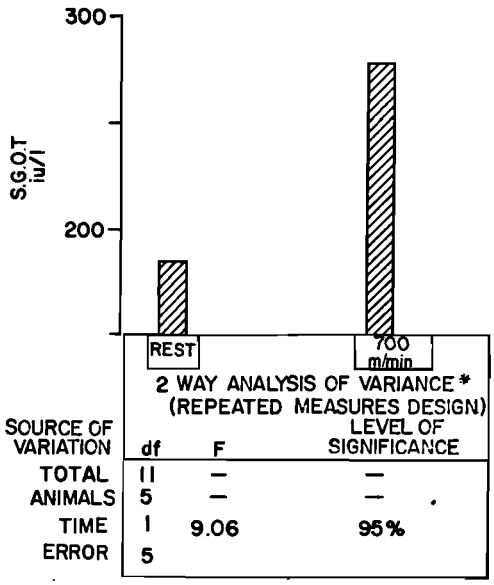


Figure 9 - S. G. O. T. from venous blood of unconditioned horses after exercise. Statistics * df = degrees of freedom.

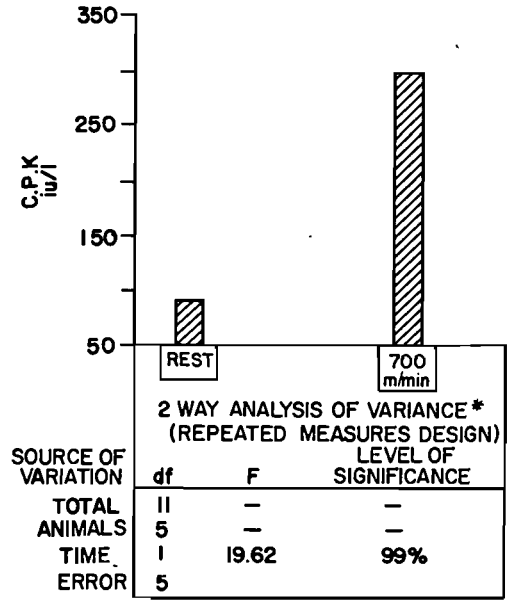


Figure 10 - C. P. K. from venous blood of unconditioned horses after exercise. Statistics * df = degrees of freedom.

heavy work. This rise in PCV and TS resulted in an increase in oxygen carrying capacity of the blood (Table 1).

The serum muscle enzymes were significantly elevated on completion of exercise (Figs 9,10). The SGOT rose 50 per cent and the CPK 227 per cent over the resting levels. Serum calcium rose significantly (13 per cent) after the heavy work but there was no significant change in serum potassium (Fig. 8).

Experiment 2

The pH and HCO_3^- decreased in arterial, venous and mixed venous blood at the heavy work load (700m/min) (Figs 11, 13). The PCO_2 fell in arterial and venous blood but was unchanged in the mixed venous blood after exercise (Fig. 12). The PO_2 increased in venous blood but showed little change in arterial and mixed venous blood over the resting levels (Fig. 14). The (a-vO_2) was calculated using one third the packed cell volume as a measure of the haemoglobin concentration. The (a-vO_2) increased with exercise (Fig. 15). The mean (a-vO_2) of the four horses was 36 m*l*/*l* at rest and 74 m*l*/*l* after exercise at 700m/min.

Experiment 3

Venous PCO_2 and HCO_3^- were significantly decreased at the completion of the ride with no associated change of pH (Table 2). The pH, however, was significantly increased at the middle of the ride. There was no significant difference in blood gases, pH or bicarbonate between those horses that completed the ride in the fastest time and those horses that completed it in the slowest time. The horse with the fastest time, subject 7A, completed the ride in 6,5 hours (200m/min) while the subject with the slowest time (29A) completed the ride in 10,5 hours (130 m/min). They both exhibited an increase in pH and a decrease in PCO_2 at the middle of the ride and a decrease in PCO_2 and HCO_3^- on completion of the

Table 2: BLOOD GASES, pH, HCO_3-CHANGES IN HORSES DURING AN 80 KM ENDURANCE RIDE

	pH			PO_2 mm Hg*			PCO_2 mm Hg*			HCO_3- mEq//		
	Pre-	Mid-	Post-	Pre-	Mid-	Post-	Pre-	Mid-	Post-	Pre-	Mid-	Post-
$\bar{X}$	7,406	7,434	7,406	36,8	39,6	40,3	43,3	41,0	35,5	26,2	26,5	21,7
±S.E.M.				± 4,1	± 4,2	± 5,0	± 3,4	± 4,2	± 3,1	± 1,8	± 2,9	± 2,9
	TWO WAY ANALYSIS OF VARIANCE FOR REPEATED MEASURES FOLLOWED BY STUDENT NEWMAN KEUL'S TEST WHEN F IS SIGNIFICANT											
F (2,30)	4,35			2,60			17,53			18,08		
	Mid. separate group from Pre. & Post.			Not Significant			Post. separate group from Pre. & Mid.			Post. separate group from Pre. & Mid.		
	P < 0,05						P < 0,01			P < 0,01		
	Critical value (F(2,30) 95% 3,32 99% 5,39											

* = 1,33 x 10^2 Pa

Table 3: P.C.V., T.S., Ca++ AND K+ CHANGES IN HORSES DURING AN 80 km ENDURANCE RIDE

	PCV %			T.S. g %			Ca++ mg %		K+ mEq//	
	Pre-	Mid-	Post-	Pre-	Mid-	Post-	Pre-	Post-	Pre-	Post-
$\bar{X}$	36	43	44	8,45	8,75	8,87	12,74	12,42	4,38	3,85
±S.E.M.	±2,44	±4,66	±6,17	± 0,45	± 0,52	±0,70	± 0,88	± 1,3	±2,4	± 3,1
	TWO WAY ANALYSIS OF VARIANCE FOR REPEATED MEASURES FOLLOWED BY STUDENT NEWMAN KEUL'S TEST WHEN F IS SIGNIFICANT									
F(2,30)	29,84			4,35			F(1,10) 0,41		F(1,10) 4,47	
	Pre- separate group from Mid. & Post			Pre. separate group from Mid. & Post			Not Significant		Not Significant	
	P < 0,01			P < 0,05						
	Critical value F(1,10) 95% 4,96 99% 10,0									
	Critical value F(2,30) 95% 3,32 99% 5,39									

ride (Table 4). Four horses had no changes in blood gases, pH or HCO_3 from the beginning to the middle of the ride and only one horse showed no change from the beginning to the end of the ride. These horses were neither the fastest nor the slowest competitors.

There was no significant change in serum potassium, although the mean potassium concentration did decrease substantially from the pre-ride value and the F statistic was very close to the critical value (Table 3). Packed cell volume and TS were significantly higher at the middle of the ride and remained high at the completion of the ride. Both PCV and the total solids increased significantly by the completion of the ride (22 per cent and 5 per cent respectively).

DISCUSSION

The data presented showed that these horses during strenuous exercise developed metabolic acidosis. This is in agreement with the findings of Krzywanek [20] and Engelhardt [8]. Nevertheless, the decline in pH was not directly correlated to the rise in blood lactic acid. Respiratory compensation resulted in a fall in venous and arterial PCO_2 to help ameliorate the low blood pH. During exercise, the decrease in bicarbonate concentration was found to be very similar to the rise in lactic acid. At moderate work (350m/min) there was no significant change in bicarbonate in spite of the 20-minute duration of exercise, while at the heavy exercise level (700m/min), the bicarbonate decrease

Table 4: BLOOD GASES, pH, HCO_3-CHANGES IN THE FASTEST AND SLOWEST HORSE DURING AN 80 km ENDURANCE RIDE

Subject	Speed m/min		pH	PO_2 mm HG*	PCO_2 mm Hg*	HCO_3- mEq/l	B.E.
7A	200	Pre	7,415	34,3	44,2	27,4	+3,4
		Mid	7,448	38,6	40,4	26,9	+4,1
		Post	7,317	46,0	38,1	18,7	- 5,9
29A	130	Pre	7,430	41,7	42,7	27,3	+3,7
		Mid	7,461	42,8	38,4	26,3	+3,9
		Post	7,404	42,4	38,5	23,4	+0,3

* = 1,33 x 10^2 Pa

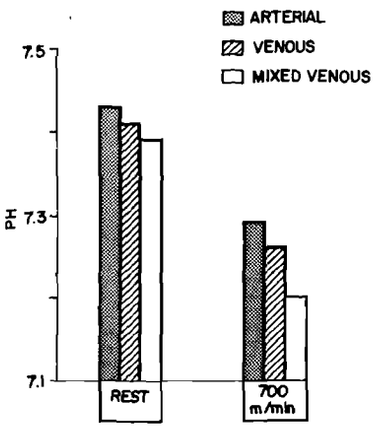


Figure 11 - Arterial, venous and mixed venous PH from the carotid artery, jugular vein and right ventricle of unconditioned horses performing at 700m/min.

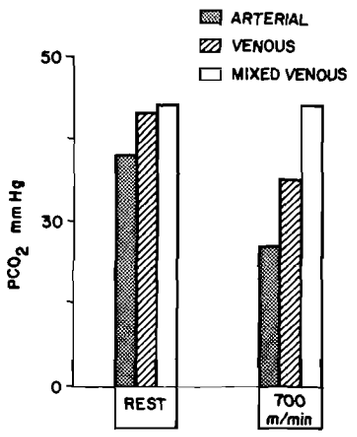


Figure 12 - Arterial, venous and mixed venous PCO_2 from the carotid artery, jugular vein, and right ventricle of unconditioned horses performing ar 700m/min.

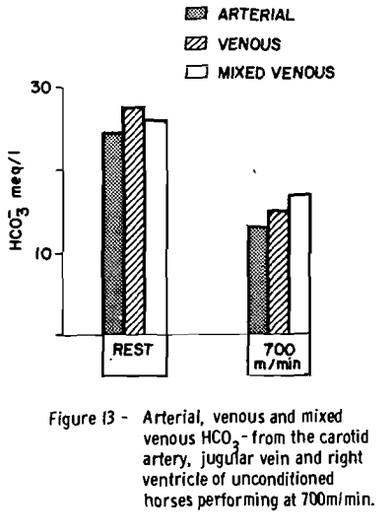


Figure 13 - Arterial, venous and mixed venous HCO_3- from the carotid artery, jugular vein and right ventricle of unconditioned horses performing at 700m/min.

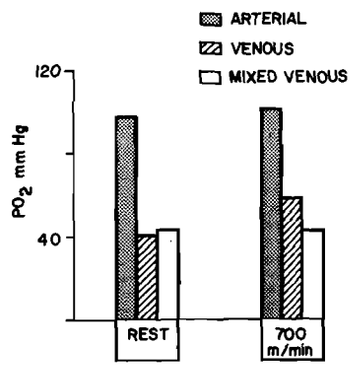


Figure 14 - Arterial, venous and mixed venous PO_2 from the carotid artery, jugular vein, and right ventricle of unconditioned horses performing at 700 m/min.

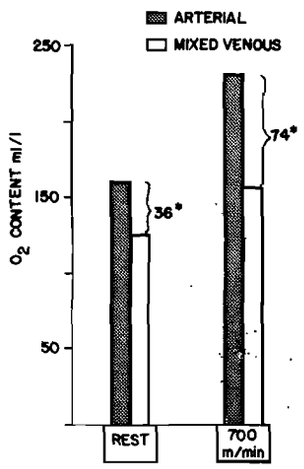


Figure 15 - Arterial, and mixed venous PO_2 content from the carotid artery and right ventricle of unconditioned horses performing at 700m/min.
* $(A\text{-}VO_2)$ difference

was a mirror image of the lactate increase. Therefore, there was no change in acid-base balance at moderate exercise and an incompletely compensated metabolic acidosis at heavy exercise. The effects of exercise on the acid-base balance and blood gases of arterial and venous blood depend on the severity of exercise. It is apparent from this study that unconditioned horses must exceed a work rate in excess of 350 m/min and approach a work rate in excess of 600m/min in order to challenge the lactic acid system of anaerobic metabolism. During rest, moderate and heavy exercise, we found a linear relation between the changes in arterial and venous pH, PCO_2 and HCO_3^-. Therefore, in these exercising horses, arterial blood pH, PCO_2 and HCO_3^- could be predicted accurately from venous blood. These findings show that venous

samples can be used to characterize arterial acid-base status during exercise, avoiding direct arterial puncture.

In the horse, the oxygen supply during exercise is increased not only by an increase in blood flow to the working muscle but also by an increase in oxygen carrying capacity of the blood[20]. This is evident by the progressive increase in PCV from moderate to heavy work in these horses. The oxygen capacity of the blood increased 8,84 vol per cent from rest to the heavy work (Table 1.).

Arterial PO_2 decreased in these horses during moderate work but returned to resting level during heavy work. In man, there is a disagreement with respect to the nature of change in PaO_2 during exercise [1 3 34]. In the horse at rest, the distribution of blood flow in the lungs is such that the uppermost parts of the lungs are very poorly perfused compared to the lower segments [14].

This is believed to be due to the effects of gravity on the blood in the pulmonary blood vessels. Alveolar ventilation is also believed to be greater in the lower segments of the lung than in the upper segments. The rate of increase in perfusion with distance down the lungs, however, is greater than the rate of increase in ventilation, resulting in a high ventilation/perfusion ration (VA/Q) in the upper zone and a low VA/Q ratio in the lower zone of the lungs. The decrease in PaO_2 during moderate work in these horses may be a result of an ever wider spread in this ventilation/perfusion ratio. Whipp's findings in man[4], indicated that during moderate exercise there is a greater increase in the blood to the upper segments of the lungs than to the lower segments which tends to result in a more even distribution to the ventilation/perfusion ratio. If moderate exercise in the horse were to result in a more even distribution of the VA/Q ratio, the distribution in ventilation/perfusion would not contribute significantly to a decrease in PaO_2. Other possible causes of this decrease in PaO_2 would be a larger right to left pulmonary vascular shunt, a diffusion limitation or a combination of the two [1 3 28 39]. The corresponding rise of PaO_2 during heavy exercise in these horses most likely represents increased return of the more highly oxygenated venous blood to the lungs. In man and dog, the mixed venous oxygen tension ($P\bar{v}O_2$) decreases during exercise resulting in an increase in the arterial-venous oxygen difference ($a\text{-}\bar{v}O_2$)[3 39]. This is believed to be the result of an increase in O_2 extraction by the working muscles. The ($a\text{-}\bar{v}O_2$) also increased in the horses studied but the PvO_2 was very similar at rest and after exercise. The rise in ($a\text{-}\bar{v}O_2$) from rest to exercise in these horses was primarily a result of the increased O_2 content of the arterial blood and not a fall in $P\bar{v}O_2$ (see footnote page 353). The rise in PvO_2 appears to represent the return of highly oxygenated venous blood from the head region.

The significant rise in blood pH on completion of the first half of the endurance ride was probably a result of the decrease in venous PCO_2 owing to hyperventilation. The hyperventilation may have occurred in order to help in the control of body temperature since it was greater than that required to handle the acid load caused by increased CO_2 production owing to increased metabolism. Ventilation is of significance in the regulation of body temperature in the horse, and environmental factors, such as the temperature and humidity, have a great influence on the respiratory rate[12]. It is conceivable that temperature regulation may have induced sufficient hyperventilation to have been responsible for the tendency towards alkalaemia in these endurance horses.

Part of the significant fall in PCO_2 on completion of the ride most likely represents respiratory compensation for the significant reduction in HCO_3^- that also was present on completion of the ride. The fall in bicarbonate is probably best explained by a corresponding rise in lactic acid as a result of anaerobic metabolism during the final portion of the ride. It has been shown in previous studies in man and this study in the horse, that bicarbonate is inversely and linearly related to lactic acid concentration in the blood of subjects performing anaerobic work [38]. A portion of the decrease in PCO_2 indirectly represents respiratory compensation for the metabolic lactic-acidosis. Since the pH did not significantly differ from the resting value, the metabolic acidosis was fully compensated for by blowing off CO_2. The fall in PCO_2 was greater than expected for the associated fall in HCO_3^-. This is based on the 95 per cent confidence limits for the expected degree of respiratory compensation to uncomplicated metabolic acidosis proposed by Winters [41]. This suggests the presence of some other stimulus to respiration besides that associated with the metabolic acidosis. This additional decrease in PCO_2 may be a result of increased ventilation as a result of heat exchange. This compensated metabolic acidosis at the completion of the ride probably occurred because many riders had their horses complete the last portion of the ride at a considerably faster rate than the rest of the ride. Speeds in excess of 600m/min cause considerable lactic-acidosis in the horse [8].

Electrolyte changes have been shown to occur in exercising man and horse. Most of the studies in the horse have been concerned with endurance events. In man, serum potassium concentrations have been shown to increase after both short duration exercise and marathon running while serum calcium increases during short-duration exercise [29 33]. The significant increase in serum calcium after heavy exercise in the horses in this study is similar to the findings in man. This rise in serum calcium most likely represents a shift of calcium out of the muscle cell as a result of acidosis [33].

Serum calcium has been shown to decrease in both man and horse during marathon running, presumably as a result of loss in the sweat [24 37]. In this study, there was no change in serum calcium. Previous studies in endurance horses by Mansmann [24] showed a significant decrease in serum Ca^{++} on completion of the 160 km endurance ride. The difference in our findings may indicate only that the two studies did not deal with similar stress with respect to environment, speed and distance.

The mean serum potassium was decreased at the end of the endurance ride but was not quite significant at the 95 per cent confidence level. In man, during short strenuous exercise and marathon running, serum potassium has been shown to rise above that which can be explained by volume contraction[1 4 21]. This rise in serum potassium has been explained by an efflux of potassium from intracellular stores in exchange for hydrogen ions during metabolic acidosis, by the diffusion of potassium from the intracellular

space when muscle and liver glycogen is reduced and by intravascular haemolysis [4 13 18]. The decrease in potassium in these endurance horses may have been from loss in the sweat.

The tendency towards alkalaemia at the middle and at the end of the ride in these horses may be important in respect to setting the stage for the development of metabolic disorders. Both alkalaemia and hypokalaemia have been found in association with synchronous diaphragmatic flutter [7 24]. The tendency towards alkalaemia and hypokalaemia in these endurance horses indicates that replacement solutions may be beneficial during competition to maintain a normal metabolic state.

Several studies in man have indicated that serum muscle enzymes rise after exercise [10 15 16]. It is believed that the increase in these enzymes is a result of increased cell membrane permeability due to hypoxia [10 11 15]. Unconditioned individuals may suffer muscle hypoxia at much less severe work loads than well-conditioned individuals. Therefore, unconditioned horses would be expected to have higher serum muscle enzyme levels after a particular work load. Cornelius [6] found that SGOT serum activity was two- and three-fold higher after strenuous exercise in unconditioned horses. In this study, we found a 50 per cent increase in serum SGOT and 225 per cent increase in CPK after heavy work. This enzyme response to a given amount of exercise may be useful as a biochemical estimate of physical fitness in the horse.

ACKNOWLEDGEMENTS

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The failure to observe a fall in $P\bar{v}o_2$ may be the result of the time of sampling after exercise.

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DISCUSSION

J.R. Gillespie: I compliment the author in his absence. It was a very worth-while paper. With regard to the question why the oxygen content increases as exercise becomes more severe, that is exactly what one would expect for the very reasons mentioned in the paper. What is happening is that the air in the lungs is going to where the blood is and, vice versa, the blood going through the lung is going where the dilatation is. So there is an evening out: the matching of diliatation and thus considerably higher efficiency of the gas chain units in the lung. I agree with every conclusion that the author made. On one point, however, I would like to sound a note of caution. If one wants to look at the total body and its respiratory

activity, the only way to do this is by looking at arterial blood. Anything short of that just will not do because one is then by-passing the lung. Whether one uses mixed venous blood or jugular blood, they both have the short-coming of having by-passed the lungs, effect on gas exchange and particularly on pH effects on the blood. This also holds true, by the way, if one wishes to consider things like lactic acid; the minute one changes the pH of the blood one is likely to change the shift between lactic acid and other acidizations of the blood. This does not mean that these other measurements do not have something to reveal how the whole body is reacting to stress.

Maureen Aitken: Could Dr Fregin or some other speakers tell us what value they consider the lactate: pyruvate ratio to be. We thought that this was a useful method of assessing anaerobic metabolism.

A. Lindholm: There is an increase in pyruvate during work but it is not very high and not that important. The muscle pyruvate concentration is very interesting. Foals have very high pyruvate concentrations; with increase in age there is a decrease in pyruvate concentration. Very little has been done on pyruvate in animals; this is especially so in the horse. I have looked at it but I did not find it that important. I think lactate is more important.

J.R. Gillespie: We must be very careful hanging our hats on lactic acid as index of anaerobic metabolism, because there are studies to show that muscles can be bathed in oxygen, with plenty of oxygen about, and they just simply will not use it. They will go ahead and begin to produce lactic acid. It may be, and we have some studies to show this, that the enzymes are being over-loaded. It may be an enzymatic problem, not at all associated with how much oxygen is present in the active muscle. I do not think we should consider exclusively lactic acid and the lactic acid:pyruvate ratio as an index of anaerobic *versus* aerobic exercise.

S.G.B. Persson: I could be wrong, but I think Dr Milne concluded that the increase in AV oxygen difference was brought about mainly by the increase of the arterial oxygen saturation. If that the increase in ($a\text{-}vO_2$) difference was brought about dings. In the horse, during exercise the ($a\text{-}vO_2$) difference is certainly to the greatest extent brought by the decrease in the venous and in the mixed venous blood, which is to be expected. Possibly this difference could have been caused by the fact that we measured these values during work, which is an advantage.

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LACTIC ACID CONCENTRATIONS AND pH VALUES IN TROTTERS · AFTER RACING

H. KRZYWANEK*

SUMMARY

In horses not exhausted by racing (1 900 to 2 500 m) at speeds from 1:22,3 to 1:31,0 kmt, the mean pH values of the jugular venous blood dropped from 7,379 before the race to 7,164 immediately afterwards, and rose to 7,213 fifteen minutes later. In those exhausted after racing the respective values were 7,379; 7,086 and 7,105.

The mean blood lactate values for horses not exhausted by the above performance were 0,63 mEq/*l* before the race, 17,99 mEq/*l* immediately thereafter and 16,92 mEq/*l* fifteen minutes later, whereas the values for horses exhausted by the race were 0,58; 23,14 and 24,37 mEq/*l*, respectively.

Resting values were attained more rapidly after the race if the horses were jogged at a speed of about 450 m/min for 2 000 m.

During the past five years we have conducted experiments on blood acidity in race-horses, at rest, as well as after submaximal and interrupted exercise; in a few instances, also, before and after races [3 4 5 6].

In another study, 173 blood analyses were carried out on 34 horses, all of them before and after races. Blood was taken from the jugular vein immediately before, and 2 and 15 minutes after the race. pH values were obtained using capillary glass electrodes (Radiometer) and lactic acid concentrations enzymatically [2]. Racing distances ranged from 1 900 to 2 500 m, running speeds from 1:22,3 to 1:31,0 kmt.** In so far as only few similar investigations have been undertaken before [1 7 9 10], the results of our studies have thrown new light upon the issue.

Table 1 shows mean values and standard deviations of all data collected in our experiments. The material was divided into two groups. Group A comprised animals that were not exhausted after the race; Group B animals that were exhausted. pH-values in Group A dropped from 7,379 at rest to 7,164 immediately after the race. Fifteen minutes later a mean value of 7,213 was found. Lactic acid concentration increased from 0,63 at rest to 17,99 mEq/*l*; after the race 15 minutes later the mean value was 16,92 mEq/*l*. Thus, pH values were almost always higher 15 minutes after the race than immediately after the completion of the competition. Lactic acid concentration, measured 15 minutes following the race, fluctuated. In some cases there were increases, in other decreases.

In Group B, pH values dropped from 7,379 at rest to 7,086 after the race. Fifteen minutes later pH values amounted to 7,105. Lactic acid concentrations increased after the race from 0,58 at rest to 23,14 mEq/*l*. Fifteen minutes later, a mean value of 24,37 mEq/*l* was still in evidence. Only in a few experiments were decreases of lactic acid concentrations seen 15 minutes after the race. The results of Group B clearly reflect the exhaustion of the horses after the races. In some cases, a remarkable degree of acidosis was found. In one horse (No. 56) the pH value dropped from 7,39 at rest to 6,97 after the race. Fifteen minutes later, a pH level of 6,94 was present. Lactic acid concentrations were 26,5 mEq/*l* after the race, 32 mEq/*l* 15 minutes later, as against 0,55 at rest. The above post-exercise value is 50 times greater than the resting value.

Table 2 shows results of studies obtained from two trotters (No. 60 and No. 71). On the left side of the table are listed pH values and lactate concentrations at rest, after the race and 15 minutes later. Horse No. 60, whose trotting performances were of average quality, ran to exhaustion in its first race. Post-exercise acidosis was high even though the running performance was only moderate. The horse failed to be placed. In a second race, the same horse placed 5th; compared with the results of the first race its kmt was better and acidosis less pronounced. In a third race, the horse won with a still better kmt and less marked acidosis.

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** Kmt = kilometres per unit of time.

Table 1: BLOOD pH VALUES AND BLOOD LACTATE CONCENTRATIONS BEFORE (B.R.) AFTER RACES (A.R.) AND 15 MINUTES LATER (A.R. + 15')

	A (n = 98)		B (n=74)	
	pH	LA [mEq/l]	pH	LA [mEq/l]
B. R.	7, 379 ± 0,02	0,63 ± 0,3	7, 379 ± 0,02	0,58 ± 0,3
A. R.	7, 164 ± 0,04	17,99 ± 2,8	7, 086 ± 0,05	23,14 ± 2,5
A. R. + 15'	7, 213 ± 0,05	16,92 ± 3,8	7, 105 ± 0,06	24,37 ± 3,4

Group A: horses not exhausted, Group B: horses exhausted.

Table 2: pH VALUES AND BLOOD LACTATE CONCENTRATIONS OF TWO HORSES (N. 60 AND NO. 71) BEFORE AND AFTER RACES

No. 60		pH	C_{Lac}
19. 11. 2000 m 1: 28,2 —	B. R. A. R. + 15'	7,34 6,99 6,97	0,35 26,59 29,83
6. 12. 2000 m 1: 26,7 V	B. R. A. R. + 15'	7,36 7,04 7,03	0,37 24,26 25,60
13. 12. 1900 m 1: 26,3 I	B. R. A. R. + 15'	7,34 7,06 7,06	0,35 21,22 21,46

No. 71		pH	C_{Lac}
23. 5. 1900 m 1: 26,1 —	B. R. A. R. + 15'	7,38 7,17 7,19	0,57 16,64 14,79
3. 6. 2000 m 1: 24,6 —	B. R. A. R. + 7,5'	7,41 7,06 7,05	1,85 21,4 24,57
17. 6. 1900 m 1: 23,0 II	B. R. A. R. + 10'	7,39 7,11 7,09	0,58 19,91 23,65
29. 8. 1900 m 1: 22,3 III	B. R. A. R. + 7,5	7,4 7,19 7,17	0,82 19,98 22,16

(For explanation of symbols see caption to Table 1).

On the right side of table 2 are shown results obtained from Horse No. 71 which had a very good racing record (it placed 6th in the German Derby in 1973 and won the St.Leger). In its first race of the season, included in our experimental series, the animal was driven with restraint. Nevertheless, it ran much faster than Horse No. 60. Blood acidosis after the race was lower. In three subsequent races, running performances of Horse No. 71 improved further, while post-exercise acidosis continued to be less pronounced.

Table 3 contains results of experiments with yet another horse (No. 73). Changes of blood acidity after the races were less pronounced than expected in terms of our previous findings with other trotters. To specify, lactic acid concentrations after six races of Horse No. 73 were 17,79; 21,52; 12,56; 10,30; 13,56 and 12,15 mEq/*l*. Corresponding blood analyses in Group B had yielded a mean value of 23,14 mEq/*l*. Evidently, blood acidity levels do not necessarily correspond to racing performances. Since degree of acidosis after races does not depend on the state of training, it reflects determinants of maximal work capacity as indicated in figure 1, in which lactate concentrations after races are plotted against running speed (kmt).

Table 3: pH VALUES AND BLOOD LACTATE CONCENTRATIONS OF HORSE NO. 73 ASSESSED BEFORE AND AFTER 6 RACES

No. 73		pH	C_{Lac}
5.12.73 1900 m Z. n. g. II	B. R. A. R. + 15'	7,40 7,20 7,22	0,65 17,79 17,60
9. 1. 74 2040 m Z. n. g. IV	B. R. A. R. + 15'	7,37 7,17 7,13	0,73 21,52 19,72
20.2.74 1900 m 1: 26,3 V	B. R. A. R. + 15'	7,37 7,20 7,26	0,69 12,56 11,73

No. 73		pH	C_{Lac}
24. 2. 74 1900 m 1: 27,5 II	B. R. A. R. + 15'	7,37 7,21 7,26	0,68 10,30 9,96
28. 3. 74 1900 m 1: 26,0 II	B. R. A. R. + 17'	7,36 7,20 7,27	0,79 13,56 10,75
11.4.74 2000 m 1: 25,8 IV	B. R. A. R. + 17'	7,40 7,28 7,34	1,05 12,15 8,89

(For explanation of symbols see caption to Table 1).

That pH values are influenced by lactic acid concentration is known. In figure 2, pH values are plotted against lactate concentrations, assessed after submaximal as well as after maximal loads; in this graph each point represents the difference between pre-load and post-load values. For lactic acid concentrations up to 12 mEq/*l*, a linear correlation with pH exists. For lactic acid concentrations of 12 mEq/*l* and above, corresponding pH values decreased significantly.

In this context it must be remembered that, during exercise, horses release blood with high erythrocyte concentration from the spleen. Thus, a conspicuous increase in haemoglobin content of the peripheral blood, accompanied by an increase of its buffer capacity, occurs. A noteworthy release of erythrocytes from the spleen occurs even during *submaximal* exertion, while at the same time lactic acid concentration does not necessarily increase. After *maximal* effort, the increase in numbers of erythrocytes and of concentration of haemoglobin is still greater but the extent to which the additional shift manifests itself is not as great as the additional shift over the previous-

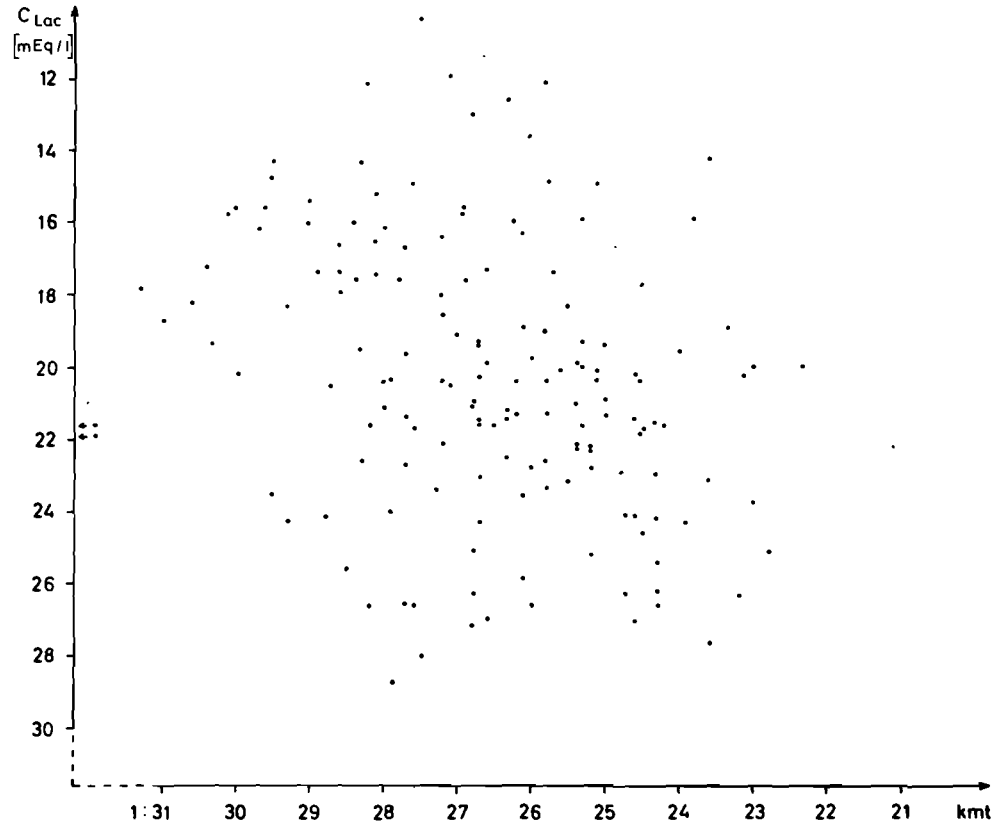
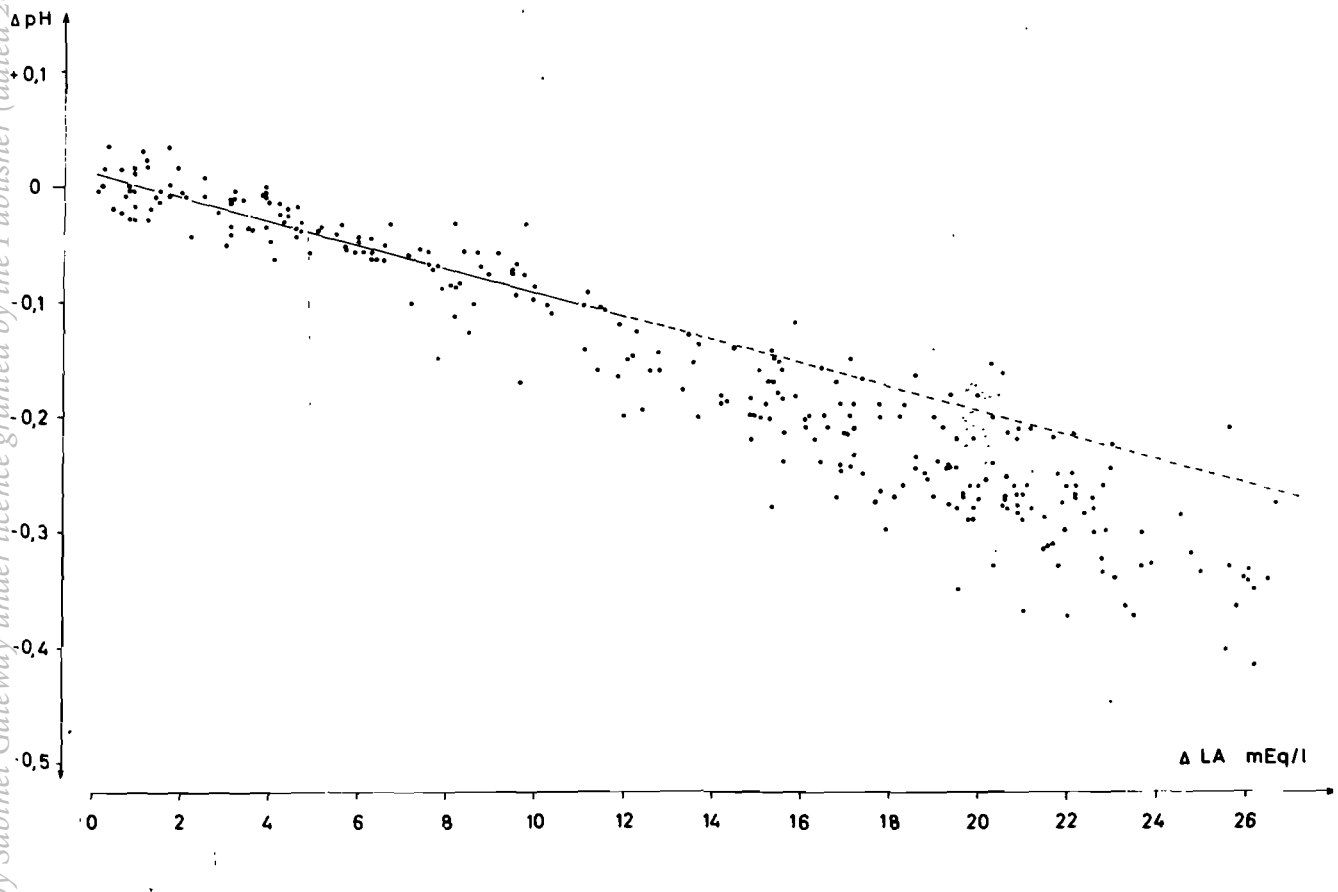


Fig. 1: Blood lactate concentrations plotted against racing speed (kmt). (Summary of 156 measurements).



2: Blood pH plotted against lactate concentration. (Each point represents the difference between resting and post-race values).

ly mentioned lactic acid concentrations. In figure 3 the alterations of haemoglobin and lactate concentrations after different running loads (300 m./min, 450 m./min and 60 m./min) are summarized. Results obtained with horses in Groups A and B are shown separately. The distribution pattern of lactic acid values plotted against pH shown in the scatter diagram (Fig. 2) seems to be explained by the findings summarized in figure 3. Haemoglobin concentrations are elevated virtually maximally already after *moderate exercise*, while lactate concentrations reach their highest values only after *races*.

The time pattern of return of blood acidity after races to pre-race levels is depicted in figure 4 (horse No. 66). Resting values can be reached more rapidly if the horses are moved after the race. To exemplify, figure 5 shows pH values and lactic acid concentrations of Horse No. 60 before and after races. In experiment a) the horse went back to the stable after the race and rested; in experiment b) the horse was harnessed again after the race and made to jog at a speed of about 450 m/min (2 000 m). pH values and lactate concentrations assessed *immediately* after the races were identical in both experiments (7,037 and

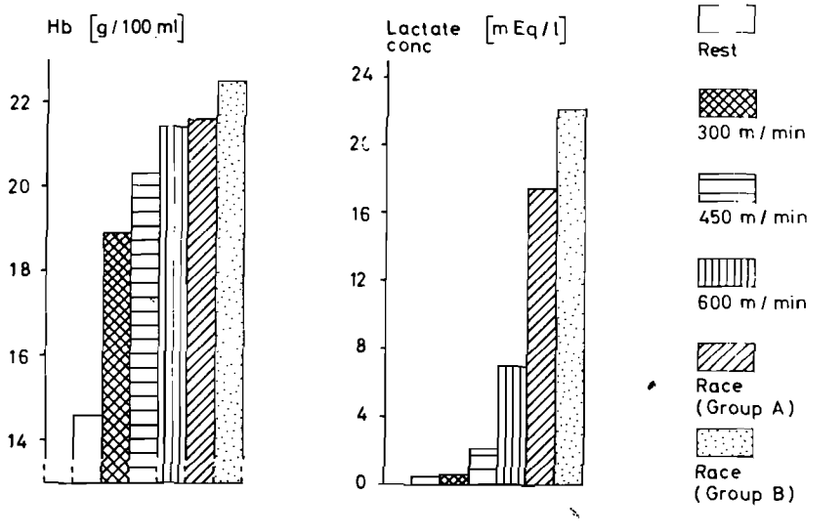


Fig. 3: Haemoglobin and lactic acid concentrations at rest and after different work loads (Columns 1 – 4). The last two columns depict results obtained from animals not exhausted (Group A) and exhausted (Group B) after races.

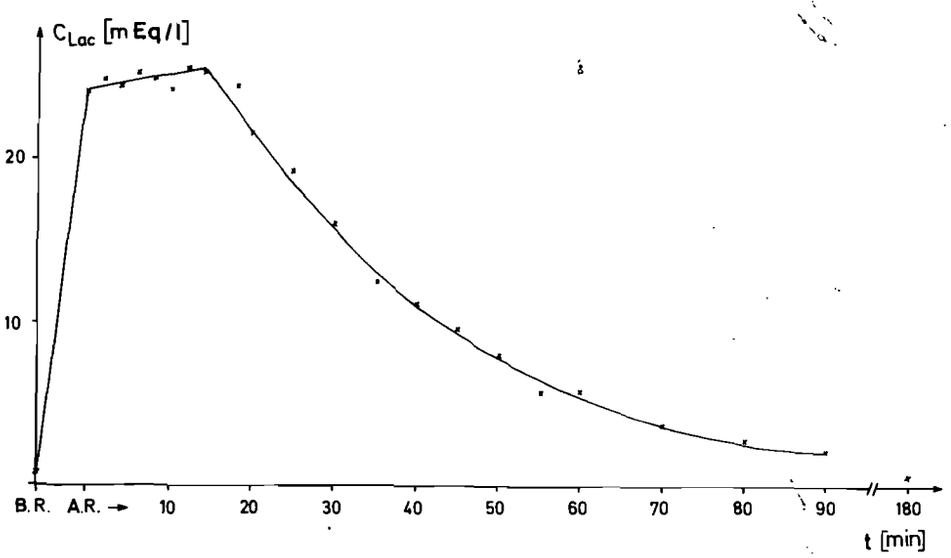


Fig. 4: Lactic acid concentrations in blood at rest, after race and during recovery (Horse No. 66).

After the races, the horses usually are led into the stables. At that time, acidosis is very high. The question arises whether high acidosis is 'dangerous'. In athletes, Osnes & Hermansen [8] found even higher lactate concentrations, accompanied by correspondingly lower pH values, after an exhausting physical performance of short duration. Such high levels of acidity are well tolerated.

26,4 mEq/*l*, respectively). In experiment a) the pH 15 minutes after the race was 7,026, which means it had remained virtually unchanged, while the lactate concentration had increased from 26,4 immediately after the race to 29,8 mEq/*l* 15 minutes later. In experiment b) the pH had increased from a post-race value of 7,037 to 7,19, whereas the lactate concentration had decreased from a post-race value of 26,4 to 18 mEq/*l*.

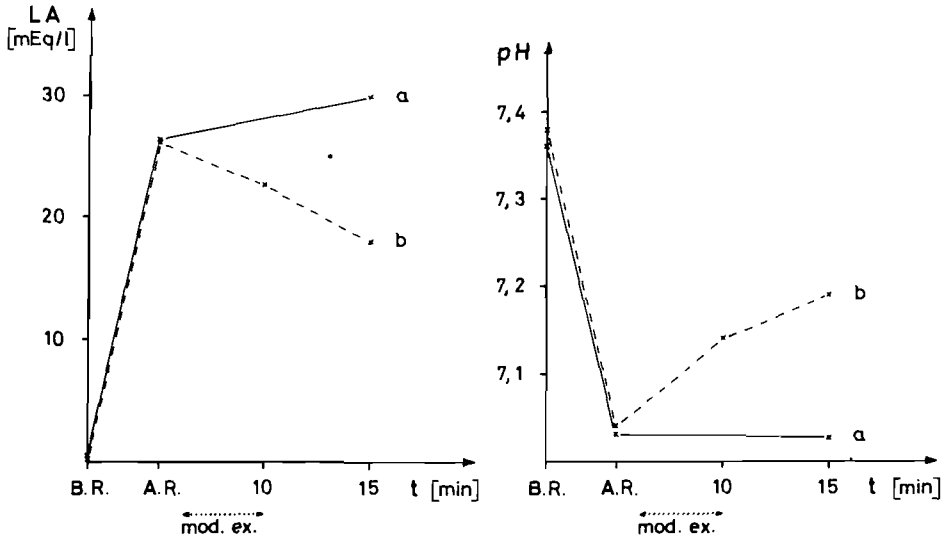


Fig. 5: Blood lactate and pH before and after races, assessed under two different experimental conditions. (For explanation see text).

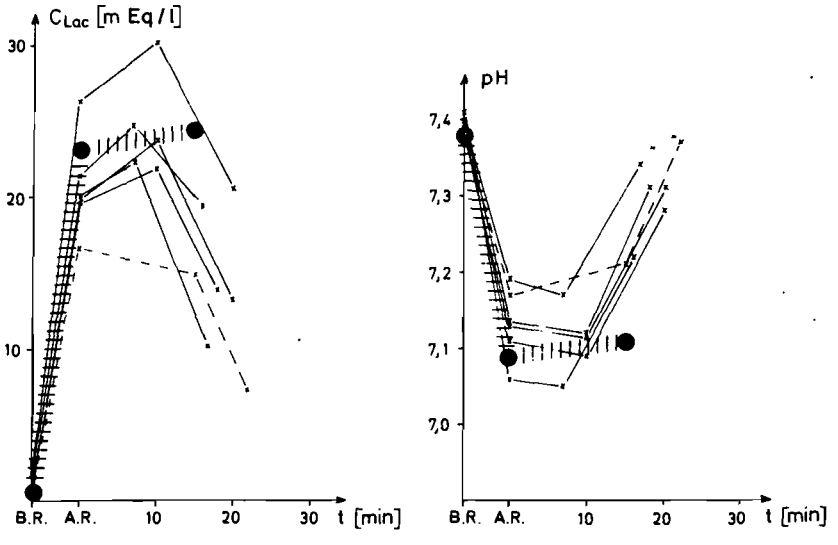


Fig. 6: The influence of moderate exercise during recovery (Horse No. 71) on lactic acid concentrations and pH-values. Symbols ● III ● give the mean values of group B; no exercise during recovery).

In figure 6 are summarized the results obtained in six experiments with Horse No. 70, which was made a jog after races. The more prompt return towards normal resting values of lactic acid concentrations and of pH is evident from a comparison with the corresponding means obtained from horses which were allowed to stand in the stable following the races.

To conclude, lactate concentrations and pH allow an evaluation of the physiological relevance of blood acidity. Our results thus yield insight into a biochemical prerequisite of the performance capacity of trotting race horses.

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DISCUSSION

J.R. Gillespie: What criteria do you use to assess exhaustion in the horse? Earlier on you separated the horses into two groups, those that were exhausted after the race and those who were not.

H.H. Krzywanek: I was always present to examine the horses. I asked the trainer's opinion. The division could not be made objectively by some exact value. Subjective criteria had to be employed. In group A we had high lactic acid values and low ones in group B.

Jl S. Afr. vet. Ass. 45(4) 361-370
(Proc. 1st int. equine vet. Conf., Pretoriuskop 1974)

CORRELATIONS BETWEEN PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS USED TO ASSESS FITNESS IN THE HORSE

MAUREEN M. AITKEN,** MARION G. ANDERSON,* GRACE MACKENZIE* AND J. SANFORD***

SUMMARY

Repeated regular performance of physical exercise can reduce the cardiac and respiratory deceleration times, the increases in blood lactate, pyruvate and lactate:pyruvate ratio and the increase in serum creatine phosphokinase concentration following exercise. Six horses of Thoroughbred and heavyweight Hunter type were subjected to three exercise programmes of varied severity. Biochemical changes were produced only by relatively severe exercise. One horse was judged physiologically and biochemically to be definitely unfit initially but its fitness was improved by training. On repetition of the exercise programmes three horses showed increased fitness as assessed physiologically.

INTRODUCTION

Physical exercise in the horse, as in other species, is accompanied by changes in physiological and biochemical parameters. The magnitude and duration of such changes may indicate the ability of an animal to produce and utilize the energy required to support increased muscular activity. This ability may be enhanced, as reflected by diminution in the magnitude of such changes, if exercise is repeated at regular intervals. An animal may thus be said to become increasingly 'fit' as a result of 'training'.

The physiological changes accompanying exercise are elevation of heart rate and blood pressure with redistribution of blood to skeletal muscle and increase in cardiac output. Respiratory rate, depth and consequently minute volume increase. The biochemical changes include elevation of blood concentrations of the metabolites glucose, lactate, pyruvate, free fatty acids and glycerol and increased serum concentrations of the enzymes lactic dehydrogenase (LDH), aldolase (ALD) and creatine kinase (CK). The rates at which heart and respiratory rates fall after exercise have been used to assess fitness in man and horse [3 14 17]. High concentrations of blood lactate and pyruvate have been observed following exercise particularly in untrained human beings [6 12 13] and the relationship between blood lactate and pyruvate levels has been used as an index of the oxygen deficiency of tissues [11 12]. Training has been found to reduce or eliminate serum enzyme increases due to exercise in man and rats [7 8].

We measured changes in the above parameters in horses of Thoroughbred and Hunter type subjected to exercise programmes of varied severity and frequency. In many cases, training effects were observed as progressive reductions in the biochemical and physiological changes accompanying the exercise. Initially the biochemical aspects of exercise in the horse were investigated extensively by one of us [2] and, in different sets of experiments, heart and respiratory rates were monitored in relation to exercise [2]. These findings will be outlined briefly. Subsequently, selected physiological and biochemical parameters were monitored simultaneously in groups of horses given fixed amounts of exercise at regular intervals. The times taken for heart and respiratory rates to fall over a predetermined range after completion of exercise were measured, and blood concentrations of lactate and pyruvate before and after exercise determined. Serum concentrations of creatine kinase (CK) were likewise monitored. CK has been shown to be the most specific of the enzymes released during exercise in being derived from skeletal muscle [2].

METHODS

Recording of ECG, heart Rate and Respiratory Rate

Electrocardiograms were recorded by radiotelemetry† using a modified bipolar chest lead as previously described [1 10 15]. A Neilson heart rate meter was used to obtain a continuous record of heart rate. Recording was carried out for five minutes before exercise, for one minute at 5-minute intervals during exercise and continuously on cessation of exercise until the heart rate had fallen to under 50 beats per minute. Simultaneously, respiratory rate was measured by observation over 30 second periods and measurements continued every minute after exercise until the respiratory rates had fallen to under 20 per minute.

Estimation of Blood Lactate and Pyruvate Levels.

Volumes of 10 m*l* blood were withdrawn from the jugular vein before and immediately on cessation of exercise. The blood was de-proteinized by mixing with 10 m*l* volumes of 0,7M ice-cold perchloric acid and centrifuging at 2 000 rpm for 10 minutes. The supernatant was stored at -15°C until lactate and pyruvate estimations were carried out.

Blood lactate levels were estimated using the Biochemica Test Combination Cat No. 15972 [5] which is based on the enzymatic method of Hohorst [9]. Extinction readings at 340 nm were made on a Unicam SP800 spectrophotometer [2].

Blood pyruvate levels were assayed using a method for the simultaneous enzymatic determination of acetoacetate and pyruvate [2 4].

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†Receiver and Transmitter: Ernes & Turner, London, England, MIE Type TM2R.

Estimation of Serum Enzyme Levels

Jugular venous blood was collected in unheparinized polystyrene tubes immediately before, immediately after and/or five hours after exercise. It was centrifuged and the upper layer removed and allowed to clot. Assays on serum were carried out immediately or within four days of storage at -15°C.

Creatine kinase was determined by the spectrophotometric method of Oliver [16] with substrate concentration, pH and temperature modified to provide optimal working conditions for horse enzymes [2].

RESULTS OF INITIAL EXPERIMENTS

Heart Rate and Respiratory Rate Deceleration

Daily lunging exercise was used in initial observations on deceleration of heart and respiratory rates after exercise. Horses, when subjected to this exercise after several weeks 'rest' at grass, showed a progressive reduction in recovery time assessed as the time for heart rate to fall to pre-exercise level. This is illustrated in figure 1. This also shows the typical rapid fall in heart rate during the minutes following cessation of exercise. Similar reductions in time for the respiratory rate to return to normal were indicated but respiration varied considerably from minute to minute in depth and in rate. This, together with the method of recording, made this parameter less useful than heart rate.

Many factors other than state of fitness influence heart and respiratory rates during and following exercise. These are summarized in table 1. Speed of performance had the greatest effect on respiratory rate in Thoroughbred type horses while environmental temperature also affected heart and respiratory rates, particularly in heavyweight Hunters. Heart rate fell more slowly in heavyweight Hunters than in Thoroughbreds. (Table 2.)

Blood lactate and Pyruvate levels

The normal levels of lactate, pyruvate and lactate/pyruvate ratios measured in nine horses on several different occasions are shown in table 3. Blood lactate and pyruvate levels and the lactate pyruvate ratio rose during exercise and began to fall when exercise ceased. Increases were greatest during galloping as shown in figure 2. Daily exercise, consisting of trotting and cantering for 13 km carried out by one horse, had a training effect as shown in figure 3. The increases decreased over a 3-week period.

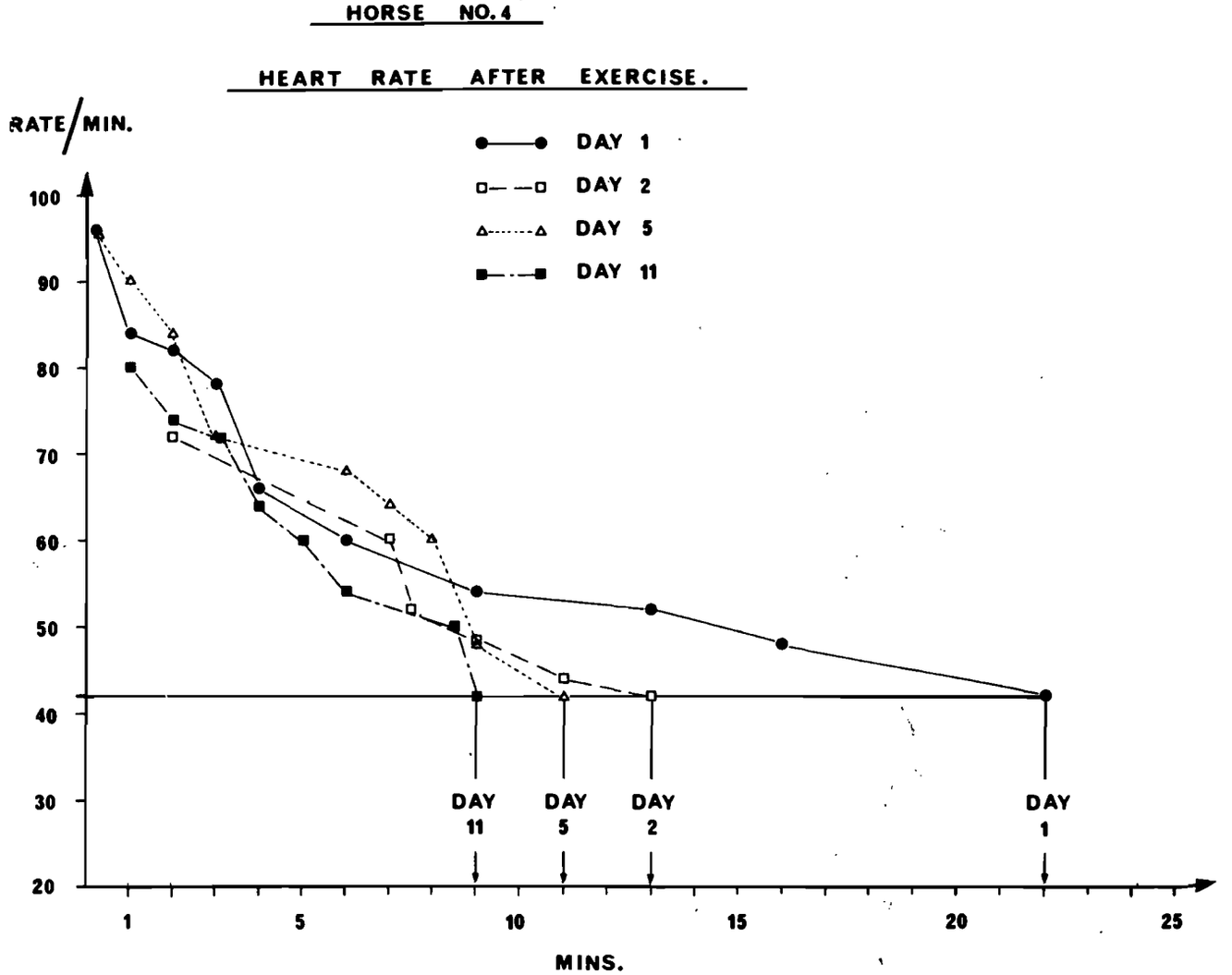

Fig. 1: Reduction in heart rate deceleration time following daily lungeing exercise.

Table 1: FACTORS INFLUENCING HEART AND RESPIRATORY RATES

Parameters	Horse No. 1 (Heavy Hunter)			Horse No. 2 (Thoroughbred)		
	n	r	p	n	r	p
1. Heart rate deceleration time/respiratory rate deceleration time	25	0,48	<0,02 >0,01	12	0,77	<0,05 >0,02
2. Heart rate deceleration time/average time per circle	28	-0,21	<0,03 >0,2	12	-0,38	<0,3 >0,2
3. Respiratory rate deceleration time/average time per circle	25	-0,09	<0,7 >0,6	12	-0,71	<0,05 >0,02
4. Heart rate before exercise/respiratory rate before exercise	28	0,18	<0,4 >0,3	12	0,52	>0,1 >0,05
5. Heart rate after exercise/respiratory rate after exercise	25	0,21	0,3	10	0,77	<0,05 >0,02
6. Heart rate after exercise/heart rate deceleration time	28	0,59	<0,01 >0,001	12	0,83	<0,02 >0,01
7. Environmental temperature/average time per circle	27	-0,15	<0,5 >0,4	12	0,44	<0,2 >0,1
8. Environmental temperature/heart rate deceleration time	27	0,44	<0,05 >0,02	12	-0,007	>0,9
9. Environmental temperature/respiratory rate deceleration time	25	0,68	<0,001	12	-0,26	<0,5 >0,4
10. Environmental temperature/sweat	27	0,49	<0,02 >0,01			
11. Average time per circle/sweat	28	0,28	<0,2 >0,1			

n = number of observations
r = coefficient of correlation

Table 2:

Horse No.	Type	Mean Average Time per circle sec.	Mean Fastest Time per circle sec.	Mean Deceleration Time Heart Rate min.
Nos. 1 & 3	Heavy Hunter	10,3	9,5	16
Nos. 2 & 4	Thorough-bred	10,4	9,7	9
	p	<0,45 >0,40	<0,3 >0,2	< 0,005

Table 3:

Metabolite	No. of Samples	Mean	SD	Range
Lactate mg./100 ml.	43	5,5	± 1,76	(2,8 – 11,0)
Pyruvate mg./100 ml.	41	0,5	± 0,19	(0,24 – 1,28)
Lactate/pyruvate ratio	39	11,4	± 3,01	(4,7 – 17,8)

(From Anderson, 1973)

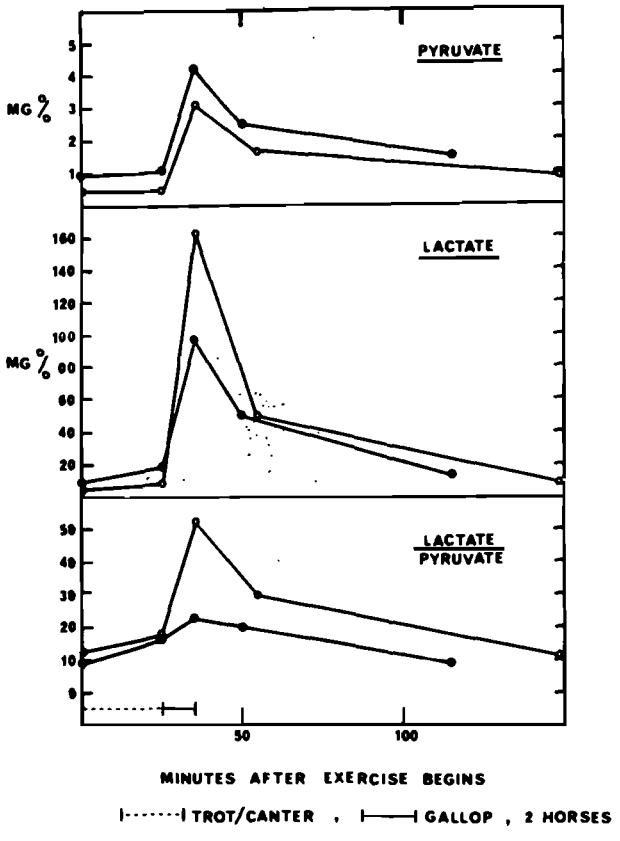

Fig. 2: Effects of exercise on blood lactate, pyruvate and lactate: pyruvate ratio. (From Anderson, 1973).

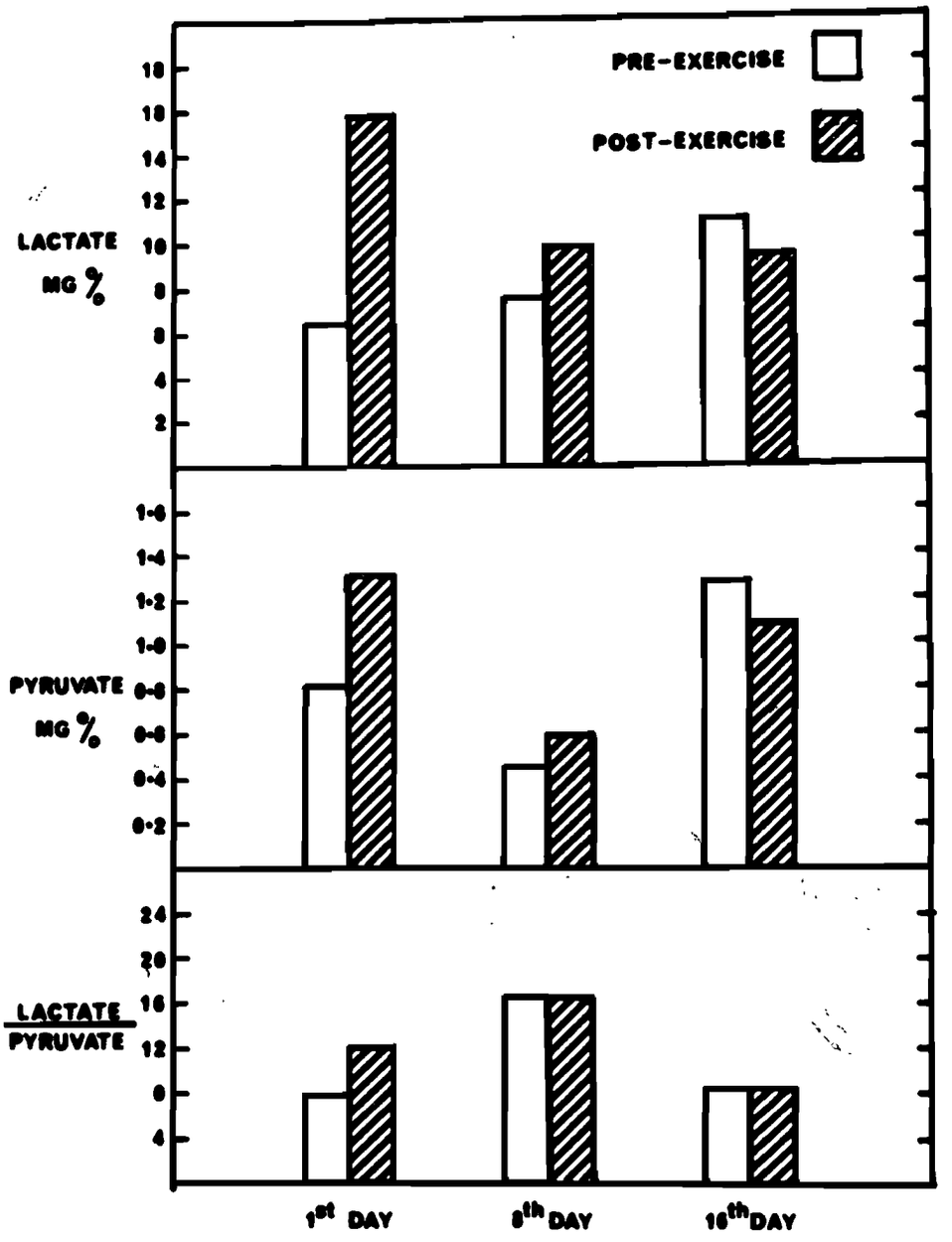

Fig. 3: Effect of training on blood lactate, pyruvate and lactate: pyruvate ratio. (From Anderson, 1973).

Serum CK Levels

The normal range for serum levels of CK in 10 horses was found to be 111 ± 33, a range of 63 to 170 mIU/m*l*. Daily variation in CK levels was found in two horses to be 58-84 and 42-75 mIU/m*l* respectively. Sampling before, during and at intervals for 72 hours after exercise revealed elevations of CK which were maximal 5-6 hours after exercise. Exercise consisting of trotting and cantering only, resulted in changes of similar magnitude to those produced when exercise included galloping.

Weekly repetition of an exercise programme involving trotting, cantering and galloping over a total distance of 20 km produced a training effect as shown in figure 4. The increase is expressed as a percentage of the initial increase.

SIMULTANEOUS PHYSIOLOGICAL AND BIOCHEMICAL OBSERVATIONS

Horses

Six clinically normal animals, four geldings and two mares of Thoroughbred or heavyweight Hunter type, aged 8 to 17 years, were used. They were housed in loose-boxes and maintained on a constant diet throughout the experiments. Daily maintenance exercise, which all animals performed, unless otherwise stated, when not carrying out the experimental exercise programmes, consisted of walking and trotting for 20-30 minutes over a distance of 3 to 5 km.

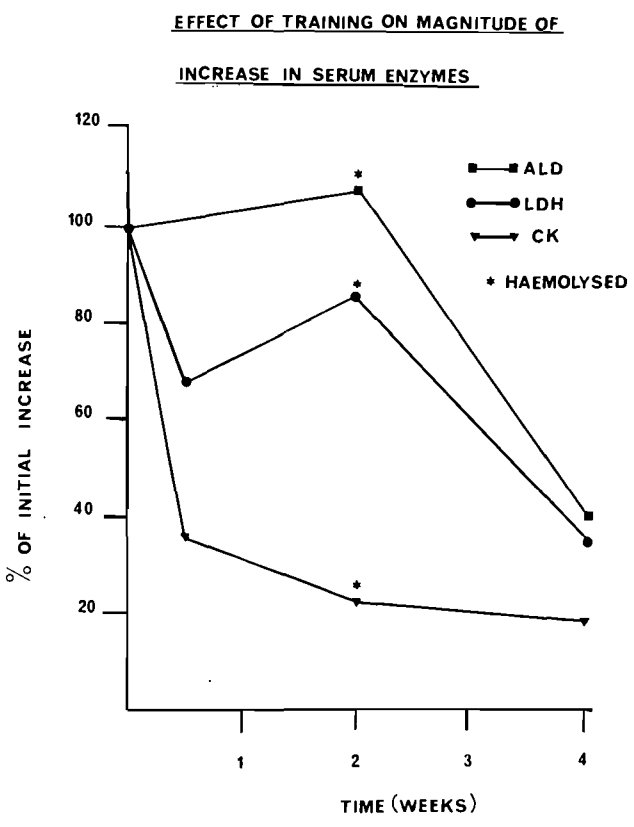

Fig. 4: Effect of training on serum enzymes. (From Anderson, 1973).

Exercise Programmes

Three experimental exercise programmes were used: all were performed in an indoor exercise unit.

Exercise A:

Ridden daily
Pace: Canter
Distance: 100 circuits of indoor arena
100 x 90 m = 9 000 m = 9 km
Duration: 27-33 min
Speed: 5-6 m/s

Exercise B:

Lunged daily
Pace: Canter
Distance: Approximately 6-8 km
Duration: 20 min
Speed: 5-6 m/s

Exercise C:

Ridden twice weekly
Pace: Trot
Distance: 20 circuits of indoor arena
20 x 90 m = 1 800 m = 1.8 km
Speed: 3,5-5 m/s
Pace: Canter
Distance: 120 circuits of indoor arena
120 x 90 m = 10 800 m = 10,8 km
Speed: 5-6 m/s
Total distance = 12,6 km
Total duration = 35 min

Table 4 lists the types, ages and sexes of the horses used, together with the experimental exercise programme which they performed. Where exercise during the weeks preceeding the experimental programme was other than the daily maintenance described, this is indicated.

Each horse performed an experimental programme on at least four occasions. Throughout the tests each horse was ridden or lunged by the same person to avoid effects due to different weights or styles of riding or lunging. The speed of cantering and trotting by each horse was maintained as constant as possible. The time taken to complete one circuit of the arena or lunge ring was measured every four minutes during Exercise A and C and every two minutes during Exercise B.

TABLE 4

Experimental Exercise	Horse No.	Exercise History	Type	sex
A	1	Maintenance	Thoroughbred	Mare
A	2	Maintenance	Heavy Hunter	Gelding
A	3	Maintenance *+ jumping	Heavy Hunter	Gelding
B	4	Lungeing	Thoroughbred	Gelding
B	5	Lungeing	Thoroughbred	Gelding
C	4	Lungeing	Thoroughbred	Gelding
C	6	Walking	Heavy Hunter	Mare

RESULTS

Exercise A

Table 5 shows, for Horses No. 1, 2 and 3, average cantering speed; maximum heart rate and respiratory rate during exercise; heart rate deceleration time, expressed as the time in minutes for heart rate to fall from 100 beats per minute to 50 beats per minute; respiratory rate deceleration time, expressed as the time for respiratory rate to fall from 70 to 25 per minute; CK concentrations in serum before and after exercise with the actual rise in CK and the rise expressed as a percentage of the pre-exercise concentration. Actual and percentage changes in lactate and pyruvate levels are similarly listed. Serum CK levels were those immediately after exercise. The relationship between heart rate deceleration and percentage rise in CK is illustrated for horses No 1 and 2 in Figure 5.

In Horse No 1, heart rate deceleration time was longest on the first day and was subsequently reduced by 60 per cent. This initial prolonged recovery did not correspond to the greatest increase in CK which occurred on Day 5. The increase in CK was smallest on Day 11. The increases in lactate and lactate/pyruvate ratio fell progressively until Day 11 when large increases ocurred.

Horse No 2. showed no improvement in heart rate deceleration over 5 days and there was no rise in CK.

Horse No. 3 consistently showed a longer heart rate deceleration time (24 to 29 min) than No 1 and 2 (7 to 9 min). This did not diminish over 12 days and was accompanied on Day 2 by a negligible rise in serum CK.

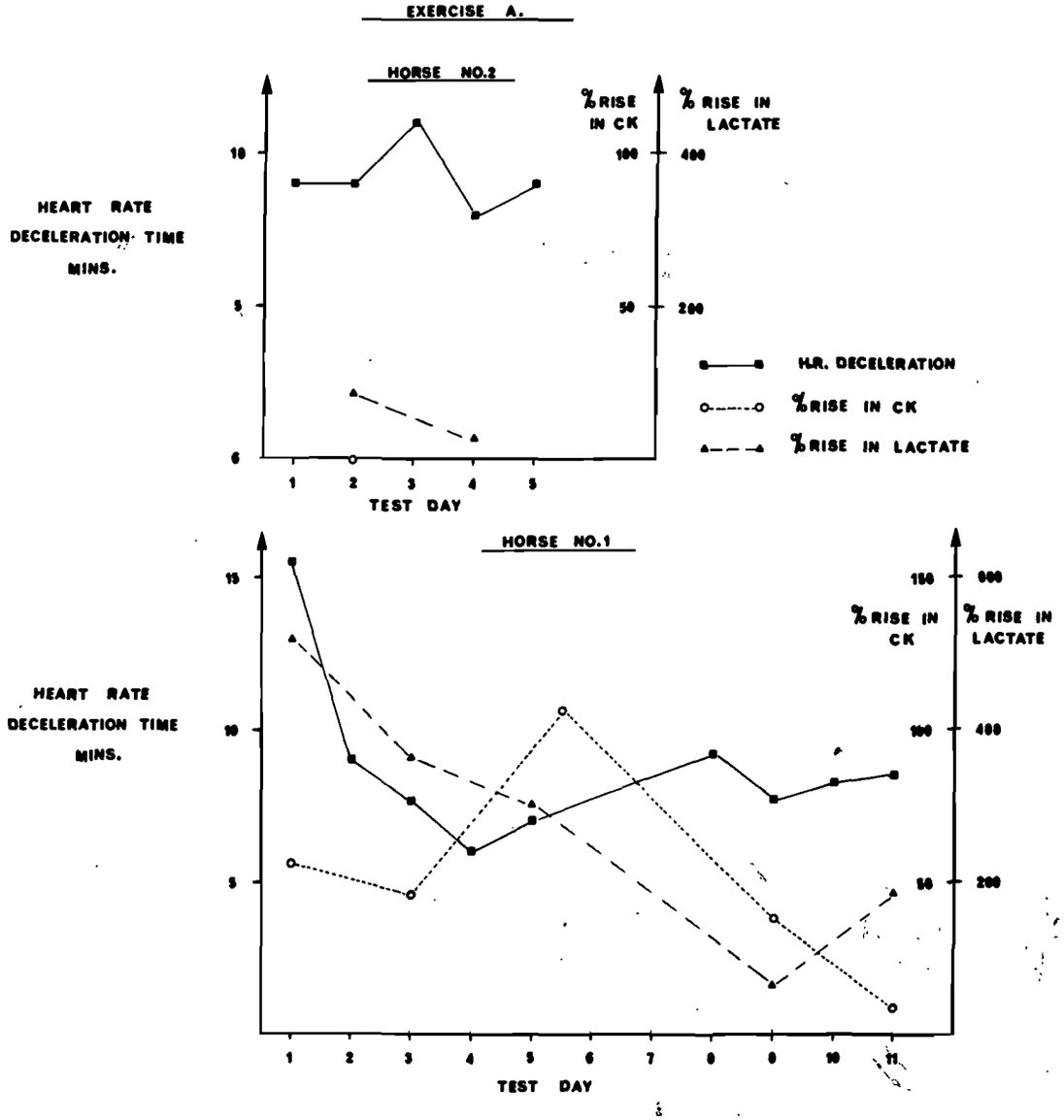

Fig. 5: Heart-rate deceleration times, changes in serum concentrations of creatine kinase and in blood concentration of lactate in two horses after cantering 9 km daily.

Exercise B

Table 6 shows the effects on Horses No. 4 and 5 of exercise on the lunge-rein.

Horse No. 4 showed a steady cardiac and respiratory deceleration time and no increase in CK immediately after exercise. This rise in lactate, however, was smaller on Day 5 than on Day 2. This is illustrated in figure 6.

Horse No. 5 showed a slight improvement in heart rate deceleration and CK increased less on Day 3 than on Day 1.

Exercise C

In this scheme, samples for CK estimation were taken five hours after exercise and metabolites were not measured. In figure 7 times for heart rate to fall from 100/min to 50/min are shown for each occasion of twice-weekly exercise, together with percentage increases in CK. Horse No 5 showed negligible changes in comparison to Horse No 6.

DISCUSSION AND CONCLUSIONS

On the basis of observations on heart rate and respiratory rate, Horse No. 2 was fit for the amount of exercise it was required to perform. Horse No. 3 showed no improvement and might also have been considered fit or alternatively as requiring a very extended period of training. Horse No. 1 showed an improvement. Biochemically, on the basis of CK changes, none of the three was clearly unfit although some improvement was indicated in No. 1. Lactate changes were not great. This might have been expected as galloping was not included in the exercise schemes. Changes in lactate/pyruvate ratio were variable. Of the three horses, No. 3 had been performing more work than had the other two prior to the experiment.

Horse No. 4 appeared fit, physiologically and on CK changes, for Exercise B. Lactate increases, although small, might have indicated some training effect. Horse No. 5 showed a slight improvement bioche-

Table 5: EXERCISE A

Test Day No.	During Exercise: Average Speed sec/circuit	Maximum Heart Rate /min	Maximum Respiratory Rate /min	Heart Rate Deceleration Time (min) 100/min →50/min	Respiratory Rate Deceleration Time 70/min →25/min	Serum: Creatine Kinase Pre mIU/ml	Serum: Creatine Kinase Post mIU/ml	Serum: Creatine Kinase Inc	Serum: Creatine Kinase % Inc	Blood: Lactate Inc mg/100ml	Blood: Lactate % Inc	Blood: Pyruvate Inc mg/100ml	Blood: Pyruvate % Inc	Blood: Lactate:Pyruvate Ratio Inc	Blood: Lactate:Pyruvate Ratio % Inc
Horse No. 1															
1	14,7		114	15,5	8,2	71	111	40	56,3	17,2	521	0,2	62,5	29,1	282,5
2	14,7		104	9	4,7	245	296	51	20,8						
3	15,4	130	108	7,7	9,5	146	213	67	45,8	15,3	364	0,02	4,3	31,5	346
4	15,9		108	6	1,2										
5	16,1	144	100	7	3,5	73	151	78	106	15,8	303	0,03	8,5	40,4	271
8	15,1		108	9,2	3,5										
9	15,1	144	108	7,7	4,5	63	87	24	38	- 2,2	- 34,3	- 0,19	- 40	1,4	10,2
10	15,6		104	8,2	4,5										
11	15,6	132	108	8,5	2,5	67	73	6	8,9	16,4	188,5	- 0,35	- 76	209,3	1107
Horse No. 2															
1	17,5		124	9	3,7	104									
2	17,7		116	9	7,5	86	85	- 1	- 1,1	2,7	84	- 0,03	- 7,3	7,7	98,7
3	16,0		116	11	6,7										
4	16,4	168	116	8	7	100				1,0	25	- 0,15	- 33	6,9	83,1
5	16,8		112	9	6										
Horse No. 3															
1	15,4		108	24	9,7	90				18,1	532	0,12	70,5	4,2	21
2	15,8		104	28	8	102									
3	15,4	144	108	43	15	91	112	21	23	24,5	340	0,72	156,5	11,2	71,3
4	15,9		104	23,5	4,7	100									
5	16,4		104	40,2	8,5	106									
8	16,3		108	18	6	102				39,1	1086	1,2	400	16,5	137,5
9	16,3		100	24	3,2										
10	16,1		104	25,5	5										
11	16,3		104	27,7	2,5	96				11,6	232	0,7	162	3,1	26,7
12	16,2		108	29,5	3,5										

Table 6: EXERCISE B

Test Day No.	During Exercise: Average Speed sec/circuit	Maximum Heart Rate /min	Heart Rate Deceleration Time (min) 100/min →50/min	Respiratory Rate Deceleration Time (min) 70/min →25/min	Serum: Creatine Kinase Pre mIU/ml	Serum: Creatine Kinase Post mIU/ml	Serum: Creatine Kinase Inc	Serum: Creatine Kinase % Inc	Blood: Lactate Inc mg/100ml	Blood: Lactate %-Inc	Blood: Pyruvate Inc mg/100ml	Blood: Pyruvate % Inc	Blood: Lactate:Pyruvate Ratio Inc	Blood: Lactate:Pyruvate Ratio % Inc
Horse No. 4														
1	9,9	108	10	3,5	121									
2	10	108	7,5	3,5	90	86	- 4	- 4,4	13,3	141	- 0,17	- 54,8	131,8	434,9
3	9,9	102	10,2	3,0										
4	9,7	102	8	2,5										
5	9,8		8,7	5,2					1,1	31,5	0,13	100	9,2	34,2
Horse No. 5														
1	8,5	114	13	9	138	187	49	35,5						
2	9	108	14	8,7	160									
3	9,2	108	9	6	150	152	2	1,3						
4	9,1	114	7,5	2										
5	9,2	114	12,5	13,5										
8	10,0	108	6,5	2										

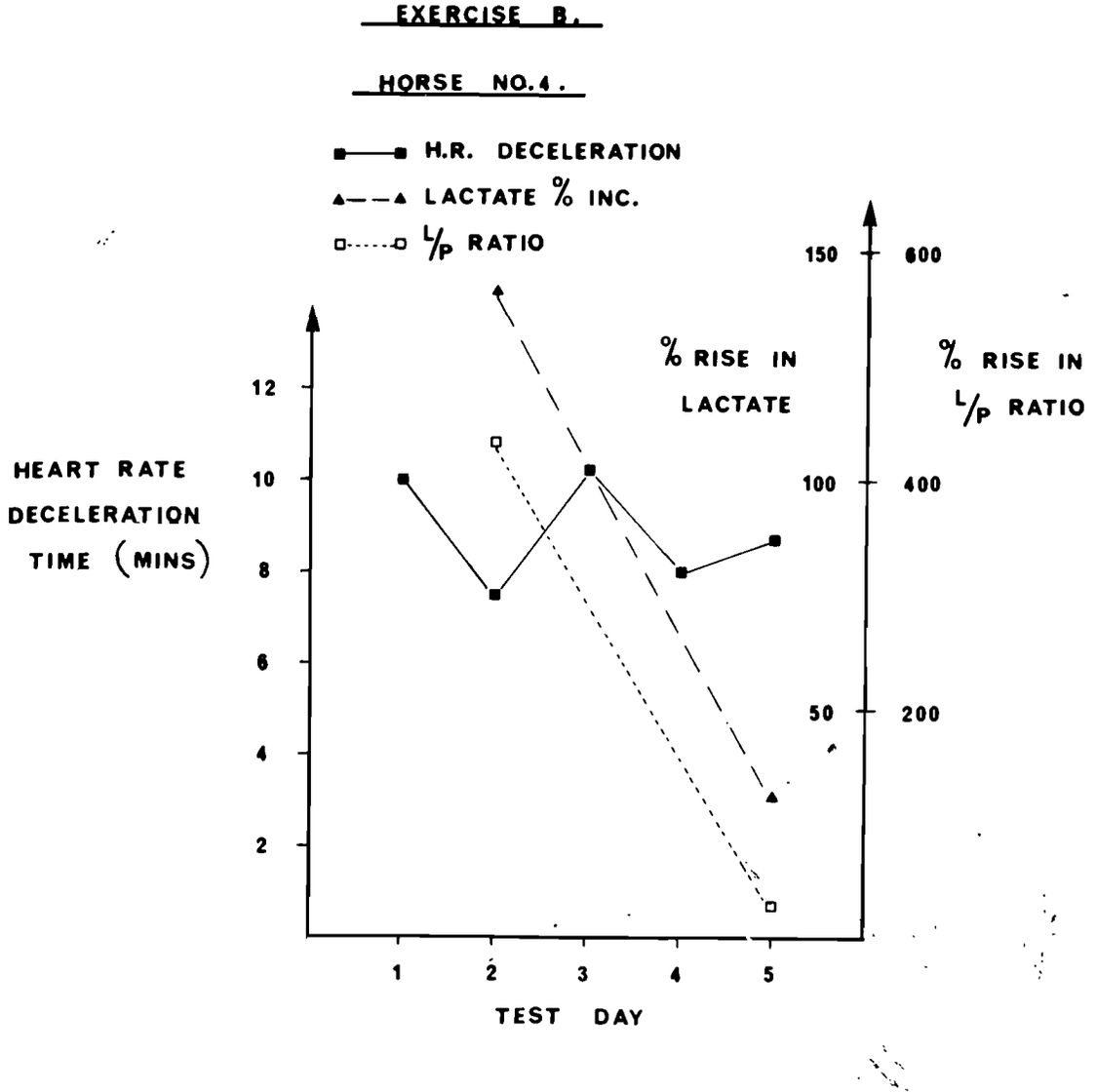

Fig. 6: Heart rate deceleration times, lactate and lactate: pyruvate ratio in one horse after cantering on the lunge-rein for 20 min daily.

mically and physiologically. Both these horses were performing regular lunging exercise prior to the experimental programme.

When Horse No. 4 began Exercise C, eleven days after completing its last performance of exercise B, it again appeared fit on the basis of heart rate deceleration. Increases in CK were greater than during Exercise B but remained steady except on Day 2 when the increase was greatest. Horse No. 6, in contrast, was judged unfit on physiological and biochemical observation. The most marked difference between the parameters measured was between Day 1 and Day 2, particularly on the basis of CK increase. This horse had performed only gentle walking exercise for two weeks prior to the experimental exercise.

CK levels were measured five hours after completion of Exercise C, whereas they were measured immediately after Exercise A and B. Also, Exercise C was performed twice weekly while Exercises A and B were carried out daily.

As CK levels had been shown to reach a peak 5 hours after exercise and to return to pre-exercise levels only within 48 hours [2], the risk of failing to detect CK increases was minimized in the case of Exercise C. Levels of CK before exercise were found to be higher on Days 2 and 3 than on Day 1 in Horse No. 1. This might have suggested a delayed, prolonged rise in CK following the first performance of Exercise A.

The difference in cardiac and respiratory deceleration times between Thoroughbred and heavy Hunter type horses previously observed was again apparent between Nos. 1 and 3 in Exercise A, and between Nos. 4 and 6 in Exercise C. Horse No. 2 performed Exercise A extremely slowly and its programme was halted when it became lame. It had been shown previously that in biochemical changes there was no apparent difference between horses of the two types [2].

We conclude that the exercise programmes used in the above experiments were not sufficiently severe to produce clearly significant changes in biochemical parameters in the horses used, with one exception, namely Horse No. 6. The CK increase even in this unfit horse was marked on the first occasion of exercise but small thereafter. The biochemical changes we measured did not appear to provide a sensitive index of gradual improvement in fitness. Rate of recovery after exercise as measured by rate of return to resting levels of heart and respiratory rates revealed the

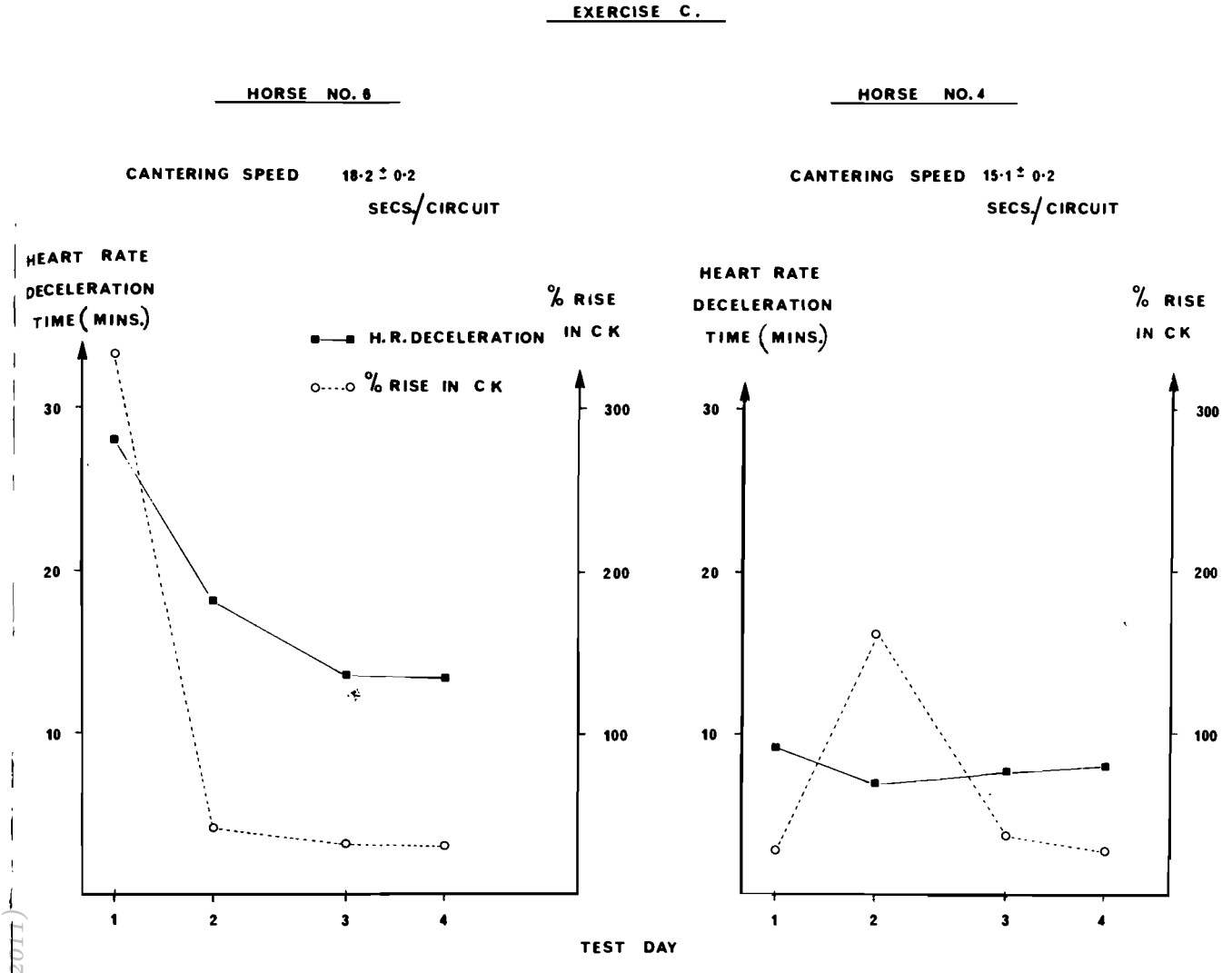

Fig. 7: Heart rate deceleration times and CK changes in two horses after trotting and cantering 12,6 km twice weekly.

degree of unfitness apparent biochemically but also revealed improvement not biochemically apparent. Nevertheless, factors other than state of fitness which may influence any parameters examined cannot be ignored.

It is accepted that a horse must be trained by exercise programmes designed for the physical work it is intended to carry out. Training results in an animal performing without becoming severely fatigued. As training progressed in the tests we have described, the efforts required of the rider or handler to maintain a steady speed in prolonged cantering exercise were reduced, but speed did not actually increase even in the lunge test where no restraint was exerted by the handler.

In daily and weekly performance of one or two furlong gallops over periods of approximately six weeks, we have not found horses to improve their speed of performance. The initial short gallop after a period of rest was in many cases the fastest performance. Training reduced variability rather than improved speed.

ACKNOWLEDGEMENTS

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DISCUSSION

S.G.B. Persson: Have you tested the reproducibility of heart rate and respiratory rate in relation to time?

Maureen Aitken: Yes, these results were really taken from experiments where I was going to look ultimately at effects of drugs on heart rate and heart rate deceleration. We only began using the drugs when we were obtaining constant, reproducible results and we found that we had to make it as low as 50, because some of the exercise programmes which we were using and some of the other performance tests subjected the animal to even less stress than they were here. We did find that if we kept the conditions very constant and carried the determinations out indoors that the results were reproducible.

J.R. Holmes: I would just like to ask you a question about cardiac deceleration. If it is accepted that in heavy weight Hunters cardiac deceleration takes a longer time than in the Thoroughbred, the question is: Why?

Maureen Aitken: That is a very good question. I think this is as might be expected from unconscious observation and observation by lay people that heavy weight Hunters are less fit than Thoroughbreds and that they do take twice as much longer to recover. It was probably badly put with no real reason apart from the fact that we expected it.

J.R. Holmes: But your horses were all unfit. All of them.

Maureen Aitken: Yes, but this is another point, I cannot remember the publication but I think someone once wondered whether a Thoroughbred was almost a natural athlete. It was suggested that Thoroughbreds never become tremendously unfit compared to other types of horses.

XX WORLD VETERINARY CONGRESS

THESSALONIKI 6-12 JULY 1975
GREECE

INVITATION

The Organizing Committee of the XXth World Veterinary Congress invites to Thessaloniki the veterinary surgeons of the whole world and all the friends of our profession, from 6 to 12 July, 1975.

At the XIXth World Veterinary Congress held in Mexico, Greece offered to organise the XXth Congress, in Thessaloniki. The Association of Greek Veterinary Surgeons feel very honoured with the acceptance of their offer. On this occasion, the splendours of the "hippiatri" of ancient Greece and the famous byzantine veterinary surgeons will be revived.

This Congress, as well as the previous ones (Madrid 1959, Hannover 1963, Paris 1967, Mexico 1971), is under the aegis of the World Veterinary Association, which comprises over 100 000 veterinary surgeons belonging to 55 countries and 11 International Organizations of Veterinary Specialists.

The city of Thessaloniki is the seat of the splendid Aristotelian University of Thessaloniki, which encompasses one of the youngest Schools of Veterinary Medicine in Europe, founded in 1950. The School will celebrate its 25th anniversary at the same time with the XXth World Veterinary Congress. Its personnel and students will take an active part in the organization of these events.

The installations of the Congress have been chosen with the object of providing the participants with every working facility and comfort. In order to facilitate group discussions and contacts among the congressists, we have tried not to overburden the scientific programme.

The Congress will offer its participants a pleasant stay in a historical city, modern and of great interest to tourists with its roman and byzantine monuments, its medieval fortifications, its modern hotels and picturesque beaches.

We believe that "xenia", the spirit of hospitality of the Greeks, world famous since ancient times, and the classic and natural beauties of Greece will leave you the best of memories.

We hope that you will be able to take part in this Congress, which will help strengthen the bonds of friendship and cooperation among the veterinary surgeons of the whole world.

Prof. E.J. Tsiroyannis
President of the Organizing Committee

Jl S.Afr. vet. Ass. 45(4) 371-373
(Proc. 1st int. equine vet. Conf., Pretoriuskop 1974)

OPEN DISCUSSION ON RESPIRATION, BIOCHEMISTRY, ELECTROLYTES AND PERFORMANCE

CHAIRMAN: W.L. JENKINS

J.R. Coffman: Regarding Dr Gillespie's warning this morning against ascribing all aspects of lactic acid acidosis to oxygen debt, there is one very straightforward factor, namely thiamin. Just as decarboxylation of pyruvate is thiamin-dependent, so is the citric acid cycle at the alpha-ketogluterate level. In sheep. lactate-induced central nervous signs may be blocked by thiamin. Have any of the speakers looked into the effect of thiamin on the conditions described in any of the papers today?

A. Lindholm: The pyruvate concentrations followed the lactic acid concentrations but were not very high in any of our experiments. As we were not interested in pyruvate per se, I cannot really throw any light on this question.

A.M. Merritt: Does Dr. Lindholm consider it possible and feasible to devise a feeding programme for horses in training which would be similar to that applied to human athletes when it is desired to pack the muscles with glycogen just before a race in order to obtain maximum utilization? Would it be practicable for the horse?

A. Lindholm: This is an important issue. For long distance racing I do not have the answer. In short distance racing of three to four minutes' duration, the muscle glycogen is sufficient to stand the drain, consequently consideration of prior glycogen loading does not apply.

I have tried giving horses carbohydrate to increase their glycogen levels but it appears as if the mechanism is different to that in human athletes, where there is an overshooting of the mark and their glycogen has to be replenished. It takes about 48 hours to replenish the glycogen stores, whether a carbohydrate-rich diet or a normal diet is provided.

For very long races it could be important to load the horse's glycogen stores as much as possible.

J.W. Evans: Have you any experience of glycogen loading by change of diet or other procedure? We have been looking at the trees instead of the forest. We have to consider increasing the horse's performance. Can we indeed change the traditional ways of training a horse, developed over all these years for the Thoroughbred, so that we can increase the efficiency of utilization and change the metabolic profile within the muscle of the animal and the enzymes that have to deal with the substrates in such a way that the horse can do a better job of running? I do not think we have addressed this question today.

D.H.G. Irwin: I have understood that lactic acid levels fell more readily after strenuous exercise when the horse was kept in motion than when it was put back into the stall.

In some parts of the world horses are hosed down after strenuous exertion. There is a belief that human athletes should not take a cold shower in the immediate post-exercise period. On the supposition that one is aiming at clearing lactic acid to avoid injury to muscle cells, which presumably will occur if a high concentration of lactic acid is allowed to remain for any length of time, will the practice of 'hosing down' horses impede the clearance of lactic acid?

H.H. Krzywanek: When horses come into the stable after a race they have a high concentration of lactic acid in their blood, in the muscle it is even higher, I believe. In our experiments we tried to determine the best time to subject the horse to moderate exercise after a race and found a pause of 10 minutes to give the best results. Taking the horses on to the track again after only two minutes was not beneficial.

The horses were washed with water and soap during the brief rest period. I do not know whether this has any effect on the lactic acid clearance. I believe a jog of about 5-6 minutes on the track after a rest of about 10 minutes is the best.

A. Lindholm: We have determined blood lactate every minute after racing. After 10 minutes the highest level was attained, then it started decreasing. Consequently the horse is at its highest blood lactate level if it is being exercised ten minutes after a race.

A. Littlejohn: Amongst the creditable and tremendous wealth of data presented here, there is not much on the sprinters, the 5 to 6 furlong horses. We have been looking at certain parameters over distances of 1 200 m, admittedly thus far only in three horses, so that conclusions as yet may not be drawn. Nevertheless the data are interesting.

During the first few hundred metres up to the 600 m mark during a 1 200 m sprint at the pretty fast rate of 12,50 m/s – about twice the speed of Dr Aitken's horses – arterial lactic acid initially is very high. At 600 m it drops some 10 to 15 per cent and then rises again to much the same levels as at the start. In view of the small number of cases studies so far, this might not be significant. Obviously there is some relationship to oxygenation, and thus there might be something to say for breath-holding.

The other interesting point is that in those horses the oxygen tension rises during a 1 200 m walk, so that it is about 10 to 12 per cent higher at the end than it was at the start of the walk.

During the trot there is not much difference in oxygen tension levels at the start and at the finish: there is a tendency, however, for it to decline. Again I have insufficient data to determine the significance of these differences. Perhaps Dr Persson could comment on the lactic acid picture.

Incidentally, after the sprint the lactic acid values fall very quickly, very much more quickly than after the 3 500 m walk periods reported. We measured lactic acid at 3 min intervals and found a very rapid drop, so that by about 12 min the lactic acid values had decreased to about one quarter of what they had been.

J.R. Gillespie: Prof. Littlejohn has made a very important contribution because it is quite a different matter measuring lactic acid, oxygen tension, carbon dioxide tension, pH, etc., when the horse is on the go than when he stops. When a horse reduces its ventilation from 2 250 *l*/min to 1 500 *l*/min as it stops racing, ± 1 600 mol of acid is added each minute. Hence CO_2 and lactic acid levels are definitely time-dependent.

Another point to consider is how fast the muscles are releasing that acid to the circulation. The body chemistry is intimately concerned here: the catecholamine level, the O_2 level, the pH at the level of the muscle. A combination of all these factors has to be considered when one looks at the lactic acid levels after the animal has stopped racing.

Maureen M. Aitken: May I ask Prof. Littlejohn whether he sampled during work?

A. Littlejohn: Yes.

Maureen M. Aitken: Our samples were taken at the end of different distances and at different speeds. On one occasion we measured 17 m/s.

S.G.B. Persson: One would expect the lactic acid level to be higher at the beginning and to decrease during a race and to increase again at the finish. At the beginning of exercise there is a delay in adaptation of the circulation. Until it adapts to the work load, the lactic acid concentration will be higher.

Dr Williamson mentioned that hypokalaemia was not very common in his material. We found that in cases of chronic diarrhoea hypokalaemia is very common and that potassium chloride by mouth is beneficial. has he had any similar experiences?

H.M. Williamson: None of the horses I included in this series suffered from a chronic disease condition. I was looking at horses clinically fit to race. I agree with Dr Persson that potassium deficiency. Although the serum analyses indicate a moderate potassium elevation, the horse has a low serum

I would like to re-emphasize that in cases of hypokalaemic alkalosis the over-all state of the horse is one of potassium deficiency. Although the serum analyses indicate a moderate potassium elevation, the horse has a low serum potassium once the alkolosis is corrected.

D.H.G. Irwin: Horses that hold their breath for a while when jumping from the gate at the start of a race are generally accepted as having a weakness in their racing ability. If so, how can breath-holding be overcome? Can a horse be taught to breath at every stride with proper training?

One fairly common problem is the horse that gets its tongue over the bit, possibly as a result of improper schooling. There is a technique for trying to prevent this. Could these two points be discussed?

J.R. Gillespie: I do not really believe that one may assume that it is necessarily a disadvantage if the horse holds its breath when jumping from the gate. The horse has a huge residual functional capacity in the lungs and gas exchange will continue quite satisfactorily for some time. I would argue that it would be more efficacious were the horse not to tie its stride by trying to breathe at that time but were to hold its breath until there is a breakthrough, just as there is in the human 220 runner. The mile or so race is very much the same for the horse as the 200 metres for the human, possibly a little longer. The human athlete holds his breath after breaking the block until about the first bend, when he will start breathing.

Purely from observations it seems that it would be a mechanical advantage to the horse were it to breathe in rhythm with its stride. The 'tucked-up' position could then be used as an expiratory force.

I should say that at least in the resting horse probably 35 per cent if not more, of the ventilatory effort is by the diaphragm. Although I did not discuss the point, I showed it on a slide that the horse's chest is very stiff. To move that chest at 100 x min appears to demand considerable work, so the longer it stays on diaphragmatic breathing the better.

One should have very good evidence about breath-holders being poor runners before accepting it as such.

A. Lindholm: Imagine the horse to take one breath every 6 seconds. Initially it is anaerobic and during the next 3 to 4 seconds all the muscle work is dependent upon ATP & CTP stores. When these are depleted, oxygen is required and the horse starts to breathe again.

M.A.J. Azzie: I find it difficult to conceive that a horse covering five furlongs in 58 or 60 seconds is going to breathe at every stride, which means at the rate of 100 respirations/min. If one allows one second for every inhalation and for every exhalation one comes to a respiratory rate of 30/min, which appears reasonable.

Does a horse actually breathe at a rate of 100/min in a gallop? I would appreciate having Dr Gillespie's opinion on this aspect of breathing in relation to stride.

J.D. Steel: I am somewhat perturbed about Dr Irwin's question concerning breath-holding, which has been going around in the horse world for the last 10 to 15 years. Until we use systems of monitoring the horse's respiration throughout exercise we will not really know whether it is actually holding its breath. A competent jockey might know. None of the jockeys I know has ever suggested to me that horses tend not to breathe at the start of a race. They often do preliminaries du ring which they become reasonably warmed up.

Study of an American slow motion film, made at, I think 450 frames/s, indicated that during galloping, at any rate, the horse would tend to breathe in at the top of the stride when all four feet are off the ground and breathe out as it comes down to the ground again. That does not necessarily imply that there has to be a respiratory cycle coinciding with every stride. There may well be a respiratory cycle with every 6 to 10 strides.

G.E. Frost: My question whether we are really sure that the horse holds its breath has already been emphasized by Prof. Steel: I concur. If it be assumed that a horse does, in fact, hold its breath when it jumps from the gate, this could conceivably affect its performance, especially in a short sprint. Unlike the human athlete, who can voluntarily adjust his breathing, the horse does not have that ability, so I believe. The horse's gait is more complex than that of man and it might well be that the moment the horse reaches a state of hypoxia and has to take a breath that that moment is inopportune and he might have to alter his stride sufficiently for him to be knocked out of the race.

S.G.B. Persson: Just a short comment on breath-holding. Trotters running on a tread-mill do breathe from the beginning; furthermore, breathing is certainly related to stride.

J.R. Gillespie: I do not think I said that horses breathe at every stride. I did say that they 'entrap' *i.e.*, they breathe with some multiple of stride frequency, at every so many strides. The same thing holds true for human athletes. What I did say, and this has been borne out by reference to the film, is that inspiration occurs when the horse is extended and expiration when he is 'tucked up'.

The best respiratory rate counts done on the horse during galloping are those by Mead. He records figures from 110-135/min. These I regard as the only ones being reasonably reliable. That again does not answer very much. If one forces a horse to take several strides before breathing, it will have to take a deeper breath because in the final instance it has to maintain minute ventilation. Hence it has to expend considerably greater effort against a stiff chest.

I am not perturbed about a horse holding its breath for several strides from the gate. He is not going to become hypoxaemic: there is sufficient oxygen for continued gas exchange. If anything, I would be concerned about CO_2. If the horse reaches the breakthrough point and starts to breathe, it will be as a result of acidosis and not hypoxaemia.

W.L. Jenkins (from the chair): The one question as yet unanswered is that of the horse getting its tongue over the bit. Has anyone any views on this matter?

H.M. Williamson: It is difficult to evaluate the observations made over many years by old-time trainers who mostly have had no scientific training but who, as a race, are pretty astute observers. Certainly some horses must get their tongues back and when they come in after the race they have their tongues over the bit. I believe that such horses have problems, certainly when their tongues are tied they do better.

C.J. Roberts: My contribution concerns the acute type of azoturia seen occasionally in the horse – one or two cases per year. The stables in New Zealand are very close to the track and the horses do not get home immediately. Such cases are seen to seize up with the typical clinical picture of Monday morning disease. One has to get to the case quickly and administer adrenaline hydrochloride – as much as 4 ml of 1/1 000 solution very slowly intravenously – on the supposition that adrenaline is a dilator of muscle capillaries and gets the circulation back to the muscles. The horses sweat and drool but return to normal in a day or two. Until we did this we found cases very difficult to treat and wasting of the gluteal muscles would sometimes supervene.

A. Lindholm: I have biopsied about 75 horses with acute rhabdomyolysis and have found lower glycogen levels in the muscles, higher lactate levels and lower CTP and ATP levels. That would indicate an hypoxic condition. I also found that upon training it was mainly the horses with low-oxidative muscle fibres that were invloved. Systematic examination of these muscles after such attacks revealed a severe damage to the muscle. Even if one tried to treat with saline as we did, one still had a damaged muscle which took several weeks to heal though the horse appeared clinically sound.

S.G.B. Persson: I would like to question Dr Lindholm on the possible significance of the myoglobin content of muscles, particularly as a possible oxygen store. If it is, what is the enzymal milieu of the muscle?

A. Lindholm: In humans much research has been done on this aspect and it is considered to be very important. It is very difficult to measure the myoglobin content of muscle, we have not done that at all. I believe it to be very important and deserving considerable investigation.

J.D. Steel: We have tried to estimate myoglobin but found it extremely difficult to obtain a satisfactory method. In a muscle biopsy specimen one has a mixture of myoglobin and haemoglobin, so that one either has to separate them or measure the myoglobin in the presence of haemoglobin. The over-all evidence seems to be that in those species which engage in high muscle activity the myoglobin levels are high. One may say with certainty that the Thoroughbred has at least twice

as much myoglobin in its muscle. Not only is myoglobin an oxygen store but it may also act by speading the inward flow of oxygen from the capillaries to the mitochondria, a suggestion that has come out in a paper by Wittenberg in Physiological Reviews. One could have myoglobin playing a considerable part in the whole question of oxygen delivery.

A. Lindholm: I forgot to mention that whale muscle, for instance, has about twice the myoglobin content of equine muscle. The question is: Why?

W.L. Jenkins: Regarding Dr Roberts' suggested therapy, we know that adrenaline is an alpha and beta agonist. It is believed that there are beta receptors in the vascular bed of skeletal muscle; these would be vasodilatatory. There is the complication of the presence of sympathetic cholinergic fibres as well, nevertheless the treatment seems rational in principle.

M.A.J. Azzie: Would anyone care to comment on the possibilities of improving the alkali reserve and thus the working capacity of the animal by doing so, *e.g.* by administration of alkalies like sodium bicarbonate orally, or lactate-Ringer, or, going to the complete extreme, administration of Darrow's solution as an electrolyte source either just before a race or say 48 hours beforehand.

M.C. Morison: In my experience horses will race more effectively when the alkali reserve has been built up. It can be done by feeding sodium bicarbonate at the rate of 1 to 2 ounces a day, 4 to 5 days prior to a race or one may inject sodium bicarbonate at the rate of about 1 *l* of a 9 per cent solution within the last 24 hours before racing. It seems to be more efficient the closer to the race it is administered.

H.M. Williamson: This would seem to confirm the human work. A reference has been quoted to the finding of enhanced endurance in human athletes after feeding sodium bicarbonate not only just before a race but for a couple of days afterwards, to obviate any severe acidotic reactions resulting from discontinuance of bicarbonate administration.

In view of my observations of horses in a state of alkalosis not racing well, I must sound a note of caution. It may be possible to tip horses over into an alkalotic state and so actually impair their performance. Until further study has been undertaken, no categorical statements can be made and only a warning mav be given.

J.R. Gillespie: It surprises me that if sodium bicarbonate were given much before a race it would not be excreted by the kidneys. I cannot reasonably conceive that much would be held in reserve and such a procedure does not make sense.

M.A.J. Azzie: I would like to add a clinical observation which supports Dr Williamson's line of thought. The idea of building up alkali reserve had been introduced into South Africa and sodium bicarbonate had been administered upon request. In no less than three cases the animals were found lying down 2 to 3 hours after bicarbonate administration. This suggests that if animals are going to race 2 to 3 hours after bicarbonate administration, it is not going to do them any good.

May I suggest that the desirability and/or dangers of administration of alkalies be investigated further, particularly when they are not indicated or not required by the animal.

W.L. Jenkins: I must throw my lot in with Dr Gillespie with regard to bicarbonate administration. It is a puzzle in a physiological sense, inasmuch as bicarbonate is quickly eliminated.

J.G. Boswell: As regards the question of handling of horses before a race, could anyone venture an opinion on the way horses should te transported, *i.e.*, sideways or facing the driver, or facing away from the line of travel: how long before the race should they be at the track; and when and what should they last be fed.

Concerning air transport of which some of your probably have had more experience than we have in South Africa, the same question concerning direction of travel arises. It was mentioned that having the horses facing the tail of the plane was not all that satisfactory and that it was much better placing them sideways across.

C.J. Roberts: In New Zealand we have a type of horse float in common use taking seven horses, the one at the tail end being placed cross-wise. It is a common belief that the latter travels best on the whole.

W.L. Jenkins: Have you any comments on the time interval: how long before a race must horses be taken to the course?

C.J. Roberts: When the tracks are fairly close, may be up to 80 to 100 miles, they are usually taken on the day of the race. Beyond that, say 200 to 300 miles, they are taken several days prior to the race as a rule. Over long distances, say 400 miles or more, trainers prefer to take them a week-end before.

H.M. Williamson: In New Zealand horses are flown in groups of six, four facing forward and two backward, all with their longitudinal axes in the direction of flight. Those that face backward appear to arrive just as well as those that face forward. Several trainers have commented that they have had their floats constructed so that the horses travel facing aft: they seemed much happier with this idea.

R.S. Thornbury: Regarding the 6-horse-float in which the one horse is placed cross-wise and the other facing the line of travel. these horses are transported this way every Saturday and usually Wednesdays as well, over distances of 50 to 60 miles. Occasionally horses are flown to Melbourne overnight, a distance of 500 miles. We are quite convinced that horses travel best cross-wise.

J.D. Steel: There is some excellent Swedish work on exercise physiology according to which a conversion of 15 per cent of haemoglobin to carboxyhaemoglobin leads to a 15 per cent decrease in work performance. A biochemist friend of mine, who is concerned with environmental pollution, commented on floats being towed hehind motor cars and the horses breathing in carbon monoxide from the exhaust. A study should be undertaken to see whether horses arrive at the race track with significant amounts of carboxyhaemoglobin in their blood, which would interfere with their racing performance. If such an observation – purely hypothetical at the moment – were to have any validity, it would support the slight preference of horses travelling side-on or even with their tails facing the touring vehicle.

M.A.J. Azzie: Despite the wide field covered, at no stage today has the oxygen consumption of the horse been considered. In South Africa routine tests are conducted on every potential mine worker. Any man that has an O_2 consumption of less than, I think, 2,8 *l*/min is not considered physically fit for the work he has to perform. Have any of you given consideration to the performance of similar O_2 consumption tests on the horse?

J.R. Gillespie: That is the essence of what we really would like to examine as far as the respiratory system is concerned. We have talked at length about our ultimate aims; the problem is to get down to doing it when the horse is going full out.

We have some ideas of how we might get at it: the problem is trying to determine O_2-consumption without interfering with breathing at full pace. I might be able to come up with an idea I believe will work.

S.G.B. Persson: Could you tackle the problem backwards by measuring the cardiac output in terms of arterio-venous oxygen tension differences and so calculating the oxygen uptake? That is what we did.

J.R. Gillespie: The problem lies in doing that with the horse going dead-out, that is quite a different proposition.

S.G.B. Persson: You could consider using a tread-mill.

J.R. Gillespie: I doubt if one could get a horse to run at absolutely full speed on a tread-mill.

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Drs. Pierre Bourdin, Felix Lucam, Danielo Codazza and Pierre Derome trying their hand at African music, after Dr. Hugh Tracey and his son Andrew's excellent lecture demonstration on this topic.

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