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THE CARDIOVASCULAR EVENTS AND ACID-BASE STATUS
OF OVERFED PONIES DURING THE ONSET OF ACUTE LAMINITIS

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THE CARDIOVASCULAR EVENTS AND ACID-BASE STATUS OF OVERFED PONIES DURING THE ONSET OF ACUTE LAMINITIS

By

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ABSTRACT

THE CARDIOVASCULAR EVENTS
AND ACID-BASE STATUS OF
OVERFED PONIES DURING THE
ONSET OF ACUTE LAMINITIS

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Twelve Shetland ponies were fed a high starch ration. Seven ponies which had a transitory metabolic acidosis developed laminitis 56 ± 3.5 hours after overfeeding. These ponies also developed a persistent hypokalemia, hyperthermia, and increased heart rate 24 hours before the onset of lameness. Serum sodium, serum chloride, hematocrit, plasma volume and blood volume were unchanged. At the onset of clinical signs of laminitis, cardiac output and blood pressure increased but total peripheral resistance was unchanged. None of the measured or calculated parameters predicted the onset of laminitis. Hypertension appeared to be a response to rather than a cause of lameness.

Three of the remaining ponies apparently died of shock 29.3 ± 2.7 hours after overfeeding. All three had severe metabolic acidosis, decreased cardiac output, systemic arterial pressure and plasma volume, and increased hematocrit, total peripheral resistance and pulmonary vascular resistance.

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LITERATURE REVIEW

Introduction

Laminitis is defined as inflammation of the ungulate foot. Although the so-called cardinal signs of inflammation (redness, heat, swelling, and pain) are often present, the cytologic and histologic signs of inflammation are inconclusive in this disease. Commonly known as "founder," laminitis has been recognized for centuries as a problem in cattle and horses, yet its etiology is still unknown. Equine laminitis was described by Zenophon, Aristoteles, Apsyrtes and Hierocles (cited by Akerblom, 1934).[39] They recorded the clinical signs and hypothesized on the etiology of the disease. Laminitis has also been reported in pigs (Nilsson, 1964; Maclean, 1968)[33] and experimentally in lambs (Morrow et al., 1973).[35] It is characterized by congestion of blood vessels in the laminae of the hoof (Adams, 1974).[2] Laminitis may be acute or chronic and may affect two feet or all four. In horses the forefeet are the most susceptible, while in cattle laminitis is more common and more severe in the hind hooves. The chronic stage of the disease often results in hoof changes. Common sequelae are rotation and osteitis of the third phalanx.

Clinical Signs

When the forefeet of the horse are affected with laminitis, the horse assumes a characteristic attitude (Adams, 1974).[2] The forefeet are placed forward and the hindfeet are carried under the body to shift the horse's weight to the heels of the feet. The foundered animal is reluctant to move in this acute stage and may show anxiety, trembling, and elevated temperature. Increased respiration and an elevated heart rate are also seen. Locally, there is a digital pulse with a very warm coronary band.

In the chronic stage there is often rotation of the third phalanx. This rotation has been suggested as being the result of the pull of the deep flexor tendon with corresponding deterioration of the laminae (Coffman *et al*., 1970).[11] In severe rotation the anterior point of the third phalanx may be pushed through the sole of the foot. Complications such as sole ulceration, a most common hoof problem in dairy cows, may be predisposed by severe, concurrent laminitis (Chew, 1972).[8] In the chronic stage, the horse has a tendency to walk on its heels, and subsequently the toe of the hoof becomes long and curled upward. Inflammation in the coronary band may also cause ring formation on the wall of the hoof.

Anatomy of Hoof Laminae

The laminae of the hoof are folds of epidermis and dermis which form a matrix in the laminar corium between the wall of the hoof and the third phalanx (Figure 1).[12]

Figure 1. Frontolateral view of the equine foot indicating projected cut-out area and histologic cross section of the laminar region. LC = laminar corium; P_I = first phalanx; P_{II} = second phalanx; P_{III} = third phalanx; c = cartilage; 1 = primary dermal lamina; 2 = secondary dermal lamina; 3 = primary epidermal lamina; 4 = secondary epidermal lamina; w = wall of hoof.

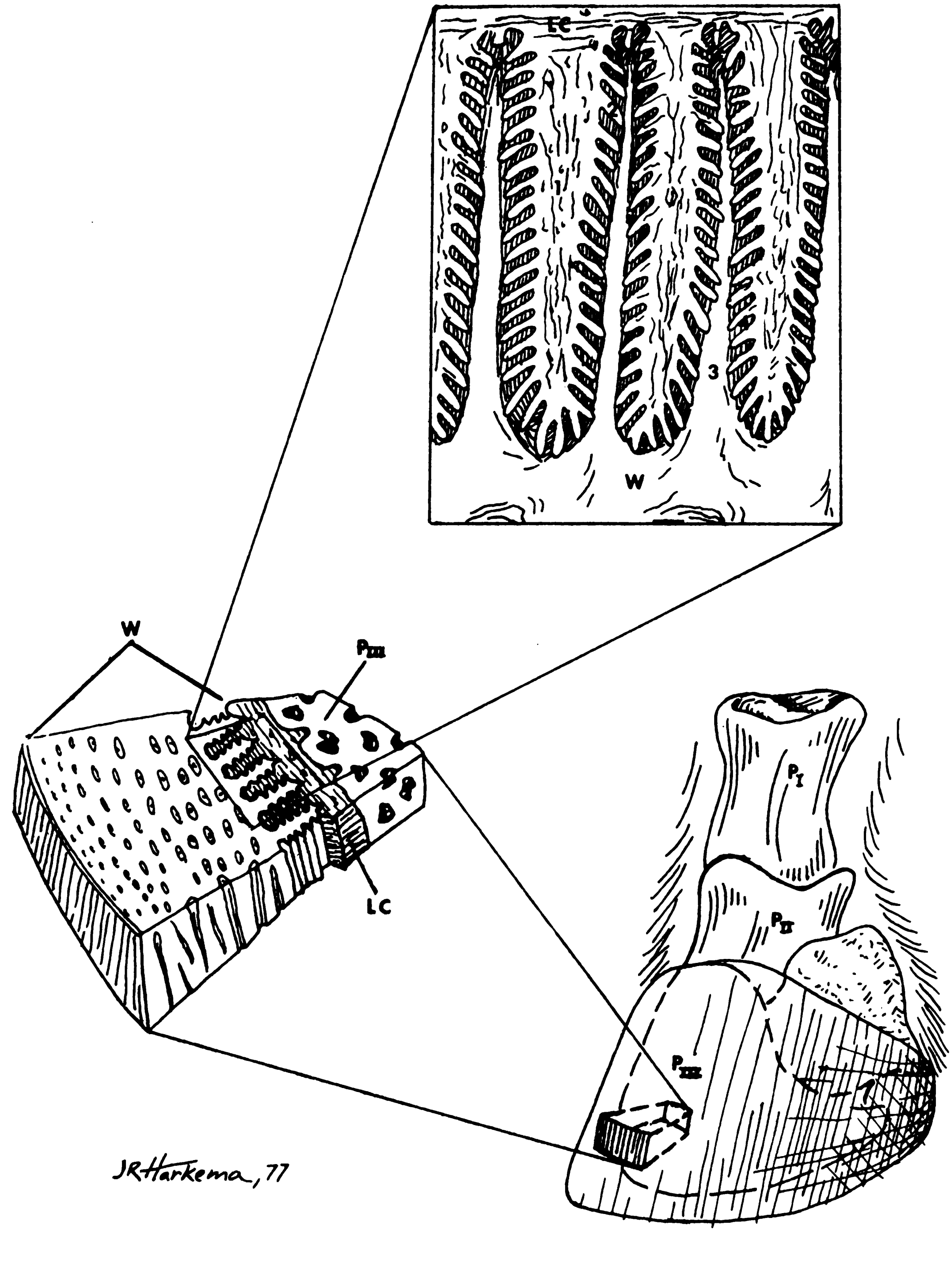


FIGURE 1

The dermal laminae contain a close-mesh network of blood vessels and nerves. Blood flow through the vessels supplies needed nutrients for the growth of the hoof and the keratinizing process of the epithelial cells. The laminae are defined as primary dermal laminae, secondary dermal laminae, primary epidermal laminae, and secondary epidermal laminae (Figure 1).[12] These latter laminae are the sites for the beginning of keratinization.

Empirical Causes

Although the pathogenesis of laminitis is still uncertain, the empirical causes of the disease have been described. It may result as a complication of endometritis or severe systemic infection. The post-parturient laminitis arises from infection caused by retention of fetal membranes or from other uterine infections. Concussion to the feet from excessive work on hard surfaces causes what is known as road founder, a traumatic type of laminitis. Water founder results from ingestion of large amounts of cold water by an overheated horse. Many miscellaneous causes of laminitis have also been recorded, e.g. viral respiratory disease and administration of exogenous corticosteroids.[2]

The most common causes of laminitis in cattle and horses are associated with digestive disturbances. In horses, eating large quantities of grain may give rise to laminitis (grain founder). This type of disease is usually associated with gastroenteritis, and the grains most commonly involved are wheat, corn, and barley.[2] Experimental induction of equine laminitis has been accomplished with a high starch diet (Adams, 1974; Carner et al., 1975; Robinson et al., 1976).[1, 17, 43] It has been proposed that changes occurring in the hoof during laminitis may be sequelae of acute gastrointestinal crisis, and the release of vasoactive toxins (e.g. histamine, lactic acid).

Since the etiology is not definite, numerous hypotheses for the pathogenesis of laminitis have been proposed. Road founder appears to be simply mechanical damage of the laminae. However, many other types of laminitis are the result of disease in areas remote from the hoof. Therefore toxins, vasoactive agents, bacterial endotoxins, and lowering of certain amino acids have been considered as possible causes of this disease.

Hypotheses on the Etiology of Laminitis

Presently there are two schools of thought on the etiology of laminitis. The first holds that acute laminitis is a primary circulatory abnormality of the laminar corium which leads to defects in keratin synthesis with subsequent separation of the hoof wall from the third phalanx. The second theory supports a primary alteration in keratin synthesis which leads to loss of structural integrity, mechanical damage to the laminar corium and circulatory changes.

The latter theory is supported by morphological studies in early acute laminitis[39] and by studies of the biosynthesis of keratin in the hoof (Larsson et al., 1956).[28] Obel (1948)[39] showed that in the early stages of acute laminitis, histopathological changes occur only in the epidermis and initially no change can be seen in the capillaries or connective tissue. Consequently, Larsson (1956)[28] theorized that the main epidermal changes in laminitis consist of a disappearance of the "onychogenic substance" (or keratin precursor) in the deep layer of the keratogenous zone in the lamellar region and in the matrix of the wall, the sole, and the frog. Through autoradiographic investigations it appeared that methionine plays an important role during keratinization in the stratum germinativum. Cystine, another important factor, was seen to be incorporated in this area when the keratin precursor had reached the keratogenous zone. Larsson showed that the richness in -S-S- groups, which characterized the definite keratin, is due not only to oxidation of -SH groups, but also to a large extent due to direct incorporation of cystine from the blood stream. Radioactive cystine and methionine studies revealed a marked decrease in arteriovenous difference in laminitis cases. This along with decreased concentration of reducing substances in the inner layers of the keratogenous zone led Larsson to conclude that there is a blocking of the metabolism of sulphur-containing metabolites in acute laminitis. The principle objection to this conclusion is that in the experiment no measurement of blood flow was made and theoretically changes in blood flow could cause changes in cystine and methionine uptake. Therefore it still remains to be determined if the decrease in uptake of these necessary amino acids is due to a change in blood flow to the laminae or a blocking of metabolism.

The other hypothesis suggests that acute laminitis is the result of a primary circulatory abnormality. Coffman et al. (1970)[12] using an angiographic technique have shown a redistribution of blood flow in acute laminitis, blood flow decreasing in the hoof and increasing in the adjacent tissues. In this study the normal foot was characterized by numerous fine vessels in the corium of the coronary band and a symmetrical net-like vascular pattern in the corium of the hoof. In the laminitis foot there was poor filling of the terminal arch, larger and fewer primary branches and irregular vascular patterns in the corium of the hoof. Coffman suggested that the decreased arterial blood flow to the laminar corium initiates acute alimentary laminitis. However, Robinson and his coworkers (1976)[43], by directly measuring digital blood flow in a group of normal and laminitis ponies, demonstrated that animals with clinical signs of laminitis have increased digital blood flow. This increase in blood flow is in apparent conflict with Coffman's angiographic studies. This paradox could be reconciled if, during laminitis, there was a marked increase in blood flow through numerous arteriovenous anastomoses at the level of the coronary band or through capillary beds proximal to the laminae. Therefore even though there is increased flow to the hoof, blood could still be shunted away from the laminae. Further local redistribution studies need to be done to resolve the problem.

Local Blood Flow and Laminitis

Since local blood flow could be changed in response to naturally occurring vasoactive agents, it has been suggested that these agents are liberated in the gut or locally in the hoof and are factors in the development of laminitis. Histamine has been suggested as one of these vasoactive agents in both cattle and horses (Akerblom, 1934, 1939; Chavance, 1946; Nilsson, 1963).[7, 39] Increased blood levels of histamine in equine laminitis have been reported by Akerblom (1934, 1939) and in subacute and chronic cases of laminitis in cattle by Nilsson (1963).[39] However, in crosscirculation bioassay experiments in ponies (Robinson et al., personal communication), no evidence of vasoactive agents could be found.

Digital vascular responses to the local perfusion of acetylcholine, epinephrine, histamine, and seratonin in normal and laminitis ponies have also been studied (Robinson, 1976).[42, 43] In these experiments the digital vascular responses were the same in both laminitis and normal ponies. Therefore, the increased digital blood flow which was observed in the laminitis ponies was not attributed to altered responsiveness to these vasoactive agents.

Overfeeding in Ruminants and Horses

Although little has been reported concerning overeating in horses or ponies, there has been extensive study of food engorgement in ruminants. Excellent descriptions and reviews of the subject have been published.[15, 32, 37, 38]

The foodstuffs which usually cause the common clinical signs of overfeeding when consumed in excess are the fermentable carbohydrates: barley, maize, oats and wheat, apples, pears, swedes, etc.[37] The clinical signs of the overfed animal include anorexia, dullness, staggery gait, labored respiration, and variable changes in heart rate and temperature.[32]

Death may occur within 24 hours or the animals may linger for six to seven days before dying.[32] In nonfatal cases recovery is uneventful, with gradual restoration of normal function of the rumen.

Major changes in the blood constituents and urine have been reported as characteristic of this syndrome. The lactate concentration in the blood is increased markedly. Dunlop and Hammond (1965)[16] have reported that both isomers of lactate are present with a predominance of the D-isomer. There is a decrease in the alkali reserve and decreased blood pH.[16] One of the first signs of a change in metabolism is a fall in the pH of urine which is latter followed by anuria.[16, 32]

Associated with ingestion of large quantities of soluble carbohydrates is a lowering of the pH of the rumen contents, together with an increase in concentration of lactic acid and volatile fatty acids (Broberg, 1960; Ryan, 1964; Scarisbrick, 1954).[32] When the pH falls to pH 5, the concentration of volatile fatty acid tends to decrease, but lactic acid concentration continues to rise, and the pH may fall below pH 4 in severe cases (Ryan, 1964).[40] With this

fall in pH various changes occur in the rumen microorganisms. A loss of protozoa occurs together with a loss of a number of bacterial species (Hungate et al, 1952; Krogh, 1960).[25, 32] The latter is replaced by massive numbers of lactobaccilli and Streptococcus bovis (Krogh, 1961). In other words, the predominantly gram-negative ruminal flora is replaced by gram-positive bacteria. The rumen wall may become necrotic and motility decreases.[27]

The alterations in the bacterial population of the rumen appear to be the result of a selective proliferation of organisms capable of fermenting carbohydrates rapidly (Hungate et al., 1964).[25] In addition, environmental changes in the rumen make it unsuitable for the survival of other organisms.[32]

Gutierrez et al. (1959) reported that the protozoa are important factors in reducing excess fermentation in the rumen.[40] They engorge themselves with granules of carbohydrate and starch, thus delaying fermentation. However, when large quantities of carbohydrate are introduced, this system is overwhelmed, and once fermentation has reduced the pH to 5, the protozoa die.

Dunlop and Hammond (1965)[16] reported that a high proportion of the less easily metabolized D(-) lactate is produced by rapid fermentation of carbohydrate in the rumen. Using the washed-out rumen of the sheep, the rate of absorption of lactic acid was found to increase in parallel with the concentration in the rumen, and it doubled when the pH was lowered from 5.2 to 4.0.[40] It is not until the rumen contents fall below pH 5 that a significant amount of free lactic acid is absorbed. Some lactate may be absorbed in the small intestine whenever there is an accumulation of lactic acid in the rumen. This absorption is probably important when high concentrations of lactic acid are formed in the rumen.[32]

The increase in the lactate concentration in the blood of these overfed animals has been attributed to at least three factors: 1) absorption of lactic acid or its salts from the alimentary tract, 2) an increase in anaerobic metabolism, either as a result of less efficient transport of oxygen by the circulation or due to tissue hypoxia caused by a histotoxic factor, and 3) an increased gluconeogenesis due to adrenocorticoid release.[32] Because of this increase in blood lactate in overfed ruminants, this metabolic disorder has been labelled as lactic acidosis.[32, 40] An increase in blood lactate has also been reported in overfed horses (Garner, 1975).[18] However no investigation into the mechanism of production in these species has been made.

The necrosis of the rumen wall and the presence of toxic factors in the digesta have been suggested by Dunlop and Hammond (1965)[16] as being responsible for the continuation of the disease after the initial 24 hours. Histamine and other amines have been implicated as possible toxic factors produced in the rumen of engorged aminals.[32] Unidentified toxic factors have also been studied in rumen fluid taken from overfed ruminants. One of these factors

was nondialyzable and heat stable, which suggests that it may be an endotoxin.[32]

Laminitis has been reported as another complication occurring after overfeeding in ruminants.[37] Morrow et al. (1973)[35] found that signs of laminitis could be created in sheep when an 85% syrup of D-L lactic acid was injected into the rumen of these animals. Since equine laminitis commonly follows overeating of soluble carboyhydrates, it is possible that the same pathogenesis following engorgement could be occurring in the horse as in the ruminant. However, since the horse does not have a rumen, the changes in microbial populations may possibly occur in the cecum or large colon since most of the microbial digestion takes place in this area of the equine alimentary tract.[3] Therefore, following overeating in the pony, the cecum and large colon concentration of lactate could be elevated as in the rumen of overfed cattle and sheep.

However, Hintz et al. (1971), using ponies with cecal and colonic fistulae, estimated that 65-75 per cent of the soluble carbohydrates were digested and absorbed pre-cecally.[3] The remainder and the insoluble and fibrous plant material are digested by bacteria fermentation in the large intestine (Elsden et al., 1946). In addition, Alexander (1972) has reported that the horse's stomach has a significant concentration of lactic acid.[3] Thus, it may be that changes in carbohydrate digestion after engorgement could occur pre-cecally in the horse.

Experimental Induction of Acute Laminitis

Alimentary laminitis has been induced in the horse with relative success (Obel, 1948; Larsson et al., 1956; Coffman et al., 1970; Garner, 1975; Robinson et al., 1976).[12, 17, 28, 39, 43] Ponies in which the disease had been induced by a dietary method often died of circulatory collapse prior to development of clinical signs of laminitis (Coffman, 1970; Adams, 1972; Robinson et al, 1976).[1, 12, 43] In 1975, Garner et al. experimentally inducted equine laminitis with a ration of 85% cornstarch and 15% wood cellulose flour.[17] The mean time lapse between administration of the gruel and the onset of lameness was 40 hours, with the time lapse ranging from 32 to 48 hours. Robinson et al. and Adams report only relative success in inducing laminitis in ponies with this dietary ration.

Cardiovascular Events of Overfed and Laminitis Animals

Although clinical characterization of the cardiovascular system at the time of acute laminitis has been extensively reported, very little exists in the literature concerning cardiovascular changes after overfeeding and prior to laminitis.

At the time of acute laminitis a hypertensive state has been reported in horses and ponies.[17, 18, 39, 43] However, cattle with acute laminitis have been reported to have hypotension.[39] No reason for this difference in species has been suggested. However, it is known that the onset of bovine laminitis seems to be less abrupt and the systemic signs less pronounced than in the case of horses. Furthermore, cattle with laminitis seldom adopt the characteristic posture of laminitis horses. Moreover, cattle often arch the back, and horses practically never do this.

Overfed ruminants have been reported to exhibit a shock-like condition, implicating a decrease in blood pressure and possible death within 24 hours of engorgement.[32] As was previously mentioned, horses and ponies that were experimentally overfed have been reported to die of cardiovascular collapse. However, Garner et al. (1975)[17] reported that experimentally overfed horses which developed laminitis had increasing arterial systolic and diastolic pressures from 16 to 56 hours after engorgement. Moderate arterial hypotension has been reported by Garner (1975)[18] during the early onset phase of engorgement in horses, but in the final prodromal phase of induced laminitis, arterial blood pressure is significantly increased.

This progressive arterial hypertension during the onset of acute laminitis has been deemed the result of a concommitant increase in cardiac output. However, this speculation did not take into account any increase in total peripheral resistance that may also contribute to the increase in blood pressure. In addition, preliminary studies in the lab of Dr. N. E. Robinson show no evidence of a progressive increase in arterial blood pressure prior to acute laminitis.

Heart rate has been reported to be elevated at the time of acute laminitis in ponies, horses and cattle. Steady increases in heart rate 16 to 56 hours after overfeeding in horses has been reported by Garner et al. (1975).[17]

Biochemical and Hematological Constituents after Overfeeding

Increased hematocrit values have been reported in experimentally overfed ruminants (Dougherty et al., 1975).[14] Maclean (1970) stated that laminitis-affected barley beef animals exhibited increased packed cell volumes.[34] In addition, Morrow (1973)[35] reported that hematocrit increased in lambs when 0.50% lactic acid was injected into their rumens. Horses that were overfed to induce laminitis showed an increase in hematocrit 24 to 40 hours after overfeeding (Garner et al., 1975).[17] This was correlated with increases in total protein. However, Nilsson (1963)[39] has reported no statistically different packed cell volumes in acute laminitis cattle compared to normal cattle.

Slightly lower than normal serum pH was observed in laminitis cattle. Experimentally engorged ruminants show decreases in blood pH to values as low as 7.1 to 7.0.[39] Lactic acidosis has also been implicated in overfed horses prior to acute laminitis (Garner, 1975).[18]

Blood levels of calcium, phosphorous, magnesium, sodium and potassium in laminitis cattle showed very little deviation from normal levels (Nilsson, 1963; Maclean, 1970).[34, 39] When laminitis was experimentally induced in lambs, significant decreases in potassium occurred two days after the time of induction. Although serum electrolyte measurements have been made during the onset of equine laminitis, no numerical data has been reported.

Shock and Laminitis

Because laminitis follows overeating and a variety of septic conditions, endotoxins and endotoxic shock are frequently mentioned as part of the pathogenesis of the condition. Shock has been defined by Wiggers as a lack of "effective circulating blood volume." Although there are numerous precipitating causes of shock, the resulting clinical manifestations are, in general, quite similar. Usually, the cause of inadequate tissue perfusion in circulation is inadequate cardiac output. The arterial pressure usually falls at the same time as cardiac output, unless nervous reflexes keep the pressure from falling. Therefore any factors that can reduce cardiac output can also cause shock (e.g. factors decreasing venous return or decreasing myocardial contractility).

A shock-like syndrome has been produced in ruminants and dogs when an unidentified toxic substance from the ruminal fluid and blood plasma of overfed sheep was administered intravenously (Dougherty and Cello, 1948).[13] After the injection of the toxic substance, blood pressure of the dogs and goats was depressed. In addition, rumen motility in sheep was inhibited and respiration was stimulated in all the intact animals. Mullenax et al.[38] injected toxic materials from rumen bacteria and rumen fluid subcutaneously and intraruminally in sheep and cattle. Immediate decreases in arterial blood pressures were followed by increases within one minute to above pretreatment levels. A decrease in rumen motility was also observed.

Following endotoxin administration, various species including rabbit, monkey, cat and dog, develop hypotension (Kuida et al., 1958, 1961).[48] Arterial pressure and cardiac output have been reported to decrease during progressive shock produced by gram-negative E. coli bacteria, but the calculated peripheral resistance remains unchanged.

Burrows (1971)[6] created E. coli endotoxemia in conscious ponies by intravenous injections of endotoxin. Immediately after administration there was a precipitous decrease in mean arterial blood pressure. Subsequently, there was a sharp increase in blood pressure above preinjection levels and then a gradual decrease until death.

Experimental Rationale

Ingestion of excessive amounts of readily-fermentable carbohydrates by ruminants, horses, and ponies results in acute indigestion. This overeating may lead to a shock-like condition and subsequent death, or acute laminitis. In ruminants, the predominately gram-negative ruminal flora is replaced by gram-positive bacteria, principally Streptococcus bovis and lactobacilli.[25, 27] These produce large quantities of lactic acid which alters the intraruminal environment and affects the acid-base status of the animal.[32] A toxic substance may also develop in the gut as a result of abrupt change in environment. These may cause sudden changes in the cardiovascular system of the overfed animal. Similar changes may be occurring in the overfed horse or pony even though anatomical differences exist. Furthermore, systemic cardiovascular changes or alterations in the acid-base status of these animals may be the cause or causes of acute laminitis.

If a toxic substance is released into the circulatory system after overfeeding and prior to laminitis, it may be reflected in the cardiovascular system during the onset of the disease. Although Garner et al. (1975)[17, 18] studied the cardiovascular events of overfed horses during the onset of laminitis, some of their results could not be duplicated in preliminary studies conducted in the lab of N. E. Robinson (Michigan State University). In addition, unanswered questions developed from that study. The cause of the progressive hypertension reported during the onset of laminitis is still unknown. It may be due to an increased cardiac output, an increased vascular peripheral resistance, or a combination of these two variables. The reason for the increase in hematocrit is also unanswered. A decrease in plasma volume or an increase in the number of circulating erythrocytes, or both, could explain this elevation. In addition, the acid-base status and the serum electrolyte status after overeating and prior to laminitis have not been thoroughly monitored and reported in the literature.

Therefore, the present study was designed to monitor the cardiovascular events and record the acid-base status of the overfed pony during the onset of laminitis. This investigation also allowed for concurrent measurements of circulating blood volumes and serum electrolytes in an effort to answer the previously proposed questions.

MATFRIALS AND METHODS

Experimental Animals

Twelve mature Shetland ponies (9 females and 3 males), weighing 162 to 255 kilograms, were brought indoors and fed grass, hay, and oats for at least one week prior to induction of laminitis.

Surgical Procedure

Twenty-four hours prior to iatrogenic induction of laminitis, the ponies were anesthetized with sodium thiamyl (10 mg/Kg of body weight, given intravenously). After intubation, anesthesia was maintained with halothane and oxygen. The left jugular vein and carotid artery were exposed and freed of fascia. In most of the ponies the left carotid artery had been surgically exteriorized to lie subcutaneously, at least two months prior to the experiment. To allow for direct measurement of arterial blood pressure and arterial blood sampling, the left carotid artery was cannulated with a saline filled No. 7 French teflon end-hole catheter secured with a silk ligature. The proximal end of the catheter was run subcutaneously, surfaced close to the dorsum of the neck, and was tied loosely with surgical tape to the mane of the animal.

Two catheters were introduced into the jugular vein. A No. 7 French balloon tipped catheter with a thermistor located near the tip (Columbus Instruments International Corporation, Columbus, Ohio) was floated through the right heart and positioned in the pulmonary artery. The catheter was used to measure body temperature, pulmonary arterial pressure, and changes in blood temperature from which cardiac output was calculated. A second No. 7 French end-hole catheter was positioned in the right atrium. This catheter was used to record right atrial pressure and served as the injection port for the bolus of cold saline used in the thermodilution determination of cardiac output. A silk ligature around the vein secured both catheters. The proximal ends of both catheters were run with the arterial catheter subcutaneously and secured at the same exposed dorsal location on the mane.

Venous catheter placements were verified by recorded pressure measurements and wave forms recorded on a multichanneled oscilloscope and recorder (VR-6, Electronics For Medicine Inc., White Plains, N.Y.).

The surgical site was closed using a simple interrupted suture of #1 Dexon suture material. After recovering from anesthesia, ponies were placed in a specially designed restraining box bedded with straw. The ponies were given free access to water and hay, and allowed to become accustomed to the box overnight, before control values were taken.

Experimental Protocol

Twenty-four hours after placement of catheters, control measurements were made of the following variables: arterial blood pressure (systolic, diastolic, and mean), right atrial pressure, mean pulmonary arterial pressure, heart rate, cardiac output, stroke volume, plasma volume, hematocrit, blood volume, total serum solids, arterial pH, arterial Pco_2, arterial Po_2, serum electrolytes (Na^+, K^+, Cl^-), and serum osmolarity.

After these control measurements were made, the ponies were given a special ration (17.6 g/kg of body weight) containing 85% starch and 15% wood cellulose (Garner et al., 1975). The special ration was diluted in water and administered by stomach tube, usually in one dose, but occasionally in two doses a half hour apart.

Every eight hours after the administration of the ration, measurements of the experimental variables were repeated until laminitis was diagnosed. The clinical signs for identification of acute laminitis included warm feet, a digital pulse, lameness, and the characteristic laminitis posture. Clinical signs were also recorded over the experimental periods prior to the disease. Euthanasia was performed with an overdose of barbituates no longer than eight hours after the disease was diagnosed.

Measurement of Blood Pressure

Blood pressure measurements were made directly using the saline filled indwelling catheters connected to a blood pressure transducer (P23 Db, Statham Instruments, Hato Rey, PR.) and recorded by the VR-6 recorder. The catheters were flushed with saline and blood was aspirated. When blood could be aspirated freely and wave forms looked normal, it was assumed that the catheters were recording accurate blood pressures.

By electronically dampening the response of the recording system, mean arterial pressures were recorded.

Measurement of Cardiac Function

The thermal dilution technique was used to measure cardiac output. Ten ml of cold physiological saline (0-2° C) were injected (manually) into the right atrium through the teflon end-hole catheter. The blood temperature change in the pulmonary artery was sensed by the thermistor at the tip of the No. 7 French balloon catheter. This precalibrated thermistor catheter was connected to a cardiac output computer (Cardiotherm-500, Columbus Instruments International Corp., Columbus, Ohio). A thermistor sensing the temperature of the cold injectate was also connected to the computer. Five cardiac output determinations were made and an average of these was recorded for each time period.

Heart rate was determined from the systemic arterial pulse trace, while stroke volume was calculated from the measured heart rate and cardiac output.

Measurement of Blood Volume and Composition

Plasma volume was measured as the volume of distribution of Evans blue dye which binds tightly to plasma albumin. Fifty ml of blood were taken with a heparinized syringe and centrifuged to obtain plasma. Ten samples of Evans blue were diluted to known concentrations (.005-.080 mg/ml) with this plasma. The optical densities of these concentrations were read on a spectrophotometer (Beckman Instruments Inc., Fullerton, California) set at 620 nm and a standard curve of optical density due to the dye vs. the concentration was constructed.

For the determination of the pony's plasma volume, about 10 ml of blood were drawn from the carotid artery. The withdrawn blood was transferred to a vacutainer collection tube with an anticoagulant (EDTA). This blood was used for determinations of the optical density of dye-free plasma (blank) and for determination of hematocrit. Using the right atrial catheter, a predetermined amount of a diluted Evans blue solution was injected and the catheter and syringe were rinsed by repeated aspirations and re-injections of blood. The catheter was then rinsed with heparinized saline.

Thirty, forty, fifty, and sixty minutes after completion of the injection of dye, blood samples were taken from the artery, for determination of the concentration of Evans blue. Samples were immediately transferred to vacutainer collection tubes with EDTA. The samples were centrifuged at

3000 r.p.m. for 10 minutes to obtain the dyed plasma. The optical densities of these samples were read at 620 nm using the Beckman spectrophotometer.

The dye obtained form the Fisher Scientific Company, Pittsburg, Pennsylvania, was used in 1% aqueous solution for nine ponies. A new lot of Evans blue from Eastman Kodak Company, Rochester, N.Y., was used in .2% aqueous solution, because of its much higher purity, for the last three ponies. The amount of injected 1% or .2% solution of Evans blue was as a rule 20 ml, corresponding to a dose which would give a spectrophotometrically determined optical density in dyed plasma within a range of .200-.800. Within this range a linear relation between the concentration of dye and the optical density due to the dye was demonstrated for the Evans blue in the plasma.

The difference between the optical density of the blank plasma (before Evans blue injection) and the optical density of a dyed plasma sample was the optical density value due to the dye. The concentration of Evans blue in that plasma sample was derived from the standard curve constructed for that pony.

The dye concentrations of the four unknown samples were plotted against time on semi-log paper and extrapolated to t_o, or injection time, and this value was used to calculate the plasma volume when the slope of the curve indicated clearance from the plasma. However, in most cases clearance was not clearly indicated, and therefore an average of the four concentrations was used to calculate the plasma volume.

This volume was calculated from the quotient $\frac{EB}{C}$, where EB is the amount of injected dye in mg and C the Evans blue concentration in mg per ml of plasma (Chien & Gregersen, 1962).[10]

The total blood volume was calculated from the equation, $\frac{PV \times 100}{100 - Hct.}$, where PV represents plasma volume and Hct. is the hematocrit. No correction was made for trapped plasma.

Total solids were measured from the blank plasma sample using a refractometer.

Measurement of Acid-Base Status

The arterial blood pH, Pco_2 and Po_2 were analyzed from a 3 ml heparinized anaerobic sample taken from the carotid artery. After withdrawal, the syringe was immediately sealed and stored in ice water. The arterial sample was analyzed within one hour of withdrawal. Pco_2, Po_2 and pH were measured with a blood gas analyzer (Blood Micro System, Type BM53, Radiometer, Copenhagen, Denmark). The pH electrode of the analyzer was calibrated before every measurement with a low pH standard buffer (pH = 6.840) and a high pH buffer (pH = 7.384). Two pH measurements were made for each sample. The blood gas electrodes were calibrated with a low standard tank (Fo_2 = 0.0%, Fco_2 = 3.02%) and a high standard tank (Fo_2 = 21%, Fco_2 = 12.05%). Two Pco_2 and Po_2 measurements were made for each blood sample.

Method of Electrolyte Determination

Serum electrolytes were measured from serum collected from a 10 ml arterial blood sample. The serum was stored frozen until the time of analysis. Sodium and potassium were measured with a flame photometer (Beckman Instruments Inc., Fullerton, California). Chloride was analyzed with a chloridometer-automatic titrator (Buchler Instruments Inc., Fort Lee, N.J.). Osmolarity of the serum samples was made with an osmometer (Advanced Instruments Inc., Newton Highlands, Massachusetts).

Statistical Analysis of Data

All data was statistically analyzed by analysis of variance, randomized complete block design, because of known or anticipated sources of variation between ponies other than experimental effects (e.g. genetic). If the F value for the time intervals was significant, then specific comparisons between treatment means were made using the Student-Neuman-Keul's test.

The data from those ponies that died of shock were analyzed as hours before death defined as zero time. Therefore control, -24, -16, and -8 hour measurements of all the variables were analyzed.

Measurements taken from the ponies of the laminitis group were analyzed in several different ways. The first analysis involved hours after the administration of the diet with overeating as zero time. Control (0 - hour), 8, 16, 24, 32, 40 and 48 hour measurements were therefore statistically analyzed. Since the laminitis ponies developed the disease at different time intervals (between 32 and 72 hours after overeating), a second analysis was performed using the time laminitis was clinically diagnosed as zero time and analyzing 40, 32, 24, 16, 8 and 0 (time of laminitis) hour measurements prior to the development of the disease.

Significant changes of certain variables were analyzed to determine their effect on the other variables measured. Since a transitory peak in base deficit was common for every laminitis pony, this event was analyzed to determine its effect on the other variables that were measured. The individual peaks in base deficit occurred at different time intervals prior to the disease, therefore zero time was defined as the peak in base deficit, and 24, 16 and 8 hour measurements before the peak in base deficit, and 8 hour measurements after the peak were analyzed. Another event that was characteristic for the laminitis ponies was a drop in PCV 8 to 16 hours prior to the signs of the disease. To determine if this drop was caused by a rise in plasma volume or a drop in cell volume of the blood, zero time was defined as the drop of PCV and 16 and 8 hour measurements before the drop and 8 hour measurements after the drop were analyzed.

TECHNIQUES

Cardiac Output Determination

The thermal-dilution technique introduced by Fegler (1954)[50] is similar in principle to the indicator dilution technique. It involves injecting a measured amount of room temperature or cooler indicator (e.g. physiological saline) rapidly into the right atrium and measuring the subsequent change in blood temperature by a thermistor in the pulmonary artery. Cardiac output is calculated from the formula, $C.O. = \frac{(T_b - T_i)V_i}{A}$, where T_b represents blood temperature, T_i represents indicator temperature, V_i represents the volume of injected indicator, and A represents the area under the dilution curve, which is measured as a temperature change in the pulmonary artery in c° x min. The accuracy and reproducibility of the thermal-dilution technique have been documented extensively in man, dog, and horse.[20, 23, 36, 49, 50] Needs for complex electronics have been minimized and immediate results are now available. The injection material (cold physiological saline or 5% dextrose) is readily obtainable and safely administered. Furthermore, the thermal-dilution curve has minimal recirculation errors since temperature of the injectate is equal to blood

temperature after one circulation time. This eliminates elaborate extrapolation.

Several practical problems exist with this technique, which were noticed in this study. Distinct fluctuation in pulmonary arterial temperature, related to cardiac and respiratory cycling, results in a fluctuating thermal base line.[50] These fluctuations may approach the magnitude of the temperature change that follows "cold" injection. Although electronic averaging provides a more stable base line, iced rather than room-temperature injectate was found to give a sufficient signal-to-noise ratio. These "cold" injections were made only when stable base lines were indicated by the unchanged microthermistor reading.

Another problem is the determination of the true temperature of the injectate as it enters the vascular system. Heat is gained by injectate from the syringe, and catheter wall. Therefore to minimize this error all injections were done as rapidly as possible.

Finally, although there is no recirculation of the thermal indicator, there is a delayed return of the vascular temperature to base line. This delay is probably due to a slow washout of "cold" from the myocardium and endothelial surfaces and the catheter.[50] Therefore, there is a variable time delay before a second injection can be made.

The cardiac output computer used in this study electronically averaged base-line fluctuations, integrated the thermal dilution curve, applied the necessary correction

factor and displayed the cardiac outputs in liters per minute within seconds after the injection. It allowed measurements to be made rapidly and accurately reproducible.

Plasma Volume Determination

The Evans blue dye dilution technique was used instead of the radioiodinated albumin technique because of its simplicity and safety when frequent determinations must be made over a period of days. This technique has some disadvantages. Since the volume of distribution of the administered dye is that of plasma albumin (Rowson, 1943)[10], any escape or metabolism of albumin during the course of the equilibrium will give an overestimation of plasma volume. These errors may be reduced by taking measurements closer to the time of injection; but if samples are taken too soon after injection of the indicator, the error from inadequate mixing increases.[41] To minimize the above disadvantages the extrapolation method was used in this experiment.

The occurrence of turbidity and residual dye in the blood are also problems. In an effort to reduce plasma turbidity, all the samples were refrigerated 24 hours and recentrifuged before optical density was measured. Since frequent determinations of PV were made and Evans blue is eliminated from the blood at a slow rate, the plasma contained residual dye from the previous determination. It was assumed that no changes in the content of residual dye occurred, other than those due to normal elimination. Therefore correction for this factor could be made by regarding the optical density of 620 nm in the blank sample as background absorption (Gibson and Evans, 1937; Hungate et al., 1963).[41]

Because this investigation dealt with a pathological condition (which in some cases lead to shock), errors in PV measurements due to poor perfusion or poor mixing may have occurred. Measurement of red cell and plasma volumes in shock have been criticized because of the failure to obtain complete time concentration curves, since it has been shown that the mixing of the injected indicator can be significantly delayed.[47] Inadequate mixing would lead to the erroneous conclusion that volume is reduced when in actuality there may be little change in absolute volume. However, Noble and Gregersen[48] have reported that sluggish blood flow did not prevent or delay the uniform mixing of the dye when it was intravenously administered. In addition they showed that dye did not escape from the circulation more rapidly in shock than in normal conditions. Therefore they concluded that this dye method of measuring plasma volume was valid, even in the presence of shock.[48]

RESULTS

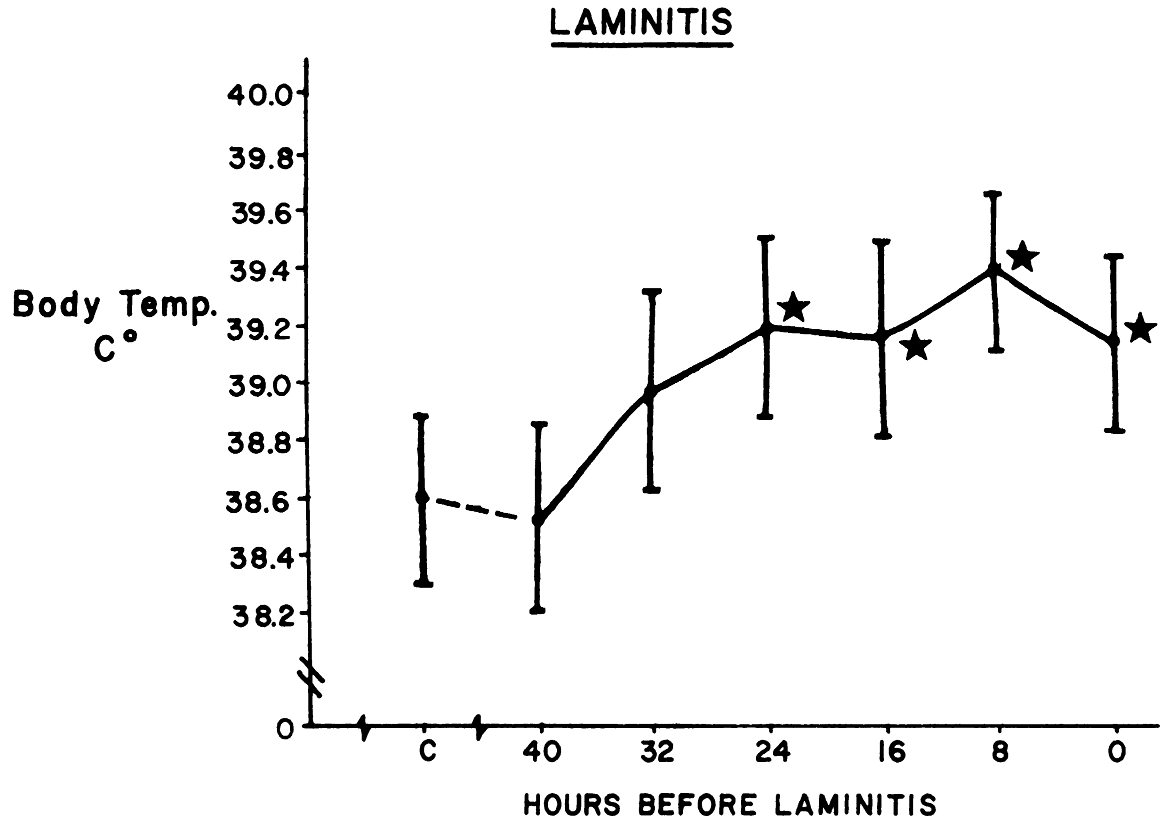
Clinical Signs of Carbohydrate Overload

Of the twelve mature Shetland ponies that were overfed, seven developed acute laminitis, three died of cardiovascular collapse (shock), one was unaffected, and one was euthanized because of other complications.

The ponies which developed laminitis (No. 1, 2, 3, 6, 10, 11, and 12) showed clinical signs of the disease at different time periods within a range of 32 - 72 hours after administration of the diet (mean of 56.0 $\pm$ 3.5 hours). Signs ranged in severity from Obel Grade II (pony moved willingly at a walking pace, but the gait was characteristic of laminitis) to Obel Grade IV (pony did not move without being forced). Ponies were clinically normal for the first 16-24 hours after administration of the diet, but had pasty green-white feces and increased intestinal sounds during the remaining periods prior to laminitis. Body temperature and pulse rate increased in these ponies and the conjunctival mucosa was injected at the time laminitis was apparent. At this time body temperature was 39.14 $\pm$ 0.31°C compared to a control value of 38.60 $\pm$ 0.29° C (Figure 2).

Ponies which died of shock (No. 5, 7, 9) had rapid pulse rates, respiratory distress, increased rectal

Figure 2. Changes in body temperature in laminitis and shock ponies prior to the disease and death respectively (mean ± standard error). C = control measurement before administration of high starch ration; ★ = statistically significant change ($P < 0.05$) from control value, using randomized complete block analysis of variance and Student-Newman-Keuls' tests.


LAMINITIS
Body Temp. C°
40.0
39.8
39.6
39.4
39.2
39.0
38.8
38.6
38.4
38.2
0
C
40
32
24
16
8
0
HOURS BEFORE LAMINITIS

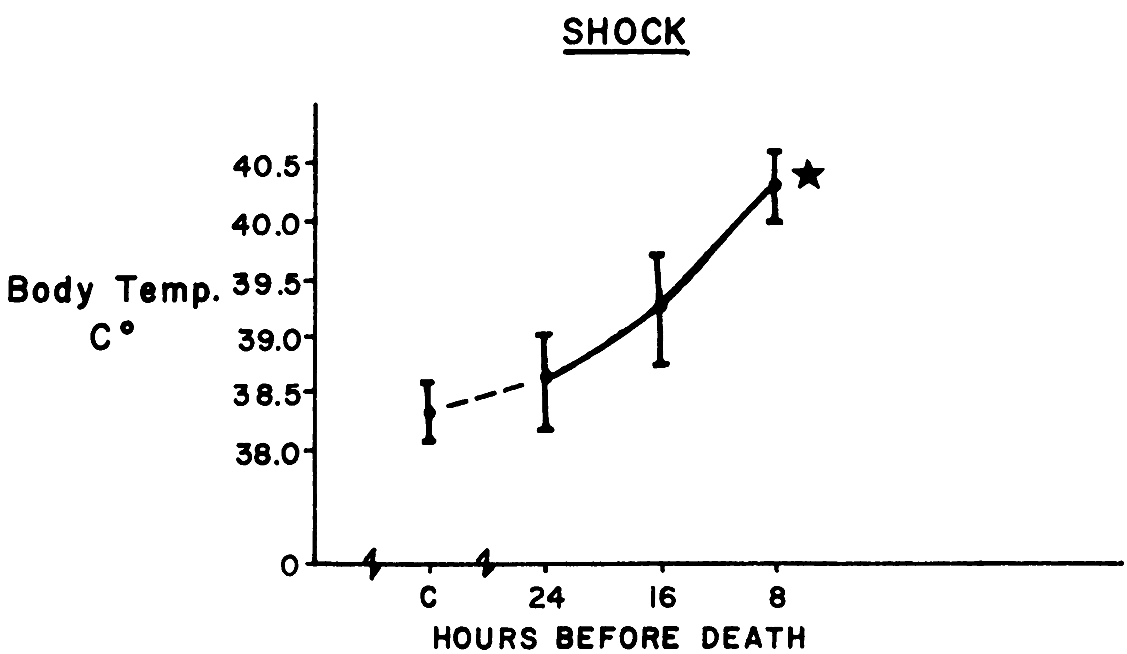

SHOCK
Body Temp. C°
40.5
40.0
39.5
39.0
38.5
38.0
0
C
24
16
8
HOURS BEFORE DEATH

FIGURE 2

temperatures, and extremities cold to the touch prior to death. Abdominal distension was apparent at least eight hours before death. Body temperature at this time was $40.30 \pm 0.30°$ C compared to the control of $38.37 \pm 0.25°$ C. These ponies died within 24-32 hours after the administration of the diet (mean of 29.3 ± 2.7 hours).

In all ponies developing laminitis there were transitory changes in base deficit, arterial blood pH and Pco_2 during the onset of the disease, and an elevation in cardiac output and arterial blood pressure at the time of laminitis. In each pony these events occurred at differing time intervals after diet administration, but at fairly constant time intervals prior to the clinical signs of laminitis. As a result, when means were calculated using overeating as zero time, many of the acid-base and cardiovascular changes were obscured (Figures 3, 4, 5). However, using the time of laminitis as zero time and taking mean values 8, 16, 24, 32 and 40 hours before laminitis, characteristic changes became apparent. For this reason all the results from laminitis ponies are analyzed using the clinical signs of laminitis as zero time.

A similar problem occurred in the ponies dying of shock. The course of shock was also highly variable. There is one endpoint--death, and two incontrovertible temporal reference points--the time of onset and the time of death. Therefore death was designated as zero time and the mean values 8, 16, and 24 hours before death were used. Since

Figure 3. Measurements of systemic cardiovascular variables in laminitis ponies before and after administration of high starch ration (mean $\pm$ standard error). $\bar{P}$ art. = systemic arterial pressure; C = control measurement before administration of ration; ★ = statistically significant change ($P < 0.05$) from control value, using randomized complete-block analysis of variance and Student-Newman-Keuls' tests.

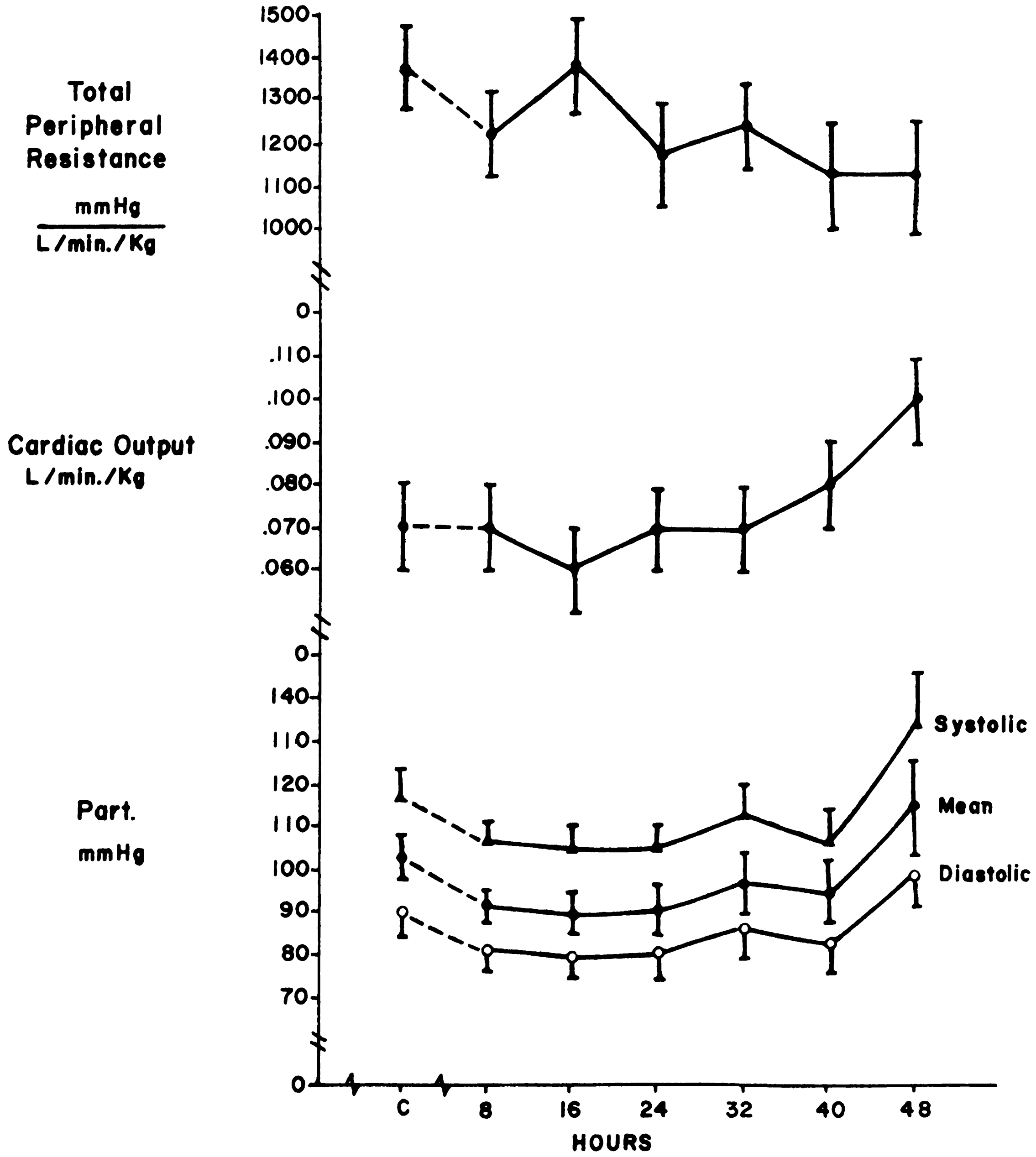

Total
Peripheral
Resistance
mmHg
L/min./Kg
1500
1400
1300
1200
1100
1000
0
Cardiac Output
L/min./Kg
.110
.100
.090
.080
.070
.060
0
Part.
mmHg
140
110
120
110
100
90
80
70
0
Systolic
Mean
Diastolic
C
8
16
24
32
40
48
HOURS

FIGURE 3

Figure 4. Measurements of cardiac variables in laminitis ponies before and after administration of high starch ration (mean $\pm$ standard error). Pra = right atrial pressure; Vs = stroke volume. C = control measurement before administration of ration; ★= statistically significant change ($P < 0.05$) from control value, using randomized complete-block analysis of variance and Student-Newman-Keuls' tests.

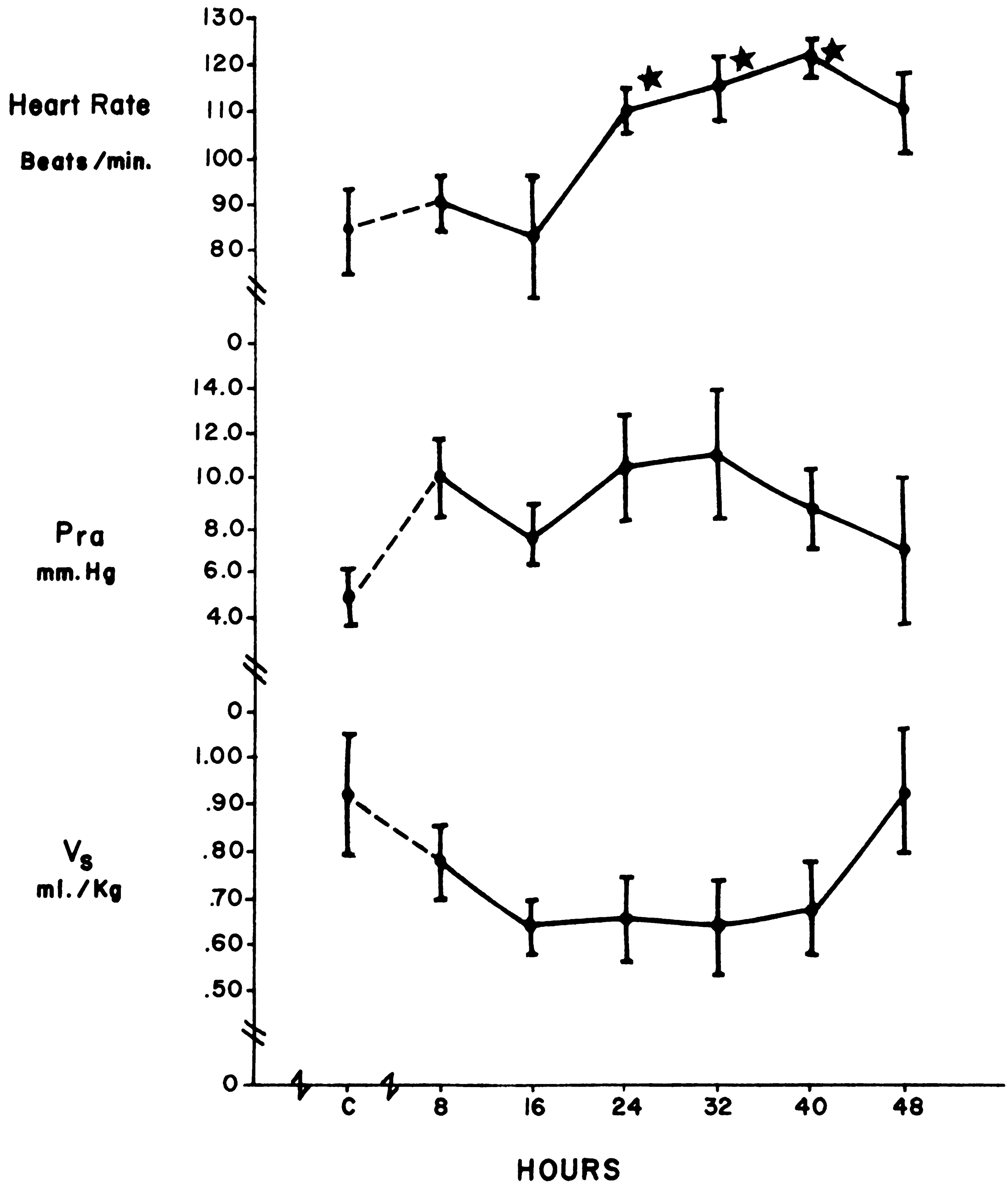

Heart Rate
Beats /min.
130
120
110
100
90
80
0
14.0
12.0
10.0
8.0
6.0
4.0
Pra
mm. Hg
0
1.00
.90
.80
.70
.60
.50
V s
ml. / Kg
0
C
8
16
24
32
40
48
HOURS

FIGURE 4

Figure 5. Measurements of acid-base status in laminitis ponies before and after administration of high starch ration (mean $\pm$ standard error). Pco_2 = partial pressure of carbon dioxide in arterial blood. C = control value before administration of ration; ★ = statistically significant change ($P < 0.05$) from control value, using randomized complete-block analysis of variance and Student-Newman-Keuls' tests.

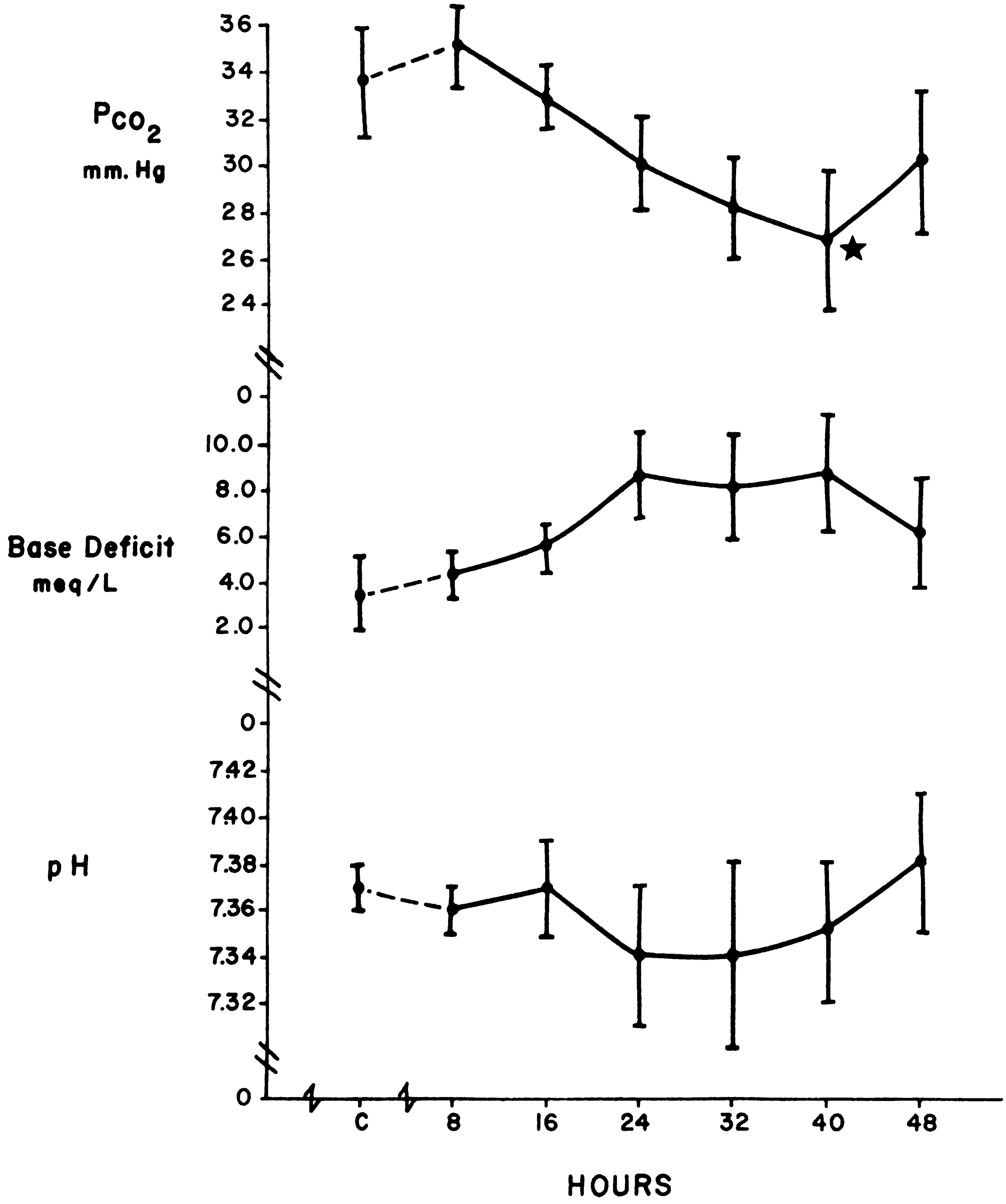

P_{CO_2}
mm. Hg
36
34
32
30
28
26
24
0
Base Deficit
meq/L
10.0
8.0
6.0
4.0
2.0
0
pH
7.42
7.40
7.38
7.36
7.34
7.32
0
C
8
16
24
32
40
48
HOURS

FIGURE 5

only three ponies died of shock, trends rather than significant changes were looked for in this data.

Effect of Carbohydrate Overload on Acid-Base Status

In both groups of ponies a metabolic acidosis developed after overeating. Acidosis was most severe in ponies dying of shock. Base deficit increased with time and eight hours before death was 21 $\pm$ 4 meq/l (Figure 6). Laminitis ponies developed a transitory base deficit (24 $\pm$ 2 hours before laminitis and 30 $\pm$ 3 hours after diet administration), and it was less severe (10 $\pm$ 2 meq/l) than in the shock group (Figure 7). When data was analyzed using the peak in base deficit for each pony as zero time and taking mean values 8 and 16 hours before the peak and 8 hours after the peak, significant decreases of arterial blood pH and Pco_2 correlated with the base deficit. Base deficit and arterial blood pH were normal when laminitis was diagnosed (Figure 8).

Effect of Carbohydrate Overload on the Systemic Circulation

Cardiac output (C.O.) and mean arterial blood pressure decreased and total peripheral resistance (TPR) increased 8-16 hours after the diet, in ponies dying of shock (Figure 9). Eight hours before death C.O. had decreased from a control value of .083 $\pm$ .005 l/min./Kg to .045 $\pm$ .004 l/min./Kg, systemic pressure decreased from 123 $\pm$ 7 mmHg to 89 $\pm$ 5 mmHg, TPR increased from 1360 $\pm$ 100 mmHg/l/min./Kg to 1820 $\pm$ 180 mmHg/l/min./Kg, and pulse pressure decreased from 39 $\pm$ 4 mmHg to 18 $\pm$ 1 mmHg.

Figure 6. Measurements of acid-base status in shock ponies before death (mean $\pm$ standard error). Pco_2 = partial pressure of carbon dioxide in arterial blood. C = control value before administration of ration; ★ = statistically significant change ($P < 0.05$) from control value, using randomized complete-block analysis of variance and Student-Newman-Keuls' tests.

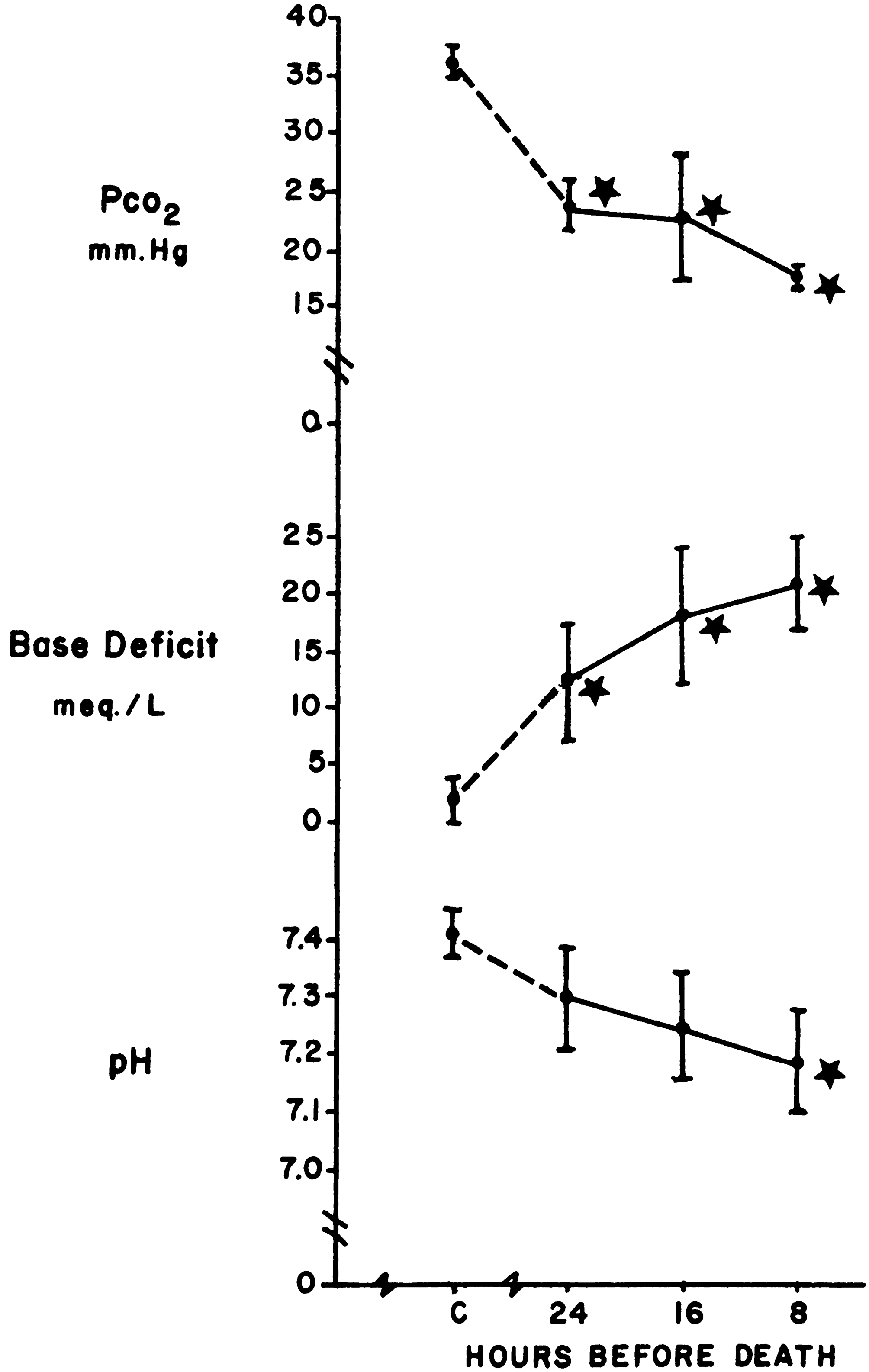

Pco2
mm.Hg
40
35
30
25
20
15
0
Base Deficit
meq./L
25
20
15
10
5
0
pH
7.4
7.3
7.2
7.1
7.0
0
C
24
16
8
HOURS BEFORE DEATH

FIGURE 6

Figure 7. Measurements of acid-base status in laminitis ponies before and during clinical signs of the disease (mean ± standard error). P_{CO_2} = partial pressure of carbon dioxide in arterial blood. C = control measurement before administration of high starch ration; ★ = statistically significant change ($P < 0.05$) from control value, using randomized complete-block analysis of variance and Student-Newman-Keuls' tests.

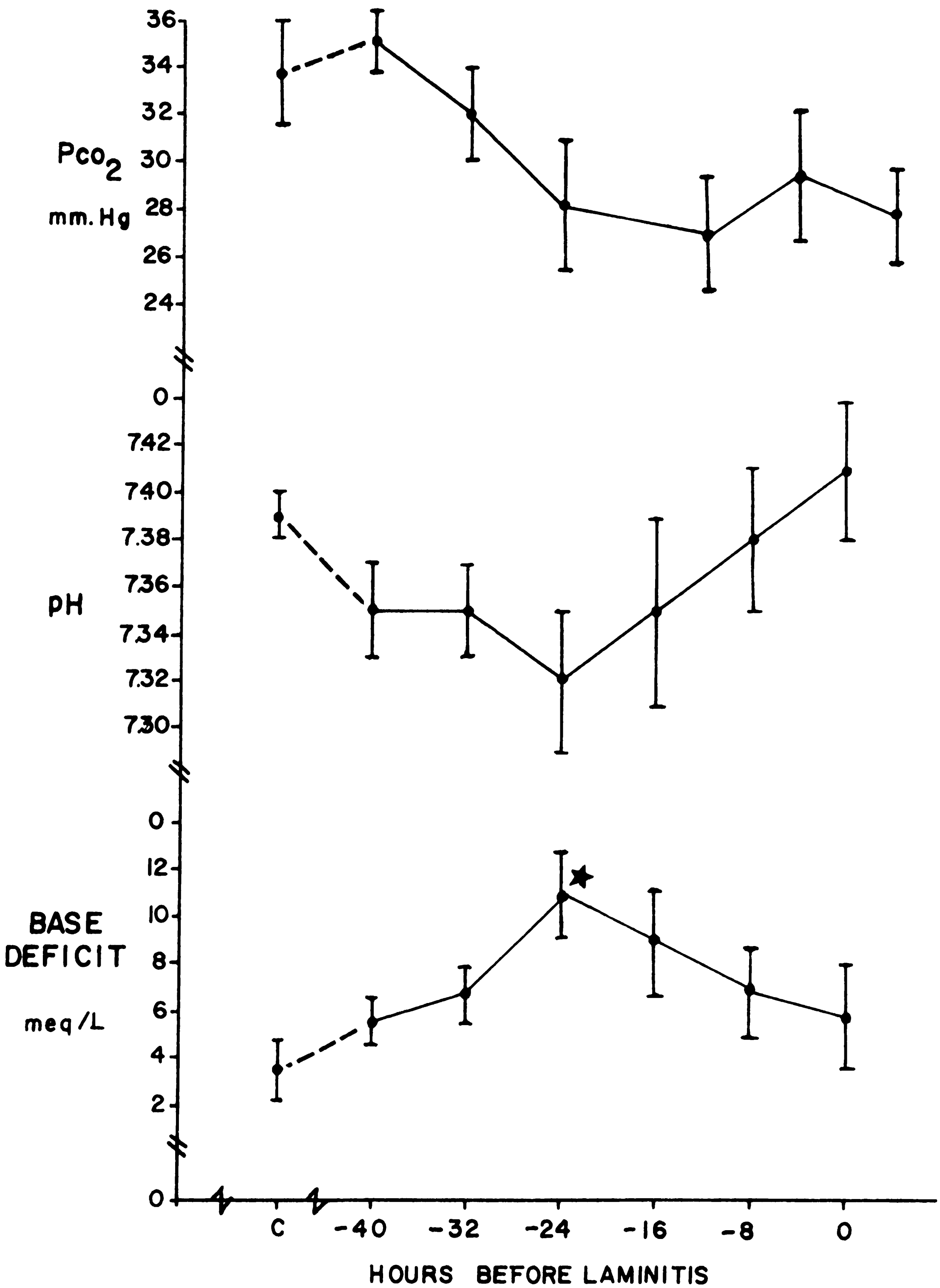

36
34
32
Pco2
30
mm. Hg
28
26
24
0
7.42
7.40
7.38
7.36
pH
7.34
7.32
7.30
0
12
BASE
DEFICIT
10
8
meq/L
6
4
2
0
C
-40
-32
-24
-16
-8
0
HOURS BEFORE LAMINITIS

FIGURE 7

Figure 8. Measurements of acid-base status before and after the peak base deficit in laminitis ponies (mean $\pm$ standard error). C = control measurement before administration of the high starch ration; ★ = statistically significant change ($P < 0.05$) from control value, using randomized complete-block analysis of variance and Student-Newman-Keuls' tests.

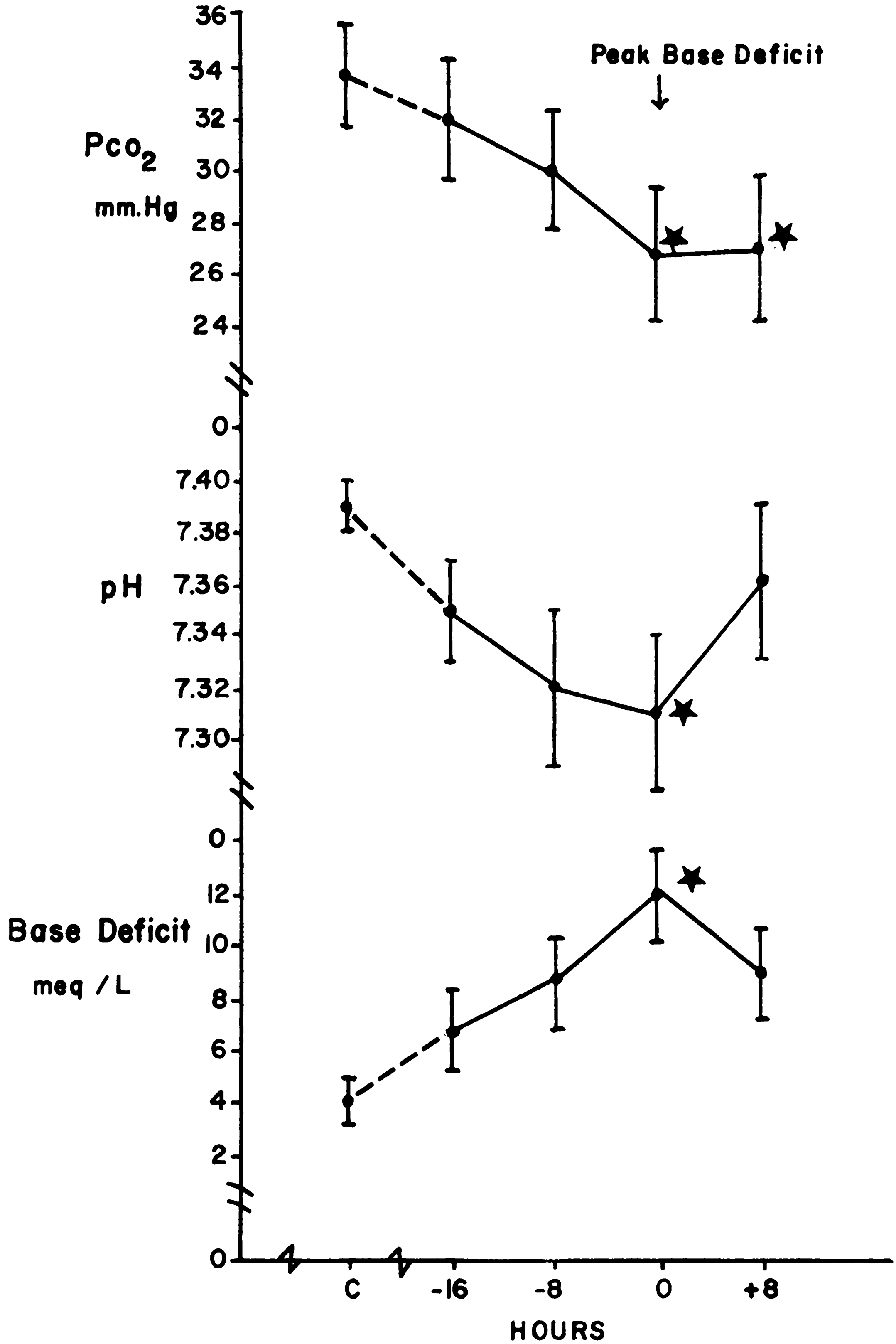

Peak Base Deficit
Pco_2
mm.Hg
36
34
32
30
28
26
24
0
7.40
7.38
pH
7.36
7.34
7.32
7.30
0
12
Base Deficit
10
meq / L
8
6
4
2
0
C
-16
-8
0
+8
HOURS

FIGURE 8

Figure 9. Measurements of systemic cardiovascular variables in shock ponies before death (mean ± standard error). TPR = total peripheral resistance; CO = cardiac output; P art = arterial blood pressure. C = control measurement before administration of high starch ration; ★ = statistically significant change ($P < 0.05$) from control value, using randomized complete-block analysis of variance and Student-Newman-Keuls' tests.

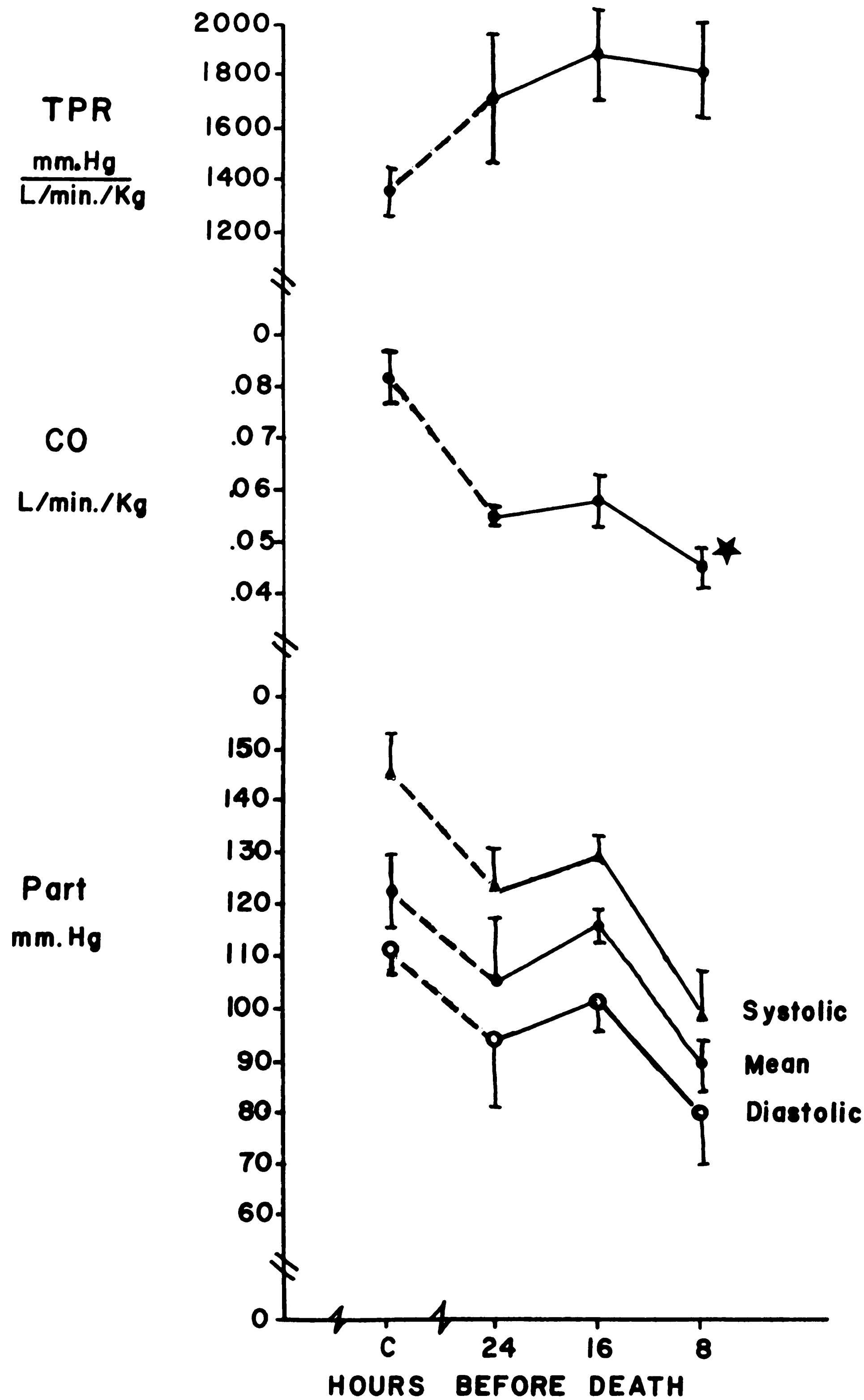

TPR
mm.Hg
L/min./Kg
2000
1800
1600
1400
1200
CO
L/min./Kg
0
.08
.07
.06
.05
.04
Part
mm.Hg
0
150
140
130
120
110
100
90
80
70
60
0
C
24
16
8
HOURS BEFORE DEATH
Systolic
Mean
Diastolic

FIGURE 9

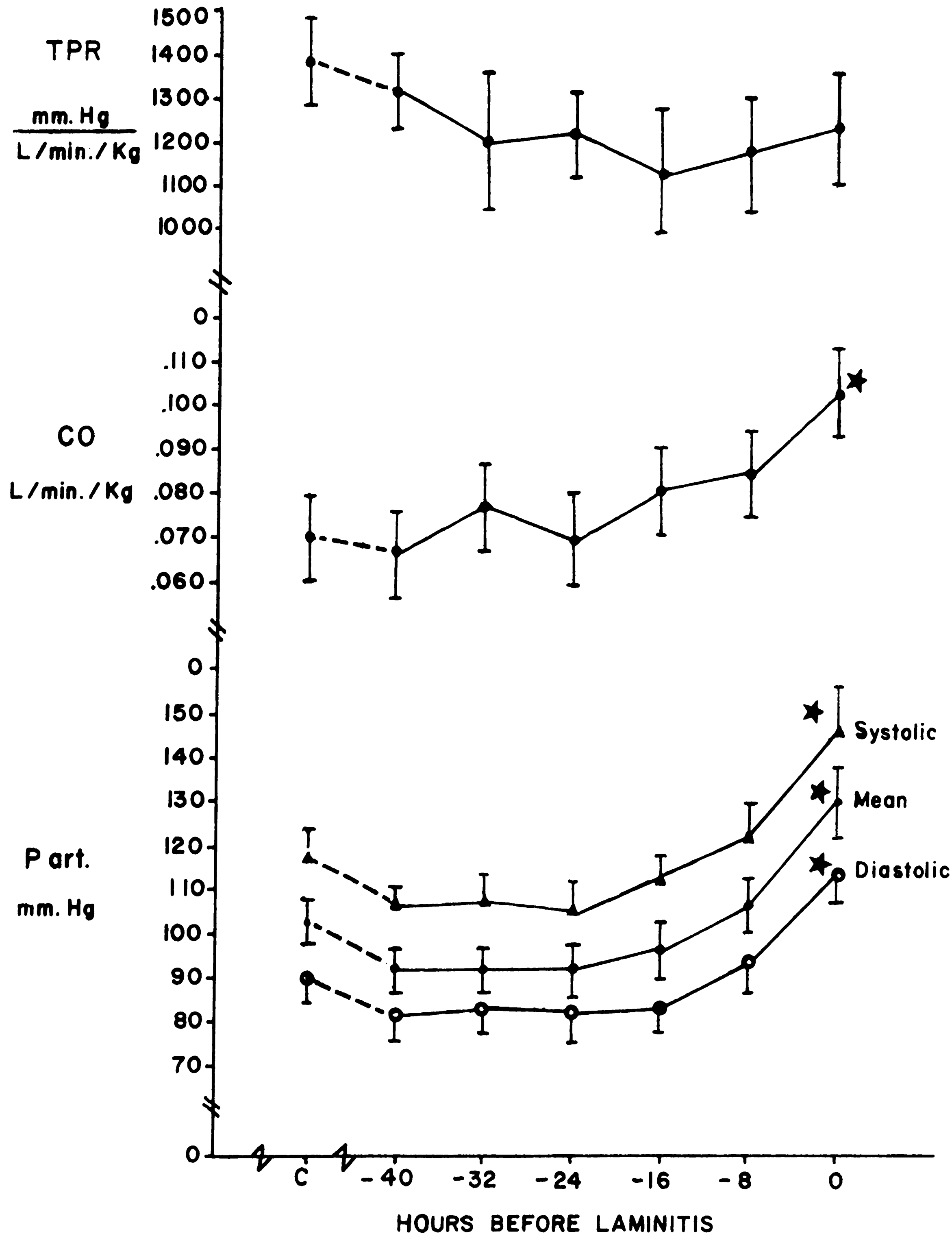
Animals developing laminitis showed no significant changes in cardiac output, arterial blood pressure or total peripheral resistance during the onset of the disease (Figure 10). At the time of laminitis both C.O. and arterial blood pressure were significantly increased above control values (Figure 10). Cardiac output increased from a control of .070 $\pm$ .010 l/min./Kg to .100 $\pm$ .010 l/min./Kg while mean arterial blood pressure increased from around 100.3 $\pm$ 4.7 mmHg to 130.1 $\pm$ 7.8 mmHg (Figure 10). Both systolic and diastolic pressure were also elevated at the time of the disease. TPR remained unchanged (Figure 10).

The transitory metabolic acidosis occurring in the laminitis ponies did not correlate with changes in blood pressure, C.O., or TPR (Figure 11).

Effect of Carbohydrate Overload on the Pulmonary Circulation

The pulmonary vascular resistance (PVR) increased with time in all the shock ponies, while those that developed laminitis showed no change in PVR during the onset of the disease (Figures 12, 13). In the shock group PVR almost doubled from the control (74% increase) eight hours prior to death. Even though the cardiac output decreased significantly in these ponies, their mean pulmonary arterial pressure remained unchanged because of the increase in PVR (Figure 12).

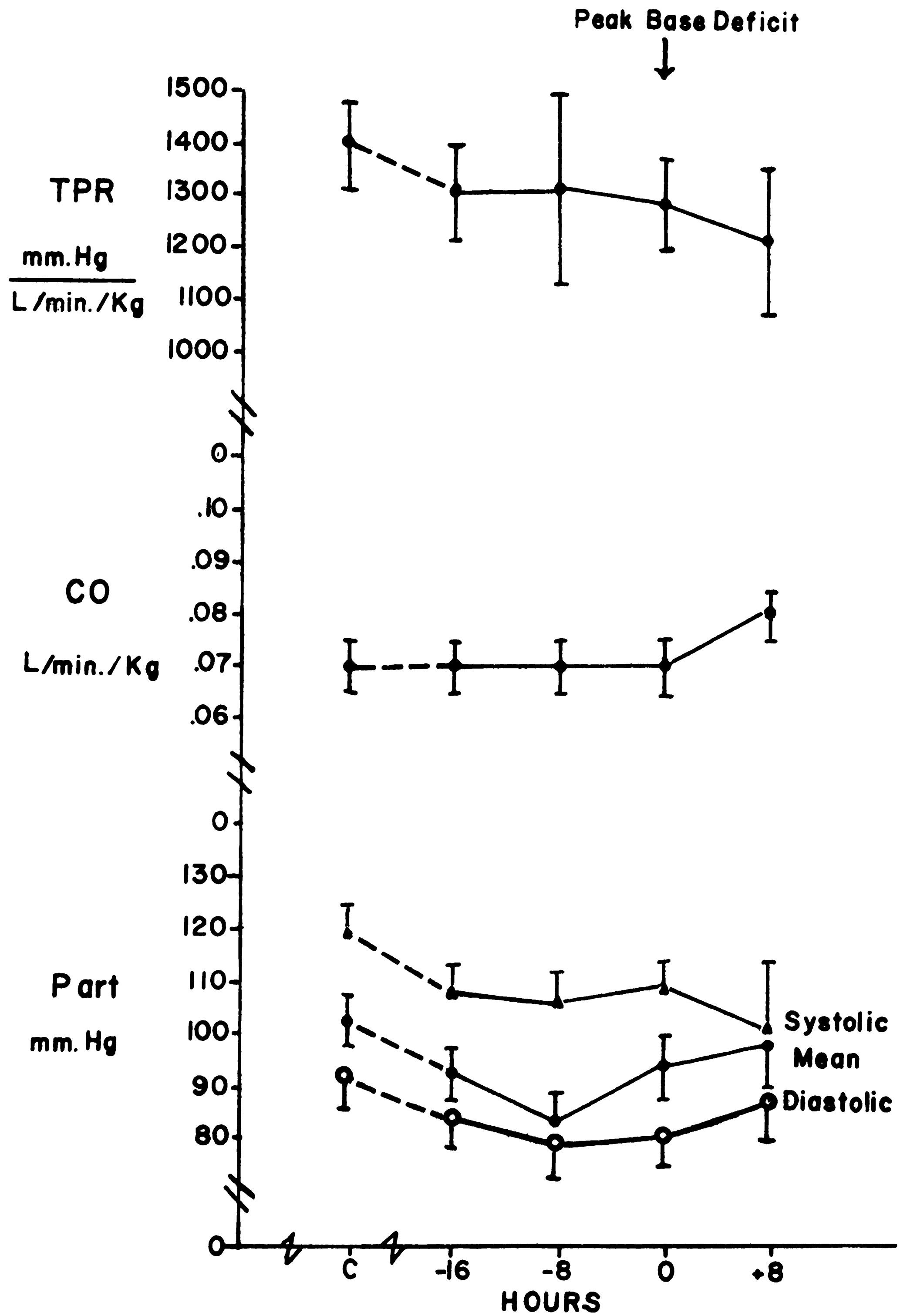
Figure 10. Measurements of systemic cardiovascular variables in laminitis ponies before and during the clinical signs of the disease (mean $\pm$ standard error). TPR = total peripheral resistance; CO = cardiac output; P art = arterial blood pressure. C = control value before administration of high starch ration; ★ = statistically significant change ($P < 0.05$) from control value, using randomized complete-block analysis of variance and Student-Newman-Keuls' tests.



TPR
mm. Hg
L/min./Kg
1500
1400
1300
1200
1100
1000
CO
L/min./Kg
0
.110
.100
.090
.080
.070
.060
P art.
mm. Hg
0
150
140
130
120
110
100
90
80
70
Systolic
Mean
Diastolic
0
C
-40
-32
-24
-16
-8
0
HOURS BEFORE LAMINITIS

FIGURE 10

Figure 11. Measurements of systemic cardiovascular variables before and after the peak base deficit in laminitis ponies. TPR = total peripheral resistance; CO = cardiac output; P art = arterial blood pressure. C = control value before administration of high starch ration; ★ = statistically significant change ($P < 0.05$) from control value, using randomized complete-block analysis of variance and Student-Newman-Keuls' tests.



Peak Base Deficit
1500
1400
1300
1200
1100
1000
TPR
mm.Hg
L/min./Kg
0
.10
.09
.08
.07
.06
CO
L/min./Kg
0
130
120
110
100
90
80
Part
mm. Hg
Systolic
Mean
Diastolic
0
C
-16
-8
0
+8
HOURS

FIGURE 11

Figure 12. Measurements of pulmonary vascular variables in shock ponies before death (mean ± standard error). PVR = pulmonary vascular resistance; CO = cardiac output; Ppa = pulmonary arterial pressure. C = control value before administration of high starch ration; ★ = statistically significant change ($P < 0.05$) from control value, using randomized complete-block analysis of variance and Student-Newman-Keuls' tests.

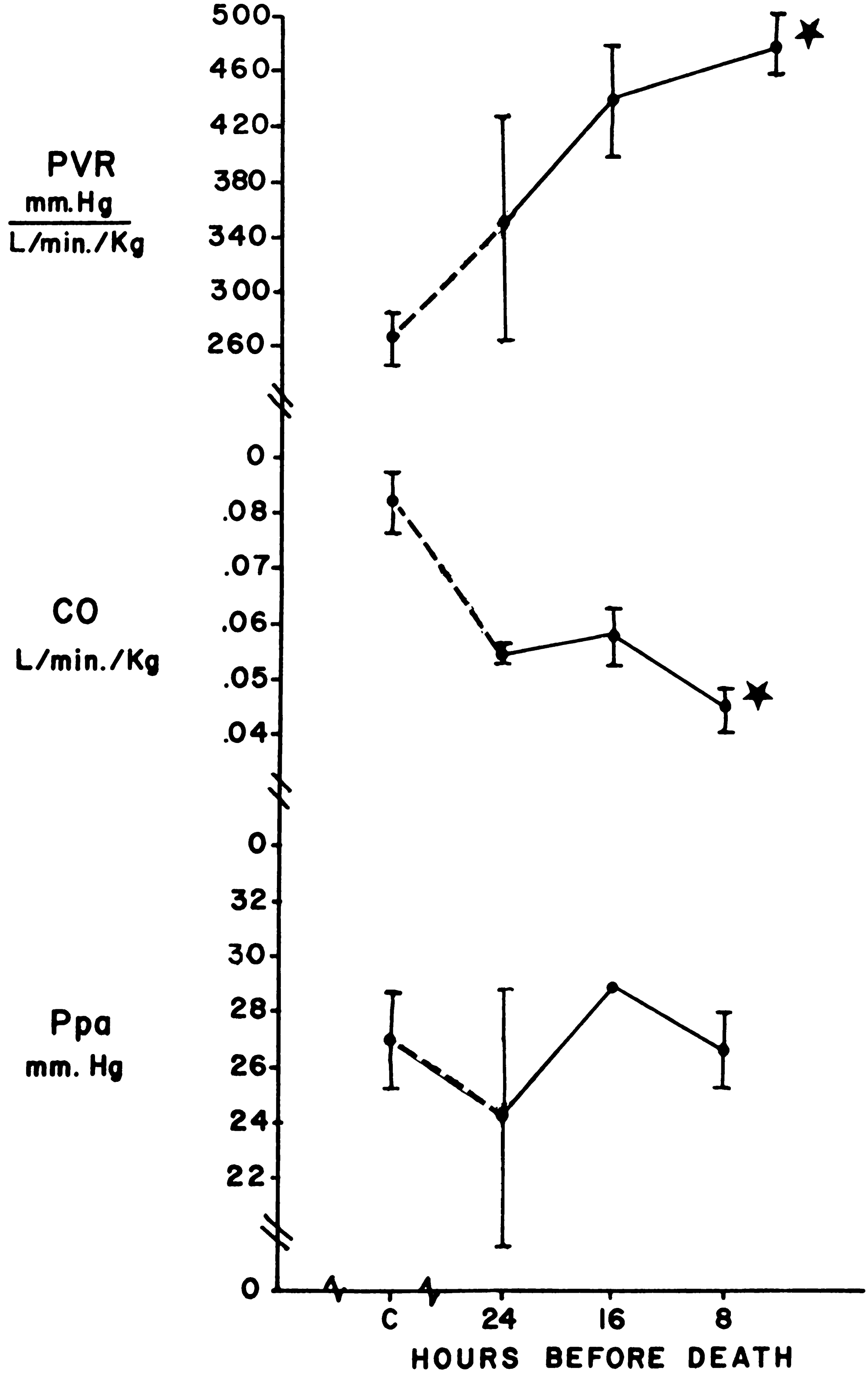

500
460
420
380
340
300
260
PVR
mm.Hg
L/min./Kg
0
.08
.07
.06
.05
.04
CO
L/min./Kg
0
32
30
28
26
24
22
Ppa
mm. Hg
0
C
24
16
8
HOURS BEFORE DEATH

FIGURE 12

Figure 13. Measurements of pulmonary vascular variables in laminitis ponies before and during the clinical signs of the disease (mean $\pm$ standard error). PVR = pulmonary vascular resistance; CO = cardiac output; Ppa = pulmonary arterial pressure. C = control value before administration of high starch ration; ★ = statistically significant change ($P < 0.05$) from control value, using randomized complete-block analysis of variance and Student-Newman-Keuls' tests.

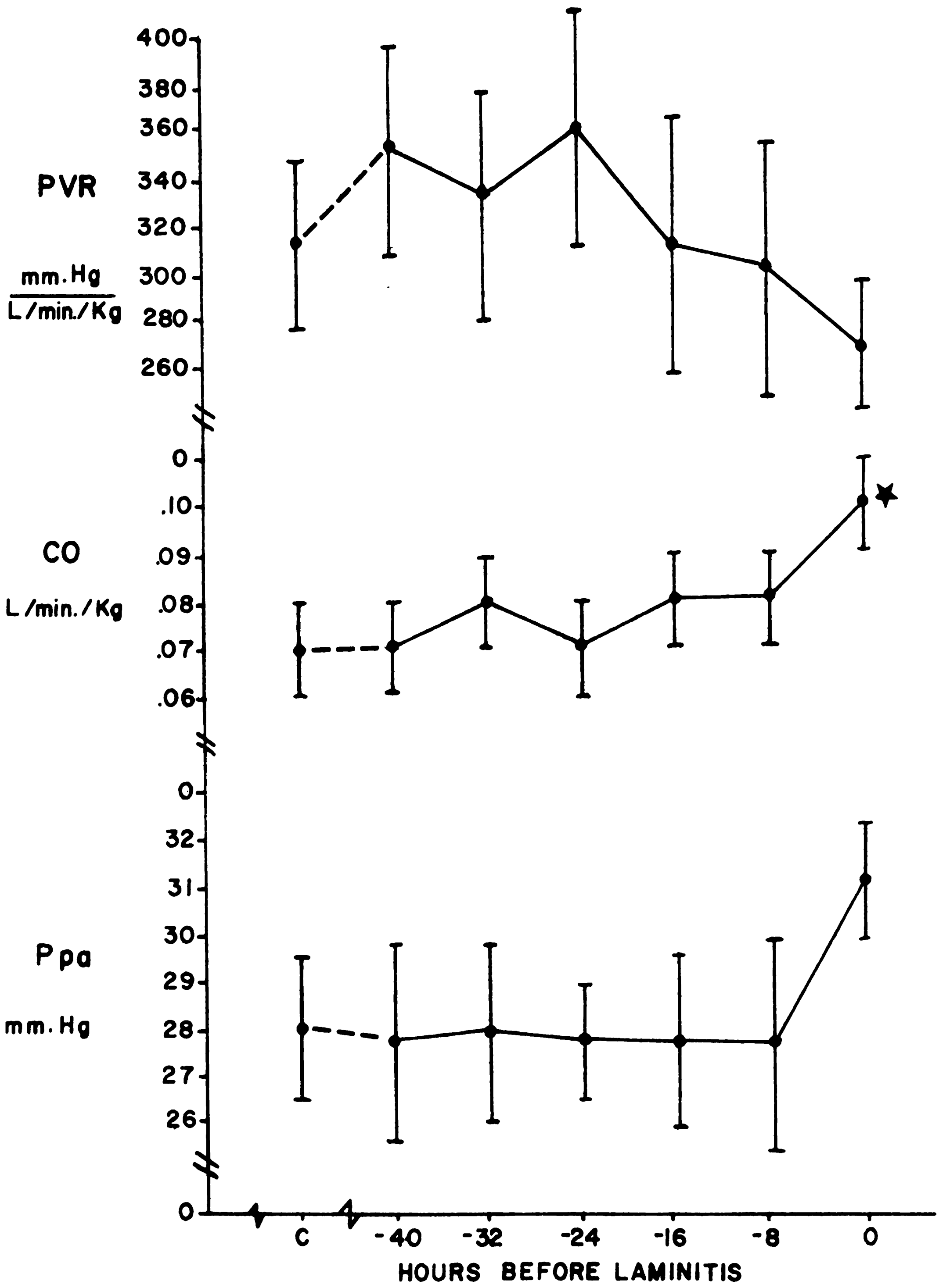

PVR
mm.Hg
L/min./Kg
400
380
360
340
320
300
280
260
CO
L/min./Kg
0
.10
.09
.08
.07
.06
Ppa
mm.Hg
0
32
31
30
29
28
27
26
0
C
-40
-32
-24
-16
-8
0
HOURS BEFORE LAMINITIS

FIGURE 13

Effect of Carboyhydrate Overload on the Variables of Heart Function

In the shock group, heart rate increased and stroke volume decreased after overeating, but right atrial pressure did not change (Figure 14). Heart rate increased 91% and stroke volume decreased 71% from control eight hours before death.

There was a transitory decrease in stroke volume in the laminitis ponies 22 ± 2 hours before laminitis (36 ± 6 hours after the diet administration) (Figure 15). This decrease was less severe (37% decrease from control) than in the ponies that died of shock (71% decrease from control). Heart rate increased significantly above control 32 ± 2 hours before the signs of the disease and remained elevated through the time of laminitis (Figure 15). Heart rates in these ponies were never as great as those in the shock ponies.

At the time of peak base deficit there was a significant increase in heart rate and a decrease in stroke volume. Stroke volume returned to normal eight hours after the peak in base deficit, while heart rate remained elevated (Figure 16).

Effect of Carbohydrate Overload on Body Fluid Volume and Composition

In all three ponies dying of shock, plasma volume was decreased eight hours before death (Figure 17). Packed cell volume increased from a normal of 38.0 ± 1.5% to 48 ± 2.5% and 54 ± 4.0%, sixteen and eight hours before death respectively. Even though plasma volume decreased, blood

Figure 14. Measurements of cardiac variables in shock ponies before death (mean ± standard error). Pra = right atrial pressure; Vs = stroke volume; HR = heart rate. C = control value before administration of high starch ration; ★ = statistically significant change ($P < 0.05$) from control value, using randomized complete-block analysis of variance and Student-Newman-Keuls' tests.

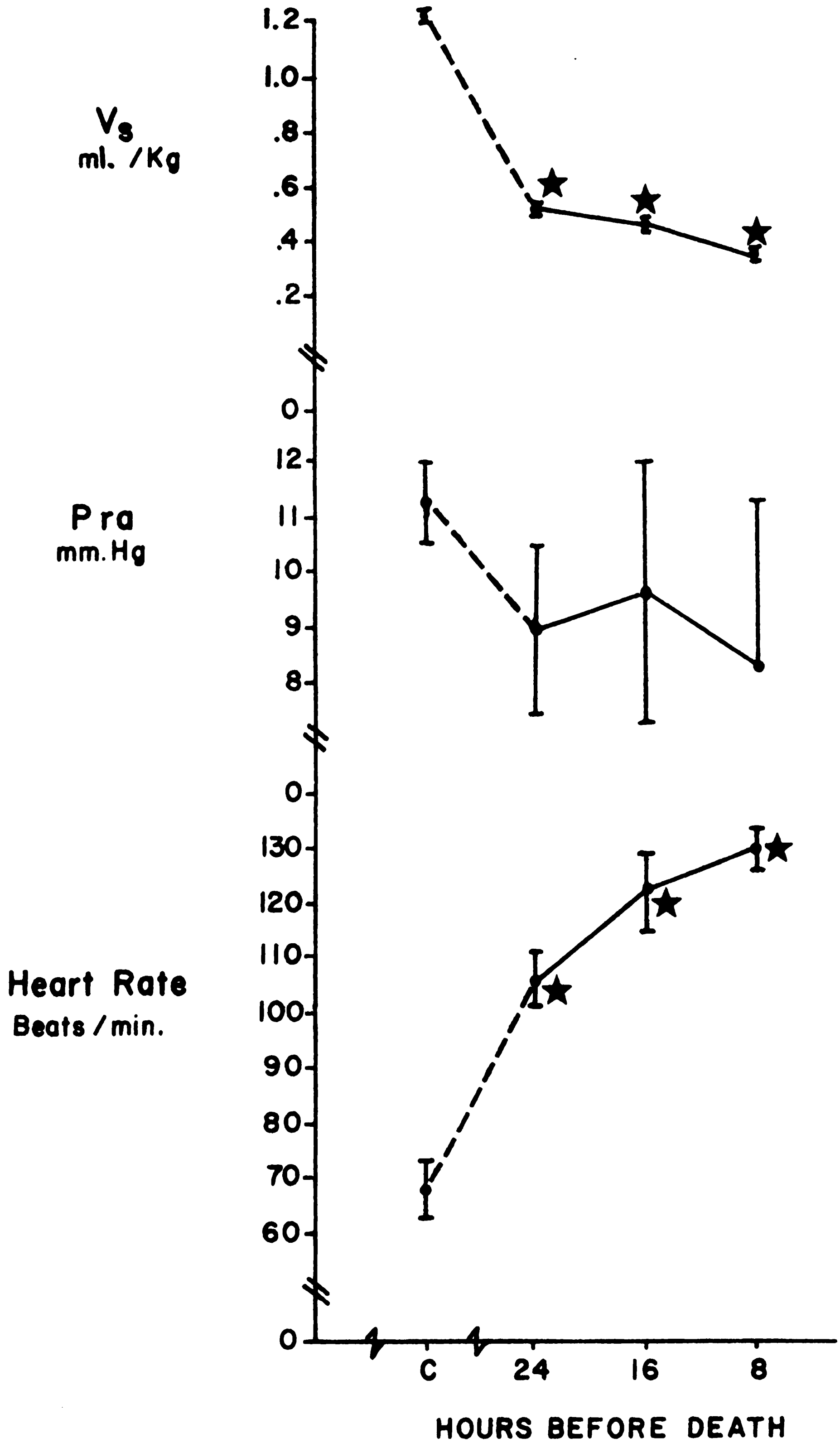

Vs
ml. /Kg
1.2
1.0
.8
.6
.4
.2
0
Pra
mm.Hg
12
11
10
9
8
0
Heart Rate
Beats / min.
130
120
110
100
90
80
70
60
0
C
24
16
8
HOURS BEFORE DEATH

FIGURE 14

Figure 15. Measurements of cardiac variables in laminitis ponies before and during the clinical signs of the disease (mean ± standard error). Pra = right atrial pressure; Vs = stroke volume; HR = heart rate. C = control value before administration of high starch ration; ★ = statistically significant change ($P < 0.05$) from control value, using randomized complete-block analysis of variance and Student-Newman-Keuls' tests.

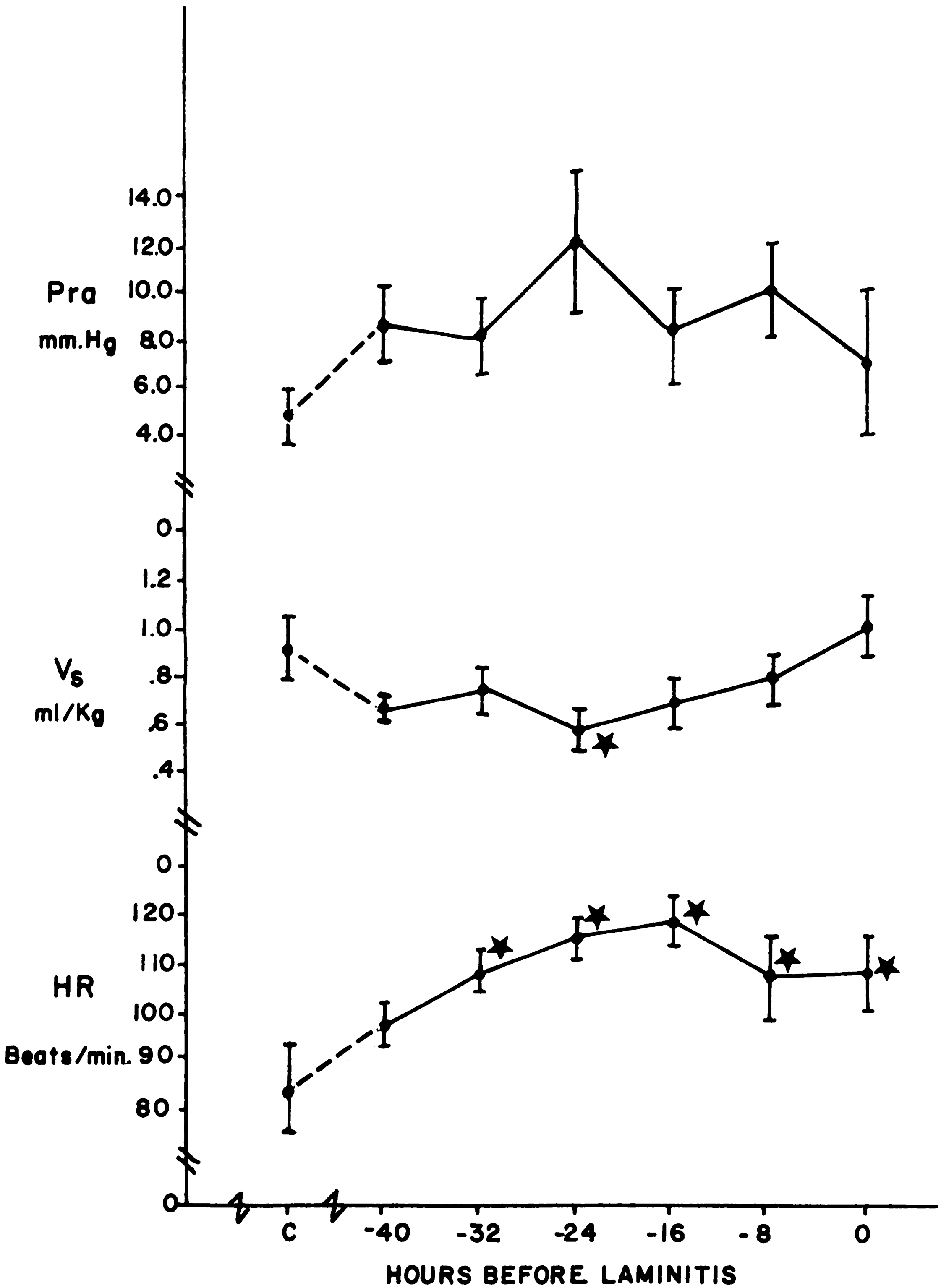

Pra
mm.Hg
14.0
12.0
10.0
8.0
6.0
4.0
Vs
ml/Kg
0
1.2
1.0
.8
.6
.4
HR
Beats/min.
0
120
110
100
90
80
0
C
-40
-32
-24
-16
-8
0
HOURS BEFORE LAMINITIS

FIGURE 15

Figure 16. Measurements of cardiac variables before and after peak base deficit in laminitis ponies (mean ± standard error). Pra = right atrial pressure; Vs = stroke volume; HR = heart rate. C = control measurement before administration of high starch ration; ★ = statistically significant change ($P < 0.05$) from control value, using randomized complete-block analysis of variance and Student-Newman-Keuls' tests.

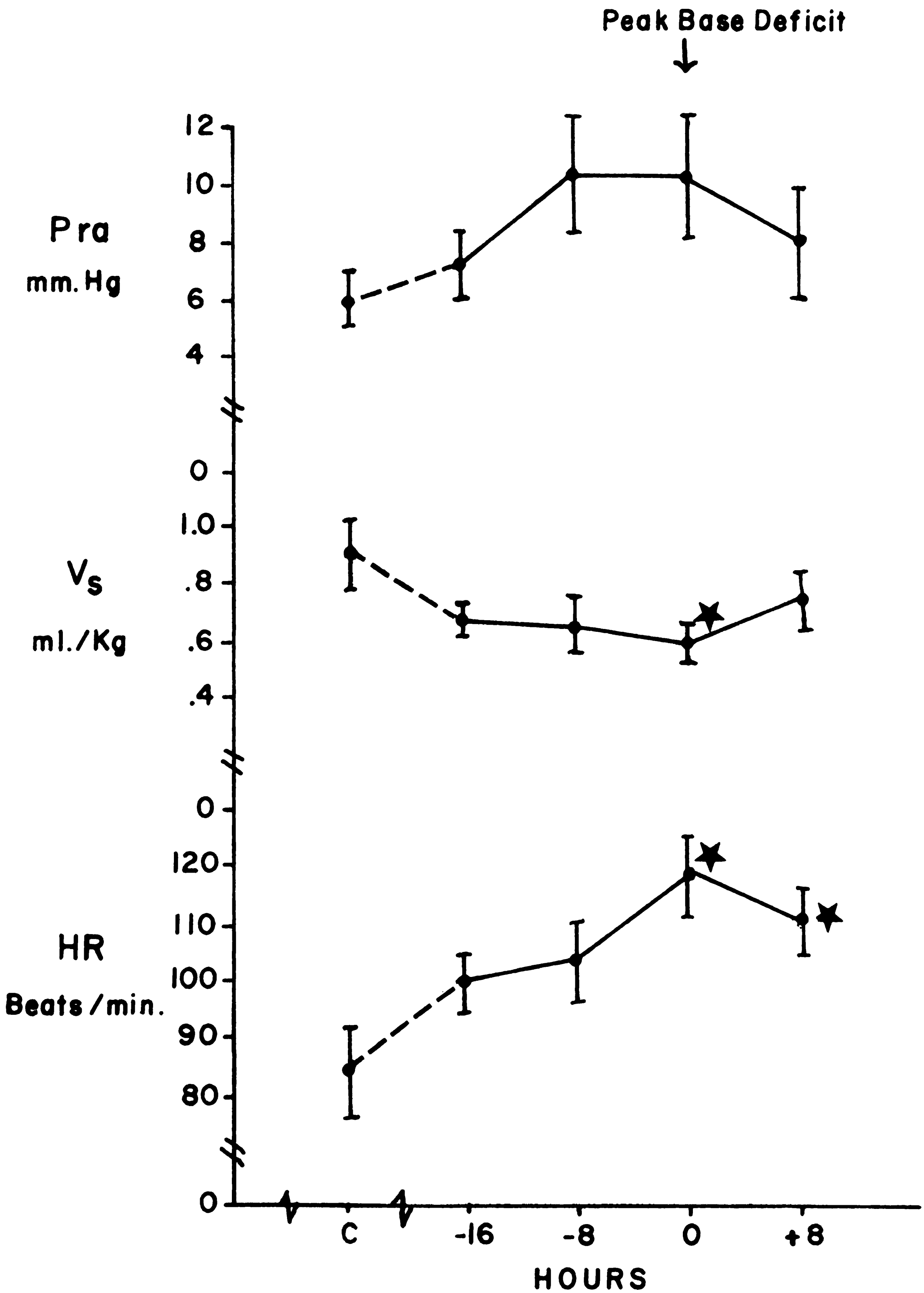

Peak Base Deficit
12
10
8
6
4
Pra
mm. Hg
0
1.0
.8
.6
.4
V_s
ml./Kg
0
120
110
100
90
80
HR
Beats/min.
0
C
-16
-8
0
+8
HOURS

FIGURE 16

Figure 17. Measurements of body fluids in shock ponies before death (mean ± standard error). TP = total protein; PCV = packed cell volume; PV = plasma volume. C = control measurement before administration of high starch ration; ★ = statistically significant change ($P < 0.05$) from control value using randomized complete-block analysis of variance and Student-Newman-Keuls' tests.

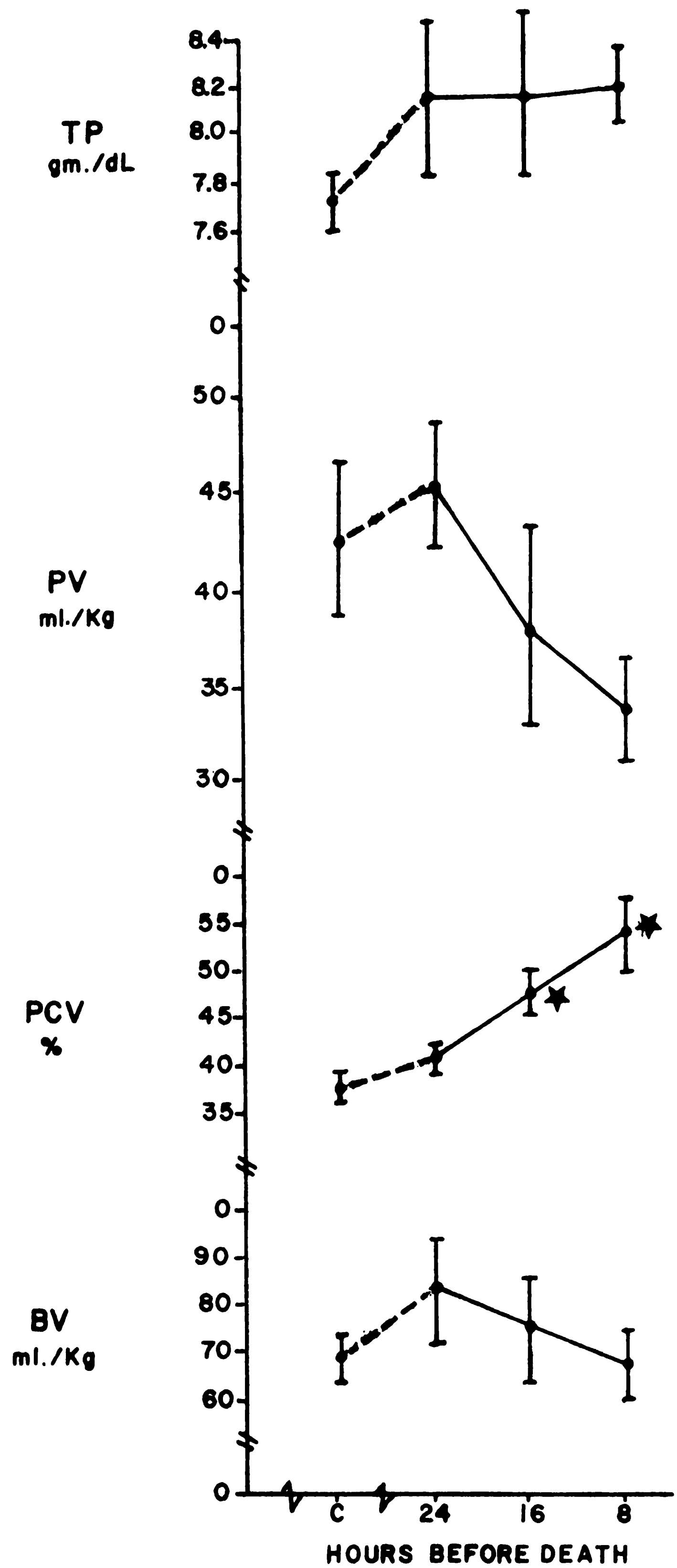

TP
gm./dL
8.4
8.2
8.0
7.8
7.6
0
50
45
PV
ml./Kg
40
35
30
0
55
50
PCV
%
45
40
35
0
90
BV
ml./Kg
80
70
60
0
C
24
16
8
HOURS BEFORE DEATH

FIGURE 17

volume remained unchanged because of this increase in PCV.

There was no significant changes in blood volume, plasma volume, or PCV during the onset of laminitis (Figure 18).

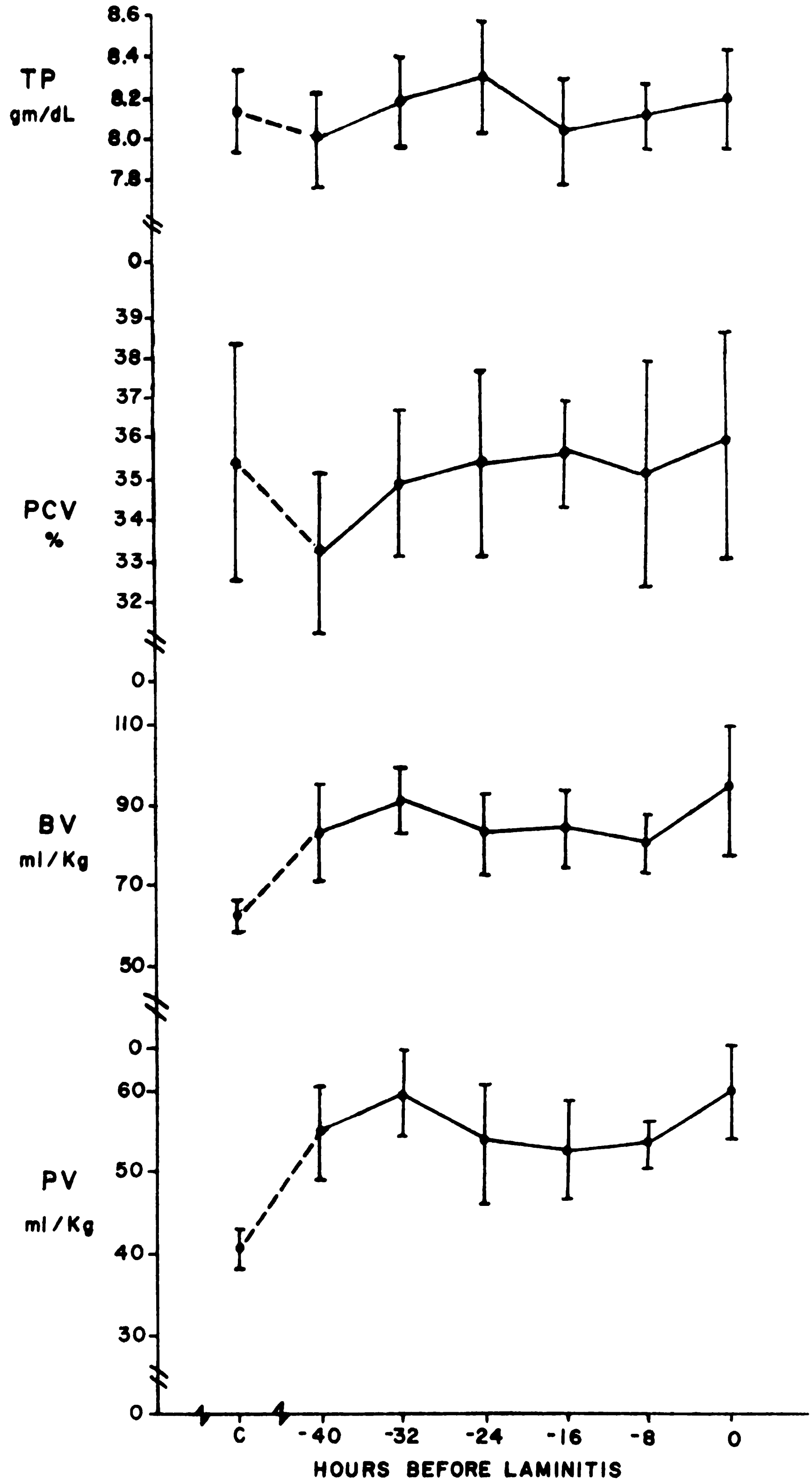
Even though no significant changes in blood volume, plasma volume, or PCV were seen when the data was analyzed in terms of hours before laminitis, in every pony that developed the disease there was either a transient decrease in PCV (4.0 $\pm$ 0.2 percentage points in ponies 1, 2, 3, and 6) or a transient discontinuation of the rise in PCV (ponies 10, 11, and 12) eight to ten hours before the clinical signs of the disease. The PCV returned to normal or was slightly elevated at the time of laminitis. Therefore, to determine the reason for this transitory event, plasma volume and blood volume measurements were analyzed with zero time defined as the transitory decrease in PCV for every pony (Figure 19). Plasma volume and blood volume, at the time of the change in PCV, decreased slightly though not significantly from control.

Effect of Carbohydrate Overload on Serum Electrolytes

There were no significant changes in potassium, sodium,or chloride in the three shock ponies 24, 16, and 8 hours before death.

In the laminitis group, potassium decreased 24, 16, 8, and 0 hours before the disease (Figure 20). At the time of laminitis potassium was 2.54 $\pm$ .14 meg/l compared to a

Figure 18. Measurements of body fluids in laminitis ponies before and during the clinical signs of the disease (mean ± standard error). TP = total protein; PCV = packed cell volume; BV = blood volume; PV = packed cell volume. C = control measurement before administration of high starch ration; ★ = statistically significant change ($P < 0.05$) from control value, using randomized complete-block analysis of variance and Student-Newman-Keuls' tests.



TP
gm/dL
8.6
8.4
8.2
8.0
7.8
0
39
38
37
36
35
34
33
32
PCV
%
0
110
90
70
50
BV
ml/Kg
0
60
50
40
30
PV
ml/Kg
0
C
-40
-32
-24
-16
-8
0
HOURS BEFORE LAMINITIS

FIGURE 18

Figure 19. Measurements of body fluids before and after drop in packed cell volume in laminitis ponies (mean $\pm$ standard error). TP = total protein; BV = blood volume; PV = plasma volume. C = control measurement before administration of high starch ration; ★ = statistically significant change ($P < 0.05$) from control value, using randomized complete-block analysis of variance and Student-Newman-Keuls' tests. +.0 = values significantly different ($P < 0.05$).

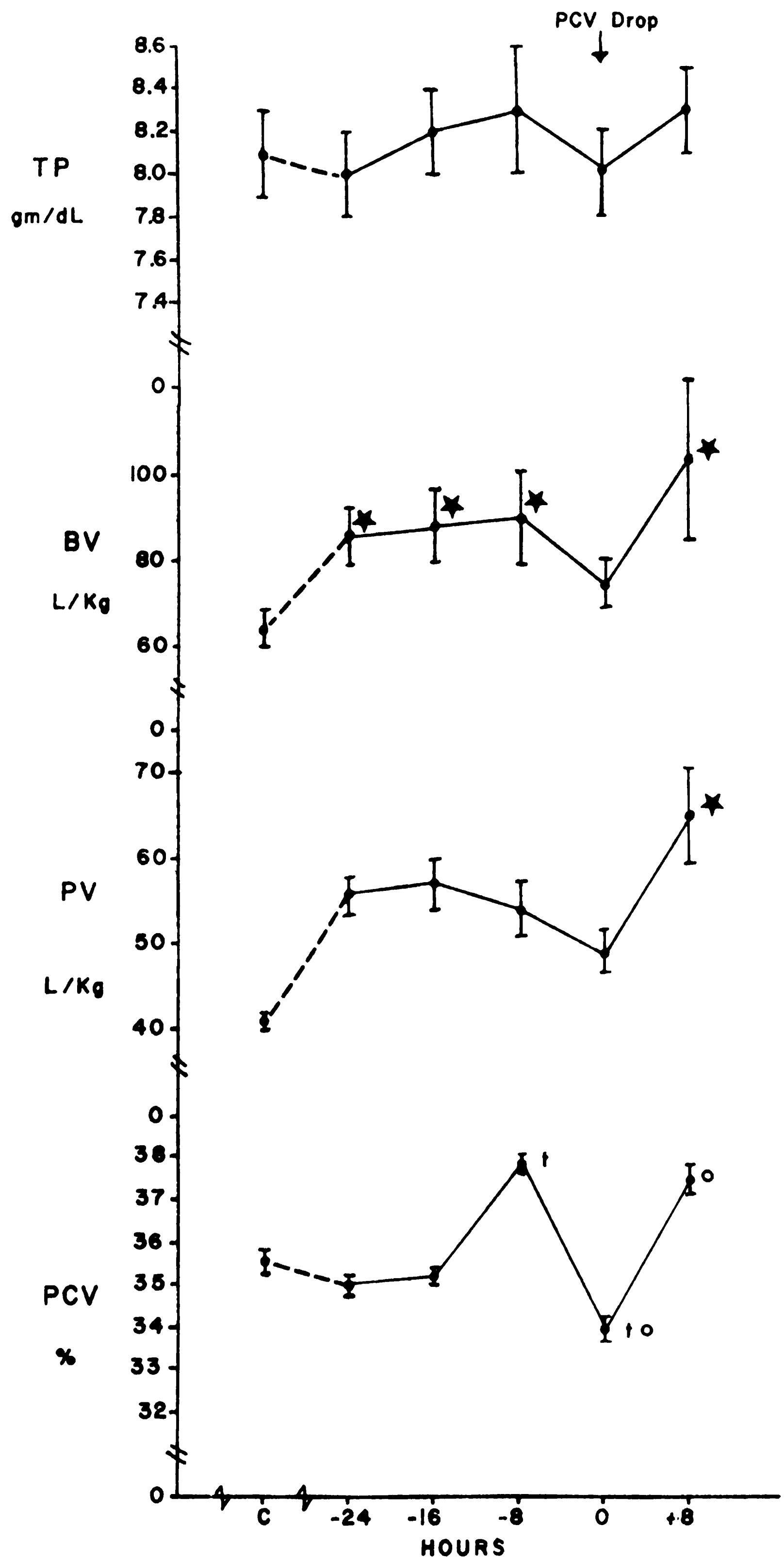
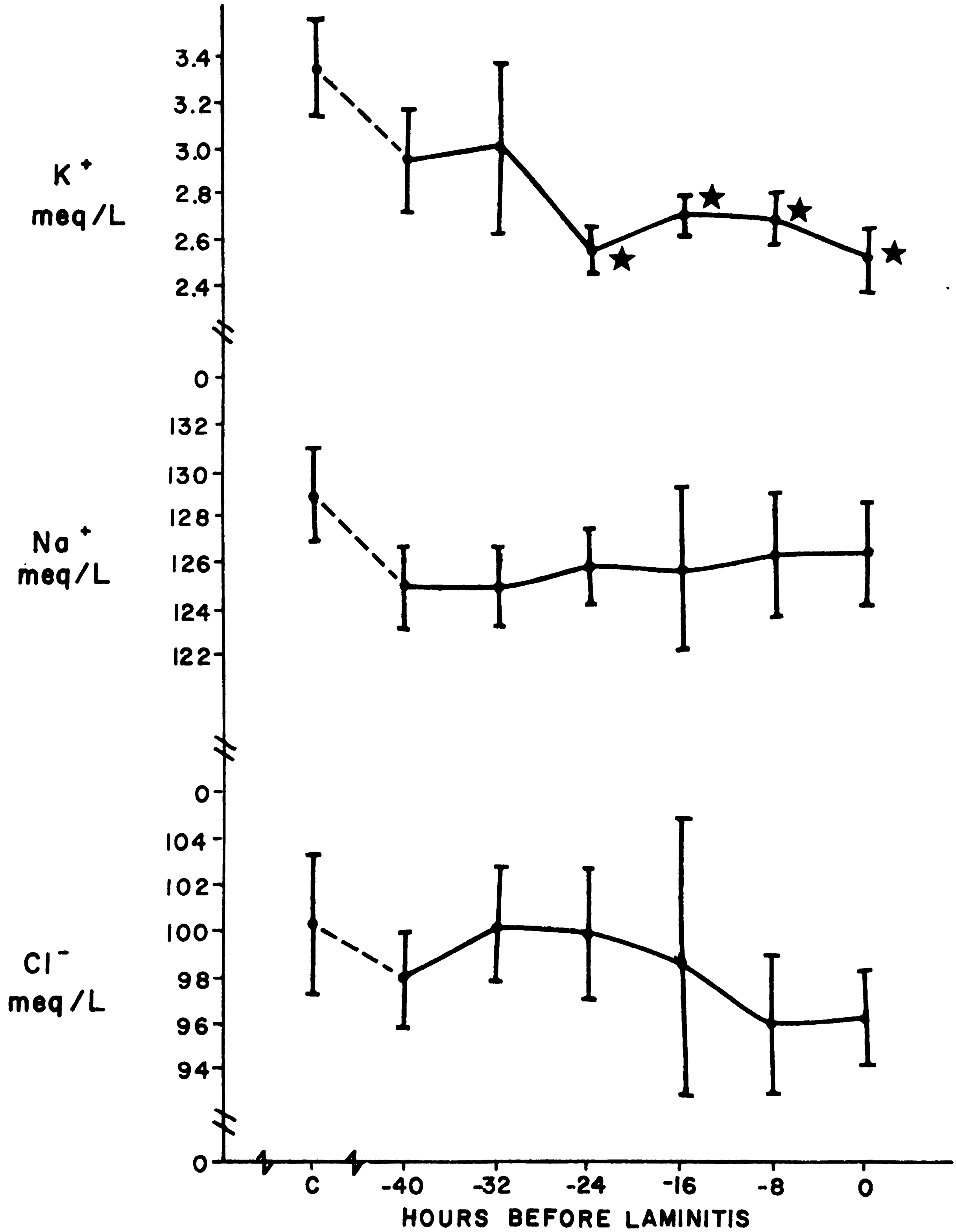

PCV Drop
TP
gm/dL
8.6
8.4
8.2
8.0
7.8
7.6
7.4
BV
L/Kg
0
100
80
60
PV
L/Kg
0
70
60
50
40
PCV
%
0
38
37
36
35
34
33
32
0
C
-24
-16
-8
0
+8
HOURS

FIGURE 19

Figure 20. Serum electrolyte measurements in laminitis ponies before and during the clinical signs of the disease (mean $\pm$ standard error). C = control measurement before administration of high starch ration; ★ = statistically significant change ($P < 0.05$) from control value, using randomized complete-block analysis of variance and Student-Newman-Keuls' tests.



3.4
3.2
3.0
2.8
2.6
2.4
K⁺ meq/L
0
132
130
128
126
124
122
Na⁺ meq/L
0
104
102
100
98
96
94
Cl⁻ meq/L
0
C
-40
-32
-24
-16
-8
0
HOURS BEFORE LAMINITIS

FIGURE 20

control of 3.36 $\pm$ .21 meq/l. Sodium and chloride in these ponies remained unchanged during the onset of the disease (Figure 20). Because of insufficient amounts of sample a complete statistical analysis of osmolarity could not be done.

DISCUSSION

Events in the systemic circulatory system after overfeeding have been suggested as causes of acute laminitis. The results from this study suggest that the major cardiovascular occurrences are responses to rather than causes of the disease. The increased arterial blood pressure at the time of laminitis results entirely from an increased cardiac output, since total peripheral resistance is unchanged. This hypertension occurred only at the time of the clinical signs of the disease and not during the onset of laminitis. The increased cardiac output was due to an increase in heart rate.

In one pony with acute laminitis, high volar nerve blocks were performed by injecting 2% lidocaine in the medial palmar nerve of all four feet. Within 10-15 minutes the elevated cardiac output of 23.8 l/min decreased to a control value of 13.4 l/min and concomitantly the mean arterial pressure decreased from 154 mmHg to 100 mmHg. This suggests that the increased cardiac output resulted from increased sympathetic outflow caused by pain in the feet.

These results are in some conflict with previously published findings where hypertension was described as a progressive development following overeating rather than an

abrupt development concurrent with clinical signs of laminitis (Garner, 1975).[17] However, in more recent studies by these investigators, arterial pressure increased only in the final prodromal phase of the disease.[18] The conflict in the time of hypertension in this study and the investigation of Garner, probably is due to the methods of statistical analysis. Since animals develop laminitis at varying intervals after overeating, using overeating as zero time in determining group means may hide many of the subtle and sudden changes occurring around laminitis. When clinical signs of laminitis are used as zero time, the onset of hypertension and other events become more obvious.

Following overfeeding with the high starch ration, the ponies either developed acute laminitis, died of shock, or remained normal. The ponies developing laminitis showed no decrease in cardiac output prior to the time of the disease. In contrast, the first event indicating the onset of shock in the overfed pony was the abrupt decrease in cardiac output 8-16 hours after the diet administration. This decrease of cardiac output appears to be due to a decrease in the contractility of the heart, since right atrial pressure remained unchanged and heart rate was acutely elevated.[22]

No consistent cardiovascular signs of transitory shock occurred in animals prior to the clinical signs of laminitis. In the present study, total peripheral resistance was unchanged at the time of laminitis. It should be noted that it has been previously reported that digital

vascular resistance was decreased at a similar time.[43] Since total peripheral resistance is the algebraic sum of the resistances of parallel systemic vascular beds, it is reasonable to assume the dilation in the digital vasculature was counteracted by constriction elsewhere. Such a differential response of vascular beds has been reported during intravenous infusion of epinephrine[22] and may well occur during an in vitro sympatho-adrenal discharge.

No changes in the pulmonary vascular resistance (PVR) occurred in those ponies that developed laminitis, but the elevation of PVR in the three shock ponies was much more pronounced than that of TPR. This corresponds to previous shock studies in the dog.[19, 21, 44] In addition, pulmonary constriction and edema of the lungs of calves in shock have been reported.[32]

The ponies' PVR during shock were twice control. The elevations of pulmonary vascular resistance probably result from an increase in viscosity (increased hematocrit and possible intravascular clotting), since very little sympathetic vascular control exists in the lungs.

Garner (1975) has reported that plasma volume decreases concomitantly with increases in cardiac output and arterial pressure after overfeeding.[18] No evidence of this type of correlation was seen in any of the overfed ponies in this experiment. Cardiac output and plasma volume decreased concomitantly in the shock ponies, while in the laminitis ponies plasma volume remained unchanged at the time cardiac output and blood pressure were elevated.

Although no changes in the vascular fluids (plasma volume, blood volume) was apparent in the laminitis group, there was a significant increase in the packed cell volumes of the shock group. Through the following calculations it was determined that there is not only a decrease in plasma volume, but also an increase in the cellular volume (CV) in the circulation of the pony in shock:

$$CV_{control} = \frac{(Hct_1)\ (PV_1)}{(1-Hct_1)}$$

$$CV_{final} = \frac{(Hct_2)\ (PV_2)}{(1-Hct_2)}$$

The calculated control CV was approximately 26.2 ml/Kg, while "eight hours" prior to death it was 39.8 ml/Kg. The increased CV implies either an increase in the number of erythrocytes in the circulation during shock or an increase in mean corpuscular volume. In several animal species including the horse, the spleen acts as a reservoir of red cells and can under sympathetic stimulation discharge blood with a high hematocrit into the circulation.[41] Splenic contraction is the most likely cause of the increased CV in the shock ponies.

Overfed ruminants have decreased plasma volume, increased hematocrit, and no change in blood volume. The increase in hematocrit and the decrease in plasma volume of overfed ruminants has been attributed to release of red blood cells into the circulation and to water movement into the rumen. It is very possible that the pony in shock, like the overfed ruminant, is losing plasma volume into the

gut and also contracting its spleen, both of which are reflected in its hematocrit.

There were no significant changes in hematocrit or plasma volume in the laminitis ponies, however, there was a transient decrease in hematrocrit 8-16 hours before the disease in each individual laminitis pony. This must be explained by a sequestration of erythrocytes rather than a transitory increase in plasma volume, since PV also was tending to decrease at this time (Figure 19). This sequestration of erythrocytes may occur in the spleen or as a result of peripheral intravascular coagulation. Some evidence of coagulopathies in laminitis has been observed (Bell, T. G., personal communication),[5] and intravascular agglutination has been implicated in shock.[47]

The metabolic acidosis seen in both groups of overfed ponies may have several causes. First, hypoxia may increase blood lactate levels, decreasing blood pH. The increase in TPR in shock causes decreased tissue perfusion which may cause stagnant anoxia. Increased lactate/pyruvate ratios occur in shock in other species.[47] Increases in the latter are thought to reflect increased anaerobic metabolism, since the increase in the lactate concentration in the blood should be accompanied by a proportional increase in pyruvate concentration if it is due solely to aerobic metabolism.[24]

Even though there was no apparent change in TPR in the laminitis ponies, this does not mean that tissue hypoxia

was absent. Redistribution of blood flow could create areas of hypoxia resulting in lactic acidosis.

Another contributor to the acidosis may be increased fermentation of carbohydrate in the gut. There are numerous reports in the literature of metabolic disorder in ruminants associated with ingestion of large quantities of carbohydrates.[32] However, little has been written about the pathogenesis of the similar acidosis in overfed ponies or horses. When cattle and sheep are overfed soluble carbohydrates, the pH of the rumen contents decreases together with an increase in ruminal concentration of lactic acid (normally indetectable) and an increase in concentration of volatile fatty acid.[32] The rumen pH may fall to below pH 4.0 in very severe cases of carbohydrate overfeeding.[16, 32] Correlated with the fall in pH is a marked increase in concentration of lactate in the blood with the D-isomer predominating. Although the absorption of lactic acid is a relatively slow process, once the pH of the rumen contents falls below pH 5 a significant amount of free lactic acid is absorbed.[32] Furthermore, it is probable that substantial amounts of lactic acid or its salts may be absorbed from the intestine. The presence of D(-) lactate in the blood indicates that sufficient absorption occurs from the alimentary tract to overload the mechanism for removing this isomer from the blood. This decreases both the alkali reserve and pH of the blood.[32, 40]

The cecum and large colon are the regions of the disgestive tract which enable the horse to utilize most of

the carbohydrates which require microbial digestion. As in the ruminant, the principle products of microbial digestion of polysaccharides appear to be volatile fatty acids[3] which can be produced in large quantities. Following overfeeding of ponies, lactate production in the cecum and large colon may increase, resulting in the increased levels of blood lactate.[18] The stomach may be a further source of lactic acid, since the stomach of the normal adult horse produces substantial amounts of lactic acid by bacterial fermentation.[3] This lactic acid which is produced in greater quantities than in pigs and rabbits[3] appears to be absorbed in the small intestine. When a pony is overfed, an increased gastric source of lactic acid could result in elevated levels of lactic acid absorbed into the blood stream from the small intestine.

Another factor adding to the acidosis may be increased gluconeogenesis due to stress related adrenocorticoid release. Increased blood glucose has been reported in overfed ruminants.[25, 32] Adrenocorticoid hormones increase gluconeogenesis, resulting in glucose accumulation with reduced pyruvate metabolism due to a lack of NAD.[32]

The control of blood pH relies on excretion of hydrogen ions in the urine or changes in ventilation. The overfed ponies appeared to urinate less frequently after the diet administration (dry bedding noticed). Oliguria has been reported in overfed ruminants. Ruminant urine is usually alkaline and the fall to pH 5 found in acidosis

represents a substantial increase in excretion of acid. The oliguria frequently observed in overfed animals would contribute significantly to the acidosis.

Finally, the increase in blood acidity could be due to the loss of bicarbonate via the bowel since diarrhea was frequently seen in the ponies that developed laminitis.

All the laminitis ponies exhibited only a transitory acidosis. A respiratory compensation occurred within eight hours after their individual peaks in base deficit (Figure 8).

The respiratory compensation of the laminitis group, whose peak base deficit was 12.0 $\pm$ 1.8 mg/l, effectively returned blood pH back to normal at the time of laminitis. When the base deficit was greater than 14 mg/l, as in the shock ponies, the respiratory system apparently was unable to compensate sufficiently to return the animal to a normal acid-base status. Acidosis presumably contributed to the events leading to death in this shock group of ponies.

Acidosis also affected the cardiovascular system by increasing heart rate and subsequently decreasing stroke volume. The increased hydrogen ion concentration of the blood causes decreased contractility of the myocardium,[26, 31, 45] which in turn decreases arterial pressure, causing reduced baroreceptor stimulation. The decrease in impulse from the baroreceptor decreases excitation of the cardioinhibitory center and reduces vagal activity, increasing heart rate.[30, 46]

Although acidosis may increase heart rate during the onset of the disease, it does not maintain the elevated heart rate at the time of clinical signs of laminitis, since the acid-base status is then normal. Other factors increasing heart rate may be fever and pain. Body temperature increased at the same time as heart rate and both remained elevated. Pain from the gut or feet may increase heart rate by increasing sympathetic outflow to the heart.

Even though both groups of ponies developed acidosis, neither group developed concurrent hyperkalemia which usually results from movement of K^+ from the intracellular to the extracellular compartment. K^+ was unchanged in shock ponies and hypokalemia developed in the laminitis group before the disease and continued until the onset of clinical signs. In the laminitis group, K^+ may have been excreted in the loose, sloppy feces faster than it left the ICF resulting in hypokalemia. Shock ponies had greater acidosis and no diarrhea. Any loss of K^+ into the gut was replaced by K^+ displaced from the ICF, resulting in normal K^+ levels.

The present study indicates that the systemic cardiovascular adjustments seen after overfeeding and prior to alimentary laminitis are responses to rather than primary causes of this hoof disease. The characteristic hypertension of acute laminitis occurred only at the time of the disease. The results of this study and our preliminary nerve block experiment suggest that pain is a primary factor in the cardiovascular response (hypertension) at the time of laminitis.

This study, however, does not indicate whether or not blood flow to the laminae was changed prior to laminitis. Even though TPR remained unchanged during the onset of laminitis, this does not exclude the possibility of redistribution of blood flow in the systemic or local circulation. If blood flow is shunted away from the laminae, this deprivation would initiate breakdown of the tissue.

Finally, this investigation dealt only with the hypothesis that laminitis is a circulatory problem. The disease may not be the result of changes in blood flow, but the direct effect of a substance (e.g. endotoxin) or a state (e.g. acidosis) on the laminae of the hoof. Even though systemic cardiovascular events did not resemble shock prior to laminitis, an endotoxin(s) may be causing direct breakdown of the laminar corium without affecting blood flow. Deleterious agent(s) may be produced at the site of the laminae or in a remote area of the body before the development of the disease. In addition, the transitory acidosis may be causing direct laminar breakdown during the onset of the disease.

SUMMARY

From the results of this investigation, the following conclusions can be made concerning the development of experimentally induced alimentary laminitis in ponies:

1. There is no evidence that the major cardiovascular events prior to laminitis are causes of the disease. The cardiovascular changes that were seen appear to be responses to rather than causes of the disease.
2. Hypertension occurs only at the time of laminitis. There was no evidence of a progressive development of hypertension. Rather, an abrupt increase in blood pressure occurred when the clinical signs of the disease were apparent. The hypertension is due to an increase in cardiac output.
3. A transient metabolic acidosis occurs prior to laminitis. However, at the time of acute laminitis, arterial blood pH is near normal.
4. Hypokalemia develops prior to the disease and continues to the time of acute laminitis. No changes in arterial sodium and chloride concentrations were apparent after overeating and prior to laminitis.

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