ABSTRACT

ENDOTOXIC SHOCK IN THE HORSE

By

Kenneth E. Gertsen

In this study 4 horses were used to study the cardiovascular effects of intravenous injection of an endotoxin of *E. coli*.

Clinical signs of endotoxic shock were noted. The horses all exhibited similar signs within 1 hour of the injection. They included depression, cold, clammy extremities, decreased capillary perfusion time, and decreased blood pressure. Hemoconcentration and acidosis were noted in the blood of each horse. Three of the horses died within 14 hours of the injection of the endotoxin. One mare survived 3 weeks before being euthanatized.

Necropsy examinations were performed on all of the horses. Hyperemia and numerous petechial and ecchymotic hemorrhages were noted grossly throughout the body. Histopathologic examination confirmed the lesions of hyperemia and hemorrhages noted on gross examination.

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By

Kenneth E. Gertsen

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INTRODUCTION

In recent reports seen in veterinary literature (Carrol *et al.*, 1965; Coffman and Bracken, 1968; Roberts, 1965) endotoxic shock in the horse has been discussed. Only one report (Carrol *et al.*, 1965) was concerned with the production of experimental endotoxic shock; the other two discussed clinical cases diagnosed as endotoxic shock (Coffman and Bracken, 1968; Roberts, 1965).

It has been suggested that endotoxins from various gram-negative bacteria play important roles in the colitis "X" syndrome.

This research was conducted to establish dosages, cardiovascular and clinical response of the horse to intravenous injection of endotoxin. The clinical responses seen were compared to signs seen in colitis "X".

With the data obtained, it should be possible to use this model of endotoxic shock to further study the syndrome, as well as to develop therapeutic techniques for use in animals in clinical shock.

REVIEW OF LITERATURE

Description of the Disease

Various writers have recognized the colitis "X" syndrome by the following clinical signs: acute onset, profuse diarrhea, peripheral circulatory collapse, rapid dehydration, and hemoconcentration (Bryans, 1963; Olson, 1966; Pickrell, 1968; Rooney *et al.*, 1963; Rooney, 1965). Most affected horses collapse, are unable to rise (Pickrell, 1968), become dyspneic (Pickrell, 1968; Rooney, 1965), and develop initial temperature rises which later drop to normal or subnormal (Olson, 1966; Rooney, 1963; Bryans, 1963). The disease usually terminates in death within 24 to 48 hours (Rooney, 1963; Rooney, 1965).

In the early stages of the syndrome the hemoconcentration is accompanied by leukopenia that is predominantly neutropenia (Bryans, 1963; Rooney, 1963). Leukocytosis may be seen later (Rooney, 1963). In most cases the blood urea nitrogen level is elevated to over 60 mg./100 ml. There is loss of serum sodium, potassium and bicarbonate (Olson, 1966).

In the usual history the affected horse has suffered from recent illness (Rooney, 1965) or stressing conditions (Bryans, 1963; Olson, 1966; Rooney, 1963). It has been suggested that the hemorrhagic colitis syndrome, which followed transportation by rail, reported in years past was the same or similar type disease (Olson, 1963; Rooney, 1963). The disease occurred in horses of all ages and was usually sporadic (Bryans, 1963; Rooney, 1963; Rooney, 1965).

The most striking necropsy lesions were in the cecum and the colon (Rooney, 1963; Rooney, 1965). The serosal surfaces were cyanotic and the mucosae were hyperemic (Rooney, 1965). Edema and hemorrhages occurred in the submucosa (Bryans, 1963; Rooney, 1963; Rooney, 1965). The liver and spleen were engorged and the adrenal glands had cortical hemorrhages (Rooney, 1963; Rooney, 1965). The lungs were emphysematous and the heart might be either normal or flabby (Bryans, 1963; Rooney, 1963). No significant lesions were found in the stomach, small intestine, pancreas, urogenital tract or central nervous system (Rooney, 1963).

Microscopic examination of the submucosa of the colon and cecum revealed dilatation of the venules and varying degrees of constriction of the arterioles (Bryans, 1963; Rooney, 1963). Necrosis with sloughing of the mucosal epithelium was seen when tissues had not undergone postmortem changes before examination (Rooney, 1963). The lymph nodes draining the cecum and colon were hyperemic with the lymphocytes undergoing necrosis (Bryans, 1963; Rooney, 1963). Rooney (1965) reported microscopic evidences of other disease processes including respiratory infection, metritis, or myocarditis.

Acute salmonellosis is one of several diseases which resembles the colitis "X" syndrome (Olson, 1962; Rooney, 1965). In both diseases affected animals have been stressed, have high BUN levels, and have elevated temperatures (Lowe, 1969; Olson, 1966). In salmonellosis the pulse is full and strong with no sign of peripheral vascular collapse. Bacteriological examination of the feces may help confirm the diagnosis of salmonellosis (Olson, 1966).

Viral arteritis is another similar sporadic disease to consider in differential diagnosis. Epizootic outbreaks are occasional. The onset is acute with temperature rise, panleukopenia, increased packed cell

volume and increased blood urea nitrogen. Some affected horses exhibit signs of respiratory disease, but others show signs of colic and diarrhea. Pregnant mares usually abort; necropsy lesions of the aborted foals are nonspecific. The mortality rate is low (Olson, 1966).

Clinically acute thromboembolic colic and torsions of abdominal viscera resemble colitis "X". Thorough clinical and necropsy examinations are essential to confirm diagnoses (Rooney, 1965).

Shock

Shock has been defined as the syndrome resulting from many circumstances in which there is impairment of the effective circulating blood volume (Collins *et al.*, 1964b). The theory of perfusion defect of tissues and vital organs in shock is widely accepted (Anderson *et al.*, 1967; Dietzman and Lillehei, 1969; Shires and Carreco, 1966; Sodeman, 1967; Spink, 1962). Hardaway (1968) discussed the normal phenomenon of blood flowing through the capillary and its ability to furnish nutrients to and carry metabolites from individual cells. He defined shock as an inadequate capillary perfusion resulting from the impairment of blood flow through the capillary. Spink (1962) agreed that in shock the microcirculation is the final common denominator -- the cell and its supporting unit, the capillary. Weil and Shubin (1967) presented a classification based on etiology which includes: hypovolemia, cardiac failure, hypersensitivity, neurogenic impediment of blood flow, endocrine failure, and bacteremic (endotoxic) shock. The purpose of the classification of shock is to aid clinicians in an organized approach to diagnosis and treatment.

Etiology

Attempts to discover the etiological agent of colitis "X" have not been conclusive (Bryans, 1963). It has been suggested that endotoxins

from various gram-negative bacteria play important roles in the syndrome (Bryans, 1963; Coffman and Bracken, 1968; Rooney *et al.*, 1963). These endotoxins have been injected intravenously and have produced signs similar to colitis "X" (Rooney *et al.*, 1963). Carrol *et al.* (1965) injected an endotoxin prepared from a culture of *Aerobacter aerogenes* intraperitoneally and noted clinical signs resembling colitis "X". The horse collapsed in 1 hour and died 8 hours after the injection.

Endotoxins

Endotoxins are lipopolysaccharides bonded to proteins in the cell wall of gram-negative bacteria (Carrol *et al.*, 1965; Gilbert, 1960; Kwaan and Weil, 1969; Lillehei *et al.*, 1967). In endotoxic shock the lipopolysaccharide has been released from the cell wall of the gram-negative bacteria (Lillehei *et al.*, 1967; Sukandan and Thal, 1965). Endotoxic shock can be reproduced experimentally by intravenous injection of the endotoxin of *Escherichia coli* in dogs (Bell and Schloerb, 1966; Duff *et al.*, 1965; Evans *et al.*, 1967; Grable *et al.*, 1963; Lillehei and MacLean, 1958; Thomas *et al.*, 1969), in rabbits (Grable *et al.*, 1963), in cats (Granway *et al.*, 1969), and in monkeys (Nies *et al.*, 1968). When viable *E. coli*, which was isolated from a case of pyelonephritis, was injected intravenously the same circulatory responses were produced in dogs and in monkeys as had been obtained when purified endotoxin was injected (Thomas *et al.*, 1969). Unlike exotoxins, endotoxins produce the same general symptoms regardless of the animal injected or the bacterium that produced the toxin (Brande, 1964; Carroll *et al.*, 1965; Fine, 1961; Weil and Spink, 1957).

Experimentally the acute signs usually include high fever, decreased blood pressure, diarrhea, muscle pains, and hyperpnea (Brande, 1964;

Weil and Spink, 1957). Endotoxins of gram-negative bacteria are said to cause their lethal effects through their potent sympathomimetic activity (Lillehei and MacLean, 1958). The basic hemodynamic alteration is severe vasoconstriction (Spink, 1962; Fine, 1968), which results in increased peripheral vascular resistance (Duff *et al.*, 1965; Lillehei *et al.*, 1967). The vasoconstriction of the hepatic veins causes pooling in the abdominal viscera with less blood available to the active circulation (Lillehei *et al.*, 1967; Weil and Spink, 1957).

There is also loss of plasma due to increased hydrostatic pressure in the congested capillaries (Lillehei *et al.*, 1967). The pooling of blood and congestion in the viscera causes decreased venous return to the heart lowering both cardiac pressure and cardiac output (Gilbert, 1960). The decreased blood flow to the kidneys causes marked decrease in urine output and resulting renal failure (Kwaan and Weil, 1969; Spink, 1962). The decreased blood flow to the viscera decreases motility and produces visceral ischemia (Fine, 1968; Lillehei and MacLean, 1967; Longerbeam *et al.*, 1962).

Necropsy findings in experimental endotoxic shock are consistent. The principal lesion is hemorrhagic necrosis of the mucosa of the gastrointestinal tract, especially the small intestine and colon (Carroll *et al.*, 1965; Evans *et al.*, 1967; Lillehei and MacLean, 1967). Petechial hemorrhages occur on the serosa of the gastrointestinal tract, the heart and the kidneys (Carroll *et al.*, 1965). Snell (1969) believes the significance of pulmonary edema is minimal, whereas Sukandan (1965) indicates it contributes to cardiac failure.

Septic (Endotoxic) Shock in Man

Because of the marked similarities, endotoxic shock has been used as a model for study of septic shock in man (Lillehei *et al.*, 1958). Shock associated with gram-negative bacterial infections of the blood stream have been suggested to be caused by the liberation of endotoxin from the bacteria (Weil and Spink, 1958). Gram-negative bacteria are the most common organisms isolated from wound infections, abscesses, urinary tract infections and septicemias in man (Dietzman and Lillehei, 1969). Septic shock is said to be second only to myocardial infarction as a cause of shock in hospitalized patients (MacLean, 1962; Weil and Spink, 1958).

Mortality from septic shock is high, ranging from 60% to 80% (Kwaan and Weil, 1969; Weil *et al.*, 1964). In prolonged shock, accumulated toxic products resulting from poor tissue perfusion and anoxia of the cells from anaerobic metabolism produce lethal effects (Sodeman, 1967). Shock can be produced in severe infections in the abscence of a bacteremia (Ebert and Abernathy, 1961). In one clinical report manipulation of the genitourinary tract and considerable trauma were considered important in producing septic shock (Weil *et al.*, 1964).

In a study of 169 clinical cases, Weil (1964) provided a clear picture of the clinical aspects of shock caused by gram-negative organisms. Initially there was fever with cold, clammy, cyanotic extremities, plus rapid, shallow breathing, vomiting and diarrhea. Concurrently, there was leukopenia, especially a neutropenia. Serum sodium and chloride levels fell and metabolic acidosis developed, and serum lactate concentration increased (Kwaan and Weil, 1969). Blood flow to the kidneys decreased, and the BUN levels rose. In one clinical study patients that died had elevated serum potassium levels (Blair *et al.*, 1969). Of the gram-negative bacteria, *E. coli*, *A. aerogenes*, *Pseudomonas aeruginosa*, and *Proteus sp.*

were those most commonly isolated (Spink, 1962; Weil and Spink, 1964; Wilson *et al.*, 1967).

Therapy in Man

No single treatment regimen has been successful in all patients in shock since the clinical manifestations, though similar, are from widely differing causes (Thal and Wilson, 1965). In treating shock it is important to restore the effective circulating volume while correcting the hemodynamic disturbance occurring in the microcirculation of the viscera (Lillehei *et al.*, 1967).

Fluids. Initially fluid therapy is the major consideration. A loss of more than 30% of the initial blood volume is critical, but there are good reserves of red blood cells and hemoglobin from which to draw (Weil and Shubin, 1967). The type of fluid administered depends on the nature of fluid loss (Thal and Wilson, 1965). Hardaway (1967) has stated that adequate fluid volume is the most important concern in therapy in non-cardiac shock, and that fluid administration should continue until there is elevation of the central venous pressure or the pulmonary artery pressure. Some of the more effective replacement fluids are colloid solutions (Lillehei *et al.*, 1967), plasma (Lillehei *et al.*, 1967; Longerbeam *et al.*, 1962; Artz and Fitts, 1962), low molecular weight dextrans (Lillehei *et al.*, 1967; Lillehei *et al.*, 1964; Artz and Fitts, 1962), and balanced electrolyte solutions (Lillehei *et al.*, 1967; Thal and Wilson, 1965).

Measuring central venous pressure has proven to be a valuable guide in monitoring fluid restoration (Anderson *et al.*, 1967; Lillehei *et al.*, 1967; Weil, 1969; Jennings, 1967). Fluids can be administered to the point of elevating the central venous pressure. The venous pressure reflects the competence of the myocardium to handle the fluid volume

returned to it. An increase in central venous pressure indicates the heart is loaded to capacity (Thal and Wilson, 1965; Weil, 1969).

Adrenergic therapy. When the body is severely stressed, the overall defense reaction may not be in the best interest of that individual (Veilleux, 1963). Endotoxins cause generalized vasoconstriction, which is not limited to the body surface; the kidneys stop producing urine; the gastrointestinal tract stops functioning; and the retention of metabolic wastes produces acidosis (Fine, 1968). The endotoxins cause their lethal effects by acting as potent sympathomimetic agents or by sensitizing the animal to levels of endogenous sympathomimetic agents which are ordinarily nontoxic (Lillehei and MacLean, 1958).

There has been some controversy over the choice of adrenergic drugs to use in the treatment of shock. Ahlquist (1948) described 2 types of receptors in the sympathetic system. The alpha receptors cause vasoconstriction, stimulate the ureter and the uterus, and promote intestinal relaxation. The beta receptors cause vasodilation and myocardial stimulation. Of the more commonly used adrenergic drugs, isoproterenol is mainly a beta stimulator, epinephrine is mainly alpha with some beta activity on the heart. Methoxyamine and phenoxybenzamine block activity at the alpha receptors (Kaiser *et al.*, 1964; Moran, 1963).

Vasopressor drugs such as epinephrine and norepinephrine are given to maintain blood pressure, but the resulting vasoconstriction and tissue anoxia may worsen rather than improve the circulation (Collins *et al.*, 1964a). Others have suggested that excessive sympathetic nervous system stimulation does more harm than good when treating shock (Brau *et al.*, 1966; Blair *et al.*, 1969; Collins *et al.*, 1964a; Hermeck and Thal, 1968; Lillehei *et al.*, 1969). In acute disease states where blood volume is

reduced, the vasomotor and adrenergic receptors are already under intense stimulation. The alphamimetic drugs will increase the blood pressure but at the expense of decreased blood flow to most organs (Hermeck and Thal, 1968). Studies have shown that epinephrine will slow blood flow in the capillaries through intense venous constriction and may even cause back-flow from the venous end of the loop (Dennis and Zimmer, 1964). It has been shown that continuous infusion of norepinephrine to maintain blood pressure can cause serious local tissue sloughs (Olgesby and Baugh, 1968). Vasopressor drugs are generally said to have limited use in septic shock (Collins, 1964b; Sodeman, 1967).

Recent clinical studies concerning septic shock have recommended the use of vasodilators rather than vasopressors (Anderson *et al.*, 1967; Bradley and Weil, 1967; Collins, 1964b; Hermeck and Thal, 1968; Lillehei *et al.*, 1964; Nickerson and Gourzes, 1962; Wilson *et al.*, 1964; Wilson *et al.*, 1967). In a study using cats the alpha blocking agents proved to be competitive antagonists to adrenaline at the adrenergic receptor sites (Birmingham *et al.*, 1967). The disadvantage of alpha adrenergic blockage is diversion of blood flow from the heart and brain, but blood pressure can be kept at an adequate level with fluid replacement (Perlroth and Harrison, 1969). Reasonable oxygenation can result during hypotension if the circulating blood volume is adequate (Brau *et al.*, 1966). Vaso-dilators must be given after adequate fluid therapy, but if given before fluid therapy, the results can be dangerous (Hardaway *et al.*, 1967; Nickerson and Gourzes, 1962; Perlroth and Harrison, 1969).

Chlorpromazine has been shown to increase peripheral blood flow significantly in dogs, but the effects were only transient (Kilman, 1969). More dogs pretreated with chlorpromazine at 25 to 59 mg./kg. survived and had fewer significant lesions on necropsy examination than those receiving

2.5 to 15 mg./kg. when both groups were given 7.5 mg./kg. of *E. coli* endotoxin (Lillehei and MacLean, 1958). Rats suffering from massive hemorrhagic shock suffered 50% lower mortality in the group pretreated with chlorpromazine (Collins, 1964b).

Phenoxybenzamine increased the number of survivors in hemorrhagic shock when dogs were pretreated before bleeding. In this study the alpha-lytic agents were of no value when there was failure to respond to replacement transfusions (Jacob *et al.*, 1956). Arbulu and Thal (1966) gave phenoxybenzamine to dogs in hemorrhagic shock thereby causing decreased total peripheral resistance with decreased left ventricular work. It was found that myocardial response to loading was enhanced after alpha adrenergic blockage. Phenoxybenzamine had no effect on the metabolic acidosis but did increase capillary perfusion in dogs given endotoxin (Abrams *et al.*, 1969). In another study dogs were given *E. coli* endotoxin at 3 to 5 mg./kg. body weight followed by phenoxybenzamine. There was increased pulmonary blood flow with falling total vascular resistance. When norepinephrine was given with phenoxybenzamine, there was markedly decreased vascular resistance with increased blood flow (Sukandan and Thal, 1965).

Phenoxybenzamine administered at the level .2 mg./kg. to 2.0 mg./kg. body weight produced overall improvement in the clinical response of patients in shock (Wilson *et al.*, 1964). The metabolic acidosis following the use of phenoxybenzamine was thought to be due to washout from muscle masses, skin, and other organs previously isolated by vasoconstriction (Hermeck and Thal, 1968). Anderson *et al.* (1967) felt that volume replacement should be the first and at times the only therapy for patients in shock. After conventional therapy has failed, phenoxybenzamine can be used to improve capillary perfusion.

Corticosteroid therapy. Massive doses of corticosteroids administered over a short period appeared to increase the overall survival rates in persons suffering from severe shock (Blair *et al.*, 1969; Melby, 1961) even though there is not adrenal cortical failure in shock (Ebert and Abernathy, 1961). Using a rat heart lung experiment, corticosteroids were shown to increase the left ventricular work index (Sayers and Soloman, 1960). Corticosteroids will protect the cell by maintaining membrane integrity and stabilization of lysosomes (Melby, 1964). Endotoxin has been found to increase lysosomal enzymes in plasma after infusion (Nies *et al.*, 1968; Weisman and Thomas, 1962). In animals treated with cortisone therapy there was definitely decreased release of lysosome enzymes (Weisman and Thomas, 1962).

Corticosteroid protection against cellular damage is nonspecific in response to noxious stimuli. The degree of protection is proportional to the concentration of corticosteroid in a given volume of tissue. Short term therapy does not alter protein or carbohydrate metabolism and there is no pituitary or adrenal cortical suppression (Melby, 1961). Cortisol increases myocardial contractibility and reduces peripheral resistance after large doses (Melby, 1964). Lillehei *et al.* (1964) reported high dosages of corticosteroids act as alpha adrenergic blocking agents. In experimental studies, massive doses of glucocorticoids such as methyl-prednisolone (195-30 mg./kg.) and dexamethasone (2-6 mg./kg.) reduced the sympathetic response to endotoxin (Dietzman and Lillehei, 1969).

In 2 separate clinical studies, more patients in gram-negative bactermic shock survived after receiving massive doses of corticosteroids (Blair *et al.*, 1969; Weil *et al.*, 1964). Wilson *et al.* (1967) recommended administering hydrocortisone at levels of 50 mg./kg. body weight to

patients in septic shock. Adrenocorticosteroids are recommended by others as part of routine shock therapy in treating septic shock (Bradley and Weil, 1967; Shires and Carreco, 1966; Spink, 1962). Phenoxybenzamine and/or cortisone in massive doses may be combined with fluids for best results (Lillehei *et al.*, 1967).

Digitalis therapy. Weil (1969) suggests a "V.I.P." approach to the patient in shock. The "V" is for ventilation of the patient, "I" is for infusion to restore volume, and "P" is for the pump-augmenting cardiac function with cardiotonic drugs. Thal and Wilson (1965) and Hardaway *et al.* (1967) suggested that digitalis be used in face of heart failure and especially if the central venous pressure remains high (Lillehei *et al.*, 1967). Shires and Carreco (1966) suggested a maximum therapeutic dosage of digitalis be given promptly when there is a rise in central venous pressure.

Antibiotic therapy. Large doses of antibiotics are recommended immediately for animals in shock (Lillehei *et al.*, 1967; Muller *et al.*, 1965; Spink, 1962; Thal and Wilson, 1965). Shires and Carreco (1966) recommended the use of 10 to 15 million units of penicillin or 2 grams of chloromycetin intravenously during the first 24 hours, but Lillehei *et al.* (1964) recommended the use of 1 gram of chloromycetin. It should be remembered that shock will persist in spite of sterilization of the blood stream (Weil and Spink, 1958). Care should be taken when administering antibiotics since the death of many bacteria may release more endotoxin thereby worsening the state of shock (Spink *et al.*, 1948).

Miscellaneous therapy. Other forms of therapy have been recommended in shock. Survival of dogs given *E. coli* endotoxin was not markedly increased when hyperbaric oxygen was given (Evans *et al.*, 1964). In another study

it was suggested that hyperbaric oxygen used in dogs in hemorrhagic shock might improve survival through better oxygenation of tissues (Navarro and Ferguson, 1968).

Hardaway and Johnson (1963) suggested that endotoxic shock is produced in part by disseminated intravascular coagulation. In studies using 1.5 ml./kg. body weight of *E. coli* endotoxin in dogs, there were prolonged Lee White clotting times, dramatic falls in platelet counts, and increased prothrombin times. The decline in fibrinogen and platelets was interpreted to have resulted from platelet clumps and the intravascular clotting process. Preheparinization did not affect mortality but did prevent the loss of clotting activity. The packed cell volumes did not increase as much in heparinized dogs as in controls (Hardaway and Johnson, 1963; West *et al.*, 1967).

Shock therapy in the horse. The syndrome of colitis "X" produces severe dehydration; consequently fluids must be given to correct the resulting fluid-electrolyte imbalance. Horses will dehydrate rapidly with colitis "X" since fluid is not only lost in the feces but there is marked accumulation of fluid within the gastrointestinal tract and fluid intake is reduced (Tasker and Olson, 1964). Sodium, potassium, and bicarbonate are suggested to be the main electrolytes lost in colitis "X"; therefore, it is recommended that balanced electrolytes be administered and that sodium bicarbonate or sodium lactate be added to these solutions (Tasker, 1967b; Tasker and Olson, 1964). Laboratory tests should be conducted during fluid therapy since too much bicarbonate or potassium can harm the patient (Tasker and Olson, 1964).

Promazine hydrochloride was used successfully as an alpha adrenergic blocking agent for part of the therapy in a horse exhibiting clinical

endotoxic shock. Coffman and Bracken (1968) reported depression following promazine administration may not be deleterious because the relief of anxiety may be desirable in any stressed horse. Coffman and Bracken (1968) and Roberts (1965) recommend massive doses of corticosteroids for horses in endotoxic shock. A pharmacological dosage of .5 mg./kg. of body weight of 9-fluoroprednisolone has been recommended and can be repeated every 4 hours for several times with no harmful side effects (Roberts, 1965).

Antibiotics are recommended to help prevent septicemia. Oral antibiotics are recommended early in the course of the disease, but later during endotoxic shock they should be administered cautiously. If large numbers of organisms die, increased levels of endotoxin are released in the intestine and are available for absorption (Coffman and Bracken, 1968).

MATERIALS AND METHODS

For this study 4 healthy horses, ranging in age from 6 to 20 years, were used. Two mares were of unknown breeding, one mare was an Appaloosa, and the fourth was a purebred Arabian gelding. All animals appeared to be in good health and had been observed at the veterinary clinic for over 2 months.

The right carotid artery of each horse was surgically exteriorized to form a permanent carotid loop (McClymont, 1950). The surgical sites were allowed to heal for at least 2 weeks before experimentation was started.

Prior to injection of endotoxin, blood was collected from 3 of the horses for bacteriologic culture. Forty cubic centimeters of blood were withdrawn by aseptic technique into a sterile heparinized syringe from the jugular vein of each horse. Blood from each horse was placed on Tryptose agar slants to which 20 cc. of brain heart infusion broth had been added. The samples were incubated for 2 weeks at 37 C. and were observed daily for growth.

Complete blood counts as well as platelet counts were performed on blood collected preinjection and at 15 minutes, 30 minutes, 1 hour, 4 hours, 8 hours, 12 hours, 36 hours, and 48 hours postinjection. A Coulter Counter* was used for couting the white blood cells.

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*Coulter Counter, Model A, Coulter Electronics, Hialeah, Fla.

Blood samples were also obtained from the jugular vein of each horse for determinations of serum sodium (Ganbrino, 1968), serum potassium (Ganbrino, 1968), serum chloride (Henry, 1964) as well as total protein (Henry, 1964) and blood urea nitrogen (Ormsby, 1942; Crocker, 1967). The clots were allowed to retract at 20° C. and the samples were centrifuged* at 5° C. to obtain clear supernatant serum. The samples were stored at -70° $\pm$ 5° C. until analyses were conducted.

A polyethylene catheter** was placed in the left jugular vein by first making a venipuncture with a 12-gauge needle. The catheter was inserted through this needle into the jugular vein and into the great veins of the thorax or the right atrium. The needle was then removed. The catheter was connected to a water manometer to record central venous pressure. By the use of a 3-way valve, venous blood samples were withdrawn through the catheter.

A 6-inch, 15-gauge intra-arterial catheter*** was placed in the carotid loop. Direct blood pressure was obtained by connecting the catheter to a pressure transducer† and recording the data on a monitor.††

A 3-way valve was connected to the blood pressure transducer which allowed for collection of samples of arterial blood for partial pressure determinations of carbon dioxide as well as oxygen saturation and pH.

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*Model PR-2, International Portable Refrigerated Centrifuge, International Equipment Co., Needham Heights, Mass.

**PE 205 Polyethylene Tubing, Clay Adams, Inc., New York, N.Y.

***Jelco IV Catheter, Jelco Labs, Raritan, N.J.

†Statham P23AC Pressure Transducer, Statham Laboratories, Inc., Hato Rey, Puerto Rico.

††Dual Trace Monitor, IR-2T, Electronics for Medicine, Inc., 30 Virginia Road, White Plains, N.Y.

Oxygen saturations were obtained using an Oximeter.* Oxygen saturations were also determined on venous blood and all samples were analyzed within 60 seconds after collection. Partial pressures for carbon dioxide and the pH of venous and arterial bloods were obtained with a blood gas analyzer.** Analyses were made within 60 seconds after collection.

Lyophilized *E. coli* endotoxin*** was used in each experiment. It was reconstituted in 10 cc. of sterile saline 1 hour before intravenous injection.

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*MicroOximeter, American Optical Co., Bedford, Mass.

**Radiometer Micro Gas Monitor, Radiometer, Copenhagen, Denmark.

***Lipopolysaccharide *E. coli* 0127:B8, Difco Laboratories, Detroit, Mich. 48210.

RESULTS

Horse #5773

Weight: 478 kg. Sex: Mare Age: 12 years

Horse #5773, an excitable and difficult to handle mare, was given 28 mg. of *E. coli* endotoxin in 10 cc. of sterile saline resulting in a dosage of .059 mg./kg. body weight. The injection extended out over a 30-second period.

Three minutes after injection of the endotoxin, the mare exhibited rapid, labored respirations and leaned heavily against the side of the stocks and tail rope; however, 7 minutes later she regained equilibrium.

The mare went down 70 minutes postinjection, but she refused to lie quietly. Restlessness consisted mostly of abortive attempts to rise, but on several occasions she was able to stand for short periods. At 7-1/2 hours postinjection, the mare stood for approximately 90 minutes. The horse continued to sway back and forth while standing and held her head low. The gingival mucous membranes were cyanotic and the capillary filling time was 8 seconds.

The mare stood at 9-1/2 hours postinjection and collapsed 1 hour later. At this time the mare began to exhibit nystagmus and the pupils were markedly dilated.

During the last few minutes of life, she became violent. She struggled trying to rise, only to collapse in extreme exhaustion. No attempts were made to restrain the mare during this time. The horse died 12-1/2 hours after the injection and a complete necropsy examination was performed.

Horse #6659

Weight: 484 kg. Sex: Gelding Age: 6 years

Horse #6659 received 27 mg. of *E. coli* endotoxin in 10 cc. of sterile saline resulting in a dosage of .056 mg./kg. body weight. Three minutes after the injection the horse began to sweat profusely and was uneasy. He supported much of his weight on tail rope, of the restraining stocks, but within 8 minutes he was much steadier.

The horse collapsed 90 minutes postinjection. The respiratory rate was increased and the movements were labored. The horse stood for the first time at 4 hours postinjection and remained standing for 45 minutes. The horse got to his feet 9 hours postinjection and remained standing for 30 minutes. He constantly shifted his weight while standing as if his legs had difficulty supporting him. The horse collapsed in extreme exhaustion but continued to make abortive attempts to rise. His death was immediately preceded by violent paddling and thrashing. The horse died 13-1/2 hours postinjection and a complete necropsy examination was then performed.

Horse #6035

Weight: 448 kg. Sex: Mare Age: 20 years

Horse #6035 received 27 mg. of *E. coli* endotoxin in 10 cc. of sterile saline, resulting in a dosage of .066 mg./kg. body weight. The injection extended over a 30-second time period.

Two minutes after injection the horse became restless. She leaned heavily against the side of the stocks and nearly fell to the floor. The respiratory movements were rapid, labored, and deep. Beads of perspiration appeared on the neck in 5 minutes, sweating became profuse, and by 10 minutes the animal was completely wet with perspiration. The mare became much steadier 10 minutes following the injection.

The mare's status remained stable for the next 90 minutes, at which time she collapsed and was moved to the casting stall. The horse lay quietly for the next 5 hours and then began abortive attempts to rise. There were deep sighing respiratory movements during the time she was in lateral recumbency. The pupils were markedly dilated.

The blood did not clot normally and bleeding continued after venipuncture and formed hematomas.

The mare died 10-1/2 hours postinjection and a necropsy examination was performed.

Horse #6162

Weight: 485 kg. Sex: Mare Age: 20 years

Horse #6162 received 19.1 mg. of *E. coli* endotoxin in 10 cc. of sterile saline, resulting in a dosage of .035 mg./kg. body weight. The injection was completed in 15 seconds.

Within 5 minutes after the injection there were urticarial plaques over her entire body. There was an initial blanching of the gingival mucous membranes, but the gums were markedly cyanotic by 3 hours postinjection. The animal was extremely lethargic and continually shifted weight from limb to limb.

After 3 hours the mare's condition began to improve. She drank approximately 2 gallons of water when it was offered at 12 hours postinjection. She ate a small quantity of hay when it was offered at 24 hours.

The mare was led to her stall 48 hours after the initial injection of endotoxin. At that time she began to show clinical symptoms of founder. She reluctantly bore weight on her forefeet and insisted on lying down. The horse otherwise appeared normal clinically and was alert with normal appetite for the next 2-1/2 weeks. The mare began to lose condition

rapidly toward the end of the third week and had trouble standing. The mare was euthanatized and a complete necropsy examination performed.

Monitored Results

Blood pH (Figures 1-4). In each of the 4 horses there was a definite drop in the pH of the blood 2 hours after the endotoxin was injected. In each of the 3 horses that died (#5773, #6659, and #6035), there was a significant drop in both the arterial and venous blood pH values ($P > .05$). Initially in each experiment there were essentially no differences between the arterial and venous blood pH levels. In the terminal stages in each there were marked differences between arterial and venous blood pH values at all times ($P > .01$).

Blood pCO_2 (Figures 5-9). Differences between the arterial and venous blood pCO_2 values were slight and not significant. The arterial blood pCO_2 definitely dropped and the venous blood pCO_2 rose except in horse #5773 in which it dropped. There were significant differences between the preinjection arterial and venous blood pCO_2 levels and those taken just before each horse died ($P > .05$).

Oxygen saturation (Figures 10-11). There were no significant changes in arterial oxygen saturation levels, but venous oxygen saturation levels fell dramatically in a pattern of falling initially, rebounding, and then steadily declining ($P > .05$). The differences between arterial and venous oxygen saturations in the control sampling were not as great as the difference seen in the terminal samples. In horse #6162 there was essentially no change in the arterial oxygen saturation level, but the venous oxygen saturation level declined slightly.

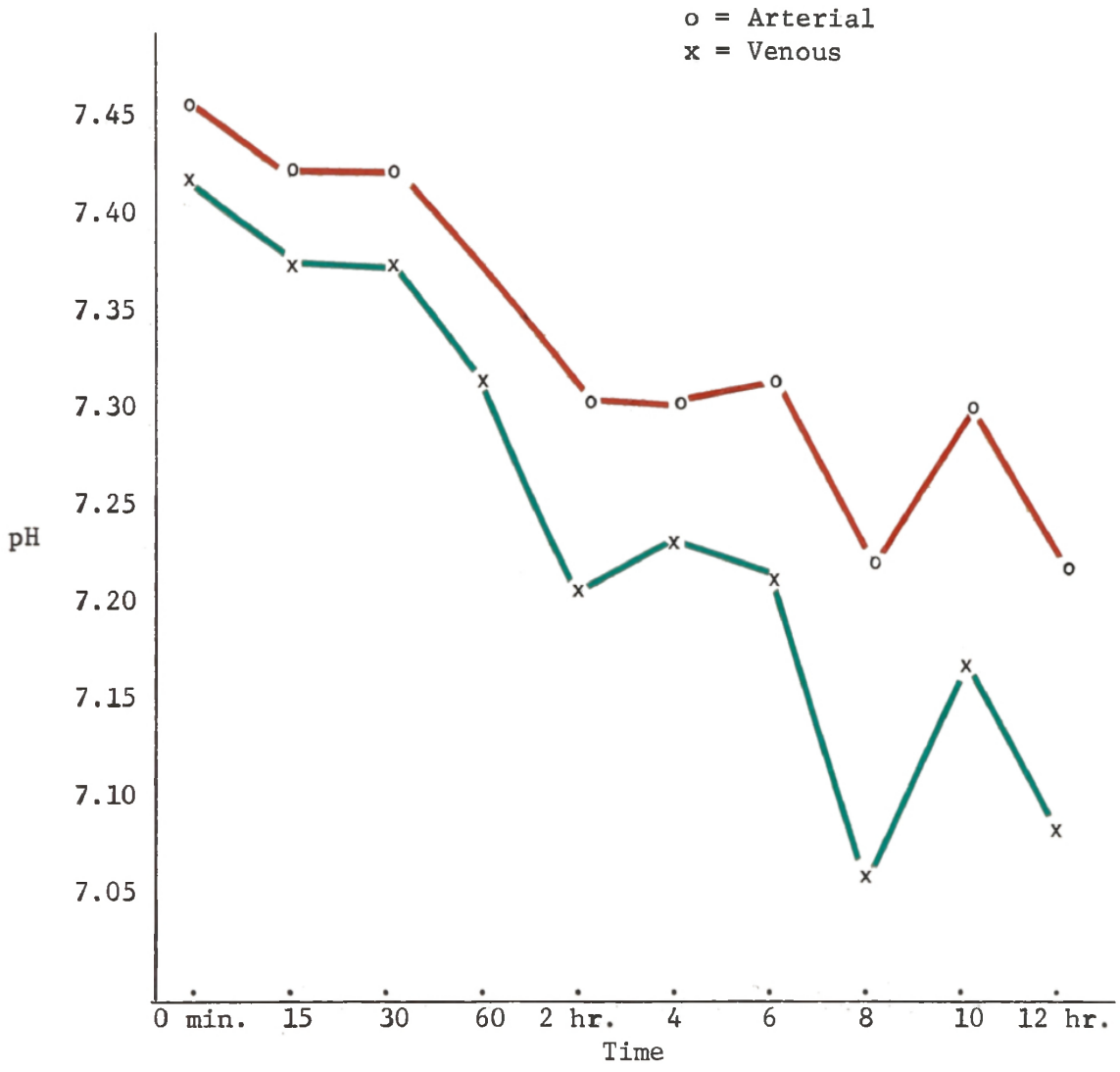

Figure 1. Blood pH of horse #5773 from preinjection to 12 hours postinjection of endotoxin.

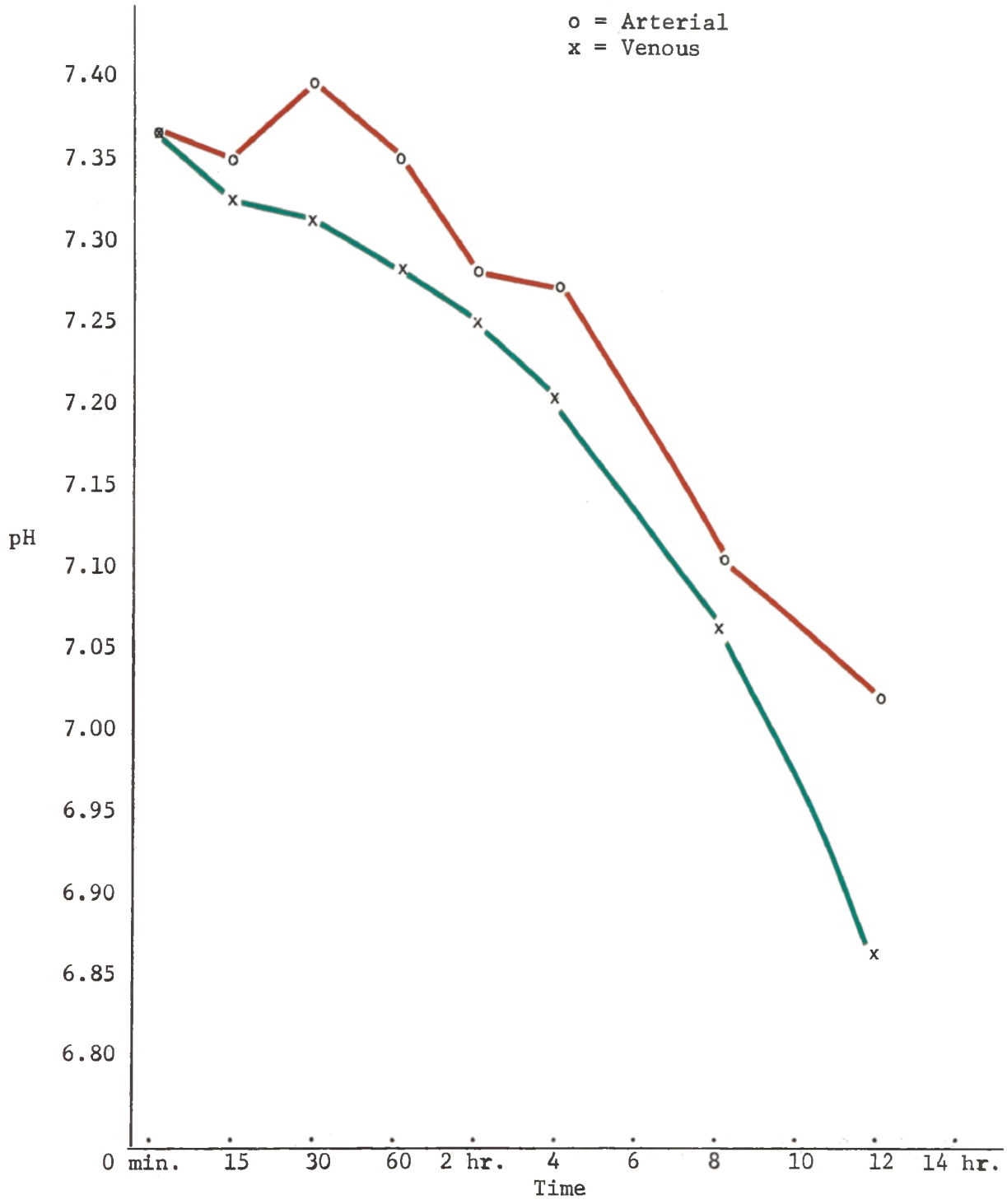

Figure 2. Blood pH of horse #6035 from preinjection to 14 hours postinjection of endotoxin.

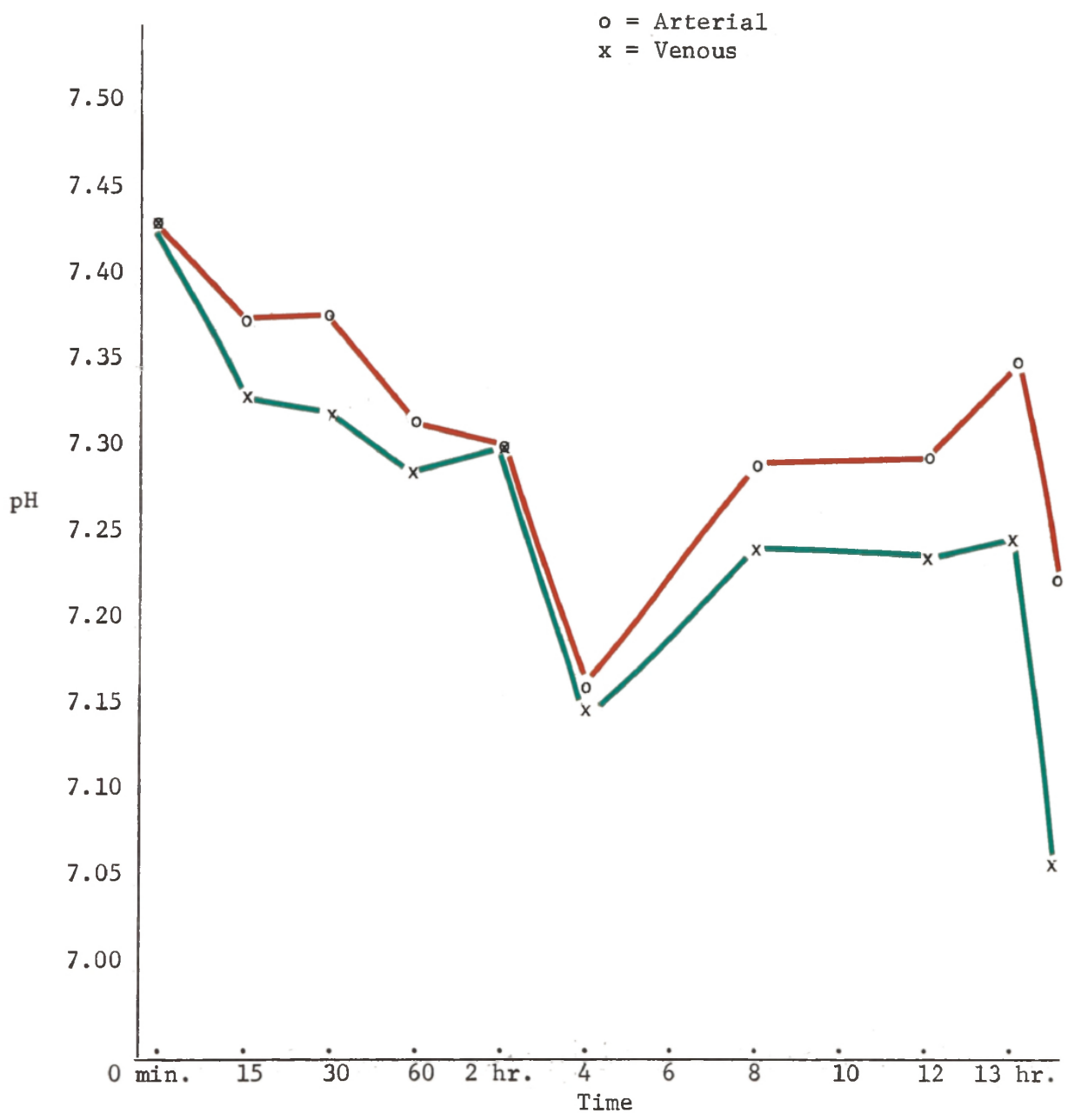

Figure 3. Blood pH of horse #6659 from preinjection to 13 hours postinjection of endotoxin.

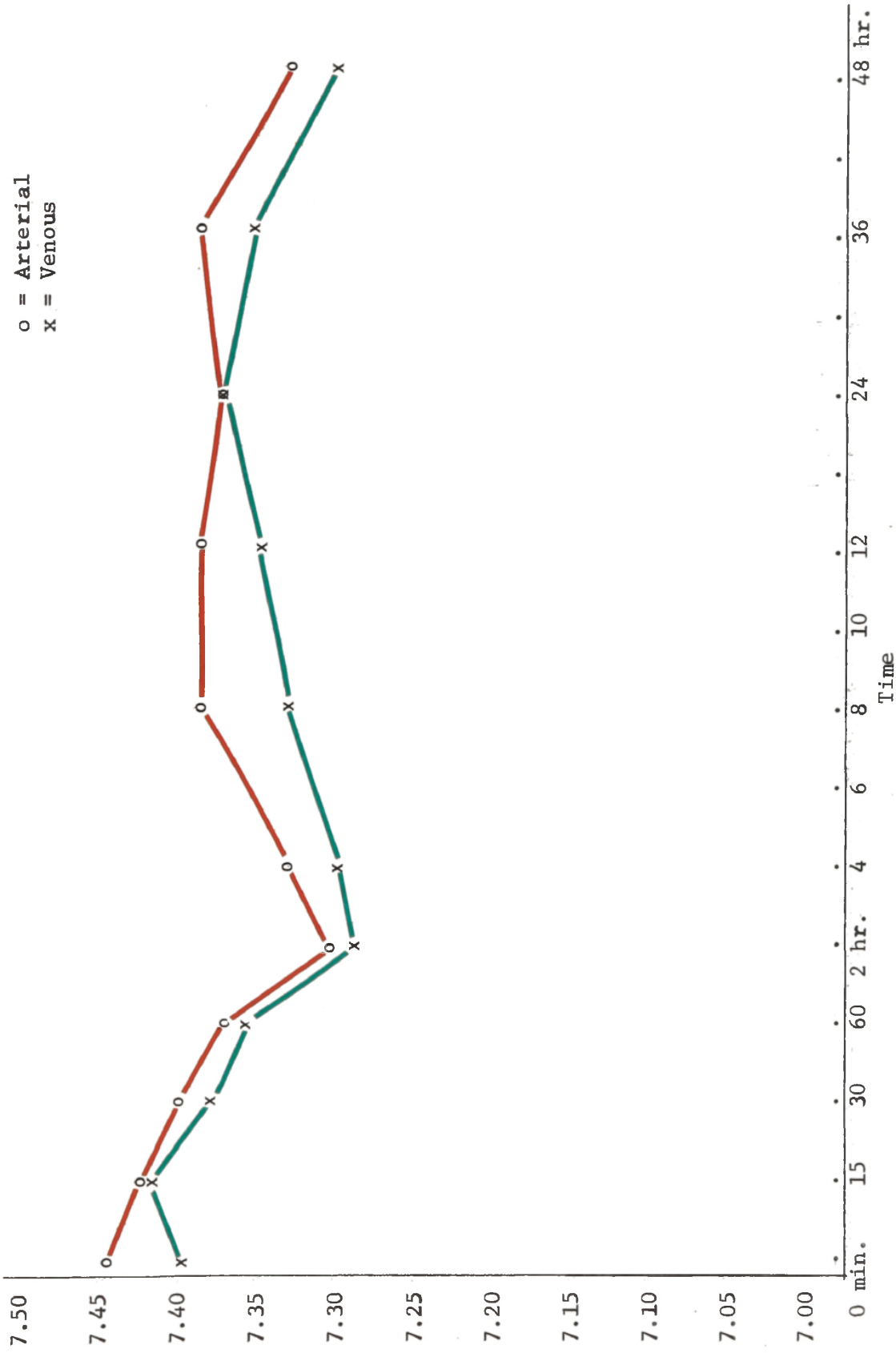

Figure 4. Blood pH of horse #6162 from preinjection to 48 hours postinjection of endotoxin.

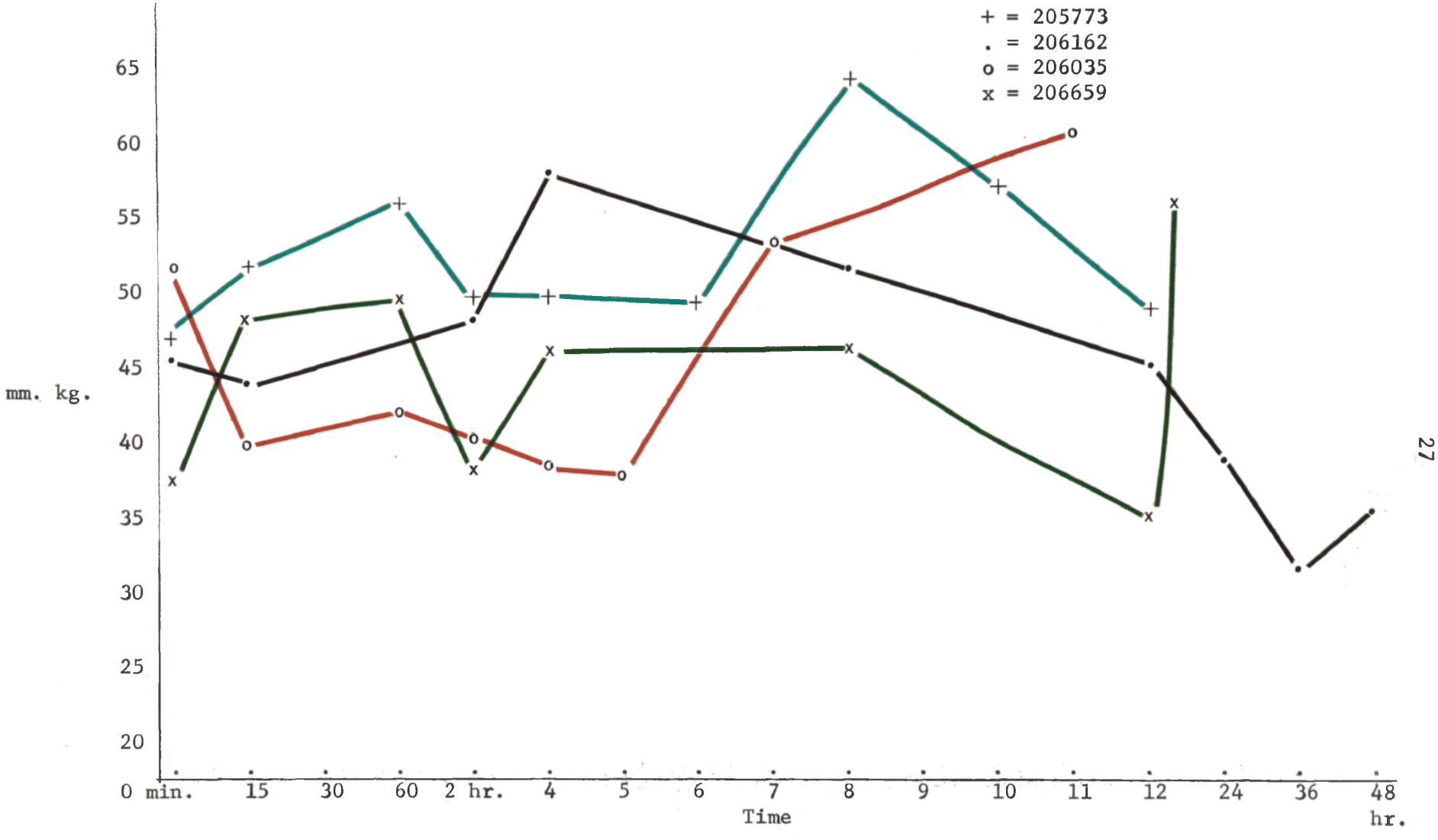

Figure 5. Venous blood pCO_2 of all 4 horses from preinjection to death of horse or 48 hours postinjection.

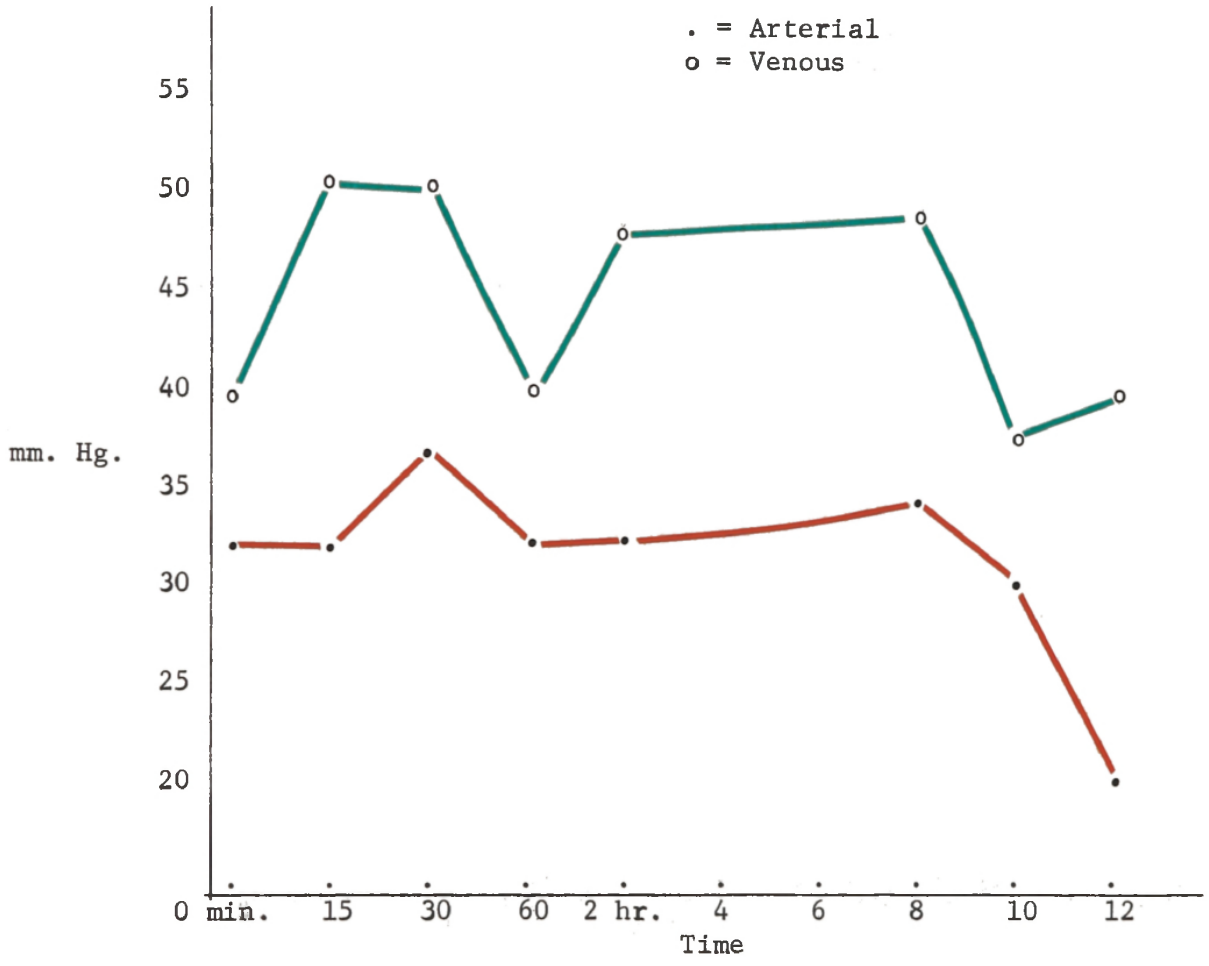

Figure 6. Blood pCO_2 of horse #6659 from preinjection to 12 hours postinjection of endotoxin.

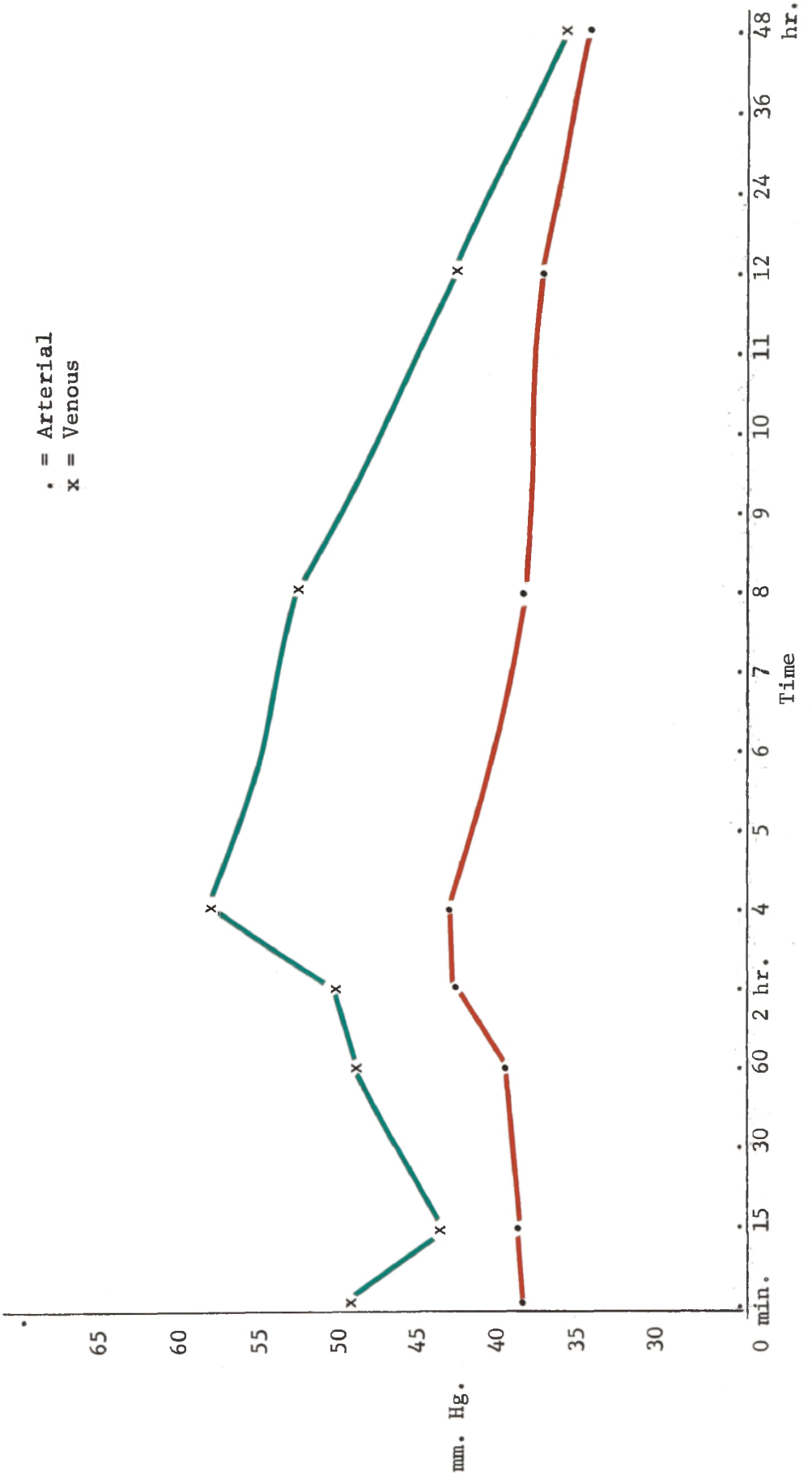

Figure 7. Blood pCO_2 of horse #6162 from preinjection to 24 hours postinjection of endotoxin.

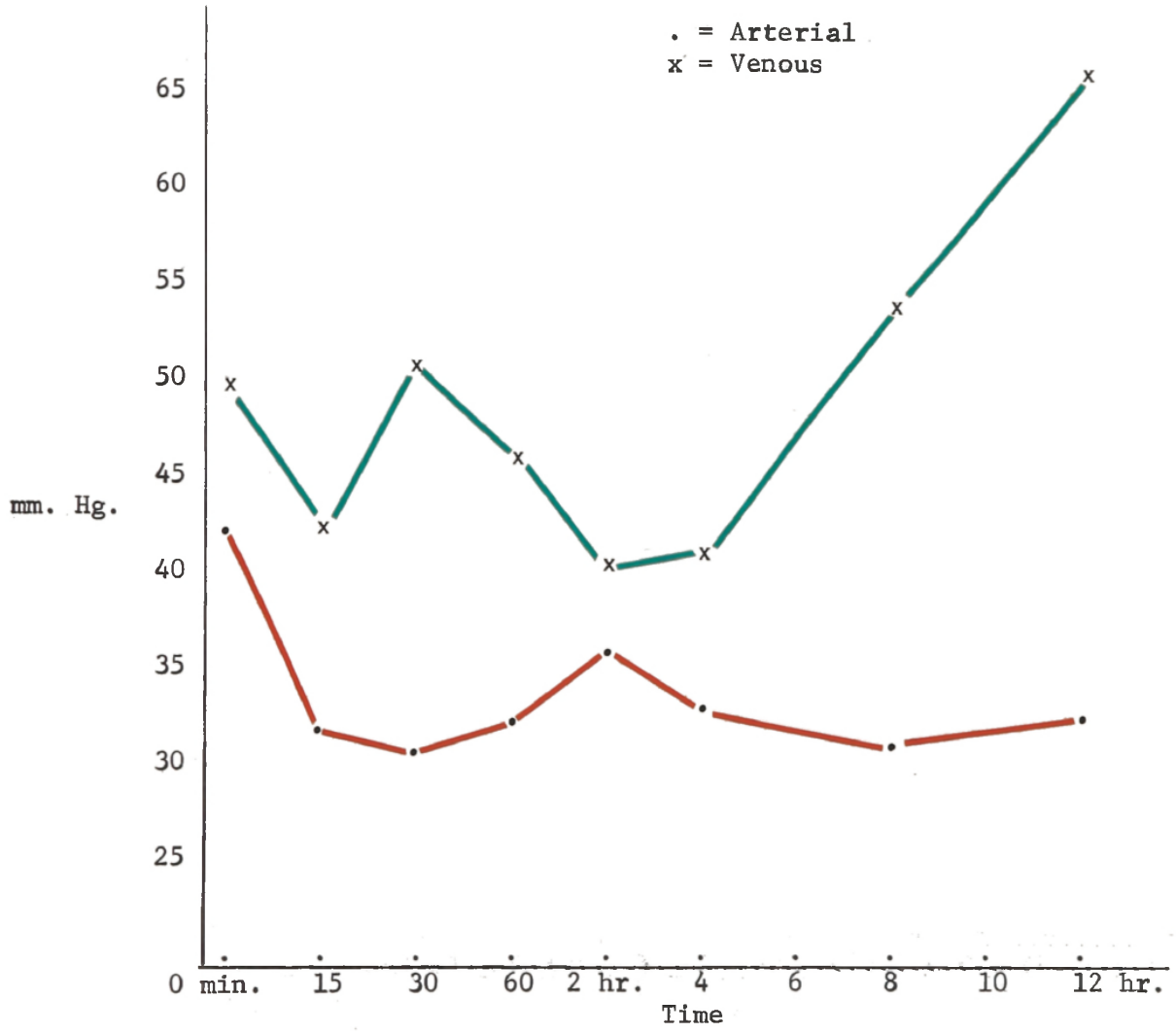

Figure 8. Blood pCO_2 of horse #6035 from preinjection to 12 hours postinjection of endotoxin.

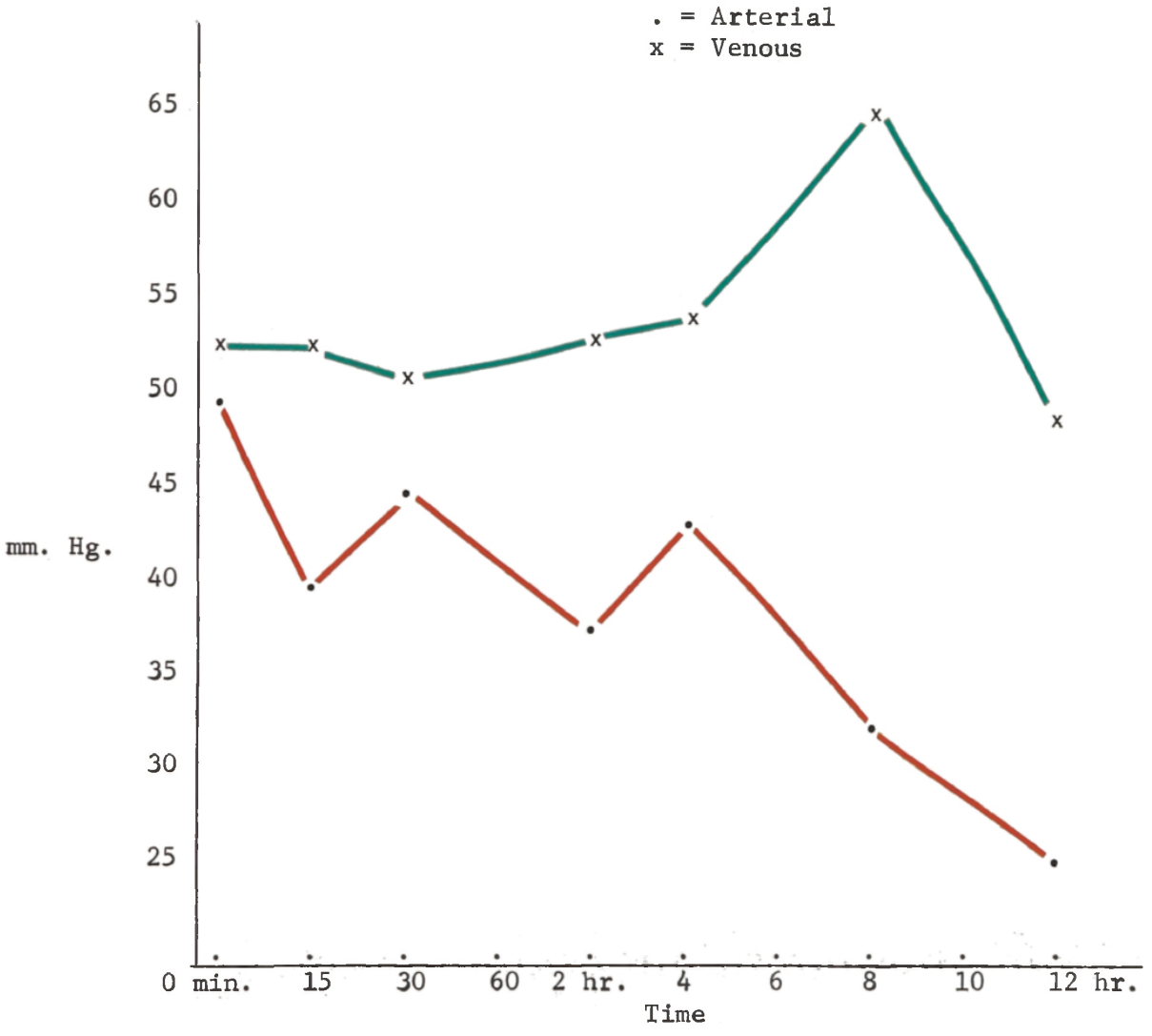

Figure 9. Blood CO_2 of horse #5773 from preinjection to 12 hours postinjection of endotoxin.

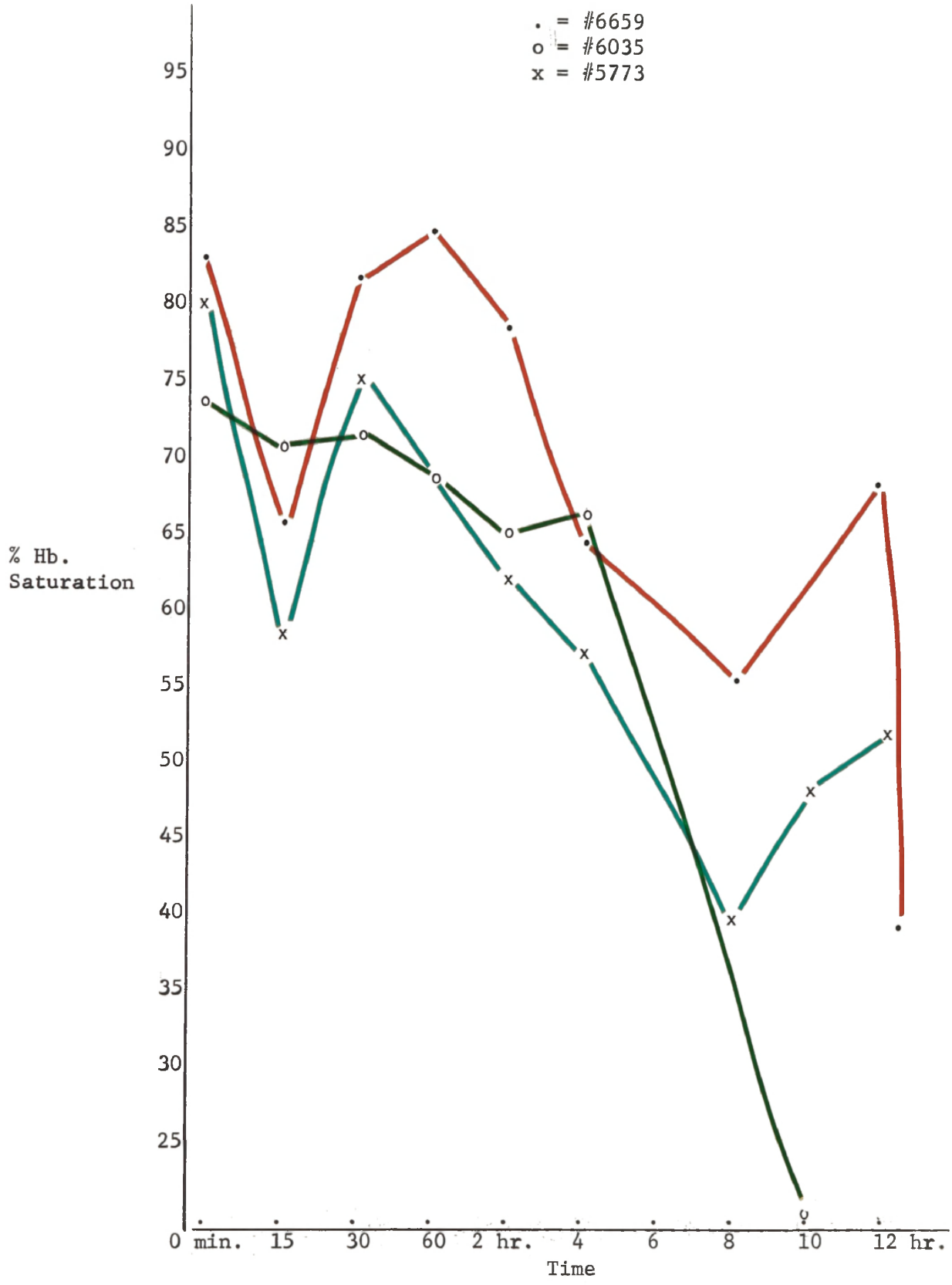

Figure 10. Venous oxygen saturation of horses #6659, #6035, and #5773 from preinjection to death of the horse.

Central venous pressure (Table 1). The central venous pressure varied within each experiment. The changes that occurred were not thought to be statistically significance, since it was difficult to keep the catheters in place as each animal struggled just before death. In 2 of the subjects (#6659 and #6035) there were gradual declines in central venous pressures followed by rising central venous pressures as death approached.

Arterial blood pressure (Figures 12-15). In each experiment after injection of the endotoxin there was an initial rise in arterial blood pressure followed by a decline within 15 minutes after injection. The blood pressure levels seemed to plateau within 2 hours after injection. There were significant differences between the preinjection and terminal blood pressure readings ($P > .05$). Horse #5773 experienced a rise at the time of the terminal sampling, but this was observed while the mare was struggling before death. The catheter was dislodged during the struggling and the horse died before it could be replaced. There were no significant changes in pulse pressures in any of the horses during the experiments.

Respiratory rate (Table 2). The respiratory rates were the most variable parameters monitored in the experiment. The changes of the respiratory movements during each trial were dramatic. No panting or shallow breathing was noted, but deep groaning respirations were frequent. Just before death respiratory movements were abdominal in nature with deep sighs.

Body temperature. Body temperatures varied slightly during the trials but rose in only 2 animals (#6659 and #5773). These rises were recorded terminally when the animals were quite excitable and were vigorously attempting to stand. Initially there were temperature drops in 3 horses (#6659, #6035, and #5773). In each this fall was significant by the sixth hour ($P > .05$), after which the temperature began to rise.

Table 1. Central venous pressure from preinjection time to 48 hours postinjection of endotoxin (mm H_2O)

Horse	#6659	#6162	#6035	#5773
Preinjection	13	2	13	-1
15 minutes	7	1	6	6
30 minutes	9	1	1	6
60 minutes	4	3	3	-4
2 hours	5	-2	15	8
4 hours	1	-1	23	0
6 hours				5
8 hours		-1	20	-4
10 hours				-5(died)
12 hours	0	+1	22(died)	
13 hours	18(died)			
24 hours		-1		
36 hours		+5		
48 hours		+2(lived)		

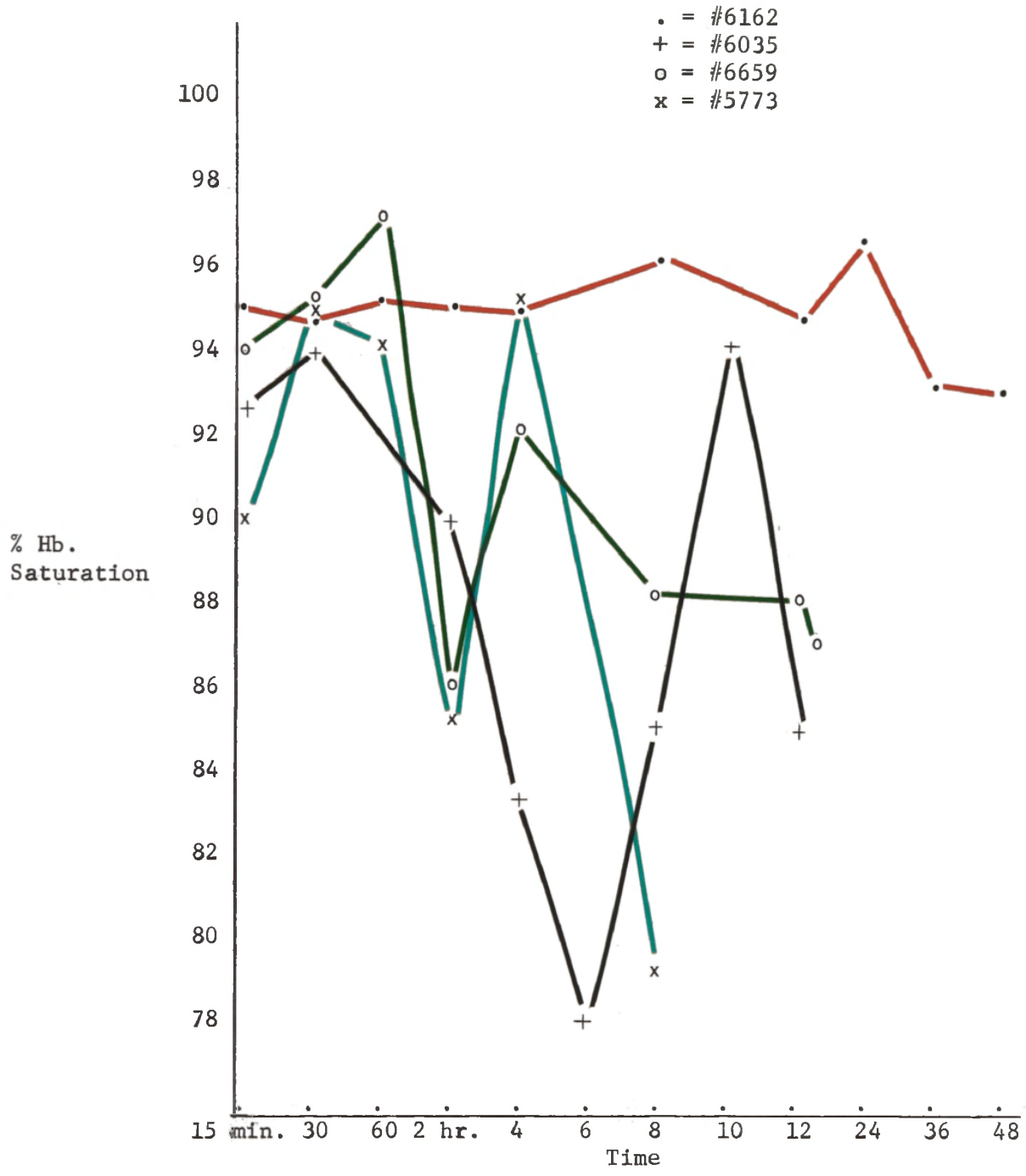

Figure 11. Arterial oxygen saturation of all 4 horses from preinjection to death of horse or 40 hours postinjection. Note the drop in saturation of all horses except #6162, who survived the 48-hour test period.

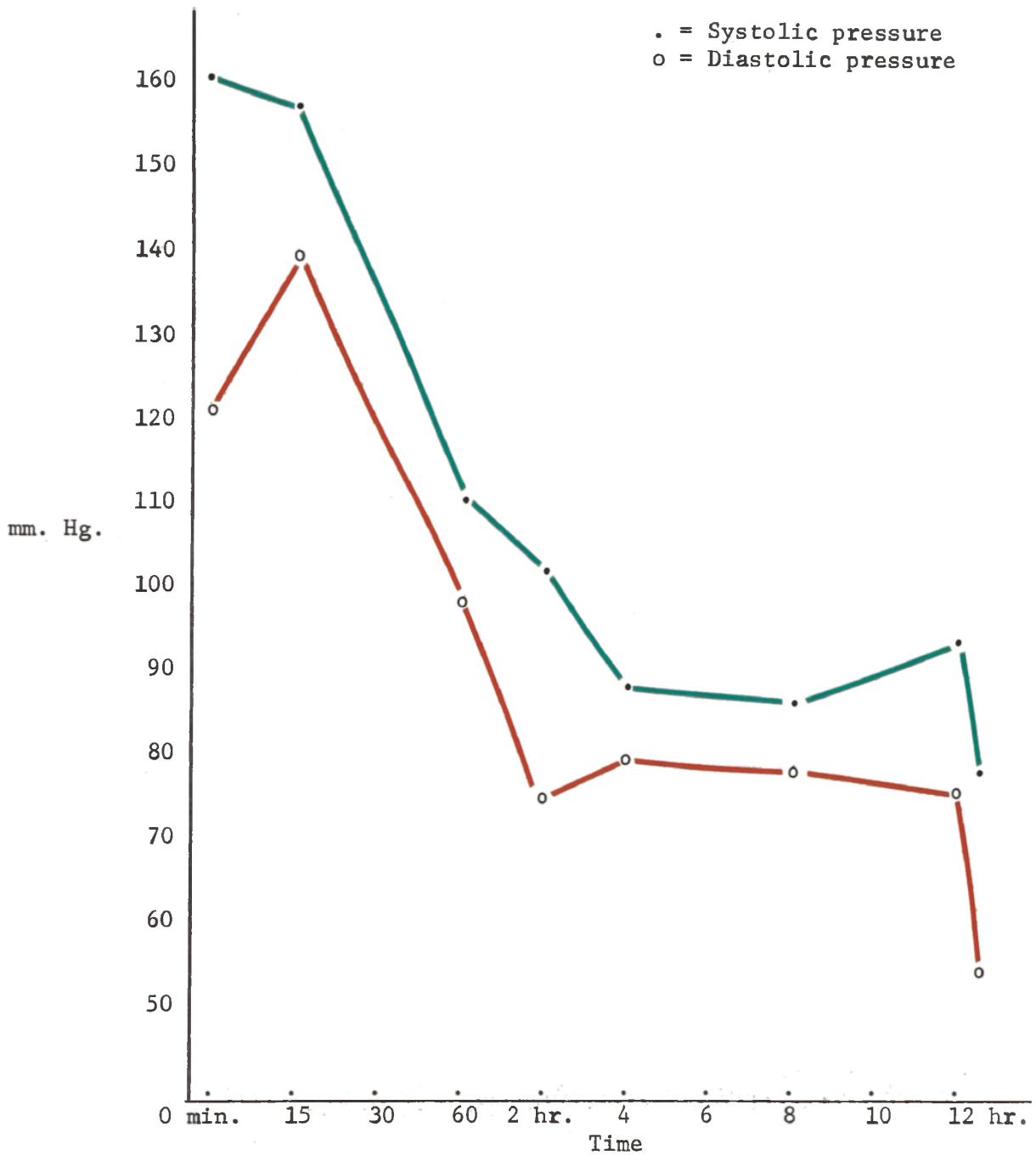

Figure 12. Arterial blood pressure of horse #6659 from preinjection to 13 hours postinjection of endotoxin.

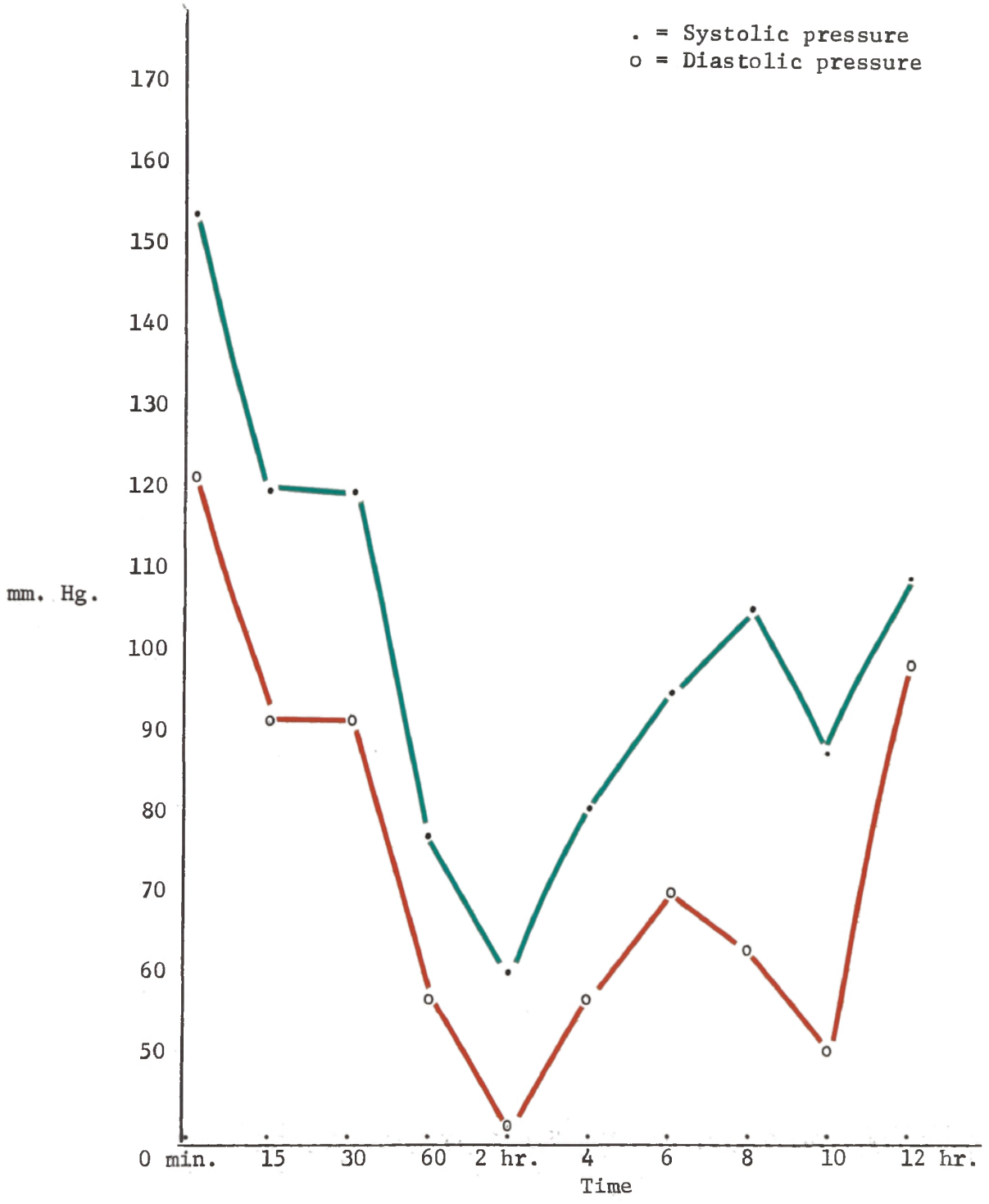

Figure 13. Arterial blood pressure of horse #5073 from preinjection to 12 hours postinjection. Note rise of pressure prior to death of animal while he was struggling.

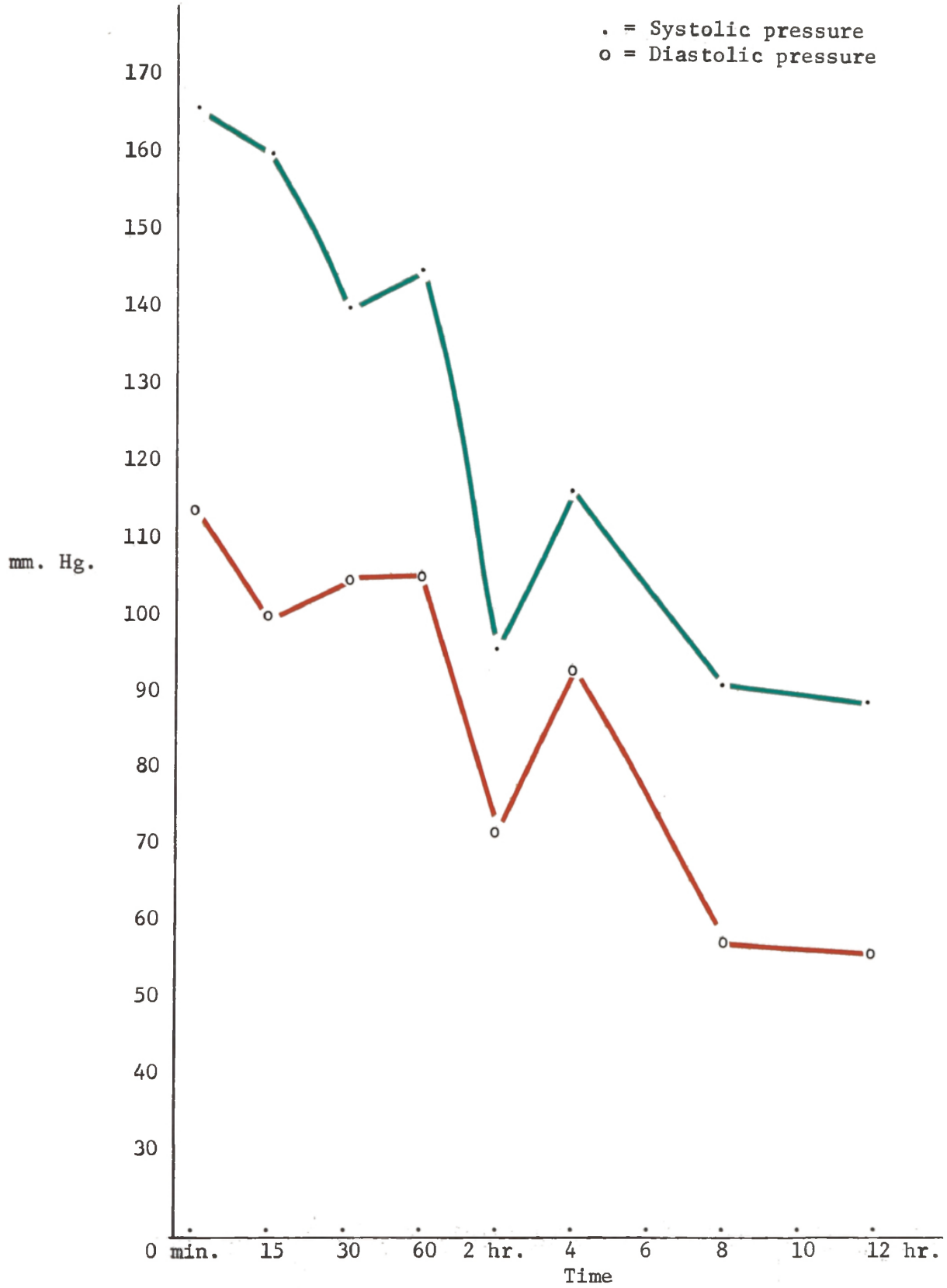

Figure 14. Arterial blood pressure of horse #6035 from preinjection to 12 hours postinjection of endotoxin.

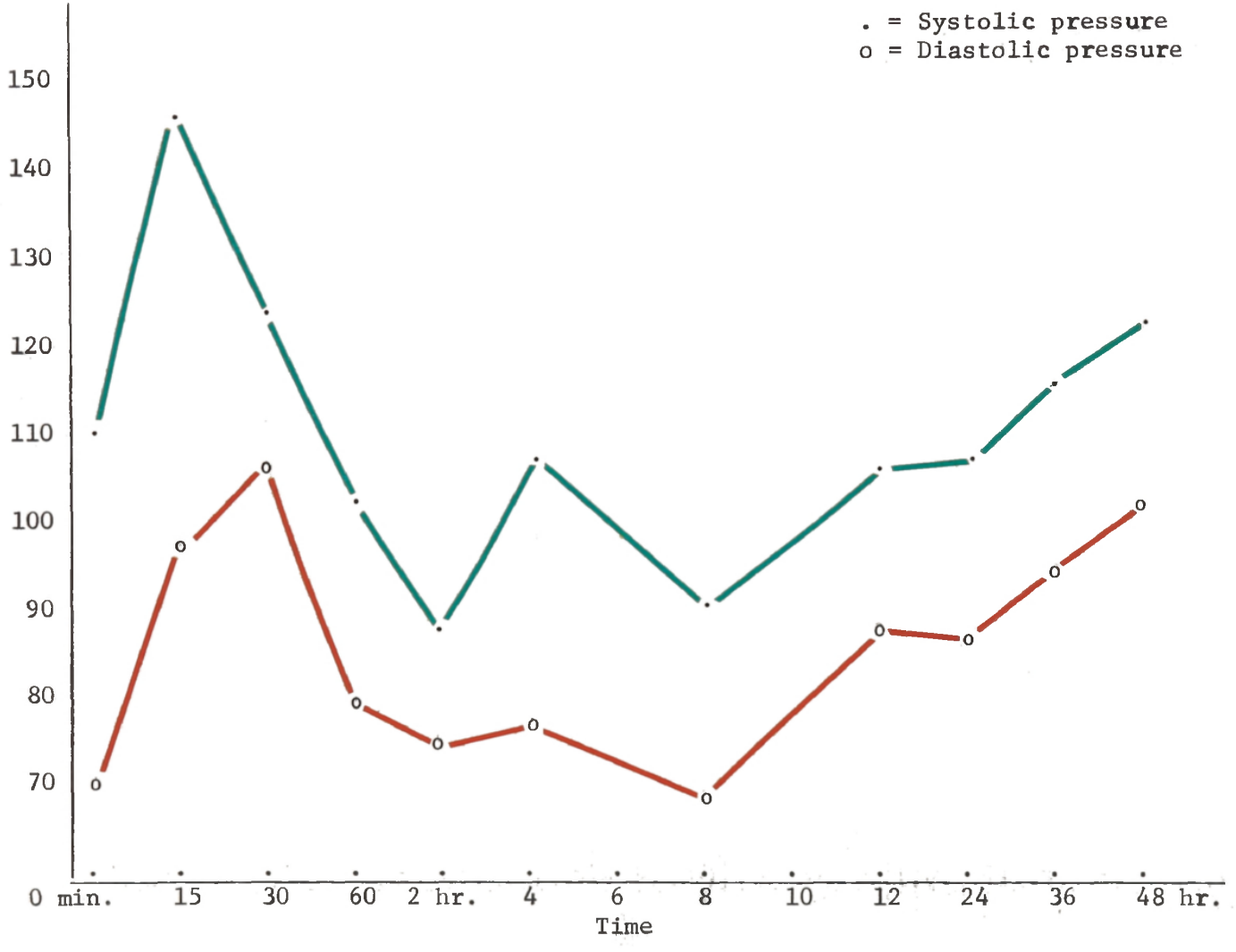

Figure 15. Arterial blood pressure of horse #6162 from preinjection to 48 hours postinjection. Note mitral increase followed by a slight drop and then a gradual rise. This horse survived the 48-hour trial period.

Table 2. Pulse rate (min.)/respiratory rate (min.)/temperature (F°) per minute from preinjection time to 48 hours postinjection of endotoxin

Horse	#6659	#6162	#6035	#5773
Preinjection	90 /25/100.4 Excited	30/13/98.8	36/20/98.8	56/16/99.5
15 minutes	60/44/100.0	30/20/100.5	40/37/99.9	68/32/
30 minutes	68/36/99.5	40/35/100.1	72/20/99.2	68/48/
60 minutes	120/28/98.4	78/30/99.2	72/18/98.5	/30/
2 hours	145/50/97.4	50/24/99.0	78/30/97.8	36/40/100.8
4 hours	70/28/96.4	40/15/99.0	/94.8	/36/98.4
6 hours			50/24 Deep sighs	/18 /96.0 Deep sighs
8 hours	96/60 /100.0 Labored	27/16/100.8	28/20/	/24 /94.6 Deep
10 hours				/24/100.8
12 hours	108/68/105.2	50/12/100.6		/32 /103.0 Labored
24 hours		64/30/100.0		
36 hours		84/22/101.4		
48 hours		72/16/100.6		

Platelets (Figure 16). Initially there were variable responses in all platelet counts in all animals, including the mare #6162 which survived for 48 hours. The levels in samples collected prior to death in 3 horses were significantly lower than the pretrial counts ($P > .05$).

Hemograms (Figures 17-21). All animals suffered hemoconcentration. The increases in packed cell volumes (PCV) and hemoglobin concentrations were significant ($P > .05$). Blood collected as each animal approached death was quite thick and viscous. It appeared to be coincidental that the ranges of PCVs and death were from 61.0 to 63.5 (number of samples insufficient for statistical analysis).

In each case there was an initial leukopenia, primarily a neutropenia, within 15 minutes after endotoxin injection. Lowest levels were reached in 4 hours ($P > .05$). In each case numbers of neutrophils tended to increase from the fourth hour postinjection until death.

Initially there were insignificant decreases in numbers of circulating lymphocytes; however, the decreases were not as marked as for the neutrophils.

The total white cell counts of 3 horses (#6659, #6035, and #5773) at the time of death were significantly different from the preinjection counts ($P > .05$). The gradual increases in the white counts as death approached reflected the increases of circulating neutrophils.

Serum protein (Table 4). The quantity of total protein in serum and the albumin globulin ratio did not change significantly in any of the horses.

Blood urea nitrogen (BUN) (Table 4). Blood urea nitrogen (BUN) levels did not increase markedly in 2 of the cases during the experiments (#5773 and #6035). Horse #6659 had an increase of 11.5 mg./100 ml. over the

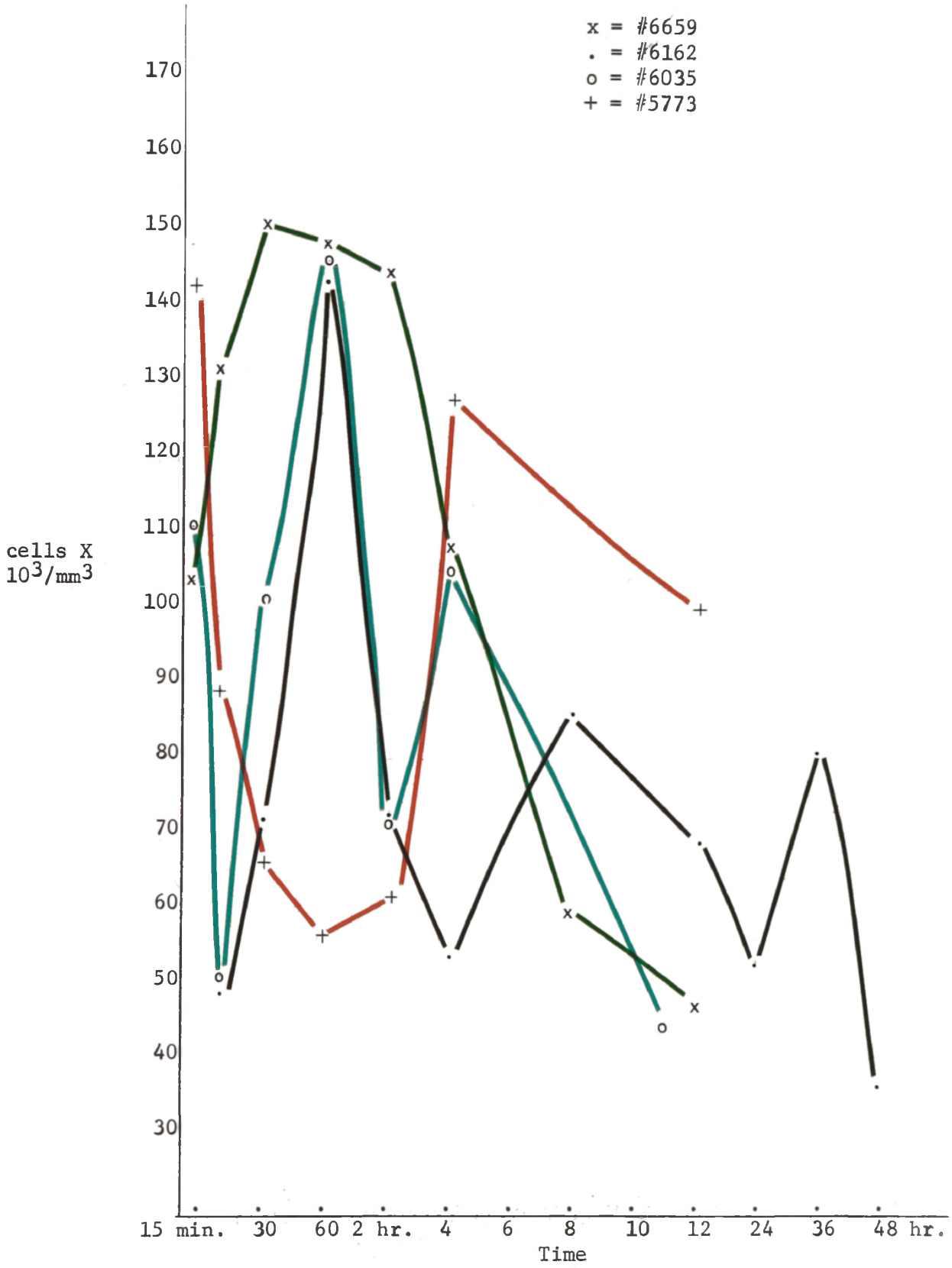

Figure 16. Platelet count of all 4 horses from preinjection to death of horse or 48 hours postinjection of endotoxin.

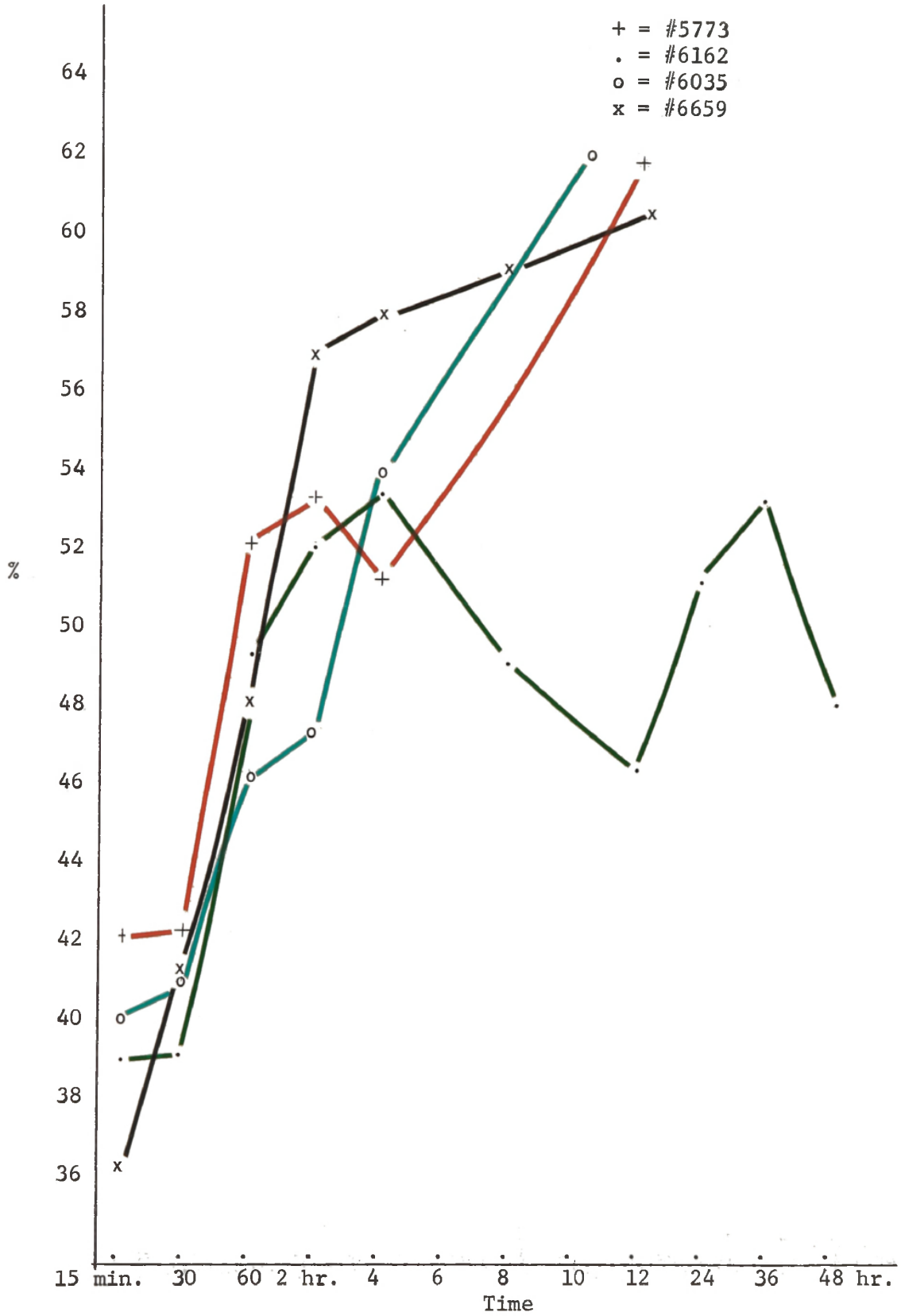

Figure 17. Packed cell volume of all 4 horses from preinjection to death of animal or the end of the 48-hour trial period. Horse #6162, which survived the 48-hour trial period, leveled out 2 hours postinjection.

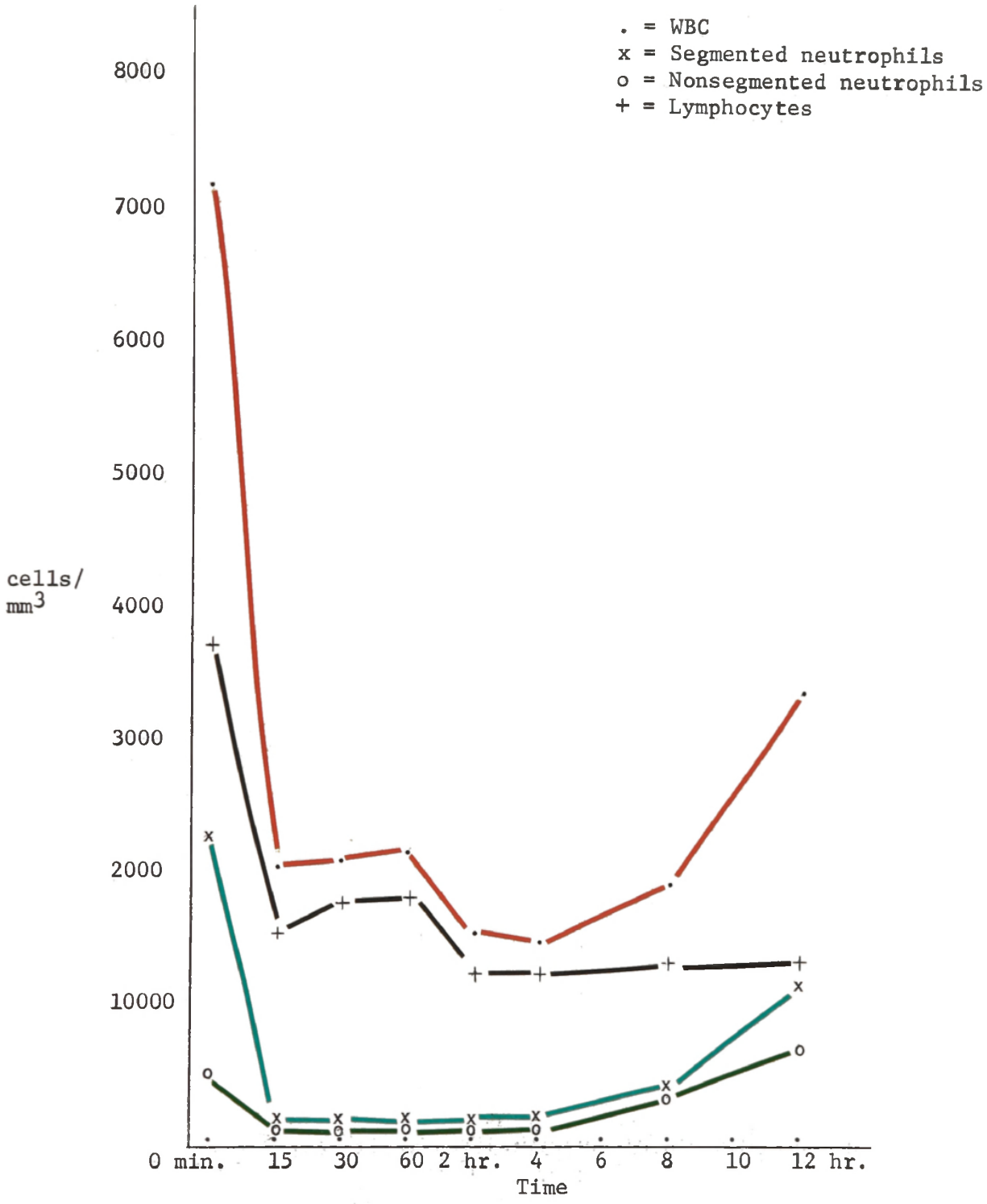

Figure 18. White blood cell count and differential of horse #6659 from preinjection to 12 hours postinjection of endotoxin.

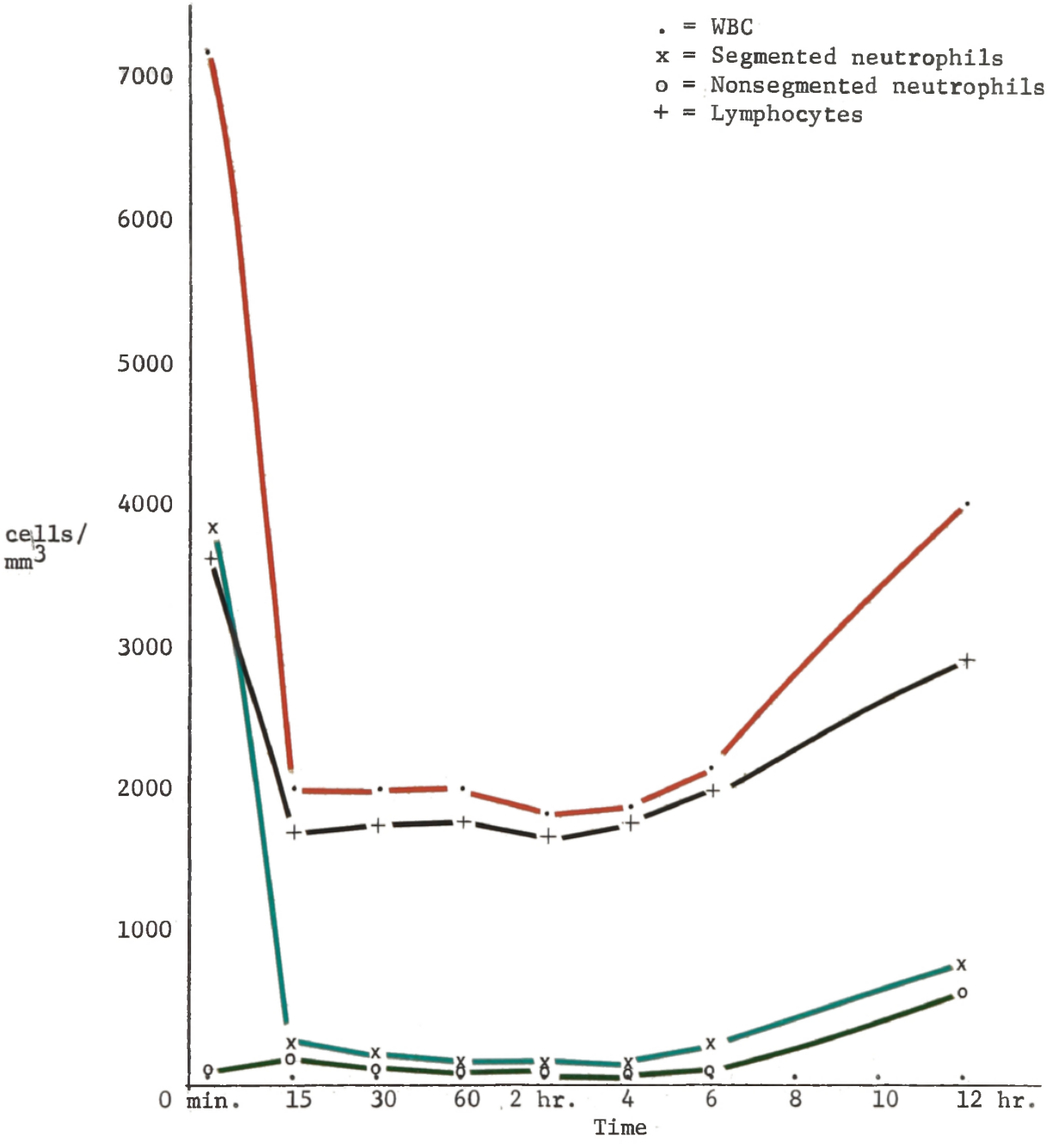

Figure 19. White blood cell count and differential of horse #5773 from preinjection to 12 hours postinjection of endotoxin.

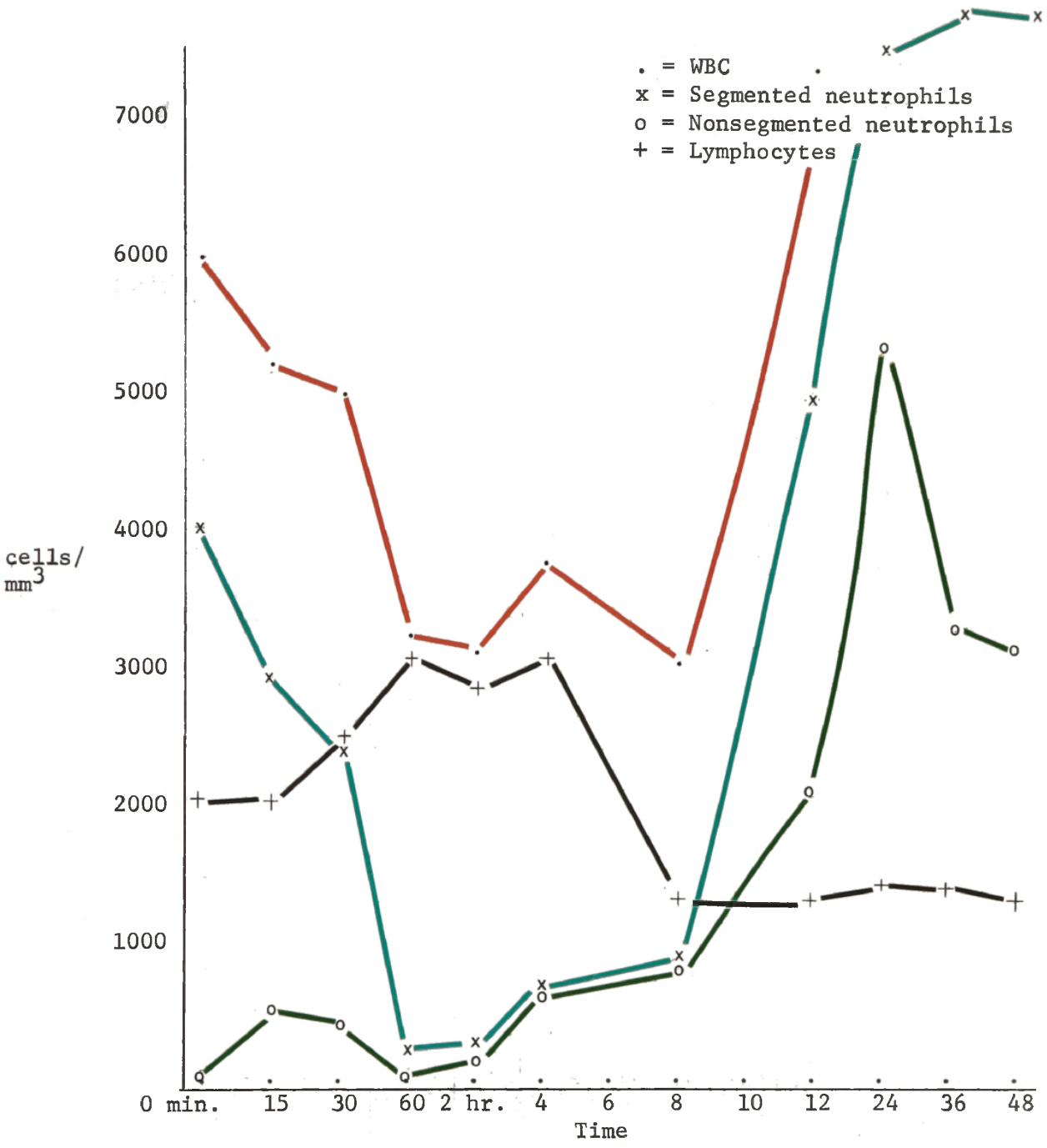

Figure 20. White blood cell count and differential of horse #6162 from preinjection to 48 hours postinjection of endotoxin.

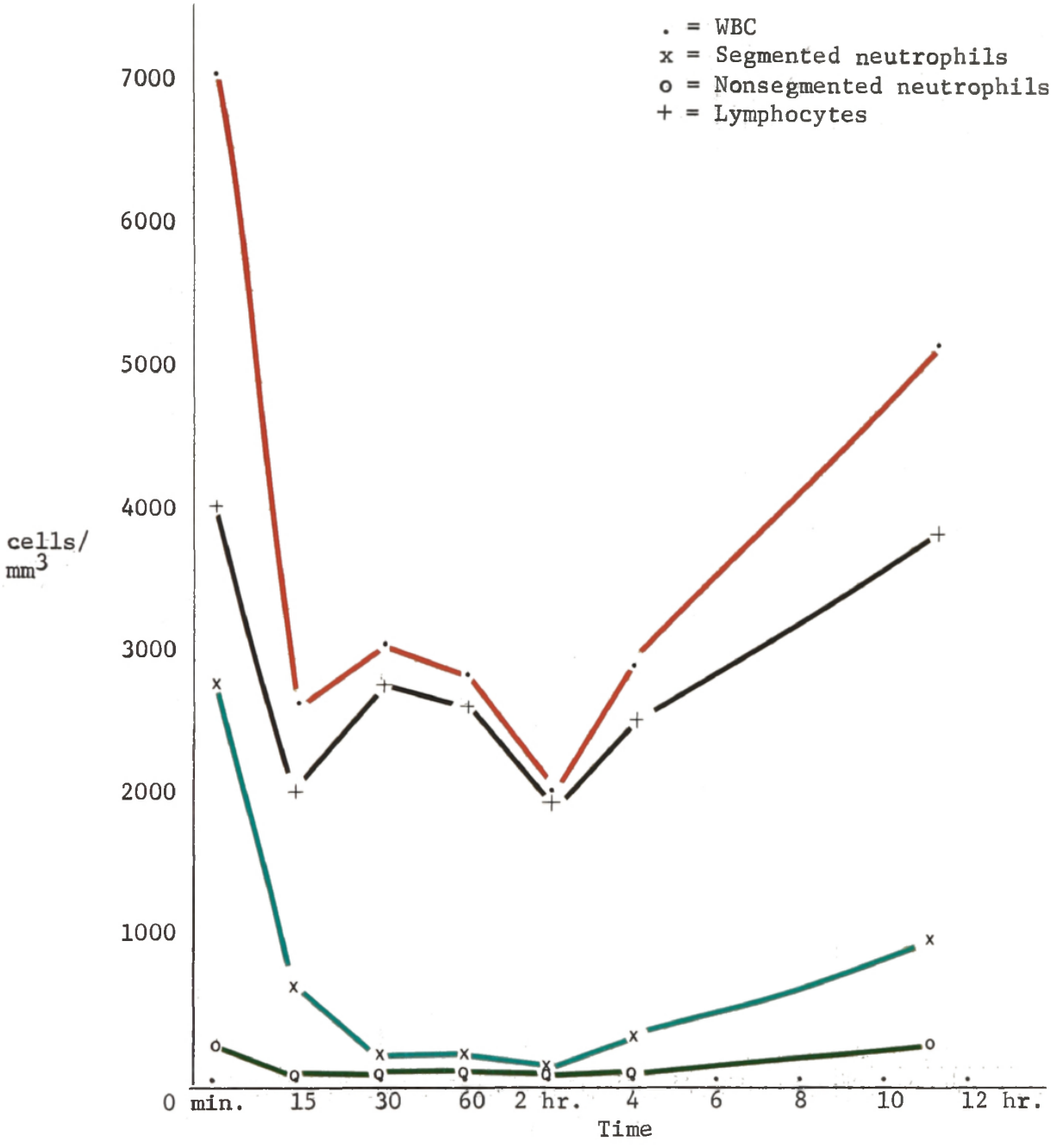

Figure 21. White blood cell count and differential of horse #6035 from preinjection to 11 hours postinjection of endotoxin.

Table 3. Serum sodium (in Eq./L.), potassium (in Eq./L.) and chloride (in Eq./L.) from preinjection time to 48 hours postinjection of endotoxin

Horse	Na (mEq./L.)				K (mEq./L.)				Cl (mEq./L.)			
	#6659	#6162	#6035	#5773	#6659	#6162	#6035	#5773	#6659	#6162	#6035	#5773
Preinjection	134	126	134	128	4.0	4.0	4.9	5.7	103.3	97.2	97.2	104.8
15 minues	135	128	128	127	3.3	3.6	5.2	4.8	101.9	101.2	100.3	97.2
30 minutes	132	123	122	123	2.7	3.3	4.6	4.5	101.9	101.2	97.2	103.4
60 minutes	132	130	130	125	2.5	2.9	4.8	4.9	103.3	100.3	99.5	98.7
2 hours	127	99	126	124	2.4	2.3	5.0	4.6	98.6	86.3	97.2	104.8
4 hours	138	126	127	129	3.3	2.9	5.5	4.2	98.6	97.2	94.8	97.2
6 hours				130				5.0				
8 hours	135	126	130		2.9	3.6	5.8		98.4	91.8	97.2	97.2
10 hours												
12 hours	136	122	134	134	3.9	3.5	7.1*	4.8	98.6	107.3	97.2	96.4
24 hours		139				3.5				105.0		
36 hours		125				5.5				107.3		
48 hours		130				7.7*						

*Hemolyzed

Table 4. Total protein (gm/100 ml.)/BUN (mg./100 ml.) from preinjection time to 48 hours postinjection of endotoxin

Horse	#6659	#6162	#6035	#5773
Preinjection	7.08/17.5	6.64/14	7.05/12	6.44/12
15 minutes	7.01/17.5	6.42/10	6.63/10.5	6.44/11.5
30 minutes	7.15/19.0	6.74/13	6.63/12	6.44/11.5
60 minutes	7.15/17.5	6.21/11	6.40/12	5.60/13
2 hours	8.58/20.5	8.70/14	6.40/13	6.74/13
4 hours	7.15/19.5	7.21/14	6.63/15	5.80/13
6 hours		6.42/		7.05/13
8 hours	8.58/24	/14	6.95/	
10 hours			/17	
12 hours	7.87/28.5	6.64/19	6.63/	7.25/14
24 hours		6.84/26		
36 hours		7.25/36		
40 hours		6.74/42		

preinjection level, and the terminal BUN value of 28.5 mg./100 ml. was 3.5 mg./100 ml. above the high normal level for horses.* In horse #6162, which lived, the BUN increased to 28 mg./100 ml. at 48 hours after endotoxin injection.

Serum Na, K, and Cl (Table 3). The quantities of serum electrolytes did not vary significantly during the experiment.

Necropsy

Grossly, the most striking lesions were hemorrhages and hyperemia. In each horse petechial and ecchymotic hemorrhages were noted on the epicardium and endocardium. Identical hemorrhages were found on the serosal surfaces of the lungs and the diaphragm.

Numerous petechial and ecchymotic hemorrhages were present in the mucosae of the small and large intestines. A few ecchymotic hemorrhages were present on the serosal surfaces of the intestines. In horse #5773 there was blood in the lumen of the small intestine.

The adrenal glands were swollen and hemorrhagic with hemorrhages primarily in the cortices with only a few extending into the medullary portions of the glands.

Histopathologic examinations confirmed the lesions of hyperemia and congestion observed on the gross examinations. The pulmonary vessels were congested and hyperemic (Figure 22). Some marginal pulmonary emphysema was seen in each of the older horses.

Microscopically hemorrhages were present throughout the myocardia. Many of the myocardial bundles were separated by an edema fluid. Hemorrhages were seen in and around the Purkinje fibers beneath the endocardium.

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*Benjamen, M. M.: *Veterinary Clinical Pathology*, Iowa State University Press, Ames, Iowa, 1961.

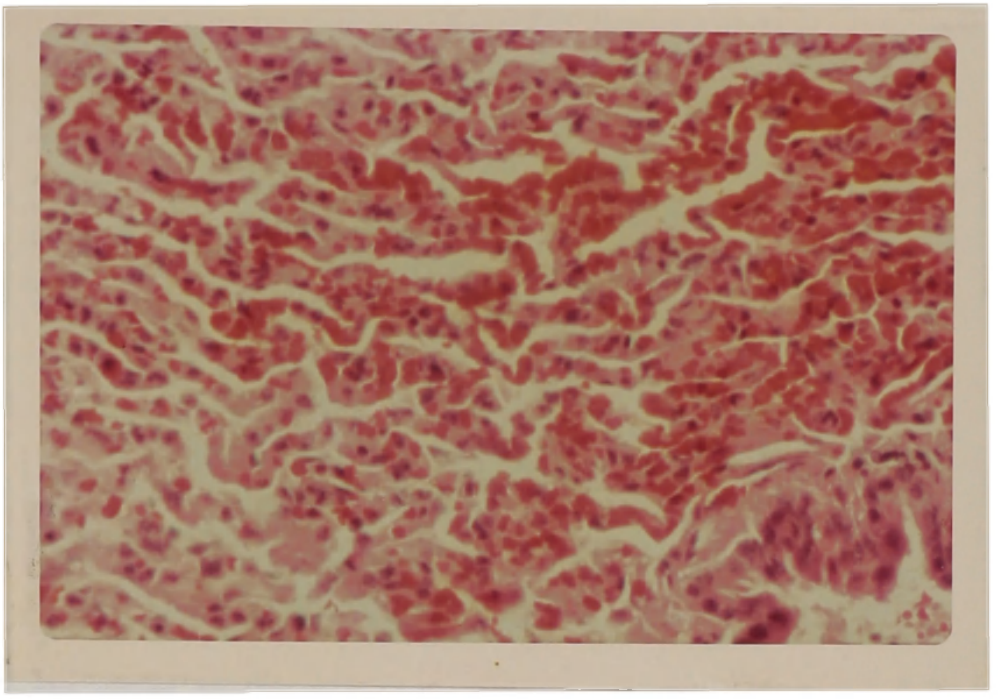


Figure 22. Lung of horse following endotoxin injection. Note marked congestion and influx of cells. Hematoxylin and eosin. x 200.

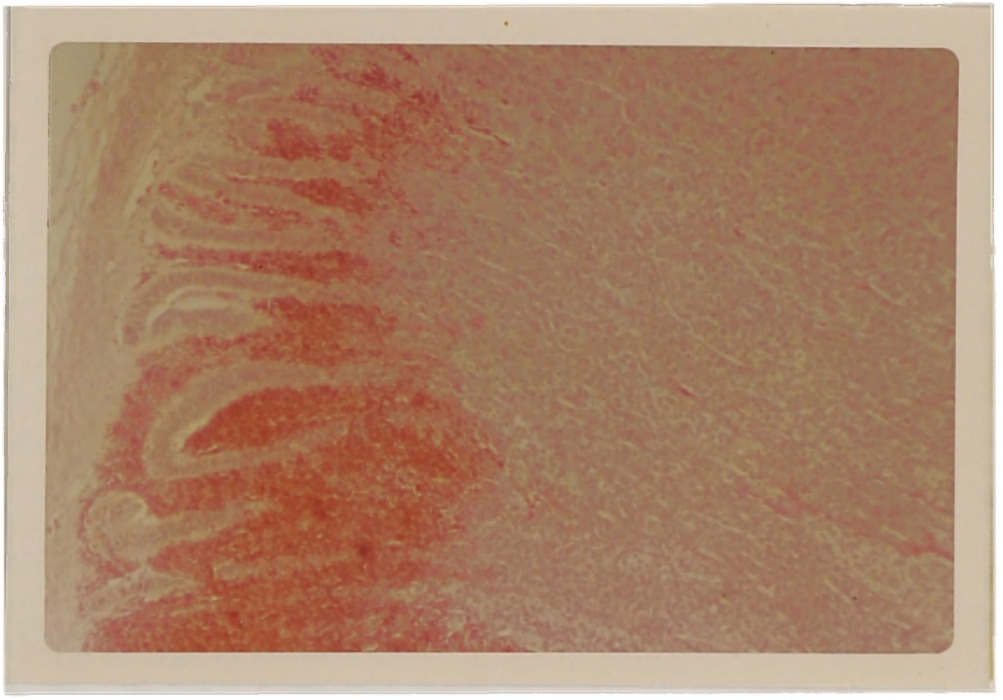


Figure 23. Adrenal gland of horse following endotoxin injection. Hemorrhage and congestion seen in zona glomerulosa. Hematoxylin and eosin. x 40.

The zona glomerulosa of the adrenal glands were hemorrhagic on microscopic examination. There were some streaks of hemorrhage into the zona fasciculata (Figure 23).

Hyperemia and congestion were seen in the cortices of the kidneys, especially in the renal glomeruli (Figure 24). Many of the collecting tubules in the kidneys contained hyaline casts, but others contained granular casts.

Horse #6162 had a mild chronic peritonitis. There was a calcified thrombus in the anterior mesenteric artery. Examination of the brain of this mare revealed an increase in glial cells in the cerebrum with occasional clumping of these cells. There were limited perivascular hemorrhages and edema in the medullary portion of the brain. There were hemorrhages and infiltrations of neutrophils into the white matter of the cerebellum. The meningeal blood vessels were congested, and the pia-arachnoid spaces mildly edematous.

Bacteriology

A beta hemolytic *Streptococcus* was isolated from the preinjection and terminal blood from horse #6659 and a *Shigella equulus* organism was also isolated from the terminal sample. No bacteria were isolated from organs obtained at necropsy.

From horse #6035 hemolytic and nonhemolytic *E. coli* bacteria were isolated from the kidney, liver, lung, and spleen specimens collected at necropsy examination. No growth was obtained from any blood culture specimens.

There was no bacterial growth on cultures prepared from any samples from horses #6162 and #5773.

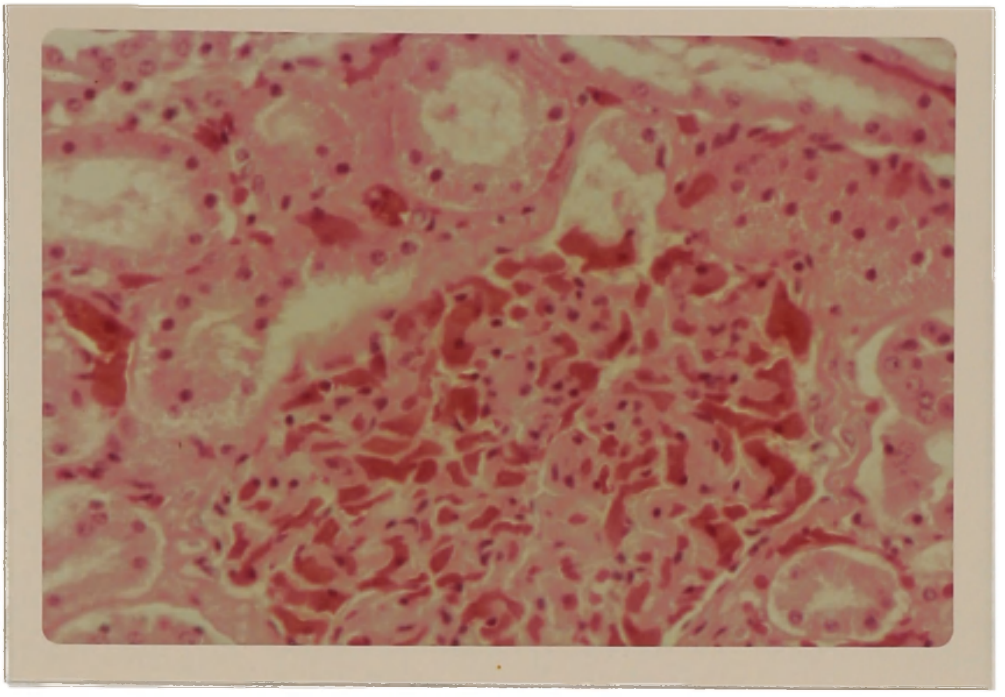


Figure 24. Kidney of horse following endotoxin injection. The glomerulus is showing congestion. Hematoxylin and eosin. x 200.

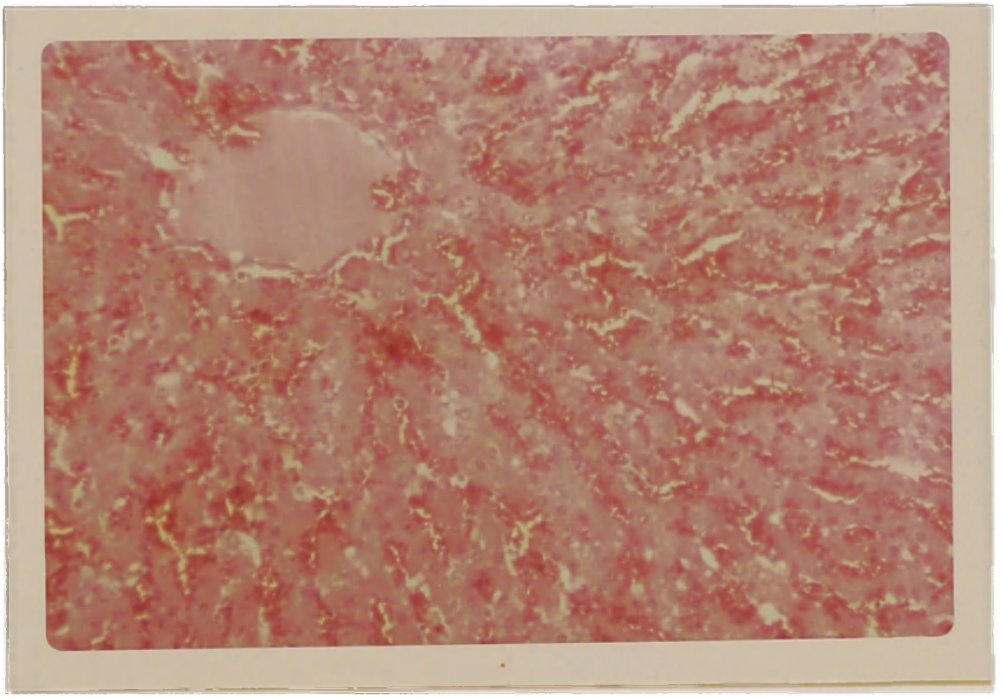


Figure 25. Liver of horse following injection of endotoxin. Note marked congestion of sinusoids. Hematoxylin and eosin. x 100.

DISCUSSION

Each of 4 horses received an intravenous injection of an endotoxin from a gram-negative bacterium (*E. coli*). Each developed signs of severe shock and was obviously stressed within 5 minutes after injection. By 1 hour each exhibited typical signs of shock, including depression and cold, clammy extremities with decreased capillary perfusion time and lowered blood pressure. The blood of each horse became hemoconcentrated and acidotic. Three of the horses died and 1 survived.

The signs produced were similar to those seen in the syndrome, colitis "X", but differed significantly in that none of the horses developed colicky signs or diarrhea or had a marked increase in the total serum protein. One constant sign was cyanosis of the gums which indicated poor capillary perfusion (Figures 26-28). This same sign was described by Coffman in a case report of endotoxic shock in a horse (Coffman and Bracken, 1968).

The cardiovascular responses in these horses were quite similar to those seen following injection of endotoxin in dogs (Duff *et al.*, 1965), cats (Granway *et al.*, 1969), rabbits (Grable *et al.*, 1963), monkeys (Nies *et al.*, 1968; Thomas *et al.*, 1969), bears (Soloman *et al.*, 1966), and coyotes (Hinshaw *et al.*, 1919). The decreases in blood pH levels accompanied by normal pCO_2 levels indicated development of severe metabolic acidosis. There were marked differences between the arterial and venous blood pCO_2 levels (Figures 6, 8 and 9) and between the arterial and venous pH readings (Figures 1-4). These variations

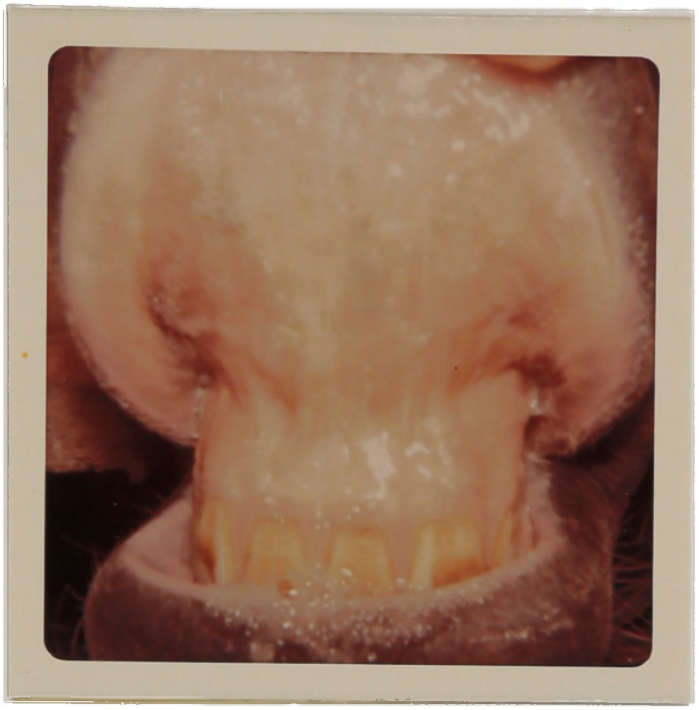


Figure 26. Pink mucous membranes of normal horse.

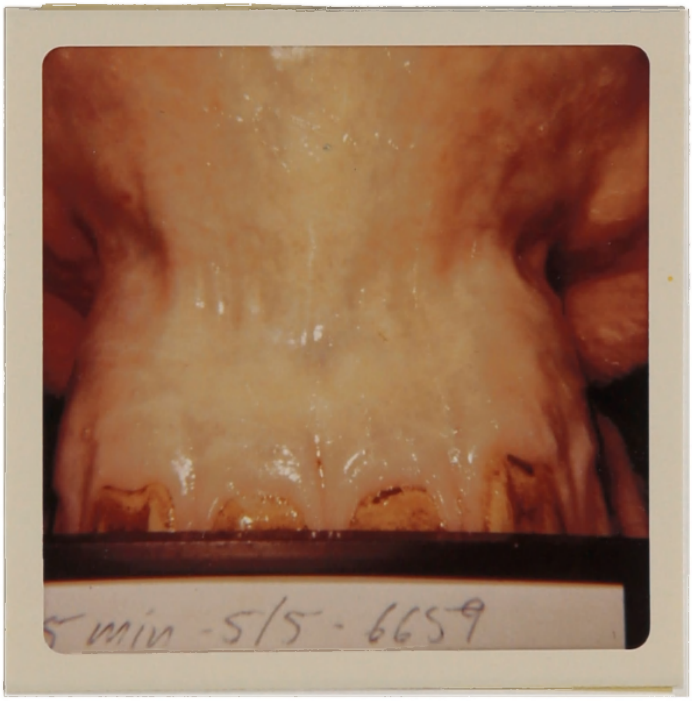


Figure 27. Mucous membranes of horse #6659, 5 minutes after injection of endotoxin. The somewhat blanched appearance is due to the potent sympathomimetic activity of the injected endotoxin.

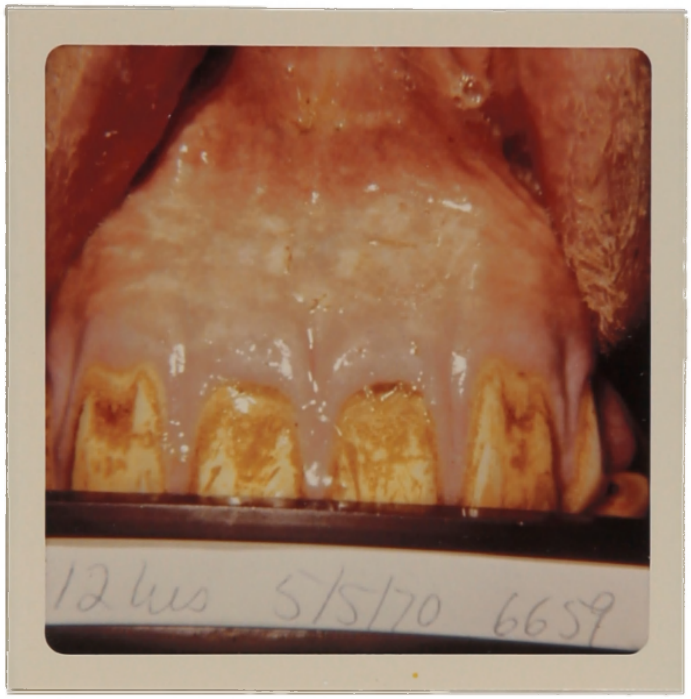


Figure 28. Mucous membrane of horse #6659, 12 hours after injection of endotoxin. Note the congested appearance and the cyanotic gum margins outlining the incisor teeth.

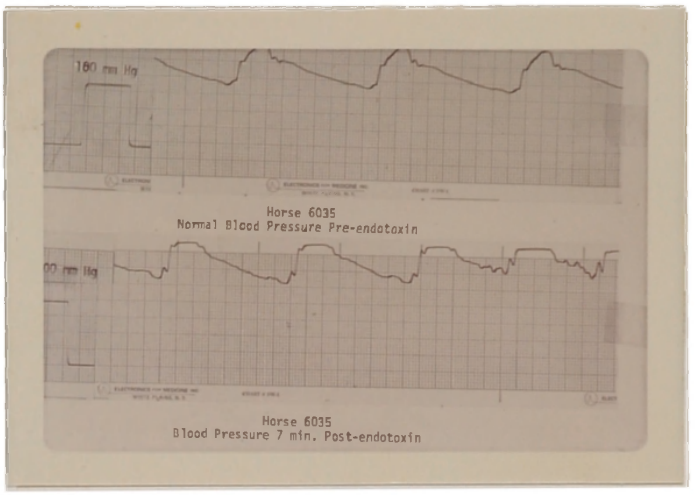
dramatically illustrated that the lungs function to maintain body homeostasis during stressing situations. The animals hyperventilated to attempt to compensate for the buildup of blood CO_2. There was no problem in maintaining adequate ventilation, but it is always imperative that the air passages are open when treating shock from any cause.

The blood pressure levels in all cases fell dramatically, as had been expected. All horses appeared to stabilize and blood pressures leveled around 2 hours after endotoxin injection. All were able to survive at blood pressures less than one-half normal levels for periods up to 10 hours before death. This suggested that during the time of extremely low blood pressure "irreversible" shock could become "reversible" with proper therapy (Figures 29-31).

The central venous pressures varied making these results inconclusive. It was noted, however, that the central venous pressures of horses #6659, #6162, and #6035 were consistent in results of the experiment. The central venous pressure of horse #6162, which was the only horse surviving the experiment. varied little, indicating the heart effectively handled the venous blood return. Horses #6659 and #6035 experienced drops in venous pressures, indicating pooling of blood in the body. Progressive rises in central venous pressures indicated heart failure. When the heart was unable to pump all of the returning blood, pulmonary congestion and central venous pressure increased until death.

Severe leukopenia was similar to that seen by Carrol *et al.* (1965). He cited Thomas (1954), who suggested that both neutrophils and thrombocytes became trapped in fine capillaries after endotoxin administration. Hardaway and Johnson (1963) indicated that initially disseminated intravascular coagulation plays a role in endotoxic shock. All observed declines in fibrinogen and platelet levels and believed these components

Figure 29. Arterial blood pressure before endotoxin injection and 7 minutes after injection in horse #6035



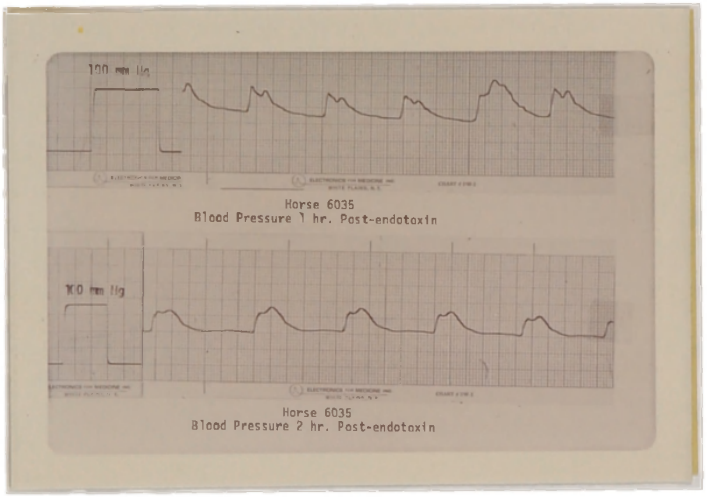
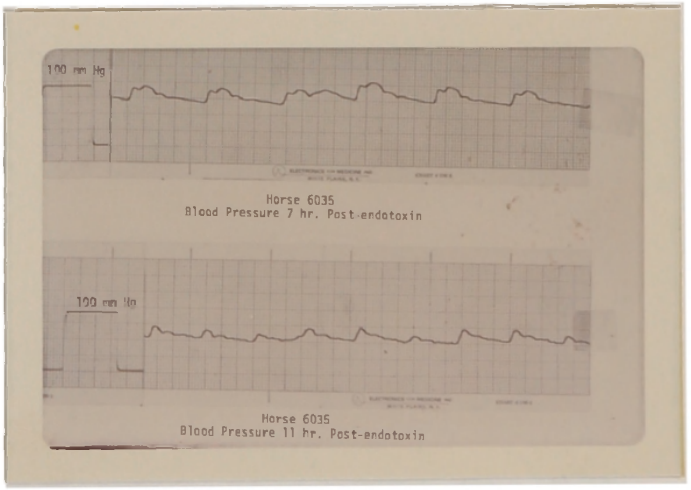


Figure 30. On horse #6035, the arterial blood pressure seen at 2 hours after endotoxin injection. Blood pressure began to stabilize at half the preinjection level.

Figure 31. Arterial blood pressure of horse #6035 at 7 hours postinjection about the same seen 2 hours postinjection. At 11 hours the pressure decreases further just before horse's death.



were being depleted in the clotting process by being bound up in platelet clumps and thrombi. Histopathologic examinations did not show any of these lesions, but the microthrombi may have been dissolved before tissues were collected. Speculations are some neutrophils could have been destroyed while attempting to neutralize the endotoxin. Compatibility of white blood cells in an endotoxin environment could conceivably be determined much as compatibility cross match tests for blood types are determined.

The development of severe hemoconcentration in all horses was dramatic. There was some fluid loss from the lungs and from sweating. No animal developed diarrhea, and only horse #6162 would drink water.

Impressions are the term hemoconcentration does not completely or accurately describe all things observed in the experimental horses. There was no increase in total serum protein in any horse (Table 4). Carrol *et al.* (1965) demonstrated a rise in the plasma protein in one horse and considered it significant. Endotoxins do possess potent sympathomimetic activity (Lillehei and MacLean, 1958). Torten and Schalm (1964) found that the spleen was capable of supplying a red blood cell volume equal to one third the circulating red blood cell mass in times of stress. Splenectomized horses were unable to respond in this way during stressing situations. Kitchen *et al.* (1965) produced dramatic rises in packed cell volumes by vigorously exercising horses. Schalm (1965) concluded that the spleen is a major reservoir of red blood cells in the horse, and that adrenalin causes it to contract to release these cells. The lack of dramatic changes in the serum electrolyte levels may be significant; therefore it is assumed there are no dramatic shifts in body water compartments. It was assumed that there was, in effect, a "red cell transfusion" from the spleen that caused the

rise in the packed cell volume, but more studies are needed to prove this conclusively.

Kitchen *et al.* (1965) found an exponential rise in blood viscosity as the packed cell volume rose. Higher blood viscosity would stress the heart and would adversely affect total cardiac function.

Interestingly, the rise in the packed cell volume of horse #6162 was as rapid as those in the other 3 horses during the first 4 hours (Figure 15) after endotoxin injection, but the rise did not continue after that time. In the other horses, the PCV values continued to rise, which was considered a poor prognostic sign. Horse #6162 did receive a smaller dosage of endotoxin which could account in part for her surviving the experiment; but clinical signs indicated she was seriously stressed.

Branch (1964) suggested that the reaction to endotoxin is allergic or anaphylactic in nature. All horses responded similarly. Especially noticeable were the development of body weals and profuse sweating. Endotoxin antibodies have been found in red blood cells of animals (Brande, 1964). It is speculated that failure to respond to exposure to endotoxin may be the result of the efficient removal of endotoxin from the blood stream by the liver and spleen. In other instances there may be a lack of exposure to the endotoxin to sensitize the animal. It is conceivable these two factors, in addition to smaller dosage, explain horse #6162's response to the endotoxin and survival. The clinical signs demonstrated the decrease in packed cell volume (Figure 16), the increase in arterial blood pressure (Figure 15) and the failure to develop an acidosis (Figure 4) indicated stress not as severe as in the other horses. Conclusions are horse #6162 experienced "reversible" shock and the other horses developed "irreversible" shock. The endotoxin affected all systems

similarly in all horse, but less severely in horse #6162.

The blood urea nitrogen values changed slightly. Hinshaw *et al.* (1967) reported unchanged blood urea nitrogen levels in coyotes following endotoxin administration. He concluded kidney function was unaffected. Definite changes were seen in the kidneys on histopathologic examination, especially in the collecting tubules. Other tests, such as measurements of urine flow and serum creatinine (Benjamen, 1961), could be used to determine kidney function.

The horses apparently did not suffer from septicemia. Bacteria were isolated from one horse from specimens collected during necropsy examination (horse #6659), but it is possible the skin was the source of contamination. Fine (1961) reported intravenous penicillin helped reduce mortality from shock, thus implying that bacteremia was a factor. McHenry (1969) reported that gram-negative bacteremic shock is frequently seen as a complication in human obstetrical patients. In analysis of 169 cases, Weil *et al.* (1964) stated *E. coli* was most frequently isolated. Conclusions were that antibiotic therapy alone yielded poor results and shock persisted in spite of sterilization of the blood. From these reports conclusions are the products of the bacteremia and not bacteria themselves produce endotoxic shock.

SUMMARY

Experiments with 4 horses indicated that endotoxic shock can be reproduced in the horse easily and consistently by intravenous injection of a toxin prepared from the bacterium *E. coli*. It appears clinical signs and death result from a combination of metabolic aberrations, which are not all clearly understood, produced by a known infected toxin or an endotoxin. It was noted in work cited previously (Kitchen , 1965; Torten and Schalm, 1964) that animals could survive with packed cell volumes over 60%. Speculations have been the spleen is probably the source of red blood cells, but further experiments in splenectomized and normal horses should be performed to provide this information.

While not all signs were identical, endotoxic shock bears striking resemblances to the clinical syndrome, colitis "X", from which affected horses seldom recover. In the latter syndrome speculations have been that the affected horse could have been previously sensitized to the endotoxin through a single or series of stressing situations. Corticosteroid therapy has been recommended for anaphylactic reactions and has been reported to produce cases of either endotoxic shock or colitis "X".

The experiment, with a few relatively simple monitors, has provided information about the horse's cardiopulmonary responses to stress. With further data from additional experimentation and carefully monitored clinical patients there is optimism that more effective therapeutic regimens for treating horses in clinical shock may be developed.

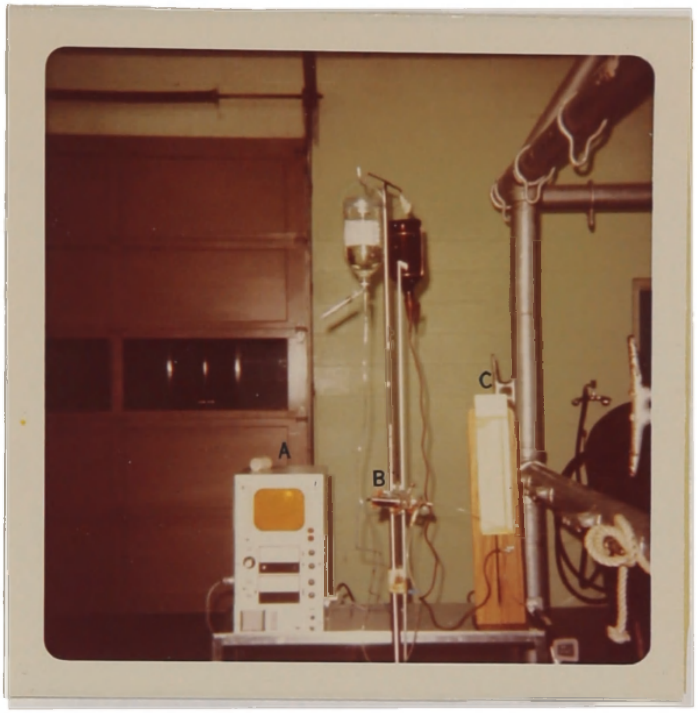


Figure 32. Dual trace recorder (A) for recording blood pressures is on left; Statham transducer (B) is on IV stand next to water manometer used to measure central venous pressure (C).

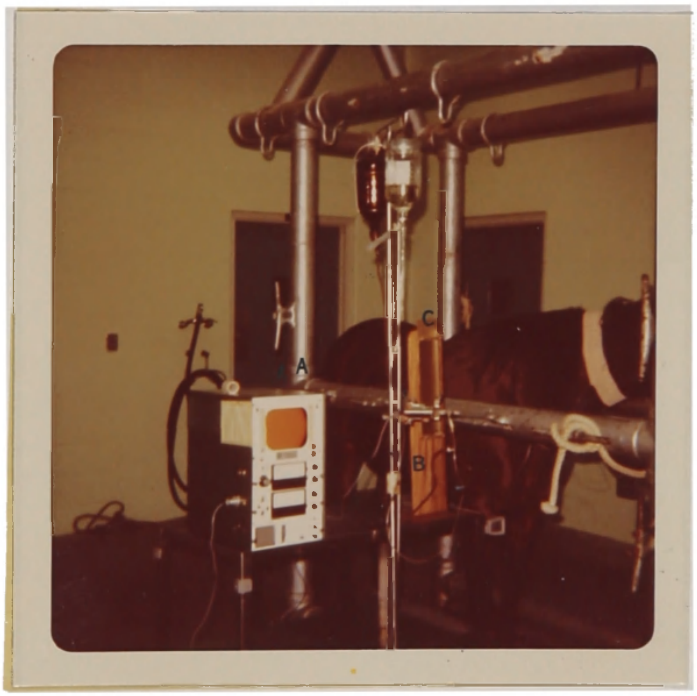


Figure 33. Arrangement of dual trace monitor (A), Statham transducer (B) and water manometer (C) next to horse in stocks prior to endotoxin injection.

Figure 34. Radiometer gas monitor (A) and American Optical Micro-Oximeter (B).

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