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Physiological and biochemical changes in serially sampled cerebrospinal fluid and blood during induced convulsions in sheep.

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PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN SERIALLY SAMPLED CEREBROSPINAL FLUID AND BLOOD DURING INDUCED CONVULSIONS IN SHEEP

by

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I. INTRODUCTION

Cerebrospinal fluid (CSF) manifests many physiological and biochemical changes that occur within the central nervous system (CNS) just as blood reflects changes in the body. It is therefore a logical starting point in the study of CNS metabolism.

There is a definite need for new approaches toward the study of CNS metabolism. Most research in this area has been conducted on anesthetized animals. This is unfortunate because such an animal is far from normal. In fact, it is erroneous to infer that biochemical and functional changes found in anesthetized animals are applicable to normal individuals. This has been done much too frequently. There are few reports in the literature on physiological and pharmaco-physiological studies of the CNS using unanesthetized subjects. However, in recent years research workers have become more aware of the necessity for using unanesthetized animals in studying CNS metabolism. The principal difficulty encountered in using unanesthetized subjects is in obtaining samples for analysis and making various physiological measurements without producing discomfort and pain, which also elicit changes.

The primary objectives of this investigation were as follows: (1) to develop a surgical technique for the establishment of a permanent, indwelling catheter in the cisterna magna or lateral ventricle of sheep to facilitate the measurement of CSF pressures, and to permit serial sampling of CSF from unanesthetized animals prior to, during and following induced convulsions.

(2) to develop a surgical technique for the placement of a permanent, indwelling catheter in the carotid artery of sheep to enable the measurement of blood pressure and to obtain serial samples of blood prior to, during and following seizures, while at the same time maintaining patency of both carotid arteries.

(3) to induce convulsive seizures in sheep using three different agents: (a) carbon dioxide (CO_2), (b) insulin, and (3) heptachlor, an insecticide capable of producing convulsions.

(4) to study the physiological and biochemical changes occurring during induced convulsive seizures, including blood and CSF pressure changes and changes in concentrations of enzymes, protein, glucose, and electrolytes.

(5) to observe the histopathological changes in the brain resulting from either the establishment of a catheter in the CSF space or associated with convulsive seizures.

Techniques for the establishment of permanent, indwelling catheters in the cisterna magna and carotid artery of sheep are described. Some of the problems encountered during these procedures as well as the advantages gained from direct catheterization of the cisterna magna are discussed. Preliminary results of measurements of biochemical and functional changes associated with convulsions are also presented.

II. REVIEW OF LITERATURE

A. Cerebrospinal Fluid

The cerebrospinal fluid (CSF) system has been one of the last to be studied physiologically. Although CSF was known to have a mechanical function of protecting the brain and cord from traumatic injuries, not until the concept of a "blood-brain barrier" came into being after the turn of the twentieth century was it given physiological significance. Investigations of the "blood-brain barrier" led to studies on the effects of introducing various drugs into the CSF. Winterstein (1961) reviewed the reports on the effects of injecting various materials at different sites along the ventriculo-cisternal-subarachnoid system.

Analyses of CSF constituents have aided in the diagnosis of various neuropathological conditions in human medicine, but have not been used extensively in veterinary medicine. The protein content of the CSF of normal and diseased patients has been studied. Goldstein et al. (1960) described methods for identification and quantification of proteins, glycoproteins, and lipoproteins in CSF. They have given normal values, variations in protein content along the cerebrospinal axis, and the effects on the protein level of obstructive and nonobstructive diseases of the ventriculo-spinal canal. Other workers described methods of detecting normal and abnormal constituents in the CSF and discussed their clinical significance as diagnostic aids (Baker 1962; Chapman and Wolf 1958; Kronholm 1961; Papadopoulos et al. 1959).

Shain (1960) reviewed the literature regarding neurohumors and other pharmacologically active substances in CSF. Substances considered as neurohumors were acetylcholine and biogenic amines including epinephrine, norepinephrine, serotonin and histamine. In his discussion he points out that inadequate procedures are now used for research studies on CSF. Two suggestions are given: (1) cisternal CSF should be obtained for study rather than relying on lumber fluid since the former may yield positive data when lumbar fluids are negative and (2) better assay methods for CSF constituents are needed.

Cerebrospinal fluid dynamics including pressure relationships to arterial and venous pressures have been studied. Davson (1956, Ch. 9) reviewed the literature pertaining to CSF dynamics. Bowsher (1960) wrote a more recent review on this subject. Most investigators concluded that body posture or position of the spinal column at the time of measurement is important in determining CSF pressure, and that venous pressure has more influence on CSF pressure than does arterial pressure (Bowsher 1960; Davson 1956; Grundy and Howarth 1957). Carmichael et al. (1937), however, showed that CSF pressure in patients sitting erect was influenced primarily by arterial pressure, rather than by venous pressure or respiration.

B. Catheterization Techniques

1. Cerebrospinal fluid catheters

The development of techniques for continuous perfusion of the ventriculo-cisternal system has provided advantages over simple injection procedures. Continuous perfusion permits lowering or elevating the

concentration of various constituents of the CSF. This allows observation of the behavior of animals subjected to a deficiency or an excess of cations such as K^+, Na^+, Ca^{++}, and Mg^{++}, and changes in CO_2 and pH. Adam et al. (1938) apparently were the first to use the perfusion method. Leusen (1948a, 1948b, 1950) was the first to carry out systematic experiments using the perfusion technique in anesthetized dogs. His experiments indicated that abnormally high concentrations of CSF K^+ or abnormally low Ca^{++} or Mg^{++} caused a sustained rise in blood pressure and augmentation of pressor reflexes in vagotomized dogs. Subsequent experiments using the same techniques have left little doubt that abnormal concentrations of CSF constituents can produce substantial autonomic effects in anesthetized animals (Leusen 1954a, 1954b, 1954c; Leusen and Lacroix 1961).

Winterstein (1961) described the various perfusion techniques used by several other investigators. Geiger (1958) reviewed the literature pertaining to brain perfusion in situ in an attempt to relate brain metabolism to function. Bartelstone et al. (1958) also described a perfusion method for dogs.

The use of permanently implanted catheters has made it possible to inject substances into the intraventricular spaces of unanesthetized animals. Feldberg and Sherwood (1954a, 1954b, 1957) were the first to use such a technique extensively. They studied the behavior of animals following intraventricular injections of various ions, drugs and hormones. They described a procedure in which permanent cannulae were introduced into one of the lateral ventricles and the cisterna magna of cats. The cannulae

were covered with a rubber membrane which allowed the injection of substances without the conscious animal being aware of it (Feldberg and Sherwood 1953). Manuilov (1952) briefly described a similar procedure using dogs. Grundy and Howarth (1957) described an elaborate perfusion technique for determining CSF pressure in cats. Davison et al. (1962) described a technique for ventriculo-cisternal perfusion in rabbits. Other workers cannulated the lateral ventricles of the Macaca monkey (Wada and Bauck 1961). Palmer (1959) modified the technique of Feldberg and Sherwood for perfusion of sheep. Pappenheimer et al. (1962) described a procedure for the perfusion of the ventriculo-cisternal system of unanesthetized goats using permanent indirect cannulae.

Winterstein (1961) stated that the perfusion method has several disadvantages. Abnormal conditions are created by the artificial perfusion fluid. It is impossible to determine the site of the effect of test materials and sometimes erroneous conclusions have been formed from such experiments.

Although permanent cannulae have been used to study the effects of injecting or perfusing abnormal concentrations of CSF constituents, drugs and hormones into the CSF space of animals, there are apparently no reports of using permanently implanted, direct, CSF catheters for detecting changes in the CSF accompanying abnormal CNS activity. Just as artificially created high or low cation concentrations in the CSF can cause CNS and systemic changes, it is probable that systemic and CNS changes are reflected by changes in CSF constituents. Indeed there are many reports of biochemical

changes in the CSF associated with such neuropathological conditions as cerebrovascular disorders, tumors, and convulsive seizures. However, there are apparently no reports of studies on CSF sampled serially prior to, during and following convulsive seizures.

2. Carotid artery catheters

Techniques for cannulation of various vessels of the body for sampling and measuring pressure changes have been described. Weeks and Jones (1960) described a method for permanently implanting polyethylene tubing in the abdominal aorta of rats to facilitate sampling and direct pressure measurements in unanesthetized animals without exciting them. These workers established the catheter through an incision into the abdominal cavity and brought the catheter out through the skin on the back of the neck. Popovic and Popovic (1960) described the permanent cannulation of the aorta and anterior vena cava of rats and ground squirrels by passing polyethylene tubing down the carotid artery and jugular vein, respectively. Barr and Soila (1960) described a technique for introducing a soft cannula into an artery by percutaneous puncture, in which the tubing served as a sleeve over a needle. Rampone (1959) cannulated the thoracic duct of dogs near its junction with the jugular vein. Blinova and Bartyzel (1956) developed a cannula for determining the lateral pressure of a vessel without obstructing its lumen.

There are relatively few reports of techniques for catheterization of the blood vessels of large animals. Jackson et al. (1960) used polyethylene

tubing to cannulate the hepatic, portal and renal veins and the renal artery and gall bladder of sheep in order to study insecticide metabolism.

Carotid loops have been extensively used in sheep and goats to provide easy access for pressure measurements and samplings of arterial blood. Linzell (1963) discussed this technique, describing the difficulties encountered and steps to overcome them.

C. Cerebrospinal Fluid Enzymes

Elevations in CSF enzyme activities have been reported in association with several CNS neuropathological conditions in humans, dogs and cats. The enzymes considered in this paper include two transaminases (glutamic oxalacetic (GOT) and glutamic pyruvic (GPT)) and one dehydrogenase, (lactic dehydrogenase (LDH)). Enzymatic transamination consists of the catalyzed reversible transfer of the alpha-amino nitrogen of an amino acid to an alpha-keto acid with the resulting synthesis of a second amino acid and a second alpha-keto acid (Braunstein and Kritzman 1937; Mason and Wroblewski 1957). This is a major reaction in the maintenance of the dynamic carbohydrate-amino acid metabolism within the body cells. LDH catalyzes the reversible oxidation-reduction reaction between lactate and pyruvate. Under normal conditions these enzymes are contained almost entirely within the cells although limited activities are normally found in the blood serum and CSF (Fleisher and Wakim 1956; Hain and Nutter 1960; Wroblewski 1957). The tissues containing the greatest amounts of these enzymes are skeletal and heart muscles, liver and brain (Mason and Wroblewski 1957). It is

generally believed that cellular degeneration results in the release of these enzymes into the tissue fluid and eventually into the blood and/or CSF.

There are conflicting reports concerning the level of activity of these enzymes in the CSF of patients and animals with neuropathological conditions. In general, they indicate that elevations in CSF GOT and LDH activities are associated with nonspecific fulminating CNS damage such as cerebrovascular accidents and with diverse conditions including thrombo-embolisms, infections, degeneration and some neoplastic conditions.

Green et al. (1959) studied enzyme activities in the CSF and brain tissue of patients with brain tumors. They found inconsistent elevations of GOT, but consistent elevations of LDH activities.

Prec et al. (1961) examined GOT and GPT activities in serum and CSF from 307 patients suffering from various nervous conditions. They found pathological elevations in some cases of vascular, inflammatory and traumatic disorders of the CNS. Most chronic diseases of the CNS produced no elevation of enzyme activities. Murawski et al. (1961) studied the CSF from 45 patients with cerebrovascular accidents. Only 31 percent showed increased enzyme activities and the elevations were not correlated with severity or the clinical course of the respective cases. Their results suggested the existence of a hemato-encephalic barrier and possibly a brain-fluid barrier for GOT.

Dioguardi et al. (1960) studied cases of human cranio-encephalic trauma, not complicated by other lesions, and concluded that neither cranial trauma or shock caused GOT and GPT elevations in the CSF.

Of special interest in relation to the significance of enzyme elevations in CSF is the controversy regarding the existence of the widely publicized "blood-brain barrier". Wroblewski et al. (1958) reported no relationship between serum and CSF LDH activities, presumably, because of a "blood-brain barrier". Workers at the Mayo Clinic showed evidence of a barrier in dogs with experimental cerebral infarction (Fleisher and Wakim 1956; Wakim and Fleisher 1956). Marked elevations in CSF transaminase activities were associated with only minor serum elevations. Conversely, high serum transaminase activities resulting from intravenous injection of the enzyme or from experimental acute hepatic necrosis were not reflected in the CSF.

Green et al. (1957a; 1957b) observed no serum enzyme elevations after cerebral infarction although moderate elevations were found in the spinal fluid from 7 of 11 patients.

Lieberman et al. (1957b), however, reported elevated serum transaminase activities in 43 percent of patients having cerebrovascular accidents but no myocardial infarction. Fleisher et al. (1957) also reported moderate elevations in serum and CSF transaminase activities in patients with cerebrovascular diseases. Lieberman et al. (1957a) reported cases of striking dissociation between levels of GOT activities in simultaneously drawn specimens of blood and CSF. This would indicate the presence of a blood-CSF barrier for GOT.

Cerebrospinal fluid and plasma enzyme levels have been studied by Lending et al. (1959) in dogs in which prolonged convulsions were induced by pentamethylene tetrazole or electroshock. Seizures of longer than 30 minutes duration resulted in plasma and CSF GOT and LDH activities that were about 3 times greater than in controls. The ratio of cisternal fluid to plasma GOT and LDH levels was similar in the control and experimental groups. This suggested that there was no major increase in permeability of the blood-CSF barrier to these enzymes. They suggested that elevated CSF activities of GOT and LDH immediately following prolonged seizures probably reflected an increase in cerebral cell membrane permeability rather than actual cellular lysis. In the same study an increased permeability of the blood-CSF barrier to albumin I^{131} was demonstrated. In a subsequent study (1961) in which puppies were subjected to prolonged hypoxia, CSF GOT and LDH activities were 5 and 3 times greater, respectively, than controls. As in the convulsion experiments, an increased blood-CSF barrier permeability to albumin I^{131} was demonstrated. There also appeared to be increased blood-CSF permeability to the enzymes, rather than actual cerebral cell necrosis.

In a clinical study on humans in which electroshock convulsions had been induced for treatment of neurologic disorders Mann et al. (1960) found significant GOT elevations in the CSF within 12 hours following seizures. The levels of activity returned to control values within 48 hours. These authors reviewed the previous reports on the effects of electroshock convulsions.

Assuming that there is an effective blood-brain barrier, elevated levels of enzymes in CSF must be due to CNS cellular degeneration or increased permeability of their membranes. If this assumption were true, then the diagnostic value of CSF enzyme levels would be much greater than that of serum enzyme determinations.

In an effort to explore changes that may affect LDH and GOT activities in human CSF, Spolter and Thompson (1962) found that these enzymes increased with age of the person and also with increased CSF protein content. They pointed out, however, that conditions which allow the leakage of protein from blood into CSF, would not result in significant elevations in enzyme activities unless the protein increase was great. Hain and Nutter (1960) had previously shown that CSF GOT and LDH activities increased linearly with age of human patients.

Although many reports have appeared concerning CSF enzyme activities associated with neuropathological conditions in humans and small animals, there are few such reports for livestock. Because of species variation the results obtained from humans and small animals may not necessarily be applicable for livestock. For instance, Cornelius et al. (1959) and Buck et al. (1961) showed that sheep and cattle did not have significant elevations in SGPT activities following acute liver necrosis as do dogs and humans.

Apparently there are no reports on values of transaminase or LDH in CSF taken during and following convulsive seizures in livestock.

D. Glutamic Acid and the Central Nervous System

Glutamic acid is very important in central nervous system metabolism, and since it is the substrate upon which GOT exerts its action, it merits discussion. The evidence that glutamic acid plays a significant role in CNS metabolism is based on the following facts: (1) Glutamic acid and its amide constitute about 40 to 80 percent of the total amino-carboxyl-N of the brain (Quastel and Quastel 1961; Roberts et al. 1958a, 1958b; Weil-Malherbe 1950). (2) Glutamic acid is the common denominator for the buffer system that maintains a balanced protein-carbohydrate metabolism in the CNS (Kini and Quastel 1959; Weil-Malherbe 1950). (3) It is a precursor of γ-aminobutyric acid, an amino acid that has been shown to be important in central nervous system inhibition (Bazemore et al. 1956, 1957; Elliott 1961; Elliott and Jasper 1959; Levin et al. 1961; Purpura et al. 1957a, 1957b; Roberts 1956, 1960; Roberts et al. 1958b; Waelsch 1949). (4) Glutamic acid has been shown clinically to antagonize convulsive seizures of various origin, and has been an effective substitute for glucose in insulin hypoglycemia (DeRopp and Snedeker 1961; Mayer-Gross and Walker 1947; Quastel and Quastel 1961; Tschirgi et al. 1949; Waelsch 1949).

The enzyme, GOT, catalyzes the reversible formation of glutamic acid from α-ketoglutaric and aspartic acids, and is undoubtedly an important link in the dynamic maintenance of glutamic acid in the CNS.

The significance of glutamic acid in the metabolism of central nervous tissue was reviewed by Weil-Malherbe (1950). The principal

chemical reactions of glutamic acid that are known to occur in the brain are: (1) deamination, (2) transamination, (3) amidation and (4) decarboxylation. The decarboxylation of glutamic acid results in the production of γ-aminobutyric acid (GABA). The role of GABA as an inhibitor in the CNS has been reviewed by Elliott and Jasper (1959) and Roberts (1960). These four methods of enzymic transformation of glutamic acid are no doubt vitally related to CNS function. Thus glutamic acid serves as a link among CNS carbohydrate and protein metabolism, the neutralization and removal of intracellular ammonia, and perhaps serves as a precursor of central chemical mediator(s).

E. Cations in the Cerebrospinal Fluid

The ionic composition of the CNS intracellular and extracellular fluid has a definite effect upon brain excitability (Davson 1956; Kini and Quastel 1959; Woodbury and Karler 1960). The effect of changes in CSF concentrations of K^+, Ca^{++} and Mg^{++} has been studied by Leusen in anesthetized dogs (1948a, 1948b, 1950). An excess of K^+ ions caused increased blood pressure and vasomotor reflexes whereas excess Ca^{++} or Mg^{++} resulted in decreased blood pressure and vasomotor reflexes. Absence of or lowered CSF Ca^{++} resulted in increased blood pressure and vasomotor reflexes, while lowered K^+ or Mg^{++} had no effect. There is evidence that an extracellular flux of K^+ is associated with increased brain excitability (Meyer et al. 1961). Krebs and Eggleston (1949) showed that glutamic acid prevented loss of K^+ from brain slices incubated in vitro. However, Brindley

et al. (1960) showed that GABA had very little inhibitory effect on intracellular loss of K^+ in the rabbit cortex.

The ratio of intracellular to extracellular concentrations of Na^+ is also considered to influence brain activity (Fois et al. 1961; Woodbury and Karler 1960). Meyer et al. (1961) measured a decrease in extracellular sodium concomitant with seizure activity in monkeys and cats.

Pappenheimer et al. (1962) breifly reported that ventriculo-cisternal perfusion of goats with an artificial CSF low in Ca^{++} caused a marked hyperexcitability.

Hurley et al. (1963) reported that rats fed a manganese deficient diet showed increased susceptibility to electroshock convulsions.

Several workers have studied the distribution of electrolytes between the plasma and CSF (Davson 1956, Ch. 8). The objective of these investigations was primarily to determine if the transfer of cations from plasma to CSF is an active process involving selective secretion by a blood-brain-CSF barrier or simple diffusion. Kemeny et al. (1961) injected excess Ca^{++}, Mg^{++}, K^+ and Na^+ ions intravenously in dogs but found little or no change in CSF concentrations. This suggested the existence of a selective mechanism which insured the stability of the cation concentrations in the CSF. Visscher and Carr (1944) previously showed that radiosodium in canine plasma slowly equilibrated with the CSF. Their experiments showed that equilibration takes place much slower in unanesthetized animals than in those under pentobarbital anesthesia. Davison et al. (1962) studied the rates of disappearance of Na^{24} and a

variety of substances from the CSF of rabbits. Rates of escape were expressed as the percentage of Na^{24} loss. There seems to be general agreement that cationic transfer between plasma and CSF is an active selective process.

Although considerable research has been directed toward determining the effect of alterations in CSF electrolytes on CNS activity, less emphasis has been given to the study of the effects of brain activity on CSF electrolyte composition. Eiduson et al. (1960) studied spinal fluid constituents following electroconvulsive therapy in 96 mental patients. They found no changes from normal values in Na^+, K^+, Ca^{++} and total protein. Fois et al. (1961) studied the CNS tissue electrolyte pattern in guinea pigs with allergic encephalomyelitis, a condition characterized by high susceptibility to convulsions. They concluded that a depletion of intracellular K^+ and increase in intracellular Na^+ and water content occurred in the cerebral cortex of affected guinea pigs. Seizures and encephalographic abnormalities appeared in strict correlation with marked increases in intracellular sodium. There was no correlation with cellular water increase or potassium decrease.

There is a paucity of information in the literature pertaining to the electrolyte composition of CSF taken serially before, during and following convulsive seizures.

F. Convulsions and Central Nervous System Activity

1. CO_2 convulsions

The effects of inhalation of high concentrations of CO_2 on the CNS

of animals have been studied by Woodbury and Karler (1960) and Woodbury et al. (1958). The inhalation of gas mixtures containing carbon dioxide produces inhibitory or excitatory responses depending on the concentration of CO_2 in the mixtures. Woodbury and Karler (1960) indicated that the action of CO_2 upon the CNS is dependent upon specific electrolyte and amino acid changes. Earlier, Leusen (1954a, 1954b, 1954c), studying the effects of perfusing CO_2-rich solutions into the ventriculo-cisternal system of anesthetized dogs, showed that CO_2 had a direct effect on the respiratory center, causing increased respiratory rate.

Meyer et al. (1961) reported on continuous measurements in cats and monkeys during convulsive seizures. They measured electrocortical activity (EEG), cortical O_2, CO_2, pH, and extracellular Na^+ and K^+. Concurrent measurements were also made on arterial blood pressure, CO_2, O_2, pH, Na^+ and K^+. Convulsive activity was produced by the application of penicillin to the cortex, by insulin hypoglycemia and by trauma. Spike activity was inhibited by producing brain acidity with CO_2 inhalation or by injecting acetazolamide or acid intravenously. Changes accompanying seizure activity included an extracellular flux of K^+ and a reduction of extracellular Na^+ presumably because electrical activity of the brain exceeded the pace at which the sodium pump removed intracellular sodium.

Mullenax (1961) and Mullenax and Dougherty (1963) studied the effects of inhalation of high concentrations of CO_2 in swine and sheep. Convulsions and immobilization were produced by allowing the animals to inhale a mixture

of 68 percent CO_2 plus 32 percent air or O_2. Changes in blood gas concentrations, blood pH, heart rate, blood pressure, respiratory frequency, electrocardiograms, electroencephalograms and muscle activity were measured. Electroencephalograms showed an increased amplitude and decreased frequency of waves during CO_2 inhalation. Return to the normal pattern required one hour. Where CO_2 was administered to animals previously anesthetized with sodium pentobarbital, EEG wave amplitude was markedly reduced during CO_2 inhalation. The effects of CO_2 on the EEG pattern in this case disappeared within 10 minutes.

2. Insulin convulsions

The effects of insulin hypoglycemia on the CNS have been studied in humans and laboratory animals in association with the use of insulin for the treatment of mental diseases. Since the original work of Stief and Tokay (1932), many investigators have observed that severe hyperinsulinism produces marked toxic effects in the CNS (Hassin 1939; Lawrence 1942; Winkelman and Moore 1940).

If the dosage of insulin is great enough to reduce blood sugar markedly below normal, depression and convulsive seizures develop in both normal and diabetic individuals. It is generally believed that the effects on the brain are due to insufficient glucose supply (Hicks 1950, 1953). Others have suggested that the damage may be produced by an accompanying brain hypoxia (Ferris et al. 1941; Liebel and Hall 1938; Tyler 1941; Tyler and Ziskind 1940).

Lesions found following insulin shock convulsions are confined primarily to the CNS. They consist of diffuse and focal types of parenchymatous degeneration of the cortex, hippocampus, basal ganglia, thalamus, medulla, cerebellum and spinal cord (Altschul and Fineberg 1949; Hassin 1939; Winkelman and Moore 1940).

Until recently convulsive seizures had not been produced in sheep and goats by injecting insulin. Bodansky (1924) was unable to cause depression of sheep blood glucose lower than 30 mg percent regardless of the dosage of insulin given. Cutler (1934) noted that large doses of insulin were required to produce shock in goats (4 to 10 units/kg). Symptoms of hypoglycemia were manifested only when the blood sugar was maintained at a level of 10 to 25 mg percent for at least 5 to 8 hours. Salivation, depression and coma, but no convulsive seizures were seen. These investigators injected the insulin intravenously. Others have noted that convulsive seizures were not observed in sheep given insulin intravenously even though severe hypoglycemia was produced (5 to 8 mg percent), (Hitchcock and Phillipson 1946; Reid 1951a, 1951b; Strand et al. 1934). However, when insulin was injected subcutaneously in sheep, Jarrett and Potter (1953) reported severe continuous convulsions. McClymont and Setchell (1955, 1956) also produced convulsive seizures by injecting insulin subcutaneously in sheep at a rate of 5 or 10 units per kg after 4 days of fasting. It is evident, therefore, that some factor or factors other than blood glucose concentration determines the type of symptoms produced in sheep and goats. One factor may be the route of

insulin administration since subcutaneous injections are more likely to produce convulsive seizures than are intravenous injections.

3. Heptachlor convulsions

Convulsive seizures have been produced in sheep and calves by oral and dermal administration of heptachlor (Radeleff et al. 1955). Heptachlor (3a, 4, 5, 6, 7, 8, 8-heptachloro-3a, 4, 7, 7a-tetrahydra-4, 7-methano-indene) is a cyclodiene chlorinated hydrocarbon insecticide, representative of the group which includes aldrin, dieldrin, chlordane, toxaphene and endrin.

Radeleff et al. (1955) and Buck et al. (1959) described the symptoms and lesions observed in animals poisoned by heptachlor. The symptoms almost exclusively involved the CNS, manifested primarily by intermittent clonic-tonic convulsions. Central depression frequently intervened between seizures. Some animals exhibited only severe depression without seizures. Other symptoms that accompanied heptachlor toxicity include extremely high temperature and drooling of high viscosity saliva. Post-mortem examinations of animals poisoned by chlorinated hydrocarbon insecticides, including heptachlor, were characteristically negative for pathognomonic lesions. Hemorrhages and congestion of the parenchymatous organs were commonly seen. The brain frequently showed engorgement of the meningeal vessels and edema.

There is a dearth of information regarding physiological and biochemical changes in the blood and CSF occurring during convulsive seizures produced by chlorinated hydrocarbon insecticides.

III. MATERIALS AND METHODS

A. Animals Used; Their Care and Management

Twenty cross-bred Columbia ewes, 1 to 2 years old, were used in this study. They were housed in an enclosed building at a fairly constant temperature of approximately 72° F. They were fed alfalfa hay and dehydrated alfalfa pellets free choice, plus whole oats during the post-operative recovery period. Water was available at all times.

The sheep were observed daily during the postoperative recovery period and almost continuously during the time of inducement of convulsions. Any abnormal attitude or symptom was noted.

B. Surgical Techniques

1. Cerebrospinal fluid catheterization

Improvements were made in catheter design, surgical technique for placement, and postoperative care over a period of 2 years in an attempt to provide a means for measuring CSF pressure and obtaining samples.

Preliminary studies were conducted on 17 mongrel dogs in an attempt to cannulate their lateral ventricles. It was assumed that changes in the CSF reflecting brain changes would first be evident in ventricular fluid. Unsuccessful attempts to establish permanent indwelling ventricular cannulae were made in 15 dogs before the idea was abandoned. Regardless of the type or design of cannula used or the method used to place it into the lateral ventricle, it would remain functional only a short time. The tips became clogged with the choroid plexus or a fibrin-like membrane. Partially

functional catheters were established in the cisterna magna of two dogs. The remainder of the surgical development and the work reported herein were with adult sheep.

Although the method of placement of a permanent, indwelling cannula in the cisterna magna of sheep was improved upon throughout the course of the investigation, some unsolved problems remain. However, it has proven satisfactory for the continuous measurement of CSF pressure and for serial sampling the CSF during any stage of activity of unanesthetized sheep.

A cannula similar to the one illustrated in Plate 1 was used. It was constructed of vinyl tubing[1] (outside diameter (o.d.) 0.088 inch and inside diameter (i.d.) 0.054 inch). The angled tip and collars were formed by heating the tubing after a stiff wire stilet was inserted into the lumen. After the catheter was formed it was washed, coated with a silicone material[2], and sterilized by autoclaving at 121° C for 20 minutes. Rubber gloves were worn during surgery because it was found that prevention of contamination of the catheter with foreign material was essential. The anchor suture, described below, was 0.3 mm nonabsorbable material[3] (Vetafil). The overall length of the cannula was approximately 6 inches.

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[1]Vinyl IV tubing, Cat. No. PV-400. Clay-Adams Inc., 141 E. 25 Street New York 10, N. Y.

[2]Siliclad(R), Clay-Adams Inc.

[3]Vetafil(R), Dr. S. Jackson, importer, Washington 11, D. C.

Each sheep was held off feed 12 hours prior to surgery. A few minutes before induction of anesthesia, atropine was injected intramuscularly at a rate of approximately 1/4 mg/kg to reduce saliva accumulation in the anesthesia system. The wool on the head and neck was clipped. Induction of anesthesia was accomplished by intravenous injection of thiamylal sodium[1] to effect. A cuffed endotracheal catheter was passed into the trachea and connected to an anesthesia machine utilizing a mixture of cyclopropane and oxygen. Anesthesia was maintained with a 1:4 mixture of these gases using a closed circle system. The sheep was positioned on its sternum and the surgical site scrubbed with a brush using a disinfectant detergent. All instruments, towels and drapes were autoclaved.

A 3-inch skin incision was made on the dorsal midline of the neck beginning at the external occipital protuberance and continuing caudally immediately over the ligamentum nuchae (Plate 1). The subcutaneous fat was divided and the ligamentum nuchae exposed. The two tendons of the ligamentum nuchae were retracted, exposing the muscles that attach to the occipital bone and atlas. These muscles were bluntly dissected from the occiput and atlas with a periosteal elevator, exposing the median occipital crest down to the alanto-occipital membrane. The muscles were dissected from this tough membrane leaving it exposed over the cisterna magna. The sheep's head was then flexed as much as possible.

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[1] Surital(R), Parke, Davis & Co., Detroit, Michigan.

A small incision was made in the atlanto-occipital membrane using a pointed scalpel. It was important that this be made exactly in the center of the triangle bounded by the "V" shaped articular surfaces of the atlas and the caudal point of the median occipital crest. Otherwise, blood vessels were invariably severed and the profuse bleeding was very difficult to control. A small hemostat was inserted through the incision in the atlanto-occipital membrane and spread to enlarge the opening. After enlargement of the incision to approximately 1/4 inch in diameter, the hemostats were used to gently extract the epidural fat, exposing the dura mater over the cisterna magna. The free ends of the anchor suture, which was attached to the upper collar of the catheter, were then loosely placed in the atlanto-occipital membrane, one on each side of the incision. With the head flexed and the dura over the cisterna magna exposed, a sterile 24 ga. needle attached to 10 cc syringe was used to withdraw as much CSF as possible. Usually, 5 to 8 cc were withdrawn. Using 3-inch artery forceps and a pointed scalpel the puncture hole in the dura was enlarged to allow the passage of the catheter. The catheter tip was then inserted through the hole in the dura and directed forward into the cisterna magna until the first collar rested against the dura mater. The anchor sutures were then tightened securing the second collar to the atlanto-occipital membrane. After placing antibiotics in the incision, the retractors were released allowing the tendons of the ligamentum nuchae to return together, and the skin was closed with mattress sutures using 0.40 mm Vetafil. All but 1 ml of the CSF previously withdrawn from the cisterna

magna was replaced through the catheter. The 1 ml retained was used for biochemical analysis and served as a control sample.

A metal Luer-lock adapter[1] was placed on the external end of the catheter to facilitate its attachment to a pressure transducer or Luer-lock syringe. When not in use, the adapter was capped and placed in the pocket of a specially made muslin collar worn by the sheep at all times.

2. Carotid artery catheterization

A catheter representative of those used to cannulate the carotid artery of sheep is pictured in Plate 1. It was made of polyethylene No. 100 tubing[1] (o.d. = 0.060 inch; i.d. = 0.034 inch). A collar was formed approximately 8 inches from one end by heating the tubing over a small flame. A stilet made of piano wire was inserted in the lumen to prevent its collapse when the tubing was heated.

The catheter was placed in the left carotid artery immediately following the placement of the cisternal catheter. The sheep was positioned on its right side, the skin disinfected, and drapes were placed over the surgical site. A skin incision was made just below the level of the jugular vein beginning at the ramus of the mandible and extending caudally 2 to 3 inches (Plate 1). The left carotid artery was exposed by blunt dissection usually disclosing two small branches about 1 to 2 inches posterior to the ramus of the mandible. The largest of these two branches, considered to be the thyrolaryngeal, was ligated approximately 1 inch from

[1]Clay-Adams, Inc., New York, N. Y.

the carotid artery. After temporarily ligating the main carotid trunk both anterior and posterior to the origin of the branch, a small incision was made in the branch about 1/2 inch from its origin. The catheter was passed through the incision into the branch and down the carotid artery toward the heart as far as the collar would allow (approximately 8 inches). The temporary ligatures were removed from the main trunk immediately before the catheter was passed allowing the blood to flow freely through the carotid artery. A size 0.30 mm Vetafil ligature was anchored to the collar and used to ligate the thyrolaryngeal branch containing the catheter immediately proximal to the collar. The anchor ligature was then passed through muscle and fascia to stabilize the catheter. Antibiotics were placed in the incision and the wound closed with mattress sutures using 0.4 mm Vetafil. Physiological saline solution containing .05 percent heparin was flushed through the catheter and a Luer-lock adapter and valve were fixed to the external end to facilitate connection to a pressure transducer or syringe. When not in use the adapter was capped and placed in a pocket on the muslin collar.

C. Postsurgical Care

The day of surgery and each day thereafter for 2 to 3 days each sheep was given intramuscular injections of penicillin and streptomycin (1,000,000 units of penicillin plus 1 gm. streptomycin) and the bandage was changed and body temperature recorded at this time. Beginning about the third day following surgery and every day or so thereafter, samples of CSF and blood were taken for biochemical analyses. Each sheep was

usually sufficiently recovered to be placed on experiment within 10 days following surgery.

D. Inducement of Convulsive Seizures

1. Carbon dioxide

A mixture of 68 percent carbon dioxide (CO_2) and 32 percent oxygen (O_2) was prepared by passing the gases from their respective cylinders through a wet-test meter[1] into a large spirometer.[2] Convulsive seizures were induced by the administration of the CO_2-O_2 mixture from the spirometer through a face mask attached to a previously described one-way breathing valve (Mullenax and Dougherty 1963). The breathing valve was connected to the spirometer by flexible tubing. The sheep were confined to a small crate during the CO_2 inhalation to facilitate the recording of CSF and blood pressures and respiratory rate during seizures. The gas mixture was administered to each animal for approximately 6 minutes.

Convulsive seizures followed by immobilization were produced in 6 sheep. Each sheep served as its own control, being subjected to the identical conditions as during the inhalation of the CO_2-O_2 mixture except that the spirometer was filled with room air.

In both the control and seizure experiments recordings were made of CSF pressure, blood pressure and respiratory rate. They were recorded

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[1]Precision Scientific Co., Chicago, Ill.

[2]Chain Compensated Gasometer (600 liter), W. E. Collins, Inc. Boston, Mass.

before placing the mask on the sheep, continuously during CO_2-O_2 inhalation, and intermittently for 2 to 4 hours following seizure production.

Blood and CSF samples were taken simultaneously from their respective catheters before applying the face mask, and at 1, 6, 15, 30, 60, and 120 or 240 minutes after beginning gas inhalation.

2. Insulin

Convulsive seizures were induced in 5 sheep by subcutaneous injections of insulin following 1 to 2 days of fasting. Three other sheep were fasted and served as controls. Ten units of regular insulin[1] per kilogram body weight were given either as a single dose or in divided doses. Blood pressure, CSF pressure and respiratory rate recordings were made and samples were taken from each sheep before and during convulsive seizures. The control animals were treated similarly.

3. Heptachlor

Seizures were induced in 4 sheep by oral administration, via stomach tube, of a 25 percent emulsifiable concentrate of heptachlor diluted with an equal volume of tap water. Doses from 150 to 300 mg/kg heptachlor were given. As in the insulin and CO_2 experiments, CSF pressure, blood pressure and respiratory rate recordings were made and samples were taken before, during and following seizures if the sheep survived.

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[1]Lente(R), 80 units per ml, Eli Lilly Co., Indianapolis, Ind.

E. Analyses of Blood and Cerebrospinal Fluid

The blood and CSF samples taken simultaneously from the sheep were subjected to determinations described below.

1. Glucose

Glucose was determined by the glucose oxidase method first proposed by Keston (1956) and developed by Teller (1956) using commercial reagents.[1] Duplicate determinations were made on heparinized blood. The filtrate was made by mixing 0.4 ml of blood, 3.6 ml distilled water, 2.0 ml of a 5 percent $BaSO_4$ solution and 2.0 ml of a 4.5 percent $ZnSO_4$ solution. This mixture was filtered through Whatman No. 1 paper. One ml of filtrate was incubated at 37° C with 4 ml of the reagent for 30 minutes. The reagent was prepared by diluting the powdered material in the chromogen and Glucostat vials to 80 ml with distilled water. After incubation, a drop of 0.4 N HCl was added and immediately mixed. After 5 minutes the optical density was measured at 400 mμ wavelength in a spectrophotometer.[2] Duplicate standards were prepared with each group of determinations.

Single determinations were made on the CSF using 0.1 ml fluid, 1.9 ml water, 1.0 ml BaOH, and 1.0 ml $ZnSO_4$ to produce the filtrate. Two (2) ml of filtrate were incubated with 2 ml of reagent. The reagent was prepared by diluting the Glucostat and chromogen to 50 ml with water. As with the blood glucose, duplicate standards were prepared with each group of determinations.

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[1] Glucostat Special(R), Worthington Biochemical Corp., Freehold, N. J.

[2] Beckman DU, Beckman Instruments Co., Fullerton, Calif.

2. Transaminase

Serum glutamic- and pyruvic-oxalacetic transaminase activities were determined by the method described by Reitman and Frankel (1957) using commercially prepared reagents[1] and Sigma instructions (1960). The 0.4 N NaOH was prepared in our laboratory. Cerebrospinal fluid activities were determined using one-half the recommended volumes.

3. Lactic dehydrogenase (LDH)

Serum and CSF LDH activities were determined by the colorimetric method described by Berger and Broida (1960) using commercially prepared reagents.[1]

4. Electrolytes

Serum and CSF sodium and potassium values were determined by flame photometry.[2] Samples were prepared in a 1:100 dilution, using a protein precipitant consisting of 5 percent trichloroacetic acid (TCA) and 10 percent isopropyl alcohol in distilled water as described by Beckman Instruments Co. (1957). Calibration curves were constructed from mock serum working standards prepared as recommended by Teloh (1959) and diluted in the same manner as the samples.

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[1]Sigma Chemical Co., St. Louis 18, Mo.

[2]Beckman Model B Spectrophotometer, Beckman Scientific Instruments Division, Fullerton, Calif.

Calcium and magnesium values were determined with a fluorometer.[1, 2]

Calcium values were determined using the method described by the Turner Company, which is based on a paper by Kepner and Hercules (1963). The method is based on the principle that the indicator, Calcein,[3] combines with Ca^{++} at pH 12 or above and will fluoresce when activated with ultraviolet light. The fluorescence is then quantitated with the fluorometer. At this pH Mg^{++} and proteins do not interfere with the determination. Glassware must be absolutely clean and free of Ca^{++}. Deionized or redistilled water must be used. The determination requires an amount of serum, CSF, or standard that contains from 1 to 5 µg of Ca^{++}. This is usually 10 to 20 µl of serum or CSF. Standards were prepared by dissolving analytical grade $CaCO_3$ in hydrochloric acid and diluting with water. A Calcein stock solution was prepared by dissolving 90 mg Calcein in 500 ml propylene glycol. A 0.5 N KOH solution was prepared and 0.2 ml of a .002 M ethylenediaminetetraacetate (EDTA) solution was added per liter to bind the Ca^{++} present in the reagents, thus reducing the blank fluorescence. Serum or CSF was diluted 1:10 or 1:100 in a TCA-isopropyl alcohol solution (5 percent TCA; 10 percent alcohol; water), mixed, and centrifuged. Duplicate aliquots of the supernatant each representing 10 to 20 µl of serum or CSF were pipetted into clean, new test tubes that

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[1]Appreciation is expressed to Drs. Harvey Diehl and Gerald Spielholtz, Department of Chemistry, Iowa State University, for furnishing the reagents and assisting in adapting these methods for serum and CSF analyses.

[2]Model 110 Fluorometer, G. K. Turner Assoc., Palo Alto, Calif.

[3]G. Frederick Smith Chemical Co., Columbus, Ohio.

were washed in tap water and rinsed 3 times in demineralized glass-distilled water. Ten ml of redistilled water were added and mixed mechanically[1] followed by 5 ml of a KOH-Calcein working solution. The latter solution was made by adding the Calcein stock solution to the 0.5 N KOH solution at the ratio of 1 to 10. After adding the KOH-Calcein solution, the sample was again mixed and transferred to clean, new test tubes (13 x 100 mm) which were used for cuvettes. The fluorescence was measured within 8 to 10 minutes as the intensity of fluorescence was not stable past that time. Standards and a blank were included with each group of determinations and were treated in the same manner as samples. Serum and CSF Ca^{++} values were estimated by comparing their intensity of fluorescence with the standard curve.

Magnesium values were determined using the method described by Diehl et al. (1963). In this procedure o,o'-dihydroxyazobenzene[2] combines with Mg^{++}, but not with Ca^{++}, at pH 10 or above (optimum pH 11.0) to form a compound that fluoresces. The intensity of fluorescence is directly proportional to the quantity of Mg^{++} present in the sample. The fluorescence is stable indefinitely, but the intensity is increased when the sample is cooled.

A stock solution of o,o'-dihydroxyazobenzene (approx. .0025 M) was prepared by dissolving 0.535 gm of the crystalline reagent in a mixture

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[1] Vortex Junior Mixer, Scientific Industries, Inc. Queens Village, N. Y.

[2] G. Frederick Smith Chemical Co., Columbus, Ohio.

of 10 ml of ethanol and 10 ml of 2 M potassium hydroxide, and diluting to 1 liter with deionized water.

A working solution was prepared by mixing the following: 200 ml stock o,o'-dihydroxyazobenzene solution, 106 ml HCl (52.5 ml concentrated HCl diluted 1:1 with deionized water), 100 ml triethanolamine, and 134 ml redistilled ethylenediamine; this mixture was diluted to 2 liters with 95 percent ethanol.

The standards and samples (containing 0.5 to 20 µg magnesium) were placed in clean 15-ml graduated centrifuge tubes. Ten (10.0) ml of working solution were added followed by deionized water at a quantity sufficient to make a total of 14 ml. The tubes were capped with plastic stoppers and inverted to mix the contents. If whole serum were used, the resulting precipitate was removed by centrifugation. The intensity of fluorescence was usually measured within 4 hours and never later than within 24 hours (excitation filter, 470 mµ peak; fluorescence filter, 580 mµ peak). To estimate the Mg^{++} content the intensity of fluorescence of the samples was compared to a standard curve which was prepared with each group of determinations. Duplicate determinations were made on each sample.

5. Total cerebrospinal fluid protein

Total CSF protein content was measured using the method of Lowry et al. (1951) as modified by Spolter and Thompson (1962). One-tenth (0.1) ml of CSF was used. Standard beef albumin was used for standards which were made up and frozen. Duplicate standards were thawed and used with each group of determinations.

6. pH

Blood and CSF pH were determined in the CO_2 experiments using a method previously described by Mullenax and Dougherty (1963).

7. Blood packed cell volume

Blood packed cell volume (PCV) was determined in the CO_2 experiments with the method of McGovern et al. (1955) using a micro-capillary centrifuge and micro-capillary tube reader.[1]

F. Recording of Physiological Data

Carotid artery blood pressures and CSF pressures were recorded by connecting the respective catheters to a pressure transducer.[2] The transducer used for measuring CSF pressure was mounted to the animal's head at the level of the cisterna magna. The transducers were connected to a polychannel direct writing recorder.[3] Respiratory rate was measured using a pneumograph.

Carotid blood pressure, CSF pressure in the cisterna magna and respiratory rate were recorded before, during and following the induction of convulsive seizures.

G. Postmortem Examination

Postmortem examinations were made on 13 sheep. The operative site and position of the catheter were specifically examined. The brain and

[1]International Centrifuge, Model MB, and Reader, Model CR, International Equipment Co., Boston 35, Mass.

[2]Pressure Transducer, Model 267B, Sanborn Company, Waltham 54, Mass.

[3]Sanborn Recorder, Model 350.

upper portion of the cord were removed and immediately placed in a 25 percent formalin solution. After fixation the brain was sectioned, stained with hematoxylin and eosin, and examined for histopathologic changes.[1] The following areas were examined: medulla, pons, inferior and superior colliculi, thalamus, caudate nucleus, lenticular nucleus, visual cortex, hippocampus, frontal lobe, and cerebellum.

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[1]Appreciation is expressed to Drs. Randall C. Cutlip and William S. Monlux, Pathological Investigations, National Animal Disease Laboratory, for making the histopathological examinations of the brains.

IV. RESULTS AND DISCUSSION

A. Surgery

1. Cerebrospinal fluid catheter

Catheters were placed in the CSF space of 19 sheep by the method described. Twelve were completely functional, 1 died apparently of meningitis, 3 became nonfunctional during the postsurgical recovery period, and 3 were only partially open during the recovery period but were placed on experiment. The sheep were considered to have recovered from the surgery when they appeared clinically normal and their CSF levels of protein, glucose and GOT returned to near control values. This usually occurred about 1 week following surgery, although protein and GOT levels often remained somewhat higher than control values. No attempt was made to determine how long the catheters would remain open and functional. However, some catheters remained open for at least 30 days. Others became closed a few days after seizure activity had been induced which apparently was a result of physical damage in the vicinity of the catheter tip due to the violent action associated with seizures.

In the first 13 sheep .05 percent heparinized[1] physiological saline was flushed into the catheter to prevent clotting. The heparin contained phenolic preservatives. This practice was discontinued because it became apparent that the heparin or the phenolic compounds were acting as an irritant causing meningitis and elevated CSF protein levels which may have contributed to the formation of a membrane over the tip of the catheter.

[1] Panheprin(R), Abbott Laboratories, North Chicago, Ill.

In some sheep in which the CSF catheter had become closed, a mixture of streptokinase, streptodornase, and human plasminogen[1] was injected into the catheter to depolymerize the fibrin clogging the catheter tip. Although this often cleared the catheter temporarily, the protein content of the CSF remained very high and the catheter became closed within a day or two. There was also evidence of severe pain to the sheep when the enzyme was injected into the CSF space. This practice was also discontinued.

There were no untoward clinical effects from the CSF catheters except in 2 sheep in which catheters were placed too far down into the cisterna magna, thus producing pressure on the spinal cord. In these sheep unilateral partial paralysis of the limbs occurred.

2. Carotid artery catheter

Catheters were placed in the left carotid arteries of 10 sheep via the thyrolaryngeal branch. Carotid arteries of the other sheep in these experiments were catheterized by tying off the carotid and inserting the cannula caudally pasts its bifurcation. The latter method was abandoned in favor of the first because it seemed inadvisable to study brain metabolism with one carotid ligated.

This method of catheterization, which allows the carotid artery to remain patent, proved highly satisfactory in this study. There was no problem of clogging with clotted blood. The lumen of the catheter (i.d. = 0.034 inch) was large enough to permit sampling and recording of blood pressure, although the small size of the catheter may have produced

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[1]Varizyme(R), American Cyanamid Company, Princeton, N. J.

slightly inaccurate pressure recordings and rounding of peak pressure tracings. However, the advantages of this technique far outweigh these disadvantages.

B. Control and Postsurgical Data

1. Cerebrospinal fluid and blood analyses

a. Presurgical Cerebrospinal fluid was taken from the cisterna magna of 9 sheep during cyclopropane anesthesia before surgical placement of the catheter. The results of biochemical analyses are presented in detail in Table 1. The following mean values were obtained: glucose 46 mg/100 ml; GOT 15 units/ml; GPT 12 units/ml; LDH 15 BB units/ml; total protein 29.5 mg/100 ml; Na^+ 154 meq/l; K^+ 3.41 meq/l; Ca^{++} 7.57 meq/l; and Mg^{++} 3.40 meq/l.

The results of analyses of venous blood serum taken from 4 sheep just prior to surgical placement of catheters and from 17 sheep held in the National Animal Disease Laboratory stock colony are given in Table 2. The following mean values were obtained: SGOT 79 units/ml; SGPT 17 units/ml; LDH 979 BB units/ml; Na^+ 144 meq/l; K^+ 4.65 meq/l; Ca^{++} 10.21 meq/l; and Mg^{++} 2.02 meq/l.

b. Postsurgical Results of biochemical analyses of CSF taken from sheep after recovering from surgery and prior to inducement of convulsive seizures are given in Table 3. The following mean values were obtained: glucose 44 mg/100 ml; GOT 28 units/ml; GPT 17 units/ml; LDH 29 BB units/ml; total protein 78.5 mg/100 ml; Na^+ 156 meq/l; K^+ 3.34 meq/l; Ca^{++} 8.12 meq/l and Mg^{++} 4.18 meq/l. Corresponding values for arterial

Table 1. Results of analyses of control CSF taken from 9 sheep

Sheep No.	15	16	17	18	19	20	21	22	23	Mean & S.D.
Glucose mg/100 ml	--	43	33	69	59	39	33	45	45	46 ± 12.5
GOT units/ml	35	15	15	16	27	8	6	7	10	15 ± 9.7
GPT units/ml	--	5	18	--	24	11	14	8	7	12 ± 6.9
LDH BB units/ml	--	--	--	--	8	15	18	18	--	15 ± 4.8
Protein mg/100 ml	32.8	29.1	21.6	31.0	27.7	34.5	31.4	28.3	28.9	29.5 ± 3.8
Na^+ meq/l	160	159	155	153	163	147	153	143	150	154 ± 6.4
K^+ meq/l	3.95	3.45	2.98	3.45	3.48	3.16	3.50	3.46	3.30	3.41 ± 0.25
Ca^{++} meq/l	7.00	8.36	8.16	8.18	8.98	7.26	7.14	7.37	5.72	7.57 ± 0.97
Mg^{++} meq/l	2.68	3.60	3.36	4.62	4.36	3.48	3.10	2.48	1.92	3.40 ± 0.95

Table 2. Results of analyses of venous blood serum taken from 4 sheep prior to surgery and from 17 normal sheep in the NADL stock colony

Sheep No.	SGOT units/ml	SGPT units/ml	LDH BB units/ml	Na^+ meq/l	K^+ meq/l	Ca^{++} meq/l	Mg^{++} meq/l
19	--	--	730	152	4.80	8.34	2.58
21	109	18	640	163	4.90	10.28	--
22	46	11	810	148	5.20	13.16	4.62
23	84	12	--	134	3.75	--	1.54
Stock colony sheep							
4	93	15	885	140	4.60	10.00	2.20
6	73	19	1155	147	5.14	14.26	1.88
7	70	20	1195	135	4.60	9.40	1.82
8	69	15	900	130	4.25	6.60	2.06
9	65	16	1160	136	4.45	12.36	1.76
10	64	14	750	143	4.70	11.16	2.02
15	107	22	1515	143	4.90	11.58	1.94
19	87	16	1920	132	4.15	8.42	1.68
27	79	20	835	146	4.78	7.66	1.96

Table 2. (Continued)

Sheep No.	SGOT units/ml	SGPT units/ml	LDH BB units/ml	Na^+ meq/l	K^+ meq/l	Ca^{++} meq/l	Mg^{++} meq/l
40	80	16	1025	142	5.40	9.88	1.58
41	70	18	1140	140	4.18	11.60	1.68
42	85	18	880	135	4.20	7.26	1.76
45	108	17	645	156	4.70	10.88	1.70
46	63	17	1030	144	4.60	9.16	1.78
47	102	15	940	155	4.70	9.18	2.18
55	42	18	555	156	5.00	10.60	1.86
74	85	13	875	142	4.60	12.56	1.86
Mean & S.D.	79 ± 19	17 ± 3	979 ± 318	144 ± 8.9	4.65 ± 0.40	10.21 ± 2.02	2.02 ± 0.66

Table 3. Results of biochemical analyses of postsurgical CSF

Sheep No.	Glucose mg/100 ml	GOT units/ml	GPT units/ml	LDH BB units/ml	Protein mg/100 ml	Na^+ meq/l	K^+ meq/l	Ca^{++} meq/l	Mg^{++} meq/l
4	40 (3)[a]	35 (3)	16 (3)	--	100.2 (3)	146 (2)	2.74 (3)	--	--
5	28 (2)	24 (2)	13 (2)	--	131.0 (1)	168 (2)	3.08 (2)	--	--
6	22 (3)	22 (3)	17 (3)	--	--	163 (2)	2.53 (2)	--	--
7	34 (2)	28 (4)	22 (4)	--	65.8 (1)	153 (2)	2.89 (2)	--	--
8	40 (1)	30 (2)	8 (2)	--	86.5 (2)	137 (2)	2.73 (2)	--	--
9	49 (6)	27 (2)	17 (2)	--	109.0 (1)	156 (3)	4.09 (3)	--	--
10	53 (3)	38 (4)	17 (4)	--	--	153 (2)	4.38 (2)	--	--
11	45 (2)	--	--	--	--	150 (3)	3.03 (5)	7.40 (1)	3.36 (2)
12	45 (1)	--	--	--	46.6 (1)	--	2.85 (2)	7.12 (1)	4.00 (2)
13	32 (1)	--	--	--	--	--	3.23 (1)	--	--
15	52 (1)	16 (1)	--	--	46.6 (1)	154 (1)	3.85 (1)	8.12 (2)	5.78 (1)
16	47 (2)	15 (4)	10 (3)	--	41.0 (4)	155 (3)	3.41 (3)	9.26 (2)	4.84 (3)
17	42 (7)	46 (4)	22 (2)	39 (3)	54.3 (4)	163 (8)	3.70 (8)	8.38 (6)	3.74 (7)
18	46 (1)	44 (2)	28 (2)	--	106.3 (2)	--	3.38 (2)	9.04 (1)	3.86 (1)
19	55 (4)	36 (3)	15 (3)	13 (2)	57.1 (3)	156 (3)	3.36 (3)	8.42 (3)	3.68 (3)
21	59 (2)	9 (2)	18 (2)	22 (2)	95.3 (2)	161 (2)	3.95 (2)	7.80 (2)	--
22	54 (4)	24 (4)	14 (3)	41 (3)	80.8 (4)	166 (4)	3.57 (4)	7.40 (4)	--
Mean & S.D.	44 ± 10.2	28 ± 10.9	17 ± 5.3	29 ± 12.3	78.5 ± 29	156 ± 8.4	3.34 ± 0.53	8.12 ± 0.75	4.18 ± 0.84

[a]Number of samples analyzed.

blood taken after recovery from surgery and prior to inducement of convulsive seizures are given in Table 4. The following mean values were obtained: glucose 55 mg/100 ml; SGOT 76 units/ml; SGPT 13 units/ml; LDH 721 BB units/ml; Na^{+} 146 meq/l; K^{+} 4.37 meq/l; Ca^{++} 8.60 meq/l; and Mg^{++} 2.83 meq/l. These presurgical and postsurgical CSF and blood data are presented graphically in Figures 8, 9 and 10.

2. Recordings of physiological data

Physiological data recorded following recovery from surgery and prior to inducement of convulsive seizures are given in Table 5. The following mean values were obtained: average blood pressure 105 mm Hg; systolic blood pressure 124 mm Hg; diastolic blood pressure 92 mm Hg; arterial pulse pressure 33 mm Hg; average CSF pressure 7.9 mm Hg; systolic CSF pressure 8.8 mm Hg; diastolic CSF pressure 6.9 mm Hg; CSF pulse pressure 2.1 mm Hg; heart rate 85 per minute; CSF rate 85 per minute; and respiratory rate 58 per minute. A representative tracing of recorded blood pressure, CSF pressure and respiratory rate is given in Figure 1. This control recording was taken from a sheep while standing. It was observed that arterial pulse, rather than respiration, influenced CSF pulse in a quietly standing sheep. However, when the sheep was lying down or was breathing from a spirometer, a secondary CSF pressure change was correlated with respiration (cf. Figures 5 and 2, respectively). The mean CSF pressure was markedly lower in the recumbent animal than in the standing animal.

Table 4. Results of biochemical analyses of postsurgical arterial blood

Sheep No.	Glucose mg/100 ml	SGOT units/ml	SGPT units/ml	LDH BB units/ml	Na^+ meq/l	K^+ meq/l	Ca^{++} meq/l	Mg^{++} meq/l
3	42 (2)[a]	49 (2)	10 (2)	--	--	--	--	--
4	50 (2)	53 (2)	10 (2)	--	--	--	--	--
5	73 (2)	54 (2)	14 (2)	--	--	--	--	--
6	47 (3)	45 (1)	11 (1)	--	--	--	--	--
7	47 (2)	64 (3)	7 (3)	--	--	--	--	--
8	--	101 (1)	10 (1)	--	--	--	--	--
9	65 (3)	63 (3)	8 (3)	--	--	--	--	--
10	70 (2)	69 (3)	13 (3)	--	--	--	--	--
11	72 (1)	81 (1)	10 (1)	--	120 (1)	2.60 (1)	--	--
12	58 (1)	91 (1)	7 (1)	--	155 (2)	4.40 (2)	--	--
13	--	--	--	--	140 (1)	4.88 (1)	--	--
15	57 (1)	120 (1)	18 (1)	--	149 (2)	4.08 (2)	--	--
16	49 (2)	72 (2)	14 (2)	--	132 (2)	3.78 (2)	--	--
17	57 (8)	72 (8)	17 (8)	602 (3)	144 (4)	4.32 (4)	8.48 (1)	2.38 (1)
18	52 (2)	80 (2)	13 (2)	--	152 (1)	4.58 (1)	--	--
19	59 (3)	117 (2)	19 (2)	698 (2)	155 (3)	5.34 (3)	8.52 (3)	2.64 (3)
21	42 (2)	50 (2)	12 (2)	685 (2)	156 (2)	4.88 (2)	9.40 (2)	3.82 (2)
22	41 (4)	118 (4)	22 (4)	898 (4)	160 (4)	4.83 (4)	8.20 (4)	2.48 (4)
Mean & S.D.	55 ± 10.7	76 ± 25.1	13 ± 4.4	721 ± 126	146 ± 12.5	4.37 ± 0.77	8.60 ± 0.52	2.83 ± 0.67

[a]Number of samples analyzed.

Table 5. Blood, CSF, and respiratory data recorded from sheep following recovery from surgery and prior to inducement of convulsive seizures

Sheep No.	Arterial blood pressure (mm Hg)				CSF pressure (mm Hg)				Heart rate per min.	CSF rate per min.	Resp. rate per min.
	Avg.	Syst.	Diast.	Pulse[a]	Avg.	Syst.	Diast.	Pulse			
3	93 (3)[b]	118 (3)	83 (3)	35 (3)	6.3 (3)	6.8 (3)	5.8 (3)	0.95 (3)	73 (3)	73 (3)	26 (3)
4	108 (4)	135 (4)	95 (4)	40 (4)	5.5 (4)	6.7 (4)	4.9 (4)	3.30 (4)	81 (4)	81 (4)	37 (4)
5	101 (2)	117 (2)	86 (2)	32 (2)	6.3 (2)	6.8 (2)	4.6 (2)	2.20 (2)	102 (2)	102 (2)	72 (2)
6	92 (2)	105 (2)	81 (2)	24 (2)	7.0 (2)	7.4 (2)	6.6 (2)	0.75 (2)	96 (2)	96 (2)	51 (2)
7	107 (3)	125 (3)	85 (3)	40 (3)	4.2 (3)	5.0 (3)	3.9 (3)	1.13 (3)	64 (3)	64 (3)	20 (3)
8	93 (3)	110 (3)	78 (3)	32 (3)	8.9 (3)	9.9 (3)	8.0 (3)	1.87 (3)	92 (3)	92 (3)	87 (3)
9	98 (7)	115 (7)	85 (7)	30 (7)	12.9 (7)	14.4 (7)	12.1 (7)	2.00 (7)	67 (7)	67 (7)	86 (7)
10	122 (3)	135 (3)	108 (3)	27 (3)	7.6 (3)	9.0 (3)	5.5 (3)	3.53 (3)	88 (3)	88 (3)	88 (3)
11	--	--	--	--	--	9.0 (1)	8.0 (1)	1.00 (1)	101 (3)	101 (3)	66 (1)
12	--	153 (4)	116 (4)	34 (4)	--	6.6 (4)	5.4 (4)	1.20 (4)	79 (4)	79 (4)	150 (4)
13	104 (1)	120 (2)	95 (2)	25 (2)	10.4 (1)	12.0 (1)	9.0 (1)	3.00 (1)	81 (2)	81 (2)	72 (2)
15	111 (3)	126 (2)	96 (2)	30 (2)	5.1 (2)	6.6 (2)	4.0 (2)	2.60 (2)	82 (2)	82 (2)	56 (2)
16	140 (1)	162 (1)	116 (1)	46 (1)	9.0 (1)	10.0 (1)	8.0 (1)	2.00 (1)	102 (1)	102 (1)	18 (1)
19	96 (1)	116 (1)	84 (1)	32 (1)	14.0 (1)	16.0 (1)	13.0 (1)	3.00 (1)	78 (1)	78 (1)	27 (1)
21	107 (2)	121 (2)	92 (2)	32 (2)	4.0 (2)	4.6 (2)	3.4 (2)	1.20 (2)	68 (2)	68 (2)	40 (2)
22	93 (2)	108 (2)	79 (2)	29 (2)	8.7 (2)	10.2 (2)	7.6 (2)	3.10 (2)	98 (2)	98 (2)	39 (2)
Mean & S.D.	105 ± 12	124 ± 16	92 ± 13	33 ± 6	7.9 ± 3.0	8.8 ± 3.2	6.9 ± 2.8	2.1 ± 1.0	85 ± 13	85 ± 13	58 ± 34

[a]Pulse pressures were obtained by direct measurements from tracings, which may account for the values not equalling systolic minus diastolic pressures.

[b]Number of recordings.

3. Postmortem examination

Examination of the brains of 13 sheep that had CSF and carotid catheters revealed a chronic diffuse lymphocytic meningioencephalitis. An occasional animal had a slight suppurative cellular reaction in the meninges. The brains from sheep catheterized early in the study showed the most severe reaction. Reactions were much less severe in those animals catheterized after the use of heparin in the CSF catheter was discontinued. Plate 2 contains photomicrographs of brain sections from 2 sheep in which heparin was injected into the CSF catheters and a comparative section from the brain of a sheep in which no heparin was used. Elevations of protein content in the CSF correlated with the severity of meningitis. Table 6 contains the postmortem findings in the brains of 13 sheep that died during experiments or that were euthanatized following an experiment.

Although the severity of meningitis was mild in those animals examined in the latter part of this study, all animals examined exhibited some meningitis. It is probable that some reaction was induced by the catheter itself. There appeared to be a mild foreign body reaction in the CNS to the vinyl catheter. Catheters made of a silicone-rubber combination[1] have been used to a limited extent and show promise.

C. CO_2 Convulsions

Inhalation of the CO_2-O_2 mixture usually induced tonic seizures in the sheep within 30 seconds and collapse occurred within 1 minute. Tonic-clonic

[1] Silastic(R), Dow-Corning Center for Aid to Medical Research, Midland, Mich.

Table 6. Postmortem changes in the brains of sheep with CSF and carotid artery catheters

Sheep No.	Treatment	Postmortem Changes
3	Control - Insulin convulsion experiment. Fasted 2 1/2 days, sacrificed by injecting $MgSO_4$ in carotid.	Severe acute diffuse lymphocytic meningitis of entire brain, slight to moderate lymphocytic perivascular cuffing throughout, especially the cerebellum and medulla oblongata. No neuronal damage was found.
4	14 units regular insulin in divided doses. Died after 12 hours of convulsive seizures.	Slight lymphocytic perivascular cuffing along a line parallel to the external capsule at the level of the caudate and lenticular nuclei and corpora quadrigemina. Slight increase in lymphocytes, neutrophils and eosinophils was present in the meninges at level of the medulla oblongata. No neuronal damage was found.
5	Control - Insulin convulsion experiment. Fasted 4 days. Sacrificed by injecting $MgSO_4$ in carotid.	Perivascular cuffing, primarily lymphocytic with an occasional neutrophil, and a generalized hyperplasia of glial cells was observed in all sections of the brain. Severe lymphocytic meningitis was also present.

Table 6. (Continued)

Sheep No.	Treatment	Postmortem changes
6	10.5 units insulin in divided doses. Died after being in convulsions 18 hours.	Generalized, subacute, diffuse, suppurative meningitis and encephalitis, with lymphocytes and neutrophils the primary infiltrating cells. No neuronal damage was evident.
7	10.5 units insulin in divided doses. Convulsions, chewing movements, stupor followed by partial recovery after 1 1/2 days. Sacrificed.	Generalized inflammation of the brain, primarily lymphocytic in anterior portion and suppurative in the posterior portion. Glial cell hyperplasia was evident. Meningitis and perivascular cuffing, primarily lymphocytic, was evident throughout. There was a focus of myelin degeneration in the white matter of the cerebrum.

Table 6. (Continued)

Sheep No.	Treatment	Postmortem changes
8	10 units/kg insulin, convulsions ceased after injecting 3 doses of 90 mg DL glutamic acid in CSF 6 hours apart. Died after trypsin, streptomycin, penicillin soln. was injected into CSF to open catheter. 2 days after recovery from insulin shock.	Chronic diffuse suppurative meningoencephalitis. The meninges were fib rotic and infiltrated with neutrophils, lymphocytes and macrophages. Lymphocytic perivascular cuffing was present in many areas of the brain and focal hemorrhages were present throughout; one small area of demyelinization was found. No changes were observed in the neurons.
9	10 units/kg insulin followed by convulsions which were stopped by injections of 5 percent dextrose. 2 units/kg insulin given, followed by convulsions and death after 24 hours.	Chronic diffuse suppurative meningoencephalitis. The meninges were thickened due to excess fibrous tissue and were infiltrated with many neutrophils, lymphocytes and macrophages. Small hemorrhages were present throughout the brain. No neuronal damage was evident.

Table 6. (Continued)

Sheep No.	Treatment	Postmortem changes
12	Convulsions induced by inhalation of 68% CO_2-32% O_2 mixture for 6 minutes. 2 weeks later 300 mg/kg heptachlor was given in divided doses which resulted in a semi-convulsive condition for 8 days. Sacrificed.	A diffuse lymphocytic meningoencephalitis, with minimal infiltration of lymphocytes was present. A diffuse gliosis, indicating a chronic irritation, was also present.
13	Convulsions induced by inhalation of 68% CO_2-32% O_2. Died after 8 1/2 minutes of breathing this mixture.	Slight lymphocytic meningoencephalitis. Multiple focal hemorrhages were found throughout the brain. A slight generalized increase in microglial cells was present.

Table 6. (Continued)

Sheep No.	Treatment	Postmortem changes
	(No heparin was injected into the CSF space of the remaining 4 sheep)	
15	Convulsions induced by inhalation of 68% CO_2-32% O_2 mixture for 6 minutes. Recovered in 2 to 3 hours. 2 days later sheep was given 200 mg/kg heptachlor. Died within 18 hours in convulsions.	As compared to brains from previously catheterized sheep, this one showed very slight alterations. A diffuse lymphocytic meningoencephalitis, with minimal infiltration of lymphocytes was observed. Neuroglial cells were in excess throughout the brain.
17	300 mg/kg heptachlor given orally in divided doses on 22nd and 23rd day post-surgery. Sheep died in seizures on 24th day post-surgery.	Chronic diffuse lymphocytic meningoencephalitis, as evidenced by generalized fibrous thickening and lymphocytic infiltration of the meninges, perivascular lymphocytic infiltration, and generalized gliosis. A chronic suppurative meningitis was present in the anterior cord region.

Table 6. (Continued)

Sheep No.	Treatment	Postmortem changes
21	150 mg/kg heptachlor orally, 10 days following surgery. Intermittent seizures for 4 days. Sacrificed on 14th day postsurgery (CSF catheter was placed too far down into the cisterna magna producing pressure on the left side of the medulla). (Catheter was made of silicone-rubber[a]).	Acute diffuse lymphocytic meningitis with a slight gliosis was present in medulla and cerebrum. Corpus quadrigemina had a subacute diffuse lymphocytic encephalitis. There was an area of liquefactive necrosis in dorsal half of the posterior part of the medulla oblongata (undoubtedly due to pressure on cord by catheter.
22	Catheter remained open 13 days. Sheep was sacrificed because catheter became occluded. (Catheter was made of silicone-rubber[a]).	Slight lymphocytic infiltration of meninges of anterior cord. General gliosis of medulla oblongata, thalamus and corpus striatum. Many blood vessels were surrounded by lymphocytic perivascular cuffs.

[a]Silastic, Dow Corning Center for Aid to Medical Research, Midland, Mich.

seizures persisted for an additional 1 to 2 minutes after which 3 sheep became comatose and 3 remained in a tonic convulsive state for the remainder of the CO_2 inhalation period.

All the sheep except one, which died, were able to rise within 10 minutes after inhalation of the gas mixture was discontinued. They were frequently unable to see or hear for 1 to 2 hours and were usually slightly incoordinated. However, they all appeared clinically normal within 3 to 4 hours.

1. Cerebrospinal fluid and blood analyses

The analyses of CSF taken from 6 sheep before, during and following convulsions produced by inhalation of the CO_2-O_2 mixture are reported in Table 7. The results of analyses of corresponding arterial blood samples are given in Table 8. These data are graphically presented in Figures 11 through 18.

Both blood and CSF glucose levels increased to at least twice the control values during the CO_2 seizures (Figure 11). The blood changes occurred sooner than those in the CSF. Glutamic oxalacetic transaminase activities were also elevated in the few sheep from which analyses were made (Figure 12). In this case the CSF changes occurred before those in the blood. A two-fold increase in serum potassium levels occurred within 1 minute after beginning CO_2 inhalation, and returned to normal within 1 hour (Figure 13). Cerebrospinal fluid potassium levels were also elevated within 1 minute but to a lesser extent than in blood. There were some elevations in serum sodium values, but CSF changes were difficult to

Table 7. Results of biochemical analyses of CSF taken before, during and following CO_2-induced convulsions in 6 sheep

	Time in minutes after beginning CO_2 inhalation						
	0 (Before inhalation)	1	6 (End of inhalation)	15	30	60	240
Glucose mg/100 ml	51 (5)[a]	52 (5)	60 (5)	68 (3)	97 (3)	103 (4)	106 (2)
GOT units/ml	16 (3)	47 (2)	32 (2)	27 (2)	42 (2)	46 (2)	46 (2)
GPT units/ml	18 (1)	19 (1)	21 (1)	23 (1)	30 (1)	26 (1)	24 (1)
LDH BB units/ml	24 (1)	20 (1)	26 (1)	7 (1)	28 (1)	65 (1)	42 (1)
Na^+ meq/l	140 (9)	148 (7)	147 (6)	145 (4)	146 (5)	158 (5)	151 (4)
K^+ meq/l	3.11 (6)	3.54 (6)	4.05 (5)	3.98 (3)	3.59 (4)	3.89 (5)	3.27 (3)
Ca^{++} meq/l	8.36 (5)	11.32 (2)	11.08 (2)	11.36 (2)	11.42 (2)	10.62 (3)	8.74 (1)
Mg^{++}	3.18 (3)	7.30 (2)	7.72 (2)	7.26 (2)	7.52 (2)	6.30 (3)	3.58 (1)
CSF pH	7.45 (2)	6.95 (2)	6.94 (2)	7.15 (2)	7.27 (2)	7.36 (2)	7.41 (1)

[a]Indicates the number of experiments from which determinations were made. Except for the Na^+ and K^+ values it also indicates the number of animals.

Table 8. Results of biochemical analyses of arterial blood taken before, during and following CO_2-induced convulsions in 6 sheep

	Time in minutes after beginning CO_2 inhalation						
	0 (Before inhalation)	1	6 (End of inhalation)	15	30	60	240
Glucose mg/100 ml	55 (3)[a]	62 (3)	99 (3)	121 (3)	118 (3)	121 (3)	106 (3)
SGOT units/ml	68 (1)	68 (1)	96 (1)	112 (1)	107 (1)	106 (1)	98 (1)
SGPT units/ml	17 (1)	--	17 (1)	24 (1)	26 (1)	28 (1)	18 (1)
LDH BB units/ml	695 (1)	733 (1)	703 (1)	735 (1)	660 (1)	663 (1)	705 (1)
Serum Na^+ meq/l	144 (6)	148 (6)	161 (6)	157 (5)	146 (5)	145 (5)	147 (4)
Serum K^+ meq/l	4.13 (6)	7.50 (6)	7.10 (6)	8.87 (5)	3.58 (5)	3.43 (5)	3.72 (4)
Serum Ca^{++} meq/l	7.72 (2)	8.28 (2)	8.52 (2)	8.08 (2)	7.80 (2)	9.30 (2)	9.64 (2)
Serum Mg^{++} meq/l	1.88 (2)	2.50 (2)	2.76 (2)	2.76 (2)	2.74 (2)	2.84 (2)	2.46 (2)
PCV %	31.7 (5)	40.3 (5)	44.2 (5)	41.5 (4)	38.5 (4)	33.2 (4)	30.9 (4)
pH	7.57 (4)	6.89 (4)	6.72 (4)	7.11 (4)	7.24 (4)	7.32 (4)	7.44 (4)

[a]Indicates the number of experiments from which determinations were made.

interpret (Figure 14). Within 1 minute after beginning CO_2 inhalation pH decreased by 0.5 to 0.7 pH unit (Figure 15). Blood PCV increased by 25 to 30 percent within 1 minute but returned to near control value within 1 hour (Figure 16). Although the CSF Ca^{++} and Mg^{++} values reported in Table 7 and in Figures 17 and 18 indicate elevations in 2 sheep studied, one showed no increase while the other showed a definite elevation. Blood Mg^{++} values were slightly elevated in the 2 experimental sheep. No definite differences from control values of GPT and LDH in either blood or CSF were noted.

The results of biochemical analyses of the CSF and arterial blood taken from the sheep during control experiments are presented in Tables 9 and 10, respectively. Note that the CSF glucose was elevated as compared to normal postsurgically recovered sheep (Table 3). These changes probably reflect the excitement associated with placing the sheep in the crate and reactions to the face mask.

2. Recording of physiological data

Physiological data recorded prior to, during and following convulsive seizures induced by inhalation of the CO_2-O_2 gas mixture are given in Table 11. The data recorded in the control experiments are given in Table 12. These data are also graphically presented in Figure 19.

Average pressures of the arterial blood and of CSF increased two-fold and three-fold, respectively, within the first minute of CO_2 inhalation. There was an accompanying increase in pulse pressures in both the blood and CSF. Heart rates were increased at 1 minute and were increased nearly

Table 9. Results of biochemical analyses of CSF taken from sheep on control CO_2 inhalation experiments[a]

	Time in minutes after putting on face mask						
	0 (Before putting on mask)	1	6 (Mask removed)	15	30	60	240
Glucose mg/100 ml	54 (4)[b]	54 (4)	58 (4)	61 (4)	65 (4)	65 (4)	67 (3)
GOT units/ml	16 (3)	31 (1)	17 (2)	17 (3)	23 (3)	23 (2)	27 (2)
LDH BB units/ml	47 (1)	10 (1)	13 (1)	67 (1)	43 (1)	125 (1)	65 (1)
Na^+ meq/l	165 (4)	159 (4)	166 (4)	160 (4)	153 (4)	156 (4)	156 (3)
K^+ meq/l	3.40 (4)	3.27 (4)	3.49 (4)	3.26 (4)	3.16 (4)	3.22 (4)	3.15 (3)
Ca^{++} meq/l	8.04 (3)	7.54 (2)	6.34 (1)	8.12 (3)	8.44 (3)	7.82 (2)	7.82 (2)
Mg^{++} meq/l	5.22 (3)	4.06 (2)	3.22 (2)	3.76 (2)	4.14 (3)	3.60 (3)	3.64 (2)
pH	7.44 (2)	7.47 (2)	7.44 (1)	7.55 (1)	7.51 (2)	7.59 (2)	7.49 (1)

[a]Sheep inhaled room air from the spirometer instead of CO_2-O_2 mixture.

[b]Indicates the number of experiments from which determinations were made.

Table 10. Results of biochemical analyses of arterial blood taken from sheep on control CO_2 inhalation experiments[a]

	Time in minutes after putting on face mask						
	0 (Before putting on mask)	1	6 (Mask removed)	15	30	60	240
Glucose mg/100 ml	65 (3)[b]	68 (2)	63 (3)	66 (3)	68 (3)	71 (3)	64 (3)
GOT units/ml	66 (1)	71 (1)	60 (1)	64 (1)	60 (1)	55 (1)	61 (1)
GPT units/ml	12 (1)	10 (1)	14 (1)	15 (1)	12 (1)	13 (1)	14 (1)
LDH BB units/ml	540 (1)	705 (1)	665 (1)	725 (1)	584 (1)	660 (1)	705 (1)
Serum Na^+ meq/l	141 (5)	152 (5)	149 (5)	144 (5)	148 (5)	152 (5)	139 (3)
Serum K^+ meq/l	4.01 (4)	4.12 (4)	3.92 (4)	4.28 (4)	4.23 (4)	4.21 (4)	4.68 (2)
Serum Ca^{++} meq/l	8.60 (1)	--	7.20 (1)	6.64 (1)	8.54 (1)	9.02 (1)	8.76 (1)
Mg^{++} meq/l	1.24 (1)	1.18 (1)	1.32 (1)	1.32 (1)	1.24 (1)	1.26 (1)	--
PCV %	30.6 (5)	31.8 (5)	30.8 (5)	31.3 (5)	30.2 (5)	31.0 (5)	30.6 (3)
pH	7.61 (2)	7.58 (1)	7.58 (1)	7.65 (2)	7.63 (2)	7.66 (2)	7.52 (1)

[a]Sheep inhaled room air from the spirometer instead of CO_2-O_2 mixture.

[b]Indicates the number of experiments from which determinations were made.

Table 11. Blood, CSF, and respiratory data recorded from sheep before, during, and following seizures induced by inhalation of CO_2-O_2 mixture

Time in minutes after beginning CO_2 inhalation	Arterial blood pressure (mm Hg)				CSF pressure (mm Hg)				Heart rate per min.	CSF[a] rate per min.	Resp. rate per min.
	Avg.	Syst.	Diast.	Pulse	Avg.	Syst.	Diast.	Pulse			
0 (Face mask on before CO_2 inhalation)	109 (5)[b]	126 (5)	95 (5)	31 (5)	9.7 (7)	11.6 (6)	9.0 (6)	2.7 (6)	89 (7)	71 89 } (6)	71 (6)
1	195 (4)	228 (4)	166 (4)	62 (4)	27.3 (6)	35.8 (5)	19.2 (5)	16.6 (5)	107 (6)	107 35 } (6)	35 (6)
6 (Stopped CO_2 inhalation; mask off)	201 (4)	225 (4)	187 (4)	38 (4)	30.8 (6)	34.0 (6)	28.3 (6)	5.7 (6)	251 (6)	21 251 } (6)	21 (6)
15	121 (3)	155 (3)	101 (3)	53 (3)	6.3 (5)	6.8 (4)	5.2 (5)	2.0 (5)	212 (5)	49 212 } (5)	49 (5)
30	110 (3)	123 (2)	100 (2)	23 (2)	5.6 (5)	4.4 (4)	3.8 (4)	1.4 (4)	203 (4)	203 (4)	111 (4)
60	123 (3)	134 (3)	111 (3)	28 (3)	5.5 (4)	6.1 (4)	4.6 (4)	1.5 (4)	180 (4)	180 (4)	82 (3)
120	115 (2)	126 (2)	108 (3)	18 (2)	4.7 (2)	4.5 (1)	4.0 (1)	0.5 (1)	120 (2)	120 (2)	33 (2)

[a]The CSF pulsation was influenced by both respiration and blood pressure. The bottom number represents the more prominent CSF pulsations.

[b]Indicates the number of animals from which measurements were made.

Table 12. Blood, CSF, and respiratory data recorded from sheep during control CO_2 inhalation experiments[a]

Time in minutes after putting on face mask	Arterial blood pressure (mm Hg)				CSF pressure (mm Hg)				Heart rate per min.	CSF[b] rate per min.	Resp. rate per min.
	Avg.	Syst.	Diast.	Pulse	Avg.	Syst	Diast.	Pulse			
0 (Before face mask)	116 (5)[c]	135 (5)	102 (5)	33 (5)	8.5 (6)	9.7 (6)	7.6 (6)	2.5 (6)	85 (6)	85 (6)	65 (6)
1	116 (5)	134 (5)	101 (5)	33 (5)	10.8 (6)	12.2 (6)	9.7 (6)	2.4 (6)	86 (6)	63 86 (3)	63 (6)
6 (Removed face mask)	115 (3)	133 (3)	101 (3)	31 (3)	10.2 (3)	11.7 (3)	8.7 (3)	3.0 (3)	84 (3)	34 84 (3)	34 (3)
15	115 (3)	127 (3)	95 (3)	32 (3)	5.5 (3)	7.0 (3)	5.3 (3)	1.7 (3)	78 (3)	78 (3)	34 (3)
30	115 (3)	129 (3)	100 (3)	29 (3)	7.5 (3)	8.3 (3)	6.7 (3)	1.6 (3)	74 (3)	74 (3)	42 (3)
60	110 (3)	127 (3)	95 (3)	32 (3)	6.3 (3)	7.0 (3)	5.5 (3)	1.5 (3)	73 (3)	72 (3)	45 (3)

[a]Sheep inhaled room air from the spirometer.

[b]When the face mask was put on, the CSF pulsation was influenced by respiration as well as blood pressure. The bottom number indicates the more prominent CSF pulsation.

[c]Indicates the number of animals from which measurements were made.

three-fold at 6 minutes. Respiratory rates were erratic but decreased at least three-fold during the CO_2 inhalation, then increased to nearly twice control rates at 30 minutes which was 24 minutes after cessation of CO_2 inhalation. There was a lesser decrease in respiratory rate in the control animals inhaling from the spirometer. The pulsation of the CSF was more correlated with respiration than with arterial pulse during CO_2 inhalation (Figure 3). However, this effect was also noted to a lesser extent in the control experiments in which room air was inhaled from the spirometer (Figure 2).

Both blood and CSF pressures returned to control values within 10 minutes after CO_2 inhalation was discontinued. In fact, CSF pressures rapidly decreased to about one-half control values. However, this same effect was noted in the control experiments and was evident after a total of 4 to 5 ml of CSF was withdrawn for biochemical analyses within a period of 15 minutes.

A representative recording of blood pressure, CSF pressure and respiratory rate of a sheep exhibiting seizures during CO_2 inhalation is shown in Figure 3. The increase in both blood and CSF pressures and rate is very prominent when compared to the representative control recording in Figure 2.

3. Postmortem examination

Postmortem findings in the brain of the sheep that died during CO_2 inhalation (Sheep 13) are given in Table 6. Multiple focal hemorrhages were found throughout the brain. The other changes were not considered

to be a result of the CO_2, but rather due to chronic irritation from the CSF catheter.

D. Insulin Hypoglycemic Convulsions

All 5 sheep given insulin exhibited convulsive seizures within 12 to 24 hours. Preconvulsive signs were manifested by weakness, salivation, trembling and twitching of the muscles about the head and neck. Two sheep exhibited bradycardia and frequent urination immediately before onset of seizures. The onset of convulsions usually occurred as a violent tonic seizure with the sheep remaining on its feet or jumping violently and landing in lateral recumbency. The convulsive seizures were predominantly of the intermittent tonic-clonic type with the sheep appearing comatose between seizures. In the early stages the sheep appeared nearly normal between seizures.

Attempts were made to counteract hypoglycemic shock by injecting 5 percent glucose into the carotid artery. Sheep that were treated after seizures were in progress for 30 minutes or more failed to respond despite the fact that CSF and blood glucose values were increased to an average of 50 and 100 mg/100 ml, respectively. Apparently hypoglycemia initiated irreversible changes which were not alleviated by the administration of glucose. Similar findings have been reported for sheep by McClymont and Setchell (1956).

Two sheep treated immediately following the first seizure did not die. In one sheep 90 mg of DL-glutamic acid were injected into the CSF in 3 equally divided doses over a period of 12 hours. The time of administration

was dictated by onset of seizures, and the treatment was repeated until no further seizures occurred. The blood and CSF glucose levels were elevated to 2 1/2 times the control levels in this sheep although no glucose was given. This was probably due to an adrenal response. A similar phenomenon has been observed in hypoglycemic laboratory animals given glutamic acid intravenously but not in adrenalectomized hypoglycemic animals (Weil-Malherbe 1950). The average dose given to the sheep (30 mg) was approximately one-twentieth the reported minimum effective dose for laboratory animals. The other sheep was given 50 ml of 5 percent glucose in the carotid artery, and the treatment was repeated in 6 hours at which time seizures occurred again. Both sheep made complete recoveries following the last treatments.

1. Cerebrospinal fluid and blood analyses

Results of biochemical analyses of the CSF and blood samples taken during insulin shock convulsions are reported in Table 13. Results of analyses of samples from 3 control sheep are given in Table 14. Cerebrospinal fluid and blood glucose and transaminase values are presented graphically in Figures 20, 21 and 22. Both blood and CSF glucose levels decreased to 10 mg/100 ml or below and seizures occurred at this time (Figure 20; Table 13). Changes in CSF glucose values paralleled those of blood at a significantly lower level throughout the experimental period. After treatment with glucose or glutamic acid, blood and CSF glucose levels were increased one- to two-fold above control levels. Other biochemical changes detected included slight increases in serum GOT (Figure 21; Table 13)

Table 13. Results of biochemical analyses of CSF and blood from 5 sheep during insulin shock

Constituent		Before fast	Fast-before insulin	Insulin-before seizures	Seizures before treatment[a]	Seizures after treatment	At death or recovery
Glucose (mg/100 ml)	CSF	33 (8)[b]	29 (3)	7 (3)	3 (4)	19 (18)	48 (6)
	Blood	51 (9)	43 (5)	12 (5)	8 (4)	29 (9)	84 (6)
GOT (units/ml)	CSF	31 (11)	45 (3)	36 (4)	45 (2)	47 (17)	64 (5)
	Blood	64 (9)	86 (4)	58 (5)	88 (4)	92 (9)	108 (6)
GPT (units/ml)	CSF	16 (13)	8 (3)	22 (3)	8 (1)	18 (20)	14 (4)
	Blood	9 (12)	6 (4)	8 (6)	10 (3)	10 (16)	13 (5)
Protein (mg/100 ml)	CSF	114 (7)	69 (2)	57 (1)	38 (1)	158 (5)	245 (1)
Na^+ (meq/l)	CSF	152 (10)	132 (3)	141 (3)	119 (1)	141 (17)	150 (4)
K^+ (meq/l)	CSF	3.09 (11)	3.18 (3)	2.65 (3)	2.70 (1)	3.20 (16)	3.66 (5)

[a]Includes 1 sheep that was given glutamic acid in cisterna magna. The other 4 were given 5% dextrose intraarterially and/or in the cisterna magna.

[b]Number of samples analyzed.

Table 14. Results of biochemical analyses of CSF and blood from 3 control sheep in the insulin shock experiments

Constituent		Before fast	After fast	At time of sacrifice
Glucose (mg/100 ml)	CSF[a]	62 (7)[b]	48 (12)	42 (3)
	Blood	45 (7)	34 (7)	34 (2)
GOT (units/ml)	CSF[a]	59 (7)	69 (12)	64 (3)
	Serum	31 (7)	46 (8)	56 (2)
GPT (units/ml)	CSF[a]	13 (7)	11 (12)	11 (3)
	Serum	17 (7)	12 (8)	10 (2)
Na^+ (meq/l)	CSF[a]	160 (4)	137[c] (4)	117[c] (1)
K^+ (meq/l)	CSF[a]	3.73 (4)	3.48[c] (4)	2.38[c] (1)
Protein (mg/100 ml)	CSF[a]	164 (2)	--	308 (1)

[a]Represents only 2 sheep.

[b]Number of samples analyzed.

[c]Represents only 1 sheep.

and, in the few samples of CSF analyzed, a slight decrease in Na^+ and K^+ (Table 13). The latter results are interesting in light of the report by Keys (1938) that high doses of insulin caused a marked decrease in serum potassium in humans and dogs. Glutamic pyruvic transaminase activities remained within the range of control values during insulin shock seizures. The apparent decrease in CSF protein is misleading partly because of the small number of samples taken before treatment of insulin shock seizures (Table 13). The values, 114 mg/100 ml before fast and 158 mg/100 ml after seizures, may be valid. It should be noted that the insulin experiments were conducted on sheep catheterized early in the study and that the CSF of these sheep contained much higher control total protein levels than did sheep catheterized later in the study. Additional experiments should be conducted before any conclusions are made regarding biochemical changes that are associated with insulin shock seizures.

2. Recording of physiological data

Physiological data were obtained from sheep before and during insulin shock, but not during seizures. These data are presented in Table 15. Representative recordings made from a sheep before and during insulin shock are given in Figures 4 and 5, respectively.

3. Postmortem examination

The postmortem changes found in the brains of sheep in the insulin experiments are described in Table 6 (Sheep 3 through 9). No degeneration of the neurons was found in the brains of 4 sheep that died during insulin

Table 15. Blood, CSF, and respiratory data recorded from 4 sheep in the insulin shock experiments

Sheep No.	Experimental stage	Arterial blood pressure (mm Hg)				CSF pressure (mm Hg)				Heart rate per min.	CSF rate per min.	Resp. rate per min.	Comment
		Avg.	Syst.	Diast.	Pulse	Avg.	Syst.	Diast.	Pulse				
7	Before insulin	108	131	87	44	7.9	8.8	7.5	1.30	60	60	21	Standing
	" "	108	126	89	37	3.8	4.5	3.6	.90	62	62	20	"
	" "	104	118	80	38	1.0	1.7	0.5	1.20	69	69	15	Recumbent on sternum
	18 hours after insulin; no symptoms	116	139	93	46	7.7	9.3	6.2	3.20	60	60	27	Standing
	42 hrs. after insulin; stupor, chewing reflex	97	114	81	43	-1.4	-1.1	-1.7	0.60	77	77	19	Recumbent
	72 hrs. after insulin; stupor, weak	109	120	85	35	1.2	1.8	0.6	1.20	68	68	14	"
8	Before insulin	93	106	81	25	10.0	10.4	9.6	0.80	93	93	63	Standing
	" "	93	110	76	34	12.8	14.3	11.3	3.00	101	101	116	"
	" "	92	113	77	36	4.0	5.0	3.2	1.80	81	81	81	"
	18 hrs. after insulin; shock but treated with glutamic acid in CSF	102	110	96	14	-3.3	-2.4	-4.2	1.80	123	120	78	Recumbent on sternum

Table 15. (Continued)

Sheep No.	Experimental stage	Arterial blood pressure (mm Hg) Avg.	Syst.	Diast.	Pulse	CSF pressure (mm Hg) Avg.	Syst.	Diast.	Pulse	Heart rate per min.	CSF rate per min.	Resp. rate per min.	Comment
9	Before insulin	100	116	88	28	7.8	10.6	8.9	1.70	60	60	34	Standing
	" "	93	111	82	29	9.8	11.5	8.9	2.60	74	74	60	"
	" "	98	120	78	42	14.5	16.1	12.9	3.20	72	72	141	"
	" "	80	100	64	36	7.7	9.3	6.2	3.10	72	72	144	Recumbent on sternum
	" "	101	114	87	37	18.8	19.5	17.5	2.00	60	60	54	Standing
	" "	103	131	103	28	22.0	23.0	20.5	2.50	75	75	99	"
	" "	108	115	92	23	10.0	10.6	9.7	0.90	57	57	72	"
	18 hrs. after insulin; shock but seizures prevented with glucose	130	148	112	36	13.8	15.3	13.4	1.90	60	60	59	"
10	Control - before fast	145	168	120	48	10.5	12.0	8.0	4.00	90	90	87	Standing
	Control - before fast	100	109	93	16	13.0	14.0	10.0	4.00	102	102	111	"
	After fasting 4 days	119	136	102	34	11.0	12.8	10.3	2.50	72	72	81	"

shock. This is surprising because all had been in convulsive seizures and coma for 12 to 24 hours before death. Most reports have indicated that parenchymatous degeneration of the brain was evident in humans and laboratory animals after having been in hypoglycemic seizures for as little as 2 to 3 hours (Altschul and Fineberg 1949; Hassin 1939; Tyler and Ziskind 1940; Winkelman and Moore 1940).

E. Heptachlor Convulsions

Each of the 4 sheep given oral doses of heptachlor exhibited convulsive seizures beginning from 4 to 48 hours after administration of the insecticide. Three died and the fourth was euthanatized after exhibiting intermittent seizures for 4 days. All sheep showed premonitory and preconvulsive symptoms similar to those described by Radeleff et al. (1955). Signs of heptachlor toxicity invariably began with a changed facial expression followed by muscle twitches about the head and neck. These signs were followed by generalized muscle spasms and convulsive seizures. Excitation of the animal or loud noises often initiated seizures.

1. Cerebrospinal fluid and blood analyses

Results of biochemical analyses of blood and CSF taken before and during heptachlor toxicity are given in Table 16. These data are also graphically presented in Figures 23 through 28. Blood and CSF glucose levels were elevated (approximately two-fold) before any signs of toxicity were observable (Figure 23). The CSF glucose levels returned to near control levels in the one sheep that became depressed but did

Table 16. Results of biochemical analyses of CSF and blood taken from 4 sheep before and during heptachlor toxicity

Sheep No.		11		15		17		21		Avg.	
Treatment		50 mg/kg. No symptoms within 72 hrs. Dose repeated at 72 hrs. 200 mg/kg. given at 96 hrs. Died at 115 hrs.		200 mg/kg. Hypersensitivity at 7 hrs. Muscle twitches at 8 hrs. Died at 20 hrs.		150 mg/kg. No symptoms at 24 hrs. Repeated dose at 24 hrs. Died at 45 hrs.		150 mg/kg. Hypersensitivity at 4 hrs. Intermittent muscle spasms and seizures during first 24 hrs. Depressed but convulsive when handled from 30 to 100 hrs. Euthanatized at 100 hrs.			
		Blood	CSF	Blood	CSF	Blood	CSF	Blood	CSF	Blood	CSF
Glucose (mg/100 ml)	Pre	52 (2)[a]	39 (2)	51 (3)	49 (3)	57 (8)	44 (7)	46 (2)	59 (2)	52	48
	24 hrs.	98 (1)	77 (1)	37 (1)	39 (1)	91 (2)	66 (2)	88 (5)	117 (5)	79	75
	48 hrs.	73 (1)	53 (1)	--	--	83 (2)	76 (2)	60 (1)	103 (1)	72	77
	72 hrs.	91 (1)	44 (1)	--	--	--	--	29 (1)	41 (1)	60	43
	96 hrs.	--	--	--	--	--	--	78 (1)	47 (1)	78	47
	120 hrs.	--	--	--	--	--	--	36 (1)	--	36	--
GOT (units/ml)	Pre	134 (2)	--	128 (2)	18 (2)	72 (8)	41 (8)	50 (2)	9 (2)	96	23
	24 hrs.	178 (1)	--	171 (1)	28 (1)	96 (1)	35 (2)	89 (5)	32 (5)	134	32
	48 hrs.	200 (1)	--	--	--	82 (2)	38 (2)	96 (1)	30 (1)	126	34
	72 hrs.	240 (1)	--	--	--	--	--	124 (1)	61 (1)	182	61
	96 hrs.	--	--	--	--	--	--	--	57 (1)	--	57
	120 hrs.	--	--	--	--	--	--	109 (1)	--	109	--

[a]Number of samples analyzed.

Table 16. (Continued)

Sheep No.		11		15		17		21		Avg	
GPT (units/ml)	Pre	17 (2)	--	22 (3)	--	17 (8)	21 (6)	12 (2)	18 (2)	17	20
	24 hrs.	34 (1)	--	27 (1)	--	22 (1)	14 (2)	21 (5)	17 (5)	26	16
	48 hrs.	41 (1)	--	--	--	20 (2)	23 (2)	25 (1)	25 (1)	29	24
	72 hrs.	51 (1)	--	--	--	--	--	22 (1)	19 (1)	37	19
	96 hrs.	--	--	--	--	--	--	--	20 (1)	--	20
	120 hrs.	--	--	--	--	--	--	23 (1)	--	23	--
LDH (BB units/ml)	Pre	--	--	--	--	602 (3)	39 (3)	685 (2)	22 (2)	644	31
	24 hrs.	--	--	--	--	620 (1)	23 (2)	973 (5)	24 (5)	797	24
	48 hrs.	--	--	--	--	729 (2)	36 (2)	960 (1)	32 (1)	849	35
	72 hrs.	--	--	--	--	--	--	1015 (1)	61 (1)	1015	61
	96 hrs.	--	--	--	--	--	--	--	57 (1)	--	57
	120 hrs.	--	--	--	--	--	--	1003 (1)	--	1003	--
Na^+ (meq/l)	Pre	--	144 (2)	--	157 (1)	144 (4)	163 (8)	156 (2)	161 (2)	150	156
	24 hrs.	--	163 (1)	--	182 (1)	--	163 (2)	156 (4)	157 (5)	156	166
	48 hrs.	--	213 (1)	--	--	--	164 (2)	166 (1)	161 (1)	166	179
	74 hrs.	--	152 (1)	--	--	--	--	151 (1)	165 (1)	151	159
	96 hrs.	--	--	--	--	--	--	155 (1)	157 (1)	155	157
	120 hrs.	--	--	--	--	--	--	164 (1)	--	164	--

Table 16. (Continued)

Sheep No.		11		15		17		21		Avg.	
K^+ (meq/l)	Pre	--	--	--	3.65 (3)	4.32 (4)	3.70 (8)	4.88 (2)	3.95 (2)	4.61	3.77
	24 hrs.	--	--	--	4.15 (1)	4.40 (1)	3.74 (2)	4.31 (4)	3.75 (5)	4.36	3.88
	48 hrs.	--	--	--	--	4.10 (2)	3.61 (2)	6.00 (1)	4.50 (1)	5.20	4.06
	72 hrs.	--	--	--	--	--	--	4.75 (1)	3.80 (1)	4.75	3.80
	96 hrs.	--	--	--	--	--	--	4.40 (1)	4.22 (1)	4.40	4.22
	120 hrs.	--	--	--	--	--	--	6.48 (1)	--	6.48	--
Ca^{++} (meq/l)	Pre	--	9.64 (1)	--	8.12 (2)	8.48 (1)	8.38 (6)	11.40 (2)	7.80 (2)	9.94	8.48
	24 hrs.	--	7.84 (1)	--	9.92 (2)	8.50 (1)	8.96 (2)	7.80 (5)	8.20 (5)	8.16	8.74
	48 hrs.	--	10.26 (1)	--	--	8.48 (2)	8.82 (2)	10.16 (1)	8.58 (1)	9.32	9.22
	72 hrs.	--	8.30 (1)	--	--	--	8.26 (1)	9.08 (1)	7.68 (1)	9.08	7.98
	96 hrs.	--	--	--	--	--	--	7.80 (1)	7.80 (1)	7.80	7.80
	120 hrs.	--	--	--	--	--	--	9.30 (1)	--	9.30	--
Mg^{++} (meq/l)	Pre	1.56 (2)	5.56 (2)	1.70 (3)	5.24 (3)	2.38 (1)	3.74 (7)	3.82 (2)	2.54 (2)	2.36	4.28
	24 hrs.	1.66	3.80 (1)	1.68 (1)	8.90 (1)	2.28 (1)	4.94 (2)	2.60 (5)	3.50 (5)	2.06	5.28
	48 hrs.	1.42	6.40 (1)	(Died)		2.46 (2)	5.10 (2)	3.34 (1)	4.04 (1)	2.40	5.18
	74 hrs.	2.10	3.88	--	--	(Died)		3.46 (1)	2.20 (1)	3.28	3.04
	96 hrs.	--	--	--	--	--	--	2.34 (1)	2.82 (1)	2.34	2.82
	120 hrs.	--	--	--	--	--	--	3.28 (1)	--	3.28	--

not die in seizures (Sheep 21). The blood glucose levels were very erratic in this sheep. Slight but consistent elevations in glutamic oxalacetic transaminase were noted in both blood and CSF (Figure 24). No increase in CSF or blood GPT activities was observed. Slight elevations in lactic dehydrogenase activities were noted in the two sheep from which samples were analyzed (Figure 25). Slight elevations in blood and CSF Na^+ occurred up to 48 hours (Figure 26). Levels of K^+ were inconsistent and of questionable significance (Figure 27). No definite changes in Ca^{++} levels were evident (Figure 28).

2. Recordings of physiological data

Physiological data were obtained from 1 of the 4 sheep in which convulsive seizures were induced with heptachlor (Sheep 21). Both blood and CSF pressures and heart rate were increased slightly during the mild seizure activity (Table 17). Representative tracings are presented in Figures 6 and 7. Figure 6 shows a control tracing. Note that CSF fluctuations are correlated with blood pressure instead of respiration. Figure 7 shows the recordings while the sheep was exhibiting intermittent whole body muscle spasms. It was noted that the CSF pressure was not elevated before or after the spasms.

3. Postmortem examination

Examination of the brains of 3 sheep in which seizures were induced with heptachlor did not disclose any neuron degeneration. The usual lymphocytic meningitis, presumably a reaction to the CSF catheters, was

Table 17. Blood, CSF, and respiratory data recorded from a sheep given heptachlor (Sheep 21)

Experimental stage	Arterial blood pressure (mm Hg)				CSF pressure (mm Hg)				Heart rate per min.	CSF rate per min.	Resp. rate per min.	Comment
	Avg.	Syst.	Diast.	Pulse	Avg.	Syst.	Diast.	Pulse				
Pretreatment	108	122	91	31	2.6	2.9	2.2	0.70	66	66	44	Standing
"	106	124	92	32	5.3	6.3	4.6	1.70	69	69	36	"
4 hrs. after heptachlor; preconvulsive muscle twitches	108	128	94	34	2.8	3.8	1.7	2.10	84	84	69	"
6 hrs. - muscle spasms	104	118	86	32	7.9	9.7	7.0	2.70	72	72	96	"
10 hrs. - intermittent spasms and twitches	108	124	90	34	4.8	6.2	4.0	2.20	75	75	66	"
20 hrs. - depressed, infrequent muscle spasms	98	114	84	30	11.5	13.1	10.7	2.40	66	66	57	"
29 hrs. - depressed (between intermittent convulsions)	98	112	84	28	10.5	12.0	8.5	3.50	63	63	60	On sternum
30 hrs. - same	88	118	63	55	-0.2	+1.2	-1.5	2.70	96	96	87	CSF had been previously excessively sampled
74 hrs. - mild convulsive seizures	138	164	122	42	9.2	11.0	8.0	3.00	135	135	36	

found. The results of the examinations of the brains are recorded in Table 6 (Sheep 15, 17 and 21).

F. Comparative Effects of Seizures Induced with CO_2, Insulin, and Heptachlor

1. Cerebrospinal fluid and blood biochemical changes

Those biochemical changes in the CSF and blood that were associated with seizures induced by inhalation of CO_2 were more rapid than in the other experiments because of the acute nature of the experiments. However, elevations in blood and CSF glucose noted during the CO_2 induced seizures were paralleled, although less acute, in those sheep poisoned on heptachlor. The opposite effect on glucose levels was noted, of course, in those sheep exhibiting insulin seizures.

Cerebrospinal fluid and blood GOT activities were elevated during the CO_2 and heptachlor induced seizures. However, CSF elevations appeared before those in the blood during CO_2 induced seizures, but blood elevations appeared before those in the CSF during heptachlor seizures. The elevations in blood serum GOT activities appeared after several hours of seizures during insulin shock, but elevations were not evident in the CSF.

Blood and CSF potassium values were definitely elevated during the CO_2 induced seizures, were not changed appreciably during the heptachlor seizures, and if anything, were decreased during insulin shock. However, no blood analyses were made during the insulin experiments.

There were slight elevations in blood and CSF Na^+ during the initial induction of CO_2 seizures and also during early stages of heptachlor

toxicity. Cerebrospinal fluid Ca^{++} and Mg^{++} levels were elevated during CO_2 seizures, but these values are questionable because one sheep showed definite elevations while the other did not. No definite changes in Ca^{++} and Mg^{++} values were evident during heptachlor toxicity.

No appreciable changes in GPT activities were detected in the CSF and blood from any of the sheep in this study.

The biochemical and physiological changes associated with convulsive seizures reported herein are preliminary. Cerebrospinal fluid obtained from the catheterized animals, even after apparent recovery from surgery, cannot be considered as entirely normal fluid. The elevations in enzyme activities and protein content in the CSF (Table 3; Figure 8) plus the histopathological findings (Plate 2; Table 6) indicate that an inflammatory reaction existed in the CNS. However, this does not necessarily invalidate the significance of changes detected during seizure activity. The chief difficulty apparently resulted from tissue reaction to the catheter or phenolized heparin; less from the technique for placement. Use of an improved type of catheter material, improved procedure for its placement, and better aftercare would further reduce the tissue response.

2. Physiological data

The striking elevations in blood and CSF pressures and in heart and respiratory rates during CO_2 seizures were not as dramatic in the insulin and heptachlor experiments. However, it should be noted that the data were recorded continuously during the CO_2 seizures. In contrast, because

of the chronic nature of the insulin and heptachlor experiments, continuous measurement of the pressures was not possible. Only occasionally were physiological data recorded during a convulsive seizure during these experiments.

A summary of maximum physiological and biochemical changes that occurred in sheep during convulsive seizures induced with CO_2, insulin, and heptachlor is given in Table 18.

3. Postmortem changes

No neuron damage was observed in any of the brains from the sheep in the entire study. The sheep that died during CO_2 inhalation had multiple focal hemorrhages throughout the brain. This was not observed in any of the other sheep.

Table 18. Summary of maximum physiological and biochemical changes during convulsive seizures[a]

Parameter	Mean control values[b]		Mean maximum response values					
			CO_2 seizures		Insulin seizures		Heptachlor seizures	
	Blood	CSF	Blood	CSF	Blood	CSF	Blood	CSF
Avg. pressure mm. Hg	105	7.9	201[c]	30.8[c]	126[d]	10.8[d]	138[e]	9.2[e]
Systolic pressure, mm. Hg	124	8.8	228[f]	35.8[f]	143[d]	12.3[d]	164[e]	11.0[e]
Diastolic pressure, mm. Hg	92	6.9	187[c]	28.3[c]	102[d]	9.8[d]	122[e]	8.0[e]
Pulse pressure mm. Hg	33	2.1	62[f]	16.6[f]	41[d]	2.6[d]	42[e]	3.0[e]
Heart and CSF rate/min.	85	85	251[c]	251[c]	60[d]	60[d]	135[e]	135[e]
Respiration rate/min.	58	--	↓ 21[c] 111[g]	--	43[d]	--	↓ 36[e]	--

[a]Those values accompanied by an arrow (↓) represent maximum decreases. The remainder represent maximum increases.

[b]Following recovery from surgery but before seizures were induced.

[c]6 minutes after beginning CO_2-O_2 inhalation.

[d]18 hours following insulin injection; insulin shock but not during seizures; sheep standing. Note: pressure and rate values were decreased when the sheep became depressed and were recumbent.

[e]Mild seizures induced with heptachlor.

[f]1 minute after beginning CO_2-O_2 inhalation.

[g]30 minutes after beginning CO_2-O_2 inhalation.

Table 18. (Continued)

Parameter	Mean control values[b]		Mean maximum response values					
			CO_2 seizures		Insulin seizures		Heptachlor seizures	
	Blood	CSF	Blood	CSF	Blood	CSF	Blood	CSF
Glucose mg./100 ml.	55	44	121[h]	106[i]	+ 08[j]	03[j]	79[k]	77[l]
GOT units/ml.	76	28	112[h]	47[f]	108[m]	64[m]	134[k]	61[n]
GPT units/ml	13	17	28[o]	30[g]	13[m]	22[p]	37[n]	24[l]
LDH units/ml.	721	29	735[h]	65[o]	--	--	1015[n]	61[n]
Na^+ mEq/l.	146	156	157[h]	158[o]	--	+ 119[j]	166[l]	179[l]
K^+ mEq/l.	4.37	3.34	8.87[h]	4.05[c]	--	+2.65[p]	6.48[q]	4.06[l]
Ca^{++} mEq/l.	8.60	8.12	9.64[i]	11.42[g]	--	--	9.30[l]	9.22[l]
Mg^{++} mEq/l.	2.83	4.18	2.84[o]	7.72[c]	--	--	3.28[n]	5.28[k]
PCV %	31.7	--	44.2[c]	--	--	--	--	--
pH	7.57	7.45	+ 6.72[c]	+6.94[c]	--	--	--	--

[h]15 minutes after beginning CO_2-O_2 inhalation.

[i]240 minutes after beginning CO_2-O_2 inhalation.

[j]During insulin shock seizures before glucose treatment.

[k]Within 24 hours following dosing with heptachlor, usually before symptoms were noted.

[l]Within 48 hours following dosing with heptachlor.

[m]At death or recovery from insulin shock.

[n]Within 72 hours following dosing with heptachlor.

[o]60 minutes after beginning CO_2-O_2 inhalation.

[p]Following insulin injection but before seizures had begun.

[q]120 hours following dosing with heptachlor.

V. SUMMARY

Surgical techniques have been developed for the placement of permanent, direct, indwelling catheters into the cisterna magna and carotid artery of sheep. Simultaneous pressure recordings and serial sampling of arterial blood and cerebrospinal fluid (CSF) were accomplished before, during and following induced convulsive seizures. Seizures were induced by 3 methods: (1) inhalation of a gas mixture containing 68 percent CO_2 and 32 percent O_2 for approximately 6 minutes, (2) subcutaneous injection of insulin, and (3) oral administration of heptachlor. Biochemical analyses performed on simultaneously taken blood and CSF samples included (1) glucose, (2) glutamic-oxalacetic transaminase (GOT), (3) glutamic-pyruvic transaminase (GPT), (4) lactic dehydrogenase (LDH), (5) pH (CO_2 induced seizures only), (6) sodium, (7) potassium, (8) calcium, (9) magnesium, and (10) CSF total protein. In addition, packed cell volume (PCV) determinations were made during CO_2 seizures. Postmortem examinations were conducted on the brains of 13 sheep that were euthanatized or that died during convulsive seizures.

Cerebrospinal fluid and carotid artery catheters were placed in 19 sheep. After recovery from surgery (approximately 10 days) the following mean values for physiological data were recorded from 17 of the sheep: blood pressure 105 ± 12 mm Hg; CSF pressure 7.9 ± 3.0 mm Hg; heart rate 85 ± 13 per minute; CSF rate 85 ± 13 per minute, and respiratory rate 58 ± 34 per minute. These data were taken from a total of 37 separate recordings of the sheep while standing quietly.

Comparisons were made between values for constituents of blood and CSF taken before the catheters were established and after the sheep had recovered from the surgery. The values for all constituents measured were essentially the same except for a two-fold increase in CSF protein, glutamic-oxalacetic transaminase, and lactic dehydrogenase. These changes indicated that an inflammation existed in the CNS. Postmortem examinations of the sheep brains corroborated this in that all showed varied degrees of meningitis and in some cases encephalitis characterized by infiltration of lymphocytes and, to a lesser extent, neutrophils. As the surgical technique for placing the CSF catheter into the cisterna magna was improved, the protein and enzymic changes became less pronounced and the meningitis became less severe.

Physiological and biochemical changes that occurred in 6 sheep during CO_2 induced seizures were very acute. Arterial blood and CSF pressures increased two- and three-fold, respectively, within 1 minute after beginning CO_2 inhalation. Heart rates increased by three-fold within 6 minutes. Respiration rates decreased during CO_2 inhalation, but increased after CO_2 administration was discontinued. Both blood and CSF glucose levels doubled during the seizures. Definite elevations in serum, and to a lesser extent CSF, potassium values occurred within 6 minutes, but returned to near normal within 1 hour. Both blood and CSF pH decreased by 0.5 to 0.7 units within 1 minute and also returned to normal within 1 hour. Glutamic oxalacetic transaminase activities were elevated in both blood and CSF of the few sheep from which analyses were made. Blood PCV increased by

25 to 30 percent within 1 minute, but returned to near control values within 1 hour. The CSF calcium and magnesium levels increased inconsistently during CO_2 seizures.

Convulsive seizures were induced in 5 sheep with insulin injections and in 4 sheep with oral heptachlor. Physiological and biochemical changes were much less acute than during CO_2 induced seizures. Blood and CSF pressures were increased during seizure activity but decreased during periods of depression. Blood and CSF glucose decreased to below 10 mg/100 ml during insulin shock seizures, but after treatment with glucose or DL-glutamic acid the values returned to above control levels. After insulin induced seizures became well advanced (30 minutes or more) all animals died, even though blood and CSF glucose levels were increased to twice those of control. Blood and CSF glucose levels increased markedly in the sheep given heptachlor, even before signs of toxicity were evident. Slight elevations in blood serum transaminase (SGOT) were noted in the sheep given heptachlor. Sodium and potassium values decreased in those sheep given insulin, but a limited number of samples were analyzed.

Changes in the other constituents during seizures induced by all 3 methods were of questionable importance. No changes in glutamic-pyruvic transaminase activities were noted in any sheep.

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VIII. APPENDIX A: FIGURES

Figure 1. Representative control tracings of blood pressure, CSF pressure, and respiratory rate in a quietly standing sheep. Note that the CSF pulsations are correlated with blood pressure and not with respiration.

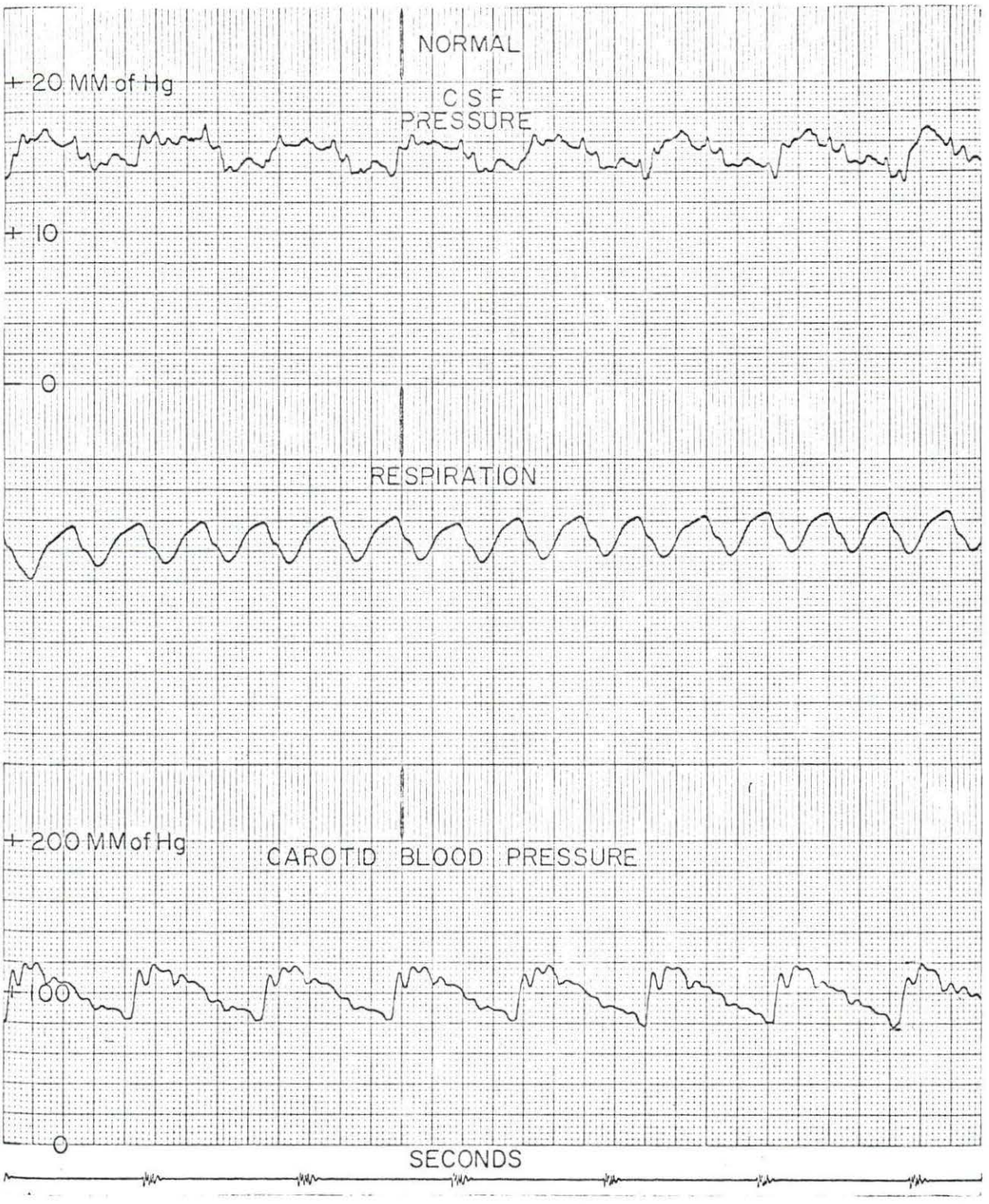

NORMAL
+20 MM of Hg
C S F
PRESSURE
+10
0
RESPIRATION
+200 MM of Hg
CAROTID BLOOD PRESSURE
+100
0
SECONDS

Figure 2. Representative control tracings of blood pressure, CSF pressure and respiratory rate in a sheep (No. 19) while standing and breathing room air from a spirometer. Note the dual correlations of CSF pulsations with respiration and blood pressure.

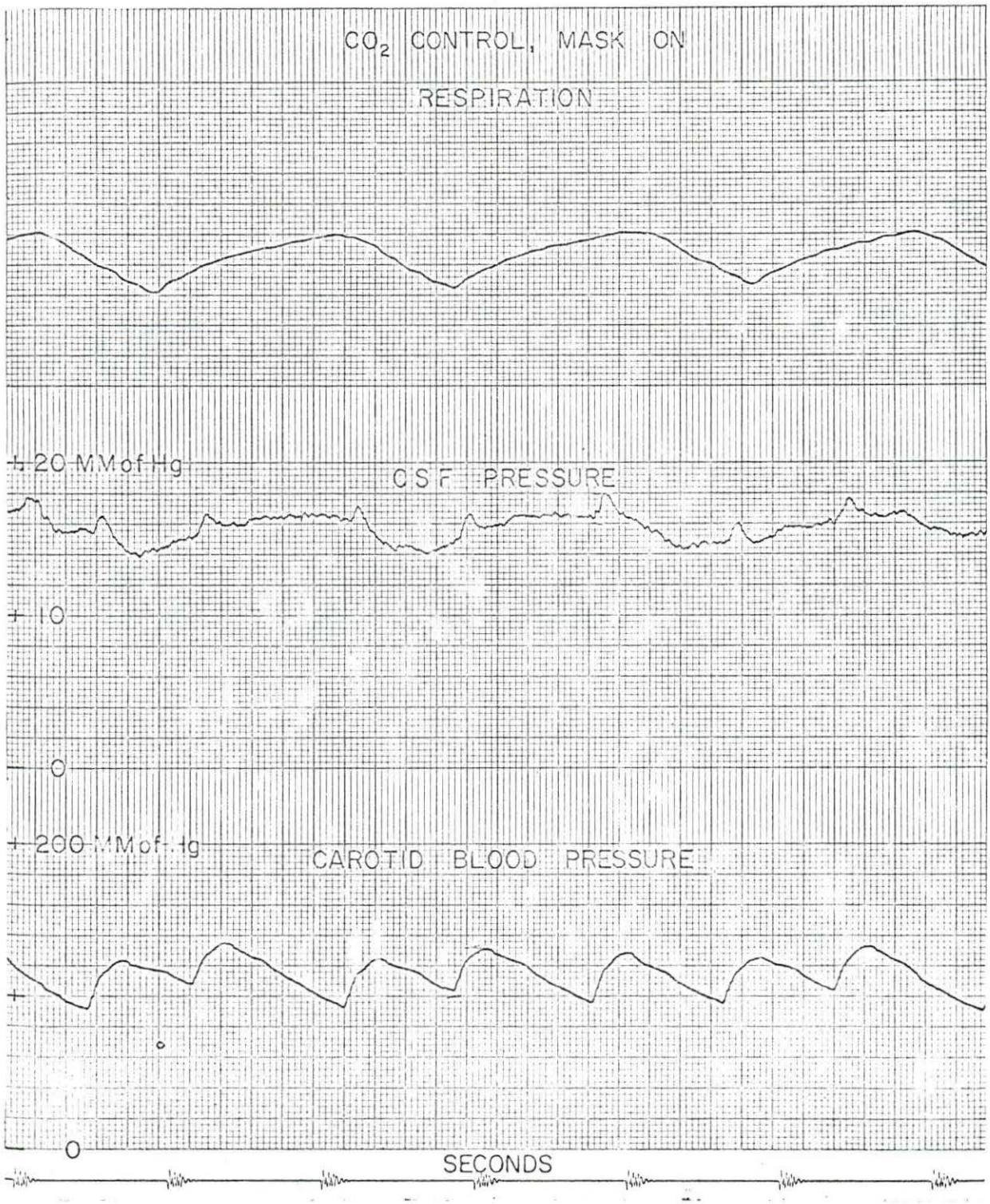

CO_2 CONTROL, MASK ON
RESPIRATION
+20 MM of Hg
CSF PRESSURE
+10
0
+200 MM of Hg
CAROTID BLOOD PRESSURE
+
0
SECONDS

Figure 3. Representative tracings of blood pressure, CSF pressure and respiratory rate from the same sheep as Figure 2 (No. 19) made approximately 3 minutes after beginning CO_2-O_2 inhalation.

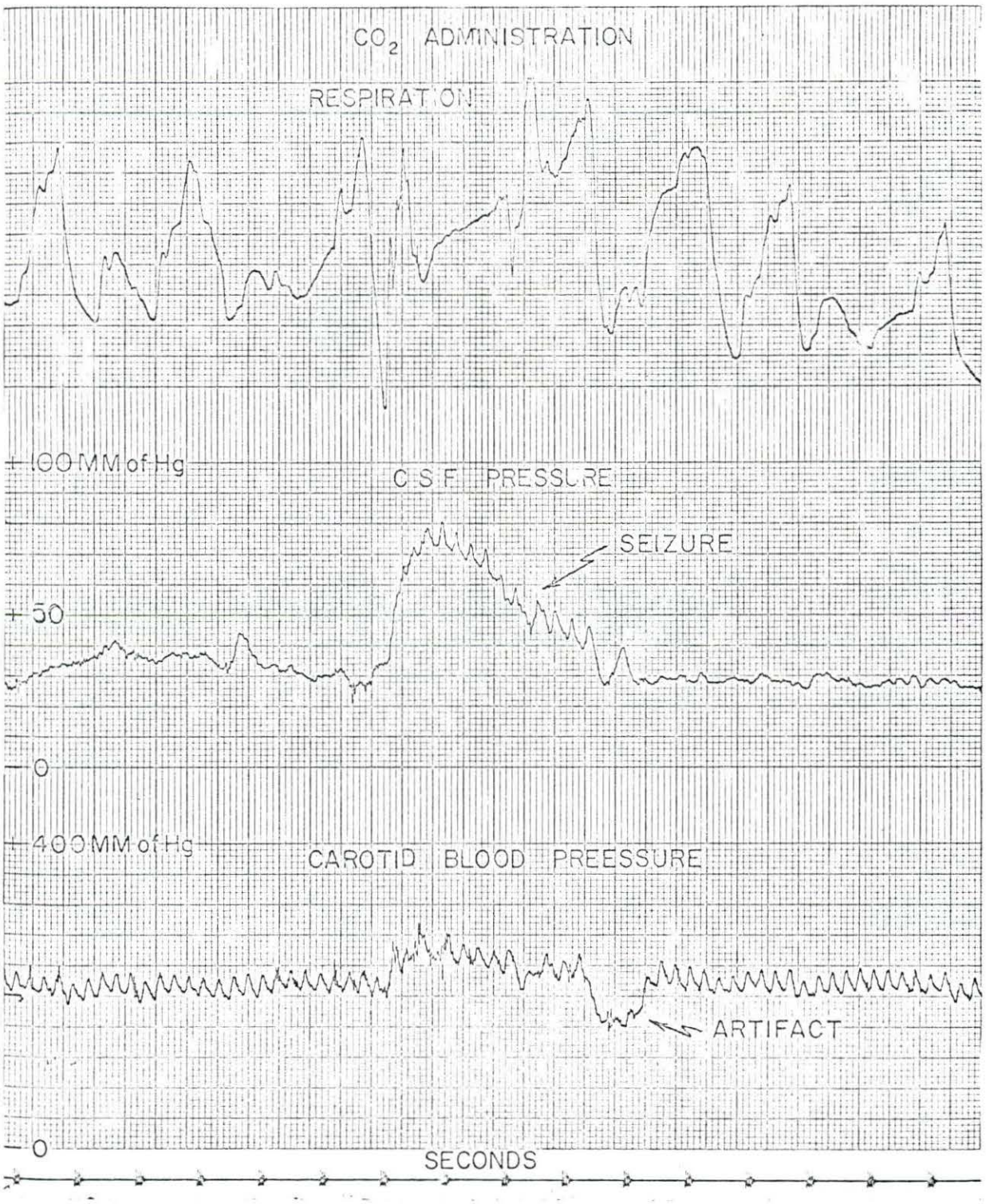

CO_2 ADMINISTRATION
RESPIRATION
+100MM of Hg
C S F PRESSURE
SEIZURE
+50
0
+400MM of Hg
CAROTID BLOOD PREESSURE
ARTIFACT
0
SECONDS

Figure 4. Representative control tracings of blood pressure, CSF pressure and respiratory rate from a sheep (No. 7) made before the insulin experiment.

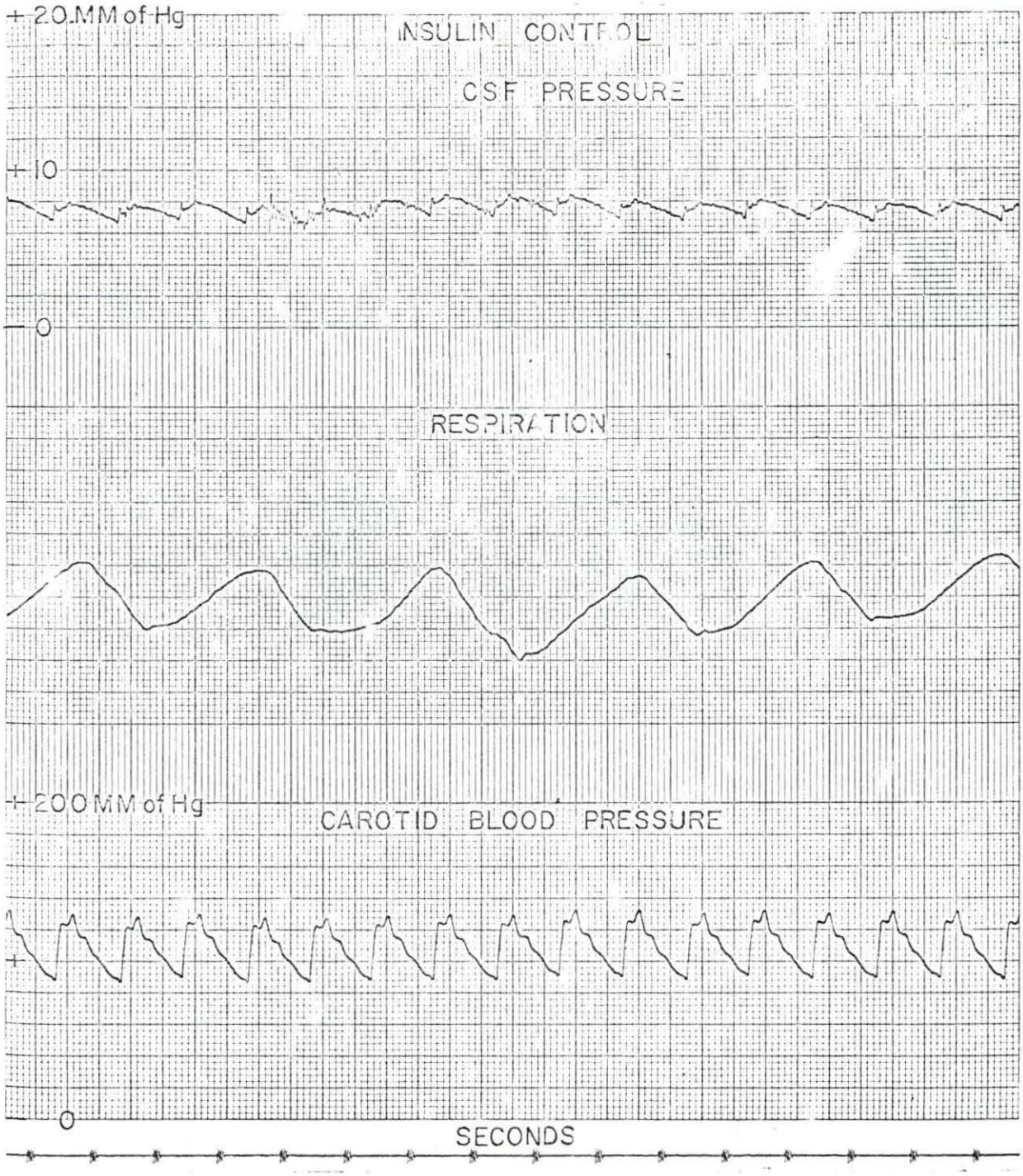

+ 20 MM of Hg
INSULIN CONTROL
CSF PRESSURE
+10
0
RESPIRATION
+200 MM of Hg
CAROTID BLOOD PRESSURE
0
SECONDS

Figure 5. Representative tracings of blood pressure, CSF pressure and respiratory rate from the same sheep as Figure 4 (No. 7) while in insulin shock stupor following seizures. Note the slight influence of respiration on the CSF pressure. The sheep was lying on its sternum.

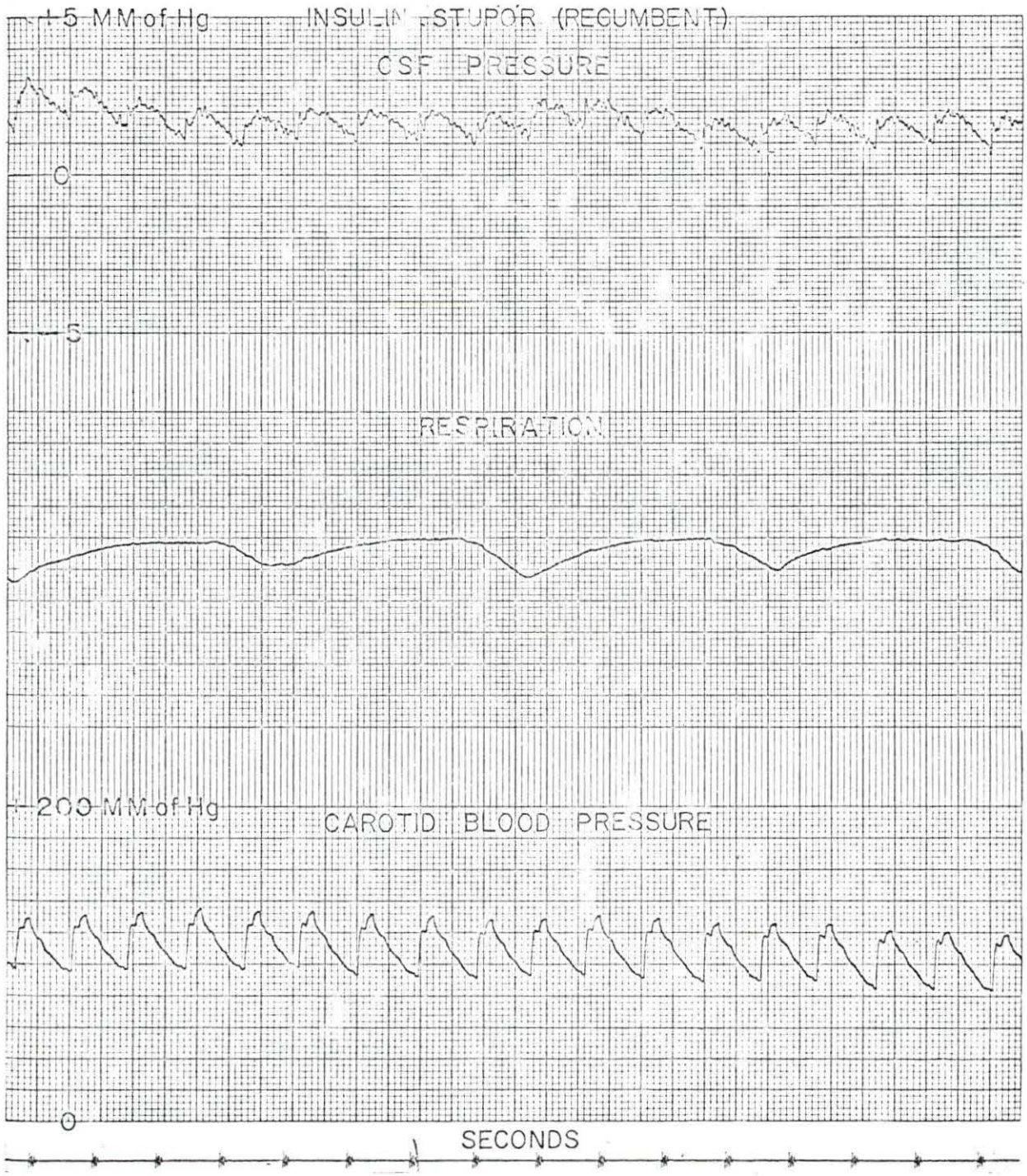

+5 MM of Hg
INSULIN STUPOR (RECUMBENT)
CSF PRESSURE
0
-5
RESPIRATION
+200 MM of Hg
CAROTID BLOOD PRESSURE
0
SECONDS

Figure 6. Representative tracings of blood pressure, CSF pressure and respiratory rate from a sheep (No. 21) before it was given heptachlor.

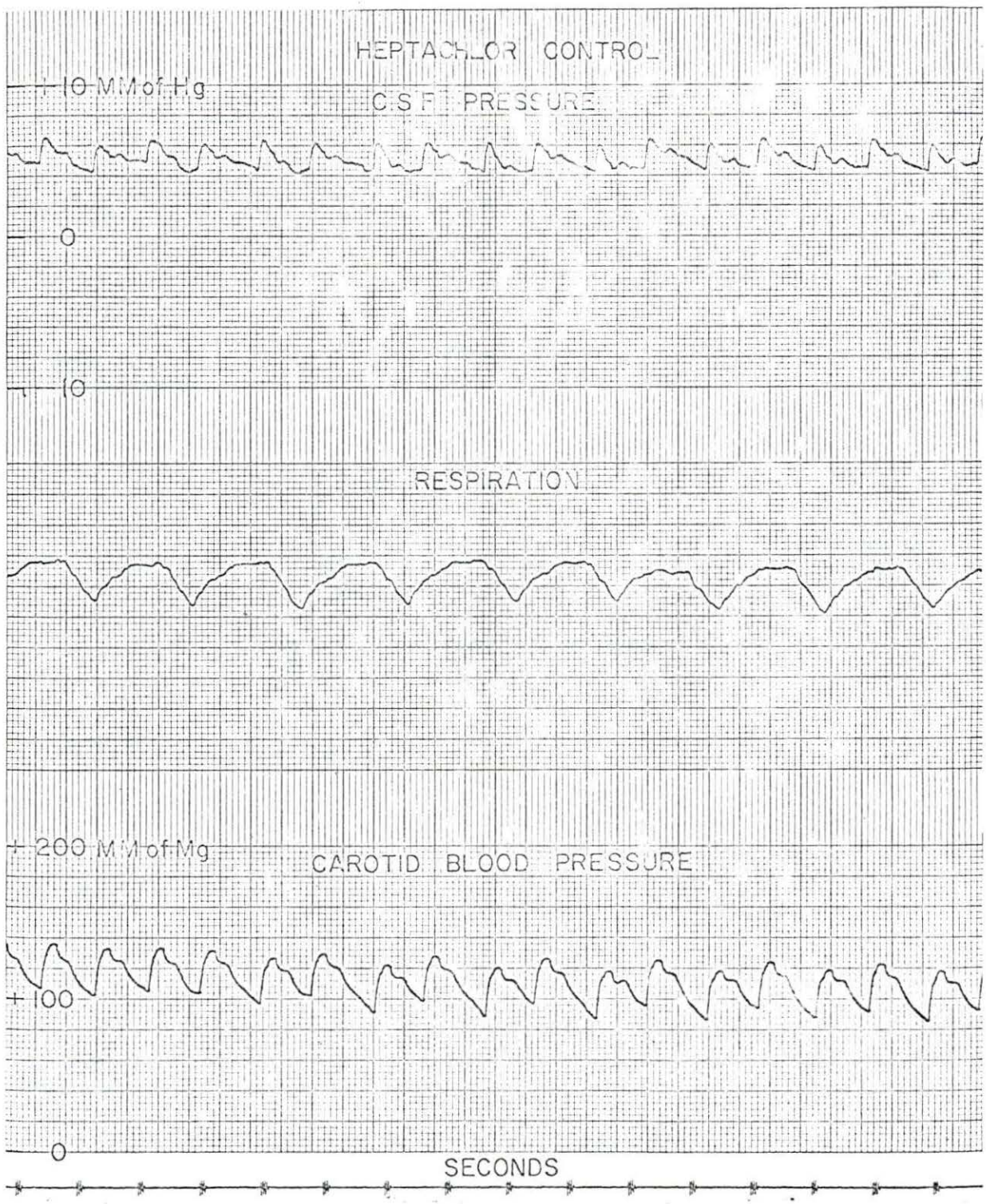

HEPTACHLOR CONTROL
+10 MM of Hg
CSF PRESSURE
0
-10
RESPIRATION
+200 MM of Mg
CAROTID BLOOD PRESSURE
+100
0
SECONDS

Figure 7. Representative tracings of blood pressure, CSF pressure and respiratory rate from the same sheep as Figure 6 (No. 21) while exhibiting intermittent whole body muscle spasms 6 hours after being given 150 mg/kg heptachlor.

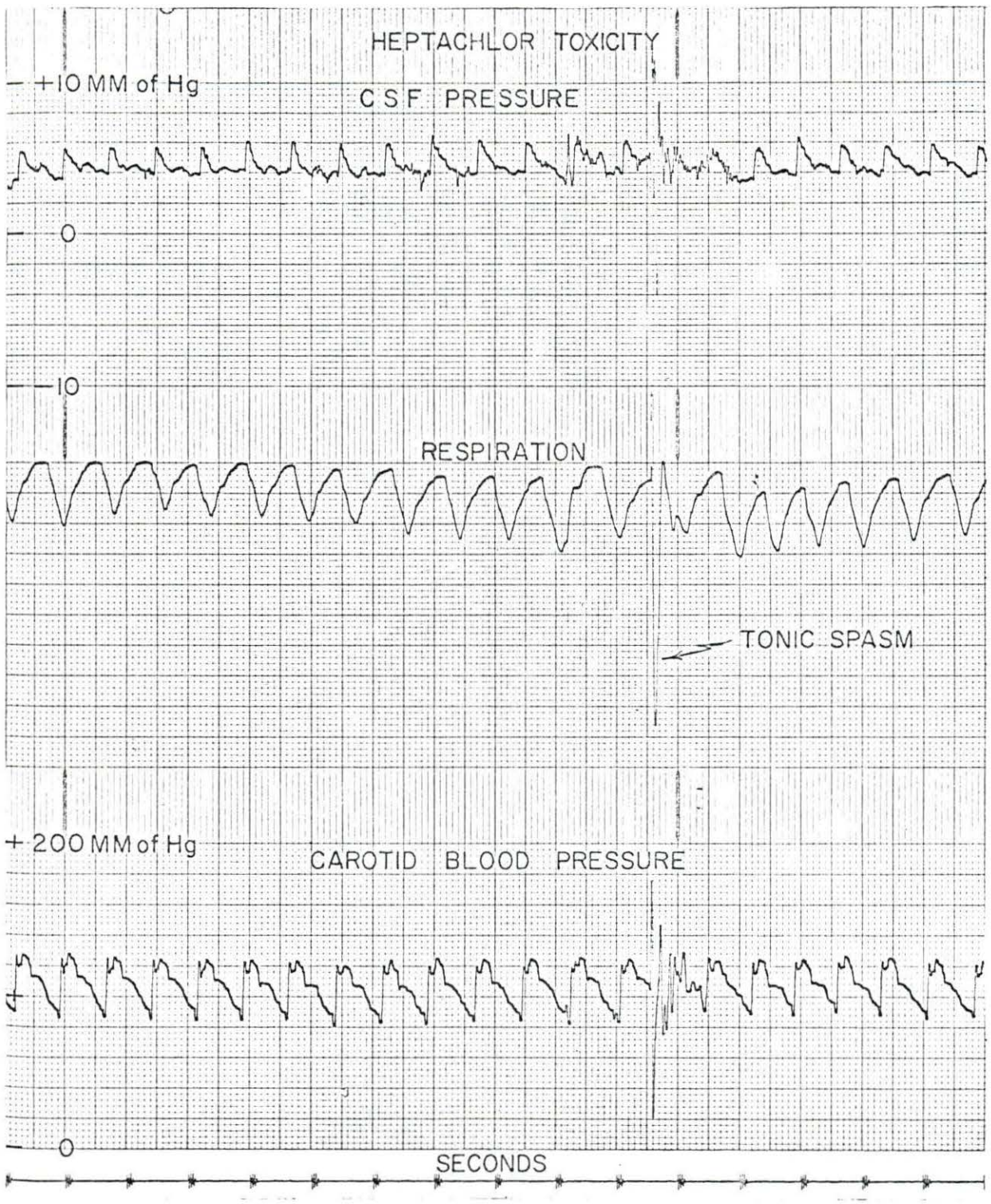

HEPTACHLOR TOXICITY
+10 MM of Hg
C S F PRESSURE
0
-10
RESPIRATION
TONIC SPASM
+200 MM of Hg
CAROTID BLOOD PRESSURE
0
SECONDS

Figure 8. Mean blood and CSF levels of glucose, GOT, GPT, and CSF protein before surgery (CONTROL) and after recovering from surgery (POST).

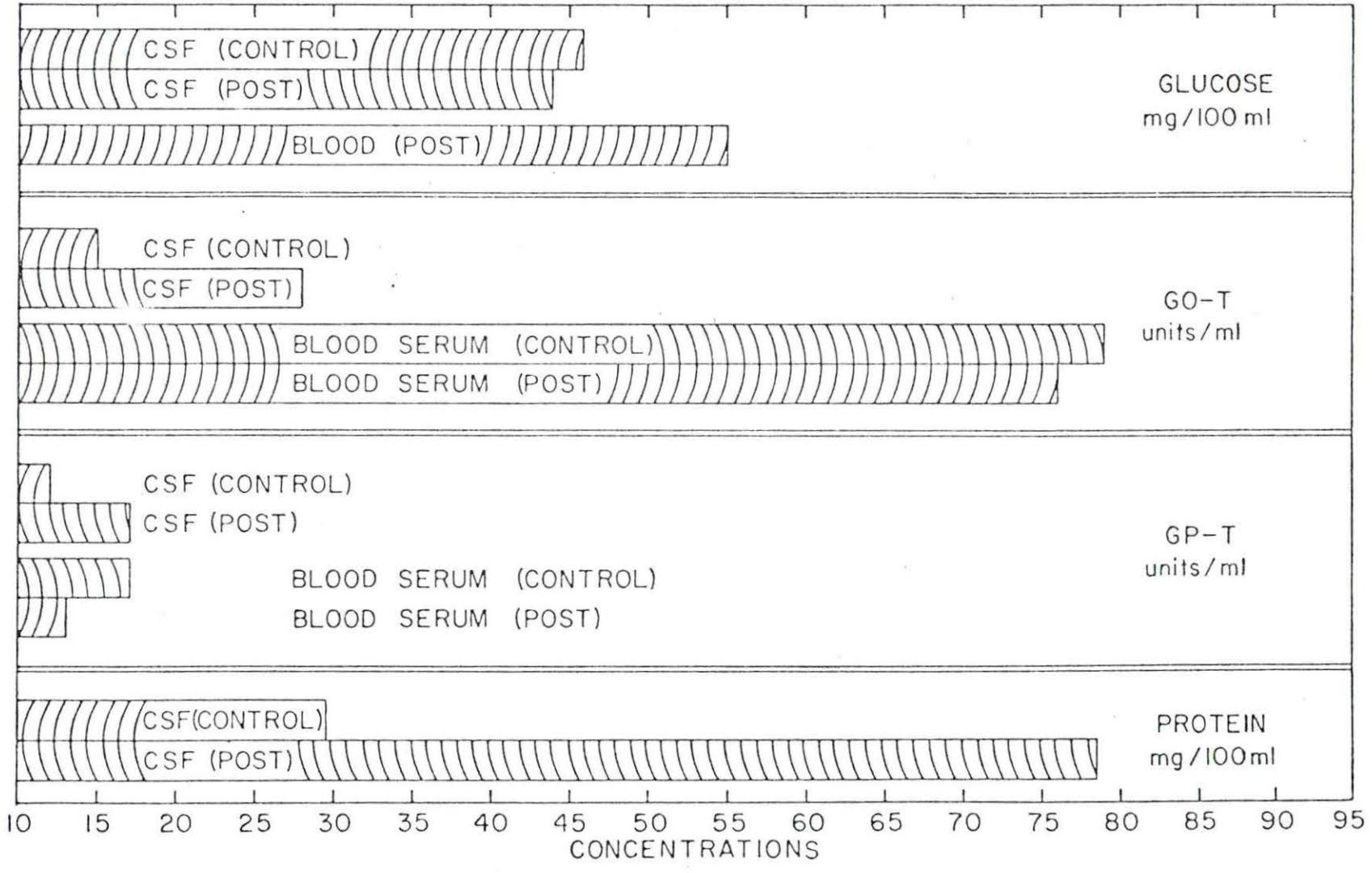

CSF (CONTROL)
CSF (POST)
BLOOD (POST)
GLUCOSE
mg/100 ml
CSF (CONTROL)
CSF (POST)
BLOOD SERUM (CONTROL)
BLOOD SERUM (POST)
GO-T
units/ml
CSF (CONTROL)
CSF (POST)
BLOOD SERUM (CONTROL)
BLOOD SERUM (POST)
GP-T
units/ml
CSF(CONTROL)
CSF (POST)
PROTEIN
mg/100ml
10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95
CONCENTRATIONS

Figure 9. Mean blood serum and CSF levels of sodium, potassium, calcium and magnesium before surgery (CONTROL) and after recovering from surgery (POST).

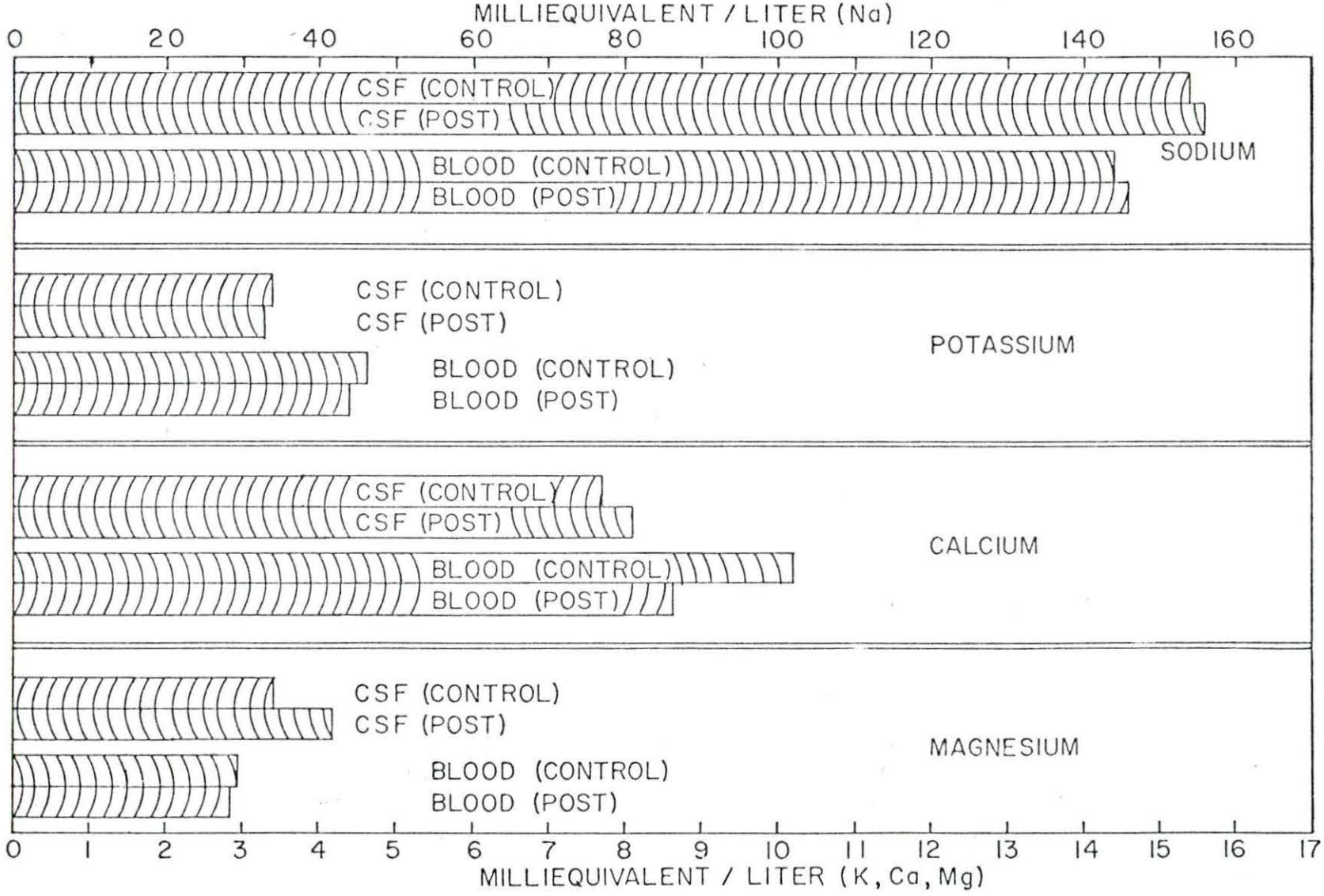
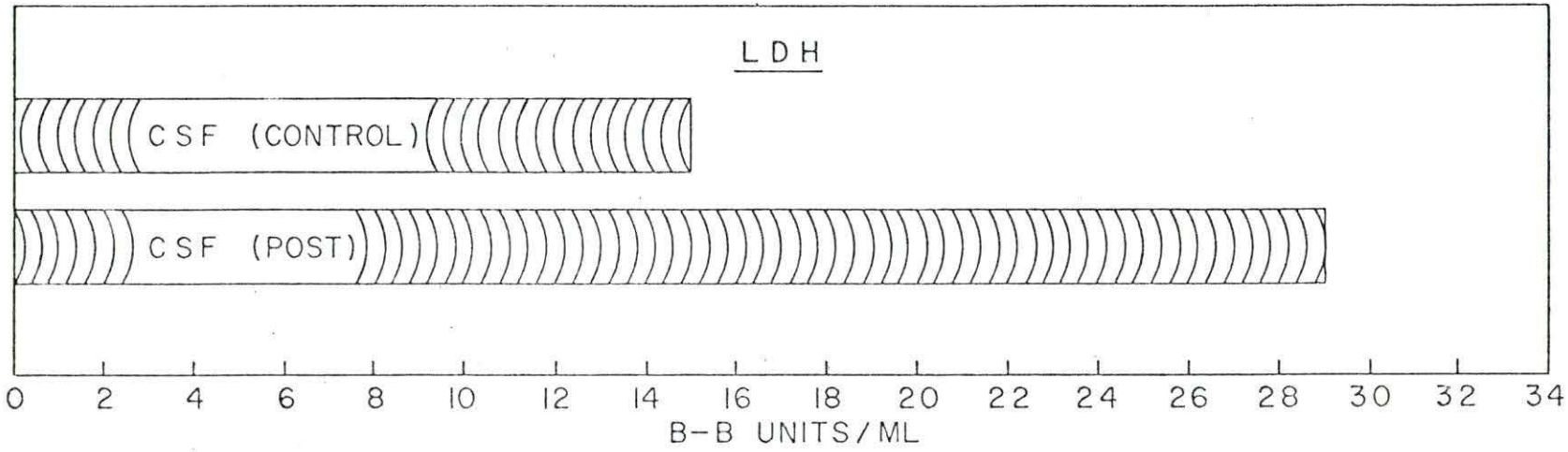

MILLIEQUIVALENT / LITER (Na)
0
20
40
60
80
100
120
140
160
CSF (CONTROL)
CSF (POST)
BLOOD (CONTROL)
BLOOD (POST)
SODIUM
CSF (CONTROL)
CSF (POST)
BLOOD (CONTROL)
BLOOD (POST)
POTASSIUM
CSF (CONTROL)
CSF (POST)
BLOOD (CONTROL)
BLOOD (POST)
CALCIUM
CSF (CONTROL)
CSF (POST)
BLOOD (CONTROL)
BLOOD (POST)
MAGNESIUM
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
MILLIEQUIVALENT / LITER (K, Ca, Mg)

Figure 10. Mean blood serum and CSF levels of LDH activity before surgery (CONTROL) and after recovering from surgery (POST).


LDH
CSF (CONTROL)
CSF (POST)
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
B-B UNITS/ML

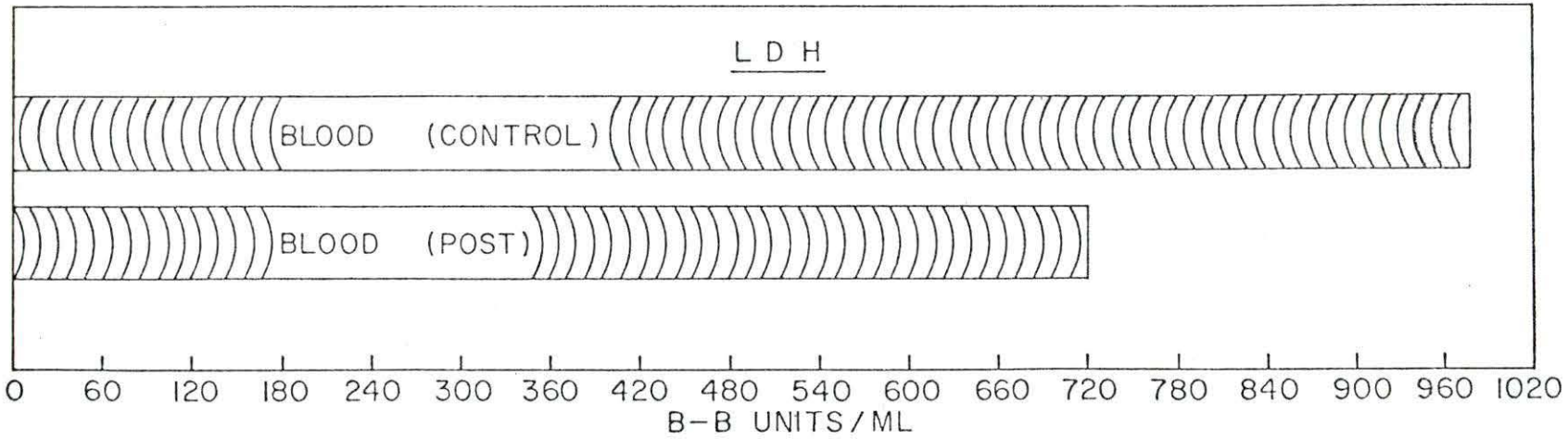

LDH
BLOOD (CONTROL)
BLOOD (POST)
0 60 120 180 240 300 360 420 480 540 600 660 720 780 840 900 960 1020
B-B UNITS/ML

Figure 11. Mean blood and CSF glucose values before, during and following inhalation of the CO_2-O_2 mixture (CO_2) and room air (CONTROL).

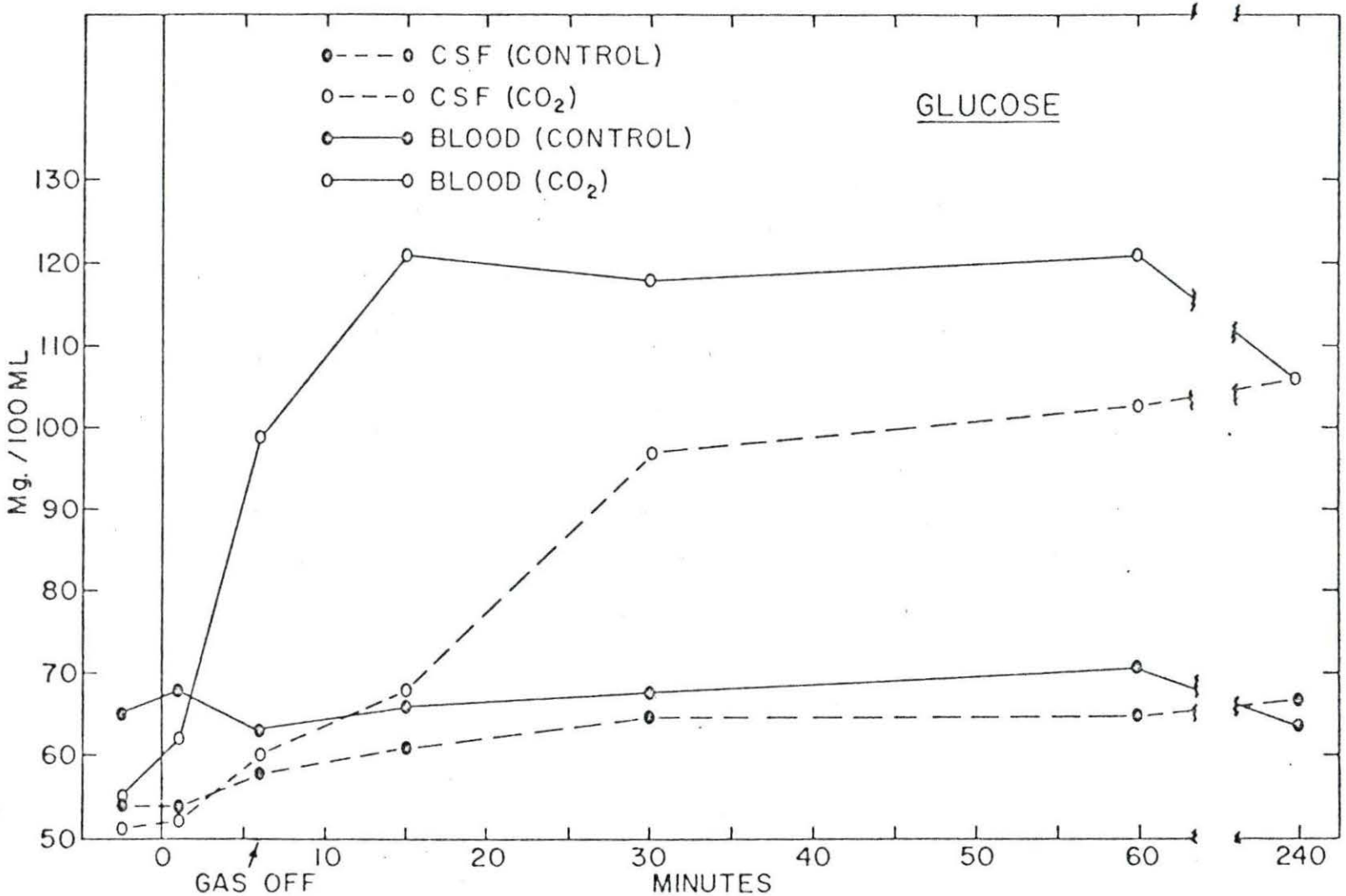

CSF (CONTROL)
CSF (CO_2)
BLOOD (CONTROL)
BLOOD (CO_2)
GLUCOSE
Mg. / 100 ML
130
120
110
100
90
80
70
60
50
0
10
20
30
40
50
60
240
GAS OFF
MINUTES

Figure 12. Mean blood serum and CSF GOT activities before, during and following inhalation of the CO_2-O_2 mixture (CO_2) and room air (CONTROL).

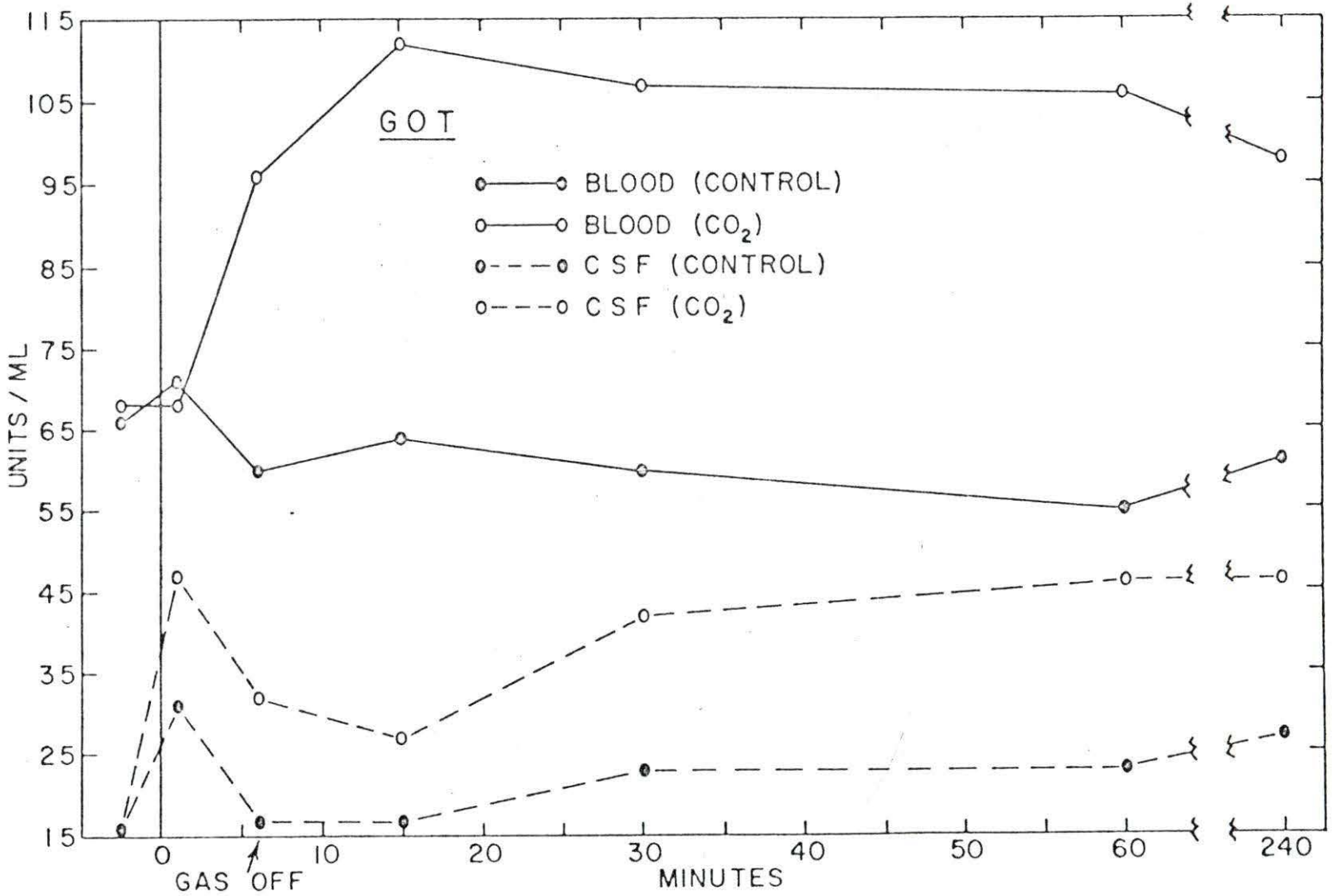

GOT
BLOOD (CONTROL)
BLOOD (CO_2)
C S F (CONTROL)
C S F (CO_2)
UNITS / ML
115
105
95
85
75
65
55
45
35
25
15
0
10
20
30
40
50
60
240
GAS OFF
MINUTES

Figure 13. Mean blood serum and CSF potassium values before, during and following inhalation of the CO_2-O_2 mixture (CO_2) and room air (CONTROL).

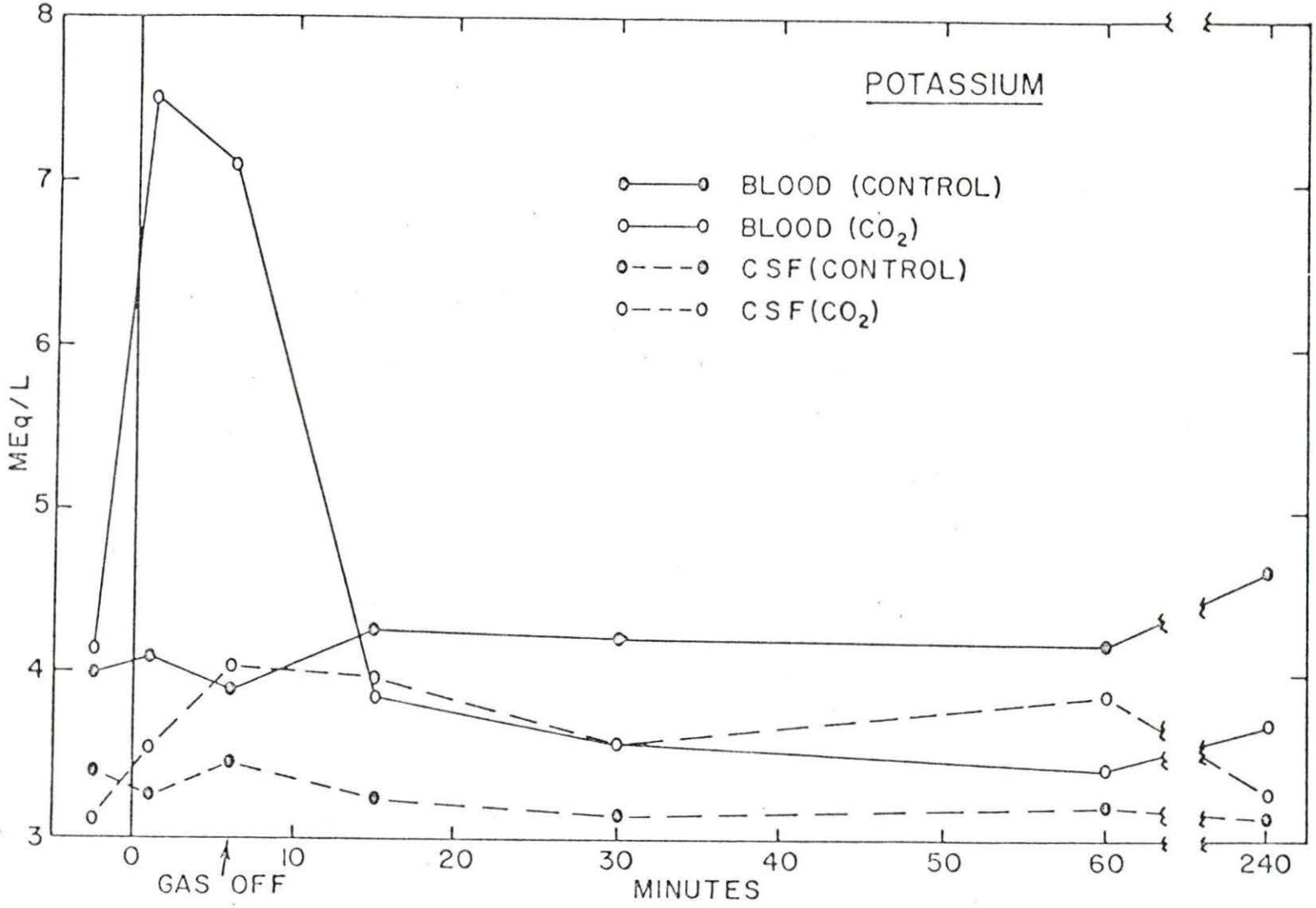

POTASSIUM
BLOOD (CONTROL)
BLOOD (CO_2)
CSF(CONTROL)
CSF(CO_2)
MEq/L
8
7
6
5
4
3
0
10
20
30
40
50
60
240
GAS OFF
MINUTES

Figure 14. Mean blood serum and CSF sodium values before, during and following inhalation of the CO_2-O_2 mixture (CO_2) and room air (CONTROL).

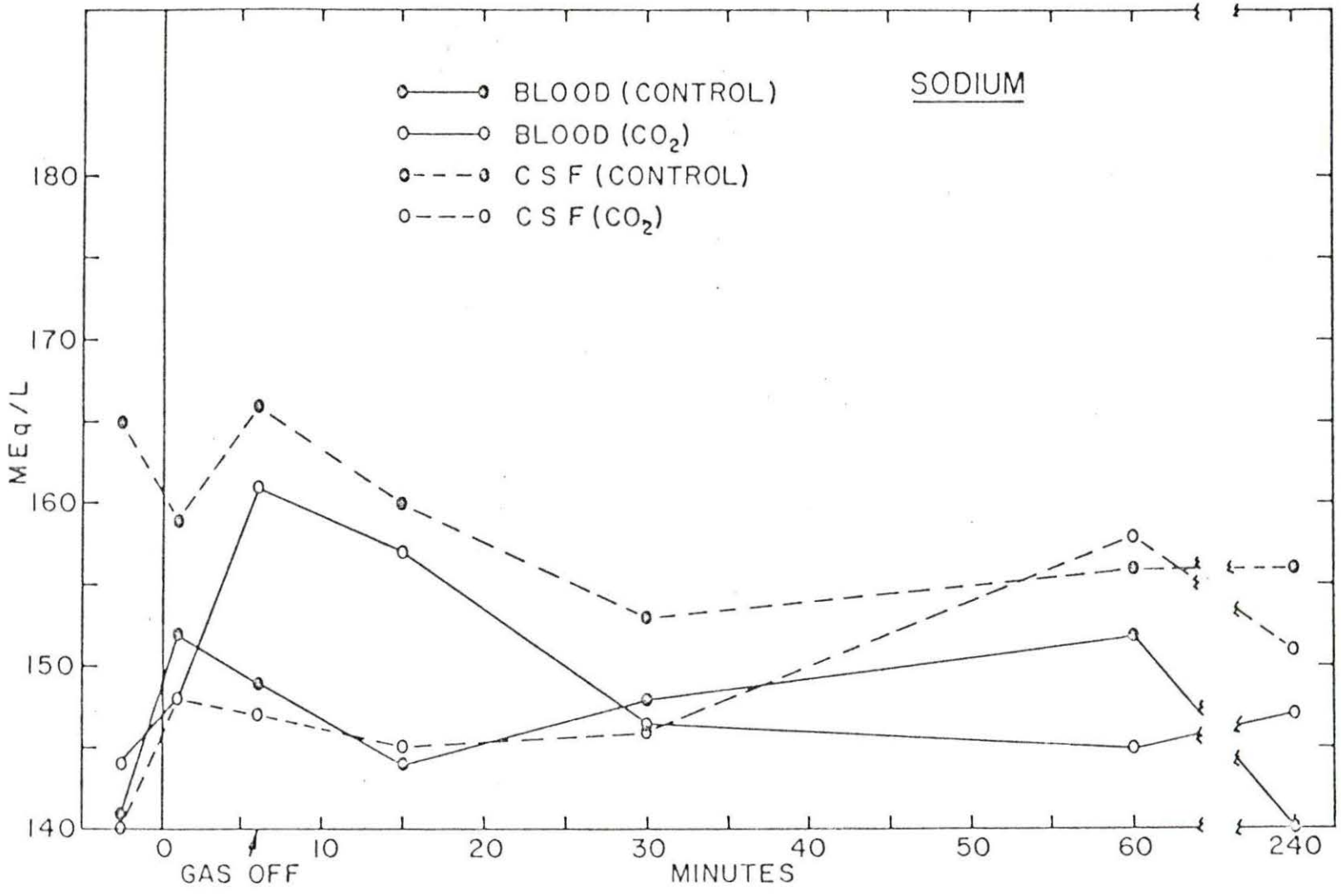

SODIUM
BLOOD (CONTROL)
BLOOD (CO_2)
C S F (CONTROL)
C S F (CO_2)
MEq/L
180
170
160
150
140
0
10
20
30
40
50
60
240
GAS OFF
MINUTES

Figure 15. Mean blood and CSF pH values before, during and following inhalation of the CO_2-O_2 mixture (CO_2) and room air (CONTROL).

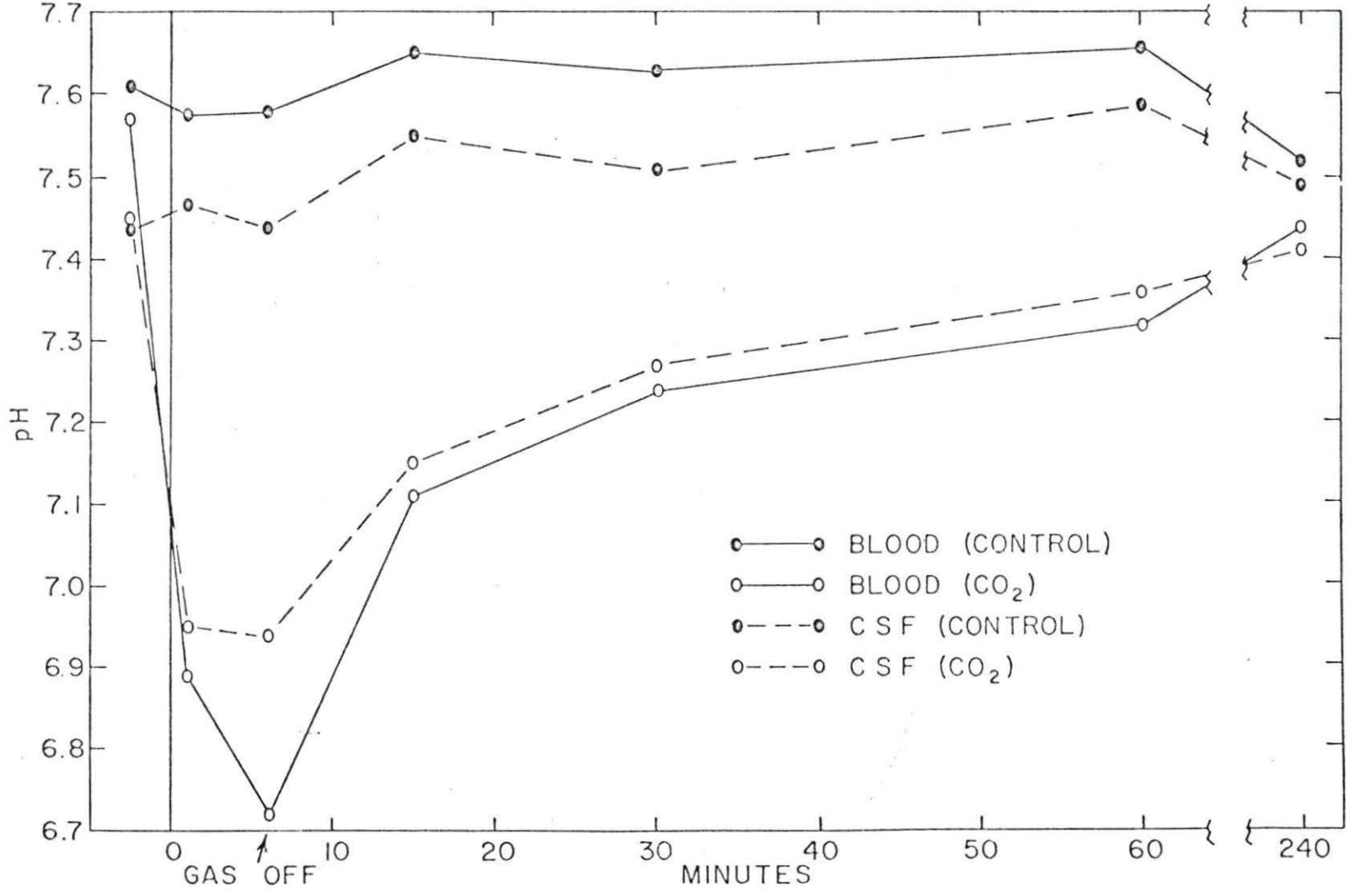

7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
pH
0
GAS OFF
10
20
30
40
50
60
240
MINUTES
BLOOD (CONTROL)
BLOOD (CO_2)
C S F (CONTROL)
C S F (CO_2)

Figure 16. Mean blood PCV percentages before, during, and following inhalation of the CO_2-O_2 mixture (CO_2) and room air (CONTROL).

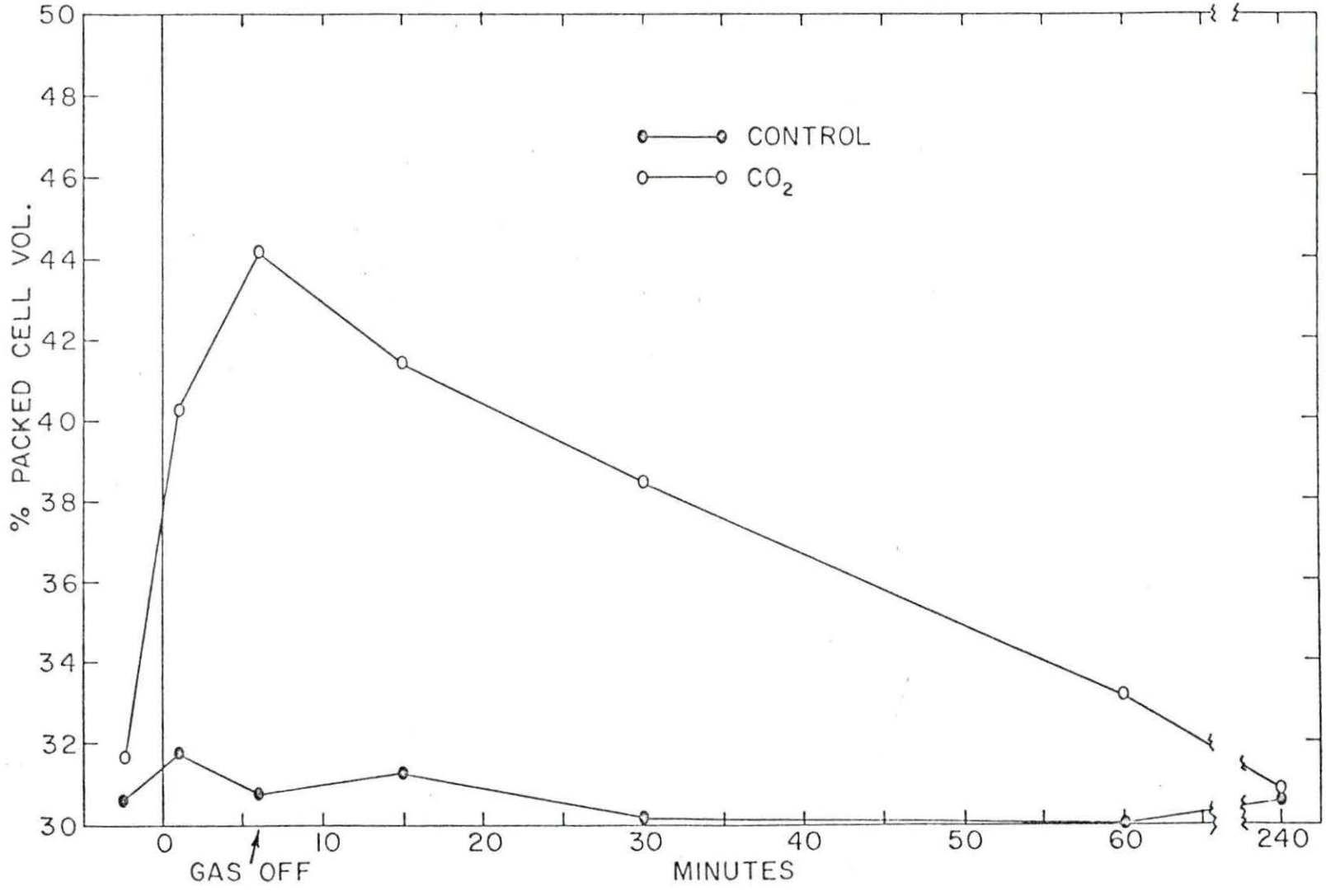

50
48
46
44
42
40
38
36
34
32
30
% PACKED CELL VOL.
CONTROL
CO_2
0
10
20
30
40
50
60
240
GAS OFF
MINUTES

Figure 17. Mean blood serum and CSF calcium values before, during and following inhalation of the CO_2-O_2 mixture (CO_2) and room air (CONTROL).

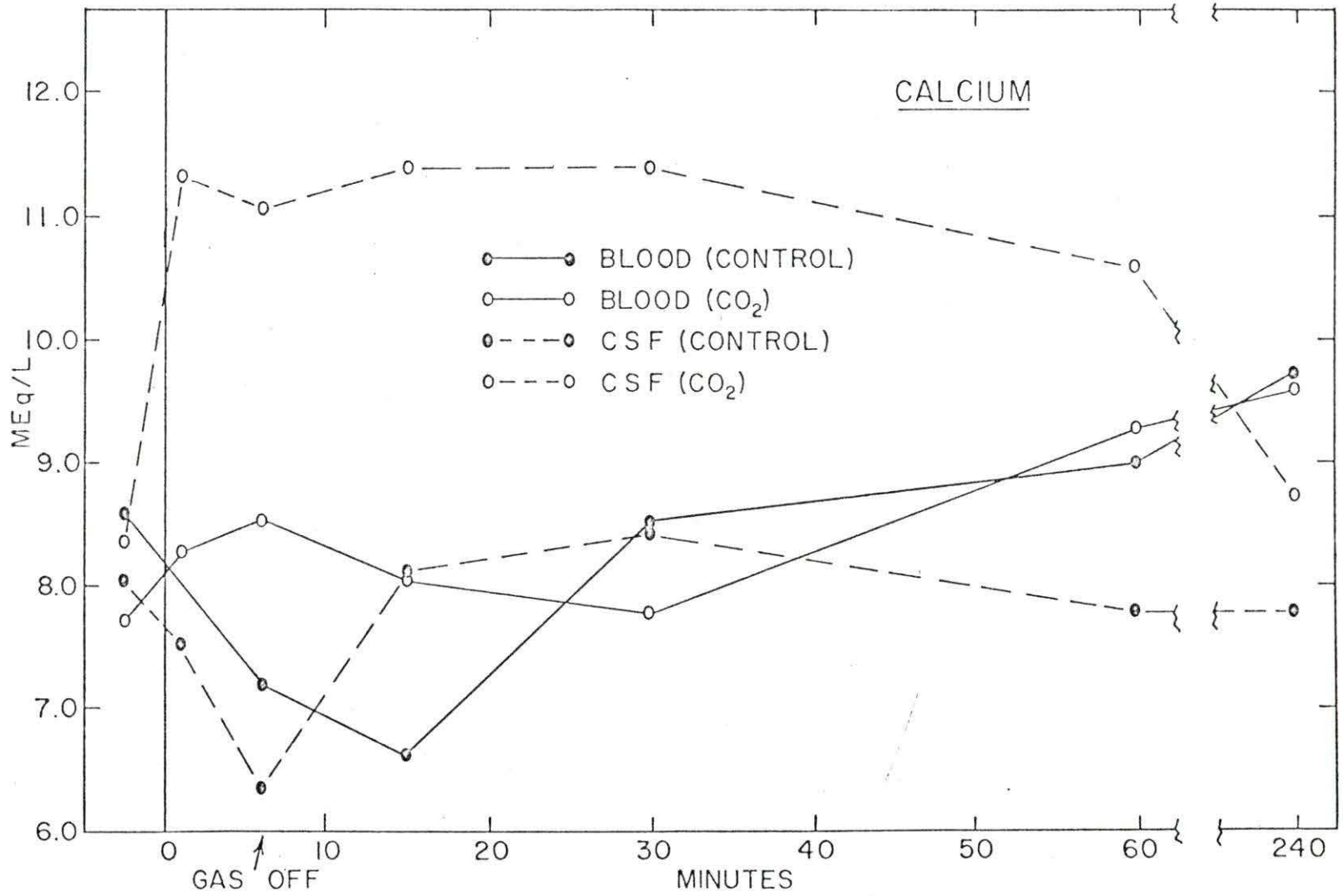

CALCIUM
BLOOD (CONTROL)
BLOOD (CO_2)
CSF (CONTROL)
CSF (CO_2)
MEq/L
12.0
11.0
10.0
9.0
8.0
7.0
6.0
0
10
20
30
40
50
60
240
GAS OFF
MINUTES

Figure 18. Mean blood serum and CSF magnesium values before, during and following inhalation of the CO_2-O_2 mixture (CO_2) and room air (CONTROL).

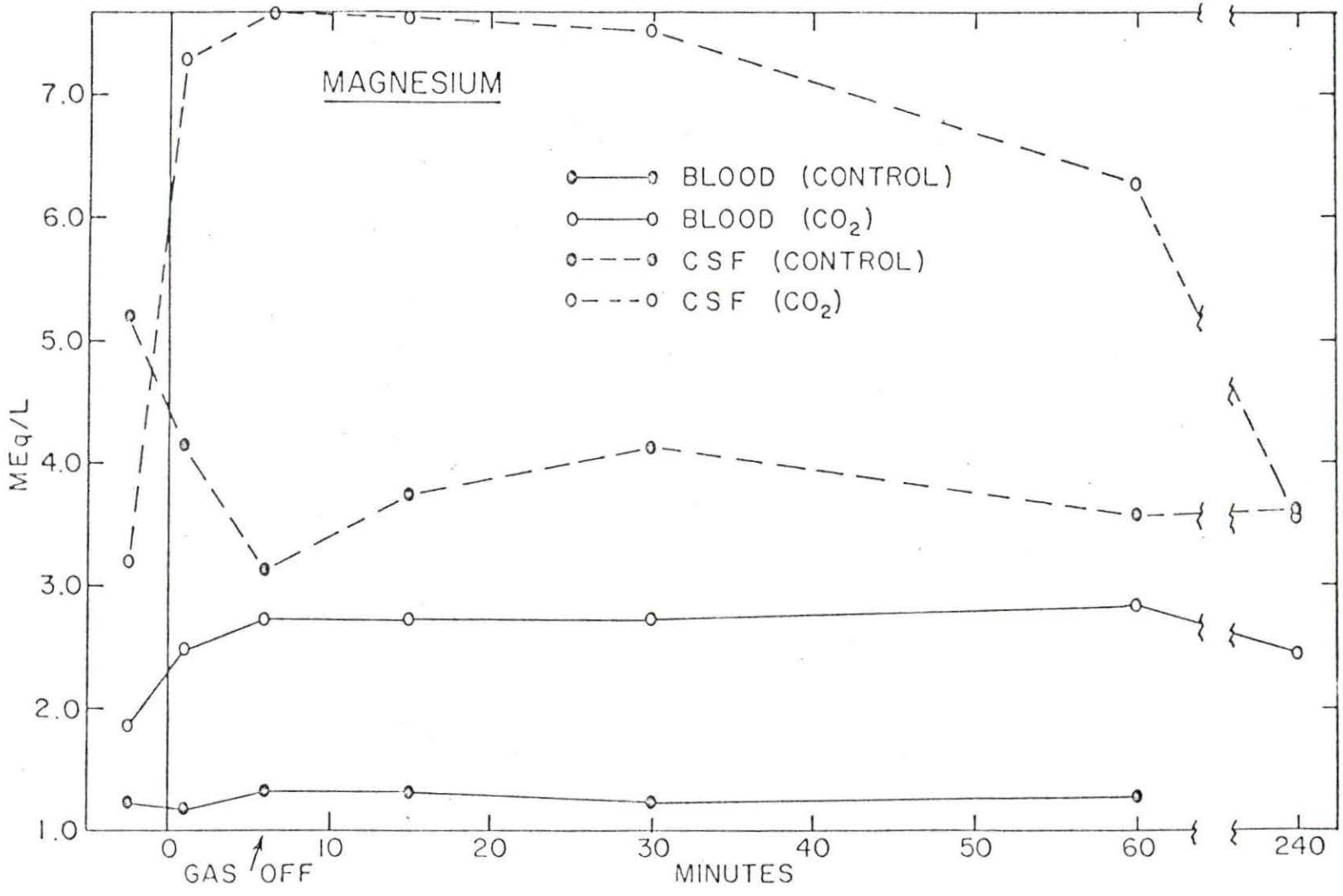

MAGNESIUM
BLOOD (CONTROL)
BLOOD (CO_2)
CSF (CONTROL)
CSF (CO_2)
MEq/L
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
10
20
30
40
50
60
240
GAS OFF
MINUTES

Figure 19. Mean blood pressure, CSF pressure, and heart and respiratory rates before, during and following inhalation of the CO_2-O_2 mixture (CO_2) and room air (CONTROL).

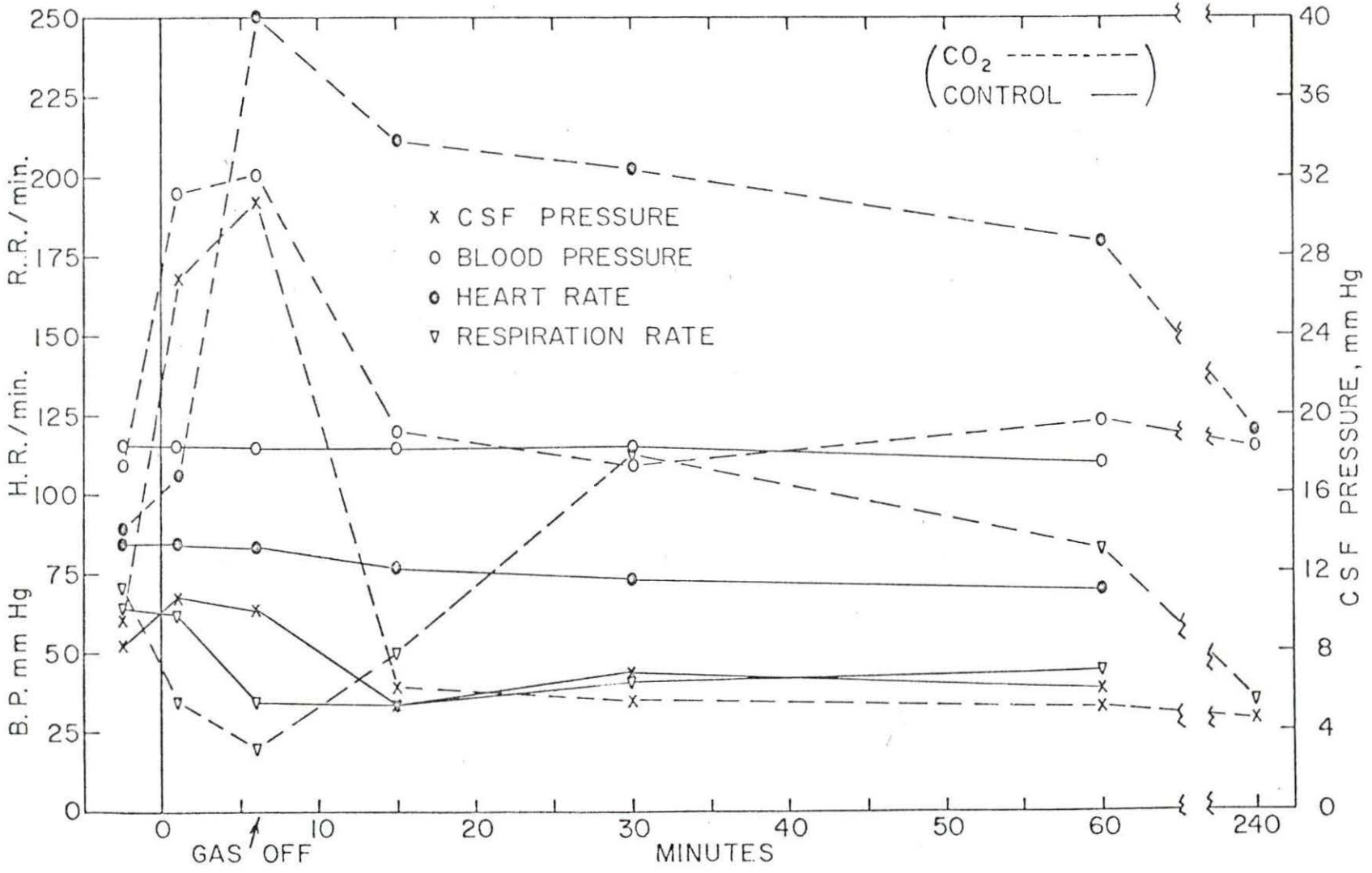

(CO2 ---------
CONTROL ——)
x CSF PRESSURE
o BLOOD PRESSURE
o HEART RATE
▽ RESPIRATION RATE
B.P. mm Hg
H.R./min.
R.R./min.
CSF PRESSURE, mm Hg
250
225
200
175
150
125
100
75
50
25
0
40
36
32
28
24
20
16
12
8
4
0
0
10
20
30
40
50
60
240
GAS OFF
MINUTES

Figure 20. Mean blood and CSF glucose values during insulin shock (INSULIN) and fasting periods (CONTROL). Experimental stages are as follows: Pre = before fast began; 1 = after fasting 24 to 48 hours and before insulin was given; 2 = after insulin was given but before seizures began; 4 = seizures after glucose or glutamic acid was given; 5 = at time of death or recovery or, in the case of controls, at time of euthanasia.

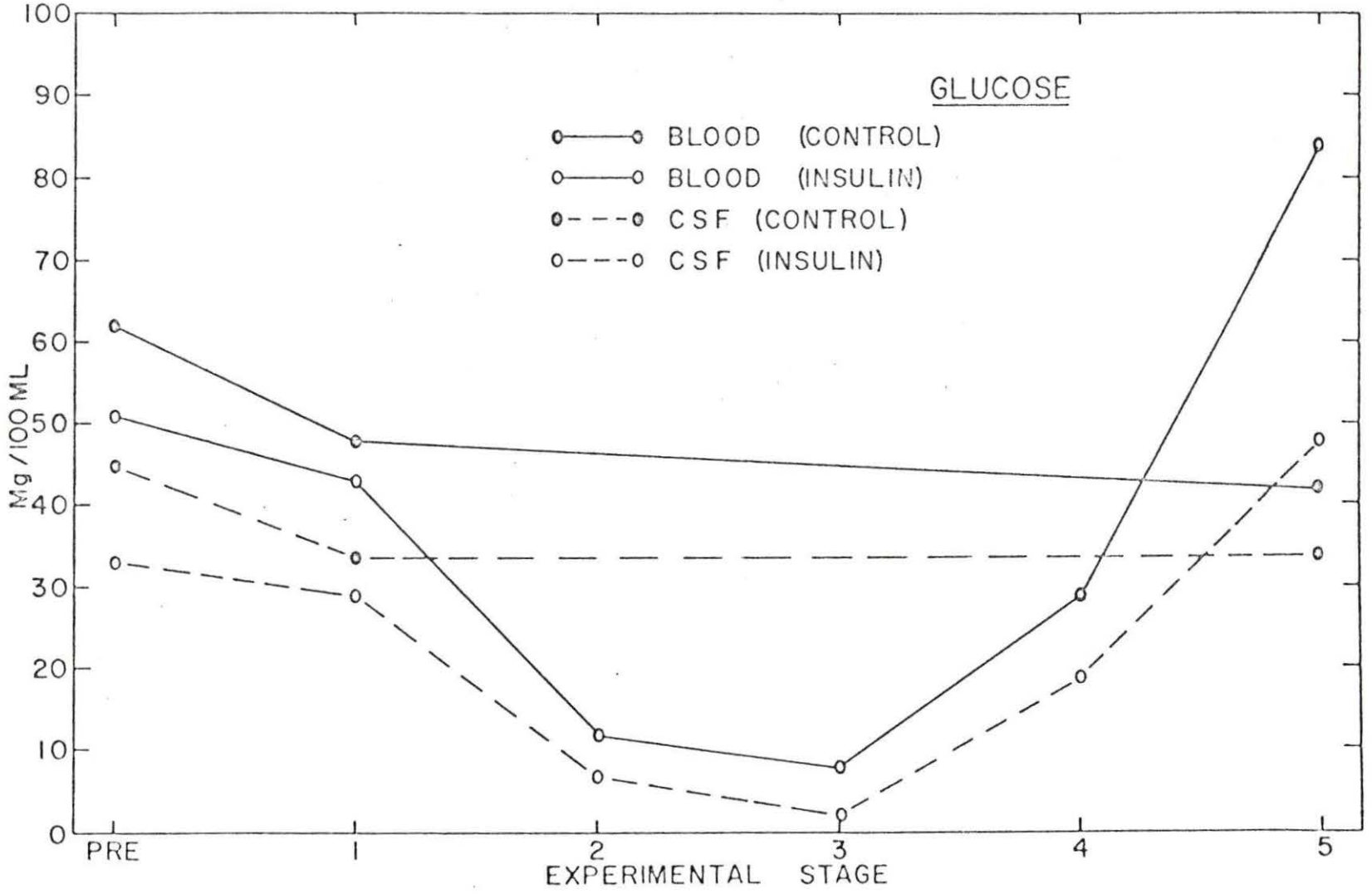

GLUCOSE
BLOOD (CONTROL)
BLOOD (INSULIN)
CSF (CONTROL)
CSF (INSULIN)
Mg/100 ML
100
90
80
70
60
50
40
30
20
10
0
PRE
1
2
3
4
5
EXPERIMENTAL STAGE

Figure 21. Mean blood serum and CSF GOT activities during insulin shock (INSULIN) and fasting periods (CONTROL). Experimental stages are the same as in Figure 20.

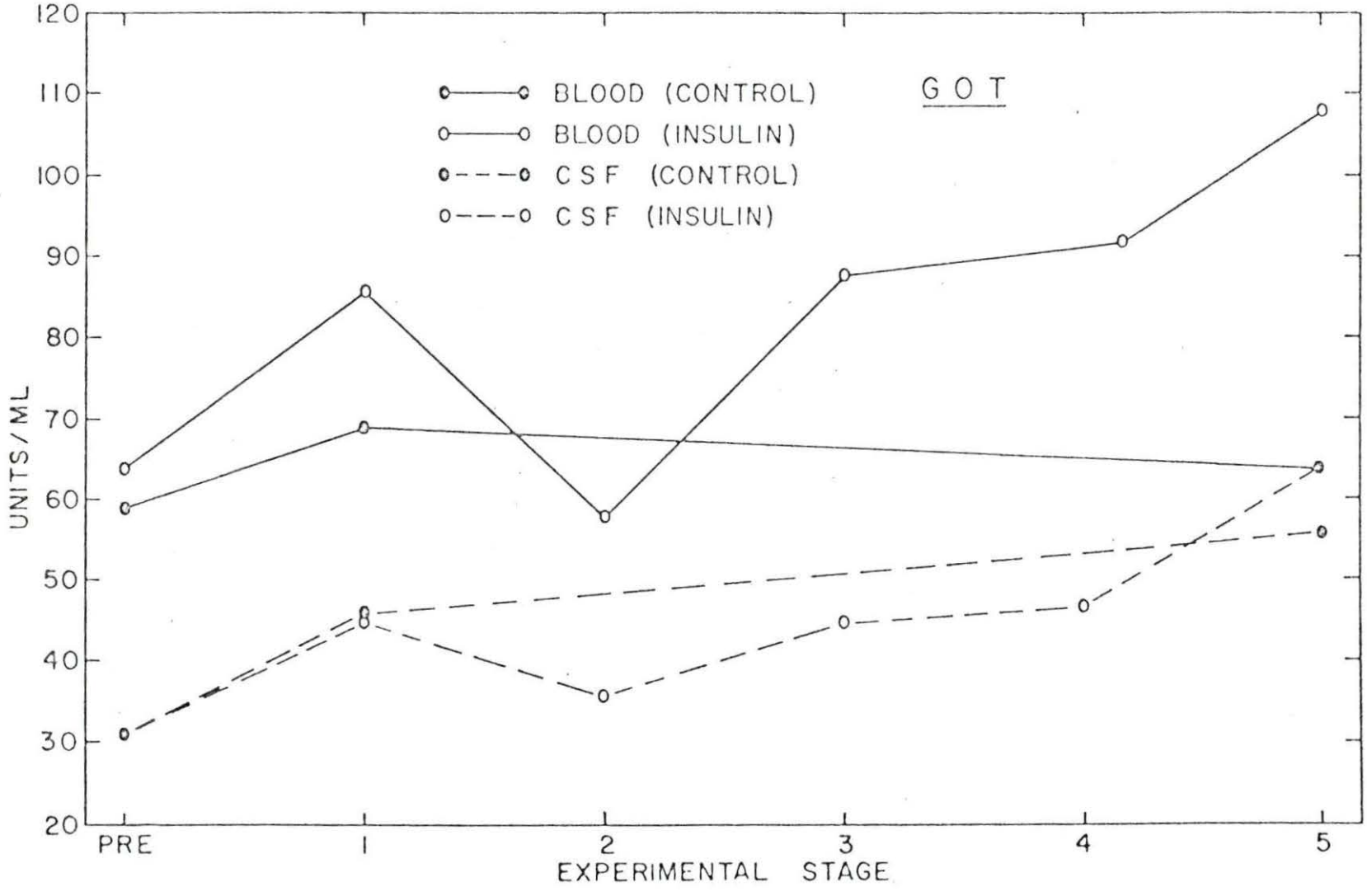

GOT
BLOOD (CONTROL)
BLOOD (INSULIN)
CSF (CONTROL)
CSF (INSULIN)
UNITS / ML
120
110
100
90
80
70
60
50
40
30
20
PRE
1
2
3
4
5
EXPERIMENTAL STAGE

Figure 22. Mean blood serum and CSF GPT activities during insulin shock (INSULIN) and fasting periods (CONTROL). Experimental stages are the same as in Figure 20.

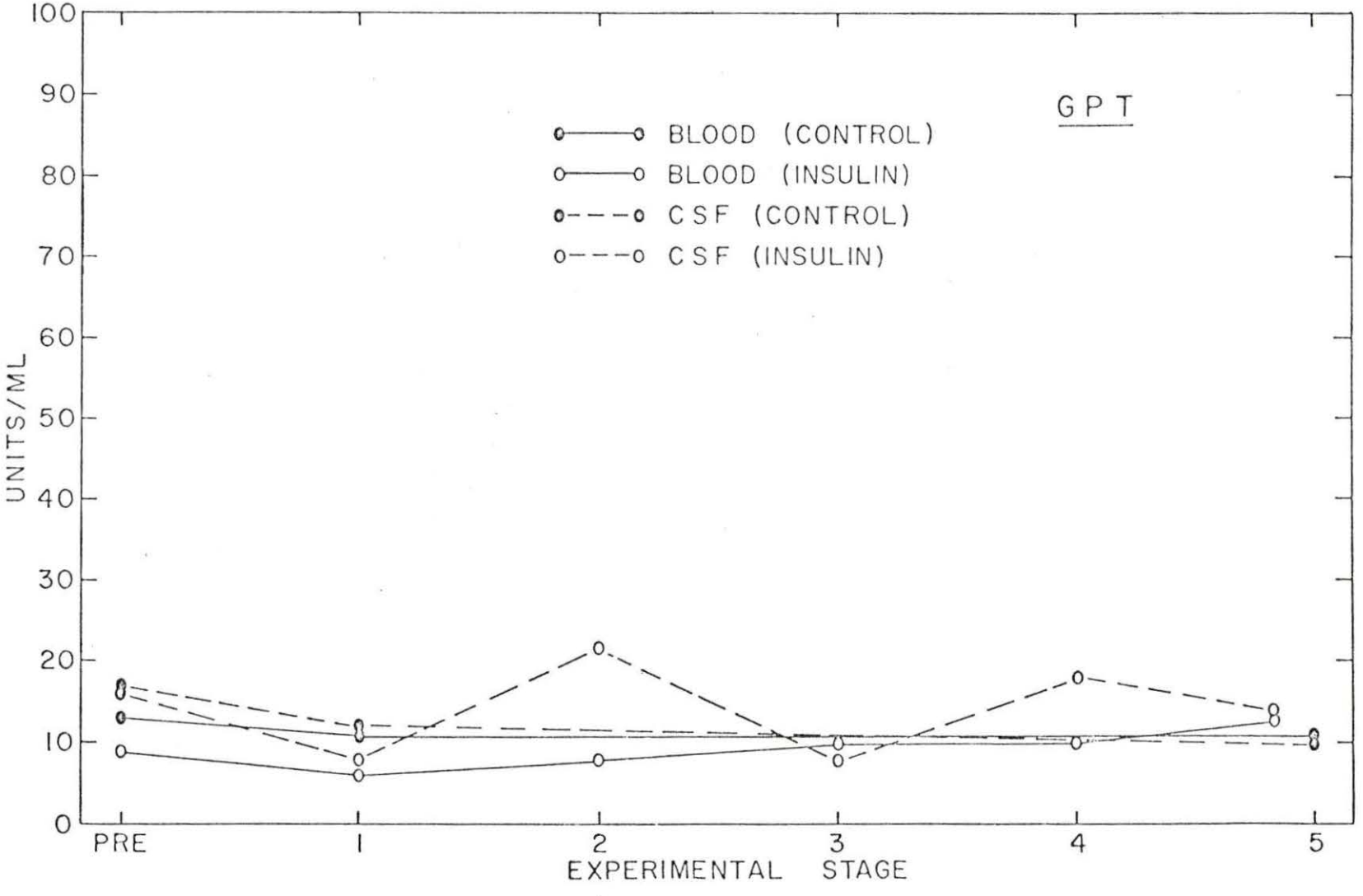

GPT
BLOOD (CONTROL)
BLOOD (INSULIN)
CSF (CONTROL)
CSF (INSULIN)
UNITS/ML
100
90
80
70
60
50
40
30
20
10
0
PRE
1
2
3
4
5
EXPERIMENTAL STAGE

Figure 23. Mean blood and CSF glucose values during heptachlor toxicity.

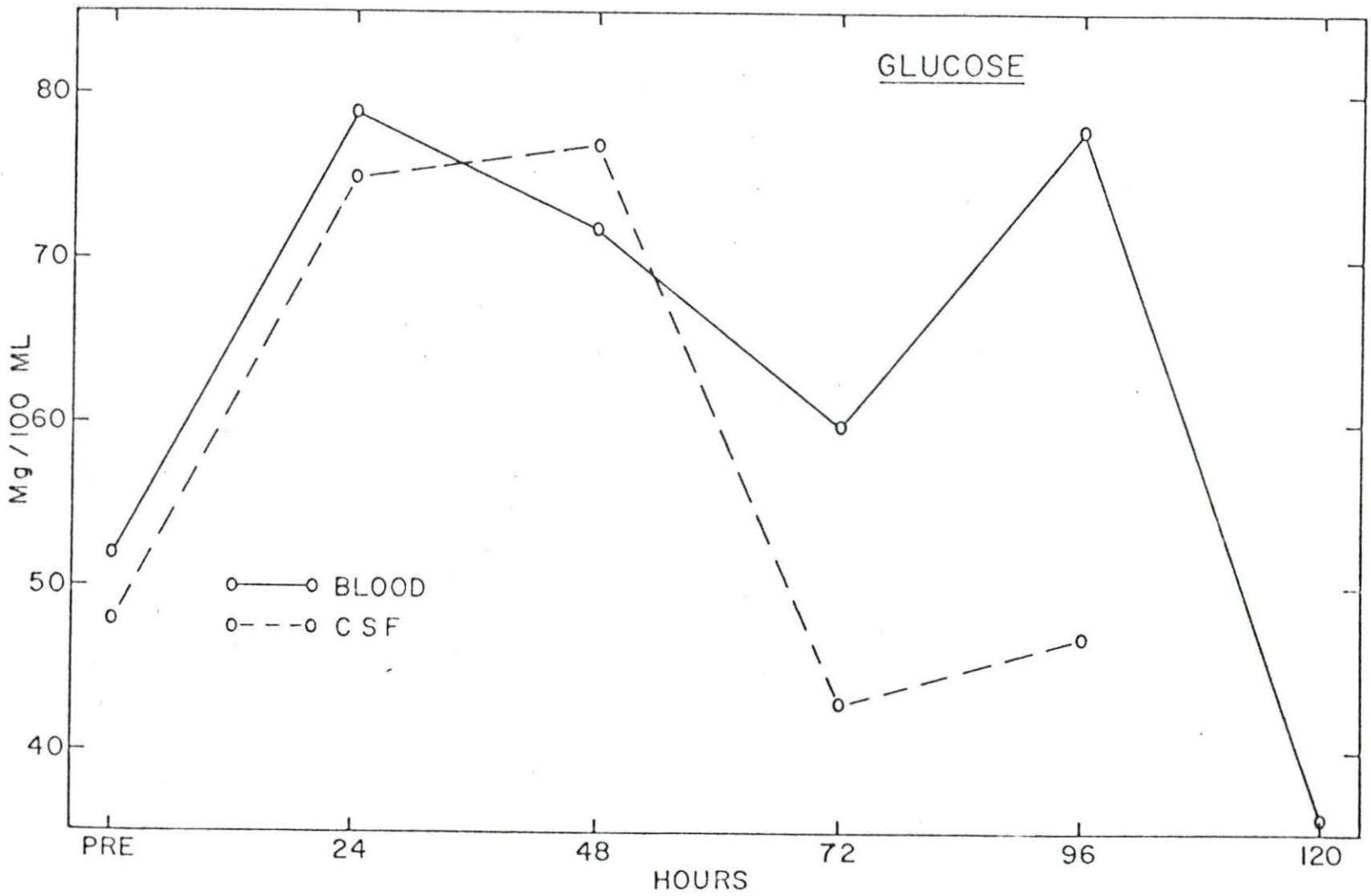

GLUCOSE
80
70
60
50
40
Mg / 100 ML
BLOOD
C S F
PRE
24
48
72
96
120
HOURS

Figure 24. Mean blood serum and CSF transaminase values during heptachlor toxicity.

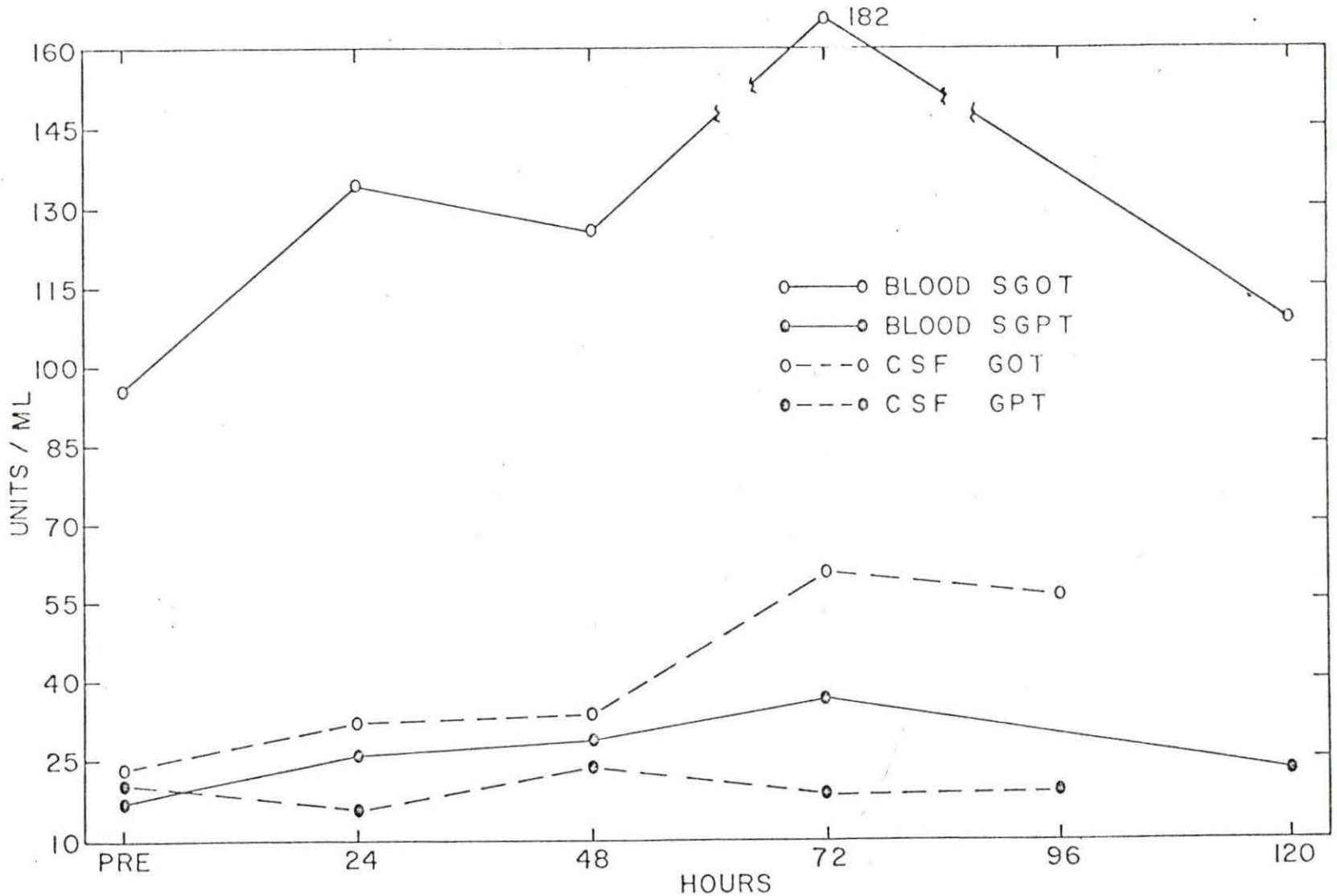

182
160
145
130
115
100
85
70
55
40
25
10
UNITS / ML
BLOOD SGOT
BLOOD SGPT
CSF GOT
CSF GPT
PRE
24
48
72
96
120
HOURS

Figure 25. Mean blood serum and CSF LDH values during heptachlor toxicity.

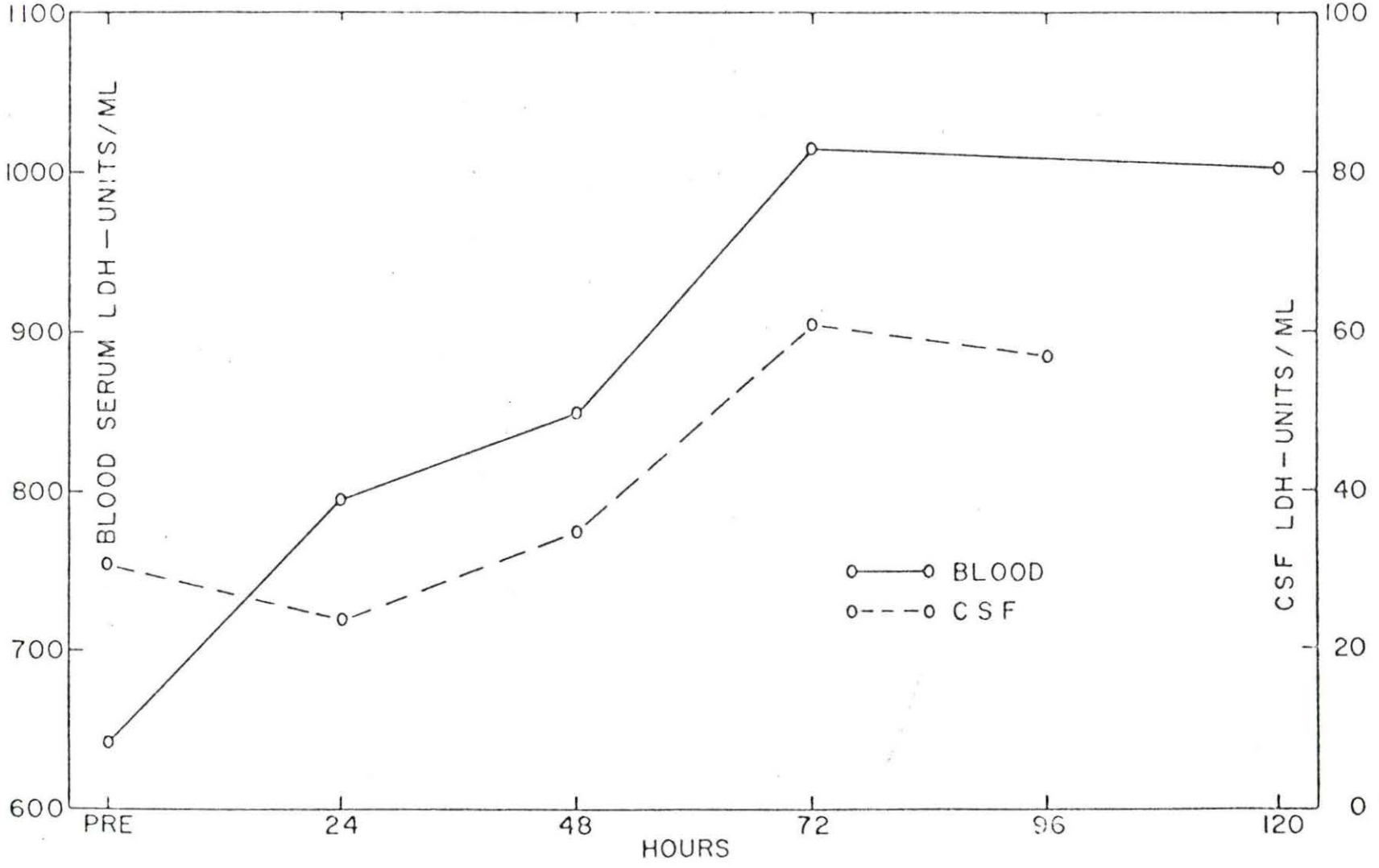

1100
1000
900
800
700
600
BLOOD SERUM LDH – UNITS / ML
100
80
60
40
20
0
CSF LDH – UNITS / ML
PRE
24
48
72
96
120
HOURS
BLOOD
CSF

Figure 26. Mean blood serum and CSF sodium values during heptachlor toxicity.

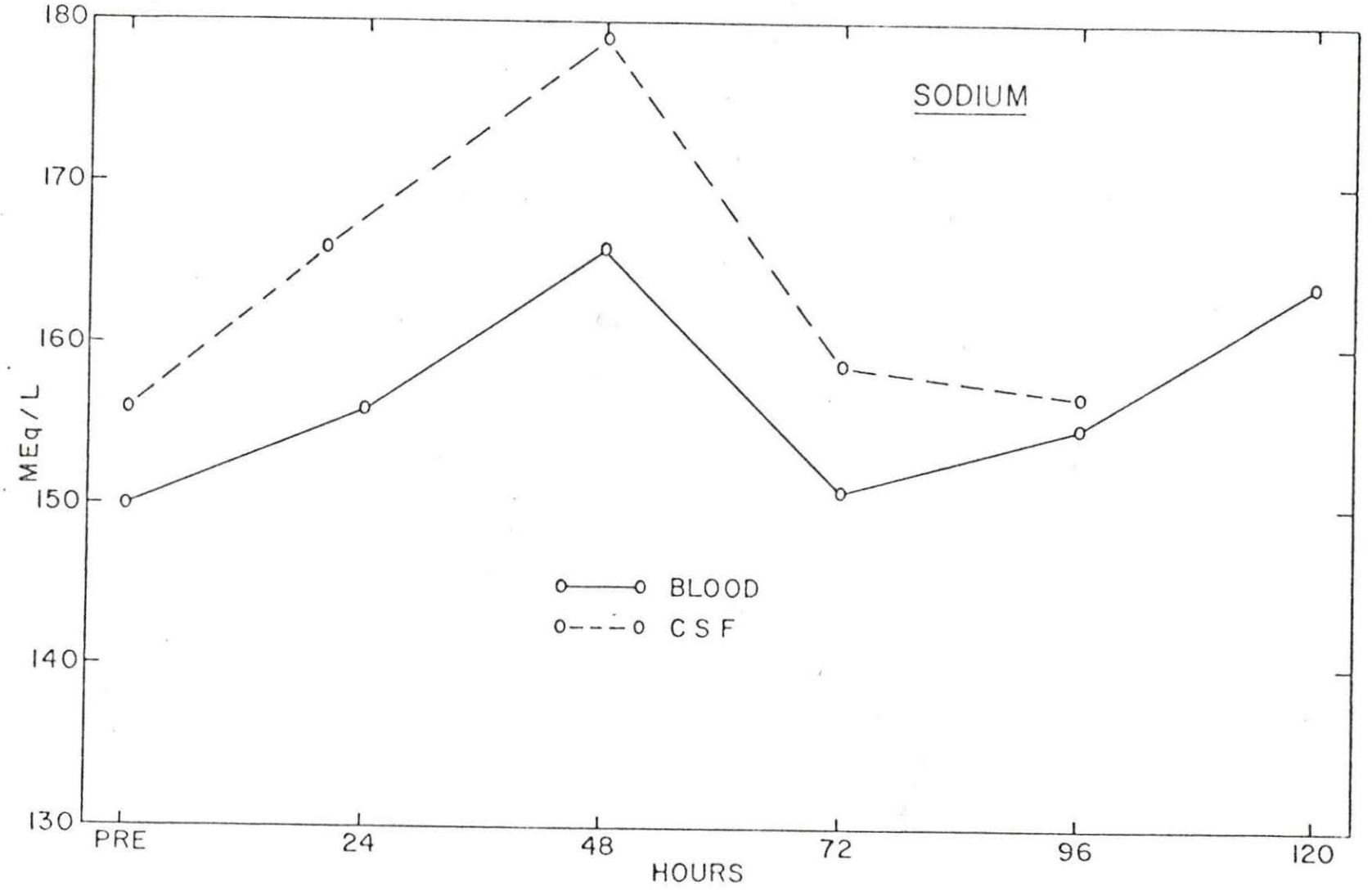

SODIUM
180
170
160
150
140
130
MEq/L
PRE
24
48
72
96
120
HOURS
BLOOD
C S F

Figure 27. Mean blood serum and CSF potassium values during heptachlor toxicity.

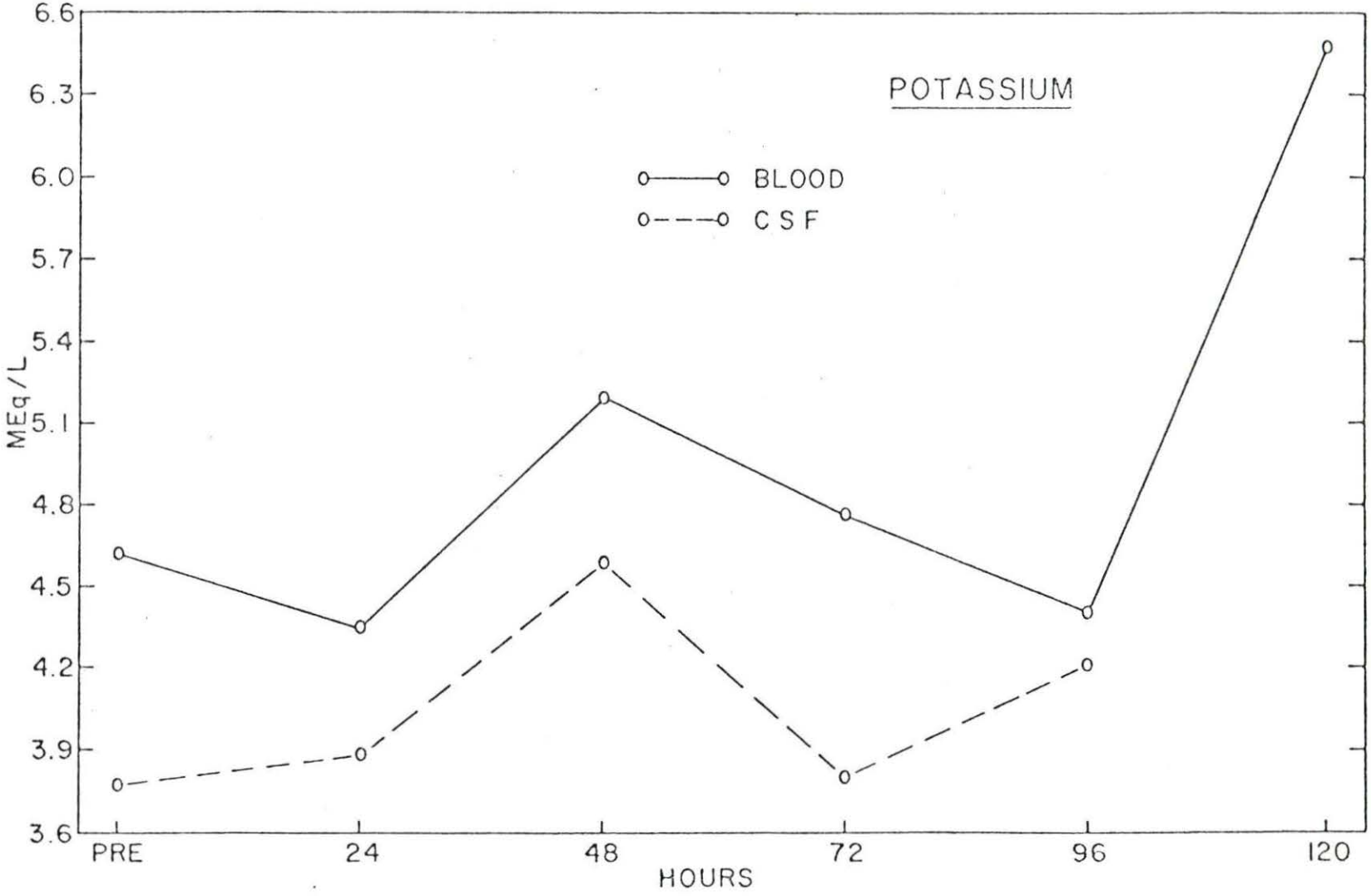
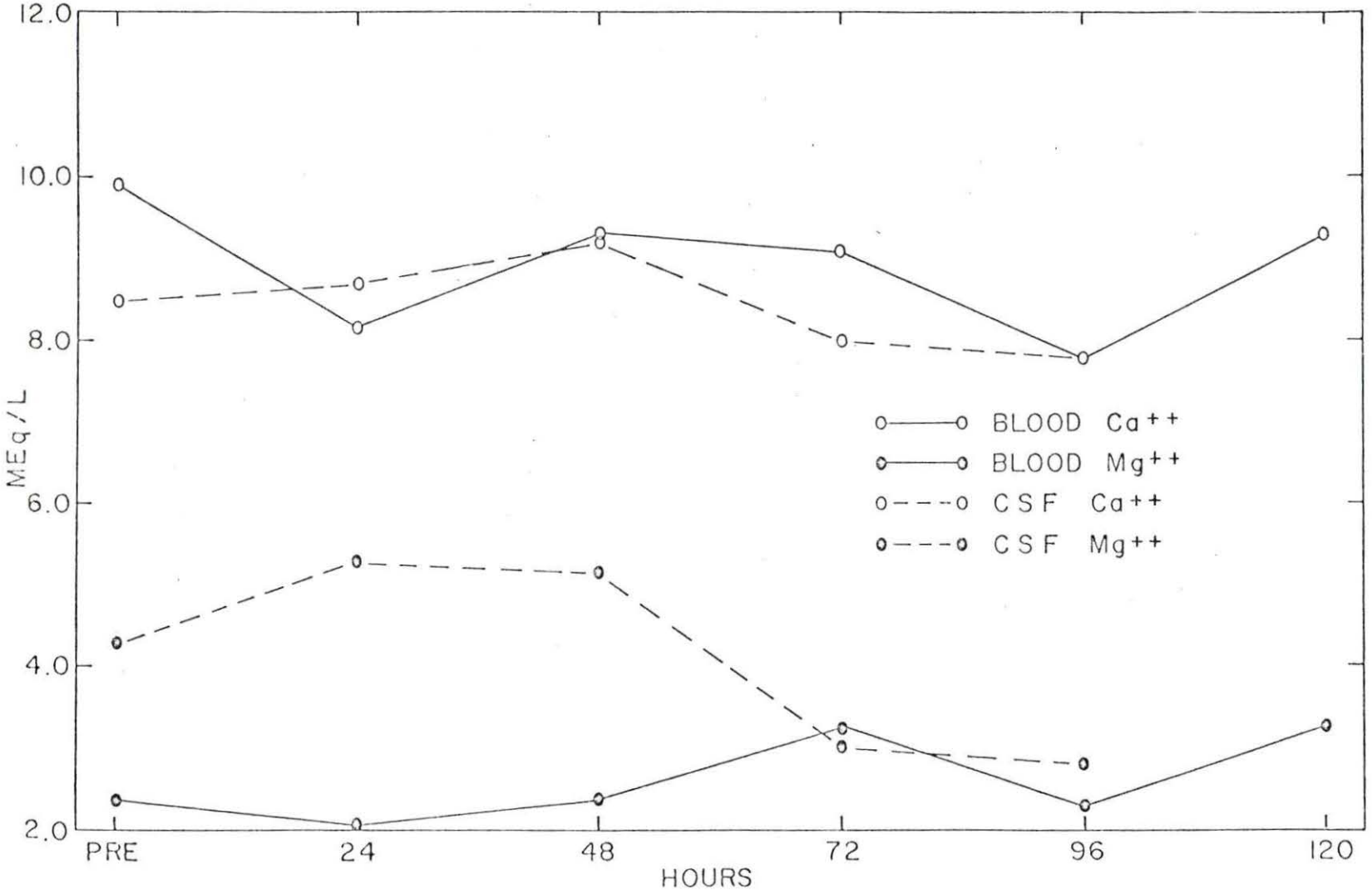

POTASSIUM
BLOOD
CSF
MEq/L
6.6
6.3
6.0
5.7
5.4
5.1
4.8
4.5
4.2
3.9
3.6
PRE
24
48
72
96
120
HOURS

Figure 28. Mean blood serum and CSF calcium and magnesium values during heptachlor toxicity.


12.0
10.0
8.0
6.0
4.0
2.0
MEq/L
PRE
24
48
72
96
120
HOURS
BLOOD Ca++
BLOOD Mg++
CSF Ca++
CSF Mg++

IX. APPENDIX B: PLATES

Plate 1. Illustration showing the relative positions of catheters in the cisterna magna and carotid artery of a sheep.

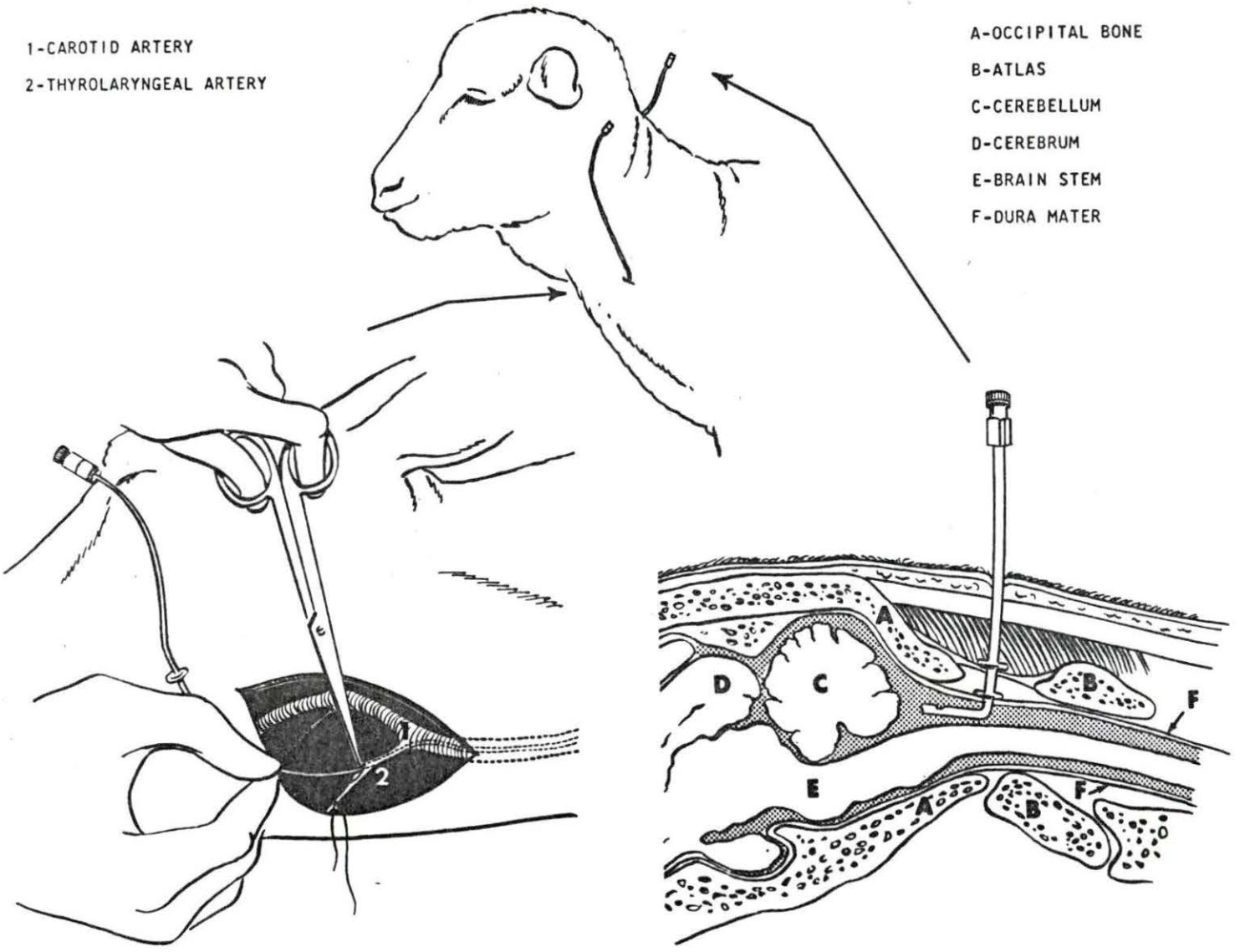

1-CAROTID ARTERY
2-THYROLARYNGEAL ARTERY
A-OCCIPITAL BONE
B-ATLAS
C-CEREBELLUM
D-CEREBRUM
E-BRAIN STEM
F-DURA MATER
1
2
A
D
C
B
F
E
A
B
F

Plate 2. Photomicrographs of sections from the medulla oblongata of sheep in which a catheter had been placed into the cisterna magna (400X magnification).

Top picture - Severe acute lymphocytic meningitis and encephalitis. There is thickening of the meninges due to infiltration of lymphocytes. General gliosis of the brain is also present. At least one neuron shows satellitosis.

Center picture - Chronic lymphocytic meningitis with extensive fibrosis of the meninges.

Bottom picture - Mild lymphocytic meningitis with moderate fibrosis of the meninges.

Note: Physiological saline solution containing 0.05 percent heparin had been injected into the CSF catheters of the 2 sheep from which the top and center sections were taken. The sheep from which the bottom section was taken received no heparin.