THE PATHOLOGY OF ORGANOMERCURIAL POISONING IN SWINE

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by
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AUTOBIOGRAPHY

The author was born in Corfu, Greece, March 26, 1931. He received his elementary and high school education in Corfu.

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During the period 1956-1958, he served in the Greek Army with the rank of Second Lieutenant Veterinarian. He then entered private practice and joined the ranks of the Veterinary Service of the Greek Ministry of Agriculture.

In 1960 he immigrated to Canada where he became employed by the Canada Department of Agriculture, Health of Animals Branch, Regina, Saskatchewan. In 1963 he entered private practice in Estevan, Saskatchewan.

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Leander Tryphonas

Abstract

The pathological and clinical features of disease in young pigs resulting from the oral ingestion of organomercurial compounds has been characterized and related to tissue levels of mercury. In three experiments, the toxic effects of methylmercuric dicyandiamide (MMD), ethylmercuric chloride (EMC) and phenylmercuric chloride (PMC) have been studied. The experimental period lasted 60-90 days and the dosage range studied was 0.19-4.56 mg. Hg/kg. daily.

In general, daily dosage levels ranging from 2.28 to 4.56 mg. Hg/kg. were toxic regardless of organic form. Lower daily dosages, 0.38 and 0.76 mg. Hg/kg. were toxic only when administered in the form of alkyl compounds (MMD and EMC).

The primary toxic effects of these mercurial compounds was manifested pathologically by degenerative changes in the susceptible cells of target organs. Alkyl mercurials caused enteric, renal and CNS disease, while aryl mercurials caused renal and enteric disease.

The analysis of levels of mercury in tissues of animals receiving the organomercurials indicates that there is a direct association between tissue level and evidence of injury.

The disease produced by MMD is largely one of the central nervous system and is related primarily to neuronal necrosis. Cortical neurons are most susceptible to injury and those in subcortical nuclei, in spinal gray matter, and in sensory ganglia are injured at higher doses. In the chronic form of toxicosis, there occurs, in

addition to neuronal necrosis, secondary gliosis, capillary endothelial proliferation and degenerative arteriopathy in leptomeningeal blood vessels supplying injured cerebral cortex.

The character of the vascular lesions in chronic MMD toxicosis is compatible with the ones of regional hypertension.

Chronic poisoning with EMC was manifested by disease of the CNS entirely similar to that described previously for MMD. The subacute form of the disease seen in animals receiving higher doses of EMC had, in addition, lesions in the large intestine characterized by edema of the mesocolon associated with degenerative arteriopathy in serosal vessels and pseudomembranous colitis and typhlitis. Analytic findings indicated that, in EMC poisoning, most tissues contained relatively high levels of mercury.

The disease occurring in PMC intoxication results from injury to the kidney and large intestine. The primary lesions are pseudomembranous colitis and typhlitis and nephrosis characterized by degeneration, necrosis, and regeneration in the epithelium of the proximal tubules. The pathology of this disease is similar to that described for mercuric chloride poisoning and reflects the ease with which phenylmercuric chloride is metabolized to release free mercuric ions.

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INTRODUCTION

Modern agriculture depends upon improved techniques to achieve its goals - increased productivity at a lower cost. The advent of organomercurial fungicides has improved grain crop yields by inhibiting the growth of parasitic fungi which destroy grain seeds.

The indiscriminate use of such substances, however, is not without hazards to man, animals or plants. The potential danger is not always realized by the uninitiated because many of these substances lack an immediately detectable deleterious effect. As a result, animals are often fed treated grain in the mistaken belief that they are harmless. If this is done for a period of time long enough to permit accumulation, overt signs of toxicosis occur and loss of animals is inevitable.

The agricultural technology has created a large number of organomercurial fungicides. Extensive studies on their effect on animals are not available to supply the pertinent information to the practicing veterinarian who is called on to protect the livestock owner's interests. The only available information is in the form of incomplete clinico-pathological reports.

The work reported in this volume concerns the pathology of organomercurial fungicides in swine. Both alkyl and aryl compounds have been used to carry out this study in an attempt to establish relationships between the basic chemical nature of the organomercurial and the type of disease it produces.

LITERATURE REVIEW

Fungicides in Agriculture

Improved growth conditions for plant crops and increased production per unit of land have resulted in improved conditions for the pests and diseases of such crops. This has created the need for methods of crop protection.

An important group of agents that endanger crops are the fungi (Schizomycetes). They constitute a serious threat to maximum yields (Sharvelle, 1962).

Fungicides were employed by man for many years. Martin (1959) states that the early history of fungicides is lost. It would appear, however, that substances which had been used as fungicides in treating animal species were tried on plants and seeds for potential fungicidal properties.

Mercurial compounds have been used in plants as fungicides and pesticides, especially following Ehrlich's lead in the development of chemotherapeutic agents, such as organic arsenic, which was used for treating human syphillis. In 1914, Riehm introduced the organic mercurials for the protection of wheat from smut (Martin, 1959).

The mercurial fungicides can be divided into two major categories:

a) Inorganic mercurials

b) Organic mercurials.

The inorganic mercurials, because of their high phytotoxicity, animal toxicity and low effectiveness, have been supplanted

by organic mercurial compounds (Sharvelle, 1962).

Martin states that the true organic mercurial derivatives are those in which the mercury atom is attached directly by one or both valency bonds, to carbon atoms. The general structure of these organic mercurials, which are found as the active constituents of seed disinfectants, is R. Hg. X., where R represents a hydrocarbon, with or without substituent groups, and X represents an acidic radical.

These compounds have undergone evolutionary changes in their specific formulae. These have been introduced by the various manufacturers in an effort to increase fungicidal activity while diminishing the hazard to plants and operators.

Fungicidal activity is influenced first by adsorption of the organomercurial to the surface of the fungus, and secondly by the degree of liposolubility (Walker, 1928).

The idea has been put forward by Daines (1936) and Booer (1936) that mercury compounds undergo reduction in the soil and liberate metallic mercury, which is the active fungicide.

The activity of organomercurial fungicides in pest control is considered to be the result of the combination of mercury with the -SH (sulphydryl) groups of susceptible enzymes (Martin, 1959).

Phytotoxicity causes disorganized hypertrophy of cereal seedlings. Characteristically, there is swelling and failure of elongation of the plumule and radical of the injured seed. Sass (1937) attributes this malformation to perturbation of mitosis.

Animal and Human Poisoning

Clinical manifestations

In swine, a chronic form of organomercurial poisoning has been observed following the advent of the use of organomercurial fungicides. The clinical signs reported by the various authors are summarized in Table 1.

If treated grain is the source of the mercurial, animals must consume it for several weeks before they manifest overt clinical signs of toxicosis.

It would appear that, in swine, the length of such a pre-symptomatic period is dependent upon the percentage of treated grain in the feed. Eveleth and Goldsby (1948) reported a case of swine poisoning in which signs of toxicosis were noted after a lapse of four weeks, or more, following feeding with Ceresan (N-(ethylmercury)p-toluene sulfonanilide)-treated grain. Taylor (1947) describes a case in which approximately six weeks elapsed before clinical signs appeared. Ceresan Jr. (hydroxymercury chlorophenol) was the compound incriminated in this instance. A latent period of three months was reported by an anonymous author (1953). In this case, pigs had been given a feed contaminated with Ceresan (N-(ethylmercury)p-toluene sulfonanilide). In 1950, McEntee reported on two different instances of organomercurial poisoning, but the compounds involved were not described. In the first case, the pigs had been on a ration composed of 50% ground corn and contaminated oats for three months before showing illness. In the second case, treated oats had comprised about

70% of the ration for approximately six weeks before the first pig manifested signs of illness.

In summary, the literature suggests that pigs have often been fed treated grain over a long period of time, either inadvertently or in the mistaken belief that the feed is harmless because of delayed onset of illness. It seems clear that the levels of organomercurials which find their way into feed grain under natural circumstances are at levels such that a presymptomatic period of one to three months is often required before any signs of poisoning appear.

Anorexia appears to be a consistent sign regardless of the kind of organomercurial involved (McEntee, 1950; Kahrs, 1968; Eveleth, 1948; Anonymous, 1953; Moore, 1948; and Loosmore, 1967). Affected animals will die following a symptomatic period which may be short or may extent for two weeks. Disturbance of the central nervous system is manifested by aimless walking, incoordination, blindness, weakness in the hind quarters and paralysis, convulsions, dysphagia and pharyngeal paralysis. In addition, some field cases of organomercurial poisoning have been characterized by diarrhea. Terminally, the animals remain in lateral recumbency and have depressed reflexes. Depressed growth is another feature of chronic organomercurial poisoning in this species. Signs which have been reported by various authors are summarized in Table 1.

The epizootiology and clinical picture of organomercurial poisoning has resulted in it being confused with hog cholera. Kahrs (1968) dealt with a herd of 44 adult swine and five litters

TABLE 1. Clinical signs of organomercurial poisoning in swine reported by various authors

Clinical Signs	Authors						
	Anonymous (1953)	Eveleth (1948)	Kahrs (1968)	Loosmore (1967)	McEntee (1950)	Moore (1948)	Taylor (1947)
Anorexia	+	+	+	+	+	+	
Dysphagia	+				+		
Pharyngeal Paralysis	+				+		
Diarrhea				+		+	
Incoordination	+	+					+
Blindness	+	+			+	+	
Aimless walking	+		+		+		
Paresis		+	+		+	+	+
Paralysis		+	+		+	+	+
Convulsions		+					

of newborn piglets which experienced an episode of fatal chronic mercury poisoning which resulted from the consumption of treated wheat. Hog cholera was suspected because neurologic signs and petechial hemorrhages were observed in the bladder, larynx and kidney, and because of recent unvaccinated additions to the herd.

Cattle are very susceptible to poisoning with mercurial compounds (Clarke and Clarke, 1967). There is literature which reports that cattle stabled in the same barn with horses treated with mercurial preparations were found dead. Absorption through the respiratory system was considered to be the route through which mercury entered the body.

In 1956, Fujimoto et al.reported on ten field cases and two experimental trials of poisoning of cattle with Ceresan (N-(ethylmercury)p-toluene sulfonanilide). Signs of illness appeared at intervals varying from two to thirty-eight days after the first treated feed was fed.

He found that increased daily intake of the mercurial appears to accelerate the appearance of toxicosis and that animals given a daily dosage of 11 and 56 mg. /kg. became ill in 36 and 23 days respectively, while animals receiving higher dosages became sick after a shorter interval. Animals receiving the same dosage do not always exhibit signs of disease simultaneously (Sonoda et al., 1956).

The signs of disease observed by Fujimoto and Sonoda et al.were comparable to the ones seen by Oliver and Platonow

(1960), but not identical. Fujimoto reported fever (about 40°) which continued for two to three weeks, severe dry cough, dyspnea, anorexia, marked lachrymation and salivation, depression of lactation, depilation, eczema, petechiae which occurred in visible mucous membranes, and death. Affected animals were four to 11 year-old cows. Oliver and Platonow worked with yearlings fed N-(ethylmercury)p-toluene sulfonanilide. Signs depended upon the dosage. The character of the signs were as follows, in the order of increasing dosage: 11 mg./kg., a primary central nervous system disturbance developed; 56 mg./kg., a combined involvment of the central nervous and gastrointestinal systems occurred; 112 mg./kg., a primary gastrointestinal syndrome occurred; 224 mg./kg., signs of a general systemic collapse occurred.

In Australia, Craig (1961) fed wheat treated with Ceresan (N-(ethylmercury)p-toluene sulfonanilide) to three sheep for a period of 84 days. All animals, except one, remained normal throughout this period. The death of this animal was attributed to factors other than mercury. Depressed growth was the only clinical sign observed. Failure of Craig's animals to manifest any overt signs of disease may be due to extremely poor palatability of Ceresan in view of Palmer's work with Ceresan in the same species. In 1963, he was able to induce organomercurial toxicosis in sheep. The amounts of organomercurial fungicide contained in 1.5 and 2.0 lbs. of wheat, treated according to manufacturers recommendations, were

fed to sheep daily in gelatin capsules with a balling gun. Signs of toxicosis appeared within 16 and six days respectively. The length of the presymptomatic period in sheep is evidently also dose-dependent.

Palmer administered Ceresan to chickens also. He found that amounts in excess of 10 mg. /kg. daily proved fatal within 12 days. Levels of 5 mg. /kg. daily were well tolerated except for a slight weight loss. Tejning and Vesterberg (1962) fed two mature hens exclusively on treated seed. One hen was maintained for 4-1/2 months on seed corn containing 14 mg. Hg/kg. in the form of methylmercury dicyandiamide (MMD). The other hen was fed treated cereals for one year containing 1 mg. Hg/kg. as MMD. The birds suffered no ill effects from this treatment. High levels of Hg were present in eggs laid by these birds (0.28 mg. Hg and 0.032 mg. Hg per egg respectively).

In man, the source of the mercurials in cases of poisonings is variable. The most common problem appears to be related to absorption occurring mainly through the respiratory system. Less commonly, poisoning has occurred via the alimentary tract, topical applications or injections. Poisoning due to ingestion of foodstuffs prepared from treated grains has been reported several times (Swensson, 1952). In Japan, ingestion of fish and shellfish contaminated with organomercurials was responsible for several fatalities in the Minamata Bay area. Industrial waste dumped into this body of water was the source of contamination (Takeuchi, 1962).

Greenfield (1963) reports that exposure to toxic levels of mercury or to organic mercury compounds causes a syndrome characterized by erethismus and tremor. Erethismus resembles the frontal lobe syndrome and is characterized by fatigue, emotional instability, and various states of depression.

Hunter reported on four patients exposed to a methylmercury compound. The clinical features were gross ataxia combined with severe concentric constriction of the visual fields (Greenfield, 1963). Similar clinical features were seen in cases of Minamata disease (Takeuchi, 1962).

Pathological findings

Gross

In swine, the reports are quite variable concerning the nature of gross lesions or organomercurial poisoning. It would appear that gross lesions may be absent or may involve any one, or all, of the central nervous, digestive or urinary systems (McEntee, 1950; Taylor, 1947).

A summary of the gross lesions observed in cases of organomercurial poisoning in swine may be found in Table 2.

Lesions reported include meningeal congestion, hyperemia of gastric mucosa, diffuse enteritis and hemorrhage and necrosis of the intestinal mucosa with ulcer formation in the colon and large intestine. In one case, petechial hemorrhages were found in the urinary bladder, larynx, external surface of kidney, and mesenteric and bronchial lymph nodes. The kidneys

are variously reported as "rubbery", "pale", or "inflamed with subperitoneal exudate" (Loosmore, 1967).

The nature of the organomercurial responsible has not always been identified by various authors; therefore, a correlation between nature of lesions and chemical structure is impossible to make.

In cattle, in the field cases described by the Japanese workers (Fujimoto et al, 1956; Mitsuo et al, 1956), petechial hemorrhages in various parts of the animal body, subacute bronchitis, edema of the body lymph nodes, increased pericardial fluid, and eczema were observed. Necrotic and hemorrhagic foci were seen in the liver. The central nervous system was not remarkably affected. Jubb and Kennedy (1963) describe moderate cerebral swelling, venous infarction and pallor as possible lesions in the cortex.

Palmer (1963) described the lesions observed in experimentally poisoned sheep. This author concluded that the gross pathologic changes were typical of metallic poisoning, and were characterized by ulceration and sloughing of the ruminal mucosa, catarrhal enteritis, pulmonary edema, and inflammation of the kidneys, lungs and spleen.

In palmer's study on chickens, poisoned birds had catarrhal enteritis, ulceration of the crop, nephritis, hepatitis, splenitis and atrial hemorrhages.

Takeuchi et al (1962) described the pathology of Minamata disease in man. They found a variety of lesions. Extreme ema-

TABLE 2. Gross Lesions of Organomercurial Poisoning in Swine Reported by the Various Authors

System	Lesions	Authors					
		Eveleth (1948)	Jubb & Kennedy (1963)	Kahrs (1968)	Loosmore (1967)	McEntee (1950)	Taylor (1947)
CNS	Congestion					+	+
Digestive	Gastric Hyperemia					+	
	Ulcerative Colitis			+	+	+	
	Diffuse Enteritis	+					
	Hemorrhage				+		
	Necrosis				+		
Urinary	Petechiae			+			
	"Rubbery" Kidneys	+					
	Pale Kidneys		+				

ciation and strong flexion of the limbs were present. In the CNS, brain swelling, total disappearance of the right lenticular nucleus, softening of the temporal lobe, atrophy of the brain with compensatory increase of cerebrospinal fluid were found. Gross atrophy of the cerebellar folia involving both lateral lobes and the vermis was prominent. In the more chronic cases, slight dilatation of the ventricles occurred. These findings are similar to the ones reported by Hunter and Greenfield (1963).

Histological

A number of cases of poisoning in animals have been investigated histologically. Table 3 summarizes the histopathological lesions reported by various authors. However, a systematic approach to such an investigation of the brain lesions is lacking. In the pig, various authors have reported edema of the white matter leading to demyelination, acute neuronal degeneration, neuronophagia, neuronal loss, and moderate gliosis, in part astrocytic, in part microglial. In one instance, hemorrhage was reported as being associated with cerebrovascular lesions characterized by adventitial proliferation and hyaline or fibrinoid degeneration of the media of meningeal arterioles (Kahrs, 1968). Hyaline degeneration has also been observed in the arterioles of the small intestine of pigs with signs of toxicosis.

In the kidney, the various components of nephrosis were seen and reported by several authors (Table 3). The histological picture described appears to be a reflection of

the particular stage of the nephrotic lesion at the time of death. Swelling and degeneration of tubular epithelium, coagulation necrosis, protein leakage in tubules, regeneration of epithelium and calcification of tubular debris were seen. In addition, interstitial edema and fibrosis occurred in the kidney of some cases.

Hepatic lesions which have been observed are centrolobular degeneration and diffuse hydropic degeneration confined to a small zone around the central vein.

In two cases, coagulation necrosis was found in the colonic mucosa. The lamina propria remained intact. In one case, polypoid masses projected from the mucosa (Loosmore, 1961).

In cattle, degeneration of the Purkinje network in the heart with histiocytic proliferation and eventual dystrophic calcification has been observed (Jubb and Kennedy, 1963). In the brain of the poisoned animals, there occurs degeneration and necrosis of cortical neurons associated with gliosis, focal areas of malacia, and perivascular cuffing with or without fibrinoid necrosis of the media of affected cortical vessels. Other lesions reported for cattle are nephrosis and hepatic congestion, accompanied by focal necrosis of hepatocytes.

Greenfield (1963) states that, in man, the histological findings of Hunter and Russell (1940) closely resemble those of Takeuchi et al (1962). The latter authors found a toxic encephalopathy affecting both the cerebral and cerebellar cortices.

Authors	Central Nervous System										Digestive System					Urinary System						
	Neurons				Glia		Vessels				Intestine			Liver		Tubules					Interstitial Edema	Fibrosis
	Degeneration	Necrosis	Neuronophagia	Loss	Microgliosis	Astrogliosis	Edema	Hemorrhage	Adventitial Proliferation	Hyaline Degeneration	Necrosis of Colonic Mucosa	Polyps in Colonic Mucosa	Degenerative Arteriopathy	Centrolobular Necrosis	Hydropic Degeneration	Coagulation Necrosis	Swelling of Epithelium	Regeneration	Protein Leakage	Calcification		
Anonymous (1953)														+		+	+					
Jubb & Kennedy (1963)	+	+		+	+	+	+			+									+			
Kahrs (1968)								+	+	+	+		+	+		+	+		+			
Loosmore (1967)											+	+					+	+		+	+	+
McEntee (1950)	+		+					+								+			+			

The most conspicuous changes were present in the cerebellum and the calcarine area. There was severe loss of granular cells in the former and loss of neurons in the latter. In the cerebral cortex, there was also extensive neuronal loss.

Fibrinoid

Current thoughts on fibrinoid and its formation

According to Gardner (1965), the term fibrinoid was introduced by Neumann in 1880 to describe the eosinophilic masses of degenerate connective tissue material which were seen in inflammations of serous surfaces, in the ruptured walls of aneurysms and in the lesions of verrucous endocarditis. Neumann considered fibrinoid to originate from degenerated collagen fibers which assumed fibrin-like staining characteristics. These views are no longer credible. More recently, the term fibrinoid has been employed to describe a type of change, or pathological material, which occurs in lesions of connective tissue and blood vessels (Montgomery and Muirhead, 1957). Fibrinoid is found in a variety of pathological processes. The exact nature of fibrinoid in such processes is not constant.

Gardner (1965) reports that Klinge, in 1933, felt that the ground substance was important in the genesis of fibrinoid. Depolymerized acid mucopolysaccharides were considered to contribute to the formation of fibrinoid by Altshuler and Angevine (1949). These authors concluded that the common feature of fibrinoid formation is the precipitation of the acid mucopolysaccharide of the ground substance of the connective tissue. The nature of the pre-

cipitant is probably an alkaline protein resulting from the necrosis of tissue or the interaction of the tissue with a damaging agent.

Montgomery and Muirhead (1954, 1957) stated that the fibrinoid seen in the walls of injured arterioles in the generalized Schwartzman reaction and in malignant hypertension was different from that found in the connective tissue diseases. Vascular fibrinoid and vascular hyalin were proven to be tinctorially and histochemically similar. They propose that a change in endothelial permeability leads to accumulation of plasma proteins within the injured tunica muscularis in the vascular diseases. The fibrinoid of connective tissue diseases and the fibrin deposited on the pericardium in inflammation were found to be tinctorially and histochemically similar but different from vascular fibrinoid and hyalin.

Immunological and histochemical studies were undertaken by Gitlin et al (1957). The data obtained by these authors indicate that the fibrinoid material found in the lesions associated with collagen diseases is, at least partly, fibrin. These authors concluded that the conventional histochemical methods are only partially reliable, since failure to detect fibrin with these methods is not to be considered as proof of the absence of fibrin. The immunofluorescent antibody method was proposed as a more reliable technique for identifying fibrin in tissue.

It is now generally accepted that fibrinoid, as found in various disease processes, is not chemically homogeneous and is not always a product of immunological reactions (Gardner, 1965).

This, then suggests that interpretation based on light microscope findings can become very tenuous. In fact, electron microscopical studies conducted by Cochran et al (1964) brought to light the morphological heterogeneity of the apparently structureless center of rheumatoid nodules.

Biava et al.(1964) reported their electron microscopic observations on the hyalinizing form of renal arteriosclerosis. They concluded that arteriolar hyalin, in this case, may constitute a deposit of substances of hematogenous origin and that it is distinct from collagen, elastic tissue, basement membrane material, fibrin and amyloid. McGee and Ashworth (1963) reported on the fine structure of the hyalin in hypertension arteriopathy. They observed an increase of extracellular material which was moderately dense, together with atrophy of smooth muscle. They concluded that, while some of the dense material was derived from elements of the blood, the majority was actually derived from increased amounts of basement membrane substance of endothelial and smooth muscle origin.

Several authors (Corner and Jericho, 1964; and Harding, 1966) have reported the presence of fibrinoid lesions in the cerebrovasculature of pig brains. Their reports are of sporadic cases and do not define the etiology and pathogenesis of these lesions. Fibrinoid vascular lesions have also been reported to occur elsewhere in the body of the pig and in a variety of diseases (Grant, 1961; Obel, 1953; and Nielsen, 1963).

In man, fibrinoid is fairly consistently found in the vessel wall in cases of malignant hypertension and polyarteritis nodosa (Allen, 1951; Boyd, 1961; and Robbins, 1967). The extent of mural involvement depends on the evolutionary stage of the disease.

In animals, inflammatory and degenerative arteriopathies with fibrinoid have been noticed in a variety of disease conditions such as malignant catarrhal fever in cattle, and Aleutian disease in mink. Occasionally, in older animals (usually four to five years for dogs and cats), hyaline degeneration is seen in splenic arteries (Montroni et al., 1949; and Jubb and Kennedy, 1963).

In 1964, Corner and Jericho reported a case of necrotizing arteritis in the brains of swine. They conducted virological and bacteriological investigations but failed to reveal the etiological agent. No mention was made in their report on whether a toxicological investigation was undertaken.

Harding (1966), in a study of vascular disease in pig brains, found wide bands of eosinophilic hyaline material beneath the endothelium of some affected vessels. He reported that the internal elastic lamina was not usually demonstrable in the lesions, and that the media was made up of rather basophilic, swollen, pale cells exhibiting karyorrhexis, or had clefts containing nuclear debris, or was infiltrated by cells resembling lymphocytes and histiocytes. Infiltration of the adventitia by a mixture of round cells and eosinophils varied from slight to extensive. In this report, pathogenesis also cannot be explained. The author speculated that,

etiologically, this condition could perhaps be attributed to a sub-clinical attack of enterotoxemia (edema disease). However, no definitive evidence was offered in support of this hypothesis.

Fibrinoid degeneration of arterioles is found in diseases of known viral etiology and have been reported in various domestic animal species. In 1957, Jones et al.described the lesions found in a spontaneous outbreak of equine viral arteritis and demonstrated the presence of a viral agent by experimental infection of other horses. Fibrinoid degeneration was considered to be an early morphologic manifestation of vascular injury. In bovine malignant head catarrh and hog cholera, vascular injury is a prominent feature. Injured vessels frequently exhibit fibrinoid degeneration.

In cattle and swine, fibrinoid necrosis of the media of cerebral arterioles was found in animals which were fed grains treated with organomercurial fungicides (Jubb and Kennedy, 1963). The authors consider that fibrinoid necrosis of the media of leptomeningeal arteries is characteristic of such poisoning.

Metabolism of Mercury and its Compounds

Grossly, the mercurial compounds may be subdivided into organic and inorganic categories. The inorganic include: elemental mercury, mercurous and mercuric compounds. The organics are mercuric and contain both alkyl and aryl compounds. This classification is necessary because the chemical structure of the mercurial entering the animal body affects, to a great extent, its absorption, distribution, retention and excretion (Swensson et al., 1959; Berlin et al., 1965; Miller et al., 1961; Swensson et al.,

1959; Berlin and Ullberg, 1963; Berlin, 1963; and Frieberg, 1959).

Absorption

Depending on the form in which mercury is presented to the animal body, mercury may be absorbed by various routes. It may gain entrance through the respiratory system when in the vapor state, through intact epithelia when applied locally in the form of ointments or suppositories, and through the gastrointestinal tract when ingested. It may also gain entrance through sites of injection (Ashe et al., 1953; Frieberg, 1959; and Berlin et al., 1963).

Elemental mercury is not significantly absorbed by the gastrointestinal tract. In man, there is clinical evidence to support such a statement (Robbins, 1967). Elemental mercury has, however, a high vapor pressure and, as a result, evaporates easily (Swensson, 1952; Goodman and Gilman, 1965). When in this form, mercury is readily absorbed by the respiratory tract and may also find its way to the brain because of its liposolubility (Ashe et al., 1953; Hughes, 1957). Kosmider (1965) states that occupational poisoning by inhalation of mercury vapor is one of the most frequent causes of mercury poisoning in man.

Mercurous compounds have a low solubility and, unless they become oxidized to the mercuric state, are not absorbed to any significant extent. This property of low solubility permits their use as cathartics, e.g. Cl - Hg - Hg - Cl, calomel.

Mercuric compounds are soluble and, as a result, are readily absorbed by the gastrointestinal tract when administered per os. Organic mercuric salts are probably absorbed unchanged. The work of Miller et al.(1961) tends to support such a view. These authors found that tissue levels of ethylmercury chloride in chickens receiving ethylmercury chloride by the oral route were similar to those where the intramuscular route was used.

Distribution and accumulation

Following absorption, the mercury compounds are transported in the blood. Immediately after absorption, the mercury content in the blood is high and distribution to various tissues and fluids takes place quickly. The rate at which mercury leaves the blood is rapid during the first five to ten minutes, but becomes lower thereafter. The rate of clearance from the blood of infused mercuric nitrate, phenylmercuric acetate and methylmercuric hydroxide has been found to be approximately the same for all three substances (Swensson et al., 1959).

Organic compounds are transported in the blood largely bound on the erythrocytes, while inorganic compounds are, to a significant extent, transported in the plasma. Following administration of alkyl and aryl compounds, 90% of blood mercury has been found in the corpuscles while, in the case of $HgCl_2$, only 50% of blood mercury has been found in the corpuscles (Berlin and Gibson, 1963; and Berlin, 1963). These authors state that the mercury turn-over rate between plasma and cells is quite low.

The pattern of mercury distribution in the body tissues changes with time. Following entry into the body, both organic and inorganic mercury compounds are distributed uniformly throughout the various tissues. Later, however, the mercury tends to accumulate in specific organs. It has been observed that accumulation of mercury in various organs correlates well with functional disturbances in these organs. Berlin and Ullberg (1963) compared the distribution of $Hg^{203}Cl_2$ and phenyl mercuric^{203} acetate injected into mice intravenously. They found that there was some correlation between the subsequent functional disturbances and the distribution pattern of mercury in states of intoxication. This correlation, however, is not absolute (Berlin et al., 1965). The affinity of mercurials for certain organs or tissues appears to be a function of the chemical nature of the compound which gains access to the animal body.

Berlin and Ullberg (1963) found that, following intravenous administration of $HgCl_2$ to mice, there was a preferential accumulation in the various body tissues in this order: kidney, liver, myocardium, mucosa of intestines, upper respiratory tract, interstitial tissue of testes and skin. The brain did contain mercury, but in exceedingly small amounts. A comparison between phenylmercuric acetate, methylmercuric hydroxide, methylmercuric dicyandiamide and the inorganic compounds $HgCl_2$ and $HgNo_3$ gave the following results. The organic compounds gave higher concentrations of mercury in the brain, liver, kidney and blood than the inorganic compounds. Generally, the ratio between mercury content of the

blood and brain seemed to be constant for each substance and indicated a distribution equilibrium (Berlin, 1963).

When phenylmercuric acetate was compared to inorganic compounds, higher concentrations of Hg were found in the liver, intestine and muscles with the former than the latter. Correspondingly smaller amounts were found in the renal cortex, bone marrow and spleen.

The affinity of various organomercurial compounds for the central nervous system varies with their chemical structure. Those with a high affinity are usually associated with CNS lesions (Hunter et al., 1940; Swensson, 1952).

Alkyl compounds enter the brain readily. Swensson (1952) injected mice intraperitoneally with methylmercuric chloride, methylmercuric dicyandiamide, ethylmercuric chloride and ethylmercuric dicyandiamide and observed that, a few minutes later, marked neurological signs consisting of ataxia and paresis appeared. This experiment illustrates how rapidly the compounds pass from blood to the central nervous system. Lesions in the CNS caused by alkyl mercury compounds have been reported by various authors (Hunter et al., 1940; Swensson, 1952).

Berlin and Ullberg (1963) studied the distribution of methylmercuric dicyandiamide (MMD) and inorganic mercury in mice. They found that accumulation in the CNS gradually occurs over a period of several days and is not correlated to the level of Hg in blood. The distribution of mercury in the brain was heterogeneous with the inorganic, but was more uniform in the case of the organic

compound. A common characteristic of both compounds was that they were taken up more by the gray matter than the white. However, the hippocampus and gray matter of the cerebellum show higher concentrations of mercury in animals receiving MMD.

The levels of accumulation of Hg in the central nervous system does not necessarily bear direct relationship to the amount of injury. The treatment of mercury poisoning with BAL (2, 3- dimercaptopropanol) is a good example. This treatment brings alleviation of symptoms in mice and swine poisoned by alkyl mercury while, at the same time, the brain level of mercury increases (Berlin et al., 1965; and Platonow, 1968). Berlin et al.(1965) explain the improvement by assuming that the increased brain mercury is in an inert state.

Swensson (1952), in his literature review, quotes Lomholt (1928) and Sollman and Schreiber (1936). These authors have stated that, in cases of poisoning with inorganic compounds, the brain contains practically no mercury.

The blood levels of mercury vary with the chemical nature of the mercurial employed. Frieberg (1959) compared methylmercuric dicyandiamide and mercuric chloride. He found that the mercury concentration in the blood was 100 times higher with the methylmercuric dicyandiamide than the inorganic. In general, for similar doses and length of administration, MMD caused a higher level of accumulation of Hg in tissues than $HgCl_2$. He also found ten times more mercury in the brain, and two times more in the liver of animals receiving MMD than in those receiving

$HgCl_2$. The mercury was found to be more firmly bound to the tissues of the animals receiving MMD. The kidneys and urine of animals receiving $HgCl_2$ had two and twenty times more mercury when compared with those receiving MMD.

Excretion

Excretion of mercury occurs mainly via the urinary and digestive systems. The chemical configuration of the various organomercurials plays an important role in the rate of elimination.

Alkyl compounds have a low excretion rate, while inorganic and aryl compounds have higher excretion rates (Swensson et al., 1969). Miller et al.(1961) studied ethylmercuric chloride in rats and chickens. They concluded that the metabolism of this compound is slower than that of phenylmercurials. Following administration of inorganic compounds, high levels of mercury are found in the colon wall (Swensson et al., 1959).

The kidney levels of mercury are high with all compounds, both organic and inorganic (Berlin and Ullberg, 1965). There appears to be no correlation, however, between the amount of mercury accumulated in the kidney and amounts excreted in the urine. Urinary excretion of mercury and blood concentration of mercury have been found to be correlated (Berlin, 1963). Jacob et al.(1963) studied urine levels of men working with a variety of mercury compounds. They concluded that urine levels of Hg are not good indicators of mercury poisoning.

Excretion of mercury following termination of exposure is slow and continuous for a long time. In man, studies have revealed that, following occupational exposure to mercuric nitrate, measurable amounts of mercury may persist in the blood and urine for as long as six years after exposure has ended.

Brown et al.(1967) studied excretion and body distribution of mercury following the inhalation of metallic mercury vapor, phenylmercuric acetate vapor and injection of mercuric chloride in rats. They noticed that the urinary and fecal mercury levels increased with exposure and steadily declined when exposure was interrupted. Animals receiving metallic mercury and mercuric chloride had higher mercury concentrations in the urine and feces than those receiving phenyl mercuric acetate. On acute exposure to metallic mercury vapor, blood and urinary levels were found to be directly related to exposure, but not to the mercury content of the organs. The mercury content in the urine had a direct relationship to exposure.

OBJECTIVES OF THE EXPERIMENTS

Several field cases of poisoning in swine caused by the ingestion of seed grains treated with organomercurial fungicides have been reported (Eveleth, 1948; Kahrs, 1968; Loosmore, 1961; McEntee, 1950; and Taylor, 1967).

The review of the literature pointed out that lesions in the nervous, digestive and urinary systems may be expected in cases of organomercurial poisoning in swine. These clinical reports did not provide information on the exact chemical nature of the organomercurial involved. Therefore, a correlation between the nature of the lesions and the nature of the organomercurial compound cannot be deducted from such reports. Only little information is available concerning the influence of dose (the percentage of treated grain in the diet) on the length of the presymptomatic period observed in cases of organomercurial poisoning in swine. In summary, existing literature does not allow correlation between the type of lesions and dosage, type of lesion and length of exposure, or type of lesions and both dosage and length of exposure.

The lack of this knowledge led to the formulation of the objectives of these experiments.

It was decided to compare alkyl and aryl mercurial poisoning. Two alkyl compounds, methylmercuric dicyandiamide and ethylmercuric chloride, and one aryl compound, phenylmercuric chloride, were used for the purpose.

The main objective was to establish the pathology of the disease caused by these two types of organomercurials in swine.

Secondly, it was desirable to correlate: a) signs and lesions to dose; b) signs and lesions to length of exposure; c) signs and lesions to amount of mercury recovered from various organs.

Thirdly, an attempt was made to establish: a) the minimum mercury level present in tissue showing lesions and b) the influence of the various radicals on the nature of lesions, e.g. dicyandiamide versus chloride (as in experiments involving methylmercuric dicyandiamide and ethylmercuric chloride).

MATERIALS AND METHODS

Rationale

The pig was chosen as an experimental animal since the literature on this subject is meager but sufficient to establish the scientific merit of the investigation, and because natural cases occur in areas where cereal grains are grown and could present a diagnostic problem. In addition, it was desirable to obtain an indication of the levels of mercury retained in the tissues of animals without clinical diseases, since such animals might be a hazard to human health.

The pathology of organomercurial poisoning in swine resulting from oral ingestion of organomercurial fungicides was studied in three experiments.

Three such compounds, methylmercuric dicyandiamide (MMD), ethylmercuric chloride (EMC) and phenylmercuric chloride (PMC) were administered orally to pigs in the appropriate dosages to result in both subacute and chronic poisoning (Table 4). These were selected on the basis of a study of field cases and a pilot study of the toxicity of Ceresan (N-(ethylmercury)p-toluene sulfonanilide).* Clinical examination was conducted daily and findings were recorded.

An attempt was made to kill half of the animals in each dosage group in an early stage, and the other half in a late stage

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* E.I. duPont de Nemours & Co., Dupont Building, Wilmington, Delaware 19801, U.S.A.

TABLE 4. Amounts of MMD, EMC, PMC and Equivalent Hg Content Administered Orally to Experimental Groups

GROUP	Experiment I			Experiment II			Experiment III		
	MMD	Hg Content	No. Pigs	EMC	Hg Content	No. Pigs	PMC	Hg Content	No. Pigs
I	2.28*	0.19*	4	0.25	0.19	5	0.29	0.19	5
II	0.56	0.38	3	0.50	0.38	5	0.58	0.38	5
III	1.12	0.76	4	1.00	0.76	5	1.16	0.76	5
IV	2.24	1.52	4	3.00	2.28	5	3.48	2.28	5
V	4.48	3.04	4	6.00	4.56	5	6.96	4.56	5
VI	----	----	5	----	----	5	----	----	5

* Expressed in mg./kg. daily.

of disease. This presented the possibility of studying the morphogenesis of the lesions. Pathological changes were determined by detailed gross and histological examination of tissues.

Standard tissue samples were collected and analyzed for mercury content with a method based on atomic absorption (Jacobs et al., 1960).

Experimental Design

Eighty-four pigs were used to carry out three experiments in which methylmercuric dicyandiamide (MMD), ethylmercuric chloride (EMC) and phenylmercuric chloride (PMC) were used. The amounts administered are shown in Table 4.

The treatments outlined in Table 4 were randomly assigned to each animal in each of the three experiments.

The levels of the organomercurials used were chosen after examination of pilot experimental data of naturally occurring cases of poisoning, and of data supplied by the various manufacturers who prepare agricultural products containing organomercurial fungicides. Consideration was given to the average daily amount of treated grain an animal might consume. A healthy young pig weighing 15 kg. is expected to eat an average of 1 kg. of cereal grain per day. This means that 1.0 mg. of Hg/kg. of body weight will be consumed daily if the grain was treated according to manufacturers directions with a commercial fungicide containing methylmercury dicyandiamide as the active ingredient. This constitutes a maximal dose under usual field

conditions. Dosages included in this experiment extend above and below such values (Table 4).

An additional replicate was carried out in the case of phenylmercuric chloride, as shown in Table 5.

TABLE 5. Replicate with PMC. Daily Dosage Rate Administered Orally to 10 Pigs.

PMC	Hg Content	No. Animals
0.58	0.38	2
1.16	0.76	2
3.48	2.28	2
6.96	4.56	2
----	----	2

Animals, Housing, Feeding

A total of ninety-four recently weaned Lacombe x York cross pigs, in four separate lots, were purchased locally. The mean body weight upon arrival for all pigs was 7 ± 15 kg.

On arrival, each lot was examined clinically, ear tagged and randomly assigned to one of six groups, each of which was

randomly placed in one of six "pig boxes". The boxes measured 8' x 3' and had a 4' wall. The floor was slatted and elevated 8" above the room floor. The slatted floor allowed the excreta to pass through readily. The boxes and pigs were washed daily in order to prevent accumulation of feces which might contain excreted mercury and, therefore, disturb the dosage schedule.

The pigs received feed and water ad libitum through automatic devices, installed in the boxes. The building in which the boxes were housed was ventilated through windows and air exchange was aided by a power fan which was inserted on the west wall of the building.

Feed was purchased from a local feed plant.* Fifty-pound paper bags of the crumbled form was usually used. The composition of this feed is described in Tables 6 and 7.

The ration illustrated in Table 6 was fed to weaned pigs daily until they reached 30 kg. in body weight. The ration in Table 7 was fed thereafter.

Clinical Examination

Clinical signs were recorded according to the scheme described by Messieri et al.(1963).

Post Mortem Procedure

Necropsies were schedules in such a way as to include, from each experimental group, animals with early and advanced

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* Federated Co-operatives, Ltd., Saskatoon, Saskatchewan, Canada.

TABLE 6. Ingredients in 18% Protein Pig Starter Pellets

Ingredient	Item	Cumul.
Ground Wheat	2200	
Ground Barley	800	
Shorts	130	3130
Soybean Meal	95	3225
Sunflower Meal	200	3425
Meat Meal	250	3675
Calcium Phosphate	20	3695
Ground Limestone	20	3715
Salt	19	3734
Staple	25	
Sugar	200	
Methionine	1 lb. 9 oz.	
Swine Tramin	2	
Vit. D_3 Premix 1MU	2	
300 S Premix	10	
Hygromix	3	
Pen. Strep.	- 8 oz.	
Tallow	20	
	3998 lbs. 10 oz.	

(Computerized)

TABLE 7. Ingredients in 16% Protein Pig Grower Pellets

Ingredient	Weight (lbs.)	
	Item	Cumul.
Ground Wheat	955	
Ground Barley	2200	3155
Ground Oats	290	3445
Rape Seed Meal	210	3655
Sunflower Meal	65	3720
Meat Meal	150	3870
Calcium Phosphate	35	3905
Ground Limestone	35	3940
Salt	20	3960
Staple	25	
325 G Premix	12 lbs. 8 oz.	
Swine Tramin	2	
Vit. D_3 Premix 1MU	2	
	4001 lbs. 8 oz.	

(Computerized)

manifestations of toxicosis. An attempt was made to have one-half of each group represent each stage of toxicosis. A complete post mortem was done on all animals.

The general post mortem technique described by Leinati (1946) was employed for all systems except the nervous system. The technique applied in examining the brain will be described under the heading of "Fixation and Removal of the Pig's Brain".

Specimens For Histological Examination

The following tissues were obtained for histological examination:

Nervous system

Cerebrum, cerebellum, medulla, spinal cord with dorsal root ganglia, Gasserian (semilunar) and celiac ganglia, eye, optic and sciatic nerves.

Digestive system

Tongue, pharynx, esophagus, salivary glands, cardia, fundus, pylorus, duodenum, pancreas, jejunum, ileum, ileo-cecal valve, cecum, spiral colon, rectum, liver.

Urogenital system

Kidneys, urinary bladder, gonads.

Cardiovascular system

Myocardium, abdominal aorta.

Respiratory system

Larynx, lungs.

Endocrine system

Adrenals, gonads, hypophysis, thyroid.

Lymphatic system

Lymph nodes, spleen, thymus.

Integumentary system

Skin.

Fixation of Tissues

Sections from the gastrointestinal tract collected during necropsy were immersed in Bouin's fixative for 24 hours and then transferred to 75% alcohol.

All other sections were immersed in 10% neutral buffered formalin.

Fixation and Removal of the Pig's Brain

The brain was fixed by perfusion *in situ* with 10% neutral buffered formalin. To this end, the animals were anesthetized with Surital (sodium thiamylal)* injected intravenously into the ear vein. Immediately thereafter, two incisions were made, one on each side of the ventral region of the neck, along the lower region of the jugular sulcus. The subcutaneous tissues and fascial planes were separated by blunt dissection along the medial aspect of the sternocephalicus muscle. The common carotid arteries were identified, brought into the incision and cannulated with tubes 40 cm. long and a diameter chosen so as to give a snug fit in the arteries. Whenever possible, an attempt was made to place the

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* Parke, Davis & Co., Ltd., 5910 Cote de Lisse Road, Montreal 9, Quebec, Canada.

cannulae within the internal carotid artery, after which they were secured in situ with a ligature applied cephalad to the point of entrance. Cannulation of the arteries was usually completed within three minutes.

At this point, an additional amount of anesthetic was injected into these cannulae and immediately connection with an infusion apparatus was established.

In situ fixation of brain was achieved by perfusion with a solution of a weak formalin made with 210 cc. of heparinized saline and 40 cc. of 10% neutral buffered formalin ("washing solution") and, following this, with 10% neutral solution of buffered formalin.

The total amount of solutions employed varied with the size of the animal. Two hundred and fifty ml. of "washing solution" and 500 ml. of neutral buffered formalin injected from a height of about one meter produced effective exsanguination and fixation in the brains of pigs up to 8 - 10 weeks of age. Older pigs required increased amounts of "washing" and formalin solutions. Six hundred cc. of "washing solution" and 1500 cc. of neutral buffered formalin were the maximum amounts used in any one pig.

Satisfactory exsanguination and fixation of the brain were achieved only when the following three procedures were carried out: 1) prompt severance of the jugular veins is imperative to prevent intravascular clotting; 2) at the time of brain perfusion, the anesthetised animals must have their heads raised considerably above the level of the rest of the body in order to facilitate drainage of the venous system (a 45^{o} angle with the horixontal or more was found satisfactory); 3) collateral circulation to the head must be prevented by clamping the common brachiocephalic trunk near the point of its origin.

The brain must be removed carefully if artifacts are to be avoided. The technique employed in this study, and described below, was used in all pigs.

Decapitation is preceded by severance of the spinal cord at the level of the occipito-atlantal joint. The head is then skinned and placed in a vice where it is clasped by the zygomatic arch. This allows precise control of the cutting instruments. Starting from the occipital foramen, and at an angle of 50^{o} to the saggital plane, a semicircular incision is made on each side of the skull. The path and angle of incision are illustrated in Figure 1. The incision is terminated at the frontal midline, the exact point depending on the stage of skull development. In the young animal, the profile of the frontal bone is raised and somewhat curvilinear. In such animals, the anterior lowest point of this profile is chosen as a point of termination of the line of incision.

In mature animals, the frontal profile is rectilinear. As a result, the point of termination of the semicircular incision has to be determined topographically. The optimal point is at the intersection of the midline and a straight line passing through the inner canthi.

The swine skeleton is not as heavily mineralized as that of other domestic species. Therefore, it offers little resistance to a saw. Vigorous sawing may result in injury of the dura and underlying brain. For that reason, it is best not to entirely transect the bone in any part of the incision line. Extreme care is required in the parietal region where the calvarium is thinnest.

The remaining bone is broken with the aid of a broad plate chisel manually operated as a lever. The cranial vault is then lifted laterally and the dura cut with scissors along the margin of the bone incision. The brain is, thus, exposed and inspected in situ. The head is removed from the vice and tilted to one side in order to expose the cranial nerves which are severed. The same process is repeated on the opposite side. Once this is achieved, the head is overturned and the brain placed adjacent to the open end of a large-mouthed container half filled with 10% neutral buffered formalin. At this point, the trigeminal, occulomotor and optic nerves and the hypophyseal infundibulum are severed. This frees the brain and allows it to fall softly into the fixative. The formalin solution is repeatedly changed during the following week and a minimum period of thirty days is allowed to pass before sectioning.

Histological Procedures

Central and peripheral nervous tissue

The brain was sectioned transversely into slices approximately 5 mm. thick with the aid of a "brain slicer".* In some brains, a slightly different technique was employed and the cerebellum and medulla were first separated from the remainder of the brain by an incision passing just rostral to the emergence of the occulomotor nerves and through the central portion of the anterior colliculi. The resulting portions were then cut into 5 mm. thick sections.

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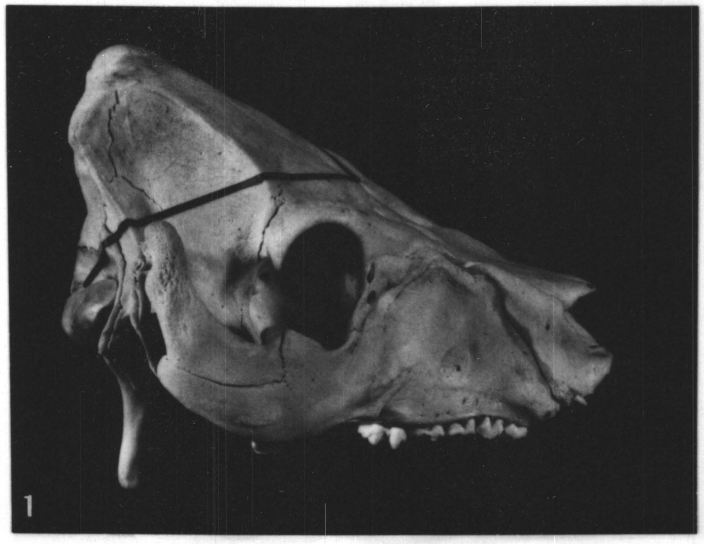
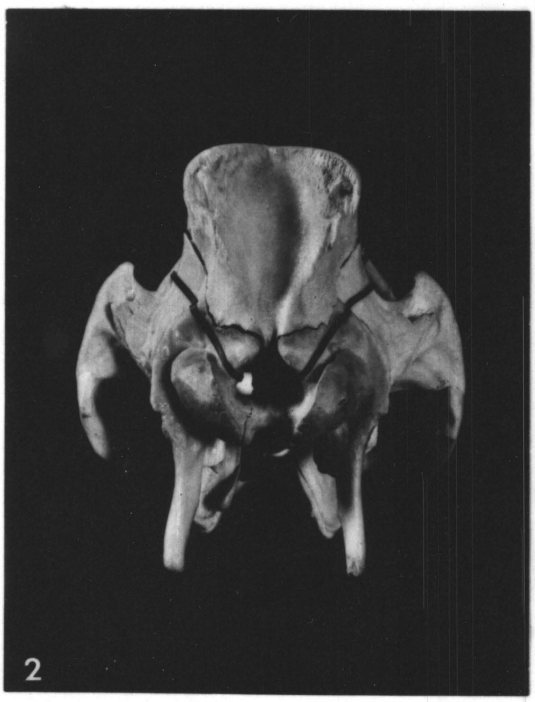
* Lipshaw Manufacturing Co., Detroit, Michigan, U.S.A.





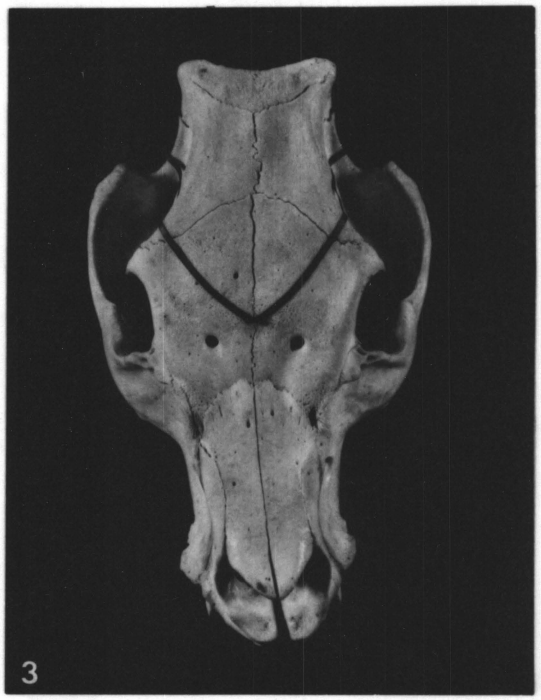


Fig. 1. Pig's skull. Pictures one to three serve to demonstrate the course of the saw cut applied to expose the brain of the pig.

All sections, except for samples from the cerebral and cerebellar cortices which were saved for analytical work, were processed histologically. Paraffin sections were cut at a thickness of 8 and stained with hematoxylin and eosin. The following stains were also employed selectively when thought to be of value: Nissl's stain for the homonymous bodies, Heidenhain's stain for myelin, Holzer's stain for astrocytes, Lendrum's picro-Mallory stain for fibrin, Masson's trichrome stain for collagen as modified for central nervous system sections, Bielschowsky's stain for axons as modified for paraffin sections and Verhoeff's stain for elastic fibers.

Other systems

Six micron thick paraffin sections were cut and stained with H & E. In addition, kidney and liver sections were stained with PAS. The procedure followed in the above techniques are those recommended by the following authors: Drury and Wellington (1967), Culling (1963) and Armed Forces Institute of Pathology (1960).

Compounds and Mode of Administration

Methylmercuric dicyandiamide, ethylmercuric chloride and phenylmercuric chloride were used in three separate experiments. The first of these compounds was kindly supplied by the Morton Chemical Company* while the Merck Company** contributed the other two. The manufacturers stated that the above

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* Morton Chemical Company, Research Department, 11710 Lake Avenue, Woodstock, Illinois 60098-815/338-1800, U.S.A.

** Merck & Co., 200 Wagaraw Road, Hawthorne, New Jersey, 00507, U.S.A.

compounds had a purity of at least 99%.

In order to increase the bulk of the chemicals to facilitate handling, the organomercurials were diluted 5:1 with lactose powder in a mechanical mixer. Since the mercurials were to be administered orally, No. 00 gelatine capsules were employed. In the case of MMD, five lots of capsules, each containing different amounts of organomercurial, were prepared as shown in Table 8.

TABLE 8. Capsule Strength in mg. of Hg in the Form of MMD.

Lots	I	II	III	IV	V
mg. of Hg	.1	.4	1.6	6.4	25.6

Such pills were used in combination to select the desired dose of mercury which was administered in accordance with the weight of the pig (Table 9). This method was found cumbersome, therefore, in the case of the other two organomercurials (EMC and PMC), the appropriate dosage was weighed into one or two capsules prior to administration.

Body weights were obtained every four days and the total amount administered to each pig was adjusted accordingly. Oral administration of pills was performed by the use of a mouth speculum and a balling gun. Gelatine capsules become sticky

when moist. In order to prevent capsules from lodging in the pharynx or the esophagus of the experimental pigs, 20 ml. of tap water was given via a plastic syringe immediately following pill administration. This procedure almost invariably induced effective deglutition reflexes.

Analytical Methods

Collection of specimens

About 50 grams of skin, fat, muscle, liver, kidney and five to 10 cc. of bile and urine samples were harvested during post mortem examination. All containers were pre-labeled. Fluid samples were placed in test tubes while solid samples were placed in plastic bags. Five ml. of blood was collected from all pigs just prior to anesthesia during the necropsy procedure. The blood was drawn from the anterior vena cava according to the technique described by Carle et al. (1942) and transferred to heparinized test tubes. All specimens above were frozen and stored below 0° C. except samples of brain and large intestine which were obtained from fixed specimens.

Repeated blood sampling during the course of Experiment II was done in animals receiving ethylmercuric chloride.

Method of mercury analysis

A modification of the method described by Jacobs et al. (1960) was used to analyze for mercury in the samples. In brief, this method involves cold incomplete digestion with sulfuric acid, oxidation with potassium permanganate, extraction with dithizone,

decomposition of the mercury dithizone complex by heating to produce mercury vapor and estimation of the mercury by atomic absorption photometry. The original method of Jacobs et al. (1960) is suitable for the detection of amounts as small as 10^{-8} to 10^{-9} gm. of mercury. This modified method detected amounts to 10^{-7} gm. A detailed description of the equipment, reagents and procedure may be found in Appendix A.

RESULTS

Alkyl Mercurial Poisoning in Swine

Studies on the pathology of methylmercuric dicyandiamide poisoning

The essential groups are described in Table 9. The dosages were selected to range from near toxic to severely toxic levels.

TABLE 9. Experimental Groups Which Received Methylmercuric Dicyandiamide

Group	No. of Pigs	Dose*	Days**
I	4	0.19	60
II	3	0.38	60
III	4	0.76	44
IV	4	1.52	30
V	5	3.04	16
Control	5	----	60

* Mg. Hg/kg. administered daily in the form of MMD.

** Maximum experimental days for this group.

In this experiment, the clinical and pathological findings will be presented separately for each dosage level. Analytical findings will be described collectively.

TABLE 10. Daily Dosage Rate, Treatment Period and Clinico-pathological Results For Each Animal Receiving Methylmercuric Dicyandiamide

Group	Dosage mg.Hg/kg. daily	Animal	Days	Signs	Lesions
I	0.19	1	60	-	-
		2	60	-	-
		3	60	-	-
		4	60	-	-
II	0.38	5	60	-	-
		6	60	-	-
		7	60	-	+
III	0.76	8	44	+	+
		9	41	+	+
		10	44	+	+
		11	46	+	+
IV	1.52	13	25	+	+
		14	28	+	+
		15	29	+	+
		16	30	+	+
V	3.04	18	19	+	+
		19	15	+	+
		20	15	+	+
		21	16	+	+
Control	----	A	20	-	-
		B	60	-	-
		C	60	-	-
		D	60	-	-
		E	60	-	-

Clinical findings

Table 10 summarizes the distribution of clinical signs and lesions among all pigs in the various experimental groups.

Group I (0.19 mg. Hg/kg. daily: All four animals in the group received methylmercuric dicyandiamide for 60 days without ill effects. Growth was not adversely affected (Fig. 2).

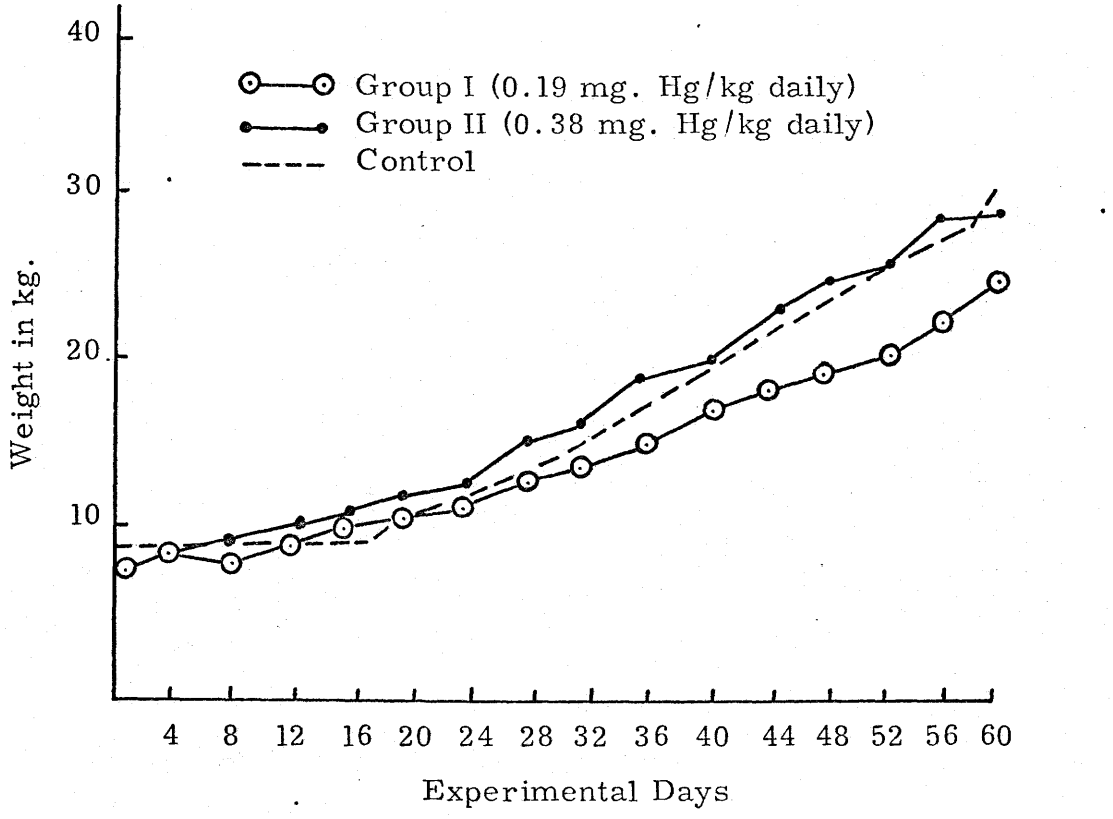


Fig. 2. This graph displays the mean growth rates in the two groups of animals receiving the lowest levels of mercury in the form of methylmercuric dicyandiamide (0.19 and 0.38 mg. Hg/kg). Note that the growth in the group receiving the higher dosage lags behind that at the lowest level.

Group II (0.38 mg. Hg/kg. daily: This group also failed to show remarkable signs of toxicosis. The growth rate was appreciably less than the group receiving the lower dosage (Fig. 2). One animal (7) developed a rectal prolapse on Day 18. It passed blood-tinged feces, probably the result of local injury. The prolapse was reduced and the problem did not recur.

Group III (0.76 mg. Hg/kg. daily: In contrast to the previous two groups, animals in this group developed unequivocal signs of illness which appeared at about the 32nd experimental day. Death occurred four to nine days following appearance of the earliest clinical sign, which was an alteration in the tone of the voice.

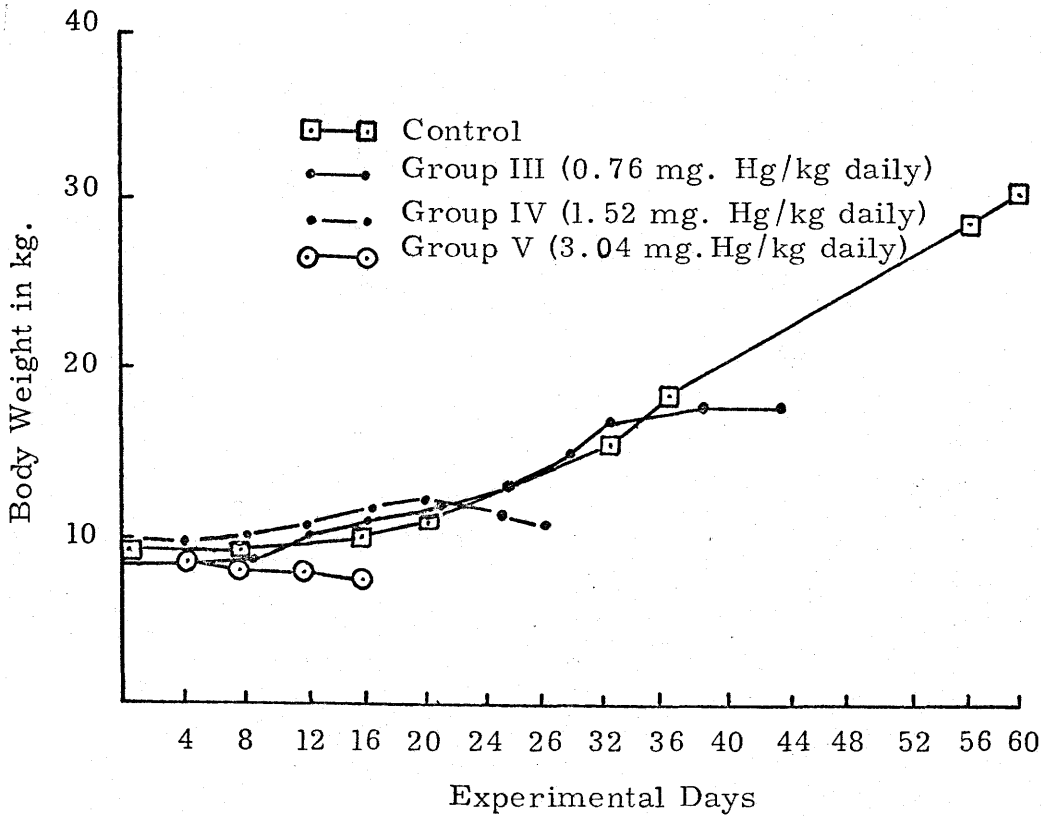
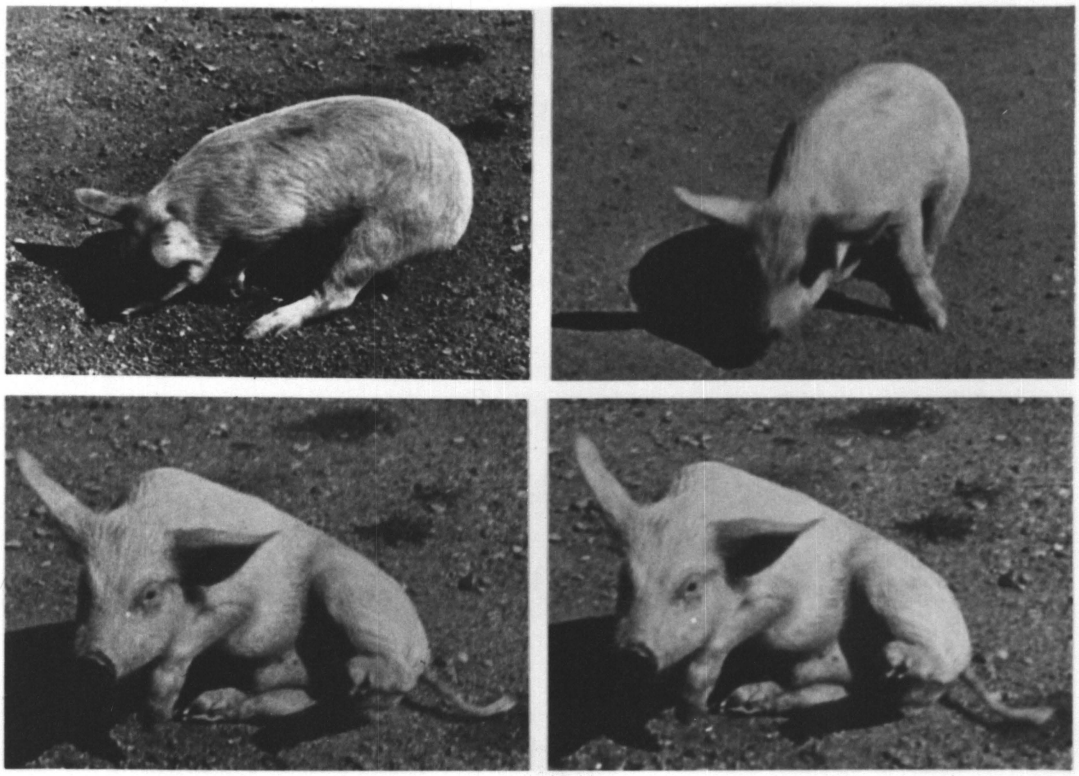


Fig. 3. Graph showing the mean body weights for Groups III, IV, V and Control during the course of MMD poisoning.

Fig. 4. This illustrates a pig with tonic contracture of masseteric musculature. This animal was unable to prehend food or reject the speculum placed in its mouth.

A B

Fig. 5. Pig with posterior paresis. This sequence of photographs illustrates lateral oscillation of the hind quarters.



C D

Fig. 6. Pig with partial paresis. Sequence shows knuckling of the front fetlocks combined with a backwards fall.

Vocal change was a sign. A change in the character of the squeal became noticeable when the animals had to be handled and restrained in connection with weighing and clinical examination. In time, the squeal became progressively more raucous and then changed to a feeble barking. A moderate degree of superficial necrotic pharyngitis was noticed in one animal.

Neurological signs. -- Three animals (9, 10 and 11) exhibited varying degrees of trismus. This was appreciated during efforts to open their mouths manually or with the aid of a mouth speculum. It was observed that affected animals held the speculum *in situ* in their mouths for extended periods of time without any attempt to reject it (Fig. 4). Incoordination and paresis were among the earliest signs to appear. Characteristically, the animals could not walk in a straight line. When walking forward, their hind quarters would oscillate laterally (Fig. 5). Partial paresis was manifested by a sudden knuckling of the fetlocks and falling (Fig. 6). Semi-paretic animals struggled immediately and regained normal posture. Such episodes were seen repeatedly and, with time, they increased in frequency. Blindness, when present, appeared to influence the nature of the ambulatory disturbance. Animals which were considered to be blind, when placed outside in unfamiliar surroundings, refused to move forward. Instead, they made extensive use of their olfactory sense, apparently, in an effort to evaluate the nature of the environment. They were seen to proceed forward only after having sniffed at every stone or plant within their reach.

Their cautiousness was manifested by a peculiar set of movements. The hind quarters were used as an anchor around which the rest of the body executed semicircular movements. Later, incoordination and paresis deteriorated beyond the limits of compensation. During the last two or three days of illness, a diffuse, fine tremor became obvious and persisted until death.

If inability to avoid natural or artificial objects is to be considered the main criterion for blindness, this sign became unequivocably manifest during the last days of life. One animal (11) was found dead on day 36. The previous morning it had walked aimlessly in the box, pushing and climbing over its penmates. Its behavior suggested blindness. Later in the evening, it behaved similarly, and,on several occasions,it was seen to climb the walls of the box in a period of excitement. When excited, it blinked its eyelids continuously.

When in the terminal stage, affected animals remained in lateral recumbency. They became hypothermic and emaciated and had depressed reflexes.

Group IV (1. 52 mg. Hg/kg. daily: Signs of toxicosis in this group were similar to those seen in Group II but were much more uniform in character and appearance.

This dosage caused signs of disease by the end of the third week. Death occurred seven to 14 days after the appearance of an altered voice and three to 11 days after the appearance of neurological signs of poisoning.

Animals with overt signs of illness lost weight rapidly (Fig. 3).

The signs are presented in chronological order of appearance and are in many ways similar to those described for the previous group. On the 16th day, one animal (14) was noted to have a raucous voice associated with a necrotic pharyngitis affecting mainly the epiglottis, but extending also to adjacent structures. External pharyngeal and laryngeal palpation proved painful to the animal. The remaining animals (13, 15 and 16) manifested vocal change within the following two days. Clinical examination of the mucosa of their pharynges revealed the presence of slight hyperemia but no necrotic process. The voice deteriorated considerably with the evolution of the disease process. Terminally, it was reduced to a feeble raucous barking. Throughout this period it was noticed that the grunt retained its normal sound.

Neurological signs. -- The first neurological manifestation of poisoning was incoordination. It was appreciated when animals were placed in an outside yard and allowed to walk freely. The earliest sign consisted of a lateral oscillation of the hind quarters as the animal proceeded forward. Paretic signs such as knuckling of the fetlock, falling, and difficulty in regaining and maintaining a standing position were either synchronous with, or followed, the appearance of incoordination. Efforts to regain posture became progressively more ineffectual and, later, were accompanied by repeated convulsive movements, cyanosis and exhaustion followed by a period of inactivity.

Next in the chronological order of appearance was the sign of trismus. This coincided with the onset of loss of body weight.

Blindness became manifested by a failure to avoid obstacles in their paths.

The animals developed a tremor which was fine, diffuse and initially continuous. It appeared two to three days prior to death. Later, when the animals were in lateral recumbency, the tremor was observed only during the brief interval between expiration and inspiration. At this stage, the legs which were uppermost in this position appeared to be most affected by the tremor.

The time spent in lateral recumbency varied from one to four days prior to death. Once in lateral recumbency, the animals lost weight and their condition deteriorated rapidly. One animal (13) kept its head extended and, when it attempted to resist manipulation, generalized convulsions occurred. Later, depression became pronounced and the reflexes, as determined by the usual clinical tests, were absent. Characteristically, such animals failed to respond to pricking of the foot with a needle. Terminally, the righting reflexes also were absent and the animals appeared to be in a coma. At this stage, the body temperature was well below 95° F.

Group V (3.04 mg. Hg/kg. daily: In this group, signs were similar to those seen in pigs receiving the lower dosage with the difference that they appeared earlier and the course of the disease was more rapid. This was partially the result of a severe diphtheroid pharyngitis which developed. The pharyngeal lesion also caused

respiratory difficulty which resulted in cyanosis when affected animals were induced to exercise or were excited during the weighing process.

Neurological signs. -- All four animals became incoordinated between the 10th and 12th experimental days. As a result, their gait was impaired thereafter. Knuckling of the anterior fetlocks was observed, both in the standing posture and during movement. This sign later progressed to the extent that support of the front quarters was impossible. Such animals were seen to crawl. When supported they would stand but, if unsupported, they tended to fall either forward or laterally.

All animals in this group became hyperesthetic and, when handled, they would experience convulsions.

Growth rate was depressed shortly after commencement of the experiment and weight loss led to terminal emaciation.

Dysphagia appeared on the 11th day and by the 16th day affected all pigs in this group.

Trismus was also clearly manifested during the same period. Food placed in their mouths failed to induce mastication. However, water was swallowed. Characteristically, when shallow water containers were placed in front of the affected animals, they tended to scoop water into their mouths and then raised their heads and swallowed.

Lateral recumbency, hypothermia, extreme emaciation, tremor and halitosis were observed terminally.

All animals died or were euthanized by the end of the third experimental week.

Controls: All remained clinically normal through the course of the experiment.

Gross pathological findings

Groups I and II (0.19 and 0.38 mg. Hg/kg. daily: No gross lesions were found in these groups.

Groups III, IV and V (0.76, 1.52 and 3.04 mg. Hg/kg. daily: Emaciation and dehydration were prominent. Complete depletion of adipose tissue was seen in animals with prolonged clinical manifestations. From such animals, it was possible to recover adipose tissue for analytical work. Dehydration was characterized by severe dryness of the skin and increased resistance to cutting during dissection. The eyes were sunken and the bony prominences of the body were obvious.

The kidneys were found to be slightly swollen, moderately pale, and grayish in color. The capsules could be detached easily. On the cut surface, the cortico-medullary contrast was diminished.

The liver was usually moderately congested but, in a few animals, this organ was pale. These variations in gross appearance could not be correlated to the dosage level.

No significant gross lesions were noticed in the gastro-intestinal tract, except for the occurrence of an occasional patch of hyperemia in the fundic stomach mucosa.

A diphtheroid inflammatory process involving the pharynx and pharyngeal portion of the larynx occurred in all animals in Group V (3.04 mg. Hg/kg. daily). Only one pig in each of Groups III (0.76 mg. Hg/kg. daily) and IV (1.52 mg. Hg/kg. daily) had

this lesion. The remainder had varying degrees of pharyngeal inflammation ranging from hyperemia to superficial necrosis.

Controls: No remarkable lesions were found in these pigs.

Histopathological findings

The important histological findings for all animals in this experiment are tabulated in Appendix B. Group I had no lesions.

Group II (0.38 mg. Hg/kg. daily):

Nervous system. -- Significant lesions were found only in one pig (7). The cerebral cortex above the sulcus rhinalis was the only area which was involved. There was diffuse loss of cerebral cortical neurons associated with micro- and astrogliosis. The various sulci contained one or two blood vessels with thickened and, on occasion, scarred walls. Only the larger blood vessels appeared involved. In a few instances, an irregular band of hyaline material occupied part of the wall and appeared to insinuate between the various cellular elements forming the wall. In many cases, it was not possible to establish whether or not the increased thickening of the wall occurred mainly at the expense of its lumen (Fig. 7). Some vessels clearly showed intimal proliferation and thickening which reduced the size of the lumen (Fig. 8). Perivascular and meningeal reaction in the sulci, in the form of histiocytosis, was present but minimal. In the medulla and spinal cord, an occasional neuron was found to be necrotic.

Other systems. -- No microscopic lesions were detected in the tissues of other organs.

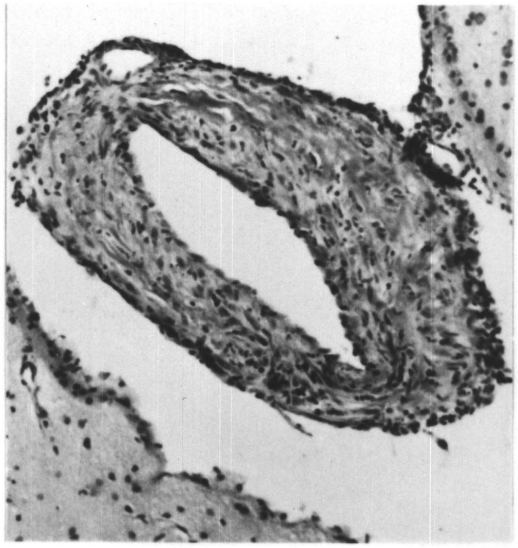


Fig. 7. Meningeal artery from Pig No. 7 which received 0.36 mg. Hg/kg. daily as MMD for 60 days. The wall contains fibrinoid material. H&E stain. x 100.

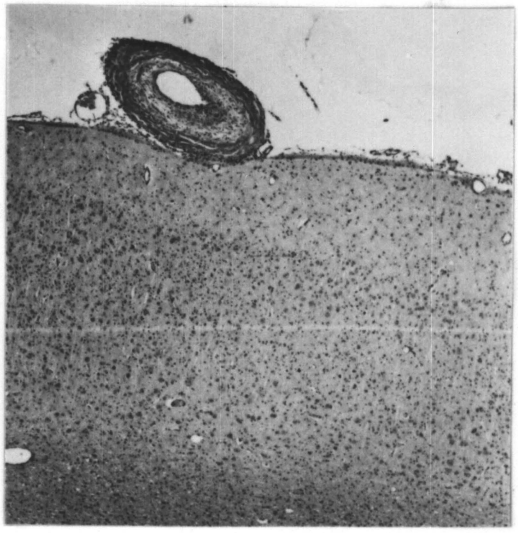


Fig. 8. Meningeal artery from Pig No. 7. Intimal proliferation with partial obliteration of lumen. Moderate gliosis in underlying brain tissue. H&E stain. x 25.

Group III (0.76 mg. Hg/kg. daily):

Nervous system. -- The severity of the brain lesions in the animals of this group was uniform. There were, however, common features related to distribution of the lesions. Those which occurred involved only the cerebral cortex above the sulcus rhinalis. In addition, the cerebro-cortical neuronal degeneration and necrosis described later had a laminar arrangement. The extent of involvement within each lamina, the number of laminae affected, and the distribution of lesions within the cortex all varied from animal to animal and also from one region to another within the same brain. In general, the occipital region appeared to be most extensively and severely affected.

One pig (8) had a severe loss of cerebro-cortical neurons, especially in the third and fourth laminae of the cortex. This neuronal loss resulted in the appearance of a distinct band of vacuoles (Fig. 9). In some areas, neuronal necrosis was extensive in the two deepest laminae (V and VI). Here vacuolation was prominent. In some degenerate nerve cells, vacuolation gave the residual cytoplasm a fenestrated appearance (Fig. 10). Several of the larger cells were mineralized.

Additional findings in the affected cortex were intense, microgliosis and astrogliosis accompanying the rarefaction induced by neuronal necrosis and slight endothelial proliferation in some capillaries.

The sulci were infiltrated very lightly with histiocytic elements. The meningeal vessels appeared uninjured.

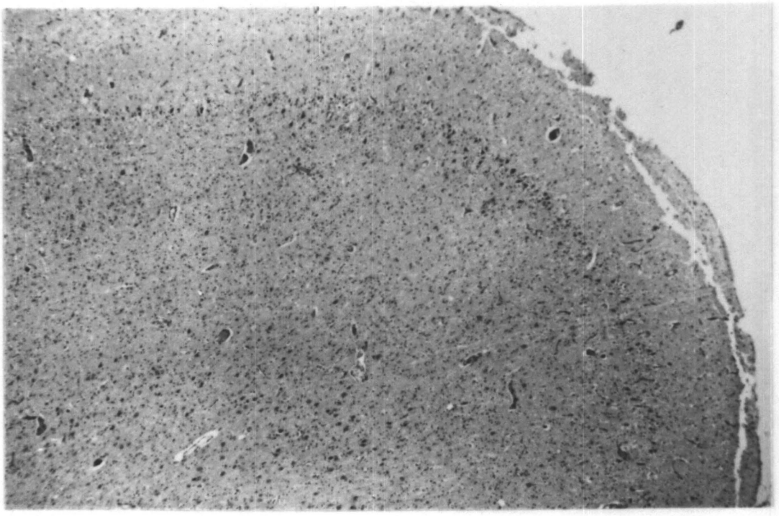


Fig. 9. Pig No. 8 which received 0.76 mg. Hg/kg. daily as MMD. Occipital region. Note the loss of neurons between the second and fifth laminae of the cerebral cortex. H&E stain. x 25.

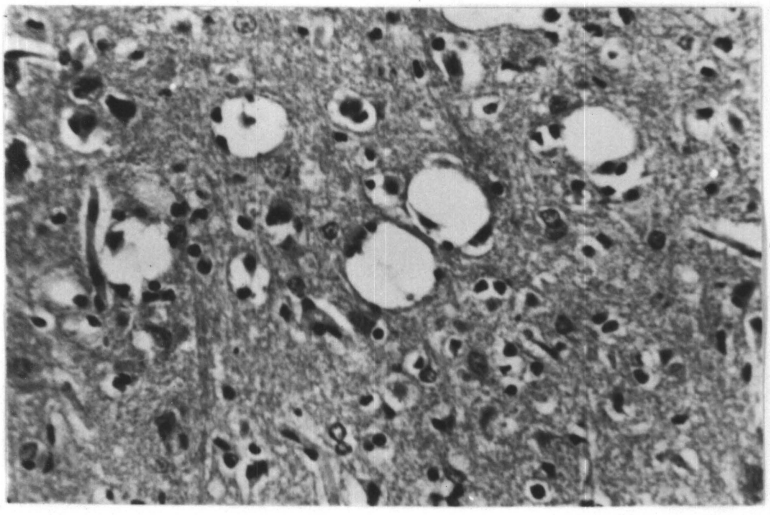
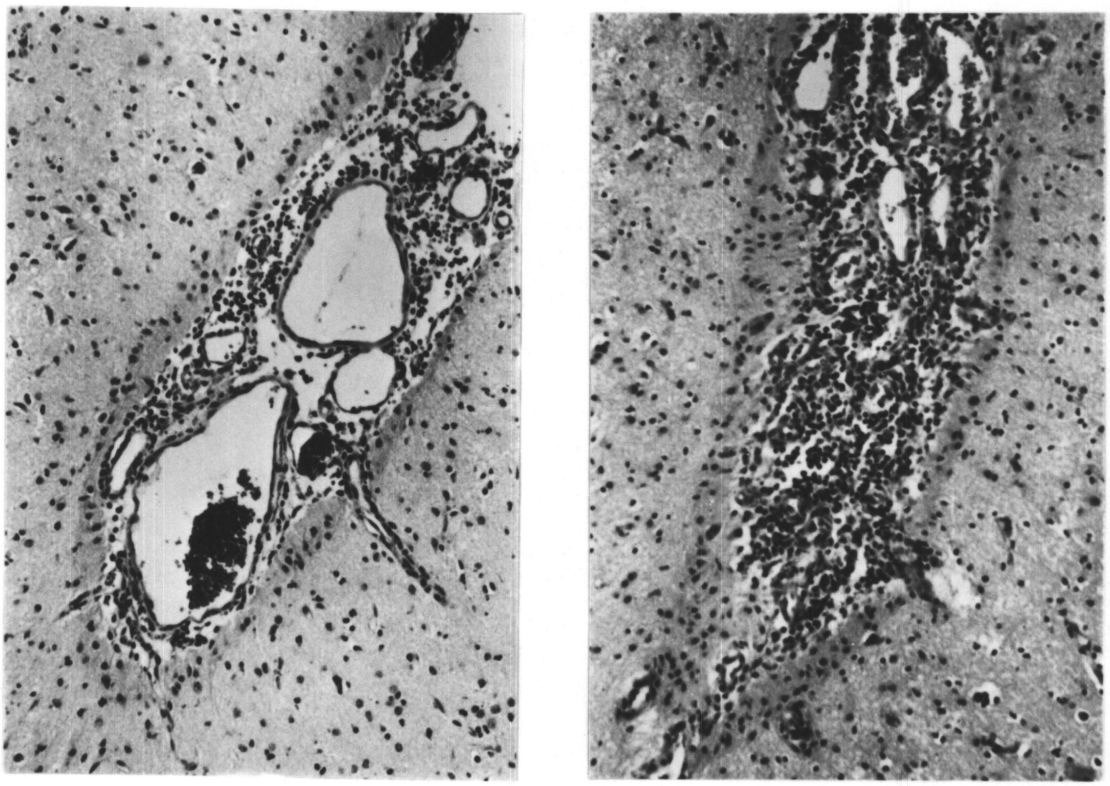


Fig. 10. Pig No. 8. There is extreme vacuolation in the cytoplasm of neurons. H&E stain. x 250.

In another pig (9), prominent features of the process in the cerebral cortex were an intense, diffuse microgliosis and a very conspicuous hypercellularity in most of the sulci. The latter represented an increase in the number of histiocytic unidentifiable mononuclear cells (Fig. 11). Eosinophils were also present but



a b

Fig. 11. Pig No. 9. Sulci of cerebral cortex (a) frontal, (b) occipital with varying degrees of meningeal hypercellularity. Microgliosis and capillary endothelial proliferation are present in the surrounding cortex. H&E stain. x 100.

their number and proportion varied from one sulcus to another. Eosinophil leucocytes were never seen in the underlying brain. Astrocytosis was also prominent and was characterized by an absolute increase in the number of single astrocytes as well as an increase in the number of clones of up to six or more such cells (Fig. 12). Cortical neurons in the affected cortex were

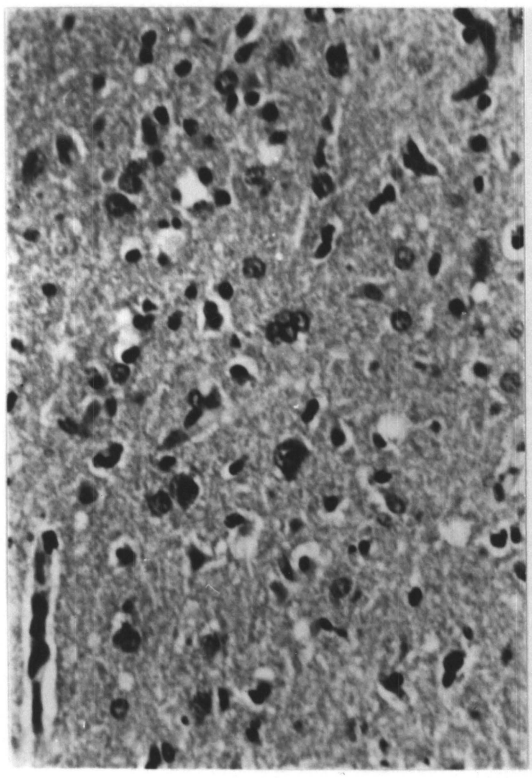


Fig. 12. Pig No. 9. Astrocytosis, microgliosis and loss of neurons in cerebral cortex. H&E stain. x 250

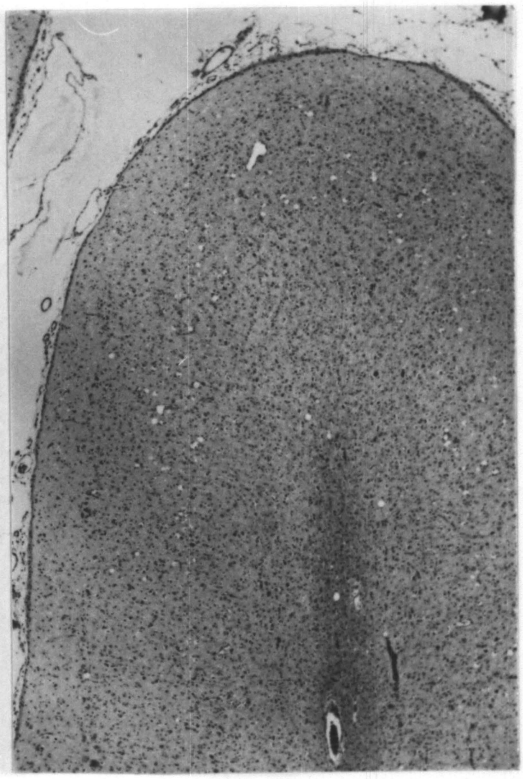


Fig. 13. Pig No. 9. Gyrus from occipital region. Neuronal loss and formation of vacuoles in laminar arrangement. H&E stain. x 25.

reduced in number. Neuronal loss resulted in the formation of vacuoles which had a laminar arrangement and were most prominent at the level of the third and fourth laminar layers (Fig. 13).

The lesions found in another pig (10) were notable because they were more severe and extensive than all other pigs in this group (see Appendix B). Extensive laminar neuronal necrosis and degeneration were present and affected the cerebral cortex unequally. The deep laminae bore the brunt of the injury (Fig. 14). Here numerous vacuoles formed as a result

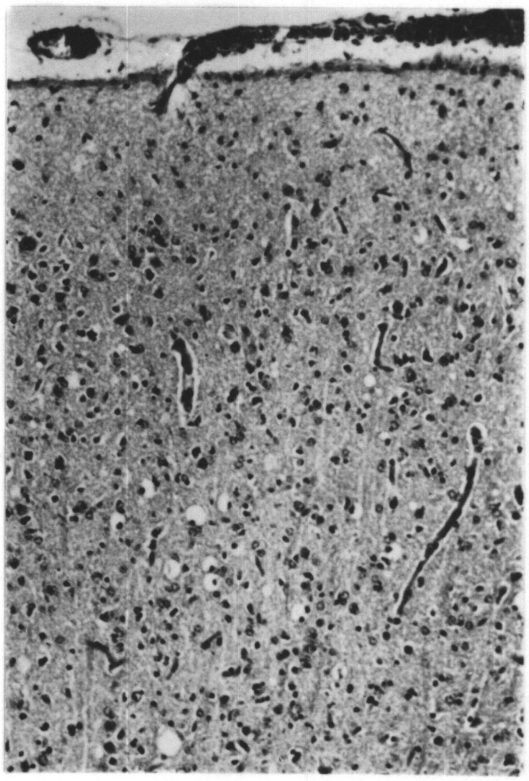


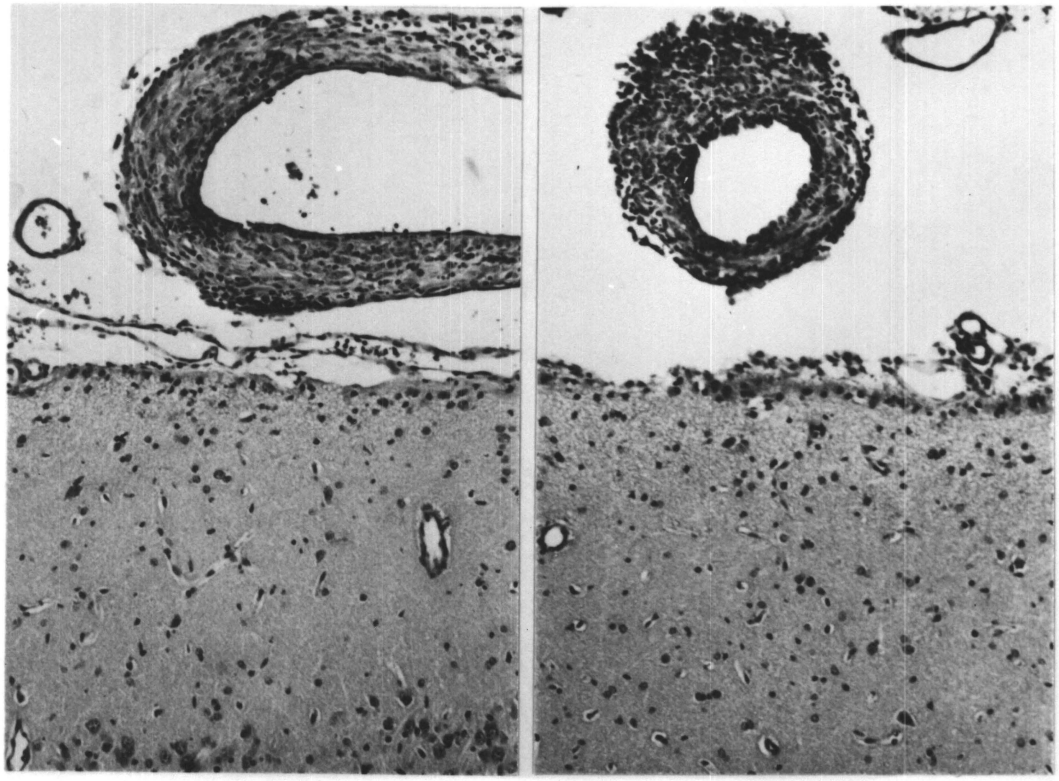
Fig. 14. Pig No. 10. Occipital cortex. Note neuronal necrosis affecting all laminae - note vacuoles associated with necrotic neurons. Microgliosis, astrogliosis and capillary endothelial proliferation are also present. H&E stain. x 100

of neuronal death and dissolution. In addition, a large number of eosinophilic granular bodies were found in the same general area and were interpreted as axon swellings.

The leptomeninges in the sulci were not infiltrated with cells except around abnormal vessels. Uniquely, in this pig, some of the larger sulcal vessels exhibited lesions consisting of pronounced thickening of the wall and scarring of the muscular layer. On occasion, hyaline material occupied part of the wall. This vascular lesion resembled that of Pig No. 7 in Group II. In some small arteries, lymphocytes and plasma cells infiltrated the vessel wall in the manner of arteritis (Fig. 15). In the nucleus ruber, a moderate degree of neuronal necrosis had occurred. No other pigs in the group had this lesion. All pigs receiving higher doses had this lesion.

The remaining pig (11) in this group had very extensive lesions in the cerebral cortex to cause substantial decortication. This resulted from a diffuse, pronounced reduction in the number of neurons and an intense gliosis involving both microglial and astroglial elements. It was impossible to distinguish clearly between the various cortical laminae. In some regions Lamina II, which, under normal circumstances, is quite obvious, was barely discernible. The cortical stroma appeared rarefied and edematous. Capillary endothelial proliferation was prominent throughout the cerebral cortex.

The sulci were variably hypercellular. This was due to an absolute increase in the number of fibroblasts, histiocytes and lymphocytes. Eosinophilic proteinaceous material was present



a b

Fig. 15. Pig No. 10. Meningeal vessels from the sulci of the temporal region. a. The vessel wall is thickened and scarred. b. Arteritis with thickening of the vessel wall. Gliosis is slight. H&E stain. x 100.

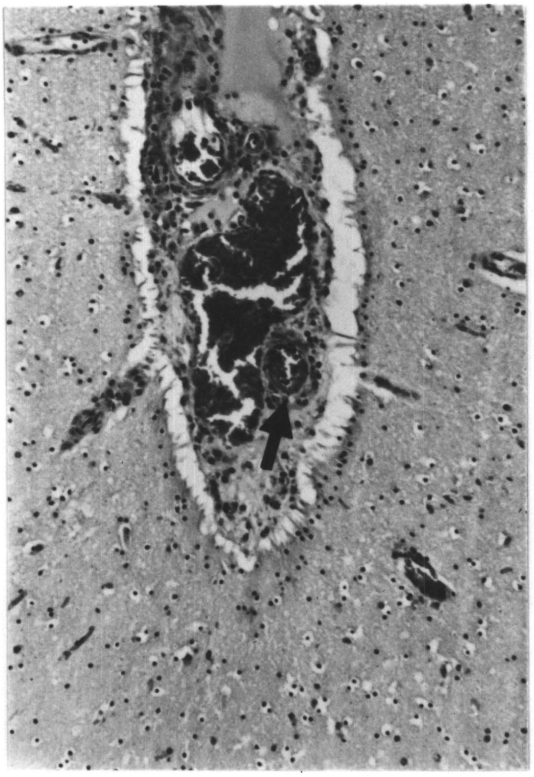


Fig. 16. Pig No. 11. Cerebral cortex sulcus showing hyalinization of the vessel wall (arrow), slight meningeal hypercellularity and proteinoid fluid in the arachnoid space. H&E stain. x 100.

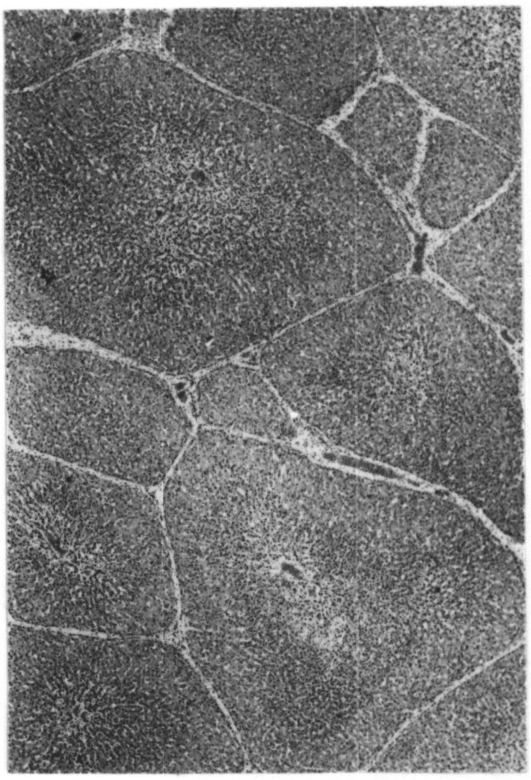


Fig. 17. Pig No. 10. Liver Centrolobular necrosis.

in several sulci and filled the subarachnoid space. This was associated with degenerative changes in the meningeal arteries and small arteries. Hyalinization of the vessel wall was a frequent feature and, in a few vessels, it was associated with an inflammatory reaction in which mononuclear cells infiltrated the wall (Fig. 16).

Other systems. -- Lesions were seen only in the digestive system. Necrotic, ulcerative pharyngitis was found in one pig (8), and centrolobular necrosis in the liver of another (10) (Fig. 17).

Group IV (1.52 mg. Hg/kg. daily):

Nervous system. -- Histological findings in the brain of all the animals in this group did not differ appreciably from one animal to another.

The nature of the lesions in this group differed markedly from those in Group III in that: 1) no large meningeal vessels were injured; 2) neuronal necrosis occurred in the dorsal root ganglia and the Gasserian ganglion; 3) neuronal necrosis occurred in several of the subcortical nuclei.

The cerebral cortex above the sulcus rhinalis was consistently injured. On occasion, the lesions were limited only to the middle and deep portions of the cerebral gyri and sulci.

Necrosis and dissolution of neurons led to the formation of vacuoles which had either a laminar distribution, mainly in the third and fifth to sixth laminae, or a random distribution throughout the various layers of the cortex (Fig. 18). Perineuronal and

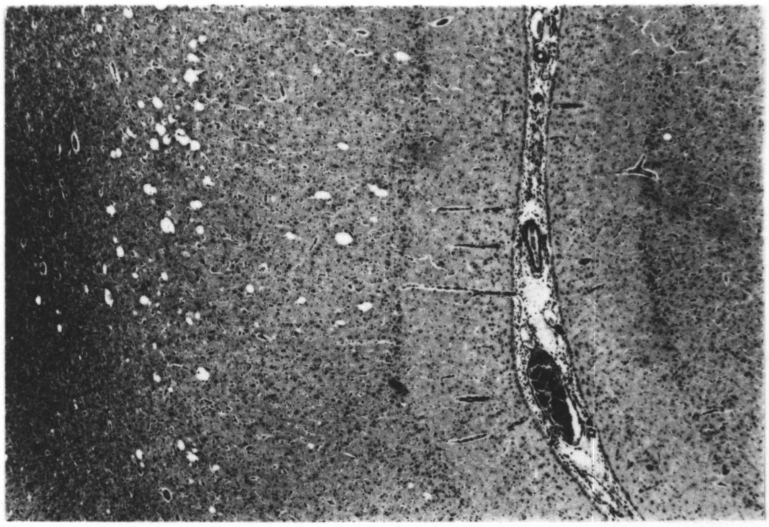


Fig. 18. Pig No. 16. Deep portion of cerebral sulcus and adjacent gyri. Hypercellularity in sulcus is moderate. The cortex is affected by diffuse gliosis, capillary endothelial proliferation and neuronal loss with vacuole formation in deeper laminae. H&E stain. x 25.

perivascular spaces were greatly enlarged and very prominent in all brains and was interpreted as edema (Fig. 19). This edematous tissue had a tendency to fracture during histological sectioning. Non-involved areas on the same sections failed to fracture.

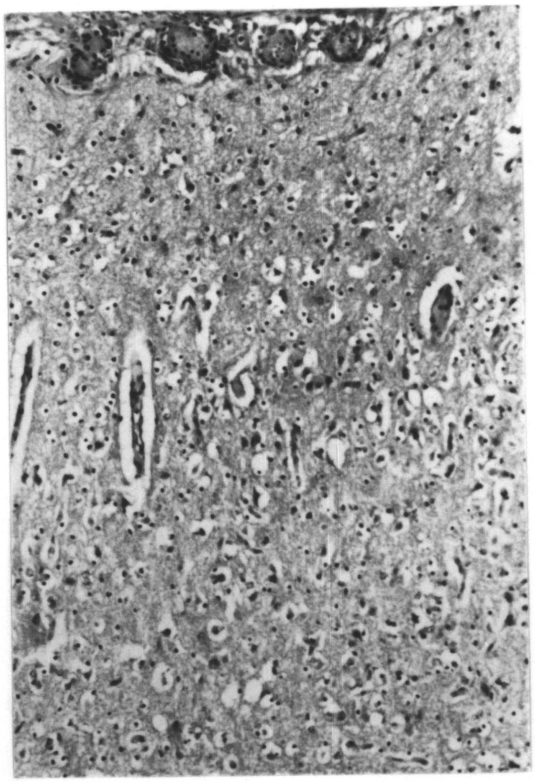


Fig. 19. Pig No. 14. Cerebral cortex near the bottom of a sulcus containing hyalinized arterioles. Edema and loss of neurons affect this cortex throughout. H&E stain. x 100.

Neuronophagia was frequently encountered in the cortex and numerous mineralized neurons were found scattered among the remaining nerve cells. These were most common at the level of the outer granular layer. Numerous eosinophilic granular bodies were present in the deepest laminar layers.

Glial reaction was prominent (Fig. 16). Severe microgliosis occurred, and mitotic figures of these histiocytic elements occurred throughout the affected cortex, but were more frequent in the subpial region and near hypertrophic capillary endothelium. Astrocytosis was also severe. A clearly detectable increase in the absolute number of astrocytes was present. These cells appeared in groups of up to six or seven. The majority of them had large vesicular nuclei. The oligodendroglia cells appeared to participate in the disease process by becoming swollen.

All leptomeninges in the sulci above the sulcus rhinalis exhibited moderate to severe hypercellularity, most frequently in the deeper aspects of the sulci (Fig. 20). This hypercellularity was due to hyperplasia of the fibroblastic and histiocytic elements in the meninges, and to an increase in the number of lymphocytes. There was active proliferation of histiocytes since numerous histiocytic cells were in mitosis. Mitotic cells were found either in the adventitial coat of blood vessels throughout the trabeculae of the subarachnoid space or along the pial lining of the brain. The histiocytic cells, when not surrounded by other cellular elements, had an ovoid shape and contained an indented hyperchromatic nucleus.

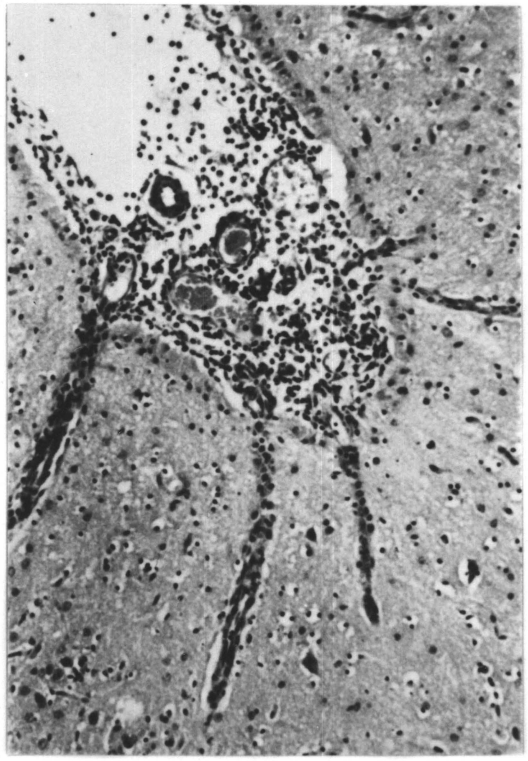


Fig. 20. Pig No. 16. Bottom of cerebral sulcus with moderate hypercellularity. Endothelial proliferation in the cortex. H&E stain. x 100.

Many arterioles and small arteries in the meninges had hyalinized walls. These were thickened at the expense of the vascular lumen in some, but not all, vessels (Fig. 21).

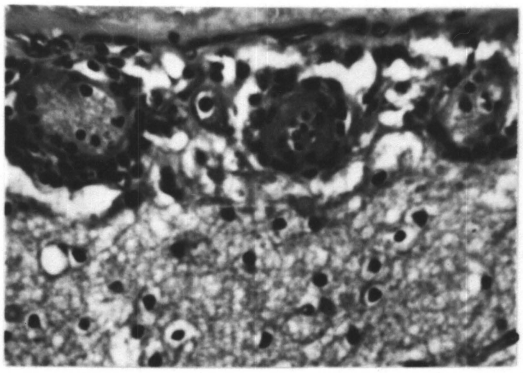


Fig. 21. Pig No. 14. Cerebral cortical meningeal vessels with hyalinized walls. Note occlusion of the lumen. H&E stain. x 250.

Variable amounts of lacey fibrin network partially filled the subarachnoid space in the sulci and, occasionally, a proteinoid amorphous eosinophilic material was also present. Such material was especially obvious in the vicinity of blood vessels (Fig. 22).

The capillaries and precapillary arterioles which entered perpendicularly the adjacent cerebral cortex had an extremely prominent endothelium (Fig. 16). The endothelial cells were hypertrophic and, on occasion, were seen in mitosis.

In Group IV, lesions were present in other parts of the brain in addition to the cortex, and were chiefly neuronal necrosis, capillary endothelial proliferation and perivascular microgliosis.

The lateral geniculate bodies were constantly affected. The ventromedial region of these structures was most severely damaged and eosinophilic granular bodies were numerous. The medial geniculate bodies were similarly involved, but to a lesser degree. The

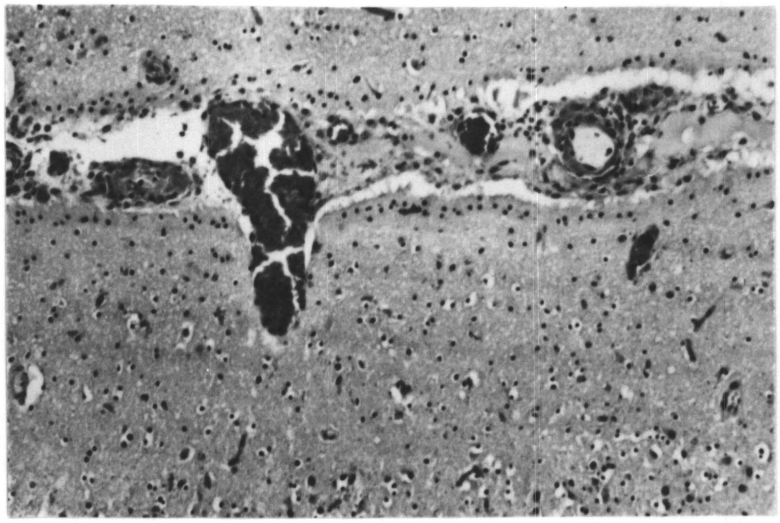


Fig. 22. Cerebral sulcus containing two partially hyalinized vessels and proteinaceous material. H&E stain. x 100.

central thalamic nuclei were also affected but the severity varied from animal to animal. The red nuclei, the pontine nuclei, and the inferior olives contained a few necrotic neurons, most of which were being phagocytosed. The spinal gray matter was affected very rarely by neuronal necrosis. The semi-lunar and dorsal root ganglia contained a significant number of necrotic neurons, most of which were undergoing neuronophagia.

In subcortical lesions, hyalinization and necrosis of arterioles and small arteries were not observed.

Digestive system. -- Lesions occurred in the digestive system. A necrotic, ulcerative, purulent pharyngitis, glossitis, laryngitis, and esophagitis (at the proximal end) were seen in three animals.

Centrolobular necrosis of liver was a constant finding in all pigs and was uniformly present throughout the organ. The processes involved about one-fifth of each lobule. Cells in juxtaposition to the necrosis were at various levels of degeneration (Fig. 23).

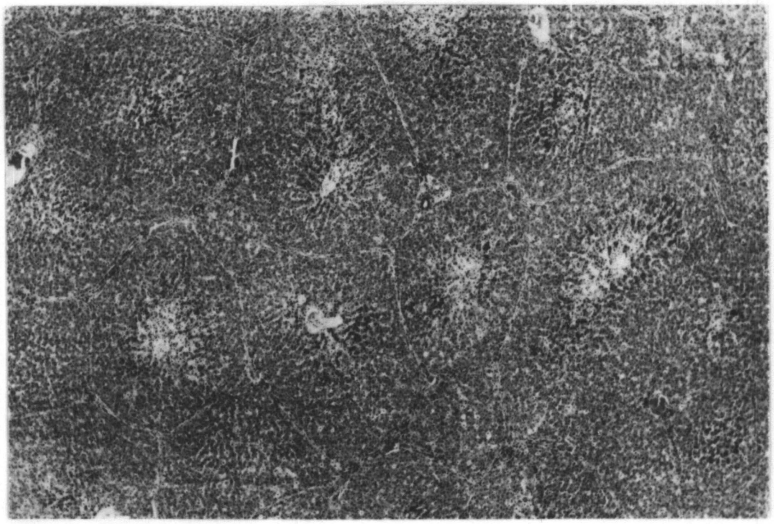


Fig. 23. Pig No. 14. Liver with centrolobular necrosis.

Lesions were also present in the kidney. Varying degrees of toxic nephrosis were seen in all kidneys and was most severe in Pig No. 14. This was characterized by hydropic degeneration of tubular cells and commonly involved the proximal tubules. Necrosis and degeneration of tubular epithelium were rare events (Fig. 24).

Fig. 24. Pig No. 14. Renal cortex with toxic nephrosis. Note severe Hydropic degeneration in proximal convoluted tubules. H&E stain. x 25.

Group V(3.04 mg. Hg/kg. daily):

Nervous system. -- No significant histological differences were detected between the brain of animals killed at an early stage of disease and those killed later.

The lesions in this group differed from those in groups receiving lower dosages of MMD in the following ways: 1) neuronal necrosis was the chief lesion and involved cortex and many subcortical nuclei; 2) glial reaction and capillary endothelial proliferation were present but much less intense; 3) leptomeningeal vessels did not show evidence of injury (Appendix B).

The distribution of lesions followed the general pattern described previously for groups receiving lesser amounts of MMD, except that additional subcortical nuclei were injured.

The cerebrum was the chief site of injury and that lying above the sulcus rhinalis was constantly involved. Lesions were usually absent from the more topical aspects of the affected gyri.

The chief lesion consisted of neuronal necrosis involving predominantly the third, fourth and fifth cortical laminar layers. Eosinophilic granular bodies were common in this area. Instances of neuronophagia were rarely encountered. Enlarged perineuronal and perivascular spaces were prominent, especially in the cortex surrounding the deeper parts of the various sulci (Fig. 25). Moderate astrogliosis and microgliosis were present throughout the cerebral cortex.

Randomly distributed glial mitotic figures were observed, especially in the molecular layer of the cerebral cortex (Fig. 26).

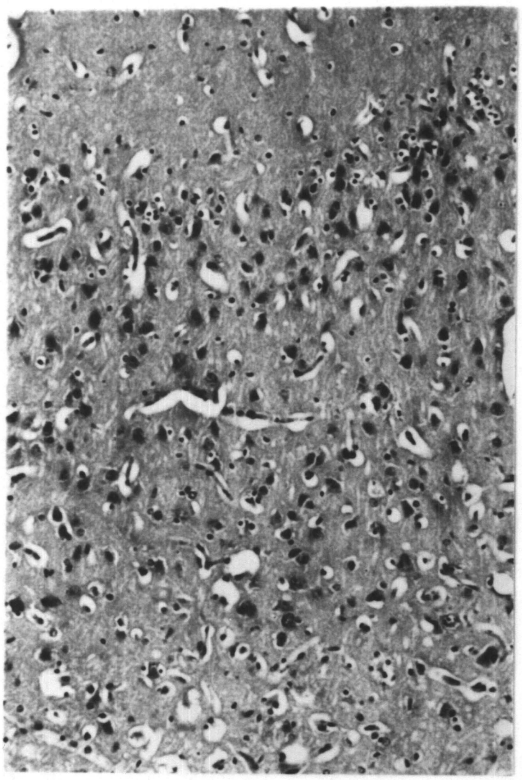


Fig. 25. Pig No. 19. Cerebral cortex below the deepest aspect of a sulcus. It appears edematous. H&E stain. x 100.

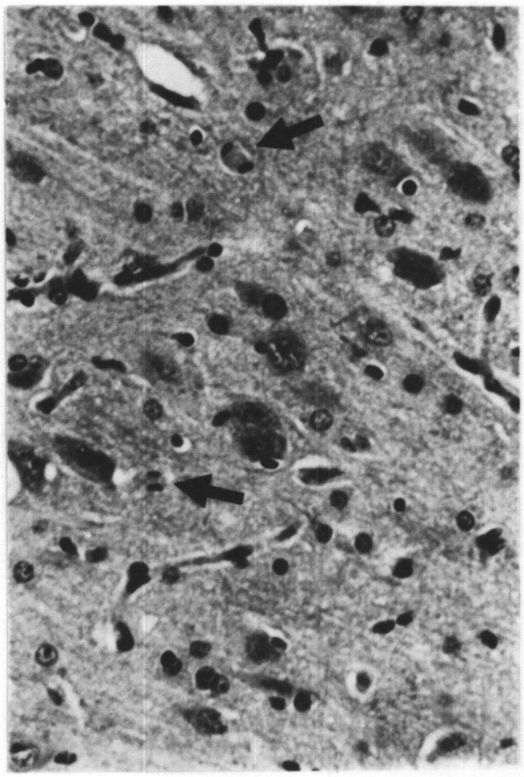


Fig. 26. Pig No. 19. Cerebral cortex. Note the two mitotic figures (arrow). H&E stain. x 250.

The subpial region at the level of the molecular layer had a hypercellular appearance which was due mainly to the increase in the glial elements. Swelling of the oligodendroglia was found in the cortical and subcortical gray matter and throughout the white matter of the brain.

A moderate increase in the number of mononuclear cells was consistently found in the leptomeninges of the sulci of the affected cortices (Fig. 27). Small arteries and arterioles

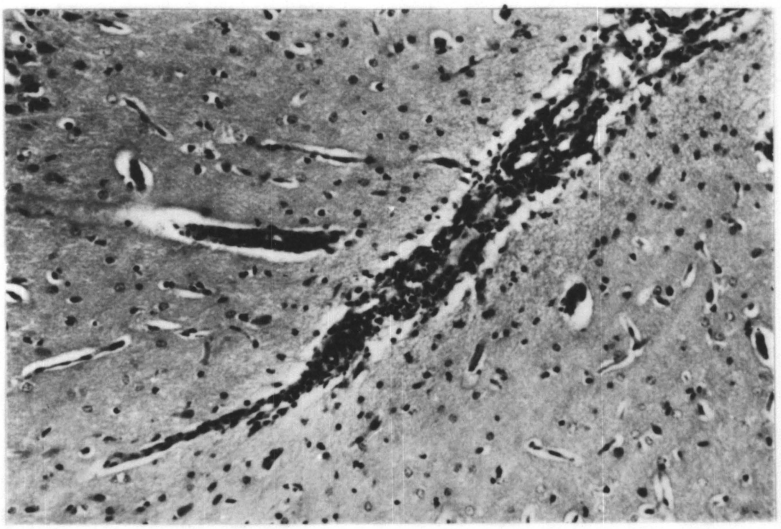


Fig. 27. Pig No. 21. Cerebral cortex. Note the hypercellularity in the sulcus. H&E stain. x 100.

appeared unaffected by hyalinization and/or necrosis in contrast to observations in pigs receiving lower dosages of MMD.

The capillary bed of the involved cortex exhibited limited endothelial cell proliferation.

The basoganglia, the central thalamic nuclei, the medial geniculate bodies, the red nuclei, the pontine nuclei, the semilunar ganglia, the dorsal root ganglia, and the spinal gray matter were affected by randomly distributed neuronal necrosis and degeneration. These lesions in the basoganglia, central thalamic nuclei, and the medial geniculate bodies were symmetrical.

The lateral geniculate bodies were symmetrically affected by neuronal degeneration and necrosis. The ventro-medial region of both lateral geniculate bodies was most severely affected. A prominent feature throughout the geniculate bodies was the presence of numerous eosinophilic granular bodies (Fig. 28).

Digestive system. -- In the liver there was a moderate number of necrotic hepatocytes which were randomly distributed within the various lobules. The lesion was more severe in one pig (21) and the usual cord-like arrangement of the hepatocytes was lost.

Urinary system. -- In the kidneys the proximal tubular epithelium had undergone the changes of hydropic degeneration. This manifestation of toxic nephrosis was most pronounced in the middle and inner cortical regions.

Controls: No significant histological lesions were detected in this group.

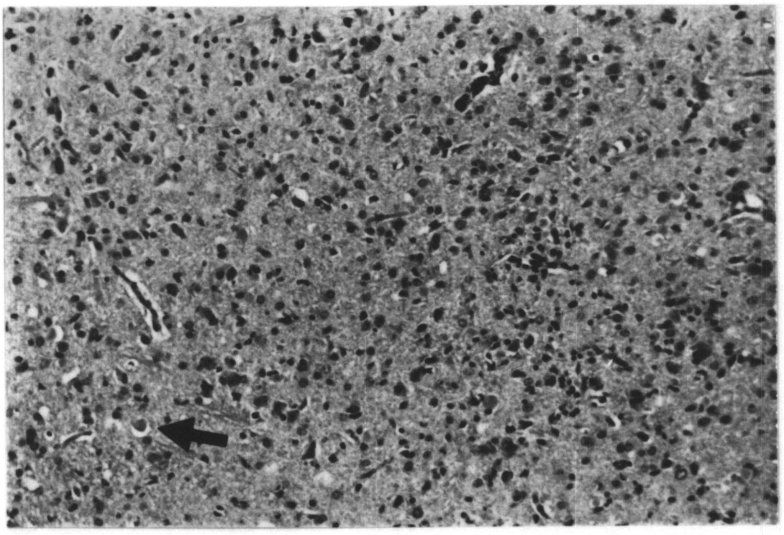


Fig. 28. Pig No. 21. Ventro-medial region of lateral geniculate body showing gliosis, neuronophagia and, in the lower area of the photomicrograph, numerous granular bodies (arrow).

Analytical findings

Reference to Table 11 will support these general observations: 1) the level of mercury which accumulated in tissues was a function of both dose and time; 2) certain tissues accumulate mugh higher levels of mercury than others; 3) the rate of accumulation of Hg in the tissues varied directly as the dosage.

The level of mercury in the tissues appeared to be directly dependent upon dosage in pigs fed at the lower levels. In groups fed the high levels of mercury, the survival time decreased and tissue levels did not exceed those found in more chronically poisoned animals in many cases. Figures 29-36 illustrate the effect of dose on the tissue level of mercury and also serve to point out the large difference between the level of accumulation in different tissues.

The rates of accumulation of mercury in each tissue of each pig were calculated by dividing the amount of mercury in the tissue by the number of days the animal received the MMD. These data are presented in Table 11. Figures 37, 38 graphically illustrate the rates of mercury accumulation for each organ when all data for each of the experimental groups are pooled. In so far as this treatment of the data is valid, the rate of mercury accumulation appears to be directly dependent upon dosage at the levels studied.

TABLE II. Levels of Hg in μg/gm or μg/ml in Tissues of Pigs Which Received Varying Doses of Methylmercuric Dicyandiamide

GROUP DOSE	PIG NUMBER	DAYS	μg Hg/ml			μg Hg/gm											
			Blood	Urine	Bile	Brain		Liver		Intestines		Kidney		Muscle		Skin	
						Hg	Hg/days	Hg	Hg/days	Hg	Hg/days	Hg	Hg/days	Hg	Hg/days	Hg	Hg/days
	1	60	0.7	0.3	---	3.0	0.05	7.9	0.13	10.2	0.17	18.2	0.30	3.1	0.05	2.3	0.03
I	2	60	0.8	0.2	---	2.0	0.03	7.0	0.12	----	----	17.1	0.28	2.6	0.04	3.4	0.05
(0.19)	3	60	0.6	0.2	---	2.1	0.04	---	----	----	----	15.3	0.26	3.7	0.05	0.9	0.02
	4	60	0.9	0.2	---	1.8	0.03	---	----	----	----	19.2	0.32	3.1	0.05	2.8	0.02
	5	60	0.9	---	---	4.5	0.08	13.6	0.23	3.4	0.06	23.4	0.38	---	----	5.3	0.09
II	6	60	0.9	0.5	---	5.3	0.08	8.9	0.15	17.1	0.28	25.5	0.42	4.4	0.07	7.3	0.12
(0.38)	7	60	1.4	0.4	1.0	6.0	0.10	13.6	0.23	----	----	58.7	0.97	---	----	6.0	0.10
	8	44	5.0	1.4	---	---	----	42.6	0.96	19.8	0.45	72.7	1.65	22.8	0.52	---	----
III	9	41	4.1	---	1.2	14.9	0.34	112.3	2.73	12.7	0.30	33.9	0.83	16.8	0.41	13.0	0.29
(0.76)	10	44	2.3	---	---	13.4	0.33	32.1	0.89	6.6	0.15	69.1	1.57	16.1	0.37	8.5	0.21
	11	46	---	0.6	---	----	----	----	----	10.1	0.28	51.0	1.42	----	----	4.8	0.13
	13	25	7.1	0.9	---	----	----	51.4	2.06	----	----	112.6	4.48	----	----	15.3	0.61
IV	14	28	---	2.9	---	----	----	49.5	1.77	11.5	0.41	94.9	3.36	29.7	1.06	10.5	0.37
(1.52)	15	29	---	0.9	---	27.3	1.00	----	----	22.1	0.76	73.8	2.54	26.2	0.90	18.0	0.62
	16	30	---	0.7	---	37.4	0.30	39.9	1.33	31.7	1.06	----	----	21.2	0.71	17.5	0.58
	18	19	4.3	0.7	---	24.0	1.26	----	----	30.6	1.61	75.5	3.97	19.3	1.00	8.8	0.46
V	19	15	6.6	1.5	---	27.6	1.80	92.4	6.16	12.1	0.80	111.1	7.40	23.3	1.55	----	----
(3.04)	20	15	8.0	1.4	---	----	----	83.1	4.16	----	----	134.5	8.30	66.1	4.40	12.8	0.85
	21	16	9.5	---	---	31.8	1.99	62.1	3.88	26.5	1.65	202.6	12.63	37.5	2.34	----	----
	A	20	0.1	0.1	0.5	0.6	----	0.9	----	0.6	----	0.7	----	0.5	----	1.1	----
	B	60	0.2	0.1	---	0.6	----	0.8	----	0.8	----	2.5	----	0.7	----	2.2	----
Control	C	60	0.2	0.2	0.5	1.4	----	0.9	----	0.8	----	2.0	----	0.6	----	2.3	----
	D	60	0.1	0.1	0.4	0.2	----	0.4	----	0.9	----	0.4	----	0.6	----	---	----
	E	60	0.1	0.1	0.5	0.4	----	0.7	----	0.9	----	0.5	----	0.5	----	---	----

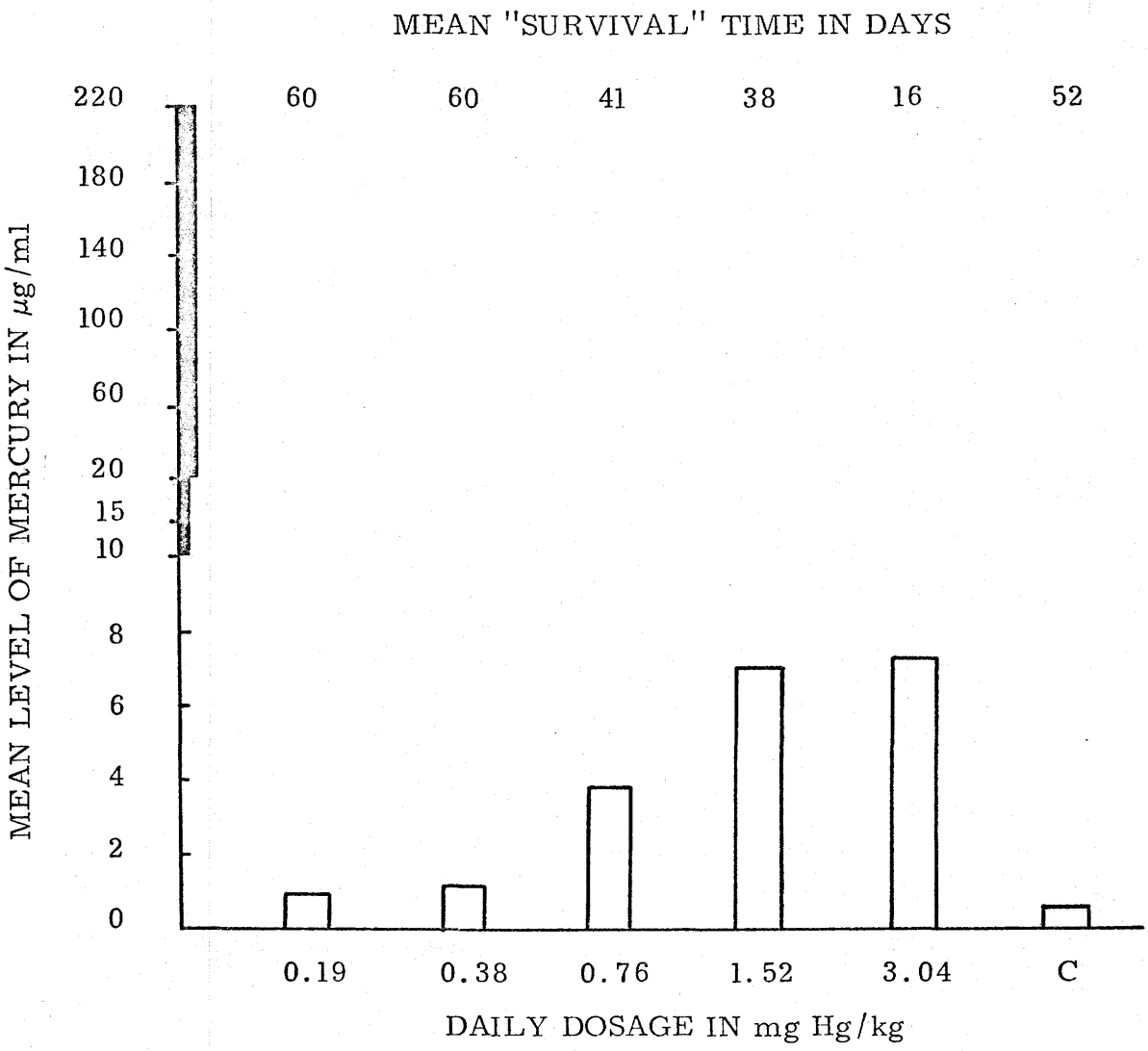


Fig. 29. Mean mercury levels in the blood and mean survival time of groups of pigs receiving increasing dosages of MMD.

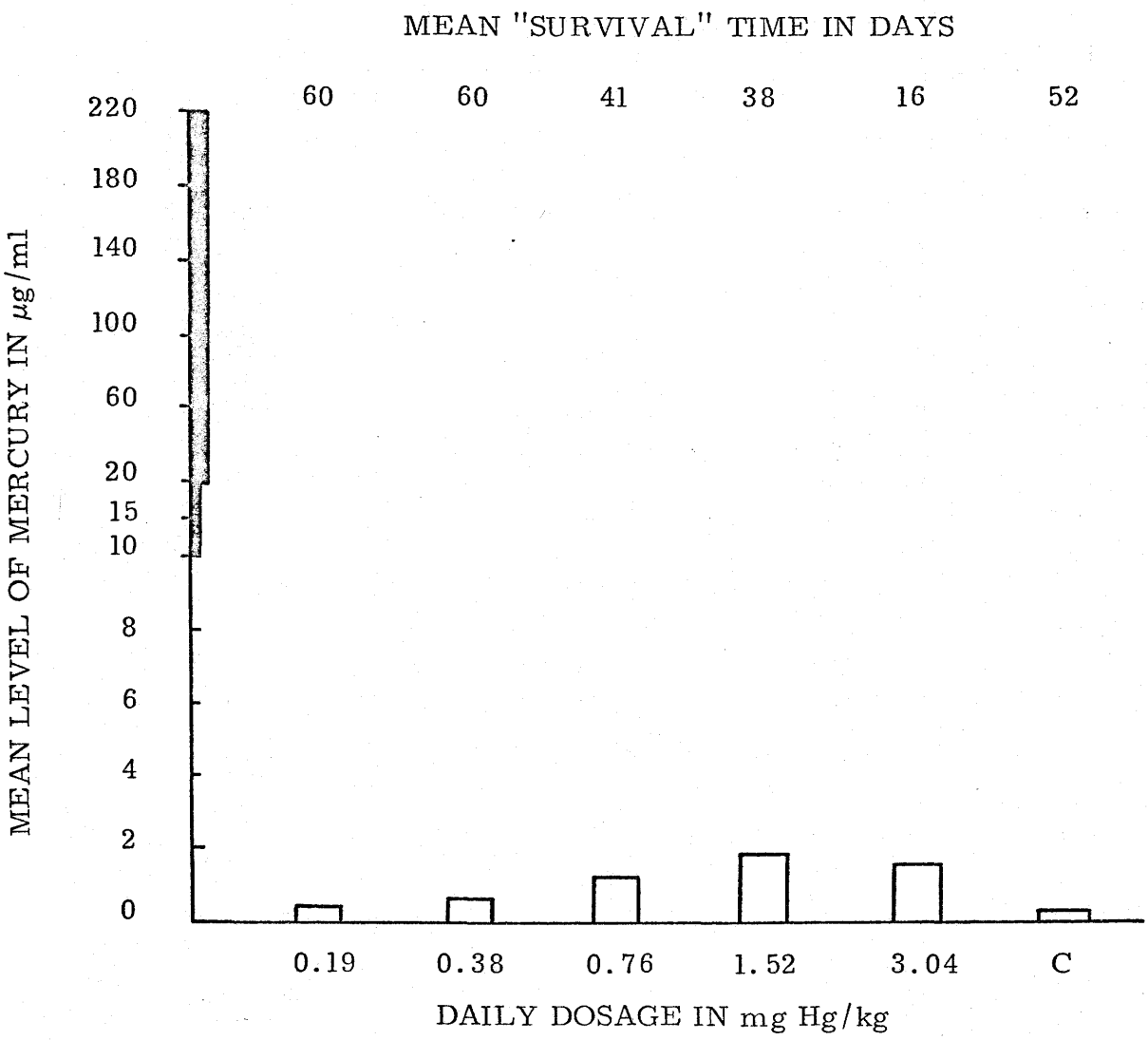


Fig. 30. Mean mercury levels in the urine and mean survival time of groups of pigs receiving increasing dosages of MMD.

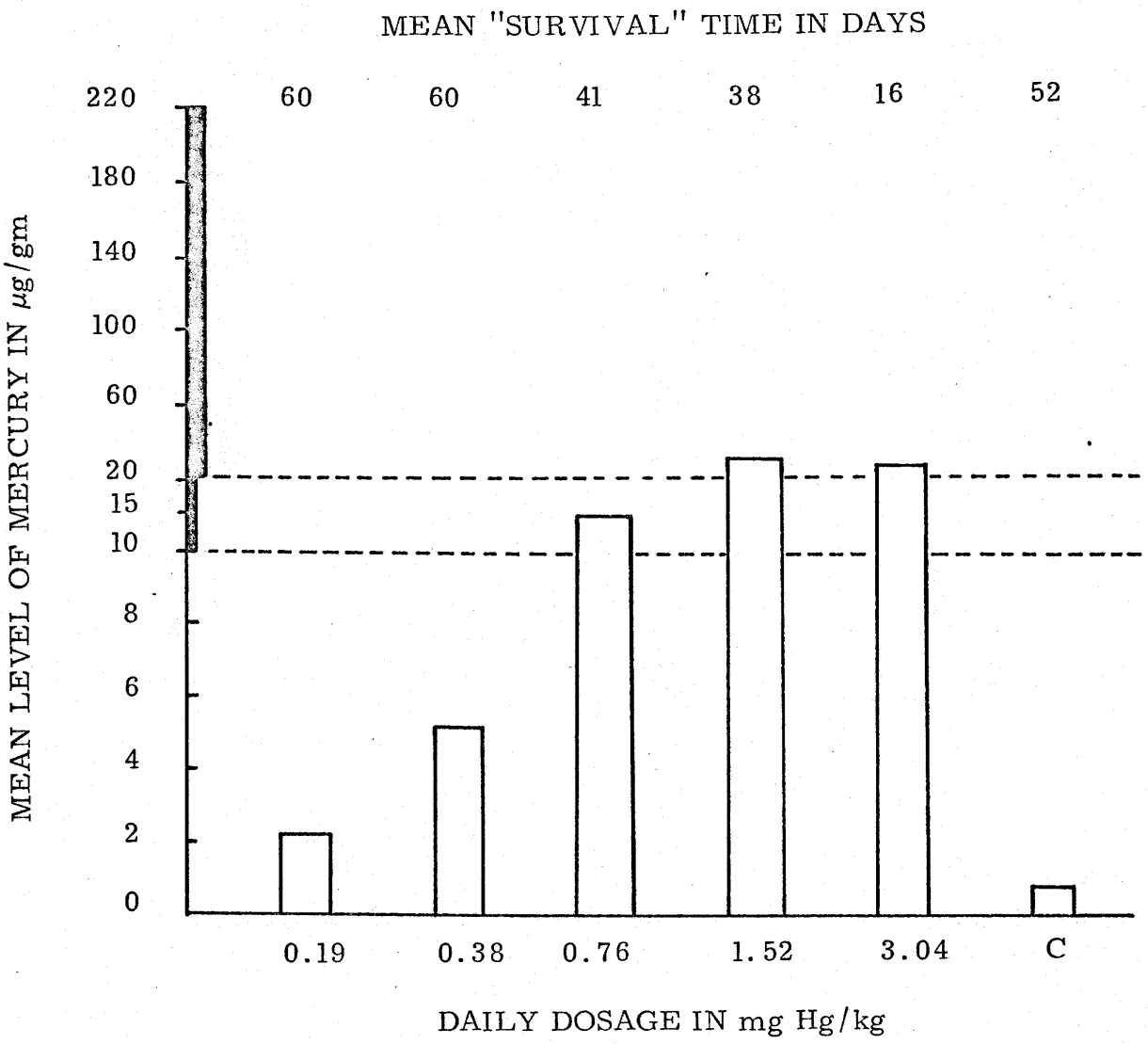


Fig. 31. Mean mercury levels in the brain and mean survival time of groups of pigs receiving increasing dosages of MMD.

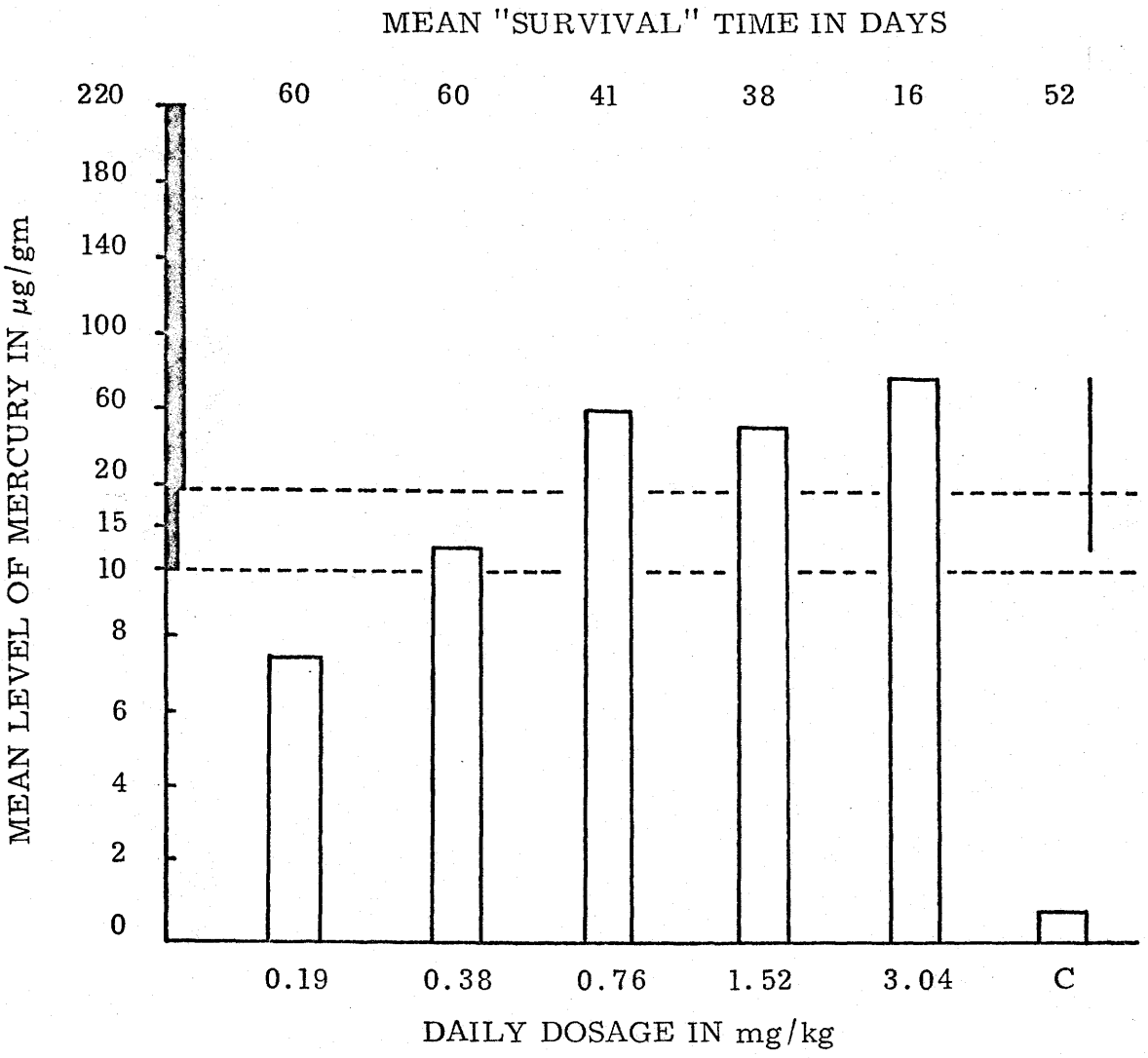


Fig. 32. Mean mercury levels in the livers and mean survival time of groups of pigs receiving increasing dosages of MMD.

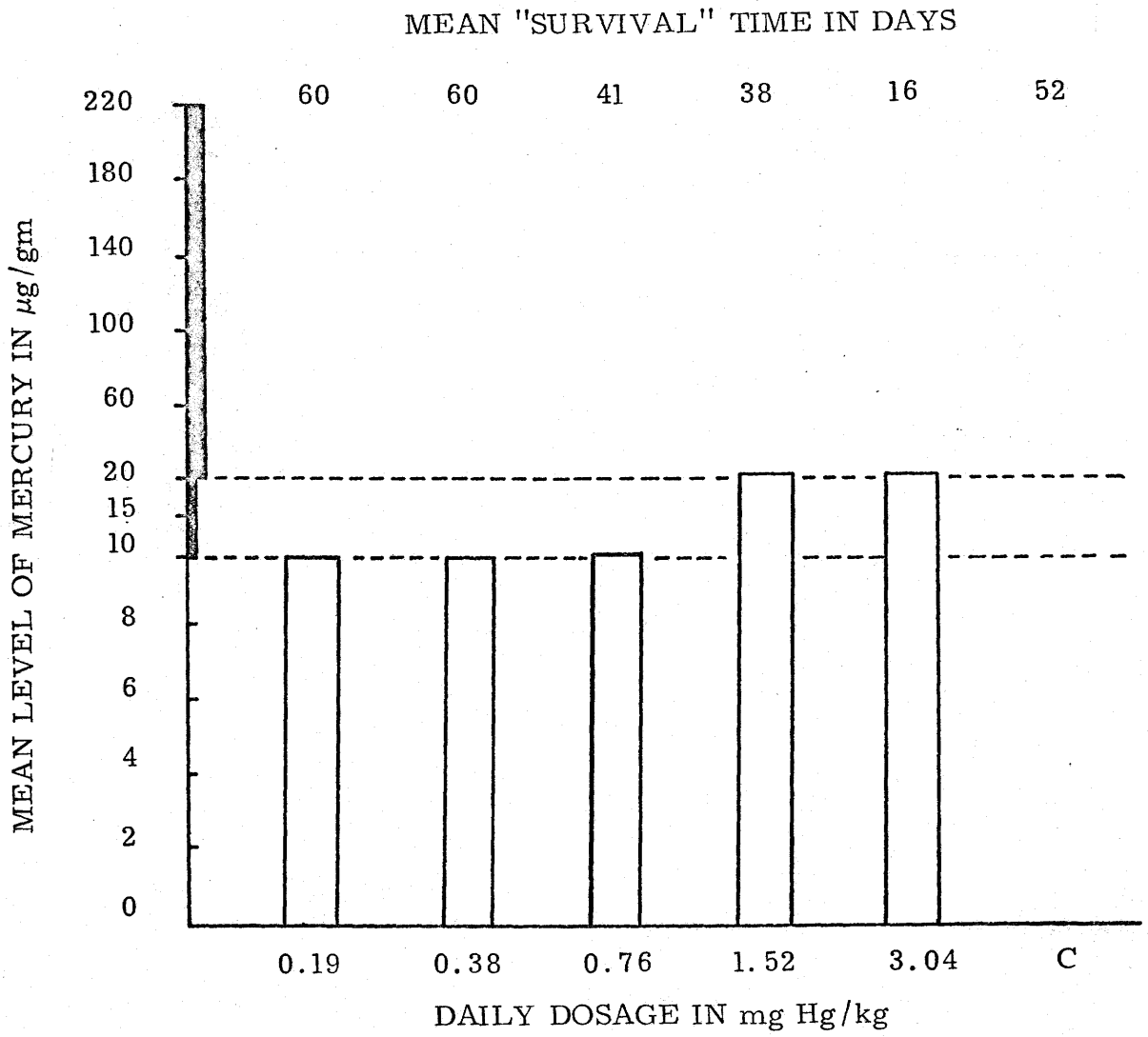


Fig. 33. Mean mercury levels in the large intestine and mean survival time of groups of pigs receiving increasing dosages of MMD.

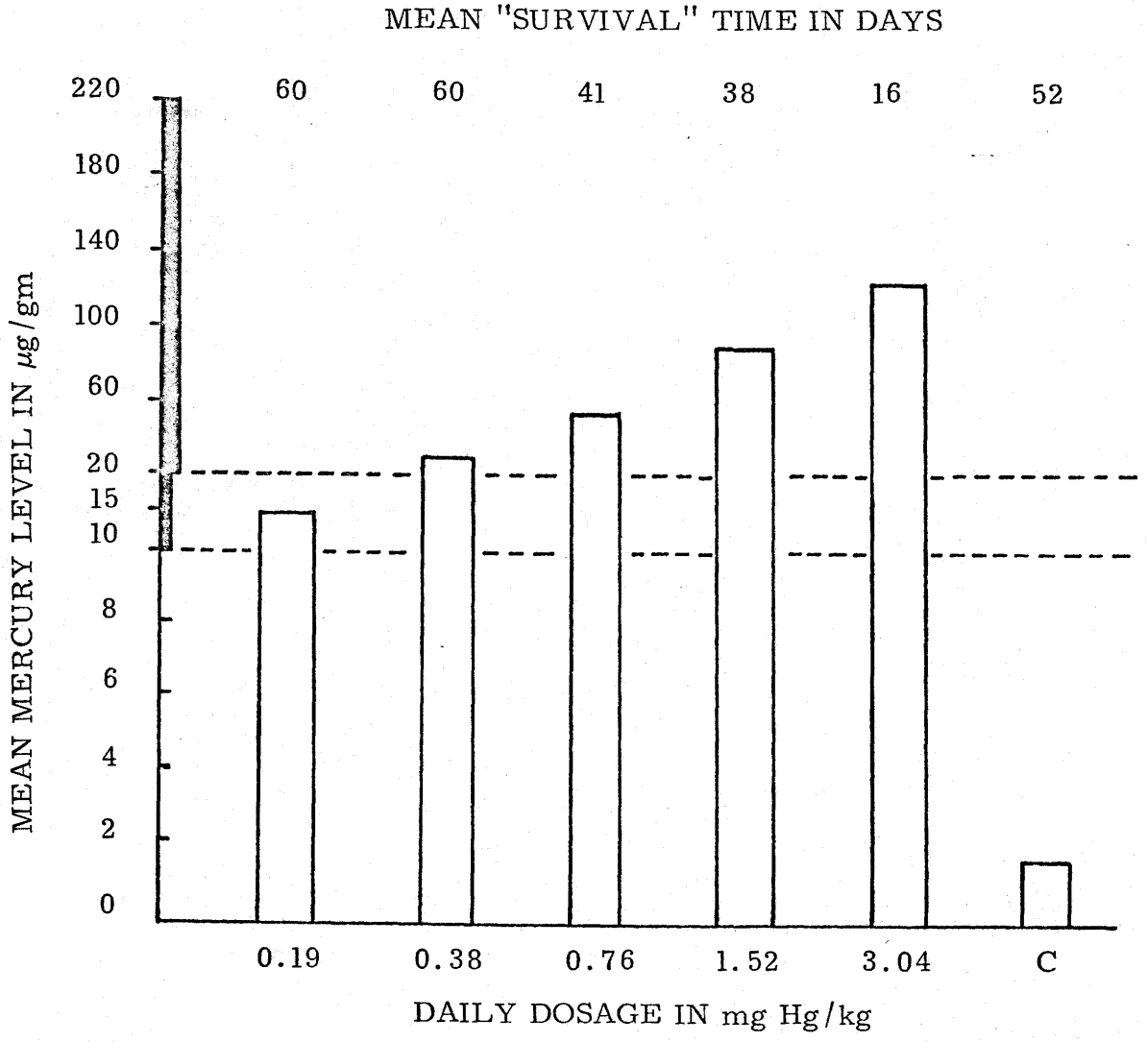


Fig. 34. Mean mercury levels in the kidneys and mean survival time of groups of pigs receiving increasing dosages of MMD.

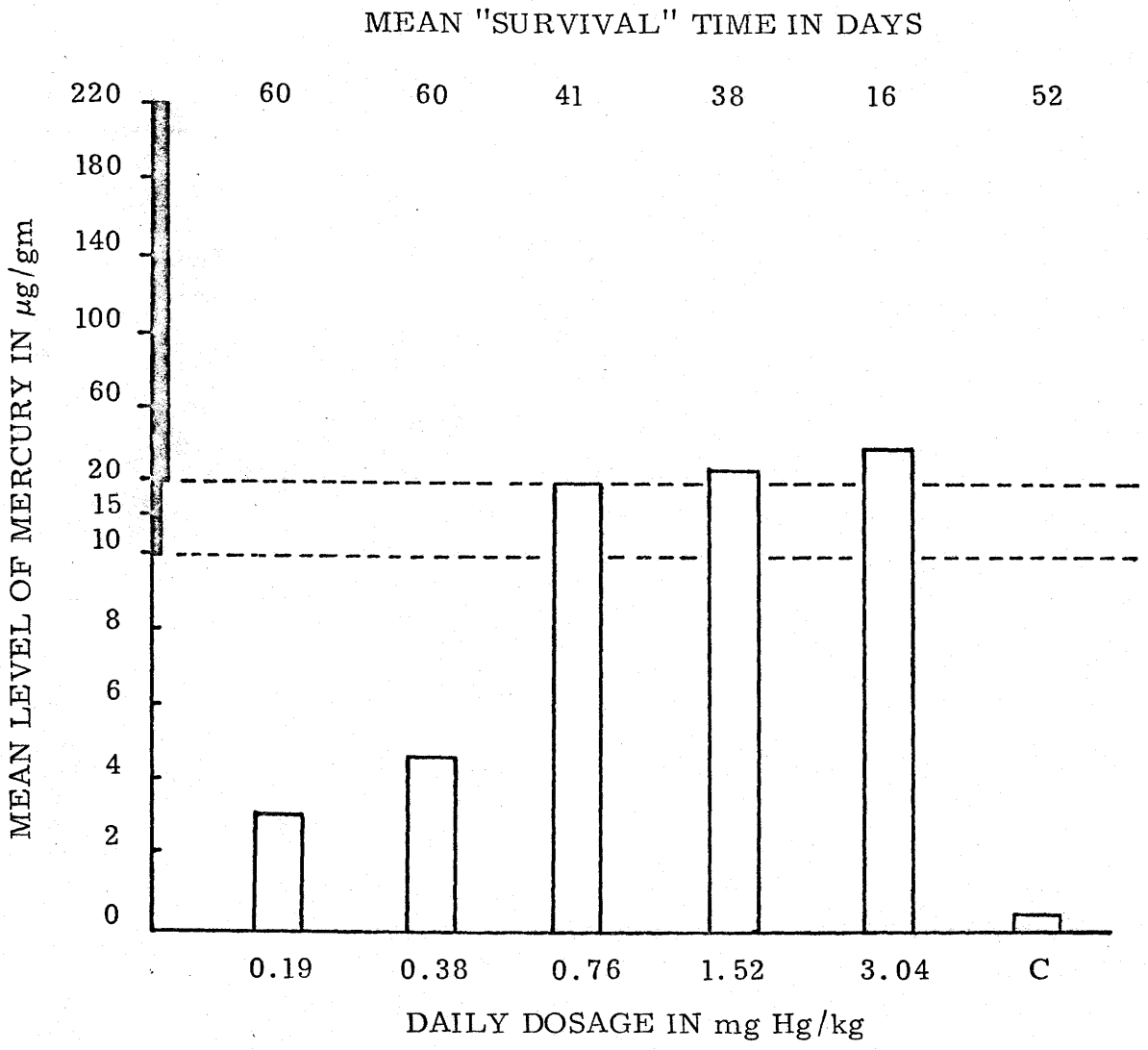


Fig. 35. Mean mercury levels in the muscle and mean survival time of groups of pigs receiving increasing dosages of MMD.

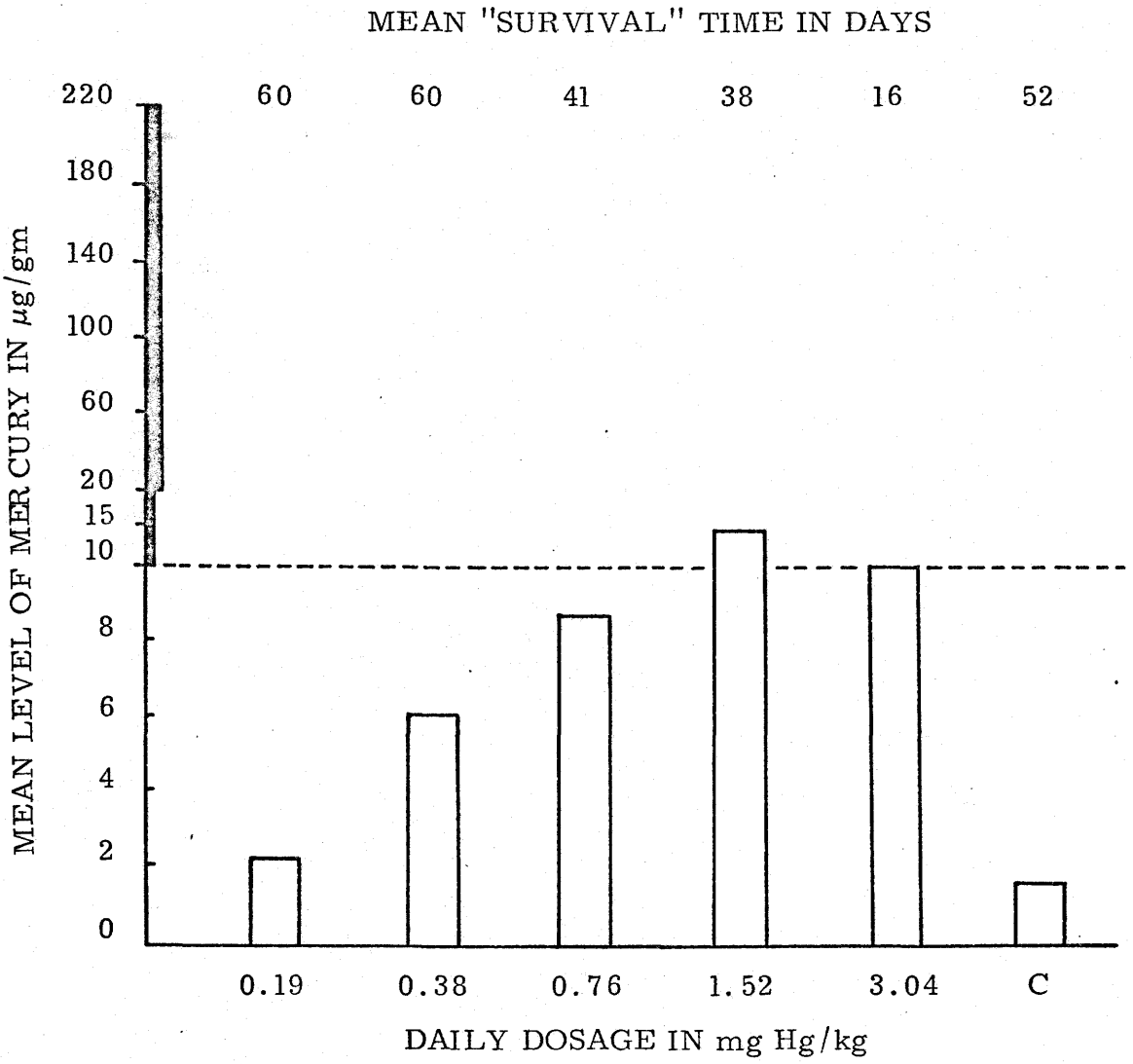


Fig. 36. Mean mercury levels in the skin and mean survival time of groups of pigs receiving increasing dosages of MMD.

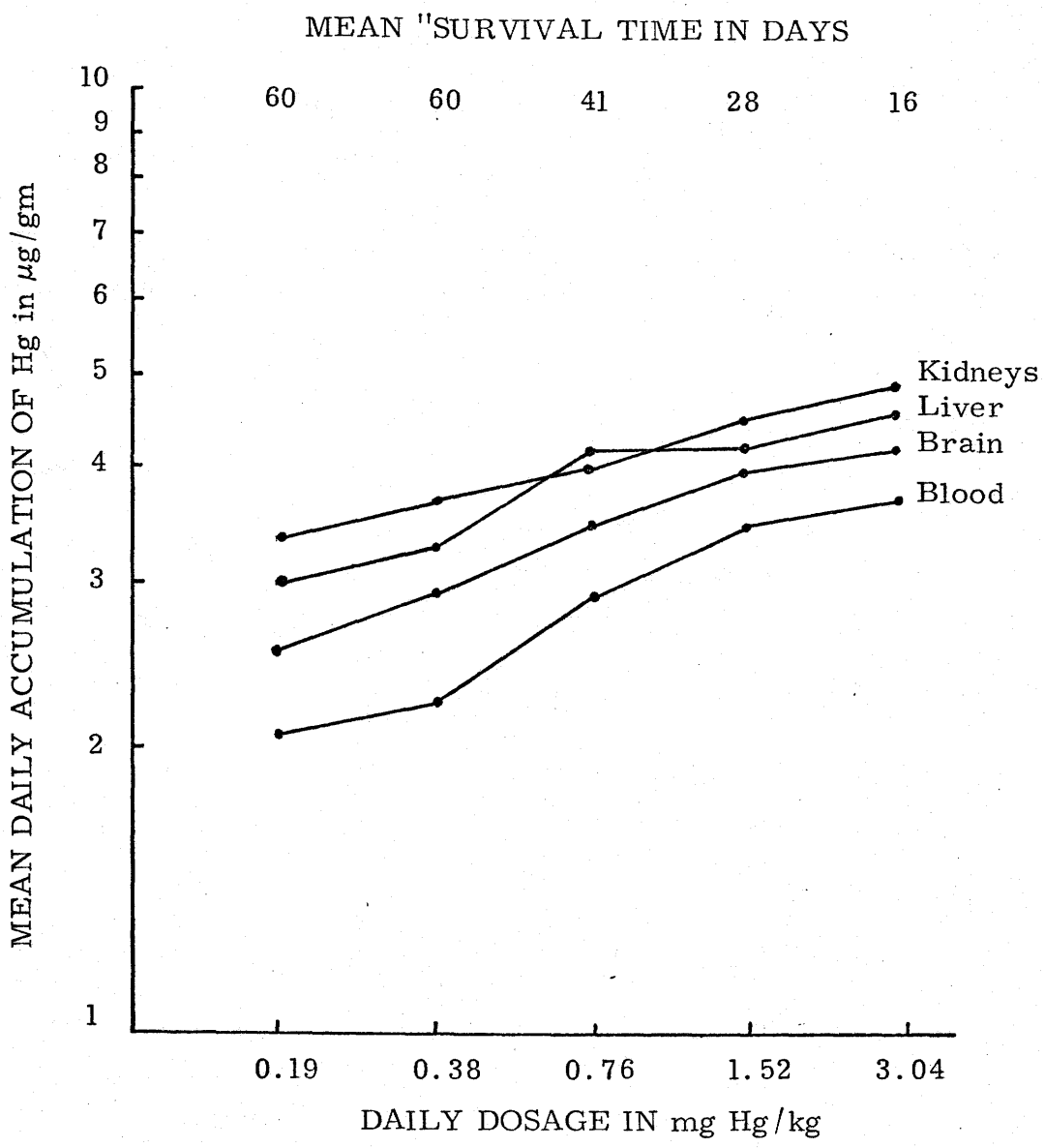


Fig. 37. Mean retention rates for brain, kidneys, liver and blood in animals receiving MMD, shown on semi-logarithmic scale.(Values in Table 11 were multiplied by 10,000 before plotting).

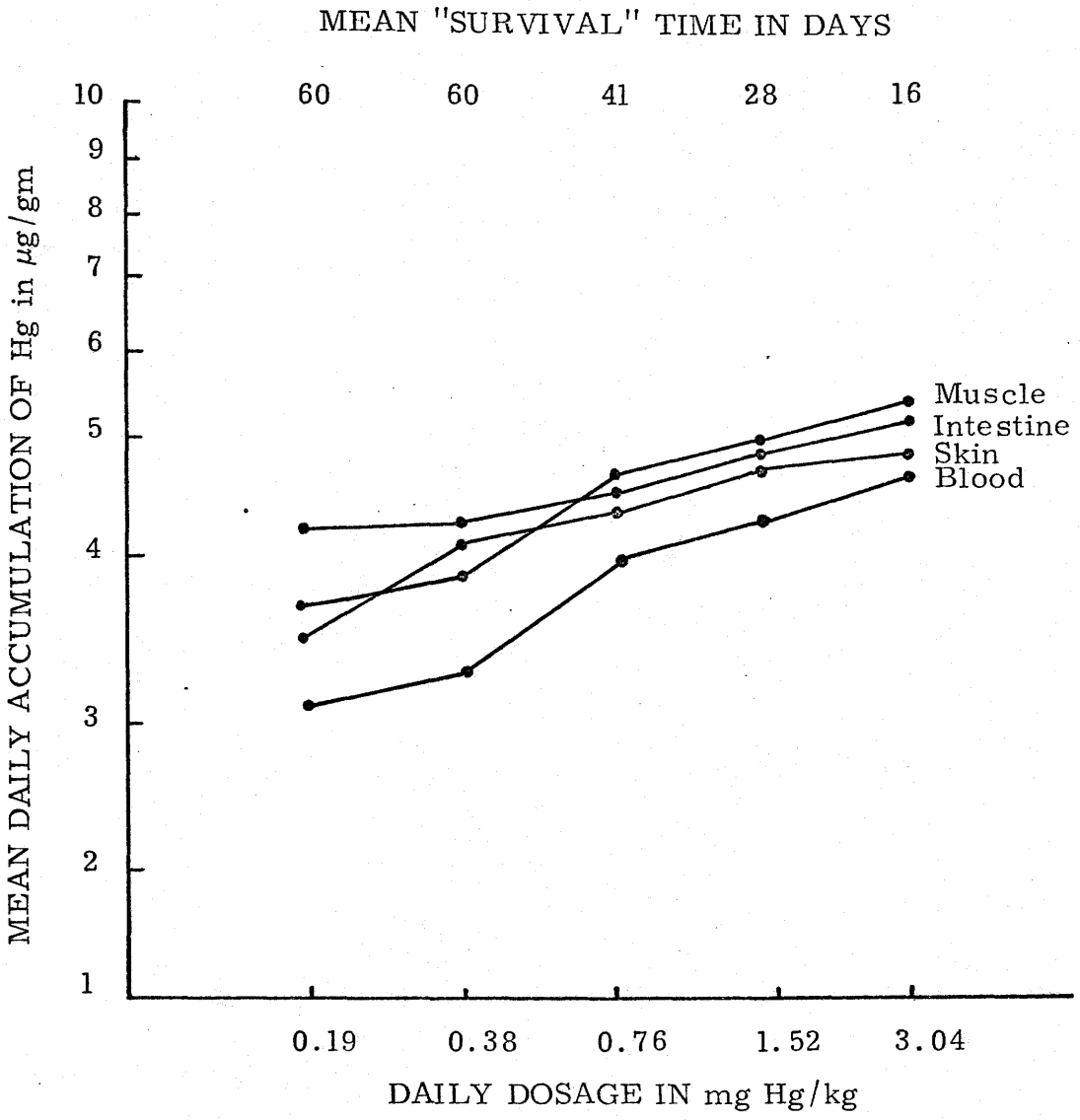


Fig. 38. Mean retention rates for intestine, muscle, skin and blood in animals receiving MMD, shown on semi-logarithmic scale.

Discussion

Mercury in the form of methylmercuric dicyandiamide is a poison in swine. Depending on the daily dosage, a disease can be produced, the course of which may be chronic, subacute and, presumably, acute; although, the acute manifestations were not produced at the dosage range studied.

The dosage range employed in this experiment, 0.19 - 3.04 mg. Hg/kg. daily as MMD, caused clinico-pathological manifestations which can be classified as chronic and subacute.

The target organs were found to be the nervous and urinary systems and the main clinical manifestations were clearly of a neurological character.

Clinical manifestations: Weight loss and failure to grow properly were constant manifestations of subacute and chronic organomercurial poisoning induced with methylmercuric dicyandiamide (MMD). This probably was largely due to dysphagia caused by any one, or all, of the following; pharyngitis, trismus, and injury to the central nervous system. Alkyl organomercurial compounds like MMD act as local irritants (Swensson, 1952; Whitehead, 1965). This property of MMD is considered responsible for the varying degrees of pharyngitis present in poisoned animals. In some animals with signs of chronic poisoning and only a mild degree of pharyngitis, a severe loss of cortical neurons probably affected the ability of such animals to feed properly. The development of trismus also prevented some animals from eating. This increased tone of the masseteric musculature is difficult to explain.

The development of trismus is correlated best with high dosage levels. Animals receiving such levels had severe neuronal necrosis and degeneration in their Gasserian ganglia. Whether this lesion alters the pattern of afferent stimuli in such a way that the resulting impulses to the masseteric musculature give rise to muscular hypertonicity is only a matter for speculation. It should be noted that the ganglionitis of rabies is associated with masseteric paralysis rather than trismus.

The length of the asymptomatic period which preceded the development of signs of toxicosis varied inversely with the dose. Presumably, this was a reflection of the time required for toxic levels of Hg to accumulate in the central nervous system. The relationship between length of presymptomatic period and daily dosage is shown in Table 12.

The data suggest that the rate of mercury accumulation in tissues is proportional to dosage.

Because the experiment was terminated at sixty days, it is not possible to give a value for the length of presymptomatic periods in Groups I and III which remained clinically normal. It is unknown whether animals in Group I would have developed signs of toxicosis. In the case of Group II, however, it could be expected that clinical signs would have precipitated within a short period after 60 days because, in Pig No. 7, there were discovered by microscopic examination, vascular lesions in the leptomeninges of the cortex. Perhaps the brains in some of the animals in the asymptomatic groups were functioning in a state of compensation. The brain has

a great capacity to compensate for lost parenchyma. There are many instances in the literature where surprisingly severe lesions were in the central nervous system of clinically normal animals. Van Bogaert, in Innes and Saunders (1962), reported on clinically silent neural lesions in monkeys. Greenfield (1963) describes many such instances in humans in relation to dementias. The same author states that, in the human, the onset of decompensation may be triggered by a variety of conditions such as physical and psychological trauma.

TABLE 12. Daily Dosage Level, Length of Presymptomatic Period and Survival Time in Animals Receiving MMD.

Group	Dose Mg./kg. daily Hg as MMD	Clinical Signs	Onset (day)	Death (day)	Lesions
I	0.19	-	-	60 (killed)	-
II	0.38	-	-	60 (killed)	+
III	0.76	+	32	41	+
IV	1.52	+	18	28	+
V	3.04	+	11	16	+
Control	----	-	-	60 (killed)	-

The neurological manifestations of toxicosis which consisted of the incoordination of the hind quarters, knuckling of the fetlocks, loss of balance, falling and inability to regain posture could result

from pathological changes in the nervous and/or the skeletal system. In this case, the evidence supports the contention that only changes induced in the nervous system are responsible for the signs.

It appeared that the distribution of lesions and the degree of injury in the various segments of the nervous system depended upon the dosage of MMD. The sites of the lesions appeared to be areas of the nervous system which are considered essential for normal muscular coordination.

In pigs which received the highest level of MMD and which had lesions in the spinal cord and dorsal root ganglia, posterior incoordination was the most consistent sign. In contrast, animals which received lower doses and which had brain lesions characterized b severe loss of neurons in the cortex and, to a lesser degree, in the corpus striatum and the red nucleus, exhibited blindness, tremor, compulsive walking and paretic phenomena. It appears, from the pathological findings, that an impairment of the statokinetic reflexes is responsible for this neurological disturbance. It is suspected that high levels of MMD injure the sensory ganglia and affect mainly the afferent route, while low levels, with their effect on the upper center, affect the efferent route.

The blindness observed is considered to be of central origin. Both in the subacute and the chronic cases, lesions were found in the central portion of the sensory visual pathway. Extensive neuronal necrosis was found in the lateral geniculate bodies of animals receiving high dosage levels, and over most of the occipital

cerebral cortex of animals receiving low dosage levels. The middle range of dosage caused lesions in both areas.

Pathology: Oral administration of toxic levels of methylmercuric dicyandiamide causes lesions in the nervous, digestive and urinary systems. The daily dosage and the length of the administration period were found to be important determinants of the level of mercury accumulation in tissues and the extent, distribution, nature and severity of the lesions induced.

Nervous system. -- An attempt was made to study the morphogenesis of brain lesions by killing and studying animals with early and terminal clinical toxicosis. In general, this approach failed to yield such information. The course of the clinical disease was quite short and compensation could have masked, in an indeterminate way, evidence of brain injury.

It is possible to classify lesions which resulted from the administration of toxic levels of MMD in these experiments as subacute and chronic. An oral dosage of 3.04 mg. Hg/kg. daily in weanling pigs induced subacute lesions and lower toxic levels induced lesions of a chronic nature.

In the subacute form, the lesions are considered to be the result of a direct toxic effect of mercury on the affected neurons. The distribution of degenerated and/or necrotic neurons was random except in the cerebral cortex where it was limited to the 3rd, 4th and 5th cortical layers. The blood vessels throughout the nervous system were unremarkable. These two findings tend to support the idea of a direct toxic effect.

In this subacute process, the glia did not undergo necrosis, but rather were stimulated to proliferate locally. Presumably, this was a response to a prolonged stimulus from neuronal necrosis. Animals with these subacute lesions contained relatively high levels of mercury in their cerebral cortices, in comparison to skin, intestine, blood and urine (Table 11). Mercury in the form of MMD appears to have an affinity for brain.

The chronic form of toxicosis is characterized by: neuronal necrosis associated with micro- and astrogliosis and capillary endothelial proliferation; and degenerative arteriopathy occurring in the sulci of the injured cortex.

The polioclastic effect of MMD is also manifest throughout most of the cerebrospinal axis in the chronic form. The severity of lesions is greatest in the cerebral cortex. The injury to the subcortical nuclei and the gray matter of the lower levels of the cerebrospinal axis depends on dose; and at the lowest dosages, most are spared from injury (see Appendix B, Table 2). The slower pace of neuronal death at this lower dosage and the sustained degradation of cellular material from necrotic neurons may provide a continuous stimulus for the mobilization of glial and capillary endothelial cells which were prominent features of the chronic disease. It is known that glia proliferate in response to neuronal death and myelin breakdown (Greenfield, 1963).

Many authors (Biggart, 1961; Greenfield, 1963; Jubb and Kennedy, 1963; Smith, 1957; and Little, 1967) have reported capillary endothelial cell proliferation in a variety of conditions associated with neuronal death.

Proliferation of the capillary endothelial cells without formation of new capillaries is believed to lead to increased resistance to blood flow. This will also result in anoxic conditions in the affected tissue if other factors, such as collateral circulation and increased blood pressure, are not able to compensate. The degenerative changes in the arterioles and small arteries are characterized by hyalinization and necrosis of the vessel wall. These changes are considered secondary to the primary neuronal injury, since this always precedes the development of the vascular lesions. The changes in the vessel wall will affect blood flow by abolishing the regulatory effect of the vessel and by impinging on the lumen. These changes will intensify the tissue anoxia which exists as a result of the capillary lesions.

The ability of the vasculature of the cortex to compensate for the effect of the lesions is limited anatomically because of lack of collateral circulation (Ranson and Clark, 1959). A vicious, self-perpetuating cycle, resulting in more tissue destruction, is thought to result.

The pathogenesis of the vascular lesion of chronic MMD poisoning could be the result of hypertension in the cerebral arterial vasculature. The lesions occurring in the arterioles and small arteries appeared to be similar to those observed in hypertensive states (Boyd, 1961; Robbins, 1957). The diffuse capillary endothelial proliferation which always preceded the development of arterial lesions could be expected to cause an increased peripheral resistance. Considering the nature of the circulation in

the cortex, this should lead to hypertension in the arterial circulation supplying the affected areas.

In spinal thrombophlebitis of man, occlusion of the venous channels leads to increased resistance to blood flow because of the inadequacy of the venous anastomoses to correct the situation (Greenfield, 1963). If this situation persists, hyaline degeneration occurs in the arterioles and varying degrees of necrosis are seen in the gray matter of the spinal cord.

The influence of length of exposure and level of mercury on the tissue becomes particularly prominent in a consideration of the development of lesions in some of the pigs in Group III receiving 0.76 mg. Hg/kg. daily. Animals with the longer survival period and lower tissue mercury levels had involvement of the larger blood vessels in the meningeal arterial system (Appendix B, Table 1).

The importance of the individual animal's susceptibility to organomercurial poisoning became apparent in Group II where only one of the animals developed lesions in the time period studied (60 days).

Administration of 0.19 mg. Hg/kg. daily for a period of sixty days failed to cause lesions in the nervous system of pigs studied. This suggests that the levels of mercury accumulated in the brains of these animals were not above the critical level for injury. It remains unknown if longer exposure at this level would induce lesions.

The basic factor which resulted in the absence of lesions in pigs fed sub-toxic levels of MMD could be either one of two

possibilities. Either the dose rate is so low that the accumulation of Hg was equal to, or less than, the excretion rate, or the accumulation of Hg in the brain tissue was progressive but at a rate so low that the toxic levels were not reached within the time period studied.

The neuronal necrosis in the thalamus, midbrain, pons and medulla oblongata, spinal cord and dorsal root ganglia are considered to be the result of a direct injury by the mercurial compound. The fact that necrotic neurones are randomly distributed is hard to reconcile with any primary vascular mechanism of injury. The highest dosage, 3.04 mg. Hg/kg. daily, caused more extensive lesions in the same structures; a fact which also argues for direct injury. The possibility that neurons which have the most fastidious metabolic requirements are more susceptible to injury by toxic factors is suspected. Also the absence of arterial lesions in the subcortical nuclei and gray matter of lower level areas suggests that here the anatomical nature of the arterial distribution system is different than in the cortex and is protected from secondary hypertension associated with capillary proliferation (Ranson and Clark, 1959).

Digestive system. -- Previous studies have shown that alkyl mercurials are readily absorbed by the intestinal tract (Miller et al., 1961). It is also known that a great variety of toxic substances can cause liver necrosis both in man and animals (Boyd, 1961; Dunne, 1964; Jubb and Kennedy, 1963; Montroni, 1949; and Robbins, 1967). Most of these authors agree that the effect of a

toxin depends both on the dose and the length of time of exposure. The present findings support this view. Daily administration of 3.04 mg. Hg/kg., in the form of methylmercuric dicyandiamide, for a maximum period of 16 days, resulted in randomly distributed necrosis of individual hepatocytes. Lower dosage (1.52 and 0.76 mg. Hg/kg. daily), administered for periods up to 30 and 44 days respectively, gave rise to mild centrolobular necrosis. Examination of the analytical data reveals that, regardless of dosage, necrosis is associated with high levels of mercury accumulation in the tissue. Tissue levels below 14 μg./gm. of wet tissue failed to cause lesions in the liver of the experimental animals. The effect of the mercury at the dose used on the liver is limited to the hepatocyte. The stroma appears to escape injury.

Urinary system. -- The review of the literature has shown that absorbed mercury is excreted mainly via the urinary and digestive systems (Swensson et al., 1959; Berlin and Ullberg, 1965; Berlin, 1963; Jacobs et al., 1963; and Brown et al., 1967). Another established fact is that, following absorption, mercury is equally distributed throughout the various organs. Subsequently, it tends to accumulate in large amounts only in specific organs, depending on the chemical nature of the mercurial. The kidney is generally one of the organs that retains large amounts of mercury. Within this organ, the renal cortex appears to be the preferred site of accumulation (Platonow, 1968). The pathology of mercurial poisoning, as it relates to kidney, has been established. The dosage and chemical form of the compound employed appear to be the main determinants of the pathological character of the

lesions. In this experiment, the influence of the dosage level was manifested by the fact that renal lesions were found only in animals receiving 1.52 or 3.04 mg. Hg/kg. daily. Such lesions were limited to the proximal convoluted tubules and were characterized by hydropic degeneration of the tubular epithelium. These findings suggest that the dosage range employed in this experiment causes minimal or no renal lesions.

Summary

Methylmercuric dicyandiamide (MMD) is highly poisonous for swine if fed for a prolonged period of time. Subacute and chronic forms of disease, depending on dosage, were studied.

The disease produced by MMD is largely one of the central nervous system and is related primarily to neuronal necrosis. Cortical neurons are most susceptible to injury and those in subcortical nuclei, spinal cord, and sensory ganglia are injured at higher doses.

In the chronic forms of toxicosis, there occurs, in addition to neuronal necrosis, secondary gliosis, capillary endothelial proliferation and degenerative arteriopathy in leptomeningeal blood vessels supplying injured cerebral cortices.

The character of the vascular lesions in chronic MMD toxicosis is comparable with the occurrence of regional hypertension.

Analysis of levels of mercury in tissues of animals receiving MMD indicate that there is a direct association with tissue level and evidence of injury.

Studies on the pathology of ethylmercuric chloride poisoning

The essential groups are described in Table 13. The dosages were selected to range from near toxic to severely toxic levels.

TABLE 13. Experimental Groups Which Received Ethylmercuric Chloride

Group	No. of Animals	Dose*	Days**
I	5	0.19	90
II	5	0.38	90
III	5	0.76	30
IV	5	2.28	18
V	5	4.56	12
Control	5	----	90

* Mg. Hg/kg. daily administered in the form of MMD.

** Maximum experimental days for each group.

Clinical and pathological findings will be presented separately for each experimental group under homonymous titles. Analytical findings will be described collectively.

TABLE 14. Daily Dosage Rate, Treatment Period and Clinico-pathological Results For Each Animal Receiving Ethylmercuric Chloride

Group	Dosage mg. Hg/kg. daily	Animal	Days	Signs	Lesions
I	0.19	751	90	-	-
		752	90	-	-
		753	90	-	-
		754	71	-	-
		755	65	-	-
II	0.38	756	90	-	+
		757	64	+	+
		758	75	+	+
		759	90	-	+
		760	44	-	+
III	0.76	761	22	-	+
		762	30	+	+
		763	30	+	+
		764	22	+	+
		765	22	+	+
IV	2.28	766	14	-	+
		767	18	+	+
		768	18	+	+
		769	14	+	+
		770	14	+	+
V	4.56	771	11	+	+
		772	11	+	+
		773	11	+	+
		774	11	+	+
		775	12	+	+
Control	----	776	44	-	-
		777	90	-	-
		778	80	-	-
		779	24	-	-
		780	50	-	-

Clinical findings

Table 14 summarizes the distribution of clinical signs and lesions among all pigs in the various experimental groups.

Group I (0.19 mg. Hg/kg. daily): None of these animals became clinically sick during the observation period. Their rate of growth paralleled that of the controls.

Group II (0.38 mg. Hg/kg. daily): Only two animals (757 and 758) developed signs. They were noticed to lag behand the others in the same group in growth rate, beginning on days 14 and 18 respectively (Figs. 39 and 40). This situation continued and, by days 53 and 64 respectively, these animals were in negative body weight balance. Anorexia became progressively more pronounced and the weight loss very dramatic.

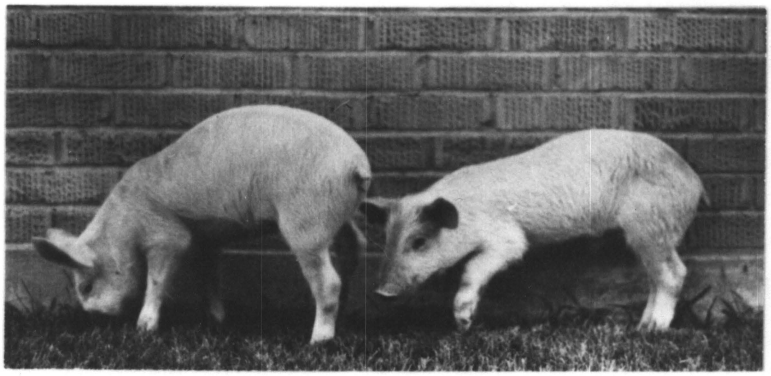


Fig. 39. Control pig 778 (left and Pig 758 (right). Picture was taken on the 14th experimental day. Depressed growth is evident in Pig 758.

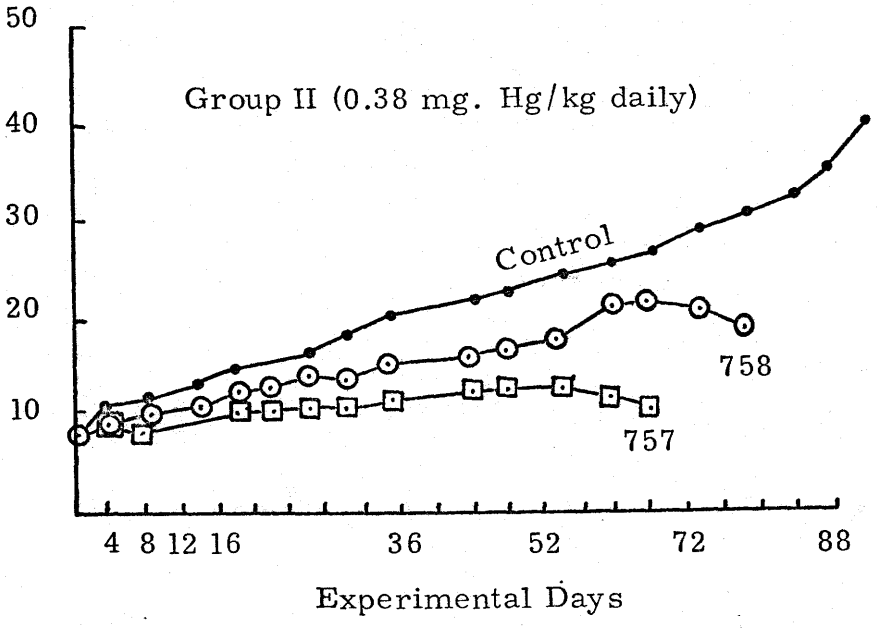


Fig. 40. Growth curves of pigs in Group II (757 and 758) with signs of toxicosis.

Neurological signs. -- Pig No. 757 became uncoordinated on day 49 and Pig No. 758 on day 56. Incoordination was associated with aimless walking which initially was intermittent and terminally became continuous in Pig No. 758. At this stage, the animals kept their heads high and failed to avoid obstacles placed in their paths. When in recumbency, the abdominal musculature was very flaccid and failed to contract, even during vigorous abdominal palpation. In fact, the animals seemed insensitive to this procedure. In contrast, when they were lifted from the ground, they went into convulsions and became cyanotic.

Animal 757, on day 51, was found with its head in the feeding box where it was seen to scoop feed into its mouth and then execute masticatory movements, during which the animal salivated abundantly.

The forelegs became weak and failed to support the body in a manner similar to that in Figure 42.

Deterioration in the clinical picture was characterized by signs of progressive cerebral deficiency. The animals appeared oblivious to their environment and failed in prehension of food and water, but would swallow fluids placed in their mouths. Finally, they remained continuously in lateral recumbency and, on many occasions, were seen to make paddling movements with their limbs. Their reflexes became depressed. The righting reflex was absent. Characteristically, the body of such an animal, when lifted by the hind legs, remained flaccid. Animal No. 758 was found dead on day 75. Animal No. 757 was euthanized on day 64.

Group III (0.76 mg. Hg/kg. daily): The clinical course of toxicosis in animals in this group was much more accelerated and uniform when compared with the previous group. Maximal duration of the experimental period was 30 days (Pigs 762 and 763). Three of the animals (761, 764 and 765) were killed on day 22, when two of this group (Pigs 764 and 765) manifested their first signs of disease. These were failure to gain weight, moderate anorexia, incoordination and knuckling of the front fetlocks (Fig. 41).

Neurological signs. -- Later, Pigs 762 and 763 developed a raucous voice which was found to be associated with suppurative and necrotic pharyngitis. Pig No. 761 was symptomless when

killed. Two pigs (762 and 763) were allowed to proceed to the terminal stages. Pig No. 763 was found dead on the 30th experimental day. These animals remained recumbent, made paddling movements, failed to feed themselves and, after becoming incoordinated, progressed to a state of complete inability to assume standing posture. While recumbent, it was noticed that their abdominal musculature was entirely flaccid. During the last four days of life, the general body condition deteriorated severely.

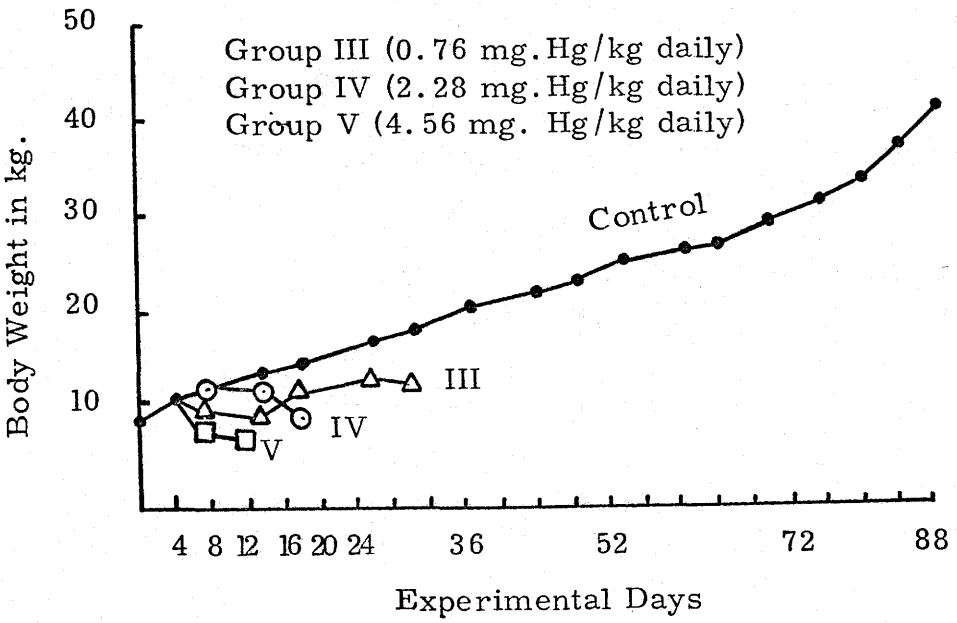


Fig. 41. Growth curves of mean body weights for Groups III, IV and V and Control during the course of EMC poisoning.

Group IV (2.28 mg. Hg/kg. daily): The clinical course of disease in animals in this group was even more accelerated than in Group III.

The animals in this experimental group survived for a maximum period of 18 days.

Two pigs (769 and 770) were killed at an early stage of disease. They were not allowed to proceed beyond the stage of incoordination and forelimb weakness. One pig (766) was killed before the appearance of any signs of poisoning.

The signs observed are presented, in the approximate order of their appearance. Moderate anorexia and dysphagia with failure to gain weight were first noticed on the eighth experimental day (Fig. 41). Concomitant signs consisted of changes in, and loss of, voice tone. This was attributed to pharyngeal irritation. Diarrhea was first noticed on day 10.

Weight loss became very pronounced during the last four days.

Neurological signs. -- Incoordination was seen on day 12 in two pigs (770 and 768). Incoordinated animals preferred to remain recumbent. When such animals were induced to stand on their feet, it was observed that their front legs failed to properly support the body. The distal part of the legs below the elbow remained extended, but the radio-humeral angle became reduced as if there were weakness affecting the anconeal musculature. As a result of this, the shoulders were noticeably below the level of the sacral region (Fig. 42). In addition, there was lordosis. The lowest point of the lordotic spine was at the thoraco-lumbar region.

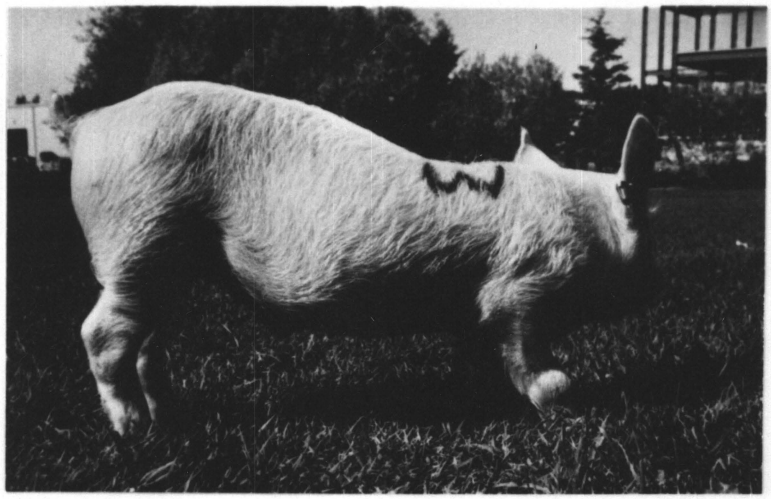


Fig. 42. Pig No. 768. Front legs fail to support the body. As a result of this, the shoulder level is below the level of the sacral region.

Animals in this stage of disease could still react to external stimuli, regain their muscular tone and even re-assume a standing posture. Once this occurred, they would also commence walking. At a later stage of disease, however, this type of response was impossible to elicit. The animals remained continuously in lateral recumbency and made paddling movements either spontaneously or following external stimulation.

A constant sign was extreme relaxation of the abdominal musculature. This sign appeared simultaneously with incoordination and remained until death. Palpation of the abdomen failed to

elicit defense contractions in the abdominal musculature.

The presence of blindness would not be ascertained.

Group V (4.56 mg. Hg/kg. /daily): The course of the disease was rapid. All animals in this group became ill at approximately the sixth experimental day. All animals died by the 11th day.

Weight loss was first noticed on the fifth and sixth days (Fig. 41). On the sixth day, there was a change in voice tone, when the squeal became raucous. The isthmus of the fauci appeared red and edematous and, within two or three days, this process developed into a purulent and necrotic pharyngitis. Anorexia and dysphagia appeared on the fifth day and was present thereafter. All of the animals, at one time or another after the appearance of the above signs, made masticatory movements and had frothy saliva drooling from their mouths.

Vomition took place at irregular intervals throughout the course of illness. The vomitus consisted of clear, yellow sero-mucinous fluid. Diarrhea was present throughout the illness. The feces were yellow in color. A foul odor emanated from all affected animals.

Neurological signs. -- Weight loss became pronounced terminally. The group's average body weight at the time of killing was approximately 2.5 kg. below the group's average recorded at the beginning of the experiment. Incoordination also appeared in this group. Initially, the affected animals preferred to remain recumbent for extended periods of time. They would rise only to

drink water or to move around for short periods of time. Abdominal relaxation, which was unaffected by external palpation, was also observed in this group. Later, ataxia became complete and was accompanied by lethargy. One pig (775) remained in lordosis for 25 minutes prior to death.

Controls: All remained normal throughout the course of the experiment.

Gross pathological findings

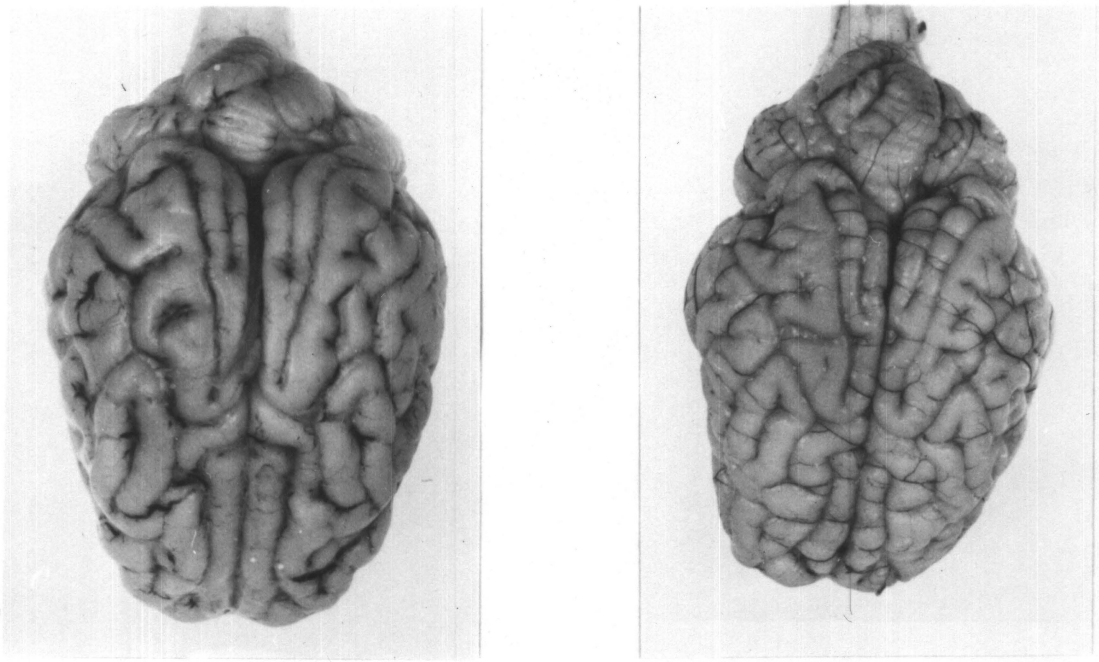
Group I (0.19 mg. Hg/kg. daily): No significant lesions were found in this group of animals.

Group II (0.38 mg. Hg/kg. daily): Lesions were found only in the two animals (757 and 758) which had clinical signs of disease.

Severe emaciation was noticed in the affected animals. The kidneys were unremarkable. The liver of clinically affected animals was pale and moderately atrophic. Findings on other organs were not contributory.

Nervous system. -- Characteristically, on opening the cranial vault, there was abundant cerebrospinal fluid running from the meningeal spaces.

Exposure of the brain revealed severe cerebral atrophy (Fig. 43). In the fresh state, the gyri were remarkably reduced in size, and sulci were prominent between the atrophic gyri. The gyri had lost their usual turgidity. It was noticed that gyri still submerged in cerebrospinal fluid could be made to move to and fro in a manner similar to the way any flexible material follows



(A) (B)

Fig. 43. (A) Control Pig 778, (B) Pig No. 758. Note atrophy in the cerebrum of Pig No. 758 and compare normal appearance of cerebellum in both animals.

the motion of the overlying fluid, e.g. marine plants. The cerebellar/cerebral weight ratio was found to be very abnormal in both pigs, especially in Pig No. 758. Figure 44 shows the degree of such deviation. It can be seen that there is a tremendous reduction in the weight of the cerebral hemisphere. Closer examination of the results in Figure 44 reveals that the difference between the mean total brain weight of the controls and that of the affected pig (758) is entirely attributable to loss of cerebral tissue. The mean brain weight of controls was calculated from a sample of 25 pigs of approximately similar age examined at random.

This sample included experimental controls, clinical post mortem material, and experimental animals which remained healthy.

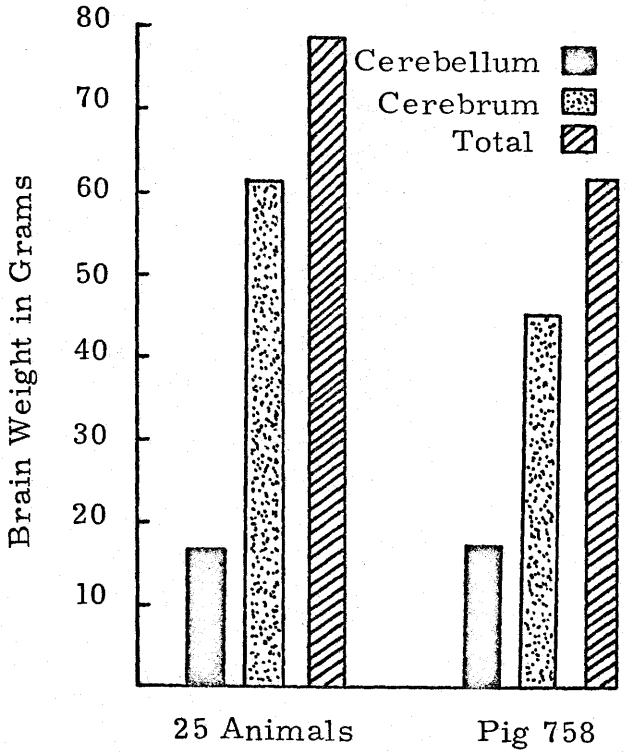


Fig. 44. A diagramatic comparison between the cerebral and cerebellar weights of Pig 758 and average of controls.

The brains of the two affected animals (757 and 758) failed to harden properly following immersion in formalin and appeared granular. These brains were difficult to cut. The tissue resisted the knife, had a tough texture and collapsed severely before cutting. It was difficult to produce gross sections with parallel surfaces.

On the cut surface of the brain, it was noticed that atrophy occurred mainly at the expense of that part of the cerebral hemisphere lying above the sulcus rhinalis (Fig. 45). Both gray and white matter were affected. The usual branching of the white matter below the overlying cortex was present, but there was a remarkable reduction in the length and width of the affected gyri. In some instances, the thickness of the cerebral cortex did not exceed 0. 5 mm. The anterior portion of the left caudate nucleus and the upper medial part of the adjacent internal capsule were malacic. Internal hydrocephalus was most severe in the brain of Pig No. 758 (Fig. 46). This was suspected when the uncut brain failed to submerge in 10% buffered formalin.

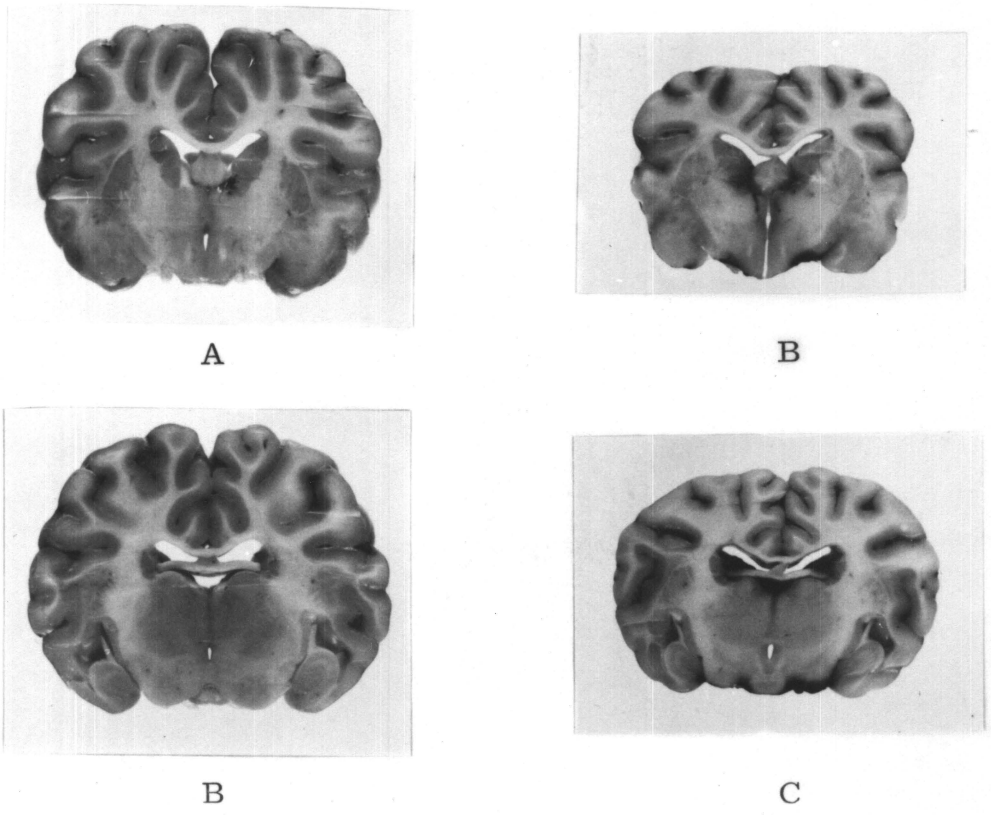
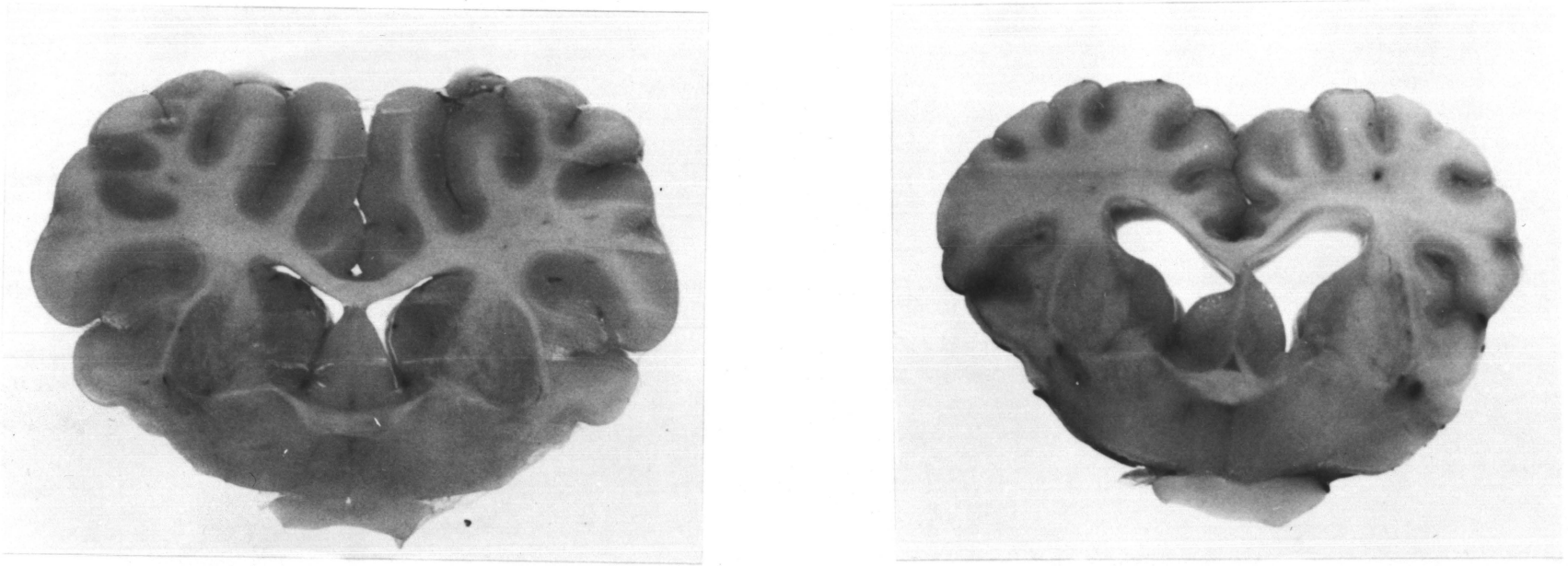


Fig. 45. (A and B) Control Pig No. 778, (C and D) Pig No. 757. Comparable transverse gross sections emphasizing the loss of cerebral tissue in Pig No. 757. x 1.



(A) (B)

Fig. 46. (A) Cross sections from brain of Control Pig No. 776, and (B) Pig No. 758 at the level of the optic chiasm. There is cerebral atrophy and internal hydrocephalus in Pig No. 758. x 2.

Group III (0.76 mg. Hg/kg. daily): All animals in this group appeared to be underweight. Two pigs (762 and 763) were emaciated. In such animals, there was almost complete absence of normal adipose tissue. In four pigs (762, 763, 764 and 765), varying degrees of serous atrophy were noticed on the epicardial fat.

Nervous system. -- The gross findings in the central nervous system were not remarkable. It should be noted, however, that brain weights were not obtained for this group.

Uro-genital system. -- No significant gross lesions were found in the urinary system. In Pig No. 763, the kidneys appeared slightly shrunken. Perirenal edema was found in two pigs (765 and 764).

Digestive system. -- The liver was mottled in one animal (763), but not significantly altered in others.

Suppurative and necrotic pharyngitis involving most of the oropharynx was found in one pig (763), and was associated with a cornified band in the esophageal epithelium. The stomach was empty in clinically advanced cases (762 and 763). Severe gastritis with a patchy distribution was seen in one pig (763) and was characterized by hemorrhagic erosion and ulceration. The ulcerated mucosa was covered by a thin necrotic membrane. The small intestine was not remarkable.

Edema of the mesentery, mesocolon and perirenal tissues was seen in two pigs (764 and 765) (Fig. 47). The latter animal

Fig. 47. Pig No. 765. Edema in mesocolon of pig which received 0.75 mg. Hg/kg. daily as EMC.

was the most severely involved and had edema in its cecal and colonic submucosa. The mucosa of the cecum was either focally hyperemic (763) or was covered by yellow, sticky fecal material. The mucosa of the spiral colon adjacent to the cecum was similarly affected. This process gradually disappeared distally in the colon.

Respiratory system. -- Enzootic pneumonia was seen in one pig (762). Excessive serous fluid was seen in the thoracic cavity of two pigs (763 and 765). Strands of fibrin were noticed in Pig No. 763.

Cardiovascular system. -- Serous atrophy of the epicardial fat (762 and 763) and increased amounts of pericardial fluid (763 and 765) were found.

Group IV (2.28 mg. Hg/kg. daily): All animals in this group appeared to be underweight. In three pigs (766, 768, 769), however, weight loss was minimal.

Nervous system. -- Gross examination of the central nervous system failed to reveal any significant changes.

Urinary system. -- The kidneys were pale, swollen and, in one pig (766), very firm. The perirenal tissue was edematous in all animals but most severely so in animal 764.

Digestive system. -- The livers were similar in all animals and had a mottled appearance and moderately friable texture.

These was acute and/or chronic pharyngitis in all animals. The inflammatory process invariably extended into the cranial part of the esophagus for approximately three to four centimeters. In two pigs (766 and 769), the process had not advanced to ulceration. Focal necrotic esophagitis was noticed at the level of the thoracic inlet in one pig (769).

The mucosa of all stomachs was hyperemic and erosions occurred in patches in the fundic area. Ulceration of the fundic mucosa was evident in two pigs (768 and 769). These lesions were covered by a pseudomembrane.

Another constant finding was the presence of edema in the mesocolon (Fig. 48). This was found either limited to the region of the cecum and spiral colon or, in some, extending up to the

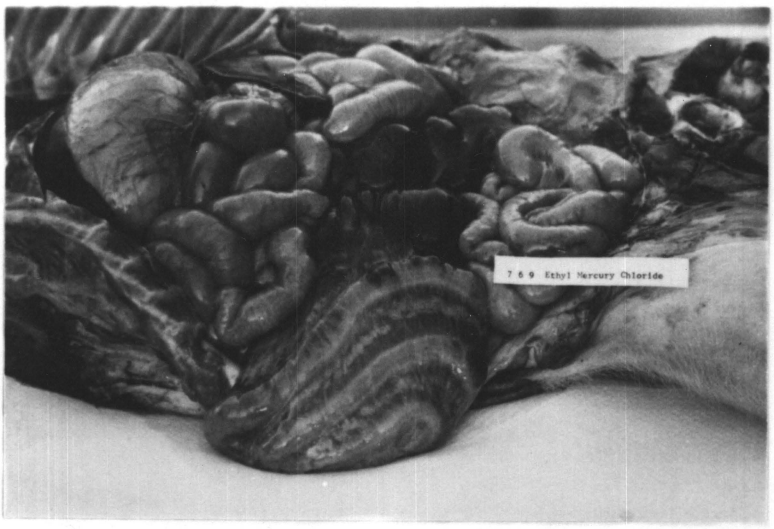


Fig. 48. Edema in the mesocolon of Pig 769 receiving 2.28 mg. Hg/kg. daily as EMC.

perirenal tissues. The cecal and first portions of the colonic mucosa were constantly found to be affected by an inflammatory process. The changes ranged from slight edema and congestion (Pig No. 766) to severe necrotic enteritis which became almost gangrenous in Pig No. 769. The lesions had a diffuse character and, where the mucosa was necrotic, a pseudomembrane had formed which adhered to the underlying tissue (Fig. 49). The gastrointestinal tract contained scant, or no, fecal material

Respiratory system. -- The lungs were affected by edema and congestion in three pigs (766, 769 and 770).

Cardiovascular system. -- Serous atrophy was noticed in the epicardial fat. There was increased pericardial fluid which contained thin strands of fibrin. Edema was found in connective tissues in the external wall of the pericardial sac.

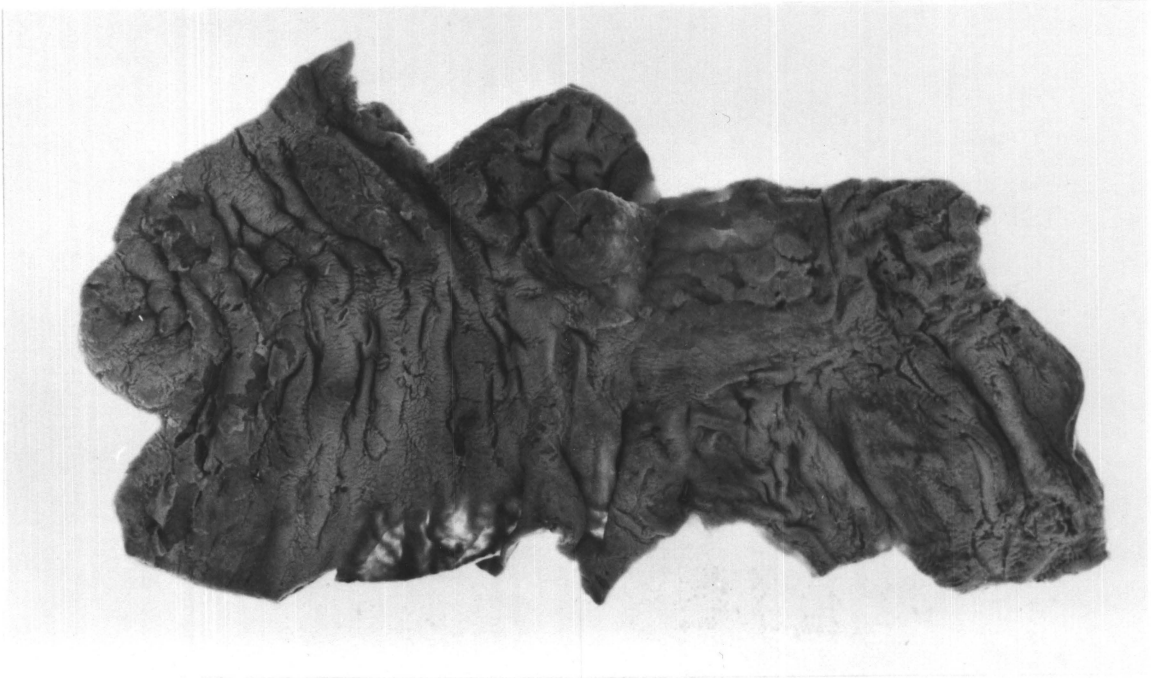


Fig. 49. Diphtheritic typhlitis (left) and colitis (right) in pig receiving 2.28 mg. Hg/kg. daily as EMC.

Group V (4.56 mg. Hg/kg. daily): Severe emaciation was prominent in all and was characterized by complete absence of recognizable fat. The skin was dry and dirty.

Nervous system. -- No significant findings were present in the brain. The leptomeningeal vessels of two animals (772 and 773) were congested. A similar finding was noticed in the saggital sinus.

Urinary system. -- The kidneys were pale, yellow and occasionally swollen.

Digestive system. -- In all animals the livers were pale, friable and mottled.

A moderate to severe inflammation occurred in the pharynges of this group. The process was purulent and necrotic in the most severely affected animals. Edema of the adjacent interstitial tissue was present in all cases. The extra-thoracic portion of the esophagus was affected in a similar manner.

The stomachs contained no ingesta. Instead, there was a variable amount of thick, bile stained, mucinous material which adhered to the epithelium. Hyperemia and, occasionally, ulceration of the fundic mucosa was found in the stomachs of all pigs.

The small intestine was segmentally affected by hyperemia. The interposed sections appeared normal.

The cecum and colon were uniformly injured. They invariably contained scant fecal material. Instead, the lumen was occupied by a thick yellow membrane which was attached to the under-lying mucosa. Fibrin clots were also found in the lumen and were either free or adhered to the intestinal wall.

Controls: No remarkable lesions were found in these pigs.

Histopathological findings

The important histopathological findings for all animals in this experiment are tabulated in Appendix B.

Group I (0.19 mg. Hg/kg. daily):

Nervous system. -- No CNS lesions were found in the pigs within this group (Note: in the brain, the granular cells

in the central folia of the cerebellum appeared reduced in number when compared to the number found in the peripheral folia. This probably is a normal finding in the pig).

Three pigs (752, 753 and 754) had acute and/or subacute inflammation in the perineural and intraneural connective tissue of the spinal nerve roots. This finding was limited to certain segments only and was characterized by a light infiltration of neutrophils and/or eosinophils.

Other systems. -- No lesions were found.

Group II (0.38 mg. Hg/kg. daily):

Nervous system. -- In the one pig which was killed on the 44th day (760), the only finding was a small area of necrosis in the anterior part of the left caudate nucleus.

The following described the lesions in the two pigs (756 and 759) which were killed on the 90th day, at which time they exhibited no signs of toxicosis. On low magnification, the cortex was diffusely or locally hypercellular (Fig. 50). The characteristic laminar arrangement of the neurons was less prominent than normal except for the second cortical layer, which was still discernible even in the most severely affected regions. It was found that more of the occipital cerebral cortex was affected than the frontal. In the frontal cortex, the lesions affected mainly the cortical layer at the deep end of the sulci. The severity of the cortical lesion appeared to be of similar degree throughout the brain, regardless of the extent of involvement. Examination of sections stained with Heidenhain's for myelin and Bielschowsky's for axons demonstrated severe loss of such structures in the

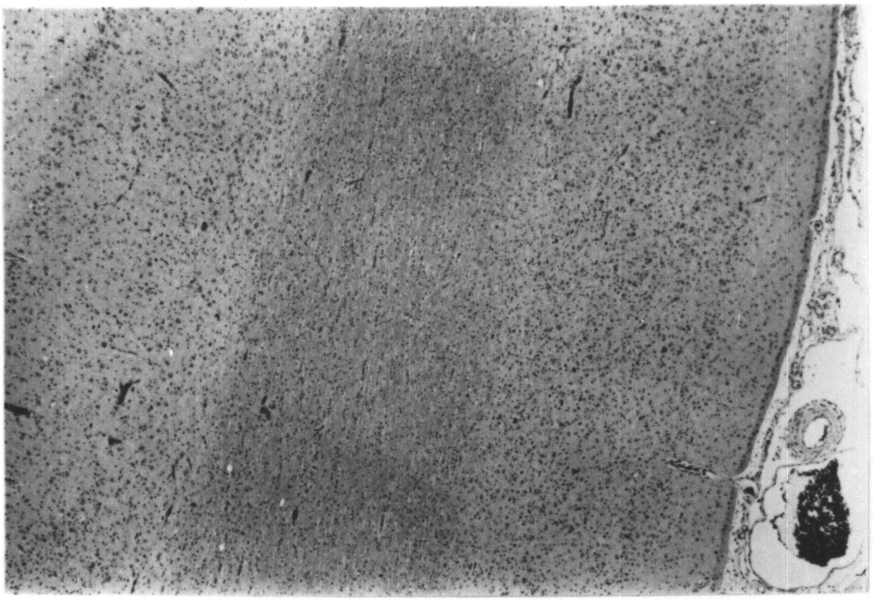


Fig. 50. Cerebral gyrus (Pig No. 756). Diffuse hypercellularity in cortex (right side). 0.38 mg. Hg/kg. daily as EMC for 90 days. H&E stain. x 25.

affected areas (Fig. 51). Thickened arterioles were found in the leptomeninges at the depth of some sulci. Affected sulci were located between, and included, the coronal and entomarginal medially, and the rhinalis laterally. The arterioles in the sulci were surrounded by variably hypercellular connective tissue, especially on the side facing the underlying cerebral cortex. Capillaries leaving the vessels in the sulci were thickened by endothelial cell proliferation and, therefore, prominent.

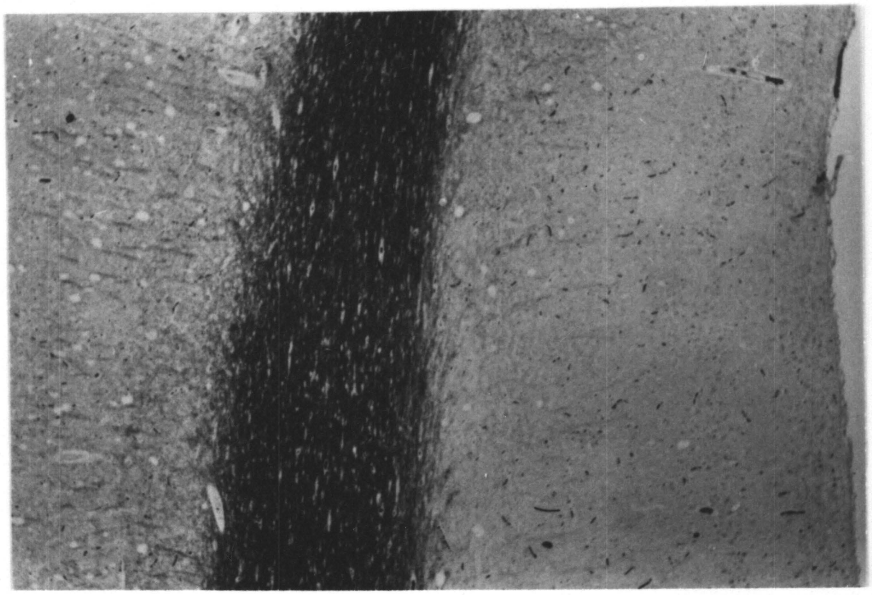


Fig. 51. Pig No. 756. Loss of axons affecting the cortex (right side). Bielschowsky's stain for axons. x 25.

Under higher magnification, it was found that the loss of normal architecture in the cortex was due to laminar neuronal necrosis and degeneration. Degenerating neurons stained uniformly red with H&E, the nuclei and cytoplasm having lost their usual basophilia. A significant number of neurons appeared shrunken and were surrounded by vacuolar spaces. Neuronal necrosis was either diffuse or, more commonly, limited to the second to fourth laminar layers. In addition, many neurons had been totally reabsorbed, leaving behind empty spaces arranged.

in a laminar pattern. Interspersed with these vacuoles, there were numerous finely granular eosinophilic masses which were interpreted as degenerating neurons or axons. Occasionally, totally calcified neurons were found in the affected areas.

An absolute increase in microglial cells was present (Fig. 52). This was associated with capillary endothelial proliferation, and a moderate degree of mitotic activity in the endothelial cells. Astrogliosis occurred and was characterized by the presence of numerous swollen vesicular astrocytes which appeared singly, in pairs, or in groups.

The trabeculae of the subarachnoid space in areas affected with cortical lesions were thickened and fibroblasts assumed embryonic characteristics. Numerous histiocytes which appeared to be locally produced, were infiltrating the meninges. In the diseases arterioles, there was proliferation of the adventitia and thickening of the intima with peripheral displacement of the interna elastica (Fig. 53).

In some instances, there appeared to be evidence of elastica reduplication.

The two pigs (757 and 758) which were affected clinically are described below.

The cerebral cortex of the whole brain was injured uniformly. In the cortex, the usual laminar neuronal stratification was destroyed, except for a faint persistence of the second laminar layer. There was a marked reduction in the

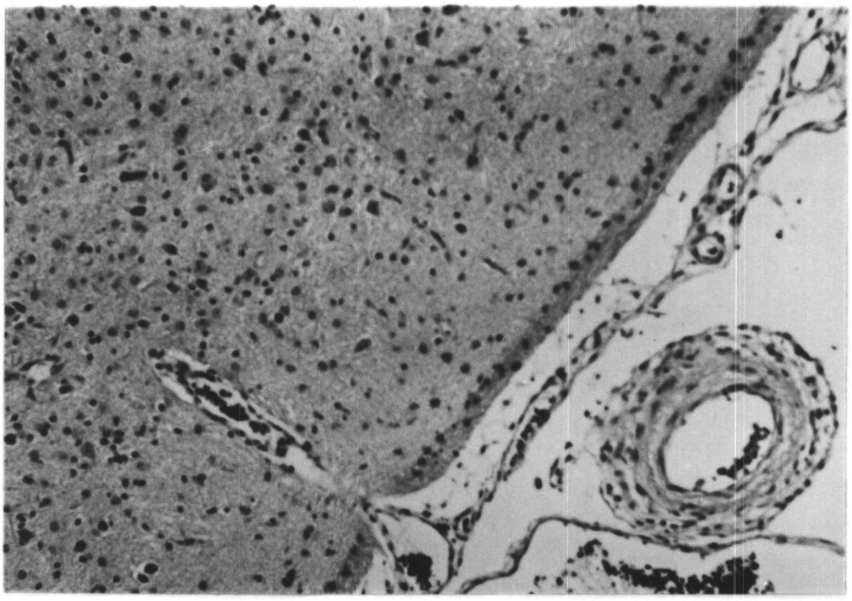


Fig. 52. Pig No. 756. Thickened vessel wall and increased cellularity in adjacent cerebral cortex due to micro- and astrogliosis. Loss of neurons is most noticeable in the outer granular layer. H&E stain. x 100.

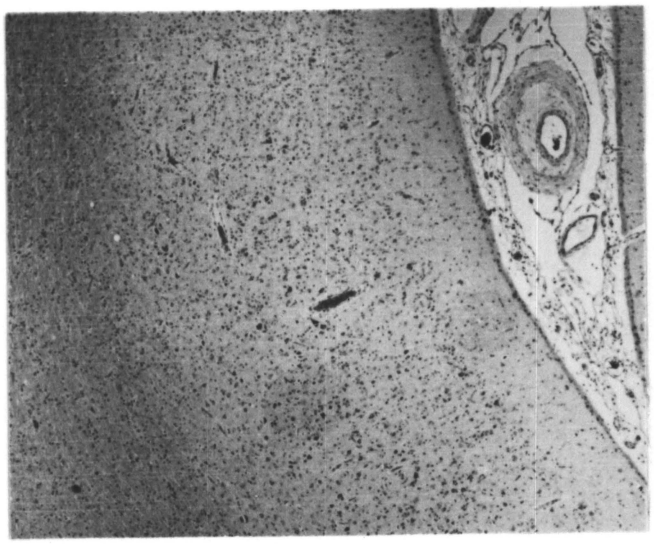


Fig. 53. Pig No. 756. Meningeal artery in entomarginal sulcus. There is thickening of the intima at the expense of the lumen. H&E stain. x 25.

number of cerebral neurons (Fig. 54). Some viable neurons persisted in the outermost aspects of the various gyri. Severe astrogliosis and microgliosis were found. The neuropile had lost its granularity and was transformed into a markedly vesiculated tissue. Intact axons were rarely seen (Figs. 56 and 57). The existing oligodendroglia appeared to have undergone acute swelling. Mineralization was found in numerous neurons (Fig. 55).

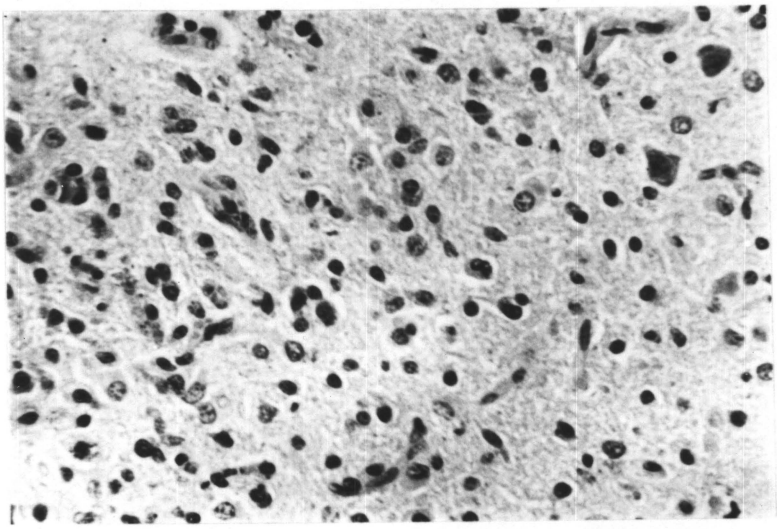


Fig. 54. Pig No. 757. Cerebral cortex with severe neuronal reduction, astrogliosis and microgliosis. Only two neurons are recognizable as such in upper right corner. H&E stain. x 250.

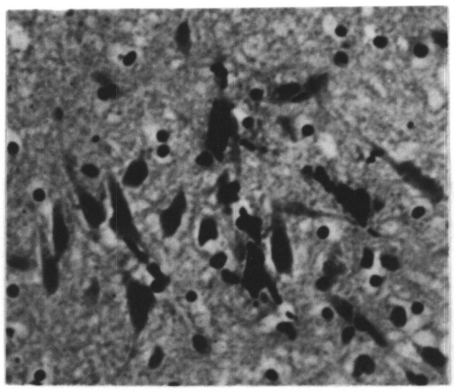


Fig. 55. Pig No. 757. Cerebral cortex, second layer. There is neuronal mineralization. H&E stain. x 250.

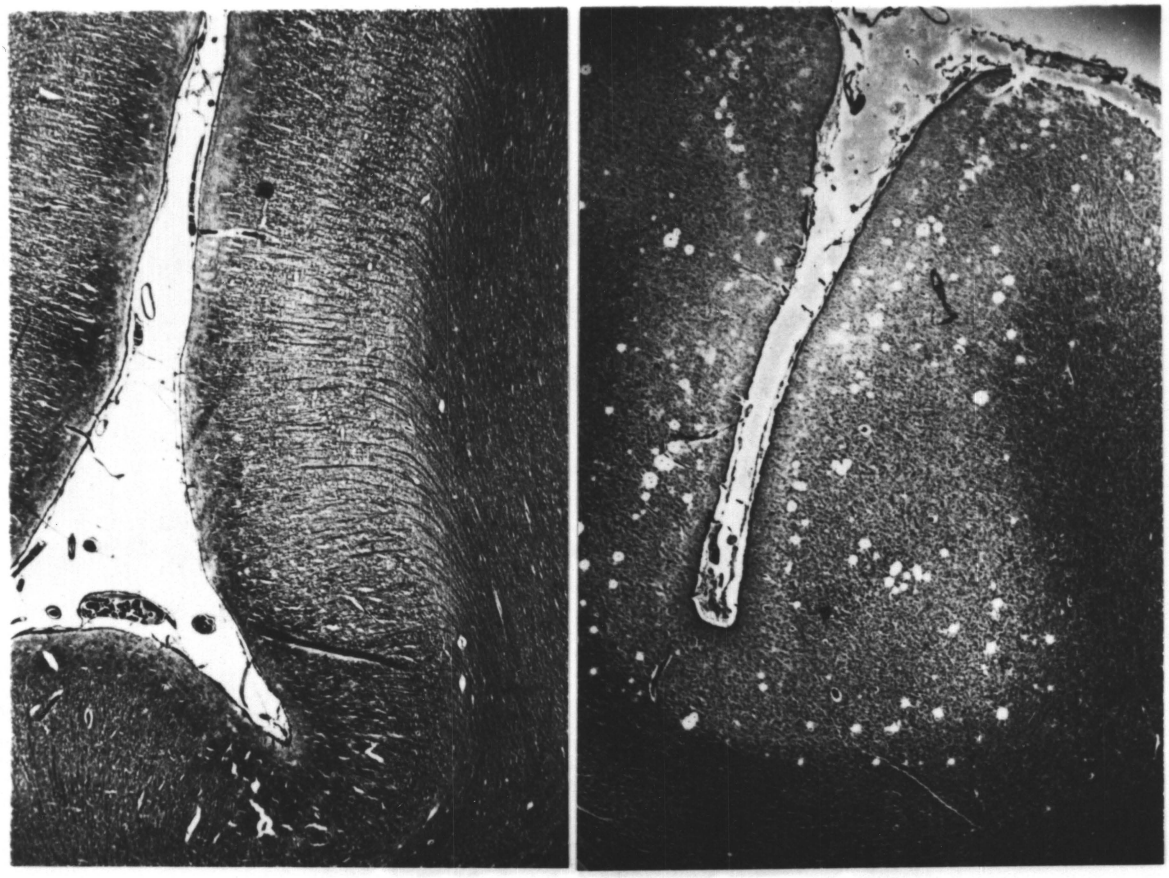


Fig. 56. Left - Control pig. Right - Pig No. 758. Occipital cerebral cortex. Severe decortication is characterized by absence of intact axons entering the underlying gyral core. Bielschowsky's stain, section thickness 20 μ. x 25.

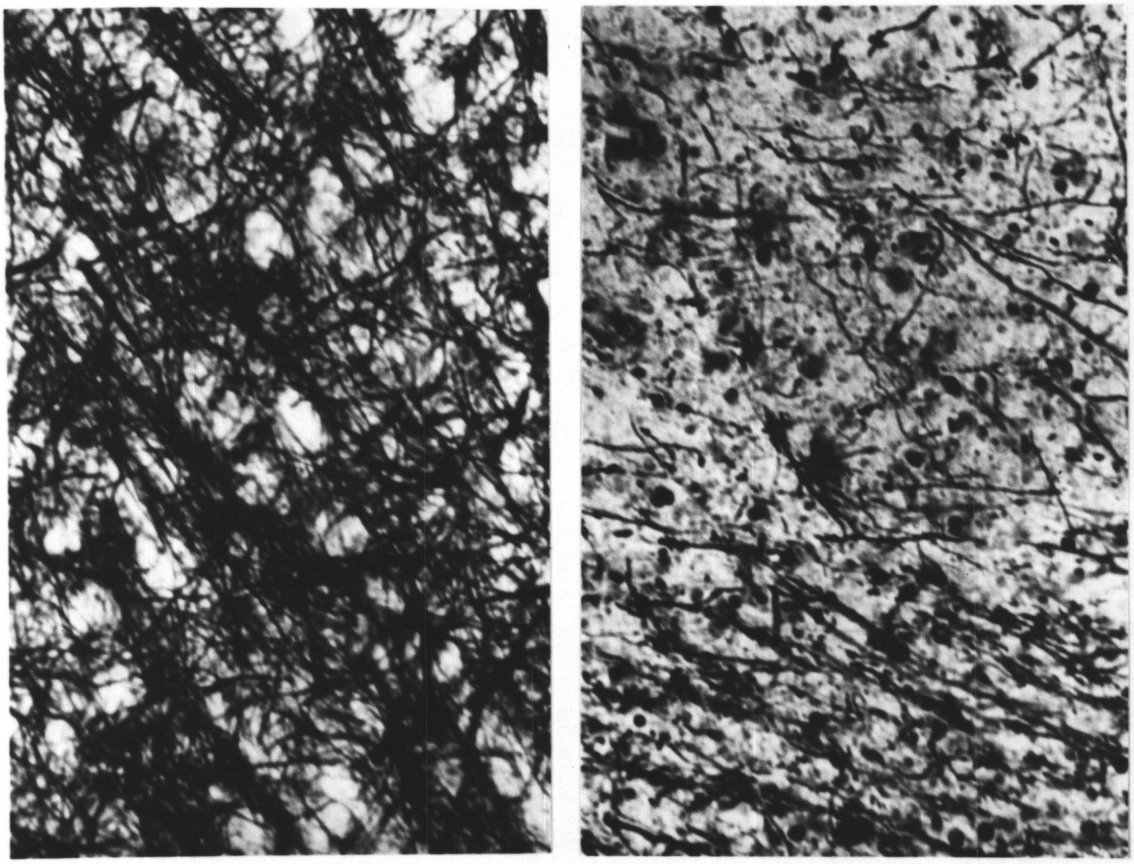


Fig. 57. Left - Control. Right - Pig No. 758. Higher magnification of cerebral cortex shown in Figure 56 to show the cortical disorganization in the affected animal. Bielschowsky's stain. x 250.

Nearly all of the leptomeningeal arterioles penetrating the sulci and covering the cerebral cortex were affected but not equally. The involvement of the larger arterial trunks appeared to be chronologically more recent.

The vessels with larger diameters were spared or slightly affected. Proliferation of the intima and moderate narrowing of the lumen was found in injured vessels. Below the intima, there was an irregular band of semi-transparent, less cellular, connective tissue which was surrounded by, or fused with, the interna elastica (Fig. 58). The elastica, at times, appeared slightly thickened, reduplicated, or even fragmented. The media of such vessels was also thickened to a certain extent by proliferation of smooth muscle cells. The adventitia occasionally was hypercellular and an occasional mitotic figure was present. This tunic, in many instances, merged with the surrounding interstitial tissue.

Smaller branches of the meningeal arterial tree had more advanced lesions. The subintimal fibrinoid band was much wider than in the larger vessel (Fig. 60). When similar sections were stained with Verhoff's elastic stain, it was seen that the interna elastica formed a significant part of the fibrinoid band. With this stain, the interna elastica appeared reduplicated, thickened, and, on occasion, fragmented (Fig. 59). In addition to the changes just described, varying degress of concentric hypertrophy of the muscular layer were prominent. The smooth muscle fibers often were separated from one another as if this tissue were edematous.

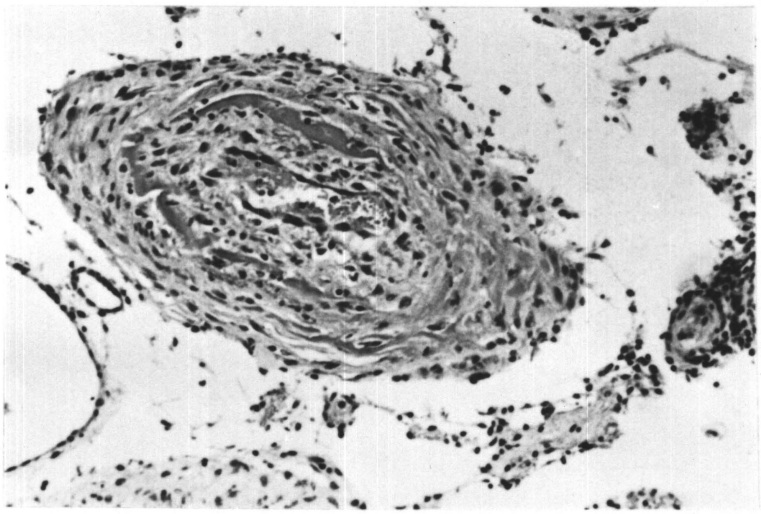


Fig. 58. Pig No. 757. Meningeal artery with intimal proliferation causing reduction of luminal size, thickening of elastica interna and degeneration of smooth muscle fibers. H&E stain. x 100.

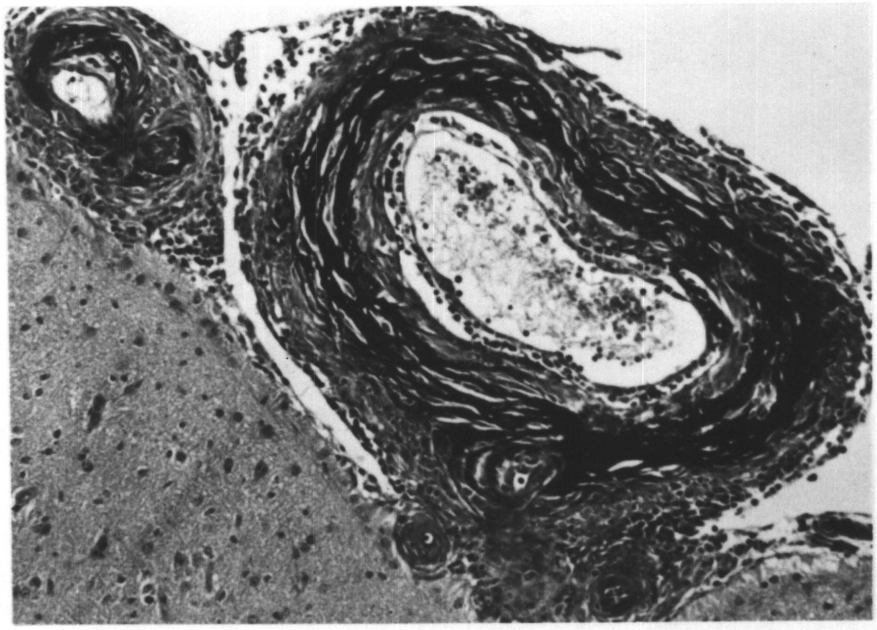


Fig. 59. Pig No. 757. Small artery and arterioles in cerebral sulcus. Thickening, reduplication and fragmentation of interna elastica. Verhoff's elastic stain. x 100.

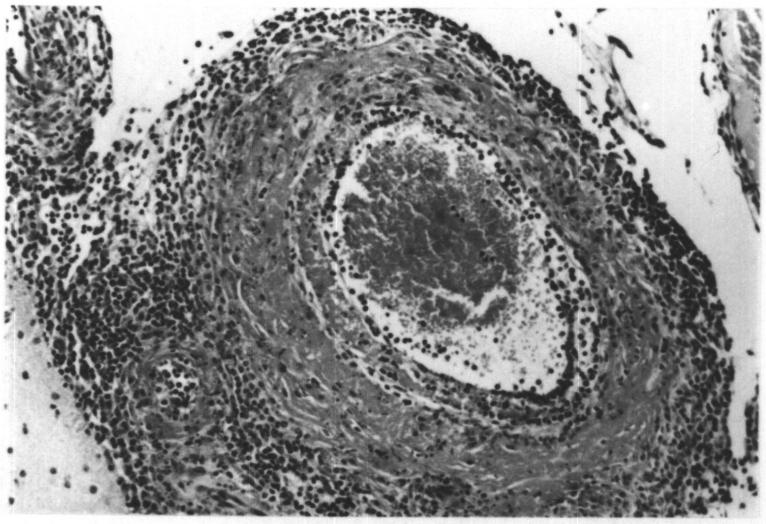


Fig. 60. Pig No. 757. Small artery and arterioles in cerebral sulcus. Interna elastica is fused with, and indistinguishable from the irregular band of fibrinoid material.

The size of the lumen of such arteries was reduced to a greater extent than in the larger vessels and, on occasion, the lumen was almost non-existent. The interna elastica was very thickened and fibrinoid material occurred in the wall of many such arteries.

The precapillary arterioles lying in the pia mater which contacted the underlying injured cerebral cortex had even more advanced lesions with their walls more extensively affected by fibrinoid degeneration. In these vessels, the fibrinoid material caused the thickness of the vascular walls to become relatively

great (Fig. 61). This arteriolar lesion began at varying distances from the point of the arteriole's origin.

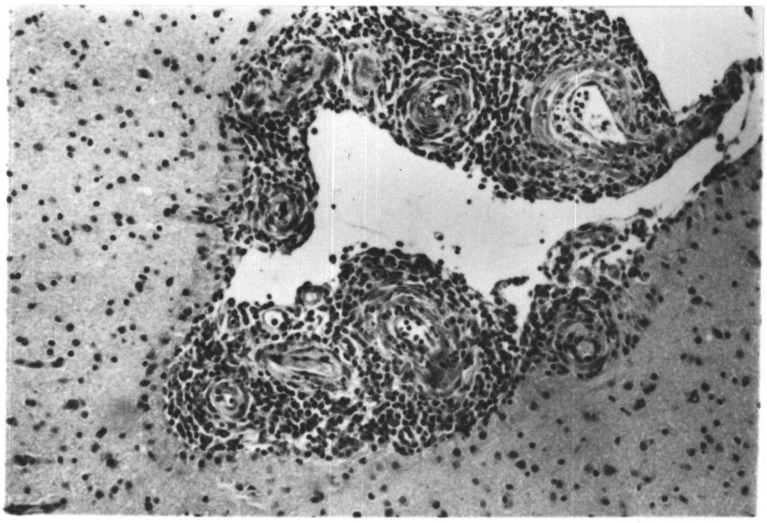


Fig. 61. Pig No. 757. Precapillary arterioles in cerebral sulcus. The thickened vessel walls are affected by fibrinoid necrosis and perivascular infiltration with lymphocytic and histiocytic elements. H&E stain. x 100.

Malacic foci were found in the basoganglia and thalamus of clinically affected animals. Vessels with degenerated walls were always associated, to some degree, with such foci. In certain instances, vessels with necrotic walls were seen in the center

of malacic foci while, in other cases, they were seen in the vicinity of the foci (Fig. 62).

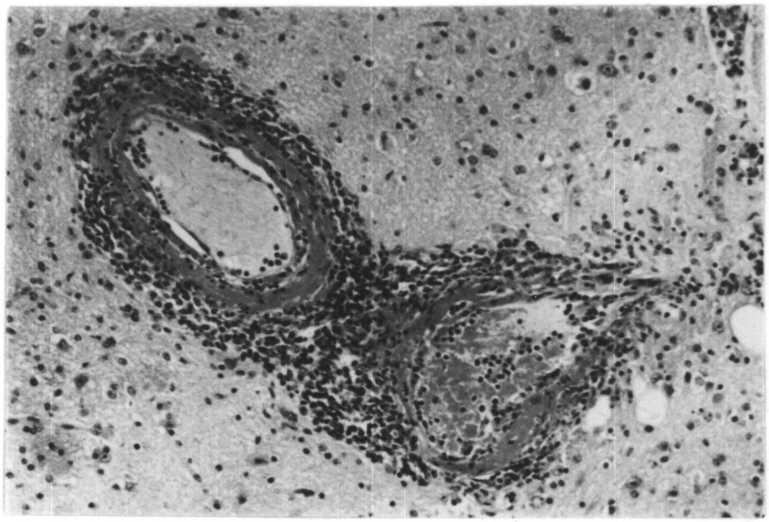


Fig. 62. Brain of Pig No. 757. Nucleus caudatus. Two vessels with fibrinoid degeneration and perivascular infiltration of lymphocytes and lipophages were found in the vicinity of a malacic focus. H&E stain. x 100.

In the brain stem of two pigs (757 and 758), there was evidence of neuronal necrosis and moderate astro- and micro-gliosis.

On H & E stained sections, degeneration in the spinal tracts in any of the pigs was not recognized.

Urinary system. -- In the kidneys, some lesions were observed. The lesions seen ranged from occasional necrosis and desquamation of the proximal tubular cells to more severe segmental necrosis of tubular epithelium associated with leakage of proteinaceous material. Varying degrees of diffuse hydropic degeneration were seen in several animals.

Digestive system. -- The livers of three pigs (756, 757 and 759) had undergone diffuse, severe, hydropic degeneration. Focal, mainly centrolobular, necrosis was noticed in Pig No. 760. Passive congestion and atrophy of hepatic cords were present in Pig No. 758.

Group III (0.76 mg. Hg/kg. daily):

Nervous system. -- Comparable lesions were found in the nervous system in all animals of this group, but were most severe in two animals in this group (762 and 763) who were allowed to enter the more advanced stage of disease.

As in other clinically affected groups, the part of the cerebral cortex lying between the coronal sulcus anteriorly, the cruciate sulcus posteriorly, and the rhinalis laterally was consistently affected. The lesions were bilaterally symmetrical and were limited in the frontal region, but became more severe toward the occipital

region. The chief lesion consisted of laminar degeneration and necrosis affecting mainly the third and fourth laminae of neurones. This lesion extended also, on occasion, either toward the topical or the deeper cortical laminae. In two animals (762 and 763), there was moderate microgliosis attended by the presence of numerous mitotic figures (presumably microglial cells), capillary endothelial cell proliferation and a moderate increase of the astrocytic population. The affected neurons were eosinophilic and a large number had no visible nucleus. A significant proportion of affected neurons had undergone dissolution. An axon was occasionally connected to the resulting vacuoles (Fig. 63). Such findings were considered to be a result of rapid neuronal death. Numerous other neurons were also eosinophilic but their nuclei retained a certain degree of basophilia. Some of these appeared shrunken and were surrounded by a perineuronal space which, at times, was traversed by thin cytoplasmic bridges. Many of the remaining neurons had severely vacuolated cytoplasm.

The size of the cerebral molecular layer was severely reduced in the dorso-lateral gyri. Lateral to this, the cortex was less severely injured and a transition to normal tissue occurred gradually.

Definite vascular involvement was seen in two pigs (763 and 762). In these, smooth muscle fiber necrosis and adventitial connective tissue proliferation which resulted in thickening of the arteriolar wall were observed. Between the various smooth muscle nuclei was scattered a moderate amount of nuclear debris. Fibrinoid was not found.

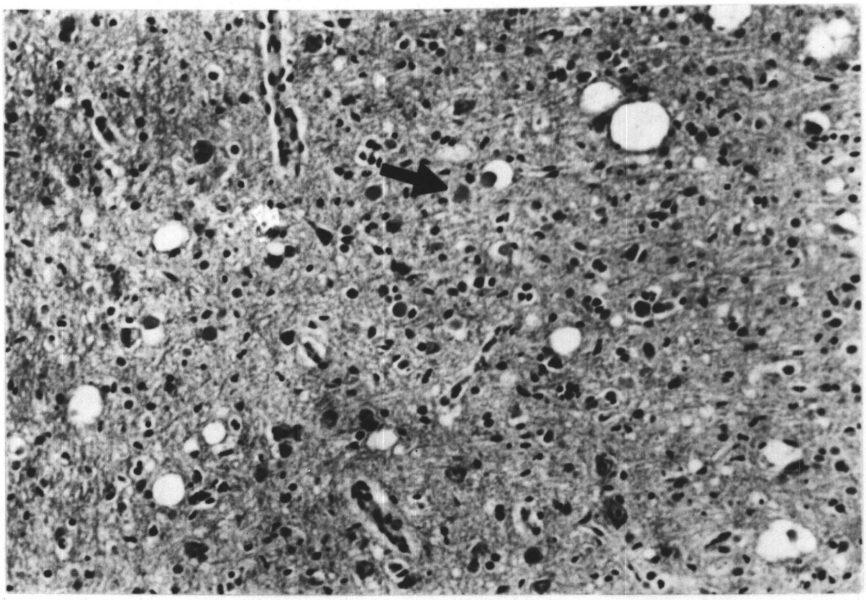


Fig. 63. Pig No. 763. Cerebral cortex with neuronal necrosis, vacuole formation, gliosis and eosinophilic granular bodies (arrow). H&E stain. x 100.

The usual linear arrangement of the various axons in the gyral cores was moderately disturbed. Severe lesions were characterized by a granular appearance of the white matter. In addition, a reduction in the number of myelinated axons entering the gyral cores was noticed at this level in sections stained for myelin with Heidenhain's stain.

In distinction to lower dosage groups, degeneration, necrosis, neuronophagia and dissolution of neurons was noticed in various areas of the thalamus (Fig. 64). One or both red nuclei,

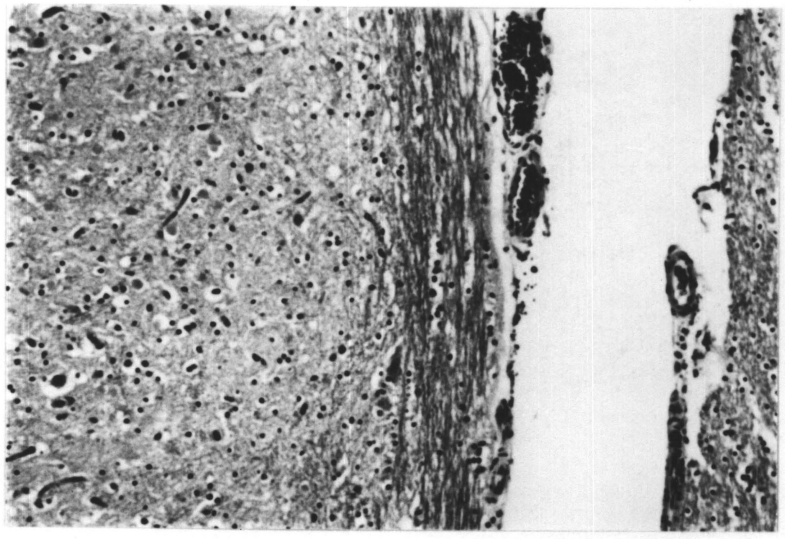


Fig. 64. Pig No. 761. Lateral geniculate body. There are numerous eosinophilic granular bodies in the deeper region (left) of the right lateral geniculate body. H&E stain. x 100.

pontile nuclei, inferior olives, semi-lunar and dorsal root ganglia were similarly affected (Fig. 65). The degree of injury, however, varied from one animal to another and, as noted earlier, animals 762 and 763 had the most severe lesions.

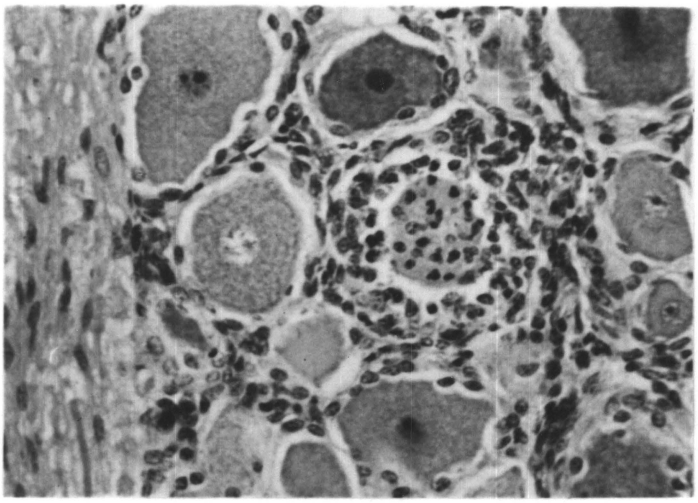


Fig. 65.
Pig No. 764.
Dorsal root ganglion with necrosis and neuronophagia in its outermost region. H&E stain. x 100.

Uro-genital system. -- The kidneys of all animals were affected by degenerative changes. The lesions appeared confined to the proximal convoluted tubules. The basic process was one of random epithelial cell necrosis associated with desquamation of necrotic cells into the lumen. Proteinaceous material formed hyalin casts in some areas. A number of proximal tubule epithelial cells were in mitosis. A mild degree of hydropic degeneration was observed in only one of the pigs. In summary, involvement was mild and did not involve the interstitial elements.

Digestive system. -- The liver of all animals was affected by varying degrees of hydropic degeneration which had a diffuse distribution. Randomly located necrotic hepatocytes were also seen. Several mitotic figures, which were most common in the mid-zone of involved lobules, gave evidence of regeneration.

A moderate degree of centrolobular congestion and hepatic cord atrophy was seen in one pig (762). Focal necrosis was seen only in one pig (765) and was random in distribution.

The esophageal mucosa of one pig (763) was ulcerated and the adjacent lamina propria was edematous. In the affected lamina propria were several arterioles with severe necrosis and hyalinization of their walls (Fig. 66). The fundic and pyloric mucosa of the stomach of the same pig had areas of superficial coagulation necrosis. Below this, the lamina propria was edematous and contained several hyalinized arterioles. Post mortem changes in this pig (763) prevented evaluation of subtle mucosal changes. The stomachs of the remaining animals in this group were not significantly affected.

In this group, the proximal part of the large intestine, namely the cecum and a small part of the spiral colon, had mild lesions. The superficial epithelium of the mucosa had undergone degeneration and some cells were necrotic. Nuclear debris accumulated at the base of the injured columnar cells and separation of the epithelial lining from the underlying lamina propria was consistently observed in areas with lesions (Fig. 67). In some lesions, a thickened eosinophilic basement membrane was prominent at the border between epithelium and underlying lamina. In one pig (762), there was severe congestion of the colonic mucosa associated with slight edema and light infiltration with acute inflammatory cells in the lamina propria. In two animals (762 and 765), several arteries were affected by hyaline degeneration and necrosis of their muscular coats (Fig. 69).

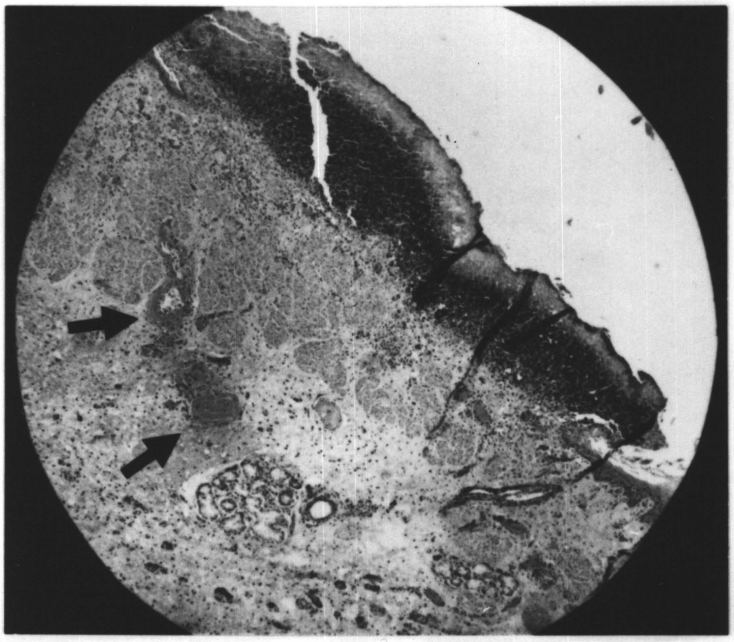


Fig. 66. Pig No. 763. Esophagus with ulceration of the epithelium, edema of the submucosa and fibrinoid necrosis of underlying small arteries (arrows). H&E stain. x 25.

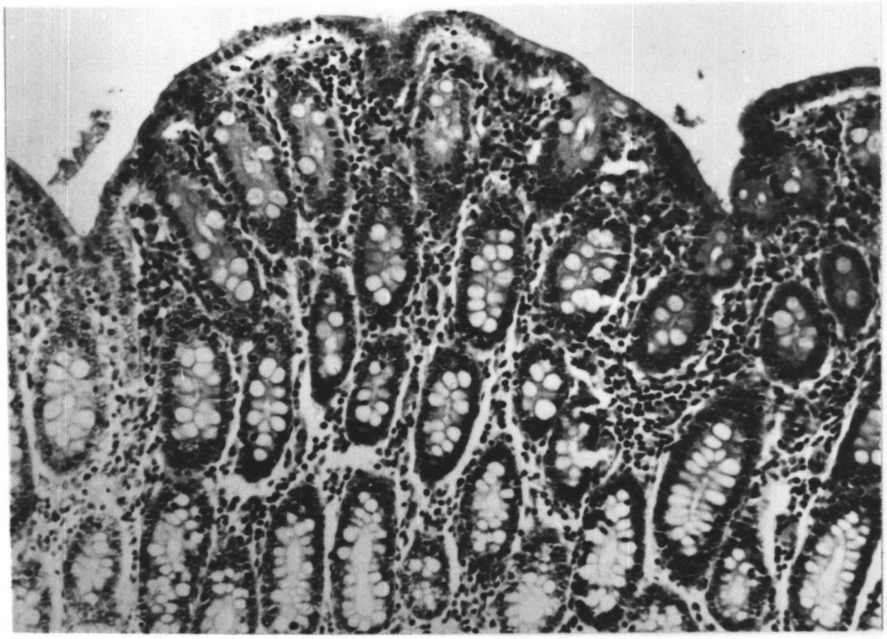


Fig. 67. Pig No. 764. Cecum with separation of the surface epithelium from the lamina propria. A small number of necrotic cells are present at the site of separation. H&E stain. x 100.

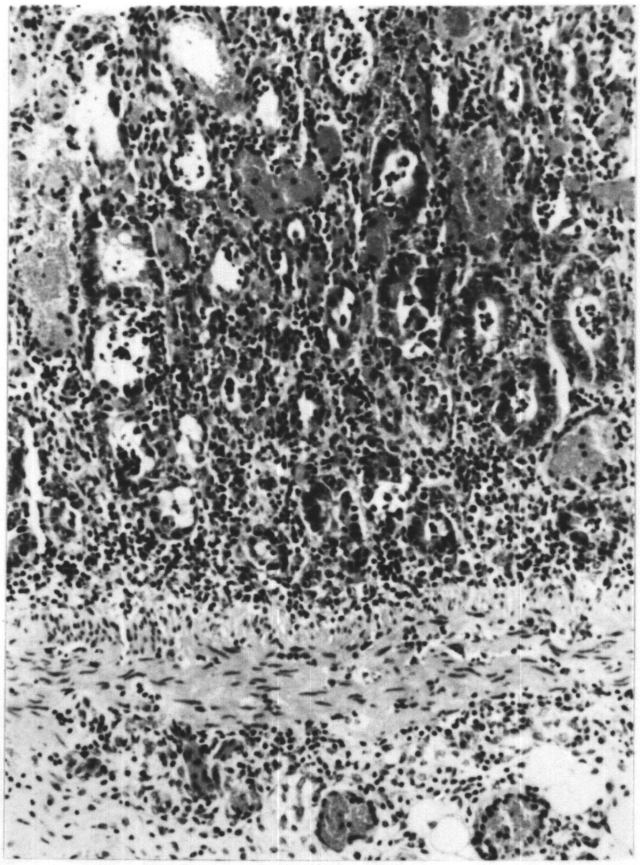


Fig. 68. Pig No. 762. Colon with congestion of the mucosa and hyaline degeneration of arterioles in the submucosa. H&E stain. x 10.

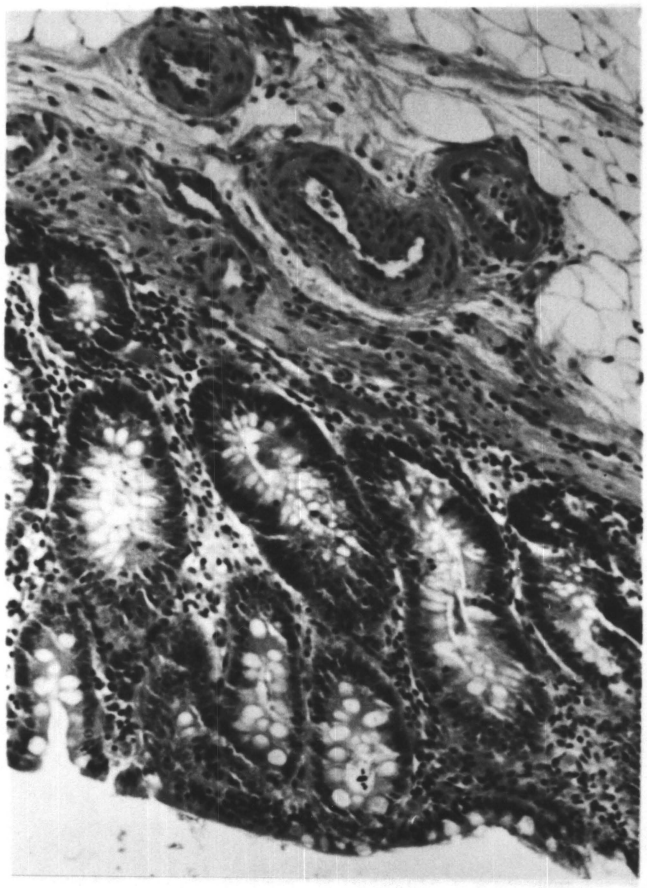


Fig. 69. Pig No. 762. Colon with hyalinized vessels in submucosa. H&E stain. x 250.

Group IV (2.28 mg. Hg/kg. daily): The lesions in this group differed markedly from those in Group III in that: 1) no large or small meningeal vessels were injured; 2) the neuronal necrosis elicited a limited glial and capillary response; 3) neuronal necrosis affected more severely several of the subcortical nuclei, gray matter of the spinal cord and the dorsal root ganglia; 4) coagulation necrosis and ulceration with formation of pseudomembranes in the cecum and colon occurred; and 5) hydropic degeneration was found in the epithelium of the proximal convoluted tubules.

Nervous system. -- Three of the animals were euthanized at an early stage of clinical disease while the remaining two (767 and 768) were allowed to develop signs of severe toxicosis.

The distribution of lesions duplicated to a great extent the one observed in the previous groups. In pigs killed early after the onset of signs (14th day), there was neuronal necrosis and dissolution of necrotic cells with formation of vacuoles. Affected neurons had a laminar distribution. The reaction of glia was minimal. In addition, there was evidence of diffuse edema throughout the cerebrum. Acute swelling of the oligodendroglia was noticed in the cortex above the sulcus rhinalis, in the basal ganglia and the thalamus. Both gray and white matter were equally affected in this respect. These lesions were more severe in animals which exhibited advanced clinical signs (767 and 768). In such animals, there was microglial proliferation and neuronophagia. Endothelial proliferation

was also observed. The number of neurons present in lateral geniculate bodies was severely reduced although the optic tract appeared normal (Fig. 70).

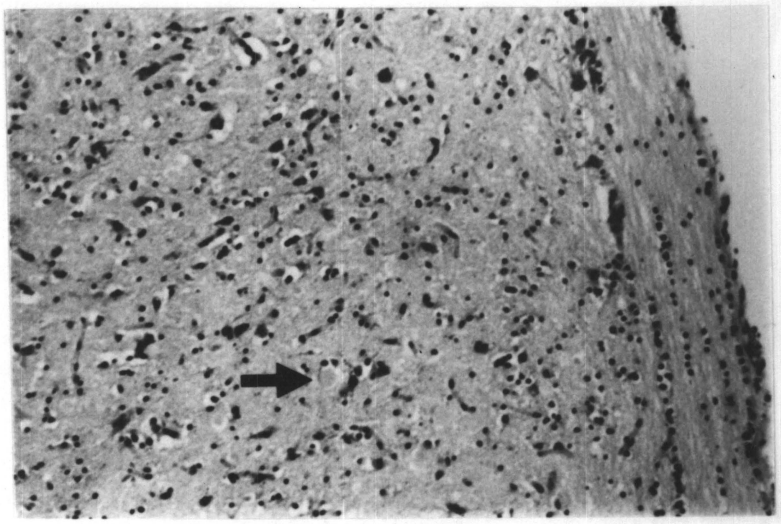
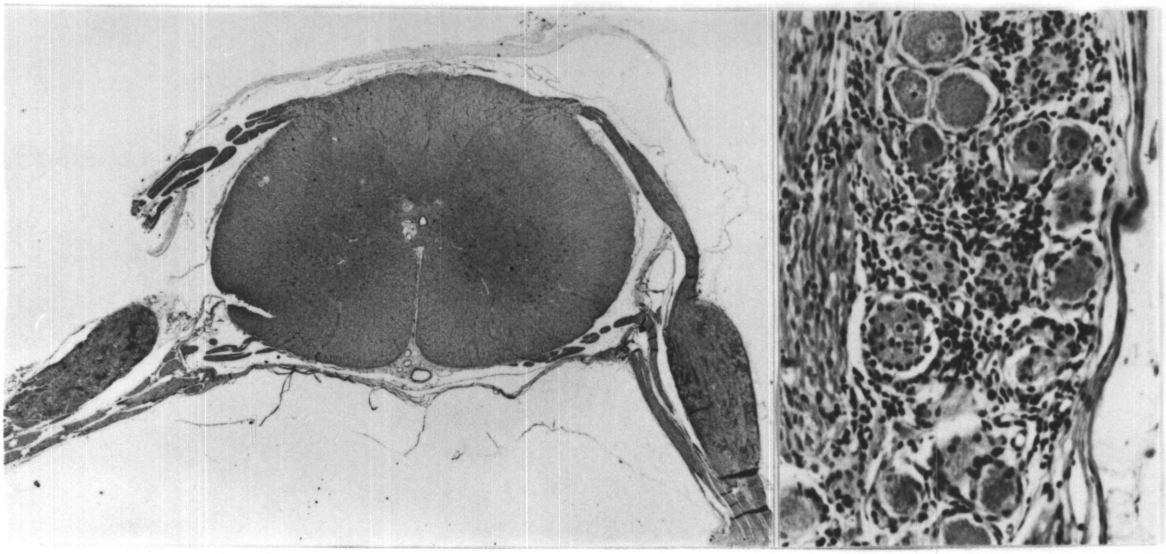


Fig. 70. Pig No. 767. Right lateral geniculate body. There are randomly scattered necrotic neurons and basophilic granular bodies (arrow). H&E stain. x 100.

The basoganglia, the central thalamic nuclei, the red nucleus, the pontile nuclei, the semi-lunar ganglia, the dorsal root ganglia in general, and the spinal gray matter were all affected by various degrees of neuronal degeneration and necrosis. The necrotic cells were randomly distributed within each of these structures (Fig. 71). The celiac ganglion remained visibly un-

affected by such a process. Capillaries having endothelial proliferation occurred in association with these areas of neuronal necrosis but, notably, the arterial vessels in the region remained normal in appearance.



a b

Fig. 71. Pig No. 766. Spinal cord, lumbar segment with neuronal necrosis (a). H&E stain. x 3.

(b) High power magnification of adjacent dorsal root ganglion. There is neuronal necrosis and neuronophagia. H&E stain. x 250.

Digestive system. -- Morphological evidence of liver damage was present and varied from animal to animal. The hepatic lobules were uniformly affected by random necrosis of hepatocytes and regeneration, or by severe diffuse cloudy swelling with periacinar areas of hydropic degeneration.

Necrotic typhlitis and colitis were present in all animals but affected most severely Pig No. 769. The minimal lesion in the mucosa consisted of congestion, light or moderate infiltration with acute inflammatory cells, and necrosis and separation of the luminal epithelium. Necrotic debris was seen below such epithelium. The more severe lesions had coagulation necrosis, congestion, and pseudomembranes containing numerous bacterial colonies.

Edema was seen in the submucosa and mesocolon. In this tissue, thickened necrotic arterioles were present. Subintimal hyaline material was stained for elastic tissue by Verhoff's stain. The internal elastica appeared less serpentine and considerably thickened in certain areas of the vessel wall.

Varying degrees of edema were present in the regional lymph nodes.

Urinary system. -- Varying degrees of hydropic degeneration was seen in the proximal convoluted tubules of the kidney (Fig. 72). Occasionally this process was more severe and was associated with necrosis of tubular epithelium. A small number of mitotic figures, two to five per 250 x optical field, was noticed in the proximal convoluted tubular epithelium. The glomeruli, the basement membrane of the tubules and the inter-

stitium appeared unaffected. The lumina of several tubules were occupied by hyaline casts. Severe edema was present in the lamina propria of the urinary bladder of Pig No. 769. This was the pig with the most severe edema in the mesocolon.

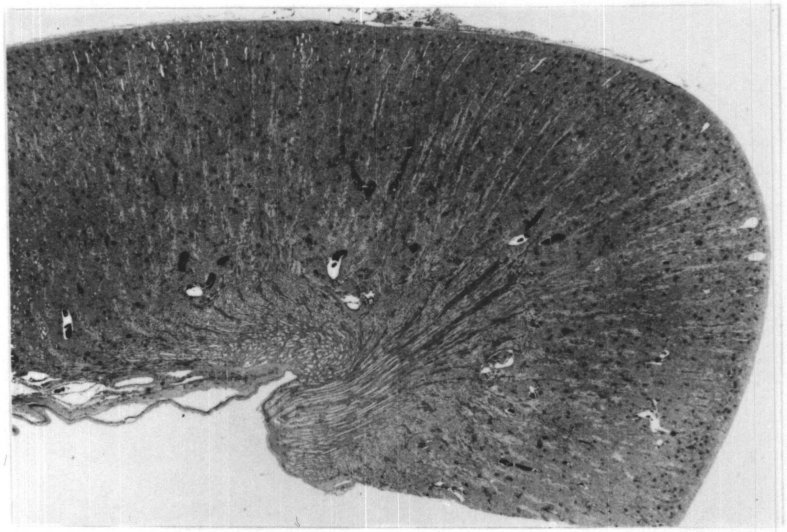


Fig. 72. Pig No. 768. Kidney with hydropic degeneration in the epithelium of proximal convoluted tubules. Affected tubules appear as slightly staining linear structures in the cortex. H&E stain. x 3.

Cardiovascular system. -- A few basophilic muscle fibers were seen in the myocardium of Pig No. 767 and were interpreted to be affected by dystrophic calcification. This was the only instance of such a lesion.

Group V (7.36 mg. Hg/kg. daily): The lesions in this group differed from those in animals of Group IV in the following ways: 1) the cerebral cortex was slightly affected; 2) the subcortical nuclei and the gray matter of the spinal cord, and the dorsal root ganglia were most severely affected; 3) the kidney was affected by hydropic degeneration; 4) the gastrointestinal tract had more severe lesions in the stomach mucosa.

Nervous system. -- Distribution of lesions in the cortex was quite similar to the previous groups. Notable in this group was the fact that lesions in the diencephalon and the spinal cord were prominent. In contrast to the cortical lesions seen in pigs poisoned at the lower dosages, segments of the dorsal cortical gyri were minimally affected by neuronal degeneration and necrosis with the exception of a few focal areas. Gliosis was also minimal or absent. Vascular lesions were seen only in Pig No. 773 and consisted largely of perivascular infiltration of histiocytic cells in the arteries of leptomeninges of the sulci of the affected cortex. Numerous mitotic figures were seen in these hypercellular areas.

The lateral geniculate bodies and the ventral thalamic nuclei were symmetrically affected by neuronal degeneration and necrosis. The degeneration of cell bodies and axons led to the formation of eosinophilic granular bodies.

Glial and vascular responses were minimal. In Pig No. 773, capillary endothelial cell proliferation and perivascular cuffing occurred in association with neuronal lesions. Extensive neuronal necrosis occurred in the red nucleus, pontine nuclei, and inferior olives (Fig. 73). In the medulla, randomly located necrotic neurons were common.

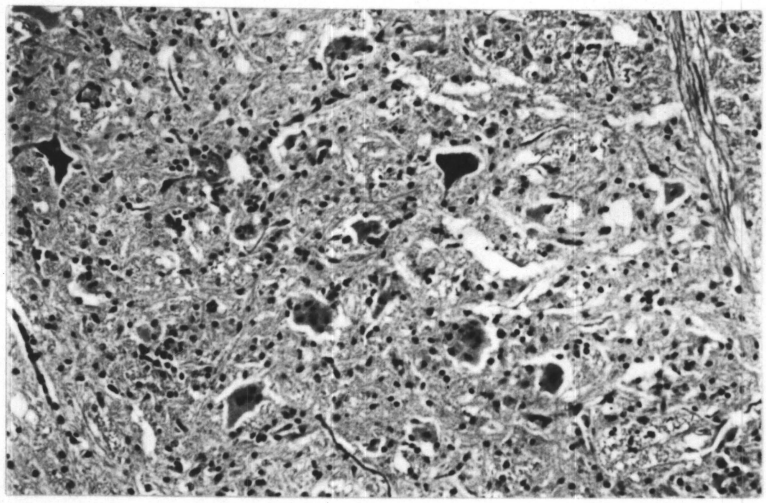


Fig. 73. Pig No. 771. Red nucleus with extensive neuronal degeneration. The cytoplasm of affected neurons has homogenization and eosinophilia. H&E stain. x 100.

The semi-lunar ganglia and the dorsal root ganglia were similarly, but more severely, affected. Approximately up to 20% of the neurons in these structures were affected by necrosis and neuronophagia (Fig. 74). The celiac ganglia failed to show any lesions.

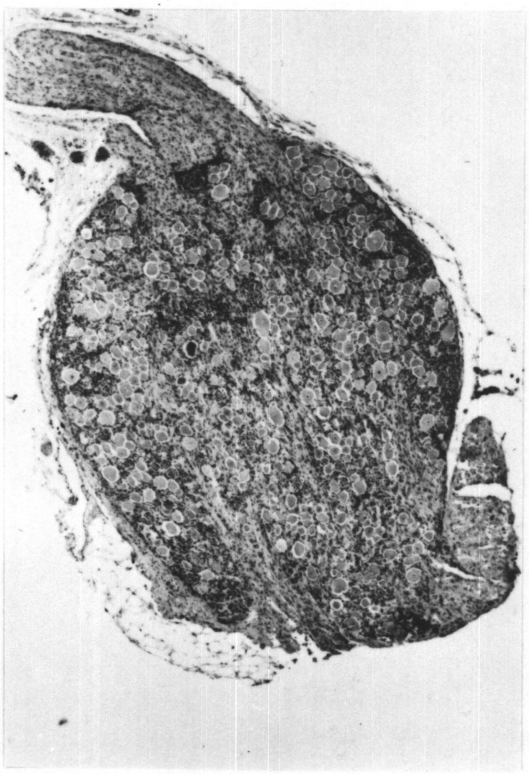


Fig. 74. Pig No. 773. Dorsal root ganglion from the thoracic region. There is severe neuronal necrosis and neuronophagia. H&E stain. x 16.

The ventral gray hornw of the spinal cord exhibited extensive neuronal degeneration, necrosis and neuronophagia.

Digestive system. -- A common finding in the liver of all animals was random necrosis of individual hepatocytes. Congestion of sinusoids and increased numbers of neutrophils were inconstant findings.

Purulent ulcerative pharyngitis was present in all animals. The degree to which the various animals were affected varied widely from one animal to another. In animals with severe involvement, the inflammatory process extended to adjacent surfaces. As a result of this, varying degrees of glossitis, rhinitis, esophagitis and laryngitis were also found. Fibrinoid necrosis of arterioles was seen in the vicinity of these lesions in one pig (772).

The gastric mucosa of all pigs was affected by an acute inflammatory process. This ranged from hyperemia and a light infiltration of neutrophils in the lamina propria to coagulation necrosis, erosion and, occasionally, ulceration of the mucosa. The upper one-third of the gastric mucosa was usually affected (Fig. 75). The fundic and pyloric regions were most consistently and severely affected. Edema of the submucosa was rarely seen.

The epithelium of the large intestine was variably, but constantly, separated from the lamina propria by inflammatory edema and a moderate amount of necrotic cellular debris. Occasionally, the crypts of Lieberkühn were dilated by necrotic debris.

Urinary system. -- The proximal tubules of the renal cortex were affected by severe hydropic degeneration associated with some epithelial cell necrosis and desquamation of dead cells. Topographically, the inner two-thirds of the cortex were most severely and consistently affected (Fig. 76). Occasionally, small hyaline casts were present in the lumina of the proximal convoluted tubules.

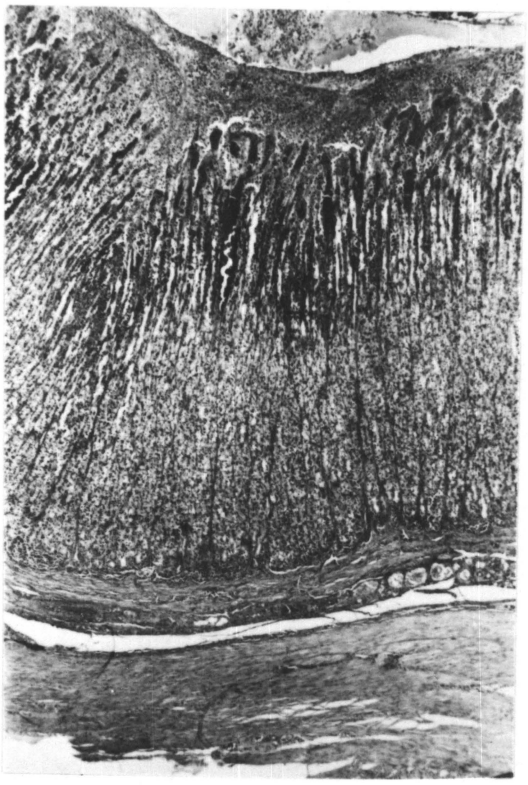


Fig. 75. Pig No. 772. Stomach, fundic region. There is congestion and necrosis in the upper part of the mucosa. H&E stain. x 25.

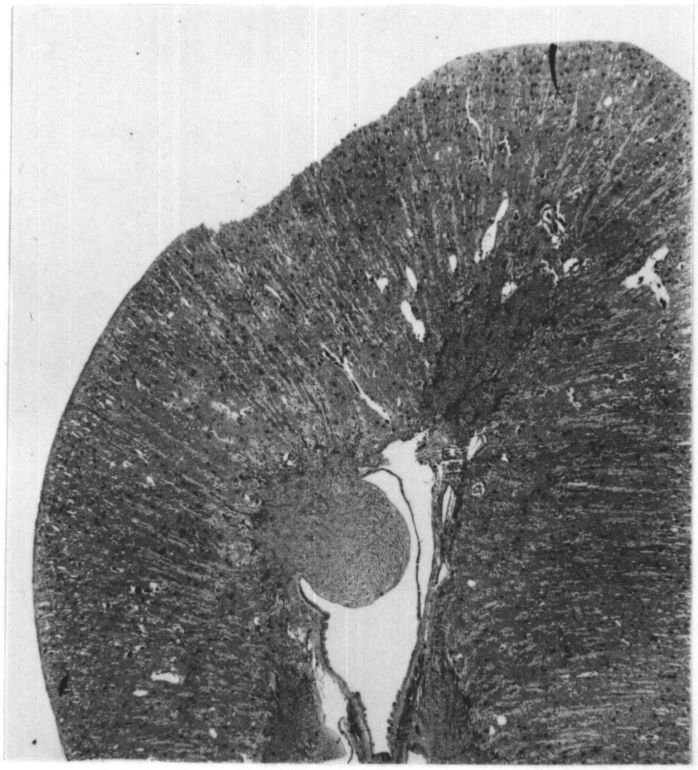


Fig. 76. Pig No. 774. Kidney with hydropic degeneration. The light staining structures represent affected tubules. There is a higher concentration of these structures in the inner portions of the renal cortex. H&E stain. x 3.

Endocrine system. -- Necrosis of cells in the adrenal cortex was a constant finding in all pigs. This process involved randomly distributed cells or, occasionally, assumed focal character.

Controls: No significant histological lesions were detected in this group.

Analytical findings

The data obtained from the analysis of tissues collected during the course of the experiment are reported in Table 15 and Figures 77 to 88.

It was found that:

1) the various organs examined accumulate different levels of mercury. In terms of the amount of mercury accumulation, the following, in diminishing order, was established; kidneys, liver, muscle brain, large intestine, skin, blood, urine and bile.

2) The level of mercury which accumulated in any one tissue was a function of either dose alone or dose and time. The levels of mercury found in skin and muscle depended directly upon dosage (Figs. 85 and 86). In the case of kidneys, the dosage level does not appear to be the only factor upon which tissue level depends (Fig. 84). Regardless of dosage, they have accumulated high levels of mercury, i.e. both high and low dosage groups have high levels in their kidneys.

3) The rate of accumulation of Hg in large intestine, muscle, bile and blood varied directly as the dosage. The level of mercury recovered from blood samples drawn on the 11th and 22nd experimental days support the above statement, since they are approximately

the same (Table 15 and Fig. 78). In the case of kidneys and brain, this was true only up to a certain dosage level. In the brain, daily dosage levels up to 2.28 mg. Hg/kg. resulted in correspondingly increased mercury accumulation, while higher doses failed to do so. The same observation was made for kidney above a daily dose level of 0.38 mg. Hg/kg.

The rates of accumulation of Hg in each tissue of each pig were calculated by dividing the amount of mercury in the tissue by the number of days the animal received EMC. The data on rate of accumulation are presented in Table 15. Figures 87 and 88 graphically illustrate the rates of mercury accumulation for each organ when all data for each of the experimental groups are pooled. In so far as this treatment of the data is valid, the rate of mercury accumulation appears to be directly dependent upon the dosage at the levels studied.

TABLE 15. Levels of Hg in μg/gm or μg/ml in Tissues of Pigs Which Received Varying Doses of Ethylmercuric Chloride

GROUP DOSE	PIG NUMBER	DAYS	μg Hg/ml								μg Hg/gm											
			Blood	Urine	Bile	Blood Series (Days)					Brain		Liver		Intestines		Kidney		Muscle		Skin	
						11	22	32	43	52	Hg	Hg/Days	Hg	Hg/Days	Hg	Hg/Days	Hg	Hg/Days	Hg	Hg/Days	Hg	Hg/Days
I (0.19)	751	90	0.3	----	0.6	0.6	0.4	0.8	0.7	0.6	5.1	0.06	5.8	0.06	4.4	0.05	-----	-----	2.9	0.03	1.1	0.01
	752	90	---	----	0.4	0.5	0.5	0.3	0.9	0.6	7.1	0.08	19.5	0.21	4.1	0.05	153.8	1.71	4.4	0.05	1.9	0.02
	753	90	0.3	----	0.6	0.3	0.3	---	0.4	0.4	6.8	0.08	10.3	0.11	2.2	0.03	122.1	1.36	3.5	0.04	1.4	0.01
	754	71	---	----	0.8	0.3	0.4	0.2	0.5	0.4	3.6	0.06	14.9	0.20	4.7	0.06	88.7	1.25	4.6	0.05	1.5	0.02
	755	65	---	0.2	0.5	0.3	0.4	0.4	0.5	0.4	---	----	16.3	0.25	3.9	0.06	146.4	2.25	1.7	0.03	0.6	0.01
II (0.38)	756	90	0.6	0.3	0.9	0.4	0.5	---	0.9	0.9	7.1	0.09	29.8	0.33	5.2	0.06	233.3	2.59	6.6	0.07	2.8	0.03
	757	60	0.8	1.6	1.2	0.9	0.9	2.1	1.9	1.6	23.2	0.36	24.7	0.39	24.6	0.04	252.0	2.94	7.8	0.12	2.7	0.04
	758	75	---	---	---	0.3	1.1	---	1.9	1.1	25.3	0.34	40.7	0.54	12.5	0.17	169.3	2.25	10.2	0.14	3.8	0.05
	759	90	0.8	0.9	0.7	0.2	0.8	0.6	0.8	0.6	6.4	0.07	31.5	0.35	3.9	0.04	248.1	2.75	9.9	0.07	1.6	0.02
	760	44	---	0.4	0.9	0.2	0.2	0.9	---	---	6.7	0.15	27.1	0.62	3.8	0.09	87.2	1.98	5.9	0.14	2.2	0.05
III (0.76)	761	22	---	---	---	0.6	---	---	---	---	5.5	0.25	15.8	0.72	4.3	0.19	118.1	5.36	7.4	0.34	2.5	0.13
	762	30	4.5	11.9	9.6	1.2	1.9	---	---	---	22.8	0.76	46.5	1.55	---	-----	213.5	7.10	27.7	0.93	7.1	0.23
	763	30	---	4.5	7.5	0.8	2.1	---	---	---	21.6	0.72	46.1	1.54	---	-----	136.4	4.53	22.1	0.73	8.7	0.29
	764	22	---	---	4.2	0.6	2.5	---	---	---	20.9	0.95	49.2	2.24	---	---	182.1	8.32	26.4	1.21	6.2	0.28
	765	22	---	7.4	3.7	1.4	0.1	---	---	---	16.2	0.74	47.2	2.15	---	---	135.9	6.18	40.6	0.83	3.6	0.10
IV (2.28)	766	14	2.7	---	2.7	1.8	---	---	---	---	28.4	2.03	66.2	4.72	20.6	1.47	149.7	16.69	30.8	2.21	7.3	0.52
	767	18	2.7	3.4	3.7	---	---	---	---	---	36.7	2.04	63.1	3.50	16.4	0.91	222.3	12.35	32.3	1.79	11.1	0.61
	768	18	4.7	5.3	3.3	5.9	---	---	---	---	30.5	1.69	81.1	4.50	28.9	1.61	164.7	9.15	34.8	1.93	10.9	0.50
	769	14	3.2	1.4	---	---	---	---	---	---	19.6	1.40	66.9	4.78	17.7	1.26	86.1	6.14	31.2	2.22	7.9	0.57
	770	14	4.9	4.2	---	3.8	---	---	---	---	32.3	2.32	80.8	5.77	16.7	1.19	124.6	8.91	40.5	2.89	10.7	0.76
V (4.56)	771	11	7.9	5.1	---	---	---	---	---	---	36.7	3.15	120.1	10.91	38.1	3.46	137.8	12.53	72.5	6.59	12.9	1.16
	772	11	8.2	2.9	---	---	---	---	---	---	27.3	2.48	45.5	8.68	32.7	2.97	105.6	9.60	45.4	4.12	14.9	1.35
	773	11	---	---	---	---	---	---	---	---	22.5	2.64	90.2	8.20	39.4	8.94	151.6	13.78	41.2	2.74	20.6	1.87
	774	11	8.1	7.1	13.5	---	---	---	---	---	33.4	3.21	98.7	8.97	72.2	6.56	100.8	9.52	51.0	4.64	11.9	1.08
	775	12	6.6	3.5	3.1	---	---	---	---	---	13.7	1.14	89.1	7.42	27.3	2.27	120.5	10.04	49.1	4.09	11.5	0.95
Control	776	49	0.1	0.1	---	0.1	0.1	---	---	---	0.6	---	0.7	---	0.2	---	0.5	---	0.6	---	0.3	---
	777	90	0.2	---	---	0.1	0.1	0.1	0.2	0.2	---	---	0.7	---	0.8	---	0.8	---	0.2	---	0.2	---
	778	80	0.1	0.6	0.3	0.1	0.1	0.2	0.2	0.2	0.5	---	1.1	---	0.7	---	0.1	---	0.3	---	0.7	---
	779	24	0.2	0.3	---	---	---	---	---	---	0.6	---	0.5	---	0.2	---	0.7	---	0.1	---	0.7	---
	780	50	0.1	0.0	0.3	0.2	0.1	0.1	0.1	0.2	---	---	0.7	---	---	---	0.4	---	0.5	---	0.8	---

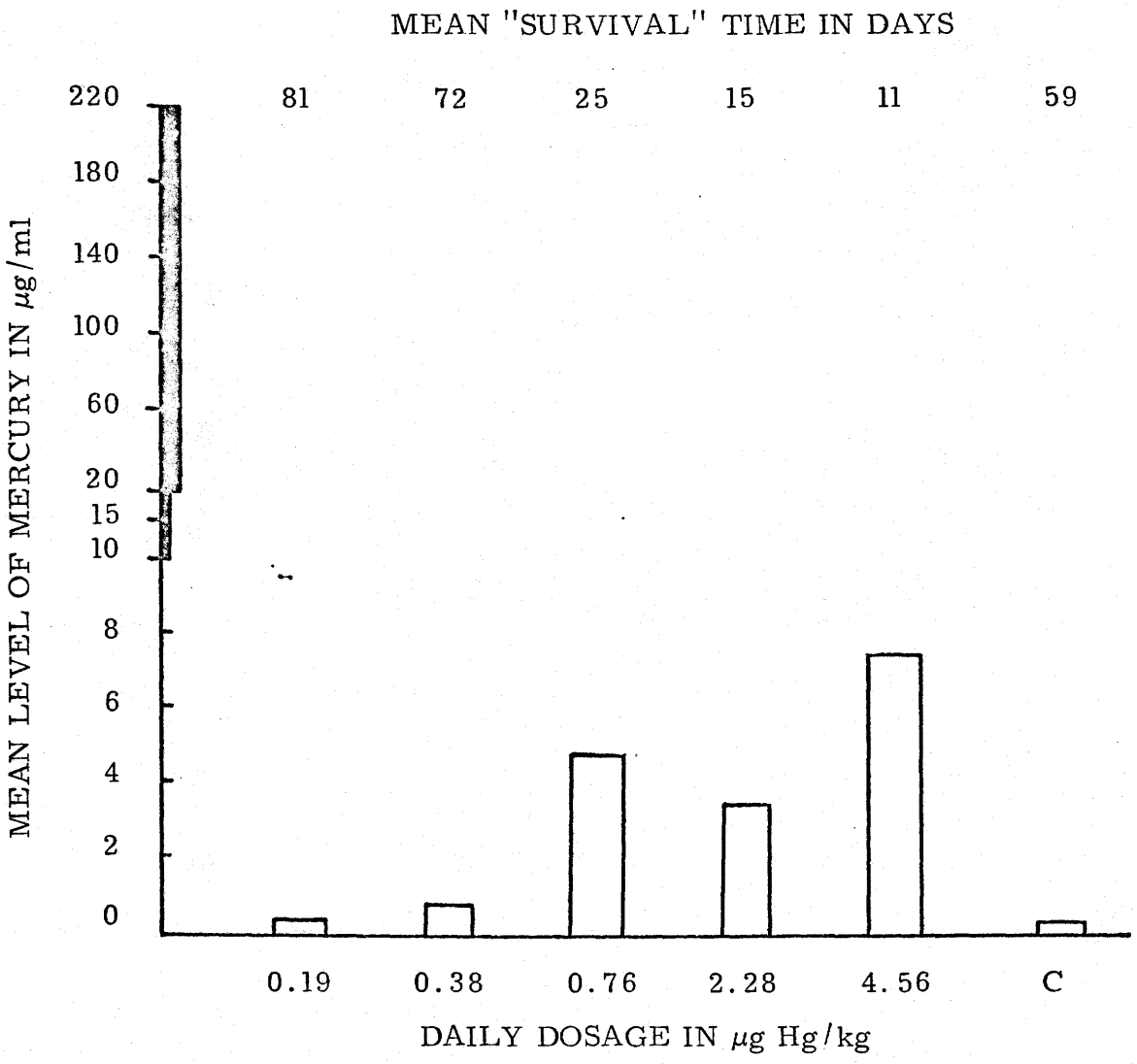


Fig. 77. Mean mercury levels in the blood and mean survival time of groups of pigs receiving increasing dosages of EMC.

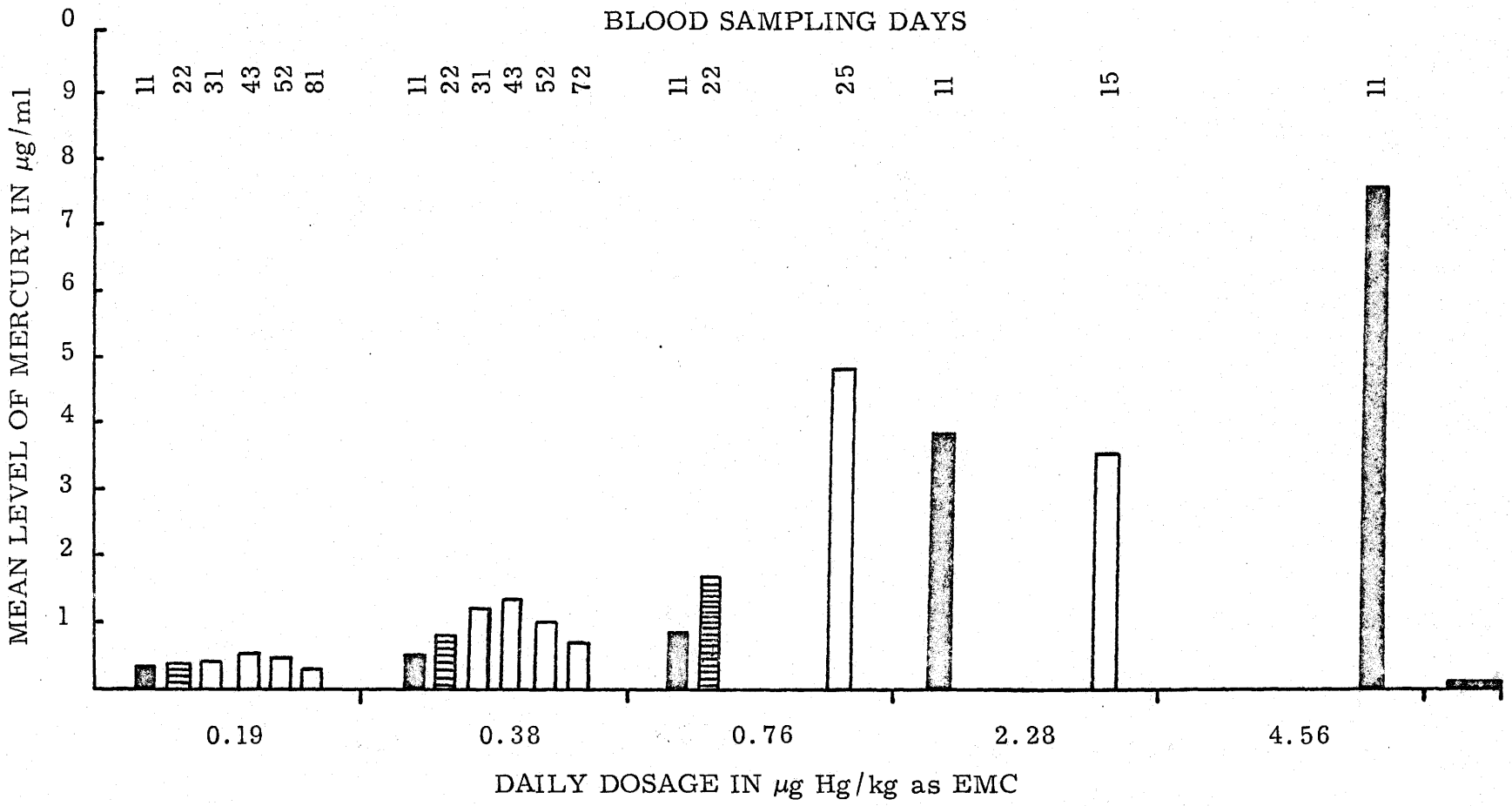


Fig. 78. Mean mercury values in serial samples of blood from animals receiving EMC.

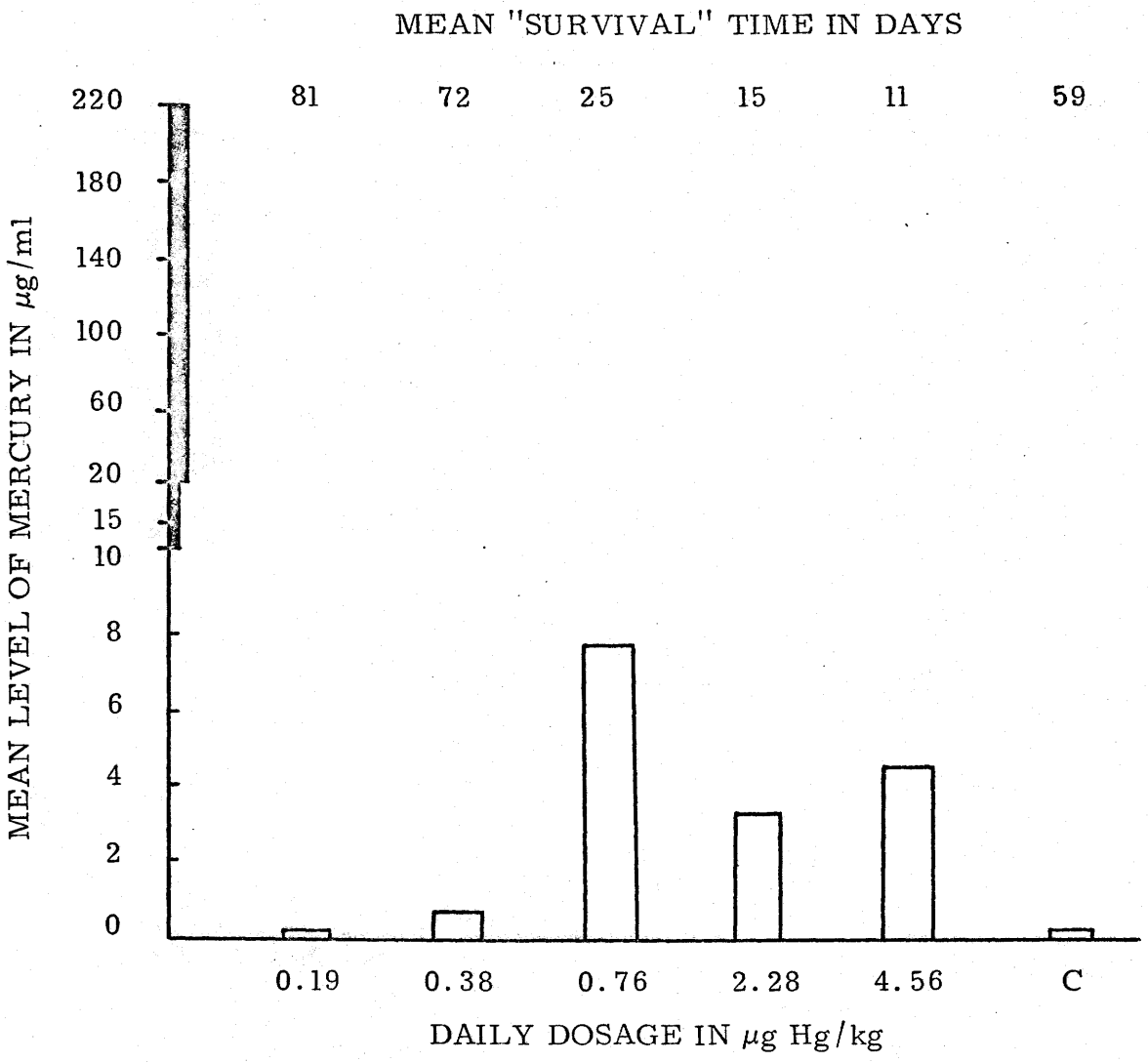


Fig. 79. Mean mercury levels in the urine and mean survival time of groups of pigs receiving increasing dosages of EMC.

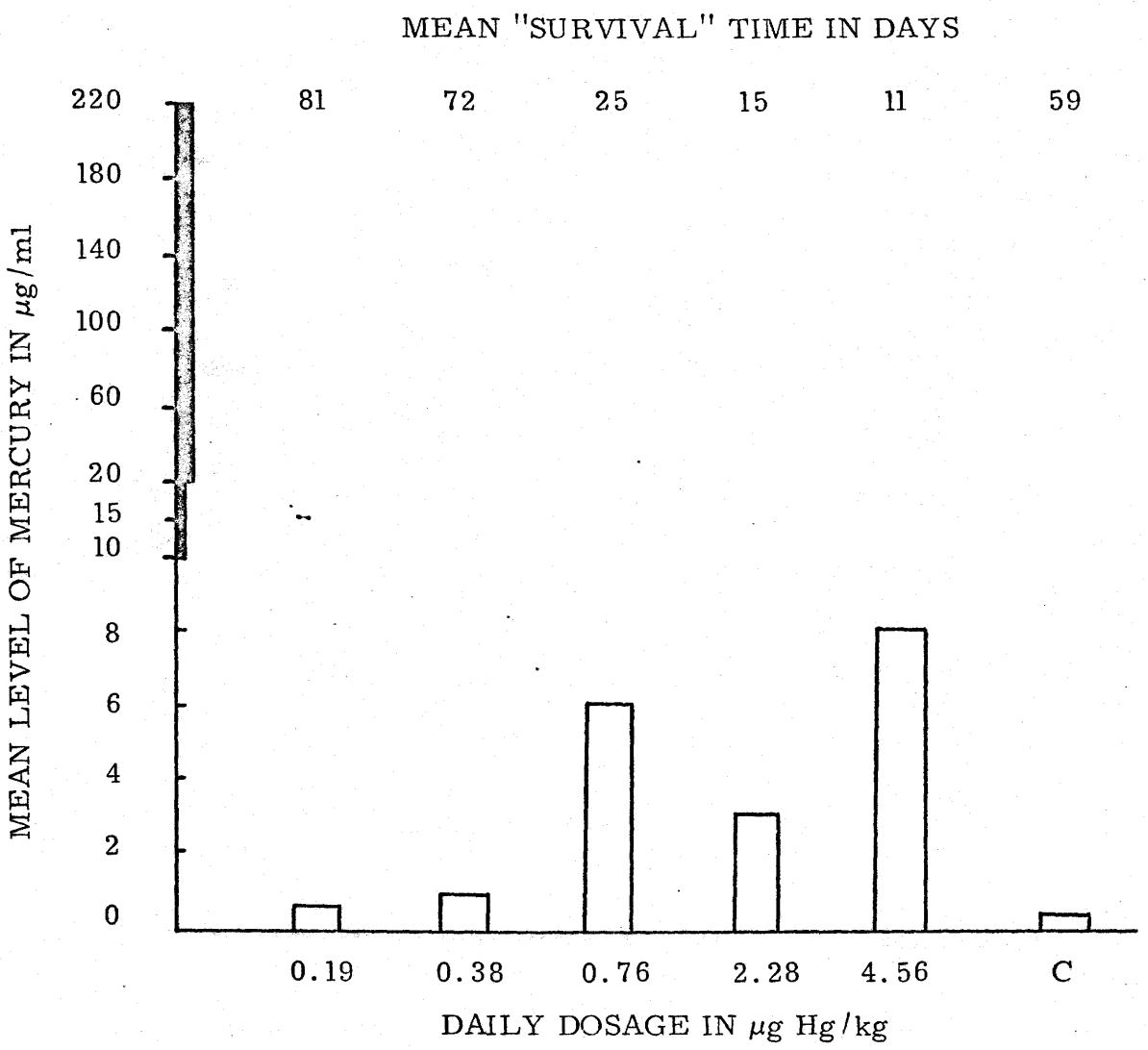


Fig. 80. Mean mercury levels in the bile and mean survival time of groups of pigs receiving increasing dosages of EMC.

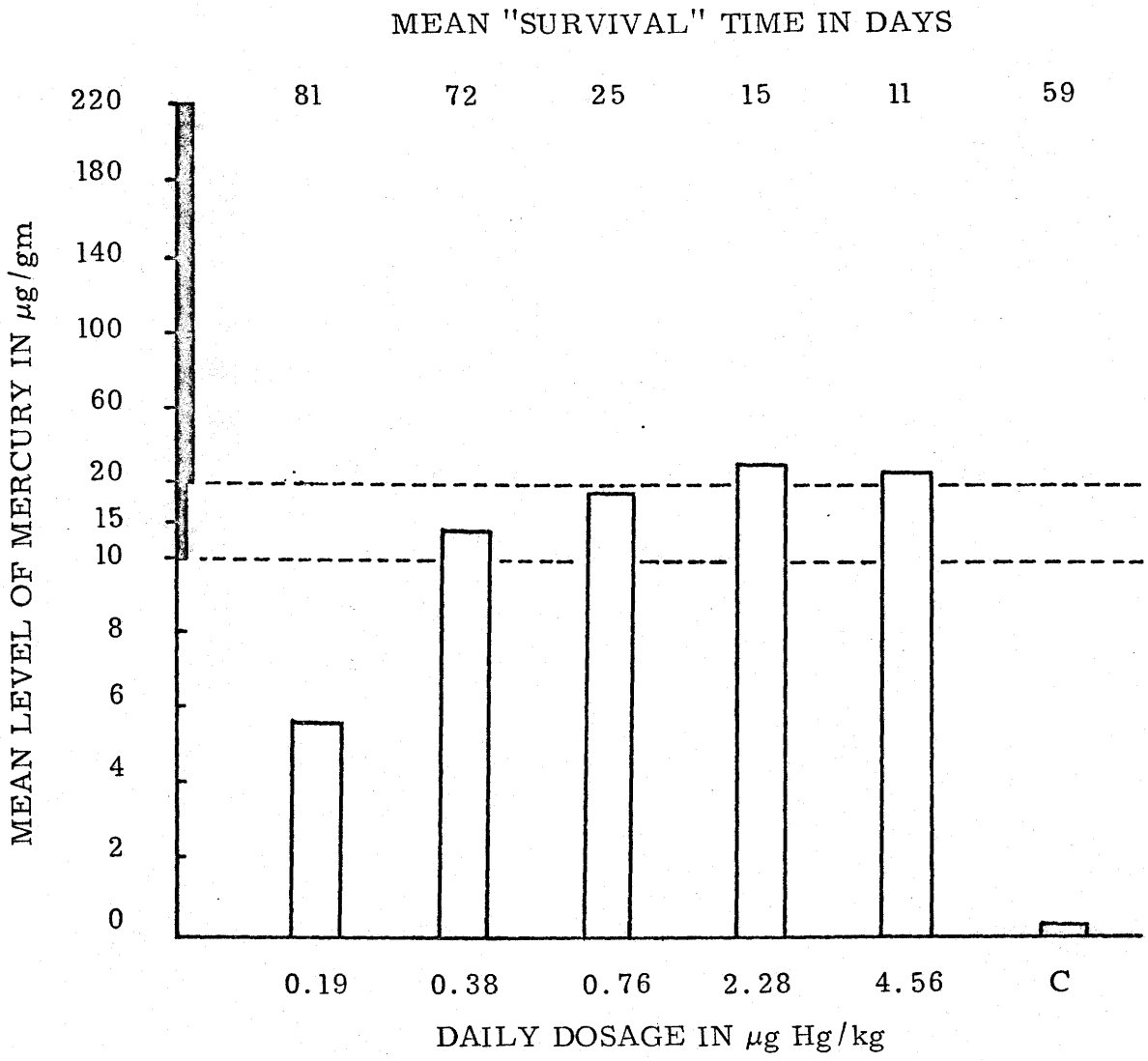


Fig. 81. Mean mercury levels in the brains and mean survival time of groups of pigs receiving increasing dosages of EMC.

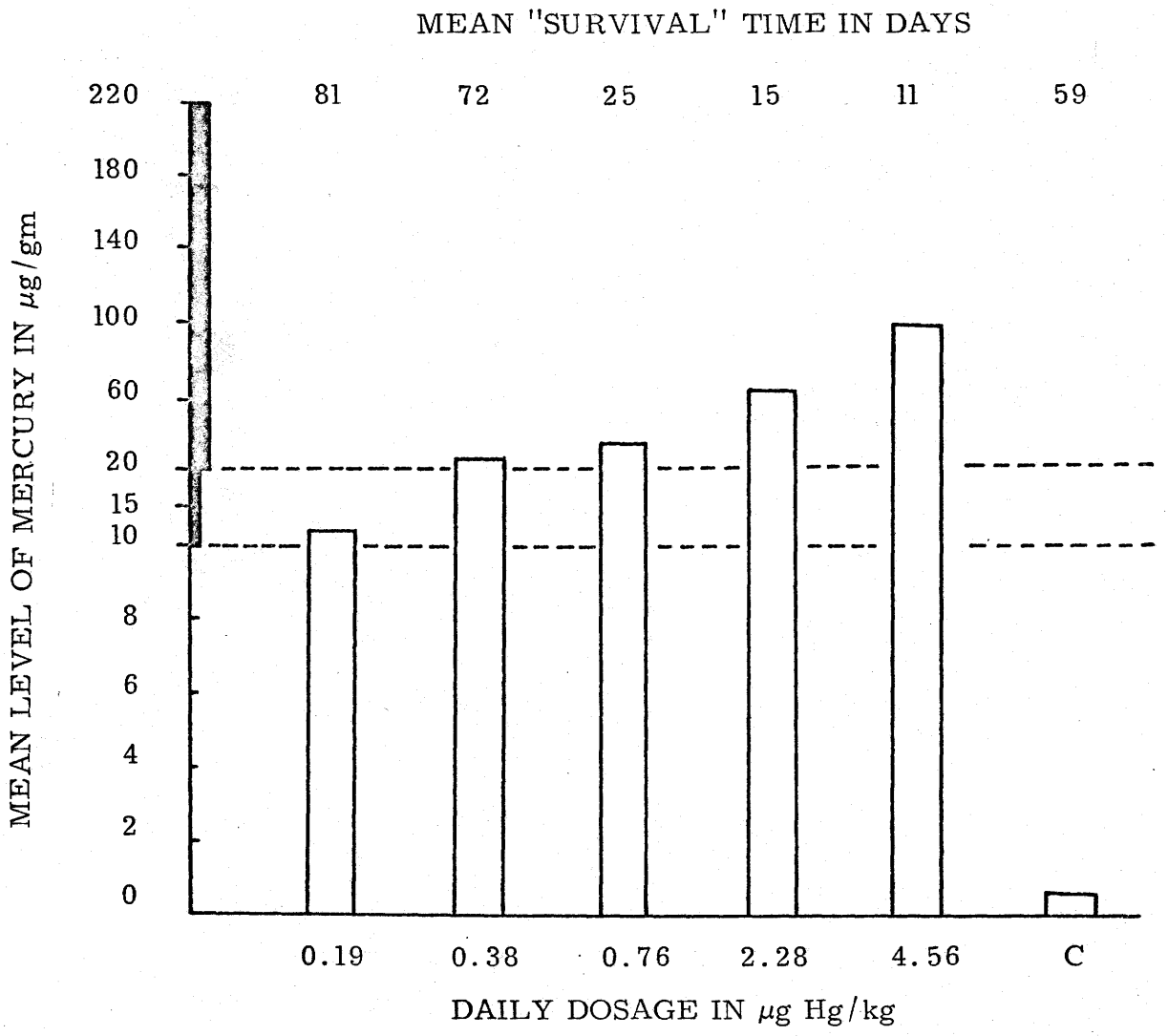


Fig. 82. Mean mercury levels in the livers and mean survival time of groups of pigs receiving increasing dosages of EMC.

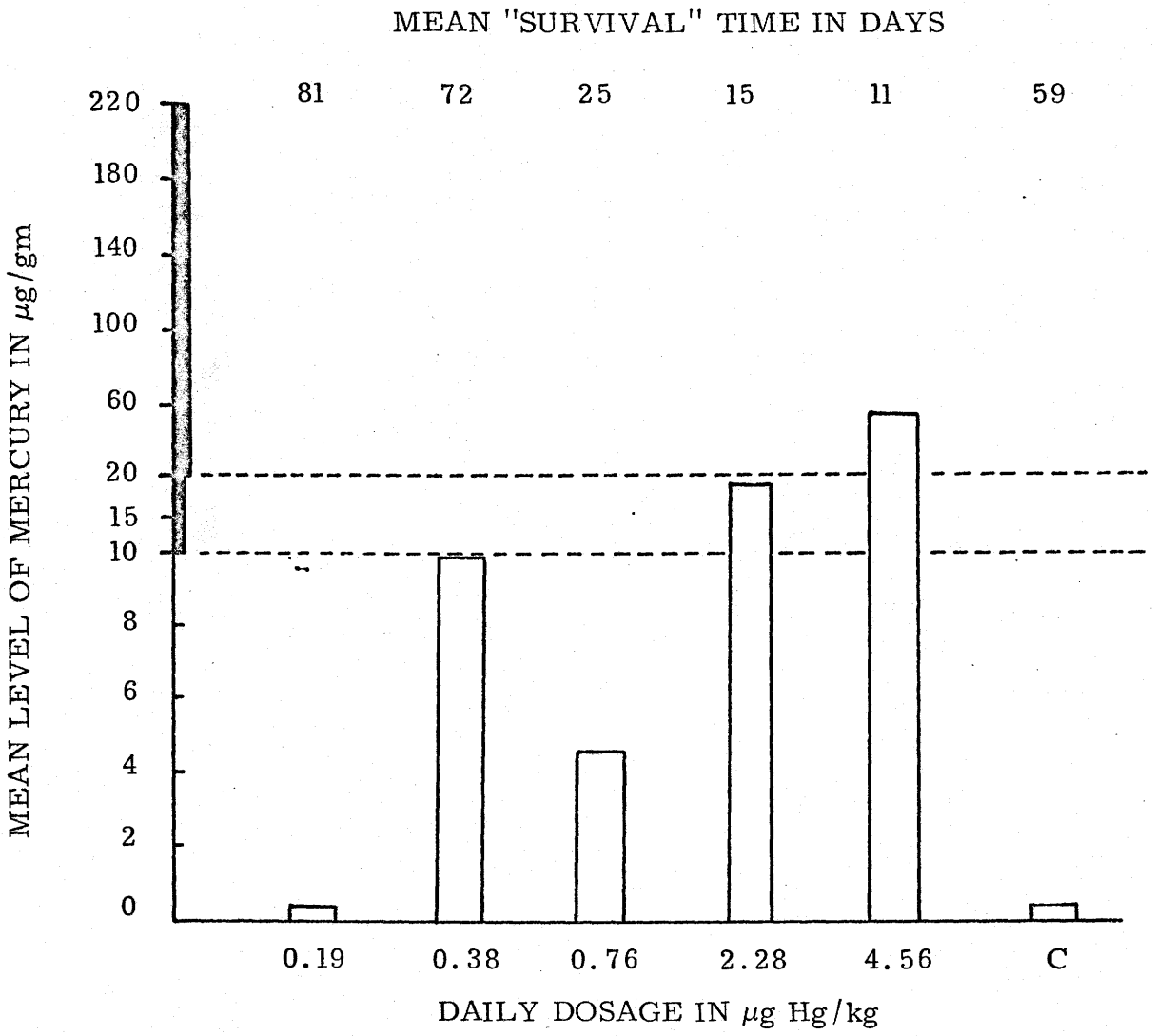


Fig. 83. Mean mercury levels in the large intestine and mean survival time of groups of pigs receiving increasing dosages of EMC.

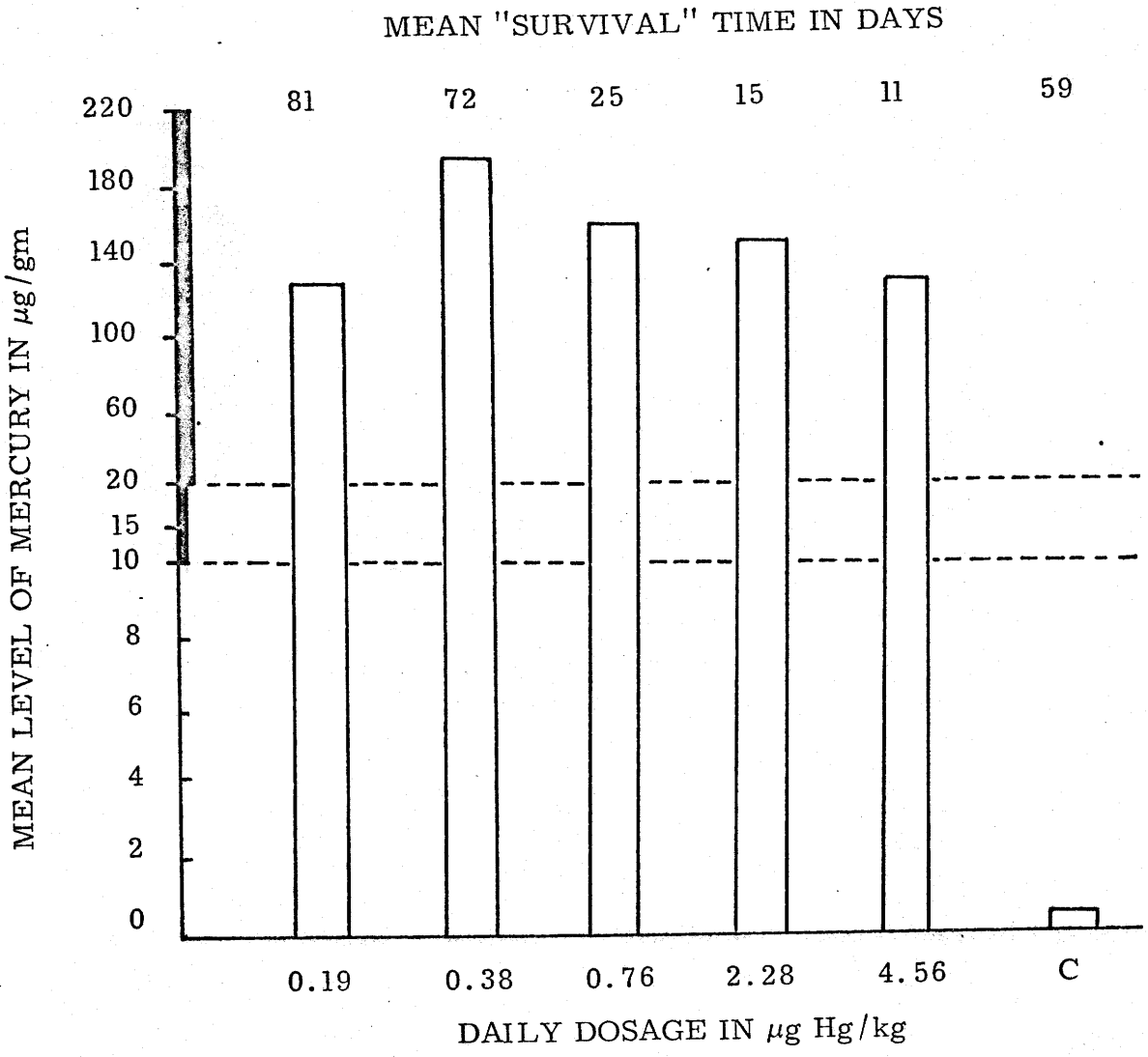


Fig. 84. Mean mercury levels in the kidneys and mean survival time of groups of pigs receiving increasing dosages of EMC.

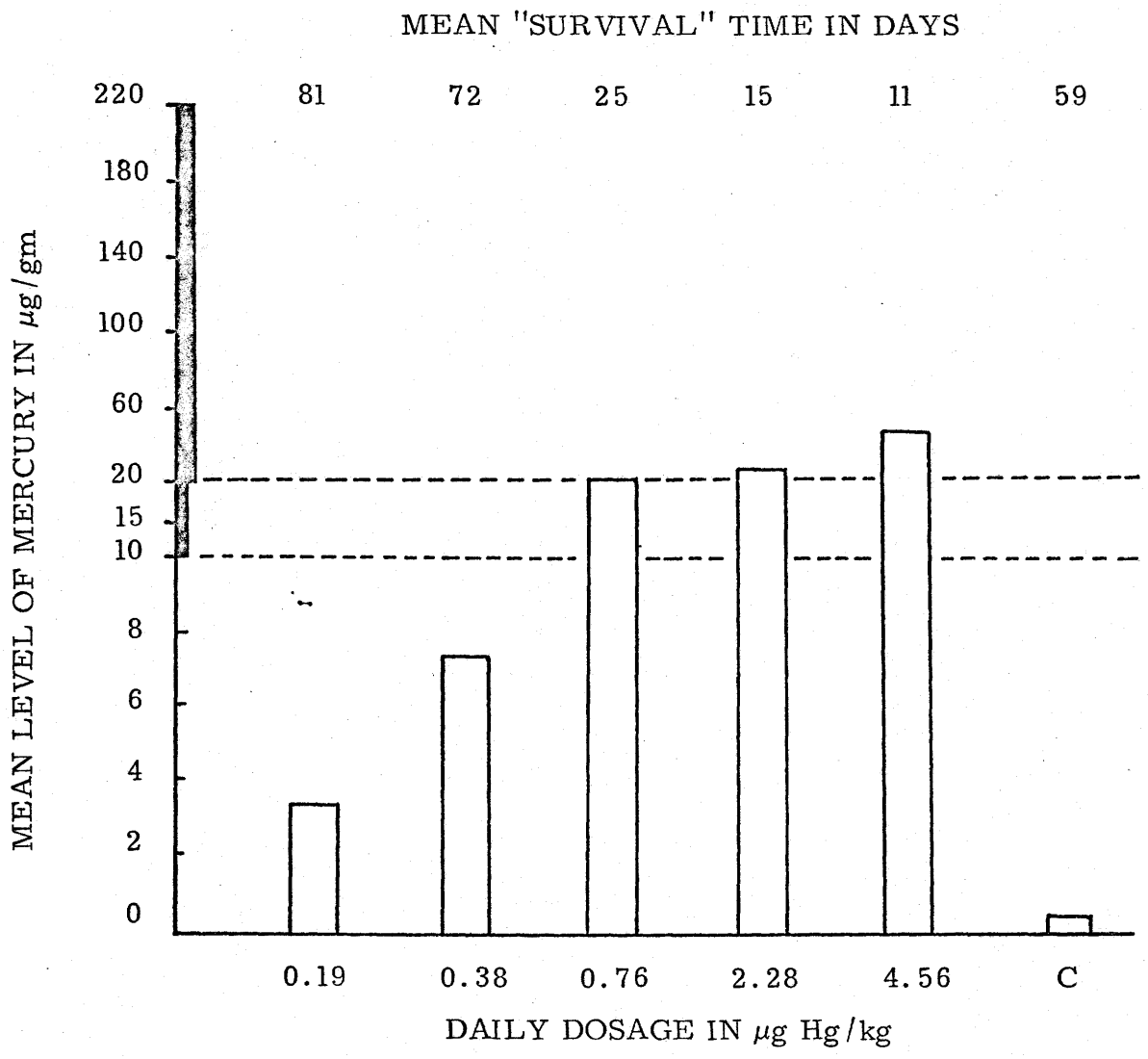


Fig. 85. Mean mercury levels in the muscle and mean survival time of groups of pigs receiving increasing dosages of EMC.

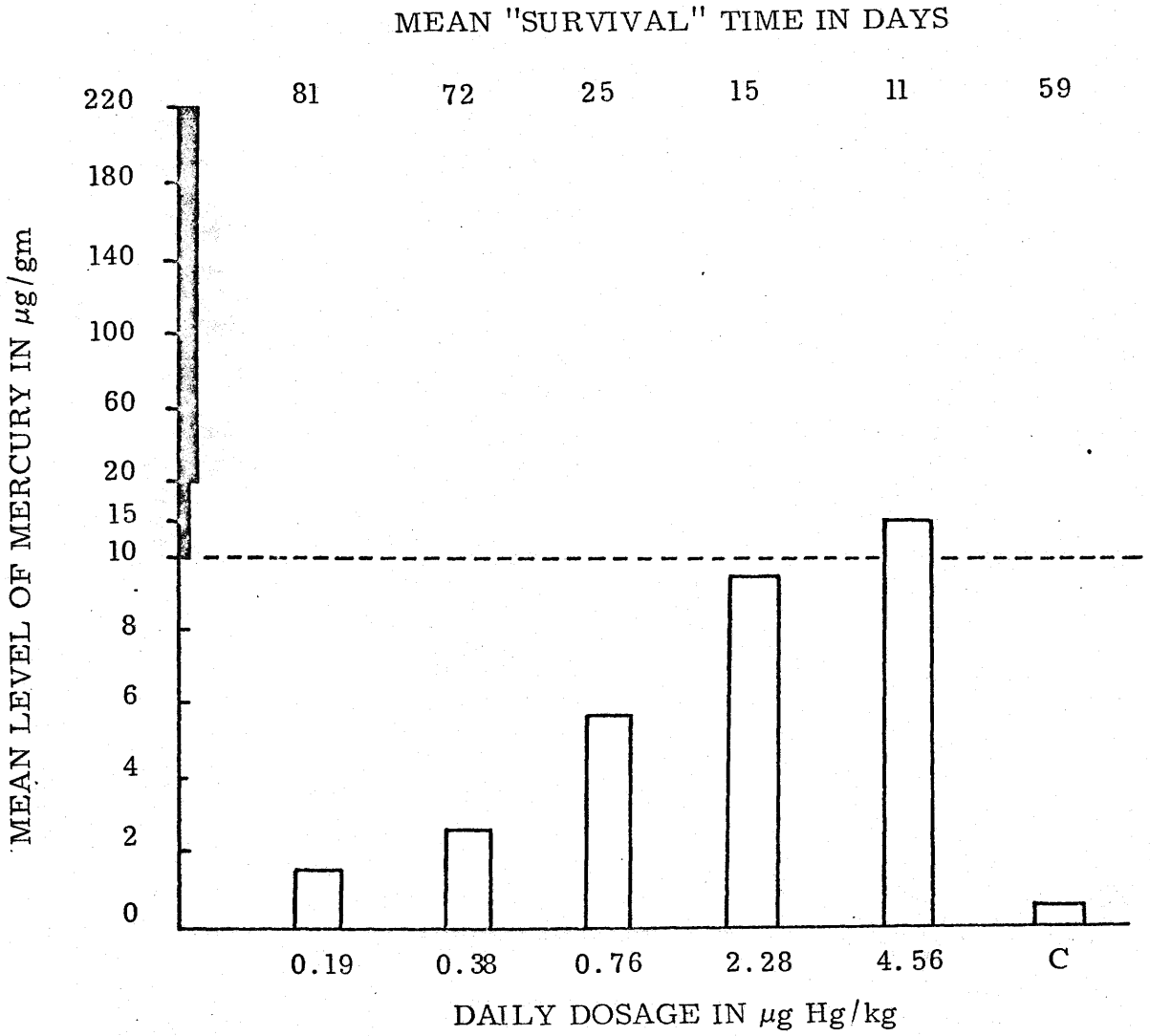


Fig. 86. Mean mercury level in the skin and mean survival time of groups of pigs receiving increasing dosages of EMC.

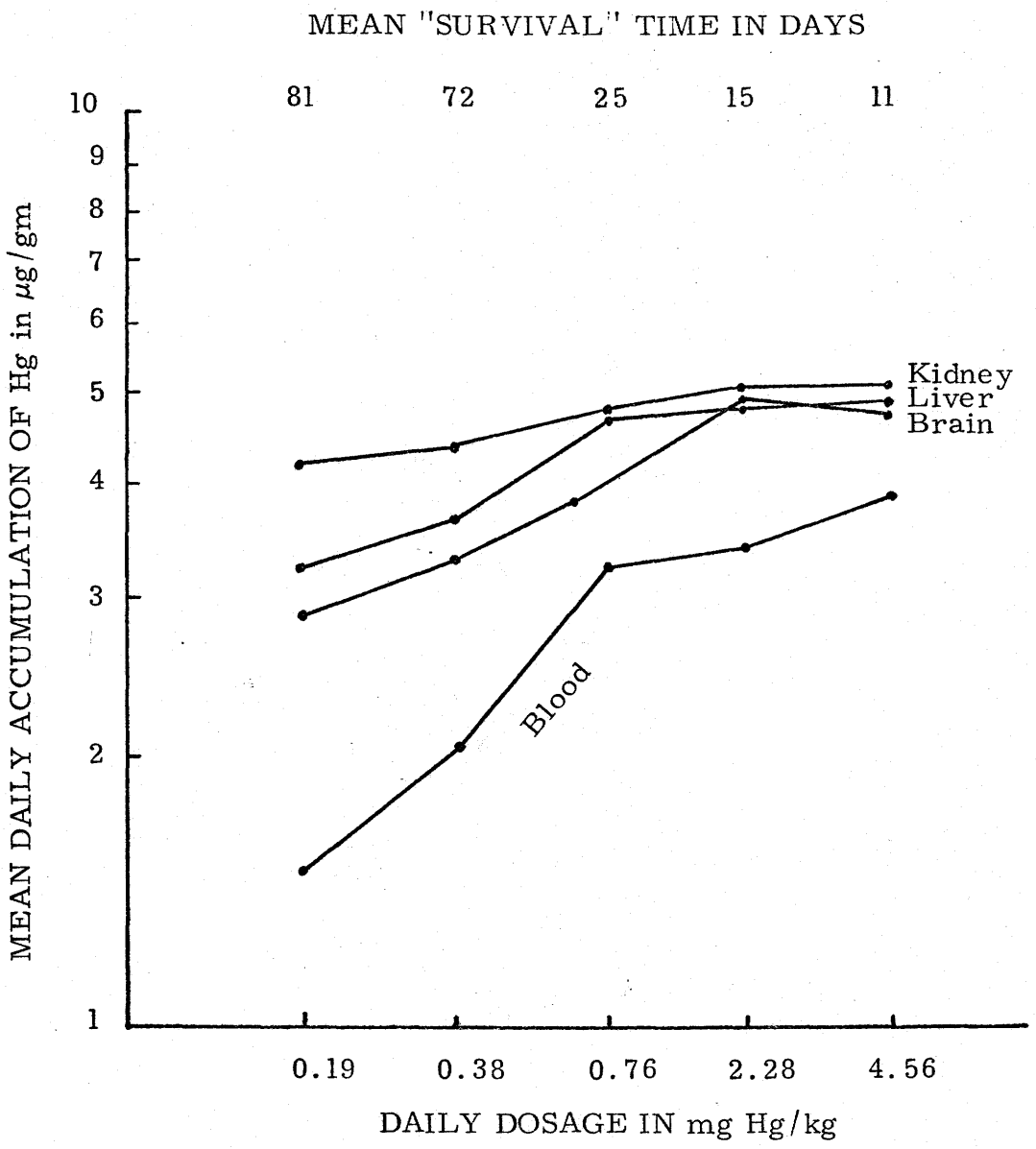


Fig. 87. Mean retention rates for kidney, liver, brain and blood in animals receiving EMC, shown on the semi-logarithmic scale.

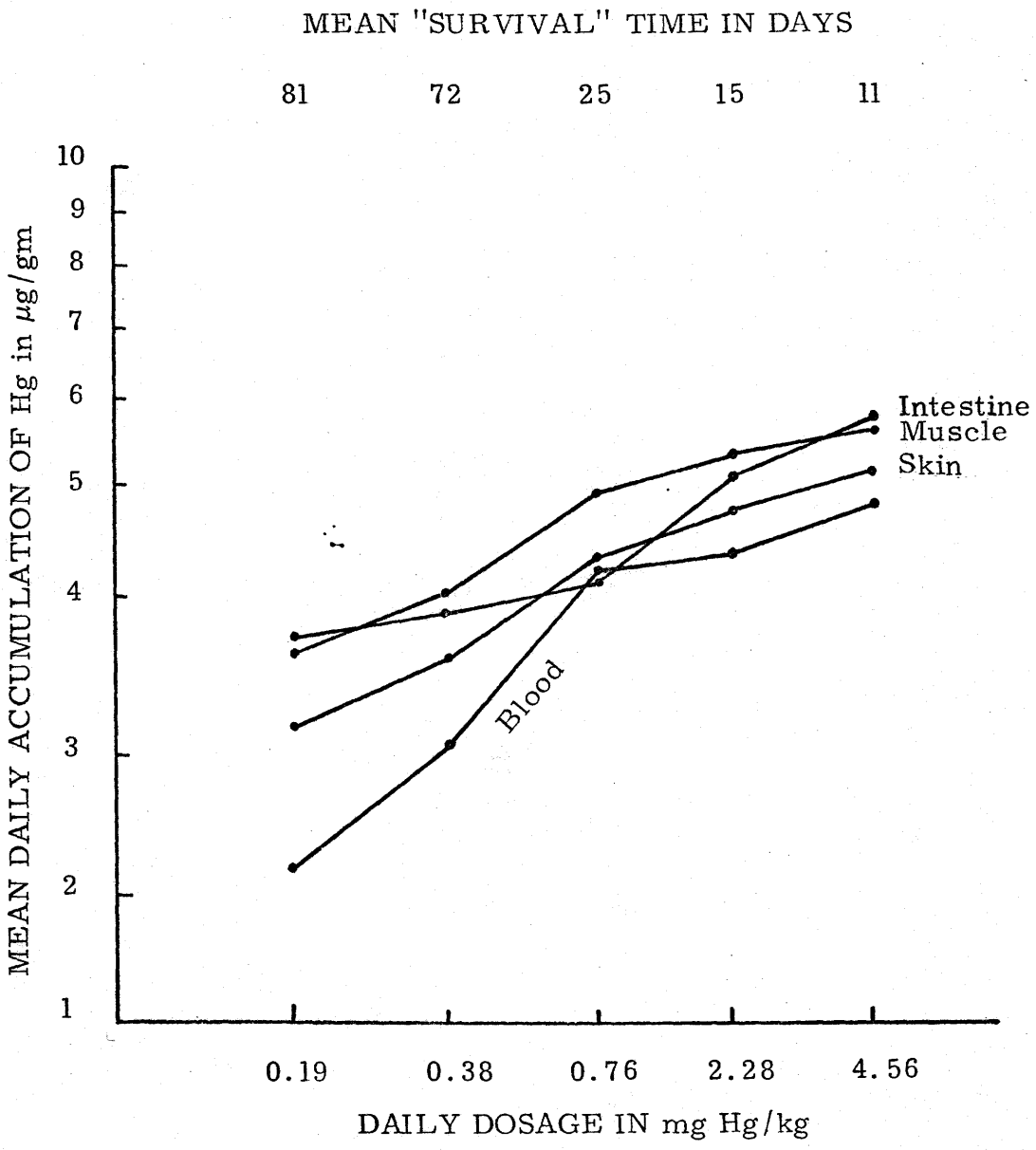


Fig. 88. Mean retention rates for intestine, muscle, skin and blood in animals receiving EMC, shown on semi-logarithmic scale.

Discussion

Ethylmercuric chloride (EMC) is poisonous to swine. Daily dosage levels ranging from 0.38 to 4.56 mg. Hg/kg. as EMC give rise to clinicopathological manifestations within a period of 90 days. Depending on the daily dosage, a disease can be produced, the course of which may be chronic, subacute and, presumably, acute; although the acute manifestations were not produced at the dosage range studied.

Anatomicopathologically, the target organs of EMC poisoning in swine are the nervous, urinary and digestive systems. Clinically, there are signs of neurologic and enteric injury.

Clinical manifestations: Depressed growth and weight loss were constant signs of organomercurial poisoning induced with ethylmercuric chloride (EMC). This probably is, to a large extent, the result of dysphagia, anorexia and diarrhea. Animals with anorexia would masticate,in an indifferent fashion, feed placed in their mouths. Dysphagia and anorexia are considered to be caused by either or both of the following - pharyngitis and injury to the central nervous system. Pharyngitis is considered to be the result of a direct local irritant effect of EMC (Whitehead, 1965). This is suggested by the fact that animals which chewed the pill containing EMC developed pharyngeal irritation within 24 hours and also by the fact that pharyngitis was a more consistent sign in animals receiving the higher dosage levels. Weight loss can also be explained by the fact that sick animals preferred to remain recumbent. In some animals with manifestations of

chronic organomercurial poisoning, a severe loss of cortical neurons probably affected their ability to feed properly. Diarrhea was a constant sign in animals receiving the two highest dosage levels (2.28 and 4.56 mg. Hg/kg. daily). This sign correlated well with injury in the musosa of the large intestine.

The length of the asymptomatic period which preceded the development of signs of toxicosis varied inversely with the dose. Presumably, this was a reflection of the time required for toxic levels of Hg to accumulate in the target tissues. The relationship between length of presymptomatic period and daily dosage is shown in Table 16. The analytical data suggest that the rate of mercury accumulation in tissues is proportional to dosage up to a certain limit (Table 15).

It is unknown whether animals in Group I would have developed signs of toxicosis at the level of EMC fed. Pigs 756 and 759 of Group II were asymptomatic, but were found to have microscopic vascular lesions. Therefore, it could be expected that clinical signs would have precipitated within a short period after 90 days. The delay in the onset of signs in Pigs 756 and 759 is probably related to their individual susceptibility and also to the apparent lack of injury severe enough to be incompatible with a state of compensation. The brain of both man and animal is known for its capacity to compensate for lost parenchyma (Glees and Cole, 1950; Bard and Macht, 1958; Adrian, 1947; Dusser de Barenne, 1934; Innes and Saunders, 1962; Swensson, 1952; and Greenfield, 1963).

The following signs of toxicosis could result from pathological changes in the nervous and/or the skeletal system: incoordination of the hind quarters, knuckling of the fetlocks, loss of balance, falling and inability to regain posture. The histopathological evidence supports the view that changes induced in the nervous system are primarily responsible for the signs.

Ambulatory disturbances were most evident in animals receiving relatively high doses of EMC. This finding correlated well with the distribution of lesions and the degree of injury in the various segments of the cerebrospinal axis. Animals receiving the high doses had correspondingly more severe lesions in the dorsal root ganglia and the gray matter of the spinal cord. In contrast, animals with brain lesions characterized by severe loss of neurons in the cortex and, to a lesser degree, in the subcortical nuclei, exhibited blindness, muscle tremor, compulsive walking and paretic phenomena. The pathological findings suggest that an impairment of the statokinetic reflexes is responsible for the neurological disturbance. It is suspected that high doses, by injuring the sensory ganglia, affect the afferent route, while low levels, with their effect on the upper center, affect the efferent route.

The blindness observed is considered to be of central origin. Lesions were found along the central portion of the visual pathways, regardless of dosage and the course of the disease. Extensive neuronal necrosis in the lateral geniculate bodies of animals receiving high dosage levels and, in most of the occipital

cerebral cortices of animals receiving low dosage levels, could account for blindness in either case.

Pathology: Toxic levels of ethylmercuric chloride (EMC), administered orally, cause lesions in the nervous, digestive and urinary systems. The daily dosage and the length of administration period were found to be important determinants of the level of mercury accumulation, the rate of accumulation, and the extent, distribution, nature and severity of lesions induced.

TABLE 16. Daily dosage level and length of presymptomatic and survival periods in animals receiving EMC.

Group	Dose mg. Hg/ kg. /day	Clinical Signs	Onset (Day)	Death (Day)	Lesions
I	0.19	-	--	90 (killed)	-
II	0.38	+	49	76 (killed)	+
III	0.76	+	22	30 (killed)	+
IV	2.28	+	12	18 (killed)	+
V	4.56	+	6	12 (killed)	+
Control	----	-	--	90 (killed)	-

Nervous system. -- An attempt was made to study the morphogenesis of brain lesions by killing and studying animals with early and terminal clinical signs of toxicosis. In general, this approach failed to yield significant information. The course of the disease was quite short and compensation for lost tissue could have prevented the early appearance of clinical signs.

The lesions induced in this experiment by the toxic dosage range (0.38 - 4.56 mg. Hg/kg. daily as EMC) may be classified as subacute and chronic. The upper dosage levels (2.28 and 4.56 mg. Hg/kg. daily as EMC) induced subacute lesions and the lower toxic levels (0.38 and 0.76 mg. Hg/kg. daily as EMC) induced lesions of a chronic nature.

The direct neurotoxic effect of mercury is demonstrated by the nature of the subacute lesions which consisted mainly of neuronal degeneration and/or necrosis. In animals with such subacute lesions, the distribution of degenerate and/or necrotic neurons was random except in the cerebral cortex where it was limited to the 3rd, 4th and 5th layers. The blood vessels throughout the nervous system were unremarkable. The rate of mercury accumulation in the high dosage level groups is rapid and is probably a decisive factor in the causation of lesions. It would appear that neurons with peculiar metabolic requirements are the first to become affected.

In the subacute process, the glia did not undergo necrosis, but rather were stimulated to proliferate locally. The degree of glial response to neuronal necrosis was much less than that observed with methylmercuric dicyandiamide (Experiment I). This probably is explained by the fact that, in the latter case, the two upper groups

(IV and V) received lower mercury levels per kilogram daily and the course of the disease resulting from the lower dosages was more protracted and, therefore, allowed more time for glial reaction.

In the chronic form of toxicosis, the direct polioclastic effect of mercury was enhanced by the secondary degenerative changes in the blood vessels of the brain. The chronic form of EMC poisoning was characterized by: neuronal necrosis associated with severe micro- and astrogliosis and capillary endothelial proliferation; and degenerative arteriopathy occurring mainly in the sulci of the injured cortex but, occasionally, affecting also the subcortical nuclei. The net effect of chronic EMC poisoning is atrophy of the cerebrum. The polioclastic effect of EMC is manifest through the rest of the cerebrospinal axis, but is comparatively very limited.

This experiment has established that there is a relationship between daily dosage level and nature, and distribution of lesions (Appendix C, Tables 1 and 2). It has also established that high dosage levels lead to high accumulation rates (Figs. 87 and 88).

The vascular lesions observed in the leptomeningeal arteries of the low level group (757 and 758) have a certain similarity to those seen in hypertensive states in man. They were always associated with neuronal necrosis, gliosis and capillary endothelial cell proliferation in the adjacent cerebral cortex. It is an accepted fact that glia proliferate in response to neuronal death and myelin breakdown (Greenfield, 1963). Varying degrees

of capillary endothelial cell proliferation have also been reported to occur in conditions associated with neuronal death (Biggart, 1957; Greenfield, 1963; Jubb and Kennedy, 1963; Smith, 1955, 1957; and Little, 1967). The explanation of the hypertensive-like changes in the meningeal arterial tree could depend on the presence of local circulatory disturbances.

Proliferation of the capillary endothelial cells without formation of new capillaries is believed to lead to increased resistance to blood flow. It will also lead to anoxic conditions in the affected tissue if other factors, such as collateral circulation and increased blood pressure, are not able to compensate.

The degenerative and proliferative changes seen in the arterioles are considered secondary to the primary neuronal injury, since this process always precedes the development of the vascular lesions. The vascular lesions will secondarily affect the blood flow by disturbing the regulatory effect of the vessel and impinging on the lumen. These changes will superimpose factors which intensify the tissue anoxia. A vicious, self-perpetuating cycle, resulting in more tissue destruction, is thought to result. The ability of the vasculature of the cortex to compensate for the interference with blood flow is limited anatomically because of a very poor collateral circulation in the area. Arterioles penetrating the cortex do not anatomose freely (Ransom and Clark, 1959).

A disease of man, spinal thrombophlebitis, provides some analogies which might be useful in assessing the pathogenesis of the arterial vascular lesion in poisoned swine. Occlusion of the venous channels leads to increased resistance to blood flow

because the venous anastomoses are not adequate to correct the situation (Greenfield, 1963). If this situation persists, hyaline degeneration occurs in the arterioles and varying degrees of necrosis are seen in the gray matter of the spinal cord.

This disease is similar to the chronic form of EMC toxicosis where degenerative arteriopathy is thought to occur secondarily to capillary endothelial proliferation, which increases resistance to blood flow.

The pathological findings reported in this experiment suggest that dosage level, survival time, tissue mercury level and animal susceptibility play an important role in the determination of lesions in the central nervous system.

The importance of the individual animal's susceptibility to organomercurial poisoning became apparent in Group II where only two of the animals developed severe lesions within the experimental period studied (90 days). The other two animals developed lesions which remained clinically silent.

The length of the survival following injury, i.e. neuronal necrosis, is a significant factor in the evolution of lesions. The course of toxicosis in pigs receiving high dosage levels of EMC was comparatively much more accelerated than in those receiving lower, but toxic, doses. Reference to Table 16 will reveal that for dosage levels 0.38, 0.76, 2.28 and 4.56 mg. Hg/kg. daily the length of clinical illness was 27, 8, 6 and 6 days respectively. Animals whose disease had a protracted course had lesions extending to the larger branches of the meningeal arterial system.

Assuming that the direct toxic effect is manifested by neuronal degeneration and necrosis, it follows that, in animals with a protracted disease, there was enough time for secondary lesions to develop.

The levels of tissue mercury determine whether or not injury occurs. It was observed that high levels of mercury were always associated with lesions. It was found that levels above 13 μg. /gm. of cerebral cortex were always associated with lesions (Table 15). The nature of the lesion induced appears to be affected more significantly by the rate of accumulation rather than by the total amount accumulated. It was seen that, while chronic lesions were associated with a daily mercury accumulation rate of about 0.20 μg. /gm., the subacute lesions were associated with a much higher rate (2.52 μg. Hg/gm.). Since the rate of mercury accumulation in the brain depends directly upon the dosage level, the higher dosage will produce a toxic level sooner.

Daily administration of 0.19 mg. Hg/kg. for a period of 90 days failed to cause lesions in the nervous system of pigs studied. This suggests that the levels of mercury accumulated and the rate of accumulation in the brains of these animals were not above the critical level for injury. It remains unknown if longer exposure at this level would lead to toxicosis. The basic factors which resulted in sub-toxic levels could be either one of two possibilities. Either the dose rate was so low that the accumulation of Hg was equal to, or less than, the excretion rate or the accumulation of Hg was progressive in the brain tissue, but at a rate so low that the toxic levels were not reached within the time period studied.

The neuronal necrosis in the thalamus, midbrain, pons and medulla oblongata, spinal cord and dorsal root ganglia were considered to be the result of a direct injury by the mercurial. The fact that necrotic neurons are randomly distributed is hard to reconcile with any primary vascular mechanism of injury. The group of pigs receiving the highest dosage (4.56 mg. Hg/kg. daily as EMC) had the most extensive lesions in these same structures; a fact that also argues for direct injury. The possibility that neurons which have the most fastidious metabolic requirements are more susceptible to injury by toxic factors is suspected.

It has been established that, with time, sustained administration of low mercury levels (0.38 mg. Hg/kg. daily) results in the involvement of larger branches of the meningeal arterial network. This might explain the malacic foci found in the basoganglia of Pigs 757 and 758. These lesions can be explained as being the result of occlusive vascular injury. It was noticed that necrotic foci were associated with vessels whose walls were affected by a degenerative process.

Digestive system. -- Both liver and colon appear to be target organs in organomercurial poisoning in swine induced with EMC.

Previous studies have shown that mercuric compounds are readily absorbed by the gastrointestinal tract (Miller et al., 1961). Hence, injury to the liver may be reasonable to expect. Morphologic manifestations of liver injury by toxic substances usually consists of degenerative processes which may include fatty and hydropic degeneration and/or necrosis (Boyd, 1961;

Dunne, 1964; Jubb and Kennedy, 1963; Montroni, 1949; and Robbins, 1967). Most of these authors agree that the effect of a toxin depends both on the dose and the time of exposure. The present findings support this view. At high dosage levels, necrosis of individual hepatocytes occurred. Lower dosage levels caused diffuse hydropic degeneration which was associated with necrosis in randomly distributed hepatocytes. This difference may be attributable to the effect of sustained undernourishment.

The analytical data presented in Table 15 suggest that daily dosage levels above 0.38 mg. Hg/kg. lead to accumulation of mercury in the liver parenchyma in amounts injurious to the hepatocyte.

Intestinal lesions. -- The excretion of mercury via the intestinal mucosa following administration of alkyl mercury compounds has been established previously (Swensson et al., 1959 and Miller et al., 1961). In this experiment, mercury levels in the wall of the large intestine varied directly as the dosage and correlated with development of lesions. Gross lesions were found in Groups IV and V, while only microscopic lesions were present in Group III. These findings suggest that accumulation of mercury in the colonic mucosa leads to injury of that tissue and it appears that the higher the level of such accumulation, the more severe the lesion induced.

The effect of EMC on the submucosal and mesocolonic vasculature is difficult to explain. The presence of edema in the latter region suggests increased vascular permeability.

Also difficult to rationalize are the hypertensive-like lesions observed in arteries of the submucosa. Histological examination of the large intestine was limited only to one or a few sections. This did not provide sufficient data to allow reconstruction of the pathological changes at that level and, therefore, formulation of constructive conclusions.

Urinary system. -- The review of the literature has shown that mercury is excreted via the urinary system (Swensson, 1959; Miller et al., 1961; Brown et al., 1967). Another established fact is that the kidney accumulates large amounts of mercury (Berlin, 1963; Platonow, 1968; Berlin and Ullberg, 1965). Berlin (1963) and Platonow (1968) have shown that the cortex is the preferred site for this. The pathological changes caused by mercury in the kidneys have been studied extensively. Dosage and chemical form of the compound employed appear to be the main determinants of the pathological character of the lesions. In this experiment, the influence of dosage level also was evident by the fact that renal lesions were found only in animals receiving 0.38 mg. Hg/kg. daily and above. Such lesions were, as with methylmercuric dicyandiamide (MMD), limited to the proximal convoluted tubules. The character of the lesions, however, was more severe with EMC than with MMD, and the severity increased with dosage. There were hydropic degeneration, epithelial cell necrosis, desquamation of necrotic cells, and proteinaceous casts in the tubular lumina. These findings suggest that the dosage range

of EMC employed in this experiment causes moderate lesions in the kidneys. Reference to Table 15 and Figure 87 will support the contention that renal lesions are as much related to the rate of mercury uptake by the kidney as to the total amount of mercury accumulated therein.

Summary

Ethylmercuric chloride is highly poisonous to swine if fed at low levels for a prolonged period of time. Subacute and chronic forms of disease were produced at the dosage studied.

The chronic poisoning was manifested by disease of the CNS very similar to that described previously in Experiment I for MMD.

The subacute form of the disease seen in animals receiving higher doses of EMC had, in addition, lesions in the large intestine characterized by edema of the mesocolon associated with degenerative arteriopathy in serosal vessels and pseudomembranous colitis and typhlitis.

Analytic findings indicated that, in EMC poisoning, most tissues had relatively high levels of mercury. Tissue levels of mercury in the dosage range studied indicated a direct relationship to dose.

Aryl Mercurial Poisoning in Swine

Studies on the pathology of phenylmercuric chloride Poisoning

In this experiment, the clinical and pathological findings will be presented separately for each dosage level.

The essential groups are described in Tables 17 and 18. The dosages were selected to range from near toxic to severely toxic levels.

TABLE 17. Experimental groups which received PMC

Group	No. of Pigs	Dose*	Days**
I	5	0.19	90
II	5	0.38	90
III	5	0.76	90
IV	5	2.28	65
V	5	4.56	34
Control	5	----	90

* Mg. Hg/kg. daily administered in the form of PMC.

** Maximum experimental days for this group.

Clinical findings

Group I (0.19 mg. Hg/kg. daily): All animals in this group remained unaffected by this dosage (Table 18).

Group II (0.38 mg. Hg/kg. daily): Clinical signs of toxicosis failed to appear in this group (Table 18).

Group III (0.76 mg. Hg/kg. daily): Moderate depression of growth was the only sign observed in this group. Table 18 outlines the duration of phenylmercuric chloride administration and the failure of the above dosage to cause clinical signs.

Group IV (2.28 mg. Hg/kg. daily): Three of the animals (989, 990, 991) failed to gain properly and suffered an abrupt weight loss during the last days of life. The remaining two pigs (987 and 988) were killed early in the course of the experiment before signs of toxicosis developed (Fig. 89).

Diarrhea was a prominent sign in all three animals which survived an extended period of time. The first clinical indication of an enteric disturbance occurred after the fifteenth experimental day, when the feces became intermittently soft. They were yellow, had a penetrating foul odor and contained fibrin strands. In time, they became permanently soft. The course of the disease was protracted. The duration varied from 20 (991) to 32 (990) days. Terminally, the animals were in lateral recumbency, weak, and in a semicomatose state. An unpleasant odor emanated from sick animals. Shivering was observed during the last two or three days.

One pig (987) exhibited petechial hemorrhages throughout the skin eight days after commencement of the experiment and had fibrin plugs in both nostrils. This caused the animal to breathe through its mouth.

Group V (4.56 mg. Hg/kg. daily): Only two pigs (982 and 986) were kept alive until the terminal stages of toxicosis developed.

Weight loss (Fig. 90) appeared early and was associated with diarrhea and inappetence.

Diarrhea was pronounced by the 10th day. Initially, it was intermittent. In this stage, the feces were pasty and they became progressively more fluid later in the course of the toxicosis. The feces were yellow, foul smelling and contained variable amounts of fibrin strands. In the terminal stages, all feces were very fluid.

Sick animals were reluctant to move about. In time, they became weak to the point where they were unable to stand. This stage was followed by lethargy, prostration and lateral recumbency.

The BUN value obtained for Pig No. 986 was found to be 40 mgm.% after 23 days of mercury administration. Terminally, the value exceeded 140 mgm.% (Fig. 91).

Controls: A hemorrhagic diathesis occurred in two pigs. This was similar to Pig No. 987 in Group IV. No enteric disturbance was noted in this group.

TABLE 18. Daily Dosage Rate, Treatment Period and Clinico-pathological Results For Each Animal Receiving Phenylmercuric Chloride

Group	Dosage mg. Hg/kg. daily	Animal	Days	Signs	Lesions
I	0.19	952	14	-	-
		953	39	-	-
		954	90	-	-
		955	90	-	-
		956	90	-	-
II	0.38	996	39	-	-
		997	90	-	-
		998	90	-	-
		999	90	-	-
		1000	90	-	-
III	0.76	951	90	-	-
		992	14	-	-
		993	90	-	-
		994	90	-	-
		995	50	-	-
IV	2.28	987	15	-	+
		988	4	-	+
		989	49	+	+
		990	63	+	+
		991	35	+	+
V	4.56	982	35	+	+
		983	6	-	-
		984	1	-	-
		985	3	-	-
		986	35	+	+
Control	----	977	14	-	-
		978	90	-	-
		979	90	-	-
		980	90	-	-
		981	70	-	-

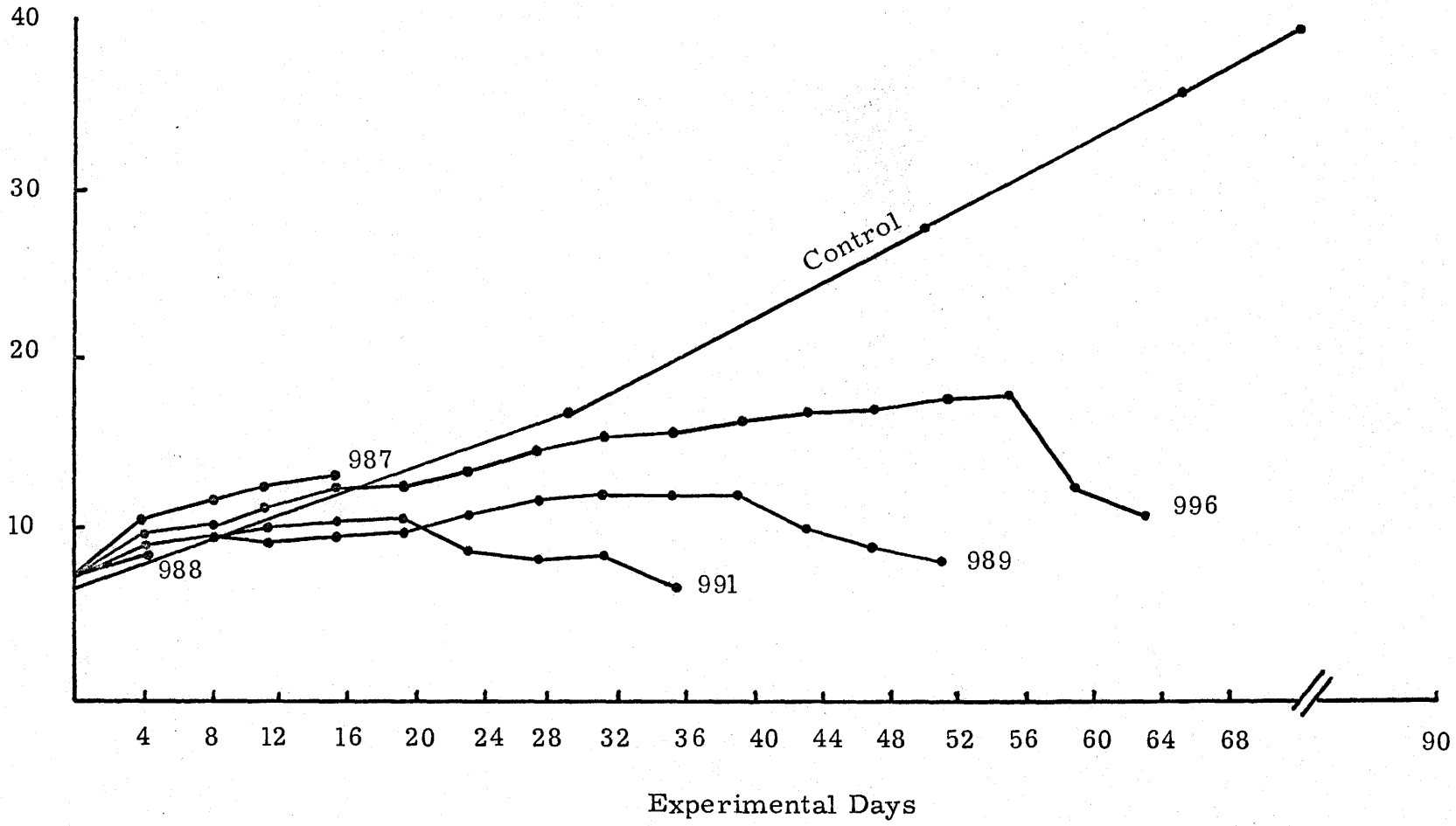


Fig. 89. Group IV. Weight loss was more pronounced in animals which developed clinical signs of poisoning.

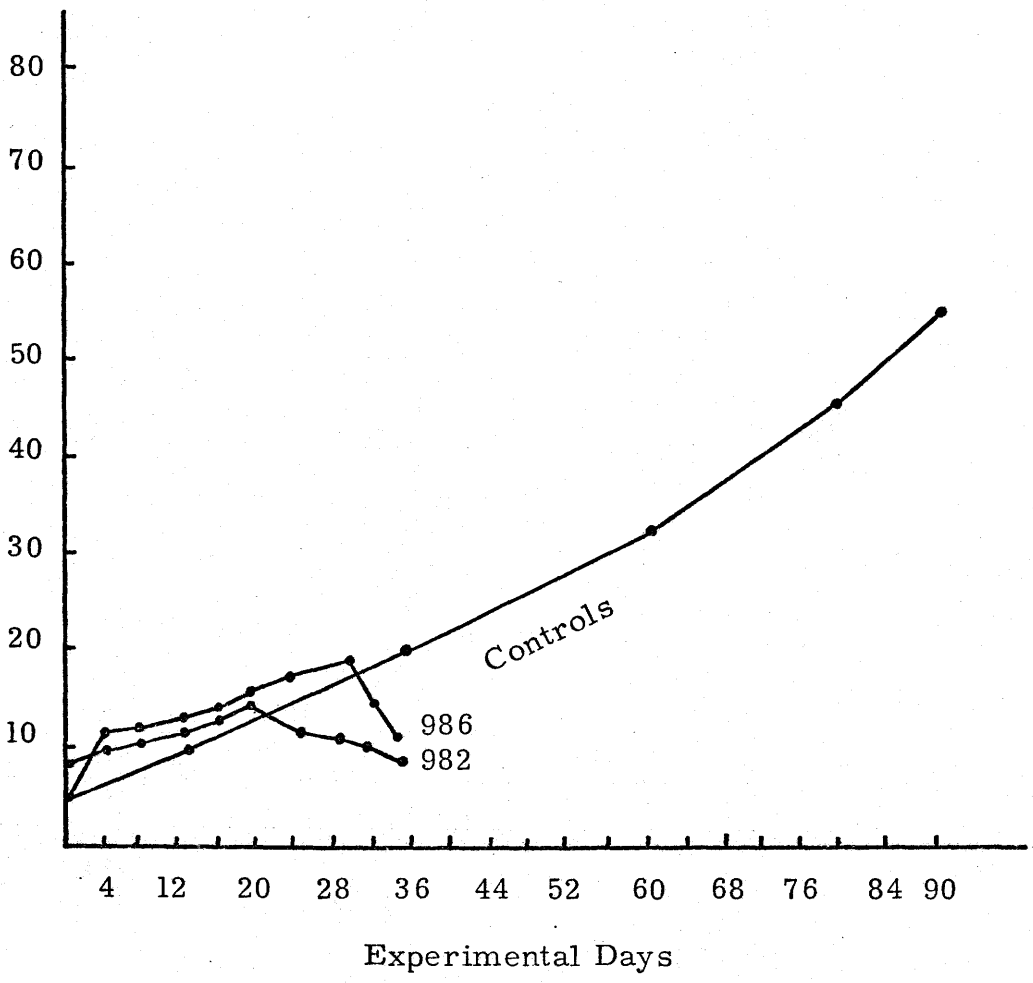


Fig. 90. Body weights of pigs 982, 986 and controls during the course of the experiment.

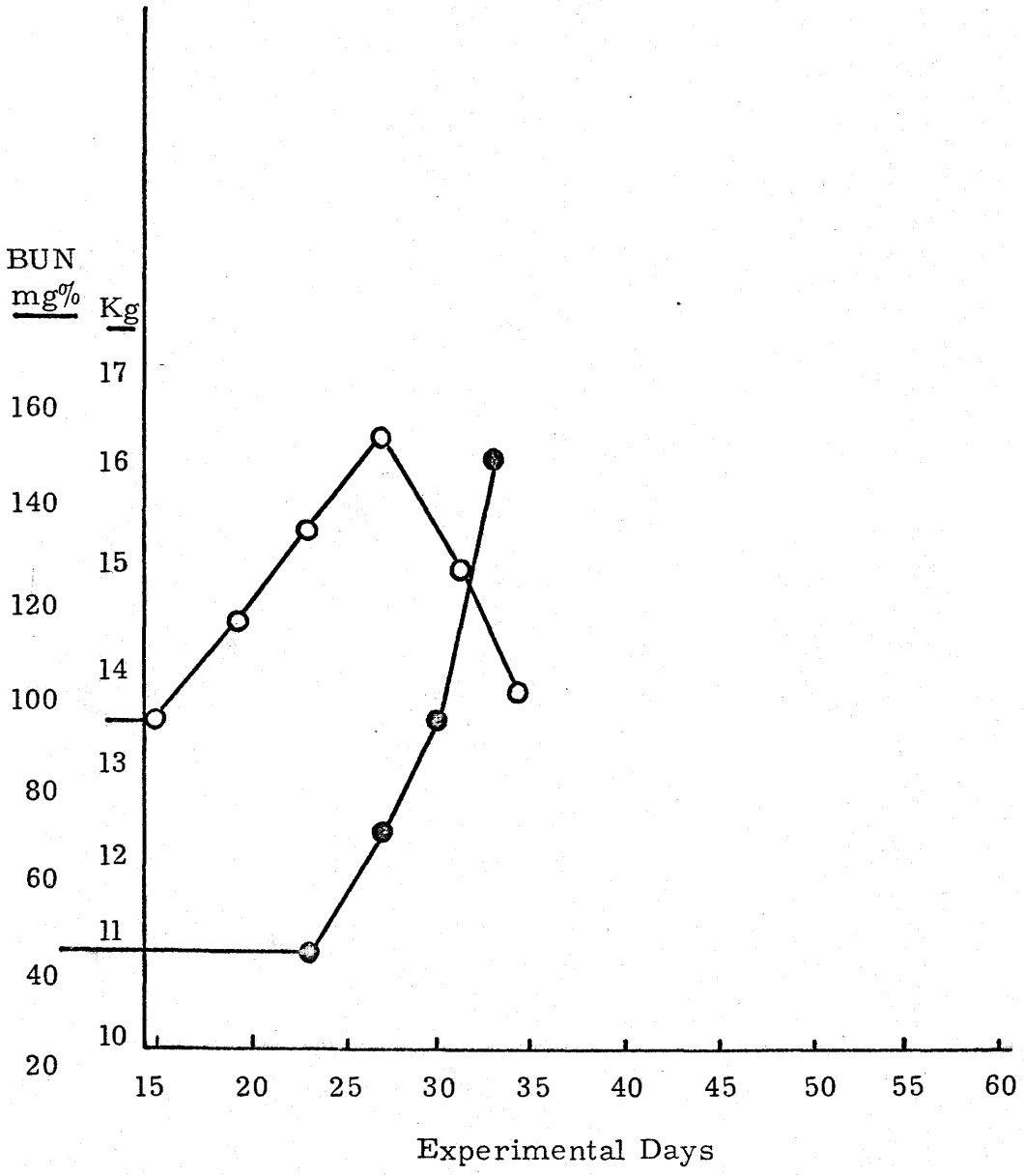


Fig. 91. Pig No. 986. BUN values in a pig with phenylmercuric chloride poisoning.

Gross pathological findings

Group I (0.19 mg. Hg/kg. daily): No significant pathological lesions were found in this group.

Group II (0.38 mg. Hg/kg. daily): No significant pathological lesions were found in this group.

Group III (0.76 mg. Hg/kg. daily): No significant pathological lesions were found in this group.

Group IV (2.28 mg. Hg/kg. daily): Emaciation was present in three of the animals which had severe clinical signs (989, 990, 991). There was nearly a total loss of adipose tissue. Varying degrees of serous atrophy in the epicardial fat were observed.

Nervous system. -- In one pig (987), there were petechial hemorrhages in the cerebrum. In the cerebellum, diffuse submeningeal hemorrhage was present. The remaining pigs in this group were free of similar lesions.

Digestive system. -- Petechial and ecchymotic hemorrhages in the serosa of various portions of the G.I. tract were seen in one pig (987).

The large intestines of three pigs which had severe diarrhea (989, 990, 991) were affected by a largely diffuse necrotic process that involved both the mucosa and submucosal layers (Appendix D, Table 2). Occasionally, in some patches, only the mucosa was involved.

The walls of the cecum and portions of the spiral colon were thickened. The lumen contained only scant amounts of fecal material. The intestinal wall was less flexible and much more rigid than normal, the result of diphtheritic inflammation of the mucosa. The internal surface of the deepest portions of the cecal and colonic sacculations were less severely affected. Adhesions between the pseudomembrane and discreet areas of the intestinal wall were found.

The proximal part of the large intestine was consistently found to be much more severely affected than the remainder. The lesions were diminished caudally and diseased tissue merged gradually with the remaining normal portion of the spiral colon (Fig. 92).

The ileo-cecal valve was similarly affected by pseudomembranous inflammation, but the ileum rarely was found to be affected.

The remaining parts of the G.I. tube were unaffected.

The livers were tawny in color but not friable. A mild degree of atrophy was found to affect the liver of all clinically sick animals.

Urinary system. -- The kidneys of the clinically affected animals (989, 990, 991) were swollen, pale yellow and had diminished cortico-medullary contrast (Fig. 93).

Group V (4.56 mg. Hg/kg. daily): Pronounced emaciation was noticed in two of the pigs (982 and 986) which were kept alive until the appearance of the terminal stages. The other pigs (983, 984 and 985) were killed before the appearance of overt clinical signs.

Nervous system. -- No lesions were found in this system.

Digestive system. -- A moderate degree of hyperemia was noticed in the pharynx of one pig (986). The esophagus, stomach and small intestine were unaffected.

The intra- and paracecal portions of the ileum were covered by a pseudomembrane. The cecum and proximal portion of the colon were severely affected by a necrotic process similar to that described for pigs in Group IV. Thick pseudomembranes covered, and in some instances replaced, the mucosa of the cecum and parts of the colon (Fig. 94). The internal surface of the sacculations in this portion of the intestine were covered by a pseudo-

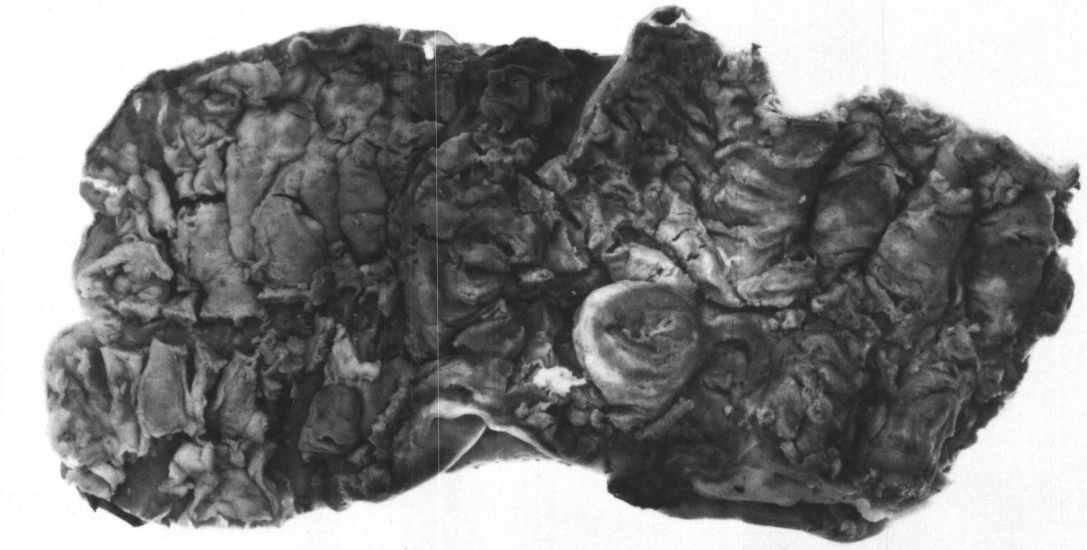


Fig. 92. Necrosis of the mucosa and formation of pseudomembranes in the cecum (left), ileo-cecal valve and colon (right).

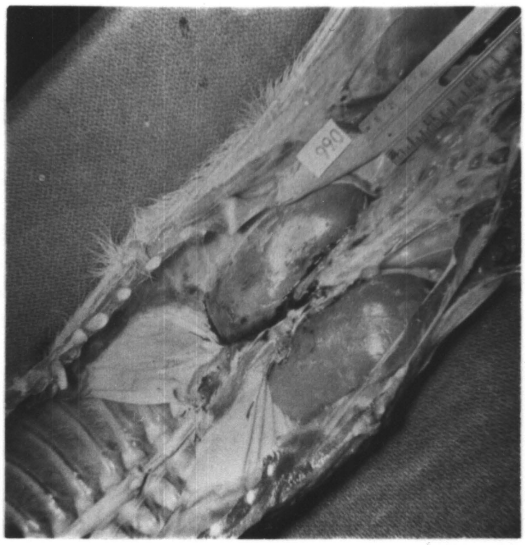


Fig. 93. Pig No. 990. The kidneys of clinically affected animals were swollen and pale yellow.

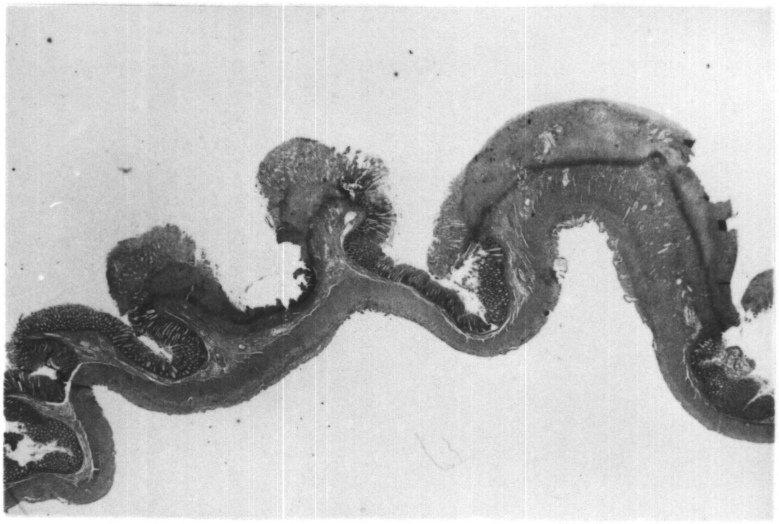


Fig. 94. Cecum. There is focal necrosis in the mucosa. Pseudomembranes adhere to affected areas. H&E stain. x 3.

membrane. Adhesions between pseudomembrane and underlying intestinal wall were present. They had a focal character and were most often found in relation to the tip of the folds in the intestinal mucosa. The lumen was occupied by variable amounts of recently formed fibrin clots which were stained yellow. The luster of the visceral peritoneum was normal in the area. In general, the affected colon and cecum had a contracted appearance when viewed in situ before dissection. The walls of the damaged portions were harder and less flexible than normal. The cecum was more severely affected by the necrotic process than the colon. The lesion in the colon extended caudally a distance of approximately 3-1/2 meters, where it gradually disappeared.

The livers were slightly atrophic and yellow or mottled.

Urinary system. -- The kidneys, like those in Group IV, were yellow and markedly swollen. The corticomedullary contrast was severely diminished.

Histopathological findings

No lesions were found in Groups I, II and III.

Group IV (2.28 mg. Hg/kg. daily): Lesions were found in the digestive, urinary and nervous systems and will be described in that order.

Digestive system. -- The liver was affected by varying degrees of hydropic degeneration (Fig. 95). This process had a diffuse character and, in two animals, was more pronounced in the periacinar region of the lobules. In general, the intensity of the process was mild.

The mucosal surface of the intracecal portion of the ileum, the cecum and the anterior colon were affected by a necrotizing process, the severity of which varied in these structures (Fig. 96). The basic lesion observed in various sections of the large intestine had some, or all, of the features to be described below.

In some areas where necrosis of the mucosa was not prominent, fibrin had exuded into the lumen and covered the surface epithelium. The exuded fibrin was mixed with degenerating or necrotic desquamated cells, leucocytes and abundant mucus. In regions where the underlying mucosa was still preserved, dilation of the crypts of Lieberkühn had occurred and, in some regions, was quite remarkable (Figs. 97 and 98). Hyperemia of the luminal region of the mucosa was an additional finding in such cases and was associated with a light infiltration of polymorphonuclear neutrophils.

In areas with coagulation necrosis of the mucosa and exposure of the lamina propria, the histological picture was different. In such areas, the surface of the intestine was covered by a structureless and finely granular eosinophilic mass which replaced the necrotic mucosa. The surface of this pseudomembrane was continuous with the fibrin layer described below. Numerous bacterial colonies were seen at the border between these two areas. Mucosal necrosis, as described above, had a focal character (Fig. 99). Necrotic areas were separated from the viable tissue by a zone of leucocytic infiltration. The leucocytes in this zone were in varying stages of degeneration.

Similar cells infiltrating the adjacent viable tissue were better preserved.

A common observation was the dilatation of the crypts. Such dilatation, on occasion, was sufficiently severe to constitute cyst formation. Ectatic crypts in many instances occurred in lymph follicles.

Edema in adjacent submucosa or tunica muscularis of the large intestine was rarely observed.

Urinary system. -- A small number of necrotic epithelial cells and mitotic figures were found in the proximal tubular epithelium, beginning after four to 15 experimental days.

Later (35-63 days post-treatment), this lesion was more intense and was characterized by epithelial cell necrosis and desquamation, which resulted in the formation of cellular casts. Regenerative attempts were indicated by development of flattened basal epithelium and numerous mitotic figures in large globoid cells (Fig. 100). In less severely injured nephrons, the proximal tubular epithelium appeared swollen and the Bowman's capsule dilated. Microscopic evidence of proteinuria was occasionally observed (Fig. 101).

Nervous system. -- The hemorrhage in the brain of Pig No. 987 appeared in two forms. The first type consisted of a diffuse submeningeal layer of extravasated blood and the second was encountered only within the brain tissue proper, as a ring of hemorrhage around vessels (Fig. 102).

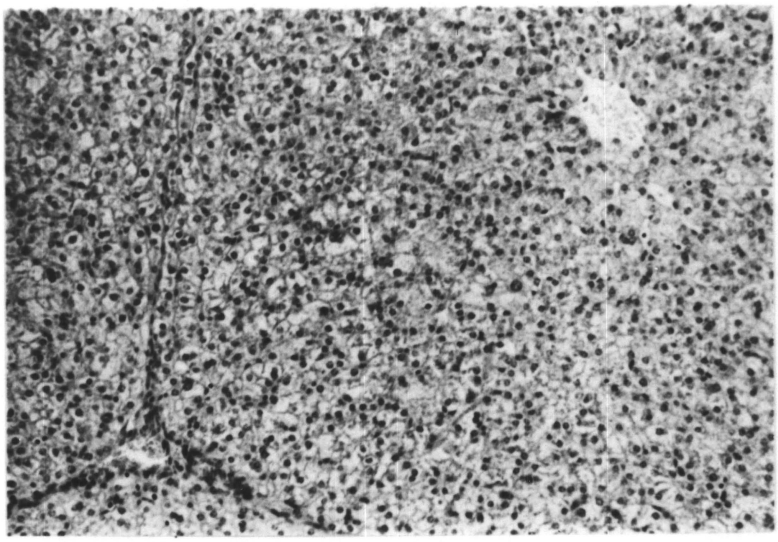


Fig. 95. Pig No. 983. Liver with hydropic degeneration affecting mainly the perilobular regions. H&E stain. x 100.

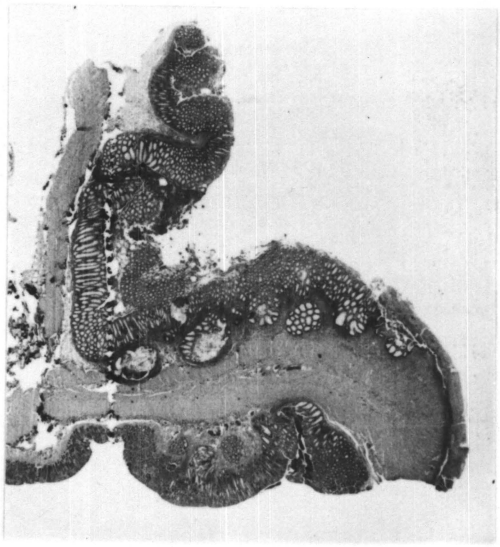


Fig. 96. Pig No. 991. Focal necrosis in the mucosa of large intestine. H&E stain. x 3.

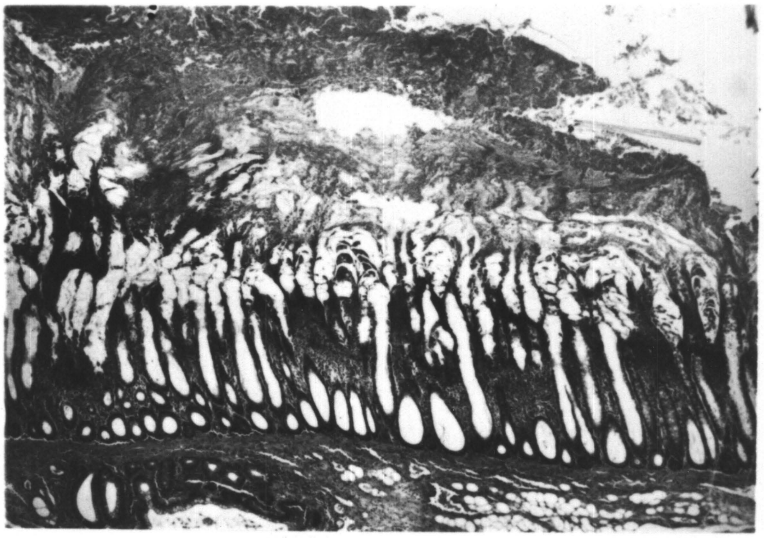


Fig. 97. Pig No. 989. Cecum. Necrotic debris in the lumen is continuous with newly produced mucus. The underlying crypts of Lieberkühn present varying degrees of dilatation in their lumina. H&E stain. x 25.

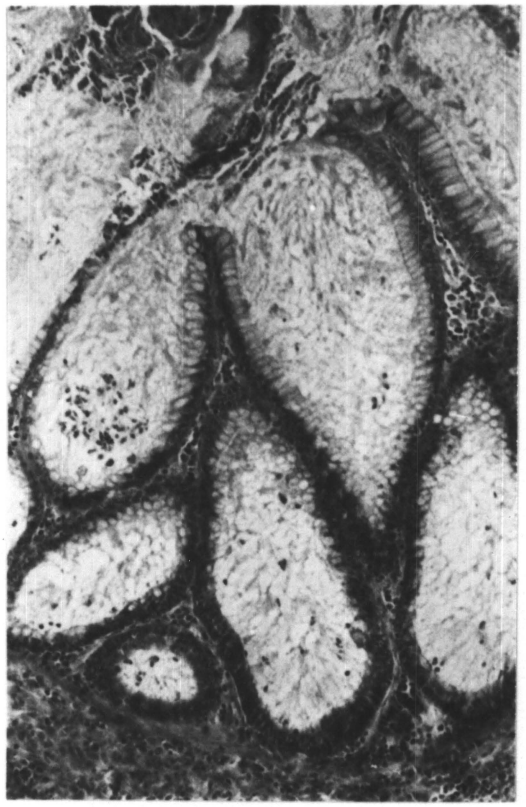
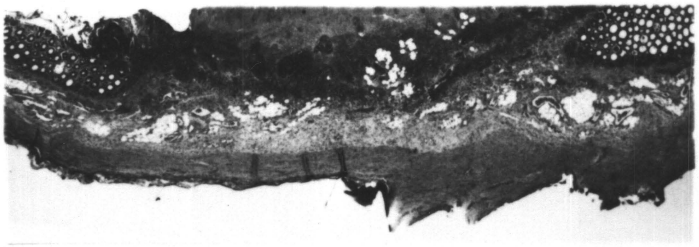
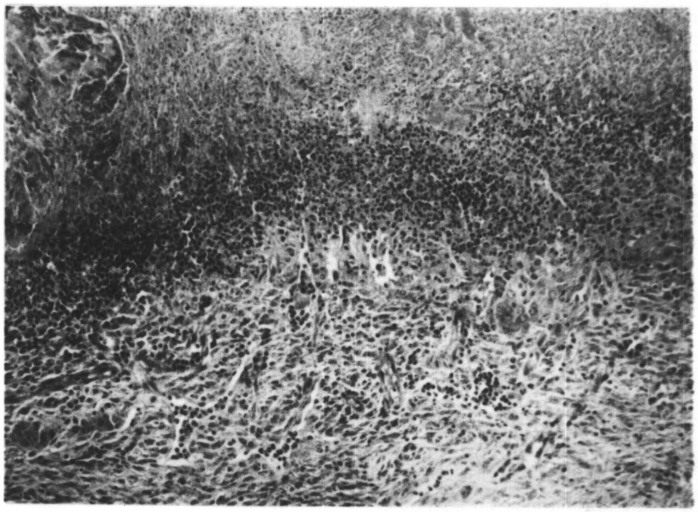


Fig. 98. Pig No. 991. Cecum. A higher power view of dilated crypts. The mucinous content is continuous with the overlying necrotic debris (upper left). H&E stain. x 250.



a



b

Fig. 99. Pig No. 990. There is ulceration of the cecal mucosa (a). H&E stain. x 3. A zone of leucocyte infiltration separates the granulation tissue from the overlying necrotic debris (b). H&E stain. x 25.

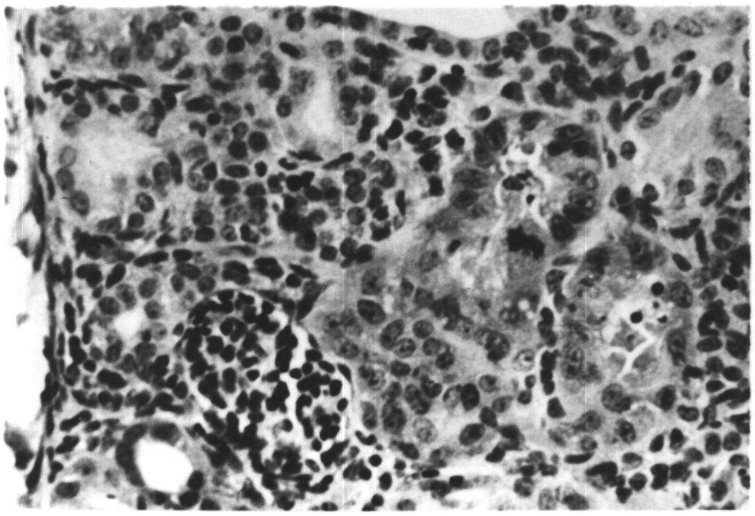


Fig. 100. Pig No. 990. Mitotic figure and desquamated necrotic cells in lumen of convoluted tubule in subcapsular region of renal cortex. H&E stain. x 250.

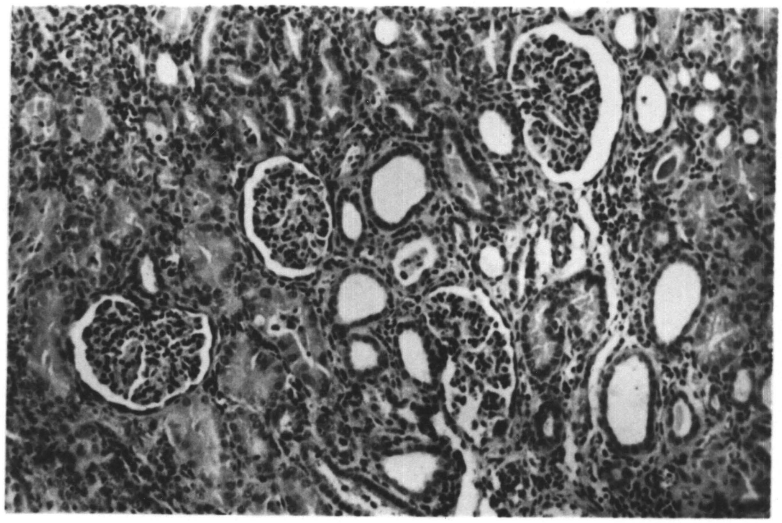


Fig. 101. Phenylmercuric chloride poisoning. There is desquamation of degenerate and necrotic cells (center), moderate swelling of proximal tubular epithelium, proteinaceous tubular fluid (center left) and dilated Bowman's capsules. H&E stain. x 100.

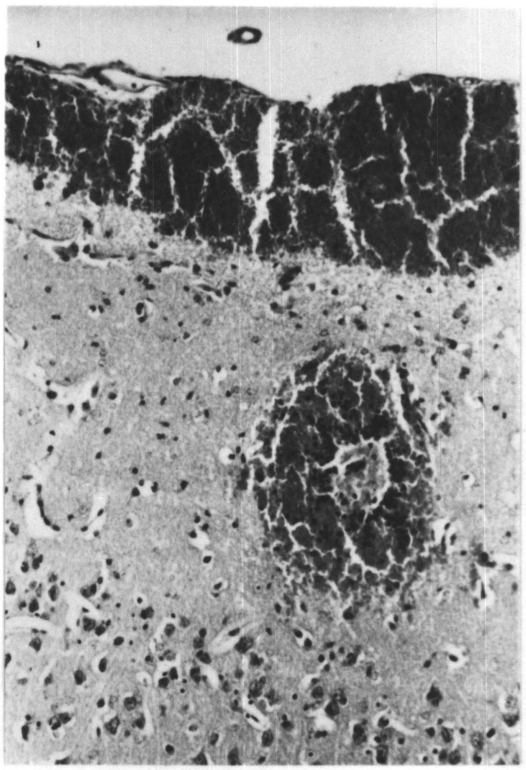


Fig. 102. Diffuse and ring form hemorrhages in the brain of an animal (Pig No. 987) which had an unexplained hemorrhagic diathesis. H&E stain. x100.

Group V (4.56 mg. Hg/kg. daily): Significant histological lesions were found only in the digestive and urinary systems. They will be described in that order.

Digestive system. -- Lesions in the intestine were present only in the two animals with the longest survival periods (35 days). They were severe and quite similar, in many ways, to the ones already described for Group IV (2.28 mg. Hg/kg. daily). The necrotic character of the lesions was more pronounced in this group than in the one receiving a lower dosage.

In appropriate sections, it was seen that the lesions were most severe at the tips of the mucosal folds (Fig. 94).

The affected mucosa was covered by a thick fibrinous layer which contained necrotic and degenerate epithelial cells, leucocytes, varying amounts of mucus and numerous bacterial colonies. The crypts of Lieberkühn in the underlying mucosa were dilated by excessive amounts of clear mucus. Occasionally, the dilated crypts could be considered large enough to be classified as cysts. These occurred in solitary lymph nodules. In areas where necrosis and destruction of the underlying mucosa occurred, the overlying membrane constituted a diphtheritic membrane which contained varying amounts of necrotic debris. Necrotic areas were limited by a zone of inflammatory cells and, on occasion, young granulation tissue (Fig. 103). Leucocytic infiltration of adjacent viable tissues was prominent. Edema was also present in adjacent tissues. This diphtheritic process was extensive and, as a result, only a small portion of the epithelial lining remained unaffected and, therefore, functional.

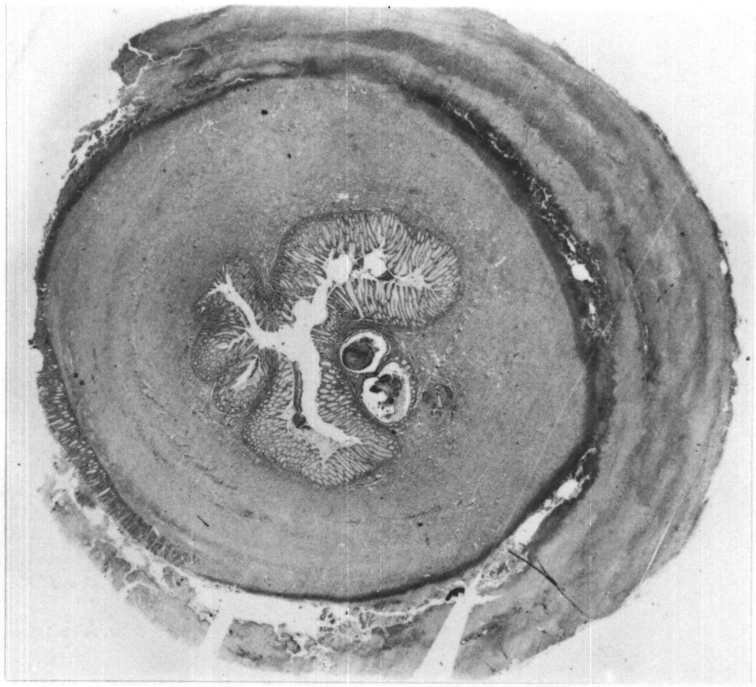


Fig. 103. Pig No. 986. Ileo-cecal valve. The necrotic debris is limited by a zone of inflammatory cells. Repair was characterized by the presence of young granulation tissue. H&E stain. x 100.

The mildest lesions were observed in the more caudal segments of the large intestine and consisted of early necrosis of the surface epithelium characterized by pyknosis and karyorrhexis. Light infiltration of inflammatory cells into the lamina propria and edema of the lamina propria. Depletion of the solitary lymph nodules was also observed (Fig. 104).

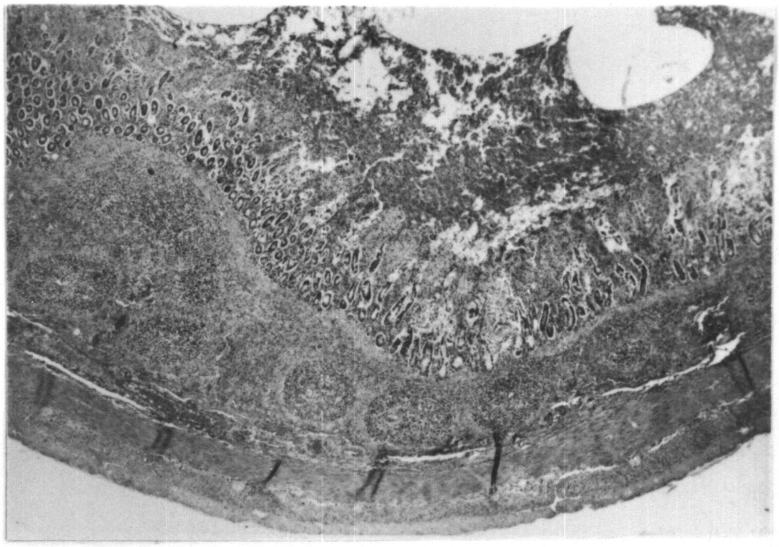


Fig. 104. Necrosis of mucosa with depletion of underlying solitary lymph nodes. H&E stain. x 25.

The liver gave evidence of injury which was related to the length of exposure to PMC. Diffuse hydropic degeneration was observed in the liver of animals with the shorter exposure periods (983, 984, 985). The extent ot the process in these animals varied with the exact length of the exposure period. Necrosis and regeneration were seen in the liver of the two animals (982 and 986) with the longer period of exposure (35 days). The necrotic and regenerative process had no distinct pattern. Degenerate and regenerating hepatocytes had a random distribution.

Urinary system. -- The lesions in the kidney were primarily confined to the tubules. The nephrons were injured in an uneven, or random, manner. The lesions were the result of the interplay between necrosis and regeneration. The proximal tubular epithelium appeared to be primarily affected.

Some proximal convoluted tubules had an irregularly swollen epithelium which, on occasion, obliterated the lumen. The cellular boundaries, however, remained distinct. Individual cells in varying stages of degeneration were either still attached to the tubular basement membrane or had fallen into the lumen (Fig. 105). Some of these cells were hyperchromatic. Desquamated cells were found singly or in groups of varying numbers. The degree of visible injury in the desquamated cells varied from fairly mild to very severe. A small number of calcified epithelial cells was found either still attached to the basement membrane or free in the lumen (Figs. 107 and 108). Mitotic figures were common in the proximal convoluted tubule epithelium (Fig. 106). Cells undergoing mitosis were large and protruded into the tubular lumen. Many tubules were lined with newly regenerated epithelium. Such epithelium was low cuboidal in shape and hyperchromic (Fig. 109).

Besides the desquamated epithelial cells, the lumen of the proximal convoluted tubules contained proteinaceous casts which were finely granular and fragmented. Some tubules were dilated with such debris (Fig. 109).

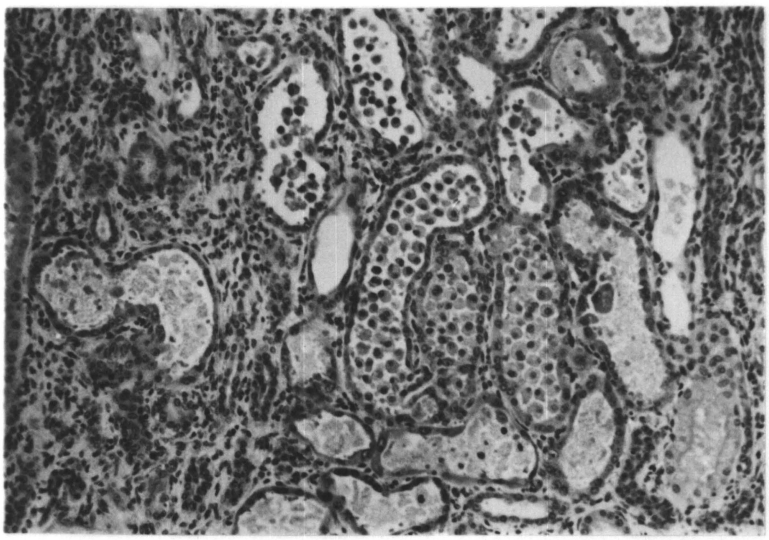


Fig. 105. Phenylmercuric chloride poisoning. Tubular epithelium in various stages of degeneration and regeneration. The tubules in the center of the picture contain numerous desquamated epithelial cells. H&E stain. x 100.

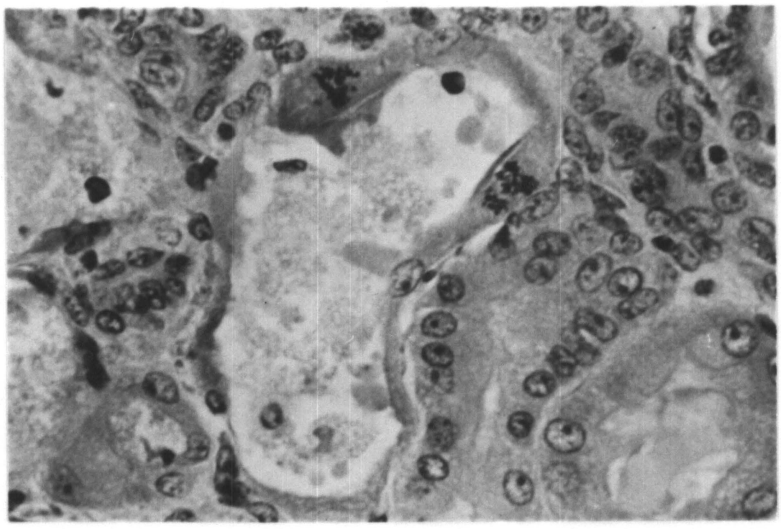


Fig. 106. Phenylmercuric chloride poisoning. Mitotic figures in tubular epithelium. H&E stain. x 250.

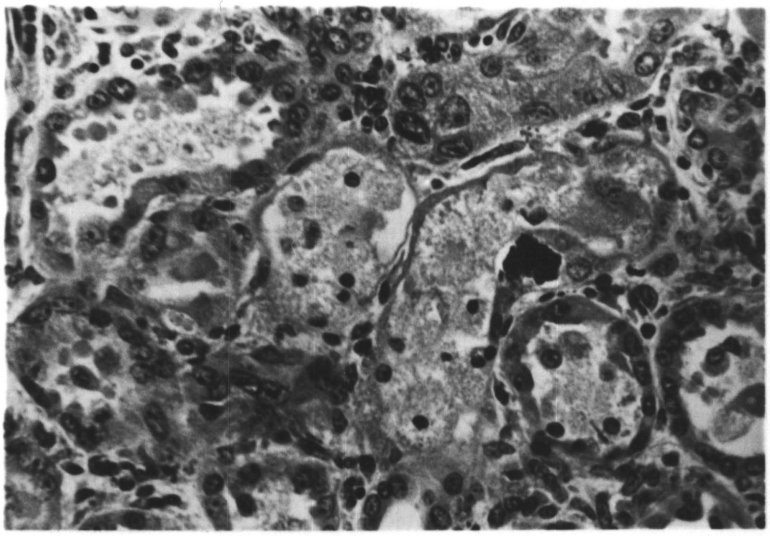


Fig. 107. Phenylmercuric chloride poisoning. Calcification of epithelial cell. H&E stain. x 250.

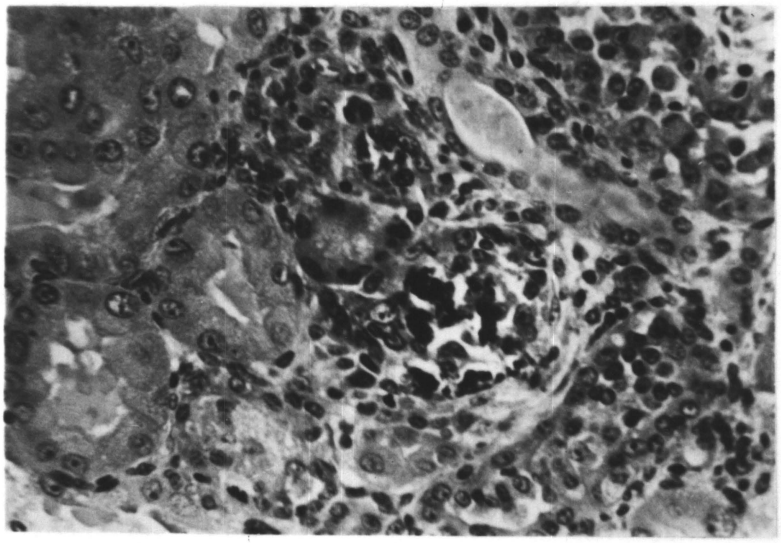


Fig. 108. Calcification of tubular contents also were found in this group. H&E stain. x 250.

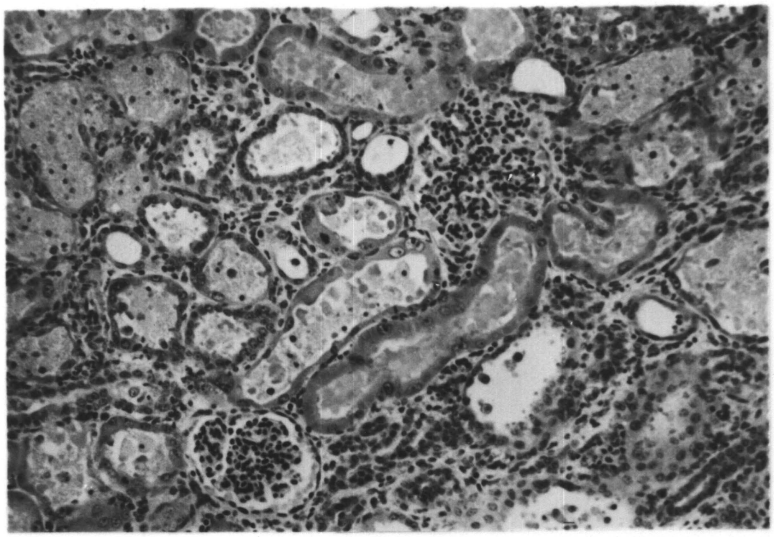


Fig. 109. Toxic nephrosis. Evidence of epithelial degeneration and regeneration is present in proximal tubular epithelium. Proteinaceous casts consisting of finely granular eosinophilic material are present in most tubules. A small amount of eosinophilic material coats the parietal layer of the Bowman's capsule. H&E stain. x 100.

The basement membrane of injured tubules remained unaffected. Also, it appeared that the interstitium and blood vessels therein were free from injury. The glomeruli were also unremarkable.

The distal parts of the nephrons were not primarily affected except for increased amounts of tubular contents which consisted of material derived from the level of the proximal convoluted tubules.

Analytical findings

The data obtained from the analysis of samples collected during the postmortem procedures on pigs poisoned with phenylmercuric chloride are tabulated in Table 19. Reference to such table will show that: 1) with the dosage range studied (0.19 - 4.56 mg. Hg/kg. daily), accumulation of mercury occurred in all tissues examined and that certain organs, especially kidney and liver, acquired much higher levels of mercury than others; 2) the level of mercury which accumulated in tissues was a function of both dose and time; 3) the rate of accumulation of mercury in the tissue varied directly as the dosage.

In terms of mercury level, the various samples which were analyzed for mercury may be arranged as follows in order of diminishing concentration: kidneys, liver, intestine, bile, brain, skin, urine, muscle and blood.

The level of Hg in a particular tissue from pigs on high dosages did not always exceed that of the same tissue from individuals in the lower groups. Animals receiving high dosages of organomercurial had relatively short survival times.

TABLE 19. Levels of Hg in μg/gm or μg/ml in Tissues of Pigs Which Received Varying Doses of Phenylmercuric Chloride

GROUP DOSE	PIG NUMBER	DAYS	μg Hg/ml			μg Hg/gm											
						Brain		Liver		Intestines		Kidney		Muscle		Skin	
			Blood	Urine	Bile	Hg	Hg/days	Hg	Hg/days	Hg	Hg/days	Hg	Hg/days	Hg	Hg/days	Hg	Hg/days
I (0.19)	952	14	0.1	0.1	0.8	0.6	0.04	5.3	0.38	6.2	0.44	30.5	2.18	0.5	0.371	0.5	0.03
	953	39	0.1	---	0.2	0.4	0.01	10.1	0.26	2.5	0.06	40.1	1.03	0.2	0.006	0.4	6.01
	954	90	0.2	0.1	0.2	0.6	0.01	3.1	0.03	3.8	0.42	39.2	0.43	0.1	0.001	0.4	0.01
	955	90	0.1	0.2	0.5	0.5	0.01	3.5	0.04	2.3	0.25	28.8	0.32	0.1	0.001	0.3	0.01
	956	90	0.1	---	0.2	2.8	0.03	3.6	0.04	2.8	----	61.1	0.68	0.1	0.000	0.04	0.01
II (0.38)	996	39	0.3	---	---	3.6	0.09	3.9	0.10	2.9	0.07	110.2	2.85	0.3	0.008	0.4	0.01
	997	90	0.1	0.5	---	0.9	0.01	7.1	0.07	2.6	0.03	155.3	1.73	0.2	0.002	0.3	0.01
	998	90	---	---	0.2	0.7	0.01	4.6	0.05	1.6	0.02	146.2	1.62	0.3	0.003	0.2	0.01
	999	90	0.2	---	0.8	0.9	0.01	6.4	0.07	7.9	0.09	137.6	1.53	0.2	0.002	0.4	0.01
	1000	65	---	---	---	1.6	0.02	4.9	0.08	3.4	0.05	146.1	2.26	0.3	0.004	0.3	0.01
III (0.76)	992	90	---	---	1.2	---	----	5.1	0.06	3.4	0.03	341.8	3.79	0.3	0.002	0.6	0.01
	993	14	0.1	0.7	5.8	---	----	18.3	1.31	56.5	4.04	110.1	8.50	0.5	0.034	0.9	0.06
	994	90	0.2	1.3	---	---	----	4.3	0.05	9.1	0.10	154.8	1.71	0.2	0.002	0.5	0.01
	995	50	---	---	---	1.1	0.02	24.1	0.48	1.2	0.02	242.5	4.85	0.4	0.007	0.7	0.01
	951	90	---	0.6	3.4	1.7	0.02	5.1	0.05	2.5	0.03	178.1	1.98	0.2	0.002	0.5	0.01
IV (2.28)	987	14	0.5	1.6	11.5	4.1	0.29	29.4	2.10	60.9	4.35	267.2	19.08	1.1	0.07	1.8	0.13
	988	3	0.3	---	0.8	---	----	7.9	2.64	4.6	1.55	78.4	26.13	1.2	0.39	1.4	0.47
	989	50	---	---	2.0	---	----	79.9	1.59	34.7	0.68	307.3	6.14	2.8	0.05	---	----
	990	65	0.1	---	---	2.1	0.03	134.5	2.07	32.3	0.49	213.3	3.28	0.8	0.01	3.8	0.05
	991	34	---	0.9	2.9	2.1	0.06	76.3	2.25	35.9	1.05	240.6	7.07	1.1	0.03	2.7	0.08
V (4.56)	982	25	---	---	16.9	---	----	160.8	6.43	---	----	157.9	6.31	1.2	0.05	4.9	0.19
	983	6	0.3	1.5	5.2	0.8	0.14	32.8	5.47	8.8	1.46	369.4	61.51	0.7	0.12	1.8	0.29
	980	1	0.2	0.7	3.5	3.3	3.27	15.3	15.31	7.9	7.90	51.9	51.96	1.3	1.31	0.8	0.75
	985	3	0.3	2.4	5.8	0.6	0.21	27.1	9.03	6.3	2.09	254.1	84.67	1.1	0.38	1.4	0.47
	986	34	2.7	0.7	4.1	2.4	0.07	121.3	3.57	115.1	3.38	258.5	7.60	1.2	0.04	2.9	0.08
Control	977	14	0.1	0.1	0.2	0.7	----	0.2	----	0.8	----	0.4	----	0.3	----	0.5	----
	978	90	0.1	0.1	0.2	0.6	----	0.3	----	1.7	----	0.4	----	0.3	----	0.2	----
	979	90	0.1	0.1	0.2	0.5	----	0.2	----	1.1	----	0.1	----	0.1	----	0.5	
	980	90	0.2	0.1	0.2	0.7	----	0.1	----	0.5	----	0.1	----	0.1	----	0.6	----
	981	90	0.1	0.1	0.2	0.9	----	0.2	----	1.8	----	0.2	----	0.2	----	0.3	----

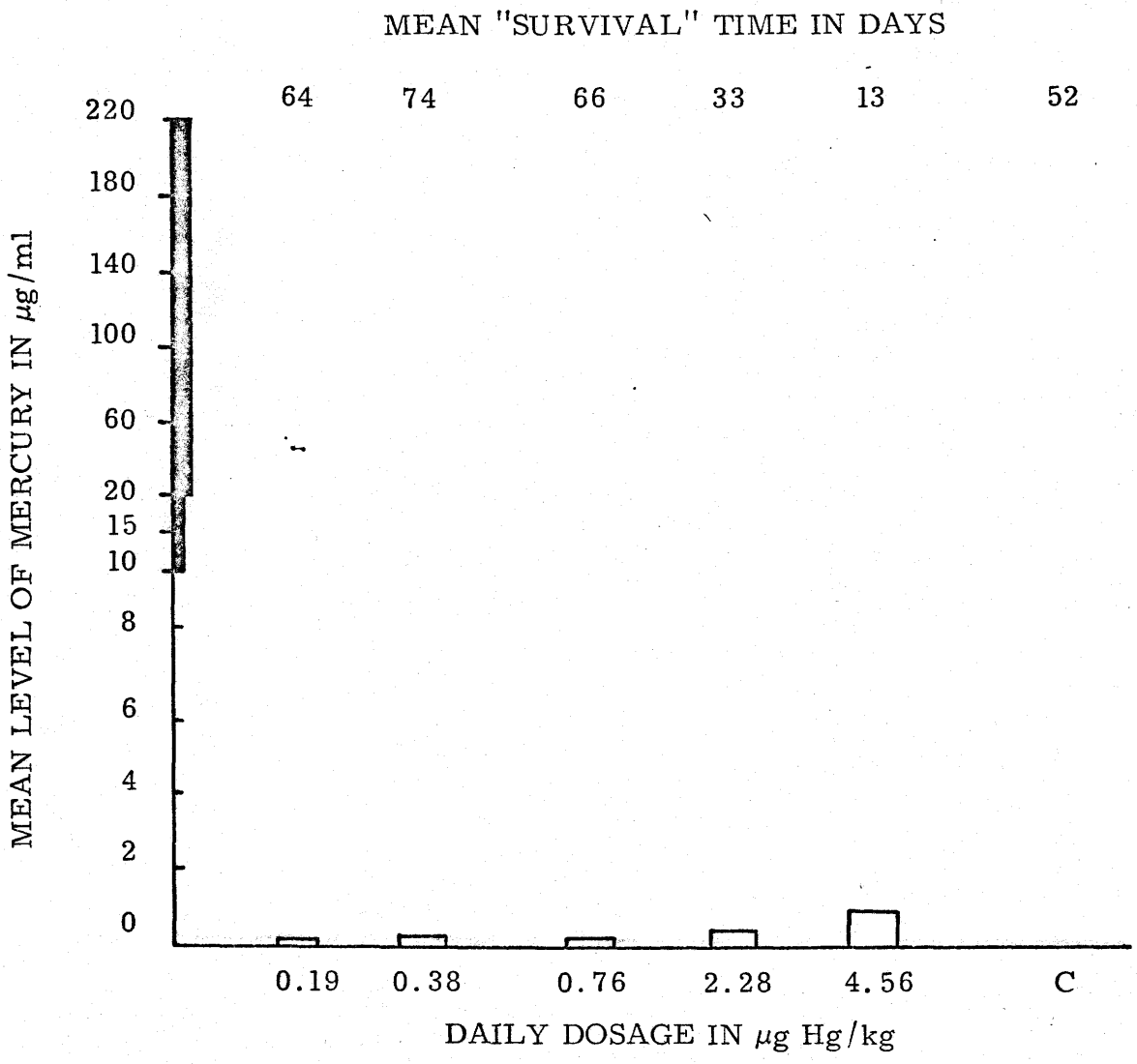


Fig. 110. Mean mercury levels in the blood and mean survival time of groups of pigs receiving increasing dosages of PMC.

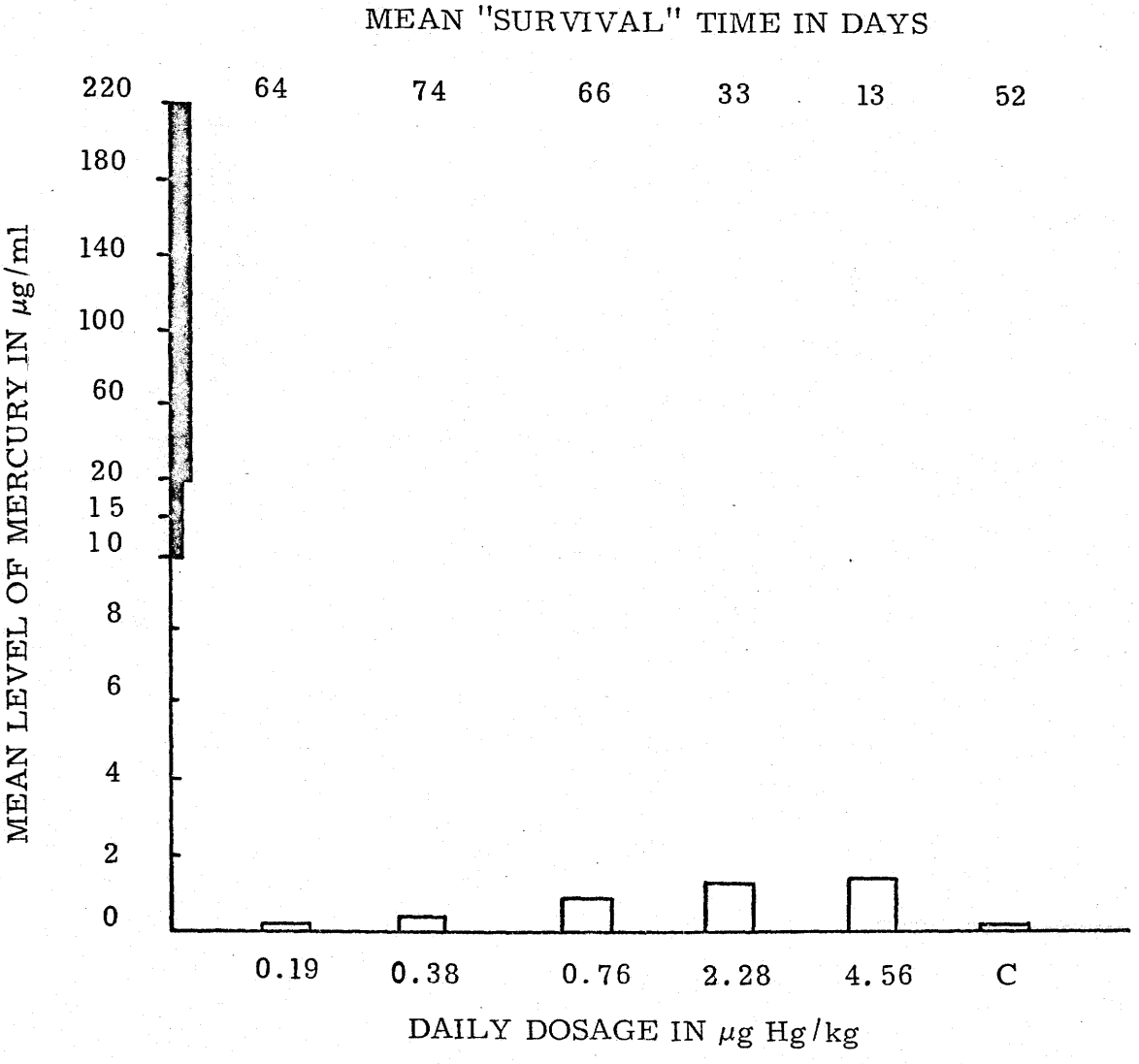


Fig. 111. Mean mercury levels in the urine and mean survival time of groups of pigs receiving increasing dosages of PMC.

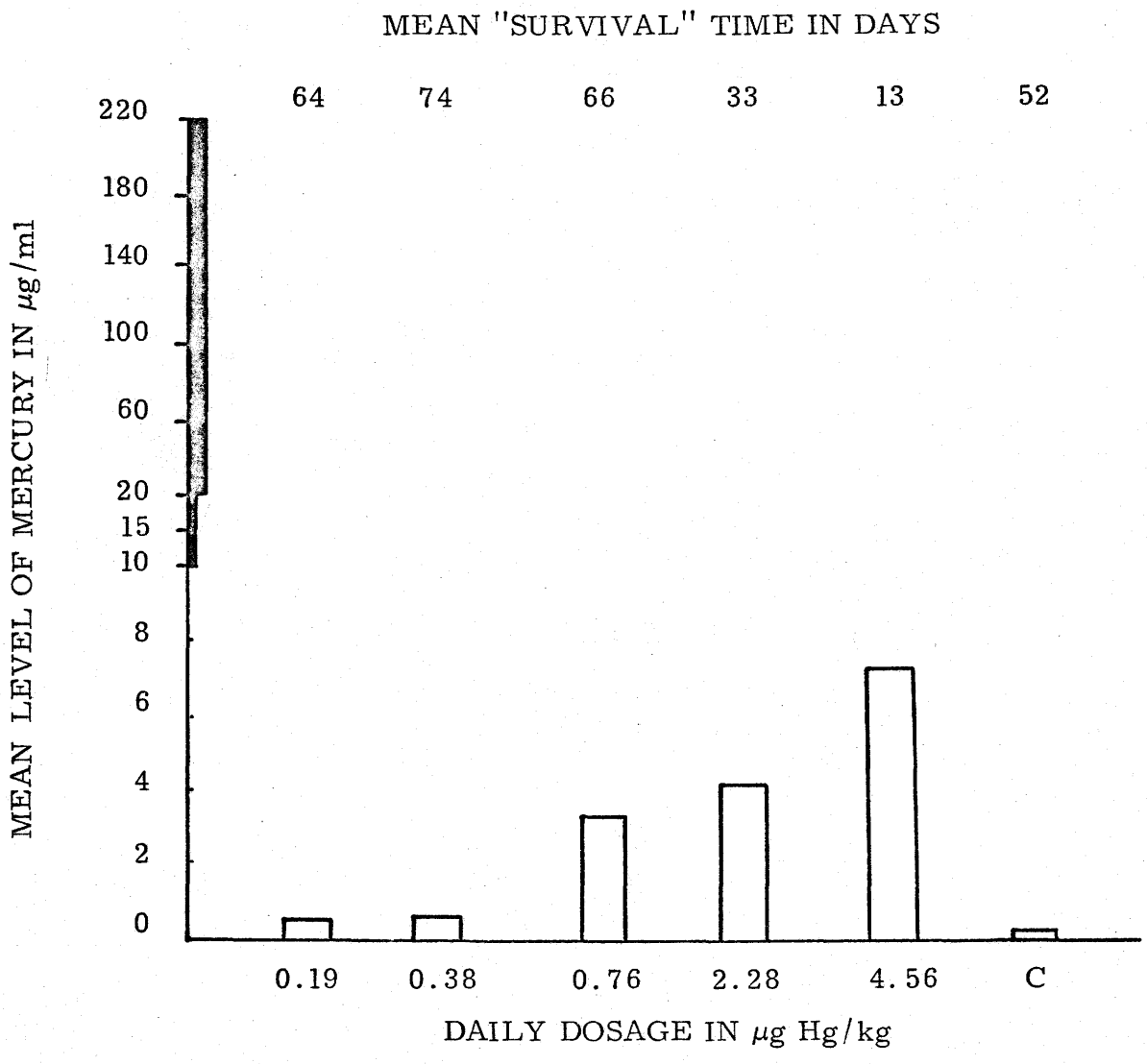


Fig. 112. Mean mercury levels in the bile and mean survival time of groups of pigs receiving increasing dosages of PMC.

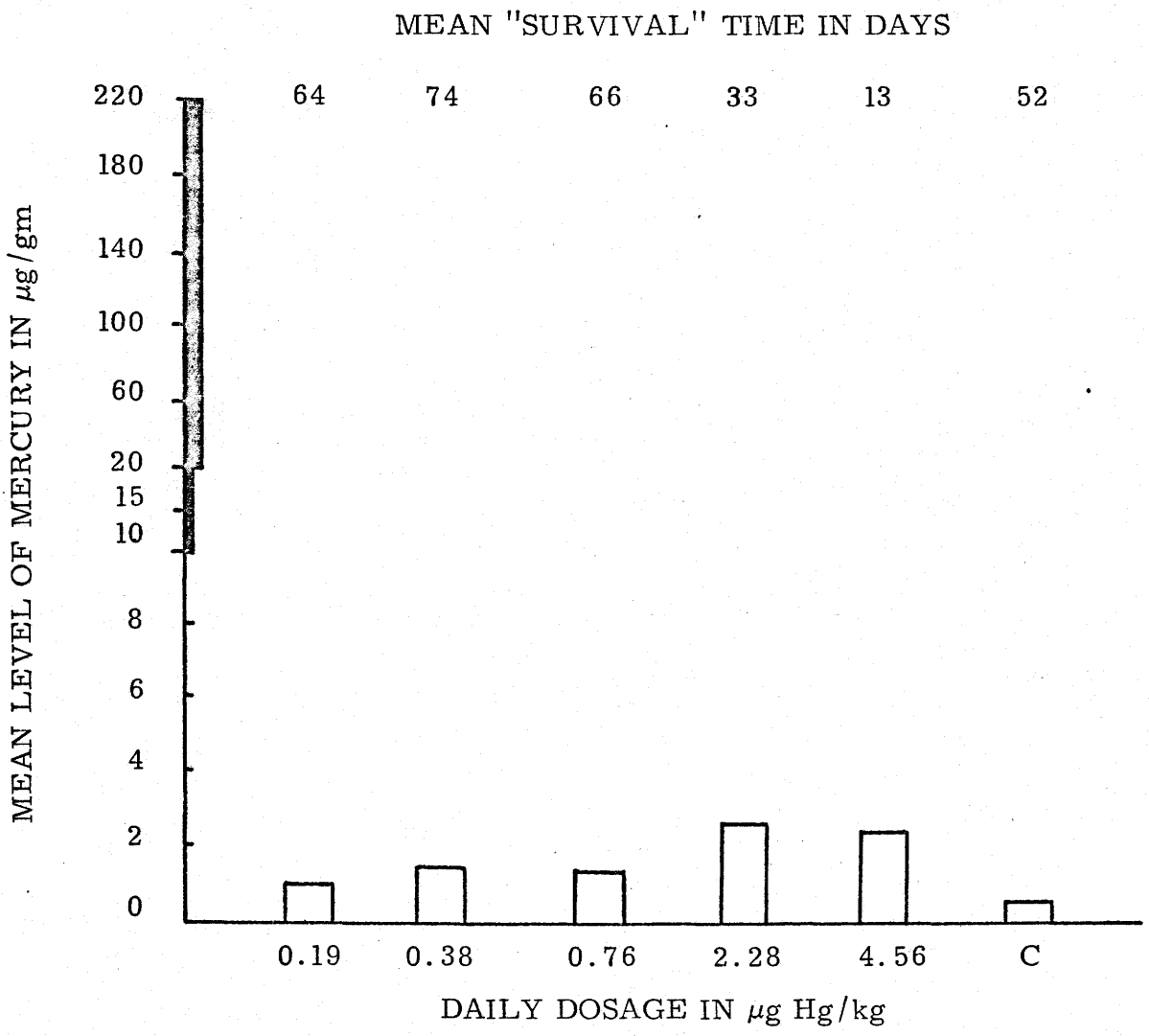


Fig. 113. Mean mercury levels in the brain and mean survival time of groups of pigs receiving increasing dosages of PMC.

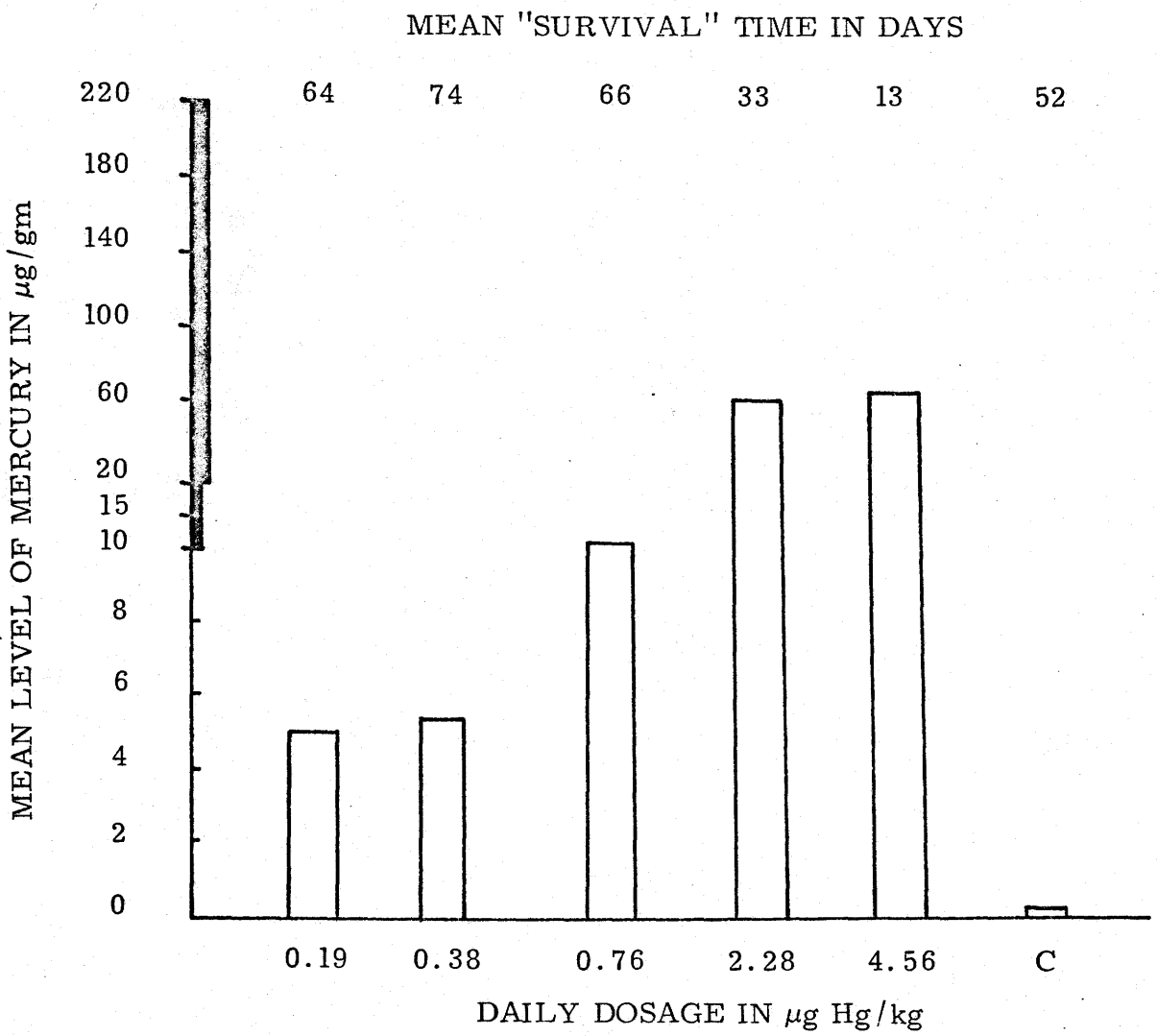


Fig. 114. Mean mercury levels in the liver and mean survival time of groups of pigs receiving increasing dosages of PMC.

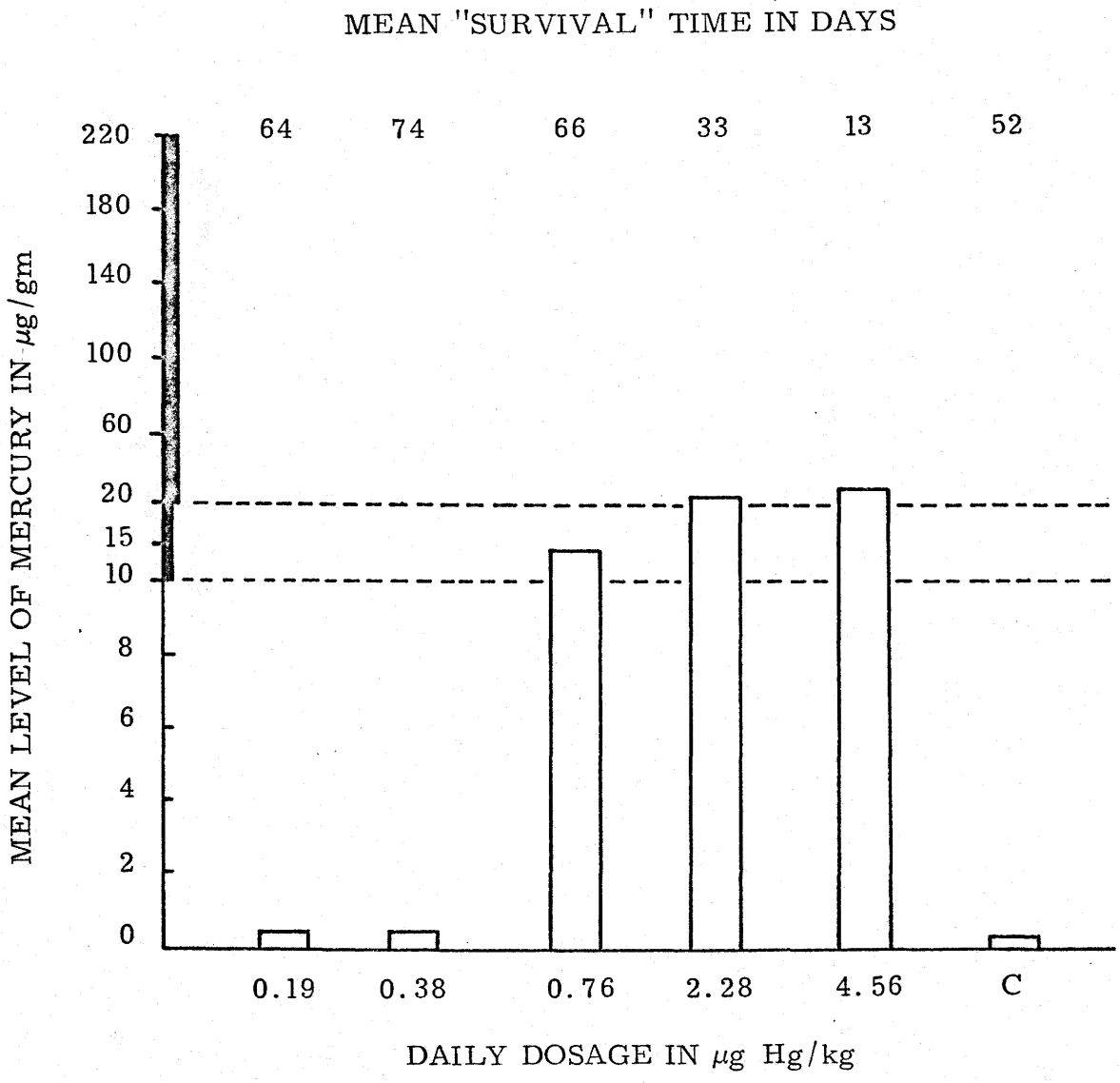


Fig. 115. Mean mercury levels in the large intestine and mean survival time of groups of pigs receiving increasing dosages of PMC.

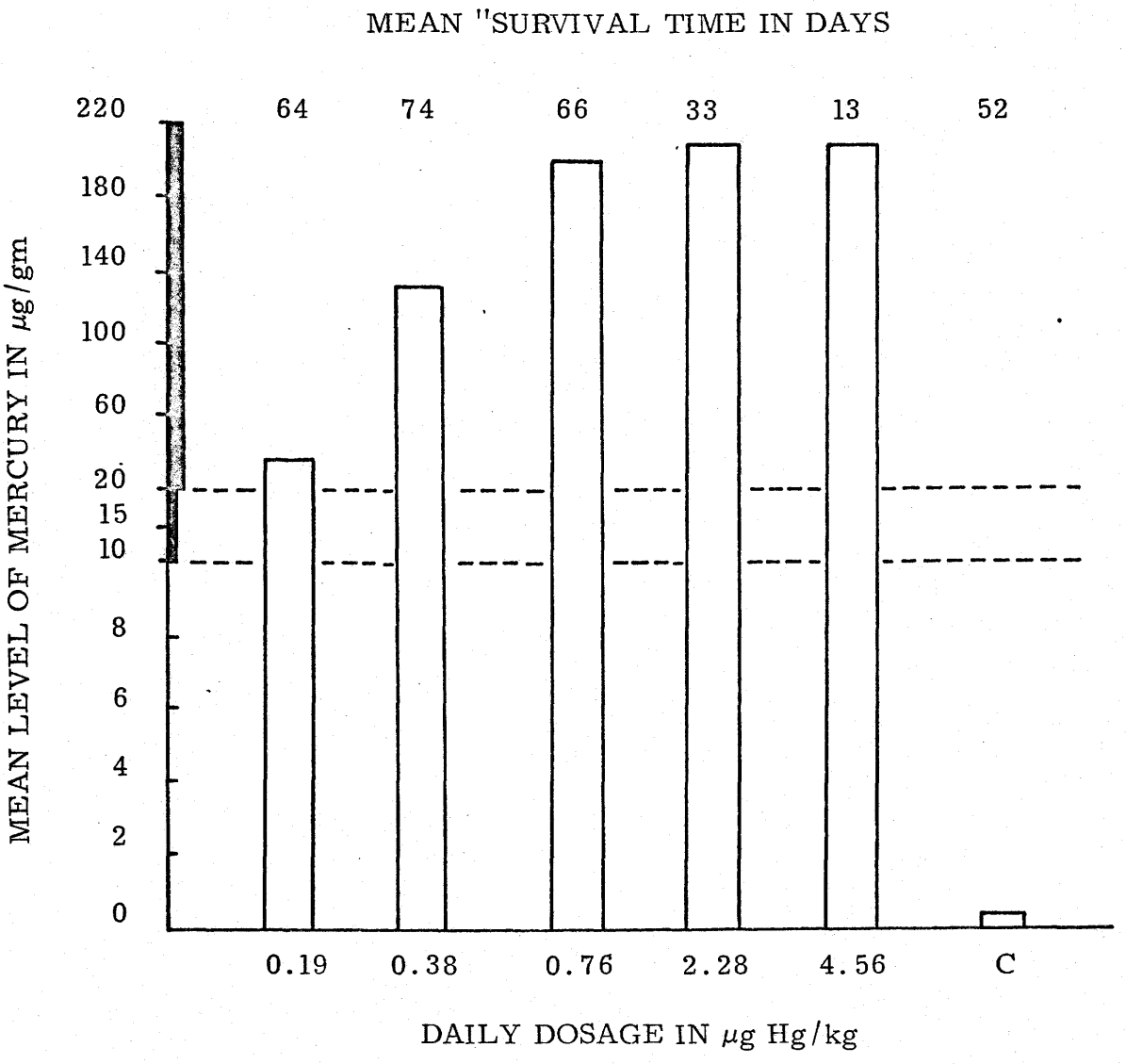


Fig. 116. Mean mercury levels in the kidneys and mean survival time of groups of pigs receiving increasing dosages of PMC.

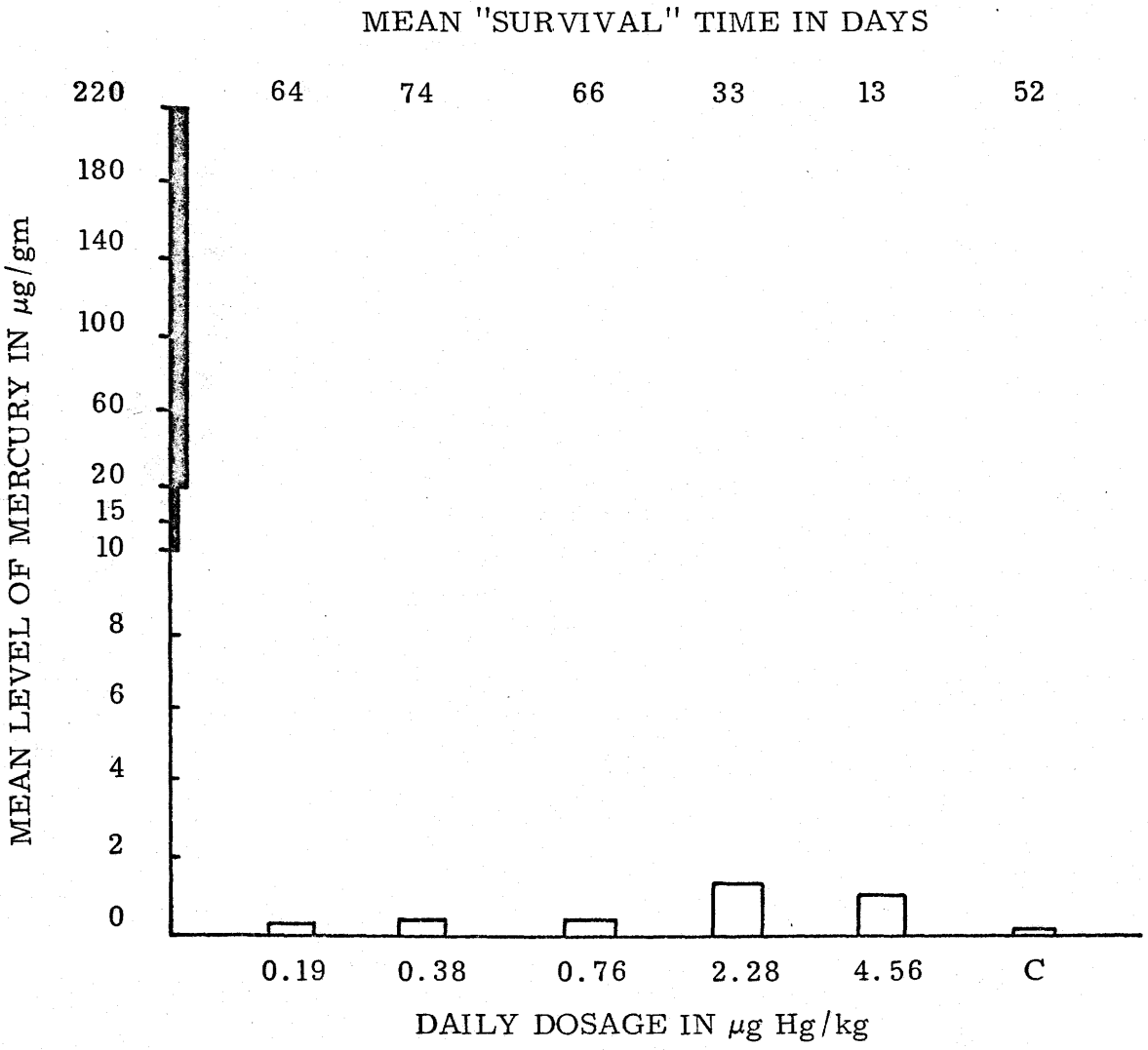


Fig. 117. Mean mercury levels in the muscle and mean survival time of groups of pigs receiving increasing dosages of PMC.

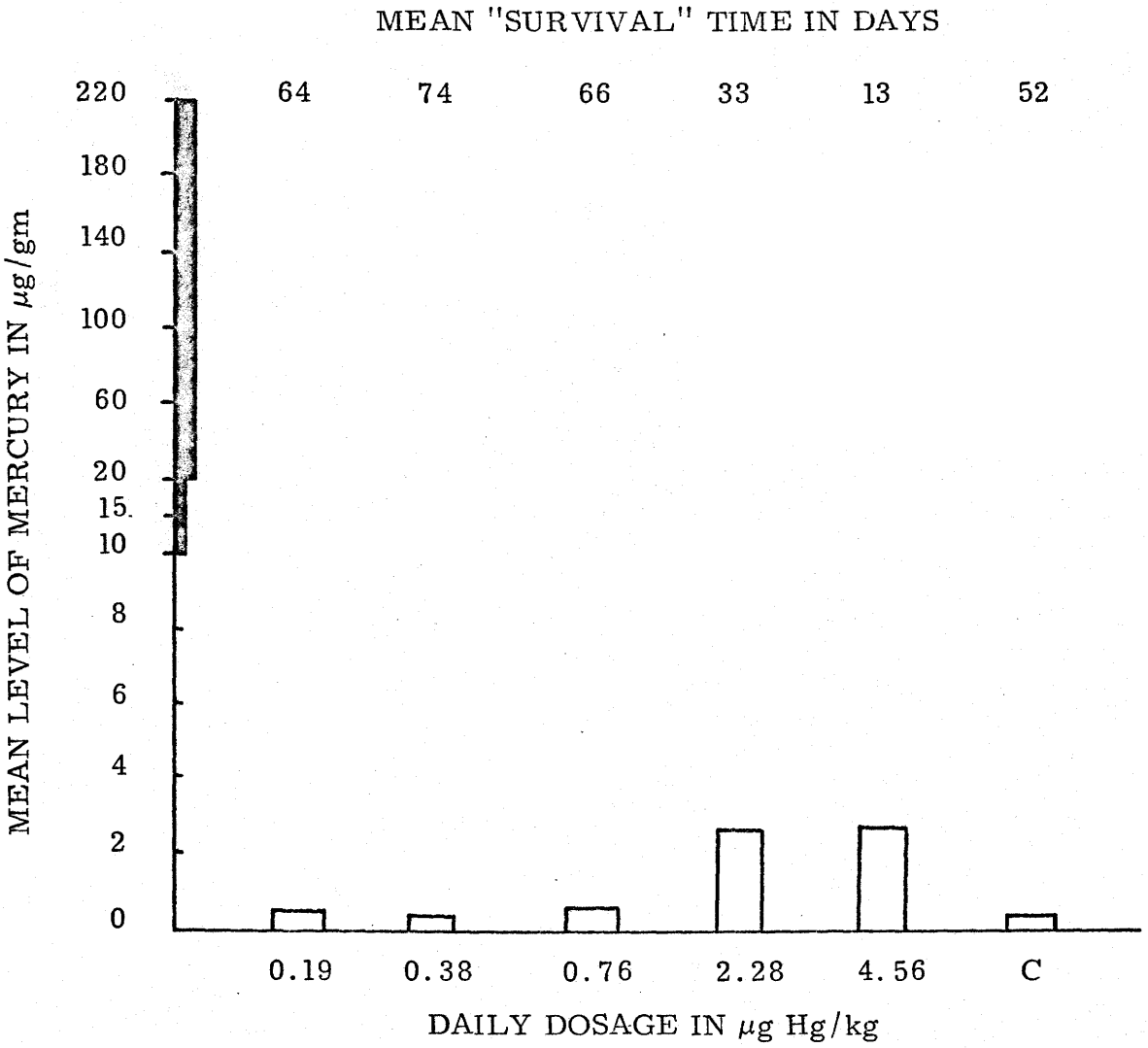


Fig. 118. Mean mercury levels in the skin and mean survival time of groups of pigs receiving increasing dosages of PMC.

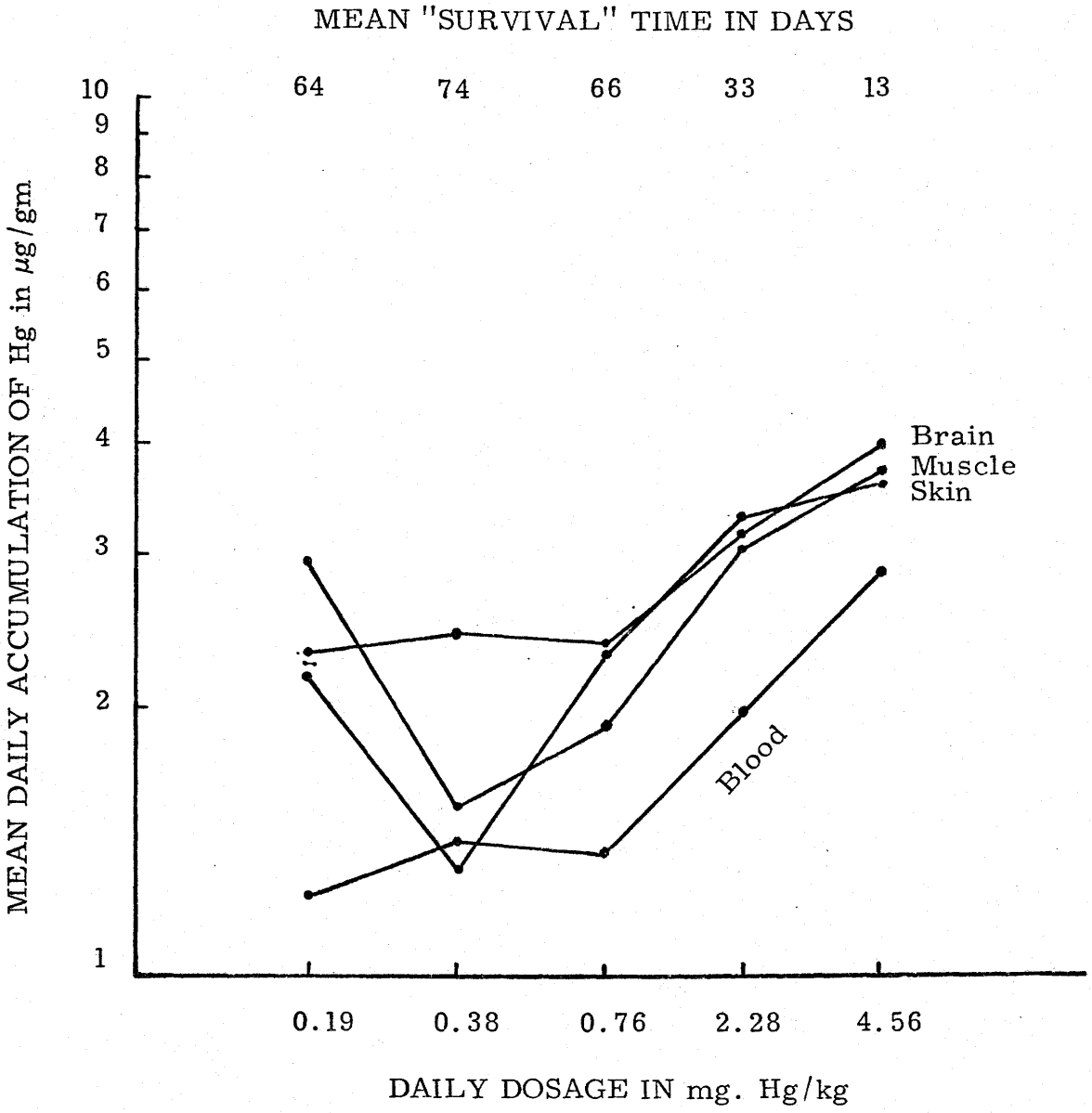


Fig. 119. Mean retention rates for brain, skin, muscle and blood in animals receiving PMC, shown on logarithmic scale.

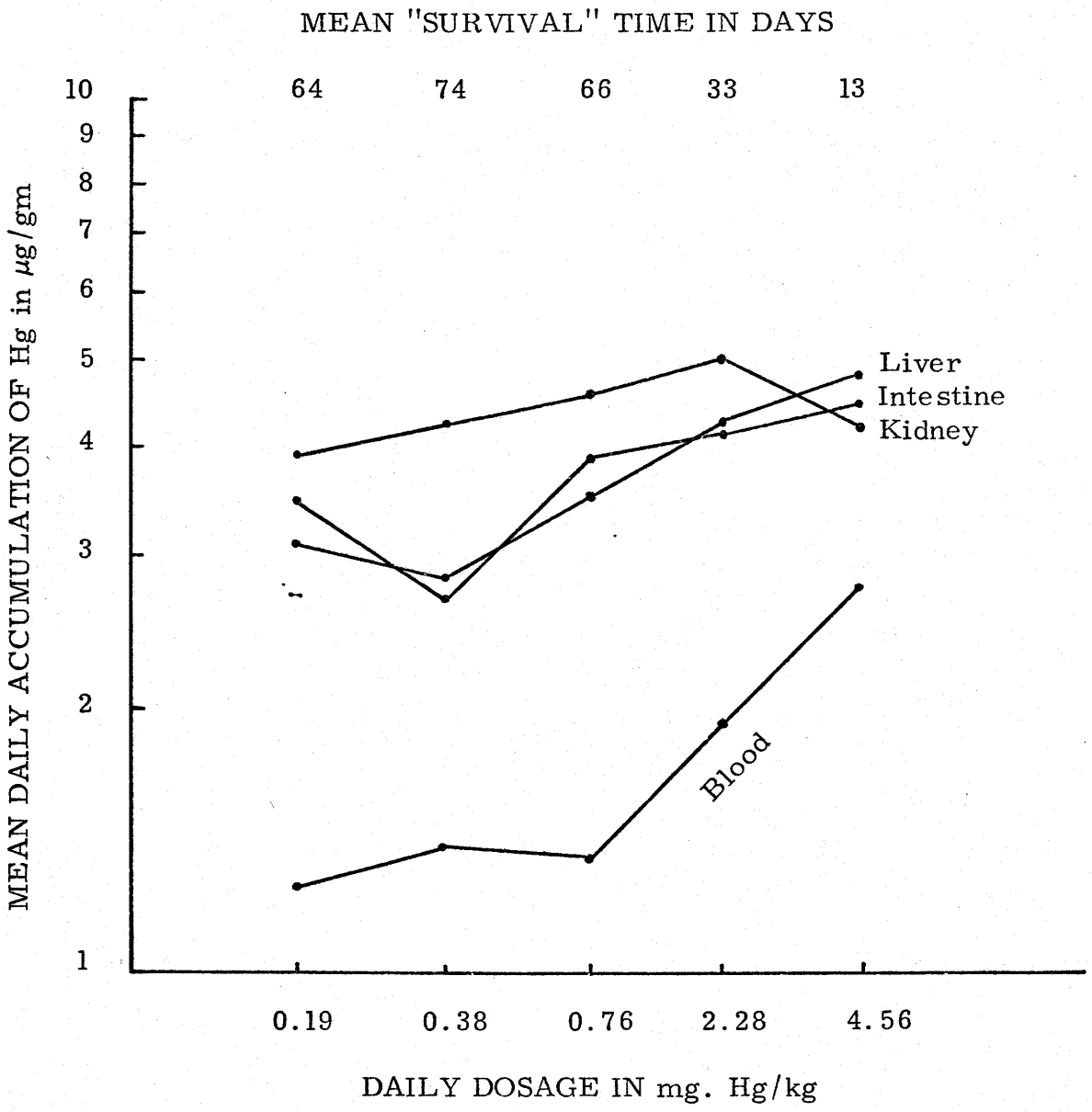


Fig. 120. Mean retention rates for kidney, liver, intestine and blood in animals receiving PMC, shown on logarithmic scale.

The rates of accumulation of mercury in each tissue of each pig were calculated by dividing the amount of mercury in the tissue by the number of days the animal received PMC. These data are presented in Table 19. Figures 119 and 120 graphically illustrate the rates of mercury accumulation for each organ when all the data for each of the experimental groups are pooled, and Figures 110 to 118, the mercury load of tissues examined. It can be seen at the dosages studied that the rate of accumulation depended on dose.

Discussion

Phenylmercuric chloride (PMC is poisonous to swine. The dosage range employed in these experiments, 0.19 - 4.56 mg. Hg/kg. daily as PMC, produced a disease with chronic clinicopathologic manifestations. The large intestine, liver and kidneys are the organs which are primarily affected. The clinical picture, dominated by enteric signs and deterioration in condition, reflects the gravity of the underlying pathology.

Clinical manifestations: Diarrhea was a consistent early sign in all affected animals. Once present, diarrhea persisted throughout the remaining experimental period and became progressively more severe. Presumably this was a reflection of progressive injury to the large intestinal tract. The anorexia and weakness were probably, in part, the result of disturbed physiology in the affected large intestine. Diarrhea can be explained by assuming failure of the large intestine to

absorb water from its contents and possibly fluid exudation. Sustained diarrhea and reduced feed intake put the affected animals in a state of negative nutritional balance and were responsible for the observed weight loss. High BUN values in affected animals had direct relationship to weight loss. This could, in part, be explained by the presence of excessive catabolism associated with weight loss. The major factor contributing to the elevated BUN values was renal injury. Terminal coma was probably the result of uremia.

Reference to Table 18 indicates that the daily oral administration of doses ranging from 0.19 to 0.76 mg. Hg/kg. fails to cause clinical signs. This suggests that the susceptible tissues of animals receiving the above dosage range fail to accumulate levels of mercury critical for injury within the period of 90 days.

The hemorrhagic diathesis encountered in some of the experimental animals is considered to be unrelated to the experiment. This statement is based on the following facts: 1) control animals were also affected at the same time; 2) a replicate of the experiment carried out with 10 pigs at a later date reproduced a chronic disease with clinicopathological features identical to the first study except that a hemorrhagic diathesis was not seen; 3) a rodenticide containing warfarin had been used in the building. It is suspected that some of this may have found its way into the feed of the affected groups.

Pathology: The toxic effect of phenylmercuric chloride (PMC was manifested pathologically by changes in the digestive and urinary systems. With the dosage range studied, the target organs were the large intestine, kidneys and liver. The lesions in these organs are considered to be the result of direct toxic effect of mercury. Support for this statement may be obtained from the following. The review of the literature has shown that aryl mercurials are catabolized in the liver and the free mercury excreted via the kidneys and large intestines. In addition, there is the pathological evidence that necrotic colitis and toxic nephrosis occur in cases of inorganic mercury poisoning in man. In these cases, high levels of mercury have been reported in the injured organs. Finally, in terms of tissue mercury levels, the highest were found in the kidney, liver and large intestine of animals receiving PMC. It is reasonable to deduce that injury to the various tissue results from the presence of mercury therein.

A closer examination of the analytical data, especially for kidney, shows that the rate of mercury accumulation plays a role in the determinism of lesions. It will be seen for, instance, that tissue mercury levels in some morphologically normal kidneys were of the same order of magnitude as in kidneys with severe lesions. It appears that in the kidney the more rapid the rate of accumulation of mercury, the more sensitive it is to injury.

The effect of mercury in the large intestine and kidney is characterized by a degenerative and necrotic reaction. In

the kidney, the injury affects the epithelial cells and not other elements. The regenerative activity observed in the proximal tubular epithelium on histological sections is a reaction to this injury.

In the intestine, the primary effect of coagulation necrosis on the epithelium of the large intestine is complicated by the intestinal bacterial flora. The formation of erosions and superficial ulcers covered by a pseudomembrane is considered to be the result of the combined activity of these two factors.

The liver necrosis is also considered to be the result of a direct toxic effect of mercury on the hepatocytes. This is based on the presence of high tissue levels in association with random necrosis of hepatocytes in the hepatic lobules.

Histological examination of tissues with ecchymoses from pigs with the hemorrhagic diathesis failed to reveal the presence of any vascular pathology or lesions other than blood extravasation.

Summary

Phenylmercuric chloride is moderately toxic to pigs if fed for prolonged periods of time.

The disease occurring in this intoxication results from injury to the kidney and large intestine. The primary lesions are pseudomembranous colitis and typhlitis and nephrosis characterized by degeneration, necrosis and regeneration in the epithelium of the proximal tubules.

The pathology of this disease is similar to that described for mercuric chloride poisoning and reflects the ease with which phenylmercuric chloride is metabolized to release free mercuric ions.

Tissue analysis for mercury suggests that only certain target organs such as the kidney and colon accumulate significantly high levels of mercury. This, presumably, results from the rapid metabolism of the compound and the excretion of the mercury ion in kidney and colon. The net effect is to spare other tissues but to injure the excretory organs when the dose level is sufficiently high.

GENERAL DISCUSSION

The experiment revealed that methylmercuric dicyandiamide (MMD), ethylmercuric chloride (EMC) and phenylmercuric chloride (PMC) are poisonous to swine. The toxicity was manifested clinically and was substantiated pathologically and analytically.

The primary toxic effect of these mercurial compounds was evidenced pathologically by degenerative changes in the susceptible cells of target organs. Alkyl mercurials caused enteric and CNS disease, while aryl mercurials caused renal and enteric disease. These findings are consistent with those of Cage (1961) who also found similar qualitative differences in the toxic effects of alkyl and aryl mercury salts. Lesions in target organs were associated with the presence of high tissue levels of mercury.

In general, daily dosage levels ranging from 2.28 to 4.56 mg. Hg/kg. were toxic regardless of organic form. Lower dosages, 0.38 and 0.76 mg. Hg/kg. daily, were toxic only when administered in the form of alkyl compounds (MMD and EMC).

The differences in toxicity are consistent with the fact that, in general, in animals fed alykl mercurials, all tissues (i.e. those analyzed) contained high levels of mercury in comparison to tissue levels in animals receiving comparable amounts of mercury as PMC. One organ, the kidney, proved to be an exception and PMC gave comparatively high levels which were injurious in some cases.

These findings are to be expected when the metabolic behavior of the phenyl mercurial is compared to the alkyl compounds (Whitehead, 1961; Berlin and Ullberg, 1963). Phenylmercuric salts are metabolized by the liver to release mercuric ions. These are excreted via the kidney and large intestine. Therefore, this poisoning mimics that seen with inorganic mercuric salts (Whitehead, 1965). In contrast the alkyl mercurials are only poorly metabolized and excreted (Miller et al., 1961). This gives rise to a persistent relatively high blood level. Also their chemical properties appear to facilitate entrance into the body cells, including those of the central nervous system (Yoshino et al., 1966). The greater toxicity of the alkyl mercurials was related to their neurotoxic properties. One twelfth of the toxic dose of Hg found for phenylmercuric chloride induced neurological disease as an alkyl compound. It is generally accepted that the chemical basis for the toxicity of mercuric ion is its affinity for the -SH groups (Barron and Kalnitsky, 1947; Goodman and Gilman, 1965; Loomis, 1968; Passow et al., 1961). It is also known that many enzymes and other intra- and extracellular substances contain such groups. It is reasonable then to assume that such a combination takes place in vivo and results in malfunction of the biological mechanisms in affected cells. It is conceivable, therefore, that the higher the level of mercury, the more severe the toxic effect. This proved to be the case with all injured organs examined in these experiments. A correlation was established between tissue dosage levels and degree of injury.

The high levels of mercury accumulated in some of the tissues suggests that human or animal consumption of tissues from animals receiving grain treated with alkyl organomercurials may be harmful if consumed for a period of time.

The differential diagnosis of organomercurial poisoning from other clinically similar entities affecting the nervous and gastrointestinal systems of pigs can be based on careful clinical evaluation and the pathological findings and should be confirmed by the analytical results. Reference to Tables 11, 15 and 19 suggest that brain, liver, kidney, large intestine and muscle may be useful to analyze for the presence of mercury when poisoning is suspected.

CONCLUSIONS

Chronic and subacute organomercurial poisoning was induced in young pigs with two alkyl mercurial compounds (methylmercuric dicyandiamide (MMD), and ethylmercuric chloride (EMC), and an aryl mercurial compound (phenylmercuric chloride (PMC)) which were administered orally. The daily dosage range used contained 0.19 - 4.56 mg. Hg/kg. daily.

It was observed that the alkyl mercurials (MMD and EMC) were more toxic than the aryl. In the time period studied (60-90 days), the toxic effect of the two alkyl compounds was manifest with dosage levels as low as 0.38 mg. Hg/kg. daily, while levels as high as 2.28 mg. Hg/kg. daily were needed with the aryl compound. Kidneys, large intestine and liver were target organs common to both classes of compounds. The brain was affected only by the alkyl mercurials.

In poisoning with the alkyl compounds, the chief gross lesions of the chronic form were cerebral atrophy, internal hydrocephalus and small malacic foci in the basal ganglia. In the subacute poisoning, there was pseudomembranous typhlitis and colitis associated with edema in the mesocolon. The chief gross lesions of PMC poisoning were severe pseudomembranous typhlitis and colitis without edema in the mesocolon and swelling of the kidneys.

Histologically, all primary lesions characteristically were degenerative in nature. With the alkyl mercurials, there occurred laminar and diffuse neuronal necrosis in the central nervous system, hydropic degeneration, and necrosis of the renal proximal tubular

epithelium, necrosis of hepatocytes and necrosis of the epithelium in the large intestine. In addition, there were degenerative changes in the cerebral vasculature characterized by fibrinoid degeneration associated, in some cases, with reduplication of the elastica in the small vessels of the leptomeningeal arterial system of the affected cortex. Diffuse gliosis was a prominent finding in the cerebral cortex of chronically affected pigs. In animals with subacute disease, the gliosis was restricted to tissue with neuronal necrosis.

Differential diagnosis of mercury poisoning from other encephalopathies and gastrointestinal disturbances is possible with proper postmortem examination and chemical analysis of brain, liver, kidneys, large intestine and muscle.

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APPENDIX A

APPENDIX A

Method For the Determination of Mercury in Animal Tissue

The method described by Jacobs et al. (1960) was modified to suit these experiments. The method, as modified, was sufficient for the purposes of these experiments, but would probably require refinement for precise toxicological studies.

In this method, the organic matter to be tested is first destroyed through a process of cold digestion brought about by concentrated sulfuric acid and a weak solution of potassium permanganate.

The mercury is then extracted by dithizone, liberated by the destructive distillation of the mercury dithizonate and estimated by the atomic absorption spectrophotometer as a gas in a closed chamber.

Equipment

The type of equipment used, along with short explanatory notes, are given hereunder.

Glassware

- Glass stoppered 125 Pyrex Erlenmeyer flasks for the digestion process.
- Measuring and volumetric pipettes.
- Pyrex separatory funnels, 125 ml.
- Pyrex graduated cylinders, 10 and 25 ml.
- Pyrex ignition tubes, 25 x 200 mm. for vaporizing the mercury dithizonate in the furnace.

Balance

A Sartorius balance with a sensitivity of 0.0001 gm. used for weighing tissue samples and reagents.

Hot Plate

Temperature controlled (0 - 600° F.) "Thermolyne" type 2200 (Fisher Scientific Company).

Separatory Funnel Rack

Wooden - simple construction made in laboratory.

Shaker

Moving platform type, set for 200 r.p.m.

Water Bath

Precision Scientific Company, Model 82.

Spectrophotometer

Atomic absorption spectrophotometer (Evans Model EEL 140). A mercury element was the light source (Froher Company, Code No. 22847).

Furnace

"Multiple Unit - Hevy-Duty". Heating Equipment Company. Temperature controlled and designed to hold a 25 x 200 mm. Pyrex ignition tube.

Gas Train

Air entering the train is passed through a charcoal filter installed in a cork fitting into the Pyrex ignition tube. This tube is

connected to a train comprising a U-tube containing non-absorbent cotton, silica gel and a few pellets of NaOH, a trap, a modified optical cell, a flowmeter, a vacuum pump and an exhaust tube leading outside the building (Fig. 1). The optic cell was 10.1 cm. long by 1.9 cm. in diameter. This size was dictated by the available space in the Evans Model EEL-140 atomic spectrophotometer used.

Recording Device

Photovolt Varicord Model 43.

Reagents

Sulfuric acid (0.25 N solution)

Prepared from concentrated reagent grade sulfuric acid.

Potassium permanganate (6% solution)

Prepared by dissolving 60 grams of reagent grade potassium permanganate in distilled water with the aid of heat and diluted to one liter with distilled water.

Dithizone extraction solution

Weigh out accurately six mg. of purified diphenylthiocarbazone and dissolve in reagent grade chloroform. Transfer to a liter volumetric flask. Make to volume with chloroform.

Hydroxylamine hydrochloride (20% solution)

Dissolve 200 gm. in de-ionized, distilled water. Transfer to a liter volumetric flask. Make to volume with water.

Sulfuric acid

Concentrated reagent grade.

Ammonium hydroxide

Concentrated reagent grade.

Chloroform

Reagent grade.

Standard solutions

Standard stock mercury solution

Exactly 0.1354 gm. of mercury chloride is dissolved in 0.25 N sulfuric acid, and diluted to 100 ml. in a volumetric flask with 0.25 N sulfuric acid. This solution contains one mg. of mercury per ml.

Standard working mercury solution

With the aid of a pipette, one ml. of the stock standard is transferred to a 100 ml. volumetric flask and diluted to the mark with distilled water. This solution contains 10 μg. of mercury per ml., and is the working standard. These solutions must be prepared fresh as required.

Procedure

Preparation of the standard curve

Transfer 1.0 ml. of the working standard (10 μg./ml.) to an Erlenmeyer flask and treat identically as the test samples. One ml. of concentrated dithizone (60 mg./liter) plus one ml. of dilute dithizone (6 mg./liter) were added to extract the mercury. After the extract was delivered into a 10 cc. graduated cylinder, it was further diluted to the 10 cc. mark with reagent grade chloroform. Concentration is now 1.0 μg./ml. This was then mixed and

volumes of 0.1, 0.3, 0.5, 0.8, 1.0 and 1.4 ml. were pipetted into the ignition tubes. These volumes were equivalent to 0.1, 0.3, 0.5, 0.8, 1.0 and 1.4 μg. /ml. of mercury. The percent transmittance readings on the chart recorded which is connected to the A.A.S. are plotted on semi-log paper versus mercury concentration in μg. /ml. (Fig. 2). A line is drawn connecting the points and the unknown are then read directly from the calibrated graph (Fig. 3).

Digestion

1. Transfer 1.0 ml. of blood, bile or urine, or weigh out 0.3 to 0.4 gm. of tissue into a 125 ml. glass stoppered Erlenmeyer flask (record weight) in an ice bath.

2. Carefully add four to five ml. of concentrated sulfuric acid and swirl to dissolve. Warm on a hot plate (300° F.) to assist in digestion, but avoid charring.

3. Replace the flask in the ice bath, chopped ice or snow, and when it is cold add 10 ml. of 6% potassium permanganate solution carefully to avoid violent reaction. Immerse in snow and swirl.

4. When reaction subsides, place on hot plate and warm to 50° F. (feel by hand), but not overheat. Place in snow, allow to cool, re-heat, swirl again and allow to stand for 40 minutes. This procedure may be repeated.

5. Dissolve the precipitate with one ml. of 20% hydroxylamine hydrochloride solution, drop by drop, if more is required to dissolve the precipitate.

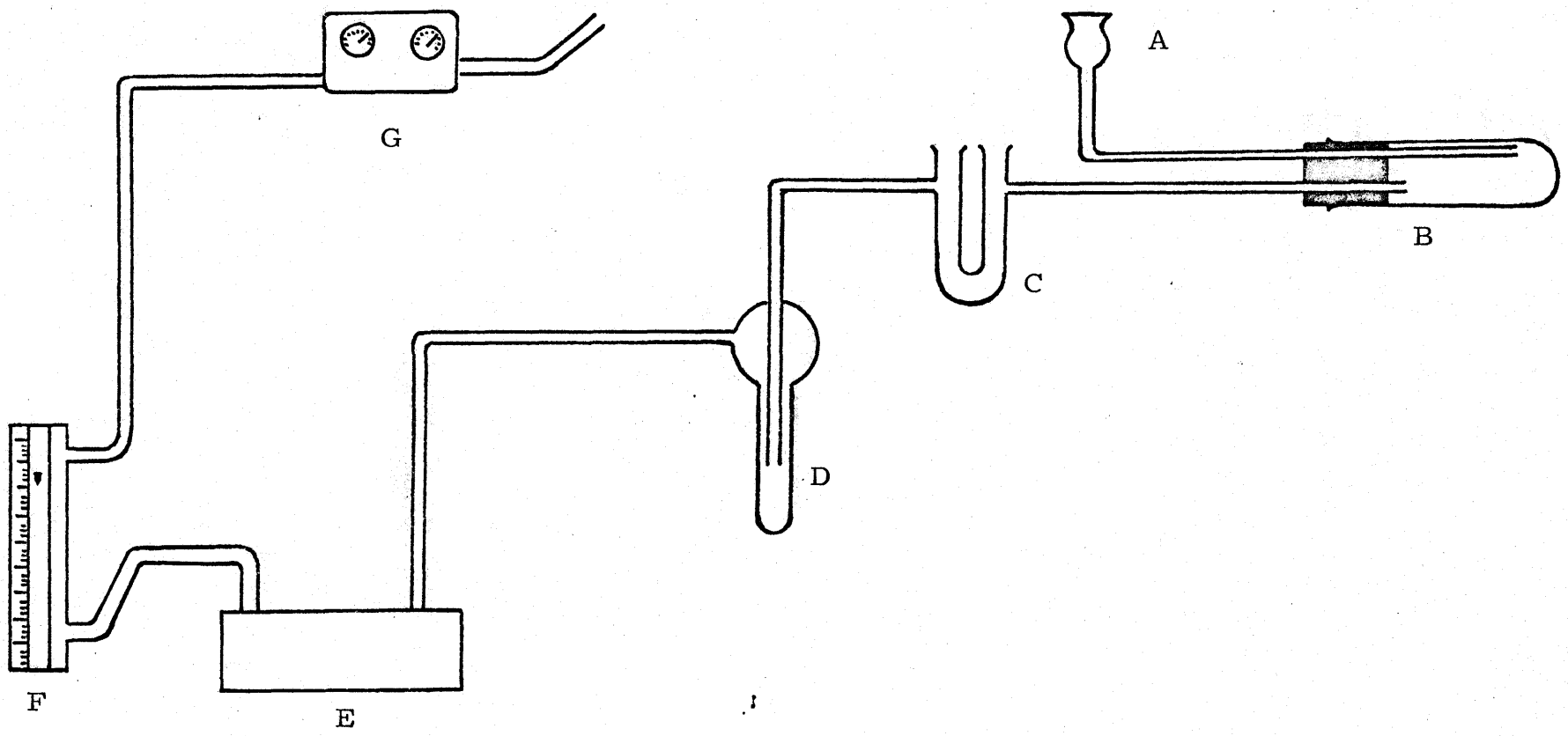

Fig. 1. A diagram showing the components of the train used to estimate mercury. A= air inlet with filter. B= Pyrex ignition tube. C= U-tube containing non-absorbent cotton, silica gel and a few pellets of NaOH. D= trap. E= modified optical cell. F= flowmeter. G= Aspiration pump.

6. Add 20 ml. distilled water, shake and transfer to dry 125 ml. separatory funnels. Wash the Erlenmeyer flask with two to three 20 ml. portions of distilled water until volume in separatory funnel is approximately 85 ml.

If pH is not between 1.0 and 1.5, the solution is adjusted with concentrated ammonium hydroxide.

Extraction

1. To the separatory funnels, add two ml. of dithizone solution of either six or 60 mg./liter. Place the funnels on the shaker (200 r.p.m.) for one minute. Release pressure slowly, allow to settle and drain off the dithizone layer. The volume and concentration of dithizone utilized is dependent on the nature of the tissue and the expected mercury concentration in that tissue, e.g., liver would require more dithizone for complete extraction than would muscle. If the dithizone layer remains green, a sufficient amount has been added; however, if the same layer turns orange, more dithizone must be added, since this color is formed when dithizone chelates with mercury and there must be an excess of dithizone to assume complete extraction. Swirl to bring down droplets of chloroform.

2. Draw off the dithizone layer very carefully into a graduated cylinder. Add two ml. of chloroform, reshake for 15 seconds to rinse the funnel of remaining traces of dithizone, and allow the phases to separate. Then the chloroform rinsing must be added to the graduated cylinder.

3. The extract volume is recorded. Depending upon the amount of mercury present, it may be necessary to dilute the extract volume with chloroform if a high mercury concentration is suspected. It may be necessary to bring the final extracted volume to a q. s. of 10, 15 or 25 ml.

4. Transfer with the aid of a pipette 0.1 to 1.0 ml., depending on anticipated concentration, of chloroform solution of mercury dithizonate to a 25 x 200 mm. Pyrex ignition tube. Place in water bath at 70° C. for one hour, or until all chloroform is evaporated. Allow tubes to cool at room temperature.

Estimation of mercury

1. Switch on the atomic absorption spectrophotometer and furnace at least 30 minutes before use. Furnace temperature must reach 1300° F.

2. Start aspirator pump and see that the air flow is approximately 3.2 liters/minute.

3. Check that instruments are properly adjusted.

4. Insert ignition tube containing mercury into the furnace to within one inch of its mouth.

5. After the peak of deflection has been reached, remove the tube from the furnace. Allow to aspirate room air for 30 seconds and repeat the procedure with the next ignition tube.

6. Run standards each time so that a graph can be calibrated from which the percent transmittance readings of the test samples may be directly used to obtain the mercury concentration.

7. The final calculation is given by:

$$\frac{\text{(Hg concentration as determined) x Extract volume from standard curve}}{\text{Sample weight x Extract sample volume}}$$

8. After samples have been processed, turn off recorder, spectrophotometer and heating chamber. Allow pump to run. Pass air through the train for five minutes.

Table 1 gives an example of levels of recovery of mercury by this method.

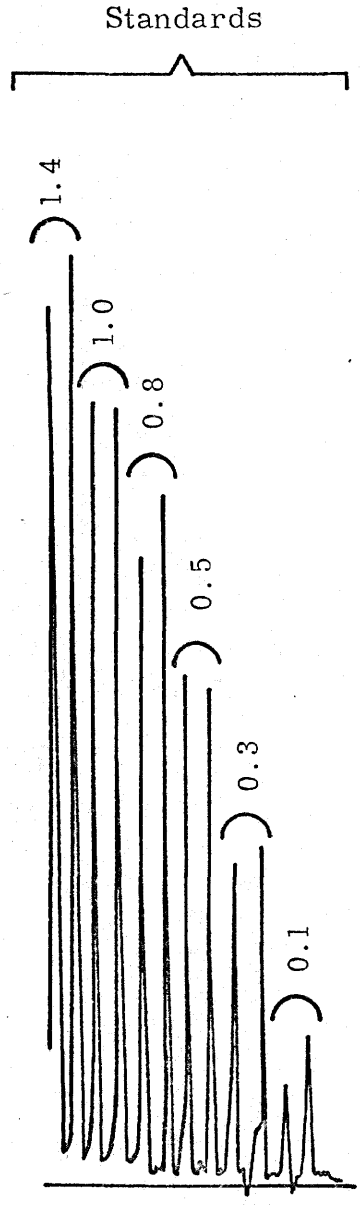

Fig. 2. Examples of atomic absorption deflection curves obtained with the Evans spectrophotometer. The peak deflection is taken as the arbitrary percent transmission.

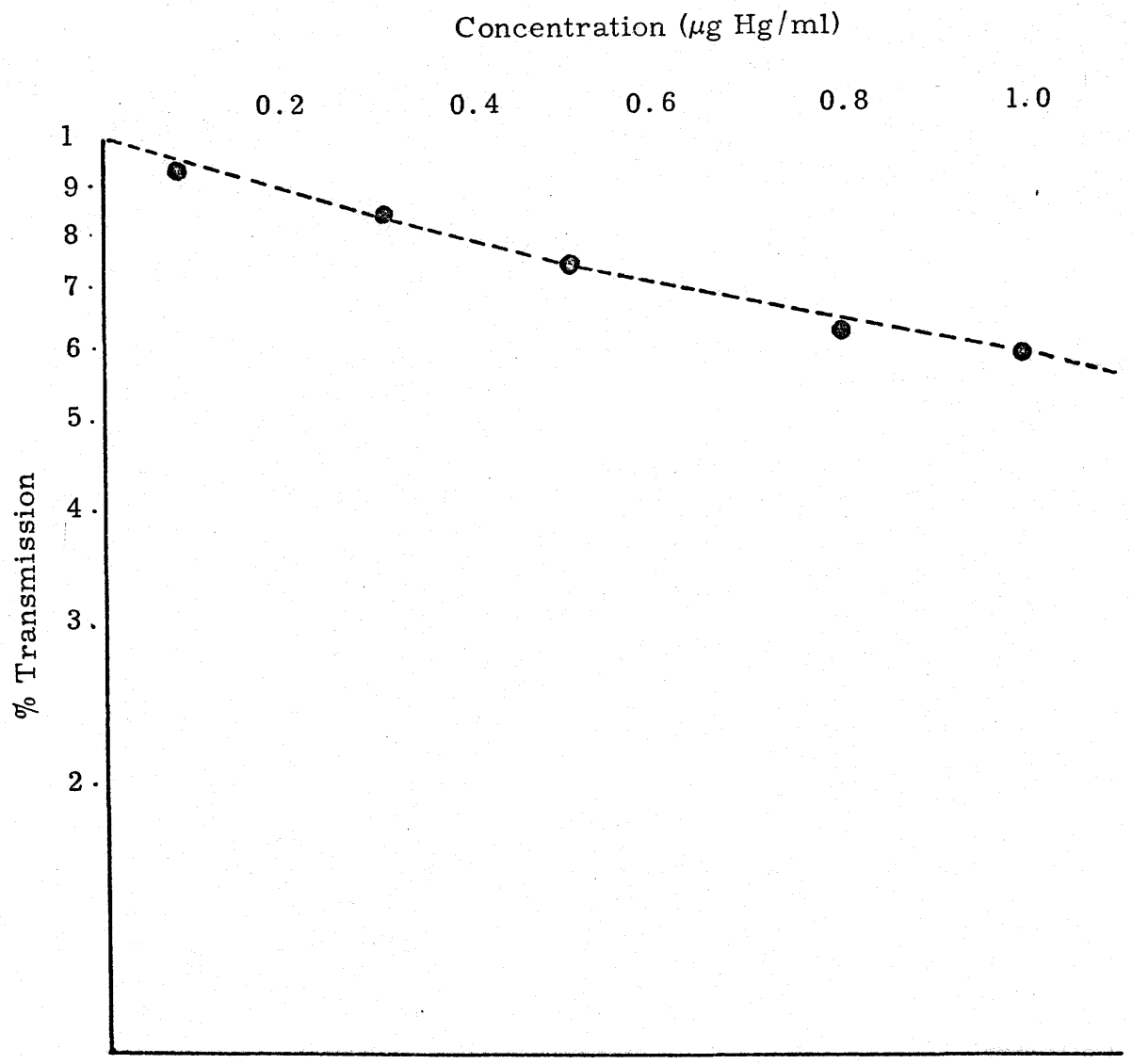

Fig. 3. Example of standard curve used for mercury recovery.

TABLE 1. The % recovery of mercury added as MMD to a sample of liver.

Sample	Liver Weight Gm.	Hg added µg. /1 ml. as MMD	Mercury recovered	
			µg.	%
1	0.351	10	9.7	97
2	0.312		9.5	95
3	0.309		9.7	97
4	0.248		9.5	95
5	0.257	50	48.5	97
6	0.223		47.5	95
7	0.337		41.5	92
8	0.385		59.4	118
9	0.235	100	91.0	91
10	0.465		80.0	80
11	0.304		92.5	92.5
12	0.282		83.5	83.5

APPENDIX B

TABLE 1. Type and Distribution of Histological Lesions in the Cerebrospinal Axis of Pigs Poisoned With Methylmercuric Dicyandiamide

GROUP DOSE	PIG NUMBER	DAYS	Neurons			Glia		Vasculature												Hypercellularity in Sulcus Leptomeninges
								Small Arteries			Large Arteries									
			Restricted Laminar Necrosis	Diffuse Laminar Necrosis	Mineralization	Astrogliosis	Microgliosis	Capillary Endothelial Proliferation	Arteriolar Fibrinoid Degeneration	Fibrinoid Degeneration	Thickening of Wall	Luminal Obstruction	Intimal Proliferation, Luminal Obstruction	Thickening and Reduplication of Elastica	Sub-intimal Fibrinois	Smooth Muscle Hypertrophy	Smooth Muscle Necrosis	Adventitial Proliferation	Periarteritis	
I (0.19)	1	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II (0.38)	5	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	60	-	●	●	●	●	●	-	●	●	●	●	●	●	●	●	●	●	●
III (0.76)	8	44	-	●	●	●	●	●	-	-	-	-	-	-	-	-	-	-	-	●
	9	41	-	●	-	●	●	-	-	-	-	-	-	-	-	-	-	-	-	●
	10	44	●	●	-	●	●	●	●	●	●	●	●	●	●	●	-	●	●	●
	11	46	●	●	●	●	●	●	●	●	●	●	●	-	●	-	-	-	-	●
IV (1.52)	13	25	●	●	●	●	●	●	●	●	●	●	●	-	-	-	-	-	-	●
	14	28	●	●	●	●	●	●	●	●	●	-	-	-	-	-	-	-	-	●
	15	29	●	●	●	●	●	●	●	●	●	-	-	-	-	-	-	-	-	●
	16	30	●	●	●	●	●	●	●	●	●	-	-	-	-	-	-	-	-	●
V (3.04)	18	19	●	-	-	●	●	●	-	-	-	-	-	-	-	-	-	-	-	●
	19	15	●	-	-	●	●	●	-	-	-	-	-	-	-	-	-	-	-	●
	20	15	●	-	-	●	●	●	-	-	-	-	-	-	-	-	-	-	-	●
	21	16	●	-	-	●	●	●	-	-	-	-	-	-	-	-	-	-	-	●
Control	A	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	D	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	E	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 2. Distribution of Neuronal Necrosis in the Cerebrospinal Axis of Pigs Receiving Varying Doses of MMD.

GROUP DOSE	ANIMALS	NEURONAL NECROSIS																							
			Basoganglia				Thalamus					Hypothalamus				Mid-Brain				Metencephalon			Spinal Cord		
		Cerebral Cortex	Nucleus Caudatus	Capsula Interna	Putamen	Globus Pallidus	Anterior Nuclear Group	Medial Nuclear Group	Lateral Nuclear Group	Pulvinar	Lateral Geniculate Body	Medial Geniculate Body	Supraoptic Nerve	Tuber Cinereum	Mamillary Body	Tectum	Cerebral Peduncles	Central Grey Matter	Red Nucleus	Pontine Nuclei	Semi-lunar Ganglion	Cerebellum	Grey Matter	Dorsal Root Ganglia	Coeliac Ganglia
I (0.19)	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II (0.36)	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7	●	-	-	-	-	-	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III (0.76)	8	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	9	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	●	-	-	-	-	-	-
	11	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IV (1.52)	13	●	-	-	-	-	-	●	-	-	●	●	-	-	-	-	-	-	●	●	●	-	-	●	-
	14	●	-	-	-	-	-	●	-	-	●	●	-	-	-	-	-	-	●	●	●	-	-	●	-
	15	●	-	-	-	-	-	●	-	-	●	●	-	-	-	-	-	-	●	●	●	-	-	●	-
	16	●	-	-	-	-	-	●	-	-	●	●	-	-	-	-	-	-	●	●	●	-	-	●	-
V (3.04)	18	●	●	-	●	-	-	●	-	-	●	●	-	-	-	-	-	-	●	-	●	-	●	●	-
	19	●	●	-	●	-	-	●	-	-	●	●	-	-	-	-	-	-	●	●	●	-	●	●	-
	20	●	●	-	●	-	-	●	-	-	●	●	-	-	-	-	-	-	●	●	●	-	●	●	-
	21	●	●	-	●	-	-	●	-	-	●	●	-	-	-	-	-	-	●	●	-	-	●	●	-
Control	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 3. Type and Distribution of Lesions in the Kidneys and Liver of Pigs Receiving Varying Doses of MMD.

GROUP DOSE	ANIMALS	Kidney								Liver			
			Proximal Tubule										
		Proteinaceous Material in Bowman's Space	Hydropic Degeneration	Proteinaceous Casts	Epithelial Cell Necrosis	Necrotic Cells in Lumen	Calcification of Necrotic Cells	Regeneration of Epithelium	Mitotic Activity	Hydropic Degeneration	Fatty Degeneration	Centrolobular Necrosis	Random Necrosis
I (0.19)	1	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-	-	-	-	-
II (0.36)	5	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-	-	-	-	-
III (0.76)	8	-	-	-	-	-	-	-	-	-	-	-	-
	9	-	-	-	-	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-	-	-	●	-
	11	-	-	-	-	-	-	-	-	-	-	-	-
IV (1.52)	13	-	●	-	-	-	-	-	●	-	-	●	-
	14	-	●	-	-	-	-	-	-	-	-	●	-
	15	-	●	-	-	-	-	-	-	-	-	●	-
	16	-	●	-	-	-	-	-	-	-	-	●	-
V (3.04)	18	-	●	-	-	-	-	-	-	-	-	-	●
	19	-	●	-	-	-	-	-	-	-	-	-	●
	20	-	●	-	-	-	-	-	-	-	-	-	●
	21	-	●	-	-	-	-	-	-	-	-	-	●
Control	A	-	-	-	-	-	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-	-	-	-	-	-
	D	-	-	-	-	-	-	-	-	-	-	-	-
	E	-	-	-	-	-	-	-	-	-	-	-	-

APPENDIX C

TABLE 1. This Table Summarizes the Type and Distribution of Histological Lesions in the Cerebrospinal Axis of Pigs Poisoned with Ethylmercuric Chloride

GROUP DOSE	PIG NUMBER	DAYS	Neurons			Glia		Vasculature												Hypercellularity in Sulcus Leptomeninges
								Small Arteries				Large Arteries								
			Restricted Laminar Necrosis	Diffuse Laminar Necrosis	Mineralization	Astrogliosis	Microgliosis	Capillary Endothelial Cell Proliferation	Arteriolar Fibrinoid Degeneration	Fibrinoid Degeneration	Thickening of Wall	Luminal Obstruction	Intimal Proliferation, Luminal Obstruction	Thickening and Reduplication of Elastica	Subintimal Fibrinois	Smooth Muscle Hypertrophy	Smooth Muscle Necrosis	Adventitial Proliferation	Periarteritis	
I (0.19)	751	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	752	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	753	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	754	71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	755	65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II (0.38)	756	90	●	●	-	●	●	●	-	-	●	-	●	●	-	-	-	●	-	●
	757	60	-	●	●	●	●	-	-	-	●	●	●	●	●	●	●	●	-	●
	758	75	-	●	●	●	●	-	-	-	●	●	●	●	●	●	●	●	-	●
	759	90	●	●	-	●	●	●	-	-	●	-	●	●	-	-	-	●	-	●
	760	44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III (0.76)	761	22	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	762	30	●	-	-	●	●	●	-	-	●	-	-	-	-	-	●	●	-	●
	763	30	●	-	-	●	●	●	-	-	●	-	-	-	-	-	●	●	-	●
	764	22	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	765	22	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IV (2.28)	766	14	●	-	-	●	●	-	-	-	-	-	-	-	-	-	-	-	-	-
	767	18	●	-	-	●	●	●	-	-	-	-	-	-	-	-	-	-	-	-
	768	18	●	-	-	●	●	●	-	-	-	-	-	-	-	-	-	-	-	-
	769	14	●	-	-	●	●	-	-	-	-	-	-	-	-	-	-	-	-	-
	770	14	●	-	-	●	●	-	-	-	-	-	-	-	-	-	-	-	-	-
V (4.56)	771	11	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	772	11	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	773	11	●	-	-	-	-	●	-	-	-	-	-	-	-	-	-	-	-	●
	774	11	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	775	12	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Control	776	49	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	777	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	778	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	779	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	780	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 2. Distribution of Neuronal Necrosis in the Cerebrospinal Axis of Pigs Receiving Ethylmercuric Chloride

GROUP DOSE	PIG NUMBER	DAYS	Neuronal Necrosis																								
			Basoganglia					Thalamus						Hypothalamus			Midbrain				Metenceph.			S. Cord		
			Cerebral Cortex	Nucleus Caudatus	Capsula Interna	Putamen	Globus Pallidus	Anterior Nuclear Group	Medial Nuclear Group	Lateral Nuclear Group	Pulvinar	Lateral Geniculate Body	Medial Geniculate Body	Supraoptic Nerve	Tuber Cinereum	Mammillary Body	Tectum	Cerebral Peduncles	Cortical Grey Matter	Red Nucleus	Pontine Nuclei	Semilunar Ganglion	Cerebellum	Grey Matter	Dorsal Root Ganglia	Celiac Ganglia
I (0.19)	751	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	752	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	753	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	754	71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	755	65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II (0.38)	756	90	●	●	-	-	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	757	60	●	●	-	-	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	758	75	●	●	-	-	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	759	90	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	760	44	-	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III (0.76)	761	22	-	-	-	-	-	-	-	-	-	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	762	30	-	-	-	-	-	-	●	-	-	●	-	-	-	-	-	-	-	●	●	●	-	-	●	-
	763	30	-	-	-	-	-	-	●	-	-	●	-	-	-	-	-	-	-	●	●	●	-	-	●	-
	764	22	-	●	●	-	-	-	●	-	-	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	765	22	-	-	-	-	-	-	-	-	-	●	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IV (2.28)	766	10	●	●	-	-	-	-	●	-	-	●	-	-	-	-	-	-	-	●	●	●	-	●	●	-
	767	18	-	●	-	-	-	-	●	-	-	●	-	-	-	-	-	-	-	●	●	●	-	●	●	-
	768	18	-	●	-	-	-	-	●	-	-	●	-	-	-	-	-	-	-	●	●	●	-	●	●	-
	769	14	●	●	-	-	-	-	●	-	-	●	-	-	-	-	-	-	-	●	●	●	-	●	●	-
	770	14	●	●	-	-	-	-	●	-	-	●	-	-	-	-	-	-	-	●	●	●	-	●	●	-
V (4.56)	771	11	●	-	-	-	-	-	●	-	-	●	-	-	-	-	-	-	-	●	●	●	-	●	●	-
	772	11	●	-	-	-	-	-	●	-	-	●	-	-	-	-	-	-	-	●	●	●	-	●	●	-
	773	11	●	-	-	-	-	-	●	-	-	●	-	-	-	-	-	-	-	●	●	●	-	●	●	-
	774	11	●	-	-	-	-	-	●	-	-	●	-	-	-	-	-	-	-	●	●	●	-	●	●	-
	775	12	●	-	-	-	-	-	●	-	-	●	-	-	-	-	-	-	-	●	●	●	-	●	●	-
Control	776	49	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	777	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	778	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	779	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	780	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 3. Type and Distribution of Histological Lesions in Kidneys of Pigs Receiving Varying Doses of Ethylmercuric Chloride

GROUP DOSE	PIG NUMBER	DAYS	Kidneys							
				Proximal Tubules						
			Proteinaceous Material in Bowman's Space	Hydropic Degeneration	Proteinaceous Casts	Epithelial Cell Necrosis	Necrotic Cells in Lumen	Calcification of Necrotic Cells	Regeneration of Epithelium	Mitotic Activity
I (0.19)	751	90	-	-	-	-	-	-	-	-
	752	90	-	-	-	-	-	-	-	-
	753	90	-	-	-	-	-	-	-	-
	754	71	-	-	-	-	-	-	-	-
	755	65	-	-	-	-	-	-	-	-
II (0.38)	756	90	-	●	●	●	●	-	-	-
	757	64	-	●	●	●	●	-	-	-
	758	75	-	●	●	●	●	-	-	-
	759	90	-	●	●	●	●	-	-	-
	760	44	-	●	●	●	●	-	-	-
III (0.76)	761	22	-	-	●	●	●	-	-	●
	762	30	-	-	●	●	●	-	-	●
	763	30	-	-	●	●	●	-	-	●
	764	22	-	-	●	●	●	-	-	●
	765	22	-	-	●	●	●	-	-	●
IV (2.28)	766	14	-	●	●	●	-	-	-	●
	767	18	-	●	●	●	-	-	-	●
	768	18	-	●	●	●	-	-	-	●
	769	14	-	●	●	●	-	-	-	●
	770	14	-	●	●	●	-	-	-	●
V (4.56)	771	11	-	●	●	●	●	-	-	-
	772	11	-	●	●	●	●	-	-	-
	773	11	-	●	●	●	●	-	-	-
	774	11	-	●	●	●	●	-	-	-
	775	12	-	●	●	●	●	-	-	-
Control	776	49	-	-	-	-	-	-	-	-
	777	90	-	-	-	-	-	-	-	-
	778	90	-	-	-	-	-	-	-	-
	779	14	-	-	-	-	-	-	-	-
	780	50	-	-	-	-	-	-	-	-

TABLE 4. Type and Distribution of Histological Lesions in the Large Intestine and Liver of Pigs Receiving Varying Doses of Ethylmercuric Chloride

GROUP DOSE	PIG NUMBER	DAYS	Large Intestine														Liver			
			Mucosa							Submucosa					Meso-colon					
			Necrosis of Surface Epithelium	Separation of Surface Epithelium	Pseudomembrane Formation	Crypt Dilatation	Erosions	Superficial Ulcers	Congestion	Edema	Round Cell Infiltration	Leukocyte Infiltration	Granulation Tissue	Fibrinoid Necrosis in Arteries	Edema	Fibrinoid Necrosis in Arteries	Hydropic Degeneration	Fatty Degeneration	Centrolobular Necrosis	Random Necrosis
I (0.19)	751	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	752	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	753	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	754	71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	755	65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II (0.38)	756	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	●	-	-	-
	757	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	●	-	-	-
	758	75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	●	-	-	-
	759	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	●	-	-	-
	760	44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	●	-	●	-
III (0.76)	761	22	●	●	-	-	-	-	-	-	-	-	-	-	-	-	●	-	-	●
	762	30	●	●	-	-	-	-	●	●	-	●	-	●	-	-	●	-	-	●
	763	30	●	●	-	-	-	-	-	-	-	-	-	-	-	-	●	-	-	●
	764	22	●	●	-	-	-	-	-	-	-	-	-	-	-	-	●	-	-	●
	765	22	●	●	-	-	-	-	-	-	-	-	-	●	-	-	●	-	-	●
IV (2.28)	766	14	●	●	●	-	-	-	●	●	-	-	-	●	●	-	●	-	-	●
	767	18	●	●	●	-	-	-	●	●	-	-	-	●	●	-	●	-	-	●
	768	18	●	●	●	-	-	-	●	●	-	-	-	●	●	-	●	-	-	●
	769	14	●	●	●	-	-	-	●	●	-	-	-	●	●	-	●	-	-	●
	770	14	●	●	●	-	-	-	●	●	-	-	-	●	●	-	●	-	-	●
V (4.56)	771	11	-	●	-	●	-	-	-	●	-	-	-	-	-	-	-	-	-	●
	772	11	-	●	-	●	-	-	-	●	-	-	-	-	-	-	-	-	-	●
	773	11	-	●	-	●	-	-	-	●	-	-	-	-	-	-	-	-	-	●
	774	11	-	●	-	●	-	-	-	●	-	-	-	-	-	-	-	-	-	●
	775	12	-	●	-	●	-	-	-	●	-	-	-	-	-	-	-	-	-	●
Control	776	49	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	777	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	778	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	779	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	780	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

APPENDIX D

TABLE 1. Type and Distribution of Histological Lesions in Kidneys of Pigs Receiving Varying Doses of Phenylmercuric Chloride

GROUP DOSE	PIG NUMBER	DAYS	Kidneys							
				Proximal Tubules						
			Proteinaceous Material in Bowman's Space	Hydropic Degeneration	Proteinaceous Casts	Epithelial Cell Necrosis	Necrotic Cells in Lumen	Calcification of Necrotic Cells	Regeneration of Epithelium	Mitotic Activity
I (0.19)	952	14	-	-	-	-	-	-	-	-
	953	39	-	-	-	-	-	-	-	-
	954	90	-	-	-	-	-	-	-	-
	955	90	-	-	-	-	-	-	-	-
	956	90	-	-	-	-	-	-	-	-
II (0.38)	996	39	-	-	-	-	-	-	-	-
	997	40	-	-	-	-	-	-	-	-
	998	90	-	-	-	-	-	-	-	-
	999	90	-	-	-	-	-	-	-	-
	1000	65	-	-	-	-	-	-	-	-
III (0.76)	992	90	-	-	-	-	-	-	-	-
	993	14	-	-	-	-	-	-	-	-
	951	90	-	-	-	-	-	-	-	-
	994	90	-	-	-	-	-	-	-	-
	995	50	-	-	-	-	-	-	-	-
IV (2.28)	987	14	-	-	-	-	-	-	-	●
	988	3	-	-	-	-	-	-	-	●
	989	50	-	-	●	●	●	-	●	●
	990	65	-	-	●	●	●	-	●	●
	991	34	-	-	●	●	●	-	●	●
	986	35	-	-	●	●	●	-	●	●
V (4.56)	982	25	-	-	●	●	●	-	●	●
	983	6	-	-	-	-	-	-	-	-
	984	1	-	-	-	-	-	-	-	-
	985	3	-	-	-	-	-	-	-	-
Control	977	-	-	-	-	-	-	-	-	-
	978	-	-	-	-	-	-	-	-	-
	979	-	-	-	-	-	-	-	-	-
	980	-	-	-	-	-	-	-	-	-
	981	-	-	-	-	-	-	-	-	-

TABLE 2. Type and Distribution of Histological Lesions in the Large Intestine and Liver of Pigs Receiving Varying Doses of Phenylmercuric Chloride

GROUP DOSE	PIG NUMBER	DAYS	Large Intestine													Liver			
			Mucosa							Submucosa					Mesocolon				
			Necrosis of Surface Epithelium	Separation of Surface Epithelium	Pseudomembrane Formation	Crypt Dilatation	Erosions	Superficial Ulcers	Congestion	Edema	Round Cell Infiltration	Leukocyte Inf'ltration	Granulation Tissue	Fibrinoid Necrosis in Arteries	Edema	Fibrinoid Necrosis in Arteries	Hydropic Degeneration	Fatty Degeneration	Centrolobular Necrosis	Random Necrosis
I (0.19)	952	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	953	39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	954	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	955	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	956	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II (0.38)	996	39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	997	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	998	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	999	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1000	65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III (0.76)	992	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	993	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	951	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	994	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	995	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IV (2.28)	987	14	●	-	●	●	●	●	●	-	-	●	-	-	-	-	●	-	-	-
	988	3	●	-	●	●	●	●	●	-	-	●	-	-	-	-	●	-	-	-
	989	50	●	-	●	●	●	●	●	-	-	●	-	-	-	-	●	-	-	-
	990	65	●	-	●	●	●	●	●	-	-	●	-	-	-	-	●	-	-	-
	991	34	●	-	●	●	●	●	●	-	-	●	-	-	-	-	●	-	-	-
V (4.56)	982	25	●	-	●	●	●	●	●	-	-	●	●	-	-	-	●	-	-	●
	983	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	984	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	985	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	986	34	●	-	●	●	●	●	●	-	-	●	●	-	-	-	●	-	-	●
Control	977	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	978	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	979	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	980	90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	981	70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-