THE EFFECTS OF ASCORBIC ACID DEFICIENCY ON METHYL MERCURY TOXICOSIS IN THE GUINEA PIG

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ABSTRACT

THE EFFECTS OF ASCORBIC ACID DEFICIENCY ON METHYL MERCURY TOXICOSIS IN THE GUINEA PIG

By

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A series of 3 experiments was conducted to study the effects of ascorbic acid deficiency on methyl mercury toxicosis in guinea pigs.

In Experiment I, 16 guinea pigs were divided equally into 4 groups, 2 of which were fed a normal diet and 2 of which were fed an ascorbic acid deficient diet. By 14 days, when ascorbic acid concentration decreased in plasma, a sublethal dose (4 mg/kg body weight) of methyl mercury dicyandiamide (MMD) was injected intraperitoneally (IP) at weekly intervals into one group of guinea pigs on each diet. The guinea pigs fed the ascorbic acid deficient diet died after the second or third injection due to extensive hemorrhagic peritonitis. There were no pathologic changes in guinea pigs fed the normal diet except one which died after the first injection as a result of a perforated colon.

In Experiment II, 20 guinea pigs were divided equally into 4 groups, as described in the previous experiment. On Day 15, 22 p.p.m. MMD was added to the diet of one group of guinea pigs fed the normal diet and one group fed the ascorbic acid-deficient diet. The main lesions in guinea pigs fed the MMD-containing, ascorbic acid-deficient

diet and which died on Days 18 and 26 were extensive hemorrhagic ulcerative gastroenteritis and coagulative necrosis of the liver. Two guinea pigs in the group fed the MMD-containing normal diet died as a result of chronic toxicosis in 150 days.

In Experiment III, the 23 guinea pigs were divided as in Experiments I and II. The design was changed in that 44 p.p.m. MMD was used instead of 22 p.p.m. and was incorporated into the appropriate diets on Day 1 instead of Day 15. The guinea pigs fed the MMD-containing, ascorbic acid-deficient diet died on Days 17 to 20 with acute neurologic signs, and the pathologic changes were mostly polioclastic. The guinea pigs fed MMD in a normal diet survived up to 38 days. The ascorbic acid-deficient controls survived up to 47 days.

The experiments indicate that the degree of ascorbic acid deficiency is important in the location and severity of clinical signs and lesions due to methyl mercury toxicosis.

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Parvaneh, Bakhtiar and Seriti

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INTRODUCTION

The alkyl compounds of mercury may present a significant human and animal hazard. These compounds are easily absorbed through the respiratory or gastrointestinal routes and are only slowly eliminated from the body. They can cause serious damage to the central nervous system.

Considerable work has been done on mercury toxicosis and its clinical and pathological effects in different species, but apparently little attention has been given to mercury toxicosis in combination with nutritional imbalances. Recent reports on the vascular lesions associated with methyl mercury toxicosis and the well known effect of ascorbic acid deficiency on vascular permeability suggest that an ascorbic acid-deficient animal might be more susceptible to methyl mercury toxicosis. If a blood vessel is more permeable during a deficiency, it is logical to postulate that the potential for greater damage by toxic substances would be increased. It is also possible that tissue surrounding such a damaged vessel would also suffer greater damage from a toxic agent than tissue surrounding an unaffected vessel.

Either a toxicosis due to methyl mercury or an ascorbic acid deficiency may not be severe enough to produce any apparent harm when occurring alone. In combination these two factors may result in the death of an individual who otherwise might have survived.

In the present research the objectives were to clarify the clinical effects of methyl mercury toxicosis in ascorbic acid-deficient guinea

pigs and to identify the gross and microscopic changes associated with that condition.

REVIEW OF LITERATURE

Mercury

It is well known that the signs and lesions of mercury toxicosis vary as to whether the mercury is in inorganic or organic form. In this review the emphasis will be on organic mercury toxicosis with considerable emphasis on the effects on the nervous system.

The most serious damage to the central nervous system in mercury toxicosis in animals and man has resulted from exposure to one form of organic mercury, the short chain alkyl mercury compounds (Hunter *et al.*, 1940; Hunter and Russell, 1954; Takeuchi *et al.*, 1962; Suzuki, 1969; Tryphonas and Nielsen, 1970).

These compounds are commonly designated by the formula R-Hg-X, where R is the short chain alkyl radical (invariably an ethyl or methyl group) and X denotes the associated anion (Dales, 1972). The nature of the anion has little effect on the subsequent metabolic behavior of the compound. Alkyl mercury has a strong affinity for amino acid sulfhydryl groups and rather quickly becomes bound to proteins or polypeptide chains (Hughes, 1957; Dales, 1972). In the body, alkyl mercury appears to pass readily into all tissue compartments (Aberg *et al.*, 1969). Gage (1964) reported that concentrations are highest in hair. In the blood, concentrations of alkyl mercury in erythrocytes are 10 times higher than plasma concentrations (Aberg *et al.*, 1969). In this respect they differ clearly from inorganic mercury salts which are bound mainly to plasma proteins (Swensson and Ulfvarson, 1963). Alkyl

mercury is found in brain tissue later than in other tissues but the bulk of the alkyl mercury in the head region is presumed to be in brain tissue (Aberg *et al.*, 1969; Berglund and Berlin, 1963).

Absorption, Metabolism and Excretion

Alkyl mercury commonly enters the body by the respiratory tract, the gastrointestinal tract or the skin (Swensson and Ulfvarson, 1963).

Data from man and experimental animals indicated that the carbon-mercury bond in these compounds is not broken to a significant extent, and most of the mercury compounds entering the body as the alkyl compound is also excreted as the alkyl mercury compound (Gage, 1964). As already mentioned, it is largely bound to sulfhydryl groups or other ligands of amino acids.

The excretion rate of the alkyl-mercury compound is slow in all species studied. Healthy human adults receiving subtoxic doses of alkyl mercury compounds excrete approximately 1% of the total body load daily. This results in an alkyl mercury biological half-life in man of 70 to 74 days (Aberg *et al.*, 1969; Löfroth, 1970). In fish the mercury biological half-life is approximately 200 days (Hammond, 1971). The gut is the main route of excretion. Only small amounts of alkyl mercury leave the body in the urine (Swensson and Ulfvarson, 1963; Aberg *et al.*, 1969). Most of the mercury apparently reaches the gut by way of the bile (Battigelli, 1960), but in mice there was evidence that the intestinal mucosa may also excrete the compound (Berglund and Berlin, 1969).

Accumulation of Alkyl Mercury in the Body

When a given dose (α) is regularly consumed over an extended period of time, body burden and excretion (β) per unit time increase

until α equals β. At that point an equilibrium is reached and body burden plateaus despite continuing intake. In fish-eating people in Sweden equilibrium is reached in 3 to 10 months (Aberg *et al.*, 1969).

One cannot pick a single critical environmental concentration for alkyl mercury. Toxic accumulation does not occur unless the rate of intake exceeds the rate of elimination from the body. The rate of intake is determined by the frequency, intensity and duration of exposure (Dales, 1972). Selenium compounds may protect the organism against the toxic effect of bivalent mercury. These effects seem to be associated with a change in the chemical reactivity and distribution of mercury in the animal (Underwood, 1971).

Estimation of the level of mercury in the body can be secured by 3 methods. They involve determining the amount of mercury in: urine (Jacobs *et al.*, 1964), blood and hair (Berglund and Berlin, 1969). Perhaps because alkyl mercury compounds have a strong affinity for red blood cells, the blood mercury level is apparently a good indicator of total body mercury load when exposure has been primarily to alkyl mercury compounds (Berglund and Berlin, 1969). They suggested that, after equilibrium has been reached in an organism, blood mercury levels can be reliably correlated with both intake of mercury and concentrations in other tissues. Calculation based on data from man and experimental animals indicated that serious neurotoxicity from alkyl mercury occurs at brain concentrations as low as 10 µg/gm, which in man corresponds to a blood mercury concentration of 50 to 100 µg/100 ml (Suzuki, 1969; Katsunuma *et al.*, 1963). Blood levels of 10 to 20 µg/100 ml have not been associated with symptoms and have been proposed as the maximum permissible blood concentration. Concentrations of mercury in hair of 400 to 500 µg/gm and above are likely to be associated with manifestations

of neurotoxicity (Berglund and Berlin, 1969). A combined Food and Agriculture Organization (FAO) and World Health Organization (WHO) committee assembled in 1966 and recommended a tentative practical residue limit of 0.02 to 0.05 mg/kg for mercury concentration in food, whatever the form of the mercury (FAO working party and the WHO expert committee, 1968).

Mercury Toxicosis

Industrial and agricultural uses for mercury have resulted in a variety of ways in which alkyl mercury compounds find their way into the environment in such a way as to cause a health hazard. Alkyl mercury compounds have been used for their fungicidal properties and have been incorporated into seed dressings, folial sprays and preservative solutions for wood, paper pulp and textiles (Swensson and Ulfvarson, 1963).

Inorganic mercury compounds have a myriad of industrial uses (Goldwater, 1963; Brieger and Rieders, 1959). It has been clearly demonstrated that microorganisms can and do convert divalent mercury to methyl mercury compounds under aerobic and anaerobic conditions. This conversion can ultimately result in the addition of alkyl mercury compounds to biologic food chains (Jernelov, 1969).

Alkyl mercury compounds have caused toxicosis in different species directly or indirectly. In Minamata, Japan (1953 to 1960), at least 121 persons were poisoned by eating fish from Minamata Bay (Takeuchi *et al.*, 1962). In 1962, 120 persons were poisoned from industrial causes in Niigata, Japan. Jalili and Abbasi (1961) described outbreaks in Iran and Iraq (1956 and 1960) in which over 300 peasants were poisoned by alkyl mercury compounds after making flour from seed treated with

the ethyl mercury compound. Bidstrup (1964) and Engleson and Herner (1952) reported similar cases in Russia and Sweden, respectively. Mercury poisoning in the United States has been caused by ingestion of contaminated pork from swine that had mercury in their food supply (Curley *et al.*, 1971).

Taylor (1947) was the first to describe a case of organomercurial toxicosis in pigs. Pathological changes due to organic or inorganic mercury toxicosis have been described in swine (McEntee, 1950; Donnelly, 1965; Tryphonas and Nielsen, 1973). Clinical signs and pathologic changes of inorganic and organic mercury toxicosis have also been described in cattle (Stevens, 1921; Turner, 1904; Boley *et al.*, 1961; Butler, 1965; Herberg, 1954; Fujimoto *et al.*, 1956; Oliver and Platonow, 1960; Herigstad *et al.*, 1972). Poisoning as a result of ingestion has been reported in dogs and cats (Green *et al.*, 1938). Edwards (1942) and Palmer (1963) reported methyl mercury toxicosis in a horse and a sheep, respectively. Jungherr in 1957 reported a case of mercury poisoning in chinchillas. Swensson and Ulfvarson (1968) studied the distribution and excretion of various mercury compounds after a single injection in poultry and rats. Diamond and Sleight (1972) described experimental acute and subchronic methyl mercury toxicosis in the rat. Herman *et al.* (1973) described the ultrastructural changes associated with methyl mercury induced primary sensory neuropathy in the rat.

Clinical Signs

Alkyl mercury compounds are capable of producing both acute and chronic effects. Local contact with skin can result in a dermatitis (Lundgren and Swensson, 1949; Hunter *et al.*, 1940). Contrary to the usual gastrointestinal manifestations of inorganic mercury intoxication

(Jubb and Kennedy, 1970; Smith, Jones and Hunt, 1972), the most serious clinical signs of alkyl mercury toxicosis are almost exclusively neurologic in nature and may begin insidiously weeks or months after the onset of exposure. The dominating clinical features of the disease in man are: paresthesia of mouth, lips, tongue, hands, feet; inability to concentrate; weakness; apathy and extreme fatigue; difficulty in swallowing; constriction of visual field; hearing difficulty; emotional instability, with depression or rage; ataxia; wholly uncoordinated movements; spasticity; tremor; paralysis; coma and death. The features are more or less similar in other species (Swensson and Ulfvarson, 1963; Kurland *et al.*, 1960; Hunter *et al.*, 1940; Jubb and Kennedy, 1970).

Pathologic Changes Due to Methyl Mercury Toxicosis

The gross lesions of methyl mercury toxicosis are not striking. Emaciation, loss of weight, cerebellar atrophy and, to a lesser extent, cerebral atrophy have been described (Takeuchi *et al.*, 1962; Hunter *et al.*, 1940; Dales, 1972). In swine with chronic alkyl mercury poisoning, Tryphonas and Nielsen (1973) described pronounced atrophy of cerebral hemispheres and communicating hydrocephalus. There was also focal erosive ulcerative gastritis.

Histopathology

The most striking lesions of methyl mercury toxicosis have been seen in the nervous system. Neuronal necrosis in the internal granular layer of the cerebellum and occipital cortex in addition to a diffuse astrocytosis in the gray matter have been described by Hunter and Russell (1954) and by Takeuchi *et al.* (1962). Changes in the central nervous system occurred after those in the peripheral nervous system. Wallerian degeneration in sensory nerves only and neuronophagia in the

spinal ganglion cells have been described (Cavanagh and Chen, 1971; Hunter and Russell, 1954; Miyakawa *et al.*, 1970). Hunter *et al.* (1940) reported that rats had severe degeneration in the peripheral nerves, dorsal roots and the trigeminal nerves. Fibrinoid necrosis of capillaries in the cerebrum was described by Diamond and Sleight (1972) and by Tryphonas and Nielsen (1973). Thickening and fragmentation of the tunica elastica interna in small arteries and perivascular accumulation of lymphocytes were also described in pigs (Tryphonas and Nielsen, 1973). They also reported that vulnerability of the cerebellar granular cells in pigs seems to be much lower than in other species, including man. They described finely granular eosinophilic bodies which were interpreted to be portions of degenerating neurons or axons and, occasionally, totally calcified neurons in the second or fourth laminae. Herman *et al.* (1973) described ultrastructural changes due to methyl mercury in spinal ganglia and sensory nerves of rats. Changes consisted of disruption of the cytoplasmic protein synthetic apparatus with relative sparing of other cellular structures.

Ascorbic Acid

Metabolism and Biochemistry

Among all the animals studied so far, only man and other primates, guinea pigs and a few other species are dependent on food sources for ascorbic acid (Roy and Guha, 1958). The ascorbic acid requirement of the guinea pig is 0.5 to 1.0 mg/day (The Vitamins, 1967).

Ascorbic acid is absorbed by the tissue of the intestinal tract (Zilva, 1935) principally in the small intestine. After oral ingestion of ascorbic acid the concentration in blood plasma rises to maximum within 1 to 1-1/2 hours. In any event the increase is only temporary.

The vitamin is transported by the blood throughout the entire organism and excess amounts are secreted in the urine within 4 to 6 hours after ingestion. Some ascorbic acid is also excreted in feces and sweat. Tissues and body fluids contain various amounts of this vitamin. Normal human blood plasma contains about 1.0 to 1.4 mg/100 ml (The Vitamins, 1967). There are no real storage organs for this vitamin, although some organs contain higher amounts. Among these, the adrenal gland contains the highest concentration. Generally tissues of high metabolic activity have the highest content (The Vitamins, 1967).

The biochemical role of this substance in the organism is not entirely clear. During the biochemical reactions, ascorbic acid is oxidized to dehydroascorbic acid which is easily reduced to ascorbic acid in the tissue. Thus it may serve as a strong reducing agent in biological systems of the body (Johnson and Zilva, 1934; Fox and Levy, 1936; Roe and Bernum, 1963; Penney and Zilva, 1943; Todhunter *et al.*, 1950; Smith, 1954; Dayton *et al.*, 1966).

Ascorbic acid has been shown to act as a co-factor in hydroxylation reactions (Schneider and Staudinger, 1964). Ascorbic acid is thought to participate in catecholamine synthesis and has been shown to function as a co-factor in dopamine β-hydroxylation (Kaufman and Friedman, 1965). Since catecholamines are the chief neurohumoral substances, they are essential for the mediation of adrenergic impulses in the autonomic nervous system. The amino acids phenylalanine and tyrosine are generally regarded as precursors of catecholamines. The main biosynthetic pathway is:

phenylalanine → tyrosine → dopa → dopamine → norepinephrine → epinephrine

In nervous tissue, where norepinephrine functions as a chemical transmitter, very little epinephrine is formed (Turner, 1966). Izquierdo *et al.* (1968) reported that ascorbic acid injected intraperitoneally into rats caused an increase in the endogenous norepinephrine level and a decrease in the dopamine in various parts of the brain. This decrease was associated with an increase in dopamine β-hydroxylase activity.

Further evidence for the importance of ascorbic acid in catecholamine synthesis was provided by histochemical and autoradiographical studies. Ascorbic and dehydroascorbic acid accumulated in peripheral nervous tissue and sympathetic ganglia (Hammarstrom, 1966) where the cell bodies of the postganglionic fibers are located and where the amine granules are thought to be synthesized (Anden *et al.*, 1969). Electron microscopic studies indicated that these granules are surrounded by membranes and appear to originate in the golgi region of the cell. They contain a very high content of catecholamines and adenosine triphosphate (ATP) (Turner, 1966). Therefore, there may be a biochemical basis for postulating that ascorbic acid deficiency may cause a lack of catecholamine synthesis. Mercury toxicosis may deplete catecholamines, since it is known that mercury derivatives which act as SH inhibitors, e.g., para-chloromercuribenzoate, exert pronounced releasing activity on granular catecholamines *in vitro* (D'Iorio, 1957). Keswani *et al.* (1969) presented evidence that SH groups arising from the protein granules serve as anchoring sites for catecholamine complexes (involving ATP and Mg^{++}). The SH inhibitors are believed to block the SH sites within the granule thus reducing the possibility of formation of an ATP-Mg^{++}-catecholamine complex. The net effect of ascorbic acid deficiency combined with mercury toxicosis may be a lack of available catecholamines.

Ascorbic Acid Deficiency

There are not many references which associate ascorbic acid deficiency with altered susceptibility to toxicoses. Kociba and Sleight (1970) reported increased susceptibility to nitrite toxicosis in the ascorbic acid deficient guinea pig. Spivey Fox *et al.* (1970) reported that 0.1 to 1% ascorbic acid in the diet almost completely protected quail against mortality, poor growth and anemia produced by cadmium toxicosis. Dietary ascorbic acid also protected quail against cadmium-inhibited spermatogenesis (Richardson and Spivey Fox, 1970). Berenshtein *et al.* (1954) reported that the subcutaneous injection of cadmium caused a considerable lowering in the content of ascorbic acid in the rabbit. Vallee *et al.* (1960) reported that ascorbic acid administration lessened the toxicity of arsanilic acid in the rat, but again no evidence was provided on the effect of a deficiency of ascorbic acid on arsanilic acid toxicosis. Fuller *et al.* (1971) reported an increased susceptibility to shock produced by intravenous injection of *E. coli* endotoxin in ascorbic acid deficient guinea pigs.

Ascorbic acid administration has been shown to be beneficial to several species of experimental animals subjected to various types of shock. Locke *et al.* (1943) observed that ascorbic acid provided protection for rabbits against gravity shock. Stewart *et al.* (1941) reported that intravenous injection of ascorbic acid prolonged the lives of cats which had 50% of their blood volume removed. Ascorbic acid given to guinea pigs was found to provide protection against traumatic hemorrhage and anaphylactic shock (Ungar, 1943; Dawson and West, 1965).

Clinical Signs and Pathologic Changes

The outstanding features of scurvy in the guinea pig are hemorrhages in almost any part of the body, particularly in intramuscular and subcutaneous areas, and a general weakness of tissues, especially in those with a comparatively high content of collagen. Other characteristic signs are loss of appetite, lessening of activity, loss of luster of eyes and hair, roughening of hair, hunching with drooping head, stiffening of hind legs and frequent outward rotation of legs and beading of ribs. In the late stages there is usually a lowering of body temperature, anemia and a tendency for diarrhea (The Vitamins, 1967).

Present knowledge indicates that ascorbic acid is essential for the production of collagen. Transformation of reticulum to collagen is retarded or stopped entirely depending on the severity of the deficiency (Hunt, 1941). In scurvy the intercellular material is fluid and amorphous. In newly formed granulation tissue of a normal animal there is an abundance of metachromatic staining material which becomes less as the scar matures. With lack of ascorbic acid this material persists and remains in a fluid or semifluid state (Dalldorf, 1938). In the muscle, either swelling or atrophy of both the sarcolemma and of muscle tissue itself may be found. There is some tendency for waxy degeneration, fragmentation, calcification, vacuolation and lysis of cells. Hemorrhage in the brain, nerve trunks and posterior root ganglion of the spinal cord may be found in the scorbutic guinea pig. The vessels have weakening of the connective tissue. Decreases in blood volume, hemoglobin and numbers of red cells and lymphocytes have been observed. The usual scorbutic lesions in skin and subcutaneous tissue are petechial hemorrhages and an increase in the water content of the skin. Degeneration of ovaries with endometrial hyperplasia,

degeneration of germinal epithelium in the testes and a tendency to fatty metamorphosis have been observed in the liver (The Vitamins, 1967).

Gore *et al.* (1968) and Majno and Palade (1951) reported an electron microscopic study of the capillaries in the scorbutic guinea pig. The lesions consisted of endothelial junctional separation and cytoplasmic disruption. Histamine or serotonin produced increased permeability of vessels by widening of the endothelial cell junction.

MATERIALS AND METHODS

General Plan

Experiments were designed to investigate the effects of ascorbic acid deficiency on methyl mercury toxicosis. The control guinea pigs were fed normal diet which consisted of pelleted guinea pig diet* supplemented with fresh cabbage daily. Commercial test diet deficient in ascorbic acid** was used to produce ascorbic acid deficiency. Methyl mercury dicyandiamide (MMD)*** was used to induce toxicosis. In all cases food and water were provided *ad libitum*.

Necropsies were performed at or soon after death. The tissues were fixed in 10% formalin-sodium acetate solution and were sectioned at 6 microns. Hematoxylin and eosin or selected special stains were

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*A. K. Zinn & Co., Battle Creek, Mich. Laboratory diet for guinea pigs. Crude protein (Min.) 20.0%, crude fat (Min.) 4.0%, crude fiber (Max.) 14.0%. Ingredients: ground wheat, ground corn, soybean oil meal, linseed oil meal, dehydrated alfalfa meal, cooked cereal meal from corn, wheat and oats, dried skimmed milk, pulverized oats, dicalcium phosphate, calcium carbonate, trace mineralized salt, animal fat preserved with butylated hydroxytoluene, corn oil, ethoxyquin (preservatives), choline chloride, choline pantothenate, d-activated animal sterol with improved stability (source of vitamin D_3), folic acid, menadione sodium bisulfite (source of vitamin K), niacin, riboflavin supplement, vitamin A palmitate with improved stability, vitamin B-12 supplement, vitamin E supplement, gelatin, thiamine mononitrate, pyridoxine hydrochloride, ascorbic acid 0.075%.

**Same as described above, excluding ascorbic acid.

***Panogen^{R15} (containing methylmercury dicyandiamide 2.2%; total mercury 1.47%). Produced by Morton Chemical Co., 110 North Wacker Drive, Chicago, Ill.

used (Weil's, Gomori trichrome, periodic acid-Schiff) according to commonly accepted methods as outlined in the Armed Forces Institute of Pathology Manual (1968). Kidney, liver and splenic tissue of selected guinea pigs were cultured on blood agar plates.

Ether anesthesia was used to facilitate the collection of blood by cardiac puncture from the guinea pigs. A 1.5 inch, 22-gauge needle was inserted caudolateral to the xiphoid cartilage toward the location of the heart. Heparin was used as anticoagulant.

Plasma levels of ascorbic acid were determined according to the Caraway modification (Caraway, 1968) of the method of Roe and Ruether (1943). The hemoglobin, packed cell volume and total and differential leukocyte counts were determined at 10-day intervals.

Experiment I

Sixteen 13-week-old guinea pigs, 11 males and 5 females, weighing approximately 525 gm each were used. Experimental design is outlined (Table 1). Two groups (A and D) were fed the normal diet and 2 groups (B and C) were given the ascorbic acid-deficient diet. Four animals were kept in each pen.

By 14 days ascorbic acid concentration in plasma had decreased to 0.25 to 0.3 mg/100 ml in Groups B and C (ascorbic acid levels in controls were 1.0 to 1.5 mg/100 ml). The mercury compound for injection was diluted with distilled water so that each milliliter contained 4 mg of MMD. Starting on Day 14 sublethal doses of MMD (4 mg/kg of body weight) were injected (IP). Injections were continued at weekly intervals for 3 weeks to surviving guinea pigs in Groups C and D. Guinea pigs in Groups A and B were similarly given injections of distilled water. Guinea pigs were examined for clinical signs of mercury

Table 1. Design of Experiment I and results after intraperitoneal administration of methyl mercury dicyandiamide (MMD) at weekly intervals to guinea pigs fed normal and ascorbic acid-deficient diets

Groups	No. of guinea pigs	Sex	Diet	Dosage of MMD (mg/kg of body weight)	Deaths after first injection	Deaths after second injection	Deaths after third injection	Mortality (%)
A	4	3 M 1 F	Normal	0	0	0	0	0
B*	4	3 M 1 F	Vit. C deficient	0	0	0	0	0
C*	4	2 M 2 F	Vit. C deficient	4 (2.66 mg Hg)	0	3 (2 M, 1 F)	1 (F)	100
D	4	3 M 1 F	Normal	4 (2.66 mg Hg)	1 (M)	0	0	25

*Groups B and C were fed an ascorbic acid-deficient diet for 14 days before injection of MMD.

toxicosis or ascorbic acid deficiency and weighed every day. All remaining guinea pigs were killed on the day that the last guinea pig in Group C died.

Experiment II

Twenty 11-week-old guinea pigs, 8 females and 12 males, weighing approximately 510 gm each were used and divided into 4 groups as shown (Table 2). The males and females in each group were kept in separate pens. After the ascorbic acid-deficient diet was fed for 14 days to Groups B and C, 22 p.p.m. MMD was added to the diets of Groups C and D. The MMD solution had been sprayed with an atomizer onto the feed in a mixer. All guinea pigs were weighed and food consumption was measured every day. They were examined for clinical signs of methyl mercury toxicosis regularly.

Experiment III

Twenty-three guinea pigs, 10 weeks old and weighing approximately 475 gm each, were used. There were 11 females and 12 males. During the experiment the guinea pigs were assigned to 3 groups of 5 animals each (A, B and C) and 1 group of 8 (D) (Table 3). The males and females were kept in separate pens for each group.

Methyl mercury dicyandiamide was added to the diet (Groups C and D) at the rate of 44 p.p.m. In contrast to Experiment II, all guinea pigs were fed normal diets until the MMD-containing diets were started.

Table 2. Design of Experiment II and results of feeding methyl mercury dicyandiamide (MMD) to guinea pigs fed a normal or an ascorbic acid-deficient diet. Total MMD consumption and time when animals were killed or died are shown

Groups	No. of guinea pigs	Sex	Diet	MMD consumption (mg/ guinea pig	Time until death or euthanasia	(Days)
A	5	3 M 2 F	Normal	0	Killed	18 (M) 22 (MF) 25 (F) 26 (M)
B*	5	3 M 2 F	Vit. C deficient	0	Killed	18 (M) 22 (F) 26 (M)
					Died	34 (M) 35 (F)
C*	5	3 M 2 F	Vit. C deficient + 22 p.p.m. MMD	9 (6 mg Hg)	Died	18 (F) 22 (MM) 25 (F) 26 (M)
D**	5	3 M 2 F	Normal + 22 p.p.m. MMD	114 (76 mg Hg)	Killed	18 (F) 22 (M) 26 (M)
					Died	150 (MF)

*Groups B and C were fed an ascorbic acid-deficient diet for 14 days before MMD was added to the diets of Groups C and D.

**Two guinea pigs in Group D continued on MMD-containing diet until they died of chronic methyl mercury toxicosis.

Table 3. Design of Experiment III and results of feeding methyl mercury dicyandiamide (MMD) to guinea pigs fed a normal or an ascorbic acid-deficient diet. Total MMD consumption and time when animals were killed or died are shown

Groups	No. of guinea pigs	Sex	Diet	MMD consumption (mg/ guinea pig)	Time until death or euthanasia	(Days)
A	5	3 M 2 F	Normal	0	Killed	17 (F) 19 (M) 20 (M) 34 (F) 38 (M)
B*	5	2 M 3 F	Vit. C deficient	0	Killed	17 (F) 19 (M) 20 (F)
					Died	46 (M) 48 (F)
C	5	2 M 3 F	Vit. C deficient + 44 p.p.m. MMD	24 (16 mg Hg)	Died	17 (MF) 19 (M) 20 (MM)
D**	8	5 M 3 F	Normal + 44 p.p.m. MMD	40 (26.6 mg Hg)	Killed	17 (F) 19 (M) 20 (M)
					Died	34 (FMM) 36 (F) 38 (M)

*Two guinea pigs in Group B continued on ascorbic acid-deficient diet and finally died of scurvy on days 46 and 48.

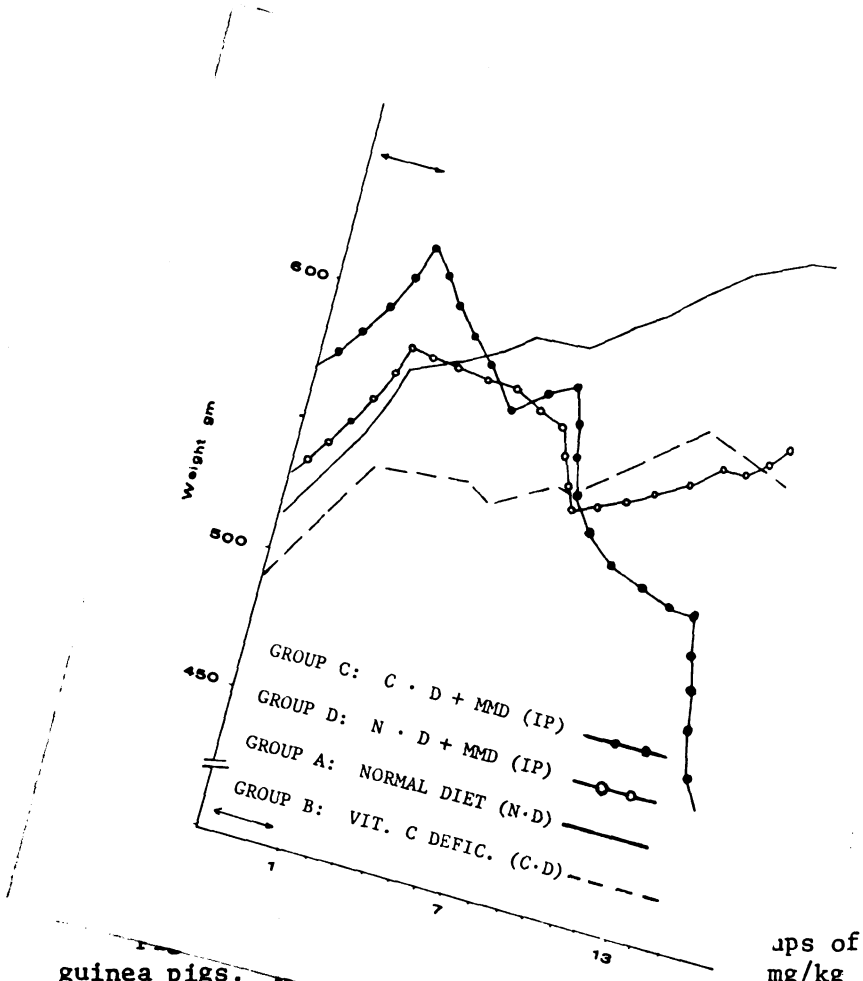
**Five guinea pigs in Group D continued on MMD-containing normal ration. They consumed 66% more MMD and survived 80% longer (average) than Group C.

RESULTS

Experiment I
Injection of Methyl Mercury Dicyandiamide

Clinical Signs

Soon after the first methyl mercury injection into guinea pigs in Groups C and D, they had ruffled hair, rapid respiration and evidence of abdominal pain. Some ran aimlessly about the cage. All except 1 of the guinea pigs in Group D appeared normal within 1 hour after injection. This guinea pig died the next day (Table 1) after evidence of severe abdominal pain and weight loss. It was 24 hours before guinea pigs in Group C appeared relatively normal. In the meantime they lost considerable weight. After the second weekly injection, all guinea pigs in Group D appeared normal within 1 hour, and 1 guinea pig in Group C appeared to be relatively normal within 24 hours. The other 3 guinea pigs in Group C stopped eating and lost weight. On the second day they were reluctant to move, had ruffled hair, closed and watery eyes and an extensively distended and painful abdomen. They had severe dyspnea, their backs were arched, and all 3 died the same day. After the third weekly injection, all guinea pigs in Group D appeared normal. The 1 remaining animal from Group C had clinical signs as described previously and died the next day. The guinea pigs in Groups A and B had no clinical signs during the experiment, except for a slight weight loss in Group B (Figure 1).



...ups of guinea pigs. ... mg/kg body weight) was give... dicated. Day 1 indicates 1st (IP) inj... 2nd injection and Day 13 the 3rd injecti... ows indicate 14-day period before the 1st injection on Day 1. Diets were as indicated.

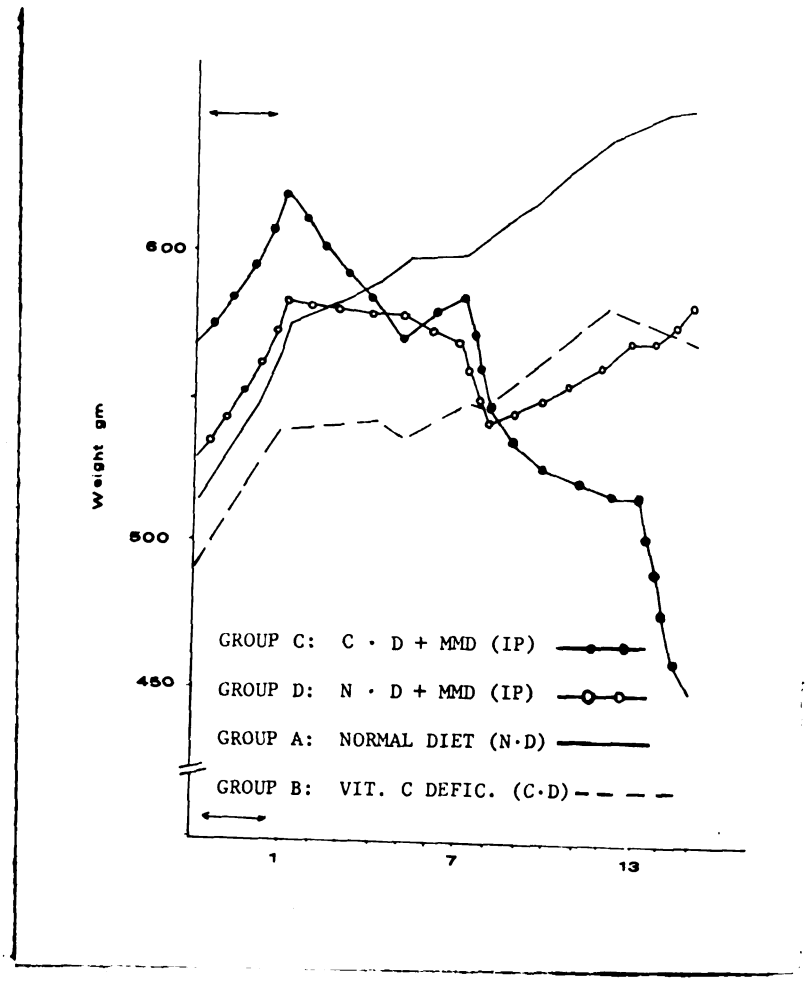


Figure 1. Average daily weights of 4 groups of guinea pigs. Methyl mercury dicyandiamide (4 mg/kg body weight) was given weekly to groups as indicated. Day 1 indicates 1st (IP) injection, Day 7 the 2nd injection and Day 13 the 3rd injection. Arrows indicate 14-day period before the 1st injection on Day 1. Diets were as indicated.

Gross Necropsy Findings

The guinea pig in Group D which died had a localized fibrinopurulent peritonitis most likely due to accidental perforation of the colon at the site of inoculation. The peritoneum was thickened and had some adhesions. All guinea pigs in Group C had extensive hemorrhagic peritonitis with blood clots throughout the peritoneal cavity. The peritoneum was grayish and dull, thickened and ulcerated at the site of injection. The mesentery and serosal surface of the intestine, urinary bladder and uterus had extensive diffuse fibrinohemorrhagic adhesions. The liver capsule was thickened, dull and adherent to the diaphragm. The guinea pigs in the other groups had a clear, transparent peritoneum and normal serosa.

Histopathology

The guinea pigs in Group C had similar histopathologic changes as follows:

CNS. There were occasional neuronal degeneration and vacuolation which were not extensive enough to relate to toxicosis.

Peripheral nerves. There were no lesions present in the sciatic or in other nerve fibers.

Liver. All the guinea pigs in Group C had moderate to severe fatty metamorphosis.

Kidney. The 3 guinea pigs in Group C which died after the third IP injection had a thickened capsule with extensive proliferation of immature fibroblasts. All the guinea pigs in Group C had foci of vacuolar degeneration and swollen epithelial cells of the proximal tubules.

Peritoneum. A diffuse hemorrhagic peritonitis with lymphocytes, plasma cells, macrophages, and fibroblasts was present (Figure 2). These changes were most extensive in the mesentery and the serosal surface of the intestine, urinary bladder, perirenal area, testicle and serosal surface of the spleen.

Intestine. A diffuse chronic serositis was present, characterized by fibroblasts, connective tissue and hemorrhage (Figure 3). The guinea pig which died in Group D had focal fibrinopurulent peritonitis.

Experiment II
22 p.p.m. Methyl Mercury Dicyandiamide in the Diet

Clinical Signs

The guinea pigs in Group C* lost weight, and retarded growth occurred as soon as the mercury-treated, ascorbic acid-deficient diet was begun. The guinea pigs continued to lose weight and became anorectic and emaciated. They became very depressed and weak, reluctant to move, and arched their back, they were dyspneic, had closed and watery eyes and salivation was increased. Terminally, bloody diarrhea, incoordination and prostration were seen. They all died between 18 and 26 days after a mercury-treated, ascorbic acid-deficient diet was instituted (Figure 4, Table 2). The guinea pigs in Group B** (Figure 4) continued to gain weight until Day 10 of the experiment. They then started to lose weight, were anorectic and depressed, and became dyspneic and incoordinated.

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*Maintained on ascorbic acid-deficient diet for 14 days before ascorbic acid-deficient and mercury treated diet was fed.

**Maintained on vitamin C-deficient diet for 14 days before experiment began and continued on the same diet.

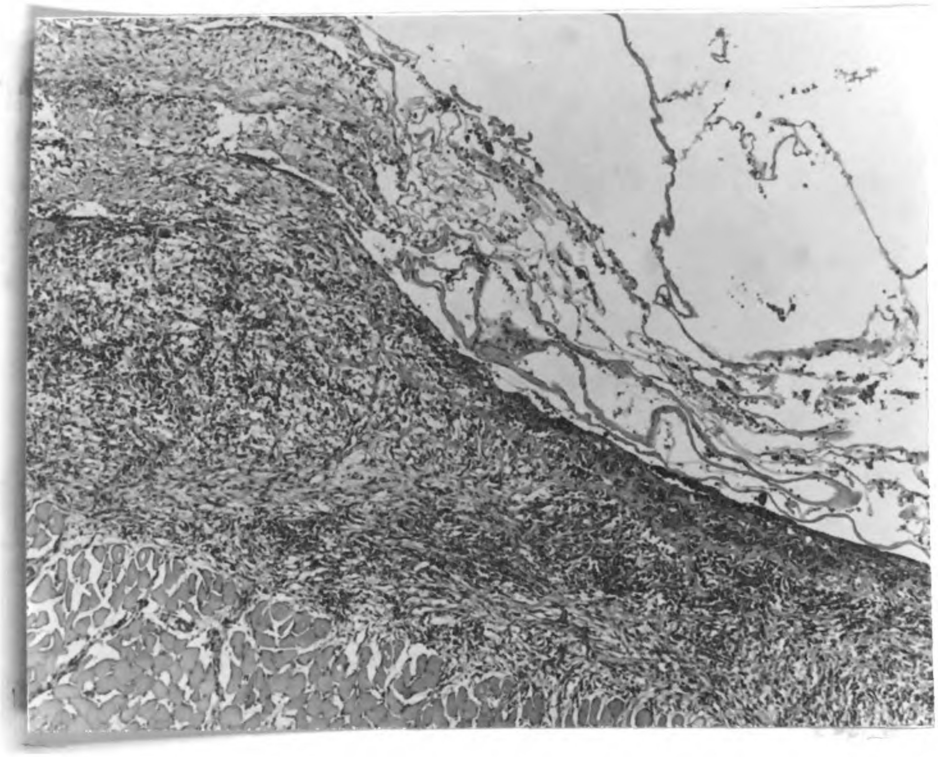

Figure 2. Chronic peritonitis in an ascorbic acid-deficient guinea pig given 3 weekly intraperitoneal injections of MMD (4 mg/kg body weight). H & E stain; X 50.

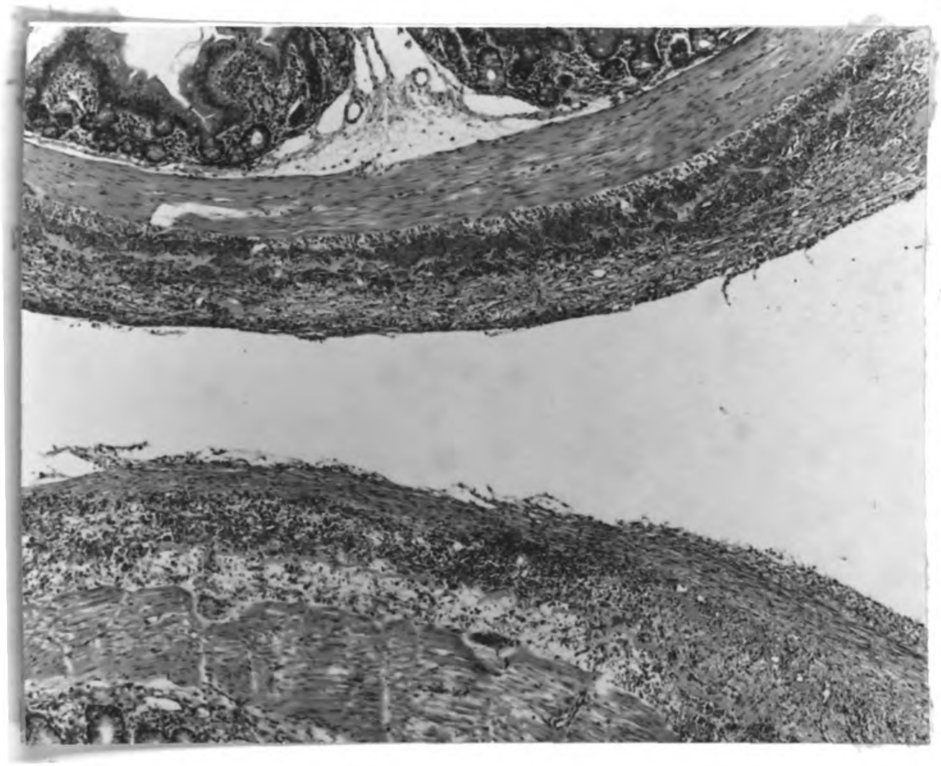

Figure 3. Diffuse chronic serositis of the intestine in an ascorbic acid-deficient guinea pig given 3 weekly intraperitoneal injections of MMD (4 mg/kg body weight). H & E stain; X 50.

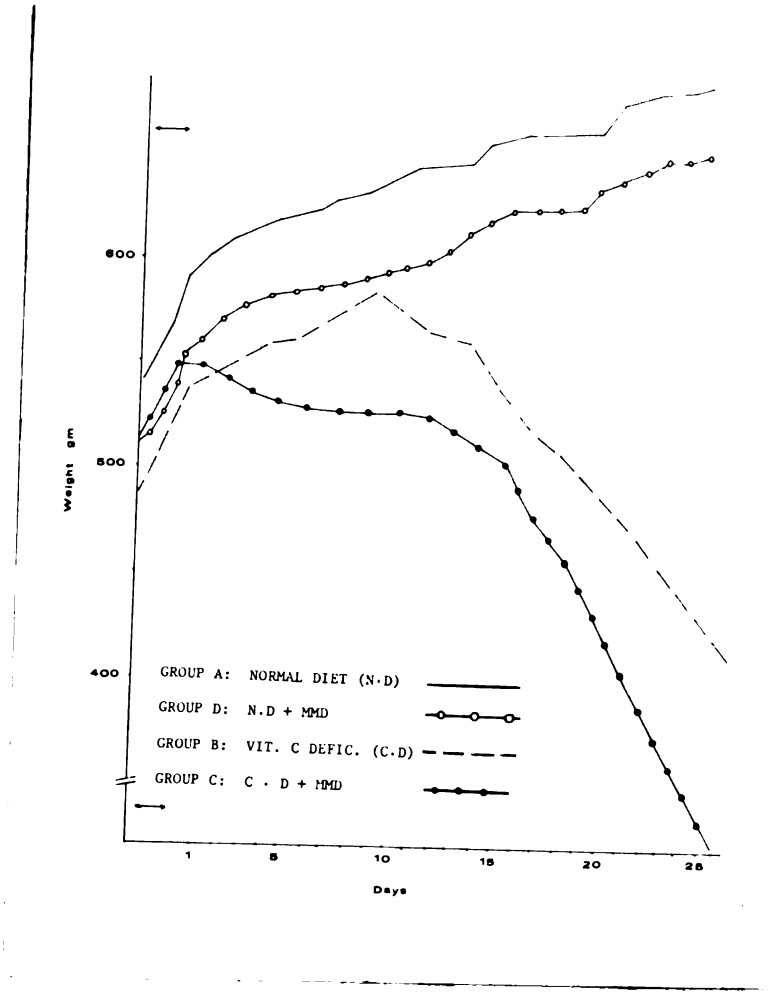

Figure 4. Average daily weights of 4 groups of guinea pigs. Methyl mercury dicyandiamide (22 p.p.m.) was added to diets of groups as indicated. Arrows indicate the 14 days in which the guinea pigs in Groups B and C were fed ascorbic acid-deficient ration and 14 days prior to the addition of MMD to the diets of Groups C and D on Day 0.

Three were killed for comparison with Group C on Days 18 through 26 and the other 2 died of scurvy on Days 34 and 35. This was 8 days after the last guinea pig in Group C had died (Table 2).

The guinea pigs in other groups were clinically normal throughout the experiment. Three guinea pigs in Group D were killed for comparison with Group C on Days 18 through 26 and the other 2 were continued on the MMD-containing normal diet. They finally died with chronic methyl mercury toxicosis on Day 150 (Table 2). Terminally the animals showed prostration and severe spastic paralysis. The legs were spread apart and the animals apparently could not move.

Gross Necropsy Findings

The most striking lesion in Group C guinea pigs was extensive gastroenteritis (Figure 5). The stomach was thickened and hemorrhagic, and edema was extensive. There was ulceration with adherent necrotic debris and ingesta. In the intestine, the duodenum and ileocecal valve had severe destructive lesions similar to those seen in the stomach. The liver was swollen and mottled with pale yellow foci on the surface. The urinary bladder was filled with bloody fluid, and there was diffuse edema with extensive hemorrhage on the mucosal and cut surface. Those guinea pigs in Group B which were euthanatized at the same time as the Group C guinea pigs had diffuse hemorrhagic foci in subcutaneous tissue, muscles and joints, and the liver had a nutmeg appearance. The 2 guinea pigs from Group D which died after 150 days with chronic toxicosis had pale friable livers, and the spleens were small and hard. The other 3 guinea pigs from Group D which were killed on Days 18 and 26 along with those in Groups A and C had apparently normal tissues.

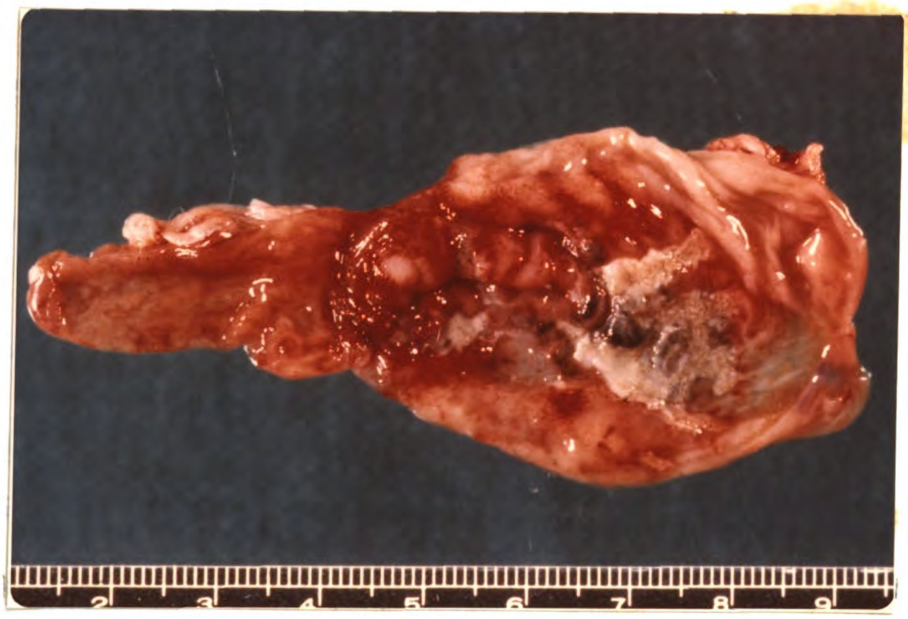

Figure 5. Hemorrhagic and ulcerative gastritis in a guinea pig fed ascorbic acid-deficient diet containing 22 p.p.m. MMD. Notice thickening of gastric wall as a result of extensive edema and hemorrhage.

Histopathology

The guinea pigs in Group C had lesions as follows:

Brain. Areas of neuronal degeneration and congestion were present. The most prominent changes were a deepening of the basophilia of the cells, chromatolysis and pyknosis. Satellitosis and neuronophagia could be seen in some areas but no consistent pattern could be established. Areas of vacuolation of gray matter were present.

Sciatic nerve. Areas of early demyelination were present. The affected nerve had swollen myelin sheaths, with small clear globular to oval shaped spaces filled with granular material or necrotic debris. These areas did not stain for myelin with Weil's staining method (Figures 6 and 7).

Liver. Focal areas of coagulative necrosis surrounded by calcification were present (Figures 8 and 9), with a moderate generalized fatty metamorphosis. Syncytial formation of hepatocytes was seen in some places.

Kidney. The renal lesions were typified by swollen tubular epithelium and glomeruli with a few randomly located necrotic cells.

Spleen. The sinusoids were dilated and filled with pinkish material and erythrocytes. A decreased number of lymphoid follicles was evident in some guinea pigs. A similar change was present in the lymph nodes of these animals.

Lung. An extensive perivascular and peribronchiolar edema with a decrease in connective tissue collagen were the main lesions in the lung.

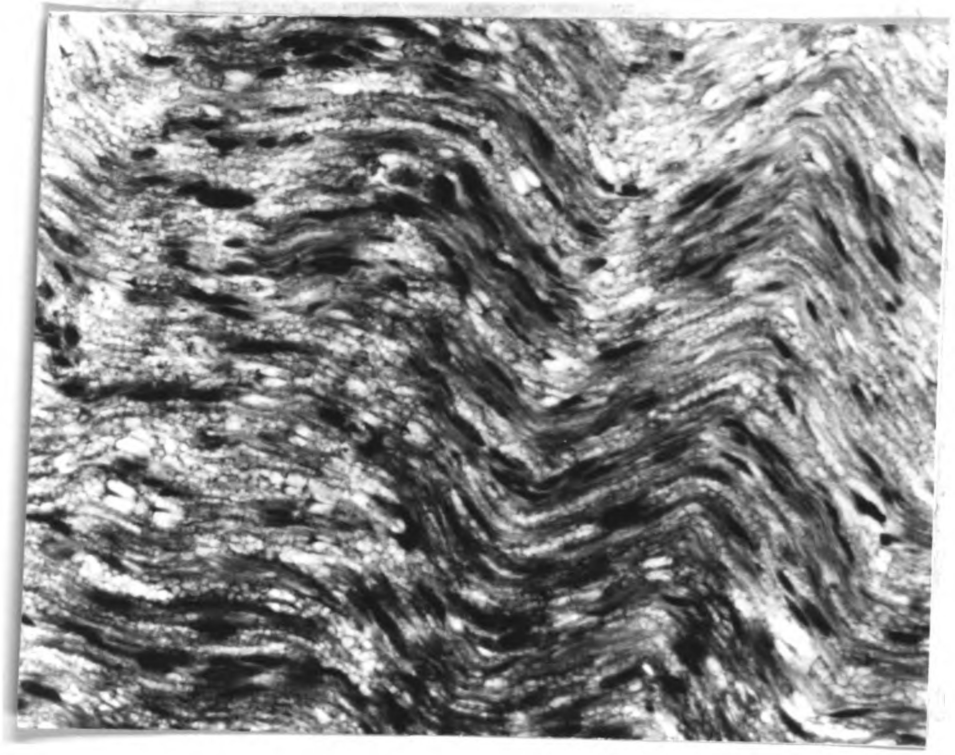

Figure 6. Sciatic nerve in a normal guinea pig. Weil's stain; X 125.

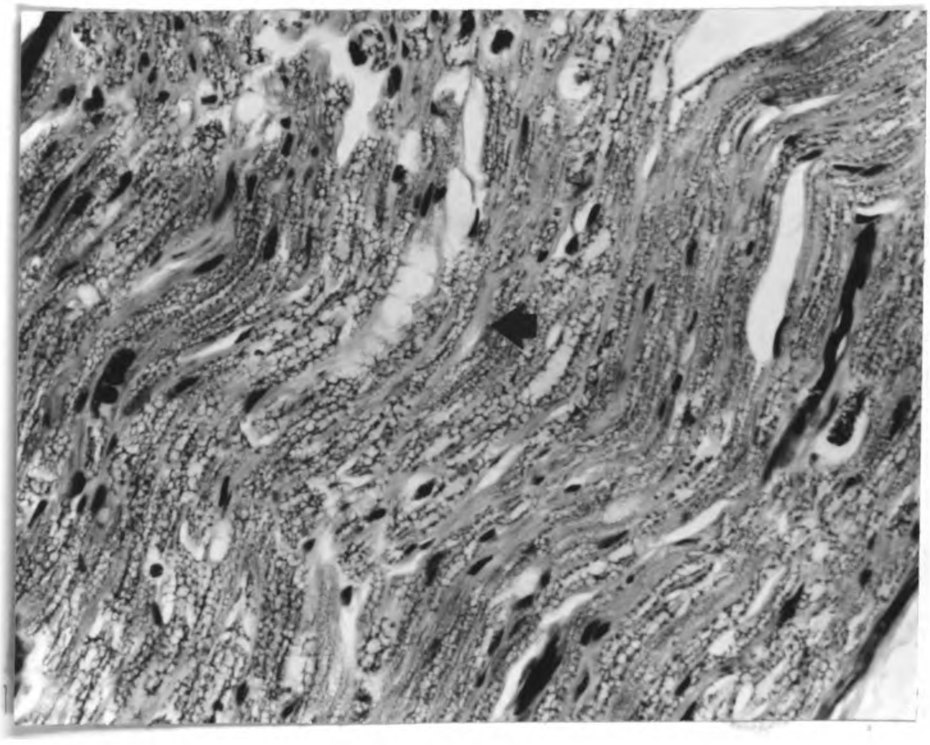

Figure 7. Sciatic nerve in a guinea pig fed an ascorbic acid-deficient diet containing 22 p.p.m. MMD. Compare with Figure 6 and notice extensive demyelination, swelling (arrow) and vacuolation of nerve fibers. Weil's stain; X 125.

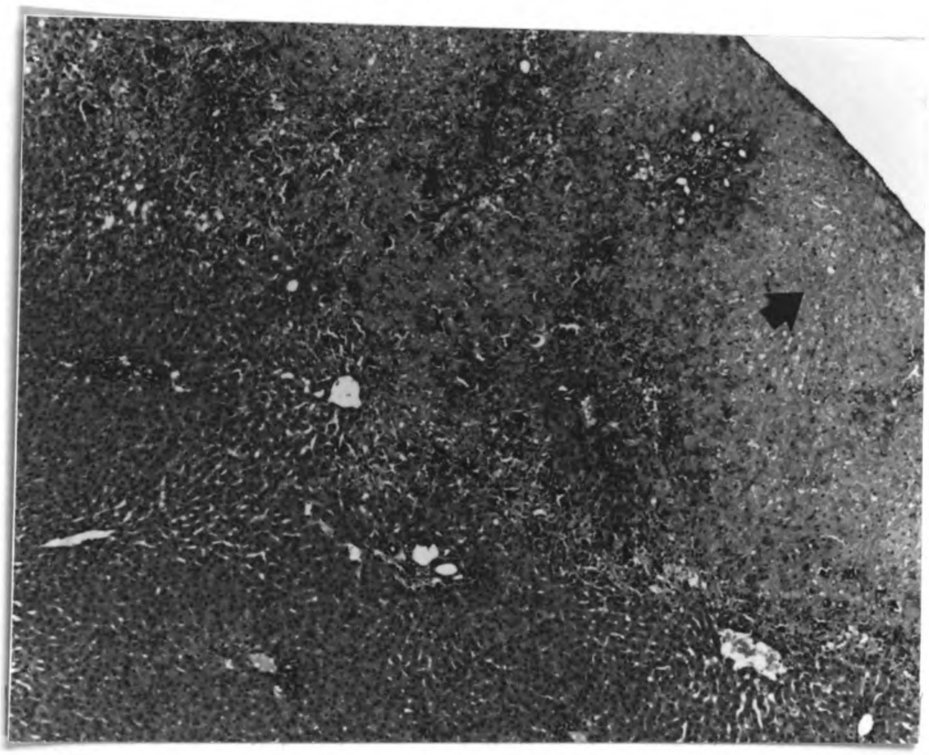

Figure 8. Areas of diffuse coagulative necrosis (arrow) in hepatic tissue of a guinea pig fed an ascorbic acid-deficient diet containing 22 p.p.m. MMD. H & E stain; X 50.

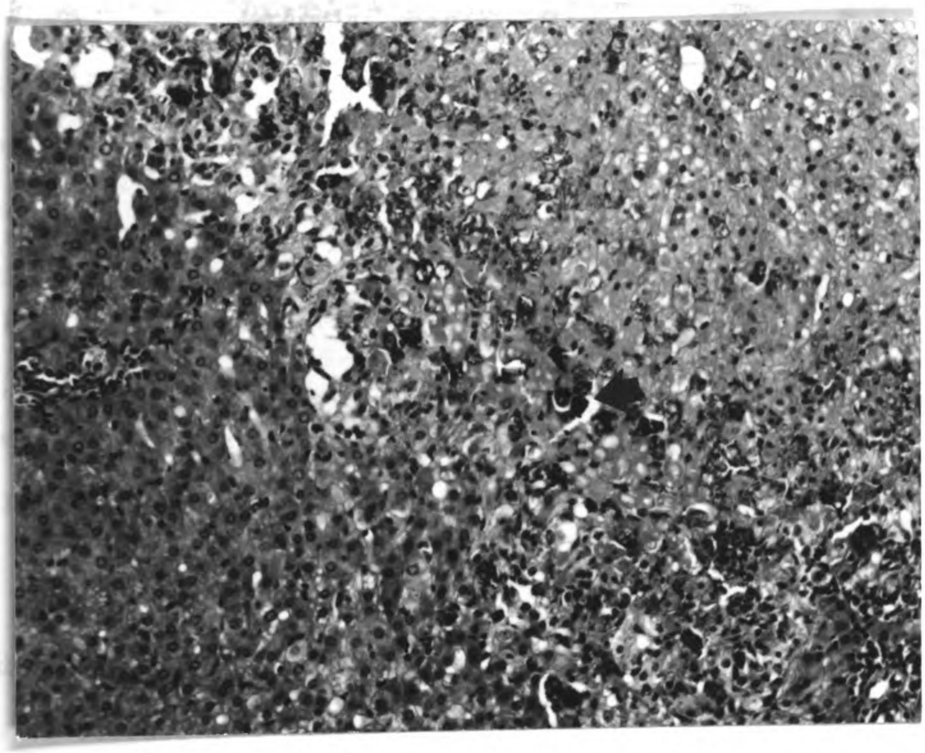

Figure 9. Higher magnification of hepatic tissue of guinea pig described in Figure 8. Dark cells (arrow) were calcified. H & E stain; X 125.

Adrenals. Focal areas of necrosis and an occasional necrotic cell were present in the cortex. Fatty metamorphosis was also seen. The medulla had extensive vacuolation and congestion.

Heart. The blood vessel walls had a disarrangement of endothelial cells. The cardiac muscle fibers in the vicinity of vessels were necrotic and had a granular appearance with vacuolation and increased cellularity (Figure 10). There were small infarcted areas and some hemorrhage. An occasional cell was necrotic, and there were empty spaces with necrotic debris and granular remnants of pre-existing muscle fibers.

Skeletal muscle. There were foci of atrophic fibers and edema in the vicinity of nerve fibers. The myelinated nerve fibers in the muscle tissue had demyelination and vacuolation.

Stomach. There was extensive submucosal edema and dilation of blood vessels and lymphatics. The mucosa was eroded and there were areas of diffuse hemorrhages. Several ulcers penetrated deeply into the muscular layer and there was necrotic debris and inflammation (Figure 11). The muscle fibers were separated and edematous with areas of hemorrhage.

Intestine. The lesions, especially in the duodenum and ileocecal valve, were essentially the same as described for the stomach. The other parts of the intestine had extensive edema, congestion, hemorrhage and erosion with submucosal calcification.

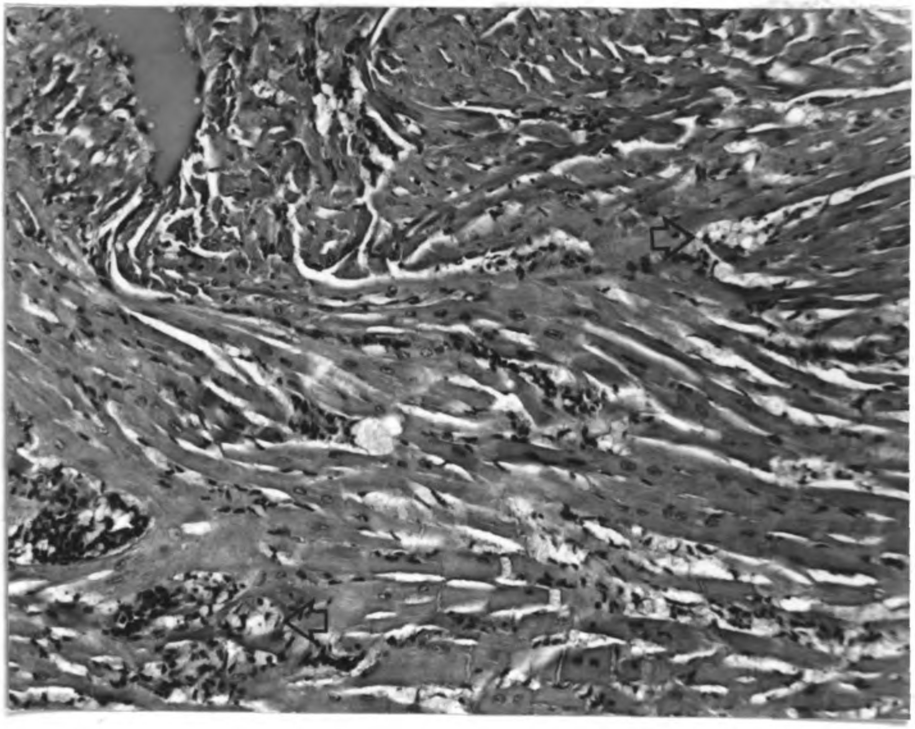

Figure 10. Cardiac muscle in a guinea pig fed an ascorbic acid-deficient diet containing 22 p.p.m. MMD. Notice focal muscular fiber necrosis (arrow) and cellular reaction. H & E stain; X 125.

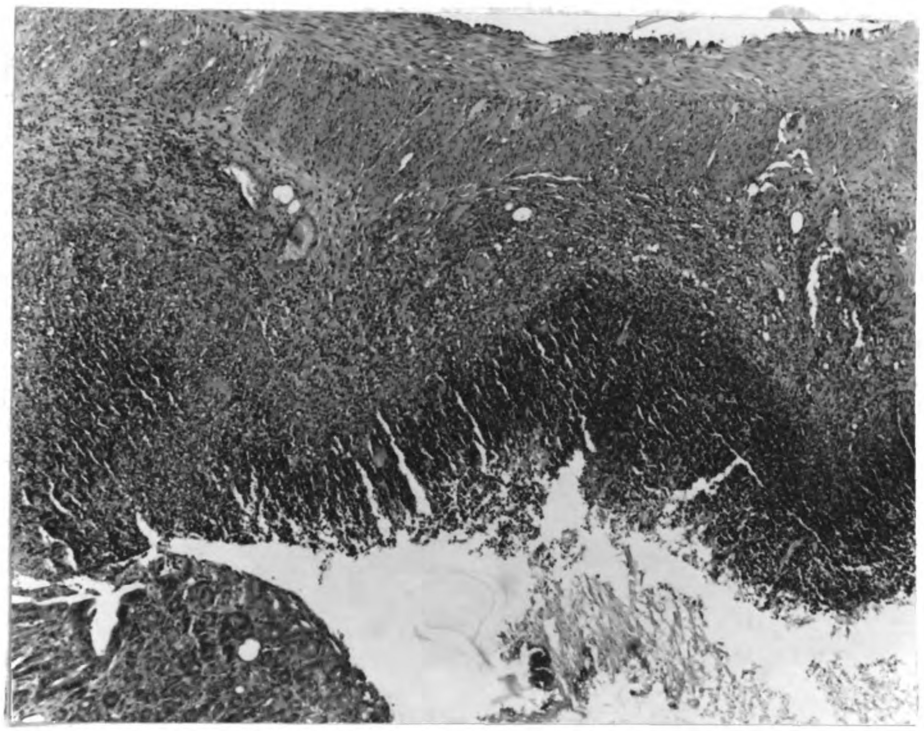

Figure 11. Ulcerated gastric mucosa in a guinea pig fed an ascorbic acid-deficient diet containing 22 p.p.m. MMD. Intact mucosal epithelium is present in the upper right corner. H & E stain; X 50.

Lesions in Guinea Pigs from Other Groups

The guinea pigs in Group B fed the ascorbic acid-deficient diet had hemorrhagic areas and edema in the dermis, muscle, gastrointestinal submucosa and lamina propria of the urinary bladder. The livers had generalized fatty metamorphosis.

The 2 guinea pigs in Group D that died of chronic toxicosis had severe lesions in the cerebrum. The lesions consisted of extensive loss of cerebrocortical neurons which resulted in the appearance of granules and vacuoles. This became more extensive toward the occipital region. An active polioclastic process was indicated by calcified neurons and granules (Figure 12) with eosinophilic hyaline-like droplets of different sizes around the vessels and in the tissue (Figure 13). The vessel walls had an extensive fibrinoid necrosis and perivascular edema (Figure 14) with severe perivascular cystic dilation and hemorrhage. The dorsal root ganglia had neuronal degeneration, gliosis and collagen formation (Figure 15). There was extensive demyelination of nerve fibers and proliferation of fibroblasts and collagen in the sciatic nerve. Severe generalized fatty metamorphosis was present in the liver with increased fibroblasts and collagen in the triads. This gave a lobular pattern to the tissue.

The 3 other guinea pigs from Group D and guinea pigs from Group A, which were killed at the same time as Group C, had no microscopic lesions.

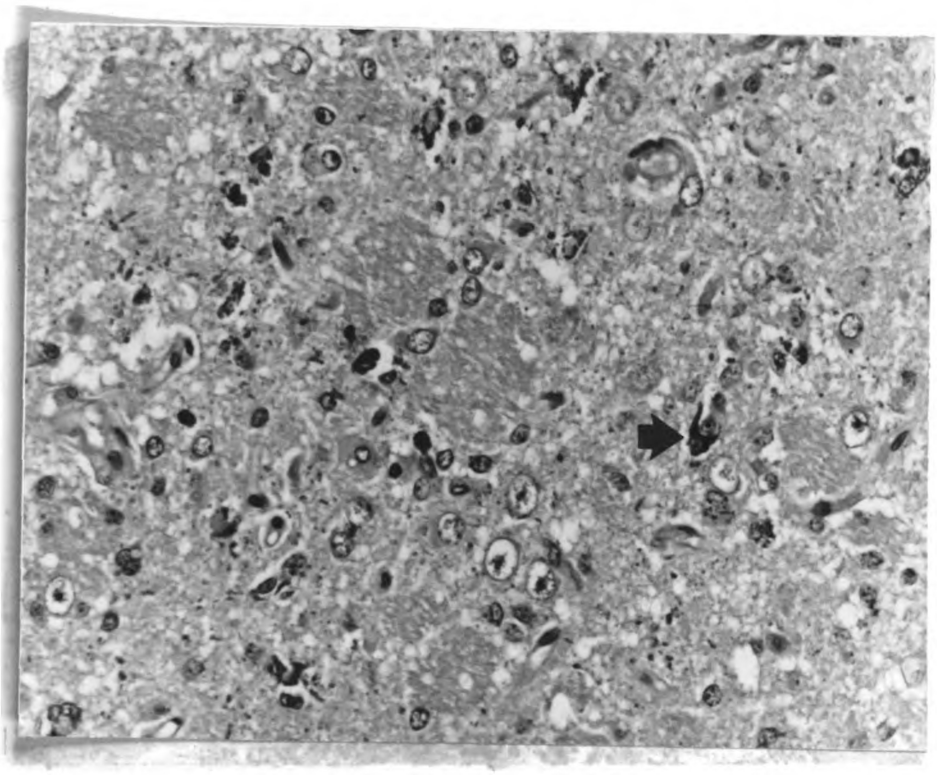

Figure 12. Cortical gray matter of a guinea pig fed normal guinea pig ration containing 22 p.p.m. MMD for 150 days. Extensive polioclastic process was shown by vacuolation, granules and calcified neurons (arrow). H & E stain; X 315.

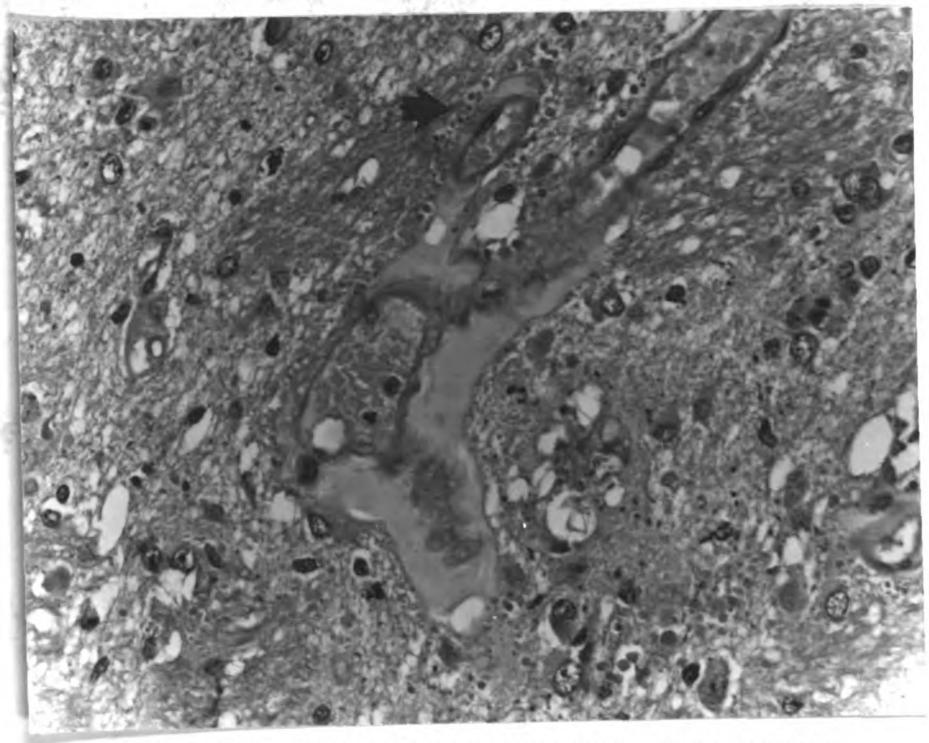

Figure 13. Extensive perivascular malacia and edema of cortical gray matter in a guinea pig fed normal guinea pig ration containing 22 p.p.m. MMD for 150 days. Notice homogeneous hyaline-like droplets in perivascular spaces (arrow) and adjacent tissue. H & E stain; X 315.

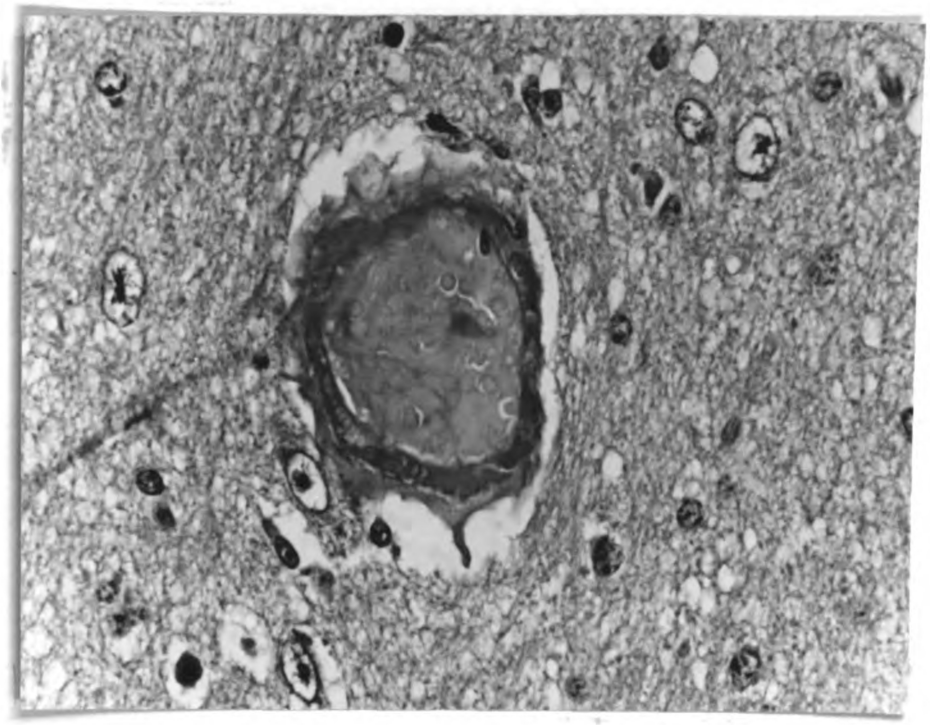

Figure 14. Severe fibrinoid necrosis of blood vessel wall and perivascular edema in the cortical gray matter of a guinea pig fed normal guinea pig ration containing 22 p.p.m. MMD for 150 days. H & E stain; X 500.

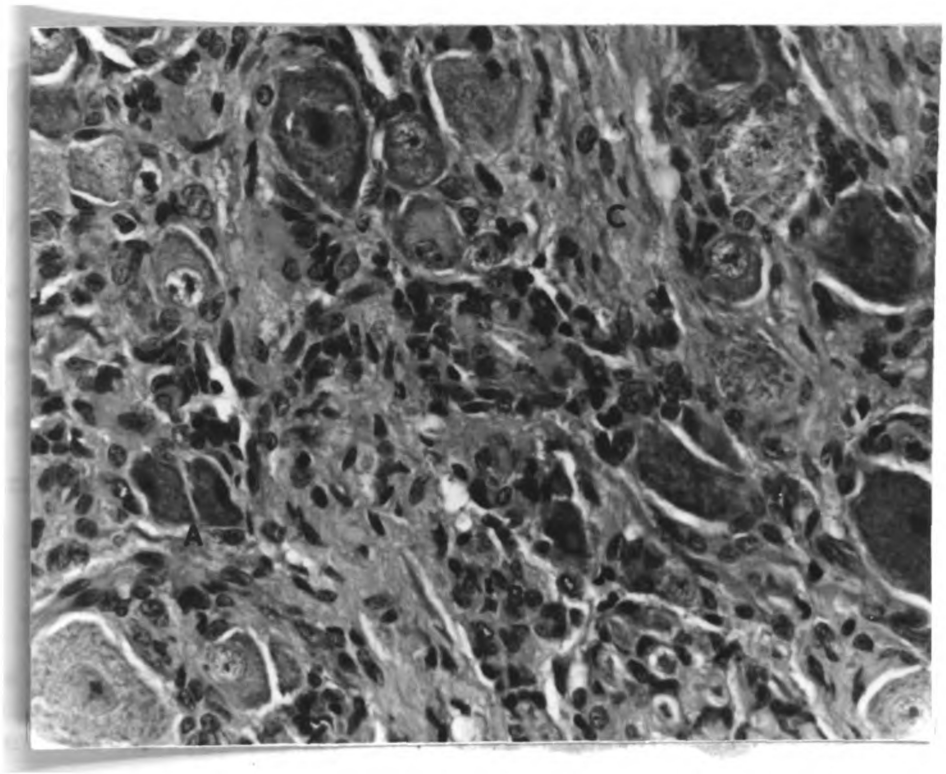

Figure 15. Dorsal root ganglion in a guinea pig fed normal guinea pig ration containing 22 p.p.m. MMD for 150 days. Notice neuronal degeneration (A), gliosis (B) and fibrosis (C). H & E stain; X 315.

Experiment III
44 p.p.m. Methyl Mercury Dicyandiamide in the Diet

Clinical Signs

The guinea pigs in Group C started to lose weight 24 hours after the beginning of the mercury-treated, ascorbic acid-deficient diet. The weight loss was rather irregular at first but became severe on Day 14 (Figure 16). By that time the plasma ascorbic acid level was 0.5 to 0.7 mg/100 ml (1.0 to 1.5 mg/100 ml in Groups A and D). On Day 17, 2 of the guinea pigs in Group C died. Initially they had a sudden onset of tremor and involuntary movements. The signs became severe in a few hours and were evidenced by aimless running and jumping. This condition was followed by a periodic recurrence of severe convulsions characterized by opisthotonos. The guinea pigs stretched their legs and necks. Muscles were spastic and rigid and the eyes were wide open for a few minutes. This was followed by an apparent loss of consciousness. The 2 guinea pigs died during a convulsive period within 2 hours after the initial signs. The other 3 guinea pigs in this group died with the same clinical signs, 1 on Day 19 and 2 on Day 20 (Table 3). The guinea pigs in the other 3 groups behaved normally during this time. Group D guinea pigs had a slower weight gain than Groups A and B (Figure 16). Three guinea pigs in Group D were killed for comparison with Group C on Days 17, 19 and 20 and the other 5 were continued on MMD-containing normal ration. They survived for 34 to 38 days (Table 3) and finally died after slowly progressive clinical signs of incoordination, paralysis, convulsion, prostration and death. Three guinea pigs in Group B were killed for comparison with Group C on Days 17, 19 and 20 and the other 2 were continued on the ascorbic acid-deficient ration and finally died on Days 46 and 48 as a result of scurvy (Table 3).

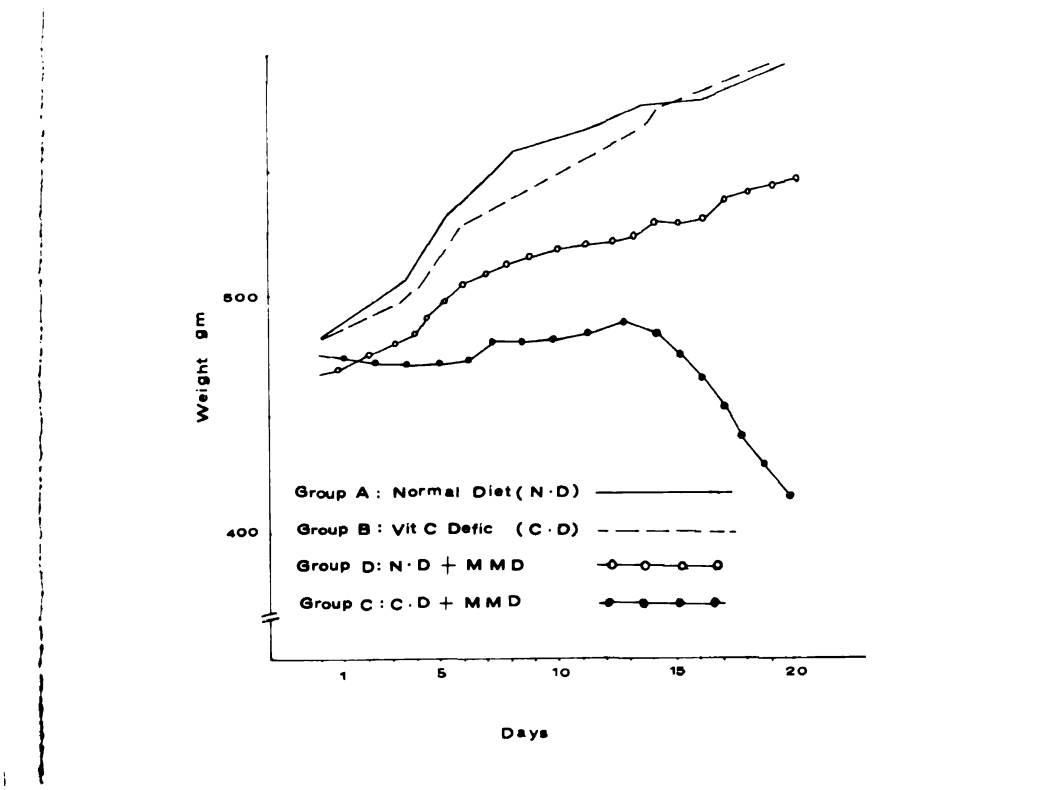

Figure 16. Average daily weights of 4 groups of guinea pigs. Methyl mercury dicyandiamide (44 p.p.m.) was added to diets of groups as indicated.

Gross Necropsy Findings

The kidneys and the livers of Group C guinea pigs were yellowish and much smaller than in other guinea pigs (Figures 17 and 18). There was little if any abdominal and subcutaneous adipose tissue. The organs from the guinea pigs in the other groups appeared to be normal.

Histopathology

The microscopic changes were similar in Group C guinea pigs, as follows.

Cerebrum. A generalized neuronal degeneration and necrosis were present throughout. The loss of cytoarchitecture was seen in different stages. Some of the degenerate neurons were swollen and had basophilic cytoplasm. Some were shrunken. Neurons in the more advanced stage of necrosis lost their cytoplasm completely and a dark stained nucleus remained in the center or periphery of a large vacuole. A large number of these vacuoles which probably indicated resorption of necrotic neurons were present. Vacuolation increased and became very severe in the region of the hippocampus. The gray matter had extensive vacuolation with disappearance of all neurons (Figures 19 and 20). A granular eosinophilic material was interspersed among the necrotic neurons and vacuoles. The walls of blood vessels were thickened. Cellularity was increased, and there was a deposition of eosinophilic homogeneous material in some vessels. Perivascular edema and hemorrhage with small foci of hemorrhage occurred in the tissue. A generalized gliosis was seen. The meninges were thickened with prominent vessels and areas of hemorrhage.

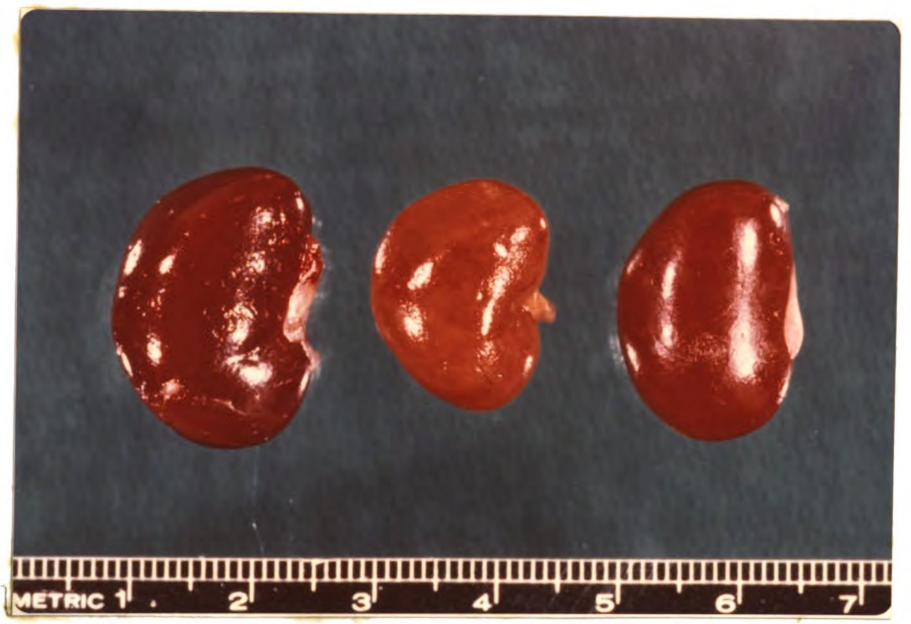

Figure 17. The kidneys (from left to right) from guinea pigs fed normal ration containing 44 p.p.m. MMD, ascorbic acid-deficient ration containing 44 p.p.m. MMD and ascorbic acid-deficient ration. Kidney from guinea pig fed ascorbic acid-deficient, MMD-containing ration was small and yellow.

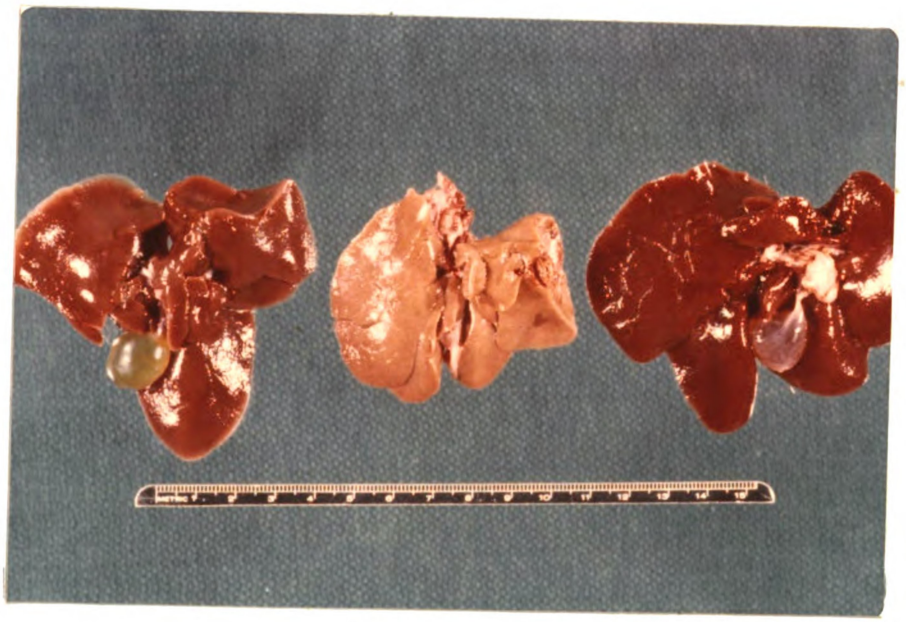

Figure 18. The livers (from left to right) from guinea pigs fed normal ration containing 44 p.p.m. MMD, ascorbic acid-deficient ration containing 44 p.p.m. MMD and ascorbic acid-deficient ration. Liver from guinea pig fed ascorbic acid-deficient, MMD-containing ration was small and pale.

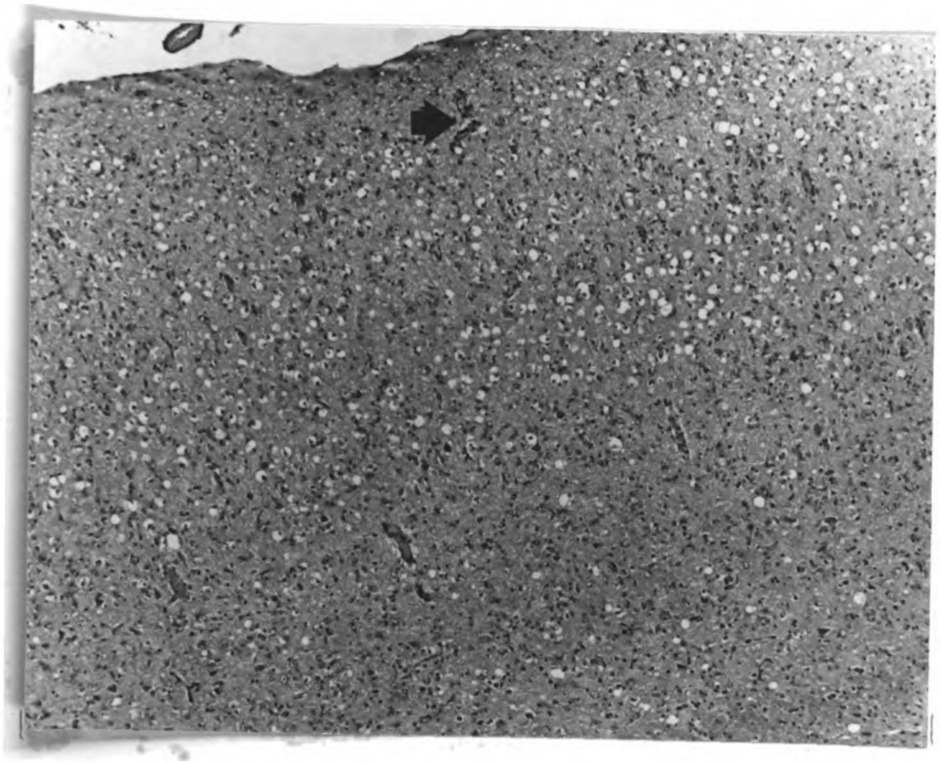

Figure 19. Extensive vacuolation and prominent vessels (arrow) of cortical gray matter in a guinea pig fed an ascorbic acid-deficient diet containing 44 p.p.m. MMD. H & E stain; X 50.

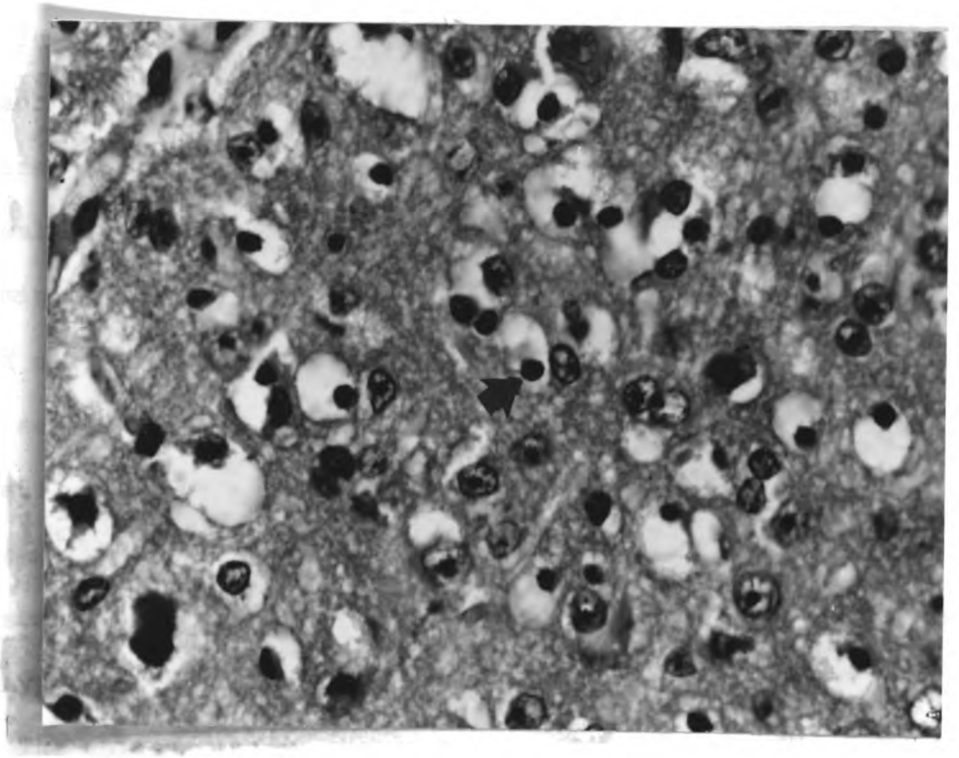

Figure 20. Higher magnification of a portion of Figure 19. The round black bodies in the vacuoles (arrow) indicate remnants of necrotic neurons. H & E stain; X 500.

Cerebellum. A reduction in the number of granular cells was seen in the cerebellar folia of Group C guinea pigs when compared to the other groups. There were vacuolation and edema with occasional degeneration of purkinje cell layers. Some of the folia had degeneration and a relative absence of purkinje cells (Figure 21).

The changes in sciatic nerve, liver, kidney, skeletal muscle, heart and adrenal gland were the same as described in Experiment II in Group C guinea pigs fed the ascorbic acid-deficient, MMD-containing diet. The wall of the aorta and large vessels in the axillary area and abdominal cavity had a degenerative change of muscle fibers with pyknotic nuclei and fragmentation of collagen bundles. These lesions were best seen by the Gomori trichrome staining method. The loose connective and adipose tissue surrounding these arteries was hemorrhagic, and there was fat necrosis. The nerve fibers in these areas were vacuolated and somewhat demyelinated. Areas of vascular damage were present in the kidney and liver.

The guinea pigs in the 3 other groups, which were killed at the same time as Group C guinea pigs were necropsied, had no apparent histopathologic changes. Five guinea pigs from Group D were continued on the MMD (44 p.p.m.)- containing normal diet. They survived for 34 to 38 days and finally died as a result of mercury toxicosis (Table 3). Lesions were seen in the cerebrum and peripheral nerve fibers. There was degeneration, necrosis and vacuolation of neurons and areas of congestion and hemorrhage in the cerebrum. Three guinea pigs had vacuolation of hepatocytes (Figure 22). The overall picture of extensive polioclastic activity which was seen in Group C was not present in this group.

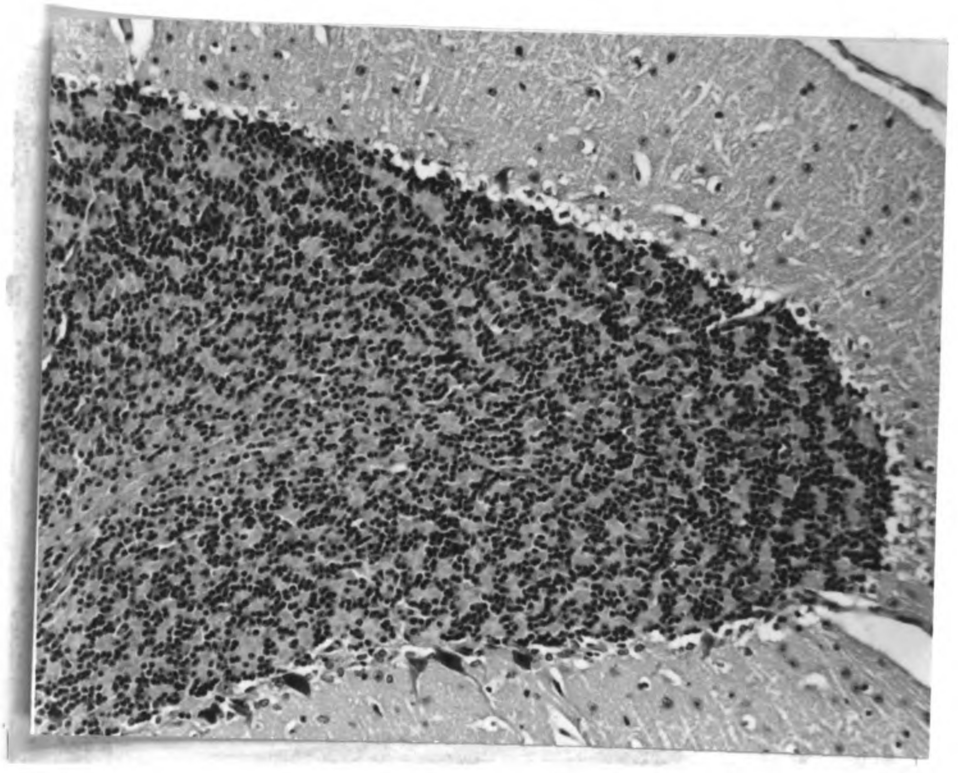

Figure 21. Cerebellum in a guinea pig fed an ascorbic acid-deficient diet containing 44 p.p.m. MMD. Notice vacuolation and necrosis and absence of purkinje cells. H & E stain; X 125.

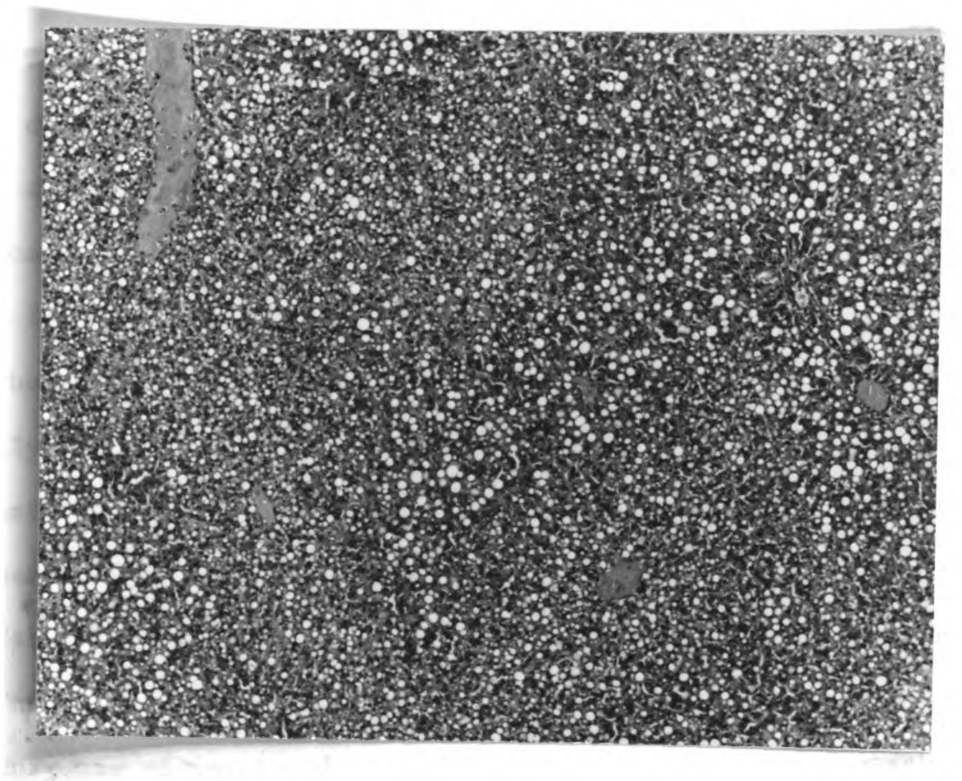

Figure 22. Hepatic tissue from a guinea pig fed normal ration containing 44 p.p.m. MMD for 34 days. Diffuse vacuolation of hepatocytes. H & E stain; X 50.

DISCUSSION

These experiments indicate that the duration and degree of ascorbic acid deficiency play a prominent role in the time of appearance, location and severity of clinical signs and lesions due to methyl mercury toxicosis.

In Experiment I and II the Group C guinea pigs were relatively scorbutic when they were given methyl mercury intraperitoneally or orally. In the scorbutic guinea pigs, diffuse hemorrhagic peritonitis or extensive ulcerative gastroenteritis indicated the destructive effect of mercury when it came into direct contact with the serosal or mucosal surface. Guinea pigs that had a diet containing adequate ascorbic acid did not have these lesions, even though in some instances much more mercury was administered. Also, there were no gastrointestinal lesions or hemorrhages seen in chronic toxicosis, even after 150 days.

Methyl mercury is a threshold substance and accumulates in the body when taken regularly even if individual doses are small. It may produce toxicosis and lesions through a cumulative effect (Suzuki, 1969). Our evidence indicates that ascorbic acid deficiency decreases the threshold (the amount of MMD required to produce toxicosis) of the body for mercury. In scorbutic guinea pigs in Experiment II, 9 mg of MMD intake in 25 days produced cerebral and peripheral nerve lesions. In contrast, it took 114 mg of MMD in 150 days to cause CNS lesions in the normally fed guinea pigs. In Experiment III, guinea pigs had normal plasma concentrations of ascorbic acid when methyl mercury was initially

fed. Guinea pigs were not in a severe scorbutic condition on Days 17 to 20 when they died of methyl mercury toxicosis. In these guinea pigs the gastrointestinal mucosa was not affected by direct contact with methyl mercury dicyandiamide (44 p.p.m.) even though the dose was twice that used in Experiment II (22 p.p.m.). However, in the nervous tissue, the ascorbic acid concentration had apparently decreased to the point that the amount of mercury required to produce lesions was lowered. This lowering of threshold may explain the extensive polioclastic effect in the CNS and destruction of peripheral nerve fibers. The guinea pigs fed a normal diet and MMD had a considerably higher threshold. They consumed 66% more MMD and survived 80% longer than the ascorbic acid-deficient, MMD-treated group.

The changes in the cerebellum in guinea pigs in methyl mercury toxicosis seem to differ considerably from those of man and such animals as the cow, cat, crow and rat (Herigstad *et al.*, 1972; Hunter *et al.*, 1954; Jubb and Kennedy, 1970; Takeuchi *et al.*, 1968; Diamond and Sleight, 1972). Vulnerability of the granular layer of the cerebellum and purkinje cells to MMD is apparently much lower in the guinea pig and a similar lack of vulnerability has been described in the pig (Tryphonas and Nielsen, 1973). In the present experiment the normal histologic structure of the cerebellum was intact in the guinea pigs with chronic methyl mercury toxicosis even though there was severe damage to the cerebrum. This pattern varied in the group fed an MMD-containing, ascorbic acid-deficient ration. This group had a variable degree of reduction of cellularity in the granular layer and degeneration, vacuolation and a partial disappearance of purkinje cells. The fibrinoid degeneration of vascular media which was observed in chronic toxicosis in guinea pigs was also reported in the rat and pig (Diamond

and Sleight, 1971; Tryphonas and Nielsen, 1973). This lesion was also present in the guinea pigs fed MMD-containing, ascorbic acid-deficient ration.

Retarded growth and loss of body weight were a constant manifestation in guinea pigs fed MMD-containing, ascorbic acid-deficient ration. Weight loss and retarded growth are clinical signs in methyl mercury toxicosis and depend on the dose. These signs are attributed to anorexia and an inability to eat as a result of CNS injury (Tryphonas and Nielsen, 1973). In the scorbutic animal, weight loss also follows anorexia. In Experiment III, the guinea pigs stopped growing and began to lose weight the day after the MMD-containing, ascorbic acid-deficient ration started. There was no anorexia present at that time and feed consumption was the same as for other groups. There was a considerable weight difference between this group and other groups on Days 17 to 20. This might be related to the combined effect of methyl mercury dicyandiamide and ascorbic acid deficiency on the cellular metabolic rate and was possibly due to a blockage of enzymatic reactions. Susceptibility to toxicosis was not affected by the sex of the guinea pigs in the experiments.

Neurologic signs have been relatively consistent in methyl mercury toxicosis. With sublethal doses, clinical signs usually progress from slight incoordination to severe paralysis and death in several days (Tryphonas, 1973; Herigstad *et al.*, 1972). The sudden onset of severe neurologic signs in Experiment III and the death of guinea pigs within a short period of time were likely related to the extensive polioclastic changes in the brain. Possibly the combination of ascorbic acid deficiency and methyl mercury toxicosis resulted in a sudden depletion of neural hormone (catecholamines) and caused disturbances in transmission of autonomic nerve impulses. There may be some other blockage in

enzymatic activity or synthesis which occurs in this condition.

Focal areas of coagulative hepatic necrosis surrounded by granular material and calcification were consistent lesions in guinea pigs fed the MMD-containing ascorbic acid-deficient diet. This type of lesion has not been described in either methyl mercury toxicosis or in ascorbic acid deficiency. A vacuolation of hepatocytes, probably fatty metamorphosis, has been described in methyl mercury toxicosis (Herigstad *et al.*, 1972; Diamond and Sleight, 1971; Tryphonas and Nielsen, 1973). The degenerative change in the aorta and in the walls of other blood vessels and hemorrhage in the surrounding loose connective and fatty tissue are thought to be the combined effect of increased permeability and fragility of vascular endothelium in ascorbic acid deficiency and to the destructive effect of circulating MMD in the blood.

It seems that the polioclastic effect of MMD in chronic toxicosis in guinea pigs is different and more destructive than in other species (Herigstad *et al.*, 1972; Diamond and Sleight, 1971; Takeuchi *et al.*, 1962). Similar but much less severe and destructive lesions were described in swine (Tryphonas and Nielsen, 1968, 1973).

SUMMARY

A series of 3 experiments was conducted to study the effects of ascorbic acid deficiency on methyl mercury toxicosis in guinea pigs.

In Experiment I, 16 guinea pigs were divided equally into 4 groups, 2 of which were fed a normal diet and 2 of which were fed an ascorbic acid-deficient diet. By 14 days, when ascorbic acid concentration decreased in plasma, a sublethal dose (4 mg/kg body weight) of methyl mercury dicyandiamide (MMD) was injected intraperitoneally (IP) at weekly intervals into 1 group of guinea pigs on each diet. The guinea pigs fed the ascorbic acid-deficient diet died after the second or third injection due to extensive hemorrhagic peritonitis. There were no pathologic changes in guinea pigs fed the normal diet except 1 which died after the first injection as a result of a perforated colon.

In Experiment II, 20 guinea pigs were divided equally into 4 groups, as described in the previous experiment. On Day 15, 22 p.p.m. MMD was added to the diet of 1 group of guinea pigs fed the normal diet and 1 group fed the ascorbic acid-deficient diet. The main lesions in guinea pigs fed the MMD-containing, ascorbic acid-deficient diet and which died on Days 18 to 26 were extensive hemorrhagic ulcerative gastroenteritis and coagulative necrosis of the liver. Two guinea pigs in the group fed the MMD-containing normal diet died as a result of chronic toxicosis in 150 days.

In Experiment III, the 23 guinea pigs were divided as in Experiments I and II. The design was changed in that 44 p.p.m. MMD was used instead

of 22 p.p.m. and was incorporated into the appropriate diets on Day 1 instead of Day 15. The guinea pigs fed the MMD-containing, ascorbic acid-deficient diet died on Days 17 to 20 with acute neurologic signs, and the pathologic changes were mostly polioclastic. The guinea pigs fed MMD in a normal diet survived up to 38 days. The ascorbic acid deficient controls survived up to 47 days.

The experiments indicate that the degree of ascorbic acid deficiency is important in the location and severity of clinical signs and lesions due to methyl mercury toxicosis.

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