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CHRONIC METHYLMERCURY TOXICOSIS IN CALVES

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
Rawlin R. Herigstad
1971

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ABSTRACT

CHRONIC METHYLMERCURY TOXICOSIS IN CALVES

By

Rawlin R. Herigstad

Six calves were used to study the experimental clinical signs, lesions and tissue residues after feeding low amounts of organic mercury over a long period of time. Weight gains tended to decrease in proportion to increasing amounts of mercury fed. Between 35 and 91 days 3 of the 6 calves developed clinical signs of toxicosis characterized by a sudden onset of ataxia and neuromuscular incoordination which progressed rapidly to convulsions and a moribund state. The histopathologic changes were primarily a reduction in the number of cerebellar granular cells and nephrosis of the proximal convoluted tubules. Mercury residues in tissues were not correlated with the amount fed but were relatively consistent within each calf when the residues in hair, liver, kidney, brain, and semitendinosus muscle were compared.

CHRONIC METHYLMERCURY TOXICOSIS IN CALVES

By

Rawlin R. Herigstad

A THESIS

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INTRODUCTION

Environmental pollution has concerned the population of the world in recent years. Mercury contamination has received great publicity especially where industrial effluents and other misuses of mercurial compounds have disturbed the ecological safety in certain areas of the world. The Great Lakes waterways have been shown to be polluted with mercury which has precipitated a concern in Michigan. This pollution has potentially endangered human lives as well as the lives of animals which man uses for food.

Although the scientific community in the United States has only recently initiated work on the mercury problem, Japanese and Swedish scientists have done considerable research on the subject during the past decade. Japanese workers have concentrated on the description and pathogenesis of organomercurial toxicosis (Minamata disease) primarily in man, while Swedish workers have investigated the effects of mercury pollution as related to the environment.

Since beef is an important constituent of American diets and only minimal information is available on the lesions and mercury residues in the tissues of calves fed organic mercury, it seemed important to determine the clinical signs and lesions of chronic methylmercury toxicosis in this species. Case reports of organomercurial toxicosis in cattle have failed to distinguish between the lesions of inorganic and organic mercury poisoning. Additional information is also needed to

differentiate organomercurial toxicosis from other diseases involving the central nervous system of cattle.

The objectives of this research in young calves were (1) to describe the clinical and morphological changes associated with methylmercury toxicosis, (2) to correlate the levels of mercury intake with residues in the tissues, and (3) to compare the encephalopathy of methylmercury toxicosis with that of other common disturbances of the central nervous system.

REVIEW OF THE LITERATURE

This review of the literature is limited to the more important and pertinent references on the history, industrial and medical uses, and physiologic and pathologic effects of mercury compounds. A comprehensive review is not intended. Additional reports can be found on toxicologic studies of mercury compounds in man and laboratory animals. While the current emphasis is directed towards organic mercury poisoning, many of the early reports did not emphasize the difference between inorganic and organic compounds in mercury toxicosis.

History

Mercury has been used by man since prehistoric times. Goldwater (1936) and later King (1957) reviewed its early uses in industry and medicine by Hippocrates, Arabian physicians, and the Chinese. Although the toxicity of mercury was known early, it was still used in medicine as a specific treatment for syphilis.

Chemistry

Mercury has the following properties and characteristics: atomic number, 80; atomic weight, 200.61; melting point, -38.9 C.; and boiling point, 356.9 C. Nearly all mercury is mined as cinnabar, HgS, which is later refined. In chemical compounds, mercury is usually present as mercurous, Hg^{+}, or mercuric, Hg^{++}, ions.

Industrial Uses

According to King (1957), mercury and its compounds are used in 80 industries and in more than 3,000 different ways. Bidstrup (1964), quoting Battigelli (1960), listed the main hazardous industrial uses of mercury as follows: cinnabar mining; felting of fur; manufacturing of scientific instruments such as thermometers, manometers, flow meters, and level regulators; manufacturing of carbon brushes in electric generators; manufacturing of mercury-vapor lamps and neon signs; producing mercury-containing paints and other mercury compounds; and using mercury in laboratories. In addition, mercury is used in amalgams, in fire-gilding gold or silver, in finger-printing powder, in running mercury-vapor turbines, in casting processes, in surgical dressings and pharmaceutical products, in dry-cell batteries, in making detonators and percussion caps, in treating fungal diseases of plants, and in the preservation of wood and textiles.

Therapeutic Uses

Although many of the inorganic and organic mercurial preparations which were used as therapeutic agents in the past have been replaced by safer and more efficient drugs today, it is regrettable that some are still found in pharmacies. Bidstrup (1964) indicated that mercurials have had a long history of use as diuretics and in treatment of syphilis and pediculosis. Today, phenylmercuric compounds are still extensively used as antiseptics and bacteriostatics.

Absorption, Distribution, Accumulation, and Excretion

Mercury and its compounds can be absorbed through the skin, gastrointestinal tract, and respiratory tract. Hughes (1957) and Battigelli (1960) stated that mercurials react with thiols, sulfhydryl groups, and

mercurial distribution in the body varies directly with its lipid solubility. Interference with thiols or sulfhydryl groups is thought to be the mechanism by which mercury inhibits enzyme systems, although the extent of this in mercury toxicosis remains unknown. Most of the thiols in plasma occur as serum mercaptalbumin which can rapidly transport mercury in the blood stream resulting in wide distribution in the tissues. Alkylmercurials with low molecular weights are the most rapid and widely distributed. At the tissue level mercurials dissolve in lipid membranes and participate in protein-mercurial complexes which allows extensive accumulation. Excretion of mercury occurs through the urine and feces, although the half life varies greatly with the individual and mercurial compound as indicated by Löfroth (1970). The half life of methylmercury is 70 days in man (Löfroth, 1970) and 200 days in fish (Hammond, 1971). All individuals have a small amount of mercury in their body normally since it is an ubiquitous element.

Mercury Toxicosis

Since mercury has produced toxicosis by passage through the food chain, the Food and Drug Administration has recently placed a 0.5 parts per million limit on mercury residues in foodstuffs for human consumption. In 1971 Ely reviewed the general nature and occurrence throughout the world of organic mercury poisoning of man from contaminated foods. He also summarized the amount of mercury residues in the common foods of Sweden and Canada as well as the toxic and nontoxic levels of mercury in human blood and hair.

In man Minamata disease has been a classic example of natural organomercurial toxicosis which resulted from eating mercury-contaminated fish and shellfish (Takeuchi *et al.*, 1962). Curley *et al.* (1971) reported the first documented case of indirect mercury poisoning in

humans in the United States caused by the ingestion of contaminated pork from swine that had consumed mercury in their food supply. Hanko *et al.* (1970) demonstrated, by direct experimental evidence, the transfer and accumulation of alkylmercury in toxic form through the food chain. They produced a toxicosis in ferrets by feeding them chickens that had consumed mercury.

Although mercury has been described as toxic in both inorganic and organic forms, most of the articles on mercury toxicosis in farm animals have been case reports. The majority of the experimental studies have used laboratory animals. Good descriptions of the pathologic changes in experimental organomercurial toxicosis are limited. Mercury toxicosis has occurred mostly in cattle and swine with only occasional reports in the other domestic animals.

The inorganic mercury compound, mercuric chloride, has caused toxicity in cattle from misuse of mercurial antiseptics and ointments (Stevens, 1921; Stevens, 1938; Turner, 1904). In 1965 Donnelly described the pathologic changes of experimental toxicosis in pigs fed mercuric chloride.

In livestock fungicide-treated grain has been incriminated in the majority of the reports of organomercurial toxicosis. In an early case report Boley *et al.* (1941) described the clinical signs and lesions in feeder calves that had been fed mercury-treated corn. There were 2 reports of dairy cows poisoned by treated seeds (Butler, 1965; Herberg, 1954).

Japanese workers reported the clinical and pathologic changes of mercury toxicosis in mature dairy cows consuming mercury-contaminated linseed meal (Fujimoto *et al.*, 1956; Sonoda *et al.*, 1956). Although there were 29 cases of toxicosis out of 171 cattle fed the contaminated

linseed meal, the majority of the affected cases had no relationship between the duration or amount of mercury-contaminated linseed meal fed. Some of the prominent clinical signs were fever, anorexia, diarrhea, dermatitis, and anemia. Hemorrhages and inflammatory reactions characterized the histologic changes in the parenchymatous organs. These workers were concerned about a complicating infectious agent. However, bacteriological and virological investigations failed to identify one.

In a pharmacologic study, Canadian workers reported the acute toxicity of an ethylmercury compound in calves (Oliver and Platonow, 1960). They recorded the cumulative effects and disposition of their compound in 5 calves given relatively large doses (4.7 to 94.4 mg. Hg./kg. body weight) for 36 days or less. Although incidental to their study, they also described the clinical signs and pathologic changes of ethylmercury toxicosis.

In 1947 Taylor recorded the first case of organomercurial toxicosis in pigs. McEntee (1950), in a detailed case report, described the pathology of organomercurial poisoning in swine. An additional report was made in 1967 (Loosmore *et al.*, 1967). Tryphonas and Nielsen (1970) reported the pathologic changes associated with experimentally produced arylmercury poisoning in swine. They stated that the lesions from phenylmercuric chloride were similar to those described for mercuric chloride. The experimental toxicity and distribution of mercury in pigs with acute methylmercurialism was recently reported, but no histological descriptions were included (Piper *et al.*, 1971).

Edwards in 1942 published a case report of mercury poisoning in a horse. In 1963 Palmer reported on the experimental production of mercury poisoning in sheep and chickens, but only the gross pathologic changes were described. Additional experimental pharmacologic reports have been

made using chickens (Miller *et al.*, 1961; Swensson and Ulfvarson, 1969). Jungherr in 1957 recorded a case report of mercury poisoning in chinchillas fed treated grain. In a toxicity study using rats, mice, and rabbits, Swensson and Ulfvarson (1969) reported that alkylmercury was excreted more slowly than other mercury compounds. Morikawa (1961), using cats, published the effects of organic mercury in pregnant queens and their offspring.

When Hunter *et al.* (1940) reported their classic description of the pathologic changes of organomercurial toxicosis in man, they mentioned that the first report of mercury-methide toxicosis was described by Edwards in 1865. English and Japanese workers have reported additional cases (Hunter and Russell, 1954; Takeuchi *et al.*, 1962). In 1964, Bidstrup reviewed numerous cases of mercury poisoning in man.

Clinical Signs

The signs of mercury toxicosis have varied with the mercury compound and the rate and duration of exposure but have been quite similar in all species. A summary of the clinical signs of acute poisoning, usually inorganic mercury poisoning, includes severe abdominal pain, weakness, depression, vomition, diarrhea, hypothermia, and collapse with death from uremia. The clinical signs of chronic poisoning, usually organic mercury poisoning, are indicated by ataxia, muscular incoordination, tonoclonic convulsions, disturbances of the central nervous system, prostration, and death. Textbooks have best delineated this difference between the clinical signs of inorganic and organic mercury toxicosis (Jubb and Kennedy, 1963; Smith and Jones, 1966).

Gross Pathology

Most of the articles in the literature have failed to make adequate differentiation between the lesions of inorganic and organic mercury toxicosis. Some reports have parroted the description of inorganic mercury poisoning when the compound was organic (Herberg, 1954; Palmer, 1963). After a critical review of the literature, Jubb and Kennedy (1963) and Smith and Jones (1966) summarized the gross pathologic changes for inorganic and organic mercury poisoning. The lesions of inorganic mercury poisoning were described as a severe hemorrhagic and coagulative gastroenteritis plus a severe nephrosis. Organomercurial toxicosis was characterized by minimal gross lesions.

Histopathology

The microscopic changes of acute inorganic mercury poisoning were coagulative necrosis of the gastrointestinal epithelium and renal tubules. The histologic lesions of organomercurial toxicosis in all species were, specifically, a selective decrease in the number of granular cells in the cerebellum and a mild to moderate toxic nephrosis in the renal tubular epithelium. Degeneration of Purkinje fibers in the bovine heart, neuronal degeneration in the cerebral cortex with moderate gliosis, degeneration of spinal neurons and cerebellar Purkinje cells, demyelination and axonal degeneration of peripheral nerves, and fibrinoid necrosis of leptomeningeal arterioles were also described in some cases (Jubb and Kennedy, 1963). These lesions were summarized from cases of organomercurial toxicosis in swine and cattle.

Summary

Although there are numerous case reports in the literature of organomercurial poisoning in the various domestic species, only meager

experimental information is available in relation to the potential magnitude of the problem. The experimental pathology and mercury residues in the tissues of calves with chronic methylmercury toxicosis are lacking. Since beef is an important constituent of American diets, the possibility of toxicosis through the food chain to man exists. Thus, studies on methylmercury toxicosis in calves are warranted because of these implications in public health.

MATERIALS AND METHODS

Six 4-week-old male Holstein-Friesian calves which were fed colostrum at birth and from the same dairy herd were used. For most of the experiment the calves were kept together in 1 lot which had a straw-bedded shed. The experiment was started in October, 1970.

The ration consisted of whole milk fed at the rate of 10% of body weight/day. The milk was equally divided between morning and evening. A small amount of grass pasture was available *ad libitum* for the first 6 weeks. During the seventh week the milk was reduced to 4 lb./day/calf, and grain, a mixture of corn, oats and protein supplement, was fed *ad libitum*. Each calf ate approximately 5 to 6 lb. of grain per day. Water was available from an automatic tank.

The calves were randomly assigned levels of mercury (Table 1). Mercury was added to the morning milk at 0, 1.25, 2.50, 5.0, 10.0, or 20.0 mg./50 kg. body weight/day. The mercury product* was an aqueous solution of a widely used seed-treating agent. The control calf was fed the mercury vehicle (Rhodamine B base dye, ethylene glycol, and ethanol) in aqueous solution.

At weekly intervals blood and hair samples were collected, the calves were weighed, and the amount of milk fed during the following week was adjusted to their weights. After 6 weeks the samples were taken

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*Ceresan L (methylmercury 2,3 dihydroxypropyl mercaptide - 2.89%, methylmercury acetate - 0.62%. Total mercury - 2.25%). Produced by E. I. duPont de Nemours & Co., Inc., Wilmington, Del.

biweekly. The blood was collected in tubes which contained 0.5 ml. of sodium citrate (200 mg./ml.). The blood samples were centrifuged, and the plasma and red blood cells were stored separately. The samples of hair were washed in mild soap, rinsed in water, and dried. At necropsy, samples of rumen contents, liver, kidney, brain, skeletal muscle, urine and feces were collected for mercury analysis. The mercury analyses of the tissues were conducted using a slightly modified procedure of flameless atomic absorption spectrophotometry (Hatch and Ott, 1968).

Prior to euthanasia, jugular blood was collected into EDTA tubes for determinations of hemoglobin, packed cell volume, total and differential leukocyte counts, and total protein. The calves were euthanatized when moribund or at the termination of the experiment. At necropsy, urine and feces were collected. The routine parameters of urinalysis were color, transparency, specific gravity, pH, acetone, albumin, bile, urobilinogen, glucose, indican, and urinary cytology.

Tissues from lung, liver, kidney, spleen, heart, skeletal muscles, abomasum, duodenum, ileum, colon, brain, spinal cord, and ulnar, brachial and sciatic nerves were collected and fixed in 10% buffered neutral formalin for histologic examination. The fixed tissues were embedded in paraffin and sectioned at 6 μ using routine procedures (Luna, 1968). The histological sections were stained with hematoxylin and eosin (H&E) and luxol fast blue.

RESULTS

Growth and Mercury Residues

Growth records of calves fed the various amounts of mercury are summarized (Table 1). As the mercury dosage per day increased, the average daily gain of the calves tended to decrease except for Calf 11. The absolute amount of mercury necessary for toxicosis increased with the duration of the experiment and with the body weight of the calves. Weekly weighings indicated individual variation in response to mercury intake, but final analysis of average daily gain was inversely related to daily mercury intake. The total dose of mercury was more important for the production of methylmercury toxicosis than the level or duration of administration in the 3 calves which developed clinical signs.

The levels of mercury in the tissues sampled from each calf are summarized (Tables 2 and 3). The mercury residues in plasma, erythrocytes and hair increased linearly at each mercury dosage during the first 5 to 7 weeks, but the rate of increase leveled off in the final 3 samplings. Although Calves 8, 9 and 11 developed the clinical signs of methylmercury toxicosis and became moribund or died, there were great differences in the amounts of mercury in the visceral tissues indicating little or no correlation with the total dose of mercury fed. Calf 8, which was fed the highest dose of mercury for the shortest time before toxicosis, had the highest tissue levels. The values in Calves 9 and 11 were not proportional to those of Calf 8. Calves 7 and 9 had approximately the same levels of mercury in their tissues but had wide differences

Table 1. Mercury dosage and weight changes of calves fed methylmercury in milk

Calf no.	Amount of mercury fed: Mercury per day (mg./kg. BW/day)	Amount of mercury fed: Total mercury (mg.)	Amount of mercury fed: Total mercury per final weight (mg./kg. BW)	Amount of mercury fed: Calculated approximation of mercury per 90% dry matter feed (mg./kg. feed)	Days fed mercury	Initial wt. (kg.)	Final wt. (kg.)	Avg. daily gain (kg./day)
10	0	0	0	0	0+	49.9	152.3	1.1
9	0.025*	1392.1	12.3	10.0	81	45.4	113.5	0.8
12	0.05	443.0	2.8	2.1	91+	52.2	159.1	1.2
7	0.1	858.2	5.7	4.1	91+	54.5	150.0	1.1
11	0.2**	2188.4	15.0	10.1	91	56.8	145.5	1.0
8	0.4	901.6	11.7	28.0	35	49.9	77.2	0.8

*Daily amount of mercury increased to 0.4 mg./kg. BW/day on experimental Day 40 which was 5 days after Calf 8 died.

**Daily amount of mercury increased to 0.4 mg./kg. BW/day on experimental Day 88 which was 7 days after Calf 9 died.

+Clinical signs of MMT did not develop during the course of the experiment which was terminated on Day 91.

Table 2. Concentrations of mercury in plasma, erythrocytes, and hair from samples taken at intervals from calves fed methylmercury (mg./kg., wet weight)

Calf no.	Mercury fed per day (mg./kg. BW/day)	Weeks on experiment 1	2	3	4	5	7	9	12	13
Plasma										
10	0	ND*	ND	ND	ND	ND	ND	ND	---	---
9	0.025(0.4)**	ND	0.1	0.2	0.4	0.6	0.8	0.9	---	---
12	0.05	ND	0.1	0.1	0.1	0.4	0.8	0.7	---	---
7	0.1	ND	ND	0.1	0.2	0.4	0.7	0.6	---	---
11	0.2(0.4)**	0.1	0.1	0.3	0.3	0.7	1.0	1.2	---	---
8	0.4	0.9	1.1	1.5	1.6	3.0	---	---	---	---
Erythrocytes										
10	0	ND	ND	ND	ND	ND	0.1	0.1	---	ND
9	0.025(0.4)**	0.5	1.0	1.5	2.0	2.1	3.2	3.8	---	---
12	0.05	ND	0.2	0.4	0.7	1.1	1.6	2.0	---	1.4
7	0.1	0.4	0.5	1.1	1.9	2.0	2.8	3.0	---	3.0
11	0.2(0.4)**	0.3	0.6	1.3	2.3	2.6	4.2	5.0	---	5.4
8	0.4	0.5	2.0	3.2	5.2	6.1	---	---	---	---
Hair										
10	0	ND	ND	ND	ND	ND	ND	0.1	ND	ND
9	0.025(0.4)**	0.1	0.2	0.4	0.8	1.1	2.4	4.2	---	---
12	0.05	ND	0.1	0.3	1.1	1.4	1.8	2.0	1.8	1.4
7	0.1	ND	0.2	0.6	1.0	2.5	3.4	3.8	3.7	3.8
11	0.2(0.4)**	0.2	1.0	3.8	6.3	11.0	11.3	12.1	13.4	12.6
8	0.4	0.6	2.0	8.2	29.0	40.8	---	---	---	---

*ND = not detectable.

**Amount of mercury fed was increased to 0.4 mg./kg. BW; Calf 9, Day 40; Calf 11, Day 88.

Table 3. Concentrations of mercury in tissues at terminal samplings from calves fed methylmercury

Calf no.	Erythrocytes	Concentrations of mercury (mg./kg., wet weight)							
		Hair	Liver	Kidney	Brain	Semitendinosus muscle	Rumen contents	Urine	Feces
10	ND*	0.1	ND	ND	ND	ND	ND	---	ND
9	3.8	4.1	4.8	3.1	2.0	2.6	1.0	---	---
12	1.4	2.0	1.9	2.0	0.6	0.6	0.1	---	0.1
7	3.0	3.8	4.1	2.6	1.5	1.9	0.4	---	1.0
11	5.4	12.6	6.2	12.3	9.4	9.4	0.6	0.7	1.0
8	6.1	40.8	29.0	27.2	20.1	24.4	4.4	2.7	---

*ND = not detectable.

in the ratios of total mercury fed to their final body weight: 12.3 to 5.7 mg./kg., respectively. Tissue levels of mercury within an individual calf were relatively consistent for all tissues sampled.

The coefficients of linear correlation and the probability of obtaining these coefficients by chance between the various tissues are tabulated (Table 4). This indicated that the mercury residues in hair, liver, kidney, brain and semitendinosus muscle were correlated linearly, but the residues in erythrocytes were not correlated with the other tissues. Therefore, as an antemortem parameter, samples of hair would best reflect the mercury status of the calf after chronic ingestion of mercury.

Clinical Signs

During the early period of the experiment, some coughing and diarrhea were present in the calves. While 1 calf (10) was given appropriate treatment, in general, this problem was not believed severe enough to modify the results. The rectal temperatures were in the normal range, and feed consumption was not impaired during this period.

Although there was a long latent period, methylmercury toxicosis was characterized by sudden onset of ataxia and neuromuscular incoordination which rapidly developed into prostration and a moribund condition in 3 to 6 days (Figure 1). The ataxia progressed from a mild incoordination characterized by swaying of the hindquarters and a stumbling, stilted gait in the forequarters to convulsions characterized by opisthotonos and tonoclonic movements. Motor incoordination gave the impression of muscular weakness. Involuntary movements of the head from side to side and twitching of the ears and neck muscles were the primary initial signs. Later, twitching of the trunk muscles became a common sign when the calves were recumbent. Hyperesthesia was present when the calves were stimulated. Failure to eat primarily resulted from spatial disorientation

Table 4. Coefficients of linear correlation and the probability of obtaining these values by chance between the various residues of mercury recorded in Table 3

	Erythrocytes	Hair	Liver	Kidney	Brain
Hair	0.645 P > 0.10				
Liver	0.622 P > 0.10	0.825 P < 0.05			
Kidney	0.689 0.10 > P > 0.05	0.825 P < 0.05	0.800 P < 0.05		
Brain	0.692 0.10 > P > 0.05	0.821 P < 0.05	0.793 P < 0.05	0.833 P < 0.05	
Semitendinosus muscle	0.689 0.10 > P > 0.05	0.830 P < 0.05	0.811 P < 0.05	0.832 P < 0.05	0.830 P < 0.05

Figure 1. Moribund Calf 8 after 34 days of feeding methylmercury.

and motor impairment of the calf in relation to its feed. Loss of appetite was not the problem. Rectal temperatures of affected calves were normal or subnormal. The poisoned calves also drooled saliva. These calves were not blind, but Calf 11 may have had decreased vision as determined by lack of response to hand movements in front of his eyes.

Clinical Pathology

The hematologic examinations did not indicate consistent changes that could be correlated with methylmercury toxicosis. All calves had a neutrophilia and/or a left shift. The urinalysis of poisoned calves revealed a 2+ to 4+ albumin and the presence of glucose. The remaining urinary parameters were normal. Fecal flotations indicated mild to moderate numbers of nematode ova and coccidial oocysts.

Gross Pathology

There were no outstanding gross lesions in any of the mercury-poisoned calves. All calves had limited consolidation and pneumonia in the anterior ventral lobes of the lungs. Calf 8 had excessive fluid over the cerebral hemispheres when the dura mater was removed. Calf 9 had focal hemorrhages in the cortices of the kidneys, and some skeletal muscles were excessively pale. Calf 11 had pale kidneys. Dehydrated feces were found in the rectum of the mercury-poisoned calves. The remaining organs of the calves with methylmercury toxicosis did not have any significant lesions. Calves 7, 10 and 12 had no gross lesions other than mild pneumonia.

Histopathology

The only significant histologic changes were a reduction in the number of granular cells in the cerebellum and a focal to diffuse toxic

proximal tubular nephrosis. The loss of granular cells in the cerebellar folia was best discernible when compared with the number of granular cells in the cerebellum of the control calf (Figures 2 and 3). The layer of Purkinje cells was edematous. Occasionally, there was degeneration of neurons in the deep layers of the cerebral cortex, but these changes were difficult to differentiate from normal attrition of neurons. The area of the visual cortex was not changed to a greater degree than other cerebral areas. The toxic proximal tubular nephrosis was characterized by granular swelling of the tubular epithelium or loss of nuclei and thinning of the epithelial cytoplasm resulting in tubular dilatation (Figures 4 and 5). Some of the Bowman's spaces and cortical tubules contained proteinaceous material. Proteinaceous casts were present in the medullary tubules. Foci of calcification were more prominent in the kidneys of calves with the clinical signs of methylmercury toxicosis. The fibers of the pale muscles of Calf 9 were fragmented, hyalinized and necrotic with a minimal inflammatory infiltrate.

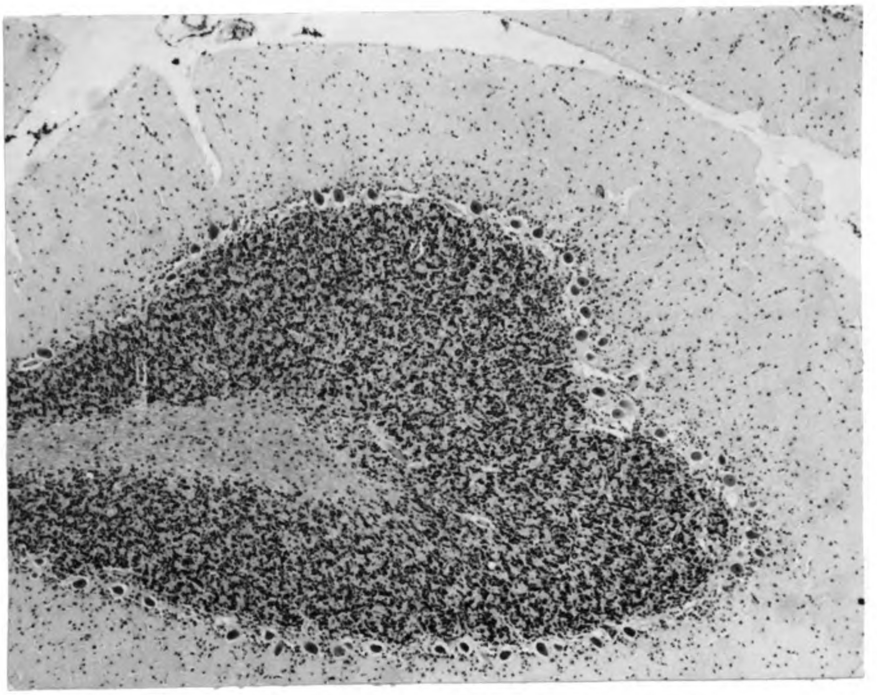

Figure 2. Cerebellum of control Calf 10. Luxol fast blue stain; x 53.

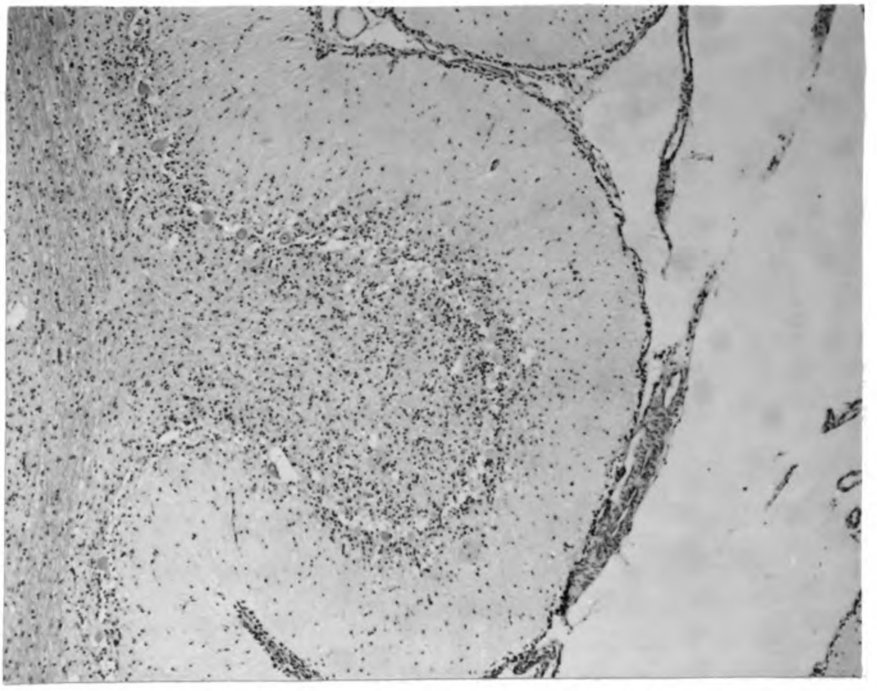

Figure 3. Cerebellum of Calf 11 fed methylmercury for 91 days. Note decrease in cells in granular cell layer in contrast to Figure 2. The number of cells along the meningeal border of the molecular layer was considered normal for a young calf. Luxol fast blue stain; x 53.

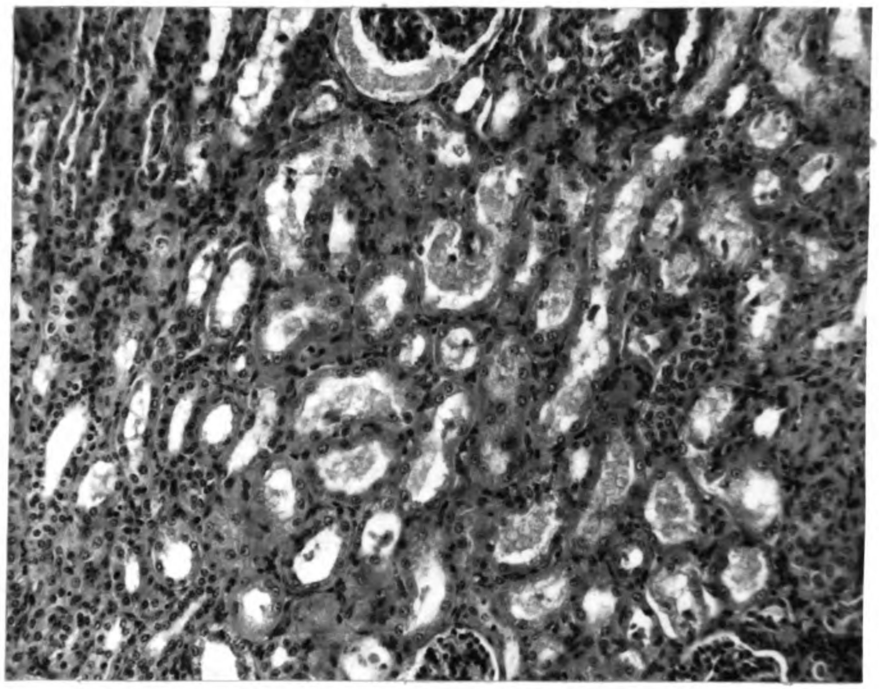

Figure 4. Kidney of Calf 11 with proximal tubular nephrosis and proteinaceous material in Bowman's space and renal tubules. H & E stain; x 136.

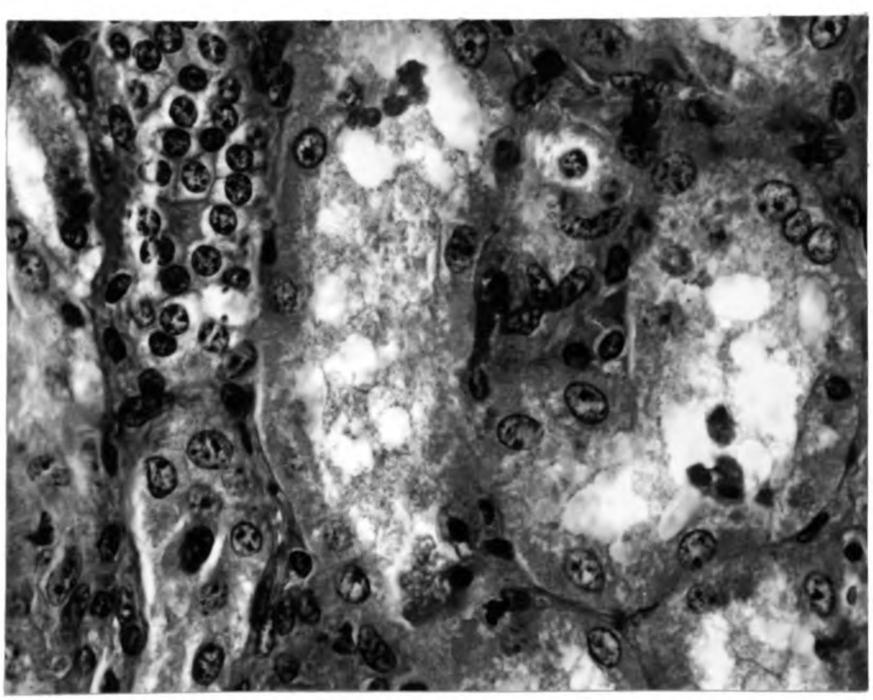

Figure 5. Higher magnification of Figure 4, showing thinning of epithelial cells and loss of nuclei. H & E stain; x 540.

DISCUSSION

The results of this experiment indicated that there was variation in the response of individual calves to a given amount of mercury consumed. This was especially evident in the variation, among calves, in residues of mercury in the tissues. Growth was increasingly inhibited by increased mercury dosage. In addition to individual variation, the use of whole milk as the feeding vehicle may have influenced the absorption of mercury since milk has long been used to reduce the toxicity of heavy metals. It was interesting to note that the average lethal amount of total mercury fed per final body weight for Calves 8, 9 and 11 was 13.0 ± 1.8 mg./kg. This would suggest the importance of the total amount of mercury fed in relation to body weight in methylmercury toxicosis.

The most consistent and specific lesion of methylmercury toxicosis in this research was a paucity of cerebellar granular cells. Also commonly observed was toxic nephrosis of the proximal convoluted tubules. In a natural outbreak of mercury toxicosis in mature cattle, Japanese workers mentioned the clinical signs of fever, anorexia, diarrhea, depilation, anemia, cardiac disturbance, and petechiae on the visible mucous membranes, and the lesions of interstitial nephritis, catarrhal bronchitis, enlargement and swelling of lymph nodes and splenic follicles, subendocardial and subepicardial hemorrhages, and focal hepatic necrosis and cirrhosis (Fujimoto *et al.*, 1956; Sonoda *et al.*, 1956). These signs and lesions were not observed in this experiment. The Japanese workers

stated that there was individual variation in response to mercury as we also observed. The Japanese investigators were suspicious of an infection that complicated their findings, but they were unable to isolate any pathogenic agents. In addition, they did not report any central nervous system signs or lesions. The neutrophilia, with the shift to the left as reported by the Japanese was also noted in our work; however, an anemia was not observed.

Canadian workers (Oliver and Platonow, 1960), using N-(ethylmercuri)-p-toluenesulfonanilide, described organomercurial toxicosis in calves, and their results were in general agreement with the present research. Although they used amounts of mercury that were approximately 100 to 2,000 times higher than those used in this report, the clinical signs and pathologic changes in general were similar. However, they reported minor gastrointestinal disturbances, and the clinical signs developed more rapidly after mercury administration. We observed that tissue residues were equally distributed throughout the visceral tissue of each individual as reported by a Swedish investigator (Löfroth, 1970), but our results were contrary to the Canadian study.

The encephalopathy observed in this study is in general agreement with an early classical report of mercury toxicosis in man, rats and a monkey (Hunter *et al.*, 1940). Our studies were also in essential agreement with a report on Minamata disease--organomercury toxicosis in man (Takeuchi *et al.*, 1962). In addition to the primary lesion of less granular cells in the cerebellum and injury to the visual cortex, the latter report also mentions degenerative changes in other cortical areas, as well as atrophy and degenerative changes in the spinal cord and peripheral nerves.

Cerebrovascular damage as described in swine was not observed in this experiment but may be important in the pathogenesis of this disease (Tryphonas and Nielsen, 1968). Mercury binds to sulfhydryl and disulfide groups and could interfere with protein metabolism (Bidstrup, 1964). In an ultrastructural study of organomercurial poisoning, damage to granular cells was a characteristic change and was believed to be a disturbance in protein synthesis, although no specific relationship was noted between vascular and granular-cell changes (Miyakawa and Deshimaru, 1969).

Differential Diagnosis

In a differential diagnosis the clinical syndrome of methylmercury toxicosis must be separated from the common encephalopathies of the bovine, especially polioencephalomalacia, thromboembolic meningoencephalitis, listeriosis, rabies, and lead poisoning.

Actually the primary incentive for this research was the similarity between the clinical signs of polioencephalomalacia in cattle and those associated with organomercuralism in other species. Two field cases of polioencephalomalacia having typical signs and lesions were submitted to the Michigan State University Diagnostic Laboratory. They had elevated levels of mercury in the brain and other tissues (1.8 to 0.1 mg. Hg/kg. wet tissue). The clinical signs of polioencephalomalacia and methylmercury toxicosis are similar except that blindness is not a primary consideration in methylmercury toxicosis. It has been reported that polioencephalomalacia responded to thiamine therapy (Jarrett, 1970), while methylmercury toxicosis did not. When the first signs of methylmercury toxicosis appeared, Calf 9 was treated intravenously with 500, 1000, and 1000 mg. of thiamine hydrochloride on 3 successive days without clinical response.

The gross and microscopic changes of polioencephalomalacia are cerebrocortical necrosis, especially in the sulci (Jensen and Mackey, 1965). Methylmercury toxicosis produced no gross lesions. A paucity of cerebellar granular cells, minimal neuronal degeneration and edema of the cerebral cortex were present microscopically.

Listeriosis, thromboembolic meningoencephalitis, and rabies are infectious diseases with clinical signs similar to methylmercury toxicosis. Listeriosis is characterized by microabscesses in the brain stem without remarkable gross lesions. *Listeria monocytogenes* has been isolated after fastidious microbiologic culturing. The lesions of thromboembolic meningoencephalitis have been described as random foci of infective thrombi and necrosis throughout the brain which can be discerned grossly and microscopically. *Hemophilus sp.* have been commonly isolated from brain and synovial fluids. Rabies is characterized microscopically by diffuse encephalitis, neuronal degeneration, and cytoplasmic inclusions (Negri bodies) in the affected neurons without any gross lesions. The virus can be identified by laboratory procedures. The gross CNS lesions of chronic lead poisoning were described as cerebrocortical softening, yellow discoloration, and cavitation. Microscopic lesions were scattered focal areas of status spongiosus and necrosis of the tips of cortical gyri (Christian and Tryphonas, 1971).

A reduction in the number of cerebellar granular cells was the characteristic microscopic lesion of chronic methylmercury toxicosis in this study. The levels of mercury in the tissues were not specifically correlated with the onset of clinical signs in the calf. Mercury concentration in the hair approximately reflected the levels in the body (Löfroth, 1970) and the level of administration.

SUMMARY

Six calves were used to study the experimental clinical signs, lesions and tissue residues after feeding low amounts of organic mercury over a long period of time. Weight gains tended to decrease in proportion to increasing amounts of mercury fed. Between 35 and 91 days, 3 of the 6 calves developed clinical signs of toxicosis characterized by a sudden onset of ataxia and neuromuscular incoordination which progressed rapidly to convulsions and a moribund state. The histopathologic changes were primarily a reduction in the number of cerebellar granular cells and nephrosis of the proximal convoluted tubules. Mercury residues in tissues were not correlated with the amount fed but were relatively consistent within each calf when the residues in hair, liver, kidney, brain and semitendinosus muscle were compared.

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APPENDIX

Table A1. Hematologic data at terminal sampling from calves fed methylmercury in milk

Calf no.	Total protein (gm./100 ml.)	Hb. (gm./100 ml.)	Hct. (%)	Total WBC	Abs. differential leukocyte counts					
					Neutro.	Stabs	Lymph.	Mono.	Eosino.	Baso.
10	6.4	12.5	38	14,300	9009	---	4290	---	901	---
9*	---	---	---	---	---	---	---	---	---	---
12	6.0	11.0	34	10,100	5454	202	4545	---	101	---
7	6.3	10.5	32	12,000	3600	600	8160	120	120	---
11	---	15.0	43	9,300	6696	372	2604	---	---	---
8**	---	13.5	42	12,900	8385	3096	3870	516	129	---

*Calf died during the night before blood sample was taken.

**BUN = 20 mg./100 ml.

Table A2. Urinary and fecal data at terminal sampling from calves fed methylmercury

Parameter	Calf no. 10	9	12	7	11	8*
Urine						
Color	yellow	brown	yellow	lt. yellow	brown	yellow
Transparency	clear	mod. cloudy	clear	clear	cloudy	clear
Sp. gr.	1.024	1.022	1.026	1.026	1.025	1.033
pH	6.0	6.0	7.0	6.0	6.0	6.5
Acetone	negative	negative	negative	negative	negative	negative
Albumin	negative	+++	negative	---	++++	++
Bile	negative	negative	negative	negative	negative	negative
Blood	negative	lg. amt.	mod. amt.	negative	lg. amt.	negative
Glucose	negative	negative	negative	negative	trace	+++
Urobilinogen (Ehrlich units)	0.1	---	---	1	0.1	1
Indican	---	---	---	---	---	negative
WBCs/hpf	occ.	10-20	0-2	3-4	0-2	occ.
RBCs/hpf	few	TNTC	---	5-6	0-2	---
Epith. cells	mod. small & lg. round	mod. small & few lg.	occ. small round	few small & lg. round	few squamous & lg. round	many squamous & few sm. round
Casts	occ. granular	occ. granular	---	---	occ. granular	---
Bacteria	---	---	---	---	trace	+
Crystals	--- few bile stained material	---	---	---	negative	--- few fat droplets

Table A2 (cont'd.)

Parameter	Calf no. 10	9	12	7	11	8*
Feces						
Direct exam	negative	negative	negative	negative	negative	negative
Flotation	light coccidia	mod. coccidia	no ova seen	light strongyles and *Nematodirus*	mod. coccidia, heavy strongyles, light *Trichuris*, heavy *Moniezia*	mod. coccidia, light strongyles

*Creatinine = 1.6 mg./100 ml.

VITA

The author was born in Glendive, Montana, on July 13, 1940, as the son of Mr. and Mrs. Raymond I. Herigstad. He was raised on their ranch. He received his primary and secondary education in the public schools of Glendive, Montana. His education was continued at Montana State University, Bozeman, Montana (1958-59, 1960-61) and Colorado State University, Fort Collins, Colorado (1961-65). He received his D.V.M. degree in June, 1965.

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The author married Chris Ann Fisher in 1964 at Genoa, Colorado. They have two daughters: Kelly May, 5, and Jamie Jo, 3.

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