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POISONING IN FERRETS BY TISSUES OF ALKYL MERCURY-FED CHICKENS

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Already several years ago investigations at the National Veterinary Institute demonstrated the occurrence of a widespread alkyl mercury poisoning in terrestrial Swedish wildlife, particularly in seed-eating and predatory birds and also in predatory mammals (*Borg* 1958, *Borg et al.* 1965, 1966, 1969). Convincing evidence indicated alkyl mercury-based seed-dressings to be the predominant source of the poisonings.

For the predatory birds and mammals involved the main source undoubtedly was the prey animals, since other sources such as atmospheric and water-borne mercury could largely be excluded; predatory birds do not normally take any water. Considering the feeding habits of the predatory animals, the mercury ingested presumably derived essentially from the tissues rather than from the digestive tract of the prey animals. Therefore, the poisonings observed in predatory animals might be regarded as truly secondary.

The purpose of the present investigations was to provide experimental evidence for the transfer of toxicity along the food chain, alkyl mercury-treated seed → seed-eating bird → predatory mammal, and further to study the clinical course, patho-anatomical features and mercury tissue distribution of secondary alkyl mercury poisoning in a mammal. To this end, methyl mercury-dressed wheat was fed to chickens, and after sacrifice musculature (and some liver) of the chickens were fed to ferrets.

EXPERIMENTAL AND RESULTS

The primary mercury source

Wheat seed commercially treated with a liquid formulation of methyl mercury dicyandiamide to give an expected mercury content of about 8 mg/kg was obtained through a regular supplier. Before feeding, the dressed wheat was mixed with a standard feed supplement to a few per cent. On analysis of nine samples of the finished feed, the mercury content was found to vary between 5.9 and 14.0 (average 8.2) mg/kg. In the calculations below, the diet was considered to contain 8 mg/kg of mercury.

The secondary mercury source

Two groups of about one year old chickens were fed the mercury containing feed described, the feed and water being freely accessible throughout the experiment.

In the first group (50 chickens, White Leghorn, about 1.4 kg) the feeding proceeded for 40—44 days (September-October, 1965) and in the second (44, Rhode Island, about 1.7 kg) for 35—38 days (February-March, 1966). The estimated average daily food intake in the two groups was about 80 and 90 g per animal, respectively, corresponding to an average mercury intake of about 0.46 and 0.42 mg/kg/day, or a total of 28 and 27 mg per animal, respectively. After the feeding period the animals were immediately sacrificed. Skeletal muscle and liver were secured and stored at —20°C until required. In all, approximately 45 kg of muscle and 6 kg of liver were obtained (roughly equal amounts from both groups).

No clinical signs of poisoning were seen in any of the chickens during the feeding period. Eggs were not laid.

The (total) *mercury contents* of the chicken muscle and liver (and of tissues of the ferrets (see below)) were determined by neutron activation analysis according to *Westermark & Sjöstrand* (1960) and *Sjöstrand* (1964) and the methyl mercury contents by gas chromatography according to *Westöö* (1966, 1967). (The analyses were performed in cooperation with the Isotope Techniques Laboratory, Inc., Stockholm). The results are summarized in Table 1. All values refer to undried samples.

From the analytical data the overall average mercury content of the chicken muscle was calculated as about 10 mg/kg. For the

Table 1. Total mercury and methyl mercury levels (wet weight) in tissues of chickens fed mercury-dressed wheat (average mercury content 8 mg/kg) for 35—44 days. Number of samples analyzed given within parenthesis.

Tissue	Total mercury mg/kg		Methyl mercury (as Hg) per cent of total Hg	
	average	range	average	range
Chest muscle, White Leghorn	12	7.3—18.6 (11)	111	92—119 (6)
„ „ , Rhode Island	8	5.9—10.9 (10)	94	65—123 (5)
Liver, mixed samples	40	39 —41 (4)	90	74—106 (2)

liver the average value was 40 mg/kg. It is evident that the tissue mercury was principally methyl mercury.

Feeding experiments with ferrets

Six adult female ferrets (polecat-ferrets, Mustela furo L. × M. putorius L.) bred at this institute were used in the experiments, four (nos. 1—4) as experimental animals divided into two groups of two, and two (nos. 5 and 6) as controls. No. 6 was used primarily to provide complementary reference material for histological examination. The experimental animals were caged individually and fed the tissue of the treated chickens as described below, the feeding being continued until the death of the animals. Water supply was unrestricted. The controls were given tissues from untreated chickens.

Group A. The animals were given muscle and liver of the treated chickens supplemented with ordinary mink feed, the average daily ration offered during the first week containing 118 g of the muscle, 7 g of the liver and 75 g of the mink feed per animal. During the second week the proportions were 93, 7 and 50 g and thereafter 68, 7 and 75 g, respectively. From the third week on, a gradually decreasing proportion of the offered food was taken, from the fourth to fifth weeks practically nothing at all. As far as possible the refused food was collected and weighed.

Taking into account the amount of food refused, the total intake of chicken muscle and liver during this experiment was estimated as not more than 2500 and 200 g per animal, respectively, corresponding to a total mercury intake of at most 33 mg per animal.

Group B. These animals received the chicken muscle and liver incorporated to about 20 and 5 %, respectively, in a special diet, composed by K.-E. Kull for ferrets. During the first week the animals were offered 150 g daily of this diet and thereafter 100 g daily. As in the preceding experiment the animals refused the food to an increasing extent.

The maximum total intake of chicken muscle and liver was estimated as 1000 and 250 g per animal, respectively, corresponding to a total mercury intake of at most 20 mg per animal.

Clinical symptoms

The appetite of the experimental ferrets gradually decreased and during the eight to ten last days of life the animals practically took no food. Weakness of the extremities, trembling, twitching of the head and incoordination appeared within about two weeks in Group A and within about three weeks in Group B. Ataxia, paralysis and apathy gradually developed. At a later stage periods of excitation were seen, the animals yelling and creeping in narrow circles.

Death occurred within 35 and 36 days in Group A and within 58 days in Group B. The controls were sacrificed after 37 days.

Morphological changes

Dead or killed animals were subjected to necropsy and specimens for *histological examination* taken from the liver, kidneys, spleen, pancreas, gonads, digestive tract, vulva, myocardium, skeletal muscles (extensor muscles of the thigh) and various parts of the central and peripheral nervous systems.

Tissues for histological examination were fixed in 10 % aqueous formaldehyde. For staining haematoxylin-eosin, van Gieson, Luxol fast blue, Mahon and gallocyanine-chrome-alum according to Einarson were used. Frozen sections were stained with scarlet red for fat.

Gross and microscopical findings were similar in all experimental animals.

Gross findings. Loss of body weight was apparent in the experimental animals (Table 2).

The nutritional state was poor in one animal (no. 4) and moderate in one (no. 3) and body fat was consistently reduced in all the experimentals. In addition, there was a pronounced generalized muscular atrophy in the experimentals.

Table 2. Body weight data, latency periods and survival times of ferrets fed chicken tissues containing physiologically incorporated methyl mercury.

Animal no.	Sex	Body weight, kg		Latency period days	Survival time days
		before	after		
Group A (dietary methyl mercury 7 mg/kg, as Hg)					
1. O 676/67	♀	0.990	0.770	approx. 14	35
2. O 692/67	♀	0.980	0.750	approx. 14	36
Group B (dietary methyl mercury 5 mg/kg, as Hg)					
3. O 1470/67	♀	0.940	0.620	approx. 21	58
4. O 1473/67	♀	approx. 0.9	0.450	approx. 21	58
Control					
5. O 700/67	♀	0.980	1.06	—	37*)
6. O 196/69	♂	—	1.35	—	37*)

*) Killed.

All experimental animals displayed a subacute cardiac ventricular dilatation, in two cases together with paleness of the myocardium.

In two animals the lungs were moderately congested, with emphysema and oedema.

In two animals (nos. 1 and 2) the liver was pale and tender but of normal size. Also the kidneys were pale. In no. 3 hepatic and renal congestion was apparent.

Haemorrhagic erosions of the gastric mucosa were seen in the animals of Group A, the ingesta consisting of a bloody mucous liquid. In the other animals the digestive tract appeared grossly normal.

In the animals in group B the vulva was enlarged, and of a whitish-grey colour.

No gross changes were seen in the central or peripheral nervous systems of any animal.

Mange of the ears (Otodectes cynotis) occurred sparsely in three experimental and one control animal. Bacteriological examination was negative in all animals.

Microscopic findings. Slight to marked, diffuse fatty degeneration was seen in the skeletal muscles of two and in the myocardium of three animals. Fatty degeneration of the liver cells and of the renal proximal tubular epithelium was seen in all

experimental animals. In one animal there was an acute waxy muscular degeneration with disintegration of the fibrils.

Hypoplasia of the lymphatic tissue of the spleen was observed in the animals of Group A; the cell content of the red pulpa was reduced and the trabeculae were prominent (Figs. 1 and 2).

Superficial haemorrhagic erosions of the gastric mucosa and slight round-cell infiltration of the intestinal mucosa were noted in two animals.

No definite changes were seen in the vulva wall except for slight connective tissue proliferation of the subcutis.

The ovaries of the animals of Group B were examined. In no. 3 one fresh luteal body, one graafian follicle and multiple primordial follicles were found, while some follicles apparently had ceased developing. In no. 4 there were multiple primordial and isolated secondary follicles but no graafian follicles or corpora lutea.

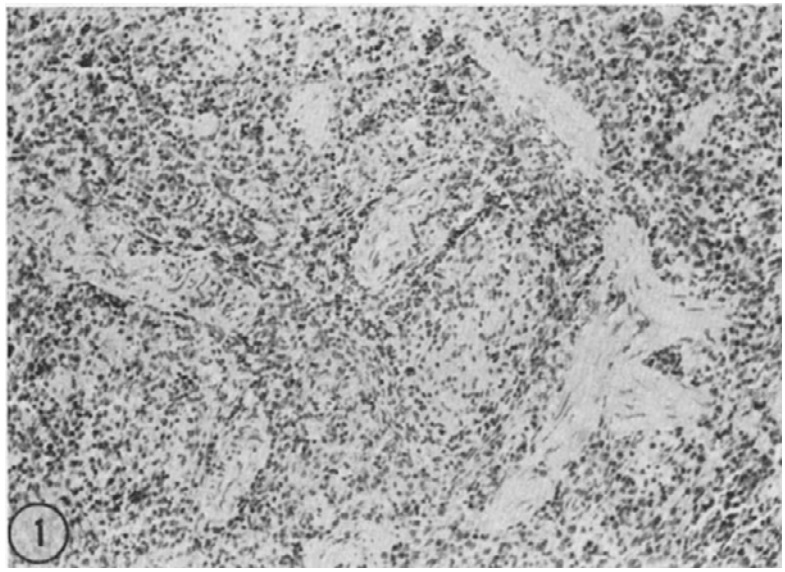
No changes were noticed in the pancreas.

The most conspicuous microscopical changes were those of the peripheral and central nervous systems, the changes being similar in all experimental animals.

In peripheral nerves (Plexus brachialis, N. ischiadicus and spinal nerves) there was a focal myelin degeneration characterized by reduced stainability, vacuolization and partial to complete disappearance of the myelin. Further, the axis cylinders were enlarged and focally disintegrated, forming fusiform or irregular fragments (Figs. 3 and 4).

In the cerebellar medulla and in the pons, areas of reduced stainability, demyelination and vacuolization occurred together with nerve cell degeneration. The latter was characterized by chromatolysis beginning around the nucleus and in advanced cases involving marginal vacuolization, homogenization of the cytoplasm and peripheral agglutination of highly stainable Nissl granules. Sometimes, the neuron degeneration was so severe that only intensely stainable pycnotic rests or vacuolized shadows of poor stainability remained. Chromatolysis was often accompanied by enlargement of the nucleoli followed by pycnosis and karyolysis (Figs. 5 and 6). These lesions were most prominent at the base of the arbor vitae but occurred in the lamellae too. In addition, slight gliosis was noted, predominantly in the pons.

In one animal (no. 1) a focal, nonpurulent lymphohistiocytic encephalitis with isolated perivascular cellular cuffings was observed.



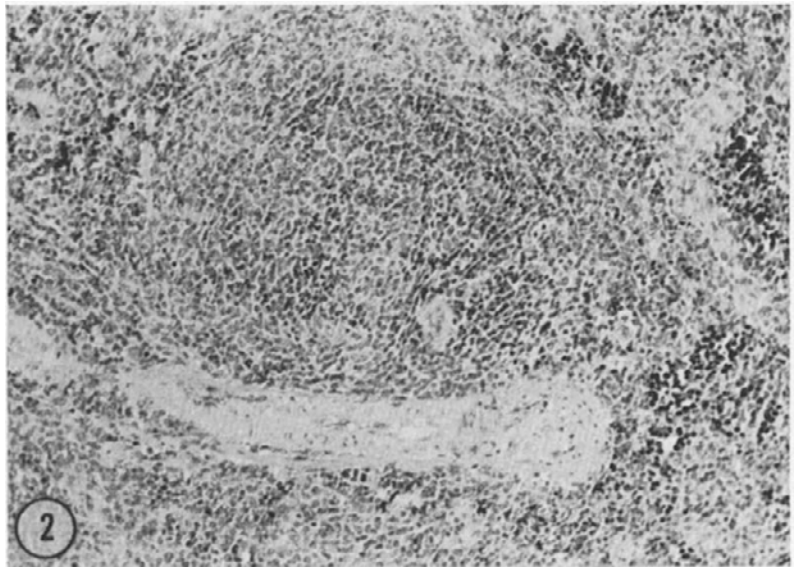
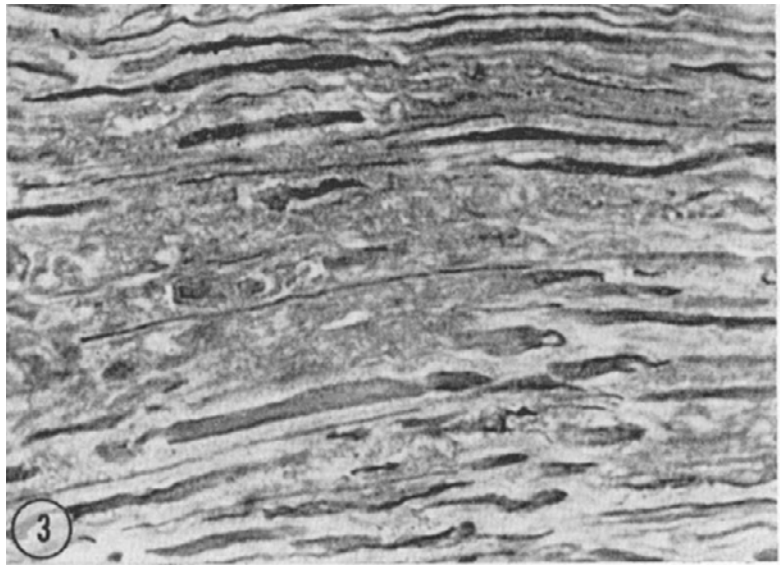


Figure 1. Spleen (ferret no. 2), hypoplasia of lymphatic tissue, H-E, × 150.

Figure 2. Spleen (ferret no. 5), normal, H-E, × 150.



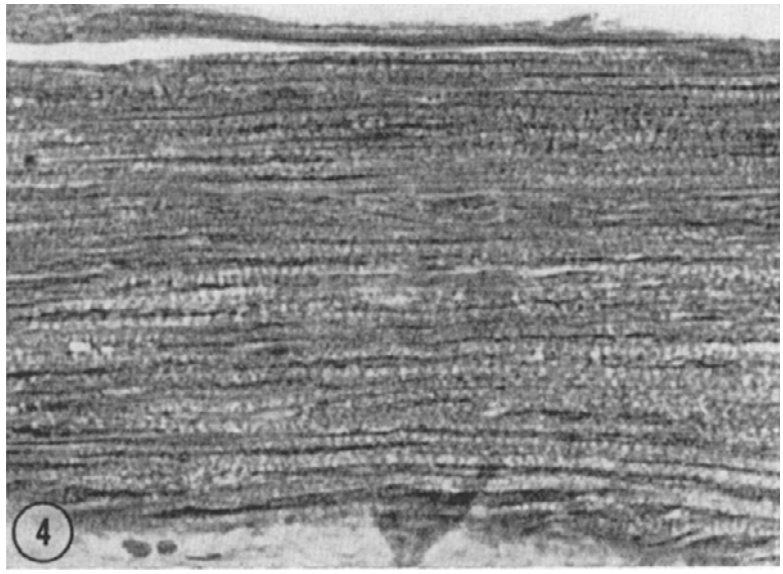
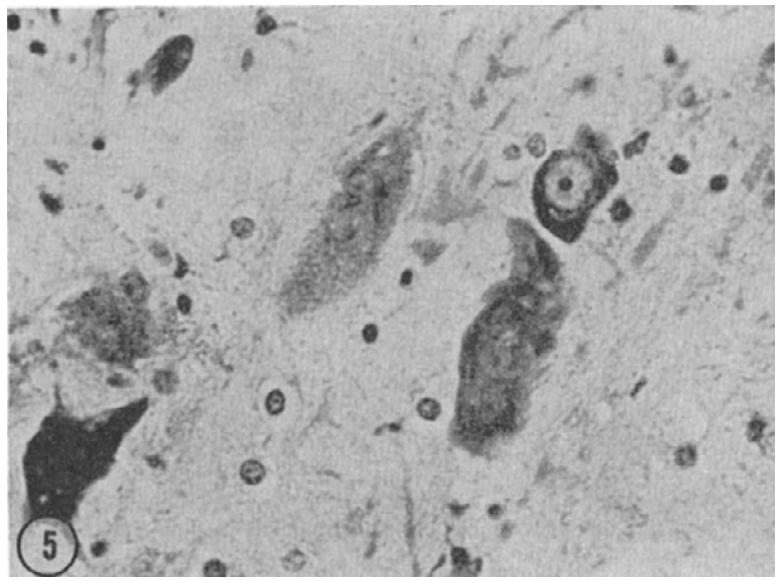


Figure 3. Peripheral nerve (ferret no. 2), myelin degeneration, Luxol fast blue, × 500.

Figure 4. Peripheral nerve (ferret no. 6), normal, Luxol fast blue, × 500.



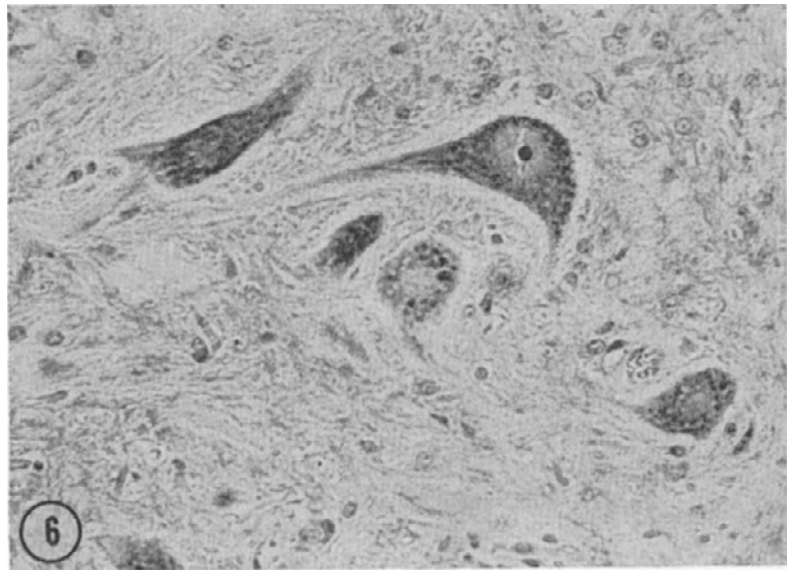


Figure 5. Pons (ferret no. 3), nerve cell degeneration, gallocyanine-chrome-alum (Einarson), × 500.

Figure 6. Pons (ferret no. 5), normal, gallocyanine-chrome-alum (Einarson), × 500.

The cerebrum displayed a slight focal myelin degeneration. Further, a very slight nerve cell degeneration was seen.

Slight focal demyelination and neuron degeneration were also seen in the spinal cord, mainly in the ventral horns.

Mercury levels

The total and methyl mercury levels found in tissue samples of the experimental and of one of the control animals are summarized in Table 3.

Table 3. Total and methyl mercury tissue levels in ferrets fed chicken tissues containing physiologically incorporated methyl mercury.

Animal no.	Total mercury (Hg) and methyl mercury (MeHg, expressed as Hg) levels, mg/kg, wet weight											
	Liver		Kidney		Skeletal muscle*)		Myocardium		Brain		Ovary	
	Hg	MeHg	Hg	MeHg	Hg	MeHg	Hg	MeHg	Hg	MeHg	Hg	MeHg
Group A (dietary methyl mercury 7 mg/kg, as Hg)												
1. O 676/67	57	45	59	35	35	23	—	—	35	34	—	—
2. O 692/67	64	44	87	50	43	35	—	—	39	70**)	—	—
Group B (dietary methyl mercury 5 mg/kg, as Hg)												
3. O 1470/67	39	28	63	47	28	12	35	15	26	33	16	—
4. O 1473/67	55	35	67	30	31	32	20	18	7	14	49	—
Control												
5. O 700/67	1.8	0.8	2.7	0.6	0.9	0.5	—	—	0.4	0.5	—	—

*) Extensor muscles of the thigh.
**) Uncertain because of small sample weight.

DISCUSSION

The course of the poisoning in the ferrets was remarkably rapid when compared with the chickens with remained clinically healthy throughout the feeding period. The dietary alkyl mercury levels and the duration of feeding were comparable in the two sets of experiments, although there might have been differences in availability of the mercury in the seed and in the animal tissues, thereby causing possible differences in absorption rate (see below).

The poisoning in the ferrets was attended by a striking loss of body weight which might be largely attributable to the pronounced and regularly occurring muscular atrophy. Although

not being pathognomonic for alkyl mercury poisoning, muscular atrophy has been associated with such poisoning also in other animal species (*Borg et al.* 1965, 1969, 1970).

Other gross changes observed in the poisoned animals were largely nonspecific and certain changes, such as those of the vulva, aetiologically unclear and possibly not related to the alkyl mercury exposition.

The most conspicuous microscopical changes were, except for fatty degeneration of parenchymatous organs, those of the central and peripheral nervous systems which afforded a morphological basis for the predominantly neurological clinical signs. The lesions were most pronounced in the cerebellum and the medulla oblongata. It might be noted that marked lesions in the occipital lobes of the cerebrum as reported for primates (*Nordberg et al.* to be published) were not observed.

Notable was lymphatic hypoplasia of the spleen in two animals. In this connection attention may be drawn to the marked accumulation of mercury in the adrenals of mice injected with ethyl mercury (*Berlin & Ullberg* 1963).

The mercury distribution pattern in the dead ferrets (Table 3) was of the same type as that observed in other species after alkyl mercury administration (*Ulfvarson* 1962, *Berlin & Ullberg, Gage* 1964, *Tejning* 1967, *Swensson & Ulfvarson* 1968 a, b), high levels being found in the liver, kidneys and central nervous system. The kidney levels did not significantly exceed those of the liver. In rats high kidney-liver ratios have been found (*Gage*), while in birds the reverse is often seen (*Borg et al.* 1965, 1969, 1970, *Tejning* 1967). The mercury levels of the skeletal muscle were high, the muscle-liver ratio being approximately 0.5—0.7, as compared with 0.2—0.4 usually seen in birds (*Smart & Lloyd* 1963, *Borg et al.* 1965, 1969, 1970, *Tejning*). The marked mercury accumulation in the gonads should be considered as physiologically important in the light of the established genetic effects of alkyl mercury (cf. *Ramel* 1967). Except for one animal (no. 4) with a low value, the brain levels were comparable to those of the skeletal musculature, or of the order of 30 mg/kg.

The analytical evidence indicated that methyl mercury constituted by far the major part of the tissue mercury in the ferrets, just as in the chickens. (In some instances the methyl mercury values exceeded the corresponding total mercury values (Tables 1 and 3), probably as a result of sample heterogeneity

— total and methyl mercury were determined in separate portions of sample). Two important conclusions can be drawn from these findings. 1) An alkyl mercury compound may be able to pass a food chain without splitting of the carbon-mercury bond. 2) Physiological incorporation into animal tissues of alkyl mercury apparently does not impair its availability to carnivorous animals.

The mercury levels attained in skeletal muscle of the poultry (10 mg/kg on an average) seem somewhat low compared with the results of other feeding experiments in birds with alkyl mercury (*Smart & Lloyd* 1963, *Borg et al.* 1965, 1969, 1970, *Tejning*). Particularly when considering that in the present experiments, unlike in the others mentioned, no mercury was excreted with the eggs and probably only small amounts with the plumage (the birds were not moulting during the experimental period), higher tissue levels could be anticipated. One possible reason for the low values is that a steady state of accumulation has not been attained during the relatively short feeding period (five to six weeks), as might be inferred by analogy from the results of *Smart & Lloyd* and *Tejning*. The differing mercury levels found in the White Leghorns and the Rhode Islands might, at least in part, be related to the different proportions in the two breeds of dark and pale chest muscle. As *Bäckström* (1969) has shown in experiments with quail, the dark (external) chest muscle retains alkyl mercury more effectively than the pale (internal) type. Taking the mercury content of the chicken feed as 8 mg/kg (and considering the different water contents of the feed and chicken muscle), the concentration factor for chicken muscle may be calculated as about 2.

In the ferrets, on the other hand, the mercury accumulation was pronounced. From dietary mercury levels of 7 and 5 mg/kg for Groups A and B, respectively, muscle levels of 40 and 30 mg/kg were built up, thus corresponding to a concentration factor of about 6. Comparable results were obtained by us in experiments with chickens and goshawks (*Borg et al.* 1970). The lower degree of accumulation in the chicken experiment probably may be attributed to a lower absorption rate of alkyl mercury from seed than from animal tissues. Incomplete absorption of alkyl mercury from treated seed was noted by *Tejning*.

The accumulation of mercury, and the secondary poisoning

as demonstrated in these experiments may have important ecological consequences. Thus, during natural conditions the accumulation is likely to be enhanced by the selection mechanisms operating in natural food chains. The selection by a predator of prey animals already debilitated by mercury poisoning undoubtedly will increase the hazards to the predator considerably. Important in this connection is further the fact that the muscle mass of a prey animal will contain the major part of the body mercury. According to *Tejning* about 75 % of the body mercury in chickens is to be found in the muscles.

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SUMMARY

Chickens were fed alkyl mercury-dressed wheat (mercury content about 8 mg/kg) for 35—44 days and were then immediately sacrificed. No signs of untoward effects were observed. Muscle of the chickens, and a minor proportion of liver, were fed to two groups of two ferrets (Mustela furo L. × M. putorius L.), the mercury content of the diet being 7 and 5 mg/kg, respectively. The ferrets of the first group died after 35 and 36 days and those of the second after 58 days.

The experimental ferrets showed a marked weight loss, attributable to muscular atrophy in addition to a reduced food intake. Clinical signs appeared in two to three weeks and were primarily neurological such as ataxia, trembling and paralysis. The signs could be correlated with pronounced degenerative changes of the central and peripheral nervous systems involving mainly the cerebellum and peripheral nerves and, to a lesser extent, the cerebrum and the spinal cord. Hypoplasia of the lymphatic tissue of the spleen and degeneration of the graafian follicles were seen as well.

High mercury levels were found in the kidneys, liver and brain and also in skeletal muscle and the gonads of the ferrets (Table 2). Methyl mercury constituted the major part of the tissue mercury in the ferrets (as well as in the chickens).

The results provide direct evidence of the transfer and accumulation of alkyl mercury in a toxic form through a food chain. The ecological implications are discussed.

SAMMANFATTNING

Förgiftning av iller med kött av alkylkvicksilverutfodrade höns.

Tamhöns utfodrades med alkylkvicksilverbetat vete (kvicksilverhalt ca. 8 mg/kg) under 35—44 dagar och avlivades omedelbart därefter. Inga tecken på förgiftning iakttogs.

Två grupper (vardera på 2 djur) av iller utfodrades med muskulatur (och en ringa tillblandning av lever) från hönsen. Den genomsnittliga kvicksilverhalten i fodret hos de två grupperna var 7 resp 5 mg/kg. Djuren i den första gruppen dog efter 35 och 36 dagar och de i den andre efter 58 dagar.

En påtaglig viktsförlust noterades hos försöksillrarna, vilken kunde tillskrivas skelettmuskelatrofi i förening med nedsatt foderintag. I övrigt iakttogs, med början efter 2—3 veckor, huvudsakligen förgiftningssymptom av neurologisk art, såsom ataxi, darrningar och förlamningar. Symtomen kunde korreleras med degenerativa förändringar främst i lillhjärna och perifera nerver men även i storhjärna och ryggmärg. Därjämte iakttogs hypoplasi av mjältens lymfatiska vävnad och follikeldegeneration i ovarierna.

Höga kvicksilverhalter påvisades i njurar, lever och hjärna och även i skelettmuskulatur och gonader hos försöksillrarna (Tabell 2). Kvicksilverinnehållet i vävnaderna hos såväl illrar som höns visades bestå huvudsakligen av metylkvicksilver.

Försöksresultaten ger positivt belägg för att alkylkvicksilver kan passera en näringskedja under anrikning och med till synes bibehållen toxicitet. De ekologiska konsekvenserna diskuteras.

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