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Effects of Cadmium on Some Clinical and Biochemical Measurements in Heifers

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Pollution with cadmium derived from industrial activities has been detected in the Kempen, an area in the province of North Brabant in the Netherlands (Tielen, 1983). Increased concentrations of cadmium have been found in soils and crops and concentrations of cadmium up to 4.3 mg and 39.0 mg/kg dry matter have been measured in the livers and kidneys respectively of cattle grazing in the area, indicating that they have been chronically exposed to cadmium.

Various clinical abnormalities such as loss of appetite, anaemia, poor growth, abortions, teratogenic lesions and renal failure have been described in cattle which have been chronically exposed to cadmium (Block, 1977; van Bruwaene et al 1984; Doyle, 1977; Lamphere et al, 1984; Miller et al, 1967; Neathery and Miller, 1976; Sharma et al, 1982; Wright et al, 1977), but there is little information about the concomitant biochemical or hematological changes. This study was designed to measure the effects of exposing cattle to measured amounts of cadmium on selected biochemical and hematological measurements in blood, urine and feces, in order to establish whether the changes could be used to diagnose cadmium toxicity or for demonstrating the effects of cadmium upon cattle.

MATERIALS AND METHODS.

Thirteen clinically normal heifers aged 18-24 months and weighing between 300 and 350 kg were used. During cadmium exposure the heifers were housed in pens and fed hay ad libitum and 2 kg concentrates per day. Before and after the experimental period, the animals were kept in pasture. The concentrations of cadmium, copper and zinc in these components of the diet are given in table 1. Nine of the heifers were pregnant and two gave birth to calves during the experiments. The doses of cadmium, and the frequency and route of administration of the doses to each heifer are detailed in table 2.

Nine animals received cadmium administered by stomach tube as aqueous solution of $CdCl_2$ twice a week. Samples of urine and faeces were taken weekly for all animals, except for animals J and K.

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Table 1: The concentrations of Cd, Cu and Zn (mg/kg dry matter) in the constituents of the diet fed during experiments.

	Cd	Cu	Zn
Hay	0.25	4.0	48
	0.44	3.1	42
	0.69	3.2	40
	3.00	2.5	56
Grass	0.20	ND	87
	0.29	ND	107
	0.13	ND	106
Concentrates	0.05	14.7	66

ND: Not Determined

During the second and third week of the experimental cadmium burden samples of ruminal fluids were taken from animals E, F, G, H and I, because of decreased ruminal motilily and a decrease of consistency of the ruminal contents on palpation. The investigation of ruminal fluid was stopped at the end of the third week. For animals J and K, samples of ruminal fluids and urine were taken during the six days following the first dose of cadmium. All feces were collected during this period. Although efforts were made to collect urine and feces separately it was impossible to completely prevent contamination of the feces by urine.
Heifer A received 100 mg cadmium as a sterile aqueous solution of cadmium chloride (0,06 mol.) administered subcutaneously in the dewlap. Heifer B received 400 mg cadmium administered in the same way.
Samples of feces and urine were taken weekly during 4 weeks.
Heifers L and M received intravenous doses of cadmium daily for five days as detailed in table 2. The total doses received were 264 mg and 165 mg of cadmium respectively. Samples of rumen fluid were taken daily and all feces were collected during the six days following the first dose; urine samples were also collected daily.
In all the experiments blood samples were taken weekly from a jugular vein of each heifer. The animals were examined daily during the experiments and the motility of the rumen was measured over a period of 5 minutes; when abnormalities in feeding behavior, rumen motility or other were noticed a complete clinical examination was performed. The food intake of each heifer was controlled daily.
The heifers were slaughtered after the experiments and examined for gross pathological lesions. Seven heifers were still pregnant. Heifer F was slaughtered after the stillbirth of a calf in reversed position and heifer G one month after the birth of a healthy male calf. The calf suckled its mother during that month.
Tissue samples were taken from the liver, kidney, masseter muscle and uterine wall of the heifers and from the liver and kidney of the fetuses for the estimation of cadmium, copper and zinc concentrations and for routine histological examination; 7 micron sections were stained with haematoxylin and eosin.

Whole blood samples were analysed for haematocrit and haemoglobin concentration, and for leucocyte and differential counts. Serum was collected and analysed for the concentrations of total proteins and the protein spectrum, urea and the activities of lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH), gamma-glutamyl transpeptidase (gamma-GT) and alkaline phosphatase (AP). In plasma samples the concentrations of cadmium, copper and zinc were measured (Breukink et al, 1974).

Table 2: The amounts routes and frequency of administration of cadmium to the heifers. The cadmium was administered as a 0.06 mol $CdCl_2$ solution in water.

Heifer	Dose frequency and route of administration	Total amount of Cd^{2+} given
A	1 x 100 mg s.c.	100 mg
B	1 x 400 mg s.c.	400 mg
C	7 x 600 mg p. os[1]	4,200 mg
D	7 x 1800 mg p. os[1]	12,600 mg
E	1 x 1800 mg p. os[1]	1,800 mg
F	3 x 1800 mg p. os[1]	5,400 mg
G	11 x 1800 mg p. os[1] and 1 x 1800 mg p. os[1]	23,400 mg
H	11 x 1800 mg p. os[1] and 4 x 3600 mg p. os[1]	34,200 mg
I	11 x 1800 mg p. os[1] and 4 x 3600 mg p. os[1]	34,200 mg
J	2 x 1800 mg p. os[1]	3,600 mg
K	2 x 1800 mg p. os[1]	3,600 mg
L	2 x 33 mg + 3 x 66 mg i.v.[2]	264 mg
M	5 x 33 mg i.v.[2]	165 mg

1) administered twice weekly
2) administered daily.

Samples of urine were tested for the presence of protein, blood, urobilinogen and glucose using test strips (Combur test; Boehringer).
Samples of ruminal fluids were judged for smell, concistency and colour and furtheron for the presence of flottation 20 minutes after collection. The sediment was judged microscopically for the presence of protozoa: their viability was judged after iodide staining.
The cadmium concentration per gram dry matter was estimated by atomic absorption spectrophotometry.
The concentrations of cadmium, copper and zinc in tissue samples were estimated by atomic absorption spectrophotometry, cadmium and zinc by the flame technique and copper by the graphite oven technique.

RESULTS AND DISCUSSION

The heifers that received more than one dose of cadmium orally showed no abnormalities except for a slight reduction in their intake of roughage and a reduction in ruminal motility to 6 move-

ments per 5 minutes during the first week of administration. Heifer E which received only one oral dose of cadmium showed no changes.
The heifers that were dosed subcutaneously developed swellings at the site of injection which were so severe that they caused pain and hindered them from eating.
The heifers dosed intravenously showed no abnormalities except for a slight reduction in their intake of roughage.

In the blood, no clear abnormalities were observed. In the heifers dosed subcutaneously there was a decrease in serum urea concentration, which may have been due to their reduced food intake, especially of the concentrates. An increase in plasma cadmium concentration was observed only in the heifers dosed intravenously. In seven of the heifers there was an increase in the copper/zinc ratio in plasma, although the concentrations of both elements stayed within their normal ranges (table 3).

Table 3: Cu/Zn ratio in the plasma of heifers before and after administration of cadmium.

Heifer	Week										
	0	1	2	3	4	5	6	7	8	9	10
A	0.77	0.67	1.01	1.06							
B	0.78	1.95	1.65	0.83							.
C	0.54	0.63	0.63	0.61							
D	0.65	0.72	0.57	0.66							
E	0.77	0.72	0.61	0.67	0.71	0.79	0.58	0.60	0.48	0.55	0,57
F	0.58	0.66	0.96								
G	0.78	0.96	1.01	0.90	1.05	0.92	0.92	0.86	0.87	0.82	0.74
H	0.66	0.85	1.17	1.08	0.85	0.91	1.06	0.84	1.43	1.46	0.89
I	0.56	0.71	0.73	0.79	0.64	0.81	0.82	0.56	0.64	0.67	0.72
J	0.72	0.89	1.03	0.68							
K	0.72	0.87	ND	0.78							.
L	0.70	1.06	0.44								
M	0.78	1.21	0.68								

ND: Not Determined

Small amounts of protein and/or blood were found in the urine of some of the heifers, probably caused by the manipulation of the bladder catheter. Very small quantities of cadmium were detected once in the urine of heifers C and D shortly after the dose had been administrated.
No changes were observed in the composition of rumen fluid.
Cadmium was detected in feces samples from all the heifers. The lowest concentrations were in the faeces of the heifers dosed subcutaneously and the highest in the feces of those dosed orally. An average of 2600 mg (72%) of the total oral dose of 3600 mg cadmium was present in the feces collected during the six days after the first administration of cadmium to heifers J and K. After intravenous administration 20% and 30% of the total dose of cadmium was present in the feces excreted by heifers L and M respectively in six days after the first dose.

Table 4: Concentrations of Cd, Cu and Zn (mg/kg dry matter) in the organs of experimental heifers, calves and fetuses.

Heifer	Liver	Kidney	Muscle	Wall	Liver	Kidney	Liver	Kidney
A	13.8	26.7	1.6	-	74.2	19.5	317	123
B	57.7	103.0	5.9	-	81.8	14.4	322	207
C	3.7	14.0	0.16	-	77.4	18.9	112	92
D	11.2	41.2	0.98	-	58.8	17.0	126	97
E	1.5	11.1	0.05	0.05	332.1	16.6	163	103
F	3.1	11.4	0.05	-	21.0	15.4	230	110
G	12.1	57.7	0.07	-	7.2	12.7	149	139
H	8.9	53.0	0.05	0.05	557.1	13.6	112	116
I	2.7	102.0	0.08	0.12	49.0	16.8	174	144
J	1.9	12.2	0.05	0.05	17.5	15.8	140	124
K	4.2	24.6	0.05	0.05	30.3	17.7	150	107
L	90.0	256.0	0.63	-	6.8	16.5	135	502
M	92.0	52.0	0.43	-	19.9	17.0	153	119
Fetus of:								
D	0.10	0.75	-	-	278.6	7.1	522	84
E	0.05	0.05	-	-	307.0	7.7	805	86
H	0.07	0.05	-	-	305.4	8.1	763	90
I	0.05	0.05	-	-	33.7	6.9	794	86
J	0.05	0.08	-	-	221.8	6.9	302	77
K	0.05	0.05	-	-	309.5	8.2	575	94
L	0.24	0.05	-	-	-	-	-	-
Calf of:								
F	0.05	0.05	-	-	-	-	-	-
G	0.07	0.43	0.05	-	-	-	-	-

No gross abnormalities were observed post mortem, and no pathological changes were observed histologically. The concentrations of cadmium in liver, kidney, muscle and uterine wall, and the concentrations of copper and zinc in liver and kidney are shown in table 4; table 5 shows the total quantities of cadmium present in the liver and kidneys and expresses the results as a percentage of the total dose of cadmium received by each heifer.

Delayed toxic effects were not observed, in contrast with the observations of Powell et al (1964) who noticed clear clinical signs after cadmium had been administered to young calves. The difference may be due to the difference in age of the experimental animals or to the greater reduction in food intake observed by Powell et al (1964). Miller et al (1967) also observed a reduction in food intake and a drop in milk production in cattle dosed with cadmium, but the animals returned to normal within a few days of the cessation of cadmium administration. It is uncertain wheter the animals would similarly have returned to normal if the dosing with cadmium had been continued for periods of days or weeks as in the present experiments.

Table 5: The total quality of cadmium in the liver and kidneys of the heifers post mortem.

Heifer	Cd(mg)		Cd (% dose)
	Liver	Kidneys	Liver and kidneys
E	1.6	1.7	0.18
G	15.0	10.4	0.11
H	11.4	9.6	0.061
I	3.3	14.9	0.053
J	2.7	2.3	0.14
K	4.9	3.5	0.23
L	142.4	54.9	74.7
M	125.4	8.8	81.3

Toxic effects have been reported in cattle dosed with organic cadmium compounds at relatively high doses for long periods (Wright et al 1977) but many workers have failed to produce acute or subacute toxic effects by the administration of cadmium to cattle (van Bruwaene et al 1982, Doyle 1977, Johnson et al, Neathery et al 1976, Schenkel and Merkle 1982, Sharma et al 1982, Vreman and van der Veen 1982).

The concentration of copper in the livers of seven of the heifers was less than the normal value of 50 mg/kg dry matter and the lowest levels were found in the heifers which had either received large cumulative doses of cadmium or had been dosed intravenously. The concentration of zinc in the livers was normal.

Plasma concentrations of copper and zinc remained within their normal ranges throughout the experiments but there were wide variations in the ratio of copper to zinc. The administration of cadmium was followed on several occasions by an increase in the Cu/Zn ratio in plasma suggesting that copper may have been expelled from liver tissue, and that there was an interaction between cadmium and copper utilisation. Such an interaction has been reported previously (Block 1977; Hill et al 1963; Neathery et al 1976) and there have been suggestions that a similar interaction exists between cadmium and zinc (Block 1977; Hill et al 1963; Lamphere et al 1984; Miller et al 1967; Powell et al 1964).

However, in the present experiments the concentration of zinc in the liver was not affected by the administration of cadmium considering the short duration of the experiments the concentrations of cadmium in the liver and kidney were high, although they remained below the toxic levels reported in the literature (Block 1977; van Bruwaene et al 1984; Krajnc et al 1983; Neathery and Miller 1976). In rats and pigs renal damage leading to proteinuria develops at levels of about 350 mg/kg dry matter (Krajnc et al 1983) and these levels are dependent upon the total amount of cadmium absorbed rather than upon the time for which the cadmium has been present.

The concentrations of cadmium in the liver and kidneys of the fetuses and calves were low, indicating that the placenta constituted an effective barrier against cadmium and that little cadmium was transferred to milk. This observation is in agreement with those of Vreeman and van der Veen (1982) on the tranfer of orally administered cadmium to milk.

The results of these experiments suggest that cadmium has a low acute or subacute toxicity for cattle more than 18 months old. Moreover, the concentrations of cadmium found in the liver, kidneys and other tissues of the experimental animals were similar to or higher than the concentrations found in animals grazing the contaminated area of the Kempen in North Brabant (Tielen, 1983). It is therefore very unlikely that animals in that area should show any signs of cadmium toxicity.

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