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Hepatic Storage Alteration of Vitamin B_{12} by Cadmium in a Freshwater Fish

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Vitamin B_{12} is an essential anti-anemic factor for man and animals. It is synthesized by microorganisms and cobalt is in one of the two major moieties of the molecule (SMITH 1962). Ruminants efficiently convert elemental cobalt into vitamin B_{12} and concentrate it in their liver and spleen. Man and fish also store the vitamin in their livers, releasing it as needed (DOSCHERHOLMEN and HAGEN 1957; YANASE 1952, 1953).

Cadmium is an antimetabolite which abnormally accumulates in the kidney and/or liver depending on the route of entry (SCHROEDER 1974). With the advent of metal pollution of land waters, experiments were performed with an edible fish from Lake Maggiore (North Italy) and tissue concentrations of Cd were determined following exposure of the fish to lake water containing 0.04 ppm $CdSO_4$ for about one month. There was 7 times more Cd in the liver and 500 times more in the kidneys of the treated fish compared with the Controls (MERLINI et al. 1971). This is in agreement with the findings of CEARLEY and COLEMAN (1974) and SMITH et al. (1976) who stated that Cd accumulated in both the liver and kidneys of bass and bluegills in the first case and in catfish in the second. The purpose of this experiment, therefore, was to determine whether or not Cd interferes with the normal physiological process of the hepatic storage of vitamin B_{12} in this fish.

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MATERIALS AND METHODS

Immature sunfish (Lepomis gibbosus, L.) were seined from Lake Maggiore (North Italy) and kept in large, outdoor tanks with running lake water. After several weeks they were acclimatized to indoor laboratory conditions and to a specially prepared food containing meat and gelatin. These fish are carnivorous, eating the soft tissues of snails which live in the lake.

The experimental group of 5 fish were pretreated for 2 weeks with 0.04 ppm Cd (as sulfate) in lake water before they and the 5 Controls were fed a single ration containing naturally labelled vitamin B_{12} with ^{58}Co obtained from Amersham, England. Pertinent information concerning Lake Maggiore water quality was given by MERLINI and POZZI (1977). The pH in this case was 7.2 ± 0.1, and each group was in 30 l of triply filtered lake water. After 5 hrs the fish were counted individually in a Packard Armac Scintillation whole body counter (Model 404), and the counts per minute per gram of fish (cpm/g) between the two groups were within 10% (Table 1).

TABLE 1

Information concerning the experimental set-up for sunfish fed a single ration containing ^{58}Co-vitamin B_{12}.

Group	Treatment	Net wet wt.	cpm/g fish 5 hours after labeled food
1	Controls - lake water only	7.86 ± 1.43	3904 ± 128
2	Treated - lake water + 0.04 ppm $CdSO_4$	6.38 ± 0.60	3709 ± 170

Following the meal with marked B_{12}, the fish were fed nonactive food for 31 days before they were sacrified. During this period the experimentals were maintained in lake water with cadmium and the controls in lake water only. The water was changed every 2 to 3 days.

Each fish was dissected into: head (plus gills), digestive tract, liver, gallbladder, kidney, gonads, and body residue. After weighing, each part was counted in a deep-well scintillation counter (intertechnique) and the counts were corrected for background decay, and geometry. The counts were elaborated as follows:

$$R = \frac{\text{cpm/g part}}{\text{cpm/g whole fish}}$$

where R is the activity of the segment relative to the whole body activity of the fish. In this manner, irrespective of the quantity of labeled B_{12} eaten, one can still determine the relative amount in each organ or tissue compared with the whole body activity on an equal weight basis.

All data are given as the mean $\pm$ standard error. An analysis of variance was calculated for each segment in the 2 groups.

RESULTS AND DISCUSSION

The results given in Table 2 show that the untreated Control fish were able to stock dietary vitamin B_{12} in the liver. By comparison, fish in water with cadmium had statistically less vitamin in their livers, more in the gallbladder, intestinal tract, and head (gills). Since the vitamin is primarily eliminated through the digestive tract, it is apparent that the presence of cadmium sulfate in the lake water accelerates this procedure by stimulating biliary excretion of the vitamin into the intestine and its elimination through the gills. The kidney was

TABLE 2

The relative concentration (R) of radioactive vitamin B_{12} in different parts of nontreated and treated fish after 31 days of nonactive food. Two asterisks = $P \ll 0.05$; one asterisk for $P < 0.05$.

Group	Liver	Digestive Tract	Gall-bladder	Kidney	Head	Body Residue
Controls	74±5**	3.1±0.6	7.2±1.8	1.7±0.6	0.30±0.07	0.22±0.05
Treated	44±7	5.7±1.6*	15.1±5.5*	2.2±1.9	0.48±0.08*	0.23±0.05

not significantly more active than the Controls because it is known that dietary B_{12} is conjugated to a high molecular weight protein once absorbed and elimination through the kidney is not possible (SMITH, 1962). Indeed, previous experiments in which food was marked with ^{58}Co alone and fed to the same species of fish showed that the kidney was one of the most active parts, indicating that kidneys could eliminate the metal alone (MERLINI and ALFASSIO-GRIMALDI, unpublished data).

Cadmium accumulates in man with age since none has been found in babies. In addition, this metal is insidious because it mimicks other diseases and only when the resistance threshold is overcome does it become truly toxic (SCHROEDER, 1974). It appears that the effect of this metal on hepatic storage of B_{12} is an excellent example of its recondite activity. Depleted of its vitamin B_{12}, fish living in water with 0.04 ppm of Cd, or less, would eventually develop anemia and in this state the more toxic effects would become operative.

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