## Uptake and Localization of Radioactive Zinc in the Visceral Complex of the Land Pulmonate *Arion rufus*

*Materials and method.* The slugs used in this experiment were taken from a stock raised and standardized in the laboratory. They were of the same post-hatching age and of approximately the same body length. The animals were kept in a plexiglass container on damp filter paper. The container was covered by a heavy glass plate sitting on cardboard riders to allow air circulation. The food was changed every other day, at the same time the container was thoroughly cleaned, lined with fresh filter paper and the slugs were washed briefly in bidistilled water.

The food consisted of a standard laboratory diet for rats, which was mixed with the dosing solution to form a mash, and was then measured out in quantities which were eaten up by the animals in about 1 day.

The radioactive isotope was Zinc 65 as $ZnCl_2$ in HCl. 0.1 ml with an activity of about 0.1 mCi were diluted in 500 ml of non-active $10^{-3}M$ $ZnSO_4$ and formed the dosing solution used in food preparation. The isotope was fed to the animals for 27 days, and was then replaced by a non-active and zinc-free diet.

The organs analyzed were liver, stomach and intestine. Sample groups consisted of 5 animals each. The livers were processed individually, the 5 stomachs and intestines respectively were taken together. The organs were placed in test tubes and vacuum-dried at the suction pump.

The radioactivity was measured with a single channel gamma spectrometer, the test tube with the dry material being introduced directly into a well-type NaJ (Tl) crystal. After the measurements, the samples were weighed and the counts were calculated as cpm/100 mg dry weight. Corrections for decay of the isotope, as well as for background count, were made on all data used herein.

For autoradiography the whole visceral complex was removed, fixed in cold neutral formol-calcium, processed according to routine, embedded in Paraplast and sectioned at 7 μ. The sections were prestained for zinc with Dithizone, heavily coated with gelatine to prevent chemigraphy, and then covered with Kodak AR-10 stripping film[1]. During exposure (74 days) they were kept in the refrigerator.

It should be noted that the isotope is not well suited for autoradiography. Zinc 65 is a relatively high-energy $\gamma$- and $\beta$-emitter with a low rate of energy loss per micron of track in a 5 μ emulsion layer. Hence the relatively long exposure time. The protective gelatine layer between source and emulsion entails serious loss in resolution and adds considerable difficulty to microphotography.

*Results. Liver.* Incorporation of radioactive zinc in the liver is extremely rapid and goes on more or less steadily until the supply is stopped, whereupon elimination sets in. The biological half-time was not reached during the experiment, so we used the elimination data obtained to calculate a decay factor $\lambda$ for maxima, minima and mean. It proved to be fairly constant:

$$\lambda_{max}: \ 0.010 \pm 0.002$$
$$\lambda_{min}: \ 0.033 \pm 0.013$$
$$\lambda_{mean}: \ 0.014 \pm 0.001$$

We therefore assume that elimination follows an exponential curve (Figure 1). The biological half-time thus obtained is 51 days, about double the time needed for the uptake. Histochemical stain and autoradiograph show that the storage of zinc in detectable amounts for light microscopy is exclusively limited to one type of liver cell, the so-called calcium cell[2] (Figure 2). The Dithizone-zinc crystals sit on the surface of the calcium spherules. Not all the spherules within one cell show the same amount of zinc, but all calcium cells in a series through one liver are zinc-positive.

For reasons mentioned above, the resolution of the autoradiograph does not go below the area of one cell, but the significantly increased grain density over the histochemically identified zinc regions connects the 2 without doubt (Figure 3 and Figure 4).

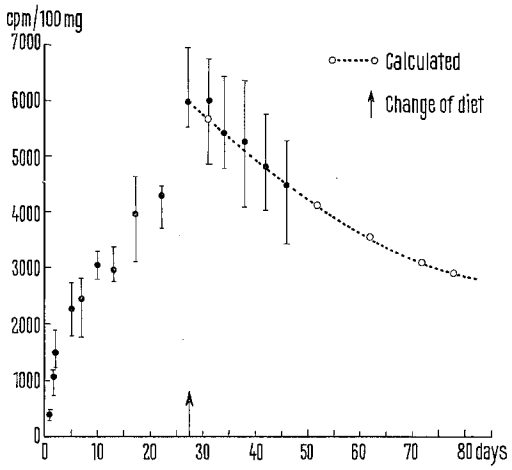


Fig. 1. Uptake and elimination of radioactive zinc in the liver of *Arion*. Maxima, minima and mean.

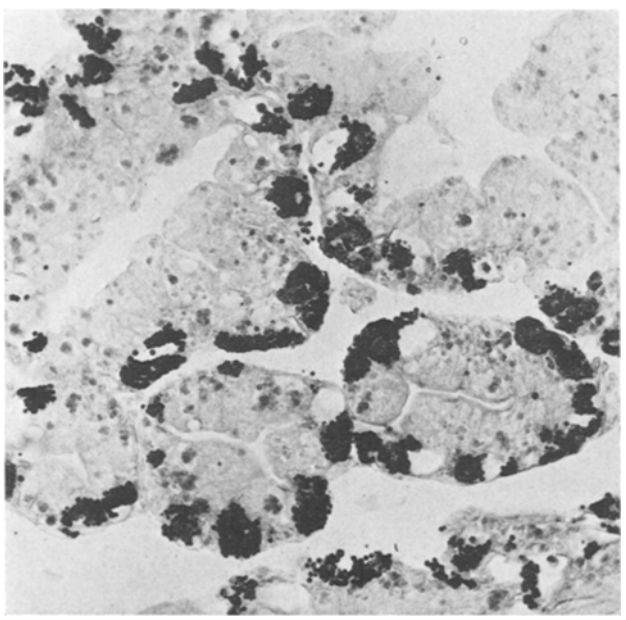

Fig. 2. Transverse section of liver acini (*Arion*). The black regions are the calcium cells filled with zinc-positive spherules. × 100.

[1] T. YOSHINAGA and N. SHIMIZU, Acta histochem. *30*, p. 90 (1968).
[2] A. T. SUMNER, Q. Jl microsc. Sci. *106*, 2 p. 173 (1965).

*Stomach and intestine.* Incorporation of zinc in stomach and intestine tissue follows a more erratic pattern than the one observed in the liver, but also shows an overall increase towards the measured maximum. The amount taken up is about 10 times less than in the liver (Figure 5).

Elimination sets in six days before the dosing diet is stopped. The decline is steady, but calculated decay factors have a high mean error, so we extrapolated the biological half-time graphically, since it lies within the measured data. It is about 10 days for the stomach and about 19 days for the intestine. Localization of the element either histochemically or on the autoradiograph is not possible; the lowest concentration limit for detection with these means is not reached in these tissues.

*Discussion.* The rapid incorporation and steady accumulation of metal ions seems to be a characteristic feature of the gastropode liver[3]. The place of storage being limited to one type of cell and within the cell to one specific structural element is interesting. During accumulation the number of these typical cells does not seem to increase, but the individual calcium cell becomes definitely larger and tightly packed with spherules entirely covered with zinc. It is sometimes impossible to distinguish any cell plasma at all.

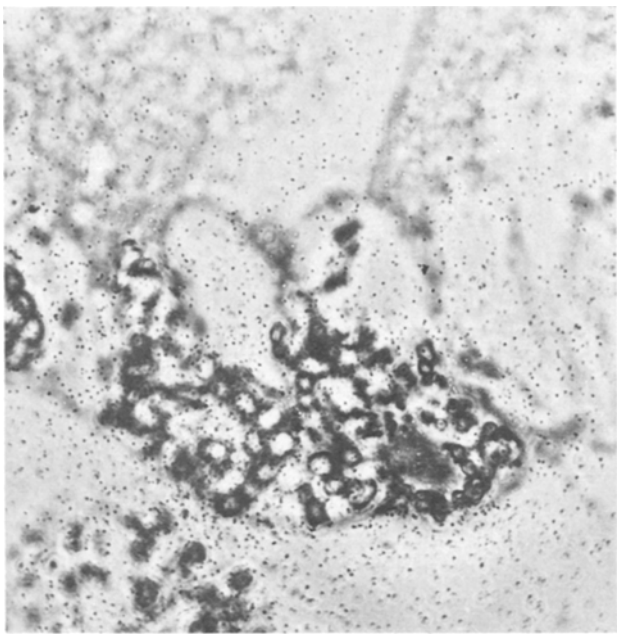

Fig. 3. Autoradiograph of 2 calcium cells in a liver acinus (*Arion*). Phasecontrast. Focus on silver grains. × 400.

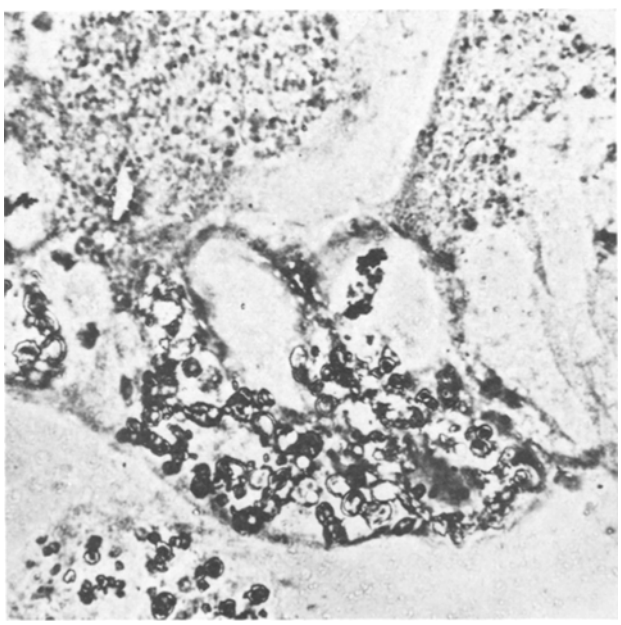

Fig. 4. As Figure 3. Focus on cells. Silver grains can be seen as phase-negative dots.

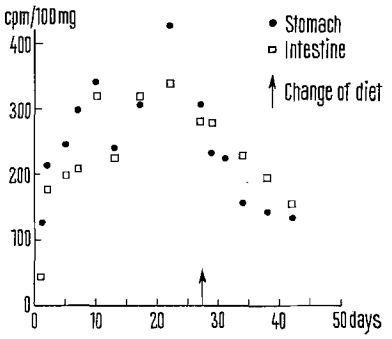


Fig. 5. Uptake and elimination of radioactive zinc in stomach and intestine of *Arion*.

The amount of metal taken up far exceeds a possible physiological need, and once the supply is stopped a slow elimination sets in. The pathway of the eliminated metal could not be found with certainty. Very few of the spherules ever turn up in the lumen of the intestine, and no other traces of zinc were visible in the excrements therein.

It is not impossible that this curious mechanism of dealing with an oversupply of metal serves to avoid toxic effects. Neither the animals nor the tissues examined showed any anomalities.

The fact that stomach and intestine start to eliminate before the diet is changed permits the assumption that these tissues relatively early reach a limit in accumulation capacity. No such limit was observed for the liver.

*Zusammenfassung.* Aufnahme und Einbau eines Metalls in die Leber von *Arion rufus* wurde mit Hilfe von 65-Zn quantitativ verfolgt. Die Lokalisation erfolgte histochemisch und autoradiographisch. Einer relativ raschen und stetigen Aufnahme von Zink und seinem Einbau einzig in den Calciumzellen bis zum Absetzen des Dosierfutters folgt eine langsamere, exponentiell verlaufende Elimination. Magen- und Darmgewebe beginnen mit dem Abbau bereits früher, während die Leber noch einbaut.

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[3] CH. M. YAGER and H. W. HARRY, Malacologia *1*, 3, p. 339 (1963).