Changes in newt brain caused by zinc water-pollution

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Summary. The zinc content of various organs of newts kept in zinc-polluted water was estimated by atomic absorption spectrophotometry. Histological examination revealed the presence of zinc-rich, unusual cells in the primordium hippocampi of chronically poisoned animals.

The present study describes the effects of an accidental, chronic zinc poisoning in the amphibian newt, *Triturus cristatus*. It includes an examination of possible effects of zinc pollution on the central nervous system.

The effects of zinc pollution on marine and freshwater animals are well documented[2-4] but the mechanisms underlying subacute or chronic zinc poisoning are not well understood yet. Crespo et al. found that dogfishes submitted to sublethal aquatic zinc pollution (15 ppm) first accumulate the metal in their gills but later develop external symptoms, apparently not related to the gill damage[5]. Other authors point out that zinc subacute toxicity involves injury to internal organs such as liver, kidneys, heart, skeletal muscle, gonads and spleen[6-8]; however, no reference to possible brain damage is given.

A few years ago we received a glass tank built with a zinc plated metal bottom, and observed that newts placed in this vessel developed a disease characterized by darkening of the skin, a loss of appetite and a slowing down of movement. This took place after 6-12 weeks, depending on the individual. Moreover, out of the 25 animals kept in this tank, 50% died after the usual artificial 'hibernation' (animals placed in a mossy covered wooden box, in a dark cold room at 4 °C, from mid-November until mid-February), while the 600 animals forming the rest of the colony recovered perfectly, with a death rate of less than 1%. The difference in the percentages of casualties was observed in two consecutive years, thus indicating the poisonous action which was investigated on the survivors.

Materials and methods. 1. The ionic contents of Zn, Cu and Fe of the water in the tank with a zinc-plated base were measured by atomic absorption spectrophotometry (Pye Unicam SP 1 900), and the concentration of Al by colorimetry with Eriochrome Cyanine R. The results obtained were compared to those for 2 control tanks made entirely of glass. Furthermore the zinc content was measured daily, for a week, by the same method, in one control tank and in the incriminated tank; each tank contained 10 animals. 2. The zinc contents of brain, liver, intestine, pancreas, kidney, skin and choanae were estimated by the same method. Samples were oven-dried in air at 100 °C until they reached constant weight, digested in 0.2 ml HNO_3 'suprapur' + 0.2 ml $HClO_4$ 'suprapur' (Merck) for 48 h at room temperature, then gently heated and diluted to 5 ml with hot (85 °C) deionized bidistilled water. Four pairs, consisting of 1 control and 1 poisoned animal of comparable size were submitted to a simultaneous analysis, to avoid possible effects of size (it is known in fishes that tolerance to zinc pollution changes with the size of the animal[2]. 3. The brains of 5 other pairs of animals were submitted to histological examination after Timm's or Bodian's stains.

Results and discussion. The ionic concentrations of Cu (7 µg/l), Fe (20 µg/l) and Al (6 µg/l) in the water of the control and the incriminated tanks were the same, but zinc concentrations were much higher in the latter than in the control (fig. 1). In the control tank the values of zinc rose in a week from 0.20 ± 0.01 mg/l to 0.26 ± 0.01 mg/l. This small zinc accumulation could be due to the daily animal

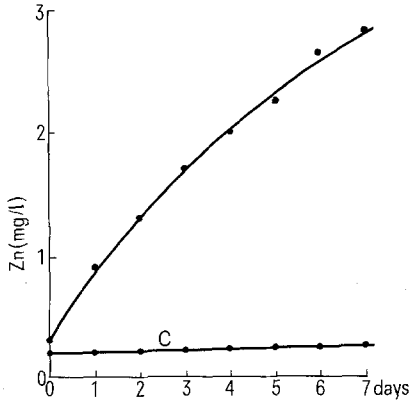

Figure 1. Daily concentration of zinc in the water of the control (C) and of the incriminated tank both filled with 15 l and accommodating 10 newts. Zinc content of the city water: 0.17 ± 0.01 mg/l.

Concentrations of Zn (µg/mg dry weight) in some organs of newt

	Controls	Poisoned	Increase (%)
Anterior brain	0.1531	0.3254	+ 112.5
	0.0579	0.1502	+ 159.4
	0.0505	0.0785	+ 55.4
	0.1619	0.1950	+ 20.4
Posterior brain	0.4324	0.5442	+ 25.9
	0.0714	0.3000	+ 320.9
	0.1012	0.1020	+ 0.8
	0.2381	0.2848	+ 19.6
Liver	0.1135	0.3333	+ 193.7
	0.0793	0.1161	+ 46.4
	0.0454	0.0829	+ 82.6
	0.0952	0.2389	+ 150.9
Intestine	0.4646	0.5373	+ 15.6
	0.2500	0.4762	+ 90.5
	0.1190	0.1681	+ 41.3
	0.3710	0.4564	+ 23.0
Kidney	0.2920	3.1405	+ 975.5
	0.2469	0.5072	+ 105.4
	0.1523	0.2647	+ 73.8
	0.3608	0.6742	+ 86.9
Pancreas	3.3918	2.6961	− 20.5
	3.4184	3.2368	− 5.3
	0.7059	3.5593	+ 404.5
	0.9231	1.9192	+ 107.9
Skin	0.6579	1.5714	+ 138.9
	0.8294	0.7042	− 15.1
	0.6818	0.3968	− 41.8
	1.5975	1.2575	− 21.3
Choanae	5.1282	2.1533	− 58.0
	1.0938	0.9067	− 17.1
	0.2941	0.6872	+ 133.7
	1.8142	1.1644	− 35.8

The anterior and posterior parts of the brain were separated by a cut through the diencephalon. For the other organs the weights of samples were of 5-15 mg (wet weight). For each sample atomic absorption spectrophotometric analyses were made in triplicate (SEM: 0.001).

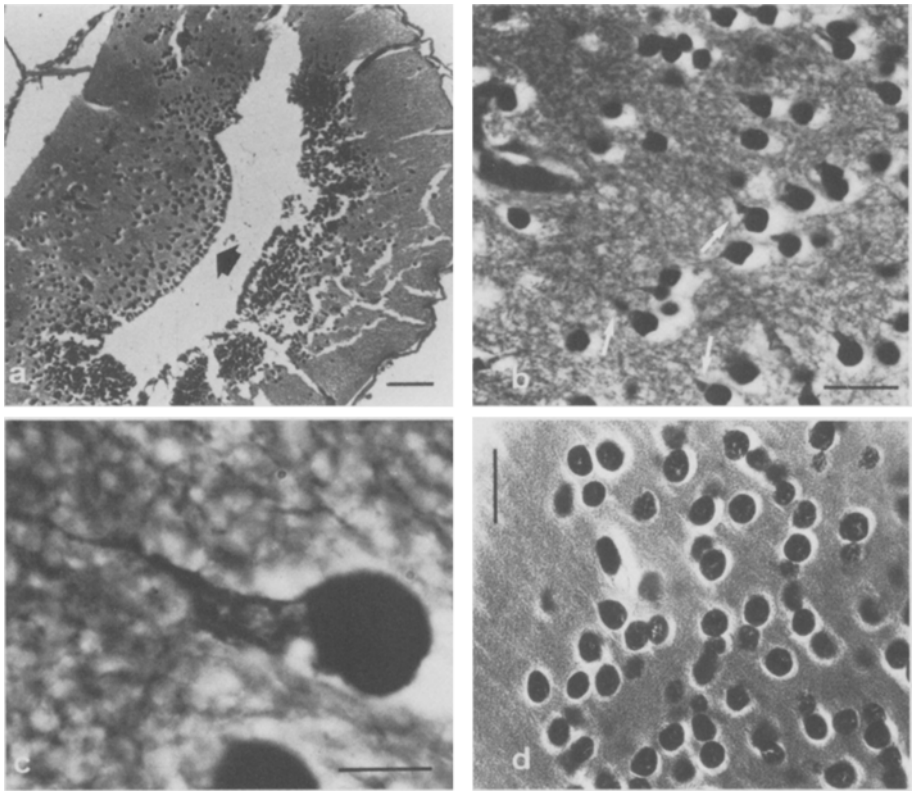

Figure 2. Brain of zinc-poisoned newt, Bodian stain. *a* Primordium hippocampi (arrow); bar: 100 µm. *b* Same preparation showing numerous cells with abnormal axonal cones; bar: 40 µm. *c* Same preparation, unusual cell with enlarged axonal cone; bar: 10 µm. *d* Brain of a control newt. Primordium hippocampi, Bodian stain; bar: 40 µm.

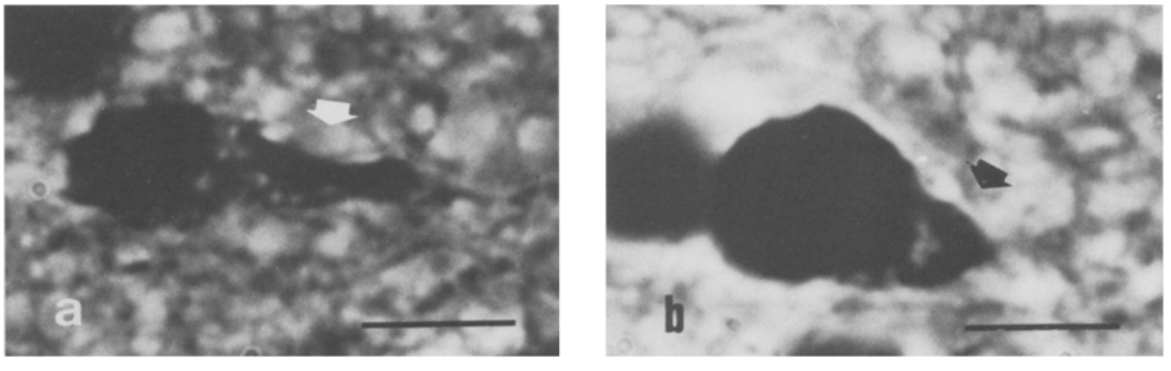

Figure 3. Zinc-poisoned hippocampal cells with lateral (a) and V shaped (b) axonal deposits. Bodian stain; bar: 10 µm.

excretion, which was thus roughly estimated at 12.5 µg (see also legend of fig. 1).

The values for zinc concentration in 7 newt organs are shown in the table. Both the anterior and posterior parts of the brains have a higher zinc content in the diseased partners than in the controls, with the percentage of increase varying from one pair to the other. Increased zinc concentrations were also noted in the livers, intestines and kidneys of all the diseased animals. Curiously in the pancreas this increase was found only in 2 out of the 4 cases and in the skins and choanae a decrease was apparent in 3 out of the 4 cases.

In vertebrates zinc is one of the most abundant divalent metals in the brain, where its concentration (of the order of 10 µg/g of wet tissue) is approximately 5 times greater than that of copper and 10 times that of manganese. Its physiological role has been correlated with the metabolism of GABA (γ-aminobutyric acid), probably the major inhibitory neurotransmitter in both the invertebrate nervous system and the vertebrate central nervous system. Moreover it was shown that the distribution of Zn^{2+} in the brain is similar to that of GAD (1-glutamate decarboxylase) and pyridoxal phosphokinase, both factors being involved with the maintenance of the steady-state level of GABA[9]. To our knowledge no such studies have been performed on the newt brain. Histological observations carried out on 5 diseased animals and 5 controls have shown a clear increase of black granular deposits (indicative of heavy metals) in the brains of the diseased newts. This increase was apparent mainly in the primordium hippocampi and in the mesencephalon, but only cells of the primordium hippocampi have shown both an increase of heavy metals (Timm) and a modified appearance (Bodian). Some of them have shown a heavy black deposit in the cell axonal cone as seen in figures 2 and 3 after application of Bodian's stain. These histological observations are suggestive of a clogging occurring at the axonal cone level, possibly due to disrupted microtubules since, according to Gaskin et al., zinc salts added to

organotypic cultures of mouse dorsal root ganglia induce disrupture of microtubules, formation of abnormal tubular structures (200–250 nm in diameter instead of the usual 20–25 nm), and accumulation of bundles of neurofilaments in some perikarya[10]. This hypothesis should be verified with an EM study.

It is known that hippocampal cells are connected with several other brain regions, including the hypothalamus[11]. It is therefore tempting to correlate the symptoms noted (darkening of the skin, loss of appetite, slowing down of the movements, inability to recuperate after hibernation), with a hypothalamic dysfunction secondary to a damage of hippocampo-hypothalamic interconnected neurons. However, zinc ions are essential for several biologically active enzymes, some of which are involved in the mechanisms of neuro-hormonal regulation. Further studies are needed to clarify the pathogenic modes of action of excess zinc in the surrounding medium, which acts as a serious pollutant factor on our animals. More specifically, one might wonder whether zinc poisoning might not impair precisely the physiological mechanisms which normally require a limited amount of this ion.

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2 P. Doudoroff and M. Katz, Sewage ind. Wastes *25*, 802 (1953).
3 G.W. Bryan, Proc. R. Soc. B *177*, 389 (1971).
4 M. Waldichuk, in: Pollution and Physiology of Marine Organisms, p. 1. Eds J. Vernberg and W.B. Vernberg. Academic Press, New York 1974.
5 S. Crespo, R. Flos, J. Balasch and G. Alonso, Comp. Biochem. Physiol. *63 C*, 261 (1979).
6 R. Flos, A. Caritat and J. Balasch, Comp. Biochem. Physiol. *64 C*, 77 (1979).
7 C.A. Crandall and C.J. Goodnight, Trans. Am. microsc. Soc. *82*, 59 (1963).
8 M.H. Wong, K.C. Luk and K.Y. Choi, Acta anat. *99*, 450 (1977).
9 C.F. Baxter, in: GABA in nervous system function. Eds E. Roberts, T.N. Chase and D.B. Tower. Kroc Fundation Series, vol. 5. Raven Press, New York 1976.
10 F. Gaskin, Y. Kress, C. Brosnan and M. Bornstein, Neuroscience *3*, 1117 (1978).
11 C.J. Herrick, in: The brain of the tiger salamander. The University of Chicago Press, Chicago 1948.

Mianserin reduces plasma levels of β-endorphin immunoreactivity in depressed patients

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Summary. Eight depressed patients were treated for 25 days with the antidepressant drug mianserin (3×20 mg/day). β-Endorphin immunoreactivity, measured in blood samples withdrawn before and after the treatment, appeared to be significantly reduced by mianserin. Since endogenous opioids can play a role in the etiology of depression, the observed effects of mianserin could be of relevance for its anti-depressant activity.

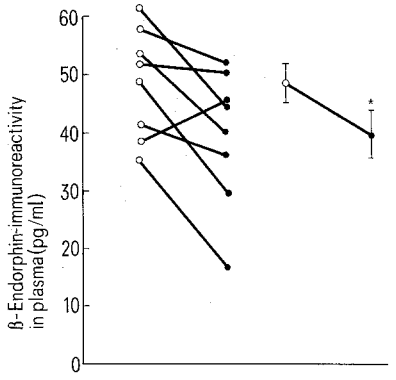
Mianserin HCl (2,3,4,10,14b-hexahydro-2-methyl-dibenzopyrazino-azepine monohydrochloride) is a clinically efficacious anti-depressant[2]. Its profile of action includes presynaptic α-adrenoreceptor blocking activity and anti-histamine properties, but not central anti-cholinergic activity[3].

Elevated concentrations of opioid material have been observed in the cerebrospinal fluid of depressed patients[4]. Treatment of such patients with lithium reduced these levels. Treatment with the opiate antagonist naloxone (Narcan®) also reduced levels of opioid material in the cerebrospinal fluid of depressed patients, though not to a clinically observable extent[5]. Although the relation between the opioid activity found in cerebrospinal fluid and brain endorphins is largely unknown, these observations have been correlated with functional disturbances in the central endorphin systems[6].

We were therefore interested in investigating the effect of mianserin on the plasma levels of β-endorphin immunoreactivity in depressed patients.

All patients (4 males and 4 females, 25–46 years old, mostly with unipolar depression) were hospitalized during the trial. The selection criterium was a minimal 2 months drug-free period before hospitalization. Mianserin (Lantanon®, Organon B.V., Oss, The Netherlands) was given orally in a daily dose of 3×20 mg, for 25 days. Blood samples were withdrawn before and after the treatment. Endorphins were extracted from plasma using Vycor-glass[7]. Assay of β-endorphin immunoreactivity in the extracts was performed using a radioimmunological method[8]. The antiserum used showed 60% cross-reactivity with β-LPH (mole/mole basis).

Results are depicted in the figure. Plasma levels of β-endorphin immunoreactivity appeared to be decreased after mianserin treatment in all patients except one (left side of the fig.). The statistical analysis of the results



Effect of mianserin on plasma β-endorphin immunoreactivity of depressed patients. Left side: effect on single patients. Right side: means (± SE) of all samples. ○, before the treatment; ●, after the treatment. * $p < 0.05$ (repeated t-test).