control animals at each dose till the 3rd day. In the 500 R exposure group the percentage of pronormoblasts and normoblasts increases significantly ($p < 0.05$) from the 5th day on as compared to the control of the same group. At 2 weeks an almost normal value is reached which is more or less maintained during the next intervals. No 2nd phase of decline is noticed at 3 weeks (table, figure). In the 1000 R, there is a rise in pronormoblasts and normoblasts percentage at 2 weeks which is slightly more than half of the normal value. This is followed by a 2nd phase of decline at 3 weeks but the value is significantly higher than that of the control of the same interval ($p < 0.002$). A further decline in percentage is observed at 4 weeks reaching the 2 weeks value of the same dose (table). Supralethally irradiated animals also show an increase in pronormoblast and normoblast percentage on the 5th day. No further observation could be made in this group as there were no survivors.

Discussion. Our findings in the lethally and supralethally irradiated control mice are in conformation with those of Hulse[6] who observed that the pronormoblasts and normoblasts become severely depleted by 1 day and completely disappear by the 3rd day. In our studies the sublethally irradiated animals also show an identical pattern of cell depletion, showing maximum reduction on 3rd day (table). The statistically significant fall in nucleated red cells at 3 weeks and renewed increase at 4 weeks is in complete agreement with the data of Brecher et al.[7] who believe that regeneration of hematopoietic tissues after X-ray injury proceeds in waves. However, the results are different in drug-treated animals. In these animals the initial decrease in percentage is less pronounced as compared with controls which received the same dose. Moreover, the recovery is also earlier and faster in the drug-treated animals especially in the sublethally irradiated group.

In the present observation, more of the pronormoblasts and normoblasts survive in MPG treated animals during the early intervals (upto 3rd day), which indicates protection of these 'blast' cells, thus maintaining a stem cell pool which can actively regenerate the marrow and bring about an early and fast recovery. An early erythropoiesis was reported earlier due to the protection afforded by MEG[8] and AET[9] to the stem cell compartment during the initial radiation damage. The present observation indicates a similar effect by MPG also. However, in the protected animals, the recovery at 4 weeks is less in higher dose (1000 R) as compared to lower dose (500 R). Thus regeneration also appears to be a dose dependent phenomenon; with a higher dose, more of the stem cells are killed and hence the regenerating pool becomes very much reduced in size, which cannot bring about complete recovery within the longest interval studied (28 days).

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Dopamine agonist performance in Planaria after manganese treatment

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Summary. Using Planaria motor performance as model, the authors confirm that Mn^{++} basically inhibits dopaminergic release with transitory hyper-release.

In man, chronic manganese poisoning produces an extrapyramidal syndrome (toxic parkinsonism[1]) with evident dystonic[2,3] and psychiatric symptoms. The metal's interference with the dopaminergic pathways is demonstrated by the considerable drop in striatal dopamine seen in man[4], as well as by the stereotyped behavior and turning produced by intrastriatal administration of manganese in rats[5].

We previously reported[6,7] that when the dopaminergic compartment of *Dugesia gonocephala s.l.* was stimulated, a distinctive motor reaction occurred, i.e. 3-dimensional screw-like movements; this was comparable to mammalian stereotyped behavior. Further research[8] confirmed the validity of this model as an alternative to the conventional experimental models using higher vertebrates.

Within the framework of our investigation of the neurobiological activity of certain metal ions[9-11], it was deemed interesting to test this model in the case of manganese poisoning also.

Materials and methods. Planaria of the species *Dugesia gonocephala s.l.* (Platyhelminthes, Turbellaria, Tricladida) in the agamous scissiparous form, bred by us (Mignone River strain), were placed in solutions of manganese sulfate and chloride (Merck) in distilled water at concentrations increasing from 15.10^{-5} to 180.10^{-4} M (table). The animals were observed in a dimly-lit environment at 18 °C once every 30 min or so for the first 24 h and thereafter about every 12 h for a total of 8 days.

Results. Solutions with a Mn^{++} content ranging from 180.10^{-4} to 18.10^{-4} M immediately or almost immediately induce screw-like movements of the type produced by dopaminergic overstimulation. These movements cease after about 15 min, later recurring spasmodically in the form of repeated 'crises' until the end. In the intercritical phase the animals lie on 1 side, curled up in a 'C'-shape. Slight luminous or mechanical stimulation at this stage leads to the immediate resumption of hyperkinetic behavior for the duration of several minutes. Death ensues in 24-48 h (unlike the situation with sodium salts, which we described earlier[12], the chloride appears to be more toxic than the sulfate, table).

	Molarity	Activity	% Deaths	(Time in days)
$MnCl_2 \cdot 4\,H_2O$	109.10^{-4}	Immediately spiralizing	100	(1)[a]
	72	Immediately spiralizing	100	(1)
	36	Immediately spiralizing	100	(2)
	18	Immediately spiralizing	100	(2)
	9	Spiralizing after 20 min	0	(8)[b]
	45.10^{-5}	Spiralizing after 30 min	0	(8)
	23	No activity	0	(8)
$MnSO_4 \cdot H_2O$	180.10^{-4}	Immediately spiralizing	100	(1)[c]
	18	Spiralizing after 10 min	0	(8)
	9	Spiralizing after 25 min	0	(8)
	45.10^{-5}	Spiralizing after 45 min	0	(8)
	15	No activity	0	(8)
NaCl	210.10^{-4}	No activity	0	(8)
$NaSO_4 \cdot 10\,H_2O$	350.10^{-4}	No activity	0	(8)

[a] pH 6.20, mOs/kg 39.04; [b] pH 7.40, mOs/kg 11.15; [c] pH 6.45, mOs/kg 33.47.

Mn^{++} concentrations between 9.10^{-4} and 45.10^{-5} M induce the same type of screw-like movements, although with a long latent period before onset (25–45 min) and with the 'crises' further apart. At these concentrations the lethal effect was zero throughout the observation period. Sodium chloride and sulfate solutions at the same anionic concentrations as those of the respective Mn salts used had no effect (table). The pH and osmotic pressure (determined cryoscopically) of the Mn^{++} solutions were all compatible with the behavior and indefinite survival of the planaria[12].

Also in the specimens treated with concentrated solutions, no significant histological modifications could be detected in the nervous system, in which, however, the metal could be identified histochemically using Timm's method[13].

Pretreatment with reserpine (8 mg/l, 24 h) prevented the Planaria from displaying screw-like movements when later placed in concentrated solutions (180.10^{-4} M).

When placed in concentrated Mn^{++} solution after pretreatment with haloperidol (1 mg/l, 24 h), the Planaria displayed screw-like behavior for only 15–20 min, after which the phenomenon ceased and did not reappear.

The semi-quantitative fluorescence method[14] usually revealed no significant differences in biogenic amine content in the nervous system between control and treated animals. However, the intensity was occasionally lower in treated animals.

Discussion. In our model Mn^{++} displays a conspicuous dopaminergic agonist activity, which it shares with Cu^{++}[9] but not with other metals[11].

This is confirmed for both metals by natural and experimental pathological data[1,10,15].

Significantly, even at high concentrations, the dopaminergic agonist activity of Mn^{++} does not extend continuously over the whole duration of the animal's survival, as was observed in the case of amphetamine, apomorphine or l-DOPA[6,7] administration, but occurs in repeated, periodic 'crises'. This behavior may be explained by a blocking of neurotransmitter release[16] which lasts until its accumulation in the presynaptic compartment becomes high enough to determine a transitory discharge of mediator, and so on. Indeed, this hypothesis has been advanced to explain the alternation of dopaminergic agonist phenomena (stereotypes, contralateral turning) with dopamine blocking (ipsilateral turning) after striatal micro-injection of Mn^{++} in rat[5] and is thus well supported by our model.

The hypothesis receives further support from the immediate appearance of the screw-like movements at high metal concentrations favouring the accumulation of high dopamine concentrations, just as happens in the rat, where high Mn^{++} doses give rise to dopaminergic agonist phenomena[5], the inhibitory effect of reserpine-induced dopamine depletion and the equally inhibitory effect produced by post-synaptic haloperidol blocking. The transitory nature of transmitter accumulation prevents it from being clearly visualized histofluorometrically.

Briefly, the experimental model we have proposed supports the idea of Mn^{++} activity being related to a blocking of presynaptic dopamine release leading to transitory hyperrelease episodes and eventually to irreversible damage to the dopaminergic cells. A molecular hypothesis to explain the phenomena observed, and particularly the harmfulness of Mn^{++} at neuron level, cannot yet be formulated, although the hypotheses[17] in which a relationship is postulated between mitochondrial deamination of the biogenic amines and the accumulation of the metal in these organelles are of considerable interest.

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