dans 75% des cas, aucune modification n'a cette fois marqué les décharges provoquées par stimulation; en particulier il n'est pas apparu de décharge sur nerf d'Ex alors que ce précurseur les favorise normalement, sans traitement par le DDC. Dans les 25% restants, l'effet de la DOPA ne s'est trouvé que diminué par rapport à la normale mais non absent, de petites décharges apparaissant encore sur les Ex après injection. Dans un cas isolé enfin, des décharges importantes en amplitude se sont développées sur Ex.

Ces expériences montrent que l'inhibition de la DA-β-hydroxylase par le DDC entraîne, sur le plan locomoteur, a) la disparition des décharges rythmiques sur nerf d'Ex, b) une absence d'effet de la DOPA, qui, sans prétraitement favorise les décharges sur cette même catégorie de nerfs. L'existence d'activités locomotrices sur Ex (ou leur accroissement) paraît en conséquence liée à la biosynthèse de NA à partir du précurseur endogène ou de la DOPA exogène. Cette biosynthèse de la NA s'effectuerait au niveau des terminaisons lombo-sacrées de voies bulbospinales noradrénergiques, l'effet de la DOPA étant encore présent chez le Lapin spinal aigu prétraité par le nialamide[4].

L'effet par fois moins net du DDC, et même absent dans un cas, paraît suggérer l'existence de différences individuelles du degré d'inhibition enzymatique par le DDC.

Ces résultats sont enfin à rapprocher d'autres faisant état de la réduction d'activité motrice spontanée chez le Rat après traitement au DDC[9]. En revanche il a été signalé la persistance, avec ce même traitement, du comportement stéréotypé induit par la DOPA[10,11]: ce comportement stéréotypé, qui consiste en reniflements, léchages ou morsures continuelles, semble par conséquent lié à des mécanismes dopaminergiques, alors que la locomotion telle que nous l'analysons dépendrait de mécanismes noradrénergiques.

Summary. Mesencephalic, curarized rabbits can develop 'locomotor' discharges with alternating phases of flexions and extensions in hind limb nerves. Moreover, DOPA was shown to exaggerate the extensor activity as compared to that in the flexor. It is shown here that diethyldithiocarbamate, a dopamine-β-hydroxylase inhibitor, suppresses discharges in extensor nerves and that a subsequent injection of DOPA is generally no more active. It is concluded that the supraspinal extensor activation in locomotion is depending upon synthesis of norepinephrine and not that of dopamine.

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Some Characteristics of *Ochromonas* Hemolysins[1]

Following the isolation of toxins from *Ochromonas* strains, HALEVY et al.[2], their biological characteristics were further investigated. Attempts to test the toxic effect of *Ochromonas malhamensis* toxins, using mice or rats as experimental animals, have generally been unsuccessful unless very large doses of toxins were injected. Toxins injected either i.p. or i.v. in amounts which, according to calculations from in vitro experiments, would have hemolysed most of the blood cells of the mice or rats, generally failed to kill the animals or even to produce an appreciable reduction in their erythrocyte count. It was suspected that the toxins were inactivated in the body. This possibility was further investigated in this report.

Table I. The protective effect of serum proteins and albumin against *O. malhamensis* EA fraction hemolysins

Tube No.	µg Toxin/ 5 ml RBC	µl Serum/ 5 ml RBC	Extent of hemolysis	µl Albumin/ 5 ml RBC	Extent of hemolysis
1	31	25.0	0	12.5	0
2	31	12.5	0	6.2	0
3	31	6.2	++	3.1	++
4	31	3.1	++	1.6	++
5	31	—	++	—	++
6	—	25.0	0	12.5	0

0.4% washed rat erythrocytes (RBC) were used. 0, no hemolysis; ++, complete hemolysis. Serum protein and albumin content was 67 and 70 mg/ml respectively. Erythrocytes, toxins and supplements were incubated at 37 °C for 1 h. EA fraction toxin was eluted from silica gel G column with ethyl acetate.

Materials and methods. Various techniques used in this study were similar to ones reported previously[2]. Toxins that were isolated from *O. malhamensis* ethyl acetate (EA) fraction were studied[2]. Hemolysis was performed using rat erythrocytes suspension of 0.4%. Incubation was done at 37° for 1 h and the result recorded[3]. Fish toxicity was determined with a small fish *Danio malabaricus* suspended in volume of 50 ml tap water. Experimenst were repeated several times. One set of data is presented.

Source of materials. Liver was obtained from white rats, rinsed with cold saline, homogenized with equal amounts of Hanks balanced salt solution pH 7. Packed washed rat erythrocytes (RBC) were diluted with equal volume of Hanks. Rat serum was diluted with equal amount of Hanks. Albumin prepared at concentration of 70 mg/ml and diluted with equal amounts of Hanks (Table II).

Incubation and extraction. Into a series of test tubes in duplicate were added 2 ml suspension of each tissue or albumin, 500 µg of *O. malhamensis* EA toxic fraction mixed and incubated with shaking at 37 °C for 60 min. Incubation of toxin in 2 ml Hanks served as a control. At the end of the incubation period to each test tube were added 9 volumes of methanol and the toxin was extracted with shaking at room temperature for 1 h. At the end of the extraction the suspension was filtered, brought to volume, an aliquot was evaporated under reduced pressure and

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Table II. Extent of hemolysis of RBC by 125 µg of toxins of *O. malhamensis* EA fraction, after incubation of the toxin with various tissues and its extraction with methanol

Tube No.	1	2	3	4
Serial dilution 0.4% RBC	5 ml	10 ml	20 ml	40 ml
Extent of hemolysis by the extracted toxins after incubation with:				
1. Hanks	++	++	++	±
2. Rat serum	++	++	++	0
3. Albumin	++	++	±	0
4. RBC	+	0	0	0
5. Liver	0	0	0	

++, Complete hemolysis; ±, partial hemolysis.

Table III. Protective effect of serum albumin and starch on the survival time of the small fish *D. malabaricus* upon the addition of *O. malhamensis* EA toxic fraction 125 µg/50 ml of tap water

Fish No.	Survival time		
	1	2	3
a) Tap water	20 min	20 min	20 min
b) Tap water + rat serum 12 µl	5 h	14 h	—
Tap water + rat serum 25 µl	18 h	18 h	over 24 h
c) Tap water + albumin 12 µl	3 h	3 h	1 h
Tap water + albumin 25 µl	18 h	18 h	24 h
d) Tap water + starch 50 µl	No protection was evident		

Rat serum protein content 67 mg/ml. Albumin and starch concentration was 70 mg/ml. Experiments were discontinued after 24 h.

tested for its hemolytic activity. The hemolysis was performed as usual with 0.4% rat erythrocyte suspension incubated for 1 h at 37 °C using serial dilutions.

Results and discussion. Addition of small amounts of protein in the form of rat serum or albumin (Bovine Albumin Powder Fraction *V*, Armour Pharmaceutical Co.) to the buffer in which erythrocytes were suspended for in-vitro studies, protected the blood cells from hemolysins as seen in Table I. About 800 µg of serum proteins and 420 µg of albumin protected the erythrocytes against 31 µg toxin.

In order to test to what extent toxin might be inactivated by various tissues, the toxin was incubated in the presence of rat homogenized liver, serum, erythrocytes and albumin. Hanks balanced salt solution served as control. Thereafter the toxin was extracted with methanol and tested to its hemolytic activity. As seen in Table II, the amount of toxin recovered after incubation with serum or albumin was only slightly inferior to that recovered from Hanks solution. While after incubation with liver homogenate there was no recovery of toxin. Almost no recovery was obtained after incubation in the presence of erythrocytes.

To test whether proteins may also protect fish against *O. malhamensis* toxins, rat serum, albumin and starch were added to water containing lethal amounts of toxins. Rat serum and albumin protected the fish. Starch did not have any effect (Table III).

Inactivation of hemolysins by the presence of serum proteins or albumin, or their possible destruction by liver enzymes, may partially explain their lack of toxicity towards mice and rats. As water in nature may contain dissolved organic matter including proteins, fish toxicity of naturally occuring *O. malhamensis* toxins may be thus reduced or eliminated. This may partly explain lack of recorded fish mortality outbreaks due to *Ochromonas* toxins.

Résumé. On a observé que les hémolysines d'*Ochromonas malhamensis* ont été tendues inactives par differentes protéines. Ce sont les enzymes du foie du rat qui ont apparemment détruit les hémolysines. Cette observation peut expliquer l'absence de mortalité chez les souris et les rats pendant l'injection d'hémolysines.

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Permeability of the Golden Hamster Placenta to Inorganic Lead: Radioautographic Evidence

The detrimental effects of lead on mammalian reproduction have long been recognized[1–5]. The now rapidly increasing level of this nonessential heavy metal in our environment has consequently been viewed with great concern by toxicologists, public health officials and ecologists[6–9] and has stimulated further research on the toxic effects of lead on reproduction. Recent breeding experiments on lead-treated rats and mice have clearly demonstrated the 'gametotoxic, intrauterine and extrauterine toxicity' of this metal leading to increased stillbirths, reduced litter sizes, growth retardation and elevated neonatal death rate[10–11]. In addition, specific teratogenic effects have been noted in both hamster[12–13] and rat[14] embryos following acute treatment of dams with inorganic lead compounds during early stages of gestation. The placental transfer of lead ions from the maternal blood into the embryonic/fetal system has been shown indirectly in older studies by ashing and chemical analytic techniques[15–17] and more recently by radioactive tracer studies[14]. At the present time, however, little is known regarding the placental sites of lead transfer, the rate at which it permeates or the specific sites within the embryo where it accumulates. The present radioautographic study utilizing ^{210}Pb[18] provides new information on these problems.

Pregnant golden hamsters at accurately timed early stages of gestation were obtained from the Lakeview Hamster Colony (Newfield, N. J., USA). These were divided into 4 groups of 10 animals each and at 08.00 h on day 7 or 8 of gestation were injected via the lingual vein with 20 or 50 µCi of $^{210}Pb(NO_3)_2$[19] either singly or in combination with a teratogenic dose (50 mg/kg) of nonradioactive $Pb(NO_3)_2$. At intervals between 15 min and 5 days postinjection the animals were killed with chloroform and the intact gestation sacs recovered and placed in Bouin's fixative for 1 week. The sacs were subsequently embedded in paraffin, serially sectioned at 5–7 µm and coated with Eastman Kodak NTB liquid emulsion by the dipping method[20]. After exposures of 2 to 5 days at 0 °C.