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.

Fig. 1. Photomicrograph of the central region of an 8-day gestation sac showing amniotic cavity (AmC), amnionic membrane (Am), developing chorioallantoic placenta (CA), decidua basalis (DB), decidua capsularis (DC), head fold stage embryo (Emb), exocelomic cavity (ExC), parietal wall of yolk sac placenta (PYS), visceral wall of yolk sac placenta (VYS) and yolk sac cavity (YSC). The mother was injected i.v. with 20 μCi/kg of $^{210}Pb(NO_3)_2$ 1 h before fixation of the tissue. Radioautogram, hematoxylin and eosin stain. ×29.

Fig. 2. Enlargement of outlined region in the previous photomicrograph showing numerous whisker-like α particle tracks emanating from the yolk sac (PYS and VYS) and amnionic (Am) membranes and the embryo (Emb) proper. Fewer tracks are visible over the maternal decidual tissue (DC) and blood spaces (MBS) and the embryonic cavities (AmC, ExC, YSC). ×155.

the coated slides were developed in D–19 and stained with hematoxylin and eosin for light microscopic examination.

Gestation sacs from all 4 groups of animals displayed considerable radioactivity within 15 min of injection. No remarkable differences in intensity or distribution of radioactivity were detected at this time among gestation sacs obtained from the different groups. Highest concentrations of lead, as evidenced by the density of α particle ionization tracks, were in the contents of the maternal blood vessels and in the parietal (outer) and visceral (inner) walls of the yolk sac placenta Lesser concentrations were present in the maternal myometrial and decidual tissues. The developing chorioallantoic placenta (chorionic-Träger plate, or ectoplacenta), the embryonic cavities (amnionic, yolk sac and exocelomic) the amnionic membrane and the embryo itself were only lightly labeled at this interval. In all labeled tissues, ionization tracks appeared to emanate mainly from nuclear and cytoplasmic regions of cells and to a lesser extent from intercellular regions.

The highest levels of α track labeling in gestation sacs from all 4 groups occurred between 1 and 4 hours after injection (Figures 1 and 2). Very heavy labeling was evident over the epithelial cells of the visceral wall of the yolk sac placenta, particularly in 8 day gestation sacs. Labeling also was intense over the parietal wall of the yolk sac, the amnionic membrane and the embryo itself. The ionization tracks were fairly evenly distributed over the different parts of the embryo. Moderately high levels of labeling were visible over the uterine tissues (mesentery, myometrium, decidua, blood vessels) and over the developing chorioallantoic placenta. Scattered ionization tracks were present in all of the embryonic cavities (yolk sac, exocelomic and amnionic).

Radioautographs of gestation sacs processed at later postinjection intervals up to 5 days exhibited a progressively decreasing intensity of labeling. This presumably was due to the maternal excretion of lead ions in urine and feces and the dilution effect of rapid cell division and growth in embryonic tissues. At the 24 h and later postinjection intervals, growth retarded, malformed, and necrotic aborting embryos were frequently encountered in the gestation sacs from animals given ^{210}Pb in combination with a teratogenic dose of nonradioactive $Pb(NO_3)_2$.

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[18] Lead-210 (radium D) has a half-life of approximately 22 years. During decay to its stable elemental form, ^{206}Pb (radium G), α particles (~ 5.3 Mev), as well as high energy β- and γ-radiations, are emitted. In radioautograms of ^{210}Pb containing tissues the alpha particles produce dense, straight tracks of ionized silver particles in the overlying photographic emulsion. These tracks can be used to determine the sites of radioactivity, i.e. ^{210}Pb, within the tissues.
[19] Obtained from New England Nuclear, Boston, Mass., USA; specific activity 8.2 μCi/mg.
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These observations are significant in that inorganic lead ions are now known to cross the placental membranes of at least one mammalian species rapidly (within 15 min of i.v. injection) and in substantial amounts even at relatively low dosage levels (less than 3 mg/kg of maternal body weight in the group of animals receiving 20 μCi of $^{210}Pb(NO_3)_2$ only). Moreover, the demonstrated period of permeability to lead ions corresponded to the most critical stages of organogenesis in this species. These findings point up the hazard of even a low level of environmental contamination with inorganic lead since potentially even very small amounts of this metal entering the maternal blood stream can be transferred to the embryonic or fetal system. What constitutes a 'safe level' of lead in the blood plasma of mammalian species becomes even more of an equivocal question.

The exceptionally high levels of radioactivity observed in the membranes of the yolk sac placenta, particularly at the earlier postinjection intervals, suggests that in the hamster this is the route by which most lead ions are transferred from the maternal blood to the embryonic compartments. Only moderate levels of radioactivity were ever observed in the developing chorioallantoic placenta. This finding supports the contention of other investigators[21–23] regarding the preeminence of the yolk sac placenta in materno-embryonic transport during the early stages of gestation in many rodent species.

The generalized distribution of radioactive lead ions within the tissues of the exposed embryos was surprising in view of the highly specific teratogenic effect of this metal on the tail bud of this species[12–13]. In fact, lead is but one of several heavy metals[24] and numerous other chemical agents[25] that produce rather distinct patterns of developmental malformations in the embryos of hamsters treated during the teratogenically critical 8th day of gestation. The reason that lead ions exert a specific deleterious effect only on the developing tail bud tissues of the embryo and not to a detectable extent on the other rapidly developing organ systems to which they gain access during this period remains an interesting problem. It is possible that at this period of embryonic development this particular system has a special affinity and sensitivity to lead ions, i.e. there exists a specific organ: teratogen interaction. The suggestion[13] that lead ions interfere with a certain enzyme or enzyme system necessary for the normal development of the tail at this time merits further attention[26].

Zusammenfassung. Trächtigen Goldhamstern wurde am 7. und 8. Tag ^{210}Pb intravenös verabreicht. Von den Placentamembranen und vom Embryo wurde radioaktives Metallion stark aufgenommen und die Einlagerung autoradiographisch verfolgt.

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Palliative Effect of Gelatine in Benign Prostatic Hypertrophy

In the course of experimentation not relevant to the present report, it was observed that supplementation of the normal diet with a total daily amount of 25 g of gelatine (Knox Gelatine Inc., Johnstown, N.Y., 12095) given in 2–3 portions as a water suspension substantially relieves the distressing symptoms associated with benign prostatic hypertrophy. Burning and urgency disappear as the stream enlarges, and the frequency of micturition is reduced. Initially, these symptoms return promptly after the gelatine supplementation is discontinued. However, after gelatine is taken for 5–6 months, a few days discontinuation is no longer accompanied by as fast a return of the symptoms as in the beginning, and the daily dose may be reduced. The effects of gelatine are specific in that dietary supplementation with an equivalent amount of meat protein is without effect. Since these observations were peripheral to other objectives they were not followed up experimentally. The following is offered tentatively.

Ingestion of protein results in higher metabolic rate, an effect which is accompanied by increased body temperature and skin blood flow[1,6]. The specific dinamic action of protein is mainly due to a few amino acids, notably glycine, alanine, phenylalanine and tyrosine. Glycine, which constitutes about 25% of gelatine, induces a sustained increase of skin blood flow when taken orally[2]. It is not clear to what extent the increased skin perfusion induced by gelatine is due to the hydrolytically relesaed glycine. The effectiveness of gelatine in the treatment of split nails[3–6] appears to be linked to its circulatory effects.

The palliative effects of gelatine in prostatic hypertrophy are thought to be due to a reduction of prostatic edema, as this factor alone could account for the prompt amelioration of urinary distress. The benefits, if any, of prolonged improvement of drainage of prostatic acini remain to be determined.

Search of the literature revealed a report that a combination of glycine, alanine and glutamic acid is of value in the relief of urinary symptoms associated with benign prostatic hypertrophy[7]. Since relief was often associated with the disappearance of manifest edema, the authors concluded that disappearance of unirary distress and reduction in the size of the prostate were due to the diuretic action of their medication.

Our observations and conclusions are in line with those of Feinblatt and Gant[7], even though it remains to be demonstrated that the mechanism of action of the amino acid combination is identical with that of gelatine. Considerations of price, availability, palatability and

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