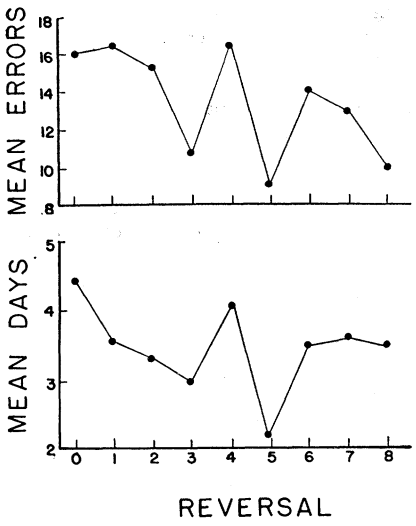

Fig. 1. Mean days and mean errors to criterion as a function of the ordinal number of reversals.

provement might be related to a "learning set" (*2*). The present report suggests that this type of learning set is very difficult to establish in the isopod. The isopod was chosen for investigation primarily because it can survive laboratory confinement for several months, a prerequisite for this type of work, which entails repeated testing of the same subject for a long period of time.

Seven isopods, *Armadillidium vulgare,* were trained on a total of eight reversals of a position habit (*3*). The apparatus consisted of a single-unit T-maze made of transparent plastic. The stem and the arms measured 3 cm in length and 15 mm in width. On the floor of each arm, 1 cm beyond the choice-point, was a grid made from copper wire. A direct current of about 7 μamperes charged the grid. On the floor of each end box was a thin sheet of sponge which was kept moist. A cover was provided for each end box, and the entire unit was painted black on the outside. Under these conditions of darkness and dampness, the isopod rarely attempted exit from the end box once it had made an entrance. Above the choice-point was mounted a 150-w light which constituted the noxious stimulus from which the animal attempted to escape. Prior to testing, all animals were given experience in a straight runway, the construction of which was similar to that of the T-maze.

All animals were required to choose one arm of the T-maze in order to gain access to the dark, moist, end box. Three animals were initially trained to choose the right arm, and the remaining four were trained to choose the left arm. Eight trials were given each day with an intertrial interval of approximately 20 seconds. If the correct arm was chosen, the animal was allowed to proceed to the end box. If, however, the incorrect arm was selected, a shock was delivered to the grid. Response to the shock almost invariably involved an abrupt withdrawal and a reversal of direction of locomotion. When the isopod reached the criterion of seven correct responses on one day, training on the following day was switched to the opposite position (first reversal). Training to this side was continued until the animal again met the criterion of seven errorless trials on one day. On the succeeding day, training was begun to the initially correct side (second reversal). Eight such reversals were given. During the course of the experiment, occasional shedding of the cuticula occurred in all animals. Performance prior to or following this condition did not appear to be impaired.

Figure 1 presents the mean number of days to criterion and the mean number of initial errors to criterion as a function of the ordinal number of reversals. Although a trend suggesting the formation of a learning set is apparent, statistical tests offered no evidence for interreversal improvement. That is, the difference between performance on the first four reversals (or first two reversals) and performance on the last four reversals (last two reversals) was not of sufficient magnitude to indicate that it did not occur by chance. Table 1 presents the individual data for all isopods involved in this study. It will be seen that only two animals (animals 1 and 9) showed definitive changes in performance with increasing reversal experience. It would seem that isopods do not show the characteristic improvement in reversal performance that has been demonstrated for the lower vertebrate forms, such as newts and turtles (*4*). Whether further reversal training would have resulted in a significant degree of improvement and whether a different invertebrate would reveal interreversal gains are problems for future work.

Table 1. Number of errors to criterion for each subject.

Animal No.	Reversal								
	0	1	2	3	4	5	6	7	8
1	24	11	7	0	19	8	9	2	6
2	0	37	1	26	15	14	9	14	4
3	0	47	6	13	17	2	13	24	12
5	34	5	8	17	17	14	6	28	19
7	17	1	16	1	5	3	7	4	11
8	19	1	3	8	7	10	28	10	8
9	17	13	66	9	34	13	25	8	9

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Uranyl Protoporphyrin: a New Uranium Complex

The purpose of this report is to describe a uranyl porphyrin compound that does not appear to be nephrotoxic to mice. Uranyl protoporphyrin is a hitherto undescribed complex of uranyl ion with protoporphyrin 9, the porphyrin ring of hemin.

Uranium in this work was assayed by radiation due to its decay and that of its daughters. Samples were counted in a scintillation well (gammas) and a flow counter (alphas and betas) and compared with appropriate standards. Uranyl protoporphyrin has been synthesized by addition of $UO_2Ac_2 \cdot 2H_2O$ or $UO_2(NO_3)_2 \cdot 6H_2O$ to aqueous protoporphyrin at *p*H 7. The complex precipitates at *p*H's of less than 5. Repeated washes with HCl at *p*H 2 soon lead to constant counts in the precipitate and none in the supernatant; uranyl acetate and nitrate are entirely soluble at *p*H 2. The porphyrin and the activity were entirely in solution after the *p*H had been raised to 7 with NaOH. Allowing the solution to stand at *p*H 7 overnight led to no precipitation of activity; unbound uranyl ion is quantitatively precipitated at this *p*H. Repeated precipitation and solution fails to remove the uranyl ion from the porphyrin ring if a *p*H higher than 8 is avoided. Apparently, sodium uranates form at higher *p*H's and thus destroy the complex. Autoclaving at *p*H 7 in 0.9 percent NaCl destroys the complex; autoclaving in water at the same *p*H does not. Excess citrate ion destroys or prevents the formation of the complex.

Uranyl protoporphyrin is more stable in acid than in alkali; indeed, it has been obtained by refluxing uranyl acetate and protoporphyrin in glacial acetic acid. The complex precipitates on addition of the reaction mixture to water.

Copper in the porphyrin ring is neither

displaced by UO_2^{++} nor does it interfere with formation of the complex. This leads one to believe that UO_2^{++} is not present in the center of the ring.

Uranyl ion forms complexes with many anions. It may be that uranyl ion is bound to protoporphyrin as a complex with the two propionic acid groups which are present on the ring. A uranyl ion might also form a complex with one propionic acid group from two different rings. The nature of this association is not yet clear; that it did not make uranyl ion freely available to the mouse is evident from the data presented in subsequent paragraphs.

It should be emphasized that an equilibrium mixture of U^{238} and its daughters, and not just U^{238} alone, was associated with the porphyrin.

Uranium-238 is an alpha emitter that decays to Th^{234}. This daughter element is a beta-gamma emitter with a half-life of 24.1 days. A sample of pure uranium has no gamma activity associated with it initially, but with time Th^{234} accumulates, and gamma activity increases, becoming constant when equilibrium is attained. Gamma activity from an aliquot of a sample of uranyl protoporphyrin which had been given to mice was found unchanged after 3 weeks. Determination of the amount of uranium present in this sample was performed by comparing its alpha and beta activity with that of a uranium standard. This assay agreed with that obtained by comparing gamma activity. These facts indicate that an equilibrium mixture of U^{238} and its daughters was associated with the porphyrin ring.

Seven white mice (Smith-Webster, 25 to 30 g each) received 9 times the LD_{50} (8 mg/kg) (*1*) of uranium as uranyl protoporphyrin by intraperitoneal injection. They were all alive and growing 30 days later.

Six mice received 3 times the LD_{50} and were all alive and growing 30 days later. Equivalent amounts of uranium as uranyl acetate or nitrate killed all 19 control mice in 3 days. In another experiment, four of five mice given 3 times the LD_{50} as uranyl protoporphyrin were alive and growing after 50 days (see Table 1). Histological studies of livers and kidneys of mice sacrificed 40 hours after they had received 3 times the LD_{50} of uranium as uranyl protoporphyrin showed no histological abnormalities. The control group, which received uranyl nitrate, had showed livers depleted of glycogen and kidneys showing marked tubular destruction typical of uranium poisoning. The uranium-damaged kidneys were obviously larger and paler than the kidneys of normal mice or of mice that had received uranyl protoporphyrin.

It is presumed that uranium as uranyl protoporphyrin has a fate in the mouse entirely different from uranium as the salt. One would expect excretion into the feces via the bile—that is, a fate similar to that of protoporphyrin (*2*).

It has been recognized since 1942 that porphyrins concentrate in tumors, in embryonic tissues, and in inflammatory tissue of human beings and animals (*3*). Since many metal ions complex with porphyrins, it seemed reasonable to expect uranyl ion to do so. One might thus have a method to localize uranium in tumors. Such concentration of uranium in tumors is desirable because neutron capture by U^{235} releases enormous amounts of energy over very short ranges in the form of fission particles. If fission were to take place in a tumor, the tumor might then be destroyed. Therapy of human tumors with neutrons from an atomic reactor is feasible (*4*). Farr *et al.* have treated glioblastoma patients with neutrons following tumor uptake of intravenously administered boron-10 as borate. In this reaction, the destructive effects are attributable to the alpha particles released. Administration of uranium salts to human beings has been found to be too nephrotoxic (*5*). A nontoxic uranium compound that might deposit in neoplastic tissue is therefore of interest.

Studies of the body dynamics of uranyl porphyrins in normal and tumor bearing animals are in progress. The use of porphyrins to prevent uranium toxicity or to decontaminate experimental animals is now being investigated. Use of uranium porphyrins in neutron capture therapy of human tumors after intravenous or local injection might be feasible if further uranium porphyrin toxicity studies are confirmatory (*6*).

Table 1. Survival of mice given equivalent amounts of uranium salts and uranyl protoporphyrin.

Amount of U^0 (LD_{50})*	As UO_2^{++}†			Uranyl protoporphyrin		
	Number of mice		Time (day)	Number of mice		Time (day)
	Survived	Dead		Survived	Dead	
3	0	6	3	4	1	> 50
3				6	0	> 30
6	0	6	3			
9	0	7	3	7	0	> 30

* The LD_{50} is 8 mg of U^0 per kilogram (intraperitoneal injection).
† As acetate or nitrate. This represents approximately 10 mg/kg, a dose far below that lethal for mice when these anions are administered as sodium salts (*7*).

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Nonphotosynthetic Light Requirement in Lemna minor and Its Partial Satisfaction by Kinetin

Gorham (*1*) was able to grow aseptic *Lemna minor* L. in darkness on minerals, sucrose, casein hydrolyzate, and yeast extract, but he found that growth was not maintained by minerals plus sucrose alone. He suggested that normal growth involved light-dependent processes other than carbohydrate production. This is a preliminary report (*2*) on the nonphotosynthetic light requirement.

Lemna minor was grown aseptically in 125-ml erlenmeyer flasks with 50 ml of Hutner's minerals and ethylenediamine tetraacetic acid (*3*), *p*H 6.3, or Gorham's minerals, plus 1 percent sucrose. Stocks kept at 23°C under 16 hours of fluorescent light (400 ft-ca) per day had a frond multiplication rate (MR) (*1*) of approximately 140. Experiments were started with at least 25 fronds per flask, and five flasks were used for each experimental treatment. Dim green light was used for counting and handling in "darkness."

After the plants have remained 5 days in darkness at 26°C, the frond multiplication rate declines to about 10. If a few minutes of red fluorescent light (*4*) are given at this time, the frond multiplication rate for the next 2 days is greatly increased. This effect of red light can be reversed by near infrared radiation (*4*), and is thus probably mediated by the system active in photoperiodism, deetio-