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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30 April 1957

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-

lation, and seed germination (*5*). In a typical experiment (with Gorham's minerals), 40 kerg/cm² (2 min) of red light induced a frond multiplication rate of 79 over the following 2 days; 2 minutes of near infrared radiation immediately following the red reduced the rate to 29, and the "dark" control rate was 8.

The frond multiplication rate in darkness drops again several days after treatment with light, but the promotion of the rate by light can be repeated. Continuous nonphotosynthetic growth on sucrose and minerals alone has now been maintained through five transfers for more than 75 days at an average frond multiplication rate of about 40 by giving 10 minutes of light (less than 200 kergs/cm²) every 3 or 4 days. Fronds so produced are white and bear very short roots. Other experiments (*6*) indicate that the slight growth in darkness implied by the frond multiplication rate of the controls in short-term experiments is in fact the result of residual light effects and of the green light used under "dark" conditions; it is not possible to demonstrate an absolute light requirement except over periods of weeks.

To determine what substances might substitute for light in this system, stock solutions were added to cultures that had been left in darkness for 5 days. Fronds were counted just before treatment and again 2 days after. Although the response to light is more rapid with Gorham's medium than with Hutner's, the latter was used because its trace-element levels and *p*H are more effectively buffered.

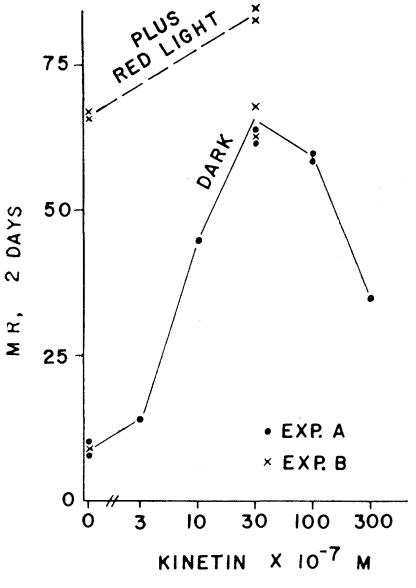

Fig. 1. Effects of kinetin and of red light on growth of *L. minor* in darkness at 26°C. Hutner's medium, *p*H 6.3, 1 percent sucrose. Values from two separate experiments. All treatments were given after 5 days in darkness. Red-light treatment (dashed line) was approximately 400 kerg/cm² (20 minutes). Each point represents a set of five flasks, with initial frond number of at least 140 per set. MR, frond multiplication rate.

Of the substances tried, only kinetin (6-furfuryl aminopurine) (Nutritional Biochemical Co.) and several related compounds produced effects similar to that of light. At the optimal level of $3 \times 10^{-6}M$ (0.645 mg/lit), the effect of kinetin was equal to that of a saturating red light dose, and the effects of kinetin and light together were much less than additive (Fig. 1). Several kinetin analogs (*7–9*) were also tested. The effect of $10^{-6}M$ 6-benzylaminopurine was equal to the optimal kinetin effect, and the effect of $3 \times 10^{-7}M$ 6-benzylaminopurine was 80 percent of the optimal kinetin effect. 6-Benzylthiopurine and 6(2-pyridylmethyl)-aminopurine gave 70 to 80 percent of the optimal kinetin effect at $10^{-5}M$, and both were inactive at 10^{-6} and $10^{-7}M$. 6-Hexylaminopurine and 6-hexylthiopurine were inactive at 10^{-7} and $10^{-6}M$, the former also at $10^{-5}M$. Adenosine at $10^{-4}M$ gave about 20 percent of the optimal kinetin effect, but it was inactive at 10^{-5} and $10^{-6}M$. Cobalt nitrate at $10^{-4}M$ (tested in Gorham's medium) gave 30 to 50 percent of the optimal kinetin effect, but it was inactive at lower levels.

The following compounds were completely inactive at the indicated molar concentrations: arginine, 5×10^{-4}; 4-chlorophenoxyisobutyric acid, 10^{-5} and 10^{-4}; cysteine, 10^{-5} and 10^{-4}; gibberellic acid (Merck), 10^{-7}, 10^{-6} and 10^{-5}; indoleacetic acid, 10^{-7}, 10^{-6}; and uridine, 10^{-5} and 10^{-4}.

These results are of interest in showing that low light doses, probably acting through the photoperiodic pigment system, can completely substitute for the complex organic supplements previously required for the heterotrophic growth of *L. minor*. The substitution of kinetin for light, at least in short-term experiments, confirms the view of Miller (*10*) that the light and kinetin effects are closely related. It seems likely that yeast extract in the complex medium cited served as a source of kinetin (*11*). These results also confirm those of the Wisconsin group (*10, 11*), of DeRopp (*12*). and of Gorton *et al.* (*13*) in showing kinetin activity at molar concentrations of about 10^{-7} to 10^{-4}. Reports have appeared (*7, 14*) of activity at 10^{-9} to $10^{-11}M$, but without comment on such unusual effectiveness. An optimum at about $10^{-9}M$ has been reported (*14*) for the same leaf-disk system that was found by Miller (*10*) to respond optimally at 10^{-5} to $10^{-4}M$.

Unlike the leaf-disk test, *L. minor* is more responsive to optimal kinetin and 6-benzylaminopurine than it is to cobalt. This, coupled with the inability of many other compounds to promote dark growth (see also *1*) suggests the use of *L. minor* for a rapid kinetin assay more sensitive and much more specific (*10*) than the lettuce-seed test (*8*). Finally, since wide differences in the dark growth of various species of Lemnaceae have been reported (*1, 15*), this group should provide valuable material with which to study the relation between light and the activity of kinetin and related substances.

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Stratigraphy of the Wisconsin Glacial Stage along the Northwestern Shore of Lake Erie

Six-year field investigations and laboratory studies of Pleistocene deposits north of Lake Erie (*1*) have provided information sufficient for preliminary conclusions on the Wisconsin stratigraphy of this area. (See Table 1 for the numbers of layers mentioned in text.)

An "early Wisconsin" *glacial cover* is represented by a sandy gray dolomitic till (No. 1) at Port Talbot. It is overlain by varved clay, also rich in dolomite. This glacial substage antedates the classical Wisconsin. Instead of using a new term, I prefer to apply the term *Early Wisconsin* to this post-Sangamon glaciation, as has been done already in Ohio (*2, 3*). This subage may correspond to the early Würm in Europe (*4*, p. 84.)

An interstadial interval followed the glacial retreat, with water level below the present one at first in the Lake Erie basin, rising slightly toward the end of the interval. Highly dolomitic silt, an erosion product of the adjoining till area, became deposited ½ mile south-