

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 pH are more effectively buffered.

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 substage 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 1/2 mile south-

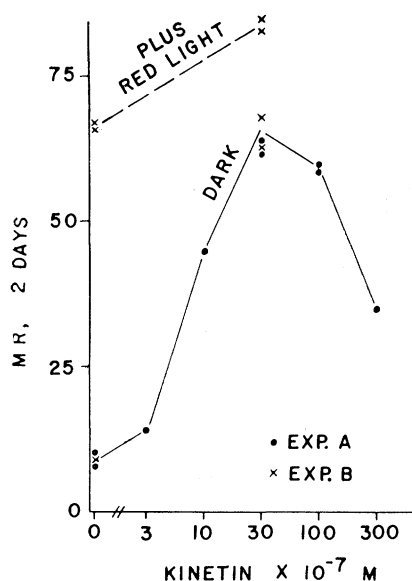


Fig. 1. Effects of kinetin and of red light on growth of *L. minor* in darkness at 26°C. Hutner's medium, pH 6.3, 1 percent sucrose. Values from two separate experiments. All treatments were given after 5 days in darkness. Red-light treatment (dashed line) was approximately 400 kerg/cm² (20 minutes).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

west of Port Talbot in shallow lake waters. This silt is intercalated by a lens of gyttja more than 39,000 radiocarbon years old (5). Results of pollen analysis indicate a tundra vegetation at the beginning of deposition of the silt, followed by forests of jack pine and spruce. Pollen spectrum of most of the Port Talbot interstadial (except for its thermal maximum, when pines with large pollen grains become abundant) suggests a cool climate with long, cold winters and short, but relatively warm, summers, similar to the present climatic conditions in the Gouin reservoir area in Quebec, lat. 48.5°N., long. 74°W. (6).

The Port Talbot interstadial is correlated with the cool climate peat ball in gravels at Amber, Ontario (7), greater than 34,000 years old (sample W-194, 8, p. 486) and with another cool climate interstadial deposit in the St. Lawrence lowland at St. Pierre, Quebec (9), older than 40,000 years (8, p. 485; 10, p. 958). If this correlation is correct, the ice sheet must have retreated north of Lake Ontario and the St. Lawrence River. This interval may correspond, at least partly, to a similar long and moderately warm interstadial in Europe that separated the early Würm from the main, or middle, Würm (4, pp. 82-83).

A readvance of glacier from the northeast blocked the outlet (the St. David's gorge?) of Lake Erie across the Niagara peninsula, the water level rose, and lacustrine calcareous clay was deposited in the proglacial lake. The glacier advanced at least as far as the central portion of the Lake Erie depression, depositing till No. 2 along its northern shore. This till is thin and discontinuous, and it probably represents a short-lived glaciation that was soon followed by a retreat or stagnation.

Fragments of larch wood and spruce (11) 24,600 to 28,200 C¹⁴ years old (see Table 1) have been found in the third layer of the lower till at Plum Point, 1 mile southwest of the Port Talbot interstadial exposure. Thus evergreen forests returned to the Lake Erie area after the retreat of glacier II. The end of this non-glacial interval occurred at least 14,000 years after the thermal maximum of the Port Talbot interstadial. If the glacial advance II that separates these two interstadials along the north shore of Lake Erie was of a short duration, it may remain unnoticed south of the lake. In such a case, the Port Talbot and the Plum Point interstadials, including the intervening glaciation II, may be considered as a single long interstadial interval farther south.

A different conclusion may be drawn if this stratigraphic problem is worked out from the north. Radiocarbon dates in agreement with those of Plum Point (24,600 and younger), are found south of Lake Erie (3, 12) but nowhere north

Table 1. Wisconsin events in southwestern Ontario along Lake Erie.

No. of glacial cover	Glacial and interstadial subages and principal events	Typical deposits observed	Radiocarbon investigations	
			C ¹⁴ age (yr) and reference	Description and location of sample
IV	<i>Main Wisconsin glaciation</i> Formation of moraines during an oscillatory retreat; lacustrine deposits in proglacial lakes. <i>Last glacial advance</i> over entire lake basin. <i>Retreat</i> of Erie lobe to the eastern portion of the lake.	Lacustrine clay, silt, sand, and gravel; clayey upper till (No. 4).	12,660 ± 440 (S-25, 16)	Evergreen log in a Lake Arkona or Whittlesey gravel bar at Ridgetown, Ont.
III	Formation of buried moraines during the retreat. <i>Main glacial advance</i> over entire area. <i>Nonglacial interval</i> in the Lake Erie basin.	Lacustrine clay and silt. Sandy lower till (No. 3); gravel.	 24,600 ± 1600 (L-217B, 5) 28,200 ± 1500 (L-185B, 5) 27,500 ± 1200 (W-177, 8, p. 485)	 Spruce wood Larch wood (Both samples in till No. 3, Plum Point, Ont.)
II	<i>Short-lived glacial advance</i> into the Lake Erie basin, preceded by a proglacial lake. <i>Port Talbot interstadial</i> . Lake Erie drainage via Lake Ontario and St. Lawrence River to Atlantic Ocean.	Gravelly lower till (No. 2); gravel; lacustrine clay. Silt; gyttja; silt.	 > 25,000 (S-7, 16) > 32,000 (W-100, 18) > 38,000 (L-185A, 5) > 39,000 (L-217A, 5)	 Gyttja at Port Talbot, Ont.
I	<i>Early Wisconsin glacial</i> subage with a proglacial lake during the retreat of the glacier.	Varved clay; sandy lower till (No. 3).		

or northeast of Plum Point. This negative evidence suggests (though it does not prove) that forests did not readvance farther northeast. Apparently the ice sheet did not retreat as far north as it did during the Port Talbot interstadial. Lithologic similarities between the tills No. 2 and 3 are also in favor of considering the glacial advance II as the beginning of the main Wisconsin glaciation.

A readvance of glacier from the northeast along Lake Erie deposited a thick layer of sandy gray calcareous till (lower till No. 3, generally called the "lower till" in previous publications).

Fabric studies in till No. 3 reveal gradual changes of the glacial movement: first it was from the southeast, then from the east northeast, and again from the southeast toward the end of deposition. The northeast-southwest movement has been recorded at widely spaced points. Heavy mineral investigations (13) suggest this as the principal regional flow during the period of glacial cover III. This flow disregards the east northeast-west southwest trend of the Lake Erie and Lake Ontario basins as guiding factors, thus possibly corresponding to the maximum of the Wisconsin glaciation.

A considerable glacial retreat followed, probably as far as the eastern portion of the lake, and lacustrine clays and silts became deposited through the central and western portions of the lake

basin. Most of them become eroded during the following advance of the Erie lobe and were incorporated in the upper till (No. 4), making it more clayey than No. 3.

The following Wisconsin glacial advance IV that covered the entire Lake Erie basin (also in northern Ohio, 14), deposited the already mentioned brown upper clayey till (No. 4). A great deal of the upper till was laid down during the retreat of the glacier, while it oscillated back and forth, forming morainic ridges. Lacustrine deposits of the proglacial lake became intercalated between two, or even three, layers of till, where the glacier advanced over them. Elevations of abandoned shore lines indicate that the retreat of the glacier, which deposited the upper till, began during Lake Maumee time. Radiocarbon dates of samples W-33 and W-71 (15, p. 469), L-217B (5), and S-25 (16) suggest that the time interval involving deposition of the tills Nos. 3 and 4 was less than 11,000 years. This means that the main Wisconsin glaciation, including even one considerable retreat, was of a relatively short duration in the central and western portion of Lake Erie, if the glacial advance II is not included.

A brief summary on the proposed Wisconsin stratigraphy for the area studied is given in Table No. 1, with temporary local terms, until it is possible to correlate the terms beyond any doubt with generally accepted units of Wis-

consin substages. A possibility is not excluded that till of the glacial advance II was the basal member of the main glacial cover III, as it was once assumed before (17), with the Plum Point wood deriving from the closing time of the Port Talbot interstadial interval.

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Effect of 3-Amino-1,2,4-triazole on δ -Aminolevulinic Acid Dehydrase Activity

The conversion of δ -aminolevulinic acid to porphobilinogen is catalyzed by an enzyme (δ -aminolevulinic acid dehydrase) described by Gibson *et al.* (1) and Shemin *et al.* (2). The activity of this enzyme is reduced in the livers of tumor-bearing animals; in the livers of C57 black mice, it is much lower than it is in the livers of other strains and is not reduced by a rapidly growing, transplanted tumor (3). These findings parallel those previously described for hepatic catalase (4). This is of particular interest, for catalase is a porphyrin-containing enzyme and δ -aminolevulinic acid dehydrase is involved in porphyrin synthesis. There is no evidence at the moment, however, that in the livers of tumor-bearing animals there is a decreased ability to synthesize protoporphyrin and hence catalase.

Table 1. Effect of 3-amino-1,2,4-triazole (AT) on activity of δ -aminolevulinic acid dehydrase in the livers of DBA mice. The mean value for liver in 47 normal DBA male mice was 47.4 ± 2.6 units (standard deviation). The mean value for liver in six normal DBA female mice was 46.7.

Item	Units (3.15×10^{-2} μ mole) of porphobilinogen per hour, per gram (wet weight) at various times (hours) after administration of AT										
	1	1½	2	3	4	8	12	24	32	36	48
<i>Dose of AT, 1000 mg/kg</i>											
Males	45.2		40.9	35.5		33.0	36.1	37.7	38.8	41.4	
Males	47.3		40.4*	34.5*		32.6	38.8	29.6	38.8	43.1	
Males	47.3					33.4	38.8	25.8			
Mean	46.6		40.6	34.8		33.0	37.9	31.0	38.8	42.3	
Females						29.1	29.1				
Females						29.9	29.2				
Females						29.6	29.9				
Mean						29.5	29.4				
<i>Dose of AT, 500 mg/kg</i>											
Males								30.8			
Males								31.2			
Males								31.2			
Mean								31.1			
Females	47.1	45.3	48.8		37.8	37.0	22.5	33.0			44.4
Females	44.9	45.3	44.5		34.4	41.0	34.4	36.1			49.7
Females	41.8	44.9	44.5			41.0	37.9	34.3			49.7
								35.7			
								38.3			
								37.4			
Mean	44.6	45.2	45.9		36.1	39.7	31.6	35.8			47.9

* These values were obtained from a homogenate of two livers. All other values were obtained from a homogenate of one liver.

Plant growth is inhibited by 3-amino-1,2,4-triazole, apparently through interference with chlorophyll synthesis (5). It was later observed that this compound lowered both plant and animal catalase (6). In rats it reduced hepatic and renal catalase activity levels, but not that of red cells, thus producing an effect similar to that observed in tumor-bearing animals.

The ability of 3-amino-1,2,4-triazole to affect two different porphyrin-containing compounds suggests a possible interference with porphyrin synthesis or inhibition of the activity of these porphyrin-containing compounds. The present study was carried out to determine whether the parallel variations of hepatic δ -aminolevulinic acid dehydrase and catalase could be caused by 3-amino-1,2,4-triazole.

DBA mice were injected intraperitoneally with an aqueous solution of 3-amino-1,2,4-triazole. Hepatic δ -aminolevulinic acid dehydrase activity was determined by the method previously described (1).

Table 1 shows that 3-amino-1,2,4-triazole reduces the level of hepatic δ -aminolevulinic acid dehydrase activity within 3 to 4 hours. Thus tumors and 3-amino-1,2,4-triazole are capable of causing a decrease in activity of both hepatic δ -aminolevulinic acid dehydrase and catalase.

There are three possible explanations

for the effect of 3-amino-1,2,4-triazole on the two enzymes: (i) the triazole or metabolic derivative might react with a chemical group common to both enzymes, resulting in inhibition of their activity; (ii) it might also interfere with porphyrin synthesis in the liver, resulting in a lowered catalase activity level, and (iii) it might interfere with a metabolic pathway necessary for the synthesis of both enzymes. The only exception to the parallel variation of these enzymes is seen after administration of Sedormid, which produces porphyria. Following administration of Sedormid, hepatic catalase activity decreases (7), whereas hepatic δ -aminolevulinic acid dehydrase activity increases (1).

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