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* Present address: department of plant biochemistry, Citrus Experiment Station, University of California, Riverside.

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"Clock" Controlled Activity Rhythms in the Fruit Fly

Recent studies in this laboratory on the daily rhythm of eclosion (*1*) in fruit flies of the genus *Drosophila* have shown that it is controlled by an interval timing device—or "clock"—that is temperature independent. It is well known that the locomotor activity of *Drosophila* adults in the field also exhibits a clear daily rhythm; activity is maximal in the evening just before sunset. A question of prime importance is whether the active period is determined by strictly exogenous factors such as light intensity, relative humidity, or temperature, or whether an internal biological timing device is involved.

Experiments in the field (*2*) have shown that temperature and humidity do not play a major role in the determination of active periods in *Drosophila.* However, it has been shown that the time and duration of active periods are correlated with definite ranges of light intensity. During the late afternoon, maximum activity occurred when the light intensity ranged between 100 ft-ca and 15 ft-ca.

To study the locomotor activity of *Drosophila* in the laboratory, a small cylindrical lucite chamber was devised with a grid of fine wires on the inside walls. Every other grid wire was connected to a common terminal. When a fly walks about in the chamber, it cannot help but short-circuit any two adjacent wires. This short circuit was detected with a high gain amplifier, and contacts per hour were translated into ink marks by an operations recorder. The virtue of this system lies in the ability of the instrument to record activity of flies under wide ranges of light intensity or even in total darkness.

Figure 1 shows the pattern of activity in *D. robusta* over a period of 7 days. Six male and six female flies were placed in the chamber with food, and the humidity of the chamber was maintained at a high level. The temperature was rigidly controlled at 21°C. For the first 4 days the flies were subjected to 12 hours of bright light (25 ft-ca incident reading) alternating with 12 hours of absolute darkness. At the onset of the fifth day, a very dim light (less than 1 ft-ca incident reading) was substituted for the bright lamp. This dim light was left on for the remainder of the experiment, and the alternating dark and bright light cycle was abolished. It can be seen that the active periods for the first 4 days were restricted to a few hours before the onset of darkness and that activity fell off abruptly when the lights suddenly went out. During the last 3 days, when the flies were in constant low light, the active periods spread to some degree, but the mean remained in phase with the previous 4 days.

These data strongly suggest that a biological "clock" is operative in determining the active periods for the flies. The absolute amount of activity declined during the last 3 days of the experiment; this was probably the result of the depressing effect of dim illumination. Previous experiments in which flies were subjected to several days in absolute darkness bear this out. The amount of activity in total darkness was so slight that no rhythm could be detected.

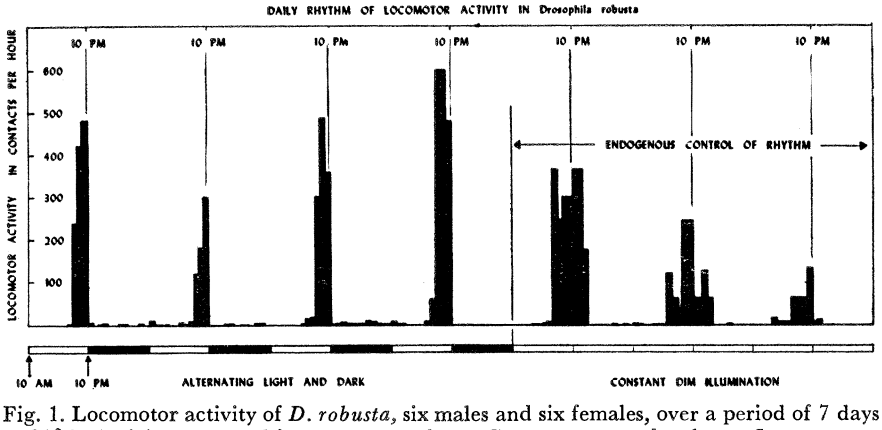

Fig. 1. Locomotor activity of *D. robusta,* six males and six females, over a period of 7 days at 21°C. Activity measured in contacts per hour. Contacts are made when a fly steps on a pair of grid wires. Under conditions of alternating light and dark, the photoperiods are 12 hours with "dawn" at 10 A.M. E.S.T.

Besides the fact that a rhythm of activity persists under constant conditions of illumination, it is also interesting to note the pattern of activity during the first 4 days of alternating light and dark. The onset of the evening peaks occurred quite regularly at about 7 P.M. without reference to any gradual fluctuation in light intensity, temperature, or relative humidity as would exist in the field. It appears then, that under alternating light conditions where the lights are turned on and off abruptly, the commencement of activity is under "clock" control.

SHEPHERD K. DE F. ROBERTS
Graduate College, Department of Biology, Princeton University, Princeton, New Jersey

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11 April 1956

Hardness of Substances in the Ideal State

The relation proposed by Tabor (*1*) between Vickers indentation hardness, H_v, and ultimate tensile strength, T_u, of an ideal plastic material, $T_u = 0.33\ H_v$, may be used to calculate a hardness of the material in the ideal state since methods are known for calculating the "ideal ultimate tensile strength." The results of such calculations are interesting even though they may be considered only as approximations.

Griffith (*2*) has measured fracture strengths as large as 9×10^5 lb/in.^{2} for fine glass fibers as compared with a theoretical strength of 1.6×10^6 lb/in.^{2} and a measured strength of 2.49×10^4 lb/in.^{2} for rods of the same glass. The fine fibers have a strength of 633 kg/mm^2 and therefore a calculated Vickers hardness of 1900. This is equivalent to the hardness of corundum or a Moh's hardness of 9 as compared with about 4.5 for ordinary glass.

Herring and Galt (*3*) have measured the elastic properties of single perfect "whiskers" of tin and found a yield stress of 200,000 lb/in.^{2} The application of Tabor's equation yields a Vickers hardness of 141 for the whisker as compared with about 21 for hard drawn tin.

Zwicky (*4*) has calculated a strength of 200 kg/mm^2 for sodium chloride. The calculated Vickers hardness is 600, equivalent to a Moh's hardness of 5. The

Moh's hardness of massive sodium chloride is 2.

Unfortunately, no data are available on the tensile strength of diamond, but, judging from the examples I have given, the ideal hardness should be at least several times the observed value. Tabor shows measured values for the indentation hardness to be 8000 to 10,000 kg/mm² for diamond. This yields a tensile strength of 4 to 5 × 10^6 lb/in.², a truly remarkable value.

It seems obvious that greatly improved abrasives might be made from relatively inexpensive substances such as aluminum oxide and silicon carbide. These materials in ideal form might be harder than diamond. The attempt to make substances in ideal form for abrasives has several attractive features as compared with an attempt to make massive pieces ideally hard, or with attempts to make superstrong metals: (i) large pieces are not required—small grains can be used as an abrasive powder; and (ii) the consequences of transformation to the non-ideal form are less severe.

ARTHUR E. NEWKIRK
Chemistry Research Department, General Electric Research Laboratory, Schenectady, New York

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Lactic Dehydrogenase Activity of Serum in Mice with Transplantable Leukemia

The finding that the serum activity of lactic dehydrogenase (LD) is elevated during acute and chronic human leukemia (*1*) prompted the study reported here of the alterations of serum lactic dehydrogenase (SLD) during the course of mouse leukemia (*2*). The purposes of this study were (i) to ascertain the relationship, if any, between SLD and the course of experimental mouse leukemia and (ii) to determine whether there was any correlation between SLD and the various types of experimental transplantable mouse leukemia.

Four lines of leukemia in mice were examined to determine whether alterations in the activity of SLD occurs during the course of the disease. Lines I, 8174, and 82B are acute stem-cell leukemias probably lymphocytic in character. Line C1498 arose spontaneously as a myeloid leukemia. Line-I leukemia, carried in C58 mice, was supplied to the Sloan-Kettering Institute by E. C. MacDowell and has since been kept in passage in F1 (C58 by BALB) mice by the intraperitoneal inoculation of 10 to 20 million cells from minced spleen of leukemic donors. The survival time of the inoculated animals is 4 to 7 days.

Table 1. Serum lactic dehydrogenase activity in mice inoculated intraperitoneally with a suspension of specified leukemic cells.

Days of inoculation	SLD* (units/ml)			
	82B	C1498	I	8174
1	1000	1200	3200	2500
2	1900	1600	6200	2900
3	3000	3300	24,000	5300
4	5400	5500	no survivors	7100
7	3700	3200		6300
8	2900	4700		10,000
9	3700	7200		15,000
10	3900	5200		18,000
11	3200	6200		40,000
15	11,000	14,000		25,000†
16	20,000	44,000†		no survivors
17	no survivors	no survivors		no survivors
Control mice	1100 ± 300 (F1)‡	1200 ± 260 (C57 BL/6)§	1100 ± 300 (F1)‡	1100 ± 300 (F1)‡

* Average of two individual determinations. † One mouse only. ‡ Average of determinations on 23 mice. § Average of determinations on 21 mice.

Leukemia 8174 arose spontaneously in an F1 (C58 by BALB) ex-breeder in the laboratory of Joseph Burchenal. It was converted to ascitic form and is passed in the same strain of mice by the intraperitoneal inoculation of ascitic fluid containing approximately 20 million cells. Survival time is 12 to 16 days. Leukemia 82B also originated in an F1 (C58 by BALB) ex-breeder in Burchenal's laboratory. It is transferred into the same strain of mice by the intraperitoneal inoculation of approximately 10 million cells from minced tumor of leukemic donors. Survival time is 12 to 16 days.

Leukemia C1498 arose as a spontaneous myelocytic leukemia in C57 BL/6 mice. At the present time, however, it is not considered histologically typical of that type of leukemia. This strain was received from Jackson Laboratory, Bar Harbor, Maine. It is transferred in mice of C57 BL/6 strain by inoculation of approximately 10 million cells from minced tumor of leukemic donors. Survival time is 10 to 16 days.

A designated number of mice were inoculated with each type of leukemia. An equal number of untreated control mice of C57 BL/6 and F1 mice were set aside. At daily intervals, two mice from each leukemic group and two from each control group were bled from the branchial artery with syringes that had been moistened with saline before use. The serum was separated from each sample, and individual analysis of SLD was made. The average of each two determinations was recorded.

Serum lactic dehydrogenase activity was measured by the method previously described (*1*). The reaction was followed in a Beckman model DU spectrophotometer using a tungsten light source. The activity is expressed as units per milliliter of serum, per minute. One unit equals a decrease in optical density of 0.001 per minute, per milliliter under the conditions described. The mean value of SLD, based on individual determinations on the serum of 23 normal F1 (C58 by BALB) mice, was 1100 ± 320. The mean value, based on the determination of the serum of 21 normal C57 BL/6 mice, was 1200 ± 260.

In mice inoculated with line-I leukemia, which is associated with the shortest survival time of those in this study, an increase in SLD was apparent within 24 hours after injection. Terminally, SLD rose to 24,000 units. Those mice inoculated with the cells of leukemia 8174 showed a slower but progressive rise in SLD activity, reaching a maximum terminally. Inoculation with C1498 and with 82B leukemia resulted in similar increments of SLD activity (Table 1).

The mechanism of the elevation of SLD during experimental mouse leukemia and human leukemia has yet to

Table 2. Comparison of lactic dehydrogenase activity of selected tissue homogenates from control and leukemic mice.

Tissue	Lactic dehydrogenase activity (units/gram wet tissue)	
	Untreated control*	Leukemic†
Serum	760	60,000
Lymph node	80,000	280,000
Liver	130,000	280,000
Spleen	100,000	230,000

* F1 mouse. † F1 mouse inoculated 16 days previously with leukemia 82B.