fusion-controlled, and this is the situation usually applicable to most systems. When $1/P$ is much greater than h/D, the dissolution rate is interfacially controlled. In order to establish the inhibitory effect of lecithin upon cholesterol monohydrate dissolution in cholate or taurocholate solutions, the dissolution rates, diffusion coefficients, and solubilities of cholesterol were determined independently in the mediums (*6*) (see Table 1). By Eq. 1, values for $(h/D + 1/P)$ were calculated by use of the measured J/A, C_s, and D. The magnitude of h was determined from the benzoic acid dissolution rate in 0.01*N* HCl, for which the rate was established to be diffusion-controlled (*7*). As shown in Table 1, $(h/D + 1/P)$ values about three times larger than h/D were found (*8*) for the dissolution of cholesterol monohydrate in taurocholate and cholate solutions not containing lecithin. However, when lecithin was present, values for $(h/D + 1/P)$ that were almost 18 times (*9*) larger than those for h/D were found. These results are in accord with an effective permeability coefficient P for the cholesterol interfacial transport of around 1.5×10^{-5} cm sec^{-1}. Lecithin effects of about the same magnitude have been observed for the dissolution of human gallstones in bile acid–lecithin mediums (*10*).

A similar lecithin effect has been observed in studies (*11*) of the transport of cholesterol from aqueous bile acid–lecithin solutions into hexadecane. It was found that the sterol transport is interfacially controlled, and the presence of lecithin markedly reduced the interfacial permeability coefficient. Oil-to-aqueous permeability coefficient values of around 10^{-7} cm sec^{-1} were found with taurocholate-lecithin mediums of the same composition as those shown in Table 1. Considering the fact that the area term in Eq. 1 is not the true area or microscopic surface area for cholesterol crystal dissolution, the real difference between the crystal-solution transport rates and the oil-aqueous transport rates of sterols should be somewhat smaller (*12*).

As demonstrated in this report, large interfacial barriers may be present during the dissolution of cholesterol monohydrate crystals in lecithin–bile acid solutions (*13*). These barriers are of such magnitude that they may be important in dissolution of cholesterol gallstones in vivo. Thus, the presence of such barriers may explain why the rates of gallstone dissolution in vivo observed by Danzinger *et al.* (*2*) are lower than those predicted by diffusion-controlled dissolution models (*14*), and the counteracting or the elimination of these barriers by appropriate measures may constitute a clinically significant goal.

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6. The following methods and materials were used. (i) Dissolution rate: The three-time recrystallized cholesterol monohydrate crystals were pressed into a pellet, with a surface area of 1.267 cm^2, and held firmly in a die. This die was then mounted at the bottom of a water-jacketed cylinder, with the pellet facing a stirring paddle inserted at the top of the cylinder. The stirring speed was maintained at 150 rev/min during dissolution. To measure the dissolution rate, the amount of cholesterol dissolved in the solvent was analyzed against time. (ii) Solubility: About 100 mg of radioactive cholesterol monohydrate was introduced into 20 ml of solvent medium in a 50-ml volumetric flask and was simultaneously flushed with nitrogen gas. The flask was shaken by a wrist-action shaker in a water bath maintained at 37°C. A 5-ml sample was filtered and assayed radioactively for cholesterol. Solubility of cholesterol monohydrate in the medium was obtained when the concentration of cholesterol in solution reached a constant. (iii) Diffusion coefficient: Measurements were made at 37°C in a diaphragm diffusion cell and by a method similar to that described by Keller *et al.* [*J. Phys. Chem.* **75**, 379 (1971)], except that the two reservoirs were separated by double silver-filter membranes (pore size, 1.2 μm; Selas Flotronics). The lower reservoir had a capacity of 3.4 ml and was completely filled with the solution of radioactive cholesterol during operation. The upper reservoir had the same capacity and was filled with solvent medium. Stirring speed in both reservoirs was maintained at 150 rev/min. A solution of 0.1*N* KCl (diffusion coefficient, 2.41×10^{-5} cm^2 sec^{-1} at 37°C) was used as a standard in the measurement. (iv) Source and preparation of cholate, taurocholate, and lecithin: Sodium cholate was used as received from Schwarz/Mann, Orangeburg, New York. Sodium taurocholate was prepared by the method of Pope [J. L. Pope, *J. Lipid Res.* **8**, 146 (1967)]. Egg lecithin was prepared by the method of Small *et al.* [D. M. Small, M. Bouyes, D. G. Gerivichian, *Biochim. Biophys. Acta* **125**, 563 (1966)] except that commercial egg lecithin of purified grade (Schwarz/Mann) was used as starting material.
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8. More recent experiments in which the rotating disk dissolution method was used confirm the existence of interfacial barriers even when lecithin is absent.
9. The 18-fold reduction factor was based on the assumption that the Nernst theory is applicable for the conditions prevailing in the dissolution experiments. Because of the empirical nature of the Nernst theory, it is perhaps instructive to consider other theories involving both diffusion and convection, such as Levich's theory on laminar flow past a plane [V. G. Levich, *Physicochemical Hydrodynamics* (Prentice-Hall, Englewood Cliffs, N.J., 1962), pp. 60–70] and Danckwerts's theory on eddy diffusion [P. V. Danckwert's, *Ind. Eng. Chem.* **43**, 1460 (1951)]. While the Nernst theory predicts a $D^{1.0}$ dependence for the dissolution rate, Levich's theory and Danckwerts's theory predict $D^{2/3}$ and $D^{1/2}$ dependences, respectively. Consideration of the lecithin effect on the cholesterol monohydrate dissolution rate in terms of $D^{2/3}$ and $D^{1/2}$ dependences leads to 40-fold and 55-fold reductions from the maximum convection or diffusion controlled dissolution rates, or both. Thus the $D^{1.0}$ dependence analysis clearly represents a conservative estimate of the importance of the lecithin effect upon the dissolution rate.
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12. Preliminary experiments (S. Prakongpan, F. Young, W. I. Higuchi, in preparation) with dissolution rates of single crystals of cholesterol monohydrate show that the intrinsic interfacial barrier permeability coefficients might be four to eight times smaller than those obtained with the present pellets when Eq. 1 is used and A is equated to pellet cross-sectional area.
13. Preliminary studies have shown that the bile acid–lecithin-cholesterol micelle participates in the rate-determining step at the crystal-solution interface (W. I. Higuchi, V. Surpuriya, S. Prakongpan, F. Young, *J. Pharm. Sci.*, in press).
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19 July 1972

Gas Exchange in Dry Seeds: Circadian Rhythmicity in the Absence of DNA Replication, Transcription, and Translation

Abstract. *A 24-hour rhythm of gas exchange has been detected in dry onion seeds. The rhythm persists in constant conditions, and its period appears to be independent of temperature. Since DNA replication, transcription, and perhaps translation do not occur in this quiescent state, it is concluded that the basic oscillation that defines circadian rhythmicity does not derive directly from these processes.*

Perhaps the most frustrating aspect of work with circadian rhythms is our lack of knowledge concerning the nature of the basic oscillation that ultimately determines the period of the fluctuation. In recent years attention has been focused on transcriptional and translational processes as the site of the basic oscillator (*1*). This line of reasoning has culminated in the "Chronon" concept (*2*), which postulates that the genome has evolved un-

der conditions of cycling light and dark to act in concert with that 24-hour cycle. The data given here describe a circadian rhythm of gas exchange in dry, quiescent onion seeds, under conditions where no DNA replication or transcription, and perhaps no translation, occurs.

Standard Warburg respirometry was used for measurements of gas exchange. Approximately 2 g of onion seeds (var. Evergreen Bunching) were layered in the bottom of each Warburg flask, which produced a layer approximately two seeds thick with no shadowing. The meniscus was equilibrated at 150 units for each manometer, and at least three thermobarometers were averaged for control fluctuations. Each determination involved at least six replicate flasks for a particular category, and curves were determined with and without KOH for CO_2 uptake. All experiments reported here were performed with KOH present to absorb CO_2, although no difference was observed when KOH was absent. It is assumed that the gas measured is O_2, and that no CO_2 is released into the atmosphere (*3*).

Various storage conditions were tried (continuous dark, light-dark cycle, light-dark cycle with cycling temperature, sealed in polyethylene, and others) and no significant differences were seen. Experiments were run in a constant-temperature room with the Warburg apparatus set for 15°, 25°, or 35°C. The standard light-dark (L : D) cycle was 12 : 12, with changes to light at 8:00 a.m. and to dark at 8:00 p.m. Light was supplied by the lamp array of the Warburg apparatus, and each experimental group was phased by two L : D cycles of 12 : 12 before exposure to constant conditions.

Figure 1A shows a typical 3-day scan under normal L : D conditions. At 25°C the rate of gas uptake by the seeds approaches 20 μl per gram of seed per 4-hour period, increasing with light exposure, and decreasing with the advent of dark. This involves a temperature effect, at least in part. Onion seeds are black, and absorb considerable radiant energy when illuminated (*4*).

Under constant dark conditions, the curve of gas uptake with time shows a distinct maximum at about 8 p.m., a secondary maximum at 8 a.m., and minima at 12 noon (primary) and 12 midnight (secondary). Figure 1B shows this relationship at 25°C. At this temperature, maxima may exceed 5 μl/g per 4 hours and minima approach zero.

Figure 1C shows a typical series of experiments in which two phasing cycles were used, with transition to constant dark conditions at 8 p.m. of day 2. The curves demonstrate the enhanced gas uptake in light, as well as the persistent 8 p.m. maximum and 12 noon minimum under constant dark conditions for 3 days following transition to constant conditions. The experiments were run at 15°, 25°, and 35°C, and show a Q_{10} of approximately 2 for amplitude differences. Period, however, is apparently independent of temperature. A 4-hour measuring interval was used here, but for the sake of clarity is not shown. Only the primary maxima and minima are represented. The substructure of each 24-hour period would mimic that shown in Fig. 1B. With the measurement

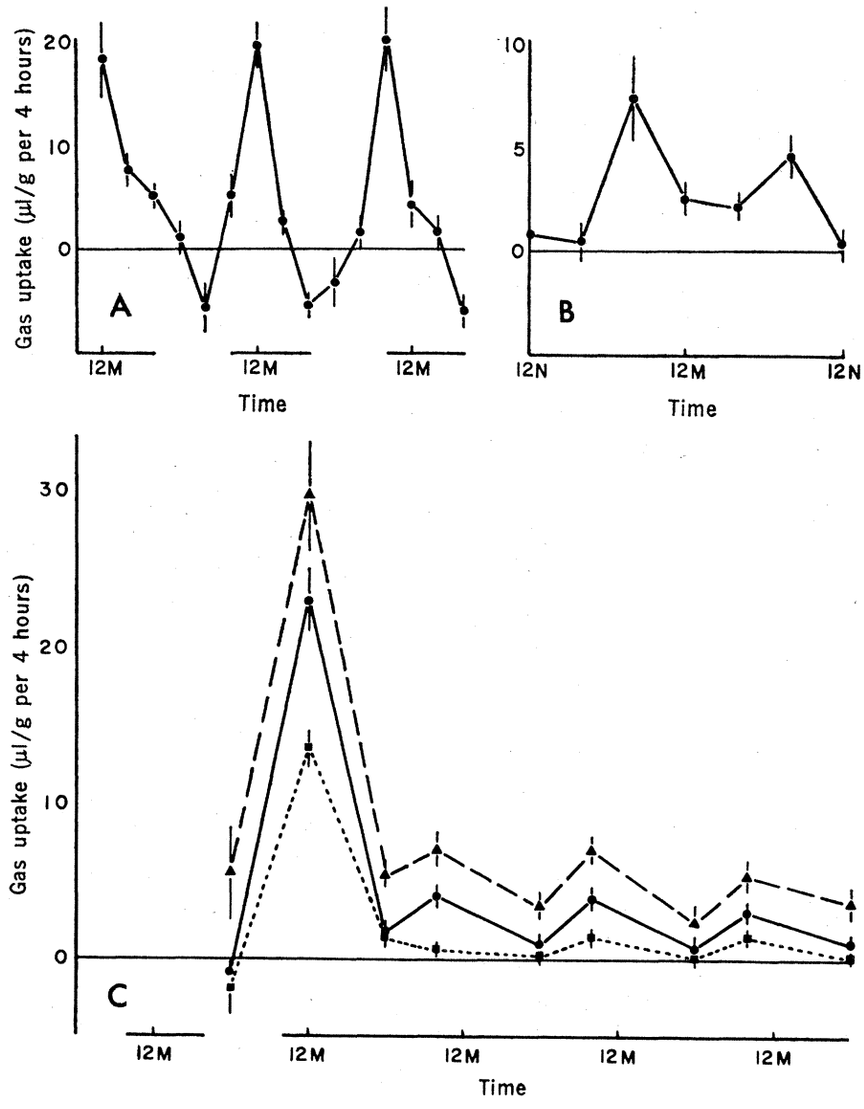

Fig. 1. (A) Gas exchange for seed under normal L : D cycle, 12 : 12. Temperature 25°C; approximately 2000 seeds. Enhanced uptake probably due to increased temperature of seeds. Continued decrease in uptake rate in the first light period probably indicates seeds are still adjusting to the cycle. Increase and decrease in subsequent cycles is phased exactly with change in illumination. Dark abscissa indicates dark period; *12M* is midnight, *12N* is noon. (B) Gas exchange for seeds under constant conditions. Temperature 25°C; approximately 2000 seeds. Only gas uptake, not release, is noted under constant conditions. (C) Three experiments, 15°, 25°, and 35°C, approximately 2000 seeds each, with two normal L : D cycles for phasing, then constant dark. With the transition to constant conditions, the 8 p.m. maximum and the *12N* minimum appear and are seen to persist for at least three cycles. The 8 a.m. and *12M* secondary maxima and minima are present, but for clarity, are not shown. Amplitude differences are in agreement with a Q_{10} of about 2. The Q_{10} for the period must vary within the limits of 0.83 to 1.17, certainly not consistent with physiological temperature dependence. Triangles, 35°C; circles, 25°C; and squares, 15°C.

interval used, Q_{10} must vary within the limits of 0.83 to 1.17.

In sum, the experiments demonstrate the existence of a rhythm of gas uptake in dry seeds which persists under constant conditions, and whose period appears to be independent of temperature. The rhythm therefore satisfies two prime requisites of circadian character and is assumed to be circadian. Allowing for the 4-hour interval, the curves resemble those already established for potato tubers and germinating beans (*5*).

Previous studies with this system have shown that DNA, RNA, and protein synthesis are initiated at discrete points following exposure to germinative conditions. The DNA replication is initiated at approximately 36 hours (*6*), RNA synthesis appears at 12 to 18 hours (*7*), and protein synthesis is detected at 12 to 18 hours of germinative conditions (*8*). Replication, transcription, and translation are not detectable in the quiescent seed. These results are in accord with those of others (*9*), which demonstrate a lack of genomic transcription in dormant seeds (*10*). Further evidence may be marshaled which shows the same sort of sequential initiation of synthesis in comparable dormant phases of other organisms (*11*). Additionally, visual evidence from electron microscopy supports the conclusions by the exclusive or near-exclusive occurrence of ribosomes in the monosomic rather than the polysomic form (*12*).

Finally, it should be emphasized that this is a demonstration of a circadian rhythm in a dormant system. The only process we know to be occurring in the dormant seed is the minimal level of respiration shown here. Since no other sequences occur to interfere with this single rhythm, one of the disadvantages of active systems is obviated. In fact, since only this phenomenon is known to occur in the dormant seed, it presents a logical argument for consideration of energy cycling as the basic oscillator. It occurs in all living cells and is coupled directly or indirectly to all metabolic pathways of the organism.

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3. This is not to say no CO_2 is released. It may simply be immediately recycled without leaving the tissue. It is conceivable on the other hand, that a mechanism (polyphenol oxidase or something akin to luminescence, for example) which does not yield CO_2 is involved.
4. Preliminary measurements indicate that seed temperature may increase to 40° or 45°C when held at constant temperature (25°C) and illuminated. Seeds heated to 120°C for 48 hours, allowed to recover for 24 hours, and then measured for gas uptake show greatly increased uptake rates which gradually (over a period of several days) decrease to normal levels. The rhythmic fluctuation is still present, which in itself would contraindicate an oscillation based on transcription-translation processes.
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10. Since the pathway of presumed gas uptake is unknown in these dry seeds, no prediction concerning the amount of transcription-translation necessary is possible. Marcus, the authority in the field, concludes "no genomic transcription." Electron microscope examination of cells from dry onion seeds shows all ribosomes to be monosomic, uniformly adsorbed to the surface of the spherosomes. No aggregates which might represent polysomes are found.
11. See, for example, *Dormancy and Survival* (proceedings of the 23rd symposium of the Society for Experimental Biology, Norwich, England), H. W. Woolhouse, Ed. (Academic Press, New York, 1969).
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13. I thank Drs. Margaret W. Bryant, D. S. Van Fleet, Hope Ritter, Goeff Magnus, and Burlyn Michel for helpful advice and discussions in the conduct of this work.

12 June 1972; revised 1 September 1972 ■

Mercury Concentrations in Recent and Ninety-Year-Old Benthopelagic Fish

Abstract. *Several species of bottom-dwelling fish from 2500 meters with similar feeding habits had mercury concentrations that differed by an order of magnitude. Within one species there was a correlation between size and concentration, with the larger individuals having mercury concentrations as high as 0.8 part per million (wet weight). The mercury content of the water in the deep-ocean habitat of these fish appears not to determine the mercury content of a particular fish; species-specific factors and size do appear to determine this concentration. The species-specific variation between the recent fish also existed between the same two species in specimens collected 90 years ago from a depth of 2000 meters, and a 90-year-old specimen fit closely the size-concentration regression curve for nine recent individuals of the same species.*

A knowledge of the concentration of mercury in the biological reservoirs of the ocean is necessary to understand the natural biogeochemical flux of this element through the marine ecosystem and to predict the consequences of cultural changes in the global flux. We measured the Hg concentration in one biological reservoir, the bottom-dwelling deep-sea fish, to provide one portion of the information needed for a predictive model.

Interest in the Hg concentrations in deep-sea fish is heightened because of the popular assumption that the deep ocean is the portion of the biosphere that has been least affected by chemical pollutants. The assumption that the deep sea is still relatively uncontaminated is almost certainly not valid for certain chemicals; for example, DDT and DDE (dichlorodiphenyltrichloroethane and dichlorodiphenyldichloroethylene) were detected in the axial muscle in most fish we collected at 2500 m (*1*). Yet, the idea that industrial and agricultural activities have not significantly increased the concentration of Hg in deep-ocean water can be supported by calculating the change in Hg concentrations which would result if the total increase in Hg flux due to human activities were to be evenly mixed into the oceans. Using this line of argument, Weiss *et al.* (*2*) have predicted that, at most, a doubling in the upper 100 m of the oceans could result from culturally enhanced degassing of Hg from disturbed land to the atmosphere and then to the oceans. If the prediction of these authors is correct, we would assume that man has not significantly increased the Hg content of deep-ocean water and, therefore, that fish living permanently at depth will have "natural" or preindustrial levels of mercury. However, an untested assumption in this reasoning is that there are no special food chain phenomena or physical mechanisms that concentrate and transport Hg rapidly from cultural sources to the deep-