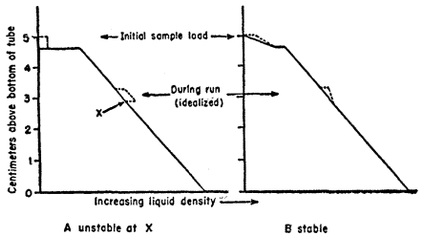

Fig. 1. Conditions for stability in a density gradient liquid column. Solid line, stabilizing gradient; dotted line, contribution due to a sample in process of analysis.

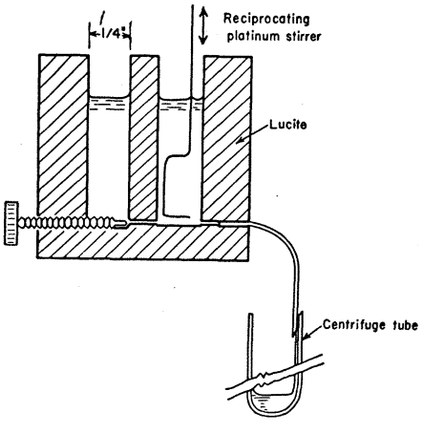

Fig. 2. Mixing chamber for the production of linear stabilizing gradients. The left chamber is filled with 2.4 ml of 5 percent sucrose, and the right, with 2.2 ml of 20-percent sucrose. After filling and after starting of the mixing motor, the center valve is opened and the exit tubing is turned down to touch the side of the centrifuge tube. The sucrose solution then runs down the wall of the tube. If the 4.6 ml are delivered in 10 to 15 minutes, the succeeding lighter solution floats on the underlying liquid with little mixing.

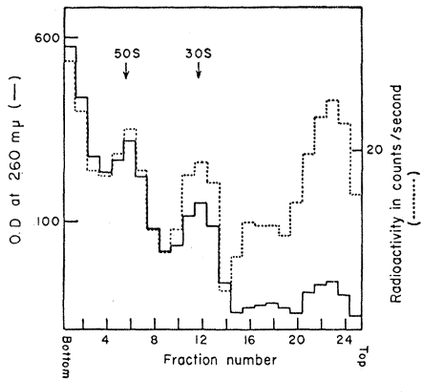

Fig. 3. Example of the use of density gradient stabilized sedimentation analysis for measurement of radioactivity incorporated into the smaller ribosomes during 1-minute exposure to $S^{35}O_4^{--}$. Solid line, optical density at 260 mμ of 0.2-ml samples diluted to 1.2 ml. Dotted line, trichloroacetic acid precipitable radioactivity.

have been used occasionally and are quite adequate.

This whole process was carried out in the cold room with solutions at 1° to 4°C. The tube was loaded into the precooled swinging bucket rotor (Spinco SW39) and centrifuged for the appropriate time. As quickly as possible at the end of the run, the tube was gently lifted out of the rotor and mounted in a device which perforated the bottom of the centrifuge tube with a hypodermic needle (ground to a short, double-sided point) located about 1 mm above the bottom of the tube. After removal of a piano wire which kept the hypodermic tubing clear and free of air bubbles, the contents of the tube were run out in 25 equal cuts by drop counting.

Figure 3 shows the result of use of this system for examination of the radioactivities of the smaller particles of *Escherichia coli* after a short period of incorporation of radioactive sulfate. A growing culture of *E. coli* was starved of sulfur for 30 minutes, and then $S^{35}O_4^{--}$ was added. After 1 minute, carrier $S^{32}O_4^{--}$, S^{32}-cystine, and S^{32}-methionine were added to displace the radioactivity of the rapidly-turning-over soluble proteins synthesized by the ribosomes (*6*). Fifteen seconds later the culture was suddenly chilled to 0°C, the cells were washed and broken, and the ribosomes were harvested in the preparative ultracentrifuge. The ribosome pellet was resuspended in appropriate buffer, and a sample was loaded in an inverted linear gradient layer, as described above. The tube was then centrifuged (37,000 rev/min, for 150 minutes) to pellet most of the 70 and 100*S* ribosomes in order to spread out the 20-to-50*S* region for a close examination of the smaller ribosomes.

The peaks in the diagram correspond closely with the peaks shown by the analytical centrifuge which was used to determine the sedimentation constants.

A series of experiments of this type (*2*) shows that the newly synthesized ribosomal protein and ribonucleic acid appear first in the smaller ribosomes (20, 30, 50*S*) and later in the larger ones (70, 100*S*).

A sample of the width discussed above has provided the best compromise between high resolution and a useful quantity for analysis, although the resolution could probably be improved to the limit set by diffusion if smaller samples and great care were used. For the example shown in Fig. 3, the full width (½ max.) due to diffusion alone would be about 1 mm or about ⅛ of the observed width. Since the width is due to causes other than diffusion, equivalent resolution could be obtained for objects very much smaller than the ribosomes. For example, serum globulin (molecular weight, 170,000, about 7*S*) would require about 12 hours of centrifugation, and the resulting diffusion width would be about 3 mm. For the preparation of large quantities of material, very broad sample gradients are useful. In the preparation of pure 30*S* particles from a mixture of 30 and 50*S* ribosomes, 2.4 ml of a very concentrated ribosome suspension are placed in the left chamber, and 2.4 ml of 20-percent sucrose, in the right chamber. In this way 10 to 20 mg of pure 30*S* particles may be obtained in a single run.

It is worth noting that, while the centrifugal force doubles from the top to the bottom of the tube in the swinging bucket rotor, the viscosity of 20-percent sucrose is about twice that of water. When correction is made for the density of the sucrose solution, it is found that the sedimentation velocity of ribosomes is very nearly constant throughout the length of the centrifuge tube.

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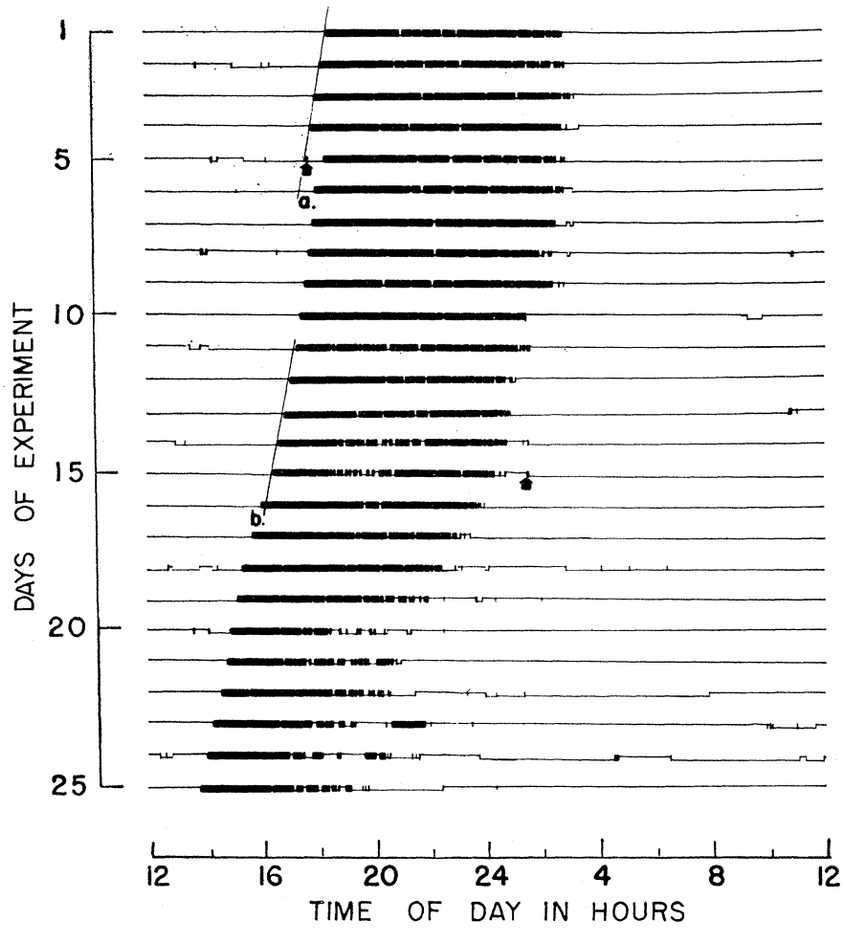
31 July 1959

Daily Light Sensitivity Rhythm in a Rodent

Abstract. Single 10-minute light periods can cause a phase shift in the rhythm of the daily locomotor activity of flying squirrels otherwise maintained in constant darkness. A daily rhythm of sensitivity to these standard light periods was found.

Recent investigations have demonstrated that many animals in isolation conditions exhibit a precise rhythm of locomotor activity which appears to be controlled by a "biological clock," characteristic in cycle length for each individual animal. Nocturnal rodents show cycles ranging from about 22 hours to about 25 hours in length for different individuals (*1*, *2*). These endogenous rhythms may be synchronized by clues



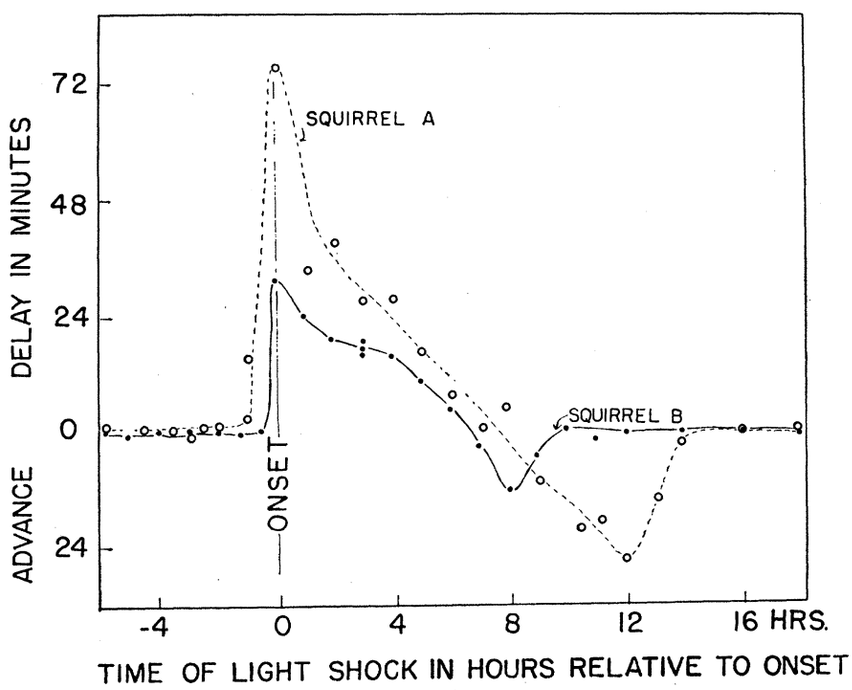


Fig. 1 (top). Activity record of a flying squirrel, showing light-shock effects: (a) delay shift in phase caused by a light shock starting 1 minute after the beginning of the daily activity period; (b) advancing phase shift caused a light shock 9 hours after the onset of activity. Arrow indicates light periods. Fig. 2 (bottom). Light-sensitivity curves for two flying squirrels, A and B.

in the environment, such as the light-dark cycle of a natural or artifical day (*1*).

It has been suggested that such synchronization is brought about by a rhythm of sensitivity of animals to light. In *Peromyscus*, feeding disturbances with a dim light during the sensitive phase caused a delay in the start of activity the following night, while a similar disturbance in the insensitive phase had no effect on the activity rhythm (*3*). With standard light exposures of several hours' duration, rhythms of light sensitivity have been shown in the hamster (*4*), and in *Euglena* (*5*). Cultures of *Drosophila*, when exposed to light, showed phase shifts dependent upon the time in their daily hatching rhythm at which light occurred (*6*).

To further describe the effect of light on the endogenous rhythm, a quantitative determination was made of the rhythm of light sensitivity; short-term "light shocks" of standard duration and intensity were used at hourly intervals during the daily activity cycle. For this study flying squirrels, *Glaucomys volans*, were housed individually in recording wheel cages. A nest chamber, supplied with excess food and water, provided free access to a running wheel mounted on a bicycle axle. Revolutions of each squirrel were indicated on an Esterline-Angus Operations Recorder. Cages were maintained individually in light-tight cabinets, in constant darkness, at a uniform temperature ± 1°F per day.

The 24-hour records for each individual were mounted in a consecutive vertical series and showed that a squirrel alternated approximately 12 hours of inactivity with 12 hours of intense running in the wheel (Fig. 1). By measuring the time between successive daily onsets of running in the wheel in constant darkness (*2*), it was possible to determine the slope of the onset of activity and thus to predict the start of activity on a particular day. At intervals of 4 to 25 days the squirrels were exposed to 10 minutes of light of an intensity of 0.5 ft-ca. A phase shift was calculated as the difference between the expected and the actual time of activity onset for the activity period following the light shock (method shown diagrammatically in Fig. 1).

These phase shifts, illustrated graphically in Fig. 2, demonstrate a marked rhythm of light sensitivity. The form of the curve followed a similar pattern for 12 squirrels tested. Maximal delay effect occurred with light at the onset of running in the wheel. A 6- to 7-hour period of gradually decreasing delay phase shifts followed. At about 7 hours after the start of running a change was made

to a short period of resetting by advancing the time of onset, until at 10 to 14 hours after the onset of activity the squirrel reached the inactive, light insensitive part of its cycle. No phase shifts were caused by light during the remainder of the inactive period except during the last hour before the start of running in the wheel.

While sensitivity curves for different squirrels showed a similar form, they differed slightly from each other in details. Striking differences in the amount of phase shifting caused by equivalent light shocks were common for different individuals. Likewise, small differences in the time relationships of the two curves are apparent in Fig. 2.

Such a rhythm of sensitivity to light has been found adequate to explain the stepwise synchronization of the active period of all flying squirrels tested, regardless of their cycle lengths, to the time of darkness in artificial or natural days (*7*). A daily rhythm of light sensitivity also serves as a basis for the interpretation of many previous studies of the effect of light upon the activity cycles of rodents (*8*).

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24 August 1959

Heat-Labile Serum Systems in Fresh-Water Fish

Abstract. Serum specimens from 18 specimens of 12 different species of fresh-water fish were examined for their ability to kill *Toxoplasma* nonspecifically. This ability was present in all sera except those of two of three great northern pike. The effect was destroyed by exposure to 53°C, 56°C, or zymosan. Complement was demonstrated in all sera except that from one great northern pike, when rabbit erythrocytes were used in the indicator system.

Specific toxoplasma antibody, when measured in the dye test (*1*), requires the presence of a heat-labile serum system (activator), similar to, if not identical with, the properdin system (*2, 3*). "Activator" can be measured quite accurately in human serum, but its detection in other species is difficult because, generally, they possess a heat-labile, nonantibody dependent factor(s) which kills *Toxoplasma*, giving them an unstained appearance in the dye test. Aside from the mouse (which has neither the "activator" nor the antiparasite effect), the heat-labile, nonspecific system has been demonstrated in the sera of rats, monkeys, cattle, sheep, horses, swine, mink, rabbits, guinea pigs, dogs, cats, pigeons, chickens, and ducks. With the exceptions noted, it thus appears to be a characteristic of most, if not all, species of mammals and fowl. Recently, an opportunity presented itself to extend these observations to several varieties of fresh-water fish. This report includes data on both nonspecific antitoxoplasmic factor and hemolytic complement in the sera of 12 species of fish.

The fish were obtained from Oneida Lake, New York, by netting (*4*). Their ages were undetermined except that all appeared to be mature specimens. Bleeding was accomplished by cardiac puncture (without anesthesia), using sterile needles and syringes. The bloods were transferred to sterile test tubes and chilled in wet ice until returned to the laboratory 1 to 2 hours later, where the sera were separated from the clots and promptly frozen and stored in dry ice. The specimens were thawed rapidly just prior to use and kept cold in an ice-water bath throughout the dilution procedure.

Testing for the nonspecific factor was accomplished by mixing 0.2 ml of either the undiluted or serial twofold dilutions of the serum with 0.05 ml of fresh mouse peritoneal exudate containing large numbers of *Toxoplasma* (RH strain); sufficient heparin was added to the exudate to make a final concentration of 1:10,000 in each tube. The tubes were incubated for 1 hour in a 37°C water bath, alkaline methylene blue was added, and the stained and unstained parasites were enumerated in the usual fashion (*1*). All serum dilutions were made with 0.85-percent salt solution. The titer of the nonspecific factor was taken to be the reciprocal of the original dilution of serum in which 50 percent of the parasites were unstained. A control tube was included in which the undiluted serum had been heated at 56°C for 30 minutes (*5*). In addition, some sera were examined after heating at 53°C (*6*) for 25 minutes; the results were almost identical. One specimen of carp serum was treated with zymosan at 17°C (*3*) and then tested for the nonspecific factor. In one experiment, the coagulation of aliquots of catfish, carp, and smallmouthed bass bloods was prevented by heparin and Versene, but these sera reacted no differently from that obtained from spontaneously coagulated blood. Hemolytic complement was assayed in the fish sera by testing with sheep erythrocytes in some instances and in others with rabbit red cells according to the recommendations of Cushing (*6*). The various data obtained from 18 of the fish (12 species) are summarized in Table 1.

It will be noted that, to varying degrees, all of the sera contained nonspecific, antitoxoplasmic factor except the sera of two of the three great northern pike. The sera of the third pike was minimally positive in a dilution of 1:2. One of the inactive sera was obtained from a mature specimen, 40 in. long and about 14 lb in weight. It is interesting that the five pike perch, a species closely related to the great northern pike, were all positive and to the same marked degree, 1:32.

In each instance, the antitoxoplasmic effect was eliminated by heating for 30 and 25 minutes at 56° or 53°C, respectively, although in a few of the latter cases, questionable activity remained in the undiluted serum. The one carp sample that was treated with zymosan

Table 1. Nonspecific heat-labile antitoxoplasmic and hemolytic complement titers of 12 species of fresh-water fish. Heat of 56°C eliminated the heat-labile, nonspecific, antitoxoplasmic factor. The effect of 53°C heat on the complement is shown below.

Titer nonspecific factor	Complement (rabbit red blood cells)	Effect of 53°C heat on complement
Bullhead (*Ameiurus nebulosus*)		
1:2	Undiluted	±
Carp (*Cyprinus carpio*)		
1:8*	1:8†	±
Catfish (*Ictalurus punctatus*)		
1:4	1:4	Eliminated
Common sucker (*Catostomus commersonii*)		
1:2	1:4	Eliminated
Dogfish (fresh-water) (*Amia calva*)		
1:4	1:4	Eliminated
Eel (*Anguilla chrysypa*)		
1:2	1:8	Eliminated
Great northern pike (*Esox lucius*)		
None found	None found	Not done
None found	1:2	Not done
1:2	1:8	+
Largemouthed bass (*Huro salmoides*)		
1:4	1:8	±
Ling (*Lota maculosa*)		
1:2	1:2	Eliminated
Pike perch (*Stizostedion vitreum*)		
1:32	1:8‡	Undiluted
1:32	Not done	Not done
1:32	Not done	Not done
1:32	Not done	Not done
1:32	Not done	Not done
Smallmouthed bass (*Micropterus dolomieu*)		
1:64	1:16	Eliminated
Yellow perch (*Perca flavescens*)		
1:16	Not done	Not done

* Eliminated by treatment with zymosan at 17°C.
† None demonstrated with sheep red blood cells.
‡ 5.5 μ/ml with sheep red blood cells.