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*).

PATRICIA J. DE COURSEY
Zoology Department,
University of Wisconsin, Madison

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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.

became negative for the nonspecific factor. In previous, unpublished studies with rabbit sera, we found that treatment with zymosan uniformly eliminates the nonspecific factor.

When complement determinations were attempted with a sheep red blood cell system, no activity was demonstrated in one carp, but one pike perch had 5.5 units/ml. These were the only two specimens examined in this manner. The remaining samples were titrated for complement with rabbit red cells (*6*) and all were positive except one of the great northern pike. Both the nonspecific and complement activities (as determined with rabbit cells) were either present or absent from the same sera, except in one northern pike.

Except for previous studies of complement in carp by Cushing (*6*), little is known of the heat-labile serum factor content of fish sera. Such sera may be readily obtained with very little practice by cardiac puncture and the fish may be kept alive for several bleedings. Some 90 ml of blood were procured in separate bleedings on two consecutive days without killing a carp. The eel presented something of a problem because it was difficult to hold, and oozed a considerable amount of oil. We believe, however, that blood, rather than a mixture of blood and oil, was obtained.

It seems reasonable to assume that the heat-labile, antitoxoplasmic system in the fresh-water fish is similar to, if not identical with, that of mammals and birds. Although toxoplasma infections have not been demonstrated in fish, the presence of the heat-labile serum activities is probably a fortuitous biologic coincidence, for it would seem unlikely that they were acquired as a specific response to any infectious agent. Properdin, complement, and Mg^{++} (*2*) make up a portion of the nonspecific system which probably also possesses other as yet unknown components, as well.

The behavior of the great northern pike is of special interest, since two of the three specimens were inactive. In fact, after the first was found to be negative, the other two specimens were acquired to check this initial experience. Since the great northern pike, pike perch, and muskellunge are closely related, it would be of considerable interest to examine the latter. This is to be attempted as specimens become available. Whether the sera of salt-water fish possess heat-labile activities comparable to those of the fresh-water fish also remains to be determined (*7*).

HARRY A. FELDMAN
LOUISE T. MILLER
Department of Preventive Medicine, State University of New York, Upstate Medical Center, Syracuse

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5. Human "activator" was added to a second control tube of similarly inactivated undiluted serum; the dye reactions were uniformly negative, indicating that the fish sera did not contain specific antitoxoplasma antibodies.
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17 July 1959

Chemically Induced Phenocopy of a Tomato Mutant

Abstract. Lanceolate, a spontaneous leaf-shape mutant which fails to produce cotyledons and plumule in the homozygous condition, shows development if supplied with either adenine or a diffusate obtained from normal seeds. Similar development occurs in a different genetic background.

The leaf of the normal tomato, *Lycopersicon esculentum,* is odd-pinnately compound. In contrast, a leaf-shape mutant found by Casseres of Costa Rica has a simple, entire, elongated leaf which we called lanceolate. Associated with the lanceolate leaf shape are small fused cotyledons, early appearance of axillary shoots, which indicate weak apical dominance, leafy inflorescence, and reduced flower size.

On selfing, lanceolate always segregated normal and lanceolate plants approximately in a 1:2 ratio. In addition, about a quarter of the seeds either failed to germinate or appeared as narrow or reduced phenotypes (Table 1). Reduced seedlings never developed beyond a cylindrical mass of green tissue about 5 cm tall and 0.2 cm in diameter, without a trace of cotyledons or plumule. When ungerminated seeds from lanceolate plants were dissected, about one quarter of the embryos were completely devoid of cotyledons and plumule. We assume that such embryos would give rise to reduced seedlings under favorable conditions of germination. Narrow plants, on the other hand, had the appearance of an extreme lanceolate. In contrast to reduced seedlings they had cotyledons and plumule; furthermore, they produced shoots, leaves, and aborted inflorescences, but never flowers. If narrow and reduced phenotypes and the ungerminated seeds are considered to be homozygous lanceolate, the segregation in the upper part of Table 1, which shows the segregation from selfed lanceolate and from the hybrid lanceolate × lanceolate, is not significantly different from a 1:2:1 ratio. That lanceolate is a heterozygote is also clear from the progeny of lanceolate crossed with normal: both lanceolate and normal plants appear in about equal proportions (lower part of Table 1). In effect, the normal allele behaves as a recessive. In accordance with long-established usage, lanceolate is considered to be a dominant allele because it produces an observably different phenotype in the heterozygous condition, and regardless of the fact that homozygous lanceolate may be expressed as an even more extreme phenotype (narrow or reduced).

It is evident that the lanceolate gene has an effect on the growth of cotyledons and leaves. One lanceolate allele reduces the size markedly; with two there is even greater reduction in leaf size (as in narrow) or complete absence (as in reduced). Thus, the lanceolate gene may be responsible for a deficiency of some growth substance. Several authors have identified substances with growth-promoting properties that may have some relevance. Went (*1*) found that there was in the cotyledons of peas a substance that promoted leaf growth. It could diffuse out of germinating seeds and stimulate growth of excised pea leaves in culture medium (*2*). Bonner and Haagen-Smit (*3*) have shown that adenine influences the growth of mesophyll but has no influence on the growth of veins. On the other hand, Went and Thimann (*4*) have shown that auxin increases the growth of veins, but not the growth of mesophyll. Finally, Miller and Skoog (*5*) concluded that the initiation and development of buds from tobacco pith cells in vitro was dependent

Table 1. Segregation in selfed and hybrid cultures of lanceolate.

	Normal	Lanceolate	Narrow	Reduced	No germ.	Total
Lanceolate selfed						
Germinated in soil	22	58	1	0	24	105
Germinated on filter paper	35	68	0	37	0	140
(F_1 lanceolate from lanceolate × broad) × lanceolate	10	33	11	0	0	54
Total	67	159		73		299
Exp. (1:2:1)	75	149		75		299
Lanceolate × normal	19	15				34
Exp. (1:1)	17	17				34