

TEMPERATURE ACCLIMATION AND THE NERVOUS SYSTEM IN FISH

By BETTY I. ROOTS* AND C. LADD PROSSER

Department of Physiology, University of Illinois, Urbana, Illinois

(Received 14 June 1962)

INTRODUCTION

The studies on temperature acclimation in fish reviewed by Brett (1956) and Fisher (1958) indicate that many types of cell are involved in the process. These reviewers point out that probably modifications occur in the nervous system; however, there appears to be little direct evidence.

The work described in this paper is based on the hypothesis that much of temperature acclimation is due to changes in the nervous system which permit normal behaviour at extreme temperatures.

SOURCES, MAINTENANCE, AND ACCLIMATION OF FISH

Goldfish (*Carassius auratus* L.), $1\frac{1}{2}$ to 2 years old and 4 to 5 in. in total length, were obtained from the Auburndale Company, Chicago (No. 1 Pool fish).

Bluegills (*Lepomis macrochirus* Rafinesque) and green sunfish (*Lepomis cyanellus* Rafinesque) were netted from local lakes by the Illinois State Natural History Survey.

Black bullheads (*Amieurus melas* Rafinesque) were obtained from a local commercial fishing lake, and through the Illinois State Natural History Survey.

The fish were kept in 10 gal. covered aquaria with up to four fish per tank at 25°, 30° and 35° C., and up to six per tank at 15° and 5° C. The water was continuously aerated and filtered through charcoal and glass wool. A complete change of water in the tank was made as required, and all water was aerated for 24 hr. before use.

The fish were kept under conditions of constant length of day, either of 16 or 12 hr., and at constant temperature. For most experiments fish acclimated to temperatures of 5°, 15°, 25° and 30° C. were used, although there were some with fish acclimated to 35° C.

Glencoe Mills Company $\frac{3}{8}$ in. pellet trout food was used. Fish at 25°, 30° and 35° C. were fed twice a day, those at 15° C. once a day, and those at 5° C. once every other day, all *ad libitum*.

On arrival at the laboratory fish were placed in water at 15° C., except where the temperature on arrival was closer to 25° or 5° C. When fish were transferred for acclimation to different temperatures they were moved in water at the same temperature as that in which they had been living which was then allowed to warm up or cool down as the case may be. The lid was left off the aquarium for the first 2 days to make the change more gradual. In this way temperature shock was avoided.

* Present address: Department of Biology, Royal Free Hospital School of Medicine, 8 Hunter Street, London, W.C. 1.

It has been shown by Hathaway (1927), Sumner & Doudoroff (1938), Doudoroff (1942) and Brett (1944, 1946) that acclimation to high temperatures takes place very rapidly, most acclimation having taken place in one day, and is complete in three days, whereas acclimation to low temperatures is a much slower process and takes 20 days for completion. Fish were not used until they had been at their temperature of acclimation for a minimum of 7 days in the case of high temperatures and of 21 days in the case of low temperatures.

COLD-BLOCKING OF SIMPLE REFLEXES

(a) *Materials and Methods*

Goldfish acclimated to 35°, 25°, 15° and 5° C. and bluegills acclimated to 30°, 25°, 15° and 5° C., all on a 16 hr. day, were used in these experiments in which the cold-blocking temperature of simple reflexes was determined. An electric shock

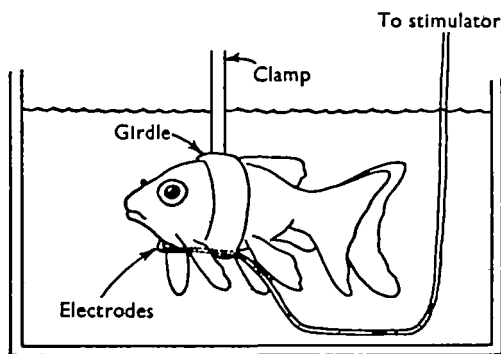


Fig. 1. Apparatus for studying a simple reflex in fish.

applied to the gular region of goldfish elicits the propeller reflex of the tail described by von Holst (1934). If the side of a bluegill is stroked with a rod the body curves towards the stimulated side.

During the experiments the fish were held in fibrous sponge girdles just behind their pectoral fins. In the case of the experiments with goldfish the stimulating electrodes were held in place by the same girdle (see Fig. 1). Recording of the response was visual in all cases as no mechanical system was found to be satisfactory. Experiments were begun at the acclimation temperature of the fish, and the temperature was lowered subsequently by 5° C. steps except that the next lower temperature below 5° C. was 1° C. which was the lowest practicable water temperature. Temperature changes were sudden. The fish were lifted out of the tank and placed in another with water at a new temperature. The fish were left for 5 min. to come to equilibrium before testing. Prosser (unpublished data) has shown that this time is adequate.

The experiments with 35° and 25° C. goldfish were repeated using fish in which the spinal cord had been transected immediately posterior to the medulla. The fish were left for 3 days to recover from the operation and were tested on the 4th day. All fish recovered from the operation.

(b) Results and conclusions

It may be seen from the results, shown in Tables 1 and 2, that the temperature at which the reflex is blocked depends upon the temperature to which the fish have been acclimated before the experiment, and that the results for goldfish and bluegills are similar. The blocking response was uniform for fish having a similar thermal history.

The results obtained with goldfish in which the spinal cord had been cut were the same as for normal fish, indicating that the brain is not involved in this response.

Muscular twitching, which in a homeotherm would be described as shivering and which was often violent, was observed in the majority of fish at the cold-blocking temperature.

Table 1. *Cold-blocking temperature of a simple reflex in goldfish*

Acclimation temperature (°C.)	Blocking temperature (°C.)	No. of fish
35	10	4
25	5	7
15	1	8
5	—	4

Table 2. *Cold-blocking temperature of a simple reflex in bluegills*

Acclimation temperature (°C.)	Blocking temperature (°C.)	No. of fish
30	10*	3
25	5	4
15	1	3
5	—	3

* Very slight response.

COLD-BLOCKING OF CONDITIONED REFLEXES

*(1) Conditioned inhibition of respiration**(a) Materials and methods*

Otis, Cerf & Thomas (1957) showed that if a conditioned stimulus, in this case light, is followed immediately by an electric shock a number of times, inhibition of respiration occurs as a conditioned response in goldfish.

Goldfish acclimated to 30°, 25°, 15° and 5° C. and kept on a 12 hr. day were used in experiments on the cold-blocking temperature of this conditioned inhibition of respiration. The fish were suspended by a fibrous sponge girdle in a small aquarium with black sides and a semi-transparent bottom. Sheets of heavy aluminium foil along two sides were used as electrodes and a light was placed beneath the tank (see Fig. 2). The light and electrodes were connected to a time-delay circuit so that either the light could be switched on alone for 5 sec., or light for 5 sec. could be followed immediately by an electric shock. The shock consisted of 25 V. square waves of 0.2 msec. duration at a frequency of 400/sec. delivered for 0.25 sec. from a Grass stimulator. The electrical resistance of the fish and tapwater between the electrodes was 20,000 Ω so the stimulus current was 0.00125 A. A wooden lever attached to a transducer rested lightly on one

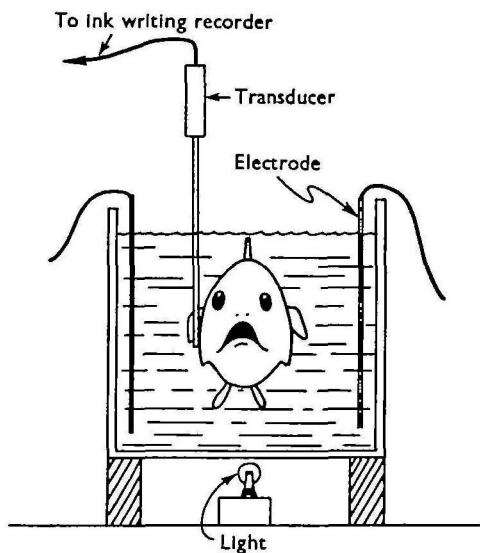


Fig. 2. Apparatus for conditioning inhibition of breathing in fish.

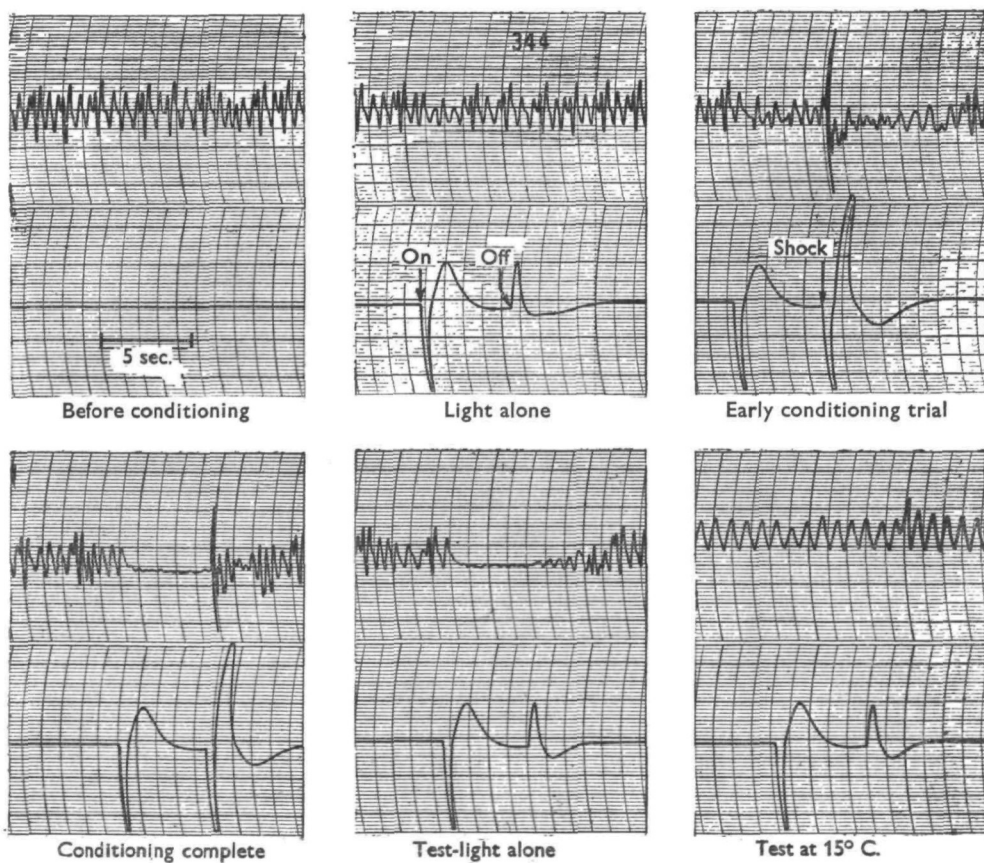


Fig. 3. Records obtained during conditioning inhibition of respiration of a goldfish acclimated to 30° C., and cold-blocking of the response at 15° C.

operculum. Opercular movements and light and shock stimuli were recorded on separate channels of an ink-writing recorder. Typical tracings are shown in Fig. 3. Fish were conditioned at their acclimation temperature. Trials were given at intervals of between 1 and 2 min. to avoid the possibility of a response to a given time interval. If the fish had not become reliably conditioned within forty trials they were discarded. (Otis *et al.* (1957) showed that a small proportion of fish failed to condition reliably even after 100 trials.) When the fish were conditioned they were lifted out of the experimental tank into another containing water at a lower temperature. The water in the experimental tank was brought to the new temperature and the fish was replaced. After 5 min. the fish was tested for retention of the response. Experiments were made in which the fish was subjected to the same procedure except that the water temperature was unchanged. No fish was ever used for more than one experiment since there is evidence (Sumner & Doudoroff, 1938) that even short exposures to another temperature have a lasting effect.

(b) Results and conclusions

Light alone does not disturb the breathing rhythm of the fish. This was tested in all cases. A typical set of records is shown in Fig. 3. The experiments in which the water temperature was not changed showed that the fish retained the conditioned response over the period of time necessary to bring about a change in temperature, and that the response was not disturbed by handling.

Table 3. *Cold-blocking temperature of conditioned inhibition of respiration in goldfish*

Test temperature (°C.)	Acclimation temperature (°C.)			
	30	25	15	5
25	+(3)	.	.	.
20	+(2)	+(3)	.	.
	-(4)			
15	-(3)	+(2)	.	.
		-(9)		
10	.	-(4)	-(4)	.
5	.	.	-(4)	.
1	.	.	.	-(3)

+, Not blocked.

-, Blocked.

Numbers in parentheses are the number of fish.

The cold-blocking temperature of this conditioned reflex is related to the acclimation temperature (Table 3) and is considerably higher than the cold-blocking temperature for simple reflexes (Table 1). It was found that more of the 5° C. fish failed to show the conditioned response within 40 trials than fish acclimated to temperatures above 5° C.

(2) Avoidance conditioning

(a) Materials and methods

Goldfish show an individual preference for the lighted or darkened side of a two-chamber aquarium and may be conditioned to avoid the side of their preference

(Prof. G. J. Thomas, personal communication). The cold-blocking temperature of this learned response was studied in goldfish acclimated to 25° C. and kept on a 16 hr. day.

The apparatus used is shown in Fig. 4. It consisted of an aquarium painted black except for an observation strip 1 in. wide left along one side. The tank could be divided into two interconnecting chambers by a unit consisting of three aluminium plates, which served also as electrodes, and two black boxes with ground-glass floors. Each box contained a 7 W. bulb which could be illuminated as required. The electrodes were connected with a Grass stimulator and the lateral end electrodes were positive with respect to the middle common electrode. Shocks consisting of 25 V. square waves of 0.2 msec. duration at a frequency of 400/sec. were used.

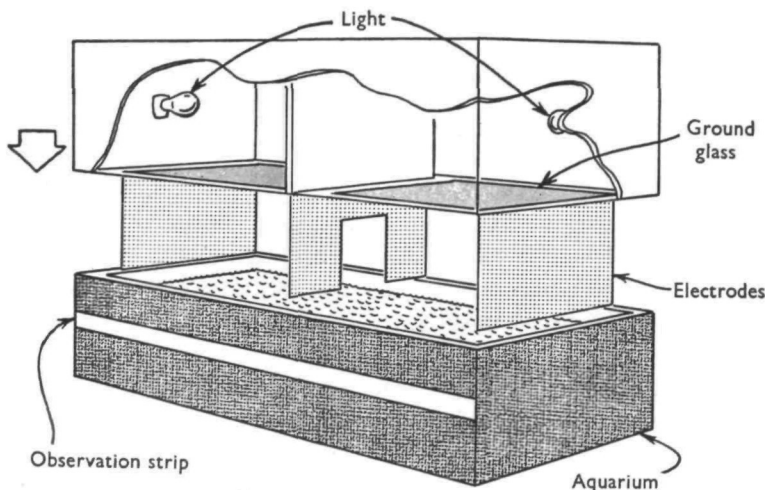


Fig. 4. Cutaway drawing of apparatus for avoidance-conditioning in fish.

Each fish was placed in the aquarium and the unit was put into place. After five minutes the fish was given six preference trials, and then avoidance-conditioning was begun. The lighting was reversed and the fish was driven over to its non-preferred side by an electric shock 15 sec. after the light change. Conditioning trials were made at intervals of between 1 and 2 min., as in the experiments on the conditioned inhibition of breathing, and if a fish moved into the other compartment between trials it was driven back by electric shock. In this way fish may be conditioned to move into either the light or the dark side within 15 sec. of reversal of the lighting. A criterion of five successive avoidance responses was adopted. The cold-blocking temperature was found by removing the trained fish to a small tank at a lower temperature, bringing the water of the experimental tank to that temperature, replacing the fish and after ten minutes testing for the avoidance response.

During the experiments the only other illumination in the room came from two 25 W. red lamps.

(b) *Results and conclusions*

Seven fish were trained and of these three were tested at 20° C. and four at 15° C. The response was blocked in all fish at 15° C. and in none at 20° C. Thus, the cold-

blocking temperature of this conditioned reflex is the same (15° C.) as for conditioned inhibition of respiration, a reflex of a lower order, and higher than that for simple reflexes.

SWIMMING

(a) Materials and methods

Green sunfish, acclimated to 30°, 25°, 15° and 5° C., and bluegills, acclimated to 5° C., all kept on a 16 hr. day, were used. The fish came from natural populations so it was thought desirable to watch their condition during acclimation and during the experimental periods. The fish were weighed and measured at intervals and their condition coefficients (K) calculated as follows:

$$K = \frac{100,000 W}{L^3},$$

where W = weight in grams, L = standard length in mm. The standard length is the length from the tip of the snout to the end of the vertebral column (Lagler, 1952).

The normal range of values for green sunfish is 2.55–4.84 (Mr W. Childers, personal communication). The value of K for all the green sunfish used in these experiments lay within this range.

The method used was similar to that described by Fry & Hart (1948). An annular Lucite trough with an inner diameter of 6 in., an outer diameter of 12 in., and 6 in. deep, mounted on an electrically driven turntable, was used. The speed of rotation could be changed by means of a Variac resistor, and a revolution counter was attached to the apparatus. Air was bubbled into the water, which was changed frequently, through a small jet.

A period of 5 min. was allowed for the fish to become accustomed to the chamber before it was rotated. On rotation, the fish swam to maintain its position with reference to objects outside the chamber. The chamber was made to rotate slowly and the speed was gradually increased until the fish was swimming steadily. After five minutes at this speed the rate of rotation was increased in steps, the change being effected in 1 min. The fish was kept swimming at each new speed for 5 min. For the purposes of these experiments the maximum swimming speed was taken as that speed at which the fish was no longer able to maintain its position. During the initial stages it was found necessary to use a glass rod to tap the fish lightly if it began to fall back. Each fish was swimming for about 25 min.

The radial position of the fish in the chamber was noted and the r.p.m. counted. Experiments with a ball of cotton wool and observation of the air bubbles showed that in this chamber used in the way described there was no slippage. The speed of swimming therefore could be calculated directly. All fish were run at their acclimation temperature, then at least three trials made at each experimental temperature. As it was not possible to obtain sufficient fish, each fish was tested at several temperatures. The sequence was at random and the fish were left at their acclimation temperatures for at least two days between trials.

(b) Results and conclusions

The maximum cruising speed of green sunfish is related to the ambient temperature but the relationship is different from that obtained for goldfish by Fry & Hart (1948) and for lake trout (*Salvelinus namaycush*) by Gibson & Fry (1954) (Table 4, Fig. 5). These authors found that there was an optimum after which the speed of swimming fell off with temperature, whereas in the present experiments the swimming rate rose to a maximum and then swimming failed abruptly. Since species as different as goldfish and trout show similar relationships, it seems unlikely that the difference between these and green sunfish is a species difference. The experimental methods differed in

Table 4. *Maximum swimming speed of fish in relation to temperature and thermal history*

Acclima- tion temperature (°C.)	Test temperature (°C.)	No. of trials	Mean speed (ft./min.)	S.D.	S.E.
Green sunfish					
30	40	3	0	—	—
	35	3	56	3.6	2.1
	30	9	48	5.3	1.8
	25	3	48	6.9	4.0
	20	3	31	6.9	4.0
	15	3	0	—	—
25	35	5	0	—	—
	30	3	46	10.6	6.1
	25	10	43	3.4	1.1
	20	4	39	9.4	4.7
	15	8	27	7.9	2.8
	10	3	0	—	—
15	30	3	0	—	—
	25	5	40	1.8	0.8
	20	3	38	1.0	0.6
	15	7	28	5.9	2.2
	10	3	22	6.0	3.4
	5	3	0	—	—
5	20	3	0	—	—
	15	3	0	—	—
	10	3	0	—	—
	5	12	0	—	—
Bluegill					
5	5	3	21	1.9	1.1

that Fry & Hart and Gibson & Fry used each fish once only whereas in the present experiments each fish was tested at several different temperatures. The difference in the relationship obtained could be explained if the brief exposures to temperatures above or below the acclimation temperature during testing had lasting effects. Sumner & Doudoroff (1938) found that a $\frac{1}{2}$ hr. exposure to 30° C. resulted in a marked increase in resistance to the lethal effects of temperature, and that the differences observed between warm- and cold-acclimated fish persist for at least 23 days. However, they did not report on the persistence of the effects of brief exposures. The experiments described above indicate that these effects persist for more than 2 days.

At both the upper and lower temperatures at which the fish did not swim there wa

impairment of balance and the fish listed. This was more marked at the upper than at the lower temperatures. At the limiting temperatures most of the fish held their dorsal-fin spines stiffly erect. This fin posture is also shown by a cornered fish, which raises them for defence, and by a displaying fish, where the erect spines probably reflect the fright component of a conflict between fear and aggression (Barlow, 1962). Swimming was possible at the upper and lower temperatures; at the upper the fish darted and tried to swim through the bottom of the trough; at the lower it swam forwards in a short burst if given a strong stimulus; but it did not swim consistently. Thus, the muscles and nerves concerned can bring about swimming movements at the limiting temperatures but the usual response is blocked.

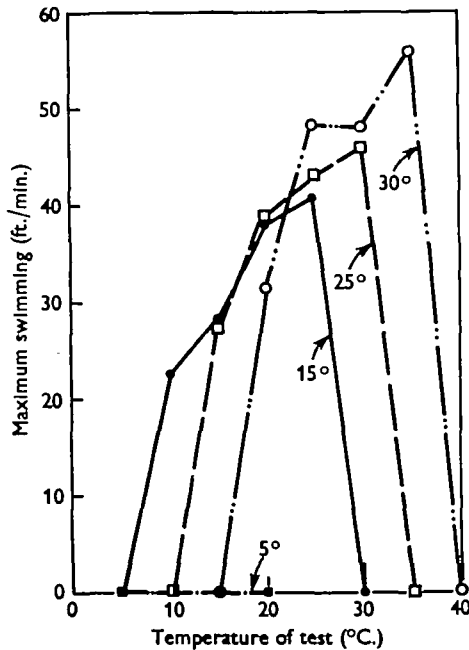


Fig. 5. Relationship between temperature and maximum swimming speed in green sunfish acclimated to different temperatures. The acclimation temperature is shown beside each curve.

The failure of the 5° C. green sunfish to swim at any temperature was investigated. They were left at 10° and 15° C. for periods up to 2 hr. before testing but cruising did not occur. The behaviour of the fish became more and more erratic with time. At 20° C. the fish were greatly distressed and bordered on heat coma. Bluegills bite much more frequently than green sunfish during hook-and-line fishing for these species through ice (Mr W. Childers, personal communication). From Table 4 it may be seen that bluegills acclimated to 5° C. can maintain a steady speed of 21 ft./min. under the conditions of these experiments. Thus the behaviour of these two species in the field is reflected by their performance in the laboratory.

PERIPHERAL NERVE

In the cold-blocking of simple reflexes the ability of peripheral nerves to conduct impulses at low temperatures could be a limiting factor. The chamber shown in Fig. 6 was designed to test this possibility. It was made of two pieces of Lucite which fitted together to make a chamber 2 mm. wide and 2 cm. long. The T-shaped top piece had a groove at either side to allow for the passage of a nerve. The bottom piece incorporated three pairs of silver-wire electrodes and had a hole through which a thermistor was inserted to lie close to the nerve. An inlet tube in the lower and an outlet

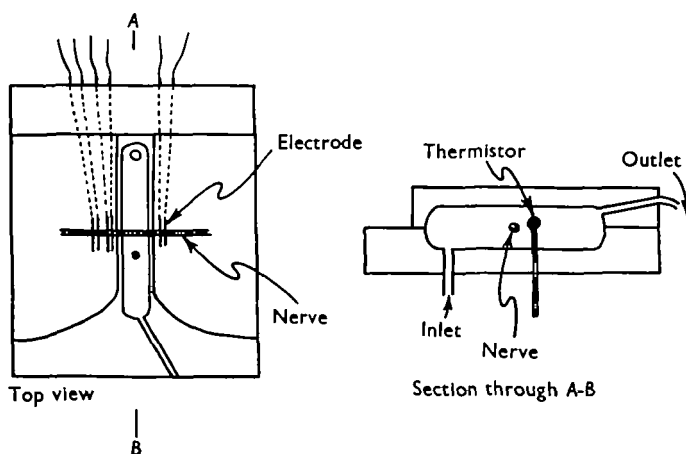


Fig. 6. Chamber for cold-blocking experiments on nerves.

tube in the upper piece allowed saline to be passed through the chamber. Sealing was effected by petroleum jelly and the whole was placed in a Lucite moist chamber. The portion of the nerve passing through the chamber could be cooled by passing cold saline through the central portion. The temperature could be lowered gradually and was continuously recorded by the thermistor.

It was intended to determine the cold-blocking temperature for one of the pectoral nerves in goldfish acclimated to 30°, 25°, 15° and 5° C. but it proved to be impossible to stimulate the nerve and record action potentials. A variety of different salines was used but this made no difference. Hook electrodes were used with the nerve *in situ* but no action potential could be recorded and a stimulus of 150 V. was necessary to produce twitches in the muscles supplied by the nerve. With such a high stimulating voltage excitation was probably due to electrotonic spread rather than to nerve impulses. Desheathing the nerve made no difference. The lateral-line nerve was tried, since Laporte (1951) recorded successfully from this nerve in the carp (*Cyprinus carpio*), but with negative results. Suction electrodes described by Easton (1962) were used to try to stimulate and record from the ends of the nerves, since Easton had used them successfully in nerves of marine fish (Dr D. M. Easton, personal communication), also with negative results. Green sunfish and bluegill pectoral nerves were tried with a little more success. Occasional preparations were obtained from which action potentials could be recorded but the stimulus required was high (3 V.). Precise cold-blocking temperatures were not determined but nerves from 25° C. acclimated fish

of both species were found to conduct at temperatures below 5° C., this being the blocking temperature of simple reflexes.

Some preliminary observations on black bullheads showed that satisfactory preparations could be made of their pectoral nerves and no difficulties were encountered.

Pieces of nerve from all four species were fixed with either formalin or Susa and transverse and longitudinal sections 10 μ thick were cut and stained with Ehrlich's haematoxylin and eosin, and with Masson's trichrome stain. No differences were observed.

BRAIN

Boycott, Gray & Guillery (1961) observed that there were differences in synapses in the brains of lizards kept at different temperatures and it was thought that similar changes might occur in fish brains. Accordingly, brains of green sunfish acclimated to 30°, 25°, 15° and 5° C. were fixed in formalin and serial sections were cut at 10 μ and stained with Bodian's stain. No differences were observed in the synapses but the overall staining was different. The sections of the 30° C. fish brains were much redder than those of the 5° C. fish and the 25° and 15° C. were intermediate so that there was a graded series. This suggests that there are differences which are as yet undefined.

Some data on the chemical composition of brains of goldfish acclimated to different temperatures will be published elsewhere.

DISCUSSION

The site of the cold-block appears to be the central nervous system. Sense organs, peripheral nerves and the central nervous system are involved in all the reflexes investigated. Cold-block of the thermal receptors may be eliminated as the limiting factor since conditioned reflexes are blocked at a higher temperature than simple reflexes. Similarly, peripheral nerve cannot be the limiting factor since what data there are show that peripheral nerves can conduct impulses at temperatures below those at which even simple reflexes are blocked. In the case of the simple reflexes studied the block must occur in the spinal cord since the results obtained with fish in which the spinal cord had been sectioned immediately behind the medulla were the same as those with intact fish.

Sanders (1940) showed that the optic tectum is concerned with learning and conditioned responses and it is probably in this area of the brain that cold-block of conditioned reflexes occurs. The cold-blocking temperature for these reflexes is higher than for simple reflexes, thus the optic tectum is more sensitive to temperature than the spinal cord.

The work of Löwenstein (1932), Löwenstein & Sand (1936) and Gray (1937) has shown that the response of fish to rotation in an annular chamber is a pseudorheotropism which is mediated by labyrinthine reflexes. These are probably reinforced by optic stimuli in the intact animal but the response is shown by blind fish. Healey (1957) quotes work which makes it clear that the optic tectum is also involved in this response. The results of extirpation experiments are contradictory and Healey suggests that removal of part of the optic tectum unbalances the system whereas removal of the whole does not. If surgical removal of the whole tectum does not affect swimming and balance, then it is likely that neither would cold- nor heat-block of the tectum. This

would explain why learned responses involving the association centres in the optic tectum are cold-blocked at higher temperatures than the pseudorheotropic response where blocking of the tectum leaves the tectospinal tracts functional.

It is clear from the experiments described that the cold-blocking temperature is related to both the acclimation temperature and the nerve pathway involved, and that the site of cold-block is in the central nervous system. This is direct evidence for the indications of Brett (1956) and Fisher (1958) that acclimation involves modifications of the nervous system which permit normal functioning at higher or lower temperatures than before acclimation. The precise nature of these changes and their relationship to metabolic changes associated with acclimation remain to be elucidated.

SUMMARY

1. The cold-blocking temperature of a simple reflex in goldfish (*Carassius auratus*) and bluegills (*Lepomis macrochirus*) was 10°, 5° and 1° C. respectively for fish acclimated to 35°, 25°, and 15° C. The response of 5° C. fish was not blocked at 1° C.
2. Similar results were obtained with goldfish in which the spinal cord had been cut immediately posterior to the medulla.
3. Conditioned inhibition of respiration in goldfish acclimatized to 30°, 25°, 15° and 5° C. was blocked at 20°, 15°, 10° and 1° C. respectively.
4. A conditioned avoidance response of goldfish acclimated to 25° C. was blocked at 15° C.
5. The cruising speed of green sunfish (*Lepomis cyanellus*) is related to the ambient temperature, and the upper and lower temperatures at which they do not swim are related to their thermal history.
6. Peripheral nerves of bluegills and green sunfish acclimated to 25° C. continued to conduct impulses at temperatures below 5° C.
7. It is concluded that the site of cold-block is in the central nervous system.

We wish to thank Mr W. Childers and Dr R. W. Larimore of the Illinois State Natural History Survey for supplying bluegills, green sunfish and black bullheads, and Professor G. J. Thomas for the sectioning of green sunfish brains.

The work was supported by a grant from the National Science Foundation to one of us (C.L.P.).

REFERENCES

- BARLOW, G. (1962). Ethology of the Asian teleost *Badis badis*. III. Aggressive behaviour. *Z. Tierpsychol.* **19**, 29-55.
- BOYCOTT, B. B., GRAY, E. G. & GUILLERY, R. W. (1961). Synaptic structure and its alteration with environmental temperature: a study by light and electron microscopy of the central nervous system of lizards. *Proc. Roy. Soc. B*, **154**, 151-72.
- BRETT, J. R. (1944). Some lethal temperature relations of Algonquin Park fishes. *Publ. Ont. Fish. Res. Lab.* **63**, 1-49.
- BRETT, J. R. (1946). Rate of gain of heat-tolerance in goldfish (*Carassius auratus*). *Publ. Ont. Fish. Res. Lab.* **64**, 5-28.
- BRETT, J. R. (1956). Some principles in the thermal requirements of fishes. *Quart. Rev. Biol.* **31**, 75-87.
- DOUDOROFF, P. (1942). The resistance and acclimatization of marine fishes to temperature changes. I. Experiments with *Girella nigricans* (Ayres). *Biol. Bull., Woods Hole*, **83**, 219-44.
- EASTON, D. M. (1960). Nerve-end recording in conducting volume. *Science*, **132**, 1312-13.
- FISHER, K. C. (1958). An approach to the organ and cellular physiology of adaptation to temperature in fish and small mammals. *Physiological Adaptation*, pp. 1-48. Ed. C. Ladd Prosser. Am. Physiol. Soc. Washington, D.C.

- FRY, F. E. J. and HART, J. S. (1948). Cruising speed of goldfish in relation to water temperature. *J. Fish. Res. Bd. Can.* **7**, 169-75.
- GIBSON, E. S. & FRY, F. E. J. (1954). The performance of the lake trout, *Salvelinus namaycush*, at various levels of temperature and oxygen pressure. *Canad. J. Zool.* **32**, 252-60.
- GRAY, J. (1937). Pseudo-rheotropism in fish. *J. Exp. Biol.* **14**, 95-103.
- HATHAWAY, E. S. (1927). Quantitative study of the changes produced by acclimatization on the tolerance of high temperatures by fishes and amphibia. *Bull. U.S. Bur. Fish.* **43**, 169-92.
- HEALEY, E. G. (1957). The nervous system. Chapter 1 in *The Physiology of Fishes*, vol. 2. Ed. M. E. Brown. New York: Academic Press Inc.
- HOLST, E. VON (1934). Studien über Reflexe und Rhythmen beim Goldfisch (*Carassius auratus*) *Z. vergl. Physiol.* **20**, 582-99.
- LAGLER, K. F. (1952). *Freshwater Fishery Biology*, p. 160. W. C. Brown. Iowa: Dubuque.
- LAPORTE, J. (1951). Continuous conduction of impulses in peripheral myelinated nerve fibres. *J. Gen. Physiol.* **35**, 343-60.
- LÖWENSTEIN, O. (1932). Experimentelle Untersuchungen über den Gleichgewichtssinn der Elritze (*Phoxinus laevis* L.). *Z. vergl. Physiol.* **17**, 806-54.
- LÖWENSTEIN, O. & SAND, A. (1936). The activity of the horizontal semi-circular canal of the dogfish (*Scyllium canicula*). *J. Exp. Biol.* **13**, 416-28.
- OTIS, L. S., CERF, J. A. & THOMAS, G. J. (1957). Conditioned inhibition of respiration and heart rate in the goldfish. *Science*, **126**, 263-4.
- SANDERS, F. K. (1940). Second-order olfactory and visual learning in the optic tectum of the goldfish. *J. Exp. Biol.* **17**, 416-34.
- SUMNER, F. B. & DOUDOROFF, P. (1938). Some experiments upon temperature acclimatization and respiratory metabolism in fishes. *Biol. Bull., Woods Hole*, **74**, 403-29.