Studies on Conditioned Responses in Fishes. Part I.

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With 19 Figures in the Text.

CONTENTS.

(A) GENERAL INTRODUCTION.

THE present work is an attempt to supply objective information upon fish behaviour in its relations to animal behaviour generally, and also has a second application as a contribution to our knowledge of the relative importance of the factors affecting the lives and migrations of fish.

Words of introduction are needed for both sides.

It may be said that out of the mass of data concerning the senses, movements, and reactions of fishes, almost the whole of it is of a subjective nature, and thereby open to conscious or unconscious bias. When rigidly applied there appears to be no sound reason to doubt the validity of subjective methods, but it is clearly more satisfactory to study any nervous reaction in an objective manner, if suitable methods can be found. It does not seem necessary for our purpose to take up any definite stand upon the psychological or philosophical implications involved, as they are discussed elsewhere by numerous writers. Washburn (**1**) gives a very readable account of most of the current views upon these points. For the various ways of approaching the problem of Behaviour generally, Driesch (**2**), E. S. Russell (**3**), and McDougall (**4**) may be consulted for the "vitalistic" or "hormic" standpoint; J. B. Watson (**5**) for the attitude of the American school of Behaviourists; Pavlov (**6**) and Bechtereff (**7**) upon the purely objective physiological "conditioned reflex" method. H. Cason (**8**) gives a very good summary of the literature on conditioned responses—which is an indispensable guide, for this literature is very scattered. C. J. Herrick (**9**) gives an account, with bibliography, of the relations between structural and functional evolution in the nervous system, which must be considered in conjunction with one another. These books are mentioned merely as an introduction—the literature is voluminous.

Now, the work of Pavlov and his collaborators, and the work of the American Behaviourists is fundamentally alike, although they differ considerably in their mode of experimentation. It seems, indeed, that the method of "conditioned reflex" formations in studying the higher nervous activities opens up to us, at last, a sound way of investigating the finer discriminatory sense of the analysers (the receptor centres) in the mammalian brain. Moreover, the method is such that the question of consciousness, which plays so large a part in current psychological discussion, does not in any way affect the results when considered solely in regard to the discriminating power of the sense receptors. Herein lies its value as applied to the study of environmental factors in their effect upon animals generally, or upon fishes, if it can be used satisfactorily with them.

With the exception of some experiments carried out recently by J. P. Froloff (**10**)*, there have been, so far as I know, very few investigations upon fishes or lower vertebrates definitely from this point of view. There has been, however, a good deal of work, mainly by American and German investigators, upon the sensory reactions of fishes and amphibians, which,

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* Hulsey Cason (**8**) states that Westerfield used the conditioned response method in studying the ability of 9 mud minnows (*Umbria limi*) to form associations with sounds. (*J. Comp. Psych.*, **1922**, vol. 2, p. 187.) The minnows were able to distinguish between vibrations of 288 and 426 per second, on a ukulele, when the low tone was associated with food and the high one with a distasteful flavour. MacDonald (same ref.) showed the same in *Pimephales notatus*. I have not yet had access to these papers.

if we could know how the experimental conditions were controlled, would probably fit in quite well. This applies particularly, of course, to training and learning experiments, similar to my own.

Before the main functional units—" unconditioned " and " conditioned " reflexes—are finally regarded as playing the enormous part in purposive behaviour assigned to them by Pavlov and by the Behaviourists, it seems imperative that their evolutionary development should be worked out and understood. It is plain that structural and functional evolution in the nervous system have gone hand in hand in phylogeny.

The physiological findings of to-day must therefore be in reasonable agreement with the anatomical findings in order to make this understanding complete ; otherwise all is confusion. At present, the exact relationships between the two are not really clear, although Herrick (**9**, p. xi), who has done so much to correlate them, states that " the evidence is biologically adequate that mind (awareness), as we know it phenomenally, is a function of a particular configuration of bodily organs." This statement seems to me still premature, unless he means it in a very broad sense, when it appears to be justified. Thus, Pavlov (**6**) regards the cerebral cortex as almost certainly the sole portion of the mammalian brain concerned in the final elaboration of conditioned reflexes, a fact which finds expression in the alternative title of his chief book. This belief is held by many others. No well-differentiated cortex is present in fishes, however ; although certain regions,—the "cortical primordia"—represent fairly definitely the places where the cerebral cortex appears in higher forms. These regions are most clearly seen in the brains of Dipnoians, and are still similar and present in almost as primitive a condition in Amphibians. (Papers by G. Elliot Smith (**11**), J. B. Johnston (**12**), Holmgren and Van der Horst (**13**).) Yet it is obvious from even a casual acquaintance with fish that they can, and do, form associations which are apparently quite comparable in nature with the conditioned responses of mammals,—certainly with those of the lower forms. It would appear, then, that subcortical regions are involved, and according to Kalischer's experiments (**14**) this takes place in dogs also, but Johnson (**15**) and others criticise his results. (See Herrick (**9**), for discussion of some of these points.)

Hence the Ichthyopsid type becomes one of the most important stepping-stones in any general study of the higher nervous activities, particularly in regard to associative memory and intentional control of behaviour and their relationships with the brain-centres.

On the other hand, and of more immediate importance to the marine biologist, it is believed that investigations on the lines here presented will offer a sounder basis for the discussion of migratory influences. Nearly all the available information relating to factors exerting an influence on fish migrations has been summarised by F. E. Chidester (**16**).

His general conclusions may be regarded as forming a summing up of the position as it now stands. After a careful examination one is forced to conclude that the foundation upon which our knowledge of the reactions of fishes to their environment rests is very slender.

Fish migrations may be divided into intentional journeyings and passive driftings. The latter do not present the peculiar difficulties to the minds of those who attempt to understand them as do the former. The denatant drift of many fish larvæ, for instance, will be completely understood when the physico-chemical conditions in the oceans are thoroughly known—a knowledge which is swiftly growing. But purposive migration, such as is undoubtedly undertaken by large numbers of fishes, will never be adequately explained, of course, until some degree of unification is achieved amongst physiologists and psychologists upon the vital questions of consciousness and biological memory. This is a reservation to be borne in mind. Certain physiological sequences are nevertheless essential in the fulfilling of any conscious or unconscious act. These, at least, can be investigated with hope of immediate practical results, and without undue insistence upon their philosophical aspect with which I am not competent to deal.

(B) An Introductory Account of Conditioned Reflex Formations.

It is necessary to give a brief account of the methods and particulars concerning conditioned reflex formations, in order that the nature of the present experiments may be more clearly understood. These details are compiled in the main from Pavlov's work on dogs (**6**). Introductory accounts are also given by Hogben (**17**), Lovatt Evans (**18**), Anrep (**19**), and others. Podkopaew (**20**) describes the practical methods in more detail.

It must be borne in mind that the whole of this literature refers solely to work on mammals, and that we are as yet ignorant of the extent to which the phenomena will be found to apply elsewhere.

Starting with Descartes' idea of the reflex, our knowledge has expanded to such a degree that it is now said we are justified in regarding all the elementary motor activities of an animal as essentially reflex. Pavlov (**6**, p. 7) summarises the idea of the reflex in the following words :—

"An *external* or *internal* stimulus (my italics) falls on some one or other nervous receptor and gives rise to a nervous impulse ; this nervous impulse is transmitted along nerve fibres to the central nervous system, and here, on account of existing nervous connections, it gives rise to a fresh impulse which passes along outgoing nerve fibres to the active organ, where it excites a special activity of the cellular structures. Thus, a stimulus appears to be connected of necessity with a definite response, as cause with effect."

In addition to the reflexes making up the sum-total of their motor activities, animals possess a limited number of inborn species reflexes, upon which their whole nervous activity primarily depends. That is to say, an animal responds from birth (or earlier) by a definite reaction when given certain stimuli, and any other individual of that species, under similar external and internal conditions, will give a like response, unless some other extraneous factor inhibits it. (Instincts, for example, may possibly be explained briefly as a whole chain of reflexes for which an inherent organisation is provided in the structure of the animal.) An example of such an inborn reflex is to be found in the mammalian salivary reflex. If food is introduced into the mouth, a secretion of saliva results. This is an inborn, elemental, or "*unconditioned*" *reflex*, due to the reaction of the stimulatory substance with the mucous membrane of the mouth. But in a normal adult animal, the sight or smell of food or of innumerable other stimuli, after sufficient association with the giving of food or of the other stimuli, will also of themselves produce a secretion of saliva, although they had previously had no such effect. These stimuli (such as sight or smell) are said to have acquired " conditioning " or " signalling " properties, and are called "*conditioned*" *stimuli*. They evoke a "*conditioned*" *reflex*. Training and education appear to be essentially the processes of formation of new conditioned reflexes, or, as Herrick, Lloyd Morgan, and others prefer to express them—*conditioned responses*. By Bechtereff they are called " combined " or " associated " reflexes, and the science of their study—reflexology.

An example of training in fishes which illustrates these points is that of Dr. Allen's experiment in which he trained fishes in the Plymouth aquarium to associate the sound of a submerged buzzer with the introduction of food. As this work was incidental to war investigations no detailed records are available, but the case is cited and interpreted by Lloyd Morgan (**21**).

The experimental formation of conditioned reflexes may be brought about in several ways. A most important general condition of the experiments is that all extraneous stimuli, other than the one undergoing investigation, should be excluded. Under such stabilised conditions the formation of conditioned reflexes proceeds with a sureness and definitiveness quite comparable with the results obtained with the spinal animal.

In building up conditioned reflexes experimentally it is necessary that the unconditioned stimulus should bring about an easily registerable response—such as a secretion of fluid—or defensive movement of a limb—or movement of the body as a whole. Food is such a stimulus, and involves both a secretory and motor reaction, either of which may be used for registration. An electric shock evokes a definite defensive motor reaction. These are thus two suitable stimuli with which to build up conditioned reflexes. An essential point is that the presentation of the *conditioning* stimulus should be applied in rigid synchrony with the *unconditioned*; this may mean either simultaneously or at any definite interval *before*, but, so far as is known at present, not after.

Conditioned reflexes are divisible into three types, depending upon their mode of formation, and upon the time relationship existing between the unconditioned and the conditioned stimuli :—(1) Simultaneous ; (2) Delayed ; and (3) Trace—reflexes. These derive their name from the time allowed to elapse before the unconditioned stimulus follows the conditioned stimulus. With *simultaneous* conditioned reflexes the conditioning stimulus is given at the same instant as, or within less than 2–3 seconds from, the unconditioned. In *delayed* reflexes the conditioning stimulus is presented from a few seconds to several minutes before the unconditioned stimulus, and is allowed to act for the whole of the intervening time. *Trace* reflexes are variants of delayed reflexes. Instead of allowing the conditioned stimulus (say, a buzzer used as a signal for food) to act continuously until the unconditioned stimulus is given, it is allowed to act for a period of ½ to 1 minute, then stopped and the unconditioned stimulus is not given until after a further definite interval of 1 to 3 minutes. Under any of these conditions, conditioned reflexes appear in dogs or in man after relatively few trials, from 1 to 30.

Conditioned reflexes after they have become firmly established may be caused to disappear by many methods—that is, they are subject to *extinction*, experimental or otherwise. This may be of the nature of an *external* or *internal*, *temporary* or *permanent inhibition*. Any strong extra stimulus falling on the animal at the time of experimentation may also cause an inhibition of the reflex then undergoing investigation. The effect may be of short or of long duration, but is usually temporary. An inhibition of this type is called by Pavlov *indirect* or *external* as it originates in a part of the brain remote from that where the reflex has its centre. Instances of this type of inhibition will be frequently revealed in the present paper.

The first type of *direct* or *internal* inhibition occurs when the positive conditioning stimuli are themselves given inhibitory properties. This may be brought about by the method of *experimental extinction*. After a conditioned reflex has become firmly established, by giving the conditioning stimulus repeatedly in succession without association with the unconditioned—the reflex becomes gradually extinguished. The exact mechanism involved is doubtful.

The second type of internal inhibition is called *conditioned inhibition*. When a previously neutral stimulus is applied repeatedly in combination with a positive conditioning stimulus and never in this combination associated with the unconditioned, it acquires inhibitory properties;

although the positive conditioned stimulus, occasionally reinforced, retains its properties when applied singly.

The third type is rather unsatisfactorily defined as *inhibition of delay*; it is involved in trace and delayed reflexes.

The fourth type, which is of the greatest interest for the investigation of sensory discriminations, is *differential inhibition*. It is brought about as follows :—A musical tone, for example, is used as a conditioned stimulus; at first, any other tone serves also as a positive stimulus, though generally with weaker effect. This is called *generalisation of stimuli*. By continuing to apply the tone first used always in association with the unconditioned stimulus and presenting any other allied tone invariably without reinforcement, the latter speedily becomes ineffective—if, of course, the animal is capable of differentiating between them. It will appear obvious that this provides a good physiological means of investigating the sense analysers.

Many more complex phenomena relating to conditioned reflexes have been investigated by Pavlov and others, but as they are outside the scope of the present article they need not be described here.

It is becoming more and more stressed that inhibition is one of the main functions of the cerebral cortex. What performs this most necessary function in fishes ?

(c) REPRESENTATION OF THE RESULTS OBTAINED IN THE PRESENT INVESTIGATION.

A uniform graphical method of recording the results has been adopted throughout the paper for the sake of brevity. Each record (cf. Figs. 3 and 4) indicates the number of associations made, their nature, and their distribution in time. Days and months are marked off with small vertical lines on the base line—the number of experiments made daily is thus seen at once. This diagrammatic picture of the progress of " conditioning " has many limitations, for it is obviously impossible to record all details in this way.

A positive conditioned response is shown by a + (plus), no response by a − (minus sign). It may be taken for granted that the conditioned stimulus was invariably reinforced by the unconditioned stimulus (food, shock, or touch, as the case may have been) except where otherwise indicated, or in tests where experimental extinction was investigated. With many of the fish, proof of their capacity to differentiate between allied stimuli was attempted. This necessitates differential indication on the graph. It has been done by the use of dots, triangles, circles, or crosses, but it has not been possible to keep the meaning of these uniform for all the records, wherefore the legends or the correlated text should be carefully noted. The " original " or " primary " conditioned response is always denoted by a plain dot whether or not differentiation was attempted. Time records—that is, of the time interval between presentation of stimulus and performance of the conditioned response—are of great interest for comparison with the learning curves of other animals, apart from their bearing on the phenomenon of the " delayed response." These are the most important omissions from the graphical records and an endeavour will be made to bring out these relationships in the text. Important intervals or events having a direct influence on the behaviour of the fish are indicated by bold arrows at the appropriate point. These are lettered, and their effect, together with any inferences to be drawn from it, is also discussed in the text. Some of the experiments lasted several months and the records are lengthy ; where one line is not sufficient they are continued in the line or lines below. This is clearly indicated in the figures, and they should be consulted carefully and constantly when reading the paper which amplifies what the records show.

SECTION A. FOOD AS UNCONDITIONED STIMULUS.

(A) THE FORMATION OF A CONDITIONED RESPONSE IN *BLENNIUS GATTORUGINE* BLOCH, TOWARDS AN INCREASE IN TEMPERATURE OF THE SURROUNDING WATER.

There is much diversity in the type of environment which fishes naturally frequent, and a very great difference in their mode of living. The blenny, *Blennius gattorugine*, was chosen for this investigation partly because of the readiness with which it adapts itself to aquarium conditions, partly because it feeds readily in captivity, but mainly because of its own particular habits. It is a natural habit of the blenny to live in a suitable hole or crevice in the rocks, about low-water mark. For this reason, if a specimen is placed in an aquarium containing any empty pots or jars it will very soon make such a pot its permanent home and only leave it when attracted by food, etc. This at once suggested to me the possibility of using a pot in which the fish could live permanently and of utilising as a registerable response its habit of coming out when it saw food placed before it. The method of doing this in the present investigation was as follows :—

The Apparatus.

This is shown in general perspective as a diagram in Fig. 1. The heavier unbroken lines indicate the part visible to the observer—mainly screening arrangements. The screen surrounds a glass aquarium (Fig. 1, A), 30 cm. × 45 cm. × 15 cm., which together with its component and contained accessories is shown in the same figure in thin dotted lines, as it is hidden from view. The aquarium rested on a wooden bench, from which it was separated by a layer of felt to lessen vibrations, if any. The box B, Fig. 1, projecting towards the observer, was the compartment from which observations were made, and was sufficiently large to take my head and shoulders. This was completely covered in by black cloth, thus forming a darkened chamber. The side of the aquarium away from the observer was close up to a north window and was not screened in any way. The whole of the aquarium and apparatus within the screen could be surveyed through the telescopic lens (C, Fig. 1), placed at a distance of 26 cm. from the thermometer T, mentioned later. Food could be introduced through the small tube (E, Fig. 1) and dropped upon a platform in the aquarium, about 20 cm. from the opening of the pot F (Fig. 1).

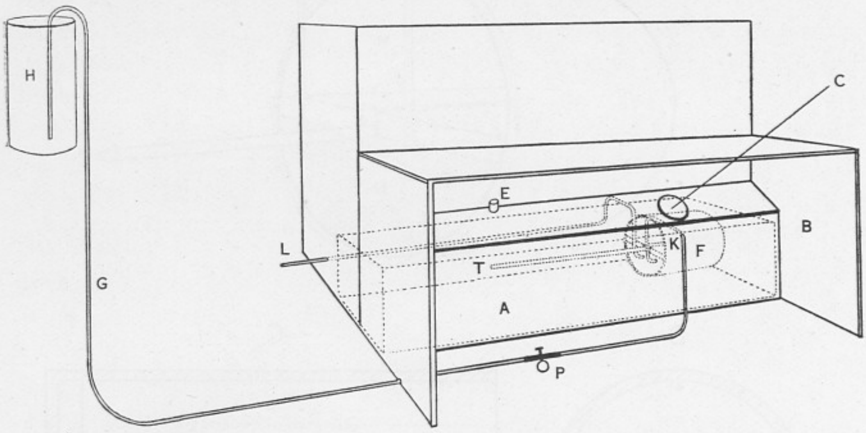

FIG. 1. General view of the apparatus used in the formation of conditioned responses in *Blennius gattorugine* towards *temperature* stimuli. Explanation in text.

The pot (Fig. 1, F) and its component fittings is the essential part of the apparatus. The general arrangement is shown by dotted lines in Fig. 1, and details are given in Fig. 2 (A) in perspective, (B) in elevation and (C) in plan. It is an ordinary 2 lb. earthenware jam jar divided longitudinally into upper and lower portions (Fig. 2 (A), M, N), separated from each other by a glass plate 1 cm. in thickness. There are two narrow gaps 0·7 cm. wide in this partition, marked *a* and *b* in Fig. 2, C. These permit of a free communication of water in the upper and lower portions. A piece of glass tubing 0·6 cm. diameter (Fig. 1, G) leads down from a small vessel (Fig. 1, H) containing hot water, placed about 200 cm. above the bench and 150 cm. away from the screen. This tube passes through the observation compartment, where it is provided with a pinch-cock (Fig. 1, P), connects with a piece of lead tube of the same bore which passes through the screen at K (Fig. 1), runs over the side of the aquarium and enters the lower compartment of the pot. Its course through the pot is shown in Fig. 2, C, as a dotted line in the form of a U-tube. On emerging from the pot the tube bends sharply up again over the further side of the aquarium and then leads away to waste (Fig. 1, L). The mouth of the lower chamber is closed by a large bung, which supports the heating tube. All joints in the pot, all crevices, and the surface of the bung, are well fixed and covered with marine glue, so that the lower part is completely closed except for the gaps leading to the upper chamber (Fig. 2 C, *a*, *b*). An accurate thermometer, graduated to 0·1° C. (Figs. 1 and 2 A, T) lies in the gap *a* (Fig. 2 C) in a horizontal position.

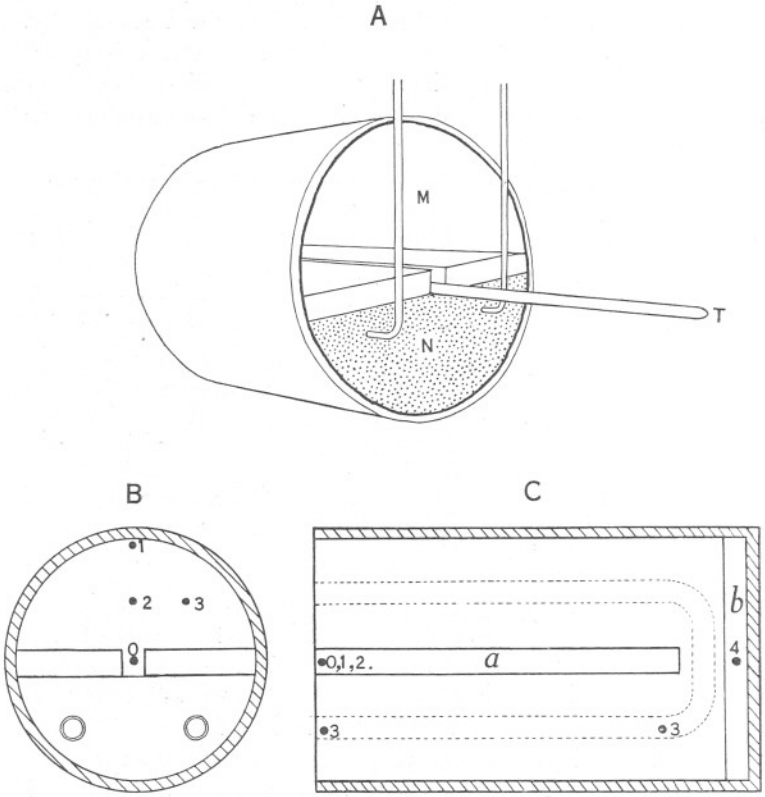

Fig. 2. Diagrams showing details of the apparatus used in the formation of conditioned responses in *Blennius gattorugine* towards *temperature* stimuli. Explanation in text.

The aquarium was filled with sufficient sea-water to cover this pot, which, as will have been surmised already from the introductory note to this section, was to be the home of the blenny.

Now, on heating the water in the lower compartment of the pot by passing hot water from the vessel H (Fig. 1) through the tube G (Fig. 1), the *heated* water finds exit through the gaps *a* and *b* (Fig. 2, C), passes straight upwards to the top edge of the pot, then forwards to enter into the main water of the aquarium. A constant temperature of 70° C. for the *heating* water was used throughout. By passing this water through the tube GL (Fig. 1) for only a few seconds, a slight rapid rise in temperature is recorded on the thermometer in the middle gap, and by varying the time of flow this rise can be made as large or small as desired (cf. Table II). It must be clearly understood that this local heating is very rapid—not prolonged, and that the temperature required is attained in 2 or 3 seconds—the thermometer column rising suddenly. As soon as the passage of hot water through G is stopped, the thermometer quickly indicates only the normal temperature of the surrounding water. For the correct interpretation of the results it is necessary to know exactly what does happen with regard to temperature increments in the upper compartment. (It is hoped, after these preliminary experiments have been completed, to repeat them, using a thermo-couple, which will give more reliable measurements.) I therefore took a series of readings at various places in the upper compartment and compared them with the reading of the fixed experimental thermometer in the gap. They gave the following results for the positions indicated in Fig. 2, B and C.

TABLE I.

Position.	Rise in temp. per 1° C. rise on recording thermometer (T).
1	0·35° C.
2	0·75° C.
3	0·09° C.
4	0·35° C.

Observations thus made show that a convection current of warm water is created, which passes vertically upwards through the two communicating spaces to the top of the pot, and then forwards to the entrance. Slight conduction takes place and probably accounts for the very small rises at position 3. These results were further confirmed by the use of carmine in suspension.

Method of Experimentation.

It has been already mentioned that it is a natural habit of the blenny to live in suitable holes, etc., and the fish normally spent all its time in the upper half of the pot.

Several times daily for a period of three weeks, yet at irregular intervals during the day, the fish was subjected to a stimulus of 3·5° C., sometimes to rises up to as much as 10° C. obtained in the manner described above. These stimuli, which were unassociated with food, caused no visible response in the fish upon any occasion. The blenny was fed at intervals during this period. The feeding times were far apart from the times when the fish was subjected to the temperature stimulus (at least 30 minutes either way). A record of these preliminary tests is unnecessary. The total number of stimuli given to the fish in this way was 42.

The experiments upon the formation of a conditioned response to a slight rise in temperature of the surrounding water as shown by the reading of the thermometer T were commenced on December 13th, 1926. They were carried out as follows :—Hot water at a temperature of 70° C. was allowed to flow through the tube GL (Fig. 1) until the thermometer T indicated the temperature desired ; this was attained in a few seconds, and the stop-cock closed, in accordance with the above description. The temperature started to fall at once. *Fifteen seconds* after the maximum temperature had been reached, a worm was dropped through E (Fig. 1). By this time the thermometer reading was back to within 0·2° C. of the original temperature. In most instances the blenny came out of its pot at once when its food, a Nereid worm, was given, snapped at it eagerly, gave a cautious glance round, and went back into the pot. This was the " unconditioned " motor response which was used in building up the *conditioned* response.

THE RESULTS.

Specimen No. 1.

The graphical record, Fig. 3, in which a dot in the lower (negative) position indicates a stimulus to which there was no response, and a dot in the upper (positive) position indicates that the fish responded by coming out of the pot before any food was given, shows at a glance certain distinctive features.* It shows an early period, lasting from the 13th December, 1926, to the first experiment on January 2nd, 1927, during which the fish showed no response to a temperature increment, no matter what its magnitude may have been. During this period the number of associations presented was 42,† although some allowance should be made for a period of eight days, December 21st to December 30th, 1926, when no experiments were made owing to my absence. What influence this may have had by retarding the progress of " learning " is not assessable. Very likely it was great, if a comparison can be made with some of the other fishes, or even the same fish, where the effect of an interval was noticed when learning had become more complete. From the 2nd January, 1927,

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* The arrows indicate the occurrence of events, such as cleaning out the apparatus, or intervals when no experiments were made, which might cause an inhibition of the response. They are discussed more fully in their appropriate place in the text.

† It is a curious coincidence only that this number is the same as the number of preliminary unassociated stimuli.

until about the 26th the proportion of the number of definite positive responses to that of no apparent responses became progressively greater. After the 26th January and until the series was completed, the fully-formed conditioned response of coming out on perceiving the temperature stimulus remained perfectly stable, except when influenced by inhibitory stimuli. These are the broad features in the progression of learning in this fish. They can now be treated in more detail.

The record, firstly, gives no information upon the temperature rises used as the conditioning stimuli—limitations of space prevent it. These are accordingly given in a separate table (Table II). The correspondence between the graphic record and the tabulated temperatures is quite straightforward :—each dot on the record represents a stimulus given, whilst the temperature used is indicated in the table in similar sequence for each day.

TABLE II.

Showing the Amount of the Temperature Rises given as Conditioning Stimuli to Specimen No. 1, Corresponding to the Record of the Results in Fig. 3.

Date.	Temperature increments given. °C.
1926.	
Dec. 13.	3·0, 3·0, 2·0, 4·0, 3·0, 3·0, 3·0.
14.	3·0, 3·0, 2·9, 3·0.
15.	3·0, 3·0.
16.	4·0, 4·0.
17.	4·0, 4·0, 4·0.
18.	5·0, 5·0, 5·0.
19.	6·0, 5·0, 5·0.
20.	6·0, 5·0, 5·0, 5·0, 5·0.
21.	5·0, 5·0, 5·0, 5·0.
30.	5·0, 5·0.
31.	5·0, 5·0, 4·5.
1927.	
Jan. 1.	5·0, 5·0, 5·0.
2.	4·0, 3·0, 3·0.
3.	4·0, 3·8, 3·6, 4·0, 3·0, 4·4.
4.	4·5, 5·0, 4·1.
5	4·0, 4·0, 3·5.
6.	2·6, 3·3, 3·8, 3·6.
8.	2·0, 2·6.
10.	5·0, 5·5, 6·0.
11.	6·5, 7·0, 6·2, 5·0.
12.	5·1.
13.	4·8, 4·2, 5·2.
14.	4·2, 4·3.
15.	3·2, 6·0.
17.	5·0, 5·0, 6·5, 6·7.
18.	5·0, 5·5, 6·5.
19.	4·0, 6·0, 4·5.
20.	4·0, 3·3, 4·0, 3·0.
21.	6·4, 4·4, 4·5, 4·5, 2·5, 3·1.
24.	4·0.
25.	4·5, 4·4, 4·6.
Jan. 26.	3·5, 2·7, 2·5, 2·6.
27.	5·5, 4·7, 4·4.
28.	4·3, 1·0, 4·0, 1·8, 2·1.
29.	1·9, 3·0, 2·1.
Feb. 3.	5·0, 4·2, 3·8, 1·0, 3·3.
4.	3·5, 3·7, 3·1, 2·4.
5.	3·2, 2·0, 2·2.
8.	3·2, 2·0, 3·0, 2·8.
9.	3·0, 6·0, 2·0, 5·0.
14.	3·0, 3·0.
17.	1·0, 1·0, 0·9, 1·2.
18.	3·0, 2·0, 2·0.
19.	2·8, 3·2, 3·0.
21.	2·5, 2·3, 2·0.
22.	3·0, 4·5, 2·5, 1·6.
23.	1·3, 3·6, 3·0, 1·5.
26.	2·3, 2·4, 2·8.
28.	2·1, 1·5, 2·5, 0·7.
March 1.	1·0, 1·5, 1·0.
2.	3·0, 0·9, 3·5.
8.	1·5, 4·0, 1·5, 2·0, 3·4.
9.	2·1, 1·0, 3·5, 1·0.
11.	1·0, 1·2.
12.	1·3, 1·5, 3·5.
14.	0·6, 0·5, 2·4, 3·7.
15.	0·5, 0·7, 3·5.
16.	1·5, 0·7, 3·5.
17.	1·5, 0·4.
18.	3·2, 0·6, 0·5.
30.	4·0, 6·4.
April 1.	3·5.
2.	3·5, 1·5, 1·1.
3.	3·5, 1·2, 3·7.

During the period when the response was becoming established, the average temperature rise given as a stimulus was 4·1° C., with a minimum of 2·5° C. and a maximum of 7·0° C. After that time the stimulus was made as generalised as possible, in order to find the lowest temperature increment to which the blenny would respond if continually reinforced by food. On January 28th, test No. 2, no response is recorded to a rise of 1·0° C., the first time such a low rise had been used; the worm was given after the 15-second interval. Later on the same day (tests 4 and 5) a positive response is recorded to a rise of 1·8° C. and 2·1° C. respectively. Increases of 1·9° C. and 2·1° C. evoked a positive response on the 29th, but again on February 3rd (test 4) a rise of 1·0° C. failed to produce a response. As it so happened, the last of these tests occurred at a time (arrow C, Fig. 3) when there was probably slight inhibition owing to disturbing external influences. The water in the apparatus had been changed and the fish had been removed to another tank for 4 days. Yet the previous stimuli of 4·2° C. and 3·8° C., being the second and third after the fish had been replaced in the apparatus, evoked a positive response, as also did the succeeding stimuli of 3·3° C., 3·5° C., etc., although the first test after its return, as invariably occurred, gave no response. Moreover, no such extraneous influence appears to have been present when 1·0° C. rise was first tried on January 28th. So that these two occasions when no response was shown towards the earliest rises of 1·0° C. may perhaps be interpreted as showing that the fish could discriminate between an increase of 1·0° C. and an increase of 2·0° C. or more, without differential training.

Some days later, after another 18 associations at which temperature stimuli had ranged from 2·0° C. to 6·0° C., with an average of 3·2° C., all of which with two exceptions had given strong positive responses, an increase of 1·0° C. was again tried (Feb. 17th, tests 1–4). Now, very strong responses were given. The stimulus had become more generalised and further evidence obtained upon the lowest temperature increase to which the fish was sensitive. A very interesting observation was recorded at this time.

Not only did the fish perform the motor response of coming out of its "home" very rapidly and eagerly, but it made very noticeable jaw movements (as though in anticipation). This has subsequently been seen in other blennies which have formed conditioned responses of a similar nature towards other conditioned stimuli, or I should consider that I have read more into this than the facts justify. It must be regarded as an additional feature of the conditioned response and seems to appear when the fishes are more hungry than usual.

Positive conditioned responses were given invariably towards stimuli of increments varying from 0·4° C. to 6·4° C. from February 17th until

April 3rd, when the experiment was stopped. The apparatus was not sufficiently delicate to give a rapid rise of less than 0·4° C. This was the lowest temperature increment given, and generalisation had become sufficiently wide to cause it to evoke a positive response when the fish was stimulated by it.

It can therefore be stated, *that this specimen of* Blennius gattorugine *was capable of perceiving, and of profiting by, a convection current giving a momentary rise of as little as* 0·4° *C. in the temperature of the water at a point on the ventral surface of the fish.**

It cannot be regarded as absolutely *proved* that the fish has formed a conditioned response towards the increase in temperature as an isolated force, for its necessary complement, the convection current, may also have been acting as the stimulus or a part of it. But it is legitimate to *infer* that the temperature was the sole stimulus, or differentiation would not have taken place in the early stages of the experiment.

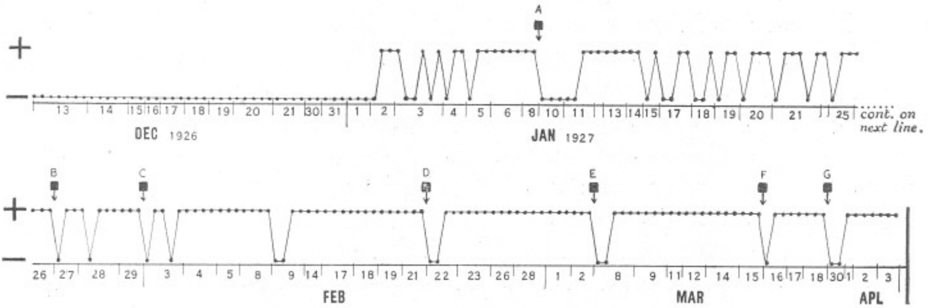

FIG. 3. Specimen No. 1.

FIG. 4. Specimen No. 3.

FIGS. 3 and 4. Records of the process of formation of conditioned responses in *Blennius gattorugine* towards *temperature* stimuli. Explanations in text.

The effect of changes in the external environment are very marked, especially when the conditioned response shows a long series of definite +s (positives). After the removal of the fish from the apparatus for

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* As the apparatus was not provided with any form of thermostat, the temperature of the water in the aquarium was always that of the room temperature at the time of the experiments. All rises are not, therefore, of equivalent meaning. The initial temperature varied from 6·5° C. to 13·0° C. in Specimen 1; but well over 75% of the experiments had an initial temperature of between 8° C. and 11° C.

cleaning purposes, whether the interval was of short (a few hours) or of long (12 days) duration, the first one or two stimuli succeeding its replacement invariably gave no response whatever. The arrows A (Jan. 10th), B (Jan. 27th), and D (Feb. 22nd) are placed against the dates when the apparatus was cleaned. An interval of 4 days without experiments occurred at C (Jan. 30th to Feb. 2nd), one of 5 days at E (March 3rd to 7th), and one of 12 days at G (March 18th to 30th). The effect is clearly obvious in the diagram. Even slight adjustments in the apparatus inhibited the response, as at F (March 16th). This seems to be clearly comparable to the phenomenon regarded by Pavlov as indirect or external inhibition.

Frequently, in order to ascertain the nature of the time-relations between the stimulus and the conditioned response, it was not followed by the unconditioned at the correct interval of fifteen seconds. When a positive response was given during the transitional, " learning," period, the time interval was longer than this—it fluctuated around 30 seconds; as " learning " progressed this became gradually reduced, until, at the last 80 presentations of the stimulus, it averaged only 10 seconds, with a range of 4 to 20 seconds. The " learning " curve is of the same general shape as those obtained in trial and error experiments of the " maze " type.

Specimen No. 2.

All conditions and methods were the same as for Specimen No. 1. The experiments lasted from April 30th, 1927, until 20th May, 1927, when the fish died. The conditioning stimulus was given in a similar manner to that described for Specimen No. 1. Fifty stimuli in all were given, but an entirely negative result was obtained. Not wishing to set up any disturbances, I had not interfered with the fish or the apparatus during this time. The fish regularly took the worms when given in reinforcement of the conditioned temperature stimulus, but on the 17th, 18th, and 19th May it never came out of the pot for food. On the 20th of May, to my regret, it was found dead with a tumourous outgrowth on the dorsal fin.

This pathological disturbance probably influenced the capacity of this fish to form the conditioned response, either by inhibiting it altogether, or delaying it for such a time that fifty stimuli were not enough to establish the response or to overcome the inhibition. Again, there is a striking parallel with Pavlov's observations on pathological disturbances in dogs, which is still further strengthened by other instances taken from my own work.

Specimen No. 3.

This specimen, for example, had been placed in the apparatus before commencing experiments with No. 2. For five days it showed great restlessness and would not remain in the pot even for a few moments when placed inside. It was hopeless trying to carry out experiments with her. This blenny was a large female very distended with eggs, large numbers of which were deposited during those five days in the tank. The day upon which she was removed, another large female very distended with eggs also, was introduced into the aquarium, and placed inside the pot. She also was given five days' trial, but behaved in the same way as No. 2. Pregnancy in dogs entirely inhibits the experimental formation of conditioned reflexes, says Pavlov. These blennies certainly appear to exhibit a similar phenomenon, for Specimen No. 3, which was kept in a main aquarium tank, laid all her eggs by the end of March, resumed normal behaviour during April, and when again placed in the apparatus on May 20th settled down at once in the pot and showed no further restlessness. Experiments upon this fish, to confirm the results on No. 1, were then started at once.

The record for this Specimen No. 3 is given in Fig. 4.

This fish formed the conditioned response remarkably quickly—after 3 days, and only 8 conditioning stimuli of an average temperature rise of 2·6° C., all of which were reinforced with food after the 15-seconds interval. It seems, as will be noticed in the record, to have become fully established suddenly. The nature of the temperature increments given is shown in Table III in exactly the same manner as that given for Specimen

TABLE III.

Showing the Amount of the Temperature Rises given as Conditioning Stimuli to Specimen No. 3, Corresponding to the Record of the Results in Fig. 4.

Date. 1927.	Temperature increments given. °C.
May 21.	3·5, 3·5.
23.	2·5, 3·0, 3·0, 2·0, 3·1.
24.	3·2, 2·3, 2·0, 2·5, 2·5.
25.	3·5, 3·5, 2·5, 2·3, 0·6, 0·5, 1·4.
26.	2·5, 2·4, 3·0, 1·0.
30.	3·0, 3·0.
June 1.	2·5, 1·0, 0·5.
2.	2·5, 1·7, 1·7.
3.	2·6, 0·8, 0·8.
5.	1·0, 0·5, 0·7, 0·6.

No. 1 in Table II. The conditioned response was given by this fish on the first applications of temperature stimuli as low as 0·5° C. and 0·6° C. (May 25th). This fish jumped out of the tank before it had been used for long (June 5th, 1927), and so it hardly gave me an opportunity to carry out the programme I had intended. Possibly the rapidity with which the response became established was due to the high initial temperature of most of the experiments. It was hot weather, and the water temperature averaged 15·4° C., considerably higher than that of the water in experiments on No. 1.

The capacity of *Blennius gattorugine* to form conditioned responses to this type of stimulus is however substantially confirmed.

(B) The Formation of a Conditioned Response in *Blennius gattorugine* Bloch, towards Salinity Changes in the Surrounding Water.

Considerable care was required in dealing with this stimulus in order to be certain that we were observing its isolated effect. It will appear obvious that any great artificial changes in the salinity of sea-water will also involve appreciable changes in the alkali reserve, hydrogen-ion concentration, and tension of dissolved gases, unless suitable precautions be taken. Moreover, as may be perceived from my study of the temperature response, the temperature of the water used as stimulus must be absolutely equal to that of the water normally flowing over the fish. Further, in giving this stimulus, changes in rate of current-flow must be guarded against. Any one of these separate altering conditions might reasonably be acting as a conditioning stimulus unless controlled and eliminated.

The Apparatus.

This was placed against the north wall of a small room adjoining the main tank-room of the Plymouth Aquarium. The amount of foot traffic four or five yards away was considerable, but the immediate vicinity of the apparatus was quite free from unusual disturbances; the constant noise of the water circulating through the main tanks was always present.

Fig. 5 shows diagrammatically the arrangement used in giving this stimulus. A and B are two 3-litre glass aspirator bottles, with outlet tubes F and E (of glass) leading by a T-piece to a single tube G which passes downwards in the manner indicated, to discharge into the end of a black, opaque, circular, plain-glass bottle (J), open at the further end. This bottle formed the permanent home of our *Blennius gattorugine*. (Cf. introductory account to temperature response.) It rested on the bottom of a small glass aquarium, 30 cm.×45 cm.×15 cm., which was entirely surrounded, save for the north side facing the window, by a wooden screen resting on the bench.

Normal sea-water entered the aspirator A through the tube C, which siphoned out water from one of the large tanks supplied with the circulating water of the Laboratory. The amount of water flowing through the bottle J over the fish into the aquarium K (provided with an overflow, not shown) was regulated by the tap G, so that its rate of flow was slightly less than that entering at C. It is obvious that water flows through F solely by virtue of the head of water in the aspirator A. A constant flow was assured by fitting a constant level overflow tube, L. This flow of Laboratory sea-water formed the normal environmental medium of the fish. The water to be used as a conditioning stimulus was run from a larger supply into aspirator B in the same way. B was surrounded completely by a jacket D, fed through the tube C with the same water as continually flowed through C into A. This kept the two solutions at identically the same temperature. The water-level in B was maintained exactly on a level with that in A. Hence, by opening stop-cock E and closing F the merest fraction of a second later, the stimulating water was caused to flow through G into J without cessation or alteration of the rate of flow.

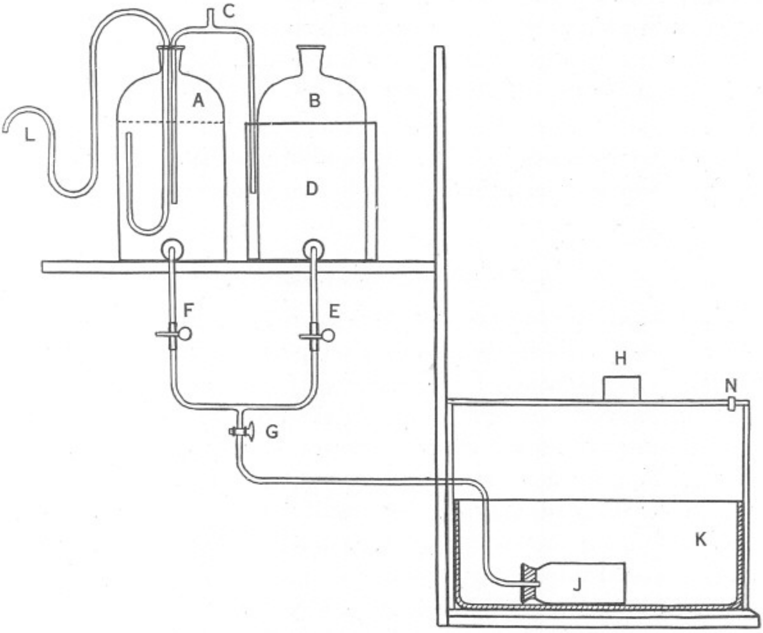

FIG. 5. Apparatus used in the experiments upon conditioned responses in *Blennius gattorugine* towards *changes in salinity*. Description in text.

A small hole 5 cm. × 3 cm. was cut out of the screen above the opening of the bottle J. Over this was placed a modified periscope arrangement (H), through which the reaction of the fish was observed. N is the opening through which food (Nereid worms, usually *Nereis diversicolor*, an estuarine form) was introduced.

The first solution to be used as a conditioning stimulus was Plymouth tap-water, as it was considered preferable to distilled water on account of the possible toxicity of the latter due to traces of copper, etc. It is practically free from all dissolved solids, except for a trace of organic matter, and has a pH of about 6·8. Fishes are probably sensitive to large differences in pH, so that after preliminary titration of the Laboratory circulating water, the experimental water was brought to the same alkali reserve by the addition of 0·421 gr. of sodium bicarbonate per 1000 c.c. of water. The water was then thoroughly mixed and agitated for 24 hours to secure a similar degree of equilibrium ; its pH, alkali reserve, and CO_2 tension now being identical with that of the circulating water.

Method of Experimentation.

The fish used in the first of these experiments had been previously used for the series of experiments with temperature changes as conditioning stimuli (Temperature, Specimen No. 1). Its normal behaviour remained unchanged—and the reactions observed were similar ones. It lived continuously and naturally in the pot J. When food was dropped in through N, the fish at once came out of the pot, swam to below the point N, took the food, and returned hastily to the pot. This action was used as the " unconditioned " motor response. The conditioning stimulus was given several times daily for a month, unaccompanied by food.

This may, in reality, be called a strong noxious stimulus, since blennies, although frequently left stranded for long periods by the tide, will not long withstand immersion in fresh water. It should perhaps be mentioned that *Blennius gattorugine*, when given any noxious or disturbing stimulus, will retreat to the most remote corner of the pot rather than leave its shelter. At no time during this period, when the preliminary effects of this stimulus were being investigated, did the fish give any sign of coming out of the pot. This strong, positive reaction towards corners (stereotropism in Loeb's sense, thigmotaxis in Doflein's wording) is exhibited by many species of blennies, and is an additional point in favour of using the " coming out " response as a measure of the conditioned response.

The routine of the tests was similar in the main to that followed in the temperature response :—The change in salinity was allowed to act for 15 seconds ; then followed an interval of 15 seconds, at the conclusion of which the worm was given. The experiments were started on April 8th,

1927. The temperature and pH of the normal Laboratory water and of the " stimulating " water were taken daily and always kept identical with each other. The extent of the change in salinity presented as a stimulus varied slightly from time to time owing to changes in the initial salinity of the circulating water which fluctuated around 36 to 37 parts per thousand.

THE RESULTS.

Specimen No. 1.

Fortunately it is here possible to represent almost all the important points in the diagrammatic record, Fig. 6. It should be remembered that the fish had been thoroughly accustomed to aquarium conditions for several months, which, one would have thought, would have made it easier to build up further responses. Three main periods are to be noted during the time when the change in salinity was as much as 37 parts per thousand. Firstly, April 8th to May 8th, covering 30 presentations of the conditioning stimulus, during which no response is recorded throughout. However, there was a long period, April 10th to 25th, when I was absent from the Laboratory and no associated trials were made, although the fish remained undisturbed in the apparatus and was regularly fed. Some allowance should undoubtedly be made for this. Frequently during this period when no responses were given the fish did not come out at once to take food when given, there being a delay sometimes of one or two minutes.

Following this, there is the transitional period during which the response became gradually established (May 8th to 16th). The conditioned response was not fully integrated at the time of its appearance, and a gradual increase in its strength took place. At the early stages, this fish came out only a short way, so far that its head and pectorals alone were visible in the periscope. Later, when the response was fully formed, the fish came right to below the food-hole and remained there till food was given, before returning. Mouth movements similar to those recorded in the temperature response, Specimen No. 1, were in the later stages frequently observed.

Then, from May 17th onwards, the presentation of the stimulus never failed to evoke a response towards the change from $37^{\circ}/_{\circ\circ}$ to $0^{\circ}/_{\circ\circ}$ (approx.)

Causing the stimulus to become as generalised as possible again seemed the best way of finding out the smallest salinity change capable of eliciting the conditioned response. Accordingly, I decided gradually to decrease the magnitude of the change without attempting to bring about any differentiation by training.

Alterations in the salinity of the " stimulating " solution were made by mixing the Laboratory circulating water from A (Fig. 5) with Plymouth drinking water in the necessary proportions. The first alteration in the magnitude of the change in salinity used as a stimulus was made on

May 26th (arrow A in the diagram Fig. 6). The diagram shows the alterations in the strength of the stimulus as having been made suddenly; actually during the first day whilst the " stimulating " or " signalling " solution was altered, the change over was made gradually, the two solutions becoming slowly mixed. Increasing the salinity of the " stimulating " solution from 0°/$_{\infty}$ to 7°/$_{\infty}$, and thus reducing the difference between the normal water and the " signalling " solution, appears to have had no effect upon the conditioned response, which was given as before. A salinity change from 37°/$_{\infty}$ to 7°/$_{\infty}$ was thus used during the period A to C (Fig. 6), and constantly evoked a positive response. No experiments were made from the 10th to 16th June, indicated by arrow B, the fish being fed occasionally without previous " stimulation." This had no effect whatever upon the strength of the " conditioned response." From arrows C to D, June 17th to July 18th, the salinity of the solution in aspirator B was still further increased to 17°/$_{\infty}$, thus giving as a conditioning stimulus a change from 37°/$_{\infty}$ to 17°/$_{\infty}$, a difference of 20°/$_{\infty}$. On June 23rd and July 11th no response at all was given on one occasion upon each day, for which no reason was apparent, but an interval of 14 days (June 23rd to July 8th) with no experiments had no weakening influence so far as could be seen. At D, July 19th, another change was made in the salinity of the " signalling " solution—the salinity being increased to 30·2 parts per thousand. Before making this alteration, how-

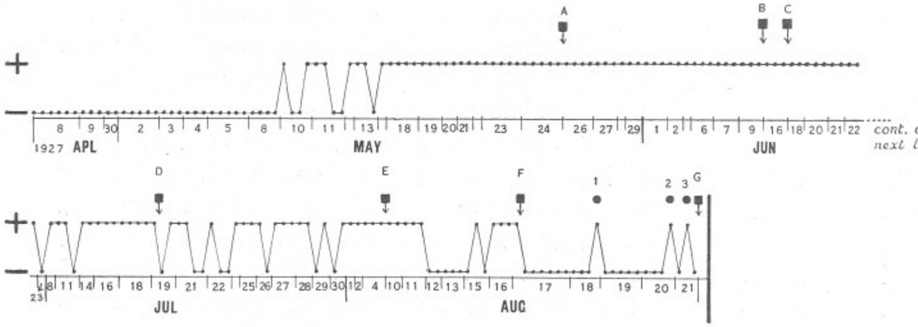

FIG. 6. Specimen No. 1.

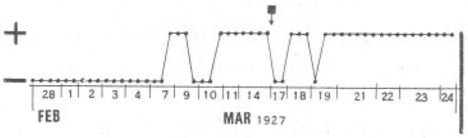

FIG. 7. Specimen No. 2.

FIGS. 6 and 7. Records of the process of formation of conditioned responses in *Blennius gattorugine* towards *changes in salinity*. Explanations in text.

ever, and in order to keep a check upon the true nature of the stimulus, I allowed the normal circulating water to flow into aspirator B (Fig. 5) and made the conditioned stimulus change from aspirator A to B in the usual way for 15 seconds, of course, without following it up with food. This was a precautionary measure to see whether movement of the stop-cocks or other manipulations were acting as stimuli. Four successive tests produced no response, and afforded a fairly definite proof that the salinity change was acting as sole stimulus. Conditioning tests were then resumed. Two days later, on July 21st (Test 3), we obtained signs of a falling off in the strength of the conditioned response. Absence of a response again occurred on the 22nd, 26th, 29th, and 30th. Upon each of these occasions the food however, when presented after the necessary interval, was itself ignored and the fish was apparently not hungry. The failure to respond does not appear to have been due to failure to discriminate the change.

The internal condition of the fish is one of the main forces of the experiment and it is a serious drawback to all discussions upon conditioned responses, that it cannot be considered in its logical relations with the situation as a whole, for we have no insight into it.

The salinity of the solution B was brought still nearer to that of the normal water on August 10th (arrow E) by being increased to 33·7°/$_{oo}$. All stimuli given by this solution on the 10th and 11th evoked a positive response, but this was followed on the 12th by signs of failure to discriminate which, on continuing, seems to have been overcome on the 16th. This may, of course, have been due to other causes.

Finally, the limits of discrimination were reached with a salinity of 34·3 parts per thousand used as the " signalling " solution, which was first used on the 17th August (arrow F). No positive response was evoked by this solution at any time during the next 4 days, 20 trials in all, although solutions of 33·7°/$_{oo}$ (arrows 1 and 2) and 17°/$_{oo}$ (arrow 3), as used before, both evoked well-marked positive responses.

This specimen of a common shore fish is therefore shown to be able to discriminate a change in the salinity of the water around it of 3 *parts per thousand, and, moreover, to be able to associate such a change or one of greater magnitude, with the acquisition of its food.*

Specimen No. 2.

(Record in Fig. 7.)

This fish jumped out of the apparatus for some unknown reason, and eventually died before I had progressed very far with the experiments. Nevertheless, it substantiated the earlier portion of the results from specimen No. 1 and deserves mention. The fish was installed on February 7th, 1927, and left to accustom itself to its surroundings until February 28th, Repeated introductions of the " strongest " conditioned stimulus without reinforcement (the change from 37°/$_{\infty}$ to 0°/$_{\infty}$) gave no response during this time. Conditioning to this stimulus commenced on February 28th, and within 7 days (18 trials) a positive response was obtained. The transitional period lasted until the 19th March. Dismantling of the apparatus may have been responsible for the failure to evoke a response on the 17th March. It was not necessary to do this at any time with Specimen No. 1, and we cannot therefore compare the effect with anything in the other fish. From the 19th until the untimely accident to the fish on the 24th March, presentation of the conditioned stimulus gave a fully-formed conditioned response on each occasion, seventeen associations in all.

(c) THE FORMATION OF A CONDITIONED RESPONSE IN THE WRASSE, *CRENILABRUS MELOPS* (L.), TOWARDS VIBRATORY (AUDITORY ?) STIMULI.

The Apparatus.

A wooden tank 100 cm.×20 cm.×20 cm. with sides 3 cm. thick contained the fish and was supplied direct with the Laboratory circulating water. The general arrangement is shown in the diagram, Fig. 8.

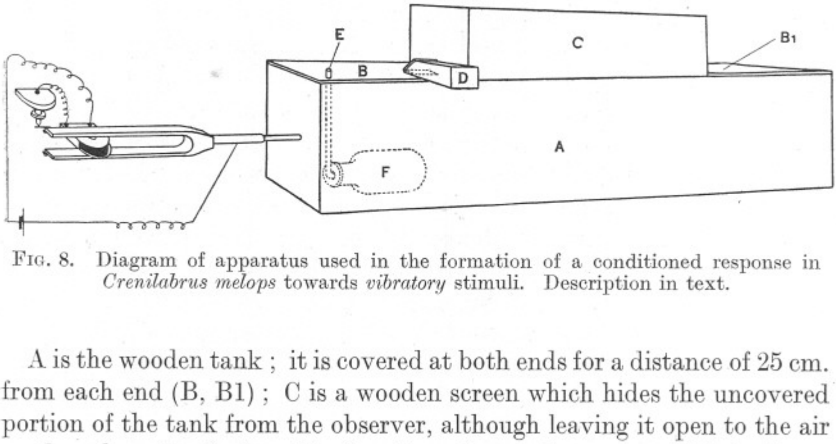

FIG. 8. Diagram of apparatus used in the formation of a conditioned response in *Crenilabrus melops* towards *vibratory* stimuli. Description in text.

A is the wooden tank ; it is covered at both ends for a distance of 25 cm. from each end (B, B1) ; C is a wooden screen which hides the uncovered portion of the tank from the observer, although leaving it open to the air on the other side, facing a blank wall, and a window. A small hole (5 cm. square) is cut in the covering piece B, and a small mirror placed over it at an angle of 45° ; this is fitted in an elongated box D, forming a modified periscope arrangement. Food (which consisted of an estuarine worm, *Nereis diversicolor*) may be introduced through the tube E and washed down into an opaque bottle F, the opening of which lies immediately below the periscope mirror. Vibratory stimuli were transmitted to the water by means of the large electrically-maintained tuning-fork, the prong of which impinged on one end of the tank. A frequency of 128 D.V.'s per second was given by this instrument.

The Experiments.

The wrasses, unlike the blennies, are active swimming fish and not sedentary forms, so that in this case the fish normally swam about more or less continually, and only entered the bottle when food was sent down into it. The experiments were carried out in the same general manner, except that the sound was allowed to act right up to the time when the fish took the food from the bottle.

The tuning-fork was sounded for 15 seconds ; a worm was then sent down into the food-bottle ; the fish at once entered and took the worm. It was desired to condition the fish to enter the food-bottle on hearing the sound, before food was given, and to wait there until it received the food. The results were, perhaps, the clearest of all that were obtained with any specimen and the record, Fig. 9, requires little comment.

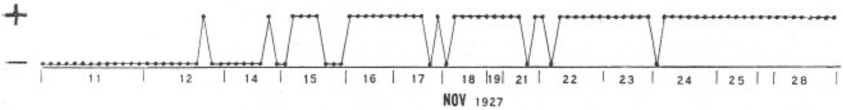

FIG. 9. Record of the process of formation of a conditioned response in *Crenilabrus melops* towards *vibratory* stimuli. Explanation in text.

A fairly large specimen of a wrasse, *Crenilabrus melops* (length 23 cm.), was used ; it was installed on November 10th, 1927, and at once became accustomed to its surroundings. Conditioning began on the 11th. After 20 associations with food, the first positive response was recorded in which the fish entered the bottle and waited until food was given. The response became speedily established, and on the 22nd November was quite complete. The same general phenomena observed in the previous fishes were thus also obtained here, but learning was relatively quicker than with most of the other fishes recorded in this paper.

At the time of writing up these results, further experiments are in progress with this fish, from which it is hoped to obtain evidence upon its capacity to discriminate between the vibrations from the tuning-fork and those from a submerged buzzer. The latter is associated with a food-bottle at the other end of the tank (B1), and an endeavour is being made to establish the correct associations between the respective sound and bottle. These results will be given in a later paper.

Other Experiments Involving the use of Vibratory Stimuli as Conditioning Stimuli, with Negative Results.

These were carried out on the same general principle as the last and previous experiments. But instead of the strong vibrations of the tuning-fork being transmitted direct to the walls of the aquarium, a submerged telephone was used. A low-resistance ex-army, field headphone was completely encased in a thin metal box, one side of which was closely applied to the diaphragm of the 'phone. This was arranged to transmit the vibrations from the same tuning-fork introduced into an electric circuit. I could myself, with the head entirely submerged, hear the " telephone " clearly at a distance of five yards under water.

The first experiment was performed on a specimen of the wrasse, *Crenilabrus melops*, in exactly the same way as that which gave a successful result with the tuning-fork impinging on one of the walls of the tank. From November 20th to December 16th, 1926, there were no indications of a positive response whatever. There were secondary complications at this time, and the result cannot be regarded as definitive. For some unknown reason this fish, as well as other species subsequently tried about the same time in the same tank, refused to take food.

In another experiment, started on January 4th, 1927, the " telephone " was introduced so as to form the closed end of a jar in which a specimen of *Blennius gattorugine* (cf. temperature and salinity responses) lived. The reverse response was here aimed at, that of conditioning the blenny to come out of the jar for food on hearing the telephone. There seemed much more likelihood of this being achieved, since the distance of the blenny from the sound was very small and more or less constant. Nevertheless, from January 4th till February 14th, 1927, during which time 109 associations were given, no positive conditioned response was recorded on any occasion.

I am fairly convinced that with the wrasse faulty technique may have accounted for the failure to obtain a positive result. No certainty can be attached to either result until more information has been obtained, but the results should be compared with those given in Section B of this paper, where those obtained with the use of an electric shock as the unconditioned stimulus are recorded.

(D) THE FORMATION OF CONDITIONED RESPONSES IN THE WRASSES *LABRUS BERGYLTA* ASC. AND *CRENILABRUS MELOPS* (L.) TOWARDS VISUAL STIMULI.

The Differentiation of Source, Intensity, and Wave-length of Light.

Specimen No. 1.

The first experiment of this series was of a very preliminary nature when begun, and improvements in apparatus and mode of treatment were being constantly made.

This, and all other investigations upon responses towards light, were carried out in a small dark room where vibrations from traffic were negligible, and where the usual noises of the Laboratory were almost absent.

The experimental fish, *Labrus bergylta*, length 12·0 cm., taken from the Laboratory tanks, was placed in a small bell-jar aquarium, 45 cm. in diameter, which was provided with ample aeration from the compressed-air supply, but not with circulating water.

Although the fish was fed very well, it was found that under these conditions the hydrogen-ion concentration of the water hardly varied over a period of at least three weeks. The temperature ranged from 10° C. to 13·5° C.

The fish was completely screened by solid wooden partitions from the observer, except for a small hole 5 cm. square, through which its movements were watched. This was easily possible, as the room was kept in total darkness during the whole period of the investigations, and it should be remembered that the fish was therefore " dark-adapted."

The food given as the unconditioned stimulus was standardised both in nature and quantity, in the same way as food given to dogs when they are used for undergoing experiments on " natural " conditioned reflexes. Small pieces of Nereis, 2 or 3 cm. in length, were found most suitable. They were introduced from the exterior through a covered glass tube, by means of a small current of water.

Method of Experimentation.

An unshielded 60-watt Fullolite gas-filled electric lamp was suspended at a height of 16 inches from the surface of the water. The turning on of this light constituted the first stimulus to which the fish was trained to react.

From October 21st to November 3rd, 1926, the food on being introduced was simply allowed to fall on to the floor of the aquarium, but invariably in the same position.

Our first experiments were directed towards seeing whether the fish would swim to this particular spot of the aquarium when the light appeared —a very simple motor response associated with feeding. For two days the sudden appearance of the light caused the fish much uneasiness, and it swam violently round and round. Nevertheless the worm was taken immediately when given. For five days there was no noticeable reaction towards the feeding-place when the light was switched on, but on the sixth day the fish showed signs of swimming to the feeding-place when the light appeared. On the seventh day, after twenty such associations, a very definite reaction was noticed. A few seconds after the light appeared, the fish, which was at first motionless, swam to the feeding-place, and remained there looking upwards at the spot where the worm was introduced. This reaction was given twenty successive times, spread over the next three days, and is in itself illustrative of the general phenomenon of learning in these fish.

After these preliminary experiments, the elicitation of a more complex form of response was commenced. The plain glass feeding-tube was removed, and a bottle, arranged as shown in the diagram, Fig. 10, was substituted in its place. A is the partition screening the fish from the observer: B is a covered tube through which the worm was introduced from without by a little water from a pipette: C is a plain glass reagent bottle, with the bottom partly cut out, leaving an opening in the shape of a segment; the lower uncut portion served as a ledge to prevent the worm from falling into the main body of water.

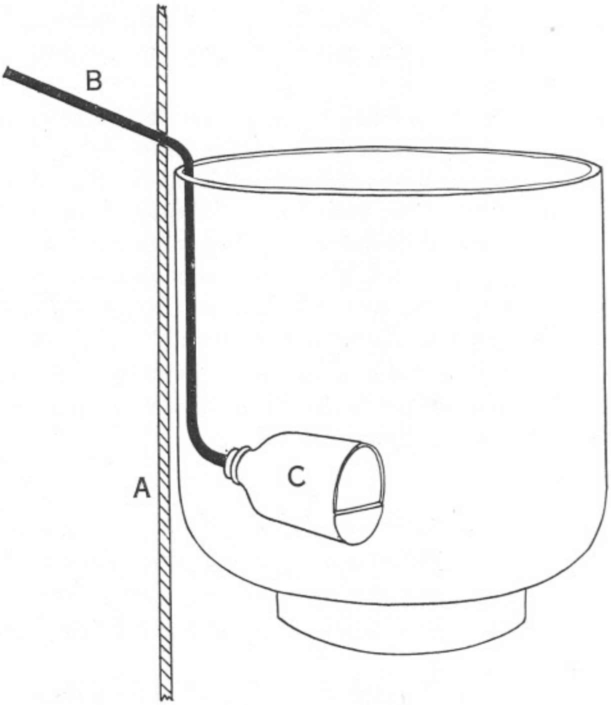

FIG. 10. Arrangement of the food-bottle in experiments on conditioned responses in wrasses towards *visual* stimuli. Explanation in text.

Next day, when the worm was introduced into the bottle 15 seconds after the light was switched on, the fish made violent efforts to seize the worm through the glass, in typical " wrasse " manner. These violent attacks alternated with slow " feeling " round the bottle, which was apparently invisible to him. After 5 hours 30 minutes, whilst " feeling " round the glass, the fish seemed suddenly to discover there was no resistance at the opening, went sharply in, and snapped the worm. The light was then switched off.

The time taken to gain entrance into the bottle became gradually reduced during the next six days, from several hours to a few seconds. But the fish gave up the useless efforts of trying to seize food through the glass bottle after five trials only. This is really only incidental to the main theme, yet it is interesting when compared with Möbius' account (**24**) of pike trying to seize minnows through glass.

On November 12th, at which date the diagrammatic record begins, the partition was taken down, and the bottle removed in semi-darkness. It was now thought desirable to eliminate all possibility that the sight of the worm was responsible for the fish's behaviour. Accordingly, the bottle was covered with plain white paper, and the whole thoroughly waxed. This rendered the bottle opaque, but left the interior sufficiently illuminated for the fish to see the worm inside when the light was switched on.

On November 15th the bottle was lowered so that it rested on the bottom of the tank. This was the last alteration in the feeding apparatus, and we may now re-state the problem attacked :—Could the fish be trained so that, when the light appeared, it would enter the bottle for food before food was introduced ? The sight of the worm under these conditions is the " unconditioned " stimulus, and the response of entering the bottle to fetch the worm is the " unconditioned " response involved in seizure of worm at sight. The appearance of the light is the " conditioning stimulus " with which we endeavour to build up a " conditioned response," involving entry into bottle when light appears, even when no food is given.

The light was left on for 15–20 seconds, and the worm then sent into the bottle. The response thus belongs to the class of " delayed responses."

THE RESULTS.

Over 500 associations with some form of light as a stimulus were made with this fish, and the diagrammatic record, Fig. 11, is thus very lengthy. It is a record of interesting successes and of interesting failures, and does not lend itself readily to a comprehensive survey.

The record has two distinct portions. The earliest, from November 12th, 1926, until January 22nd, 1927, being concerned with the results obtained upon the capacity of the fish to differentiate by training between the light from one 60-watt electric light bulb and that from two separate such bulbs. In other words, it gives information upon its *visual acuity*,

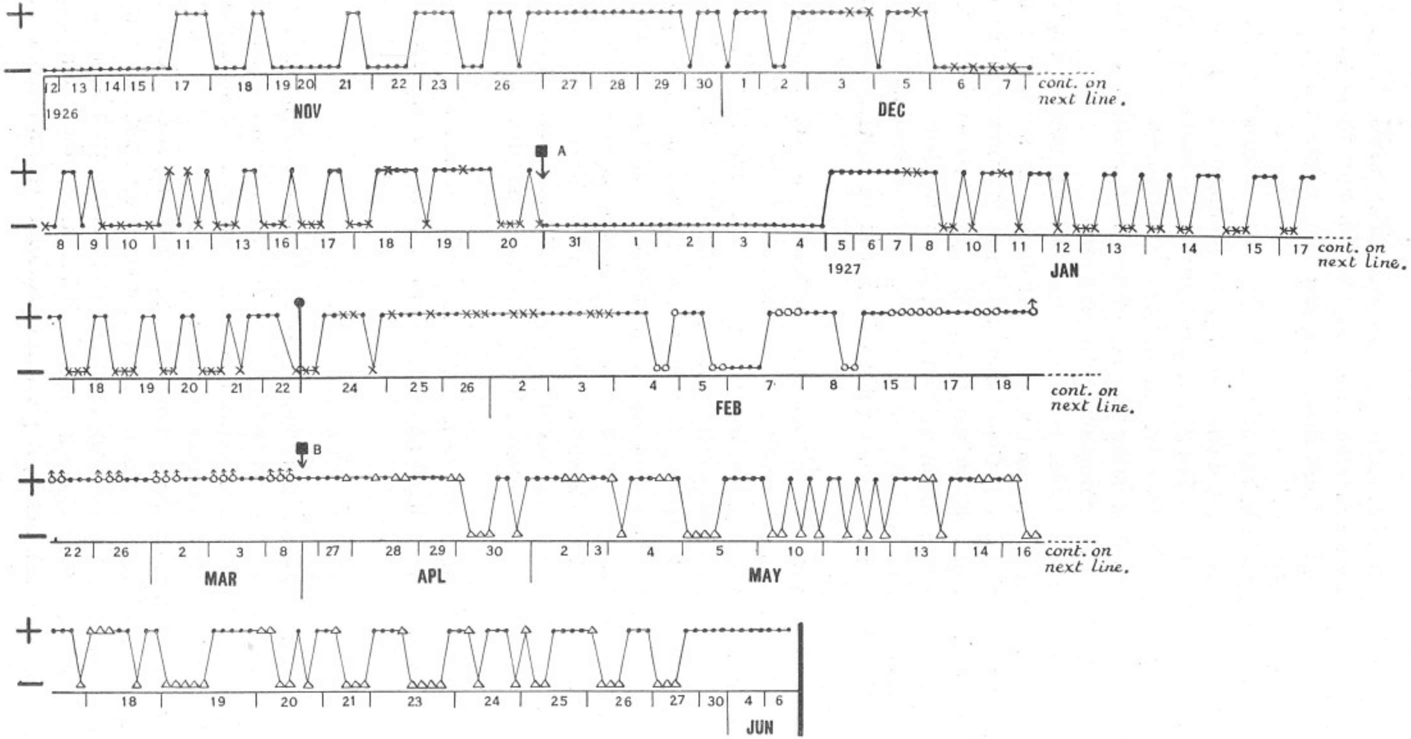

Fig. 11. Record of the process of formation of a conditioned response in *Labrus bergylta* towads *visual* stimuli. Specimen No. 1. Explanation in text.

as distinct from its *visual sensitivity;* that is, upon its capacity to discriminate the size, form, location, a number, etc., of such lights. Its capacity to differentiate between varying degrees of intensity in the light used as a conditioning stimulus is revealed in the second portion of the record, following January 22nd, 1927.

It has been necessary to use the same type of dot and cross for both portions, but they are not analogous. In the first portion a dot indicates that one unshaded lamp was used as a stimulus, a cross, that two lamps were used. In the second portion a dot represents one unit of *intensity*, the details of which will be given later, and the other signs indicate different multiples of this.

From the 12th to the 17th November, 1926, there were no signs of the building up of the response (which was here of the type described in the section on vibratory stimuli where a wrasse was used), and the fish showed a good deal of restlessness when the light was switched on. Four successive times on the 17th the fish entered the bottle before food was given. Entry into the bottle involved a somewhat sidewards inclination of the body of the fish on account of the narrowness of the opening. A gradual increase in the proportion of positive responses to those in which no conditioned response occurred is shown from the 17th to 26th November, by which time the conditioning appears to have become complete and to have remained so until December 3rd.

Additional features of the response soon became pronounced—much more so than those recorded for *Blennius gattorugine* in the temperature and salinity experiments. They seemed to be of the nature of anticipatory movements, and included waving of the tail and a peculiar side to side motion of the fish whilst in the bottle. So marked were they that during the later stages in differentiation they were of much help in determining the effect of a stimulus—the primary stimulus (of one lamp, or unit intensity) always evoking such movements whilst other stimuli being used frequently failed to do so, even though the fish performed the response of entry into bottle.

The only trials which failed to evoke a response during the period 26th November to 3rd December appear to have been influenced by internal conditions, for in my notes I recorded the fish as very sluggish at these times, and as ignoring the food also. As the response was now well established, I started discrimination tests. Another electric light similar to the first was placed alongside the latter on December 3rd; the two were so arranged that they could be switched on simultaneously or separately. The method of denoting this in the record has been mentioned above. The stimulus of two lights (represented by a cross in Fig. 11) was never followed by food, whilst the individual light (represented in the record by a dot) was invariably reinforced as before. No differentiation was shown at first (Dec. 3rd and 5th). Indeed, for some days (6th to 16th) the original response to the primary stimulus became considerably weakened, and some features peculiar in the response to the two lights became also temporarily present in that shown towards one light. Such features included hiding reactions—the fish tried to hide beneath the bottle, these movements alternating with periods of restless swimming. Possibly, as the fish was *dark-adapted* (a point to be remembered), the two lights were sufficiently bright to cause discomfort.

From the 16th December onwards, the original positive response to the one light became re-established and remained stable until experiments were temporarily suspended on December 20th, 1926.

Here followed an interval of importance, up till December 29th (marked by arrow A in the record), during which the fish was removed from the tank in the dark room and placed in one of the main aquarium tanks, an absence from experimental conditions of 10 days. On my return, the fish was replaced in the experimental room, and left undisturbed in total darkness for 48 hours. Upon resumption of the tests, the primary conditioned response towards one light was found to have become completely lost, and it required 30 stimuli, constantly reinforced, to establish it once more (Jan. 5th, 1927). Further, it was not before January 19th that the additional features of the primary response described above as " anticipatory " movements, were again observed.

From January 7th to the 22nd a systematic attempt was again made to establish the differentiation which had begun before the vacation, and this time with entire success. Only 3 times in all, once on the 7th, 8th, and 11th, respectively, did the wrasse give a positive response towards " two lights," whilst at the remaining 32 trials, with this stimulus, complete absence of a response was recorded. During the earlier stages (7th to 11th Jan.) hiding reactions were shown towards the " two lights." These afterwards gave way to slow swimming movements only, accompanied by no attempt to enter the bottle, although the stimulus of " two lights " was allowed to act for 2 minutes.

The evidence shown in portion (1) of the record, November 12th, 1926, to January 22nd, 1927, together with that given in the text, is sufficiently definite to justify the statement that *this specimen could, by the method of differential inhibition as defined by Pavlov* (cf. Introductory (b)), *discriminate between one light and two lights* (*used together*) *when used as reinforced and non-reinforced stimuli respectively.*

When the evidence given in the second portion is considered, it appears likely that the fish was *not* discriminating between the different degrees of illumination, but between one and two points of light.

Here followed the change over to the " intensity " experiments.

An elongated wooden box (30 cm. × 30 cm. × 75 cm.) painted dull black inside, was fitted up over the bell-jar. Across the lower opening of the box was fitted a translucent screen consisting of a piece of parchment paper between two pieces of glass. A 100-watt lamp fixed inside the box at a distance of 8 cm. from the screen gave an illumination at the screen approximately equal to that given by the original 60-watt lamp at the same distance from the bell-jar. When light of this intensity was used as a conditioning stimulus it is denoted in the record (Fig. 11) by the same type of dot used to denote the one lamp originally used. Another similar 100-watt lamp fastened to a piece of wire which could be moved up and down within the box, from outside the compartment, was used to provide any other intensity desired. Both lamps used simultaneously and at equal heights from the screen thus gave "2 × the original intensity." Very small intensities were obtained by pulling up the movable light away from the screen to the distance necessary.

It would have been quite rational to suppose that as the fish could differentiate between the light from one and that from two lamps used simultaneously, on being presented with a stimulus differing so widely in form as did the square illuminated screen from the unshaded lamp, the fish would have given no response. Initial generalisation of stimuli seems to be very wide, however, and except for three isolated occasions (on the day of commencement) when no responses were given the fish responded in a manner quite unchanged. It will be remembered that the "two lights" when first tried evoked a positive response, and that it was only after some time that the differentiation was obtained. Non-reinforcement of the stimulus undergoing differentiation actually inhibited the primary response upon that occasion. Now, although "2× unit intensity" was never reinforced, as it was desired to obtain evidence upon discrimination, "unit intensity" itself was constantly reinforced from the commencement. The initial generalisation was thus never broken down when "unit intensity" was used as stimulus and a constant positive conditioned response invariably resulted from its use up to the end of the experiments. It became, in fact, the new primary response.

Upon the 3rd February, as no signs of discrimination had been shown by the fish between "unit" and "2× unit intensity," I started an attempt to bring about discrimination between "unit" and "half-unit intensity" (denoted by a small circle in the record, Fig. 11), the latter, of course, not being reinforced by food. Distinct evidence for discrimination was shown at first. The "half-unit intensity" gave no response when first tried (Feb. 4th, tests 5 and 6); also upon the 5th (tests 4 and 5) and 8th (tests 5 and 6). For a short time (Feb. 7th) the fish was rather "sickly," and only gave very slow responses to the primary stimulus. It was put under circulating water for a short time and soon recovered. From the 8th to the 14th there were no experiments performed, but, contrary to the experience in December, 1926, this interval had no inhibitive effect. The initial discrimination between the " unit " and " half-unit intensity " then seems to have become lost, and a positive response was given invariably to both stimuli. Another factor was brought in upon the 22nd to see if the discrimination could be re-established when some inhibitive or disturbing influence was associated with the " half-unit intensity." When this combined stimulus was presented at the tests denoted in the record (Fig. 11) by the sign ♂, the food-bottle was given a violent jerk, which, under normal circumstances, would certainly have startled the fish. Although the fish actually was startled on each occasion, in a few seconds it entered, and would try to do so even whilst the bottle was being agitated. The conditioned response was obviously very powerful.

No evidence for discrimination between these stimuli could be obtained, and the use of this stimulus was concluded on March 8th, 1927. A very long gap then occurred in the experiments—up till April 26th (indicated by the arrow B). During part of this time the fish was again placed in one of the main aquarium tanks in normal daylight. On replacing it in the experimental tank it was left undisturbed, as before, for 48 hours. In spite of this prolonged absence from experimental conditions (8 weeks), no reduction was observable in the strength of the conditioned response. " Unit intensity," when given as stimulus and not even reinforced, gave the typical response unchanged.

We may state now, therefore, *that when the response has not been long established* (as at Christmas, 1926), *an interval of several days without tests causes an extinction of, or a great weakening of, the conditioned response in fishes ; whilst when it is firmly established* (as on March 8th) *even a lengthy absence from experimental conditions brings about no perceptible influence.*

A very low intensity was now brought into use with the object of making the difference between the two intensities used in discrimination as great as possible. If discrimination could then be established, we could work backwards and find the limits. A small triangle (△) is used to denote trials where this intensity was used as stimulus. A full statement of the various degrees of illumination will be given later. No signs of discrimination were obtained until April 30th ; positive conditioned responses entirely similar to those given towards " unit intensity " as stimulus having been given. The record then shows a preponderance of negatives with this stimulus, denoting that no responses were obtained, but the positive responses shown can hardly be called true positive responses. Thus, from the 30th April onwards, when the wrasse was given △ as stimulus, even when it entered the bottle (denoted as positive), the anticipatory movements were quite absent. The entry into the bottle was very slow and very distinct from the quick entry given towards the primary stimulus. Later (from the 16th May onwards) it is seen that this semi-positive response was given when △ was first tried each day, but subsequent trials on the same days evoked discrimination and no response. It appears that the comparison between the two had to be offered within a fairly limited period. The fish died on June 6th, 1927, and cut short any further discrimination, but the evidence is clear *that it only differentiates with difficulty between even very great differences in intensity of illumination.*

Whether these experiments should be regarded as throwing light upon discrimination of intensity of *illumination* of a surface, or upon *brightness* of a surface, is not certain ; with the type of screen used the terms have almost the same meaning.

The illuminations used in these tests were as follows :—By the term " unit intensity " as used in this paper is meant the *illumination* at the under surface of the illuminated screen when using one 100-watt Mazda lamp, on a 210-volt lighting circuit, at a distance of 8 cm. from that screen.

This equals approximately 7500 metre-candles, the others (2×) and (half-unit) being derived directly from this. Intensity △ (the lowest tried) was approximately 80 metre-candles.

The time-relations observed in this fish are interesting. The interval between presentation of stimulus and performance of response, which before conditioning had really begun averaged several minutes, became reduced in the earlier stages when the conditioned response had been formed to about 20 seconds. This time (20 secs.) was the latent period fixed by me as the time-interval between stimulus and food. Later, however, when the conditioned response was thoroughly established, the interval became reduced to a few seconds (about 5) ; indeed, the fish went into the bottle with great rapidity immediately the light appeared, and remained there during the remaining 10 or 15 seconds until food was given, before coming out. On May 6th and 7th no organised experiments were made, but the primary stimulus (unit intensity) was repeatedly given to demonstrate the nature of the conditioned response to representatives present at a Challenger Society meeting. None of these tests was followed by food, yet on each occasion the response was typical. The time-interval, however, between stimulus and response gradually lengthened to 50 seconds and indicated that, if this had been continued, the conditioned response would have undergone considerable weakening by " extinction." When reinforced with food, the response at once regained its normal time-relationship.

Specimens No. 2, 3, 4, and 5.

Introductory. These fishes were all very small, immature specimens of *Crenilabrus melops* or *Labrus bergylta* (about 6·5 cm. long), and were used in the formation of conditioned responses towards light of varying *wavelengths.* I was particularly unfortunate with all of them, for they died before I had time to get absolutely conclusive evidence upon their sense of colour-discrimination as revealed by this method. Valuable information was however obtained, and they all serve to illustrate general phenomena relating to learning in fishes. The same method was followed as in Specimen No. 1, but the apparatus was on a smaller scale and there were minor differences connected with the method of illumination, in order to allow of the ready interposition of a Wratten light filter between the source of light and the vessel containing the fish.

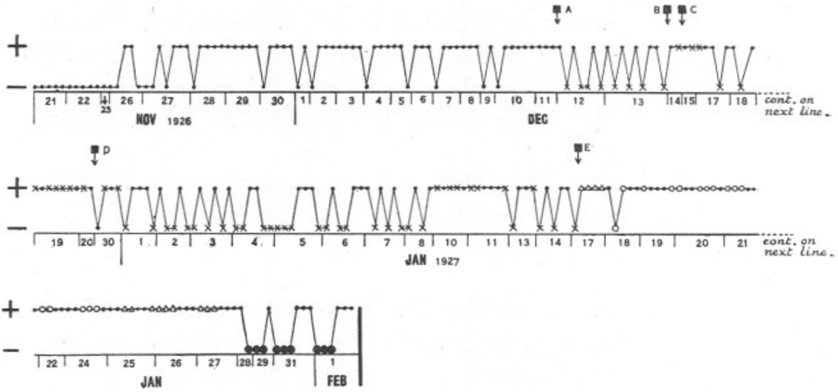

Fig. 12. Record of the process of formation of a conditioned response in *Crenilabrus melops* towards *visual* stimuli. Specimen No. 2. Explanation in text.

The time-interval and the nature of the response evoked were also the same.

Specimen No. 2 (Record in Fig. 12). This fish, *Crenilabrus melops*, was first conditioned to the unshielded light from a small 2-volt lamp worked by battery. As will be seen from the record, learning was relatively quick, the response having become well established in 7 days; it first appeared after 14 associated trials. There were occasions when no response was given. Such was the result when the first test was made on December 1st, 2nd, etc. On December 12th (arrow A, Fig. 12) tests were started with a Wratten's K3 light filter (yellow) interposed between the light from a similar 2-volt lamp of the same intensity placed alongside the first. This was used as a differential stimulus and never reinforced by food. If I had known then what I have since learnt from Specimen No. 1, that this left a loophole for misinterpretation of the results, I should not have used two separate sources of light, although contiguous with each other. This objection was afterwards removed, and is absent from other experiments in this series. There are periods, such as from the 12th to 13th December, 1926, and the 1st to 8th January, 1927, during the time when two separate sources of light were used, which show unmistakable signs of discrimination, either between source or between the actual colours, white and yellow. But there are other periods, 14th to 17th December ; 19th to 20th December ; and again from the 10th to 13th January, 1927, when no discrimination was shown. Why ? Upon the 14th December (arrow B) the position of the yellow and white lights was reversed, and on the 15th (arrow C) changed back again. This gave rise to the first confused period, which, except for two instances on the 17th and 18th December, remained confused at several trials up to the 20th. Whether it be considered that the fish had been discriminating between yellow and white, or between the relative positions of the sources of illumination, the confusion was to be expected, for a hitherto unreinforced stimulus became, on these occasions, reinforced by food. The fish would thus speedily react to either white or yellow—or to either light—as they were now apparently positive stimuli. Then followed an interval of 10 days, December 20th to 29th (arrow D), during which no experiments were carried out. This caused a slight weakening of the conditioned response, and moreover, on resuming, there was again no discrimination (cf. also with Specimen No. 1). By continuing to reinforce the white light and not reinforcing the yellow, in their original positions, discrimination was later established and remained stable for a considerable time. The lack of discrimination on the 10th and 11th January is unaccounted for.

Arrow E (Fig. 12) indicates the date when a 60-watt Fullolite gas-filled lamp, worked from the main 210-volt lighting circuit, was fitted up in the illumination compartment which had contained the white light in its original position. Wratten light filter No. 70 (red*) was interposed on the occasions denoted by a △, whilst this light unshielded was given as a stimulus at the trials shown with an open circle o. The original stimulus was placed in the other illumination compartment, previously occupied by the yellow filter. Continuing differentiation as shown, no discrimination between these new stimuli was obtained between 17th and 28th January, a positive conditioned response being evoked by all three.

Insertion of Wratten light filter No. 76 (violet) in the path of the light from the 60-watt lamp, gave, however, no signs of a positive response whenever it was given as a stimulus (denoted by a large black dot ●, on Jan. 28, 29, 31, and Feb. 1st, 1927).

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* Full details of these filters, showing their selectivity, maximum transmission, wavelength, etc., may be obtained from Kodak Ltd., Wratten Division, Kingsway, London, W.C. 2.

Specimen No. 3 (Record in Fig. 13). This was a wrasse of the same species and of the same size as No. 2.

The conditioned response was evoked towards the monochromatic green light obtained by interposing Wratten light filter No. 74,* in the path of the light from a 60-watt lamp, arranged as shown in Diagram 16 (T and H, light and filter respectively).

An exceedingly long time was required to make this fish " at home " in the tank. From March 27th, 1927, until April 11th the fish would not eat or move unless strongly disturbed. I was absent then for two weeks and the fish was removed for this period to one of the main tanks, where I have no record of its behaviour. On being returned to the experimental tank the same difficulty was again experienced, but by May 1st the fish had become more accustomed to its surroundings and took food readily when given. It proved the most difficult of all the specimens investigated to " condition." Between April 26th and May 24th 134 associations of stimulus+food were given without evoking a positive response, but it then became called out very rapidly and completely (May 25th *et seq.*). The record after that is quite a straightforward one. Differential training was started early (May 27th). It will be seen that, as in Specimen No. 2,

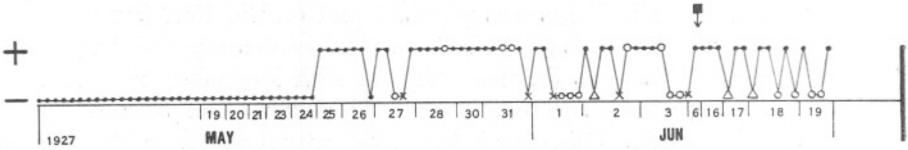

FIG. 13. Specimen No. 3.

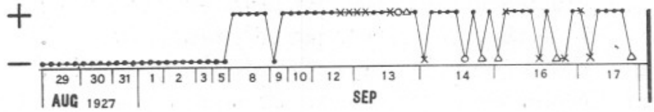

FIG. 14. Specimen No. 4.

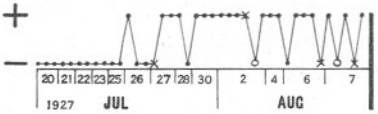

FIG. 15. Specimen No. 5.

FIGS. 13, 14, 15. Records of the process of formation of conditioned responses in wrasses towards *visual* stimuli. Explanation in text.

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* Full details of these filters, showing their selectivity, maximum transmission, wavelength, etc., may be obtained from Kodak Ltd., Wratten Division, Kingsway, London, W.C. 2.

no signs of a conditioned response were given at any time towards the violet light obtained with Wratten filter No. 76 (denoted by a × in the record). The evidence that the fish could discriminate by the method of differential inhibition between the original green (Wratten filter No. 74) and a monochromatic red light (Wratten filter No. 70), not reinforced by food (denoted by a small circle o in Fig. 13), is not very powerful, but definitely suggests that it probably could. When presented at three separate trials (June 2nd, 17th, and 18th) with the non-reinforced stimulus of unfiltered white light from the 60-watt lamp (△ in the record), a complete absence of a response resulted. An interval of nine days, when no experiments were performed (June 6th to 16th), had no effect upon the strength of the conditioned response.

Specimen No. 4 (Record in Fig. 14). A small example of *Labrus bergylta* (length 6·5 cm.). The conditioned response was here evoked by the same type of stimulus to which No. 3 was " conditioned," but using monochromatic yellow light obtained by using Wratten light filter K3 and a 60-watt lamp as source. The record requires little amplification. Twenty-three trials were sufficient to bring about the formation of the conditioned response (Aug. 29th to Sept. 5th, 1927) which became established rapidly. The evidence obtained upon colour-discrimination is inconclusive, owing to the limited nature of the record. Red, violet, and green (shown on the record by the marks △, o, and × respectively) were all discriminated on several trials, but positive responses towards these colours were also given.

Specimen No. 5 (Record in Fig. 15). A specimen of *Crenilabrus melops* (8·0 cm. long). The conditioned response was evoked by monochromatic red light, using Wratten light filter No. 70, with a 60-watt lamp as source.

No difficulty was experienced in forming the response, which, as will be seen in the record, became established fairly rapidly. A positive response first appeared after twelve trials (20th to 26th July, 1927). The fish died before sufficient evidence upon discrimination was obtained. Violet light from Wratten filter No. 76 (denoted in the record by a small circle, o), however, when tried on two occasions, produced a negative result as in the other specimens. A green filter (Wratten No. 74) was used at the trials shown in the record by a ×.

SECTION B. ELECTRIC SHOCK AS UNCONDITIONED STIMULUS.

(A) Introductory.

The method of carrying out these experiments was evolved with the intention of repeating Froloff's experiments (**10**) and (**22**) in a more natural manner. At the same time it eliminates other possible interpretations of the reactions he recorded. A brief résumé of his method will make these points clearer. His experiments appear to have been carried out with great care and he lays stress upon the necessity for scrupulous standardisation of these experiments. Such standard conditions he maintained throughout. The fish was isolated in one room and the investigator remained in an adjoining room, all connections being carried through the separating wall and stimuli given cautiously and quietly. Of two wires from the secondary coil of an induction coil one was sunk to the bottom of the aquarium containing the fish being experimented upon. The other—a very light thin wire—was attached to a small wire clamp fastened into the dorsal fin of the fish. The same wire was further attached to the underside of a Marie capsule—thus suspending the fish. Any movements the fish then made were immediately registered on the Marie registration disc in the experimenter's compartment.

If left in the dark, and so long as there was no incidence of extraneous stimuli, the fishes used appear to have taken up a restful position in a few minutes from the time of suspension. When this restful condition had been maintained a few moments, one of the conditioning stimuli (light, sound, etc.) was presented, and after a definite interval the electric circuit closed. This at once brought about a registerable "flight-reaction." After a sufficient number of synchronous presentations (between five and thirty) of the conditioned and unconditioned stimuli, the fish reacted to the former alone, giving a "flight-reaction."

I tried in the first place to repeat these experiments exactly as described by Froloff, but without success or confirmation of his results. In my experiments the fish simply would not remain still when suspended in this manner.* A few moments' pause—then vigorous movement—pause again, perhaps for minutes—and so on for hours—was the invariable result. It was clearly impracticable to suspend them in this way without great disturbance of the results. As, however, I have substantially confirmed his conclusions, although in a different way, that fishes do form conditioned responses to light and vibratory (auditory ?) stimuli, it is possible that my duplication of his method was not exact. Under the circumstances, I concluded that it was desirable, both on humane as well as practical grounds, to eliminate the necessity for fastening the fish in any way whatever. This led to the construction of the present type of apparatus, which is essentially derived from Lillie's method (**23**) for sending non-polarised currents through Echinus eggs. Polarisation is better eliminated, as its effect, if present, would be an influence of uncertain character.

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* The fishes tried included *Cottus bubalis*, *Gobius paganellus*, *Crenilabrus melops*, *Labrus bergylta*, *Blennius gattorugine*, *Motella* sp., *Pleuronectes flesus*, and *P. limanda*, *Leuciscus rutilis*, *Leuciscus leuciscus*, and *Cyprinus carpio*.

The Apparatus.

This is shown diagrammatically in Fig. 16. A is a shallow glass dish (30 cm. × 10 cm. × 5 cm.) placed inside a wooden box (C) (40 cm. × 17 cm. × 17 cm.) which has some 10 cm. of loose cotton-wool (B) on the bottom to lessen any vibrations from any external source. External to the box, at either end, are the two the non-polarisable electrodes. These consist of glass dishes (D) containing a saturated solution of zinc sulphate, in each of which is immersed a large plate of zinc (E), connected up with the terminals of the secondary of an induction coil (G). The glass vessel A is filled with sea-water of the required amount and contains the experimental fish, which is allowed to remain quite free and not fastened or restrained in any way. A continuous aeration is maintained. Current is conveyed through the sea-water in A (and hence also through the fish) by means of bridges of agar-agar jelly (F), connecting with the electrodes D at either end. The current actually passed through the fish is extremely small, and the responsive movements are also small but decisive. Movements of the fish are recorded by the pointer of the pivoted reed (L). A piece of waxed silk thread is attached to this by one end ; the other end

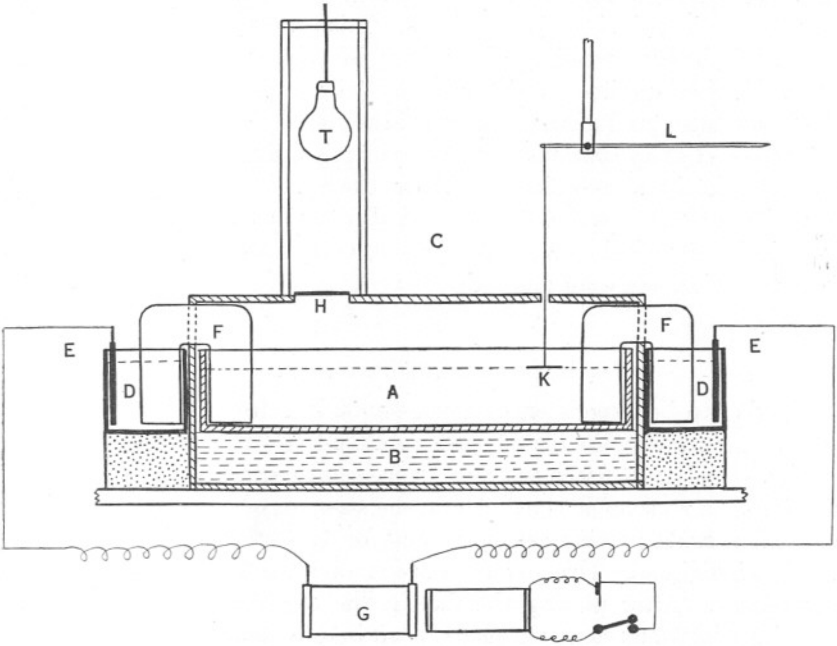

FIG. 16. Diagram of apparatus used in experiments upon conditioned responses in fishes in which an electric shock is used as an unconditioned stimulus. Explanation in text.

is fastened to the centre of a microscopic cover-glass, $1\frac{3}{4}$ in. × $\frac{7}{8}$in. (K) which has been covered with a thin film of vaseline on both sides. This floats on top of the water. The vaselined cover-glass has a very high surface tension, and sufficient stress is maintained on the thread to keep the pointer horizontal. The slightest body movement of even very small fish contained in the water (such as *Gobius ruthensparri*) is registered by this means. The thread passes through a small hole in the box surrounding the dish A. Another small square opening in the top of the box, 5×5 cm., allows for the introduction of any required apparatus to be used as a conditioned stimulus. The diagram (Fig. 16) shows how this was adapted for introducing visual stimuli. T is a source of light, movable in a vertical plane, within a black box; H is a Wratten or other light filter. In the experiments with auditory (vibratory) conditioned stimuli the instrument producing sound was also placed through this opening. The whole of the apparatus, with the exception of the recording needle, was completely surrounded by black cloth before each experiment. Experiments were carried out in a small dark basement room, remote from Laboratory noises and interference, every care being exercised to keep conditions constant and quiet.

Method of Experimentation.

The fish to be experimented upon was taken from one of the main tanks and placed in the dish A with approximately 800 c.c. of sea-water. It was found that fishes vary in their behaviour towards an induced shock, but a reasonable constancy in the strength of the response in any individual could be obtained by the use of a constant amount of water for that individual. This amount varied more with the species than with individuals. After placing the fish in the apparatus and completely covering with black cloth, the fish was left in total darkness for a period of at least fifteen minutes. Under these conditions the fish usually became quiescent almost at once and made no further movement. (It is not likely that very active fish would do this, but only sedentary forms were used for this reason.)

The conditioning stimulus was then presented at intervals varying from one minute to five minutes, with an average of three minutes, and immediately followed, upon each occasion, by the "unconditioned" electrical stimulus, consisting of a single make-and-break induction shock. The latter at once evoked a decisive movement, which was indicated by oscillations of the pointer.

The object of the experiment is to see whether the fish will form the "shock-reaction" when given a stimulus, visual, auditory, or any other, to which it had been previously indifferent and to which it had shown no signs of a motor response until " conditioned " to do so. All signalling or conditioned stimuli used were tried at least twenty times with the fish before the process of conditioning began.

It should be noted that these responses belong to the type described by Pavlov as " simultaneous," and in this respect they differ from the previous experiments.

The strength of the shock used was the lowest that would give a measurable response. This could be adjusted to a nicety either with the coil or by varying the amount of water in A. There was a definite quantity of water in which the fish could be placed, so that, when the make-and-break were made, no shock-response was given. Decreasing the amount of water by a further 20–50 c.c. and so reducing the ratio of the resistance of the fish to the total resistance, was then sufficient to give the slight decisive response required. There is a certain minimal strength of stimulation which will evoke a decisive movement when the head of the fish points towards the kathode, but not if it is orientated towards the opposite pole, when reversed and placed in such a poisiton. The amount of current required to cause a movement in a fish appears to bear some relation to the nature of the epidermal coverings. Both these points deserve further investigation.

The number of associations made daily in the course of the experiments averaged 8, and varied from 6 to 14. Fifteen minutes or more after the last daily test had been made the fish was returned to the main tank from which it was taken.

This method of investigating conditioned responses in fishes is much more in line with Pavlov's method for dogs, though it is not necessarily a more convenient one for the present purpose. Neither is it likely to give such good results with fishes as with mammals on account of the difficulty of keeping them. A great desideratum for this work is a harmless unconditioned response which will evoke in fishes a registerable response of a quantitative nature.

The possibilities of this method have only been lightly explored as yet.

THE RESULTS.

(B) The Formation of a Conditioned Response in *Blennius gattorugine* towards Visual Stimuli.

Specimen No. 1.

The conditioning stimulus was the light from a 60-watt Fullolite gas-filled lamp on the main lighting circuit passed through Wratten light filter No. 74 (green) and allowed to act for 2 seconds. The record is given in Fig. 17.

The conditioned response became established after 20 associations (Sept. 13th, 1927, trial 6). Upon the 14th a completely negative result was obtained ; the reason for which was immediately apparent when the apparatus was uncovered at the conclusion of the day's experiments, and it was discovered that the air circulation had become stopped byaccident. This had completely inhibited the response, but the effect did not persist long on the morrow. Upon the 15th, 16th, 17th, and 19th September the earliest trials made during the day evoked no positive conditioned response, but later trials on the same days evoked a positive reaction, gradually increasing in magnitude. No diminution in the strength or constancy of the response having occurred from the 19th to 23rd, experiments upon the capacity for colour discrimination were commenced. The × in the record denotes trials in which the same light source was used, but the green filter was removed and Wratten light filter No. 70 (red) was interposed. This stimulus was not reinforced. At first no discrimination was shown, the positive shock-reaction being given towards the red light just as to the green. After 9 trials, not reinforced, the red light failed to evoke a positive response (Sept. 26th, trials 4, 5, and 6). Tested immediately afterwards (Sept. 26th, trial 7) with the primary stimulus (green), a strong positive conditioned response was obtained. Experiments on September 27th began with the use of a green filter and a constant positive response was still given ; on being presented with the differential stimulus, red light

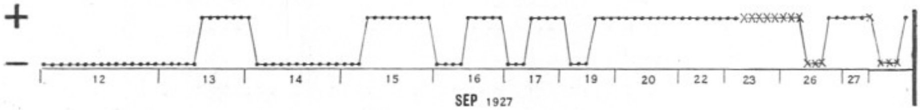

FIG. 17.

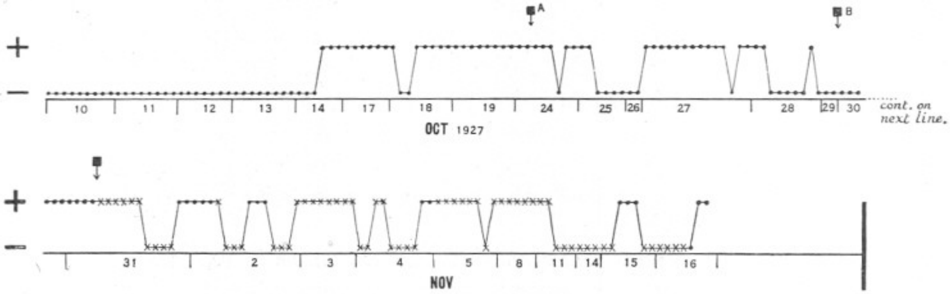

FIG. 17. Specimen No. 1. FIG. 18. Specimen No. 2.

FIGS. 17 and 18. Records of the process of formation of conditioned responses in *Blennius gattorugine* towards *visual* stimuli. Explanation in text.

(trials 4 to 7), discrimination was at once shown. A trial with the green followed two minutes later and gave an unchanged positive response.

Specimen No. 2.

Conditioning stimulus, as No. 1, but using monochromatic red light obtained through Wratten light filter No. 70. Procedure also as in No. 1. Record in Fig. 18.

Thirty-five associations, spread over five days, were required to establish the conditioned response in this specimen, but once formed, it did not suffer diminution daily as in No. 1. It remained exceedingly stable and on October 24th (see arrow A) I decided to see if it could be "extinguished" or caused to disappear by simple non-reinforcement. The course of this phenomenon was erratic (see Oct. 24th to 29th), and there was no gradual diminution. It became a zero response on the 28th and remained so on the 29th, and at the first trials on the 30th. It was then decided to rebuild the response by again reinforcing it with the unconditioned electric stimulus (arrow B). Three trials only were necessary, though the response did not immediately regain its maximum. On the 31st October the conditioned response was very marked. An attempt was now made to obtain evidence upon the capacity of the fish to discriminate the primary stimulus of red from one or more allied shades of grey (shown by a × in the record). These were obtained by interposing photographic lantern slides in the path of the light. To obtain these tints, grey No. 1 was exposed for 5 seconds, grey No. 2 was exposed for 10 seconds at a distance of 30 cm. from a 30-watt lamp, and both developed simultaneously for 10 minutes. Grey No. 1 gave a brightness equal approximately to that of the red filter No. 70 as judged by my own eye, dark adapted ; grey No. 2 was rather less bright. Very definite evidence for discrimination is shown on the record. The differentiation being complete from the 11th to 16th November, 1927. The greater part of these differential trials represents the result obtained with grey No. 1, but grey No. 2 (darker) was also presented at tests No. 8, 9, and 10 of October 31st and tests No. 6 and 7 of November 15th, with a negative result.

(c) THE FORMATION OF A CONDITIONED RESPONSE IN THE EEL, *ANGUILLA VULGARIS* TURTON, TOWARDS VIBRATORY STIMULI.

A large number of experiments were carried out by this method, using, in place of the visual stimulus, an electric buzzer enclosed in a small glass rectangular jar. This was placed in the vessel A (Fig. 16) and rested on the bottom at the end F. Procedure was the same as with visual stimuli, but a period of five seconds, during which time the buzzer was kept sounding, was allowed to elapse before giving the shock stimulus. The only fish which gave indisputable evidence of forming the conditioned response to this sound was a small fresh-water eel, 16 cm. long. Fig. 19 shows the progress of the experiment.

There are three typical periods. A period of 30 trials (10th to 16th Aug., 1927), during which there were no signs of a response to the sound ; a second period (16th and 17th Aug.) in which the response became established ; and the final period (19th to 24th) when the conditioned response was firmly established. Owing to the shallowness of the dish, on the three occasions denoted by arrows in the record, the fish wriggled out after receiving the shock, and being so slimy it was rather difficult to recapture it. It will be noticed that these strong disturbances resulted in no responses being given at the trials immediately following the return of the eel to the apparatus.

Other fishes tried with this stimulus included *Gasterosteus aculeatus*, *Cottus bubalis*, *Pleuronectes platessa*, and *Gobius minutus*. The results from these are of an indecisive nature, all of them having responded on occasions to the sound, but only in an erratic sequence which seemed to bear no relation to the progress of conditioning. It is most likely that the nature of the sound stimulus was not a suitable one for the purpose.

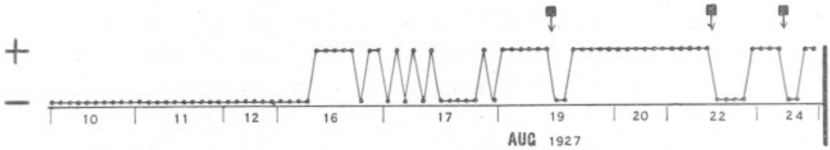

FIG. 19. Record of the formation of a conditioned response in *Anguilla vulgaris* towards *vibratory* stimuli. Explanation in text.

Experiments of a similar nature were also carried out in which a key D tin-whistle was used as a stimulus. *Blennius gattorugine* was the fish tried. All the holes in the whistle were closed and the bottom sealed with a very thin sheet of brass ; this end was immersed in the water. The whistle was connected with the compressed-air supply and a constant note was obtained by regulating the pressure ; it was sounded by removing a damper placed over the throttle. Eighty-three associations given between October 7th and 19th, 1927, were not sufficient to produce the slightest indications of a conditioned response to the sound of the whistle in the specimen tried. I had previously satisfied myself that this note could be heard plainly with the head completely submerged at a distance of four yards from the whistle when sounded in the same manner.

Considered in conjunction with the experiment upon this species of blenny recorded in Section A (cf.), in which a submerged telephone was used, it appears probable that this fish is incapable of forming conditioned responses to sound produced in either of these two ways. It would be foolish to infer from these premisses alone that the fish cannot hear. It did not prove difficult to establish the response towards vibratory stimuli with the wrasse, and Dr. Allen states that only a relatively short time was required to produce such a response in pollack (*Gadus pollachius*) and rudd (*Scardinius erythropthalmus*). The possibility that the blenny really cannot hear this type of sound or vibration is thus increased.

ACKNOWLEDGMENTS.

This work was suggested to me in the first place by Dr. E. J. Allen, F.R.S., as a general problem to be attacked. I have especially to thank him for his interest and help throughout, and for the loan of the Wratten light filters used in the experiments on visual stimuli. The work was carried out during the holding of a Student Probationership in the Plymouth Laboratory of the Marine Biological Association during the years 1926 and 1927.

My wife has rendered me invaluable service.

SUMMARY.

It has been shown by experiments formulated upon the " conditioned response " principle that the blenny, *Blennius gattorugine*, is able to perceive and to profit by very small changes in its environment. This fish is able to form conditioned motor responses using food as unconditioned stimulus, towards a momentary increase of 0·4° C., or more, in the *temperature* of the surrounding water.

It is also able to form similar conditioned motor responses towards a momentary decrease in the *salinity* of the surrounding water, of as little as 3 parts per 1000, or towards a change of greater magnitude (up to 37 parts per 1000).

Conditioned responses have been established in the wrasses, *Crenilabrus melops* and *Labrus bergylta*, towards *visual* stimuli of varying kinds. It appears that these fishes can discriminate after differential training between one or two sources of light, and between monochromatic red, green, yellow, or violet light, but not readily between even comparatively large differences in intensity of a luminous source. These results are those obtained upon " dark-adapted " fishes. More extensive experiments will be necessary before a final statement is made upon their capacity for colour discrimination.

Using an electric shock as an unconditioned stimulus it has been shown that *Blennius gattorugine* can also form *visual* conditioned responses towards monochromatic red and towards monochromatic green light; and that it can, by the method of " differential inhibition," distinguish red from green, and red from closely allied shades of grey.

Conditioned responses have been formed in the wrasse, *Crenilabrus melops*, towards *vibratory* stimuli, using a tuning-fork of 128 D.V's. per second, and food as unconditioned stimulus. A conditioned response towards the vibrations from an electric buzzer has been formed in the common eel, *Anguilla vulgarius*, using an electric shock as unconditioned stimulus. Up to the present time it has not proved possible to establish conditioned responses in *Blennius gattorugine* towards the vibrations produced by the tuning-fork, a submerged telephone transmitting the same note, or towards the basic note of a key D tin-whistle, arranged as a closed pipe.

Many of the phenomena relating to conditioned reflex formations in mammals are shown to occur during the formation of similar responses in fishes.

Froloff's observations have been confirmed in most respects.

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