

THE NERVES AND MUSCLES OF MEDUSAE

VI. THE RHYTHM

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Previous papers in this series contained references to the swimming rhythm of medusae, in particular to the temporary change in frequency which can follow stimulation of the subumbrellum. The present paper is a collection of observations on the normal rhythm as determined by a single marginal ganglion, and the varied effects of stimulation thereon. Sufficient data is not yet available for the formulation of a comprehensive theory of the origin and control of the rhythm; however, some of the more readily observable data which bears on this topic can now be given. The term *marginal ganglion* refers to the concentration of nerve cells on the stalk and round the base of the *tentaculocyst*, which is the anatomical term for the whole organ including mesogloea and a terminal mass of crystals. For *Aurelia* the structures were described by Schäfer (1878).

Several features of the rhythm emerge from the work of previous authors. Romanes (1877) established that the rhythm originates in the tentaculocysts, provided strong evidence that it first appears in nervous tissue and found that the frequency is modified by stimulation of distant regions of the bell. In one particular experiment Romanes showed that in *Aurelia* the efferent motor excitation could be initiated in the tentaculocyst by afferent excitation which was accompanied by a wave of contraction of the marginal tentacles. Later workers extended these findings; Horstmann (1934*a, b*) concluded that sensory excitation is converted to motor impulses in the ganglion. Pantin & Vianna Dias (1952) deduced that the pacemaker is within the through-conducting limits of the nervous pathway which co-ordinates the symmetrical contraction at each beat; Horridge (1956*a*) concluded that the ganglion contains a polarized junction at which the diffuse (= *primary* or multipolar) nerve net modulates the frequency of the pacemaker in the giant fibre motor net, but at which excitation does not pass in the reverse direction. Other workers, e.g. Fränkel (1925), Bozler (1926*a, b*) have emphasized that sensory cells within the ganglion region (on and around the stalk of the tentaculocyst) also influence the frequency of the rhythm. However, in all these accounts the character of the rhythm as a time series is not recorded, and the actual modification of the rhythm by artificial means has not been described.

The intervals between the beats of a single isolated ganglion are not uniform and therefore a statistical treatment is necessary. A decision must then be taken as to the treatment of the variability between the eight or more ganglia of one individual

and between individual animals of the same species. Ganglia may show various modes of activity whose separate realities may be demonstrated by statistical tests. Some of these modes are atypical, or known to be the result of stimulation, but those ganglia which fail to respond in a typical way do not invalidate conclusions drawn from the typical mode of activity.

METHODS

Some records of the rhythm were made by the standard kymograph technique. The lever was attached to the mesogloea so that the primary nerve net was not mechanically stimulated by the hook or clip. But as a rule neither intact animals nor parts bearing a tentaculocyst beat normally under such circumstances since they are necessarily constrained by pins, stretched or placed in an abnormal orientation. Later observations were made on isolated freely floating segments 2–4 cm. across, each containing a single tentaculocyst, and the intervals were recorded on cash-register paper by a pen relay. A second pen was used in experiments in which a control ganglion was simultaneously recorded. The relay was operated by an observer with a morse tapper key. The key was pressed at the instant when a particular part of the bell passed a readily observable part of its traverse at each contraction; this arrangement ensured that an irregular rhythm did not take the observer by surprise. The method was satisfactory only because the intervals between beats were greater than 3 sec. The observer did not know the expected result of the experiment. Each diagram of this paper shows a different method of plotting the results.

THE RHYTHM OF A SINGLE GANGLION

A segment, which is an eighth of a jellyfish and bears a single tentaculocyst, continues to beat for days in a dish of aerated sea water. Several hours after isolation the rhythm becomes free from cyclical changes. This may be checked later by statistical methods, but in practice when the number of beats per minute becomes reasonably constant and cyclical changes are not noticed, the ganglion is tentatively considered suitable for experimentation.

Examples of such a rhythm in *Aurelia* are shown in Figs. 1*a* and 2*a*. A characteristic of the rhythm of a single undisturbed ganglion of *Aurelia*, *Cassiopea*, *Chrysaora*, *Cyanea* and *Pelagia* is that the intervals are far from being of equal duration and the average frequency is less than half the maximum frequency at which the ganglion may discharge when stimulated. The standard deviation of interval is usually 20–30% of the mean; therefore many observations are necessary to establish that differences of mean interval are significant. Previously this point has not been reported.

The rhythm is a time series which can be considered as beginning at any point when a large number of intervals are taken; the first-order serial correlation coefficient (r_1) is often significantly less than zero. Figures of $r_1 = -0.3$ to -0.4 are frequently found: out of ten likely records tested, eight had significantly negative values of r_1 between -0.25 and -0.4 . A negative first-order serial correlation coefficient means that long intervals tend to be followed by short intervals and vice

versa; the correlation is rather low, and to establish a correlation coefficient of -0.25 at the 95 % level sixty intervals must be recorded and 100 at the 99 % level. The second-order serial correlation coefficient (r_2) has not been observed to be significantly negative. However, in three cases r_2 was significantly positive when r_1

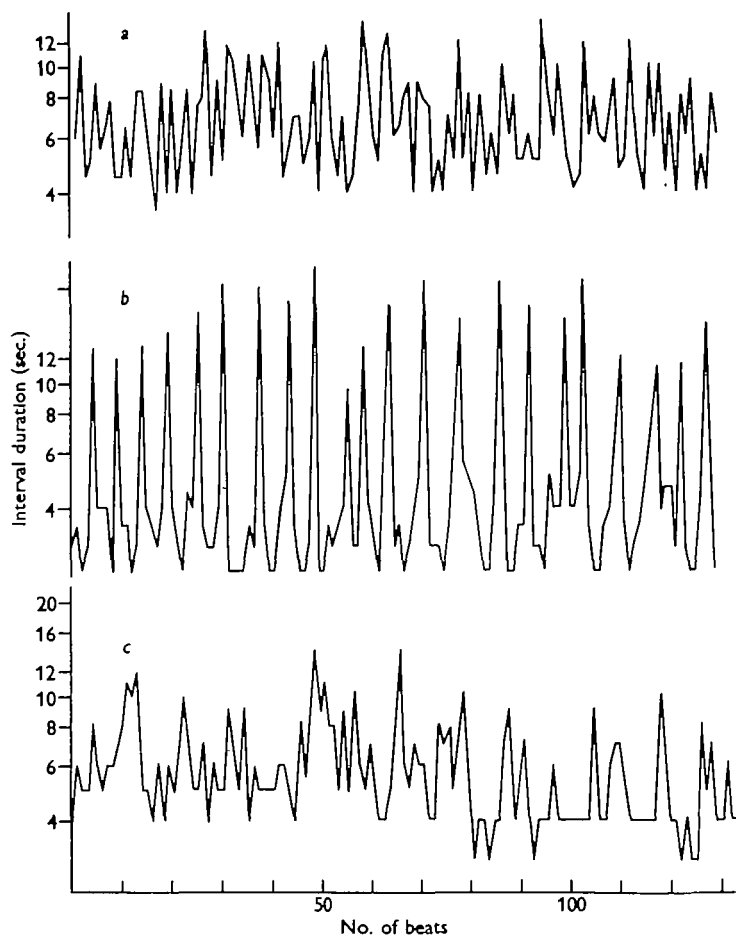


Fig. 1. (a) The normal experimental rhythm of a ganglion of *Aurelia*. The duration of each interval is plotted upwards on a logarithmic scale and the points thus placed at equal distances from left to right are joined by straight lines. Mean interval, 7.1 sec.; σ , 2.51 sec.; r_1 , -0.287 ; N , 130. (b) The same ganglion immediately following a mechanical stimulus (a pinch at the bell margin). Long intervals alternate with bursts of shorter intervals. The numerical constants are not given since they are meaningless in such an example. (c) The same after 1 hr. showing a recovery from the cyclical rhythm to a pattern that temporarily has positive first-order serial correlation. Mean interval, 5.8 sec.; σ , 2.5 sec.; r_1 , $+0.176$; N , 180.

was significantly negative. A positive second-order coefficient means that the interval is similar to the next but one, whether long or short. For example, a single ganglion of *Aurelia* gave the following figures: number of observations (N) 67, mean interval (m) 7.68 sec., standard deviation (σ) 2.9 sec., first-order serial correlation coefficient

(r_1) -0.38 , second-order coefficient (r_2) $+0.23$ (significant at 95 % level). The figures for the *Aurelia* ganglion in Fig. 1 (a) are $N=130$, $m=7.1$, $\sigma=2.51$, $r_1=-0.287$ (significantly less than zero at 99.9 % level), $r_2=+0.12$ (significantly greater than zero at 90 % level).

The pattern of the rhythm described above appears to be that to which an intact ganglion of *Aurelia* settles down after some hours of isolation. For reasons that will be discussed below, experiments on the rhythm must be made with a single ganglion and not with the whole animal. This rhythm will therefore be called the *normal experimental rhythm* of a single ganglion. This is the optimum state for experiments on the modification of the rhythm, and appears to correspond with the natural rhythm of an intact animal.

Other types of rhythm occur. Commonly a single ganglion attached to a kymograph lever shows a cyclical pattern similar to that of Fig. 1 (b). This is a result of severe mechanical stimulation or of continual stimulation of the diffuse nerve net. Such a rhythm may settle down in a matter of minutes, but sometimes cyclical changes persist for several hours. As the cyclical changes slowly disappear the variability falls and the first-order serial correlation coefficient goes from positive to negative. An example of slow recovery is shown in Fig. 1 (b) and (c).

A regular rapid rhythm at a frequency of 0.3–0.5 per sec. is usually a consequence of stimulation; a preparation attached to a kymograph lever may continue to beat in this way for several hours but if removed from its attachment often slows considerably. The disturbance of the rhythm caused by attaching the mesogloea to the lever may persist for the duration of the experiment. Such preparations may give information of some possible responses of a ganglion but they are not characteristic of the intact animal.

STIMULATION OF THE GANGLION VIA THE PRIMARY NERVE NET

Romanes (1877) observed that a temporary acceleration of the rhythm follows the removal of a piece of the bell. He also showed that the ganglion can initiate a beat in response to a distant mechanical stimulus. These observations have been confirmed by later workers.

The normal experimental rhythm of an *Aurelia* ganglion is shown in Fig. 2a, together with ten repetitions following electrical stimulation of the primary nerve net (Fig. 2, b–k). The stimulus was a short burst of twelve shocks at 4 per sec. applied to the tentacular margin of the bell outside the area of the giant fibre net. The stimulus did not produce a contraction wave directly. Intervals of half an hour between the experiments appeared to be sufficient for a full recovery. The diagrams, typical of many experiments, show that the rhythm is accelerated for about ten beats following the stimulus. The initial rapid rhythm then either slows down or is terminated abruptly by a long pause. After this the rhythm is slower and more variable than normal. Fig. 2 contains the whole of the data; some of this information is lost if the results are combined, but the result is then easier to visualize. In Fig. 3 the mean instantaneous frequency of this ganglion is plotted

for each second following the stimulus. The instantaneous frequency is the reciprocal of the interval between consecutive beats. Each point on the graph is a mean of the ten measurements of instantaneous frequency from the data illustrated in Fig. 2. The standard deviations represented by vertical lines are drawn only at 10 sec. intervals. The average frequency of the normal experimental rhythm is shown by the horizontal line, together with its standard deviation based on 70 intervals. Fig. 3 shows the same result as given above in Fig. 2 but most of the unusable data is excluded. At the 95% level of significance points on the right-hand side of the curve show that the rhythm has not recovered its normal average frequency at 90 sec. after the stimulus.

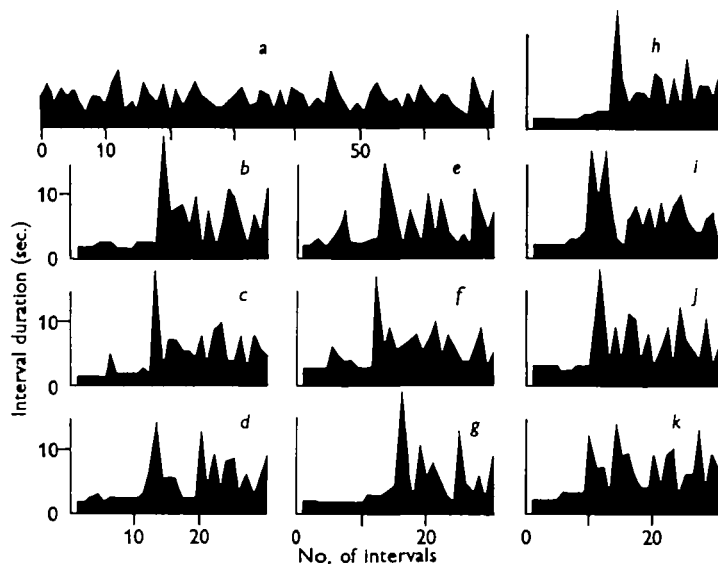


Fig. 2. (a) The normal experimental rhythm of a ganglion of *Aurelia*. The duration of each interval is plotted upwards on a linear scale and the points thus placed at equal intervals from left to right are joined by straight lines. (b) – (k) The rhythm of the same ganglion following an electrical stimulus to the primary nerve net, as described in the text.

The initial acceleration is followed by a definite slowing. The instantaneous frequency 50 sec. after the stimulus is 0.1 ± 0.04 per sec. compared to the normal 0.24 ± 0.075 per sec. and the difference is highly significant (better than 99.9%). Following this slower period the intervals are more variable: calculations from the raw data show that from the fifteenth to the thirtieth beat following the stimulus the standard deviation of the intervals was 7.3 ($N=150$) compared to a normal of 2.35 ($N=70$); the increase is significant (better than 99%). Over the same period, corresponding with the aggregate of right-hand halves of the diagrams (b) to (k) of Fig. 2, the first-order serial correlation coefficient increases from -0.21 to -0.41 , a change which is significant at 95% level. The second halves of the experimental records (b) to (k) subjectively appear more variable than normal and appear to have sharper peaks with more indentations; the calculations show these appearances to be significant.

In contrast to the usual behaviour, a small proportion of the ganglia temporarily slow down following stimulation of the primary net. Some examples of both *Aurelia* and *Cyanea* ganglia consistently showed this effect following a mechanical stimulus at a distance from the ganglion. The ephyra larvae at once stop beating if one arm touches a food particle and do not beat during the course of the maintained contraction of an arm in the feeding response. *Nausithoe* and many Hydro-medusae behave similarly. The ganglia of adult *Aurelia* and *Cyanea* which behave in this way are therefore not outstanding exceptions, though they appear to be uncommon.

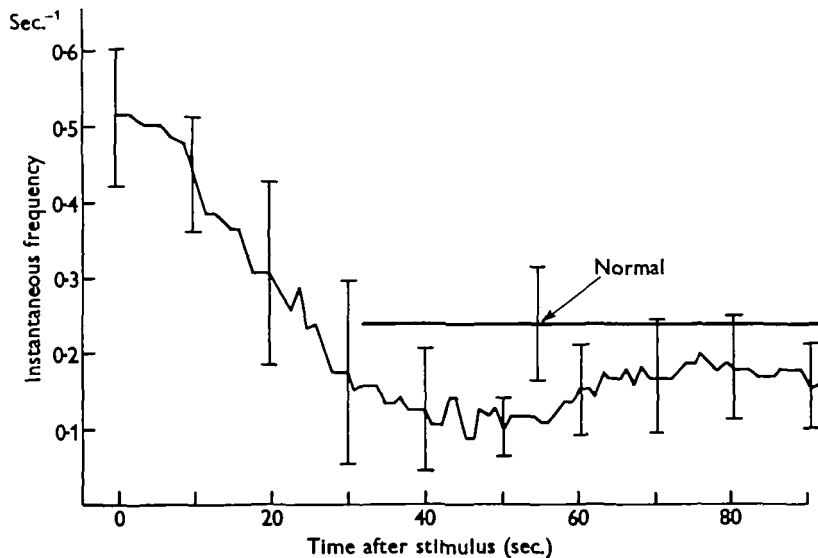


Fig. 3. The average change in frequency with time following an excitation of the primary nerve net. This figure is derived from the data of Fig. 2 (*b-k*) by calculating the average frequency over the ten experiments at each second following the stimulus. The vertical lines represent standard deviations for each 10 sec. The previously observed mean is also shown together with its standard deviation based on 70 intervals, as in Fig. 2 (*a*).

Repeated stimulation of a ganglion often produces a rhythm in which relatively long intervals are interspersed with bursts of rapid beating as in Fig. 1(*b*). This cyclical rhythm reverts to a more normal state after a few hours unless the ganglion is damaged. It bears no relation to the fishing behaviour of *Gonionemus* (Agassiz, 1862), which periodically inverts and sinks from the surface without beating. I have not observed alternate bursts and long pauses in intact Scyphozoa in the sea.

STIMULATION VIA THE GIANT FIBRE NET

(a) Previous work

The traditional view, common to almost all workers, of the origin of the rhythm of medusae is that a state of excitation builds up to a threshold at which the pace-maker fires off an impulse. Such a rhythmical system is described as a relaxation

oscillator: this continues to be an acceptable hypothesis because no likely alternative theory has been suggested. The only experimental data so far reported which has any bearing on this theory is the effect of an extra beat artificially introduced into the rhythm.

Working with *Rhizostoma*, Bethe (1903) described the interval which follows the extra beat as longer than normal, suggested that the longer interval sometimes *compensated* for the previous shorter one, and compared the scyphozoan rhythm with that of the frog heart. However, the compensatory pause of the frog heart appears because there is always an impulse in transit in the fibres which conduct towards the ventricle. An artificial stimulus to the ventricle produces an antidromic impulse which cancels with a spontaneous impulse and never reaches the place of origin of the rhythm in the pacemaker. After an extra beat the rhythm of the heart is therefore in step with its former rhythm.

Pantin & Vianna Dias (1952) re-examined the compensatory pause of *Aurelia* and made two new observations: (a) that the stimulus which produces an extra beat may cause a change in the frequency, and (b) that the interval which follows an intercalated beat is usually about as long as a normal interval. They conclude that the compensatory pause is not truly present in medusae but that it sometimes appears accidentally when there is an appropriate change in frequency. The second observation, (b) above, agrees with a theory that the basis of the rhythm is a relaxation oscillator in which a pacemaker repeatedly charges to a threshold and discharges again. An antidromic impulse would then discharge the ganglion from whatever level it had reached and would be followed by an interval of normal duration if no other factors enter into the situation. This is described as a resetting of the rhythm, and according to Pantin & Vianna Dias is the usual outcome of this experiment in *Aurelia*. If an artificially induced extra beat can reset the rhythm the impulses of the normal rhythm of the most rapid ganglion presumably also reset the rhythms of the other ganglia.

Experiments with one or more antidromic impulses (at intervals of 2 sec.) have now been repeated. Ganglia which are discharging rapidly and regularly usually show only a reset of the rhythm at the same frequency as before, i.e. the interval following the extra beat is similar to the other intervals. This confirms Pantin & Vianna Dias; however, such preparations are in my experience stimulated to saturation via the diffuse nerve net or they would not beat so rapidly. Further stimulation of the primary nerve net or of its continuation in the ganglion has little effect; an antidromic impulse is then followed by a pause of the usual length. Such preparations are readily observed in kymograph experiments; an example is shown for *Cassiopea andromeda* (Fig. 4a, b), and *contra* Bethe (1903) the same effects are usually observed in *Rhizostoma* (Ross, private communication). In these circumstances the origin of the rhythm is within the through-conducting giant fibre net. The argument for this conclusion is fully set out by Pantin & Vianna Dias (1952). However, different results which do not agree with the theory of a simple reset of a pacemaker are also found, at least in *Aurelia*, *Cassiopea* and *Cyanea*.

(b) *An apparent compensatory pause*

A single ganglion of *Cyanea* was observed continuously for 4 hr. for most of which time it beat with an interval of 3.0 ± 0.25 sec. Experience with other preparations indicates that the persistence of this rapid rhythm was probably in part a consequence of periodic experimental stimulation. During the 4 hr. sixty observations of the effect of an extra single shock were made. One observation was rejected because the interval following the extra beat was in this case four times the usual length. Over this series of fifty-nine experiments the mean interval following the extra beat was significantly longer than either of the two control intervals recorded before the extra beat (see Table 1, series 1). The difference between 2.94 ± 0.24 sec.

Table 1. *The effect of extra beats on the rhythm*

Mean interval lengths, averaged over the number of repetitions shown, are given in lines A, B and C for the three normal intervals preceding the stimulus. The interval of line D is that curtailed by the stimulus, which initiates the number of extra beats shown. The mean intervals following are shown in E, F, G and H. The figures at the foot of the table which show a statistically significant change are in black.

Series ... Species ...	1 <i>Cyanea</i>	2 <i>Aurelia</i>	3 <i>Aurelia</i>	4 <i>Cyanea</i>	5 <i>Cyanea</i>	6 <i>Cyanea</i>
Repetitions of the sequence	59	20	10	8	10	10
Sequence of preceding mean intervals:						
A	3.0 ± 0.21	3.92 ± 0.84	4.05 ± 0.85	3.0 ± 0.25	19.3 ± 9.1	—
B	2.96 ± 0.23	3.85 ± 0.63	3.8 ± 1.18	3.13 ± 0.20	19.4 ± 11.2	19.5 ± 9.0
C	2.94 ± 0.24	3.85 ± 0.8	4.3 ± 0.93	2.93 ± 0.16	22.2 ± 8.0	20.2 ± 10.3
Mean curtailed interval: D	1.55 ± 0.25	1.85 ± 0.8	2 sec. intervals	2 sec. intervals	2 sec. intervals	2 sec. intervals
No. of extra beats	1	1	8	3	10	20
Sequence of the following mean intervals:						
E	3.68 ± 0.43	5.22 ± 1.32	6.8 ± 6.3	14.25 ± 8.1	31.5 ± 16.8	51.0 ± 25.6
F	2.9 ± 0.24	5.22 ± 1.32	11.0 ± 4.3	3.25 ± 0.75	33.5 ± 14.9	38.0 ± 13.9
G	2.97 ± 0.2	4.27 ± 1.0	5.75 ± 1.7	5.88 ± 5.1	37.5 ± 13.2	20.2 ± 11.1
H	—	—	4.45 ± 1.25	3.13 ± 0.20	18.5 ± 8.2	17.7 ± 6.5

and 3.68 ± 0.43 sec. for $N=59$ is significant to better than 99.9%. As for the pause being compensatory, $3.68 + 1.55$ cannot be shown to be different from $2.94 + 2.96$ on the above evidence. Here lies the fallacy of trying to demonstrate a compensatory pause. The data, by their very nature, cannot show that the pause compensates for the previous shorter interval because this is the null hypothesis, i.e. in instances of apparent compensation the only statement that can be made is that there is no significant difference between the sum of the means $B+C$ and the sum $D+E$ (the letters refer to those of Table 1). Compensation can never be demonstrated. Some examples of Scyphozoa ganglia give a significant difference; i.e. $B+C$ may be greater or less than $D+E$. Similarly, it cannot be shown that the rhythm is

reset at *the same* frequency; i.e. that E is equal to A , B and C , for this again is a null hypothesis; certainly in some ganglia E cannot be shown to be different from A , B or C , particularly when the rhythm is rapid, but it cannot be shown to be the same.

The example of *Cyanea* rhythm set out as series 1, Table 1, shows a slowing of the rhythm for one interval. A record which shows that the rhythm may be slowed for longer periods is set out as series 2 of Table 1. While the experiment was in progress the rhythm appeared to slow after each stimulus. A later test for significance showed that E is not greater than the mean intervals before the extra stimulus.

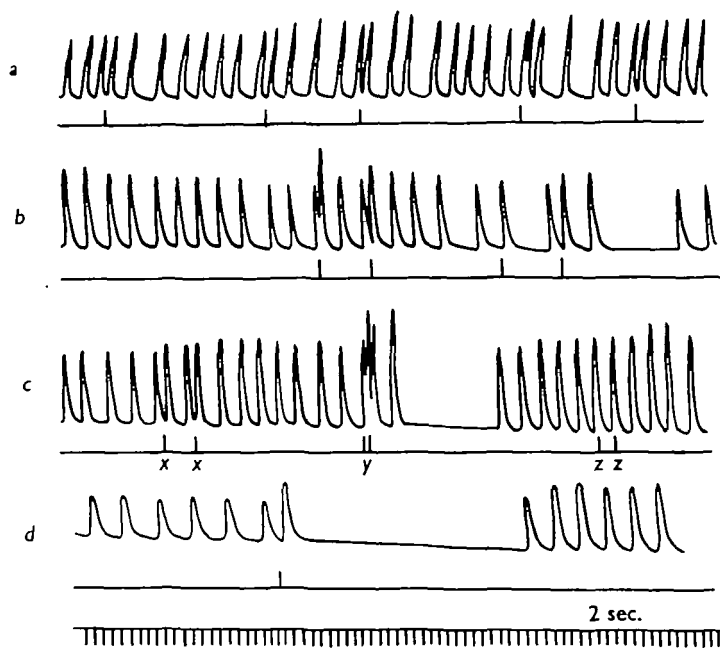


Fig. 4. The effects of an antidromic impulse on the rhythm of *Cassiopea*. This is a tracing from a kymograph record; for further explanation see text.

However, the sum of lines E and F is significantly greater than the sum of lines A and B (99 % level of significance). Therefore the slowing of the rhythm can be shown over the aggregate period of two intervals. The increase in standard deviation for lines E and F is also significant (99 % level). Therefore the rhythm becomes more variable. Twenty repetitions of this experiment just give a sufficient level of significance in face of the variability of the rhythm.

From *Cassiopea andromeda* I have available the results of about fifty experiments in the form of smoked-drum records, four of which are shown in Fig. 4. The segment of the *Cassiopea* bell was attached to a kymograph lever by the jelly, but nevertheless the rhythm was usually rapid and regular.

An extra beat introduced into the rhythm is followed by a variety of possible effects. Fig. 4(a) shows four instances in which the interval following an extra beat

was apparently normal and one instance in which it was shorter. In (b) the first two extra beats were followed by apparently normal intervals; the third and fourth were followed by a slowing of the rhythm. In (c) the results show the inconsistency of experiments on the rhythm. At X two single extra beats were injected and were followed by apparently normal intervals. At Y two extra beats were injected together. The following interval is normal but the next is longer. At Z two shocks of the same strength as before were given while the muscle was in the refractory period of a spontaneous beat. There was no effect on the rhythm though the extra height of the following contractions shows that the primary net was stimulated. In (d) a single extra beat was followed by a long pause and the regular rhythm suddenly started again at a slightly higher frequency than before.

The idea of a compensatory pause was based on experiments with *Rhizostoma*, and Bethe found that the pause was by no means always exactly compensatory. Pantin & Vianna Dias (1952) worked with *Aurelia* in Brazil; Horstmann (1934), who also found no evidence of a compensatory pause, worked on *Aurelia* of the North Sea. These results are the only primary data available. Bozler (private communication) tells me that he made no kymograph records of medusae. Ross (private communication) found the typical result for rapidly beating *Rhizostoma* to be a reset of the rhythm. We must conclude therefore that an apparently compensatory pause cannot be found, although an unexplained pause in the rhythm is consistently observed in most specimens.

(c) Several antidromic impulses

A number of beats may be artificially introduced into the rhythm, thereby forcing a higher frequency of discharge by an external pacemaker. The intervals following the extra beats are usually longer than normal; the effect is more marked than in experiments with one extra beat, i.e. fewer repetitions are required for a given level of significance and the rhythm continues for more than one interval at a frequency lower than normal.

Typical results are shown for *Aurelia* and *Cyanea* in Table 1. Following eight forced beats at 2 per sec. (series 3) *Aurelia* showed a slowing of the rhythm for three intervals following the final forced beat. The example of *Cyanea* shown in series 4 was beating rapidly before each experiment; the examples 5 and 6 were beating very slowly but the results do not appear to differ. There is a similar significant temporary slowing and increase in variability.

DIRECT CURRENT STIMULATION

Between each pair of radial muscles of *Cyanea* lies a muscle-free area in which there are many large bipolar cells running predominantly from the ganglion towards the muscles. This anatomical arrangement makes it possible to stimulate muscle plus nerve nets, nerve nets alone or ganglion plus nerve nets. As would be expected, condenser shocks in the above situations produce a contraction wave. A shock applied to the primary net where it occurs alone, as in the marginal lappets, can initiate a beat only indirectly from the ganglion after a marked delay of up to 3 sec.

The above experiments have been repeated with direct-current stimulation, using non-polarizable $\text{Zn}/\text{ZnSO}_4/\text{Agar}$ electrodes.

When the ganglion has been removed, d.c. stimulation of the area of the giant fibre net may produce a contraction wave when the current is switched on but repeated contractions do not follow. The result is the same if the current flows through an area which includes muscle fibres. If there is a repetitive response of the giant fibre net it must be completed before the muscle recovers from its refractory period of about 2.0 sec. With the ganglion intact, d.c. stimulation of any region outside the ganglion may influence the rhythm, exactly as described for other stimulation; presumably the stimulus acts on the primary net.

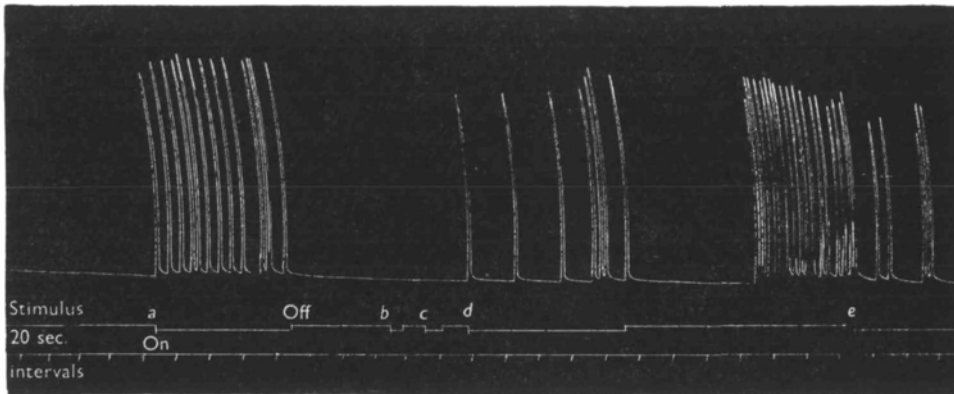


Fig. 5. *Cyanea*. The effect of d.c. stimulation in the region of the ganglion (a) subumbrellum (SU) positive; current of 0.08 mA./mm.^2 . (b) SU negative; current 0.08 . (c) SU negative; current 1.2 . (d) SU negative; current 2.2 ; (e) SU negative; current of 2.2 mA./mm.^2 now slows down a spontaneous rhythm.

With the current passing through the stalk region of the ganglion the rhythm is accelerated when the negative electrode is on the exumbrellar side. With the subumbrellar electrode negative a greater current density is required and results are less consistent: an existing spontaneous rhythm may be stopped and repetitive discharges can also be evoked. Three effects on the ganglion are shown in Fig. 5 as demonstrated in one preparation. The current density required to show these effects is of the order of 0.1 mA./mm.^2 . The distribution of current between the inside of the nerve cells and the outside is unknown, and it is therefore impossible to assess conditions under which the nerves are stimulated.

An attempt to explain these results is partly frustrated by the action of the primary nerve net. The directional effect indicates an orientation of the pacemaker, but could equally well arise from orientated sensory cells in and around the ganglion. When the ganglion itself is stimulated by d.c. the effects persist for many beats; stimulation of the ganglion via the extraganglionic primary nerve net has only a transitory effect. However, the repetitive discharge of the stimulated ganglion may still originate indirectly in the ganglionic sensory cells which have shown themselves

to have a maintained effect on the rhythm in responses to light and gravity. At present I cannot show that d.c. stimulation of the ganglion has a primary effect on the pacemaker though the observations do not contradict this view.

RESPONSES TO GRAVITY

(a) *Compensatory movement*

When tilted from an even keel most Scyphozoa (not *Aurelia*) respond by adding an asymmetrical component to their normally symmetrical contractions. The uppermost edge relaxes more slowly and has a feebler stroke than the lower edge. Bozler (1926) found that the response depends on sensory cells in the region of the marginal ganglia; Horridge (1956*b*) inferred that the asymmetrical extra component of the contraction is transmitted via the primary (diffuse) nerve net and not by the giant fibre net. The compensatory response is not necessarily accompanied by changes in the frequency of the rhythm.

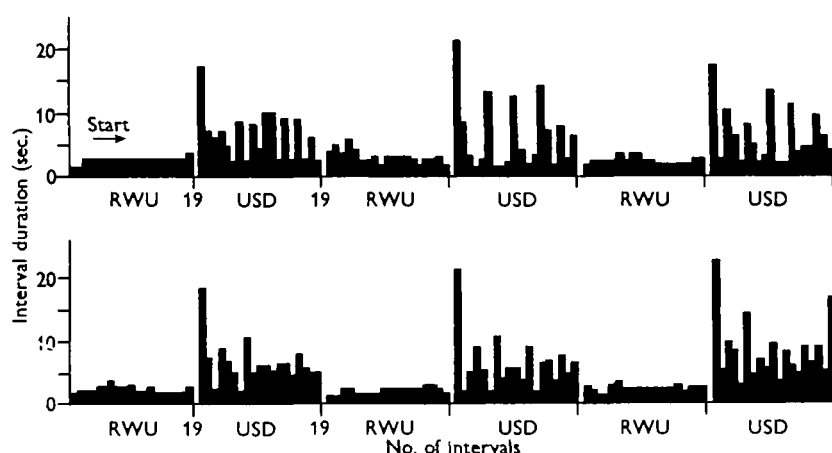


Fig. 6. The effect of inversion on the rhythm of *Aurelia*. The ganglion was alternately upside down (USD) and right way up (RWU) for 20 beats in each position. The durations of successive intervals are plotted upwards as columns of equal width reading from left to right.

(b) *The effect of inversion on the rhythm*

The rhythm of a segment of *Aurelia* containing a single ganglion was recorded, and after every twenty beats the preparation was turned over so that the sub-umbrellar surface was alternately facing upwards and downwards. The frequent changes stimulate the rhythm so that the results cannot be compared with the normal experimental rhythm. Out of twenty preparations eleven consistently showed a slower and more variable rhythm when inverted. A typical record is plotted in Fig. 6. For at least the first twenty beats the rhythm was rapid and regular when the preparation was right way up; slower and irregular when inverted. However, when the same ganglion was allowed to remain right way up for longer, the rhythm progressively became slower and less regular. Starting to count after

30 min., the mean interval between beats was 5.1 ± 2.1 sec. ($N=100$) compared with a mean interval of 6.9 ± 2.9 sec. ($N=100$) of the inverted ganglion, similarly starting to count after 30 min. Such behaviour is not always found; of twenty ganglia from three *Aurelia* only eleven clearly showed a difference similar to that shown in Fig. 6. However, these preparations each behaved consistently, and the change of frequency must be considered as part of the possible behaviour of the ganglion.

The rhythm of an intact animal is determined by its fastest ganglion and therefore the acceleration of a single ganglion on turning right way up could accelerate the rhythm and hence the movement through the water. As yet there is no evidence that *Aurelia* has a steering mechanism; however, if it were turned haphazardly in all directions by water currents or by irregularities of the bell it would nevertheless tend to swim upwards. The above observations on the rhythm of a single ganglion suggest that *Aurelia* maintains its position in the sea by a *kinesis* in the sense defined by Fränkel & Gunn (1940).

THE ACTION OF LIGHT

Horstmann (1934*a*) describes the action of light on the rhythm of *Aurelia*. When daylight was allowed to enter a darkened room the frequency increased by a factor of 1.1–2.0. The number of beats per minute was recorded for up to 30 min. In eleven out of fifteen ganglia this effect of light was definite. I can now confirm Horstmann's observation in that several single ganglion preparations of *Aurelia* slowed their rhythm when shaded from daylight, for example a mean interval 5.1 ± 1.85 sec. in daylight slowed to 7.2 ± 1.92 sec. in the dark, measured for 40 beats.

Variation of light intensity appears to have no action on the ganglia of *Cyanea*, which differ from those of *Aurelia* in having no pigment spots (Horstmann, 1934*a*). The effect of an increased light intensity on *Aurelia* is an acceleration of a symmetrical beat, and the response of the intact animal is apparently a change in rate, not in direction of swimming.

The response to light of the ephyra larva of *Cassiopea andromeda* has a very marked directional character. When the subumbrellar surface is illuminated the animal turns through 180° in as few as four beats. The asymmetry of the contraction at each beat is similar to that found in the compensatory response to tilting of the axis (e.g. *Cyanea*, Horridge, 1956*b*) and the local component of the asymmetrical movement must presumably be controlled via the double innervation of the bell muscles.

THE ACTION OF TRYPTAMINE AND OTHER DRUGS

Following Ross's (1957) discovery that tryptamine is physiologically active on anemone neuromuscular preparations I tried its effect on the scyphozoan ganglion. The procedure was designed to take account of the following conditions. The rhythm must be observed for several minutes before a significant effect can be detected, but the control ganglion does not necessarily keep a constant rate for long periods; measurement of the duration of every interval is an unnecessary labour

when only the effect on the average rate is sought, but some measure of the variability is required.

A segment (*A*) of *Cyanea* bearing a single ganglion was placed in a large dish of sea water as control. Another segment (*B*) of the same animal with a rhythm similar to the first was placed in a similar dish which contained tryptamine diluted in sea water. The number of beats per minute was recorded for each preparation. In the

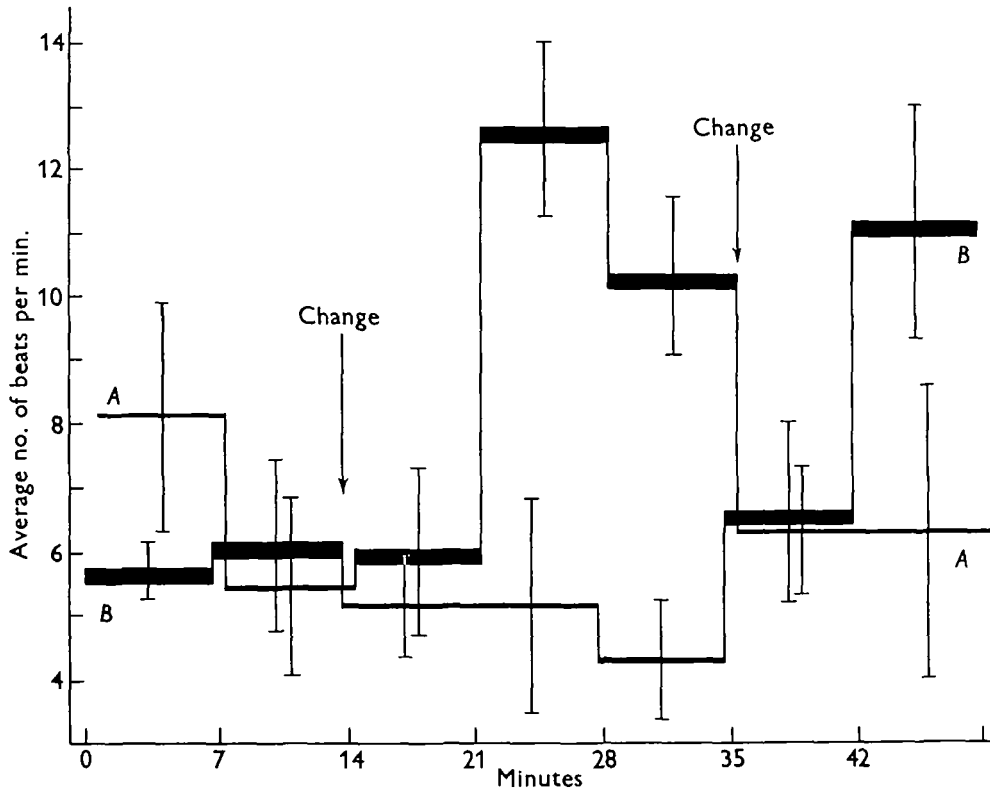


Fig. 7. The effect of tryptamine in concentration 3×10^{-6} g./ml. on the rhythm of two segments of *Cyanea*, *A* and *B*. The number of beats each minute was counted. The mean for each period of 7 min. is represented by a horizontal line; the standard deviation between seven observations each of 1 min. is given for each 7 min. period. A thick line indicates that the preparation is in the tryptamine solution, a thin line indicates that it is in sea water.

example set out in Fig. 7 this was continued for 14 min. The segments were then changed over, each into the other dish, and the rate per minute was recorded for 21 min. The segments were then changed back and observed for another 14 min. The drug acts slowly and there may be contamination between the dishes but both these objections can only reduce the effect of the drug as compared with the control. For convenience the results have been arranged (Fig. 7) as the average number of beats per minute for each 7 min. period, with standard deviation between minutes based on 7 min. in each case. The thick lines of the figure indicate that the ganglion

was temporarily immersed in the tryptamine solution. In each period except the first the treated ganglion beat faster, but the results become significant only when the treated ganglion has been immersed for at least 7 min.

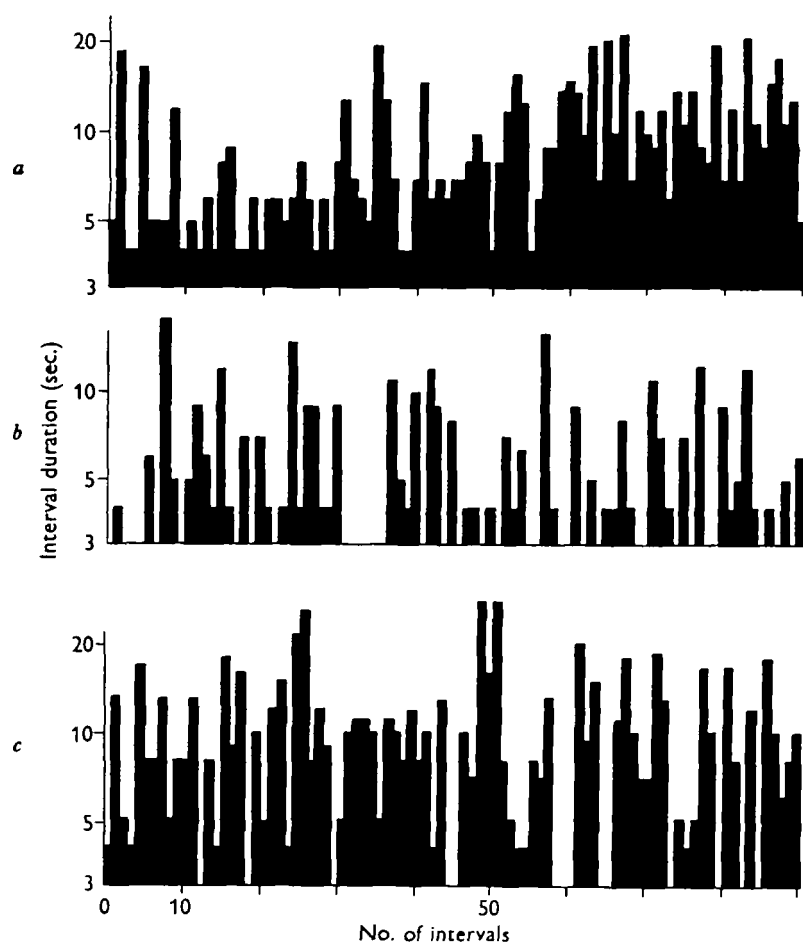


Fig. 8. The effect of tryptamine in concentration 2.5×10^{-5} g./ml. on the character of the rhythm of *Cyanea*. The durations of successive intervals are plotted upwards on a logarithmic scale as columns of equal width reading from left to right. The arbitrary base line was determined by the shortest interval, which happened to be 3 sec. (a) Initially in sea water, (b) after 12 min. in tryptamine 10^{-4} g./ml., (c) on return to similar sea water.

Tryptamine accelerates the rhythm and has been effective on *Cyanea* in concentrations as low as 10^{-5} g./ml. of sea water. *Aurelia* is 10–100 times less sensitive. The effect is completely reversible. No effect on the rhythm has been detected apart from the increased rate. A record of intervals for a typical example is shown in Fig. 8. In particular, analysis of three likely records failed to detect any effect on the first-order serial correlation coefficient. Apparently the drug acts directly on the pacemaker.

By use of the above method of interchanging the control and the treated ganglia between two dishes the effects of the following drugs at concentrations of 10^{-3} g./ml. were tested but no significant influence on the average frequency was discovered: acetylcholine with and without physostigmine, adrenaline, curare, ephedrine, histamine, 5-hydroxy-tryptamine. These observations suggest that, like the coelenterate neuromuscular junction, the pacemaker is pharmacologically different from analogous mechanisms in other phyla.

THE RHYTHM OF AN INTACT ANIMAL AND OF A SINGLE GANGLION

Several attempts to demonstrate some degree of co-ordination between the rhythms of the eight to thirty-two ganglia round the margin of the bell have been reported. Two mutually compatible views can be found in Romanes (1877). He suggests that the rhythm originates from afferent excitation which comes into each ganglion from all parts of the bell, and also that the fastest ganglion fires off all the others and superimposes its rate on them. Horstmann (1934*a, b*) comes to the conclusion that continuous sensory excitation is converted in the marginal ganglia to rhythmical motor excitation. He also notes that the rhythm of the whole animal is more regular than that of a single ganglion. To account for the latter fact he suggests that a variation of frequency of one ganglion is compensated by an appropriate opposite variation of other ganglia which he supposes to be co-ordinated with it. Another suggestion of Romanes is that the rhythm is co-ordinated by some property, such as refractory period, of the muscle sheet, a suggestion which could only apply at frequencies higher than those considered in this paper. Fränkel (1925) suggests that the regularity of the beat has its origin in the movement of the bell itself because the tentaculocysts swing in unison and so maintain exact synchrony.

The above statements must be revised in the light of more recent work on the two overlying nerve nets of Scyphozoa. Excitation in each of the two nets has a particular effect on the rhythm of a ganglion. The action of the giant fibre net in resetting the rhythm of all but the fastest ganglia is experimentally unavoidable, so that the potential rhythms of the slower ganglia are never observed unless the ganglia are isolated. Horstmann found that first one ganglion then another initiates a contraction wave and supposed that some mechanism other than the contraction wave itself was responsible. However, he ignored the standard deviation between intervals and the other characters of the rhythm now described. A further objection to any theory of co-ordination between ganglia is raised by the observation that in favourable specimens of *Aurelia* the excitation in each conducting system is accompanied by a visible wave of contraction, in one case of the bell muscle, in the other of the marginal tentacles; yet during normal swimming the tentacles are relaxed and extend passively to their full length. This indicates that the primary (diffuse) net does not participate in co-ordination of the ganglia. Lastly, Fränkel's suggested mechanical mechanism of co-ordination has not been tested by experiment; for example, the bell might be clamped in various ways to bring out the effect of different movements.

If the ganglia are separately observed in isolation, each will show a normal experimental rhythm. Fig. 9(a) shows a typical example of the distribution of the various intervals between beats for one typical ganglion. If eight such ganglia were co-ordinated by a pathway with the properties of the giant fibre net then the mean interval between beats would be shorter than that for the isolated ganglion. This is

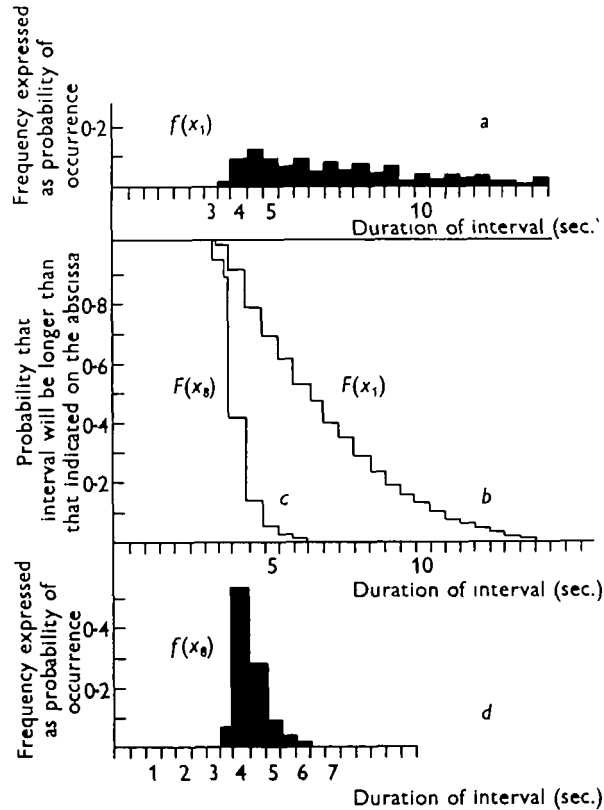


Fig. 9. Derivation of the theoretical distribution function (d) of intervals for a jellyfish of eight similar ganglia from the observed distribution (a) of intervals of one of the ganglia. For further explanation see text.

because the probability of any particular interval being short is increased by the presence of several ganglia, and the short interval determines the rate for all. The calculation of the distribution of intervals for eight connected ganglia is as follows:

The distribution of intervals for a single ganglion is drawn, $f(x_1)$ (Fig. 9a). From this the cumulative distribution function $F(x_1)$ is drawn out by summing the occurrences from $P=0$ for the longest intervals to $P=1$ for the shortest intervals. The ordinate is the probability that values will be greater than the readings on the abscissa (Fig. 9b). The probability that a particular interval will be greater than a particular value x will be $F(x)$. When n ganglia are considered the probability that *all* will have an interval longer than x will be $(F(x))^n$. This means that the long intervals become less likely, for $(F(x))^n$ is less than $F(x)$: therefore short intervals are more

likely. In Fig. 9(c) is drawn $F(x_8)$ for eight ganglia and from the differences between the heights of the steps of $F(x_8)$ the distribution $f(x_8)$ is drawn in Fig. 9(d).

Comparison of $f(x_8)$ with $f(x_1)$ shows that the rhythm of the eight combined ganglia is less variable than that of a single ganglion, although there is no separate excitation which co-ordinates the ganglia. In this example the mean observed interval is 6.93 ± 2.5 sec. for a single ganglion and the mean calculated interval for eight ganglia is 4.23 ± 0.63 sec. ($N=122$ in each case).

This theory is complicated by several factors. The negative serial correlation coefficient r_1 means that short intervals will not occur as often as expected on the above theory because they will tend to be followed by long ones in all the connected ganglia. However, since r_1 is small the effect has been disregarded. Secondly, the independent rhythms of the ganglia differ. This only means more labour in that $F(x_1).F(x_2).F(x_3)$. etc. is substituted for $(F(x))^n$.

THE REVERSAL OF THE COMPENSATORY MOVEMENT

Fränkel (1925) first showed that the beat of the jellyfish *Cotylorhiza* is asymmetrical when the animal is tilted from an even keel. This species always tended to swim upwards in his experiments. The *Rhizostoma* which he observed swam horizontally and failed to give a compensatory movement towards the vertical. However, he adds that in 'special physiological states' *Rhizostoma* shows a compensatory movement.

During observations of several species of Scyphozoa certain findings suggest something comparable to Fränkel's 'physiological states'. For example, specimens of *Pelagia noctiluca* at Naples in 1957 swam upwards when left undisturbed and downwards when held by a clip for better observation. Bozler (1926*b*) found that his specimens of *Pelagia* swam downwards when stimulated, though in the previous year they swam upwards. *Cassiopea andromeda* normally rests subumbrellar surface uppermost on the bottom in shallow water. When disturbed it swims in the normal way upwards and then turns over and swims to the bottom upside down. *Cyanea* specimens usually swim upwards in the laboratory but when disturbed they can be induced to turn over and swim downwards, and when left in water some individuals persistently hit the exumbrellar surface on the bottom of the tank. The mechanism of the reversal of the compensatory movement is unknown; presumably it resides in the marginal ganglia.

CONCLUSIONS

The regulation of the rhythm

The sequence of intervals is not haphazard, and one particular regularity is shown by the negative first-order serial correlation coefficient; long intervals tend to be followed by short ones and vice versa. A self-regulating action of the ganglion is also shown by the temporary slowing which follows a period of abnormally high frequency; a series of artificially initiated beats has a greater effect than a single extra beat. The same regulatory mechanism could account for both of the above effects and the pause which follows a series of forced beats is the overshoot of an

inadequately damped regulator. A similar overshoot is seen in the return to normal frequency in Fig. 3. If such a regulatory system becomes over-compensatory the result could be a regular cyclical series of bursts of beats; and in fact a cyclical rhythm is commonly observed in over-stimulated or damaged preparations. The rhythm is normally controlled at a frequency below the maximum.

Organization of the ganglion

The marginal ganglion of the jellyfish is a concentration of nerve cells on the stalk of the tentaculocyst and in the neighbouring epithelium. It appears to be a relatively simple nervous centre whether considered in terms of histological structure or functional capabilities. The ganglion is connected to the rest of the animal by two physiological conducting systems which are both nerve nets (Horridge, 1956 *b*). A study of the marginal ganglia of the *Aurelia* ephyra revealed only four types of neurones (Horridge, 1956 *a*), and though sensory cells are more numerous in the ganglion of the adult there is no physiological indication that the organization is fundamentally different. The ganglion has two characteristics of higher central nervous systems; it produces impulses spontaneously and it integrates several kinds of sensory excitation, each of which alone can influence the rate of the spontaneous rhythm. The ganglion was considered by Jordan (1912) to be a rudimentary reflex centre, but this term is avoided here on account of its associations with the study of the vertebrate nervous system.

The data now presented show the responses of the ganglion to a variety of stimuli so far as an influence on the rhythm has been noted. The results do not conflict with the histological and physiological results previously set out for the ephyra larva; a physiological model of the ganglion can be partially correlated with a histological map of neurone connexions. Histologically, in both the adult and the ephyra ganglia, there are central endings of motor cells of the giant fibre net. The most satisfactory physiological model locates the pacemaker at these endings, with a polarized junction separating it from the sensory inputs. At this junction the sensory excitation from several distinct sources would be integrated together to modulate the rhythm. The physiological results can be interpreted in terms of one such integrating junction and one pacemaker in each ganglion. However, to account for the large number of nerve cells in the tentaculocyst, a reduplication of pacemakers in the ganglion is suggested, with the fastest setting the pace as for the interaction between ganglia.

The information available from the present study relates only to the rhythmical output of the intact ganglion. Such observations cannot demonstrate the internal organization but may be used to test a theory suggested on other grounds. Experimental confirmation of the mechanism must spring from observations on the components of the ganglion. The theory suggested above has no reference to the self-regulatory mechanism which is inferred from the observations of the sequence of intervals. Here we meet a difficulty in the analysis of the normal action of the ganglion. Experiments that separate the pacemaker from its regulator will produce an abnormal rhythm; but on the other hand the system as a whole defies analysis.

This is a property of any self-regulating mechanism; that this feature would appear in such an elementary central nervous system was unexpected.

SUMMARY

1. Features of the rhythm of isolated ganglia of *Aurelia* are the high variability of the intervals between beats and their negative first-order serial correlation coefficient.
2. A regular rapid rhythm or a cyclical rhythm is a consequence of stimulation.
3. The effect of stimulation of the primary (diffuse) nerve net is usually a transient acceleration of the rhythm.
4. A rapid, uniform rhythm is usually reset at a similar frequency by an artificially induced beat; a normal rhythm usually shows a slight pause, which is accentuated if several antidromic impulses are initiated.
5. The pause which follows forced beats, the negative serial correlation coefficient and the time course of the return to normal after stimulation indicate a self-regulating mechanism within the ganglion.
6. Stimulation of the ganglion with direct current can stop or accelerate the rhythm but throws no light on the possible mechanism.
7. When the ganglion is inverted the rhythm becomes slower and more variable.
8. Tryptamine accelerates the rhythm. The minimum effective concentration in the bathing sea water was 10^{-5} g./ml. Acetylcholine, adrenaline, curare, ephe-drine, histamine, 5-hydroxy-tryptamine and physostigmine have no effect.
9. The theoretical relation between the rhythm of one ganglion and of eight connected ganglia shows that the apparent redundancy of ganglia gives a more regular rhythm.

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