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## THE INFLUENCE OF PRESSURE, TEMPERATURE AND URETHANE ON THE LUMINESCENT FLASH OF *MNEMIOPSIS LEIDYI*<sup>1</sup>

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The luminescent flash response, induced by electrical stimulation of small segments of excised meridional canals of *Mnemiopsis leidyi*, has been recently analyzed in relation to temperature and certain other factors (Chang, 1954). This flash, as well as that of the firefly (Chang, 1956), has been found to resemble, in important respects, the contraction response of directly stimulated muscle fibers. Temperature relations of muscular contraction and various other biological processes, including specific enzyme action, bacterial luminescence, cell division, nerve activity, etc., are subject to modification by increased hydrostatic pressure. Moreover, temperature-pressure relations may be influenced by the presence of narcotics such as alcohol or urethane as well as other chemical agents (*cf.*, Johnson, Eyring and Polissar, 1954; Johnson, 1957; Brown, 1957; Marsland, 1957; Tasaki and Spyropoulos, 1957; Spyropoulos, 1957a, 1957b).

Since studies of pressure-temperature-inhibitor relations have proved a useful approach to understanding certain aspects of the chemical and physiological control of biological processes, and since studies incorporating all three variables are yet available with respect to relatively few processes, the present investigation of the *Mnemiopsis* flash was undertaken. Unfortunately, no separate biochemical components of the luminescent system have been obtained thus far from this organism, and it does not secrete a luminous slime, so the pressure-temperature relations could not be studied in regard to the luminescence of both whole organs and the reaction system *in vitro*, as was recently done with *Chaetopterus* (Sie, Chang and Johnson, 1958). More than 8000 individual flashes of the excised *Mnemiopsis* organs, however, have been accurately measured and carefully analyzed to constitute the basis of this study.

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## MATERIALS AND METHODS

*Mnemiopsis leidyi* collected around Woods Hole, Massachusetts, and kept in large aquaria with very slowly running sea water for not more than two days, were used for this study. As previously shown (Chang, 1954), reproducible responses to electrical stimulation are obtained only with small portions of the photogenic organs, which are closely associated with the meridional canals. For experiments, the canals, with their closely adjacent tissues, were carefully excised. A small piece, measuring from 1.5 to 4 mm. in length, and including from one to four paddle plates, was cut out for the test material. This piece was then placed in a lucite chamber which in turn was sealed in a pressure bomb with a glass window as previously described (Sie, Chang and Johnson, 1958) for the purpose of stimulation at normal or under increased hydrostatic pressures.

A pair of Ag-AgCl electrodes in the specimen chamber was connected to an electronic stimulator which had controllable parameters of pulse amplitude, duration, repetition frequency and synchronization delay. The flash response was recorded by means of a stabilized photomultiplier-amplifier unit described by Chang (1954). The two beams of a dual-beam cathode-ray oscillograph were fed respectively by the output of the light detection unit and by the stimulus signal, and were photographed on a continuously moving film or with a single-frame camera.

Increased pressure was applied by means of an oil-filled hydraulic pump operated by hand. Pressures up to 10,000 pounds per square inch (psi) could be applied within approximately one second.

## RESULTS

*The time course of the flash response*

The time course of luminescent intensity in the *Mnemiopsis* flash has been shown to remain unaltered with increasing flash maxima due to increasing strength of stimulation (Chang, 1954). Results obtained in the present study show that, with a given strength of stimulation, increased pressure reduces the flash maxima but the time course of intensity again remains essentially the same. Figure 1 illustrates superimposed tracings of oscilloscope records from a single specimen under different pressures up to 1000 psi at room temperature. With this specimen, higher pressures diminished the flash intensity so much that the form of the response was hardly analyzable.

Temperature has a marked effect on the time course of the responding flash, which becomes progressively prolonged as the temperature is lowered (Chang, 1954). At a given constant temperature, between 35 and 15.5° C., however, the time course was found to remain unaltered by increased pressure.

*Latent period*

According to a limited amount of data obtained in the present study with respect to the latent period between the time of stimulation and the onset of luminescent response, no significant variation was induced by pressure. While a critical study of this relationship would require additional experiments specifically designed for this purpose, it appears likely that the differences in the latent period under normal and increased pressures would be quite small, if any.



*Pressure versus flash height at constant temperature*

The initial effect of increased pressure was always to reduce the intensity of the flash, and remarkably small amounts of pressure were required to produce a detectable decrease in flash height, so small in fact that they could not be read accurately on the hydraulic pump's gauge, which was not calibrated for pressures less than 200 psi. Moreover, when applied suddenly, as little as 1000 to 1500 psi

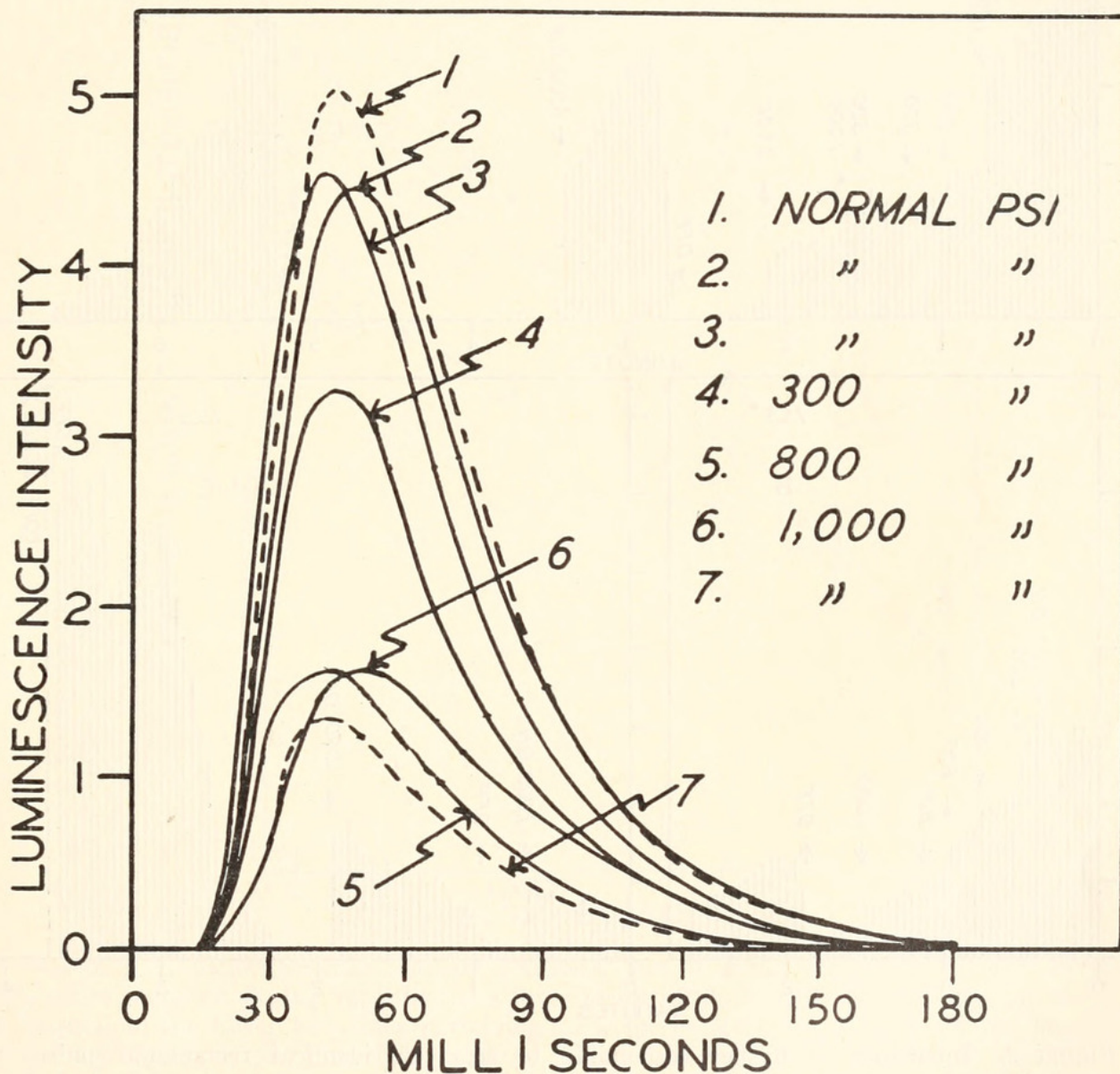


FIGURE 1. Superimposed tracings of oscillograph records of luminescent responses of a single specimen at 22° C., under normal and various increased pressures, applied in a step-wise series. The time was measured from the front edge of the square pulse used for stimulation.

often caused a virtually complete inhibition of the luminescent response (Fig. 2, A and C). Frequently, though not invariably, however, a process of adaptation under a sustained pressure took place, whereby during continued stimulation at a given frequency the flash reappeared and facilitated to successively higher maxima, sometimes reaching intensities several times greater than the highest intensity observed with identical stimuli prior to compression (Fig. 2, C). Apparently, this same



process of adaptation occurred to various extents during step-wise application of pressure in small increments, inasmuch as such step-wise increases up to a given pressure were considerably less inhibitory than a sudden increase to that pressure (Fig. 2).

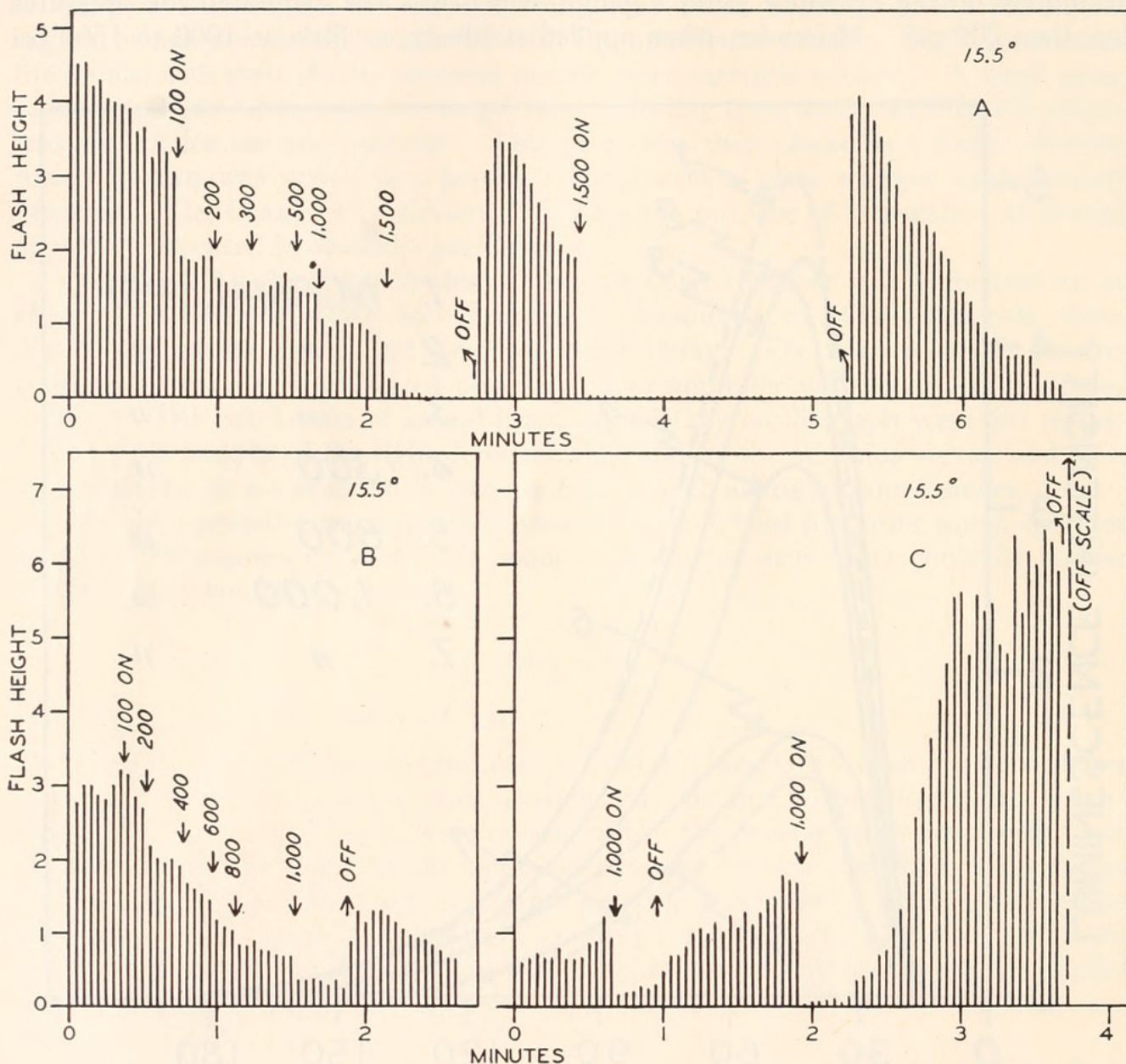


FIGURE 2. Intensities of flashes responding to repeated, identical rectangular pulses at the rate of one every 3 seconds. B and C were taken from the same specimen, and A from another. The downward arrows represent the time of application of the various pressures indicated in psi, and upward arrows represent decompression to normal pressure. The flashes that went off scale in C reached a height of 16 or above on the relative scale of the figure when measured at a lower sensitivity of the phototube.

The initial effect of decompression was essentially always an increase in flash maxima over those occurring while under pressure, or in some instances those occurring prior to compression (Fig. 2, A, B, C). Such increases sometimes attained dramatic proportions, especially in those instances wherein adaptation under pressure had taken place to a very marked extent, as indicated in Figure 2, C and



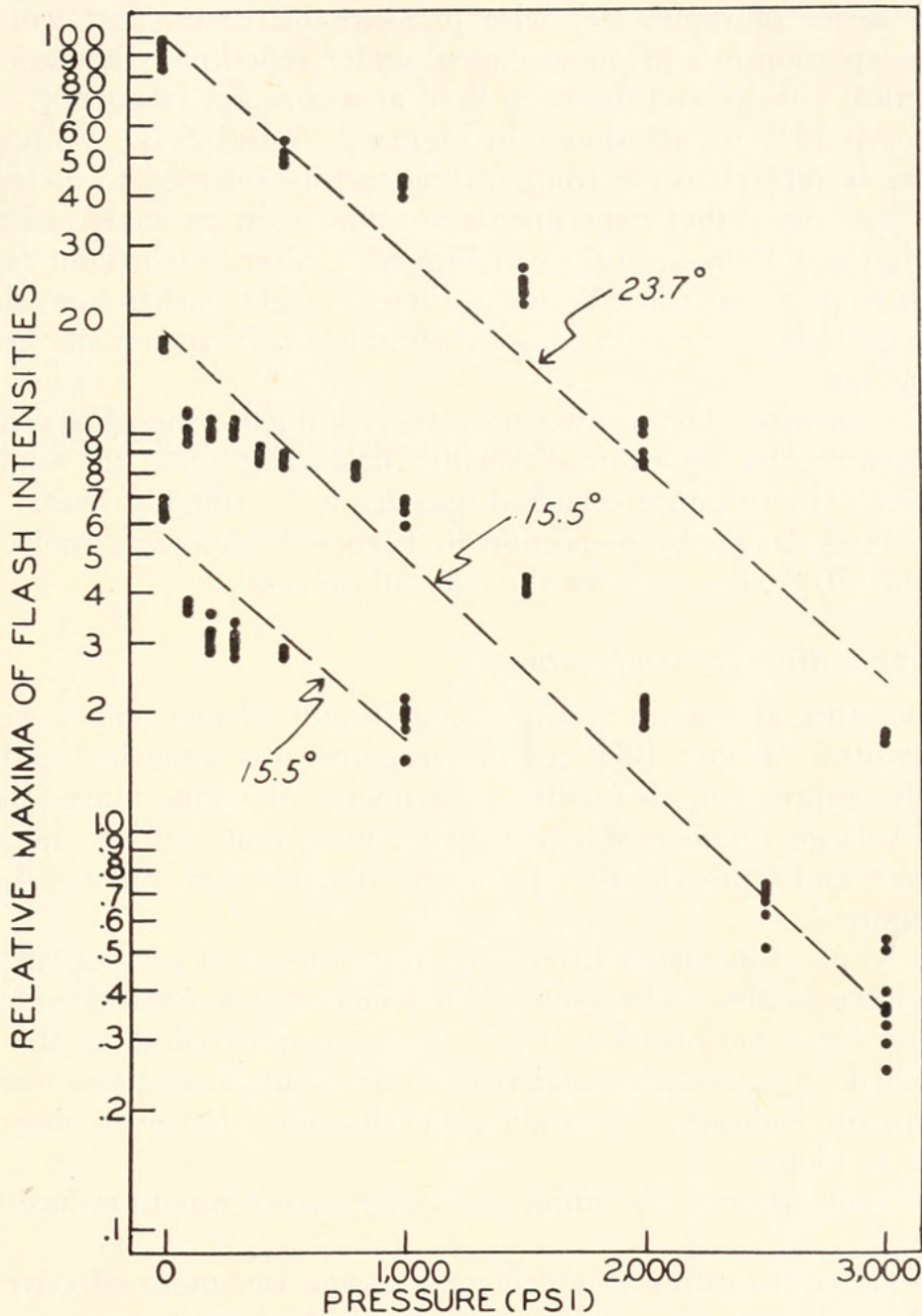


FIGURE 3. Relation between pressure and logarithm of flash intensity, in a step-wise series of pressure increases during repetitive responses to identical stimuli. Dashed lines were drawn by inspection, the lower two lines pertaining to a specimen in sea water, and the uppermost line to a specimen in sea water containing 0.1 *M* urethane.

illustrated more clearly in later figures. The only exceptions to increasing flash maxima following decompression occurred when, for unknown reasons, the specimen deteriorated under pressure with complete loss of excitability (Fig. 6, A).

Because of a wide variability in the quantitative effects of pressure on different specimens, and the phenomenon of adaptation that occurred to various extents at unpredictable rates, reliable data concerning the relation between amount of pressure and of effect produced are obviously difficult to achieve. The physiological state of the specimen at the moment of the experiment was evidently an important factor in the results obtained. The most feasible approach to investigating the quantitative relation between amount of pressure and effect produced appeared to



be through a series of rapid, step-wise pressure increases, that would permit a minimum of adaptation in a given specimen, under repetitive stimulation by square pulses of identical voltage and duration fired at a constant frequency. The results of such a series at 15.5° C. are shown in Figure 2, A and 2, B. Although analysis of these results is subject to the complicating factors referred to above, data from Figure 2 and from two other experiments are plotted in an analytical manner (*cf.*, Johnson, Eyring and Polissar, 1954) in Figure 3, where each point represents the height of an individual flash in a series of three to eight flashes immediately before or after a change in pressure, at a constant stimulation frequency of one every three seconds throughout.

Despite the numerous factors that potentially influence the observed results, the relationship between the logarithm of relative flash height and the amount of pressure under which the response occurred appears to be roughly linear. The slopes of the dashed lines drawn by inspection in Figure 3 indicate a molecular volume change of about 170 cc. per mole for the over-all process.

#### *Pressure effects at different temperatures*

At a temperature as low as 5° C., strong stimuli elicited only a weak response at normal pressure. Under 1000 psi the response was abolished and it failed to return after decompression, so further experiments at temperatures this low were abandoned. A large number of experiments were done within the range 15 to 36° C., however, and representative results are illustrated in Figures 4, 5, and 6, in addition to Figure 2.

Qualitatively, no pronounced differences in the effects of pressure at the different temperatures were found. The same phenomena, and same sort of variability as described above for experiments at 15.5°, were encountered at all the higher temperatures studied. Quantitative differences are difficult to make certain of, for the reasons already indicated. Certain generalizations, however, may be adduced from the data, as follows.

First, at all temperatures the initial effect of pressure was to reduce the intensity of the flash.

Second, at all temperatures a sudden compression was more effective in reducing the flash intensity than was a more gradual or step-wise increase in pressure.

Third, adaptation and facilitation under pressure varied unaccountably. Out of the total number of experiments performed, they failed to occur in a larger number of instances than they did occur. In some instances they failed to occur during reasonably long periods of sustained pressure (Figs. 2, A; 4, A; 5, B; 6, A) even though excitability was not destroyed, as shown by recovery after decompression. In other instances, they occurred readily, sometimes resulting in flash intensities greatly exceeding those at normal pressure as already noted (Fig. 2, C, 1000 psi), or at pressures as high as 3000 psi (Fig. 5, A), 4000 psi (Fig. 6, B) and 5000 psi (not illustrated). Moreover, adaptation and facilitation sometimes occurred promptly on raising the pressure from a given high pressure, where they had not appreciably occurred, to a still higher pressure, *e.g.*, after raising from 2000 psi to 3000 psi (Figs. 4, A and 6, B).

Fourth, although sudden decompression always led to an increase in flash intensity, the pattern of changing maxima in successive flashes varied considerably.



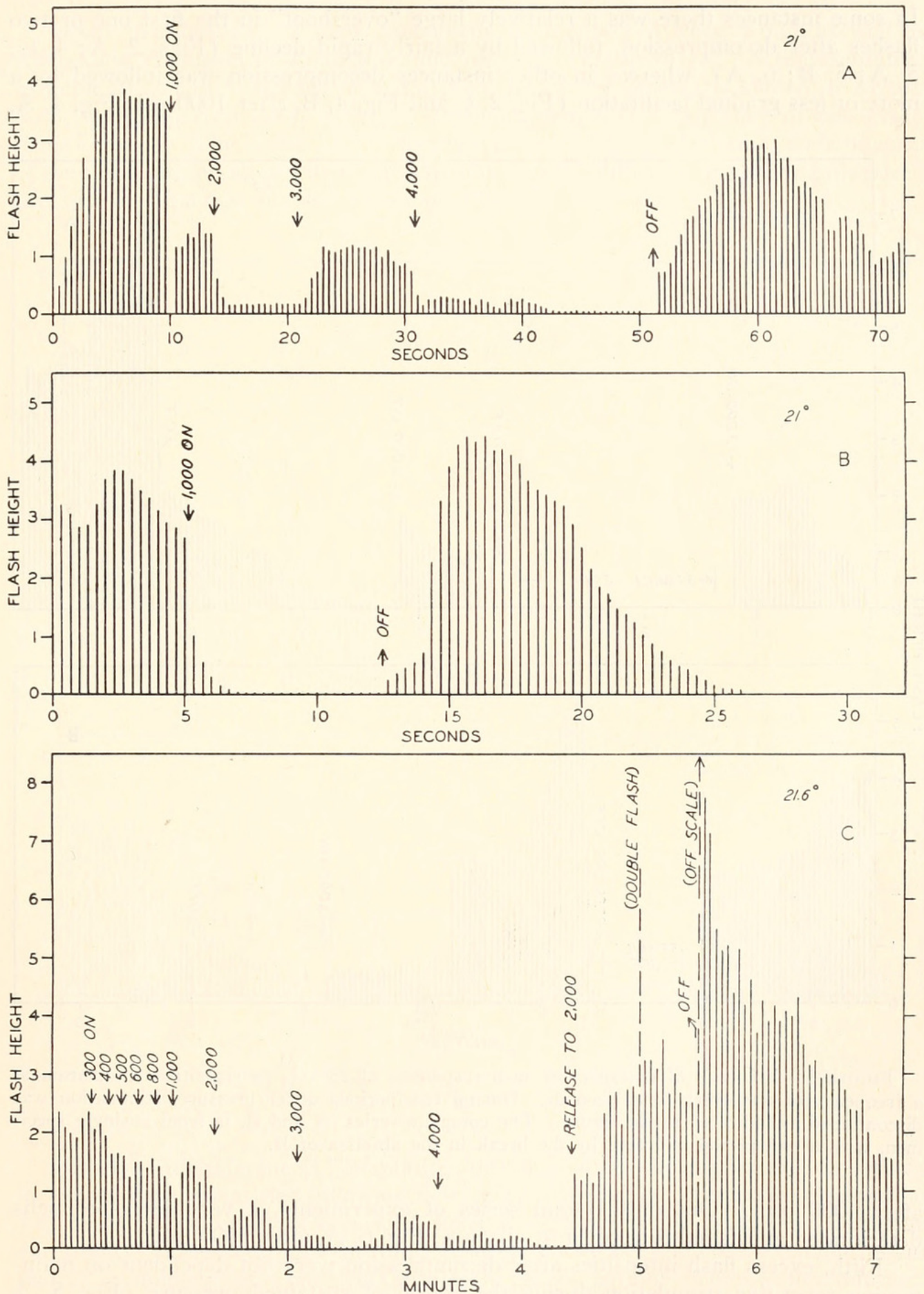


FIGURE 4. Intensities of flash response to repeated identical stimuli, at the rate of one every 3 seconds (A and C) and of 3 per second (B). Arrows represent the time of applying or of releasing, pressure indicated as psi. Room temperature.



In some instances there was a relatively large "overshoot" in the first one or two flashes after decompression, followed by a fairly rapid decline (Figs. 2, A; 4, C; 5, A; 5, B; 6, A), whereas in other instances decompression was followed by a more or less gradual facilitation (Fig. 2, C and Fig. 4, B, after 1000 psi; Fig. 4, A,

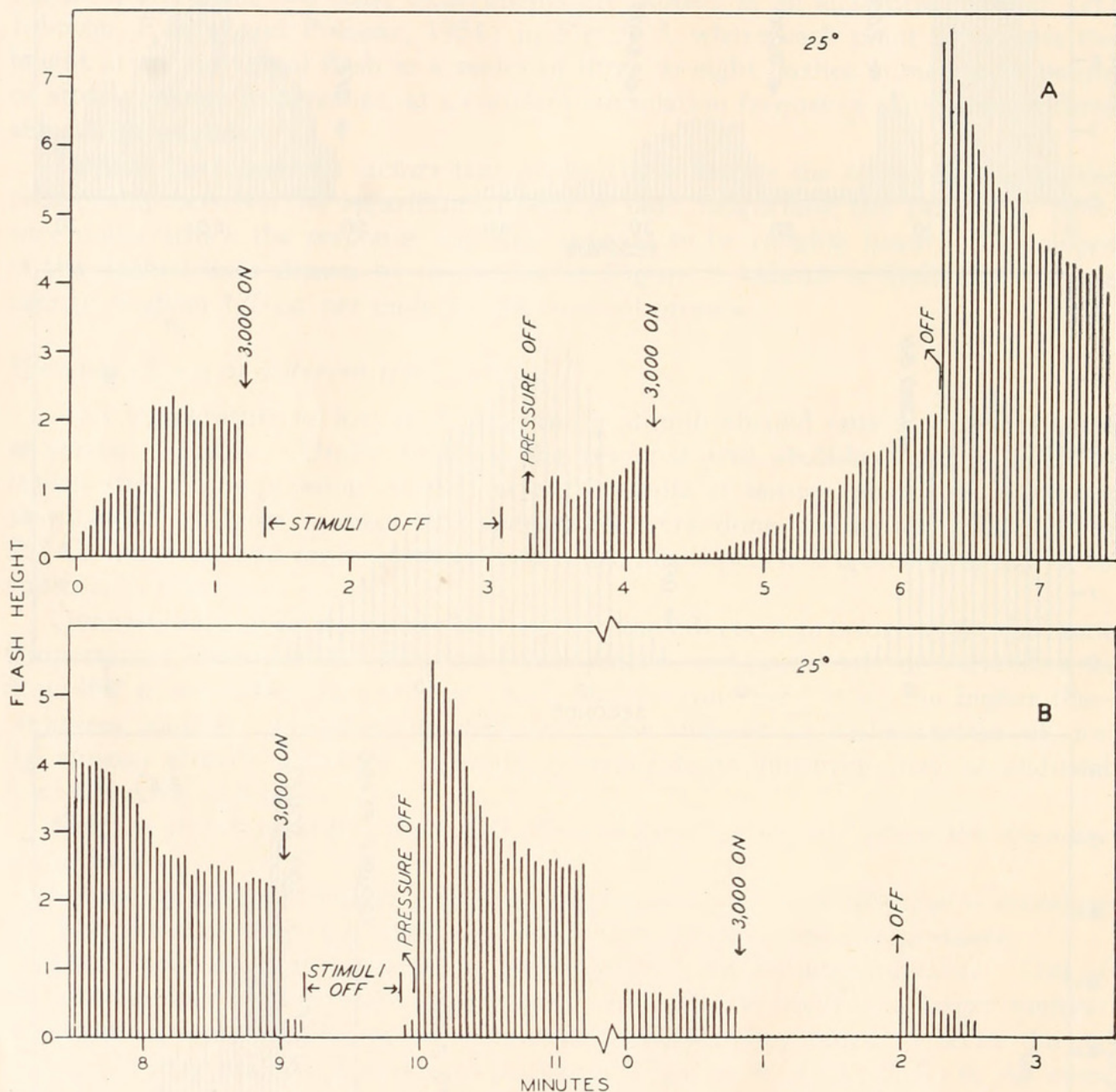


FIGURE 5. Influence of pressure on flash responses, at 25° C., to repetitive stimulation at a frequency of one every three seconds. During two periods under pressure, stimulation was discontinued as indicated in the figure. The complete series, A and B, is from a single specimen, with a rest period indicated by the break in the abscissa of B.

after 4000 psi). Out of the total series of experiments, a very few specimens deteriorated and failed to recover at all (*e.g.*, Fig. 6, A, after 5000 psi).

Fifth, excess flash intensities after decompression were not dependent on maintaining repetitive stimulation during the period of sustained pressure (Fig. 5, A and B).



Sixth, qualitatively the same phenomena were observed when a high as well as when a low frequency of stimulation and response were involved. A representative example of a high frequency of stimulation, *i.e.*, 3 per second, is shown in Figure 4, B, for comparison with the more commonly employed frequency of one every three seconds. The higher frequency was inconvenient as a routine, both because of the rapid fatigue always associated with it, and the difficulty of applying a desired pressure in a fraction of a second between flashes.

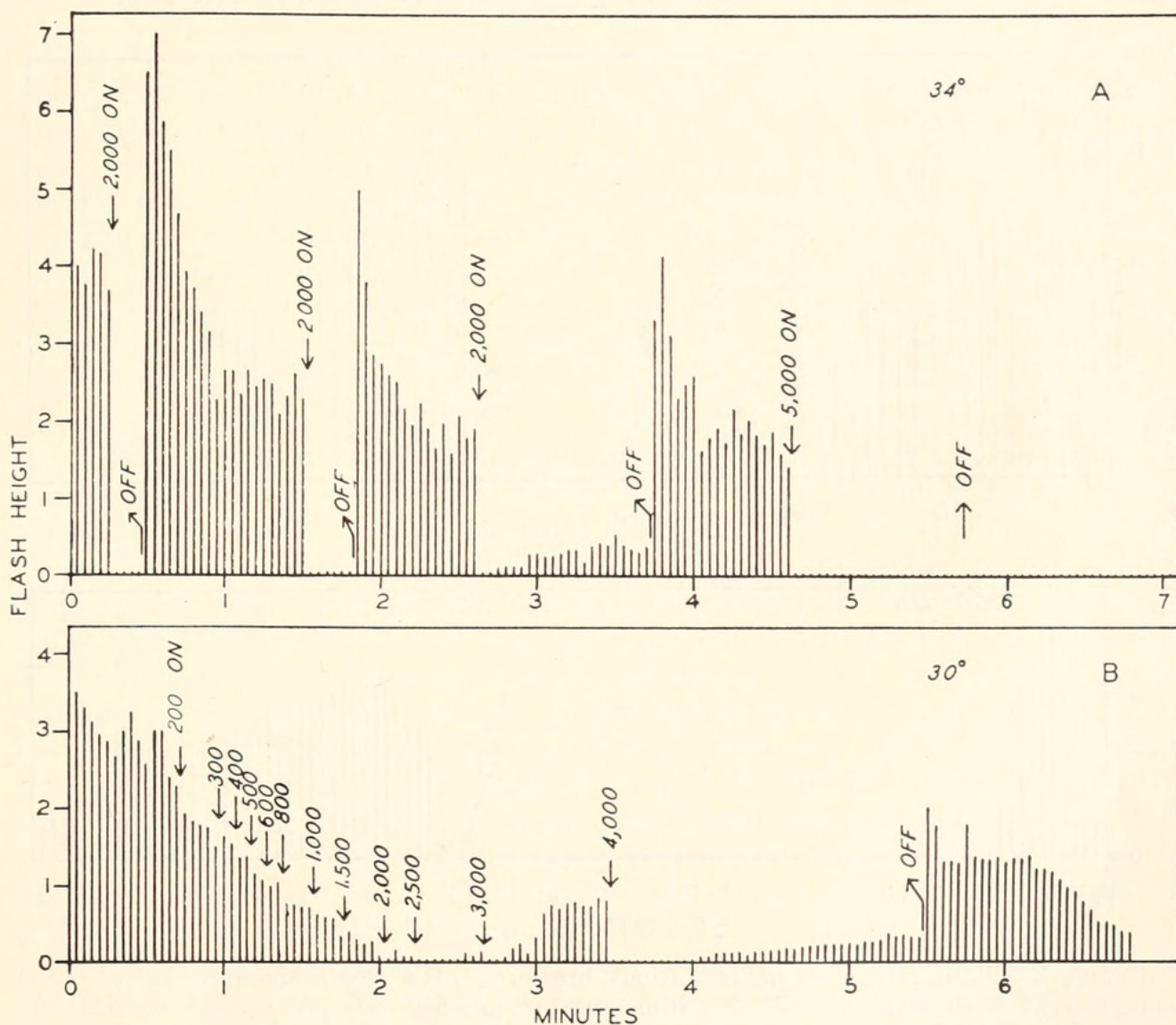


FIGURE 6. Flash responses under various pressures, at 34° (A) and 30° C. (B), at a stimulation frequency of one every three seconds. Two different specimens.

Finally, with reference to adaptation and facilitation under pressure, as well as to excess flash intensities following release of pressure, a noteworthy observation was made on a number of occasions, namely, that specimens which had lost their excitability, through fatigue or other causes, could be rendered excitable again merely by holding them under 3000 to 5000 psi for periods of one to five minutes. Such treatments were not invariably successful, of course, inasmuch as deterioration beyond the possibility of recovery sometimes occurred.



*Urethane and pressure*

At room temperature, 1.0 *M* urethane in sea water quickly abolished the luminescent response. Lower concentrations of 0.5 down to 0.05 *M* caused inhibitions that varied in extent with the individual specimen, the amount of adherent jelly, and duration of exposure to the drug. Although some specimens gave luminescent flashes, at reduced intensity, in 0.5 *M* urethane, at least for a short period of time, other specimens very rapidly lost their excitability in 0.25 *M*. In 0.2 to 0.15 *M* urethane the response of excised canals disappeared after a few

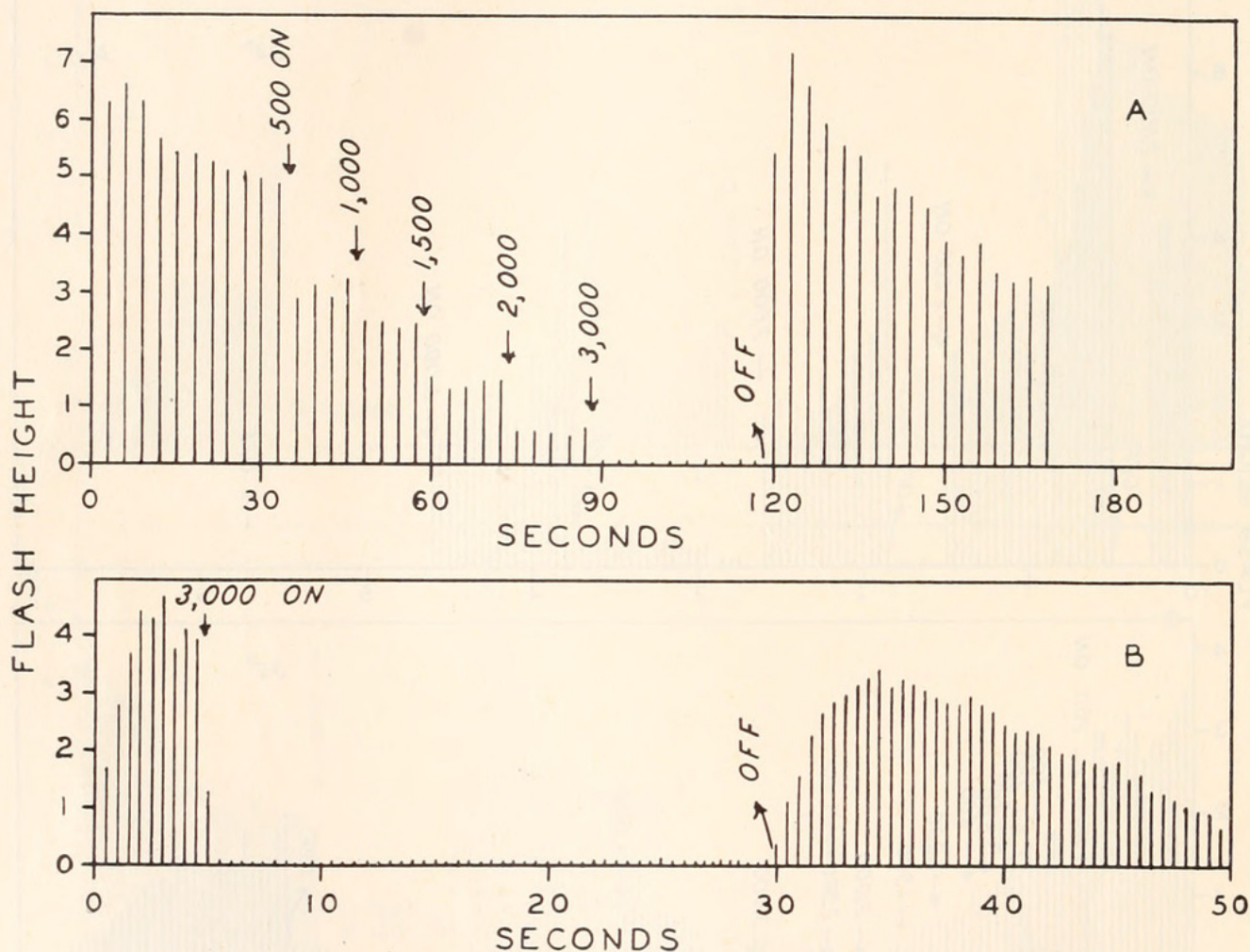


FIGURE 7. Flash responses, under various pressures, of a single specimen in sea water containing 0.1 *M* urethane at 23.7° C. Stimulation frequencies were one every 3 seconds (A) and 2 per second (B).

minutes and the cilia stopped beating, although in sea water without urethane the luminescence and ciliary action would often persist for a couple of days. In 0.1 *M* urethane the response was lost after about half an hour, but in 0.05 *M* it persisted dimly for a longer time. Thus, urethane causes a progressively increasing inhibition of the flash response, at a rate depending on the concentration added, being faster the greater the concentration. In studying the influence of pressure, therefore, the experiments were carried out within a short period of time after adding the drug.

The results showed that the generalizations described in the preceding section with respect to the action of pressure in absence of urethane are qualitatively ap-



plicable in the presence of urethane. Representative data are illustrated in Figure 7, for 0.1 *M* urethane and two different frequencies of stimulation. The instability of the system in the presence of urethane, especially in the higher concentrations or at higher temperatures, made satisfactory experiments difficult to carry out, and the quantitative significance of the results uncertain. The data indicate, however, that no marked difference in volume change for the over-all process of the flash is caused by urethane (see Fig. 3).

### DISCUSSION

The *Mnemiopsis* flash is obviously limited by two types of processes, namely, physiological excitation that leads to a luminescent response, and biochemical reactions involved in light emission itself. While detailed information is not available with respect to either of these processes, it is reasonable to believe that pressure can alter the response by influencing the state of the activating system in the photogenic organ just prior to, or at the time of stimulation, as it does in muscle (Brown, 1957). Moreover, it may be assumed with considerable assurance that the process of light emission is limited by the activity of one or more essential enzymes. The probability that *Mnemiopsis* luminescence is directly dependent on more than one enzyme is suggested by the somewhat complicated effects of pressure described in this paper, as well as by the inability to demonstrate a simple "luciferin-luciferase reaction," *i.e.*, light emission on mixing a boiled and cooled aqueous extract of the triturated photogenic organs with a cold-water extract of similar organs. Even with a single limiting enzyme, pressure may affect the over-all process through several mechanisms. Two mechanisms of potential importance are (1), by influencing an equilibrium between catalytically active and reversibly inactivated states of the enzyme, the actual state depending upon temperature and the conditions of the chemical environment such as presence of drugs, ions, or other agents that act on the equilibrium, and (2), by influencing the catalytic process itself, *i.e.*, the change from normal to activated states of the reactants. Where consecutive reactions are responsible for the measured results, the effects of increased pressure are liable to become considerably more complicated, and transitory changes from one steady-state to another can assume a variety of patterns, such as an initial augmentation followed by an inhibition, or vice versa, with a converse pattern associated with the release of pressure.

In the present experiments the immediate effects of changes in pressure are attributable to an immediate change in one or more specific reaction rate constants, or possibly equilibrium constants. The phenomena of adaptation and facilitation under pressure, as well as "overshoot" following decompression, are indicative of changes in steady state concentrations of reactants, although effects on slowly changing states of equilibria cannot be ruled out. Such equilibrium changes could pertain, *a priori*, either to the process of physiological excitation or to enzymes involved in light emission, but the fact that "overshoot" and excess luminescence following a period under pressure are not contingent on continued stimulation during that period argues against the process of physiological excitation as the major site of action.

The pronounced instability of the *Mnemiopsis* system at high temperatures or in



the presence of urethane makes it unusually difficult to find evidence of any reversible, inactivation equilibrium change, analogous to those which limit other processes that undergo less rapid destruction under the influence of these factors (Johnson, Eyring and Polissar, 1954). In *Mnemiopsis*, if such equilibria exist, they are obscured by the essentially irreversible processes, and the effect of pressure on these equilibria becomes correspondingly difficult to detect.

Finally, some basic similarities should be pointed out with respect to the influence of pressure on the electrically stimulated flash in excised segments of the *Mnemiopsis* organ, and on the much slower flash in excised notopodia of *Chaetopterus* (Sie, Chang and Johnson, 1958). Thus, both exhibit initial reductions in flash maxima, followed by adaptation and facilitation under pressure, as well as "overshoot" and excess luminescence on release of pressure. Parenthetically, in view of the fact that the vertical distribution of certain ctenophores extends to depths of some 2500 meters (Chun, 1903), where the pressure amounts to 250 atmospheres or about 3500 psi, it is interesting to surmise that the phenomenon of adaptation of the flash response to increased pressure might enable a luminescent ctenophore to descend gradually from the surface of the sea to such depths without having the intensity of its flash reduced by the increase in pressure.

The similarities between the flash of *Mnemiopsis* and that of *Chaetopterus*, as well as similarities in the effects of pressure on the two, are more impressive than the differences, which are largely quantitative. The flash of the latter organism is of the order of 100 times longer in duration, but in both organisms the decay after peak intensity is exponential, and in both organisms the time-course becomes longer with decrease in temperature. The *Chaetopterus* flash, as well as luminescence intensity of the secreted slime, are somewhat less sensitive to increased pressure than is the *Mnemiopsis* flash, while the effects of increased pressure are more sensitive to temperature and to the presence of urethane.

The foregoing remarks are necessarily somewhat general. Further and more specific interpretation of the observed phenomena must await more detailed knowledge than is presently available concerning the total process of the flash response. The effects of pressure on the luminescence of homogenates ("squeezates") of *Mnemiopsis* have been studied and will be made the subject of a later communication.

#### SUMMARY

Using small segments of excised meridional canals of *Mnemiopsis leidyi*, luminescent flashes induced by square wave electrical pulses of precise voltage and duration have been accurately recorded with the aid of a photomultiplier-amplifier and dual beam cathode ray oscillograph. Analyses of more than 8000 flashes, under various conditions of temperature, hydrostatic pressure, urethane concentration, and frequency of repetitive stimulation, have led to the following generalizations.

1. The time course of luminescence intensity in an individual flash at a given temperature is not appreciably altered by increased pressures which greatly reduce the flash maximum.

2. The latent period between time of stimulation and onset of response is likewise not significantly altered by pressure, within the sensitivity of the methods employed.



3. In a series of consecutive flashes, at frequencies of one per 3 seconds to 4 per second, the initial effect of increased pressure is always to reduce the maximum intensity of the flash; detectable reductions are caused by relatively slight pressures, of less than 100 psi.

4. A series of pressure increases in increments of several hundred psi is less inhibitory on flash intensities than a sudden increase to the highest pressure involved. Sudden increases of 1000 to 5000 psi temporarily abolish the flash, whereas with gradual increases to these pressures, the flash may persist, though at reduced intensity.

5. Under a sustained pressure, a process of adaptation frequently occurs, whereby on continued repetitive stimulation the initially inhibited flash recovers and then facilitates, sometimes to much higher intensities than prior to compression.

6. On sudden decompression, part way or all the way to atmospheric pressure, the initial effect is always an increase in flash intensity over that occurring under pressure, or sometimes over that occurring prior to compression. The only exception occurs when excitability has disappeared completely, as occasionally happens.

7. The recovery process after pressure assumes a variety of unpredictable patterns; in some instances the first one to three flashes are excessively high ("overshoot"), followed by rapidly decreasing flash maxima, whereas in other instances a gradual facilitation and decline take place.

8. Excess luminescence intensity in the recovery phase is independent of maintaining repetitive stimulation during the preceding period under pressure.

9. Excitability that has been lost through fatigue or unknown causes can be restored in some instances by subjecting the specimen to pressures of 3000 to 5000 psi for periods of 1 to 5 minutes.

10. Qualitatively the same results of pressure are observed at various temperatures between 15 and 35° C. Any definite influence of temperature on the effects of pressure is obscured by variations in the quantitative effect of a given pressure on different specimens and on the same specimen in different physiological states.

11. Urethane, in concentrations between 0.05 and 0.5 *M*, causes a progressively increasing reduction of flash maxima with duration of exposure of the drug, and at rates that increase with drug concentration and temperature. Qualitatively the same phenomena are observed with respect to the influence of pressure on the flash in the presence as in the absence of urethane.

12. The *Mnemiopsis* photogenic system is particularly sensitive to destructive effects of urethane and of elevated temperatures, thereby obscuring the possible existence of reversible thermal inactivation reactions and the possible influence of pressure thereon.

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