the 1-hour period following exposure to the three temperatures during each of the eight 3-day periods. During the first period, the rankings are exactly the reverse of what would be expected on the basis of heat production and caloric need in the cold. By the third period, however, the rankings are in accord with this expectation. An analysis of variance performed on these data showed that differences among temperatures and periods attained the .001 level of significance.

A second study—this one of response to 20-minute exposures to temperatures of either −10° or 20°C for six consecutive days—showed, during an 18-day period (which allowed for two reversals), results similar to those obtained in the first experiment. Both food intake and frequency of response at the lower temperature tended to increase for about 3 days and then to level off.

One criterion of acclimatization is a change in behavior that is more adaptive in one particular situation than in another. In the present situation, acclimatization would be evinced by a greater response frequency at lower than at higher temperatures (provided that bar-pressing is not inefficient as a method of producing and conserving heat for a limited period) and by an increase in the amount of food ingested after exposure. While the former did not occur, there was still a considerable rise in frequency of bar-pressing from the original level for the two lower temperatures, and I think it fair to call both the changes in bar-pressing behavior and in food consumption evidence of acclimatization—or, at least, a form of acclimatization.

The other feature of the data that seems pertinent to acclimatization is the retention of adaptation in the face of constant shuffling of the exposure temperature. Perhaps most striking is the specificity that soon appears in food intake. This short-term adaptation of energy intake to energy output is one of the three types of energy regulation postulated by A. Mayer (*5*), who, in the rabbit, found this short-term regulation to be the most precise of the three types. It must be emphasized once more, however, that the findings I am reporting are based on very brief exposures.

These data do not warrant making any statements about the ultimate mechanism of regulation of food intake. It would seem, though, that day-to-day regulation, at least with this feeding schedule, is quite sensitive to changes in temperature and that there is a short latency of response to its effects.

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2. These data were obtained in the laboratories of the Department of Experimental Psychology, U.S. Air Force School of Aviation Medicine. They were reported earlier at the 1957 meetings of the Federation of American Societies for Experimental Biology.
3. Only the first four periods are included in this graph because the animals were changed to a 3-minute periodic reinforcement schedule, beginning with the fifth period, and the data for the last four periods are therefore confounded with the response to the changed program.
4. B. F. Skinner, *The Behavior of Organisms* (Appleton-Century-Crofts, New York, 1938).
5. This work has been reviewed by J. Mayer [*Ann. N.Y. Acad. Sci.* **63**, 15 (1955)].

17 October 1957

Apparent Exception to the "All or None" Law in Cardiac Muscle

The "all or none" law as applied to muscle states that the strength of the contraction is independent of the strength of the stimulus. Recently my coworkers and I observed an apparent contradiction to this principle. We were repetitively stimulating, at threshold voltage, an excised cat papillary muscle in a saline (*1*) bath by means of mass platinum electrodes lying alongside the muscle. When the stimulus voltage was suddenly doubled, the contractions, after a few seconds' delay, became gradually more forceful until they were about 100 percent above the previous level (Fig. 1*A*). When the voltage was returned to the threshold value, the contractions, after some delay, returned to the base line. The phenomenon was repeatable, and all cat papillary muscles (18 in all) have shown this response in varying degrees. With a tenfold increase in stimulus voltage, the augmentation was much more pronounced.

These observations suggested that some fibers, quiescent at the lower voltages, were activated by the stronger stimulation—that is, that the papillary muscle was not a functional syncytium. The delay in the onset of the augmentation could be explained by assuming that some time was required before the recruited fibers reached maximum tension (treppe phenomenon). To test this suggestion the muscle was speared with a KCl-filled glass microelectrode having a maximum diameter at the tip of 0.5 μ (*2*). Resting and action potentials were recorded from 697 individual cells in 12 cat papillary muscles during electrical stimulation at threshold voltages (*3*). No cells were found that showed a resting potential but no action potential, such as might be expected to exist if the nonsyncytial hypothesis were correct. Accordingly, it was abandoned.

Results from eight isolated strips of cat atria suggested another hypothesis. In this tissue, under similar conditions, strong stimulation produced an initial depression, then a post-high-voltage rise in the contraction strength (Fig. 1*B*). Since acetylcholine would be expected to depress the contractions and would be expected to be present in the atria, it seemed possible that the strong stimulation had caused the release of this autonomic mediator. Indeed, atropine consistently blocked the depression and unmasked the augmentation produced by strong stimulation (Fig. 1*C*). These observations were consistent with the view that, in the atrium, both acetylcholine and a potentiating substance were released by the high voltage. When the stronger stimulation was terminated, the release of both substances diminished (or ceased), but the rapid hydrolysis of the acetylcholine permitted the post-stimulation augmentation by the potentiating substance. The possibility that acetylcholine is responsible for both depression and augmentation cannot be overlooked. [Burn (*4*) cites several examples of the potentiation of contractions by low doses of acetylcholine.] If this were the case, however, one would expect that some augmentation would precede the depression, but this did not occur.

Since it seemed reasonably certain that acetylcholine was the depressing agent, it followed that epinephrine or norepinephrine might be the potentiating substance in both the atria and the papillary muscle. In addition to these considerations and the fact that these hormones are known to be present in heart muscle (*5*), there were other indications that implicated the epinephrines. For instance, spontaneous activity commonly

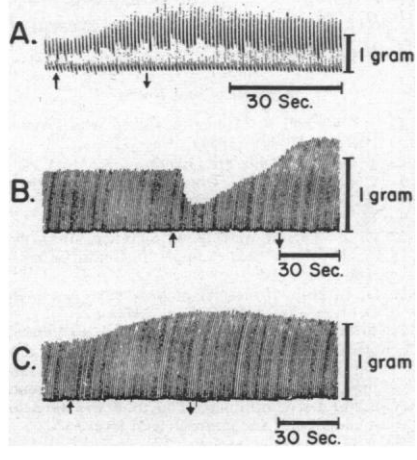

Fig. 1. *A*, Isometric contractions of driven papillary muscle of cat. At upright arrow, stimulus voltage was suddenly increased from 40 to 80 v; at inverted arrow, voltage was reduced to 40 v (threshold). *B*, Isometric contractions of left atrium of cat; arrows same as *A*. *C*, Same as *B*, 5 minutes after the addition of 10 μg of atropine to 8 ml bath.

occurred a few seconds after the strength of the stimulus was reduced to the threshold value. Incidentally, the spontaneity could usually be stopped by again increasing the voltage. Also, the threshold for electrical stimulation fell during and shortly after a period of high-voltage stimulation. The similarity of the responses to strong stimulation and the effects of adding epinephrine or norepinephrine is striking. Finally, large amounts (150 $\mu g/cm^3$) of the sympatholytic drug dibenamine abolished or minimized the augmentation in both the atria and the papillary muscle.

It has been tentatively concluded that acetylcholine is the depressor substance and that one of the epinephrine compounds is the potentiating substance. The relative amount of each substance released will determine whether depression or potentiation will predominate. Thus, the lack of a significant depressor effect in the papillary muscle is explained by a relative absence of acetylcholine. In the atria, released acetylcholine overwhelms the concomitant release of the potentiating substance.

It is obvious that this phenomenon should be considered in interpreting the results of experiments which involve electrical stimulation of cardiac tissue. In pharmacological studies on isolated muscles, at least, it is quite possible that certain drugs may exert their effects by altering the threshold for the release of these substances. Further, if different areas of the heart contain different amounts of the two substances, as suggested here, uniformity of results with different tissues could hardly be expected.

More significance might be attached to these results if it could be demonstrated that these substances are released in the spontaneously beating heart strip or in one stimulated by point electrodes at threshold voltage. This seems a likely possibility in view of the fact that acetylcholine and the epinephrines are released in the spontaneously beating perfused heart (*4*). If these substances are released with each normal contraction, a number of previously described phenomena might be explained. For example, the treppe phenomenon and post-stimulation potentiation could well be explained on this basis (see *6*). Also, the opposite effect ("negative" treppe) seen in the rat ventricle could be due to a relative absence of the potentiating substance. Indeed, preliminary studies indicate a crude correlation between the magnitude of the high-voltage potentiation and the extent of the treppe phenomenon and of post-stimulation and extrasystolic potentiation (*7*).

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4 November 1957

Long-Term Recording from Single Neurons in Brain of Unrestrained Mammals

Much of our knowledge of the activity of single neurons in the central nervous system has been concerned with the study of phenomena of short time course —milliseconds to hours—and has involved the use of anesthetized animals, animals with brain lesions, or animals restrained by curarization. Some information is beginning to appear on the activity of single neurons in unanesthetized normal animals, but this is limited to activity of, at most, a few hours for the same neuron (*1*).

It has been found possible to record discharges of single neurons for periods of a week or more by implantation of several stainless-steel wires, 80 μ in diameter, with insulation exposed at the cross section of the tip. The animal in use is the California ground squirrel, *Citellus beecheyi*, which is being used in a study of brain mechanisms in hibernation.

The implantation technique is fully described elsewhere (*2*), and since it involves many details necessary for the particular measurements made in hibernating squirrels, only the details pertinent to this report are described here. The anesthetized squirrel was placed in a specially designed stereotaxic instrument, and holes were drilled and tapped in the skull for four 0-80 stainless-steel machine screws; smaller holes (approximately 340 μ in diameter) were drilled at desired sites for the 80-μ stainless-steel insulated wires. The single 80-μ wires were lowered into the brain through the latter holes with the aid of a manipulator or, for short distances, merely by hand. A dental cement was applied around the base of the electrode and allowed to run onto one of the screws; after the cement had hardened, the electrode holder was loosened and withdrawn. Ordinarily, four to six of these particular electrodes were implanted in the brain of each animal. About 2 mm of the free end of the 80-μ wire was carefully stripped of its insulation and a No. 28 gauge color-coded flexible lead was soldered to it. Several more layers of the dental cement were applied until the solder junction was firmly imbedded. The leads were all brought together, tied at intervals, and passed through a flexible plastic tube, which was then imbedded in cement at its base.

Connection to the unanesthetized, unrestrained squirrel was made by merely plugging in the appropriate leads; these ran out of the animal's cage, and handling of the animal was not involved. Conventional amplifying and recording equipment was used, and the potentials at the tip of the electrode were referred to one of the screws on the skull, which was grounded. With the method of connection used it has been possible to study unit patterns during intervals when the squirrel was falling asleep, waking, and in the alert state.

Figure 1 illustrates the discharge of a unit with a predictable pattern when the squirrel is alert and, in this case, curled up in its nest. The room was dark and the squirrel had been made immune to camera noises by puncturing of both eardrums and plugging of the external meati with cotton at the time of electrode implantation. Three long impulse trains of consecutively shorter duration are shown, with relative silence between trains. Each train is further characterized by a high-frequency outburst close to the start of the train. The slow waves have been filtered out; they are normally

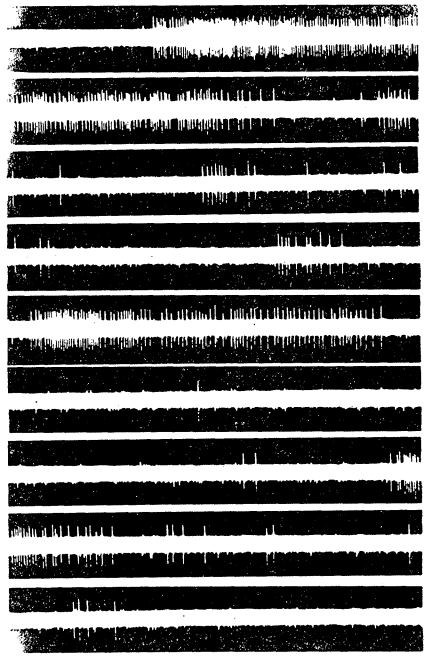

Fig. 1. Pattern of a unit in the mesencephalic reticular formation of an alert, deafened squirrel, resting in the dark, recorded on the eighth day after implantation of the electrode. Time for each strip is 4.6 sec; spikes average 60 μv; time constant, 2 msec.