sterone synthesis (*10*). A variation in the amount of precursor substrates or cofactors would be equally likely to influence the adrenal cycle.

The conversion of added substrate progesterone-4-C^{14} to corticosterone is not constant but this conversion apparently follows a different time course from that of the in vitro ACTH response (*6*). Since a rhythm of adrenal response to ACTH added in vitro can be observed in the absence of the glands' nervous, hormonal or circulatory controls during the 2-hour incubation periods, use of this technique may further reveal the roles of intrinsic adrenal, pituitary, and other factors in the adrenal cycle.

The timing of the reactivity rhythm to ACTH, demonstrated in vitro, can be compared with that of the serum corticosterone rhythm of the adrenal donor mice, also shown in Fig. 2. Means and the standard errors are shown for each time of adrenal removal for the incubation study. Peak adrenal responsiveness to ACTH, added in vitro, is grossly out of phase with the peak of serum corticosterone found in the absence of all intentional external stimulation other than that by alternating 12-hour periods of light and darkness.

The rhythms of serum levels as well as adrenal content of corticosterone should reflect, in part, spontaneous cyclic adrenal activity. If so, these results demonstrate a significant difference between the timing of endogenous cyclic adrenal activity under basal conditions and the timing of the glands' ability to respond to increasing doses of ACTH. Extrapolation of a phase difference between rhythms in adrenal activity and the glands' reactivity to ACTH beyond the inbred C mouse and the dose range of the ovine rather than murine ACTH studied would not be justified without additional evidence. From a methodologic viewpoint, the demonstration of differences in the in vitro response of an organ coincident with predictable changes in physiologic state emphasizes the usefulness of circadian rhythm analysis as a powerful tool for biological investigation, particularly for bioassay, and in the general area of endocrinology (*11*).

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11. The valuable technical assistance of Joanne McNally, Orville L. Weiszhaar and Robert J. Ertel is gratefully acknowledged. This work was supported in part by grants from the U.S. Public Health Service (A-4661) and the American Cancer Society (E-155C). A further exposition of these experiments and the statistical design will be published later by Dr. R. McHugh and Mrs. R. Loewenson of the University of Minnesota.

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Conditioning of Extrasystoles in Humans with Respiratory Maneuvers as Unconditional Stimulus

Abstract. Extrasystoles are known to occur in some healthy individuals during common respiratory maneuvers such as holding the breath. Evidence is presented which shows that under controlled laboratory conditions this kind of extrasystole can be conditioned. Conditional stimuli signaling inspiration and expiration acquired the properties of the respiratory maneuvers by inducing extrasystoles during normal breathing.

Recent studies in the field of cardiovascular conditioning have revealed that changes in the configuration of the electrocardiogram can occur as a conditional reflex. Bykov and collaborators (*1*) have shown that electrocardiographic changes induced by morphine, strophanthin, or nitroglycerin can be conditioned after a given conditional signal, such as the sound of a trumpet, has been paired with the injection of one of these drugs. Perez-Cruet and Gantt (*2*) have shown conditioning of the changes in amplitude of the T-wave on the electrocardiogram to a tone previously paired with an intravenous injection of bulbocapnine.

Most investigations on conditioning of the electrocardiogram have been performed in dogs. In humans evidence of this basic principle is not available. Some knowledge of electrocardiographic conditioning in animals provided the basis for the design of the present study, whose main purpose it was to investigate some of the mechanisms by which the brain can induce extrasystoles through conditional reflexes.

One hundred and five volunteers with negative history of cardiac disease were studied. There were 91 males and 14 females, ranging in age from 17 to 43 years (average age was 26). The subjects were placed in a recumbent position on a ballistocardiograph or sitting on a chair. They were instructed to look at a rectangular board (3 by 4 feet) on which were mounted eight lights in serial arrangement. All subjects were instructed that when a given set of three lights was on they should take a deep breath and hold it until another set of eight lights was on, at which time they should exhale slowly and completely, and resume normal breathing again when the eight lights were off.

In 13 subjects the lights plus the respiratory maneuvers induced one isolated extrasystole in one out of ten trials. In six other subjects the mean values (41 to 93 trials) of unconditional extrasystoles per trial induced by respiratory maneuvers were: S_1, 5.2; S_2, 1.9; S_3, 0.6; S_4, 1.0; S_5, 0.3; and S_6, 0.2. The percentages of trials with unconditional extrasystoles during the same conditions were: S_1, 92.7; S_2, 69.0; S_3, 61.0; S_4, 46.5; S_5, 23.7; and S_6, 15.1. Extrasystoles occurred during the inspiratory and expiratory phases of respiratory maneuvers, except in three subjects where extrasystoles occurred only at the beginning of expiration. Almost all extrasystoles were of ventricular origin, but some premature auricular beats were observed in young subjects. Figure 1*A* illustrates an example of unconditional extrasystoles induced by the respiratory maneuvers in subject 4.

In view of the high incidence of extrasystoles during respiratory maneuvers in subjects 1 to 6, these six individuals were selected for the attempted conditioning of extrasystoles. The conditioning techniques consisted in pairing the same lights signaling inspiration and expiration, namely, the conditional stimuli, with the respiratory maneuvers. Average duration of the conditional stimuli was 39 seconds. Subjects were allowed to breathe normally after each trial for at least 38 seconds (intertrial interval). After the subjects had been exposed to the lights plus the respiratory maneuvers 15 or more times, instructions were

given that they should keep looking at the rectangular board, but should maintain a normal respiratory rhythm. The same sets of lights were presented again, but in the presence of a normal respiratory rhythm (extinction period). The frequency of the response during extinction, that is, the appearance of extrasystoles during the presentation of the same lights but in the presence of a normal respiratory rhythm, was used as the main criterion for conditioning, provided the same lights had been previously reinforced with the respiratory maneuvers. Conditioning was also evaluated in terms of rate of formation, rate of extinction, latency of response, external inhibition, and restoration.

It was found that only subjects 1, 2, and 4 showed evidence of conditioning of extrasystoles. The mean values (10 to 32 trials) of conditional extrasystoles per trial induced by the conditional stimuli were: S_1, 2.2; S_2, 2.7; S_3, 0; S_4, 0.9; S_5, 0.3; and S_6, 0. The percentages of trials with conditional extrasystoles during the extinction period were: S_1, 64.7; S_2, 81.3; S_3, 0; S_4, 27.3; S_5, 19.0; and S_6, 0. The frequency of conditional extrasystoles during the presentation of the conditional stimuli was definitely higher than during the intertrial interval preceding it; the statistical analysis of this difference, with paired t tests (*3*) was very significant in S_1 ($p < .01$) and S_2 ($p < .001$). On this basis, it was concluded that in subjects 1 and 2 the induction of conditional extrasystoles by chance during the presentation of the conditional stimuli was very unlikely. Figure 1*B* illustrates the appearance of conditional extrasystoles during the presentation of the conditional stimuli in the presence of a normal respiratory rhythm. In subject 4, one isolated conditional extrasystole was usually induced during the late phase of the conditional stimuli and it was followed by a series of extrasystoles during the intertrial interval. This fact indicates that in this subject the latency of the conditional reflex was prolonged and that the conditioning was manifested as a delayed response. Subjects 3, 5, and 6 did not show conditioning of extrasystoles. Subject 3 had ventricular and auricular extrasystoles which occurred always at a fixed point in the beginning of expiration during the respiratory maneuvers.

Extinction of conditional extrasystoles occurred readily after ten nonreinforced trials in subject 4, but it was more difficult in subjects 1 and 2. Successive inhibition was observed in subjects 1, 2, and 4 during the extinction period. Physical exercise with outdoor sports completely inhibited the development of extrasystoles induced by respiratory maneuvers as well as the conditional ones.

The mechanisms involved in the induction of conditional extrasystoles are undoubtedly complex. It appears conceivable that the unconditional extrasystoles elicited in some subjects by respiratory maneuvers depends primarily upon "reflex" mechanisms in the lower brain stem reticular formation, activated by sensory impulses originating in the peripheral respiratory apparatus, and discharging to visceral motor neurons probably including those of the vagus nerve (*4*). It is, however, virtually certain that such more elementary mechanisms are influenced by more rostal levels of the central nervous system: extrasystoles have been elicited by electrical stimulation at the level of the hypothalamus (*5*) as well as in certain mesencephalic structures known to be directly connected with the hypothalamus (*6*). Hypothalamic mechanisms, in turn, can be assumed to be highly dependent upon variations in the functional state of the so-called limbic system of the cerebral hemisphere; in fact, extrasystoles have been observed in the course of electrical stimulation of certain limbic structures (*7*). Finally, neocortical mechanisms, judging from the known efferent connections of the neocortex, could affect virtually all of the above-mentioned levels of the central nervous organization. The question, at which levels the closure is made between the conditional stimulus and the effector mechanisms of extrasystoles, cannot yet be answered. In this general problem area further systematic animal experimentation would seem necessary and promising.

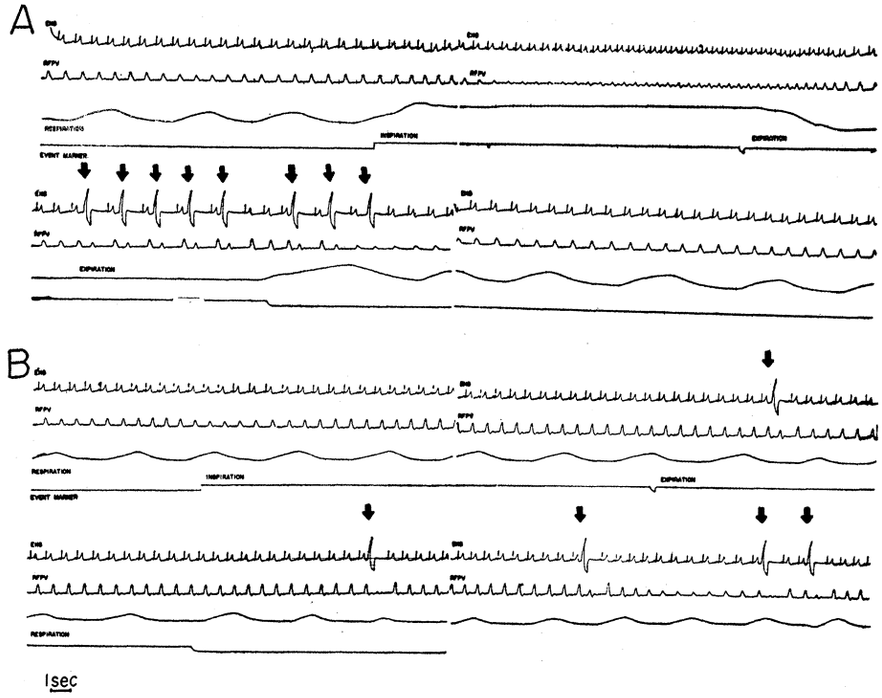

Fig. 1. Continuous record of electrocardiogram, finger pulse volume, and respiration, showing: *A*, unconditional extrasystoles (arrows) induced by respiratory maneuvers; and *B*, conditional extrasystoles (arrows) induced by conditional stimuli during normal respiratory rhythm.

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