ELECTRICAL STIMULATION OF ISOLATED HEART PREPARATIONS FROM PERIPLANETA AMERICANA[1]

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INTRODUCTION

In order to study adequately the effects of insecticides upon the various tissues and functional processes of insects, particularly insects which it is desired to control, it is necessary to have more knowledge of the manner in which the tissues and organs of insects function under normal and abnormal conditions. This paper is a report of experiments which yielded further information regarding the functional processes of the insect-cardiac mechanism.

In utilizing the mechanocardiographic method of studying the effects of nicotine and other solutions upon a perfused, isolated heart preparation from the American cockroach (*Periplaneta americana* (L.)) it is necessary to consider the effects of possible contractions by other than cardiac-muscle fibers. In addition to the cardiac-muscle fibers, the cockroach isolated heart preparation previously used (*4, 5, 6, 7*)[2] possessed alary muscles (of the heart), dorsal body muscles, and, in the thoracic region, especially of heart preparations from the adult insect, portions of the muscles involved in the somatic wing mechanisms.

It has been shown previously (*4*) that the systolic rise of curve in the mechanocardiogram taken from the whole isolated heart preparation of *Periplaneta americana* is often immediately preceded by a sudden depression, the presystolic notch. It has been suggested (*4*), on the basis of certain unpublished evidence, that the presystolic notch might result from an increased intracardiac pressure (hydrostatic) produced by a propagated wave of heartbeat in a cardiac region other than that from which the mechanocardiographic record is being taken. Although under certain conditions the presystolic notch may originate in this manner, the possibility still remains that under other conditions it might originate from a rhythmic presystolic contraction of the alary-muscle fibers of the heart, especially in the segment from which the record is being made. If the presystolic notch originates in accordance with this hypothesis, it should be possible to obtain supportive evidence by artificially stimulating the isolated heart preparation, especially the alary-muscle fibers in the segment in which the heart lever is attached.

The experiments herein reported were performed with this in mind, but also with the general objective of determining how the isolated heart preparation of *Periplaneta americana* responds to single and repeated faradic stimuli and how these responses compare with the better known results of artificial stimulation of the vertebrate heart.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 137.

METHODS

As described below, four different types of isolated heart preparations were used in these experiments.

Type A, the entire isolated heart preparation that was utilized in previous investigations (*5, 6, 7*) was used in some of these experiments. The preparation consisted of the beheaded insect's dorsal body wall (terga), dissected from the rest of the body but retaining the cardiac mechanism. It contained the cardiac tube, the alary-muscle-fiber groups, the external and internal dorsal body muscles, the dorsal diaphragm, the fat bodies, trachea, and other incidental structures associated with the dorsal diaphragm and the terga, and, in the thorax, the severed somatic wing muscles.

Type B, the second preparation, consisted of only the abdominal region of the heart, prepared either by transverse section across preparation A between the third thoracic and the first abdominal segments or by separating by similar cross section the abdomen from the thorax of the living insect and subsequently isolating the abdominal heart preparation by dissection. Whichever procedure was followed, the resulting preparation was the same and contained the entire abdominal but not the thoracic portions of the cardiac mechanism. In this preparation the longitudinal internal and external dorsal body muscles of the abdomen remained intact and retained their normal origins and insertions.

Type C, the third preparation, consisted of a single abdominal segment separated by transverse section with a safety-razor blade from adjacent posterior and anterior segments of either preparation A or preparation B (usually the latter) but cut in such a way that the adjacent margin of the adjacent overlapping segment remained attached to the segment being isolated. This preparation of a single abdominal segment contained a single pair of alary muscle-fiber groups and only that length of cardiac tube that lay between their cardiac terminations. The internal dorsal body muscles were transectioned, and only the ends attached to the isolated segment remained in the preparation. The external dorsal body muscles were not severed.

Type D, the fourth preparation, was made in the same way as preparation C except that the adjacent margin of the adjacent overlapping segment was dissected away and the connections of the external dorsal body muscles therefore destroyed. This preparation was thus completely separated from both anterior and posterior adjacent segments.

During some of these experiments the isolated heart preparation was kept moist by occasional flooding with approximately 0.11 Lévy's (*3*) stock saline solution (prepared without the buffers). This saline was composed of 11.78 gm. of sodium chloride, 0.92 gm. of potassium chloride, and 0.66 gm. of calcium chloride per liter of solution. In the other experiments the heart preparation was continuously perfused with a stream of another saline solution that was made to flow over the exposed tissues. The formula for the composition of this saline solution was arrived at in the following way: Mixtures were made containing known proportions of the 0.11 Lévy's solution described above and of a saline (composed of 3.26 gm. of sodium chloride, 7.39 gm. of potassium chloride, 2.54 gm. of calcium chloride, and 1.69 gm. of magnesium chloride per liter of solution)

prepared by Babers on the basis of his analysis (*1*) of the blood of the mature larva of the southern armyworm (*Prodenia eridania* (Cram.)). These mixtures were used to perfuse the isolated heart preparation of *Periplaneta americana*, and that mixture was chosen which sufficed to maintain the perfused heart preparation in the most steady state with respect to both rate and amplitude of cardiac contractions. The selected mixture was found by calculation to have the composition 10.93 gm. of sodium chloride, 1.57 gm. of potassium chloride, 0.85 gm. of calcium chloride, and 0.17 gm. of magnesium chloride per liter of solution. This solution will be referred to in this paper as the magnesium saline, the 0.11 Lévy's solution will be referred to as Lévy's saline. The perfusion saline was oxygenated by bubbling oxygen through the reservoir bottle before and during the experiment.

The isolated heart preparation was held by pins in a beeswax container so constructed that the perfusion saline flowed over the exposed cardiac mechanism into a surrounding drainage depression from which it flowed into a collecting jar. The drainage depression of the wax container surrounded a central wax elevation or plateau one edge of which was higher than the other. Drainage was facilitated by laying narrow strips of wet lens paper in the drainage depression. The heart preparation used in each experiment was pinned to the wax plateau with the external dorsal surface down and the internal cardiac mechanism (dorsal diaphragm) up. The rate of flow of perfusion fluid over the exposed cardiac tissues was adjusted by means of a stopcock until it was as rapid as possible without mechanically interfering with the hair attachment or the mechanocardiogram. This method of perfusion was somewhat different from that used in previous studies of the rate of insect heartbeat (*5, 6, 7*).

The apparatus used to stimulate the heart preparation electrically consisted of the well-known student-type inductorium, key, signal magnet, dry cells, and a specially made pair of microelectrodes with tungsten tips. The signal magnet was included in the primary circuit and placed so that its lever would intercept the light passing through the end of the camera slit upon closure of the key of the primary circuit. Thus the shadow of the signal magnet appeared in the mechanocardiograms as lower marginal bands during the times the key of the primary circuit was closed. When single induced shocks were used they were applied to the tissue at times indicated by the beginning and the end of the marginal band. When the inductorium was so wired as to supply tetanizing shocks they were applied to the tissue in rapid succession throughout the duration of the period indicated by the marginal band. The time record, consisting of lines extending completely across the photographic paper, were made by a Lieb watch timer, the lever of which was made to cast a shadow across the entire camera slit at determined time intervals (1 second in these experiments).

The complete mechanocardiographic set-up of apparatus was essentially the same as that previously described (*4*). The electrocardiographic camera, however, has been remodeled to yield paper speeds ranging from about 0.5 to about 100 mm. per second. In order to adapt intensity of light to paper speed, the beam of light was passed through suitable filters. The records were made upon a roll of bromide photographic paper. Most of the experiments were

performed at room temperature, which was maintained fairly constant and usually at a temperature lying within the range 25° to 30° C.

Unless otherwise stated, the lever contact was with the dorsal diaphragm immediately over the cardiac tube. As the cardiac-muscle fibers contracted, the dorsal diaphragm and lever attachment were lowered and the lever moved so as to produce an upward deflection of the mechano-cardiographic curve and, conversely, when the diaphragm wa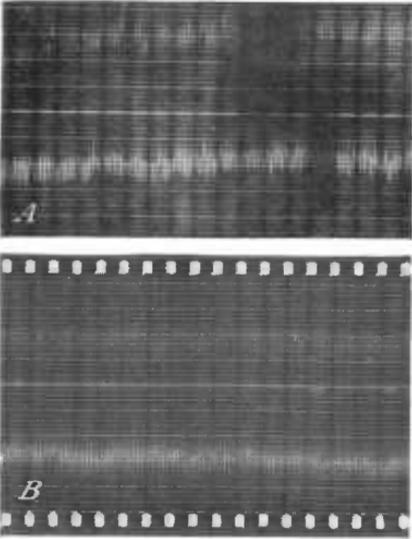s raised the curve was depressed. Similarly, upward or downward deflections of the lever shadow on the photographic paper were produced when the diaphragm was lowered or raised by any other cause, as for example by movement of the preparation resulting from contractions of the dorsal body muscles. The position of the electrodes was changed from time to time as indicated in the legends of the illustrations and under the heading Results. The stimuli applied consisted of single induced shocks, induced shocks repeated by opening and closing the key of the primary circuit by hand, and series of induced tetanizing shocks applied at a rate determined by the vibrator of the inductorium. Because of current leakage through the saline from one electrode to another, a rather strong stimulating current was employed.

FIGURE 1.—*A*, Mechanocardiogram from a completely isolated fourth abdominal segment (preparation D) perfused with the oxygenated magnesium saline. Room temperature 29° C.; record taken 105 minutes after beginning of perfusion; lever over heart; electrodes in place on distal extremity of alary-muscle group; paper speed about 0.5 mm. per second; time markings in seconds; no electrical stimuli. *B*, Similar record from an incompletely isolated third abdominal segment (preparation C). Room temperature and perfusion as in *A*; preparation 105 minutes old; lever over heart; no electrodes.

All the mechanocardiograms obtained in these experiments are to be read from left to right.

RESULTS

EXTRASYSTOLES AND SUMMATION OF CONTRACTIONS

Figure 1, *A*, shows the regularity in rate and amplitude of heart contraction when the completely isolated segment (preparation D) was perfused continuously with the magnesium saline solution. *A* and *B* (preparation C) are parts of records taken at low paper speed in different experiments after each of the preparations had been perfused for about 105 minutes.

The results of applying single and repeated faradic stimuli to the completely isolated fourth abdominal segment (preparation D) are shown in figures 2, 3, and 4. Figure 2 (*A, a; B, a*) shows the extrasystoles produced by stimulating with single induced shocks during diastasis (the rest period of the cardiac cycle). These extrasystoles are a little greater than the spontaneous contractions (fig. 2, *A, c* and *d; B, c* and *d*).

In figure 3 the extrasystoles at *A*, *a* and *b*, and at *B*, *a*, were produced by single shocks applied at different times (*A*, *c* and *d* and *B*, *b*)
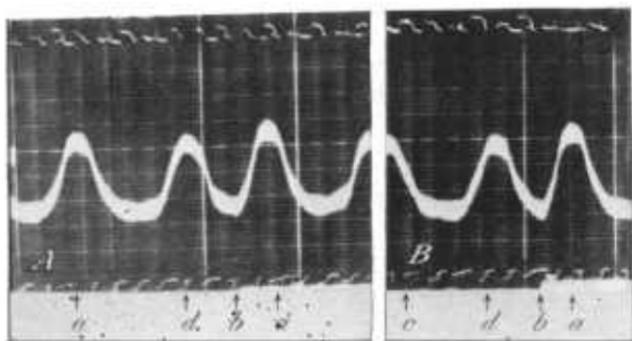

FIGURE 2.—Mechanocardiograms from a completely isolated fourth abdominal segment (preparation D), 145 minutes old. Room temperature 29° C.; perfusion with magnesium saline; lever over heart; electrodes over lateral extremity of alary-muscle group; time markings in seconds; and stimuli single induced shocks. *A*, Extrasystole at *a* produced by stimulus applied during late diastasis at *b*; *c* and *d* are spontaneous contractions. *B*, Extrasystole at *a* produced by stimulus applied during early diastasis at *b*; *c* and *d* are spontaneous contractions.

during diastole (relaxation period). The summation effect in *B* is greater than that in *A*. In figures 2 and 3 the electrodes were at the lateral extremity of an alary-muscle group.
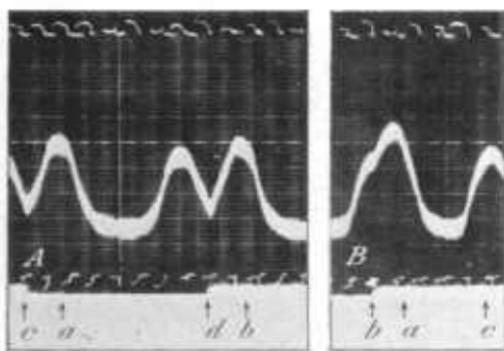

FIGURE 3.—Mechanocardiograms from a fourth abdominal segment (preparation D). Room temperature 29° C.; perfusion with magnesium saline; time markings in seconds; lever over heart; electrodes over lateral extremity of alary-muscle group; single induced shocks applied. *A*, Extrasystoles at *a* and *b* caused by single shocks applied during late diastole at *c* and *d*, respectively. *B*, Extrasystole at *a* caused by a single shock applied at *b* during the beginning of the diastole of a spontaneous beat; *c* is a spontaneous contraction.

Similarly figure 4 shows the effect of applying single shocks (electrodes medially over an alary-muscle group) at different times during systole (contraction period). Apparent summation effects are produced. In figure 4, *B*, *d*, summation at the height of contraction is very evident. The shock applied at *A*, *c*, may have fallen during the refractory period, which would account for the contraction height being more nearly that of the spontaneous contractions *e* and *f*.

Figure 5 shows the production of extrasystoles by single induced shocks applied during late and early diastole to an abdominal heart preparation (type B) when the electrodes were on the dorsal diaphragm over the heart in the fifth abdominal segment and the lever over the heart in the fourth abdominal segment. The strength of the stimulus was not sufficient to cause interfering contractions of the body muscles. Summations of contractions are

shown, particularly at *f* and *h*, when the shocks *e* and *g* fell at the height of the contraction curve.

COMPENSATORY PAUSE

No recognizable compensatory pauses have been observed to follow the extrasystoles produced in these experiments.

TETANUS OF THE HEART

Figure 6 shows the result of applying to the completely isolated single fourth abdominal segment (preparation D) a series of single
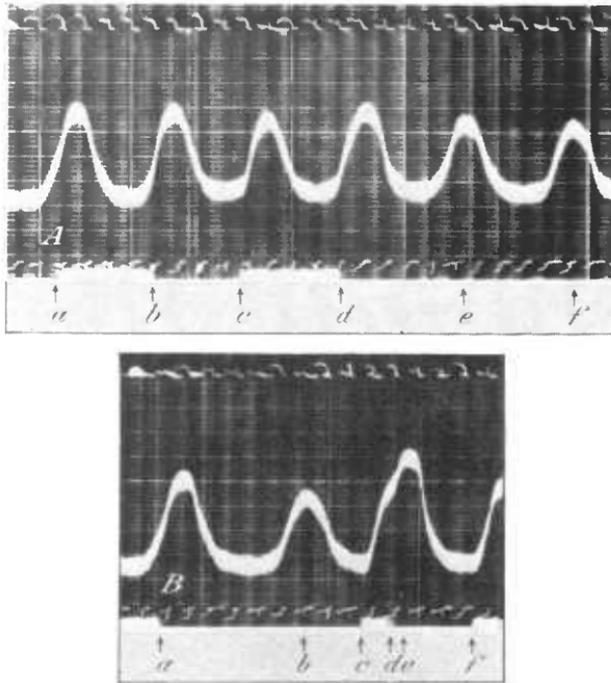

FIGURE 4.— Mechanocardiograms from a fourth abdominal segment (preparation D). Room temperature 29° C.; perfusion with magnesium saline; lever over heart; electrodes in medial position over one alary-muscle group; single induced shocks applied; time in seconds. *A*, Single induced shocks were applied during systole at *a*, *b*, and *d*. The shock at *c* fell either at the end of diastasis or at the beginning of systole; *e* and *f* are spontaneous beats. *B*, Shocks *a* and *d* fall respectively in early and very late systole; *c* and *f* fall during diastasis; *b* is a spontaneous contraction; *e* is a marked summation of contractions.

induced shocks at gradually decreasing time intervals when both the lever and the electrodes are in contact with the dorsal diaphragm over the cardiac tube. *B* is a continuation of *A*, and *C* of *B*. At figure 6, *A*, *d*, summation effects appear and increase until, at *e*, a complete tetanus is developed, which persists until the cessation of stimuli at *B*, *f*. In *B*, between *f* and *g*, there occurs a rapid relaxation, followed by a quick appearance of spontaneous rhythm in which the systoles appear to increase gradually in magnitude as further apparent relaxation occurs. At *C*, *l*, complete recovery has apparently been made.

In the same way, in figure 7, *A* and *B* show cardiac tetanus produced by similarly applying a series of single induced shocks to the abdominal heart preparation (type B) when the lever was on the dorsal diaphragm over the heart in the fifth abdominal segment and the electrodes were over the lateral extremity of the insect's left alary-muscle group. Cardiac tetanus begins at *A, e,* lasts to the cessation of stimulation at *B, f,* and is followed by rapid relaxation from *f* to *g*. This is followed by a period of inhibition, broken by the spontaneous contractions *h, i,* and *j,* of increasing magnitude. Complete recovery is evident at *j*. The record of tetanic contraction of the heart, seen from *A, e,* to *B, f,* is not a smooth curve, as in figure 6, *A, e,* to *B, f,* but has superimposed upon it a series of irregularities the
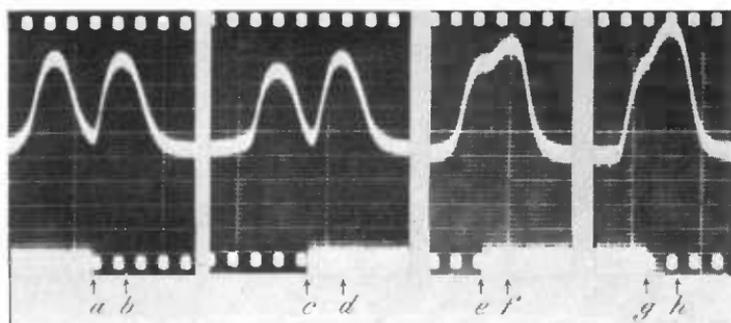

FIGURE 5.—Mechanocardiogram from an isolated abdominal preparation (type B). Room temperature 25° C.; heart preparation kept moist with 0.11 Levy's saline; lever over heart in fourth abdominal segment; electrodes over heart in fifth abdominal segment; time in seconds. Single induced stimuli applied at *a, c, e,* and *g* produced extrasystoles *b, d, f,* and *h,* respectively. The shocks were applied during late and early diastole.

larger of which are produced by interfering contractions of the dorsal body muscles given in response to the applied series of stimuli. The minor variations are like those seen in *A, a* to *b,* and were caused by mechanical vibrations of the apparatus that had nothing to do with tissue response.

In figure 7, *C,* is shown the first part of a cardiac tetanus similarly produced by stimulating an entire isolated heart preparation (type A). The marked interference of body-muscle contractions with the record of cardiac tetanus is shown.

STAIRCASE EFFECT

Apparent staircase phenomena have been observed, especially when spontaneous cardiac rhythm begins after the temporary inhibition following cardiac tetanus (fig. 6, *B, C,* and fig. 7, *B*), and when heart preparations begin spontaneous contractions after certain other periods of diastolic standstill not associated with cardiac tetanus.

REFRACTORY PERIOD

Results of the application of stimuli during various parts of systole (fig. 4) seem to indicate that the stimuli were effective during the greater portion of the contraction period and therefore, if the absolute refractory period occurs, it is confined to at least the early part of systole.

MECHANICAL STIMULATION OF THE HEART

Microscopic observations showed that when the dorsal diaphragm of an isolated heart preparation was mechanically stimulated in the region of the cardiac tube by touching it firmly (but without injury to the tissues) with the point of a dissecting needle, the response of the cardiac tube was a more or less sustained contraction.   The con-
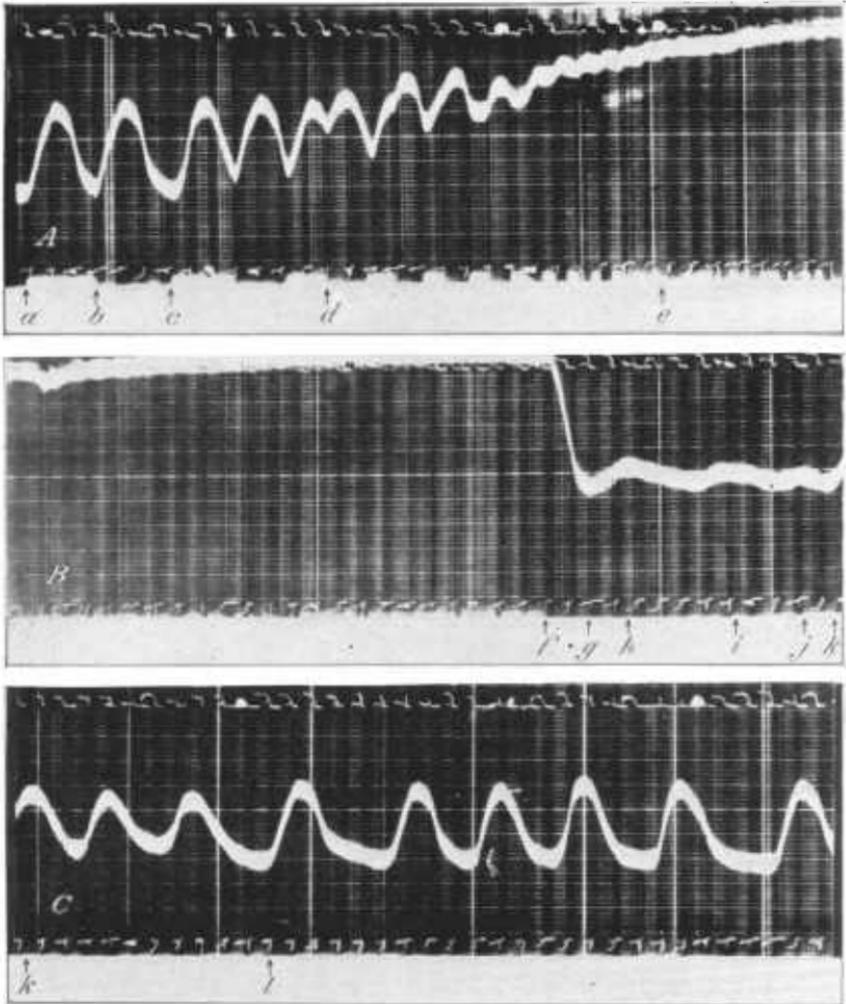

FIGURE 6.—Mechanocardiogram from a completely isolated fourth abdominal segment (preparation D).   Room temperature 29° C.; perfusion with magnesium saline; lever and electrodes over heart; time in seconds.   *A*, Effects of a series of single induction shocks applied with decreasing time intervals at *a*, *b*, *c*, *d*, and so on.   At *e*, complete tetanus is produced.   *B*, Continuation of *A*. Stimuli cease to be applied at *f*, and relaxation is completed at *g*; at *h*, *i*, and *j*, incomplete spontaneous beats occur; *k* is part of the first beat *k* shown in *C*. *C*, Continuation of *B*.   At *l*, recovery is complete.

traction did not always involve the entire cardiac tube but often was confined to the cardiac regions close to the point of stimulation.   This localized contraction has been observed to persist while the spon-

tancous rhythm of other regions of the heart continued without interruption. Whether the contraction was of the nature of a cardiac tetanus, a cardiac contracture, or a different type of response is not known.

In these experiments, isolated heart preparations have often temporarily ceased to exhibit spontaneous rhythm apparently because of mechanical stimuli applied in the process of adjusting the contact
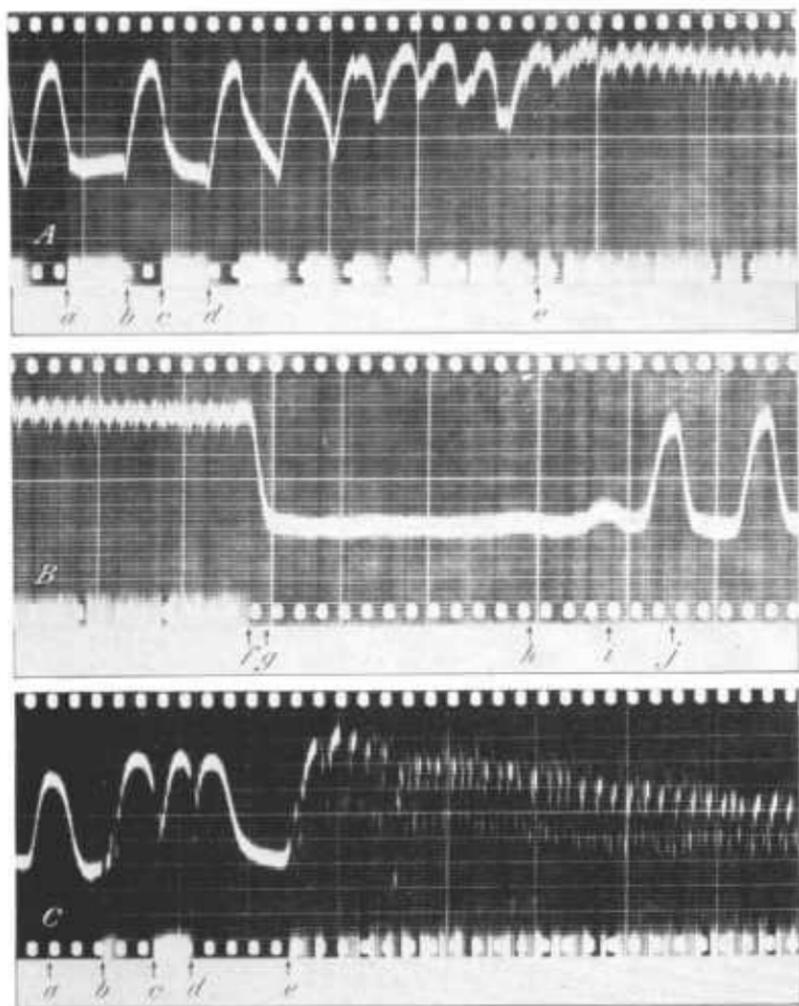

FIGURE 7.—*A* and *B*, Mechanocardiogram from an isolated abdominal heart preparation (type B). Room temperature, 25° C.; heart preparation kept moist with 0.11 Lévy's saline; lever over heart in fifth abdominal segment; electrode on distal extremity of insect's left fifth abdominal alary-muscle group; time in seconds. *B* is a continuation of *A*. Single induced shocks applied at *a*, *b*, *c*, and so on, with decreasing intervals. At *e*, complete cardiac tetanus is produced, but the curve is disturbed by dorsal body-muscle contractions. The minor vibrations (as from *a* to *b*) are mechanical and have nothing to do with tissue response. *C*, Mechanocardiogram from an entire heart preparation (type A). Single induced shocks applied at *a*, *b*, *c*, and so on, and cardiac tetanus produced at *e*. The tetanus curve is disturbed by contractions of dorsal body muscles. Time in seconds.

between lever and dorsal diaphragm. Under these conditions the cardiac response was very probably the same as the response observed to follow noninjurious mechanical irritation with a dissecting needle.

CONTRACTIONS OF THE DORSAL BODY MUSCLES

Figure 8, *A*, shows the effect of repeatedly stimulating an isolated abdominal heart preparation (type B) kept moist with Lévy's saline, when the lever contact was over the heart in the fifth abdominal segment and the electrodes were on lateral extremities of a fifth abdominal alary-muscle group. The paper speed was low. The downward trend of the contractions shown in *A* between *c* and *d* was caused by interfering body-muscle contractions. The single shocks applied at *a*
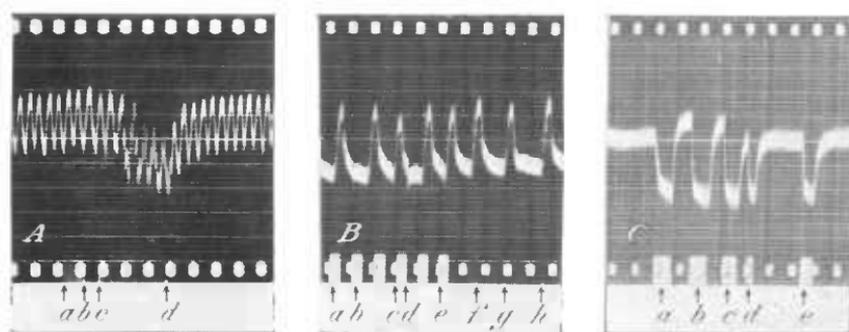

FIGURE 8.—*A* and *B*, Mechanocardiograms from an isolated abdominal heart preparation (type B). Room temperature 29° C.; heart preparation kept moist with 0.11 Lévy's saline; lever over heart in fifth abdominal segment; electrode over lateral extremity of alary-muscle group in fifth abdominal segment. *A*, Single induced shocks at *a* and *b*, and a series of single shocks applied between *c* and *d*. *B*, Single induced shocks applied at *a*, *b*, and so on to *e*, with make shock applied at *c* and break shock applied at *d*, and so on. The disturbing effects of dorsal body-muscle contractions are shown. At *f*, *g*, and *h*, are undisturbed spontaneous beats. The stimulating current did not affect the heart, which continued to contract spontaneously. *C*, Record from same preparation after the heart had been severed longitudinally and the fourth, fifth, and sixth left alary-muscle groups removed. Lever medially and electrodes laterally in region where left alary-muscle group had been. Induced tetanizing shocks applied at *a*, *b*, and so on to *e*. Contractions of the dorsal body muscles depressed the curve during stimulation.

and *b* produced no marked effects in this record made with a low paper speed. In figure 8, *B*, are shown the effects of applying single induced shocks to the same preparation at different times in the cardiac cycle when greater paper speed was used. The interference of body-muscle contractions with the mechanocardiogram at the times of stimulation is evident. All the cardiac contractions are spontaneous, and those at *f*, *g*, and *h* exhibit no body-muscle effects.

The dorsal diaphragm and the cardiac tube of this preparation were then severed with a safety-razor blade and the fourth, fifth, and sixth left alary-muscle groups and associated dorsal diaphragm removed. The lever and electrodes were then placed in contact with the exposed dorsal body muscles of the left side, the lever medially and the electrodes laterally. The record thereupon obtained by faradic tetanizing stimulation is shown in figure 8, *C*. The stimuli, applied at *a*, *b*, *c*, *d*, and *e*, resulted in marked depressions of the otherwise straight-line

record. After the removal of the entire heart and the right alary-muscle groups, stimulation gave results similar to those shown in figure 8, *C*.

Figure 9 shows distortions of the mechanocardiogram caused by dorsal body-muscle contractions made in response to single induced
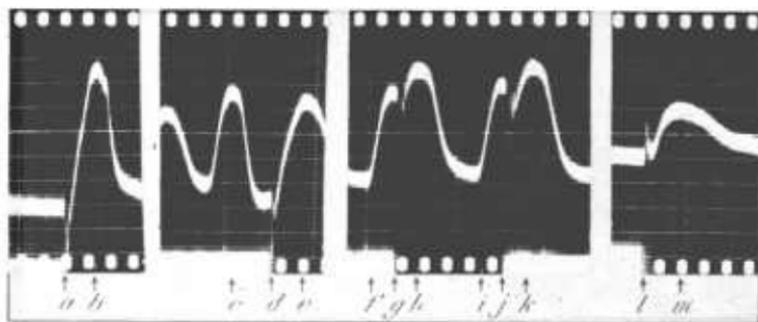

FIGURE 9.—Mechanocardiograms from a whole-heart preparation (type A). Lever and electrodes in an abdominal segment. Single induced shocks applied at *a*, *d*, *g*, *j*, and *l*. At *b*, *e*, *h*, *k*, and *m*, extrasystoles were thus produced. At *h* and *k*, summation effects are recorded. At the time of the stimulus distortions of the curve by dorsal body-muscle contractions can be seen. The upward deflection, at *l*, is unusual; dorsal body-muscle deflections were usually downward. Time in seconds.

shocks applied to the whole heart preparation (type A). Lever and electrode contacts were in an abdominal segment. The contractions *b* and *e* were given in response to stimuli applied at *a* and *d*, respectively, and were preceded by interfering body-muscle contractions
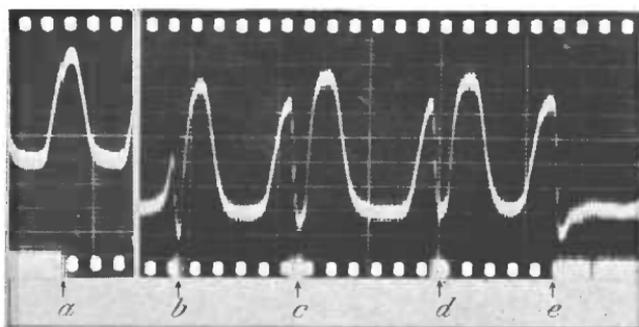

FIGURE 10.—Mechanocardiograms from an isolated abdominal heart preparation (type B). Room temperature 25° C.; heart preparation kept moist with 0.11 Lévy's saline; lever over heart in fifth abdominal segment; electrode over distal extremity of insect's left fifth abdominal alary-muscle group; time in seconds. At *a*, a single induction shock was applied. At *b*, *c*, and *d*, short tetanizing stimuli were applied. At *e*, a more prolonged tetanizing stimulation was begun. The marked effects of dorsal body-muscle contractions on the mechanocardiograms are shown.

whose records somewhat resemble presystolic notches. Similar distortions, however, occurred during other parts of the cardiac cycle at *g*, *j*, and *l*. Usually the body-muscle contractions produced depressions of the curve, but at *l*, the deflection was upward.

Figure 10 shows similar results of applying short tetanizing stimuli to an abdominal heart preparation (type B). The lever was over the heart in the fifth abdominal segment, and the electrodes were over the lateral extremities of the left alary-muscle group of the same segment. The very small vibrations were mechanical and had nothing to do with tissue response. Marked disturbances of the heartbeat record are shown.

Figure 11 shows depressions of an otherwise straight-line record obtained from an incompletely isolated abdominal segment (preparation C) from which the heart and both alary-muscle groups had been removed. The lever was in the region where the heart had been, and the electrodes were placed laterally where one of the alary-muscle groups had been located. Series of single induced shocks were applied between *a* and *b* and between *c* and *d*. During stimulation the otherwise straight-line record was depressed by contractions of the dorsal body muscles. The external dorsal body muscles are considered to be responsible for this movement.

When the completely isolated third abdominal segment (preparation D) from which the heart and both alary-muscle groups had been removed was similarly stimulated with induced shocks, applied either singly or in rapid succession, or with tetanizing stimuli, no appreciable effect of body-muscle contraction upon the otherwise straight-line record was observed.
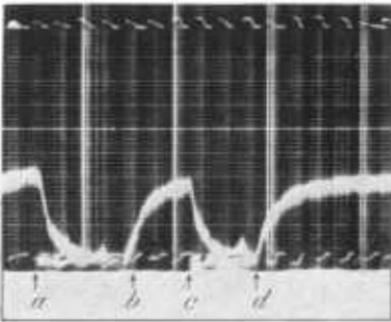

Figure 11.—Mechanocardiogram from an incompletely isolated third abdominal segment (preparation C) about 5 hours old. Room temperature 29° C.; preparation perfused with the magnesium saline; time in seconds. This record was obtained after cardiac tube and both alary-muscle groups had been removed. Lever on midregion where cardiac tube had been; electrodes lateral to lever, in region where one alary-muscle group had been. From *a* to *b* and from *c* to *d*, a series of single induced shocks were rapidly applied. The contractions of the dorsal body muscles depressed the curve.

SUMMATION OF STIMULI

It was observed that when slightly subminimal single shocks were rapidly applied by manipulating the key of the primary circuit or when tetanizing stimuli of subminimal strength were applied, contractile responses of the heart and of the dorsal body muscles could be obtained. This indicates that summation of stimuli occurred in these muscles.

ALARY MUSCLES

No convincing evidence was obtained that the application of single induced shocks or of tetanizing stimuli to the alary-muscle fibers caused them to give responsive contractions. When the alary muscles were stimulated while they were under low-power microscopic observation they were not observed to respond with visible contraction either in the form of twitches or of visible tonus changes.

DISCUSSION

It is generally recognized [3] that the vertebrate heart (especially frog and mammalian ventricles) can respond to applied stimuli with extrasystoles, particularly when the stimuli are strong and are applied during late diastole or during diastasis. Because it possesses an absolute refractory period that persists throughout the systole and a relative refractory period that extends into at least the earlier portion of diastole, vertebrate cardiac muscle is not responsive to stimulation while it is contracting and responds to only unusually strong stimuli while it is relaxing, especially during early diastole. For the same reasons and because it exists in syncytial form, vertebrate cardiac muscle does not normally exhibit well-defined summation of contractions in response to single shocks or complete tetanic contractions when stimulated with tetanizing shocks. It can display staircase phenomena, compensatory pauses, and response to mechanical as well as to electrical and other stimuli. Its response to mechanical stimulation, applied by touching the ventricle with the point of a needle, appears as a twitch, not as a sustained contraction.

Very little has been known of the responses to electrical stimulation of the insect heart, especially the isolated insect-heart preparation. From the results of these experiments it is evident that in the perfused isolated-heart preparation of *Periplaneta americana* the cardiac responses differ considerably from the responses usually reported to be given by vertebrate hearts. The cockroach heart can respond to single induced shocks with extrasystoles during both diastasis and diastole. The extrasystolic contractions are more or less summated, the degree of summation being greatest at the height of the contraction curve, that is, at the very beginning of diastole and perhaps at the very end of systole. These results would seem to indicate either (1) that the cockroach cardiac muscle does not react syncytially as does vertebrate cardiac muscle, but behaves more like skeletal muscle, in which the individual fibers, but not the muscle as a whole, give the all-or-none response, or (2) that the cockroach cardiac muscle functions syncytially but fails to obey the all-or-none law and gives graded contractions. They also indicate that at the height of contraction and in early diastole the insect cardiac muscle is not in an absolute refractory state and probably is not in a marked relative refractory state. If, furthermore, the slight increase in contraction height resulting from the application of a single shock during the early, middle, or late portion of systole is to be interpreted as a summation of contractions of the cardiac-muscle fibers [4] given in response to the stimulus, it would seem that the insect cardiac muscle fails to exhibit absolute refractoriness throughout all except perhaps the very early part of systole.

The cockroach heart gives a well-defined tetanus in response not only to tetanizing stimuli but also to single shocks rapidly applied by opening and closing the key of the primary circuit by hand. In this response the cockroach heart differs from the vertebrate heart. The

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[3] See various textbooks, manuals, and reviews of vertebrate physiology for prevailing opinions as to the usual characteristics of vertebrate hearts and cardiac muscle, for example, books by Howell, Starling, Bayliss, MacLeod, and others.

[4] If the increased contraction height of the curve is not caused by additional cardiac-muscle contraction, it seems that it would have to result from the alary-muscle fibers responding to the stimulus with relaxation, since contractions of the alary-muscle fibers would be expected to depress, and the contractions of the dorsal body muscles have been found nearly always to depress the curve of heartbeat. This would be contrary to the way muscle fibers are now known to respond to stimuli.

cardiac tetanus thus obtained serves as additional evidence that at the height of the contraction curve the insect cardiac muscle is not in a state of absolute refractoriness.

The cockroach heart also differs from the vertebrate heart in that no recognizable compensatory pauses have been found to follow extrasystoles. Although occasionally certain extrasystoles have been followed by diastatic periods somewhat longer than those preceding the same extrasystoles, the observed increase of diastasis is not significantly great when the variations in duration of diastasis that commonly occur spontaneously are considered. The lack of occurrence of compensatory pauses by the cockroach heart suggests that stimulating this heart is analogous to stimulation not of the ventricle but rather of the sinus venosus, for example, of the frog heart.[5] If this analogy is correct, it implies that in stimulating the cockroach heart, as was done in these experiments, the stimuli act upon something corresponding to pacemaker tissue or to a tissue that sets its own pace. This would not be unexpected where, as in most of these experiments, a strong stimulus is applied directly to the cardiac muscle of an isolated segment of the heart mechanism.

Comparison of the results obtained with the different types of isolated heart preparation used in these experiments shows that movement of the preparation resulting from contractions of the dorsal body muscles is a factor that must be brought into consideration when experiments are undertaken in which insect heart preparations are subjected to various stimuli, either electrically or by the application of certain dissolved substances.[6] It is evident that one way to eliminate interfering movements of the dorsal body muscles is to utilize preparation D (see Methods) in which the internal and external dorsal body muscles are either severed or torn from their insertions.

The movements caused by contractions of the dorsal body muscles may affect the mechanocardiogram in more than one way, but in these experiments the effect was nearly always to depress the record of heartbeat. With strong single shocks, sharp, rapid, momentary depressions at the time of stimulus are superimposed on the mechanocardiogram and, when occurring just before systole, may somewhat resemble the presystolic notch. This resemblance may be quite marked when the stimulus is a very short tetanizing current. The fact that through stimulation the body-muscle depressions can be made to occur at other times during the cardiac cycle than just prior to systole indicates the difference between these depressions and the spontaneously occurring presystolic notches. This is shown also by comparison of the forms of the body-muscle depressions and the presystolic notches. It is hardly to be expected that dorsal body-muscle contractions are the cause of the presystolic notch since this would have the improbable meaning that the dorsal body muscles contract rhythmically in exact synchronism with the heart rhythm. The origin of the presystolic notch, therefore, is probably to be sought among other causes.

[5] A compensatory pause often follows an extrasystole of the frog heart produced by stimulation of the ventricle. The pause is thought to be caused by the next excitatory impulse from the sinus venosus (pacemaker) reaching the ventricle when the latter is in the refractory state produced during the extrasystole. The ventricle has to wait for still another excitatory impulse to arrive from the pacemaker before it contracts. Stimuli applied to the sinus venosus (pacemaker), therefore, produce extrasystoles that are not followed by compensatory pauses.

[6] Since nicotine can produce contraction or contracture in skeletal muscle (*2*), its effect upon insect dorsal body muscle deserves consideration, particularly when high or toxic concentrations are used.

In none of these experiments has evidence been obtained that the applied stimuli produced recognizable contractions of the alary-muscle fibers. It is possible that the alary-muscle fibers contracted when stimulation caused interfering contractions of the dorsal body muscles, but when these interfering movements were not produced, the alary muscles exhibited no signs of undergoing contraction, even when the stimulating electrodes were over the alary-muscle fibers themselves and the stimulus was strong enough to cause contraction of the cardiac muscle. Neither did the alary-muscle fibers show any recognizable shortening when observed microscopically during stimulation. Although this evidence does not constitute proof that the alary-muscle fibers are incapable of contraction, it does render more probable the hypothesis that the alary-muscle fibers of *Periplaneta americana* tend to maintain a tonus and to apply a steady pull upon the wall of the cardiac tube rather than to produce diastole by rhythmic twitches given synchronously with the rhythm of the cardiac muscle. These facts would also indicate that even should contractions of the alary-muscle fibers occur during, and be undetected because of, interfering body-muscle contractions, the threshold of excitability of the alary-muscle fibers is considerably higher than that of the cardiac muscle in the same abdominal segment.

The failure to obtain recognizable contraction of the alary-muscle fibers in these experiments also lends weight to the hypothesis (4) that the presystolic notch results not from the rhythmic presystolic contraction of the alary-muscle fibers but probably from a presystolic increase of intracardiac hydrostatic pressure resulting from the contraction of the heart in some segment other than that from which the mechanocardiogram is being recorded.

These isolated heart preparations no doubt contained intrinsic nervous mechanisms (5) that probably have to do with cardiac regulation. What effect these structures had in the response of the heart to stimulation in these experiments is not known, but, since leakage of electrical current through the perfusion saline from one electrode to the other necessitated the use of strong stimuli, it was assumed that the cardiac, alary, and dorsal body muscles were subjected to direct stimulation.

SUMMARY AND CONCLUSIONS

Four different types of isolated heart preparations from the American cockroach (*Periplaneta americana* (L.)) have been electrically stimulated and mechanocardiograms of their responses recorded. The four types were: Type A, the whole isolated heart preparation, including both thoracic and abdominal portions; type B, the abdominal heart preparation; type C, the single abdominal segmental preparation, incompletely separated from the adjacent margin of the adjacent overlapping segment but completely separated from the adjacent overlapped segment; and type D, the single abdominal segment completely separated from both adjacent segments. The preparations were occasionally flooded or continuously perfused with saline solutions.

The applied stimuli consisted of single induced shocks, induced shocks rapidly repeated by opening and closing the key of the primary circuit by hand, and induced tetanizing shocks consisting of a series of

shocks applied at the rate determined by the vibrator of the inductorium.

The mechanocardiograms obtained show that the heart of this insect can respond to single shocks, applied during diastasis and diastole, with extrasystoles that are more or less summated, particularly during early diastole; that it gives an apparent summation response when the shock falls during systole; and that the extrasystoles thus obtained are not followed by compensatory pauses.

It is also shown that the heart can respond to tetanizing stimulation with a complete cardiac tetanus and can rapidly relax and quickly its original spontaneous rhythm after the cessation of stimulation. Posttetanic standstills often occur.

During recovery from posttetanic standstill and during recovery from standstill produced by certain other causes, the cockroach heart exhibits apparent staircase phenomena.

The responses of this heart to single shocks applied at different times in the cardiac cycle and to tetanizing stimuli show that this insect's cardiac muscle is not absolutely refractory during diastole, diastasis, and, probably, late systole. They also indicate that, if the absolute refractory period exists in this cardiac muscle, it is probably confined to early systole.

This insect heart can respond to mechanical stimulation with a more or less prolonged contraction, which may be localized in that part of the heart tube lying near the point of stimulation.

Contractions of the dorsal body muscles may cause variations to be superimposed on the heartbeat record. The variations may stimulate the presystolic notches when they are caused by shocks eliciting extrasystoles or when they happen to fall immediately prior to systoles.

The heart of this insect differs from the vertebrate heart (particularly frog and mammalian ventricles) in its ability to respond to stimulation with summated extrasystolic contractions, in its failure to exhibit compensatory pauses, in its apparently shorter absolute refractory period, and in its ability to respond to a mechanical stimulus with a more or less prolonged contraction.

Stimulation of the alary-muscle fibers (of the heart) with strong single, repeated, or tetanizing shocks yielded no evidence that they responded with recognizable contractions in the form of either twitches, tetanus, or tonus changes. These negative results lend weight to the hypothesis that cardiac dilation during diastole is not produced by rhythmic contractions of the alary muscles but rather that the latter tend to exert a steady tension on the cardiac walls. They also support the view that the presystolic notch is produced not by presystolic contractions of the alary-muscle fibers but by some other agency (probably increases of intracardiac hydrostatic pressure).

Interfering contractions of the internal and external dorsal body muscles may interfere with mechanocardiograms obtained from heart preparations of types A, B, and C. These can be eliminated by using the type D preparation.

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