SOME OBSERVATIONS ON CARDIAC AUTOMATISM IN CERTAIN ANIMALS*

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ABSTRACT

Certain aspects of the acetylcholine hypothesis of cardiac automatism have been tested in vitro with spontaneously beating cardiac tissue from rabbits, rats, clams, and hagfish. The beat of atria from rabbits and rats may be depressed or excited by acetylcholine, depending upon the state of the tissue. Proguanil and cocaine inhibition of the beat in the rat may be antagonized by acetylcholine so that reversal of the depression occurs. The action of acetylcholine on the hearts of clams was found to be strictly inhibitory. Proguanil and cocaine, in contrast to their action on mammalian atria, exert a stimulatory effect on the heart of the molluscs studied. In fact, cocaine stimulated these hearts when they were inhibited by acetylcholine.

Studies on the non-innervated hagfish heart revealed that this tissue is completely insensitive to the action of acetylcholine. Extracts prepared from beating hearts of this species will accelerate hypodynamic hearts of the hagfish as well as of the mussel. An extract of the neurogenic lobster heart was without effect on the hagfish heart. Proguanil was likewise ineffective in concentrations which produced inhibition and excitation in rat and clam hearts respectively.

It was concluded that acetylcholine does not play a role in the myogenic automatism of all species, and that another mechanism is responsible is suggested on the basis of results obtained in the hagfish hearts.

INTRODUCTION

Cardiac automaticity is a prime example of the spontaneous rhythmic contractility exhibited by many tissues throughout the animal kingdom. The type of pacemaker responsible for this activity is generally considered to be in one of two categories: neurogenic or myogenic, depending upon the source of the

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impulse driving the contractile machinery. The classic experiments on the
origin of the heart beat in the ganglionic tissue of Limulus polyphemus (Carlson,
1904 a) may be cited to illustrate neurogenic automatism, whereas the early
chick embryo heart illustrates a non-innervated yet spontaneously contractile
organ (Patten, 1949). In the latter case, the myocardial elements may be con-
sidered chemoreceptors as well as effectors, and thus are individually sensitive
to the effects of locally produced metabolites.

Little is known about the detailed mechanism of stimulus production in
myogenic tissues. Although a number of workers suggested that acetylcholine
might play a role in this process, none was able to demonstrate an excitatory
as well as an inhibitory action of this compound in mammalian tissues. It re-
mained for Burn and his colleagues at the University of Oxford to investigate
this problem systematically. During a study of the pharmacological properties
of the antimalarial proguanil (paludrine), Burn and Vane (1949) noted that
under the influence of this compound, acetylcholine inhibition of the sponta-
neous beat of isolated rabbit auricles was gradually changed to a stimulatory
action. Next, in order to eliminate the effect of any drug on the heart, auricles
were allowed to beat in vitro until they became quiescent (Bülbring and Burn,
1949). In 63 per cent of the experiments it was possible to restart the auricular
beat with acetylcholine. In addition, studies on the choline acetylase system
indicated that enzyme activity, hence acetylcholine synthesis, was directly
related to mechanical activity. Observations on the ciliary movement in frog
esophageal membrane, rabbit trachea, and gill plates of Mytilus edulis (Kordik,
Bülbring, and Burn, 1952; Bülbring, Burn, and Shelley, 1953) also led to the
conclusion that acetylcholine produced locally in the tissue was responsible for
initiating the rhythmic activity. These and other studies resulted in the hy-
pothesis that acetylcholine is a local hormone, and that the spontaneous ac-
tivity of the tissue is dependent upon the balance between a rhythmic syn-
thESIS by choline acetylase and destruction by cholinesterase of the compound
(Burn, 1953; Burn and Kottegoda, 1953; Bülbring, Kottegoda, and Shelley,
1954).

In view of the fundamental importance of this hypothesis, it was considered
worthwhile to confirm some of these observations, and to extend these studies
to several other species. The present report deals with experiments conducted
upon the isolated myocardial tissue of rabbits, rats, clams, and hagfish. Clams
(Venus mercenaria) were chosen because of the myogenicity of the beat (Krijgs-
man and Dívaris, 1955) and the extreme sensitivity to acetylcholine exhibited
by their hearts (Welch and Taub, 1948). Hagfish (Polistotrema stouti) were of
great interest, especially as the systemic heart of the adult is extrinsically non-
inervated and has no intrinsic ganglionic tissue (Greene, 1902; Carlson, 1904 b;
Augustínsson et al., 1956). Since this is the only chordate known which ex-
hibits this condition as an adult, these primitive fish afford a unique oppor-
tunity for investigation of myogenic automaticity in an organ sufficiently large to permit gross experimentation.

**General Methods**

All studies were performed on spontaneously beating isolated heart preparations. A double-walled glass cylinder served as the heart chamber; water circulating from a constant temperature bath through the outer jacket kept the medium surrounding the heart at ± 0.1°C. during any study. The hearts were washed with fresh medium of appropriate temperature through an inlet located in the bottom of the cylinder. A constant volume of 10.0 ml. was maintained, and the medium was gassed from the bottom of the chamber.

The rate and isotonic force of the heart beat were recorded continuously by means of a Statham strain gage coupled to an Esterline-Angus recording millimeter.

If a study extended over a period of several days, fresh medium was prepared daily. Sterile technique was not employed. However, in the mammalian experiments inclusion of small amounts of penicillin, or tetracycline hydrochloride (Pfizer) in the solution was found to be helpful in combating bacterial action. No effects on the heart beat were noted when these compounds were included.

Drugs were made up as concentrated stock solutions immediately before use and were equilibrated to the temperature of the bath before addition. Prior to adding any compound to the chamber, an equivalent volume of fluid was withdrawn; concentrations given are after dilution. Throughout these studies the concentrations of the drugs are expressed on a weight:volume basis; thus 1 mg./10 ml. = 1:10⁴ (or 1:10,000). The weights employed were of the salts.

The following compounds were employed; their sources are also indicated: acetylcholine iodide, Eastman Organic Chemicals, Rochester, New York; cocaine hydrochloride, New York Quinine and Chemical Works, Inc., New York; ergonovine maleate, Mann Research Laboratories, New York; lysolcithins (50 per cent α'-monooleyl L-α-glycerylphosphoryl choline and 50 per cent β-monooleyl L-α-glycerylphosphoryl choline), Dr. Erich Baer, University of Toronto; proguanil hydrochloride (paludrine: 1-(p-chlorophenyl)-4-isopropylbiguanide hydrochloride), Ayerst Laboratories Inc., New York; serotonin creatinine sulfate, Abbott Laboratories, Chicago.

This report deals with experiments carried out on a total of 104 individual heart preparations. Whenever feasible, several studies were carried out on the same heart; thus the experimental and control observations were recorded on the same preparation.

**RESULTS**

The hearts employed for these studies were obtained from seven species which embraced two animal phyla: Chordata and Mollusca.

**Mammalia**

(a) *Rabbit Experiments.*—Young adult male New Zealand white rabbits were used for these studies; the average body weight was 3.5 kg.

The atria were prepared for recording by the method of Bühring, Kottegoda,
and Shelley (1954), and then placed in the equilibrated chamber containing
Locke's solution. The gas used in all experiments was 95 per cent O₂-5 per cent
CO₂, and the pH of the medium was 7.4. Excellent spontaneous beat of the
isolated auricles was obtained in all cases reported here, and this could be
maintained for from 24 to 48 hours. Thirteen rabbits were used.

The purpose of these experiments was to determine whether or not acetyl-
choline would stimulate as well as inhibit the heart beat. The preparations were
allowed to become hypodynamic spontaneously in vitro without addition of
drugs or any other agents.

In eight out of thirteen preparations (62 per cent) a clear-cut stimulatory
response to acetylcholine was observed. Of the twenty-eight positive responses
recorded, this effect ranged from regularization of the beat, increases in rate
and/or force, to restarting of completely quiescent preparations. An example

of the latter case is illustrated in Fig. 1. Twenty-four and one-half hours after
the atria were placed in the bath they had been at a standstill for several hours.
Addition of acetylcholine, 1:10⁶, to the bath caused resumption of the beat
after about 1 minute. Approximately 5 minutes after the contractions started
the acetylcholine was washed out and the beat stopped. Alternate treatment
of this preparation with acetylcholine (1:10⁷ to 1:10⁸) and washing with fresh
medium enabled the heart to be started and stopped six more times. In one
case it was restarted (after an interval of 13½ hours) by acetylcholine, 1:10⁶,
and it beat steadily for 2 hours, only to stop again when the acetylcholine was
removed. During this interval, further additions of acetylcholine (to a final
concentration of 3:10⁸) did not significantly alter either the rate or force of the
beat.

It is worth noting that in freshly extirpated atria, acetylcholine, 1:10⁷ to
1:10⁸, exerted only an inhibitory effect on rate and force; later, in the same
preparation an identical dose of the compound was ineffective or only slightly
so, while still later the effect was changed to a positive response.

The temperature at which these studies were carried out (20°C.) apparently
influenced the results only insofar as maintenance of the integrity of the auricles was concerned; that is, when the auricles were allowed to beat continuously at 28°C, their survival as evidenced by a later positive response to acetylcholine administration was less likely. In contrast, when the temperature of the medium was lowered, say to 20°C, for at least half of the time during which they were beating in vitro, positive results were much more likely.

These results are in good agreement with those reported earlier (Bülbring and Burn, 1949).

(b) Rat Experiments.—Adult male rats (350 to 400 gm.) of the Long-Evans strain were used for these studies. The atria were prepared for recording as in the rabbit experiments, except that Tyrode's solution pH 7.5 (Holtz and Westermann, 1955) was used for the medium.

Auricular preparations from twenty animals were employed. In addition to spontaneous hypodynamia (nine experiments), proguanil hydrochloride (nine experiments) and cocaine hydrochloride (two experiments) were used to limit the contractility of the atria.

In the spontaneously hypodynamic preparations acetylcholine stimulation was observed in five of the nine atria (55 per cent). With the same concentration of acetylcholine, the effect ranged, as in the rabbit, from initial inhibition, to no effect, to a definite increase in rate and force as the experiment progressed. The concentrations of acetylcholine studied ranged from 1:10⁷ to 1:10⁸. Again it was noted that maintenance of the preparations for long periods at about 28°C. apparently exerted a deleterious effect on their continued survival and it was found that a temperature of 20°C. was most effective.

In the experiments using proguanil, it was found when this compound in a concentration of 1:10⁴ was allowed to act on the beating auricles from 1 to 2 hours, that the contractions would cease. (Normally under these conditions the preparations would beat with undiminished vigor for about 4 hours.) In six of the nine preparations studied (67 per cent), it was possible to restart the auricles after periods of quiescence ranging from 15 minutes to 1 hour by the addition of acetylcholine, 1:10⁷ to 1:10⁵ (twelve observations).

In two experiments, using fresh auricular preparations, it was found that cocaine would inhibit and acetylcholine would restart the beat. This is illustrated in Fig. 2. Approximately 30 minutes after the experiment was started, addition of acetylcholine, 1:10⁸, produced a 50 per cent reduction in the force of the beat. After washing out the acetylcholine and allowing sufficient time for recovery (15 minutes), cocaine hydrochloride, 1:10⁴, was added. Two and one quarter minutes later, the heart came to a complete standstill, and remained quiescent for 45 minutes at which time acetylcholine, 2:10⁴, was added. Within 30 seconds, resumption of the beat occurred, and a second addition of acetylcholine, 2:10⁴, 3 minutes after contractions began produced no further effect. Fifteen minutes later, cocaine and acetylcholine were both washed out, and the beat continued. These observations were repeated eight more times on...
this heart and it was also noted that after the beat was restored, acetylcholine,
$1:10^6$, would inhibit the beat once again, in a manner similar to the observation recorded in Fig. 2 A.

These results were confirmed in a second atrial preparation.

**Pelecypoda**

Since the spontaneously beating hearts of certain marine molluscs, notably the quahog (*Venus mercenaria*), are extraordinarily sensitive to inhibition by very low doses of acetylcholine (Welch and Taub, 1948), it appeared of interest to test this compound for excitatory activity on hypodynamic clam hearts.

For these studies, twenty-five heart preparations from *V. mercenaria*, two from the Pismo clam (*Tivela stultorum*), and one from a gaper clam (*Schizothaerus nuttalli*) were used.

The hearts were prepared for recording by the method of Welch and Taub (1948). Survival *in vitro* of from 1 to 3 days was obtained by placing the isolated hearts in sea water buffered to pH 7.6, aerated with 95 per cent O$_2$–5 per cent CO$_2$, and maintained at 16°C.

As in the rat auricles, paludrine, $1:10^4$, was used in seven experiments in an attempt to render the spontaneously beating ventricles hypodynamic. In marked contrast to the rat however, it was found in all cases that this compound actually stimulated the rate and force of these preparations. In fact, proguanil would induce a regular beat in an almost quiescent heart. This observation is illustrated in Fig. 3 in which a weakly beating preparation was markedly stimulated by the addition of 1 mg. of proguanil to the bath. In view of this finding, proguanil was useless as an agent for limiting the spontaneous activity of these hearts.

On twenty spontaneously hypodynamic clam hearts, acetylcholine ($1:10^4$ to $1:10^9$) was completely ineffective in producing or stimulating a beat (thirty-eight observations). The threshold for inhibition in a fresh heart was about $1:10^6$ in *Venus*, and about $5:10^9$ in *Tivela* and *Schizothaerus*. In all species studied, cocaine ($1:10^6$, $5:10^6$) and ergonovine maleate ($1:10^4$, $1:10^6$) were markedly stimulatory.

Fig. 4 illustrates a marked inhibition by acetylcholine, $1:10^6$, on an isolated ventricle of *T. stultorum*. While the ventricle was inhibited by this compound, addition of cocaine hydrochloride ($1:10^6$) in the presence of the acetylcholine induced a sharp stimulation of the force of the beat with a concomitant slight diminution of rate. These results are in diametric opposition to those obtained in the rat auricle where cocaine inhibited and acetylcholine restarted the beat.

**Cyclostomata**

In view of the contrast between the results obtained with acetylcholine on mammalian and molluscan hearts, it was considered of interest to test the effect
Fig. 2. Effects of ACH and C on spontaneously beating rat atria. A, inhibition produced by ACH, 1:10^4. B, after wash and 15 minutes' recovery, C, 1:10^4, induced cessation of the beat. C, 45 minutes later (no wash), ACH, 2:10^4, stimulated the preparation; a second addition of ACH, 2:10^4, after 3.5 minutes had no effect.

Fig. 3. Stimulatory effect of P, 1:10^4, on the isolated heart of the quahog clam, Venus mercenaria.

Fig. 4. Inhibition by ACH, 1:10^4, and stimulation by C, 1:10^4, in an isolated heart of the Pismo clam, Tivela stultorum.
of this compound on hypodynamic contractile tissue known to be aneural. Survey of the literature revealed that the systemic heart of a primitive marine chordate, the hagfish, lacked extrinsic or intrinsic innervation (Greene, 1902; Carlson, 1904 b; Augustinsson et al., 1956), and thus was admirably suited for this investigation.

The California hagfish, *Polistotrema stouti*, was used. As 15°C. is the approximate environmental temperature of these animals, it was chosen for the experiments. Average size of the animals was about 35 cm., body weight 45 gm.

Although the hagfish possesses three hearts, only the systemic organ was employed in this investigation. The preparation consisted of the joined auricle and ventricle.

The isolated hearts would beat spontaneously for 1 to 3 days in any of several media: whole sea water, sea water diluted one-third by distilled water, or the artificial sea water of Lyman and Fleming (1940). Later, an artificial medium was devised empirically which contained: NaCl, 20.0 gm.; MgCl₂·6H₂O, 5.0 gm.; CaCl₂ (anhydrous), 2.25 gm.; KCl, 0.70 gm., made up to 1 liter with distilled water. This solution could be diluted to 75 per cent of full strength without exerting any noticeable effect on the rate or force of the heart beat. The pH of the various media tested ranged from 6 to 8, but no differences in the beat were noted. Air was used to gas the medium.

In all, forty-three heart preparations were used for these studies. Acetylcholine (1:10⁶ to 1:10²) exerted no effect on the rate or force of the spontaneous heart beat in over fifty observations. This lack of effect had previously been noted, but only to a concentration of 1:10⁶ (Östlund, 1954). This is illustrated in Fig. 5. Six hours after the start of this experiment, 100 mg. of acetylcholine were added to the bath (1:10⁶) and the beat continued unchanged for 15 minutes. After the compound was removed by washing, the beat continued with the same rate and force as before. Similarly, when the beat slowed as the length of time the heart was isolated increased, acetylcholine failed completely to exert any effect. Finally, systemic hearts which had ceased beating spontaneously were completely unaffected by the addition of acetylcholine to the bath, although ten out of twelve hearts studied in this way responded to electrical stimulation by a sharp contraction, indicating that the contractile mechanism was still capable of response.

Proguanil (1:10⁹) did not affect the spontaneous heart beat in several preparations. Cocaine was ineffective also, until the concentration of 8:10⁴ was reached, at which point atrioventricular block occurred, each chamber beating independently of the other. This was readily reversed by washing.

In view of certain recent studies on a digitalis-like effect of certain lysolecithins in the frog heart (Titus, Weiss, and Hajdu, 1956; Hajdu, Weiss, and Titus, 1957), it was of interest to determine whether this material would influence the heart beat of the hagfish. In six experiments a preparation of mixed
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synthetic α- and β-lysolecithins was homogenized in the medium to form a colloidal suspension and was added to the chamber at a final concentration of 1:10⁶. Neither stimulation nor depression was observed on fresh or partially hypodynamic hearts.

Serotonin creatinine sulfate was tested in concentrations of 1:10⁷ to 1:10⁵; no effect was observed in any experiment.

The hagfish heart appeared quite refractory to the drugs studied: nothing that had been tested appeared to influence the rate, hence pacemaker activity, of the heart beat. Several reports in the literature on myogenic automaticity in different species (Arvanitaki and Cardot, 1933; Hykes and Hykesova, 1932) prompted the author to attempt stimulation of a partially hypodynamic hagfish heart with extracts prepared from cardiac tissue of living hagfish. The preliminary results of these studies are considered to be of sufficient importance to warrant their inclusion here.

The systemic hearts from a number of fish were dissected free of blood vessels, connective tissue, etc., rinsed quickly in distilled water, blotted, and while still beating frozen quickly in a vial chilled by dry ice. (The average hagfish systemic heart weighs roughly 125 mg.) The pooled hearts from a number of animals were kept frozen at -10°C, until used. Tissue stored in this manner for up to 2 months did not lose its efficacy.

The extract was prepared by placing a sufficient mass of tissue in a glass homogenizing tube so that after addition of a proper volume of medium each milliliter of the resulting homogenate represented about 300 mg. of fresh muscle (wet weight). The hearts were homogenized by hand with a teflon pestle while ice cold; mild centrifugation was then employed to remove large particles and connective tissue. The resulting clear pinkish supernate was used for the experiments.

The pH of the extract approximated that of the medium and before addition to the test heart, the extract was brought to the temperature of the bath.

Repeatedly, it was found that the extract had no effect on either rate or force of freshly isolated hagfish hearts beating at a frequency of 12 to 20 times per minute. In eight preparations however, whose rate had slowed markedly but whose force of contraction was still strong, addition of 2 or 3 ml. of the extract produced a slight but definite increase in the rate but not in the force of the beat after a latent period varying from 2 to 8 minutes. This increase was maintained until the material was removed by washing and then the earlier rhythm was resumed. Fig. 6 illustrates this finding. The initial heart rate was 14/minute. Twenty-six hours after the beginning of the experiment, the spontaneous rate had slowed to 3/minute and the pattern of the beat was irregular although the force was strong. Addition of 2.0 ml. of the extract induced a doubling of the rate to 6/minute about 3 minutes later which was maintained for 20 minutes. Fifteen minutes after washing, the rate was again 3/minute.
Fig. 5. Lack of response to ACH, 1:10⁹, by the isolated spontaneously beating heart of the California hagfish, Polistotrema stouti.

Fig. 6. Effect of HHE on the isolated spontaneously beating heart of the California hagfish, Polistotrema stouti. A, control record, start of experiment. B, 26 hours later; addition of 2.0 ml. of HHE produced doubling of the rate which was maintained for 20 minutes until the extract was removed by washing. C, 15 minutes after washing.
This result was obtained five more times in this preparation and repeatedly confirmed in the other experiments. In no case to date, however, would the extract restart a completely quiescent heart. It merely accelerated or regularized the beat of hearts which had slowed spontaneously.

Results obtained from heating the extract in boiling water for 5 minutes have been inconclusive to date. In one study acceleration occurred after heating; in two others the results were negative. This point requires further investigation.

An extract was prepared by the method already described from several neurogenic hearts of the spiny lobster, Panulirus interruptus. The results of these studies on three hypodynamic hagfish hearts were completely negative; the pattern and rate of the beat remained unchanged by addition of the extract of this tissue.

The hagfish heart extract was also tested on a fresh but non-beating heart of the mussel, Mytilus californianus, maintained as described under "Mollusca" (see Fig. 7). Acetylcholine, 1:10⁶, 1:10⁵, and 5:10⁵, failed to induce a spontaneous beat in this preparation; however, addition of 2.0 ml of the extract induced a slow (4/minute) but regular beat 4 minutes after addition of the material to the bath. Washing 15 minutes later resulted in immediate cessation of the beat. This observation was repeated five times on this heart and it was found that acetylcholine, 1:10⁶, would inhibit the beat which was induced by the hagfish heart extract. Therefore, this effect is apparently not specific for the one species.

Discussion and Conclusions

The gradual decrease of rhythmic activity which occurs in an isolated heart may be considered to represent a type of heart failure, produced under relatively well controlled environmental conditions. The fact that acetylcholine is capable of stimulating certain mammalian myocardial tissue suffering this type of failure is most interesting, but does not necessarily imply that this compound has a role in automaticity. Demonstration of a stimulatory effect has not as yet been achieved in the range of physiological temperatures and this
factor may prove to be of the utmost importance in this regard. A possible explanation of the role of acetylcholine in mammalian cardiac automatism rests on experiments reported by Marshall and Vaughan Williams (1956). These workers found that cooling spontaneously beating rabbit atria below 20°C. would result in cessation of the contractions; however, even after there was no detectible mechanical activity, it was possible to find small non-propagated localized pacemaker potentials in the vicinity of the sinus node in nineteen of twenty-five experiments. Addition of acetylcholine to the heart at this point resulted in propagated action potentials and mechanical contraction once more. Thus, acetylcholine was considered to have restored the link between pacemaker and contractile activity which was broken by cooling. Additional evidence favoring this idea was published recently by Burn and Rand (1957). Using the isolated rabbit atrium–vagus nerve preparation of McEwen (1956), these workers demonstrated that as the temperature was lowered, vagal stimulation produced only inhibition. However, when the temperature reached the point at which contractions ceased (about 15°C.) vagal stimulation now produced contractions. Thus the excitatory and inhibitory actions of acetylcholine may be mediated through the sinoauricular node, rather than in a general fashion directly upon the myocardium as a whole.

The marked contrast among species in their response to the agents investigated in the present study is most interesting. Proguanil and cocaine inhibition of the mammalian tissue and subsequent acetylcholine stimulation are evidence of antagonism between these compounds. The fact that proguanil is capable of stimulating molluscan cardiac tissue is most surprising, and it indicates, at least superficially, basic differences in the organization of the different tissues. Further evidence for this view rests on the observation that acetylcholine was only inhibitory in the clam hearts studied, yet the fact that cocaine would stimulate in the presence of acetylcholine inhibition is again in direct contrast to the experiments on the rat heart. Especially interesting are the studies on the aneural hagfish heart. Since no organized, localized, innervated pacemaker is apparent in the myocardium of this species, nervous intervention is precluded in the interpretation of the effects of drugs. These hearts, whether fresh or hypodynamic, are completely insensitive to acetylcholine; in this regard they closely resemble other myogenic hearts such as Fundulus (Armstrong, 1935), chick (Markowitz, 1931), and Limulus (Prosser, 1942) embryos prior to innervation as well as adult Anopheles (Jones, 1956), Artemia and Eubranchipus (Prosser, 1942). Apparently innervation and acetylcholine sensitivity are linked; if this be so, then it is quite difficult to visualize how acetylcholine can mediate automaticity in non-innervated structures, and it is concluded that this compound does not assume a role in the rhythmic activity of such tissues.

The evidence presented indicates the presence in the hagfish heart of a substance (or substances) capable of stimulating the rate, hence pacemaker func-
Acetylcholine may be ruled out. Catecholamines (adrenalin and nor-adrenalin), although present in the hagfish heart in relatively high concentrations, exert no influence on its normal activity (Östlund, 1954). Serotonin was likewise ineffective, as were lysolecithins. Further investigations on the nature of the cardiac active material present in the hearts of this species are being conducted at present.

In conclusion, it is felt that the rhythmic activity of myogenic tissues does not depend upon an acetylcholine-cholinesterase mechanism, but rather upon another biochemical process which may act upon and within the cell membranes themselves, perhaps through changes in permeability and ionic transport.

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