# THE PHARMACOLOGY OF THE INSECT HEART

## I. The action of Adrenalin and Acetylcholine on the heart of the Water-bug (*Lethoceros*)

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### INTRODUCTION

The present state of the pharmacology of the Insect heart is unsatisfactory, as BEARD (1953, p. 263) recently pointed out. The problem, according to this author, has been investigated in the intact or in the isolated heart and drugs have been tested at randon or without respect to their modes of action on other systems and there is no reason to believe that the response to drugs is the same in all Insects. Yet, he complains, comparative studies are few and generalizations accordingly cannot be safely made. What is known is the result of two approaches, one with the purpose of explaining normal heart action through the use of drugs having a known action on other animals, the other involving the use of the heart and circulatory responses as a criterion of mode of action of compounds having insecticidal value.

During the course of the class-room work in our Department, with the purpose of showing the heart beat of an Insect, a large water-bug of the genus *Lethoceros* was once dissected and proved to be an excellent material for that kind of study. Even after this first and perhaps not too careful exposure of the heart, the organ immediately resumed its beating and in such a way that I was soon led to record *in situ* the heart beat with the procedure commonly used in the case of the frog heart. The recording was in all respect satisfactory, and therefore the heart of the water bug was chosen for a series of studies of the response of the organ to drugs. In this first paper, the results obtained with adrenalin and

acetylcholine, after preliminary observations on the pH and the saline, are reported.

The action of Adrenalin upon the insect heart was studied by DAVENPORT (1949) in *Stenopelmatus* and he reported that the drug at $10^{-6}$ retards and at higher concentrations arrests the organ. KRIJGSMANN & KRIJGSMANN (1950), on the contrary, found out that adrenalin stimulates the isolated heart of *Periplaneta*. Acetylcholine was found to accelerate the heart rate of *Melanoplus* (HAMILTON 1939), *Blatta* (PROSSER 1942), *Apis* (PROSSER, l. ci.,), *Stenopelmatus* (DAVENPORT, l. c.) and *Periplaneta* (KRIJGSMANN & KRIJGSMANN, l. c.). HAMILTON (l. c.) in *Melanoplus* observed immediate stimulation with acetylcholine, but also irregularities due to the development of slow rhythmic contractions of the allary muscles. Isolated segments of the heart, however, showed similar types of response and this would indicate that ACh does not act upon a single localized center. DAVENPORT (l. c.) in *Stenopelmatus* found that sensitive heart preparations can be stimulated even with $10^{-6}$ACh and that prior treatment with physostigmine makes less sensitive preparations also sensitive to $10^{-6}$ACh. Higher concentrations produced a marked acceleration in rate, increased the tonicity and induced a transitory systolic tetany. *ACh* would also restore some activity in fatigued or depressed hearts. Other choline derivatives have also been tested. Acetyl-beta-methylcholine, although less effective than ACh, also stimulates the heart of *Stenopelmatus*. Carbaminoylcholine may induce a systolic tetany similar to that caused by ACh. In view of some cholinesterasic activity of *Melanoplus* heart extracts (MEANS 1942) and of the fact that physostigmine potentiates the action of ACh in *Stenopelmatus*, DAVENPORT (l. c.) suggested that the heart action in insects might represent a cholinergic system.

## MATERIAL AND METHODS

Mostly large representatives of the genus *Lethoceros*, measuring from 6 to 9 cm in length were employed in the experiments. In some cases specimens belonging to the genus *Belostoma* were also used. The animals, captured in the surroundings of S. Paulo, were

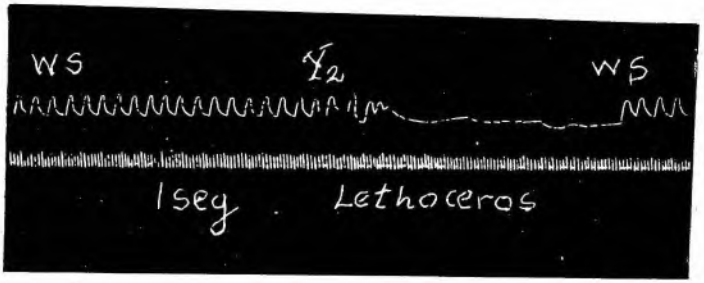
kept in the laboratory in large crystallizers containing pond or simply tap water. In the experiments, the first step consisted in severing the sting and legs at their basis, after previously sectioning the nerve cord in the region between the head and the thorax, behind the subesophagical ganglion. An incision followed separating the abdominal sternites from the tergites. The animal was then pinned down on its backs to a wax tray and the strip of sternum, digestive tract and gonad were carefully lifted away, exposing the dorsal vessel. The preparation from then on was continuously bathed with fresh saline, the wax tray being clamped at an angle so that the perfusion fluid flowed gently over the heart in a posteroanterior direction. Only those preparations which after perfusing with saline for some minutes exhibited a regular heart beat were used in the experiments. In the case of *Lethoceros,* to record the beatings a delicate hook to which a long thread of woman's hair was attached was carefully cramped on the wall of the dorsal vessel and the opposite end of the thread connected with a long (ca. 20 cm) and light aluminium lever. This finally inscribed the beatings on a smoked drum of a kymograph. In the case of *Belostoma,* much smaller in size, the heart rate was checked visually with a stopwatch. Drugs were administered directly to the heart from a pipette, after stopping the continuous flow of saline, and in such a quantity that the remaining traces of plain saline surrounding the heart were completely flushed away and replaced by the drug solution. Acetylcholine chloride from Roche Products Co. and adrenalin chloride from Parke, Davis and Co. were used. The former was dissolved in saline from the solid state and the latter diluted from $10^{-3}$ aqueous solution contained in the ampules.

## RESULTS

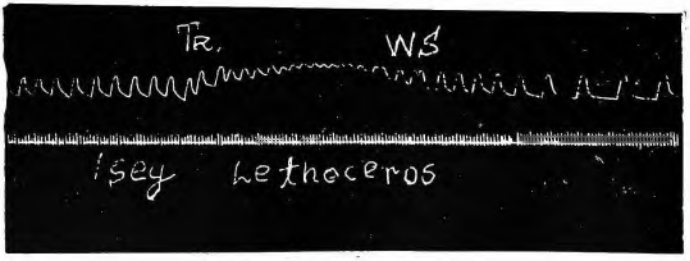
a. *The saline and the pH.* The physiological saline according to WILDER AND SMITH (1938) was used. Its composition is the following: NaCl, 5.5 gm; KCl, 0.14 gm; $CaCl_2$, 0.12 gm and distiled water to a liter, with a pH of 5.5 Acetylcholine dissolved in this unbuffered saline increased the pH, which at $10^{-7}$ was 5.9, at $10^{-6}$ 6.2, at $10^{-5}$ 6.2 and at $10^{-4}$ 6.3. Adrenalin, on the contrary, decreased the pH, which at $10^{-4}$ was 3.7, but regularly

increased with further dilutions. In some experiments, the pH of WILDER AND SMITH's saline was adjusted to 7.3 with M/2 $Na_2HPO_4$, but, as a rule, unbuffered saline was used, since it proved to be more efficacious in restoring the beat of hearts arrested in consequence of traumas or after drug test which caused depression or arrest of the organ. Tracing n. 5 of fig. 3 shows the ability of unbuffered WILDER AND SMITH's saline in restoring the heart beat after severe administration of ACh. Since the pH of this unbeffered saline is 5.5, the suggestion is made that the optimal pH for *Lethoceros* heart is on the acid side of the neutral point.

Another insect saline which was used by YEAGER (1939) in the roach heart and blood and composed of: NaCl, 10.93 gm; KCl, 1.57 gm; $CaCl_2$, 0.85 gm and $MgCl_2$, 0.17 gm, was also tested. This saline caused an immediate stop of the heart beat, which was promptly washed out with WILDER AND SMITH's saline. Even when deprived of $MgCl_2$, the YEAGER's saline could not be used as shown in the tracing 1 of fig. 1. Tracing 2 of fig. 1 shows the action of a saline used in the heart of the Brazilian fresh-




Tracing 1: The effect of YEAGERS's saline deprived of Mg($Y_2$) on the heart of the waterbug previously bathed with WILDER & SMITH's saline (WS).




Tracing 2: The effect of a saline (for the Brazilian fresh water crab *Trichodactylus*, Tr) with less Na than WILDER & SMITH's on the heart of the water bug.

water crab *Trichodactylus* (VALENTE, in the press), with a low concentration of NaCl.

b. *The action of adrenalin*. Fig. 2 shows four tracings recorded *in situ* of the heart of *Lethoceros* under the action of different concentrations of adrenalin. It can be clearly seen that with $10^{-7}$ and $10^{-6}$ there is stimulation of the heart beat, the frequency principally being increased. With $10^{-5}$ a tendency to systolic tetany appears and with $10^{-4}$ this systolic tetany becomes almost complete.

Similar results were obtained with *Belostoma*, the pronounced tendency to systolic tetany being always present with $10^{-5}$ and $10^{-4}$ Ad.

c. *The action of acetylcholine*. Fig. 3 shows five tracings recorded *in situ* of the heart beat of *Lethoceros* under the action of acetylcholine solutions from $10^{-6}$ to $10^{-2}$. The tracings indicate that with $10^{-6}$ there were no changes in frequency, although the amplitude seems to be slightly increased. This increase in amplitude, however, can be considered as a mere hydrostatic effect, since there was an interval between the removal of plain saline and administration of the drug, during which the organ remained wet, but not under liquid pressure. This hydrostatic pressure can clearly be seen in the displacing of the base line of the tracings. With $10^{-5}$, $10^{-4}$ and $10^{-3}$ the situation was not significantly different and one could hardly speak of essential modifications in the frequency. $10^{-2}$ ACh was tested to find out just how far could the water bug heart remain indifferent to the drug. An immediate diastolic block was then observed, which was, however, promptly reversible on washing with saline.

The tracings of the figures refer to experiments performed with unbuffered saline. In those using buffered saline, similar results were obtained. As to *Belostoma*, the results were not so uniform, in a few cases a little stimulatory effect of ACh seeming to be present and in a few others depressory effects being observed. As a rule, however, no effect at all was observed.

d. *The cholinesterase activity of the water bug heart*. The cholinesterase activity of extracts of *Belostoma's* and *Lethoceros'* hearts was checked according to the technique of AMMON (1934).

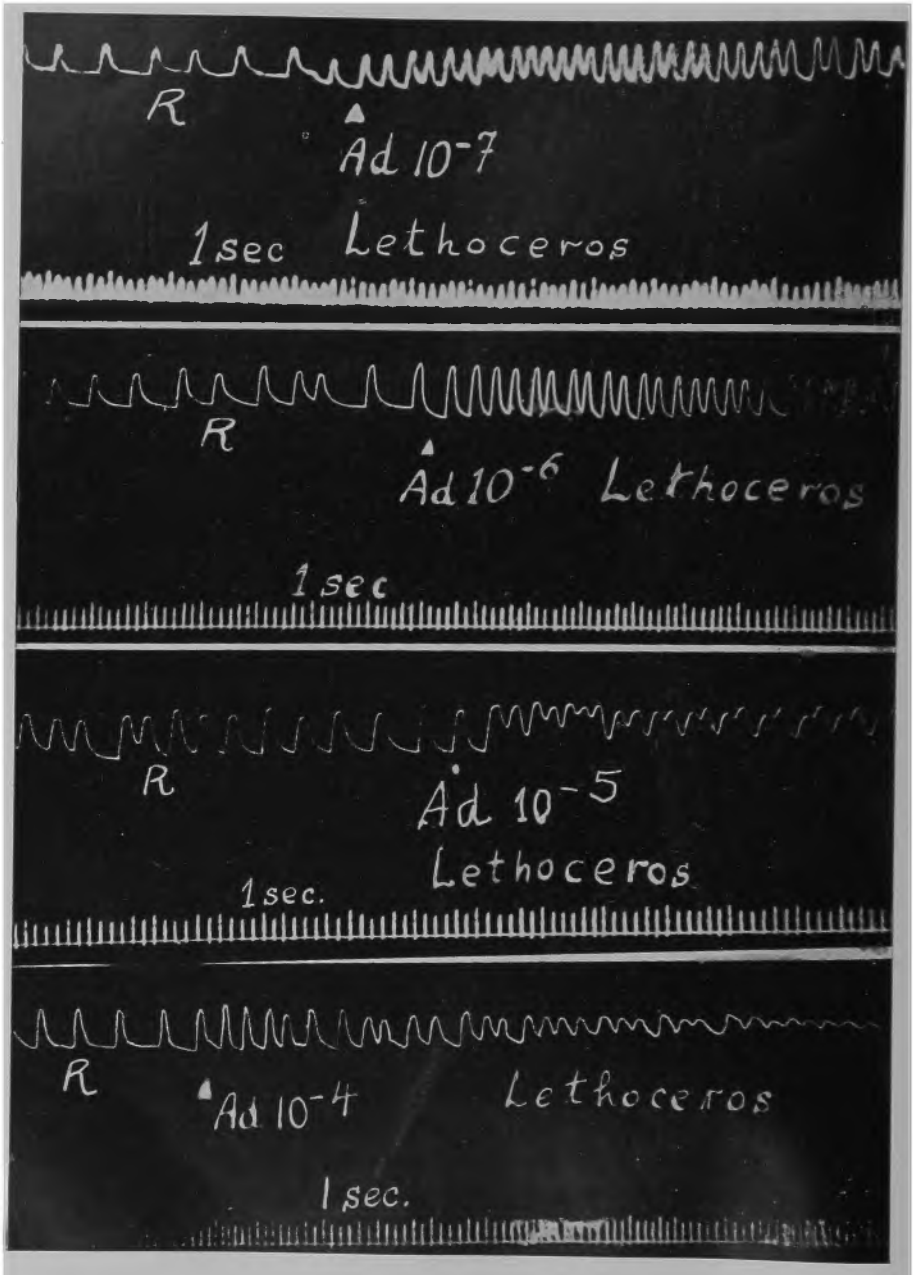

R
Ad 10-7
1 sec Lethoceros
R
Ad 10-6 Lethoceros
1 sec
R
Ad 10-5
Lethoceros
1 sec.
R
Ad 10-4
Lethoceros
1 sec.

Figura 2

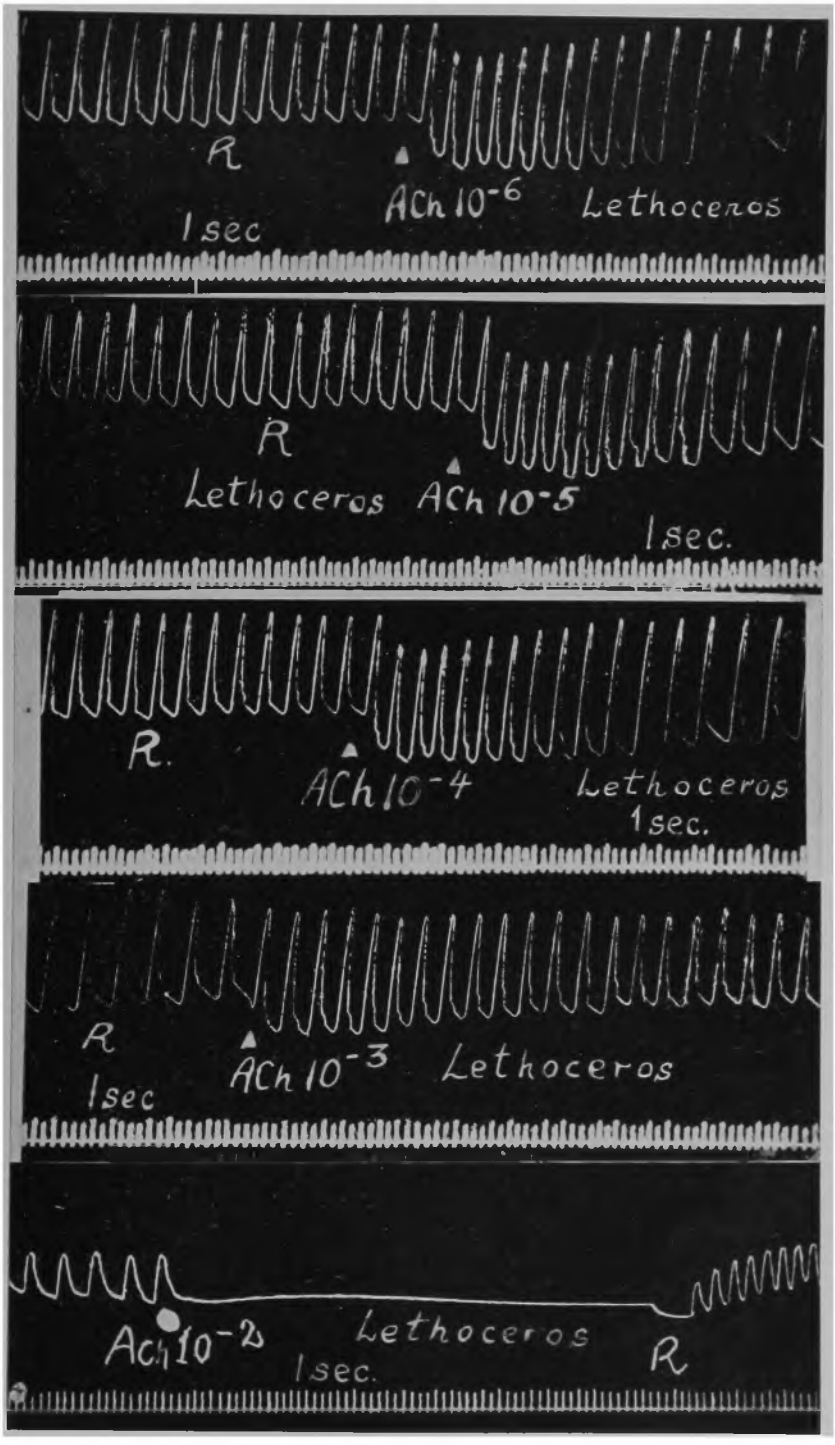

R
ACh 10-6
Lethoceros
1 sec
R
Lethoceros
ACh 10-5
1 sec.
R.
ACh 10-4
Lethoceros
1 sec.
R
1 sec
ACh 10-3
Lethoceros
ACh 10-2
Lethoceros
1 sec.
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**Figura 3**

Extracts of 0.5 mg of heart in WILDER & SMITH's saline buffered with bicarbonate were put against 0.1 mg acetylcholine. The heart tissue was obtained by severing the allary muscles under the binocular with an iridectomic scissor at their insertion points. In no case decomposition of acetylcholine could be detected.

## DISCUSSION

a. Althcugh it was not the purpose of this work the study of the action of ions on the water bug's heart, some preliminary conclusions can be drawn from the results obtained with WILDER AND SMITH's and YEAGER's salines. These salines differ (see table I) significantly in Na/K and (Na + K)/Ca ratios and in the fact that the latter has MgCl2 whereas the former has not. YEAGER's saline arrested immediately the heart of both *Belostoma* and *Lethoceros*. In DAVENPORT's experiment (l. c.), neither LEVY's, nor MALOEUF's, nor YEAGER AND HAGER's (1934 apud DAVENPORT l. c., p. 23) saline, which I presume has a composition similar to that used by YEAGER AND GAHAN (1937) were able to maintain the heart beat of *Stenopelmatus*. Frog saline, which except for the presence of bicarbonate, is qualitatively and quantitatively similar to WILDER AND SMITH's saline (also a modified Ringer Solution) was then successfully used.

Table I

NaCl, KCl and $CaCl_2$ contents of some salines used in insect heart studies.

| | gm NaCl | KCl | $CaCl_2$ | water | Na/K | (Na+K)/Ca |
|---|---|---|---|---|---|---|
| Levy 1928 | 9.00 | 0.70 | 0.46 | 1 l. | 12.8 | 47.0 |
| Maloeuf 1935 | 9.00 | 0.20 | 0.20 | 1 l. | 45.0 | 47.5 |
| Yeager & Gahan 1937 | 9.82 | 0.77 | 0.50 | up to 1 l. | 7.0 | 14.7 |
| Wilder & Smith 1938 | 5.00 | 0.14 | 0.12 | up to 1 l. | 33.9 | 47.0 |
| Yeager 1939 | 10.93 | 1.57 | 0.85 | up to 1 l. | 12.6 | 21.2 |
| Davenport 1949 | 6.70 | 0.15 | 0.12 | up to 1 l. | 44.7 | 45.7 |

The effects of Na, K and Ca on the insect heart were studied by BERGERARD (1947) in *Gryllus* and DREUX (1950) in *Galleria*. Solutions with Na/K ratios less than 8 stop the heart in

systole. Higher ratios increase the rate of beat but decrease the amplitude. Solution with (Na + K)/Ca ratios of less than 3 cause diastolic arrest, higher ratios retard the rhythm and decrease the amplitude. As to magnesium, FISZER (1950 a and b) reported that when it replaces calcium in a perfusing saline the heart of *Gryllus* is arrested in diastole, but that there is neither synergism nor antagonism between these two ions. Rather, their actions are distinct. There is, however, antagonism between magnesium and potassium. Magnesium is found in insect hemolymph in higher concentration than in man and often (LEVENBOOK 1950 in *Gastrophilus*, FLORKIN 1943 apud BUCK 1953 p. 160 in *Hydrophilus* and BIALASZEWICZ & LANDAU 1938 apud Buck, l. c., p. 160 in *Bombyx*) in strikingly high concentration. FLORKIN (1949) pointed out that Mg is much higher in proportion to the other cations than in most other animals except marine invertebrates and METCALF (1935) suggested that it derives mainly from chlorophyll. The concentration of magnesium in the blood of insect is high enough to induce anesthesia in most non-marine animals. LEVENBOOK (1949 apud BUCK, l. c., p. 160) injected magnesium into the body of adult *Locusta* in a concentration approximating that already present in the blood. Notwithstanding, it rapidly produced a cataleptic condition. After subsequent injection of calcium, which is known to abolish magnesium anesthesia in other animals, the insects recovered quickly. Injection of calcium alone, however, was fatal. LEVENBOOK tentatively concluded that free magnesium as well as free calcium are toxic and that probably they do not occur as such in the insect blood, but bound to protein. On the basis of these results, the failure of LEVY's, of YEAGER & HAGER's salines in maintaining the heart beat of *Stenopelmatus* might be explained on account of relatively low Na/K ratios and perhaps excessively high concentrations of NaCl, KCl and $CaCl_2$ as compared with WILDER AND SMITH's or frog saline (KOZHANTOCHIKOV 1932 apud BEARD, l. c., p. 269 obtained in *Blatta* a systolic standstill with hypertonic Ringer solutions). The failure of YEAGER's saline, on the other hand, in maintaining the heart beat in the water bug can be attributed to the presence of Mg ions, and the low Na/K ratio.

The fact that the optimal pH for the water bug heart seems to be on the acid side of the neutral point can not be considered as astonishing, since it is well known that eight five per cent of the values for the hydrogen-ion concentration of insect blood fall slightly on the acid side of neutrality (BUCK 1953).

b. The responses of both *Belostoma* and *Lethoceros* hearts to adrenalin reveal that the organ is sensitive to the drug. Adrenalin can stimulate the heart beat ($10^{-7}$ and $10^{-6}$) or even induce a systolic tetany ($10^{-5}$ and $10^{-4}$). These results agree with those obtained by KRIJGSMANN & KRIJGSMANN (l. c.) on the isolated heart of *Periplaneta* where stimulation was also obtained with adrenalin. They are not in agreement with those of DAVENPORT (l. c.) reported for another Orthopteran, *Stenopelmatus*, namely, that the drug at $10^{-6}$ retards and at higher concentrations arrests the heart in diastole.

c. From the results obtained when $10^{-6}$ up to $10^{-3}$ acetylcholine solutions were pipetted upon the water bug heart, it seems that little or even nothing can be said in favor of any particular action of the drug. These results are in complete disagreement with the those of previous authors, which all found stimulatory effects of acetylcholine, as already mentioned. The question naturally arises: Are these differences in effect caused by differences in intrinsic nervous mechanisms of the animals studied? ALEXANDROWICZ (1926) reported the presence of ganglionic cells in the lateral heart nerves of *Blatta*. DAVENPORT (l. c.) found in *Stenopelmatus* ganglia and nerves closely invested to the heart muscle by the surrounding connective tissue. As to *Melanoplus*, *Apis* and *Periplaneta* in which HAMILTON (l. c.), PROSSER (l. c.) and KRIJGSMANN & KRIJGSMANN (l. c.) respectively found stimulatory effects of acetylcholine, no reference is made in their papers to the presence or absence of ganglia in the heart. From experiment with the water bug *Belostoma flumineum* MALOEUF (l. c.) suggested that in this Hemipteran the heart beat and rhythm are probably independent of posible rhythmic impulses dispatched from ganglionic cells. May be this is also the case in the water bugs used in the present work and that would explain the results

obtained with acetylcholine since according to PROSSER (l. c.) hearts unaffected by this drug are noninnervated.

d. No cholinesterase activity could be detected in extracts of the heart of both *Belostoma* and *Lethoceros*, using the AMMON (l. c.) technique. HAMILTON, using the responses of the frog and turtle hearts to the grasshopper brei also could not detect cholinesterase in this Orthopteran. However, using the Cartesian Diver technique, MEANS (1942) demonstrated that the grasshopper heart extracts has a small (as compared with the nervous and muscular estructures) QCH.E of 0.40. HAMILTON (l. c.) recognized that the absence of cholinesterase in *Melanoplus* rendered difficult a natural function of acetylcholine in that animal's nervous system and, although he obtained acceleration of the heart beat with acetylcholine, he states that "it seems very improbable that it could be of importance in the nerve conduction of this insect", since, among other things, the intact grasshopper is relatively insensitive to acetylcholine. On the basis of the results of MEANS (l. c.), however, and from his own results with physostigmine, DAVENPORT (l. c.) suggested that the heart action in insects might represent a cholinergic system. Although complementary studies are still necessary, the present evidence in the case of the water bug does not seem to support this view.

SUMMARY

1. The action of acetylcholine and adrenalin on the heart of two aquatic Hemipterans (*Belostoma* and *Lethoceros*) was studied.

2. Preliminary tests in order to find out the suitable physiological saline for the experiments indicated that WILDER & SMITH' saline, with Na/K and (NA + K)/Ca ratios similar to those of frog saline used by DAVENPORT in his insect heart studies, was to be adopted. YEAGER's saline which contains Mg $Cl_2$ stopped the heart of the water bug. A brief discussion of the action of ions on the heart action is given. The fact that the water bug heart beat was better maintained in unbuffered saline (pH ca.5.5) than in saline adjusted to pH: 7.3 with a buffer,

suggests that the pH optimal for the heart action in these insects seems to be on the acid side of the neutral point.

3. The heart beat was recorded *in situ* in the case of the large *Lethoceros* and checked visually with a stopwatch in the case of smaller *Belostoma*. The average heart rate in the former was 24 beats per minute and in the latter 36, at ca. 20°C.

4. Adrenalin acts upon the water bug heart, increasing the frequency in concentrations equal to $10^{-7}$ and $10^{-6}$. At $10^{-5}$ it induces a systolic tetany, which can be almost complete with $10^{-4}$.

5. Acetylcholin produced no modifications on the heart beat of the water bug when used in concentrations from $10^{-6}$ up to $10^{-3}$. When $10^{-2}$ ACh was tested an immediate diastolic block was observed.

6. No cholinesterase activity was detected when, using the AMMON technique, heart extracts of *Belostoma* and *Lethoceros* were put against acetylcholine solutions.

7. From the lack of action of acetylcholine, from the absence of cholinesterase activity of heart extracts and from the fact that probably in the water bug the heart and rhythm are independent of possible rhythmic impulses from ganglionic cells (MALOEUF in *Belostoma flumineum*) it is here suggested that at least in the Hemipterans studied the heart action does not seem to represent a cholinergic system.

SUMÁRIO

1. Foi estudada a ação da acetilcolina e da adrenalina sôbre o coração de dois insetos hemipteros aquáticos (*Belostoma* e *Lethoceros*), conhecidos por baratas d'gua.

2. Provas preliminares a fim de encontrar o líquido fisiológico mais apropriado para as experiências indicaram que a solução de WILDER e SMITH, cujas relações NA/K e (Na + K)/Ca são semelhantes às do Ringer de Anfíbio usado por DAVENPORT nos seus estudos sôbre o coração dos insetos, era de se adotar. A solução de YEAGER, que contém $MgCl_2$, parou o coração da barata d'água. O fato do coração dêstes insetos se manter

melhor em solução fisiológica não tamponada (pH ca. 5.5) do que na de pH ajustado com tampão a 7.3 sugere que o pH ótimo para a atividade cardíaca parece estar no lado ácido do ponto neutro.

3. O batimento cardíaco foi registrado *in situ* no caso dos grandes exemplares de *Lethoceros* e contados com um cronômetro no caso dos espécimes menores de *Belostoma*. A taxa cardíaca média nos primeiros foi de 24 batimentos por minuto e nos segundos de 36, a 20°C.

4. Adrenalina age sôbre o coração da barata d'água aumentando a freqüência, quando em concentrações de $10^{-7}$ e $10^{-6}$. A $10^{-5}$ induz tetanía sistólica, que se torna quase completa a $10^{-4}$.

5. Acetilcolina não produziu modificações no batimento cardíaco da barata d'água quando usada em concentrações de $10^{-6}$ até $10^{-3}$. Quando $10^{-2}$ foi experimentada o coração parou imediatamente em diástole.

6. Não se registrou atividade colinesterásica quando, usando-se a técnica de AMMON, colocaram-se extratos do coração de *Belostoma* e *Lethoceros* em contacto com soluções de acetilcolina.

7. Com base na falta de atividade da acetilcolina, na ausência de poder colinesterásico de extratos de coração de barata d'água e finalmente no fato de que provàvelmente nesses hemipteros o batimento e o ritmo cardíaco independem de impulsos ritmicos advindos de células ganglionares (MALOEUF in *Belostoma flumineum*), sugere-se aqui que, pelo menos no caso dos hemipteros estudados, a atividade cardíaca não represente um sistema colinérgico.

## LITERATURE

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