CEYLON J. MED. SCI. Vol. XV No. 2, (December 1966)

The Estimation of 5-Hydroxytryptamine and Acetylcholine using the Isolated Hearts of *Geloina erosa* and *Meretrix lusoria* (Röding)

by

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There are several biological test preparations very sensitive to 5-hydroxytryptamine (5HT) and others which are suitable for the estimation of acetylcholine (ACh). Among these, the isolated hearts of molluscs, which are highly sensitive to both 5HT and ACh, have been used for the estimation of these substances; 5HT stimulates and ACh inhibits the contraction of these hearts. Molluscan hearts have the advantage over the other preparations because, unlike other preparations, they are almost insensitive to histamine, adrenaline, substance P etc., which may be found with 5HT and ACh in tissue extracts.

The isolated heart of the bivalve mollusc, *Venus mercenaria*, which is readily available in the U.S.A., was first used by Welsh in 1943 for the estimation of ACh (see Welsh, 1954). The same preparation was used for the determination of 5HT in tissue extracts by Twarog and Page (1953). The action of ACh on this preparation is suppressed by benzoquinonium. Since *Venus mercenaria* is not easily available in Great Britain, Gaddum and Paasonen (1955) searched in British waters for a suitable substitute for the estimation of 5HT. They showed that the isolated heart of the bivalve, *Spisula solida*, which is relatively abundant in the sea near Britain, was most useful in this respect. In a similar search for a mollusc whose heart is sensitive to ACh, Hughes (1955) found that the heart of *Mya arenaria*, a bivalve easily obtainable in the British Isles, had a high specificity and sensitivity to ACh.

None of these molluscs, *Venus mercenaria*, *Spisula solida*, or *Mya arenaria*, is readily obtainable in Ceylon. Hence a search was made in Ceylon waters for a suitable substitute. The work described in this paper is a continuation and an extension of work briefly reported earlier (Kottegoda, 1959).

Two varieties of brackish water molluscs were tested. The isolated hearts of both of these were found to be sensitive to 5HT and ACh. These two, *Geloina erosa* and *Meretrix lusoria* (Röding) (Fig. 1*a* and *b*), are found in abundance in the Negombo lagoon near its connection with the sea. The smaller of the two, *Geloina erosa*, (average length 6.5 cms) could be readily collected at low tide from beneath the sand on the east side of the Pitipana bridge, while the larger, *Meretrix lusoria*, (average length 8.5 cms) is found on the opposite bank of the lagoon about one mile inland. Both molluscs are edible.

Either variety could be kept alive for about 2-4 weeks at room temperature (30°C) in a tank containing lagoon water which was continuously aerated during the day. *Meretrix lusoria* was the hardier of the two; some specimens of the latter survived in the tank for as long as 8 weeks. It was also found that specimens of *Meretrix lusoria* could be kept alive for 2 weeks or so when wrapped in cloth moistened with lagoon water and stored in the refrigerator (4°C). It was our impression that the sensitivity of the hearts diminished after the molluscs had been in captivity for over two weeks. If any died, the tank was cleaned up as early as possible and the water changed.

METHODS

The dissection for the exposure of the heart in either variety was essentially the same as that described by the previous authers for other bivalve molluscs except that it was not found necessary to crack the shell with a hammer to expose the heart. One of the shells was loosened by severing the byssus muscles with a scalpel. After the separation of the pericardium ligatures were applied to either side of the ventricle (Fig. 2*a* and *b*), the heart removed and suspended in a 2 ml bath containing lagoon water which was aerated. The changing of the bath fluid was done by overflow suction. A light straw lever was used to record the contractions on a smoked drum. In some experiments a spring was used on the lever. Artificial sea water (Welsh and Taub, 1948) was found to be a satisfactory substitute for lagoon water. The heart of *Meretrix lusoria* regularly gave a more powerful contraction than that of the smaller *Geloina erosa*. The latter commenced regular contractions within about 15 minutes of setting it up in the bath, the former took longer, and sometimes one hour elapsed before the contractions became regular. Once regular contractions were established they lasted for 2-4 hours during the experiment. Some isolated hearts, which had been left overnight unaerated in the bath after an experiment, commenced regular beating the next morning when aeration was resumed.

RESULTS

Acetylcholine

Both preparations showed a high sensitivity to ACh which depressed the contractions. The action of ACh was suppressed by benzoquinonium (Fig. 3). The lowest concentration which produced an effect was 10^{-9}, whilst most hearts were sensitive to 2×10^{-8}. Both hearts were more or less equally sensitive to ACh.

5-hydroxytryptamine

The hearts of both varieties were stimulated by low doses of 5HT. The action of 5HT was blocked by LSD which itself regularly caused some increase in the amplitude of the contractions (Fig. 4*a* and *b*). The highest sensitivity to 5HT was at a concentration of 10^{-9}, whilst most hearts were appreciably stimulated by a concentration of 10^{-8}.

Adrenaline, histamine and potassium

Concentrations of adrenaline greater than 10^{-7} were required to stimulate these isolated hearts. The response to adrenaline was qualitatively different to that obtained with 5HT. Histamine in a concentration of 10^{-6} had no action. A concentration of 10^{-3} potassium chloride produced no effect on the hearts (Fig. 5).

Tissue extracts

The hairs of the common Ceylon nettle *Tragica involucrata* (N.O. Euphorbiaceae) has been shown to contain acetylcholine and histamine (Kottegoda, 1959). Extracts of these hairs made with lagoon water and tested on the hearts showed an ACh-like effect which was blocked by benzoquinonium (Fig. 6).

Extracts of the pulp and peel of one of the Ceylon plaintains (bananas), anamalu, which have been found to contain 5HT (Dharmatilake and Kottegoda, 1966) were tested on the isolated hearts and these extracts gave a 5HT-like effect (Fig. 7). The venom of the scorpion, *Leiurus quinquestriatus*, has been shown to contain 5HT (Adam and Weiss, 1959). The venom of the large Ceylon scorpion, *Palamnaeus indicus*, was extracted by electrical stimulation of the telson and applied to the isolated heart. There was a 5HT-like action (Fig. 8).

Of the two varieties of molluscs used in this investigation, it was felt that the heart of the larger one, *Meretrix lusoria* (Röding) was more suitable than that of *Geloina erosa*. The former was the hardier of the two, its heart is larger and therefore easier to dissect and although it took a longer time to give a regular contraction this was maintained for a longer period. There did not appear to be any difference between these two hearts in respect of their sensitivity to 5HT or ACh.

SUMMARY

The isolated hearts of two brackish water bivalve molluscs, *Meretrix lusoria* (Röding) and *Geloina erosa*, readily available in Negombo, Ceylon, have been shown to be suitable for the estimation of 5HT or ACh.

Of these two, the heart of *Meretrix lusoria* was larger and also easier to set up and gave regular contractions over a longer period. These hearts are not sensitive to histamine or potassium and relatively insensitive to adrenaline.

These isolated hearts were sensitive to concentrations of 5HT or ACh as low as 10^{-9}.

ACKNOWLEDGEMENTS

Our thanks are due to Dr. F. P. Luduena of the Sterling-Winthrop Research Institute for a generous gift of benzoquinium and to Mr. I. Wickramasuriya for the illustration, Fig. 2. We are especially grateful to Mr. P. Kirthisinghe, former Reader in Zoology, University of Ceylon, for the identification of the molluscs.

REFERENCES

ADAM, K. R. AND WEISS, C. (1959). The Occurence of 5-Hydroxytryptamine in Scorpion venom. *J. exp. Biol.*, **35**, 39—42.

DHARMATILAKE, R. M. AND KOTTEGODA, S. R. (1966). 5-Hydroxytryptamine (5HT, Serotonin) in Edible Fruits. *Proc. Ceylon Ass. Advmt Sci.*, **1**, 8.

GADDUM, J. H. AND PAASONEN, M. K. (1955). The Use of Some Molluscan Hearts for the estimation of 5-Hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **10**, 474—483.

HUGHES, B. (1955). The Isolated Heart of *Mya arenaria* as a sensitive Preparation for the Assay of Acetylcholine, *Br. J. Pharmac. Chemother.*, **10**, 36—38.

KOTTEGODA, S. R. (1956). Substances present in the Sting of *Tragica involucrata*. *Proc. Ceylon Ass. Advmt Sci.*, **1**, 4—5.

KOTTEGODA, S. R. (1959). Use of some Ceylon Molluscs for Estimation of 5-hydroxytryptamine. *Proc. Ceylon Ass. Advmt Sci.*, **1**, 5.

TWAROG, B. M. AND PAGE, I. H. (1953). Serotonin Content of Some Mammalian Tissues and Urine and a Method for its Determination. *Am. J. Physiol.*, **175**, 157—161.

WELSH, J. H. AND TAUB, R. (1948). The Action of Choline and Related Compounds on the Heart of *Venus mercenaria*. *Biol. Bull. Mar. Biol. Lab., Wood's Hole*, **95**, 346—353.

WELSH, J. H. (1954). Marine Invertebrate Preparations useful in the Bio-assay of Acetylcholine and 5-Hydroxytryptamine. *Nature, Lond.*, **173**, 954—956.

EXPLANATION OF PLATES

PLATE I

Fig. 1 (a) *Geloina erosa* (b) *Meretrix lusoria*, Röding

PLATE II

Fig. 2 (a) Separation of shells to expose heart (b) exposure of the heart

PLATE III

Fig. 3 Action of Ach on the heart of *Meretrix lusoria;* 2ml bath. The action is blocked by benzoquinonium (B)

PLATE IV

Fig. 4 (a) Action of 5HT on the heart of *Meretrix lusoria;* 2 ml bath.

PLATE V

Fig. 4 (b) Blocking by LSD of the action of 5HT; 2ml bath

PLATE VI

Fig. 5 Comparison of the effect of KCl and 5HT on *Geloina erosa* heart; 2ml bath

Fig. 6 Action of the extract of the Ceylon nettle (K) on the heart of *Meretrix lusoria*. There was an Ach-like action which was blocked by benzoquinounim (B)

PLATE VII

Fig. 7 Extracts of banana (anamalu) (A) giving a 5HT-like effect on the heart of *Geloina erosa*

Fig. 8 Similarity between the action of 5HT and that of the venom of the Ceylon scorpion S on the heart of *Meretrix lusoria*.

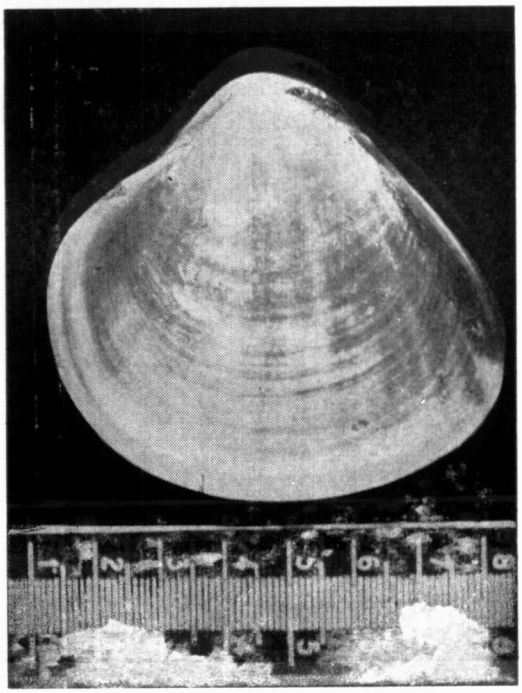

Fig. 1 (a)

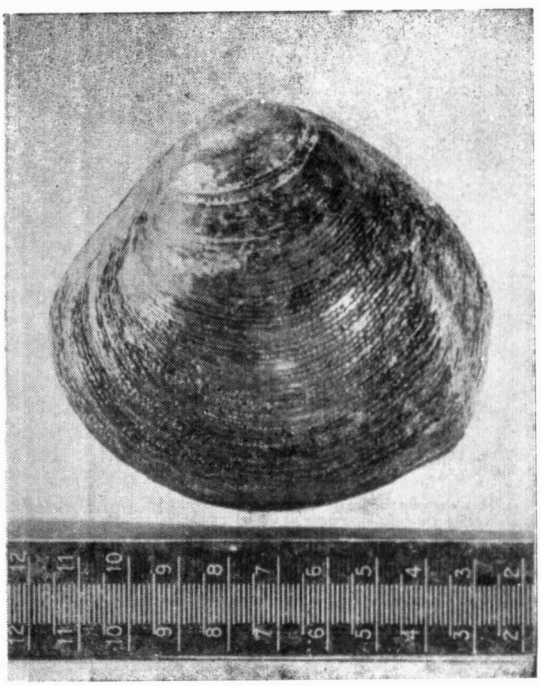

Fig. 1 (b)

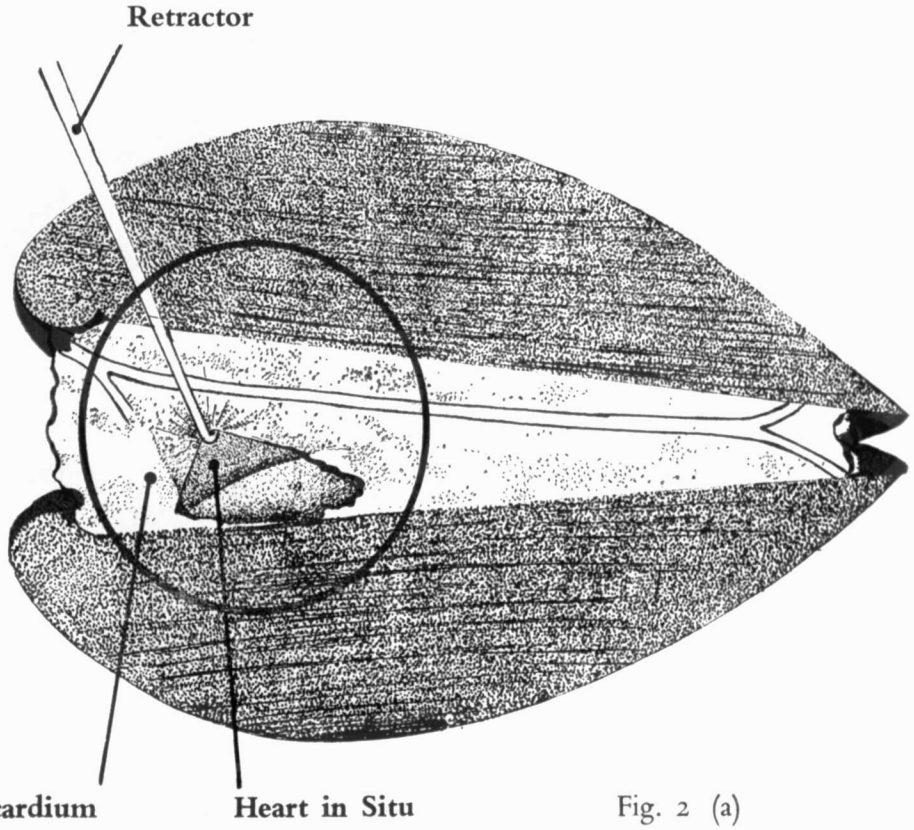
Retractor
Pericardium
Heart in Situ

Fig. 2 (a)

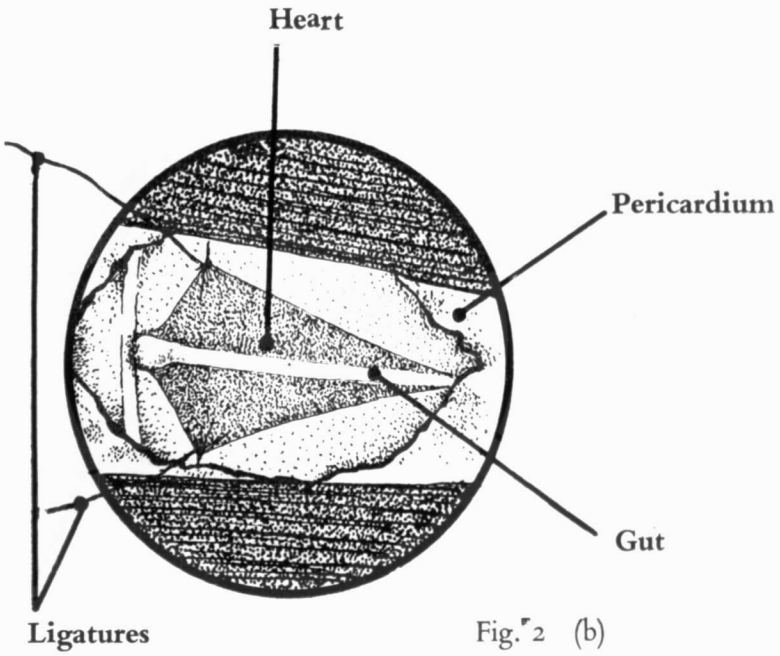
Heart
Pericardium
Gut
Ligatures

Fig. 2 (b)

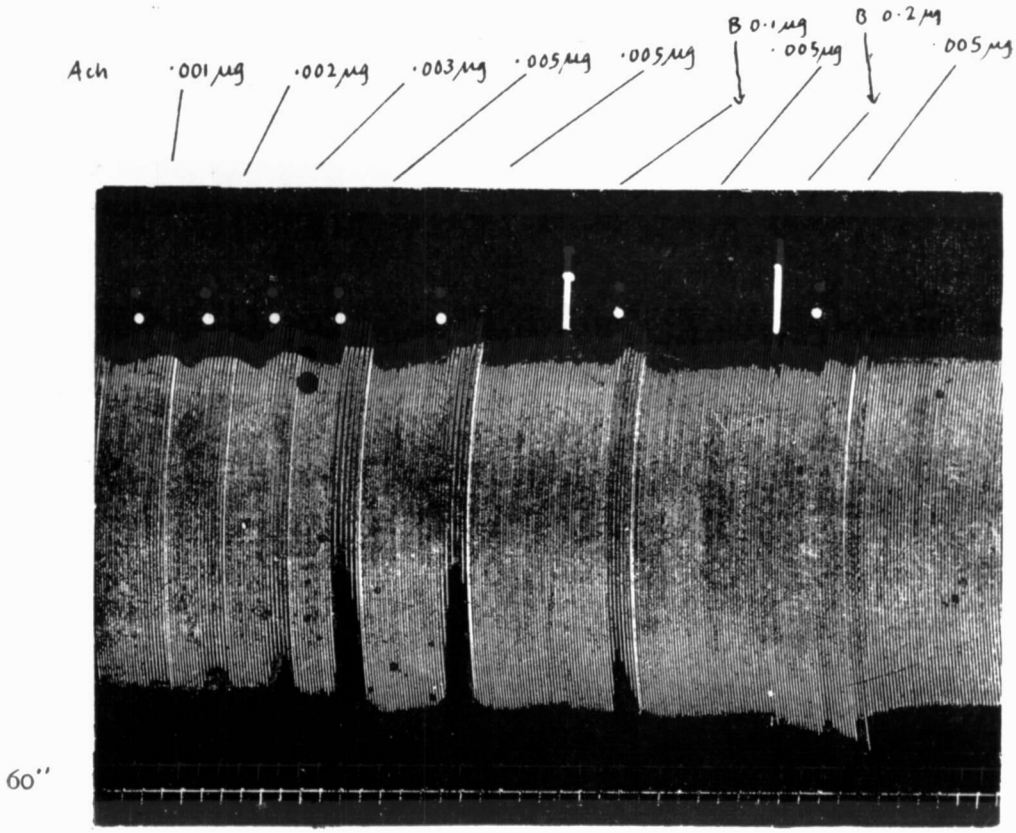
Ach
·001 µg
·002 µg
·003 µg
·005 µg
·005 µg
B 0·1 µg
·005 µg
B 0·2 µg
·005 µg
60"

Fig. 3

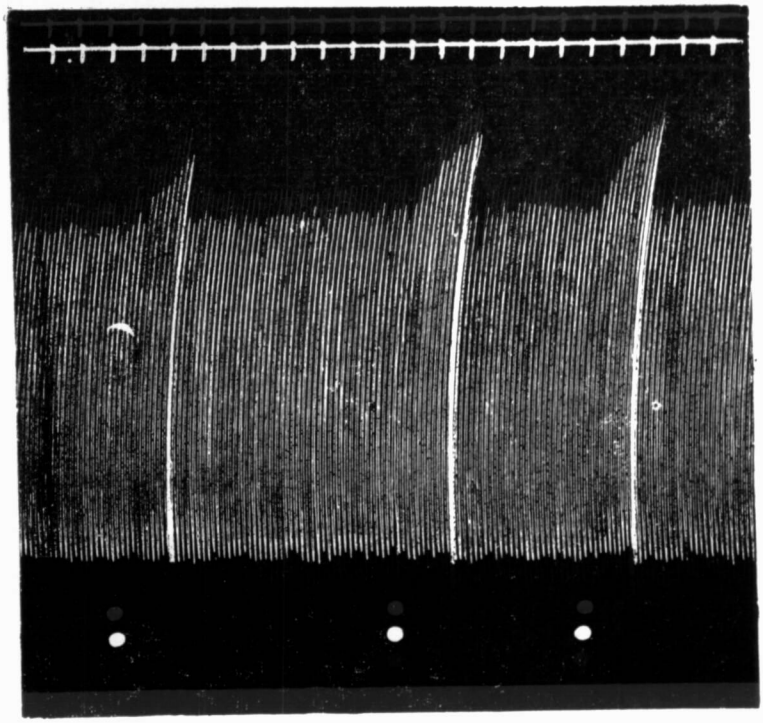

Fig. 4 (a)

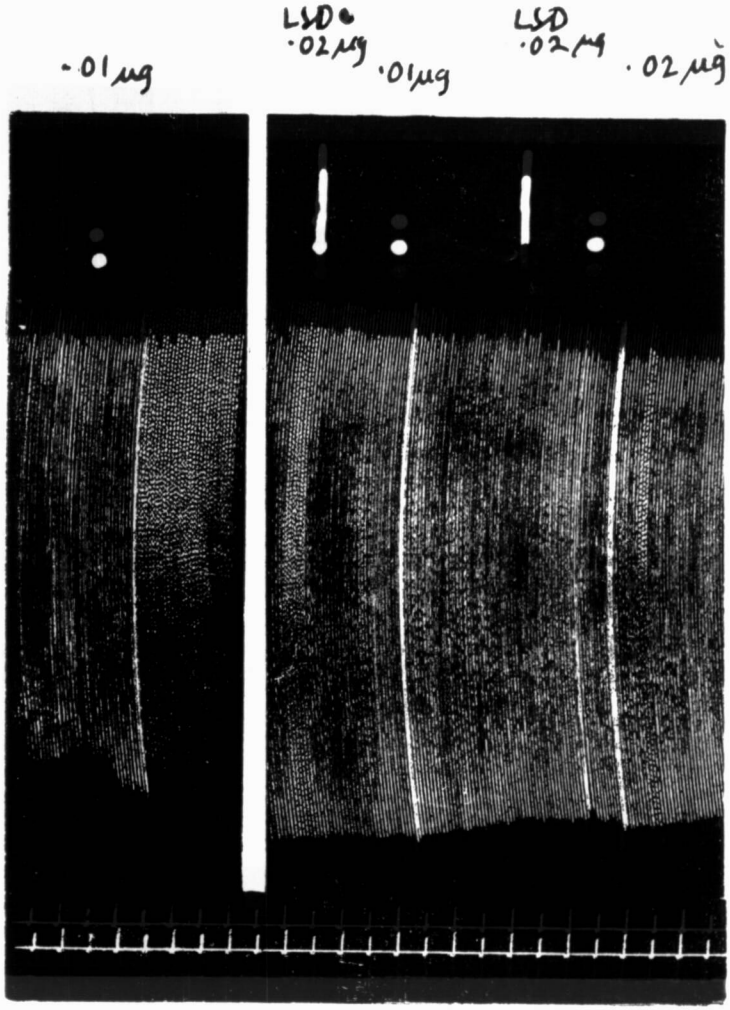

Fig. 4 (b)

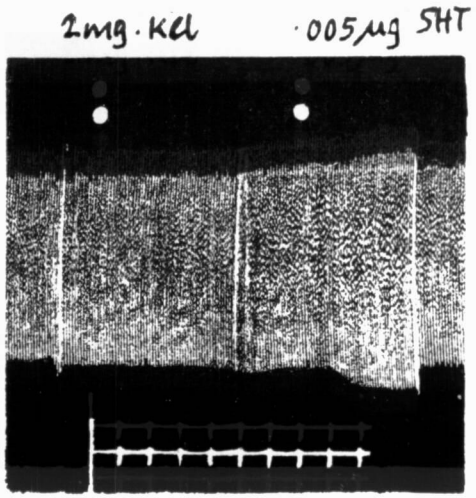

Fig. 5

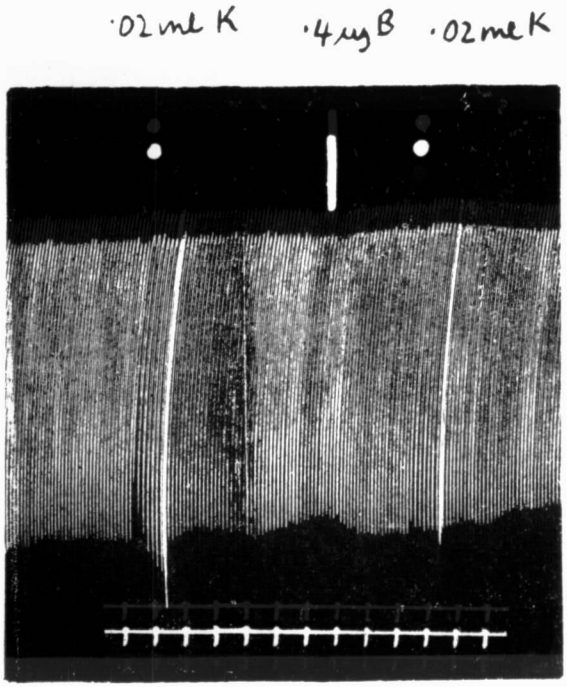

Fig. 6

The extract K was from 30 leaves made up to 5 ml lagoon water

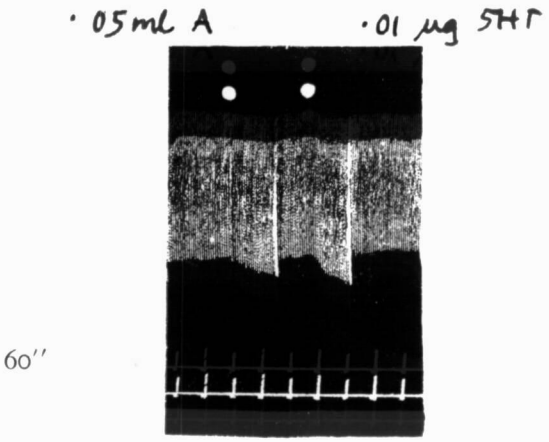

Fig. 7

The extract was equal to 0.5 G. banana per ml

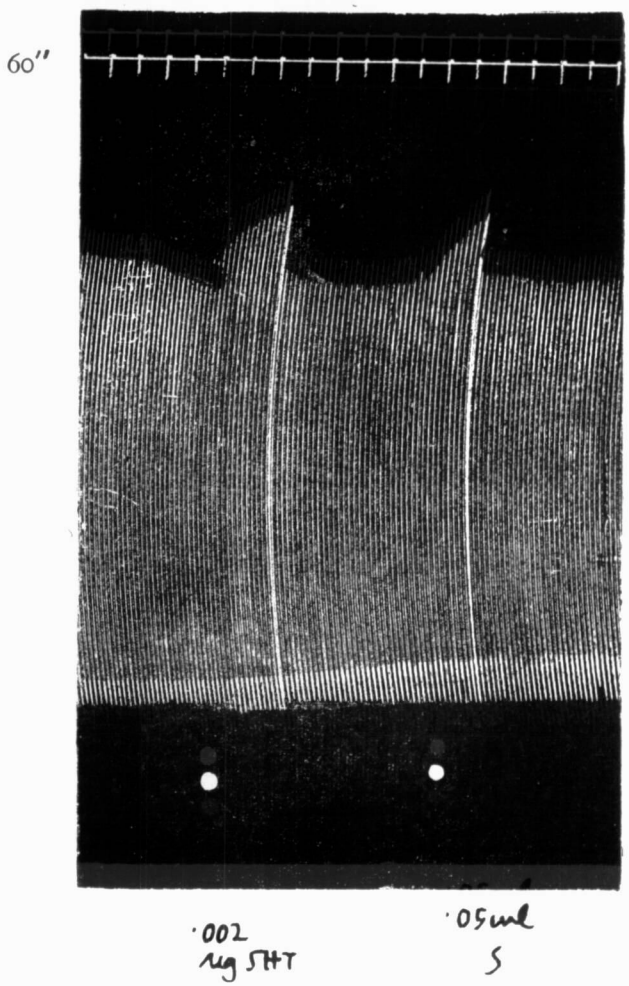

Fig. 8