STUDIES ON INTERNAL SECRETION III.—THE ACTION OF PITUITARY EXTRACT AND ADRENALINE ON CONTRACTILE TISSUES OF CERTAIN INVERTEBRATA.*

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1. Introduction.

The present investigation was undertaken by the senior author in the first place to shed further light on two questions which emerged in the course of investigations on the rôle of the pituitary in the regulation of colour response (Hogben and Winton, 1922-23, Hogben, 1923-24). These were: (*a*) how far the action of pituitary extract is a specific one for the uterus or extends to contractile tissues generally; (*b*) with what justification the action of adrenaline on an effector organ may, in the absence of direct evidence, be regarded as indicative of sympathetic innervation. Study of the action of vertebrate autocoids on the contractile tissues of Invertebrata was therefore undertaken; and after preliminary experiments directed to select suitable materials it was decided to confine attention to the isolated heart of the crab (*Maia squinado*), the perfused heart of the scallop (*Pecten maximus*), the isolated crop of the sea-slug (*Aplysia*), and the isolated pharynx of the sea-mouse (*Aphrodite*). The first was suggested by Carlson's work (1905) on Limulus, the only arthropod in which an isolated heart

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appears to have been employed for experimental purposes. The technique of perfusion and recording of the heart rhythm in the Lamellibranch mollusc Pecten is described by Mines (1912). Brucke (1905) first drew attention to the suitability of the crop of the Gasteropod for the study of muscular contraction. The rhythmical activity of the pharynx in annelids is well known, though we are not aware that isolated preparations have been made by previous workers. Aphrodite was selected as the largest representative of the phylum obtainable at Plymouth.

Though a careful study of the action of electrolytes on the heart of Pecten has been made by Mines, and of the action of drugs on the heart of Helix by Lovatt Evans (1912), the crop of Aplysia by Straub (1907), and a wide range of invertebrate hearts by Carlson (1905-7), there have been hitherto very few observations relating to the effect of vertebrate autocoids on invertebrate muscle. Carlson mentions the fact that adrenaline has an excitatory action on the heart of Limulus but does not give graphical records. Brucke and Satacke (1912) refer to a single experiment on the effect of adrenaline on the blood-pressure of the lobster. Elliott (1904) describes a single experiment with negative results on the heart of the crayfish. Gaskell (1918) obtained indications of excitatory action of adrenaline on the contractile blood-vessels of the leech. Gaskell has also found in the central nervous system of those annelids possessing contractile blood-vessels, cells that give the chrome-staining reaction of Henle. But since the method employed by him to test the physiological activity of extracts prepared from these ganglia was not altogether conclusive, the existence of adrenaline-secreting cells in the invertebrate phyla remains an open question.

2. Methods.

In experiments on the heart of Pecten a glass vein-tube was inserted and tied with silk in the auricle as suggested by Mines, whose method was supplemented by a simple device described by Shanks for the maintenance of a constant head of pressure (fig. 1). Two reservoirs for control and experimental fluids were connected by rubber tubing with screw clips

to a T-piece inserted in the mouth of a perfusion cannula connected with the vein-tube and provided with a lateral limb, which, acting as an overflow, maintains the fluid contained therein at the same level, when the clips are adjusted. By adjusting the screw clips the fluid being perfused can be changed without any disturbance of pressure in the auricle. The ventricle was connected by a silk thread furnished with a glass hook to the recording lever. In the experiments on Maia, Aplysia, and Aphrodite a modification of the uterus bath was employed as indicated in the diagram, the saline medium

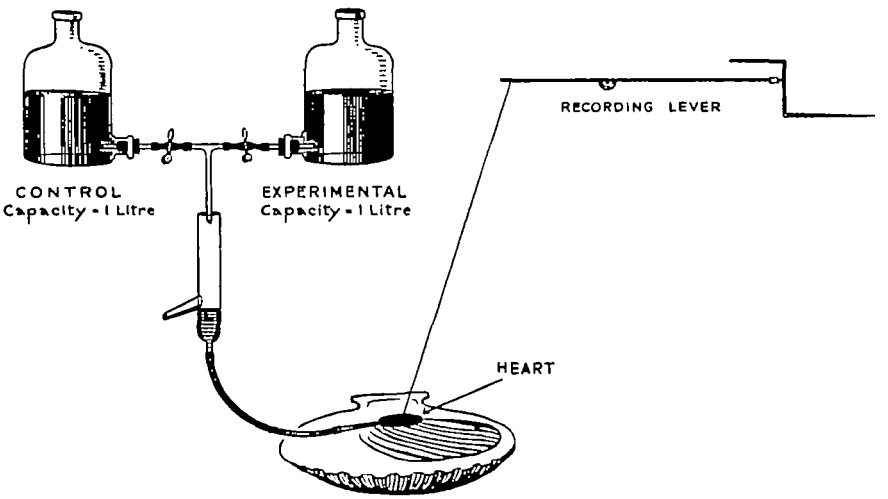

FIG. 1.—Perfusion of Heart of Pecten.

being aerated in all experiments (fig. 2). Change of the saline was effected either by adjusting the clips and taps leading from the reservoirs to the muscle bath, or by adding with a pipette to a known quantity of the fluid in the latter a measured amount of the reagent. In some experiments a frontal writing point was employed, in others the older type of recording lever.

The saline media whose formulæ are given below are purely empirical. The preparations were selected because they would show isolated rhythm in sea-water of the appropriate reaction. By varying the proportions of the constituents of artificially prepared sea-water as a basis, it was possible to prescribe media in which a more regular and protracted

rhythm could be maintained. All the reagents which were employed were made up in the same media as that in which the preparations were perfused, and always brought to the same P_H by addition of Na_2CO_3 or Sörensen's standard Na_2HPO_4 buffer solution. It proved convenient to employ a uniform P_H in all experiments, all testing and perfusing fluids being made up to P_H 6.8 to 7.0 on the Brom-thymol-blue series. The preparation of adrenaline employed was that made in 0.001 g. tabloids by Messrs Burroughs Wellcome. All the

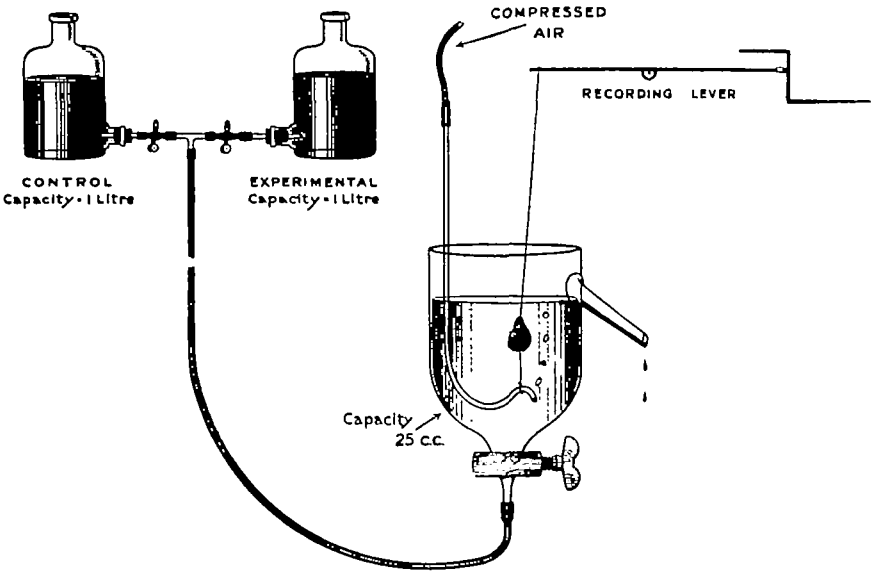

FIG. 2.

experiments with adrenaline were supplemented by testing with the closely allied base epinine (BW). Two samples of pituitary extract were used; Burroughs Wellcome's "Infundin" and a laboratory product specially prepared for researches (Hogben, Schlapp, and Macdonald) on the quantitative comparison of pressor activity. The latter was a sterile saline extract of ox glands collected while still warm in ice-cold acetone and extracted for six hours with alcohol after desiccation. Suprarenal extracts were made from ox glands in the appropriate fluid acidified with glacial acetic and subsequently neutralised. The experiments on the action of adrenaline were repeatedly performed in every case.

3. Experimental Data.

The action of adrenaline and of pituitary extract was tested in all the preparations employed, and the results were singularly uniform. Reference may best be made to the characteristic features of each by describing them individually.

a. **The Heart of Maia.**—In sea-water properly aerated and reduced to a suitable P_H by addition of HCl the isolated heart of the spider crab beats with a somewhat irregular rhythm and with a frequency which varies very considerably in different individuals. The authors are not aware that experiments on the isolated Crustacean heart have hitherto been made. The actual formula for the perfusion of the heart was in proportions by volume of $\frac{5}{8}$ molar solutions, NaCl 200, $MgCl_2$ 30, KCl 0.5, $CaCl_2$ 2, dextrose 1.5, with Na_2HPO_4 to P_H 7.0.

In this medium the heart would beat with regularity and with little diminution of amplitude for two or more hours. For excising the heart the following procedure was adopted. The dorsal surface of the carapace is removed by means of a stout pair of scissors and carefully separated from the subjacent pigmented dermis. The latter is then dissected from the roof of the pericardium, exposing a powerful muscular heart with its two large pairs of ostia. Before further dissection the pericardium is immediately washed out with the perfusing fluid, and glass hooks for suspending the preparation in the perfusion bath are inserted in the heart muscle at the anterior and posterior extremities. When the tendons suspending the heart have been severed along with the large arteries, it is ready to transfer to the bath. Perfusion of the heart is ensured by the opening of the ostia.

The action of adrenaline, 1/40,000, of suprarenal extract 2 per cent. and of epinine 1/400,000 repeatedly gave uniform results. There was a remarkable acceleration of the rhythm accompanied by increased tonus (figs. 3 and 4). The latent period between the addition of the autocoid and the onset of the characteristic response was usually somewhat protracted, a minute at least intervening in most experiments (fig. 4). As a similar delay is seen in the action of other reagents (*e.g.* excess of KCl), and since the heart continues to beat for

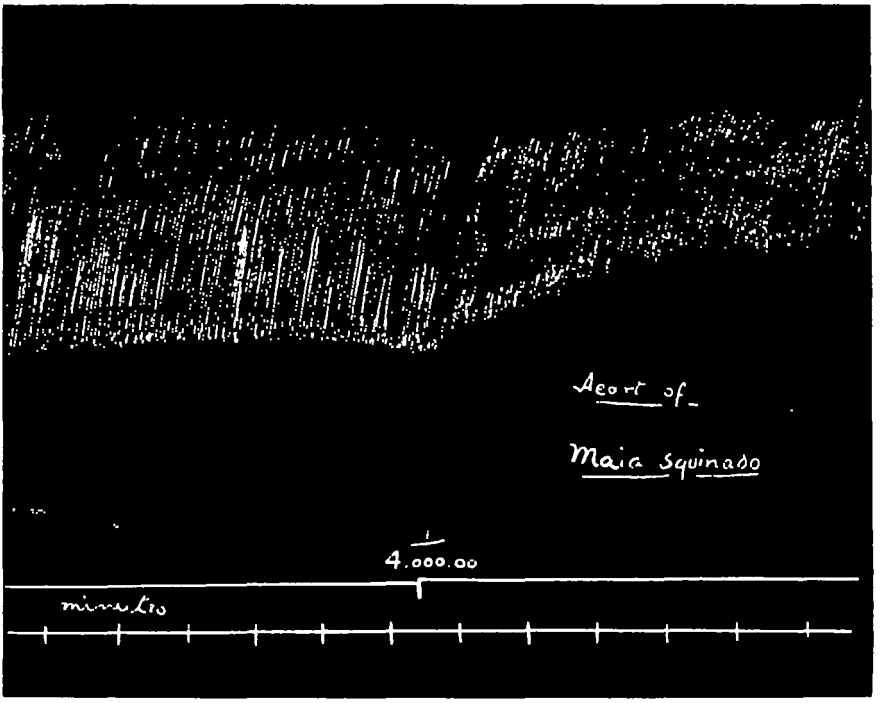

FIG. 3.—Action of Epinine (Burroughs Wellcome) on the Isolated Heart of the Spider Crab (Maia).

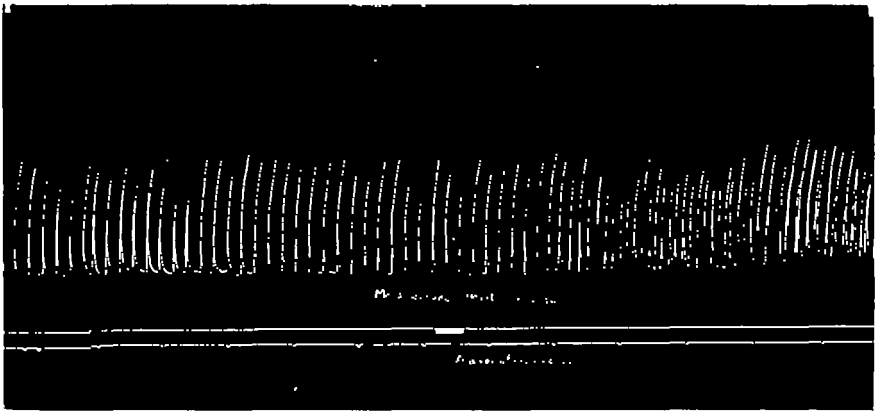

FIG. 4.—Action of Adrenaline on Heart of Maia. (Time interval one minute.)

more than half a minute when transferred to a fixing reagent such as Bouin's fluid, it is probable that this is due to the slow rate at which the autocoid is absorbed by the heart muscle. Another circumstance seen in fig. 4 is the multiphasic character of the accelerated rhythm produced by adrenaline. Other reagents which influence the rate of beat were found to affect the regularity of the rhythm, and this is possibly attributable to differential action on the contractile elements which do not appear to form a continuous myocardium. Pituitary extract (depressor-free) in concentration equivalent to 2 per cent. of the fresh gland (ox) substance evoked no response in the heart of *Maia squinado* under the conditions in which this investigation was carried out.

b. **The Heart of Pecten.**—As Mines (1912) has previously shown, the heart of the bivalve Pecten will beat with regularity if perfused *in situ* with fresh sea-water at a $P_H = 7.0$. It was not therefore thought necessary to employ an artificial medium for testing the action of pituitary extract and adrenaline.

Pituitary extract in the same concentration as above (2 per cent.) produced no modification of the heart-beat in these experiments. Adrenaline and epinine in concentrations of 1/500,000 evoked a very characteristic response. A marked increase in tone with final arrest of the heart in systole is well seen in figs. 5 and 6. With very dilute solutions marked acceleration of the slowly beating heart was found to follow perfusion with adrenaline or epinine, but the acceleration was in general obscured by the immediate tonic contraction of the ventricle. It is to be noted in connection with the theoretical aspect of the question that according to Carlson's (1905) observations the innervation of the heart in the Lamellibranchs is of a purely inhibitory character.

c. **The Crop of Aplysia.**—Brucke made observations on contraction of the isolated crop of the Gasteropod Aplysia using a plethysmographic method of recording. In these experiments the anterior third of the crop was prepared in a manner analogous to the course usually adopted with the guinea-pig's intestine or pig's ureter, the segment being ligated with silk at each extremity and connected by one end with the writing lever. The isolated crop of Aplysia will often beat for

a considerable time—twenty-four hours—in sea-water. The rhythm in this medium is, however, a very irregular one. For maintaining a rhythm of more uniform amplitude and frequency,

FIG. 5.—Heart of Pecten—Action of Adrenaline. At first signal perfusion fluid changed from sea-water at $P_H = 7.0$ to $P_H = 6.8$. Time interval one minute.

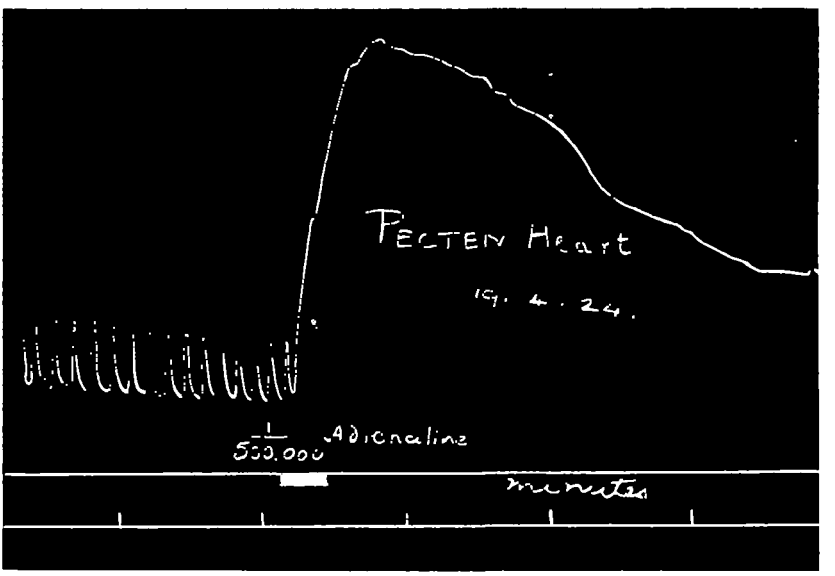

FIG. 6—Heart of Pecten—Action of Adrenaline.

a formula found to be satisfactory for this investigation was in proportions by volume of $\frac{5}{8}$ molar solutions, NaCl 100, $MgCl_2$ 20, $CaCl_2$ 2, KCl 5, dextrose 3, with Na_2HPO_4 to P_H 7.0.

As in the experiments with the heart of Maia the action of adrenaline and epinine on the isolated crop of the sea-slug were supplemented with tests on the action of 2 per cent. suprarenal extract. In each case the characteristic action was excitatory and comparable with the effect of adrenaline on the uterus of the mammal. Adrenaline produced maximal contraction of the crop of Aplysia in dilutions of 1/50,000. In the case of epinine

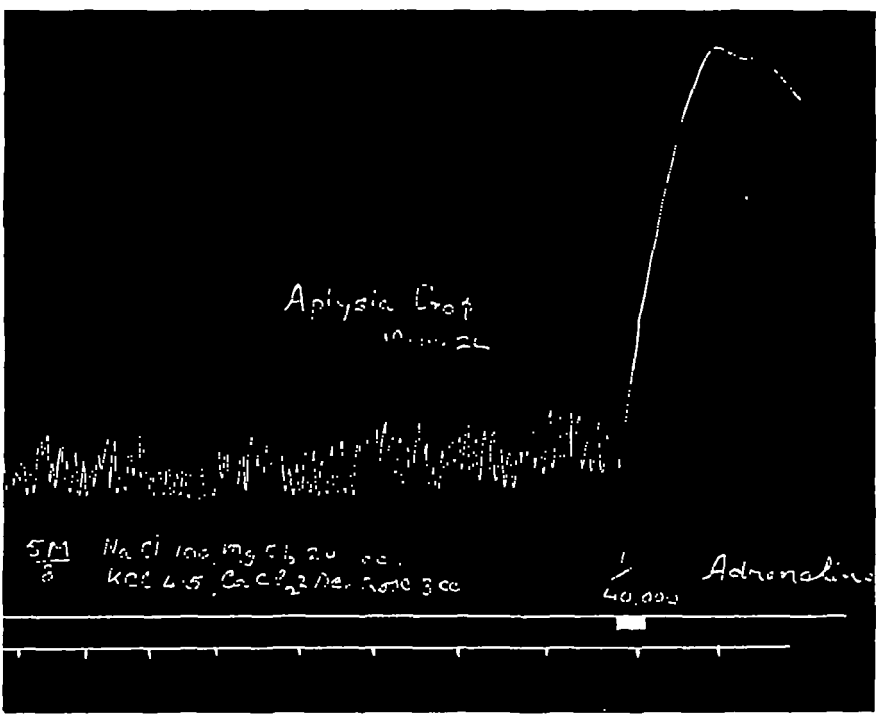

FIG. 7.—Action of Adrenaline on the Crop of Aplysia.

a series of six experiments showed uniformly maximal contractions with dilutions of 1/1,000,000. In another experiment maximal response was obtained with a dilution of 1/10,000,000 and perceptible effects were obtained subsequently with 1/25,000,000. Epinine is stated by the manufacturers to be ten times less active than adrenaline: presumably this refers to experiment on mammals. Though the minimal dose for adrenaline was not determined with accuracy, there is little doubt that adrenaline is not more active than epinine in its effect on the crop of Aplysia.

An interesting point, referred to below, was noted in experiments with pituitary extract. The depressor-free extract evoked no response in concentrations of 2 per cent. Corresponding dosage of commercial extract (B.W. Infundin) which contains the depressor constituent produced a definite increase of tone, a result which could also be produced by histamine.

d. **The Pharynx of Aphrodite.**—The pharynx of Aphrodite is a powerfully muscular structure which contracts with a slow rhythm in sea-water. In a few experiments the entire organ was placed in the bath, but to simplify the analysis of the records it was subsequently decided to make use of transverse rings about half a centimetre in width. The saline medium which was ultimately employed was prepared thus: in proportions by volume of $\frac{5}{8}$ molar solutions NaCl 260, $MgCl_2$ 48, KCl 24, $CaCl_2$ 40, with Na_2HPO_4 to P_H 7.0. Adrenaline and epinine in dilutions of 1/50,000 to 1/200,000 produced acceleration of the contractile rhythm with increased tone. The actual limits of sensitivity were not determined. No effects were registered in experiments carried out to test the action of pituitary extract.

4. Discussion.

Two points of general interest arise out of the observations recorded in this communication :—

a. **Action of Adrenaline.**—The materials investigated show considerable diversity both from the histological and phyletic standpoint, and it may be questioned whether in future great importance should be attached to the action of adrenaline as an indicator of the presence of a sympathetic nervous supply in the absence of direct evidence. It is true that Gaskell's speculations leave the way open for an interpretation which would conserve a functional relation between the action of adrenaline and of sympathetic neurones. Since Anderson and Elliott have shown that the effects of pilocarpine and adrenaline are not vitiated by degenerative section of parasympathetic and sympathetic nerves, it has only been possible to conserve the idea that such reagents act on the motor end-organs by postulating a hypothetical myoneural junction supposed to be

localised beyond the visible nerve endings. It now appears that the action of adrenaline is much more widely spread than has been recognised in the past, and it is perhaps legitimate, therefore, to comment upon a doctrine which, if less generally accepted than was formerly the case, is still current in physiological literature. The fact that drugs like atropine and apocodeine exclude both the action of pilocarpine and adrenaline on the one hand and of parasympathetic and sympathetic stimulation on the other, naturally suggests that both effects are referable to some common process inherent in the contractile mechanism, but there seems no need to envisage the common factor as structural in the morphological sense or a separate chemical unit as implied in the term "receptor substance." Perhaps it may even be questioned whether such terms as *parasympathomimetic* and *sympathomimetic* would have been employed extensively, if the recognition of the action of muscarine on the heart of Helix and of adrenaline on the crop of Aplysia had preceded that of the action of pilocarpine on the heart of the frog and of adrenaline on the ileum of the cat.

b. **Action of Pituitary Extract.**—Dale, who discovered the action of pituitary extract on the uterus of the mammal and introduced the oxytocic method of standardisation was not able to record a corresponding effect on the isolated mammalian gut or bladder. Several later investigators have, however, claimed that pituitary extract is a general excitant of plain muscle, especially in the case of the intestine. The experiments on which this conclusion is founded are either lacking in quantitative treatment, as in the recent work of Dixon (1922), or suggest like the experiments of Young (1915) that pituitary extract only exerts its action in quantities that could have no physiological significance, and might well be referred to the presence of plain muscle excitants found in other tissue extracts and in blood. It has recently been emphasised (Hogben and Schlapp; Hogben, Schlapp, and Macdonald) that the depressor component of pituitary extract is like histamine in its physiological behaviour. Experiments carried out by Dr Macdonald (not yet published) showed that depressor-free extracts of the pituitary have no appreciable excitatory action on the intestine, that the depressor residue like histamine acts powerfully on the

gut, and that *fresh* extracts only produce effects in quantities commensurate with those in which other tissue extracts behave similarly. It has been pointed out elsewhere (Hogben and Schlapp) that the effect of pituitary extract on arterial rings has not yet been studied with depressor-free extracts. Until

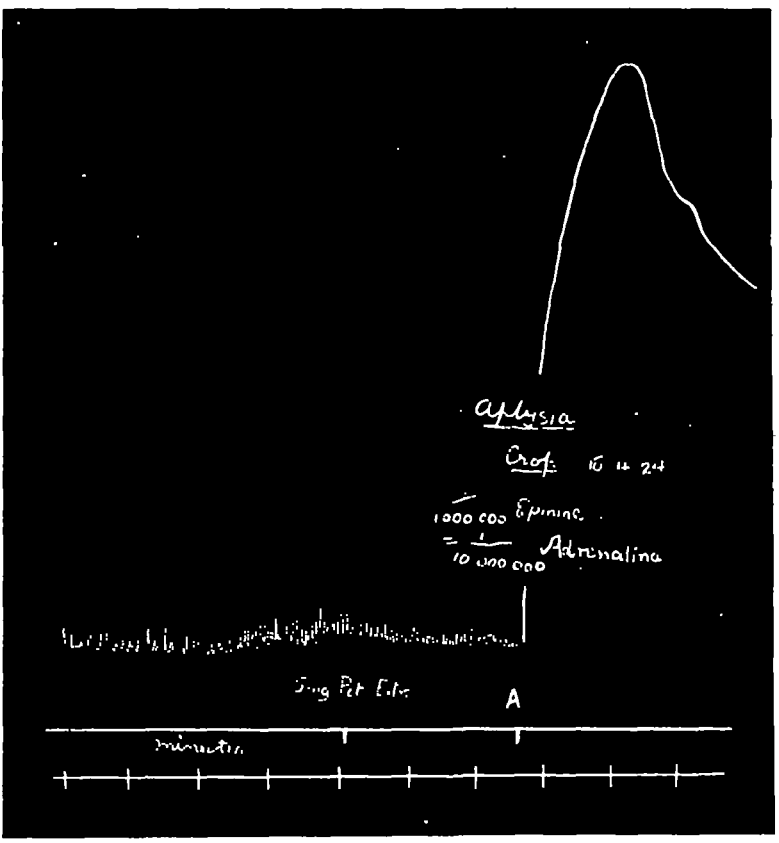

FIG. 8.—Action of Pituitary Extract and Epinine on the Crop of Aplysia. At first signal 5 mg. desiccated depressor-free ox pituitary substance was added to the bath (40 c.c. capacity). At A one in a million epinine. Epinine is stated to be one-tenth as active as adrenaline in the mammal.

comparative quantitative comparisons of the action of fresh and depressor-free extracts of the pituitary and other tissue extracts have been carried out simultaneously on the uterus and on other forms of plain muscle, there is inadequate evidence to support the view that pituitary extract acts specifically on plain muscle other than uterine. It is therefore not surprising that depressor-free extracts in the quantities employed in these

experiments produced no effect on the contractile structures which have been described, nor that large doses of commercial pituitary extract had, like histamine, a slight excitatory action on the crop of Aplysia.

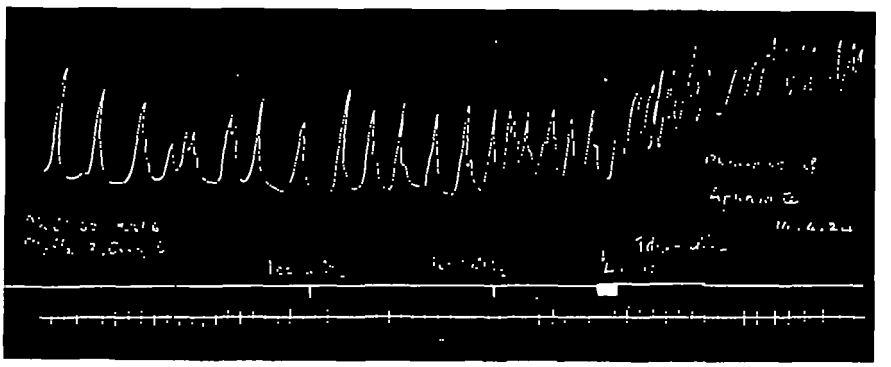

FIG. 9.—Action of Calcium and Adrenaline on the Pharynx of Aphrodite.

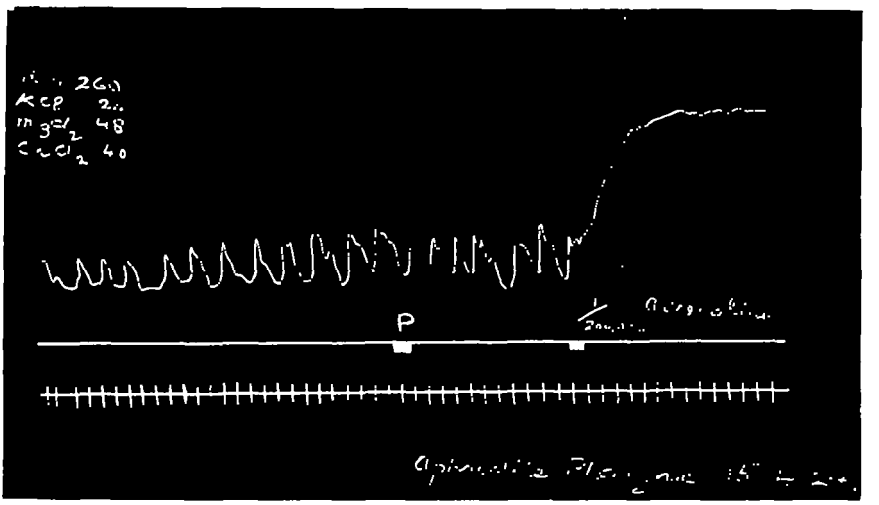

FIG. 10.—Action of Pituitary Extract and Adrenaline on the Pharynx of Aphrodite. At P 5 mg. desiccated (depressor-free) ox pituitary in 40 c.c. At second signal adrenaline $\frac{1}{1000000}$.

5. Summary.

1. The action of adrenaline and pituitary extract has been studied on the isolated heart of the crab *Maia*, the perfused heart of the bivalve *Pecten*, the isolated crop of the Gasteropod mollusc *Aplysia*, and the isolated pharynx of the annelid *Aphrodite*.

2. Methods for the study of the physiology of rhythmical contraction in these invertebrate preparations are described.

3. Pituitary extract in quantities comparable with those which excite the mammalian uterus was not found to have any action on invertebrate muscle.

4. Adrenaline (and epinine) in all cases produced a very pronounced increase in tone accompanied (in Maia, Aphrodite, and Pecten) with acceleration of the normal rhythm.

5. The specificity of the oxytocic action of pituitary extract and the parallelism between the action of adrenaline and of sympathetic excitation in vertebrates are discussed.

It is a pleasure to express the very great debt which the authors owe to Dr Allen and the staff of the Marine Biological Laboratory at Plymouth, who spared no pains in placing every available facility at their disposal. Acknowledgment is also due to the British Association and the University of London for the use of a table in the Laboratory. The expenses of the research were defrayed by a grant from the Government Grants Committee of the Royal Society.

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