# STUDIES ON THE PITUITARY

## VII. THE SEPARATE IDENTITY OF THE PRESSOR AND MELANOPHORE PRINCIPLES.

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(With Seven Text-figures.)

### I. INTRODUCTION.

In the course of experiments undertaken in this department to throw further light on the co-ordination of pigmentary changes in Amphibia, various indications have arisen to suggest that the pressor and melanophore components of extracts of the pituitary gland are not one and the same substance. The separate identity of the pressor and oxytocic components first indicated by Dale and Dudley (1921) has now been established beyond reasonable doubt by the work of Schlapp (1925) and of Draper (1926) who have availed themselves of a method of pressor assay devised by Hogben, Schlapp and Macdonald (1924) to permit comparison with a precision of the same order as that of Dale's oxytocic standardisation. By the methods of separation used by Schlapp and a third method used by Dreyer and Clark (1924), it is not possible to state with certainty that the melanophore and pressor activities are referable to separate substances. Dale and Dudley have shown that the oxytocic and pressor substances are destroyed by normal soda in the cold. Smith (1924) has recorded that this is not the case with the melanophore stimulant. But no final conclusion can be drawn from this discrepancy, until we have at our disposal a satisfactory method of assay for the melanophore principle, and have carried out simultaneous pressor and melanophore assays on the same samples subjected to the same treatment. With the aid of a new method for the assay of the melanophore principle, we have carried out experiments with this end in view.

### II. THE STANDARDISATION OF MELANOPHORE ACTIVITY.

Methods employed to assay the melanophore activity of extracts of the pituitary are based on the determination of the minimal dose requisite either for whole animals, as originally proposed by Hogben and Winton (1922), or the perfused limb (Fenn, 1924). In experienced hands this method is capable of giving consistent results, but the order of precision is questionable.

a further analysis of the control of Amphibian colour change, Slome and Hogben have shown that colour change can be described quantitatively by assigning arbitrary numerical symbols to different configurations of the pigmentary effector organs with very consistent, and illuminating, results, as compared with crude observation of macroscopic changes; and the use of this method has made it possible to analyse the time relations of pigmentary response in *Xenopus laevis*, the South African clawed toad. By adopting this procedure it has been shown that colour change in *Xenopus* (and probably all Amphibia) is regulated by two distinct endocrine systems, one being the secretion of what has hitherto been called the melanophore stimulant of the pituitary, and the other being connected with the anterior lobe of the same gland. This investigation has involved comparison of the behaviour of operated and normal animals treated with different quantities of pituitary extracts; and the present method of assay was first devised for that purpose, but has not been described hitherto.

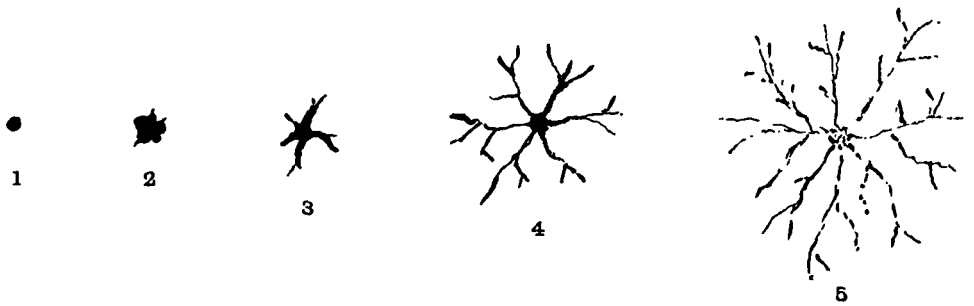



Fig. 1.

The melanophores of *Xenopus* lend themselves more readily to symbolical description than those of *Rana*, and its chromatic reactions are more easy to control. The use of this method in class work has shown that observations of different and inexperienced observers lead to surprisingly consistent results. The scale employed is indicated in Fig. 1. In carrying out an assay it is first necessary to find roughly the threshold dose for a submaximal effect, and then to proceed with matching the samples to be compared in quantities round about 20 per cent. less than it. Curves showing the intensity against the duration of the response are then plotted as follows.

For constructing a curve six animals which have been placed in optimum conditions for pallor till equilibrium is attained are injected with the same quantity of test fluid. The ordinates represent the mean values of six individuals whose melanophores have been placed on the scale indicated in Fig. 1. Needless to say the web of the hind foot is used for this purpose. Observations are repeated hourly for the first 6 hours, and additional records may be taken at 12 and 24 hours after injection. Comparison of the duration intensity curves of two samples provides two criteria for relative potency; and thus gives a much higher order of discrimination than can be obtained by methods based upon the minimal dose. In Fig. 2 three curves each based on six medium-sized males weighing 35·2 gm. are shown. One series

was injected with a dilution of 1 : 10 of a pituitary extract subject to the treatment described below for destroying pressor activity, the other two curves are based on batches injected respectively with a dilution of 1 : 15 and 1 : 20 of identically the same solution. A fourth batch injected with 1 : 30 gave hardly any response at all. The figure shows consistent discrimination between the various doses, and a much finer sensitivity would have been obtained, if working within the range 1 : 30 to 1 : 20. In the hands of a novice the method gives convincing results to an order of 20 per cent., but it is capable of achieving a much higher refinement with experience.

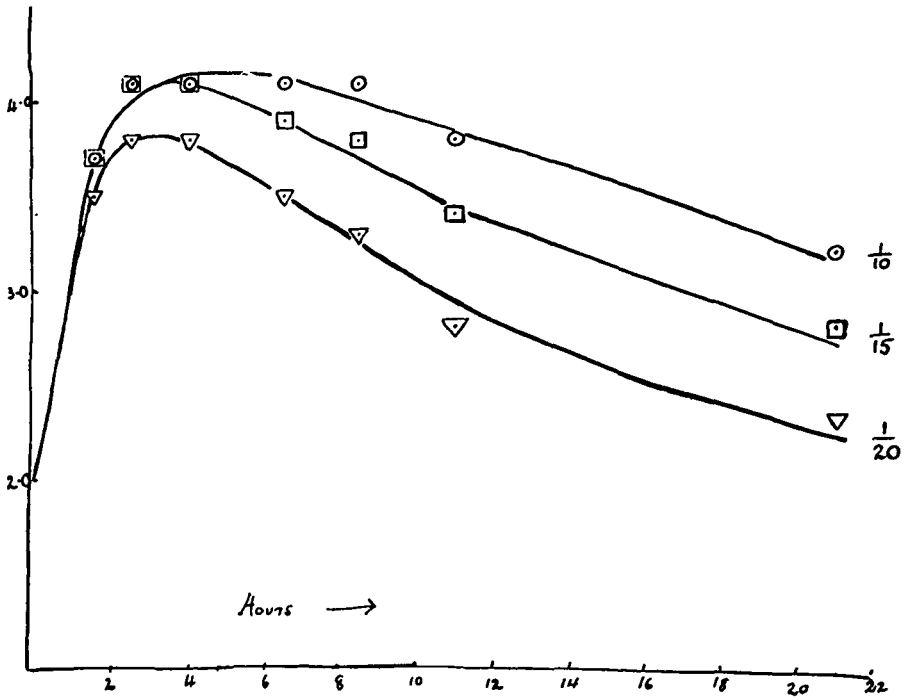



Fig. 2.

## III. COMPARISON OF MELANOPHORE AND PRESSOR ACTIVITY.

A comparative assay of pituitary extracts before and after treatment with cold sodium hydroxide was carried out with two types of extracts.

(1) An aqueous extract made from fresh posterior lobes of the ox, sterilised by boiling was prepared in the laboratory. The original solution contained 7 gm. of fresh substance in 20 c.c. To this 20 c.c. of 2·7 molar sodium hydroxide was added, and the mixture divided into two equal portions *A* and *B*. *A* was neutralised immediately with the necessary quantity of molar hydrochloric acid, and *B* was allowed to stand for 3 hours before neutralisation. A first rough assay of the melanophore activity of the two samples was made on batches of three toads, three

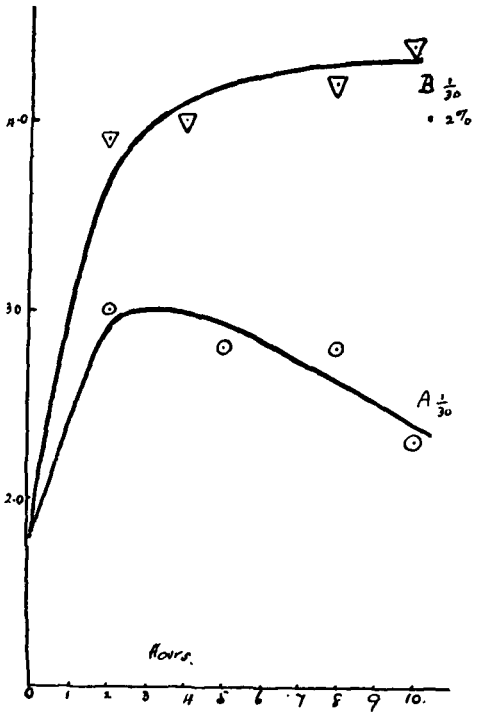
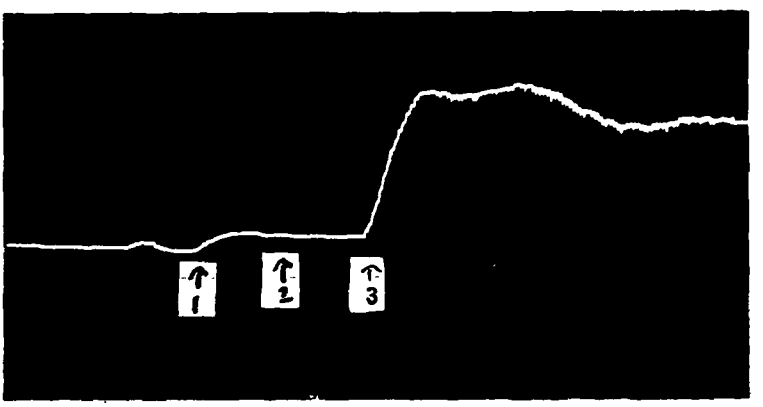



Fig. 3. Same sample as Figs. 4 and 5.




At 1, 1 c.c. *B* + 1 c.c. saline. At 2, 2 c.c. saline. At 3, 1 c.c. *A* + 1 c.c. saline.

Fig. 4. Same sample as Fig. 3. Spinal cat carotid, b.p.

dilutions of each solution being employed. The results referred to the same in Fig. 1 are given in Table I, time being measured in hours.

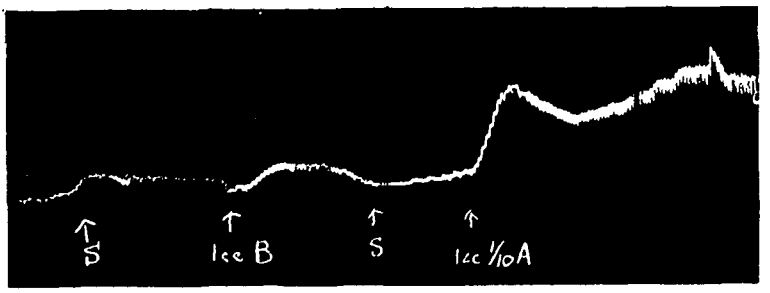
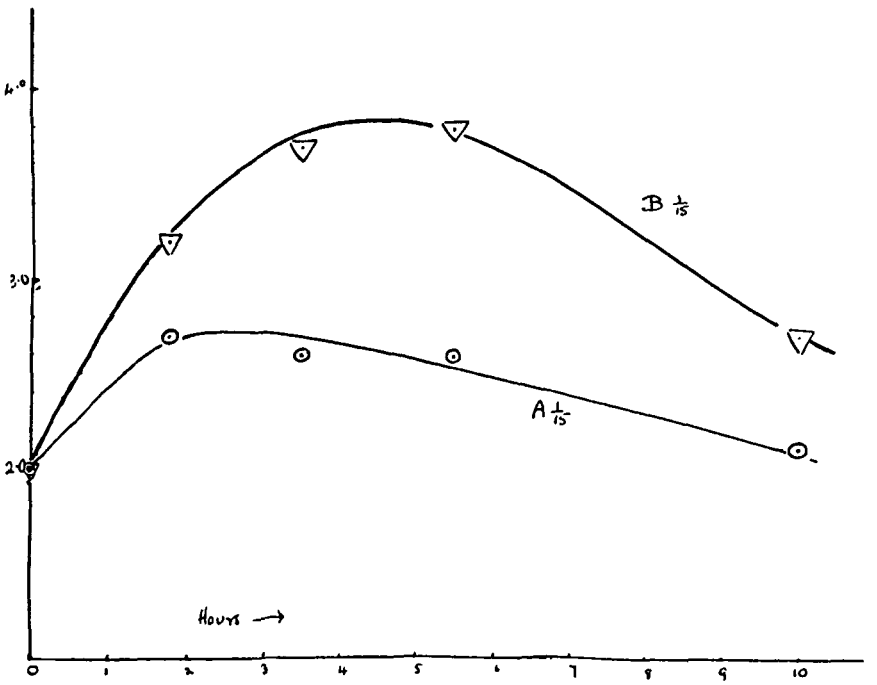



Fig. 5. Same sample as in Figs. 3 and 4. Cat (spinal) carotid, b.p.



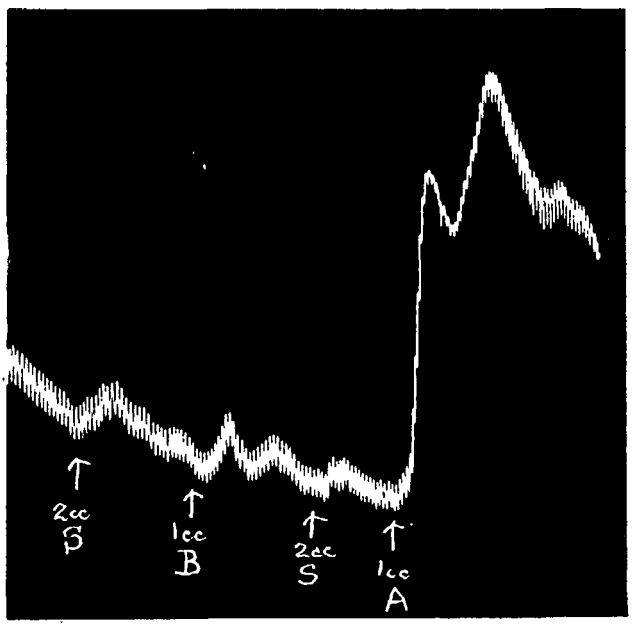

Dose = ·26 pressor unit.

Fig. 6. Parke Davis "pitressin."

In using small batches of animals as in the foregoing experiment experienced workers will have no difficulty in obtaining results consistent with larger samples, if intermediate stages of 2·5, 3·5, etc., on the scale of Fig. 1 are employed.

Table I.

| Time (hours) | Dilution | | | | | |
|---|---|---|---|---|---|---|
| | 0·7 % | | 0·07 % | | 0·007 % | |
| | *A* | *B* | *A* | *B* | *A* | *B* |
| 0 | 2·0 | 2·0 | 2·0 | 2·0 | 2·0 | 2·0 |
| 2 | 4·0 | 4·0 | 3·5 | 4·0 | 2·5 | 2·5 |
| 5 | 4·5 | 4·5 | 3·5 | 4·5 | 2·5 | 2·5 |
| 10 | 3·0 | 4·5 | 2·8 | 4·0 | 2·0 | 2·5 |
| 15 | 2·5 | 4·5 | 2·0 | 3·8 | 2·0 | 2·5 |
| 21 | 2·5 | 4·25 | 2·0 | 3·8 | 2·0 | 2·5 |
| 26 | 2·0 | 3·5 | 2·0 | 2·5 | 2·0 | 2·0 |




*A* 1 c.c. = 4 pressor units.

Fig. 7. Parke Davis "pitressin." Spinal cat carotid b.p.

The final comparison is given in Fig. 3, each curve based on six individuals. From this it is seen that the melanophore activity of a sample exposed to the action of 1·35 molar sodium hydroxide for 3 hours increases. This result is easily explicable in the light of what ensues: for the constriction of the vessels by the intact pressor substance of sample *A* will render access of the melanophore stimulant to the melanophores more difficult. Two pressor tests were carried out on the same days as the above, the spinal cat being used. In Fig. 4 it is seen that 1 c.c. of *B* h[illegible]o effect, while 1 c.c. of *A* gives a pronounced one. In Fig. 5 it is seen that a very definite reaction to one-tenth of a c.c. of *A* is given. It follows that more than

nine-tenths of the pressor activity is destroyed by exposing the extract to the action of 1·35 molar sodium hydroxide at room temperature.

(2) The next comparison was made with the separated pressor extract supplied by Parke Davis under the commercial name "pitressin." This does not contain the oxytocic principle in appreciable quantity: "pitocin" the separated oxytocic extract of the same firm has little melanophore activity. Two solutions *A* and *B* were treated in a manner exactly analogous to *A* and *B* in the preceding series of tests, *i.e.* *A* was not subjected to prolonged action of soda and *B* was. The melanophore assay is shown in Fig. 6, the dose being 1 c.c. of a 1 : 15 dilution of the solution used for pressor assay (Fig. 7). Again *A* gave a positive and *B* a negative pressor reaction in all doses tested. The results obtained with commercial samples of concentrated pressor extract are thus in entire agreement with those obtained with the laboratory preparations.

## IV. CONCLUSIONS.

1. When pituitary extracts are subjected to prolonged action of sodium hydroxide above normal concentration the destruction of pressor activity is accompanied by an appreciable increase in melanophore activity.

2. The increase in melanophore activity is easily explicable, because constriction of the vessels by the pressor component would tend to mask the activity of the melanophore stimulant.

3. There is thus adequate proof for the view that the melanophore, pressor and oxytocic activities of pituitary extracts are referable to distinct entities.

In using the term entity one must not eliminate the possibility that these three activities are referable to different radicles in the same molecule, since the experimental results are not incompatible with the view that the separation of the three activities involves the fragmentation of a complex molecule. In the absence of direct evidence for this alternative, it is an economy of hypothesis to conclude that three separate autacoids exist in the gland.

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