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THE ACTIVE PRINCIPLES OF THE POSTERIOR LOBE OF THE PITUITARY BODY. By WALTER SCHLAPP. From the Department of Physiology of the University of Edinburgh. (With fifteen figures in the text.)

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THE ACTIVE PRINCIPLES OF THE POSTERIOR LOBE OF THE PITUITARY BODY.

By WALTER SCHLAPP.[1] From the Department of Physiology of the University of Edinburgh.

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CONTENTS.

1. INTRODUCTION.

IN recent years controversy has centred round the question of whether the many physiological responses known to be evoked by extracts of the posterior lobe of the pituitary body are to be attributed to one or to more than one active principle. The solution of this problem has been sought along two lines. The first method, which consists in attempting to localise the sources of the active principles in the histologically distinct parts of the posterior lobe, has been followed by HERRING, who states that the oxytocic and pressor substances can be traced to different parts. This statement has recently been confirmed by HOGBEN and DE BEER. The second method, which seeks to separate from extracts by physical or chemical means fractions which produce one form of response to a preponderating degree, has been tried with varying results by a number of investigators, and since it is along such lines that the investigations recorded here have proceeded, it seems desirable to give a brief review of the results which have hitherto been published.

These results fall naturally into two categories: one category is in favour of the view that a single substance is responsible for all the physiological activities of posterior lobe extracts, and another appears to indicate that these activities are manifestations of the presence of more than one active principle. The former view seems to be the prevalent one in

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America, and finds its chief support in the work of ABEL and his collaborators. In the course of certain experiments designed to isolate an active substance in a pure form from extracts of the posterior lobe of the pituitary body, ABEL has carried out a variety of chemical operations, consisting largely of precipitations, with the view of eliminating impurities from the extracts. He has found that it is possible to isolate a fraction having an oxytocic activity 1000 to 1250 times greater than that of histamine, which also exhibits melanophore, diuretic, and pressor activity in a marked degree. From the fact that the activities are manifested to a progressively increasing extent by the fractions as they are purified, he argues that they must be attributed to one and the same substance. Apart from the fact that the results argue only for the possibility of such an assumption, it is significant that the pressor response of Abel's purified preparation is characterised by an inversion effect on second injection. The observation of SCHAFER and VINCENT, who showed that it is possible to separate the depressor substance from posterior lobe extracts by alcoholic extraction, has, however, been recently confirmed by HOGBEN and SCHLAPP, who have emphasised the similarity of the response evoked by this substance to that produced by histamine in both the decerebrated and anæsthetised carnivore. Since this substance is obviously present in ABEL'S most purified preparation, the possibility still remains that the oxytocic, melanophore, and diuretic activities are also manifestations of distinct substances which the precipitation procedure has failed to separate. The view that the pressor and oxytocic responses are attributable to a single substance has, however, been independently advanced by SMITH and M'CLOSKY, who have submitted extracts to the process of diffusion. They were able to show that the ratio of oxytocic to pressor activity does not change during diffusion through collodion sacs. They have also shown that these two activities diminish at the same rate in extracts which have been submitted to temperatures above normal for varying periods of time.

The second of the two views mentioned above has been supported largely by DALE and his collaborators. The contention advanced by these workers is that if a single method exists by which it is possible to separate any two of the active principles, no further demonstration of their divergence is necessary. DALE and DUDLEY submit extracts of posterior pituitary to continuous extraction with butyl alcohol. The major part of the oxytocic activity passes into the butyl alcohol, while only about 50 per cent. of the pressor activity goes along with it. Experiments by FENN, who applied this and other methods to the pressor, oxytocic, and melanophore substances, confirm these results, and FENN further states that the melanophore stimulant constitutes a third active principle. That this latter differs from the oxytocic principle seems to have been clearly demonstrated by DREYER and CLARK, who showed

that the melanophore principle is retained during ultrafiltration through collodion membranes, while the oxytocic principle passes the filter.

In repeating certain of these experiments and in attempting new methods of separation, the present writer has had two considerations particularly in view. In the first place, it seemed to him that the results of previous experiments were to some extent vitiated by the fact that no steps had been taken to remove from the pituitary material the depressor substance which has been referred to above. The presence of any quantity of this substance would give an increased oxytocic and a decreased pressor value in any given standardisation. The second consideration has been the use of a proved method of pressor standardisation. Since the development of the technique of standardisation of pressor activity by HOGBEN, SCHLAPP, and MACDONALD, it has become possible to deal satisfactorily with this aspect of the work.

2. METHODS.

(1) *The Preparation of Desiccated Pituitary Material.*

The pituitary bodies were removed from oxen immediately after slaughter and placed in the refrigerator at $-10°$ C. Within six hours the capsule of each body was removed and the posterior lobe dissected out while still in the frozen condition. These lobes were then dehydrated in a large volume of cold acetone, and after two hours were transferred to ether, in which medium they were stored for transport to the laboratory. The lobes were next cut in pieces with scissors and dried in an oven at 50° C., after which they were ground to a fine powder in a mortar. This material was extracted continuously with absolute alcohol in Sohxlet's apparatus for twelve hours in order to remove all depressor substance, dried in the oven at 50° C., and stored in a stoppered bottle.

(2) *The Preparation of Extracts.*

Except in experiments A.H., '24, II. and III., the extracts have been deproteinised as far as possible before use. The procedure followed was that adopted by DUDLEY, who heats a quantity of the material with distilled water on the boiling-water bath for 10 minutes, adds a few drops of 5 per cent. acetic acid and heats a further 10 minutes. The extract is then filtered clear and a portion " titrated " with colloidal ferric hydroxide until the protein is precipitated and the supernatant liquid remains clear. The requisite amount of colloidal ferric hydroxide is then added to the main bulk of the solution and the precipitated protein filtered off. This procedure was modified in later experiments to the extent that the addition of acetic acid to the extracting water

was omitted and a double extraction with half quantities of distilled water substituted. It was found that the extracts thus produced were at least as potent in all respects as those in which acetic acid had been employed. The solution of colloidal ferric hydroxide was found to have a content of 4·9 per cent. Fe_2O_3.

(3) *Preservation.*

Since it proved impossible for one individual to carry out the various standardisations simultaneously, it was found necessary to preserve the extracts after they had undergone the various processes to be described, by boiling and sealing in sterile glass ampoules. Under these conditions there was no observable diminution of activity, even after several months.

(4) *Standardisation.*

Oxytocic.—In the estimation of oxytocic activity the uterus method of Dale and Laidlaw was used. The apparatus was identical with that described by Burn and Dale, and the writer is indebted to Dr J. H. Burn for an opportunity of seeing the test carried out.

Pressor.—The method employed has been that developed by Hogben, Schlapp, and Macdonald.

Melanophore.—In the estimation of melanophore activity the procedure recommended by Hogben and Winton has been followed, in preference to that described by Fenn, which proved less satisfactory in the writer's hands.

3. The Effect of Boiling Posterior Lobe Extracts with Dilute Hydrochloric Acid.

In the experiment A.H., '24, I., 30 c.c. of an extract corresponding to a concentration of 15 mgm. of desiccated substance per c.c. were prepared. A sufficient quantity of concentrated hydrochloric acid was added to make the concentration of the whole 1/10 molar, and a 5 c.c. sample (I.) set aside for testing purposes. This was immediately neutralised with solid sodium bicarbonate, using methyl red as indicator, and sealed in glass ampoules. The remaining 25 c.c. were set to boil under a reflux condenser ; 10 c.c. were removed at the end of 20 minutes' boiling (Sample III.), and the remaining 15 c.c. at the end of 40 minutes (Sample V.). As in the case of Sample I., these portions were neutralised and sealed in glass ampoules. Tests for pressor and oxytocic activity were then undertaken.

Pressor Activity.

For this test the following dilutions were found necessary :—

I. 1 part of extract added to 9 of Ringer's solution.
III. 1 ,, ,, ,, 5 ,, ,,
V. 2 parts ,, ,, 1 ,, ,,

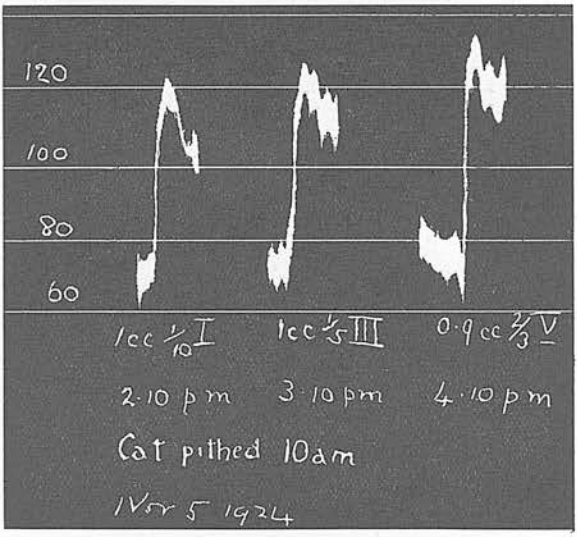

Fig. 1.

The following equivalence of pressor activity was established on a decerebrated cat (fig. 1): 1 c.c. I.=1 c.c. III.=0·9 c.c. V. Hence, taking the pressor activity of I. as equal to 100, the activities of III. and V. are represented by 50 and 17 respectively.

Oxytocic Activity.

The following dilutions were found necessary for the uterus test :—

I. 1 part of extract added to 199 of Ringer's solution.
III. 1 ,, ,, ,, 99 ,, ,,
V. 1 ,, ,, ,, 24 ,, ,,

Equivalent doses were found to be as under (fig. 2): 1 c.c. I.=1 c.c. III.=0·65 c.c. V.

Again taking the oxytocic activity of I. as equal to 100, the activities of III. and V. are represented by 50 and 19 respectively.

In experiment A.H., '24, III., an identical procedure was adopted. The acid concentration was, however, 1/20 molar and the times of boiling were doubled.

The following equivalence of pressor activity was established (fig. 3): 1 c.c. I./10=0·8 c.c. II./5=8·8 c.c. $\frac{3}{5}$ of III.

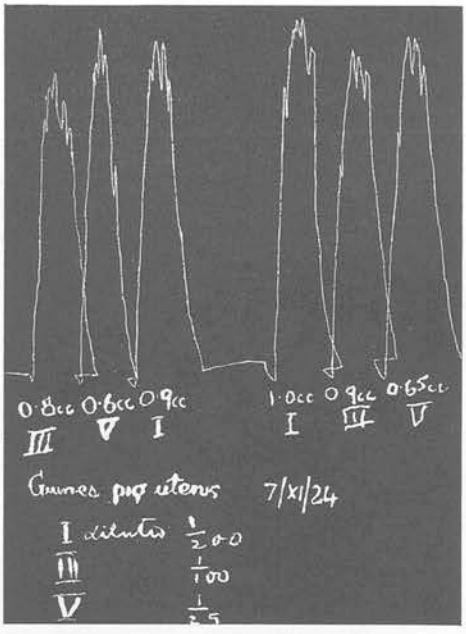

FIG. 2.

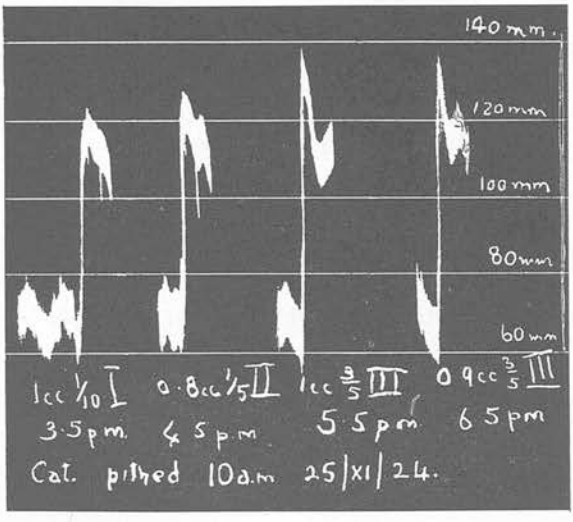

FIG. 3.

Hence, if the activity of I. is taken as 100 as before, the activities of II. and III. are 63 and 21 respectively.

In the uterus test equal doses were found to be 1 c.c. I./200=0·67 c.c. II./100=0·9 c.c. III./40 (fig. 4).

Here the activities of I., II., and III. are as 100, 56, and 22 respectively.

In a further experiment in which the extract had been deproteinised,

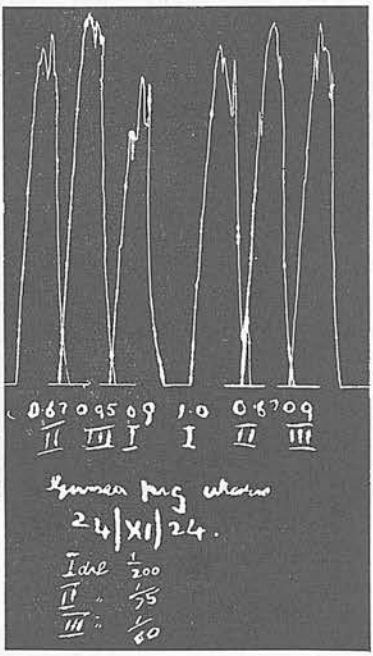

Fig. 4.

no difference in the rate of destruction of pressor and oxytocic principles could be detected.

Experiments upon the pressor and melanophore activities were also undertaken. The protocol of only one is given here. The procedure was identical with that followed in experiment A.H., '24, I., except that deproteinised extracts were used. Only one sample was tested after 30 minutes' boiling.

Pressor Activity.

The unboiled extract (A) was diluted with 13 parts of Ringer's solution, and the boiled extract (B) with 4. 1 c.c. of A was found to be equal to 1 c.c. of B, and hence the activity of B is 35 per cent. of that of A (fig. 5).

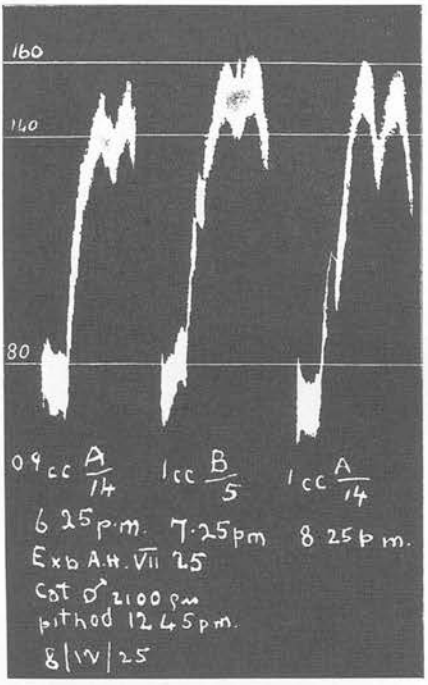

FIG. 5.

Melanophore Activity.

The figures for the final standardisation of melanophore activity are given below :—

A.

Wt. of frog in gm.	23	24	26	26	27	28
Amount of A in c.c.	·0003	·00025	·0002	·00015	·0001	·00005
Condition of melanophores	S	S	S	C	C	C

B.

Wt. of frog in gm.	23	25	26	27	27	29
Amount of B in c.c.	·00027	·0004	·00054	·00065	·0008	·0009
Condition of melanophores	S	S	S	C	C	C

S = stellate. C = contracted.

It is clear therefore that

·00015 c.c. of A = ·00054 c.c. of B.

Hence the melanophore activity of B is 28 per cent. of that of A.

In a further experiment 1/20 molar acid was used and the extract boiled for 10 minutes. No appreciable diminution of either pressor or melanophore activity could be found.

The results of the experiments with boiling acids are summarised in the following table :—

Strength of acid.	Time of boiling.	Pressor activity.	Oxytocic activity.
M/10	0 min.	100	100
	20 ,,	50	50
	40 ,,	17	19
M/20	0 min.	100	100
	40 ,,	63	56
	80 ,,	21	22
M/10	0 min.	100	100 *
	30 ,,	34	28 *

* Melanophore activity.

4. Experiments with Butyl Alcohol.

For the experiments now to be described, a quantity of deproteinised extract, usually 30 c.c. of a concentration of 10 mgm. of desiccated substance per c.c., was prepared. Of this 5 c.c. diluted to 10 were set aside for assay. (In the calculation of the activity it is therefore necessary to double the dilution of the control in any particular standardisation.) The remainder was extracted continuously for 18 hours in Dakin's apparatus with N-butyl alcohol, or alternatively was shaken with three successive portions of the alcohol in a shaker for periods of 30 minutes. The watery and alcoholic layers were then separated by means of a separating funnel. These portions were distilled at 40° C. and 15 mm. of mercury pressure in order to remove all traces of the alcohol. When this had been accomplished, each portion was made up to the original volume of 25 c.c. with distilled water, and sealed in sterile ampoules. Tests for pressor oxytocic and melanophore activity were thereupon undertaken.

The results of three such experiments are now given.

Experiment B.A., III. '25.

In this experiment the letter B refers to the control, C to the alcoholic layer, and D to the watery layer.

Pressor Activity.

A comparison of B, C, and D showed that the following quantities were approximately identical in their pressor effects (fig. 6): 1 c.c.

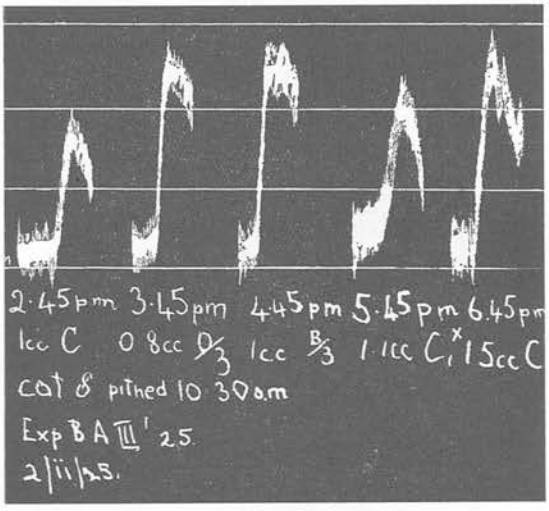

Fig. 6.

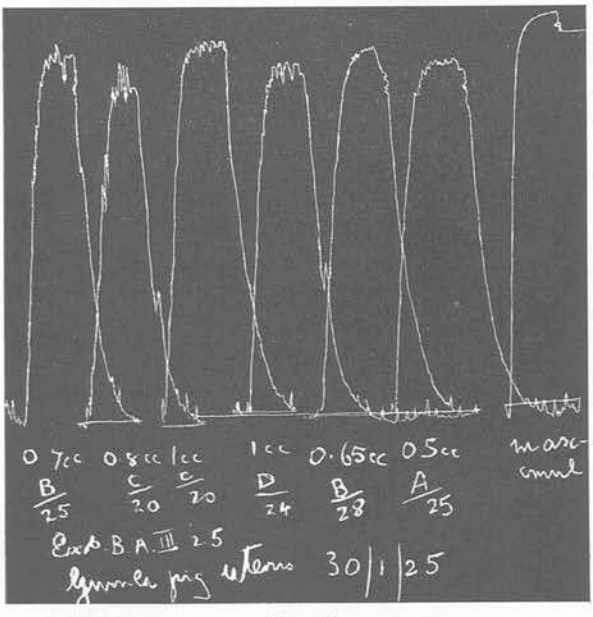

Fig. 7.

B/6; 0·8 c.c. D/3; 1·5 c.c. C. Hence B is 9 times as potent as C and 1·6 times as potent as D in pressor activity.

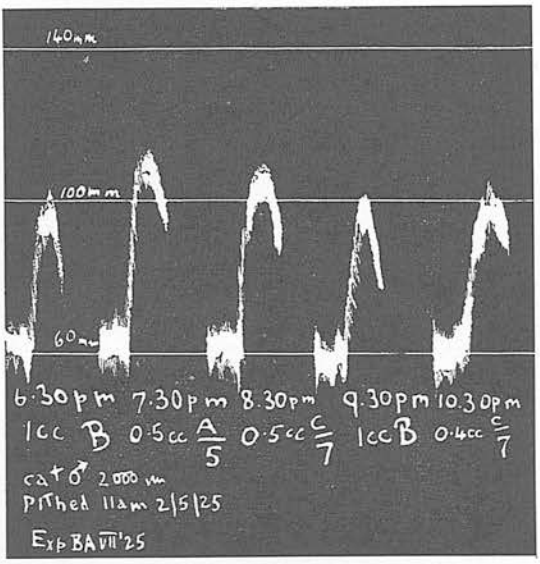
160mm
100mm
60mm
6.30pm 7.30pm 8.30pm 9.30pm 10.30pm
1cc B 0.5cc A/5 0.5cc C/7 1cc B 0.4cc C/7
cat ♂ 2000 gm
Pithed 11am 2/5/25
Exp BA VII '25

FIG. 8.

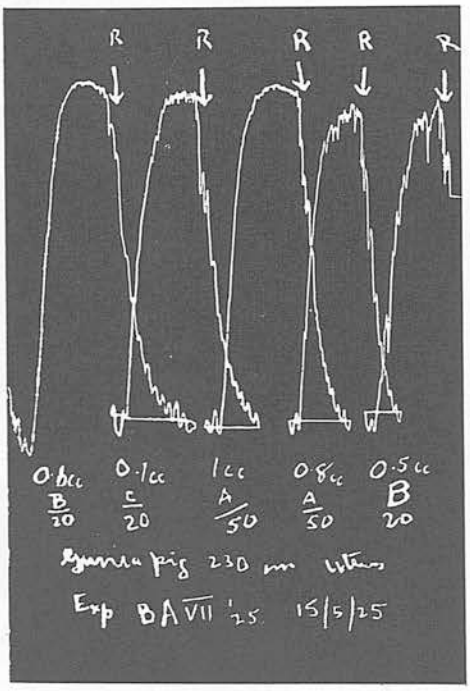
R R R R R
0.1cc 0.1cc 1cc 0.8cc 0.5cc
B/20 C/20 A/50 A/50 B/20
Exp BA VII '25 15/5/25

FIG. 9.

Oxytocic Activity.

The following equivalence was established (fig. 7) :—0·7 c.c. B/50 = 1 c.c. C/20 = 0·8 c.c. D/24. Therefore B is 3·6 times as potent as C and 2·4 times as potent as D in oxytocic activity.

No melanophore standardisation was undertaken.

Experiment B.A., VII. '25.

Here A refers to the control, B to the alcoholic, and D to the watery layer.

Pressor Activity.

Equal doses were found to be as follows (fig. 8) : 0·5 c.c. A/10 and 0·5 c.c. C/7 ; 1 c.c. B and 0·4 c.c. C/7. Hence A = 24 B = 1·4 C.

Oxytocic Activity.

The standardisation (fig. 9) shows that 1 c.c. A/100 = 0·6 c.c. B/20 = 0·7 c.c. C/20.

It is clear therefore that in respect of oxytocic activity A is 3 times as potent as B and 3·5 times as potent as C.

Melanophore Activity.

The final figures are given :

A.

Wt. of frog in gm.	40	31	30	26	26	24
Amount of A in c.c.	·000025	·00003	·000035	·00004	·000045	·00005
Condition of melanophores	C	C	C	S	S	S

B.

Wt. of frog in gm.	38	31	30	26	25	23
Amount of B in c.c.	·000031	·000037	·000044	·00005	·000055	·000062
Condition of melanophores	C	C	C	C	S	S

C.

Wt. of frog in gm.	38	31	29	28	24	23
Amount of C in c.c.	·001	·0012	·0014	·0016	·0018	·002
Condition of melanophores	C	C	S	S	S	S

Hence the equivalence for melanophore activity is as follows :— ·000035A=·00005B=·0012C, or A is 24 times as potent as B and 1·4 times as potent as C.

Experiment B.A., VIII. '25.

In this experiment the letters A, B, and C refer to the control, alcoholic, and watery layers respectively.

Oxytocic Activity.

The standardisation reproduced in fig. 10 shows that 0·4 c.c. A/60 = 0·55 c.c. B/25 = 0·8 c.c. C/29. Hence A is 3·3 times as potent as B and 6 times as potent as C in oxytocic activity.

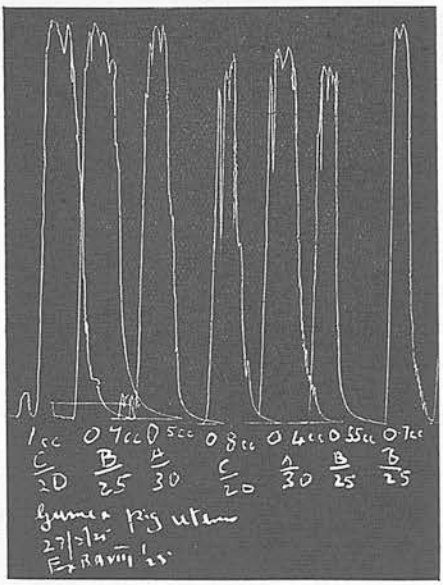

Fig. 10.

Pressor Activity.

The pressor standardisation, fig. 11, shows that 0·7 c.c. A/16 = 1 c.c. B = 0·6 c.c. C/6.

It is clear, then, that A is 13 times as potent as B and 1·6 times as potent as C in pressor activity.

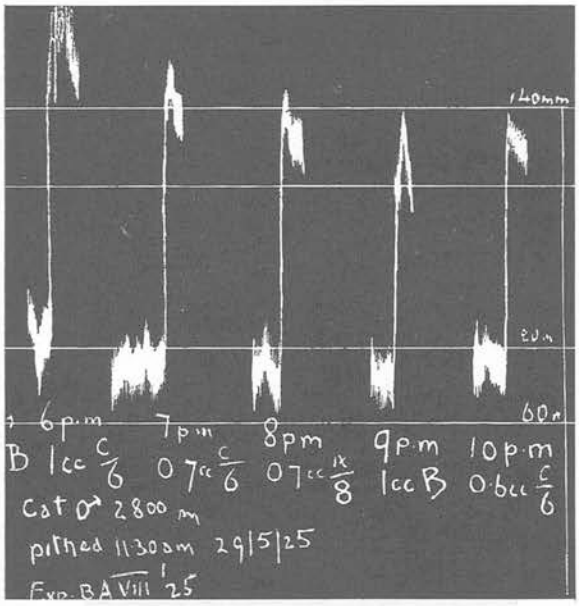

FIG. 11.

Melanophore Activity.

The figures given are for the final standardisation.

A.

Wt. of frog in gm.	40	39	37	36	30	30	29
Amount of A in c.c.	·0001	·0002	·0003	·0004	·0005	·0006	·0007
Condition of melanophores	C	C	C	S	S	S	S

B.

Wt. of frog in gm.	39	39	37	37	34	30	30
Amount of B in c.c.	·001	·002	·004	·006	·008	·01	·012
Condition of melanophores	C	C	C	S	S	S	S

C.

Wt. of frog in gm.	39	38	37	35	32	30	28
Amount of C in c.c.	·0002	·0004	·0006	·0008	·001	·0012	·0004
Condition of melanophores	C	C	S	S	S	S	S

C = contracted. S = stellate.

Hence it is clear that 0·0004 c.c. A = 0·006 c.c. B = 0·006 c.c. C, and thus A is 15 times as potent as B and 1·5 times as potent as C in melanophore.

The results of these experiments with butyl alcohol are tabulated below. The activity of the control is always taken as 100, the activities of the watery and alcoholic fractions being thus expressed as percentages of the activity of the original extract in any particular respect.

	Oxytocic activity.	Pressor activity.	Melanophore activity.	
Control . .	100	100	100	
Alcoholic layer .	28	11	..	Exp. B.A., III. '25.
Watery ,, .	41	62	..	
Recovered .	69%	73%	..	
Alcoholic layer .	33	4	4	Exp. B.A., VII. '25.
Watery ,, .	28	71	71	
Recovered .	61%	75%	75%	
Alcoholic layer .	33	8	7	Exp. B.A., VIII. '25.
Watery ,, .	17	62	66	
Recovered .	50%	70%	73%	

5. Experiments on the Adsorption of the Active Principles to Lead Sulphide.

Guggenheim was the first to show that the active principles (pressor and oxytocic) of the posterior lobe of the pituitary body are adsorbed to precipitates of lead sulphide produced in extracts. The experiments described here involved the addition of lead acetate to extracts and the subsequent precipitation of lead sulphide by the passage of washed sulphuretted hydrogen gas. Preliminary experiments showed that all

three activities could be almost completely removed by such a precipitation, although the mere passage of hydrogen sulphide gas left them unchanged. The precipitation of lead sulphide in an extract containing 1 per cent. of lead acetate reduced the pressor, oxytocic and melanophore activities so much that insufficient activity remained for quantitative work. For the purpose of obtaining a degree of adsorption which would discriminate between the active substances it was found necessary to employ a concentration of 0·01 per cent. of lead acetate. The experimental procedure was as follows: 18 c.c. of deproteinised extract (10 mg. per c.c.) were prepared and divided into two equal portions, A and B; 1 c.c. of distilled water was added to A, and 1 c.c. of 0·1 per cent. lead acetate solution to B. Washed sulphuretted hydrogen gas was then passed through B for 10 minutes, and the dissolved gas subsequently removed by aerating the solution for 20 minutes. The precipitated lead sulphide was then filtered off and the extract sealed in ampoules. Portion A was submitted to the identical procedure, including filtration.

The results of two experiments are given below :—

Experiment Pb., III. '25.

Pressor Activity.

A is at least 10 times as potent as B (fig. 12).

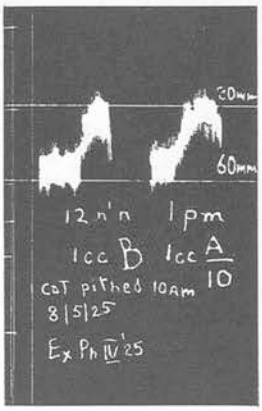

Fig. 12.

Oxytocic Activity.

0·8 c.c. A/25 is equivalent to 0·7 c.c. B/10 (fig. 13). Hence A = 2·2 B.

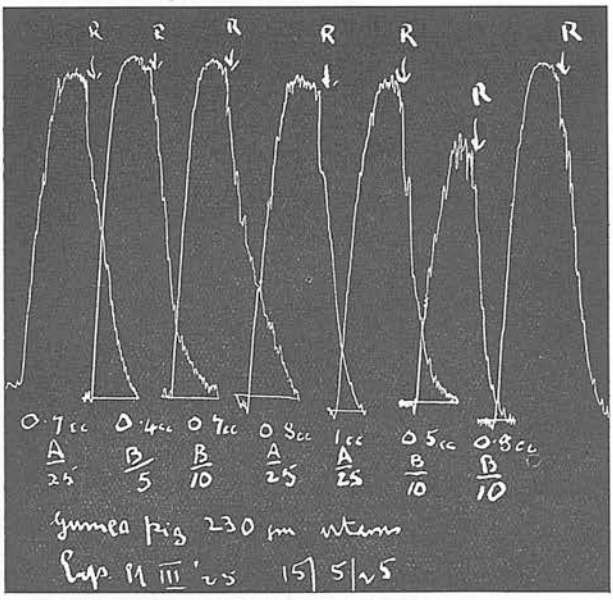

Fig. 13.

Melanophore Activity.

Final standardisation.

A.

Wt. of frog in gm.	25	24	22	21	20	18
Amount of A in c.c.	·0002	·0004	·0006	·0008	·001	·0012
Condition of melanophores	C	C	C	S	S	S

B.

Wt. of frog in gm.	25	23	22	20	19	17
Amount of B in c.c.	·004	·008	·012	·016	·02	·024
Condition of melanophores	C	C	S	S	S	S

Hence ·0006 A = ·008 B, or A = 13 B.

Experiment Pb., IV. '25.

Pressor Activity.

1 c.c. B/7 = 1 c.c. C, hence B = 7 C (fig. 14).

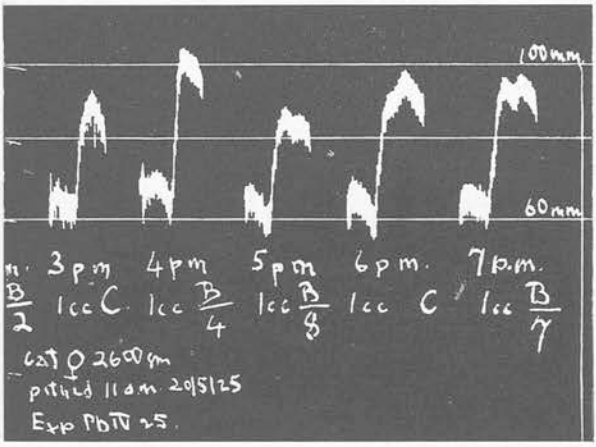

Fig. 14.

Oxytocic Activity.

0·7 c.c. B/40 = 0·6 c.c. C/10 (fig. 15). Hence B = 3·4 C.

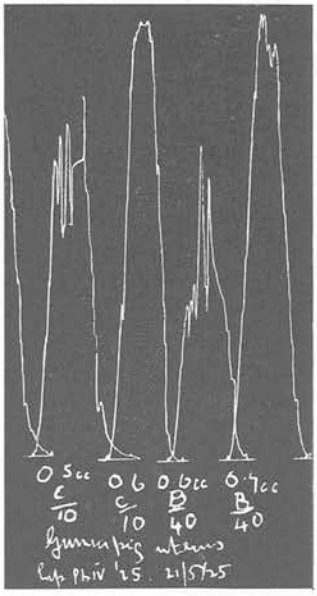

Fig. 15.

Melanophore Activity.

B.

Wt. of frog in gm.	20	18	17	16	14	12	12
Amount of B in c.c.	·0003	·0004	·0005	·0006	·0007	·0008	·0009
Condition of melanophores	C	C	C	S	S	S	S

C.

Wt. of frog in gm.	20	18	17	16	16	15	14
Amount of C in c.c.	·004	·006	·008	·010	·012	·016	·016
Condition of melanophores	C	C	C	C	S	S	S

Hence ·0005 B = ·01 C, or B = 20 C.

These results are tabulated below, showing the percentage of the active principles adsorbed.

	Exp. Pb., III. '25.		*Exp. Pb., IV.* '25.	
	Control.	Exp.	Control.	Exp.
Press. . .	0	90	0	86
Mel. . .	0	93	0	95
Oxytoc. . .	0	55	0	70

6. Discussion.

The results of this investigation in so far as they are concerned with the pressor and oxytocic principles are compatible only with the view that the responses must be attributed to different substances. In an earlier paper, dealing with pressor standardisation, the writer, together with others, stated that " We cannot regard Dudley's conclusions as fully established until his fractions have been standardised (for pressor activity) by a method of comparison . . . based upon the comparison of successive responses of the same character and the same order of magnitude as the rise evoked by initial injection "—a criticism which would apply equally to the results published by Fenn. While the present writer still adheres to this view, the results of the experiments with butyl alcohol described in this paper make it quite clear that it is possible to effect such a separation, and that while his methods are still open to criticism, neither the fundamental truth of Dudley's contention nor the justice of his criticism of Abel's work can be denied. The work of Smith and McClosky may to a certain

extent be borne out by the experiments with boiling hydrochloric acid here described, since in both cases there is no discrimination between the active principles. No results of this kind can, however, be regarded as having any weight in favour of identity when a separation has been effected. There cannot now remain any doubt as to the correctness of DUDLEY'S observations; further, the use of an accurate method of pressor standardisation has revealed another method of separating these two principles—namely, by adsorption to lead sulphide. Under these circumstances the view that the pressor and oxytocic responses of extracts of the posterior lobe of the pituitary body are due to one and the same substance can no longer be maintained.

A similar state of affairs exists with regard to the oxytocic and melanophore stimulants. The separation reported by DREYER and CLARK has been confirmed by FENN, and the results of the experiments with lead sulphide and with butyl alcohol described in this communication are in complete agreement with the view advanced by these workers, namely, that the oxytocic and melanophore responses are attributable to distinct substances.

With regard to the statement by FENN that the melanophore stimulant constitutes a third active principle, the present writer has been unable to detect during boiling with hydrochloric acid, extraction with butyl alcohol, or adsorption to lead sulphide, such a difference as would enable him to substantiate this conclusion. The explanation of the difference of opinion may lie in the fact that FENN'S quantitative data are not complete. In a separation by butyl alcoholic extraction he finds that the residue is 3 times as potent in pressor activity, 10 times as potent in melanophore activity, and $\frac{1}{8}$ as potent in oxytocic activity as the extract. The significance of these figures depends, however, upon the degree of recovery which can be attained; this is not given by FENN, but if it be 70 per cent. of the control (which is the highest recovery the present writer has attained) the difference in solubility in butyl alcohol of the pressor and melanophore principles lies within the limits of error of such methods of standardisation as he was able to employ. Under these circumstances there does not as yet appear to the present writer to be sufficient evidence for the view that the pressor and melanophore responses are due to distinct substances, and it is perhaps better to preserve the economy of hypotheses and to regard them as identical until they are definitely proved to be otherwise.

7. SUMMARY.

1. When depressor-free extracts of the posterior lobe of the pituitary body are boiled with dilute hydrochloric acid, the oxytocic, pressor, and melanophore responses evoked by them are destroyed slowly and simultaneously.

2. By treating depressor-free extracts with N-butyl alcohol, it is possible to separate a fraction into which the greater part of the oxytocic activity has passed.

3. By producing precipitates of lead sulphide in extracts by the passage of sulphuretted hydrogen gas through suitable concentrations of lead acetate all the active principles are to some extent adsorbed. But the quantity of pressor and melanophore substance adsorbed exceeds that of oxytocic adsorbed by a significant amount.

4. With regard to the oxytocic response on the one hand and the pressor and melanophore on the other, the results show that these activities must be attributed to distinct substances.

5. The experiments provide no positive evidence that the pressor and melanophore responses are due to distinct substances.

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