THE PHOSPHATE CONTENT AND THE BIOLOGICAL ACTIVITY OF THE ANTERIOR LOBE PITUITARY

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(With Two Text-figures.)

Qualitative and quantitative studies of the active substance in the anterior lobe of the pituitary gland stimulating amphibian metamorphosis have suffered considerable restriction on account of the limitations of the animal test, in which the specific response—the production or acceleration of metamorphosis—is confined to a small range, and the lack of information upon its chemical nature and relationships (Spaul, 1930). Attempts have been made in recent work to overcome these difficulties by seeking for an interpretation of this biological activity in chemical terms, and so establish a chemical test having wider application. Little progress has been made so far, but a correlation of the biological activity with chemical methods, providing also a means of studying chemically the activity itself, has been discovered (Spaul and Myddleton, 1930).

A precipitate is obtained by the addition of iodine solution to active extracts of the anterior lobe. This appears to be a complex association of iodine with the active substances and possibly others, since the removal of the iodine with alcohol and the solution of the dried product in acetic acid gives a biologically active extract. The precipitate can be regarded within certain limits as a measure of the biological activity. Also similarly prepared extracts of the posterior lobe of the gland, which do not assist metamorphosis, give a precipitate with iodine solution. These precipitates appear to be the same, but, apart from differences in their biological activity, further examination reveals a higher phosphate content of the anterior lobe precipitate. MacArthur, in his tissue analysis of the pituitaries of cattle, found a close similarity in the nature of the proteins of the two lobes, but the phosphate phosphorus higher in the anterior lobe.

It would seem, therefore, that some relationship might exist between the phosphate content and the biological activity of the anterior lobe. These investigations supply evidence which allows fuller consideration of this possibility.

EXPERIMENTAL OBSERVATIONS.

Details of the preparation of extracts, the biological tests (metamorphic and melanophore) and the chemical test have been given in previous papers (Spaul, 1928, 1930; Spaul and Myddleton, 1930). Ox pituitaries were used.

1. QUANTITATIVE ESTIMATIONS.

A grading of the volume of the precipitate obtained in the chemical test by the addition of *N*/20 iodine solution (6·35 gm. I_2 and 15 gm. KI per litre) to the extracts prepared with varying strengths of acetic acid has been found to agree more with that of the biological activity than with the concentration of the acid (Spaul and Myddleton, 1930). The phosphate phosphorus content of the precipitates shows an even closer approximation.

The precipitates obtained with 10 c.c. of 0·5, 0·25 and 0·125 per cent. acetic acid extracts were washed free from iodine by small successive washings with absolute alcohol in a centrifuge tube and the phosphate phosphorus determined. The average figures were:

Table I.

Extract	Ppt. with iodine in c.c.	Phosphate P— % of fresh gland
20 % anterior lobe in 0·5 % acetic acid	3·1	0·0028
20 % anterior lobe in 0·25 % acetic acid	2·6	0·0024
20 % anterior lobe in 0·125 % acetic acid	1·3	0·0014
20 % posterior lobe in 0·5 % acetic acid	3·2	0·00015
20 % posterior lobe in 0·125 % acetic acid	1·4	0·00015

0·5 per cent. acetic acid extracts are approximately twice as active as the 0·125 per cent. acetic acid extracts and only slightly more than 0·25 per cent. acetic acid extracts.

In Fig. 1 the biological activity, the volume of the iodine precipitate, and the phosphate content of the anterior lobe extracts (20 per cent.) in different strengths of acetic acid are compared. The 0·125 per cent. acetic extract is taken as the standard, and for the purposes of constructing comparable curves the value of each, in this extract, taken as unity.

The iodine precipitates, when washed free from iodine with alcohol and then re-dissolved in 0·125 per cent. acetic acid, give a melanophore reaction, but this is extremely small in the anterior lobe compared with the posterior lobe, whose reaction is the same as a Ringer's extract (of that portion) giving no iodine precipitate. Hence the melanophore stimulant does not appear to be directly concerned with the phosphate content of the iodine precipitate.

The addition of 78 c.c. of absolute alcohol to 10 c.c. of an extract gave a precipitate which was filtered off after boiling, washed with alcohol and ether and extracted in a soxhlet with alcohol, and then with chloroform. After extraction lasting 30 hours the phosphate phosphorus was determined in the residue.

Table II.

Extract	Phosphate P in alcohol ppt.
20 % anterior lobe in 0·5 % acetic acid	0·0107 % of fresh gland
20 % anterior lobe in 0·125 % acetic acid	0·0089 % ,,
20 % anterior lobe in Ringer's extract	0·0076 % ,,

Compared with the figures obtained from the iodine precipitate the 0·5 and 0·125 per cent. acetic acid extracts contain an amount of phosphate insoluble in alcohol, chloroform, and ether, other than that precipitated with iodine, and almost the same as the total found in the Ringer's extract, which is unable to induce any metamorphic change and gives no iodine precipitate.

20 per cent. anterior lobe extract in 0·5 per cent. acetic acid: 0·0107 per cent. – 0·0028 per cent. = 0·0079 per cent (cf. Ringer's = 0·0076 per cent.).

20 per cent. anterior lobe extract in 0·125 per cent. acetic acid: 0·0089 per cent. – 0·0014 per cent. = 0·0075 per cent. (cf. Ringer's = 0·0076 per cent.).

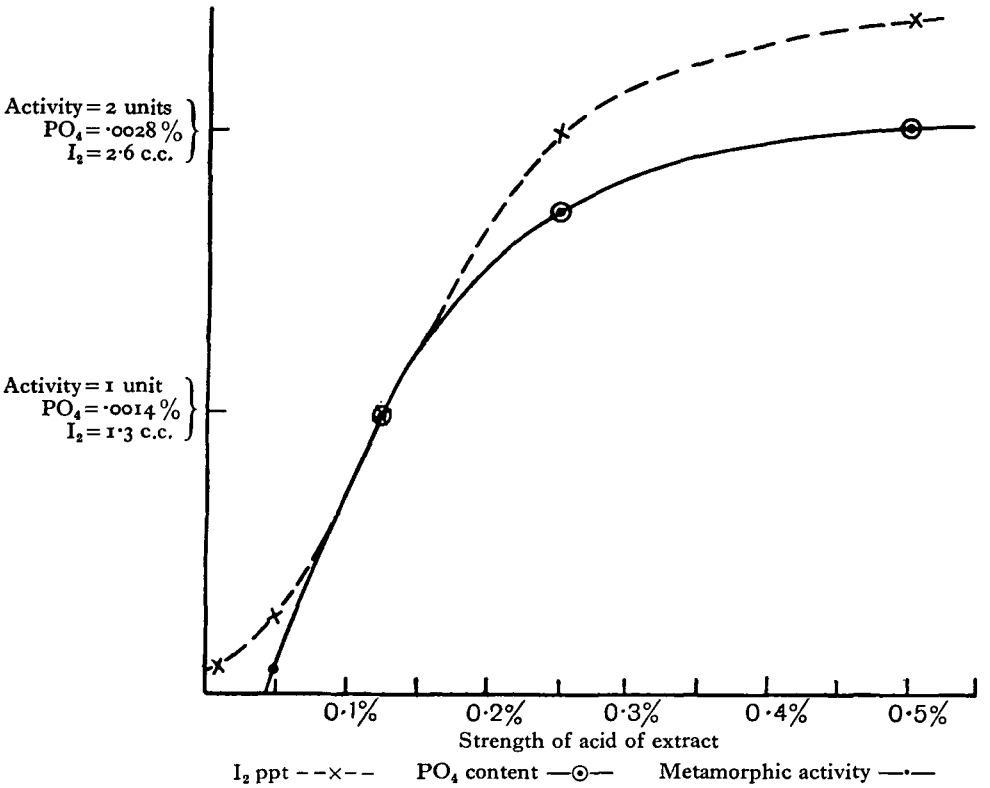

Fig. 1.

Ringer's extracts are not acid, and in earlier work (Spaul, 1930) these extracts were acidified by the addition of acetic acid to test whether their failure was due to the presence of the factor influencing metamorphosis in an inactive condition. However no change was induced, and the difference in the phosphate contents noted above offers an explanation. It would seem, therefore, that some definite association exists between the phosphate content of the iodine precipitate and metamorphic activity.

An examination of extractions from the fresh gland for phosphate provides further supporting evidence. Weighed amounts of the fresh gland were extracted

with alcohol and ether, then ground and extracted with boiling water, and finally extracted in a soxhlet for 30 hours with alcohol and 10 hours with chloroform.

The following results were obtained:

Anterior lobe: Phosphate P extracted by water (*i.e.* insoluble in alcohol and ether) = 0·020 per cent. of fresh gland.

Phosphate P insoluble in water, alcohol, ether and chloroform = 0·0031 per cent. of fresh gland (soluble in acetic acid).

Whole gland: Phosphate P extracted by water (insoluble in alcohol and ether) = 0·0194 per cent. of fresh gland.

Phosphate P insoluble in water, alcohol, ether and chloroform = 0·0025 per cent. of fresh gland (soluble in acetic acid).

Posterior lobe: Phosphate P extracted by water (insoluble in alcohol and ether) = 0·0043 per cent. of fresh gland.

Phosphate P insoluble in water, alcohol, ether, and chloroform = 0·0002 per cent. of fresh gland (soluble in acetic acid).

Water extracts of the anterior lobe are inactive (Spaul, 1930). The agreement between the figures for the phosphate phosphorus insoluble in water, alcohol, ether and chloroform, and the phosphate content of the iodine precipitate obtained from 0·5 per cent. acetic acid extracts of the anterior lobe is significant since 0·5 per cent. acetic acid extracts are the most active in metamorphosis that have been so far prepared. MacArthur (1919) describes the total residue insoluble in alcohol, ether and chloroform as "protein" and has not observed the presence of phosphate phosphorus. This "protein" contains the active metamorphic principle. A third phosphate or free phosphoric acid soluble in alcohol is also present in all extracts.

The alcohol used here was neutralised by prolonged shaking with lime followed by distillation, the ether neutralised with metallic sodium and then distilled and the chloroform neutralised by standing with anhydrous potassium carbonate followed by distillation.

Estimation of phosphates. In view of the well-known sources of error in the estimation of phosphorus as phosphate, two different methods were employed and in several cases a third method was used as a check. In extracts and in solutions made by re-dissolving precipitates, the proteins were removed as far as possible from 2 or 5 c.c. of extract by adding 2 c.c. of a 20 per cent. trichloracetic acid, or 2 c.c. of a saturated solution of ammonium sulphate containing a few drops of hydrochloric acid. The clear centrifuged solution was transferred to another tube and the phosphate precipitated as phosphomolybdate by adding a few drops of dilute nitric acid, 2 c.c. of a 50 per cent. ammonium nitrate solution and 3 c.c. of a 5 per cent. solution of ammonium molybdate, and warming for 20 minutes in a water bath at 60°–70° C.

The phosphomolybdate was centrifuged and washed twice with 15 c.c. of a 1 per cent. solution of ammonium nitrate, and then with 15 c.c. distilled water. The precipitate was then dissolved in a measured excess of standard NaOH, and the excess alkali immediately titrated back with standard H_2SO_4 to phenol phthalein. The acid was standardised against pure sodium phosphate under identical conditions.

The values obtained by titration were checked in each case by adding to each solution after titration a measured volume of 5 per cent. ammonium molybdate in $5N$ H_2SO_4 and a measured volume of hydroquinone in 20 per cent. sodium sulphite, and making up to standard volume in a Nessler tube. The blue colour produced by reduction of the phosphomolybdate in 1 hour was matched against standards containing pure sodium phosphate.

In a third method 2 to 5 c.c. of the extract were mixed with 2 c.c. of a 5 per cent. solution of ammonium molybdate containing a little nitric acid, made up to 10 c.c. with water and allowed to stand for 5 minutes. 1 c.c. of a saturated solution of benzidine acetate was then mixed with the solution, and from 2 to 4·5 c.c. of $2N$ ammonium hydroxide added according to the acidity of the solution. The evanescent blue colour which developed at once was matched with standards freshly prepared.

With care a close agreement is possible in these tests, and this has been attained in all recorded values.

The total phosphorus was determined in fresh gland tissue and in precipitates by digesting with a mixture of nitric acid and sulphuric acid, and precipitating as phosphomolybdate after neutralising with ammonia and re-acidifying with dilute HNO_3 in the usual manner. The precipitate was weighed in a glass-matte filter, where the quantity of the precipitate allowed of this procedure. In all cases the phosphomolybdate was dissolved in alkali and made up to a standard volume. A measured volume of this solution was reduced with hydroquinone as already described, and the blue coloration matched against standards.

2. THE CHEMICAL RELATIONSHIPS OF THE PHOSPHATE CONTENT AND BIOLOGICAL ACTIVITY.

Extracts of the anterior lobe in 0·125 per cent. solutions of HCl and H_2SO_4 do not affect metamorphosis, but they give a large precipitate with the iodine solution (HCl, 4·5; H_2SO_4, 5) and a melanophore response. The precipitate in the case of HCl is soluble in alcohol. The iodine precipitate from the extract in 0·125 per cent. H_2SO_4 was washed free from iodine by repeated centrifuging with successive volumes of absolute alcohol and the residue dried *in vacuo*. A weighed amount of the solid was dissolved by warming in the requisite amount of 0·125 per cent. acetic acid to give approximately the equivalent of a 20 per cent. extract. This solution had a threshold value of 0·005 c.c. in the melanophore test, and the volume of the iodine precipitate obtained was 0·8 c.c. The solid contained no phosphorus and its sulphur content was 0·220 per cent. The phosphate phosphorus present in the iodine precipitate of a 20 per cent. extract of the anterior lobe in 0·125 per cent. acetic acid extract amounted to 0·443 per cent., about twice the quantity of sulphate sulphur in the precipitate from the 0·125 per cent. H_2SO_4 extract (see diagram). Further centrifuging of this precipitate with three successive amounts of 15 c.c. absolute alcohol left the phosphate content unchanged. At this stage no theoretical assumption upon this relationship is justified. An extract prepared from the dried precipitate of the acetic acid extract, after the removal of the iodine in the usual way,

assisted metamorphosis, and had a melanophore reaction almost equivalent to that of the original extract.

12 c.c. of *N*/20 iodine solution were added to 20 c.c. of the 20 per cent. extract of the anterior lobe in 0·125 per cent. acetic acid containing five drops of 30 per cent. H_2SO_4, and the precipitate washed free from iodine by centrifuging with absolute alcohol (120 c.c.). The dried residue was dissolved in 10 c.c. of 0·125 per cent. acetic acid. This extract induced no change in the rate of transformation of tadpoles

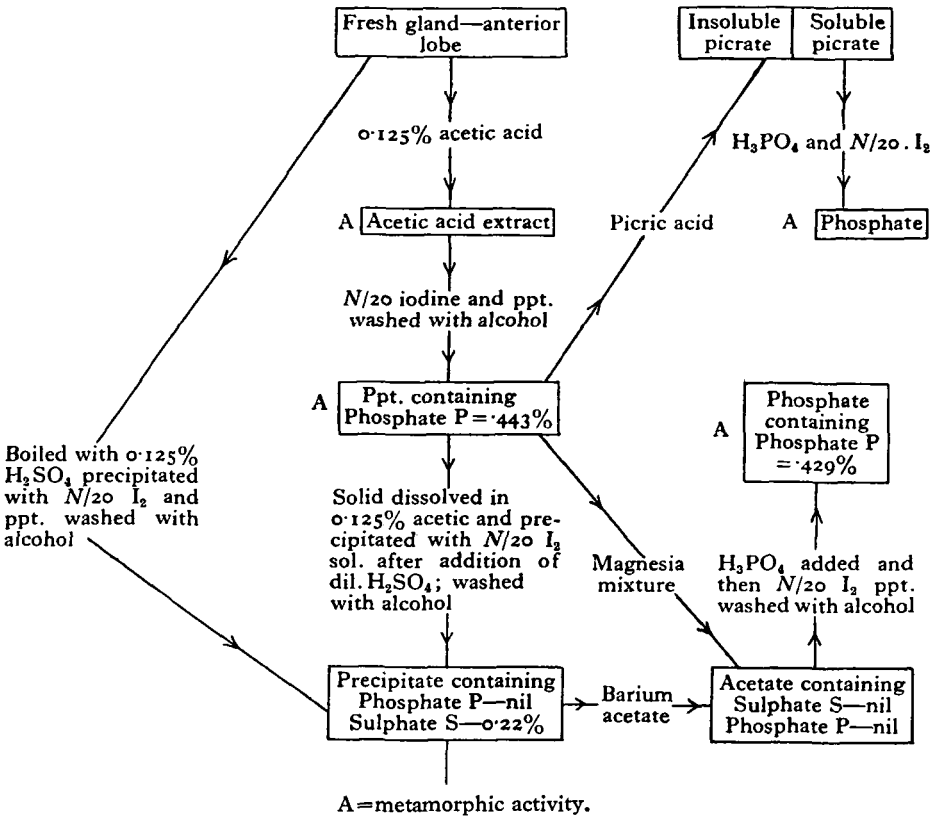

Fig. 2. Diagram illustrating the chemical relationships of the phosphate content of the iodine precipitate.

to adult frogs, whilst the threshold value of the melanophore stimulant approximated with that of the original extract (0·002 c.c.). Further the precipitate in the chemical test contained no phosphate phosphorus, but about the same quantity of sulphate sulphur as the dilute sulphuric acid extracts (see diagram).

N/20 barium acetate solution was next added, with warming, to 5 c.c. of this solution until the sulphate was just completely precipitated. The barium sulphate was separated by centrifuging. The liquor containing the acetate was made up to 10 c.c. with 0·125 per cent. acetic acid and precipitated with *N*/20 iodine. The precipitate, washed free from iodine with alcohol, was dried and then dissolved in

10 c.c. of 0·125 per cent. acetic acid. This extract had no effect upon metamorphosis but showed a melanophore reaction (threshold value = 0·0015 c.c.). The solid contained no sulphur or phosphorus (see diagram).

The failure of this extract in metamorphosis is significant in view of the reduced activity in metamorphosis obtained in the fresh gland extract in dilute acetic acid when the *p*H is increased by the addition of sodium or potassium acetate (Spaul, 1930). It would appear that the hydrogen-ion concentration is not primarily concerned with the loss, but the gradual displacement of the phosphate content with the increasing concentration of acetate is actually the responsible factor. There is a reduction of the iodine precipitate to a minimum, whilst the melanophore response is undisturbed. The reduction in the rate of change of tadpoles produced by alteration in the *p*H by means of potassium acetate cannot be observed so readily on account of slight toxic effects.

To the remaining 5 c.c. of the solution of the iodine precipitate from the extract acidified with H_2SO_4, barium acetate was added to precipitate sulphate. The liquor, after centrifuging off the barium sulphate, was made up to 10 c.c. with 0·125 per cent. acetic acid, 0·5 c.c. phosphoric acid (sp. gr. 1·75) added, and then 6 c.c. iodine solution. The precipitate was centrifuged and washed free from iodine. The residue was dried and dissolved in 0·125 per cent. acetic acid. This extract had a threshold value = 0·01 c.c. in the melanophore test, and appeared to stimulate metamorphosis. The solid contained 0·429 per cent. phosphate (see diagram).

Further quantities of the iodine precipitate from the 0·125 per cent. acetic extract were washed free from iodine, dissolved in 0·125 per cent. acetic acid and precipitated with a 10 per cent. solution of picric acid. The precipitate was filtered off, then warmed with dilute alcohol and the solution filtered hot. On cooling, a precipitate was formed in the filtered solution. This precipitate was re-dissolved in dilute alcohol by warming, and allowed to cool. The precipitate, which formed on cooling the solution, was dissolved in water, and a few c.c. of phosphoric acid added. The free picric acid was extracted from the solution by shaking once or twice with ether. Ether was removed from the aqueous solution under diminished pressure. *N*/20 iodine solution was added to the aqueous solution, and the precipitate centrifuged and freed from iodine by absolute alcohol. The residue was dried *in vacuo* and dissolved in 0·125 per cent. acetic acid. This extract showed signs of metamorphic activity, but the reaction was not entirely satisfactory. It is possible that some disturbance of the normal complex, linked with the phosphate in the active factor, occurred during treatment. A threshold value = 0·055 c.c. was obtained in the melanophore test (see diagram).

The reduction in the quantity of melanophore stimulant observed in this and the preceding extract is of interest, as it suggests a possible means of separating the melanophore and metamorphic factors by chemical means.

Again 10 c.c. of the 20 per cent. extract of the anterior lobe in 0·125 per cent. acetic acid and 2 c.c. of magnesia mixture were warmed to 70° C. and allowed to stand 3 hours. The precipitate was centrifuged off and iodine solution added to the clear liquor. The resultant precipitate was washed free from iodine with alcohol

and dried. The residue was dissolved in 10 c.c. of 0·125 per cent. acetic acid (see diagram). This extract, free from phosphate, was found to be inactive so far as metamorphosis was concerned, but showed a melanophore response (threshold value = 0·0018 c.c.). The *p*H of all these extracts was noted and, as they corresponded with that of the original fresh gland extract in acetic acid, a consideration of possible variations due to *p*H differences was not necessary.

The results of these experiments emphasise the essential nature of the phosphate content of the iodine precipitate and, coupled with the quantitative determinations, indicate its importance in relation to the influence of the anterior lobe extract upon amphibian metamorphosis. At the same time these experiments leave no doubt as to the independence of the phosphate content from the melanophore stimulant.

DISCUSSION.

The evidence collected here by quantitative and qualitative analysis directs attention especially to the particular rôle of a small fraction of the total phosphate content of the anterior lobe, but it is necessary to consider other relationships of this portion before an appreciation of its significance is complete.

In the first place the active phosphate associated with the iodine precipitate has been converted into a crystalline picrate free from phosphorus. The crystals have not yet been obtained free from extraneous matter. They are slightly soluble in water. After several re-crystallisations followed by re-conversion into phosphate, the iodine method fails to produce a precipitate. Considerable amounts of iodine are adsorbed on the precipitate from fresh extracts, and it must be assumed that the re-actant is a protein-like body which is extensively removed by re-crystallisation of the picrate.

Brailsford Robertson (1916) isolated an active principle from the anterior lobe pituitary called "Tethelin," having growth-promoting properties. It is prepared by the extraction with alcohol after desiccation but, although containing 1·4 per cent. of phosphorus, it is soluble in water, alcohol, ether and chloroform, and hence could hardly be identical with the metamorphic factor which is insoluble in these liquids and requires dilute acetic acid for extraction. The growth autacoid or hormazone of Smith (1920) is not found in either water or alcohol extracts of the anterior lobe, but remains in the residue after extraction with water and boiling alcohol, and hence differs from Tethelin. Growth results from feeding this substance, whereas it is necessary to inject the metamorphic factor, since it is gradually destroyed by pepsin (Spaul, 1930; Spaul and Myddleton, 1930). More investigations are required before further differentiation is possible. Further, the anterior pituitary substance concerned with the regulation of the ovary is water-soluble, insoluble in lipoid solvents, destroyed at 60° C. (Zondek and Ascheim, 1928) and inactive when administered by mouth (Smith, 1927). This substance, therefore, does not appear to be the same as the metamorphic factor.

As regards the proportion of phosphate concerned with metamorphic activity, a comparative study of the distribution of the total phosphate content in the gland,

before and after extraction, gives some indication of the relation between the amounts involved. 20 per cent. extracts of the anterior lobe in Ringer's, 0·125 per cent. acetic acid and 0·5 per cent. acetic acid, and a 20 per cent. extract of the posterior lobe in 0·5 per cent. acetic acid were used in this comparison. The results were as follows:

Table III.

	Anterior lobe		Posterior lobe	
Total phosphorus in fresh gland ...	0·270 %		0·283 %	
Total phosphate in fresh gland	0·039 %		0·026 %	
Ratio: total phosphate/total phosphorus	1 : 6·92		1 : 10·85	
	Ringer's anterior lobe	0·125 % acetic anterior lobe	0·5 % acetic anterior lobe	0·5 % acetic posterior lobe
Weight of fresh gland taken for extract	8 gm.	17 gm.	27 gm.	10 gm.
Weight of residue after extraction ...	1·6265 gm.	3·48 gm.	4·14 gm.	1·1862 gm.
Ratio: residue/fresh gland	1/4·92	1/4·88	1/6·52	1/8·43
Total phosphorus in residue	0·774 %	0·453 %	0·549 %	0·677 %
Phosphate P in residue	0·127 %	0·083 %	0·007 %	0·059 %
Ratio: phosphate P/total phosphorus ...	1/6·09	1/5·46	1/78·4	1/11·47
Total P extracted by 10 c.c.	2·325 mg.	3·547 mg.	3·717 mg.	4·060 mg.
Phosphate P extracted by 10 c.c. (calc.)	0·265 mg.	0·440 mg.	0·758 mg.	0·420 mg.
Phosphate found in 10 c.c. of extract ...	0·290 mg.	0·420 mg.	0·758 mg.	0·373 mg.
Ratio: PO_4 extracted/total P extracted ...	1/8·77	1/8·06	1/4·9	1/9·67
Phosphate P pptd. with I_2 ppt. in 10 c.c.	—	0·03 mg.	0·058 mg.	0·003 mg.
Ratio: I_2 ppt.: PO_4/PO_4 of extract ...	—	1/14·7	1/13·07	1/140

These figures and ratios show that: (1) The posterior lobe contains more phosphorus but less phosphate than the anterior lobe which agrees with the findings of MacArthur (1919). (2) The residues after extraction with 0·5 per cent. acetic acid are smaller—particularly as regards the posterior lobe—with little phosphate, which is exceptionally low in the case of the anterior lobe. (3) Larger amounts of phosphorus are extracted by 0·5 per cent. acetic acid, especially from the posterior lobe, but only in the case of the anterior lobe is the phosphate content high; Ringer's solution extracts less phosphorus and phosphate than the acid; 0·5 per cent. acetic acid extracts double the amount of phosphate from the anterior lobe extracted by 0·125 per cent. acetic acid and precipitated by iodine, as already shown, but not twice the amount of other phosphates. The amount of phosphate extracted from the posterior lobe is smaller than that in the 0·125 per cent. acid extracts of the anterior lobe, but the extremely small amount precipitable with iodine would in part account for this. Hence, it is evident, distinct differences exist in the chemical combinations of the phosphates of the two lobes.

The phosphate precipitated with iodine in posterior lobe extracts may indicate post-mortem diffusion of the metamorphic factor, although, compared with the rate of diffusion of the melanophore stimulant, the quantity appears to be excessive (Spaul, 1925, 1927) (threshold value in c.c.: anterior lobe, 0·002; posterior lobe, 0·0000005). Probably a small portion is normally present. The cone of Wulzen consists of tissue similar to that of the anterior lobe, and shows the iodine leucobase

reaction, unlike the remainder of the posterior lobe (Spaul and Howes, 1930). Unfortunately the metamorphic test cannot be successfully applied to the posterior lobe extracts, since a relatively high concentration of the factor is required to produce any effect, whilst the posterior lobe itself inhibits metamorphosis. A further possibility is the existence of other combinations of phosphate, capable of extraction and precipitable with iodine in a manner similar to that associated with the active factor of metamorphosis. These latter explanations receive some support from an examination of the phosphate contents of the iodine precipitates of the extracts of both lobes of the pituitaries of different breeds of cattle sent from South America. The results are tabulated:

Table IV.

	Aberdeen Angus		Hereford		Shorthorn	
	Anterior pituitary	Posterior pituitary	Anterior pituitary	Posterior pituitary	Anterior pituitary	Posterior pituitary
Vol. of iodine ppt.	1·7	1·9	1·2	1·7	1·3	1·5
Phosphate content in mg. per 10 c.c. ...	0·065	0·059	0·035	0·035	0·021	0·014

Previous investigations have shown the effectiveness in metamorphosis of extracts of the anterior lobes of both Aberdeen Angus and Hereford, and the failure of the Shorthorn (Spaul and Myddleton, 1930). The melanophore tests showed post-mortem diffusion, especially in the Shorthorns, which to some extent explained the results in metamorphosis. The posterior lobes of these glands compared with the anterior lobes were relatively large, and hence with bigger areas in contact diffusion was inevitably more pronounced. The values of the phosphate content, however, show effective differences due to the breed. The total amount in the whole gland as well as the quantity in the anterior lobe is greatest in the Aberdeen Angus and least in the Shorthorn. The large phosphate content of the posterior lobe cannot be due to post-mortem diffusion alone, but whether the bulk consists of the active substance normally present, or another similar one, cannot be decided until further knowledge has been gained or the isolation of these fractions achieved.

Lastly, the distribution of the phosphate content in the anterior lobe affords still further confirmation of its association with metamorphic activity. The middle region of the lobe has been found to be most active, the peripheral region least and that portion adjoining the posterior lobe slightly less than the middle (Spaul and Howes, 1930). The phosphate content of the iodine precipitate of extracts of these regions shows practically identical grading with the activity. The total phosphate of the extract, however, is graded from the inner to the outer region, whilst the total phosphorus is graded in the reverse direction.

If the anterior lobes are exposed at ordinary temperatures for any length of time diffusion occurs with re-distribution of the phosphates.

The information obtained so far is scanty and remains to be completed, but sufficient has been collected to allow the formulation of some definite view upon the chemical aspect of the metamorphic activity of the anterior lobe. It is apparent that

the chemical composition of the responsible factor is characterised by the association of phosphate phosphorus with a protein complex, and it depends upon the phosphate for the full expression of its activity. This phosphate portion differs from the remaining phosphate present in the lobe by reason of its insolubility in alcohol, ether, chloroform and water and the need for dilute acid (acetic) for its extraction, and the addition of iodine solution for its separation in these extracts. These features distinguish it from the growth factor—Tethelin—and further will assist in the comparison with other activities of the anterior pituitary as more detailed and accurate knowledge of their chemical properties accumulates. At the same time an advance in the knowledge of the constitution of the factor itself will be possible.

SUMMARY.

1. Quantitative estimations directly associate the phosphate phosphorus precipitated by iodine solution from active extracts of the anterior lobe pituitary in dilute acetic acid with the metamorphic activity of the lobe.

2. Substitution of the phosphate content of the iodine precipitate by other radicles (sulphate, acetate, picrate, etc.) results in the loss of activity.

3. The active factor is insoluble in alcohol, chloroform and ether, which distinguishes it from Robertson's growth-promoting tethelin.

4. The melanophore stimulant is independent of the phosphate content.

5. There is a small quantity of phosphate precipitated by iodine solution from posterior lobe extracts which indicates, apart from post-mortem diffusion, either the presence normally of the active factor in this lobe (Cone of Wulzen) or the existence of some other phosphate combination precipitated by iodine, but ineffective in metamorphosis.

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