REFERENCES

- Alles, G. A. & Hawes, R. C. (1940). *J. biol. Chem.* **133**, 375.
 Ammon, R. (1933). *Pflüg. Arch. ges. Physiol.* **233**, 486.
 Augustinsson, K. B. (1948). *Acta physiol. scand.* **15**, suppl. 52.
 Augustinsson, K. B. & Grahn, M. (1952). *Ark. Kemi*, **4**, 277.
 Chang, C. C. & Lee, C. Y. (1955). *J. med. Ass., Formosa*, **54**, 103.
 Chaudhuri, D. K. (1950a). *Ann. Biochem.* **10**, 65.
 Chaudhuri, D. K. (1950b). *Ann. Biochem.* **10**, 71.
 Markwardt, F. (1953). *Naturwissenschaften*, **40**, 341.
 Mounter, L. A. (1951). *Biochem. J.* **50**, 122.
 Mounter, L. A. & Whittaker, V. P. (1953). *Biochem. J.* **53**, 167.
 Nachmansohn, D. & Lederer, E. (1939). *Bull. Soc. Chim. biol., Paris*, **21**, 797.
 Wilson, I. B. (1954). *The Mechanism of Enzyme Action*, p. 642. Baltimore: The Johns Hopkins Press.
 Wilson, I. B. & Bergmann, F. (1950). *J. biol. Chem.* **186**, 683.

Purification and Amino Acid Analysis of Melanophore-expanding Hormone from Hog and Ox Pituitary Glands

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The preparation of highly purified, if not pure, melanophore-expanding hormone from hog pituitary has recently been reported by Lerner & Lee (1955). The present writers, with the aid of counter-current distribution technique, have succeeded in preparing highly purified melanophore-expanding material, virtually free from adrenocorticotrophic (ACTH) activity, from both hog and ox pituitary. The products from the two sources are not chemically identical. The amino acid composition of the material from hog pituitary differs considerably from that reported by Lerner & Lee (1955). The successful segregation of the melanophore-expanding principle and ACTH along with other evidence indicates the non-identity of the two hormones.

EXPERIMENTAL

Preparation of material for countercurrent distribution

(a) *Hog-pituitary extract.* A quantity (150 g.) of acetone-dried and powdered posterior pituitaries, assaying from 6 to 10 units of melanophore-hormone activity per mg., was divided into 10 g. lots and each lot was extracted with 25 ml. of acetone and 160 ml. of glacial acetic acid at 70°. The material was left in contact with the solvent at this temperature for 10 min. The extracts were centrifuged, the sediment of tissue fragments was removed and washed with 20 ml. each of cold acetone and glacial acetic acid and the washing was added to the previous extract. To the combined supernatant fluids were added 1 ml. of saturated NaCl and a quantity of acetone equal to half the volume of the extract. The resulting precipitate was removed by centrifuging and to the supernatant fluid was added an equal volume of diethyl ether. The precipitate was collected by centrifuging, washed three times with acetone and placed in a desiccator. The total yield of this partially purified material from 150 g. of posterior-pituitary lobes

was 27 g. The melanophore-hormone activity was 30 units/mg., thus representing a recovery of 67.5% of the activity of the original material. Further purification of the product was achieved by dissolving 27 g. of the ether precipitate in 750 ml. of 0.1N acetic acid. Oxycellulose (4.5 g.), previously washed successively with 1N-HCl, water and 0.1N acetic acid, was added and the mixture stirred for 40 hr. at room temperature. The oxycellulose was collected by centrifuging, washed successively with 0.1N acetic acid and water, and then suspended twice in 100 ml. of 0.1N-HCl and stirred for 30 min. to elute the hormone. The oxycellulose was removed by centrifuging and the pH of the combined supernatant fluids was adjusted to 6.5 with aq. N-NH₃. The resulting precipitate was removed by centrifuging and to the supernatant fluid 5 vol. of acetone were added. The resulting precipitate was washed with acetone and stored in an evacuated desiccator. The yield was 660 mg. of a powder which assayed 800 units/mg., representing a recovery of 44% of the activity of the original material. This procedure was based on the methods of Payne, Raben & Astwood (1950), and Astwood, Raben, Payne & Grady (1951), originally developed for the purification of ACTH, first used by Raben, Rosenberg & Astwood (1952) for the purification of melanophore hormone and later modified for this purpose by Landgrebe & Mitchell (1954). The present authors have introduced only slight modifications.

(b) *Ox-pituitary extract.* A quantity of purified powder which had been prepared from ox posterior pituitary lobes by Stehle (1944) was available. Stehle's method involved fractionation of an acetic acid extract of the tissue with organic solvents, and final isolation of the active material by precipitation with picric acid. The powder assayed 150 units/mg.

Countercurrent distribution

For the segregation and purification of the melanophore-expanding substance and ACTH a 100-tube (each 200 ml. capacity) all-glass Craig apparatus was used. For analytical purposes a 24-unit (20 ml.) stainless-steel Craig instrument

was used. At the end of the countercurrent run the solvent mixture from each tube was homogenized by the addition of a small quantity of ethanol. The optical density of the solution at 2775 Å was determined in a Beckman DU spectrophotometer. This method has been used extensively for following the distribution of ACTH in counter-current extraction (Kuehl, Meisinger, Brink & Folkers, 1953). The solute was recovered by concentrating the solution, under reduced pressure in an atmosphere of nitrogen, to about 1 ml. volume, transferring the concentrate to a 100 ml. graduated cylinder with the aid of 4 ml. of methanol, and precipitating the solute by addition of 95 ml. of ethyl acetate. The precipitate was collected by centrifuging and dried in a desiccator.

Potentiation of the melanophore-expanding activity by treatment

A sample (1 mg.) of the purified product was dissolved in 1 ml. of 0.1 N-NaOH and placed in a boiling-water bath for 15 min. The solution, cooled to room temperature, was neutralized to pH 7 with 0.1 N-HCl and assayed with appropriate dilution for melanophore-expanding potency.

Assay of melanophore-expanding activity

The potency of the material, after appropriate dilution, was assayed in the intact frog (*Rana pipiens*). Each preparation was assayed three times on four frogs at various dose levels. The degree and the duration of the darkening of the skin colour were taken as criteria of the response. A small, known quantity of the solution, diluted to 1 ml. with 0.8% NaCl, was injected into the dorsal-lymph sac. A solution of the International Standard Posterior Pituitary Powder, prepared according to the stipulations of the British Pharmacopoeia, was used as the standard. In keeping with the definition of the unit of oxytocic and vasopressor activity, 1 mg. of this standard powder is considered to be equivalent to 2 i.u.

ACTH assay

The assays for adrenocorticotrophic potency of the fractions were performed by Saffran & Schally (1955), with their *in vitro* method. Before being assayed the material prepared from hog pituitary was treated with hydrogen sulphide to insure against loss of activity (Kuehl *et al.* 1953).

Determination of amino acid composition

A small quantity (2–4 mg.) of the powder was dissolved in 0.2–0.4 ml. of 6 N-HCl in a 1 ml. ampoule. The ampoule was sealed and heated at 100° for 22 hr. The contents were evaporated *in vacuo* over solid sodium hydroxide and anhydrous calcium chloride. The residues were dissolved in sufficient water to give a concentration of 0.15 mg./0.02 ml. of solution. By the use of this quantity of the solution the amino acids were separated by paper chromatography as described in a previous publication (Benfey, 1953), with 0.5% ninhydrin in absolute ethanol as the indicator. After a period of 24 hr. for colour development, the stained areas were cut out and transferred to 15 ml. centrifuge tubes containing 7 ml. of 50% *n*-propanol to elute the colour. The optical density of the solution was determined at 5700 Å for all the amino acids except proline, which was

read at 3500 Å. As standards for comparison solutions of pure amino acids in quantities equivalent to 0.07 μmole of amino acid were chromatographed. Three chromatographic runs were carried out with each solvent system, each unknown being accompanied by a standard mixture according to the method of Wellington (1952). With limited facilities this procedure affords satisfactory quantitative estimations of the amino acids. Tryptophan was determined according to the method of Spies & Chambers (1948).

RESULTS

Hog-pituitary extract

A plot of the spectrophotometric absorption of the contents of the individual units of the counter-current apparatus in our preliminary runs revealed two peaks. The fractions represented by one peak were found to contain the melanophore-expanding material and those corresponding to the other peak the adrenocorticotrophic activity. When 660 mg. of the purified hog-pituitary powder was used, 180 transfers were applied in the large Craig apparatus with equal volumes (100 ml.) of 0.5% trichloroacetic acid and *sec.*-butanol as the solvent system. The units (tubes), 69–71 inclusive in this series, were found to contain the highest melanophore activity. The calculated partition ratio (*K*) of the contents was 0.64, and after removal of the solvents 16.2 mg. of material was obtained. The

Fig. 1. Analytical redistribution of hog melanophore-expanding hormone between 0.5% trichloroacetic acid and *sec.*-butanol. ●, Experimental; ○, calculated.

melanophore-expanding activity of the product was 2500 units/mg. and the ACTH potency was less than 0.1 i.u./mg. By analysis of the material in the 24-tube apparatus [with equal volumes (8 ml.) of the above-mentioned solvents] the plot shown in Fig. 1 was obtained.

Comparison of the experimental curve with the theoretical calculated curve indicates a purity of 80–85% for the sample of melanophore hormone examined. The material from the 'peak' tubes (nos. 6–10) of the 24-tube transfer after removal of the solvents was used for the amino acid determinations and the biological assay. The amino acid composition of the melanophore-expanding material from hog pituitary is indicated in Table 1.

jected to 100 transfers with equal volumes (100 ml.) of the above-mentioned solvents. Tubes 41–44 inclusive in this series contained the highest melanophore potency. The partition ratio was 0.74. The combined contents of the tubes, on evaporation of the solvents, yielded 18 mg. of dry residue which assayed 450 units/mg. An ACTH assay was not made on the material but, considering that a previous assay (Benfey, Saffran & Schally, 1954) on Stehle's preparation had indicated an ACTH potency of only 0.065 i.u./mg., it is safe to conclude that the activity of the fraction obtained after countercurrent purification probably was negligible. The plot of the data obtained from an analytical redistribution of the most potent material

Table 1. *Amino acid composition of the melanophore-expanding hormone from hog and ox pituitary*

Amino acid	Hog-pituitary extract			Ox-pituitary extract		
	% by wt.	Molar ratio	No. of residues/mol.	% by wt.	Molar ratio	No. of residues/mol.
Alanine	0.2	0.2	—	2.6	2.9	3
Arginine	4.2	1.9	2	5.2	3.0	3
Aspartic acid	5.8	3.5	4	4.1	3.1	3
Cystine	0.3	0.1	—	1.6	0.7	1
Glutamic acid	7.3	4.0	4	7.5	5.1	5
Glycine	2.2	2.3	2	2.5	3.3	3
Histidine	2.9	1.5	2	4.2	2.7	3
Isoleucine	—	—	—	0.9	0.7	—
Leucine	0.3	0.2	—	3.7	2.8	3
Lysine	6.7	3.7	4	5.8	4.0	4
Methionine	1.6	0.9	1	0.8	0.5	1
Phenylalanine	3.7	1.8	2	5.3	3.2	3
Proline	4.9	3.4	3	3.8	3.3	3
Serine	2.2	1.7	2	2.3	2.2	2
Threonine	—	—	—	1.5	1.3	1
Tryptophan	5.3	2.1	2	3.7	1.8	2
Tyrosine	3.8	1.7	2	2.7	1.5	2
Valine	0.5	0.3	—	6.1	5.2	5

The tubes 166–168 inclusive in this series, on the other hand, were found to contain the highest ACTH activity. The combined contents of these tubes yielded 47 mg. of dry material which assayed only 20 units of melanophore-expanding potency/mg. and 14–15 i.u. of ACTH activity/mg. The plot of the data obtained from an analytical redistribution of the material between equal volumes (8 ml.) of 0.1% trichloroacetic acid and *sec.*-butanol in the small Craig apparatus revealed that the material did not behave as a single substance. The partition ratio in this solvent system was approximately 1.

Ox-pituitary extract

A plot of the spectrophotometric data obtained with Stehle's ox-pituitary powder after countercurrent distribution between 0.1% trichloroacetic acid and *sec.*-butanol revealed a single broad peak. In a larger run 317 mg. of the material was sub-

jected to 100 transfers with equal volumes (8 ml.) of a 0.1% trichloroacetic acid and *sec.*-butanol in the small Craig unit is shown in Fig. 2. The amino acid analysis of material from the 'peak' tubes (nos. 9–13) of the 24-tube transfer is indicated in Table 1. The partition ratios of the various preparations are given in Table 2.

It is of interest that treatment of the highly purified fraction from hog pituitary with alkali produced no increase in the activity whereas with the highly purified fraction from ox-pituitary alkali treatment produced approximately a threefold increase in the potency.

DISCUSSION

Our ox and hog preparations are clearly different. One difference is the behaviour of the two preparations towards weak alkali. (As yet there is no explanation why the activity of certain melano-

phore-hormone preparations might be increased by treatment with alkali.) Secondly, the potency of the ox preparation (450 units/mg.) is much lower than that of the hog preparation (2500 units/mg.). This is approximately a fivefold difference in potency. The same ratio is seen in the starting acetone powder. Another striking difference is in the partition ratios between trichloroacetic acid and *sec.*-butanol. Further it seems that, from the amount of material which can be accounted for by the amino acid analysis (Table 1), the hog preparation contains more trichloroacetic acid than the ox preparation. (The preparations are in the form of trichloroacetic acid salts. Removal of the bound acid would accordingly result in increased potency/unit weight of material.) On the basis of the amino acid data the molar ratios have been calculated (Table 1). From these values it may be inferred

that the minimum molecular weight of the hog preparation is approximately 3800 and that of the beef preparation 5600. Finally, the amino acid composition of our two preparations is different. Major differences between the two melanophore preparations are evident in the amounts of the amino acids alanine, leucine, threonine and valine. These differences between the two preparations may be due to different methods of production. It is possible that the method of purification influences the final product, since peptides are highly sensitive material. But it is also possible and probable that more than one peptide which exhibits melanophore potency may be isolated from pituitary tissue.

In comparing our hog preparation with that of Lerner & Lee (1955), major differences in the amounts of the following amino acids are evident: alanine, cystine, histidine, leucine, methionine, threonine and valine. Although the two preparations appear to possess similar potencies, the differences in their amino acid composition lead the authors to conclude that the two preparations are not alike.

It has been claimed that the melanophore-expanding and the adrenocorticotrophic hormone are identical, and supposedly pure ACTH preparations, including corticotropin B, have been reported to contain a high degree of melanophore potency (Brink *et al.* 1953; Winter, Brink & Folkers, 1953; Bell, 1954). Our countercurrent-distribution experiment with the hog material achieved a clear separation of the two biological activities. In addition, our ox-melanophore hormone contains only a trace of ACTH activity. This evidence leads us to conclude that the two hormones are not identical. [Raben *et al.* (1952) indicated that a separation of melanophore hormone and ACTH is possible by solvent partition, but this note has never been followed by any detailed description.] Apparently, in isolating corticotropin B, Kuehl *et al.* (1953), were not able to separate the two activities. As can be seen from Table 2, the partition ratio of our hog preparation is quite similar to that of corticotropin B (which is obtained from corticotropin by pepsin hydrolysis). Our ACTH preparation, on the other hand,

Fig. 2. Analytical redistribution of ox melanophore-expanding hormone between 0.1% trichloroacetic acid and *sec.*-butanol. ●, Experimental; ○, calculated.

Table 2. Partition ratios of melanophore-expanding and adrenocorticotrophic hormone preparations between 0.1 and 0.5% trichloroacetic acid and *sec.*-butanol

	0.1% trichloro- acetic acid	0.5% trichloro- acetic acid
Hog melanophore-expanding hormone	—	0.45–0.64
Corticotropin B (Kuehl <i>et al.</i> 1953)	—	0.5–0.6
Hog adrenocorticotrophic hormone	1	7
Corticotropin (Kuehl <i>et al.</i> 1953)	1.31	7
Ox melanophore-expanding hormone	0.74–0.85	7

Table 3. *Amino acid composition of melanophore-expanding and adrenocorticotrophic hormone preparations*

Molar ratios calculated for an arbitrary total of 50 amino acid residues per molecule.

Amino acids	Melanophore hormone			Corticotrophic hormone	
	Ox	Hog*	Hog†	Corticotropin B hog‡	Corticotropin A hog§
Alanine	2.9	0.3	2.1	1.8	4.0
Arginine	3.0	3.2	3.0	5.2	4.0
Aspartic acid	3.1	6.0	3.8	3.3	3.0
Cystine	0.7	0.2	2.9	—	—
Glutamic acid	5.1	6.8	4.7	4.6	7.8
Glycine	3.3	3.9	4.1	4.8	4.0
Histidine	2.7	2.6	0.5	1.6	1.7
Isoleucine	0.7	—	—	—	—
Leucine	2.8	0.3	1.7	—	3.0
Lysine	4.0	6.3	4.7	6.4	5.3
Methionine	0.5	1.5	—	1.4	1.0
Phenylalanine	3.2	3.1	3.7	2.3	3.8
Proline	3.3	5.8	4.2	4.1	3.8
Serine	2.2	2.9	4.2	3.4	2.9
Threonine	1.3	—	1.4	—	—
Tryptophan	1.8	3.6	2.6	3.0	1.9
Tyrosine	1.5	2.9	3.0	3.1	1.9
Valine	5.2	0.5	3.3	4.7	3.2

* From Table 1, multiplied by 1.7.

† Percentage as given by Lerner & Lee (1955), multiplied by 131 and divided by the molecular weight of the amino acids.

‡ Data given by Brink *et al.* (1953), for the preparation isolated by countercurrent distribution, multiplied by 0.8.

§ Data given by Hays & White (1954) for the preparation 384-6-I, multiplied by 1.9.

behaves like unhydrolysed corticotropin. From this it may be inferred that the hog-melanophore hormone cannot be separated from corticotropin B in this solvent system but can be separated completely from corticotropin. This may account for the fact that corticotropin B possesses a high melanophore potency. (It has been known for many years that anterior pituitary lobes, which serve as source material for the isolation of ACTH, contain some melanophore activity.)

Most authors, including Lerner & Lee (1955) and Brink *et al.* (1953), report melanophore potency in terms of arbitrary units which are not related to Posterior Pituitary Standard Powder. This makes it difficult to compare the melanophore data of different authors.

The molar ratios of the component amino acids of various melanophore-hormone and ACTH preparations are listed in Table 3. For purposes of comparison they are recalculated to give an arbitrary total of 50 amino acid residues/molecule. From this it can be seen that our hog preparation differs from corticotropin B mainly by the absence of alanine and valine. Apart from these two amino acids the preparations are quite similar. In general, as can be seen from Table 3, melanophore and ACTH preparations show many common features in their amino acid composition.

SUMMARY

1. The melanophore-expanding hormone has been isolated in highly purified form from ox and

hog pituitary glands by countercurrent distribution. The materials are virtually free from adrenocorticotrophic hormone, which has also been segregated.

2. An amino acid analysis and other evidence reveals numerous differences between the preparations from the two sources.

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REFERENCES

- Astwood, E. B., Raben, M. S., Payne, R. W. & Grady, A. B. (1951). *J. Amer. chem. Soc.* **73**, 2969.
- Bell, P. H. (1954). *J. Amer. chem. Soc.* **76**, 5565.
- Benfey, B. G. (1953). *Brit. J. Pharmacol.* **8**, 435.
- Benfey, B. G., Saffran, M. & Schally, A. V. (1954). *Nature, Lond.*, **174**, 1106.
- Brink, N. G., Boxer, G. E., Jelinek, V. C., Kuehl, F. A. jun., Richter, J. W. & Folkers, K. (1953). *J. Amer. chem. Soc.* **75**, 1960.

- Hays, E. E. & White, W. F. (1954). *Recent Prog. Hormone Res.* **10**, 265.
- Kuehl, F. A. jun., Meisinger, M. A. P., Brink, N. G. & Folkers, K. (1953). *J. Amer. chem. Soc.* **75**, 1955.
- Landgrebe, F. W. & Mitchell, G. M. (1954). *Quart. J. exp. Physiol.* **39**, 11.
- Lerner, A. B. & Lee, T. H. (1955). *J. Amer. chem. Soc.* **77**, 1066.
- Payne, R. W., Raben, M. S. & Astwood, E. B. (1950). *J. biol. Chem.* **187**, 719.
- Raben, M. S., Rosenberg, I. N. & Astwood, E. B. (1952). *Fed. Proc.* **11**, 126.
- Saffran, M. & Schally, A. V. (1955). *Endocrinology*, **56**, 523.
- Spies, J. R. & Chambers, D. C. (1948). *Analyt. Chem.* **20**, 30.
- Stehle, R. L. (1944). *Rev. canad. Biol.* **3**, 408.
- Wellington, E. F. (1952). *Canad. J. Chem.* **30**, 581.
- Winter, C. A., Brink, N. G. & Folkers, K. (1953). *Proc. Soc. exp. Biol., N.Y.*, **82**, 365.

Studies on the Skeletal Tissues

4. THE RENEWAL OF INORGANIC PHOSPHATE IN BONES OF VARIOUS SPECIES OF SMALL MAMMAL AS A FUNCTION OF TIME*

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The uptake of ^{32}P and ^{45}Ca by bones varies considerably with type, area and age of the tissue. Differences according to bone type (diaphysis, metaphysis, alveolus) are well established (Rogers & Weidmann, 1951); the faster rate of phosphorus renewal in *spongiosa* as compared with *compacta* was shown to be statistically significant in various species of small mammals (Rogers, Weidmann & Jones, 1953). Variations in the absorption of isotopes in the different areas of bone shaft were clearly demonstrated by radioautography (Amprino, 1952; Engfeldt, Engström & Zetterström, 1952) and also by measurements of radioactivity (Rogers *et al.* 1953). The age of bone greatly influences the rate of isotope incorporation. The uptake of minerals is much quicker in the developing bones of the young than in the mature bones of the adult animal (Leblond, Wilkinson, Bélanger & Robichon, 1950).

These variations were attributed to local structural differences in the bone tissue, such as differences in micro-structure (Amprino, 1952), crystal size (Hevesy, Levy & Rebbe, 1940), vascularity (Neuman & Riley, 1947), but also to differences in the chemical composition, e.g. degree of calcification (Hevesy *et al.* 1940; Amprino, 1952).

Isotope incorporation is comparatively rapid immediately after the administration of the tracer, and slows down markedly within a few hours. Hevesy *et al.* (1940) explained the initial phase as an ion-exchange reaction and the subsequent slower phase as a biological recrystallization. It was thought that the rate of reaction in the initial

phase decreased with the exhaustion of the easily exchangeable fraction of bone-salt crystallites, and that the second phase was entirely governed by the rate of recrystallization. Neuman & Neuman (1953) defined recrystallization more explicitly as an ionic-diffusion through the solid crystal. One of the main reasons for regarding the initial uptake as an ion-exchange absorption was that the reaction was too fast to be accounted for by new bone formation and could, therefore, only indicate that an equilibrium was quickly established between the exchangeable ions of the surface area of the bone and the blood minerals. Bauer (1954), however, explained the decrease in turnover rates of bone salt by the rapid fall of isotope concentration in the blood. Since bone and blood minerals are in a dynamic equilibrium, bone deposits formed in the early phase of the reaction, when the tracer concentration of the blood is still high, contain more isotope than those formed in the later stages.

Radioautographic pictures showed heavy concentrations of ^{32}P and ^{45}Ca on the outside surfaces of the shaft, and fewer deposits in the Haversian systems. The areas of high tracer concentration coincided with areas of low mineral-salt content. It was suggested (Amprino, 1952; Engfeldt *et al.* 1952) that the turnover rate is proportional to the relative amount of recently formed but not yet fully calcified bone. The radioautographic findings were later confirmed by radioactivity measurements in bone-powder samples taken from the various regions of the shaft bone of the rabbit (Rogers *et al.* 1953). Significant differences were found in the turnover rates of phosphorus between subperiosteal, 'middle' and endosteal layers. These

* Part 3: Rogers, Weidmann & Jones (1953).