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## Extracts of Anterior Pituitary Growth Hormone.

AMOS E. LIGHT, EDWIN J. deBEER AND CHARLES A. COOK. (Introduced by L. Reiner.)

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The solvent action of phosphate buffer, urea and sodium hydroxide solutions in extracting the growth principle of the anterior pituitary gland was investigated with the aid of a comparatively simple bioassay method.[1] The potency of each extract was determined and expressed as mg per cc of a standard anterior lobe powder. The percent yield was calculated by dividing the potency by the number of mg of powder used per cc in making the extract.*

To facilitate comparison, all extracts were prepared in as uniform a manner as possible from the same desiccated, powdered anterior lobe substance† which also served as the standard for all assays. For example, phosphate buffer extract No. 1 (Table I) was prepared by extracting 25 g of powder with 200 cc of a solution (pH 8) containing 5.616 g of anhydrous $Na_2HPO_4$ and 0.302 g of anhydrous $KH_2PO_4$ per liter. After 3 hours of continuous stirring at 10°C, the insoluble residue was separated by centrifuging and successively extracted with three 125 cc volumes of buffer solution. The combined extract, which had a pH of 7.5, was filtered through cellulose and asbestos pads, sterilized by filtration through a Seitz apparatus and stored at 10°C in sterile rubber-capped bottles. After

[1] Light, A. E., deBeer, E. J., and Cook, C. A., in press.

* Inspection indicates that the dose-response curve (Fig. 1) for the extracts resembles that for the powder suspension. An experiment sufficiently elaborate to establish the linearity of the relationship between log dose and response will be reserved for a selected extract since it is obviously impractical to do this for each extract. However, at least 2 and often 4 graded doses of each extract were given (intraperitoneally) thus establishing the slope for each extract curve. The standard was employed in each assay. In the following example, the standard contains 5 mg of powder per cc.

| Standard | | Extract 8D | |
|---|---|---|---|
| Dose cc/100 g rat | Response % gain in wt | Dose cc/100 g rat | Response % gain in wt |
| 0.05 | 1.3 | 0.01 | 2.0 |
| 0.10 | 3.0 | 0.02 | 3.4 |

Therefore, 1 cc of extract containing about 10 mg of protein is equivalent to 6.4 cc or 32 mg of standard powder.

† Burroughs Wellcome & Co. (The Wellcome Foundation, Ltd.), London.

TABLE I.
Extracts of Anterior Pituitary Growth Hormone.

| No. | Preparation | Mean potency* mg/cc | Mean Yield % | Limits of error as % of mean Lower % | Limits of error as % of mean Upper % | Protein Trichlor-acetic acid % | Total solids % | Ash % | Nitrogen (Kjeldahl) % |
|---|---|---|---|---|---|---|---|---|---|
| | Desiccated anterior lobe (standard) | | | | | | 88.0 | 5.0 | 11.5 |
| 1 | Phosphate buffer | 50.0 | 100 | 58 | 173 | 1.18 | 1.57 | 0.52 | 0.13 |
| 2 | Phosphate buffer | 39.2 | 78 | 51 | 196 | 1.43 | 1.80 | 0.70 | 0.14 |
| 3 | NaOH | 25.8 | 52 | 61 | 165 | 1.47 | 1.42 | 0.40 | 0.14 |
| 4 | 1% Urea | 23.4 | 47 | 69 | 145 | 1.00 | | | |
| 5 | 5% " | 20.0 | 40 | 58 | 172 | 1.00 | | | |
| 6 | 10% " | 50.0 | 100 | 59 | 168 | 0.99 | | | |
| 6D | 10% " | 36.8 | 74 | 53 | 189 | 0.99 | | | |
| 7 | 20% " | 30.5 | 61 | 64 | 156 | 1.15 | | | |
| 8D | 10% " | 32.0 | 64 | 69 | 145 | 1.21 | 1.63 | 0.54 | 0.32† |
| 9 | 10% " (pH 9) | 35.4 | 71 | 66 | 151 | 1.63 | | | |
| 9D | 10% " | 33.8 | 68 | 75 | 134 | 1.12 | | | 0.31† |
| 10‡ | 10% " | 19.2 | 77 | 58 | 173 | 1.00 | | | |
| 11 | 1% Guanidine | 20.3 | 41 | 56 | 177 | | | | |

* Mean potency determined in terms of mg of standard powder per cc of extract.
† 0.28% urea still present.
‡ Double volume.
D Dialyzed to remove urea.

several weeks a precipitate appeared in this extract. The high yield indicates that most of the active material was extracted. About 1% of the extract was organic matter, probably protein. This figure was approximated by 3 different methods, *i.e.*, by trichloracetic acid precipitation, by calculating the total N as protein and by subtracting the ash, which was largely due to buffer salts, from the total solids.

In contrast to the buffer extracts, the gelatinous nature of the extracts prepared with dilute aqueous NaOH solutions made them very difficult to clarify by filtration. Furthermore, a considerable precipitate formed after one week of storage. In preparing this type of extract, NaOH was added at intervals in order to maintain the pH at 7.5. These extracts were not as uniform in potency as those obtained with phosphate buffers.

Urea, in high concentrations has been shown to bring about remarkable changes in the chemical and physical properties of certain proteins.[2, 3] Accordingly, a study was made of the properties of phosphate buffer extracts containing 1%, 5%, 10%, and 20% urea. (Table I.) These preparations were of about the same potency as the phosphate buffer extracts. The addition of urea in concentrations as low as 1% or 5% retarded precipitation for several months, particularly if the urea were added just before filtration through a Seitz apparatus. When the concentration of urea was 10% or 20%,

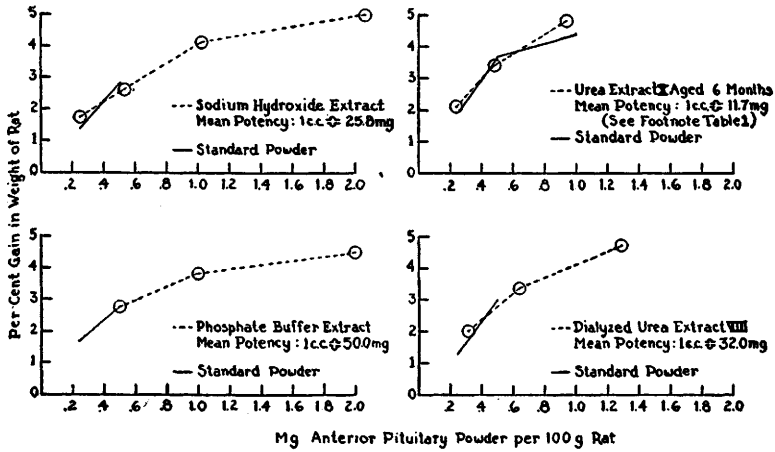


FIG. 1.

**Dose response curves for anterior pituitary extracts in terms of standard powder and compared with standard powder curves.**

[2] Steinhardt, J., *J. Biol. Chem.*, 1938, **123**, 543.

[3] Greenstein, J. P., *Ibid.*, 1939, **128**, 233.

the extracts were still clear at the end of 6 months. When extracts 6 and 9 were dialyzed through cellophane membranes about 95% of the urea was removed and precipitates appeared in the corresponding preparations, 6D and 9D, in about 3 weeks.

As extracts 9 and 10 indicate, it appeared unnecessary to increase either pH or volume in order to improve the efficiency of the extraction. The 1% guanidine extract yielded an amount of hormone similar to that of the 1% urea.

*Summary.* Phosphate buffer extracts of growth hormone were highly active when assayed in terms of anterior pituitary powder. This method of extraction permitted a careful control of pH and gave high yields of hormone. These extracts had less tendency to form precipitates than those prepared with sodium hydroxide. The addition of urea retarded such precipitation.

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## Biological Assay of Anterior Pituitary Growth Hormone.

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Normal animals have been employed in the assay of growth hormone by Evans *et al.*,[1] Van Dyke and coworkers[2] and Lee.[3] Inherent limitations of such an assay method have emphasized the importance of statistical treatment of the data. Bülbring,[4] working with hypophysectomized rats, has utilized the rapidly rising portion of a dose-response curve and has reported results which indicated relatively low limits of error.

In order to avoid the complex metabolic derangements associated with an extirpation of the entire pituitary gland, an assay procedure was developed in which groups of normal rats were used to determine the increased body weight resulting from administration of

1 Evans, H. M., Uyei, N., Bartz, Q. R., and Simpson, M. E., *Endocrinology*, 1938, **22**, 483.

2 Chou, C., Chang, C., Chen, G., and Van Dyke, H. B., *Ibid.*, 322.

3 The Pituitary Gland, Proc. Assn. for Research in Nervous and Mental Disease, **17**, Williams & Wilkins, Baltimore, 1938, 216.

4 Bülbring, E., *Quart. J. Pharm. and Pharmacol.*, 1938, **11**, 26.