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.

## 11398

## Biological Assay of Anterior Pituitary Growth Hormone.

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(Introduced by L. Reiner.)

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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.

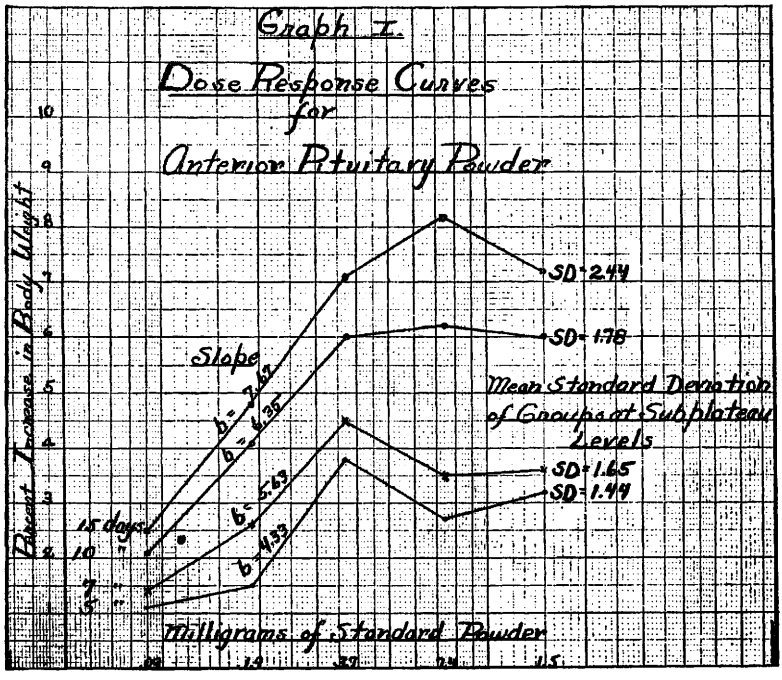
3 The Pituitary Gland, Proc. Assn. for Research in Nervous and Mental Disease, **17**, Williams & Wilkins, Baltimore, 1938, 216.

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

growth hormone preparations. Groups of 15 normal, plateaued, female rats, approximately 5 months of age, were selected with respect to strain (Yale), weight (240-300 g) and diet (Fox Chow). Three preliminary weighings during a 5-day period were necessary to establish the fact that a plateau level in the normal growth curve, essential for the assay method, had been reached. To accustom the animals to the standardized routine treatment, injections were also made at these times with saline, phosphate buffer (pH 8) or 10% urea in 0.04 molar buffer solution.

Well-defined dose-response curves were obtained when daily, graded doses of a saline suspension of desiccated anterior lobe substance* were injected intraperitoneally (Graph I). A sufficient quantity of this material was also available to serve as a standard for subsequent assays. Statistical analysis of a curve based on results obtained with 165 rats revealed that on the steeply ascending portion of the curve, below the plateau level, the per cent increase in body weight was a linear function of the logarithm of the dose.

In routine assays, 2 groups were injected with sub-plateau doses




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

of the standard powder* and two with the experimental extract. For each preparation the ratio of the greater dose to the smaller should be at least 2 to 1. The standard powder was suspended in a saline, buffer or urea solution and the assay data from 5 daily injections were used to calculate the potency of experimental preparations in terms of milligrams of the standard powder. Final group weights were recorded 120 hours after the initial injections of either the standard powder or the extracts. The groups were also weighed daily at the time of injection for evidence of any irregularity in body weight. Control groups injected with saline, 10% buffer solution or various doses of an inactive pituitary extract showed no significant changes in body weight.

Gaddum[5] has shown that the statistical evaluation of biossays involving a linear relationship and the use of a standard substance can be greatly simplified by following the plan given below. Using 4 groups, each containing an equal number of animals, let $X_{11}$, $X_{12}$ be 2 doses of the standard preparation and $X_{21}$, $X_{22}$ the doses of the test preparation, and the corresponding mean responses of each group be $Y_{11}$, $Y_{12}$, $Y_{21}$ and $Y_{22}$. The responses were expressed as per cent gain in body weight. Let d, the log of the ratio of the greater to the smaller dose, *i. e.*, $\frac{X_{22}}{X_{21}}$, be the same for each preparation. The following equation will then express the potency of the test preparation in terms of cc of the standard solution.

$$\text{1 cc of test solution} = \frac{X_{11}}{X_{21}} \text{ antilog } \left(\frac{Y_{21} + Y_{22}}{2b} - \frac{Y_{11} + Y_{12}}{2b}\right)$$

The term b, or slope, serves to convert each average response, *i. e.*, $\frac{Y_{21} + Y_{22}}{2}$ and $\frac{Y_{11} + Y_{12}}{2}$, into the logarithms of the corresponding average doses. Since the resulting terms are logarithmic, the difference between the two values represents the antilog of the ratio of the two average doses. The average slope, b, is given by the expression,

$$b = \frac{1}{2}\left(\frac{Y_{22} - Y_{21}}{d} + \frac{Y_{12} - Y_{11}}{d}\right).$$

The standard error of the assay, expressed logarithmically, is $\text{S.E.} = \frac{\text{S.D.}}{b}\frac{1}{\sqrt{n}}$, where S.D. is the standard deviation, b is the slope and n is the number of animals in a single group. When restricted to the sub-plateau levels, the standard deviation was independent of the size of the dose. The limits of error within which the calculated

[5] Gaddum, J. H., Med. Res. Council, Special Report Series No. 183, 1933, 31.

potency should fall 21 out of 22 times, can be obtained by dividing the potency by the antilog of 2 S.E. for the lower limit and multiplying the potency by the antilog of 2 S.E. for the upper limit.

As shown in Graph I, a longer period of treatment results in an increased slope of the sub-plateau portion of the curve. This would tend to narrow the limits of error but it is partly offset by an increase in the standard deviation. The limits of error when calculated as described above were 67-149, 70-142, 71-140 and 68-147% of the mean potency for the 5-, 7-, 10- and 15-day periods of treatment, respectively. These limits of error have been confirmed by additional assays with normal rats. The average of 31 standard deviations was 1.6 for the 5-day assay. In view of these results, the 5-day period was used for routine assays. Bülbring's data from hypophysectomized rats gave limits of 49-206% for the 7-day period when calculated as above. These wider limits are largely due to the fact that Bülbring used only 5 animals per group instead of 15 as used in the present study, since the ratio $\frac{\text{S.D.}}{b}$ is approximately the same for both sets of data. Bülbring's ratio is $\frac{4.95}{14}$ or 0.354. The corresponding figures from Graph I are $\frac{1.65}{5.63}$ or 0.293. The actual values for standard deviation and slope are not comparable since Bülbring expresses the response in grams, while in Graph I response is given in per cent.

In comparing 2 preparations assayed against the same standard, but at different times, the standard error, by the formula[6] for the standard error of the difference of 2 means is

$$\text{S.E.}_{(M_1 - M_2)} = \sqrt{(\text{S.E.}_{M_1})^2 + (\text{S.E.}_{M_2})^2}$$

is the $\text{S.E.}_{M_1}$ is the standard error of one assay and $\text{S.E.}_{M_2}$ where standard error of the other assay. Since these values are approximately equal, the standard error is the $\sqrt{2}$ or 1.41 times the standard error of a single assay, which for a 5-day period gives limits of about 57-174%. This means that for 2 preparations, each assayed against the same standard powder, one must be nearly twice as potent as the other in order to be considered significantly different. Evidence is being accumulated as to the stability of the hormone in the anterior pituitary powders and in experimental extracts.

[6] Coward, K. H., *Biological Standardisation of the Vitamins*, Balliere, Tindall and Cox, London, 1938, 166.

*Summary.* A selected anterior lobe powder from ox pituitary was used successfully as a standard of reference for one year in an assay procedure employing normal rats. By restricting the biological comparison to the steeper portion of the dose-response curves it was possible to obtain relatively low limits of error.

## 11399 P

## Application in Man of Method for Continuous Reciprocal Transfusion of Blood.*†

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Purified heparin, by prolonging the coagulation time of blood, has reduced the technical difficulties and made feasible the continuous reciprocal transfusion of blood in man. Thalhimer, Solandt and Best,[1] using a uremic and a normal dog and employing purified heparin as an anticoagulant, conducted a reciprocal transfusion for 27 hours, reducing the blood urea from high levels to normal without injury to the normal dog. Prinzmetal[2] carried out exchange transfusions in the investigation of arterial hypertension in patients with inoperable cancer. We have been unable however, to find any instance in which the work of Thalhimer *et al.* was applied in man.

Heparin (10,000 Toronto units in 1,000 cc normal saline) was given intravenously at approximately the rate of 40 drops per minute to the normal donor and to the patient for 20 minutes before and throughout the transfusion. An additional 2,000 units were given intravenously to each individual as the transfusion was started, and repeated 30 minutes later. This maintained the blood coagulation time between 20 and 30 minutes. The median basilic veins of the

* The authors are indebted to Dr. William A. Wolff, Chemist to the Pennsylvania Hospital, for valuable suggestions on the chemical aspects of this problem.

† Since this paper was submitted for publication a transfusion was carried out in which 26,770 cc were exchanged in approximately 5 hours. The total nitrogen excretion in the urine increased from 548 mg per hour before to 851 mg per hour during the tranfusion.

1 Thalhimer, W., Soldant, D. G., and Best, C. H., *Lancet*, 1938, **2**, 554.

2 Prinzmetal, M., Friedman, B., and Rosenthal, N., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 545.