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A Proposed Modification in the Official Method for the Assay of Posterior Pituitary Solution*

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INTRODUCTION

In compliance with a request of the United States Pharmacopœia Revision Committee, this laboratory has undertaken a critical evaluation of the official guinea pig uterine method (1) for the assay of posterior pituitary extracts. This is essentially the same as the method of Dale and Laidlaw (2). That portion of the present U. S. P. monograph on Solution of Posterior Pituitary, which describes the assay of the preparation, is inadequate in several respects. The objection, which has been raised by many, is that the description of the procedure and apparatus lacks detail.

Those portions of the procedure which stand most in need of amplification or revision are: (*a*) the preparation of the Standard Solution, (*b*) the description of the guinea pig uterus best suited for assay purposes, (*c*) the description of the apparatus, (*d*) the technique involved in performing the assay, and (*e*) the definition of what constitutes an assay.

Improvements in the technique of the assay, itself, must await completion of investigations which are now in progress. This report deals only with the question of the definition of what constitutes an assay. The present Pharmacopœial monograph specifically requires that an assay shall consist of "equal, submaximal contractions in two successive pairs of contractions." This requirement fails to provide any means of testing the submaximal character of the contractions constituting the assay, and furthermore demands that they be equal without defining what shall be considered equal by law. In order to correct these defects a new and a more precise definition of what should constitute a Pharmacopœial assay is proposed. This suggested change in definition takes account of biological variation and also brings about greater uniformity in technique employed by different workers.

PROCEDURE

For the purpose of obtaining the desired information concerning the reliability and practicability of

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a new definition of what constitutes a posterior pituitary assay and to gather the opinions of experienced pituitary analysts regarding this definition, the coöperation of six other laboratories was requested. In this laboratory a posterior pituitary solution was prepared, according to Pharmacopœial specifications, from a powder of unknown potency and assayed for its oxytocic activity. This assay was carried out by the present official method, using the procedure as described with the single exception that the series of contractions considered as constituting the assay was obtained as follows:

> The doses of the standard and of the unknown solutions were determined, which, when administered alternately, elicited a series of four contractions of approximately the same height. Then a third dose of the standard solution 25 per cent greater than that used to produce the two preceding standard contractions was administered. The height of each of the five contractions was measured. The first four contractions were considered *submaximal* and *equivalent*, if the difference in height between the highest and lowest of these four was less than half the difference in height between the lowest of the four and the contraction resulting from the increased dose of the standard.

An example of such an assay is shown in Fig. 1. It may be seen that an assay obtained in this way shows that the first four contractions are submaximal and furthermore precisely defines the word *equivalent* as used in relating these contractions.

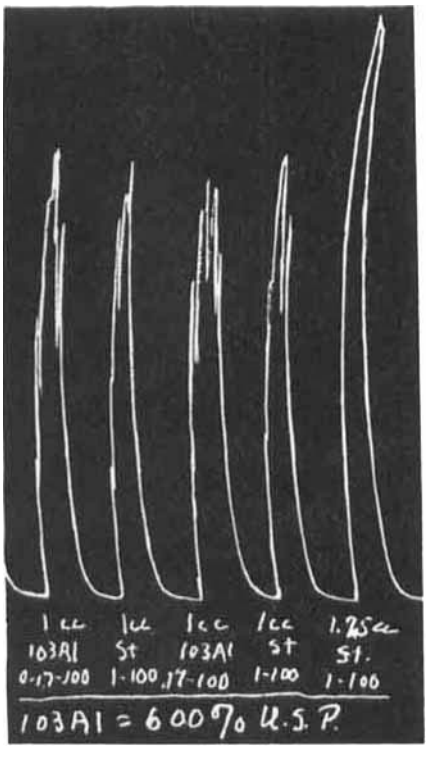

Fig. 1.—Kymograph Tracings—Pituitary Assays.

Portions of the unknown pituitary solution, prepared and assayed in this laboratory, and labeled *Q*, and also portions of the standard solution of this laboratory, labeled *S*, were sent to the coöperating assayists with the following requests: (*a*) an oxytocic assay of *Q* by the described method, using *S* as a standard; (*b*) a comparison of the standard solution of this laboratory with that of the coöperating laboratory, also by the described method; (*c*) a supply of the laboratory standard solution for comparison with our standard; (*d*) photostatic copies of the records of successful assays; and (*e*) an opinion of the reliability and feasibility of the suggested method.

It was thought that the data received in reply to these requests would supply information concerning: (*a*) the consistency of assay results obtained by the refinement of method under trial, and (*b*) the comparison between the potency of standard solutions prepared and used in various laboratories.

The results of the assays carried out according to the proposed procedure by the coöperating laboratories are set forth in Table I.

Table I.—Results of Coöperative Assays Obtained by Following the Proposed Technique[a]

Laboratory	Oxytocic Assays of *Q* (Per Cent of Standard)	Outside Assays of Standards with *S* as Reference (Per Cent)	U. of C. Assays of Standards with *S* as Reference (Per Cent)
1	75–80	100	100
2	77	100	100–110
3	75–80	94–100	100
4	85	. . .	100
5	80	100	100
6	85	. . .	. . .

[a] The following laboratories were asked to participate in the coöperative assay: Abbott Laboratories; Armour and Co.; Food and Drug Administration; Eli Lilly and Co.; Parke, Davis and Co.; The University of Chicago; Wilson Laboratories. One of these failed to report.

It may be noted from a study of the results obtained that the assays of the unknown *Q* carried out by the various laboratories checked very closely. The greatest variation between any two of the assays was 12 per cent. The standard solutions of all the coöperating laboratories were found to be of practically the same potency.

Each of the laboratories which had reported at the time of this communication stated that in their opinion the proposed method would give reliable results and would aid the analyst in obtaining an official assay.

In view of the evidence submitted of the reliability and practicability of the method described it has been recommended that an official posterior pituitary assay should be defined as follows:

> Determine the quantity of the standard and of the unknown solution which, when two doses of each are administered alternately, will elicit a series of four contractions of approximately the same height. Then administer a third dose of the

standard solution 25 per cent greater than that used to produce the two preceding standard contractions. Measure the height of each of the five contractions. The first four contractions are to be considered submaximal and equivalent if the difference in height between the highest and lowest of these four is less than half the difference in height between the lowest of the four and the contraction resulting from the increased dose of standard. Two such series of five contractions shall constitute an assay.

We wish to emphasize the fact that the record of an assay obtained in this way furnishes proof of the submaximal character of the first four contractions. Furthermore the definition makes clear exactly what is required by the term equivalent as used in regard to these four contractions.

It is further suggested that the wording in the rubric in regard to potency should be changed from "One cc. of Solution of Posterior Pituitary produces an activity upon the isolated uterus of the virgin guinea pig corresponding to not less than 80 per cent and not more than 120 per cent of that produced by 0.005 Gm. of the Standard Powdered Posterior Pituitary" to the following wording: "One cc. Solution of Posterior Pituitary produces an activity corresponding to that produced by 0.005 Gm. of the Standard Powdered Posterior Pituitary." A note worded as follows should then be added at the end of the assay procedure: "*Note*—Owing to the many variable factors in the assay of solution of posterior pituitary, evidence of potency in all assays of solution of posterior pituitary to within 20 per cent above or below the standard, is acceptable."

Further study of the problem of the assay of posterior pituitary extracts is now under way in this laboratory. Among the points under consideration are the following: (*a*) the composition of the bathing fluid; and (*b*) alternative or subsidiary methods of assay, namely, the pressor assay for the pressor principle using the anesthetized dog (3); a modification thereof using the anesthetized cat; and the blood pressure depressor assay of Coon (4) for the oxytocic principle using the anesthetized chicken. This latter method has been shown to be simple, speedy and reliable, and devoid of the many technical difficulties involved in the present official method.

SUMMARY

1. A refinement of the definition of what constitutes an official assay of solution of posterior pituitary is proposed.

2. The results of a coöperative study of the proposed definition carried out by six laboratories testify to its reliability and practicability.

3. A change is recommended in the wording of the rubric in regard to potency of solution of posterior pituitary.

4. A plan of future work on posterior pituitary assay is outlined.

REFERENCE

(1) "The Pharmacopœia of the United States of America," Eleventh Decennial Revision (1936), page 217.

(2) Dale, H. H., and Laidlaw, P. P., *J. Pharmacol.*, 4 (1912), 75.

(3) Hamilton, H. C., JOUR. A. PH. A., 1 (1912), 1117.

(4) Coon, J. M., *Arch. Int. de Pharm. et de Therap.*, 62 (1939), 79.

Laboratory Apparatus and Procedure for Preparing Permanent Records of Biological Vitamin D Assays*

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INTRODUCTION

The United States Pharmacopœia (1) supplies detailed information for conducting the official biological assay of cod liver oil and related substances for vitamin D. However, no suggestions are offered for preparing permanent records of the results of vitamin D assays. Since the results of the assays should be available for considerable periods of time subsequent to the conclusion of the assay, it is essential that these results be preserved in a permanent form, as free as possible from the personal factor of the assayist. Obviously, whenever there is a possibility of the results of vitamin D assays being used in a legal controversy or in court action their availability in a permanent form is of supreme importance. Accordingly, it seems desirable to describe the procedure and equipment used in this laboratory for preparing permanent records of vitamin D assays both to assist others who are conducting such assays and to assist, particularly, those who are just establishing vitamin D assay procedures.

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