THE STANDARD FOR ASSAY OF PITUITARY SOLUTION U. S. P. X.*

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The Bureau of Chemistry, through its Pharmacological Laboratory, has undertaken to provide Bio-Assay standards. These are to be prepared as described in U. S. P. X. and to be made available some time before it goes into effect. The standard pituitary powder has been prepared and is now ready for distribution.

On August 11, 1924, there were received from the laboratory of one of the large packing houses 115 grams of fresh posterior lobes of pituitary glands in acetone. These glands were at once worked up according to the suggested method of preparation of the official standard as follows:

The glands were cut into small bits with scissors, placed in about 1500 cubic centimeters of dry acetone and allowed to stand over night. The following day the acetone was decanted off, the material placed on watch glasses and dried in vacuum desiccators over fused calcium chloride at a temperature of 25° C. The following morning the dry material was rubbed up in a small agate mortar until it passed through a No. 40 sieve. For the large amount of glands used this was a somewhat laborious process. For smaller amounts it would not be so tedious. There was a small residue of connective tissue that did not grind up well and was discarded; this weighed 0.63 gram. The powdered material was again placed in the vacuum desiccators and dried over night. The following day it was transferred to two extraction thimbles and extracted for three hours with dry acetone in Soxhlet extractors. The powder was then spread on a large watch glass and dried in the vacuum desiccator over calcium chloride. For the present it has been preserved in this manner, with the desiccator kept in a light-proof cupboard. Later the powder is to be divided into small lots and placed in ampuls. The yield from 115 grams of fresh gland was 17.9 grams of powder.

This material was then compared with earlier preparations made by the same method, as follows:

On August 18, 1924, a solution of this powder was made in the following manner: Twenty-five milligrams were weighed out and transferred to a small agate mortar. A burette was filled with 0.25% acetic acid and the reading noted. One or two drops of acid were added to the powder from the burette and the mixture rubbed thoroughly with the pestle until it became of an "impalpable frothy consistency." It is necessary to be careful not to add too much acid at the beginning or the material will not rub up easily. After the material was thoroughly triturated, more acid was added until a uniform suspension resulted; three or four cubic centimeters of acid are sufficient for this purpose. The suspension was then transferred quantitatively to a small beaker, washing out the mortar with successive portions of dilute acid from the burette. Finally enough acid from the burette was added so that there was exactly one cubic centimeter for each milligram of powder taken. The beaker was covered with a watch glass and the contents brought to a boil. The

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suspension was then filtered through a small moist filter paper into a graduated cylinder. After cooling, 0.25% acetic acid was added through the filter to wash and to bring the solution exactly up to the original volume. There is a possibility of slight loss at this point by the retention of some active material in the fluid that remains in the paper. By using the dilute acid to wash and at the same time to replace the fluid lost by evaporation during boiling, the loss becomes exceedingly small. One cubic centimeter of this solution contains the activity of one milligram of the standard powder. One cubic centimeter portions of the clear fluid were then measured carefully into hard glass ampuls, which were sealed and sterilized in free steam for twenty minutes on three successive days. At the same time and by the same method, solutions were made of a similar powder prepared by the senior author in the Pharmacology Laboratory of the University of Michigan, December 17, 1923[1], and from one of the original powders of Smith and McCloskey,[2] received by the Bureau of Chemistry about January, 1924. When solutions of these three powders were compared by the isolated uterus method of assay, it was found that they were uniform in strength.

The uniformity of the various preparations made by Smith and McCloskey had of course been pointed out by them in their original publication[2] and was confirmed by Nelson[1], who also demonstrated the feasibility of repeating the process and obtaining powders of similar strength. It was in fact this possibility of reduplication that led to the recommendation that this preparation be adopted as the U. S. P. standard.[1] The fact, that by working with relatively large amounts of material it has again been possible to reduplicate this powder as shown by the fact that solutions of it have the same activity as similar solutions of the powders made by Smith and McCloskey, and by Nelson in another laboratory, is additional important confirmation of the conclusions previously reached, and should add to the confidence with which this standard is received by the manufacturer.*

It might be pointed out that the actual yield of dry powder per gram of fresh gland is the same as that obtained by Smith and McCloskey. They give as the average of seven preparations that one gram of powder is obtained from 6.4 grams of fresh gland. In this laboratory the yield from 115 grams was 17.9 grams of powder or one gram of powder for 6.4 grams of fresh gland by weight, exactly the figure obtained by the earlier authors. One gram of powder corresponds by assay to a little more than seven grams of fresh gland, instead of 6.4 grams. The U. S. P. X. requirement, that one cubic centimeter of finished pituitary solution shall have the same activity as that possessed by five milligrams of powder, then means that it shall contain the activity of about 35 milligrams of fresh gland, or be a 3.5% solution of fresh posterior lobe.

Summary.—There has been prepared in the Pharmacology Laboratory of the Bureau of Chemistry according to the proposed directions for the U. S. P. X. about eighteen grams of desiccated, defatted, powdered posterior lobe of the pituitary gland. This material corresponds in activity with other available samples prepared in other laboratories by the same method. It is hoped and believed that

[1] E. E. Nelson, JOUR. A. PH. A., XIII, 426, 1924.

[2] M. I. Smith and W. T. McCloskey, "Public Health Report," March 16, p. 493, 1923.

* Dr. H. H. Dale, in personal communications to Dr. C. W. Edmunds, reports that he finds a uniformity in successive preparations of this powder prepared in his laboratory.

these results will add to the confidence of the manufacturers of pituitary solution in the proposed U. S. P. X. standard and method of assay.

BIO-ASSAY OF VERATRUM PREPARATIONS.

BY L. W. ROWE.

Within recent years it has been concluded by several authorities that the chemical estimation of the alkaloidal content of veratrum preparations is not indicative of corresponding therapeutic value and that a physiological test should be used. As far back as 1905 Houghton and Hamilton (1) published an article on a new aqueous veratrum preparation called "Veratrone" which was "adjusted to one-fourth the strength of the U. S. P. fluidextract *by determining the M. L. D. per Gm. body weight for frogs* of the same species and weight kept under uniform conditions, comparing the results with those obtained from the injection of known quantities of the U. S. P. fluidextract of veratrum viride." Twelve years later, in 1917, Pilcher (2) reported a large number of physiological experiments and concluded that, for practical purposes, the fatal dose for frogs would seem to be satisfactory.

Since 1905, Veratrone has been standardized by the physiological method proposed by Houghton but there are some disadvantages to this method, the chief of which is the indefiniteness of the end-point. Frogs injected with a lethal dose of veratrone may still be just alive 18 or even 24 hours after being dosed. A whole series of frogs given graded doses may be found 15 hours later to be more dead than alive, but still they are not dead and even a skilled technician hestitates about drawing any conclusion. Such a condition necessitates the use of a large number of frogs before a conclusion can be reached and thus renders the assay very expensive in amount of material used and the time required.

The comparative inexpensiveness of the white mouse first caused us to consider it as a suitable substitute for the frog in standardizing veratrum preparations. After using this animal for testing a number of preparations in comparison with the frog several advantages were discovered, the chief one being the rapidity and definiteness with which an assay can be carried out when white mice are used.

The mice are carefully weighed to within 0.5 Gm. and injected intraperitoneally with such a dilution that the total volume of the dose is not greater than 1 cc. The mice are very quickly affected and final results may be read within 30 minutes after injection, as it is very rare that a mouse dies after that period and then it is doubtful whether reliance could be placed in such delayed results.

The table on the following page gives the M. L. D. found on frogs and white mice for a number of veratrum preparations:

This series of tests has shown several facts when the results are carefully analyzed.

The M. L. D. of an average or standard F. E. Veratrum Viride should be 0.00025 cc. per Gm. body weight of white mouse administered intraperitoneally. Veratrone, an aqueous solution of veratrum adjusted to one-fourth fluidextract strength should have an M. L. D. for white mice of 0.0010 cc. per Gm.