

4. A phytosterol glucoside (phytosterolin) has been separated whose characteristics indicate it to be identical with the synthetic sitosterol-*d*-glucoside of Salway.

5. Glycerol has been identified.

6. Indications have been found of the presence of ceryl alcohol, a small amount of resin and carotinoid coloring matter.

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## A PROPOSED PHYSIOLOGICAL STANDARD FOR PITUITARIUM, U. S. P.\*

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Glandular products are finding their way into therapeutics and some of them have found their way into the U. S. Pharmacopœia. "Pituitarium is the cleaned, dried and powdered posterior lobes obtained from the pituitary body of domesticated animals which are used for food by man. It is a yellowish or grayish amorphous powder, having a characteristic odor, and is only partially soluble in water" (10). Its official preparation is **Liquor Pituitarii**. No mention is made in the Pharmacopœia as to the physiological strength of Pituitarium. It is known that many manufacturers use Pituitarium to make Liquor Pituitarii. Confronted with this, how can the manufacturer know how much powder to use to make Liquor Pituitarii of the strength mentioned in the Pharmacopœia? Variations in manufacturing methods may cause differences in the physiological potency of Liquor Pituitarii, irrespective of the raw material used. By standardizing the potency of the Pituitarium powder only one of the causes of variation is removed, even though it may be the greatest. It is still necessary for the manufacturer to assay each lot of finished pituitary solution.

The potency of desiccated posterior pituitary powders is most important in considering the recommendation of the Second International Conference on Biological Assays held in Geneva in 1925 (5), that a dried posterior powder be adopted in every Pharmacopœia to be used as the basis for the preparation of all commercial pituitary solutions. This suggestion was not adopted by the Third Conference held at Geneva in 1928 (6), because there was some doubt whether such a powder would retain the diuretic action as well as the pressor and oxytoxic potency. Reports by Mackensie (3), Smith and McClosky (8) and Kestranek, Molitor and Pick (2) all indicate that the diuretic action of the posterior pituitary gland is quantitatively preserved in preparing a desiccated powder by the U. S. P. X method.

A number of samples of commercial posterior powders were obtained directly from the manufacturers, representing their current output, and a study of their activities made. Extracts were made to contain the activity of 1 mg. powder per cc. and 10 mg. powder per cc., and were prepared as follows: A desired amount

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of powder was carefully weighed, placed in a small agate mortar and moistened with a few drops of distilled water containing 0.25 per cent of acetic acid. The moistened powder was triturated thoroughly until the whole was of an impalpable frothy consistency. A few cc. of the 0.25 per cent acetic acid solution were added and the mixture stirred thoroughly. The mixture was transferred to a hard glass beaker; the small agate mortar was rinsed with acetic acid solution and the rinsings added to the pituitary mixture; then enough 0.25 per cent acetic acid was added to make the final volume of the mixture as above stated. This mixture was heated to the boiling point for not more than one minute and filtered. This solution was placed in hard glass ampules and sterilized by fractional sterilization for twenty minutes on three successive days at a temperature not exceeding 100° C., and then stored in the ice box until used.

The method of preparation as outlined is described by Smith and McClosky (7), and is the method specified in the U. S. Pharmacopœia (10), for the preparation of the test solution of the official standard posterior pituitary powder.

A supply of Standard Powdered Pituitary for this work was made according to the specification of the U. S. Pharmacopœia, Tenth Revision, and was assayed against the Standard Powdered Pituitary as supplied by the Bureau of Chemistry. It was found to check exactly with the official powder.

The uniformity of the above posterior pituitary powder, when compared with the official standard powder, further confirms the work of Smith and McClosky (7), as also the results of Nelson and Munch (4), and Swanson (9).

In carrying out the assay of pituitary extracts we have used the method and general type of apparatus for studying the activity of the isolated smooth muscle of mammals described by Dale and Laidlaw in 1912 (1), with some modifications. The apparatus must have an accurate temperature regulating device. The chamber in which the uterus is suspended should have a capacity of not less than 100 cc. We have used throughout this work young female guinea-pigs weighing between 250 Gm. and 300 Gm., which have not been pregnant or in heat, and have been segregated at the time of weaning and kept, therefore, out of sight and smell of the males.

The animal is killed by a blow on the head, and at once both horns of the uterus are removed and placed in Locke's solution. One entire horn, freed from the broad ligament, ovary and fallopian tube is suspended in the bath of Locke's solution. The temperature of the bath should be held within  $\frac{1}{10}$  of a degree of 38° C. throughout the whole assay. After complete relaxation, which takes from 15 to 30 minutes, the weights upon the uterus are so adjusted as to write a base line with but slight spontaneous movements. The extracts to be tested are always pre-diluted with glass redistilled water so that the dose added to the bath of 100 cc. is usually about 0.3 cc. to 0.5 cc. and never exceeds 1.0 cc. The dilutions are usually made of such strength as to require nearly equal volumes of standard and unknown to elicit equivalent reactions. Each dose of standard and unknown is carefully measured with pipettes graduated to 0.01 cc. After an equivalent dose of unknown is found for a given dose of standard, the respective doses are then increased or decreased, or both, by 20 per cent. At the end of the experiment a large dose of standard is given to show that all previous reactions are due to sub-maximal contractions.

The following is the composition of the Locke's solution used in this work:

Sodium chloride	9.000 Gm.
Calcium chloride (anhydrous)	0.240 Gm.
Potassium chloride	0.420 Gm.
Magnesium chloride	0.005 Gm.
Sodium bicarbonate	0.500 Gm.
Dextrose	0.500 Gm.
Glass redistilled water to make	1000.00 cc.

The Locke's solution is freshly prepared each day from stock solutions of twentyfold concentration exclusive of the dextrose and sodium bicarbonate. The dextrose and sodium bicarbonate are weighed out each time as required. Oxygen used to aerate the bath should be bubbled through a 2 per cent solution of sodium bicarbonate to keep the tone and irritability of the uterine muscle at a uniform level, as suggested by Smith and McClosky (7).

The results of the assays are shown in the following table:

#### ASSAY OF COMMERCIAL POSTERIOR PITUITARY POWDERS.

P. C. No.	Original conc. prepared mg./cc.	Conc. found by U. S. P. X bioassay mg./cc.	Mg. com. powder equiv. to 1.0 mg. U. S. P. X std. powder.	% Activity std. powder.
1469	10.0	0.15	66.7	1.5
1544	1.0	0.25	4.0	25
1483	10.0	3.00	3.3	30
1548	10.0	3.60	2.8	36
1607	10.0	3.60	2.8	36
1556	10.0	5.00	2.0	50
1517	10.0	6.00	1.67	60
1463	10.0	7.50	1.33	75

From the figures reported the activity of the majority of the powders assayed is between 30 per cent and 50 per cent of the standard posterior pituitary powder, U. S. P. X and the recommendation is hereby made that 50 per cent be established as the standard of activity for "Pituitarium."

#### SUMMARY.

A wide variation in the physiological activity of eight samples of commercial Pituitarium which represent the current commercial output has been found. Physiological assay by the method outlined in the U. S. P. X showed that these products ranged from 1.5 per cent to 75 per cent of the physiological activity of the official U. S. P. X posterior pituitary powder.

A majority of the powders show an activity between 30 per cent and 50 per cent of the standard posterior pituitary powder.

The recommendation is made that the manufacturers be required to market a preparation for commercial purposes showing an activity of 50 per cent of the official standard powdered pituitary described in the U. S. P. X.

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## A COMPARATIVE PRECIPITATION METHOD FOR THE QUALITATIVE IDENTIFICATION OF EACH OF THE COMMON GUMS.

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The common gums are rapidly attaining a wide use in commerce. With the exception of a number of specific tests for tragacanth, there is no easy, rapid and definite method for the differentiation of one gum from another.

We found, by use of the following method, that the precipitate given by any one of the gums when precipitated from an aqueous solution by alcohol was distinctly different than that given by any other gum.

A 1% test solution of each gum was made, except for agar-agar and Indian gum in which case a more dilute solution was made. To 20 cc. of the test solution, 70 cc. of 95% alcohol was added drop by drop, with constant stirring until precipitation was complete. The texture, quality and the characteristics of the precipitate, as well as the point at which definite precipitation began, were noted.

The gums used were acacia, tragacanth, Irish moss, quince seed, Indian gum and agar-agar. Acacia, tragacanth and agar-agar dissolved directly in the required amount of water. Two Gm. of Irish moss, obtained as the seaweed, were placed in 100 cc. of water and allowed to stand on the steam-bath for one hour. The swelled mass was filtered by pressure through flannel and the resulting filtrate was used as the test solution. Indian gum was permitted to stand on the steam-bath for four hours and the upper layer was used as the test solution. One Gm. of ground quince seed was placed in 100 cc. of water and allowed to stand for one hour on the steam-bath. It was then filtered through filter paper and the filtrate was used as the test solution.

The experiments were then repeated on test solutions of different concentrations and similar results were obtained. In no case could the precipitate given by one gum be confused with that given by any other gum.

A description of the behavior of each precipitate follows and a table, in which the differences in the precipitates may be seen at a glance, is appended. The terms, cloudy and clear, as applied to the supernatant liquid, indicate its appearance after the alcohol solution has been standing for four hours.

*Acacia*.—When 40 cc. of the alcohol had been added a very fine, flocculent precipitate appeared which became more dense on further addition of alcohol. It settled very slowly leaving a cloudy supernatant liquid. It distinctly did not adhere to either the stirring rod or the beaker.

*Tragacanth*.—When 10 cc. of alcohol had been added a precipitate first ap-

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