SCIENTIFIC SECTION, AMERICAN PHARMACEUTICAL ASSOCIATION

PRELIMINARY NOTE ON THE VALUE OF BETA-IMINAZOLYLETHYLAMINE HYDROCHLORIDE AS A STANDARD FOR TESTING PITUITARY EXTRACTS.*

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In a paper on "Pituitary Extract" in the *Journal of the American Medical Association*, August 8, 1914, page 476, Dr. George B. Roth suggested the use of the above histidin derivative as a standard for testing pituitary extracts. The U. S. P. Revision Committee later incorporated the following standard in the U. S. P. IX for Liquor Hypophysis:

"One mil of Solution of Hypophysis, diluted 20,000 times, has the same activity on the isolated uterus of the virgin guinea pig as a 1 to 20,000,000 solution of beta-iminazolylethylamine hydrochloride when tested as directed by the United States Hygienic Laboratory."

Before adopting a complex substance like the above as a standard for adjusting the strengths of commercial preparations, it would have been better, perhaps, to make a thorough study of a number of problems such as the following:

1—Degree of uniformity in the physiologic action of different available samples of the proposed standard substance.

2—Rate of deterioration of solutions of this substance.

3—Effect of sterilization on solutions of this substance.

4—Rate of deterioration of the substance itself.

5—Effect of repeated doses on the isolated uterus.

6—The toxicity of the substance as compared with Pituitary Extract.

7—The relative activity of a Pituitary Extract of the strength proposed by the U. S. P. IX and that of the commercial extracts as supplied by the leading pharmaceutical manufacturing houses.

As it would be impracticable as well as rather expensive to prepare a fresh 1:20,000,000 solution of the standard substance for each test, we thought it advisable to determine whether or not it would be possible to prepare a 1:1,000 dilution of the salt, put this up in ampuls and then dilute the contents of one of these ampuls to 1:20,000,000 for each test. In order to know whether or not it would be safe to do this it was necessary to determine the rate of deterioration of the solution when kept under different conditions.

Accordingly, a little over a year ago we made a solution of the salt, divided it into twelve parts and stored them for one year in the following manner:

(*a*) Sterilized { 1—amber ampuls / 2—flint ampuls } Kept in *light*.

* Read before the Scientific Section of the American Pharmaceutical Association, Atlantic City, September, 1916.

(*b*) Unsterilized { 1—amber bottles; 2—flint bottles; 3—amber ampuls; 4—flint ampuls } Kept in *light*.

(*c*) Same as (*a*) but kept in the *dark*.

(*d*) Same as (*b*) but kept in the *dark*.

After one year had elapsed tests were started in order to determine the relative activity of the solutions kept under different conditions. These tests have not been completed, but to date the following results have been obtained:

1—The solution in the *unsterilized amber ampuls* kept in the *light* was of exactly the same activity as the solution in the *unsterilized flint ampuls* kept in the *light*.

These ampul solutions, however, were 2.6 times as active as fresh solutions made from the same sample of the standard substance as was originally employed.

2—The solution in the *sterilized amber ampuls* kept in the *light* was of exactly the same activity as the solution in the *sterilized flint ampuls* kept in the *light*.

3—The solution in the *sterilized amber ampuls* kept in the *light* was of exactly the same activity as the solution in the *sterilized amber ampuls* kept in the *dark*.

4—The solution kept in the *flint bottles* in the *light* was of exactly the same strength as a new solution of the substance but only about one-third as active as the solutions which had been kept in the ampuls.

5—The solution kept in the *amber bottles* in the *light* showed practically no activity.

While it is hard to draw positive conclusions from this set of tests, and without endeavoring to explain the entire loss of activity of the material in the amber bottle kept in the light, it would seem that the solutions in ampuls whether kept in amber or in flint and whether kept in the light or in the dark all retain their activity. The surprising part of the result, however, was the fact that new solutions of the original substance possessed only about one-third of the activity of the solutions prepared one year before from the same sample. Did the dry salt lose physiologic activity at a greater rate than solutions of the salt preserved in ampuls or did the latter break down into substances possessing greater physiologic activity? That the former is probably the true explanation is indicated by comparisons with some commercial ampul solutions of beta-iminazolyl-ethylamine hydrochloride which are described further on in this communication.

In order to determine whether or not sterilization influences the activity of these solutions a 1:40,000 dilution was prepared and then divided into two portions. One portion was filled into ampuls, sealed and sterilized for 15 minutes at 115° C., after which it was tested against the unheated portion. Repeated tests showed that both portions possessed the same activity. In other words, sterilization does not affect the activity of solutions of this substance.

The next step was to compare the activity of different available samples of the proposed standard substance.

It was impossible to satisfactorily carry out this set of experiments because due to the European War it was impossible to obtain new samples of the standard substance to compare with the one which we used in carrying out our experiments. We were, however, able to obtain ampuls containing a 1:1,000 dilution of the salt bearing two different laboratory numbers which we presume were made from two

different lots of the salt. Repeated tests showed that these two solutions were of exactly the same activity as a corresponding solution of our standard substance which was purchased a little over a year before. The original activity of this substance we do not know but the results given above show that the ampul solutions prepared from it one year ago are about three times as active as new solutions prepared from the same sample. This would tend to show that the substance used in preparing the ampul solutions which were supplied to us by the manufacturers of the substance must have been only about one-third as active as the substance which we used one year ago. Perhaps the manufacturers prepared these ampul solutions from some of the original lot from which we obtained our supply and their stock deteriorated at exactly the same rate as the sample in our possession.

In our preliminary work with this standard substance the uterus seemed to "play out" or become insensitive to the substance more quickly than when testing pituitary extract alone. This led us to believe that this substance might be more toxic than corresponding amounts of pituitary extract. Later experiments, however, had a tendency to dispel this idea. In order to prove this point we determined the minimum lethal dose of both preparations on guinea pigs. The results of these tests showed that the M. L. D. for a standard Pituitary Extract (H. K. M. Co.) is 5.75 mils per 250 Gm. body weight of animal. The M. L. D. of beta-iminazolyl-ethylamine hydrochloride was found to be 2.0 mils of a 1:1,000 dilution per 250 Gm. body weight of animal.

According to the proposed standard for the U. S. P. IX a 1:20,000,000 dilution of the standard substance equals a 1:20,000 dilution of pituitary extract, or in other words, the standard substance itself is 1,000 times as active as pituitary extract.

According to our results on the toxicity of the two substances the standard is 2,875 times as toxic as pituitary extract. When used in the proportions employed in the isolated uterus test, therefore, their relative toxicities would be as 1 is to 2.87. When we consider the variations in the size of the dose of the unknown, however, the difference in toxicity is practically negligible.

We find, however, that the standard adopted by the U. S. P. IX is very low because by comparison we find that the commercial extracts, prepared by the leading pharmaceutical houses, which have been on the market for several years and to which physicians have become accustomed as to dosage, etc., are from 3 to 5 times as active as an extract of the new U. S. P. standard strength. This is unfortunate, as there is no reason why a weaker preparation than the ones to which physicians have become accustomed should be placed on the market. It is probable that manufacturers will continue to endeavor to supply pituitary extracts of the same strengths that they have been supplying in the past, marking such preparations "different in strength from the U. S. P." or perhaps "stronger than the U. S. P." Before it becomes necessary to revise the Pharmacopoeia again it is to be hoped that definite requirements can be drawn up for the test substance itself and that an accurate coördination of the required U. S. P. strength and of the common pharmaceutical practice may be secured.

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