SCIENTIFIC SECTION

INTERPRETATION OF BIOLOGIC ASSAYS.*

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Most laboratory men know the behavior of drugs which can be chemically tested and can therefore interpret these results and intelligently apply the assay results to the proper adjustment of the products assayed.

Most of these men, however, have not had sufficient practical experience with Bio-Assays to know the behavior of the various drugs to which the Biologic Assay must be applied.

Therefore, they do not have the necessary experience to properly interpret and apply the results obtained by the assay.

The Biologic Assays themselves can be mastered after limited experience but the proper application and interpretation of the results must be backed by years of experience.

In order to market Biologically standardized preparations which will satisfactorily meet the new U. S. P. Standards, more is required, therefore, than just a working knowledge of the various Biologic Assay methods.

This knowledge must be backed by a sufficient amount of practical experience to enable one to properly interpret and apply the information gained from the assay, in issuing instructions for the proper adjustment of the product assayed.

In other words, one should have sufficient experience to know if a certain preparation tests 200% whether or not it should be diluted 100% and whether a re-assay is necessary after the dilution. Theoretically it should be diluted 100% and a re-assay should not be necessary. Practical experience, however, has shown that this is not always the case.

Whether or not a preparation should be given the full theoretical dilution or concentration as based upon the assay depends upon several factors.

First.—The permanency of the preparation (its rate of deterioration).
Second.—The percentage over or understrength.
Third.—The age of the preparation at the time of the assay.

1. THE PERMANENCY OF THE PREPARATION.

Preparations of Cannabis, for example, are practically permanent and, therefore, are usually as active after one or two years as they were the day tested.

In the case of such preparations it is safe to dilute to the limit and still be assured that the preparations will be of standard strength when they reach the consumer.

On the other hand, such drugs as Digitalis, Ergot[1] and Aconite deteriorate more or less rapidly and dilutions should not be carried too close to the theoretical.

Digitalis.—Some tinctures lose practically no activity within a year, others

* Read before the Scientific Section of the Maryland Pharmaceutical Association, Buena Vista Springs, Pa., June 30, 1927.

[1] "A New and Reliable Method for the Preservation of Ergot Preparations," by Paul S. Pittenger and C. E. Vanderkleed, JOUR. A. PH. A., August 1922.

lose 15 to 30% within two to three months. In most cases, therefore, preparations of digitalis should not be diluted to less than 110 to 115% of standard (Higher U. S. P. limit).

Ergot.—A freshly prepared preparation of Ergot in most cases loses from 25 to 30% within three months. After 4 to 6 months' aging the preparation will be found to be almost permanent.

The dilution recommended for Ergot preparations, therefore, should depend largely upon the age of the preparation at the time of assay.

Aconite.—Deterioration is very rapid. Preparations of this drug are sometimes almost inert within a year.

If 2% Acetic Acid is added to the percolating menstruum the preparation is much more permanent.

Preparations of this drug should not be diluted to less than 110 to 120% of the average standard (Higher U. S. P. Limit).

2. THE PERCENTAGE OVER OR UNDERSTRENGTH.

If the preparation assays within 100 to 150% of standard, you can usually depend upon the diluted preparation assaying in direct proportion to the dilution. In cases, however, where the strength of the preparation runs as high as 175 to 250% or in other words where high dilutions are necessary, the diluted preparation will not always test in exact proportion to the dilution.

In these cases it is always advisable to give only about three-quarters the theoretical dilution and re-assay.

This is the safest procedure and usually not more expensive because the increased yield due to the high dilution pays many times over for the re-assay.

It is a good practice in cases where preparations assay 150 to 200% of standard not to dilute the entire lot at once. This usually produces 50 to 100% over estimated yield and thus a proportionate overstock which may deteriorate to below standard before being sold. It is best to dilute only sufficient for immediate demand and then re-assay the remaining portion and adjust as required.

Deterioration of Dilutions.—High dilutions of some preparations tend to increase their rate of deterioration.

Highly diluted Fluid Extract of Ergot is in this class and a 100% dilution of a 200% Fluid Extract of Ergot is not advisable.

Concentrations.—In the case of *understrength* preparations the question always arises as to the best method of fortifying. Should the product be concentrated or an overstrength preparation added?

Here again *sufficient experience* is required to judge each individual case. For example, if the case be a S. E. Digitalis as low as 15 or 20% to be used for manufacturing purposes, it is satisfactory to use 5 times the usual formula quantities. On the other hand, if it be an Ergot preparation which had deteriorated to 20 or 30%, it is not advisable to use five times the usual amounts because of the possibility of the presence of toxic decomposition products.

If a preparation is only slightly *understrength* and an *overstrength* preparation is not at hand it is usually advisable to reduce the volume by taking the required amount of the preparation and reducing it in a high vacuum to an extract. This extract is then dissolved in the remainder of the preparation.

But here again it is necessary to have a knowledge of the behavior of the drug under consideration. Aconite, for example, cannot be treated in this manner because the slight amount of heat, required to concentrate, even in vacuum, splits the Aconitine into its decomposition products—Aconine and Benzaconine which are practically physiologically inert. If, however, the *preparation is very markedly understrength*, the above is not a profitable procedure, and would in most cases seriously affect the color and consistency of the preparation.

In these cases it is advisable to prepare a new preparation using an excess of drug in order to obtain an overstrength preparation which can be diluted with the understrength product.

In some cases this is not practicable as it would produce too large a yield. It is, therefore, advisable to make it a practice not to dump the drug from the percolater until after the assay, as the drug may not have been completely exhausted. Then, if necessary, add additional drug and re-percolate using the understrength preparation as menstruum.

Diluting with Understrength Preparations.—The use of understrength preparations for diluting those that are overstrength is usually practicable but there are cases in which it is not good policy.

In the case of *Pituitary Extract*, for example if the preparation is understrength immediately after making it is satisfactory for use in diluting overstrength lots. In some cases, however, you may have a preparaion which originally tested 100% and has deteriorated more rapidly than usual. Experience has shown that it is *not advisable to use such a preparation for dilution purposes*, because such lots have a tendency to start rapid deterioration in the new lot and it is, therefore, usually more profitable to discard the deteriorated lot.

3. THE AGE OF THE PREPARATION AT THE TIME OF ASSAY.

As before stated some preparations deteriorate more rapidly than others. In most cases, however, experience has shown that the most rapid deterioration takes place during the first three to four months. Some preparations which deteriorate rapidly during this period are comparatively permanent thereafter. This is the reason for the U. S. P. requirement that the standard F. E. Ergot supplied by the Bureau of Chemistry be aged 6 months before standardizing.

The author has also proven that practically all digitalis preparations deteriorate the most during the first three months after manufacture.[1,2]

In the summary of the results of one of these experiments[2] the following statement appears:

> "The tabulated results show the average deterioration in 9 to 13 months of 43 samples to be 18.8 per cent. The average deterioration of 38 samples during the first three or four months being 4 per cent per month while the average deterioration of 32 samples after the first three or four months was 2.4 per cent per month."

Therefore, in the case of drugs which deteriorate the *age* of the preparation must be taken into consideration when ordering dilutions. A fresh preparation should not be diluted as close to the theoretical as an aged preparation.

[1] "The Deterioration of 'U. S. P.' and 'Fat-Free' Tinctures of Digitalis," by Paul S. Pittenger and H. K. Mulford, Jr., JOUR. A. PH. A., March 1918.

[2] "The Deterioration of Tincture of Digitalis," by Paul S. Pittenger, JOUR. A. PH. A., December 1918.

RE-ASSAYS.

It is also important to know when re-assays are necessary in order to be assured that stocks meet the official requirements.

This depends entirely upon the permanency of the preparation. As before stated, Cannabis for example, is very stable. Therefore, once standardized usually need not be re-assayed if properly stored.

On the other hand, stock of preparations of such drugs as Ergot, Aconite and Ditigalis should be assayed at least every three months and readjusted to standard before offering for sale.

In cases were dilutions of 10 to 50% are made based upon physiologic assay, a re-assay of the dilution is usually not necessary. *Re-assays, however, are always necessary in the case of high dilutions and after all concentrations.*

NECESSITY FOR SUPPLYING ASSAYIST WITH ALL POSSIBLE INFORMATION IN REFERENCE TO SAMPLES.

From this brief description of some of the factors which influence the intelligent interpretation of Biologic assays and their practical application, the necessity is apparent for supplying the assayist with all possible information in reference to the product being assayed.

This information should include age, whether a new preparation or a re-assay of an old lot, information as to deviations from U. S. P. formulas such as an addition of Acetic Acid to the menstruum in Aconite preparations, etc. In other words, the assayist should have all available information.

Pharmaceutical manufacturers who do not have their own biologic laboratory and must select one to do this class of work for them should carefully consider the following:

1. The experience of the operator in practical biologic assay work.
2. The experience of the operator in the interpretation and practical application of assay results.

It is also important to select a laboratory in which information in reference to products submitted for assay is kept in the strictest confidence.

After selecting such a laboratory or submitting samples to your own analyst, confidence should be placed in the laboratory to the extent that all possible information will be furnished in reference to the product submitted.

This is the only way in which the full benefits of Biologic Assays may be obtained.

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THE VITAMINS OF CITRUS FRUITS.

Stanley G. Willimott and Frank Wokes, pharmaceutical chemists reported on some of the constituents of Citrus fruits at the recent meeting of the British Pharmaceutical Conference. In view of the theory that certain constituents of orange, lemon and grape fruit are produced by photo-synthetic action in the flavedo, a comparsion was made by the authors between their concentration in the flavedo and in the juice. Vitamins were found in all three fruits. Vitamin C is present to a greater extent in the *juice* of the grape fruit; Vitamin B is concentrated mainly in the flavedo; Vitamin A is present in greater amount in the orange, both in peel and juice.