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 uterus.

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

7. The relative toxicity 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.

The results of experiments are then given which tend to prove that the standard substance deteriorates quite rapidly and 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 the physicians have become accustomed as to dosage, etc., are from three to five times as active as an extract of the new U. S. Pharmacopoeia standard strength. This is unfortunate, as there is no reason why a weaker preparation than the one to which physicians have become accustomed should be placed on the market." The findings of the author as reported in the above paper have since been corroborated by Eckler[7] and Hamilton.[8]

It is to be hoped, therefore, that before it becomes necessary to revise the Pharmacopoeia again 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.

PHARMACODYNAMIC LABORATORY,
H. K. MULFORD COMPANY,
August 15, 1917.

ON THE DETERIORATION OF CRUDE INDIAN CANNABIS.*

BY C. R. ECKLER AND F. A. MILLER.

It has long been known that crude Indian Cannabis loses its activity quite rapidly, and Marshall[1] and others have shown that the deterioration is due to oxidation of the active principles, but the rate of deterioration during commercial storage has not been determined, and this was of particular interest to us. For the purpose of learning something on this point, two sets of experiments were carried out, our intention being to imitate the different conditions under which the crude drug might be kept.

One lot of drug was stored in a cool basement in three portions, one portion sealed in alcohol, one portion sealed dry, and one portion unsealed dry. Another lot was stored in a warm attic in four portions, one portion, granulated, sealed;

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[7] Eckler, *Amer. Journ. of Pharmacy*, May 1917, p. 195.

[8] Hamilton, *Amer. Journ. of Pharmacy*, Feb. 1917.

* Read before Scientific Section, A. Ph. A., Indianapolis meeting, 1917.

[1] Marshall, "Experiments on the Cause of the Loss of Activity of Indian Hemp," *Pharm. Jour.*, Vol. 82, p. 418 (1909).

one portion, granulated, unsealed; one portion, whole, sealed; one portion, whole, unsealed.

METHOD OF EXTRACTING SAMPLES.

All samples were finely granulated and made into fluidextracts according to the U. S. P. method except that no heat was used in the process, the final percolate being evaporated under an air jet.

METHOD OF TESTING.

The method of assay on pure bred fox terriers, essentially as described by us previously,[2] was employed in this work. In all cases the drug was administered in the form of a fluidextract.

EXPERIMENTS ON BASEMENT STORED DRUG.

A lot of drug, supposed to be of the 1911 crop, was received December 28, 1911. The assay of this drug was finished February 14, 1912, and it was found to possess approximately 75 percent standard activity. It was set aside for aging May 28, 1912, in a cool basement, as follows:

One portion of 69 pounds, granulated drug, was sealed in an alcohol barrel with enough alcohol to keep it well moistened.

One portion of 74 pounds, whole drug, was sealed in an alcohol barrel dry.

One portion of 74 pounds, whole drug, was left unsealed in the original box in which it was received.

February 14, 1917, these portions were assayed and gave results as follows:

The sample sealed in alcohol seemed not to have lost appreciably in activity.

Both dry portions seemed to have lost fully 60 percent of their original activity. (The drug at this date possessed from 25 to 30 percent standard activity.) No difference could be noticed between the activity of the dry portions.

EXPERIMENTS ON ATTIC STORED DRUG.

A lot of drug was received December 27, 1912, claimed by the drug merchant to be of the 1912 crop. The assay of this drug was finished January 1, 1913, and it was found to possess approximately standard activity. March 17, 1913, four portions were set aside for aging, each portion consisting of ten 500 Gm. packages, as follows:

One portion, granulated for percolation, in muslin bags.

One portion, granulated for percolation, in amber bottles with paraffined corks.

One portion, whole drug, in muslin bags.

One portion, whole drug, in amber bottles with paraffined corks.

These portions were stored in an attic room of the laboratory where the temperature varied from 65 to 105° F., the average temperature for fall, winter, and spring being about 75° F., and for the warm summer months about 90 to 95° F.

It was our intention to assay these samples at least once each year, but the pressure of other work hindered to such an extent that we made, aside from the

[2] Eckler and Miller, "A Study of American Grown Cannabis in Comparison with Samples from Various Other Sources," *Eighth International Congress of Applied Chemistry*, Vol. 17, p. 23.

original testing, only three assays over a period of about 50 months. A carefully prepared fluidextract made from the same drug was used for comparison. This fluidextract was preserved in two well-filled, one-pint, amber bottles with paraffined corks, until the first aging period was over, that is, until the aged samples were assayed for the first time. After this time, the fluidextract was kept in well-filled, one-ounce, amber bottles with paraffined corks. These small bottles were placed in a glass cupboard in the laboratory, and a fresh one was opened for each succeeding assay.

March 31, 1914, first assay of aging samples. (Approximately $14^1/_2$ months from date of first assay.) At this time no decided deterioration could be noticed with certainty. A few of the results suggested a very slight lessening of activity.

December 14, 1915, second assay of samples. (Approximately 35 months from date of first assay.) All samples had apparently lost about 60 percent of their original activity. No decided difference could be noticed between them. The results on three out of eight dogs seemed to be slightly in favor of the sealed samples.

April 4, 1917, third assay of samples. (Approximately $50^1/_2$ months from date of first assay.) All samples seemed to have lost considerably over 90 percent of their original activity, in fact, ten times the originally active dose caused not more than barely perceptible symptoms in some of the dogs, while in the others, no decided effects were noticeable.

The fluidextract used for comparison still compared favorably with recently prepared preparations from fresh drug which were considered of standard strength.

REMARKS AND CONCLUSIONS.

From the results of the tests on the attic stored samples, the loss in activity was practically 100 percent in about 50 months. (The drug at the end of the aging period was, however, about 55 months old from date of harvest.) This would give an average loss in activity of about 2 percent per month. Apparently, however, the deterioration did not proceed so rapidly at first, for in the first period of about 14 months not more than a very slight deterioration was noticeable, while during the next period of about 21 months there was a deterioration of nearly 60 percent of the original activity, and during the last period of about 15 months there was apparently a loss of approximately 40 percent.

The dry samples stored in the basement lost in about 60 months, approximately 60 percent of their original activity, or about 1 percent each month on the average. (This drug at the end of the aging period was about 65 months old from date of harvest.) These results in connection with those of the preceding paragraph, would seem to suggest that the warmer temperature of the attic was influential in increasing the rate of deterioration.

Drug stored in sealed containers in a dry state did not retain its activity appreciably longer than when stored in unsealed containers, not did it retain its activity appreciably longer when stored whole than when granulated.

Granulated drug sealed in a tight barrel and well moistened with alcohol seemed to retain its full activity for at least 60 months.

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INDIANAPOLIS, INDIANA.

ABSTRACT OF DISCUSSION.

C. R. ECKLER: We carry this method out in a specially constructed kennel where the animals are in stalls in pairs, and so arranged that they cannot see each other or anything about the room which might attract their attention or excite them. We determine the smallest amount of the drug which will just produce incoördination of the muscles. It is a method that must be studied out individually and one must become thoroughly acquainted with the susceptibility of the dogs to the drug.

H. C. HAMILTON: I was not sure in most of his work whether Mr. Eckler was speaking of the crude drug that had deteriorated greatly, or the extract.

C. R. ECKLER: I was speaking of the crude drug. Every time I made an assay, I extracted a sample of the crude drug and made a fluid extract. In my second experiment I made all my comparisons with a fluid extract which was made at the beginning, from the crude drug. That was carefully preserved as a standard for comparison, but at each assay I extracted a new sample of the crude drug.

H. C. HAMILTON: This report is very decidedly different from the results that I have obtained. I have tested old samples of Cannabis Indica that have been preserved with no particular care—I distinctly remember one sample ten years old that I found accidentally preserved —it was simply chucked away on the back part of a shelf. While, of course, I cannot say that it had not lost any of its activity, I can say that it was fully up to the standard of the ordinary drug; and that would rather be contrary to the results that Mr. Eckler obtained.

There is another point I would like to bring up, and that is just a question about the method. This subject is getting rather threadbare, but the original method as proposed by Cole, of Houghton's method—said nothing whatever about the breed of dogs. We do not use any particular breed. We select a dog that is susceptible, regardless of what breed, nor do we determine the smallest dose that will produce incoördination. We have a standard test dose, which does not agree with that of the U. S. P. We have a standard test dose and detect with it the difference in the activity of the drug, not by having in one case shown activity and in another case not, but that it shows differences in degrees. Those are two points in the Houghton Method, that the original publication of it was rather specific on, I think. I have not found Cannabis in any form to deteriorate appreciably, except in the powdered extract form. We are now using a standard solid extract for comparison, the sample is at least seven years old, and it is as good as the average sample—better than some—and just a little less active than some of the very best samples that are imported.

C. R. ECKLER: I should imagine that the deterioration of the samples reported on might differ from others. As to the deterioration of crude Cannabis, I might say, that this work was stimulated by the fact that some years ago we became rather overstocked with Cannabis and one lot in particular deteriorated to such an extent before it could be used up, that it was discarded at a loss of several hundred dollars. Fluid extracts and solids carefully prepared retain activity for a very long time but I have had fluids that deteriorated very materially. We also to some extent take into account in this work, as Mr. Hamilton has said, the depth of symptoms, but where we give dogs large doses, ten times the original dose, and fail to see any symptoms, we conclude, of course, that the activity is gone.

HAROLD GRAY: As I understand it, there was no deterioration of the fluidextract?

C. R. ECKLER: No, I did not say that, but I say that carefully prepared fluidextracts kept in small containers, well filled and not opened, will retain activity for a long time. I do not say there is no deterioration, but I have had preparations for five years that were still approximately up to their original activity. But I have seen fluids that did lose materially in activity.