AN AID IN THE BIOLOGIC ASSAY OF CANNABIS PREPARATIONS.

BY BENJAMIN WHITE AND JOSEPH CIANCIARULO.

Anyone who has performed biologic assays of cannabis preparations is familiar with the difficulty of determining the various degrees of muscular incoördination produced by borderline doses of the drug. The present Pharmacopœial method specifies that an average dose of a standard preparation should so disturb the dog's power of coördination that, during the second hour following the oral administration of the drug, the animal will manifest a slight swaying of the body, or some ataxia when it runs about. While there is a considerable variation in the susceptibility of different dogs to cannabis, there are other circumstances which make it difficult to determine to what extent the animal is actually affected. The appetite of the dog, his friendliness to the observer, and his reaction to his surroundings all tend to mask minimum effects. An alert, hungry and friendly dog will overcome a partial loss of muscular control, which would be plainly noticeable in a sluggish or sulky animal. Furthermore the surroundings in which the dog is observed will affect different dogs to varying degrees.

A sharp end-point, that is, the least degree of muscular incoördination that can be definitely noted, is greatly to be desired, because it would add to the accuracy of the assay. In order to achieve such an end-point it was felt that it might be possible to train dogs to perform some muscular feat, so that the minimum effects of the drug would be manifest while the influence of environment would be diminished. This idea led to training the test animal to stand or walk on its hind legs when offered some tempting morsel of food. This simple means has greatly facilitated the assay of cannabis preparations and it is reported here in the hope that it may be of value to others.

The method used was the one specified in the Ninth Edition of the United States Pharmacopœia. The animal was a female mongrel fox-terrier weighing about 7 kilos. The dog was trained to stand on its hind legs, to maintain this position and to walk erect when offered raw meat. The dog was always fasted for twenty-four hours and weighed before each test. The drug was administered orally in gelatin capsules. In all the tests reported the same preparation of fluidextract of *Cannabis sativa*, U. S. P. IX, was used. This preparation had been used for six months as a standard in other cannabis assays and its potency had been accurately determined. During the experiment the animal was kept alone in a quiet room, and observations were made every half-hour.

When a reacting dose of the drug was given, the first symptoms observed were those of mild excitation. At this stage it would eagerly stand erect or jump for the proffered meat. Later, as the effect of the drug became more pronounced, the dog would make an eager but unsuccessful attempt to stand erect and later its efforts became more and more listless. Sometimes during the latter part of this stage it was necessary to coax the dog to induce it to make any effort to arise. These effects would usually wear off during the third hour. With larger doses, the dog, after passing more rapidly through these stages, would become lethargic and show no desire for food if any effort were required to secure it.

Incoördination was considered to be established when the dog was unable to stand erect.

The results of a number of experiments are shown in the following table:

No. of assay.	Date of assay.	Weight of animal in kilos.	Dose per kilo in cc.	Total dose administered in cc.	Results.
1	Jan. 24	6.78	0.040	0.27	Positive
2	Jan. 31	6.48	0.030	0.19	Positive
3	Feb. 7	6.35	0.020	0.13	Negative
4	Feb. 11	6.61	0.0295	0.20	Positive
5	Feb. 18	6.72	0.0250	0.17	Negative
6	Feb. 21	6.41	0.0270	0.18	Negative
7	Feb. 28	6.15	0.0285	0.18	Negative
8	Mar. 20	6.54	0.0290	0.18	Positive
9	Mar. 27	6.81	0.0280	0.19	Negative
10	Mar. 31	7.37	0.0290	0.22	Positive
11	Apr. 3	6.69	0.0290	0.20	Positive
12	Apr. 7	6.74	0.0290	0.20	Positive
13	Apr. 17	7.15	0.0285	0.20	Negative
14	Apr. 21	7.03	0.0290	0.20	Negative
15	Apr. 24	7.03	0.0295	0.20	Negative
16	Apr. 28	7.06	0.030	0.21	Negative
17	May 5	6.92	0.030	0.20	Positive

The animal after a succession of doses of the preparation administered at short intervals developed a certain temporary tolerance, but when the interval was again lengthened to one week, the tolerance apparently wore off.

These experiments seem to show that a more definite end-point in the determination of muscular incoördination, and, therefore, a more exact estimation of the effective dose of cannabis preparations, can be determined by this method than the one in the Pharmacopœia.

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SWEETENING EFFECT OF AMMONIA ON FLUIDEXTRACT OF LICORICE.*

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Independent investigations conducted during the last few years have quite conclusively shown that the use of ammonia in the extraction or preparation of fluidextract or extract of licorice is not only unnecessary but that it produces undesirable effects of gelatinization and precipitation.

By eliminating ammonia, the preparation, from a standpoint of physical elegance, was undoubtedly improved and manufacturing difficulties removed, but some doubt was entertained as to the effect the elimination of ammonia would have on the taste.

In order to observe if ammonia added to fluidextract of licorice aided in producing a sweeter or better tasting preparation, the following experiments were made:

To each of three 50-cc. quantities of fluidextract licorice was added ammonia water 28%—0.25 cc., 0.5 cc., and 1 cc., respectively.

These 3 samples, together with a sample of the same fluidextract to which no ammonia had been added, were marked arbitrarily and submitted to several people with request that they express their preference as to flavor or taste.

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