polarized light. The results can best be compared by a study of the photomicrographs shown in Figs. 1, 2 and 3.

Slides were made of hydrastine in two per cent sulfuric acid, and picric acid solution. The crystals formed were entirely unlike those of coptine and picric acid solution. Photomicrographs of these, and of the crystals obtained with berberine in two per cent sulfuric acid and picric acid, show the marked differences in the three, as seen in Figs. 4 and 5. These tests conclusively set coptine apart from berberine and hydrastine.

## SUMMARY AND CONCLUSIONS.

1. *Coptis occidentalis* contains coptine, an ether-soluble alkaloid which is the same as the one found in *Coptis trifolia*. This alkaloid produces a marked purple fluorescence when dissolved in ether solutions.

2. Coptine is hydrolyzed slowly by dilute solutions of sulfuric acid to a form which gives no characteristic alkaloidal tests.

3. Microscopic examinations are practical to identify coptine and to distinguish it from two related alkaloids, hydrastine and berberine; the crystals formed with picric acid solution and coptine in sulfuric acid being particularly efficient for this identification.

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# BIOASSAY OF LAXATIVES ON MONKEYS (RHESUS) AND ON LOWER MAMMALIANS (DYEMEAL METHODS).*

BY S. LOEWE.[1]

The potency of many of the laxative preparations in use cannot be measured by chemical methods. Biological procedures must be employed, but the need for dependable bioassay methods for laxatives is unsatisfied.

In 1925, Loewe and Faure (1) devised a "dyemeal" procedure for measuring the passage of the intestinal contents, and applied this procedure to the assay of laxatives. Since that report, these studies have been continued with the objects of improving the bioassay methods implicated in those observations and of finding the most suitable test-animal for laxatives as well as the most appropriate test-function.

In the search for test-animals, our attention was originally directed to the smaller laboratory animals, particularly the albino mouse, which is most appropriate for dyemeal methods. As these studies went on, it became apparent that for many purposes the monkey was superior to all other test-animals. From the numerous manifestations of laxative action, increase in rate of intestinal progression was finally found most appropriate for the dyemeal assays in the mouse, and change in stool consistency for the assay in the monkey. Therefore, the present report on useful routine methods omits all our attempts with other devices of testing the functioning of the intestine, and is restricted to the applications of the dyemeal

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methods on smaller rodents and to the method developed for laxative bioassay in the rhesus monkey.

### A. DYEMEAL METHODS.

*Principle.*—The underlying principle of the dyemeal methods is to measure the rate of progression of intestinal contents. A dyemeal is employed to provide an easily traceable material. The rate of progression is measured in one of two ways, either by recording the time necessary for the meal to traverse a given distance ("time method") or by measuring the distance traversed in a given time ("distance method"). In either case, a precise starting time is secured by administering the dyemeal through a stomach tube. In the time method, the distance employed is that from the pyloric sphincter to the anus, the arrival of the dyemeal at the latter point being indicated by the evacuation of the first dyed stool. In the "distance method" the time selected for killing the animals was either 120 or 160 minutes, and the distance measured immediately after death was that from the pyloric sphincter to the most advanced position of the dyemeal. The "distance method" is the procedure of choice because the "time method" involves a second variable, namely, the defecation reflex. It has been found that a considerable laxative effect can be masked by delayed defecation.

*Technique.*—The albino mouse is the species of preference. The meal consists of a non-absorbable dye. No significant difference was found between India ink, colloidal charcoal and carmine. For the purpose of decreasing the considerable individual variation in intestinal motility in mice, the meal may contain a mucilage (1% to 2% salep or tragacanth, or 10% to 20% gum acacia). The test dose is added to the dyemeal, if the drug acts sufficiently rapid. Otherwise the drug is administered 30 to 120 minutes prior to the dyemeal, generally by stomach tube. The total volume of the dyemeal is 0.3 cc. Each dose of the test substance is given to a series of at least 7, preferably 10 to 15, animals. Two control series are run, one consisting of a group of mice receiving only the dyemeal, and another group receiving suitable doses of an appropriate reference preparation.

*Interpretation of Results.*—The results are expressed in terms of (relative) potency, *i. e.*, the ratio of equi-effective doses of test substance ($T$) and reference preparation ($R$):$R/T$. In the "time method" those doses are equi-effective which cause an equal shortening of defecation time; in the "distance method," those doses which allow the dye to reach an equal position in the large intestine.

*Applications and Restrictions of the Dyemeal Methods.*—The dyemeal methods fundamentally can be employed with any species of laboratory animals. In addition to mice, rats (2) and guinea pigs have been used. These two species, however, have no advantage over the mouse; the mouse has the advantage of low cost and small absolute dose. The susceptibility of all these animals to laxatives, nevertheless, is rather low in comparison to humans; large doses relative to body weight must be employed, and rodents, as all the species below the primates, are insensitive to several laxatives effective in man. Those drugs which have been tested in mice are the following, in order of decreasing effectiveness: physostigmine, cholin esters, castor oil, salines, anthraquinones, colocynth, calomel, diphenolisatines and phthaleins.

### B. MONKEY METHOD.[1]

#### *I. Principle.*

Williams, Abramowitz and Killian (1933) (3) showed that phenolphthalein is an effective laxative for the rhesus monkey (*Simia rhesus*). The method described here resulted from a continuation of the work of these authors. To date, 4500 experiments have been performed on 128 monkeys.

Early in the course of this study, it became obvious that the susceptibility to laxatives varies greatly in different monkeys, as well as in the same animal at different times. For example, the average effective doses as determined for individual monkeys may differ as much as ±98% from the group average. Also, for a particular monkey, at different times variations may occur in the response to doses extending over a range of ±60% of the average dose for that animal.

[1] These monkey experiments have been sponsored by Ex-Lax, Inc., Brooklyn, N. Y.

The details of susceptibility of the monkey which will be reported in a separate communication are necessarily of great significance for the development of any procedure for assaying unknown laxatives. Any bioassay has the purpose of determining the dosage relation between the test preparation and a reference preparation. This relation is usually expressed in terms of (relative) potency, *i. e.*, in the ratio $R/T$ (see page 428) of equi-effective doses of the two preparations. In the rhesus monkey, because of the above individual and group variations, equi-effectiveness offers no definite measure of potency. It is much more conclusive to consider the relation of inequi-effective doses in a procedure of "bioassay by approximation" (4). In applying this procedure to the rhesus monkey, the following principles were adopted:

1. Different preparations may only be compared with each other on the same test-animal. The average dose for an entire group is of no value.

2. Only "effectiveness" and "ineffectiveness" may be considered in evaluating the laxative response. The finer grades of intensity of action are observed, but not considered too significant, because of the fluctuating susceptibility.

3. Ineffective doses of the test preparation may only be referred to effective doses of the reference preparations, and effective test doses only to ineffective reference doses. With the aid of these cross-comparisons, from which border values of maximum and minimum potency are obtained, the range of potency may be determined by approximation.

4. Only animals in which the range of reference has been carefully determined may be used for evaluations. The accuracy of the assay depends upon thorough "calibration" of the animal with respect to the reference preparation.

## *II. Care of the Experimental Animals.*

*Animals.*—Adult animals (1500 to 5500 Gm.) are used. Weight and temperature are biweekly controlled. Sex has been found to be relatively unimportant. The number of stock animals ranged from 45 to 60.

*Housing.*—The monkey house is a glass-brick building with ample daylight and air-conditioned to an average of 75° F. Each animal is placed in an individual all-round-metal screen cage measuring 10 cubic feet. Interchangeable screen-covered metal pans allow the stools to be collected, and aid in keeping the cages clean. Cages are placed on wheel stands to facilitate cleansing and exposing the animals to open air and sunlight.

TABLE I.—DIET.

| | Monday. | Tuesday. | Wednesday. | Thursday. | Friday. | Saturday. | Sunday. |
|---|---|---|---|---|---|---|---|
| Forenoon: | One slice of wheat bread and butter, and: | | | | | 2 Slices of wheat bread and butter | |
| | 1 orange | 1/2 apple | 1 orange | 1/2 apple | 1 orange | | |
| Afternoon: | 100 Gm. lettuce, 100 Gm. boiled potato, 1 hard boiled egg | 100 cc. milk, 100 Gm. carrots, 100 Gm. boiled potato | 100 Gm. lettuce, 1/2 cup "monkey food," 100 Gm. boiled potato | 100 Gm. raw or cooked oats or rice, 100 Gm. tomato pulp with bone- and blood-meal | Same as on Monday | 100 Gm. carrots, 100 cc milk | 100 Gm. lettuce, 1 apple |

*Diet.*—Two meals are fed daily, 9:30 A.M. and 4:30 P.M. The diet schedule is shown in Table I. Water is offered liberally.

*Stool Records* —Records are kept of the number of stools per day and of the stool characteristics, the latter in numerical designations—lacking (0), solid (2), medium (4), soft (6), liquid (8) or intermediate between these (3, 5 or 7, respectively)—and summarized for each test monkey in a continual "stool curve" (Fig. 1). Monkeys fed the above diet consistently passed "hard" (2) stools.

## *III. Technique of Bioassays.*

*Administration of Laxatives.*—Only animals with hard stools are used. Doses are weighed individually with a maximum error of 1.5%. Most laxatives are easily fed as powder or solution, by introducing them into a slice of banana, apple or potato. The dose is usually administered before the afternoon meal, and must be consumed without delay.

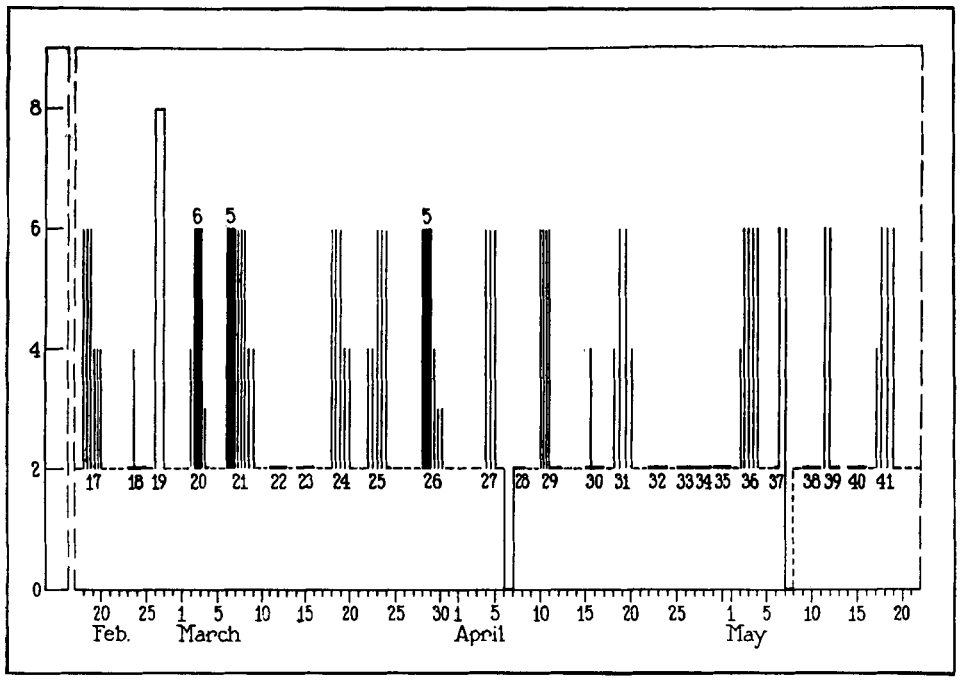


Fig. 1.—Part of Stool Curve of monkey No. 352.—Abscissa: calendar days; ordinate: stool consistency (see text for explanation of figures). Figures at ordinate level 2 are Prot. Nrs. of laxative experiments performed; figures at ordinate level 6: number of stools on particular day.

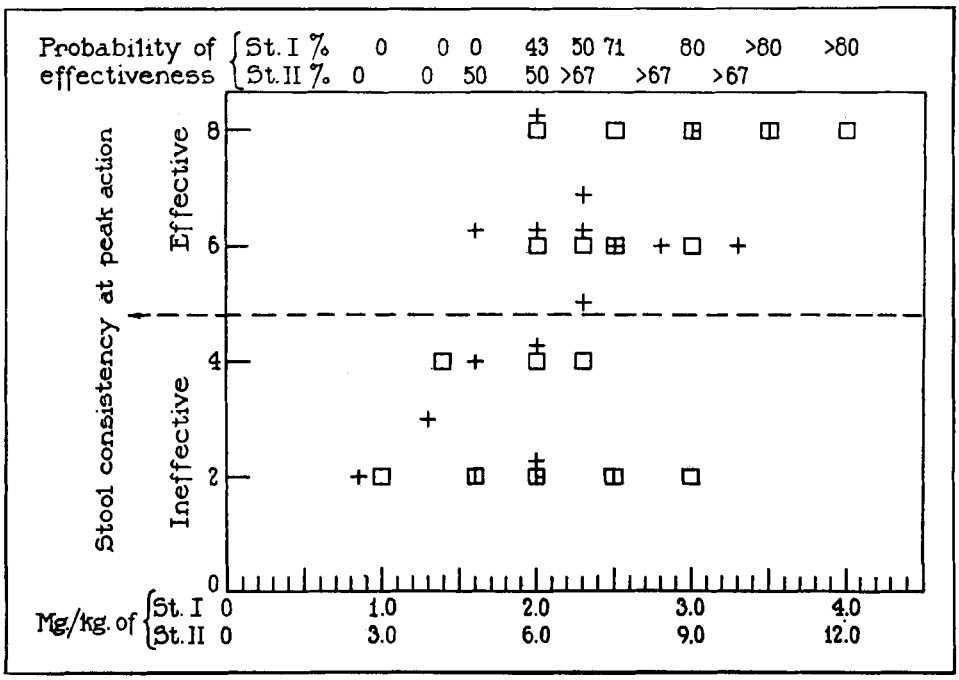


Fig. 2.—Calibration Graph of monkey No. 282.—□ = one, ⊡ = two, ⊟ = three, ⊞ = four experiments with reference preparation No. I ("St. I"); + = single experiments with reference preparation No. II ("St. II").

*Determining the Laxative Action.*—The action is recorded in the "stool curve" (see Fig. 1). The final result is determined by the stool consistency during the period of greatest deviation of the stools from the normal, results 0 to 4 are designated as "ineffective," 5 to 8 as "effective."

*Evaluating Individual Susceptibility (Calibration of Animals).*—For a precise calibration of each monkey, it is necessary to examine a wide range of doses of an appropriate reference substance repeatedly before and after examining the unknown laxative. When assaying laxatives from various chemical classes, a well-defined preparation from any of these, when assaying different lots of the same laxative, a single lot of suitable potency is selected as the reference preparation. Our animals were calibrated simultaneously with a highly purified U. S. P. phenolphthalein, a commercial phenolphthalein and a pure diacetyldiphenolisatine. Monkeys, when satisfactorily calibrated, have an individual record of 22 to 50 calibration experiments which is summarized in the form of a "calibration graph" with an abscissa of mg. per Kg. for the reference preparations, and an ordinate of the responses recorded by stool consistency, as in Fig. 2.

The task of calibration is to ascertain the "Maximum Ineffective Dose," $L$, and the "Minimum Effective Dose," $H$, of the reference preparation, valid for the particular monkey pending revision through subsequent re-calibration. $L$ is the dose with an incidence of not more than 10% of positive, $H$ that with not more than 10% negative responses; if the number of experiments is insufficient for direct determination, either of these doses may be calculated by interpolation.

*Performance of the Bioassay.*—Administer orally graduated doses of the test preparation to 7 to 10 well-calibrated monkeys. Observe subsequent evacuations for at least 2 days, and determine if the dose was "effective" or "ineffective" (see above). Calculate for each single experiment either the maximum potency by dividing the reference dose, $H$, of the same animal by the ineffective test dose, $d$; or the minimum potency by dividing the reference dose, $L$, by the effective test dose, $D$. Group each of these two series of potency values in arithmetical progression, and find the potency as the mean value between the lowest maximum ($H/d$) and the highest minimum ($L/D$) figure (4). If the range between these two values varies by more than $\pm 15\%$ of its mean value, or is determined by less than four experiments, perform an additional series of tests with appropriate doses.

For the sake of comparison, many of the bioassays performed have also been calculated by referring the test dose to a "Reference Standard Dose," $R_{50} = {}^1/_2 (L + H)$, of the same animal. The results reached by this procedure were naturally found to be less conclusive, because of the considerable individual variation. Any type of "Unit Dose" is an inadequate reference dose unless the validity of this figure is defined by considering the range of deviation (compare Fig. 2).

*Applications and Advantages of the Monkey Method.*—The method presented here has been found applicable to all three purposes of bioassay, namely, to compare active principles from different pharmacological classes, different preparations of the same active principle or different lots of the same preparation. Laxatives from the following classes have been comparatively evaluated with phenolphthalein and diacetyldiphenolisatine as reference substances: salines, anthraquinones, isatines and phthaleins. Various preparations of the same active principle have been compared with each other, *e. g.*, extracts from senna and esters from the isatine and phthalein groups. Finally, the differences in potency between batches of the same preparations have been determined, *e. g.*, of diphenolisatine-esters and phenolphthalein.

When assays of the same test substance were repeated after varying intervals with the use of different monkeys, the results always agreed within a range of $\pm 15\%$. This is a degree of reproducibility rarely exceeded in any bioassay method.

The rhesus monkey responded to all the numerous laxatives tested. The ease with which the rhesus can be used as a test animal for phenolphthalein is a decided advantage in the pharmacological investigation of this drug hitherto hampered by the lack of an appropriate test-animal.

The monkey is one of the very few animals which is similar to humans in its responses to laxatives in doses comparable to those used clinically. This is illustrated by the following example of parallel experiments on the rhesus and on humans, details of which will be reported in a separate paper:

Four laxatives (I, II, III and IV) of different potency were prepared, all containing phenolphthalein. They were first carefully assayed in the monkey. Four groups of healthy human volunteers were used, each consisting of between 272 and 593 individuals. As can be seen in

Table II, the figures for relative potencies obtained by assay on the monkey show a surprising correlation with those found by human assay.

TABLE II.

Potencies of four test-substances I, II, III and IV in the rhesus monkey and in healthy humans (number of "watery" responses per hundred "soft" responses). Relative Potencies as referred to that of substance I = 1.0.

| Test-Substance No. | I. | II. | III. | IV. |
|---|---|---|---|---|
| Relative potency found: | | | | |
| (*a*) in the monkey: | 1.00 | 1.74 | 2.22 | 2.67 |
| (*b*) in humans: | 1.00 | 1.70 | 1.90 | 3.50 |

SUMMARY.

1. Two techniques for bioassaying laxatives are described: (*a*) the dyemeal methods on smaller laboratory animals; (*b*) a method which uses the monkey as test-animal. The advantages and restrictions of the dyemeal methods are briefly discussed.

2. A method of "bioassay through approximation" is recommended for the evaluation of laxatives. The rhesus monkey is the test-animal, and the method is based upon a thorough study of the individual and group variations in the susceptibility in this species.

3. In more than 4500 experiments on 128 monkeys, the method was found valuable for the bioassay of laxatives from different pharmacological groups as well as for comparing the potency of various preparations of the same active principle and different batches of the same preparation. The error of the method is not over $\pm 15\%$ and usually less.

4. The method was found to be far superior to any existing bioassay method for laxatives. The monkey manifests susceptibility to a large number of laxatives and its sensitivity to these is comparable to that of humans. By assaying four different laxative preparations on monkeys and on several thousand healthy humans, the same values of relative potency were found.

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## PHARMACEUTICAL SOCIETY OF GREAT BRITAIN.

The 98th annual meeting of the Pharmaceutical Society of Great Britain was held May 17, 1939, President Thomas Guthrie presiding. Under Public Services he referred to the efforts of the Society to secure adequate pharmaceutical services and discussed the actions of the members in air raid precautions.

The President discussed his membership during the past twenty-three years, in the Council, and stated that he is now retiring as a member. A tribute was paid him.

An important discussion ensued, relating to students of Pharmacy and conscription; it was the opinion that students should not be taken from their last year of study for six months; the matter has been discussed with the Ministry of Labor, but the decision of the latter has not been received.—From the *Pharmaceutical Journal.*