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TWO NEW METHODS FOR PHARMACOLOGICAL COMPARISON OF INSOLUBLE PURGATIVES.*

BY DAVID I. MACHT AND J. BARBA-GOSE (of Barcelona).

INTRODUCTORY.

Although the pharmacodynamics of intestinal movements has been studied by many eminent physiologists and pharmacologists such as Magnus (1), Cannon (2) and others, the methods for quantitative comparative evaluation of laxatives and purgatives at the disposal of the pharmacologist are still very inadequate, particularly in respect to those drugs which are not soluble in water or physiological solutions. When dealing with powerful alkaloids and other active principles readily soluble in water, it is a simple matter to follow their effects either on surviving segments of the intestine or isolated strips of muscle or even on the whole intestinal tract *in situ*. When, however, the investigator wishes to ascertain the action of laxative oils, resins and other insoluble substances, the problem is of a very different nature, and the commonest method of approach has hitherto been to feed the various materials to large animals, which had been previously given a dry diet, and then to note the frequency, the quantity and the consistency of the stools. Such experiments have usually been conducted on dogs, cats and rabbits. Sollmann (3) in his comprehensive Laboratory Guide in Pharmacology, can suggest no better method for comparing purgatives than a personal trial of representative specimens from various groups of laxatives by the students themselves. For economical reasons a number of German investigators after the World War attempted to utilize the white mouse for studying intestinal peristalsis. Thus, Laqueur (4) used the whole length of the excised intestine of the white mouse for demonstrating the effects of a number of active principles, which, however, are not used as purgatives. Fühner (5) devised an interesting method of roughly comparing the number of laxatives by feeding them to mice in the form of small pills and, following his work, Loewe and Faure (6) devised a more elaborate method of tracing the passage of ingesta by feeding mice with various laxatives together with small quantities of India ink. The latter method has not been satisfactory in the experience of the present writers but suggested the use of finely divided carbon in the first method to be described.

In connection with a study of the oil of *Ruvettus pretiosus*, or "castor-oil fish," the present authors began an extensive investigation on the comparative laxative

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effects of various oils and a number of synthetic chemically pure insoluble compounds, for which all the methods previously employed in the comparative study of laxatives were found to be unsuitable (7). The problem was to devise a practical method by which various oils and other insoluble liquids or solids could be quantitatively compared in respect to their purgative action on animals and, furthermore, in view of the great scarcity of certain synthetic chemical compounds of which not more than one or two cubic centimeters were available, to devise a micropharmacological method in which even very small quantities of the material could be used to advantage, yielding definite and quantitative pharmacodynamic results. After numerous futile attempts, the authors finally succeeded in elaborating two procedures which proved to be quite satisfactory for such purposes. One of these utilizes the animals as a whole while the other involves the employment of isolated surviving loops of the intestine.

THE FIRST METHOD.

An excellent method for studying the effect of purgatives on intact animals can be carried out on white rats. The authors found these animals much more suitable in many respects for such work than white mice. In the white rat the passage of intestinal contents from the pylorus to the rectum usually requires from one and a half to two hours and sometimes even less. Full-grown white rats, of the genus *Mus norvegicus*, have been used by the authors exclusively. These animals are first kept for some time in the laboratory and fed on a standard dry diet, the composition of which is as follows:

Wheat—25 grams
Ground maize—25 grams
Rolled oats—28½ grams
Flaxseed meal—10 grams
Casein or whole milk (dried)—10 grams
Sodium chloride—1 gram
Calcium carbonate—0.5 gram.

Before a series of experiments, a number of rats are selected and allowed to fast from fifteen to twenty hours so as to have the stomach and small intestine free from food. In order to determine the time required for passage of ingesta through the intestinal tract, a number of the animals are given by "stomach tube" a specially prepared emulsion containing finely divided animal charcoal. An assistant holds the animal wrapped in a towel; its jaws are pried open with a pair of forceps; and the experimenter introduces the tube into the stomach. The "stomach tube" consists of the distal portion of such a fine silk ureteral catheter as is employed for cystoscopy by urologists and gynecologists. The passage of the tube requires considerable experience, and a beginner will often introduce the catheter, or "stomach tube," so clumsily as to produce trauma, pierce the esophageal walls, and injure the lungs. From 0.5 to 1 cc. of a suspension of finely divided charcoal is injected into the stomach through the tube by means of a tuberculin syringe provided with a thick bore needle, which fits snugly into the catheter. With practice the passage of the "stomach tube" and introduction of the suspension can be accomplished very easily without discomfort to the animals. The suspensions which the authors have been using were so prepared as to be thick enough not to flow too readily and

yet thin enough to be injected through the large needle fitting into the catheter. The formula generally employed was as follows:

Tragacanth	2.0 gm.
Purified animal charcoal	12.0 gm.
Water	130.0 cc.

In some cases a small quantity of powdered nutgalls, added to the mixture described above, made an even more satisfactory emulsion.

After introducing the charcoal suspension into the stomach, the time is noted and the animal is placed in a cage and allowed to eat of the ration mentioned above *ad libitum*. At the end of a given period, conveniently, from 40 to 50 minutes, the animal is taken from the cage and killed as quickly as possible with chloroform, care being taken to use a liberal amount of the anesthetic so as to minimize, or eliminate entirely, the stage of excitement. The body is then rapidly opened and the entire

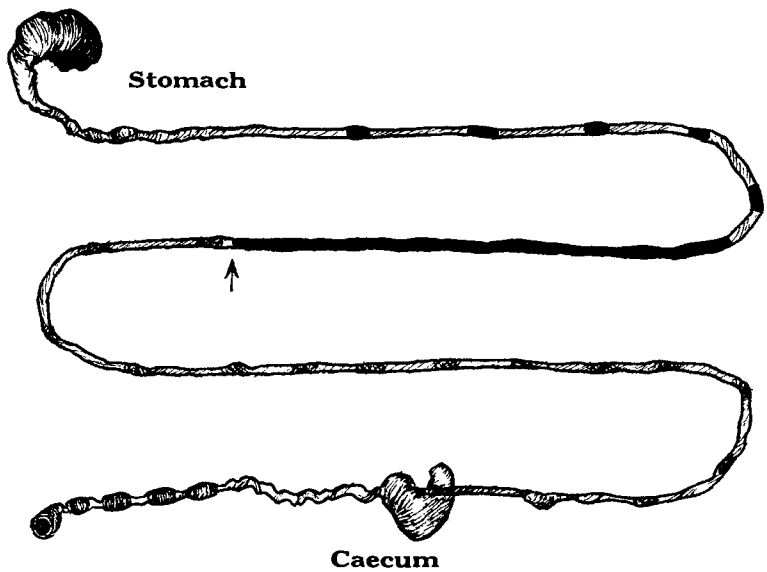

Fig. 1.

gastrointestinal tract, from the esophagus to the anus, is excised and stretched out on a table. The total length of the intestinal tract from the pylorus to the end is measured in centimeters. Examination of the intestine plainly reveals how far the carbon suspension has moved from the time of its introduction into the stomach until the time of death. The carbon particles can be detected by their black color showing through the intestinal walls. The exact position of the small particles, of course, can be more accurately ascertained by making an incision into the lumen of the gut. The distance the carbon particles have traversed from the pylorus is also measured with a centimeter tape and expressed in the form of percentage of the total length of the intestinal canal. Thus, it has been found that the usual distance traversed in fifty minutes by the contents containing carbon particles is from 45 to 50 per cent of the total length of the intestinal tract. The normal time of passage of contents through the intestines having been established, other animals from the same group are selected and given the substance to be tested in a similar way.

Thus, for instance, the effect of castor oil is studied as follows: the stomach tube is introduced by the method described above and 0.5 cc. of the charcoal suspension is injected into the stomach. This is followed by 0.1 cc. of castor oil and the drug is followed again by 0.5 cc. of the carbon suspension. In a similar manner as little as 0.1 cc. of an oil or other insoluble liquid to be tested for its laxative effect can be administered to rats by "sandwiching" it between two doses of the charcoal suspension. This method was found to be preferable to mixing the drug thoroughly with the suspension before injection. It is needless to state that when a number of drugs are studied at the same time in the manner described above, the stomach tube and catheter, syringe and needle must be thoroughly cleansed between the various injections. Figure 1 is a diagramatic illustration of the method employed. It represents the whole greater intestinal tract of a rat. The carbon mixture with food contents is colored black, and the arrow shows the farthest point reached by the carbon suspension at the end of a given period of time. The distance from the pylorus to this point, expressed as a percentage of the total length of the intestinal canal from the pylorus to the anus, gives a numerical index for the speed of passage through the intestines of the particular mixture administered.

The authors have performed a great many experiments by this method and found it exceedingly useful, especially for the comparative study of the laxative effects of various oils and insoluble liquid chemical compounds. Thus, for instance, in connection with an extensive study of the oil from *Ruvettus pretiosus*, or "castor-oil fish," the following comparative figures were obtained, and it will be noted that while olive oil is but very slightly laxative, castor oil and *Ruvettus* oil are very much more so, the latter being of about the same efficiency. It will be further noted that it is the unsaponifiable fraction of *Ruvettus* oil which possesses most of the laxative effect.

TABLE I.

		Average distance traversed (per cent of total length).			Average distance traversed (per cent of total length).
I	Controls	45	IV	*Ruvettus* oil	68
II	Olive oil	55	V	Saponifiable fraction	63
III	Castor oil	70	VI	Unsaponifiable fraction	77

THE SECOND METHOD.

The method usually employed in studying intestinal pharmacodynamics *in vitro* consists of suspending either a segment of live intestine or strips of surviving muscle in physiological saline solution kept at body temperature and oxygenated. The effect of drugs on such preparations is studied by introducing various quantities of the substances into the physiological saline in which the segment is suspended and observing the contractions of the muscle as inscribed by the lever on the kymograph. Such a method is obviously applicable only to the study of drugs or chemicals which are more or less soluble in water or physiological saline solutions, and they are inadequate for the study of oils, resins and other insoluble solids or liquids. The following very simple procedure devised by the authors was found to give valuable information in connection with the study of purgative oils and some related insoluble chemicals. A cat is anesthetized with ether and a laparotomy is performed. A segment of the small intestine, whether it be the ilium or jejunum, is selected and a loop of any desired length is tied off. The mesenteric vessels leading

to the loop are ligated and the segment is excised with the ligatures intact. This closed segment of the intestine is placed in the Locke or Tyrode solution to be used as the material for pharmacological investigation. The intestinal loop is taken out without allowing the Locke solution to penetrate the lumen and a piece of the intestine, one or two inches long, is cut off and one end of this segment is ligated so as to completely occlude the lumen. Into the free and open end of the segment is introduced a specially constructed glass cannula (C), which is presently to be attached to the short arm of a recording lever. The occluded or ligated end of the segment

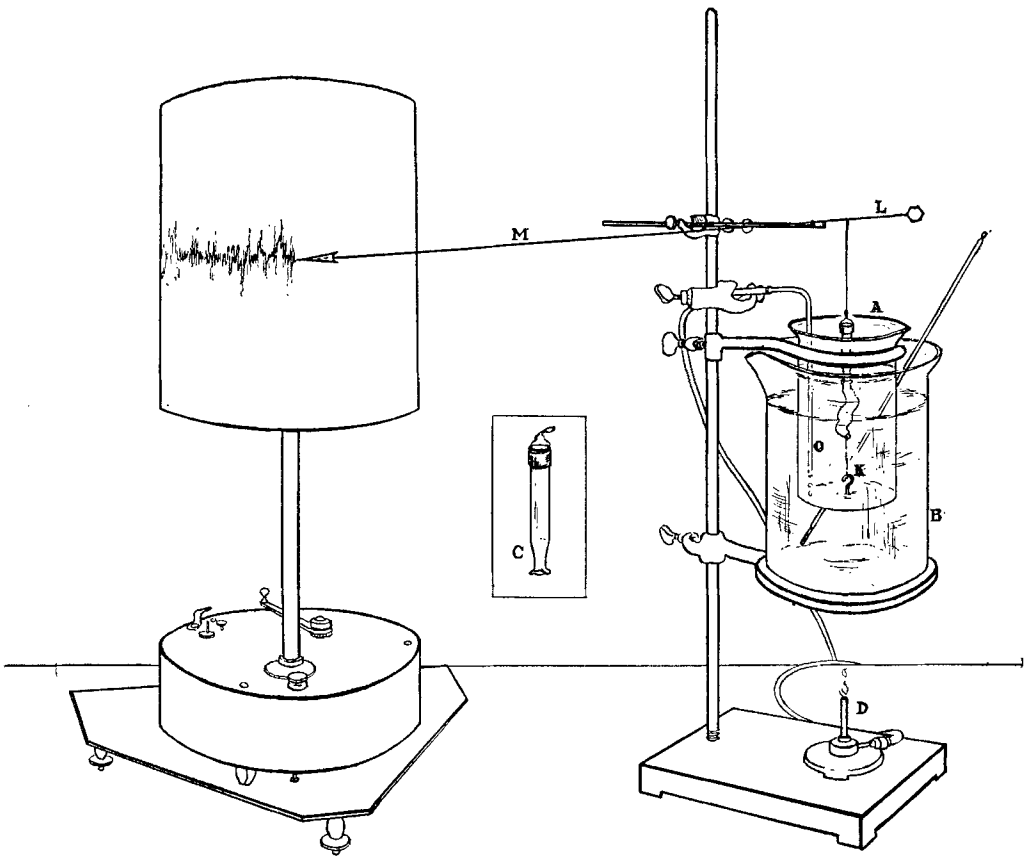

Fig. 2.

is attached to a glass hook (K) fused into the bottom of a glass beaker (A), containing physiological saline oxygenated through a tube (O). The free end of the segment attached to the glass cannula or collar is fastened to the short arm of the lever (L). The collar or cannula is made of such a length that even when the intestinal segment is contracted to a high degree its opening will still be above the level of the physiological saline solution. The chamber (A), containing the preparations thus suspended in oxygenated physiological saline, is kept at a constant temperature being immersed in a water-bath (B) heated by a Bunsen burner (D). The long arm of the level (M) is weighted sufficiently to keep the lever writing in a

horizontal position under normal conditions. In this way, by the use of a very slowly revolving kymograph, the normal contractions of the intestinal segment are first allowed to be recorded for an hour or more. When such a tracing is fairly uniform and level, minute quantities of an oil or other insoluble liquid to be tested are carefully introduced with a fine pipette through the glass cannula into the lumen of the intestinal preparation. If the weight of the drug thus introduced is sufficient to change slightly the level of the long arm of the lever, a counterweight sufficient to offset this is added. The preparation is then allowed to write upon the slowly moving drum at a constant temperature for from one to two hours, or longer. In this way the oil or other insoluble liquid brought in contact with the surface of the intestinal mucus membrane is allowed to be acted upon, if possible, by whatever traces of digestive enzymes may be present in the villi and to be absorbed. As a matter of fact, to the surprise of the experimenters, castor oil and other laxative oils, as well as various insoluble liquid chemicals, after remaining for an hour or two, and sometimes longer, inside the intestinal segment under the conditions described

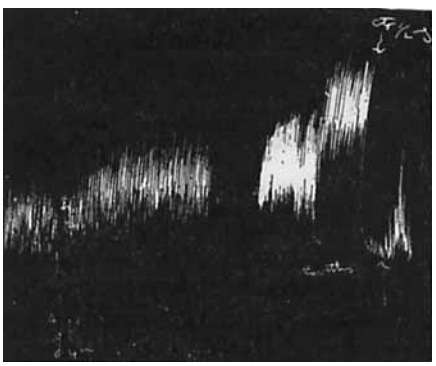

Fig. 3.—Effect of olive oil, Ruvettus oil and atropine sulphate on segment of cat's jejunum.

above, showed a pharmacodynamic effect, some of them producing stimulation of the contractions, others increasing the tonus of the preparation, and still others having an inhibitory or paralytic action. An illustration of the curves obtained by the method described is shown in Fig. 3. Here we see the relative effects of olive oil, oil of *Ruvettus* and atropine sulphate on two isolated segments of cat's intestine.

COMMENT.

The two methods described above have been employed by the authors especially in studying the oil of *Ruvettus pretiosus*, or the so-called "castor-oil fish," and comparing it with olive oil, castor oil, croton oil, mineral oil and certain other oils. They were furthermore employed for testing small quantities, 0.1 to 0.2 cc., of chemicals obtained by Professor E. Emmet Reid and Dr. Warren M. Cox on fractionating *Ruvettus* oil in order to analyze in greater detail its exact mechanism of action, to be described in a fuller pharmacological study elsewhere. It was found, in general, that the results obtained by both methods completely agreed with each other. These results, obtained by the devices described above,

were also corroborated by roentgenological studies made by the authors on rats with the kind assistance of Dr. M. Ostro, externe in roentgenology, Johns Hopkins Hospital, and roentgenologist, Sinai Hospital, Baltimore. In view of these gratifying results, it is deemed worth while to publish a description of the methods of experimentation for the benefit of those interested in this line of work. While the procedures employed may not be ideal, they were found to be quite adequate, and further investigation along these lines may lead to greater improvements of technique in a domain of pharmacology which has not yet been satisfactorily developed.

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A STATISTICAL STUDY OF THE PHARMACOPŒIAL CONSTANTS OF OLEUM CHENOPODII.*

BY JAMES C. MUNCH AND WILLIAM F. REINDOLLAR.

During the five-year period from 1925 to 1929, inclusive, authentic samples of normal and high-test wormseed oil were collected at the stills. The ascaridol content, specific gravity at $\frac{25°}{25°}$ C., specific rotation, refractive index at 20° C. and solubility in 70% alcohol were determined by the methods of the tenth Pharmacopœia (6). Pertinent data upon thirty-nine samples of normal and seven samples of high-test oil have been subjected to statistical analysis. Because of the difficulty of obtaining authentic oils a larger number of results could not be obtained. A detailed study has been made of the normal oils, but because of limited data less attention has been paid to high-test oils.

The values for ascaridol and the physical constants of the normal oils are given in Table I. The sums of the individual values of each determination have been divided by the number of samples to obtain the "mean." The deviation of each individual value from the mean has been squared. The sum of these squares has been divided by the number of observations to obtain the average of the squares. The square root of this average value is termed the "Standard Deviation." The standard deviation is a statistical measure of the accuracy of a series of determinations, and is expressed in the same order of magnitude as the mean. In order to obtain figures by which comparisons of the accuracy of different determinations may be made the "Coefficient of Variation" is calculated. This is obtained by dividing the standard deviation by the mean and multiplying the quotient by one hundred. In other words it is that per cent of the mean which is represented by the standard deviation. A series of results which differs but slightly from the mean, such as those of specific gravity or refractive index, show small values for

* Scientific Section, A. PH. A., Baltimore meeting, 1930.