EVALUATION OF CATHARTICS.*†

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INTRODUCTION.

It has been pointed out by the students of human afflictions that in this country approximately 90% of the people—at one time or another—suffer from constipation. The discomforts and toxic complications, as a result of such constipation, are too well known to require enumeration or discussion.

The certain and speedy removal of the clogged-up waste and food material (or the removal of agents causative of indigestion and diarrhea) from the intestinal canal is of paramount importance, as such clearance alone prevents further resorption of toxic substances and excretions.

Since the danger of using drastic intestinal irritants is also well known, methods and medicaments must be selected for treatment which are least, if at all, harmful to one or all body organs and tissues.

An adequate bio-assay method is therefore needed, permitting one to determine the physiological value of the multitude of cathartic substances, natural or synthetic, all more or less highly recommended as laxatives. This need becomes all the more urgent in view of the fact that our knowledge of the chemical nature of our most common vegetable drugs, as aloes, cascara, rhubarb, senna and podophyllum, is still too vague to permit their chemical evaluation.

The author, after a brief historical review, aims to emphasize the significance of additional results, obtained with his daphnia method (1–5), since these findings permit quantitative comparisons, made possible through the use of a uniform chemical reference standard.

HISTORICAL.

An interpretive review of results obtained upon testing of laxatives on various animals and man, has been given most recently by Munch (6) from whose book we quote the following:

"Fish: The reports (with cathartics) do not suggest any characteristic response by the fish; accordingly tests upon these animals do not seem to offer possibilities for quantitative assays.

"Mice: Mice stored and fed under laboratory conditions showed enormous variations in the normal rate of passage of food along the gastro-intestinal tract. In a series of preliminary examinations concordant results were not obtained in testing castor oil, aloin, podophyllum or fluidextract of cascara.

"Rabbits: Rabbits appear too sensitive to cathartics to be used for bio-assays.

"Cats: Though cats were found to be very serviceable for testing cathartics, the sensitivity of different animals varied greatly. The minimum effective dose of each cathartic was essentially constant when tested upon the same animals at weekly intervals over a period of several months. Differences in dosage of 25 to 50 per cent could be detected without difficulty. Smaller differences in dosage were detected upon sensitive cats.

"Dogs: Dogs have proven more variable and more difficult to standardize than cats. The possibility of constipation complicates their use for assay purposes.

"Man: Because of the great variation in activity of the gastro-intestinal tract of the same person at different times, as well as of different persons at the same time, marked differences in response to cathartics have been observed.

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** A. O. A. C. Referee on Daphnia Methods.

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"Conclusion: Tests upon cats have given more satisfactory and consistent results than tests upon mice, dogs or men. The accuracy of results (testing the same cat?) is usually between 20 and 50 per cent."

Since then Williams, Abramowitz and Killian (1934) (7), testing white and yellow phenolphthalein on various animals, found that rats failed to respond to a second and larger dose, furnishing further evidence of the inconsistency of the laxative action of phenolphthalein in lower mammals; that only repeated doses proved effective to cats, that a dog (18 Kg.) showed no laxative action, after having received a dose of 10 Gm. of yellow phenolphthalein.

Monkeys (Marcacus rhegus) of 2 Kg to 3.5 Kg. body weight were found most suitable for the study of phenolphthalein, the laxation becoming evident from 3 to 22 hours, usually within 6–12 hours. The minimum dose for different monkeys varied quite widely (as does the dose for human subjects) namely, from 2.1 mg. to 6.8 mg./Kg. body weight for the white and from 0.6 mg. to 3.0 mg./Kg. body weight for the yellow phenolphthalein.

Green, King and Beal (1936) (8), working with cascara and its constituents, concluded that bio-assay methods used were not definitely quantitative; rats were too undependable with the present technique; guinea pigs could be used to interpret the results of feeding various compounds of the bark with those of pure compounds. To permit readjustment of animals to normal at least one-week intervals were arranged between experiments.[1]

To a limited extent, especially selected human beings are said to have been found satisfactory as test objects, provided they are given prolonged rest periods. However, no published data are available.

Realizing the limitations of the test animals, found unsatisfactory for biological testing of cathartics, the advantages and disadvantages of using the cat were considered. The use of the cat is suggested by the comparatively favorable report, cited above, as well as its employment in Vieth's experiments in which the effects of oxyanthraquinones were determined in a systematic survey.

Of interest in this connection is the fact, that hexaoxyanthraquinone, found ineffective to the cat, proved laxative to man. Therefore Oswald in his book (1924) on "Chemical Constitution and Pharmacological Effect" concludes that the results obtained with the cat are not to be transferred without reserve to human beings, and urges a renewed systematic survey.

The disadvantages of using cats were:

1. The need of selecting specially sensitive animals.
2. The need of using the same sensitive animals.
3. The need of weekly intervals and continuance of the experiments over a period of several months.
4. The limited accuracy (at best about 20 to 50 per cent).
5. The restriction to a limited number of animals.
6. The difficulty of controlling such factors as uniform age, food consumption and vitality, deemed necessary for comparative studies.

Commissioned in 1934 with the testing of several aloes, aloin and the resinous residue remaining after its extraction (a problem in which the U. S. P. Revision Committee was interested), this author had chosen a new test animal, the transparent crustacean Daphnia magna. The disadvantages, cited above for cats were overcome and the tests for the evaluation of aloe gave highly satisfactory results (9).

BIOLOGICAL AND CHEMICAL REAGENTS AND METHODS.

Daphnia Magna.—The propagation of daphnia for experimental use has been reported by this author and his associate (10–11). In order to achieve standardization of the animals, the following cultural methods have been adopted.

The media are prepared from well-ærated tap water by adding 125 mg. soy-bean flour (Cellu) and 25 mg. of urea to each gallon, or at most 0.1% dried shredded cow manure (Bovung) or sheep manure (Whizard Brand), the latter usually yielding a darker pigmented and thus more

[1] The same authors, continuing their studies, determined the degree of catharsis by the rate of fecal output (expressed in Gm. per hour), JOUR. A. PH. A., 27, 95–100 (1938).

conspicuous contents in the food canal. A p_H of 7.8–8.1 is maintained by the addition of marble (in pieces) and a uniform temperature of 70±1° F. The problem of successful breeding consists in frequent feeding, freedom from worms and other parasites (through sterilization of the culture medium, if necessary) and subsequent inoculation of fodder organism from established cultures; freedom from molds of the saprolegnia type stimulated to abundant growth by acid media; freedom from chlorine and from an excess of carbon dioxide, which is indicated by numerous gas bubbles formed near surface; freedom from excess of hydrogen sulfide, detected by its odor, particularly when the culture jar is stirred; absence from excessive pollution with bacteria or infusions which causes a cloudy turbidity and lack of oxygen in the medium.

Chemical Reference Standard.—As a dependable yardstick representing a uniform constant standard measure for the degree and speed of evacuation, specially purified crystalline elaterin has been found to be most satisfactory in the standardization of evacuation. The commercial substance elaterin is a combination of two crystalline compounds, elaterin α in prisms and melting above 200° C. (230° C. or less) levorotatory, physiologically inactive, not readily soluble in absolute alcohol; and elaterin β, crystallizing in plates melting below 200° C. (194–197° (?)) dextrorotary, readily soluble in absolute alcohol and physiologically a very active cathartic (12).

TESTING METHOD.

While the complete separation has not been effected, the mixture, upon suspension in culture water as well as the filtrate[1] of this suspension, has caused speedy evacuation effected generally in from ten to twenty minutes, markedly exceeds that time observed previously for curacao aloe in a concentration of 0.05% in culture water.

A tentative assay is suggested below for the biological evaluation of rhubarb and rhaponticum, in particular and for laxative drugs in general (4). Consideration was given to.

I. The standardization of conditions as:

1. Number of animals.
2. Amount of culture water.
3. Composition including the p_H concentration of the culture water.
4. Temperature of the culture water.
5. Pretreatment of the animals (pure line, uniform sex, age and vitality)
6. Technique of administration of the preparation to be tested.

II. Determination of the normality spread of normal deviation.

1. The best time interval of observation.
2. Intensity of effect at a certain prefix time.

III. Unit of effect—fixed in terms of time and extent of evacuation.

IV. Variation of reaction of animals.

For observation of the laxative effect flat Vie-tubes or chambers, specially designed by the author, are used; the first, holds about 1 cc.–2 cc. depending upon the number of animals placed in one tube and the method of examination, the second, may be used in the projector for observation on the transparent screen or like the tubes inspected at magnification varying from 6–100 and over, with handlens or microscope.

ASSAY METHOD FOR THE EVALUATION OF LAXATIVES.

(Submitted to the A. O. A. C.) (4).

Test Object.—Daphnia (Daphnia magna). Age 10 days. Reared from pure strains, at temperatures between 68–72° C. in culture water of known p_H concentration (p_H 7.8–8.1); female sex (without eggs or young in the brood sac) from the same colonies, kept under identical conditions and with 100% filled food canal. Use 30 daphnia for each test.

Procedure.—Prepare a suspension of reference elaterin (Merck) in concentration of 0.1 Gm. in 100 cc. of culture water of p_H 7.8–8.1 and filter. (Preferably use crystalline elaterin β, melting point 197° C., 0.02 Gm. in 100 cc. culture water as above and filter.) If the solution ob-

[1] A fairly coarse filter, permitting the passage of fodder organisms was used.

tained is to be preserved, check it for potency against freshly prepared solutions at least every month and discard it if not of standard potency.

Add the standard in 0.5-cc. quantities to the test animals, placed singly in special observation tubes (flat, 3″ long, 0.5″ wide) freed from decantable culture water.

Observe the speed and extent of evacuation at low magnification (6–10), and check, if desirable, with higher magnification (100). Record the time of approximately 50% and 100% evacuations.

To test the preparation, dissolve or suspend it in amounts of 0.1 cc. in 10, 50 and 100 cc. of the same culture water as specified above and filter. Add this test solution in 0.5-cc. quantities to the test animals. Proceed as directed for the standard solution.

Determine the concentration of the test and standard solutions producing the same degree of response.

SUMMARY AND CONCLUSIONS.

The chemical evaluation is not possible of any of the common organic cathartics, as their chemical composition is not fully understood.

The author's daphnia method of physiological evaluation with Daphnia magna, worked out for the evaluation of curacao and cape aloe, aloin, and remaining resins, has been successfully applied to other organic cathartics, as rhubarb, rhaponticum, cascara, jalapin, podophyllum (13), elaterin, yohimbine (14), etc. The laxative effect of white and yellow phenolphthalein was verified by Messrs. Tinsley from Coll. of Medicine, University of Illinois and Prof. Bonisteel of Coll. of Pharmacy, Fordham University.

As a dependable yardstick, representing a uniform constant standard measure for the degree and speed of evacuation, specially purified crystalline elaterin (preferably β) has been found most satisfactory. Thus it should be possible to express the efficiency of any organic cathartic in terms of laxative units, using standardized female daphnia, 10 days old, with filled food canal, as the biological reagent, and the pure organic chemical elaterin as a standard substance for comparison.

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