An Evaluation of the Cathartic Action of Bile Salts Following Acute and Chronic Administration to Mice*

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A survey of the literature regarding the cathartic action of bile and bile salts reveals that most of the observations are based on acute experiments. Fubini and Luzzati (1) found that bile increases peristaltic movements of the small intestine, and Horrall, *et al.* (2), observed that bile and bile salts have a temporary local stimulant effect on the motility of the small intestine in anesthetized dogs. According to Haney, *et al.* (3), the application of bile to the mucous surface of the small intestine of dogs results in an increase of propulsive motility which is attributed to the influence of bile salts. Van Liere and Northup (4) reported a decrease in the emptying time of the stomach in humans following administration of bile acids. Broun, *et al.* (5), observed that large amounts of dog bile act as a purgative following oral administration to dogs. Whipple and Smith (6) found an increase in bile salt secretion following daily bile feeding to dogs, and they observed diarrhea in the animals which received large quantities (21 Gm.) of bile by mouth.

The present investigations are based on the effect of acute and chronic administrations of bile salts to mice. With slight modifications, the method for the bioassay of senna described by Geiger (7) was adapted to this procedure.

EXPERIMENTAL

Geiger's method consists of the bioassay of cathartic drugs in mice by the following procedure. After expression of urine from the bladder, the animals are placed in individual one-liter beakers,[1] the bottoms of which are covered with a double layer of filter paper held in place by a piece of one-third inch wire mesh. Food is supplied during the course of the experiment. After observation for about one-half hour, those mice whose stools are sufficiently moist to stain the filter paper are rejected. The balance are suitable for the evaluation of cathartic action in the following manner.

A solution of the drug is administered by stomach tube in 0.5-cc. volumes, using 16 or 18 gage hypodermic needles to the blunted end of which is fixed a small tear drop of solder. After replacing the mice in the beakers the stools are observed at regular intervals. Stools sufficiently soft to moisten the paper and leave a clearly visible stain are an indication of catharsis and are designated as a positive action.

Preliminary experiments confirmed Geiger's observation that 0.5 cc. of distilled water, *per se*, did not cause catharsis.

The term "bile salts," as used in this report, designates a product commercially known as Bile Salts Compound,[2] which contains not less than 75% of the following combined salts: sodium glycocholate, sodium glycodesoxycholate, sodium glycohyodesoxycholate, sodium taurocholate, sodium taurodesoxycholate and sodium taurohydrodesoxycholate. The remainder consists mainly of inorganic matter and salts of free fatty acids present in bile. In addition, there may also be included small amounts of protein, fat, pigment and other constituents of normal bile.

Stock aqueous mixtures containing 80 mg. of bile salts per cc. were prepared and all other concentrations made from these by dilution with distilled water.

The temperature of the animal room should be kept above 20° C., since lower temperature are likely to cause diarrhea (7).

All animals were given Purina Dog Chow Checkers and water, *ad lib.* The cages were constructed with wire mesh bottoms, and to permit daily gross observation of the nature of the stools, the dropping pan was covered with paper.

A group of young male mice was used in preliminary studies to determine the effectiveness of bile salts in producing catharsis. Attempts in this direction, using small doses (2 and 4 mg.) and observing the animals for a period of one hour, were unsuccessful. However, chronic administration of 2 mg. of bile salts per mouse per day was begun in

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[1] In this laboratory it was found very convenient to employ frog assay tank compartments elevated about one-half inch above the filter paper.

[2] Acknowledgment is made of the courtesy of the American Ferment Company in supplying this drug.

Table I.—Determination of Threshold Cathartic Dose for Bile Salts in Adult Mice

No. Mice	Bile Salts Mg./Mouse	Results 1st Hr. +	1st Hr. −	2nd Hr. +	2nd Hr. −	3rd Hr. +	3rd Hr. −	4th Hr. +	4th Hr. −
34[a]	4	7[b]	27	..	..	..	..	..	..
5	10	0	5	0	5	0	5	0	5
11	15	1	10	1	10	1	10	1	10
9	30	0	9	1	8	2	7	2	7
8	40	0	8	2	6	3	5	4	4

[a] Young male mice, all others adult.
[b] Observation period one hour.

Table II.—Results of Assays in Control and Bile-Salt Treated Mice at Approximately Thirty-Day Intervals

No. Mice	Daily Bile Salts, Mg./Mouse	Chronic Administration, Days	Assay Dose, Mg./Mouse	Results 1st Hr. +	1st Hr. −	2nd Hr. +	2nd Hr. −	3rd Hr. +	3rd Hr. −	4th Hr. +	4th Hr. −	Positive, Per Cent
28	2–30 days											
	4–30 days	60	40	13	16	20	8	25	3	27	1	96
25	Controls	60	40	7	18	11	14	15	10	15	10	60
22	20	90	30	7	15	13	9	16	6	18	4	82
27	Controls	90	40	9	18	18	9	20	7	23	4	85
22	20	120	30	9	13	15	7	18	4	19	3	86
20	Controls	120	40	6	14	9	11	10	10	11	9	55
19	20	157	40	12	7	15	4	17	2	18	1	94
24	Controls	154	30	8	16	14	10	16	8	17	7	70
20	0–8 days	165	30	5	15	6	14	11	9	11	9	55
20	Controls	165	30	0	20	3	17	5	15	6	14	30

a group of 30 young male mice, with a comparable number of animals serving as controls.

In view of the ineffectiveness of small doses of bile salts, the original procedure based on senna was duplicated for further study of the method. One-half cc. volumes of a 5% infusion of senna were administered to each of 15 additional mice which were observed for a period of two hours. At the end of 30 minutes two were positive, at one hour four were positive, while after two hours 14 were positive. The fluidity of the stools at this time was so marked that readings could be made at a glance. In view of the difference between these results and those obtained previously with bile salts, the action of bile salts was reinvestigated using larger doses and longer periods of observation. Under these conditions a degree of catharsis comparable to that induced with senna was obtained, thus making it possible to establish a T. C. D. (threshold cathartic dose—the dose which will produce catharsis in approximately 50% of the animals) for bile salts. Although the animals were supplied with water and left over night, it was observed that no further positive results were obtained after four hours, and this was accepted as a suitable period of observation. The results of these determinations are summarized in Table I. Based upon these experiments, 40 mg. was selected as the T. C. D. for the assay at the end of the 60-day period of chronic administration. This group of animals had received daily doses of 2 mg. for 30 days and 4 mg. for 30 days. The last daily dose was administered approximately 24 hours before the assay period. The results are shown in Table II. It will be noted that cathartic action occurred in 96% of the mice which had received daily doses of bile salts and in only 60% of the controls, which receive no daily bile salts.

The experimental animals were continued for an additional period of 30 days (90 days, total) using a daily dose of bile salts representing approximately one-half the T. C. D., *i. e.*, 20 mg. per mouse. At the end of this time the T. C. D. (40 mg.) was given to the controls. The animals which received the daily dose of bile salts were given 30 mg. per mouse instead of 40 mg. as in the previous assay. The results are summarized in Table II.

Following another 30-day period (120 days, total), during which the experimental group of mice continued to receive the daily dose (20 mg.), the assay was repeated as above: 30 mg. to experimental animals, 40 mg. to controls. Table II summarizes the results of this assay. As indicated in the 90-day assays the per cent of positive results for the experimental animals (82% at 30 mg.) and for the controls (85% at 40 mg.) closely approximate each other. In the 120-day assay, however, there is a greater difference in the per cent of positive actions between the experimental animals (86% at 30 mg.) and the controls (55% at 40 mg.). The average weight of experimental and control animals at this time was 20.0 Gm. and 24.0 Gm., respectively.

Daily administration of bile salts (20 mg.) was continued for another period of 30 days (150 days, total). At the end of this time the assay was repeated using 0.5-cc. volumes of 1:15 dilution (the approximate T. C. D., see Table III) of fluidextract of senna instead of bile salts. From the results obtained (Fig. 1 and Table III) it is apparent, within the time limitations of the test, that (*a*) the onset of active catharsis from comparable doses of bile salts is more rapid than that from senna, and (*b*) the cathartic effect of senna remains comparatively unchanged as a result of chronic administration of bile salts to this group of mice. It should be

Table III.—Determination of the Approximate T. C. D. of Fluidextract of Senna, in 0.5-Cc. Doses, and the Effect of Daily Oral Administration of Bile Salts upon its Cathartic Action in Mice

No. Mice	Conc. of Fldext.	Bile Salts Daily Dose in Mg.	Bile Salts No. of Days	Results 1st Hr. +	1st Hr. −	2nd Hr. +	2nd Hr. −	3rd Hr. +	3rd Hr. −	4th Hr. +	4th Hr. −	Positive, Per Cent
8	1:10	Controls	143	0	8	4	4	5	3	8	0	100
7	1:15	Controls	143	0	7	1	6	2	5	5	2	71
7	1:20	Controls	143	1	6	2	4	2	4	2	4	33
21	1:15	20	150	1	20	7	14	10	11	13	8	61
24	1:15	Controls	150	1	23	7	17	10	14	14	10	58

emphasized, however, that data are needed from other dosage levels before general conclusions can be drawn on these phases of the problem. The average weight of experimental and control animals at this time was 22.5 Gm. and 23.8 Gm., respectively.

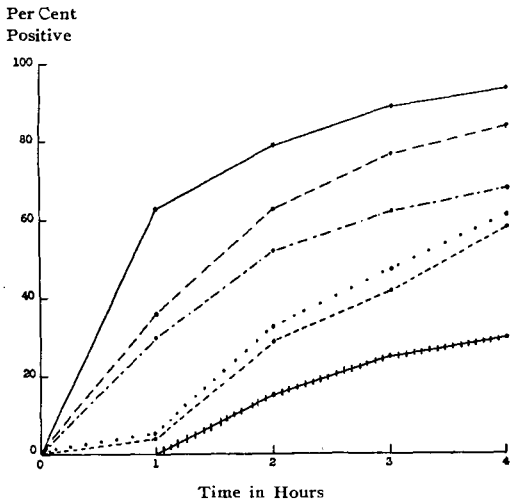

Fig. 1.—Relationship of Positive Reactions in Mice of All Age Groups When Assayed with Bile Salts at 30- and 40-Mg. Levels, and the Influence of Daily Administration of Bile Salts on Cathartic Effect of Senna.

|—|—|—|—| Controls—Bile Salts 30 Mg.
— · — · — · Controls—Bile Salts 40 Mg.
— — — — Bile Salts Daily 20 Mg.—Bile Salts 30 Mg.
———— Bile Salts Daily 20 Mg.—Bile Salts 40 Mg.
- - - - - - Controls—Fldext. Senna, 1:15
. . . . . Bile Salts Daily 20 Mg.—Fldext. Senna, 1:15

Thirty-four days (154 days, total) following the last assay with bile salts, the control mice received 30 mg. of this preparation. The results are shown in Table II. The difference between these results and those obtained from the 90- and 120-day experimental animals at the same dosage levels, and from the 120-day controls at 40 mg., would seem to indicate that the susceptibility of the controls to bile salts has shown a considerable increase. However, the results obtained in a subsequent assay (Fig. 1 and Table II, 166-day controls), in which only 30% of animals were positive at the same dosage level (30 mg.), would seem to discount the possibility of an increased susceptibility to bile salts in the controls. The administration of fluidextract of senna to this group only four days previously may have influenced these results. Further investigation of this phase of the problem is under consideration.

Following a 37-day period (157 days, total), the assay with bile salts was repeated in the experimental animals, using the T. C. D. as the assay dose. The results (Table II) compare favorably with those obtained from the 60-day experimental group which also received 40 mg. as the assay dose. Following this assay daily administration of bile salts was discontinued. Eight days later an assay dose of 30 mg. was given to these animals. The results are shown in Table II. The response was approximately 30% less than when these animals were receiving bile salts daily, and was also less than the average response of the control animals following 40-mg. doses.

The percentage relationship of the positive reactions of control and experimental animals when assayed with bile salts at 30- and 40-mg. levels, is shown in Fig. 1. The influence of time as an important factor in the assay is emphasized since Fig. 1 clearly shows that a comparatively long period of time is required for accurate evaluation of the cathartic action of bile salts by this method.

DISCUSSION

The results of the experiments presented in this report indicate that mice are desirable animals for the study and evaluation of effects produced by chronic oral administration of cathartic drugs. Based upon the methods described, the acute and chronic cathartic effects of bile salts were investigated.

The animals employed in these studies exhibited no tolerance to threshold doses following chronic daily administration of bile salts. Indeed, it would appear that the susceptibility of mice to threshold doses of the drug is increased inasmuch as the per cent of positive results in treated animals following a 40-mg. assay dose was always greater than in the controls at the same assay dose, and the results from 30-mg. assay doses in the experimental animals were comparable to 40 mg. in the controls. Some

of the more obvious causes of increased susceptibility to the cathartic action of bile salts are:

1. Accumulation of the drug in the body.
2. A difference in weight of the control and experimental animals when a fixed dose is administered upon a weight per mouse basis.
3. A moderate evacuation of the bowels insufficient to cause fluidity of the stools.
4. A mild, repeated, non-specific irritation of the gastro-intestinal mucosa, due to daily application of bile salts.
5. A specific cellular phenomenon by which the susceptibility of the gastro-intestinal tract to bile salt action is increased.
6. A greater combined total of bile constituents in the intestine due to the probable choleretic effect of daily administration of bile salts (6, 8, 9).

Accumulation of the drug in the body is not responsible for the increased susceptibility since daily administration of relatively small doses (2 and 4 mg.) and daily administration of 20-mg. doses of bile salts, representing one-half the T. C. D., induce equivalent increases in suceptibility (Table II). Furthermore, the daily administration of approximately one-half the T. C. D. for a period of 90 days did not induce active catharsis in the animals, indicating that a minimum of 50% of the T. C. D. of bile salts is essentially eliminated within 24 hours. Since an interval of 24 hours was allowed to elapse between the last daily dose and the administration of any assay dose, it is apparent that the acute effects of the last daily dose upon the effect of the assay dose is negligible.

The average weight of both control and experimental animals, which fluctuated between 20 and 24 Gm. throughout the duration of these studies, apparently did not affect the results. This observation has previously been made by Geiger (7), who employed mice weighing between 20 and 24 Gm.

A moderate evacuation of the bowels insufficient to cause fluidity of the stools does not appear to be a causative factor in the production of increased susceptibility to bile salts. If such an action of the drug brought about a more intimate contact of the drug with the intestinal mucosa, the response of threshold doses of senna presumably would be greater in those animals treated with bile salts than in the controls. Figure 1 and Table III indicate that this is not the case.

Evidence cited above would indicate that a mild, repeated, non-specific irritation of the gastro-intestinal mucosa, due to daily application of bile salts, is not produced, since there is no difference in the response of the two groups to senna.

Either or both of the two remaining factors (5 and 6, above) appear to be the mechanisms responsible for the increase in susceptibility to bile salts. Data obtained thus far in this investigation do not distinguish between the two. It is interesting to note that a comparison of the results presented in Tables II and III, and Fig. 1, shows that the increased susceptibility is specific for bile salts to the extent that the approximate T. C. D. of senna is not influenced. From an analysis of data presented in Table II, it appears that increased susceptibility is a factor of continuous application of bile salts inasmuch as after a period of eight days, during which no bile salts were given, the response to the 30-mg. assay dose was appreciably diminished.

CONCLUSIONS

1. A method for chronic study of cathartic drugs in mice is described.
2. Bile salts in sufficient quantity will induce active catharsis in mice and this action may be evaluated by the method described.
3. The threshold cathartic dose of bile salts for mice was determined to be approximately 40 mg. per mouse. One-half of this dose administered daily for relatively long periods induces no active catharsis.
4. Daily administration of sub-threshold doses of bile salts to mice for a period of about 150 days induces no tolerance to the cathartic effect of the threshold dose of this drug, given 24 hours after the last daily administration.
5. Within the limits of these experiments, it appears that such daily administration induces an increased susceptibility

to the cathartic action of bile salts. The probable causes of this phenomenon and its dependency on daily application are discussed.

6. The results of a single assay, using a 1:15 dilution of fluidextract of senna, indicate that its cathartic effect remains practically unchanged following daily administration of bile salts to mice.

7. Approximately four hours are required to develop maximum cathartic action of bile salts in mice.

8. Bile salts induce catharsis more rapidly than senna under the conditions described.

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Book Reviews

Anhydrous Aluminum Chloride in Organic Chemistry, by CHARLES ALLEN THOMAS in collaboration with MARY BALUK MOSHIER, HERBERT E. MORRIS and R. W. MOSHIER. xiii + 972 pages. 6 x 9 in. 1941. American Chemical Society Monograph No. 87. New York: Reinhold Publishing Corporation. $15.00.

The spectacular achievements in the field of synthetic organic chemistry, exemplified especially by the syntheses of vitamins, hormones, chemotherapeutic agents and rubber substitutes are known to everyone. Less spectacular, and consequently not so well known or appreciated, are the discoveries in the purely academic phases of chemistry; however, without these developments, products such as those which have been mentioned never could have been synthesized.

The applications of aluminum chloride in organic chemistry were initiated and studied quite extensively by the two collaborators, the Frenchman, Friedel, and the American, Crafts, purely as an academic project. Subsequent discoveries by hundreds of other investigators have increased the range of usefulness of aluminum chloride to an astonishing extent. The collection of the vast amount of data in this monograph, and its arrangement under pertinent chapter headings, have made information readily available; the laborious task, performed by the author and collaborators, is one which must be recognized with gratitude not only by the professional chemist, but also by the layman who benefits by the industrial exploitation of chemical syntheses.

This monograph deals not only with Friedel-Crafts syntheses but, as the title indicates, with all types of transformations of organic compounds in which aluminum chloride can play a role.

It is stated in the preface that "Every effort has been made to make it complete and to include all references of published material, both of a purely scientific and industrial nature, including the available patent literature;" these objectives have been realized to an admirable degree. Furthermore, the usefulness of the book has been increased greatly by the inclusion of numerous tabulations of reactions and reaction products and a patent and author index, in addition to the customary subject index.

In view of the obvious importance of the subject, and its excellent and exhaustive treatment, it seems hardly necessary to state that this monograph will be recognized as an indispensable book for every organic chemist.

The binding, printing and paper are of the same fine quality found in the other American Chemical Society Monographs.—F. F. BLICKE.