normal growth was obtained on all diets used in these experiments.

Analytical data were obtained on a representative sample of mixed soy sterols by the Schoenheimer-Sperry procedure, using a sample of pure cholesterol‡ as a standard of reference. The color intensity of the soy sterols was found to be, on the average, 77.3% of that of an equal weight of cholesterol. When saponification and the digitonin precipitation was used an average of 89% recovery was obtained with the mixed soy sterols. Thus, if any appreciable absorption of the mixed soy sterols occurred it would probably have been detected by the analytical method used. The data shown above (Fig. 1) indicate that there could be very little absorption of the constituents of the soy sterols in their original state.

The data presented in this report do not exclude the possibility that the effects described may be produced by some minor constituent of the sterol mixtures fed.

Summary. Evidence is presented that the addition to the chicks' diet of mixed soybean sterols and of purified sitosterols in the presence of cottonseed oil and cholesterol causes markedly lower plasma and liver levels of cholesterol than the addition to the diet of a mixture of cholesterol and cottonseed oil alone. The possibility is discussed that one or more of the constituents of the mixed soybean sterols interferes with the absorption of cholesterol.

The author wishes to acknowledge his indebtedness to Dr. Ruth Okey of the University of California for her interest and suggestions.

Received September 4, 1951. P.S.E.B.M., 1951, v78.

‡ Difco Standardized Cholesterol m.p. 149.5-150°C (corr.)

Quantitative Estimation of Antispasmodic Potency Using Isolated Guinea Pig Gall Bladder—Common Duct Preparation.*† (19003)

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Reuse(1) evaluated the antihistaminic activity of pyrilamine and Phenergan with the isolated guinea pig gall bladder-common duct preparation. The data presented indicated that the log-dose response was linear but the method was not developed as a quantitative procedure for estimating antispasmodic potency.

In view of the fact that the antihistaminic drugs have varying degrees of antispasmodic activity(2), we have investigated their effects upon acetylcholine spasm of the guinea pig gall bladder-common duct *in vitro*. Statistical evaluation of the results indicates that this tissue preparation is useful for screening drugs for antispasmodic activity.

Experimental. Guinea pigs, of either sex, weighing 343 to 958 g (average 607 g) were killed by a blow on the head. The gall bladder and common duct were removed, emptied of bile and arranged for recording contractions by the Magnus method. The bathing fluid

* The authors wish to thank Parke, Davis and Co. for the Benadryl and Ambodryl; Merck and Co. for the Phenergan; Bristol Laboratories for the C-5581-H; Winthrop-Stearns for the Thenfadil; Burroughs-Wellcome and Abbott Laboratories for the Chlorocyclizine; Schering Corp. for the Chloro-Trimeton; and Medico Chemical Specialties Corp. for the Anthallan used in this investigation.

† This article is based on work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.

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TABLE I. Antispasmodic Activity of Anthallan.

Spasmogen	Conc. in mM	Anthallan activity: Counteraction ED_{50} and range in mM	Counteraction Slope and range	Prevention ED_{50} and range in mM	Prevention Slope and range
Histamine	12	4.1 (2.1–8)	2.59 (.66–10.1)	5.2 (2.6–10)	2.59 (.66–10.1)
$BaCl_2$	80	60 (54–312)	2.19 (.61–7.8)	150 (62–360)	2.2 (.61–7.9)
Acetylcholine chloride	.04	39 (15–100)	4.1 (1.5–8.5)	33 (12–86)	4.1 (1.5–8.5)

was Tyrode's solution of the following composition: NaCl, 8.0 g; KCl, 0.2 g; $MgCl_2$, 0.1 g; $CaCl_2$, 0.2 g; Na_2HPO_4, 0.05 g; $NaHCO_3$, 1.0 g, and dextrose, 1.0 g per liter. Maximum sensitivity of the tissue was obtained by allowing it to remain in the bathing fluid for 1½ hours after removal from the animal. The bathing fluid was changed every half hour during this interval. The spasm was produced by adding 0.5 cc of the spasmogen to the bath (see Table I for concentrations used). The antihistaminic solutions were added to the bath in a constant volume of 0.5 cc in concentrations varying from 0.0002 to 40 millimoles per liter. The drugs were evaluated for their ability to counteract as well as prevent the spasm, using five preparations for each drug. The results obtained were statistically analyzed by the Litchfield-Wilcoxon(3) method.

Results. Table I shows the results obtained when Anthallan was tested against musculotropic and neurotropic spasmogens. From these data it is quite evident that the guinea pig gall bladder-common duct preparation is more sensitive to neurotropic stimulus than to a musculotropic one. It can be seen that Anthallan is approximately 6 to 10 times more potent as an antihistaminic than it is as an antispasmodic. Against any of the spasmogens it was observed that the log dose-response curve was linear within a 10-fold dilution range, *i.e.*, 4 to 40 mM.

Inasmuch as the antihistaminic potency of the various drugs had been well established (2), our other evaluations were confined to establishing their antispasmodic potency compared to atropine. The results obtained are given in Table II. It is quite evident that although the antihistaminic drugs can counteract or prevent acetylcholine spasm, their potency in this respect is of a very low order. Phenergan is the exception, being less potent than atropine in counteracting such spasms but being equal to atropine in preventing them. Although it would appear from the ED_{50} values that Ambodryl, C-5581-H and Thenfadil have equal potency in counteracting acetylcholine spasm, calculation of their potency ratios compared to atropine shows that Ambodryl is more potent than the other compounds. Similarly such calculations indicate that Thenfadil is more potent than C-5581-H in preventing such spasms. The drugs are rated in the following order in counteracting or preventing acetylcholine spasm: Atropine, Phenergan, diphenhydramine, Ambodryl, Thenfadil, C-5581-H, chlorocyclizine, chloroprophenpyridamine and Anthallan. The antihistaminic drugs gave linear log dose-response curves within a 100-fold dilution range, *i.e.*, 0.04 to 4 mM.

Discussion. Loew(4) has shown that the antihistaminic drugs can counteract histamine, acetylcholine or barium spasm of the isolated guinea pig ileum. The dose of antihistamine required is dependent upon the particular spasmogen used, being lowest with histamine and highest with barium. Our data on Anthallan (Table I) confirm these findings and show that the isolated guinea pig gall bladder-common duct preparation gives results quantitatively equal to the ileum preparation now commonly employed for evaluating antihistaminic and antispasmodic potency. Furthermore, the lack of spontaneous rhythmic contractions by the gall bladder-common duct preparation eliminates one possible

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TABLE II. Antispasmodic Activity of Antihistaminics.

Drug	Chemical name	Molecular wt base	Counteraction: ED_{50} and range in mM	Counteraction: Potency compared to atropine	Prevention: ED_{50} and range	Prevention: Potency compared to atropine
Atropine	Tropine ester of tropic acid	289.2	.007 (.001–.032)	100	.0042 (.0009–.019)	100
Phenergan	[N-dimethylamino isopropyl-thio-diphenylamine]	284.4	.046 (.008–.25)	31	.005 (.001–.018)	100
Diphenhydramine	β-dimethylamino-ethyl benzhydryl ether	255.4	.37 (.06–2.41)	21	.68 (.11–4.08)	6
Ambodryl	β-dimethylamino-ethyl-(4-bromo-benzhydryl)-ether	334.3	.74 (.08–6.3)	13	1.25 (.14–11.25)	4
C-5581-H Bristol	2-benzylphenoxy-N, N-dimethyl-ethanolamine]	255.4	.72 (.24–2.2)	2	.8 (.2–3.2)	.9
Thenfadil	[N'-(2-pyridyl)-N'-(3-thenyl)-N, N-dimethyl ethylenediamine]	259.4	.6 (.13–2.7)	2	3 (.43–21)	1.6
Chlorocyclizine	[N'-methyl-N'-(4-chlorobenzhydryl) piperazine]	300.8	.8 (.16–4.1)	1	1.41 (.25–7.8)	.6
Chloroprophenpyridamine	[1-(4-chlorophenyl)-1-(2-pyridyl)-3-dimethylamino propane]	274.8	4.3 (1.4–12.9)	.3	2.1 (.9–5.9)	.3
Anthallan	3-di(n-butyl)-amino-methyl 4,5,6-trihydroxyphenyl phthalamide	323.2	39 (15–100)	.07	33 (12–86)	.05

source of error common to ileum preparations. The response of the gall bladder-common duct preparation to the various spasmogens and the concentrations of these agents required is of the same order of magnitude as required to elicit a similar response from the ileum preparation. The linearity of the log dose-response curves and the dosage range is similar for both preparations.

Previous reports have shown that the antihistaminic drugs have only a slight antispasmodic activity as compared to atropine(2,4-8). Sharp(8) found that diphenhydramine had a greater antispasmodic activity than Ambodryl. The results herein presented confirm these findings.

Summary. It has been shown that the isolated guinea pig gall bladder-common duct preparation is suitable for the evaluation of antispasmodic and antihistaminic activity. The log dose-response curve is linear over a wide range. The drugs studied are rated in the following order in counteracting or preventing acetylcholine spasm: Atropine, Phenergan, diphenhydramine, Ambodryl, Thenfadil, C-5581-H, chlorocyclizine, chloroprophenpyridamine and Anthallan.

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Received September 4, 1951. P.S.E.B.M., 1951, v78.