Further Investigation of Reserpine and Deoxycholic Acid Potentiation

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Abstract □ Intravenous administration of reserpine acetate and oral deoxycholic acid showed no increase in blepharoptotic potency relative to intravenous reserpine acetate alone. Since this experiment eliminates physicochemical interactions within the gastrointestinal tract, it suggests that the potentiation of reserpine taken orally as reserpine-bile acid coprecipitates is by physicochemical rather than by physiopharmacological means.

Keyphrases □ Reserpine-deoxycholic acid—potentiation □ Potentiation—reserpine by deoxycholic acid □ Interactions—possible potentiation of reserpine acetate by deoxycholic acid

The oral administration of reserpine as a deoxycholic acid coprecipitate was shown by Malone *et al.* (1) to increase its relative blepharoptotic potency as well as to hasten its onset of action and prolong its duration. This phenomenon was also seen using coprecipitates of reserpine with other cholanic acid derivatives (2). No attempt was made by these authors to elucidate the mechanism of action for this potentiation.

Gibaldi *et al.* (3) studied the dissolution properties of varying molar ratios of reserpine–deoxycholic acid coprecipitates and attributed the correlation of increased dissolution rates with the *in vivo* ptotic activities to a decrease in the particle size of the administered reserpine. Recently, Stoll *et al.* (4) studied such mixtures in regard to micellar solubilization, reduction of interfacial tension, and particle size, all of which could increase the dissolution rate of the insoluble reserpine particles. Surface-tension determinations of the coprecipitates in a pH 6.4 buffer eliminated micellar formation as a factor, since they did not exhibit a break in the surface tension *versus* concentration curve typical of the CMC for micelle formation. There was, however, a qualitative relationship between the compounds' abilities to lower interfacial tension and to potentiate ptotic activity. A near rank correlation was found for increased dissolution rates of the coprecipitates and the 4–10-hr. postadministration rise in potency. This was felt to be due to the decreased particle size achieved during the coprecipitation procedure and the surface tension-lowering abilities of the bile acids which facilitate wetting of the insoluble reserpine particles. Possible physiological effects of the cholanic acid derivatives such as changes in gastric motility and emptying, competition for protein binding sites, changes in membrane permeability, and involvement with enterohepatic circulation were considered by these authors to play a minor role, if any, in the potentiation phenomena, since a physical mixture of reserpine with deoxycholic acid was shown to produce only a slight rise in ptotic activity compared to the coprecipitates (1). Physiological factors, however, were not eliminated.

The present paper investigates the effect of intravenous reserpine after the oral dosing of deoxycholic acid. This experiment should eliminate physicochemical interactions within the gastrointestinal tract but not affect any physiopharmacologic mechanisms.

Table I—Apparent Intravenous Potencies of Reserpine Acetate in Mice Receiving Orally Administered Deoxycholic Acid Relative to Intravenous Reserpine Acetate in Mice Receiving Dosage Vehicle Only Orally

Hours after Dosage				
2	4	6	10	24
1.01[a] (0.80–1.28)	0.98 (0.82–1.22)	1.02 (0.83–1.22)	1.11 (0.86–1.18)	1.62 (0.45–2.22)

[a] The range of figures within parentheses indicates the calculated 95% confidence limits for the listed potency.

EXPERIMENTAL

Materials—Deoxycholic acid[1] was suspended for dosing in 0.25% agar.[2] Reserpine acetate was prepared by solubilizing reserpine[1] in glacial acetic acid and diluting with distilled water to a final acetic acid concentration of 0.029%.

Assay Procedure—Male albino mice[3] (15–26 g.) were maintained in this laboratory on Purina laboratory chow and tap water *ad libitum* for at least 1 week prior to testing. The animals were removed from food 18 hr. before dosing and placed back on food after the +6-hr. ptotic readings had been completed. At −1 hr. the test mice were dosed orally with the deoxycholic acid suspension (30 ml./kg.), while the reference animals received only 0.25% agar vehicle (30 ml./kg.). This was followed at 0 hr. with a 20-sec. intravenous injection of reserpine acetate (10 ml./kg.). The intravenous dosages used for the 3 × 3 assay (0.125, 0.25, and 0.50 mg./kg., calculated as reserpine base) are ptotically equivalent to the oral dosages of reserpine base (3.0, 6.0, and 12.0 mg./kg.) originally used (1) to document the reserpine–deoxycholic acid potentiation. The oral deoxycholic acid dosages used in the present study (20.7, 41.5, and 82.9 mg./kg.) correspond to the dosages used in the original studies with the coprecipitates. Therefore, the 1:257 *M* ratio of reserpine to deoxycholic acid in this study should be pharmacologically equivalent to the 1:16 *M* ratio in the coprecipitate studies (1, 2). In all other respects, the blepharoptotic assays were carried out exactly as described by Malone *et al.* (1).

RESULTS AND DISCUSSION

Reserpine had to be administered intravenously as the acetate salt due to its low solubility in aqueous media. It was shown previously that acetate salt formation does not significantly alter the slope of oral dose–response curves as compared to reserpine base (2). The statistical treatment for the 3 × 3 assays (132 mice, 22 animals/dosage level) involved analysis of variance, factorial analysis, and calculation of potency with its 95% confidence limits as described by Bliss and Calhoun (5).

As illustrated in Table I, the intravenous potencies of reserpine acetate in combination with oral deoxycholic acid when compared to intravenous reserpine acetate as a standard ranged from 0.98 to 1.11 for the first 10 hr. of the experiment. The average λ value (s/b) for these four assays is 0.25, which agrees well with the assay char-

[1] Nutritional Biochemical Corp., Cleveland, Ohio.
[2] Difco Laboratories, Detroit, Mich.
[3] Obtained from E. G. Steinhilber Inc., Oshkosh, Wis.

acteristics of 0.27 and 0.28 reported earlier (1, 2). Waning of ptotic activity at +24 hr. decreased the slope of the dose–response curves, causing a wide range in the 95% confidence limits of the potency and an increase in the calculated λ value (0.96) for this assay. From these results, it can be seen that the potency of the reserpine–deoxycholic acid combination was equivalent to 1.0 over the entire time-course spectra of activity.

To detect if there was any acceleration of activity, ptotic readings were also collected at the +1-hr. interval. However, because of the characteristic slowness of the reserpine response, ptotic activity was seen only with the top doses of both the test and standard treatments. The mean responses were equivalent. Controls receiving a 20-sec. intravenous injection of 0.029% acetic acid, with and without orally administered deoxycholic acid, were without ptotic activity.

The absence of reserpine potentiation with the concomitant administration of reserpine acetate and deoxycholic acid by different routes lends further support to the theory that the mechanism of potentiation with orally administered reserpine–bile acid coprecipitates is by physicochemical rather than by pharmacological means. A physical mixture of 1:16 *M* reserpine and deoxycholic acid was shown to cause a slight but significant rise in potency (maximum of 1.8×) when given orally (1). Although such a mixture does not allow the intimate combination of ingredients found in a coprecipitate and does preclude reduction in particle size during precipitation, there still exists the possibility of local surface-tension lowering by the bile acids. As both drugs enter the circulation in the present experiment, actual physical contact in the gastrointestinal tract is eliminated, yet any true pharmacologic potentiation should occur. The only chance for physical interaction in the gastrointestinal tract is the possibility of enterohepatic excretion of the intravenous reserpine into the intestine. The absence of potentiation, therefore, also precludes an enterohepatic recirculation phenomenon as a potentiation mechanism for the reserpine–deoxycholic acid coprecipitates.

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Fluorescent Analysis of Primary Aliphatic Amines by Reaction with 9-Isothiocyanatoacridine

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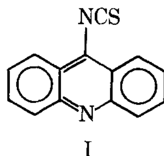
Abstract ☐ Primary aliphatic amines are reacted with 9-isothiocyanatoacridine to yield the corresponding thiourea derivatives. These derivatives, after reaction in base, yield new compounds of intense fluorescence. Conditions have been developed so that it is possible to relate the fluorescence of the final reaction mixture to the concentration of original amine or amine salts without separation of products, even in the presence of excess fluorescent reagent. This method of analysis is successful at the microgram level and can be extended to the nanogram level by TLC separation and direct TLC fluorimetry of the reaction product.

Keyphrases ☐ 9-Isothiocyanatoacridine reagent—fluorescent analysis, primary aliphatic amines ☐ Amines, primary aliphatic—fluorescent analysis using 9-isothiocyanatoacridine ☐ TLC fluorimetry—analysis

These laboratories are interested in the development and evaluation of fluorescent isothiocyanate compounds as new fluorescent protein-labeling agents (1). Such compounds are also being evaluated as functional group reagents for the analysis of trace amounts of amines by formation of fluorescent thiourea derivatives. For example, 9-isothiocyanatoacridine (I) has been found useful for the detection of traces of penicillin (2).



In the course of developing assays based upon fluorescent thiourea compounds from 9-isothiocyanatoacridine, it was observed that base treatment of the initial reaction product resulted in an increase in fluorescence of the reaction mixture. This increase was also accompanied by a change in the activation and fluorescent wavelengths of the mixture. These conditions are advantageous for assay of traces of amines based upon the measurement of a reaction product, even in the presence of excess original fluorescent reagent. For example, when the procedure is applied to *n*-butylamine, activation for the final reaction mixture is at 300 nm. with fluorescence at 490 nm. These are compared to an activation wavelength of 410 nm. and fluorescence at 440 and 460 nm. for the butylthiourea derivative of 9-isothiocyanatoacridine and also for the reagent *per se*. The purpose of this paper is to report the development and results of such an assay.

EXPERIMENTAL

Development of Reaction Conditions—Fluorescence intensity as a function of the period of heating was studied. Samples containing 1.5012 mg. of 9-isothiocyanatoacridine (I) and 0.0339 mg. of *n*-butylamine in 10 ml. of absolute alcohol were shaken in a 60°