# Factorial Study of Thiourea Adjuvants on Quinine and Quinidine Absorption

By PATRICK W. RAGOZZINO* and MARVIN H. MALONE

**A factorially designed experiment using the single perfusion technique in rats indicated that thiourea consistently enhanced absorption of quinine from the intestine while being ineffective for quinidine. *N,N'*-Diethylthiourea increased absorption of both alkaloids. 1-Phenyl-2-thiourea was an inhibitor of alkaloidal absorption, except when tested in fasted animals. With this exception, fasting routinely decreased the ability of the animals to absorb these isomers. Strong and weak buffer systems did not interfere with alkaloidal absorption.**

PERFUSION STUDIES conducted by Ragozzino and Malone (1) indicated that thiourea, *N,N'*-diethylthiourea, 1-phenyl-2-thiourea, and 2-benzyl-2-thiourea were all effective as adjuvants in increasing the absorption of quinine from the small intestine of anesthetized rats. *N,N'*-Diethylthiourea was the only significant adjuvant reported for quinidine. These adjuvants appeared to be specific and apparently operating on some enzyme-dependent transport process. Most absorption studies that have appeared in the literature (2) utilized experimental animals that had been fasted for 20 to 24 hours prior to experimentation. In view of reports indicating that fasting for as little as 16 hours nullified certain enzyme-dependent detoxication systems (3), it was considered important to study the effect of fasting on the enzyme-dependent adjuvant transport system. A factorial experiment was designed to examine the relative effects of thiourea, *N,N'*-diethylthiourea, 1-phenyl-2-thiourea, fasting, ionic strength, and some of their interactions on the absorption of quinine and quinidine as measured by the disappearance of these drugs from the rat small intestine.

## EXPERIMENTAL

**Methods and Materials.**—Wistar strain albino rats of each sex were obtained from E. G. Steinhilber, Oshkosh, Wis., and housed separately in the animal quarters of this laboratory for at least 1 week prior to use, with Purina Lab Chow and water being allowed *ad libitum*. Weights of the animals varied from 120 to 160 Gm. at the time of testing. When fasting was specified, food was withheld from the rats for 24 hours prior to experimentation, but water was permitted *ad libitum*. Animals were housed in cages having wide-mesh screen floors to prevent coprophagy. The rats were anesthetized lightly with pentobarbital sodium, 50 mg./Kg. i.p., to implant the perfusion cannulae surgically into the duodenal and ileal ends of the small intestine. The stomach and cecum were closed by ligatures.

The perfusion experiments with quinine and quinidine were carried out according to the "single perfusion technique" described by Schanker, *et al.* (2), with the following minor changes. Inulin recoveries were based on the colorimetric method of Smith (4). Six experiments were run concurrently so that each of six rats would receive exactly the same treatment. Three of these animals received perfusate warmed to 37° from waterbath *A* and the remaining three from waterbath *B*. A Phipps and Bird perfusion pump was utilized, delivering fluid at the constant rate of 1.5 ml./minute. The perfusion solution termed "Buffer A" in these experiments consisted of the drug in a concentration of 1 mmole/L. in a weakly buffered saline solution containing the following salts in millimoles per liter: NaCl, 145; KCl, 4.56; $CaCl_2$, 1.25; $Na_2HPO_4$, 1.33; and $NaH_2PO_4$, 0.33. "Buffer B" differed only with respect to the concentration of ingredients: $Na_2HPO_4$ was changed to 72 mmole/L. and $NaH_2PO_4$ to 28 mmole/L., so that the total phosphate concentration would be 0.10 *M*. Sodium ion concentration was maintained at 145 mmole/L., and the chloride concentration reduced in proportion to the added buffer anions. The initial pH of all perfusion solutions was adjusted to 7.2 ±0.01 before experimentation. After perfusion, the pH of solutions containing Buffer A was 6.6 and for perfusion solutions containing Buffer B:7.1.

Quinine and quinidine were estimated spectrophotometrically using a modification of the method of Brodie (5). Following the extraction of the alkaloid with the polar solvent ethylene dichloride, the alkaloid was returned to the aqueous phase by the addition of 0.1 *N* $H_2SO_4$ and re-extracted with a less polar solvent, benzene. This procedure was repeated twice, since it was found that complete extraction of the alkaloids without their metabolic or degradation products was achieved. Using this double extraction procedure, the biological blank was negligible in every case.

In analyzing the results of this study, the general statistical methods used were those described by Snedecor (6), except that the analysis of variance was performed as described by Cochran and Cox (7). These experiments were planned to have a $2^4 \times 4$ factorial design involving five factors, four of them at two levels each, and the fifth one at four levels. In

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most cases the low level of a factor occurring at two levels indicated that none of that factor was present. In the case of the four-level factor, each level actually represented a different mode of administration of the treatment. Only one-half of the 64 possible combinations were used. However, the 32 chosen combinations were selected in such a way that information on the effects of interest would not be sacrificed. The following scheme (Table I) lists the factors studied together with the "levels" of each. In the *Code* column, the customary notation for a two-level factorial is used, the presence or absence of the code letter for a particular factor indicating the level of that factor. In the case of the four-level factor, two code letters are required to specify the level.

TABLE I.—FACTORIAL DESIGN OF PERFUSION STUDY

| Factor | Level | Code |
|---|---|---|
| Drug | Quinine | a |
| | Quinidine | No a |
| Thiourea | Present | b |
| | Absent | No b |
| *N,N'*-Diethylthiourea | Present | c |
| | Absent | No c |
| 1-Phenyl-2-thiourea | Present | d |
| | Absent | No d |
| Mode of administration | Buffer A (weak) | No e, no f |
| | Buffer B (strong) | e alone |
| | Fasted 24 hours | f alone |
| | Not fasted | e and f |

TABLE II.—EFFECT OF VARIOUS ADJUVANTS ON RAT INTESTINAL ABSORPTION OF QUININE AND QUINIDINE: A FACTORIAL EXPERIMENT

| Test Group Code[a] | % Absorbed[b] |
|---|---|
| ab | 49.9 (48.9 to 51.1)[c] |
| ad | 20.7 (17.9 to 25.5) |
| ac | 30.2 (28.9 to 31.5) |
| abcd | 47.9 (45.7 to 50.4) |
| abde | 47.6 (46.1 to 49.2) |
| acde | 32.9 (31.2 to 37.0) |
| ae | 15.2 (13.0 to 18.7) |
| abce | 53.1 (53.0 to 53.2) |
| abdf | 52.6 (49.6 to 55.0) |
| acdf | 30.0 (27.2 to 32.5) |
| af | 13.1 (12.3 to 13.9) |
| abcf | 53.4 (51.6 to 55.2) |
| abef | 29.0 (15.4 to 39.5) |
| acef | 31.0 (28.9 to 33.4) |
| adef | 19.9 (9.9 to 27.3) |
| abcdef | 50.5 (46.4 to 54.2) |
| [d] | 17.2 (16.6 to 18.2) |
| bc | 99.5 (98.7 to 100.0) |
| bd | 17.6 (16.3 to 19.2) |
| cd | 99.1 (98.7 to 100.0) |
| de | 20.6 (17.3 to 23.4) |
| bcde | 88.7 (87.8 to 89.6) |
| be | 16.8 (12.9 to 19.1) |
| ce | 99.0 (97.4 to 100.0) |
| df | 14.8 (11.1 to 18.1) |
| bcdf | 80.4 (77.8 to 83.4) |
| cf | 99.6 (98.7 to 100.0) |
| bf | 17.0 (15.4 to 19.1) |
| ef | 17.1 (15.2 to 18.4) |
| bcef | 99.5 (98.4 to 100.0) |
| bdef | 16.7 (12.5 to 31.7) |
| cdef | 98.4 (96.4 to 100.0) |

[a] For identification see Table I. [b] The per cent absorbed is expressed as the mean for a total of six animals run concurrently, three males and three females. Four determinations were made per animal, one each for the four 10-minute samples collected after the 30-minute drug perfusion period which followed the initial 30-minute drug-free cleansing perfusion. [c] Observed range of the mean values per group. [d] No coded factor present.

TABLE III.—ANALYSIS OF VARIANCE

| Source of Variation | Degrees of Freedom | Mean Square | F Ratio |
|---|---|---|---|
| Experiments | 1 | 24,708.34 | 10.3[a] |
| Main effects | | | |
| *A:* Quinine *vs.* quinidine | 1 | 19,436.63 | 8.1[a] |
| *B:* 1-Phenyl-2-thiourea | 1 | 32,701.03 | 13.7[a] |
| *C:* Thiourea | 1 | 37,282.14 | 15.6[a] |
| *D: N,N'*-Diethylthiourea | 1 | 35,350.56 | 14.8[a] |
| *M:* Methods of treatments | 3 | 55,205.26 | 23.1[a] |
| Interaction between main effects | | | |
| *A* × *B* | 1 | 1,119.25 | 0.4 |
| *A* × *C* | 1 | 21,053.34 | 8.8[a] |
| *A* × *D* | 1 | 15,678.31 | 6.5[b] |
| *B* × *C* | 1 | 2,032.12 | 0.8 |
| *B* × *D* | 1 | 6,526.80 | 2.7 |
| *C* × *D* | 1 | 13,484.89 | 5.6[b] |
| *A* × *M* | 3 | 1,196.87 | 0.5 |
| *B* × *M* | 3 | 12,650.52 | 5.3[b] |
| *C* × *M* | 3 | 5,765.63 | 2.4 |
| *D* × *M* | 3 | 1,541.73 | 0.6 |
| Interactions with experiments | | | |
| *A* × Experiments | 1 | 6,186.34 | 2.5 |
| *B* × Experiments | 1 | 3,154.03 | 1.3 |
| *C* × Experiments | 1 | 629.74 | 0.2 |
| *D* × Experiments | 1 | 4,584.63 | 1.9 |
| *M* × Experiments | 3 | 1,076.92 | 0.4 |
| Remainder or pooled error | 30 | 2,385.81 | ... |
| Further comparisons | | | |
| *AC:* quinine with thiourea | 1 | 74,569.57 | 31.2[a] |
| Quinidine, quinidine with thiourea, quinine without thiourea | 2 | 719.71 | 0.3 |
| *AC* × *M* × Experiments | 3 | 84,244.23 | 35.3[a] |
| *AC* × *M* | 3 | 2,926.47 | 1.2 |
| *AC* × Experiments | 1 | 7,309.13 | 3.0 |

[a] Statistically significant at the 1% probability level. [b] Statistically significant at the 5% probability level.

TABLE IV.—INTERACTION BETWEEN QUININE AND QUINIDINE AND PRESENCE *versus* ABSENCE OF THIOUREA EXPRESSED IN ABSOLUTE UNITS

| Thiourea | Quinidine | Quinine | Av. | Difference |
|---|---|---|---|---|
| Absent | 144.82 | 143.50 | 144.16 | 44.24 |
| Present | 154.97 | 221.82 | 188.40 | |
| Av. | 149.90 | 182.66 | | |
| Difference | 32.76 | | | |

## RESULTS AND DISCUSSION

**Interpretation of the Factorial.**—The results of these experiments are shown in Table II. The mean per cent absorbed for each group represents a total of 24 separate determinations. Amount absorbed was calculated from the difference in the concentration entering and leaving the intestine. The analysis of variance in Table III shows that all the main effects were statistically significant as well as four of the first-order interactions. These will be considered separately.

For the purpose of analysis, the data could be arranged so that an internal comparison could be made concerning the duplicatability of the results. The results of the analysis indicated that the three

rats on one side of the perfusion apparatus supplied by water bath *A* responded to a different degree than the three rats on the other side of the apparatus supplied by water bath *B*, possibly due to slight but inherent differences between the two constant temperature water baths employed. To determine whether this effect was significant, the analysis of variance was determined as if two concurrent experiments had been carried out—three rats per run or a total of 12 analyses per group. For the simplicity of calculation and to demonstrate more dramatically the variation or similarity in response of the various factors and levels, all the data were coded so that the values represented absolute units ranging from 0 to 400.

The main effect *A* (quinine *versus* quinidine) and the main effect *C* (presence *versus* the absence of thiourea) were studied together because their interaction, $A \times C$, is statistically significant. The 2 × 2 table or Table IV represents the four averages involved, each value being an average of 16 items, eight from one experiment and eight from the other experiment. The explanation of the significant main effects (*A*, *C*) and the interaction $A \times C$ is now apparent. Averaged over the quinidine and the quinine, the addition of thiourea caused a mean increase of 44.24 units. Quinine was higher than quinidine by 32.76 units, when averaged over the presence and absence of thiourea. However, the difference between thiourea and lack of thiourea was much greater with quinine than with quinidine, and the difference between the quinine and quinidine existed only when thiourea was present—thus causing the significant $A \times C$ interaction. Further inspection of Table IV confirms this conclusion since there appears to be no difference between the two isomers when thiourea is absent, and there appears to be no effect of thiourea on quinidine. This suggests that instead of looking at the *A*, *C*, and $A \times C$ effects, one should examine the effect of quinine with thiourea contrasted with the other three combinations (quinidine, quinidine with thiourea, and quinine without thiourea) averaged together. This comparison is listed at the bottom of the analysis of variance in Table III. This comparison is, in fact, statistically significant to a high degree (F ratio = 31.2), while the variation among the remaining three means is well within experimental error (F ratio = 0.3).

Considering the effect resulting from the combination of quinine and thiourea as the one of greatest interest, it seemed worthwhile to determine if this effect would be exhibited by both experiments and with all four modes of treatment. For this purpose, the remaining comparisons listed among the *Further comparisons* in Table III were made. There is a moderately large but not significant interaction between this effect and *Experiments* and also a significant (F ratio = 35.3) second-order interaction among this effect, *Experiments* and the methods of treatments. The reason for this is shown in Table V. The combinations of quinine and thiourea when averaged over the four methods of treatments was 50.81 units better than the others in *Experiment* 1, and 97.19 units better in *Experiment* 2. Thus, the two experiments agree on the superiority of this combination, but they disagree on its magnitude. This is the cause of the rather large interaction of $AC \times$ Experiments. However, this interaction was not

TABLE V.—INTERACTION BETWEEN QUININE–THIOUREA AND METHODS OF TREATMENTS EXPRESSED IN ABSOLUTE UNITS

| Factor | Food | Fasting | Buffer A | Buffer B | Av. |
|---|---|---|---|---|---|
| *Experiment 1:* | | | | | |
| Quinine with thiourea | 177.01 | 118.45 | 242.19 | 206.04 | 185.93 |
| Others | 171.64 | 44.63 | 192.10 | 132.04 | 135.12 |
| Differences | 5.37 | 73.82 | 50.09 | 74.00 | 50.81 |
| *Experiment 2:* | | | | | |
| Quinine with thiourea | 356.15 | 180.72 | 230.10 | 262.75 | 257.63 |
| Others | 159.82 | 87.05 | 216.53 | 178.61 | 160.44 |
| Differences | 196.33 | 93.67 | 13.57 | 84.14 | 97.19 |

TABLE VI.—INTERACTION BETWEEN METHODS OF TREATMENTS AND PRESENCE *vs.* ABSENCE OF 1-PHENYL-2-THIOUREA IN ABSOLUTE UNITS

| 1-Phenyl-2-thiourea | Food | Fasting | Buffer A | Buffer B | Av. |
|---|---|---|---|---|---|
| Absent | 243.87 | 75.68 | 229.58 | 200.92 | 187.51 |
| Present | 137.94 | 97.90 | 195.19 | 149.15 | 145.05 |
| Differences | 105.93 | −22.22 | 34.39 | 51.77 | 42.46 |

statistically significant and the interaction $AC \times$ methods of treatments was not significant. Observation of the individual differences listed in Table V indicates that in *Experiment* 1 the superiority of the combination of quinine and thiourea was uniformly substantial, except for the nonfasted animals. On the other hand, in *Experiment* 2 there was a large difference for the nonfasted animals, but the difference was rather small with Buffer A. This discrepancy between the results of the two experiments was the cause of the significant $AC \times M \times$ Experiments interaction. It should be noted that the mean values listed in Table V for quinine with thiourea are averages of only two items, and they are subject to large experimental errors.

The reason for the statistically significant main effect *B* (presence *versus* the absence of 1-phenyl-2-thiourea) is that the average of the 32 items with 1-phenyl-2-thiourea absent was 187.51 units (Table VI) compared with 145.05 units for the average of 32 results with 1-phenyl-2-thiourea present. Apparently this adjuvant was detrimental to the absorption of the alkaloids. However, there is a significant interaction between this effect and the methods of treatments. Table VI shows averages of eight items per entry. The reason for the significant interaction with methods of treatments is evident—the 1-phenyl-2-thiourea was not detrimental when administered to fasted animals but was detrimental when given with Buffer A, Buffer B, and to rats that had not been fasted.

The presence *versus* the absence of *N,N'*-diethylthiourea, or the main effect *D*, was statistically significant according to the analysis of variance (Table III). In Table VII the average of 32 items with this adjuvant present was 188.31 units; the average of 32 items without *N,N'*-diethylthiourea was 144.21 units. This is a difference of 44.10 units in favor of the addition of this adjuvant. However, there is a significant $C \times D$ interaction, indicating that the effect of this adjuvant may depend upon whether thiourea is present. This interaction is shown in Table VII, which indicates that the effect

TABLE VII.—INTERACTION BETWEEN PRESENCE *vs.* ABSENCE OF *N,N'*-DIETHYLTHIOUREA AND PRESENCE *vs.* ABSENCE OF THIOUREA

| *N,N'*-Diethylthiourea | Thiourea | No Thiourea | Differences |
|---|---|---|---|
| Present | 196.86 | 179.83 | 17.03 |
| Absent | 179.93 | 108.40 | 71.53 |
| Differences | 16.93 | 71.43 | |

TABLE VIII.—EFFECTS OF METHODS OF TREATMENTS ON ALKALOID ABSORPTION EXPRESSED IN ABSOLUTE UNITS

| Methods | Av. |
|---|---|
| Food | 190.95 |
| Fasting | 86.79 |
| Buffer A | 212.39 |
| Buffer B | 174.99 |

of *N,N'*-diethylthiourea is greater when thiourea is absent (71.43 units contrasted to 16.93). There is a large statistically significant effect for the interaction $A \times D$. Gross inspection of the lower half of Table II indicates that there is a large effect between *N,N'*-diethylthiourea and quinidine as previously reported (1), and this is the reason for the significant $A \times D$ interaction.

The *Methods of treatments* comparison was statistically significant as indicated in the analysis of variance of Table III. Table VIII indicates that this was almost entirely due to the 24-hour fasting of the rats prior to the test. Fasting definitely hinders absorption of the alkaloids in these experiments. Each figure in Table VIII is the mean of 16 items. The inhibitory effect of fasting can be seen as a uniform phenomenon in Tables V and VI. Inspection of Table VI indicates that 1-phenyl-2-thiourea in a fasted animal encourages alkaloidal absorption as reported earlier (1); yet in a nonfasted animal its presence actually discourages absorption.

## SUMMARY

A factorial experiment was carried out in rats to study the effects of thiourea, *N,N'*-diethylthiourea, 1-phenyl-2-thiourea, ionic strength, *ad libitum* food, and 24-hour fasting on the absorption of quinine and quinidine. Thiourea administered in equal weight with quinine consistently gave enhancement of absorption, while absence of effect from thiourea was observed when given with quinidine. 1-Phenyl-2-thiourea administration resulted in lowering the rate of absorption in nonfasted animals while increasing absorption in fasted animals. *N,N'*-Diethylthiourea routinely increased the absorption of both quinine and quinidine. There was no noticeable interference with alkaloidal absorption from either the strongly buffered or the weakly buffered vehicles. Fasting exerted a strong inhibitory effect on quinine and quinidine absorption.

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# Influence of Adrenergic Receptors on Blood Sugar and Lactic Acid Levels in the Rat

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**Six treatment combinations were compared for their production of blood sugar and lactic acid in rats. The challenging drugs—saline, epinephrine, and levarterenol-isoproterenol combined—were tested against saline (without adrenergic blockers) and against DCI combined with hydergine. Epinephrine and levarterenol-isoproterenol, the challenging amines, were both effective in increasing the production of blood sugar and lactic acid. The adrenergic blockade produced by DCI combined with hydergine was effective in inhibiting both hyperglycemia and hyperlacticacidemia. Since a specific blockade of both $\alpha$ and $\beta$ adrenergic receptors prevented the glycogenolytic effects of epinephrine and levarterenol-isoproterenol, it is concluded that this effect is mediated through these receptors.**

IT IS WELL known that the sympathetic amines will cause an increased production of both blood sugar and lactic acid (1); there is, however, some uncertainty as to which adrenotropic receptors, if any, are responsible for the mediation of this response. Glycogenolysis was first attributed to the $\alpha$ receptors by Ahlquist (2), then to both receptors by Van der Pol (3) and Claasen and Noach (4). Mayer, *et al.* (5), and McCutcheon (6) took exception to this and suggested that the $\beta$ receptors—or possibly some unknown receptors

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