Beckman IR-5 or Perkin-Elmer 221 spectrophotometer as KBr pellets.

Hydroxyalkyl-2-thioureas—To 0.2 mole of an alkyl or aryliso-thiocyanate in 100 ml. of ethanol was added portionwise 0.2 mole of an amino-alcohol, and the resultant solution was heated for 0.5 hr. on a steam bath. In some cases, the hydroxyalkyl-2-thioureas crystallized from the cooled solution; in other instances, the solutions were concentrated or evaporated to dryness to induce crystallization.

1-(Hydroxyalkyl)-3-benzoyl-2-thioureas—These compounds were prepared essentially by the method of Douglass and Dains (4). To 44.6 g. (0.55 mole) of sodium thiocyanate dissolved in 500 ml. of acetonitrile or acetone was added slowly 70.3 g. (0.50 mole) of benzoyl chloride, and the resulting mixture was heated on a steam bath for 10 min. The mixture was then cooled, and the amino-alcohol (0.50 mole) was added with constant stirring. The resultant mixture was heated on a steam bath for 15 min. and poured into a large volume of water. The oily layer generally crystallized on cooling to give the 1-(hydroxyalkyl)-3-benzoyl-2-thiourea (Table I).

1-(2-Hydroxyethyl)-2-thiourea (Ia)—To 22.4 g. (0.1 mole) of 1-(2-hydroxyethyl)-3-benzoyl-2-thiourea was added 100 ml. of 6% NaOH solution, and the resulting solution was heated on a steam bath for 10 min. The cooled solution was then made slightly acidic with 6 *N* H_2SO_4; the benzoic acid, which separated, was removed by filtration. The filtrate was adjusted to neutrality with dilute NaOH solution. The solvent was removed under reduced pressure, giving a residue which was extracted with three 100-ml. portions of hot CH_3CN. The CH_3CN extracts were evaporated to dryness, and the Ia was recrystallized (Table I).

Reaction of Hydroxyalkyl-2-thioureas with Methyl Iodide—To 1-(hydroxyalkyl)-2-thiourea (0.05 mole) dissolved in 40 ml. of acetonitrile or ethanol was added methyl iodide (0.06 mole), and the solution was heated under reflux. When methyl mercaptan evolution was very faint or not detected within 0.5 hr., the solution was refluxed for 2 hr. and cooled or concentrated under reduced pressure to give the *S*-methylthiopseudourea hydriodide. The latter gave a positive nitroprusside test (1) when heated with base (Table II).

When a copious evolution of methyl mercaptan was detected within the first 0.5 hr. of refluxing, heating was continued until methyl mercaptan evolution virtually ceased, generally within 6–12 hr. The solution was cooled or concentrated, causing separation of the 2-amino-2-oxazoline derivative (or 2-amino-5,6-dihydro-4*H*-1,3-oxazine derivative) as the hydriodide salt. The IR spectra of these compounds showed a characteristic peak at 5.9–6.1 μ (C=N) (Table III).

2-Amino-2-oxazoline (IIIa)—A mixture of 1.0 g. (0.0031 mole) III*f* in 10 ml. of 1:1 HCl was agitated and heated under reflux for about 2 hr. until solution was complete. The benzoic acid, which separated on cooling, was removed and the filtrate was concentrated several times with water to remove the excess acid. The syrup was treated with a saturated ethanolic solution of picric acid to give 0.45 g. (46%) of 2-amino-2-oxazoline picrate as fine needles (from water), m.p. 192–195° [lit. (5) m.p. 186–188°].

2-Amino-5,6-dihydro-4*H*-1,3-oxazine—2-Benzamido-5,6-dihydro-4*H*-1,3-oxazine hydriodide (III*k*, 1.0 g., 0.0030 mole) was treated as described previously to give 0.37 g. (37%) of 2-amino-5,6-dihydro-4*H*-1,3-oxazine picrate as fine needles (from water), m.p. 207–208° [lit. (6) m.p. 200°].

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Comparison of Effects of 1,8-Dihydroxyanthraquinone and 1,5-Dihydroxyanthraquinone on Different Segments of Rabbit Gastrointestinal Tract

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Abstract □ Using an isolated muscle bath technique, five different segments of the rabbit gastrointestinal tract (duodenum, jejunum, ileum, and ascending and descending colon) were used to record the effects of two doses of 1,5-dihydroxyanthraquinone. Transducer-recorded tracings were made of contractions per minute, interval between contractions, and amplitude of contractions. The results were evaluated; statistical tests among comparisons of the three parameters between the two drug doses, control values, and previous values for 1,8-dihydroxyanthraquinone again indicated the parameter of interval between contractions to be a statistically significant measurement of activity. No statistically significant differences in effects were shown between the two drugs.

Keyphrases □ 1,8- *versus* 1,5-Dihydroxyanthraquinone—effect on gastrointestinal tract, rabbit □ Gastrointestinal tract, rabbit—effects, 1,8- *versus* 1,5-dihydroxyanthraquinone

The normal activity of different segments of the rabbit gastrointestinal tract has been previously delineated, using an isolated muscle bath technique (1).

The effects of 15- and 30-mg. doses of 1,8-dihydroxyanthraquinone on these different segments of rabbit intestinal tract have been studied with this technique and statistically compared with the standards (2). The results of the statistical analyses of the transducer-

Table I—Summary of Averages and Standard Deviations of Measurements of Rabbit Intestinal Activity Obtained with 15 mg. 1,5-Dihydroxyanthraquinone

Segment	No. of Measurements	Contractions/min.	Interval between Contractions, sec.	Amplitude of Contractions, mm.
Duodenum	18	16.0 ± 0.89	1.8 ± 0.1[a]	0.93 ± 0.63
Jejunum	18	13.8 ± 1.68	2.2 ± 0.3[a]	2.22 ± 1.87
Ileum	18	11.1 ± 1.42	2.7 ± 0.1[a]	4.26 ± 4.01[a]
Ascending colon	18	7.9 ± 1.34[a]	3.7 ± 1.1[a]	1.03 ± 1.10
Descending colon	18	5.9 ± 0.90	5.2 ± 1.2[a]	1.62 ± 0.43

[a] Significantly different from normal averages at $p = 0.05$.

Table II—Summary of Averages and Standard Deviations of Measurements of Rabbit Intestinal Activity Obtained with 30 mg. 1,5-Dihydroxyanthraquinone

Segment	No. of Measurements	Contractions/min.	Interval between Contractions, sec.	Amplitude of Contractions, mm.
Duodenum	18	15.6 ± 1.26	1.9 ± 0.1[a]	1.88 ± 1.72
Jejunum	18	12.4 ± 0.49	2.4 ± 0.2[a]	1.69 ± 1.21[a]
Ileum	18	10.1 ± 1.18	2.9 ± 0.2[a]	3.65 ± 2.31[a]
Ascending colon	18	6.2 ± 0.69[a]	4.8 ± 0.7[a]	0.65 ± 0.33
Descending colon	18	8.6 ± 1.97[a]	3.7 ± 0.9[a]	1.06 ± 1.78

[a] Significantly different from normal averages at $p = 0.05$.

recorded tracings indicated that the parameter of interval between contractions was a statistically significant measurement of activity.

Therefore, the purposes of this study were to observe the effects of 1,5-dihydroxyanthraquinone on the different segments of the rabbit gastrointestinal tract and to compare these statistically with the previous normal and 1,8-dihydroxyanthraquinone figures to determine the value of the parameter of interval between contractions as a significant measurement of activity for drug-effect studies.

EXPERIMENTAL

Adult albino rabbits were anesthetized with ether, and 2–3-cm. segments of duodenum, jejunum, ileum, and ascending and descending colon were expediently removed and maintained in oxygenated Tyrode's solution in the same manner as in the previously delineated standards and 1,8-dihydroxyanthraquinone studies (1, 2).

As in the previous studies, the Tyrode's solution in glass distilled water again contained sodium chloride, 0.8%; potassium chloride, 0.02%; calcium chloride, 0.02%; magnesium chloride, 0.01%; sodium bicarbonate, 0.1%; sodium diphosphate, 0.005%; and glucose, 0.1%; 15 ml. was used to bathe the intestinal segment in the isolated muscle bath chamber (1, 2).

Similar to the standard and 1,8-dihydroxyanthraquinone studies, the temperature was maintained at 38°, and the preparation was oxygenated using an air flow slowly (2–3 bubbles/sec.) bubbled through the solution (1, 2). The segment was attached to the muscle hooks, and the intestinal activity was again recorded *via* transducer (E & M isotonic myograph) and physiograph recorder (E & M) (1, 2).

The segments were allowed to acclimate to the muscle bath environment, following which normal intestinal activity for the different segments was recorded. Six different rabbits were used to provide six different samples of duodenum, jejunum, ileum, and ascending and descending colon for each of the two doses of the 1,5-dihydroxyanthraquinone in the same manner as in the 1,8-dihydroxyanthraquinone studies (2).

The anthraquinone was 1,5-dihydroxyanthraquinone.[1] The drug was placed in Tyrode's solution so as to provide 0.1 and 0.2% concentrations, providing 15 and 30 mg./15 ml. isolated muscle bath chamber, respectively, for the low and high doses.

The effects of each dosage were recorded for a minimum of 15 min. for each of the six different samples of the five different segments and were completely repeated at the second dosage for six different samples of the five different segments, identical to the design of the 1,8-dihydroxyanthraquinone studies (2).

From each recording of each individual gut segment, amplitude of contraction, interval between contractions, and contractions per minute were measured and averaged as in the two previous studies (1, 2).

Standard deviations were calculated; analyses of variance with F test and Scheffe's S method tests were made between combinations of treated and control intestinal segments (3).

[1] Eastman Organic Chemicals, P 2246.

RESULTS

Table I summarizes the averages and standard deviations of the amplitude of contraction, interval between contractions, and contractions per minute obtained with the low dose (15 mg.) of 1,5-dihydroxyanthraquinone.

Table II summarizes the averages and standard deviations of the amplitude of contraction, interval between contractions, and contractions per minute obtained with the high dose (30 mg.) of 1,5-dihydroxyanthraquinone.

Table III summarizes the similar parameters obtained with the low dose (15 mg.) of 1,8-dihydroxyanthraquinone, and Table IV summarizes those obtained with the high dose (30 mg.) of 1,8-dihydroxyanthraquinone.

Table V summarizes the averages and standard deviations of the amplitude of contraction, interval between contractions, and contractions per minute obtained with the normal untreated intestinal segments.

Of the 150 possible comparisons of the three parameters between drug-treated and normal averages, 31 were significantly different statistically at $p = 0.05$, including the 15 from the 1,8-dihydroxyanthraquinone (2).

All 10 comparisons of the interval between contractions for both the 15 and 30 mg. 1,5-dihydroxyanthraquinone and normal averages were significantly different statistically at $p = 0.05$. There were no significant differences between any of the comparisons between the 15- and 30-mg. doses of 1,5-dihydroxyanthraquinone; there was one significant with the 1,8-dihydroxyanthraquinone, the ileum comparisons of amplitude of contractions (2).

The remaining six significant comparisons were jejunum *versus* jejunum amplitude of contractions between 30-mg. dose and normal averages (this is compared to the 15-mg. dose of the 1,8-dihydroxyanthraquinone which was significant); ileum *versus* ileum amplitude of contractions between both treatments and normal averages; ascending colon *versus* ascending colon contractions per minute between both treatments and normal averages; and the descending colon *versus* descending colon contractions per minute between 30-mg. dose and normal averages.

Table III—Summary of Averages and Standard Deviations of Measurements of Rabbit Intestinal Activity Obtained with 15 mg. 1,8-Dihydroxyanthraquinone

Segment	No. of Measurements	Contractions/min.	Interval between Contractions, sec.	Amplitude of Contraction, mm.
Duodenum	18	14.4 ± 1.05	1.9 ± 0.3[a]	0.73 ± 0.36
Jejunum	18	12.4 ± 1.65	2.5 ± 0.4[a]	1.05 ± 0.46[a]
Ileum	18	9.8 ± 2.04	3.2 ± 0.6[a]	1.52 ± 1.35[a,b]
Ascending colon	18	8.1 ± 2.75[a]	4.5 ± 2.5[a]	4.62 ± 5.79
Descending colon	18	7.2 ± 1.54	4.4 ± 0.9[a]	1.51 ± 1.63

[a] Significantly different from normal averages at $p = 0.05$. [b] Significantly different from 30-mg. dose average at $p = 0.05$.

Table IV—Summary of Averages and Standard Deviations of Measurements of Rabbit Intestinal Activity Obtained with 30 mg. 1,8-Dihydroxyanthraquinone

Segment	No. of Measurements	Contractions/min.	Interval between Contractions, sec.	Amplitude of Contraction, mm.
Duodenum	18	15.2 ± 1.41	1.9 ± 0.2[a]	2.84 ± 1.92
Jejunum	18	13.4 ± 0.90	2.3 ± 0.2[a]	2.87 ± 2.44
Ileum	18	10.3 ± 1.10	2.7 ± 1.0[a]	7.04 ± 2.06[b]
Ascending colon	18	7.7 ± 1.55[a]	3.5 ± 0.9[a]	0.18 ± 0.13
Descending colon	18	6.4 ± 1.69	4.3 ± 1.2[a]	0.35 ± 0.22

[a] Significantly different from normal averages at $p = 0.05$. [b] Significantly different from 15-mg. dose average at $p = 0.05$.

Table V—Summary of Averages and Standard Deviations of Measurements of Normal, Untreated Rabbit Intestinal Activity

Segment	No. of Measurements	Contractions/min.	Interval between Contractions, sec.	Amplitude of Contraction, mm.
Duodenum	21	16.7 ± 1.68	3.9 ± 0.1	2.56 ± 2.50
Jejunum	21	12.4 ± 2.31	5.0 ± 1.5	6.53 ± 3.46
Ileum	21	10.5 ± 1.27	6.2 ± 1.1	9.17 ± 0.68
Ascending colon	15	2.1 ± 0.83	15.0 ± 8.2	1.68 ± 0.57
Descending colon	15	4.3 ± 1.68	13.4 ± 5.0	3.91 ± 2.38

SUMMARY AND CONCLUSIONS

Five different segments of the rabbit intestinal tract—duodenum, jejunum, ileum, and ascending and descending colon—were utilized in recording the activity of these segments in response to 15- and 30-mg. doses of 1,5-dihydroxyanthraquinone, using an *in vitro* isolated muscle bath technique.

Contractions per minute, interval between contractions, and amplitude of contraction were recorded, determined, and averaged; standard deviations were calculated; and analyses of variance with *F* test and Scheffe's *S* method tests were performed on segment combinations between the 1,5-dihydroxyanthraquinone-treated and normal segments and the 1,8-dihydroxyanthraquinone-treated segments.

Of the 150 comparisons of the three parameters between drug-treated and normal averages, 31 were significantly different statistically at $p = 0.05$ and included all comparisons of interval between contractions for both drug treatments and normal averages.

Comparing the actual figures (averages) of the three parameters for the five segments of the high dose of the 1,5-dihydroxyanthraquinone and the normal averages, 5/5 values for interval between contractions for the high dose were less than the normal figures and all showed statistically significant differences.

Comparing the actual values of the three parameters for the five segments of the low dose of the 1,5-dihydroxyanthraquinone and the normal averages, 5/5 values for interval between contractions for the low dose were less than the normal figures and all again showed statistically significant differences.

Comparisons of effects between the high and low doses of 1,5-dihydroxyanthraquinone on the three parameters showed no statistically significant differences. Comparisons of the activity between the high doses of both drugs on the three parameters and comparisons between the low doses of the two drugs showed no statistically significant differences.

The statistical tests between figures of both drugs *versus* normal show considerable agreement in the numbers of statistically significant differences and agree much of the time with specific segments.

The results from this *in vitro* study and their statistical comparisons show again the parameter of interval between contractions to be a statistically significant measurement of activity.

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