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A STUDY OF ADVERSE GASTROINTESTINAL EFFECTS OF P-AMINOQUINOLINE--ETC(11)

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of 8-Aminoquinolines

FINAL SCIENTIFIC REPORT

by

ROBERT S. TEAGUE, M.D., Ph.D.

ROY L. MUNDY, Ph.D.

and

EDWARD R. SEIDEL, Ph.D.

Report date: October 1980

(For the period 1 May 1973 to 1 June 1979)

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

FORT DETRICK, FREDERICK, MARYLAND 21701

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University of Alabama Medical Center

Birmingham, Alabama 35294

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Investigations were carried out to elucidate the mechanism of adverse effects of experimental antimalarial compounds on the gastrointestinal tract. Most of the experimental work used primaquine as a prototype 8-aminoquinoline. This compound was found to have anticholinergic and motility inhibiting effects on the gut from several species. A method for the assessment of the relative potency of these effects was developed and 12 compounds were compared.		

Section 20.

→ All compounds tested had significant depressant effect on the motility of the isolated rabbit intestine. These quantitative results may allow comparison of the discomfort producing capacity of the 8-aminoquinoline series when testing in man is possible.

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FOREWORD

Period Covered: 1 May 1973 to 1 June 1979

Animal Experimentation: In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

Disposition Instructions: Unclassified report. When this document has served its purposes, DESTROY IT.

SUMMARY

This report is a final summary of information contained in two Annual Reports from this laboratory; one for the period 1 May 1973 to 1 June 1975 and a second for the period 1 May 1976 to 1 June 1977 plus nine interim reports made to the Pharmacology Department, Walter Reed Army Medical Center during the period 1 May 1978 to 1 June 1979. These reports are a part of this final report.

The report encompasses the entire period 1 May 1973 to 1 June 1979 and each study done on the adverse effects of 8-Aminoquinolines is summarized as follows:

1. Experiments were carried out which indicate that primaquine has the ability to decrease the sensitivity of the smooth muscle of the guinea pig ileum to acetylcholine. This is true when the drug is administered intraperitoneally and the gut removed for study two hours later or when the primaquine is added directly to the ileum strip in a muscle bath. The question of whether this attenuation of acetylcholine-induced contractions is anticholinergic or nonspecific in nature has not been answered.

2. It was found that intraperitoneal injection of large doses of primaquine decreased the gastrointestinal transport of a carbon meal in mice. The agent is some 5 to 6 times less potent than dextroamphetamine in this respect. Atropine alone did not inhibit transport of a carbon meal in the mouse but when combined with small doses of primaquine it became potent in this respect. Ageing of primaquine solutions in room light intensified its ability to depress gastrointestinal transport. Degradation of primaquine may intensify some of its biological activities.

3. Large, cumulative concentrations of primaquine, applied *in vitro*, caused relaxation of the guinea pig ileum ($1-5.5 \times 10^{-5}$ and 1×10^{-4} g/ml). Baseline tension was reduced by the larger doses. Single applications of primaquine to the guinea pig ileum often led to baseline tension increase and development of spontaneous rhythmic activity. This activity was often larger in amplitude than in control preparations, which developed spontaneous activity occasionally, and sometimes reached an amplitude of 50 percent of the maximum acetylcholine-induced contractions. Since some of the control preparations developed rhythmic activity and the development of activity could not be proven to be dose-related, the system was abandoned.

4. Primaquine caused a dose-related depression in spontaneously occurring intraluminal pressure waves and in height of cyclic contractions of the isolated rabbit ileum. It also relaxed muscles which had been placed under tension by mechanical or chemical means.

5. Pamaquine, another 8-aminoquinoline, at cumulative doses of up to 1×10^{-5} g/ml did not affect baseline tension or motility of the guinea pig ileum. At low doses (7×10^{-6} g/ml) it did not influence methacholine-induced contractions but at higher levels (7×10^{-5} g/ml) it did significantly inhibit these responses. Pamaquine did not alter histamine-induced contractions of the ileum.

6. Although relatively low doses of primaquine caused signs of nausea followed by vomiting in conscious dogs, lethal, oral doses caused no other indication of abdominal discomfort. One dog developed an intussusception of the jejunum. Since this dog gave no indication of abdominal discomfort after the initial bout of emesis, it is unlikely that gross observation will allow assessment of "pain" in the dog.

In the anesthetized dog it was possible to increase the tonus and amplitude of intestinal contractions by applying unbuffered solutions of primaquine either to the mucosal or serosal surface of the gut after laparotomy. These responses were not always produced by drug application and did not appear to be dose-related. When increased activity was elicited it could not be blocked by atropine. In studies with the dog jejunum in vitro, no increase in tone or activity was induced by cumulative additions of primaquine. It is concluded that primaquine may act directly on the gut but an intact nervous system is required for the increased activity to take place.

7. Primaquine, administered orally to the rat did not cause gross ulcerations or other visible forms of tissue damage to the mucosa of the stomach or small bowel of this species. Evan's Blue dye escaped from the circulation into the lumen of the stomach of 2 of 3 animals treated with 80 mg/kg of primaquine for 4 consecutive days but also appeared in the stomachs of 1 of 6 control animals.

Work in this laboratory has brought us to the conclusion that experiments on isolated muscle preparations or in vivo measurements in anesthetized animals are not likely to produce a model suitable for assessment of the adverse effects of 8-aminoquinolines on the gastrointestinal tract.

The thrust of the program should now be centered on the conscious animal of a species other than the rodent. In order to observe altered tone or activity in such an animal, it will be necessary to use surgically isolated segments of the gastrointestinal tract or to attach strain gauge transducers to the stomach or intestine.

The fact that an intact central nervous system appears to be needed for the production of altered intestinal activity, however sporadic, again points out the possible importance of examining the emetic potential of a series of 8-aminoquinolines in the conscious cat or dog. It is possible that the intricate nervous outflows which coordinate the complex act of vomiting may also alter gastrointestinal activity in such a way as to produce discomfort.

8. Primaquine, when administered orally to two lightly restrained, unanesthetized rats with chronically implanted duodenal recording catheters, failed to produce a consistent alteration of Type I activity.

9. Acutely or chronically implanted extraluminal strain gauge transducers were used to study the effects of primaquine on the motor activity of the GI tract of the dog. In neither anesthetized nor unanesthetized animals did primaquine cause any consistent effects on the musculature of the stomach, duodenum, jejunum, ileum or proximal large bowel.

10. Primaquine diphosphate was injected i.p. in mice to see if it produced writhing like acetic acid or phenylquinone. Primaquine (10^{-3} M, 0.02 ml/gm) failed to produce writhing. When injected simultaneously with acetic acid, primaquine displayed analgesic activity attenuating acetic acid-induced writhing. The mechanism of this protective action was not explained.

11. The hypothesis that primaquine might cause epigastric pain by producing spasm of the gall bladder musculature was tested in the isolated gall bladder. The results did not support the thesis. In fact, primaquine was shown to be a weak competitive antagonist ($pA_2 = 4.57$) of acetylcholine in the preparation.

12. The interaction of primaquine and the C-terminal octapeptide of cholecystokinin was studied in the isolated gall bladder. Primaquine was shown to be a competitive antagonist of the peptide. The paper also reports a potentiating action of cholecystokinin on acetylcholine and oxotremorine in the preparation.

13. Two congeners of primaquine were studied in isolated smooth muscle preparations. The 6-hydroxy-8-aminoquinoline derivative (WR 6890) neither contracted nor relaxed the isolated ileum or gall bladder. The compound had no effect on the sensitivity of either tissue to cumulative doses of acetylcholine. The 6-methoxy-8-aminoquinoline derivative was insoluble in aqueous solvents and therefore inappropriate for use in the isolated organ preparation.

14. In many experiments done in this laboratory on the effect of primaquine diphosphate on the tone and contractile force of intestinal tissues, an inhibition of spontaneous activity has been observed. A test system to quantitate the inhibitory activity of primaquine on the spontaneous rhythmic activity of the rabbit ileum has been developed in order to evaluate the potency of inhibitory effects of candidate antimalarial agents. The rationale for the work is based on the fact that local inhibition and dilation of intestinal segments may lead to abdominal discomfort.

Primaquine diphosphate was applied cumulatively at 1.6, 3.2, 6.4, $\times 10^{-5}$ and 1.3 and 2.6×10^{-4} molar concentrations to six rabbit ileum preparations in an isolated muscle bath. Primaquine diphosphate caused dose-related decrease in the spontaneous activity of the rabbit ileum. The depression ranged, on average, from 43 percent of control activity at the lowest concentration tested to 84 percent at the highest. When these data were plotted as percent inhibition against the logarithm of the molar concentration of primaquine they fitted a straight line. Analysis gave an ID 50 (concentration needed to reduce activity by 50 percent) of 4.1×10^{-5} M.

The mechanism of the depressant action of primaquine is quite likely related to a membrane or receptor locus of action because recovery of activity occurs promptly once the chemical is washed from the preparation. The weak antimuscarinic activity of primaquine may explain its depression of the spontaneous activity of the rabbit ileum.

15. Compound WR 181023AG, a primaquine analog, was tested at cumulative dose intervals of 1.28, 2.56, 5.12×10^{-5} and 1.02 and 2.05×10^{-4} M on six rabbit ileum preparations in an isolated muscle bath. A dose related depression of the spontaneous activity of the muscle preparation was obtained. The lowest concentration depressed activity by 26 percent, on average, while the highest dose caused an 83 percent decrease.

When the individual responses were transformed to probits and plotted against the agent concentrations as the minus logarithm of molar strength, the results fitted a straight line (ANOVA, $P < 0.01$). The concentration of WR 181023AG required to reduce the activity of the rabbit ileum to fifty percent of original activity (ID-50) was calculated to be 3.94×10^{-5} M with a 95% CL of 3.33 to 4.55×10^{-5} M.

When the compound was washed from the muscle bath the activity of the muscle recovered rapidly and reached 89 percent of control activity within five minutes following washout.

16. Compound WR 225448 has been tested at 1.0, 1.5, 2.3, 3.4 and 5.1×10^{-5} molar concentrations on six ileal preparations of the rabbit. The compound causes a dose-dependent depression of spontaneous activity of the rabbit intestine. The concentration needed to depress spontaneous activity to 50 percent of control (ID 50) is 2.79×10^{-5} M and the 95 percent confidence of the analysis is $2.64 - 2.94 \times 10^{-5}$ M. The data were fitted to a straight line and yielded a correlation coefficient of 0.721 and analysis of variance showed the regression was significant at the $P = < 0.01$ level.

The compound is not as soluble as the parent 8-aminoquinoline primaquine. In two of the six experiments reported here, there was evidence of formation of cloudiness in the muscle bath at the highest concentration used. The precipitation was noted as "colloidal" clouding in the bath when the highest concentration was added.

The muscles did not regain spontaneous activity when Compound WR 225448 was washed from the preparation. In only two of the six preparations was any activity observed and this was difficult to measure accurately with the system described in Interim Report 1A.

17. Compound WR 224097 AB, 8-(5-amino-2-pentylamine)-5-(4-fluorophenoxy) -6-methoxy quinaldine, monofumarate, a primaquine analog, was injected cumulatively at 1.0, 2.0, 4.0, 8.0×10^{-5} M and 1.6×10^{-4} M concentrations into six rabbit ileum preparations in an isolated muscle bath. A dose related depression of spontaneous activity of the muscle preparations was observed. Lower concentrations of the compound caused stimulation of the muscles, however, as the concentration was increased a dose related depression which was not relieved when the compound was washed from the bath was seen.

The compound depressed the muscles to 50 percent of original activity (ID 50) at 3.28×10^{-5} M concentration. The 95 percent confidence of the inhibitory effect was $3.04 - 3.52 \times 10^{-5}$ M. When the activity decrements were transformed to probits and plotted versus dose levels coded arbitrarily as 0-4 dose units a formula describing the regression was generated, $Y = 4.07 + 0.56X$. The data had a correlation coefficient of 0.770 and analysis of variance showed that the regression was significant at the $P < 0.01$ level.

Compound WR 224097AB is slightly more potent as a depressant of ileal activity than primaquine, however, there was no statistical significance between the results obtained for the 8-aminoquinolines.

18. Compound WR 232956 has been tested at 2.5, 5.0×10^{-6} and 1.0, 2.0 and 4.0×10^{-5} molar concentrations on six ileal preparations of the rabbit. The compound stimulates the ileum at low concentrations and then causes a dose dependent depression of spontaneous activity of the rabbit intestine at higher dose levels. The concentration which depressed spontaneous activity to fifty percent of that in control preparations (ID 50) was 1.42×10^{-5} M and the 95 percent confidence limits of the analysis was $1.40 - 1.45 \times 10^{-5}$ M. The data fitted a straight line when the responses were transformed to probits and plotted versus the coded dose of compound (arbitrarily 0-4) and yielded a correlation of 0.803. Analysis of variance for the regression showed that the regression was significant at the $P = < 0.01$ level.

Compound WR 232956 was difficult to place in solution at the requisite concentration to permit attainment of the final bath concentrations reported here. From 30 to 45 minutes of stirring was needed to produce a solution, however, the Compound appeared to be in a "true" solution in each of the experiments we carried out.

"Recovery" of the activity of muscles treated with Compound WR 232956 reached approximately 50 percent that of controls within five minutes after it was washed from the bath. Two of six muscles exhibited excellent recovery (110 and 86 percent) while three of six attained less than 25 percent of control activity. One of six muscles became erratic after washout of the chemical and no activity record could be obtained.

19. Compound WR 212579 was tested at 1.6, 3.2, 6.4×10^{-5} and 1.3 and 2.6×10^{-4} M concentrations on six ileal preparations from the rabbit, in vitro. The compound showed a potent depressant activity on spontaneous activity in the preparations. The concentration required to reduce activity to 50 percent of control activity (ID 50) was 1.52×10^{-5} M. Because the dose range used was too high, it was not possible to calculate 95 percent confidence limits for the assay. When the percent inhibition values were transformed to probits and plotted against the doses employed (arbitrary dose units, 0-4) a formula was generated $Y = 4.71 + 0.68X$. Analysis of variance revealed that there was a positive regression $P = < 0.01$ and the correlation coefficient for the data was 0.835.

Although the muscle preparations were depressed over 95 percent in activity, when they were allowed to recover for 30 minutes after washout of the WR 212579, five of the six regained, on average, some 80 percent of original activity.

These experiments were not completely satisfactory in that the calculated ID 50 fell below the lowest dose of compound injected. This shortcoming could have been resolved by reducing the beginning concentration of the agent to 4 and 8×10^{-6} molar.

In summary, Compound WR 212579 is a potent, slowly reversible depressant of spontaneous activity in the rabbit ileum in vitro.

20. Compound WR 221527 was injected cumulatively into six rabbit ileal preparations at concentrations of $1.6, 3.2, 6.4 \times 10^{-5}$ and 1.3 and 2.6×10^{-4} molar. The compound strongly depressed spontaneous activity of the preparations and one injection at the 1.3×10^{-5} molar concentration and three of the injections at the 2.6×10^{-4} concentration were not made because of strong depression of activity in the muscles at lower concentrations.

The muscles were observed for 30 minutes after the chemical was washed from the muscle bath and regained, on average, 90 percent of their control activity. While the recovery of the muscles was not rapid as with the prototype compound primaquine, the depression was certainly reversible. It should be noted that the depression of activity was severe in these muscles as compared to the primaquine experiments (93.7% vs 84%) and that the longer recovery period may simply reflect a more complete saturation of some receptor or membrane locus.

The concentration needed to reduce the spontaneous activity to fifty percent of control activity (ID 50) was 2.9×10^{-5} M. When the responses were transformed to probits and plotted against arbitrary dose units (0-4) a formula was generated, $Y = 4.51 + 0.54X$. Analysis of variance showed that the regression differed from zero at $P = < 0.01$. The correlation coefficient of the regression was 0.811.

The compound is highly soluble in water and there was no problem in making concentrations of sufficient strength to reach the desired final molar concentrations in the bath.

21. Compound WR 6021 (Isopentaquine) was tested at cumulative doses of 1.0, 2.0, 4.0, 8.0×10^{-5} and 1.6×10^{-4} molar on eight (8) preparations of rabbit ileum as described in Interim Report 1A.

The concentration required to inhibit spontaneous activity of the ileum by fifty percent (ID 50) was 2.57×10^{-5} M. The ninety-five percent confidence limits for the determination were 2.16 to 2.97×10^{-5} M. When the data was transformed to probits of percent inhibition and regressed against the arbitrary dose designations zero to four, the regression was represented by the equation $Y = 4.33 + 0.52X$. The correlation coefficient for the regression was 0.681. The regression differed from zero, ANOVA $P = < 0.01$.

When the compound was washed out of the preparation and activity measured either at 5 minutes (N-6) or 10 minutes (N-2) following washout the muscles has regained, on average, 64 percent of control activity.

Compound WR 6021 is very soluble and presented no problem in the preparation of samples of sufficient concentration to permit the injection of the concentrations used in the experiments.

22. Compound WR 215296 was very poorly soluble in deionized water and would not allow the preparation of a solution of the requisite concentration to attain adequate muscle bath concentrations for testing.

Compound WR 4234 (Pamaquine) was also of insufficient solubility to allow testing in the protocol described in Interim Report 1A.

Compound WR 233195 was supplied in a very small amount (milligrams) and there was insufficient chemical to allow testing.

Ricinoleic Acid (No Known WR Number) is known to inhibit the tone of intestinal muscle in the dog and to inhibit water absorption from the gut¹. Since this action has been advanced for the mechanism of the gastrointestinal action of ricinoleic acid we have tested this compound in the system described in Interim Report 1A at 4.8 and 7.2×10^{-5} and 1.0 and 1.6×10^{-4} M in five isolated rabbit ileum preparations. The ID 50 was 1.0×10^{-4} M and the 95% confidence limits of the estimate were 8.7×10^{-5} to 1.1×10^{-4} M. This compound is significantly less potent than the 8-aminoquinolines in its ability to depress the spontaneous activity of the rabbit ileum in vitro.

One graduate student, Dr. Edward R. Seidel, was supported for a portion of his graduate training by participating in the work on this Contract. His publications related to the project are as follows:

E. R. Seidel, R. L. Mundy and R. S. Teague, Antimuscarinic Activity of the 8-aminoquinoline Antimalarial Drug, Primaquine, (Abstract). Abstracts of Papers for 17th Annual Meeting, Society of Toxicity, San Francisco, CA, March 12-16, 1978, p 79.

E. R. Seidel and R. L. Mundy, Potentiation of the Gall Bladder Response to Cholinergic Agents by the C-Terminal Octapeptide of Cholecystokinin (O-CCK), (Abstract) The Pharmacologist (Fall), 1978.

E. R. Seidel and R. L. Mundy, Serotonomimetic Properties of Primaquine in the Isolated Colon, Submitted for publication, Europ. J. Pharmacol., 1979.

E. R. Seidel and R. L. Mundy, Modulation by Cholecystokinin of Guinea Pig Gall Bladder Acetylcholine Sensitivity and Muscarinic Receptor Density, Submitted for publication, J. Pharmacol. Exptl. Therap., 1980.

E. R. Seidel, Modulation of Cholinergic Sensitivity of the Guinea Pig Gall Bladder by the C-Terminal Octapeptide of Cholecystokinin, Doctorial Dissertation, The University of Alabama in Birmingham, 1980.

REFERENCE: ¹Steward, J. J., Gaginella, T. S., Olsen, W. A. and Bass, P., "Inhibitory Actions of Laxatives on Motility and Water and Electrolyte Transport in the Gastrointestinal Tract," J. Pharmacol. Exptl. Therap. 192: 458-467, 1975.



the University of Alabama in Birmingham

the Medical Center / DEPARTMENT OF PHARMACOLOGY

THE DEPRESSANT ACTION OF PRIMAQUINE ON SPONTANEOUS
ACTIVITY OF THE RABBIT ILEUM IN VITRO

ROY L. MUNDY

CONTRACT NUMBER

DADA-17-67-C-7136

INTERIM REPORT NUMBER 1A

ABSTRACT

In many experiments done in this laboratory on the effect of primaquine diphosphate on the tone and contractile force of intestinal tissues, an inhibition of spontaneous activity has been observed. A test system to quantitate the inhibitory activity of primaquine on the spontaneous rhythmic activity of the rabbit ileum has been developed in order to evaluate the potency of inhibitory effects of candidate antimalarial agents. The rationale for the work is based on the fact that local inhibition and dilation of intestinal segments may lead to abdominal discomfort.

Primaquine diphosphate was applied cumulatively at $1.6, 3.2, 6.4, \times 10^{-5}$ and 1.3 and 2.6×10^{-4} molar concentrations to six rabbit ileum preparations in an isolated muscle bath. Primaquine diphosphate caused a dose-related decrease in the spontaneous activity of the rabbit ileum. The depression ranged, on average, from 43 percent of control activity at the lowest concentration tested to 84 percent at the highest. When these data were plotted as percent inhibition against the logarithm of the molar concentration of primaquine they fitted a straight line. Analysis gave an ID 50 (concentration needed to reduce activity by 50 percent) of $4.1 \times 10^{-5} \text{M}$.

The mechanism of the depressant action of primaquine is quite likely related to a membrane or receptor locus of action because recovery of activity occurs promptly once the chemical is washed from the preparation. The weak antimuscarinic activity of primaquine may explain its depression of the spontaneous activity of the rabbit ileum.

INTRODUCTION

Primaquine diphosphate causes disturbing gastrointestinal side-effects in service personnel who must take it prophylactically (Clayman et al., 1952). Primaquine is well tolerated at the recommended therapeutic dose in most subjects, however, some complain of anorexia, nausea, abdominal cramps and other vague gastrointestinally related symptoms. The Department of Pharmacology, University of Alabama in Birmingham, in conjunction with the Pharmacology Department, Walter Reed Army Institute of Research, has carried out extensive animal studies in an attempt to elucidate the mechanism responsible for the effects of 8-aminoquinolines upon gastrointestinal motility and tone (Teague and Mundy, 1975a). One reproducible effect has been the ability of 8-aminoquinolines to cause depression of spontaneous activity and/or muscle tone (Teague and Mundy, 1975b). Depression of smooth muscle tone and activity has been observed by Bass and coworkers in the case of ricinoleic acid which is known to cause gastrointestinal distress (Stewart, 1973; Stewart et al., 1975). A test system has been developed to quantitate the gastrointestinal muscle depressant potential of 8-aminoquinolines. It is the objective of the work reported here to provide information on the depressant action of primaquine diphosphate upon rabbit ileum in an in vitro test system. The hope is that the depressant potential of primaquine when combined with clinical testing, may generate an index to the gastrointestinal toxicity of other members of the 8-aminoquinoline series. Subsequent reports will give results for individual 8-aminoquinolines.

METHODS

Adult, male, New Zealand White strain rabbits weighing approximately 2.5 kilograms were used. The animals were housed in animal quarters provided by the Division of Animal Services of the University of Alabama in Birmingham. This is

an accredited animal care facility and day-to-day animal care complies with Federal Regulations. The animals had continuous access to Wayne Rabbit Ration and water until they were removed from the animal quarters to our laboratory.

The rabbits were selected at random from the holding area, transported to our laboratory, and killed by cervical dislocation. A midline incision was made along the linea alba in the abdominal wall for approximately 6 to 8 centimeters in order to expose the intestines. The ileocecal junction was identified and the ileum was traced anteriorly for 10 centimeters. Two contiguous segments of ileum, 3 centimeters long, were removed and the intestinal contents removed by gentle flushing from a syringe filled with Tyrode's solution (Description follows). Stainless steel hooks were inserted into each end of the segments for attachment to a stationary attachment point in a muscle bath and via a 000 silk suture to an isometric strain gauge transducer. The tissue was immersed in Tyrode's solution during the time from removal from the animal until it was placed into the muscle chamber. The tissue was at room temperature during this preparatory period. It took from four to six minutes for preparation.

The tissue was suspended in Tyrode's solution in a jacketed muscle bath, (75 ml capacity) which was held at 37°C by circulating water through the external jacket from a thermostatically controlled 10 gallon aquarium. Tyrode's solution for replenishing the bath during washing of the tissue, was held in a flask which was immersed in the aquarium. The prewarmed Tyrode's solution was forced from the flask into the muscle bath by air pressure and removed from the chamber by suction when the tissue was washed. The Tyrode's solution in both the holding flask (reservoir) and muscle bath was aerated constantly with 95% oxygen and 5% carbon dioxide mixture.

Resting (baseline) tension of the muscle segment was set at 1 gram when it was placed into the muscle bath by mechanically adjusting the height of the

transducer above it. This level of tension was maintained by minor mechanical adjustments throughout a 45 minutes control period and the tissue was washed two times during this period with 200 ml of fresh Tyrode's solution.

The Tyrode's solution is made as follows (Pharmacological Experiments on Isolated Preparations, 1968).

NaCl	160 grams
KCl (10% solution)	40 ml
MgSO ₄ (anhydrous, 4.88% solution).	51.8 ml
NaHCO ₃	20 grams
NaH ₂ PO ₄ (5% solution).	20 ml
CaCl ₂ (1 Molar).	36 ml
Glucose.	1 gm/l (added at use)
Deionized Water q.s. ad	20 l

As soon as spontaneous activity began in the ileal segment, the strain element of the isometric transducer (Grass FT-10) was deformed by contractions of the muscle. Distortion of the strain element changed electrical conductivity in the element in proportion to the strength of contraction. This conductivity change was amplified and the amplified signal fed to the galvanometer of a Grass Model 5 Polygraph. Paper speed of the oscillograph was set at 0.25 millimeters per second. The sensitivity of the electrical system was calibrated at the beginning of each experiment by hanging weights of known value on the stainless steel hook to which the ileum was later attached. The vertical axis of the chart record is calibrated in grams of weight as millimeters of upward pen travel. The signal from the amplifier was also fed into an integrator (Grass UI-1) for five minutes during the last five minutes of the 45 minute control period and for five minutes exactly five minutes after each primaquine injection. The integrator collected and summed the electrical charge from the amplifier. It was adjusted so that no charge was accepted at the 1 gram baseline and was set

to discharge and return to the baseline after a total charge has been accepted which drives the pen upward 4 centimeters. One pen deflection of 4 centimeters and a drop back to baseline is called a ramp. The number of ramps occurring during a five minute period was counted from the chart paper and divided by five to give a value of ramps/minute (Figure 1). This value was used as a measure of "activity" of the preparation. After the five doses of primaquine were injected, the bath was washed out with 200 ml of fresh Tyrode's solution and the activity was determined for five minutes after the wash-out had been completed five minutes earlier. This measurement was termed "recovery".

The primaquine diphosphate (Bottle Number AU 29317) used in this study came from the Pharmacology Department, Walter Reed Army Institute of Research with quality control data that defined the physical and chemical characteristics of the compound. It was stored in a refrigerator, in the dark, from the time of receipt until used. Diagram number 1 shows a flow sheet describing how primaquine diphosphate was weighed, dissolved in deionized water and diluted to make solutions which were injected into the muscle bath to produce the final molar concentrations used in this study. In control experiments an identical volume of deionized water was injected instead of primaquine on the same time schedule, as described above, for drug injection.

The ID 50 value and confidence limits was calculated by the method of Bliss (Bliss, 1952). Molar concentrations of primaquine were transformed to the negative logarithm, base 10, for the statistical computations. Actual molar concentrations used are shown in Diagram 1 and the logarithmic transformation of these concentrations is shown in Table 1 and Figure 2.

RESULTS

Figure 1 shows a photograph of a typical polygraph record from experiment Number 49. The upper tracing is inscribed from the contractions of the ileum while the lower trace shows the ramps from the integrator output. In this particular experiment the activity as measured in ramps/minute was not decreased very strongly as the concentrations of primaquine were increased (see percentages in Table 1) until $1.3 \times 10^{-4}M$ was reached, however, primaquine markedly inhibited activity at the two highest concentrations. The preparation recovered fully from the inhibition when the primaquine was washed from the bath as shown by the recording marked "recovery".

Results obtained with 18 control preparations, in which distilled water was injected into the bath instead of the primaquine solution, were analyzed by regression analysis and yielded a regression equation of $Y = 115 + 3.3 X$. The response values entered in the calculation were the average values obtained from each of the five injections and the arbitrarily chosen numbers 1-5 represented the X axis or numbers of injections made. The correlation coefficient for the analysis was 0.901 and analysis of variance for regression gave an F ratio of 13.3. This means that the regression is significantly different from zero at the $P = < 0.05$ level. The muscles increased spontaneous activity by 16.5 percent during the 50 minute experimental period. At no time period was there a decline in activity below that recorded in the control period. See Figure 3.

Table 1 lists the individual responses from the six experiments at five dose levels expressed as percent inhibition of the muscles from control activity. The table also shows the percent recovery of the muscles, again using the control activity as 100 percent, five minutes, after primaquine diphosphate was washed from the bath. In experiment 48, this measurement was not made because of technical difficulties. On average, the muscles were back to 81 percent of control activity five minutes after washout. The range of recovery was 59 to 103

percent of control activity. The ID 50 for primaquine was calculated to be $4.1 \times 10^{-5}M$ with 95 percent confidence limits of 2.4 to $7.0 \times 10^{-5}M$. Analysis of variance showed that the regression line differed from zero with a confidence of $P = < 0.05$. When average values of the responses at the five dose levels were tested for regression against the negative logarithm of the doses used there was a correlation coefficient of 0.819. However when all of the values were included in the calculation the correlation coefficient decreased to 0.612 accenting the wide spread of individual values.

Primaquine diphosphate is freely soluble in deionized water and presented no difficulty in the preparation of a solution of the salt of sufficient concentration to reach effective inhibitory doses.

DISCUSSION

The test system measured depression of activity caused by primaquine diphosphate. However, the measurement system has spontaneous fluctuations which appear to be intrinsic to the rabbit ileum.

In the experiments where deionized water was injected instead of primaquine, regression analysis indicated that there was a small increase in the integral of activity with time. This argues that the depression of activity which occurred when primaquine was injected was a result of the action of primaquine and that the calculation of an ID 50 should provide a reliable index of its potency as an intestinal depressant.

It is necessary to put the ileum under tension in order for spontaneous activity to be measured. In numerous trials it was found that under the conditions in our laboratory that one gram of tension gave the most activity. More tension reduced the strength of the contractions and less tension resulted in failures to contract spontaneously. It was also true that not all segments set up spontaneous rhythms (approximately 3 percent). When this occurred a very

small dose of acetylcholine chloride (approximately 10^{-8} molar) would always initiate spontaneous activity which continued after washout of the agonist. This fact would suggest a cholinergic link in the production of spontaneous activity in the isolated rabbit ileum.

In several of our experiments, lower concentrations of primaquine (10^{-8} - 10^{-6} molar) caused an increase in activity of the ileal segment. These increases often reached 150 to 200 percent of control activity. A possible explanation may be that primaquine is a serotonergic agonist. Hong and Pardo reported in 1966 that quipazine, a quinoline, contracts the guinea pig ileum via serotonin receptors and Seidel and Mundy reported in 1979 that primaquine contracts the guinea pig colon and that the contractions are antagonized by methysergide. It seems highly likely that the 8-aminoquinolines may share the agonistic properties of quipazine.

The depressant action of larger doses of primaquine may be explained by its weak anticholinergic action (Seidel, Mundy and Teaque, 1978). When acetylcholine is used as the agonist and atropine or primaquine are the antagonists the pA_2 of atrophine is 8.57, while that of primarquine is 4.57.

The fact that the muscles regained a major portion of their original activity soon after the primaquine was removed from the bath argues for a membrane, perhaps receptor, mode of action for the agent. Membrane or receptor binding must be relatively non-stable because of the rapid recovery noted.

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TABLE 1

INHIBITORY EFFECT OF PRIMAQUINE ON ACTIVITY OF RABBIT ILEUM IN VITRO

<u>LOG DOSE (Molar)</u>	<u>PERCENT INHIBITION^Δ</u>						<u>X ± S.E.M.</u>
	<u>EXPERIMENT #</u>						
	<u>45</u>	<u>46</u>	<u>47</u>	<u>48</u>	<u>49*</u>	<u>50</u>	
-4.79	57	38	73	38	17	37	43 ± 8
-4.49	56	66	58	56	22	43	50 ± 6
-4.19	51	48	57	75	17	34	47 ± 8
-3.89	50	69	52	66	31	47	53 ± 6
-3.59	91	79	88	88	81	79	84 ± 2

PERCENT RECOVERY FIVE MINUTES AFTER REMOVAL OF PRIMAQUINE

WASHOUT	65	59	81	ND	103	99	81 ± 9
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Δ100 - (Treated Activity/Control Activity X 100) = Percent Inhibition.

ID 50 = $4.1 \times 10^{-5}M$

95% CL = $2.4 - 7.0 \times 10^{-5}M$

Formula of Regression Line: $Y = 173 + 28X$

ANOVA P = <0.05

ND = Not determined

*Experiment illustrated in Figure 1.

FIGURE 1

INHIBITORY EFFECT OF PRIMAQUINE DIPHOSPHATE ON THE RABBIT ILEUM
IN VIVO. EXPERIMENT 49.

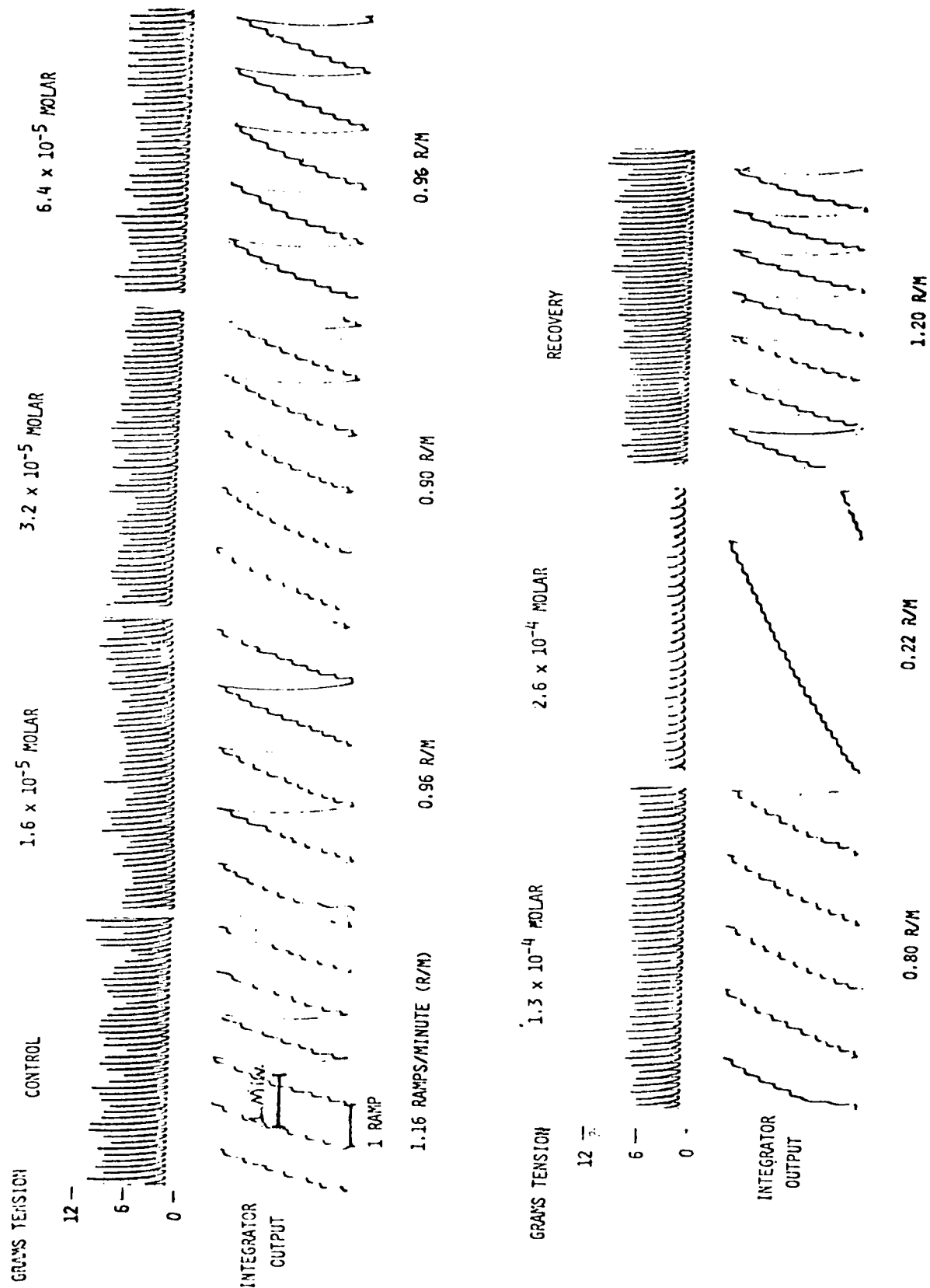
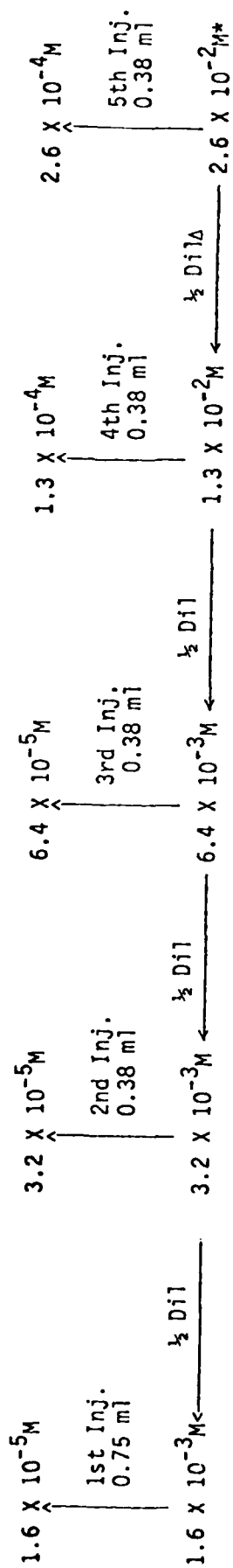


DIAGRAM 1

DIAGRAM OF DILUTION AND INJECTION FORMAT



* 0.1183 Gms. Primaquine Diphosphate to 10 ml deionized water.
 $\Delta = \frac{1}{2}$ Dilution, 1 ml stock or drug solution plus 1 ml deionized water.

FIGURE 2

INHIBITORY EFFECT OF PRIMAQUINE DIPHOSPHATE ON THE RABBIT ILEUM IN VITRO

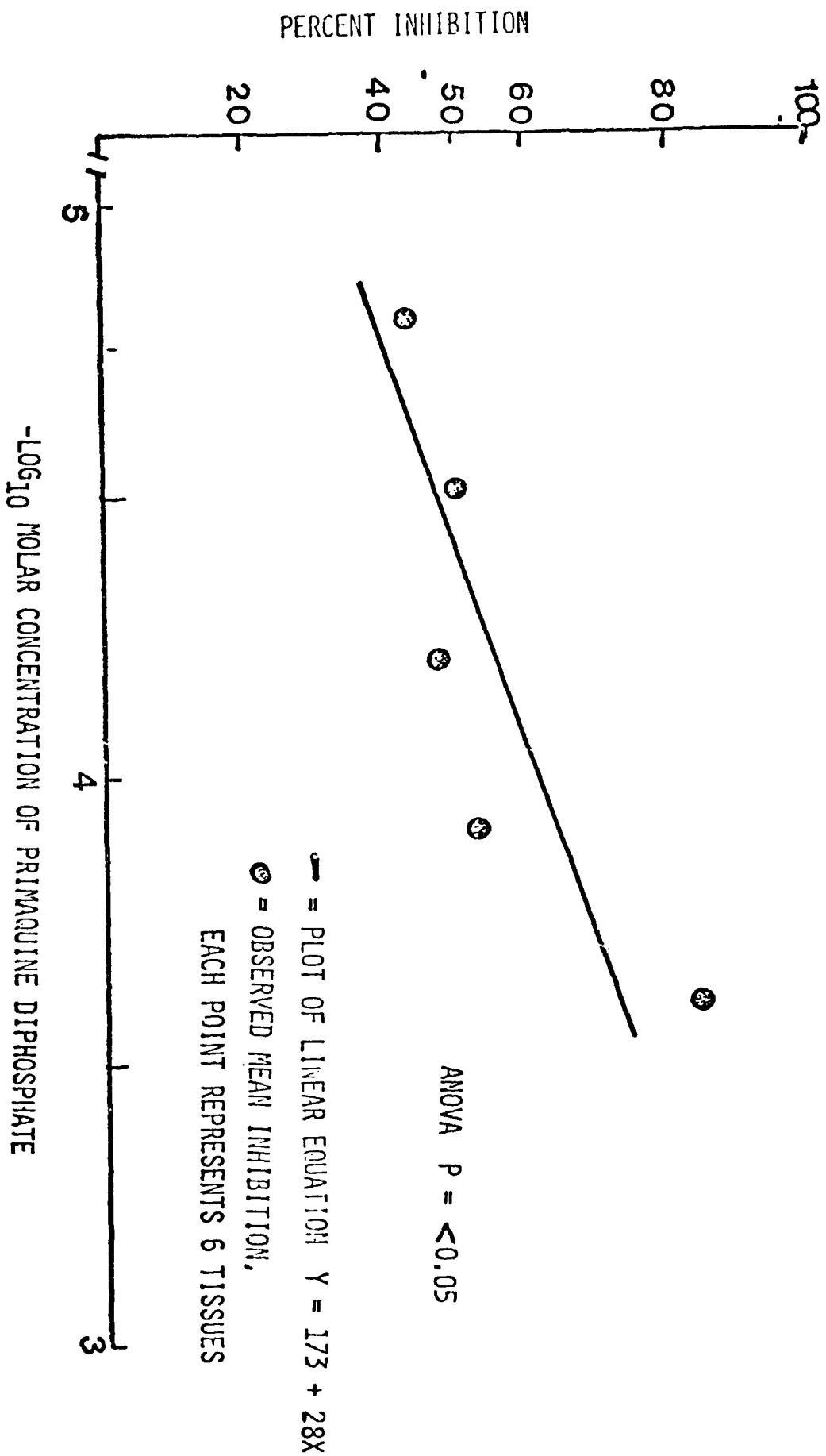
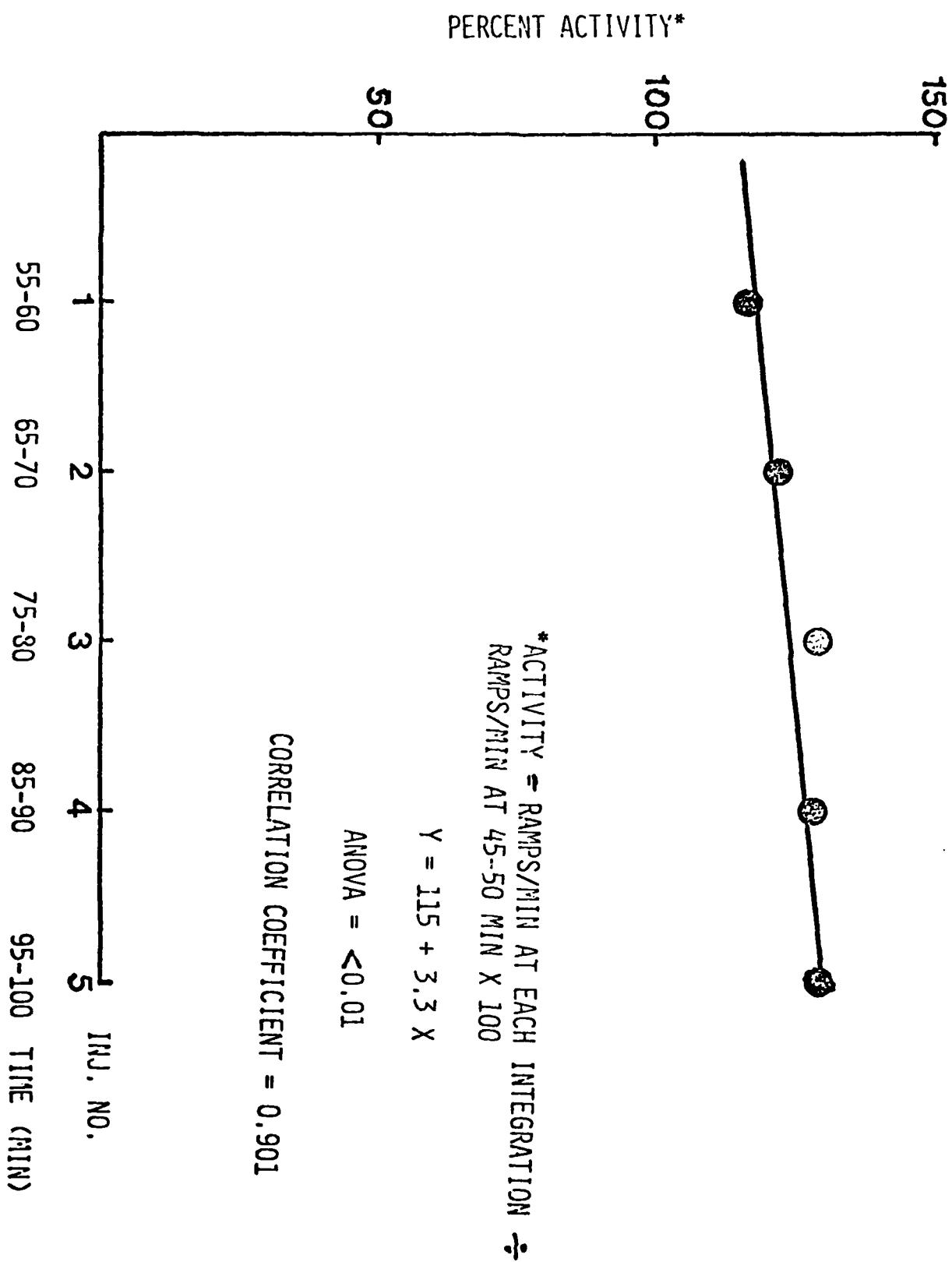


FIGURE 3 EFFECT OF TIME AND THE SECTION OF DEIONIZED WATER ON SPONTANEOUS ACTIVITY OF RABBIT ILEUM IN VITRO*





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THE DEPRESSANT SECTION OF WR 181,023 AG ON SPONTANEOUS
ACTIVITY OF THE RABBIT ILEUM IN VITRO

ROY L. MUNDY, Ph.D.

CONTRACT NUMBER

DADA-17-67-C-7136

INTERIM REPORT NUMBER 2A

ABSTRACT

Compound WR 181023AG, a primaquine analog, was tested at cumulative dose intervals of 1.28, 2.56, 5.12×10^{-5} and 1.02 and $2.05 \times 10 \times 10^{-4}$ M on six rabbit ileum preparations in an isolated muscle bath. A dose related depression of the spontaneous activity of the muscle preparation was obtained. The lowest concentration depressed activity by 26 percent, on average, while the highest dose caused an 83 percent decrease.

When the individual responses were transformed to probits and plotted against the agent concentrations as the minus logarithm of molar strength, the results fitted a straight line (ANOVA, $P < 0.01$). The concentration of WR 181023AG required to reduce the activity of the rabbit ileum to fifty percent of original activity (ID-50) was calculated to be 3.94×10^{-5} M with a 95% CL of 3.33 to 4.55×10^{-5} M.

When the compound was washed from the muscle bath the activity of the muscle recovered rapidly and reached 89 percent of control activity within five minutes following washout.

INTRODUCTION

WR 181023 AG is a close analog of primaquine which differs in chemical structure by substitution of a methyl group in the carbon 4 position. Its chemical name is 8-(5-Amino-2-pentylamino)-6-methoxylepidine diphosphate. It is quite soluble in water and presented no problem in testing on the isolated rabbit ileum preparation described in Interim Report 1A.

When the compound was tested according to the protocol described, it produced a dose-related depression of spontaneous activity in the muscle. Its potency in this respect was not different from that of primaquine. When the compound was washed from the muscle preparation there was a rapid recovery of spontaneous activity and in five minutes after the washout, on average, the muscles had regained over eighty percent of their original (control) activity.

METHODS

The methods used in these experiments were identical in all respects to those described in Interim Report 1A.

RESULTS

The results are tabulated in Table 1 and illustrated in Figure 1. The ID 50 of the compound was 3.94×10^{-5} M with a 95 percent confidence level of 3.3 to 4.55×10^{-5} M. The data fitted a straight line with a confidence ANOVA, $P = < 0.01$. The correlation coefficient of the least squares analysis was 0.662.

The tissues recovered within five minutes following washout of the chemical to 89 percent of control activity with a S.E.M. of 10.4 percent.

WR 181023 AG resembles the action of primaquine in the described test system. There was no significant difference in the potency of this chemical and its parent 8-aminoquinoline. Rapid recovery, as in the case of primaquine indicates that a membrane or receptor locus of action is probably responsible for the action of this compound.

TABLE 1

INHIBITORY EFFECT OF WR 181023 AG ON ACTIVITY OF RABBIT ILEUM IN VITRO

LOG DOSE (Molar)	<u>PERCENT INHIBITION*</u>						$\bar{X} \pm$ S.E.M.
	EXPERIMENT #						
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	
-4.89	1	9	24	12	44	64	25.7 $\pm$ 9.8
-4.59	7	19	41	42	65	65	39.8 $\pm$ 9.6
-4.29	9	37	57	66	78	75	54.0 $\pm$ 10.9
-3.99	35	68	85	81	97	100	77.7 $\pm$ 9.7
-3.69	64	78	92	97	--	--	82.8 $\pm$ 7.4

PERCENT RECOVERY FIVE MINUTES AFTER REMOVAL OF WR 181023 AG

WASHOUT	106	100	102	88	49	--	89.0 $\pm$ 10.4
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*100-(Treated Activity/Control Activity x 100) = Percent Inhibition.

ID 50 = 3.94×10^{-5} M.

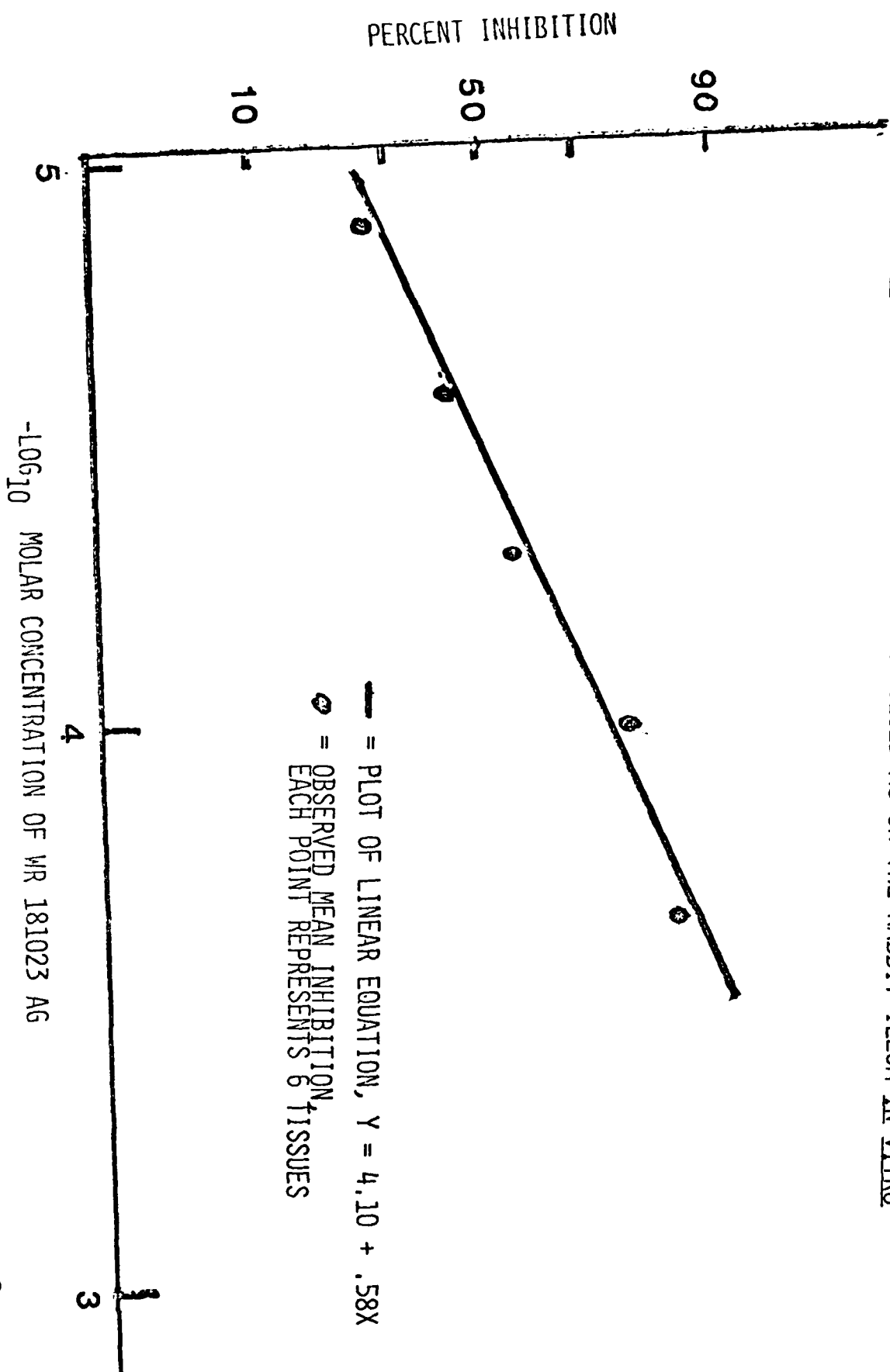
95% CL = $3.33 - 4.55 \times 10^{-5}$ M.

Formula of Regression Line: $Y = 4.10 + .58X$. (Probit response vs coded dose, 0-4.)

ANOVA P - < 0.01.

-- = Not determined.

FIGURE 1 INHIBITORY EFFECT OF WR 181023 AG ON THE RABBIT ILEUM IN VITRO





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the Medical Center / DEPARTMENT OF PHARMACOLOGY

THE INHIBITORY EFFECT OF COMPOUND WR 225448 ON SPONTANEOUS
ACTIVITY OF THE RABBIT ILEUM IN VITRO

ROY L. MUNDY, Ph.D.

CONTRACT NUMBER

DADA-17-67-C-7136

INTERIM REPORT NUMBER 3A

ABSTRACT

Compound WR 225448 has been tested at 1.0, 1.5, 2.3, 3.4 and 5.1×10^{-5} molar concentrations on six ileal preparations of the rabbit. The compound causes a dose-dependant depression of spontaneous activity of the rabbit intestine. The concentration needed to depress spontaneous activity to 50 percent of control (ID 50) is 2.79×10^{-5} M and the 95 percent confidence of the analysis is $2.64 - 2.94 \times 10^{-5}$ M. The data were fitted to a straight line and yielded a correlation coefficient of 0.721 and analysis of variance showed the fit to be significant at the $P = < 0.01$ level.

The Compound is not as soluble as the parent 8-aminoquinoline primaquine. In two of the six experiments reported here there was evidence of formation of cloudiness in the muscle bath at the highest concentration used. The precipitation was noted as "colloidal" clouding in the bath when the highest concentration was added.

The muscles did not regain spontaneous activity when Compound WR 225448 was washed from the preparation. In only two of the six preparations was any activity observed and this was difficult to measure accurately with the system described in Interim Report 1A.

INTRODUCTION

Compound WR 225448 was studied in the rabbit ileum preparation described in Interim Report 1A. There were no variations from the protocol described in that report.

METHODS

As described in Interim Report 1-A.

RESULTS

The responses from the six experiments are tabulated in Table 1 and shown graphically in Figure 1.

DISCUSSION

Compound WR 225448 appears to be slightly more potent as a depressant of spontaneous activity in the rabbit ileum than is primaquine. However, the overlap in 95% confidence limits between the two assays makes it clear that there is no significant difference in this activity. The one striking difference in the two compounds is that WR 225448 causes a non-reversible depression of the muscle.

TABLE 1

INHIBITORY EFFECT OF WR 225448 ON ACTIVITY OF RABBIT ILEUM IN VITRO

LOG DOSE (Molar)	PERCENT INHIBITION*						$\bar{X} \pm S.E.M.$
---------------------	---------------------	--	--	--	--	--	----------------------

EXPERIMENT

	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	
-4.9999	0	1	10	11	a	6	7.0 $\pm$ 2.3
-4.8239	34	9	53	23	a	12	26.2 $\pm$ 8.0
-4.6383	66	52	71	38	2	13	40.3 $\pm$ 11.5
-4.4685	84	57	98	54	36	43	62.2 $\pm$ 9.9
-4.2924	90	80	--	I	52	I	74.0 $\pm$ 11.4

PERCENT RECOVERY FIVE MINUTES AFTER REMOVAL OF WR 225448

WASHOUT	0	0	6	0	34	0	20.0 $\pm$ 14.0
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*100 - (Experimental Activity/Control Activity X 100).

a = Stimulation noted - no depression.

-- = No injection made.

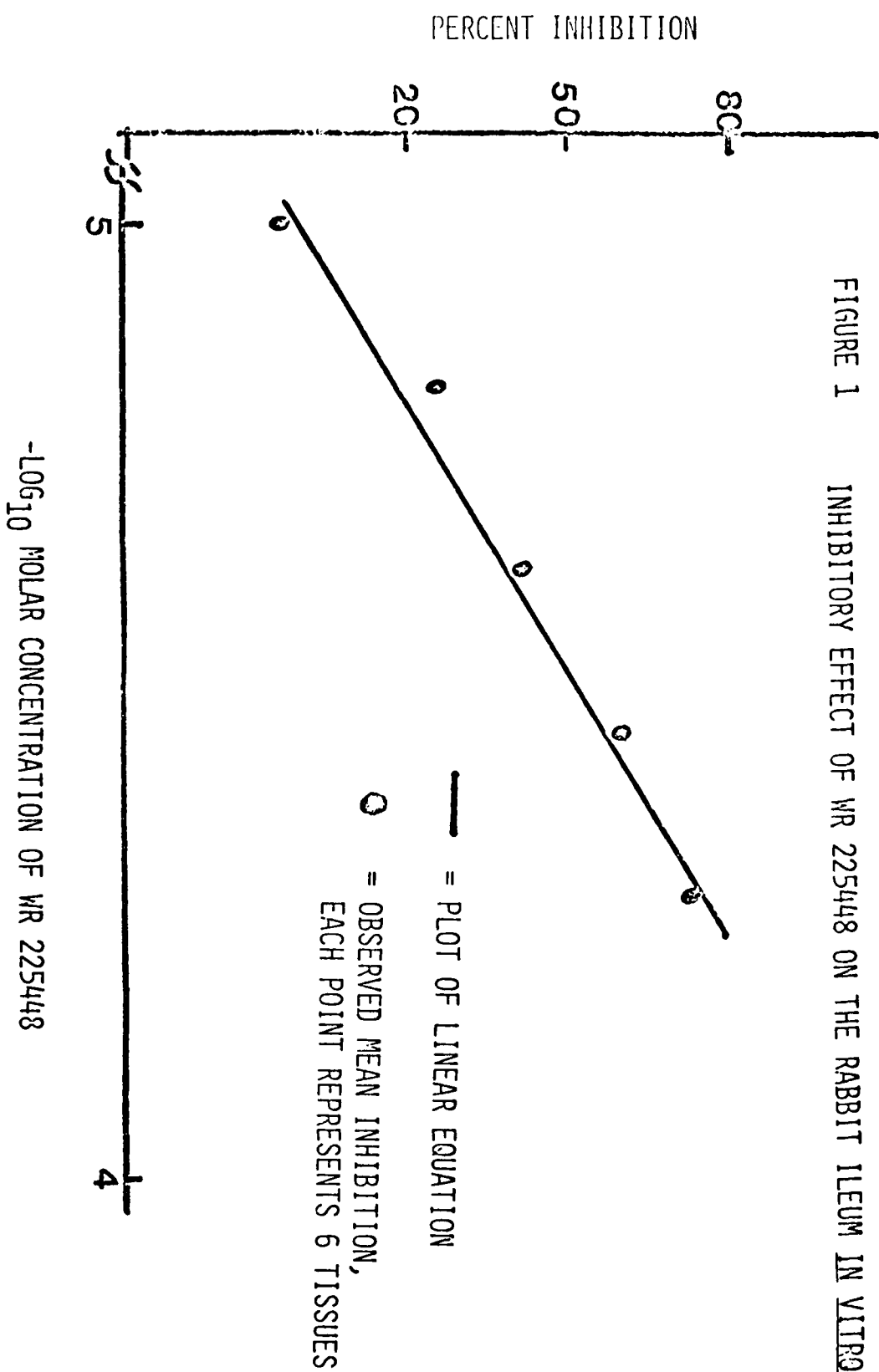
I = Compound precipitated in bath.

ID 50 = 2.79×10^{-5} M.95% CL = $2.64 - 2.84 \times 10^{-5}$ M.Formula of the Regression Line: $Y = 3.53 + 0.59 X$. (Probits of response vs. coded doses 0-4).

ANOVA P = < 0.01

Regression Coefficient = 0.721

FIGURE 1 INHIBITORY EFFECT OF WR 225448 ON THE RABBIT ILEUM IN VITRO





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the Medical Center / DEPARTMENT OF PHARMACOLOGY

THE INHIBITORY ACTION OF COMPOUND WR 224097 AB ON THE
SPONTANEOUS ACTIVITY OF THE RABBIT ILEUM IN VITRO

ROY L. MUNDY

CONTRACT NUMBER

DADA-17-67-C-7136

INTERIM REPORT NUMBER 4A

ABSTRACT

Compound WR 224097 AB, 8-(5-amino-2-pentylamino)-5-(4-fluorophenoxy)-6-methoxy quinaldine, monofumarate, a primaquine analog, was injected cumulatively at 1.0, 2.0, 4.0, 8.0 x 10⁻⁵M and 1.6 x 10⁻⁴M concentrations into six rabbit ileum preparations in an isolated muscle bath. A dose related depression of spontaneous activity of the muscle preparations was observed. Lower concentrations of the compound caused stimulation of the muscles, however, as the concentration was increased a dose related depression which was not relieved when the compound was washed from the bath was seen.

The compound depressed the muscles to 50 percent of original activity (ID 50) at 3.28 x 10⁻⁵M concentration. The 95 percent confidence of the inhibitory effect was 3.04 - 3.52 x 10⁻⁵M. When the activity decrements were transformed to probits and plotted versus dose levels coded arbitrarily as 0-4 dose units a formula describing the regression was generated, $Y = 4.07 + 0.56 X$. The data had a correlation coefficient of 0.770 and analysis of variance showed that the fit was significant at the $P < 0.01$ level.

Compound WR 224097 AB is slightly more potent as a depressant of ileal activity than primaquine, however there was no statistical significance between the results obtained for the two 8-aminoquinoline lines.

INTRODUCTION

Compound WR 224097 AB is a congener of the 8-aminoquinoline antimalarial primaquine and differs from it by the substitution of a 4-fluorophenoxy group on carbon 5 of the parent compound. It was tested for its depressant activity on the isolated rabbit ileum in vitro in the test system described in Interim Report 1A. There were no deviations of the protocol for testing outlined in that report.

METHODS

As described in Interim Report 1A.

DISCUSSION

Compound WR 224097 AB appears to be a slightly more potent depressant of ileal muscle than its parent compound, primaquine. However, the wide range of the 95 percent confidence for the assays does not allow the minor difference in potency to be called significant. There are two other differences noted in these experiments which might be worthy of note. First, WR 224097 AB caused stimulation of the muscles at the two lowest concentrations applied. Second, when the compound was washed from the muscle bath at the end of the experiment there was no rapid recovery of muscle activity.

TABLE 1

INHIBITORY EFFECT OF WR 224097 AB ON ACTIVITY OF RABBIT ILEUM IN VITRO

LOG DOSE (Molar)	PERCENT INHIBITION*							
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	$\bar{X} \pm S.E.M.$
-4.9999	a	a	15	a	8	a	a	11.5±3.5
-4.6990	39	a	40	a	60	34	20	38.6±6.4
-4.3979	66	53	69	44	47	67	65	58.7±3.9
-4.0969	61	54	88	67	95	84	98	78.1±6.5
-3.7959	66	47	99	69	91	90	98	81.4±7.2

PERCENT RECOVERY FIVE MINUTES AFTER REMOVAL OF WR 224097 AB

WASHOUT	17	--	--	9	25	--	9	15.0±3.8
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* = $100 - (\text{Experimental Activity} / \text{Control Activity} \times 100)$.

a = Stimulation noted - no depression.

-- = No recovery of muscle at 5 minutes post washout.

ID 50 = $3.28 \times 10^{-5} M$.

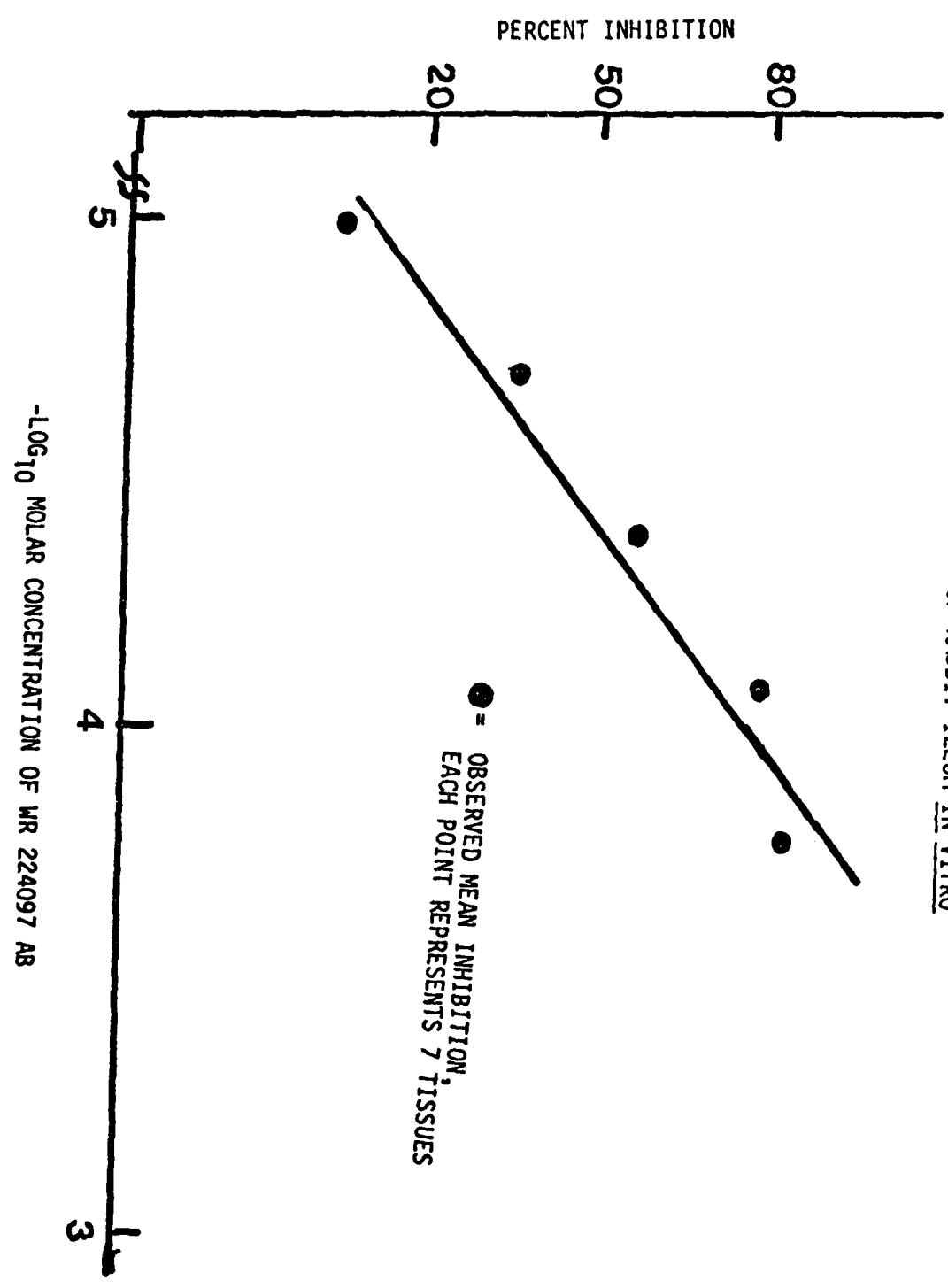
95% CL = $3.04 - 3.52 \times 10^{-5} M$.

Regression Line: $Y = 4.07 + 0.56X$. (Probit of response vs coded doses 0-4).

ANOVA P = <0.01

Regression Coefficient = 0.770.

FIGURE 1
INHIBITORY EFFECT OF WR 224097 AB ON THE SPONTANEOUS
ACTIVITY OF RABBIT ILEUM IN VITRO





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THE INHIBITORY EFFECT OF COMPOUND WR 232956 ON SPONTANEOUS
ACTIVITY OF THE RABBIT ILEUM IN VITRO

ROY L. MUNDY, Ph.D.

CONTRACT NUMBER

DADA-17-67-C-7136

INTERIM REPORT 5A

AN AFFIRMATIVE ACTION / EQUAL OPPORTUNITY EMPLOYER

ABSTRACT

Compound WR 232956 has been tested at $2.5, 5.0 \times 10^{-6}$ and 1.0, 2.0 and 4.0×10^{-5} molar concentrations on six ileal preparations of the rabbit. The compound stimulates the ileum at low concentrations and then causes a dose dependent depression of spontaneous activity of the rabbit intestine at higher dose levels. The concentration which depressed spontaneous activity to fifty percent of that in control preparations (ID 50) was 1.42×10^{-5} M and the 95 percent confidence limits of the analysis was $1.40 - 1.45 \times 10^{-5}$ M. The data fitted a straight line when the responses were transformed to probits and plotted versus the coded dose of compound (arbitrarily 0-4) and yielded a correlation of 0.803. Analysis of variance for the regression showed that the regression was significant at the $P = < 0.01$ level.

Compound WR 232956 was difficult to place in solution at the requisite concentration to permit attainment of the final bath concentrations reported here. From 30 to 45 minutes of stirring was needed to produce a solution, however, the Compound appeared to be in a "true" solution in each of the experiments we carried out.

"Recovery" of the activity of muscles treated with Compound WR 232956 reached approximately 50 percent that of controls within five minutes after it was washed from the bath. Two of six muscles exhibited excellent recovery (110 and 86 percent) while three of six attained less than 25 percent of control activity. One of six muscles became erratic after washout of the chemical and no activity record could be obtained.

INTRODUCTION

Compound WR 232956 was tested in the rabbit ileum preparation described in Interim Report 1A. There were no deviations from the protocol described in that report.

METHODS

As described in Interim Report 1A.

RESULTS

The response from the six experiments are tabulated in Table 1 and shown graphically in Figure 1.

DISCUSSION

Compound WR 232956 is a more depressant compound on the rabbit ileum than is primaquine. It also appears to have more stimulatory activity at lower doses than the parent 8-aminoquinoline.

The muscles did not recover from the depressant effects of this chemical with anywhere near the rapidity of recovery following washout of primaquine. The potency of this compound to stimulate and depress the rabbit intestine may mean that it will show more toxicity to the G.I. tract than the now available 8-aminoquinoline antimalarial, primaquine. That theory will have to await human testing.

TABLE 1

INHIBITORY EFFECT OF WR 232956 ON ACTIVITY OF RABBIT

ILEUM IN VITRO

LOG DOSE (Molar)	PERCENT INHIBITION*						$\bar{X} \pm S.E.M.$
	EXPERIMENT NUMBER						
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	
-5.6021	11	a	a	9	7	a	9.0 $\pm$ 1.2
-5.3010	9	11	3	24	20	a	13.4 $\pm$ 3.8
-4.9999	47	33	25	55	57	a	43.4 $\pm$ 6.2
-4.6990	63	53	48	69	78	12	53.8 $\pm$ 9.5
-4.3979	--	66	71	65	--	49	62.8 $\pm$ 4.8

PERCENT RECOVERY FIVE MINUTES AFTER REMOVAL OF WR 232956

WASHOUT	b	110	13	31	21	86	52.2 $\pm$ 19.3
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* = 100-(Experimental Activity/Control Activity X 100).

a = Stimulation noted - no depression.

-- = No injection made.

ID 50 = 1.42×10^{-5} M.

95% CL = 1.40 - 1.45×10^{-5} M.

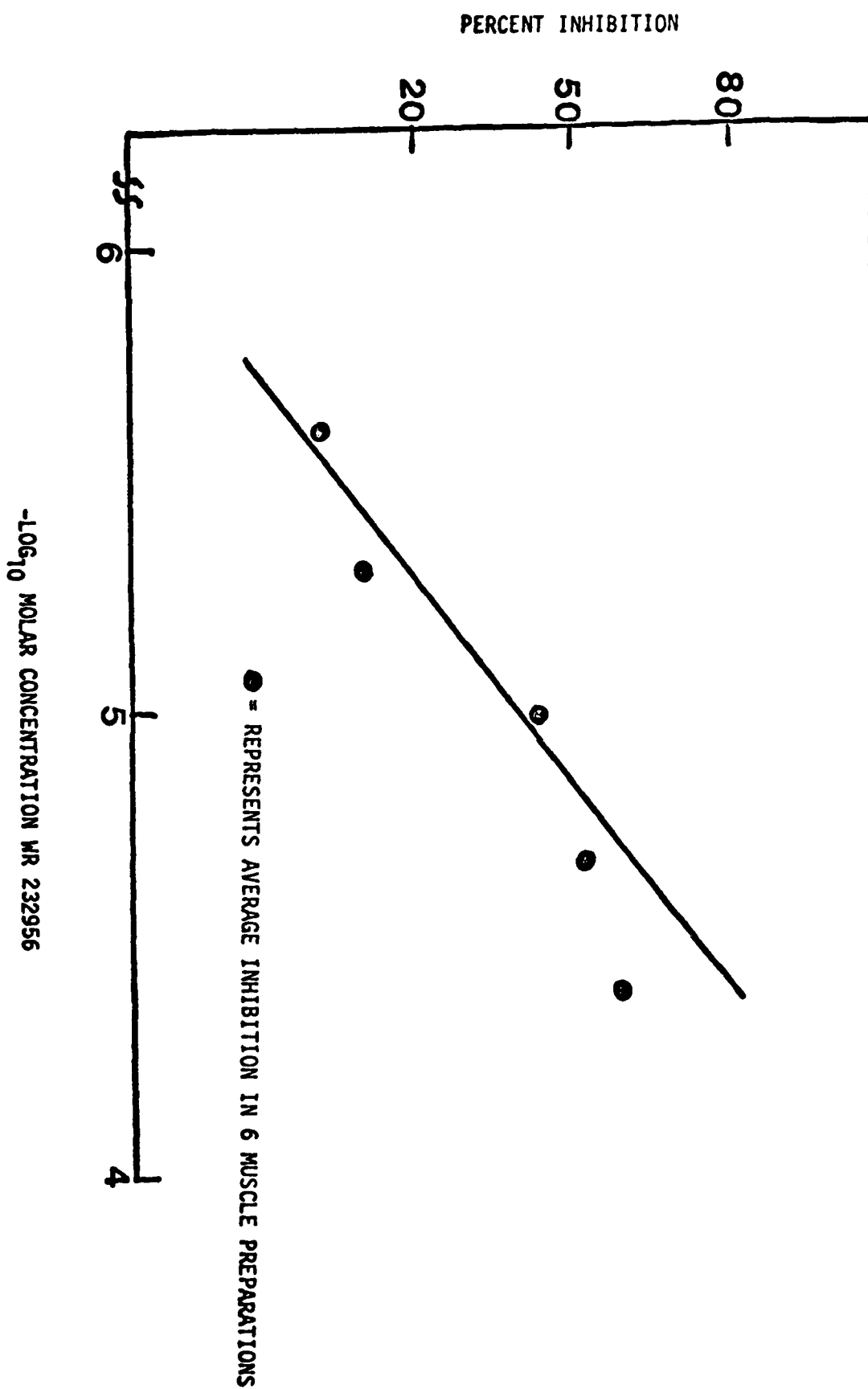
Formulation of the regression: $Y = 3.59 + 0.48X$. (Probits of response vs coded doses 0-4).

ANOVA P = < 0.01

Correlation Coefficient = 0.803

b = erratic behaviour of tissue did not allow recording.

FIGURE 1. INHIBITORY EFFECT OF COMPOUND WR 232956 ON THE SPONTANEOUS ACTIVITY OF THE RABBIT ILEUM IN VITRO





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the Medical Center / DEPARTMENT OF PHARMACOLOGY

THE INHIBITORY ACTION OF COMPOUND WR 212579 ON THE
SPONTANEOUS ACTIVITY OF THE RABBIT ILEUM IN VITRO

ROY L. MUNDY, Ph.D.

CONTRACT NUMBER

DADA-17-67-C-7136

INTERIM REPORT NUMBER 6A

AN AFFIRMATIVE ACTION / EQUAL OPPORTUNITY EMPLOYER

ABSTRACT

Compound WR 212579 was tested at $1.6, 3.2, 6.4 \times 10^{-5}$ and 1.3 and 2.6×10^{-4} M concentrations on six ileal preparations from the rabbit, in vitro. The compound showed a potent depressant activity on spontaneous activity in the preparations. The concentration required to reduce activity to 50 percent of control activity (ID 50) was 1.52×10^{-5} M. Because the dose range used was too high, it was not possible to calculate 95 percent confidence limits for the assay. When the percent inhibition values were transformed to probits and plotted against the doses employed (arbitrary dose units, 0-4) a formula was generated $Y = 4.71 + 0.68X$. Analysis of variance revealed that there was a positive regression $P = < 0.01$ and the correlation coefficient for the data was 0.835.

Although the muscle preparations were depressed over 95 percent in activity, when they were allowed to recover for 30 minutes after washout of the WR 212579, five of the six regained, on average, some 80 percent of original activity.

These experiments were not completely satisfactory in that the calculated ID 50 fell below the lowest dose of compound injected. This shortcoming could have been resolved by reducing the beginning concentration of the agent to 4 and 8×10^{-6} molar.

In summary, Compound WR 212579 is a potent, slowly reversible depressant of spontaneous activity in the rabbit ileum in vitro.

INTRODUCTION

Compound WR 212579 was tested for depression of spontaneous activity of the rabbit ileum in vitro by the procedure detailed in Interim Report 1A. Doses of 1.6, 3.2, 6.4 X 10⁻⁵ and 1.3 and 2.6 X 10⁻⁴ M were injected into six muscle preparations.

METHODS

One significant change in the protocol listed in Interim Report 1A was made. The muscles were observed for 30 minutes following washout of the compound. In all other experiments the recovery values given are for recovery, or lack of it, at 5 minutes.

RESULTS

Results are listed for each experiment in Table 1 and shown graphically in Figure 1. Please note that the recovery values are given for 30 minutes after washout of the chemical.

DISCUSSION

Compound WR 212579 is very soluble in water and gave no problem in the preparation of effective concentrations. The Compound appears to be at least as potent as primaquine as a depressant of spontaneous activity of the preparations although a quantitative statement of relative potency cannot be made because of poor selection of test doses in our experiments. It can be seen that WR 212579 is a potent depressant which is slowly reversible.

TABLE 1

INHIBITORY EFFECT OF WR 212579 ON ACTIVITY OF RABBIT ILEUM IN VITRO

LOG DOSE (Molar)	PERCENT INHIBITION*						$\bar{X} \pm \text{S.E.M.}$
	EXPERIMENT NUMBER						
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	
-4.7959	48	49	49	49	13	41	41.5 $\pm$ 5.8
-4.4949	75	76	67	60	51	49	63.0 $\pm$ 4.0
-4.1938	90	86	83	82	75	71	81.2 $\pm$ 2.9
-3.8861	91	100	98	99	94	91	95.5 $\pm$ 1.6
-3.5850	95	--	--	--	--	--	-- --

PERCENT RECOVERY THIRTY MINUTES AFTER WASHOUT OF WR 212579

WASHOUT	109	68	65	69	95	a	81.2 $\pm$ 8.8
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* = 100-(Experimental Activity/Control Activity X 100)

a = not determined, technical difficulty

-- = not injected, depression already severe.

ID 50 = 1.52×10^{-5} M

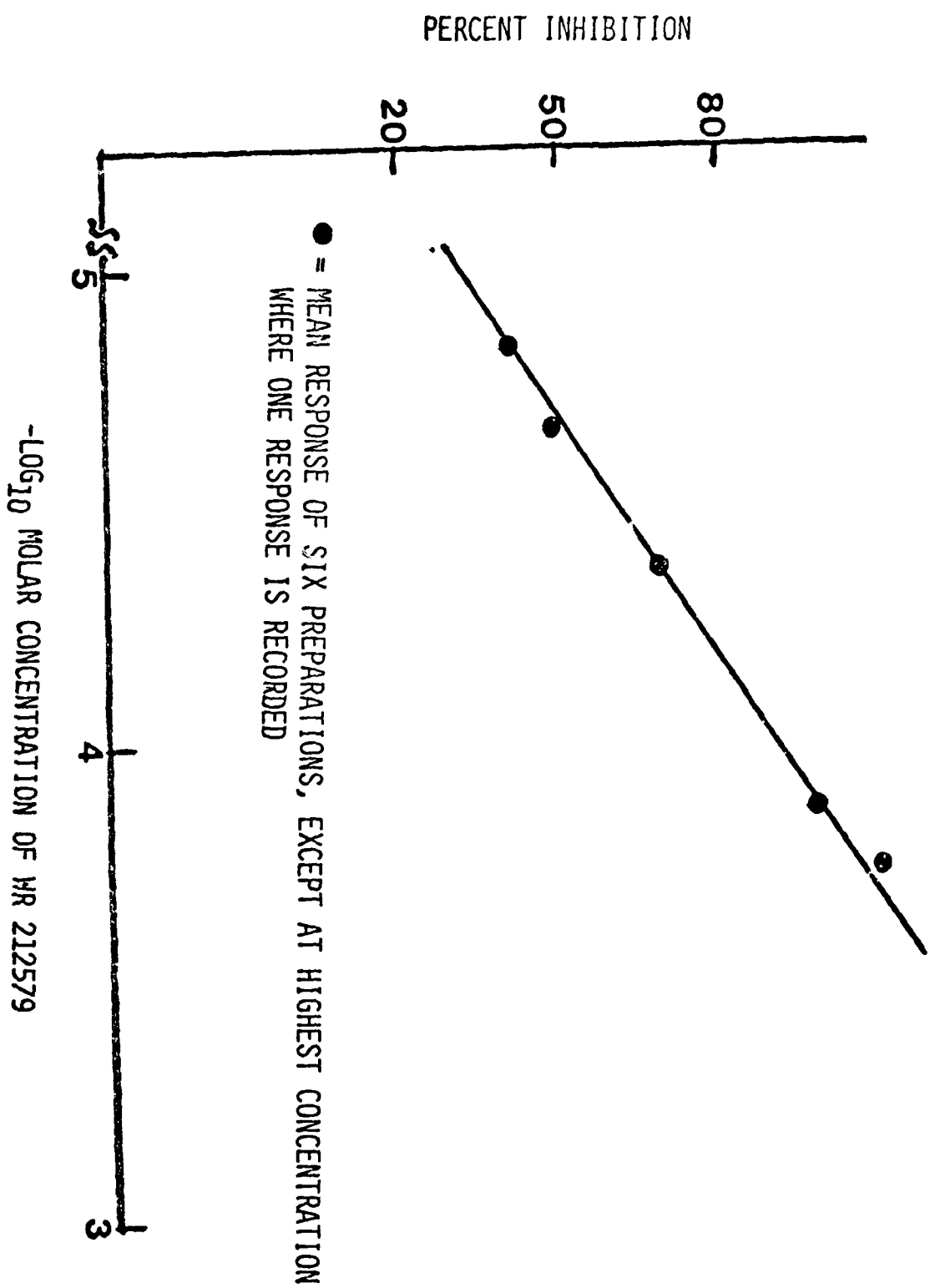
95% CL = not determined

Regression Equation: $Y = 4.71 + 0.68X$. (Probit of response vs coded doses 0-4)

ANOVA P = < 0.01

Regression Coefficient = 0.835

FIGURE 1. INHIBITORY EFFECT OF COMPOUND WR 212579 ON THE SPONTANEOUS ACTIVITY OF THE RABBIT ILEUM IN VITRO





the University of Alabama in Birmingham / UNIVERSITY STATION / BIRMINGHAM, ALABAMA 35294

the Medical Center / DEPARTMENT OF PHARMACOLOGY

THE INHIBITORY ACTION OF COMPOUND WR 221527 ON THE
SPONTANEOUS ACTIVITY OF THE RABBIT ILEUM IN VITRO

ROY L. MUNDY, Ph.D.

CONTRACT NUMBER

DADA-17-67-C-7136

INTERIM REPORT NUMBER 7A

ABSTRACT

Compound WR 221527 was injected cumulatively into six rabbit ileal preparations at concentrations of 1.6, 3.2, 6.4×10^{-5} and 1.3 and 2.6×10^{-4} molar. The compound strongly depressed spontaneous activity of the preparations and one injection at the 1.3×10^{-5} molar concentration and three of the injections at the 2.6×10^{-4} concentration were not made because of strong depression of activity in the muscles at lower concentrations.

The muscles were observed for 30 minutes after the chemical was washed from the muscle bath and regained, on average, 90 percent of their control activity. While the recovery of the muscles was not as rapid as with the prototype compound primaquine, the depression was certainly reversible. It should be noted that the depression of activity was severe in these muscles as compared to the primaquine experiments (93.7% vs 84%) and that the longer recovery period may simply reflect a more complete saturation of some receptor or membrane locus.

The concentration needed to reduce the spontaneous activity to fifty percent of control activity (ID 50) was 2.9×10^{-5} M. When the responses were transformed to probits and plotted against arbitrary dose units (0-4) a formula was generated, $Y = 4.51 + 0.54X$. Analysis of variance showed that the regression differed from zero at $P < 0.01$. The correlation coefficient of the regression was 0.811.

The compound is highly soluble in water and there was no problem in making concentrations of sufficient strength to reach the desired final molar concentrations in the bath.

INTRODUCTION

Compound WR 221527 was tested for its depressant activity on the isolated ileum by the test system detailed in Interim Report 1A. The Compound was freely soluble and the tests did not differ significantly from those described in Interim Report 1A except that a longer observation period was used to study recovery of the muscles.

METHODS

As described in Interim Report 1A. The muscles were allowed to recover in the bath for 30 minutes after WR 122527 was washed from the preparation.

DISCUSSION

Compound WR 122527 is a potent, reversible depressant of spontaneous activity in the rabbit ileum in vitro. The potency of this compound would appear to be approximately the same as for primaquine, the prototype 8-aminoquinoline. It is obvious from inspection of the data in Table 1 that the doses of WR 221527 used in these studies were too high. Beginning doses of 3.2 and 6.4×10^{-6} molar would have given a better dose-response.

TABLE 1

INHIBITORY EFFECT OF WR 221527 ON ACTIVITY OF RABBIT ILEUM IN VITRO

LOG DOSE (Molar)	PERCENT INHIBITION*						$\bar{X} \pm \text{S.E.M.}$
	EXPERIMENT NUMBER						
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	
-4.7959	64	4	22	29	47	22	31.3 $\pm$ 8.6
-4.4949	81	71	37	43	57	46	55.8 $\pm$ 7.1
-4.1938	94	86	54	58	58	72	70.4 $\pm$ 6.8
-3.8861	98	--	78	87	74	91	85.6 $\pm$ 4.3
-3.6383	--	--	96	95	90	--	93.7 $\pm$ 1.9

RECOVERY AT 30 MINUTES AFTER WR 221527 REMOVED FROM BATH

WASHOUT	25	143	68	109	122	74	90.2 $\pm$ 17.5
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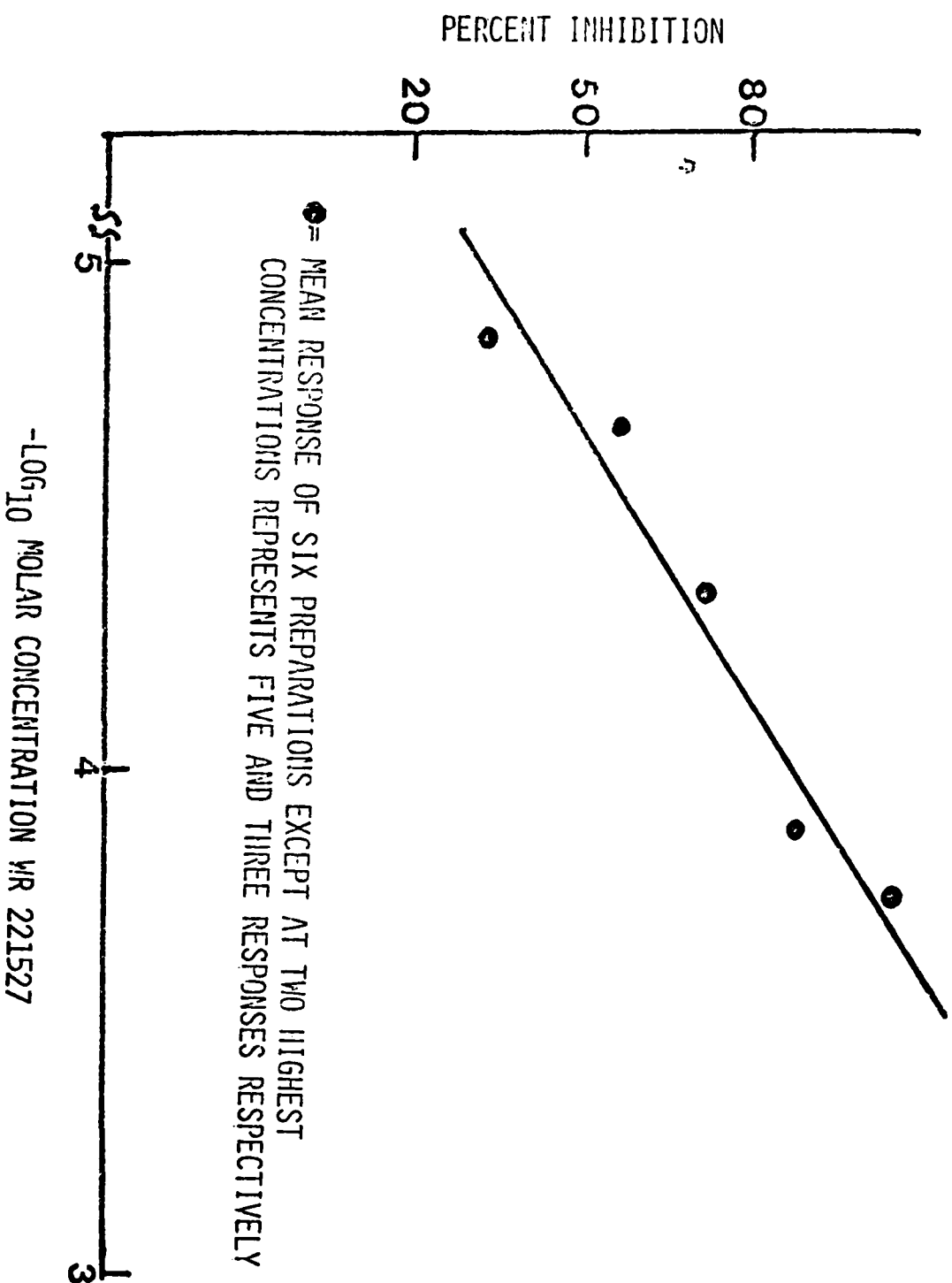
* = 100-(Experimental Activity/Control Activity X 100).

-- = Dose not injected, muscle already seriously depressed.

ID 50 = 2.9×10^{-5} M.95% CL = $2.1 - 3.7 \times 10^{-5}$ M.Regression: $Y = 4.51 + 0.54X$. (probit of response vs coded doses 0-4).ANOVA $P = < 0.01$

Regression Coefficient = 0.811

FIGURE 1. INHIBITORY ACTION OF COMPOUND WR 221527 ON THE SPONTANEOUS ACTIVITY OF THE RABBIT ILEUM IN VITRO



THE INHIBITORY ACTION OF COMPOUND WR 6021 (ISOPENTAQUINE)
ON THE SPONTANEOUS ACTIVITY OF THE RABBIT ILEUM IN VITRO

ROY L. MUNDY, Ph.D.

CONTRACT NUMBER

DADA-17-67-C-7136

INTERIM REPORT NUMBER 8A

ABSTRACT

Compound WR 6021 (Isopentaquine) was tested at cumulative doses of 1.0, 2.0, 4.0, 8.0×10^{-5} and 1.6×10^{-4} molar on eight (8) preparations of rabbit ileum as described in Interim Report 1A.

The concentration required to inhibit spontaneous activity of the ileum by fifty percent (ID_{50}) was 2.57×10^{-5} M. The ninety-five percent confidence limits for the determination were 2.16 to 2.97×10^{-5} M. When the data was transformed to probits of percent inhibition and regressed against the arbitrary dose designations zero to four, the regression was represented by the equation $Y = 4.33 + 0.52X$. The correlation coefficient for the regression was 0.681. The regression differed from zero, ANOVA $P = < 0.01$.

When the compound was washed out of the preparation and activity measured either at 5 minutes (N-6) or 10 minutes (N-2) following washout the muscles has regained, on average, 64 percent of control activity.

Compound WR 6021 is very soluble and presented no problem in the preparation of samples of sufficient concentration to permit the injection of the concentrations used in the experiments.

INTRODUCTION

Compound WR 6021 (Isopentaquine) was tested on 8 isolated rabbit ileal preparations as described in Interim Report 1A.

METHODS

Methods of procedure were as described in Interim Report 1A except that recovery of the muscle following washout of the chemical was followed for 10 minutes rather than for five minutes as per the protocol in two of eight experiments. These experiments are designated Numbers 6 and 7 in Table 1.

RESULTS

The results of the individual experiments are tabulated in Table 1 and shown diagrammatically in Figure 1.

DISCUSSION

Isopentaquine depresses the spontaneous activity of the isolated rabbit ileum. Its potency in this respect is no different from that of primaquine the prototype 8-aminoquinoline used in these studies. The muscles showed considerable recovery from the depressant effect of isopentaquine when it was washed from the bath.

TABLE 1

INHIBITORY EFFECT OF COMPOUND WR 6021 ON THE SPONTANEOUS
ACTIVITY OF THE RABBIT ILEUM IN VITRO

LOG DOSE (Molar)	PERCENT INHIBITION*								X <u>+</u> S.E.M.
	EXPERIMENT NUMBER								
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	
-4.9999	51	35	16	33	39	39	50	47	38.8 <u>+</u> 4.0
-4.6990	59	42	38	33	41	49	50	37	43.7 <u>+</u> 3.0
-4.3979	67	45	29	33	41	52	60	49	47.0 <u>+</u> 4.5
-4.0969	78	49	57	43	53	75	80	77	64.0 <u>+</u> 5.3
-3.7959	91	68	86	76	100	100	94	100	89.4 <u>+</u> 4.2

PERCENT RECOVERY FIVE MINUTES AFTER REMOVAL OF WR 6021
(Experiments 6 and 7 recovery at 10 minutes)

WASHOUT	44	39	66	86	69	43	65	100	64.0 $\pm$ 7.6
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* = 100-(Experimental Activity/Control Activity X 100).

ID 50 = 2.57×10^{-5} M.

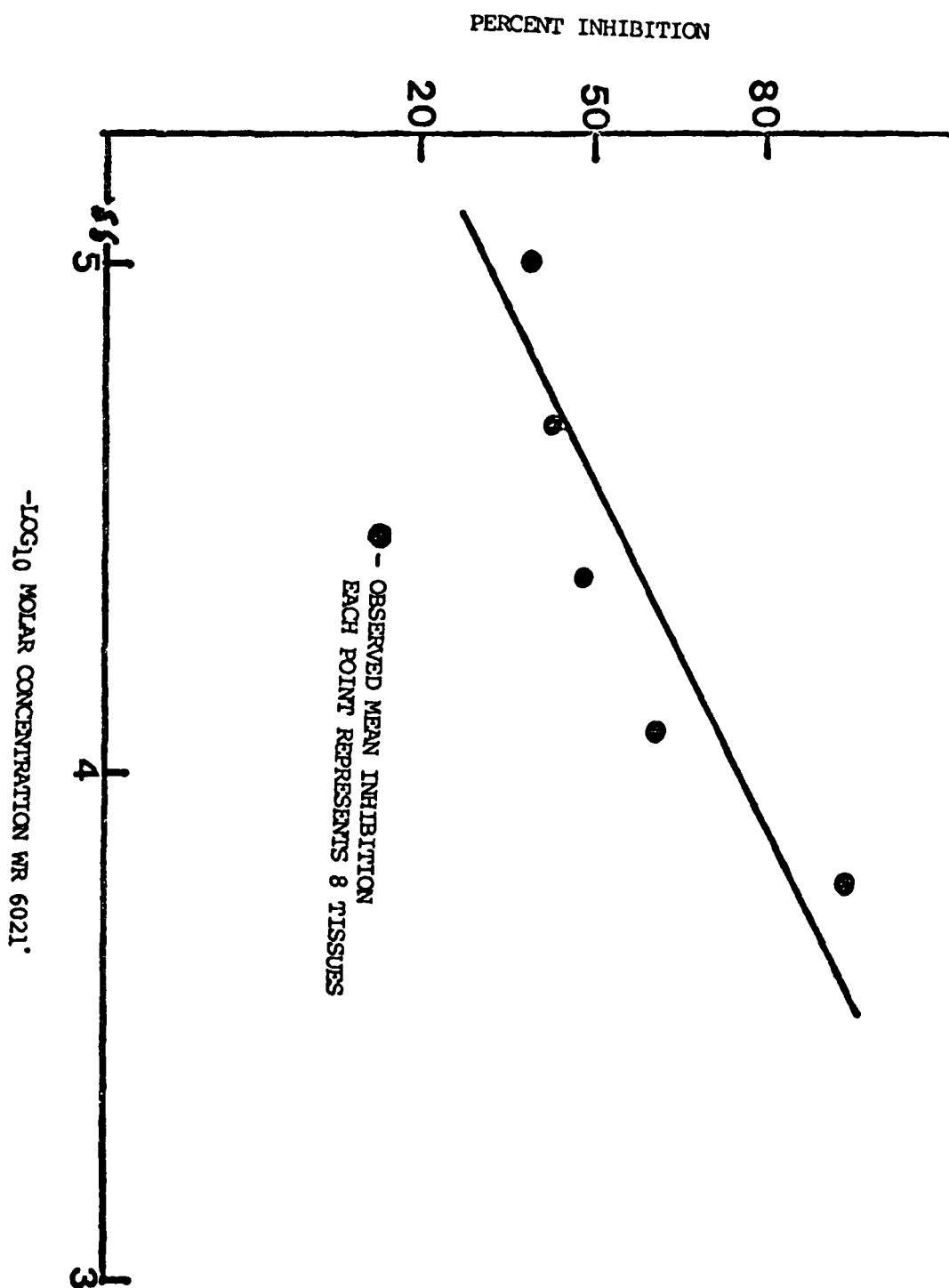
95% CL = $2.16-2.97 \times 10^{-5}$ M.

Regression: $Y = 4.33 + 0.52X$. (Probit of response vs coded doses 0-4).

ANOVA $P = < 0.01$.

Regression Coefficient = 0.681.

FIGURE 1. INHIBITORY EFFECT OF COMPOUND WR 6021 ON THE SPONTANEOUS ACTIVITY OF THE RABBIT ILEUM IN VITRO





The University of Alabama in Birmingham
Department of Pharmacology
205 934 4517

August 14, 1980

INFORMATION ON MISCELLANEOUS WR COMPOUNDS SUBMITTED FOR
STUDY ON THE SPONTANEOUS ACTIVITY OF THE RABBIT ILEUM IN VITRO.

ROY L. MUNDY, Ph.D.

CONTRACT NUMBER

DADA-17-67-C-7136

INTERIM REPORT NUMBER 9A

University Station / Birmingham, Alabama 35294
An Affirmative Action / Equal Opportunity Employer

ABSTRACT

Compound WR 215296 was very poorly soluble in deionized water and would not allow the preparation of a solution of the requisite concentration to attain adequate muscle bath concentrations for testing.

Compound WR 4234 (Pamaquine) was also of insufficient solubility to allow testing in the protocol described in Interim Report 1A.

Compound WR 233195 was supplied in a very small amount (milligrams) and there was insufficient chemical to allow testing.

Ricinoleic Acid (No Known WR Number) is known to inhibit the tone of intestinal muscle in the dog and to inhibit water absorption from the gut(1). Since this action has been advanced for the mechanism of gastrointestinal action of ricinoleic acid we have tested this compound in the system described in Interim Report 1A and 4.8 and 7.2×10^{-5} and 1.0 and 1.6×10^{-4} M in five isolated rabbit ileum preparations. The ED_{50} was 1.0×10^{-4} M and the 95% confidence limits of the estimate were 8.7×10^{-5} to 1.1×10^{-4} M. This compound is significantly less potent than the 8-aminoquinolines in its ability to depress the spontaneous activity of the rabbit ileum in vitro.

REFERENCE:

1. Steward, J.J., Gaginella, T.S., Olsen, W.A. and Bass, P., "Inhibitory Actions of Laxatives on Motility and Water and Electrolyte Transport in the Gastrointestinal Tract," J. Pharmacol. Exptl. Therap. 192: 458-467, 1975.

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