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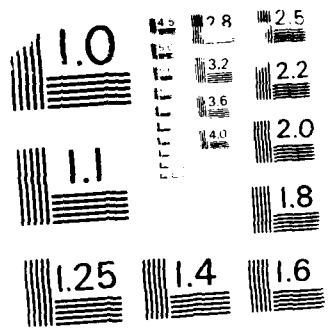
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Development of Microcomputer Methods for  
Analysis and Simulation of Clinical  
Pharmacokinetic Data Relevant to New  
Drug Development

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Annual Report

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Robert Wagoner, B.S.

January 16, 1984

Supported by  
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Duke University  
Division of Clinical Pharmacology  
Durham, North Carolina 27710

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| 20. ABSTRACT (Continue on reverse side if necessary and identify by block number)<br><br>The research proposed under this contract is a feasibility study in the development of applications of new microcomputer graphics technology to the analysis and interpretation of clinical pharmacological data. This involves continuing development of comprehensive programs for analysis, interpretation, and simulation of pharmacokinetic data, dose-response kinetic data, and other data relevant to new drug development, for use with the Tektronix 4052 Microcomputer Graphics System. The combination of such modern analytical and |                       |  |

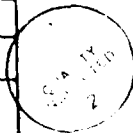
illustrative methods in clinical pharmacology, based on new high-speed micro-computers and associated graphics, are thought to greatly reduce both cost and time involved in the overall process of clinical evaluation of new drugs in the U.S. Army Drug Development Program.

The work performed during the past twelve months of the contract includes the following:

1. Continuing development of a non-linear pharmacokinetic data fitting program to analyze drug data where non-linear processes are responsible for some of the drug disposition pathways.
2. Development of a pharmacokinetic and pharmacodynamic data simulation program to simulate both time courses of drug concentrations and pharmacologic effect.
3. Continuing development of a statistical program package applicable to problems in clinical pharmacology, involving both parametric and non-parametric tests.
4. Development of a pharmacokinetic simulation program based on differential equations, intended to simulate time courses of concentrations of drugs exhibiting non-linear kinetics.
5. Initial development of a quantal (probit) dose-response analysis program.
6. Special analysis of data from WRAIR, one on a comparative bioavailability study of mefloquine, the other on methemoglobinemia induced by primaquine and related compounds.

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## SUMMARY

The research proposed under this contract is a feasibility study in the development of applications of new microcomputer graphics technology to the analysis and interpretation of clinical pharmacological data. This involves continuing development of comprehensive programs for analysis, interpretation, and simulation of pharmacokinetic data, dose-response kinetic data, and other data relevant to new drug development, for use with the Tektronix 4052 Microcomputer Graphics System. The combination of such modern analytical and illustrative methods in clinical pharmacology, based on new high-speed microcomputers and associated graphics, are thought to greatly reduce both cost and time involved in the overall process of clinical evaluation of new drugs in the U.S. Army Drug Development Program.

The work performed during the past twelve months of the contract includes the following:

1. Further development of a non-linear pharmacokinetic data fitting package to analyze drug data in systems where some non-linear processes are responsible for transport or metabolism of the drug (e.g. Michaelis-Menten kinetics). This is a continuation of work begun last year. Some of the causes for the disappointing slowness of the original programs have been identified, as well as some limits on the degree of improvement which is achievable.

2. Creation of a pharmacokinetic and pharmacodynamic data simulation program which enables the user to simulate the time course of drug concentration and effect. This program extends the capabilities of our previously developed pharmacokinetic simulator to allow the examination of effect vs time relationships for various drug dosing regimens. The user can now have graphs of the predicted concentration and/or effect data resulting from any type of dosing regimen, with the same ease of use and flexibility available from the original pharmacokinetic simulation program.

3. Additional work on statistical programs for clinical pharmacology. One of our main efforts in the statistical package was the implementation of several multiple-comparison tests. These tests are particularly useful in simultaneous comparative studies of several drugs, allowing comparison of all possible sets of competing treatments in order to quickly isolate the most promising for further study. This enables more efficient use of time and money in testing new experimental courses of treatment, reducing the development cost of new drugs.

4. Development of a pharmacokinetic simulation program based on differential equations. This program is intended to be used as a companion to the non-linear pharmacokinetic data analysis package, allowing the prediction of time courses of drug concentrations due to various dosing regimens in systems characterized by rate-limited processes of drug transport and/or metabolism. It provides functions similar to those available in our linear pharmacokinetic simulator, which was previously developed.

5. Work on a probit (quantal) dose-response analysis program. This type of pharmacodynamic analysis is commonly used in studies involving responses of a discrete, categorical nature, rather than the continuous effect data which is dealt with by our previous dose-response analysis program. The two techniques can overlap somewhat if the discrete responses are converted to percentages of some total available response. If this is done, the two programs produce very similar results for the ED-50, or median effective dose, but the bounds placed on this estimate are sometimes quite different. This discrepancy between the two methods is a matter we intend to examine further during the coming year.

6. Special analysis of data from the Walter Reed Army Institute of Research. Two requests were received for assistance with data analysis in connection with drug development experiments being performed at WRAIR. One was from LTC Charles Pamplin, asking that we analyze the results from a comparative bioavailability study of two formulations of mefloquine which was performed on 12 normal subjects using a cross-over experimental design. This experiment was complicated by the fact that one of the subjects was mistakenly given the two treatments in the wrong order, resulting in an unbalanced study. We have managed to successfully overcome this difficulty in the analysis, and are currently preparing a final report on the results.

The other special request came from CPT John Anders, who has performed experiments on the degree of methemoglobinemia in dogs given primaquine and several new antimalarial compounds under development. One of his questions involved the pooling of results from the primaquine-treated groups in two different experiments, in order to obtain a larger sample and thus greater statistical power. The analysis of his data also involved the use of some of the multiple comparison methods we have developed this year, which are discussed in this report.

## BACKGROUND

The process of drug development has been both complicated and facilitated by the trend toward early application of the methods of clinical pharmacokinetics. Current practice and existing and proposed new Food and Drug Administration Regulations demand sophisticated evaluation of drug bioavailability and descriptive pharmacokinetics in Phase I clinical studies. This requires development of assay methods for new drugs and their metabolites and methods for evaluation of concentration vs time data to obtain relevant parameters to characterize drug behavior. Of similar importance is knowledge of characteristics of relationships between dose or concentrations and pharmacological response. The information thus obtained from pharmacokinetic and pharmacodynamic studies relates directly to the optimal design of dosing schedules of new drugs in man, including individualization of therapy due to disease processes or other factors which may affect drug behavior. In this regard the eventual course of development through Phase II and Phase III clinical studies is rendered less empirical and ultimately more efficient in both time and cost, by minimizing the use of scarce resources.

Modern computer technology has greatly enhanced the capability of these methods and made possible their applications to clinical pharmacokinetics. A number of available computer programs have been frequently employed for this purpose. Those which have been developed for use specifically in clinical pharmacokinetics are based on compartmental methods of analysis and yield estimates of parameters associated with preselected compartmental models. While useful and informative, they lack the ease of use and cognitive appeal of direct graphic simulations and graphics-assisted data analysis. It is in the interest of developing general purpose programs with the advantages of computer graphics that the present contract was initially pursued.

Previous experience with the Tektronix 4051 and 4052 Microcomputer Graphics Systems indicated that this was an especially suitable microcomputer system for our purposes. While similar systems are now available from a variety of sources, it is in the interest of conformity with existing systems in the U.S. Army Drug Development Program at the Walter Reed Army Institute of Research that the Tektronix System has been employed. The present Tektronix 4052 System in our laboratory and for which the programs to be described were developed is identical to that now in use at Walter Reed. A data communications interface has been installed to facilitate direct transmission of programs and data between these two facilities.

## PROGRESS

### A. Non-linear Pharmacokinetic Data Analysis Package Using Differential Equations.

This program package, on which work was begun last year, is intended to be used for fitting data to a model involving some non-linear kinetic processes. Such systems cannot be analyzed by model-independent methods such as our linear pharmacokinetics program package, developed earlier under this contract. Because it is important to be able to characterize these data in cases where a new drug shows evidence of some rate-limited or time-dependent kinetic behavior, this package has been designed to allow the fitting of a model described by a system of differential equations, which need not be restricted to the linear, first-order models used for simpler pharmacokinetic analysis. In developing this package, we have tried to retain many of the "user-friendly" aspects of our other pharmacokinetics package, including extensive graphic interaction for verification of data values and initial estimates of the model parameters. The detailed statistical analysis of the results of the fitting procedure has also been retained, although not as much information can be derived by the program with regard to clearance terms, volumes of distribution, or half-times, since the program has no knowledge of the meaning of the model parameters other than time and concentration in the sampling compartment.

The principal reason for our dissatisfaction with the program last year was the fact that complete analysis of one set of data took much longer than a similar problem would using the linear pharmacokinetic analysis package. A number of possible solutions for this difficulty were tried, specifically including testing several different algorithms for the numerical integration of the system of differential equations which describes the model. These attempts were only somewhat successful, for reasons which became apparent after trying many of the different methods. One of the main difficulties in numerically solving a differential equation system is the choice of an appropriate step-size to use in moving from one observed data point to the next. Sophisticated methods exist for adjusting the step-size so as to maintain the desired level of accuracy without taking more steps, and time, than necessary. However, due to the unequal spacing frequently found between successive observations in pharmacological studies, automatic step-size adjustment becomes impractical, because the solution must be restarted for each new interval, and the resulting increases in overhead and bookkeeping in the program waste most of the time saved by using the more elaborate algorithm. A further cause for the slowness of the program is the fact that the numerical integration of the model with respect to time must be followed by a stage of numerical differentiation with respect to all the other parameters. This is in marked contrast to the linear pharmacokinetics program, which uses analytic derivatives which have been written

into the code. We feel that this may be the most time-consuming step in the solution process, and that further attempts to reduce the running time would not be productive enough to justify the effort. Therefore, to simplify the program, the complex predictor-corrector method which was being used has been replaced by a fourth-order Runge-Kutta integration technique, along with a simple means of determining step size. These changes reduce the size of the program significantly, and should also make it easier to maintain and modify in the future.

#### B. Pharmacokinetic and Pharmacodynamic Simulation Program.

This program is an extension of our earlier pharmacokinetic simulator to add the capability of predicting the time course of effect of a drug, based on the predicted concentration and on a concentration-effect relation like the one used in our program for pharmacodynamic analysis. The resulting program allows the user to request that the predicted effect be plotted against time alone, or with the predicted concentrations as well. If concentrations are to be graphed, they may be shown on linear or logarithmic scales, as in the original program. The effect axis is always linear and will be automatically scaled by the program, but the user may override the choice of endpoints and tic intervals if desired, another feature which has been retained from the earlier program. A new capability with this version, which is most useful when drawing graphs using the Tektronix plotter, is the pause feature, which stops the pen after drawing the axes and labels, and again after the concentration curve and before the effect curve, allowing the changing of pen colors for different sections of the plot. In addition to these changes, some other improvements have been added to reduce the drawing time of the program, resulting in a more uniform line width on the paper, which is less likely to smear. This program should prove most useful in designing drug dosage regimens on the basis of some desired range of effectiveness, such as to maintain a minimum effective level, or to avoid a level of effect which is associated with negative drug reactions.

#### C. Statistical Program Package for Clinical Pharmacology.

Most of the development of the statistical program package this year has been concentrated on the problem of performing multiple comparisons among several groups of data without inflating the apparent significance of the results. This problem can occur frequently in pharmacology research in situations where a pilot experiment is performed on a number of new drugs or methods of treatment in order to determine which is the most promising for further investigation. A similar situation arises when an established standard compound or formulation is to be compared against several new ones, to see if there are differences in quality. The difficulty with this type of comparison is that the various subsets of the data that are to be contrasted are not generally independent, and this

can lead to stated probability levels which are much more significant than the actual test result ("false positives"). The techniques used for avoiding this problem all involve making the individual comparisons at more stringent probability levels, so that a statement can be made about all the tests simultaneously at an overall significance level of 0.05 or 0.01 or whatever level is desired. Procedures for doing this have now been added to our non-parametric analysis of variance program, the Kruskal-Wallis test for one-way classifications, and the Friedman test for data in a two-way layout. Additionally, we have written a program for the parametric one-way analysis of variance with multiple comparisons, using the Bonferroni method for adjusting the significance levels. Two other tests are being investigated for the parametric analysis of variance program: Dunnett's test for comparing all other treatments vs a control or standard; and Duncan's for all possible comparisons. Because these methods currently require the use of tabular values in order to apply the tests, we are hoping to find a way to have the necessary critical values computed within the program, making it more useful. One other development for the statistical program package has been the beginning of a method for analyzing data from unbalanced cross-over design studies. This problem was brought to our attention in connection with the analysis of mefloquine bioavailability data requested by LTC Charles Pamplin. Because a cross-over design may easily be upset by accident, or by uncooperative subjects, the ability to analyze data resulting from an unbalanced experiment is most important in order to learn as much as possible from the completed sections of the study. We intend to do further development work on this program in the coming year.

#### D. Non-linear Pharmacokinetic Simulation Program Using Differential Equations.

This program complements the capabilities of the non-linear pharmacokinetic data fitting package by allowing the prediction of concentration vs time curves for the same kinds of kinetic models defined in terms of sets of differential equations. In this, it may be even more valuable than our linear kinetic simulator in helping people to visualize the effects of changes in dosage regimens, since the non-linear models are more difficult for most people to grasp intuitively. As much as possible, we have tried to make this program as easy to use as the corresponding linear one, with the same options for such features as titles, axis ranges, and log/linear scaling. In addition, this program also has the pre-computation of the entire time-course before drawing the graph in order to reduce the drawing time and produce a more uniform looking graph. The only significant feature not yet available in the non-linear simulation program is the ability to make effect predictions based on the sample compartment concentration. This section has been left out of the current version of the program not because of any problem with implementation, but instead because we do not know of any good source of data concerning concentration-effect relations in non-linear kinetic models. Without such a source, this portion of the program cannot be properly tested.

E. Quantal Dose-Response Analysis Program using Probits.

The purpose of this program is to allow the analysis of dose-response data in situations where the measured response is discrete or categorical, rather than the continuously variable responses dealt with by our earlier pharmacodynamic analysis program. Categorical data can arise in many different circumstances, including experiments where the response can only be roughly measured and those in which the response is an event which has an all-or-nothing character, such as death in an LD-50 study. The method used for analyzing such data involves converting the percentage responding at each dose level into probability units, or probits, and then performing a linear regression of probit response vs log dosage. This has the effect of fitting a cumulative normal probability curve to the dose-response data. Because of the nature of this curve, the initial fitting is not usually sufficiently accurate to fully describe the data. To correct this, weights are assigned to each data point based on its position on the initial regression line, and a new weighted regression is computed. This process is then repeated, with new weights drawn from the new regression line. This cycle is repeated until the values at two successive stages of the iteration are judged sufficiently close. The program runs fairly quickly, with convergence to a final answer typically occurring after four to eight cycles of this re-weighted regression. The resulting line corresponds to a normal probability curve, with the mean representing the ED-50 and the standard deviation giving a measure of the sensitivity of the effect to changes in the dose. During comparison of this program to the logistic pharmacodynamic data analysis program, we obtained very consistently comparable estimates of the ED-50.

F. Special Data Analysis Requests from Walter Reed.

Two major analysis projects were performed this year at the request of researchers from the Walter Reed Army Institute of Research. One, requested by LTC Charles Pamplin, involved the treatment of data from a cross-over design comparative bioavailability study of two tablet formulations of mefloquine. This was somewhat similar to an earlier analysis of data which we performed in the previous contract year. The second analysis project, for CPT John Anders, was an examination of measured methemoglobinemia in dogs, caused by dosing with primaquine or with new experimental antimalarial compounds. Details on both of these analyses follow.

LTC Pamplin asked us to analyze the data from a study performed by Bio-Med, Inc. for WRAIR on the comparative bioavailability of two tablet formulations of mefloquine, lot E555 prepared by Lafayette Pharmacal and a second prepared by Hoffman-LaRoche. The study was intended to be a balanced cross-over experiment, with 12 normal subjects receiving both preparations, with half the subjects to be given the tablets in one order

and the other half in the reverse order. Since it was assumed that the volume of distribution and rate of elimination of mefloquine in a given individual would be independent of the formulation given, our analysis focused on possible differences in the rate of absorption and in the fraction of the total dose which was actually available. This analysis was complicated by two factors. First, due to the withdrawal of one of the original test subjects during the study, a replacement was added, but he was inadvertently given the formulations in the reverse order of the individual who left the study. This resulted in an unbalanced cross-over design, which complicated the statistical analysis. Second, due to the long half-life of mefloquine, a significant amount of residual drug from the first dose was still present in the subjects when the second formulation was administered. This fact complicated the pharmacokinetic analysis. Nevertheless, we were able to perform a detailed analysis of the data, as summarized below.

The data regarding dosing order, times of administration, body weight, measured concentrations during the study, etc. were transmitted through telephone lines from WRAIR to Duke, using the data communications interface of our Tektronix 4052. After receiving this set of data, we performed some extensive reformatting to enable the subsequent pharmacokinetic analysis steps to access all the data. Two principal steps were taken in the kinetic analysis of the concentration vs time data. First, the area under the curve was calculated using a combination of the trapezoidal rule and extrapolation beyond the last data point based on the terminal exponential curve. For each subject, this calculation was performed in such a way as to force the exponential slopes after the first and second doses to be identical. In addition, the area under the second dosing interval curve was corrected for the carry-over of the exponential tail from the first dosing interval. Second, a non-linear regression analysis was performed, fitting the concentration data to a model which allowed different absorption rates and fractional availabilities for the first and second doses, but kept the volume of distribution and elimination rate terms identical during both dosing intervals. The resulting collection of pharmacokinetic parameters was then statistically analyzed, using an analysis of variance model for an unbalanced cross-over design. The results of this analysis showed a consistent and statistically highly significant pattern of differences between the two formulations. The data conclusively showed that the Hoffman-LaRoche preparation was more rapidly and more completely absorbed. In addition, this project showed that useful data can be obtained from bioavailability studies in which there is some interference between successive stages, and from imperfect experimental designs. Preliminary results from this study have already been sent to WRAIR, and a final report is currently being prepared for submission.

CPT Anders requested our assistance in analyzing data from two experiments which dealt with methemoglobinemia in dogs caused by primaquine or by one of several new experimental antimalarials. In the first experiment, primaquine was compared with WR238605, WR225448, and WR242511. In the second, primaquine was compared with WR6026. In both studies, molar equivalent doses of the drugs were given. The objective of this analysis was to compare all the drugs to determine if any statistically significant differences in methemoglobinemic potential existed. Prior to making such a comparison, however, we had to verify that the primaquine data in the first experiment was comparable to that in the second, so that pooling of these two sets of data would be legitimate.

Data from a total of 19 dogs were presented for analysis. The desired comparison was to be on the basis of percent methemoglobinemia (Mhb) caused by administration of one of the antimalarial compounds in four daily doses. Because of the significantly non-zero baseline (pre-dose) values of methemoglobin in the dogs, corrections had to be made in order to compare only the effect of the drug. This was achieved by fitting each dog's Mhb vs time data to a model based on a one-compartment kinetic system with first-order input, modified to have a non-zero baseline as one of the model parameters. The results of this analysis were then treated with both parametric and non-parametric analysis of variance procedures with multiple comparison testing, as described earlier in this report. The parameters tested were baseline Mhb, rate of increase during dosing, rate of decrease after dosing, theoretical initial Mhb level, and total area under the curve above baseline. Preliminary comparison of the primaquine data from the first and second experiments showed no significant differences, so these two groups were pooled for all subsequent analyses. The results showed no pattern of differences between groups for baseline, nor for rate of Mhb increase or decrease. Statistically significant differences were found in the theoretical initial Mhb level and in the area under the curve, with WR242511 being significantly higher than any of the other groups. No other significant differences were detected by this procedure. In particular, the new compound which resulted in the lowest Mhb levels, WR6026, was not significantly higher than primaquine, which was lowest, under direct comparison. A detailed report on the results of this analysis has been submitted, and a copy is attached as an appendix to this report.

Technical Report

Statistical Comparison of Methemoglobin-Producing  
Potential of Five 8-Aminoquinoline Antimalarial Drugs

September 21, 1983

Robert Wagoner

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Duke University Medical Center  
Durham, NC 27710

## INTRODUCTION

CPT John Anders of the Walter Reed Army Institute of Research presented data from two experiments, involving a total of 19 subjects (male beagles), concerning the methemoglobinogenic potential of five different 8-aminoquinoline antimalarial drugs. The purpose of these experiments was to compare primaquine, WR2975, with four new compounds, WR238605, WR225448, WR242511, and WR6026, with respect to the degree and duration of drug-induced methemoglobinemia. The analysis of the data was complicated by the fact that there were significant levels of baseline methemoglobin which had to be taken into account in order to compare the different drugs. An additional concern dealt with the fact that control groups in each of the two experiments were given primaquine, but it was not known whether these two groups could be pooled together. These problems were solved, using nonlinear regression analysis to describe the production and elimination of methemoglobin in each dog, and using parametric and nonparametric statistical methods for comparing multiple groups. The results showed some very important differences among the drugs, and indicated that two of them, WR238605 and WR6026, would be the most promising for further investigation.

## METHODS

The initial problem faced in analyzing this data was the choice of an appropriate model to describe the time course of methemoglobin formation and elimination in the individual dogs. Because no information was available on the drug concentrations, it was not possible to perform a simultaneous pharmacokinetic and pharmacodynamic analysis to describe the time course of drug concentration and the concentration-effect relationship between drug concentration and methemoglobin level. This forced the choice of a simpler pharmacokinetic model to fit to the data. In addition, the model had to be modified to take into account the baseline levels of methemoglobin, which were quite high in some cases, and which varied widely among different animals. The model finally chosen was a single-compartment multiple-dose kinetic system, with first-order input and output, with an additional constant term to correct for baseline. The parameters of this model were baseline,  $C_0$ ,  $k_a$ , and  $k_{el}$ . The baseline term reflects the initial pre-dose level of methemoglobin, based on one week of baseline data, as well as the limiting terminal concentrations at the end of the experiment.  $C_0$  is usually interpreted as the theoretical concentration at time 0, if instantaneous drug absorption were to take place. In this case,  $C_0$  represents the initial percentage methemoglobinemia which would result if both drug absorption and drug-caused methemoglobin production took place instantly.  $k_a$  is normally a measure of drug absorption rate; in this model it probably reflects both the absorption rate of the drug and the rate of formation of methemoglobin.  $k_{el}$  is usually taken to represent the drug elimination rate; in this case it may be a measure of elimination of drug or methemoglobin, or a combination of both processes. Using this model, nonlinear regression analysis was performed on each individual animal's methemoglobin measurements. The resulting fitted curves were examined to see if the model adequately described the data. The results were surprisingly good, showing no pattern of deviations that would suggest a more complex model was needed. The parameters describing these individual curves were then tabulated and summarized to prepare for the statistical comparisons to be performed. An additional descriptive parameter was computed, the area under the curve (AUC). This is the integral of the level of methemoglobin due to drug across time, after correcting for the baseline values. AUC provides a measure of the total methemoglobinogenic impact of a particular drug, since increases in level or duration of effect will cause increases in AUC.

After computing the best-fitted curves for each set of data and collecting the resulting parameters, statistical testing was performed, using both parametric and nonparametric techniques. The primaquine control groups from the first and second experiments were compared and found to be well-matched, so these groups were pooled together for all subsequent comparisons against the other drugs. The data were classified into groups based on which drug had been given, and these groups were then

tested for any significant difference using the classical one-way analysis of variance and its nonparametric equivalent, the Kruskal-Wallis test. These procedures for detection of overall differences were augmented by multiple comparison procedures to allow pair-wise testing of groups in order to isolate the areas where important differences were found. These methods are designed to control the likelihood of a so-called Type I error, that is, concluding that a significant difference exists when the actual cause is random variation. In this study, the possibility of a Type II error, failure to detect a real difference, was also a great concern. Therefore, additional tests were performed in order to assure that those groups which were declared "not significantly different" were truly comparable.

## RESULTS

The individual parameters from the curve-fitting procedure are listed in Table 1, along with groups means and standard deviations and overall means and standard deviations. The results of the statistical tests by parametric and nonparametric methods were almost identical, with both approaches finding significant effects in  $C_0$  and AUC, but no systematic differences in any of the other parameters. Baseline methemoglobin levels seemed to be a random characteristic of each dog, and  $k_a$  and  $k_{el}$  showed no patterns of being faster or slower for any particular drug. The multiple comparison procedures showed that WR242511 caused significantly higher methemoglobin levels, as measured by  $C_0$  and AUC, than primaquine, WR238605, and WR6026. There was also a smaller significant difference between WR225448 and primaquine. When individual tests were performed to maximize the power of detecting small differences, no statistically significant differences were found between primaquine and WR238605, nor between primaquine and WR6026. These two compounds would appear to be the most promising for further study, since when given in equimolar amounts as primaquine, they cause no significantly higher levels of methemoglobinemia.

Table 1: Summary of parameters of fitted curves describing the time courses of methemoglobin levels in individual animals.

|          | Baseline | C <sub>0</sub> | k <sub>a</sub> | k <sub>el</sub> | AUC    |
|----------|----------|----------------|----------------|-----------------|--------|
| WR2975   |          |                |                |                 |        |
| 2A30     | 0.4321   | 1.432          | 0.07981        | 0.008743        | 163.8  |
| 2A20     | 1.0458   | 1.320          | 0.04248        | 0.009592        | 137.6  |
| 2A21     | 4.5192   | 2.741          | 0.05370        | 0.006564        | 417.6  |
| 2D93     | 6.3253   | 3.030          | 0.02039        | 0.020385        | 148.6  |
| 2D95     | 0.4711   | 0.958          | 0.07285        | 0.008483        | 112.9  |
| 2D96     | 2.6197   | 1.903          | 0.13725        | 0.002596        | 733.1  |
| Mean     | 2.5689   | 1.897          | 0.06775        | 0.009394        | 285.6  |
| S.D.     | 2.4167   | 0.828          | 0.04020        | 0.005938        | 246.2  |
| WR238605 |          |                |                |                 |        |
| 2A23     | 0.3714   | 10.824         | 0.01487        | 0.006170        | 1754.3 |
| 2A26     | 0.3487   | 5.565          | 0.00690        | 0.006896        | 806.9  |
| 2A31     | 1.9596   | 2.993          | 0.02122        | 0.001357        | 2205.6 |
| Mean     | 0.8932   | 6.460          | 0.01433        | 0.004808        | 1588.9 |
| S.D.     | 0.9236   | 3.992          | 0.00718        | 0.003010        | 713.9  |
| WR225448 |          |                |                |                 |        |
| 2A25     | 2.2463   | 7.470          | 0.05045        | 0.001673        | 4465.5 |
| 2A22     | 1.4132   | 13.387         | 0.01012        | 0.005126        | 2611.8 |
| 2A27     | 0.2983   | 5.513          | 0.01764        | 0.003728        | 1478.8 |
| Mean     | 1.3193   | 8.790          | 0.02607        | 0.003509        | 2852.0 |
| S.D.     | 0.9774   | 4.100          | 0.02145        | 0.001737        | 1507.7 |
| WR242511 |          |                |                |                 |        |
| 2A28     | 1.2719   | 15.193         | 0.06037        | 0.004330        | 3508.5 |
| 2A24     | 0.9122   | 17.584         | 0.02744        | 0.004116        | 4272.3 |
| 2A29     | 0.9842   | 18.623         | 0.01665        | 0.006048        | 3079.0 |
| Mean     | 1.0561   | 17.133         | 0.03482        | 0.004831        | 3619.9 |
| S.D.     | 0.1903   | 1.759          | 0.02278        | 0.001059        | 604.4  |
| WR6026   |          |                |                |                 |        |
| 2D94     | 4.2800   | 5.042          | 0.07176        | 0.004314        | 1168.9 |
| 2D97     | 4.1263   | 6.792          | 0.07178        | 0.004271        | 1590.2 |
| 2D98     | 0.3562   | 7.188          | 0.03571        | 0.007487        | 960.0  |
| 2D99     | 4.2543   | 2.574          | 0.07952        | 0.004431        | 580.9  |
| Mean     | 3.2542   | 5.399          | 0.06469        | 0.005126        | 1075.0 |
| S.D.     | 1.9332   | 2.102          | 0.01966        | 0.001576        | 420.9  |
| -----    |          |                |                |                 |        |
| Mean     | 2.0124   | 6.849          | 0.04689        | 0.006122        | 1589.3 |
| S.D.     | 1.8269   | 5.642          | 0.03341        | 0.004143        | 1405.2 |

TECHNICAL REPORT

BIO-MED EXPERIMENT #10

COMPARATIVE BIOAVAILABILITY OF TWO TABLET FORMULATIONS OF MEFLOQUINE HYDROCHLORIDE

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## Introduction

A series of oral formulations of mefloquine were produced during the development of this new antimalarial drug. The first to be used in Phase II therapeutic trials was a 250 mg (mefloquine-HCl) capsule manufactured by Lafayette Pharmacal. Results of these initial clinical trials suggested limited bioavailability of the capsule formulation. A subsequent 250 mg (mefloquine-HCl) tablet formulation, E443, also manufactured by Lafayette Pharmacal was more successful therapeutically.<sup>1</sup>

A later 250 mg (mefloquine-HCl) tablet formulation, B512, was prepared by InterX with in vitro dispersion superior to that of E443. Extended Phase I safety, tolerance and pharmacokinetic studies with the new formulation, B512, suggested that at doses in excess of 1000 mg, it was not as well tolerated as E443 had been in earlier studies. A comparative oral bioavailability study with B512 and E443 demonstrated that the former was more rapidly and possibly more completely absorbed than the latter.<sup>2</sup>

A new lot, E555, of 250 mg (mefloquine-HCl) tablets was prepared by Lafayette Pharmacal according to the same formulation as E443. A tablet containing 250 mg mefloquine base (approximately 275 mg mefloquine-HCl) was also prepared by Hoffmann-LaRoche, Basel, Switzerland (HLR) for use in clinical trials supported by Hoffmann-LaRoche and under the auspices of the Steering Committee on the Chemotherapy of Malaria (CHEMAL) of the UNDP World Bank WHO Special Program for Research and Training in Tropical Diseases (TDR). The present study was conducted to compare the oral bioavailability in normal healthy volunteers of the E555 and HLR tablet formulations.

### Study Design and Methodology

This was to have been a standard randomized balanced cross-over study in which the safety, tolerance and oral bioavailability of a single 1000 mg (mefloquine·HCl) dose of the E555 formulation (4 tablets) would be compared with a single 1100 mg (mefloquine·HCl) dose of the HLR formulation (4 tablets). Both formulations were to be administered in random sequence to each of 12 volunteers in three groups of 4, with an interval of 28 days between the two doses. One of the participants (#263) failed to return for the second dose and was replaced by a 13th individual (#273). However, the replacement, #273, was given the two formulations in the opposite sequence assigned to the original participant, #263. Therefore, the resulting study was an unbalanced cross-over. While the data are still amenable to analysis there is a loss of power to detect significant differences because of this circumstance.

The sequence of administration to each of the volunteers is shown in Table 1, where A designates the HLR and B the E555 formulations. It should be noted that in this study the dose of mefloquine administered to each individual was not exactly the same for the two formulations. This was due to the fact that HLR was manufactured to contain 250 mg of mefloquine base and E555 to contain 250 mg of mefloquine·HCl. The administered dose was 4 tablets in both cases resulting in a total dose of 1100 mg for A and 1000 mg for B of mefloquine·HCl. These were the values employed in the subsequent data analysis.

Details of volunteer selection, drug administration and the blood sampling schedule for drug assays are described in the Final Clinical Report, Experiment Number 10, Bio-Med Inc. (January 9, 1979), a copy of which is appended (Appendix I). Whole blood concentrations of mefloquine were determined by a high pressure liquid chromatographic method reported previously.<sup>3</sup>

Compliance with the schedule of blood samples for drug assay was excellent for all of the 12 volunteers who completed the study. The exact sampling times and drug concentrations for each participant are presented in Appendix II.

#### Data Analysis

These data were evaluated in a number of different ways to compare the rate and relative extent of systemic absorption from the two formulations. Non-linear curve fitting to standard pharmacokinetic models\* was performed in a Tektronix 4052 Graphics Computer System with programs developed in the Division of Experimental Therapeutics, Walter Reed Army Institute of Research and later modified in the Division of Clinical Pharmacology, Department of Medicine, Duke University. Statistical analyses were performed using the Statistical Analysis System (SAS) at the University of North Carolina.

A simple comparison of the peak concentrations of mefloquine (Table C) was made using the analysis of variance (ANOVA) for a cross-over design with terms in the model for treatment, subject and period effects. These peak concentrations were corrected according to the dose administered (A=100 mg, B=1000 mg) but not for body weight or estimated residual following the first dose.

A similar comparison of the areas under the concentration-time curve following each formulation was made. In this case the areas were calculated by the trapezoidal rule from time 0 to the final measured sample plus the extrapolated terminal portion of the curve based on a single exponential regression of the points on the curve beyond 96 hours. In addition, the area under the second concentration-time curve (Period 2) in each case was corrected by subtracting the extrapolated area under the first curve beyond the time of the second dose (i.e., residual area from Period 1). The plotted time concentration data and fitted terminal exponential regression in each case are shown in Appendix III.

From the areas thus estimated an apparent clearance term was calculated by dividing the area into the dose administered in each case:

$$Cl_{app} = \frac{D}{Area}$$

These clearance terms, shown in Table 3, were then compared by ANOVA for a cross-over design. Based on the assumption that the volume of distribution (V) and elimination rate (Kel) of mefloquine is the same regardless of the formulation in which it is administered, and the following relationship:

$$Cl_{app} = Kel \cdot V \cdot F$$

significant apparent differences in clearance between the two formulations are directly attributable to differences in fractional absorption (F).

Finally, all of the concentration-time data following administration of both formulations were fitted for each participant to a model which assumed that the volume of distribution and elimination rate constants for both formulations were the same but that the absorption rate constant and fractional absorption could vary independently. The parameters of this model, therefore, were:

- $V$  = Volume of distribution
- $k_{el}$  = Elimination rate constant
- $k_{aB}$  = Absorption rate constant for B (i.e., E555)
- $k_{aA}$  = Absorption rate constant for A (i.e., HLR)
- $F_B$  = Fractional absorption for B (E555)
- $F_A$  = Fractional absorption for A (HLR)

The clearance terms (see Table 4) thus obtained were similarly compared by ANOVA for a cross-over design, again to test indirectly the hypothesis that  $F_B = F_A$ . In addition, the mean ratio of  $F_B/F_A$  was calculated and its standard variance from unity tested by a one-sample t-test. The mean ratio of  $k_{aB}/k_{aA}$  was similarly evaluated. Graphs of these fitted curves are shown for each individual in Appendix III.

### Results

The corrected peak concentrations for each individual following formulation A (HLR) and B (E555) are shown in Table 1. The ANOVA comparing these values (Appendix IV) shows a significant difference among subjects (p=0.007) but no significant period effect (p=0.540). There was also a significant treatment difference in peak concentrations (p=0.001) favoring formulation A.

The clearance terms computed from areas obtained by the trapezoidal rule plus exponential extrapolation of terminal points were similarly compared. These corrected clearance terms for each formulation are shown in Table 3. The ANOVA comparing these values (Appendix VI) shows a significant difference among subjects ( $p=0.0025$ ), no significant period effect ( $p=0.9229$ ) and a significant difference between treatments ( $p=0.0150$ ) favoring a higher fractional absorption for Formulation A.

The fitted curves (see Appendix IV) provided a set of parameter estimates for each participant including an elimination rate constant from which the half-life was calculated. These values are shown for each subject in Table 4. The mean half-life in these 10 volunteers was approximately 12 days which corresponds well with previously reported results.<sup>5</sup> The clearance terms computed from the fitted curves are shown in Table 5. The ANOVA for these values (Appendix VII) similarly shows a significant difference among subjects ( $p=0.0011$ ), no significant period effect ( $p=0.4464$ ), and a significant difference between treatments ( $p=0.0134$ ), again favoring a higher fractional absorption for Formulation A.

The values for the absorption rate constants ( $k_a$ ) estimated independently for each formulation and their ratio (Formulation A/Formulation B) are shown in Table 6. The mean ratio was 1.5589 and was significantly greater than 1.0 ( $p=0.0099$ ).

The values for fractional absorption ( $f$ ) as a function of the volume of distribution ( $V_d$ ) are shown in Table 7, based on the assumption that the volume of distribution for each drug is the same regardless of the formulation in which it is administered. The ratio of fractional absorption of the two forms

ratios (A/B) can be obtained by dividing these terms. The mean ratio was 1.3735 and was significantly greater than 1.0 ( $p=0.0012$ ).

### Conclusions and Discussion

Analyses of the blood concentration-time data from this comparative oral bioavailability study, by a variety of statistical and pharmacokinetic models, all indicate that the HLR tablet formulation containing 250 mg of mefloquine base was more rapidly and completely absorbed than was the Lafayette Pharmacal E555 tablet formulation containing 250 mg of mefloquine-HCl. Corrections were made in these analyses for the difference in dose (1000 mg versus 1100 mg of the HCl salt), but not for differences in body weight of the individual participants. There is some controversy about whether or not such a correction should also be made, but in this case a correlation analysis of the parameter of interest (clearance) with body weight of the individual participants showed that no such correlation existed (coefficient of correlation = 0.16,  $p=0.1217$ ).

In a previous comparative bioavailability study formulation E-43, which was also manufactured by Lafayette Pharmacal and was identical to the present E555, was compared with Interyl formulation E542. The latter was more rapidly and possibly more completely absorbed. Prior clinical experience suggested the more frequent occurrence of particularly CNS side effects with E-43 than had been the case with E443. Since the HLR formulation also produced significantly higher peak blood concentrations of mefloquine with more rapid and complete absorption of the drug than does the E555 formulation, a higher than expected frequency of adverse experiences may be observed with the use of the HLR formulation.

The methods employed in this comparative oral bioavailability study demonstrate that it is not absolutely essential to allow sufficient time to elapse between doses to reach nondetectable blood concentrations of the drug. In the case of a drug like mefloquine, with a half-life greater than 10 days, this would clearly be impractical. It is nevertheless quite possible to compare the fractional absorption of two formulations.

TABLE 1

A = HLR tablets 250 mg mefloquine base

B = E555 tablets 250 mg mefloquine·HCl

| <u>Volunteer #</u> | <u>Period 1</u> | <u>Period 2</u> |
|--------------------|-----------------|-----------------|
| 261                | B               | A               |
| 262                | A               | B               |
| 263                | A               | --              |
| 264                | B               | A               |
| 265                | B               | A               |
| 266                | B               | A               |
| 267                | A               | B               |
| 268                | A               | B               |
| 269                | A               | B               |
| 270                | A               | B               |
| 271                | B               | A               |
| 272                | B               | A               |
| 273                | B               | A               |

Note: There are five individuals with sequence AB and seven with BA. The cross-over is, therefore, unbalanced.

TABLE 2

Peak Concentration ( $\mu\text{g}/\text{ml}$ ) of Mefloquine  
(Corrected to 1000 mg Dose)

| <u>Subject</u> | <u>FORMULATION</u> |          |
|----------------|--------------------|----------|
|                | <u>A</u>           | <u>B</u> |
| 261            | 0.737              | 0.383    |
| 262            | 0.750              | 0.802    |
| 264            | 1.229              | 0.628    |
| 265            | 1.054              | 0.928    |
| 266            | 0.811              | 0.611    |
| 267            | 1.211              | 1.025    |
| 268            | 0.636              | 0.498    |
| 269            | 0.623              | 0.678    |
| 270            | 0.629              | 0.403    |
| 271            | 0.932              | 0.873    |
| 272            | 0.838              | 0.312    |
| 273            | 0.957              | 0.615    |

TABLE 3

Total Body Clearance of Mefloquine  
Dose Divided by Area Under Concentration-Time Curves by Trapezoidal Rule  
and Extrapolation of Single Exponential Fit of Terminal Data  
(beyond 96 hours)

| <u>Subject</u> | <u>FORMULATION</u> |          |
|----------------|--------------------|----------|
|                | <u>A</u>           | <u>B</u> |
| 261            | 3.339              | 5.283    |
| 262            | 3.607              | 3.307    |
| 264            | 1.274              | 2.168    |
| 265            | 2.297              | 3.367    |
| 266            | 3.887              | 4.407    |
| 267            | 1.338              | 2.292    |
| 268            | 4.891              | 5.252    |
| 269            | 3.565              | 3.930    |
| 270            | 5.126              | 1.474    |
| 271            | 3.737              | 4.262    |
| 272            | 3.626              | 4.267    |
| 273            | 4.774              | 5.027    |

TABLE 4

| <u>Subject</u> | <u>Kel</u> | <u>t<sub>1/2</sub> (days)</u> |
|----------------|------------|-------------------------------|
| 261            | 0.001531   | 18.86                         |
| 262            | 0.002065   | 13.99                         |
| 264            | 0.001410   | 20.48                         |
| 265            | 0.002403   | 12.02                         |
| 266            | 0.002219   | 13.02                         |
| 267            | 0.001539   | 18.77                         |
| 268            | 0.002559   | 11.29                         |
| 269            | 0.002192   | 13.18                         |
| 270            | 0.005081   | 5.68                          |
| 271            | 0.003021   | 9.00                          |
| 272            | 0.003004   | 9.61                          |
| 273            | 0.003768   | <u>7.66</u>                   |
| Mean           |            | 11.96                         |

TABLE 5

Total Body Clearance (ml min)  
Obtained from Fitted Regression Curves:  
Biexponential Model

| Subject | Formulation |       |
|---------|-------------|-------|
|         | A           | B     |
| 261     | 3.158       | 4.582 |
| 262     | 2.940       | 3.653 |
| 264     | 1.558       | 2.497 |
| 265     | 2.633       | 3.495 |
| 266     | 3.761       | 3.916 |
| 267     | 1.735       | 1.525 |
| 268     | 6.342       | 6.305 |
| 269     | 1.713       | 3.443 |
| 270     | 1.247       | 4.351 |
| 271     | 1.432       | 4.352 |
| 272     | 1.148       | 4.175 |
| 273     | 1.371       | 2.160 |

TABLE 6

## Absorption Rate Constants

| <u>Subject</u> | <u>Formulation</u> |          | <u>Ratio</u>  |
|----------------|--------------------|----------|---------------|
|                | <u>A</u>           | <u>B</u> | <u>A/B</u>    |
| 261            | 0.1690             | 0.1516   | 1.1148        |
| 262            | 0.3892             | 0.2137   | 1.8212        |
| 264            | 0.4952             | 0.5382   | 0.9201        |
| 265            | 0.2603             | 0.2579   | 1.0093        |
| 266            | 0.3291             | 0.1408   | 2.3374        |
| 267            | 0.2303             | 0.1240   | 1.8573        |
| 268            | 0.2228             | 0.1842   | 1.2096        |
| 269            | 0.1735             | 0.0958   | 1.8111        |
| 270            | 0.4068             | 0.3208   | 1.2681        |
| 271            | 0.2565             | 0.2118   | 1.2110        |
| 272            | 0.2678             | 0.3250   | 0.8240        |
| 273            | 0.2409             | 0.0725   | <u>3.3228</u> |
| Mean           |                    |          | 1.5589        |

TABLE 7

## Ratio of Fractional Absorption

| <u>Subject</u> | <u>Formulation</u> |          | <u>Ratio</u> |
|----------------|--------------------|----------|--------------|
|                | <u>A</u>           | <u>B</u> | <u>A/B</u>   |
| 261            | 0.4848             | 0.3341   | 1.4511       |
| 262            | 0.7024             | 0.5653   | 1.2425       |
| 264            | 0.9049             | 0.5647   | 1.6024       |
| 265            | 0.9126             | 0.6875   | 1.3274       |
| 266            | 0.5900             | 0.5666   | 1.0413       |
| 267            | 0.6624             | 0.6094   | 1.0852       |
| 268            | 0.4343             | 0.4058   | 1.0702       |
| 269            | 0.5871             | 0.5489   | 1.0692       |
| 270            | 0.5647             | 0.3540   | 1.5952       |
| 271            | 0.7684             | 0.6226   | 1.2342       |
| 272            | 0.6395             | 0.3105   | 2.0596       |
| 273            | 0.7764             | 0.6213   | 1.2588       |
| Mean           |                    |          | 1.3735       |

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APPENDIX I

Final Clinical Report  
Experiment Number 10  
Bio-Med Inc.  
January 9, 1979

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January 9, 1979

FINAL REPORT

ANTIMALARIAL DRUG PROJECT


EXPERIMENT NUMBER 10

TITLE: COMPARATIVE BIOAVAILABILITY  
AND PHARMACOKINETICS OF  
WR 142,490·HCl (MEFLOQUINE  
HYDROCHLORIDE) AND MEFLOQUINE  
HYDROCHLORIDE·HLR

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## FINAL REPORT

### EXPERIMENT NUMBER 10

COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF  
WR 142,490·HCl (MEFLOQUINE HYDROCHLORIDE) AND  
MEFLOQUINE HYDROCHLORIDE·HLR

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FINAL CLINICAL REPORT

## EXPERIMENT NUMBER 10

COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF  
WR 142,490·HCl (MEFLOQUINE HYDROCHLORIDE) AND  
MEFLOQUINE HYDROCHLORIDE·HLR\*ABSTRACT

Mefloquine hydrochloride, a substituted quinoline methanol, has been shown to be an effective single dose agent in the treatment of chloroquine-resistant *P. falciparum* malaria and effective for prophylaxis. The drug administered to human subjects in single doses up to 1000 mg was well tolerated. Intolerance at higher doses was manifested by temporary light-headedness, diarrhea, abdominal cramps, occasional nausea and/or vomiting. Symptoms were considered dose related and mild in all cases.

A variety of formulations have been used in previous tolerance and therapeutic studies. Clinical results in infected volunteers and blood level determinations in a limited number of pharmacokinetic studies indicate considerable variation in the bioavailability of these formulations. The use of a new formulation in field studies, such as the HLR formulation, must therefore be supported by prior demonstration of adequate bioavailability.

A study for comparative bioavailability of the HLR formulation and an established effective formulation, WR 142,490·HCl, was done. A classical two way balanced crossover design including 3 groups of 4 subjects each was used. Symptoms were absent or mild and temporary. Symptomatology attributed to drug ingestion included gastrointestinal symptoms and headache following ingestion of both formulations. Lightheadedness occurred only following administration of the WR formulation. No significant changes in physical examination or laboratory values attributed to drug administration were observed. In conclusion, WR 142,490·HCl and the F. Hoffmann-La Roche, & Co. formulation were both well tolerated under conditions of this study. Drug assay analysis is not yet available and will be reported separately at a later date by the responsible institution.

\*F. Hoffmann-La Roche, & Co.

FINAL REPORT

## EXPERIMENT NUMBER 10

COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF  
WR 142,490·HCl (MEFLOQUINE HYDROCHLORIDE) AND  
MEFLOQUINE HYDROCHLORIDE·HLR\*INTRODUCTION:

Mefloquine hydrochloride, a substituted quinoline methanol, has been shown to be an effective single dose agent in the treatment of chloroquine-resistant *P. falciparum* malaria. Its prophylactic effectiveness against chloroquine-resistant *P. falciparum* malaria inoculated by infected mosquitoes has also been demonstrated. Clinical studies in humans showed that 10 subjects who received 250 mg of WR 142,490·HCl weekly for 8 weeks were protected when exposed to mosquitoes heavily infected with multi-drug-resistant *P. falciparum*. The drug administered to human subjects in single doses up to 1000 mg was well tolerated. Intolerance at higher doses was manifested by temporary light-headedness, diarrhea, abdominal cramps, occasional nausea and/or vomiting. Symptoms were considered dose related and mild in all cases.

In addition, 12 healthy young males have completed a year long study during which each subject received a single weekly dose of 500 mg of WR 142,490·HCl without significant adverse clinical or laboratory effects. It appears that this anti-malarial is well tolerated and deserving of additional clinical investigations in man.

A variety of formulations have been used in previous tolerance and therapeutic studies. Clinical results in infected volunteers and blood level determinations in a limited number of pharmacokinetic studies indicate considerable variation in the bioavailability of these formulations. The use of a new formulation in field studies (such as the HLR formulation) must therefore be supported by prior demonstration of adequate bioavailability.

This study was designed to compare specific bioavailability parameters of WR 142,490·HCl and the HLR preparation of Mefloquine·HCl following single oral dose administration to healthy human male subjects, i.e.: peak blood levels, time to peak level, blood level-time patterns and area under concentration-time curve.

\*HLR and WR as used in the text designate the F. Hoffmann-La Roche, & Co. and Walter Reed preparation respectively. Following a code number the letters designate the preparation administered.

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The IND with Supplements and Clinical Summary were available at all processing committee meetings and were available at all times in the Office of the Clinical Director of BIO-MED, Inc.

#### METHODS AND MATERIALS:

##### Methods Subject Selection - Acceptability Criteria:

Thirteen healthy male subjects, 21 to 38 years of age, weighing 56-87 kg and within 10% of their ideal body weight, were employed for the study. They were recruited from the Washington, D.C. metropolitan area. Candidates were hired by BIO-MED, Inc. as temporary employees for study purposes.

Candidates for employment were screened to obtain the subjects for study. The medical evaluation included a comprehensive history and physical examination, chest X-ray, electrocardiogram, urinalysis, white blood cell and differential count, red blood cell count, hemoglobin, hematocrit, MCV, MCH, MCHC, platelet count, glucose, BUN, creatinine, sodium, potassium, chloride, carbon dioxide, uric acid, total protein, albumin, globulin, calcium, phosphate, cholesterol, triglycerides, alkaline phosphatase, SGOT, SGPT, LDH, total bilirubin, and G6PD.

Subject acceptability criteria are based upon the precept that the risks of participation should be slight, and comparable for all subjects. Following this guideline, certain subjects were rejected routinely: for example, subjects with organic heart murmurs, splenomegaly or active lesions on chest X-ray. The presence of conditions which did not increase risk or potentially compromise the validity of the study, as illustrated by epidermophytosis, "shotty lymphadenopathy", or scarred tympanic membranes, was not ordinarily cause for rejection. Deviations of laboratory values of 3 standard deviations or more from the mean were generally cause for rejection dependent upon the particular test and associated clinical and laboratory observations. For example, a serum sodium of 153 mEq/L of itself would not be a cause for rejection, whereas a serum calcium of 11.2 mg/dl would.

Whenever doubt existed concerning the eligibility of a subject a decision was made following consultation with fellow M.D. investigators and other specialists, as appropriate. In this manner, questionable candidates were given full consideration and the integrity and ethics of the Research Team protected.

Qualified candidates were presented with a complete explanation of the background and procedures to be used in the study and all details of the protocol as it involved the individual subjects. They were interviewed in a group and individually in the presence of an investigator and a member of the Human Use Committee. Each participant was given the opportunity to ask questions. Following this, the consent form was read and those wishing to participate signed it in the presence of a witness, an investigator and a member of the Human Use Committee.

During the first 5 days of each study interval the subjects were housed in a controlled environment on Nursing Unit 5-W at the Washington Hospital Center. Thereafter, they reported according to the protocol schedule (page 4).

Subject Assignment - Drug Administration:

A classical 2 way balanced crossover design was used. Each subject was randomly assigned to 1 of 2 possible sequences of drug administration, within the limitations of the design.

Three groups of 4 subjects each were admitted to the study sequentially. Within each group the 2 formulations were administered at a dose of 4 tablets\* to 2 subjects each, by random assignment. Following a "wash out" period of 4 weeks, each subject was given 4 tablets\* of the alternate formulation. Follow-up and sampling times following each administration are listed in the tables on page 11.

On the day of drug administration, subjects were permitted to drink water ad lib until 1 hour prior to and 2 hours after drug administration. Breakfast was withheld. The drug was administered in the presence of a member of the investigating team. Subjects returned to a regular diet 6 hours after drug administration.

The clinical and laboratory evaluation of the subjects is outlined on the following page:

\*The 4 tablets for the Walter Reed formulation constituted a single dose of 1000 mg of Mefloquine·HCl, and for the F. Hoffmann-La Roche, & Co. preparation a single dose of 1100 mg Mefloquine·HCl. WR Lafayette Pharmacal lot number E-555; HLR lot number 21-5998-001-01.

SCHEMATIC STUDY PLAN FOR EACH DOSING

| STUDY DAY                     | 0* | 1* | 2* | 3* | 4* | 5 | 7 | 8 | 14 | 21 |
|-------------------------------|----|----|----|----|----|---|---|---|----|----|
| Dose                          |    | X  |    |    |    |   |   |   |    |    |
| Physical Exam                 | X  |    | X  |    |    |   | X |   |    | X  |
| Interview                     | X  | X  | X  | X  | X  |   | X |   |    | X  |
| Vital Signs                   | X  | X  | X  | X  | X  |   | X |   |    | X  |
| ECG                           | X  |    | X  |    |    |   | X |   |    | X  |
| Laboratory Tests <sup>+</sup> | X  |    | X  | X  |    |   | X |   |    | X  |
| Drug Assay <sup>++</sup>      |    | X  | X  | X  | X  | X | X | X | X  | X  |

\*Controlled Environment in Study Unit 5-W.

<sup>+</sup>Glucose, BUN, Creatinine, Sodium, Potassium, Chloride, Carbon Dioxide, Uric Acid, Total Protein, Albumin, Globulin, Calcium, Phosphate, Cholesterol, Triglyceride, Alkaline Phosphatase, SGOT, SGPT, LDH, Total Bilirubin, CBC (differential and indices), Platelets, Urinalysis. Additional studies were done as clinically indicated.

<sup>++</sup>Drug Assay - Blood drawn on each subject immediately prior to drug administration and after dosing at 1,2,4,6,8,10,11, 12,13,15,20,24,28,34,48,56,63,72,76,80 hours and on days 5, 7,8,14, and 21.

The schedule for hematologic and biochemical blood tests is indicated. For these tests 27 ml venous blood was obtained while the subject was fasting before breakfast. Seven ml blood was used for determination of white blood cell and differential count, red blood cell count, hematocrit, hemoglobin, MCH, MCHC, MCV, and platelet count. Twenty ml of the venous blood specimen was centrifuged and the serum separated. The serum was divided into 2 samples. One sample was stored in the refrigerator as a "back-up" until the biochemical lab report was received. Thereafter it was stored in the freezer until released by the investigator. The other sample was used on the day obtained to determine the values for serum glucose, BUN, creatinine, sodium, potassium, chloride, carbon dioxide, uric acid, total protein, albumin, globulin, calcium, phosphate, cholesterol, triglycerides, alkaline phosphatase, SGOT, SGPT, LDH, and total bilirubin.

On the last study day for each subject final physical and laboratory evaluation was done. All abnormal findings caused follow-up until normalcy, stabilization, or proper medical disposition was secured.

Drug Assay Specimen Collection and Time Schedule:

Specimen collection for drug assay was as follows: 6 ml venous blood was obtained in a heparin rinsed syringe. It was placed in 16 x 125 mm glass test tubes with screw-on teflon lined caps. Specimens were stored at -20°C pending transport to Department of Pharmacology, Walter Reed Army Institute of Research, for drug assay. A Specimen Collection Worksheet (BMI-WS-1) was completed by the staff nurse on each subject participating in the study. Twenty-four hour urine specimens were collected for drug assay on days 0, 1, and 2 from 8:01 a.m. to 8:00 a.m. (See the tables and Specimen Collection Worksheet on pages 11 and 12 respectively.)

RESULTS:

Subject code no. 263 withdrew from the study before administration of the WR preparation. Subject code no. 273 was substituted and the sequence of drug administration was inadvertently reversed.

Symptoms:

All symptoms are tabulated in Table I (pages 18 through 20). Three subjects (code nos. 265, 268, and 270) had symptoms possibly drug related following administration of each formulation. Four subjects (code nos. 261, 264, 266, and 271) had symptoms only after ingestion of the HLR preparation and 2 subjects (code nos. 262 and 272) only following administration of the WR formulation. Therefore, 7 of 13 administrations of the HLR formulation and 5 of 12 administrations of the WR preparation were associated with minor, possibly drug related symptoms.

Gastrointestinal:

The most frequent symptoms involved the gastrointestinal tract. Nausea variously described as a "queasy" feeling and "stomach ache" was associated with the administration of the HLR formulation on 2 occasions (subject code nos. 264 and 265) and the WR formulation on 3 occasions (subject code nos. 262, 265, and 268). Two other subjects receiving the HLR preparation had nausea not attributed to drug: 4 days after ingestion of the HLR preparation, subject code no. 262 had nausea

with 2 episodes of emesis. He was anorexic and dysphoric for approximately 1 week. Subsequently, a severe dental infection and abscess was found which was considered causal. Subject code no. 268 had transient lightheadedness and nausea associated with bloodletting 20 hours after administration of the HLR preparation not considered drug induced.

Loose stools occurred in seven subjects. Four subjects (code nos. 261, 265, 268, and 271) only following administration of the HLR preparation and 2 only following administration of the WR formulation. One subject had loose stools after receiving each formulation. Three subjects had onset of loose stools within 2 hours of drug administration: subject code nos. 261 and 268 after HLR and subject code no. 272 after the WR formulation. For subject (code no. 271) having 2 loose stools on the day of administration of the HLR preparation, the time of onset was not documented. One subject (code no. 266) had 1 loose stool 8 hours after receiving the WR formulation. Another subject (code no. 265) had 1 watery stool 48 hours after ingesting the HLR preparation. In 2 subjects with recurrent loose stools (code nos. 268 HLR and 270 WR) the frequency was 1 to 3 per day and the duration was 6 days except that subject code no. 268 HLR had abdominal cramps and passed 7 watery stools during the first 12 hours after onset at 45 minutes after dosing. In all other subjects the duration was of less than 24 hours or (code no. 265) only 1 loose stool was evacuated. The passage of watery stools was not associated with abdominal cramps except at onset in 1 subject (code no. 268 HLR).

In summary, 12 of 25 drug administrations were associated with mild, non-incapacitating gastrointestinal symptoms potentially attributable to drug administration: 7 episodes following HLR and 5 episodes following WR formulation administration.

#### Neurologic:

Eight of 25 drug administrations were associated with headache, lightheadedness, syncope, vasovagal reactions, or nightmares. However, only 2 subjects had symptoms considered potentially drug related: subject code no. 265 had headache and lightheadedness following administration of the HLR and WR formulation respectively. Additionally, subject code no. 268 had both symptoms only after receiving the WR preparation. Potentially drug related lightheadedness occurred as follows: subject code no. 265 WR noted onset of vague "dizziness" 4 hours after dosing with duration of 9 hours. The other subject with lightheadedness (code no. 268 WR) had been typing and reading constantly. Seven hours after dosing, he noted headache, lightheadedness, and nausea of 3 hours duration dissipating rapidly upon discontinuation of typing and reading.

Light-headedness not attributed to drug administration occurred on 3 occasions. Subject code no. 273 experienced light-headedness and syncope associated with bloodletting 30 minutes before dosing. Similarly, 2 subjects (code nos. 268 HLR and 272 WR) experienced transient vasovagal reactions associated with bloodletting 20 and 15 hours after dosing, respectively.

Three subjects in addition to subject code no. 268 WR noted headaches. One subject (code no. 265 HLR) experienced a possibly drug related headache. He noted onset of a mild right temporal headache 6 hours after dosing which persisted for 8 hours. Two other subjects had headaches not attributable to drug: 1 subject (code no. 261 WR) had a mild right temporal headache starting prior to dosing, of less than 24 hours duration. The other subject (code no. 263 HLR) had onset of a mild headache of approximately 15 minutes duration 6 days after dosing. These latter 2 subjects stated the headaches were similar to those they experienced commonly without obvious precipitants.

One subject (code no. 268 HLR) had vivid dreams and nightmares possibly but not probably drug related starting 56 hours after dosing and recurring nightly for 4 nights.

In summary, 1 subject (code no. 268) had dizziness, headache, and nausea of 3 hours duration with onset 7 hours after receiving the WR formulation, possibly precipitated or aggravated by typing and reading. Another subject (code no. 265) had vague "dizziness" for approximately 9 hours starting 4 hours after receiving the WR preparation. The same subject had a mild right temporal headache of approximately 8 hours duration starting 6 hours after receiving the HLR formulation. In both subjects the neurologic symptoms potentially drug related were mild and temporary. Neurologic symptoms otherwise were not considered potentially drug related.

#### Miscellaneous:

Three subjects (code nos. 264, 272, and 273) had symptoms of the common cold during the interval of study. Three other subjects (code nos. 262, 267, and 268) had symptoms and signs not considered related to drug administration which are presented in Table I and the individual subject final summaries.

#### Physical Findings:

No changes attributed to drug administration occurred in any subject.

### Laboratory Values:

The range ( $\bar{x} \pm 2$  SD) of laboratory values for the BIO-MED, Inc. normal population ( $n > 100$ ) is presented as Table IIA (page 21). All laboratory values outside the normal range are included in Table IIB (pages 22 through 24). All subjects had 2 or more values outside the normal range reported. Minimal serum carbon dioxide content elevation reported in 9 subjects was the most frequently reported abnormality. The elevations were minimal and inconsistent, occurring before and after administration of both formulations without discernable pattern. This observation of minimal, inconsistent deviations of laboratory values in a random manner is characteristic of most of the abnormalities reported and not considered drug related. In some subjects values for a given determination clustered about the upper or lower limits of the range for that determination. Subject code no. 264 exemplifies this occurrence for serum creatinine and albumin values. Subject code no. 273 demonstrated a persistent minimal elevation of serum alkaline phosphatase before and after administration of both formulations. In the absence of other laboratory or clinical findings to suggest an active disease process, the observation of a fixed pattern of deviation before and after drug administration is not considered drug related.

Two subjects (code nos. 261 and 266) demonstrated minimal SGPT elevations following administration of the HLR formulation: an isolated elevation to 56 U/L occurred 6 days after dosing in subject code no. 261 and elevations to 52 U/L and 48 U/L occurred 2 and 20 days after dosing respectively in subject code no. 266 with a normal value of 38 U/L reported 6 days after dosing. The upper limits for this determination is 47 U/L. These minimal and inconsistent deviations may be considered possibly, but not probably drug related.

### Electrocardiograms:

No significant changes in serial rhythm tracings occurred in any subject.

### DISCUSSION:

The 2 formulations of mefloquine were well tolerated at the dose levels administered. Five of 12 administrations of the WR formulation and 7 of 13 administrations of the HLR preparation were associated with symptoms. Three subjects had symptoms following administration of each drug, four only associated with the HLR formulation, and 2 only associated

with the WR preparation. The character and frequency of symptoms suggest no significant difference in clinical response to the 2 formulations. Therefore, symptoms potentially drug related occurred in 13 of the 25 trials. The symptoms were mild and did not interfere with normal activity.

The absence of gastrointestinal symptoms in previous WR 142,490·HCl safety and tolerance studies at comparable dose levels suggests their frequency in the current study may be attributed in part to the requirements of the experimental protocol. The subjects in this study ingested the drug following an overnight fast and continued to fast for 6 hours following drug administration. During this interval frequent bloodletting was required for specimen collection. These factors may have been causal or contributory to the gastrointestinal symptomatology described.

Light-headedness, headache, and nightmares were observed in association with WR 142,490·HCl administration in previous studies at dose levels higher than those administered in this study. Their frequency in this study was 3 potentially drug related involving 2 subjects in 25 trials: 2 after administration of the WR formulation and 1 after administration of the HLR preparation. The symptoms were mild, temporary, and not incapacitating. The conditions of the study rather than drug effect may have been causal or contributed to the neurologic symptoms.

Intercurrent illnesses and medical conditions unrelated to drug administration occurred with the frequency expected in a study of this duration.

Similarly deviations of laboratory values not attributable to drug administration were frequent. Two subjects receiving the HLR preparation had minimal elevation of SGPT values reported considered possibly, but not probably drug related.

In summary, the study has been completed without significant deviations or adverse reactions and the specimens for assay are in custody of the responsible organization.

CONCLUSIONS AND RECOMMENDATIONS:

This study was performed for pharmacokinetic purposes. Noted as an integral part of monitoring was the occurrence of mild and temporary gastrointestinal and neurologic symptoms considered related primarily to prolonged fasting and frequent bloodletting.

The drug assay results will be reported at a later date by the responsible organization.

EXPERIMENT 10: COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF  
of WR 142,490·HCl (MEFLOQUINE HYDROCHLORIDE) and  
MEFLOQUINE HYDROCHLORIDE·HLR

DRUG ASSAY COLLECTION

| DAY OF THE STUDY | SPECIMEN NUMBER | BLOOD SPECIMEN TIME | BLOOD VOLUME (ml) |
|------------------|-----------------|---------------------|-------------------|
| 1                | 1               | 0 Prior to dosing   | 6                 |
|                  | 2               | 1 hr 9:00 AM        | 6                 |
|                  | 3               | 2 hr 10:00 AM       | 6                 |
|                  | 4               | 4 hr NOON           | 6                 |
|                  | 5               | 6 hr 2:00 PM        | 6                 |
|                  | 6               | 8 hr 4:00 PM        | 6                 |
|                  | 7               | 10 hr 6:00 PM       | 6                 |
|                  | 8               | 11 hr 7:00 PM       | 6                 |
|                  | 9               | 12 hr 8:00 PM       | 6                 |
|                  | 10              | 13 hr 9:00 PM       | 6                 |
|                  | 11              | 15 hr 11:00 PM      | 6                 |
| 2                | 12              | 20 hr 4:00 AM       | 6                 |
|                  | 13              | 24 hr 8:00 AM       | 6                 |
|                  | 14              | 28 hr NOON          | 6                 |
|                  | 15              | 34 hr 6:00 PM       | 6                 |
| 3                | 16              | 48 hr 8:00 AM       | 6                 |
|                  | 17              | 56 hr 4:00 PM       | 6                 |
|                  | 18              | 63 hr 11:00 PM      | 6                 |
| 4                | 19              | 72 hr 8:00 AM       | 6                 |
|                  | 20              | 76 hr NOON          | 6                 |
|                  | 21              | 80 hr 4:00 PM       | 6                 |

HOME

|    |    |            |               |   |
|----|----|------------|---------------|---|
| 5  | 22 | 96-98 hr   | 8:00-10:00 AM | 6 |
| 7  | 23 | 144-146 hr | 8:00-10:00 AM | 6 |
| 8  | 24 | 168-170 hr | 8:00-10:00 AM | 6 |
| 14 | 25 | 312-314 hr | 8:00-10:00 AM | 6 |
| 21 | 26 | 480-482 hr | 8:00-10:00 AM | 6 |

TOTAL 156 (ml)

URINE SPECIMENS

|   |   |                    |
|---|---|--------------------|
| 0 | 1 | 8:01 AM to 8:00 AM |
| 1 | 2 | 8:01 AM to 8:00 AM |
| 2 | 3 | 8:01 AM to 8:00 AM |

TOTAL AMOUNT OF BLOOD WITHDRAWN FOR EACH STUDY SUBJECT  
FOLLOWING EACH DRUG ADMINISTRATION

| DAY   | HEMATOLOGY | CHEMISTRY | BLOOD ASSAY | TOTAL (ml) |
|-------|------------|-----------|-------------|------------|
| 0     | 7          | 20        |             | 27         |
| 1     |            |           | 66          | 66         |
| 2     | 7          | 20        | 24          | 51         |
| 3     | 7          | 20        | 18          | 45         |
| 4     |            |           | 18          | 18         |
| 5     |            |           | 6           | 6          |
| 7     | 7          | 20        | 6           | 33         |
| 8     |            |           | 6           | 6          |
| 14    |            |           | 6           | 6          |
| 21    |            |           | 6           | 6          |
| TOTAL |            |           |             | 264 (ml)   |

Specimen Collection Worksheet

Experiment #10: COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF  
WR 142,490·HCl (MEFLOQUINE HYDROCHLORIDE) AND  
MEFLOQUINE HYDROCHLORIDE·HLR

Name: \_\_\_\_\_ Code: \_\_\_\_\_ Age: \_\_\_\_\_ Ht: \_\_\_\_\_ cm. Wt: \_\_\_\_\_ k

First Dosing

Drug Formulation: \_\_\_\_\_  
Total Dose: \_\_\_\_\_ mg Dose(mg/kg): \_\_\_\_\_  
Date & Time Dosed: \_\_\_\_\_

Second Dosing

Drug Formulation: \_\_\_\_\_  
Total Dose: \_\_\_\_\_ mg Dose(mg/kg): \_\_\_\_\_  
Date & Time Dosed: \_\_\_\_\_

Whole Blood Collection

| Spec. No. | P.D.*      | Sched | Actual |
|-----------|------------|-------|--------|
| 1         | -15 min.   |       |        |
| 2         | 1 hr       |       |        |
| 3         | 2 hr       |       |        |
| 4         | 4 hr       |       |        |
| 5         | 6 hr       |       |        |
| 6         | 8 hr       |       |        |
| 7         | 10 hr      |       |        |
| 8         | 11 hr      |       |        |
| 9         | 12 hr      |       |        |
| 10        | 13 hr      |       |        |
| 11        | 15 hr      |       |        |
| 12        | 20 hr      |       |        |
| 13        | 24 hr      |       |        |
| 14        | 28 hr      |       |        |
| 15        | 34 hr      |       |        |
| 16        | 48 hr      |       |        |
| 17        | 56 hr      |       |        |
| 18        | 63 hr      |       |        |
| 19        | 72 hr      |       |        |
| 20        | 76 hr      |       |        |
| 21        | 80 hr      |       |        |
| 22        | 96-98 hr   |       |        |
| 23        | 144-146 hr |       |        |
| 24        | 168-170 hr |       |        |
| 25        | 312-314 hr |       |        |
| 26        | 430-482 hr |       |        |

Whole Blood Collection

| Spec. No. | P.D.*      | Sched | Actual |
|-----------|------------|-------|--------|
| 1         | -15 min.   |       |        |
| 2         | 1 hr       |       |        |
| 3         | 2 hr       |       |        |
| 4         | 4 hr       |       |        |
| 5         | 6 hr       |       |        |
| 6         | 8 hr       |       |        |
| 7         | 10 hr      |       |        |
| 8         | 11 hr      |       |        |
| 9         | 12 hr      |       |        |
| 10        | 13 hr      |       |        |
| 11        | 15 hr      |       |        |
| 12        | 20 hr      |       |        |
| 13        | 24 hr      |       |        |
| 14        | 28 hr      |       |        |
| 15        | 34 hr      |       |        |
| 16        | 48 hr      |       |        |
| 17        | 56 hr      |       |        |
| 18        | 63 hr      |       |        |
| 19        | 72 hr      |       |        |
| 20        | 76 hr      |       |        |
| 21        | 80 hr      |       |        |
| 22        | 96-98 hr   |       |        |
| 23        | 144-146 hr |       |        |
| 24        | 168-170 hr |       |        |
| 25        | 312-314 hr |       |        |
| 26        | 480-482 hr |       |        |

24 Hr. Urine Collections

| STUDY DAY | TIME  |      | TOTAL |      |          |
|-----------|-------|------|-------|------|----------|
|           | Begin | End  | Hrs.  | Min. | Vol (ml) |
| 0         | +     | 0800 |       |      |          |
| 1         | 0801  | 0800 |       |      |          |
| 2         | 0301  | 0800 |       |      |          |

24 Hr. Urine Collections

| STUDY DAY | TIME  |      | TOTAL |      |          |
|-----------|-------|------|-------|------|----------|
|           | Begin | End  | Hrs.  | Min. | Vol (ml) |
| 0         | +     | 0800 |       |      |          |
| 1         | 0801  | 0800 |       |      |          |
| 2         | 0801  | 0800 |       |      |          |

\* Post Dose

+Variable fraction of 24 hour collection due to starting times.

BMI-WS-1

BIO - MED, Inc.

Tel: (202) 882-0977

EXPLANATION FOR POTENTIAL SUBJECTS  
ANTIMALARIAL DRUG PROJECT  
EXPERIMENT NUMBER 10  
Comparative Bioavailability and Pharmacokinetics  
of WR 142,490·HCl (Mefloquine Hydrochloride)  
and Mefloquine Hydrochloride·HLR

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GENTLEMEN:

This document explains the nature of the study, its purpose, procedures, risks and benefits. You will be given the opportunity after reading it to ask additional questions. If you then choose to participate as a research subject, you will be asked to initial the last page signifying that you have read and understand its contents prior to obtaining your formal written consent to participate in the study. The subject will participate for a total of 50 days.

This study involves taking by mouth the drug mefloquine hydrochloride. Mefloquine is similar to quinine and has been approved by the Food and Drug Administration for investigational studies. The drug has been administered to more than 100 subjects to determine its safety and tolerance, and ability to prevent and cure malaria.

Previous studies with this drug established that it was well tolerated in single doses up to 1000 mg. Doses of 1250, 1500, and 1750 mg occasionally caused transient light-headedness or gastrointestinal symptoms. At 1250 mg only mild diarrhea lasting for  $\frac{1}{2}$  to 3 hours after taking the drug was reported. There was no associated nausea, vomiting or abdominal pain. At 1500 mg two subjects reported a transient sense of light-headedness, two subjects had mild diarrhea without other symptoms, and one subject vomited five minutes after taking the drug. At 1750 mg there was mild to moderate diarrhea, with the number of stools varying from 1 to 6 over a period of 35 minutes to 6 hours after taking the drug. Occasional nausea and mild abdominal cramps were also reported by some subjects.

This study is to compare the bioavailability of two different formulations. It will be conducted using one tablet formulation provided by Walter Reed Army Institute of Research, and the other provided by F. Hoffman-La Roche, & Co. The dose to be administered in both cases is 4 tablets\*. You may experience some of the symptoms discussed above. No other symptoms or long term effects are anticipated.

\*The four tablets for the Walter Reed formulation will constitute a single dose of 1000 mg of Mefloquine·HCl, and for the F. Hoffman-La Roche, & Co. preparation a single oral dose of 1100 mg Mefloquine·HCl.

Research Facility: George Hyman Research Building • 110 Irving Street, N. W. • Washington, D.C. 20010  
Mailing Address: 1295 Lavall Drive • Gambrills, Maryland 21054

We will study these two preparations by using a two way cross-over design. You will be assigned in a random manner to one of 3 groups designated Group I through III, each group containing 4 subjects. You will receive one drug preparation on one occasion and four weeks later the other formulation. You will be required to remain on the research unit for five days at the beginning of each period.

On the day the drug is to be administered, breakfast will be withheld. You will be permitted to drink water as you wish except for 3 hours encompassing the time of drug administration. The drug will be administered in the presence of a member of the investigating team at 8:00 AM. At 2:00 PM you may resume the normal diet you select from the hospital menu until you are discharged from the unit.

The purpose of the present study is to accurately determine the pattern in which the drug appears and disappears from your body. Blood (6 ml) will be drawn at the times specified in Table II after you take the drug to measure the amount of drug present. You will notice that the frequency of specimens is much greater during the early days of the study. In addition, we will collect all of your urine during the first 3 days encompassing the time of drug administration. You will be admitted to the research unit for the first 5 days and seen on brief visits for a period of 3 weeks. On the fourth week you will be readmitted to the research unit for a second 5 day period, after which you will be seen on brief visits thereafter per schedule. The long period of follow-up is required because of the expected slow release of the drug from the body. It is important that the blood be obtained as nearly as possible to the times specified in Table II and that you eat a light breakfast (i.e. cereal, milk, juice, coffee, bread -- no eggs or bacon) on the days you come in for blood drawing. It is also important that you avoid taking any other medication during the entire period and avoid the use of alcohol. Such factors as time of day, meals, alcohol, other drugs, and lack of proper sleep may affect the level of drug in your blood on any given day.

It is expected that the amount of drug remaining in your blood on the last day of the study will be very low. However, the late specimens are just as important as the early specimens in obtaining an overall accurate assessment of the way the drug is handled by the body. Therefore, please do not start the study if you anticipate difficulty in adhering to the schedule.

C  
The amount of blood to be withdrawn for the entire study will be 555 ml, which is obtained over a seven week period and is about 39 ml more than a unit of whole blood that many people donate at American Red Cross Center Blood Banks. Instead of repeated venipunctures we will place a small teflon catheter in an arm vein and obtain the blood samples from it during the first fifteen (15) hours. In this way repeated venipunctures may not be necessary. The specifics for the study are presented in the schematic on the following page.

SCHEMATIC STUDY PLAN FOR EACH DOSING

| DAY OF STUDY                  | 0* | 1* | 2* | 3* | 4* | 5 | 7 | 8 | 14 | 21 |
|-------------------------------|----|----|----|----|----|---|---|---|----|----|
| Dose                          |    | X  |    |    |    |   |   |   |    |    |
| Physical Exam                 | X  |    | X  |    |    |   | X |   |    | X  |
| Interview                     | X  | X  | X  | X  | X  |   | X |   |    | X  |
| Vital Signs                   | X  | X  | X  | X  | X  |   | X |   |    | X  |
| ECG                           | X  |    | X  |    |    |   | X |   |    | X  |
| Laboratory Tests <sup>+</sup> | X  |    | X  | X  |    |   | X |   |    | X  |
| Drug Assay <sup>++</sup>      |    | X  | X  | X  | X  | X | X | X | X  | X  |

\*Controlled Environment

<sup>+</sup>Glucose, BUN, Creatinine, Sodium, Potassium, Chloride, Carbon Dioxide, Uric Acid, Total Protein, Albumin, Globulin, Calcium, Phosphate, Cholesterol, Triglyceride, Alkaline Phosphatase, SGOT, SGPT, LDH, Total Bilirubin, CBC (differential and indices), Platelets, and Urinalysis. Additional studies will be done as clinically indicated.

<sup>++</sup>Drug Assay - Each subject immediately prior to drug administration and after dosing at 1, 2, 4, 6, 8, 10, 11, 12, 13, 15, 20, 24, 28, 34, 48, 56, 63, 72, 76, 80 hours and on days 5, 7, 8, 14, and 21.

On the days indicated by \*, the participants in the study will remain in a controlled environment. The entire group will remain together with a member of the Research Unit Staff and will function according to their direction. Facilities provided while participating in the study include room and board with a study-lounge area. Recreation (tennis and basketball) is also provided if weather conditions permit and supervision is available.

You have already had many of the examinations listed on the schematic as a part of your qualification examination. The laboratory tests require urine collections and venipunctures to obtain blood specimens. This will not affect you except for temporary discomfort associated with obtaining blood from your arm vein.

On the last study day for each subject final physical and laboratory evaluation will be done. All abnormal findings will cause follow-up until normalcy, stabilization or proper medical disposition is secured.

The Human Use Committee members are also looking after your safety. They insure that you are not subjected to undue risk and discomfort. A member of this committee will be available to speak with you at the Washington Hospital Center. After members of the investigating team and Human Use Committee are satisfied that you understand the study and written informed consent form you will be permitted to sign it. No subject may participate without a signed consent. By signing the informed consent you signify that the study has been explained to you with regard to its risks and requirements and you wish to participate.

It should be clear that your participation in this study is of no therapeutic value to you personally. The benefit, rather, is to others who live in parts of the world where malaria is a serious problem, and to Americans, civilian and military, who may travel to these areas. For this reason especially, your participation must be entirely voluntary with full knowledge of the personal risks and general benefits involved. Furthermore, you retain the right to withdraw your consent at any time without prejudice.

If after reading this document, you have any additional questions, please ask them before affixing your initials below and signing the consent form.

Initials of Participant

SUBJECT AGREEMENT

CONSENT TO PARTICIPATE AS A STUDY SUBJECT

I, \_\_\_\_\_, hereby give my informed consent to participate as a study subject in the study entitled, "Comparative Bioavailability and Pharmacokinetics of WR 142,490·HCl (Mefloquine Hydrochloride) and Mefloquine Hydrochloride·HLR."

The implications of my voluntary participation; the nature, duration and purpose; the methods by which it is to be conducted and the inconveniences and hazards which may reasonable be expected have been explained to me by Dr. \_\_\_\_\_, and are set forth in the document entitled, "EXPLANATION: ANTIMALARIAL DRUG PROJECT EXPERIMENT NUMBER 10, Comparative Bioavailability and Pharmacokinetics of WR 142,490·HCl (Mefloquine Hydrochloride) and Mefloquine Hydrochloride·HLR," which I have signed.

I understand that with all drug administration and clinical investigation there are associated potential discomforts and risks. The discomforts and potential risks of participation as a subject in this study have been explained to me and I freely and voluntarily accept them. I understand that I will attain no direct therapeutic benefits from participation in the study.

All questions and inquiries I have made regarding the study have been answered to my satisfaction and I understand that I have the right to ask questions concerning the study at any time and have them answered to my satisfaction. Further, I understand I am free to withdraw, without prejudice, my consent and participation from the project at any time; however, I may be requested to undergo further examination, if in the opinion of the attending physician, such examinations are necessary for my health or well-being.

I consent to the taking and publications of any photographs in the course of the study for the purpose of advancing medical science, provided that my identity will remain confidential.

I certify that I have read and understand the above consent and that the explanations therein were made to me and that all inapplicable paragraphs, if any, were stricken before I signed.

\_\_\_\_\_  
Date

\_\_\_\_\_  
Witness - Human Use Comm. Cert.

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Investigator Certification

\_\_\_\_\_  
Address

\_\_\_\_\_  
Witness

REAFFIRMATION OF CONSENT:

\_\_\_\_\_  
Date

\_\_\_\_\_  
Witness

\_\_\_\_\_  
Signature

EXPERIMENT NO. 10: COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF  
 WR 142,490-HCl (MEFLOQUINE HYDROCHLORIDE) AND MEFLOQUINE HYDROCHLORIDE-HLR

TABLE I: SYMPTOMS SUMMARY

| GROUP | CODE NUMBER | FORMULATION |          | Nausea                   |      | Loose Stool              |   | Headache               |        | Light-headedness |   | Other†                      |  |        |
|-------|-------------|-------------|----------|--------------------------|------|--------------------------|---|------------------------|--------|------------------|---|-----------------------------|--|--------|
|       |             | 1st Dose    | 2nd Dose | A*                       | B†   | A                        | B | A                      | B      | A                | B | A                           | B  |        |
| I     | 261         |             | HLR†     |                          |      | Watery stools x 3<br>2.0 |   |                        |        |                  |   |                             |  |        |
|       |             | WR†         |          |                          |      |                          |   | Right temporal<br>-1.0 |        |                  |   |                             |  |        |
|       | 262         | HLR         |          | 96.0                     | 72.0 |                          |   |                        |        |                  |   | Emesis x 2<br>108.0         | 122.0  |        |
|       |             |             | WR       | 1.0                      | 5.0  |                          |   |                        |        |                  |   | Anorexic, dysphoria<br>96.0 | 168.0  |        |
|       | 263         | HLR         |          |                          |      |                          |   | Chronic<br>144.0       | ≈ 0.15 |                  |   |                             | Pale, hyperventilation, after shower<br>54.0 | 0.3    |
|       |             |             |          |                          |      |                          |   | NOT DOSED              |        |                  |   |                             | Pain, dental abscess<br>336.0                | 192.0  |
|       | 264         | HLR         |          | "Queasy" feeling<br>0.45 | 1.15 |                          |   |                        |        |                  |   |                             |  |        |
|       |             | WR          |          |                          |      |                          |   |                        |        |                  |   |                             | Mild coryza<br>504.0                         | ≈ 96.0 |

\*A = Onset hours . minutes after dosing: (-) before dosing

†B = Duration hours . minutes after onset

†See Individual Subject Final Summary for details:

-HLR is designated as Formulation A

-WR is designated as Formulation B

TABLE I: SYMPTOMS SUMMARY (cont.)

| GROUP | CODE NUMBER | FORMULATION |          | Nausea |                            | Loose Stool                      |                            | Headache    |                         | Light-headedness           |       | Other† |  |
|-------|-------------|-------------|----------|--------|----------------------------|----------------------------------|----------------------------|-------------|-------------------------|----------------------------|-------|--------|--|
|       |             | 1st Dose    | 2nd Dose | A*     | B*                         | A                                | B                          | A           | B                       | A                          | B     | A      | B                                      |
| II    | 265         |             | HLR†     | -1.0   | "Queasy stomach"<br>≈ 8.0  | 48.0                             | Watery stool x 1<br>< 0.05 | 6.0         | Right temporal<br>≈ 8.0 |                            |       |        |  |
|       |             | WR†         |          | 3.30   | Stomach ache<br>≈ 0.20     |                                  |                            |             |                         | "Dizziness"<br>4.0         | ≈ 9.0 |        |  |
|       |             | HLR         |          |        |                            |                                  |                            | NO SYMPTOMS |                         |                            |       |        |  |
|       | 266         | WR          |          |        |                            |                                  | Watery stool x 1<br>≈ 8.0  |             |                         |                            |       |        |  |
|       | 267         | HLR         |          |        |                            |                                  |                            |             |                         |                            |       |        |  |
|       | 268         | HLR         |          | 20.0   | 2° to bloodletting<br>0.05 | 7 Day 1, then<br>1-2/day<br>0.45 | 144.0                      |             |                         | 2° to bloodletting<br>20.0 | 0.05  |        | Perianal pain -<br>fissures<br>≈ 168.0 |
|       |             | WR          |          | 7.0    | 3.0†                       |                                  |                            |             | 7.0                     | 3.0†                       |       |        | Vivid dreams,<br>nightmares<br>56.0    |
| III   | 269         | HLR         |          |        |                            |                                  |                            |             |                         |                            |       |        | Pain in<br>inguinal ring<br>60.0       |
|       |             |             | WR       |        |                            |                                  |                            |             |                         |                            |       |        | ≈ 2.0                                  |

\*A = Onset hours . minutes after dosing: (-) before dosing  
 #B = Duration hours . minutes after onset

†See Individual Subject Final Summary for details:  
 -HLR is designated as Formulation A  
 -WR is designated as Formulation B

TABLE I: SYMPTOMS SUMMARY (cont.)

| GROUP | CODE NUMBER | FORMULATION |          | Nausea |                                      | Loose Stool                            |             | Headache    |   | Light-headedness |   | Other† |   |
|-------|-------------|-------------|----------|--------|--------------------------------------|--|-------------|-------------|---|------------------|---|--------|---|
|       |             | 1st Dose    | 2nd Dose | A*     | B*                                   | A                                      | B           | A           | B | A                | B | A      | B   |
| III   | 270         | HLR†        |          |        |                                      |  |             |             |   |                  |   |        |   |
|       |             |             | WR†      |        |                                      | Watery stool x 1<br>6.0 < 0.05         |             |             |   |                  |   |        |   |
|       |             |             |          |        | Recurrent<br>2-3x / day<br>6.0 144.0 |  |             |             |   |                  |   |        |   |
|       | 271         |             | HLR      |        |                                      | Watery stools x 2<br>unrecorded < 24.0 |             |             |   |                  |   |        |   |
|       |             |             |          | WR     |                                      |  | NO SYMPTOMS |             |   |                  |   |        |   |
|       | 272         |             | HLR      |        |                                      |  |             |             |   |                  |   |        | Mild coryza &<br>sore throat<br>-24.0 144.0   |
|       |             |             |          | WR     |                                      |  |             |             |   |                  |   |        | Vaso-vagal reaction<br>at bloodletting<br>15.0 0.6  |
| I     | 273         |             | HLR      |        |                                      |  |             |             |   |                  |   |        |   |
|       |             |             |          | WR     |                                      |  |             | NO SYMPTOMS |   |                  |   |        | Mild coryza<br>"Common cold"<br>144.0 < 168.0<br>Vaso-vagal reaction<br>at bloodletting<br>-0.50 < 0.30 |

\*A = Onset hours . minutes after dosing: (-) before dosing

\*B = Duration hours . minutes after onset

†See Individual Subject Final Summary for details:

-HLR is designated as formulation A

-WR is designated as Formulation B

TABLE IIA: LABORATORY VALUES, NORMAL RANGE

April - June 1975

AMDP Subjects

Age: 21-45 Sex: Male Normal Population: n &gt; 100

Laboratory: National Health Laboratories

|                              | $\bar{x}$ | S.D. | $\pm 2$ S.D. |
|------------------------------|-----------|------|--------------|
| Glucose (mg/dl)              | 90        | 12   | 66-114       |
| BUN (mg/dl)                  | 15        | 3.1  | 9-21         |
| Creatinine (mg/dl)           | 1.1       | 0.16 | 0.8-1.4      |
| Sodium (mEq/L)               | 144       | 3.5  | 137-151      |
| Potassium (mEq/L)            | 4.4       | 0.41 | 3.6-5.2      |
| Chloride (mEq/L)             | 104       | 3.0  | 98-110       |
| Carbon Dioxide (mEq/L)       | 27        | 1.9  | 23-31        |
| Uric Acid (mg/dl)            | 6.0       | 1.0  | 4-8          |
| Total Protein (g/dl)         | 7.2       | 0.41 | 6.4-8.0      |
| Albumin (g/dl)               | 4.6       | 0.25 | 4.1-5.1      |
| Globulin (g/dl)              | 2.6       | 0.42 | 1.8-3.4      |
| Calcium (mg/dl)              | 10        | 0.44 | 9.0-10.9     |
| Phosphate (mg/dl)            | 3.5       | 0.50 | 2.5-4.5      |
| Cholesterol (mg/dl)          | 176       | 33   | 110-242      |
| Triglyceride (mg/dl)         | 111       | 58   | 0-207        |
| Alkaline Phosphatase (U/L)   | 60        | 17   | 26-94        |
| SGOT (U/L)                   | 23        | 12   | 0-47         |
| SGPT (U/L)                   | 17        | 15   | 0-47         |
| LDH (U/L)                    | 153       | 40   | 73-233       |
| Total Bilirubin (mg/dl)      | 0.58      | 0.34 | 0.0-1.3      |
| Hematocrit (Vol %)           | 45        | 2.52 | 40-50        |
| Hemoglobin (GMS %)           | 15        | 0.84 | 13.3-16.7    |
| WBC (thous/cu mm)            | 6.3       | 1.6  | 3.1-9.5      |
| RBC (million/cu mm)          | 5.0       | 0.36 | 4.3-5.7      |
| Lymph (%)                    | 39        | 9.8  | 19-59        |
| Seg. Neutrophils (%)         | 58        | 11   | 36-80        |
| MCV (cu microns)             | 90        | 3.85 | 82-98        |
| MCHC (%)                     | 33        | 2.0  | 29-37        |
| MCH (micro micro GM)         | 30        | 1.45 | 27-33        |
| Platelet Count (thous/cu mm) | 264       | 81   | 102-426      |

EXPERIMENT NO. 10: COMPARATIVE BIOAVAILABILITY AND PHARMACOKINETICS OF  
WR 142,490-HCl (MEFLOQUINE HYDROCHLORIDE) AND MEFLOQUINE HYDROCHLORIDE-HLR

TABLE IIB: ABNORMAL BIOCHEMICAL and HEMATOLOGY VALUES

| GROUP-<br>CODE<br>NUMBER | FORMULATION     |                  | TESTS                  | FIRST DOSE |           |      |      |       |           |      | SECOND DOSE |      |           |               |   |   |    | NORMAL RANGE |
|--------------------------|-----------------|------------------|------------------------|------------|-----------|------|------|-------|-----------|------|-------------|------|-----------|---------------|---|---|----|--------------|
|                          | 1st<br>Dose     | 2nd<br>Dose      |                        | Screen     | STUDY DAY |      |      | 7     | STUDY DAY |      |             | 0    | STUDY DAY |               |   | 7 | 21 |              |
|                          |                 |                  |                        |            | 0         | 2    | 3    |       | 0         | 2    | 3           |      | 0         | 2             | 3 |   |    |              |
| I-<br>261                | WR <sup>†</sup> | HLR <sup>†</sup> | Glucose                | 97         | 105       | 107  | 119* | 97    | 96        | 103  | 124*        | 108  | 92        | 66-114 mg/dl  |   |   |    |              |
|                          |                 |                  | Triglyceride           | 88         | 117       | 153  | 167  | 188   | 210*      | 128  | 182         | 284* | 220*      | 0 - 207 mg/dl |   |   |    |              |
|                          |                 |                  | SGPT                   | 40         | 37        | 35   | 30   | 42    | 32        | 33   | 35          | 56*  | 39        | 0 - 47 U/L    |   |   |    |              |
|                          |                 |                  | Eosinophiles           | 5          | 2         | 4    | 5    | 2     | 3         | 7*   | 3           | 3    | 4         | 0 - 5 %       |   |   |    |              |
| I-<br>262                | HLR             | WR               | Carbon Dioxide         | 35*        | 33*       | 34*  | 33*  | 31    | 32*       | 33*  | 34*         | 29   | 28        | 23 - 31 mEq/  |   |   |    |              |
|                          |                 |                  | Total Protein          | 7.2        | 6.8       | 6.3* | 6.3* | 7.1   | 7.2       | 6.5  | 7.5         | 7.1  | 7.1       | 6.4 - 8.0 g/  |   |   |    |              |
|                          |                 |                  | Albumin                | 4.6        | 4.3       | 3.9* | 4.0* | 4.5   | 4.2       | 4.0* | 4.6         | 4.4  | 4.4       | 4.1 - 5.1 g/  |   |   |    |              |
|                          |                 |                  | White Blood Cell Count | 6.7        | 5.4       | 7.3  | 6.8  | 11.0* | 6.2       | 5.8  | 5.9         | 6.8  | 11.2*     | 3.1-9.5 th/c  |   |   |    |              |
| I-<br>263                | HLR             | -                | Carbon Dioxide         | 28         | 30        | 29   | 28   | 32*   | -         | -    | -           | -    | -         | 23 - 31 mEq/  |   |   |    |              |
|                          |                 |                  | Uric Acid              | 4.6        | 5.2       | 5.5  | 4.5  | 3.6*  | -         | -    | -           | -    | -         | 4 - 8 mg/dl   |   |   |    |              |
|                          |                 |                  | Albumin                | 4.3        | 4.0*      | 4.2  | 4.2  | 4.2   | -         | -    | -           | -    | -         | 4.1 - 5.1 g/  |   |   |    |              |
| I-<br>264                | WR              | HLR              | Creatinine             | 0.7*       | 0.7*      | 0.5* | 0.5* | 0.6*  | 0.6*      | 0.6* | 0.5*        | 0.7* | 0.5*      | 0.8 - 1.4 mg/ |   |   |    |              |
|                          |                 |                  | Albumin                | 4.2        | 4.2       | 3.8* | 4.1  | 4.5   | 4.4       | 4.0* | 4.3         | 4.4  | 4.2       | 4.1 - 5.1 g/  |   |   |    |              |
| II-<br>265               | WR              | HLR              | Carbon Dioxide         | 33*        | 29        | 35*  | 33*  | 33*   | 33*       | 30   | 34*         | 32*  | 34*       | 23 - 31 mEq/  |   |   |    |              |
|                          |                 |                  | Total Protein          | 6.5        | 6.5       | 6.5  | 6.0* | 6.4   | 6.6       | 6.8  | 6.4         | 6.6  | 6.5       | 6.4 - 8.0 g/  |   |   |    |              |
|                          |                 |                  | Albumin                | 4.2        | 4.3       | 4.2  | 4.1  | 4.0*  | 4.3       | 4.5  | 4.1         | 4.2  | 4.1       | 4.1 - 5.1 g/  |   |   |    |              |
|                          |                 |                  | Cholesterol            | 232        | 242       | 258* | 210  | 207   | 243*      | 249* | 234         | 226  | 236       | 110 - 242 mg/ |   |   |    |              |
|                          |                 |                  | Alkaline Phosphatase   | 113*       | 100*      | 97*  | 93   | 99*   | 101*      | 107* | 97*         | 104* | 95*       | 26 - 94 U/L   |   |   |    |              |
|                          |                 |                  | LDH                    | 205        | 192       | 194  | 146  | 190   | 194       | 168  | 173         | 182  | 235*      | 73 - 233 U/l  |   |   |    |              |
| II-<br>266               | WR              | HLR              | Eosinophiles           | 1          | 0         | 2    | 1    | 3     | 1         | 6*   | 2           | 0    | 4         | 0 - 5 %       |   |   |    |              |
|                          |                 |                  | Carbon Dioxide         | 31         | 27        | 31   | 31   | 30    | 28        | 27   | 27          | 29   | 32*       | 23 - 31 mEq/  |   |   |    |              |
|                          |                 |                  | SGPT                   | 20         | 24        | 36   | 36   | 20    | 25        | 37   | 52*         | 38   | 48*       | 0 - 47 U/L    |   |   |    |              |

\*denotes abnormality

†HLR is designated as Formulation A and WR as Formulation B in Individual Subject Final Summaries

TABLE IIB: ABNORMAL BIOCHEMICAL and HEMATOLOGY VALUES (cont.)

| GROUP-<br>CODE<br>NUMBER | FORMULATION |             | TESTS                  | FIRST DOSE<br>STUDY DAY |      |      |       |       |       |       | SECOND DOSE<br>STUDY DAY |      |              |                 |           |    |    | NORMAL RANGE |
|--------------------------|-------------|-------------|------------------------|-------------------------|------|------|-------|-------|-------|-------|--------------------------|------|--------------|-----------------|-----------|----|----|--------------|
|                          | 1st<br>Dose | 2nd<br>Dose |                        | Screen                  | 0    | 2    | 3     | 7     | 0     | 2     | 3                        | 7    | 21           |                 |           |    |    |              |
|                          |             |             |                        |                         |      |      |       |       |       |       |                          |      |              | 26              | 32*       | 28 | 31 |              |
| II-<br>267               | HLR†        | WR†         | Carbon Dioxide         | 33*                     | 26   | 32*  | 28    | 31    | 28    | 28    | 32*                      | 29   | 34*          | 23 - 31 mEq     |           |    |    |              |
|                          |             |             | Hemoglobin             | 16.0                    | 16.0 | 16.4 | 16.9* | 14.9  | 16.0  | 16.8* | 17.1*                    | 15.5 | 16.4         | 13.3-16.7 g%    |           |    |    |              |
|                          |             |             | White Blood Cell Count | 7.2                     | 7.6  | 8.3  | 9.0   | 6.9   | 10.4* | 8.3   | 7.6                      | 6.6  | 6.7          | 3.1-9.5 th/     |           |    |    |              |
|                          |             |             | Lymphocytes            | 24                      | 20   | 23   | 20    | 17*   | 23    | 18*   | 16*                      | 16*  | 23           | 22              | 19 - 59 % |    |    |              |
|                          |             |             | Eosinophiles           | 6*                      | 1    | 8*   | 2     | 3     | 2     | 4     | 6*                       | 6*   | 2            | 6*              | 0 - 5 %   |    |    |              |
| II-<br>268               | HLR         | WR          | Carbon Dioxide         | 34*                     | 27   | 31   | 32*   | 31    | 28    | 28    | 30                       | 28   | 34*          | 23 - 31 mEq     |           |    |    |              |
|                          |             |             | Phosphate              | 2.7                     | 2.3* | 2.3* | 2.6   | 3.4   | 2.7   | 2.6   | 2.8                      | 2.8  | 4.1          | 2.5 - 4.5 m%    |           |    |    |              |
|                          |             |             | Hematocrit             | 47.8                    | 45.4 | 48.8 | 50.7* | 43.7  | 43.9  | 48.0  | 47.4                     | 45.3 | 45.3         | 40 - 50 Vol     |           |    |    |              |
|                          |             |             | Eosinophiles           | 0                       | 0    | 2    | 2     | 1     | 4     | 6*    | 0                        | 0    | 2            | 0 - 5 %         |           |    |    |              |
|                          |             |             | % ATL                  | 0                       | 0    | 0    | 0     | 0     | 0     | 0     | 0                        | 0    | 0            | 8*              | 0 %       |    |    |              |
| III-<br>269              | HLR         | WR          | Glucose                | 74                      | 84   | 85   | 80    | 72    | 50*   | 82    | 84                       | 92   | 66 - 114 mg% |                 |           |    |    |              |
|                          |             |             | Carbon Dioxide         | 34*                     | 32*  | 31   | 32*   | 30    | 30    | 28    | 29                       | 30   | 25           | 23 - 31 mEq     |           |    |    |              |
|                          |             |             | Triglyceride           | 132                     | 161  | 89   | 139   | 110   | 219*  | 142   | 139                      | 103  | 218*         | 0 - 207 mg/100  |           |    |    |              |
| III-<br>270              | HLR         | WR          | Carbon Dioxide         | 34*                     | 34*  | 30   | 32*   | 31    | 30    | 27    | 29                       | 33*  | 23 - 31 mEq  |                 |           |    |    |              |
|                          |             |             | Phosphate              | 2.5                     | 2.3* | 2.8  | 2.7   | 2.6   | 2.5   | 2.7   | 2.7                      | 2.9  | 2.6          | 2.5 - 4.5 m%    |           |    |    |              |
|                          |             |             | Eosinophiles           | 6*                      | 3    | 2    | 2     | 7*    | 7*    | 3     | 5                        | 5    | 4            | 0 - 5 %         |           |    |    |              |
| III-<br>271              | WR          | HLR         | Carbon Dioxide         | 30                      | 29   | 30   | 32*   | 27    | 28    | 27    | 30                       | 29   | 30           | 23 - 31 mEq     |           |    |    |              |
|                          |             |             | Albumin                | 4.5                     | 4.7  | 4.3  | 4.4   | 4.2   | 4.0*  | 4.2   | 4.7                      | 4.3  | 4.4          | 4.1 - 5.1 g/100 |           |    |    |              |
|                          |             |             | LDH                    | 223                     | 220  | 164  | 166   | 174   | 202   | 168   | 182                      | 188  | 246*         | 72 - 233 U/1    |           |    |    |              |
|                          |             |             | Total Bilirubin        | 0.7                     | 0.5  | 1.0  | 0.8   | 1.4*  | 0.3   | 0.5   | 1.1                      | 0.6  | 0.7          | 0.0 - 1.3 m%    |           |    |    |              |
|                          |             |             | Lymphocytes            | 28                      | 30   | 28   | 28    | 30    | 28    | 32    | 14*                      | 28   | 26           | 19 - 59 %       |           |    |    |              |
|                          |             |             | Segmenters             | 68                      | 64   | 60   | 70    | 68    | 68    | 58    | 84*                      | 68   | 73           | 36 - 80 %       |           |    |    |              |
| MCH                      | 32.8        | 31.1        | 32.5                   | 32.6                    | 31.5 | 31.6 | 32.8  | 33.1* | 32.3  | 32.6  | 27-33 micro              |      |              |                 |           |    |    |              |

\*denotes abnormality

†HLR is designated as Formulation A and WR as Formulation B in Individual Subject Final Summaries



APPENDIX II

Sample Times and Blood Concentrations  
of Mefloquine

PHSD 84 0083  
DW 15-8 18

Experiment #10

Deepak Bakhu #261 Age 21 Ht 5' 10" Wt 150 lbs

First-Order Formulation M. Lafayette  
 (based on 1-31-78 0015)

| Lead No. | Time Post Role | Actual Time | Count (No. of 100) |
|----------|----------------|-------------|--------------------|
| 1        | 0:15 min       | 0805        | 30                 |
| 2        | 1:00 time      | 0815        | 31                 |
| 3        | 1:00 hr        | 0915        | 57                 |
| 4        | 1:00 hr        | 1015        | 71                 |
| 5        | 1:00 hr        | 1107        | 100                |
| 6        | 1:00 hr        | 1407        | 100                |
| 7        | 1:00 hr        | 1610        | 103                |
| 8        | 1:00 hr        | 1812        | 104                |
| 9        | 1:00 hr        | 1905        | 105                |
| 10       | 1:00 hr        | 2015        | 140                |
| 11       | 1:00 hr        | 2100        | 100                |
| 12       | 1:00 hr        | 2240        | 100                |
| 13       | 1:00 hr        | 2350        | 100                |
| 14       | 1:00 hr        | 2810        | 107                |
| 15       | 1:00 hr        | 3155        | 105                |
| 16       | 1:00 hr        | 3200        | 103                |
| 17       | 1:00 hr        | 3307        | 115                |
| 18       | 1:00 hr        | 3400        | 106                |
| 19       | 1:00 hr        | 3500        | 110                |
| 20       | 1:00 hr        | 3600        | 110                |
| 21       | 1:00 hr        | 3700        | 117                |
| 22       | 1:00 hr        | 3800        | 110                |
| 23       | 1:00 hr        | 3900        | 110                |
| 24       | 1:00 hr        | 4000        | 110                |
| 25       | 1:00 hr        | 4100        | 110                |
| 26       | 1:00 hr        | 4200        | 110                |
| 27       | 1:00 hr        | 4300        | 110                |
| 28       | 1:00 hr        | 4400        | 110                |
| 29       | 1:00 hr        | 4500        | 110                |
| 30       | 1:00 hr        | 4600        | 110                |
| 31       | 1:00 hr        | 4700        | 110                |
| 32       | 1:00 hr        | 4800        | 110                |
| 33       | 1:00 hr        | 4900        | 110                |
| 34       | 1:00 hr        | 5000        | 110                |
| 35       | 1:00 hr        | 5100        | 110                |
| 36       | 1:00 hr        | 5200        | 110                |
| 37       | 1:00 hr        | 5300        | 110                |
| 38       | 1:00 hr        | 5400        | 110                |
| 39       | 1:00 hr        | 5500        | 110                |
| 40       | 1:00 hr        | 5600        | 110                |
| 41       | 1:00 hr        | 5700        | 110                |
| 42       | 1:00 hr        | 5800        | 110                |
| 43       | 1:00 hr        | 5900        | 110                |
| 44       | 1:00 hr        | 6000        | 110                |
| 45       | 1:00 hr        | 6100        | 110                |
| 46       | 1:00 hr        | 6200        | 110                |
| 47       | 1:00 hr        | 6300        | 110                |
| 48       | 1:00 hr        | 6400        | 110                |
| 49       | 1:00 hr        | 6500        | 110                |
| 50       | 1:00 hr        | 6600        | 110                |
| 51       | 1:00 hr        | 6700        | 110                |
| 52       | 1:00 hr        | 6800        | 110                |
| 53       | 1:00 hr        | 6900        | 110                |
| 54       | 1:00 hr        | 7000        | 110                |
| 55       | 1:00 hr        | 7100        | 110                |
| 56       | 1:00 hr        | 7200        | 110                |
| 57       | 1:00 hr        | 7300        | 110                |
| 58       | 1:00 hr        | 7400        | 110                |
| 59       | 1:00 hr        | 7500        | 110                |
| 60       | 1:00 hr        | 7600        | 110                |
| 61       | 1:00 hr        | 7700        | 110                |
| 62       | 1:00 hr        | 7800        | 110                |
| 63       | 1:00 hr        | 7900        | 110                |
| 64       | 1:00 hr        | 8000        | 110                |
| 65       | 1:00 hr        | 8100        | 110                |
| 66       | 1:00 hr        | 8200        | 110                |
| 67       | 1:00 hr        | 8300        | 110                |
| 68       | 1:00 hr        | 8400        | 110                |
| 69       | 1:00 hr        | 8500        | 110                |
| 70       | 1:00 hr        | 8600        | 110                |
| 71       | 1:00 hr        | 8700        | 110                |
| 72       | 1:00 hr        | 8800        | 110                |
| 73       | 1:00 hr        | 8900        | 110                |
| 74       | 1:00 hr        | 9000        | 110                |
| 75       | 1:00 hr        | 9100        | 110                |
| 76       | 1:00 hr        | 9200        | 110                |
| 77       | 1:00 hr        | 9300        | 110                |
| 78       | 1:00 hr        | 9400        | 110                |
| 79       | 1:00 hr        | 9500        | 110                |
| 80       | 1:00 hr        | 9600        | 110                |
| 81       | 1:00 hr        | 9700        | 110                |
| 82       | 1:00 hr        | 9800        | 110                |
| 83       | 1:00 hr        | 9900        | 110                |
| 84       | 1:00 hr        | 10000       | 110                |

Experiment 11

Deepak Bakshi #241 - continued

Second\_Dosage formulation 1 Hoffmann-La Roche  
 Dosed on 1/28/98 12:00

| Dose no. | Time Post Dose | Actual Time | Area Under Curve |
|----------|----------------|-------------|------------------|
| 1        | 15 min         | 0740        | 0                |
| 2        | 0 time         | 0800        | 0                |
| 3        | 1 hr           | 0900        | 139              |
| 4        | 2 hr           | 1005        | 187              |
| 5        | 4 hr           | 1100        | 213              |
| 6        | 6 hr           | 1207        | 221              |
| 7        | 8 hr           | 1305        | 230              |
| 8        | 10 hr          | 1315        | 237              |
| 9        | 11 hr          | 1307        | 244              |
| 10       | 12 hr          | 1401        | 251              |
| 11       | 13 hr          | 1401        | 257              |
| 12       | 15 hr          | 1500        | 264              |
| 13       | 17 hr          | 1604        | 271              |
| 14       | 19 hr          | 1706        | 278              |
| 15       | 21 hr          | 1805        | 285              |
| 16       | 24 hr          | 1907        | 292              |
| 17       | 26 hr          | 2000        | 299              |
| 18       | 28 hr          | 2100        | 306              |
| 19       | 30 hr          | 2200        | 313              |
| 20       | 32 hr          | 2300        | 320              |
| 21       | 34 hr          | 2400        | 327              |
| 22       | 36 hr          | 2500        | 334              |
| 23       | 38 hr          | 2600        | 341              |
| 24       | 40 hr          | 2700        | 348              |
| 25       | 42 hr          | 2800        | 355              |
| 26       | 44 hr          | 2900        | 362              |
| 27       | 46 hr          | 3000        | 369              |
| 28       | 48 hr          | 3100        | 376              |
| 29       | 50 hr          | 3200        | 383              |
| 30       | 52 hr          | 3300        | 390              |
| 31       | 54 hr          | 3400        | 397              |
| 32       | 56 hr          | 3500        | 404              |
| 33       | 58 hr          | 3600        | 411              |
| 34       | 60 hr          | 3700        | 418              |
| 35       | 62 hr          | 3800        | 425              |
| 36       | 64 hr          | 3900        | 432              |
| 37       | 66 hr          | 4000        | 439              |
| 38       | 68 hr          | 4100        | 446              |
| 39       | 70 hr          | 4200        | 453              |
| 40       | 72 hr          | 4300        | 460              |
| 41       | 74 hr          | 4400        | 467              |
| 42       | 76 hr          | 4500        | 474              |
| 43       | 78 hr          | 4600        | 481              |
| 44       | 80 hr          | 4700        | 488              |
| 45       | 82 hr          | 4800        | 495              |
| 46       | 84 hr          | 4900        | 502              |
| 47       | 86 hr          | 5000        | 509              |
| 48       | 88 hr          | 5100        | 516              |
| 49       | 90 hr          | 5200        | 523              |
| 50       | 92 hr          | 5300        | 530              |
| 51       | 94 hr          | 5400        | 537              |
| 52       | 96 hr          | 5500        | 544              |
| 53       | 98 hr          | 5600        | 551              |
| 54       | 100 hr         | 5700        | 558              |
| 55       | 102 hr         | 5800        | 565              |
| 56       | 104 hr         | 5900        | 572              |
| 57       | 106 hr         | 6000        | 579              |
| 58       | 108 hr         | 6100        | 586              |
| 59       | 110 hr         | 6200        | 593              |
| 60       | 112 hr         | 6300        | 600              |
| 61       | 114 hr         | 6400        | 607              |
| 62       | 116 hr         | 6500        | 614              |
| 63       | 118 hr         | 6600        | 621              |
| 64       | 120 hr         | 6700        | 628              |
| 65       | 122 hr         | 6800        | 635              |
| 66       | 124 hr         | 6900        | 642              |
| 67       | 126 hr         | 7000        | 649              |
| 68       | 128 hr         | 7100        | 656              |
| 69       | 130 hr         | 7200        | 663              |
| 70       | 132 hr         | 7300        | 670              |
| 71       | 134 hr         | 7400        | 677              |
| 72       | 136 hr         | 7500        | 684              |
| 73       | 138 hr         | 7600        | 691              |
| 74       | 140 hr         | 7700        | 698              |
| 75       | 142 hr         | 7800        | 705              |
| 76       | 144 hr         | 7900        | 712              |
| 77       | 146 hr         | 8000        | 719              |
| 78       | 148 hr         | 8100        | 726              |
| 79       | 150 hr         | 8200        | 733              |
| 80       | 152 hr         | 8300        | 740              |
| 81       | 154 hr         | 8400        | 747              |
| 82       | 156 hr         | 8500        | 754              |
| 83       | 158 hr         | 8600        | 761              |
| 84       | 160 hr         | 8700        | 768              |
| 85       | 162 hr         | 8800        | 775              |
| 86       | 164 hr         | 8900        | 782              |
| 87       | 166 hr         | 9000        | 789              |
| 88       | 168 hr         | 9100        | 796              |
| 89       | 170 hr         | 9200        | 803              |
| 90       | 172 hr         | 9300        | 810              |
| 91       | 174 hr         | 9400        | 817              |
| 92       | 176 hr         | 9500        | 824              |
| 93       | 178 hr         | 9600        | 831              |
| 94       | 180 hr         | 9700        | 838              |
| 95       | 182 hr         | 9800        | 845              |
| 96       | 184 hr         | 9900        | 852              |
| 97       | 186 hr         | 10000       | 859              |
| 98       | 188 hr         | 10100       | 866              |
| 99       | 190 hr         | 10200       | 873              |
| 100      | 192 hr         | 10300       | 880              |

Experiment #1

Gene Skop #262 Age 41 wt 134 lb wt 150 lb

First dosing terminated at 10:15 am - 10:15 am  
 Based on 1971 13 1000

| Time | Time | Cost | Dose | Actual time | Cost |
|------|------|------|------|-------------|------|
|      | 15   | min  |      | 1835        | 100  |
|      | 0    | time |      | 1800        | -    |
|      | 1    | hr   |      | 1900        | 100  |
|      | 2    | hr   |      | 2000        | 100  |
|      | 4    | hr   |      | 2100        | 200  |
|      | 5    | hr   |      | 2400        | 250  |
|      | 8    | hr   |      | 2558        | 348  |
|      | 10   | hr   |      | 2804        | 458  |
|      | 11   | hr   |      | 2900        | 508  |
|      | 12   | hr   |      | 3030        | 558  |
|      | 13   | hr   |      | 3070        | 608  |
|      | 15   | hr   |      | 3150        | 658  |
|      | 16   | hr   |      | 3200        | 708  |
|      | 14   | hr   |      | 3200        | 708  |
|      | 18   | hr   |      | 3300        | 758  |
|      | 4    | hr   |      | 3400        | 808  |
|      | 18   | hr   |      | 3500        | 858  |
|      | 16   | hr   |      | 3600        | 908  |
|      | 3    | hr   |      | 3700        | 958  |
|      | 17   | hr   |      | 3800        | 1008 |
|      | 1    | hr   |      | 3900        | 1058 |
|      | 4    | hr   |      | 4000        | 1108 |
|      | 14   | hr   |      | 4100        | 1158 |
|      | 10   | hr   |      | 4200        | 1208 |
|      | 11   | hr   |      | 4300        | 1258 |
|      | 12   | hr   |      | 4400        | 1308 |

Experiment #10

Drug: 5-ASA      #202      cont. used

Second dosing formulation: Caracetin  
Dosed on 1/18/13 2010

| Time (hr) | Time Post-Dose | Actual Time | Dose (mg) |
|-----------|----------------|-------------|-----------|
| 0         | 15 min         | 0745        | 0         |
| 0         | 0 time         | 0810        | 0         |
| 1         | 1 hr           | 0908        | 100       |
| 2         | 2 hr           | 1010        | 100       |
| 3         | 3 hr           | 1100        | 100       |
| 4         | 4 hr           | 1200        | 100       |
| 5         | 5 hr           | 1300        | 100       |
| 6         | 6 hr           | 1400        | 100       |
| 7         | 7 hr           | 1500        | 100       |
| 8         | 8 hr           | 1600        | 100       |
| 9         | 9 hr           | 1700        | 100       |
| 10        | 10 hr          | 1800        | 100       |
| 11        | 11 hr          | 1900        | 100       |
| 12        | 12 hr          | 2000        | 100       |
| 13        | 13 hr          | 2100        | 100       |
| 14        | 14 hr          | 2200        | 100       |
| 15        | 15 hr          | 2300        | 100       |
| 16        | 16 hr          | 2400        | 100       |
| 17        | 17 hr          | 2500        | 100       |
| 18        | 18 hr          | 2600        | 100       |
| 19        | 19 hr          | 2700        | 100       |
| 20        | 20 hr          | 2800        | 100       |
| 21        | 21 hr          | 2900        | 100       |
| 22        | 22 hr          | 3000        | 100       |
| 23        | 23 hr          | 3100        | 100       |
| 24        | 24 hr          | 3200        | 100       |
| 25        | 25 hr          | 3300        | 100       |
| 26        | 26 hr          | 3400        | 100       |
| 27        | 27 hr          | 3500        | 100       |
| 28        | 28 hr          | 3600        | 100       |
| 29        | 29 hr          | 3700        | 100       |
| 30        | 30 hr          | 3800        | 100       |
| 31        | 31 hr          | 3900        | 100       |
| 32        | 32 hr          | 4000        | 100       |
| 33        | 33 hr          | 4100        | 100       |
| 34        | 34 hr          | 4200        | 100       |
| 35        | 35 hr          | 4300        | 100       |
| 36        | 36 hr          | 4400        | 100       |
| 37        | 37 hr          | 4500        | 100       |
| 38        | 38 hr          | 4600        | 100       |
| 39        | 39 hr          | 4700        | 100       |
| 40        | 40 hr          | 4800        | 100       |
| 41        | 41 hr          | 4900        | 100       |
| 42        | 42 hr          | 5000        | 100       |
| 43        | 43 hr          | 5100        | 100       |
| 44        | 44 hr          | 5200        | 100       |
| 45        | 45 hr          | 5300        | 100       |
| 46        | 46 hr          | 5400        | 100       |
| 47        | 47 hr          | 5500        | 100       |
| 48        | 48 hr          | 5600        | 100       |
| 49        | 49 hr          | 5700        | 100       |
| 50        | 50 hr          | 5800        | 100       |
| 51        | 51 hr          | 5900        | 100       |
| 52        | 52 hr          | 6000        | 100       |
| 53        | 53 hr          | 6100        | 100       |
| 54        | 54 hr          | 6200        | 100       |
| 55        | 55 hr          | 6300        | 100       |
| 56        | 56 hr          | 6400        | 100       |
| 57        | 57 hr          | 6500        | 100       |
| 58        | 58 hr          | 6600        | 100       |
| 59        | 59 hr          | 6700        | 100       |
| 60        | 60 hr          | 6800        | 100       |
| 61        | 61 hr          | 6900        | 100       |
| 62        | 62 hr          | 7000        | 100       |
| 63        | 63 hr          | 7100        | 100       |
| 64        | 64 hr          | 7200        | 100       |
| 65        | 65 hr          | 7300        | 100       |
| 66        | 66 hr          | 7400        | 100       |
| 67        | 67 hr          | 7500        | 100       |
| 68        | 68 hr          | 7600        | 100       |
| 69        | 69 hr          | 7700        | 100       |
| 70        | 70 hr          | 7800        | 100       |
| 71        | 71 hr          | 7900        | 100       |
| 72        | 72 hr          | 8000        | 100       |
| 73        | 73 hr          | 8100        | 100       |
| 74        | 74 hr          | 8200        | 100       |
| 75        | 75 hr          | 8300        | 100       |
| 76        | 76 hr          | 8400        | 100       |
| 77        | 77 hr          | 8500        | 100       |
| 78        | 78 hr          | 8600        | 100       |
| 79        | 79 hr          | 8700        | 100       |
| 80        | 80 hr          | 8800        | 100       |
| 81        | 81 hr          | 8900        | 100       |
| 82        | 82 hr          | 9000        | 100       |
| 83        | 83 hr          | 9100        | 100       |
| 84        | 84 hr          | 9200        | 100       |
| 85        | 85 hr          | 9300        | 100       |
| 86        | 86 hr          | 9400        | 100       |
| 87        | 87 hr          | 9500        | 100       |
| 88        | 88 hr          | 9600        | 100       |
| 89        | 89 hr          | 9700        | 100       |
| 90        | 90 hr          | 9800        | 100       |
| 91        | 91 hr          | 9900        | 100       |
| 92        | 92 hr          | 10000       | 100       |

Experiment #10

Fred D. Amosani #263 Age 56 Ht 58" Wt 145 lbs

First dosing formulation - Hoffmann-La Roche  
 Dose on 1/31/53 1953

| Time | Time Post Dose | Urine Volume | Radioactivity |
|------|----------------|--------------|---------------|
|      | 15 min         | 0.10         | 0             |
|      | 0 time         | 0.05         | 0             |
| 0000 | 1 hr           | 0.15         | 0             |
|      | 1 hr           | 0.05         | 100           |
|      | 4 hr           | 0.05         | 100           |
|      | 5 hr           | 0.05         | 50            |
|      | 8 hr           | 0.05         | 111           |
|      | 10 hr          | 0.05         | 148           |
|      | 11 hr          | 0.05         | 200           |
|      | 12 hr          | 0.05         | 10            |
|      | 13 hr          | 0.05         | 20000         |
|      | 15 hr          | 0.05         | 100           |
|      | 16 hr          | 0.05         | 535           |
|      | 14 hr          | 0.05         | 543           |
|      | 18 hr          | 0.05         | 574           |
|      | 14 hr          | 0.05         | 593           |
|      | 18 hr          | 0.05         | 593           |
|      | 18 hr          | 0.05         | 148           |
|      | 18 hr          | 0.05         | 507           |
|      | 13 hr          | 0.05         | 1000          |
|      | 17 hr          | 0.05         | 100           |
|      | 16 hr          | 0.05         | 100           |
|      | 18 hr          | 0.05         | 100           |
|      | 148-150 hr     | 0.05         | 100           |
|      | 148-170 hr     | 0.05         | 100           |
|      | 112-114 hr     | 0.05         | 100           |
|      | 107-104 hr     | 0.05         | 100           |

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