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Influence of Pamaquine and Atabrine on the Enzymatic Degradation of Quinine.

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The purpose of the present investigation is (1) to devise a procedure for the quantitative evaluation of the activity of the enzyme which destroys quinine and (2) to study the effect of pamaquine and atabrine on the degradation of quinine by the enzyme.

Materials and Methods. Suspensions of fresh rabbit's liver were prepared by homogenization with Ringer-Locke solution in a Waring blender. They were centrifuged at 1200 r.p.m. for 15 minutes; the supernatant only was used in the following experiments. The mixture and the supernatant were found to give the same enzyme activity and the same relationship between concentration and effect. However, more consistent results were obtained with the latter.

The enzymatic digestion was conducted at 39°C in an incubator. The enzyme preparation and the quinine solution of Ringer-Locke were kept in an incubator for half an hour before mixing. As a rule, 0.5 ml of the former was pipetted into 3.5 ml of the latter in a 30 ml centrifuge tube. At appropriate times equal volume of 4% NaOH was added to the digestion medium to terminate the reaction.

The determination of quinine was carried out by a method which has previously been described.[1] In brief, quinine was extracted from the digestion medium at an alkalinity of 2% NaOH with ethylene dichloride. In control experiments, with and without heating on a steam bath for an hour, the amount of quinine extracted with ethylene dichloride at this concentration of NaOH was found to be the same. The concentration of quinine was measured fluorometrically with the Coleman photofluorometer (model 12). Pamaquine does not give any fluorometric reading with B_1, PC_1 filters. Atabrine shows a slight fluorescence through these filters; it does not, however, interfere with quinine determination. A blank value for atabrine is subtracted from the total fluorometric readings when quinine is determined in the presence of atabrine.

Results and Discussion. The graphs in Fig. 1 indicate the relationships between the percentage degradation of quinine and the concentration of liver. The rate of the reaction is shown by curves in Fig. 2. Except for low concentrations and during the beginning of the experiment, the degradation of quinine is in a linear relationship with the concentration of liver or with the time of reaction in logarithms. The linearity in rate was found to

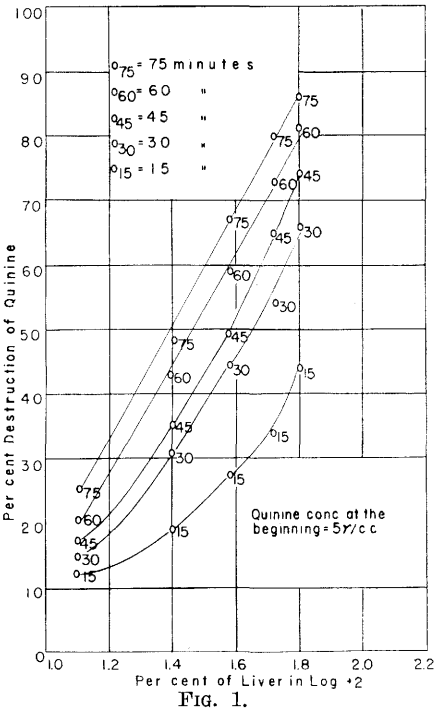

FIG. 1.

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1 Chen, G., and Geiling, E. M. K., *J. Pharm. and Exp. Therap.*, 1944, **82**, 120.

hold as long as 8 hours for a digestion medium containing 5 μg/ml of quinine and 0.25% of liver. Whether or not this relationship holds true for the pure enzyme and quinine awaits confirmation. If so, it may reveal the reaction mechanism of the enzymatic process.

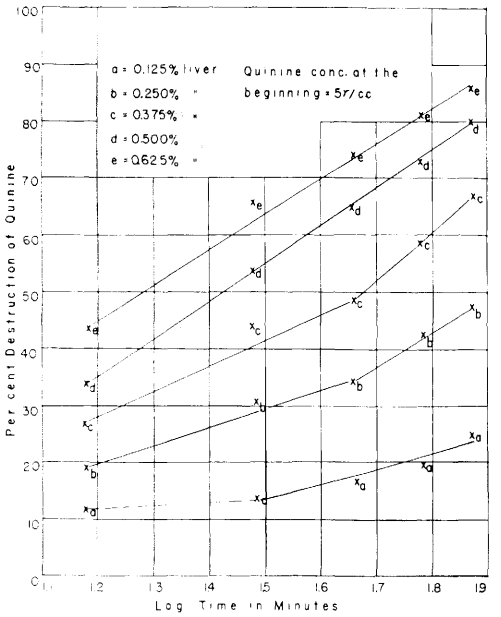

FIG. 2.

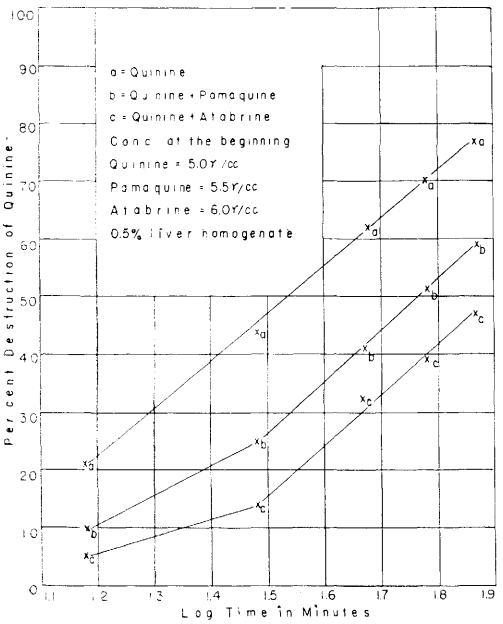

FIG. 3.

Clearly the linear relationship between concentration and effect for a certain duration of reaction, as shown in Fig. 1, may be utilized to determine quantitatively the enzyme activity of a preparation. The duration of one hour was chosen in the present work. The relationship deviates from linear at shorter intervals. The line for one hour will be taken as a reference standard in subsequent calculations for the enzyme activity of a sample by locating the corresponding value on the abscissa to the per cent degradation of quinine on the ordinate. For convenience in calculations, the value one on the abscissa will be considered as 10 units of enzyme activity.

The effect of pamaquine and the effect of atabrine on the enzymatic destruction of quinine were carried out under identical conditions as for the experiment in obtaining the reference standard; *i.e.* in a medium containing the supernatant of a 0.5% liver homogenate, 5 μg per ml of quinine and a molecular equivalent quantity of pamaquine or atabrine. Data in Fig. 3, obtained with the same sample of liver homogenate, indicate that the enzymatic destruction of quinine was decreased by the addition of pamaquine or atabrine. There is, however, no difference in the rate of the reaction. By interpolation from the line for one hour in Fig. 1, the enzyme activities as represented by the curves in Fig. 3 for the same period are as follows: for quinine alone, 50.1 units; quinine plus pamaquine, 30.2 units and for quinine plus atabrine, 22.4 units. The suppression of enzyme activity by pamaquine or atabrine is thus 39 and 54% respectively. It is not understood whether the effect of these two compounds is on the enzyme or on the substrate in the enzyme system. As judged by the rate of the reactions with and without pamaquine or atabrine, the effect of the two compounds is probably on the enzyme.

Summary. A procedure has been devised for the quantitative evaluation of the activity of the enzyme which degradates quinine. Pamaquine and atabrine were found to suppress the enzymatic degradation of quinine by a liver homogenate.