[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

The Effect of Pamaquin on the Oxygen Consumption of Liver Slices[1]

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Interest in Pamaquin (Plasmochin) and related 8-aminoquinoline drugs has been stimulated recently by confirmation of the fact that these substances exert an unique curative action against relapsing malaria (*Plasmodium vivax*) when administered in conjunction with quinine.[3,4] Various theories have been proposed to account for this unique action of Pamaquin. These are discussed in some detail elsewhere.[3] As part of a broad general program dealing with the mechanism of antimalarial action of the 8-aminoquinolines, a study of the effect of Pamaquin on the oxygen uptake of liver tissues *in vitro* has been undertaken.

In the only previously published study of this nature Nandi[5] has reported that "the drug (Pamaquin) always stimulates the oxygen uptake or the respiration of the normal tissues." This report is so at variance with observations along similar lines dealing with suppressive types of drugs such as Quinacrin[6] and the naphthoquinone antimalarials[7] as well as with the reported inhibitory effect of Pamaquin and other antimalarials upon *d*-amino acid oxidase activity[8] that it was felt that a further investigation of the problem might furnish significant information concerning the specific action of the 8-aminoquinolines.

Accordingly we have investigated the effect of Pamaquin on the oxygen uptake of slices of rat and guinea pig livers using the conventional Warburg manometric techniques. The results are summarized in Table I.

When concentrations of drug of the order of 1–6 $\times 10^{-4}$ *M* were used the effect on the oxygen uptake of guinea pig liver slices appears to be in sharp disagreement with the observations reported by Nandi.[5] In no case were we able to observe the marked stimulation of oxygen uptake reported by him. It appears that the results reported by Nandi may be peculiar to the rather unusual conditions of his experiments. The "Mammalian Ringer" medium prepared according to Nandi was found to have a *p*H of 4.53; the gas atmosphere in his experiments appears to have been air. These conditions probably account for the very low values of the Q_{O_2} in his controls (200, 185, 135), and the apparent great stimulations of the Q_{O_2} in the presence of Pamaquin. In the present work, carried out under more nearly physiological conditions, the *p*H was buffered at 7.4 and the atmosphere was oxygen. Using guinea pig liver slices, no discernible effect on Q_{O_2} was noted with drug concentrations up to 6.4 $\times 10^{-4}$ *M*. At higher concentrations a definite inhibition of oxygen uptake became apparent.

With rat liver slices oxygen uptake at drug concentrations which produce no effect on guinea pig liver is definitely stimulated. At higher concentrations inhibition occurs. The reason for this anomalous behavior is not apparent. The fact that the degree of stimulation of the oxygen uptake is independent of the concentration of the drug over the pertinent range of concentration suggests that the drug plays a catalytic role in this case.

The inhibitory action of 2–4 $\times 10^{-3}$ *M* Pamaquin on the respiration of liver slices presents a somewhat clearer picture. Unpublished work from this Laboratory has indicated that the effect

TABLE I

THE EFFECT OF PAMAQUIN AT VARIOUS CONCENTRATIONS ON THE OXYGEN UPTAKE OF LIVER SLICES

Concentration of drug, *M*	(Control)	1.6×10^{-4}	3.2×10^{-4}	4.8×10^{-4}	6.4×10^{-4}	2×10^{-3}	3×10^{-3}	4×10^{-3}
Guinea Pigs								
Q_{O_2}[a]	322 ± 17[b]	319 ± 12	334 ± 9	337 ± 16	335 ± 11	249 ± 12	235 ± 17	224 ± 15
% change[c]		0	0	0	0	−22	−27	−30
Animals	22	11	11	11	11	11	11	11
Rats								
Q_{O_2}[a]	727 ± 12[b]	996 ± 55	955 ± 41	986 ± 38	971 ± 48	614 ± 36	558 ± 49	492 ± 28
% change[c]		+38	+37	+36	+34	−16	−23	−32
No. of animals	16	11	11	11	11	4	4	6

[a] Q_{O_2} = μl.O_2 taken up per 100 mg. dry weight of tissue per hour. [b] The deviations are mean deviations (A. D.). [c] The percentage coefficients indicated have been calculated on the basis of the differences of the Q_{O_2}'s. The coefficient is calculated by taking the difference between the control and experimental values, and expressing this difference as a percentage of the former value.

(1) The work here reported was done under a grant from the National Institutes of Health to Columbia University.

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(3) Wiselogle, "Survey of Antimalarial Drugs," Vol. I, Edwards Bros., Ann Arbor, Mich., 1946.

(4) Berliner, *et al.*, *J. Clin. Investigation*, **27**, 108 (1948); Alving, *et al.*, *ibid.*, **27**, 34 (1948).

(5) Nandi, *J. Malaria Inst. India*, **3**, 475 (1940).

(6) Wright and Sabine, *J. Biol. Chem.*, **155**, 315 (1944).

(7) Ball, Anfinsen and Cooper, *ibid.*, **168**, 257 (1947).

(8) Hellerman, Lindsay and Bovarnick, *ibid.*, **163**, 553 (1946).

at these concentrations is observed under a variety of conditions. The fact that the respiration of liver slices is practically unaffected during the first hour of the experiment is interesting. There may be a delayed penetration of the tissues by the drug. Ball and co-workers[7] have suggested such a scheme to explain a similar delayed inhibitory effect on liver tissue respiration by the naphthoquinones. A second possibility, which we are inclined to favor on the basis of information presently at hand, is that the drug is converted in the presence of the tissue enzyme systems into one or more degradation products which are the actual inhibitory agents. A study of the degradation of Pamaquin and related drugs in the presence of tissues is under way at present.

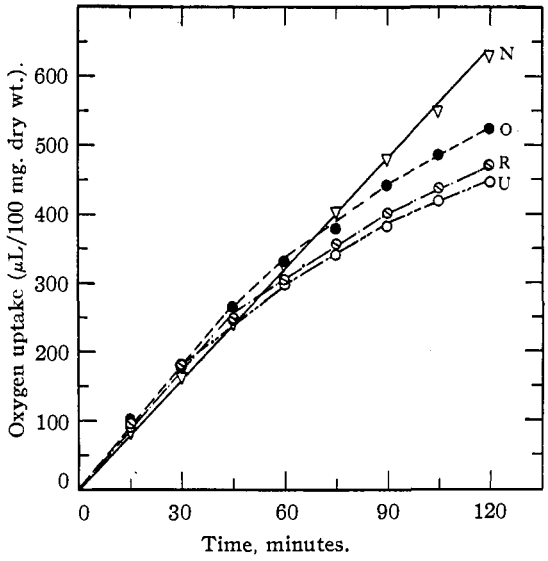

Fig. 1.—The effect of Pamaquin upon the oxygen consumption of guinea pig liver (the points represent the arithmetical mean of 11 observations in each case): ▽, N, control; ●, O, 2×10^{-3} *M*; ⊘, R, 3×10^{-3} *M*; ○, U, 4×10^{-3} *M*.

Experimental

Male guinea pigs (Albany strain) weighing approximately 250-350 grams and male white rats (Sherman strain) weighing approximately 150-200 g. were used. The animals were killed by a blow over the occiput and the blood was drained from the internal organs by decapitation. The liver was quickly removed and was kept in a beaker surrounded by ice until slicing. The same lobe (largest) of the liver was used in all experiments, and cross section slices (0.4-0.5 mm. thick) were made. The slices were suspended in Krebs-Ringer (0.1 *M* phosphate solution[9] (adjusted to *p*H 7.4) containing 0.2% glucose. Dry weights were determined at the end of the experiment by placing the tissues in vials, rinsing once with distilled water, and drying overnight at 105°. The time elapsing between the death of an animal and the start of the equilibration period was approximately thirty minutes.

The tissues (approximately 40 mg. final dry weight of guinea pig liver, and 22 mg. final dry weight of rat liver) were placed in the Ringer phosphate-glucose solution in Warburg flasks of approximately 15 ml. volume with a side arm and central well. A solution of Pamaquin citrate in the Ringer phosphate-glucose medium was placed in the side arm, and 0.1 ml. of 20% potassium hydroxide (and filter paper roll) was placed in the center cup. In all cases, the over-all volume of liquid was 3.0 ml.

The flasks were flushed with oxygen for one minute and, after a ten-minute equilibration period in the thermostat bath (at 37 ± 0.1°) the drug solutions were tipped in. Equilibration was continued for another five minutes before the start of measurements. Readings were taken every fifteen minutes for a period of two hours. The rate of shaking was 120 times per minute.

The concentrations of Pamaquin given are the concentrations finally present in the solutions. In the more dilute solutions, where an attempt was made to duplicate the concentrations employed by Nandi,[5] it was assumed that Nandi's "parts per thousand" expressed the grams of free drug base per thousand grams of solution.

The results are summarized in Table I. In Fig. 1 rate curves are given for the oxygen uptake noted in several experiments.

Summary

The presence of 1.6 to 6.4×10^{-4} *M* Pamaquin has been found to have no effect upon the oxygen uptake of guinea pig liver slices. The same concentration of drug causes an apparent stimulation in the oxygen uptake of rat liver slices.

The oxygen uptake of both guinea pig and rat liver slices has been shown to be inhibited by concentrations of 2 to 4×10^{-3} *M* Plasmochin. The respiration was found to be practically unaffected during the first hour of the experiment; the inhibitory action, proportional to the concentration of drug, began to appear at the end of this time.

NEW YORK 27, N. Y. RECEIVED NOVEMBER 17, 1948

(9) Krebs and Henseleit, *Z. physiol. Chem.*, **210**, 33 (1932).