warm 40-45°C. Twenty to 30 seconds were required to complete each injection. At the beginning of each injection there was an aching pain which was in every way similar to that experienced during aspiration. This pain diminished before the injection had been completed. There was no temperature sensation experienced by the subject with either the hot or the cold saline solution.

The second and third subjects did not experience the constant deep burning pain noted by the first, but they did experience pain during the initial seconds of saline injection. Neither of these subjects experienced any temperature sensation with the injection of 1.5 ml of isotonic saline ranging in temperature from 16°C to 45°C. No sensations of pressure were felt by the subjects during any of the procedures.

We conclude from these observations that structures in the sternal marrow cavity are pain sensitive while being insensitive to temperature within the range studied. The pain results from traction and pressure upon these sensitive structures without giving rise to a sense of pressure or fullness.

Summary. Pain and temperature sensitivity of the structures in the sternal bone marrow cavity was tested on three normal subjects. Four injections of 1 ml of hot (40-45°C) and cold (16-22°C) saline were made through the anesthetized skin and periosteum of each subject. No temperature sensations were reported although a deep burning pain and an aching pain were reported at the beginning of each injection.

Received July 12, 1950. P.S.E.B.M., 1950, v74.

Metabolites of Pamaquine in Urine.* (18065)

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Pamaquine, (8-(4-Diethylamino-l-methylbutylamino)-6-methoxyquinoline), a derivative of 8-aminoquinoline, exerts a curative action in mosquito-transmitted vivax malaria presumably by attacking a tissue form of the plasmodium which is responsible for relapses (1-4). Two pieces of evidence suggest that the compound exerts its activity *in vivo* through a metabolic transformation product. One, there is no correlation between the mean plasma concentration of pamaquine and its antimalarial effect(3) and two, the drug, although it exerts considerable activity against plasmodia *in vivo*, is relatively inactive in growing cultures of erythrocytic parasites(5).

The usefulness of pamaquine is limited by the frequent occurrence of toxic effects with doses in the therapeutic range. The adverse reactions include the regular production of methemoglobin and the occasional occurrence of acute hemolytic anemia. Since the drug does not produce methemoglobin nor hemolysis *in vitro*, except in concentrations far in excess of those achieved in plasma

* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and New York University.

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on therapeutic dosage, it appears likely that its toxicity also is mediated through a metabolically derived product.

Although pamaquine is known to be extensively metabolized in the body(6) little is known concerning its pathway of metabolism. A recent paper by Hughes and Schmidt(7) indicates that pamaquine and its homologues are transformed, in part, in the rhesus monkey to water soluble derivatives with acidic properties. These substances were not identified. In the present work, a preliminary study has been made of certain of the basic metabolic products of pamaquine in urine and plasma. The specific purpose of these studies was to acquire information which bears on the possibility that such metabolic transformation products might account for the toxic or therapeutic effects of pamaquine.

Experimental. It was observed that a few drops of urine or plasma of humans or dogs who were receiving pamaquine produced methemoglobin when added to hemolysed red cells, and hemolysis when added to intact red cells. Erythrocytes of dogs were much more susceptible to the hemolytic action than were those of humans. Below pH 5-6, urine stored at low temperature retained these properties for considerable periods of time but lost them with progressively increasing rapidity as the pH was raised. The loss in oxidative ability almost exactly paralleled the loss in ability to lyse erythrocytes, suggesting that the two properties were possessed by the same substance or substances.

Partial purification of the material responsible for the effects on blood was accomplished by extracting it from cooled urine at pH 10 into cooled chloroform and then returning it to a pH 6 phosphate buffer. This procedure resulted in the loss of a considerable portion of the activity because of the instability of the material at the alkaline pH necessary for its extraction. The extracted material appeared yellow and showed an absorption maximum at 460 mu. However, this absorption may have been due to the presence of other metabolic transformation products. Attempts at further purification were unsuccessful because of the instability of the substance.

8-Aminoquinoline administered to dogs also imparted to the urine the two properties of hemolysing red cells and converting hemoglobin to methemoglobin. 8-Aminoquinoline induced these effects only *in vivo,* but 5-hydroxy-8-aminoquinoline was found to hemolyse red cells and to form methemoglobin *in vitro.*

The urine and plasma of patients and dogs receiving pamaquine contained another transformation product which was highly fluorescent in acid solution. This substance was found to be stable in acid and alkaline solution. It possessed neither methemoglobin-forming nor hemolytic properties. Unlike pamaquine it did not couple with diazotized sulfanilic acid. The substance was partly purified as follows: It was extracted from alkaline urine into ethylene dichloride, and then returned to 0.1N H_2SO_4. The acid solution was made alkaline and extracted several times with heptane. The basic metabolite in the heptane was then returned to 0.1N H_2SO_4. The acid phase was then adjusted to pH 6 and extracted several times with petroleum ether. This served to remove pamaquine, while leaving the metabolic substance in the aqueous phase. The absorption spectrum of the substance was measured and was found to be distinctly different from that of pamaquine (Fig. 1). The sharply defined peak of the absorption curve at 250 mu suggests the presence of a single absorbing substance, though it is possible that it is representative of a mixture of two or more products. No attempt was made to isolate the material in crystalline form because of the minute amounts of material involved.

The fluorescent metabolite in urine could be roughly estimated by the methyl orange reaction(8) on the assumption that its molecular weight is about the same as that of the parent drug. Subjects receiving 60 mg per day of pamaquine excreted 0.3-2 mg of the

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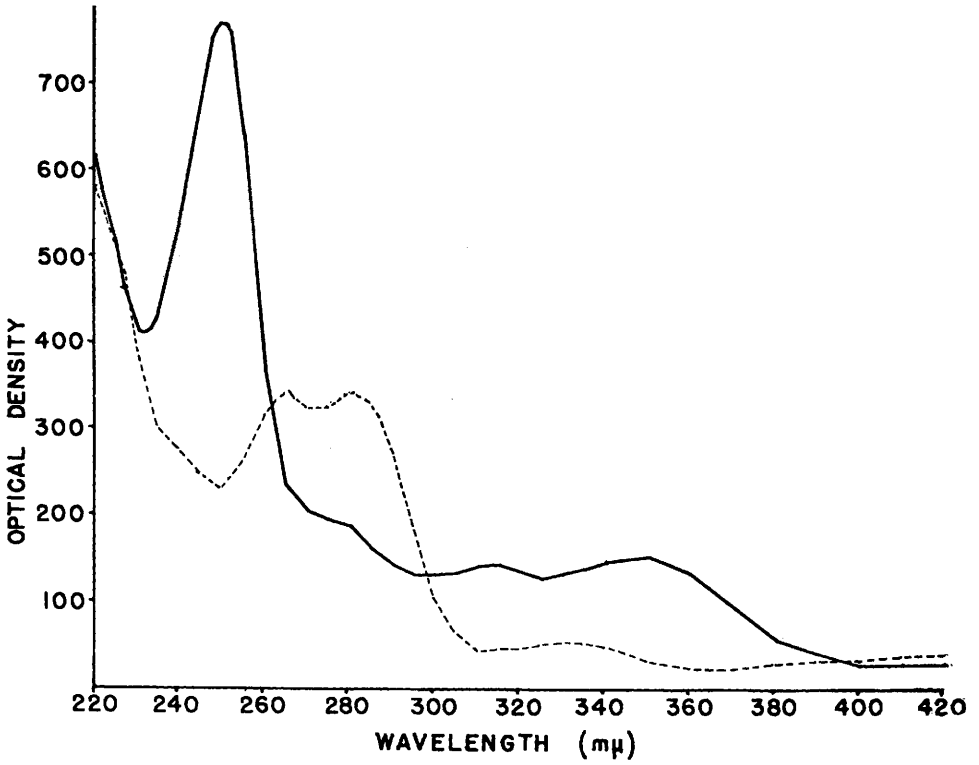

FIG. 1.
The absorption spectra of pamaquine (broken line) and the fluorescent metabolic product of pamaquine (solid line) in 0.1 N H_2SO_4. Concentration of pamaquine was 10 μg per ml; that of the metabolite was unknown. Cell thickness = 1 cm.

metabolite in the urine per day, and exhibited mean plasma levels of 5-30 μg per liter. These plasma levels are considerably lower than those of the parent compound(6). One subject was unusual in that he attained a plasma level of the substance of 350 μg per liter and excreted about 10 mg per day.

A homologue of pamaquine, 8-(3-diethylaminopropylamino) - 6 - methoxyquinoline, which differs only in the composition of the side chain, was administered to dogs and was also found to transmit to the urine a fluorescent substance and a substance or substances with hemolytic and methemoglobin-producing properties. Both these materials had solubility characteristics similar to the ones formed from pamaquine.

Discussion. Since neither of the two pamaquine metabolites was isolated in crystalline form, it was not possible to identify them. The fact that 8-aminoquinoline forms methemoglobin and lyses erythrocytes *in vivo*, but not *in vitro*, whereas its 5-hydroxy derivative is active *in vitro*, suggests that 8-aminoquinoline and its derivatives, such as pamaquine, may be converted in the body to 5-hydroxy derivatives, which then act as methemoglobin formers through reversible conversion to a quinonimine (Blanchard and Schmidt)(9).

It is possible that the oxidant metabolic material found in plasma and urine is directly responsible for at least some of the noxious effects of pamaquine in man. Its presence in blood suggests a direct link with the formation of methemoglobin. Its connection with the occurrence of acute hemolytic anemia is not clear since the latter occurs only seldom, whereas methemoglobinemia results uniformly. It may be that this substance causes hemolytic anemia when some other predisposing factor is also present.

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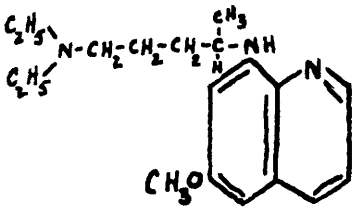
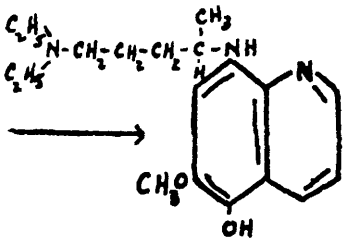
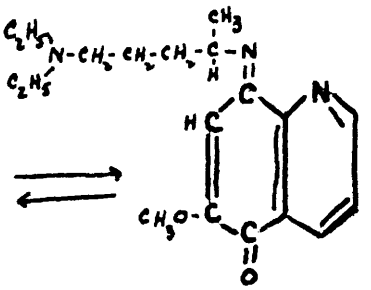


Pamaquine



5-Hydroxy Pamaquine



Quinonimine of Pamaquine

The possibility exists that both the toxicity and therapeutic effects of pamaquine are mediated through the same metabolic derivative. This is an important consideration in assaying the chances of obtaining an 8-aminoquinoline which is curative in malaria without being toxic. It is of interest that Greenberg, Taylor and Josephson have found that whereas pentaquine, (8-(Isopropylaminoamylamino)-6-methoxyquinoline) exerts a negligible effect on the erythrocytic form *P. gallinaceum in vitro,* its 5-hydroxy derivative exerts considerable activity on this parasite(10).

Summary. Two metabolites of pamaquine have been demonstrated in the plasma and urine of humans and dogs receiving pamaquine. One of these is an unstable compound which can, *in vitro,* convert hemoglobin to methemoglobin and lyse erythrocytes. The other is a stable compound which is highly fluorescent. Neither of these substances has been isolated in crystalline form.

It is possible that the substance with the oxidant and lytic properties is responsible for the toxic actions of the parent drug. The answer as to whether it also accounts for its therapeutic effect awaits definition of its antimalarial activity.

A homologue of pamaquine which differs only in its substitution on the side chain, yields metabolic products with properties similar to those obtained with pamaquine.

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Received July 12, 1950. P.S.E.B.M., 1950, v74.

Histochemical Alterations Revealed by Tetrazolium Chloride in Hypertensive Kidneys in Relation to Renal VEM Mechanisms.* (18066)

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Tetrazolium salts provide a new tool for the study of metabolic activity of living cells (1-3). The compound used in the present

* Supported by grants from the New York Heart Association, the Josiah Macy, Jr., Foundation, Eli Lilly and Company, the National Institute of Health, United States Public Health Service, and the Postley Hypertension Fund, and by a grant from the Leukemia Research Foundation, Inc., to one of us (M. M. B.).