degenerated rapidly in tissue cultures whether infected or not. Some of the testes in our series had markedly depressed germinal cell activity *in vivo*. Therefore it was probably not the germinal cells that were infected. The Sertoli cells, the interstitial cells of Leydig, and the fibroblasts remain to be considered. The susceptibility of interstitial cells to certain other viruses injected into the intact testis has been well demonstrated.

The capacity of testicular tissue from monkeys, bulls, hamsters, guinea pigs and mice to support multiplication of Lansing virus *in vitro* has been explored to a limited extent. At the present time it appears that Lansing virus has not multiplied in any of these experiments except possibly those in which tissue of cynomolgus monkeys' testes were employed.‡

Summary. The Lansing and Hof. strains of poliomyelitis virus multiplied extensively in tissue cultures of testes from men 57 to 93 years old, most of whom had carcinoma of the prostate gland. Lansing virus was carried through 8 successive tissue cultures and Hof. virus was carried through 7 cultures. Several less extensive series were completed with each virus. The amount of Hof. virus in these tissue cultures per milligram of tissue was approximately the same as was obtained in infected monkey spinal cords.

‡ In experiments completed after this paper was submitted for publication poliomyelitis virus multiplied readily in tissue cultures of testicular tissue from both rhesus and cynomolgus monkeys.

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A Metabolic Intermediate of Pamaquine from Chickens. (18599)

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(Introduced by N. B. Eddy)

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Berliner(1) observed that pamaquine (8-(4 - diethylamino - 1 - methylbutylamino)-6-methoxyquinoline), though markedly plasmodicidal *in vivo*, was relatively inactive when added to cultures of *Plasmodium falciparum in vitro*. Geiman(2) made essentially the same observation for the related 8-aminoquinolines, pentaquine and isopentaquine, using *in vitro* cultures of *P. knowlesi*. Berliner (1) also found that a solution of pamaquine, which had aged for several years, was highly active *in vitro*. Zubrod, Kennedy and Shannon(3) found that pamaquine was so extensively metabolized in the vertebrate body that extremely little of the drug was recovered in the excretory products of treated patients. Brodie and Udenfriend(4) and Hughes and Schmidt(5) have described metabolic intermediates of pamaquine and related 8-aminoquinolines, but none of these have been fully characterized and their antimalarial activities have not been determined. In an earlier report by three of us(6) it was shown that pamaquine, when added to *in vitro* cultures of *P. gallinaceum*, did not possess the marked antimalarial activity associated with the drug *in vivo*. In further trials (unpublished data) it was found that concentrates from the blood, tissues and droppings of chickens treated with pamaquine possessed antimalarial activity *in*

1. Berliner, R. W., personal communication.

2. Geiman, Q. M., Proceedings of the Fourth International Congresses on Tropical Medicine and Malaria, 1948, v1, 618.

3. Zubrod, C. G., Kennedy, T. J. and Shannon, J. A., *J. Clin. Inv.*, 1948, v27, 114.

4. Brodie, B. B., and Udenfriend, S., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 845.

5. Hughes, H. B., and Schmidt, L. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 581.

6. Greenberg, J., Josephson, E. S., and Taylor, D. J., *J. Inf. Dis.*, in press.

vitro which could not be accounted for on the basis of the pamaquine present in these concentrates. The foregoing suggested that the antiplasmodial and possibly the toxic, methemoglobin-forming properties of pamaquine are the result of metabolic transformation products of the drug. We, therefore, began our studies of the metabolites of pamaquine with the hope that we might be able to isolate a product or products which possessed antimalarial activity and, at the same time, be without toxic properties.

This report deals with the description of an intermediate obtained from chickens treated with pamaquine.

Methods. The product to be described has been obtained by essentially the same method from homogenized tissues (pooled blood, spleen, kidney, lung and pancreas) of treated chickens and from their droppings. However, since the amounts recovered from tissue homogenates were extremely minute, this report will be confined to the description of the product obtained from chicken droppings. Male and female New Hampshire Red chickens weighing 1500-2500 g were each given a single oral dose of pamaquine citrate (20 mg/kg body weight). Droppings were collected over an 18-hour period. Each 100 g of droppings were extracted with 600 ml of ethylene dichloride by shaking for 30 minutes. The organic phase, after separation of the solid residue by filtration, was washed twice for 15 minutes with approximately one-half volume of pH 8.8 buffer. After the second washing the final product was concentrated into about 1/100 volume of aqueous 0.1 N HCl. Partial purification was done with a 9 plate countercurrent distribution between ethylene dichloride and pH 7.6 buffer. Further purification and crystallization were impossible because of the small amounts of material available. The final extract was tested for the presence of pamaquine by the method of Brodie, Udenfriend and Taggart (8). Practically no measurable pamaquine was discovered in the chicken droppings. In fact, no material which would couple with diazotized sulfanilic acid was found in the crude ethylene dichloride extracts of the droppings of pamaquine-treated chickens. This would indicate that there was no pamaquine or ethylene dichloride soluble 8-aminoquinoline with an unsubstituted hydrogen in the 5 position present in the droppings. Ultraviolet absorption spectra were obtained, using the Beckman spectrophotometer Model DU. To test for the antimalarial activity of the degradation product *in vitro, P. gallinaceum* was used. Insufficient amounts were obtained to test the material *in vivo*. A method for the maintenance of *P. gallinaceum in vitro* and the methods employed for the assay of drug activity in such cultures have been described in detail(6,7). At the end of the 24-hour incubation period of the test material with *P. gallinaceum* the amount of methemoglobin present in the cultures was estimated by Wendel's adaptation(9) of the method of Evelyn and Malloy to the Coleman Junior Spectrophotometer, Model 6A. Additional changes in the procedure were necessitated by the fact that the chick erythrocyte in the cultures is resistant to hemolysis and contains a nucleus. Complete hemolysis with distilled water required one hour and the nuclear material and stroma were settled after centrifuga-

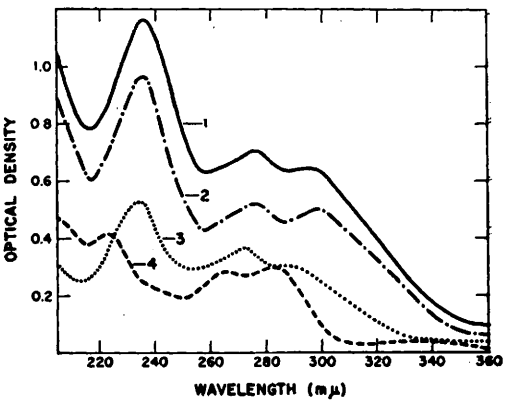

FIG. 1.

Absorption spectra of a metabolite of pamaquine from chickens (Curve 1), a product from the oxidative degradation of pamaquine by irradiation with ultraviolet (Curve 2), 8-amino-5,6-quinolinequinone (Curve 3), and pamaquine (Curve 4) in 0.1 N HCl.

7. Taylor, D. J., Greenberg, J., Josephson, E. S., and Ray, A. P., *J. Inf. Dis.* in press.

8. Brodie, B. B., Udenfriend, S., and Taggart, J. V., *J. Biol. Chem.*, 1947, v168, 327.

9. Wendel, W. B., personal communication.

tion for ten minutes at 3000 r.p.m.

Experimental. The ultraviolet absorption spectrum of the metabolite of pamaquine in 0.1 N HCl is shown in Fig. 1 (curve 1). Concentrates of the metabolite in 0.1 N HCl are orange-red. Dilute solutions of the metabolite in 0.1 N HCl are yellow. When the aqueous acid solution was frozen, the metabolite separated out as an orange-red oil. It was stable for at least 2 weeks when stored in the frozen state in aqueous acid solution at −18°C. The metabolite did not form a colored complex with diazotized sulfanilic acid, its absorption from wavelength 400 to 500 mμ being the same whether it was treated with sulfanilic acid or diazotized sulfanilic acid.

Estimations of the concentration of the metabolite in aqueous acid were based upon a comparison of its absorption of light at wavelength 420 mμ with that of pamaquine. This assumes that the molecular weight of the two compounds was roughly equal and that molecule for molecule they both absorb equally at 420 mμ. On this basis the metabolite was significantly active *in vitro* at concentrations of 625 μg/liter, while pamaquine was inactive at concentrations of less than 10,000 μg/liter (Table I). Furthermore, the metabolite produced significant methemoglobin at concentrations of 1250 μg/liter, while pamaquine produced none at concentrations below 20,000 μg/liter (Table I).

Discussion. It would be well to compare our metabolite with those obtained recently by several investigators studying the metabolic fate of 8-aminoquinolines. Brodie and Udenfriend(4) found 2 metabolites of pamaquine in the urine and blood of treated humans and dogs. One of these was stable and non-toxic as determined by its ability to lyse red cells and produce methemoglobin; the other was unstable and toxic by the same criteria. The ultraviolet absorption spectrum of their stable degradation product does not correspond to our metabolite, from which it also differs in that it does not produce methemoglobin *in vitro*. Since they have not been able to characterize fully their unstable, methemoglobin-inducing substance, comparison with our product is impossible. How-

TABLE I. *In vitro* Antimalarial and Methemoglobin-producing Properties of Pamaquine, a Metabolite of Pamaquine from Chickens, a Product from the Ultraviolet Irradiation of Pamaquine and 8-amino-5,6-quinolinequinone (DR 15,897).

Conc. in culture (μg/l)	No. of parasites surviving 24-hr exposure*				Methemoglobin in cultures after 24 hr. % total hemoglobin†			
	Pamaquine	Metabolite of pamaquine from chickens	Irradiation product from pamaquine	DR 15,897	Pamaquine	Metabolite of pamaquine from chickens	Irradiation product from pamaquine	DR 15,897
300	—	—	4×10^8	—	—	—	—	—
625	—	2.5×10^7	5×10^6	4×10^8	—	5.9	7.9	—
1250	—	5×10^6	1.25×10^6	2×10^8	—	19.4	6.9	—
2500	—	3.75×10^6	5×10^5	5×10^6	—	28.6	12.4	20.6
5000	2.5×10^8	1.25×10^5	5×10^4	5×10^4	—	42.9	35.2	29.0
10000	2.0×10^5	—	—	—	5.6	—	—	—
20000	5.0×10^3	—	—	—	6.6	—	—	—
40000	<1	—	—	—	22.4	—	—	—

* 5×10^8 parasites were initially present in each culture. Numbers of parasites in control cultures surviving 24 hr incubation ranged between 5×10^7 and 4.5×10^8. Differences less than 10-fold in numbers of viable parasites surviving treatment are not considered significant.

† In control cultures methemoglobin (% total hemoglobin) ranged between 3.8 and 7.4.

ever, it should be noted that our material is relatively stable in aqueous acid solutions.

Hughes and Schmidt(5) described degradation products of 8-aminoquinolines from plasma of monkeys, which differ among themselves in their solubility characteristics, but all of which form colored complexes with diazotized sulfanilic acid. Our degradation product obviously does not fit in their group of aqueous alkaline soluble, ethylene dichloride insoluble, compounds. Nor, does it fit in their group of ethylene dichloride soluble compounds which couple with diazotized sulfanilic acid. It should be mentioned that in our experience 8-aminoquinolines are degraded into a number of substances or complexes, so that it is possible for different investigators to select for characterization several metabolites whose properties are markedly different. Elderfield and Werble(10) obtained an 8-amino-5,6-quinolinequinone from the destructive irradiation of pamaquine with ultraviolet light, which they kindly sent us for testing. Their product corresponds quite closely to our metabolite in antimalarial activity and methemoglobin-forming properties *in vitro* (Table I). Their material also does not couple with diazotized sulfanilic acid and the ultraviolet absorption spectrum of their product and ours are almost identical (Fig. 1, curve 2). Finally, it should be mentioned that Drake and Pratt(11) have synthesized 8-amino-5,6-quinolinequinone (DR 15,897) as a possible degradation product of pentaquine. This material resembles our metabolite in its absorption characteristics over most of the ultraviolet range (Fig. 1, curve 3). However, it differs in its solubility characteristics, being much less soluble in aqueous acid solutions than our metabolite. DR 15,897 corresponds with our metabolite in *in vitro* antimalarial activity and methemoglobin-inducing properties from 2500 to 5000 μg/liter (Table I). Below this concentration the antimalarial activity of DR 15,897 decreased sharply, so that it was essentially inactive at 1250 μg/liter. Data on methemoglobin-inducing properties below 2500 μg/liter were not obtained. The properties of this synthetic quinolinequinone and the metabolite are similar enough to suggest a close structural relationship. Our metabolite is probably a quinolinequinone with a relatively undegraded 8-position sidechain as compared with DR 15,897. A difference in the structure of the sidechain could account for the discrepancies in solubility characteristics and absorption in the ultraviolet. If our product were a quinolinequinone it would substantiate the theory of Schönhöffer(12) which postulates that 6-methoxy - 8 - aminoquinolines are degraded through hydroxyquinolines to quinolinequinones.

It should be noted that while our product appears to be 16 times as active and toxic *in vitro* as pamaquine, the ratio of minimal antimalarial concentrations to minimal methemoglobin-forming concentrations is the same (1:4) for both compounds. It is possible that pamaquine is degraded in minute amounts in our culture vessels to yield active metabolites. However, the hope of obtaining an active metabolite which is non-toxic has not been realized in our preparation.

Summary. A metabolite of pamaquine hitherto undescribed has been obtained from the tissues and droppings of treated chickens. The *in vitro* antimalarial and methemoglobin-producing properties of this metabolite are approximately 16-fold those of pamaquine. There are striking similarities in ultraviolet absorption spectra and *in vitro* antimalarial and methemoglobin-inducing behavior of the metabolite and the two 8-amino-5,6-quinolinequinones studied.

10. Elderfield, R. E., and Werble, E., personal communication.

11. Drake, N. B., and Pratt, Y., *J. Am. Chem. Soc.* in press.

12. Schönhöffer, F., *Z. physiol. Chem.*, 1942, v274, 1.

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