compared with that of an oil solution of the same preparation. The dosages of the suspensions were varied from 50 I.U. to 2,000 I.U. Uterine weight or estrus smear were used as the criteria of effectiveness of the estrogens. 800 rats were used in the vaginal smear studies.

With the larger sized particles (60 and 100 mesh) 100 I.U. were as effective as 10,000 I.U. in oil solution. When the dosage level of 400 I.U. was used, estrus was maintained in 50% of the rats for 19 days with 60 mesh particles, 14 days with 100 mesh particles, 8 days with 200 mesh particles and 5-6 days with 325 mesh particles. Further increase in dosage levels up to 2,000 I.U. did not increase the duration of the estrus. This indicates the primary importance of particle size with this estrogen in controlling the duration of its effectiveness.

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Metabolism of Various 8-Aminoquinolines in Rhesus Monkey.* (17751)

HETTIE B. HUGHES AND L. H. SCHMIDT.

From The Christ Hospital Institute of Medical Research, Cincinnati, Ohio.

The following observations on metabolic transformations of various 8-aminoquinoline derivatives were an unexpected outgrowth of routine studies on the subacute toxicity of 2-methoxy -8- (3-aminopropylamino) -quinoline (CN 1100) for the rhesus monkey. These studies showed that, as compared with some 200 other 8-aminoquinolines which had been examined previously(1,2), CN 1100 had remarkably low toxicity, producing no untoward reactions when administered orally in repeated daily doses as large as 384 mg base per kg body weight.† Since the concentrations of this quinoline in the plasma were unusually low, even at doses as large as the above, and since less than 0.5% of the administered drug could be recovered from the urine, the question was raised whether the lack of toxicity of CN 1100 merely reflected poor absorption from the gastrointestinal tract. This possibility led to a study of the toxicity of CN 1100 administered parenterally. It was found that the drug had little toxicity when given intramuscularly in single doses as large as 48 mg per kg, suggesting that the low toxicity observed previously was a real characteristic of CN 1100 rather than an artifact resulting from defective absorption.

As part of the above study on parenteral toxicity, measurements were made of the rate of disappearance of CN 1100 from the plasma. The 8-aminoquinoline concentrations were determined by the method of Brodie(3) which employs primary extraction of the quinoline with an organic solvent, followed by re-extraction and coupling of the drug with diazotized sulfanilic acid to yield a colored complex. In anticipation of the results to be obtained by this procedure, one of us (HBH) added diazotized sulfanilic acid directly to diluted samples of plasma and noted the development of colors which appeared far more intense than those obtained later in the analysis of the ethylene dichloride extracts. This impression was confirmed by quantitative studies which showed clearly that the plasma of monkeys receiving CN 1100 contained considerable quantities of an aqueous alkali-soluble, ethylene dichloride-insolu-

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1. Wiselogle, F. Y., Survey of Antimalarial Drugs, 1941-45. J. W. Edwards, Ann Arbor, Mich., 1946.

2. Unpublished observations, this laboratory.

† Limitations in the supply of CN 1100 precluded studies at higher doses.

ble degradation product (or products).

Current interests in the metabolic fates of certain 8-aminoquinolines made it important to determine whether the *in vivo* formation of alkali-soluble, ethylene dichloride-insoluble degradation products is a property peculiar to the 2-methoxy-8-aminoquinolines, of which CN 1100 is a representative, or whether the reaction is common to the 6-methoxy-8-aminoquinolines now employed as curative antimalarial drugs. Studies have been carried out with representative groups of both series of compounds. The results are outlined below.

Experimental. A. Metabolic transformations following intramuscular administration. Pamaquine [6-methoxy-8-(4-diethylamino - 1 - methylbutylamino)-quinoline], isopentaquine [6-methoxy-8- (4-isopropylamino-1-methylbutylamino)-quinoline], primaquine [6-methoxy-8-(4 - amino-1-methylbutylamino)-quinoline], pentaquine [6-methoxy-8-(5-isopropylaminopentylamino)-quinoline], SN 13,058 [2-methoxy-8-(3-diethylaminopropylamino) - quinoline], and CN 1100 were administered in doses of 6 mg base per kg body weight to rhesus monkeys (*Macaca mulatta*), weighing 4.0 to 6.0 kg.‡ In addition CN 1100 was administered in doses of 12 and 48 mg per kg. Each of these compounds was employed as the soluble acid salt, pamaquine as the monocitrate, isopentaquine as the monooxalate, primaquine and pentaquine as the diphosphates, SN 13,058 as the dihydroiodide, and CN 1100 as the monophosphate. Aqueous solutions of these salts, containing 10 mg base equivalent per ml, were injected into the thigh muscles. Oxalated blood samples, drawn so as to prevent hemolysis, were obtained from the various monkeys prior to drug administration and ¼, ½, 1, and 2 hours thereafter. Plasma samples were separated immediately and were analyzed for their contents of 8-aminoquinoline derivatives soluble in ethylene dichloride by the Brodie method(3), modified by using ethylene dichloride in place of petroleum ether as the organic solvent.§ The samples were also analyzed directly for their contents of total water-soluble 8-aminoquinoline derivatives which would couple with diazotized sulfanilic acid. The procedures used in the latter analyses were as follows. A 0.4 ml aliquot of plasma was added to 5 ml of diazotized sulfanilic acid reagent(3) and allowed to react at room temperature for the appropriate period to insure maximum color development. This color was measured in a Klett photoelectric colorimeter, using as blanks the pretreatment specimens of plasma which had been reacted with the diazotized sulfanilic acid reagent precisely as the post-treatment specimens. The equivalent 8-aminoquinoline concentrations of the plasma samples were calculated by comparing the above colorimetric readings with those obtained by analysis of plasma to which known concentrations of the respective drugs had been added. Studies carried out prior to the animal experiments showed that each of the compounds could be recovered quantitatively from plasma by either of the above methods.

The results of these experiments, summarized in Table I, show that each of the 8-aminoquinolines gave rise to alkali-soluble, ethylene dichloride-insoluble degradation products. On the 6 mg per kg doses, CN 1100 yielded the largest amount of degradation product, SN 13,058 the smallest amount.

The time relationships between the concentrations of substances soluble and insoluble in ethylene dichloride deserve comment. In all cases the peak concentrations of ethylene dichloride-soluble substances (unchanged drug-?) were reached ¼ to ½ hour after in-

‡ We are indebted to the following for the compounds used in this study: to Dr. Robert C. Elderfield, Columbia University, for pamaquine and CN 1100; to Dr. Nathan Drake, University of Maryland, for pentaquine; to Dr. J. B. Koepfli, California Institute of Technology, for SN 13,058; and the Lilly Research Laboratories, Eli Lilly and Company, for isopentaquine and primaquine.

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

§ A 30-minute reaction period was required for full color development of the diazonium complexes of pamaquine, isopentaquine, primaquine, and pentaquine. The resulting yellow color was measured in a Klett colorimeter using a No. 47 filter. A 3-hour reaction period was required for full color development of the diazonium complexes of SN 13,058 and CN 1100. The pink color of these complexes was measured with a No. 54 filter.

TABLE I.
Concentrations of Various 8-Aminoquinolines and Their Metabolic Products in Plasma Following Intramuscular Administration.

Monkey No.	Drug	Dose, mg/kg	Mg of 8-aminoquinoline* per liter of plasma — A Water-soluble substances — Hours after treatment: ¼	½	1	2	B Ethylene dichloride-soluble substances — Hours after treatment: ¼	½	1	2	C Alkali-soluble ethylene-dichloride-insoluble derivatives (A-B) — Hours after treatment: ¼	½	1	2
2912	Pamaquine	6	†	†	5.47	4.51	†	†	2.99	0.93	†	†	2.48	3.58
2925	Isopentaquine	6	2.94	4.70	5.29	5.88	2.82	3.10	3.10	1.67	0.12	1.60	2.19	4.21
2670	Primaquine	6	2.77	3.32	4.43	6.09	1.10	1.16	1.15	0.89	1.67	2.16	3.28	5.20
2926	Pentaquine	6	1.90	3.17	4.12	5.07	0.81	1.29	0.89	0.55	1.09	1.88	3.23	4.52
2928	SN 13,058	6	—	1.84	2.07	3.91	1.30	1.47	1.31	1.13	—	0.53	0.76	2.78
2657	CN 1100	6	7.07	14.14	16.02	5.82	1.64	1.56	0.92	0.05	5.43	12.58	15.10	5.77
2952	,,	12	9.85	15.88	14.12	3.82	0.99	1.20	0.53	<0.01	8.86	14.68	13.59	3.82
2607	,,	48	23.82	63.97	87.32	53.83	2.61	3.54	2.97	0.66	21.21	60.43	84.35	53.17

* Expressed as parent drug equivalent.
† Animal in convulsions at this period; unable to obtain blood samples.

jection of the 8-aminoquinoline and declined substantially thereafter. In contrast to this, the concentrations of the alkali-soluble, ethylene dichloride-insoluble substances (degradation product or products) were lowest at the ¼hour period and, with the exception of CN 1100, rose steadily throughout the period of observation to reach an apparent peak at 2 hours. In the case of CN 1100, peak concentrations were attained at 1 hour.

It should be mentioned, also, that the peak concentrations of degradation products derived from any of the 8-aminoquinolines were always as great as the peak concentrations of unchanged drug and were considerably greater in some instances, notably with primaquine, pentaquine, and CN 1100. In the 2 monkeys, Nos. 2657 and 2607, receiving the latter compound, the peak concentrations of degradation product were 10 and 24 times the concentrations of unchanged drug. The absolute values of these concentrations were so great as to suggest that the degradation products may have greater chromogenic powers than the parent drug. The only other possible explanation for these high concentrations would be preferential localization of the drug in the plasma; this suggestion is not supported, however, by existing information on the tissue distribution of 8-aminoquinolines(4).

B. Metabolic transformations following oral administration. The above findings made it of interest to determine whether similar patterns of degradation occurred following oral administration of the various 8-aminoquinolines, also whether the degradation products accumulated in the plasma. In this experiment, pamaquine, isopentaquine, primaquine, pentaquine, SN 13,058, and CN 1100 in daily doses of 12 mg base per kg body weight were given to monkeys for 4 days. The daily doses were administered via stomach tube in equally divided portions at 8 A.M. and 5 P. M. Samples of oxalated blood were obtained on the final day just prior to the 8 A.M. treatment and 2 hours thereafter. Plasma samples therefrom were analyzed for their contents of water-soluble and ethylene dichloride-soluble 8-aminoquinoline derivatives, according to meth-

4. Unpublished observations, this laboratory.

TABLE II.
Concentrations of Various 8-Aminoquinolines and Their Metabolic Products in Plasma Following Oral Administration.

Monkey No.	Drug*	Mg of 8-aminoquinoline† per liter of plasma — A: Water-soluble substances — Hours after treatment: 2	A: 15	B: Ethylene dichloride-soluble substances — Hours after treatment: 2	B: 15	C: Alkali-soluble, ethylene-dichloride-insoluble derivatives (A-B): 2	C: 15
2912	Pamaquine	2.17	.17	.550	.103	1.62	.07
2922	Isopentaquine	4.28	.92	.292	< .017	3.99	.92
2738	Primaquine	9.07	2.59	1.737	.172	7.33	2.42
2924	Pentaquine	4.79	.50	.187	.031	4.60	.47
3012	SN 13,058	9.16	.24	3.565	< .012	5.60	.24
3025	CN 1100	3.46	< .11	< .010	< .010	3.46	.0

* In all cases the dose of drug was 6 mg per kg, administered at 8 A.M. and 5 P.M. daily for 4 days.
† Expressed as parent drug equivalent.

ods described above.

The results of this experiment, summarized in Table II, show that degradation products soluble in aqueous alkali but insoluble in ethylene dichloride were formed after oral administration of the various 8-aminoquinolines as well as after parenteral administration. The data also indicate that, with the possible exception of primaquine, there was no significant accumulation of these products in plasma.

C. Nature of metabolic products. Preliminary experiments, designed to elucidate the nature of the degradation products, have been carried out with drug CN 1100. Although these studies have not yet led to definition of the structures of the degradation products, they have shown that comparatively large quantities of alkali-soluble, ethylene dichloride-insoluble degradation products are eliminated in the urine of monkeys treated with CN 1100, also, that these degradation products may be extracted quantitatively from acidified urine with butanol.

The following experiment is characteristic of those which led to the above conclusions. Forty mg of CN 1100 base were administered to a monkey intramuscularly, after which urine was collected for a period of 18 hours. This urine contained 0.056 mg of ethylene dichloride-soluble 8-aminoquinoline derivatives (unchanged drug-?)—the equivalent of only 0.14% of the material administered. On the other hand, alkali-soluble, ethylene dichloride-insoluble substances (degradation products) amounted to 21.07 mg—equivalent to 52.7% of the injected dose. Following acidification of the urine to pH 2, this product was extracted quantitatively with n-butanol. This solubility suggests that the material (or materials) is acidic in nature. Such a property could be acquired either by stepwise oxidation of the CN 1100 side chain to yield a carboxyl group or by cleavage of the quinoline nucleus to yield an anthranilic acid type structure. Work on these possibilities is in progress.

Discussion. The data set forth in the preceding experiments indicate clearly that certain 2-methoxy-8-aminoquinolines are degraded by the monkey to alkali-soluble, ethylene dichloride-insoluble derivatives. Observations with the 6-methoxy-8-aminoquinolines in current use as curative antimalarial drugs suggest, grossly at least, that the degradation of these compounds follows a similar pattern.

Although the nature of the degradation products has not yet been established, the present observations may be useful in pointing the way for future studies on the metabolism of the 8-aminoquinolines. Numerous groups of investigators, including our own, have pursued this general problem, believing that the elucidation of the metabolic fate of these compounds will materially assist the rational development of more effective and less toxic curative antimalarial drugs. Unfortunately, the possibility that the parent drugs are degraded to acidic substances has re-

ceived little attention from the laboratories engaged in these studies. The results of the present work focus attention on this particular group of degradation products.

Summary. Studies on the metabolism of the 8-aminoquinolines, pamaquine, isopentaquine, primaquine, pentaquine, SN 13,058, and CN 1100, have been carried out in the rhesus monkey. As shown by differential analyses of the 8-aminoquinoline contents of plasma, each of the above compounds is degraded to alkali-soluble, ethylene dichloride-insoluble derivatives. This degradation follows either oral or intramuscular administration of the drugs. The extent of degradation of the different compounds varies markedly, being greatest with CN 1100 and least with SN 13,058. Preliminary studies on CN 1100 have indicated that the degradation products of this compound are present in the urine in considerable quantity.

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Distribution of Mercury in Rats Following Oral and Intravenous Administration of Mercuric Acetate and Phenylmercuric Acetate.* (17752)

C. S. PRICKETT, EDWIN P. LAUG, AND FRIEDA M. KUNZE.
(Introduced by A. J. Lehman.)

From the Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D.C.

Chronic toxicity investigations in rats over a 2-year period have shown that mercury in the form of the phenylmercuric acetate salt is far more toxic than mercury in the form of the mercuric acetate salt(1). The following experiments were undertaken in an effort to trace the fate of the 2 mercurials following oral and intravenous introduction into the rat.

Method. Young adult male rats, ranging from 250 to 300 g body weight were used in these experiments. Equivalent quantities of mercury, in the form of aqueous solutions of phenylmercuric acetate and mercuric acetate were administered under very light ether anesthesia, either by the femoral vein or by stomach tube. In all but a few preliminary experiments, the dose of mercury was 120 μg per animal. For the intravenous injection the dose was contained in 0.25 ml volume, for the oral, in 2.0 ml volume. The animals were sacrificed at intervals of 2, 6, 24, 48 and 96 hours after treatment; tissues, blood, and excreta were analyzed for mercury. In the case of the excreta, urine and feces were collected separately, and the contents of the bladder and of the cecum and large intestine at the time of sacrifice were considered part of the respective voided samples. Mercury analyses were made by the method of Laug and Nelson(2).

Results. Table I shows the tissue distribution of mercury 24 hours after intravenous injection of the metal in the form of the 2 salts under investigation. It can be seen that the kidney is the most important storage depot for mercury and that the amounts found in this tissue are fairly well graded to the quantities of mercury injected. This storage response of the kidney after intravenous injection of mercury is, as might be expected, similar to that obtained when the metal is introduced by other routes, such as the dermal(3), vaginal(4), and oral(1).

* A part of these data was presented at the meetings of the Federation of American Societies for Experimental Biology, March, 1949.

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