[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH AND THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

IDENTIFICATION BY DISTRIBUTION STUDIES. IX. APPLICATION TO METABOLIC STUDIES OF 4-AMINOQUINOLINE ANTIMALARIALS[1]

ELWOOD O. TITUS, LYMAN C. CRAIG, CALVIN GOLUMBIC, HAROLD R. MIGHTON, IRIS M. WEMPEN, AND ROBERT C. ELDERFIELD

Received July 11, 1947

Although the capacity of the animal organism for causing transformations of drugs has been recognized, many studies of the pharmacological or therapeutic activities of various substances appear in which this factor has not been taken into consideration. Thus frequently, the observed effect has been related to the dosage of a given drug or to drug levels in various parts of the body as determined spectrophotometrically. By the latter method, confusion may occur between the identification of the drug administered and metabolic products of the drug which, while actually being the active agent or agents, still show spectrographic properties closely similar to the drug itself. Often this has been the only feasible approach because of the difficulty in the detection, isolation and identification of the small amounts of the transformation products carried in the complicated mixture present in the biological fluids. The need for such information, often realized in the past, is obvious for the proper evaluation or interpretation of the underlying cause of the desired effect.

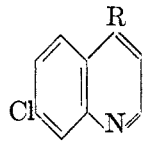
The method of counter-current distribution (1) is an approach which should prove helpful for the problem as stated, because of the quantitative nature of the process and other advantages mentioned in previous publications (2). An excellent opportunity to test this thesis came with the appraisal of the 4-aminoquinoline group of drugs as among the most effective suppressive antimalarials, and the need for knowledge of the fate and physiological disposition of representative members of the series. It was thought that information on the mechanism of detoxification of the drugs could prove of value both as a guide to more efficient administration of the substances and as a source of leads for the synthesis of perhaps even more highly useful drugs.

In the present investigation, the metabolic fate of three typical 4-aminoquinolines as indicated by the nature of the degradation products isolated from the urine of normal human volunteers receiving the drugs has been the subject of a preliminary study. Unfortunately, termination of hostilities prevented accumulation of sufficient material to enable the studies to be carried as far as might have been desired. The drugs investigated were 4-(4-diethylamino-1-methylbutylamino)-7-chloroquinoline (chloroquine) (SN-7618) (I),[2] 4-(3-di-

[1] The work described in this paper was done in part under contracts, recommended by the Committee on Medical Research, between the Rockefeller Institute for Medical Research and Columbia University, and in part under a grant from the United States Public Health Service (National Institute of Health) to Columbia University.

[2] The prefix, SN, identifies a drug in the "Survey of Antimalarial Drugs," Edwards Bros., Ann Arbor, Mich., 1946. The numbers will be used frequently for convenience hereinafter.

ethylamino-2-hydroxypropylamino)-7-chloroquinoline (oxychloroquine) (SN-8137) (II) and 4-(3-diethylaminopropylamino)-7-chloroquinoline (SN-9584) (III).



I. R = $NHCH(CH_3)(CH_2)_3N(C_2H_5)_2$ (SN-7618)
II. R = $NHCH_2CH(OH)CH_2N(C_2H_5)_2$ (SN-8137)
III. R = $NH(CH_2)_3N(C_2H_5)_2$ (SN-9584)
IV. R = $NHCH(CH_3)(CH_2)_3NHC_2H_5$ (SN-13,616)
V. R = $NHCH_2CHOHCH_2NHC_2H_5$
VI. R = $NH(CH_2)_3NHC_2H_5$ (SN-13,588)

Since the potentialities of the method of counter-current distribution had been but incompletely explored at the time this investigation was begun, a preliminary part of the present work involved a study of the most efficient means of applying the procedure to the two types of mixtures encountered, *i.e.* mixtures of typical quinoline bases extractable from urine with chloroform, and mixtures of water-soluble amphoteric substances extractable with butanol.

At the outset the method was subjected to a critical test in which the homogeneity of the drugs administered as determined by the counter-current technique was checked against the homogeneity as determined by the solubility method of Northrup and Kunitz (3).[3] The latter method appeared to be the most suitable of the methods previously available for determining purity, and also offered certain theoretical advantages as a test of the sensitivity of the distribution process. By such a check of the purity of the original drugs, the possibility of the appearance of degradation products as artifacts was eliminated.

In analysis by distribution, a mixture is subjected to a series of stepwise extractions between portions of two immiscible solvents moving counter-currently. Components, the higher partition coefficients of which favor concentration in the upper layer, will naturally move more rapidly through the apparatus in the direction in which this phase is traveling, so that after a time the several substances in a mixture appear as isolated bands moving through the apparatus at different rates in a manner somewhat analogous to the spreading of bands on a chromatographic column. Each operation in which the two phases are equilibrated, allowed to settle, and transferred to new positions is termed a plate, in view of the analogy with other counter-current fractionation processes. After a suitable number of plates have been applied, the contents of each tube are analyzed, and the concentrations plotted as a function of the position of the tube in the apparatus. The stepwise nature of the procedure has made possible a

[3] A detailed and rigorous comparison of the two methods will appear shortly. Craig and co-workers, in press.

simple mathematical treatment which permits the calculation of theoretical distribution curves for pure substances as a function of their distribution constants (1, 4). This has enhanced the usefulness of the technique as a means of estimating homogeneity, since the quantity of impurity may be estimated by summation of the differences between the theoretically and experimentally obtained ordinates of a distribution curve. When, as in these determinations, the two substances are separated more or less completely, the relative amounts of the components of the mixture can be estimated by summation of the ordinates in each band.

The usefulness of the technique obviously depends on the extent to which the components of a mixture differ in distribution constant, and upon the availability of a suitable analytical method which will reveal all the impurities, so that it seemed desirable to check the results by an independent method.

Such a method is available in the Northrop and Kunitz technique in which a series of samples of increasing weight are equilibrated with a fixed volume of solvent, and the concentrations of the resulting solutions are plotted against sample weight. It follows from the phase rule that, for a pure substance, such a curve should rise at a 45° slope from the origin provided the ordinates are properly chosen, indicating complete solution of the sample, until at the saturation point it becomes a horizontal straight line, indicating the constant solubility of a pure substance. With a mixture, the first break in the rising curve occurs when the solution becomes saturated with one component. The curve then rises at a lesser slope, becoming horizontal only when sufficient quantity of sample is taken to saturate the liquid phase with all of the components of the mixture. Determination of the samples and concentrations by weight makes the results independent of any particular analytical method, and, except in the improbable instance of two components with identical solubilities, any type of mixture should be revealed.

When samples of the three drugs were compared by the two methods, the quantity of impurity, estimated in terms of equivalents of acid required by titration of the contents of each tube of the Craig apparatus, was found to be consistently a little higher than the amount revealed by the solubility technique. The results were of the same magnitude, and in no case was there sufficient impurity to cast doubt on the relationship between degradation products and starting materials.

The efficient separation of small quantities of impurities from the drugs in the homogeneity studies encouraged the use of the same two phase systems of organic solvents and concentrated buffers for the isolation of degradation products, since it appeared that molecules with but minor structural differences might differ widely in distribution constants in such systems. This follows naturally from the nature of the equilibria involved. The over-all partition coefficient which determines the behavior of a substance on distribution may be considered to result from the contributions of several equilibria: the distribution of the free base between solvent and aqueous phase; the molecular association, if any, of the compound in the buffer or organic solvent; and the ionization of the base in the

aqueous phase (2). The ionization constant determines the percentage of the base existing in the ionic form in the aqueous phase. By fixing the *p*H with buffer an over-all partition ratio in the vicinity of one can be obtained. Within this range the distribution constant changes rapidly with *p*H; and conversely, in systems buffered to constant *p*H, structural changes which affect *p*Ka values should have decided effects on distribution constants. This has proved to be the case with the drugs and their degradation products encountered in this investigation as well as with numerous other quinoline bases examined by this technique, details of which have been reported elsewhere (5). From experience to date, the conclusion appears justified that for substances of this type, the distribution constant in a well-defined system of buffer and solvent can be safely regarded as a characteristic physical constant.

With the fundamental reliability of the counter-current method apparently established for the separation of mixtures of the type likely to be encountered, attention was turned to the problem at hand. The collected urine of the volunteers was made ammoniacal and extracted with chloroform and the quinoline bases obtained. The amounts thus secured accounted for about 40% of the drug administered in the case of SN-7618, 50% of the administered SN-8137 and 15% of the administered SN-9584 on the assumption of an output of one liter of urine per day per subject.

As pointed out by Craig (2), counter-current distribution is most efficient when the partition coefficient between the two phases is unity. Therefore systems of organic solvent and buffer between which the components of the mixtures of degradation products extracted with chloroform distributed equally were chosen. The contents of the tubes were analyzed spectrophotometrically by measuring the optical density of the solutions at 330 mμ, at which point a characteristic maximum of derivatives of 4-amino-7-chloroquinoline occurs. The complete absorption curve for SN-7618 has been reported by Drake and coworkers (6). None of the substances isolated from this chloroform extractable fraction showed significant deviations from this characteristic absorption curve.

Distribution of the chloroform extractable mixtures from the urine of subjects receiving each of the three drugs resolved each into two fractions, one the recovered starting material, and the other, a degradation product which proved in each case to be the monoethylamino compound (IV, V, VI) resulting from the loss of one ethyl group from the terminal diethylamino group of the side chain. De-ethylation, apparently a general detoxification mechanism for this class of compound, has not been frequently observed in the cases of other substances, although it has been noted with N-ethylglycine (7) and with N-ethylbarbital (8). Oxidation of the 2-position of the quinoline nucleus to yield the carbostyril, which might have been expected in the light of the data of Mead and Koepfli (9) concerning the degradation of quinine, was not observed. The extent of de-ethylation was found to vary with the side chain; the monoethyl compound accounted for roughly 25% of the chloroform extractable bases from urine in the case of SN-7618, 60% with SN-8137, and 20% with SN-9584.

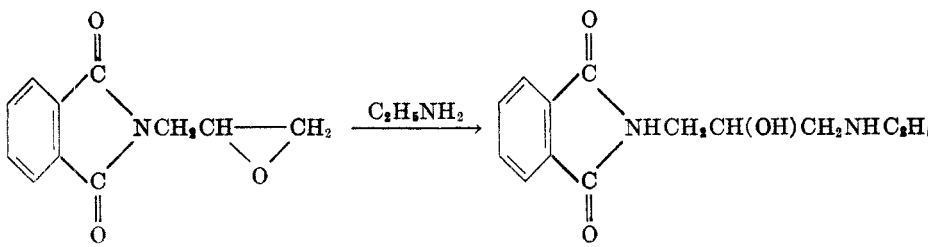
In only one instance was a degradation product other than the de-ethylated

compound isolated from the chloroform extracts. Occasional batches of urine from subjects receiving SN-9584 contained a high-melting substance, which appeared in the same fraction as the monoethyl compound, VI. Although difficultly separable by distribution, the two were readily separated by fractional crystallization. Analytical data suggested the possibility of a hydrated hydroxylamine derivative of the monoethyl compound, VI, and the absorption spectrum indicated that the quinoline nucleus had not been modified. Unfortunately, the few urine specimens in which this compound was found provided only enough for the analytical data and it was not definitely identified.

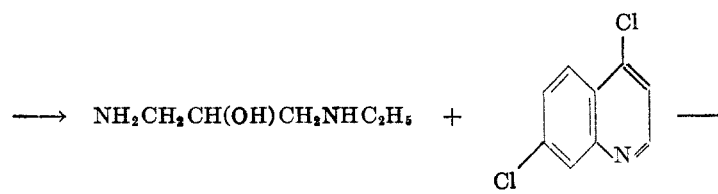
Although both SN-7618 and SN-8137 contain asymmetrical carbon atoms, only in the case of the former was there evidence of preferential degradation of one isomer.

On the basis of the above information, particularly the indication from N-ethyl determinations that the three degradation products were indeed the substances represented by IV, V, and VI, *dl*-4-(4-ethylamino-1-methylbutylamino)-7-chloroquinoline (IV) (SN-13,616) and 4-(3-ethylaminopropylamino)-7-chloroquinoline (VI) (SN-13,588) were synthesized elsewhere (10, 11). 4-(3-Ethylamino-2-hydroxypropylamino)-7-chloroquinoline (V) has been synthesized in the course of the present work. Final identification of the degradation products with the synthetic substances was on the basis of distribution constants and mixed melting points in the cases of V and VI. In the case of IV, the preponderance of an optical isomer in the degradation product rendered the melting points of little use, although the agreement of the distribution constants was satisfactory.

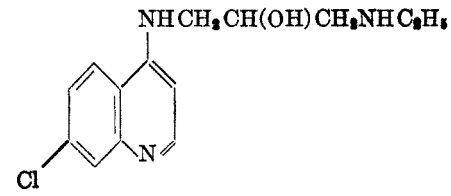
For the synthesis of the monoethyl compound, V, reactions VII–V were used. Epoxypropylphthalimide (VII) (12) was treated with ethylamine to yield 1-ethylamino-2-hydroxy-3-phthalimidopropane (VIII) which was hydrolyzed to 2-hydroxy-3-ethylaminopropylamine (IX). The latter was condensed with 4,7-dichloroquinoline according to the general method of Pearson, Jones, and Cope (13) to yield the monoethyl compound, V.



VII VIII



IX



V

The behavior of SN-13,616 and SN-13,588 in the human furnished interesting confirmation for the evidence here presented that these two substances represent at least one possible mode of elimination of the two parent drugs, SN-7618 and SN-9584. Although both monoethyl derivatives showed a high order of activity against avian malaria,[2] absorption studies showed that both drugs were poorly absorbed in man and eliminated unchanged to a great extent (14).

After removal of chloroform extractable degradation products from the ammoniacal urine of subjects receiving SN-8137 and SN-9584, further extraction of the urine with butanol yielded a series of more water-soluble degradation products. Separation of these substances from the urea and other constituents of normal urine was partially achieved by cautious addition of chloroform to methanolic solution of residues from the butanol extract. The mother liquors remaining after the separation of the urea were evaporated and the residues fractionated further by distribution.

The slight solubility in ordinary organic solvents of these amphoteric substances limited the solvent pairs in which distribution was practical to systems of highly polar solvents such as butanol and water. The necessity of recovering distributed material by evaporation of both upper and lower phases made the use of buffers impractical if contamination of the products with salt was to be avoided. Despite the fact that these restrictions appeared to eliminate many of the factors which made the distribution constants of related substances more sensitive to differences in structure, it was possible to achieve efficient separation of the degradation products by taking advantage of their amphoteric properties and distributing them first in butanol and dilute ammonia and subsequently in butanol and dilute acetic acid.

Use of the first system served chiefly to remove contaminants since distribution of the products of either drug yielded similar patterns. These revealed two main fractions, one a band of material of low distribution constant containing about 80% of the total solids, and the other, a fraction near the center of the distribution curve in which were concentrated most of the substances the absorption of which at 330 mμ indicated their quinoline nature. This central fraction was redistributed in the second pair of solvents, butanol and 10% acetic acid, which proved to be most effective for the separation of the quinoline derivatives from each other. The distribution curves obtained with this system revealed the strong influence of the nature of the side chain on the mode of elimination of the 4-aminoquinolines.

The hydroxylated side chain of SN-8137, which renders possible the formation

of water-soluble conjugates, apparently favors elimination of the drug as a uronide, possibly a glycuronide, largely to the exclusion of further degradation. Such a uronide, which was found by the butanol-acetic acid distribution to account for the major part of the material extracted from the urine by butanol, was freed from non-quinoline contaminants by repeated redistributions and finally obtained as an amorphous solid. Analytical data suggested the formulation of this substance as a uronide of the N-oxide of the original drug, and the behavior of its hydrolysis products suggested that the conjugate was more than a simple glycuronide of the original drug. Optimal acid hydrolytic conditions were established by counter-current distribution of the reaction products from several runs made under increasingly severe conditions. The mildest conditions which gave complete conversion of the uronide from the water-soluble derivative to a chloroform-soluble base were thereafter used. This acid hydrolysis split the compound into water-soluble material which gave naphthoresorcinol color reactions typical of uronic acids, and a chloroform-soluble base, the absorption spectrum and behavior of which upon distribution in solvent-buffer systems were identical with those of the pure drug SN-8137. However, when an attempt was made to distill this chloroform-soluble base, extensive decomposition occurred in contrast to the behavior of SN-8137 on distillation. Likewise the picrate prepared from the base was a mixture from which, after reduction, the characteristic stubby rods of the picrate of SN-8137 could be separated from a picrate crystallizing as thin needles by fractional crystallization. Apparently the quinoline compounds originally present in the conjugate consisted of a mixture of conjugates of SN-8137 and a closely related substance. In the light of the experience with the oxygenated derivative of the de-ethylated SN-9584, which was difficult to separate from the parent compound by distribution, this might be the N-oxide.

The second fraction, which appeared in small amounts in the same butanol-acetic acid distribution as the conjugate, yielded a small amount of crystalline product melting at 163–165°. This was obtained in too small a quantity for analysis.

Much more extensive degradation was revealed when the butanol extractable quinolines from the degradation products of the relatively simple diethylaminopropylamino quinoline (SN-9584) were distributed in the butanol-acetic acid system. Three major fractions were observed, but crystalline material was obtained from only one. The fraction which appeared in the same region of the distribution as had the conjugate of SN-8137 was not obtained pure. It appeared to have suffered alteration of the quinoline nucleus, as the typical absorption spectrum was distorted. It was not a conjugate of the original drug, since no chloroform-soluble material could be obtained after prolonged hydrolysis.

A second fraction appearing in the center of the distribution curve was isolated by repeated redistribution and recrystallization from water, and identified as N-(7-chloroquinolyl-4-)-β-alanine, a product to be expected from the oxidative deamination of the side chain. This compound was readily prepared by con-

densation of 4,7-dichloroquinoline with β-alanine in phenol solution. At the same time the next lower homolog was similarly prepared from glycine in the hope that it might prove to be identical with the material which melted at 163–165° isolated from the degradation products of SN-8137. However, N-(7-chloroquinolyl-4-)-glycine decomposed at 251–253°.

Both acids were characterized by methylation with diazomethane, which yielded the methyl ester of the betaine in each case, in agreement with the observations of Kuhn and Brydowna on the methylation of zwitterions with this reagent (15).

EXPERIMENTAL[4, 5, 6]

Homogeneity of the drugs and test of the counter-current method. (a) *Use of the solubility method.* Samples of SN-7618, SN-8137, and SN-9584 as the diphosphates were examined by the method of Northrop and Kunitz (3). A series of samples ranging from 20 to 300 mg. was equilibrated at 0° for twenty-four hours with 3 ml. of solvent in an apparatus similar to that of Ing and Bergmann (16) and Moore and Stein (17). The solubility was determined by weighing the residue after centrifuge filtration and the per cent of impurity was estimated from the slope of the curve obtained by plotting solubility in mg. per ml. versus the weight of the original sample. A representative curve obtained with SN-7618 is shown in the top part of Figure 1 and the data obtained with all three drugs are summarized in Table I.

(b) *Use of the counter-current method.* Samples of approximately 100 mg. of the same drugs were subjected to "counter-current distribution" between chloroform and 2 molar phosphate buffers in a 20-tube Craig apparatus (2). Upon completion of the distribution, the aqueous layer of each tube was made alkaline with sodium hydroxide and the organic bases were extracted by shaking into the chloroform phase, which was then separated. Three milliliters of water was added to each chloroform solution and the quantity of base in each tube was estimated by titration of the chloroform-water mixture with 0.1 *N* hydrochloric acid using sodium alizarin sulfonate as indicator. The mixture was agitated for twenty to thirty seconds after each addition of acid to ensure complete extraction of base into the aqueous phase containing the indicator. The number of mls. of hydrochloric acid required for the titration of each tube was plotted against the tube number. The two maxima in the resulting distribution curve indicated the separation of a small quantity of contaminant from the main body of the material.[7] The sums of the ordinates of the points within the two parts of the curve were taken as a measure of the relative amounts of drug and contaminant.

Typical data obtained by the two methods with a sample of SN-7618 are compared in Fig. 1 and the results for all three drugs are summarized in Table I. The inhomogeneity present as indicated by the counter-current method was consistently slightly higher (0.5%) than that indicated by the solubility method. This deviation was not sufficiently large to interfere with the later use of the former method.[8]

---

[4] Except where otherwise noted, the experiments were carried out in the laboratories of the Rockefeller Institute for Medical Research.

[5] All melting points were taken on a heated stage microscope.

[6] Microanalyses were done by Mr. D. Rigakos.

[7] All distribution curves are plotted to show the fractions of higher distribution constant at the right. In all cases the sample was first added to the tube numbered O, but since distribution is effected by transfer of the upper layers in the Craig apparatus and by the lower layers in separatory funnels, it is necessary to number the curves in opposite directions in order for curves obtained by the two techniques to be comparable.

Isolation of the degradation products of 4-(4-diethylamino-1-methylbutylamino)-7-chloroquinoline (SN-7618). (a) *Recovery of undegraded drug.* Three-liter batches of the pooled urine of normal human volunteers receiving 400 mg. per day of SN-7618 were made ammoniacal and extracted with 2 one-liter portions of chloroform. The combined chloroform

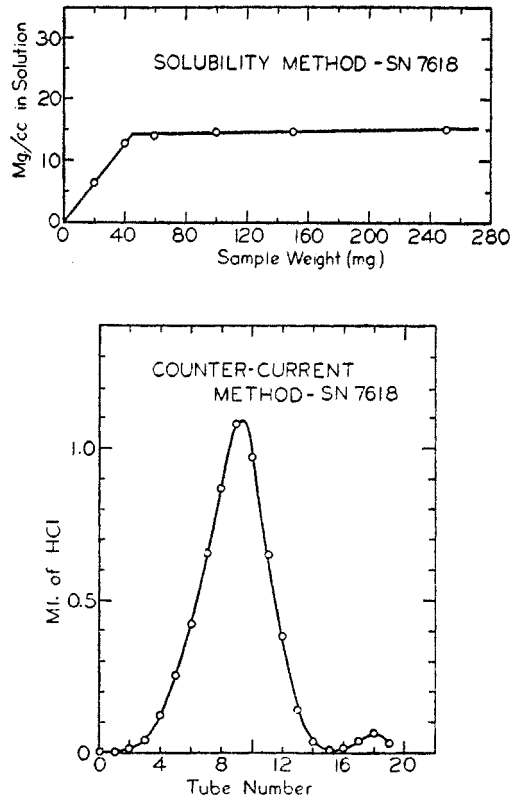


FIG. 1

TABLE I

COMPARATIVE INHOMOGENEITY DETERMINATIONS

DRUG	SOLUBILITY METHOD		COUNTER-CURRENT METHOD	
	Solvent	% Inhomogeneity	*p*H	% Inhomogeneity
SN-7618	Acetone-water 1:1	1.6	6.55	2.6
SN-8137	Ethanol-water 2:1	0.4	6.70	1.0
SN-9584	Acetone-water 1:2	0.2	6.70	0.5

extracts were then extracted with 250 ml. of 1.0 *N* sulfuric acid, the acid solutions were made ammoniacal, and the quinoline bases were removed by extraction into fresh chloroform. Evaporation under vacuum of the extracts yielded from 85 to 100 mg. of an oil per liter of urine. This was further fractionated by counter-current distribution between 8-ml. layers of chloroform and 2-molar phosphate buffer of *p*H 6.36 in a 25-tube Craig apparatus. Upon completion of the distribution, each tube of the machine was emptied into a separatory

funnel and the material from both phases concentrated into the chloroform layer by the addition of alkali and shaking. The optical density of each chloroform solution at 330 mμ was measured with a Beckman quartz spectrophotometer using a 1-cm. cell and plotted as a function of the number of the tube (Curve 1, Fig. 2).

The absorption curve of the material in the tubes containing the larger fraction, about 50% of the total, in tubes 6 to 15 of Fig. 2, proved identical with that of the original drug. The value of 0.71, calculated from Fig. 2, Curve 1 by the method of Williamson and Craig (4), for the coefficient of distribution between chloroform and the 6.36 *p*H buffer was in good agreement with the value of 0.70 observed with authentic SN-7618 in the same system.

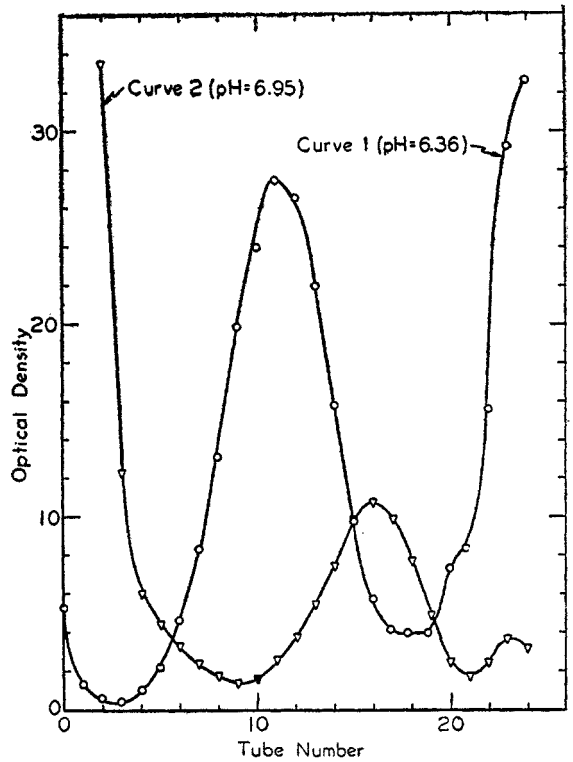


FIG. 2. DISTRIBUTION OF CHLOROFORM-EXTRACTABLE DEGRADATION PRODUCTS OF SN 7618 IN $CHCl_3$ AND 2-MOLAR PHOSPHATE

A sample of 70.7 mg., obtained by evaporation of several of the more concentrated chloroform solutions, melted at 83–89°. Recrystallization from ether yielded 14 mg. melting at 85–90°. A mixed melting point with a sample of the original drug (base) melting at 88–90° was 87.5–90°. The material showed slight optical activity, $[\alpha]_D^{25}$ + 7.5°, (c = 0.015 in chloroform).

Anal. Calc'd for $C_{18}H_{26}ClN_3$: C, 67.6; H, 8.2.
Found: C, 67.8; H, 8.3.

(b) *Isolation of the monoethyl compound, IV.* Another aliquot of the same material, the distribution of which was illustrated in Fig. 2, (Curve 1) was similarly distributed in chloroform and a 2 molar phosphate buffer of *p*H 6.95. An optically active base amounting to roughly 25% of the total material was isolated by evaporation of the solutions in tubes 13 to 18 of Fig. 2 (Curve 2). Redistribution of the material in the same system indicated

homogeneity, the partition coefficient being 1.7 as compared with the value of 1.5 observed with an authentic sample of 4-(4-monoethylamino-1-methylbutylamino)-7-chloroquinoline (10) (SN-13,616) (IV).

Molecular distillation at a bath temperature of 130° and 0.05 mm. yielded a clear distillate which slowly solidified on standing and melted at 80–95°; $[\alpha]_D^{25}$ +145°, (c = 0.0145 in methanol). The absorption spectrum in chloroform was identical with that of the original drug.

Anal. Calc'd for $C_{16}H_{22}ClN_3$: C, 65.7; H, 7.6; N, 12.1.
Found: C, 65.3; H, 7.9; N, 12.1.

An authentic sample of SN-13,616 was distilled in the same manner as the degradation product to yield a distillate which crystallized on standing and melted at 95–100°. A mixed melting point with the degradation product was 85–100°.

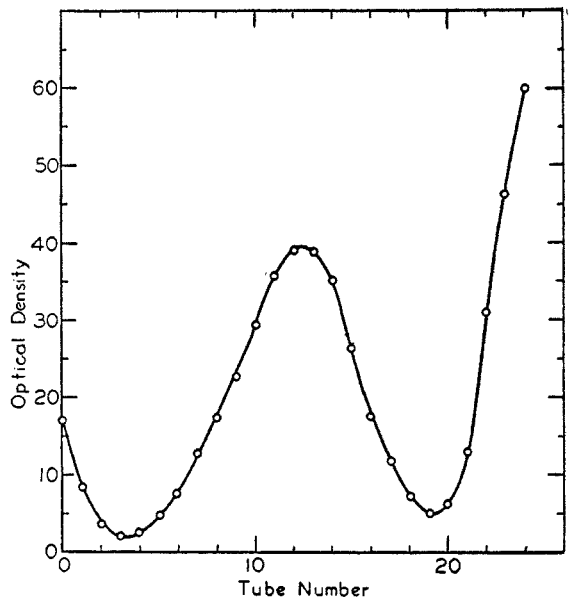


FIG. 3. DISTRIBUTION OF $CHCl_3$-EXTRACTABLE DEGRADATION PRODUCTS OF SN 8137 IN $CHCl_3$ AND 2-MOLAR PHOSPHATE (pH = 6.70)

A dipicrate of the degradation product prepared in acetone and recrystallized from the same solvent melted at 123–133°, then resolidified at 143–158°, and finally melted at 203–206°.

Anal. Calc'd for $C_{28}H_{28}ClN_9O_{14}$: C, 44.8; H, 3.8.
Found: C, 45.2; H, 4.0.

A dipicrate similarly prepared from the authentic sample melted at 120–130°.

Anal. Found: C, 45.0; H, 4.0.

Although direct identification of the degradation product with SN-13,616 was rendered difficult because of the appearance of optical activity, a comparison of the N-ethyl values obtained with SN-7618 and the degradation product support the interpretation given. This determination commonly gives consistently low results, but the comparative figures are significant.

Anal. Calc'd for $C_{18}H_{26}ClN_3$ (SN-7618): C_2H_5, 18.2. Found: C_2H_5, 10.9.
Calc'd for $C_{16}H_{22}ClN_3$ (degradation product): C_2H_5, 10.0. Found: C_2H_5, 6.0.

Isolation of the degradation products of 4-(3-diethylamino-2-hydroxypropylamino)-7-chloro-

quinoline (SN-8137). (a) *Recovery of undegraded drug.* Urine from subjects receiving 400 mg. per day of SN-8137 was extracted as described above to yield from 100 to 130 mg. of oily residue per liter of urine. A 24-plate distribution with chloroform and a 2-molar phosphate buffer of *p*H 6.70 separated two fractions as illustrated in Fig. 3. The unchanged drug was recovered as an oil upon evaporation of the solutions in tubes 9 to 16 of Fig. 3. A dipicrate could be prepared in acetone in nearly quantitative yield. The melting point, 222–224°, was not depressed upon mixture of the material with the dipicrate prepared from authentic SN-8137 which also melted at 222–224°.

Anal. Calc'd for $C_{25}H_{28}ClN_9O_{15}$: C, 43.8; H, 3.7.
Found: C, 43.6; H, 3.5.

(b) *Isolation of the monoethyl compound (V).* The monoethyl compound, which comprised about 60% of the total oil obtained above, crystallized readily in rosettes of short stout needles upon evaporation of tubes 22–24 of Fig. 3. This substance melted at 178–180° and the mixed melting point with a synthetic sample of 4-(3-ethylamino-2-hydroxypropylamino)-7-chloroquinoline, melting at 178–179°, was 178–180°.

The dipicrate crystallized from acetone in compact plates, melting at 231–235°.

Anal. Calc'd for $C_{26}H_{24}ClN_9O_{15}$: C, 42.4; H, 3.3.
Found: C, 42.6; H, 3.4.

Synthesis of 4-(3'-ethylamino-2'-hydroxypropylamino)-7-chloroquinoline: (Columbia). (a) *Epoxypropyl phthalimide.* A mixture of 100 g. of potassium phthalimide and 200 ml. of epichlorohydrin in a flask equipped with stirrer and reflux condenser was heated in a bath warmed to 160° for eight hours, during which the original pasty yellow mass was gradually transformed into a suspension of finely divided powder in dark brown liquid. Longer heating had no appreciable effect on the yield. The epichlorohydrin was distilled from the reaction mixture under water-pump vacuum, and the residue was taken up in 400 ml. of hot ethanol. The insoluble inorganic residue was filtered from the hot solution by suction and washed with 100 ml. of hot ethanol. When cooled, the combined ethanol solutions deposited 120 g. of white crystalline epoxypropylphthalimide, m.p. 89–92.5°. Recrystallization from 100 ml. of ethanol yielded 72 g. (63%) m.p. 96–98°. Weizmann and Malkowa, who prepared the substance from the bromohydrin, give the m.p. as 93–94° (12).

(b) *N-ethyl-1,3-diamino-2-propanol.* Fifty grams of epoxypropylphthalimide was added over a period of fifteen minutes to 500 ml. of a cold (−5°), vigorously stirred 33% solution of ethylamine in water. The mixture was stirred for one hour at 0 to −5°, allowed to stand for twenty hours at 10°, and then concentrated under vacuum on the steam-bath to a volume of 100 ml. after which 150 ml. of hydrochloric acid (sp. gr. 1.19) was added, and the solution was gently refluxed for two hours.

After cooling, the precipitate of 33.2 g. of phthalic acid was filtered off by suction and washed once with a little cold water. The filtrate and washings were evaporated under vacuum to less than 100 ml., cooled, and filtered free of a further small precipitate of phthalic acid. The filtrate was made alkaline with 6 *N* sodium hydroxide, evaporated nearly to dryness, and taken up in absolute ethanol. The alcoholic solution was filtered free of salts and evaporated under vacuum to a thick oil which was distilled through a 15-cm. Vigreux column. A considerable amount of resinous material proved undistillable; 5.65 g. (23%) of distillate, b.p. 117–119° at 18 mm., was obtained. Since further purification of the hydroxy amine by repeated distillations did not prove satisfactory, it was used directly as obtained for the subsequent step.

(c) *4-(3'-Ethylamino-2'-hydroxypropylamino)-7-chloroquinoline (V).* A mixture of 4.0 g. of once-distilled N-ethyl-1,3-diaminopropanol-2 and 6.80 g. of 4,7-dichloroquinoline (18) was heated according to the procedure of Pearson, Jones, and Cope (13). An exothermic reaction began when the bath temperature reached 148°, and the temperature of the reaction mixture rose rapidly to 160°. It was quickly cooled and maintained at 125–135° for two hours. The brittle solid reaction product was pulverized, triturated with several 100-ml. portions of ether to remove unreacted dichloroquinoline, and taken up in 30 ml. of hot

ethanol. The alcoholic solution was made alkaline with sodium hydroxide and gradually diluted with an equal volume of water. The red oil which separated from the solution was removed, taken up in approximately 300 ml. of benzene, and dried by evaporation of the benzene solution to 200 ml. Cooling of the benzene precipitated a solid contaminated with red, gummy material. Trituration of this with a little acetone left 2.86 g. (36%) of white crystals, m.p. 177–178°. After two recrystallizations from acetone, the substance melted at 178–179°.

Anal. Calc'd for $C_{14}H_{18}ClN_3O$: C, 60.2; H, 6.5.
Found: C, 60.4; H, 6.6.

(d) *Isolation of degradation products extractable from urine by butanol.* Three-liter batches of the urine remaining from the chloroform extractions were twice re-extracted with 1200-ml. portions of butanol and the extracts were evaporated under a vacuum, leaving usually from 1 to 3 g. of residue per liter of urine. This was dissolved in hot methanol, using approximately 3 ml. per g., and some of the urea and other constituents were precipitated by the slow addition of two volumes of chloroform to one of the hot methanol solution. After standing several hours, the precipitated solids were filtered off. Negligible quantities of material absorbing in the ultraviolet at 330 mμ were lost in this way. The mother liquor was evaporated under a vacuum to yield about 500 mg. of oily residue per liter of urine.

A four-plate distribution in separatory funnels (2) using 20 ml. layers of 50% aqueous methanol and chloroform served to remove some chloroform-soluble impurities which interfered with later fractionation. Tubes 0 and 1 of this distribution, in which occurred those substances more soluble in water, contained practically all of the material which absorbed light of 330 mμ wave length. These were evaporated under a vacuum and the oily residues, after material from two batches had been combined, were then distributed between 20 ml. layers of butanol and 3% ammonia. Because of the very stable emulsions formed in this system, it was necessary to centrifuge each tube for fifteen to thirty minutes after equilibration in order to separate the layers. The distribution was accordingly made in 100-ml. glass-stoppered conical centrifuge tubes rather than in the conventional separatory funnels, and the lower layers were siphoned from tube to tube. Upon completion of the distribution, which was usually carried out for 16 plates, 10 ml. of methanol was added to each tube to make the two phases mutually miscible and the concentration of quinoline was estimated with the Beckman spectrophotometer. By plotting the optical densities of each fraction at 260 and 330 mμ as ordinates against the tube numbers as abscissae it was possible to estimate the extent to which the quinoline fractions, absorbing at both wave lengths, had been separated from the constituents of normal urine which showed slight absorption at 260 mμ (Fig. 4).

Two quinoline fractions were separated by this procedure, the one occurring in tubes 0 and 1 varying from a negligible quantity to roughly 20% of the total, depending on the batch. Most of the normal constituents of urine were removed at this stage, since fractions 11 to 15 contained about 80% of the weight distributed.

The residue from the evaporation under vacuum of tubes 4 to 9 of Fig. 4 was further resolved by distribution in a 24-plate Craig apparatus between 8-ml. layers of butanol and 10% acetic acid. After completion of the distribution, the two layers in each tube were united as before by the addition of 4 ml. of methanol and the concentration measured spectrophotometrically at 260 and 330 mμ (Fig. 5).

Further purification of the main constituent in tubes 0 to 5 of Fig 5 by redistribution in the same system required the application of about 60 plates for complete separation of the quinoline from an impurity which did not absorb in the ultraviolet. Since the preceding distribution had removed from these tubes all material of partition coefficient greater than about 0.2, none of the remaining material could advance beyond the first four or five tubes in one revolution of the apparatus. It was therefore possible to apply 60 plates in the second distribution by rotating the machine two and one half times without fear of overlapping of the ends of the distribution curve.

A convenient final purification of this material was achieved by 8-plate distribution

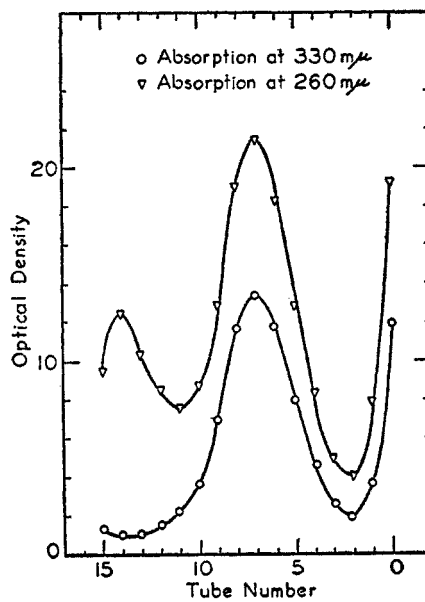


FIG. 4. DISTRIBUTION OF BUTANOL-EXTRACTABLE DEGRADATION PRODUCTS OF SN 8137 IN BUTANOL AND 3% AMMONIA

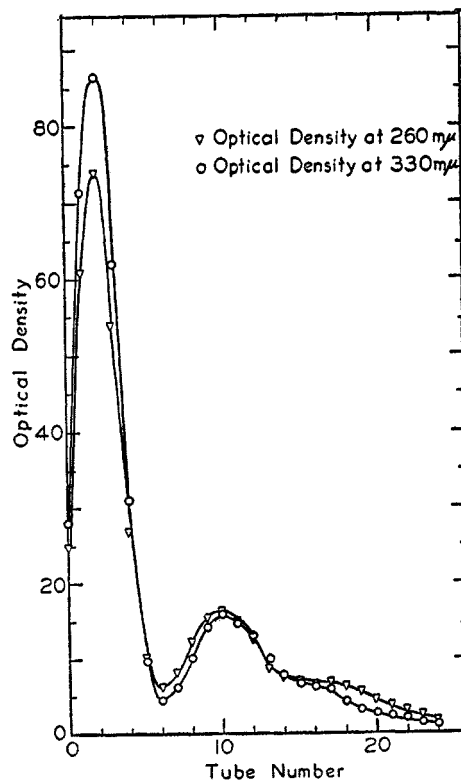


FIG. 5. DISTRIBUTION OF TUBES 4–9 OF FIGURE 4 IN. BUTANOL—10% HOAc

between 10-ml. layers of butanol and 3% ammonia, centrifuging each tube after equilibration and adding methanol as described above. Evaporation of the more concentrated fractions, 2, 3, and 4 in Fig. 6, yielded an ammonium salt as a clear brittle resin, soluble in water, slightly soluble in methanol and insoluble in non-polar solvents. Since attempted recrystallizations from water and various mixtures of water, acetone, and methanol yielded only amorphous powders, samples of the resin which had been redistributed until the experimental curves showed agreement with the theoretical distribution of a homogeneous substance were submitted for analysis. The substance contained no sulfur or phosphate. It gave a positive ninhydrin reaction. A solution of one mg. in a few drops of water after warming gently with a drop of dilute sodium hydroxide and readjustment to neutrality no

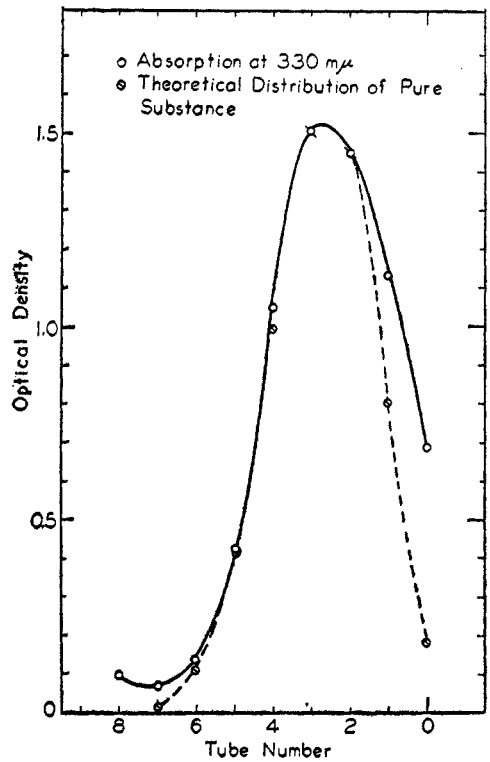


FIG. 6. DISTRIBUTION OF CONJUGATED DERIVATIVE OF SN 8137 IN BUTANOL AND 3% AMMONIA

longer gave a positive ninhydrin test. Analytical figures obtained agreed fairly well with those demanded for the N-oxide of a glycuronic acid derivative of the original drug.

Anal. Calc'd for $C_{22}H_{33}ClN_4O_8$ (the N-oxide of a glycuronide of SN-8137): C, 51.1; H, 6.4; N, 10.8; Cl, 6.9; Mol. wt. 516.8.

Calc'd for $C_{22}H_{33}ClN_4O_7$ (a glycuronide of SN-8137): C, 52.7; H, 6.6; N, 11.2; Cl, 7.1; Mol. wt. 500.8.

Found: C, 52.3; H, 6.3; N, 11.4; Cl, 6.5; Mol. wt. (from Cl analysis), 545.

The conjugation of the quinoline nucleus with a carbohydrate moiety, suggested by the analytical data, was confirmed by subjecting 10-mg. samples of the pure resin to a series of increasingly severe acid hydrolyses. After each attempt, the reaction mixture was evaporated to dryness under vacuum and the extent of hydrolysis determined by a distribution of the products between butanol and 3% ammonia.

Curve 1 of Fig. 7, in which the concentration of quinoline as determined spectrophotometrically is plotted against the tube number, illustrates the distribution of pure resin before hydrolysis; curve 2 indicates the partial liberation of the quinoline moiety by two hours refluxing in 2 *N* hydrochloric acid; and curve 3, the complete hydrolysis obtained upon heating for one hour at 130° in a sealed tube with 6 *N* hydrochloric acid. Under the latter conditions, all of the quinoline became soluble in organic solvents and was retained largely in the butanol layers upon distribution.

The presence of a uronic acid in the conjugate could be demonstrated by the naphthoresorcinol test as described by Hanson, Mills, and Williams (19) for glycuronides, except that the reagents were heated at 130° for one hour in a sealed tube before the two-hour heating at 100°.

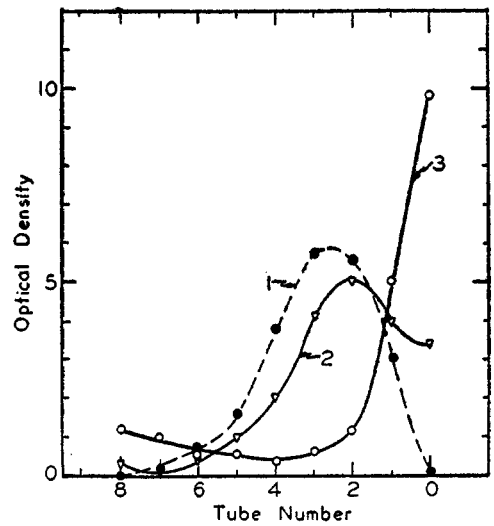


FIG. 7. Curve 1(●)—Pure SN 8137 conjugate. Curve 2(▽)—Partially hydrolyzed SN 8137 conjugate. Curve 3(○)—Completely hydrolyzed SN 8137 conjugate.

The aqueous reaction mixture from the hydrolysis of the conjugate was filtered free of charred decomposition products, made ammoniacal and extracted with chloroform. Evaporation of the extract yielded a base which accounted for one-half of the weight of the original conjugate and all of the basic material absorbing in the ultraviolet at 330 mμ. This material appeared to be a homogeneous substance closely related to SN-8137 when subjected to counter-current distribution. In chloroform and 2 molar phosphate buffer at *p*H 6.70 it exhibited partition coefficients of 0.39 and 0.49 in two separate runs. A known sample of 4-(3-diethylamino-2-hydroxypropylamino)-7-chloroquinoline when similarly distributed had a partition coefficient of 0.44. In isopropyl ether and 2-molar phosphate at *p*H 7.45 the constants were 1.40 for the hydrolysis product and 2.0 for the original drug (Fig. 8). Evaporation of the most concentrated, and presumably the purest, fractions from a distribution of this hydrolysis product in the latter system yielded a hygroscopic oil whose absorption curve in 95% ethanol proved identical with that of the original drug.

In spite of the excellent agreement with the theoretical distribution of a pure substance shown by the central band in Fig. 8, material isolated by evaporation of the more concentrated fractions proved to be a mixture, since treatment with two equivalents of picric acid in acetone yielded a mixture of picrates which crystallized poorly from acetone or acetone-ethanol mixtures and melted at 177–189°. The dipicrate of SN-8137 is readily crystallized from acetone and melts at 222–224°.

An attempted molecular distillation of 39 mg. of this mixture at 120° and 10^{-4} mm., under which conditions SN-8137 distills readily, yielded only 7 mg. of distillate, colored by decomposition, and some decomposition of the residue was noted.

Except for the mixed picrates of the hydrolysis product and the difficulties in distillation, the data would indicate that the original resin was the ammonium salt of the glycuronide of SN-8137, a possibility not excluded by the analytical data.

In view of the discrepancies noted in the preparation of the picrate and in the attempted distillation, the aminoquinoline portion of the conjugate would appear to have undergone some modification of the side chain. Since SN-8137 is recovered unchanged when subjected to the above hydrolytic conditions, decomposition of the drug base could not have accounted for the mixture of picrates. Although other possible alternative transformation products may be imagined, the most reasonable assumption appears to be that the conjugate is largely a glycuronide of the N-oxide of SN-8137 particularly in view of the later

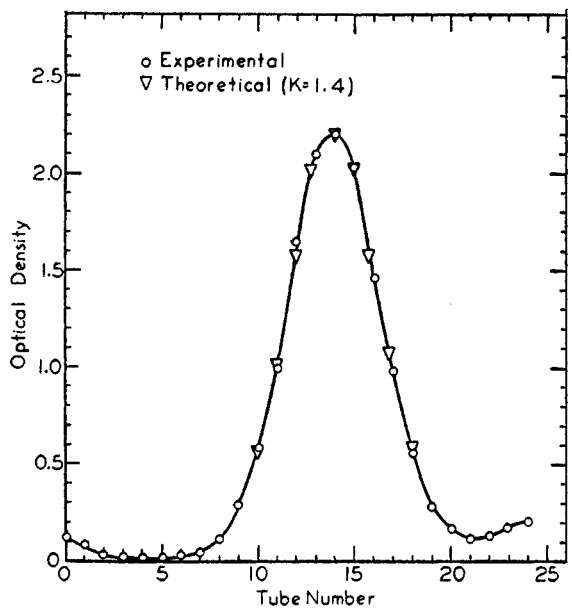


FIG. 8. DISTRIBUTION OF HYDROLYTIC PRODUCT OF SN 8137 CONJUGATE IN ISOPROPYL ETHER 2-MOLAR PHOSPHATE BUFFER (*p*H = 7.45)

experience with SN-9584. The inhomogeneous nature of the hydrolytic products may be due either to contaminants of the glycuronide or to formation of other decomposition products during the hydrolysis.

If the drug were present in the conjugate as an N-oxide, the mixture of quinoline bases isolated after hydrolysis might be expected to consist of the N-oxide together with decomposition products formed during the hydrolytic reaction. In such a case, reduction of the mixture with a mild reducing agent should make it possible to isolate the orignal diethylaminohydroxypropylaminochloroquinoline (SN-8137) by reduction of any amine oxide present. With this possibility in mind, a 10-mg. sample of the substance isolated from the above hydrolysis was dissolved in one ml. of 10% acetic acid and reduced by the gradual addition of 300 mg. of 2% sodium amalgam. The solution was then decanted from the mercury, the mercury washed several times with a little water, and the combined washings made ammoniacal and extracted with three 5-ml. portions of chloroform.

After evaporation of the chloroform, counter-current distribution of the product in-

chloroform and a 2-molar phosphate buffer at *p*H 6.58 indicated a substance 90 to 95% pure. The contents of each of the more concentrated tubes was evaporated to dryness, taken up in a few drops of acetone, and to each was added two equivalents of picric acid in a few drops of acetone. After standing several hours in the cold, a crystalline picrate melting at 186–194° was filtered off and recrystallized. About three times the volume of acetone from from which it had precipitated was required to redissolve this material. Cooling overnight deposited thin yellow needles melting at 196–198°, obtained in too small a quantity for analysis.

The combined mother liquors after partial evaporation yielded the short stubby rods characteristic of the dipicrate of SN-8137 melting at 220–223°. A mixed melting point with an authentic sample melting at 222–224° was 222–224°.

Anal. Calc'd for $C_{28}H_{28}ClN_9O_{15}$: C, 43.7; H, 3.7.
Found: C, 43.7; H, 3.7.

A second degradation product, appearing in tubes 8 to 13 of the butanol-acetic acid distribution illustrated in Fig. 5, occurred in amounts which varied with different urine samples from negligible quantities up to about one-tenth of the total butanol-extractable quinolines. Combination of all available material yielded about 30 mg. (from 12 liters of urine). An eight-plate distribution in butanol and 10% acetic acid disclosed approximately 10% of impurities when the optical density of each fraction at 330 mμ was plotted against tube number. This was eliminated by discarding the end fractions, tubes 0, 1, 7, and 8. The remaining material was combined, evaporated to dryness under vacuum, and subjected to an eight-plate distribution in butanol and 3% ammonia as previously described. Spectrophotometric analysis as before indicated the presence of about 20% of inhomogeneity in tubes 0 to 2. Evaporation of the most concentrated fractions, tubes 3, 4, and 5, yielded a solid soluble in dilute ammonia and acids, slightly soluble in hot water, and almost insoluble in methanol and organic solvents. Several recrystallizations from water were required to free the material from colored contaminants. Tubes 3 and 4 yielded white crystals melting at 163–165° in quantities too small for analysis. From tube 5 was obtained 1.5 mg. melting at 156–160°. Due to lack of material these fractions were not investigated further.

Isolation of the degradation products of 4-(3-diethylaminopropylamino)-7-chloroquinoline (SN-9584). (a) *Recovery of undegraded drug.* Three-liter batches of urine from patients receiving 400 mg. per day of SN-9584 were made ammoniacal and extracted with chloroform as previously described, yielding from 25 to 35 mg. of basic oils per liter of urine. These could be separated by a 24-plate counter-current distribution in the Craig apparatus using chloroform and 2-molar phosphate at *p*H 6.70, into recovered drug (tubes 0 to 8 of Fig. 9), and a smaller fraction in tubes 20 to 24 containing degradation products. Evaporation of the more concentrated fractions from the first portion of the curve yielded crystals which, after one recrystallization from ether, sintered at 72° and melted at 74–75°. The melting point 76° for an authentic sample of the drug was not depressed by mixture with this material.

Anal. Calc'd for $C_{16}H_{22}ClN_3$: C, 66.0; H, 7.6.
Found: C, 65.9; H, 7.6.

(b) *Isolation of the monoethyl compound (VI).* The fraction from tubes 21 to 24 of Fig. 9 behaved as a homogeneous substance with a partition coefficient of 1.8 upon redistribution in chloroform and 2-molar phosphate at *p*H 7.09. Evaporation of the more concentrated fractions from such a run, however, occasionally left a mixture of two compounds, resolvable only with difficulty by counter-current distribution in the system used, because of their identical partition coefficients, but readily separable by crystallization. In a typical run, 27 mg. of the mixture was recrystallized from acetone at 0° to yield 6.2 mg. of crystals melting at 195–200°. A second recrystallization from acetone containing a trace of methanol gave 4.5 mg. of leaflets melting at 201–203°.

Anal. Calc'd for $C_{14}H_{20}ClN_3O_2$: C, 56.5; H, 6.7; N, 14.1.
Found: C, 56.4; H, 6.2; N, 14.0.

From 2.4 mg. of this substance was obtained 4 mg. of a picrate which crystallized from acetone at room temperature as rosettes of short compact needles, m.p. 232–236°.

Anal. Calc'd for $C_{20}H_{23}ClN_6O_9$: C, 45.6; H, 4.4.
Found: C, 45.4; H, 4.3.

The absorption spectrum in chloroform of this substance was not changed from that of the original drug, making it somewhat unlikely that there was any modification of the original 4-amino-7-chloroquinoline nucleus. The analytical data would suggest then that the extra oxygen to be accounted for had in some way entered the side chain of the major degradation product, possibly to form a hydrated hydroxylamine derivative. This compound was isolated in quantities sufficient only to secure the analytical data. In certain later batches of urine only the monoethylaminopropylamino-7-chloroquinoline (VI) appeared in the second fraction, while in others a small amount of the above compound was present.

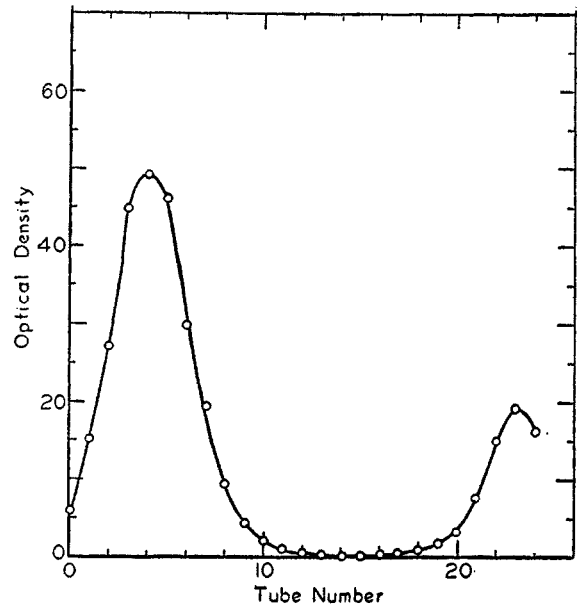


FIG. 9. DISTRIBUTION OF $CHCl_3$-EXTRACTABLE DEGRADATION PRODUCTS OF SN 9584 IN CHC AND 2-MOLAR PHOSPHATE (*p*H = 6.70)

The mother liquors from the crystallization of the unknown derivative described above were evaporated to dryness and the residue was redistributed in butanol and a 2-molar phosphate buffer at *p*H 6.8, in which system the material behaved as a homogeneous substance with a partition coefficient of 1.27. An authentic sample of 4-(3-ethylaminopropylamino)-7-chloroquinoline (SN-13,588), showed a constant of 1.20 when distributed in the same system.

The fractions near the maximum of the distribution curve (Fig. 9) were evaporated taken up in a little chloroform and freed from traces of butanol and buffer by several washings with water. Evaporation of the chloroform solution left a hygroscopic crystalline solid melting at 65–79°. Recrystallization of a sample weighing 13.7 mg. from dilute acetone at 0° yielded 8.3 mg. of plates, m.p. 65–75°. A sample of the synthetic product treated identically melted at 65–78°. Distillation of these substances at 100° and 10^{-3} mm. did not change the melting point, which was not depressed upon mixing the two samples.

The dipicrate of the degradation product crystallized from acetone in small rosettes, m.p. 251–253°.

Anal. Calc'd for $C_{26}H_{24}ClN_9O_{14}$: C, 43.3; H, 3.3.
Found: C, 43.6; H, 3.1.

A dipicrate of authentic SN-13,588 melted at 251–253°. The melting point was not depressed on mixing with the picrate of the degradation product.

(c) *Isolation of degradation products extractable from urine by butanol.* Urines which had been extracted with chloroform were further extracted with butanol as described previously for the isolation of the excreted derivatives of SN-8137. The same procedure of precipitation with methanol and chloroform and distribution between chloroform and 50% methanol was followed. Fractions 0 and 1 of the chloroform-methanol distribution were

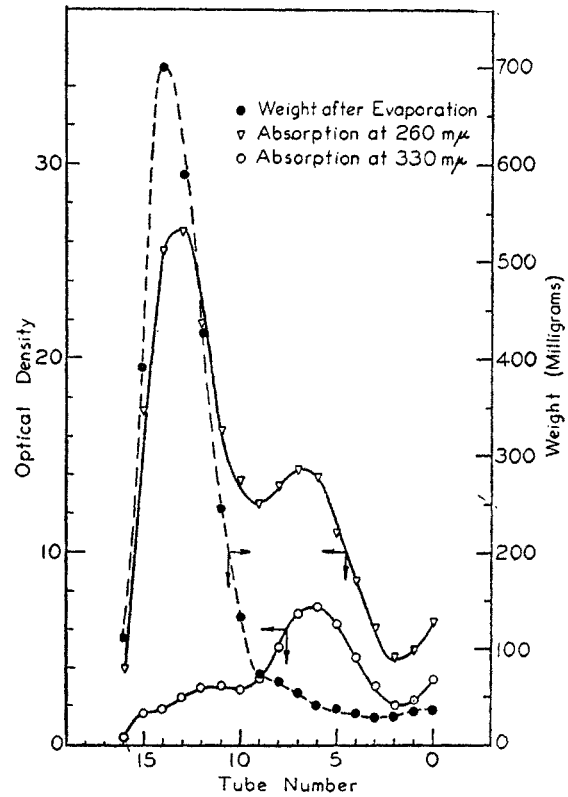


FIG. 10. DISTRIBUTION OF BUTANOL-EXTRACTABLE FRACTION FROM SN 9584 IN BUTANOL AND 3% AMMONIA

combined, evaporated, and the residue distributed in butanol and 3% ammonia exactly as before, centrifugation again being required after each equilibration to overcome the heavy emulsification. Eighty-six per cent of the total weight of the mixture was concentrated into tubes 10 to 16 of this distribution (Fig. 10), permitting the isolation in tubes 2 to 9 of a quantity of quinoline compounds corresponding to about 12 mg. per liter of urine.

This fraction was further resolved into three components upon evaporation and redistribution in a Craig apparatus with butanol and 10% acetic acid. As before, methanol was added to each tube of the machine upon the completion of 24 equilibrations and the optical densities of the resulting homogeneous solutions measured at both 260 and 330 mμ (Fig. 11). The decided difference in intensity of absorption noted at 260 and 330 mμ indicated considerable contamination of the fractions occurring in maximum concentration in tubes 12 and 19 with constituents of normal urine, since the intensity of absorption at these two wave

lengths approached the same value in the butanol, methanol, acetic acid solution as the material approached homogeneity.

Some improvement in the separation of the fractions from each other and from contaminants could be achieved by the application of a greater number of plates, using the withdrawal technique as described by Craig (1). A total of thirty-nine plates was applied to the mixed degradation products of SN-9584, as illustrated in Fig. 12. Because of the series of withdrawals, this curve is not continuous, the portion to the right of the discontinuity representing the fractions withdrawn. Tube 39 represents the first fraction withdrawn and would correspond to the twenty-fourth tube of a twenty-four-plate distribution. Tube 38 corresponds to the twenty-fourth plate of a twenty-five-plate distribution, etc. The fractions to the left of the discontinuity, which were those remaining in the machine at the

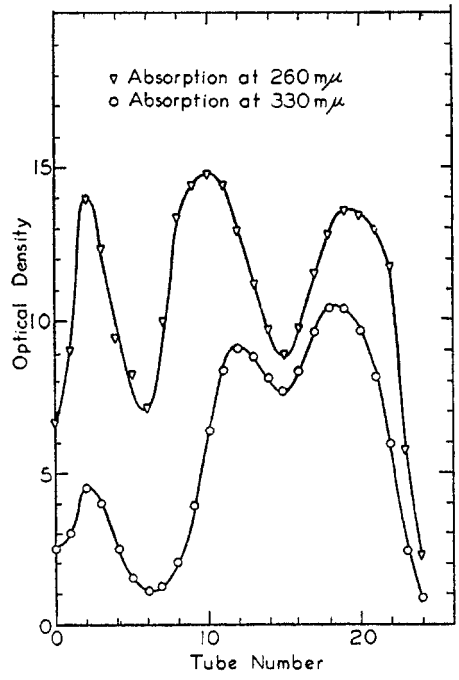


FIG. 11. CONTENTS OF TUBES 2-9 OF FIGURE 10 DISTRIBUTED IN BUTANOL AND 10% ACETIC ACID

time of the last withdrawal, have all been subjected to the full thirty-nine equilibrations.

(d) *Isolation of N-(7-chloroquinolyl-4)-β-alanine.* The combined contents of tubes 10 to 23 of the 39-plate (Fig. 12) distribution were evaporated and freed of most remaining contaminants by 16-plate distribution in 10-ml. layers of butanol and 3% ammonia in centrifuge tubes as previously described. The purest material from tubes 6 to 8, amounting to approximately 18 mg., was freed of impurities, which did not absorb in the ultra-violet, by several recrystallizations from hot water to yield about 6 mg. of colorless crystals which melted and resolidified from 130–145° and finally melted at 267–269°. A synthetic sample of N-(7-chloroquinolyl-4)-β-alanine behaved identically and the melting point was not depressed on mixture of the samples.

Anal. Calc'd for $C_{12}H_{11}ClN_2O_2$: C, 57.5; H, 4.5.
Found: C, 57.1; H, 4.5.

Synthesis of N-(7-chloroquinolyl-4)-β-alanine: (Columbia). A solution of 10 g. of 4,7-dichloroquinoline and 9 g. of β-alanine in 40 g. of phenol was heated with vigorous stirring

for one hour at 160°. When the brown reaction mixture had cooled, it was shaken with 150 ml. of 10% potassium carbonate solution and 250 ml. of ether. The ether layer was decanted from the heavy emulsion which settled in the lower layer. A second extraction with 200 ml. of ether gave a clean separation of the clear layers. After a third ether extraction, the combined extracts were back extracted with several 100-ml. portions of 10% potassium carbonate solution.

The combined aqueous solutions, which showed a blue fluorescence, were neutralized to *p*H 7 with 65 ml. of 6 *N* hydrochloric acid. The finely divided precipitate which formed slowly on standing overnight in the refrigerator was filtered off yielding 8.5 g. (63%) of material which melted, after preliminary melting and resolidification between 130° and 145°, at 265–268°. This material could be recrystallized from boiling water, using about 200 ml. per g., with a recovery of 85%. A 1-g. sample recrystallized twice yielded 729 mg. which first began to melt on the hot stage at 130°, had completely resolidified at 145°, and finally melted at 267–269°.

Anal. Calc'd for $C_{12}H_{11}ClN_2O_2$: C, 57.5; H, 4.5.
Found: C, 57.1; H, 4.5.

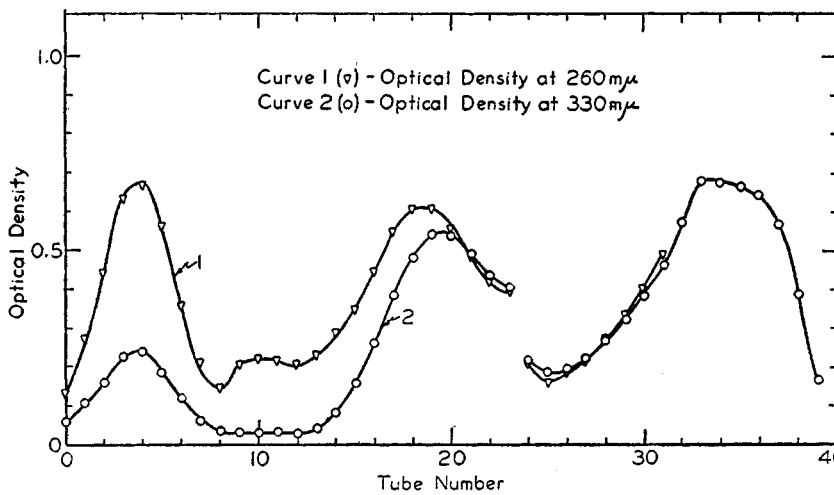


FIG. 12. DISTRIBUTION OF BUTANOL-EXTRACTABLE DEGRADATION PRODUCTS OF SN 9584 IN BUTANOL AND 10% ACETIC ACID

Another sample of the first precipitate obtained above was distributed in an 8-plate run with butanol and 10% acetic acid, using 10-ml. layers in separatory funnels. After the last equilibration, 5 ml. of methanol was added to each tube, and the concentration was estimated spectrophotometrically. The contents of the most concentrated fraction, tube 4, was evaporated under vacuum and the dry residue analyzed.

Anal. Found: C, 57.2; H, 4.6.

Preparation of the methyl ester with diazomethane apparently resulted in the simultaneous methylation of the nitrogen at the 4 position. To 6 ml. of 40% potassium hydroxide solution and 15 ml. of ether at 0° was added 1 g. of N-nitrosomethyl urea. The yellow ether layer was decanted and to it was added about 500 mg. of N-(7-chloroquinolyl-4)-β-alanine.

The finely divided solid was washed into the ether with approximately 10 ml. of methanol and the solution was allowed to stand overnight in the refrigerator during which time most of the suspended solid dissolved. Evaporation of the solvent left an oil which crystallized to a yellow-white solid after standing several hours. This was taken up in hot 95% ethanol. Cooling precipitated colorless rectangles melting at 148–149°. After two recrystallizations from ethanol, the material still melted at 148–149°.

Anal. Calc'd for $C_{14}H_{15}ClN_2O_2$: C, 60.3; H, 5.3.
Found: C, 59.8; H, 5.3.

Preparation of N-(7-chloroquinolyl-4)glycine: (Columbia). A mixture of 10 g. of 4,7-dichloroquinoline, 8.25 g. of glycine and 30 g. of phenol in a 500-ml. flask equipped with stirrer, thermometer, and air-cooled reflux condenser was heated for one hour at an internal temperature of 165–170° with stirring. After cooling, the reaction mixture was shaken between 200 ml. of ether and 200 ml. of 10% sodium carbonate solution and each layer was back extracted with 200 ml. of the other solvent. The aqueous solution was warmed with 5 g. of decolorizing carbon (Norit), filtered and neutralized with 110 ml. of 1 *N* sodium hydroxide solution to *p*H 6.5. On standing overnight in the cold, 7.8 g. of finely divided white precipitate separated, melting at 253–255° with decomposition. The material when tested for homogeneity by counter-current distribution in butanol and 10% acetic acid behaved as a homogeneous substance, but contained about 1% of ash, from which it was liberated by recrystallization from water.

Anal. Calc'd for $C_{11}H_9N_2O_2$: C, 55.82; H, 3.8.
Found: C, 55.5; H, 3.6.

The product was methylated with diazomethane as before yielding a dimethyl compound which melted at 214.5–215° after two recrystallizations from ethanol.

Anal. Calc'd for $C_{13}H_{13}ClN_2O_2$: C, 59.9; H, 5.01.
Found: C, 59.4; H, 5.2.

The two fractions in tubes 0 to 5 and 18 to 24 of Fig. 12 remain to be investigated. That they are degradation products of SN-9584 is indicated by the fact that normal urine when carried through an identical fractionation procedure gave no fraction corresponding to them.

Material isolated from tubes 0 to 4 of Fig. 12 was redistributed in butanol and 10% acetic acid, but only oils were obtained upon evaporation of the most concentrated fractions. No chloroform extractable quinoline bases were liberated from this material when it was subjected to acid hydrolysis under conditions comparable to those used with the conjugate of SN-8137.

SUMMARY

Attempts to isolate degradation products from the urine of men receiving representative antimalarials of the 4-aminoquinoline (chloroquine) type have revealed a series of transformation products. The quinoline nucleus is apparently not involved in these transformations. An ethyl group attached to the terminal nitrogen of the side chain is first removed probably by oxidation. Apparently oxidation of the terminal nitrogen to an oxide type of compound then results and finally complete removal of the terminal basic group results with the formation of an acid.

Another type of transformation involves the formation of a conjugate of the uronic acid type. In this type of transformation oxidation of the terminal nitrogen also appears to be involved.

NEW YORK 21, 27, N. Y.

REFERENCES

(1) CRAIG, *J. Biol. Chem.*, **155**, 519 (1944).
(2) CRAIG, *et al.*, *J. Biol. Chem.*, **161**, 321 (1945).
(3) NORTHRUP AND KUNITZ, *J. Gen. Physiol.*, **13**, 781 (1930).
(4) WILLIAMSON AND CRAIG, *J. Biol. Chem.*, **168**, 687, (1947).
(5) ELDERFIELD, *et al.*, *J. Am. Chem. Soc.*, **68**, 1516, 1524 (1946).

(6) DRAKE *et al.*, *J. Am. Chem. Soc.*, **68,** 1914 (1946).
(7) BUSH AND BUTLER, *J. Pharmacol.* **68,** 278 (1940).
(8) HANDLER, BERNHEIM, AND KLEIN, *J. Biol. Chem.*, **138,** 211 (1941).
(9) MEAD AND KOEPFLI, *J. Biol. Chem.*, **154,** 507 (1944).
(10) CARMACK *et al.*, *J. Am. Chem. Soc.*, **68,** 1220 (1946).
(11) TARBELL *et al.*, *J. Am. Chem. Soc.*, **68,** 1217 (1946).
(12) WEIZMANN AND MALKOWA, *Bull. soc. chim.*, [4], **47,** 356 (1930).
(13) PEARSON, JONES, AND COPE, *J. Am. Chem. Soc.*, **68,** 1225 (1946).
(14) Private communication from Dr. Allen N. Butler, Massachusetts General Hospital, Boston, Mass.
(15) KUHN AND BRYDOWNA, *Ber.*, **70,** 1333 (1937).
(16) ING AND BERGMANN, *J. Biol. Chem.*, **129,** 203 (1939).
(17) MOORE AND STEIN, *J. Biol. Chem.*, **150,** 113 (1943).
(18) PRICE AND ROBERTS, *J. Am. Chem. Soc.*, **68,** 1204 (1946).
(19) HANSON, MILLS, AND WILLIAMS, *Biochem. J.*, **38,** 274 (1944).