[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF COLUMBIA UNIVERSITY]

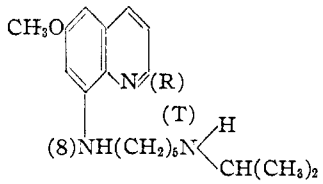
On the Physiological Disposition of Pentaquine Labeled in the Side Chain with N^{15} [1]

BY ROBERT C. ELDERFIELD[2] AND LELAND L. SMITH

RECEIVED JUNE 10, 1952

When pentaquine labeled at either of the side chain nitrogens is fed to monkeys the breakdown of the drug is prompt and substantially complete. Apparently the entire side chain is cleaved from the quinoline nucleus and deamination occurs.

As a part of a general program dealing with the physiological disposition of the 8-aminoquinoline antimalarials in these laboratories and elsewhere, a study of the distribution of isotopic nitrogen in the urines of Rhesus monkeys to which were fed pentaquine labeled in various positions has been made. The positions of the various isotopic labels are shown in the formula



The terminal nitrogen of the side chain will be designated N(T); the 8-amino nitrogen N(8); and the ring nitrogen N(R). The N(T)-labeled pentaquine monophosphate[3] was enriched by 20.5 atom % excess N^{15} and the N(8)-labeled pentaquine monophosphate was enriched by 20.0 atom % excess N^{15}. The particular labeled position was thus enriched about 60 atom % excess N^{15}. The N(R)-labeled drug was not available for this study and its synthesis is described in an accompanying paper.[4]

The drugs were fed to Rhesus monkeys and the urines were collected at Christ Hospital Institute of Research, Cincinnati, Ohio, through the cooperation of Dr. L. H. Schmidt to whom we express our appreciation. The pooled urines were acidified and promptly frozen and were shipped to New York frozen and stored frozen until used.

Results with N(T)-Labeled Pentaquine.—Excretion of N^{15} was followed in the urines in two experiments. (1) The drug was fed at a dose level of 12 mg. of free base per kg. of body weight during which the urines were collected for eight days and for one additional day after stopping the drug. (2) The drug was administered for four days at the same dose level and the urine was collected for four days after stopping the drug. Collection of the urine began at the start of drug administration and each days output was examined separately. In Expt. 1 the total N^{15} fed was 57.11 mg.; in Expt. 2 it was 28.46 mg.

The excretion of total nitrogen and urea nitrogen by days together with data for the excretion of N^{15} for Expt. 1 is shown in Table I. Similar data for the period in Expt. 2 after stopping the drug are shown in Table II. The data in Expt. 2 obtained during drug administration paralleled those of Expt. 1. Thus, during nine days of drug administration about 50% of the total N^{15} fed was excreted, and during the four days immediately following stopping the drug an additional 15% of the N^{15} administered was excreted.

TABLE I

EXCRETION OF TOTAL NITROGEN AND UREA NITROGEN

N(T)-Labeled pentaquine. Expt. 1. Total isotope excreted, 28.711 mg.; total isotope administered, 57.11 mg.; total isotope in urea nitrogen, 4.176 mg.

Day	Wt. urine, g.	Total N, mg.	Excess N^{15}, atom %	N^{15}, mg.	Urea N, mg.	Excess N^{15}, atom %	N^{15}, mg.
1	1161.97	5503	0.111	6.108	4439	0.015	0.666
2	897.06	4336	.097	4.206	3668	.011	.403
3	561.87	2968	.079	2.344	2838	.015	.426
4	887.81	3940	.095	3.743	3115	.015	.470
5	736.56	4147	.059	2.447	3403	.013	.442
6	842.42	4081	.097	3.958	3757	.016	.601
7	1016.59	3491	.090	3.142	2633	.012	.316
8	748.41	2239	.067	1.500	2096	.021	.440
			Drug stopped				
9	371.30	2745	0.046	1.236	2287	0.018	0.412

TABLE II

EXCRETION OF TOTAL NITROGEN

N(T)-Labeled pentaquine. Expt. 2. After stopping drug

Day after stopping drug	Total wt. urine, g.	Total N, mg./g. urine	Total N, g.	N^{15} excess, atom %	Total N^{15} mg.	Fraction of dose, %
1	1552.80	7.230	11.227	0.014	1.572	5.52
2	1145.05	4.411	5.051	.028	1.414	4.96
3	1452.00	3.956	5.744	.017	0.975	3.42
4	960.90	11.201	10.763	.002	0.215	0.76
					4.176	14.7

The amount of N^{15} excreted as measured by the ratio of total N^{15} excreted to N^{15} fed is a maximum on the first day and about 85% of the N^{15} fed was found in the urine of the first 24 hours. This ratio dropped off during succeeding days and became constant at about 52% of the N^{15} fed. When feeding of the drug was stopped an increase in the relative amount of N^{15} excreted was noted. This trend is shown graphically in curve 1 of Fig. 1; the data for which and method of obtaining them are given in Table III.

The excretion of N^{15} as urea nitrogen was followed for nine days with N(T)-labeled pentaquine (Table I). The N^{15} found as urea nitrogen accounted for about 15% of the total N^{15} excreted or about 7.5% of the N^{15} fed. These data are presented in Fig. 2 from which it is obvious that the excretion of N^{15} in urea is fairly constant through-

(1) This investigation was carried out under a grant to Columbia University from the National Institutes of Health.

(2) Department of Chemistry, University of Michigan, Ann Arbor, Mich.

(3) This drug and the N(8) labeled drug were prepared by A. H. Blatt and Norma Gross, THIS JOURNAL, **75**, 1245 (1953).

(4) R. C. Elderfield, L. L. Smith and E. Werble, *ibid.*, **75**, 1245 (1953).

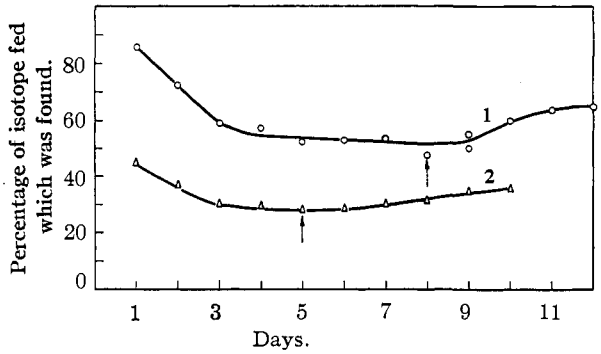


Fig. 1.—Excretion of N^{15}: curve 1, from N(T)-labeled pentaquine; curve 2, from N(8)-labeled pentaquine. Arrows indicate cessation of drug feeding.

TABLE III

RELATIVE EXCRETION OF TOTAL N^{15}

N(T)-Labeled pentaquine. Expts. 1 and 2

Day (i)	N^{15} found, mg. (Ai)	Cumulative N^{15} found, mg. (ΣAi)	Cumulation N^{15} administered (iC)	N^{15} found, % ($\Sigma Ai/iC \times 100$)
1[a]	6.108	6.108	7.14	85.5
2[a]	4.206	10.314	14.28	72.3
3[a]	2.344	12.658	21.42	59.0
4[a]	3.743	16.401	28.56	57.4
5[a]	2.447	18.848	35.70	52.8
6[a]	3.958	22.806	42.84	53.4
7[a]	3.142	26.948	49.98	54.0
8[a]	1.500	27.448	57.11	48.2
		Drug stopped		
9[a]	1.263	27.448	(57.11)	50.3
9[b]	1.572	15.802[c]	(28.46)	55.4
10[b]	1.414	17.216	(28.46)	60.5
11[b]	0.975	18.191	(28.46)	63.8
12[b]	0.215	18.406	(28.46)	64.5

[a] Data from Expt. 1. [b] Data from Expt. 2. Since the drug was stopped in Expt. 2 after the fourth day the ratio of total N^{15} excreted to N^{15} fed will have reached the constant value of about 52%. The data from the two experiments have therefore been combined. [c] In order to compare the results for the period following stopping the drug, 50% of the total N^{15} fed (28.46 mg. in Expt. 2) was added to the summation of N^{15} found. These values are not in perfect agreement with those of the period of drug administration. However they do serve to indicate the trend in excretion of the isotope.

out the course of the experiment with a slight tendency to increase relative to the total N^{15} found toward the end of the experiment. The excretion of N^{15} as urea nitrogen in Expt. 2 after stopping the drug was of about the same magnitude. Although no weights of isotope were determined, figures for enrichment of urea collected in the four days after stopping the drug were 0.023, 0.012, 0.016 and 0.012 atom % excess.

Excretion of unchanged pentaquine was checked by the Brodie method.[5] Negligible amounts of the drug (assuming the Brodie method to be specific for pentaquine and not for its metabolites) were excreted each day (270–312 γ/l./day). With the amount of material available isotope analysis by days was not possible. However, one pooled sample showed 9.35 atom % excess enrichment of

(5) B. B. Brodie, S. Udenfriend and J. V. Taggart, *J. Biol. Chem.*, **168**, 327 (1947).

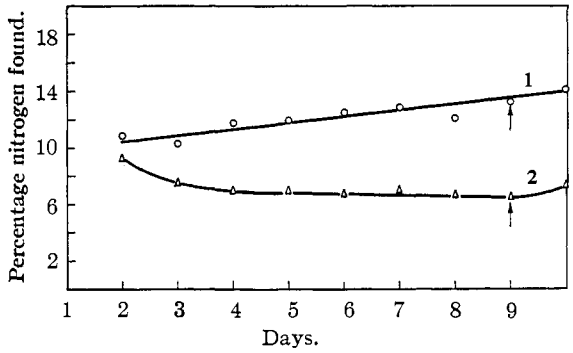


Fig. 2.—Excretion of N^{15} as urea nitrogen from N(T)-labeled pentaquine curve 1, per cent. of total N^{15} found as urea N^{15}; curve 2, per cent. of total N^{15} fed found as urea-N^{15}. Arrows indicate cessation of drug feeding.

N^{15}. The excretion of 8–9 γ of N^{15} per day from 20.5 atom % enriched pentaquine accounts for but 0.002% of the daily dose of labeled drug.

A series of extraction procedures was investigated in the hope that a metabolic product or products of the drug might be isolated. When urine acidified to *p*H 1 was extracted with ethyl acetate, hippuric acid and an intractable, evil smelling resin were obtained. The hippuric acid, isolated in the amount of 250 mg. per liter of urine, was enriched by 0.01 atom % of excess N^{15} which is of the same order of magnitude as the enrichment of the urea. Inasmuch as the isolation of the hippuric acid was not quantitative it is not possible to estimate the absolute amount of N^{15} excreted.

The resinous material gave positive naphthoresorcinol tests for glucuronic acid both before and after acid hydrolysis. This resin, isolated in the amount of 1071 mg. per liter of urine contained 3.86 mg. of total nitrogen per liter of urine which was enriched by 0.013 excess atom % of N^{15}.

Extraction of urine made alkaline with ethylene dichloride, hexane, ether, *n*-butyl alcohol and isoamyl alcohol afforded small amounts of material which was significantly enriched with N^{15}, but the quantities were uniformly too small for further isolation attempts.

Results with N(8)-Labeled Pentaquine.—In general these paralleled the experience with N(T)-labeled pentaquine. The pertinent data for total N^{15} excreted are summarized in Table IV. In this experiment (Expt. 3) drug feeding was maintained for five days and collection of urine was continued for five days after stopping the drug. However the amount of N^{15} excreted both during feeding of the drug and in the subsequent period was significantly less than was the case with the N(T)-labeled drug. Thus, whereas 60–65% of the N^{15} fed as N(T)-labeled drug was excreted during the period of Expt. 1, only about 35% of the N^{15} fed as N(8)-labeled drug was excreted during the period of Expt. 3. The qualitative rate of excretion was the same with both drugs (curve 2, Fig. 1).

The urea excreted in Expt. 3 was not examined in as great detail as was done in Expts. 1 and 2. Atom % enrichments of urea nitrogen for the first four days of drug feeding were: 0.015, 0.017, 0.013 and 0.019. These values compare favorably with similar figures obtained in Expt. 1 (Table I).

TABLE IV

EXCRETION OF TOTAL NITROGEN

N(8)-Labeled pentaquine. Expt. 3

Isotope excreted during feeding, 7.804 mg., 28.0%; isotope excreted after feeding, 2.106 mg., 7.5%; total isotope excreted, 9.910 mg., 35.5%

Day	Wt. urine, g.	Total N, mg./g. urine	Total N, g.	N^{15} excess, atom %	Total N^{15} mg.
1	1467.45	2.414	3.542	0.071	2.515
2	1021.70	3.153	3.221	.049	1.578
3	1482.45	1.881	2.789	.037	1.032
4	1105.65	2.123	2.347	.061	1.432
5	1213.10	4.112	4.989	.025	1.247
		Drug stopped			
6	1300.45	3.000	3.901	0.005	0.195
7	761.60	4.968	3.784	.016	.605
8	1274.35	1.624	2.070	.011	.228
9	917.90	5.045	4.961	.015	.744
10	1210.75	3.454	4.172	.008	.334

In one determination of total urea, for the fourth day, the amount of N^{15} found in the urea was 0.377 mg. as compared with 0.470 mg. excreted with the N(T)-labeled drug. It may therefore be assumed that the total N^{15} excreted as urea nitrogen in Expt. 3 is about 15% of the total N^{15} excreted.

The hippuric acid isolated in Expt. 3 was enriched to the same extent (0.01 atom % excess) as was that isolated from Expt. 1. However the resin obtained along with the hippuric acid in Expt. 3 differed significantly from that obtained in Expt. 1. Inconclusive naphthoresorcinol tests for glucuronic acid were given by this material. Further the total nitrogen found in this material as well as the enrichment were greater by a factor of ten compared to the corresponding material from Expt. 1. We were unsuccessful in isolating any chemical individual from this resin.

Conclusions

From the results obtained one conclusion can be drawn regarding the physiological disposition of N(T)- and N(8)-labeled pentaquine. The breakdown of the drug is prompt and substantially complete. The data suggest that the entire side chain is detached from the quinoline nucleus and that further rupture of the side chain with deamination occurs. The nitrogen thus liberated finds its way into the general nitrogen pool of the body from which it is slowly eliminated.

It is felt that no further useful information can be obtained from these two drugs. It is hoped that some clue to the fate of the quinoline nucleus may be gained from a similar study of the N(R)-labeled drug.

Experimental

Nitrogen Analyses.—The usual micro-Kjeldahl method with modifications developed for the aminoquinolines was used.

For quantitative determinations digestion of the sample (of size to give 0.2–1.0 mg. of nitrogen) with 2 ml. of concd. sulfuric acid and 1.5 g. of a mercury oxide–selenium catalyst[6] for six hours gave quantitative recovery of nitrogen. The digestion mixtures were made alkaline with 10 ml. of 40% sodium hydroxide solution, and, after addition of 10 ml. of 14% sodium thiosulfate solution, the ammonia was distilled into 5 ml. of 4% boric acid containing a drop of methylene blue–methyl red indicator.[7] The distillate was titrated with 0.01 *N* hydrochloric acid.

For the preparation of ammonia for conversion to nitrogen for isotope analysis the same procedure was used except that the sample size was chosen to give 1 mg. of nitrogen and the digestion time was 18–24 hr.[8] The ammonia, collected as ammonium sulfate, was converted to nitrogen for analysis for isotopic content by treatment with sodium hypobromite according to Rittenberg.

Two mass spectrometers were used: a Nier 60° type instrument[9] and a commercial 180° instrument.[10] Both spectrometers gave concordant results. The presence of air in the samples was checked by the argon peak and tank nitrogen was always run with the unknown samples. The enrichment of the samples was usually so small that only the 28 and 29 peaks could be read.

Determination of Urea.—This was done by the Van Slyke–Cullen method[11] with urease from Arlington Chemical Co., Yonkers, N. Y. After 30 minutes the ammonia was aspirated into 4% boric acid for titration or into 0.07 *N* sulfuric acid for isotope analysis.

Xanthydrol precipitation[12] was used for non-quantitative isolation of urea for isotope analysis. The results were consistent with those obtained with urease.

Isolation of Hippuric Acid.—The *p*H of 2750 ml. of urine was adjusted to 1.55 with hydrochloric acid, and the urine was extracted with four 500-ml. portions of ethyl acetate. After centrifuging to break emulsions, the extracts were concentrated at reduced pressure under nitrogen until crystallization began. After treatment with decolorizing carbon and cooling, 686.5 g. of crude hippuric acid, m.p. 172.5–182° separated. Successive recrystallization from ethanol, water and acetone raised the m.p. to 186–188°. The mixture m.p. with a known sample was not depressed. From the filtrate from the first crop, the evil smelling resin described above was obtained on concentration.

NEW YORK 27, N. Y.

(6) J. O. Cole and C. R. Parks, *Ind. Eng. Chem., Anal. Ed.*, **18**, 61 (1946).

(7) T. S. Ma and G. Zuazaga, *ibid.*, **14**, 280 (1942).

(8) D. Rittenberg in D. W. Wilson, A. O. C. Nier and S. P. Riemann, "Preparation and Measurement of Isotopic Tracers," J. W. Edwards, Ann Arbor, Mich., 1948, p. 31.

(9) We wish to acknowledge the help and coöperation of Professor T. I. Taylor, of this Department, in the operation of this instrument.

(10) We wish to acknowledge the help and coöperation of Dr. D. Rittenberg, of the College of Physicians and Surgeons, in the operation of this instrument.

(11) D. D. Van Slyke and G. E. Cullen, *J. Biol. Chem.*, **19**, 141, 181, 211 (1914); **24**, 117 (1916).

(12) R. Fosse, *Compt. rend.*, **158**, 1076, 1432, 1588 (1914).