Figure 7—*Effects of disopyramide on membrane responsiveness in dog Purkinje fibers. The control curve is one characteristic of a normal Purkinje fiber, with the maximum rate of rise (dV/dt) of about 600 v./sec. It is evident that 2 mg./l. disopyramide caused a shift of the membrane responsiveness curve to the right, and there was also depression of dV/dt to around 400 v./sec., while the maximum resting potential remained unchanged (90–95 mv.). The measurements were made 45 min. after exposure to disopyramide. Key: ●—●, control; and ×- - -×, after 2 mg./l. disopyramide.*

tively speaking, the dog Purkinje fibers used in the present study are perhaps at least 5 times more sensitive to the depressant effect of disopyramide than the rabbit atrial muscle fibers. This is consistent with our own data showing that human atrial muscle fibers are more resistant to disopyramide than dog Purkinje fibers. These differences in sensitivity to disopyramide, if not due to species difference, could be explained by the well-known fact that the specialized ventricular conduction system is more sensitive to drugs than ordinary cardiac muscle fibers.

Like quinidine, disopyramide also depresses myocardial contractility and left ventricular function. It would be an undesirable property if the depressant effect of disopyramide on myocardial performance, which outlasts its electrophysiological effects in the dog heart, is also found in man.

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Pharmacokinetics of Iodochlorhydroxyquin in Man

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Abstract ☐ A method of extracting iodochlorhydroxyquin from plasma and quantitating by GLC was developed and used to study the pharmacokinetics in man. A clearcut dose–plasma concentration relationship was obtained, and no evidence of accumulation was found.

Keyphrases ☐ Iodochlorhydroxyquin—pharmacokinetics in man, dose–plasma concentration relationships, GLC determination ☐ Quinolines (iodochlorhydroxyquin)—GLC analysis of human plasma samples, pharmacokinetics ☐ Pharmacokinetics--iodochlorhydroxyquin half-life, dose–plasma concentration relationships, GLC determination ☐ GLC—analysis of iodochlorhydroxyquin in human plasma samples

Recently two publications (1, 2) appeared dealing with the GLC of halogenated quinolines using flame-ionization detection. Neither publication was concerned with the analysis of extracts derived from biological material.

To study the pharmacokinetics of iodochlorhydroxyquin, 5-chloro-7-iodo-8-hydroxyquinoline, a sensitive and specific method of analysis was needed; the presence of two halogen atoms in the molecule made GLC using an electron-capture detector an obvious choice. Acetylation at the 8-position of the quinoline ring system provided a derivative of sufficient volatility to allow 50-ng./ml. concentrations to be measured.

EXPERIMENTAL

By using the developed method, the kinetics in man were studied as follows.

Single-Dose Administration—Six volunteers were given single oral doses of 250 and 1500 mg. of iodochlorhydroxyquin powder[1]

[1] Iodochlorhydroxyquin was administered as a powder with the addition of 7% *N*-stearoyl-*N'*,*N'*-diethylethylenediamine (Sapamine). This formulation is available commercially as Entero-Vioform.
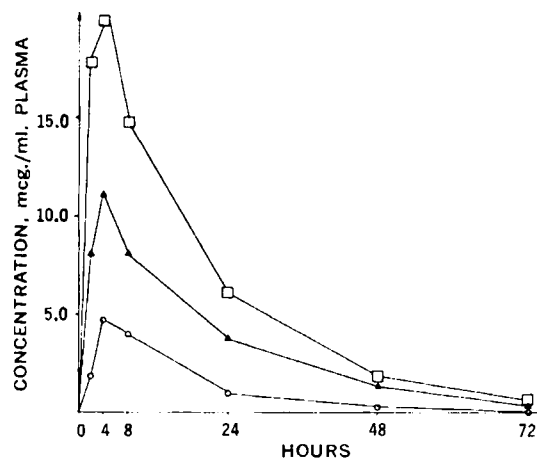
Figure 1—*Mean plasma levels of iodochlorhydroxyquin (micrograms per milliliter) after single oral doses of 250 (O), 750 (▲), and 1500 (□) mg. iodochlorhydroxyquin powder (six subjects per dose).*

in a crossover study, three subjects beginning with the 250-mg. and three with the 1500-mg. dose with 2 weeks between the two administrations.

Another group of six volunteers took a single oral dose of 750 mg. Blood was withdrawn from all subjects immediately before and 2, 4, 8, 24, 48, and 72 hr. after administration.

Multiple-Dose Administration—Six volunteers were given 500 mg. of iodochlorhydroxyquin powder three times daily at 8:00 a.m., 2:00 p.m., and 8:00 p.m. for 7 days followed by 250 mg. three times daily at the same times for an additional 7 days. Blood samples were withdrawn immediately before the first dose of the day on Days 1–5 and 8–12 and at 8:00 a.m. on the first 4 days following the end of administration.

Heparin[2] was added to all samples, which were then immediately centrifuged, and the plasma was stored at −20° until analysis.

Methods and Apparatus—GLC was carried out using a chroma-

Table I—Analysis of Plasma Samples Containing Iodochlorhydroxyquin

Amount Added, ng./ml.	Amount Found, ng./ml.
54	50
	60
107	92
	110
533	580
	490
1065	1152
	1044

tograph[3] equipped with an electron-capture detector ([63]Ni source) operated with a pulse space of 150 μsec. A 3.04-m. (10-ft.) glass column, 0.31 cm. (0.125 in.) i.d. filled with 3% OV-17 on Gas Chrom Q, 100–120 mesh[4], was used. The column oven temperature was kept at 205° with the injector block and detector oven temperatures at 230 and 340°, respectively. Nitrogen at a flow of 50 ml./min. was used as carrier gas. Under these conditions, the retention times of the internal standard 5,7-dichloro-8-acetoxyquinoline and of 5-chloro-7-iodo-8-acetoxyquinoline were 6.0 and 14.6 min., respectively.

Extraction—To correct for losses during the extraction, 5,7-dichloro-8-hydroxyquinoline (1000 ng.) was added to each plasma sample (up to 2 ml. used) before extraction. Water (14 ml.) was then added and the sample was extracted three times with 2-ml. portions of methylene chloride–ether (1:4). After centrifugation, the organic phase was removed and evaporated, and 200 μl. of acetic anhydride and 50 μl. of pyridine were added. The sample was placed in a water bath at 70° for 1 hr. and, after the addition of 4
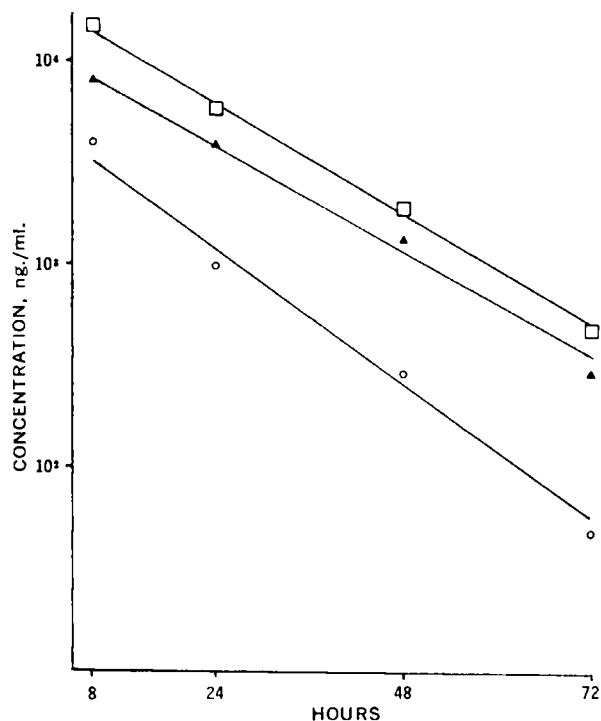

Figure 2—*Elimination of iodochlorhydroxyquin from plasma after single oral doses of 250 (O), 750 (▲), and 1500 (□) mg. iodochlorhydroxyquin powder (six subjects per dose).*
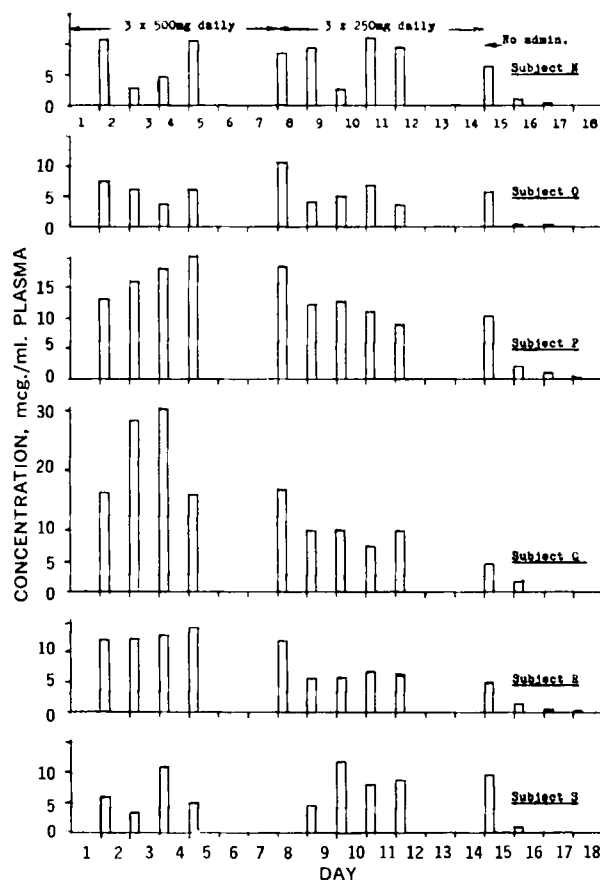

Figure 3—*Plasma levels of iodochlorhydroxyquin during multiple-dose administration of iodochlorhydroxyquin powder.*

[2] Liquemine, Roche.

[3] Pyc 104.
[4] Applied Science Labs., Inc., State College, Pa.

Table II—Plasma Levels of Iodochlorhydroxyquin, Micrograms per Milliliter, after Single Oral Doses of Iodochlorhydroxyquin Powder

Dose, mg. Subject	250						750						1500					
	G	H	I	K	L	M	A	B	C	D	E	F	G	H	I	K	L	M
Hours																		
0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2	1.2	1.0	1.7	4.4	2.0	1.5	4.8	2.7	18.0	8.7	8.3	6.5	6.2	12.9	19.5	34.8	9.2	24.8
4	3.8	1.4	1.6	8.0	6.9	7.1	8.6	4.2	23.2	10.5	9.8	10.3	16.6	14.6	33.5	24.7	14.7	16.9
8	4.0	3.0	1.5	8.3	3.8	3.5	5.4	3.6	15.8	8.9	6.6	9.3	16.7	14.2	15.2	25.4	10.9	6.5
24	0.6	1.0	0.7	1.7	1.3	0.7	4.1	2.9	5.8	3.1	3.4	4.2	11.7	6.2	5.7	7.8	2.9	3.1
48	0.2	0.3	0.1	0.5	0.5	<0.05	0.6	1.0	1.5	1.9	1.2	2.4	0.6	1.6	0.8	4.5	2.6	1.3
72	<0.05	<0.05	<0.05	0.2	0.2	<0.05	0.1	0.1	0.2	0.3	0.8	0.5	0.1	0.2	0.1	2.6	0.8	0.3

Table III—Standardized Areas under Plasma Concentration Curves, (Micrograms per Milliliter) (Hour)

Dose, mg./kg.	Subject												Mean ± SEM
	A	B	C	D	E	F	G	H	I	K	L	M	
3.33	—	—	—	—	—	—	60	63	38	171	79	76	81 ± 19
10.00	189	175	246	240	265	207	—	—	—	—	—	—	220 ± 14
20.00	—	—	—	—	—	—	447	438	356	594	238	310	397 ± 51

ml. of water, extracted with 3 × 1 ml. ethyl acetate. The ethyl acetate was washed once with 2 ml. of saturated sodium bicarbonate, evaporated, and redissolved in 100 μl. ethyl acetate for injection.

In cases where more than 0.5 ml. of plasma was taken, it was necessary to add a TLC step. The ethyl acetate extract (100 μl.) was applied to a silica gel plate with a reference compound on either side, and the plate was developed in hexane–ether (1:3). The reference compound may be the 8-acetoxy derivative of either the 5,7-dichloro or 5-chloro-7-iodo compound, since they both have the same R_f value in this system. After development, the appropriate region is noted, scraped off, shaken with 1 ml. of saturated sodium bicarbonate, and extracted (3 × 0.5 ml.) with ethyl acetate. After centrifugation, the organic phase is evaporated and redissolved in 100 μl. ethyl acetate for injection. The use of 5,7-dichloro-8-hydroxyquinoline, both as an internal standard for the extraction and as the 8-acetoxy derivative for GLC, permitted the amount of iodochlorhydroxyquin present in the sample to be calculated from the peak area ratio of the two acetoxy derivatives. Plasma samples

Table IV—Plasma Levels of Iodochlorhydroxyquin (Micrograms per Milliliter) Measured Immediately before the Morning Dose (8:00 a.m.) during Multiple-Dose Treatment with Iodochlorhydroxyquin Powder, 3 × 500 mg. Daily for 7 Days Followed by 3 × 250 mg. for an Additional 7 Days

Day	Subject					
	N	O	P	Q	R	S
3 × 500 mg. Daily						
1	—	—	—	—	—	—
2	10.7	8.1	13.5	16.1	12.2	5.4
3	2.7a	6.1	15.7	28.0	12.2	3.0
4	4.5	3.6	18.1	30.0	12.9	10.7
5	10.5	6.6	20.0	15.7	14.1	4.7
6						
7						
3 × 250 mg. Daily						
8	8.3	10.2	18.5	16.8	11.9	<0.05
9	9.3	3.9	11.7	9.9	5.5	4.3
10	2.5	4.6	13.0	9.8	5.6	11.6
11	11.0	6.6	10.7	7.1	6.6	7.7
12	9.5	3.4	8.7	9.9	6.4	8.5
13						
14						
No Administration						
15	6.5	5.6	10.0	4.5	4.6	9.1
16	0.7	0.1	1.7	1.5	1.5	0.9
17	0.1	0.06	0.7	<0.05	0.6	<0.05
18	<0.05	<0.05	0.1	<0.05	0.08	<0.05

a Dose not taken the evening before.

were prepared with added iodochlorhydroxyquin and analyzed using this method (Table I).

RESULTS AND DISCUSSION

Single-Dose Administration—The results of the single-dose study are given in Table II and illustrated in Fig. 1. Maximum plasma concentrations were reached around 4 hr. after administration for the three doses studied and then decreased with an apparent half-life of between 11 and 14 hr. (Fig. 2).

For the average plasma concentration curves, the time of maximal concentrations observed and the half-life values obtained allow the calculation of the range of the absorption rate constant, k_a, which was found to be between 0.63 and 0.69 hr.[−1].

The areas under the plasma curves can be calculated and standardized for the body weight of the individual according to the following formula:

$$\text{standard area} = \frac{Wi}{75} \int_0^{72\ \text{hr.}} c\,dt \ (\text{mcg./ml.})(\text{hr.}) \quad (\text{Eq. 1})$$

where Wi = individual body weight, and 75 kg. is taken as the standard body weight. The area under the plasma curve values so obtained (Table III) clearly reflect the different dosages. Comparison of the area under the plasma curve values of Subjects G–M showed no statistically significant difference in the relative absorption of the two dosages of 250 and 1500 mg.

Metabolic studies using ^{14}C-iodochlorhydroxyquin showed that approximately 25% of a 750-mg. oral dose was excreted in the urine over 72 hr., demonstrating a substantial absorption of the drug from the GI tract. Urine samples collected in this study were examined using the GLC method, and no free iodochlorhydroxyquin was found. Heating the urine samples for 1 hr. at 60° in hydrochloric acid liberated some iodochlorhydroxyquin, which accounted for less than 1% of the administered dose. No attempt was made to estimate glucuronide-conjugated iodochlorhydroxyquin. These findings agree well with a study (3) where 12–24% of the administered dose (250 mg.) was found in the urine over 10 hr. No free iodochlorhydroxyquin was found.

Multiple-Dose Administration—The plasma levels measured immediately before the first dose of the day are given in Table IV and illustrated in Fig. 3. Equilibrium between the amount absorbed and the amount eliminated per 24-hr. period appears to be reached by the 5th day of treatment at the latest. After discontinuation of administration, the plasma levels fall to undetectable values after approximately 3 days, which is in accord with the single-dose findings. Urine samples collected over 1 hr. immediately before the first dose of the day were analyzed when no free iodochlorhydroxyquin was found. No evidence of the accumulation of iodochlorhydroxyquin in the plasma was found.

SUMMARY

A GLC method, capable of measuring plasma levels as low as 50 ng./ml. of iodochlorhydroxyquin, was developed. Volunteers given single oral doses of 250, 750, and 1500 mg. of iodochlorhydroxyquin powder exhibited a clear dose–plasma concentration relationship. Calculation of the area under the plasma concentration curves showed that the relative absorption of the three doses was similar. The half-life was between 11 and 14 hr. Volunteers given orally 3 × 500 mg. of the powder daily for 7 days followed by 3 × 250 mg. daily for an additional 7 days had blood samples withdrawn 24 hr. after the daily dose. Equilibrium between the amount absorbed and the amount eliminated per 24-hr. period appeared to be reached by the 5th day of administration. After discontinuation of treatment, the plasma levels fell to undetectable levels after 3 days, a finding that agreed well with the results of the single-dose study. No evidence of the accumulation of iodochlorhydroxyquin was found.

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Blood Level Profile in Man following Chronic Oral Administration of Flurazepam Hydrochloride

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Abstract □ The blood level profile of flurazepam and its major metabolites was determined in man following the oral administration of 30 mg. daily for 2 weeks. The levels of the intact drug were below the sensitivity limit of the assay (<3–4 ng./ml.). The hydroxyethyl metabolite was measurable only during the early hours after a 30-mg. oral dose and was not detectable after 24 hr. The major metabolite in blood was N-desalkyl-flurazepam, which reached steady-state (plateau) levels after 7 days. The half-life of elimination of the N-desalkyl metabolite ranged from 47 to 100 hr. No measurable amounts of the N-desalkyl-3-hydroxy metabolite were seen in blood.

Keyphrases □ Flurazepam hydrochloride and metabolites—blood level profile in man following 14 days administration, half-lives □ Blood level profile—flurazepam hydrochloride and metabolites after 14 days administration, man □ Biotransformation—flurazepam hydrochloride after chronic administration, blood level profile including metabolites, half-lives □ Benzodiazepines, flurazepam hydrochloride—biotransformation, blood level profile, metabolites

Flurazepam[1], 7-chloro-1-[2-(diethylamino)ethyl]-5-(*o*-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one dihydrochloride (I), was synthesized by Sternbach *et al.* (1) and is marketed as a hypnotic for the treatment of insomnia (2). Studies on the metabolism of flurazepam (3) showed that it was extensively metabolized in both man and dog to yield measurable amounts of the hydroxyethyl (II) and N-desalkyl (III) metabolites in the blood. The latter was further metabolized to the 3-hydroxy analog (IV), which appeared in trace amounts in urine as a glucuronide conjugate. In addition, they showed that both flurazepam and the hydroxyethyl

metabolite were metabolized extensively in the dog to an acidic compound, flurazepam-N-yl-acetic acid (V), by the oxidation of the alcohol side chain to a carboxylic acid. Flurazepam-N-yl-acetic acid is a minor urinary metabolite in man.

The characterization of these metabolites and the synthesis of authentic reference compounds were reported previously (4, 5). The objective of the present study was to define the blood level profile of flurazepam and its major biotransformation products when the drug is administered once daily for 14 days at the usually recommended therapeutic dose of 30 mg./day.

PROCEDURE

Blood levels of flurazepam and its major biotransformation products were analyzed by a spectrofluorometric method (6), which involves selective extraction of flurazepam and its metabolites (II, III, and IV) from blood buffered to pH 9.0 into ether, back-extraction into 6 *N* HCl, and hydrolysis to their benzophenones followed by cyclization to the highly fluorescent 9-acridanone derivatives. These derivatives are extracted into ether and separated by TLC, and their fluorescence is determined in methanol–0.1 *N* HCl (80:20) (after elution from the silica gel) at their respective activation and emission maxima.

The sensitivity of the assay is of the order of 3–10 ng. of each compound/ml. of blood using a 4-ml. specimen per analysis. The spectrofluorometric assay does not specifically differentiate N-desalkyl-flurazepam from the N-desalkyl-3-hydroxy metabolite if present, since they both give rise to the same 9-acridanone derivative (6). However, the presence of N-desalkyl-flurazepam as the major metabolite in the blood of these subjects was confirmed by electron-capture GLC analysis[2], which completely resolves the N-