possessing a higher ratio, *i.e.*, thiopental and thiamylal, become concentrated to a higher degree in the body lipids than do barbital or metharbital and might be expected to be ultrashort acting.

In considering these solubility data in total, an approximate correlation has been observed between the lipophilic nature of the various barbiturate analogs and their therapeutic action. One must view this study with proper perspective in relation to the numerous other physical and chemical properties as well as the various biopharmaceutical parameters which all contribute to the variation in the final therapeutic activity possessed by the members of this series. The net result of the complex interaction of these and other factors determines the type and degree of the pharmacological activity involved.

The underlying concept of the pH-partition hypothesis as an approximate model would seem to be confirmed. However, such phenomena as binding, detoxification, active or passive diffusion, and complexation would play important roles in biological readouts which were not patternized or aberrant in behavior.

It is anticipated that several studies on the barbiturates and sulfonamides relative to solubility and partitioning will be undertaken in these laboratories and will be the subject of future communications.

REFERENCES

(1) A. N. Paruta, *J. Pharm. Sci.*, **58**, 204(1969).

(2) "Remington's Pharmaceutical Sciences," part V, No. 67, Mack Publishing Co., Easton, Pa., 1965.

(3) C. Hansch and S. M. Anderson, *J. Med. Chem.*, **10**, 745 (1967).

(4) A. N. Paruta and S. A. Irani, *J. Pharm. Sci.*, **55**, 1060 (1966).

(5) S. A. Khalil and A. N. Martin, *ibid.*, **56**, 1225(1967).

(6) A. N. Paruta, B. J. Sciarrone, and N. G. Lordi, *ibid.*, **51**, 704(1962).

(7) N. G. Lordi, B. J. Sciarrone, T. J. Ambrosio, and A. N. Paruta, *ibid.*, **53**, 463(1964).

(8) *Ibid.*, **53**, 1349(1964).

(9) R. E. Stuckey, *J. Pharm. Pharmacol.*, **14**, 217(1941).

(10) L. Reber and C. Pathamanon, *Amer. J. Pharm.*, **139**, 234 (1967).

(11) System/360 Scientific Subroutine Package (360A-CM-03X), Version III, Programmers Manual, International Business Machines Corp., White Plains, N. Y., 1968, p. 408.

(12) B. Ostle, "Statistics in Research," Iowa State College Press, Ames, Iowa, 1954, chap. 6.

(13) T. C. Daniels and E. C. Jorgensen, in "Textbook of Organic Medicinal and Pharmaceutical Chemistry," 4th ed., C. O. Wilson and O. Gisvold, Eds., J. P. Lippincott, Philadelphia, Pa., 1962, pp. 334, 336–340.

(14) S. K. Sharpless, in "The Pharmacological Basis of Therapeutics," 3rd ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N. Y., 1968, p. 107.

ACKNOWLEDGMENTS AND ADDRESSES

Received September 2, 1969, from the *College of Pharmacy, University of Rhode Island, Kingston, RI 02881*

Accepted for publication May 11, 1970.

Abstracted in part from a dissertation presented by Thomas L. Breon to the Graduate School, University of Rhode Island, in partial fulfillment of the Master of Science degree requirements.

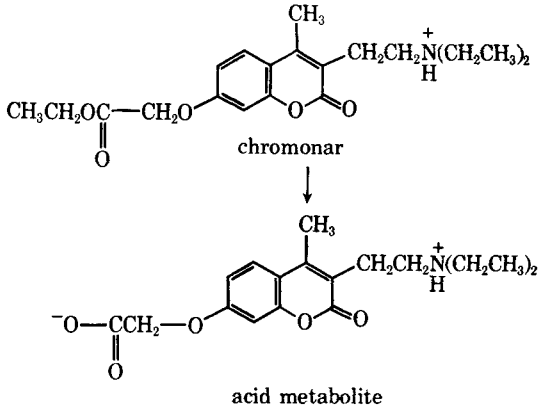
Metabolism and Excretion of Chromonar and Its Metabolite in Dog and Man

YVONNE C. MARTIN and RONALD G. WIEGAND

Abstract □ A simple method for the detection of chromonar and its acid metabolite by fluorescence techniques is described. Following oral or intravenous administration, chromonar is rapidly hydrolyzed to its metabolite, the corresponding acid. No further metabolism is observed. The metabolite distributes according to a single-compartment model. The plasma half-life is 1 hr. in man and dog. Excretion of the metabolite into the bile accounts for approximately 25% of the dose, and excretion into the urine accounts for the remainder.

Keyphrases □ Chromonar—metabolism, excretion □ Metabolite, chromonar—*in vitro, in vivo* determination □ Blood levels, chromonar and acid metabolite—human, dog □ Excretion—chromonar acid metabolite □ TLC—separation, identification □ Fluorometry—analysis

The coumarin compound chromonar[1] is used for the treatment of angina pectoris in Germany and Japan. The early report by Klarwein and Nitz (1) demonstrated that when the drug comes in contact with biological tissues, it is rapidly hydrolyzed to the corresponding acid (Scheme I), which exists as a zwitterion at the pH of the blood and urine. The present authors, therefore expected that the tissue distribution and further metabolism of the compound would be minimal and that the study of plasma and urine concentrations of the acid metabolite at various times after dosage would provide the information necessary to assess the way that chromonar is handled by the body.



Scheme I—Metabolism of Chromonar

[1] 3-(β-Diethylamino-ethyl)-4-methyl-7-carbethoxy-methoxy-2-oxo-(1,-2-chromene). Also known as Cassella 4489, Abbott-27053, and Intensain hydrochloride. Chromonar is marketed by the Cassella Co. of Germany.

Table I—Kinetic Parameters ($\pm SD$) of the Appearance and Disappearance of the Acid Metabolite Following Intravenous Administration of Chromonar to Dogs[a]

Dog	Dose, mg./kg.	Approximate Half-Life, hr., Chromonar	Conversion Constant, Chromonar to the Acid Metabolite, $hr.^{-1}$	Metabolite Disappearance Constant, $hr.^{-1}$	Metabolite Half-Life, hr.	Metabolite Apparent Relative Volume of Distribution, V_d', l./kg.
1	2	0.04	18.6 ± 3.6	1.11 ± 0.02	0.62	0.356 ± 0.022
	5	0.02	85.3 ± 13.1	0.76 ± 0.10	0.92	0.482 ± 0.018
	9	0.01	167 ± 44	0.94 ± 0.20	0.74	0.541 ± 0.036
2	2	0.04	23.2 ± 3.8	0.89 ± 0.12	0.78	0.500 ± 0.033
	5	0.04	20.6 ± 3.7	0.85 ± 0.14	0.81	0.429 ± 0.029
	10	0.04	25.7 ± 5.1	0.36 ± 0.11	1.94	0.563 ± 0.034
3[b]	10	0.02	7.1 ± 1.5	1.09 ± 0.21	0.64	0.408 ± 0.050
4[b]	10	0.02	6.5 ± 1.0	0.95 ± 0.13	0.73	0.458 ± 0.039
5	5			0.87	0.80	
6	5			0.91	0.76	
Mean		0.03		0.87	0.87	0.467

[a] Calculated from the plasma levels of the metabolite and whole blood levels of chromonar. [b] These dogs received 10 mg./kg. chromonar intravenously 6 days/week for 25 months prior to this study.

Table II—Kinetic Parameters ($\pm SD$)[a] of Disappearance of the Metabolite from Plasma Following Oral Administration of Chromonar to Dogs

Dog	Dose, mg./kg.	Lag, hr.	Metabolite Disappearance Constant, $hr.^{-1}$	Metabolite Half-Life, hr.	Metabolite Apparent Relative Volume of Distribution, l./kg.
7	50	0.25	0.71 ± 0.04	0.97	5.78 ± 0.19
8	150	0.25	0.48 ± 0.05	1.44	7.35 ± 0.07
9	150	0.25	0.65 ± 0.04	1.06	2.80 ± 0.11
Mean			0.616	1.16	5.31

[a] Estimated by the method of Wiegand and Sanders (2), the variance was minimized by manipulation of the time lag.

Since both compounds are highly fluorescent, it was possible to modify the original assay method of Klarwein and Nitz (1) to make it more sensitive. To determine the amount of chromonar in a sample, it was extracted immediately after collection into ether from basic solution; then it was reextracted into acid, and the fluorescence of the acid phase was determined. The amount of the acid metabolite in a sample was determined by the fluorescence of the supernatant solution after trichloroacetic acid precipitation.

EXPERIMENTAL

Reagents—Chromonar and the acid metabolite[1] were used. The standards used in the analytical procedures were homogeneous on TLC in Solvent Systems I and II. The chromonar hydrochloride, which was administered to animals and to human subjects, contained 0.6% of 3-(β-diethylamino-ethyl)-4-methyl-7-hydroxy-2-oxo-(1,2-chromene), which is the immediate chemical precursor of chromonar. The fluorescence spectrum of this compound did not interfere with the determination of chromonar or the acid metabolite.

Diethyl-*p*-nitrophenyl monothiophosphate[2] was the commercial product. All other reagents were commercial products of reagent grade.

Fluorescence Analysis of Chromonar—Within 5 sec. after withdrawal from the subject, the blood sample (usually 1 ml.) was shaken vigorously by hand for 1 min. in a 50-ml. ground-glass-stoppered centrifuge tube with 1 ml. 1 *M* tris chloride buffer, pH 8.6, and 25 ml. diethyl ether. These ether extracts are stable for several hours. When a convenient number of samples had been extracted, within 1–3 hr. of the original extraction, the tubes were centrifuged at low speed and 20 ml. of the ether phase was transferred to a second tube which contained 3 ml. 0.1 *N* HCl. The samples were then mechanically shaken for 10 min. and centrifuged. The ether layer was discarded, and the fluorescent intensity of the aqueous layer was determined in an Aminco-Bowman spectrophotofluorometer. The excitation wavelength was 334 mμ and the emission wavelength was 400 mμ. A tissue blank, standard chromonar solution, and tissue blank plus standard were analyzed with each set of samples.

Fluorescence Analysis of Chromonar plus Its Acid Metabolite—When the total of chromonar plus the acid metabolite is to be analyzed, there is no need for the pH 8.6 extraction. Consequently, the sample is treated with trichloroacetic acid to precipitate proteins, and the fluorescence is read on the supernatant solution.

Plasma or urine samples were used as such or diluted with isotonic saline. Feces were homogenized with five volumes of isotonic saline and then further diluted with saline if necessary. Samples could be stored at −15° for several weeks with no apparent degradation of the acid metabolite.

For the analysis, 2 ml. of 5% trichloroacetic acid was added with vigorous shaking to 0.1 ml. of the sample. The fluorescence of the clear supernatant layer was determined after the samples were centrifuged. A tissue blank, standard, and tissue blank plus standard were included with each set of samples.

TLC of Urine, Bile, and Fecal Samples—The methods and solvent systems were similar to those used by Klarwein and Nitz (1). Following sample applications the TLC plates (Analtech silica gel G, 200 μ thick) were first developed with water, then dried for 1 hr. at 100°, and then redeveloped with one of the following systems: I, ethanol–chloroform–water in a 10:2:1 volume ratio; II, ethylacetate–isopropyl alcohol–ammonium hydroxide in a 9:7:4 volume ratio; or III, chloroform–isopropyl alcohol–acetic acid–water in a 4:2:1:1 volume ratio. The location of fluorescent spots was detected under long wavelength UV light by comparison with authentic compounds; chromonar and the acid metabolite emit a characteristic yellow-green color.

To quantitate the acid metabolite after chromatography of a known volume of sample, the spots with the same R_f as the standard were scraped off and extracted with several portions of 95% ethanol. The samples were diluted with water, and the metabolite

[2] Parathion, K&K Laboratories, Inc., Plainview, N. Y.

content was then determined in 5% trichloroacetic acid solution as previously described.

***In Vitro* Conversion of Chromonar to Its Metabolite by Human Plasma**—Freshly collected human plasma from three subjects was incubated with chromonar. At 1, 2, 3, 4, and 5 min., samples were removed and analyzed for the concentration of unchanged drug.

Dog Blood Level Studies—The plasma concentration of chromonar and its acid metabolite was determined after intravenous administration of chromonar in isotonic saline to six dogs. Dogs 1 and 2 were 10-kg. female mongrels which had been anesthetized by 3 mg./kg. s.c. morphine followed in 15 min. by 250 mg./kg. sodium barbital. Three doses were given to each animal, with intervals of 2 hr. Blood samples were taken from the jugular vein. Dogs 3 (female, 9.5 kg.) and 4 (male, 9.6 kg.) were on a chronic toxicity study for 25 months at the same dose. They were not anesthetized. Blood samples from these dogs were taken from the femoral vein. Female mongrels, Dogs 5 (5.5 kg.) and 6 (14 kg.), were anesthetized as described. The ureters and common bile ducts were cannulated, and the total urinary and biliary output was collected for 4 hr.

The plasma concentration of the acid metabolite was also determined after oral administration of chromonar in a capsule. Dog 7 was a 14.5-kg. male. Dogs 8 (female, 7.1 kg.) and 9 (male, 13.8 kg.) had been on a chronic toxicity study for 25 months at the same dose. None of these dogs was anesthetized. Blood samples from these dogs were taken from the femoral or jugular vein.

Human Blood Level Studies—In a multiple-dose study, 12 normal volunteers were divided into two groups of six. The first group, Subjects 1–6, took 150 mg. of chromonar orally on Day 1 and, for the following 6 days (2–7 inclusively), 150 mg. orally, t.i.d. On the 8th day of the experiment, they were given a final oral dose of 150 mg. In addition, Subjects 1 and 2 received an intraduodenal dose of 150 mg. prior to Day 1. The second group was given 40 mg. of chromonar intravenously on Day 1; for the subsequent 6 days, they received 150 mg. of the drug orally, t.i.d. On the 8th day, they were given a second 40 mg. of chromonar intravenously. Plasma samples were collected from blood taken from the femoral vein on Days 1 and 8 at appropriate times. Urine samples were collected prior to and 0–4, 4–8, and 8–24 hr. following drug administration.

Six additional normal adult subjects were given three 150-mg. oral doses of chromonar tablets with 6 hr. between doses. Plasma was obtained from blood drawn at 0, 1, 2, 4, 6, 8, 10, 12, 14, and 24 hr.

Excretion of the Acid Metabolite by Humans—To quantitate the amount of acid metabolite recovered in urine and feces, four subjects (19–22) were given a single 150-mg. tablet of chromonar. Urine and feces were collected for 4 days after drug administration.

Calculation of Kinetic Constants from Blood Level Studies—For the calculation of the disappearance constant and the apparent relative volume of distribution of the metabolite, the computer program of Wiegand and Sanders (2) was used. The curve of the plasma levels of the metabolite, which was determined following the oral administration of chromonar to dogs, often indicated an apparent delay in absorption of 10–15 min. In these cases the kinetic constants were recalculated using several time lags. The lag which resulted in the best fit (lowest sum of squares of deviations of experimental points from the calculated curve) was chosen, and these values are reported in the tables. For the case of blood levels of the metabolite following oral administration of chromonar to humans, data were not sufficient for use of this program. In the case of intravenous administration of chromonar to dogs, the first-order constant that describes the increase in plasma concentration of the metabolite relates to the conversion of chromonar to its metabolite rather than to absorption of drug, as in the original formulation of the program (3).

For intravenous administration of chromonar to humans, plasma samples were not taken until 0.5 hr. after the dosing. Thus, the conversion to the acid metabolite was complete. Since the disappearance curve suggested a single-compartment model, the plasma half-life of the metabolite was calculated for these subjects, assuming an exponential decline of plasma levels with time. The slope of the least-squares line of the logarithm of concentration against time gives the first-order disappearance constant, k_d. The plasma half-life, $t_{1/2}$, is related to k_d by the expression $t_{1/2} = \ln 2/k_d$. The intercept of this plot is c_o, the theoretical initial plasma concentration. However, this theoretical initial concentration is also equal to the dose of the drug, a_o, divided by the apparent relative volume of distribution of the drug V_d'. Thus, V_d' was evaluated as $V_d' = a_o/c_o$.

Table III—Recovery of the Acid Metabolite 4 hr. after the Intravenous Administration of 5 mg./kg. Chromonar to Dogs

Dose, %	Dog 5	Dog 6
Urine	53.2	75.3
Bile	27.4	16.6
Total recovery, %	80.6	91.9

Calculation of Area under the Blood Level Curves—Areas under the acid metabolite plasma level curves for the subjects who had received chromonar orally were calculated by the addition of the area of each triangular or trapezoidal segment of the actual curve. The areas on Day 8 were corrected for the area due to the initial plasma concentration of the metabolite.

Areas under the metabolite blood level curve following intravenous administration were calculated from the extrapolated theoretical initial plasma concentration of the metabolite. The relationship is: area $= c_o/k_d$.

RESULTS

Analytical Methods—Klarwein and Nitz (1) reported that diethyl-*p*-nitrophenyl monothiophosphate could be used to prevent the hydrolysis of chromonar. However, in preliminary investigations, it was observed that when diethyl-*p*-nitrophenyl monothiophosphate was used, a slight excess of this inhibitor (twice the concentration necessary for inhibition of the serum esterase) quenched the fluorescence of the chromonar in the final solution. Thus, diethyl-*p*-nitrophenyl monothiophosphate was not used in the present method; instead, all samples analyzed for the concentration of chromonar were extracted immediately (within 5 sec.) from the basic solution with ether. With this procedure, 95–100% of the standard chromonar which had been added to a blood or tissue homogenate sample was recovered. A typical blank blood sample corresponded to 0.02 mcg. chromonar/ml.

In the assay method for the total of chromonar and its acid metabolite, the simple trichloroacetic acid precipitation to remove most of the interfering substances from plasma, urine, bile, and feces was practical because of the intense fluorescence of the drugs. A relatively selective and sensitive spectrophotofluorometer is also necessary. In this procedure, a typical plasma blank in the determination of either chromonar or its acid metabolite corresponded to 0.01 mcg./ml.

For both methods the range of concentrations over which the fluorescence is linear with respect to concentration was 0.04–10.0 mcg./ml. sample. Above this range the fluorescence decreased with concentration. Since the concentration of drug in certain urine and bile samples exceeded 10 mcg./ml., appropriate dilutions were necessary.

The intensity of fluorescence of chromonar and its metabolite depends on the pH of the sample (1). It was, therefore, essential that the samples, standards, and blanks were prepared in the same way. If a sample exhibited too high a fluorescence, it was not further diluted with water but rather with the same concentration of acid that had been used in the original determination.

***In Vitro* Conversion of Chromonar to Its Metabolite by Human Plasma**—The half-life ($\pm SD$) was calculated to be 2.68 ± 0.96 min.; thus, chromonar is extremely short-lived in human plasma.

Blood Levels in Dogs—Calculations derived from the plasma concentration of chromonar and its metabolite at various times after an intravenous dose of chromonar are summarized in Table I. The half-life of chromonar is very short in the dog; essentially none remained in the blood after 10 min. (0.17 hr.). The plasma half-life of the metabolite averaged 0.87 hr. This relatively short half-life represents either efficient excretion or rapid metabolism of the metabolite. The decline in plasma levels of the metabolite parallels the decline of coronary vasodilation.[3] The half-life of the metabolite was not significantly shorter in Dogs 3 and 4, which had been on a chronic study for 25 months prior to this study.

[3] T. D. Darby and Y. C. Martin, unpublished observations.

Table IV—Calculations from Plasma Levels of the Metabolite in Six Subjects Given 40 mg. Chromonar Intravenously

Subject	Sex	Dose, mg./kg.	Day	Metabolite Disappearance Constant, hr.,$^{-1}$ $\pm SD$	p^a	Metabolite Half-Life, hr.	Intercept C_o, mcg./ml.	Area, hr. mcg./ml.	Metabolite Apparent Relative Volume of Distribution, l./kg.
7	M	0.48	1	0.670 ± 0.071	0.50	1.03	1.20	1.79	0.40
			8	0.735 ± 0.052		0.94	1.36	1.85	0.35
8	F	0.70	1	0.858 ± 0.102	0.26	0.81	0.89	1.04	0.79
			8	0.716 ± 0.043		0.96	1.33	1.86	0.53
9	M	0.52	1	0.903 ± 0.106	0.63	0.76	1.51	1.67	0.34
			8	0.804 ± 0.019		0.86	1.20	1.49	0.43
10	M	0.52	1	0.843 ± 0.026	0.79	0.82	1.46	1.73	0.36
			8	0.856 ± 0.038		0.81	1.33	1.55	0.39
11	F	0.77	1	0.855 ± 0.033	0.31	0.81	1.75	2.05	0.44
			8	0.905 ± 0.032		0.77	1.92	2.12	0.40
12	F	0.66	1	0.957 ± 0.053	0.50	0.72	1.53	1.60	0.43
			8	0.882 ± 0.088		0.79	1.81	2.05	0.36
Mean			1	0.861 ± 0.063	0.68	0.80	1.39	1.65	0.46
Mean			8	0.830 ± 0.043		0.84	1.49	1.82	0.41
Mean			1 and 8			0.82	1.44	1.74	0.44

[a] Probability value, which should be less than 0.05 if slopes are significantly different on Days 1 and 8 in same subject.

With oral administration of chromonar, a 0.25-hr. time lag in absorption was observed (Table II); this is approximately the disintegration time of the gelatin capsules used to administer the drug. The mean half-life of the metabolite in blood was 1.16 hr.; this is somewhat longer than that determined after intravenous administration.

In the case of oral dosage, the V_d' term as calculated also includes the fraction of the drug that is not absorbed. The ratio of the intravenous to oral volumes of distribution, 0.088, indicates that in the dog 8.8% of an oral dose of chromonar is absorbed.

Excretion of the Metabolite by Dogs—Within 4 hr. after a single intravenous dose of 5 mg./kg. of chromonar, an average of 86% of the dose was recovered in the urine and bile as the metabolite (Table III). The identity of the fluorescent material was confirmed by quantitative TLC. At least 90% of the fluorescence was cochromatographic with the authentic sample of the acid metabolite. Since the plasma half-life of the metabolite is the same in these dogs (Dogs 5 and 6) as in the others studied, one may conclude that the major route of disappearance of the metabolite from blood is excretion into the urine and bile. There is no evidence for further metabolism or tissue accumulation of the drug. In these cases the bile was collected as it was excreted. Since the plasma half-life of the acid metabolite was not shortened by this procedure, enterohepatic circulation apparently does not occur.

Human Blood Level Studies—The mean half-life of the acid metabolite following the intravenous administration of chromonar was 0.80 hr. (Table IV). There were no apparent differences between males and females. When these six subjects (7–12) were given 150 mg. chromonar, t.i.d., orally for 6 days and the intravenous study was repeated, the half-life was not significantly altered. There was also no significant change in the intercept, the area under the plasma curve, or the apparent relative volume of distribution. Thus, as in the case of the dog, there is apparently no metabolic adaptation to chromonar after continued administration.

The actual values for the plasma concentration of the metabolite following intravenous administration of chromonar to Subject 11 are plotted in Fig. 1. This is graphic illustration of the lack of metabolic adaptation by repeated doses of chromonar. The plot of the logarithm of concentration against time is linear, which confirms the appropriateness of using the single-compartment model for distribution of the metabolite.

The standard deviation of the values calculated for the first-order disappearance constants of the plasma levels in each subject is usually approximately 10% of the value of the constant. This also indicates that the data are fit very well by a single-term equation or a single-compartment model. There was no indication of a deviation from simple first-order disappearance in any of the data.

The six subjects (1–6) who were given oral doses of chromonar separated by 6 days of 150-mg. t.i.d. dosage showed no consistent change in the area under the metabolite plasma curve (Table V). The area under the curve is a measure of availability of drug by the oral route. The two subjects (1 and 2) who received an intraduodenal dose showed essentially the same area under the plasma curve after this dose as after oral drug, indicating that chromonar may be absorbed from the intestine.

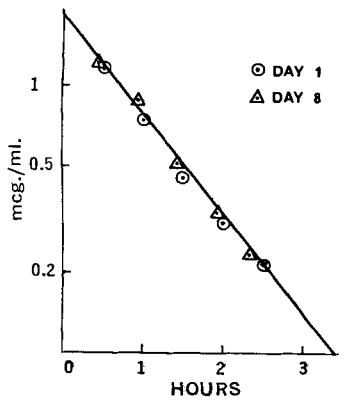


Figure 1—*Plasma levels of the acid metabolite after single 40-mg. i. v. doses of chromonar to Subject 11 (Table IV). On Days 2–7, 150 mg. chromonar t.i.d. was given orally.*

Table V—Calculated Areas under the Metabolite Plasma Level Curves in Six Subjects Given 150 mg. Chromonar Orally

Subject	Sex	Dose, mg./kg.	Day	Area, hr. mcg./ml.
1	M	2.23	0(duodenal)	1.48
			1	2.33
			8	1.58
2	M	2.13	0(duodenal)	1.01
			1	1.33
			8	1.73
3	M	2.06	1	2.52
			8	1.10
4	F	3.00	1	2.46
			8	1.52
5	F	1.99	1	1.50
			8	1.63
6	F	2.28	1	2.44
			8	2.11
Mean ($n=6$)			1	2.10
			8	1.61

Table VI—Human Urine Recoveries of the Metabolite Following Administration of Chromonar

Subject	mg. Acid Metabolite Recovered in Urine: Day 1 0–4 hr.	Day 1 4–8 hr.	Day 1 8–24 hr.	Day 1 %	Day 8 0–4 hr.	Day 8 4–8 hr.	Day 8 8–24 hr.	Day 8 %
				Oral				
1 duod.	18.0	1.3	1.5	20.5				
oral	33.8	5.0	0.2	26.0	18.6	9.7	0.5	19.2
2 duod.	25.4	2.7	0.4	19.0				
oral	25.4	5.9	1.1	21.6	43.3[a]	3.6[b]	0.6	31.7
3	9.4	4.2	1.3	9.4	31.8	3.2	1.4	24.3
4	21.2	12.5	0.0	22.5	25.2	11.5	1.6	32.2
5	27.4	9.4	0.2	25.7	2.07	8.97	0.74	7.87
6	47.9	8.9	0.0	37.9	36.0	4.7	0.0	27.2
			Mean	23.8			Mean	23.8
				Intravenous				
7	27.0	2.9	0.3[c]	78.0	18.6[d]	2.5[e]	0.9	55.0
8	19.5	6.2	1.7[e]	68.5	33.0	2.6	0.8[c]	91.0
9	11.8	2.2	4.1[c]	45.3	22.2	1.5		59.3
10	19.0	1.5[f]	0.2	54.3	24.3[g]	1.0	1.6	67.3
11	14.8	0.5	0.3	39.0[h]	18.3[c]	1.3	0	49.0
12	24.5	6.4	1.9	82.0	33.9	2.9	2.7	98.8
			Mean ($n=5$)	65.6			Mean ($n=6$)	70.1

[a] 0–6 hr. [b] 6–13 hr. [c] Might be overlap with next dose or previous. [d] 0–2 hr. [e] 2–6 hr. [f] 4–10.5 hr. [g] 0–5 hr. [h] Refrigerated, but not frozen, for 4 days; not included in mean.

Table VII—Recovery of the Metabolite in Urine and Feces Following an Oral Dose of 150 mg. Chromonar

Recovery of the Metabolite, hr.	Subject 19	20	21	22
		Urine, mg. Recovered		
0–24 hr.	25.3	0.2	43.4	58.2
24–48 hr.	5.4	40.9	1.2	0.0
48–72 hr.	1.2	0.2	0.0	0.0
72–96 hr.	0.0	0.2	0.2	0.2
Total	32.8	41.5	44.8	58.2
		Feces, mg. Recovered		
0–24 hr.	0.5	0.0	23.6	0.0[a]
24–48 hr.	44.0	39.6	21.0	7.6[a]
48–72 hr.	24.9	31.4	25.4	7.6[a]
72–96 hr.	12.9	10.5	2.1	3.2[a]
Total	82.3	81.5	72.1	18.4
Total g. samples	327	253	276	94
Total mg. excreted	115.5	123.0	116.9	76.6
% of dose recovered as the metabolite[b]	83	89	84	55

[a] Low weights of fecal samples in this subject (see text). [b] Corrected for difference in molecular weights and purity of administered drug.

The actual serum levels of the metabolite following oral administration of chromonar to Subject 2 are plotted in Fig. 2. Note that the rate of decline of serum levels of the metabolite is approximately the same as that of Subject 11 to whom the dose was administered intravenously. The variation in the amount of chromonar absorbed by the same subject on different days is often seen.

A comparison of the areas under the plasma curves after intravenous and oral dosage allows estimation of the fraction of the dose absorbed orally. Correction for the dose by each route must be made. Calculated on this basis from the data in Tables IV and V, 32% of the drug was absorbed on the 1st day of oral administration; on the 8th day, 25% was absorbed. Thus, the overall average absorption was 28%.

After a 6-day (t.i.d.) regimen of chromonar orally, the mean plasma concentration of the metabolite was 0.06 mcg./ml. 10 hr. following the last dose. (This compares with a mean of 0.9 mcg./ml. at the peak following an oral dose.) Thus, there was no appreciable accumulation of the metabolite.

The plasma concentrations of the metabolite when the drug was given at 6-hr. intervals (Subjects 13–18) are summarized in Fig. 3. The pattern of absorption does not appear to vary from one dose to another. There was also no accumulation during the day, and by 24 hr. the plasma levels of the metabolite were essentially zero. The plot of the mean plasma concentration against time was fit very well by a calculated curve in which first-order absorption and elimination were assumed. A value of 1.60 hr.$^{-1}$ was used for the

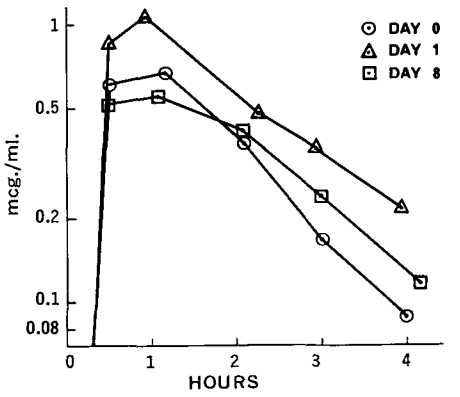


Figure 2—*Plasma levels of the acid metabolite after single duodenal (Day 0) or oral (Days 1 and 8) doses of 150 mg. chromonar to Subject 1 (Table V). On Days 2–7, 150 mg. chromonar t.i.d. was given orally.*

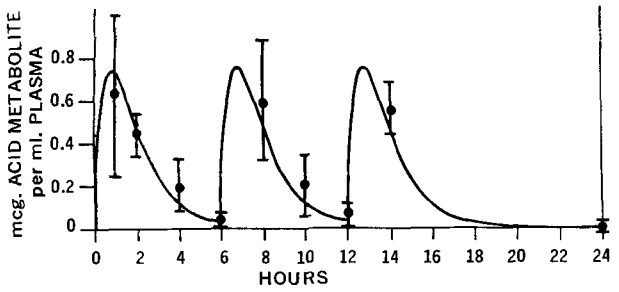


Figure 3—*Plasma levels of the acid metabolite. Three 150-mg. tablets were given with 6 hr. between doses.*

absorption constant, 0.820 hr.$^{-1}$ for the disappearance constant, 1.53 l./kg. for the apparent volume of distribution, and 2.28 mg./kg. for the dose.

From the intravenous studies the apparent volume of distribution of the acid metabolite in man was 0.44 l./kg. (Table IV).

Human Excretion Studies—The urinary excretion data from Subjects 1–12 are summarized in Table VI. Excretion of the metabolite occurred primarily in the first 4 hr. after administration. In the second 4-hr. period, relatively less drug was excreted after intravenous dosage than after oral dosage, which is consistent with the plasma curves and with the expected delay after oral administration due to the absorption phase. When the drug was administered intravenously, an average of 68% of the dose of chromonar was excreted into the urine as the metabolite, with the remainder probably excreted into the bile. From the urinary excretion data for these subjects, it is also possible to calculate the percent of an oral dose which was absorbed. Calculated from the average percent of the doses excreted in the urine, absorption was 36% on the 1st day and 34% on the 8th day, for an overall average of 35%. This agrees very well with the figure of 28% absorption determined from the plasma levels of the same subjects.

Following a single oral dose of chromonar, an average of 77% of the dose is recovered as the acid metabolite in urine plus feces (Table VII). The fecal weights from Subject 22 were very low; excluding this subject, the recoveries averaged 85%. The identity of the fluorescent material in the samples was confirmed by TLC in Solvent Systems II and III.

Bishydroxycoumarin, ethyl biscoumacetate, and warfarin are well-known drugs which also contain the coumarin ring system. All three of these drugs are completely metabolized, although the coumarin portion of the molecules is usually not degraded (4–6). The apparent multicompartment distribution reported for bishydroxycoumarin (7) is not seen with the acid metabolite of chromonar.

SUMMARY

Following the oral or intravenous administration of chromonar, the drug is rapidly hydrolyzed to the corresponding acid. It is largely in this form that the drug circulates in the blood and is excreted into the bile and urine. In dogs as well as humans, the plasma half-life of the acid metabolite is approximately 1 hr. The decline in plasma levels conform to a single-compartment model. Excretion, not further metabolism, accounts for the decline in blood levels of this metabolite. Biliary excretion is an important factor; in the dog an average of 22% of an intravenous dose was recovered in the bile and 64% in the urine. There is no evidence of accumulation of the metabolite when chromonar is administered on a chronic basis.

REFERENCES

(1) M. Klarwein and R. E. Nitz, *Arzneim.-Forsch.*, **15**, 555(1965).

(2) R. G. Wiegand and P. G. Sanders, *J. Pharm. Exp. Ther.*, **146**, 271(1964).

(3) A. Goldstein, L. Aronow, and S. Kalman, "Principles of Drug Action," Harper & Row, New York, N. Y., 1968, p. 322.

(4) R. Nagashima, G. Levy, and E. J. Sarcione, *J. Pharm. Sci.*, **57**, 1881(1968).

(5) R. T. Williams, "Detoxication Methods," Wiley, New York, N. Y., 1959, p. 631.

(6) R. A. O'Reilly, P. M. Aggeler, M. S. Hoag, and L. Leong, *Thromb. Diath. Haemorrh.*, **8**, 82(1962).

(7) R. Nagashima, G. Levy, and R. A. O'Reilly, *J. Pharm. Sci.*, **57**, 1888(1968).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 11, 1969, from *Chemical Pharmacology Department, Abbott Laboratories, North Chicago, IL 60064*

Accepted for publication April 23, 1970.

The technical assistance of Erna Scherfling and Harold Brondyk is gratefully acknowledged.