Comparative Study in Man of the Absorption and Excretion of Amobarbital-^{14}C from Sustained-Release and Nonsustained-Release Dosage Forms

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Amobarbital was tagged with a radioactive carbon atom and incorporated into sustained-release and nonsustained-release dosage forms to obtain comparative absorption and excretion data. Radioactivity levels in plasma and urine showed that the sustained-release formulation released about one-third of its amobarbital promptly and the remaining portion gradually, whereas the nonsustained-release dosage form showed a steady decrease in plasma concentration after 1 hr. and a higher urinary excretion rate during the first 12-hr. period. These performance characteristics indicate that a moderately short-acting drug, such as amobarbital, can be pharmaceutically modified so as to be a candidate for use with longer-acting drugs, such as trifluoperazine, in a single dose unit.

TESTS IN ANIMALS showed that a combination of amobarbital and trifluoperazine has a broader spectrum of activity than the individual drugs, but combining them in a single dose unit posed a pharmaceutical problem because amobarbital is a moderately short-acting drug (1) while trifluoperazine is long acting. Incorporating the amobarbital component into a sustained-release formulation would solve the problem if a formulation could be prepared that would release amobarbital promptly and gradually over an extended period. This report describes the specific sustained-release dosage form and how it was evaluated in man.

EXPERIMENTAL

Preparation of Dosage Forms—Based on the results of preliminary clinical studies, a combination of 30 mg. of amobarbital and 2 mg. of trifluoperazine was chosen for testing. However, conventional bioanalytic techniques to measure levels of amobarbital and its metabolites in blood and urine are not sufficiently sensitive to accurately measure drug concentration from a 30-mg. dose, so it was necessary to label the amobarbital with a radioactive carbon atom. The utility of this technique for evaluating pelleted sustained-release preparations has been reported previously (2–4).

Based on human safety considerations, an upper limit of 50 μc. of ^{14}C was selected for the 30-mg. amobarbital dose. To obviate the problems involved in uniformly mixing labeled and unlabeled drug, the 5-ethyl-5-isoamyl barbituric acid-2^{14}C was synthesized with a specific activity of 1.57 μc./mg.

The dosage forms for this study were prepared by a method similar to that reported previously (2, 4). One kilogram of nonsustained-release amobarbital pellets was prepared in a 12-in. stainless steel coating pan located in a disposable glove-box inside a walk-in fume hood fitted with special filters. A portion (150 Gm.) of these nonsustained-release pellets was set aside and the remaining pellets were coated with a wax-fat coating.

Six separate groups of sustained-release pellets with approximately 6, 7, 8, 9, 10, and 11% wax-fat (WF) coating were prepared to insure a sufficient spread in patterns to facilitate blending for the *in vitro* release pattern selected on the basis of controlled clinical studies. This desired *in vitro* pattern was 35, 60, 75, and not less than 80% released at the 0.5, 2, 4.5, and 7-hr. intervals, respectively.

The *in vitro* release patterns of the six experimental groups are shown in Table I; they were determined by the method of Souder and Ellenbogen (5). In these groups, it was found that the blend consisting of 35% nonsustained-release pellets, 10% WF-1, 20% WF-2, 15% WF-3, 10% WF-4, and 10% WF-5 gave a calculated pattern of 35, 56, 77, and 86% released at the desired time intervals.

The *in vitro* patterns of this blend assayed before and after it was encapsulated with 2 mg. of nonsustained-release trifluoperazine are shown in Table II. These data indicated that the *in vitro*

TABLE I—*In Vitro* RELEASE PATTERNS OF AMOBARBITAL-^{14}C WAX-FAT COATED GROUPS

Group	% *In Vitro* Release at Time Interval 0.5 hr.	2 hr.	4.5 hr.	7 hr.
WF-1	0	72	88	91
WF-2	0	51	75	84
WF-3	0	23	62	77
WF-4	0	9	51	63
WF-5	0	4	41	57
WF-6	0	3	20	51

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TABLE II—BLEND AND CAPSULE *In Vitro* RELEASE PATTERNS (AMOBARBITAL-^{14}C)

Material	% *In Vitro* Release at Time Interval 0.5 hr.	2 hr.	4.5 hr.	7 hr.
Blend	34	54	80	86
Sustained-release amobarbital-^{14}C, trifluoperazine capsule	34	53	77	83

release pattern of the experimental capsule closely approximated the desired pattern.

The nonsustained-release pellets of labeled amobarbital, previously set aside, were encapsulated with 2 mg. of nonsustained-release trifluoperazine.

In Vivo Protocol—Two dosage regimens, *A* and *B*, were compared in 10 normal subjects, using a cross-over design: (*A*) sustained-release amobarbital-^{14}C, 30 mg., nonsustained-release trifluoperazine, 2 mg., given at 0 hr.; (*B*) nonsustained-release amobarbital-^{14}C, 30 mg., nonsustained-release trifluoperazine, 2 mg., given at 0 hr.

These regimens were tested to determine whether the sustained-release amobarbital formulation provided a prompt initial dose, whether the remaining portion of amobarbital was delivered gradually, and whether there was any significant reduction in the total amount of amobarbital made available from the sustained-release formulation.

Prior to the tests, the subjects were examined and found to be in good health. All underwent studies to rule out impairment of renal, hepatic, and hematopoietic function. The subjects were then divided into two groups of five and each group was studied on alternate weeks over a 4-week period. The order in which the test formulations were given was alternated within each group.

Each morning of the study, the subjects reported in a fasting state and were given a test dose with fruit juice. They received no food or drink for 90 min.; then they resumed their normal diets. For the first 12 hr., this consisted of a free selection of hospital cafeteria food.

Plasma and Urine Sampling—A blood specimen was taken from each subject before drug administration and at hourly intervals after it for a total of 12 hr., by means of an indwelling anticoagulated venous needle system (4). The plasma was separated and immediately frozen until assayed.

Complete urine specimens were collected for 96 hr. after drug administration. The collection intervals were 0–3, 3–6, 6–9, 9–12, 12–24, 24–48, 48–72, and 72-96 hr. Urine specimens were also frozen until assayed.

It was also decided to determine the pH of all urine specimens passed in the first 12 hr. of each segment of this cross-over study. The authors did not intend to try to establish a relationship between urinary pH and drug excretion (6, 7). An increase in the excretion of a weak acid like amobarbital (pKa 7.96), or its principal metabolite hydroxyamobarbital, could be expected only in an alkaline urine and Elliot *et al.* (8) have found that only 1% of urine specimens have a pH of 7.2 or more. Rather, the authors wanted to determine whether pH profiles would be similar on two separate occasions. Information of this sort is not presently available and would be useful in the design of dosage form performance studies involving drugs which *are* influenced by urinary pH fluctuations.

Analysis of the Specimens—Plasma and urine samples were assayed for radioactivity using a method previously used to assay dextroamphetamine-^{14}C (4). The pH of the urine specimens was determined immediately after voiding, using a Beckman model G glass electrode pH meter.

RESULTS AND DISCUSSION

Plasma Data—These data, as shown in Fig. 1, are reported as micrograms of amobarbital per ml. of plasma, but include radioactivity that originated from the metabolites as well as from the parent compound. A comparison of the two regimens at the first hour after administration shows that the plasma level of radioactivity resulting from the sustained-release formulation was 36% of the amount resulting from the nonsustained-release formulation. This value for the sustained-release formulation agrees closely with the *in vitro* release value of 34% at 0.5 hr.

The plasma levels of radioactivity following the administration of the nonsustained-release regimen were consistently and significantly higher ($P < 0.01$) than the levels of the sustained-release regimen. And there was a statistically significant difference ($P < 0.01$) in the patterns: the nonsustained-release regimen showed a steady decrease in plasma

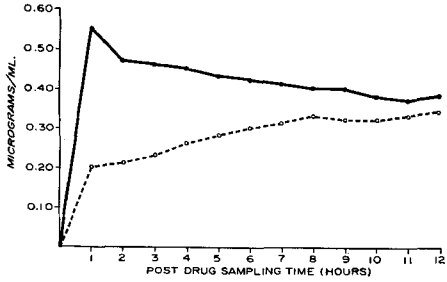

Fig. 1—Plasma levels of radioactivity reported as micrograms of amobarbital/ml. of plasma. Each line represents the averages of 10 volunteers. Key: – – – –, (A) sustained-release amobarbital-^{14}C, 30 mg., nonsustained-release trifluorperazine, 2 mg.; ————, (B) nonsustained-release amobarbital-^{14}C, 30 mg., nonsustained-release trifluoperazine, 2 mg.

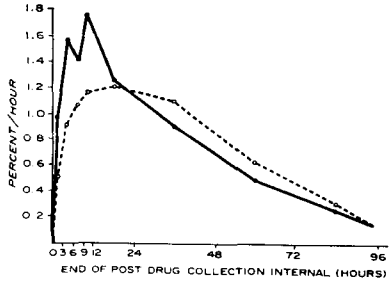

Fig. 2—Urinary excretion rate of radioactivity reported as per cent of dose excreted per hour. Each line represents the averages of 10 volunteers. Key: – – – –, (A) sustained-release amobarbital-^{14}C, 30 mg., nonsustained-release trifluoperazine, 2 mg.; ————, (B) nonsustained-release amobarbital-^{14}C, 30 mg., nonsustained-release trifluoperazine, 2 mg.

TABLE III—AVERAGE CUMULATIVE URINARY RECOVERY OF RADIOACTIVITY REPORTED AS PER CENT OF ADMINISTERED DOSE

Regimen, %	Cumulative Period, hr.							
	0–3	0–6	0–9	0–12	0–24	0–48	0–72	0–96
A	1.6	4.4	7.7	11.3	26.2	53.4	69.0	77.4
B	3.0	7.8	12.2	17.7	32.1	57.1	69.8	76.2

TABLE IV—URINARY pH PROFILES FOR TWO 0–12-hr. PERIODS

Subject	Regimen							
	A				*B*			
	Intervals, hr.				Intervals, hr.			
	0–3	3–6	6–9	9–12	0–3	3–6	6–9	9–12
1	5.42	7.45	7.14	6.70	5.37	6.31	6.32	6.75
2	5.45	5.42	5.57	5.46	6.18	5.95	6.23	5.85
3	7.21	6.95	6.36	6.07	5.68	6.02	6.90	6.80
4	7.21	7.70	7.80	6.95	6.83	7.17	7.42	6.54
5	6.34	5.80	6.29	6.62	5.50	7.20	7.52	6.35
6	5.52	6.45	6.70	6.20	5.55	6.76	6.22	5.35
7	5.50	5.28	5.28	5.46	5.96	6.35	6.20	4.93
8	5.30	6.50	5.75	5.70	5.50	6.65	5.48	6.85
9	5.51	6.51	7.50	6.55	6.06	6.38	6.30	5.60
10	5.71	7.50	7.05	7.08	5.35	6.38	5.42	6.17
Av.	5.92	6.56	6.54	6.28	5.80	6.51	6.40	6.12
S.D.	0.738	0.860	0.843	0.595	0.465	0.428	0.705	0.665

concentration after 1 hr.; in contrast, the sustained-release regimen showed a slowly rising pattern after 1 hr. All these data indicated that the sustained-release formulation made approximately one-third of its amobarbital content available for absorption promptly and that it delivered the remaining portion gradually.

Urine Data—The radioactivity excretion rates for the two regimens were computed by dividing the per cent of radioactivity excreted per collection-interval by the number of hours in the interval. Each value was then plotted at the midpoint of the collection interval, as shown in Fig. 2.

An analysis of the plots for the two regimens showed a statistically significant difference ($P < 0.01$). Furthermore, the data showed that less amobarbital was excreted from the sustained-release regimen than from the nonsustained-release regimen during the first 24-hr. period. But the reverse was true thereafter; more amobarbital was excreted from the sustained-release regimen. These findings are consistent with the plasma findings and give further proof that the sustained-release amobarbital formulation makes its drug gradually available for absorption.

The cumulative urinary recovery data, reported in Table III as the per cent of administered dose in each collection interval, are essentially identical for the two regimens over a 96-hr. period and indicate that amobarbital in the sustained-release formulation was as available for absorption as amobarbital in the nonsustained-release dose.

Urinary pH Data—The 0–12 hr. urinary pH profiles for each of the 10 subjects during the two test periods are reported in Table IV. The profiles were very similar and, as could be expected, showed a peak in the 3–6-hr. interval consistent with the afternoon postprandial tide and range reported by Elliot *et al.* (8). But the most interesting finding was the similarity in the averages of the profile values: the figures for the two test periods varied by less than 0.2 pH units.

SUMMARY

This study in man was undertaken to determine the performance characteristics of a sustained-release amobarbital formulation. To obtain comparative absorption and excretion data, amobarbital was tagged with a radioactive carbon atom and incorporated into sustained-release and nonsustained-release dosage forms. Data from plasma and urine studies showed that the sustained-release formulation made about one-third of its amobarbital content promptly available for absorption and that the remaining portion was absorbed gradually. This was in contrast to the nonsustained-release dosage form, which showed a steady decrease in plasma concentration after 1 hr. and had a higher urinary excretion rate during the first 12-hr. period. Furthermore, there was no reduction in drug availability from the sustained-release formulation: the cumulative urinary recovery of radioactivity from both dosage forms after 96 hr. was about 75%. These data indicate that it is possible to combine this sustained-release amobarbital formulation in a single dose unit with a long-acting drug like trifluoperazine.

During this study, urinary pH data were also recorded which showed that 0–12-hr. urinary profiles taken on two separate occasions were very similar and that profile averages varied by less than 0.2 pH units.

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