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

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The absorption and excretion of dextroamphetamine-^{14}C sulfate in both sustained-release and nonsustained-release dosage forms were evaluated in identical studies in man and dog. A direct comparison of the results of these studies showed that the various dosage forms performed in a similar way in both man and dog. These results provided information on the design and evaluation of *in vivo* studies intended to indicate which preliminary sustained-release formulations merit objective human testing.

IN THE ABSENCE of an accurately measurable clinical effect, an objective way to evaluate the performance of sustained-release dosage forms in humans is to compare how a drug is absorbed and excreted from this dosage form and from the regimen it is intended to replace. There are many problems involved in conducting such studies: the need for a substantial number of humans; the need for specialized analytical techniques to measure the drug levels in body tissues; the need for knowledge not only of the statistical considerations in experimental design, but also of individual drug kinetics; and the need for professional surveillance to insure faithful adherence to dosage regimens, food and liquid intake, and a set schedule of sample collections.

Because these problems exist, only the most promising experimental formulations merit such objective human testing. So there is a need for *in vivo* animal studies that will provide data comparable to those obtained in human studies and thus indicate which preliminary formulations merit such testing.

The authors recently conducted an absorption and excretion study in humans comparing a dextroamphetamine-^{14} C sulfate pelleted sustained-release product with similarly labeled nonsustained-release regimens (1). It was necessary to tag the drug with the radioactive carbon atom because ordinary biochemical methods are not adequate to measure the low concentration of dextroamphetamine sulfate that appears in blood.

It was decided to carry out an identical study in dogs to obtain directly comparable data, reasoning that a comparison of the data would serve as a useful reference for the design and evaluation of future formulation screening studies in the dog.

This study is a report of the data on the absorption and excretion of various dosage forms of dextroamphetamine-^{14} C sulfate in both man and dog, as well as an analysis of how well these data compare with each other.

EXPERIMENTAL

Preparation of Dosage Forms—Based on human safety considerations, the highest dose of dextroamphetamine sulfate to be administered (15 mg.) was tagged with a maximum single radiation dose of 45 μc. To obviate the problems involved in uniformly mixing labeled with unlabeled drug, we chose to synthesize the labeled drug with a specific activity of approximately 3 μc./mg. The method of preparation described by Blackburn and Burghard was used (2).

The pelleted sustained-release dosage form of the labeled drug was prepared by a method similar to that reported previously by Rosen and Swintosky (3). One kilogram of medicated nonsustained-release pellets was prepared in a 12-in. coating pan located in a disposable glove-box inside a walk-in fume hood fitted with special filters. A portion (135 Gm.) of these nonsustained-release pellets was set aside and the remaining pellets were coated with a wax-fat coating. Six separate groups with approximately 7, 9, 11, 13, 15, and 17% wax-fat coating were prepared. This was done to insure that there would be sufficient spread in patterns to facilitate blending for the *in vitro* pattern of the commercial product.

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 (4).

As reported (4), the average *in vitro* pattern of 15 consecutive commercial lots of the specific 15 mg. dextroamphetamine sulfate sustained-release product we chose to simulate was 39, 62, 80, and 90% at the 0.5, 2, 4.5, and 7 hr. time intervals,

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respectively. In the six experimental groups it was found that the blend consisting of 25% noncoated pellets, 55% W-F3, and 20% W-F2 pellets gave a calculated pattern of 37, 57, 81, and 97% at the same time intervals.

The *in vitro* patterns of this blend before and after encapsulation are shown in Table II.

These data indicated that the *in vitro* release pattern of the experimental ^{14}C-labeled capsule closely approximated that of the commercial product.

The 5-mg. and 15-mg. nonsustained-release capsules which were used in the *in vivo* studies were prepared by filling capsules with nonsustained-release pellets of dextroamphetamine-^{14}C sulfate.

In Vivo Protocols—The following four dosage regimens of labeled drug in man and dog were compared: *A*, 15-mg. sustained-release dosage form given at 0 hr.; *B*, 15-mg. nonsustained-release dosage form given at 0 hr.; *C*, 5-mg. nonsustained-release dosage form given at 0 hr.; *D*, 5-mg. nonsustained-release dosage form given t.i.d., at 0, 4, and 8 hr.

These particular regimens were chosen because the authors wanted to determine five performance criteria: whether the sustained-release formulation provided a prompt initial dose; whether it was similar to t.i.d. administration; whether it was dissimilar to an equivalent nonsustained-release dose; whether equal doses in different dosage forms were equally effective in making the drug available for absorption; and whether there was any significant variability among subjects receiving the various regimens.

The earlier study in humans utilized 16 subjects in a crossover sequence; the study plan is shown in Table III.

Radiation safety considerations precluded the giving of each subject all four dosage regimens. Instead, each subject received two regimens, one week apart. Because four subjects were studied at one time, all four regimens were given on each day of the study. All possible combinations and permutations were covered in the first three groups, so the authors utilized the last group to repeat the most significant direct comparisons—that of *A* with *D*, and *B* with *C*.

The plan for the dog study is shown in Table IV. Eight female purebred beagle dogs ranging in weight from 9 to 11 Kg. were used in this study. They were given no food after 4:30 p.m. the day prior to dosing and the prescribed dosage regimen was administered, with no fluid, starting at 10 a.m. the next morning. The animals were then fed and watered 3.5 hr. after receiving the drug. Each animal received all four dosage regimens.

Blood Sampling—Human blood specimens were collected at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 32, and 48 hr. To accomplish this with a minimum of discomfort to the subject, the first seven specimens were collected through an indwelling anticoagulated venous needle system, as illustrated in Fig. 1. Before each collection, 5 ml. of blood was aspirated into a syringe; this flushed the system with fresh venous blood. Then the syringe was isolated from the system and the required 10-ml. specimen was collected through a heparin vacuum tube inserted in the stopcock. When the specimen had been withdrawn, the system was again flushed with the anticoagulant-blood mixture by returning 5 ml. to the subject. The plasma was removed from each blood sample and frozen until assayed.

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

Group	% Wax-Fat	% *In Vitro* Release at Time Interval 0.5 hr.	2 hr.	4.5 hr.	7 hr.
WF-1	7	46	100	100	100
WF-2	9	34	69	100	100
WF-3	11	20	50	84	100
WF-4	13	4	18	51	87
WF-5	15	2	3	21	70
WF-6	17	2	3	7	33

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

	% *In Vitro* Release at Time Interval 0.5 hr.	2 hr.	4.5 hr.	7 hr.
Blend	34	56	79	92
15-mg. sustained-release capsule	33	57	80	91

TABLE III—HUMAN STUDY PLAN OF VARIOUS DOSAGE REGIMENS[a]

Subject	Initial	1 Wk. Later
1	*A*	*B*
2	*B*	*C*
3	*C*	*D*
4	*D*	*A*
5	*A*	*C*
6	*B*	*D*
7	*C*	*A*
8	*D*	*B*
9	*A*	*D*
10	*B*	*A*
11	*C*	*B*
12	*D*	*C*
13	*A*	*D*
14	*B*	*C*
15	*C*	*B*
16	*D*	*A*

[a] *A*, 15-mg. sustained-release dosage form given at 0 hr.; *B*, 15-mg. nonsustained-release dosage form given at 0 hr.; *C*, 5-mg. nonsustained-release dosage form given at 0 hr.; *D*, 5-mg. nonsustained-release dosage form given t.i.d., at 0, 4, and 8 hr.

TABLE IV—DOG STUDY PLAN OF VARIOUS DOSAGE REGIMENS[a]

Dog	Wk. of Study 1	2	3	4	5	6	7	8
101	*A*		*D*		*C*		*B*	
102	*B*		*C*		*A*		*D*	
103	*C*		*B*		*D*		*A*	
104	*D*		*A*		*B*		*C*	
201		*A*		*D*		*C*		*B*
202		*B*		*C*		*A*		*D*
203		*C*		*B*		*D*		*A*
204		*D*		*A*		*B*		*C*

[a] *A*, 15-mg. sustained-release dosage form given at 0 hr.; *B*, 15-mg. nonsustained-release dosage form given at 0 hr.; *C*, 5-mg. nonsustained-release dosage form given at 0 hr.; *D*, 5-mg. nonsustained-release dosage form given t.i.d., at 0, 4, and 8 hr.

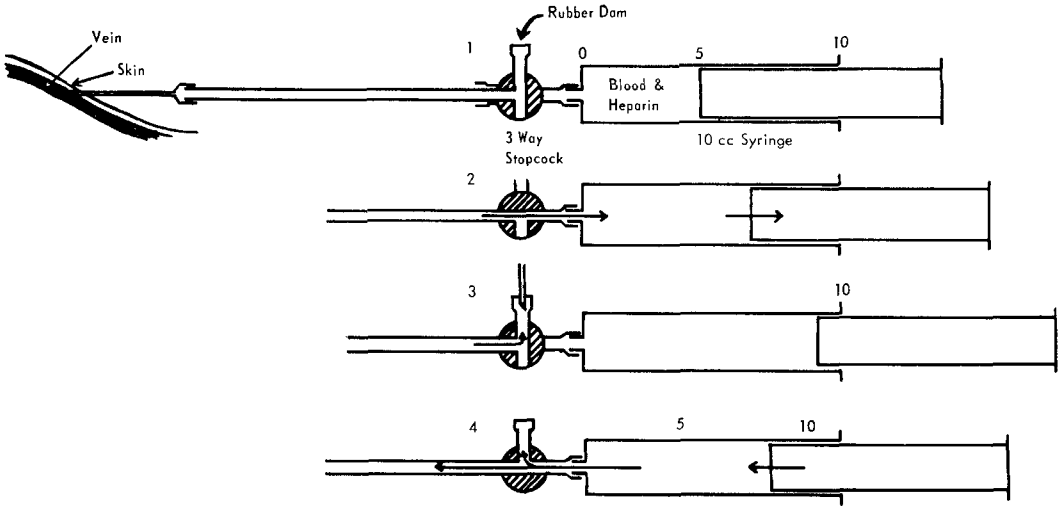

Fig. 1—Technique of blood collection. Key: 1, *needle is inserted in vein;* 2, *specimen collection is begun by aspirating 5 ml. of blood to flush system with fresh blood;* 3, *specimen is collected* via *needle puncture of rubber dam after isolation from syringe;* 4, *following collection, system is flushed with heparin solution to prevent clotting. Stopcock is then closed to await next collection.*

Blood samples from the dog were taken using a 5-ml. heparinized vacuum tube, and the time periods were the same as those in the human study. These plasmas were also separated and frozen until assayed.

Urine Sampling—Urine was collected from all humans for 48 hr. following ingestion of the drug. The collection intervals were 0–3, 3–6, 6–9, 9–12, 12–24, 24–32, and 32–48 hr. Aliquots of these samples were frozen until assayed.

Urine was collected from the dogs by catheterization at the same time intervals as those in the human study, and these samples were also stored frozen until assayed.

Analysis of the Specimens—Samples of plasma and urine ranging from 0.2 to 0.5 ml. were placed in a liquid scintillation counting solution with the following formula: dimethyl POPOP [2,2-*p*-phenylenebis(5-phenyloxazole)], 50 mg.; PPO (2,5-diphenyloxazole), 7-Gm.; naphthalene, 80 Gm.; toluene, 400 ml.; *p*-dioxane, 400 ml.; diluted to a total of 1000 ml. with absolute alcohol.

The samples were counted using a Tri-Carb[1] liquid scintillation spectrometer, and all measurements were corrected for background, quench, and dilution. The efficiency of the counter was approximately 50%.

RESULTS AND DISCUSSION

The human data have been reported previously (1), but because they are an integral part of this comparative study, they are repeated here. In this regard, note that two minor errors were found after reporting these data and, although they in no way alter the original interpretation, they have been corrected in Fig. 2 (ordinate scale) and Table V (*0–48 hr. data column*).

Plasma Data—The radioactivity appearing in the plasma of the humans following the administration of the four dosage regimens is shown in Fig. 2.

[1] Model 314-DC, Packard Instrument Co., Inc., La-Grange, Ill.

Because of the low order of radioactivity, the average plasma data are reported as counts per minute per milliliter. Of course, the authors were measuring the levels of radioactivity not only from the parent compound but also from its metabolites.

The 15-mg. sustained-release dosage form and the 5-mg. capsule given at 0, 4, and 8 hr. produced roughly similar curves, but the sustained-release preparation showed a higher level during most of the first 8 to 10 hr. after administration, with none of the "staircase" effect or 12-hr. peaking seen with the t.i.d. regimen. The 15-mg. nonsustained-release dosage form gave different results. It showed a high peak at 2 hr. that was maintained for at least 8 hr.; this plot paralleled the one for the 5-mg. capsule given once, but at a proportionately higher level.

The radioactivity appearing in the plasma of dogs following the administration of the four regimens is shown in Fig. 3.

Although the plots for the 15-mg. sustained-release dosage form were very similar in man and dog, the plots for the 5-mg. capsule given t.i.d. differed. In the dogs, this plot showed a rapid rise after the first two doses but, unexpectedly, there was no rise after the third dose. And there was no peak at the 12th hr., causing a plateau from the 6th to the 12th hr.

The 15-mg. nonsustained-release dose showed a peak at 2 hr. that was maintained until the 4th hr.; then the level started to drop. As in the human study, this plot paralleled that of the 5-mg. capsule given once, but at a proportionately higher level.

Urine Data—The urinary excretion plots for the humans are shown in Fig. 4; those for the dogs are shown in Fig. 5. Although the excretion values are expressed as milligrams of drug to which the ^{14}C is equivalent, as mentioned before, the radioactivity reflects not only the parent compound but also its metabolites.

The urinary findings in man and dog were consistent with the plasma findings. The 15-mg.

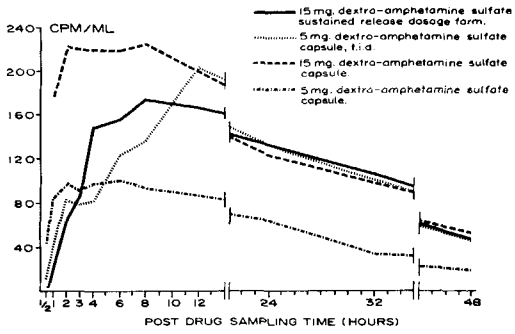

Fig. 2—Adjusted average human plasma levels. Each line represents eight subjects per regimen in a balanced incomplete block crossover design.

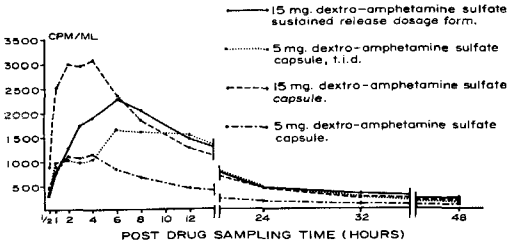

Fig. 3—Adjusted average dog plasma levels. Each line represents eight dogs per regimen.

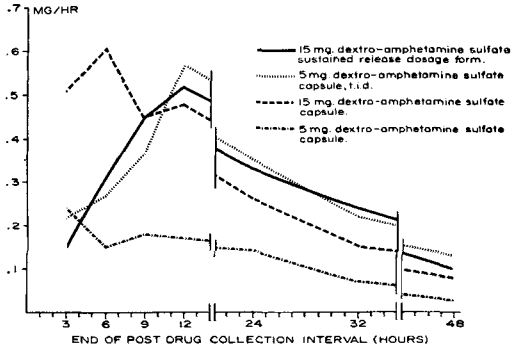

Fig. 4—Adjusted average human urinary excretion rates. Radioactive counts are expressed as average milligrams of dextroamphetamine sulfate per collection interval divided by the number of hours in each interval

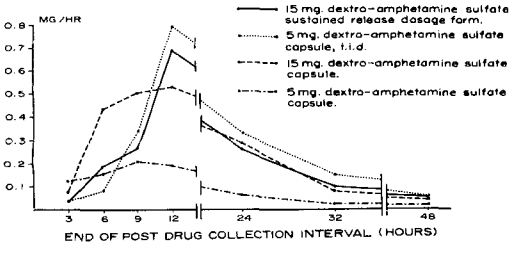

Fig. 5—Adjusted average dog urinary excretion rates. Radioactive counts are expressed as average milligrams of dextroamphetamine sulfate per collection interval divided by the number of hours in each interval.

sustained-release dosage form gave a curve similar to that of the 5-mg. capsule given three times a day. And, just as the plasma data for the 3–10-hr. period showed more drug being absorbed from the sustained-release dosage form, so the urinary data showed that during this same period the excretion was greater from the sustained-release form than from the t.i.d. regimen. The plots of the 15 and 5-mg. plain capsules again paralleled each other at proportionate levels.

The cumulative urinary excretion data for the humans are shown in Table V. These data indicated that in the first 12 hr. following administration of the drug, the sustained-release and t.i.d. regimens made approximately the same amount of drug available for absorption. On the other hand, the 5-mg. capsule given once made significantly less of the drug available and the 15-mg. capsule given once made significantly more of the drug available. Cumulative urinary excretion data for the 24th hr. through to the 48th hr. showed that the three 15-mg. regimens made the same amount of drug available for absorption. Almost proportionately equivalent amounts were made available from the 5-mg. single dose.

TABLE V—ADJUSTED AVERAGE[a] URINE RECOVERIES IN HUMANS, mg. OF DEXTROAMPHETAMINE SULFATE EQUIVALENT

Dosage Form	Collection Interval, hr. 0–12	0–24	0–48
5-mg. capsule	2.18	3.90	4.89
15-mg. sustained-release capsule	4.30[b]	8.24[b]	11.78
5-mg. capsule, three times each day	4.32	8.48	12.30
15-mg. capsule	6.14	9.31	11.86

[a] Each figure is the average for eight humans. [b] No figure included in a brace is significantly different from any other figure included in that brace ($P < 0.05$).

The cumulative urinary excretion data for the dogs are shown in Table VI. In the 0–12 hr. interval, the three highest and three lowest values were not significantly different from each other; only the extreme values showed a significant difference. And, as in the human study, the data in this interval for the sustained-release and t.i.d. regimens showed excellent agreement. It should be noted that although both humans and dogs excreted similar amounts after the 5-mg. acute dose, the dogs excreted less than the humans after the three 15-mg. regimens. It is assumed that this finding is related to the lower total absorption seen in the dogs. All three 15-mg. regimens again made the same amount of drug available by the 24th hr., and through to the 48th hr., with significantly less drug excreted from the 5-mg. single dose.

Although the most valid way to compare drug availability from various dosage forms is to compare cumulative urinary recovery data, plotting plasma data is also informative. When the authors plotted plasma level *versus* time, the area under the 15-mg. acute dose plot was significantly greater than the area under the 15-mg. sustained-release plot or the 5-mg. t.i.d. plot, but the urinary recoveries for all three regimens were similar. And this was true for both man and dog. To explain this apparent paradox,

we assume that the 15-mg. acute dose did not achieve distribution equilibrium during the early time periods.

TABLE VI—ADJUSTED AVERAGE[a] URINE RECOVERIES IN DOGS, mg. OF DEXTROAMPHETAMINE SULFATE EQUIVALENT

Dosage Form	Collection Interval, hr. 0–12	0–24	0–48
5-mg. capsule	2.02[b]	2.74	3.23
15-mg. sustained-release capsule	3.50[b]	6.62[b]	8.28[b]
5-mg. capsule, three times each day	3.77[b]	7.71[b]	9.76[b]
15-mg. capsule	4.62	9.76	9.33

[a] Each figure is the average for eight dogs. [b] No figure included in a brace is significantly different from any other figure in that brace ($P < 0.05$).

Coefficient of Variation Data—Table VII shows the coefficient of variation for the first 8 blood specimens and for the cumulative urine collections in the human study; Table VIII shows these data for the animal study.

In both humans and animals, these data indicated that there was a relationship between the variation at any particular time and the average recovery result at that time, with low intrinsic values showing the highest degree of variation. At most data points, the three 15-mg. regimens showed equivalent variability; in other words, the variability was related to the average ^{14}C recovered at each sampling time or collection interval, and not to the dosage forms studied.

The design of the experiments provided internal checks of its validity by including dose response relationships. In man and dog, the 15 and 5-mg. plain capsules gave plasma and urine plots that paralleled each other at proportionate levels; this fact validated our methodology.

SUMMARY

The study in man and dog was undertaken to determine certain performance criteria for four dosage forms of dextroamphetamine-^{14}C sulfate. The experimental data indicated the following conclusions.

1. In man and dog, the initial 3-hr. portions of the plasma plots for the sustained-release dosage form, the t.i.d. regimen, and the 5-mg. single dose were similar. Therefore, the sustained-release formulation did provide a prompt initial dose.

2. In man and dog, the plasma and urine plots for the sustained-release dosage form and the t.i.d. regimen were similar.

3. In man and dog, the plasma and urine plots for the sustained-release dosage form and the 15-mg. plain capsule were dissimilar.

4. In man and dog, the sustained-release dosage form made as much drug available for absorption as the other two 15-mg. regimens.

5. In man and dog, the variation in plasma and urine data is not greater with the sustained-release dosage form than with the t.i.d. regimen.

Because the preceding five statements can be applied to both man and dog, this example study serves to promote confidence in the selection of sustained-release formulations worthy of objective human testing based on comparative dosage form performance criteria obtained in the dog.

TABLE VII—COEFFICIENTS OF VARIATION[a] FOR HUMAN PLASMA AND URINE DATA

Study Time, hr.	15-mg. Sustained-Release Capsule	15-mg. Capsule	5-mg. Capsule	5-mg. Capsule Given Three Times a Day
	Plasma Results			
0.5	48	46	82	88
1	53	39	58	60
2	44	35	38	20
3	34	30	33	22
4	34	20	33	17
6	22	25	22	22
8	11	23	26	19
12	18	22	23	15
Collection Interval, hr.	Urine Results			
0–12	25	20	20	34
0–24	17	18	12	18
0–48	9	16	9	9

[a] The coefficient of variation is the standard deviation of a set of data divided by the average of the same set, expressed as a percentage.

TABLE VIII—COEFFICIENTS OF VARIATION[a] FOR DOG PLASMA AND URINE DATA

Study Time, hr.	15-mg. Sustained-Release Capsule	15-mg. Capsule	5-mg. Capsule	5-mg. Capsule Given Three Times a Day
	Plasma Results			
0.5	72	108	80	91
1	31	52	24	30
2	27	33	28	16
3	23	34	26	17
4	25	15	58	51
6	28	19	39	26
8	19	24	43	34
12	25	35	51	39
Collection Interval, hr.	Urine Results			
0–12	50	63	48	65
12–24	30	20	35	18
24–48	19	14	27	15

[a] The coefficient of variation is the standard deviation of a set of data divided by the average of the same set, expressed as a percentage.

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