PREPARATION OF A ^{35}S LABELLED TRIMEPRAZINE TARTRATE SUSTAINED ACTION PRODUCT FOR ITS EVALUATION IN MAN

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Multi-pellet sustained action capsules containing radiochemically labelled drug were prepared by a pan coating procedure. Pellets of varying lipid thickness were prepared, and mixed in different proportions to obtain products having variable *in vitro* release characteristics. Capsules were prepared from these pellets for administration to man. Oral doses of 5 mg. of labelled trimeprazine were given in a non-sustained release form at 0, 4, and 8 hours. Other subjects received single 15 mg. oral doses of labelled trimeprazine in sustained action capsules. The ^{35}S activity was determined in blood and urine at selected times. Sustained action capsules were prepared which when evaluated in man gave data for ^{35}S activity in blood and urine which were comparable to that observed for three divided doses of non-sustained release drug administered at 0, 4, and 8 hours.

This study was undertaken to establish a procedure for incorporating ^{35}S trimeprazine tartrate [(±)-10-(3-dimethylamino-2-methylpropyl)-phenothiazine tartrate], an antipruritic drug, into a sustained action capsule; and to acertain, by *in vivo* studies, its *in vitro* release characteristics.

The introduction of sustained action dosage forms of drugs has necessitated the adoption of standards for control of manufactured batches. However, it is apparent that different *in vitro* tests are necessary to evaluate different types of preparations, and that they have no value as an indication of sustained release *in vivo* unless their results can be related to their biological response[1]. Before clinical trials, animal studies may provide qualitative sustained release data; however, ultimate proof rests with human testing. The selection of a suitable objective test depends in part on the methods of analysis that can be applied to the drug. For example, blood concentrations and urinary excretion data are applicable for this purpose with some chemotherapeutic agents[2–4]. Direct measurement of pharmacological or physiological responses has also been useful[5].

For trimeprazine tartrate none of these approaches was found particularly suitable. Therefore, a radiochemical approach was selected, because preliminary studies indicated that it would permit detection and quantitative measurement of ^{35}S in blood serum and urine. In addition the detection of the ^{35}S of trimeprazine tartrate and its metabolities would not be complicated by the host of substances in the urine which frequently complicate other analytical methods.

Since one of the objectives of the work was the direct comparison of sustained release and multiple dose drug administration, satisfactory data could be obtained by measuring only the radioactivity appearing in the blood and urine of subjects receiving these dosage regimens of labelled

drug. This approach assumes that the human body metabolises trimeprazine tartrate in the same manner whether administered in conventional or sustained release form.

Methods and Materials

Source of ^{35}S Trimeprazine Tartrate

(±)-10-(3-Dimethylamino-2-methylpropyl)phenothiazine 9-^{35}S tartrate with a specific activity of 5·6 μc/mg. was made in these laboratories, under the direction of Dr. D. W. Blackburn.

Preparation of Dosage Forms

A disposable glove-box made of plywood and plexiglass and fitted as depicted in Figure 1 was placed in a fume hood with a draft of 1,400 cu. ft./min. The hood was provided with filters to trap particles.

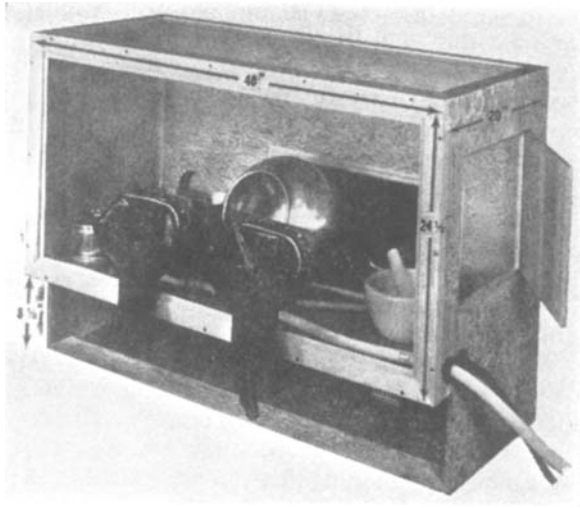

FIG. 1. Picture of glove-box showing position of equipment.

A flexible rubber sheet was inserted in the back wall of the glove-box to provide an air lock entry port for the drive shaft of the motor driving a 12-in. stainless steel coating pan. The glove-box was maintained under a slight positive static pressure of 0·2 in. of water by an air flow of about 2 cu. ft./min. The air was removed through a filter in the back of the box.

Materials Used in Preparation of the Medicated Pellets

31·2 g. of ^{35}S trimeprazine tartrate.
58·8 g. of a 1:1 mixture of starch, U.S.P. and powdered sucrose, U.S.P.
900 g. of U.S. No. 16 to 20 mesh sugar pellets.

Water-alcohol gelatin adhesive:

gelatin, U.S.P., 10 per cent w/v.; hydrochloric acid, U.S.P., 0·5 per cent v/v; water, 30 per cent v/v; Alcohol (90 per cent ethanol, 10 per cent methanol) 70 per cent v/v.

Wax-fat Solution:

glyceryl monostearate, N.F., 11 per cent w/w; glyceryl distearate, 16 per cent w/w; white wax, U.S.P., 3 per cent w/w; carbon tetrachloride, U.S.P., 70 per cent w/w.

The 900 g. of sugar pellets were placed in the coating pan. The 31·2 g. of ^{35}S trimeprazine tartrate and 58·8 g. of starch-sugar mixture were placed in the glove-box before sealing the entry port. The water-alcohol gelatin adhesive was placed in the Binks Pressure Vessel under 35 lb./sq. in. gauge pressure. Shoulder length rubber gloves were attached to the glove ports, and the operator transferred the labelled drug to the mortar. The drug was triturated to a fine powder, mixed with the starch-sugar diluent and divided into three approximately equal portions, each in a separate container. Then air was circulated through the box, the coating pan set in motion and the pellets sprayed with the gelatin adhesive. When just wet, after about 2 seconds of spray, using a Binks Flow Gun Model 31V (Binks Manufacturing Co., Chicago, Illinois) fitted with a No. 5 nozzle, the spraying was stopped and the pellets were allowed to roll for about 30 seconds to distribute the adhesive. One of the three portions of powder was then sprinkled on the pellets and the mass mixed by means of a rubber paddle. After 2 minutes of mixing the pellets had taken up all of the powder, the pan was stopped, and the pellets were dried for 5 minutes. The two remaining portions of coating powder were applied in the same way and the pellets were dried for 1 hour, then removed from the pan and screened through a U.S. No. 12 onto a U.S. No. 25 standard mesh sieve to remove lumps and "fines". After screening, the pellets were removed from the glove-box, the motor disconnected from the pan, and the glove-box sealed and removed from the hood. 967 g. of pellets designated Group A were recovered from the screening operation. 750 g. of these were placed in the 12-in. stainless steel coating pan in the fume hood for further processing. The wax-fat solution was placed in the Binks Pressure Vessel fitted with a heating mantle. This solution was maintained under 35 lb./sq. in. gauge pressure at 40°. Using the Binks Flow Gun fitted with a No. 3 nozzle, the wax-fat solution was sprayed at 7 second intervals on the pellets rotating in the pan. Two-minute drying intervals followed each 7-second spraying period. This procedure was repeated 38 times, until the pellets had increased in weight by 105 g. or 14 per cent of the starting weight. This material was designated Group B. 703 g. of Group B pellets were subsequently returned to the pan and a 1·3 per cent increment of wax-fat added. These pellets were designated Group C. A 1·6 per cent increment of wax-fat was then applied to 562 g. of Group C pellets. These pellets were designated Group D. A 2 per cent increment of wax-fat was then

applied to 419 g. of Group D pellets. This material was designated Group E. Each group of pellets was screened through U.S. No. 12 onto U.S. No. 25 standard mesh sieves to remove lumps and fines and sampled by the Sample Splitter described by Souder and Ellenbogen[6]. Representative samples of each group were assayed for ^{35}S trimeprazine spectrophotometrically by ultra-violet absorption over the range 270 to 240 mμ. Samples were then tested for *in vitro* release using USP XV Simulated Gastric and Intestinal Fluids by the method of Souder and Ellenbogen[6].

From these results capsules of the desired release characteristics were made by mixing the various groups. A desired composition was filled by hand into No. 0 hard gelatin capsules. Several capsules were taken at random to be assayed for ^{35}S trimeprazine and *in vitro* release.

Studies on Man

Fourteen adult male inpatients were used, in several of whom one dosage regimen was crossed-over to another. The drug was administered either at 0 hour in the once a day regimen or at 0, 4, and 8 hours in the three divided doses regimen. The dosage regimens were as follows:

5 mg. of non-sustained release ^{35}S trimeprazine, single dose.
15 mg. of non-sustained release ^{35}S trimeprazine, single dose.
5 mg. of non-sustained release ^{35}S trimeprazine, 3 doses in one day.
15 mg. of ^{35}S trimeprazine in sustained release capsules, single dose.

Blood samples were withdrawn at $\frac{1}{2}$, 1, 3, 6, 9, 12, 15, 24 and 48 hours by venipuncture of the anticubital veins. Total urinary output collections were made at 3, 6, 9, 12, 15, 24 and 48 hours. Measurements of radioactivity on the blood serum were by the method of Chen[7]. Serum and urine samples were counted in the Packard Tri-Carb Model 314-DC Liquid Scintillation Spectrometer (Packard Instrument Co., La Grange, Illinois) with the counting chamber set at $-8°$.

Results of the Pan Coating Formulation Technique

Release and assay data for the five bulk pellet groups are given in Table I.

Previous experimentation with laboratory animals, and subjective clinical studies, suggested that a 2:9:9 pellet ratio of groups A, B, and E, respectively, might provide a useful release characteristic in encapsulated pellets. Experimentally, this bulk composition was found to assay 11·3 mg. of ^{35}S trimeprazine per g. (three observations) with release of 18, 37, 70, and 88 per cent by $\frac{1}{2}$, 2, $4\frac{1}{2}$, and 7 hours.

Individual 442 mg. portions of this bulk pellet mixture, equivalent to 5 mg. trimeprazine base, were placed into No. 0 hard gelatin capsules. Each capsule contained approximately 475 pellets. Four capsules from 75 were selected randomly for assay. Average assay results indicated 11·0 mg. trimeprazine per g. (six observations), and a release of 21, 38, 67, and 88 per cent at the respective time intervals.

Capsules of Group A non-lipid coated pellets, each containing 5 mg. of ^{35}S trimeprazine, served as controls for non-sustained release ^{35}S trimeprazine.

Results and Discussion

The preparation of sustained release capsules described in the MacDonnell patent[8] is a coating pan procedure which does not lend itself to the precise laboratory preparation of a single dosage unit. Since the preparation of the medicated pellets before application of the lipid coating is a dusting procedure, it was conducted in a "closed" inexpensive disposable system.

The selection of 900 g. of starting sugar pellets and the concentration of drug in the coating powder were based on the following considerations. Lipid coated groups of less than 400 g. result in unpredictable batch to batch variations of *in vitro* release. Selection of a relatively low dose of ^{35}S trimeprazine (5 mg.) for inclusion in a relatively large capsule results in a low concentration of drug on the pellets and minimises the amount

TABLE I

Release and Assay Data for the Five Bulk Pellet Groups

Pellet groups	Per cent ^{35}S trimeprazine released				Assay* mg./g.	No. of observations
	½ hr.	2 hr.	4½ hr.	7 hr.		
A	100	—	—	—	14·1	8
B	31	59	92	93	12·0	2
C	11	29	86	92	10·2	2
D	10	22	74	90	10·1	2
E	0	12	47	89	9·9	3

* A S.D. of ±0·5 mg./g. was obtained from pooled results of 26 assays.

of radioactivity used in the experiment. Application of coating powder in three or more coats produces a uniform coating of the pellets and good batch to batch reproducibility. The preparation of a minimum of four primary sustained release groups and one non-sustained release group offers the best chance of providing material which can be selected for mixing to give the desired *in vitro* release pattern.

Patient Safety Considerations

Preliminary tests in rats with labelled trimeprazine tartrate showed that over 95 per cent of the ^{35}S administered orally was excreted in the urine and faeces within 96 hours (unpublished). Tests in human subjects receiving unlabelled trimeprazine orally showed that approximately 30 per cent of the administered dose was excreted as phenothiazines, in just the ether extracted portion of the urine, after 24 hours. Therefore, it was considered safe to administer a dose of less that 50 μc to man.

Blood and Urine Studies

The objective studies in man were made by Dr. Philip C. Johnson, Veterans Administration Hospital, Oklahoma City, Oklahoma. Details of the blood, urine, and faecal data after both oral and intravenous administration of ^{35}S trimeprazine tartrate will be published elsewhere.

The following is a summary of the results. The serum levels of radioactivity after administration of four different regimens are plotted in Figure 2 as arithmetic average mg. of trimeprazine. Although we recognise that the radioactivity measured in the blood and urine may emanate from both ^{35}S trimeprazine and its metabolites, we have chosen for convenience to express the values as mg. of trimeprazine to which the ^{35}S is equivalent. Inspection of these curves reveals that after peak serum levels are attained, after single 5 and 15 mg. oral doses of non-sustained release drug, ^{35}S disappearance from the serum follows distinctly similar die-away patterns for both doses. Also it is of interest to note that the areas under these two curves in the 0 to 24 time interval are in the ratio

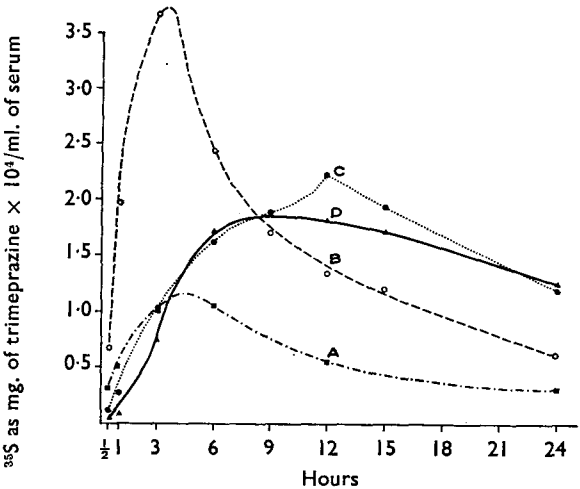

FIG. 2. Average ^{35}S serum levels of adult human subjects after oral administration of labelled trimeprazine: A, 5 mg. once daily to 4 subjects; B, 15 mg. once daily to 4 subjects; C, 5 mg. three times a day every 4 hours (total of 15 mg.) to 5 subjects; and D, 15 mg. sustained release capsules to 5 subjects. The drug was administered at 0 hour in the once a day regimens and at 0, 4 and 8 hours in the three divided doses regimen.

of 1 : 3, indicating a similar percentage absorption from these two regimens. Figure 2 also illustrates the similarity between ^{35}S serum levels observed for a single 15 mg. dose of sustained release drug and three 5 mg. doses of non-sustained release drug given at 0, 4, and 8 hours.

Figure 3 shows arithmetic average cumulative urinary recoveries represented as mg. of the administered doses. Over a 24-hour period the curves for the 5 mg. non-sustained release dose administered at 0, 4, and 8 hours, and the single 15 mg. sustained release dose are nearly straight lines, which indicates a fairly constant rate of ^{35}S urinary elimination over this period. There is no significant difference in the per cent urinary recovery of ^{35}S between the four dosage regimens.

The combined results in Figures 2 and 3 indicate that the ^{35}S serum and urine patterns are very different when one compares the single 15 mg. doses, where one dose is sustained release and the other is not. The 15 mg.

sustained release dose results in a much lower peak serum level, and ^{35}S activity is maintained at a much more uniform level. Also it is apparent that administration of the sustained release product closely parallels the t.i.d. divided dose therapy in ^{35}S serum and urine levels. The composite data of Figures 2 and 3 strongly suggest the therapeutic equivalence of this single 15 mg. sustained release dose administered once in 12 hours, to three 5 mg. non-sustained release doses given at 4 hour intervals. These objective studies have been supported with subjective clinical studies in 460 human subjects receiving unlabelled trimeprazine tartrate sustained release capsules having similar *in vitro* release characteristics.

It is apparent from this study that when a labelled drug can be incorporated into sustained and non-sustained action dosage forms, the development pharmacist has a new useful tool for evaluating performance

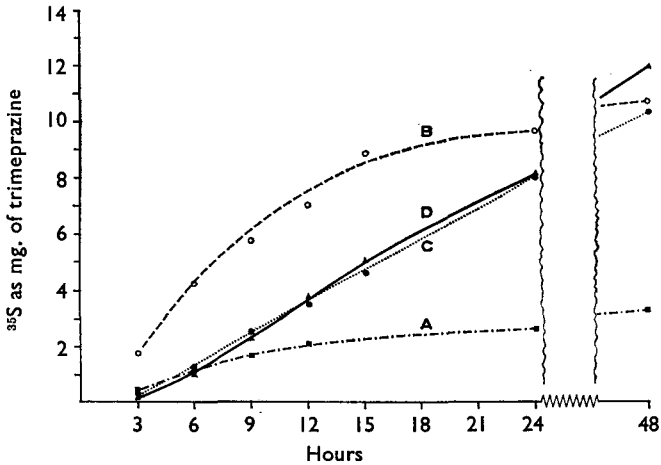

FIG. 3. Cumulative average ^{35}S urinary excretion of adult human subjects after oral administration of labelled trimeprazine: A, 5 mg. once daily to 4 subjects; B, 15 mg. once daily to 4 subjects; C, 5 mg. three times a day every 4 hours (total of 15 mg.) to 5 subjects; and D, 15 mg. sustained release capsules to 5 subjects. The drug was administered at 0 hour in the once a day regimens and at 0, 4 and 8 hours in the three divided doses regimen.

of such products. The biochemical measurements of a drug and its metabolites in human blood and urine are especially valuable in relative comparisons of objective performance. When the development pharmacist has access to such human testing procedures in man, drug product performance can be evaluated quantitatively at the biochemical or pharmacological level, and if these last can be related to therapeutics, establishment of rational *in vitro* release specifications can be accomplished with some confidence.

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After Dr. Swintosky presented the paper there was a DISCUSSION.