Sustained-Release Principle in Human Subjects Utilizing Radioactive Techniques

By R. L. BOGNER and J. M. WALSH

Radioactive tracer studies were conducted in six human volunteers utilizing tritium-labeled phenylephrine to determine tritium blood levels obtaining at various time intervals after oral administration of the usual dosage form, the hydrochloride salt of the drug, and of a sustained-release preparation containing the drug as a protocolloid tannate complex. The results obtained from three subjects receiving each preparation showed that a sustained-release form provided a plateau-shaped blood level curve, whereas the hydrochloride salt rapidly yielded a high peak concentration which dropped comparatively sharply thereafter. The technique of incorporating a radioactive drug in different dosage forms and tracing the radioactivity in humans can assist the development of sustained-release drug preparations.

DURING THE PAST several years, an increased interest in long acting oral therapeutic agents has developed. The theoretical benefits derived from long acting medication are extension of optimal therapeutic effects and better dosage control and more uniform blood levels, with a consequent reduction in the incidence of undesirable side effects from intermittently high and low levels. Several types of oral sustained-release preparations have been devised in attempts to achieve these goals. However, the inadequacy of quantitative studies of drug absorption after orally administered sustained-release preparations have prompted criticism of the claims made for some of these products. Much of the supportive evidence have been projected from subjective observations in human therapy. Animal studies are of limited value in predicting gastrointestinal absorption in man, particularly over an extended number of hours.

The present report describes results obtained using the radioactive tracer technique to follow the appearance of a drug in plasma and urine of humans after oral administration of a sustained-release preparation based on the use of protocolloidal tannate complexes of therapeutic amines. In practice, an amine tannate, usually in combination with polygalacturonic acid, serves to provide the drug reservoir. Physical and chemical characteristics and *in vitro* studies of this type of therapeutic amine complex have been described (1).

The drug chosen for this study was phenylephrine made radioactive by random tritium labeling. Comparisons were made of plasma levels of tritium radioactivity following oral administration of tablets containing tritium-labeled phenylephrine as the hydrochloride and as the protocolloid tannate complex. The radioactive technique provides a sensitive and specific means of following absorption from the gastrointestinal tract into the systemic circulation and excretion *via* the kidneys.

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EXPERIMENTAL

Radioactive Phenylephrine Hydrochloride.—Phenylephrine hydrochloride was randomly labeled by the Wilzbach (2) gas exposure technique. A 5-Gm. quantity of solid, finely divided compound was exposed at 27° to 10 curies of tritium gas at a pressure of 0.39 Atm. for 2 weeks. Labile and adsorbed tritium atoms were exchanged by dissolving the initial tritiated product in water (5 Gm. 30 ml.) and removing the water with vacuum at room temperature. This exchange in a hydroxylic solvent was repeated, and the specific activity of the product was found to be 74.2 μc. per mg. Subsequently, an acidic methanol solution of the amine salt was chromatographed on Amberlite CG-45 type I resin, eluted with acidified ethanol and recrystallized from isopropyl alcohol. Repeated recrystallizations yielded a product with a constant specific activity of 11.4 μc. per mg., m.p. 143–144°. Paper chromatography and radioscanning demonstrated one radioactive peak as shown in Fig. 1.

Radioactive Phenylephrine Tannate.—A 0.3-Gm. quantity of phenylephrine-H^3 hydrochloride was dissolved in 2 ml. of distilled water, and a solution of 60 mg. sodium hydroxide in 2 ml. of distilled water was added to liberate phenylephrine-H^3 base which was kept from precipitating by warming the solution. A solution of 0.57 Gm. of tannic acid N.F. in 3 ml. of water was added to the warm solution of amine base; a brown gummy precipitate formed. This precipitate was ground in a mortar with ice, the mixture was filtered, and the finely dispersed solid material was washed repeatedly with cold water. The product was dried *in vacuo* at approximately 60° to yield a dry, free-flowing, light tan powder with a specific activity of 4.14 μc. per mg. and containing 29.8% phenylephrine-H^3 base.

Radioactive Tablets.—The tritium-labeled forms of phenylephrine hydrochloride and phenylephrine tannate were processed into radioactive tablet dosage forms by Irwin, Neisler and Co. on a single-punch experimental tablet machine. The radioactive

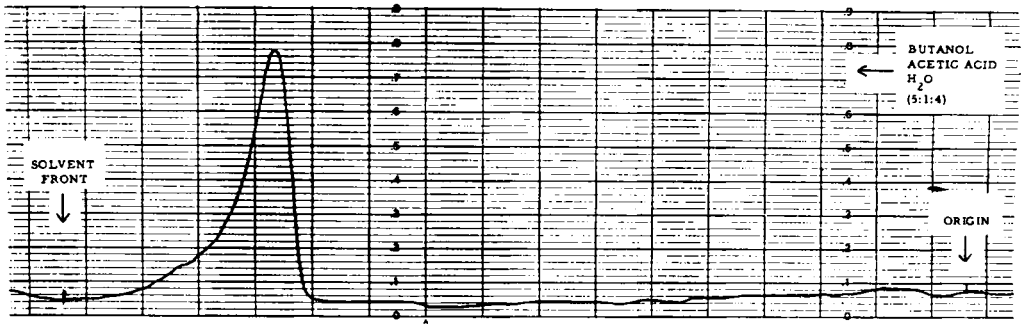

Fig. 1.—Radioscan of paper chromatogram of phenylephrine-H^3 hydrochloride.

TABLE I.—FEATURES OF SUBJECTS

Subject	Sex	Age	Radioactive Tablet
A	Female	39	Phenylephrine-H^3 tannate
B	Male	75	Phenylephrine-H^3 tannate
C	Male	54	Phenylephrine-H^3 tannate
D	Female	50	Phenylephrine-H^3 hydrochloride
E	Male	63	Phenylephrine-H^3 hydrochloride
F	Male	29	Phenylephrine-H^3 hydrochloride

tannate tablets were identical to commercial forms,[1] except that the stable nonradioactive content of the tannate of phenylephrine was replaced by 26.3 mg. (7.84 mg. phenylephrine-H^3 base) of the radioactive form of the drug representing 109 μc. The hydrochloride of radioactive phenylephrine was utilized in an analogous tablet for comparison in which 9 mg. of hydrochloride provided 7.39 mg. phenylephrine-H^3 base equivalent to 102.5 μc.

Preliminary Animal Studies.—Preliminary studies on plasma levels and urinary clearances of radioactive phenylephrine were carried out with two dogs after administration of the hydrochloride and tannate forms of the drug. The hydrochloride, as expected, was more rapidly both absorbed and eliminated during the test periods. Data from this preliminary screening served as bases for obtaining the required authorization to perform the human studies.

Administration to Human Volunteers.—The radioactive tablets were administered to six volunteers by Dr. Campbell Moses, Addison H. Gibson Laboratory, University of Pittsburgh. The features of the subjects are recorded in Table I.

All subjects had been admitted to the hospital for observations and diagnosis; subsequent studies revealed no abnormalities associated with disorders of absorptive or excretory functions.

Three subjects (*A*, *B*, and *C*) were each given a single tablet of phenylephrine-H^3 tannate and three other subjects (*D*, *E*, and *F*) were each given a single tablet of phenylephrine-H^3 hydrochloride. All doses were given on an empty stomach at 8 a.m. with 4 ounces of water following an overnight fast. Breakfast was served at 9 a.m., lunch at 1 p.m., and dinner at 5 p.m. Blood samples were obtained from each subject at 1, 2, 4, 6, 8, 12, 24, and 48 hours post-ingestion; plasma was immediately separated by centrifugation. Urine was collected as voided, and cumulative 24-hour collections were obtained.

[1] The product Rynatan contains 25 mg. phenylephrine tannate, 8 mg. chlorpheniramine tannate, 25 mg. pyrilamine tannate, and 32 mg. polygalacturonic acid plus inert excipients

TABLE II.—PLASMA LEVELS OF TRITIUM RADIOACTIVITY FOLLOWING ORAL ADMINISTRATION OF PHENYLEPHRINE-H^3 TANNATE AND PHENYLEPHRINE-H^3 HYDROCHLORIDE TABLETS

Subject	Time, hr.	Plasma Tritium, d.p.m./ml.	Equivalent Phenylephrine,[a] mcg./ml.
Tannate			
A	1	955	0.031
	2	2190	0.071
	4	2785	0.091
	6	1935	0.063
	8	1000	0.032
	12	880	0.029
B	1	990	0.032
	2	1960	0.064
	4	2800	0.091
	6	2660	0.086
	8	1950	0.063
	12	1440	0.047
C	1	785	0.025
	2	3550	0.115
	4	4160	0.135
	6	4860	0.158
	8	3380	0.110
	12	3400	0.111
Hydrochloride			
D	1	5300	0.172
	2	2860	0.074
	4	4105	0.133
	6	3750	0.122
	8	3450	0.112
	12	100	0.003
E	1	4500	0.146
	2	5290	0.171
	4	1500	0.049
	6	1450	0.047
	8	2200	0.071
	12	100	0.003
F	1	8570	0.278
	2	7470	0.242
	4	6350	0.206
	6	4670	0.152
	8	4100	0.133
	12	900	0.029

[a] Calculation based upon the assumption that the specific activity of the radioactive material in the plasma was identical to the specific activity of the administered material.

Analyses.—The total tritium radioactivity of each plasma sample was determined by a liquid scintillation counting technique. The procedure involved addition of 0.1 to 0.2 ml. plasma to 1 ml. of hydroxide of Hyamine 10-X[2] and subsequent addi-

[2] 1 *M* solution in methanol. Marketed by Rohm and Haas, Philadelphia, Pa.

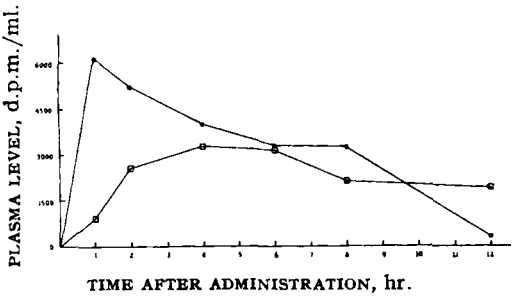

Fig. 2.—Average plasma levels of tritium radioactivity following oral administration of phenylephrine-H³ hydrochloride and tannate tablets. Key: ●, hydrochloride; □, tannate.

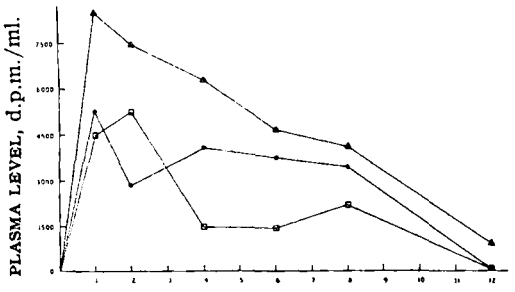

Fig. 3.—Plasma levels of tritium radioactivity following oral administration of phenylephrine-H³ hydrochloride tablets. Key: ●, subject *D*; □, subject *E*; Δ, subject *F*.

tion of 0.5 ml. ethanol and 15 ml. toluene-phosphor (0.6% PPO[3] and 0.02% POPOP[4]). The samples were counted in a Packard Tri-Carb liquid scintillation spectrometer with counting chamber set at 3°, discriminator set at 10–100, and at voltage corresponding with the peak tritium counting rate. All sample counts were corrected for background and counting efficiency as determined from internal standards. Results were expressed as disintegrations per minute per milliliter (d.p.m./ml.).

Urine concentrations of tritium were also determined by liquid scintillation counting. Aliquots of 1 ml. of urine were placed in counting vials with 15 ml. dioxane-phosphor (0.7% PPO, 0.005% POPOP, and 8% naphthalene). All sample counts were again corrected for background and efficiency.

RESULTS

The plasma levels of tritium radioactivity in each of three humans following oral administration of phenylephrine-H³ hydrochloride tablets are listed in Table II. The tritium levels include the contributions from tritium-labeled phenylephrine as well as any tritium-containing metabolites. The tritium levels are also represented in terms of "equivalent phenylephrine concentrations" based upon the assumption, for purposes of calculation, that the specific activity of the radioactive material in the plasma was identical to the specific activity of the administered phenylephrine-H³.

Table II also shows corresponding plasma levels of tritium radioactivity and "equivalent phenylephrine concentrations" in humans following oral ingestion of phenylephrine-H³ tannate tablets.

The average plasma levels of the subjects receiving the hydrochloride and of those receiving the tannate are plotted in Fig. 2; the individual responses are shown in Figs. 3 and 4.

The 24-hour urine excretion of tritium radioactivity from each subject may be seen in Table III.

DISCUSSION

Examination of the average curves and the individual curves reveals relationships which are significant to the action of the sustained-release principle. Phenylephrine provided as the usual rapidly soluble salt form (hydrochloride) was quickly absorbed from the human gastrointestinal tract with resulting high blood levels attained by the first and second hours; appreciable levels were still present after 8 hours, but between the eighth and twelfth hours, the concentrations dropped off rapidly. The tannate complex provided a more gradual rise in tritium blood levels with a plateau rather than a sharp peak in the 2 to 8-hour range and with remarkably stable maintenance of blood level continued through the twelfth hour. Consistent individual blood levels were obtained in these humans with the sustained-release preparation, although the hydrochloride showed wide individual variations. Despite the use of only six humans, the measurement technique relates the variations in readings clearly to individual variations in absorption and elimination.

Figure 2 should be considered as a reflection of the relative absorption characteristics of drug from the two preparations and not as a suggestion of recommended absolute drug levels. The quantitative blood values obviously can be varied by variation in the absolute dosage. The shape of the two curves relative to one another is the point of primary significance.

The excretion data illustrated even more dra-

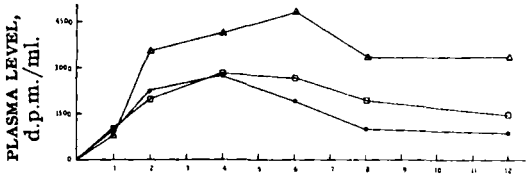

Fig. 4.—Plasma levels of tritium radioactivity following oral administration of phenylephrine-H³ tannate tablets. Key: ●, subject *A*; □, subject *B*; Δ, subject *C*.

TABLE III.—URINARY EXCRETION OF TRITIUM RADIOACTIVITY

Subject	Drug Form	Administered Radioactivity Excreted in 24 hr., %
A	Phenylephrine-H³ tannate	12.4
B	Phenylephrine-H³ tannate	15.9
C	Phenylephrine-H³ tannate	13.3
D	Phenylephrine-H³ hydrochloride	37.2
E	Phenylephrine-H³ hydrochloride	20.0
F	Phenylephrine-H³ hydrochloride	69.0

[3] 2,5-Diphenyloxazole.
[4] 1,4-Bis-2-(5-phenyloxazolyl-benzene).

matically than did the blood levels that absorption and excretion of phenylephrine from the hydrochloride show very wide individual variations, whereas the tannate complex sustained-release form showed an unusual degree of consistency of performance.

SUMMARY

Tritium-labeled phenylephrine hydrochloride and tannate were prepared and incorporated into ordinary and sustained-release type dosage forms, respectively.

Blood levels of tritium radioactivity in humans following oral ingestion of the ordinary hydrochloride dosage form followed a characteristic pattern of rapid rise and fall; blood levels following administration of a tannate complex dosage form rose more slowly but appeared to be more sustained.

REFERENCES

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Electrochemical Oxidation of Chlorpromazine Hydrochloride

By F. HENRY MERKLE and CLARENCE A. DISCHER

The electrolytic oxidation of chlorpromazine hydrochloride has been studied. Controlled potential coulometry has shown the chlorpromazine undergoes a two-electron oxidation in dilute aqueous acid media. In 9 *N* sulfuric acid two successive one-electron oxidations, involving a stable semiquinone free radical intermediate, are identifiable. Polarographic studies with a rotating platinum electrode confirm the occurrence of two separate one-electron oxidation steps in 9 *N* sulfuric acid. The coulometrically determined *n* values are in agreement with these observations. Reaction mechanisms are proposed for the electrolytic oxidation of chlorpromazine under various conditions of acidity. Spectral absorbance curves are presented as supporting evidence for the indicated reaction mechanisms.

THE OXIDATION of chlorpromazine hydrochloride[1] by cerium (IV), iron (III), permanganate, and hydrogen peroxide as well as by photo-irradiation has been reported (1–3). It has also been shown that the intermediate species in the oxidation of chlorpromazine exists as a free radical (4). More recently, the existence of stable semiquinone free radicals of chlorpromazine and other *N*-substituted phenothiazine derivatives has been demonostrated (5). However, because of the inherent difficulties in the quantitative determination of the free radical species, their study has been restricted considerably.

This study was undertaken to establish an oxidation mechanism for chlorpromazine hydrochloride in aqueous media. Since chlorpromazine and its oxidation products are electrolytically active, controlled-potential coulometry offered a direct approach for this study. The oxidation at a platinum anode has proved to be an extremely useful method for the production of the intermediate free radical species. Moreover, this study indicates the possible use of controlled-potential coulometry as a quantitative analytical technique for chlorpromazine and its oxidized forms in the presence of one another.

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[1] Chlorpromazine hydrochloride and chlorpromazine 5-oxide were supplied by Smith Kline and French Laboratories, Philadelphia, Pa.

INSTRUMENTATION

An electronic controlled-potential coulometric titrator, model Q-2005 ORNL, was used to perform the electrolyses (6). Oxidations were performed in a cell designed to accept a cylindrical wire mesh rotating platinum electrode (1 in. diameter × 2 in. height) and approximately 100 ml. of solution. The reference (S.C.E.) and auxiliary (working cathode) electrodes were separated from the sample solution by agar plugs and fritted glass diaphragms. A triplet vacuum tube voltmeter, model 805, with a 7-in. scale served as the readout device.[2] Polarograms were obtained on the Sargent model XXI recording polarograph. An H-type cell was used with a rotating platinum microelectrode. A saturated calomel reference electrode was separated from the sample portion by an agar plug and fritted-glass diaphragm.

PROCEDURE AND RESULTS

Controlled Potential Data.—When chlorpromazine hydrochloride is electrolyzed at a potential of approximately +0.70 v. *versus* S.C.E. in 1 *N* sulfuric acid under an atmosphere of nitrogen, the solution gradually assumes a deep red. As the electrolysis is

[2] In the latter stages of this study readout voltages were measured with a Non-Linear Systems model 484 A digital voltmeter.