Biochemical Pharmacology, Vol. 18, pp 2331–2338. Pergamon Press. 1969. Printed in Great Britain

# CHLORPHENESIN METABOLISM IN THE RAT AND DOG*

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(*Received* 18 *October* 1968; *accepted* 28 *March* 1969)

**Abstract**—The absorption, distribution and metabolic fate of chlorphenesin (3-*p*-chlorophenoxy-1,2-propanediol) were studied in the rat and dog. After oral ingestion of chlorphenesin-$^{14}$C, the drug was rapidly absorbed by the rat, and radioactivity reached a peak blood concentration in 30 min. The half-life of serum radioactivity was about 140 min. A distribution study of 3-*p*-chlorophenoxy-1,2-propanediol-1,3-$^{14}$C (chlorphensin-1,3-$^{14}$C) in the rat showed that over half the dose was excreted in the urine after 4 hr, the remainder being principally in the gastrointestinal tract and carcass. A small portion of radioactivity was also recovered as respiratory $CO_2$. Five urinary end-products were identified after administration of the drug. They were (1) 3-*p*-chlorophenoxylactic acid, (2) *p*-chlorophenoxyacetic acid, (3) unchanged drug, (4) a conjugate of chlorophenol, and (5) a conjugate of chlorphenesin.

THE ABILITY of chlorphenesin (3-*p*-chlorophenoxy-1,2-propanediol) and other phenoxy propanediols to selectively suppress passive cutaneous anaphylaxis elicited in guinea pigs with penicillin conjugates was recently reported from these laboratories.[1] Although chlorphenesin has been available since 1949, no metabolic studies of the fate of this drug have appeared in the literature. Recently, studies of the metabolism of the carbamate derivative of chlorphenesin in the rat[2] and dog[3] were reported. The major urinary metabolite in both species was the *O*-glucuronide of the unchanged drug, chlorphenesin carbamate. In addition, 3-*p*-chlorophenoxylactic acid, *p*-chlorophenoxyacetic acid and *p*-chlorophenol were identified in the urine of these animals. In this communication we have summarized our observations on the absorption, distribution and metabolic fate of chlorphenesin.

## METHODS

*Synthesis of 3-*p*-chlorophenoxy-1,2-propanediol-1,3-$^{14}$C (chlorphenesin-1,3-$^{14}$C)*

Hydrogen chloride was bubbled into 2·07 g of glycerol-1,3-$^{14}$C at room temperature until 0·85 g of the gas had reacted. An aqueous solution, containing 3·5 g of the gas and 1·06 g of sodium hydroxide in 20 ml, was slowly added to the glycerol chlorohydrin, and the reaction mixture was refluxed for 2 hr. After cooling, the two phases were separated, and the lower aqueous phase was extracted three times with 10 ml chloroform. The chloroform extracts and the organic phase from the reaction mixture were combined, washed with water and dried over calcium sulfate. The chloroform solution was concentrated to about half its volume *in vacuo*. The addition

* A preliminary report of this paper was presented at the 1967 Spring Meeting of the American Society for Pharmacology and Experimental Therapeutics.

of pentane caused the separation of an oil which crystallized upon cooling. This crude product was recrystallized from benzene to yield 1·5 g of chlorphenesin-1,3-$^{14}$C; m.p. 78·5–79·5°, sp. act. 2·46 × $10^5$ dpm/mg. An infra-red spectrum of this material was identical to that obtained from an authentic sample of chlorphenesin; in addition, this compound was homogenous by thin-layer chromatography in the system described below.

*Synthesis of chlorphenesin-UL-ring-$^{14}$C*

This compound was prepared from a reaction mixture containing 1·08 g *p*-chlorophenol-UL-ring-$^{14}$C (Mallinckrodt Nuclear Chemicals, Orlando, Fl.), 0·94 glycerol chlorohydrin, and aqueous sodium hydroxide by the procedure described above. A yield of 953 mg of chlorphenesin-UL-ring-$^{14}$C was obtained; m.p. 77·5–79·0°, sp. act. 5·92 × $10^5$ dpm/mg. This material was chromatographically homogeneous.

*Blood concentration in the rat*

Four fasted, male Sprague–Dawley rats, weighing approximately 200 g, each received, by stomach tube, 16·7 mg of chlorphenesin-1,3-$^{14}$C dissolved in physiological saline. Blood samples were taken from the tail at appropriate intervals, digested as described below and assayed for radioactivity by liquid scintillation counting. The scintillation fluid consisted of 7 g of 2,5-diphenyloxazole, 250 mg of 1,4-*bis*-[2-(4-methyl-5-phenyloxazolyl)]-benzene and 125 g of naphthalene dissolved in 1·0 l. of *p*-dioxane.

*Distribution of chlorphenesin-1,3-$^{14}$C in the rat*

A male rat of the Sprague–Dawley strain, weighing approximately 200 g, received 15·2 mg of chlorphenesin-1,3-$^{14}$C i.p. The animal was sacrificed after 4 hr and the organs were removed. The tissues and the remainder of the carcass were homogenized with 6 vol. of 1 M Hyamine hydroxide (Packard Instrument Co., Downers Grove, Ill.) and heated at 50° until dissolved. An aliquot of the digested tissue or body fluid was then assayed for radioactivity. The carbon dioxide of respired air was trapped in 2·5 M sodium hydroxide and a portion of this solution was counted.

*Thin-layer chromatography*

Plates coated with a 250 micron thick layer of SilicAR 7G were developed in chloroform–ether–acetic acid (20:25:1). Chlorphenesin and its metabolites were detected under short wavelength ultraviolet light.[9]

*Isolation of metabolites from dog urine*

Two male beagle dogs were each given 500 mg/kg of nonradioactive chlorphenesin orally and their urine was collected under toluene for 24 hr. The pooled urine, approximately 300 ml, was acidified to pH 1 with concentrated hydrochloric acid and continuously extracted with ethyl acetate for 48 hr. The ethyl-acetate solution was evaporated to 50 ml and the concentrate was washed five times with 25-ml portions of 1 N sodium hydroxide. The ethyl-acetate phase was evaporated to dryness; the residue was dissolved in hot water and allowed to cool. The solid which crystallized was identified as chlorphenesin.

The combined basic washes of the ethyl-acetate extract were treated with Darco G-60 charcoal, acidified to pH 1 with concentrated HCl and continuously extracted with ethyl acetate for 18 hr. The organic phase was concentrated *in vacuo* to give an

oily residue which was dissolved in methanol. The addition of water to the methanolic solution gave a precipitate. This solid, recrystallized from hot water, was 3-*p*-chlorophenoxylactic acid.

Two male beagle dogs were each given two i.p. injections, 6 hr apart, of 250 mg/kg of nonradioactive chlorphenesin and their urine was collected under toluene for 24 hr. The urine (approximately 1400 ml) was pooled, made alkaline with 50% sodium hydroxide, and saturated with sodium chloride. The resultant mixture was continuously extracted with ether overnight and the ether phase was discarded. The aqueous phase was adjusted to pH 1 with concentrated hydrochloric acid and again extracted with ether overnight. This ether extract was evaporated to dryness; the residue was dissolved in acetone and the solution was streaked on two glass plates coated with a 2-mm thick layer of SilicAR 7G (Analtech, Wilmington, Del.). These plates were developed in chloroform–ether–acetic acid (20:25:1) and the zone of adsorbent, having a fluorescing band around $R_f$ 0·6, was removed from the plate. This material was extracted overnight with ether in a Soxhlet extractor. The solvent was removed *in vacuo* and the residue was dissolved in 10 ml of 0·5% sodium hydroxide. After treatment with Darco G-60 charcoal, the filtrate was acidified to pH 2 with concentrated hydrochloric acid and evaporated to approximately 0·5 ml. The crystalline product which formed was removed by filtration and identified as *p*-chlorophenoxyacetic acid.

*Quantitation—Inverse isotope dilutions*

Typical inverse isotope dilution experiments were carried out as follow:

A. 3-p-*Chlorophenoxylactic acid, chlorphenesin and* p-*chlorophenoxyacetic acid.* A male Sprague–Dawley rat received i.p. 30·8 mg chlorphenesin-1,3-$^{14}$C in 1·0 ml of polyethylene glycol 400. Urine (6·8 ml) was collected over a period of 22·5 hr and assayed for radioactivity. Isotope dilutions were carried out on 1·0-ml aliquots of this urine as follows: (1) Nonradioactive 3-*p*-chlorophenoxylactic acid, 1000 mg, was added to the first aliquot, followed by the addition of 50% sodium hydroxide until a clear solution was obtained. Acidification of the mixture with concentrated hydrochloric acid caused the precipitation of 3-*p*-chlorophenoxylactic acid. The precipitate was separated and recrystallized from methanol–water to constant specific activity; m.p. 137–138°. (2) Nonradioactive chlorphenesin, 1000 mg, was added to the second aliquot of urine and the mixture was dissolved in methanol. Sufficient distilled water was added to precipitate chlorphenesin. It was separated and recrystallized from benzene to constant specific activity; m.p. 78–78·5°. (3) Nonradioactive *p*-chlorophenoxyacetic acid, 1000 mg, was added to a third aliquot of urine and the mixture was made homogeneous by adding methanol and heating gently. Upon cooling, *p*-chlorophenoxyacetic acid crystallized. This material was recrystallized from methanol–water to constant specific activity; m.p. 156–157°.

B. *Conjugated metabolites.* Four male rats each received 30 mg of chlorphenesin-UL-ring-$^{14}$C, i.p. and urine was collected for 24 hr. A 1·0-ml aliquot of each urine was added to an equal volume of concentrated hydrochloric acid and the mixtures were heated on a steam bath for 10 min.[4] One gram of nonradioactive *p*-chlorophenol was added to two of the hydrolyzed urines. The mixtures were made homogenous by the addition of 3 N sodium hydroxide. The resulting solutions were neutralized with 3 N hydrochloric acid, saturated with solid sodium bicarbonate, and

continuously extracted with ether for 18 hr. The ethereal extracts were washed with 5% w/v sodium bicarbonate, distilled under vacuum, and the phenol fractions were collected. The distillates were washed with sodium bicarbonate and then allowed to react with excess chloroacetic acid.[5] The resulting derivatives were isolated, recrystallized to constant melting point (156–158°) and their radioactivity was measured.

To the other two hydrolyzed rat urine aliquots, 1 g of nonradioactive chlorphenesin was added and procedure (2) above was followed.

*Quantitation—Thin-layer chromatography*

Two male beagle dogs were each given 30 mg of chlorphenesin-1,3-$^{14}$C by capsule. Their urine was collected for 24 hr under toluene and an aliquot of the urine was subjected to thin-layer chromatography. After development, the relative radioactivity corresponding to each of the major metabolites was determined quantitatively with a Vanguard model 880 autoscanner equipped with an integrating recorder.

Three male Sprague–Dawley rats were each given 30 mg of chlorphenesin-1,3-$^{14}$C i.p. and their urine was collected for 24 hr and analyzed for radioactivity in the same manner.

## RESULTS AND DISCUSSION

*Absorption and distribution in the rat.* The gastrointestinal absorption of chlorphenesin was investigated in the rat after oral administration of radioactive drug. Peak-blood radioactivity was obtained 30 min postadministration, indicating rapid drug absorption (Fig. 1). At that time about 4·1 per cent of the administered radio-

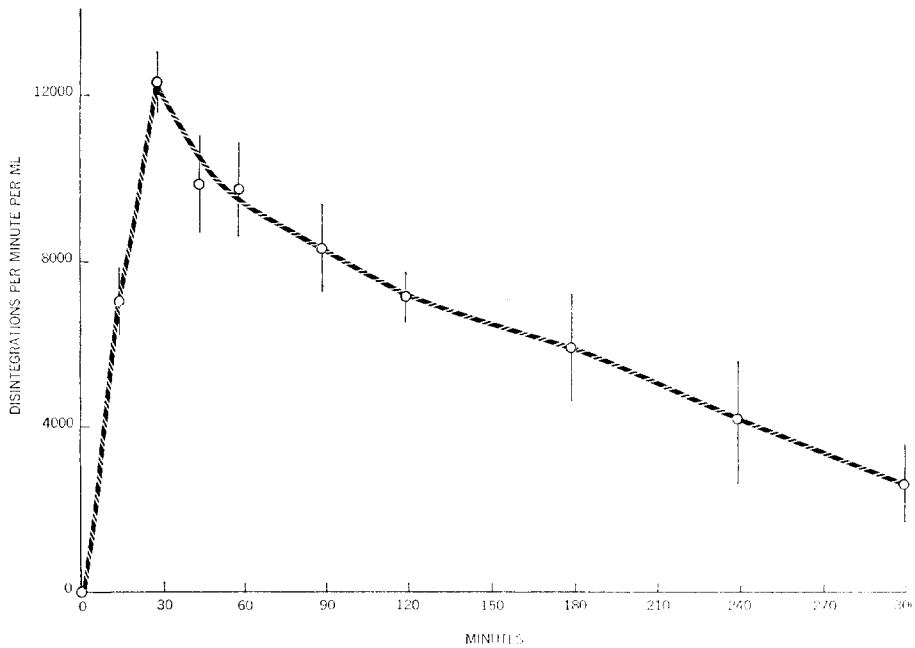


FIG. 1. Blood concentration of radioactivity after oral administration of chlorphenesin-1,3-$^{14}$C to rats.

activity was circulating in the blood. The half-life of blood radioactivity was approximately 140 min.

This elimination was via the kidneys since 4 hr after the i.p. administration of chlorphenesin-1,3-$^{14}$C to the rat over half of the administered radioactivity was present in the urine, including bladder contents. A distribution study of carbon-14 in this animal showed that there were also significant amounts of radioactivity in the gastrointestinal tract, carcass, liver, body cavity fluid and expired air (Table 1). Only trace amounts of radioactivity were present in the kidneys, lung, heart or spleen.

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN A RAT 4 hr AFTER i.p. ADMINISTRATION OF CHLORPHENESIN-1, 3-$^{14}$C

| Specimen analyzed | Activity recovered (% of dose) |
|---|---|
| Urine, including bladder contents | 55·50 |
| Carcass | 20·75 |
| Stomach and intestines | 17·91 |
| Carbon dioxide, respired | 6·38 |
| Liver | 2·46 |
| Body cavity fluid, including blood | 2·22 |
| Kidney | 0·73 |
| Lung | 0·16 |
| Heart | 0·10 |
| Spleen | 0·08 |
| Total recovery | 106·29 |

*Identification of urinary metabolites.* Thin-layer chromatography of urine from either rats or dogs that had received chlorphenesin-1,3-$^{14}$C showed the presence of three radioactive substances in significant amounts. These urinary end-products of chlorphenesin were identified as (1) 3-*p*-chlorophenoxylactic acid, (2) *p*-chlorophenoxyacetic acid, and (3) unchanged drug. The identity of these substances was established by means of their $R_f$ values in thin-layer chromatography, inverse isotope dilution and by isolation of these compounds from the urine of dogs receiving the nonradioactive drug.

Urine from rats that had received chlorphenesin-UL-ring-$^{14}$C contained small quantities of two additional metabolites. These were identified as a conjugate of chlorphenesin and a conjugate of *p*-chlorophenol. The presence of a chlorphenesin conjugate was established by the increase in the amount of chlorphenesin present in the urine after acid hydrolysis. The *p*-chlorophenol was identified in acid-hydrolyzed urine by inverse isotope dilution.

*Isolation of urinary end-products.* 3-*p*-Chlorophenoxylactic acid, obtained from the aqueous basic wash of the ethyl-acetate extract of the acidified dog urine, was identified by its chromatographic properties, its melting point, and its infra-red spectrum, which were identical to those of an authentic sample of this compound.

*p*-Chlorophenoxyacetic acid, obtained by ether extraction of acidified dog urine followed by thick-layer chromatography, was identified by its thin-layer chromatographic properties, its melting point and its infra-red spectrum, which were identical to those of authentic *p*-chlorophenoxyacetic acid.

Unchanged chlorphenesin was isolated from dog urine by ethyl-acetate extraction.

$R_f$ values in thin-layer chromatography, melting point and infra-red spectral analysis confirmed the identity of the isolate as chlorphenesin.

*Quantitation of urinary end-products—Inverse isotope dilution.* Urinary end-products excreted in the rat after the administration of radioactive chlorphenesin were quantitated by inverse isotope dilution. The results are shown in Table 2. 3-*p*-Chlorophenoxylactic acid was the major metabolite, while unchanged chlorphenesin and *p*-chlorophenoxyacetic acid were also present in significant amounts.

TABLE 2. QUANTITATION OF RADIOACTIVE URINARY END-PRODUCTS IN THE URINE OF RAT GIVEN CHLORPHENESIN-$^{14}$C

| Urinary end-product | Position of $^{14}$C | No. of animals | % of Urinary radioactivity Average* |
|---|---|---|---|
| 3-*p*-Chlorophenoxylactic acid | 1,3 | 3 | 45·6 |
| *p*-Chlorophenoxyacetic acid | 1,3 | 3 | 22·5 |
| Chlorphenesin, free | 1,3 + ring | 5 | 24·7 |
| Chlorphenesin, conjugated† | ring | 2 | 3·8 |
| *p*-Chlorophenol, conjugated | ring | 2 | 8·3 |

* Values obtained by inverse isotope dilutions.
† By difference, before and after acid hydrolysis.

Conjugated derivatives of chlorophenol and chlorphenesin were present only in minor amounts. Within the lower limit of our procedure, 0·03 per cent, no free *p*-chlorophenol could be detected. These results are consistent with the findings reported by Spencer and Williams[6] on the metabolism of chlorophenol and with the investigations of the metabolism of chlorphenesin carbamate.[2,3] The quantity of conjugated chlorphenesin was obtained by determining the difference in the amount of free chlorphenesin present before and after acid hydrolysis.

The *p*-chlorophenoxyacetic acid found in the urine after the administration of chlorphenesin-1,3-$^{14}$C contains only one radioactive carbon atom, while the other major urinary end-products, chlorphenesin and 3-*p*-chlorophenoxylactic acid, contain two radioactive sites. For comparative purposes, it is more meaningful to express the concentration of these urinary end-products $\mu$M/ml of urine. As determined by inverse isotope dilution, the amounts of 3-*p*-chlorophenoxylactic acid, *p*-chlorophenoxyacetic acid and chlorphenesin excreted in the urine of the rat were, respectively, 7·94, 7·83 and 5·08 $\mu$M/ml.

*Quantitation of urinary end-products—Thin-layer chromatography.* Relative quantitation of the three major metabolites of the dog and rat which had received chlorphenesin-1,3-$^{14}$C was determined by thin-layer chromatography. These results are shown in Table 3. Under these experimental conditions the low content of conjugated chlorphenesin did not permit quantitation and any *p*-chlorophenol conjugates present would not be radioactive.

*Comparison with the metabolism of related drugs.* The nature of the urinary metabolites formed from chlorphenesin could be anticipated from the investigations reported by Buhler *et al.*[2,3] These investigators found that chlorphenesin carbamate is

TABLE 3. QUANTITATION AND $R_f$ VALUES IN THIN-LAYER CHROMATOGRAPHY OF URINARY END-PRODUCTS IN THE URINE OF ANIMALS RECEIVING CHLORPHENESIN-1, 3-$^{14}$C

| End-product | $R_f$ | % of Urinary radioactivity | |
|---|---|---|---|
| | | Dog* | Rat† |
| 3-*p*-Chlorophenoxylactic acid | 0·38 | 57·3 | 41·8 |
| Chlorphenesin, free | 0·15 | 30·4 | 35·5 |
| *p*-Chlorophenoxyacetic acid | 0·62 | 12·0 | 22·8 |

* Average of two dogs.
† Average of three rats.

excreted as (1) unchanged drug, (2) conjugated drug, (3) *p*-chlorophenoxyacetic acid, (4) 3-*p*-chlorophenoxylactic acid and (5) conjugated *p*-chlorophenol. The major urinary end-product of the carbamate derivative is the *O*-glucuronide, and only small amounts are excreted in the acid and free phenol forms.[2, 3] Our findings indicate, however, that chlorphenesin is conjugated during metabolism to a lesser extent than chlorphenesin carbamate, the major portion of the drug being excreted either unchanged or in the form of its lactic acid and acetic acid oxidation products. These differences in chlorphenesin and chlorphenesin carbamate metabolism may be due to the protection afforded by the carbamate group to the vulnerable primary hydroxyl of chlorphenesin. It has been shown in studies with mephenesin carbamate,[7] meprobamate[8] and mebutamate[9] that the carbamate moiety is not readily degraded *in vivo*.

The investigations of Riley,[10] Titus *et al.*[11] and Wyngaarden[4] have shown that mephenesin is excreted as unchanged mephenesin, a conjugated form of mephenesin, 3-*o*-toloxylactic acid and to a minor extent β-(4-hydroxy-2-methylphenoxy)lactic acid. Comparison of the metabolites formed from mephenesin and from chlorphenesin show a number of similarities and several differences. Both drugs are excreted as a conjugate, as a lactic acid derivative and in an unchanged form. Riley,[10] however, did not find any significant amounts of either *o*-cresol or *o*-toloxyacetic acid, although one would expect these metabolites to be formed by analogy with the findings made with chlorphenesin and chlorphenesin carbamate. The more sensitive techniques currently available would no doubt be helpful in resolving this apparent anomaly. The hydroxylation which occurs to a minor extent with mephenesin cannot occur with chlorphenesin or its carbamate because of the presence of a chlorine atom in the critical para position of the aromatic ring.

*Acknowledgement*—The authors are grateful to Mr. M. Meyer for obtaining and interpreting the infra-red spectra referred to in this manuscript and to Mrs. M. Krumins and Mr. A. Schlosser for their skillful technical assistance.

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