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Metabolic Fate of *p*-Toluoyl Chloride Phenylhydrazone (TCPH) in Sheep. The Nature of Bound Residues in Erythrocytes

Prem S. Jaglan,* Ronald E. Gosline, and A. William Neff

Ten days after a single oral therapeutic dose of 50 mg/kg [^{14}C]TCPH (phenylhydrazine label), 93% of the radioactivity was recovered, 19% in urine and 74% in feces. The ^{14}C residues were higher and persisted longer in blood and blood rich organs such as liver, lung, kidney, and spleen compared to other tissues. Detailed examination of blood indicated ^{14}C residues were largely present in the hemoglobin. The ^{14}C residues could neither be extracted into organic solvents nor separated from hemoglobin by dialysis, gel filtration, or electrophoresis. Administration of [^{14}C]TCPH (carboxyl labeled) resulted in a lower concentration of ^{14}C in the blood. Most of the ^{14}C residue in the blood was found in the plasma rather than in the erythrocytes which demonstrated that only the phenylhydrazine part of the molecule was bound to erythrocytes. Chromic acid oxidation of heme or globin from the phenylhydrazine label experiment produced [^{14}C]benzoic acid, further demonstrating that only the phenyl part of TCPH was bound to hemoglobin and that the carboxyl carbon of benzoic acid came from heme or globin.

A potential anthelmintic, *p*-toluoyl chloride phenylhydrazone (TCPH), was demonstrated to exhibit a broad spectrum activity against a wide variety of gastrointestinal nematodes and cestodes of ovines (Folz and Rector, 1973, 1974). Although metabolism of hydrazine derivatives has recently been reviewed (Colvin, 1969), no in depth studies regarding the transport of phenylhydrazones in blood and the degree with which these were associated with the proteins have been made previously (Gillette et al., 1974). This report describes the distribution, tissue residues, and excretion of TCPH in sheep. Novel metabolism of TCPH in sheep blood using two labeled forms of TCPH has been studied and the nature of covalent binding to hemoglobin is discussed.

MATERIALS AND METHODS

Synthesis of [^{14}C]TCPH. Metabolism studies were carried out using [^{14}C]TCPH (Figure 1), labeled uniformly in the phenylhydrazine ring (TCPH-I) or labeled at the carboxyl position (TCPH-II). The compound was prepared in 65–70% yield. In a typical run, 50 ml of chilled pyridine was added to 0.04 mol of phenylhydrazine hydrochloride containing 8 mCi of [U-^{14}C]phenylhydrazine hydrochloride, cooled in an ice bath, followed by addition of 0.04 mol of *p*-toluoyl chloride dropwise. The contents were stirred for 2 days at room temperature after which 400 ml of ice water was added and the solids were collected and crystallized from ethanol and water to give 0.032 mol of *p*-toluic acid phenylhydrazide (TAPH). TAPH (0.026 mol) was chlorinated by dissolving in 100 ml of carbon tetrachloride and refluxing with 0.03 mol of phosphorus pentachloride for 2 h. After cooling to 0 °C, 0.14 mol of phenol was added and stirred for 1 h. The solvent was evaporated to dryness in a roto-evaporator at 40 °C and the compound crystallized from methylene chloride and Skelly B. Similarly, TCPH-II was prepared using ^{14}C-labeled *p*-toluoyl chloride (carboxyl label).

The labeled compounds were more than 98% pure as determined by TLC and radioautography on silica gel GF plates in hexane–acetone (7:3, v/v). The labeled drug was diluted with cold TCPH to give the desired specific activity.

Treatment of Sheep and Sampling. Crossbred feeder lambs (female) freshly sheared and in good physical condition were acclimated in metabolism cages for 5 days. Food and water were provided ad libitum. A catheter was used to collect urine separately from feces. The dose was prepared by mixing radioactive TCPH with cold TCPH and grinding with the inerts of formulation for 5 min in a tissue homogenizer to obtain a uniform particle size (specific activity of the dose was 40.7 μCi/mmol). A single oral dose of 50 mg/kg was given by stomach tube to ensure its delivery into the rumen. Daily records of food consumption, excretion, body temperature, and general health were maintained. Urine and feces were collected separately once daily. Cages were washed with water and washes combined with feces. Daily heparinized blood samples (20 ml) were collected via the jugular vein. The sheep were slaughtered at various intervals following treatment. Liver, kidney, lung, spleen, brain, heart, muscle, tongue, fat, bone marrow, bile, and total intestinal contents were collected for residue determination.

Radiotracer Techniques. All samples were counted in triplicate along with blank samples in a Packard Tri-Carb Liquid Scintillation Spectrometer Model 3375 at a counting efficiency of ~80% in low potassium glass vials (Packard Instrument Co.) with Teflon lined caps. The samples were counted long enough to reduce statistical error to less than 2%. Each sample was corrected for

Agricultural Division, The UpJohn Co., Kalamazoo, Michigan 49001.

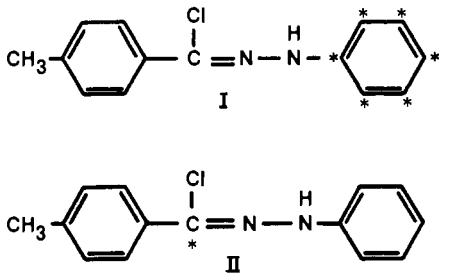

Figure 1. *p*-Toluoyl chloride phenylhydrazone (TCPH), ^{14}C labeled.

background and quenching by determining counting efficiency by using [^{14}C]toluene as an internal standard. Urine, about 1 g, was counted directly in 10 ml of Insta Gel (Packard Instrument Co.). Solvent extracts were transferred into the counting vials and evaporated under nitrogen and counted in 15 ml of Diotol (New England Nuclear). Feces were combined with cage wash and homogenized for 5 min in a Polytron homogenizer. An aliquot of 0.5 to 1.0 g of the slurry was weighed in visking bags, dried overnight, and combusted in Schöniger flasks. The combustion products were absorbed in 10 ml of phenethylamine solution (2 N in methanol) and a 5-ml aliquot was removed, diluted with 10 ml of scintillator solvent (450 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene and 27.7 g of 2,5-diphenyloxazole per gal of toluene), and counted. Aliquots (0.5 g) of blood, plasma, erythrocytes, and other aqueous solutions were processed similar to feces.

A 10-g sample of fresh tissues (except fat and bone marrow) was diluted with 5 vol of water and homogenized for 5 min in a Virtis Macro-45 homogenizer (Virtis Instrument Co.). An aliquot of about 1 g of slurry was combusted and counted as described under feces. Fat and bone marrow (10 g) were first extracted with 50 g of warm hexane. About a 1-g aliquot of hexane was transferred to a counting vial and counted in 15 ml of Diotol. The drug concentration equivalent of TCPH was obtained by dividing disintegrations per minute per gram of fresh tissue by the specific activity of the dose.

Comparative Blood, Plasma, and Erythrocytes ^{14}C from Two Labeled Forms of TCPH. Daily blood samples from two different sheep treated with a single oral dose of TCPH-I and TCPH-II at the rate of 50 mg/kg were analyzed for ^{14}C. The blood was centrifuged at 10 000*g* for 30 min and the plasma aspirated. The weight of plasma and erythrocyte was noted and an aliquot was combusted and counted (Figure 2).

Fractionation of Blood. Blood was fractionated by the method of Krowke et al. (1971) to measure the incorporation of ^{14}C into lipids, proteins, nucleic acids, and polysaccharides.

Preparation of Hemoglobin, Heme, and Globin. Hemoglobin, heme, and globin were prepared by the method of Anson and Mirsky (1930) as modified in Figure 3. An aliquot of each fraction was counted after Schöniger combustion. Concentration of radioactivity was expressed as parts per million of TCPH equivalent based on actual weights of blood, plasma, and erythrocytes and dry weight basis of hemoglobin, globin, and heme.

Electrophoresis of Hemoglobin. The electrophoresis of an aliquot was carried out at 10 °C in a Brinkman continuous flow apparatus (Brinkman Instrument Technical Bulletin No. 11, 1962) with volatile buffers in the separation cell and electrode compartment (0.1 M acetic acid and ammonium hydroxide, pH 8.7). Conditions

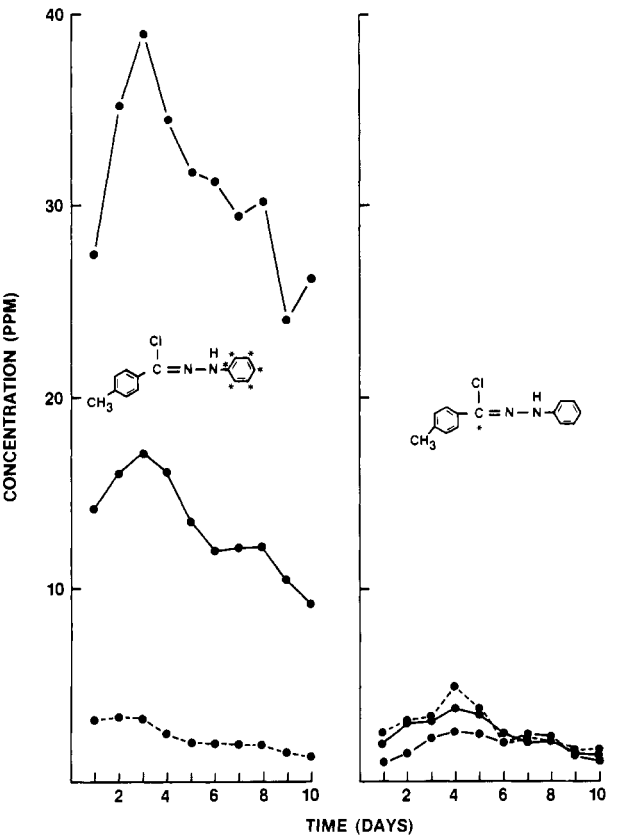

Figure 2. Concentration of ^{14}C in whole blood, plasma, and erythrocytes from treatment of sheep with a single oral dose of TCPH-I and TCPH-II: (•—) erythrocytes; (•—) whole blood; (•- - -) plasma.

of pH, sample application, and flow rate were optimized so that hemoglobin was collected in the middle (tubes 20–28), so that free, unbound, and uncharged molecules were separated in tubes 1–5. Ion exchange membranes (Heptin CRGI at anode and Nepton ARIII at cathode) were used to reduce losses of sample due to migration into the electrode compartment. An aliquot from each tube was analyzed at 280 and 413 nm for uv and visible adsorptivity, respectively, with a Cary-15 uv–visible spectrophotometer and total absorptivity was calculated for each tube. An aliquot of each tube was also counted for radioactivity.

Oxidation of Heme and Globin. The heme filtrate from 25 ml of heparinized blood (Figure 3) was dried on a rotary evaporator (Gosline et al., 1969), the residue dissolved in 80 ml of 25% sulfuric acid (v/v) and cooled to 0 °C, and 25 ml of 10% chromium trioxide was added dropwise with stirring. The solution was then stirred for 16 h at room temperature, and the pH adjusted to 1.5 with 50% sodium hydroxide. The solution was extracted with 200 ml of ether and the ether layer washed with 200 ml of distilled water twice. The ether was dried over anhydrous sodium sulfate and evaporated to dryness and the residue dissolved in 1 ml of methanol and an aliquot counted for ^{14}C radioactivity. The [^{14}C]globin residue was similarly treated.

Thin-Layer Chromatography. A 50-μl aliquot of the ether extract was spotted on a silica gel GF 250 μm (Analtech, Inc.) plate 1.5 cm above the lower edge of the plate and developed in benzene, dioxane, and acetic acid (90:25:4) to 12 cm in saturated glass tanks lined with Whatman paper. Benzoic acid, which was suspected to be the derivative, was chromatographed parallel to the sample. The plate was air dried, visualized under uv at

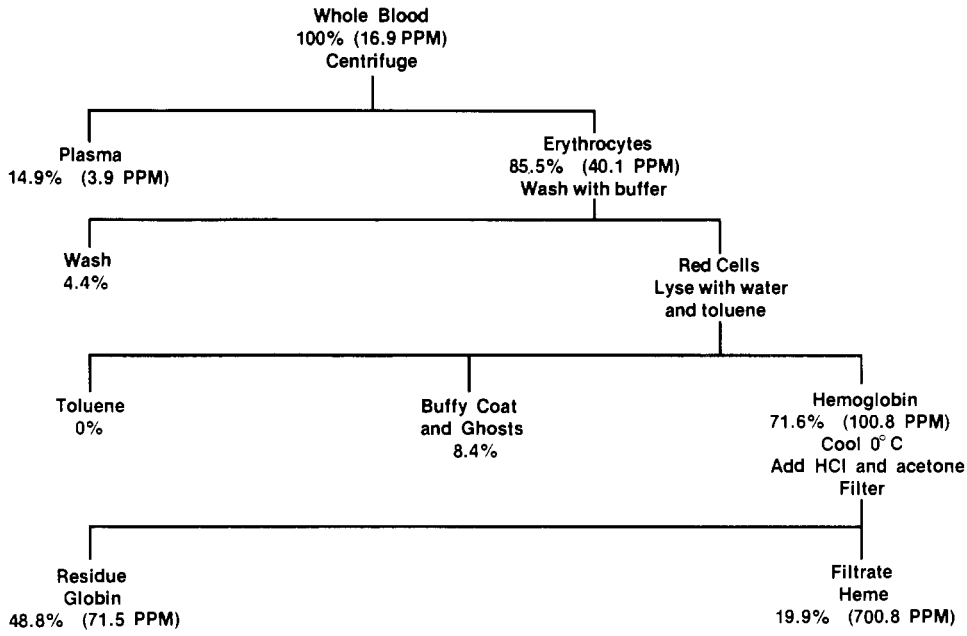

Figure 3. Schematic diagram of fractionation of blood.

Table I. Cumulative Excretion of Radioactivity from Sheep after an Oral Dose of TCPH

Days after treatment	TCPH-I				TCPH-II			
	Urine	Feces	Total excreted	Cumulative excreted	Urine	Feces	Total excreted	Cumulative excreted
1	8.8	21.7	30.5	30.5	5.0	2.1	7.1	7.1
2	4.4	20.3	24.7	55.2	6.1	4.3	10.4	17.5
3	4.1	13.8	17.9	73.1	5.7	13.0	18.7	36.2
4	0.9	15.1	16.0	89.1	4.5	5.7	10.2	46.4
5	0.2	1.7	1.9	91.0	2.3	18.9	21.2	67.6
6	0.1	0.4	0.5	91.5	0.9	17.6	18.5	86.1
7	0.1	0.2	0.3	91.8	0.2	6.2	6.4	92.5
8	0.1	0.2	0.3	92.1	0.1	1.7	1.8	94.3
9	0.0	0.2	0.2	92.3	0.1	0.4	0.5	94.8
10	0.1	0.1	0.2	92.5	0.0	0.5[a]	0.5	95.3
Total	18.8	73.7	92.5		24.9	70.3	95.3	

[a] Includes GI content at slaughter.

254 nm, and divided into 1-cm increments from the bottom to top and silica gel scraped. Each 1-cm increment scraping was counted in 15 ml of Diotol and the percent radioactivity computed.

Derivatization of Ether Extracts. An aliquot of ether extract was derivatized by reaction with diphenyldiazomethane (Fieser and Fieser, 1967). TLC of an aliquot of the derivative was done on silica gel GF 250-μm plates in hexane–ethyl acetate (9:1) as described above and radioactivity in each fraction was determined. A diphenyl methylbenzoate standard prepared by the above procedure was chromatographed parallel to the sample. A second aliquot of the ether extract was methylated with diazomethane by the procedure of Stanley (1966).

GLC–Mass Spectrometry. An LKB Model 9000 gas chromatograph–mass spectrometer was used. The resolution of the instrument was about m/e 1000, the temperature of the ion source was 290 °C, and electron energy was 70 eV. Acceleration voltage was 3.5 kV and the scan limits were 0 to 500; the scan speed used was 6 (m/e 0 to 500 in 16 s) and the uv oscillograph chart speed was 5 cm/s. Effluent from the gas chromatograph was monitored by a total ion current detector. The instrument was equipped with a coiled borosilicate glass column (0.3 cm i.d. and 2.0 m length) which was packed with Gas-Chrom Q 100–120 mesh coated with 3% SE 30. The gas chromatograph was operated isothermally at 50 °C, with flash heater at 210 °C. Helium was used as the carrier gas and the flow rate was 25 ml/min. The recorder was operated with an input signal of 1 mV cm^{-1}.

RESULTS AND DISCUSSION

TCPH was efficacious against gastrointestinal nematodes and cestodes of ovines following a single oral dose of 30–50 mg/kg. A number of experiments were therefore conducted in sheep to study the metabolic disposition, excretion, and retention of radioactivity from a single oral dose of 50 mg/kg. The sheep developed mild diarrhea and anorexia 8–10 h after treatment which lasted for 2–4 days after which the sheep regained their normal health. No ill effects were observed 1 week after treatment.

Excretion of ^{14}C Radioactivity. A single oral dose of 50 mg/kg of TCPH-I or -II cleared the gastrointestinal tract of sheep over a 10-day period following treatment (Table I). The radioactivity observed in the feces was 3–4 times that found in urine. The overall excretion pattern for the two labeled forms was similar. Although only residual levels of radioactivity were observed in the urine and feces 10 days after treatment, it was apparent from the size of the dose and the accountability data that the tissues contained measurable levels of radioactivity.

Distribution of ^{14}C Residues in Tissue at Slaughter. Examination of a variety of tissues taken at selected intervals after treatment with TCPH-I indicated that the

Table II. Concentration of ^{14}C (Expressed as Parts per Million of TCPH Equivalent) in Sheep Tissues after an Oral Dose of [^{14}C]TCPI-I or -II

Tissues	TCPH-I at days after treatment						TCPH-II, 10 days after treatment
	3	5	7	10	14	21	
Blood	15.7	14.3	11.9	9.3	10.1	5.3	1.3
Liver	13.1	8.6	6.1	3.9	5.0	2.8	2.3
Kidney	6.1	4.6	3.7	3.0	2.6	1.7	1.2
Lung	4.2	2.9	2.4	2.0	1.8	2.3	0.6
Spleen	3.8	5.0	4.7	2.2	4.1	1.9	<0.5
Heart	1.8	1.4	1.4	0.9	0.7	0.5	<0.5
Tongue	1.8	0.9	0.8	0.5	0.6	<0.5	<0.5
Brain	0.5	<0.5	0.7	0.5	<0.5	<0.5	<0.5
Muscle	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Fat	2.1	0.9	0.8	0.6	<0.5	<0.5	0.7
Bone marrow	2.0	1.0	1.2	<0.5	<0.5	<0.5	<0.5
Bile	6.6	1.2	0.5	<0.5	<0.5	<0.5	1.0

highest residues were present in blood and blood rich organs such as liver, lung, kidney, and spleen (Table II). The data showed a relatively slow depletion of radioactivity from these tissues with time. Comparison of the residues from a TCPH-I treated sheep with a TCPH-II treated one (slaughtered at 10 days post-treatment) showed a distinct difference in residue levels, particularly in the blood, 9.3 ppm for TCPH-I and 1.3 ppm for TCPH-II.

Comparative Blood, Plasma, and Erythrocyte ^{14}C Residues following Treatment with TCPH-I or -II. The comparative ^{14}C levels expressed as parts per million of TCPH equivalent in whole blood, plasma, and erythrocytes plotted as the parts per million vs. time (Figure 2) for both labels emphasized the difference in residues due to the position in which the ^{14}C label is placed. Although plasma levels were about equal from both labels, TCPH-I residues were much higher in erythrocytes. With the passage of time, ^{14}C declined uniformly in both plasma and erythrocytes from the TCPH-II treated animal and reached about 1 ppm. On the other hand, ^{14}C levels in erythrocytes of the TCPH-I treated animal were 15 times higher than in the TCPH-II treated animal and radioactivity persisted much longer. These data suggested that molecular cleavage of TCPH had taken place and only the phenylhydrazine portion of the molecule was responsible for the high blood residues.

Nature of ^{14}C Residues in the Erythrocyte from TCPH-I Treated Sheep. Preliminary work showed that very little radioactivity was extracted from blood by ethyl acetate. TLC of these extracts on a silica gel plate developed in hexane–acetone (7:3) showed that radioactivity was separated into several spots. There was no radioactivity corresponding to TCPH. However, some of the radioactivity ran parallel to TAPH, but no effort was made to characterize this further because it constituted only 0.4% of the total blood residues.

Fractionation of blood by the procedure of Krowke et al. (1971) showed that the major part of the radioactivity was associated with proteins (66%). The rest of the radioactivity was diffused into several components, viz., RNA, 4%; carbohydrates, 6%; glucosaminglycon, 3%; and lipids, 19%.

Distribution of radioactivity from the TCPH-I treatment into various blood fractions (Figure 3) showed the concentration of the radioactivity in erythrocytes was ten times that of plasma. Both heme and globin were labeled. Although 49% of blood radioactivity was present in the globin as compared to 20% in heme, the concentration of radioactivity was 700 ppm relative to heme in contrast to 72 ppm in globin.

Nature of ^{14}C Activity in Hemoglobin. No more than 10–15% ^{14}C activity could be extracted from hemoglobin with organic solvents under different pH conditions.

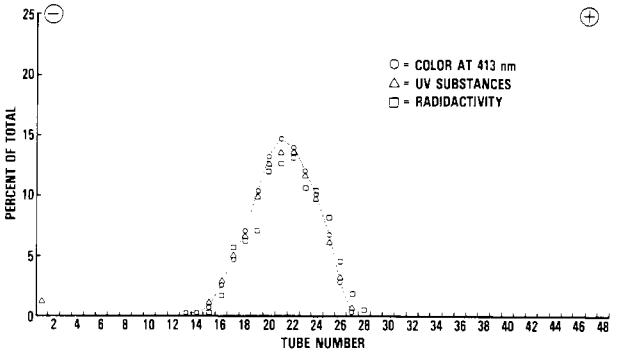

Figure 4. Electrophoresis of sheep hemoglobin treated with a single oral dose of TCPH-I.

Organic solvents with a high dielectric constant like formamide are good protein solvents (Mahler and Cordes, 1966) and extracted more ^{14}C than other solvents. The radioactivity in hemoglobin was not dialyzable. It eluted with hemoglobin from Sephadex G-25. Electrophoresis data (Figure 4) indicated that the radioactivity was associated with hemoglobin for all the parameters measured, viz., color and uv absorption matched with the radioactivity.

Characterization of the Bound Radioactivity in Globin and Heme. After hydrolysis of globin with Pronase, trypsin, or pepsin, the radioactivity remained associated with the aqueous phase and very little radioactivity partitioned into hexane, methylene chloride, and ether. Hydrolysis in 6 N HCl (Moore and Stein, 1963), however, generated ~20% chloroform extractable radioactivity. Although concentration of radioactivity in the globin was low making characterization difficult, TLC of the hydrolyzed globin produced a number of compounds with TLC characteristics similar to known aromatic amino acids. These observations suggested covalent binding of the TCPH-I phenyl group to amino acids.

Chromate oxidation of heme generated a radioactive fragment of the metabolite, most of which was extractable into ether at pH 2. All of the radioactivity extracted into ether had an R_f on silica gel TLC identical with authentic benzoic acid. Derivatization of the ether extractable radioactive compounds with diphenyldiazomethane and silica gel TLC showed that the derivative had the same R_f as authentic diphenyl methylbenzoate (Figure 5). The identity of benzoic acid was further confirmed by esterification of an aliquot with diazomethane. GLC–mass spectrometry of the ester had the same retention time and fragmentation pattern as methylbenzoate, viz., m/e 136 (molecular ion) m/e 105 M – 31 (loss of CH_3O), m/e 77 M – 59 (loss of $CH_3OC{=}O$). Identification of radioactive benzoic acid demonstrated that the phenyl group was

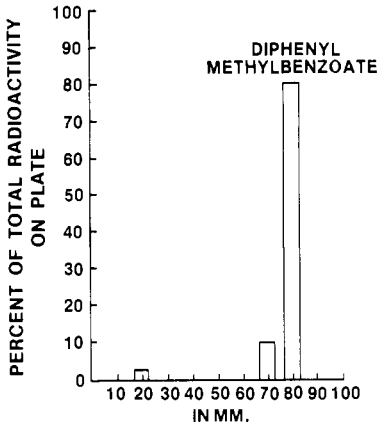

Figure 5. TLC of heme oxidation products after derivatization with diphenyldiazomethane.

derived from TCPH whereas the carboxyl group came from the heme fraction. The above data suggested that radioactive benzoic acid should be generated also from oxidation of globin. Indeed, [^{14}C]benzoic acid was produced. However, specific activity was about 1.5% of the theory. Globin has endogenous precursors such as phenylalanine, which were also oxidized to benzoic acid and consequently diluted the resulting [^{14}C]benzoic acid. It is possible that reaction of the phenyl group might occur with the sulfhydryl and/or amino group of amino acids. Oxidation of these bound groups would not yield benzoic acid.

CONCLUSIONS

Factors that affect covalent binding of drugs to macromolecules have recently been reviewed (Gillette et al., 1974). Preferential uptake of radioactivity by the erythrocytes and its covalent binding to the hemoglobin from the TCPH treatment were not unique, however. Aromatic sulfonamides have a very high affinity for erythrocytes and selectively inhibit carbonic anhydrase (Maren et al., 1960), whereas aromatic amines, especially aniline and its metabolite phenylhydroxylamine (Brodie and Axelrod, 1948), bound specifically to heme in the red cells to produce methemoglobinemia (Crick and Jackson, 1953; McLean et al., 1969). McIsaac et al. (1958) reported 10% of the dose of [^{14}C]phenylhydrazine was present in rabbit erythrocytes from an oral dose of 50 mg/kg; however, the nature of the binding was not investigated and the authors assumed that it probably was associated with red cell constituents as a kind of phenylhydrazone.

In this study, the nature of binding by TCPH in the red cell was found to be different from that of aromatic sulfonamides. When a hemoglobin free extract of red cells was prepared according to Meldrum and Roughton (1934), who used the method to extract and concentrate carbonic anhydrase, only 3% of the blood radioactivity was found in this fraction. Most of the radioactivity was bound to the hemoglobin and not to carbonic anhydrase or other proteins as judged by electrophoresis.

TCPH was easily hydrolyzed to TAPH which could be hydrolyzed further to phenylhydrazine and *p*-toluic acid or to aniline and *p*-toluamide. Preferential uptake of radioactivity from TCPH-I and not from TCPH-II showed that either phenylhydrazine or some other intermediate was binding to the erythrocytes. Although aniline and phenylhydroxylamine are classical compounds which cause methemoglobinemia (Crick and Jackson, 1953; McLean et al., 1969), no methemoglobinemia was observed from TCPH treatment even from a dose of 197 mg/kg. An equivalent dose of aniline (75 mg/kg) and phenylhydrazine (87 mg/kg) produced extensive methemoglobinemia (Glenn, 1972).

Whether metabolism of TCPH and phenylhydrazine was similar in the erythrocytes is conjectural at this time, but identification of radioactive benzoic acid from oxidation of heme or globin was significant, which showed that the phenyl group of benzoic acid was derived from TCPH and the carboxyl group from heme or globin and further supported the covalent nature of binding of TCPH metabolite to hemoglobin. It further demonstrated that the two nitrogen atoms have been metabolized away from the TCPH; their fate is unknown at this time.

The problem of determining the specific nature of the bound residue can be realized by calculating the molar ratio of ^{14}C residue to hemoglobin and amino acids. Knowing that 5.3 ppm of TCPH equivalent residue in blood was equal to 1.7 ppm of phenyl radical at withdrawal after 3 weeks, and that blood contained 12.4% hemoglobin and the molecular weight of hemoglobin was 65 000 and 71.6% of blood radioactivity was present in the hemoglobin, it could be shown that only 1 out of 120 hemoglobin molecules contained residue. Knowing that 576 amino acids were present in each molecule of hemoglobin, one could calculate that there was only 1 mol of residue per 69 000 mol of amino acids which made their characterization difficult.

The metabolism of TCPH-I in blood probably occurred as postulated in Figure 6. TCPH or TAPH or some metabolite in the presence of oxygen and hemoglobin gave rise to the phenyl radical, which interacted with hemoglobin. The fractionation of hemoglobin into heme and globin and their oxidation gave rise to radioactive benzoic acid. Beaven and White (1954) have also demonstrated that phenylhydrazine could be degraded in vitro by ox-

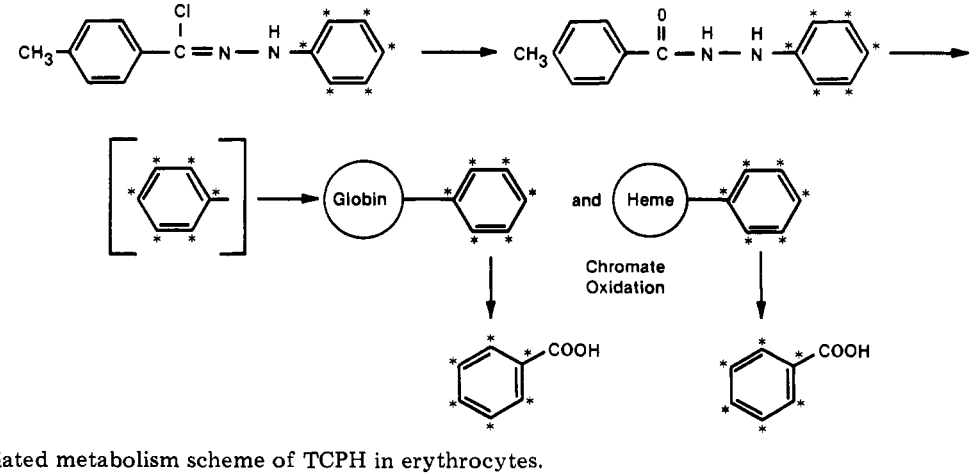

Figure 6. Postulated metabolism scheme of TCPH in erythrocytes.

yhemoglobin to benzene and molecular nitrogen which also supports the above conclusion.

ACKNOWLEDGMENT

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Comparison of Extracted Peel Oil Composition and Juice Flavor for Rough Lemon, Persian Lime, and a Lemon–Lime Cross

Philip E. Shaw* and Charles W. Wilson, III

A hybrid (lem'n lime) from rough lemon and Persian lime fruit was evaluated. Composition of its peel oil extract was compared with that of each parent. Flavor of hybrid juice was compared with flavors of good quality lemon and lime juices. Lem'n lime peel oil extract differed from that of either parent, primarily by the presence of thymol and thymol methyl ether and the absence of neral and geranial. These differences might explain its strong, non-citrus-type aroma. A taste panel judged the juice as lemon–lime in character and mild in flavor. Thus, this lemon–lime cross will probably be an acceptable alternative to lemon–lime mixtures only when mild flavoring properties are desired.

Lemon and lime essential oils bring a premium price in flavor markets because of their desirable flavoring properties in many products such as juice drinks and carbonated beverages. A hybrid from a cross between lemon and lime with essential oil and juice having combined flavoring properties of lemon and lime would be a potentially valuable new commodity.

A lemon–lime hybrid of *Citrus jambhiri* Lush. × *Citrus latifolia* Tanaka C.V. Persian (rough lemon and Persian lime) has been developed recently and designated "lem'n lime" (Lucerne, 1973). One reason for selecting the rough lemon parent was to develop a hybrid with mild flavoring properties (Lucerne, 1975). A comprehensive analytical and flavor study of the peel oil and juice from this hybrid was needed to help assess its potential. No systematic study has been carried out comparing the composition of peel oil from a citrus hybrid with composition of oils from those two parents. For many citrus hybrids, however, composition has been systematically compared between juice sac lipids of hybrids and of parents (Nagy and Nordby, 1972, 1974; Nordby and Nagy, 1974a,b). Tatum et al. (1974) compared flavonoids and coumarins in leaves between citrus hybrids and the parent plants. In those studies, hybrids generally showed lipid and flavonoid–coumarin compositions intermediate between the two parents, although some exceptions were noted.

We have evaluated a hybrid of rough lemon and Persian lime. We compared composition of a hexane extract of its peel oil with that for each parent and flavor of its juice with flavor of good quality lemon and lime juices.

Citrus and Subtropical Products Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Southern Region, Winter Haven, Florida 33880.

EXPERIMENTAL SECTION

Samples of lem'n lime and Persian lime fruit were obtained from the Citcross Corporation, Homestead, Fla. Rough lemon fruit was obtained from Mr. Don Bridges, Florida State Department of Agriculture, Winter Haven, Fla. Commercial frozen concentrated lemonade was purchased from a local market.

Peel Oil Extraction. Whole fruit (2.3 kg each of rough lemon, lem'n lime, and Persian lime) was grated on a kitchen grater to remove the flavedo (outer portion of the peel) which was immediately placed in a beaker and covered with hexane. The flavedo–solvent mixture was filtered through a Buchner funnel and the filtrate concentrated under reduced pressure at 45 °C to remove the bulk of the solvent. Residual oils were stored at 5 °C. Oil yield was about 2.2 ml/kg of fruit.

Analytical Procedures. Oils were qualitatively analzyed with a Varian Model 1400 gas–liquid chromatograph (GLC) coupled to a DuPont Model 21-490 mass spectrometer (MS) operated at 70 eV with a source temper-