Sesame (*Sesamum indicum*) Carbohydrates

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White and black sesame (processed, unprocessed, and dehulled seeds) have been analyzed for the total carbohydrate composition. Sesame contained D-glucose (3.63%), D-galactose (0.40%), D-fructose (3.43%), sucrose (0.17%), raffinose (0.59%), stachyose (0.38%), planteose (0.23%), and sesamose (0.14%). In addition, penta- and hexasaccharides (0.12 and 0.04%, respectively) obtained were shown to be the higher homologues of the planteose series. The 70% alcohol-insoluble residue on alkali extraction furnished hemicellulose A and hemicellulose B in 0.58–2.34 and 2.71–2.59% yields, respectively. Hemicellulose A was found to be an acidic glucan containing galacturonic acid and glucose in a ratio of 1:12.9, while hemicellulose B contained galacturonic acid, glucose, arabinose, and xylose with trace galactose in a ratio of 1:3.8:3.8:3.1, respectively. The alkali-insoluble residue contained only glucose and probably represents the associated cellulose fraction.

Sesame (*Sesamum indicum*) is one of the chief sources of vegetable oils in India. Even though many varieties are known, only two commercial varieties, white and black, are cultivated in India on a large scale. The chemical composition of sesame has been the subject of investigation for many years. There are some reports on the nature of free sugars occurring in sesame (Hatanaka, 1959; Gloria and Gustavo, 1969), but the polysaccharides have not been investigated.

The objectives of the present study were to isolate, identify, and quantitate the simple sugars and constituent monosaccharides in polysaccharides of sesame seeds. The ethanol-soluble mono- and oligosaccharides and acid hydrolysates of polysaccharides were fractionated and characterized by paper, thin-layer, and gas–liquid chromatography.

EXPERIMENTAL SECTION

Three batches of sesame (white and black, varieties Tankar and Chowk) were purchased in the local market during November 1974 and January–February 1975, respectively. Dehulled (by alkali treatment at 96 °C for 1 min) seeds (Shamanthaka Sastry et al., 1969) and processed (dehulled, expeller pressed, and solvent extracted) sesame cake were those available in the Institute.

Locally purchased seeds were ground and defatted with chloroform–*n*-hexane (2:1) at a temperature below 70 °C. The defatted materials were ground to 60 mesh and extracted with 70% alcohol (v/v, 10 g/100 ml) until the phenolsulfuric acid reaction was negative (three overnight extractions with shaking were required) and centrifuged to remove the insoluble residue. The alcohol extracts were pooled and (after dilution to 50% alcohol concentration) passed through ion exchange resins [(Dowex-1 (Cl^-) and Dowex-50 (H^+)] to remove amino acids and minerals. The extracts were then evaporated to dryness under vacuum at 40 °C.

The dried material was redissolved in distilled water and the solution was placed on a charcoal–Celite column [2:1 by weight, 31.0 × 4.0 cm (Whistler and Durso, 1950)]. The column was eluted successively with water and aqueous alcohol (increasing from 2 to 25%). Pure fractions were isolated by rechromatography on filter sheets and were characterized by chromatographic (paper, thin-layer, and gas–liquid chromatography) comparison, high-voltage electrophoresis at different pH values, 9.2 and 10.0, by optical rotation measurements, by partial hydrolysis (0.5 N H_2SO_4, 80 °C for 4 h), and also by identifying the cleavage products on enzymic hydrolysis.

α-Galactosidase (contaminated with invertase) was extracted from *Agave vera cruz*; invertase was commercially purchased (BDH, Poole, England).

The alcohol-insoluble residues were extracted with sodium phosphate buffer (0.02 M; pH 7.5) containing 1 M sodium chloride solution. The residues were then subjected to proteolysis with Pronase (Kaken Chemical Co., Ltd., Japan) in phosphate buffer (pH 7.5). After centrifugation the residues were extracted with 10% carbonate-free NaOH solution under nitrogen atmosphere (Whistler and Feather, 1965). Hemicellulose A was precipitated from the alkali extract by adjusting the pH to 5 with dilute acetic acid, and collected by centrifugation. Addition of ethanol (3 vol) to the clear centrifugate gave hemicellulose B.

The flour, residues, and hemicelluloses were hydrolyzed into their constituent sugars with 7% H_2SO_4 at 100 °C for 6 h. The hydrolysates were neutralized to pH 6.5 with solid $BaCO_3$, passed through Dowex-50 (H^+), and evaporated to dryness under vacuum at 40 °C. The dried materials were redissolved in water for further analyses by paper and gas–liquid chromatography. Nitrogen in the flour and residues was determined by the micro-Kjeldahl procedure (AOAC, 1970).

Preliminary identification of sugars in the ethanol-soluble extracts and hydrolysates was obtained by descending paper chromatography using 1-propanol–ethanol–water (7:1:2) and butanol–acetic–water (4:1:5, organic phase) as the solvent systems. Thin-layer chromatograms were run using butanol–ethanol–water (2:1:1) and ethyl acetate–isopropyl alcohol–water (11:4:2) solvent systems. High voltage electrophoresis was done in borate buffers at pH 9.2 and 10.0. The visualization of sugars on chromatograms and electropherograms was achieved with *p*-anisidine hydrochloride (Hough et al., 1950), alkaline $AgNO_3$ (Partridge, 1948), and urea–HCl (Dedonder, 1952) spray reagents. Total sugar, pentose, and uronic acid contents were estimated by the standard methods. Unless otherwise stated optical rotations were observed for water solutions at ca. 26 °C.

Final identification of sugars in hydrolyzed and non-hydrolyzed carbohydrate fractions was achieved by GLC

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Table I. Composition[a] of the Alcohol-Soluble Solids of Defatted Sesame Flours and the Content of Mono-, Di-, and Oligosaccharides[b]

Composition		Unprocessed flour 1	Unprocessed flour 2	Processed flour 2[c]	Dehulled flour 1	Dehulled flour 2
Yield of alcohol-soluble solids, g		11.26 ± 0.28	10.36 ± 0.27	7.70 ± 0.19	9.20 ± 0.18	9.20 ± 0.18
Protein (N × 6.25)		1.89 ± 0.10	2.21 ± 0.12	3.06 ± 0.11	3.50 ± 0.12	2.20 ± 0.12
Moisture		0.30 ± 0.006	0.36 ± 0.006	3.01 ± 0.006	0.40 ± 0.005	0.30 ± 0.004
Ash		0.10 ± 0.00	0.10 ± 0.00	0.40 ± 0.00	0.30 ± 0.00	0.10 ± 0.00
Total sugar[d]		96.10 ± 2.12	95.70 ± 1.78	89.00 ± 1.13	92.00 ± 1.12	96.00 ± 1.09
Sugars detected[e]	Glc	3.60 ± 0.22	3.66 ± 0.12	2.53 ± 0.35	3.13 ± 0.31	3.35 ± 0.30
	Gal	0.44 ± 0.04	0.36 ± 0.02	0.01 ± 0.00	0.04 ± 0.00	0.07 ± 0.00
	Fru	3.49 ± 0.33	3.38 ± 0.15	1.98 ± 0.09	2.47 ± 0.08	2.79 ± 0.10
	Suc	0.20 ± 0.01	0.14 ± 0.02	0.15 ± 0.00	0.15 ± 0.01	0.19 ± 0.00
	Raf	0.65 ± 0.06	0.54 ± 0.04	0.22 ± 0.03	0.21 ± 0.02	0.27 ± 0.02
	Sta	0.43 ± 0.02	0.34 ± 0.03	0.20 ± 0.01	0.17 ± 0.01	0.29 ± 0.01
	Pla	0.27 ± 0.01	0.19 ± 0.01	0.54 ± 0.00	0.55 ± 0.05	0.62 ± 0.05
	Ses	0.13 ± 0.00	0.15 ± 0.01	0.37 ± 0.01	0.38 ± 0.03	0.39 ± 0.02
	Pen	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.15 ± 0.01	0.16 ± 0.00
	Hex	0.04 ± 0.00	0.05 ± 0.00	0.09 ± 0.01	0.08 ± 0.00	0.09 ± 0.00

[a] Composition on a moisture-free basis expressed as percentages of defatted sesame flour. (Each value represents the mean of three determinations on a dry basis and the standard deviation from the mean.) [b] The values in columns 1 and 2 refer to white and black sesame, respectively. [c] The material from white sesame was not available. [d] Percentage of sugars in the alcohol extract. [e] Computed from the weights of the fractionated sugars. Abbreviations used are: Glc, glucose; Gal, galactose; Fru, fructose; Suc, sucrose; Raf, raffinose; Sta, stachyose; Pla, planteose; Ses, sesamose; Pen, pentasaccharide; Hex, hexasaccharide.

in which the relative retention times of the Me_4Si derivatives of sesame sugars were compared with those of authentic sugars and by cochromatography with Me_4Si derivatives of authentic sugars. The monosaccharides were silylated at room temperature by shaking with 1 ml of Tri-Sil (Pierce), whereas the oligosaccharides required incubation at 65 °C for 1 h before injection into the gas chromatograph. The Me_4Si derivatives were chromatographed on a Varian Aerograph series 1400 gas chromatograph equipped with a flame ionization detector and a stainless steel column (5 ft × $^1/_8$ in. o.d.) packed with 3% OV-1 on 80–100 mesh Chromosorb W (HP) (Pierce). The flow rate for the carrier gas, N_2, was 30 ml/min and the sample injected was 2 μl. The detector and injection port temperatures were maintained at 300 °C and the oven was operated at a combination of isothermal–programmed–isothermal temperatures as shown in Figures 1a and 1b. Inositol was used as the internal standard.

RESULTS AND DISCUSSION

Three different batches of sesame (white and black, as well as dehulled and processed materials) were used in the present study. In the first experiment, the defatted materials were extracted with 70% alcohol. The total amount of solids in the ethanol extracts as well as its composition are presented in Table I. By preparative chromatography on a charcoal–Celite column individual sugars were isolated and characterized by a combination of PC, TLC, and GLC. It should at the very outset be stated that the seeds of the two types and pressed cake were not grown under similar conditions and hence the results may not be comparable. However, the results represent analytical values in triplicates. Thus, on an average the total monosaccharides constituted 7.41 and 7.82% (for white and black sesame, respectively) of dry weight of the flour and were identified as D-glucose, D-galactose, and D-fructose, while the total oligosaccharides–sucrose, raffinose, stachyose, planteose, and sesamose along with two higher oligosaccharides–represented only 1.84 and 1.57% of the flour solids. The higher oligosaccharides were found to be penta- and hexasaccharide by judging from their chromatographic mobilities. Although the sugar pattern was the same for both white and black sesame considerable variations were observed in their individual proportions (Figures 1a and 1b). As Kinman and Stark (1954) have reported both variety and location of growth appear to exert marked influence on yield and composition.

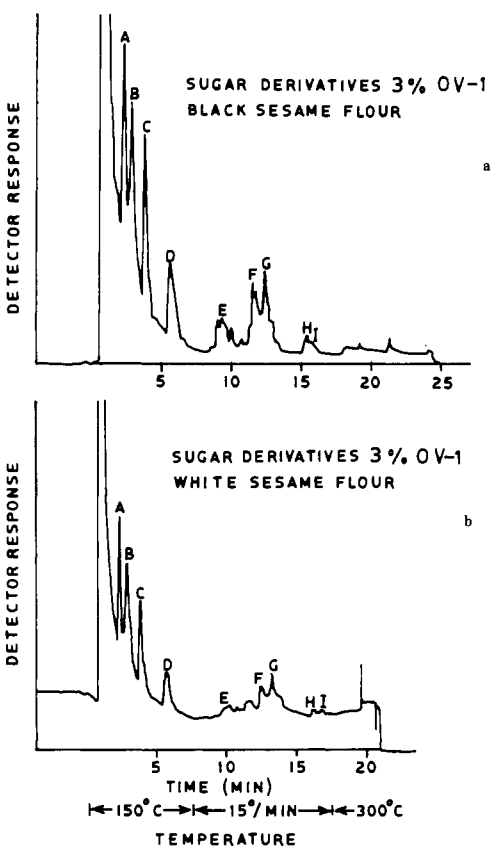

Figure 1. Chromatograms of Me_4Si derivatives of sugars in ethanol extracts from black sesame (a) and white sesame (b) flours. The peak identities are: (A) fructose; (B) galactose; (C) and (D) glucose; (E) sucrose; (F) raffinose; (G) planteose; (H) stachyose; (I) sesamose.

The extracts from dehulled as well as processed sesame also had the same sugar composition. Galactose was, however, noticed in trace amounts and also a decrease in the proportion of individual sugars was observed. These

Table II. Composition[a] of 70% Alcohol-Insoluble Residue from Sesame Flour

Composition		Unprocessed flour 1	Unprocessed flour 2	Processed flour 2[b]	Dehulled flour 1	Dehulled flour 2
Yield (dry solids)		89.7 ± 2.38	88.9 ± 2.48	92.0 ± 2.13	90.6 ± 2.04	90.0 ± 1.82
Protein (N × 6.25)		61.7 ± 1.82	60.8 ± 1.62	58.9 ± 1.28	60.1 ± 1.12	58.9 ± 1.20
Moisture		5.9 ± 0.06	5.9 ± 0.07	6.7 ± 0.06	6.4 ± 0.07	6.7 ± 0.07
Ash		6.6 ± 0.01	6.9 ± 0.01	6.0 ± 0.00	6.0 ± 0.01	6.6 ± 0.01
Total sugar		23.0 ± 0.82	22.0 ± 0.78	24.0 ± 0.79	22.4 ± 0.80	23.0 ± 0.83
Sugars detected[c]	GalA	1.9 ± 0.12	1.8 ± 0.10	1.8 ± 0.09	1.8 ± 0.10	1.6 ± 0.09
	Glc	10.6 ± 0.21	10.1 ± 0.31	10.4 ± 0.21	9.9 ± 0.20	9.2 ± 0.30
	Ara	8.1 ± 0.00	8.6 ± 0.01	7.6 ± 0.00	6.2 ± 0.08	6.8 ± 0.06
	Xyl	1.6 ± 0.00	1.4 ± 0.00	1.7 ± 0.00	1.8 ± 0.00	1.6 ± 0.00

[a,b] See Table I. [c] Abbreviations used are: GalA, galacturonic acid; Glc, glucose; Ara, arabinose; Xyl, xylose.

variations may be due to the complete or partial destruction of sugars during processing (because of amino acid–carbohydrate interaction). Since dehulling of the seeds also involved mild heat treatment (1% alkali at 96 °C for 1 min) a decrease in sugar content was noticed in the respective extracts.

It is of interest to note the marked variation in the sugar composition of sesame with that reported earlier. According to Hasegawa et al. (1954) sucrose and an unknown trisaccharide other than raffinose are the only free sugars present in sesame. Hatanaka (1959) reports the occurrence of glucose (trace), sucrose (1%), trisaccharide (0.5%), tetrasaccharide (0.1%), and a trace of pentasaccharide. The possible occurrence of raffinose has also been speculated. According to Gloria and Gustavo (1969) glucose (2.6 and 1.55%), galactose (1.1 and 0.65%), fructose (0.42 and 0.24%), saccharose (0.57 and 0.34%), and planteose (0.1 and 0.06%) in addition to raffinose (trace) are the alcohol extractable sugars from two varieties of sesame, viz. Trockenen Prepluchen and Trochenen samen, respectively. These results are quite contrary to those reported in the present paper, wherein glucose and fructose (3.6 and 3.4%, respectively) are present in high proportions. The results also indicate marked differences in the relative proportion of other sugars. However, it is not always clear how far the products of different workers are comparable for, as Aspinall and Meek (1956) have pointed out, the composition may be seriously affected by the origin of the sample, method of preparation, etc., and it is often uncertain to what extent particular products are mixtures. Mikolajczak et al. (1970) reported that sunflower meal contained 9.7% of di- and oligosaccharides, mainly sucrose, trehalose, and raffinose; on the other hand, Sabir et al. (1975) reported that mono- and oligosaccharides in the ethanol extract of sunflower flour and head constituted 9.8 and 6.2%, respectively, of the flour solids. Tharanathan et al. (1975) have reported about 14.0% of sucrose in the alcoholic extract of groundnut. Cotton seed is reported (Altschul, 1958) to contain 10% of extractable sugars of which raffinose amounts to the extent of 4 to 9%. However, sucrose was reported to be the principal sugar in all cases, which is quite contrary to the results reported here.

Partial hydrolysis of the planteose fraction, $[\alpha]_D$ +129.6° (c 5), yielded glucose, a ketose disaccharide, and a trace amount of unhydrolyzed oligosaccharide. The disaccharide on complete hydrolysis (1 N H_2SO_4, 100 °C for 4 h) revealed galactose and fructose in the ratio of 1:1 and thus was found to be planteobiose. Under these conditions the sesamose fraction, $[\alpha]_D$ +155° (c 4), was cleaved exclusively to glucose and di- and trisaccharide which had the same chromatographic mobility as that of planteobiose and planteose, respectively. Complete hydrolysis of these oligomers furnished glucose, galactose, and fructose in equal proportions. The pentasaccharide, $[\alpha]_D$ +176° (c 2.5), on similar hydrolysis was cleaved to planteobiose, glucose, galactose, and fructose, while the hexasaccharide, $[\alpha]_D$ +186.6° (c 1.5), gave an oligosaccharide together with galactose, glucose, and fructose in equal concentrations. The oligosaccharide had the same mobility as that of planteose and on further hydrolysis gave sucrose and galactose. Thus, the penta- and hexasaccharides may be the higher homologues of the planteose series.

Further confirmation for the presence of planteose and sesamose was obtained by identifying the cleavage products formed by treatment with α-galactosidase. Thus, planteose on α-galactosidase action gave galactose and sucrose along with unhydrolyzed planteose, while similar treatment with sesamose revealed galactose and planteose together with a trace amount of sucrose. Faint spots due to glucose and fructose were also noticed on the chromatograms.

It is very interesting to note the occurrence of planteose together with raffinose and stachyose. These oligomers have been reported to occur together in some plant materials (Cerbulis, 1955). Hence it was necessary to determine the existence of these oligomers by enzymatic assay. Incubation of a concentrated solution of the extract with invertase in acetate buffer (pH 5.6 at 37 °C for 12 h) revealed the presence of melibiose and a faint spot due to manninotriose.

In the second experiment, the alcohol-insoluble residues were used for the extraction of hemicelluloses. This residue on complete acid hydrolysis (Tharanathan and Anjaneyalu, 1972) revealed galacturonic acid, glucose, arabinose, and xylose with trace galactose (Table II). As reported earlier (Tharanathan et al., 1975) the removal of protein from the residue was found to be beneficial for the complete extraction of the polysaccharides. Like soybean (Altschul, 1958) and sunflower (Goldovskii and Bozhenko, 1933), sesame contained no starch, as revealed by negative iodine color reaction. Furthermore, digestion with amyloglucosidase released no reducing sugar and perchloric extraction (Hassid and Neufeld, 1964) was also found to be negative. However, Bally (1932) has reported that the seed cells contain starch. Much of the protein in the residues was extracted by treatment (twice) with phosphate buffer (pH 7.5) containing 1 M sodium chloride solution, followed by proteolysis with Pronase. The resulting residue still contained 9 and 13% (for white and black sesame, respectively) protein. The yield of the residue was around 47–48% of the starting material. Extraction of the residue with NaOH solution under a nitrogen atmosphere (Whistler and Feather, 1965) gave the two hemicelluloses A and B in 0.58 and 2.34 and 2.71 and 2.59% yields, for white and black sesame, respectively. The yield of the alkali-insoluble residue amounted to 40% of alcohol-extracted residue (Table III).

Hemicellulose A, from both sources, was a fibrous material insoluble in water, and was found to be an acidic

Table III. Composition[a] of Residues and Hemicelluloses

Composition	Residue left after protein extraction at pH 7.5		Pronase-treated residue		Hemicellulose A		Hemicellulose B		Alkali-insoluble residue	
	1	2	1	2	1	2	1	2	1	2
Yield (dry solids)	50.3 ± 2.11	54.4 ± 2.23	48.0 ± 1.94	48.0 ± 1.89	0.6 ± 0.00	2.3 ± 0.12	2.0 ± 0.00	2.6 ± 0.00	44.4 ± 1.38	41.0 ± 1.34
Protein (N × 6.25)	12.1 ± 0.55	17.6 ± 0.48	9.0 ± 0.40	13.5 ± 0.42	8.6 ± 0.11	7.4 ± 0.18	2.1 ± 0.00	4.2 ± 0.09	1.9 ± 0.12	6.6 ± 0.10
Moisture	5.2 ± 0.50	5.0 ± 0.41	5.2 ± 0.43	5.3 ± 0.08	6.1 ± 0.09	5.0 ± 0.09	3.0 ± 0.06	3.3 ± 0.06	5.1 ± 0.19	5.3 ± 0.00
Ash	8.9 ± 0.51	9.2 ± 0.54	8.6 ± 0.50	8.7 ± 0.53	9.3 ± 0.28	8.8 ± 0.11	2.1 ± 0.08	2.2 ± 0.00	7.1 ± 0.23	9.1 ± 0.64
Total sugar	72.6 ± 3.18	63.3 ± 2.45	74.6 ± 2.29	68.3 ± 2.16	71.0 ± 2.11	75.2 ± 2.18	89.8 ± 3.09	56.7 ± 1.21	81.2 ± 3.12	76.6 ± 2.48
Pentose	28.3 ± 0.67	26.0 ± 0.58	31.0 ± 0.62	30.3 ± 0.55	11.3 ± 0.11	11.6 ± 0.34	53.8 ± 1.42	52.7 ± 0.89	4.7 ± 0.21	5.7 ± 0.26
Uronic acid	10.7 ± 0.59	10.4 ± 0.61	12.7 ± 0.58	12.1 ± 0.49	3.7 ± 0.05	2.7 ± 0.08	11.6 ± 0.51	10.7 ± 0.38	6.0 ± 0.21	6.3 ± 0.39
Sugars detected	GalA 5.2 ± 0.27	4.1 ± 0.38	5.8 ± 0.43	5.1 ± 0.40	5.1 ± 0.48	6.4 ± 0.58	7.6 ± 0.71	6.2 ± 0.60	Glc	Glc
	Glc 30.6 ± 0.68	27.1 ± 0.58	32.0 ± 0.69	30.2 ± 0.74	65.8 ± 1.10	69.1 ± 1.09	28.8 ± 0.72	27.2 ± 0.74		
	Ara 22.2 ± 0.51	20.7 ± 0.49	23.1 ± 0.54	21.7 ± 0.58			29.3 ± 0.81	25.2 ± 0.76		
	Xyl 16.1 ± 0.72	9.2 ± 0.61	11.8 ± 0.83	10.0 ± 0.88			24.2 ± 0.69	24.9 ± 0.74		

[a] See Tables I and II.

glucan as it contained galacturonic acid and glucose. On the other hand hemicellulose B from black sesame was easily soluble in water and consisted of galacturonic acid, glucose arabinose, and xylose with trace galactose, while that from white sesame was only partially soluble in water. The principal sugars in the water-soluble fraction were galacturonic acid, glucose, galactose, arabinose, and xylose and in the water-insoluble fraction they were glucose, arabinose, and xylose. The alkali-insoluble residue contained glucose only and thus probably represents the cellulosic fraction.

The presence of glucose in hemicellulose B might have arisen from the hydrolysate of a cellulose-containing mucilage or fraction in the residue (alkali insoluble). Smith and Montgomery (1959) have demonstrated the presence of cellulose-containing mucilages in the seed of flax and white mustard. Characteristically these mucilages contain cellulose, also termed as colloidally dispersed cellulose, solubilized in some fashion by the associated mucilagenous polysaccharides (as well as by the presence of other sugar moieties). It has been claimed, in some cases, that solubilization may occur by the noncovalent encapsulation of the cellulose by the associated polysaccharides (Grant et al., 1969).

Thus, it is apparent from the above studies that the hemicellulose portion of sesame is a composite aggregate of at least two to three different polysaccharides which are held firmly by strong noncovalent bonds.

Preliminary fractionation studies on hemicellulose B revealed that they are highly heterogeneous in that they contain an araban, araboxylan, and an acidic hexosan–pentosan complex. Work on these aspects is in progress.

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

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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

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