TABLE II. Effect of Sodium Salicylate on Tissue Mg Contents (meq/kg Wet Wt).

	6 control animals receiving no salicylate	14 animals given salicylate for 6 days, 40 mg/kg I.S. daily	6 animals given salicylate, 300 mg/kg I.V. within 2 hr
Bone cortex	319.9 ±10.00†	261.5 ±10.15*	271.3 ± 8.65*
Bone marrow	10.8 ± 1.46	6.2 ± 1.01	6.9 ± .90
Brain	12.6 ± .35	9.2 ± .19*	9.9 ± .38*
Adrenal	12.5 ± 1.32	8.4 ± .37*	13.3 ± 1.91
Appendix	20.3 ± .59	14.2 ± .73*	16.2 ± .59*
Heart	15.4 ± .24	11.3 ± .56*	13.4 ± .31*
Kidney	16.5 ± .42	10.7 ± .43*	11.9 ± .48*
Liver	16.8 ± .60	9.5 ± 1.01*	14.0 ± .59*
Lung	11.5 ± .43	7.5 ± .39*	9.2 ± .29*
Muscle	21.6 ± .84	15.0 ± 1.01*	19.9 ± .22
Pituitary	15.3 ± .85	15.0 ± 1.37	
Skin	5.2 ± .37	2.8 ± .16*	4.9 ± .30
Spleen	17.5 ± 1.70	13.7 ± .65	17.0 ± 2.55
Stomach	20.3 ± .62	13.7 ± .81*	17.1 ± 1.48
Testis	11.8 ± .34	9.4 ± .44*	10.3 ± .50

* Statistically significant when compared with control values. † Standard error.

The exact enzymatic mechanisms affecting magnesium metabolism remain to be established.

Summary. Intravenous administration of sodium salicylate, 150 mg/kg twice within 2 hours to normal rabbits, resulted in a significant decrease in the tissue magnesium content of bone cortex, brain, appendix, heart, kidney, liver, and lung. The turnover of magnesium, as measured by Mg^{28}, was increased in the bone marrow, kidney, and liver.

In animals given sodium salicylate in daily doses of 40 mg/kg for 6 days, tissue magnesium content was significantly decreased in bone cortex, brain, appendix, adrenal, heart, kidney, liver, lung, muscle, skin, stomach, and testis. The relative specific activity was increased in the brain, appendix, heart, kidney, lung, and stomach. In rabbits, sodium salicylate depletes the tissues of magnesium and increases the turnover of this element in the body.

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Evidence for the Pentose Cycle in *Nocardia corallina*.* (31280)

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Nocardia corallina is a typical actinomycete which undergoes cyclic morphological changes during normal growth; the mechanisms behind these changes are unknown. is to search for answers at the metabolic level. One approach toward eventual explanations

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Previous work(1) provides evidence for the Krebs cycle and for formation of adaptive permeases correlated with specific stages of the growth cycle. The pentose cycle (see Horecker(2) for a review) occurs widely in many species and was chosen for further study because of its relationship to fat metabolism which is known to fluctuate during the growth cycle of this organism(3).

Materials and methods. *Nocardia corallina* (ATCC 4273) was grown at 29°C on nutrient agar plus 0.5% fructose and transferred at 48-hour intervals. Cell-free extracts were prepared from cultures harvested in 0.9% saline and washed 3 times with 0.01 M phosphate buffer, pH 7.3. Cells were disrupted for 20 minutes at 4°C in a Mickle disintegrator with approximately 33% (V/V) beads (Ballotini, No. 12). The resultant suspensions were centrifuged twice at 7,000 × *g* and 4°C.

The method of DeMoss(4) was used to measure dehydrogenase activity. Sedoheptulose was detected by the procedures of Axelrod *et al*(5) and Klevstrand and Nordal(6). Ketosugars were detected by the method of Dische and Borenfreund(7) as modified by Axelrod and Jang(8). The products formed during the incubation of cell-free extracts with ribose 5-phosphate were determined using the orcinol reagent as modified by Brown(9) and quantitated by procedures similar to those of Horecker(10). Reaction mixtures were prepared as described by Rao *et al*(11). Protein was determined using the biuret reaction as described by Layne(12) with crystalline bovine albumin as the standard.

Results. The first two enzymes of the pentose cycle, glucose 6-phosphate dehydrogenase and gluconate 6-phosphate dehydrogenase, were found to be present in extracts using the procedures described in(4). The dehydrogenases were NADP (nicotinamide-adenine dinucleotide phosphate) specific. Extracts from all stages of the growth cycle tested were active with maximum activity in coccoids and lowest activity in fragmenting forms (Table I). Evidence for other enzymes in the pentose cycle was obtained by incubating cell-free extracts with ribose 5-phosphate and appropriate cofactors which are detailed in Table II. After incubation, the reaction mixtures were analyzed for known intermediates of the pentose cycle by the procedures listed in *Materials and methods.* Typical results of analysis by the cysteine-carbazole procedure(7,8) are shown in Table II. The rapid development of an absorption maximum in the complete system at 540 mμ is indicative of ketopentose formation from ribose 5-phosphate(7).

TABLE I. Glucose 6-Phosphate Dehydrogenase Specific Activity in Cell-Free Extracts from Various Stages of the Growth Cycle.

Cell type	Culture age (hr)	Specific activity*
Short hyphae	8	12.7
Long "	22	11.6
Fragmentation forms	24	3.9
Uniform coccoids	48	21.9

* Dehydrogenase activity was recorded as units of optical density change per min per mg protein, using assay procedures described by DeMoss(4).

Table III shows results of analysis by the procedure of Axelrod *et al*(5). The appearance of an absorption maximum near 505 mμ is evidence for the formation of heptulose(13). The procedure does not distinguish between gluco-, manno-, and sedoheptulose. Heptulose formation was confirmed by ascending paper chromatography using the orcinol spray(6) for detection. Hexose

TABLE II. Evidence for Formation of Ketopentoses by the Cysteine-Carbazole Procedure.

Analysis mixture*	Length of incubation after addition of color reagents: 5 min	30 min	12 hr	Absorption maximum (mμ)†
Control A	.008	.025	.083	540
" B	.000	.023		560
Complete reaction	.032	.235	.290	540

* Complete reaction mixture contained: Tris buffer (0.1 M, pH 7), 0.6 ml; thiamine hydrochloride (0.15 M), 0.2 ml; $MgSO_4 \cdot 7H_2O$ (0.1 M), 0.2 ml; cell-free extract from 48-hr-old culture (14.4 mg protein), 0.9 ml; ribose 5-phosphate (0.025 M), 0.9 ml; volume adjusted to 3.0 ml with deionized water. The reaction was incubated at 30°C for 2 hr, and 0.1 ml was used for analysis. Controls were identical with the complete reaction mixture except that in Control A, the cell-free extract was omitted and in Control B, ribose 5-phosphate was omitted.

† Rapid development of an absorption maximum at 540 mμ is indicative of presence of ketopentoses (7).

was synthesized, as indicated by the increase in optical density at 600 mμ.

Quantitation by the orcinol procedure of the products formed after various intervals of incubation of ribose 5-phosphate with cell-free extracts is shown in Fig. 1. There was a decrease in pentose, an increase in hexose and the appearance of heptulose. Percentage recovery, based on sugar analysis at zero time, is included at the top of the graph. The formation of triose occurs in the pentose cycle and could account for the decrease in percentage recovery during the first 30 minutes of incubation.

Discussion. The preceding results provide evidence for the major steps of the pentose pathway in *Nocardia corallina*. The differences in glucose 6-phosphate dehydrogenase activity in cell-free extracts from various stages of growth suggest that pentose cycle activity may vary throughout the growth cycle. Previous work(3) has shown that fluctuations in stainable fat are correlated with the morphological changes of the growth cycle. Pentose cycle activity is known to be linked to fat synthesis through production of $NADPH_2$ by the former and requirement for $NADPH_2$ by the latter, and the possibility that lipogenesis may be controlled by availability of reduced NADP has been proposed(14,15). However, recent work has cast doubt on this theory(16-18). *Nocardia corallina* appears to be a useful tool for further study of control mechanisms in lipogenesis since changes normally occur in fat metabolism during the growth cycle and a pentose cycle is operational with fluctuations in the $NADPH_2$ producing steps.

TABLE III. Evidence for Heptulose and Hexose Synthesis from Ribose 5-Phosphate by Cell-Free Extracts.*

Incubation time (min)	Optical density change at: 505 mμ	600 mμ
10	.370	.180
120	.527	.191

* The procedure of Axelrod *et al*(5) was used for analysis. The peak at 505 mμ is evidence for heptulose(13) and the peak at 600 mμ is indicative of hexose(5). Reaction mixtures were as described for Fig. 1 except that cell-free extract from an 18-hr culture containing a total of 13.2 mg protein was used.

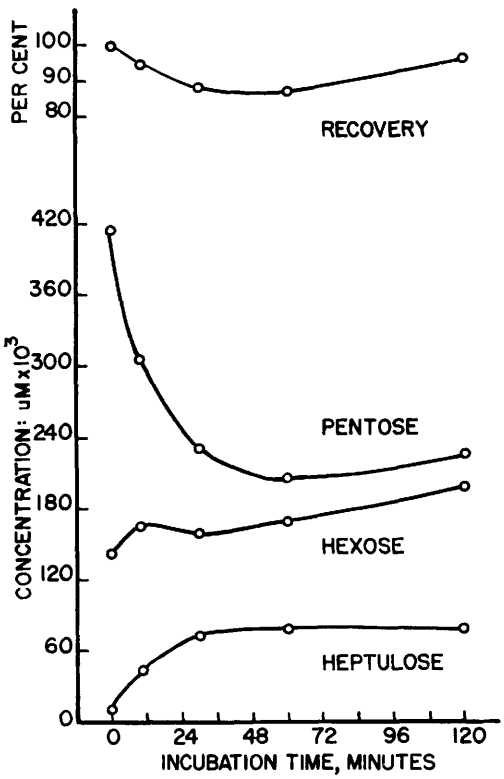

FIG. 1. Quantitation of sugars present in reaction mixtures after various intervals of incubation. Cell-free extract from a 20-hr culture was incubated at 30°C with the cofactors listed in Table II. One ml of a 1/20 dilution was analyzed at the indicated intervals by the orcinol procedure(9,10).

Summary. Cell-free extracts of *Nocardia corallina* were shown to contain glucose 6-phosphate and gluconate 6-phosphate dehydrogenases which were NADP specific as well as enzymes for the conversion of ribose 5-phosphate into ketopentose, heptulose, and hexose. The results were interpreted as evidence for the major steps of the pentose pathway in *N. corallina*. Cells from various stages of the growth cycle differed in glucose 6-phosphate dehydrogenase specific activity which suggests that changes in pentose cycle activity may be correlated with differences in fat metabolism and the life cycle previously reported for this organism.

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Identification of 5-Hydroxytryptophol as a Serotonin Metabolite In Man.* (31281)

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Although the metabolism of serotonin (5-HT) has been investigated intensively, the metabolic fate of this amine has not been defined completely. In man, the major metabolic route for 5-HT is oxidative deamination to the intermediate aldehyde and then oxidation to 5-hydroxyindoleacetic acid (5-HIAA)(1).

Evidence for a reductive pathway of 5-HT metabolism to the corresponding alcohol has recently been demonstrated in animals. Thus, Kveder *et al* found that 5-hydroxytryptophol (5-HTOH) was formed from 5-HT by rat liver slices and that 5-HTOH-O-glucuronide was a major 5-HT metabolite in rat urine(2). Bartholini, Pletscher, and Bruderer showed the formation of 5-HTOH from 5-HT during the incubation of isolated rabbit platelets with reserpine(3). Subsequently, Feldstein and Wong detected both the aldehyde and the alcohol derivatives of 5-HT in rat liver homogenates incubated with 5-HT-C^{14}(4).

This communication reports the isolation and identification of free 5-HTOH and 5-HTOH liberated from its glucuronide and sulfate conjugates in human urine and documents the *in vivo* conversion of 5-HT-C^{14} to 5-HTOH-C^{14} in man.

Extraction of free and enzymatically-released 5-hydroxytryptophol. Aliquots (75 ml) of 24-hour urine collections from patients with carcinoid tumors were adjusted to pH 11 with sodium hydroxide. Solid barium chloride was added to precipitate inorganic phosphates and sulfates which interfere with subsequent sulfatase hydrolysis. The samples were centrifuged to sediment the barium precipitates. The supernatant urine was then filtered and adjusted to pH 7. Separate 20 ml aliquots of the urine were saturated with sodium chloride and the free 5-HTOH was extracted with two volumes of ether. The ether extracts were then washed with 3 ml of 0.5 M phosphate buffer, pH 7, to remove traces of 5-HIAA.

The ether extracted urine was pooled and incubated with 15,000 units of bacterial

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