Presence and Biosynthesis of a Polyprenol-type Compound in *Ascaris lumbricoides* (Human)

STEROLS are important constituents of the non-saponifiable lipids in *Ascaris lumbricoides*, the human intestinal parasite (roundworm). *Ascaris suum*, a similar species of worms present in the pig intestine, is found to be incapable of synthesizing sterols and is completely dependent upon exogenous sterols[1]. The work reported here shows that in *Ascaris lumbricoides* (human), mevalonate-2-^{14}C, which is a well-known precursor for isoprenoid compounds, is incorporated into a non-saponifiable lipid component, which has the characteristics of a polyprenol.

Roundworms were obtained from patients dosed with 'Helmacid', an anthelmintic drug containing piperazine phosphate. After collection the worms were kept in a modified Tyrode solution[2] (NaCl 0.8%, KCl 0.02%, $CaCl_2$ 0.02%, $MgCl_2$ 0.01%, $NaHCO_3$ 0.015%, Na_2HPO_4 0.05% and 0.5% glucose) at 37° C for 4 or 5 days, before use. The worms were transferred to fresh medium each day and could be kept alive for thirty to forty days by this method. It is known that piperazine salts narcotize the worms and such worms regain movement when transferred to fresh medium[2]. Only active worms in the weight range of 1.5 to 2 g were used for experiments.

Mevalonate-2-^{14}C, 0.1 μCi (5.8 μCi/μmol), was injected into each worm and the worms kept in the same medium at 37° C for 2 h. After incubation the worms were cut into pieces and saponified with ethanolic sodium hydroxide containing pyrogallol. The non-saponifiable lipids were extracted with light petroleum (40–60° C) and fractionated by chromatography on alumina column deactivated with 5% water[3]. The elutions were carried out with light petroleum containing increasing concentrations of diethyl ether. Radioactivity was measured in a 'Beckman Model LS-100' liquid scintillation counter, using 0.5% diphenyloxazole in toluene as fluor. The distribution of radioactivity in various fractions is shown in Table 1.

Table 1 Incorporation of Mevalonate-2-^{14}C into Non-saponifiable Lipids of *Ascaris lumbricoides*

Fraction	Type of compounds	Radioactivity c.p.m. g^{-1} wet weight of worms
Non-saponifiable lipids	—	33,000
Light petroleum	Hydrocarbons	135
5% ether in light petroleum	Ubiquinone	1,050
10% ether in light petroleum	Prenols, rhodoquinone	27,500
20% ether in light petroleum	Sterols	350

Values represent means of three independent analyses carried out with three worms in each set.

Results indicate that more than 80% of the radioactivity is recovered in the 10% ether fraction, which elutes compounds with polarity characteristics of polyprenols. This fraction was chromatographed on thin layer silica gel plates using benzene–chloroform (60:40) as the developing solvent. On exposure to iodine vapours three compounds could be detected on the chromatogram. One of the compounds with R_F 0.53 was identified as rhodoquinone by its purple colour and ultraviolet absorption spectrum. Radioactivity was found to be associated only with one compound having the R_F 0.25. This compound also gave a purple colour on spraying with anisaldehyde–sulphuric acid reagent, which is generally used to detect isoprene compounds[4]. It was further purified by repeated thin layer chromatography to give a single homogeneous spot. The infrared spectrum of this purified compound showed a clear O-H stretching at 3,310 cm^{-1} similar to that of solanesol. The O-H stretching in sterols was in the region of 3,450 cm^{-1}, distinct from that of isoprene alcohols. Comparison of this compound with known prenols on thin layer chromatography showed that it is different from geraniol, farnesol and solanesol having 2, 3 and 9 isoprene groups, respectively. Reverse phase thin layer chromatography indicated that the molecule is probably smaller in size than solanesol.

This is the first report on the presence of a polyprenol type of compound in this organism and its rapid synthesis suggests that it may have some important role in its metabolism. It is conjectured that this prenol might function as a sugar carrier[5] in the biosynthesis of mucopolysaccharides, which form a part of the structural material of the cuticle of this parasite (R. Kaleysa Raj, unpublished observation). Hence compounds capable of inhibiting the biosynthesis of this isoprenoid compound might prove to be effective anthelmintics.

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Influence of Folic Acid on Excitable Tissues

THE occurrence of folic acid deficiency anaemia, complicating the use of a wide range of anti epileptic drugs, has led to the suggestion that these compounds produce their effect by antagonizing folic acid in the brain[1]. This implies that folic acid can act as a cerebral excitant. However, administration of pteroyl monoglutamate (PGA) and formyl tetrahydrofolate (f-THF) orally or intraperitoneally to rats and mice produced no alteration in their convulsive threshold[2]. I describe here experiments to examine this suggestion further.

Intracerebral injections of PGA ($0.2–10.0 \times 10^{-8}$ mol in 4 μl.) and f-THF ($0.2–2.0 \times 10^{8}$ mol in 4 μl.) were given to white Wistar rats weighing 170–200 g, and to SAS/A-strain mice weighing 20–27 g. Control animals were injected with 4 μl. of isotonic saline intracerebrally. In rats 1.0×10^{8} mol f-THF and 10.0×10^{-8} mol PGA, and in mice 0.5×10^{-8} mol f-THF and 3.4×10^{-8} mol PGA (each made up to 4.0 μl.), produced a similar sequence of events. During 30–50 s after injection the animal appeared normal; this interval was followed by a variable period of 10 s to 2 min during which the animal became hyperkinetic. In this phase there was much preening activity interspersed with rapid exploratory-type walking. The next phase lasted from 1–65 min and consisted of running and jumping. The mice jumped approximately 20–30 cm vertically, standing, not running jumps, and the rats jumped in a similar fashion up to 30–50 cm. With these doses of f-THF and PGA the mortality rate was approximately 70% and death occurred at 10–65 min, preceded by tonic and clonic convulsions.