The significance of phenol coupling in soils has not been assessed; however, it has been shown that pesticides and their transformation products can be readily bound to soil components[8]. Enzymatic oxidative coupling may be an important reaction that results in covalent bonding of phenolic intermediates to soil organic polymers. Anilinic residues of pesticides may also be similarly bound, since peroxidases are active in the oxidation and coupling of aromatic amines. Whether the binding reactions with phenolic pesticide intermediates should be viewed as resulting in the formation of recalcitrant molecules of unknown toxic properties or as mechanisms of environmental detoxication is still open to debate.

Our results form the basis for future investigations with agrochemicals in soils in which the degree of polymerization of these chemicals as well as the extent of their incorporation into soil organic matter may be determined under various conditions. It is our suggestion that the role of phenol oxidases in determining the fate of xenobiotic chemicals in the soil environment has been widely overlooked and probably underestimated.

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Synthesis and some characteristics of [^{13}C]-specially enriched tetragastrin and the related compound[1]

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Summary. [^{13}C]-enriched tetragastrin and the related compound were synthesized in solution. Conversion of S-[^{13}C]-methylated tetragastrin to the enriched tetragastrin gave 10.5 ppm upfield chemical shift of C^{ε} resonance. The potency of the synthetic tetragastrin to stimulate gastric acid secretion was virtually identical with that of pentagastrin (ICI).

Gastrin, a gastrointestinal hormone, is an acidic heptadecapeptide amide and exists in 2 forms, which are gastrin I with Tyr residue in position 12 and gastrin II with sulfated Tyr residue in the position[3]. In both forms, 4 amino acid residues in the C-terminal, i.e., H-Trp-Met-Asp-Phe-NH_2 (tetragastrin), are known to be responsible for the full range of physiological activity of the parent hormone[4]. A number of analogues of tetragastrin have been synthesized and subjected to the investigation of structure-function relationships in the active site of gastrin. Morely[5] assumed that the Trp, Met and Phe positions in the tetragastrin participate significantly in binding the molecule with the site of physiological action. The ^{13}C-NMR-spectroscopy has become a useful tool for the study of conformations of peptides and proteins in solution. Recently, Bleich et al.[6] reported ^{13}C- and ^{1}H-NMR-studies of the tetragastrin at natural abundance using perdeuterio dimethyl sulfoxide solution. In this paper an attempt was made to synthesize the tetragastrin in which the methyl group of the methionine residue was specially enriched with [^{13}C]: H-Trp-[S-methyl^{13}C]Met-Asp-Phe-NH_2 (**I**). For comparison, the [^{13}C]-enriched tripeptide amide H-[S-methyl^{13}C]Met-Asp-Phe-NH_2 (**II**) was also synthesized. Specific enrichment with ^{13}C of the C^{ε} of the methionine residue in the peptide was performed by a method that modified the one by Jones et al.[7], which consists of methylation at the methionine sulfur with [^{13}C]-enriched methyl iodide followed by random demethylation. For the synthesis of compound **I**, protected tetragastrin, t-Boc-Trp-Met-Asp-Phe-NH_2[8], was first constructed by stepwise procedure starting from H-Phe-NH_2. The synthetic route is shown in figure 1.

The protected tetragastrin was methylated in dimethylformamide with 10fold equivalent of 90% methyl-[^{13}C] iodide. The reaction mixture was kept at pH 7.0 and allowed to stand at room temperature in dark for 24 h. An-

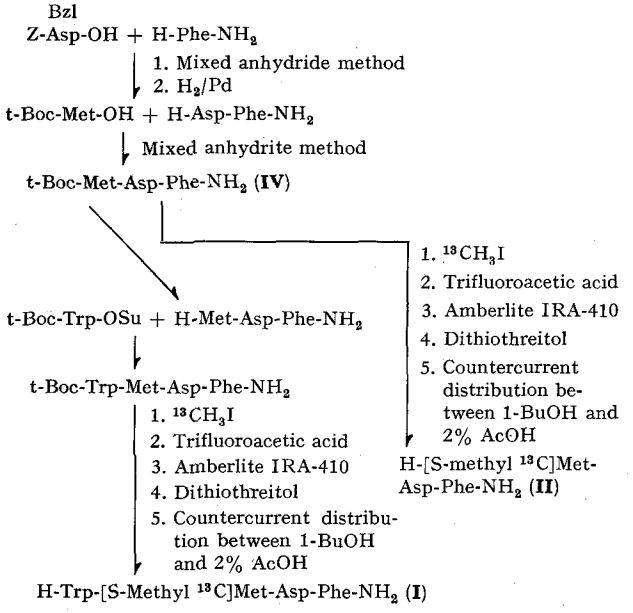

Fig. 1. Synthesis of [^{13}C]-enriched gastrin-related peptides.

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8 Abbreviations used are: t-Boc: tertiary butoxycarbanyl; BuOH: butanol; AcOH: acetic acid; DMSO-d_6: perdeuteriodimethyl sulfoxide; Bzl: benzyl ester; OSu: N-hydroxy succinimido ester; R_f^{I}- and R_f^{II}-values refer to the solvent systems: 1-BuOH-AcOH-H_2O(4:1:5) (upper layer) and 1-BuOH-pyridine-AcOH-H_2O(30:20:6:24), respectively.

hydrous ether was added to the mixture and the particpate, after drying, was treated with trifluoroacetic acid to remove the α-amino protecting group. The resulting S-[^{13}C]-methylated compound, which was kept with 5fold equivalent of dithiothreitol at pH 9.0 for 36 h at room temperature, gave crude preparation of compound **I**. Purification of the crude material by countercurrent distribution between 1-BuOH and 2%-AcOH gave pure compound {$[\alpha]_D^{29}$ −35.2 (c 1.0, DMF); R_f^I 0.46; R_f^{II} 0.65; amino acid ratios in acid hydrolysate, $Asp_{1.02}Met_{0.94}$ $Phe_{1.04}$ (recovery 91%) (Trp was just determined)}. The R_f-values and the optical rotation of the [^{13}C]-enriched tetragastrin showed good agreement with those of the original one.

Compound **II** was prepared by S-[^{13}C]-methylation of t-Boc-Met-Asp-Phe-NH_2 (**IV**) followed by deprotecting and demethylation in the same manner as described for compound **I**. The protected tripeptide amide (**IV**) is an intermediate in synthesizing compound I. ^{13}C-enrichment of methionine residue was also similarly carried out with methyl-[^{13}C] iodide to give homogenous [^{13}C]-enriched tripeptide (**II**) {$[\alpha]_D^{21}$ + 1.92 (c 0.52, 50% AcOH); R_f^I0.35; R_f^{II}0.54}. In addition, the amino acid analyses of the acid hydrolysates in both of the enriched peptides indicated the complete demethylation of S-methylated methionine residue.

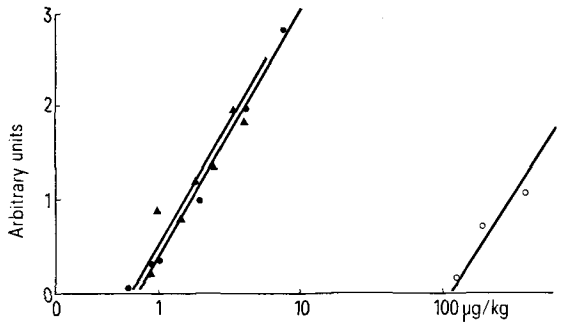

Fig. 2. Dose-response curve of gastrin-related peptides on gastric acid secretion. On the abscissa is the dose (µg/kg), and on the ordinate is the area of pH-reduction due to gastric acid secretion of the peptide. The symbols are as follows: ● pentagastrin (ICI); ▲ [^{13}C]-enriched tetragastrin; ○ [^{13}C]-enriched tripeptide.

Biological activities of compound **I** and **II** were compared with that of pentagastrin (ICI) in terms of potency to stimulate gastric secretion which was measured by Schild's rat method[9,10]. The activity of compound **I** was found to be essentially identical with that of pentagastrin, while that of compound **II** was only 1% of the reference peptide shown in figure 2.

NMR-spectra were obtained on a JEOL-PFT-100 system operating in the Fourier transform mode. ^{13}C-spectra were proton decoupled and obtained at room temperature (24 ± 1 °C) controlled by JEOL temperature controller. A deuterium internal lock was provided by means of D_2O capillary which was inserted into the material. The data were accumulated using 90° pulse with 6250 Hz bandwidth, and 200–500 transients were accumulated per spectrum. Chemical shifts were referenced to carbon disulfide (CS_2). The chemical shift of CS_2 from tetramethylsilane (TMS) was obtained at 193.98 ppm ($\delta = 198.98\text{-}\delta_{cs_2}$). S-[^{13}C]-methylated tetragastrin (16 mM) in DMSO-d_6 showed a single, narrow ^{13}C-resonance at 167.79 ppm (= 26.19 ppm from TMS). When it was reduced by dithiothreitol, the chemical shift of ^{13}C-resonance of the enriched tetragastrin shifted upfield 10.57 ppm, i.e., at 178.36 ppm. This change in the chemical shift was in good agreement with that reported by Jones et al.[11]. These ^{13}C-enriched compounds with well-defined structure may serve as substrates suitable for NMR-studies on the binding of gastrin with its receptor.

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Red cell metabolism in red and grey kangaroos

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Summary. Glucose utilization, lactate production and glutathione regeneration were measured in the red blood cells of 2 species of Australian Marsupials, Eastern grey kangaroo (*Macropus gigantus*) and red kangaroo (*Macropus rufus*), and were found to be significantly lower in the red blood cells from grey than that of red kangaroos.

Red cells of Eastern grey kangaroo (*Macropus gigantus*) have considerably higher activity of several red cell enzymes incluring those of glucose-6-phosphate dehydrogenase, glucose-6-phosphate isomerase and lactate dehydrogenase, than those of red kangaroos (*Macropus rufus*)[3]. Just the opposite is true for the level of reduced glutathione (GSH) which is higher in the red cells of red kangaroos than those of grey kangaroos[4]. In continuation of these studies we have now measured glucose utilization, lactate production, and certain aspects of GSH metabolism in the red blood cells of these 2 species of Australian marsupials.

Materials and methods. Blood was obtained from lateral tail veins of kangaroos and collected into heparinized tubes. The red cells were washed 3 times in saline and a cell suspension giving a haematrocrit of about 30% was made in Krebs' Ringers buffer pH 7.4 containing 8 mM glucose. The cell suspension was incubated ar 37 °C and aliquots were removed at hourly intervals for the mea-

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