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.

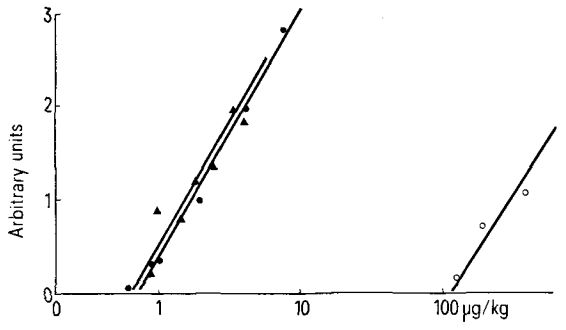

Fig. 2. Dose-response curve of gastrin-related peptides on gastric acid secretion. On the abscissa is the dose (µg/kg), and on the ordinate is the area of pH-reduction due to gastric acid secretion of the peptide. The symbols are as follows: ● pentagastrin (ICI); ▲ [^{13}C]-enriched tetragastrin; ○ [^{13}C]-enriched 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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surement of glucose (Sigma Technical Bulletin No. 510) and lactate[5]. Previously described methods were used for the measurements of regeneration of GSH by intact red cells[6] and the stability of GSH in the presence of acetylphenylhydrazine[7].

Results and discussion. The results (table) show that glucose consumption averaged 4.99 ± 0.24 μM/h gHb in the cells of red kangaroo compared to 3.36 ± 0.29 in those of grey kangaroos, the difference being statistically significant ($p < 0.01$). Differences in the lactate production by the red cells were also significant between the 2 species ($p < 0.002$).

Metabolism of glucose and reduced glutathione (GSH) in the red blood cells of red and grey kangaroos

	Red kangaroos (N = 5)	Grey kangaroos (N = 5)	p
Glucose consumption (μM/h gHb)	4.99 ± 0.24	3.36 ± 0.29	0.01
Lactate production (μM/h gHb)	10.87 ± 0.72	5.88 ± 0.35	0.002
GSH regeneration (μM/min gHb)	0.275 ± 0.029	0.038 ± 0.017	0.001
GSH stability (μM/h gHb)	0.052 ± 0.007	0.040 ± 0.008	NS

Results are means ± SEM.

GSH regeneration in the red cells of both the species of kangaroo proceeded linearly; more than 90% of the original GSH was regenerated in less than 30 min in red kangaroo and about 60 min in grey kangaroo. Mean GSH regeneration of 0.275 ± 0.029 μM/min gHb in the red cells of red kangaroos was significantly higher ($p < 0.001$) than 0.038 ± 0.017 obtained in grey kangaroos (table). As shown in the table, there was no significant difference in the GSH stability between the 2 species of the kangaroo.

The activity of glucose-6-phosphate dehydrogenase and several other enzymes of glucose metabolism is higher in the red blood cells of grey than those of red kangaroos and yet, as shown in the table, glucose utilization, lactate production, and GSH regeneration rates are significantly lower in the red blood cells of grey than those of red kangaroos. The reason for this paradoxical relationship between enzyme activity and glucose metabolism is not yet clear but it may possibly be related to the level of glycolytic intermediates and/or differences in permeability of the red cells to glucose. Studies in this direction may help understanding the evolutionary interrelationships between the different species of marsupials.

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Peroxidase-Isoenzyme in Kressekeimlingen (*Lepidium sativum* L.) und ihre Hemmung durch Silybin, Silydianin und Silychristin

Peroxidase isoenzymes in cress seedlings (*Lepidium sativum* L.) and their inhibition by silybin, silydianin and silychristin

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Summary. Seedlings of *Lepidium sativum* L. contain 4 peroxidase isoenzymes. Their de-novo-synthesis is significantly depressed in the presence of $3 \cdot 10^{-3}$ moles/l silybin, silydianin and silychristin in the incubation medium respectively.

In früheren Mitteilungen[1,2] über unsere Untersuchungen zur Funktion von «Silymarin» in der Pflanze hatten wir berichtet, dass Silybin (**I**), Silydianin (**II**) und Silychristin (**III**), die zusammen das biologisch aktive Prinzip der Früchte der Mariendistel (*Silybum marianum* Gaertn.) darstellen[3], das Wachstum von Kressekeimlingen in spezifischer Weise beeinflussen. Die Wirkungen von **I** bis **III** sind dosisabhängig und lassen sowohl bei der Aktivität der einzelnen Verbindungen als auch bei der Kombination derselben untereinander markante Unterschiede erkennen[1]. Als Ursache für diesen Effekt von **I** bis **III** hatten wir eine Interaktion der Wirkstoffe mit dem Metabolismus des primären Phytohormons Indolessigsäure (IAA) in Betracht gezogen[4] und diese Vermutung auch experimentell gestützt[2,4]. So erweisen sich **I–III** in vitro als spezifische Inhibitoren der Meerrettich-Peroxidase[2], eines Enzyms, das mit der Indolessigsäure-Oxidase (IAA-Oxidase) der Pflanzen nahe verwandt ist[5]. Der durch die Inhibition der IAA-Oxidase verminderte Abbau der IAA muss daher zwangsläufig zu einer Verstärkung der speziellen Wirkungen des Phytohormons führen.

In dieser Arbeit führen wir den Nachweis, dass unser Testobjekt[6], Keimlinge der Gartenkresse (*Lepidium sativum* L.), mehrere Peroxidase-Isoenzyme enthält und dass die De-novo-Synthese derselben durch die Testsubstanzen **I–III** signifikant herabgesetzt wird.

Material und Methodik. Testsubstanzen und Testsystem. Zur Anwendung kamen stabile, wasserlösliche Derivate von **I** bis **III** (Na-Salze der Dihemisuccinate)[7]. Als Testobjekt wurden Keimlinge von *Lepidium sativum* L., Sorte «cresson frisé», benutzt. Eine genaue Beschreibung dieses Testsystems findet sich an anderer Stelle[6]. Folgende

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