figure shows the more detailed distribution of both enzyme activities in the duodenal mucosa. The highest Mg^{2+}-HCO_3^--ATPase activity was found in the 1st part of the duodenal mucosa (within 3 cm of the pylorus). Carbonic anhydrase activity was also highest in the same area as Mg^{2+}-HCO_3^--ATPase; however, its specific activity was very low.

Discussion. In the present experiment it was demonstrated clearly that the Mg^{2+}-HCO_3^--ATPase activity in the duodenal mucosa, especially in its microvilli, is higher than in the other segments of the gastrointestinal tract investigated. On the other hand, carbonic anhydrase activity was higher in the gastric and large intestinal mucosa, and these distribution patterns were essentially the same as those reported by Carter and Parsons[11]. These phenomena seem to be related to the neutralization of gastric acid. The role of these enzymes may be as follows: CO_2 produced in the duodenal lumen by the neutralization of HCl with $NaHCO_3$ diffuses into the epithelial cells where carbonic anhydrase converts this CO_2 to H_2CO_3 by hydration. H_2CO_3 dissociates to H^+ and HCO_3^-. The former may exchange with Na^+ existing as NaCl in the duodenal lumen and HCO_3^- exchanges with Cl^- through the brush border membrane. Mg^{2+}-HCO_3^--ATPase may mediate this HCO_3^--Cl^- exchange process. Since exchanges of HCO_3^--Cl^- and $H^+ - Na^+$ through the jejunal mucosa have been documented by various investigators[12,13] a similar exchange process may also be expected in the duodenal mucosa. Although the specific activity of carbonic anhydrase in the duodenal mucosa was low, it is interesting to consider that the CO_2 diffusion through the membrane is stimulated by the presence of carbonic anhydrase[14,15].

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0014-4754/83/080872-02$1.50+0.20/0

Contribution to knowledge of the biosynthesis of cyclosporin A

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Summary. ^3H- and ^{13}C-NMR spectroscopic investigations on the structure of labeled cyclosporin A were performed after feeding of appropriate precursors. The 6 N-methyl groups and the methyl group in position 4 of the ε,ζ-unsaturated amino acid No. 1 (Mebmt) are introduced as intact CH_3-units from methionine. Four head-to-tail acetate units are involved in the biosynthesis of the 8-carbon chain of the olefinic amino acid.

Cyclosporin A (fig. 1), a cyclic peptide with antifungal and immunosuppressive properties, is produced as the major product, together with several minor analogue peptides, in cultures of *Tolypocladium inflatum* Gams. Information on production[2], directed biosynthesis[3], chemistry[4-6] and pharmacology[7,8] has been reported. In feeding experiments with several tritium and carbon-14 labeled precursors [methyl-^3H]methionine proved to be the most suitable compound for the biosynthetic preparation of radiolabeled cyclosporin A for pharmacokinetic and metabolic studies[9]. The analysis of the corresponding ^3H-NMR spectrum (fig. 2), which showed 6 signals for the tritiated 7 N-methyl groups and the methyl group in the γ-position of the olefinic amino acid No. 1, initiated additional more detailed studies on the biosynthesis of cyclosporin A. Accordingly, in a series of experiments the incoorporation of carbon-13 and deuterium from sodium [1-^{13}C]acetate, sodium [2-^{13}C]acetate, [methyl-^{13}C]methionine and [methyl-(^{13}C, $^2H_3$)]methionine was investigated.

Methods. The following nutrient media were used to cultivate the producing organism. Medium 1: malt extract 20 g, yeast extract 4 g, agar 20 g, water to 1 l, pH = 5.7. Medium 2: maltose 50 g, caseinpeptone 10 g, KH_2PO_4 5 g, KCl 2.5 g, water to 1 l, pH = 5.3. A subculture of a strain of *Tolypocladium inflatum* Gams was propagated on medium 1 for 2 weeks at 27 °C and cultivated in medium 2 as earlier described[3]. Filter-sterilized solutions of the precursors were added 2 h after inoculation of the production medium. The cultures were incubated on a rotary shaker for 10 days at 27 °C.

Cyclosporin A was extracted from the broth with 1,2-dichloroethane, purified by gel filtration on sephadex LH 20 with methanol, and separated from minor cyclosporins by repeated HPLC on μ Bondapak Phenyl, 5 μm at 1000 psi with acetonitrile-water 6:4.

Precursors. a) 600 mg/l of sodium [1-^{13}C]acetate at 90 atom % C-13, b) 600 mg/l of sodium [2-^{13}C]acetate at 80 atom % C-13, c) 500 mg/l of [methyl-^{13}C]methionine at 92 atom % C-13, d) 500 mg/l of [methyl-(^{13}C,$^2H_3$)methionine at 92 atom % C-13, 98 atom % ^2H, and e) 75 mg/l of [methyl-^3H]methionine at a sp.act. of 5 Ci/mmole.

Analytical techniques. The incorporation of carbon-13 was determined by ^{13}C-NMR spectroscopy on a Bruker WH-360 spectrometer of solutions of about 250 mg of the corresponding labeled cyclosporin A in 2.5 ml of $CDCl_3$. The relevant signals were assigned by using characteristic

chemical shifts, multiplicities, and single-frequency proton decoupling[6].

^{13}C-enrichment ratios E were calculated from the peak integrals of the ^{1}H-inverse-gated-decoupled spectra[10,11], $E = I_i/I_m$, where I_i is the peak intensity of the labeled carbon atom i and I_m is the mean peak intensity of the unlabeled carbon atoms of the enriched sample. The use of the inverse-gated-decoupling method guarantees a depression of the NOE's. The saturation problem was eliminated by a sufficiently long delay (60 sec) between the scanning pulses.

In the feeding experiment with [methyl-^{3}H]methionine, the localization of the incorporated tritium was detected by proton decoupled Fourier transform ^{3}H-NMR spectroscopy[12] on a Bruker HX 90 E instrument (7 mCi, 30 mg of [^{3}H]cyclosporin A in 250 µl $CDCl_3$, 3000 scans). The radioactivity data were determined by LSC in a Tri-Carb liquid scintillation spectrometer (Packard Instr. Mod. 3375).

Results. The ^{13}C-NMR spectrum of the enriched cyclosporin A derived from [1-^{13}C]acetate (fig. 3a) showed 4 enhanced signals of approximately double intensity (table) for

^{13}C-chemical shifts (ppm) and enrichment factors for the carbon atoms of N-methyl-(4R)-4-but-2E-en-1-ul-4-methyl-threonine (amino acid No. 1) and the 7 N-methyl groups

Carbon atom	Chemical shift	Enrichment factor
C-1	170.4	2
C-2	58.8	2
C-3	74.7	2
C-4	36.0	3
C-5	35.6	3
C-6	129.7	2
C-7	126.3	2
C-8	17.9	3
C-4-CH_3	16.8	9
1-N-CH_3	33.9	10
3-N-CH_3	39.4	8
4-N-CH_3	31.3	9
6-N-CH_3	31.5	11
9-N-CH_3	29.5	11
10-N-CH_3	29.7	11
11-N-CH_3	29.7	11

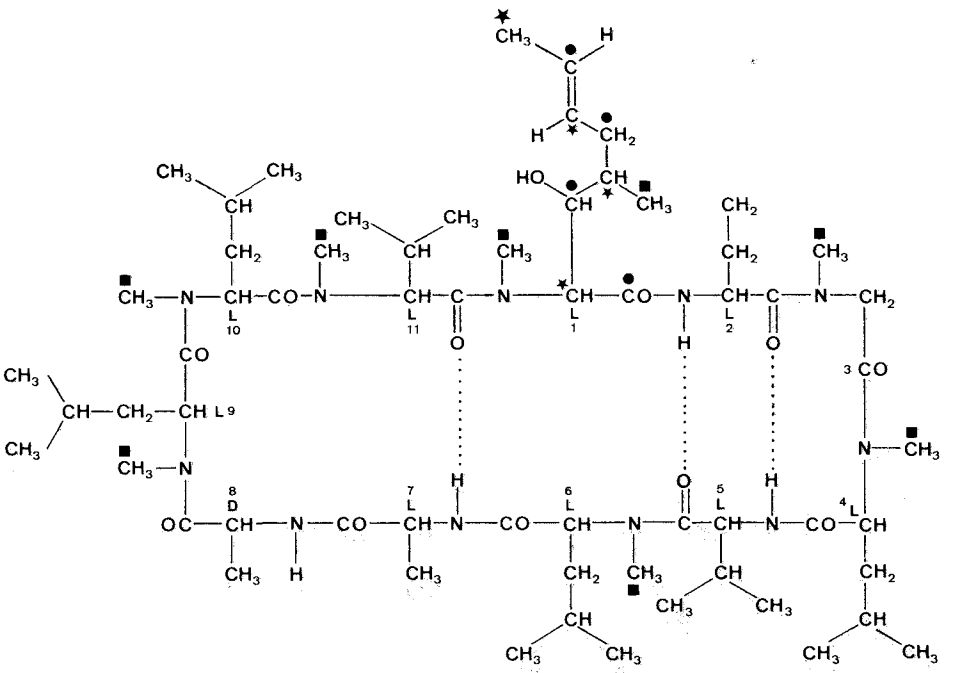

Figure 1. Structure of cyclosporine A and distribution of ^{13}C after addition of [1-^{13}C]acetate ●, [2-^{13}C]acetate * and [methyl-^{13}C]methionine ■.

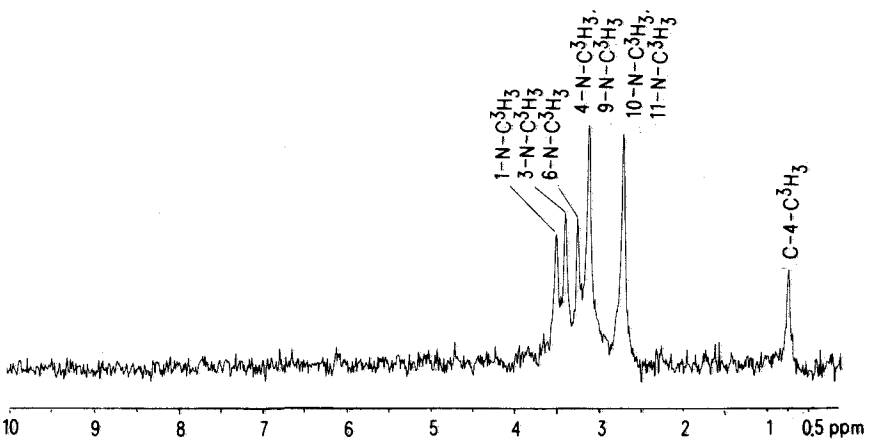

Figure 2. ^{3}H-NMR spectrum of [^{3}H]cyclosporin A in $CDCl_3$. Precursor: [methyl-^{3}H]methionine. The assignments are based on ^{1}H double resonance and NOE experiments.

the carbon atoms 1, 3, 5 and 7 of the N-methyl-(4R)-4-but-2E-en-1-yl-4-methyl-threonine (Mebmt)[13] (amino acid No. 1, see fig. 1). Equal ^{13}C-enrichments compared to the unlabeled positions were observed for the carbon atoms 2, 4, 6 and 8 (fig. 3b), when [2-^{13}C]acetate was used as the precursor. For all other amino acids no ^{13}C-incorporation was found. Consequently, the biosynthesis of cyclosporin A proceeds via the formation of an 8-carbon polyketide chain by head-to-tail couplings of 4 C_2-units, yielding the skeleton of the olefinic amino acid. As already mentioned, methylation of the chain in position 4 as well as the N-methylation of 7 amino acids by an appropriate C_1-donator are further processes as demonstrated by the feeding experiments with [methyl-^{3}H]methionine (fig. 2) and [methyl-^{13}C]methionine (fig. 3c). From the ^{13}C-NMR data a 10-fold enrichment was calculated for all [^{13}C]methyl incorporations (table). In contrast, the relative intensities of the [^{3}H]methyl signals in the ^{3}H-NMR spectrum (fig. 2) indicate that the methylation of the amide groups is slightly favored by a factor of 1.5 in relation to the c-methylation of the C_8-skeleton of the olefinic amino acid.

Compared to the ^{13}C-NMR spectrum of [^{13}C]cyclosporin A (fig. 3c), the corresponding N-$^{13}C^2H_3$ and C-$^{13}C^2H_3$ signals of the $^{13}C^2H_3$-enriched substrate (fig. 3d) showed a splitting of 20 Hz and an isotopic highfield shift of about 1 ppm[14] due to the geminal deuterium atoms. The lack of a ^{1}J-proton coupling for the C-$^{13}C^2H_3$-signal at 11.4 ppm points out, that the methyl-transfer to the polyketide chain occurs via

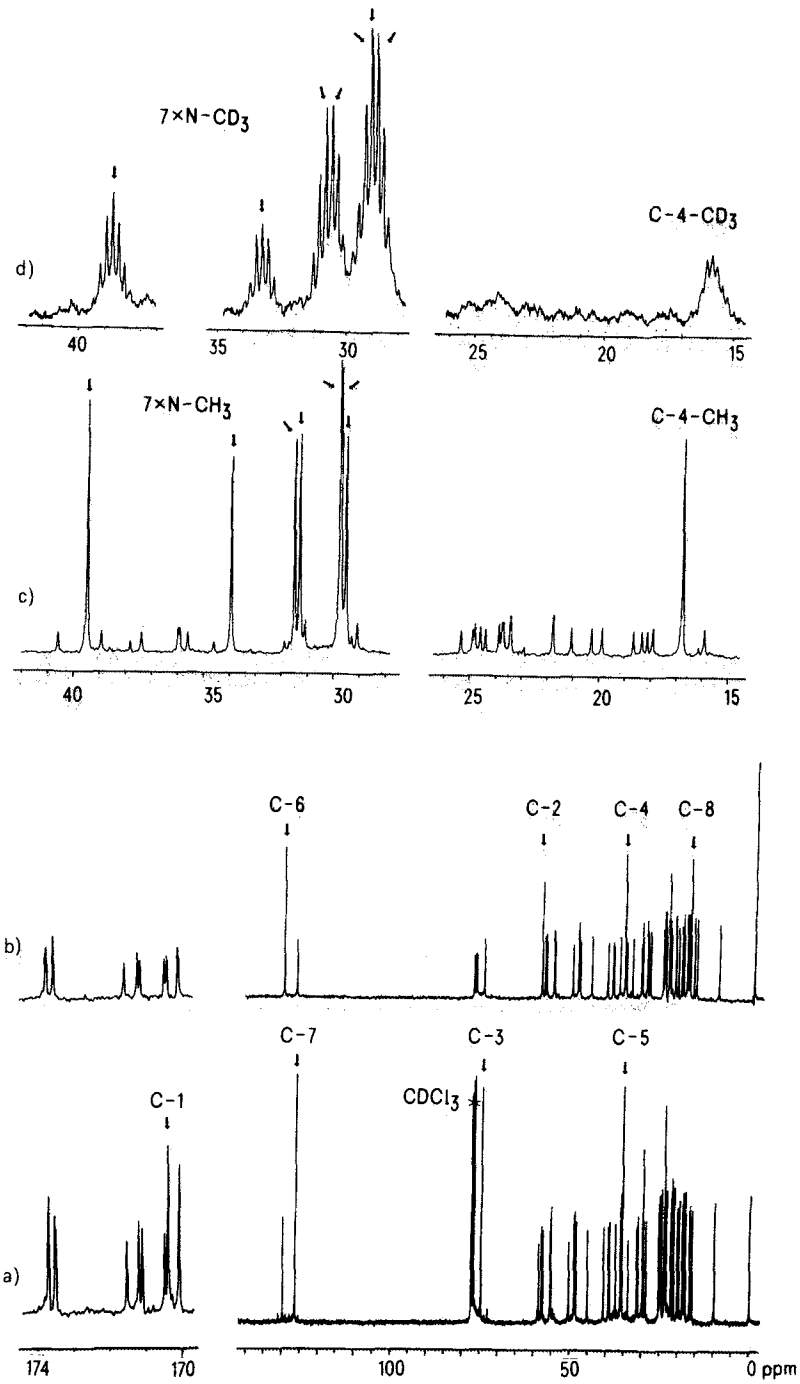

Figure 3. The ^{1}H-inverse-gated-decoupled ^{13}C-NMR spectra of ^{13}C-enriched cyclosporin A in $CDCl_3$. *a* Precursor, [1-^{13}C]acetate; *b* Precursor, [2-^{13}C]acetate; *c* Precursor, [methyl-^{13}C]methionine; *d* Precursor, [methyl-(^{13}C,^{2}H$_3$)]methionine, ^{1}H-undecoupled.

intact C^2H_3-units possibly by nucleophilic attack of an appropriate enolate and precludes a possible mechanism via cyclopropane or methylene intermediates.
The incorporation of the different labeled atoms in cyclosporin A is schematically shown in fig. 1.

1 Acknowledgments. We thank Prof. Dr D. Arigoni (Federal Institute of Technology, Zürich) for helpful discussions, Miss S. Bogner and Messrs S. Mangin, R. Masero and R. Reimann for their excellent technical assistance.
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0014-4754/83/080873-04$1.50+0.20/0

Immunocytological study of the distribution of C-cells calcitonin in the thyroid gland of the normal adult gerbil

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Summary. The distribution of C-cells in the thyroid gland of the gerbil *(Meriones inguiculatus)* was studied on serial sections in the cranio-caudal direction. C-cells were visualized by immunofluorescence with an anti-calcitonin immune serum. A very heterogeneous distribution was found. C-cells first appear in the posterio-interior position. Then they progressively occupy the whole posterior part and finally are confined to the posterio-exterior position. No C-cells at all were detected in the upper and lower poles. According to the species, there is a wide variation in the relative distribution of C-cells in the thyroid gland. Our results add to the information available about the distribution of C-cells and its bearing on phylogenic evolution.

The parafollicular C-cells have been ascribed as the site for synthesis, storage and release of the hypocalcemic factor calcitonin[2,3]. Their distribution has been described in some vertebrate species, including man[4-8]. There is no such description for the thyroid gland of the gerbil *(Meriones inguiculatus)*, an animal more and more frequently used for research[9-15].
The purpose of this paper is to illustrate the distribution of C-cells in the thyroid of the adult Gerbil.
Material and methods. Sheep antiserum to synthetic human calcitonin (Ciba-Geigy) was obtained by repeated intradermal injections of this antigen coupled to ovalbumine (Sigma) with glutaraldehyde and emulsified with Freund's complete adjuvant.
Adult gerbils were sacrificed at 4 weeks: their thyroids were excised, fixed in aqueous Bouin's solution, dehydrated and embedded in paraffin. The thyroid gland was along its entire length in a plane perpendicular to the long axis, and sections (6 µm thick) were taken every 3 mm.
Deparaffined sections were used for an indirect immunofluorescence technique. The reaction was performed with sheep anti-human calcitonin immune serum (dilution 1:10) and fluorescent conjugate of goat anti-sheep gammaglobulin (Institut Pasteur). The staining period was 2 h at room temperature. Evans Blue (0.01%) was used as a counter stain. The tissue sections were examined with a Leitz fluorescence microscope with a vapour mercury lamp for epi-illumination, BG12 exciter filter and K530 barrier filter.
The immunofluorescent reaction detected a moderate number of cells within the thyroid. No fluorescence was present when the conjugate was used alone, or when the specific antibody was saturated by synthetic human calcitonin. Thus, the specificity of these antisera and cross-reaction between human and gerbil thyrocalcitonin can be assumed.
Results. The C-cells were irregularly distributed through 1 section and between 2 other sections. Depending on the follicule, one or several fluorescent cells were visualized around it (fig. 1). The localization was referred to the

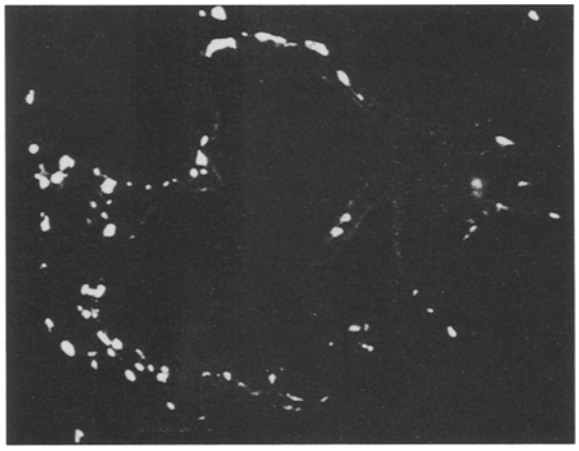

Figure 1. C-cell distribution in gerbil thyroid gland is visualized by immunofluorenscence with antihuman calcitonin sera. Note their parafollicular position.