tically unaltered when the starting fragment length is increased from 0.2 to 2 kb. Here, intermediately repetitive sequences seem to be interspersed only with highly repeated ones.

As shown in figure 2, the previously suggested inverse correlation between the extent of sequence interspersion and genome size in bird nuclear DNA is confirmed. With increasing genome size, from 1.6 to 1.9 pg DNA per haploid genome, less single copy DNA appears to be adjacent to repeated sequences on 2–2.7 kb long fragments. Since the absolute amount of unique DNA shows no significant increase with increasing genome size, our result is interpreted as reflecting an increased average length of repetitive sequences, neighbouring single copy sequences in the larger genomes. It is not possible to say whether the genome size variations in bird genomes are due mainly to additions or loss of repetitive sequences relative to a common ancestral genome. Tandem duplication would serve as a likely mechanism to increase repetitive sequence length. A mechanism reducing repetitive sequence length, on the other hand, if not a totally random process, but rather depending on sequence structure, could be facilitated by repetitive sequence subrepetitivity. The mosaic structure of the long repeats in *Drosophila* DNA, some subrepeats of which are considered as transposable elements[20], may serve as a model for repetitive sequence evolution in birds. There is evidence that the long repeats at least in chicken DNA have a subrepeat composition[21].

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2 Present address. City of Hope Research Institute, Division of Biology, 1450 East Duarte Road, Duarte, CA 91010, USA.
3 Arbeitsgruppe Biologie für Mediziner der Universität Heidelberg, Im Neuenheimer Feld 328-104, D-6900 Heidelberg.
4 R.J. Britten and D.E. Kohne, Science *161*, 529 (1968).
5 J. Schmidtke and J.T. Epplen, Hum. Genet. *55*, 1 (1980).
6 E.H. Davidson, B.R. Hough, C.S. Amenson and R.J. Britten, J. molec. Biol. *77*, 1 (1973).
7 E.H. Davidson and R.J. Britten, Science *204*, 1052 (1979).
8 W.R. Crain, F.C. Eden, W.R. Pearson, E.H. Davidson and R.J. Britten, Chromosoma *56*, 309 (1976).
9 R.C. Ullrich, B.D. Kohorn and C.A. Specht, Chromosoma *81*, 371 (1980).
10 C.K. French and J.E. Manning, J. molec. Evol. *15*, 277 (1980).
11 W.R. Crain, E.H. Davidson and R.J. Britten, Chromosoma *59*, 1 (1976).
12 J.T. Epplen, M. Leipoldt, W. Engel and J. Schmidtke, Chromosoma *69*, 307 (1978).
13 J.T. Epplen, U. Diedrich, M. Wagenmann, J. Schmidtke and W. Engel, Chromosoma *75*, 199 (1979).
14 S. Ohno, Evolution by Gene Duplication. Springer, Berlin-Heidelberg-New York 1970.
15 R.J. Britten, D.E. Graham and B.R. Neufeld, Meth. Enzymol. *29E*, 363 (1974).
16 J. Cairns, Cold Spring Harb. Symp. quant. Biol. *28*, 43 (1963).
17 R.B. Goldberg, W.R. Crain, J.V. Ruderman, G.P. Moore, T.R. Barnett, R.C. Higgins, R.A. Gelfand, G.A. Galau, R.J. Britten and E.H. Davidson, Chromosoma *51*, 225 (1975).
18 R.R. Arthur and N.A. Straus, Can. J. Biochem. *56*, 257 (1978).
19 F.C. Eden and J.P. Hendrick, Biochemistry *17*, 5838 (1978).
20 P.C. Wensink, S. Tabata and C. Pachl, Cell *18*, 1231 (1979).
21 F.C. Eden, J. biol. Chem. *255*, 4854 (1980).

Prostaglandin-like substances in *Propionibacterium acnes*. VI. Characterization of the lipid fraction by gas chromatography in conjunction with mass spectrometry[1]

S. Abrahamsson, L. Hellgren, J.G.A.M. Raaijmakers and J. Vincent

Department of Structural Chemistry, Faculty of Medicine, University of Göteborg, Göteborg (Sweden); Department of Dermatology, Faculty of Medicine, University of Trondheim, Trondheim (Norway), and Research Laboratories, Gist-Brocades, Haarlem (The Netherlands), 3 April 1981

Summary. The lipid fraction of *P. acnes* was submitted to stepwise purification followed by bioassay in order to localize the prostaglandin-like material. GC-MS analysis revealed the occurrence of substances having a part but not a total molecule in common with the prostaglandin family, suggesting that prostaglandin-like substances represent a new group of prostaglandin compounds.

Our investigations on the lipids of *Propionibacterium acnes* revealed the occurrence of prostaglandin-like substances (PLS) in these bacteria[2]. Furthermore, we could demonstrate that these substances mimic E-prostaglandins in various biological situations in vitro (gerbil colon bioassay[3], human blood vessels[4]) or in vivo (hamster cheek pouch). PLS induces an acceleration of cyclic AMP synthesis in rat ovaries[5] and possesses significant chemotactic properties[6]. In spite of great similarities in the biological response between PGE_2 and PLS, our results point to the fact that PLS seems to differ from PGE_2. This was especially apparent in the bioassay with a human utero-tubal junction[7] as well as in cascade superfusion bioassay[8]. Moreover, PLS also demonstrated a potent inhibitory effect on human platelet aggregation[8]. In order to establish whether PLS contain PGE_2, investigations with reversed-phase chromatography and gaschromatography-mass spectrometry were performed[9]. These analyses could clearly demonstrate that PLS were not identical with PGE_2.

This investigation is an attempt to determine the structural composition of the lipid fraction from *P. acnes* with special reference to the localization of PLS.

Material and methods. PLS from *P. acnes* were extracted as described in an earlier communication[2]. In the first purification step prostaglandin-like compounds were separated from other lipids via dry-column chromatography (Woelm). As large samples were necessary for further work, we designed a new dry-column system (unpublished data). This system allows a rapid work-up with a minimum of losses. A solvent system I[10] was used. The biologically active fraction was then submitted to preparative TLC. An automatic liner developed in our laboratory, allowing rapid narrow-band application of samples dissolved in large solvent volumes, was used to apply the samples to precoat-

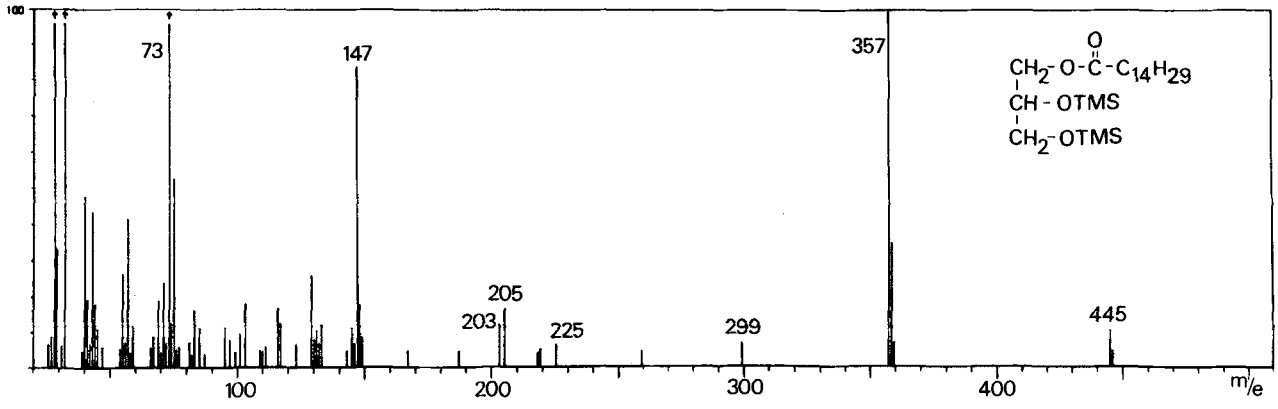

Figure 1. Mass spectrum of the bis-trimethylsilyl derivative of pentadecanoic-1-monoglyceride.

ed plates (0.25 mm, Merck). After development in a solvent system E[11] the main zone was scraped off and eluted with chloroform-methanol (1:1, v/v). The residual zones of the plate were eluted in the same manner. Gerbil colon bioassay revealed exclusive localization of the biologically active compound in the main zone. This fraction was further purified by reversed phase HPLC[12] using Waters Ass. μBondapak C-18 column, solvent system (methanol/water 65:35, v/v+0.01% acetic acid adjusted to pH 5.6 with ammonium hydroxide). The smooth muscle-contracting activity was checked as previously. The final specimen was derivatized with a silylating mixture[13].

The sample was then injected into a gas chromatograph (Varian, model 1440, equipped with a 20 m × 0.3 mm inside diameter glass capillary column coated with SE 54; carrier gas: helium, oven temperature: 250 °C) coupled with a Varian MAT 311A mass spectrometer and Varian SS 166 data system.

Results. The lipid fraction of *P. acnes* is very complex. Determination of most peaks, in spite of the high separating power of the capillary GC-column, was impossible, because most peaks seem to consist of more than one component. However, branched odd-carbon acids (iso and anteiso-C_{15} respectively C_{17}), normal C_{15}-C_{18} and $C_{18:1}$ acids were identified. Comparison of the spectra allowed further ascertainment of the following types of substances: hydroxy acids, monoglycerides and other hydroxy compounds, some of them containing an additional keto group. Two of the peaks were shown to correspond to monoglycerides of pentadecanoic acid; the mass spectrum of 1 of them is shown in figure 1.

The total ion current with deletion of masses lower than m/e 250 of the purified and silylated PLS specimen is shown in figure 2. The mass chromatograms demonstrate a presence of m/e 173, 199 and 129 (not shown in the figure), which are diagnostic for the aliphatic chain of prostaglandins, prostacyclin and tromboxanes. However, the corresponding mass spectra were not similar to known compounds in the prostaglandin family.

Discussion. Investigations concerning the lipid composition of *P. acnes* are sparse. Lipids, especially fatty acids, were dealt with as possible chemical markers for classification and identification purposes. Bacteria related to the coryneform group, such as *P. acnes*, do not contain substantial amounts of unsaturated acids but have high proportions of iso and anteiso branched-chain acids in addition to the straight-chain saturated acids. Moore and Cato[14] compared some strains of *P. acnes* and *Propionibacterium* species and found that *P. acnes* consistently fermented lactate to propionic acid. Consequently, they stated that *P. acnes* does fulfil the diagnostic criteria for members of the genus *Propionibacteria*. Also the presence of other short-chain fatty acids (C_2-C_6) was reported[15,16]. A recent analysis of volatile bacterial metabolites by head-space gaschromatographic technique confirms these observations[17].

However, the most detailed analyses of the cellular fatty acids in *P. acnes* were performed by the Moss group[18-20].

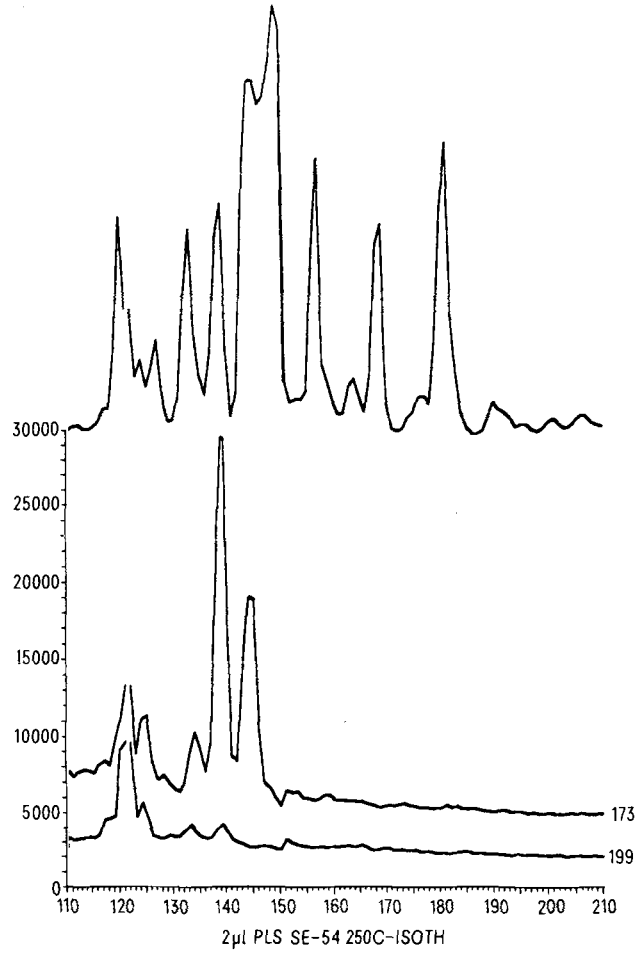

Figure 2. Part of selected mass chromatograms of the silylated PLS sample from *P. acnes* (last purification step). 1 min corresponds to 30 scale divisions.

They found the C_{15}-saturated branched chain acid as a most abundant acid (24–49%). Smaller amount of acids were C_{16}, C_{15} and C_{17}-saturated branched-chain acid. Also the normal saturated fatty acids (C_{12}, C_{14}, C_{18} and C_{20}) as well as unsaturated acids ($C_{16:1}$, $C_{18:1}$) were detected, but only at low levels. Mycolic acids i.e. long-chain 3-hydroxycarboxylic acids having a long alkyl branch on C-2, formed in some corynebacteria, were absent in *P. acnes*[21].

Our GC-MS analysis confirms the previous pharmacological finding that the bioactive compounds in *P. acnes* are prostaglandin-like. Furthermore, we have been able to present evidence about the structure of the common part of these molecules, namely the aliphatic chain. In spite of the fact that this chain is a general feature of the prostaglandin family, it was not possible to find any other member of this group with the same spectral pattern for the remaining part of the molecule. These data agree with recent proposal of Kuehl et al.[22] that, in contrast to early concepts, other prostanoids (i.e. products derived from PGG_2) than classical prostaglandins play a key role in the etiology of inflammation.

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2 S. Abrahamsson, L. Hellgren and J. Vincent, Experientia *34*, 1446 (1978).
3 S. Abrahamsson, N. Engström, L. Hellgren and J. Vincent, in manuscript.
4 E. Berlin, L. Hellgren, O. Thulesius and J. Vincent, Experientia *36*, 197 (1980).
5 L. Hellgren, G. Selstam and J. Vincent, Experientia *35*, 1096 (1979).
6 J. Belsheim, A. Dalen, H. Gnarpe, L. Hellgren, O.-J. Iversen and J. Vincent, Experientia *35*, 1587 (1979).
7 L. Hellgren, B. Lindblom, J. Vincent and L. Wilhelmsson, Experientia *35*, 1298 (1979).
8 S. Abrahamsson, R.J. Gryglewski, L. Hellgren, J. Splawinski, J. Vincent and B. Wojtaszek, Experientia *37*, 164 (1981).
9 S. Abrahamsson, K. Gréen, L. Hellgren and J. Vincent, Experientia *36*, 58 (1980).
10 K.C. Strivastava and V.K.S. Shukla, Z. analyt. Chem. *293*, 45 (1978).
11 J.M. Bailey, E.W. Bryant, S.J. Feinmark and A.N. Makheja, Prostaglandins *13*, 479 (1977).
12 R.C. Murphy, S. Hammarström and B. Samuelsson, Proc. natl Acad. Sci. USA *76*, 4275 (1979).
13 J.G.A.M. Raaijmakers, J. Chromat. *138*, 355 (1977).
14 W.E.C. Moore and E.P. Cato, J. Bact. *85*, 870 (1963).
15 Z. Strapatsaki-Cocovini, Annls Inst. Pasteur, Paris *93*, 647 (1957).
16 K. Takizawa, J. Derm. *4*, 193 (1977).
17 L. Larsson, P.-A. Mårdh and G. Odham, J. clin. Microbiol. *7*, 23 (1978).
18 C.W. Moss and W.B. Cherry, J. Bact. *95*, 241 (1968).
19 C.W. Moss, V.R. Dowell, Jr, D. Farschtchi, L.J. Raines and W.B. Cherry, J. Bact. *97*, 561 (1969).
20 C.W. Moss, V.R. Dowell, Jr, V.J. Lewis and M.A. Schekter, J. Bact. *94*, 1300 (1967).
21 M. Goodfellow, M.D. Collins and D.E. Minnikin, J. gen. Microbiol. *96*, 351 (1976).
22 F.A. Kuehl, Jr, J.L. Humes, G.C. Beveridge, C.G. Van Arman and R.W. Egan, Inflammation *2*, 285 (1977).

Low pH in fungal bud initials

G. Turian

Laboratoire de Microbiologie générale, Université de Genève, Place de l'Université 3, CH-1211 Genève 4 (Switzerland), 11 August 1981

Summary. Quenching of fluorescence of the pH probe, 4-methylesculetin, in bud initials of *Allomyces* hyphae and yeast *(Saccharomyces)* vegetative cells confirms the cytoplasmic acidity (pH not more than 5) in such amitochondrial structures.

Budding is a common morphogenetic process, continuous in filamentous fungi and cyclic in yeasts[1].

We have recently shown by semivital staining with pH indicators that the ultimate, budding tips of filamentous fungi are more acidic than the subapical, mitochondria-rich zone[2]. As this suggested a decreasing pH gradient toward the apices of elongating hyphae, we have attempted to confirm such results using the more refined technique of a fluorescence pH probe. Gerson and Burton[3] have already used with success the fluorescence of 4-methylesculetin (6,7-dihydroxy-4-methylcoumarin) for ascertaining pH in moving plasmodia of the slime mold *Physarum*. We thought that the wide outgrowing apices, budding laterally below differentiated zoosporangia of *Allomyces*, as well as the emergent buds from vegetative cells of *Saccharomyces*, would be especially fitted for an extension of our previous work.

The *Allomyces arbuscula* cultures were obtained from germinating zoospores which produce young mycelia after 24 h of culture at 25 °C in liquid glucose-casein hydrolysate-yeast extract (GCY) medium[4]. Bunches of apically differentiated hyphae, starting to bud new hyphal branches laterally, were transferred on quartz slides into drops of fresh medium saturated with 4-methylesculetin (Senn Chemicals). The fluorescence of this reagent fades below 5.5[3] and is therefore practically extinct in the GCY medium (pH 5.2).

Using exciting UV-light at 350 nm and filters for emitted fluorescent light in the band of 420–450 nm on an O-lux Leitz microscope[5], we observed a vivid greenish-grey fluorescence in the differentiating zoosporangia (relatively alcaline pH) and their supporting hyphae, in sharp contrast to the total lack of fluorescence in the young apices which budded laterally (full extinction in their tip, fig. a). Such quenching of fluorescence of 4-methylesculetin in outgrowing apices, and especially at their ultimate tips indicates that their cytoplasmic (cytogel?) pH is not above 5.0. Alizarin yellow S (sulphonated) stained similar apices orange yellow, a color indicative of a pH-value also around 5; this is in full agreement with previous results obtained with bromocresol purple[2]. In comparison, the subapical cytoplasm and especially its mitochondrial rodlets, stained pinkish violet with alizarin, were most fluorescent with 4-methylesculetin (pH more than 6.0).

From these data with outgrowing hyphae of *Allomyces*, we can tentatively surmise the existence of a decreasing pH gradient from the subapical, mitochondrial zone of the hyphae (average pH at least 6) to the apical hyaloplasm, a presumed cytogel[2], at a pH close to 5.

In a 2nd set of experiments, buds emerging cyclically from