Table 5. *Cocos nucifera:* Left- and right-spiralled palms at LPTI Farms, Manado

Location	Spirality of palms		(L+R)	(L−R)	$\frac{(L-R)^2}{L+R}$
	Lefts	Rights			
Paniki seed garden:					
Talls	1293	1331	2624	−38	0.5503
Dwarf	1544	1415	2959	129	5.6239
Kima Atas - Talls	552	471	1023	81	6.4135
Mapanget - Talls	372	312	684	60	5.2632
Kayuwatu - Talls	286	284	570	2	0.0070
Total	4047	3813	7860	234	6.9664

Table 6. *Cocos nucifera:* Numbers of leaflets on halves of leaves

No. of Palms	Spiral	No. of leaves	Mean length of lamina	Longest leaflets		Mean leaflets on halves			Difference
				Left	Right	Left	Right	Total	
6	Left	36	4.28 m	1.37 m	1.37 m	118.94	116.95	235.99	1.99
6	Right	36	4.39 m	1.34 m	1.37 m	117.14	120.03	237.17	2.89

verify this, 6 leaves each from 6 left-spiralled and 6 right-spiralled palms were lopped and the numbers of leaflets on halves counted. To determine the left and right halves, the leaves are always held vertically with the tip above. The left-hand side of the leaf as it appears to an observer viewing the abaxial surface (lower) is regarded as left half, and the other, the right half. The data are presented in table 6.

When the 5 spirals are considered, in a left-spiralled palm the left half of the leaf bears more leaflets than the right half, and similarly, the right half bears more leaflets in a leaf of a right-spiralled palm.

1 We thank Ir. Hasman Asiz, LPTI, for the facilities and encouragement received for completing this work, and Prof. T. Antony Davis, UNDP/FAO Coconut Agronomist, for the technical guidance in this investigation.
2 T.A. Davis, J. Genet. *58*, 42 (1962).
3 T.A. Davis, J. Genet. *58*, 186 (1963).
4 T.A. Davis, Nature *204*, 496 (1964).
5 T.A. Davis, J. Palm Soc. *15*, 63 (1971).
6 R. Child, Coconut, 2nd ed. Longman, London 1974.
7 J.S. Patel, The coconut. Monograph, 1938.
8 K.P.V. Menon and K.M. Pandalai, The coconut palm. Monograph, 1960.
9 Y. Fremond, R. Ziller and M. de Nuce de Lamothe, The coconut palm. 1966.
10 H. Sudasrip, H. Kaat and T.A. Davis, Phillip. J. Coconut Studies *3*, 5 (1978).
11 T.A. Davis, Proc. Indian Acad. Sci. *40* B, 424 (1974).

The effect of acidic polysaccharides and prostaglandin-like substances isolated from *Propionibacterium acnes* on granulocyte chemotaxis

J. Belsheim, A. Dalen, H. Gnarpe, L. Hellgren, O.-J. Iversen and J. Vincent

Department of Clinical Bacteriology, Gävle Hospital, Gävle (Sweden), Department of Microbiology and Department of Dermatology, University of Trondheim, Trondheim (Norway), 19 March 1979

Summary. Three acidic polysaccharide (AP) fractions and the prostaglandin-like substances (PLS) isolated from *P. acnes* were investigated regarding their chemotactic activities on polymorphonuclear leukocytes. Both AP's and PLS induced a significant chemotactic response, which suggests their involvement in inflammatory acne vulgaris.

Chemotactic agents released within the comedones have been suggested to contribute to the inflammatory reactions in acne vulgaris. It has been well established that *Propionibacterium acnes* are the most important organisms present in the complex comedone microflora, and furthermore, that the whole bacteria are chemotactic for human polymorphonuclear leukocytes[1].

Earlier investigators have maintained that the bacterial lipase is the prime chemotactic factor in *P. acnes* culture supernatants[2]. This has, however been contradicted by Puhvel and Sakamoto[3], who studied different chemotactic preparations of *P. acnes* (i.e. whole cell suspensions, soluble lipopolysaccharides, fresh culture supernatants, and extracellular products obtained from dialyzed cultures) as well as other comedonal components. They found the highest cytotaxin activity to be present in the undialyzed culture supernatants. The lipopolysaccharides did not exhibit a significant chemotactic activity in the concentrations tested. However, all chemotactic activity in comedones was related to bacteria. Both *P. acnes* and its extracellular products were effective as cytotaxigens.

The purpose of the present investigation is to increase our understanding of the chemoattractant properties of *P. acnes*. We evaluated the leukocyte chemotactic response called forth by three acidic polysaccharide (AP) fractions of *P. acnes*, and the prostaglandin-like substances (PLS) extracted from lipid moieties of the bacterial cells.

The AP fractions as well as the PLS were isolated from *P. acnes*. The AP's are released from the cell wall during growth. They can be separated as 3 molecular entities by

Table

Chemoattractant		Spontaneous migration (%)	Directed migration (%)
E. coli lipopolysaccharide	20 mg/ml	100	100
AP fraction I	0.1 mg/ml	100	83
AP fraction II	0.1 mg/ml	101	93
AP fraction III	0.1 mg/ml	102	95
PGE_2 incubated with cells, *E. coli* lipopolysaccharide as chemotactic factor	1 µg/ml	97	96
	10 µg/ml	100	100
	50 µg/ml	99	86
PLS incubated with cells, *E. coli* lipopolysaccharide as chemotactic factor		75	67
PLS as chemotactic factor		100	95

Chemoattractiveness of acidic polysaccharide (AP) fractions I–III in a concentration of 0.1 mg/ml, and prostaglandin-like substances (PLS) with biological activity equivalent to 50 ng PGE_2. PGE_2 in concentrations of 1, 10 and 50 µg/ml were used as a reference to PLS. Spontaneous migration is measured with Medium 199 as chemotactic factor, and expressed as 100± 2.8% (2 SD). Directed (stimulated) migration is expressed as percent of the normal response elicited by stimulation with 20 mg/ml *E. coli* lipopolysaccharide. Normal is expressed as 100± 7.8% (2 SD).

molecular weight (~ 600000, 200000, 50000). But are antigenically identical. The largest of the AP's (fraction I) sensitizes sheep erythrocytes through a single binding site. A high incidence of antibodies to the AP antigens has been demonstrated among healthy blood donors (unpublished data).

The presence of PLS in the lipid fraction of *P. acnes* is to our knowledge the 1st report about the occurrence of these substances in microorganisms[4]. They possess a potent stimulatory effect on ovarian cyclic AMP[5] and the biological response in the human utero-tubal junction bioassay was similar, although not identical to PGE_2[6]. Bioassays on gerbil colon[7] as well as on isolated human umbilical arteries[8] verified that PLS from *P. acnes* mimics the effects of E-prostaglandins. Likewise, in an in vivo investigation (hamster cheek pouch), these compounds induce a PGE-like response[9].

Antigens. P. acnes organisms were grown for 1 week in brain heart infusion broth and on artificial media under aerobic and anaerobic conditions, respectively. Bacteria-free supernatants were dialyzed against tap water and freeze-dried. The material was redissolved in a small volume of physiological saline and applied to a Sepharose 6B-CL column (Pharmacia, Uppsala). The AP containing fractions were identified by double diffusion in agarose gel using specific antisera. Each of the 3 separate fractions were collected, dialyzed against distilled water and freeze-dried. Dried material dissolved in physiological saline (0.1 mg/ml) was used for the chemotactic assays. Extraction of PLS was performed as described earlier[4]. The biological activity of PLS used in this investigation was assayed by gerbil colon bioassay and corresponded to approximately 50 ng PGE_2. Different concentrations of PGE_2 were run in parallel as a reference.

Chemotactic assay. A migration under agarose method, described in detail elsewhere[10], was used. The granulocyte spontaneous migration was determined as the migration called forth when the chemotactically inert Medium 199 was used as the chemotactic factor. The normal value, expressed as 100% ± 2.8% (2 SD), was based on more than 400 individual observations. The normal value for directed migration was obtained by stimulation with the reference *E. coli* lipopolysaccharide at a concentration of 20 mg/ml, and was expressed as 100% ± 7.8% (2 SD). This was based on more than 200 separate observations. All factors known to influence leukocyte migration were kept constant throughout the investigation. Figures shown in the table (referring to directed migration), are based on 12–36 individual observations, using either 2 or 3 leukocyte donors. The relative chemotactic activity of the investigated substances is also demonstrated in the table.

The chemotactic activity of the AP fractions was somewhat lower than the standard *E. coli* lipopolysaccharide reference. However, the concentrations of AP's used were only 0.1 mg/ml and we have seen earlier[11] that the standard *E. coli* reference at such a low concentration elicits an undetectable chemotactic response in our system. Therefore, it is apparent that all 3 fractions were stronger chemoattractants than the *E. coli* reference. The differences seen between the high molecular weight fraction I and the other 2 AP fractions may be due to its ability to bind to cells, and perhaps to a lower diffusion rate in agar.

When the cells were incubated with PLS from *P. acnes* the spontaneous migration was depressed below the normal level. Directed migration could, however, be stimulated by the *E. coli* reference up to 67% of the standard, which would appear to be a significant depression. When PLS was used as the chemoattractant, spontaneous migration was not affected, and the directed migration was found to be only slightly below the 2 SD 'normal range' set out by using *E. coli* lipopolysaccharide as chemoattractant. Considering the vast discrepancy in the concentration of PLS and the reference *E. coli* standard, the PLS was a much more potent chemoattractant than the reference *E. coli* lipopolysaccharide.

Incubation of the PGE_2 reference with the leukocyte suspension caused no depression of lipopolysaccharide-stimulated migration at a concentration of 10 µg/ml, and at a concentration of 50 µg/ml it caused a depression to 86% of normal. The results of our investigation would suggest that the PLS as well as the AP fractions isolated from *P. acnes* are much more potent as depressants of chemotactic migration when they are in direct contact with the granulocyte than PGE_2. The PLS may have an effect similar to PGE_2 with respect to some biologic effects, but would seem to have a different effect on granulocyte migration. The depression of spontaneous migration by incubation with PLS at such a low concentration substantiates this. However, the PLS and AP fractions appear to have a much greater potency as mediators of the chemotactic response than PGE_2.

P. acnes organisms are not unique in the production of cytotaxins acting as chemoattractants, i.e. most enterobacterial lipopolysaccharides are known to stimulate leukocyte chemotaxis. The whole bacterium has been reported to attract monocytes and macrophages in vitro; however, it

has not previously been shown to have a significant effect on the neutrophil[12]. The chemoattractant material appears to be a lipid, released spontaneously into surroundings from fibrillar structures which lie on the outside of the bacterial cell[13]. The data obtained from this investigation suggests that the AP fractions and the PLS from *P. acnes* are important as chemotactic factors, and supports the concept of lipids as attractants. The potent chemoattractant effect of AP's and PLS, in addition to the depressive effects of PLS in direct contact with the granulocytes may be an essential factor in the development of the inflammatory lesions seen in acne vulgaris.

1 D.J. Gould, W.J. Cunliffe and K.T. Holland, J. invest. Derm. *68*, 251 (1977).
2 W.L. Lee and A.R. Shalita, J. invest. Derm. *70*, 219 (1978).
3 M.S. Puhvel and M. Sakamoto, J. invest. Derm. *71*, 324 (1978).
4 S. Abrahamsson, L. Hellgren and J. Vincent, Experientia *34*, 1446 (1978).
5 L. Hellgren, G. Selstam and J. Vincent, Experientia *35*, 196 (1979).
6 L. Hellgren, B. Lindblom, J. Vincent and L. Wilhelmsson, Experientia *35*, 1298 (1979).
7 S. Abrahamsson, N. Engström, L. Hellgren and J. Vincent, in preparation.
8 E. Berlin, L. Hellgren, O. Thulesius and J. Vincent, Experientia, in press (1980).
9 L. Hellgren, M. Romanus and J. Vincent, in preparation.
10 H. Gnarpe, J. Belsheim and S. Persson, Infection *6*, suppl 1, 98 (1978).
11 H. Gnarpe and J. Belsheim, in manuscript.
12 P.C. Wilkinson, G.J. O'Neill and K.G. Wapshaw, Immunology *24*, 997 (1973).
13 R.J. Russell, R.J. McInroy, P.C. Wilkinson and R.G. White, Immunology *30*, 935 (1976).

Net ATP synthesis by running the red cell calcium pump backwards

A. Wüthrich, H.J. Schatzmann[1] and P. Romero

Veterinär-Pharmakologisches Institut, Universität Bern, Länggass-Strasse 124, CH-3000 Bern (Switzerland) and Departamento de Biologia Cellular, Universidad Central de Venezuela, Caracas (Venezuela), 30 August 1979

Summary. Ca^{2+} loaded inside-out vesicles from human red blood cells, yielding Ca^{2+} into a Ca^{2+} free medium with 4 mM EGTA, 2 mM ADP and 10 mM phosphate, produced an excess of 14.9 pmoles · min^{-1} · (mg protein)$^{-1}$ of ATP compared to controls in which the transmembrane Ca^{2+} gradient was abolished by the ionophore A 23 187.

The human red blood cell membrane is equipped with an ATP-fuelled Ca pump[2-4] which under physiological conditions extrudes Ca^{2+} ions from the cytosol against a gradient of 10^3–10^4. Its normal performance can be symbolized as:

$$n\,Ca_i^{2+} + ATP \rightarrow n\,Ca_0^{2+} + ADP + PO_4^{3-}$$

(Ca_i^{2+}, Ca_0^{2+} = internal and external calcium). It is of considerable interest to know whether such an active transport system is reversible as a whole, i.e. whether an (electro) chemical gradient (of Ca^{2+}) can run down in such a way that the energy is not dissipated but appears as bond energy in a chemical compound (ATP). In order to test this point one must measure the change in ATP concentration after having made some (or all) concentrations on the right larger, and those on the left smaller than normal. The Na pump[5-8] and the Ca pump of the sarcoplasmic reticulum[9] have been run backwards successfully. Confirming an earlier report[10] Rossi et al.[11] recently demonstrated that in ATP depleted, PO_4^{3-} enriched human red cells 2 mM external Ca^{2+} concentration is sufficient to induce $^{32}PO_4^{3-}$ incorporation into ATP. However, in those experiments the net ATP concentration decreased significantly during the process, which leaves room for doubt whether the energy for ADP^{32}P synthesis was indeed derived from the Ca^{2+} gradient rather than from concomitant ATP hydrolysis.

In the present experiments inside-out vesicles were prepared from fresh human red cells essentially according to Steck and Kant[12] (see legend to table). After separation on a dextran cushion 60% of the material was present as closed inside-out vesicles as judged from latent acetylcholinesterase[12]. The vesicles were first loaded with Ca by pump action in a medium containing 20 or 40 μM Ca^{2+}, 2 mM ATP and excess Mg^{2+}. They were subsequently washed in buffered KCl solution. This procedure resulted on average in 21.5±2.4 mM (SEM, n=21) internal Ca^{2+} concentration (for an estimate of vesicle volume[17]. The pellet was resuspended by vigorous shaking in a Na-free medium containing 10 mM PO_4^{3-}, 2 mM ADP, 4 mM EGTA, 5 mM Mg^{2+} and 20 μM P^1, P^5-di(adenosine-5′-)pentaphosphate, a potent adenylate kinase inhibitor[13], and incubated for 15 min at 37 °C. (When necessary, contaminating ATP in ADP (Boehringer) was removed by eluting 200 μmoles at a time from a 15×1.2 cm column of Dowex SBR (type I) anion exchange resin with 0.02 N HCl in the cold, followed by neutralisation with KOH and freeze-drying). ATP concentration was measured by the luciferin-luciferase method, comparing samples with standards having the same composition except for ATP concentration. Crude firefly extract (Sigma) was chromatographed on a 100×2.6 cm G 100 Sephadex column[14,15] to free it from adenylate kinase. Luminescence was assayed by photon counting either with an EMI 6097 photomultiplier tube without wavelength discrimination or on a Packard scintillation spectrometer. Calibration curves were exponential in the first and linear in the second case.

The conditions used (described in detail in legend to the table) seemed appropriate to induce reversal of the pump direction since addition of ADP caused an extra leak of Ca^{2+} of 239±76 pmoles · min^{-1} · (mg protein)$^{-1}$ (6 experiments, P=0.024) from the vesicles during 15 min at 37 °C. The table summarizes experiments demonstrating that this ADP dependent Ca^{2+} movement was accompanied by net ATP synthesis. After loading the vesicles with Ca^{2+} the suspension was divided into 2 portions which were treated identically except that one (control) was treated with 10 μM A 23 187, a rather Ca-specific ionophore, in order to abolish the Ca^{2+} gradient across the membrane. It may be seen from the table that in the presence of a Ca-gradient across the membrane an extra ATP production of 14.9 pmoles · min^{-1} · (mg protein)$^{-1}$ took place. There was a large basal ATP formation, probably attributable to incomplete adenylate kinase inhibition. On the other hand there must have been some ATP consumption by the Mg^{2+} stimulated ATPase. Its activity in vesicle preparations was 0.85 nmoles · min^{-1} · (mg protein)$^{-1}$ at 100 μM ATP (8 experiments).