may also be interpreted as an adaptation to the highly specific and homogeneous laboratory habitat.

Natural selection forces can be assumed to be smallest in populations 1, at least with respect to fertility and mating success, and largest in populations 3, where opportunity is given for natural selection to act on all fitness components; populations 2 represent an intermediate situation. It has been proposed[8] that pupa weight of natural populations of *T. castaneum* is subject to centripetal (stabilizing) selection, the genes controlling the expression of the trait acting in an essentially additive manner and showing overdominance for fitness. Under this model, a relaxation of natural selection forces in the short term should occur when transferring wild populations to the laboratory. Consequently, an increase of the genetic and phenotypic variances should follow without necessarily implying a change of the mean. This is in agreement with our results. On the other hand, it has also been proposed[9] that fitness may be determined by the trait itself; our observations are also consistent with the consequences of this alternative model. It seems difficult to discriminate experimentally between both models although they are conceptually very different[10,11]. In the long term, the domestication process may result in an increase of the mean pupa weight, as suggested by the estimates found for our laboratory populations. This increase can be interpreted as the consequence of directional selection towards a new optimum which is subsequently maintained by centripetal selection. This process is better observed in our populations 3 where the action of natural selection should be more intense.

1 We wish to thank Prof. F.J. Ayala for useful discussions in the preparation of this paper.
2 A. Robertson, in: Population Biology and Evolution, chapter 1. Ed. R.C. Lewontin. Syracuse University Press, Syracuse, N.Y. 1968.
3 F. Orozco, A.E. Bell, Can. J. Genet. Cytol. *16*, 49 (1974).
4 A.E. Bell, C.H. Moore, Egypt. J. Genet. Cytol. *1*, 92 (1972).
5 M.A. Toro, Thesis, Universidad Complutense de Madrid 1978.
6 C. López-Fanjul, W.G. Hill, Genet. Res. *22*, 69 (1973).
7 J.F. McDonald, F.J. Ayala, Nature *250*, 572 (1974).
8 P.K. Kauffman, F.D. Enfield, R.E. Comstock, Genetics *87*, 327 (1977).
9 K. Mather, Symp. Soc. exp. Biol. *7*, 66 (1953).
10 A. Robertson, in: Heritage from Mendel, chapter 14. Ed. R.A. Brink. University of Wisconsin Press, Madison 1967.
11 A. Roberston, in: Genetics Today, vol.3, p.527. Ed. S.J. Geerts. Pergamon Press, Leyden 1965.

Prostaglandin-like substances in *Propionibacterium acnes*. III. Differential contractile effects on smooth muscle layers of the human utero-tubal junction[1]

L. Hellgren, B. Lindblom, J. Vincent and L. Wilhelmsson

Department of Dermatology, University of Trondheim, Trondheim (Norway); Departments of Dermatology, Obstetrics and Gynecology, Sahlgren's Hospital and Department of Structural Chemistry, Faculty of Medicine, University of Göteborg, S-400 33 Göteborg 33 (Sweden), 10 January 1979

Summary. The biological activity of a lipid fraction extracted from *P. acnes* was tested on isolated smooth muscle strips from the human utero-tubal junction. The bioassay experiments support the concept that prostaglandin-like substances (PLS) occur in *P. acnes*. However, in the bioassay system used, the effect of PLS was different from that of $PGF_{2\alpha}$ and PGI_2 but similar, although not identical, to that of arachidonic acid and PGE_2.

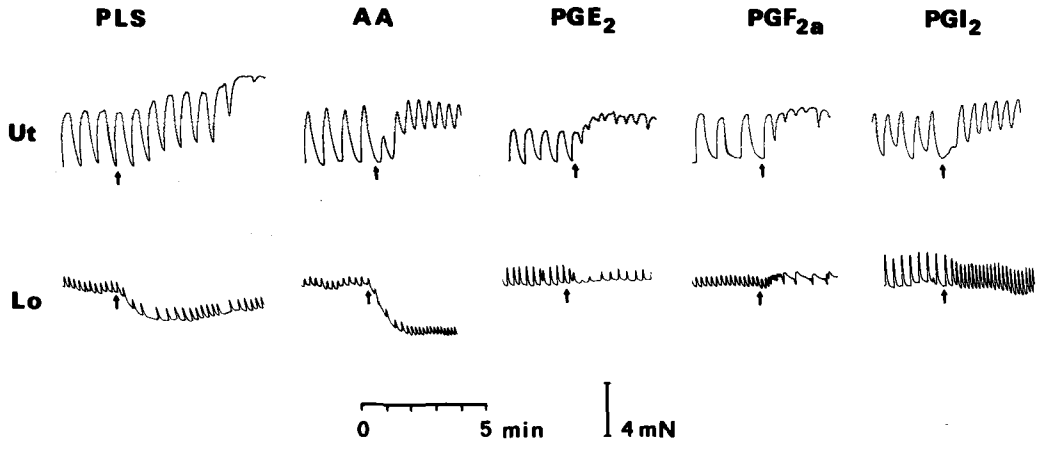
Acne vulgaris is prerequised by the presence of sebum and *Propionibacterium acnes*. The relationship between sebum excretion rate and clinical severity of the disease is now well established[2]. However, the exact mechanism by which androgens enhance sebum excretion rate, and the further development of an inflammatory acne lesion, is still not fully understood. The fatty acids produced by microbial lipolysis of sebaceous triglycerides have been suggested as initiators of a toxic inflammatory reaction[3]. Recent investigations claim that these fatty acids are only mildly inflammatory when injected in physiological concentrations into human skin[4]. With guinea-pig sensitization tests it has been shown that especially the short and middle-chain fatty acids may act as potent allergens[5]. The precise role of *P. acnes* as a trigger factor in the development of the initial inflammatory lesions is yet to be determined. Deleterious bacterial products are probably not only lipase, but might also be other enzymes, antigens or hitherto unidentified metabolites acting as terminal inflammatory mediators.

At the beginning of 1977, prostaglandin-like substances (PLS) of the E-type were isolated from the lipid fraction of *P. acnes*[6]. Recently it has also been demonstrated that these substances stimulate the formation of cyclic AMP in rat ovaries[7]. In addition, bioassay experiments on gerbil colon[8], as well as on human umbilical artery[9], verify that the PLS from *P. acnes* mimic the effects of prostaglandins of the E-type. Likewise, in an in vivo study (hamster cheek pouch) these compounds induced a PGE-like response[10].

Natural prostaglandins such as PGE_2 and $PGF_{2\alpha}$ may be bioassayed by studying their contractile effects on 3 different animal muscle tissues: the rat stomach, the chicken rectum and the rat colon[11]. The 3 muscle layers of the human utero-tubal junction (UTJ) react differently to diverse prostaglandins and may offer an alternative possibility for detection of specific prostaglandin compounds[12].

The aim of this investigation was to elucidate the biological activity of PLS from *P. acnes* by comparing the contractile effects with those of arachidonic acid (AA), PGE_2, $PGF_{2\alpha}$ and PGI_2. The in vitro technique utilized is a modification of a method which was originally developed to elucidate the effect of various biologically active substances on isolated circular and longitudinal smooth muscle at the ampullary-isthmic junction of the human Fallopian tube[13].

Material and methods. The spontaneous contractile activity of small strips from the external (uterine) and the inner (longitudinal) smooth muscle layers of the human UTJ was recorded as described earlier[12]. Tissue specimens were obtained from cycling women undergoing sterilization or hysterectomy. The strips were approx. 4 mm long with a cross sectional area of about 1.0 mm^2. 1 'uterine' and 1 'longitudinal' strip was mounted in each organ chamber, which was filled with 50 ml of Krebs bicarbonate buffer



Typical tracings illustrating the stimulatory and inhibitory effects of the test substances used in the assay on muscle strips from the external spiral-shaped uterine layer (*Ut*) and the inner, longitudinal layer (*Lo*) at the UTJ. PLS = prostaglandin-like substances from *P. acnes* (3 µl, corresponding to a concentration of 0.06 µl/ml), AA = arachidonic acid (10 µg/ml), PGE_2, $PGF_{2\alpha}$ and PGI_2 (100 ng/ml). The uterine specimens are stimulated by all the substances but the response elicited by PLS developes more slowly than the effects of the other substances, which are more acute in character. In the longitudinal layer, the effect of PLS is very similar to that of AA, with a marked relaxation as the most striking feature. The addition of each sample to the organ chamber is indicated by an arrow.

(pH 7.34) and continuously areated with a mixture of 96% O_2 and 4% CO_2 at 37 °C. The bioassays were performed with a lipid fraction isolated from *P. acnes* according to a previously described technique[5]. The procedure applied represents an established method for the recovery of prostaglandins from biological material and involves careful extraction with ethyl acetate followed by thin-layer resp column chromatographic separation. The reference substances AA, PGE_2, $PGF_{2\alpha}$, and PGI_2 were added to the organ chamber either as a single injection or in gradually increasing amounts, in order to estimate the threshold concentration.

Results. Addition of less than 1 µl of the lipid fraction to the organ chamber did not alter the spontaneous contractile activity of the strips. Higher concentrations, 1–10 µl, induced a pronounced response in both preparations. The response of strips from the longitudinal layer was inhibitory and characterized by reduced basal tonus and decreased frequency of contractions (figure). In contrast, strips from the uterine muscle layer reacted with an increase in tonus and at volumes of ≥ 3 µl or more, the spontaneous activity developed into a contracture. The response of the longitudinal layer appeared within 1 min after addition of the substance and lasted for more than 20 min. In the uterine layer, the response was delayed and the maximal effect was not obtained until 5 min after the injection. The effect lasted for at least 20 min.

Among the reference substances, AA caused an immediate stimulatory response with increased frequency and amplitude of contractions when tested on uterine preparations. In the longitudinal layer, AA caused a dramatic reduction of tonus, sometimes accompanied by decreased frequency and amplitude. The qualitative responses of PGE_2, $PGF_{2\alpha}$ and PGI_2 on uterine specimens were generally uniform, whereas the effects of these prostaglandines on the longitudinal layer exhibited certain differences. PGE_2 acted inhibitory, while $PGF_{2\alpha}$ and PGI_2 elicited clear-cut stimulatory responses[12].

Discussion. The present findings support the concept that PLS occur in the lipid fraction of *P. acnes*. The effects of PLS in this system seemed to be reasonably similar to those of AA and PGE_2, although not identical. The stimulatory effect of PLS on the uterine layer occurred with a certain delay, which was not the case for AA or PGE_2. The effects of PLS, AA and PGE_2 on the longitudinal layer were qualitatively identical, although the inhibition caused by PGE_2 was not accompanied by a reduced tone. The delay in response of the uterine layer to PLS may indicate a metabolic transformation of these substances during the course of the assay.

It would have been preferable to determine the potency of PLS in terms of added amount. However, the extract contained very small amounts of soluble lipid material, and quantification, *e.g.* by dry weight determination, was not feasible in our experimental situation. If known volumes of the added PLS fraction, extracted from known number of bacterial cultures (incubated under identical conditions and time) are compared to known amounts of added PGE_2, it appears that 3 µl of the lipid fraction corresponded to 5 µg of PGE_2 in the present bioassay system.

It may be concluded that the lipid extract tested in the present bioassay system contains prostaglandins or potent transient derivatives. The effects of PLS resemble those of PGE_2 but do not seem to be identical. Definite characterization of the structure of the compound or compounds in question requires further purification and analysis by mass spectrometric methods.

1 Acknowledgments. We are greatly indebted to Ass. Prof. Krister Gréen and Prof. Nils Wiqvist for critical review of the manuscript.

2 W.J. Cunliffe and S. Shuster, Lancet *1*, 685 (1969).
3 J.S. Strauss and A.M. Kligman, Archs Derm. *85*, 779 (1960).
4 S.M. Puhvel and M. Sakamoto, J. Invest. Derm. *68*, 93 (1977).
5 L. Hellgren and J. Vincent, Curr. Med. Res. Opin. *5*, suppl. 2, 42 (1978).
6 S. Abrahamsson, L. Hellgren and J. Vincent, Experientia *34*, 1446 (1978).
7 L. Hellgren, G. Selstam and J. Vincent, Experientia *35*, 196 (1979).
8 S. Abrahamsson, N. Engström, L. Hellgren and J. Vincent, in manuscript.
9 E. Berlin, L. Hellgren, O. Thulesius and J. Vincent, in manuscript.
10 L. Hellgren, M. Romanus and J. Vincent, in manuscript.
11 S.H. Ferreira and J.R. Vane, Nature *216*, 868 (1967).
12 L. Wilhelmsson, B. Lindblom and N. Wiqvist, Fert. Steril., in press.
13 B. Lindblom, L. Hamberger and N. Wiqvist, Fert. Steril. *30*, 553 (1978).