female flies, respectively, at 20% RH and 0.009 and 0.03 for flies in an atmosphere with 100% RH. These results show that males were slightly more active than females, but that flies in damp conditions became less active than those in drier situations. Furthermore these differences in activity level suggest that flies should tend to aggregate in moist microhabitats where the degree of activity is least, whereas in fact they tend to be distributed throughout the whole of a gradient and also show slight statistical preferences for the wet end of the gradient. These apparent contradictions can be resolved if it is assumed that the reactions of individual flies depend upon their physiological condition, which in turn is determined by the conditions under which the flies have previously been kept. Further work showed that flies kept at 20% and 100% RH under similar conditions, initially had the same water content, but after 48 h the flies kept at the lower humidity had a lower water content than the flies kept in moist air; while the fat content of flies kept at a RH of 20% ranged from 2.6 to 39.1% (a range of 36.5%) of the b.wt compared with a range of 15.8 to 26.1% (i.e. a range of 15.8%) for the flies kept in a moist atmosphere. Buxton and Lewis[2] found that tsetse flies kept in a low humidity metabolize fats to maintain their water content. These experiments show that *G. morsitans* loses water and metabolizes more fat when kept in low humidities and that these physiological changes are associated with a rise in the level of activity, which would initially cause them to move away from existing conditions. Over a period, these various factors may encourage flies to aggregate in moist situations, where the activity level is minimal, but they are probably also responsible for producing a wide range of reactions within a given fly population. Hungry flies are attracted to mammalian hosts by their form and smell[5] radiant heat[6]. The above experiments show that gorged flies are initially attracted to dry microhabitats and then show an increased tendency to settle in moister situations.

These experiments show that the reactions of *G. morsitans* to humidity and light gradient are more complex than has previously been supposed and that the validity of previous ecological investigations needs to be reassessed in the light of these findings.

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Prostaglandin-like substances in *Propionibacterium acnes* II. Stimulatory effect on ovarian cyclic AMP

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Summary. The prostaglandin-like substances (PLS) from *Propionibacterium acnes* increased the ovarian tissue levels of cyclic AMP (cAMP) approximately 2-fold. The lipid material extracted from *P. acnes* thus behaved like PG's of the E-type, and since it is unlikely that other known stimulators of the ovarian cAMP system can be present in the bacterial lipid fraction, these experiments give further evidence in favour of the occurrence of PLS in *P. acnes*.

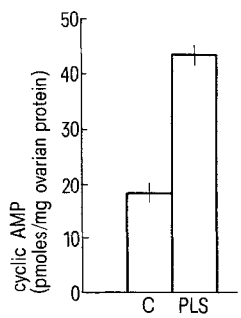
P. acnes is acknowledged to play a pivotal role in the inflammatory reactions in acne vulgaris. On studying lipids of *P. acnes*, we discovered the occurrence of prostaglandin-like substances (PLS)[1,2]. The bacteria were cultured on chemically defined media, carefully extracted and the lipid fraction was further purified by several chromatographic procedures. The combined gas chromatographic-mass spectrometric analysis is now in progress. However, to investigate the biological properties of these substances, bioassays were performed on smooth muscle of the utero-tubal junction (UTJ), as well as on gerbil colon. The UTJ bioassay demonstrated that these compounds possessed a prostaglandin-like effect on the spontaneous contractility of the smooth muscle strips[3]. In the gerbil colon bioassay contractile effect was recorded[4]. Thus, in both systems, the PLS of *P. acnes* mimics prostaglandins of the E-type (PGE). Lately, these compounds has been shown to elicit the PGE-like response in hamster pouch vessels[5].

It is well-known that PGE are potent stimulators of the cyclic AMP (cAMP) system in the prepubertal rat ovary (for references see Selstam et al.[6]). To characterize more closely these new substances extracted from *P. acnes*, their effect on the ovarian cAMP system was studied.

Animals. Rats of the Sprague-Dawley strain, 23 days old, obtained from Anticimex Ltd, were used. They were deprived of food 24 h before the experiments, but allowed to drink tap water ad libitum.

Chemicals. Tritiated cAMP (^{3}H-cAMP) with a specific activity of 31 Ci/mmole was purchased from New England Nuclear Co., Boston, Mass., USA. Cyclic 3′, 5′-AMP-dependent protein kinase and protein kinase inhibitor for the cAMP assay were obtained from Sigma Ltd. All other chemicals were of analytical grade and purchased from Merck Co. or Sigma Ltd.

Experimental procedures. 4 rats were sacrificed by cervical fractures and the ovaries were rapidly removed and placed in ice-chilled buffer. Each ovary was trimmed free from extraneous tissues, rinsed, blotted on a filter paper, weighed (range: 6.9–9.1 mg) and put into a 'preincubation' flask. The preincubation period lasted for 60 min in a medium



Effect of prostaglandin-like substances (PLS) from *P. acnes* on the cAMP content in the prepubertal rat ovary when compared with control (C). For details see the text.

consisting of 1 ml Krebs bicarbonate buffer with half the normal calcium concentration (1.25 mM) and containing glucose (1 mg/ml). The ovaries were then transferred to new flasks containing fresh medium with the addition of 50 µl of extracted substances. Incubations were performed at 37 °C in a shaking water bath and with 95% O_2+5% CO_2 as gas phase. At the end of the incubation, the ovaries were immediately frozen in Frigen 11 (chilled with dry ice).

Analysis of cAMP. The ovaries were stored at −80 °C until analysis. The tissue content of cAMP was determined according to Gilman[7]. Total ovarian protein content was determined according to Lowry[8].

Statistical methods. Values are given as mean±SEM. Significance was tested with Student's t-test. A p-value of less than 0.05 was obtained and considered as significant.

The effect of PLS from *P. acnes* on prepubertal ovary is shown in the figure. As can be seen, PLS increased the cAMP content approximately 2-fold. The known stimulators of the cAMP system are gonadotropins (for references see Selstam et al.[9]), catecholamines (for references see Condon and Black[10]) and prostaglandins (for references see Selstam et al.[6]). Since a lipid fraction of *P. acnes* was investigated the above mentioned substances, except prostaglandins, can be excluded. Therefore, the present study gives further evidence that the lipid fraction of *P. acnes* contains prostaglandins or prostaglandin-like substances. If these substances interfere with the surrounding tissue, there is a new possibility to explain, at least in part, the inflammatory response to *P. acnes*. The hypothesis that can be put forward is that substances such as PLS, produced by the bacteria, may, besides a reaction to the bacteria itself, play a role in the inflammation. Prostaglandins are known to possess many diverse biological effects, including their role as important inflammatory mediators[11] and may therefore also directly contribute in the inflammatory response in acne vulgaris.

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Decreased cyclic GMP levels in rat cecum mucosa during adaptive stimulation of Na-K-ATPase[1,2]

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Summary. In rat cecal mucosa, Na-K-ATPase specific activity and sodium and fluid absorption were increased by giving polyethylene glycol administration with the drinking water. Whereas cyclic AMP levels were unchanged, cyclic GMP was reduced by about 50%. This finding suggests a regulatory role of cyclic GMP in intestinal sodium and fluid absorption.

Active sodium absorption in the intestine is stimulated in acute experiments by several alpha-adrenergic and muscarinic cholinergic agonists[4]. Under these conditions, the guanosine 3′:5′-monophosphate (cGMP) concentration of the cell increased, whereas the concentration of adenosine 3′:5′-monophosphate (cAMP) was unaffected[5]. On the basis of these results, a functional connection between active sodium absorption and the cGMP content of the transporting cell has been considered[5]. The hypothesis, however, has not been tested in other situations involving stimulation of active sodium transport in the intestine.

In rat cecum mucosa, active sodium absorption and the specific activity of the Na^+-K^+-activated adenosine triphosphatase (Na-K-ATPase) increase persistently when the nonabsorbable polymer, polyethylene glycol (PEG), is added to the diet[6-9]. The effect is significant after a few days and maximal after about 2 weeks, and is not due to the concomitant increase in cell number, i.e., the hyperplasia[6,7]. In the present communication, we report that the intracellular concentration of cGMP in the mucosa fell parallel with this adaptive increase in active sodium transport and Na-K-ATPase, whereas the cell content of cAMP remained unchanged.

Materials and methods. Male Wistar rats (160–200 g) were used. Control animals received tap water ad libitum. In experimental rats, polyethylene glycol 4000 (160 g/l, Serva, Heidelberg) was added to the drinking water. After 4 to 56 days, rats were anaesthetized with sodium thiobarbital (Inactin®, 80 mg/kg). The cecum was excised and washed with ice-cold 0.9% (w/v) NaCl solution. The mucosa was gently scraped off as described previously[8] and transferred to a tube containing 4 ml of 1 N perchloric acid, about 0.1 pmoles ^{3}H-cAMP (38.4 Ci/mmole, NEN) and 0.2 pmoles ^{3}H-cGMP (21 Ci/mmole, Amersham) for determination of cyclic nucleotide recoveries. After homogenization and centrifugation, cyclic nucleotides were purified by chromatography on a 0.65×2.2 cm column of aluminium oxide (90 neutral, activity grade I, Merck, Darmstadt)

Influence of the time interval between cecum excision and mucosa homogenization on cAMP levels in rat cecum

Time (sec)	cAMP (pmoles/mg protein)
20–29	6.7± 2.6 (3)
30–39	8.0± 0.6 (23)
40–49	11.5± 0.9 (6)
50–59	25.1± 6.0 (6)
60–69	25.1±13.4 (4)
70–99	21.6± 3.5 (7)

Data are means±SEM with the number of observations in parentheses.