the cage top. Continuous access to food was provided via a trough which protruded downward from the cage top about 7 cm. In each case, the trough was constructed of the same material as the cage top.

The vertical electric field was generated by grounding the metal cage top and applying the appropriate voltage to an insulated metal plate which was placed under the plastic cage. The horizontal electric field was generated by employing a suitably mounted capacitor in which neither the energized plate nor the grounded plate made physical contact with the plastic cage. The relatively high strength vertical and horizontal electric fields employed resulted in electric field induded vibration in the vicinity of the cages of about 2.5×10^{-8} cm/sec, which was smaller than the ambient vibration in the absence of the electric fields.

The results are given in the Table. In the 1st generation, males and females reared in both the horizontal and vertical electric field were significantly smaller than the controls when measured at 35 days postpartum. Larger depressions in average body weight were seen in the 2nd generation at 35 days postpartum, while at 10 weeks postpartum the differences between the experimental and control weights had narrowed considerably. A very large mortality rate in the vertical field mice during the 8-35 day postpartum period was also noted. A large mortality rate was again seen in the vertical groups in the 3rd generation, however the only group whose body weights were significantly affected were the males exposed to the vertical electric field.

The mice exposed to the electric fields demonstrated obvious effects compared to the equivalent control mice. The most severe effects were seen in the males and females exposed to the vertical field, possibly due to the greater intensity of the vertical field. Alternatively, a direction-dose factor may be involved. In the vertical field experiments, a relatively constant dorsi-ventral exposure vector existed, particularly for the central nervous system, regardless of the movement of the mice. In the horizontal field, the relationship between the mice and the field direction was constantly changing as a result of their movement. The increased severity in the vertically exposed mice may therefore indicate the existence of a directionally sensitive sensing mechanism within the mouse which initiates a response proportional to the time the electric field is along a certain axis.

The vertically exposed mice experienced (after weaning) microcurrents of the order of 5 μA when eating or drinking, because both acts necessitated touching grounded conductors. The horizontally exposed mice experienced much less microcurrent because their entire cage was constructed of plastic. The possibility must therefore be considered that the greater weight depressions and the increased mortality in the vertical mice may be related to the grounding microcurrents.

Long term exposure to altered environmental conditions may lead to adaption via a variety of mechanisms including exclusion of susceptable individuals from the genetic pool by death prior to maturity or by favoring the survival of those genetically constituted to better resist the altered circumstances. The elevated 8–35 days mortality rate in the 2nd generation, and the decreased severity of the weight differentials between the experimental and control mice in the 3rd generation may be interpreted as evidence for such a mechanism. On the other hand, the elevation of the 8-35 day mortality rate in the 3rd generation is some evidence to the contrary. More extensive studies are necessary to explore this possibility, as well as to explore the basic causative factors for the effects described herein.

Cytokinin Contents and cAMP Metabolism During Growth of *Escherichia coli*[1]

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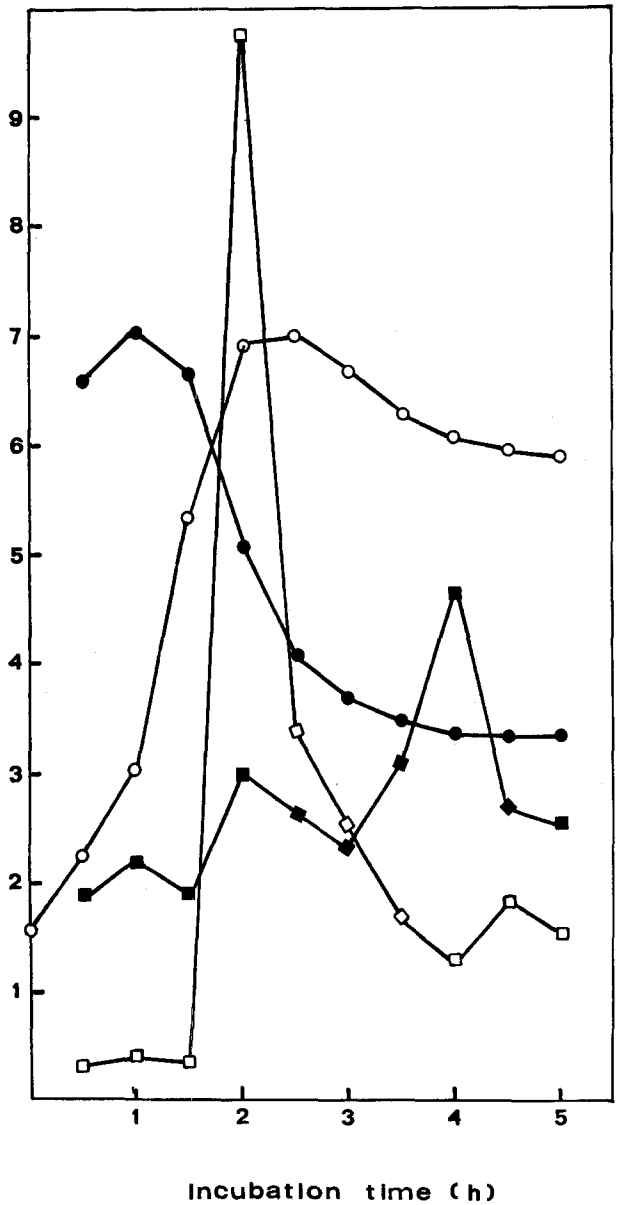
Summary. During *Escherichia coli* growth, we found an inverse correlation between free cytokinin content and cAMP level. The rates of synthesis of adenylate-cyclase and cAMP-phosphodiesterase were practically constant.

At present our knowledge of the role of cytokinins (N^6-derivatives of adenine) in microbial physiology is still scarce[3–5]. Recent evidence has shown, for a wide range of biologically active compounds, including cytokinins[6–9], an action correlated to the adenosine, 3′:5′ monophosphate (cAMP) system[10]. Since cytokinins do not induce as evident effects on microorganisms as on animals and higher plants[11], in order to verify a possible interaction between cytokinins and cAMP in microbial metabolism, as a first approach, we measured cytokinin and cAMP levels together with the rate of synthesis of the enzymes responsible for cAMP metabolism during a cultural cycle of *Escherichia coli*.

Materials and methods. Escherichia coli, B/b strain, kindly provided by Dr. M. L. BARNETT, Cambridge University, England, was used. Bacteria were aerobically grown in M9 Salts Medium[12] at 37 °C. Growth was measured turbidimetrically and by direct counts in a Petroff-Houser chamber. Intra- and eso-cellular cAMP was measured according to BUETTNER et al.[13], using the protein-binding assay of GILMAN[14]. Presentation of intracellular concentrations of cAMP in units of molarity is based on an accessible volume of 4.7×10^{-12} ml/bacterium during the first 120 min of growth, and on a volume of 4.28×10^{-12} ml/bacterium for the residual time. At 30 min intervals, 5 l of culture were rapidly cooled to 1 °C and centrifuged by a continuous MSE H.S.18 apparatus. The extraction of free cytokinins from known weights of wet cells was carried out according to EINSET and SKOOG[15]. The cytokinin activity of the diluted extracts was measured according to VAN ONCKELEN and VERBEEK[16]. For the enzyme assays, washed cells were added to glass beads, 2 parts in weight, and 60 m*M* pH = 7.5 *Tris*-HCl buffer, 3 parts in volume, and disrupted in a Braun Supercell-homogeneizer. The homogenate was centrifuged for 30 minutes at $30{,}000 \times g$, and the fluid supernatant was directly employed in enzyme reactions. The same extract was used in blank reactions after a 3 min treatment in boiling water. Adenylate-cyclase assays were carried out according to BÜRK[17], by recording ^{14}C-cAMP increases. In the cAMP-phosphodiesterase assays the reaction mixture contained, in 60 m*M* pH = 7.5 *Tris*-HCl, 0.5 m*M* ^{3}H-cAMP, 2 m*M* $MgCl_2$, 2.5 m*M* dithioerytritol; ^{3}H-cAMP decreases were controlled.

Results and discussion. During the fast multiplication phase, free cytokinin content of *Escherichia coli* B/b is the highest, while cAMP level is the lowest. During the late-log-phase and the reduced multiplication phase, free cytokinins progressively decrease to minimal values, while cAMP reaches the highest level (Figure). These results allow us to generalize to a certain extent that cAMP level is inversely correlated to cellular growth rate[13,18–22]. On the other hand, free cytokinin content, because it is higher during the exponential phase and lower in the stationary phase, may be considered directly correlated to the growth rate. We pointed out an analogous correlation in *Saccharomyces cerevisiae*[23].

The results reported in the Table show that the two enzymes involved in cAMP metabolism, i.e. adenylate-cyclase and cAMP-phosphodiesterase, are synthesized at a level which does not change very much during cell growth. Thus a possible regulatory action of the two enzymes on cAMP level can occur mainly through a modulation of their activity. Since cytokinins generically stimulate cellular growth and cAMP inhibits it[17,22,24,25], we hypothesize that free cytokinins may be involved in the regulation of the activities of the two enzymes responsible for biosynthesis and degradation of cAMP. We are in the process of trying to verify this hypothesis.



Relationship of growth and cAMP concentrations to the cytokinin contents of *Escherichia coli* B/b in M9 salts medium (30 min monitoring).
○—○, Cells/ml ($\times 10^9$); ●—● free cytokinins per g of cells ($\times 100$ ng); □—□, intracellular cAMP ($\times 10^{-5}$ *M*); ■—■, extracellular cAMP ($\times 10^{-7}$ *M*).

Adenylate-cyclase and cAMP-phosphodiesterase activity from *Escherichia coli* B/b cells

Age of culture (min)	Adenylate-cyclase (% of radioactivity supplied as ^{14}C-ATP and found out as cAMP)	cAMP-PDE (% of radioactivity supplied as ^{3}H-cAMP and degraded)
30	40.15	85.25
60	43.65	86.40
90	42.05	86.00
120	40.53	86.35
150	39.70	86.25
180	39.80	86.63
210	40.05	85.21
240	39.15	85.45
270	38.85	86.05
300	39.15	85.55

Values obtained after 20 min reaction time.

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[3] M. L. Gefter and R. L. Russell, J. molec. Biol. *39*, 145 (1969).
[4] F. Skoog and D. J. Armstrong, A. Rev. Plant Physiol. *21*, 359 (1970).
[5] M. D. Litwack and A. Peterkofsky, Biochemistry *10*, 994 (1971).
[6] R. C. Gallo, S. M. Hecht, J. Wang-Peng and S. O'hoop, Biochim. biophys. Acta *281*, 488 (1972).
[7] H. N. Wood, M. C. Lin and A. C. Braun, Proc. natn. Acad. Sci., USA *69*, 403 (1972).
[8] S. M. Hecht, R. D. Faulkner and S. D. Hawrelak, Proc. natn. Acad. Sci., USA *71*, 4670 (1974).
[9] U. Kull and B. Kuhn, Z. Naturforsch. *30C*, 69 (1975).
[10] A. Robison, R. W. Butcher and W E. Sutherland, *Cyclic AMP* (Academic Press, London 1971).
[11] S. Coppola, G. Percuoco, A. Zoina and G. Picci, Atti Colloquio sui *Rapporti piante-microrganismi* (Int. Soc. Soil. Sci. Pisa 9–10 giugno 1972).
[12] R. C. Clowes and W. Hayes, *Experiments in Microbial Genetics* (Blackwell Scientific Publishing, Oxford and Edinburg 1968).
[13] M. J. Buettner, E. Spitz and H. V. Richenberg, J. Bact. *114*, 1068 (1973).
[14] A. G. Gilman, Proc. natn. Acad. Sci., USA *67*, 305 (1970).
[15] J. W. Einset and F. Skoog, Proc. natn. Acad. Sci., USA *70*, 568 (1973).
[16] H. A. Van Onckelen and R. Verbeek, Phytochemistry *11*, 1677 (1972).
[17] R. R. Bürk, Nature, Lond. *219*, 1272 (1968).
[18] R. S. Makman and E. W. Sutherland, J. biol. Chem. *240*, 1309 (1965).
[19] M. Abou-Sabé, M. Burday and J. Gentsch, Biochim. biophys. Acta *385*, 289 (1975).
[20] A. Peterkofsky and C. Gazdar, Proc. natn. Acad. Sci., USA *68*, 2794 (1971).
[21] J. Otten, G. S. Johnson and I. Pastan, Biochem. biophys. Res. Commun. *44*, 1192 (1971).
[22] M. M. Buerger, B. B. Bombik, B. McL. Breckenridge and J. R. Sheppard, Nature, Lond. *239*, 161 (1972).
[23] S. Coppola, G. Tucci and G. Picci, G. Microbiol. *19-20-21*, 13 (1971).
[24] J. R. Sheppard, Proc. natn. Acad. Sci., USA *68*, 1316 (1971).
[25] N. D. Judewicz, E. M. De Robertis and H. N. Torres, Biochem. biophys. Res. Commun. *52*, 1257 (1973).