pregnancy also prepare the host for the allogeneic challenge of mating. Such a relationship between the ovary and thymus would add further support to the concept of a thymic-ovarian interaction[30].


1 Erickson, R.P., in: Immunobiology of Gametes, p. 85. Eds M. Edidin and M.H. Johnson. Cambridge University Press, Cambridge and London 1977.
2 Beer, A.E., and Neaves, W.B., Fert. Steril. *29* (1978) 3.
3 Vojtiskova, M., Polackova, M., and Pokorna, Z., Folia biol. (Praha) *15* (1969) 322.
4 McLean, J.M., Shaya, E.I., and Gibbs, A.C.C., J. reprod. Immun. *1* (1980) 285.
5 McLean, J.M., Mosley, J.G., and Gibbs, A.C.C., J. Anat. *118* (1974) 223.
6 Gleicher, N., Deppe, G., and Cohen, C.J., Obstet. Gynec. *54* (1979) 335.
7 Brabin, B.J., Rev. infect. Dis. *7* (1985) 579.
8 Svendsen, P., Stigbrand, T., Teisner, B., Folkersen, J., Damber, M.G., von Schoultz, B., Kemp, E., and Svehag, S.E., Acta path. microbiol. scand. *86* (1978) 199.
9 Chaouat, G., Monnot, P. Hoffman, M., and Chaffaux, S., Am. J. reprod. Immun. *1* (1980) 18.
10 Gill, T.J., and Repetti, C.F., Am. J. Path. *95* (1979) 465.
11 Grossman, C.J., Endocr. Rev. *5* (1984) 435.
12 Sulke, A.N., Jones, D.B., and Wood, P.J., Br. med. J. *290* (1985) 884.
13 Behrman, J., Br. med. J. *283* (1981) 88.
14 Stockard, C.R., and Papanicolaou, G.N., Am. J. Anat. *22* (1917) 225.
15 Long, J.A., and Evans, H.M., Mem. Univ. Calif. *6* (1922) 1.
16 Rogers, A.W., in: Techniques of Autoradiography, p. 211. Elsevier, Amsterdam, London and New York 1969.
17 Ford, W.L., in: Handbook of Experimental Immunology, p. 2315. Ed. D.M. Weir. Blackwell Scientific Publications, Oxford 1978.
18 Wagner, H.P., Cottier, H., Cronkite, E.P., Cunningham, L., Janzen, C.R., and Rai, K.R., Exp. Cell Res. *46* (1967) 441.
19 Winer, B.J., in: Statistical Principles in Experimental Design. McGraw Hill, New York 1971.
20 Bierman, E.L., and Hirsch, J., in: Textbook of Endocrinology, p. 907. Ed. R.H. Williams. Saunders, Philadelphia 1981.
21 Nequin, L.G., Alvarez, J., and Schwarz, N.B., Biol. Reprod. *20* (1979) 659.
22 Astwood, E.B., Am. J. Physiol. *126* (1939) 162.
23 Boettiger, E.G., J. cell. comp. Physiol. *27* (1946) 9.
24 Butcher, R.L., Collins, W.E., and Fugo, N.W., Endocrinology *94* (1974) 1704.
25 Ross, G.T., and van de Weile, R.L., in: Textbook of Endocrinology, p. 355. Ed. R.H. Williams. Saunders, Philadelphia 1981.
26 Franks, C.R., Perkins, F.T., and Bishop, D., Br. J. Cancer *31* (1975) 100.
27 Luster, M.I., Hayes, H.T., Korach, K., Tucker, A.N., Dean, J.H., Greenlee, W.F., and Boorman, G.A., J. Immun. *133* (1984) 110.
28 Nossal, G.J.V., Am. J. Anat. *170* (1984) 253.
29 Marsh, P.J., and McLean, J.M., J. Anat. *140* (1985) 525.
30 Ivanova, M., Bourneva, V., Gitson, L., and Angelova, Z., Am. J. reprod. Immun. *6* (1984) 99.


0014-4754/87/091010-03$1.50 + 0.20/0


## Lunar soil and Zagami meteorite inhibit biosynthesis of itoic acid, a siderophore

T. Ito

*Department of Biology, East Carolina University, Greenville (North Carolina 27858-4353, USA), 5 March 1987*

*Summary.* Lunar soil, Zagami meteorite, and Gorda Ridge ocean basalt inhibited the synthesis of iotic acid and coproporphyrin III in iron-deficient *Bacillus subtilis* cultures. Synthetic ilmenite did not. The results indicated that the three natural samples served as sources of iron for the organism, but the ilmenite did not.
*Key words.* Siderophore; *Bacillus subtilis;* itoic acid; porphyrin; lunar soil; meteorite; ilmenite.

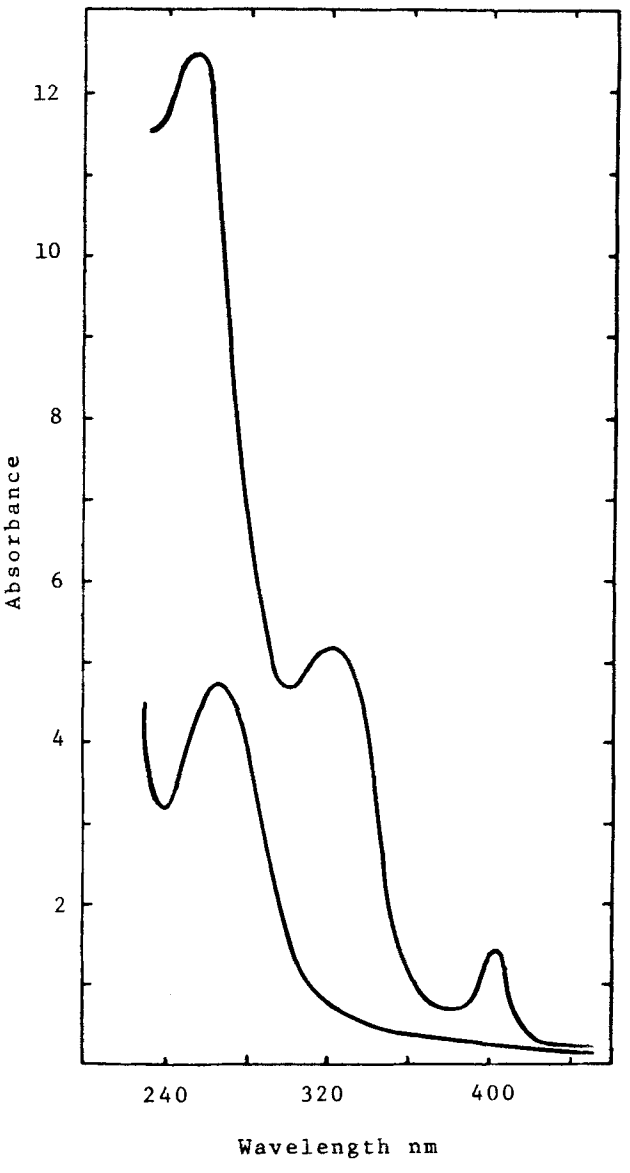
Since Fe(III) is extremely insoluble in water, almost all microorganisms, especially under iron-deficient growth conditions, excrete iron-binding compounds known as siderophores that chelate iron, and transport the bound iron into the cells[1,2]. *Bacillus subtilis,* for instance, when grown in an iron-deficient growth medium[3] (FeDGM), excretes 2,3-dihydroxybenzoylglycine (itoic acid), the first chemically identified siderophore[4], and coproporphyrin III[5] into the culture medium. Excretion of these two compounds is extremely sensitive to iron concentration in the growth medium, and stops as iron concentration rises[4-7]. The present study was undertaken to test whether extraterrestrial minerals and terrestrial minerals of a similar elemental composition serve as sources of iron for the organism and inhibit thereby the excretion of these compounds.

*Materials and methods.* Samples of lunar soil (10084,151), Zagami meteorite, Gorda Ridge ocean basalt, Quebec ilmenite, and synthetic ilmenite were provided by the Solar System Exploration Division of the Johnson Space Center, Houston, Texas. These mineral samples (10 mg) were autoclaved in 250-ml flasks containing 50 ml FeDGM. The flasks were inoculated with five drops of a freshly grown *Bacillus subtilis* (ATCC 15933) in FeDGM, and incubated for three days at 30 °C in a gyratory shaker at about 50 cycles/min. The cells were removed by centrifugation at 10,000 g for 10 min, and the absorbance of each supernatant, diluted five-fold, was read at 320 nm and 405 nm for the measurement of itoic acid and corproporphyrin III, respectively[4,8]. All flasks containing FeDGM were previously rinsed thoroughly with 6 N HCl in order to remove any possible iron contamination on the inner wall of the flasks.

*Results and discussion.* A typical spectrum of the supernatant of the 3-day-old *Bacillus subtilis* culture grown in FeDGM showed two peaks which were not observed with the addition of iron (fig.). The peak near 320 nm was indicative of itoic acid and another at 405 nm of coproporphyrin III. The lunar

Effect of extraterrestrial and terrestrial minerals on biosynthesis of itoic acid and coproporphyrin III by *Bacillus subtilis*. Each number represents absorbance of the 3-day-old culture supernatants at the approximate absorption maxima of itoic acid (320 nm) and coproporphyrin III (405 nm). The numbers without parentheses are the mean absorbance of four determinations, and those in parentheses the ranges. The addition of the minerals was at 200 mg/l of FeDGM. The minerals were virtually insoluble, and stayed as solids at the bottom of the culture flasks.

| Minerals added to FeDGM | Absorbance of 3-day-old culture supernatants at 320 nm | 405 nm |
|---|---|---|
| None | 5.00 (5.32 –4.70) | 1.07 (1.23 –0.925) |
| Lunar soil | 0.661 (0.690–0.625) | 0.216 (0.225–0.210) |
| Zagami meteorite | 0.584 (0.615–0.555) | 0.196 (0.205–0.190) |
| Gorda Ridge basalt | 0.663 (0.680–0.655) | 0.215 (0.225–0.200) |
| Quebec ilmenite | 1.14 (1.24 –1.05) | 0.615 (0.830–0.500) |
| Synthetic ilmenite | 3.17 (3.53 –2.71) | 1.58 (2.09 –1.13) |




Spectra of the 3-day-old culture supernatants of *Bacillus subtilis* grown in FeDGM (upper) and in FeDGM plus 100 µg iron ($FeCl_3$)/l (lower).

soil (10084.151), collected during the Apollo 11 mission[9], the Zagami meteorite, postulated to be of Mars origin[10], and the Gorda Ridge basalt[11], a representative of the most abundant volcanic rock on earth, all completely inhibited excretion of itoic acid and coproporphyrin III. No inhibition of coproporphyrin III excretion and slight inhibition of itoic acid excretion were observed with the synthetic ilmenite. The Quebec ilmenite inhibited excretion of both, but not to the same extent as the other three natural samples (table). The result indicated that iron was released from the extraterrestrial and terrestrial materials, and transported into the organism through the 'low affinity'[2] itoic acid-independent transport system. However, the iron must have come from such sources as pyroxenes and olivines, but not from ilmenite, all of which are abundant in the lunar soil, the Zagami meteorite and the ocean ridge basalt. Failure of the synthetic ilmenite to release iron for utilization by the organism reflected the tight crystalline structure of ilmenite. The inhibition of excretion of these two compounds by the Quebec ilmenite indicated that this ilmenite sample was somewhat contaminated with other iron sources. It is interesting to note that ash from Mt St. Helen inhibited biosynthesis of rhodotorulic acid, a siderophore of *Rhodotorula pilimanae*[12].

Acknowledgment. I thank NASA Johnson Space Center, Houston, Texas for the samples and for laboratory space, and the American Society for Engineering Education for a summer fellowship.

1 Neilands, J. B., in: Microbial Iron Metabolism, p. 3. Ed. J. B. Neilands. Academic Press, New York 1974.
2 Neilands, J. B., A. Rev. Biochem. *50* (1981) 715.
3 Garibaldi, J. A., and Neilands, J. B., J. Am chem. Soc. *77* (1955) 2429.
4 Ito, T., and Neilands, J. B., J. Am. chem. Soc. *80* (1958) 4645.
5 Garibaldi, J. A. and Neilands, J. B., Nature *177* (1956) 526.
6 Peters, W. J., and Warren, R. A., J. Bact. *95* (1968) 360.
7 Neilands, J. B., in: Inorganic Biochemistry, vol. 1, p. 167. Ed. G. L. Eichhorn. Elsevier Scientific Publishing Co., Amsterdam/London/New York 1973.
8 Neilands, J. B., and Garibaldi, J. A., Biochem. Prep. *7* (1960) 36.
9 Morris, R. V., Score, R., Dardano, C., and Heiken, G., Handbook of Lunar Soils, Part I: Apollo 1–15. Planetary Materials Branch Publication, vol. 67, p. 10. NASA Johnson Space Center, Houston 1983.
10 Nyquist, L. E., Bogard, D. D., Wooden J. L., Wiesmann, H., Shih, C.-Y., Bansal, B. M., and McKay, G., Meteoritics *14* (1979) 502.
11 Kay, R., Hubbard, N. J., and Gast, P. W., J. geophys. Res. *75* (1970) 1585.
12 Akers, H. A., and Magee, K. P., Experientia *41* (1985) 1354.

0014-4754/87/091012-02$1.50 + 0.20/0


## Differential effects of prostaglandins and isoproterenol on cAMP content and Na, K pump activity in rat submandibular acini

J. R. Martinez, N. Cassity and S. Barker

*Departments of Child Health and Physiology, University of Missouri School of Medicine, Columbia (Missouri 65212, USA), 26 February 1987*

*Summary.* The β-adrenergic agonist isoproterenol and prostaglandins $E_1$ and $E_2$ (but not $F_{2\alpha}$) increased the cAMP content of rat submandibular acini in vitro, but only isoproterenol enhanced ouabain-sensitive $^{86}Rb$ (K) uptake. These findings suggest that cAMP is not involved in the activation of the Na, K pump in salivary cells.
*Key words.* Submandibular acini; isoproterenol; prostaglandins; cAMP; K uptake.

Several lines of evidence indicate that activation of a Na, K pump localized in the basolateral cell membrane[1] is an important consequence of autonomic stimulation in the salivary glands[2–4]. Enhanced pump activity is observed with both cholinergic[5] and β-adrenergic[2] stimuli, but the specific mechanism in each case is not entirely clear, as the two types