phosphate into the cell is against the concentration gradient. Phosphate uptake in relation to the substrate concentration yielded a hyperbolic curve (fig. 3) suggesting the entry of phosphate by a saturable system. When these data were replotted as a Lineweaver-Burk plot a K_m value of 45.45×10^{-5} M was found. The results satisfy the requirements of an active transport system.

Over the past few years considerable attention has been given to a new class of protein called binding proteins. It is now a well-established fact that the transport of many substances in bacterical cells requires proteins of a specific type, present in the periplasmic region of cells, from which they can be selectively released by a cold osmotic shock treatment[20-22]. We have presented evidence for the presence of binding proteins for phosphate[12] and glucose[11,16] in *A. nidulans*. Binding proteins for tryptophan[15] and sulphate[23] have also been reported in *N. crassa* and *A. nidulans*, respectively. To examine the possible role of binding protein in the phosphate uptake in *Claviceps* sp. strain SD-58, cells were subjected to osmotic shock treatment, which caused a significant decrease in the uptake of phosphate. Partial restoration of uptake was observed by the addition of shock fluid to shocked cells (fig. 4). On analysis of shock fluid, the phosphate binding activity was reduced by 80–90% on incubation with protease. This suggested that the component involved in transport of phosphate which is released by an osmotic shock is a protein.

The protoplast preparation of cells was found to be retarded in phosphate uptake ability by about 80%, which confirms the involvement of periplasmic binding protein.

Several transport systems have been shown to be regulated by inhibition of uptake by high concentration of substrate or the operation of distinct transport systems depending upon the concentration of substrates[15,19,24,25]. The phosphate uptake system in *Claviceps* sp. strain SD-58 was not inhibited by high concentration of phosphate (10 mM), nor did it show the existence of two transport systems (fig. 3). Thus, under conditions of high phosphate concentration, the cell will continue to accumulate phosphate. Accumulation of a high amount of phosphate in the cell will in turn affect the cell physiology leading to a higher rate of TCA cycle operation[7] and a greater biomass, with concomitant inhibition of alkaloid synthesis.

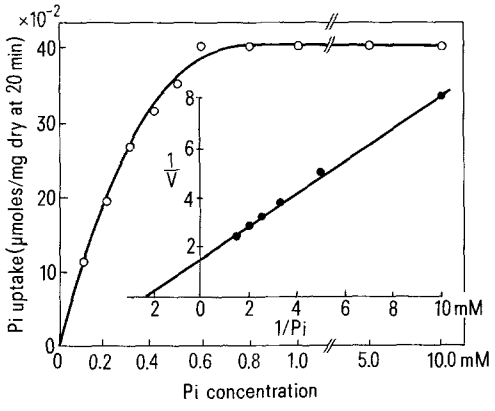

Figure 3. Substrate dependent uptake of phosphate by *Claviceps* sp. strain SD-58. Inset figure: Line weaver – Burk plot of phosphate uptake.

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1 Arcemone, F., in: Biologically active substances Exploration and exploitation, p. 49 Ed. D.A. Hems. John Wiley and Sons, Inc., New York 1977.
2 Floss, H.G., Robbers, J.E., and Heinstein, P.F., Recent Adv. Phytochem. *8* (1974) 141.
3 Demain, A.L., and Inamine, E., Bact. Rev. *34* (1979) 1.
4 Demain, A.L., J. appl. Chem. Biotechnol. *22* (1972) 345.
5 De Waart, C., and Taber, W.A., Can. J. Microbiol. *6* (1960) 675.
6 Vaidya, H.C., and Desai, J.D., Indian J. Biochem. Biophys. *20* (1983) 222.
7 Vaidya, H.C., and Desai, J.D., Indian J. exp. Biol. *20* (1982) 475.
8 Vaidya, H.C., and Desai, J.D., Indian J. exp. Biol. *19* (1981) 829.
9 Vaidya, H.C., and Desai, J.D., Folia microbiol. *28* (1983) 12.
10 Desai, J.D., Desai, A.J., and Patel, H.C., Appl. envir. Microbiol. *45* (1983) 1694.
11 Desai, J.D., and Modi, V.V., Indian J. exp. Biol. *12* (1974) 438.
12 Desai, J.D., Misra, J., and Modi, V.V., Indian J. exp. Biol. *14* (1976) 198.
13 Brown, C.E., and Romano, A.H., J. Bact. *100* (1969) 1198.
14 Mark, C.G., and Romano, A.H., Biochim. biophys. Acta *249* (1971) 216.
15 Wiley, W.R., J. Bact. *103* (1970) 658.
16 Desai, J.D., and Modi, V.V., Experientia *31* (1975) 160.
17 Robbers, J.E., Cheng, L., Anderson, J.A., and Floss, H.G., J. nat. Prod. *42* (1979) 537.
18 Fiske, C.H., and Subba Row, Y.J., J. biol. Chem. *66* (1925) 376.
19 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
20 Pardee, A.B., Science *162* (1966) 632.
21 Wetzel, B.K., Spicer, S.S., Dvork, H.F., and Heppel, L.A., J. Bact. *104* (1970) 529.
22 Neu, H.C., and Heppal, L.A., J. biol. Chem. *240* (1965) 3685.
23 Moore, B.G., and Spencer, B., Biochem. J. *127* (1972) 27.
24 Kepes, A., in: The cellular function of membrane transport, p. 155. Ed. J.F. Hoffman. Prentice Hall, Englewood Cliffs, N.J., 1964.
25 Higgins, C.F., Ardeshir, F., and Ames, G.F., membrane transport, vol. 2, p. 100. Ed. A.N. Martonosi. Plenum Press, New York 1982.

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Endogenous splenic colonies and megakaryopoiesis in methylcellulose treated irradiated mice[1]

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Summary. Endogenous splenic colonies are increased in methylcellulose-treated irradiated mice, 10 days after sublethal irradiation (450 R). The spleen shows an enhancement of megakaryopoiesis, especially localized around foam-cell foci. This suggests that the macrophage system, activated through phagocytic activity against methylcellulose, affects megakaryopoiesis by a microenvironmental mechanism.

Key words. Mouse, irradiated; irradiation, sublethal; mouse spleen; splenic colonies, endogenous; methylcellulose treatment; megakaryopoiesis; macrophage system.

Table 1. Spleen weight and endogenous splenic colony number at varying times after irradiation. C = Control group. MC = Methylcellulose-treated group. *p = 0.05. The analysis of covariance was employed for the statistical evaluation of the endogenous splenic colony number. In order to control splenic weight variability, this parameter was assumed as covariate. Since the variable studied was represented by frequencies (number of colonies per spleen), original data X were transformed according to the formula: $X_t = \sqrt{X + 0.5}$.

Day	4		6		8		10	
Group	C	MC	C	MC	C	MC	C	MC
(n of mice)	(5)	(5)	(5)	(5)	(7)	(7)	(9)	(9)
Spleen weight								
mg	20.4	105.8	29	127	39	171	75	114
mean ± S.E.	± 3.26	± 8.24	± 8.8	± 15.7	± 2.02	± 19.9	± 12.1	± 7.8
N of colonies								
per spleen: mean	0	0.2	4.4	3.2	8.4	6.5	10.1	17.1*
(range)	–	(0-1)	(0-10)	(0-10)	(3-12)	(2-9)	(5-18)	(6-27)

Repeated intraperitoneal injections of methylcellulose (MC) in rats and mice induce spenomegaly caused by phagocytosis of MC by the splenic macrophages[6,7], which assume the appearence of foam-cells (FC). In rats blood alterations are detectable, related to increased spleen size[8]; mice differ from rats in hematologic response to MC treatment in that MC-treated splenectomized mice show changes similar to nonsplenectomized ones, caused by blood cell destruction in organs other than the spleen[7]. MC-treated mice show an increase in CFU-GM in the marrow and in the spleen and a fourfold enhancement of CFU-S in the spleen but not in the marrow[7]; finally they develop hepatic hemopoiesis in response to the increased blood cell demand[9].

We compared the pattern of endogenous splenic colonies (ESC) in MC-treated mice and the histological findings in spleen, femural bone marrow and liver with that in a control group, in order to evaluate the splenic hemopoietic process in mice, in a microenvironment modified by phagocytosis of MC by the macrophage system.

Materials and methods. Mice were male, 40 days old, C 57 Bl/6N (Charles River); water and food were available ad libitum. One group of mice was injected intraperitoneally with 0.5 ml of a 2.5% sterile solution of MC (Tylose MH 300 – Fluka AG) in saline 3 times a week for 2 weeks; a control group was treated at the same time with saline. The day after the last injection all mice were exposed to beta radiation generated from a linear accelerator (450 R, 22 MeV).

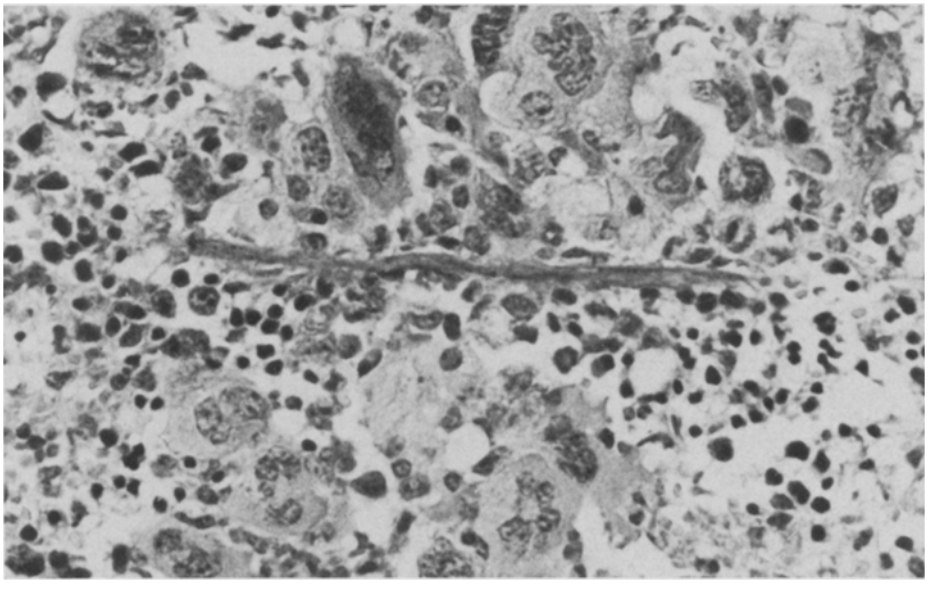
On day 4, 6, 8, 10 after the irradiation animals were killed by cervical dislocation. Spleens were removed, weighed and ESC macroscopically counted; histological sections were prepared from spleens, femurs and livers by paraffin embedding and hematoxylin-eosin, Giemsa and PAS staining.

Results. Over the period of 4–10 days we observed a rising trend in the number of ESC both in the MC-treated group and in the control (table 1). On day 10 there was a statistically significant increase ($p = 0.05$) in the number of ESC in the MC-treated group.

The histological examination of the spleen and the liver of MC-treated animals showed a large number of FC aggregates. Splenic erythropoiesis and granulocytopoiesis was increased in MC-treated mice as compared to controls; however, the topographical distribution of the hemopoietic areas and their ratio were unmodified.

The most important finding was a very large increase in splenic megakaryopoiesis in the MC-treated group. We counted the number of megakaryocyte clones greater than three cells, and the number of megakaryocytes per clone, in two midline sections for every spleen from the animals sacrificed on the day 10 after irradiation (table 2). The megakaryocyte morphological appearence, as observed in standard histological preparations, was confirmed by the acetylcholinesterase staining[10] performed in some sections from both the experimental groups. Megakaryocytic clones were distributed randomly throghout the splenic parenchyma, but they appeared to be in close connection with FC aggregates (fig.). Sometimes mixed erythro-megakaryocytic colonies were detectable.

Femural bone marrow showed fewer and comparatively smaller FC aggregates than the spleen. There were no differ-



Megakaryocytes around foam-cell foci in the spleen of a MC-treated mouse. × 320; hematoxilin-eosin staining.

ences between the megakaryopoietic pattern of MC-treated femurs and that of the controls; some megakaryocytes were detectable, but they were not in any particular arrangement; they were not grouped, but distributed singly throughout the marrow.

The histological examination of the liver showed some FC aggregates, but in our model we could not demonstrate hepatic hemopoiesis in any group.

We measured the cellular and nuclear area of megakaryocytes from both experimental groups by an electronic planimetric system (Videoplan, Kontron. At least 50 cells evaluated for each day and each group). We could not demonstrate any difference at various times between the MC-treated and control groups. Moreover there was no difference in nuclear lobulation number. Taken together, all these data do not allowed us to postulate any difference in megakaryocyte ploidy, at the different times, between the experimental groups.

Discussion. Our study, performed using the ESC technique, demonstrated on day 10 a statistically significant increase in ESC number in the MC-treated group, which is in line with observations in a preceding study[7], carried out by the exogenous CFU-S technique.

As a matter of fact, data are available in the literature suggesting that functional conditions of RES may affect the hemopoietic process. Blockade of RES by administration of carbon particles[11] or polystyrene latex particles[12] before sublethal irradiation in mice, as well as RES stimulation by bacterial endotoxins[13], have been shown to increase the number of ESC. In carbon-treated mice this effect seems to depend on some humoral activity present in the serum[14].

The large increase in splenic megakaryocyte production in MC-treated mice should be accounted for a splenic and reticulo-endothelial trapping of platelets, but this hypothesis is in contrast to the observation in bone marrow, where we could not demonstrate an increase in megakaryocytes in the MC-treated group. Ebbe reported furthermore that chronic thrombocytopenia at the time of irradiation appears to increase the radiosensitivity of thrombopoietin-responsive cells[15]. On the other hand, Crandall and Boggs observed in MC-treated irradiated mice that megakaryopoietic foci are more resistant than the erythroid ones[16].

We want to emphasize that in the femural bone marrow of MC-treated mice we observed a small number of FC in comparison to the large number detectable in the spleen. The constant topographical relationship between FC groups and megakaryocytic clones observed in the spleen suggests the possible role of macrophages, activated by phagocytosis, in the control of megakaryopoiesis at a microenvironmental level. Recently it was observed that monocyte-macrophage lineage cells elaborate an activity enhancing murine megakaryopoiesis in vitro[17], and that could support our hypothesis.

Table 2. Number of megakaryocytic clones per section and megakaryocytes per clone evaluated on day 10. Two midline sections for every spleen were completely scored. C = Control group. MC = Methylcellulose treated group. *$p < 0.01$. Original data were transformed: $X_t = \sqrt{X + 0.5}$ for the analysis of variance.

Group (n of mice)	C (9)	MC (9)
N of meg. clones mean ± SE	6.5 ± 2.03	31 ± 6.7*
N of meg. per clone mean ± SE	4.6 ± 0.9	5.31 ± 0.57

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2 Divisione Ematologia.
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6 Palmer, J.G., Eichwald, E.J., Cartwright, G.E., and Wintrobe, M.M., Blood *8* (1953) 72.
7 Stang, H.D., and Boggs, D.R., Am. J. Physiol. *233* (1977) H234.
8 Teom, T.B., J. Path. Bact. *81* (1961) 33.
9 Pfirmer, W., Joice, R.A., Turner, R., and Boggs, D.R., Blood *52* (1978) 610.
10 Karnovky, M.J., and Roots, L., J. Histochem. Cytochem. *12* (1964) 219.
11 Mori, K.J., Radiat. Res. *56* (1973) 494.
12 Mori, K.J., Seto, A., and Ito, Y., Experientia *31* (1975) 112.
13 Smith, W.W., Brecher, G., Budd, R.A., and Fred, S., Radiat. Res. *27* (1966) 610.
14 Mori, K.J., Nakamura, S., Seto, A., and Ito, Y., Radiat. Res. *18* (1977) 225.
15 Ebbe, S., and Stohlman, F., Jr, Blood *35* (1970) 783.
16 Crandall, T.L., and Boggs, D.R., Exp. Hemat. *8* (1980) 25.
17 Williams,. N., Jackson, H., Ralph, P., and Nakoins, I., Blood *57* (1981) 157.

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Chemical control of eucaryotic and blue-green algae in anaerobic photoreactors culturing Rhodospirillaceae

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Summary. To control the growth of eurocaryotic and blue-green algae in anaerobic reactors of photosynthetically grown Rhodospirillaceae, the effect of algae inhibitors with different modes of action was examined. Tests were performed with mixed populations of green algae and blue-green algae, besides strains of the purple nonsulphur bacteria *Rhodopseudomonas capsulata, Rhodospirillum rubrum* and *Rhodomicrobium vannielii.* Chloroxuron, a urea-derivative, was found to inhibit completely growth of green and blue-green algae at 5 ppm. When it was applied to the Rhodospirillaceae cultures, growth was not reduced and nitrogenase activity was not inhibited.

Key words. Algae, green; algae, blue-green; Rhodospirillaceae; algae inhibitors; algae control.

Since hydrogen production was observed in *Rhodospirillum rubrum* by Gest and Kamer[1,2] the hydrogen evolving photosynthetic bacteria have been studied in detail, as hydrogen is considered a promising fuel for the future. Many strains belonging to the Rhodospirillaceae convert different organic acids into hydrogen with high efficiency using light energy[3–5]. Recently, applied studies aimed at producing hydrogen over longer periods in outdoor conditions were reported[6–9]. The