late colonies. In this way indirect evidence was obtained for the presence of *M. arthritidis* antigens upon the surface of 7-day CFUs. To this could be attributed loss of spleen-seeding efficiency ('f' fraction) of 7-day CFUs of *M. arthritidis*-treated mice: if, as seems likely, membrane-associated mycoplasmas are expressed upon the surface of transplanted CFUs, the homing pattern of the latter might be affected.

The data presented here provides additional evidence which supports the hypothesis that the observed ability of *M. arthritidis* to activate viral murine leukemogenesis[1,5,11] in mice may result from direct action of mycoplasma organisms upon the hematopoietic target cells, causing stimulation and an increase in their numbers and/or sensitivity to R-MuLV.

1 Sanin, A.V., Kaulen, D.R., Khorobrykh, V.V., Pronin, A.V., Rakovskaya, I.V., and Kagan, G.Y., Bull. exp. Biol. Med. *88* (1979) 874.
2 Sanin, A.V., and Khorobrykh, V.V., Bull. exp. Biol. Med. *94* (1982) 1567.
3 Wallner, S.F., in: Current Concepts in Erythropoiesis, p. 209. Ed. C.D.R. Dunn. Wiley & Sons 1983.
4 Sanin, A.V., Khorobrykh, V.V., and Kaulen, D.R., Symposium on Proliferation and Differentiation of Stem Cells, p. 39. Prague 1982.
5 Kagan, G.Y., Rakovskaya, I.V., Morgunova, T.D., and Postnikova, Z.A., Vop. Virus. (Russ.) *2* (1975) 171.
6 Okunewick, J.P., Biomedecine *26* (1977) 152.
7 Till, J.E., and McCulloch, E.A., Radiat. Res. *14* (1961) 213.
8 Siminovitch, L., McCulloch, E.A., and Till, J.E., J. Cell comp. Physiol. *62* (1963) 327.
9 Magli, M.C., Iscove, N.N., and Odartchenko, N., Nature *295* (1982) 527.
10 Harris, R.A., Hogarth, P.M., Wadeson, J.J., Collins, P., McKenzie, I., F.C., and Penington, D.G., Nature *307* (1984) 638.
11 Sanin, A.V., Khorobrykh, V.V., and Kaulen, D.R., Bull. exp. Biol. Med. *90* (1980) 1184.

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Effects of puromycin on rat embryos in vitro

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Summary. Somite-staged rat embryos were exposed to varying concentrations of puromycin for 48 h in vitro. Medium concentrations below 0.92 μM had no significant effects, while concentrations above 1.84 μM were lethal. Between these extremes, there were concentration dependent increases in the incidence of malformations in a close relationship to growth retardation.
Key words. Rat embryo; in vitro; puromycin; microbial toxin; teratogenicity; lipids.

A number of microbial toxins have been shown to be animal teratogens[1]. The ubiquitous occurrence of toxigenic microbes in the environment makes these compounds potential human teratogens. Many microbial toxins are potent inhibitors of protein synthesis[2]. Puromycin, an antibiotic produced by the soil actinomycete *Streptomyces alboniger*, is a well-characterized inhibitor of protein synthesis which acts by disrupting RNA translation[3]. Much of the data on the teratogenicity of puromycin is conflicting. It has been suggested that puromycin and other similar protein synthesis inhibitors produce embryolethality but few or no overt terata[4]. One explanation put forth for these findings is mediation of the toxic effects of puromycin through interference with placental function rather than direct effects on the embryo. The whole rodent embryo culture system developed by New and his co-workers (for review, see New[5]) has found considerable use in experimental teratology, since it allows one both to isolate the embryo from maternal effects and to examine possible mechanisms of teratogenesis. One proposed mechanism of teratogenesis is alteration of cell membrane structure or function[6]. Changes in phospholipids and cholesterol, which are important structural lipid components of cell membranes, have been associated with normal embryonic differentiation and development[7]. The present studies examine the direct effects of puromycin, a model microbial toxin, on the development of rat embryos in

Effect of puromycin on organogenesis-staged rat embryos in vitro[a]

	Puromycin (μM)				
	0	0.46	0.92	1.38	1.84
Viable/total embryos	14/14	15/16	16/16	14/18	14/20*
Impaired yolk sac/total viable embryos	0/14	0/15	0/16	5/14*	10/14*
Crown-rump length (mm)	3.26 ± 0.05 (n = 14)	3.14 ± 0.05 (n = 15)	2.87 ± 0.09* (n = 16)	2.54 ± 0.06* (n = 14)	2.11 ± 0.10* (n = 14)
Head length (mm)	1.67 ± 0.03 (n = 14)	1.65 ± 0.03 (n = 15)	1.44 ± 0.05* (n = 16)	1.31 ± 0.04* (n = 14)	0.98 ± 0.06* (n = 14)
Somites	23.4 ± 0.2 (n = 14)	23.3 ± 0.2 (n = 15)	22.2 ± 0.5 (n = 14)[b]	20.3 ± 0.7* 1(n = 7)[b]	20.7 ± 1.5* (n = 3)[b]
Protein (μg/embryo)	146.3 ± 9.1 (n = 12)	140.9 ± 9.6 (n = 13)	111.4 ± 10.3* (n = 14)	78.3 ± 5.5* (n = 12)	50.3 ± 6.6* (n = 13)
DNA (μg/embryo)	17.2 ± 1.7 (n = 12)	17.0 ± 1.3 (n = 13)	13.5 ± 1.3* (n = 14)	9.1 ± 0.9* (n = 12)	4.9 ± 0.9* (n = 13)
Phospholipid (nmol/embryo)	26.1 ± 1.8 (n = 7)	22.2 ± 2.3 (n = 7)	22.2 ± 2.6 (n = 12)	16.8 ± 1.6* (n = 12)	12.7 ± 1.8* (n = 11)
Cholesterol (nmol/embryo)	24.4 ± 1.7 (n = 7)	23.4 ± 1.3 (n = 7)	21.5 ± 0.6 (n = 12)	21.9 ± 1.8 (n = 12)	19.3 ± 1.5 (n = 11)
Abnormal/total embryos	1/14	3/15	10/16*	13/14*	13/14*

[a] Embryos were grown over gestation days 9–11 in 4 ml medium containing puromycin at the indicated concentration. Values are means ± SE. [b] Somites could not be accurately counted in some embryos in these groups. * Significantly different from control ($p < 0.05$).

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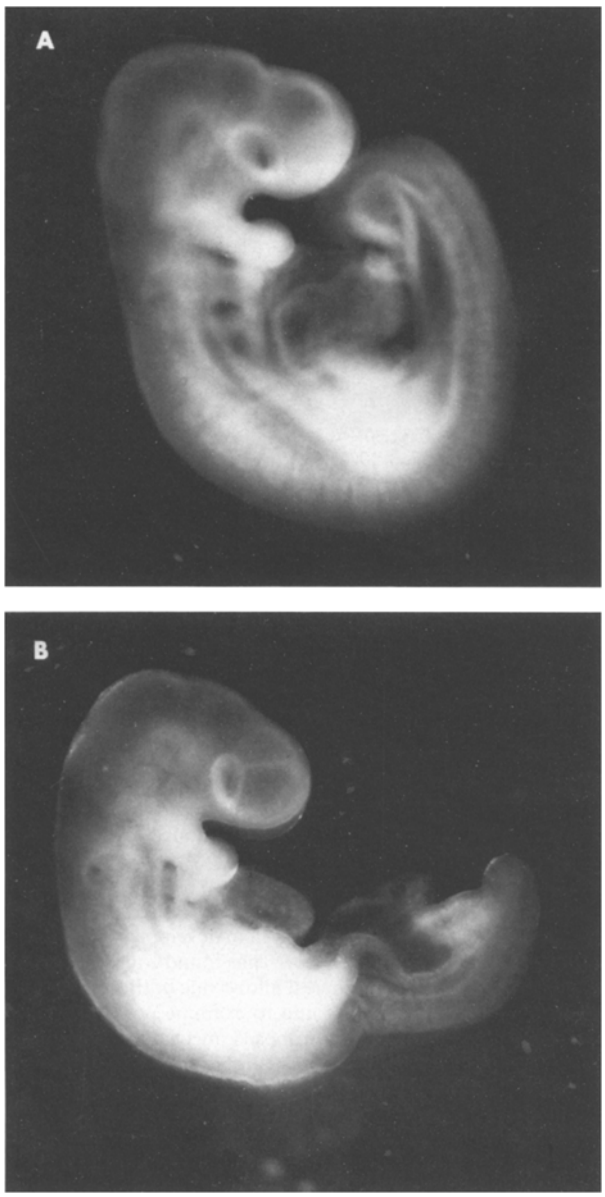

Photomicrographs of unfixed day-11 rat embryos after 45 h in culture. Both pictures were taken at the same magnification. *A* Control; *B* 1.38 μM puromycin.

vitro, and assess whether any abnormal development caused by this compound is reflected in alterations in embryonic phospholipid or cholesterol contents.

Materials and methods. Embryo culture. All rats used in this study were from the Osborne/Mendel strain maintained in the FDA breeding colony. Headfold-stage embryos were explanted on gestation day 9.5 (day of positive vaginal smear is 0.5) into Tyrode's solution, which was prepared with a glucose concentration of 2 instead of 1 g/l, using the procedures described by New[5]. Embryos were cultured in 30-ml disposable polyethylene serum bottles (Wheaton, No. 224033, Millville, NJ) fitted with disposable rubber stoppers (Wheaton, No. 224094, Millville, NJ). Before use, all bottles were thoroughly rinsed in deionized water, and all rubber stoppers were boiled for 1 h; inconsistent results were obtained when bottles and stoppers were not so treated. The culture medium, 4 ml total volume, was adult male rat serum that was prepared by immediate centrifugation[8]. The medium was diluted 10% with deionized water[9], and supplemented with penicillin, 50 IU/ml, and streptomycin, 50 μg/ml. Aqueous solutions of puromycin dihydrochloride (P-L Biochemicals, Milwaukee, WI) were added at the beginning of culture. Two embryos were cultured per bottle. All bottles were gassed initially with a mixture of 5% oxygen, 5% carbon dioxide and 90% nitrogen. Bottles were regassed 20 and 28 h later with mixtures in which oxygen was increased to 20 and 40%, respectively, carbon dioxide was kept constant at 5% and nitrogen provided the balance. Bottles were rotated horizontally at 30–40 rpm in an incubator at 37.5 °C for 45 h. Experiments were conducted in five individual runs spread over a 3-month period. Controls were included in all runs, but some runs did not include all concentration levels of the test compound. Analysis of variance showed that there were no significant between-run variations, so data from all runs were pooled.

Evaluation of embryos. Embryos were initially evaluated for viability. Embryos with obvious signs of physical trauma (e.g., torn yolk sac) were excluded from further study. Embryos without an active heart beat or yolk sac circulation were recorded as nonviable. All viable embryos were evaluated under a dissecting microscope equipped with an eyepiece reticle for crown-rump and head lengths, somite number and gross abnormalities. The embryos were then washed three times in saline, transferred in a 1-ml volume to storage vials and homogenized with a Heat-Systems (Plainview, NY) sonifier at 20 W for 15 s with a microprobe tip. Total protein in embryo homogenates was measured by the method of Lowry et al.[10]. Total DNA in the homogenates was determined fluorometrically[11] with H 33258 reagent (Calbiochem, San Diego, CA). Lipids were extracted[12] from the remainders of the embryo homogenates, and total phospholipids[13] and total cholesterol[14] in the embryo extracts were quantitated fluorometrically.

Statistical methods. All data analysis was performed on a computer, using programs developed by the SAS Institute (Cary, NC). Data for embryo measurements, somite numbers and biochemical contents were analyzed by using analysis of variance. Significant differences from control values were identified by Tukey's highly significant difference test. Data for incidences of abnormalities and embryo viability were analyzed by using a one-tailed Fisher's exact test. The significance level was set at $p < 0.05$.

Results. Medium puromycin concentrations of 3.68 μM or higher were lethal to all embryos tested. The table summarizes the effects of nonlethal concentrations of puromycin on cultured embryos. Embryos cultured in medium containing 0.46 μM puromycin were not significantly different from controls in any of the parameters examined. The viability rate at 1.84 μM was significantly reduced from control levels, and the incidence of impaired yolk sac circulation was significantly increased in the groups treated with 1.38 and 1.84 μM. At puromycin concentrations of 0.92 μM and higher, treated embryos had significant decreases in all growth and biochemical indices except total phospholipid, for which the decrease became significant at 1.38 μM, and total cholesterol, which was not significantly decreased at any puromycin level. The number of abnormal embryos was significantly higher at puromycin concentrations at or above 0.92 μM. The most consistent defects observed were those related to embryonic rotation. Abnormalities in flexion, ranging from slight kinks to a dorsiflexed posture, were noted. Defects in the posterior neural tube (caudal to the rhombencephalon), such as open neural folds or delayed closure of the posterior neuropore were more common than defects of the cranial neural tube. Figures 1 and 2 show photographs of representative control and puromycin-treated embryos.

Discussion. The results show that puromycin is directly toxic to rat embryos, independent of maternal effects. However, puromycin did not induce abnormalities in the absence of significant overall growth retardation. This response is consistent with that of an embryotoxin rather than that of a teratogen[15]. In a review[4] of teratology data for a number of protein synthesis inhibitors,

including puromycin, none of the compounds reviewed except L-asparaginase led to a significant increase in malformations, but rather caused generalized toxic responses such as fetal resorption or weight reduction. Although human exposure to puromycin would not be expected to be significant, puromycin may serve as a model compound for microbial toxins that do pose environmental hazards to humans. Many mycotoxins are reported to be embryotoxic[1], and many of these are known to act by inhibiting protein synthesis[2].

Total embryonic phospholipid content was reduced by puromycin in a way that did not appear to be independent of overall growth inhibition. However, cholesterol content did not decrease significantly. Cytotoxic agents can interfere with the feedback control of cellular cholesterol synthesis with a subsequent intracellular accumulation of cholesterol and its esters[16]. It is possible, then, that increases in cholesterol caused by increased cytotoxicity partially offset decreases in cholesterol caused by reduced embryo size. Measurable changes in individual phospholipid species in embryos exposed to a teratogenic insult have been reported[6]. However, the total embryo phospholipid content used in the present study may not be as sensitive a parameter as ratios of individual phospholipid species for detecting embryotoxic-related changes. Schmid et al.[17], in comparing the effects of cyclophosphamide and acrolein on cultured rat embryos, were able to differentiate the teratogenic effects of the former compound and embryolethal effects of the latter compound. The present study reinforces the utility of rodent embryo culture in making this distinction, and it demonstrates the potential of this system for evaluating some of the underlying mechanisms of teratogenesis.

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1 Hayes, A.W., Mycotoxin Teratogenicity and Mutagenicity, p.41. CRC Press, Boca Raton, Florida 1980.
2 Ueno, Y., CRC Crit. Rev. Toxic. *14* (1985) 99.
3 Yarmolinski, M.B., and de la Haba, G.L., Proc. natn. Acad. Sci. USA *45* (1959) 1721.
4 Ritter, E.J., in: Handbook of Teratology, vol.2, p.99. Eds J.G. Wilson and F.C. Fraser. Plenum Press, New York 1977.
5 New, D.A.T., Biol. Rev. *53* (1978) 81.
6 Chepenik, K.P., and Waldman, S.A., in: Handbook of Experimental Pharmacology. Teratogenesis and Reproductive Toxicology, vol. 65, p.93. Eds E.M. Johnson and D.M. Kochhar. Springer-Verlag, Berlin 1983.
7 Waldman, S.A., Chepenik, K.P., and Waite, B.M., Int. J. Biochem. *16* (1984) 593.
8 Steele, C.E., and New, D.A.T., J. Embryol. exp. Morph. *31* (1974) 707.
9 Klein, N.W., Vogler, M.A., Chatot, C.L., and Pierro, L.J., Teratology *21* (1980) 199.
10 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
11 Labarca, C., and Paigen, K., Analyt. Biochem. *102* (1980) 344.
12 Bligh, E.G., and Dyer, W.J., Can. J. Biochem. Physiol. *37* (1959) 911.
13 Schiefer, H.-G., and Neuhoff, V., Hoppe-Seyler's Z. physiol. Chem. *352* (1971) 913.
14 Albers, R.W., and Lowry, O.H., Analyt. Chem. *27* (1955) 1829.
15 Schmid, B.P., Xenobiotica *15* (1985) 719.
16 Siperstein, M.D., J. Lipid Res. *25* (1984) 1462.
17 Schmid, B.P., Goulding, E., Kitchin, K., and Sanyal, M.K., Toxicology *22* (1981) 235.

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Interferon-β can induce the production of plasminogen activator by cultured human cancer cells

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Summary. Three cultured human cell lines, renal cancer cells (ACHN), bladder cancer cells (EJ), and fibroblasts transformed in culture by Co-60 gamma rays (KMST-6), when treated with interferon-β, produced 1.5 to 4 times as much plasminogen activator as the untreated control cultures. This enhanced production of PA was inhibited by cycloheximide or actinomycin D.
Key words. Plasminogen activator; interferon; cultured human cancer cells.

As an antiviral agent, interferon (IFN) was discovered by Isaacs and Lindenmann[1]. Since then interferons have been found to have various biological activities other than antiviral action, such as enhancement of host immune response[2], induction of the major histocompatibility complex class I[3] and class II antigen[4], induction of cell differentiation[5], inhibition of cell growth[6], and antiproliferative activity against tumor cells[7]. In addition, IFNs induce the cellular production of some biologically active substances such as prostaglandin E in fibroblasts[8], colony-stimulating factors in macrophages[9], and fibronectin, also in macrophages[10]. Although various mechanisms have been proposed for IFN's activities as reviewed by Sen[11], the precise mechanisms of the various actions of IFNs are not yet clearly understood. In this communication, we describe the effects of IFNs on the production of plasminogen activator (PA) by cultured human cancer cells.

Materials and methods. We used the following human cells: a renal carcinoma cell line (ACHN)[12], a urinary bladder tumor cell line (EJ)[13], and human fibroblasts transformed in culture by Co-60 gamma rays (KMST-6)[14]. These cell lines were maintained in Eagle's minimum essential medium (MEM, Nissui Seiyaku, Tokyo) supplemented with 10% FBS. When PA was assayed, we used a serum-free defined medium described by Namba et al.[15], which consisted of a 1:1 mixture of Dulbecco's modified MEM (Nissui) and Ham's F12 (Nissui) supplemented with 0.1% bovine serum albumin fraction V (Sigma, St. Louis, MO), 10 μg/ml of transferrin (Collaborative Research, Waltham, MA), 1 μg/ml of insulin (Sigma), and 5 μg/ml of oleic acid (Sigma). These additives were used in place of FBS in order to avoid complications due to possible activators and/or inhibitors of PA in serum. We detected neither activators nor inhibitors of PA in this defined medium. The cells could grow well in this medium.

We used three types of human IFNs: IFN-β produced in fibroblasts (Mochida Pharmaceutical Co., Tokyo), recombinant IFN-β, (rIFN-β, Kyowa Hakko Co. Ltd, Tokyo), and recombinant IFN-γ (Japan Roche Co., Tokyo).

For assay of PA produced by cells into culture medium, cells were seeded into plastic dishes (35 mm in diameter) in 2 ml of MEM with 10% FBS at a density of 10^5 cells/dish. Twenty-four hours later, this medium was removed, the dishes were rinsed twice with PBS, and 2 ml of the defined medium was added to cultures with IFN, whose concentrations are indicated in Results. We harvested the assay media at 72 h after the start of the culture and centrifuged them to remove cells or cell debris. The assay of PA was carried out using a chromogenic substrate