this, a microanalysis of the electron dense cytoplasmic granules, contained in the epithelial cell of the branchial chamber, and mucous present on the cell surface in direct contact with sea-water also showed total absence of vanadium. Quantitative analysis of the metal has not yet been done. Thus the present ultrastructural microanalysis of vanadium presents some new aspects concerning the distribution and metabolism of this metal in the ascidian blood cells. The present report demonstrates a direct localization of vanadium in the various types of blood cells. Earlier reports analized vandium with NMR[5] and with crude biochemical procedures in the blood cells in toto[10,11] and described a single value which according to several authors represented prevalently vanadium content of the vanadocytes. Furthermore it has also been shown here that the vacuole membrane of the blood cells examined possesses a high selectivity for the uptake of vanadium from sea-water. This is probably by simple diffusion through the branchial epithelium and against the concentration gradient in the vacuoles of blood cells. At last, these data showed scanty vanadium content in the vanadophores of vanadocytes. Contrary to claims of several earlier authors[3,7,8], the electron density of vanadium complex formed cannot be used as a marker in later stage of histogenesis of the blood cells, since the vanadophores of vanadocytes of *Phallusia mamillata* and *Ciona intestinalis* show a high electron density but very low vanadium content. This cellular type can no longer be considered as the main element for the selective uptake of vanadium. Vanadocytes could be destined to other metabolic functions[12] and probably also be the seat of catabolism of haemovanadin, as proposed earlier[13].

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Peritoneal macrophages from adjuvant arthritic rats enhance tumour cell growth in vitro

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Summary. Peritoneal macrophages from rats with adjuvant arthritis enhance the incorporation of ^{3}H-thymidine in 2 tumour cell lines in vitro. Maximal enhancement is found during the development of the secondary lesions, and it is suggested that the immunologic commitment of the macrophages could interfere with their regulation of tumour cell proliferation in vivo.

Macrophages appear to play an important role in the control of tumour cell growth[2]. Nonspecific activation of macrophages by i.p. administration of live organisms, such as *Corynebacterium parvum*, BCG or *Listeria monocytogenes*, elicits cytotoxic effects against a large variety of tumour cells, both in vitro and in vivo[3,4]. Recently Salmon and Hamburger[5] proposed that macrophages may also play a role as tumour promoters, and in the present report we show that, during the development of rat adjuvant arthritis, a chronic inflammatory disease triggered by immunologic mechanisms[6], peritoneal macrophages do enhance the growth of 2 rat compatible tumour cell lines in vitro, as compared to similar numbers of macrophages from non-arthritic rats.

Materials and methods. Adjuvant arthritis was induced in female, inbred Lewis rats (190–210 g) by injecting 0.3 mg of heat-killed *Mycrobacterium butyricum* (Difco) suspended in 0.1 ml of mineral oil, into the foot pad of the right hind paw. Saline-injected animals matched for sex and age served as controls. Peritoneal macrophages were collected from control rats and from arthritic rats 4, 7, 14, 21 and 28 days after the induction of adjuvant arthritis, by injection of 10 ml sterile saline into the peritoneal cavity. Cells from 5 control and 5 arthritic rats were poooled, respectively, and the cell suspensions were adjusted to 1×10^6, 5×10^5 and 1×10^5 cells/ml in culture medium (RPMI 1640, Gibco), supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin. All cell suspensions were kept at 4 °C to avoid loss of adherent cells. Aliquots of 0.5 ml were pipetted into the flat-bottomed wells of tissue-culture treated multidishes (Flow Laboratories, cat. No. 75-033-05). The cultures were incubated at 37 °C in 5% CO_2 in air, nonadherent cells were removed 2 h later by washing. After overnight incubation, the medium was removed and 0.5 ml fresh medium containing 2×10^5 tumour cells/ml was added to each well. Cultures without macrophages were run in parallel. 24 h later 1 µCi/ml of ^{3}H-thymidine (^{3}H-TdR, 2500 mCi/mmole, Amersham, England) was added to each well, and the cultures were incubated for 4 h at 37 °C. At the end of incubation, the tumour cells were transferred into ice-cold trichloroacetic acid (12% TCA) and the precipitates were washed 3 times with 6% TCA, dissolved in 0.5 NaOH and counted in a liquid scintillation counter. The adherent cells left in the wells were dissolved in 0.5 N NaOH for determination of residual ^{3}H-TdR incorporation. All samples were performed in quadruplicate and the results were calculated as dpm/well ±SEM. Percent change in ^{3}H-TdR incorporation was calculated, compared to cultures with control macrophages. Tumour cell viability was assessed by the eosin exclusion method. The tumour cells used were the Yoshida Sarcoma cells, propagated in vivo in Lewis rats and the Ascites Hepatoma AH-13 cells, propagated in vitro for at least 20 years. These tumour cells were originally induced in Donryu rats by feeding with o-amino-azotoluene[7]. I.p. injection of $2\text{–}4\times10^7$ cells of each type in Lewis rats led to death in 8–10 days.

Results and discussion. Yoshida Sarcoma cells, propagated in vivo, were harvested from the peritoneal cavity of Lewis rats, inoculated 5 days previously with 2×10^7 living tumour

cells. The tumour cell suspension was depleted of adherent host cells by culture for 2 h before use. 2×10^5 cells/ml were cultured alone or on macrophage monolayers, prepared from 1×10^6, 5×10^5 and 1×10^5 peritoneal cells/ml. Macrophages from control rats did not significantly alter the incorporation of ^{3}H-TdR in Yoshida Sarcoma cells: mean uptake in the absence of macrophages was found to be 362, 926 ±74,601 dpm/well in 3 separate experiments, in cultures with macrophages the range of uptake was 327, 279 to 405,300 dpm/well, regardless of the number of macrophages. In contrast, figure 1 shows that macrophages from arthritic rats, collected 14 and 21 days after the induction of adjuvant arthritis, consistently enhanced the incorporation

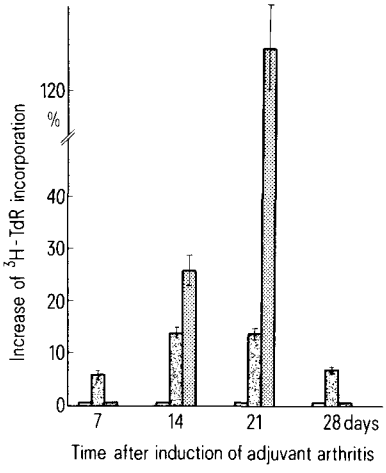

Fig. 1. Cocultivation of macrophages and Yoshida Sarcoma cells. Yoshida Sarcoma cells, 2×10^5 cells/ml, were cultered on macrophage monolayers, prepared from suspensions of 1×10^5 (□), 5×10^5 (▨) and 1×10^6 (▩) peritoneal cells/ml from control and arthritic Lewis rats. Each suspension consisted of cells pooled from 5 rats. The cultures were incubated for 24 h at 37 °C, 1 μCi/ml of ^{3}H-TdR was added for the last 4 h. The Yoshida Sarcoma cells were removed from the cultures and their incorporation of ^{3}H-TdR was measured. All samples were performed in quadruplicate. The percent increase in ^{3}H-TdR incorporation (±SD) in cultures with macrophages from arthritic rats was calculated, as compared to the corresponding cultures with control macrophages.

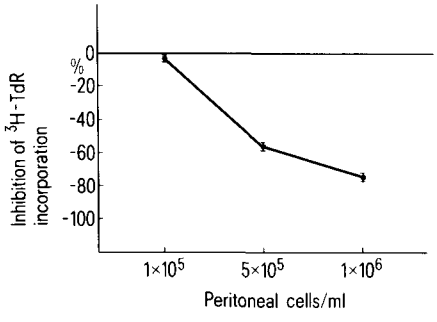

Fig. 2. Effect of control macrophages on the ^{3}H-TdR incorporation of ascites hepatoma AH-13 cells. Ascites hepatoma AH-13 cells, 2×10^5 cells/ml, were cultured alone or on macrophage monolayers, prepared from 1×10^6 peritoneal cells/ml pooled from 5 control Lewis rats. The cultures were incubated for 24 h at 37 °C, 1 μCi/ml of ^{3}H-TdR was added for the last 4 h. After removal of the AH-13 cells from the cultures, the ^{3}H-TdR incorporation was measured and the percent inhibition of ^{3}H-TdR incorporation in cultures with control macrophages was calculated, as compared with the corresponding cultures without macrophages. Each value is the mean ±SEM of 5 separate experiments, all samples were performed in quadruplicate.

of ^{3}H-TdR in Yoshida Sarcoma cells, dependent on the number of macrophages in the culture. The greatest enhancement was seen with 1×10^6 peritoneal cells/ml on day 21 of adjuvant arthritis (+127%); no effect was seen with 1×10^5 cells/ml. No differences in viability of the tumour cells were seen, all cultures contained > 90% viable cells at the end of the experiment.

Uptake of ^{3}H-TdR by the adherent macrophage monolayer was found to represent less than 3% of the uptake by the tumour cells, consistent with the reported lack of thymidine kinase in macrophages[8]. Although the Yoshida Sarcoma cells were depleted of adherent host cells before use, nonadherent host cells might still interfere with the macrophage/tumour cell assay. In order to exclude this possibility, the above experiments were repeated, using another rat compatible tumour cell line, the ascites hepatoma AH-13, propagated in vitro for at least 20 years[7]. The AH-13 cells were harvested from cell cultures during logarithmic growth. In contrast to the results obtained with the Yoshida cells, macrophages from control rats inhibited the ^{3}H-TdR incorporation of the AH-13 cells (figure 2), dependent on the number of macrophages in the culture. In the presence of macrophages from arthritic rats, however, enhancement of ^{3}H-TdR incorporation was observed (figure 3). Increases in ^{3}H-TdR incorporation over the level of that observed with AH-13 cells cultured without macrophages were not seen. Maximal enhancement was found with 1×10^6 and 5×10^5 peritoneal cells/ml, collected on day 14 and 21 after the induction of adjuvant arthritis. No enhancement was found in cultures with 1×10^5 cells/ml, all cultures contained > 90% viable cells.

The present results indicate that macrophages from rats with adjuvant arthritis have a decreased ability to deal with tumour cells. These findings are based on increased uptake of ^{3}H-TdR by the tumour cells, and the relevance of using this uptake as a measure of tumour cell growth has been studied by Nathan and Terry[9]. They found an extremely close correlation between the number of nonadherent,

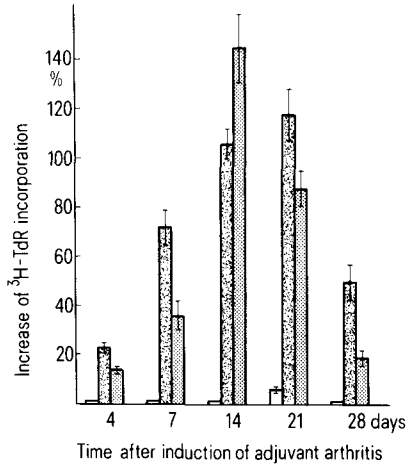

Fig. 3. Cocultivation of macrophages and ascites hepatoma AH-13 cells. Ascites hepatoma AH-13 cells, 2×10^5 cells/ml, were cultured on macrophage monolayers, prepared from suspensions 1×10^5 (□), 5×10^5 (▨) and 1×10^6 (▩) peritoneal cells/ml from control and arthiritic Lewis rats. Each suspension consisted of cells pooled from 5 rats. The cultures were incubated for 24 h at 37 °C, 1 μCi/ml of ^{3}H-TdR was added for the last 4 h. The AH-13 cells were removed from the cultures and their incorporation of ^{3}H-TdR was measured. All samples were performed in quadruplicate. The percent increase in ^{3}H-TdR incorporation (±SD) in cultures with macrophages from arthritic rats was calculated, as compared to the corresponding cultures with control macrophages.

esterase-negative tumour cells and the thymidine uptake by the cultures. Maximal enhancement was found 14 to 21 days after the induction of the arthritis, concomitant with the appearance and the development of the secondary lesions, generally believed to be a cell-mediated immune response[10,11], where the macrophages are likely to be involved. This immunological commitment might result in altered macrophage control of tumour cell growth, possibly under the influence of lymphokines or serum factors, as suggested by Hibbs et al.[12]. Alternatively, since the macrophages are a heterogenous cell population, a shift in macrophage subpopulations may occur during the course of adjuvant arthritis. Lee and Barry[13] have in fact recently presented evidence for the existence of 2 discrete subpopulations of macrophages involved in tumour cell killing and in promotion of immune responses. It is tempting to suggest that similar mechanisms are involved in the reported tumour growth enhancement in vivo of Krebs-2-carcinoma in mice immunized with BCG[14] and of hepatoma in guinea-pigs treated with Freund's complete adjuvant[15].

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Polyadenylate-polyuridylate enhancement of 7,12-dimethylbenz-anthracene skin carcinogenesis

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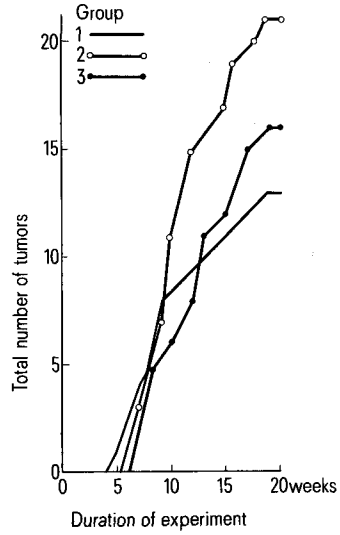
Summary. Double-stranded polynucleotide polyadenylate-polyuridylate (Poly AU) enhanced skin tumor formation in Swiss mice by 75% when injected prior to a single application of 7,12-dimethylbenzanthracene (DMBA). When given after the carcinogen application Poly AU did not significantly enhance tumor formation.

The immune competence of animals has been shown to be important in the induction and progression of chemical carcinogenesis in skin and other tissues[2-6]. Chemical carcinogens have been shown to induce a specific antibody in animals[7,8] and the presence of carcinogen antibody enhances carcinogenesis[8]. Polyadenylic-uridylic acid (Poly AU), a double-stranded polynucleotide, stimulates humoral antibody formation and cell-mediated immunity[9-13]. This study was designed to examine the effect of stimulation of the immune response by polyadenylate-polyuridylate (Poly AU) on DMBA induced skin tumor formation.

Material and methods. Swiss mice, 8-week-old females, received 100 µg of 7,12-dimethylbenzanthracene (DMBA) (Aldrich Co., Milwaukee, Wisc.) once on the interscapular area of the back skin. The hair had previously been removed with electric clippers. Group 1 received only DMBA, group 2 received 100 µg of Poly AU (Sigma Corp., St. Louis, Mo.) i.p. each day for 5 days before the 100 µg application of DMBA on the skin. Group 3 received Poly AU, 100 µg i.p. daily for 5 days starting the day after the application of DMBA. The mice were examined weekly and all tumors recorded. At the end of 20 weeks the animals were killed and autopsied. The skin as well as internal tissues were studied microscopically.

Results and discussion. Poly AU injections, both before and after DMBA applications, enhanced skin tumor formation as shown in the figure. Group 1 (DMBA only) had a total of 12 tumors (27% of the animals with tumors) while group 2 (Poly AU before DMBA) had a total of 21 tumors (60% of the animals with tumors) and group 3 (Poly AU after DMBA) had a total of 16 tumors (40% of the animals with tumors. Poly AU administered to animals before the application of the chemical carcinogen DMBA on the skin significantly increases skin tumor formation ($p < 0.02$, group 2 vs. group 1). The increase in DMBA skin tumor formation when Poly AU was given after DMBA was not



Effect of Poly AU on DMBA skin tumor formation. Group 1 received 100 µg of DMBA. Group 2 received 100 µg of Poly AU i.p. each day for 5 days before a 100 µg application of DMBA on the skin. Group 3 received 100 µg of DMBA followed by daily injections of 100 µg of Poly AU for 5 days starting the day after DMBA application.