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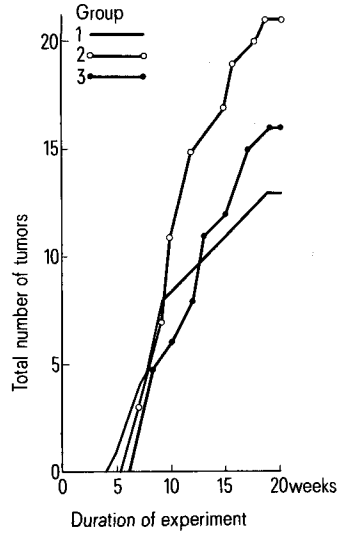
Summary. Double-stranded polynucleotide polyadenylate-polyuridylate (Poly AU) enhanced skin tumor formation in Swiss mice by 75% when injected prior to a single application of 7,12-dimethylbenzanthracene (DMBA). When given after the carcinogen application Poly AU did not significantly enhance tumor formation.

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.

Group	Treatment	N° of animals	Tba*	Histologically verified Tumors	Papillomas	Squamous cell carcinomas	Regressing tumors
1	DMBA only	30	8	12	6	-	6
2	Poly AU before DMBA	30	18	21	11	-	10
3	Poly AU after DMBA	30	12	16	5	2	9

*Tba = tumor bearing animals.

statistically significant ($p > 0.05$), group 3 vs. group 1) using the χ^2 test. Histological analysis (table) of the tumors showed most tumors to be papillomas with some of them regressing prior to termination of the studies. The only malignant tumors were seen in animals receiving Poly AU after DMBA (group 3).

Previous studies have shown that poly AU enhances both intracellular levels of cyclic AMP[13] and immune responses[9-13]. Cyclic AMP, like Poly AU, stimulates DMBA carcinogenesis when administered with DMBA or preceding carcinogen treatment[14]. Recently, another polycyclic aromatic hydrocarbon carcinogen, benzo(a)pyrene, was shown to induce an antibody in mice. The presence of antibody stimulated tumor development[8]. The enhancement of tumor formation seen when the immune response is stimulated before or at the time of carcinogen administration may be due to heightened carcinogen antibody formation.

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An uptake of fluorescein isothiocyanate labeled neocarzinostatin into the cancer and normal cells[1]

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Summary. An uptake of fluorescein isothiocyanate labeled neocarzinostatin into normal and cancerous epithelial cells from bladder was investigated. Results showed that neocarzinostatin traversed the cell membrane into cytosol and nuclei, and it appeared to have a preferential cytotoxicity for the cancer cell.

Neocarzinostatin (NCS) is an unique proteinacious antibiotic of known amino acid sequence[2] and is used for cancer treatment in man[3]. It inhibits DNA synthesis in bacteria and mammalian cells and arrests mitosis[4,5]. Although most of the molecular mode of action of NCS have been clarified as DNA strand scission at primarily thymidinyl residue followed by phosphorylation at both 3′ and 5′ sites which result in the inhibition of DNA synthesis[6-10], it is still unclear at the cellular level whether NCS actually enters into the cell to exert its effects. We have investigated this point by means of fluorescence microscopy using fluorescein isothiocyanate labeled NCS (F-NCS) and tumor cells obtained from human bladder cancer (transitional cell carcinoma histologically) as well as normal cells.

Materials and methods. F-NCS was prepared as described previously and retained original biological activity[11]. Bladder tumor and normal bladder epithelium were taken from transurethral biopsy and/or surgery. The tissues were minced and treated with 0.25% trypsin for 30 min at 37 °C. After filtration through a platinum mesh (No. 80), freed cells were washed twice with 0.01 M phosphate buffered 0.15 M saline (PBS, pH 7.0) and subcultured in RPMI 1640 supplemented with 10% foetal calf serum for 4 h to allow repair of the damaged membrane. Then, cells were washed twice with glucose Hanks' balanced salt solution (pH 7.2) and suspended in it to give a concentration of 10^6/ml. Aliquots of 0.5 ml of cell suspension were mixed with 0.2 ml of F-NCS (OD490 nm = 0.165) and incubated at 37 °C. After varied intervals, a small drop of cell suspension was placed on a slide glass and examined by a fluorescence microscope directly. In order to verify the incorporation by cancer cells, cells incubated with F-NCS for 1.5 h were washed 3 times with PBS, fixed with ethanol and then xylene, and embedded in solid paraffin followed by slicing with microtome into thin section (5 μm). The sliced thin section was placed on a slide glass and washed with xylene to remove paraffin before fluorescence microscopy.

Results and discussion. During initial 30 min, an uptake of F-NCS was slow and not distinct. Fluorescence was observed as sparse granules in cytoplasm as well as in nuclei. An enhanced incorporation of F-NCS by cancer cells was observed after 1 h. After 1.5–2 h of incubation with F-NCS, the uptake reached a maximum. As shown in figure 1, apparent fluorescence was observed in cytoplasm as well as in nuclei. Furthermore, in the cytoplasm, F-NCS was distributed homogenously and its uptake was thus definite. Furthermore, by examining the sliced sections, the uptake of F-NCS was also confirmed both in cytoplasm and nuclei, although it was less distinct than that of unsliced materials (figure 2). When uptake was compared with that of normal cells, the incorporation by the tumor cells was higher and faster than that by the normal blood