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Subcutaneous Injections of Vaccine Adjuvant DEAE-Dextran induce Local Sarcomas in Mice

THE water-soluble, cationic polymer diethylaminoethyl (DEAE) dextran, when added to many animal virus preparations, apparently facilitates the adsorption and penetration of viral particles into cells, and thereby increases the titre. This has led to the suggestion[1] that DEAE-dextran might usefully be incorporated into vaccines, at least those designed for veterinary use. This proposal has received considerable attention recently in the investigation of effective immunization procedures against foot and mouth disease[2–5]. We report here a series of experiments in which DEAE-dextran preparations were injected subcutaneously into mice, and unexpectedly gave rise to fibrosarcomas at the site of injection. These results provide a warning that injection of vaccines containing DEAE-dextran may give rise to tumours in other species.

Subcutaneous sarcomas were first observed in female Swiss–Webster mice which had received a single intraperitoneal injection (1 μmol/g body weight) of the carcinogen 1-ethyl-1-nitrosourea (ENU) in trioctanoin at 5 weeks of age, followed by weekly or twice-weekly subcutaneous injections of 0.5 ml. of neutral, buffered saline containing 500 μg DEAE-dextran (Pharmacia Corporation, Uppsala; nitrogen content 3.2%; made from dextran of molecular weight 2×10^6), alone or combined with 50 μg of the interferon inducer poly I·poly C[6]. These subcutaneous injections were continued for either 10 or 20 weeks, and a total of eighteen sarcomas eventually were seen in 127 mice, which were alive at the time when the first tumour was observed, 25 weeks after the first injection. No sarcomas developed in forty-two control mice which received ENU alone. All survivors were killed 40 weeks after the first injection. A second experiment was then carried out in which no poly I·poly C was used. Sarcomas were again observed when an intraperitoneal injection of ENU was followed by repeated subcutaneous injections of DEAE-dextran in saline, but not when the subcutaneous injections consisted of the electrically neutral dextran from which the DEAE-dextran had been prepared, or of saline alone. Sarcomas also developed during the first year of life in four of forty-eight mice given DEAE-dextran injections, but no ENU. This demonstrated that ENU pretreatment was not essential for sarcoma induction by DEAE-dextran. The incidences of other tumour types including mammary carcinoma, thymic lymphoma, and pulmonary adenoma were not significantly affected by repeated subcutaneous injections of DEAE-dextran in either ENU-treated or normal mice. These results will be presented in detail elsewhere.

Since the dextran component of DEAE-dextran is not oncogenic, and the diethylaminoethyl moieties closely resemble in structure many compounds which have been tested as non-reactive analogues of the nitrogen mustards without demonstrable oncogenic effect, it seems reasonable to conclude that DEAE-dextran is a non-reactive chemical carcinogen, with local but not systemic oncogenicity for rodent tissues. In this respect it closely resembles insoluble plastic films[7]. The chemically most closely related material to DEAE-dextran which is known to be oncogenic is the iron oxide–dextran complex, which is used in man to treat refractory iron-deficiency anaemia. This material also gives rise to local sarcomas at the site of repeated subcutaneous injection in mice and rats[8]. Whether the mechanisms of action of iron-dextran and DEAE-dextran are similar remains to be investigated.

Murine sarcoma viruses are among those whose infectivity is enhanced by DEAE-dextran[9,10], and it is conceivable that oncogenesis in mice by this chemically unreactive polymer is dependent on chronic infection by a member of the murine leukaemia–sarcoma complex. Efforts to isolate viruses with the properties appropriate to this hypothesis are currently in progress, using tissue cultures derived from DEAE-dextran-induced sarcomas.

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Protein Deficiency and Mucosal Granulocytes

SCRIMSHAW *et al.*[1] have reviewed the evidence relating protein deficiency and bacterial infections. Guggenheim and Buechler[2] showed decreased *in vitro* bactericidal activity of peritoneal fluids from protein-deficient rats while Newberne *et al.*[3] demonstrated impaired clearance of salmonellae from circulation by rats fed low-protein diets.

The intestinal mucosa usually contains numerous peroxidase-containing neutrophils and eosinophils in the lamina propria[4], the bactericidal activity of which is well documented[5–7]. Fasting for a brief period (1–2 days) decreases the number of granulocytes in gastric mucosa[8], and prolonged protein deficiency causes a leucopenia and reduction in the myeloid–erythroid ratio of bone marrow with increased numbers of immature myeloid forms[9–10]. We have investigated whether the granulopenia of chronic protein deficiency is associated with reduced numbers of tissue granulocytes in the intestinal mucosa.

Male Sprague-Dawley rats weighing 70–80 g were fed on the protein-deficient and control diets of Edozien[11] for 8 weeks.