following tissues; brain, eye, tooth, vibrissae, maxillary sinus, oral mucosa, tongue, oropharynx, salivary glands, bone marrow, lung, heart, the walls of the blood vessels, muscular tissues, thyroid gland, adrenal gland, thymus, esophagus, stomach, small and large intenstine, liver, pancreas, kidney, peritoneum, periostium, cranio-spinal nerves and ganglia, and the skin. In some of these tissues, most of the cells are composed of the neural crest cell derivatives, and in other tissues the neural crest cells are scattered among other cells, and in the suckling mice the undifferentiated neural crest cells are present there[10]. The excessive cell proliferation or tumors and heterotopic melanin pigmentation occurred in the sites where neural crest cells may be present. Tumors and heterotopic melanin pigmentation were conspicuous in the orofacial region and their occurrence corresponds with the fact that the neural crest cells are widely and densely distributed in the orofacial region[5]. The multiple tumors were composed of atypical cells such as spindle-shaped cells or elongated cells, round or oval cells and irregular-shaped cells. Furthermore, the spindle-shaped cells which lost their staining capacity were scattered in suckling mice injected with endotoxin as well as in the suckling mice injected with mitomycin C[11]. Some of the tumors, composed of the spindle-shaped or elongated cells, showed mostly very characteristic features: a) an interlacing wavy network, b) a tight interlacing pattern of the stream. Further, multiple excessive proliferation of melanin-producing and -containing cells or melanoma were often seen. The histological figures of these tumors closely resembled those in the suckling mice injected with mitomycin C[11].

It is said that a tumor induced by a single agent in a single cell type can have a multiplicity of phenotypes[12]. Endotoxin belongs to the mitogens. It is said that endotoxin increases the cyclic AMP level[13] and phosphorylation of nucleoproteins[14]. On the other hand, it is said that DNA-histone interaction is affected when the histone is phosphorylated and consequently depression of the activity of the DNA template occurs[15], and that histone act as the regulator of transcription of the DNA template[16]. It is thought the alternation of transcription and translation of the genome into the phenotype is responsible for carcinogenesis[17]. It is assumed in our laboratory that DNA nucleotide sequences in the neural crest cells may be specific and that neural crest cells may have something to do with cyclic AMP[4], and that macrophages or Kupffer cells may be of neural crest origin[5]. On the other hand, it is said that macrophages[8] or Kupffer cells[18] take up endotoxin. From the above evidence, it is speculated that multiple excessive cell proliferation or tumors and heterotopic melanin pigmentation caused by endotoxin may have something to do with the neural crest cells.

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Soft versus hard water as a factor in the incidence of anencephalic foetuses in litters from trypan blue treated mice

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Summary. In an experiment designed to test the possible correlation between hard water and neural tube anomalies, high calcium intake was found to increase the number of exencephalic foetuses in litters from trypan blue treated mice. This is a reversal of the suggested trend in man where soft water and anencephaly may be correlated.

Regional variation in the incidence of anencephaly in England and Wales has suggested a possible correlation with water hardness[1-4]. For obvious reasons, such a correlation is difficult to establish in man, and it was thought that an experimental approach using a laboratory mammal might be informative. Female BALB/c/Gr mice were divided at weaning into 3 groups. The first was fed standard rat cake (Oxoid Pasteurised 41B) and tap water ad libitum. The second was given rat cake plus 1% calcium acetate solution buffered to pH 7.2 (the pH of local tap water) with a little NaOH. The third was placed on 1% calcium acetate until mating, then transferred to tap water. Mice were mated with BALB/c/Gr males a minimum of 6 weeks after weaning. Mice with a vaginal plug were given an i.p. injection of 1 ml 1% trypan blue (Gurr) in 0.93% saline on day 8 of pregnancy. Females were killed on day 14 of pregnancy and foetuses examined after fixation in Bouin's fluid.

All groups of foetuses contained exencephalics. Mice raised on hard water were less fertile than controls raised on tap water (table) but had larger litters, with more implants and a similar degree of resorbtion (solid moles). They also produced more exencephalic foetuses. Mice transferred from hard to tap water at mating fared badly,

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Exencephaly in litters of foetuses from mice raised on tap or hard water

Maternal water supply	No. of mice	No. pregnant	Total implants	Total litter size	No. resorbtions	Normal foetuses	Exencephalic foetuses
Tap water (2.8 mg % Ca)	32	16 (50%)	98	6.1	32 (42%)	64	6 (13%)
Hard water (109 mg % Ca)	78	32 (40%)	240	7.9	94 (44%)	124	22 (18%)
Hard → soft at mating	48	10 (21%)	66	6.5	24 (36%)	26	16 (33%)

with lowered fertility (although litter size and number of resorptions were similar to those of controls) and more exencephalics.

In man, it has been suggested that neural tube defects such as anencephalus and spina bifida might be correlated with softness of drinking water[1-3], although Fielding and Smithells[4] were unable to demonstrate this correlation in South West Lancashire. In this short series in the mouse, a correlation does seem to be present, but in reverse, with more neural tube defects in the offspring of mice exposed to hard water. The effect of transfer from hard to soft water at mating, designed to test whether the effect was upon the foetus or the maternal physiology, seems merely to add an extra trauma.

Preparation of fixed antigenic, non-oncogenic and protective neoplastic cells

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Summary. Transplantable spontaneous mammary adenocarcinoma and sarcoma P 1798 cells were incubated with vibrio cholera neuraminidase (VCN), then fixed cells demonstrated ability to exclude trypan blue dye and to immunized animals and produce cytotoxic sera of high titers. The fixed neuramindase-treated tumor cells became non-oncogenic and protected the host against high doses of fresh untreated homologous tumors.

Experiments with in vitro assays have established[1] that most human malignant cells contain cell-surface antigens which are either absent or present at very low concentrations on the surface of normal cells of the corresponding adult tissue. Some of these antigens can evoke a specific cell-mediated immunity directed against malignant cells of corresponding tissue type[2]. Tumor cells treated with vibrio cholera neuraminidase (VCD) have been used to immunize mice[3,4] and to cause remission of small but progressively growing tumors[5-7]. Therefore, the present investigations were aimed at the preparation of fixed, antigenic, non-oncogenic cells that immunologically protect the host against spontaneous mammary adenocarcinoma and against sarcoma P 1798.

Materials and methods. Tumors Sarcoma P 1798 and spontaneous mammary adenocarcinoma were carried on in 2 strains, CFW_1 and BALB/c, 18–20 g inbred, brother-sister white mice. Doses of 10^3 viable sarcoma cells, or 10^4 viable adenocarcinoma cells administrered s.c., developed into solid tumors in all the inoculated mice and killed their hosts in 21–28 days. So far, no regression was observed in mice inoculated with each of these 2 strains.

Cell preparations. Transplantable mouse sarcoma P 1798 and spontaneous mammary adenocarcinoma cell suspensions in Eagle's minimum essential medium (MEM) were prepared from fragments of tumor tissue as described in an earlier communication[8]. For frozen cell preparation, cells were quickly frozen in liquid nitrogen. For vacuum dried cells, cells were quick-frozen in acetone-dry ice bath and dried under vacuum while frozen. Formalinized cell preparations were made by washing the cells with 0.85% NaCl solution and centrifuged at 1500 rqm for 10 min. 10 volumes of 2% buffered formalin were added to the packed cells which were suspended uniformly by gentle stirring at room temperature for 10 h. Cells were then washed twice with 0.85% NaCl solution and stored at 10°C until ready for use. The 2% buffered formalin contained per l 20 ml of 40% formaldehyde, 4g $NaH_2PO_4 \cdot H_2O$, 6.5 g Na_2HPO_4 and pH adjusted to 7.2. Glutaraldehyde cell preparations were made by washing the cells with 0.85% NaCl solution, then centrifuged at 1500 rpm for 10 min. 10 volumes of 0.2% buffered glutaraldehyde were added to the packed cells which were suspended uniformly by gentle stirring at room temperature for 1 h. Cells were then washed twice with 0.85% NaCl solution and stored at 10°C unitil ready for use. The 0.2% buffered glutaraldehyde was made in phosphate buffer and adjusted to pH 7.2.

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