Survey on carcinogenesis experiments in Sprague-Dawley rats by administration of commercial camembert- and roquefort-cheeses and mold mycelium

Group	Mean life expectancy in months	Malignomas N	Percent	Mean induction time t_{50} in test months
A1	22 ± 6	5 *a, a, a, b, c,*	6.3	19 ± 9
A2	18 ± 8	2 *b, b*	3.3	20 ± 23
A3	22 ± 7	4 *c, c, c, d*	6.6	25 ± 4
A4	21 ± 7	5 *a, b, b, b, c*	8.3	26 ± 4
B1	21 ± 6	5 *a, a, a, b, b*	8.3	15 ± 5
B2	20 ± 6	3 *a, a, c*	5.0	18 ± 13
B3	28 ± 5	9 *a, a, b, b, b, b, c, d, e*	15.0	25 ± 3
B4	18 ± 8	1 *b*	1.7	23
C1	20 ± 7	6 *a, a, b, b, b, c*	10.0	17 ± 4
C2	19 ± 6	7 *a, a, a, b, b, c, c*	11.6	22 ± 4
C3	27 ± 5	6 *a + e, b, b, c, c, e*	10.0	24 ± 5
C4	14 ± 6	3 *b, b, c*	5.0	19 ± 3
Total control	17 ± 8	3 *a, a + b, c*	2.7	11 ± 6

a Carcinomas (mamma, bile duct, uterus, abdominal cavity, kidney, forestomach, ovary, adrenal cortex, tail). *b* Sarcomas (pylorus, mediastinum, abdominal cavity, stomach, ovary, uterus, lung, subcutaneous, kidney, thigh, neck, vagina). *c* Leukoses and reticuloses. *d* Haemangioendotheliomas (small intestine mesenterium, kidney). *e* Malignant thymonas (lung hyllus).

The results of the carcinogenicity experiments are summarized in the table. The observed malignomas are grouped according to type, incidence in percent and mean induction times. These results show clearly that by feeding commercial cheeses in extreme dosages no statistically significant higher tumor rate could be observed in the test groups compared to the control groups. The same statement applies to the oral application of mycelium-suspensions, as well as to their s.c. application.

The higher tumor incidence in experiment B3, which could be shown by common statistical methods (t-test), is apparent only, since these animals had a much longer life span than the control animals, and on grounds of their higher life expectation could be expected to show a higher tumor incidence. The same applies in this connection to test groups C1–C3.

It is remarkable that after s.c. application of mycelium-suspensions only 2 animals showed sarcomas locally at the site of application. On account of the very low incidence and the frequent injections, we are inclined to regard these kinds of tumor as non-specific, and to assign no local carcinogenic effect to the mycelium-suspensions themselves. The lack of carcinogenic effects in our experiments is further proved by the fact that the mean induction times of malignomas in all experimental groups were much higher in comparison with the controls. A chronic-toxic effect, manifesting itself in the experimental animals as shortening their mean life expectancy, has not been observed. The above results allow that a carcinogenic effect can be assigned neither to the starters used in German cheese technology, nor to their products.

Multiple APUD system (neural crest) tumors caused by endotoxin in suckling mice

T. Nozue and T. Kayano

Department of Anatomy, Tokyo Medical and Dental University, 5–45, 1-Chome, Yushima, Bunkyo-ku, Tokyo (Japan), 13 September 1976

Summary. In suckling mice injected i. p. with endotoxin on the 1st day after birth and surviving up to the 4th day, multiple tumors and heterotopic melanin pigmentation occurred in the sites where the neural crest cells may be present.

Enterochromaffin cells of the gut, argyrophil cells of the bronchioles, islet of the Langerhans, and parafollicular cells of the thyroid gland are all derived from the neural crest[1–6]. All these cells belong to the amine precussor uptake and decarboxylase (APUD) system[1,2,3,7]. It has been shown that histamine or serotonine increase in amount in animals treated by endotoxin[8], and further that macrophages proliferate remarkably[9]. On the other hand, it is assumed in our laboratory that macrophages may be of neural crest origin[5]. Accordingly, it is speculated that the neural crest cells may have specific sensitivity to endotoxin, and react to endotoxin stimulation by proliferation. The following experiments were designed to demonstrate this reaction.

Materials and methods. Lipopolysaccharide B.E. coli 026:B6 (Difco Laboratries, Detroit, Mich., USA) were used as endotoxin. 100 suckling mice of the ICR-JCL strain were used as experimental animals. 90 mice were injected i.p. with endotoxin at a dose of 50 mg/kg body weight on the 1st day after birth and 16 suckling mice survived up to the 3rd day after injection. 10 mice not treated with endotoxin, were used as the control specimens. The specimens were fixed with 10% neutral formalin and decalcified with Plank-Rychlo's fluid and embedded in paraffin. Transverse, frontal and sagittal sections of the total bodies of the mice of 7 μm in thickness were prepared serially and stained with hematoxylin-eosin and ferric chloride stain for melanin.

Results and discussion. In the suckling mice injected i.p. with endotoxin on the 1st day after birth and surviving up to 3rd day after injection, multiple excessive cell proliferation or tumors occurred in all individuals, and further, heterotopic pigmentation by melanin was seen. Tumors or pigmentation were seen in certain sites of the

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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

D. R. Johnson

Department of Anatomy, School of Medicine, University of Leeds, Leeds LS2 9NL (West Yorkshire, England), 29 September 1976

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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