Investigations on carcinogenic effects of Penicillium caseicolum and P. roqueforti in rats[1]

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Summary. A feeding test over the whole life span of 800 Sprague-Dawley rats with commercial starters for camembert– and blue-cheese, with commercial cheese and test cheese, as well as subcutaneous application of the mycelia, did not yield any indication for a detrimental or carcinogenic effect.

The results published by Gibel et al.[4] in 1971 gave rise to the suspicion that camembert molds used in food technology might lead to an increased cancer rate, if given in long-term tests orally or s.c. to rats. Kurata[5], Kanota[6] and Wei and coworkers[7,8] reported on the formation of different mycotoxins by several isolates of Penicillium roqueforti, but there is no evidence of a possible carcinogenic effect yet. For this reason we started a long-term study (748 animals) with Sprague-Dawley rats of both sexes of the age of about 100 days, which were fed both mold species, as well as cheeses produced by them, to investigate possible hazards and cancer development.

We used 2 different commercial starters for camembert manufacture, the strain Penicillium camemberti var. candidum III C3, used by Gibel et al. and a commercial starter for the manufacturing of blue-cheese. With the camembert starters NaCl-free cheeses with a large surface and little cheese mass ($30 \times 13 \times 1.5$ cm, test camembert A and F, respectively) were produced as is common in cheese manufacturing. Mycelium-suspensions for oral and s.c. application of all 4 mold strains were produced by culturing them on wort agar with 2.5 g wet mycelium in 100 ml physiological saline, respectively and stored until use at –25 °C.

Cheese or mold mycelium was given according to following administration schedule:

A. *Feeding tests for life* (5 days cheese, 2 days Altromin®-pellets, water ad libitum)

1. 5 types of commercial camembert or brie, respectively – 12–45% fat in dry matter – in weekly alternation	80 animals
2. 1 type of commercial blue-cheese – 30% fat in dry matter	60 animals
3. Test-camembert A – 5.6% fat in dry matter	60 animals
4. Test-camembert F – 5.6% fat in dry matter	60 animals
5. Control: Altromin®-pellets	21 animals

B. *2.5 ml mold-suspension, once weekly per gavage for life*

1. Camembert-culture A (M = 4.95 g mycelium/animal)	60 animals
2. Camembert-culture F (M = 4.10 g mycelium/animal)	60 animals
3. P. camemberti var. candidum III C 3 (M = 6.23 g mycelium/animal)	60 animals
4. Blue-cheese-culture (M = 4.80 g mycelium/animal)	60 animals
5. Control: 0.9% NaCl-solution	44 animals

C. *2.5 ml mold-suspension, once weekly s.c. for 52 weeks*

1. Camembert-culture A (M = 2.94 g mycelium/animal)	60 animals
2. Camembert-culture F (M = 2.75 g mycelium/animal)	60 animals
3. P. camemberti var. candidum III C 3 (M = 2.63 g mycelium/animal)	60 animals
4. Blue-cheese-culture (M = 2.93 g mycelium/animal)	60 animals
5. Control: 0.9% NaCl-solution	44 animals

The average amount of cheese consumed by 1 animal during the complete test period was 12.7 kg with test cheese A, 12.5 kg with test cheese F, 9.5 kg with commercial camembert and 8 kg with commercial blue-cheese. The lower consumption of commercial cheeses can probably be explained by their higher fat proportions. With gavaging, the mean applied overall doses were in experiment B1 = 198 ml/animal, B2 = 164 ml/animal, B3 = 249 ml/animal, B4 = 192 ml/animal, B5 = 126 ml/animal in relation to the suspension amount administered. The corresponding data for experiments with s.c. application of mold-suspensions are: C1 = 118 ml/animal, C2 = 110 ml/animal, C3 = 105 ml/animal, C4 = 117 ml/animal, C5 = 115 ml/animal.

The animals were observed for life, autopsied after death and conspicuous findings histologically examined. The rats showed no acute-toxic lesions during the treatment. Their weight development was in the normal range. Unfortunately some rats of the control group, as well as some animals of the test group C4, died intercurrently with viral infection.

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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

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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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