Activation of a Mammary Tumour Virus in O20 Strain Mice by X-irradiation and Urethane

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In mouse mammary tumours often a virus particle, called a B particle by Bernhard & Guérin (1958), is found which is associated with the genesis of these tumours (Hageman, Links & Bentvelzen, 1968). Three distinct biological entities show B particles, viz. the Bittner virus (Hageman et al. 1968), the nodule-inducing virus (Nandi, 1966), and the Mühlbock virus (Bentvelzen, 1968). The general opinion about chemical and physical induction of mammary tumours in mice is against the idea of potentiation of latent mammary tumour viruses (cf. Bittner, 1960). This contrasts with concepts on similar modes of induction of leukaemia in mice (see for a review Gross (1961) and Kaplan (1967)). We have found that injection of carcinogenic chemicals or whole body irradiation of B-particle-free mice leads to the release into the serum of antigens which cross-react with a soluble antigen of the Bittner virus (Daams et al. 1968, Bentvelzen et al. 1968). Biological evidence supporting the hypothesis of activation of a mammary tumour virus by such treatment is presented below.

The Ozo strain of mice is highly resistant to so-called spontaneous development of mammary tumours. No biological evidence for the presence of a mammary tumour virus was found by Mühlbock & van Rijssel (1954). We found no B particles in electron microscopic preparations of fifteen mammary tumours, induced by the extreme hormonal stimulation of pituitary isografts under the kidney capsule (Boot et al. 1962), though at least 300 thin sections of each mammary tumour were examined.

The Ozo strain is also resistant to the Bittner and the Mühlbock virus: mammary tumours appear at a late age. Dux & Mühlbock (1966) found this resistance to be mainly localized in the target organ itself. The Bittner virus grows poorly in the Ozo strain (Mühlbock & van Rijssel, 1954), and according to Bentvelzen (1968) this may be the cause of the resistance. Hybrids of the C3 Hf mouse strain, carrying the nodule-inducing virus and the Ozo strain seldom show B particles in their mammary tumours (Mühlbock & Bentvelzen, 1968).

Two-month-old female Ozo mice were given 200 rad. whole body irradiation (Siemens Isomatix; 250 kv, 8 ma, 1 mm. Cu, FSD 30 cm.; 53.5 rad./min.), while the ovarian region was shielded by a lead girdle (2 cm. broad, 3 mm. thick). Two days later forced breeding of the animals was started and urethane was given continuously in the drinking water. As controls one group was force-bred while receiving urethane in drinking water and another group was force-bred and received ordinary tap water. In spite of the lead girdle six out of 13 animals proved to be sterile and were excluded from the experiment. Of the seven remaining mice four developed a mammary tumour before 1 year of age, two developed lymphatic leukaemia and one developed a uterine tumour. In the control groups no mammary tumours were found before one year of age (Table 1).

Small pieces of one of the four mammary tumours were fixed in 2% osmium tetroxide in phosphate buffer, pH 7.1, for 2 hr and embedded in a mixture of Epon
and Araldite. Thin sections were stained with uranyl acetate and lead hydroxide and studied with a Philips EM 200 electron microscope. This tumour contained budding and mature B particles (Pl. I a).

Cell-free filtrates of two other of these induced mammary tumours were made in the following way: tumours were homogenized at 4° in a solution of 0·61% NaCl+ 0·04% bovine serum albumin+ 0·02 M McIlvaine’s phosphate-citrate buffer. Ten ml. of buffer was added to each g. of tumour and the material was homogenized again in the same volume of fresh buffer. The homogenate was centrifuged at 1500 g for 5 min. The supernatant fluids were combined and centrifuged at 10,000 g for 15 min. The upper half of the supernatant fluid was used as cell-free filtrate and contained the equivalent of 1 g. tumour in 20 ml. buffer. Half a ml. of this fluid was injected intraperitoneally into 4-week-old female BALB/c mice. These mice were subjected to forced breeding.

### Table 1. Development of mammary tumours in forced breeding of O20-strain mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Effective no. of animals</th>
<th>No. with mammary tumour before 1 year</th>
<th>Average tumour age in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>64</td>
<td>0</td>
<td>225 ± 19</td>
</tr>
<tr>
<td>Urethane in drinking water</td>
<td>14</td>
<td>0</td>
<td>--</td>
</tr>
<tr>
<td>Whole body irrad.+urethane in drinking water</td>
<td>7</td>
<td>4</td>
<td>225 ± 19</td>
</tr>
</tbody>
</table>

One filtrate induced mammary tumours before one year of age in eight out of ten mice; the other in four out of ten. The average tumour ages were 193 ± 21 and 225 ± 18 days, respectively. In 447 force-bred BALB/c females no tumour arose before that age. Of 38 BALB/c mice carrying the Bittner virus 100% developed a tumour at 172 ± 30 days average.

Cell-free filtrates of two tumours induced in BALB/c mice by the O20-extract were prepared. These were injected into virus-free BALB/c mice. Although the experiments are still in progress, several tumours have already been observed.

Three mammary tumours, induced in BALB/c by the O20 tumour extract, have been examined with the electron microscope. In these tumours B particles were present (Pl. I c), whereas they were not seen in eight BALB/c tumours induced by extreme hormonal stimulation. This indicates that irradiation + urethane activates a B particle virus in mice of the O20 strain, and that this virus can be transmitted by cell-free filtrates.

In two of these tumours also C particles (Pl. I c) were present; these are often associated with the development of leukaemia (Bernhard & Guérin, 1958). It is not clear whether these C particles are involved in the genesis of mammary tumours or that they are only present as passengers (Calafat, 1968).

In another experiment sixteen O20 females were injected with 0·25 ml. of a 10% urethane solution in distilled water and then force-bred. Two of them developed a mammary tumour before one year of age; they were studied with the electron microscope and contained B particles (Pl. I b).

It is clear that carcinogenic treatments can induce both a B particle virus and mammary tumour inciting virus in O20 strain mice, which are ordinarily free of them.
(a) Thin section of a mammary tumour of an O2o mouse induced by irradiation and urethane in the drinking water. Mature (B) and budding (BB) B-particles can be seen in the acinar lumen.

(b) O2o mammary tumour induced by an intraperitoneal injection of urethane. Mature (B) and immature (IB) B-particles are present in the acinar lumen. Intracytoplasmic A-particles (A) are also visible.

(c) BALB/c mammary tumour produced by injection of a cell-free extract of an O2o mammary tumour induced by irradiation and urethane in the drinking water. Many B and C particles can be seen together in the acinar lumen. Inserts: B-particle with an external envelope formed by a double membrane and an eccentric nucleoid surrounded by a thin membrane; C-particle with a central located nucleoid, wrapped by a thin, smooth outer membrane.

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It is likely that both entities are identical. It is also very likely that the gene-complex of the $O_{20}$ strain, which inhibits the manifestation of its own mammary tumour virus, also interferes with the manifestation of the nodule-inducing virus in ($C_{3}Hf \times O_{20}) F_{1}$ mice and furthermore causes resistance to superinfection with either Mühlbock or Bittner virus by interfering with the replication of these viruses.

**Department of Biology**

A. TIMMERMANS  
P. BENTVELZEN  
PHILOMENA C. HAGEMAN  
JERO CALAFAT

**Department of Electron Microscopy**

Antonie van Leeuwenhoekhuis  
The Netherlands Cancer Institute  
Amsterdam, The Netherlands

**REFERENCES**


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(a) Autoradiogram of a region of a tobacco mosaic virus infected protoblast following incubation in 125I-labelled TMV antibody, developed with D19B. The antibody is detectable as silver grains which are predominantly over regions of cytoplasm containing groups of virus particles. Part of the large central vacuole (V) is visible.

(b) Region of (a) (arrowed) at higher magnification showing virus particles.

(c) Autoradiogram similar to (a) developed with Microdol X. The silver grains are smaller, obscuring less detail of the virus.

M. A. Mayo and E. C. Cocking

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