Response of mice to combined exposure of Friend leukemia virus (FLV) and methyl methane sulfonate

Mouse strain	Treatment	% of mice that died of leukemia in the course of experiment*	Mean time of leukemia deaths (days after virus)	Mean spleen weight at death**
SJL/J	Sham injected	0 (20)	-	0.3441 (0.2894–0.3988)
	MMS only	0 (22)	-	0.3265 (0.2884–0.3646)
	Virus only	65.4 (26)	104.4 (81.6–127.1)	1.1871 (1.0011–1.3731)
	MMS and virus	93.3 (45)	51.9 (43.6–60.3)	1.3797 (1.2281–1.4713)
$B10SJF_1$	Sham injected	0 (14)	-	Normal Weight 0.3 g
	MMS only	0 (21)	-	Normal Weight 0.3 g
	Virus only	17.9 (28)	143.0 (112.7–173.3)	2.0135 (1.4954–2.5316)
	MMS and virus	80.0 (34)	85.5 (81.1–90.0)	3.2527 (3.0479–3.4575)

Mice were injected with 100 mg MMS/kg b. wt and 5 h later with FLV (0.1 SED). They were followed for 300 days.
* The number in parenthesis equals the total number of mice in group. ** Mice that did not die of leukemia were sacrificed after 300 days.

the data presented in this paper. Because $B10SJF_1$ has a higher degree of FLV resistance than SJL/J, its response to FLV coupled with MMS is especially notable. In our previous studies we found that the time interval between exposure to MMS and FLV is a critical factor in the demonstration of any possible potentiating effect of the chemical carcinogen[2]. MMS given 5 h before FLV had the maximum effect. This time interval agrees closely with the optimum necessary for MMS potentiation of adenovirus transformation of CHO cells as reported earlier by Casto et al.[6]. Although it is not yet certain how MMS produces viral leukemia potentiation in vivo, our previous data demonstrated a rapid appearance and disappearance of the effect in vivo[2], similar to that seen by Casto et al.[5] in vitro, which they suggest may indicate a direct effect on the DNA of the target cell. Relative to this, Regan and Setlow[7] reported that MMS produces direct short strand breaks in DNA similar to those caused by X-rays[4,6,8], and earlier studies with X-rays have shown that radiation is a potentiator of viral leukemias[10,11]. According to Regan and Setlow[7] the DNA damage caused by MMS is quickly repaired. This would be in accord with the rapid disappearance of the potentiating effect of the virus. It is thus possible that potentiation of viral leukemogenesis by MMS may be dependent upon the DNA breaks caused by the chemical and/or their subsequent repair.

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A transmission and scanning electron microscopic study of cytoplasmic threads of dividing neuroepithelial cells in early chick embryos[1]

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Summary. Cytoplasmic threads found on the luminal surface of the developing chick neuroepithelium contain a remnant of the spindle complex and an electron-dense midbody. Most threads become long and thin and eventually split at the midbody. This finding suggests that the midbody plays a role in the final separation of daughter cells.

The developing chick neuroepithelium, like many other embryonic epithelia, exhibits interkinetic nuclear migration which results in the accumulation of mitotic figures near its luminal surface[2–5]. Mitotic cells are rounded and remain adjacent to the lumen from late prophase through early telophase. By late telophase, forming daughter cells start to elongate towards the base of the neuroepithelium[6]. During cytokinesis, connections between daughter cells may persist for a relatively long time. As a result, long protoplasmic bridges (=cytoplasmic threads) may be formed. These thread-like structures have been described previously[6–8], but very little is known about the sequence of their formation and function. As part of our interest in the development of the chick neuroepithelium, we have used transmission and scanning electron microscopy to examine dividing neuroepithelial cells with emphasis on their cytoplasmic threads.

Materials and methods. Fertile White Leghorn eggs were incubated at 37.5 °C to obtain embryos at stage 8+ of development[9]. Stage 8+ embryos were chosen because all phases of neurulation are represented in a single embryo[6]. For transmission electron microscopy (TEM), embryos were fixed in 1% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) containing 0.2% tannic acid for

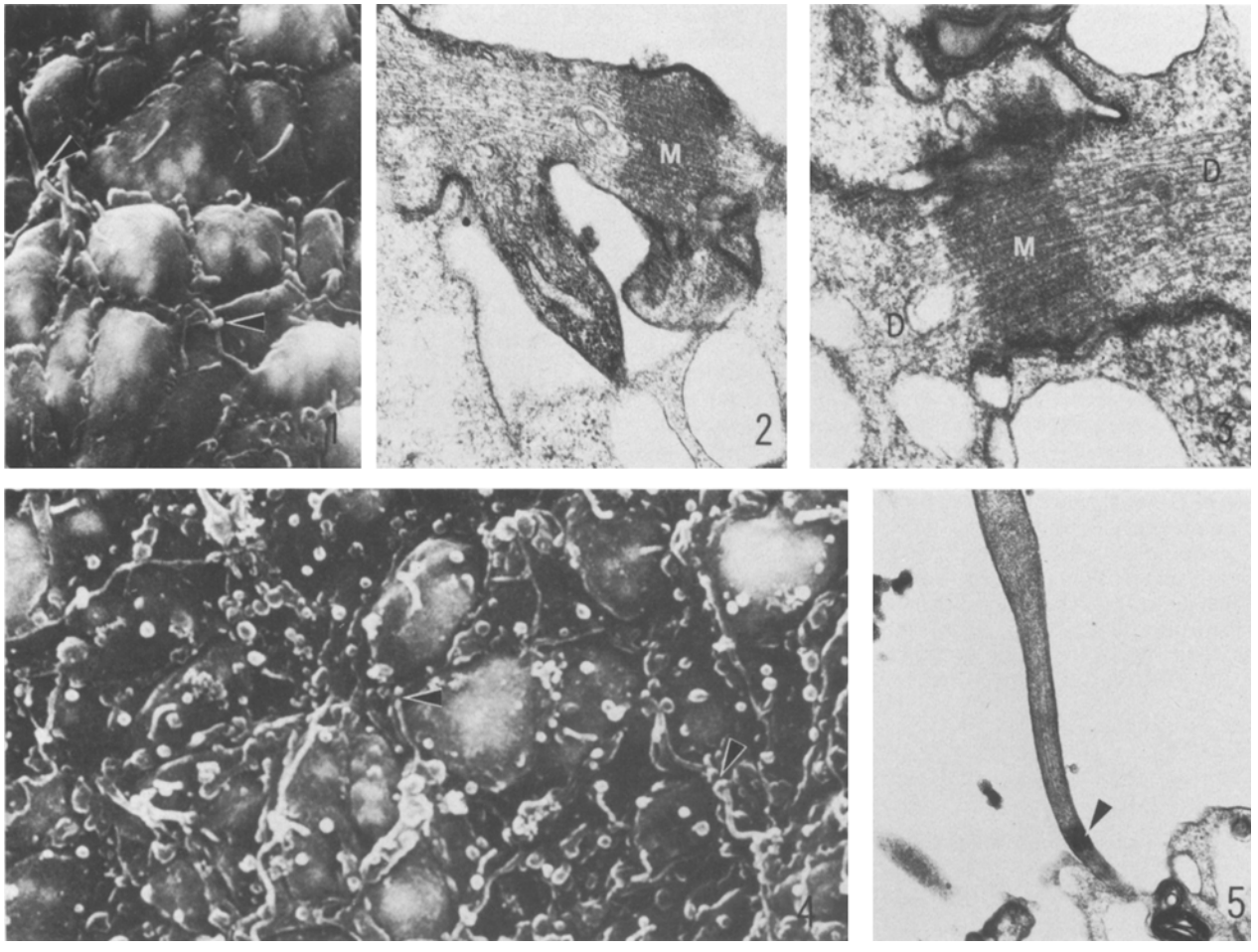

Fig. 1. Dorsal surface of the neural plate region showing several short threads with midbodies (arrows) at about the middle of their length. Margins of the apical surfaces of cells are revealed by the presence of short cytoplasmic extensions. ×2800. Fig. 2. Longitudinal section through a short thread. The thread is filled with microtubules of the spindle remnant and a dense region which is the midbody (M). Small cytoplasmic granules and vesicles are often intermingled with the microtubules. ×23,000. Fig. 3. Frontal section through the apical regions of several neuroepithelial cells showing a short connection (=forming cytoplasmic thread) between daughter cells (D) with a midbody (M). ×30,000. Fig. 4. Dorsal surface of the neural plate region showing threads which are severed at the level of the midbody (arrows). ×3600. Fig. 5. Longitudinal section through a thread showing several electron-dense regions along its length. Electron-dense area is most conspicuous where the thread shows abrupt bending (arrow). ×17,000.

1 h at room temperature, washed thoroughly in buffer, and postfixed for 40 min in 1% osmium tetroxide in the same buffer. Embryos were stained 'en bloc' with 1% uranyl acetate for 1 h, dehydrated in a graded series of increasing concentrations of ethanol, and embedded in Epon. Thin sections (silver/pale gold) were contrasted with uranyl acetate and lead citrate and examined with an RCA EMU-4 electron microscope. For scanning electron microscopy (SEM), specimens were prepared by the procedure described by Nagele et al.[10]. Briefly, isolated embryos were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 2 h and postfixed in 1% osmium tetroxide in the same buffer for 0.5–1 h. They were then dehydrated in a graded series of increasing concentrations of ethanol. After completion of dehydration, all specimens were affixed to a round coverslip (diameter=12 mm, Bellco Glass, Inc.) with Duco cement (E.I. du Pont de Nemours & Co.) and dried by Freon 13 in a Denton critical point drying apparatus. Coverslips with dried specimens were fastened to the surface of a stub with silver conducting paint (Ernest Fullam, Inc.). Specimens were coated with gold/palladium in an Edwards evaporator and examined with an AMR-900 scanning electron microscope.

Results and discussion. As shown in figure 1, cytoplasmic threads are found on the luminal surface of the developing neuroepithelium. They project from the margins of apical cell surfaces and have never been observed in the intercellular spaces or on the basal surface of the neuroepithelium. These threads have an electron-dense midbody and are filled with microtubules which represent a remnant of the spindle complex (figure 2). The midbody is often evident in SEM micrographs as a central swelling of the thread (figures 1 and 4). In stage 8+ chick embryos, threads are most numerous in the region where the margins of the neural plate are slightly elevated as neural folds. Bancroft and Bellairs[7] have suggested that the number of threads in any given area of the neuroepithelium reflects the mitotic activity of that region.

SEM reveals that 1. threads vary considerably in length (4–45 μm) and in width (0.1–0.4 μm) and 2. an inverse relationship appears to exist between the length and width of threads. The persistence of threads after the completion of mitosis and the fact that longer and thinner threads usually extend over several cells suggest that daughter cells may be displaced with respect to each other within the neuroepithelium following their division[6]. As daughter cells

separate, threads become progressively longer and thinner. Our previous study[6] has shown that when the polar migration of chromosomes is nearly complete, the cytokinetic ring of microfilaments begins to form between daughter cells. Although spindle microtubules tend to disorganize and disappear in other parts of the cells, they persist and may even increase in number in the region of the cytokinetic ring. By late telophase, daughter cells are somewhat elongated towards the base of the neuroepithelium. Whether this elongation is active or passive is not yet clear. Nevertheless, the cytoplasmic mass flows basally until daughter cells remain connected only at their apical corners by a short cytoplasmic bridge (= thread) filled with spindle microtubules and enveloped by the cytokinetic ring (figure 3). The present study also shows that small cytoplasmic granules and vesicles contribute to the formation of an electron-dense midbody (figures 2 and 3). The fact that the midbody is usually found in close association with the cytokinetic ring (figures 2 and 3) suggests that the constricting effect of furrow microfilaments may trap small cytoplasmic granules and vesicles and press them between the microtubules within the forming thread. Arnold[11] has suggested that a) the cytokinetic ring itself cannot completely pinch apart the 2 daughter cells and b) final cell separation occurs at the midbody. Confirmation of this view has proved to be difficult due to the rapidity of the separation event. However, we have often found threads split at the level of the midbody (figure 4). Some of the longer threads, which show rather abrupt bending, often possess additional electron-dense regions (figure 5) and may be broken in more than one place. This finding further supports the idea that midbodies form as a result of trapping of cytoplasmic material, although we cannot yet rule out the possibility that multiple separation points are due to breakage during tissue processing.

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The effect of Valium® anaesthesia on the radiosensitivity of the skin of the mouse foot[1]

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Summary. Valium® anaesthesia significantly reduces the effect of large single doses of X-rays on the mouse foot skin as compared with Nembutal® anaesthesia. The radioprotective action of Valium may be attributed to a direct effect on the cells of the skin.

Radiobiological studies on the effects of ionizing radiation on normal or malignant tissues often necessitate general anaesthesia of the animal in order to position it accurately in the radiation beam. Most of these experiments are carried out on mice which are anaesthetized by an i.p. injection of sodium pentobarbitone (Nembutal®) causing a general anaesthesia of about 60 min duration. A recent single-dose study of the radiation-induced skin reaction[3,4] with negative pions at low dose-rate (0.2–0.3 gray/min) resulted in long irradiation times of up to 5 h; for that reason pentobarbitone had to be replaced by diazepam (Valium®) which allows the immobilization of the mice during 5–6 h. In this paper we report on the effect of diazepam on the radiation sensitivity of the mouse foot skin.

Materials and methods. The experiment was carried out on female NMRI mice weighing 23–26 g at the time of the irradiation. The anaesthesia was performed either with 30 mg/kg diazepam or with 70 mg/kg sodium pentobarbitone; the drugs were injected i.p. 5–8 min before the irradiation. The X-rays (35, 40, 45 gray) were delivered by a Picker unit (250 kVp, 0.9 mm half-value layer in Cu, 2.5 gray/min). The method for assessment of the skin damage and for the presentation of the results has been published in full detail elsewhere[5]. Briefly, the skin reaction on the irradiated foot was scored daily by 2 independent observers and recorded on an arbitrary scale.

As an index of skin damage the average of all daily means of observations (day 8–30 post irradiation) on each dose-group was calculated and plotted against the radiation dose.

Results. The foot skin of 2 series of mice, of 3 dose-groups each, has been irradiated with single doses of X-rays. In the first series Nembutal was used as the anaesthetic, whereas in the second series the anaesthesia was performed with Valium. The figure shows the skin damage as a function of the radiation dose. At each dose the Valium series manifests a significantly lower level of skin damage as compared with the reference series ($p < 0.01$, Mann-Whitney-U statistics). A dose modification factor (DMF) of about 0.8 has been estimated from the 2 curves by comparing the doses which produce a skin damage index of 1.5. This DMF value below unity illustrates the decreased radiosensitivity of the skin when diazepam is used as an anaesthetic.

Discussion. It has been reported that pentobarbitone has no significant influence on the radiosensitivity of the mouse foot skin[6–8]. Thus, from the present data it can be concluded that Valium can induce radioprotection of the skin. Psychotropic drugs (Valium®, Librium®) elicit hypothermia in mice and exert a radioprotective effect against lethal wholebody doses of X-rays[9]. Hypothermia has been found both in Valium- and Nembutal-treated[10,11] animals. The depression of the core temperature was significantly more pronounced in the mice treated by Nembutal[10]. It therefore seems probable that hypothermia represents a concomitant