Induction of lymphomas by urethane in combination with diethylstilboestrol in adult male CFLP mice

| Treatment* | n | Animals with lymphomas | Average latency period of lymphomas (days ± SD)** |
|---|---|---|---|
| DES and after 14 days urethane | 22 | 4 (18.1%) | 172 ± 15.8 |
| DES and urethane simultaneously | 25 | 8 (32.0%) | 161 ± 46.7 |
| Urethane and after 14 days DES | 25 | 11 (44.0%) | 186 ± 70.3 |
| Urethane | 48 | No lymphomas within 300 days | |
| DES | 40 | 2 (5.0%) | On the 223rd and 229th day |
| Untreated controls | 45 | 1 (2.2%) | On the 270th day |

*Dose of urethane: 1000 mg/kg i.p., dose of DES: 50 mg/kg s.c.; **the survivors were killed and examined on the 300th day of the experiment.

which had shown that 1000 mg/kg of urethane could induce lung adenomas in CFLP mice in 11 weeks with a mean tumour number of 7.43 per animal[3].

The transplantation of the induced lymphomas into newborn CFLP mice was successfully performed in 6 cases in spite of the genetic heterogenicity of CFLP mice. The successive passage of lymphomas from CFLP mice into newborn BALB/c, C3H/He-mg, CBA/Ca and AKR mice was also positive.

All the induced and transplanted lymphomas seemed histologically to be lymphoblastic lymphosarcoma, a frequent type of malignant diseases of hematopoietic organs in mice[4,5]. The original structure of the thymus and lymph nodes completely disappeared due to tumour proliferation. The tumour cells were relatively monomorphic atypic lymphoblastic elements with large and rounded nuclei and prominent nucleolus, the cytoplasm margin was narrow. Several mitosis also occurred. Primarily in the thymus tumours, sporadic cells with wide clear cytoplasm reminescent of the 'starry sky' were observed. The tumour cells infiltrated the liver, spleen, kidney, bone marrow and even the lung adenomas sometimes. Electron microscopy also showed that the tumour cells correspond to lymphoblasts. In the induced thymomas, intracellular A type and extracellular C type virus particles could be observed in the transplanted lymphomas C type particles. Our morphological findings showed a good agreement with that of other chemically induced lymphomas in mice[6,7].

According to our results, urethane in combination with not only natural oestradiol but also with synthetic DES induced lymphomas in a low-leukemia colony of mice. Separately, the carcinogenic effect of urethane[8] and DES[9] in animals is well known. Our results indicate that in combination their carcinogenic potential may be enhanced. Human epidemiological data show that DES and urethane can be considered certain and possible carcinogens in man, respectively[10]. However, on the basis of a single experimental observation, the carcinogenic risk of their combination to man cannot be estimated yet.

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## Quantitative histological study of spinal afferent innervation on the ventral surface of the cat stomach by horseradish peroxidase (HRP) method

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*Summary.* Retrograde axonal transport of horseradish peroxidase (HRP) was applied to the ventral surface of the cat stomach. We investigated the number, size and distribution of HRP-positive cells in spinal ganglia. The unexpected finding was the wide distribution of these cells from T3 down to L3. This would result in a diffuse pattern of referred pain.

Much of the work on visceral afferent innervation has been based on electrophysiological activation of afferent fibres[1] and histological anterograde degeneration method[2]. These methods gave us the information qualitatively, but exact quantitative observation is still obscure. Recently retrograde axonal transport of horseradish peroxidase (HRP) was demonstrated in peripheral afferent fibres and its usefulness was revealved[3-5]. In our study HRP was applied to the ventral surface of the cat stomach. We investigated the number, size and distribution of HRP-positive cells in spinal ganglia.

*Material and methods.* The cats weighing about 2-3 kg were laparotomized and injected 200 mg of HRP (Type II: Sigma Chemical Co.) diluted to 33.3% in physiological saline, into the ventral wall of the stomach via multiple penetrations with Hamilton syringe under Nembutal anesthesia. Figure 1 shows the injection sites of HRP. We took care not to leak HRP out of the wall of the stomach at the

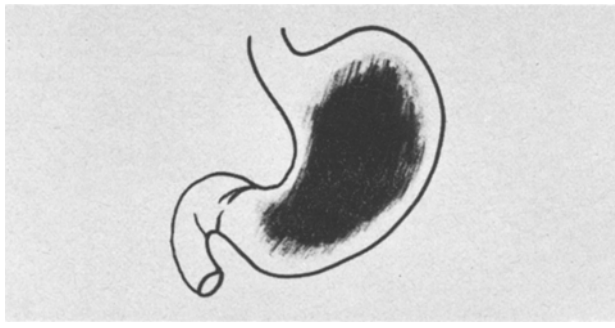

Fig. 1. The injection 200 mg of HRP (Type II: Sigma Chemical Co.) diluted to 33.3% in physiological saline into the ventral wall of the cat stomach via multiple penetrations. Black indicates injection sites.

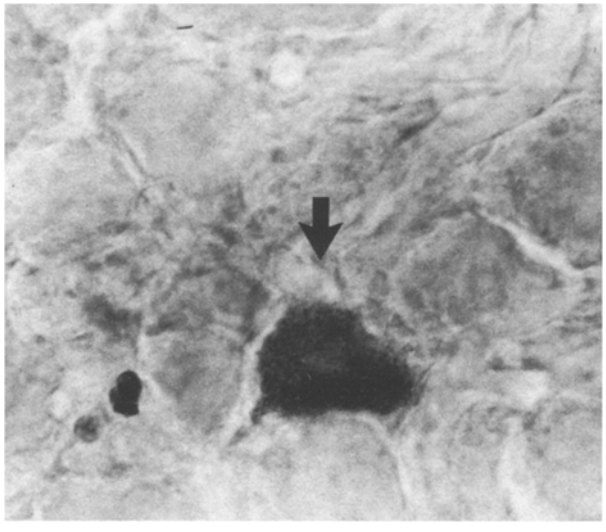

Fig. 2. Spinal ganglion (left T8) section 3 days after injection of HRP into the ventral wall of the cat stomach. HRP-positive cell is seen (arrow). Section counterstained with cresyl violet. × 400

time of injection. The other cat received 30 mg of HRP (Type II: Sigma Chemical Co.) i.v. as a control, described by Yamamoto et al.[6]. After a 3-day survival period, the cats were perfused transcardially with 2.5% glutaraldehyde in 0.15 M phosphate buffer (pH 7.4).

Spinal ganglia (T3-L3) were rapidly dissected out. They were kept in the same fixative for 4 h and then washed several times in 0.1 M phosphate buffer (pH 7.4) containing 5% sucrose overnight. All ganglia were serial sectioned (30 µm thick) on a cryostat and incubated by HRP-method according to Malmgren and Olsson[7,8]. HRP-positive cells were selected according to the criteria of Nauta et al.[9].

*Results.* As shown in figure 2, HRP-positive cells were clearly demonstrated in spinal ganglia. HRP-positive cells

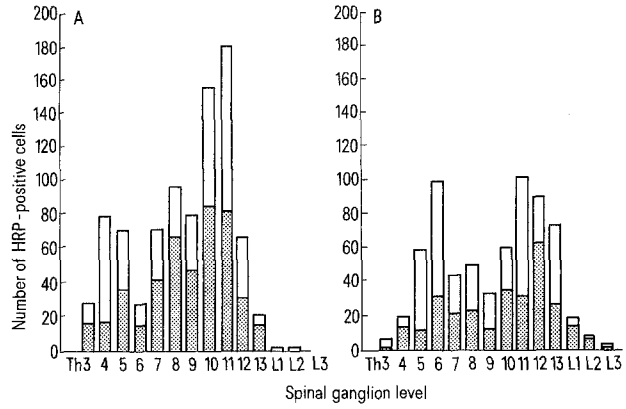


Fig. 3. The distribution of HRP-positive cells in spinal ganglia after injection of HRP into the ventral wall of the cat stomach (cat A and cat B). Survival period, 3 days. The black column shows number of HRP-positive cells of left side and the open column right side.

were distributed to the left and right sides of ganglia. This indicates that the stomach receives afferent innervation bilaterally[10]. Interesting findings were the number, size and distribution of HRP-positive cells, although there were some differences in number and distribution of positive cells between cats. Figure 3 shows the distribution of HRP-positive cells in spinal ganglia of 2 cats (A and B). Holmes and Davenport reported[11] that ganglia T3-L3 contain 8000–12,000 cells per ganglion in the cat. The majority of positive cells was found at level T 11 (right) of cat A and its number was 100. This means that positive cells were about 1% of total number of ganglion cells. The diameter range was 30–80 µm in all spinal ganglion cells. Most of HRP-positive cells were 30–40 µm. Therefore HRP-positive cells were the smallest in spinal ganglion cells[5]. An unexpected finding was the wide distribution of HRP-positive cells in spinal ganglia as shown in figure 3. In cat B positive cells were observed from T3 down to L3. No HRP-positive cell was found in the control cat which had received 30 mg of HRP i.v. Moreover we checked the diffusion of HRP to adjacent structures such as esophagus and duodenum. No reaction product was seen in these regions.

*Discussion.* In this work, small number and wide distribution of HRP-positive cells in spinal ganglia were observed. Besides, positive cells were small in size. Visceral pain from the gastrointestinal tract is believed to be mediated over spinal afferent fibres and not over the vagus[12]. Recently the small cells in spinal ganglia have been suggested to be nociceptive[13,14]. Our results, taken together with these observations, suggest that sensation, including pain from spinal afferent fibres of the stomach, is feeble and referred pain is a diffuse pattern. At present, however, we must remember the limitation of quantitative evaluation by HRP method, in so far as we have not as yet more sensitive methods.

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