Gastric cancer in rats after chronic intraperitoneal application of sap of green parts of potatoes (*Solanum tuberosum* L.)

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Summary. Chronic i.p. application of the sap of green potato plants (potato-tops) induced carcinomas of the stomach in 4 out of 12 treated rats.

As part of our investigations on the carcinogenic effect of certain plants and natural substances, we applied fresh sap of the green parts of potatoes (*Solanum tuberosum* L.) chronically, i.p. to BD IX rats. In a 1st experimental arrangement described here we investigated the effect of the sap of green potato tops. The sap of potato tubers was administered in a 2nd experiment. These results will be later published in separate paper.

The green potato tops were gathered in August 1974 and thoroughly pressed through gauze. The resulting sap was centrifuged roughly and the supernatant filtered until it was free of bacteria. It was then stored in small portions at −35 °C. Once a week, 12 BD IX rats were given an i.p. injection of 0.25 ml per animal. After an induction period of about 850 days, we found carcinomas of the stomach in 4 rats which had survived for the longest time. In 2 cases, the original carcinomas were located in the glandular stomach and had metastasised into the liver. Pathohistologically, the carcinomas were little differentiated adenocarcinomas. The cells were polymorphous with ample cytoplasm and with nuclei, mostly contain a nucleolus. The cells had gland-like formations. 2 other animals died of papillomas and squamous cell carcinomas of the forestomach. The remaining 8 rats of the test group died of pneumonia without any having tumors after the median induction time of 850±20 days.

As a control, we used 20 rats of the same strain. They were given 0.25 ml 0.9% NaCl solution per animal i.p. once a week. The solution was filtered and then stored at −35 °C, as described above. All 20 rats of the control group died after a medium age of 880±30 days, without developing tumors.

Carcinomas of the forestomach and of the glandular stomach are practically unknown 'spontaneous tumors' in BD-rats. The chemical analysis of the sap of green potato tops, which we had used for the experiment, carried out in our institute (Kann et al., unpublished), showed the presence of several volatile N-nitroso-compounds. We cannot yet decide whether the described carcinogenic effect is due to these N-nitroso-compounds. We have already begun to study the effects of chronic administration, in order to show whether carcinomas of the stomach in rats are also caused after oral application of this potato sap.

Chlorpromazine causes vesicle population changes in the monoaminergic synapse of the rat caudate nucleus

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Summary. The population of monoaminergic synaptic vesicles in the rat caudate nucleus remained unchanged or slightly decreased 3 h after chlorpromazine (CP) administration, and clearly increased after 24 h. The diameter of synaptic vesicles became smaller when the vesicles increased. These findings suggest that CP causes presynaptic blocking in part of its actions and leads to a condition in which neural transmission is inactive. In the control animals, population of the vesicles tended to fluctuate following the circadian rhythm.

In order to shed more light on the action mechanism of chlorpromazine (CP), synaptic vesicles in the caudate monoaminergic bouton were morphometrically studied.

Material and methods. 9 male albino rats (Wister strain), weighing 180–200 g, were kept in natural light and divided into 3 groups of 3 animals each. For adaptation to experimental manipulation, i.p. injection of physiological saline solution was performed for 1 week at 11.00 h. Then each group was administered i.p., 5 ml/kg physiological saline solution, 5 mg/kg CP solution (1 mg/ml) and 25 mg/kg CP solution (5 mg/ml) at 11.00 h, respectively. After 3, 12 and 24 h, all animals in the respective groups were decapitated under ether anesthesia and frontal slices were fixed in ice-cold Dalton's solution for 1 h, and processed for EM. Block stain of uranium acetate was done. About 75 nm ultrathin sections, doubly contrasted with lead citrate, were examined with JEM T7. 3 investigators, each of whom had examined 3 experimental animals from 1 of 3 groups of rats decapitated at the same time, photographed 100 pictures per rat at a final magnification of 40,000. In another study[1] we identified 2 kinds of monoaminergic vesicles, which take up α-methylnoradrenaline and show granular vesicles by potassium permanganate fixation by the method initiated by Hökfelt[2]. The A type bouton seems to have an asymmetric synaptic contact and is composed of a relatively large axon terminal and a dendritic spine (figure 1, a). The presynaptic area has 1 or 2 small mitochondria and scattered, pleomorphic vesicles. The B type has a smaller presynaptic site than a postsynaptic dendrite, and is characterized by a symmetrical membranous contact and pleomorphic vesicles with a diameter of 40–80 nm. Although there are some differences in the synaptic features, depending on fixation by potassium permanganate or osmium, we chose these synapses from the tissues fixed by Dalton's solution and subjected them to morphometrical study. The B type synapse was further classified into B_1 and B_2 in the photos. The B_1 type has a more prominent enlargement of the synaptic cleft than B_2, and its synaptic complex sometimes has rather constricted parts. Cisternal structures are often seen in the vicinity of the postsynaptic membrane (figure 2, a). The B_2 synapse has a discrete synaptic contact and no cisternal structure in the postsynaptic dendrite (figure 3, a). The population of vesicles in these synapses was measured in 2 ways: first, all vesicles in a bouton were counted. The area of the bouton was measured with a planimeter and the

area occupied by mitochondria was substracted. Then, the number of vesicles per bouton area was calculated. Second, squares of 1 cm were drawn on each photographic print along a synaptic complex in a bouton (figure 1, a). All vesicles contained within or touching the lines were totalled. At a magnification of 40,000, the actual synaptic area examined was equal to 0.06 μm^2 per bouton. The diameter of synaptic vesicles were measured with a rule graduated to 0.02 mm with a magnifying glass.

Results and discussion. The results are shown in figures 4–6. In the control animals, the population of vesicles in synapses of all types obviously fluctuated in accordance with a circadian rhythm[3]; that is to say, synaptic vesicles increased at 23.00 h and decreased at 11.00 h. There were no significant differences of vesicle population between CP administered animals and control after 3 and 12 h except for some synapses. At these times the density of vesicles decreased in some boutons given CP. After 24 h the density of vesicles in all types of bouton given CP increased, especially in the vicinity of the synaptic complex (figure 5). The diameter of vesicles became smaller when vesicle population increased.

Whether the changes in synaptic vesicles were due to the effects of CP on the chemical neurotransmission, or the manifestation of physiological events, was previously discussed[4]. The present authors have shown, furthermore, that promethazine, an analogue structually similar to CP which also has a cell membrane-stabilizing effect[5] and no antipsychotic actions, caused no significant changes in synaptic

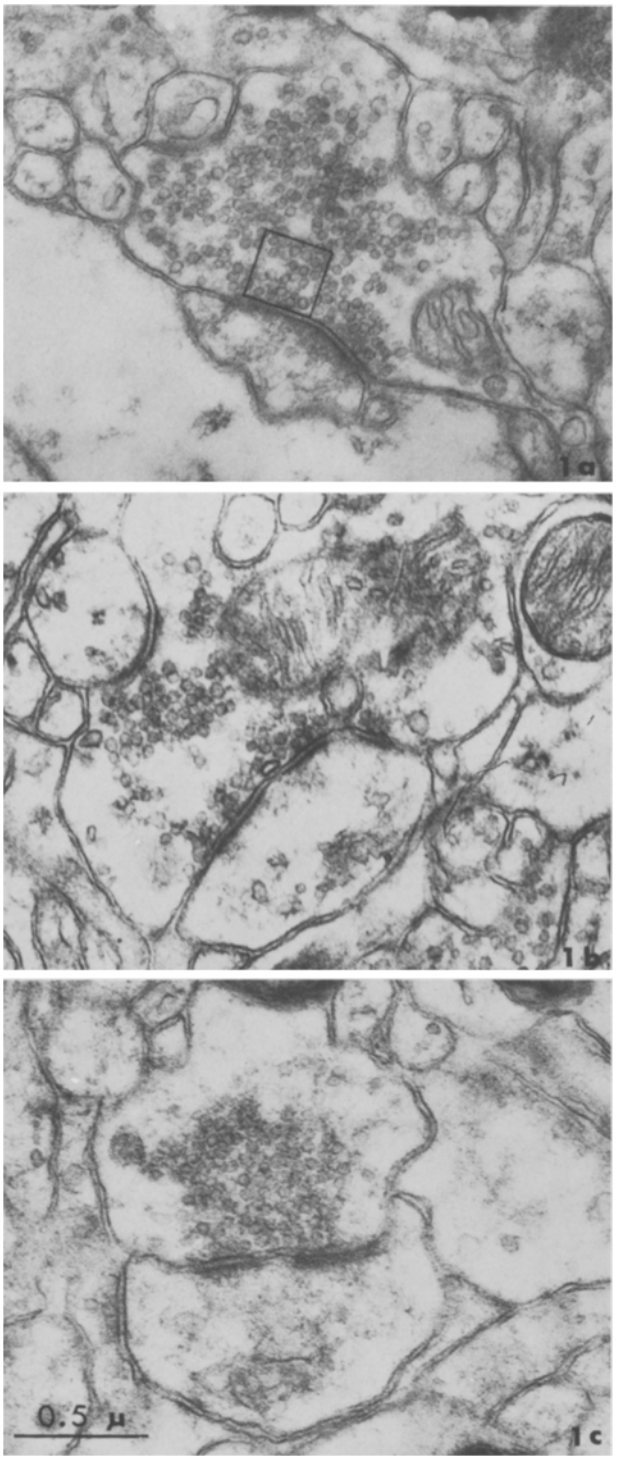

Fig. 1. Synapse A: *a* control; *b* 3 h after 25 mg/kg CP administration; *c* 24 h after 25 mg/kg CP administration.

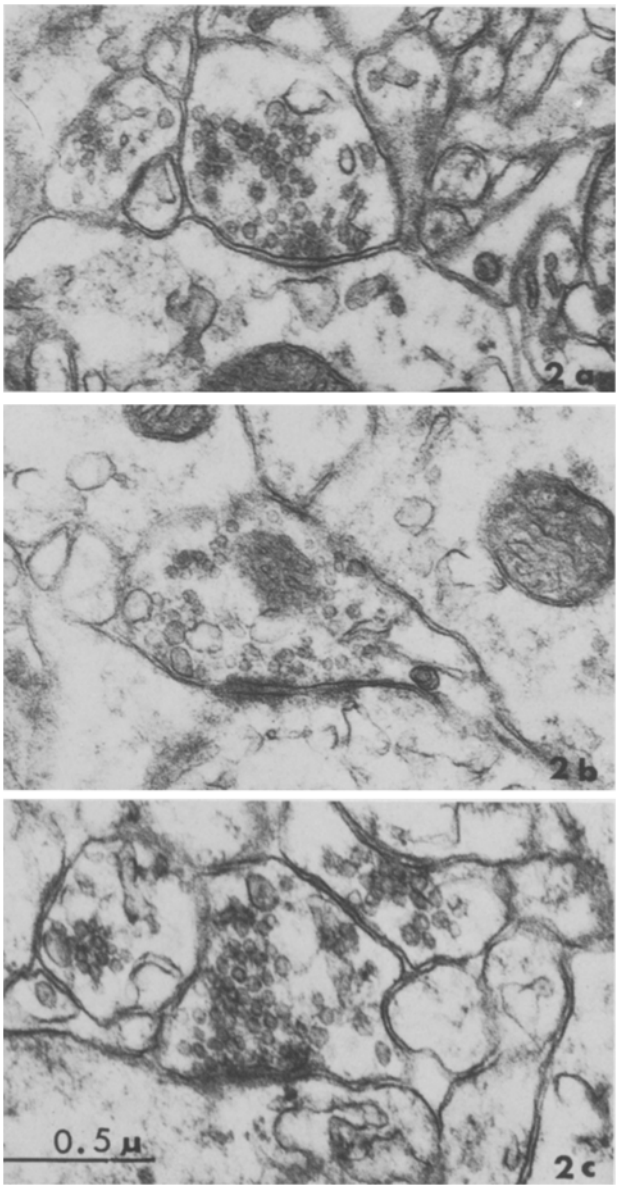

Fig. 2. Synapse B_1: *a* control; *b* 3 h after 5 mg/kg CP administration; *c* 12 h after 5 mg/kg CP administration.

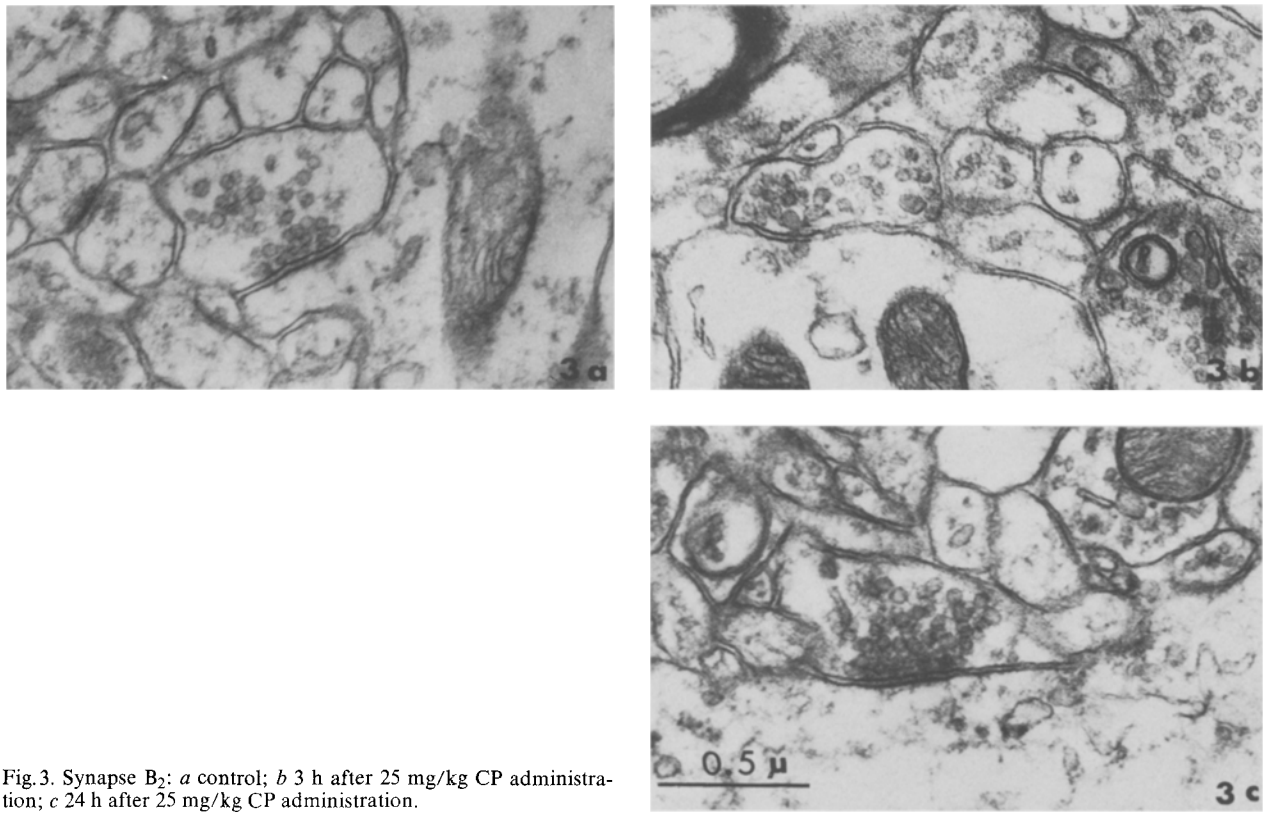

Fig. 3. Synapse B_2: *a* control; *b* 3 h after 25 mg/kg CP administration; *c* 24 h after 25 mg/kg CP administration.

vesicles in the caudate nucleus[6]. Therefore, changes of synaptic vesicle may be regarded as the result of CP actions on the neurotransmitters in the caudate nucleus. Although the exact relationship between each monoaminergic neurotransmitter and the type of synapse is, to date, obscure, the dominant monoamine in the caudate nucleus is dopamine. Turnover rate of catecholamine forms a peak in 3 or 4 h after CP administration[7]. This metabolic change corresponds to the feature of synaptic vesicles in the present study according to which density of vesicles decreased slightly or remained unchanged, and the diameter of vesicles became larger. It seems that synaptic vesicles become

Effects of CP on the synaptic vesicles

	3 h after treatment (14.00 h)			12 h after treatment (23.00 h)			24 h after treatment (11.00 h)		
	Control	5 mg/kg	25 mg/kg	Control	5 mg/kg	25 mg/kg	Control	5 mg/kg	25 mg/kg
Population of synaptic vesicles per bouton area (X 16.7/µm²)									
B 1 synapse	6.0±0.7 (16)	4.1±0.3 (25)	5.3±0.6 (12)	8.4±1.1 (12)	6.4±0.4 (14)	5.6±0.5 (16)	4.0±0.3 (21)	5.2±0.6 (9)	4.5±0.6 (14)
B 2 synapse	5.7±0.4 (42)	5.8±0.4 (36)	6.3±0.4 (40)	6.1±0.5 (18)	5.4±0.5 (10)	6.6±0.4 (30)	4.3±0.6 (15)	5.9±0.6 (23)	5.8±0.7 (23)
A synapse	6.0±0.5 (24)	5.2±0.3 (30)	6.9±0.4 (23)	10.3±0.9 (10)	8.4±0.6 (21)	8.7±0.5 (11)	5.0±0.6 (17)	6.6±0.6 (22)	8.6±1.0 (12)
Population of synaptic vesicles per 0.06 µm² - along synaptic complex									
B 1 synapse	10.5±0.6 (31)	9.0±0.5 (28)	10.1±0.6 (29)	12.9±1.0 (11)	13.7±0.8 (17)	12.7±0.8 (16)	9.5±0.4 (20)	14.8±0.8 (11)	12.7±1.0 (10)
B 2 synapse	13.7±0.5 (46)	10.7±0.6 (40)	11.7±0.5 (35)	13.6±0.9 (19)	13.5±0.6 (14)	14.5±0.8 (30)	12.0±0.7 (20)	14.1±1.2 (16)	14.0±1.0 (26)
A synapse	16.2±0.8 (29)	15.1±0.5 (60)	14.8±0.5 (37)	17.4±0.7 (22)	17.5±0.7 (24)	18.5±0.9 (19)	12.8±0.6 (40)	17.5±0.7 (24)	19.1±1.2 (23)
Diameter of synaptic vesicles (X 2.5 nm)									
B 1 synapse	19.3±0.4 (34)	21.1±0.7 (29)	21.8±0.6 (27)	18.9±0.5 (10)	21.2±0.8 (17)	19.5±0.3 (16)	24.5±1.0 (20)	23.0±1.0 (11)	21.3±0.6 (10)
B 2 synapse	19.4±0.4 (46)	19.7±0.3 (37)	20.8±0.3 (29)	20.0±0.3 (19)	20.3±0.5 (12)	19.6±0.3 (30)	21.6±0.6 (20)	21.6±0.6 (16)	20.8±0.7 (26)
A synapse	18.2±0.2 (29)	19.3±0.3 (57)	19.7±0.2 (37)	18.4±0.2 (23)	19.0±0.3 (23)	19.1±0.8 (19)	21.5±0.4 (40)	20.7±0.4 (24)	18.8±0.2 (23)

All values are expressed as mean ± SE. Numbers of experiments in parenthesis.

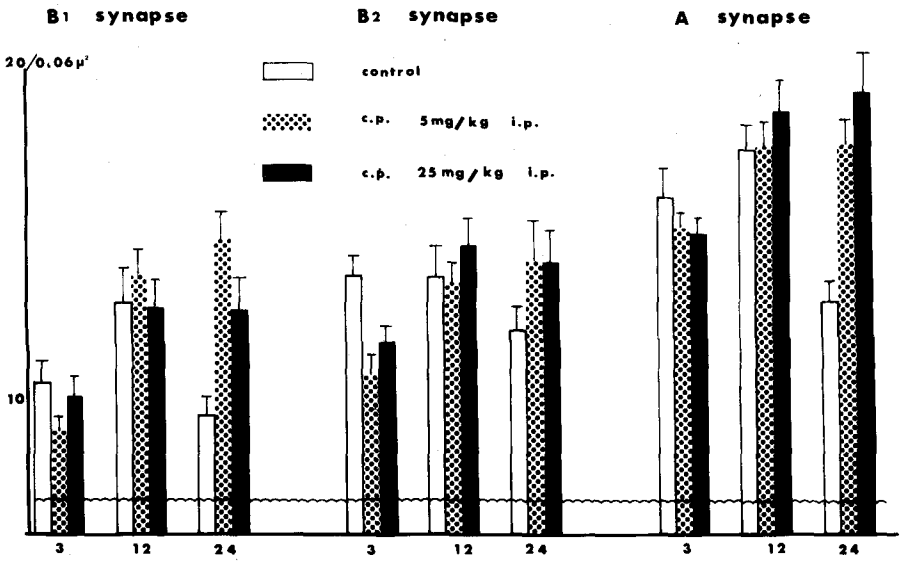

Fig. 4. Population of synaptic vesicles per bouton area (mean ±SE).

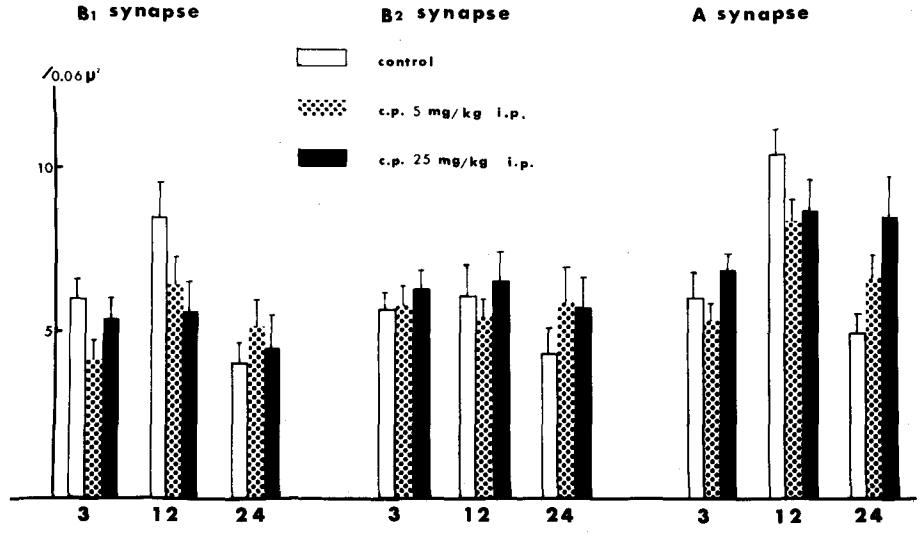

Fig. 5. Population of synaptic vesicles per 0.06 μm^2 along the synaptic complex (mean±SE).

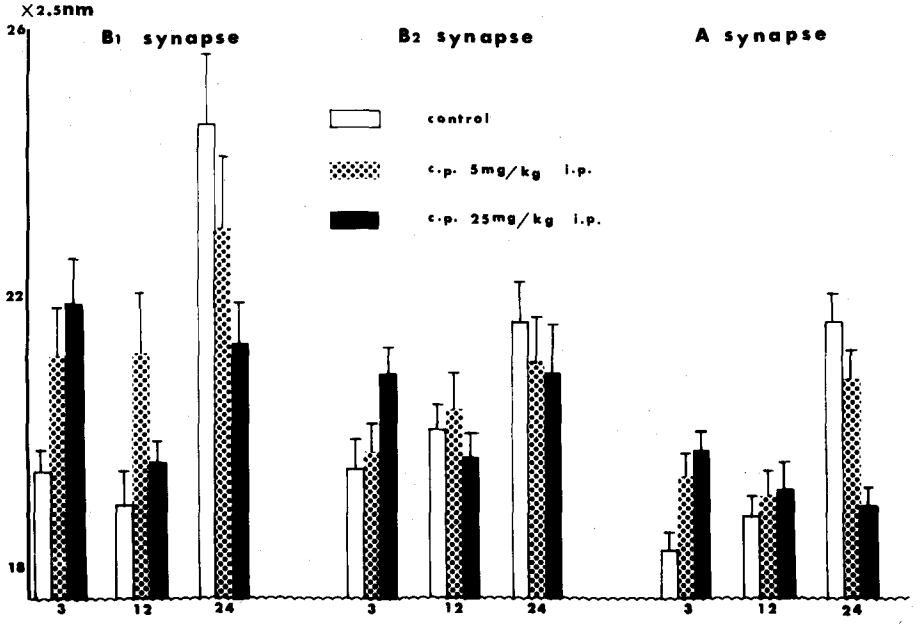

Fig. 6. Diameter of synaptic vesicles.

larger as the chemical transmission is more active. The findings up to 12 h after CP administration support some previous studies. Various neuroleptics accelerate loss of fluorescence of dopaminergic terminals in the caudate nucleus when tyrosine hydroxylase is inhibited[8]. Increase of synaptic vesicles in 24 h after CP administration suggests a possibly inhibited release of transmitter or accelerated reuptake or synthesis of monoamine for its usual consumption. Because CP prevents uptake of monoamine to synaptosome[9], and inhibits the electrically stimulated release of H^3-dopamine from rat striatal slices[10], the present findings in 24 h after CP administration suggest that CP will lead to a condition in which neural transmission is inactive. In other words, CP is thought to cause clinical effects through the presynaptic blocking in part of its actions.

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Are intermediate filaments of vertebrate smooth muscle cells and tonofilaments of epithelial cells identical cell structures?

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Summary. In epithelial and smooth muscle cells of the urinary bladder of the frog, a class of filaments exists which is partly disintegrated by glycerol treatment and very resistant to potassium solutions of high ionic strength.

There are some indications that the intermediate filaments found in different muscle types and the tonofilaments described in a number of non-muscle cells are similar cell structures. Both have a diameter of about 100 Å[2-8], are resistant to various extraction procedures[5,9] and cannot be decorated with heavy meromyosin[4]. Since, however, investigations were made either on muscle or on non-muscle cells using different species and procedures, the results are not really comparable. In the present report, a tissue is used which contains muscle and epithelial 100 Å filaments in immediate vicinity. Therefore, fixation and extraction procedures were carried through under identical conditions.

Material and methods. The tissue employed was the urinary bladder of the frog, *Rana temporaria*. Small strips of the bladder were first incubated for 20 h in ice-cold 50% glycerol, 10^{-2} M $MgCl_2$, 10^{-2} M Tris-HCl (pH 7.2) solution. For salt extraction, glycerinated tissue was placed in one of the following extraction solutions for 5 h[10]: A 4 mM EGTA, 4 mM $MgCl_2$, 5 mM ATP, 0.05 M KCl, histidine

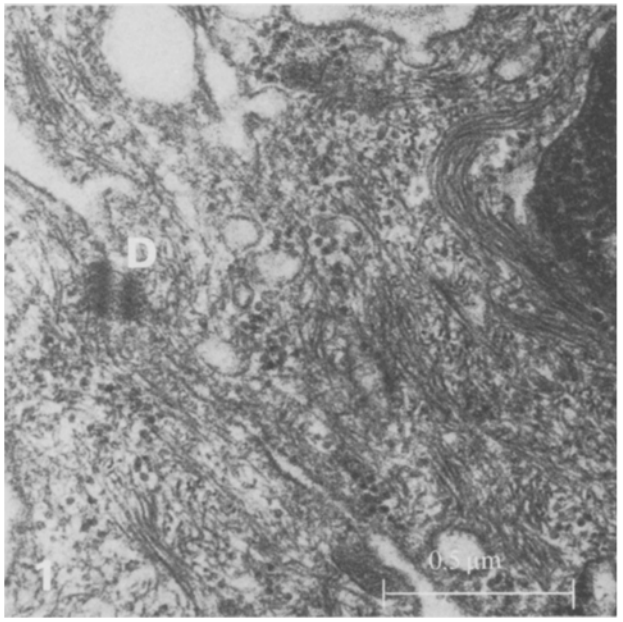

Fig. 1. Epithelial cells of the urinary bladder of the frog. The cells contain numerous tonofilaments, running through the cytoplasm at all angles. D = desmosome.

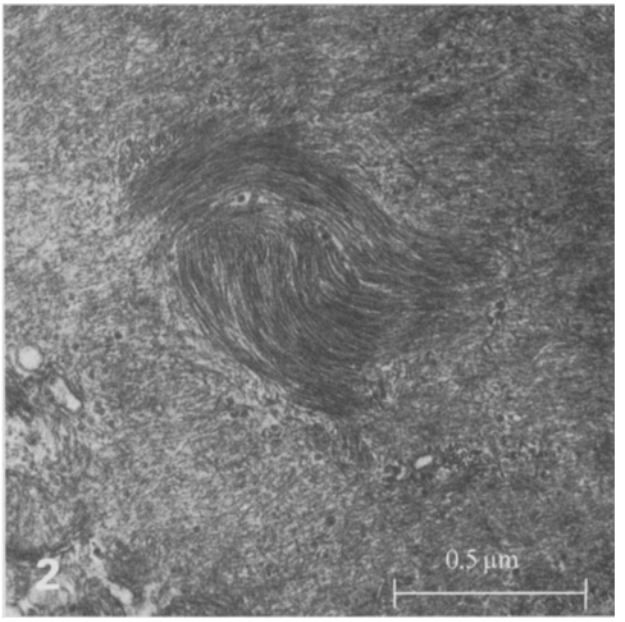

Fig. 2. Oblique section through a smooth muscle cell of a bladder. In the centre of the cell a large bundle of darkly-stained intermediate filaments is seen.