of definite membrane were the criteria used). Only those cells with obvious degenerative changes were considered necrotic. Counts were corrected by Aberchrombie's method to eliminate errors due to variation in nuclear diameter.

Results and discussion. The variations in the population dynamics of spermatids in control and MPG-treated mice 5 days after exposure to 500, 1000 and 1500 R are presented in the table. At 5-day-interval, the numbers of spermatids in control mice are found to be 60.50, 43.00 and 30.00 at 500, 1000 and 1500 R respectively whereas in MPG-treated mice the counts observed are 84.00, 65.45 and 50.00 respectively and these data are statistically highly significant. Our observations on the number of spermatids clearly show that MPG largely protects the radiation-induced lesions produced in the primary spermatocytes. Our data are highly significant as studied up to 1500 R dose. Histopathological studies at 500 R, reveal that a higher number of necrotic spermatocytes was observed in diakinesis metaphase 1 in nonprotected groups, as compared with control mice[8]. Irradiation damage of primary spermatocytes remain latent until the cell enters meiotic metaphase and anaphase. Many abnormal figures typical of chromosomal aberrations thus occur, and nuclei of resulting spermatids show abnormal size variations (giant cells). These giant spermatids are formed by spermatocytes irradiated in pacyhtene, and are greater in number in nonprotected mice with increased doses of irradiation of 1000 R and 1500 R (unpublished data). There is considerable evidence that DNA is the major target of ionizing radiations in the living cells[9,10]. Significant chemical protection of DNA-molecule provide significant protection against radiation death[5]. It is not known whether these chemical protectors protect DNA directly or indirectly (by preventing radiation-induced release of DNA-destructive enzymes) or protect against radiation-induced destruction of DNA repair system.

Effect of MPG on the survival of spermatids* 5 days after whole body exposure of Swiss albino mice to different doses of gamma-radiation**

Doses		Total No. of tubule cross-section counted	Total number of spermatids	Mean number of spermatids ± SE
500 R	Control	20	1210	60.50 ± 3.10
	Drug-treated	20	1560	84.00 ± 2.50
				$p < 0.001$
1000 R	Control	20	871	43.00 ± 2.00
	Drug-treated	20	1389	65.45 ± 3.30
				$p < 0.001$
1500 R	Control	20	560	32.25 ± 2.00
	Drug-treated	20	1005	50.00 ± 2.80
				$p < 0.001$

* No. of spermatids in normal nonirradiated mouse testis = 104 ± 3 per tubule cross-section.

** Sensitivity of primary spermatocytes scored by counting the number of spermatids in stage I and II of tubules, 5 days after post-irradiation.

The most plausible explanation with regard to the mechanism of protection relates to the possibility that some –SH compounds partially or completely inhibit the deleterious action of the free radicals normally found in X-irradiated tissue; or they may even modify the nature of these radicals in such a manner as to render them less harmful[11–13]. It is also thought possible that the effective –SH carrying compounds may combine temporarily with some radiosensitive enzymes, making them radio-resistant[11]. The exact mechanism of MPG protection is not known; however, these explanations may also apply to our findings.

The present results indicate that MPG very likely protects the chromosomes in the pachytene of meiosis, thus allowing more spermatocytes to survive and become normal spermatids. This is possible either by reducing the number of radiation-induced chromosome breaks or by causing a restitution of the broken chromosomes. Further, investigations with a view to understanding this mechanism is in progress in our laboratory.

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Histochemical studies on two types of cells containing catecholamines in sympathetic ganglia of the bullfrog

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Summary. The excitation/emission spectra maxima obtained from orange fluorescing small cell clusters and greenish yellow fluorescing ganglion cell bodies were at 400–410/490–560 nm and at 400–410/470–500 nm, respectively.

Azuma et al.[3] reported from a viewpoint of biochemical determination that the sympathetic ganglion chain of the bullfrog contained catecholamines, such as noradrenaline and adrenaline, in a relatively large quantity.

On the other hand, Kojima[4], using formaldehyde-induced fluorescence microscopy[5], mentioned that there were 2 types of cells, namely the small cell clusters and the ordinary ganglion cell bodies showing specific fluorescences in the sympathetic ganglia of the bullfrog. According to his work, it was suggested that the small cell clusters and the ganglion cell bodies would contain mainly a primary catecholamine (dopamine or noradrenaline) and a secondary catecholamine (adrenaline), respectively.

A further experiment was attempted to demonstrate the cellular catecholamines in bullfrog sympathetic ganglia by means of fluorescence microspectrophotometry[6] and

the chromaffin reaction with potassium iodate for histochemical demonstration of noradrenaline[7].

The isolated fresh sympathetic ganglia of the bullfrog (*Rana catesbeiana*) were used. The specimens were then freeze dried and processed according to the Falck-Hillarp method[5]. The formaldehyde vapour treatment was performed at 80 °C for 3 h using paraformaldehyde equilibrated at 75% relative humidity. All sections (8 µm) were mounted in liquid paraffin after removal of paraffin. Several fresh specimens were treated with a 10% potassium iodate solution for 5 min according to Hillarp and Hökfelt[7] in order to demonstrate noradrenaline-containing chromaffin cells. A fluorescence microspectrophotometer (Nikon, SPM-RFL SYSTEM) was used for measurements of fluorescence (excitation and emission) spectra and fluorescence intensity.

When the bullfrog sympathetic ganglia were examined by means of fluorescence microspectrophotometry, the small cell clusters showing an orange fluorescence and the ganglion cell bodies showing greenish yellow fluorescence showed excitation/emission spectra with peaks at 400–410/490–560 nm and at 400–410/470–500 nm, respectively. Typical excitation and emission spectra obtained from these 2 types of cells are shown in figure 1. The solid and the dotted lines represent excitation/emission spectra obtained from an orange fluorescing small cell cluster and a greenish yellow fluorescing ganglion cell body, respectively. These spectra maxima obtained from the orange fluorescing small cell cluster and the greenish yellow fluorescing ganglion cell body were almost similar to those obtained from the noradrenaline-containing chromaffin cell cluster[8] and the adrenaline-containing acidophilic summer cell[8] observed in sections of bullfrog adrenals treated with formaldehyde vapour of 75% humidity equilibration at 80 °C for 3 h, respectively.

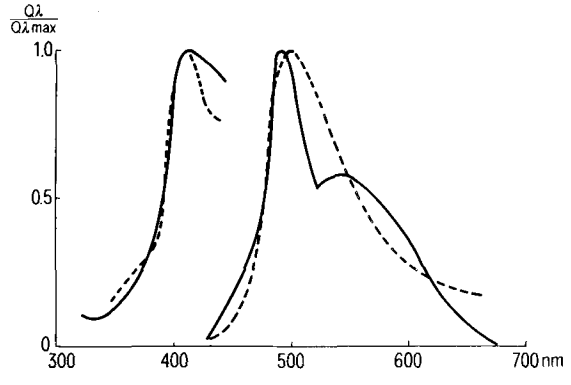

Fig. 1. Typical excitation and emission spectra obtained from the small cell cluster (—) and the ganglion cell body (---), showing excitation/emission maxima at 410/490–540 nm and 410/500 nm, respectively. The specimen was treated with formaldehyde vapour of 75% humidity equilibration at 80 °C for 3 h.

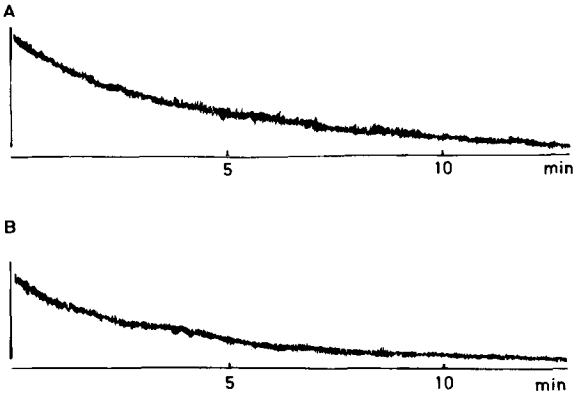

Fig. 2. Both the orange fluorescing small cell cluster *A* and the greenish yellow fluorescing ganglion cell body *B* exhibited a photodecomposition upon irradiation with blue-violet light.

The photodecompositions of these 2 types of cells showing different catecholamine fluorescences in bullfrog sympathetic ganglia were observed by means of fluorescence microspectrophotometry (figure 2). Both the orange fluorescence in the small cell cluster (figure 2, A) and the greenish yellow fluorescence in the ganglion cell body (figure 2, B) decreased upon exposure to blue-violet light. After the treatment with a solution of potassium iodate for 5 min, the small cell clusters were coloured dark brown, while the ganglion cell bodies developed either no colour or a very weak yellow-brown one.

These results obtained from the present experiment indicate that 2 types of specific fluorescences in the sympathetic ganglia of the bullfrog are derived from catecholamines. The orange fluorescing substance in the small cell clusters appears to be a primary catecholamine (mainly, noradrenaline), because it has been confirmed[4] that it easily appears after the formaldehyde vapour treatment at reduced temperature (50 °C) and of short duration such as 30 min, and because they are intensely positive to the chromaffin reaction. The fact that the emission maximum obtained from the orange fluorescing small cell cluster shifted 40–60 nm to the long emission spectrum side as compared with that obtained from dried protein microdroplets containing noradrenaline (1 mg/ml)[6] suggests that a primary catecholamine, particularly noradrenaline, would be localized in high concentrations in the small cell clusters in the sympathetic ganglia of the bullfrog.

Furthermore, concerning a greenish yellow fluorescence in the ganglion cell bodies, it was shown by the measurements of the excitation/emission spectra that this fluorescence was derived from catecholamine. Besides, the report[4] that the ganglion cell bodies in the sympathetic ganglia of the bullfrog developed a greenish yellow fluorescence when the formaldehyde vapour treatment was prolonged at high temperature (80 °C) for 3 h, suggests that most of fluorescing substance in the ganglion cell bodies would be a secondary catecholamine (adrenaline). However, the fact that some ganglion cell bodies were coloured a very weak yellow-brown after the treatment with a solution of potassium iodate indicates that they also contain a small amount of noradrenaline.

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