Results of a typical experiment of mutagenicity testing of cis- and trans-isomers of 1,3-dichloropropene (DCP) with Salmonella thyphimurium TA 1535, with and without S9

Mutagen	Concentration (µl/ml top agar)	Survivors ($\times 10^8$) Mean value of 3 plates	%Survival	Revertant colonies per plate Mean value of 3 plates ± SE
cis-DCP	S9 Absent			
	–	0.96	100	15 ± 2.64
	0.1	0.70	72.9	215 ± 10.59
	0.5	0.38	39.6	456 ± 15.55
	1.0	0.04	4.2	146 ± 29.68
	S9 Added			
	–	0.81	100	11 ± 1.73
	0.1	0.81	100	72 ± 3.71
	0.5	0.62	76.5	287 ± 2.02
	1.0	0.29	35.8	434 ± 21.2
trans-DCP	S9 Absent			
	–	0.96	100	15 ± 2.64
	0.1	0.92	95.8	110 ± 8.14
	0.5	0.64	66.7	288 ± 8.02
	1.0	0.22	22.9	359 ± 7.12
	S9 Added			
	–	0.81	100	11 ± 1.73
	0.1	0.87	107.4	32 ± 4.58
	0.5	0.78	96.3	110 ± 6.11
	1.0	0.49	60.5	217 ± 2.08

The bacteria were grown in nutrient broth, shaken for 12 h at 37 °C, and 0.1 ml was then added to the molten top agar, with and without 0.5 ml of 'S-9-mix'[12]. This mix contained per ml: 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP, 100 mM sodium phosphate (pH 7.4) and 0.3 ml of liver homogenates (S-9) (9000 × g supernatant) from male Wistar rats (of about 250 g each) which were induced by a single i.p. injection of a polychlorinated biphenyl (PCB) mixture (Aroclor 1254), diluted in corn oil to a concentration of 200 mg/ml. A dosage of 500 mg/kg was given to each rat 5 days before sacrifice. DCP was diluted 100fold in dimethylsulfoxide (DMSO) and equivalent volumes to those listed in the table were added directly to the top agar. Triplicate petri plates containing Vogel-Bonner E medium[13] were overlayed with this mixture and incubated at 37 °C. After 48 h the revertant colonies were counted. For determination of survival rates, the top agar mixture was poured on triplicate petri plates containing Vogel-Bonner E medium with 8% nutrient broth. In this case, the bacteria have been diluted by the factor of 10^8 in 0.9% NaCl before addition to the top agar. The colonies of surviving bacteria were counted after 24 h of incubation at 37 °C.

Surprizingly there is not only no enhancement but even a marked reduction of the rate of back mutations after addition of microsomes. Moreover, the cytotoxicity of both isomers is also drastically reduced. The reason for this unexpected finding still has to be revealed. To make sure that the S9-mix used was enzymatically active, a control series of 6 plates with Vogel-Bonner E medium was overlayed with top agar containing 40 µg of 2-aminoanthracene (in 100 µl of DMSO), with and without S9-mix, and treated in the same way as the other plates. 721 ± 9.36 colonies of revertants were counted on the (triplicate) plates with S9-mix, 11 ± 2.21 colonies on those without it. We anticipate a direct alkylating reactivity of DCP because, according to preliminary experiments, both isomers of DCP rapidly react with 4-nitrobenzpyridine.

The data shown strongly suggest further investigation on 1,3-DCP as a potential carcinogen and demonstrate that it is a potent mutagen under the experimental conditions as described.

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Intracellular localization of calcitonin in the C cells of the rat[1]

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Summary. Specific localization of C cells at the electron microscope level was achieved by an indirect immunoperoxidase technique. The hormone is present in the electron dense granules. The presence of granules apparently devoid of calcitonin was also detected.

Specific localization of calcitonin is a perequisite for the study of the secretion of the hormone at the ultrastructural level. We have shown that antibodies, raised in the the rat, directed towards human calcitonin can be used for the specific localization of calcitonin producing cells (C cells) in the rat using a double immunofluorescence technique[3]. In the present work, we have studied the ultrastructural localization of calcitonin in the rat C cells, using a double immunoperoxidase technique.

Material and methods. 1-mm³ blocks of thyroid glands of male Wistar rats, 100 g b.wt were fixed for 1 h at 4°C in 2.5% glutaraldehyde in phosphate buffer 0.1 M pH 7.2,

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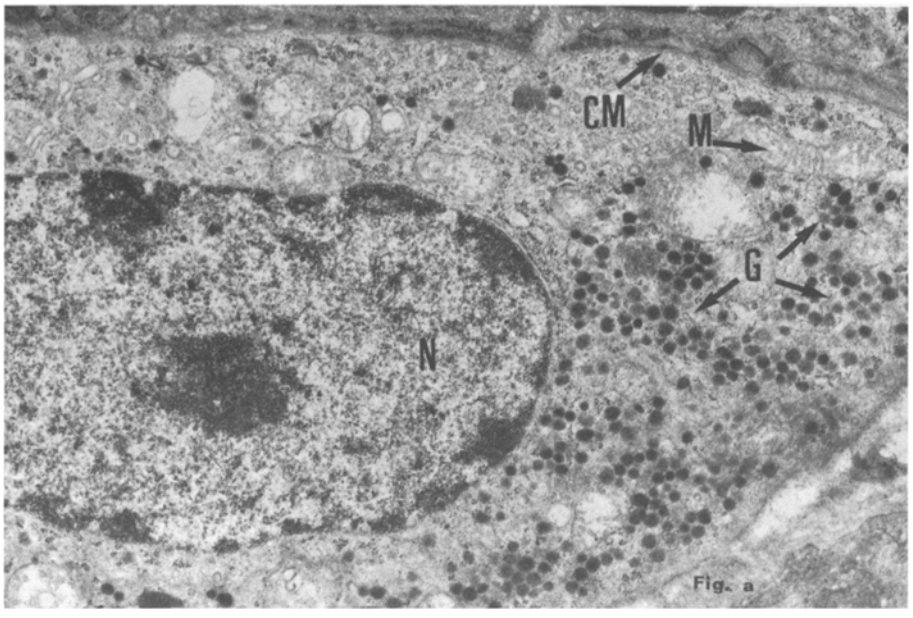

Fig. a. Electron micrograph of C cell rat's thyroid. N, nucleus; M, mitochondria; CM, cellular membrane; G, granule. Double fixation and stained with uranyl acetate and lead citrate. ×5600.

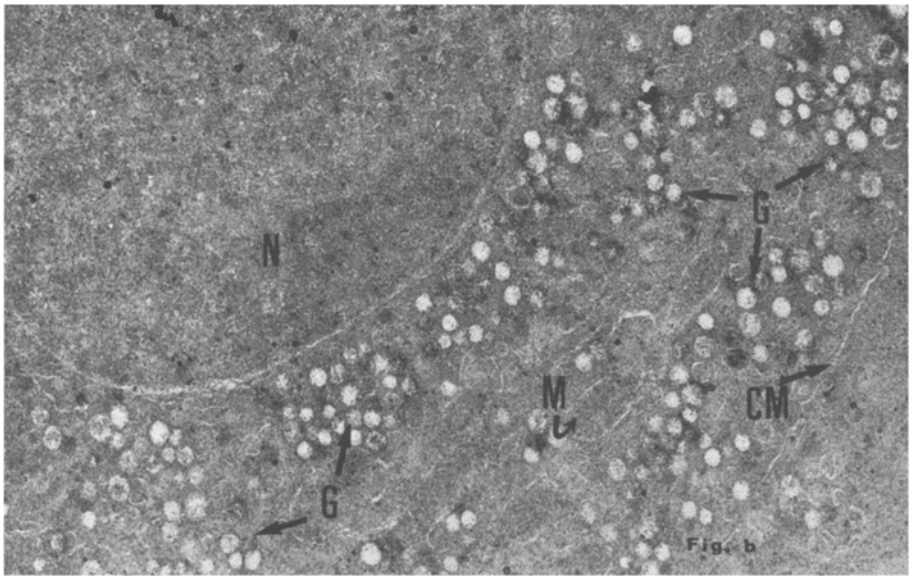

Fig. b. Electron micrograph of a C cell control section; no staining after incubation with saturated antibody. ×8300.

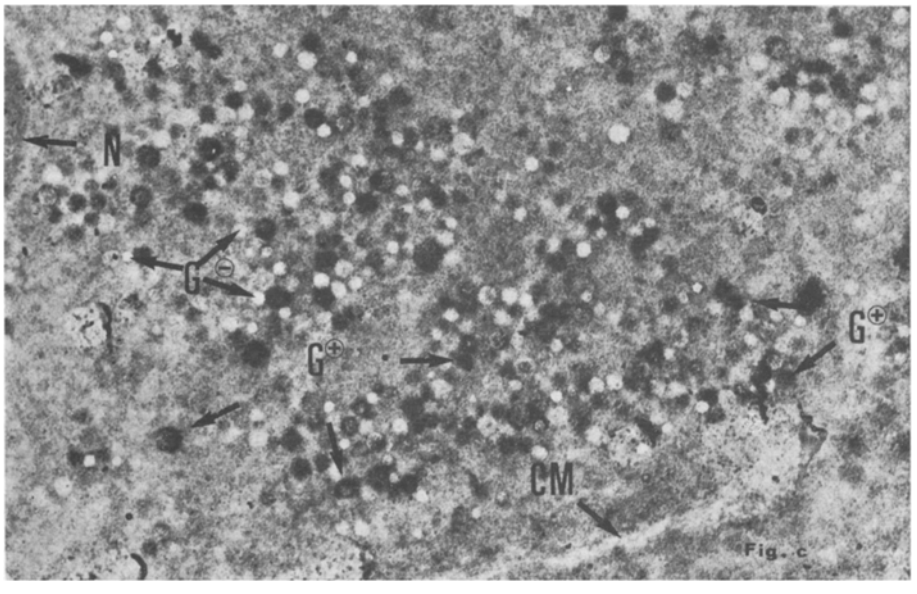

Fig. c. Electron micrograph of a C cell double immunoperoxidase technique section. Positive reaction G (+), negative reaction G (–). ×8300.

and postfixed in 1% osmium tetraoxide in the same buffer. After dehydration in a gradient of alcohol water, and alcohol propylene oxide, the blocks were embedded in epon 812 and polymerized at 60 °C.

Gold sections were mounted on nickel grids, and floated for 20 min on a drop of 10% hydrogen peroxide solution, in order to eliminate the excess osmic acid.

The sections were extensively washed with distilled water and incubated on a drop of antibody directed towards human calcitonin diluted 1/50 in phosphate buffer 0.2 M ph 7.5 containing 0.2% human albumin and 1% sodium merthiolate. Excess antibody was removed by repeated washing of the sections in phosphate buffered saline (PBS) and the washed sections were incubated on a drop of anti-rat Ig immunoglobulin (rabbit) labelled with peroxidase and purified by molecular sieving[4]. After further washings with PBS, the sections were stained for peroxidase by incubation with continuous agitation for 20 min in the following substrate: phosphate buffer 0.2 M pH 7.2 containing 0.075% diamino benzidine and 0.001% hydrogen peroxide. The treated sections were washed twice with distilled water and post-fixed in 1% osmic acid before examination in an electron microscope[5,6].

The following controls were used in order to establish the specificity of the immunoperoxidase stain: a) sections incubated in the presence of normal rat serum, b) sections incubated in the presence of specific anticalcitonin antibody saturated with synthetic human calcitonin, c) sections directly incubated in the substrate for the control of normally occurring peroxidase in the thyroid gland.

Results. In sections of the thyroid gland treated with lead and uranium salts, C cells are easily demonstrated by the presence of numerous electron dense granulations (figure a). Treatment with hydrogen peroxide destains the cellular organellae, the membranes and the electron dense granules. In the thyroid sections stained by the double immunoperoxidase technique, the C cells contain numerous granules revealed by the presence of a dark precipitate of reaction product (figure c). This reaction is specific as sections incubated with normal serum or with the antibody saturated with synthetic calcitonin do not show a positive reaction (figure b). Naturally occurring peroxidase is limited to the mast cells and red blood corpuscles.

Discussion. In electron microscope studies, C cells have been identified principally by their high content of electron dense granules. The work presented here confirms that these granules do contain specifically immunoreactive calcitonin. The fact that a part of these granules do not show a positive reaction with the immunoperoxidase technique may be due to a partial denaturation of the calcitonin affecting its immunoreactivity, or to the presence of a second type of granules containing a polypeptide-s of unknown nature synthetized by the C cells. The naturally occurring peroxidase present in the thyroid does not hinder specific labelling of the granules of the C cells, as the enzyme occurs only in the mast cells and red blood corpuscles which are morphologically quite distinct from the C cells. The calcitonin content of the C cells is located in the granules, and separation of cellular components, by density gradient centrifugation, showed that calcitonin is present predominantly in the granular fraction[7].

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Eye lens development and γ crystallins in Discoglossus pictus (Anura)

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Summary. The ontogeny and localization of the γ crystallins in Discoglossus pictus lens development has been determined. Using antibody specific for amphibian γ crystallins in the immunofluorescence technique, it was found that γ crystallins first appear in primary lens fibre cells in the lens rudiment, and continue to be restricted to the fibre area as lens development progresses. Thus the role of γ crystallins as indicators of a differentiated state remains constant in amphibian evolution, having been demonstrated in the most archaic anuran superfamily, as well as in others more recently evolved.

Discoglossus pictus, the painted frog, is a member of the family Discoglossidae and native to southwestern Europe and northwestern Africa. Although ranid in appearance and size, this anuran is considered to be evolutionarily primitive. Previous studies to detect the time of first appearance and localization of the γ crystallins, those structural lens proteins specific for lens fibre formation in amphibians, have consistently associated their appearance with the differentiation of primary lens fibre cells in the embryonic lens rudiment[3–5]. In order to determine the presence of this association in the archaic anurans, the otogeny and localization of the γ crystallins in D. pictus development was elucidated by means of the immunofluorescence technique.

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