Summation of the effects of dietary antioxidants on MCA induced cutaneous carcinogenesis

Diet	Parameters	Week 12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	33	37
Regular	Animals with tumors or lesions (%)	33	61	56	67	72	67	61	83	78	94	83	94	94	89	94	94	100
	Animals with tumors (%)	0	0	0	11	17	28	28	22	38	56	44	50	44	56	50	66	72
	No. lesions/animal	0.50	0.89	1.00	1.39	1.83	1.33	1.72	1.83	1.78	2.00	1.67	2.11	2.17	1.89	2.89	2.76	2.33
	No. tumors/animal	0.00	0.00	0.00	0.17	0.22	0.39	0.50	0.61	0.78	0.89	0.78	0.83	0.72	0.89	0.83	1.12	1.67
	No. lesions + tumors/animal	0.50	0.89	1.00	1.56	2.06	1.72	2.22	2.44	2.56	2.89	2.44	2.94	2.89	2.78	3.72	3.88	4.00
Special	Animals with tumors or lesions (%)	15	23	23	69	62	62	77	62	69	85	100	85	77	85	92	85	77
	Animals with tumors (%)	0	0	8	15	15	31	38	23	46	38	46	62	54	46	54	46	54
	No. lesions/animal	0.15	0.38	0.54	0.92	1.08	0.85	1.38	0.46	0.62	1.08	1.23	1.15	0.85	1.23	1.15	1.15	1.38
	No. tumors/animal	0.00	0.00	0.08	0.23	0.23	0.38	0.54	0.77	0.62	0.54	0.77	0.92	0.85	0.85	0.69	0.62	0.77
	No. lesions + tumors/animal	0.15	0.38	0.62	1.15	1.31	1.23	1.92	1.23	1.23	1.62	2.00	2.08	1.69	2.08	1.85	1.77	2.15

Free radicals have been implicated in a number of pathological conditions[12,13]. Increased levels of free-radicals and lipid peroxidation have been reported in skin following UVL-radiation[14,15], Many anticancer compounds exhibit free-radical inhibitory effects[16,17]. Indeed, some of these have recently been shown to inhibit chemically-induced skin carcinogenesis[18]. Whether free-radicals are involved in either UVL or chemically-induced skin carcinogenesis is yet unknown. However, the results of this study indicate that antioxidants are effective in allaying the deleterious effects of topically-applied 3-methylcholanthrene and suggest that there may be a confluence in the developmental steps of both chemical and UVL-induced carcinogenesis at which antioxidants act.

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The endocrine nature of the paraganglia of man

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Summary. Brightly fluorescent paraganglia were found in the retroperitoneal tissue of adult man. The histofluorescence properties of the paraganglia indicate the presence of tryptophyl peptides, which might be of endocrine importance.

The extra-adrenal catecholamine storing and syntethizing cells are widely distributed along and within the sympathetic and parasympathetic nervous system[1-3]. These cells, including the small, intensely fluorescent (SIF) cells of the sympathetic ganglia[4-7] are described as paraganglia (PG)[1-8].

The PG of man dominate during the fetal period[1,2], while only a few observations on their postnatal fate are available[1,9-11]. To elucidate the nature of the human adult PG, tissues obtained from vascular, gynaecological and urological surgery were analyzed systematically using the formaldehyde induced fluorescence (FIF) method[2,4] for catecholamines to trace the PG.

As acknowledged in the preliminary reports[12,13], brightly yellowish to orange fluorescent PG were regularly found embedded in the para-aortic and retroperitoneal connective tissue (figures 1 and 2). Microspectrofluorimetric recordings of the emission spectra of the fluorophore showed emission maximum at 480 nm typical to catecholamines (figure 3). The PG are considered as APUD cells[14] characterized by Pearse[15,16]. The 2 most important common characteristics of the APUD cell series are

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Relative intensity of the fluorescence

Specimen	FIF	FIF+HCl
Models		
Noradrenaline	100	143
Adrenaline	48	29
Tryptophane	35	130
Tissues		
Adrenal medulla, rat, NA-cells	100	134
Adrenal medulla, rat, A-cells	82	58
Adrenal medulla, man	106	104
Paraganglion, man	98	330

The summary of the recordings of the fluorescence intensities of the models and tissues studied. The measurements were carried out at the emission maximum of the compound. The models consisted of microdroplets of 2×10^{-3} M of the amine or amino acid in 1% polyvinylpyrrolidone buffered at pH 7.4 with 0.1 M phosphate buffer; 0.02% L-alanine was added to promote the formaldehyde condensation reaction. The tissues were sectioned serially at 5 µm and the measurements were made from the same sections. The effect of photodecomposition has been considered. The measurements were performed using objective 25 × and a spot of 10 µm in diameter. The values presented are means of 200 microdroplet measurements and 47 acidification experiments in which 50 measurements/section were made before and after treatment.

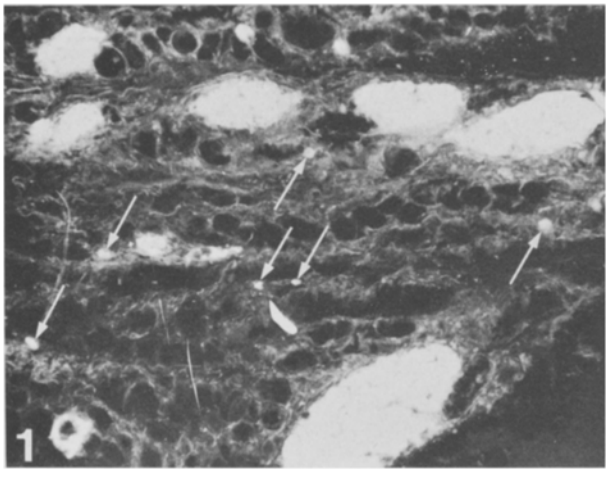

Fig. 1. A low magnification fluorescence micrograph from the retroperitoneal space, near the aorta. Brightly fluorescent flock of PG of varying size is observed. The arrows point out the smallest 'micro-PG' consisting of only a few cells. × 150.

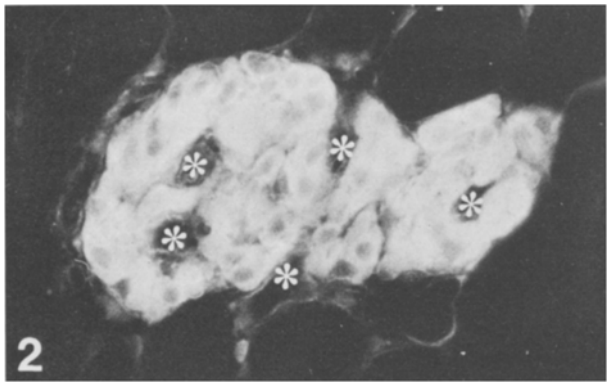

Fig. 2. Typical abdominal PG surrounded by fat tissue form the interrenal retroperitoneal space near the renal artery. The cells form coiling cords, which are in close connection with the capillaries (stars). × 440.

a) the capability to synthesize and store catecholamines and b) to produce a peptide hormone[15, 16], although the peptide has not been recognized in all the cells exhibiting the other cytochemical characteristics. The tryptophane containing peptides of several APUD cells (ACTH-, MSH-, Gastrin-, Glucagon-) can be demonstrated treating the FIF-specimens with HCl[17–21].

The paraganglia including SIF-cells and adrenal medulla of man as well as adrenal medulla and pancreas of the rat as controls were processed parallelly for the FIF. The results from sections were compared with models containing amino acids or monoamines (figure 3, table). The specimens were treated with vapour of 25% HCL for 5 min at 22 °C in closed glas jar[17]. The emission spectra of the specimens and the intensity of the FIF were recorded before and after the acidification using microspectrofluorimetric equipment (figure 3). Figure 3 shows the effect of HCl on the PG and models containing tryptophane. The FIF of the PG changes like the fluorescence of the tryptophane models. The relative intensities of the fluorescence are summarized in the table. The most interesting feature is the 3–4fold increase in the intensity of the fluorescence of the PG, with the simultaneous shift in the emission maximum to 495 nm. This change cannot be done to catecholamine-fluorophores. Neither of these changes were observed in the adrenal medulla of rat or man. The same magnitude of the increase in the intensity was also observed in tryptophane fluorescence. Noradrenaline fluorescence, on the other hand, was about 1.3–1.4 times as strong after HCl gassing in both the model and the adrenal medulla of the rat, though adrenaline fluorescence weakened (Table).

The emission spectra of formaldehyde-induced fluorescence of noradrenaline in the model, in the noradrenaline cells of the adrenal medulla of the rat, and in the paraganglia of man showed a maximum at 480 nm. After acidification, both the noradrenaline model and the noradrenaline cells of the adrenal medulla still exhibited a maximum at

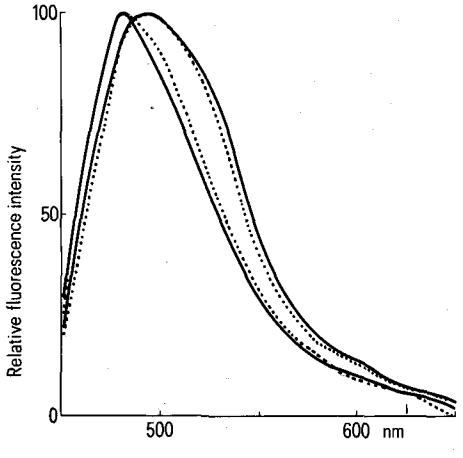

Fig. 3. The emission spectra of the tissues and models before and after HCl-treatment. The curves after treatment have been extrapolated to the same intensity as the control to show the change in the emission maximum. The Leitz-MPV 2 microspectrophotometer was equipped with HBO 100 (Osram) and the filters TAL 405, TK 455 as well as Veril B-60 bandinterference filter for the emission curves. The emission curve of noradrenaline model before (left, ———) and after HCl treatment (left ....) show the same maximum at 480 nm. The right hand pair of curves, tryptophane fluorescence from the pancreatic acinar cells of the rat after formaldehyde-HCl treatment (right ———) and the PG after the same treatment (right .....), show also identical spectral characteristics, but emission maximum at 495–500 nm. – The spectra, which are uncorrected instrumental values, are given as relative fluorescence intensity versus wavelength.

480 nm, though the maximum of the fluorescence of the paraganglia was shifted to 495 nm (figure 3). For control purposes, formaldehyde-HCl-induced fluorescence of tryptophan in the tissue proteins was measured from the zymogen granules of the pancreatic acinar cells known to contrain chymotrypsinogen and trypsinogen with a relatively high number of tryptophane residues, which exhibit a bright fluorescence with this method[19, 21].The emission maximum of the fluorescence was at 495 nm, and the shape of the curve was similar to that recorded from the paraganglia of man (figure 3). After HCl treatment, intensely fluorescent granules could be observed in the cytoplasm of the PG-cells against the homogenous background fluorescence.

The 3fold increase in the formaldehyde-induced fluorescence of the paraganglia of man after acidification strongly suggests that there is a protein containing a relatively high number of tryptophane residues in the cytoplasmic granules. The molecular basis of the acid-catalyzed formaldehyde-induced fluorescence of tryptamines including tryptophane has been suggested to be the promotion of the formation of strongly fluorescent dihydro-β-carboline compounds[19]. Various modifications of the acid-catalyzed formaldehyde condensation reaction have been used for the localization of tryptophane-containing peptide hormones in the endocrine cells and of enzymes stored in the cytoplasmic granules in the exocrine cells[13–18]. Further evidence for the presence of tryptophane-containing protein was gained with the studies concerning the emission spectra, which can be used for the differentiation of the formaldehyde-induced fluorescence of various biogenic monoamines and amino acids at the cellular level[19]. The amino acid composition of the major protein constituents of the chromaffin granules of the adrenal medulla of several species is known and tryptophane has been found to be missing from these proteins[22]. Our observations suggest that there is a hitherto unknown protein in the granules of the paraganglionic cells of adult man. Considering the general characteristics of APUD-cells the results suggest, that the PG of man, which are widely distributed and well vascularized clusters of cells, do produce and store both catecholamines and tryptophane containing protein possibly of an endocrine nature.

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Serum activity inhibiting specific simian virus 40-induced transplantation resistance and its correlation with primary SV40 tumors appearance in hamsters

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Summary. Using the modified technique of transplantation test, ITR serum activity was found in most (14 out of 21) individual hamster sera obtained during the latent period of primary SV40 carcinogenesis (60 days after virus infection when newborn). On the other hand, as a rule, no ITR activity was observed in the sera of the same hamsters after tumor appearance and during their growth. ITR activity rapidly disappeared from sera of hamsters neonatally infected with SV40 after their successful immunization with the same virus during the latent period. There appears to be a correlation between the presence of ITR serum factor during the latent period and the subsequent primary SV40 tumor appearance in hamsters.

The factor(s) blocking lymphocytes cytotoxic activity in sera of individuals bearing primary and transplantable tumors have been demonstrated mainly with in vitro assays[1–8]. The interest of these factors, however, is connected with their supposed and unfavourable role for the organism in vivo. The studies of the role of humoral factors, and especially blocking serum activity in vitro and in vivo in hamsters bearing SV40[9] induced tumors, are scanty and contradictory[10–15]. Therefore, the present in vivo studies were carried out in order to investigate possible influence on tumor cells of the sera samples obtained from individual Syrian hamsters during different periods of primary SV40-induced carcinogenesis. For this purpose, the sera samples, as well as control NHS, were used for the pretreatment of SV40 test-tumor cells in vitro and subsequent challenge of such pretreated cells in immune (SV40 virus-inoculated) and normal adult hamsters.

Materials and methods. Syrian hamsters of both sexes were used. Their origin has been described[16]. SV40 virus, strain No. 128, with a titer of $10^{7.4}$–$10^{7.5}$ $TCID_{50}$/1.0 ml, was used for infection of newborn hamsters and for immunization of adult animals. Newborn hamsters not older than 18 h were infected S.C. with 0.2 ml of the undiluted SV40 virus. Some of the animals neonatally infected with SV40 were immunized by reinfection with the same virus (in a volume of 0.5 ml) i.p. during the latent period (60 days after birth). Such immunization can effectively prevent tumor appearance in hamsters inoculated by SV40[17–20]. As transplantable test-tumor throughout these studies hamsters SV40-induced sarcoma was used. From this tumor, an in vitro tissue culture cell line designated as E-1 was established. The origin and tumorigenicity of the E-1 cell line have been reported[16, 21]. In these studies E-1 cells from the 6th to the 100th in vitro passages were used. Sera from each hamster neonatally infected with SV40 were obtained repeatedly at the different stages of primary SV40 carcinogenesis. Control sera were obtained from untreated hamsters of the same age. All sera were prepared by the retroorbital sinus or cardiac puncture, heat-inactivated at 56 °C for 30 min and stored at –20 °C until use. For pretreatment of the E-1 test-tumor cells with hamster sera in vitro 0.05 ml of undiluted serum was added to the known number of serially 10-fold-diluted E-1 cells. The mixtures of the E-1 cells pretreated either with sera tested or with NHS were incubated at 37 °C for 45 min and challenged both in 4–5 immunized and 4–5 normal adult hamsters s.c. Modified technique of transplantation test was used. Details of the technique