and 132,000. Although not positively identified, their molecular weights suggested that they might be α_1-lipoprotein and ceruloplasmin respectively. The change in the 132,000 protein appeared to be qualitative and consisted of changes in the migration patterns of 3 apparent forms.

Discussion. The present work demonstrates that certain serum macromolecules are depleted preferentially from the culture medium by developing rat embryos. The uptake of proteins to the visceral yolk sac and embryo was not studied. Recently it has been suggested that albumin is taken up by the visceral yolk sac and digested before passage to the embryo[16].

In the only other report that examined protein utilization by cultured embryos, a 125,000 and 2 greater than 200,000 mol.wt proteins were depleted in the culture medium[6]. It is not clear whether any of these correspond to any of the depleted proteins we detected. It should be noted that our electrophoretic system was non-dissociating and it is likely that the actual molecular weight of the 2 unidentified proteins differ from the apparent molecular weights in our system. However, it supports the contention that certain serum macromolecules are selectively taken up by the conceptus, and may be helpful in defining the protein requirements of developing embryos. Transferrin in a defined medium has recently been shown to support the differentiation of mouse kidney tubules in vitro[17]. Likewise, α_2-M and α_1-acid glycoprotein have been shown to be sufficient to support the growth of primary embryo fibroblasts[18,19]. Whether a concoction of several or all of the above proteins in an artificial medium will be sufficient to support normal whole-embryo development remains to be determined.

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Dietary carotenoids block photocarcinogenic enhancement by benzo (a)pyrene and inhibit its carcinogenesis in the dark[1]

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Summary. The carotenoids β-carotene (C) and canthaxanthine (CX), with and without pro-vitamin A activity, respectively, when perorally administered to mice, markedly prevent benzo(a)pyrene photocarcinogenic enhancement (BP-PCE), continue to block such BP-PCE and protect significantly against BP carcinogenesis in mice maintained in the dark. These results appear relevant to both the pathogenesis of chemical carcinogenesis and rational programs of skin cancer prevention in humans.

In the last decade, experimental trials have suggested that a diet rich in red carrots inhibits the appearance of tumors induced by dimethyl-benz-[a]-anthracene in mice[3] and that injection or peroral administration of carotenoids delays skin tumor induction in hairless mice exposed to UV-B (290–320 nm) irradiation[4,5]. We approached the study of a possible skin cancer modulation by carotenoids, applying our experimental model which proved that non-carcinogenic long UV-irradiation (300–400 nm) strikingly enhances skin benzo(a)pyrene (BP) carcinogenesis in mice[6]. This suggested that in the epidemiology of skin cancer in humans, where chronic sun exposure is recognized to be the major etiologic factor[7], concurrent chemical factors, regardless of their exogenous or endogenous origin, should play an important role. Our experimental trial demonstrated that β-carotene (C) and canthaxanthine (CX), 2 carotenoids, respectively, with and without pro-vitamin A activity, when perorally administered to mice, inhibit and later block BP photocarcinogenic enhancement (PCE)[8]. In the present report the comprehensive results of such study are referred to point out their implication with regard to both the pathogenesis of BP carcinogenesis and possible rational programs of skin cancer prevention in humans.

Materials and methods. C and CX were perorally administered to female Swiss albino mice, strain 955, with daily diet (2.5 mg of C or CX in 5 g of pellets, the latter being the amount of food consumed daily by 1 mouse weighing about 25 g). One month later, the mice were given additional administrations of C and CX, dissolved in arachidic oil, by catheter twice a week (25 mg per 0.250 kg of b.wt).

2 h after C and CX administration, the mice were painted with BP (100 µg) acetone solution on a clipped mid-dorsum area and were either exposed to long UV-light (Philips bulb W 125 W, 300–400 nm with maximum output at 365 nm, negligible 313 nm band, with flux at animal level: 5.89×10^3 erg cm^{-2} sec^{-1}) for 2 h or kept in the dark. In this experiment, 16 groups of animals, 75 per group, were employed. Of these, 6 groups were used to investigate the activity of C and CX on BP photocarcinogenesis. The other groups were controls with respect to light, solvent and carotenoids, and were either exposed to UV or kept in the dark.

For each experimental group the cumulative percentage of mice bearing one or more tumors was calculated using conventional statistical methods based on adjustment for mice that died during each 2-week period of observation.

Results. As shown in figure 1, BP-PCE was evident soon after the beginning of tumor onset. It was maximum (7-4 times in 'BP + UV' vs 'BP-dark' group) at 28–36 weeks after initial UV exposure, when the percentage of mice with tumors in the irradiated group was in the range of 50. At this stage, BP-PCE was also clearly inhibited to the same extent by C and CX treatments. In the dark, however, C and CX did not show any significant inhibition of carcinogenesis.

With the progress of the experiment, BP-PCE decreased, since the values of carcinogenicity in the 'BP-dark' group were increasing. Nevertheless, from the 44th to the 60th week, C and CX completely blocked the kinetics of BP-PCE at tumor incidence values of 40 and 54–64, respectively. Thus, C was more active than CX.

At this stage, the values of carcinogenicity in the 'BP-dark' group became higher than those in the 'BP + C + UV' group and similar to those in the 'BP + CX + UV' group. Furthermore, C and CX exerted a significant inhibitory activity also on carcinogenesis in the dark. In this case, CX was more efficient ($p = < 0.01$) than C ($p = < 0.05$).

To better evaluate C and CX efficiences on BP-PCE, their percent inhibition coefficients were computed with respect to BP carcinogenesis in the dark.

In figure 2 such inhibition coefficients, plotted against time, are expressed by 2 linear functions. Values of almost 200 for C and greater than 100 for CX were found at the end of the experiment. In this representation the 100 value indicated the shifting point from the inhibiting to the blocking effect of carotenoids on BP-PCE kinetics.

All the control animal groups did not show any tumor onset except for the 'no BP – no C or CX' group exposed to light; 8% of mice had tumors (papillomas + some epitheliomas) at the end of the experiment. This was expected, although the long UV-light is generally considered non-carcinogenic on the basis of previous data[9].

Discussion. These are original data demonstrating that carotenoids, with and without pro-vitamin A activity, have antitumorigenic properties.

The above data result from the effects of a carcinogen and the action of UV-A light (320–400 nm) which is generally considered non tumorigenic, and a negligible 313 nm tumorigenic band which is present in the sun spectrum at earth level. The energy output of the tumorigenic band during the experiment, however, was 100 times less than the minimum UV energy requirement for skin carcinogenesis[10]. Therefore, the low incidence of skin neoplastic growth observed in the control group ('no BP – no C or CX' exposed to light), can be considered a long term effect of near UV-light, perhaps by exciting endogenous photodynamic substances.

The mechanism of the carotenoid antitumorigenic action may be explained assuming that BP initiates a carcinogenic process by oxyradical formation, as was first observed with hematoporphyrin excited by light[11]. Indeed, both these substances behave in the same fashion in a photodynamic reaction[11] and are photocarcinogenic in mice[6,12]. Excited states are considered also possible in the dark via interaction of molecules with endogenously produced free radicals such as $O_2^{\cdot}$, $OH\cdot$, or singlet oxygen[13]. The properties of carotenoids as free radical scavenger and singlet oxygen quencher, rather than as UV screening agents, as was observed by pigment measurement in the skin of mice and humans fed C[14,15], may explain their antitumorigenic activity.

The remarkable tumor inhibition exerted by C and CX, up to blockage of the kinetics of BP-PCE, in experimental conditions that may mimic the pathogenesis of skin cancer in humans, appears to be relevant in occupational medicine. Some countries recommend that outdoor workers (roadworkers, life-guards on the ocean beaches, etc.) use external protective measures such as clothing and sunscreen preparations ('sun oils') spread on skin surfaces exposed to light. All these preparations contain compounds (derivatives of salicylic acid, p-aminobenzoic acid, cinnamic acid, benzimidazole, etc.) which have maximum absorption in the UV-B, whereas the actinic damage is primarily due to UV-A. Only a few substances (i.e. specific derivatives of benzophenone) available act as UV-A filters and do not irritate the skin[16]. Furthermore, the oil medium of the sunscreening preparations may undergo oxidation, thus becoming a potential carcinogen. Our experimental data suggest that peroral administration of carotenoids should be considered in the development of rational programs of

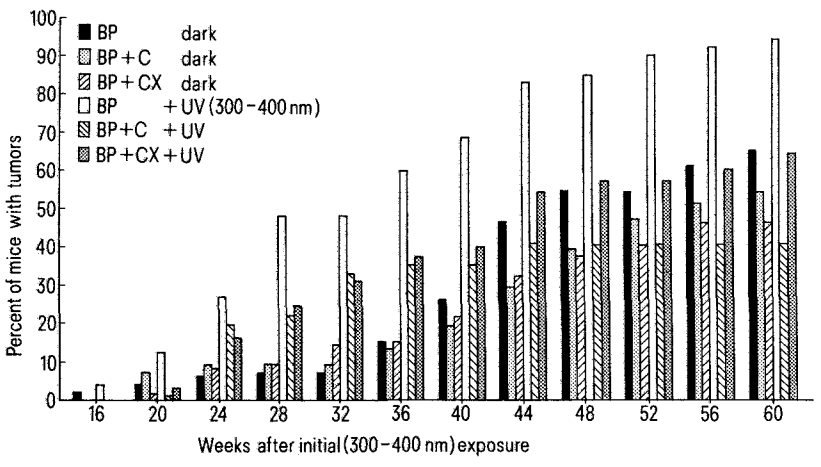

Figure 1. Tumor (papilloma + squamous epithelioma) incidence in Swiss female albino mice, strain 955, painted with BP and kept in the dark or exposed to UV (300–400 nm) light; painted with BP, fed C or CX and kept in the dark or exposed to UV. 75 mice per group. At the 40th week 22% of all tumors in the light and 8% in the dark were epitheliomas; at the end of the experiment they were 80–90% of all tumors independently of light exposure.

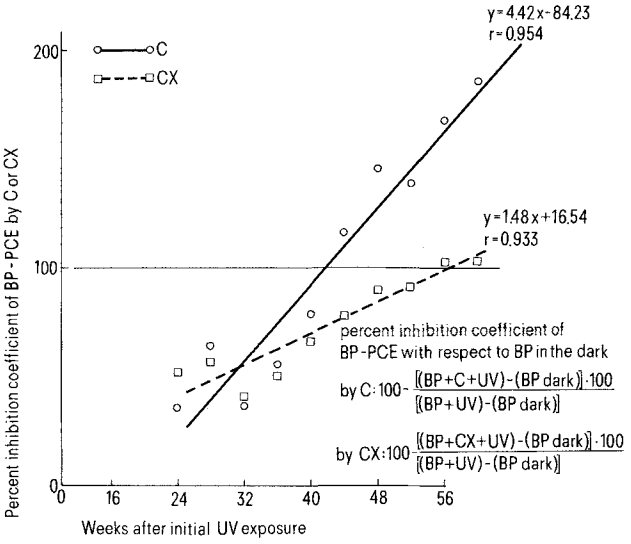

Figure 2. Percent inhibition coefficient of BP-PCE by C and CX against weeks after initial UV exposure. The value 100 indicates the shifting point from the protective to the blocking effects of carotenoids on BP-PCE.

skin cancer prevention for outdoor workers, especially when they use materials such as tar which are well-known photocarcinogens.

Finally, these experiments may support the hypothesis that dietary vitamin A may lead to a reduction of human cancer rates[17]. Our results may also address such stimulating issues as those presented in a review article on dietary β-carotene and human cancer[18], since carotenoids may exert their antitumorigenic action independently of their pro-vitamin A activity[5,8]. Furthermore, attention should be given to carotenoids due to their lack of toxicity in humans; in contrast, retinoids may be harmful to the liver and inactive on skin tumors[5]. Most recent data suggest that C may also exert a therapeutic action in tumor-transplanted mice[19,20].

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Exocrine pancreas: difference in the amylase content of the dorsal and ventral regions[1]

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Summary. The amylase content of the acinar tissue is higher in the splenic region of the rat pancreas containing glucagon-rich islets than in the duodenal region harboring pancreatic polypeptide-rich islets.

The pancreatic gland displays different levels of heterogeneity. 1st, the islets of Langerhans constituting the endocrine pancreas are scattered within the acinar or exocrine tissue[2]. 2nd, in the exocrine pancreas, the cells forming the periinsular halos differ from the teleinsular acinar cells by tinctorial and functional criteria[3]. 3rd, the islets of Langerhans contain at least 4 different types of endocrine cells secreting insulin, glucagon, pancreatic polypeptide and somatostatin, respectively[2]. 4th, 2 distinct populations of islets, rich in glucagon- or pancreatic polypeptide-producing cells are non-randomly located in the dorsal (or splenic) and ventral (or duodenal) pancreatic regions, respectively[2]. These 2 pancreatic regions also differ from one another in their embryogenesis, vascularization and exocrine drainage[2]. The present work shows that, in the rat, the hydrolase content of acinar cells is also different in the dorsal and ventral moieties of the pancreatic gland.

Small pieces of pancreatic tissue (wet wt: 98 ± 3 mg; $n = 36$) removed from the dorsal and ventral pancreatic regions[2] of fed albino rats were homogenized in 1.0 ml of distilled water. The protein[4], amylase, lipase and chymotrypsinogen content[3] of each homogenate was measured by methods described elsewhere.

Relative to the wet weight of tissue, the protein content of