Carcinogenic activation of benzo(a)pyrene by iodine and ferric chloride in the respiratory tract of Syrian golden hamsters[1]

F. Stenbäck and J. Rowland

Department of Pathology, University of Oulu, Kajaanintie 52 D, SF-Oulu 22 (Finland), and Eppley Institute for Research in Cancer, University of Nebraska Medical Center, 42nd and Dewey Ave., Omaha (Nebraska 68105, USA), 4 July 1977

Summary. Benzo(a)pyrene (0.5 mg), in itself weakly carcinogenic, when given in combination with ferric chloride (0,16 mg) or iodine (0,2 mg), alone noncarcinogenic, induced a number of tumors of different parts of the respiratory tract of Syrian golden hamsters.

The chemical binding of polycyclic aromatic hydrocarbons to cellular targets, a process induced by hydroxylating enzyme systems, presumably represents the key event in the carcinogenic process of these compounds[2,3]. Iron and copper are an integral and essential part of several purified hydroxylase enzymes[4], suggesting that a metal may play a role in hydrocarbon activation. Therefore, some exogenous ions might activate aromatic hydrocarbons similarly and, thereby enhance their carcinogenicity. The goal of the present study is to evaluate the significance of iodine (I_2) or ferric chloride ($FeCl_3$) as chemical activators of B(a)P in inducing respiratory tumors in Syrian hamsters.

Materials and methods. Male and female Syrian golden hamsters from the Eppley colony were randomly divided and distributed 6/cage. They were kept in plastic cages on sterilized San-i-celt bedding and fed Wayne pelleted diet and tap water ad libitum. B(a)P, I_2, and $FeCl_3$ were obtained from Aldrich Chemical Company (Milwaukee, Wis.) and saline (0.9% NaCl, sterile, nonpyrogenic) from Baxter Laboratories, Inc., (Morton Grove, Ill.). B(a)P was ground in a mullite motor for 8 h to obtain fine particles. I_2 and $FeCl_3$ dusts were added to the fine particles of B(a)P and 'ball milled' in saline suspension for 17 h. Hamsters, under barbiturate anesthesia, in groups of 24 males and 24 females received intratracheal instillation of 0.2 ml of the saline suspension once weekly for 20 weeks, as described by Saffiotti et al.[5]. Treatment by group was as follows: group 1, 0.5 mg B(a)P; group 2, 0.2 mg I_2; group 3, 0.5 mg B(a)P plus 0.2 mg I_2; group 4, 0,16 mg $FeCl_3$; group 5, 0.5 mg B(a)P plus 0.16 mg $FeCl_3$ and group 6, 0.2 ml saline.

Hamsters were weighed weekly, checked twice daily; those in poor health were isolated and left to die normally or killed when moribund. All animals except for a few which were cannibalized were autopsied. The technique used for autopsy and fixation of the lungs was as previously described[5]. The respiratory organs, as well as other tissues showing macroscopic abnormalities, were examined histologically.

Results and discussion. The survival rates of the experimental groups are presented in table 1. B(a)P when given alone, was weakly effective, inducing 1 laryngeal and 1 tracheal papilloma. Squamous metaplasia of tracheobronchial epithelium was more frequent. In peripheral alveoli, bronchiolar proliferation was rare. However, 1 animal had an accumulation of squamous cells arranged in a glandular

Table 1. Survival rate and number of tumor-bearing hamsters treated intratracheally with B(a)P, I_2, B(a)P and I_2, $FeCl_3$, B(a)P and $FeCl_3$, and saline

Group	Treatment*	Total No. of animals	Effective No. of animals	No. of survivors at week				Total No. of TBA**	Total No. of resp. TBA
				20	40	60	80		
1	0.5 mg B(a)P	48	48	45	30	5	0	7	3
2	0.20 mg I_2	48	47	42	30	6	0	5	0
3	0.5 mg B(a)P and 0.20 mg I_2	48	48	41	34	12	0	19	17
4	0.16 mg $FeCl_3$	48	48	45	32	8	0	2	0
5	0.5 mg B(a)P and 0.16 mg $FeCl_3$	48	48	46	33	6	0	12	6
6	0.20 ml saline	48	48	40	31	4	0	1	0

* Received intratracheal instillation once weekly for 20 weeks. ** TBA = tumor-bearing animals.

Table 2. Number and morphologic types of respiratory tumors induced by B(a)P, I_2, B(a)P and I_2, $FeCl_3$, B(a)P and $FeCl_3$, and saline

Site and tumor type	Number of tumors by treatment					
	B(a)P	I_2	B(a)P and I_2	$FeCl_3$	B(a)P and $FeCl_3$	Saline
Larynx						
Papilloma	1	0	2	0	0	0
Squamous cell carcinoma	0	0	0	0	0	0
Trachea						
Papilloma	1	0	19	0	6	0
Squamous cell carcinoma	0	0	2	0	0	0
Bronchi and lung						
Papilloma	0	0	2	0	0	0
Squamous cell carcinoma	0	0	2	0	0	0
Adenoma	0	0	2	0	0	0
Adenosquamous lesions	0	0	6	0	0	0
Peripheral adenomatoid lesions	0	0	9	0	6	0
Others	7[a]	5[b]	9[c]	2[d]	8[e]	2[f]

[a] 2 forestomach papillomas; [b] 4 forestomach papillomas, 1 pancreatic carcinoma; [c] 9 forestomach papillomas; [d] 2 forestomach papillomas; [e] 6 forestomach papillomas, 1 adrenal hemangioma, 1 adrenal cortex adenoma; [f] 2 forestomach papillomas.

fashion and a tracheal papilloma. Alone I_2 did not induce any respiratory tract tumors, but given in combination with B(a)P induced 2 papillomas of the larynx and 19 papillomas in the trachea. These papillomas were composed of a fibrous stalk covered by proliferating epithelium. The 1st papillomas were seen in this group in an animal that died 8 weeks after the start of the treatment. Alveolar epithelial proliferation was also common, although the condition was not always of a neoplastic nature. Benign epithelial growth in the bronchiolar region resulted in tumors composed of small cuboidal cells arranged in a glandular fashion. Peripheral adenomatoid lesions were common, along with adenosquamous lesions, 2 adenomas and 2 squamous cell carcinomas of the lung were seen. In B(a)P/$FeCl_3$ treated animals, an activating effect of the dust was noticed, although not as strongly as with B(a)P/I_2. $FeCl_3$ alone was ineffective, but in combination with B(a)P induced 6 tracheal papillomas. Tumor distribution is given in table 2.

The carcinogenic polycyclic aromatic hydrocarbons are relatively inert as far as reactions with cellular components are concerned. The specific chemical binding of hydrocarbons with biological macromolecules[6,7] – probably the 1st essential step in cancer induction – requires that the compounds be activated by cellular hydroxylating enzymes[1]. Several hydroxylases require transitional metals (iron is the most commonly found) for enzymic activity[2], and this suggests that metals play a role of prime importance in the hydroxylation reaction. A covalent binding of B(a)P and other carcinogenic hydrocarbons to nucleic acids[8] is also produced by I_2, hydrogen peroxide, hydrogen peroxide plus ferrous ion.

Previous studies have mainly involved the physical characteristics of the dust. One of the main functions of the carrier agent was considered to be an increased retention time of the dust, compared to B(a)P alone[9,10]. Chemical activation by metals of polycyclic hydrocarbons has been reported in experiments in mouse skin[11]. Since atmospheric pollution may contain, and cigarette tobacco tars do contain, both various metal ions and aromatic hydrocarbons, the results presented here, may be relevant to the etiology of pulmonary cancer in man.

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Pigment granules in choroidal melanophores of the albino goldfish[1]

I.K. Takeuchi, Y.K. Takeuchi and T. Kajishima[2]

Department of Embryology, Institute for Developmental Research, Aichi Prefectural Colony, Kasugai, Aichi 480-03 (Japan); and Biological Institute, Faculty of Science, Nagoya University, Nagoya 464 (Japan), 20 February 1978

Summary. Pigment granules in choroidal melanophores of the albino goldfish contained fine particulate materials which were in various degrees aggregated in clumps. Tyrosinase was considered to be present in an inhibited state in these pigment granules.

In the melanin-synthesizing cells of many vertebrate species, melanin pigments are synthesized and stored in pigment granules called 'melanosomes'. In higher vertebrates, premelanosomes, the unpigmented precursors of melanosomes, have a highly organized internal structure composed of numerous coiled filaments, which are arranged parallel to each other and oriented along the long axis of the granule[3]. This internal structure of the premelanosome has also been reported in dermal melanophores and retinal pigment cells of some species of Reptilia[4], Amphibia[5,6], and Pisces[7]. Recently, however, we have reported that the premelanosomes in melanophores of the guppy and retinal pigment cells of the goldfish contained only fine particulate materials[8,9]. On the other hand, Turner et al.[10] claimed that the premelanosomes in melanophores of the goldfish are multivesicular-type in their inner structure. To clarify this, pigment granules in choroidal melanophores of the albino goldfish were examined electron microscopically in the present study. The albino mutant is known to show complete or incomplete absence of melanin synthesis in melanosomes, and is very suitable material to study the premelanosome inner structure[11].

From the adult goldfish (*Carassius auratus*) of the albino strain (gene symbol, *pp,cc*[9]), the eyes were removed intact and immersed in a Karnovsky's solution. In this solution, choroidal layers of the eyes were dissected out and cut into small pieces. Fixation and embedding into epoxy resin were according to the same method indicated in the previous paper[12].

Cytoplasm of choroidal melanophore was filled with numerous pigment granules. Most of these granules were ellipsoidal in shape, about 0.2 μm in diameter and 0.4 μm in length, and contained fine particulate materials which were in various degrees aggregated in clumps (figure, a). However, no granules whose cores had been completely occupied with these materials were encountered. These observations indicated that melanization was only partially stopped in these pigment granules. Pigment granules were sometimes found in which fine particulate materials were only lightly deposited. This type of granule was always smaller in size than the densely pigmented one (figure, b). Thus, it was considered to be the precursor of the densely pigmented one, that is, the goldfish premelanosome. Granules with a multivesicular-type core were not found in the present study.

For the detection of tyrosinase activity, the tissues were first incubated with Dopa according to the method described by Ide[13]. This treatment resulted in no ultrastructural changes in the pigment granules (figure, c). Secondly, the tissues were incubated with Dopa and iodoacetoamide according