INCREASE IN TYPE A VIRUS PARTICLES INDUCED IN BALB/c MOUSE EPIDERMIS DURING CHEMICAL CARCINOGENESIS

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Summary.—Electron microscopic observations of normal BALB/c mouse epidermis revealed the presence of isolated intracisternal A particles. Hyperplasia, papilloma and carcinoma formation induced by topical application of the carcinogenic polycyclic hydrocarbons benzo(a)pyrene (B(a)P) and 3-methylcholanthrene (MC) is accompanied by an increase in the number of A particles. Topical application of a non-carcinogenic irritant a-pinene (aP) failed to provide comparable changes. Examination of the nuclei indicated occasional electron dense granules in the nucleoplasmin which became more common throughout the progression of carcinogenesis.

Intracisternal A particles (Bernhard, 1960) have been discovered in both normal and neoplastic mouse tissue (Wivel and Smith, 1971), in a wide variety of transplantable tumours (Kuff et al., 1972) and in early mouse embryos (Biczysko et al., 1973). A previous investigation of MC-induced carcinogenesis in Leaden strain (C57L) mice reported the presence of intracisternal A particles in squamous cell carcinomata but noted their absence from normal epidermis and small papillomata (Kakefuda, Roberts and Suntzeff, 1970). The present preliminary work with BALB/c mice records the presence of A particles in normal epidermis and an increase in their number throughout the early changes in carcinogenesis induced by B(a)P and MC.

MATERIALS AND METHODS

Three-month-old male mice from an inbred BALB/c colony were each housed in a separate box and were isolated in an air conditioned room.

Approximately 24 h before the commencement of treatment, and subsequently when required, the hair from a strip of skin about 1.5 cm wide along the dorsal midline of the mice from the nape of the neck to the tail was removed by electric clippers. The shaved area of the backs of 60 mice was painted with 0.3 ml aliquots of acetone containing either 300 µg B(a)P or 300 µg MC on Mondays and Thursdays for a period of 15 weeks. Thirty mice were similarly treated with 0.3 ml aP/acetone (1/1) and 60 mice were used as untreated or acetone treated controls. One animal from each treatment was killed 3 h after each application and skin from the treated area was removed for electron microscopy. Thirty additional mice were similarly treated with 300 µg MC for 20 weeks, after which time carcinomata were removed for electron microscopy.

Pieces measuring 1 mm² were fixed in 3% gluteraldehyde in 0.2 mol/l phosphate buffer at 4°C, rinsed in buffer and post fixed in 1.33% osmium tetroxide. They were subsequently placed in 2% uranyl acetate, dehydrated in alcohols and embedded in TAAB C resin (TAAB Laboratories, Reading). Sections were cut with a Reichert OMU-3 ultramicrotome, collected on copper grids and stained with lead citrate and uranyl acetate. They were examined on a Philips 301 electron microscope at an accelerating voltage of 80 kV.

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RESULTS
Examination of untreated and acetone treated skin revealed the presence of occasional intracisternal A particles in the epidermis. They were approximately 65 nm in diameter and the typical doughnut shape associated with A particles. The 2 concentric shells enclosed a comparatively electron lucent centre, the inner shell having a diameter of approximately 40 nm. Particles were observed within cisternae of both rough and smooth endoplasmic reticulum (ER). Characteristically the intracisternal particles form by budding at the ER (Fig. 1). Occasionally small electron-dense granules were present in the epidermal cell nucleoplasm (Fig. 2). These granules varied from ovoid to rectangular in cross section, being approximately 40 nm in length and 25 nm in width. They were predominantly confined to the periphery of the nucleus.

Polycyclic hydrocarbon treatment resulted in initial inflammation accompanied by hair loss. Sparse hair regeneration followed and papillomata began to appear during the 11th week of treatment. Twelve mice out of 30 had developed squamous carcinoma after 20 weeks' treatment with MC.

Examination of sequential stages in the carcinogenic process with the electron microscope indicated an increase in A particles. In hyperplastic epidermis the particles were individually scattered throughout the cytoplasm and ER (Fig. 3). Large numbers were observed in papillomata, as single individual particles or often in conspicuous groups (Fig. 4). There was no substantial difference in the distribution of A particles between B(a)P and MC induced papillomata. Eight of the 12 carcinomata examined contained a large proportion of cells which were packed with A particles (Fig. 5). A slight increase was observed from the second week of treatment with aP although in none of the animals examined was their incidence as great as in poly-

Fig. 1.—Type A virus particle budding at the ER. × 72,000.
FIG. 2.—Electron dense granules in nucleoplasm of an untreated epidermal cell. × 79,000.

FIG. 3.—Distribution of A particles in an epidermal cell in B(a)P induced hyperplasia. × 72,000.
cyclic induced papillomata and carcinomata. No intercellular particles were observed at any stage during carcinogenesis, even in carcinoma, where large spaces occur between individual cells. After polycyclic treatment there is an increase in the number of nuclei containing electron dense granules in their periphery. This increase appears in parallel with the build up in the number of A particles within the cells. Occasionally papilloma and carcinoma cell nuclei contain a large number of these granules (Fig. 6). There was no increase in these granules after αP treatment.

DISCUSSION

In the present investigation intracisternal A particles have been detected in normal epidermis of BALB/c mice. These particles increase in number throughout the early stages in polycyclic hydrocarbon induced carcinogenesis. Kakefuda et al. (1970) did not detect them in normal or hyperplastic epidermis of Leaden strain (C57L) mice after MC treatment. A number of attempts to demonstrate biological activity associated with A particles have been unsuccessful. However, comparisons of labelling kinetics (Okano et al., 1973) for type A and type C virions in cell culture supported the idea that type A virions are precursors for type C. In the present instance, however, no intercellular particles or intracellular C particles were detected. This supports the current view that in the formation of most type B and all type C particles no true intercellular type A particle is ever involved (Dalton, 1972). Biczysko et al. (1974), investigating the possible morphological sequences of the spontaneous production of an endogenous virus as represented by type A virus particles, described the involvement of round granular structures in the nucleoplasm. Occasionally, similar granules have been detected in the present instance in nuclei of both untreated and polycyclic treated epidermis but have not been observed between the inner
Fig. 5.—A particles in MC induced squamous carcinoma. $\times$ 72,000.
Fig. 6.—Dense nuclear granules in MC induced papilloma.  × 38,000.
leaflets of nuclear membrane. Whether
the granular material observed on ultra-
structural examination of the nucleus
represents a viral precursor and whether
the observed increase in granules is
related to the increase in the number
of A particles induced by polycyclic
hydrocarbon treatment are interesting
problems. A direct relationship between
the proliferation of A particles and
neoplastic transformation of BALB/c
mouse epidermis appears likely as short-
term non-carcinogenic irritant treatment
does not appreciably alter their incidence.

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