(figures 2 and 3). At level with the 3rd somite, we observed in notochord lateral face, 2 primordial germ cells, covered with a net which continued with perichordal material (figure 3). Distal to 4th somite, we did not observe primordial germ cells on notochord.

Clawson et al.[10] described the primordial germ cells at stage 8 close to the notochord. At stage 9, these cells can be observed in the mesenchyme surrounding the notochord and neural tube (notochord-neural tube complex)[11] (figure 1).

In chick embryo it has been described that 2 perichordal microfibrils types unite the notochord with the neural tube: the smaller fibrils are specially concentrated near the notochordal boundary membrane. These fibrils have a diameter of about 10 nm and are digestible with hyaluronidase and alpha amylase; they may be precursors of the larger type, which is about 20 nm in diameter and is digestible with collagenase[12-17]. The collagen and proteoglycan secretion by chick axial organs, has recently been made obvious[18-23]. The immunological role of proteoglycan in promoting cell recognition is now well established[24]. It seems possible, therefore, that, when the proteoglycan component of the notochordal comes into contact with the primordial germ cells, it directs their movement toward the notochord, thus promoting a chemostatic response, permitting primordial germ cells migration. According to our 1st observations, we can postulate that, as in amphibians, the notochord exhibits attraction on primordial germ cell. Our projected experiments will permit us to confirm such a postulate.

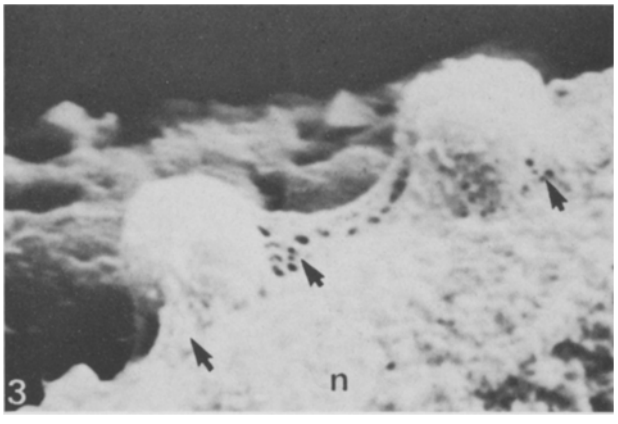


Fig. 3. Scanning electron micrograph of a chick embryo of 7 somites. The arrows show the perichordal substance surrounding 2 primordial germ cells. × 6000.

1 J.D. Gipoulux, C.r. Acad. Sci., Paris *257*, 1150 (1963).
2 J.D. Gipoulux, C.r. Acad. Sci., Paris *257*, 2719 (1963).
3 C. Hamburger and H.L. Hamilton, J. Morph. *88*, 49 (1951).
4 T.F. Anderson, Trans. N.Y. Acad. Sci. *13*, 130 (1951).
5 R.D. Lillie, Stain Technol. *26*, 163 (1951).
6 D.B. Meyer, Anat. Rec. *139*, 314 (1961).
7 D.B. Meyer, Dev. Biol. *10*, 154 (1964).
8 R.W. Mowry, J. Histochem. Cytochem. *4*, 407 (1956).
9 T. Barka and P.J. Anderson, in: Histochemistry: Theory, Practice and Bibliography, p.65. Harper and Row Publishers, New York 1965.
10 R.C. Clawson and L.V. Domm, Am. J. Anat. *125*, 87 (1969).
11 W.J. Schwartz and L.V. Domm, Am. J. Anat. *135*, 51 (1972).
12 F.N. Low, Anat. Rec. *160*, 93 (1968).
13 F.N. Low, Am. J. Anat. *128*, 45 (1970).
14 J.J. O'Connel and F.N. Low, Anat. Rec. *167*, 425 (1970).
15 E.C. Carlson and F.N. Low, Am. J. Anat. *130*, 331 (1971).
16 R.G. Frederickson and F.N. Low, Am. J. Anat. *130*, 347 (1971).
17 E.C. Carlson, Am. J. Anat. *136*, 77 (1973).
18 T.N. Kvist and C.V. Finnegan, J. exp. Zool. *175*, 221 (1970).
19 T.N. Kvist and C.V. Finnegan, J. exp. Zool. *175*, 241 (1970).
20 S. Bazin and G. Strudel, C.r. Acad. Sci, Paris *275*, 1167 (1972).
21 T.F. Linsenmayer, R.L. Trelstad and J. Gross, Biochem. biophys. Res. Commun. *53*, 39 (1973).
22 C.K. Lauscher and E.C. Carlson, Anat. Rec. *182*, 151 (1975).
23 M. Brancroft and R. Bellairs, J. Embryol. exp. Morph. *35*, 383 (1976).
24 S. Roth, E.J. McGuire and S. Roseman, J. Cell Biol. *51*, 536 (1971).

Acetic acid pretreatment of initiated epidermis inhibits tumour promotion by a phorbol ester

A.W. Murray[1,2]

School of Biological Sciences, Flinders University of South Australia, Bedford Park (South Australia 5042), 9 March 1978

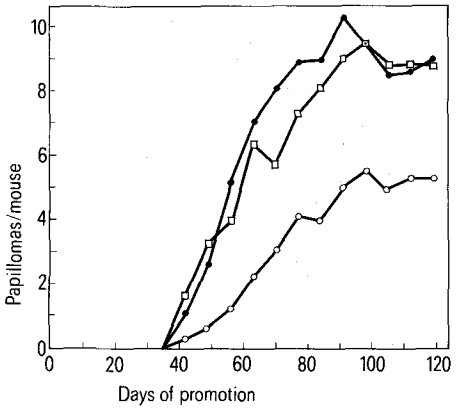
Summary. Mouse skin initiated with 7,12-dimethylbenz(a)anthracene and then exposed to multiple treatments of acetic acid, shows a decreased papilloma yield on subsequent promotion with croton oil.

The role of hyperplasia in promotion during 2-stage tumorigenesis[3] is not understood. It has been generally observed that although all promoters induce epidermal hyperplasia, not all hyperplastic agents are promoters[4,5]. For example, a recent paper has reported that acetic acid is only a weak promoting agent despite its ability to induce intensive epidermal hyperplasia[4]. This type of result has been assumed to imply that promoters must induce biochemical changes in epidermis which are unrelated to hyperplasia[4-6]. It was the purpose of the present study to determine whether cytotoxicity of compounds such as acetic acid could be an alternative explanation for their inability to act as tumour promoters.

Materials and methods. Female Swiss albino mice were used and were maintained as described before[7]. Each treatment group consisted of 25 animals. All animals were initiated with 25 µg 7,12-dimethylbenz(a)anthracene (DMBA). 1 week after initiation, 1 group was treated with 167 µmoles of acetic acid, another with 500 µmoles of acetic acid and a 3rd control group with acetone (all treatments were applied to the dorsal skin as solutions in 0.2 ml acetone). The treatments were repeated 4 times at a rate of 2 applications per week. 4 days after the final acetic acid treatment, all groups were promoted with twice-weekly applications of croton oil (0.2 ml of a 0.5% solution in acetone). The mice were examined twice-weekly for papilloma formation.

Results and discussion. As shown in the figure, pretreatment of initiated animals' skin with 5 applications of 500 µmoles acetic acid resulted in a tumour yield during subsequent promotion only about 50% of that in the control group. This decrease is unlikely to be a consequence of interference with DMBA-initiation as acetic acid treatment was not begun until 1 week after DMBA application. Consequently,

the simplest explanation is that acetic acid treatment results in the death of a proportion of the initiated cells. Previous studies have shown that a single application of 400[8] or 500[4] µmoles of acetic acid to mouse skin causes a marked initial inhibition of thymidine incorporation into DNA. This result suggests that acetic acid is cytotoxic at these doses.



Development of papillomas in mice initiated with DMBA and promoted with croton oil. 1 week after initiation with 25 µg DMBA, groups were treated 5 times at a rate of 2 applications per week with either acetone (●), 167 µmoles of acetic acid (□) or with 500 µmoles of acetic acid (○). 4 days after the final acetic acid or acetone treatment, all groups were promoted with croton oil (see materials and methods).

Application of a higher dose of acetic acid to mouse skin (1000 µmoles) causes superficial ulceration[4].

In a similar way, cell death could explain the observation that acetic acid inhibited 12-0-tetradecanoyl phorbol-13-acetate promotion in mouse epidermis[4].

The results emphasise the dangers inherent in interpreting experiments showing the inability of substances like acetic acid to act as efficient promoters. Such experiments leave open the possibility that these hyperplastiogenic substances induce all of the biochemical changes in skin necessary for promotion, but do not assay as promoters because of cytotoxicity. Consequently the observation that not all hyperplastic agents act as tumour promoters[4,5] does not, by itself, eliminate the possibility that epidermal hyperplasia is a sufficient condition for promotion.

1 Acknowledgment. The author thanks Mr M. Froscio for excellent technical assistance.
2 This investigation was supported by the Australian Research Grants Committee, the Australian Tobacco Research Foundation and the University of Adelaide Anti-Cancer Foundation.
3 R.K. Boutwell, Prog. exp. Tumor Res. *4*, 207 (1964).
4 T.J. Slaga, G.T. Bowden and R.K. Boutwell, J. nat. Cancer Inst. *55*, 983 (1975).
5 T.J. Slaga, J.D. Scribner, S. Thompson and A. Vaije, J. nat. Cancer Inst. *57*, 1145 (1976).
6 T.G. O'Brien, Cancer Res. *36*, 2644 (1976).
7 A.W. Murray and M. Froscio, Cancer Res. *37*, 1360 (1977).
8 L. Krieg, I. Kühlmann and F. Marks, Cancer Res. *34*, 3135 (1974).

Effect of tannic acid on the Ehrlich ascites tumor cells

E.C. Chew[1]

Department of Anatomy, University of Hong Kong, Hong Kong (Hong Kong), 20 March 1978

Summary. Tannic acid was used to fix and stain Ehrlich ascites tumor cells in suspension. An increase in surface microvilli and cytoplasmic blebs in the tumor cells was observed. The mechanism of tannic acid induced surface morphological changes in tumor cells and the formation of a precipitate of protein-tannic acid-heavy metal complex are discussed.

Tannic acid (TA) was recently applied as an additional fixative[2-4] as well as a staining substitute[5-7] for biological specimens in electron microscopy. Simionescus and Simionescus[8] reviewed the action of TA-glutaraldehyde mixture on fresh tissues and stated that these preparations are usually affected by a) the unsatisfactory penetration of cells; b) the formation of interstitial precipitates, and c) the extraction or precipitation of some tissue constituents. The present communication reports the effect of TA on Ehrlich ascites (EA) tumor cells in suspension. The surface morphological changes of EA cells after TA treatment are presented and the possible mechanism of these alterations is discussed.

Material and methods. The Ehrlich ascites tumor cells in suspension were kindly provided by Dr Y.C. Kong of the Department of Biochemistry, the Chinese University of Hong Kong. The tumor is carried in this laboratory by s.c. and i.p. transplantations in WHT/HT (Swiss) mice.

In all the experiments performed, tumor cell pellets were made from peritoneal fluid containing tumor cells and were fixed in 2.5% glutaraldehyde with or without 4 or 8% tannic acid at 20 °C. Some specimens were post-fixed in 1% osmium tetroxide. Routine procedures were followed for dehydration and embedding in Epon 812. Sections were examined with or without uranyl acetate and lead citrate staining and studied with a Philips EM 300 at 60 kV. Thick sections of about 1 µm were cut and stained with paragon[9] for light microscopic observations.

Results. Under the light microscope, the tumor cell pellet showed that it contained mainly EA cells with only a few blood cells and a small amount of cell debris. The tumor cells appeared viable and mitotic figures were frequently seen (figures 1 and 2). In the control group, i.e. without the addition of tannic acid to the fixative, the tumor cells were round and the surface was smooth (figure 1). When tannic acid was added to the fixative, the cell membrane looked very thick and always contained numerous microvilli. Heavy metal deposits were found on the cell membrane as well as in the interstitial spaces (figure 2).

The fine structure of the EA tumor cells have been described by many investigators[10-15]. Again, under the electron microscope, tumor cells in the control group always possessed a smooth surface with only a few microvilli. The cell membrane was well defined. When tannic acid was added to the fixative, the cell membrane appeared to be very dense and irregular. An increase in microvilli and cytoplasmic blebs was an obvious feature. Heavy metal particles were found in the interstitial space and on the cell membrane (figures 3-5). When the specimen was fixed in TA-glutaraldehyde mixture, but without post-fixing in osmium tetroxide, the results were rather similar to that just described. However, the contrast was much less. The cellu-