matters arising

Two-stage transformation *in vitro*

THE evidence for malignant transformation of cells *in vitro* by the combined action of benzo(a)pyrene (initiator) and phorbol ester (promoter), presented by Lasne *et al.*[1] is very striking. I feel, however, that they fail to bring out the true nature of their results.

The benzo(a)pyrene treatment of the primary rat fibroblasts was at the third passage, yet the dramatic increase in the percentage of transformed colonies was delayed until the thirty-fourth passage, when the incidence became 20.5% (benzo(a)pyrene alone) and 33.0% (with phorbol). By this time the 'primary' fibroblast culture was becoming senescent. This was indicated by the rise in frequency of transformed colonies in the controls to 11.5% by the fortieth passage.

It seems that we are not observing an increase in the rate of transformation but rather the selection of already transformed clones in a mixed population of cells. The phorbol seems to increase the selective advantage of transformed cells by 50%. There is no sign so far of two-stage transformation.

Two-stage transformation becomes apparent only when 2-d-old rats were injected with 2×10^6 cells. At the fortieth passage, although 42.5% of the cells were transformed by *in vitro* standards, they produced no tumours on injection. At the same time, cells also exposed to phorbol produced tumours in every rat. There has been a malignant transformation of the phorbol-treated cells which only becomes apparent on injection into animals.

The 'two stages' of malignant transformation are therefore, first, the transformation in colony morphology as seen *in vitro*, which is induced by benzo(a)pyrene. After 30 passages these transformed cells have a selective advantage and grow at the expense of the untransformed cells. The selective advantage is slightly enhanced by phorbol. Second, the transformation of stage 1 cells which is cryptic *in vitro*, but apparent on injection into young rats by the production of tumours. This is induced, or perhaps merely accelerated, by phorbol.

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DR LASNE REPLIES—Thank Dr Bateman for the analysis of our results in our paper. I think however, that the problem is not so simple.

So far as we know, the increase in the percentage of transformed colonies (11.5% by the fortieth passage) in tissue culture, does not necessarily indicate senescence. On the contrary, it shows and confirms the beginning of an *in vitro* spontaneous cell transformation[1–3].

When Dr Bateman speaks about the senescent cell selection theory, he perhaps thinks of the Prehn's 'cloning selection theory'[4]. In this case, we refer him to reports[5–7] that in chemical carcinogenesis, no selection of pre-existing transformed cells has happened.

Concerning the action of phorbol ester, and in general the action of the other promoters, on the growth of pre-existing transformed cells, we wonder whether the author is aware of studies on carcinogensis *in vivo*. Our results are interpreted in correlation with the model established *in vivo*. In previously initiated animals, the promoter increases the tumour incidence, and shortens the latent period[8]. *In vitro*, the phorbol ester gives similar results increasing the number of transformed colonies, and accelerating the tumour development after inoculation of the cells in animals. But we have not discussed the general theory of cocarcinogenesis, which requires greater attention. Our paper merely raises this question.

Lastly, these results have been obtained and presented to the scientific public. Each investigator may present his own interpretation. We have proposed one.

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The Musgrave Block-Amadeus Basin Complex

DAVIDSON[1] suggested that a plate tectonic model, involving continental collision, could explain the features observed in the Musgrave Block-Amadeus Basin area of central Australia. Several significant features of the area are not accounted for in his model and these are, I believe, of sufficient importance to warrant modification of the model.

In Davidson's model the Giles Complex is considered to represent part of an ophiolite thrust belt, originating in layers 2 and 3 of the oceanic lithosphere. Bearing in mind the form of ophiolite sequences, however (see, for example, the discussions at the Penrose Field Conference[2]), there is no indication that rocks of the Giles Complex are in fact ophiolites, even if the absence of cherts and pillow lavas in the area is ignored. There is a marked absence of olivine rich rocks, a deficiency of ultramafic rocks in general, and all parts of the Complex show some features characteristic of stratiform intrusions, such as cumulate textures, cyclic units, rhythmic layering and associated 'sedimentary type' structures[3,4]. Repetition within the sequence of mafic–ultramafic units is largely a result of the igneous layering and cyclic units. Some intrusions in the central part of the Complex show features characteristic of crystallisation under high pressure (that is, near the base of the crust[4]) and such features are incompatible with an oceanic ophiolite type origin. Furthermore, there is evidence that the separate bodies of the Giles Complex show a depth stratification[4] which is inconsistent with Davidson's model.

Several structural aspects and relationships are also inconsistent with that model. The bodies of the Giles Complex were intruded into granulite facies rocks—chilled margins occur in places, and unfaulted boundaries are transgressive to the earliest recognisable folds in the granulites[4,5]. Relatively minor, local low-angle faults have affected some intrusions such as Gosse Pile[6] and Kalka[4]. The faulting occurred under conditions of high pressure and temperature, equivalent to granulite facies[7], and should not be confused with the much larger scale mylonitic faulting such as occurred in Woodroffe, Hinckley and Davenport. The low-angle fault-