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## Cellular Division and Cellular Volume Distribution in the Presence of 2-Phenyl-ethanol and some of its Derivatives

AGENTS that inhibit DNA synthesis in cells of higher animals are comparatively rare. Following on our earlier work[1], we have tested mouse lymphoma cells in order to determine the rate of division and change in volume brought about by the presence of various concentrations of 2-phenyl-ethanol (PEA) and some of its derivatives.

Mouse lymphoma cells *L* 5178 *Y* were used, and were grown in a 10–20 per cent horse serum medium. At the beginning of an experiment, 30,000 cells/ml. were inoculated into fresh medium. Five millilitres in 25 ml. roller tubes were brought to the required concentration of the compound to be tested and maintained at 36·5° C and 1 r.p.m. until the controls had reached about 300,000 cells/ml.—this usually took 2 days. Ten tubes were run in parallel at each step of the investigation. At the end of the experiment the number of cells in each tube was determined by means of a Coulter counter model *B* with size distribution plotter. The values were averaged and the standard deviations calculated. Parts of the volume of each tube were pooled for each set of ten and their volume distribution was plotted. The mean value was determined in each set.

Each compound to be tested was set up in eight concentration steps from 0 to 0·1 per cent. The curves relating concentrations to the number of cells/ml. at the end of the experiment were plotted and the 50 per cent inhibition estimated.

The concentrations of PEA and some of its derivatives that reduce cell concentrations to half the control values are given in column *c* of Table 1. The activity of PEA in this respect is about the same as was shown earlier by *Escherichia coli C* 600 when tests were carried out for viable bacteria or enzyme induction[2]. From this we suggest that similar inhibitory mechanisms prevail. The necessary concentration for 50 per cent inhibition in this experiment is one-tenth of that which reduces DNA-polymerase activity to 36 per cent (ref. 1). We did not find significant changes of the cellular volume at any of the concentration levels studied. Such a situation would be expected with unbalanced growth.

Table 1. INHIBITION OF CELLULAR DIVISION IN MOUSE LYMPHOMA CELLS IN CULTURE

| *a* | *b* | *c* | *d* | *e* |
|---|---|---|---|---|
| 1 | 2-phenyl-ethanol | 0·04 | 50·0 ± 8·8 | 50 |
| 2 | β-phenyl-ethylamine | 0·004 | 50·0 ± 5·9 | 20 |
| 3 | 2-(*m*-chlorophenyl)-ethanol | 0·015 | 50·0 ± 4·3 | 10 |
| 4 | 2-naphthyl-ethanol | 0·0035 | 50·0 ± 9·5 | 10 |
| 5 | *p*-fluoro-cinnamic alcohol | 0·008 | 50·0 ± 5·6 | 10 |
| – | control | – | 100·0 ± 4·5 | 90 |

Column *a*, current number of compond tested; *b*, name of compound; *c*, concentration of compound that brings about 50 per cent inhibition; *d*, average inhibition with standard deviation in per cent; *e*, number of portions tested.

In PEA where the hydroxyl group has been replaced by the amino group the inhibitory activity is increased by a factor of ten (Table 1, No. 2). This increased inhibition in cell culture cannot be caused by an increased inhibition of the polymerase system[3]. Binding a halogen to the PEA ring (No. 3) increases its activity in cell culture by a factor of about three, whereas the polymerase showed the strongest response of all the compounds tested. The replacement of the phenyl ring in PEA by the naphthyl ring (No. 4) causes its inhibitory powers in cell culture to increase by a factor of ten. In *p*-fluoro-cinnamic alcohol (No. 5) the side chain as well as the ring portion has been altered, and this has resulted in a five-fold increase in inhibition.

Comparing the changes of activity for cell culture and for polymerase system with the alterations in the PEA molecule it seems likely that the polymerase system of the cell is not exclusively affected. The different derivatives may attack the functional parts of the cells in a very different manner. The experiments will be continued.

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## Induction of Thymine Dimers in Synchronized Populations of Chinese Hamster Cells

RECENT evidence indicates that the response of Chinese hamster cells *in vitro* to ultra-violet radiation changes during the difference phases of the cell cycle[1,2,4]. Humphrey *et al.*[1] have shown that cells synthesizing deoxyribonucleic acid (DNA) at the time of irradiation using ultra-violet rays (*S* phase cells) sustain more chromosome damage than cells which were in the period before DNA synthesis ($G_1$) or cells in the period after DNA synthesis ($G_2$). Sinclair and Morton have demonstrated that the response to ultra-violet radiation in synchronous populations of cells as measured by cell death also fluctuates with the age of the irradiated cells; the fraction of surviving cells falls as the cells move from $G_1$ into *S* phase, and rises as they move from *S* into $G_2$ phase[2]. In an effort to explain these changes in radiation response during the cell cycle, the production of an ultra-violet photoproduct of DNA, the thymine dimer[3] has been measured in synchronized population of Chinese hamster cells[4].

*B*-14 Chinese hamster cells with a generation time of 12 h were subcultured into 150 mm² Petri dishes ($10^7$ cells/Petri dish) containing McCoy's 5*a* medium supplemented with 10 per cent calf serum[5]. Cells were grown for 20 h at 37° C in a medium containing tritiated thymidine (Schwarz BioResearch, Inc., 1 μc./ml., 13 c./mmoles) to obtain labelled cells for the determination of thymine dimers. Labelled cells were incubated for 1·5 h in a medium containing 0·06 γ/ml. 'Colcemid' in order to accumulate the cells in metaphase[6]. 'Colcemid' was omitted in one series of experiments. Mitotic cells were detached from the glass Petri dishes[7] by shaking the cultures on a wrist action shaker and collected by pooling the media[2]. Cells collected