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# THE TOXICITY OF SPERMINE AND SPERMIDINE TO CELLS IN CULTURE

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**Abstract**—Spermine tetrahydrochloride and spermidine trihydrochloride were examined for toxic effect on primary human embryo lung fibroblasts and on H.Ep.2 cells. Both substances inhibited the primary cells more than the H.Ep.2 cells and in each case a higher concentration of spermidine than of spermine was required to produce the same effect on cell morphology and replication. Neither substance influenced glucose uptake from the medium by either cell type during the first 24 hr of contact. Comparison of the toxicities towards some bacteria and towards these cultures of human cells leads to the conclusion that neither spermine nor spermidine is likely to be suitable for antibacterial chemotherapy.

THIS work was carried out as part of a programme to investigate the toxicity of antibacterial substances of animal origin towards mammalian cells. The aliphatic amine, spermine, $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$, occurs in various tissues but is particularly abundant in the posterior lobe of the prostate gland and in prostatic secretion.[1–8] Spermidine, $NH_2(CH_2)_3NH(CH_2)_4NH_2$, is also found in several tissues,[2,9,10] but differences in the detailed distribution of the two polyamines in the brain have been noted.[3] Spermine is known to inhibit some bacterial cultures, especially strains of *Staphylococcus aureus*, at concentrations as low as 10 $\mu$g/ml,[7,8] and spermidine at concentrations as low as 70 $\mu$g/ml.[8]

Toxicity studies of spermine using cultured mammalian cells have used, with one exception, a culture medium including calf serum. It has been shown that the enzyme spermine oxidase, present in ruminant serum converted spermine to the more toxic acrolein,[11] and thus gave an exaggerated figure for the toxic levels of spermine. Similar work on spermidine has also used bovine serum.[12] Goldstein,[13] without giving details of methods, mentioned that the $ID_{100}$* of spermine was 15 $\mu$g/ml for Walker 256 carcinosarcoma cells in a medium containing horse serum, i.e. without spermine oxidase.

It seemed therefore desirable that the toxicity of these substances should be further examined and established, using other cell types and other indices of toxicity, with a view to assessing their therapeutic potential.

## MATERIALS AND METHODS

### *Materials used*

Spermine tetrahydrochloride and spermidine trihydrochloride were obtained from

* Presumably the dose completely inhibiting the culture's growth as measured by total protein estimations.

Sigma London Chemical Co. Ltd., London. H.Ep.2 cells were obtained from Burroughs Wellcome & Co., London, and grown in 90% Medium 199 with 10% horse serum (both obtained from Flow Laboratories, Ltd., Irvine, Ayrshire) + 10 i.u./ml penicillin and 25 μg/ml streptomycin. The primary human embryo lung fibroblasts (here termed H.E.L. cells) were obtained from Flow Laboratories, as was the medium in which they were grown—90% Eagle's Basal Medium with 10% horse serum. Glucose oxidase and peroxidase were obtained from British Drug Houses, Ltd., Poole, Dorset, and all other reagents were of analytical quality. The flasks in which the cells were grown were Falcon Tissue Culture Flasks obtained from Gateway International, Los Angeles, California.

*Methods*

*Antibacterial studies.* Loopfuls of overnight cultures of *Staphylococcus aureus* (N.C.T.C. 6571) were inoculated into bottles of either Medium 199 or Eagle's Basal Medium, with horse serum, both being without antibiotics, but containing spermine or spermidine. This confirmed the minimum antibacterial concentrations of the two substances, which in these media were 50 μg/ml for spermine and 400 μg/ml for spermidine.

*Morphological studies.* Cells from stock cultures were trypsinised and suspended in media as above, the volume being adjusted to give about $10^5$ cells per ml. Aliquots of 3 ml were pipetted into Falcon flasks and the cells allowed to settle during overnight incubation at 37°. The media were then replaced with media containing spermine or spermidine, and controls without these substances set up simultaneously. The cells were observed by phase-contrast illumination, using a Zeiss inverted microscope. On the fifth day the condition of the cells was graded according to the recommendations of Toplin.[14] Time-lapse cinemicrographic observations were also made, using the apparatus previously described.[15–17] The resulting film has been shown elsewhere.[18]

*Cell replication studies.* The cells were suspended as before and 1 ml aliquots were inoculated into 120-ml medicine bottles which had been previously charged with 19 ml culture medium containing spermine or spermidine. The bottles were incubated flat to permit monolayer growth, and the cell numbers were estimated after 1, 3 and 5 days of culture. The cell counts were performed by the method described by Paul,[19] using a Coulter Counter model B and a 100-μ orifice (Coulter Electronics, Inc., Hialeah, Florida).

*Glucose uptake measurement*

Cells were trypsinised from the stock bottles and 1-ml aliquots containing approximately $10^6$ cells in suspension were added to 60-ml medicine bottles containing 10 ml of media. The bottles were incubated at 37° for 2 days to allow a flourishing culture to establish itself. The media were then decanted and 5 ml fresh media containing spermine or spermidine were added. The bottles were again incubated and 0·1-ml samples of media removed for analysis after 4, 8·5 and 24 hr. The glucose content of the samples was determined by the method of Huggett and Nixon[20] after deproteinisation in 1 ml of 2% perchloric acid.

## RESULTS

*Morphological studies.* Neither substance produced visible signs of toxicity until several days had passed. The cells appeared to lose much of their intracellular

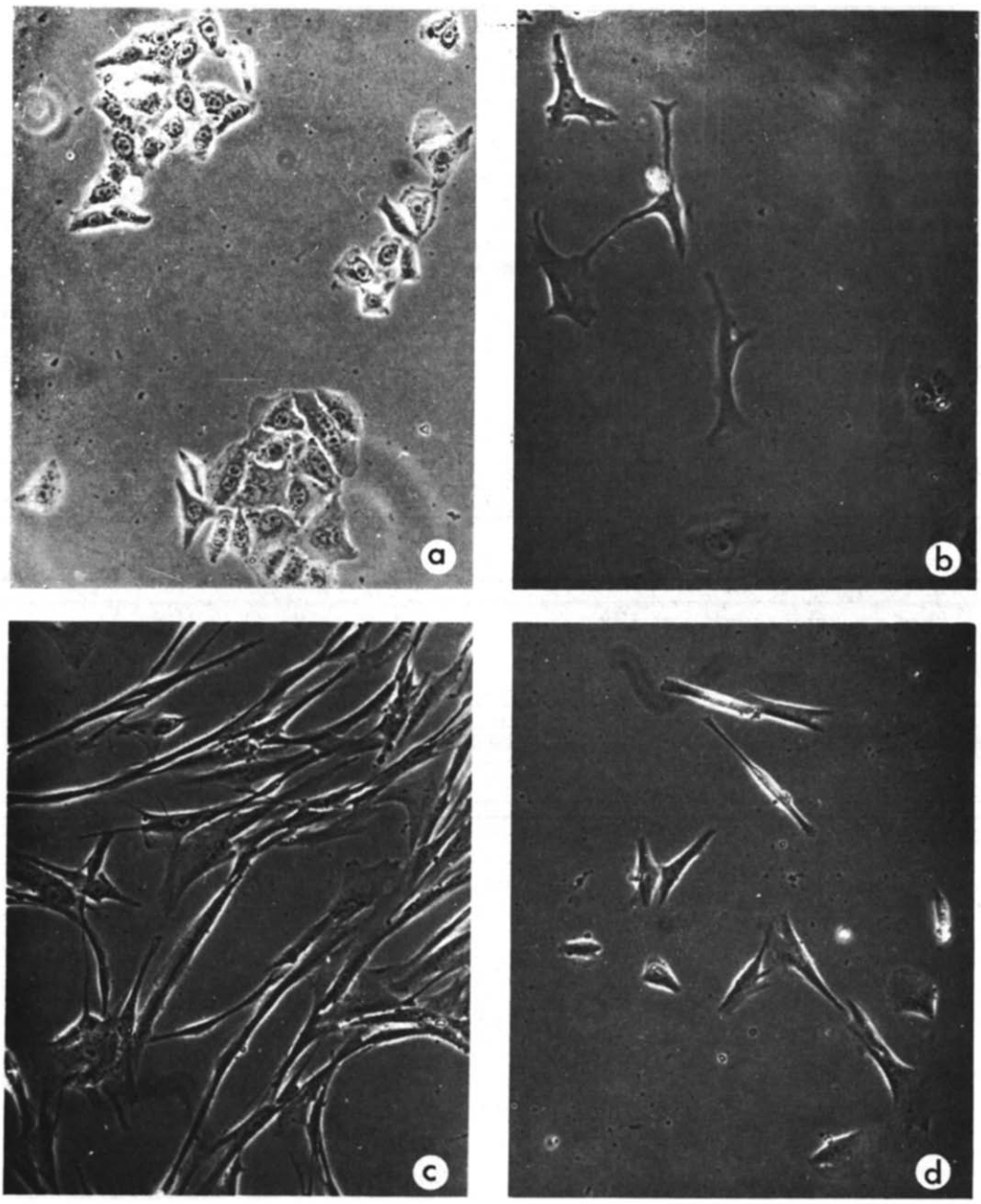


FIG. 1. The photographs show the typical appearance of both cell types in the presence of toxic levels of spermine. The effect of spermidine is similar.
(a) H.Ep.2 cells after 5 days in Medium 199
(b) H.Ep.2 cells after 5 days of contact with 100 $\mu$g/ml of spermine
(c) H.E.L. cells after 5 days in Eagle's Medium
(d) H.E.L. cells after 5 days of contact with 40 $\mu$g/ml of spermine.

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organisation, and became much diminished in size. Their rate of atrophy was dependent on the dose of the substances. Vacuoles, although present in a few cases, were not a predominant feature of the toxic symptoms. The typical appearance of such cells under the influence of spermine is shown in the plate. The results with spermidine are similar, apart from being produced by higher concentrations of the latter. The Toplin classification of the cells (Table 1) shows that the H.E.L. cells are more sensitive than are the H.Ep.2 cells to both spermine and spermidine.

TABLE 1. CYTOTOXIC AND LETHAL END-POINTS (μg/ml) OF SPERMINE AND SPERMIDINE FOR H.Ep.2 AND H.E.L. CELLS

| Cell type | Spermine | | Spermidine | |
|---|---|---|---|---|
| | Cytotoxic end-point | Lethal end-point | Cytotoxic end-point | Lethal end-point |
| H.Ep.2 cells | 100 | 170 | 300 | 400 |
| H.E.L. cells | 30 | 40 | 100 | 200 |

*Cell replication studies.* A significant effect was not seen with either substance on either cell type, at the dose levels used, until after 5 days. With both substances inhibition of cell replication increased with increasing concentrations. However, with spermidine the transition from slight to complete inhibition occurred over a smaller range of doses with both cell types. For both substances, the H.E.L. cells were found to be more sensitive than the H.Ep.2 cells. The growth curves are shown in Fig. 2. From these counts the $ID_{50}$ values were calculated—i.e. the concentrations inhibiting by 50 per cent the cell number increase. These $ID_{50}$ values are shown in Table 2.

TABLE 2. $ID_{50}$ VALUES FOR SPERMINE AND SPERMIDINE AFTER 5 DAYS OF CULTURE (μg/ml)

| Cell type | Spermine | Spermidine |
|---|---|---|
| H.Ep.2 cells | 35 | 60 |
| H.E.L. cells | 25 | 40 |

The values were obtained from the weighted log dose–inhibition curves drawn from six separate results at each concentration of drug.

*Glucose uptake measurement.* During the first 24 hr of culture, the requirement of the cultures for glucose is apparently unaffected by the presence of either substance in the media in the dose levels used. In control experiments the amount of glucose removed after 24 hr of contact was 0·15 mg per ml of medium. This figure is a mean of eight experiments with a coefficient of variation of 9 per cent. The experiments where spermine or spermidine were incorporated in the media gave figures for glucose removal not significantly different from 0·15 mg.

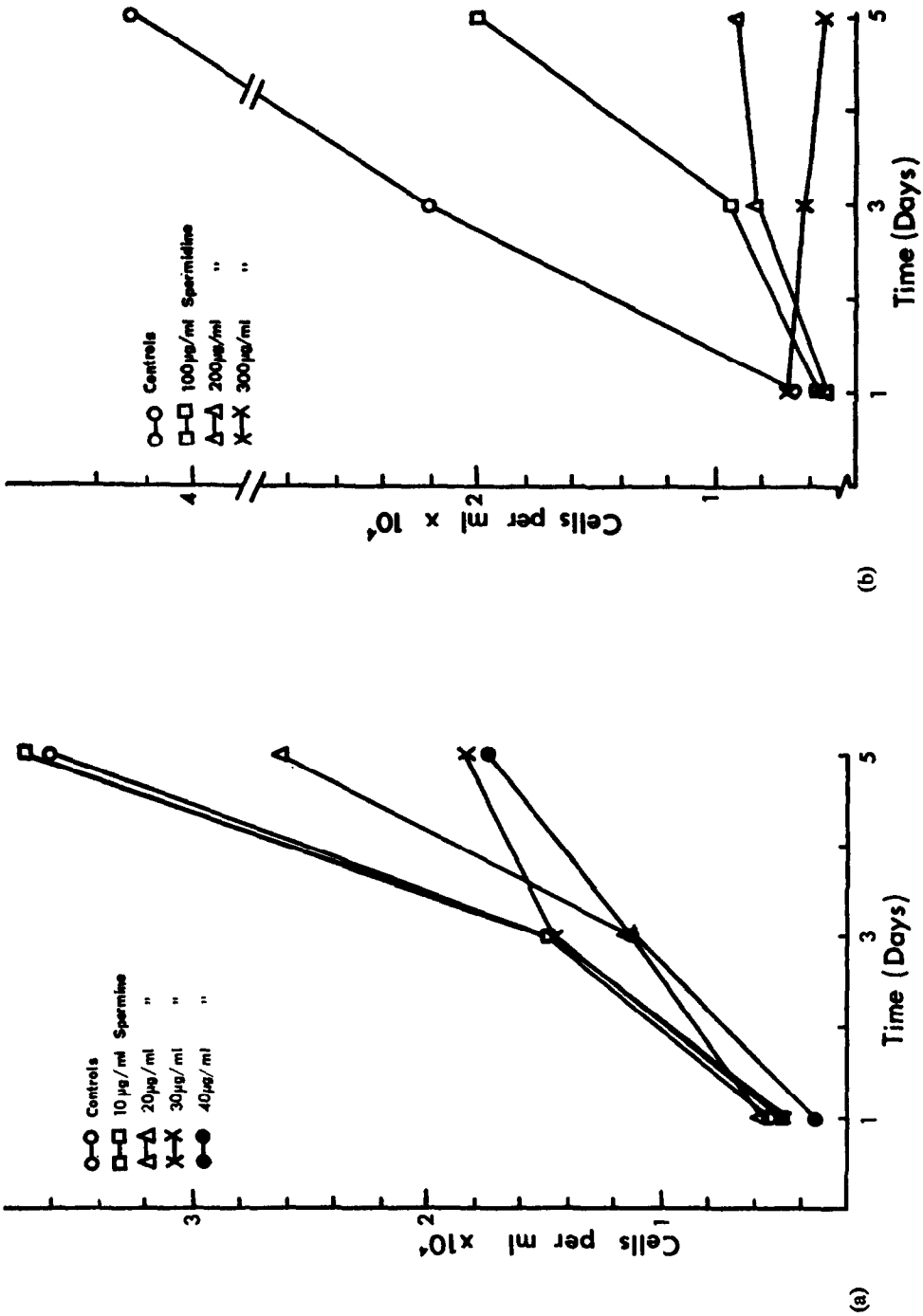
Cells per ml x 10⁴
Time (Days)
Controls
10 µg/ml Spermine
20µg/ml "
30µg/ml "
40µg/ml "
(a)
Cells per ml x 10⁴
Time (Days)
Controls
100µg/ml Spermidine
200µg/ml "
300µg/ml "
(b)

FIG. 2

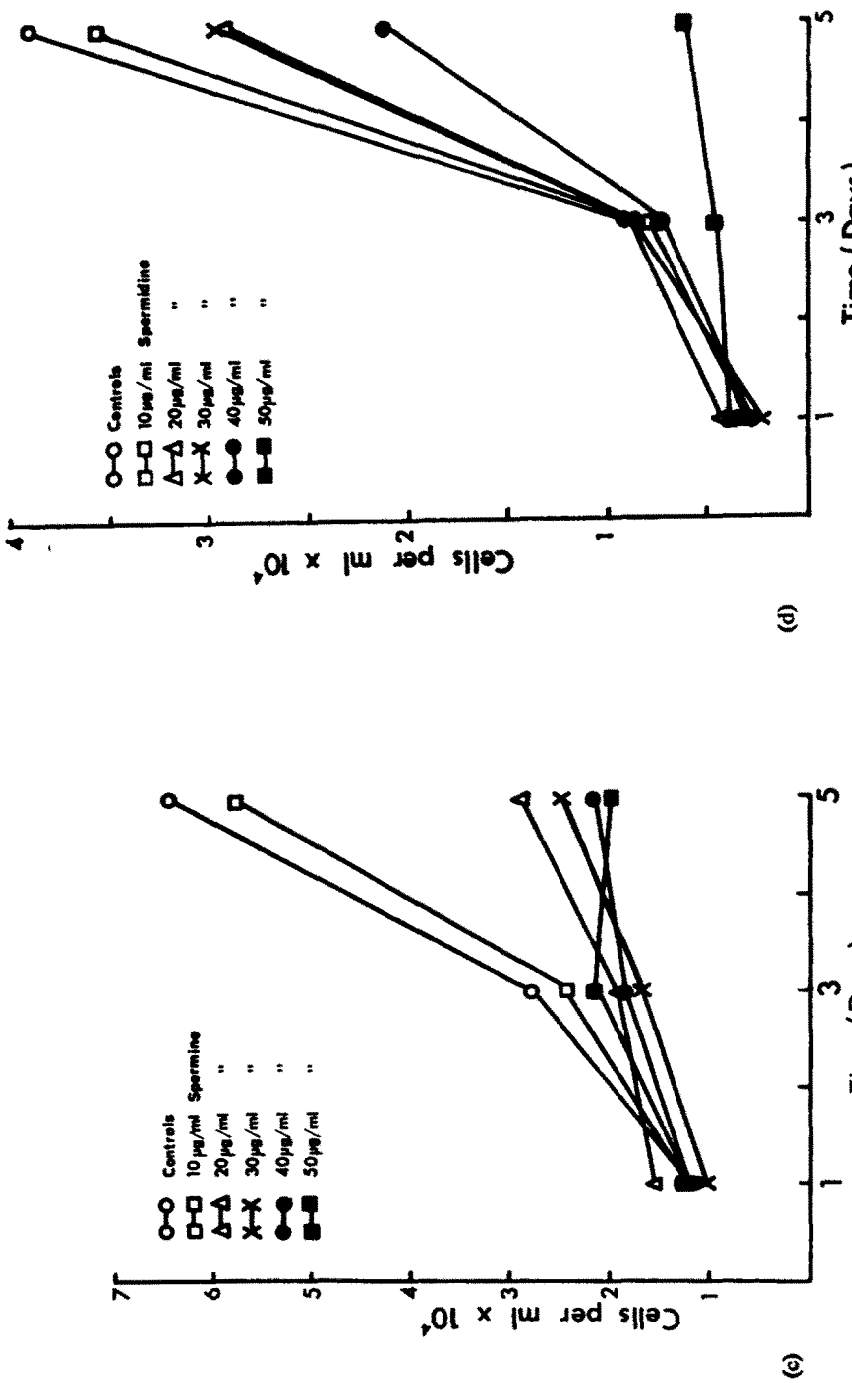


FIG. 2. Typical increase in cell numbers with time. Each point is the mean cell number obtained from three bottles.
(a) H.Ep.2 cells with spermine (b) H.Ep.2 cells with spermidine
(c) H.E.L. cells with spermine (d) H.E.L. cells with spermidine.

## DISCUSSION

The onset of signs of toxicity of spermine and spermidine to human cells requires the passage of some time. The evidence of the morphology studies, both static and cinemicrographic, indicates a slow wasting of the cell substance and interference with normal mitosis rather than the more drastic direct disruption of cell structure exhibited by some other compounds, e.g. nitrogen mustards. The inference here is that toxicity consists of an interference with a metabolic process. There is plentiful evidence that spermine and spermidine by their affinity for nucleic acids interfere with nucleic acid function,[21-29] and Goldstein[13] has shown that spermine inhibits protein synthesis in cultured cells. The appearance of the intoxicated cells in the present work does not contradict this opinion.

The greater resistance of H.Ep.2 cells to both substances is worthy of note. These cells have also been noted to be more resistant to some antibiotics[30] while fibroblast sensitivity to steroids is well documented.[31-40] In contrast, Kharisanova,[41] found no difference in sensitivity to many substances between these cell types. There is considerable uncertainty about the changes which occur in cultivated cells when they become an established cell line,* and about the validity of extrapolating results obtained from such cells to the intact animal or even cells freshly derived from it. The bulk of the work on *in vitro* cellular toxicity and pharmacology has involved the use of established cell lines.[42] These have been found convenient in practice because they are readily available in relatively large amounts, giving a prospect of reduced variation among replicate experiments. However, there is now an accumulation of somewhat contradictory reports on the long-term stability of such lines[44-60] with reference to antigenicity, tumorigenicity and chromosome picture. Such changes associated with adaptation to life *in vitro*, supported by the evidence of different sensitivity observed in the present work, would suggest that the use of an established cell line in routine drug toxicity testing was inadvisable in the present unsatisfactory state of knowledge.

## CONCLUSION

The studies indicate that both spermine and spermidine have a therapeutic index so low as to render them unsuitable for use as chemotherapeutic agents in bacterial infections.

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* As defined by the Committee on Terminology, American Tissue Culture Association.[43]

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