

Over the last 50 years, researchers have frequently thought they had evidence relating EEG patterns to mental activities, only to find that their conclusions were unwarranted because of inadequate controls or insufficiently sensitive analysis (17). Our negative results pin down the inadequacies of the current research paradigm that attempts to correlate abstract psychological constructs with simple measures of the mass electrical activity of the nervous system.

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## Differential Killing of Normal and Cystic Fibrosis

### Fibroblasts by Dexamethasone

Kurz *et al.* (1) claimed that neither dexamethasone nor dibutyryl adenosine 3',5'-monophosphate (cyclic AMP) kills normal human diploid fibroblasts. This contradicts our previous finding that the two drugs reduce the plating efficiency of normal human diploid fibroblasts but are less toxic to fibroblasts from individuals with cystic fibrosis (CF) (2-6). Although Kurz *et al.* claimed to have reproduced our methods, there were serious differences which render their results invalid.

In our studies, we distinguished between normal and CF fibroblasts through the use of a cell survival assay. This assay requires that each cell, in order to be scored as a survivor, express its reproductive capacity by doubling at least six times after exposure to cytotoxic agents. The correct use of this method requires that stringent standards be adhered to in order to ensure that cells scored as survivors (in the form of colonies) are indeed capable of prolonged proliferation after exposure to a cytotoxic agent. In our earlier reports, we had stated that cells must form colonies of at least 50 cells (2), but that figure was later increased to 65 to 75 cells (6). To ensure that only true colonies were counted, cells in all our dishes are counted visually and then scanned under the microscope. Colonies found not to contain the minimum number of cells are excluded from the count.

When appropriate minimum standards

are not adhered to in scoring colonies, invalid results are obtained since many cells are able to perform four or five divisions after exposure to a cytotoxic agent before reproduction ceases. When colonies of less than a certain number of cells are counted as survivors, the result is that the actual toxicity of a particular agent is underestimated. (Control dishes have few or no abortive colonies, whereas dishes exposed to cytotoxic agents, especially at high doses, have many abortive colonies. Therefore, when survival is corrected for plating efficiency, the result is falsely elevated.) Kurz *et al.*'s claim that 78 to 134 percent of normal fibroblasts survived in  $10^{-5}M$  dexamethasone phosphate was based on counts of colonies that contained "about 30 or more cells." This would require only five cell doublings and thus would include a fair number of abortive colonies. We have seen many cells divide up to five times after drug exposure and then cease dividing as determined by daily microscopic observation. To illustrate the consequences of including abortive colonies in the analysis, we are providing data from an experiment with human cells that were exposed to ethylmethane sulfonate (EMS), a potent cytotoxic mutagen (Table 1). The effect of including small colonies is to make EMS seem less toxic than it actually is.

Another issue raised by Kurz *et al.* is the effect of cell number on the plating

Table 1. Cell survival after exposure to ethylmethane sulfonate.

Drug concentration	Cells plated per 100-mm dish	Colonies with $\geq 75$ cells*	Survival† (%)	Colonies with $\geq 30$ cells*	Survival† (%)
None	1500	54	100	57	100
50 $\mu g/ml$	2000	42	59	67	88
100 $\mu g/ml$	3000	46	43	97	85

\*Numbers are averages. †Corrected for plating efficiency.

efficiency of human fibroblasts. In some experiments, we varied the number of cells plated per dish; Kurz *et al.* suggested that our results might therefore be an artifact of cell density ("cloning efficiency decreases at higher cell densities") rather than a consequence of the toxicity of dexamethasone. They reported that, with their methods, increasing the number of cells plated per dish from 100 to 1000 resulted in a 50 percent decrease in plating efficiency in two fibroblast strains. In our experiments, 1500 to 8000 cells are typically plated per 100-mm dish (in the absence of the drug, the range is 500 to 2000 cells). Table 2 shows that there is no decrease in plating efficiency with increasing numbers of cells plated. We had determined long before starting our CF experiments that, in the ranges given above, the number of cells plated per dish has no significant effect on the plating efficiency. Furthermore, the results in tables 1 and 2 of Kurz *et al.* were based on plating a maximum of 300 cells per 100-mm dish. We have never worked with such a low number of cells per dish because too few colonies are obtained for statistical reliability. In addition, we have performed experiments in which killing by dexamethasone was observed when nearly equal numbers of cells were exposed to various dexamethasone concentrations (Table 3). These data reveal that killing by dexamethasone is not an artifact of the number of cells plated.

Although Kurz *et al.* apparently examined many relatively minor parameters in detail, they failed to establish proper colony criteria, one of the most important aspects of the survival assay method. In addition, they failed to work within the range of cell numbers specified in our reports. We believe that these factors contributed significantly to their results and invalidate their claim of having replicated our experiments. Also, inasmuch as their results were obtained with normal human fibroblasts only and not CF fibroblasts, we are concerned that their interpretations of the results were too broad.

Finally, they stated that they "know of no other evidence showing that human fibroblasts are killed by glucocorticoids." However, Weichselbaum *et al.* (7), who are experienced in the use of the survival assay method, recently observed killing of normal human diploid fibroblasts with glucocorticoids. Gibbs (8), using our method, not only found that dexamethasone kills normal human diploid fibroblasts, he found that it kills fibroblasts from obligate CF hetero-

Table 2. Plating efficiencies with different numbers of cells plated.

Cells per 100-mm dish	Colonies counted per replicate dish*	Plating efficiency (%)
500	43, 41, 42	8.4
1000	76, 73, 81, 80	7.8
1500	126, 123, 106, 129	8.1
2000	159, 163, 172	8.3

\*Each colony had  $\geq 75$  cells.

Table 3. Survival of skin fibroblasts subjected to various concentrations of dexamethasone after being plated in nearly equal numbers.

Drug concentration (M)	Cells plated per 100-mm dish	Colonies counted per replicate dish*	Survival (%)†
0	1500	208, 200, 185, 216, 197, 207	100
$10^{-10}$	1500	117, 108, 134	59
$10^{-9}$	1500	97, 78	43
$10^{-8}$	1500	63, 88, 71	37
$10^{-6}$	2000	36, 29, 47	14

\*Each colony had  $\geq 75$  cells. †Corrected for plating efficiency.

zygotes at a lower rate. The fact that other laboratories have confirmed our results suggests that the technique is valid when properly utilized.

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## Clomid Administration in Rats

We have reviewed the slides selected by McCormack and Clark (1) as most representative of the histologic changes that they observed after administering Clomid to pregnant rats.

Independent review of the slides by

pathologists with knowledge of neoplasia in the female genital tract revealed squamous metaplasia and inflammation but no evidence of cellular atypia or neoplasia. Furthermore, there were no histologic changes suggestive of those observed in offspring born to women exposed to diethylstilbestrol during pregnancy.

We are expressing our opinion because of the implications of this animal study for humans who require Clomid for ovulation induction.

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We did not describe our findings as evidence for neoplasia; however, the teratology that we observed cannot be denied. Uteri that contain 90 to 100 percent metaplastic epithelium are not normal. Likewise, gross abnormalities, such as large uterine masses that contain necrotic remains of pups, are not normal.

We have studied hundreds of slides from both normal and abnormal tissues, and are familiar with the histology of the reproductive tract of the rodent. The abnormalities that we reported are similar to, if not identical to, those caused by neonatal or fetal exposure to estrogens (1). Similar findings have been observed as a result of neonatal treatment with Tamoxifen, a drug similar to Clomid that is used in the treatment of breast cancer (2). In our opinion and in the opinion of Chamness *et al.* (2) these changes are atypical and abnormal. Whether they occur in humans is unknown; however, the clinical implications remain clear.

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## Differential killing of normal and cystic fibrosis fibroblasts by dexamethasone

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