# COLCHICINE BINDING ACTIVITY OF MAMMALIAN CELL CULTURES GROWN IN ATTACHED AND SUSPENSION CULTURE

J. R. WARR and Clare DOLAN
*Department of Biology, University of York, Heslington, York Y01 5DD, England*

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## 1. Introduction

Ostlund and Pastan [1] observed that mouse L cells grown attached to the substratum contained >5-times as much tubulin (as measured by colchicine binding activity) as cells grown in suspension culture. They found that the increase did not simply reflect differences arising from growth in suspension medium, since cells grown in attached culture in normal or suspension medium had similar levels of colchicine binding. It was suggested [1] that the large accumulation of tubulin on attachment to the substratum may be consistent with their observation that virus-transformed cells had less tubulin per unit protein than untransformed cells, since it has been suggested that neoplastic cells may grow invasively because of deficient attachment to the substratum.

On the basis of the data in [1], it appeared that the switch from suspension to attached culture may provide a valuable model for the regulation of tubulin synthesis in mammalian cells. We initially chose to investigate the phenomenon in Chinese hamster ovary (CHO) cells since this cell type has been very widely used for genetical and related cell biological studies. Our immediate aim was to characterise the kinetics of changes of tubulin levels on attachment to the substratum. However, we do not observe the large change reported [1], either in CHO or in L cells and we suggest here that this large change is not a general phenomenon in mammalian cells.

## 2. Materials and methods

### 2.1. *Cells and media*

Chinese hamster ovary cells (CHO-K1) were kindly provided by Dr R. Wilson of the Genetics Department, University of Glasgow. Mouse L cells (NCTC clone 929) were purchased from Gibco Bio-cult Ltd. and were originally from the American Type Culture Collection. Cells were checked for absence of mycoplasma infection by the uracil/uridine uptake method [2]. Attached cell cultures were grown in 500 ml roller bottles containing 50 ml medium. Attached culture medium contained 100 ml 10 × concentrate MEM medium Glasgow modification (Flow Labs.), 850 mg sodium bicarbonate, 292 mg L-glutamine, 115 mg L-proline, 30 mg streptomycin and 100 ml foetal calf serum (Flow Labs.) made to 1 l with glass-distilled water (pH 7.4). Suspension cultures were grown either in 50 ml in 250 siliconised flasks rotating at 100 rev./min (CHO cells) or in 100 ml in spinner culture vessels (L cells). Suspension culture medium contained 10.81 g 'Autopow' MEMS Eagle modified medium (Flow Labs.), 850 mg sodium bicarbonate, 292 mg L-glutamine, 115 mg L-proline, 30 mg streptomycin, 100 ml foetal calf serum and 10 ml Pluronic F68 (Ugine Kuhlmann, Paris) 10% stock solution all made to 1 l, at pH 7.4.

### 2.2. *Colchicine binding*

Cells were harvested by trypsinisation from attached culture or directly from suspension culture by centrifugation at 400 g for 15 min and washed twice with 5 ml cold 50 mM sodium pyrophosphate buffer (pH 6.5) containing 2.5 mM $MgCl_2$, 0.1 mM GTP and 0.25 M sucrose (buffer A). The cells were resuspended in 3.5 ml of the same buffer and sonicated for 3 × 15 s. Cell breakage was confirmed microscopically. Triplicate colchicine binding assays were set up at zero time and also after 1, 2 and 3 h preincubation

at 37°C. (Preincubation of samples enables decay of colchicine binding activity to be plotted and extrapolated back to the start of the incubation period [3]. The decay line was obtained from the data using a linear regression programme.) Aliquots (0.2 ml) of the sonicated samples were mixed with 0.1 ml 1 μM [*ring C-methoxyl*-$^3$H]colchicine (spec. act. 4 Ci/mmol, Radiochemical Centre, Amersham, batch 23) in buffer A excluding sucrose (buffer B). After incubation for 1 h at 37°C the colchicine binding reaction was stopped by placing the samples on ice, adding 2 ml cold 2% TEAE cellulose suspension in buffer B. The samples were mixed and left for a further 5 min on ice. Samples were collected by filtration through 1.8 mm discs of Whatman GF/C glass fibre filter paper and washed with 30 ml buffer B excluding GTP, and counted after subsidence of chemiluminescence in a 1:1 mixture of Triton X-100 and 1% PPO in toluene.

## 3. Results and discussion

The colchicine binding activity of cell extract of CHO cells grown in attached culture was compared with that of cells grown in suspension culture. We consistently observed that the binding activity was only slightly greater in attached cells than from suspension culture (table 1). In contrast with the observation [1] of a >5-fold increase, none of our preparations showed as much as a 2-fold increase and the mean increase was only 53%. The binding of cell extracts from the 2 growth conditions was, however, significantly different at the 0.1% probability level by a *t*-test. The slight difference between the attached and suspension cultures was not due to differences between the media used, since attached cells grown in suspension medium gave similar binding activity to the attached cells grown in attached medium.

Since cells in suspension culture are slightly smaller than those in attached culture, data is also given on a per cell basis in table 1. The difference in cell size makes the difference in binding per cell between the two cultures closer to 2-fold. (Ostlund and Pastan [1] did not present data on this basis for comparison.)

In [1] single measurements of colchicine binding activity were made after 1 h incubation. Colchicine binding activity of tubulin from animal sources decays with first order kinetics (e.g., fig.2 in [3]). To allow for this decay of colchicine binding activity which occurs during the period of incubation it is preferable to determine the initial binding capacity by preincubating aliquots for different times in the absence of colchicine and then determining the colchicine bound during a fixed incubation period. Extrapolation of the decay curve back to the beginning of the incubation period gives the initial binding activity. The colchicine binding activities given here

Table 1
Colchicine binding activity of cell extracts from CHO cells grown in attached and suspension culture and in different media

| | No. of observations | Colchicine bound | | Half-life of binding (h) |
|---|---|---|---|---|
| | | cpm/mg protein | cpm/$10^6$ cells | |
| Attached culture in attached medium | 9 | 64 404 (6212) | 12 169 (1853) | 5.53 (1.76) |
| Suspension culture in suspension medium | 9 | 42 085 (5882) | 6135 (136) | 9.07 (2.40) |
| Attached culture in suspension medium | 3 | 72 074 (17 454) | 11 219 (447) | 4.64 (0.92) |

Values for colchicine binding and for half-lives are given as means followed by standard deviations in brackets

Table 2
Colchicine binding activity of cell extracts from L cells grown in attached and suspension culture

| | No. of observations | Colchicine bound | | Half-life of binding (h) |
|---|---|---|---|---|
| | | cpm/mg protein | cpm/$10^6$ cells | |
| Attached culture in attached medium | 2 | 30 141 | 6941 | 3.73 |
| Suspension culture in suspension medium | 2 | 16 596 | 4056 | 4.78 |

Values for colchicine binding and for half-lives are means of 2 determinations

are all initial binding activities derived in this way. If the suspension binding activity decayed much more rapidly than attached binding activity, this could explain how Ostlund and Pastan [1] observed a large difference between the two types of cell preparation on their single time point (after 1 h incubation) whilst we observe very little difference on extrapolation back to zero time. However it can be seen from table 1 that the half-life of the binding activity from suspension cultures appears greater than that from attached cultures, although the two are not significantly different at the 1% probability level. The difference between the data in [1] and our data cannot, therefore, be explained on this basis.

Ostlund and Pastan [1] were working with L cells, whereas the work described above was carried out with CHO cells, which obviously could be the basis of the discrepancy between our results. We therefore repeated the work with L cells and observed essentially similar results (table 2) to those which we had obtained previously with CHO cells.

In summary we find that the colchicine binding activity of attached cells is only slightly higher than that for suspension cultures both for CHO cells and L cells. This finding disagrees with that in [1] and suggests that the very large difference which they observed may not be a general phenomenon. Since we observe consistent results with both CHO and L cells it seems unlikely that the discrepancy between our results and theirs with L cells is simply due to a difference in cell type. It seems likely that the discrepancy is due to some unidentified difference in culture technique between their laboratory and ours. Whatever this may be, it does appear that the very large difference between colchicine binding activities in attached and suspension culture which they reported, is not simply attributable to the cells being attached to the substratum. Finally under the conditions which we have employed, the system does not seem to be as convenient for studying the regulation of tubulin synthesis in mammalian cells as had been anticipated.

## Acknowledgement

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## References

[1] Ostlund, R. and Pastan, I. (1975) Biochemistry 14, 4064–4068.
[2] Pedex, K. W. C. (1975) Experimentia 31, 1111–1112.
[3] Wilson, L. (1970) Biochemistry 9, 4999–5007.