J. Cell Sci. 15, 217–220 (1974)
Printed in Great Britain

SOME EFFECTS OF ANTIMETABOLITES ON CELL DETACHMENT

L. WEISS AND D. HUBER
Department of Experimental Pathology,
Roswell Park Memorial Institute,
Buffalo, New York 14203, *U.S.A.*

SUMMARY

Actinomycin D, cycloheximide, puromycin and ouabain facilitated the detachment of Ehrlich ascites tumour cells from glass surfaces. The results suggest that the altered detachment pattern is due to impaired synthesis of constituents of the cell periphery, which results in a decrease in its mechanical strength.

These reagents have been shown by others to reduce the rate of cell aggregation in various shaker-incubators, and observations of this type have generally been accepted as evidence of diminished cell adhesion. The present results support the alternative interpretation that the reagents act through their effects on cell separation, which is independent of adhesion rate.

INTRODUCTION

In an earlier communication (Weiss & Chang, 1973) it was noted that actinomycin D, cycloheximide and puromycin significantly increased the rates of adhesion of Ehrlich ascites tumour (EAT) cells to protein-coated glass and plastic surfaces. These results were in *apparent* contrast to the work of others (Dunn, Owen & Kemp, 1973; Moscona & Moscona, 1963), which ostensibly showed in general, that various antimetabolites decreased the rate of cell adhesion.

In this communication we describe the effects of a number of antimetabolites which have been shown to increase the rates of EAT cell adhesion to protein-coated glass, on the detachment of these cells from similar substrata. The results will be discussed ·n terms of mechanisms of cell adhesion, and the current practice of its measurement.

MATERIALS AND METHODS

Cell culture

Ehrlich–Lettre hyperdiploid ascites carcinoma (EAT) cells were grown at 37 °C in suspension culture at densities of 2–6 × 10^5 per ml, in synthetic medium RPMI 1630 (Moore, Sandberg & Ulrich, 1966) supplemented with 5 % foetal calf serum. Cells were removed from cultures, washed once in medium plus serum, and then diluted to give a final concentration of approximately 10^4 per ml.

Reagents

The following reagents were added to aliquots of cell suspension to give the final concentrations indicated in parentheses: cycloheximide (1 μg/ml); actinomycin D (1 μg/ml); puromycin (10^{-5} M) or ouabain (10^{-3}, 10^{-4} or 10^{-5} M). The cells were then incubated for 30 min at 37 °C, 3-ml aliquots were next removed and added to the shearing chambers described below.

Shearing

The apparatus is described in detail elsewhere (Weiss, 1961*a*); 3 ml of cell suspension (*ca.* 10^4 per ml) were added to each shearing vessel. The bottoms of the vessels consisted of glass disks with 2-mm diameter circles scratched out on a 1-cm radius, on the side not coming into contact with the cell suspension.

After 2 h of incubation at 37 °C, in medium supplemented with antimetabolites, the cells adherent to the glass over the inscribed circles were enumerated. A stainless steel cylinder with a 1°-coned face, rotating at a known speed was introduced into the fluid covering the cells for a known number of revolutions, and then removed. The distance between the bottom of the rotating cylinder and the glass surface to which the cells adhere, was predetermined with a micrometer stop. The cylinder itself did not come into direct contact with the cells, which were thus exposed to a shearing force transmitted through the culture medium.

After shearing, the culture vessels were inverted, the residual adherent cells lying on the inscribed circles counted again, and the percentage detachment calculated.

The shearing force F, to which the cells were exposed is given by

$$F = \frac{\omega . \eta . r}{h} \mathrm{N\,m^{-2}},$$

where r is 0·01 m, ω is the angular velocity of the rotating cylinder (31·5 rad $s^{-1} \equiv$ 300 rev/min); η is the viscosity of the medium ($9{\cdot}5 \times 10^{-4}$ N s m^{-2}) and h is the distance between the bottom of the cylinder and the glass (1×10^{-4} m). In the present experiments, the cells were exposed to estimated shearing pressures of approximately 3 N m^{-2} for 2 min. Although these estimates are approximate, the pressures are highly reproducible.

RESULTS

The results which are summarized in Table 1, show that exposure to each of the reagents for a period of approximately 2·5 h facilitates the cell detachment produced by a shearing pressure of approximately 3 N m^{-2} for 2 min.

Table 1. *Cell detachment in the presence of various reagents*

Reagent	% of cells detached ± S.E. (no. of observations): Controls	Experimental	*t*-test
Cycloheximide	8·4 ± 1·8 (48)	25·0 ± 2·4 (53)	$P < 0{\cdot}001$
Puromycin	11·5 ± 2·2 (24)	23·4 ± 2·7 (31)	$0{\cdot}01 > P > 0{\cdot}001$
Actinomycin D	5·2 ± 1·1 (55)	27·1 ± 1·5 (64)	$P < 0{\cdot}001$
Ouabain, 10^{-3} M	7·7 ± 1·8 (31)	21·2 ± 2·1 (32)	$P < 0{\cdot}001$
Ouabain, 10^{-4} M	7·9 ± 1·2 (32)	28·6 ± 2·5 (32)	$P < 0{\cdot}001$
Ouabain, 10^{-5} M	11·7 ± 2·0 (24)	14·7 ± 2·1 (24)	$0{\cdot}4 > P > 0{\cdot}3$

It should also be noted that at the concentrations used here, under similar experimental conditions, cycloheximide, puromycin (Weiss & Chang, 1973) and ouabain (Weiss, 1972) diminished the incorporation of ^{14}C-amino acids by EAT cells. However, as assessed by trypan blue exclusion tests and/or measurements of reproductive integrity, this treatment produced no detectable changes in cell viability over the period of 2·5 h.

DISCUSSION

It has been shown previously that under conditions similar to those used here, in the presence of serum-containing medium, the rate of EAT cell adhesion to (protein-coated) glass and plastic surfaces was increased by the antimetabolites cycloheximide, puromycin and actinomycin D (Weiss & Chang, 1973) and by ouabain, valinomycin and gramicidin (Weiss, 1972) which interfere with transmembrane movements of ions. The present experiments show that in the case of cycloheximide, puromycin, actinomycin D and ouabain, the cells which adhered at an increased rate, were also detached more easily.

It has been amply demonstrated that when cells are detached from glass surfaces by the present shearing technique (Weiss, 1961*b*; Weiss & Combs, 1963; Weiss & Lachman, 1964) and various non-quantitative detachment procedures (Rosenberg, 1960; Marin, 1965; Bolund, Darzynkiewicz & Ringertz, 1970; Poste *et al.* 1973), cellular material is left behind on the substratum. Measurements of cell detachment as described here, in part at least, provide an index of the mechanical strength of this residual material which lies between the major permeability barrier of cells and the substrata to which they adhere (Weiss, 1961*b*), and which constitutes the 'fuzz' of electron microscopists. The production of this material has been shown to be dependent on cellular metabolism and is inhibited by actinomycin D, and cycloheximide (Poste *et al.* 1973). The results given in Table 1 therefore probably indicate that by interfering with the synthesis and replacement of external material, the various reagents facilitate cell detachment.

Although our previous experiments showed that various antimetabolites increased the rate of EAT cell adhesion to glass and plastic surfaces, others have shown that the same reagents decreased the rate of aggregation of embryonic cells in gyratory shakers (Moscona & Moscona, 1963; Dunn *et al.* 1973). A partial explanation of the apparent difference between the results is that the EAT cells used by us were taken directly from suspension culture, whereas the embryonic cells were obtained by tryptic dissociation of embryos, and this type of enzyme treatment has been shown to affect the later response of both EAT (Weiss & Chang, 1973) and embryonic (Weiss & Maslow, 1972) cells to the reagents.

In the absence of direct measurement of the *strength* of adhesion between cells and other substrata, it has often been assumed that the *rate* is in some way proportional to the strength of cell adhesion. Strength of adhesion is often erroneously, but operationally, defined as the ability of cells to resist separation (Weiss, 1967). The present work shows that rate of adhesion is not proportional to resistance to separation.

In experiments made with cells in gyratory shakers and other dynamic procedures, adhesion is assessed either by aggregate size or by the number of residual free cells. In such experiments, the free cells are subjected to forces tending to bring them together, and aggregates are subjected to forces tending to pull them apart. The effects of any reagent which either reduces aggregate size or increases the number of free cells, could therefore depend on its activities in decreasing cell adhesion or, alternatively in facilitating cell detachment. The present studies suggest that cycloheximide, puromycin, actinomycin D and ouabain act by promoting detachment rather than by inhibiting adhesion. Failure to discriminate between adhesion and separation can therefore lead to confusion in the interpretation of this type of experiment. Recognition of these differences accounts for the apparent differences between our results and those of others.

This work was partially supported by Grant No. BC-87F from the American Cancer Society, Inc.

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(*Received* 16 *October* 1973)