Evaluation of the Adhesion to Glass of Mammalian Cells by an Electronic Particle Counter

The measurement of the adhesion of cells to a glass surface is one of the several contact phenomena which can be studied in an attempt to understand better the mechanisms regulating tissue organization, and the migrational behaviour of normal and neoplastic cells.

TAYLOR[1] and NORDLING[2] have studied the adhesion of cultured cells on flat surfaces of different materials by the method of adhesion chambers. Technically this method is time-consuming for microscopic counts and it gives no quantitative information about the cytotoxicity of pharmacological treatments intended to modify adhesion. In the last few years, the wide utilization of electronic particle counters has provided a fast and reliable method for counting cells released from monolayers by trypsin treatment[3, 4]. This paper proposes a method for evaluating quantitatively the rate of adhesion of cells cultivated in vitro, based on the utilization of an electronic particle counter apparatus.

Materials and methods. KB cells were used throughout all the experiments. The growth medium was Eagle MEM based on Hank's balanced salt solution plus 10% fresh non-clumping calf serum[5]. Test tubes were Bellco borosilicate disposable tubes washed as described by NORDLING[6]. Cell suspensions were obtained by a 10 min treatment with 0.25% Difco Trypsin in calcium and magnesium free Hank's solution (CMFHS).

Cells were centrifuged and resuspended in growth medium, at concentration of 2×10^5/ml in a spinner flask, with continuous stirring. From this pool of cells, aliquots of 1 ml of cell suspension were distributed in test tubes slanted in a rotating drum. The 2 populations of adhering and non-adhering cells were evaluated in each tube by counting the non-adhering cells in the supernatant medium, and releasing by a 10 min incubation in trypsin solution the adhering cell population. A Coulter Counter mod. B was used to evaluate the number of cells. The total number of cells in suspension was measured by counting every particle over the threshold line, appearing in the frequence distribution (Figure 1).

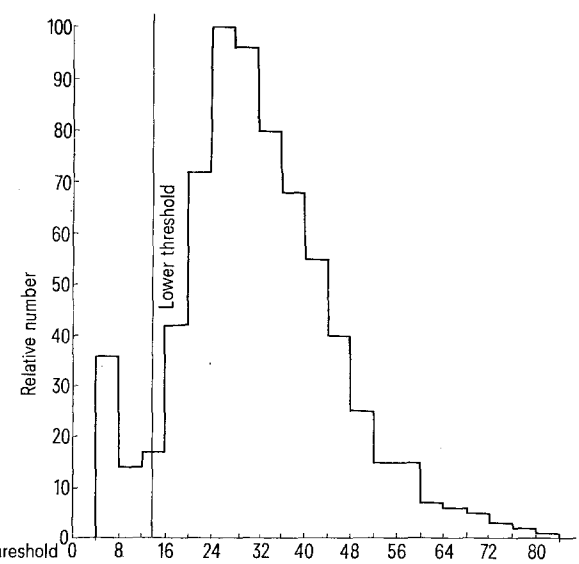

Fig. 1 Size distribution of KB cells. Coulter Counter mod. B with automatic plotter. Pore size 100; matching switch 64; amplification 4; intensity 0.707. The lower threshold used was 14 corresponding to a particle volume of 161 μm^3. No upper threshold was included. Impedance on CMFHS was 22 kr.

Heat-killed cells were obtained incubating for 30 min at 56 °C the cell suspension. Formalin-fixed cells were obtained treating the cell suspension with 10% of formalin for 24 h. Cells were then washed several times in 0.85% NaCl over a 24 h period, and resuspended in growth medium.

Results. Figure 2 shows that the percentage of non-adhering cells A) decreases as an exponential function in relation to time. This phenomenon lasts only through the 1st h when it reaches a value close to the 'noise' of the counting apparatus. On the other hand, the adhering cells B) increase in an almost complementary way during the 1 h period that is considered as the true adhesion period. Their rate of increase is, however, slower than the rate of decrease for non-adhering cells. Curve C) represents the sum of A) and B). A cell loss, due to the trypsin treatment of adhering cells, is evident during the 1st h.

The plateau reached by curve B) and C) between the 1st and 2nd h indicates that adhesion was completed by most of the population before cell growth would begin. Experiments on heat-fixed cells are plotted in Figure 3 where a sharp decrease of the total cell number begins immediately and remains almost constant for 2 h. The adhering cells (curve B) do not change from a 20% value

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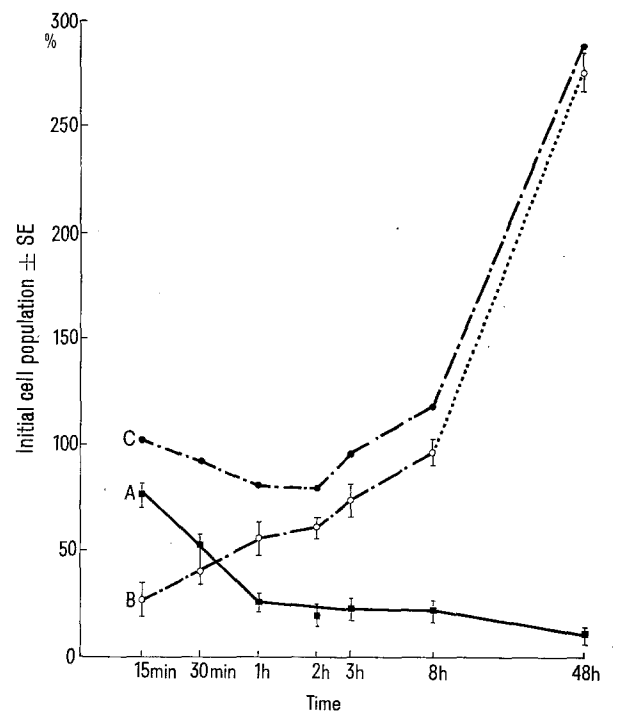

Fig. 2. Percentage of the initial population as a function of time (log scale). Living cells. A) Non-adhering cells collected in the supernatant medium. B) Adhering cells recovered from glass by 10 min treatment with trypsin. C) Total recovery of cells. Vertical bars represent the standard error of the mean ($n = 5$).

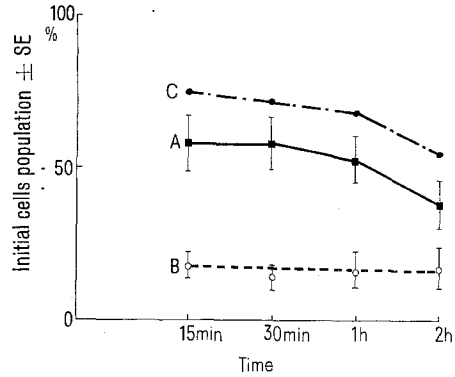

Fig. 3. Percentage of the initial population as a function of time (log scale). Heat-fixed cells. A) Non-adhering cells. B) Adhering cells. C) Total recovery.

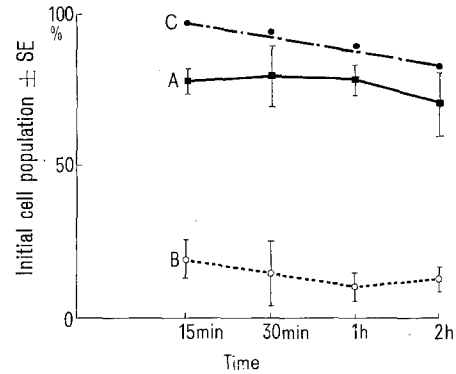

Fig. 4. Percentage of the initial population as a function of time (log scale). Formalin-fixed cells. A) Non-adhering cells. B) Adhering cells. C) Total recovery.

that is maintained constant during the whole experiment. The slow decrease of non-adhering cells (curve A) after 30 min is parallel to the decrease of the total cell population and is unrelated to curve B. Formalin-fixed cells (Figure 4) decrease in number slowly C) without any relation between the non-adhering A) and the adhering B) fraction.

Discussion. The decrease in the number of non-adhering cells and the parallel increase of those which adhere describes a change in the condition of the cell population, that follows an exponential function in relation to time.

The absence of adhesion in cell populations killed by heat or by formalin does not agree with the data of TAYLOR[1] and NORDLING[2], who found that dead cells did adhere to the glass at a faster rate than living cells. An explanation may be that with strongly adhering cells, such as KB cells, 2 phases of adhesion can be distinguished. The first may be assumed to be a rather weak bond, that is to say if we consider the DERJAGUIN-LANDAU-VERWEY-OVERBEEK theory of colloid stability[7, 8] as applied to cells. Then due to a reduction in the electrostatic potential between the cells and the glass surface[9, 10] some adhesion will occur, the rate being proportional to the potential. The possibility also has to be considered that the cells are held to the glass in a 'secondary minimum' or by 'macromolecular bridges'.

After this first contact is established, the cells spread, and due to the large mutual interfacial area they become then firmly bound to the glass, in which case they may be only detached by rupturing the cells or by fracturing the membrane[11].

It seems that the above method is more likely to leave among adhering cells only the population entering into this second phase of adhesion. This capacity of cells to adhere in a firm way to the glass surface[12] is a specific marker of viability[13, 14].

Riassunto. La velocità di adesione al vetro di cellule KB è stata studiata utilizzando un contatore di particelle elettronico. I risultati mostrano che è possibile studiare l'adesione di cellule al vetro come fenomeno vitale collegato ai fenomeni di attaccamento attivo e di riproduzione cellulare.

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[13] This work has been supported by a grant from the G. A. and L. Pfeiffer Research Foundation, New York, USA.
[14] We wish to thank Dr. D. WILKINS, Battelle Institute, Geneva, for his helpful discussion and advice.

CORRIGENDUM

D. A. J. GOODLAD and C. M. CLARK: *Effect of Pregnancy and Feeding Pattern on Tryptophan Pyrrolase in the Rat*, Experientia *28*, p. 207 (1972). On page 208 the first sentence should read as follows: The level in non-pregnant rats was, however, significantly **increased** by restricting their food intake.

F. J. OELSHLEGEL JR. and G. J. BREWER: *New Positive, Tetrazolium-Linked, Staining Method for the Use with Electrophoresis of Phosphoglycerate Kinase*, Experientia *28*, p. 116 (1972). On page 117 in the 3rd paragraph, the quantity of $MgCl_2$ should be **0.011 *M*** instead of 0.11 *M*. The G6PD should be 0.5 mg per **100 ml** and the HK should be 0.1 mg per **100 ml** instead of per ml.