

ON THE PHYSICO-CHEMICAL BASIS OF CELL ADHESION

K. VASUDEVA RAO, JOHN V. GEORGE AND D. E. S. TRUMAN*

Department of Zoology, University of Delhi, Delhi-7

ABSTRACT

Reaggregation of rat liver cells has been studied under various experimental conditions. It has been found that binding $-SH$ groups of the cells abolishes cellular adhesiveness which can be restored by a treatment with cysteine. Inhibitors of cellular respiration, synthesis of RNA and the absence of Ca^{++} and Mg^{++} do not affect cellular adhesivity. Non-viable (dye permeating) cells also have the same adhesiveness as healthy cells. It is concluded that the formation of cell-cell bonds in shaking suspensions is a physico-chemical process, dependent on the properties of the cell surface and independent of cellular metabolism.

INTRODUCTION

UNDERSTANDING the mechanism by which cells adhere to each other is of interest in several biological and biomedical subjects. The orderly manner in which cells bind with each other and assume the characteristic tissue architecture is obviously regulated by complex mechanisms. Mixed heterotypic cell aggregates are known to sort out and assume new spatial arrangements by selective cell adhesions¹⁻². The reaggregation of disaggregated cells in shaking suspensions has been widely used as a suitable experimental model for the study of cellular adhesiveness³. It is assumed that the cells suspended in shaking media collide with each other and reaggregate. Evidence has been obtained recently⁴ that sulfhydryl (thiol, $-SH$) groups on the cell surface play an important role in cell-cell adhesion. Using chick embryonic liver and kidney cells it has been found that binding $-SH$ groups of the cell surface by carboxypyridine disulfide (CPDS) results in a total inhibition of cellular adhesivity which can be restored by a subsequent treatment with certain thiols. Whether CPDS binds only the surface groups or interferes with any other metabolic activity of the cell is not clearly understood. Mehrishi and Grassetti⁵ have presented evidence to indicate that CPDS binds only the surface $-SH$ groups. However, the possibility exists that in case CPDS enters the cells it could interfere with a variety of metabolic processes, especially those involving enzymes dependent on $-SH$ groups for normal function. The present investigation was therefore undertaken to see if the adhesion of cells in shaking suspensions is a mere physico-chemical process or depends on the metabolic activities of the cells.

MATERIAL AND METHODS

Liver from adult male albino rats was excised into Ringer saline, chopped into small pieces and

incubated in calcium-magnesium-free (CMF) medium 199, buffered at pH 7.3 by HEPES⁶ for 30 minutes and successively passed through hypodermic needles of 18, 20, 22 and 24 gauge. The suspension was centrifuged for 2 minutes at 500 rpm. The supernatant containing erythrocytes and some broken cells was removed; the lightly pelleted cells were resuspended in medium 199 and passed through a hypodermic needle of gauge 26. The cell suspension thus prepared contained 95% single cells.

Reaggregation of cells.—Two ml cell suspensions containing 8×10^5 cells per ml were shaken in 50 ml Erlenmeyer flasks on a reciprocating shaker

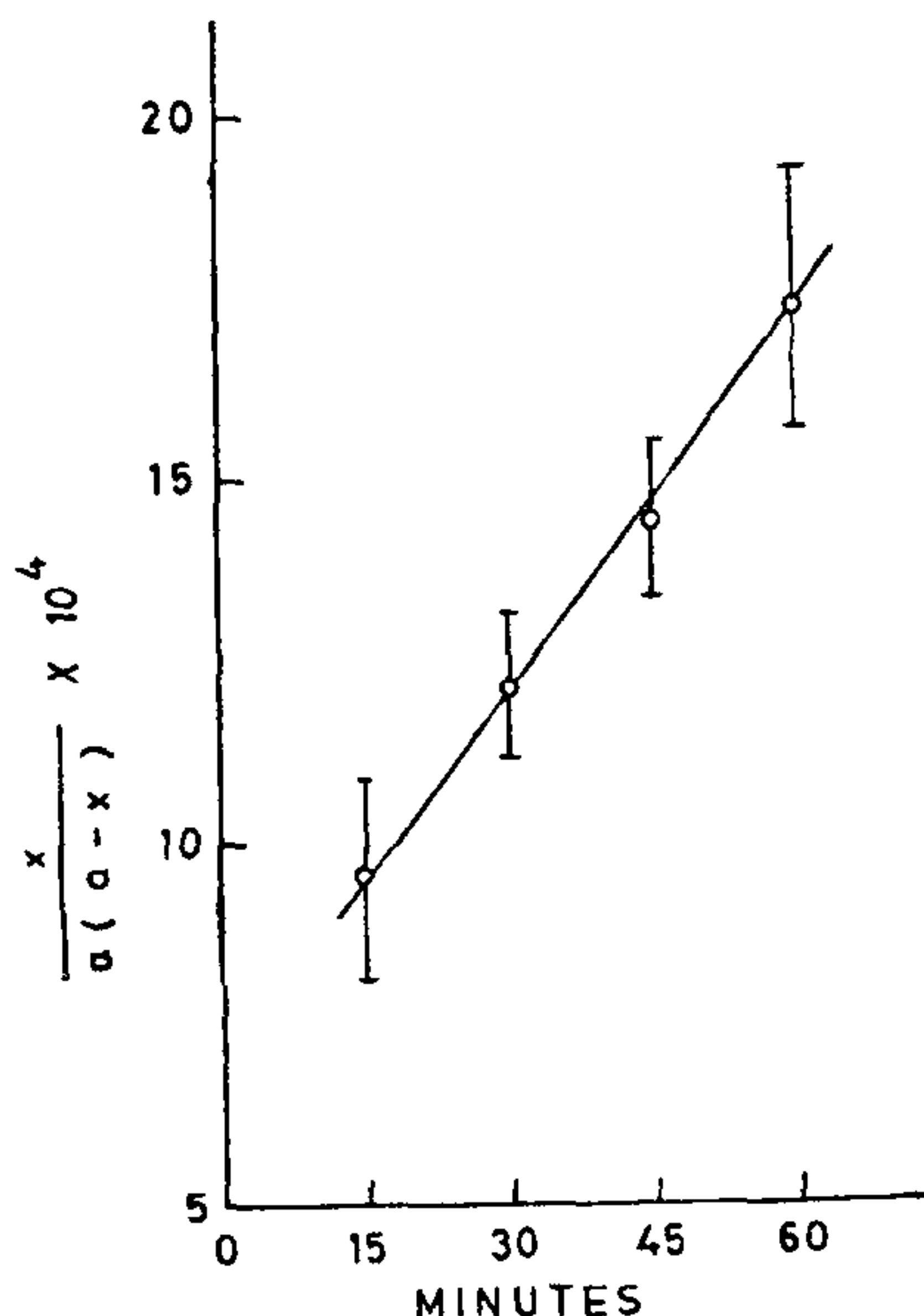


FIG. 1. Depletion of single hepatocytes (ordinate) in shaking suspensions plotted against time (abscissa), x depletion of single cells from the original population, a .

* Permanent address: Institute of Animal Genetics, West Mains Road, Edinburgh EH9 3JN, Scotland, U.K.

at 60 strokes per min. Extraneous factors such as density of cells and speed of shaking which influence the rate of reaggregation of cells were kept constant in all experiments. Cell viability was monitored with the dye (Trypan blue) exclusion test till the termination of the experiments. Cells prepared as described above were viable to the extent of 98–100% through the duration of all the experiments.

RESULTS AND DISCUSSION

1. *Reaggregation of untreated hepatocytes.*—When disaggregated cells are shaken in a suspension they collide against each other and some of the collisions are effective in making adhesive bonds, resulting in a depletion of the single cells from the original population. The rate of depletion of single cells is a measure of the adhesivity of the cells⁷. It has been shown recently⁴ that the kinetics of

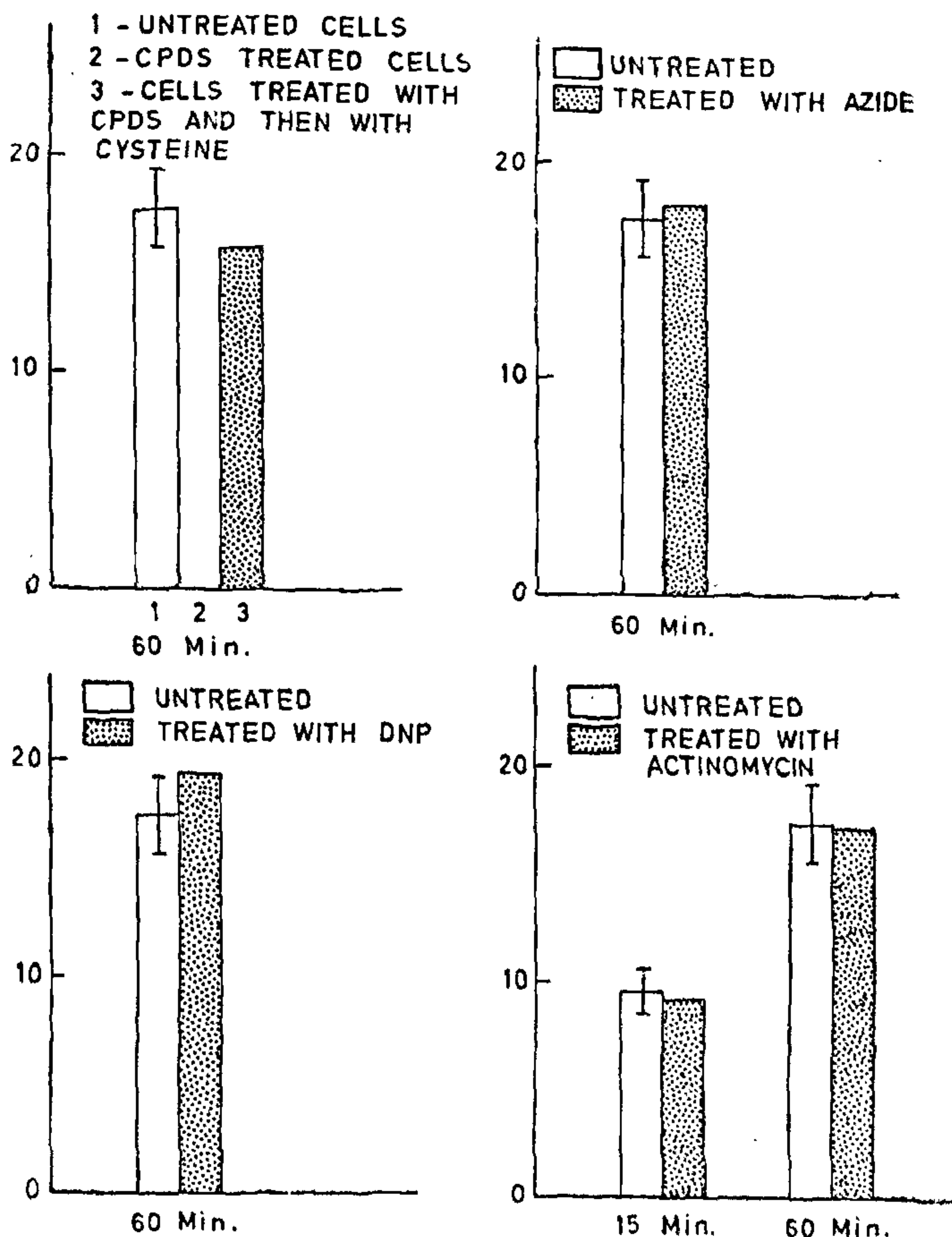


FIG. 2. Effect of chemical treatments on cell adhesion. The scale on the ordinate is as in Fig. 1. Expt 1, protocols: *top left*, cells were treated with 2×10^{-4} M CPDS for 15 min., washed, divided into two groups: one treated with 1 mM cysteine for 15 min., washed and reaggregated in medium 199; the other group was reaggregated in medium 199 without any subsequent treatment; *top right*, cells reaggregated in medium 199 with or without 3 mM sodium azide; *lower left*, cells reaggregated in medium 199 with or without 2 mM 2-4-dinitrophenol; *lower right*, cells incubated in 5 μ g/ml actinomycin D for 30 min. and reaggregated in medium 199 in its presence; control cells were reaggregated in medium 199 after a sham treatment. The duration of reaggregation is indicated in each case.

the population of cell aggregates becomes very complex when reaggregation has progressed for a long time. However, the rate of depletion of single cells from the original population shows a definite time correlation initially and thus can be used as a measure of cell adhesivity. The rate of depletion of single hepatocytes was found to be constant up to 1 hour (Fig. 1). The determination of cell adhesivity by this rapid quantitative method is sensitive enough to assess the results obtained in this study.

2. *The effect of binding -SH groups.*—CPDS is known to bind the cell surface -SH groups⁵ and to inhibit adhesiveness. The inhibition is reversed by a variety of thiols. Rat hepatocytes treated with 2×10^{-4} M CPDS and washed to remove the excess chemical lost adhesiveness which could be restored by a subsequent treatment with cysteine (Fig. 2). The molecular mechanism of the inhibition and its reversal is, however, not clear. While binding of the cell surface -SH groups is an obvious mechanism, there are other possibilities. One has to visualize a wide spectrum of physiological activities which may be affected by binding -SH groups. The most obvious metabolic process which could be affected by binding -SH groups is cell respiration, but there is evidence that other cell functions like RNA⁸ and protein⁹ synthesis may also be affected. It was therefore considered desirable to see if the inhibition of cellular adhesion by CPDS is mediated through its action on any metabolic activity. Inhibition of respiratory

enzymes with 2 mM 2-4-dinitrophenol or 3 mM sodium azide did not affect reaggregation of the hepatocytes (Fig. 2; the experimental protocols are described in the legends to the figures). Similarly 5 μ g/ml actinomycin did not alter cell adhesiveness (Fig. 2). These findings lead to the conclusion that cell adhesion is neither an energy driven process nor dependent on the synthesis of RNA.

3. *Reaggregation of non-viable cells.*—When cells are disaggregated using a CMF saline instead of the CMF medium 199 they show dye permeation within 30 minutes. These cells are obviously unhealthy and non-viable, probably due to leaking out of important diffusible substances from the cytoplasmic pool. However, even these cells show the same rate of reaggregation as normal cells (Fig. 3) indicating that the formation of cell-cell bonds is a mere physico-chemical process and is independent of cellular metabolism.

4. *Reaggregation of cells in a CMF medium.*—It is known that the divalent cations Ca^{++} and Mg^{++} are important in cell adhesion¹⁰. However, their precise role is not clear. In this study it has been found that the rate of reaggregation is not affected by the absence of Ca^{++} and Mg^{++} in the medium (Fig. 3).

CONCLUSIONS

The present study indicates that the formation of cellular aggregates in shaking suspensions is a physico-chemical process, chiefly dependent on the cell surface properties and apparently independent

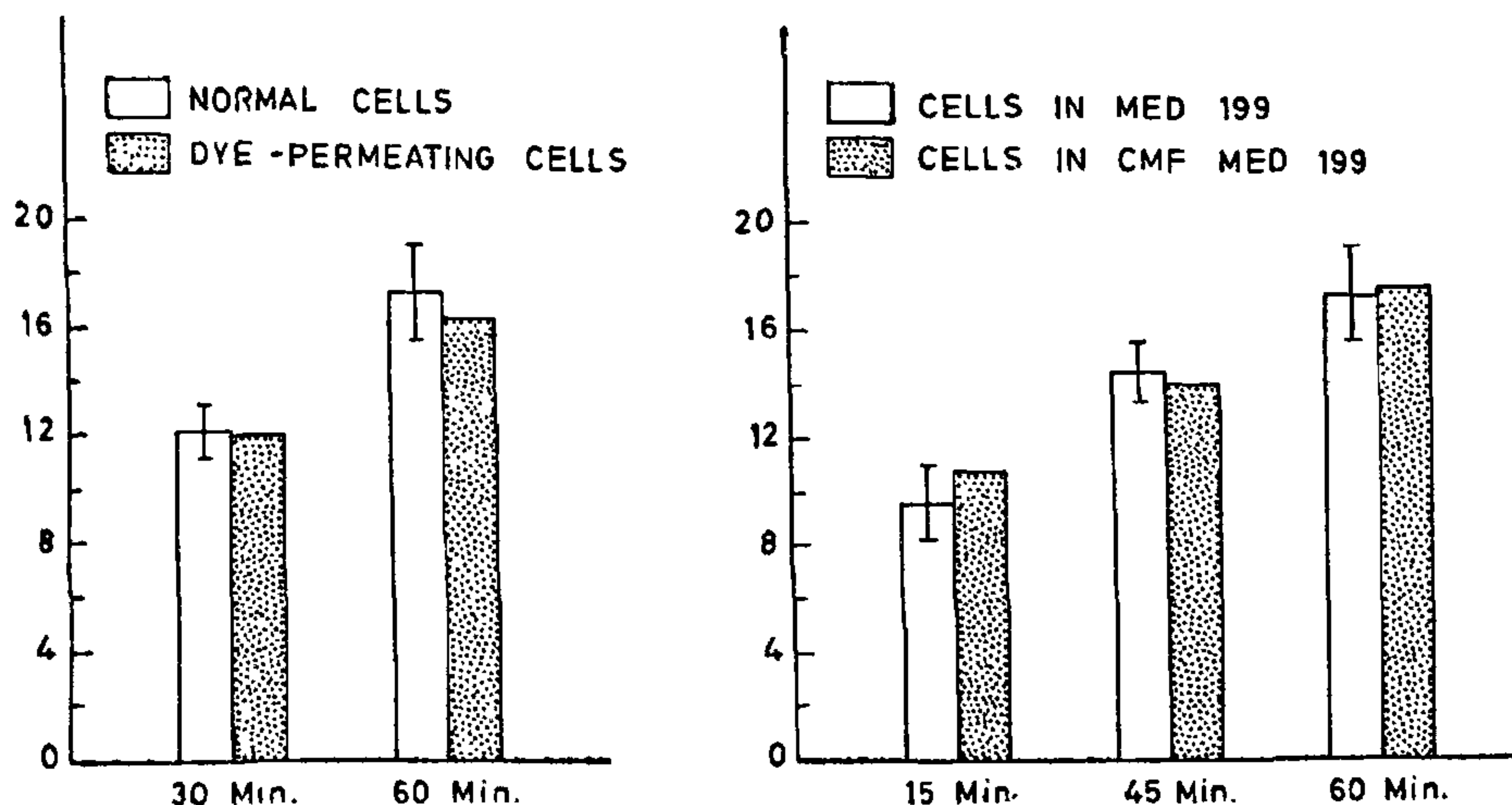


FIG. 3. *Left:* Normal cells prepared as described under material and methods; non-viable (dye-permeating) cells prepared by using CMF saline. Reaggregation in medium 199. *Right:* Reaggregation of cells in normal and CMF medium 199. The scale on the ordinate is as in Fig. 1. The duration of reaggregation is indicated in each case.

of cellular metabolic activities. Further, it appears that -SH groups on the cell surface are directly involved in the mechanism bringing about cell-cell bonds. It is generally accepted that cell adhesion plays a significant role in morphogenetic processes. It is also known that -SH groups are important in a variety of morphogenetic processes^{11,12}. In view of these facts the present findings seem to be interesting.

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EFFECTS OF ACTINOMYCIN D ON GROWTH, HETEROCYST FORMATION, AND NUCLEIC ACID SYNTHESIS IN THE BLUE-GREEN ALGA, *ANABAENA DOLIOLUM*

V. V. S. TYAGI*

Department of Botany, University of Udaipur, Udaipur

ABSTRACT

Actinomycin D promotes growth and decreases heterocyst frequency at low concentrations. Carotene/chlorophyll ratio is also increased. At higher concentrations, the antibiotic inhibits RNA synthesis but not DNA synthesis. These concentrations, however, fail to inhibit heterocyst differentiation. This indicated that heterocyst formation does not depend upon the availability of freshly formed RNA.

INTRODUCTION

A LARGE number of antibiotic substances have been found to inhibit the growth of the blue-green algae¹. Kumar² studied the effects of some common antibiotics on the unicellular blue-green alga, *Anacystis nidulans* and found them to be growth inhibitory. The evidence for specific inhibition by antibiotics is, however, lacking. Talpasayi and Kale³ reported the inhibition of heterocyst formation in *Anabaena ambigua* by chloramphenicol and believed it to be due to inhibition of protein synthesis. Importance of actinomycin D as specific inhibitor of DNA dependent RNA synthesis has been currently established⁴, although no study seems to have been done on the blue-green algae with this antibiotic. The present study was undertaken with a view to discovering the action of actinomycin D on the blue-green algae and the role of the sensitive process in growth and heterocyst formation.

MATERIAL AND METHODS

Anabaena doliolum—a heterocystous nitrogen fixing blue-green alga with characteristic spores⁵, grows well in Allen and Arnon's⁶ medium. During growth in the nitrate-free medium, heterocysts are formed sequentially with almost equal spacings. No heterocysts are, however, formed in potassium nitrate (0.02 M) supplemented medium and the filaments continue vegetative growth.

Fresh and healthy spores were used as inoculum, and the antibiotic was mixed in the medium after visible growth appeared. The effect on growth was recorded in terms of optical density of extracted pigments and that on heterocysts in terms of frequency. Heterocyst frequency is expressed as numbers of heterocysts per hundred vegetative cells in a filament. Pigment extraction was done with 80% aqueous acetone and extinction was measured in a Spectronic-20 spectrophotometer at 660 nm.

The effect of actinomycin on nucleic acid synthesis was studied under nitrogen fixing conditions. Cultures were raised from spores in potassium

* Present address: Department of Botany, Government College, Dholpur (Raj.).