which would result from the decomposition of 1 to 10 percent of the hydroxyurea administered, and the formation of a corresponding amount of hydroxylamine. This is well below the mouse LD_{50} for hydroxylamine (*11*), which has also been shown to have antitumor activity (*12*). In patients receiving hydroxyurea intravenously and orally, recovery of the hydroxyurea in the urine averaged about 50 percent and in no case exceeded 80 percent of the administered dose (*6*).

In one case the red-cell fraction was treated by the procedure used for plasma after the cells were lysed by freezing and thawing, and this fraction also contained acetohydroxamic acid. Assuming that the red cell contains 64 percent water (*13*), the concentration in relation to red-cell water was the same as that in the serum, suggesting that the metabolite is freely diffusible through cell membranes.

From a biochemical viewpoint, the major metabolic effect of this type of drug interference would be diminution in oxidative phosphorylation, with consequent effects due to a diminished total concentration of adenosine triphosphate (ATP), or perhaps specific lowering of mitochondrial ATP (*14*). Although it seems surprising that interruption at such a central point of cell catabolism should be peculiarly effective in chronic myelogenous leukemia, the same situation obtains in the unique value of arsenite in the therapy of this disease. This agent, which is now known to block the formation of acetyl-coenzyme A by preventing the participation of α-lipoic acid in the pyruvic oxidase reaction (*15*), was once widely used and highly recommended in the treatment of this blood dyscrasia (*16*) and remains valuable experimentally (*17*).

The acetyl-coenzyme A cleavage formulation does not exclude the additional attack by the hydroxylamine generated upon other areas of cell function. It has been reported that hydroxyurea simulates the action of hydroxylamine in fragmenting mammalian and bacteriophage DNA (*18*); this type of action may be particularly pertinent in explaining the occasional case of megaloblastosis that develops during treatment with large doses of hydroxyurea (*19*). Hydroxylamine is also well known as a very potent inhibitor of catalase (*11*).

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23 September 1963

Inhibition of Adhesiveness and Aggregation of Dissociated Cells by Inhibitors of Protein and RNA Synthesis

Abstract. *Mutual adhesiveness and aggregation of dissociated embryonic retina cells in suspension cultures are suppressed rapidly and reversibly by puromycin, an inhibitor of RNA-dependent protein synthesis; and at a slower rate, irreversibly, by actinomycin D, an inhibitor of RNA formation. The results imply that biosynthetic processes are involved in the mechanism of reattachment of these cells.*

Clarification of mechanisms controlling mutual adhesion and aggregation of embryonic cells is an essential step towards detailed analysis of cell interactions, differentiation, and histogenesis. Critical studies on various aspects of cell contact have become possible through procedures for reaggregation of single cells, in suspensions prepared by tryptic dissociation of embryonic tissues (*1*). Evidence from such studies pointed to a correlation between adhesiveness of the dissociated cells and metabolic activity (*2*) and suggested that their histological reattachment and aggregation might require resynthesis of cell-binding constituents removed from the cell surface or from intercellular spaces in the course of dissociation (*3*). The indications that adhesive properties of cells are influenced by changes in the metabolic states raised the question whether suppression of biosynthetic processes in dissociated embryonic cells might not affect their adhesiveness. The data summarized here provide evidence that the capacity of dissociated embryonic cells to readhere and to aggregate is indeed inhibited by suppressing protein synthesis at the RNA level with puromycin (*4*) and with actinomycin at the DNA-RNA level (*5*).

Cell suspensions were obtained from the neural retinas of 10-day chick embryos by our method of tryptic dissociation (*1*). The dispersed cells were rinsed in a calcium- and magnesium-free salt solution with deoxyribonuclease (30 μg/ml) added to minimize incidental clotting (*3, 6*). The cells were aggregated in flasks by the rotation procedure (*1*) whereby the cells are rapidly swept into contact independently of individual mobilities or proliferation; mutually adhesive cells aggregate, others remain single or form small, loose clusters. Aggregability in terms of number, size, and rate of formation of aggregates provides a measure of cell adhesiveness with reference to the particular conditions (*1, 3*). Samples (3 ml) of cell suspension (2×10^6 cells/ml) in culture medium (Eagle's basal medium with 1 percent glutamine, 10 percent horse serum, and penicillin-streptomycin) with and without the inhibitors were distributed into 25-ml erlenmeyer flasks, gassed with a mixture of 5 percent CO_2 and air, and rotated on a gyratory shaker at 70 rev/min. All cultures were incubated at 37°C and scored after 1, 2, 4, 6, 12, and 24 hours of rotation. The results are based on more than 200 cultures.

Figure 1 shows the increase in size of aggregates in control cultures (*a*), and the suppression of cell aggregation by puromycin (*b*) and actinomycin D (*c*). In puromycin the block to aggregation occurred within the first

hour; 1-hour cultures contained mostly free cells and small, loose clusters and there was no further aggregation during the rest of the cultivation period. Concentrations of puromycin as small as 0.5 μg/ml (the lowest tested) were inhibitory. The effect was concentration dependent. The puromycin block was reversible in that cells exposed for 6 hours to the inhibitor resumed aggregation, after a short lag period, when transferred to normal medium (Fig. 1*c*). To test whether the block to aggregation might not be due primarily to interference by puromycin with the repair of possible dissociation damage to cells, cells were cultured in normal medium before exposure to the inhibitor. Puromycin added to 6-hour cultures, when aggregation was already well advanced, stopped reversibly further aggregation in about 90 minutes (Fig. 1*d*). Addition of adenosine triphosphate (ATP) ($4 \times 10^{-3}M$) did not prevent the inhibitory effect of puromycin.

It appears that unimpaired protein synthesis is required for progressive reattachment and aggregation of these cells. The finding that cell aggregation is also reversibly inhibited by ribonuclease (*3*) seems relevant here. The evidence suggests therefore that, under our experimental conditions, RNA-dependent synthesis is essential for regeneration of mutual adhesiveness of the cells and that the products involved turn over, change, or become inactivated rather rapidly, at least as long as the cells remain free. Formation of the initial small clusters by some of the cells, prior to the onset of the inhibition plateau, could be due to their residual reserves of the essential constituents, to the time required for an effective suppression of protein synthesis by puromycin, or to factors not susceptible to the inhibitor.

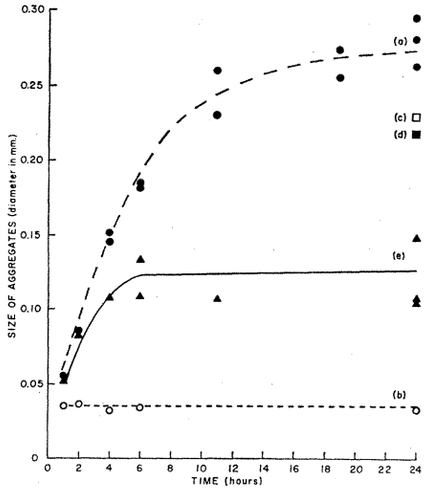

Fig. 1. Size of aggregates in suspension cultures of trypsin-dissociated neural retina cells from 10-day chick embryos: (*a*) controls; (*b*) in medium with puromycin (5 μg/ml); (*c*) cultures maintained for 6 hours in puromycin and then transferred to normal medium for the duration of the incubation period; (*d*) cultures maintained for 6 hours in normal medium and then transferred to medium containing puromycin; (*e*) in medium with actinomycin (0.5 μg/ml). Each point represents an average of measurements on several cultures in one experiment.

In considering further the prompt suppression of cell aggregation by blocking RNA-dependent protein synthesis it was of interest to examine whether inhibition of synthesis of new RNA by actinomycin would affect aggregation of these cells. It was found that actinomycin inhibited aggregation, albeit at a rate significantly slower than puromycin (Fig. 1*e*). In the presence of actinomycin D (0.5 μg/ml or higher concentrations) the cells continued to aggregate for about 4 hours, though at a declining rate as compared with controls; thereafter no increase in size of aggregates was detectable. The same effect was obtained after 1-hour exposure of cells to the inhibitor. Actinomycin added to 6-hour cultures in normal medium blocked further aggregation after about 5 hours. The time required for the full effect of actinomycin could reflect the life time of the existing RNA involved in the processes tested; however, it could also be due to the rate of binding of the drug with DNA. Under the conditions studied the effect of actinomycin was irreversible.

The usefulness of our exploratory results is that they suggest a hitherto unexploited and potentially promising approach to various aspects of cell-contact and cell-association phenomena. An adequate interpretation of these data and their comparison with other work bearing on this topic (*7*) require further information, particularly with reference to protein and RNA turnover and to other types of cells. The present findings agree with the postulation that biosynthetic processes are involved in the attachment mechanisms and contact properties of embryonic cells; hence they suggest that cell contact phenomena may be subject to controls which regulate these processes. It is conceivable that production of extracellularly functioning materials with specific cell-binding roles may be a major aspect of this matter; the recent isolation of aggregation-promoting materials from live sponge cells (*8*) and other evidence (*3*) point in this direction. Accordingly, the possibility exists that the block to aggregation by inhibitors of protein synthesis might be due to interference with formation of such materials. Their susceptibility to inhibition would be likely to vary with different kinds of tissues, cells, and functional or developmental states of cells (*9*).

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Melatonin Synthesis in the Pineal Gland: Control by Light

Abstract. *In rats placed in continuous darkness for 6 days, there is a striking increase in the activity of melatonin-synthesizing-enzyme (hydroxyindole-O-methyl transferase) in the pineal gland, but no change in the activity of monoamine oxidase. Since melatonin appears to have a hormonal role in mammals, and its synthesis is confined to the pineal gland, the inhibition of hydroxyindole-O-methyl transferase by light may constitute a mechanism of neuroendocrine regulation.*

Melatonin (5-methoxy-*N*-acetyltryptamine) is produced in the mammalian pineal gland by the *O*-methylation of *N*-acetylserotonin (*1*, *2*). Although the *O*-methylating enzyme, hydroxyindole-*O*-methyl transferase (HIOMT), has