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4. Simultaneous intraarterial studies have confirmed that median systolic blood pressure measured by this cuff system is equal to median intraarterial systolic blood pressure (unpublished data from our laboratory).
5. Separate analyses of variance were computed for the initial five control sessions in all patients and for differences between the initial five and final five control sessions in four patients. Although possible, it is unlikely that more than 15 or 16 control sessions would yield significantly lower systolic blood pressures.
6. The data were treated as a three-factor repeated measures experiment with two levels of the first factor (control-conditioning); five levels of the second factor (sessions); and 25 levels of the third factor (trials). For the significant main effect of control-conditioning, d.f. = 1/6; for the significant control-conditioning × trial interaction, d.f. = 24/144. Computed by the Biomed 08V Analysis of Variance Program on an IBM 360 computer.
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9. Since completion of this investigation on systolic blood pressure, a method for determining diastolic blood pressure by using the constant cuff-pressure technique has been developed. Studies are now under way with hypertensive patients to attempt to lower diastolic blood pressure through similar conditioning techniques.

10. We acknowledge the competent technical contributions of Miss Barbara R. Marzetta.
11. Supported in part by NIH (HE 10539-04, SF 57-111, SF 57-135, and grant RR-76 from the General Clinical Research Centers Program of the Division of Research Resources); Hoffmann-LaRoche, Inc., Nutley, N.J. 07110; the Council for Tobacco Research; National Institute of Mental Health (MH-08853; Research Scientist Award K5-MH-20,476; and MH-04172); and Office of Naval Research (Physiological Psychology Branch) N00014-67-A-0298-0024.
12. A preliminary report of these experiments was presented at the May 1971 meeting of the American Society for Clinical Investigation.

27 May 1971

Cell Dissociation: Univalent Antibodies as a Possible Alternative to Proteolytic Enzymes

Abstract. *Univalent antibody fragments directed against special membrane antigens dissociate multicellular bodies of the cellular slime mold* Dictyostelium discoideum *completely into single cells. This provides a gentle method for cell dissociation and demonstrates that a nonenzyme protein can disintegrate a tissue by binding to specific cell surface sites.*

Proteolytic enzymes have been shown to be the most effective agents for dissociating tissues into single cells (*1*). Enzymes or other macromolecules may interfere with cell-to-cell binding in two ways, either by sterically blocking or by degrading cell surface sites. It is open to question whether enzymatic activity is absolutely essential for a protein to dissociate cells. Therefore we tried nonenzyme proteins which specifically bind to certain cell surface structures. Univalent antibody fragments (Fab) prepared by papain digestion of immunoglobulin G (see *2*) were selected because of their specificity, because of their relatively low molecular weight (50,000) which restricts their direct action to a small target area, and because each Fab molecule binds to only one antigenic site. Furthermore, use of Fab avoids cell agglutination and other side effects of complete antibody action which follow from cross-linking of cell-membrane structures (*3*).

As a test tissue, fragmented multicellular bodies of the cellular slime mold *Dictyostelium discoideum* were used. In the "slug" stage of this organism, cells are intimately associated forming a tissue that can be dissociated only partially by ethylenediaminetetraacetic acid (*4*), and by proteolytic enzymes only in presence of 2,3-dimercaptopropanol or other sulfhydryl compounds (*5*, *6*). Earlier (*7*, *8*), we reported that, in *D. discoideum*, cell aggregation that precedes slug formation can be completely inhibited by Fab directed against special membrane sites. These sites are thought to be identical with structures that participate in cell adhesion.

The aggregation-inhibiting Fab was prepared from an antiserum produced by immunizing rabbits with a particulate fraction of aggregation-competent *D. discoideum* cells. To ascertain whether this Fab dissociates the slug tissue, slugs were cut into pieces of approximately 50 μm in diameter and incubated in barbital buffer, *p*H 7.3, containing 32 mg of Fab per milliliter (*8*). Dissociation was observed in 10-μl samples spread on a cover slip and covered with a thin, O_2-permeable Teflon mem-

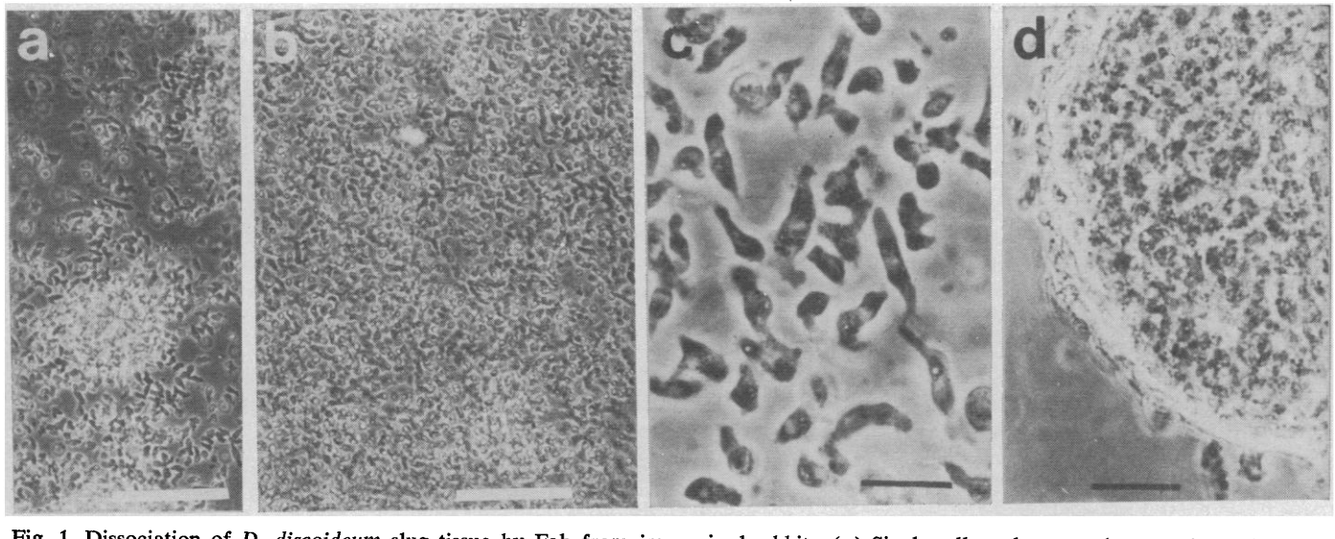

Fig. 1. Dissociation of *D. discoideum* slug tissue by Fab from immunized rabbits. (a) Single cells and groups that remained after incubation for 3 hours; (b and c) completely dissociated slug fragment after an additional 3-hour incubation period; (d) control: undissociated slug fragment incubated for 6 hours with Fab from nonimmunized rabbits. White scales, 50 μm; black scales 10 μm.

brane (*9*). Without any mechanical agitation, the fragmented slugs were loosened at the periphery, and gradually they fell into single cells. After 3 hours only the central parts of the pieces were intact (Fig. 1a), which eventually became also completely dissociated (Fig. 1, b and c). The tips of the slugs were dissociated last. This corresponds to the observation that the presumptive stalk cells that form this part of the slug adhere more strongly to each other than the presumptive spores (*6*). No dissociation was observed in controls containing 32 mg of Fab per milliliter from nonimmunized rabbits (Fig. 1d).

The slow dissociation rate indicates that at a given time only a small portion of the Fab receptor sites is accessible to being blocked by Fab. It remains to be seen whether Fab can break already established bonds between associated cells, or whether it seizes only newly synthesized or detached groups, thus hindering them from contributing to cell adhesion. In this case cell dissociation by Fab would depend on turnover or exchange of its receptor sites.

The dissociated cells were actively motile and began to reaggregate approximately 5 hours after the removal of unbound Fab; they also aggregated in the Fab solution about 12 hours after the beginning of incubation.

For the organism used in this study, application of univalent antibody fragments provides a gentle and efficient method of cell dissociation. This method may be advantageous also in other systems, especially for experiments in which degradation of the cell surface has to be avoided or when the dissociated cells should be kept for longer periods in the single cell state.

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9. A sample of the Teflon membrane was provided by Prof. Fischer, Freiburg/Br.
10. Supported by the Deutsche Forschungsgemeinschaft and the Stiftung Volkswagenwerk.

9 March 1971

Histamine Augments Leukocyte Adenosine 3′,5′-Monophosphate and Blocks Antigenic Histamine Release

Abstract. *Extracellular histamine stimulates accumulation of adenosine 3′,5′-monophosphate in human leukocytes and prevents antigenic release of histamine from cells of allergic donors. Both effects occur at histamine concentrations that can be achieved by antigenic release of the amine in vitro.*

Leukocytes from human donors allergic to ragweed release histamine after exposure to ragweed antigen E (*1*). This effect, mediated by cell-bound reaginic (IgE) antibody, serves as a useful model in vitro for human allergy, inasmuch as the sensitivity of the leukocytes to antigen E reflects the clinical severity of the allergic diathesis (*2*). Inferential evidence, based on the effects of catecholamines and methylxanthines, suggests that elevated intracellular concentrations of adenosine 3′,5′-monophosphate (cyclic AMP) inhibit IgE-mediated release of histamine from human leukocytes and human or primate lung tissue (*3*). Histamine itself stimulates production of cyclic AMP in some tissues, such as brain (*4*). We have examined the possibility that histamine might stimulate production of the cyclic nucleotide in leukocytes as well. If so, extracellular histamine should prevent the release of intracellular histamine triggered by exposure to specific antigen. The experiments reported here were designed to test this hypothesis.

Human leukocytes were isolated and suspended in an isotonic, tris-buffered artificial medium (tris-A). In some instances, 0.6 m*M* Ca^{2+} and 1.0 m*M* Mg^{2+} were added (tris-ACM) (*1*).

Synthesis of cyclic AMP by intact leukocytes was measured as described elsewhere (*5*). Cells suspended in tris-A were incubated with [^{3}H]adenine (6 c/mmole, 1 μc per 10^7 cells) for 40 minutes at 37°C, centrifuged, and resuspended in either tris-A or tris-ACM containing appropriate concentrations of histamine and theophylline, $10^{-2}M$ (*6*), and incubated for a further 10 minutes at 37°C. Histamine caused a dose-related increase in accumulation of cyclic [^{3}H]AMP in leukocytes (Fig. 1), usually maximal at $10^{-4}M$ and detectable at about $10^{-6}M$. The maximal effect, expressed as percentage of increase over basal [^{3}H]nucleotide accumulation, varied considerably from subject to subject. In other experiments (not shown), histamine stimulated adenyl cyclase activity in broken leukocytes, as measured by the method of Krishna *et al.* (*7*).

Histamine release from leukocytes of ragweed-sensitive donors was measured by adding fractions of a cell suspension in tris-ACM to a series of tubes containing a constant amount of antigen E and variable concentrations of exogenous histamine. The reaction mixtures were then incubated at 37°C for 60 minutes, and the percentage of the total cellular histamine released into the fluid phase was determined fluorimetrically (*1*, *3*). The percentage of inhibition caused by histamine was calculated from the formula $[(C-E)/C] \times 100$, where C and E stand for the percentage of histamine release in the control and histamine-containing tubes, respectively.

Exogenous histamine caused a dose-dependent inhibition of antigenic histamine release from the cells of allergic donors (Fig. 2, left). Extracellular histamine at concentrations ranging from 10^{-7} to $10^{-6}M$ caused 50 percent inhibition of release of intracellular histamine in all subjects tested. These

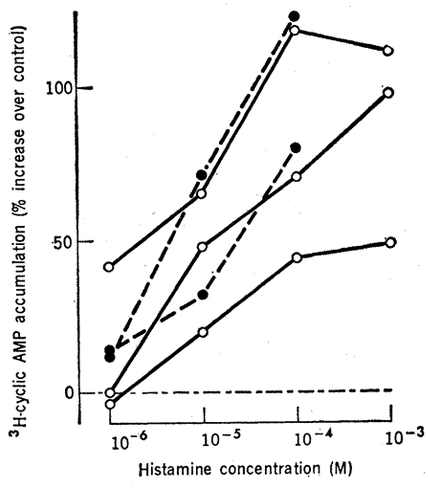

Fig. 1. Conversion of [^{3}H]adenine to intracellular cyclic [^{3}H]AMP by intact leukocytes (ordinate) as a function of extracellular histamine concentration (abscissa). Each line represents a single subject. Incubation medium was either tris-ACM (solid circles) or tris-A (open circles). Accumulation of cyclic [^{3}H]AMP is represented as percentage of increase over basal accumulation (in the absence of extracellular histamine), which averaged 0.28 $\pm$ 0.14 percent of [^{3}H]adenine added per 10^5 leukocytes per 10 minutes of incubation (*5*).