References and Notes

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3. Copies of detailed reports of all experiments may be obtained from Peter L. Carlton, Graduate Psychology Laboratories, Rutgers University, New Brunswick, New Jersey. Funds for duplication and mailing of these reports have been made available by Rutgers University.
4. Material supplementary to this paper has been deposited as Document No. 8990 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington, D.C. 20025. A copy may be secured by citing the Document number and by remitting $12.50 for photoprints, or $4.25 for 35-mm microfilm. Advance payment is required. Make checks or money orders payable to Chief, Photoduplication Service, Library of Congress.
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Antigen-RNA Complexes

Hashem (*1*) reports studies which suggest that ribosomal RNA extracted from human lymphocytes previously incubated with specific sensitizing antigens will induce cytological transformation and mitoses in cultured autologous lymphocytes. Hashem's conclusions are based essentially on an enumeration of the percentage of transformed cells and mitoses appearing in the cultures; the prevention of mitoses by treating the active RNA fractions with ribonuclease; and the lack of transformation in cultures incubated with RNA extracted from autologous nonstimulated lymphocytes. The cells were grown in minimum-essential medium supplemented with 15 percent fetal calf serum and antibiotics (penicillin and streptomycin); results were considered positive if after 5 days of culture there were more than 3 percent transformed cells and over 0.1 percent mitoses.

We are not told the specific percentage of transformation obtained in each culture or the range of transformation obtained with each antigen and with the RNA from antigenically stimulated cells. It is therefore impossible to compare the efficacy of RNA as a blastogenic agent with the antigen from which it presumably derived its transforming properties. Furthermore, it is difficult to evaluate the significance of 3 percent transformation in view of the reports from several laboratories that far greater transformation may occur in the absence of phytohemagglutinin or specific antigens. Johnson and Russell (*2*) noted 5 to 48 percent transformation in cultures of human peripheral lymphocytes grown with media supplemented with fetal calf serum. I have observed 5 to 40 percent transformation in a similar culture system (*3*) and Hirschhorn *et al.* (*4*), report 5 to 10 percent blastoid cells in their control cultures grown with fetal calf serum.

Johnson and Russell reduced the degree of transformation by substituting autologous for fetal calf serum. Elimination of penicillin and streptomycin from the medium resulted in further reduction, an indication that the cells may have been responding to several components of the culture medium. Johnson and Russell obtained between 1.9 and 5.3 percent transformed cells even when they used autologous human serum and eliminated antibiotics.

Hashem reports that ribonuclease completely abolished the mitosis-stimulating activity of RNA, but makes no statement concerning its effect on the lymphocyte-transforming properties of the active RNA preparations. Friedman and co-workers (*5*), who induced antibody formation in rat lymph node cultures with RNA extracted from macrophages incubated previously with bacteriophage T2, found that ribonuclease reduced but did not completely abolish the ability of RNA to induce antibody synthesis. Thus the transforming properties attributed by Hashem to RNA may actually be due to specific sensitizing antigens which have formed complexes with the active RNA fractions.

Friedman and co-workers have convincingly demonstrated that several antigens of phage T2 are present in those RNA preparations which are capable of inducing antibody synthesis, and they also speculate that this activity is specifically due to the presence of these antigens.

That macrophages may also be important in peripheral lymphocyte transformation is evident from the work of McFarland and Heilman (*6*). Starting with peripheral blood, partially purified of polymorphonuclear leukocytes, they observed the gradual appearance of macrophages, each of which became surrounded by clusters of small lymphocytes. These cells firmly attached themselves to the central macrophage and subsequently underwent blastoid transformation. The authors speculate that such a contact, if accompanied by a cytoplasmic connection, could provide a means of transfer of instructive material needed for transformation or antibody synthesis. Hashem also observed occasional cytoplasmic connections between small lymphocytes and transforming cells. Although macrophages are not specifically mentioned in Hashem's report, in view of the observations of McFarland and Heilman (*6*) and the importance of macrophages in the induction of primary immune responses in vitro (*5*, *7*) it seems possible that at least some of the RNA capable of inducing transformation may have been extracted from macrophages which had previously encountered the specific antigen.

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