in those glands that recovered from inhibition.

In other experiments tricyamp (50 μg) and actinomycin D (0.5 μg) (*5*) were offered simultaneously for 2 hours, and the glands were then incubated with tritiated uridine in the presence of these substances. Controls were incubated with actinomycin alone or with neither substance. As reported earlier (*6*), actinomycin by itself almost totally suppressed uridine incorporation into nucleolar RNA and greatly inhibited the chromosomal incorporation (the effect on cytoplasm could not be studied since it is not appreciably labeled in the present incubations, Fig. 1). In the presence of tricyamp the nuclear incorporation was restored to the level of the controls. In parallel experiments tricyamp also relieved the rather specific inhibition of chromosomal incorporation of uridine or guanosine caused by dichlororibofuranosylbenzimidazole (*7*). The observations point in part to the action of tricyamp as probably stimulating DNA-dependent RNA synthesis, but whether this applies to the nucleolus as well as chromosomes is unknown.

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Antibody Production by Nonimmune Spleen Cells Incubated with RNA from Immunized Mice

Abstract. *RNA isolated from the spleens of B_6AF_1 mice immunized to sheep red blood cells was incubated with the spleen cells of nonimmunized mice. The number of antibody-forming cells detected by the Jerne plaque technique was significantly higher among the cells incubated with the RNA from immune animals than among cells incubated without RNA, or with RNA from nonimmune animals.*

There have been several reports concerning "transformation" or induction of mammalian cells by the addition of specific informational RNA depleted of protein. The production of mature viral protein after the addition of polio virus RNA to cultures of HeLa cells may be considered a transformation, although the infectious RNA is assumed to have unique properties of stimulating self-replication (*1*). Niu *et al.* have reported a series of experiments in which RNA obtained from an organ with highly specific function is capable of providing information for the synthesis of specific protein by cells less well differentiated (*2*).

There have been at least two reports which suggest the transformation of lymphoid cells with RNA obtained from cells with a prior exposure to antigen. Fishman (*3*) produced antibody to bacteriophage and Mannick and Egdahl (*4*) produced reactivity to transplantation antigens. In our work, RNA isolated from the spleen of mice immunized with sheep red blood cells converted a small percentage of nonimmune isologous mouse spleen cells into antibody-forming cells. Antibody production by the converted cells was detected by the plaque-assay method (*5*).

We used B_6AF_1 male mice (Jackson Laboratories, Bar Harbor, Maine) 8 to 10 weeks old. The donor mice were immunized by intraperitoneal injections of 50 percent washed sheep red blood cells in 0.1 ml phosphate buffered saline (*p*H 7.4) over a period of 6 to 16 days. Two to three days after the last injection, the mice were killed by cervical dislocation and their spleens were removed for RNA extraction by a modification of the method of Scherrer and Darnell (*6*). The spleens were homogenized in equal parts of 90 percent phenol (containing 0.1 percent 8-hydroxyquinoline) and a mixture of 0.01*M* acetate buffer (*p*H 5.0); 0.5 percent sodium dodecyl sulfate, and 10 μg of bentonite per milliliter. After homogenization, the solution was heated to 60°C for 4 minutes, and was cooled rapidly to 10°C in a bath composed of alcohol and dry ice. After centrifugation at low speed to separate the phases, the aqueous layer containing the RNA was removed. The phenol layer was again extracted with buffer as before, and the aqueous phases were combined and treated three times with the 90 percent phenol mixture, heating and cooling each time. Finally, 2.5*M* sodium acetate was added to the aqueous phase to a final concentration of 0.3*M*, and the RNA was precipitated with two volumes of absolute ethanol. The white precipitate was washed three times with 75 percent ethanol and after the final wash, the RNA preparation was dissolved in Eagle's basal medium (*7*) (without added serum or antibiotics) at *p*H 7.4. The dissolved RNA was added to a cell suspension made from the spleens of nonimmunized B_6AF_1 mice.

The RNA extracted from spleens of 20 donors was added to a pooled cell suspension obtained from spleens of five recipients in 1.5 ml of Eagle's basal medium. Donor RNA was added to the recipient cells and the suspensions were incubated at 37°C for 30 minutes in a water bath equipped with a shaking device. At the end of this period, 0.2 ml of the cell suspension was added to each of a series of tubes containing 0.15 ml of 25 percent washed sheep red blood cells in 2 ml of 0.7 percent agar in Earle's basic salt solution with glucose at 38° to 40°C. Immediately after the addition of the cell suspension to the melted agar, the warm mixtures were overlaid on a solidified base of 1.4 percent agar in Earle's basic salts contained in 100-mm petri dishes.

After a few minutes at room temperature to allow the overlay to solidify, the dishes were incubated at 37°C for 3 hours. After incubation, fresh frozen guinea pig serum was diluted 1:1 with Eagle's basal medium, and 1.5 ml was added to each petri dish. The plates were incubated for an additional 30 minutes at 37°C and were stored at 4°C overnight. Each plate was examined grossly and microscopically for specific zones of hemolysis (Fig. 1) by two observers who were not aware of the group to which

Table 1. Plaque formation by nonimmunized spleen cells treated with RNA in four experiments with 20 mice each. The mean of 11 such experiments is also given.

Treatment	Number of plaques			
	Expt. 1	Expt. 2	Expt. 3	Expt. 4
Immunized				
RNA alone	65	15	117	60
RNA + ribonuclease		2	32	
RNA + NaOH				23
Nonimmunized				
RNA alone	25	4	14	
RNA 3.5 hr after antigen*	9			
RNA + antigen	14			
No RNA	15		43	10
Mean of 11 experiments† with RNA from immunized mice				
With RNA	94 ± 21.2			
Without RNA	20 ± 5.4			
$p < .01$				

* Given intravenously. † Separate RNA isolate for each experiment.

the plate belonged nor the score of the other observer. For 80 percent of the plates, the readings of the two observers were identical. For the others, the higher of two readings was always recorded. Approximately 10 million recipient cells were plated onto each dish, and 8 to 12 dishes per group were examined. Estimates of the number of plaque-forming cells in donor spleens averaged 5000 per spleen.

The results of four experiments are summarized in Table 1. When cells from nonimmune mice were incubated with RNA from immunized mouse spleens the number of plaques formed was significantly higher than that formed by the same number of control cells incubated with RNA from 20 nonimmune mouse spleens, or than that formed by cells incubated with medium alone. Prior treatment of the RNA with 25 μg of ribonuclease per milliliter for 1 to 24 hours or with 0.3*N* NaOH for 24 hours (adjusted to *p*H 7.3 before adding to cells) reduced the number of plaques formed to control numbers.

There were additional controls. In the first, RNA was isolated from the spleens of 20 mice which had received 0.1 ml of a 50-percent suspension of sheep red blood cells intravenously 3.5 hours before they were killed. In the second, 1.9 ml of the 50-percent suspension of sheep red blood cells was added at the beginning of the extraction of the RNA from the spleens of 19 nonimmunized mice. The number of plaques formed by each group was no higher than the number observed in controls receiving buffer.

The appearance of plaques in plates receiving cells from nonimmune animals, or the same cells after incubation with RNA from nonimmune animals is a corroboration of the results reported by Jerne and Nordin (*5*). Presumably, these areas of lysis represent naturally occurring antibody produced by a few cells present in the spleens of nonimmune animals. The significantly greater numbers of plaques in the experimental groups after incubation with RNA from immunized mice indicate that some cells have been induced either to synthesize or to release antibody.

Several interpretations of these findings have been considered. Possibly, a small amount of antigen that formed a complex with RNA was stable during the isolation procedure and this complex induced either antibody formation in the recipient cells or the release of antibody already formed. However, results of injection of antigen 3.5 hours before the animals were killed and the addition of excess antigen to the extraction procedure do not support this explanation.

It is also conceivable that the RNA contains a factor other than antigen which stimulates the release of antibody already present in a few recipient cells. This factor would have to be sensitive to ribonuclease and not be present in RNA isolated from spleens of nonimmune mice.

Another explanation is that donor messenger RNA is picked up nonspecifically by spleen cells containing ribosomes and provides the information for the synthesis of antibody. The RNA from 20 donor spleens containing approximately 10^5 antibody-forming cells and 2×10^9 cells not forming antibody was added to approximately 2×10^8 nucleated spleen cells. Thus there was a 2000-fold dilution of the amount of antibody-specifying RNA per cell and a 20,000-fold dilution of antibody-specifying RNA with other RNA. That 75 to 115 of the recipient cells would nonspecifically capture enough antibody-specifying RNA to form a plaque is questionable for two reasons. Probably, there would not be enough informational RNA per cell if this were distributed randomly among all the nucleated cells, and any cell with unusual potential to pick up RNA likely would be swamped with nonspecific information. These factors have led to the consideration of another possible explanation, namely that cells with prior commitment to make a specific antibody are capable of recognizing and selectively capturing the corresponding informational RNA. This would explain antibody production by relatively few cells in the presence of a large dilution of antibody-specifying RNA.

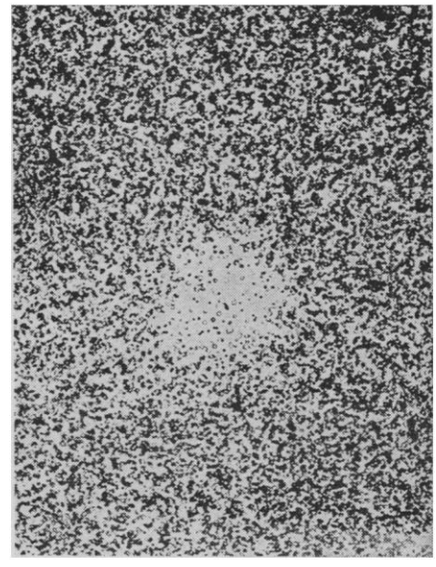

Fig. 1. Plaque of sheep red blood cell lysis formed by a spleen cell taken from a nonimmunized mouse. The spleen cells from the mouse were incubated with RNA isolated from the spleens of immunized mice. (× 120)

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