implanted cells against the recipient. Likewise, regenerated thymus tissue of lethally irradiated $B1F_1$ mice injected with C57BL bone marrow cells induced a fatal wasting disease on implantation in sublethally irradiated $B6D2F_1$ mice. It was concluded, therefore, that regenerated thymus tissue in lethally irradiated $B1F_1$ mice injected with C57BL bone marrow was repopulated by immunologically competent cells derived from the donor marrow.

1. Murray, R. G., in *Histopathology of Irradiation*, edited by W. Bloom, McGraw-Hill, New York, 1948, 446.
2. Hirsch, B. B., Brown, M. B., Nagareda, C. S., Kaplan, H. S., *Radiation Res.*, 1956, v5, 52.
3. Urso, P., Congdon, C. C., *Blood*, 1957, v12, 251.
4. Kaplan, H. S., Nagareda, C. S., Rosston, B. B., Brown, M. B., PROC. SOC. EXP. BIOL. MED., 1958, v98, 384.
5. Congdon, C. C., *Blood*, 1956, v12, 746.
6. Gengozian, N., Urso, I. S., Congdon, C. C., Conger, A. D., Makinodan, T., PROC. SOC. EXP. BIOL. MED., 1957, v96, 714.
7. Dehart, R. L., Cosgrove, G. E., Upton, A. C., *Lab. Invest.*, in press.
8. Popp, R. A., *J. Nat. Cancer Inst.*, 1961, v26, 629.
9. Trentin, J. J., PROC. SOC. EXP. BIOL. MED., 1956, v92, 688.
10. Uphoff, D. E., *J. Nat. Cancer Inst.*, 1957, v19, 123.
11. Uphoff, D. E., Law, L. W., *ibid.*, 1959, v22, 229.
12. Kaplan, H. S., Rosston, B. H., *Stanford Med. Bull.*, 1959, v17 (2), 77.
13. Congdon, C. C., Duda, D. B., *Arch. Path.*, 1961, v71, 311.

Received August 9, 1961 P.S.E.B.M., 1961, v108

Effect of Ribonuclease Upon Immunologically Active Cells.* (26997)

BRANISLAV D. JANKOVIC AND HAROLD F. DVORAK† (Introduced by Byron H. Waksman)
Department of Bacteriology and Immunology, Harvard Medical School, and Neurology Service, Massachusetts General Hospital, Boston, Mass.

Brent *et al*(1) have demonstrated that lymphoid cells of homograft sensitized animals provoke a cutaneous reaction of the delayed type (the "transfer reaction") when injected intradermally in the antigen donor. The specificity of this reaction is consistent with 2 possibilities: that the transfer reaction is dependent on "cell-bound antibodies" or "endoantibodies" or that humoral antibodies are produced in such small quantities that their effect can be detected only in the immediate vicinity of the cells which produce them(2). The character of the reaction itself, which is an indurated inflammatory lesion, implies either that host cells infiltrate the injection site or that the transferred cells increase in mass by multiplication and/or synthesis of new cytoplasmic material. All these features require an intact protein synthesis.

In the present experiments we have applied a biochemical technique to this immunological model to evaluate the role of protein synthesis by immunologically active cells in producing the reaction. Ribonuclease provides a useful tool for investigating this problem. Allfrey *et al*(3) demonstrated in experiments with microsomes that the integrity of ribonucleic acid may be deleteriously affected by ribonuclease with a resulting inhibition of protein synthesis, and Brachet(4), and Ledoux and Baltus (5) have shown that ribonuclease penetrates many living cells and is able to slow protein anabolism without killing the target cells.

Materials and Methods. Dutch rabbits were immunized with slices of New Zealand rabbit ear skin implanted subcutaneously. Ten days later draining lymph nodes were recovered, minced in cold Hanks' solution and a cell suspension prepared. Active ribonuclease (5 × crystallized, Nutritional Biochemicals Corp.) or ribonuclease inactivated by heat (30 min at 100°C) were added to aliquots containing equal numbers of cells to give a final concen-

* This study was carried out with support of grant from Nat. Inst. of Allergy and Infect. Dis.
† Post-Sophomore Fellow, Nat. Inst. of Health.

TABLE I. Transfer Reaction with Lymphoid Cells Exposed to Ribonuclease.

Concentration of ribonuclease (mg/ml)	Time of incubation at 37°C (min.)	No. of living cells per 0.1 ml (million) before incubation	after incubation	Skin reaction 24 hr	48 hr	72 hr
		Rabbit 1				
4	0	2.5		sl.ery.	+	+
"	60	"	2.1	" "	0	0
in.RNAse*	0	"		++	++++	+++ N
"	60	"	1.9	++	+++	+++ N
—	—	2.5 million cells only (control)		++	+++	++++ N
		Rabbit 2				
4	0	2.5		sl.ery.	+	+
"	60	"	2.2	" "	+	0
in.RNAse	0	"		+	+++	+++ N
"	60	"	1.7	+	+++	+++ N
—	—	2.5 million cells only (control)		++	++++	+++ N
		Rabbit 3				
4	0	2.5		+	+	±
"	60	"	2.4	0	0	0
in.RNAse	0	"		+	+++	+++
"	60	"	2.1	+	++	+++
—	—	2.5 million cells only (control)		++	+++	++++

*in.RNAse = ribonuclease inactivated by heat; sl.ery. = slight erythema; ±, +, ++, +++ and ++++ represent different degrees of induration; N = necrosis.

tration of 4 mg/ml. The cells were injected intradermally in the original New Zealand antigen donor immediately after addition of active or inactivated ribonuclease, and again after 60 min of incubation at 37°C. Approximately 2.5 million viable cells were injected in 0.1 ml. Cells were counted at zero time and again after 60 min of incubation using the trypan blue method as a measure of cell viability. The sites of cell injection were examined daily for erythema, induration and necrosis, and the skin reactions graded on an arbitrary scale ranging from ± to ++++.

Results. The results (Table I) suggest that ribonuclease directly affects the immunological activity of lymphoid cells from a sensitized rabbit, depriving them of their ability to produce the transfer reaction when injected into the skin of the antigen donor. The fact that cells injected immediately after addition of ribonuclease still give positive reactions indicates that the action of this enzyme is directed against injected cells, not against host cells migrating to the site of reaction. This inference is further supported by the fact that cells incubated with ribonuclease and then washed to remove the enzyme before injection into the donor lose the ability to produce a transfer reaction. Cells treated with biologically inactive enzyme retain their immunological competence and give a transfer reaction of the same intensity as nontreated (control) cells. The exposure of cells to 4 mg/ml of ribonuclease for one hour at 37°C does not kill the cells, as judged by the trypan blue method or subsequent survival of treated cells in Millipore filter chambers. Smears of treated cells stained with methyl-green-pyronin showed a recognizable decrease in cytoplasmic basophilia of certain cell types as compared with smears of control cells.

Discussion. Although the exact mechanism of ribonuclease activity upon living cells is not known, it has been postulated that this enzyme acts by breaking down soluble ribonucleic acid or by forming a complex with this acid and thus inactivating it(6). A variety of cells treated with ribonuclease show abnormal incorporation of amino acids into protein molecules, impairment of protein synthesis, and depression of cell growth and mitotic activity (cf. 7). It is not unlikely that ribonuclease exerts a similar effect on the lymphoid cells used in our experimental model. Since our data show that the specific activity of immune lymph node cells is inhibited, it is probable that the trans-

fer reaction is ultimately bound to the protein anabolism of immunologically active cells, *i.e.* intact protein synthesis is essential for expression of the reaction. However, it remains to be determined whether this is an increased synthesis of specific protein or antibody in situ ("local secondary specific protein or antibody response") on second contact of immunologically active cells with specific antigen, or the increased synthesis of protein accompanying increased cell size and/or proliferation.

Summary. Incubation of homograft sensitized lymphoid cells with ribonuclease inhibits their ability to produce the "transfer reaction" on intradermal injection into the antigen donor. Ribonuclease in the concentration used does not kill the cells as determined by trypan blue staining and culture in Millipore filter chambers. The data indicate that intact protein synthesis of transferred immunologically active cells is essential for the expression of the "transfer reaction."

1. Brent, L., Brown, J. B., Medawar, P. B., *Lancet*, 1958, v2, 561.
2. ————, in *Biological Problems of Grafting*, 1959, Blackwell Scientific Publ., Oxford, p64.
3. Allfrey, V. G., Daly, M. M., Mirsky, A. E., *J. Gen. Physiol.*, 1953, v37, 157.
4. Brachet, J., *Nature*, 1954, v174, 876.
5. Ledoux, L., Baltus, E., *Experientia*, 1954, v10, 500.
6. Brachet, J., *Nature*, 1960, v186, 194.
7. ————, *Biochemical Cytology*, 1957, Academic Press Inc., New York, p264.

Received August 11, 1961 P.S.E.B.M., 1961, v108

Effect of Solvents on Eosinophile-Stimulating Substance of Erythrocyte Stroma.* (26998)

JOHN S. CHAPMAN (Introduced by D. W. Seldin)
Department of Internal Medicine and William Buchanan Laboratory, University of Texas Southwestern Medical School

It has been shown that blood is capable of exciting in animals a peritoneal reaction which in its later stages is characterized by the appearance of eosinophiles in definitely elevated proportions. It was then established that the component of blood responsible for this reaction was neither plasma nor hemoglobin but the stroma and membranes of erythrocytes (1,2).

The present studies were undertaken as the first steps toward isolation and identification of the eosinophile-stimulating substance (E.S.S.), if such exists.

Materials and Methods. Erythrocyte stroma was prepared as previously described.

Procedure 1. 2.5 ml of stroma was shaken repeatedly in 7.5 ml ether at room temperature, allowed to stand over night, reshaken and allowed to sediment. After filtration the soluble fraction was evaporated in partial vacuum to dryness. The residue was suspended with repeated shaking in sterile 0.7% saline to make 10 ml, and 1 ml of the suspension was injected into the peritoneal cavity of each of 10 male albino mice. The insoluble portion was likewise suspended in 0.7% sterile saline to make 10 ml, and 1 ml was injected intraperitoneally into each of 10 male albino mice.

Procedure 2. 2.5 ml of erythrocyte stroma was suspended in 7.5 ml petroleum ether and was treated in the same manner as described in procedure 1. One ml of the hexane soluble fraction suspended in 0.7% sterile saline was injected intraperitoneally into each of 10 male albino mice, and the insoluble portion was similarly injected.

Procedure 3. This experiment was carried out in the same way as procedure 1, except that chloroform was used as the initial extraction agent. Both soluble and insoluble fractions were resuspended and injected as before.

Procedure 4. Except for the use of acetone as the initial solvent this procedure was iden-

* This study was supported originally by a grant from the Dallas Tuberculosis Assn. and has been extended under grant from Dept. of HEW.