Table 1. Virucidal effect of the peroxidase–halide–hydrogen peroxide system. The reaction mixture (final volume 1.0 ml) contained 20 μmole of sodium lactate buffer (*p*H 4.5) for poliovirus and 20 μmole of sodium-potassium phosphate buffer (*p*H 6.0) for vaccinia virus, either poliovirus [$10^{5.2}$ $TCID_{50}$/ml or 3.0×10^6 plaque-forming units (PFU) per milliliter, as indicated] or vaccinia virus ($10^{4.2}$ $TCID_{50}$/ml), and the supplements as follows: MPO, 30 units; LPO, 30 units; H_2O_2, 0.1 μmole; NaI, 0.1 μmole; NaBr, 0.1 μmole; and NaCl, 10 μmole. The total number of monolayer cultures inoculated at each virus dilution is shown in parentheses. Statistical analysis was performed as described by Finney (*9*).

Supplements	$TCID_{50}$/ml ± S.E.		PFU/ml poliovirus
	Poliovirus	Vaccinia virus*	
None	$10^{5.2\pm0.07}$ (48)	$10^{4.2\pm0.2}$ (12)	3.0×10^6 (8)
MPO+H_2O_2+NaI	$10^{0.8\pm0.09}$ (24)†	$10^{1.4\pm0.1}$ (8)†	< 100 (10)†
MPO(heated)+H_2O_2+NaI	$10^{4.9\pm0.18}$ (8)		2.7×10^6 (6)
MPO+H_2O_2	$10^{4.8\pm0.2}$ (8)	$10^{3.1\pm0.2}$ (12)†	2.8×10^6 (4)
MPO+NaI	$10^{5.1\pm0.27}$ (8)	$10^{3.7\pm0.1}$ (8)	2.6×10^6 (10)
H_2O_2+NaI	$10^{5.1\pm0.08}$ (8)	$10^{4.0\pm0.2}$ (12)	2.5×10^6 (10)
MPO+H_2O_2+NaBr	$10^{0.8\pm0.14}$ (12)†		
MPO+H_2O_2+NaCl	$10^{1.2\pm0.22}$ (12)†		
LPO+H_2O_2+NaI	$10^{0.7\pm0.10}$ (20)†	$10^{0.8\pm0.1}$ (8)†	< 100 (6)†
LPO(heated)+H_2O_2+NaI	$10^{5.3\pm0.23}$ (8)		2.8×10^6 (4)
LPO+H_2O_2	$10^{5.0\pm0.21}$ (12)	$10^{2.6\pm0.1}$ (12)†	2.5×10^6 (6)
LPO+NaI	$10^{5.4\pm0.21}$ (8)	$10^{4.1\pm0.2}$ (8)	3.6×10^6 (4)
LPO+H_2O_2+NaBr	$10^{1.1\pm0.38}$ (8)†		
LPO+H_2O_2+NaCl	$10^{5.0\pm0.19}$ (8)		

* Vaccinia virus was used at *p*H 6.0 due to its inactivation in buffer alone at a more acid *p*H.
† Value significantly different from that of the control ($P < .01$).

occurs under conditions in which a bactericidal effect is observed, and thus the two may be causally related (*2*). A similar mechanism may explain the virucidal effect of the peroxidase–iodide–hydrogen peroxide system since iodination of virus particles can occur and may result in a loss of infectivity (*11*). Myeloperoxidase and H_2O_2 have an antimicrobial effect in the absence of halide under certain conditions (*3, 12*). A significant but reduced virucidal effect on vaccinia virus (but not on poliovirus) in the absence of halide was observed here. The addition of a halide with the virus preparation or with the other reagents cannot be excluded.

The components of the peroxidase–halide–hydrogen peroxide virucidal system are present in mammalian tissues and extracellular fluids. Peroxidases are found in the mammary, salivary, lacrimal, and harderian glands and their secretions (lactoperoxidase), in neutrophils (myeloperoxidase), in eosinophils, and in the thyroid. Hydrogen peroxide is formed in the polymorphonuclear leukocyte after phagocytosis (*13*), in certain extracellular fluids (for example, by the xanthine–xanthine oxidase system in milk), and by microbial metabolism either within leukocytes or extracellularly. The halides are widely distributed in extracellular and intracellular fluids although their concentration varies.

Many viruses are engulfed by neutrophils, mononuclear cells, and tissue macrophages (*14–16*). Some investigators have emphasized the protection afforded by intraleukocytic residence of viruses against specific antibodies and other nonspecific viral inhibitors of blood and have pointed to phagocytosis as a means of disseminating viral particles (*15*). However, others have reported a rapid decrease in the titer of certain viruses following ingestion by leukocytes (*16*). The nature of the intraleukocytic virucidal systems is not known. Peroxidase-mediated systems may exert a virucidal effect in leukocytes and in certain extracellular fluids (for example, saliva, milk, tears, and areas of inflammation) and may thus contribute to the host defense against viral infection.

MELVIN E. BELDING
SEYMOUR J. KLEBANOFF
U.S. Public Health Service Hospital, Seattle, Washington 98114, and
Department of Medicine, University of Washington School of Medicine, Seattle 98105
C. GEORGE RAY
Department of Pediatrics and Microbiology, University of Washington School of Medicine

References and Notes

1. S. Baron, *Mod. Trends Med. Virol.* **1**, 77 (1967); E. F. Wheelock, R. P. B. Larke, N. L. Caroline, *Progr. Med. Virol.* **10**, 286 (1968); M. R. Hilleman, *Science* **164**, 506 (1969).
2. S. J. Klebanoff, *J. Exp. Med.* **126**, 1063 (1967).
3. ———, *J. Bacteriol.* **95**, 2131 (1968).
4. R. I. Lehrer and M. J. Cline, *Clin. Res.* **17**, 121 (1969).
5. K. Agner, *Acta Chem. Scand.* **12**, 89 (1958).
6. M. Morrison and D. E. Hultquist, *J. Biol. Chem.* **238**, 2847 (1963).
7. S. J. Klebanoff, *Endocrinology* **76**, 301 (1965).
8. The $TCID_{50}$ (tissue culture infective dose 50 percent effective) is the amount of virus which produces cytopathic effect in 50 percent of the tube monolayer cultures.
9. D. J. Finney, *Statistical Methods in Biological Assay* (Hafner, New York, 1952), pp. 523–530.
10. Human myeloperoxidase supplied by J. Schultz.
11. H. Fraenkel-Conrat, *J. Biol. Chem.* **217**, 373 (1955); ——— and M. Sherwood, *Arch. Biochem. Biophys.* **120**, 571 (1967); M. L. Anson and W. M. Stanley, *J. Gen. Physiol.* **24**, 679 (1941); Y. Hsu, S. Nomura, C. W. Krusé, *Amer. J. Epidemiol.* **82**, 317 (1966).
12. R. J. McRipley and A. J. Sbarra, *J. Bacteriol.* **94**, 1425 (1967).
13. G. Y. N. Iyer, D. M. F. Islam, J. H. Quastel, *Nature* **192**, 535 (1961); M. Rechcigl and W. H. Evans, *ibid.* **199**, 1001 (1963); J. Roberts and Z. Camacho, *ibid.* **216**, 606 (1967); B. Paul and A. J. Sbarra, *Biochim. Biophys. Acta* **156**, 168 (1968); M. Zatti, F. Rossi, P. Patriarca, *Experientia* **24**, 669 (1968).
14. E. Kovács, R. K. Baratawidjaja, A. Walmsley-Hewson, N. A. Labzoffsky, *Arch. Ges. Virusforsch.* **14**, 143 (1963); E. Nelson, H. Hager, E. Kovács, *Amer. J. Pathol.* **44**, 29 (1964); R. G. Sommerville and P. S. MacFarlane, *Lancet* **1964-I**, 911 (1964); R. K. Baratawidjaja, L. P. Morrissey, N. A. Labzoffsky, *Arch. Ges. Virusforsch.* **17**, 273 (1965); R. G. Sommerville, *ibid.* **19**, 63 (1966).
15. I. Gresser and D. J. Lang, *Progr. Med. Virol.* **8**, 62 (1966).
16. R. G. Sommerville, *ibid.* **10**, 398 (1968).
17. Supported by PHS grants AI-07763 and AM-1000. We thank B. Sinnott for technical assistance and E. Perrin for help in the statistical analysis.

29 August 1969; revised 3 November 1969

Antigen Competition: Antigens Compete for a Cell Occurring with Limited Frequency

Abstract. *Experimentally altered ability of transferred spleen cells to generate hemolytic plaque-forming cells provided evidence that antigens compete for a type of multipotential cell that contributes to the formation of immunologically competent units. Delay of exposure of transferred spleen cells to antigen provided results which suggest that different types of cells interact to form competent, antigen-reactive units even in the absence of antigen.*

Concurrent primary antibody formation against an unrelated antigen quantitatively reduces the primary immune response to a given test antigen. Immunologists have known of antigen competition for many years (*1*; for review, see *2*) but have no satisfactory explanation of the manner in which antigens compete. Competition occurs between soluble antigens (*3*), particulate antigens (*4–6*), a soluble and a particulate antigen (*7*), and between chemically defined haptens (*8–10*). Response to a particulate antigen may prolong survival of allografts (*5, 7*). Sev-

eral authors conclude that antigens do not compete at the level of immunocompetent cells (*6, 11, 12*), but competition probably involves some type of humoral factor (*6*). Others (*4, 5, 8, 9*) have interpreted their studies as indicating competition for some type of multipotential cell—either an antigen-processing cell or an ancestor of antibody-producing cells.

Most of our studies on competition of antigens (*13*) concern production of hemagglutinating antibodies. The pertinent aspects of these studies are: (i) Competition of antigens is displayed by cells derived from the nonprimed animal but not by cells from animals preprimed with the test antigen; (ii) competition is not the result of physical blockade that might prevent the test antigen from reaching sites of immunologically competent cells; (iii) competition is greatest when 3 to 4 days separate administration of competing and test antigens; and (iv) a high dose of competing antigen virtually eliminates response to the test antigen when the number of immunologically competent cells in the intact mouse is nonspecifically reduced by x-rays (200 r) only to the extent that the remaining fraction of cells does not exceed the number responsive to an optimum dose of test antigen (*14*). In this report, we describe an analysis of antigen competition in which the production of hemolysin-forming cells was assessed by the procedure of Jerne *et al.* (*15*). Our results and conclusions differ from those in similar studies by Radovich and Talmage (*6*).

Our procedure was to assess the immune response of varying numbers of immunologically competent cells in the presence or absence of a competing antigen. Recipient mice (*16*) were x-irradiated (850 r) and immediately injected intravenously with spleen cells from unprimed, syngeneic donors. Competing antigen, when used, was mixed with the spleen cells in vitro and the mixture injected into recipient mice. Donor spleen cells were administered in a graded series of doses.

Horse erythrocytes (Hrbc) were used as the competing antigen and sheep erythrocytes (Srbc) as the test antigen, both in near-maximum quantities. There is little cross-reaction of antibodies directed against these antigens (*6*). When the competing antigen was used, each mouse received 2×10^9 Hrbc. Three days later, the Srbc were injected, and the response to Srbc was assessed in groups of mice killed daily to determine the peak production of hemolytic-plaque–forming cells (PFC). The 3-day separation of the injection of the two antigens assured maximum competition (*4, 6, 13*). Measurements of production of PFC against Srbc as a function of the number of donor cells transferred to recipients gave positive control data. The Srbc were injected 3 days after spleen cell transfer.

Figure 1 shows the peak production of PFC as a function of the number of donor spleen cells for noncompetitive and competitive conditions, and also the background control level of PFC produced by transferred spleen cells not stimulated with Srbc. For both competitive and noncompetitive conditions, the relation between the logarithms of the yield of PFC and of the number of spleen cells transferred is monophasic. Both lines extend to background level without inflections. A two-fold increase in the inoculum of transferred cells, over a 100-fold range, doubles the output of PFC.

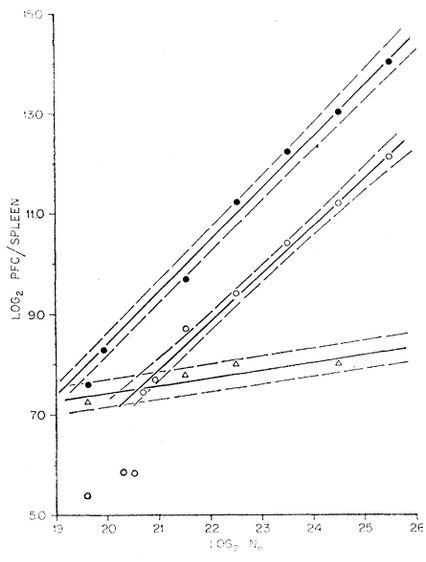

Fig. 1. Production of hemolytic-plaque–forming cells by varying inoculums of nonprimed donor mouse spleen cells (N_0) transferred into irradiated, syngeneic recipients. Response to 2×10^9 sheep erythrocytes injected 3 days after transfer of spleens (●) or a mixture of spleen cells and competing antigen, horse erythrocytes (○); broken lines, 95 percent confidence belts. Controls (△) consisted of spleen-cell inoculums exposed to either horse erythrocytes or no antigen at all; broken lines, twice the standard errors of estimate. Slopes of lines for noncompetitive and competitive responses are not significantly different from 1.0 and not different from each other. Lines were fitted to points by the least-squares method by use of the estimating equation, $\log_2 Y = a + b \log_2 x$. Points represent logarithms of the geometric means of 5 to 20 replicates of each inoculum of spleen cells cultured in irradiated, syngeneic recipient mice.

Clearly, for any given spleen-cell inoculum, prior exposure of the cells to a competing antigen reduces the response to the test antigen to approximately 30 percent of the noncompetitive level (Fig. 1)—a highly significant effect as shown by the 95 percent confidence belts. Among the possible explanations for this result, the most interesting to us was that competition involves access of the antigens to some type (or types) of cell present in limited numbers.

Null response was tested for competitive and noncompetitive conditions with inoculums of spleen cells calculated to give negative and positive responses. We decided what yield of PFC per spleen of recipient mice constituted a positive response based on the following analysis. Varying inoculums of spleen cells were cultured in recipient animals and either not exposed to antigen or exposed to Hrbc only (shown as background in Fig. 1). The slope of the line is 0.15. We tested the veracity of this relation by extrapolating the line to $\log_2$ dose $= 0$ and comparing the intercept on the ordinate with the number of PFC per spleen obtained in control experiments in which irradiated mice received neither spleen cells nor antigen. This comparison showed that the value of the intercept of the extrapolated line is not significantly different from the background number of PFC per spleen in the irradiated mouse. A response was assumed to have occurred when an individual value of PFC produced by a given inoculum, less the standard error obtained from all the replicate samples of that inoculum, exceeded the upper limit obtained when twice the standard error of the estimate was added to the fitted value for the background controls. When this criterion was applied to the values of all samples obtained over a range of inoculums (for competitive and noncompetitive conditions), the frequency of positive responses could be analyzed by a maximum-likelihood method (*17*). The frequency of cell units competent to respond to the test antigen (Srbc) was three times as great in the absence of a competing antigen as in its presence, agreeing very well with the reduction in yield of PFC by a competing antigen.

The null-response data (frequency of

nonresponders) is shown in Fig. 2, where the log proportion of nonresponders is plotted as a function of the spleen cell inoculums, as in target analysis. This plot indicates a difference in mechanism underlying noncompetitive and competitive responses. That is, the noncompetitive response may be described as a "single-hit" process and the competitive response as "multihit."

The monophasic relation between the production of PFC and the size of the inoculum of spleen cells (Fig. 1) is distinctly different from the biphasic relation found by previous investigators in this laboratory (*18, 19*). The difference is explained by a difference in experimental procedure, namely, the time of injection of antigen relative to spleen-cell inoculation. In the previous investigations, spleen cells were mixed with antigen at the time they were transferred to irradiated recipients. In our experiments, 3 days separated the transfer of spleen cells from the time of injection of test antigen.

The biphasic relation has been interpreted as revealing the necessity for interaction of two distinct cell types in formation of an immunologically competent unit (*19*). One phase of the relation has a slope of about 2, suggesting that this phase typifies the interaction of antigen-reactive cells with ancestral precursors of functional antibody-forming cells. The antigen-reactive cells are considered to have multiple binding sites for these precursor cells; but the binding sites may become saturated with these progenitor cells and, when this occurs, the relation between PFC yield and the spleen-cell inoculum changes to a slope of 1. These conclusions are well documented (*19*) and agree with the conclusions of other investigators (*20*).

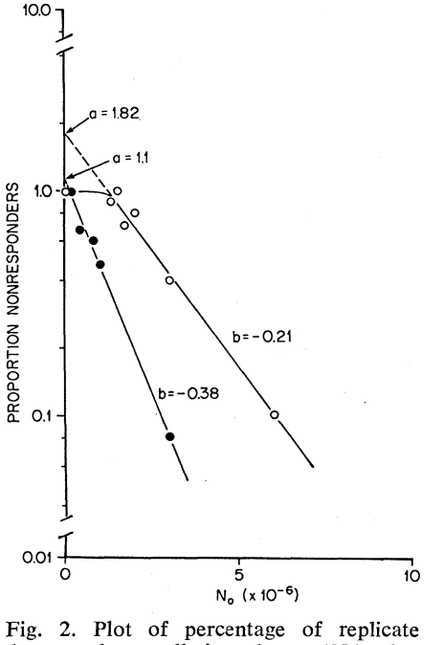

Fig. 2. Plot of percentage of replicate donor spleen cell inoculums (N_0) that failed to produce a significant number of hemolytic-plaque–forming cells above the background control number. Lines fitted to points for noncompetitive (●) and competitive (○) responses by the least-squares method by use of the estimating equation, $\log_{10} Y = a + bx$. Each point represents 5 to 20 replicates of each inoculum of spleen cells cultured in irradiated, syngeneic recipient mice.

Since the relationship of Fig. 1 shows no indication of a phase with slope greater than 1, it can be concluded that the 3-day interval before antigen stimulation is sufficient for precursor cells to saturate the binding sites on antigen-reactive cells. If correct, this would mean that the interaction of the two types of cells to form a unit occurs in the absence of specific antigen. It would also suggest that the effect of a competing antigen is to uncouple the association between antigen-reactive cells and precursor cells.

Our data indicate that antigens compete with each other in activation of immunologically competent units. This competition is revealed in both the reduction of the yield of PFC per inoculum of a given magnitude and the decreased frequency of competent units resulting from the presence of the competing antigen. Inasmuch as the antigen-reactive cells are the cells occurring in limiting frequency (*19*), they are probably the cells that may be preempted by competing antigen. This interpretation is somewhat belied by the "multihit" relation for the competitive condition shown in Fig. 2. Precise interpretation of this figure is not yet clear, but the shoulder on the competitive curve indicates a threshold event in the course of establishing response to the test antigen. This effect might be termed competition pressure.

From studies similar to those reported here, Radovich and Talmage (*6*) concluded that competition of antigens involved action of a humoral factor. Their conclusion was based on the observation that competition was marked for a given inoculum of spleen cells but not revealed by an inoculum one-fifth as large. Their assessment of PFC was made at a single time after antigen stimulation. We find, as others (*18*) have, that the time of peak production of PFC varies with the size of the inoculum. We assume that our care to determine peak production of PFC by each inoculum accounts for the difference between our results and those of Radovich and Talmage.

Investigations of antigen competition in in vitro, antibody-forming systems revealed no evidence of competition for immunologically competent cells (*11, 12*; but see *21*). The competition may not be demonstrable with the systems of dispersed cells presently used because of the requirement for a suitable length of time between introduction of competing and test antigens. The functional life of such in vitro systems seems too short.

J. F. Albright
Thomas F. Omer
Julia W. Deitchman
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37803

References and Notes

1. L. Michaelis, *Deut. Med. Wochenschr.* **28**, 733 (1902); *ibid.* **30**, 1240 (1904); L. Hektoen and A. K. Boor, *J. Infect. Dis.* **48**, 588 (1931); M. Björneboe, *Z. Immunitaetsforsch. Exp. Ther.* **99**, 245 (1941).
2. F. L. Adler, *Progr. Allergy* **8**, 41 (1964).
3. ———, *J. Immunol.* **78**, 201 (1957); P. Abramoff, M. A. Zickes, C. A. Joyce, *Proc. Soc. Exp. Biol. Med.* **107**, 949 (1961); C. Stiffel, S. Ben-Efraim, M. F. Perramant, P. Liacopoulos, *Ann. Inst. Pasteur Paris* **111**, Suppl., 94 (1966).
4. J. F. Albright and T. Makinodan, in *Molecular and Cellular Basis of Antibody Formation*, J. Sterzl, Ed. (Czechoslovak Academy of Sciences, Prague, 1965), p. 427.
5. D. Eidinger, S. A. Khan, K. G. Millar, *J. Exp. Med.* **128**, 1183 (1968).
6. J. Radovich and D. W. Talmage, *Science* **158**, 512 (1967).
7. J. Miller, C. Martinez, R. A. Good, *J. Immunol.* **93**, 342 (1964).
8. A. A. Amkraut, J. S. Garvey, D. H. Campbell, *J. Exp. Med.* **124**, 293 (1966).
9. I. Schechter, *ibid.* **127**, 237 (1968).
10. N. I. Brody, J. G. Walker, G. W. Siskind, *ibid.* **126**, 81 (1967).
11. R. W. Dutton and R. I. Mishell, *Cold Spring Harbor Symp. Quant. Biol.* **32**, 407 (1967).
12. D. Osoba, *J. Exp. Med.* **129**, 141 (1969).
13. J. F. Albright, J. W. Deitchman, T. F. Omer, T. W. Evans, in preparation.
14. It is known [T. Makinodan, M. A. Kastenbaum, W. J. Peterson, *J. Immunol.* **88**, 31 (1962)] that an autoregulatory process prevents participation in a given response of all the cells in the intact animal that are capable of responding to a given antigen.
15. N. K. Jerne, A. A. Nordin, C. Henry, in *Cell Bound Antibodies* (Wistar Inst. Press, Philadelphia, 1963), p. 109.
16. Experimental animals were hybrid mice derived from matings of C3H Anf/Cum ♀ × 101 Anf/Cum ♂; obtained from Cumberland View Farms, Clinton, Tenn.
17. E. H. Porter and R. J. Berry, *Brit. J. Cancer* **17**, 583 (1963).
18. M. J. Bosma, E. H. Perkins, T. Makinodan, *J. Immunol.* **101**, 963 (1968).
19. D. L. Groves, W. E. Lever, T. Makinodan, *ibid.*, in press.
20. G. F. Mitchell and J. F. A. P. Miller, *J. Exp. Med.* **128**, 821 (1968); D. E. Mosier and L. W. Coppleson, *Proc. Nat. Acad. Sci. U.S.* **61**, 542 (1968); D. E. Mosier, *J. Exp. Med.* **129**, 351 (1969).
21. N. E. Cremer, *J. Immunol.* **90**, 685 (1963).
22. Sponsored by AEC under contract with Union Carbide Corporation. We are indebted to Dr. T. Makinodan and Dr. D. L. Groves for helpful discussions and suggestions. Dr. W. E. Lever provided valuable advice concerning the statistical analyses.
12 August 1969