Table 1. Immune response of guinea pigs to derivatives of oligotyrosines and tyrosine as antigens; PCA, passive cutaneous anaphylaxis.

Days after sensitization	Animals (No.)		Delayed skin reaction, average diameter (mm)
	Responding	With PCA-positive serums	
p-Azobenzenearsonate–hexa-L-tyrosine			
10	10/10	0/10	12
18	9/10	1/10	16
25	7/8	6/7	17
p-Azobenzenearsonate–tri-L-tyrosine			
10	9/10	0/10	10
18	10/10	0/10	11
25	10/10	7/10	16
p-Azobenzenearsonate–N-acetyl-L-tyrosine amide			
10	2/5	0/5	6
18	5/5	0/5	9
25	5/5	1/5	9
32	5/5	3/4	12
p-Azobenzoate–hexa-L-tyrosine			
10	0/5	0/5	0
18	0/5	0/5	0
p-Azobenzenesulfonate–hexa-L-tyrosine			
10	0/5	0/5	0
18	0/5	0/5	0

peptides of low molecular weight, such as tris-2,4-dinitrophenyl-bacitracin (molecular weight, 1928) (*12*) and α-2,4-dinitrophenyl-hepta-L-lysine (molecular weight, 1080) (*13*), were also reported to be antigenic. Substances of even lower molecular weights are known to be capable of inducing allergic states in humans and in experimental animals; these act as haptens and form complete antigens either by direct chemical combination with body proteins in vivo [for example, picryl chloride (*14*) and penicillin (*15*)] or by undergoing certain metabolic changes that result in the formation of one or more substances that subsequently react with proteins of the host, such as *p*-phenylenediamine dyes (*16*). Available evidence indicates that in such cases the bond between hapten and protein is covalent (*17*). Cases have been reported, however, of immune response to substances of low molecular weight that are not known to form covalent bonds with proteins, such as arsphenamine (molecular weight, 367) (*18*) and neoarsphenamine (molecular weight, 466) (*19*).

In order to determine whether or not binding between the oligotyrosine conjugates and guinea pig proteins may be responsible for initiating the immune response to these low-molecular-weight substances, 10 mg of iodine-131–labeled *p*-azobenzenearsonate–hexa-L-tyrosine was kept in vitro with 5 ml of normal guinea pig serum for 1 hour at room temperature and then overnight at 4°C. Subsequently we fractionated the mixture on a diethylaminoethyl-cellulose column, using the concentration gradient with a 0.02*M* to 0.3*M* K_2HPO_4-KH_2PO_4 buffer system at *p*H 8 (*20*).

Some of the labeled material was associated with serum fractions, mainly in the prealbumin and albumin regions (Fig. 1). The identity of the proteins involved was confirmed by paper electrophoresis (0.025*M* borate buffer, *p*H 9) of the isolated fractions. Most of the labeled conjugate was eluted in an unbound form. Fractionation of guinea pig serums that had been kept under analogous conditions with iodine-131–labeled hexatyrosine or with its iodine-131–labeled *p*-azobenzoate derivative, both of which are nonimmunogenic, yielded similar results.

Thus it seeems that, while non-covalent binding to proteins may be a factor in the sensitizing capacity of *p*-azobenzene-arsonate–oligotyrosines, alone it cannot sufficiently explain the mechanism of sensitization to these substances. Whatever the mechanism involved, our evidence shows that certain substances that are of small molecular size (molecular weight as low as 450) and do not form covalent bonds with proteins are nevertheless immunogenic.

FELIX BOREK
YEHUDIT STUPP
MICHAEL SELA

Section of Chemical Immunology, Weizmann Institute of Science, Rehovoth, Israel

References and Notes

1. E. Katchalski, M. Sela, H. I. Silman, A. Berger, in *The Proteins*, H. Neurath, Ed. (Academic Press, New York, 1964), vol. 2, p. 405; M. Sela, in *Advances in Immunology*, F. J. Dixon, Jr., and J. H. Humphrey, Eds. (Academic Press, New York, in press), vol. 5.
2. F. S. Kantor, A. Ojeda, B. Benacerraf, *J. Exp. Med.* **117**, 55 (1963).
3. S. Leskowitz, *ibid.*, p. 909.
4. F. Borek and Y. Stupp, *Immunochemistry*, in press, and unpublished data.
5. Donated by R. Arnon; prepared by condensation of *N*-carbobenzoxytrityrosine with trityrosine methyl ester by the *N,N*-dicyclohexylcarbodiimide method, removal of the carbobenzoxy group with HBr in glacial acetic acid, and alkaline hydrolysis of the ester group.
6. Both obtained from Yeda Research and Development Co., Ltd., Rehovoth.
7. D. Kupke, *Compt. Rend. Trav. Lab. Carlsberg* **32**, 167 (1961).
8. M. Tabachnick and H. Sobotka, *J. Biol. Chem.* **235**, 1051 (1960).
9. We thank S. Leskowitz for this information.
10. M. Sela, S. Fuchs, R. Arnon, *Biochem. J.* **85**, 223 (1962).
11. G. Berglund, *Nature* **206**, 523 (1965).
12. J. G. Abuelo and Z. Ovary, *J. Immunol.* **95**, 113 (1965).
13. S. F. Schlossman, A. Yaron, S. Ben Efraim, H. A. Sober, *Federation Proc.* **24**, 319 (1965).
14. K. Landsteiner and M. W. Chase, *J. Exp. Med.* **66**, 337 (1937).
15. F. R. Batchelor, J. M. Dewdney, D. Gazzard, *Nature* **206**, 362 (1965).
16. R. L. Mayer, in *Progress in Allergy*, P. Kallos, Ed. (Karger, Basel-New York, 1954), vol. 4, p. 79.
17. H. N. Eisen, in *Cellular and Humoral Aspects of the Hypersensitive States*, H. S. Lawrence, Ed. (Hoeber-Harper, New York, 1959), p. 89.
18. K. Landsteiner and J. L. Jacobs, *J. Exp. Med.* **64**, 717 (1936).
19. W. Frei, *Klin. Wochenschrift* **7**, 539, 1026 (1928).
20. J. L. Fahey and A. P. Horbett, *J. Biol. Chem.* **234**, 2645 (1959).
21. Supported by U.S. Air Force grant 64-22. One of us (F.B.) received a fellowship (1-F3-AI-19, 910-02) from NIH.

22 June 1965

Bacterial Stimulation of Sporangium Production in Phytophthora cinnamomi

Abstract. *Bacteria, notably* Chromobacterium violaceum, *stimulate initiation of production of sporangia by* Phytophthora cinnamomi, *a plant pathogen which does not produce this asexual stage in ordinary agar or liquid culture.*

Reproduction in fungi and in other microorganisms is affected in various ways by a number of environmental and nutritional factors. This report concerns asexual reproduction by sporangia in *Phytophthora cinnamomi* Rands, a cosmopolitan plant pathogen with a large host range (*1*), and in two other species of the genus. *Phytophthora cinnamomi* does not produce either sporangia or oospores in ordinary agar or liquid culture.

In 1935 Mehrlich (*2*) stimulated sporangium production in *P. cinnamomi* with a nonsterile soil extract. The basis for the effect was not elaborated beyond presentation of evidence that heating the extract to 121°C for 20 minutes destroyed the stimulatory principle.

In connection with studies of *P. cinnamomi* as the casual agent of a serious root rot of avocado trees in California and Latin America, a modification of Mehrlich's method has been used in our laboratory as follows. Ten grams of moist soil are added to 1000 ml of deionized water; and the mixture is shaken vigorously and filtered through Whatman No. 42 filter paper in a Buchner funnel under a slight vacuum. The filtered extract may then be stored in a dark bottle at

room temperature; under such conditions, the extract has retained activity over 1-year storage periods. To test sporangium production, disks of mycelium and agar from the margin of a culture on V-8 juice agar are placed in the soil extract in petri dishes.

Formation of sporangia was reported in 1959 (*3*), and I presented further data on stimulation in 1962 (*4*).

Data reported in 1959 and from subsequent experiments with nonsterile soil extract indicated the involvement of a microbial agent in asexual sporulation. Sterilization of the extract by autoclaving [15 lb (7 kg) for 20 minutes] or filtration (Seitz or Berkefeld filters) removed the stimulatory factor. This factor was thermolabile; it was eliminated from the soil extract by incubation at 45°C for 5 days or at 50°C for 10 minutes, but not by freezing. Results with dilution and dialysis also supported the microbial theory.

The nonsterile soil extract has also stimulated formation of sporangia in the opposite (A_1) mating type of *P. cinnamomi* (*5*) and increased production of sporangia in *P. citrophthora* and *P. palmivora*.

Subsequently many soil bacteria were isolated and tested for influence on sporangium development by *P. cinnamomi* in sterile extract. Stimulation was obtained in Australia in the spring of 1964 in a number of tests with a purple-pigmented bacterium identified as *Chromobacterium violaceum* (Schroeter) Bergonzini (*6*). This bacterium was first isolated from South Australian soils by the soil-dilution plate method in 1964–65. This organism was also isolated from soil in South Australia by the rice-grain method (*7*); it produced a deeply pigmented culture, the pigment was not soluble in water, the bacterium did not grow at 37°C, and no endospores were produced.

When *C. violaceum* was added to soil extract sterilized by autoclaving, sporangium production in *Phytophthora cinnamomi* ranged from 5 to 40 percent of that in the nonsterile soil extract. Usually the bacteria were suspended in sterile distilled water; then 0.05 to 0.10 ml of this suspension was pipetted into 8 ml of sterile soil extract in a small petri dish. Stimulation of sporulation was more consistent and of higher magnitude when a culture of *Chromobacterium violaceum* which had been recently isolated from soil, or recently transferred, was used. No stimulation occurred in several tests with bacterial cultures several weeks old, including an isolate of *C. violaceum* from California soils (*8*). The *C. violaceum* freshly isolated on rice grains from California soils was effective in inducing formation of sporangia. Corpe (*9*) reported considerable variation in this group. Soils from different areas varied considerably in stimulation of sporangium production; this could reflect differences in bacterial population.

Several other bacteria and combinations of bacteria have given occasional stimulation of sporangium production in *Phytophthora cinnamomi*, in tests in California and in Australia. Washings from dilution plates containing a number of different bacteria, when added to sterile soil extract containing disks of mycelium of *P. cinnamomi*, have consistently caused sporangia to be produced. Thus, *Chromobacterium violaceum* is not the only microorganism involved in the phenomenon. This is indicated also by reports on the stimulation of sporulation in *Phytophthora cinnamomi* by bacteria or diffusates from nonsterile soil extract (*10*, *11*); in one case "aseptic" sporangia were reported on the side of Millipore membranes opposite a soil extract (*11*).

Chromobacterium violaceum, although not readily isolated from soil, is a common inhabitant of many soils throughout the world, and it also is commonly found in water. Thus this organism could well be one of the important biotic agents providing the stimulus for production of this essential spore stage by *Phytophthora cinnamomi* and other species of the genus.

That several tests of spring water and other water sources for stimulation of sporangium production have given positive results further substantiates the probable importance of an organism such as *Chromobacterium violaceum*. Another link in the involvement of this bacterium in sporulation is provided by the data on inactivation of the factor or agent at temperatures of 45° to 50°C. This correlates with the fact that *C. violaceum* does not grow at 37°C and does not produce spores.

There are indications that the substance or substances influencing production of sporangia are intimately associated with the bacterial cell, for removal of bacteria from the soil extract by filtration through a Millipore filter (0.45-micron pore size) also prevented development of sporangia. Filtration of the extract through Millipore filters with pore sizes of 3.0 or 1.2 microns did not remove bacteria; these filtrates stimulated production of sporangia. Undoubtedly *C. violaceum* and other microorganisms in the soil are producing some metabolite or metabolites that are essential for triggering sporangium production in *Phytophthora*.

GEORGE A. ZENTMYER
Department of Plant Pathology, University of California, Riverside

References and Notes

1. G. A. Zentmyer and A. O. Paulus, *Calif. Agr. Expt. Sta. Circ. 465* (1957).
2. F. P. Mehrlich, *Phytopathology* **25**, 432 (1935).
3. G. A. Zentmyer and L. A. Marshall, *ibid.* **49**, 556 (1959).
4. Conference on *Phytophthora cinnamomi*, 14–15 April 1962. Proceedings edited by G. A. Zentmyer, issued in duplicated form, 1963; 12 pp.
5. J. Galindo A. and G. A. Zentmyer, *Phytopathology* **54**, 68 (1964).
6. Culture identified by J. Harris, Commonwealth Scientific Industrial Research Organization, Adelaide, South Australia.
7. W. A. Corpe, *J. Bacteriol.* **62**, 515 (1951).
8. Isolate provided by J. P. Martin, Department of Soils and Plant Nutrition, University of California, Riverside.
9. W. A. Corpe, *J. Bacteriol.* **66**, 473 (1953).
10. K. H. Chee and F. J. Newhook, *New Zealand J. Agri. Res.*, in press; W. J. Manning, *Phytopathology* **55**, 505 (1965).
11. D. H. Marx and F. A. Haasis, *Nature* **206**, 673 (1965).
12. Supported in part by NSF grant GB-658. A portion of the research was done at the Waite Agricultural Research Institute, Adelaide, South Australia, while I held a Guggenheim fellowship. I thank M. V. Bumbieris, Waite Agricultural Research Institute, Adelaide, and J. Castro and S. M. Mircetich, Department of Plant Pathology, University of California, Riverside, for technical assistance.

24 September 1965

Quenching of DNA Phosphorescence

Abstract. *Paramagnetic cations quench the phosphorescence of DNA at concentrations well below one ion per DNA phosphate. The order of quenching efficiency is copper, nickel, cobalt, and manganese.*

Low concentrations of Mn^{++} quench the phosphorescence of DNA (*1*). We now report that Mn^{++}, while itself a good quencher, is inferior to other paramagnetic cations tested. The quenching efficiency from highest to lowest is in the order Cu^{++}, Ni^{++}, Co^{++}, Mn^{++}.