represents the radioactive globulin, was less than 0.2 percent of the corresponding specific activity in the serum; for this calculation it was assumed that 0.01 mg of IgA per milliliter was present in the serum. This concentration (0.01 mg/ml) of IgA was the maximum amount of IgA which could be present and not detected by the techniques used; if a lower serum concentration of IgA were present, the discrepancy between specific activity of IgA in serum and that in saliva would be even greater. Thus the IgA in the saliva of these patients cannot originate solely from transport from the serum and must arise from another source, presumably in a salivary tissue.

The deficiency of IgA in the serum of the patients studied can be explained by a severe defect of IgA synthesis. However, the immunofluorescent evidence of IgA in the bone marrow suggests that the synthetic defect is incomplete. Other mechanisms such as impaired IgA release or increased catabolism may contribute to the deficiency in serum. Whatever the factors leading to this serum abnormality, there was no apparent defect of IgA production in salivary gland.

D. E. McFarlin
*National Institute of Neurological Diseases and Blindness, Bethesda, Maryland*

W. Strober
R. D. Wochner
T. A. Waldmann
*National Cancer Institute, Bethesda, Maryland*

### References and Notes

1. M. Mannik and H. G. Kunkel, *Bull. Rheumat. Dis.* **13**, 309 (1963); R. Ceppellini, *Immunochemistry* **1**, 145 (1964).
2. T. B. Tomasi, E. M. Tan, A. Solomon and R. A. Pendergast, *J. Exp. Med.* **121**, 101 (1965).
3. St. Thieffry, M. Arthuis, J. Alcardi, G. Lyon, *Rev. Neurol.* **105**, 390 (1961); R. R. Young, K. F. Austin, H. W. Moser, *Clin. Res.* **12**, 240 (1964); R. D. Peterson, W. D. Kelly, R. A. Good, *Lancet* **1964-I**, 1189 (1964); P. Fireman, M. Boseman, D. Gitlin, *ibid.*, 1195 (1964).
4. J. L. Fahey and E. M. McKelvey, *J. Immunol.* **94**, 89 (1965).
5. W. Strober, R. D. Wochner, T. A. Waldmann, M. Barlow and D. McFarlin, in preparation.
6. A. Solomon, J. L. Fahey, R. A. Malmgren, *Blood* **21**, 403 (1963).
7. Hyland Laboratory—#70-898. The globulin fraction containing antiserum to IgA was isolated by diethylamine ethyl cellulose chromatography and conjugated with fluorescein isothiocyanate. Excess fluorescein was removed by dialysis and Sephadex G-25 chromatography.
8. These patients have NIH numbers 12715, 12714, 23530, 60424, and 60639.
9. We thank Dr. W. K. Engel for the privilege of studying his patients and for advice during this study.

29 July 1965

## Immunogenicity and Role of Size: Response of Guinea Pigs to Oligotyrosine and Tyrosine Derivatives

Abstract. *Guinea pigs injected with 100 micrograms of* p-*azobenzenearsonate derivatives of hexa-*L-*tyrosine, tri-*L-*tyrosine, or* N-*acetyl-*L-*tyrosine amide, in complete Freund's adjuvant, developed, after 10 to 19 days, delayed-type hypersensitivity to these substances. This was shown by skin reactions, followed by the formation of circulating antibodies that were detectable by passive cutaneous anaphylaxis. Experiments with* p-*azobenzenearsonate–hexa-*L-*tyrosine labeled with iodine-131 showed that this substance was bound in vitro to proteins of normal guinea pig serum. Binding was similar with the nonantigenic hexa-*L-*tyrosine and its* p-*azobenzoate derivative.*

Synthetic polypeptides composed of only one type of amino acid are generally nonimmunogenic (*1*). On the other hand, chemical combination of organic compounds of low molecular weight with homopolymers like polylysine (*2*) or polytyrosine (*3*) may result in antigenic materials.

In a study of the role of the extent of coupling and of the degree of polymerization of various conjugates of poly-L-tyrosine with regard to their immunogenicity in guinea pigs, we found that lightly coupled *p*-azobenzenearsonate–polytyrosine was antigenic even when the polymer had an average degree of polymerization as low as 6, while similar coupling with diazonium derivatives of *p*-aminobenzoic acid and *p*-sulfanilic acid resulted in nonimmunogenic materials (*4*).

We have now examined closely the immunological properties of *p*-azobenzenearsonate derivatives of hexa-L-tyrosine (*5*), tri-L-tyrosine, and *N*-acetyl-L-tyrosine amide (*6*). The lightly coupled (*4*), colored products were purified by dialysis in preheated cellophane tubing (*7*) and lyophilized; *p*-azobenzoate and *p*-azobenzenesulfonate derivatives were similarly prepared and purified. Agar-gel electrophoresis showed that the products were homogeneous in 0.025*M* barbital buffer, *p*H 8.2; each derivative moved toward the anode as a discrete spot. In both hexa- and trityrosine derivatives, an average of one tyrosine residue per molecule was found to be coupled with the haptenic group, as determined colorimetrically (*8*).

Guinea pigs injected in the footpads with single 100-$\mu$g doses of *p*-azobenzenearsonate derivatives of hexa-L-tyrosine, tri-L-tyrosine, or *N*-acetyl-L-tyrosine amide, in complete Freund's adjuvant, developed delayed hypersensitivity to these substances after 10 to 18 days; this was shown by skin reactions, followed by formation of circulating antibodies that were detectable in the serum by passive cutaneous anaphylaxis (Table 1). In all cases studied, the presence of complete Freund's adjuvant in the sensitizing injection was found necessary to effect a positive response. Similar antigenic properties of a *p*-azobenzenearsonate derivative of L-tyrosine were observed by Leskowitz (*9*).

The smallest synthetic polypeptide antigen previously reported was a linear copolymer of tyrosine, glutamic acid, and alanine with a molecular weight of 4000 (*10*). Fibrinopeptide B (molecular weight, 1400) produced antibodies when adsorbed on acrylic plastic particles (*11*). Derivatives of

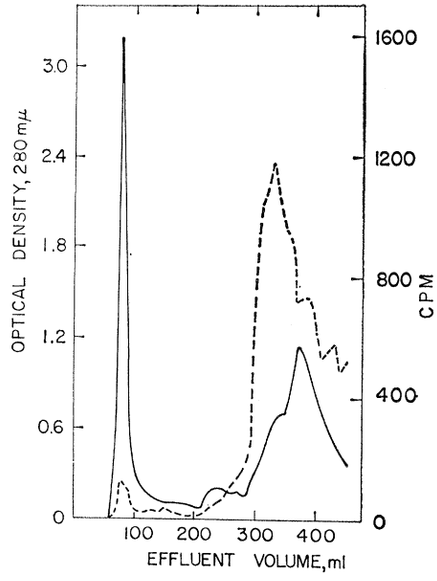


Fig. 1 Elution of normal guinea pig serum (5 ml) mixed with *p*-azobenzenearsonate–hexatyrosine (10 mg) labeled with iodine-131. A linear concentration gradient of 0.02 to 0.3*M* phosphate buffer, *p*H 8, was used on a column of diethylaminoethyl-cellulose. Solid line, ultraviolet absorbancy; broken line, radioactivity; *CPM*, count per minute.

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 $\alpha$-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