

Fig. 1. Sticky board traps used for nitidulid attractant studies. (Left) Control; (right) baited with paint primer.

test plots where partially decayed, fallen fruit became heavily infested with both larvae and adults. Subsequent infestation occurred in cull tomatoes and melons that had been distributed throughout the area, and lure traps were placed near them as the infestations occurred.

Results attained during the week of 20 July revealed an acrylic primer, a Dupli-Color product (DP-GM-3), to be the attractant for S. geminata. Some individual traps collected more than 3000 beetles.

Later tests made with a series of paint pigments, concealed vials, and colored papers confirmed that the response was to odor, not to color. Periodic testing of the paint primer during July, August, and September indicated that timing of application will be of major concern when the primer is used in a control program.

Field testing of the individual components of the primer during the latter part of August failed to indicate the active component. However, the season had advanced beyond the period of peak flight, and the response to our standard acrylic primer was negligible.

During our field investigation, two dipterous species also responded. An undetermined species responded to the acrylic primer, and Olcella parve Adams, reported by Jantz and Beroza (10) as being attracted to caproic acid, responded to amyl butyrate and ethyl butyrate.

> ORLO K. JANTZ **RICHARD F. GERTZ*** MARCUS T. WELLS, JR.*

Agricultural Research Service, U.S. Department of Agriculture, and Department of Entomology, Michigan State University, East Lansing

19 MAY 1967

References and Notes

- 1. Weekly Insect Report for Michigan Agricul-ture, compiled by A. C. Dowdy (Michigan State Univ., East Lansing, 1965–66), vols. 3 and 4.
- 2. A. H. Howitt, personal communication.
- 3. Lawrence Phillips, personal communication.
- Dupli-Color touch-up car paints, Dupli-Color Products Co., Inc., 1601 Nicholas Boulevard, Elk Grove Village, Illinois. W. A. Connell, Delaware Univ. Agr. Exp. 5.
- Sta. Bull. 318 (1956).
- D. M. Daugherty and C. H. Brett, N. Carolina State Coll. Agr. Exp. Sta. Tech. Bull. 171 (1966).
- J. F. Illingworth, Proc. Hawaiian Entomol. Soc. 7, 254 (1929); C. T. Schmidt, Ann. Entomol. Soc. Amer. 28, 475 (1935).
- W. A. Connell, J. Econ. Entomol. 49, 539 (1956); G. F. Knowlton, ibid. 35, 105 (1942). 9. C. K. Dorsey and J. G. Leach, ibid. 49, 219
- (1956) 10. O. K. Jantz and M. Beroza, J. Econ. En-
- O. K. Jantz and M. Beroza, J. Econ. Entomol. 60, 290 (1967).
 We thank Mr. Wirt V. Dunlop, president, and Mr. Stanley E. Kendall, vice president, of the Dupli-Color Products Company, for their cooperation. We thank Dr. Melville Hatch, University of Washington, and John Newman and Thomas F. Hlavac, Michigan State University for incast indexification. State University, for insect indentification. This report is part of a cooperative project between the Entomology Research Division and the Crops Research Division, Agricultural Re-search Service, United States Department of Agriculture, and the Departments of En-tomology and Crop Science, Michigan State University, East Lansing. Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal Article No. 3969.
- Graduate students, Department of Entomology, Michigan State University.

11 April 1967

Two Types Of Lambda Polypeptide Chains In Human Immunoglobulins

Abstract. Two antigenic subtypes of human lambda polypeptide chains were distinguished by rabbit antiserum produced to a lambda Bence Jones protein. Lambda Bence Jones proteins and G myeloma proteins with lambda light chains were identified as being in one or the other subtype. The Oz (+) lambda chain subtype is present in light chains from pooled normal human immunoglobulin G and in whole normal immunoglobulin G molecules.

Knowledge of the heterogeneity within the immunoglobulin system has been advanced through immunochemical studies of Bence Jones proteins and related immunoglobulins. Heterologous antiserum has been useful for identifying subclasses of gamma polypeptide chains in man (1) and mouse (2) and in identifying genetic factors within the heavy-chain subclasses (3). Heterologous (rabbit) antiserum also has been used to identify two major types (κ and λ) of light polypeptide chains in human and mouse immunoglobulins (4). We found rabbit antiserum valuable for identifying two subtypes of lambda light polypeptide chains in human immunoglobulins.

Rabbits were immunized with λ -type Bence Jones proteins emulsified in Freund's complete adjuvant. Antiserums to 14 different Bence Jones proteins were tested, and only one antiserum (R98), prepared to Bence Jones Oz, distinguished two subtypes of lambda Bence Jones proteins. The subtype specificity was present only in blood collected over a 2-month period from this rabbit. The antiserum was rendered specific for lambda determinants by absorption with hypogammaglobulinemic serum and G myeloma protein, type K.

The specific anti- λ serum was tested against a panel of 22 lambda Bence Jones proteins which had been purified by precipitation with 80 percent ammonium sulfate, followed by zone electrophoresis, anion-exchange chromatography, or Sephadex gel filtration, or a combination of these techniques. Only the 4S peaks from gel filtration were used, eliminating the possibility that differences could be attributed to comparison of whole Bence Jones protein with fragments of the protein (5). Antigenic differences were noted on Ouchterlony analysis where the precipitin lines formed by some Bence Jones proteins, Oz (+), spurred over the precipitin lines of adjacent Oz (-) proteins (Fig. 1, left).

The observation of antigenic differences among the lambda Bence Jones proteins was extended by regrouping the proteins and comparing them in separate groups. When the Oz (+) proteins which formed spurs (Fig. 1, left) were placed in neighboring wells, reactions of identity were seen. A similar pattern was observed when the Oz(-)proteins, that is, those without spurs, were in adjacent wells. These findings indicate the existence of two categories of Bence Jones protein, those with an antigen detected by antiserum R98, Oz (+), and those without this antigen, Oz (-).

The antigenic relations of these two categories of lambda Bence Jones protein were further investigated by individual absorption with 18 Bence Jones proteins and testing of the capacity of the absorbed R98 antiserum to react with the panel of Bence Jones proteins. Absorption with a typical Oz (-) Bence Jones protein removed the ability of the antiserum to precipitate Oz (-) protein, while Oz (+) proteins continued to show strong precipitin lines (Fig. 1, right). Absorption with eight Oz (+) Bence Jones proteins removed Table 1. Comparison of subtypes urinary lambda Bence Jones protein with the serum paraprotein abnormality. The last category ("not known") includes proteins from patients for whom there are no data concerning serum immunoglobulin abnormality.

– Serum paraprotein	Urinary Bence Jones proteins		
	Oz (+)	Oz (-)	Inter- deter- minate
Bence Jones protein only	5	3	
G myeloma protein	3	1	
W macroglobulin	1	2	
A myeloma protein		1	
D myeloma protein			1
Not known	3	3	

the anti-Oz (+) activity from the rabbit antiserum. A nonprecipitating reaction occurred between some Oz (+) Bence Jones proteins and the absorbed R98 antiserum. Four Bence Jones proteins did not show spur formation over adjacent Oz (-) proteins but did remove Oz (+) activity in the absorption test. Further investigation of these four proteins revealed that they inhibited the precipitin lines of Oz (+) proteins in adjacent wells. This finding of proteins that give nonprecipitating reactions with anti-Oz (+) antibodies on Ouchterlony tests emphasized the need for absorption tests or appropriate inhibition tests. In a collection of 23 purified lambda Bence Jones proteins, 12 (55 percent) were Oz (+), while ten (45 percent) were Oz(-); one could not be classified (Table 1).

The subtypes of lambda chains could be detected in 7S immunoglobulin molecules. Purified G myeloma proteins with lambda light chains were tested by Ouchterlony analysis. Eight were Oz

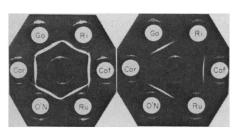


Fig. 1. Ouchterlony double-diffusion tests of λ -type Bence Jones proteins. (Left) Unabsorbed anti-\lambda-type Bence Jones protein Oz (R98) in the center well shows that three of the λ -type Bence Jones proteins give spurs over the precipitin lines of adjacent proteins. (Right) The same antiserum has been absorbed with an antigenically deficient λ -type Bence Jones protein. The antiserum no longer reacts with antigenically deficient proteins and is specific for the Oz (+) determinant.

(+), and 14 were Oz (-). As with the Bence Jones proteins, some of the G myeloma proteins were identified as Oz (+) by means of inhibition reactions.

The frequency of Oz (-) G myeloma proteins is apparently higher than that of Oz (-) Bence Jones proteins. Because of the possibility that negative reactions in the myeloma proteins were due to blocked antigenic determinants, caused by steric hindrance or other effects of light-chain combination with heavy polypeptide chains in the immunoglobulin G (IgG) molecules, five pairs of homologous G myeloma proteins and Bence Jones proteins were analyzed. In all cases the myeloma protein reacted in the same way as the Bence Jones protein from the same patient. These findings suggest that the G myeloma proteins are not Oz (-) because of a blocked determinant.

The lambda-chain subtype was compared to the heavy polypeptide chain subclass of the G myeloma proteins (6). Eleven IgG 1 (γ_{2b} ; We), five IgG (γ_{2a}, Ne) , four IgG 3 (γ_{2e}, Vi) , 2 and one IgG 4 (γ_{2d} , Ge) myeloma proteins were tested. Both Oz (+) and Oz (-) proteins were found in the IgG 1 and IgG 2 subclasses. The four IgG 3 myeloma proteins were Oz (-), and one IgG 4 protein was Oz (+). Further testing of IgG 3 and IgG 4 proteins is required to exclude definitely a relation between these subclasses and Oz type. No relation between Gm factors of these myeloma proteins and Oz type was demonstrated. Similarly, lambda Bence Jones protein subtypes showed no apparent correlation with the absence or the presence or type of anomalous serum immunoglobulin (see Table 1).

Normal human light chains, obtained by reduction and alkylation of chromatographically purified, polled IgG, were tested by Ouchterlony analysis with subtype-specific anti-Oz (+) serum and reacted at a concentration of 2 mg/ml. Intact, pooled, normal human IgG gave an inhibition reaction at 6 mg/ml. Forty normal human serums were also tested, and each serum showed either precipitin bands or inhibition reactions. These experiments indicate that the Oz (+)antigenic determinants are present in the serum immunoglobulins of most normal people. Quantitative studies will be required to determine the percentage of normal immunoglobulin molecules that contain Oz(+) lambda light chains.

Evidence for antigenic heterogeneity

of lambda Bence Jones proteins has been presented. Nachman et al. (7) investigated seven lambda Bence Jones proteins and found one rabbit antiserum which detected two categories of lambda Bence Jones proteins. Two of the Bence Jones proteins lacked antigens which were present in the other five. The specific antigens, however, were not identified on normal light chains. Epstein and Gross (8) with human agglutination systems also found antigenic differences in lambda Bence Jones proteins but encountered steric hindrance to the detection of their antigens in intact IgG molecules. These two studies apparently detected antigens different from the Oz (+) antigens because of our evidence that Oz (+) antigens are present on normal IgG molecules and normal light polypeptide chains.

Our immunochemical studies led to chemical studies of the human lambda light-chain subtypes. These investigations indicate that an amino acid interchange at one specific position in the "common region" of lambda Bence Jones proteins is associated with serological differences (9). It is important to determine if a genetic variation in the lambda chains, similar to the Inv phenotype system of the kappa light chain (10), is being detected in this immunochemical system.

DANIEL EIN JOHN L. FAHEY

National Cancer Institute, Bethesda, Maryland 20014

References and Notes

- 1. S. Dray, Science 132, 1313 (1960); W. D. S. Diay, believe 10s, 1913 (1960), W. D. Terry and J. L. Fahey, *ibid.* 146, 400 (1964); H. M. Grey and H. G. Kunkel, J. Exp. Med. 120, 253 (1964).

- (1964); H. M. Grey and A. C. J. Exp. Med. 120, 253 (1964).
 2. J. L. Fahey, J. Wunderlich, R. Mishell, J. Exp. Med. 120, 243 (1964).
 3. H. G. Kunkel, W. J. Yount, S. D. Litwin, Science 154, 1041 (1966); N. L. Warner and L. A. Herzenberg, J. Immunol. 97, 525 (1966); J. E. Coe, Science 155, 562 (1967).
 4. M. Mannik and H. G. Kunkel, J. Exp. Med. 117, 213 (1963); J. L. Fahey, J. Immunol. 91, 448 (1963); K. R. McIntire, R. M. Asofsky, M. Potter, E. L. Kuff, Science 150, 361 (1965).
- 111, 213 (1903); J. L. Faney, J. Immunol. 91, 448 (1963); K. R. McIntire, R. M. Asofsky, M. Potter, E. L. Kuff, Science 150, 361 (1965).
 5. H. F. Deutsch, Immunochemistry 2, 207 (1965); A. Solomon, J. Killander, H. M. Grey, H. G. Kunkel, Science 151, 1237 (1966); D. Cioli and C. Baglioni, J. Mol. Biol. 15, 385 (1966).
 6. W. D. Terry, J. L. Fahey, A. G. Steinberg, J. Exp. Med. 122, 1087 (1965). The G myeloma proteins used in this study were made available by Dr. William Terry.
 7. R. L. Nachman, R. L. Engle, Jr., S. Stein, J. Immunol. 95, 295 (1965).
 8. W. V. Epstein and D. Gross, J. Exp. Med. 120, 733 (1964).
 9. E. Appella and D. Ein, Proc. Nat. Acad. Sci. U.S., in press.
 10. C. Ropartz, J. Lenoir, J. Rivat, Nature 189, 586 (1961).

- 586 (1961). 11. We thank Dr. William D. Terry for his advice.

27 March 1967

SCIENCE, VOL. 156