calls, the scores were significantly higher ($P < .05$, two-tailed) when a tape was parental than when it was foreign; for orientation to the nonspeaker end the scores were higher when a tape was foreign than when it was parental ($P < .05$, two-tailed); position scores for the nonspeaker side and scores for sitting were not significantly different between a tape that was parental and one that was foreign.

A laughing gull chick's immediate response, in the field, to arrival of one of its parents is usually orientation toward the parent, calling, and approach. In the proximity of an adult other than the parent the chick, particularly if it is outside the family territory, usually orients away from the adult, crouches silently, or flees. Such selective responsiveness by the chick is evident in the field as early as 6 days after hatching. Whatever the total range of characteristics on which the chick can base this selective responsiveness, it is clear from my experiment that individual characteristics in the calls of the adults are sufficient for it, at least after a certain age or degree of experience. What these individual characteristics are, when and how a chick's discrimination of them develops, and what the consequences are of the development of such discriminations for the later behavioral development and social relationships of a chick, are not known.

Recognition by chicks of individual characteristics of the voices of parents is not unique to the guillemot, although the ability in laughing gull chicks may not be established prior to hatching, as Tschanz (*3*) has shown is the case in the guillemot. The laughing gull chick, unlike the guillemot chick, does not hatch where it is immediately confronted with proximity of numerous adults other than its parents to which it must react selectively. Only after several days when the parents begin leaving them unattended for periods and they start wandering away from the nest, do laughing gull chicks come into frequent close contact with adults other than their parents; and there is reason to believe that ability to recognize the parents develops during the early days after hatching as a consequence of positive conditioning in which association of the parental calls with feeding plays a prominent part (*2*). In the black-billed gull it seems that chick recognition of the parents' voice is not manifest until after at least 1 day after hatching (*4*).

Although the details of its development and adaptive significance may vary from species to species, individual recognition by young of voices of their parents may be widespread in colonially breeding species of birds (*4–6*). Recognition of their young by parents (*10*) and of one adult by another (*11*) on the basis of individual characteristics of voice may also be more common than is presently realized.

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References and Notes

1. A. C. Bent, *Smithson. Inst. Bull.* **113**, 154 (1921).
2. C. G. Beer, in preparation.
3. B. Tschanz, *Z. Tierpsychol.*, Suppl. 4 (1968).
4. R. M. Evans, in preparation.
5. R. E. Hutchinson, J. G. Stevenson, W. H. Thorpe, *Behaviour* **32**, 150 (1968).
6. B. Stonehouse, *Falklands Island Dependencies Survey Report 23* (1960).
7. The chicks were taken from the gullery in the Brigantine National Wildlife Refuge, New Jersey, and the experiment was carried out in the Field Station of the Institute of Animal Behavior, Rutgers University, which is situated in the Refuge.
8. S. Siegel, *Nonparamatric Statistics* (McGraw-Hill, New York, 1956).
9. The calls given by the chicks in the experimental situation were harsh in quality. Sonagrams show more or less unstructured bands extending from 1 to 7 khz. The calls were either disyllabic ["chiz-ik" according to M. Nice, *Trans. Linnaean Soc. N.Y.* **8**, 1 (1962) and J. P. Hailman, *Behaviour*, Suppl. 15 (1967)] or polysyllabic ("chirirah" according to C. G. Beer, in preparation).
10. S. J. J. F. Davies and R. Carrick, *Austral. J. Zool.* **10**, 171 (1962).
11. J. S. Weeden and J. B. Falls, *Auk* **76**, 343 (1959).
12. Supported by PHS grant GM 12774 and by a grant from the Rutgers Research Council (07-2208). The cooperation and hospitality of the U.S. Fish and Wildlife Service and the personnel of the Brigantine National Wildlife Refuge are gratefully acknowledged. Contribution No. 58 from the Institute of Animal Behavior, Rutgers University.

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Elution of Glomerular Bound Antibodies in Experimental Streptococcal Glomerulonephritis

Abstract. *Immunoglobulin G, eluted from glomeruli of rats with streptococcal glomerulonephritis, reacts with type 12 M protein of the streptococcus but not with other streptococcal or renal antigens. Therefore, this disease may be mediated by fixation of antigen-antibody complexes consisting of streptococcal M protein and type-specific antibody.*

Clinical and immunopathologic findings associated with poststreptococcal glomerulonephritis in man suggest that this disease is mediated by the fixation of antigen-antibody complexes in the region of the basement membrane of the glomerulus (*1*). Which, if any, streptococcal antigen or antigens is involved in the antigen-antibody complex is not certain. The elution and characterization of the bound γ-globulins would provide a direct assessment of their reactivity. However, such studies have not been performed and are limited by the necessity for more tissue than can be obtained by the usual biopsy techniques.

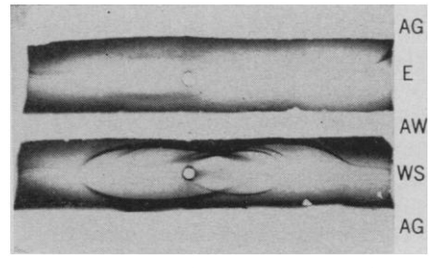

Fig. 1. A comparison by immunoelectrophoresis of serum proteins with those present in the acid eluate of the kidney. *E*, eluate; *WS*, whole rat serum; *AG*, goat antibody to rat globulins (Hyland Labs., lot No. S228Cl); and *AW*, goat antibody to whole rat serum (Hyland Labs., lot No. 822DOO1A1).

The availability of an experimental model which fulfills many of the epidemiological, clinical, and laboratory features of the disease, as seen in man, offers an accessible alternative opportunity to study this aspect of the pathogenesis of the disease (*2*). In this model of experimental streptococcal glomerulonephritis in rats, the disease is restricted to animals exposed to a nephritogenic strain of group A, type 12 streptococcus; it is characterized by proteinuria and by tissue-bound γ-globulin and streptococcal M protein in the region of the glomerular basement membrane. Our characterization of the fixed γ-globulins provides direct support for the hypothesis that streptococcal M protein and type-specific antibody are the immunologic reactants in the experimentally induced disease.

Twenty-six Sprague Dawley rats were exposed to the nephritogenic (A12N) strain of type 12 streptococcus and 15 to the nonnephritogenic (A12) strain (*2*). All animals were killed at 65 days. There was no difference in the amounts of type 12 specific hemagglutinins in

Table 1. Antibody activity in the acid eluates. Indirect hemagglutinins (IHA) are expressed as the reciprocal of the titer of type 12 specific hemagglutinins. Bacterial agglutinins (BA): C, complete; 0, no agglutination of type 12 (T12) and type 6 (T6) streptococci. Indirect fluorescent antibody (IFA) assay: degree of fluorescence is expressed as none (0), definite but dull (1+), and maximum brilliance (4+).

Eluate	Protein (mg/100 ml)	IHA T12	BA		IFA		
			T12	T6	T 12	T 6	Kidney
A-12	112	<4	0	0	1+	0	0
A-12N	35	32	C	0	4+	0	0

the serums of animals in the two groups. Significant deposits of fixed γ-globulin were present in the glomeruli of 20 out of the 26 rats exposed to A12N strain, but no deposits were present in the 15 rats exposed to A12 strain. The tissues from the rats exposed to the two strains were pooled separately. The renal cortices were separated from the medullas, weighed, and ground in a Ten Broeck tissue homogenizer. Dissociation of the fixed γ-globulins was attempted in alkaline (*p*H 9.0) and acid (*p*H 3.2) borate buffers (*3*).

The presence and specificity of antibody in the eluates was assessed with indirect hemagglutination (IHA), bacterial agglutination (BA), and indirect fluorescent antibody (IFA) techniques. In the IHA test we used red cells sensitized with purified M protein and a soluble inhibitor to block cross-reactive antibodies that were not type-specific (*4*). Bacterial agglutinins were assayed as follows. A culture of the test strain was grown overnight in trypticase soy broth. The viable bacterial sediment was washed and resuspended in saline to the original volume. The viable suspension (0.5 ml) was incubated with the eluate (1 ml) at 37°C in a water bath for 1 hour. Wet mounts were prepared and examined with a darkfield microscope for complete, partial, or no agglutination. The IFA test consisted of fixation of the washed test strain of bacteria or of 4.0-μ sections of rat cortex to glass slides and was performed with fluorescein-labeled rabbit antiserum to rat γ-globulin (Antibodies, Inc.) (*2*).

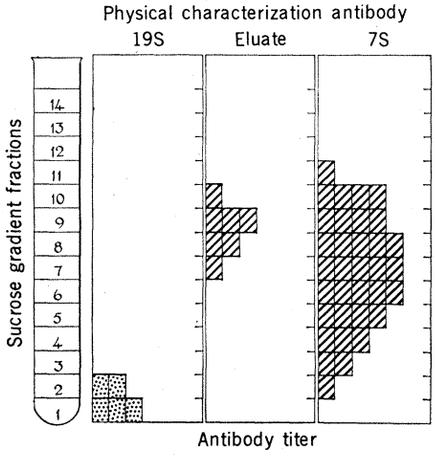

Fig. 2. A comparison of the sedimentation rate of the type 12 specific hemagglutinins in the acid eluate of the kidney with 7*S* and 19*S* human hemagglutinins for the lipopolysaccharide of *Escherichia coli* O4. The stippled and cross-hatched blocks represent twofold dilutions of the gradient fractions starting at a dilution of 1 : 2.

Immunoglobulins were recovered only in the acid-eluted fraction and were specific for streptococcal M protein. Although the final protein concentration of the nonnephritogenic eluate was at least three times greater than that of the nephritogenic eluate, only the latter contained appreciable amounts of antibody (Table 1). The specificity of this antibody for type 12 M protein was demonstrated by its reactivity with purified type 12 M protein (IHA test) and with group A, type 12 bacteria (BA and IFA test) and by its failure to react with a strain of group A, type 6 (BA and IFA tests). In addition, this antibody did not react with renal antigens as assessed by the indirect fluorescent method.

These antibodies were characterized further by immunoelectrophoresis (*5*) and sedimentation on a sucrose gradient (*6*). A single protein band was found on immunoelectrophoresis of the eluate and corresponded to the IgG band of normal rat serum (Fig. 1). Separation of the eluate on a sucrose gradient revealed that the immunoglobulins sedimented in the same range as 7*S* human immunoglobulins (Fig. 2).

Immunoglobulins eluted from the kidneys of rats with experimental streptococcal glomerulonephritis are of the IgG class and react with type 12 streptococcal M protein but not with other streptococcal antigens or with renal antigens. This observation directly implicates type 12 M protein and type-specific antibody as the immunologic reactants in the production of this disease. Because of the similarity of this experimental model to the disease observed in man, it seems possible that these same reactants may also mediate the production of the disease in man. Still undetermined is the difference between nephritogenic and nonnephritogenic strains which leads to the formation and fixation of such complexes only in the kidneys of animals and humans exposed to the nephritogenic strains.

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References and Notes

1. K. L. Vosti, *The Medical Clinics of North America* (Saunders, Philadelphia, 1965), pp. 1717–1728; R. C. Mellors and L. G. Ortega, *Amer. J. Pathol.* **32**, 455 (1956); P. Freedman, J. H. Peters, R. M. Kark, *Arch. Intern. Med.* **105**, 524 (1960); G. A. Andres, L. Accini, K. C. Hsu, J. B. Zabriskie, B. C. Seegal, *J. Exp. Med.* **123**, 399 (1966); R. T. McCluskey, P. Vassalli, G. Galli, D. S. Baldwin, *N. Engl. J. Med.* **274**, 695 (1966); A. F. Michael, K. N. Drummond, R. A. Good, R. L. Vernier, *J. Clin. Invest.* **45**, 237 (1966); G. Treser, M. Semar, M. McVicar, M. Franklin, A. Ty, I. Segal, K. Lange, *Science* **163**, 676 (1969).
2. L. H. Lindberg, K. L. Vosti, S. Raffel, *J. Immunol.* **98**, 1231 (1967).
3. I. L. Spar, W. F. Bale, D. E. Wolfe, R. L. Goodland, *ibid.* **76**, 119 (1956).
4. K. L. Vosti and L. A. Rantz, *ibid.* **92**, 185 (1964).
5. J. J. Scheidegger, *Int. Arch. Allergy Appl. Immunol.* **7**, 103 (1955).
6. K. L. Vosti and J. S. Remington, *J. Lab. Clin. Med.* **72**, 71 (1968).
7. Supported by PHS grants T1-AI-185 and AI-06964. L.H. is a National Science Foundation science faculty fellow. We thank M. Klopp and N. Steingold for technical assistance.

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Diversity and Composition of Abyssal Benthos

Sanders and Hessler (*1*) present some important data obtained by use of their anchor dredge and epibenthic sled, and by application of their rarefaction procedure to assess diversity independent of sample size. However, some of their conclusions are not unequivocal and others have been proposed previously.

The theory that "the deep sea harbors a qualitatively restricted fauna" has been adequately disproved (*2*, *3*, and others). Sanders and Hessler say that they did not expect to find so many deposit-feeding species. Research on deep-sea mollusks (*3*) has already shown that deposit feeders are dominant in the deep sea.

The statement, ". . . the implication, at least for bivalves is that faunal composition is far more sensitive to change in depth than to the effects of distance" is insufficiently documented. The accompanying discussion also omits any reference to pertinent previous work