weeks (Fig. 3); the symptoms of diabetes such as polyuria and polydypsia were markedly reduced.

These findings indicate that islet isograft survival and amelioration of the diabetic state can be obtained by intraperitoneal implantation of microencapsulated islets into rats with chemically induced diabetes. The microencapsulated islets remained morphologically and functionally intact for as long as 4 months in culture in vitro at 37°C. The microencapsulation technique described herein offers certain advantages over other transplantation methods. For example, the maintenance of viable islets or dispersed endocrine tissues in culture for long periods can contribute materially to the collection of sufficient islets for implantation. It also avoids the problems associated with diffusion chambers and hollow fiber units. These problems include (i) the small surface area of the devices, which necessitates the use of large units that may be too bulky and complex; (ii) the surgery required for implantation or shunt connection with risks of clotting or infection; and (iii) the relatively high diffusion resistance inherent in the filter membranes and hollow fibers, which results in long transit time (*18*).

Microencapsulation also provides more options for the method and site of implantation. Unlike the bulky hollow fiber units and diffusion chambers, microcapsules are small enough to pass through a gauge 18 or 19 hypodermic needle. Therefore, several options are available for method of implantation. The preferred manner of introduction of the microcapsules, for example, through surgical incision openings at one or multiple sites or through direct hypodermic injections, remains to be determined. Currently, placement of islets or dispersed pancreatic tissues in the liver by way of the portal vein is highly favored. Sutherland *et al.* (*2*) have also shown that injection into the splenic pulp is effective. Other studies have indicated that the intramuscular route may be as effective as the intraperitoneal route (*19*). Further studies should provide significant information in all of these areas and may lead to the use of islet transplantation in the treatment of human diabetes.

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References and Notes

1. P. E. Lacy, J. M. Davie, E. M. Finke, *Science* **204**, 312 (1979).
2. D. E. R. Sutherland, A. J. Matas, J. S. Najarian, *Surg. Clin. North Am.* **58**, 365 (1978).
3. Y. S. Mullen, W. R. Clark, I. G. Molnar, J. Brown, *Science* **195**, 68 (1977).
4. R. L. Strautz, *Diabetologia* **6**, 306 (1970).
5. R. J. Gates, M. I. Hunt, R. Smith, N. R. Lazarus, *Lancet* **1972-II**, 567 (1972).
6. R. J. Gates and N. R. Lazarus, *ibid.* **1977-II**, 1257 (1977).
7. W. L. Chick, A. A. Like, V. Lauris, *Science* **187**, 847 (1974).
8. W. L. Chick, J. J. Perna, V. Lauris, D. Low, *ibid.* **197**, 780 (1977).
9. W. J. Tze, F. C. Wong, L. M. Chen, S. O'Young, *Nature (London)* **264**, 466 (1976).
10. A. M. Sun, W. Parisius, G. M. Healy, I. Vacek, H. G. Macmorine, *Diabetes* **26**, 1136 (1977).
11. P. E. Lacy and M. Kostianovsky, *ibid.* **16**, 35 (1967).
12. F. Lim and R. D. Moss, paper presented at the Fourth International Symposium on Microencapsulation, Miami, Fla., 1979; *J. Pharm. Sci.*, in press.
13. W. B. Ennis, Jr., and D. T. James, *Science* **112**, 43 (1950).
14. R. E. Sparks, R. M. Salemme, P. M. Meir, M. H. Litt, O. Lindan, *Trans. Am. Soc. Artif. Intern. Organs* **15**, 353 (1969).
15. P. E. Lacy, E. H. Finke, S. Conant, S. Naber, *Diabetes* **25**, 484 (1976).
16. L. A. Sternberger, *Immunochemistry* (Prentice-Hall, Englewood Cliffs, N.J., 1974).
17. C. N. Hales and T. J. Randle, *Biochem. J.* **88**, 137 (1963).
18. N. A. Theodorus and S. L. Howell, *Transplantation* **27**, 350 (1979).
19. K. Vermitsky, F. X. Pi-Sunyer, K. Reemstma, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 873 (Abstr. 3421) (1979).
20. We thank S. B. Weir for immunohistochemical and photographic work. We thank R. D. Moss, B. Lockwood, C. Coe, and G. Malam for technical assistance. Supported by research grants for Damon Corp. P. J. Vasington of Damon was responsible for the initiation of this work.

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Inhibition of Biological Activity of Mouse β-Nerve Growth Factor by Monoclonal Antibody

Abstract. *A continuous hybrid cell line was derived that secretes monoclonal antibody capable of inhibiting the biological activity of mouse β-nerve growth factor (β-NGF). Results obtained with monovalent fragments indicate that the monoclonal antibody inhibits activity by interfering with the direct interaction between β-NGF and the cell membrane receptor rather than by precipitating the dimeric form of β-NGF. This monoclonal antibody binds to an antigenic determinant common to mouse β-NGF, snake venom (*Naja naja*) β-NGF, and human β-NGF. These antibodies should provide specific molecular probes for a variety of studies of nerve growth factor including its tissue distribution and mechanism of action.*

Considerable data concerning the structure and function of nerve growth factor (NGF) have been obtained with the use of specific antiserums to mouse β-nerve growth factor (β-NGF). Developmental immunosympathectomy (immunological interruption of sympathetic nerve pathways) can be effected in various mammals by treating them after birth with rabbit antiserum specific for mouse β-NGF (*1*). Immunological comparisons among mammalian NGF's indicate that although some antigenic determinants are evolutionarily conserved, many are not (*2*). Precise immunochemical characterization of these determinants is not possible with polyclonal antibody reagents. Specific radioimmunoassays have been developed by using affinity purified antibodies that are sensitive to about 0.25 ng of β-NGF per milliliter (*3*, *4*). The limits of sensitivity and specificity have been reached in methods in which conventional antibody reagents are used, and it has been suggested that more sensitive assays will require the preparation of monoclonal antibodies (*5*). In this report we describe the derivation of a hybridoma that secretes monoclonal antibody capable of inhibiting the biological activity of mouse β-NGF.

From the submaxillary glands of adult male CF1 mice we isolated 7*S* NGF and separated the β subunit by carboxymethyl cellulose ion-exchange chromatography (*6*). Human β-NGF was isolated from term placenta according to the method of Mobley *et al.* (*7*). The purity of β subunit preparations was assessed by polyacrylamide gel electrophoresis at *p*H 10.3 (*8*) and by isoelectric focusing in a *p*H gradient (*p*H 3 to 10) in 7.5 percent acrylamide gels (*9*). A 5-week-old male Lewis rat was immunologically primed with an intraperitoneal injection of 500-μg of glutaraldehyde-polymerized β-NGF emulsified in complete Freund's adjuvant. Three weeks later the same rat was injected intraperitoneally with 300 μg of polymerized β-NGF in incomplete Freund's adjuvant.

Four days after the secondary immunization, spleen cells were obtained from the rat and suspended in RPMI 1640 medium. These cells were mixed with P3 × 63Ag8 mouse plasmacytoma cells at a ratio of 5:1 in a small petri dish and centrifuged at 1700 rev/min for 5 minutes (*10*). The fusing agent, PEG-1000 (polyethylene glycol; Baker Chemical Co.) dissolved 35 percent, weight to volume, in Dulbecco's modified Eagle's medium (DMEM) at 37°C, was carefully added to the resulting monolayer of cells over a 1-minute period. One minute later DMEM was slowly added and the supernatant

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carefully aspirated. This washing procedure was repeated twice, after which 8 ml of complete DMEM (DMEM containing 20 percent fetal bovine serum) was added to the monolayer. The cells were incubated overnight at 37°C in 5 percent CO_2, and then adjusted to 5×10^5 per milliliter in hypoxanthine-aminopterin-thymidine medium (*11*) and dispersed into 96-well plates (150 μl per well) in order to clone the hybrids by limiting dilution. After 10 days about 720 clones were visible.

At this time, the supernatant (50 μl) was collected from each visible clone and assayed for anti β-NGF activity with an enzyme-linked immunosorbent assay (ELISA) with alkaline phosphatase (*12*). Only 20 of the 720 clones proved to be specific for β-NGF, and after 1 month six clones had been subcloned and were stable. Clone 8 expanded much sooner than any other clone, and the studies described here are concerned only with clone 8. Rats were chosen as the source of immune spleen cells to circumvent the problem of eliciting an immune response in mice to mouse NGF. The fact that a significant immune response was elicited in a rat (a titer of 10^4 with the ELISA on serum obtained after secondary immunization) is in agreement with other studies (*2, 5*) that indicate that some antigenic determinants have rapidly diverged during evolution.

Inhibition of NGF bioactivity was determined by means of a modification of the bioassay method of Greene (*13*), in which dissociated sympathetic ganglia from 11-day-old chick embryos provide target cells. Fifty microliters of hybridoma supernatant or antibody (purified on protein A-Sepharose CL-4B; Pharmacia) diluted in complete DMEM (5 percent horse serum) was incubated with 50 μl of 7*S* NGF for 1 hour at room temperature. To this, 100 μl of ganglionic cells (5×10^4 cells per milliliter in complete DMEM, 5 percent horse serum) were added. After 18 to 24 hours of incubation (37°C, 60 percent humidity, 5 percent CO_2) the number of neurite-bearing cells per well scan was counted by using a phase-contrast microscope. Triplicate cultures were scored for each NGF concentration.

Ouchterlony analysis of concentrated supernatant revealed that clone 8 antibody was in the immunoglobulin G (IgG) class. Monoclonal antibody from clone 8 was purified by protein A-Sepharose CL-4B affinity chromatography at *p*H 8.0. These two facts taken together indicate that this clone secretes either the IgG1 or IgG2c subclass (*14*). Rat IgG for controls was also purified on protein A-Sepharose CL-4B. The Fab fragment of clone 8 was prepared by papain (Sigma) cleavage (*15*). Intact antibody was removed by passage through protein A-Sepharose CL-4B and the Fab fragment isolated by gel filtration on Sephadex G-150. The Fab fragment was judged to be free of divalent antibody fragments or whole antibody by sodium dodecyl sulfate polyacrylamide gel electrophoresis under nonreducing conditions (*16*); furthermore, the NGF-binding activity of the Fab fragment was confirmed by the ELISA (*12*) with the use of rabbit antibody to rat kappa light chains conjugated to alkaline phosphatase as the second antibody reagent.

The effect of the clone 8 supernatant on 7*S* NGF biological activity is shown in Fig. 1A. The concentration of NGF required for a half-maximal response was 10 times greater when the NGF had been incubated with 50 μl of clone 8 supernant divalently), we found it necessary to with control supernatant (from a Lewis rat-P3 × 63Ag8 hybridoma that lacks specificity for β-NGF).

To determine whether this monoclonal antibody inhibited activity by interfering with receptor binding of β-NGF or simply by precipitating β-NGF dimer (or other oligomeric forms of NGF which might present a certain β-NGF determinant divalently), we found it necessary to prepare and assay Fab fragments from clone 8 IgG. Figure 1B shows the biological responses to NGF in the presence of intact clone 8 antibody, nonspecific rat IgG, clone 8 Fab fragments, and complete DMEM. The curves for rat IgG and complete DMEM are comparable: inhibition of the response by intact clone 8

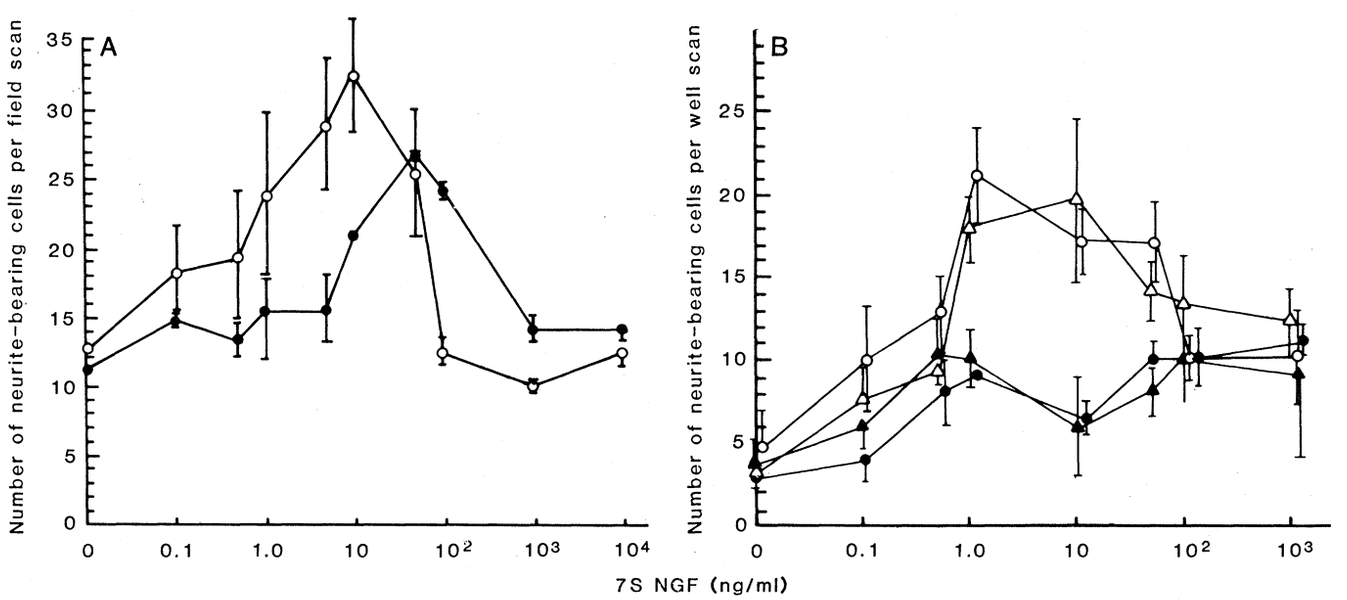

Fig. 1. (A) Number of neurite-bearing cells per field scan with clone 8 supernatant (●) and with control supernatant from hybridoma lacking specificity for NGF (○). Supernatants were incubated with various dilutions of 7*S* NGF for 1 hour at room temperature before dissociated ganglionic cells were added. In the presence of clone 8 supernatant the biological response plot is shifted to the right, requiring a tenfold greater concentration of NGF to effect biological response. Vertical bars indicate the standard deviation of triplicate determinations. (B) Number of neurite-bearing cells per well scan with clone 8 antibody (▲), clone 8 Fab fragment (●), control rat antibody (△), and medium alone (○). All antibodies were purified by protein A-Sepharose CL-4B affinity chromatography and added to a final concentration of 250 μg/ml. However, only 5 μg of the clone 8 antibody per milliliter was rat IgG as determined by two-site enzyme-linked immunoassay. P3 × 63Ag8 antibody, of the murine IgG1 subclass, does not bind protein A (*14*) and thus is removed in these preparations. Most of the IgG was presumably bovine from the calf serum (15 percent) in the culture medium. Vertical bars indicate the standard deviation of triplicate determinations.

antibody and clone 8 Fab fragments was nearly identical.

With such a large quantity of antibody present (Fig. 1A) it is important to consider why biological activity remains at all. Smaller quantities of polyclonal antibody are capable of completely abolishing biological activity in similar experiments (*17*), perhaps by precipitating β-NGF through multiple determinants. Since the Fab fragment of clone 8 antibody cannot remove NGF from soluble phase dynamic equilibrium, the addition of target cell receptors to the system would be expected to shift the equilibrium away from the antigen-antibody interaction. This is thermodynamically favorable: the high-affinity binding constant of the NGF-target cell interaction is on the order of 10^{11} liter per mole (*18*), whereas average association constants of antigen-antibody interactions are generally 10^2- to 10^4-fold lower. These results are consistent with an antibody-NGF affinity constant on the order of 10^8 liter per mole.

It is possible that the α or γ subunit could mask or sterically inhibit binding of clone 8 antibody to its determinant while not interfering with the receptor binding of β-NGF. This possibility has been excluded because the α and γ subunits do not inhibit the binding of clone 8 antibody to β-NGF in the ELISA, even at concentrations of 0.5 mg/ml. The clone 8 antibody does not bind to the α or the γ subunit because the ELISA gave negative results with these antigens.

There is evidence that the active site of β-NGF is well conserved evolutionarily (*5*), whereas other determinants may have diverged rapidly (*2*). This monoclonal antibody reacted with mouse β-NGF, snake venom (*Naja naja*) β-NGF, and human β-NGF in the ELISA, indicating that the antigenic determinant recognized has been strictly conserved during evolution. This conflicts with other studies (*19*) which failed to demonstrate cross-reactivity between mouse β-NGF and human β-NGF.

The production of a monoclonal antibody that is directed toward a determinant at or near the active site of the β subunit of NGF may be extremely fortunate, since polyclonal antiserums appear to be directed mainly toward less specific antigenic determinants (*5*). The availability of high-affinity monoclonal antibodies that specifically inhibit the biological activity of mouse β-NGF will permit studies on the similarity of active sites between mouse and other species, the distribution of NGF among various tissues, the interaction between β-NGF and its receptor, and the mechanism of action of the hormone factor. It will also be of interest to define the structural determinants to which various monoclonal antibodies bind on each of the subunits and, hence, the nature of the interaction within the 7*S* NGF complex.

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References and Notes

1. R. Levi-Montalcini and P. U. Angeletti, *Pharm. Rev.* **18**, 619 (1966).
2. C. S. Bailey, B. E. C. Banks, J. R. Carstairs, D. C. Edwards, F. L. Pearce, C. A. Vernon, *Biochim. Biophys. Acta* **437**, 259 (1976).
3. I. A. Hendry, *Biochem. J.* **128**, 1265 (1972).
4. K. Suda, Y. A. Barde, H. Thoenen, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4042 (1978).
5. G. P. Harper and H. Thoenen, *J. Neurochem.* **34**, 5 (1980).
6. S. Varon, J. Nomura, E. M. Shooter, *Biochemistry* **6**, 2202 (1967); R. W. Stach, B. J. Wagner, B. M. Stach, *Anal. Biochem.* **83**, 26 (1977).
7. W. C. Mobley, A. Schenker, E. M. Shooter, *Biochemistry* **15**, 5543 (1976).
8. A. P. Smith, L. A. Greene, H. R. Fiske, S. Varon, E. M. Shooter, *ibid.* **8**, 4918 (1969).
9. A. C. Server and E. M. Shooter, *J. Biol. Chem.* **251**, 165 (1976).
10. G. Kohler and C. Milstein, *Nature (London)* **256**, 495 (1975).
11. K. Horibata and A. W. Harris, *Exp. Cell Res.* **60**, 61 (1970).
12. E. Engvall and P. Perlman, *J. Immunol.* **109**, 129 (1972).
13. L. A. Greene, *Neurobiology* **4**, 286 (1974).
14. G. A. Medgyesi, F. Füst, J. Gergely, H. Bazin, *J. Immunochem.* **15**, 125 (1978).
15. R. R. Porter, *Biochem. J.* **73**, 119 (1959).
16. W. F. Studier, *J. Mol. Biol.* **79**, 237 (1973).
17. E. L. Fenton, *Exp. Cell Res.* **59**, 383 (1970).
18. A. Sutter, R. J. Riopelle, R. M. Harris-Warrick, E. M. Shooter, *J. Biol. Chem.* **254**, 5972 (1979); S. P. Banerjee, S. H. Snyder, P. Cuatreacasas, L. A. Greene, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2519 (1973).
19. P. Walker, M. E. Weicshel, D. A. Fisher, *Life Sci.* **26**, 195 (1980).
20. We thank N. R. Woodruff for his interest in this work and for helpful discussions and M. C. Meyer and R. Graziano for their technical assistance. S.W. is a predoctoral fellow of the American Heart Association. This work was supported by American Cancer Society grant BC-249 and NIH grants A110148 and CA27915.

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Transporting Renal Epithelium: Culture in Hormonally Defined Serum-Free Medium

Abstract. *A hormonally defined medium was used to isolate a homogeneous epithelioid cell population from canine kidney. Monolayers of these cells form domes, an indication of active ion transport, and this process is inhibited by ouabain. This technique allows the isolation of primary cultures of renal epithelial cells, free of fibroblasts, for the characterization of biochemical and physiological properties related to renal function.*

Models have been used to study the biochemical events associated with the hormonal regulation of ion transport by the kidney. These models include the urinary bladders of amphibia and reptilia and, most recently, cells in tissue culture (*1*). Active ion transport occurs in a number of different epithelioid cell cultures (*2–10*) and is characterized by the formation of structures designated as hemicysts or domes. These domes represent areas of localized detachment of the monolayer caused by the accumulation of fluid between the basolateral membrane and the culture dish (*11*) as the result of active ion transport from the apical to the basolateral surfaces of the cells. Dome formation is inhibited by agents that block transepithelial sodium transport, such as ouabain (*6, 12, 13*), and is thus presumed to be associated with transport processes related to normal renal function.

Normal cells grown in a tissue culture environment, which provides control of the external milieu and cell homogeneity, would be useful for the study of biochemical and physiological mechanisms related to kidney function and their hormonal regulation. Attempts at the isolation of normal epithelioid cell lines from the kidney have been generally unsuccessful, however, because of persistent problems with the elimination of contaminating fibroblastic cells, the isolation of a specific cell type, and the definition of a medium in which specific cells can proliferate while maintaining differentiated properties. A number of investigators have therefore used continuous epithelioid kidney lines (for example, MDCK), to investigate biochemical mechanisms related to renal function. All of these continuous cell lines exhibit aberrant biochemical or physiological properties when compared to cells that have not undergone transformation. Therefore, a primary kidney cell culture system should be of significantly greater value in the study of transport mechanisms. We have used a hormonally defined medium (*14, 15*) to isolate and grow, free of fibroblasts, a morphologically uniform epithelioid canine kidney cell population that forms domes.

Seven healthy mongrel dogs, four male and three female (4.3 ± 0.9 years old, weighing 27.5 ± 0.9 kg), were

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