References and Notes

1. J. Daly, *Cyclic Nucleotides in the Nervous System* (Plenum, New York, 1977).
2. J. W. Phillis, J. P. Edstrom, G. K. Kostopoulos, J. R. Kirkpatrick, *Can. J. Physiol. Pharmacol.* **57**, 1289 (1979).
3. B. L. Ginsborg and G. D. Hirst, *J. Physiol. (London)* **224**, 629 (1972); M. L. Michaelis, E. K. Michaelis, S. L. Myers, *Life Sci.* **24**, 2083 (1979); C. Hollins and T. W. Stone, *Br. J. Pharmacol.* **69**, 107 (1980).
4. A. Sattin and T. W. Rall, *Mol. Pharmacol.* **6**, 13 (1970).
5. S. H. Snyder, J. J. Katims, Z. Annau, R. F. Bruns, J. W. Daly, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3260 (1981).
6. D. van Calker, M. Muller, B. Hamprecht, *J. Neurochem.* **33**, 999 (1979); C. Londos, D. M. F. Cooper, J. Wolff, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2251 (1980); R. F. Bruns, *Can. J. Physiol. Pharmacol.* **58**, 673 (1980).
7. R. F. Bruns, J. W. Daly, S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5547 (1980).
8. U. Schwabe and T. Trost, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **313**, 179 (1980); M. Williams and E. Risley, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6892 (1980).
9. R. R. Goodman, M. J. Cooper, M. Gavish, S. H. Snyder, *Mol. Pharmacol.*, in press.
10. M. Rodbell, *Nature (London)* **284**, 17 (1980); M. E. Maguire, E. M. Ross, A. G. Gilman, *Adv. Cyclic Nucleotide Res.* **8**, 1 (1977); R. J. Lefkowitz and L. T. Williams, *ibid.* **9**, 1 (1978); S. H. Snyder and R. R. Goodman, *J. Neurochem.* **35**, 5 (1980).
11. T. Pfeuffer, *J. Biol. Chem.* **252**, 7224 (1977); D. Cassel and Z. Sellinger, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3307 (1977).
12. L. T. Williams, D. Mullikin, R. J. Lefkowitz, *J. Biol. Chem.* **253**, 2984 (1978); B. M. Rouot, D. C. U'Prichard, S. H. Snyder, *J. Neurochem.* **34**, 374 (1980); D. C. U'Prichard and S. H. Snyder, *ibid.*, p. 385.
13. C. B. Pert and S. H. Snyder, *Mol. Pharmacol.* **10**, 868 (1974); D. A. Greenberg, D. C. U'Prichard, P. Sheehan, S. H. Snyder, *Brain Res.* **140**, 378 (1978); R. S. L. Chang and S. H. Snyder, *J. Neurochem.* **34**, 916 (1980).
14. L. J. Pike, and R. J. Lefkowitz, *Mol. Pharmacol.* **14**, 370 (1978); R. J. Lefkowitz, personal communication.
15. S. K. Smith and L. E. Limbird, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4026 (1981).
16. M. Gavish, R. S. L. Chang, S. H. Snyder, *Life Sci.* **25**, 783 (1979); L. Toll and S. H. Snyder, in preparation.
17. L. S. Schleifer, J. C. Garrison, P. C. Sternweis, J. K. Northrup, A. G. Gilman, *J. Biol. Chem.* **255**, 2641 (1980); J. K. Northrup, P. C. Sternweis, M. D. Smigel, L. S. Schleifer, E. M. Ross, A. G. Gilman, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6516 (1980).
18. A. Levitski and A. M. Tolkovsky, *Biochemistry* **17**, 3811 (1978).
19. G. Koski, W. F. Simonds, W. A. Klee, *J. Biol. Chem.* **256**, 1536 (1981).

20. We thank G. Mack for excellent technical assistance, D. C. Hanks for manuscript preparation, and R. F. Bruns and J. Daly for helpful suggestions. Supported by PHS grants MH-18501, DA-00266, and NS-16375, grants from the McKnight Foundation, the International Life Sciences Institute, and by research scientist award DA-00074. R.R.G. is supported by medical scientist training program grant GM-7309.

* Present address: Department of Pharmacology, Israel Institute of Technology, Bat Galim, Haifa.

† To whom correspondence should be addressed.

5 October 1981; revised 7 December 1981

Impulse-Coded and Analog Signaling in Single Mechanoreceptor Neurons

Abstract. *Although most sensory neurons convey temporally coded impulses to the central nervous system, certain nonspiking receptors use only graded afferent signals. Each of three large nerve fibers from the lobster oval organ, a mechanoreceptor subserving ventilation, carry both impulses and graded potentials. Thus, both impulse frequency and receptor potential amplitude are available for information transfer.*

In a variety of sense organs, adequate stimuli impinging upon the sensory receptor terminals are transduced into a common type of membrane response, the receptor potential, the characteristics of which have been extensively documented (*1*). It is a graded, nonpropagated potential limited in its electrotonic spread by the passive cable properties of the cell membrane. Usually an electrically responsive region separate from the transduction site converts this analog signal into a frequency-modulated train of nerve impulses, which, in turn, relay the sensory information to the central nervous system.

However, studies on the thoracicocoxal muscle receptor organ (the T-C MRO) and other stretch receptors at the base of the walking legs (*2, 3*), swimmerets, and uropods (*4*) in decapod Crustacea, and on certain invertebrate photoreceptors (*5, 6*) have shown that some sensory neurons lack the usual spike-initiating zone and electrically excitable membrane, but have sufficiently large length constants to permit the electrotonic spread of the receptor potential itself right into the ganglionic neuropil. This "slow" analog signal evidently causes graded release of chemical transmitter at identified synaptic zones, thereby evoking corresponding postsynaptic potentials (*7*) and consequent impulse activity in associated motoneurons (*8*). Several other examples of such graded, nonspiking transmission have recently been found, in both sensory and interneuronal systems (*3, 9*). The experiments reported here show that certain sensory nerve fibers have both large length constants and active spike-supporting membranes and are capable of carrying both the analog and impulse signals over long distances. This raises the possibility that single afferent fibers can use both methods of transmission for conveying sensory information.

The oval organ (*10*) lies within the ventilatory appendage (the second maxilla) of all decapod crustaceans so far studied, and its position and anatomy suggest that it is a proprioceptor capable of monitoring the beat of the gill bailer (the scaphognathite). It consists of a rich arborization of dendrites arising from three afferent fibers of large diameter, supported by a conical array of connective tissue strands which span the region of maximum flexion and extension near the base of the gill bailer. The cell bodies of the three sensory neurons lie centrally in the subesophageal ganglion, adjacent to the motoneurons innervating the ventilatory muscles, as has recently been revealed by cobalt backfills (*11*).

The nerve trunk of an isolated preparation from the lobster (*12*) (Fig. 1) was removed from its sheath and securely pinned to a Sylgard platform with cactus spines, so that controlled stretching of the connective tissue strands of the oval organ caused no movement of the nerve fibers beneath the recording electrodes. The three sensory fibers were readily distinguishable—both from other nerve fibers in the same trunk and from one another—by their large diameter and relative positions; they were labeled X, Y, and Z in descending order of size (*13*). All other fibers were discarded except two motor axons going to a muscle intrinsic to the gill bailer; these two axons (MN1 and MN2 in Fig. 1), whose diameters were similar to those of Y and Z, served as controls.

Intracellular recordings from the individual fibers, made 3 to 8 mm from the oval organ, showed that each of the three sensory fibers, but not the two motor axons, responded to a ramp-and-hold pull stimulus in a characteristic way. Each response comprised, in differing proportions, two distinct components: overshooting action potentials and an underlying graded potential (Fig. 1a). The responses of fiber X had the fewest spikes (one to six) and the largest graded potentials, yielding depolarizations as much as 35 mV from the resting membrane potential in the unstretched state. Fiber Y responded with a sustained, slowly adapting discharge superimposed upon a smaller graded potential. Fiber Z showed the smallest graded potentials but an intermediate degree of spike adaptation.

Addition of tetrodotoxin (TTX) to the saline bathing a preparation undergoing standard pulls at 1-minute intervals produced first a progressive diminution and finally the complete disappearance of all spikes, but left the graded potentials unchanged (Fig. 1b). Thus, while the overshooting action potentials evidently depend on fast sodium channels (as in the majority of known nerve cells), the graded potentials are independent of

0036-8075/82/0326-1635$01.00/0

spiking and involve other membrane channels.

In the TTX-treated preparations, spiking never recovered, even after repeated washing with fresh saline. The fibers retained their full graded responsiveness, however, thus allowing an examination of the slow depolarizations free from the superimposed active processes. From the close correspondence between the parameters of the stretch stimuli and the resulting membrane depolarizations, we conclude that the graded potentials are receptor potentials, electrotonically conducted along the large-diameter afferent fibers. None of the three fibers displayed prominent dynamic components, but the receptor potentials of Y and Z showed adaptation to an extent typical of other crustacean tonic mechanoreceptors (*14*). In all three, the amplitude of the static component, after adaptation, showed a simple, almost linear relationship with the amplitude of the stimulus (Fig. 2c), which fits the oval organ for a role as a position or displacement receptor.

Length constants (λ) were calculated from steady-state amplitudes of stretch-evoked receptor potentials, recorded simultaneously from two microelectrodes inserted several millimeters apart in the same fiber. We assumed that the fiber behaves as an infinite cable. Mean and maximum values of λ and the number of preparations of each fiber were: X, 8.0 and 8.5 mm, $N = 3$; Y, 7.2 and 10.2 mm, $N = 4$; and Z, 10.1 and 10.7 mm, $N = 2$. The values ranged widely, probably because of various degrees of damage to the fibers by the dual impalements. They are therefore likely to be underestimates (*2, 3*).

By using an estimate of input resistance calculated from the slope of voltage-current curves (obtained from TTX-treated fibers), a high specific membrane resistance of the order of 100 kilohm-cm^2 is indicated. Thus both length constant and membrane resistance of the oval organ fibers are consistent with values reported for other fibers conducting graded signals over comparable distances (*2, 3, 5–7*).

It has not so far been possible to record from the oval organ fibers within the ganglionic neuropil. If we assume a length constant of 1.0 cm (or more), however, receptor potentials evoked by maximum pulls, though undoubtedly attenuated over the distance between sensory endings and ganglion (1 to 1.5 cm), could arrive centrally with amplitudes of 12 mV or more. Graded potentials of comparable amplitudes recorded peripherally and centrally in afferent fibers of the crustacean T-C MRO and other nonspiking sensory neurons can elicit strong reflex activity in postsynaptic motoneurons (*4–8*). Furthermore, in arthropods, respiratory interneurons and locomotor premotor interneurons communicate with motoneurons by graded changes in membrane potential ranging from 10 mV to 2 mV or less (*15, 16*). Thus the receptor potentials arriving at the central terminals of oval organ fibers could be expected to have significant postsynaptic effects over the physiological range of the receptor. Preliminary experiments have provided evidence of tonic reflexes

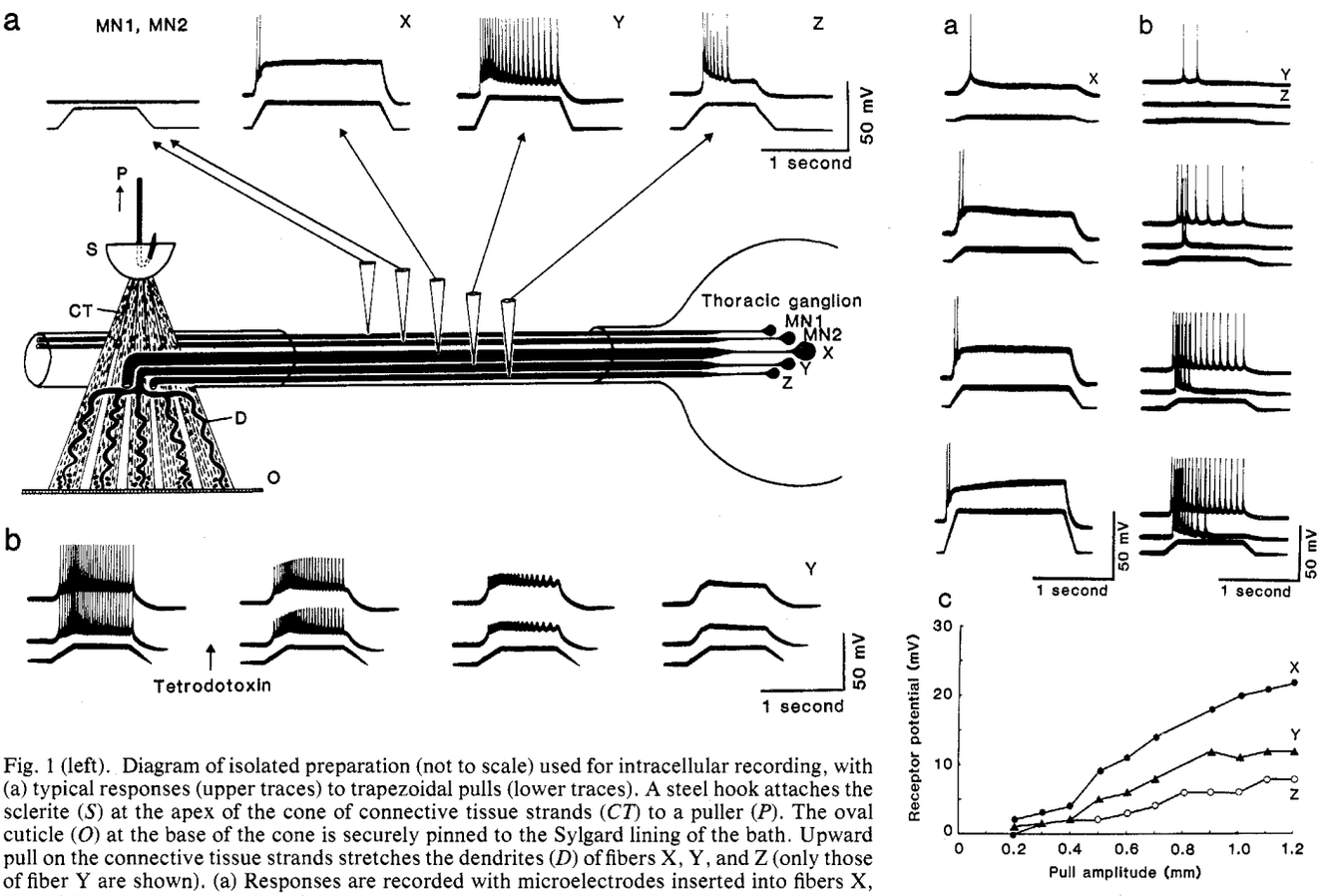

Fig. 1 (left). Diagram of isolated preparation (not to scale) used for intracellular recording, with (a) typical responses (upper traces) to trapezoidal pulls (lower traces). A steel hook attaches the sclerite (*S*) at the apex of the cone of connective tissue strands (*CT*) to a puller (*P*). The oval cuticle (*O*) at the base of the cone is securely pinned to the Sylgard lining of the bath. Upward pull on the connective tissue strands stretches the dendrites (*D*) of fibers X, Y, and Z (only those of fiber Y are shown). (a) Responses are recorded with microelectrodes inserted into fibers X, Y, and Z; no responses are seen in the motor axons MN1 and MN2 which run in the same nerve trunk. (b) Recordings from two microelectrodes inserted into another fiber Y taken before and after the addition of tetrodotoxin (bath concentration, $8 \times 10^{-7} M$). Responses are to successive pulls delivered at 1-minute intervals. Distal electrode 3.6 mm, proximal electrode 5.8 mm from confluence of sensory dendrites. Fig. 2 (right). Intracellular responses of sensory fibers to increasing amplitude of stretch. (a) Responses of fiber X. (b) Responses of fibers Y (upper traces) and Z (middle traces). Trapezoid pulls (lower traces) were delivered at 1-minute intervals. (c) Graph showing relationship between amplitude of the adapted graded potentials (after 1 second of pull) and the amplitude of stretch [data are taken from the three fibers of a single oval organ, from a different preparation from the one shown in (a) and (b)].

in scaphognathite motoneurons which could be due to the slow nonimpulsive component of the oval organ input (*17*).

In fibers Y and Z, the spikes superimposed upon the receptor potentials also reflect the stimulus parameters. For example, the number of spikes evoked by a step pull of constant duration is proportional to the pull amplitude over at least a major part of the response range of both Y and Z (Fig. 2b). Their frequency increases during the dynamic phase (ramp) of a constant velocity pull and varies with its velocity. The threshold amplitude of fiber Y is lower than that of fiber Z, both with rapid pulls of graded amplitude (Fig. 2b) and in the responses to large pulls at low velocities. At suprathreshold amplitudes, fiber Z fires initially at higher frequencies than Y, but adapts more rapidly, probably because of the apparently faster decline of its underlying receptor potential (Fig. 2b).

Thus, at least fibers Y and Z are capable of conveying sensory information normally, by means of trains of impulses, in addition to that coded in the graded potentials. The brief, highly phasic discharge of one or at most a few spikes in fiber X is not likely to have much significance for encoding amplitude, but it probably has other functions. For example, it may provide timing cues about the onset or phase of, or disturbances to, rhythmic movements of the gill bailer (*10, 15*).

We believe this is the first report of a sensory receptor whose afferent fibers carry both spikes and graded depolarizations, over a distance of 1 cm or more, into the central nervous system. Similar dual signaling is known in molluscan photoreceptors (*18*), amacrine cells of the vertebrate retina (*19*), and in motoneurons of the lobster stomatogastric ganglion (*20*); it may be present in a range of cell types in vertebrate and invertebrate nervous systems. The lobster oval organ may thus prove to be a useful model for studying the cellular properties of such neurons.

VALERIE M. PASZTOR
Department of Biology, McGill University, Montreal, Quebec, Canada H3A 1B1
BRIAN M. H. BUSH
Department of Physiology, University of Bristol, Bristol BS1 5LS, England

References and Notes

1. *Cold Spring Harbor Symp. Quant. Biol.* **30** (1965), whole volume; D. Mellon, Jr., *The Physiology of Sense Organs* (Freeman, San Francisco, 1968); W. R. Loewenstein, Ed., *Handbook of Sensory Physiology*, vol. 1, *Principles of Receptor Physiology* (Springer-Verlag, New York, 1971).
2. B. M. H. Bush, in *Structure and Function of Proprioceptors in the Invertebrates*, P. J. Mill, Ed. (Chapman & Hall, London, 1976), pp. 115–151; in (*3*), pp. 147–176; _____ and A. Roberts, *J. Exp. Biol.* **55**, 813 (1971); M. Mirolli, *ibid.* **78**, 1 (1979).
3. A. Roberts and B. M. H. Bush, Eds., *Neurones Without Impulses* (Cambridge Univ. Press, New York, 1981).
4. W. J. Heitler, *J. Exp. Biol.*, in press; D. H. Paul, *Science* **176**, 680 (1972).
5. A. J. Hudspeth, M. M. Poo, A. E. Stuart, *J. Physiol. (London)* **272**, 1 (1977); S. R. Shaw, *ibid.* **220**, 145 (1972); in (*6*), pp. 275–295; F. Zettler and M. Jarvilehto, *J. Comp. Physiol.* **85**, 89 (1973).
6. F. O. Schmitt and F. G. Worden, Eds., *The Neurosciences: Fourth Study Program* (MIT Press, Cambridge, Mass., 1979).
7. A. R. Blight and R. Llinas, *Philos. Trans. R. Soc. London Ser. B* **290**, 219 (1980); D. Oertel and A. E. Stuart, *J. Physiol. (London)* **311**, 127 (1981).
8. B. M. H. Bush and A. Roberts, *Nature (London)* **218**, 1171 (1968); A. J. Cannone and B. M. H. Bush, *J. Exp. Biol.* **86**, 275 (1980); *ibid.*, p. 305.
9. F. O. Schmitt, P. Dev, B. H. Smith, *Science* **193**, 114 (1976); K. Pearson, in (*6*), pp. 145–157.
10. V. M. Pasztor, *Can. J. Zool.* **47**, 435 (1969); *Zoomorphologie* **193**, 171 (1979).
11. Cobalt diffused overnight into the cut central ends of the oval organ afferents or the ventilatory motor axons (or both); the cobalt sulfide precipitate was intensified with silver [R. M. Pitman, C. D. Tweedle, M. J. Cohen, *Science* **176**, 412 (1972); J. P. Bacon and J. S. Altman, *Brain Res.* **138**, 359 (1977)].
12. The subesophageal ganglion, the oval organ with its supporting structures, and the connecting nerve trunk were dissected from the anterior thorax of the lobster (*Homarus gammarus*) and maintained in a bath of lobster saline (507 m*M* NaCl, 8.4 m*M* KCl, 15 m*M* $CaCl_2$, 7.5 m*M* $MgCl_2$, 10 m*M* tris, 5 m*M* maleic acid; *p*H 7.4); the bath was chilled, aerated, and flowing. Stretch stimuli were delivered to the organ with an electromagnetic puller and monitored with a strain-gauge transducer. Intracellular responses of the sensory (or motor) fibers were recorded with micropipettes filled with 3*M* KCl (18 to 30 megohms).
13. Average fiber diameters were: X, 41 μm; Y, 32 μm; Z, 22 μm; motor axons MN1 and MN2, 25 μm. Mean nerve length, 12.5 mm (range 10.8 to 15 mm).
14. C. Eyzaguirre and S. W. Kuffler, *J. Gen. Physiol.* **39**, 87 (1955); S. Nakajima and K. Onodera, *J. Physiol. (London)* **200**, 161 (1969).
15. R. E. Young and P. E. Coyer, *J. Exp. Biol.* **82**, 53 (1979); A. J. Simmers and B. M. H. Bush, *Brain Res.* **197**, 247 (1980); A. J. Simmers, in (*3*), pp. 177–198.
16. M. Burrows and M. V. S. Siegler, *J. Physiol. (London)* **285**, 231 (1978); M. Burrows, *Science* **204**, 81 (1979); *J. Neurophysiol.* **42**, 1108 (1979).
17. V. M. Pasztor and B. M. H. Bush, in preparation.
18. D. L. Alkon and M. G. F. Fuortes, *J. Gen. Physiol.* **60**, 631 (1972); R. Chase, *J. Exp. Biol.* **60**, 721 (1974).
19. J. E. Dowling, in (*6*), pp. 163–181.
20. J. A. Raper, *Science* **205**, 304 (1979); K. Graubard, J. A. Raper, D. K. Hartline, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3733 (1980); P. S. Dickinson, F. Nagy, M. Moulins, *J. Neurophysiol.* **45**, 1125 (1981).
21. We thank S. Best for assistance in early experiments and S. Best, A. Cannone, F. Clarac, R. DiCaprio, and J. Simmers for helpful comments on the manuscript. Supported by research grants from the Royal Society and the Science Research Council (United Kingdom).

8 July 1981; revised 21 September 1981

Inhibition of Idiotype–Anti-Idiotype Interaction for Detection of a Parasite Antigen: A New Immunoassay

Abstract. *Described in this report is an immunoradiometric assay of general applicability that is based on a new principle: the inhibition of the interaction between monoclonal antibodies by an antigen. The advantages of this assay are that it measures concentrations of single epitopes, purified antigen is not required, and the reagents can be obtained in unlimited amounts and are homogeneous. Its features are particularly attractive when the antigen has not been purified and is a minor component of a complex mixture of molecules.*

A monoclonal antibody (3D11) was recently produced in BALB/c mice against a 44,000-dalton membrane protein (Pb44) of sporozoites of *Plasmodium berghei*, a rodent malaria parasite (*1, 2*). This antibody (of the immunoglobulin IgG1, κ chain isotype), injected intravenously into mice protects the animals against infection with sporozoites.

Here we report the isolation of a second monoclonal antibody against the idiotype of 3D11, and describe an immunoassay based on the specific inhibition of the interaction between the two monoclonal antibodies by Pb44. This assay has been named 4i, which stands for inhibition of idiotype–anti-idiotype interaction. It does not require purified antigen and should have general applicability.

The monoclonal antibody to 3D11 was obtained by injecting BALB/c mice at various skin sites with 3D11 cross-linked with rabbit IgG by treatment with glutaraldehyde (*3*). A total of 1 to 2 mg of protein was given per mouse over a period of 10 weeks. The spleen cells of two animals were used for fusion with the plasmacytoma cell line SP2 (*4*) as described in (*5*). We screened the supernatants of the cultured cells for an activity that would inhibit the reaction between ^{125}I-labeled 3D11 and a conventional rabbit antiserum to the 3D11 idiotype (*6*). From a total of 700 wells originating from two fusions we found one supernatant that contained antibodies with the desired properties. The cells from the positive well were expanded and cloned by limiting dilution. The resulting hybridoma (2D12) was injected intraperitoneally into pristane-treated mice to obtain ascites fluid. A monoclonal antibody (IgG2a, κ chain) was present in this fluid at concentrations of 2 to 4 mg/ml.

We next studied the effect of the Pb44 antigen on the 3D11/2D12 interaction.

0036-8075/82/0326-1637$01.00/0