acetate or potassium chloride was able to promote [^{35}S]methionine incorporation into cataractous lenses, indicating the importance of the cation in this process. Optimum incorporation of [^{35}S]methionine into the crystallins was observed between 30 m*M* K^+ (90 m*M* Na^+) and 60 m*M* K^+ (60 m*M* Na^+). At best [^{35}S]methionine incorporation into the crystallins of the cataractous lens fiber cells was approximately 30 percent of the incorporation in the normal lens fiber cells. The failure to obtain greater incorporation of [^{35}S]methionine into the crystallins may be due to decreased amounts of adenosine triphosphate (ATP) or to reduced specific activities of the intracellular pool of [^{35}S]methionine in the Philly (*4*, *8*) and Nakano (*3*, *7*) lenses. The differences in [^{35}S]methionine incorporation could not be accounted for by differences in uptake of the labeled amino acid.

Further evidence indicating the importance of the intracellular cation environment for optimal crystallin synthesis was obtained in ouabain-treated lenses from 13-day-old normal mice. Ouabain is an inhibitor of Na^+,K^+-adenosine triphosphatase (*14*) and increases the Na^+/K^+ ratio in cultured lenses (*15*). As reported previously (*7*), ouabain treatment (2×10^{-3} *M*) reduced crystallin synthesis at least 95 percent in the cultured lenses. Approximately 80 percent recovery of crystallin synthesis was obtained when the K^+ concentration was increased to 50 m*M* and the Na^+ concentration was decreased to 89 m*M* in the lenses cultured in the presence of ouabain (data not shown).

The above experiments indicate that the reduction in crystallin synthesis during the early phases of cataractogenesis in the Philly and the Nakano lenses occurs principally in the fiber cells and is due to underutilization rather than degradation of crystallin mRNA's. We do not know whether or not the crystallin mRNA's in the fiber cells are confined to the clear regions of the cataractous lenses or for how long the crystallin mRNA's remain in the Nakano or Philly lens. The fact that changes in the intralenticular concentrations of Na^+ and K^+ may stimulate crystallin synthesis in the cultured lens of the Nakano or Philly mouse or in the ouabain-treated lens of the normal mouse supports the idea that alterations in the concentration of these cations contribute to the reduction in crystallin synthesis in osmotic cataracts (*5*, *6*). It is likely that other factors besides ions also contribute to the limitation of crystallin synthesis in the Nakano and Philly lens since we were only able to obtain partial recovery of crystallin synthesis by changing the concentrations of Na^+ and K^+ in the cultured lenses; much better results were achieved in the ouabain-treated normal lenses. Taken together, our findings show that the impairment of crystallin synthesis during the onset of cataractogenesis in Nakano and Philly mice is due to poor utilization of crystallin mRNA's in the lens fiber cells.

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References and Notes

1. J. H. Kinoshita, *Invest. Ophthalmol.* **13**, 713 (1974).
2. J. H. Kinoshita and L. O. Merola, *ibid.* **3**, 577 (1964); D. V. N. Reddy, D. Schwass, B. Chakrapani, C. P. Lim, *Exp. Eye Res.* **23**, 483 (1976); V. E. Kinsey and K. R. Hightower, *ibid.* **26**, 521 (1978).
3. S. Iwata and J. H. Kinoshita, *Invest. Ophthalmol.* **10**, 504 (1971).
4. P. F. Kador *et al.*, *Exp. Eye Res.* **30**, 59 (1980).
5. J. Piatigorsky and T. Shinohara, *Science* **196**, 1345 (1977); T. Shinohara and J. Piatigorsky, *Nature (London)* **270**, 406 (1977).
6. J. Piatigorsky, in *Current Topics in Eye Research* J. A. Zadunaisky and H. Davson, Eds. (Academic Press, New York, 1980), vol. 3, pp. 1-39.
7. J. Piatigorsky, H. N. Fukui, J. H. Kinoshita, *Nature (London)* **274**, 558 (1978).
8. J. Piatigorsky, P. F. Kador, J. H. Kinoshita, *Exp. Eye Res.* **30**, 69 (1980).
9. P. F. Kador, J. S. Zigler, Jr., J. H. Kinoshita, *Invest. Ophthalmol. Visual Sci.* **18**, 696 (1979).
10. J. J. Cenedella and G. B. Bierkamper, *Exp. Eye Res.* **28**, 673 (1979).
11. W. H. Garner and A. Spector, *Doc. Ophthalmol.* **18**, 91 (1979).
12. R. G. Ham, *Exp. Cell Res.* **29**, 515 (1963).
13. R. Reszelbach, T. Shinohara, J. Piatigorsky, *Exp. Eye Res.* **25**, 583 (1977).
14. J. L. Dahl and L. E. Hokin, *Annu. Rev. Biochem.* **43**, 327 (1974).
15. J. H. Kinoshita, H. L. Kern, L. O. Merola, *Biochim. Biophys. Acta* **47**, 458 (1961); H. L. Kern, P. Roosa, S. Murray, *Exp. Eye Res.* **1**, 385 (1962); S. L. Bonting, *Invest. Ophthalmol.* **4**, 723 (1965); J. E. Harris and B. Becker, *ibid.*, p. 709.
16. P. Zelenka and J. Piatigorsky, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1896 (1974).
17. We thank Drs. Jin H. Kinoshita, Peter F. Kador, and Henry N. Fukui for providing the Nakano and Philly mice; and R. Waldman for technical assistance, Dr. Howard M. Jernigan, Jr., for help in the Na^+ determinations with the atomic absorption spectrometer, and Deborah Carper for a gift of the crystallin antiserums.

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Dorsal Root Ganglion Neurons Are Destroyed by Exposure in utero to Maternal Antibody to Nerve Growth Factor

Abstract. *Rats and guinea pigs, when immunized with mouse nerve growth factor, produce antibodies that cross-react with their own nerve growth factor. The antibodies reach developing offspring of these animals both prenatally (rats and guinea pigs) and postnatally (rats). Depriving the fetus of nerve growth factor in this way results in the destruction of up to 85 percent of dorsal root ganglion neurons as well as destruction of sympathetic neurons. Sensory neurons of placodal origin in the nodose ganglion were not affected. These data demonstrate that dorsal root ganglion neurons go through a phase of nerve growth factor dependence in vivo.*

Nerve growth factor (NGF) is required for maintenance and survival of sympathetic neurons. The most compelling evidence for this is that heterologous antibodies to NGF, when passively transferred to newborn animals, destroy sympathetic neurons [immunosympathectomy (*1*)]. It has also been shown that NGF is required for the survival of sympathetic neurons in vitro. Similarly, NGF enhances the survival in vitro of dorsal root ganglion (DRG) neurons taken from embryonic chicks (*2*), embryonic rodents (*3*), newborn rodents (*4*), and human embryos (*5*). However, antibodies to NGF, when administered to newborn rodents, do not destroy sensory neurons (*1*). Hence, a physiological role for NGF in the development of sensory neurons in vivo is not yet established. In a recent study in which we used an experimental autoimmune approach, we found that there may be a permanent effect on dorsal root ganglia in rats exposed in utero to maternal antibodies to mouse NGF (*6*). We now report that rats and guinea pigs immunized with mouse NGF produce antibodies that cross-react with their own NGF. Antibodies to NGF, transferred to the offspring in utero, caused massive destruction of both peripheral sympathetic neurons and DRG sensory neurons. In the rat, exposure to maternal antibodies to NGF postnatally in milk destroyed sympathetic neurons, but not dorsal root ganglion neurons.

Adult female Sprague-Dawley rats and outbred guinea pigs were immunized with mouse NGF (*7*). These two species were chosen for study because they represent species in which antibody is passively transferred to offspring at different times during development. The rat receives small amounts of antibody prenatally and greater quantities postnatally via the milk, whereas the guinea pig receives large amounts of maternal antibody prenatally (*8*). For initial immunizations, 100 μg (rats) or 200 μg (guinea

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pigs) of mouse NGF in complete Freund's adjuvant was injected into the footpads. Boosters of 15 percent of the initial dose of mouse NGF in complete Freund's adjuvant were given at approximately monthly intervals. Serums from mothers and from offspring were assayed in the chick dorsal root ganglion by a modification (*6*) of a method previously described (*9*). Titers were defined as being greater than or equal to the reciprocal of the highest serum dilution that blocked the outgrowth of neurites in the bioassay. Rats were killed at maturity, and guinea pigs were killed shortly after birth; the animals were either perfused with 10 percent Formalin before death or were immersed in 10 percent Formalin after death. Ganglia were removed, and cell counts and size-frequency histograms were determined (*10*).

Initial experiments were carried out in rats. Considerable variation was found among litters born to different rats immunized with NGF (*11*). Therefore, a female rat (titer, 500 to 1000) that had previously produced seriously affected litters was selected for breeding. Offspring born to this NGF-immunized rat were exchanged at birth with offspring of a control rat. Thus, animals were exposed to antibodies to NGF in utero only or in milk only. Offspring born to the NGF-immunized female rat had serum titers of antibodies to NGF at birth (10 to 25), confirming their exposure to the antibody during prenatal development. Rats born to a normal rat, but nursed by the NGF-immunized female, had higher serum titers during the first 2 weeks of life (~ 50). Rats exposed to antibodies to NGF grew normally, and the only external sign of abnormality in these animals was ptosis (drooped eyelids indicative of sympathectomy). Exposure to antibodies to NGF in utero only or in milk only produced a 90 percent decrease in neurons in the superior cervical sympathetic ganglia (Table 1). The number of neurons in the eighth cervical DRG was decreased 70 percent in animals exposed to antibodies to NGF in utero, but was not decreased in animals exposed to the antibodies in milk. These results are consistent with our previous data showing a 70 to 80 percent decrease in the retrograde transport of ^{125}I-labeled NGF in rats exposed in utero to maternal antibodies to NGF (*6*) and demonstrate that NGF is required for the survival of sensory neurons in vivo. The lack of effect of postnatally administered antibodies to NGF on DRG neurons indicates that either DRG neurons lose their dependence on NGF or that postnatally administered antibodies to NGF do not gain access to the NGF supplied to peripheral sensory neurons.

Table 1. Effects of exposure to maternal antibodies to NGF on neuronal numbers in peripheral ganglia of offspring. Animals were the offspring of control or NGF-immunized females. Rats were examined at maturity (16 to 20 weeks of age). The "in utero only" group was born to an NGF-immunized rat and nursed by a control rat; the "in milk only" group was born to a control rat and nursed by an NGF-immunized rat. Guinea pigs were examined shortly after birth.

Species	Exposure	Cell numbers in ganglia*		Nodose
		Superior cervical	Eighth cervical DRG	
Rat	None (control)	25,400 ± 1,940 (3)	5,650 ± 220 (3)	
	In utero only	1,690 ± 40 (3)	1,750 ± 110 (3)	
	In milk only	2,300 ± 270 (3)	5,870 ± 220 (3)	
Guinea pig	None (control)	57,600 ± 5,080 (3)	15,500 ± 180 (3)	15,900 ± 980 (3)
	In utero			
	Litter 1	280 ± 130 (5)	3,120 ± 96 (3)	15,500 ± 1,530 (3)
	Litter 2		2,280 ± 156 (3)	

*Means ± standard errors, with number of animals in group given in parentheses. Cell numbers were determined as described in (*8*). Blank spaces indicate cell numbers were not determined.

As mentioned above, developing rats received more antibodies postnatally via the milk than rats that were exposed to antibodies prenatally. More profound and consistent effects of prenatal exposure to maternal antibody would be expected in a species in which greater amounts of maternal antibody are passively transferred to the offspring prenatally. This occurs in the guinea pig (*8*). The guinea pig offers the additional advantage that guinea pig NGF can be isolated and purified from the prostate (*12*). Hence, antibodies to mouse NGF in the guinea pig show reactivity to both mouse and guinea pig NGF, and cross-reactivity can be determined in vitro. Two female guinea pigs that were immunized against mouse NGF showed serum titers of antibody against mouse NGF of 4000 to 8000. Antiserums from both animals neutralized guinea pig NGF at dilutions equal to a titer of 2000 or greater, thus directly demonstrating cross-reactivity of the antibodies with guinea pig NGF. Serums of newborns from these guinea pigs had titers greater than 1000 against guinea pig NGF and mouse NGF (titers about two orders of magnitude higher than that of newborn rats). At birth the guinea pigs appeared normal; however, by 2 days of age, all had bilateral corneal keratitis (opacities). Most of the offspring died within a few days of birth. All offspring failed to gain weight and had obvious sensory deficits, including insensitivity to heat, cold, pinpricks, pinching, and corneal irritation. Such stimuli elicited brisk responses from age-matched control guinea pigs. One offspring of a guinea pig producing antibodies to NGF survived for 2 weeks, during which time growth was stunted (weight of 89 g compared to a control weight of 210 g). In spite of the limited number of animals examined it is clear that guinea pigs exposed to antibodies to NGF prenatally are severely affected. Cell counts were performed on three peripheral ganglia in guinea pigs fixed shortly after birth (Table 1). The superior cervical sympathetic ganglia were barely recognizable as expansions of attenuated sympathetic trunks, and the number of neurons was reduced by more than 99 percent. The eighth cervical DRG was reduced in neuronal number by 80 percent in one litter and by 85 percent in another. The nodose ganglion, a sensory ganglion (tenth nerve) of placodal origin, rather than of neural crest origin, was not affected.

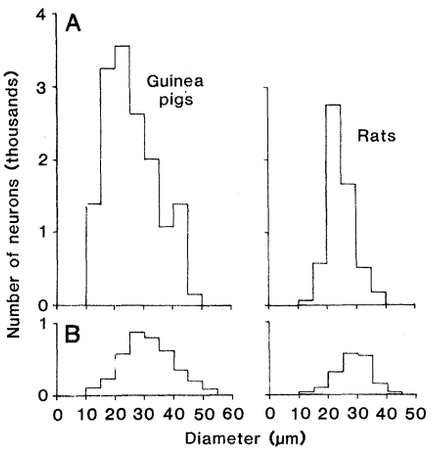

Fig. 1. Size-frequency histograms of neurons of the eighth cervical DRG in (A) normal animals and (B) animals exposed in utero to maternal antibodies to NGF. Guinea pigs were killed within 2 days of birth. Rats were killed at 16 to 20 weeks of age.

Sensory neurons of the DRG are heterogeneous in size and subserve different sensory modalities. Size-frequency histograms were generated from the DRG of both guinea pigs and rats (Fig. 1). Histograms from control guinea pigs and guinea pigs exposed to antibodies to NGF were similar, indicating that the antibodies affected cells of almost all

sizes. The histograms of the guinea pigs exposed to antibodies to NGF tended to shift toward larger cell diameters; this shift is more pronounced in the rat (Fig. 1), in that the decrease in cell numbers appears to be due to the loss of cells having diameters less than 30 μm. The shift toward cells of larger diameter in the histograms of both species may indicate either that the largest neurons are less susceptible to antibodies to NGF or that surviving neurons become hypertrophied. It is perhaps more likely that the larger neurons, which develop earlier in gestation (*13*), pass through the phase of NGF dependence before the antibody reaches the fetus [antibody levels in the fetus increase in later stages of gestation (*8*)].

These experiments indicate that most of the sensory neurons in dorsal root ganglia depend for survival on NGF during prenatal development. Our data are quantitatively consistent with experiments showing that most embryonic DRG neurons will survive in vitro in the presence, but not in the absence, of NGF (*3*). The survival of 15 to 20 percent of DRG neurons in the guinea pig may be interpreted as an incomplete destruction of NGF-sensitive neurons, resulting either from an insufficient titer of antibody or access of antibody to their source of NGF, or from a temporal difference in their dependence on NGF. Alternatively, some DRG neurons (up to 20 percent) may not require NGF at any time.

It is not known whether sensory neurons in cranial ganglia are affected by exposure to antibodies to NGF. Deficits observed in the guinea pig (for example, absence of corneal reflex in response to irritants) suggest that at least some of the sensory functions mediated by the trigeminal ganglion are affected. Since sensory neurons in the nodose ganglion are not decreased in number, neurons derived from the placodes, rather than from the neural crest, do not appear to require NGF. This is consistent with the inability of nodose ganglion neurons to retrogradely transport ^{125}I-labeled NGF (*14*). Further studies will be required to resolve the issue of whether cranial sensory ganglia and sensory ganglia that are derived all or in part from the placodes undergo a period of NGF dependence during development.

These data demonstrate that exposure in utero to maternal antibodies to NGF, in addition to producing immunosympathectomy, destroys sensory neurons. Because the developmental anomalies observed in sympathetic and sensory ganglia in familial dysautonomia (*15*) are similar to those observed in the animals described in this report, we suggest that these animals may be a useful model of this human pathological condition. At a more fundamental level, the experimental autoimmune approach should make possible a more precise determination of the time and degree of dependence of different populations of sensory neurons on NGF. The ability to ablate prenatally a significant percentage of the peripheral sensory nervous system will make possible the study of retrograde and anterograde effects of sensory lesions.

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References and Notes

1. R. Levi-Montalcini and P. V. Angeletti, *Physiol. Rev.* **48**, 534 (1968).
2. ———, *Dev. Biol.* **7**, 653 (1963).
3. S. M. Crain and E. R. Peterson, *Brain Res.* **79**, 145 (1974).
4. S. Varon, C. Raiborn, and E. Tyszka, *ibid.* **54**, 51 (1973).
5. S. M. Crain, E. R. Peterson, M. Liebman, H. Schulman, *Exp. Neurol.* **67**, 205 (1980)
6. P. D. Gorin and E. M. Johnson, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5382 (1979).
7. V. Bocchini and P. U. Angeletti, *ibid.* **64**, 787 (1969). Mouse NGF was kindly supplied by the laboratory of R. Bradshaw.
8. F. W. Brambell, *Frontiers of Biology* (Elsevier, New York, 1970), vol. 18.
9. E. L. Fenton, *Exp. Cell Res.* **59**, 383 (1970).
10. Paraffin sections were cut at 6 μm on a calibrated microtome. In every 20th Nissl-stained section, all neurons containing nucleoli were counted. The total number was obtained by multiplying the summed sample by 20 and correcting for "split" nucleoli by the expression $t/(t + d)$, where t is the thickness of the section and d is the mean nucleolar diameter [E. W. Konigsmark, in *Contemporary Research Methods in Neuroanatomy*, W. H. Nauta and S. O. E. Ebbesson, Eds. (Springer-Verlag, New York, 1970), p. 315]. Multiple nucleoli were accounted for by multiplying the correction by the mean number of nucleoli in a sample of 25 nuclei. Mean nucleolar diameter was corrected for random sectioning by the expression $4\bar{d}/\pi$. [E. R. Weibel, in *Principles and Techniques of Electron Microscopy*, M. A. Hayat, Ed. (Van Nostrand, New York, 1973) p. 237]. Final correction factors ranged from 0.51 to 0.63. Neuron diameters were minimum chords of cells containing nucleoli measured at random when they "passed" a filar micrometer as a mechanical stage was moved in a series of linear sweeps. Pooled samples of 150 measurements from three ganglia were used to construct each distribution histogram.
11. P. D. Gorin and E. M. Johnson, *Dev. Biol.*, in press. The effect on offspring of female rats with comparable antibody titers against mouse NGF shows marked variability as assessed by the size of sympathetic ganglia and tyrosine hydroxylase activity in the ganglia at birth. Possible reasons for this include variability in cross-reactivity of the antibodies to mouse NGF with rat NGF or variability in the time during gestation when antibody is transferred to the fetus. However, in any individual female rat, the severity of effects is consistent from litter to litter. Therefore, the offspring chosen for the analyses reported here were from a female rat that had previously delivered severely affected litters.
12. G. P. Harper *et al.*, *Nature* (*London*) **279**, 160 (1979). The guinea pig NGF used in these experiments was prepared by J. Rubin and R. Bradshaw by a modification of the procedure in (*5*).
13. S. N. Lawson, K. W. T. Caddy, T. J. Biscoe, *Cell Tissue Res.* **153**, 399 (1974).
14. K. Stoeckel, G. Guroff, M. Schwab, H. Thoenen, *Brain Res.* **109**, 271 (1976); E. M. Johnson, R. Y. Andres, R. A. Bradshaw, *ibid.* **150**, 319 (1978).
15. C. M. Riley, R. L. Day, D. M. Greeley, W. S. Langford, *Pediatrics* **3**, 468 (1949); J. Pearson and B. Pytel, *J. Neurol. Sci.* **39**, 47 (1978); ———, N. Grover-Johnson, F. Axelrod, J. Dancis, *ibid.* **35**, 77 (1978).
16. We thank P. Osborne for assistance. This work was supported by the National Foundation–March of Dimes, the Familial Dysautonomia Foundation, and by grants HL20604 and HD12260 from the National Institutes of Health. E.M.J. is an established investigator of the American Heart Association.
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Fourier-Transformed Infrared Photoacoustic Spectroscopy of Biological Materials

Abstract. *A new technique for measuring the infrared spectra of solids has been developed. The photoacoustic spectra of hemin, hemoglobin, protoporphyrin IX, and horseradish peroxidase show how this technique can be used to obtain structural information about biological materials which cannot readily be studied by normal transmission infrared spectroscopy. The method requires milligram quantities of material and involves no sample preparation.*

It is common in the study of biochemicals that an infrared spectrum of a lyophilized material or an insoluble material is required. Until now, this has been a requirement that could be met only by grinding the material into a KBr wafer or pellet. The possibility of structural alterations resulting from such a procedure is great.

The new technique of Fourier-transformed infrared (FTIR) photoacoustic spectroscopy offers a versatile and convenient solution to this problem. This technique, described briefly below and more fully elsewhere (*1–5*), can be employed for studying the properties of such biological materials. Photoacoustic spectroscopy (PAS) of solid materials in the visible and ultraviolet spectral regions was originally developed in 1881 by Tyndall (*6*), Röntgen (*7*), and Bell (*8*) and has recently been brought back to the forefront of research by Rosencwaig (*9*) and others (*10–13*). However, visible and ul-

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