5. L. Feeney-Burns, E. R. Berman, H. Rothman, *Am. J. Ophthalmol.* **90**, 783 (1980).
6. R. W. Young and D. Bok, *J. Cell Biol.* **42**, 392 (1969).
7. B. W. Streeten, *Arch. Ophthalmol.* **66**, 391 (1961); H. Kolb and P. Gouras, *Invest. Ophthalmol.* **13**, 487 (1974); L. Feeney, *Invest. Ophthalmol. Visual Sci.* **17**, 583 (1978); G. L. Wing, G. C. Blanchard, J. J. Weiter, *ibid.*, p. 601.
8. J. S. Bellin, *Photochem. Photobiol.* **4**, 33 (1965); R. S. Becker, *Theory and Interpretation of Fluorescence and Phosphorescence* (Wiley-Interscience, New York, 1969), p. 34.
9. Microscope photometer system (Leitz MPV 1.1); photomultiplier tube (Hamamatsu R928) in a Pacific system (housing, amplifier, and model 204 power supply); light source, 100-W mercury arc (Osram, HBO); Ploempak 2.3 epi-illumination unit with filter modules; ×40 oil immersion apochromatic objective (numerical aperture, 1.00).
10. The PMT was cross-calibrated to a multiply calibrated photodiode (EG&G HUV4000B); filters were calibrated on a spectrophotometer (Cary 210). See also V. J. Ferrans, L. M. Buja, W. C. Roberts, D. S. Fredrickson, *Am. J. Pathol.* **64**, 67 (1971).
11. The emission unit of the Turner 430 spectrofluorometer, including the PMT (Turner Associates, No. 430-340), was calibrated with an L-101 spectral irradiance standard (Electro Optics Associates) [J. Lee and H. H. Seliger, *Photochem. Photobiol.* **4**, 1015 (1965)].
12. Human RPE cells were brushed from fresh, unfixed eyes within 24 hours of the donor's death and pooled in distilled water under nitrogen and lyophilized.
13. A. L. Tappel, in *Free Radicals in Biology*, W. A. Pryor, Ed. (Academic Press, New York, 1980), vol. 4, p. 8.
14. C. C. Farnsworth and E. A. Dratz, *Biochim. Biophys. Acta* **443**, 556 (1976).
15. Cellulose HPTLC plates (Eastman, No. 13255) were developed with a mixture of butanol, acetic acid, and water (12:3:5).
16. Silica gel HPTLC plates (E. Merck, No. 11845) were activated and developed with a mixture of *n*-heptane, chloroform, methanol, and acetic acid (76:49:13:2).
17. The postnuclear fraction was layered on a discontinuous density sucrose gradient and centrifuged; the yellow fluorescent band was collected. Electron microscopy showed virtually pure lipofuscin granules (G. E. Eldred and L. Feeney-Burns, in preparation).
18. Silica gel was activated and developed with hexanes, chloroform, methanol, and acetic acid (76:49:13:2).
19. C. E. White and R. J. Argauer, *Fluorescence Analysis, A Practical Approach* (Dekker, New York, 1970), p. 30; C. A. Parker, *Photoluminescence of Solutions* (Elsevier, New York, 1968), p. 252.
20. K. R. Brizzee, J. M. Ordy, B. Kaack, *J. Gerontol.* **29**, 336 (1974).
21. H. Hyden and B. Lindstrom, *Discuss. Faraday Soc.* **9**, 436 (1950).
22. H. Barden, F. Aviles, W. Rivers, in *Pigment Cell*, vol. 4, *Biologic Basis of Pigmentation*, S. N. Klaus, Ed. (Karger, Basel, 1979), p. 263; H. Barden, *J. Neuropathol. Exp. Neurol.* **39**, 598 (1980).
23. R. D. Taubold, A. N. Siakotos, E. G. Perkins, *Lipids* **10**, 383 (1975).
24. J. G. Bieri, T. J. Tolliver, W. G. Robison, Jr., T. Kuwabara, *ibid.* **15**, 10 (1980); W. G. Robison, Jr., T. Kuwabara, J. G. Bieri, *Invest. Ophthalmol. Visual Sci.* **19**, 1030 (1980).
25. K. S. Chio and A. L. Tappel, *Biochemistry* **8**, 2827 (1969); V. G. Malshet and A. L. Tappel, *Lipids* **8**, 194 (1973); B. L. Fletcher, C. J. Dillard, A. L. Tappel, *Anal. Biochem.* **52**, 1 (1973); V. G. Malshet, A. L. Tappel, V. M. Burns, *Lipids* **9**, 328 (1974).
26. C. J. Dillard and A. L. Tappel, *Lipids* **6**, 715 (1971); M. Minssen and K. D. Munkres, *Biochim. Biophys. Acta* **291**, 398 (1973); I. D. Desai, B. L. Fletcher, A. L. Tappel, *Lipids* **10**, 307 (1975); B. D. Goldstein and E. M. McDonagh, *J. Clin. Invest.* **57**, 1302 (1976).
27. A. Tappel, B. Fletcher, D. Deamer, *J. Gerontol.* **28**, 415 (1973); A. S. Csallany, K. L. Ayaz, L. C. Su, *J. Nutr.* **107**, 1792 (1977).
28. Supported by NIH grants EY 05456 (G.E.E.), EY 03408 (W.S.S.), and EY 03274 (L.F.B.); by NSF grant BNS 76-11921 (W.S.S.); and by grants-in-aid from Research to Prevent Blindness, Inc. (L.F.B.) and the National Society to Prevent Blindness (G.E.E.). Tissues were provided through the Eye Research Foundation and the Lions Tissue Eye Bank, Columbia, Mo.

28 December 1981

Early Chronic Low-Level Methylmercury Poisoning in Monkeys Impairs Spatial Vision

Abstract. *Five monkeys were treated from birth with oral doses of mercury as methylmercury (50 micrograms per kilogram of body weight per day); concentrations in the blood peaked at 1.2 to 1.4 parts per million; and declined after weaning from infant formula to a steady level of 0.6 to 0.9 part per million. There were no overt signs of toxicity. When tested between 3 and 4 years of age under conditions of both high and low luminance, treated monkeys exhibited spatial vision that was impaired compared with that of control monkeys.*

In the outbreaks of human poisoning from methylmercury in Japan and later in Iraq, one of the most consistent signs in adults was deficits in visual function. The deficit that has received the most emphasis is constriction of the visual fields, although other visual deficits, particularly changes in visual acuity, have been reported with equal frequency (*1*). Human data suggest that the fetus or neonate exposed to methylmercury in breast milk may be at greater risk from methylmercury poisoning than the adult and that the signs may be different from those of adult methylmercury poisoning (*2, 3*).

The visual system of macaque monkeys resembles that of humans (*4*) and exhibits the same signs and pathological lesions as that of humans when exposed to methylmercury (*5–7*). Macaques are therefore excellent models for testing the effects of methylmercury on the visual system. We separated cynomolgus monkeys (*Macaca fascicularis*) from their mothers within 12 hours after birth and raised them in a primate nursery (*8*). They were given oral doses of mercury (0 or 50 μg per kilogram of body weight per day) as methylmercury starting at birth and continuing throughout the period of testing. Blood concentrations peaked at approximately 1.2 to 1.4 ppm and dropped after withdrawal of infant formula at 200 days of age to a steady level between 0.6 and 0.9 ppm (*9*). None of the monkeys showed any overt signs of toxicity. Food intake and weight gain were indistinguishable from those of control subjects and routine hematological measures were normal. No abnormalities were detected during regularly scheduled clinical neurological examinations, and treated monkeys were as agile in the large exercise cages as controls.

Optic head and macula appeared nor-

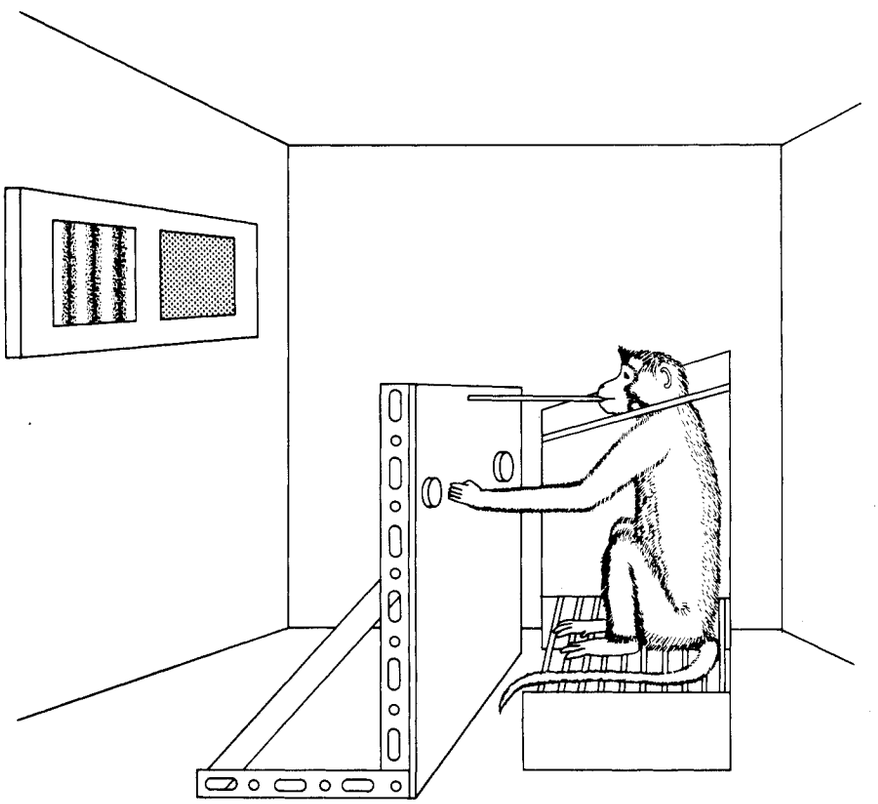

Fig. 1. Diagram of the apparatus.

0036-8075/82/0514-0759$01.00/0

mal in all monkeys. Streak retinoscopy revealed that most of the monkeys' eyes were slightly hyperopic (+0.25 to +0.50 diopters), as is normal for young primates. One control monkey had slight astigmatism in the plane of testing in one eye, and the other was slightly myopic in both eyes (−0.50 diopters). One treated monkey had slight astigmatism 90° to the plane of testing in one eye, and another was hyperopic in one eye (+2.50 diopters). No monkey had refractive errors severe enough to interfere with the task. These monkeys exhibited no constriction of visual fields when tested in a perimeter that measured to approximately 80°.

When the monkeys were between 3 and 4 years of age, their ability to see the various frequency components of objects was determined (*10*). The seated monkey faced two oscilloscopes at a distance of 114 cm; each scope subtended 4° of visual angle horizontally (Fig. 1). On each trial, one oscilloscope (chosen randomly) displayed a stimulus of a vertical sine wave grating, while the other was a blank screen of equal average luminance. The contrast (*11*) and spatial frequency (cycles per degree) of the gratings were controlled by an on-line computer. The monkey was required to press the button corresponding to the oscilloscope displaying the grating in order to receive apple juice through a drinking spout (*12*). For each of a number of spatial frequencies, the contrast at which the monkey responded with 70 percent correct choices was determined and considered to be the threshold for that frequency (*13*). Monkeys were tested at two average luminances: 5 foot lamberts in the photopic (cone vision) range, and 5×10^{-4} foot lamberts in the scotopic (rod vision) range (1 foot lambert = 3.4263 candela/m^2). The latter luminance was achieved by inserting neutral-density filters between the monkey and the oscilloscopes; monkeys were dark-adapted for 20 minutes before being tested.

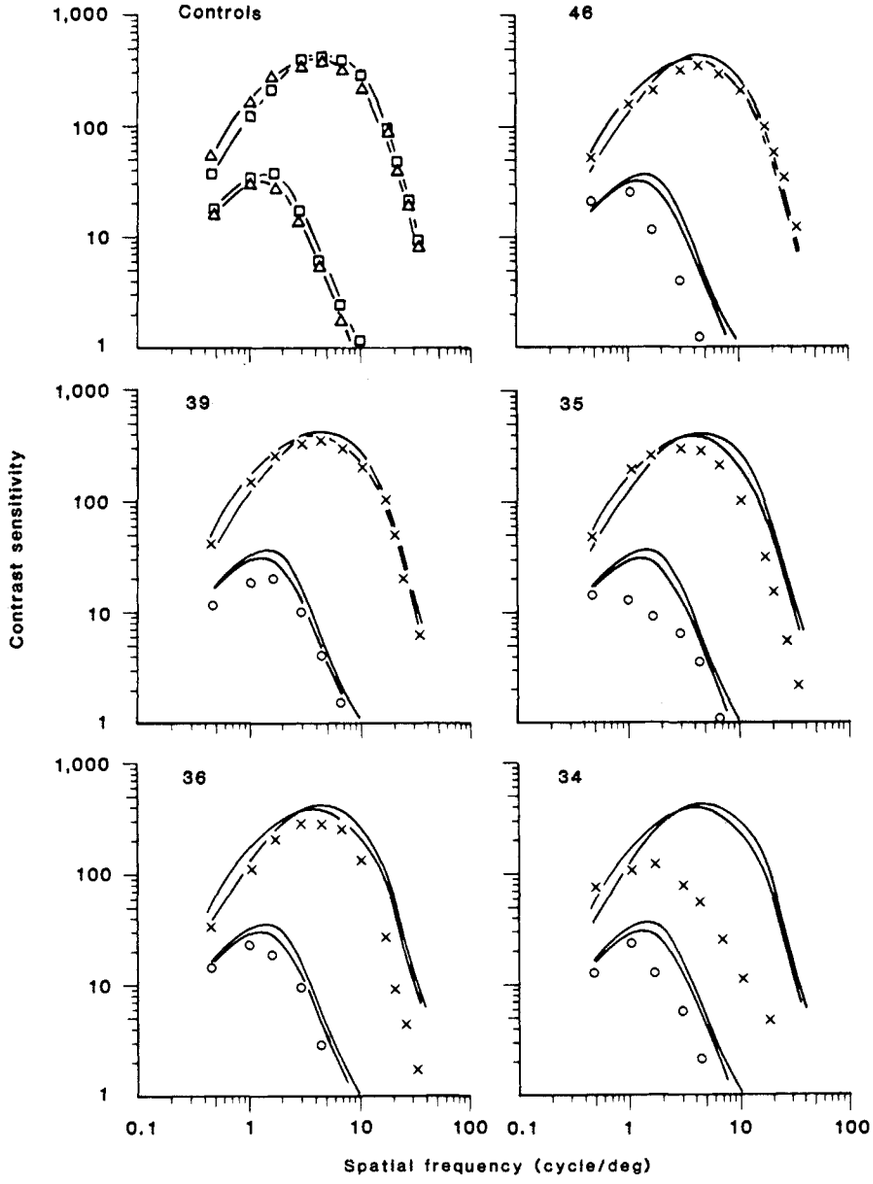

Fig. 2. Spatial contrast sensitivity functions for two control and five methylmercury-treated monkeys under conditions of high and low luminance. For the control monkeys, the squares and triangles represent the individual animals. For each treated monkey, the x's represent threshold at each frequency under high luminance conditions, and circles the thresholds at low luminance. Solid lines on each graph represent envelope of thresholds for control monkeys.

The contrast sensitivity functions for the two control monkeys were very similar to those published for other macaque monkeys (*4*). The shape of the psychometric function was similar for control and treated monkeys (*14*), indicating that factors other than ability to detect the grating (such as motivation and attention) did not differ between treated and control monkeys. All five monkeys treated with methylmercury exhibited impairment of spatial visual function compared with the two control monkeys (Fig. 2). At the high luminance, two of the treated monkeys (46 and 39) had contrast sensitivity functions indistinguishable from those of the controls, two (35 and 36) were impaired at high frequencies (and slightly at middle frequencies), and the fifth (34) was severely impaired at all but very low frequencies. At the low luminance, all treated monkeys were impaired, but patterns of deficit varied among monkeys. Two (46 and 34) were substantially impaired at frequencies greater than 1 cycle per degree, one (39) was somewhat impaired at the lower frequencies, one was most impaired at middle frequencies (35), and the fifth was moderately impaired at all but the lowest frequency (36). The deficits of individual monkeys under low luminance conditions were not correlated in any obvious fashion with their deficits at the high luminance.

In the human as well as the monkey, deficits in vision produced by methylmercury are thought to be central in origin (*15*). The most severe pathological changes in the adult are in calcarine fissure, whereas infant poisoning results in a wider distribution of damage throughout the visual cortex. Our study indicates that impairment of visual acuity may occur independently of constriction of visual fields in infantile methylmercury poisoning and that impairment of acuity may be a more sensitive indicator of exposure. Whether this also may be the case in the adult is unclear, al-

though adult Minamata patients in Japan were found to have middle- but not low-frequency deficits (high frequencies were not tested) with little or no constriction of visual fields (*16*).

Although deficits in peripheral vision, especially constriction of visual fields, may be the most conspicuous and extensive visual lesion in methylmercury poisoning, other visual changes seem to occur at the same time and possibly earlier under certain conditions.

DEBORAH C. RICE
STEVEN G. GILBERT
Toxicology Research Division, Bureau of Chemical Safety, Health Protection Branch, Health and Welfare Canada, Ottawa, Ontario K1A 0L2

References and Notes

1. S. Sabelaish and G. Hilmi, *Bull. WHO* **53** (Suppl.), 83 (1976); S. F. Al-Damluji and Clinical Committee on Mercury Poisoning, *ibid.*, p. 65; H. Rustam and T. Hamdi, *Brain* **97**, 499 (1974).
2. L. Chang, *Environ. Res.* **14**, 329 (1977).
3. Y. Harada, in *Minamata Disease, Study Group of Minamata Disease*, M. Kutsuna, Ed. (Kumamoto University, Kumamoto, Japan, 1968), pp. 73 and 93; T. Takeuchi, in *ibid.*, p. 141; P. Pierce *et al.*, *J. Am. Med. Assoc.* **22**, 1439 (1972); L. Amin-Zaki, S. Elnassani, M. Majeed, T. W. Clarkson, R. Doherty, M. R. Greenwood, *Pediatrics* **541**, 587 (1970).
4. R. de Valois and K. de Valois, *Annu. Rev. Psychol.* **31**, 309 (1980); W. Merigan, *Vision Res.* **20**, 953 (1980).
5. H. Evans, V. Laties, B. Weiss, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 1858 (1975); C.-M. Shaw, K. Mottet, R. L. Body, E. S. Luschei, *Am. J. Pathol.* **80**, 451 (1975).
6. M. Berlin, C. A. Grant, J. Hellberg, J. Hellstrom, A. Schutz, *Arch. Environ. Health* **30**, 340 (1975).
7. R. Garmon, B. Weiss, H. Evans, *Acta Neuropathol.* **32**, 61 (1975).
8. R. Willes, P. Kressler, J. Truelove, *Lab. Anim. Sci.* **27**, 90 (1971).
9. Total blood mercury was determined by flameless atomic absorption spectrophotometry [F. Iverson, R. H. Downie, H. L. Trenholm, C. Paul, *Toxicol. Appl. Pharmacol.* **27**, 1 (1974)]. Sampling frequency was every 14 days until 450 days of age, once monthly until 3 years of age, and quarterly thereafter.
10. It is believed that the spatial detection capacity of the visual systems is by means of multiple spatial frequency channels, each sensitive to a portion of the total spectrum [F. W. Campbell and J. G. Robson, *J. Physiol. (London)* **197**, 551 (1968); F. W. Campbell, in *The Neurosciences: Third Study Program*, F. O. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, Mass., 1974), p. 95]. One can predict the detectability of any pattern on the basis of a Fourier analysis of the pattern and the sensitivity of the visual system (of an individual) to sine waves of different frequencies. Thus a determination of the contrast sensitivity function of an individual describes the spatial detection ability of that individual, in the plane of testing.
11. Contrast is defined as difference in luminance between the lightest and darkest part of the sine wave divided by their sum.
12. The first lever on which the monkey made three presses (fixed ratio 3) was considered to be the response choice. Correct choices were reinforced with juice; juice delivery was preceded by a 0.7-second tone. Incorrect choices resulted in a 7-second time-out period, which was signaled by a clicking noise. Each reinforcement or time out was followed by a 3-second intertrial interval. Responses during the time out or intertrial interval reset the component to its initial value. Monkeys were tested 5 days per week.
13. In each session, a series of five contrast values differing by 1-dB steps was tested for each of 20 frequencies. Contrasts were presented in a random order. The series of contrast values for the next session was chosen on the basis of each monkey's performance. Threshold was considered to have been reached when the threshold determination did not vary by more than one contrast level for four consecutive sessions. Frequencies were tested in sequential order to allow maximum practice effect.
14. The psychometric function for any particular frequency is represented as a plot of contrast versus percent correct. The steepness of the slope indicates how well the animals' behavior is under schedule control. A reduced slope suggests poorer control over the monkey's behavior, while a shift in the function to the right with no difference in slope indicates visual impairment.
15. *WHO Environmental Health Criteria I, Mercury* (World Health Organization, Geneva, 1976).
16. S. Ishikawa, R. Okamura, K. Mukuno, *Nippon Ganka Gakkai Zasshi* **83**, 336 (1979); K. Mukuno, S. Ishikawa, R. Okamura, *Br. J. Ophthalmol.* **65**, 284 (1981).

8 June 1981; revised 19 October 1981

Global Carbon Monoxide Fluxes: Inappropriate Measurement Procedures

Bartholomew and Alexander have calculated (*1*) that the global CO uptake by soil is 4.1×10^{14} g/year. Although we should be satisfied to see Seiler's earlier estimates (*2*) confirmed, we are deeply concerned about the experimental procedure used by Bartholomew and Alexander (*1*) and what we feel are misquotations from Seiler's earlier paper (*2*). Their calculation is based on laboratory experiments that represent disturbed conditions, whereas our experiments are based on measurements made in the field under natural conditions. We are also concerned about the application of an unrealistically high CO mixing ratio of 3 parts per million by volume (ppmv) and the use of the radiotracer technique with ^{14}CO as the tracer.

The CO uptake depends on concentration, and observed ambient CO mixing ratios in the lower troposphere are of the order of 0.05 to 0.30 part per million (ppm); thus reported uptake rates based on mixing ratios of 3 ppmv must be overestimated by approximately one order of magnitude. Furthermore, the report by Bartholomew and Alexander totally neglects the fact that CO is not only destroyed but also produced in soil. In summer at high soil surface temperatures and ambient CO mixing ratios ($\leq$ 0.30 ppmv), the production sometimes exceeds the destruction. Under these conditions the soil acts as a source of atmospheric CO. Extrapolation of results obtained at 3 ppm to low ambient mixing ratios, however, indicates that the soil is always a net sink, an incorrect generalization.

The existence of simultaneous production and destruction of CO by different processes in the soil clearly demonstrates the inapplicability of a radiotracer technique that measures only the oxidation of ^{14}CO to $^{14}CO_2$ and not the production of CO by soil. The use of ^{14}CO is therefore inappropriate for the determination of the CO net flux between the soil and atmosphere. Thus we feel that agreement between Seiler's earlier data and those obtained by Bartholomew and Alexander (*1*) is fortuitous.

The global CO uptake rate of 5×10^{14} g/year (*2*) is based on in situ measurements carried out under ambient natural conditions, different types of soil, and different seasons and weather conditions, covering soil temperatures of 3° to 50°C. This is well documented in (*2*); we therefore do not understand the statement of Bartholomew and Alexander that Seiler's estimate is based on "measurements of a few European soils in the laboratory" and "multiplying the average uptake rate of a few soils at 15°C."

W. SEILER
R. CONRAD
Max-Planck-Institut für Chemie, D-6500 Mainz, West Germany

References

1. G. W. Bartholomew and M. Alexander, *Science* **212**, 1389 (1981).
2. W. Seiler, in *Environmental Biogeochemistry and Geomicrobiology*, W. E. Krumbein, Ed. (Ann Arbor Science, Ann Arbor, Mich., 1978), vol. 3, p. 773.

5 October 1981

We are surprised that Seiler and Conrad missed the crucial difference between their studies and ours. Atmospheric scientists accept the fact that the properties and behavior of CO, SO_2, and N_2O are different, and they would not assume that the average values of some properties of five gases could be used to predict the properties of all gases. If it is inappropriate to conclude that, "once you have seen one atmospheric component, you have seen them all," it would also seem reasonable to suggest that soils differ markedly, and that averaging numbers for five soils, all from one geographical area, does not provide a meaningful mean value for all soils. Soil taxonomists have labored long and hard to develop a meaningful and useful scheme for the categorization of soils, and it is incumbent upon atmospheric scientists, microbiologists, and others to base their extrapolations on, or at least link them to, the classification systems thus devel-

0036-8075/82/0514-0761$01.00/0