ing skeletal muscle atrophy (10-15), fibrillation (16), increased numbers of extrajunctional ACh receptors (17), and other effects (9, 18). Although our studies indicate that surgical and pharmacological denervation have profound effects on the susceptibility of skeletal muscle to Coxsackievirus infection, they do not specify the nature of the changes that permit infection at the muscle cell level. The susceptibility of muscle cells must depend on their ability to support the entire sequence of steps in the virus replication process, including virus binding, entry, uncoating, and synthesis and release of viral components. Studies with poliovirus (another related picornavirus) have demonstrated that host cell susceptibility occurs relatively early in the infectious cycle (before synthesis of viral components) (19). Further studies are needed to determine which step in the infection sequence is limiting in innervated muscle and is altered after denervation to permit Coxsackie A2 virus infection.

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## **References and Notes**

- G. Dalldorf, J. Exp. Med. 106, 69 (1957); R. J. Goldberg and R. L. Crowell, J. Virol. 7, 759 1971)
- (1971).
   W. P. Rowe, Science 117, 710 (1953); T. Sato and S. M. Chou, Neurology 28, 1232 (1978).
   D. M. Fambrough, Physiol. Rev. 59, 165 (1979); C. Edwards, Neuroscience 4, 565 (1979); Thesleff and L. C. Sellin, Trends Neurosci. 4, 122 (1980); D. B. Drachman, A. Pestronk, E. F. Stanley, in Disorders of the Motor Unit, D. L. Scheiland, Ed. (Wiley, New York, 1982), pp. Schotland, Ed. (Wiley, New York, 1982), pp. 107-117.
- Coxsackie  $A_2$  virus stock (American Type Cul-ture Collection lot Cox 82VR ET L1184A 792162) was prepared by two serial passages through Swiss mice less than 24 hours old. The 4. mice were inoculated intraperitoneally and sub-cutaneously with 75  $\mu$ l of a 1:10 dilution of the original virus preparation in phosphate-buffered saline. Seven days later the mice were killed and their limb muscles were homogenized with a Potter-Elvehjem tissue grinder and centrifuged for 10 minutes at 2500g. The supernatant was for 10 minutes at 2500g. The supernatant was inoculated into a second litter of neonatal mice. When moribund (1.5 to 2 days), these animals were killed and their limb muscles were homogenized and centrifuged as above. The supernatant solution was collected, assayed, and stored at  $-70^{\circ}$ C until being used as stock virus for infection of mice
- 5. In the Coxsackievirus assay rhabdomyosarcoma cells (supplied by R. L. Crowell) were cultured in Eagle's minimum essential medium containing sodium bicarbonate ( $\rho$ H 7.3) and 10 percent fetal bovine serum at 37°C in air with 5 percent CO<sub>2</sub>. Microtiter plates were seeded with  $^{1}$  sector  $^{10}$  CO<sub>2</sub>. Microtiter plates were seeded with  $^{1}$  ×  $^{10}$  cells per well and grown to 90 percent confluence over 24 hours. Each muscle to be assayed was homogenized in 2.0 ml of medium containing 0.2 percent fetal bovine serum in a mortar cooled to  $-70^{\circ}$ C. Serial tenfold dilutions of the homogenate were made and 0.25 ml of each dilution was added to quadruplicate microtiter containing rhabdomyosarcoma cells. s were examined at 4 to 6 days for a wells Plates cytopathic effect. The dilution yielding 50 percent positive and 50 percent negative responses was estimated by interpolation of the data, and

the resultant virus titer was expressed in multiples of  $TCID_{s0}$  units per milliliter of muscle homogenate. Statistical analysis by Student's *t*-text for the statistical analysis by Student's *t*-text. test for two samples was used to compare results for different groups of animals. 6. After excision the muscles were pinned to a stiff

- card at resting length and quick-frozen in iso-pentane cooled with solid CO<sub>2</sub>. Multiple 6- $\mu$ m sections were made of each muscle in a cryostat. After staining with hematoxylin and eosin the slides were coded and examined microscopical-
- 7. A. C. Guvton and M. A. MacDonald, Arch. Neurol. Psychiatry 57, 578 (1947); V. B. Brooks, J. Physiol. (London) 134, 264 (1956); S. G. Cull- Candy, M. Lundt, S. Thesleff, *ibid.* 260, 177
   (1976); I. Kao, D. B. Drachman, D. L. Price, *Science* 193, 1256 (1976); D. W. Pumplin and T. S. Reese, *J. Physiol. (London)* 273, 443 (1977); Polak, L. C. Sellin, S. Thesleff, ibid. 319 (1981); L. L. Simpson, *Pharmacol. Rev.* 33, 155 (1981)
- S. Thesleff, J. Physiol. (London) 151, 598 (1960); 8. Inestell, J. Physiol. (London) 151, 596 (1960);
   L. W. Duchen, J. Neurol. Sci. 14, 47 (1971); A. J. Harris and R. Miledi, J. Physiol. (London) 217, 497 (1971); S. S. Freeman, A. G. Engel, D. B. Drachman, Ann. N.Y. Acad. Sci. 274, 46 (1972) (1976)
- 9. D. B. Drachman, Ann. N.Y. Acad. Sci. 228, 160 (1974)
- M. B. Giacobini-Robecchi, G. Giacobini, G. 10. Filogamo, J. P. Changeux, Brain Res. 83, 107 (1975).
- D. B. Drachman, E. F. Stanley, A. Pestronk, D. L. Price, J. W. Griffin, J. Neurosci. 2, 232 (1982).
- (1982).
  H. L. Fernandez and B. U. Ramirez, *Brain Res.* **79**, 385 (1974); S. Ochs, *Ann. N.Y. Acad. Sci.* **228**, 202 (1974); L. Guth and E. X. Albuquerque, *Physiol. Bohemoslov.* **27**, 401 (1978); L. Guth, V. F. Kemerer, T. A. Samaras, J. E. Warnick,

## **Residential Firewood Use**

In their report on the use of residential firewood in the United States, Lipfert and Dungan (1) used the New England Fuelwood Study (2) results in developing an equation to estimate national firewood consumption by air pollution control regions. Although the degree of fuelwood-induced air pollution is partially a function of the concentration of fuelwood use in any given region, I strongly disagree, both theoretically and statistically, with their estimation method. I will limit my discussion to the following two points: (i) the issue of causality is ignored and (ii) the extrapolation of New England household woodburning habits to the rest of the country is questionable.

The Lipfert-Dungan estimation equation states that the amount of residential fuelwood burned per degree day is dependent on population density. They also imply in their reference 5(1) that this variable, since it is important in air quality analysis, is also important in explaining the number of cords of wood a household might consume. While population density may be an appropriate index for air quality analysis, Lipfert and Dungan fail to substantiate their claim that it is important in predicting household fuelwood consumption. By not discussing the causal relations between cords of wood consumed, climatic conditions (degree days), and population denE. X. Albuquerque, *Exp. Neurol.* **73**, 20 (1981).
 D. A. Mathers and S. Thesleff, *J. Physiol.* (London) **282**, 105 (1978); J. J. Bray, J. W. Forrest, J. I. Hubbard, *ibid.* **326**, 285 (1982).
 E. E. Schelwingd, D. D. Darshen, *Paris Psychology* **1**, 2000

- E. F. Stanley and D. B. Drachman, Brain Res. 261, 172 (1983).
- 15. D. B. Drachman, Science 145, 719 (1964); I. D. B. Drachman, Science 145, 719 (1964), 1.
  Jirmanova, M. Sobotkova, S. Thesleff, J. Zelena, *Physiol. Bohemoslov*. 13, 467 (1964).
  J. O. Josefson and S. Thesleff, *Acta Physiol. Scand*. 51, 163 (1961). 16
- Scand. 51, 163 (1961).
  17. S. Thesleff, J. Physiol. (London) 151, 598 (1960); D. A. Tonge, *ibid.* 241, 127 (1974); A. Pestronk, D. B. Drachman, J. W. Griffin, Nature (London) 264, 787 (1976); L. L. Simpson, J. Pharmacol. Exp. Ther. 200, 343 (1977).
  18. D. B. Drachman, in Biology of Cholinergic Function, A. M. Goldberg and I. Hanin, Eds. (Raven, New York, 1976), pp. 162–186.
  19. J. J. Holland, L. C. McLaren, J. T. Syverton, J. Exp. Med. 110, 65 (1959); L. C. McLaren, J. J. Holland, J. T. Syverton, *ibid.* 109, 479 (1959).
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- was begun in collaboration with 1. the development Weiner; some of the results were reported previ-ously [I. Kao *et al.*, *Neurology* **27**, 344A (1977)]. To whom requests for reprints should be addressed.

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sity, their extrapolation of New England data to the country as a whole is not defendable.

The New England Fuelwood Survey found a number of socioeconomic variables that were significant in explaining residential fuelwood consumption patterns. These included cost of conventional energy in comparison with wood energy, woodburning apparatus used, percentage of owner-occupied households, and household location in relation to firewood supplies and wood-using industries. These variables were not included by Lipfert and Dungan. Their unsuccessful attempt to explain the divergence of a number of state consumption patterns from their best-fit line as well as a recently completed nationwide residential fuelwood consumption survey (3), which estimated that average consumption of fuelwood per household is greater in the southwest region than in the north central region, provide ample evidence that there are serious weaknesses in Lipfert and Dungan's model specifications. The equation is not only inadequate but also inappropriate for estimating national fuelwood consumption rates by region.

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## **References and Notes**

- 1. F. W. Lipfert and J. L. Dungan, Science 219,
- 1425 (1983) 2. M. R. Bailey, P. R. Wheeling, M. I. Lenz, Wood M. R. Balley, P. K. Wheeling, M. I. Leitz, *wood and Energy* (Economic Research Service, Department of Agriculture, Washington, D.C., 1982 and 1983). Six reports, for each of the New England States (Maine, New Hampshire, Vermont, Massachusetts, Connecticut, and Rhode Island), make up the New England Fuelwood Study Study
- 3. E. Skog and I. A. Waterson, Residential Fuel-wood Use in the United States: 1980-1981 (Forest Products Laboratory, Department of Agri-culture Forest Service, Madison, Wis., in press).

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The Skog and Waterson (1) survey data on the distribution of residential fuelwood use support not only our estimate of total national consumption but the relation we described for the distribution of usage across the country in terms of heating degree days and population density as well. With respect to the issue of causality, we stated that the purpose of our inquiry was to derive an empirical relation for the spatial density of fuelwood use because of concern about air quality impacts. For this reason, population density (at the county level) was a useful explanatory variable. We also had to restrict our analysis to those variables for which data were readily available for the whole country.

We did not examine why wood fuel was used. Economic factors play a large role in these causal relationships (2) as do esthetic and life-style factors. Bailey is correct in that we did not consider these factors, and some may indeed be sources of variability in usage patterns. However, several factors that Bailey cited are collinear with population density-percentage of owner-occupied households and household location in relation to firewood supplies-and would not greatly change the predictive power of a regression. We used population density because of the purpose to which the regression equation would be put and the availability of data nationwide from counties.

Finally the Skog and Waterson data (1)support the validity of our extrapolation of the New England data to the rest of the country in the following ways.

1) Our estimate of total national usage for the 1978-1979 heating season was  $34.7 \times 10^6$  cords. Their estimate for the 1980–1981 heating season was  $42 \times 10^6$ cords, for both primary and secondary homes. This must be considered excellent agreement, since the Department of Energy estimates indicate at least a 20 percent increase for the 2 years (3).

2) With regard to distribution, a comparison of our state estimates of total usage with those of Skog and Waterson (1) for the 37 states for which estimates were reported gave a correlation coefficient of 0.79. This is about the same as we had reported in our original analyses (R = 0.81 for 18 states).

3) Bailey's statement that Skog and Waterson (1) "estimated that average consumption of fuelwood per household is greater in the southwest region than in the north central region," providing "ample evidence that there are serious weaknesses" in our model is in error. Their report states that the total consumption in the South (12 states from Florida and the Carolinas to Texas and Oklahoma) is about the same as that for 13 north central states (from the Dakotas to the Great Lakes), as are the numbers of households in the two regions, and explains this finding as a trade-off between less urbanization and higher heating degree days (1), precisely the two factors used in our model. When the individual state estimates for these two regions are compared with our model predictions, we find that consumption was overestimated in 2 of the 13 northcentral states and underestimated in 4 of the 12 states in the South. However, it is possible that the Skog and Waterson survey may be biased toward a disproportionate representation of rural households (4).

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## Notes and References

- 1. E. Skog and I. A. Waterson, Residential Fuelwood Use in the United States: 1980–1981 (For-est Products Laboratory, Department of Agri-culture Forest Service, Madison, Wis., in
- press). 2. F. W. Lipfert and J. Lee, *Air Pollution Implica*tions of Increasing Residential Firewood Use, BNL33906 (Brookhaven National Laboratory, Upton, N.Y., October 1982).
- 3. The 95 percent confidence limit on our national estimate was  $31.1 \times 10^6$  to  $38.2 \times 10^6$  cords. Skog and Waterson (1) report a standard error of "up to 10 percent" which would give approxi-mate confidence limits from  $34 \times 10^6$  to  $50 \times 10^6$  cords; the difference between the esti-mates is not statistically significant European 50 × 10° cords; the difference between the esti-mates is not statistically significant. Further-more, a Department of Energy survey (DOE/ EIA Report 0341, Department of Energy, Wash-ington, D.C., 1982) indicates a 22 percent in-crease in residential wood energy consumption, from 1978 to 1981. This factor would increase our 1978–1979 estimates to 42.3 × 10<sup>6</sup> cords.
- 4. Equal numbers of urban and rural dwellers were
- polled, but "urban" was defined as applying to a village or city with more than 2500 people. Present address: Marine Biological Laboratory, Woods Hole, Mass. 02503.

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