useful information can be gathered from the experimental curves once the throw is reduced from the low-field value. For the case of americium it was possible to detect the beginning of flux penetration into the sample (H_{c1}), but it was not possible to measure H_{c2} (*13*). We were not able to detect H_{c1} for protactinium for technical reasons. We shall, however, show later that for protactinium we can measure H_{c2} adequately down to $\sim$ 1/2 T_c before this distortion of the cooling curves becomes severe.

The tetragonally shaped polycrystalline sample showed a superconducting transition at 0.45 $\pm$ 0.02 K, slightly higher than the first sample. While this slightly higher T_c is within experimental error of the first, there are two rather obvious possible causes for the difference. First, the early Los Alamos work (*1*, *3*) showed that impurities can either raise or lower the T_c, since all of the transitions were measured for the body-centered tetragonal modification of protactinium. So perhaps our second sample is less pure. The second and more interesting cause may be that internal strains effectively pressurize the sample, thus raising T_c. A positive pressure effect was reported in (*3*), and this observation is probably correct because protactinium should somewhat resemble uranium, which has a dramatic pressure effect (*14*). We note that no matter how well annealed a sample may be, if it is noncubic and polycrystalline it will be significantly strained by thermal contraction after cooling to low temperatures. Thus, our measurements on the polycrystalline protactinium sample were obviously made on a strained sample.

The applied field curves for this second sample were similar to those in Fig. 1. However, the signal-to-noise ratio was improved since the sample was larger. Also, the DPE did not appear until the applied field was $\sim$ 10 Oe because of defects from the strains that should inhibit the Meissner effect somewhat. As expected (*12*), no DPE was observed in the curves taken with the Helmholtz coils since the signal coils were then oriented perpendicular to the applied field.

Figure 2 shows the critical field points for the first sample where we have taken the T_c's as the onsets of the transitions in the applied fields. The polycrystalline sample had a slightly higher H_{c2} but the curves became difficult to interpret at a higher temperature than for the single crystal. That is, the data was more like that of americium (*13*). For the highest-temperature points in Fig. 2 there is a small departure from a proper critical field dependence. This feature is not uncommon and is usually considered to be due to compositional variations (*15*). We feel that, once again, the self-heating and strains can interact to simulate such compositional variations in protactinium. As done in (*15*), we will exclude such points from further consideration.

The curve in Fig. 2 is a least-squares fit of the data to

$$H_{c2}(T)/H_{c2}(0) = 1 - T^2/T_c^2 \qquad (1)$$

Because of the rather limited temperature range of useful data, there is little reason to consider a fit of the data to a more sophisticated function or to consider the deviations of the data from a Bardeen-Cooper-Schrieffer curve. Such considerations involve departures from Eq. 1 of a few percent—of the same magnitude as the possible errors due to sample heating. A few self-consistent parameters based on the fit are

$$T_c = 0.430 \text{ K}$$

$$H_{c2}(0) = 56 \text{ Oe}$$

$$(dH_{c2}/dT)_{T_c} = -260 \text{ Oe/K}$$

We feel that until measurements such as resistivity and heat capacity are made on high-purity protactinium, further pursuit of superconducting parameters is too speculative. At best, we can say that the Ginsburg-Landau parameter κ is of the order of unity, and conclude that protactinium is type II.

We have found the superconducting properties of protactinium to be in the appropriate place between those of thorium [T_c = 1.4 K (*5*)] and those of uranium [$T_c < 0.1$ K (*5*, *14*)]. There is no longer any doubt that a smooth variation of superconducting properties in the early actinides can be caused by the increasing f character in the energy bands as the early actinide elements slowly acquire their electrons (*16*). It then seems quite likely that many of the interesting properties of uranium (*14*) that are not seen in thorium may be observed to some extent in protactinium.

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References and Notes

1. R. D. Fowler, B. T. Matthias, L. B. Asprey, H. H. Hill, J. D. G. Lindsay, C. E. Olsen, R. W. White, *Phys. Rev. Lett.* **15**, 860 (1965).
2. M. J. Mortimer, *Harwell Rep. AERE-R 7030* (1972).
3. R. D. Fowler, L. B. Asprey, J. D. G. Lindsay, R. W. White, in *Proceedings of the 13th International Conference on Low Temperature Physics*, K. D. Timmerhaus, W. J. O'Sullivan, E. F. Hammel, Eds. (Plenum, New York, 1974), p. 377.
4. R. O. A. Hall, J. A. Lee, M. J. Mortimer, *J. Low Temp. Phys.* **27**, 305 (1977).
5. H. H. Hill, *Physica* **55**, 186 (1971).
6. B. Johansson, *Phys. Rev. B* **11**, 2740 (1975).
7. J. Bohet and W. Müller, *J. Less Common Met.* **57**, 185 (1978).
8. D. Brown, T. C. Tso, B. Whittaker, *J. Chem. Soc. Dalton Trans.* **22**, 2291 (1977).
9. J. C. Spirlet, *J. Phys. (Paris)*, in press.
10. J. L. Smith and R. G. Haire, *Science* **200**, 535 (1978).
11. J. L. Smith and R. O. Elliott, in *Proceedings of the 2nd International Conference on the Electronic Structure of the Actinides*, J. Mulak, W. Suski, R. Troć, Eds. (Ossolineum, Wrocław, 1977), p. 257.
12. R. A. Hein and R. L. Falge, Jr., *Phys. Rev.* **123**, 407 (1961).
13. J. L. Smith, G. R. Stewart, C. Y. Huang, R. G. Haire, *J. Phys. (Paris)*, in press.
14. S. D. Bader, N. E. Phillips, E. S. Fisher, *Phys. Rev. B* **12**, 4929 (1975).
15. T. L. Francavilla and F. L. Carter, *ibid.* **14**, 128 (1976).
16. H. H. Hill and E. A. Kmetko, in *Heavy Element Properties*, W. Müller and H. Blank, Eds. (North-Holland, Amsterdam, 1976), p. 17; H. L. Skriver, O. K. Anderson, B. Johansson, *Phys. Rev. Lett.* **41**, 42 (1978).
17. We thank B. T. Matthias, G. R. Stewart, F. de la Cruz, and C. Y. Huang for many discussions and R. B. Roof for x-ray studies. Work at Los Alamos was performed under the auspices of the Department of Energy.

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A Nodule-Specific Plant Protein (Nodulin-35) from Soybean

Abstract. *Nodulin-35, a 35,000-molecular-weight protein, is present in soybean root nodules developed by different strains of* Rhizobium japonicum, *irrespective of their effectiveness in fixing atmospheric nitrogen. This protein is not detected in uninfected plants and bacteroids or in free-living* Rhizobium *and appears to be synthesized by the plant during the formation of root nodules.*

Development of root-nodule symbiosis between legumes and *Rhizobium* species, which results in effective fixation of atmospheric nitrogen, requires the specific expression of both host (*1*) and *Rhizobium* (*2*) genes. Through classical genetic experiments, several plant genes have been linked to effective nodulation, but, with the exception of leghemoglobin (*3*), no other plant gene product that is obligatory for the development of symbiosis has yet been identified. We report here the presence in soybean root nodules of a 35,000-mo-

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lecular-weight protein (referred to as Nodulin-35) that is formed only as a result of infection with *Rhizobium*, irrespective of whether the nodules are effective in nitrogen fixation. This protein is not detectable in control root tissue or in any other part of the plant; it is also absent from free-living *Rhizobium* and bacteroids. It is generally believed that the formation of root nodules (nodulation) depends upon the expression of a specific class of plant genes, some of which are under the control of *Rhizobium*. Nodulin-35 may represent one of the products of this class of genes.

There are three apparent stages of development in the formation of root nodules: (i) infection of roots by *Rhizobium*, (ii) nodulation, and (iii) the expression of *nif* genes leading to effective symbiotic nitrogen fixation. A mutation in plant (*1*) as well as in *Rhizobium* genes (*2*) could affect the development of the nodules at any of the three stages. In soybean, four genes (R_{j1} to R_{j4}) that affect nodulation have been identified (*4*). Mutation in R_{j1} does not allow infection of roots to take place, while mutation in R_{j2}, R_{j3}, or R_{j4} results in the development of nodules that cannot fix nitrogen. It is not known how mutations in *Rhizobium* that prevent infection or cause ineffective nodules affect the expression of plant genes.

In an effort to understand more about the expression of "nodule-specific" plant genes, we examined cytoplasmic proteins from both effective and ineffective root nodules of soybean as well as from uninfected roots, free-living *Rhizobium*, and bacteroids. Soybean (*Glycine max* L. var. Prize) was infected with *Rhizobium japonicum* (strain 61A76), which forms effective nodules, and grown under conditions described previously (*5*); root nodules, harvested 3 to 4 weeks after inoculation, were stored in liquid nitrogen. Bacteroids were prepared from 2- to 3-week-old nodules (*6*). Free-living *Rhizobium* were grown in liquid cultures (*7*) and also were induced for nitrogenase activity by restricted aeration (*8*), as shown by the acetylene reduction assay (*9*) (data not presented). Soluble cytoplasmic proteins (105,000*g* supernatant) were prepared as outlined in the legend to Fig. 1 and examined electrophoretically on polyacrylamide gels containing sodium dodecyl sulfate (SDS) (*10*).

Figure 1 shows that, while there are several plant proteins common to nodules and uninfected root tissue, a polypeptide having a molecular weight of approximately 35,000 is present in root nodules (lanes b and d) but is not detectable in uninfected roots (lane a), bacteroids (lane c), or free-living *Rhizobium* (lane k); nor is it detectable in *Rhizobium* induced for nitrogenase (lane l). This protein (Nodulin-35) represents about 4 percent of the total cytoplasmic fraction and can be enriched severalfold by ammonium sulfate fractionation. Precipitation of Nodulin-35 is virtually complete at 30 percent saturation and hence it facilitates purification of this polypeptide (see below). Glycoprotein staining (*11*) indicated that this is a nonglycosylated polypeptide. In order to determine whether this protein is present in nodules that do not fix nitrogen, cytoplasmic proteins were prepared from ineffective root nodules developed by several mutants of *R. japonicum* (strains SM3, SM4, SM5, and 61A24) and analyzed on SDS polyacrylamide gels (Fig. 1, lanes e to h). The presence of a common protein band at molecular weight 35,000 in nodules and its absence in bacteroids (lanes i and j) demonstrated that Nodulin-35 is a nodule-specific protein, and its appearance is not related to the effectiveness of the nodules in nitrogen fixation.

Nodulin-35 was purified to homogeneity by means of preparative SDS gel electrophoresis (Savant), and antibodies raised to this protein were tested for monospecificity by immunoelectrophoresis and double immunodiffusion (*12*). A 30 percent ammonium sulfate–precipitated fraction prepared from both effective and ineffective nodules, along with non-nodulated roots (used as a control), was reacted with the antiserum to Nodulin-35. Figure 2 shows a common precipitation line between cytoplasmic proteins obtained from nodules developed by different strains of *Rhizobium*, whereas Nodulin-35 is not detectable in uninfected roots. Furthermore, extracts from free-living *Rhizobium* (including

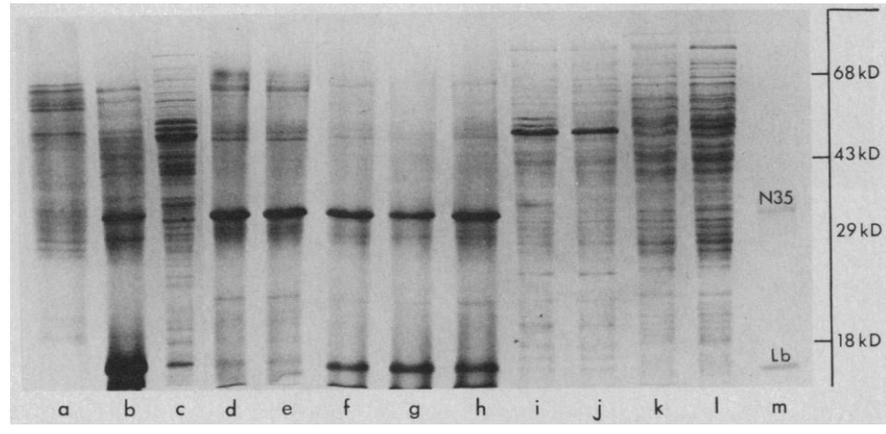

Fig. 1. SDS gel electrophoresis of cytoplasmic proteins extracted from soybean roots, nodules, bacteroids, and free-living *Rhizobium*. Total cytoplasmic proteins from (a) uninfected roots; (b) nodules induced by an effective strain of *Rhizobium japonicum* (strain 61A76, wild type); (c) wild-type bacteroids; (d to h) ammonium sulfate–precipitated fraction (30 percent saturation) of cytoplasmic proteins from nodules induced by *Rhizobium* strains 61A76, SM3, SM4, SM5, and 61A24, respectively; (i) wild-type bacteroids; (j) bacteroids from *Rhizobium* strain SM4; (k) free-living wild-type *Rhizobium*; (l) *Rhizobium* induced for nitrogenase; and (m) molecular weight markers: *N35*, Nodulin-35; and *Lb*, leghemoglobin. Control roots and 3-week-old nodules were ground with liquid nitrogen in a mortar and pestle, transferred into a buffer containing 50 m*M* tris-HCl (*p*H 8.7), 20 m*M* KCl, and 10 m*M* $MgCl_2$, and homogenized at 4°C. After removal of cell debris at 20,000*g* centrifugation, the supernatant was recentrifuged at 105,000*g* for 2 hours. The supernatant obtained after separation of ribosomes was either lyophilized and dissolved in the sample buffer [100 m*M* dithiothreitol, 80 m*M* tris-HCl (*p*H 6.8), 2 percent (weight to volume) SDS, 10 percent (by volume) glycerol, and 0.2 percent (weight to volume) bromophenol blue] or fractionated by ammonium sulfate (30 percent saturation). The precipitate was collected by centrifugation, resuspended in 10 m*M* tris-HCl (*p*H 7.8), dialyzed, and, following lyophilization, dissolved in the sample buffer. Samples containing approximately 100 μg of protein were boiled for 2 minutes in the sample buffer and electrophoresed. Free-living *Rhizobium* (strain 61A76) were cultured in liquid media as reported (*5*); bacteroids were extracted from nodules as described by Sutton *et al.* (*6, 7*). To isolate cytoplasmic proteins from free-living *Rhizobium* and bacteroids, the cells were suspended in a small volume of 80 m*M* tris-HCl (*p*H 6.8), sonicated on ice for 30 seconds (Sonifier Cell Disruptor, model W140D; Ultrasonics), and, following removal of cell debris at 20,000*g*, the extract was recentrifuged at 105,000*g* for 2 hours. The resulting supernatant was prepared for gel electrophoresis as outlined above. Cultures of *Rhizobium* induced for nitrogenase were grown at a low oxygen tension (*8*), examined for nitrogenase activity by the acetylene reduction assay (*9*), and analyzed electrophoretically. Electrophoresis was carried out on discontinuous SDS slab gels [17.5 percent acrylamide and 0.07 percent (weight to volume) bisacrylamide] in the buffer system described by Laemmli (*10*). The gels were stained in 0.2 percent (weight to volume) Coomassie brilliant blue R in 50 percent methanol and 10 percent acetic acid and destained in 30 percent methanol and 7 percent (by volume) acetic acid.

cultures induced for nitrogenase) and from bacteroids do not cross-react with the antiserum when examined by the double immunodiffusion test. The identity of this 35,000-molecular-weight protein was further determined in nodules induced by different strains of *R. japonicum* by analyzing peptide maps of purified Nodulin-35 on SDS gels (*13*). All profiles of proteolytic digestion of this protein are similar, suggesting that it is the same protein and its presence in different nodules is not simply a result of electrophoretic comigration or cross-reactivity with antibody (Fig. 3). The apparent structural identity of the 35,000-molecular-weight protein in nodules developed by various strains of *Rhizobium* also suggests plant rather than bacterial origin. That Nodulin-35 is synthesized by the host plant is shown by immunoprecipitation of the translation product of the nodule polysomes and its inhibition of synthesis with cycloheximide (Fig. 4). No immunoreactive material was obtained with the translation product of control root polysomes (data not shown). It is not certain whether this protein is involved in the structure or the function of nodules. Since the nodules formed by various effective and ineffective strains of *Rhizobium* contain Nodulin-35, the presence of this protein may not be related to the function of the nodules in fixing nitrogen.

It is apparent that the development of true symbiosis is obligatory for the effective fixation of atmospheric nitrogen. Failure to develop such symbiosis may result in a parasitic/saprophytic state and the nitrogen fixed under these conditions may be of no use to the host (*14*). Further characterization and identification of nodule-specific plant proteins (Nodulins) should promote an understanding of the specific requirements for developing symbiotic association with *Rhizobium*. This capacity is primarily restricted to legumes. *Trema* is the only nonlegume presently known to develop symbiosis with *Rhizobium* (*15*); it must harbor some of the genes obligatory for the development of the *Rhizobium*-legume symbiosis. Studies of plant gene expression in different mutants of soybean (*4*) could also greatly contribute to our knowledge of the role of the plant in developing association with *Rhizobium* leading to symbiotic nitrogen fixation.

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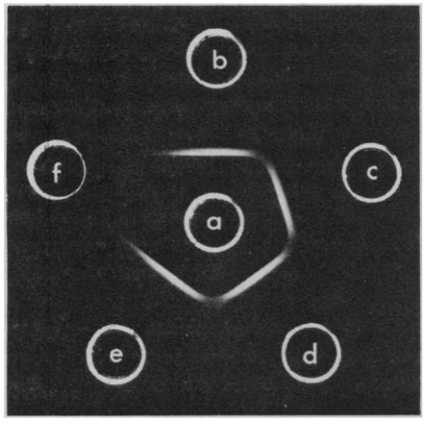

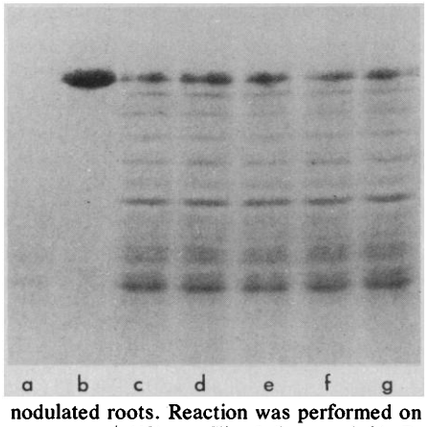

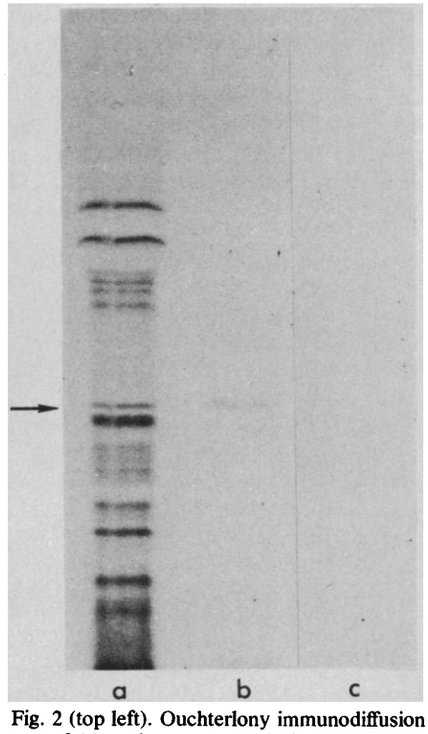

Fig. 2 (top left). Ouchterlony immunodiffusion test of (a) antiserum to Nodulin-35, with the 30 percent ammonium sulfate–precipitated fraction of cytoplasmic proteins from root nodules formed by *Rhizobium* strains (b) 61A76, (c) SM4, (d) SM5, (e) 61A24, and (f) from nonnodulated roots. Reaction was performed on Hyland agarose immunodiffusion plates at room temperature (*12*). Fig. 3 (bottom left). Peptide maps of Nodulin-35 isolated from effective and ineffective root nodules. The pure protein was digested with α-chymotrypsin during SDS slab gel electrophoresis (*13*). (a) α-Chymotrypsin, (b) undigested Nodulin-35, and (c to g) digestion products of Nodulin-35 from nodules induced by *Rhizobium* strains 61A76, SM3, SM4, SM5, and 61A24, respectively. Fig. 4 (right). SDS slab gel electrophoresis of in vitro translation products from soybean polysomes (autoradiofluorogram). In vitro translation of polysomes was carried out in micrococcal nuclease-treated wheat germ translation system (*16*): (a) total translation product, (b and c) immunoprecipitation of the translation product with antibody against Nodulin-35, and (c) translation in presence of 1 μg of cycloheximide per milliliter, which gave 89 percent inhibition of total trichloroacetic acid–precipitable counts. Arrow indicates the position of Nodulin-35 run as a marker in the parallel gel. Polysomes were isolated from 3-week-old nodules induced by *Rhizobium* strain 61A76 and translated in the presence of [^{3}H]leucine as described by Verma *et al.* (*3*). After translation, the ribosomes were removed by ultracentrifugation (105,000*g* for 2 hours) and the supernatant containing released polypeptides was prepared for electrophoresis as given in the legend to Fig. 1. Immunoprecipitation reaction was carried out on TCA-precipitated product as described by Lingappa *et al.* (*17*) using staphylococci-bound protein A to precipitate antigen-antibody complexes. After staining and destaining, the gel was treated with dimethyl sulfoxide (Me_2SO) and 20 percent 2,5-diphenyloxazole (New England Nuclear) in Me_2SO, dried, and exposed to prefogged x-ray film (Kodak RP-X-omat) for quantitative autoradiofluorography (*18*). In order to resolve Nodulin-35 from the band running just below it, the gels were overrun for 1 hour; as a result leghemoglobin is not visible in these gels.

References and Notes

1. F. B. Holl and T. A. LaRue, in *Proceedings of the First International Symposium on Nitrogen Fixation*, W. E. Newton and C. J. Nyman, Eds. (Washington State Univ. Press, Pullman, 1976), vol. 2, pp. 391–399.
2. R. J. Maier and W. J. Brill, *J. Bacteriol.* **127**, 763 (1976).
3. D. P. S. Verma and A. K. Bal, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3843 (1976); D. Baulcombe and D. P. S. Verma, *Nucleic Acids Res.* **5**, 4141 (1978).
4. L. F. Williams and D. L. Lynch, *Agronomy* **46**, 28 (1954); B. E. Caldwell, *Crop Sci.* **6**, 427 (1966); ———, K. Hinson, H. W. Johnson, *ibid.*, p. 495; B. E. Caldwell and G. Vest, *ibid.* **8**, 680 (1968); G. Vest, *ibid.* **10**, 34 (1970); ——— and B. E. Caldwell, *ibid.* **12**, 692 (1972).
5. D. P. S. Verma, D. T. Nash, H. M. Schulman, *Nature (London)* **251**, 74 (1974).
6. W. D. Sutton, N. M. Zepsen, B. D. Shaw, *Plant Physiol.* **59**, 741 (1977).
7. W. D. Sutton, *Biochim. Biophys. Acta* **366**, 1 (1974).
8. Y. J. Avissar and K. D. Nadler, *J. Bacteriol.* **135**, 782 (1978).
9. R. W. F. Hardy, R. D. Holsten, E. K. Jackson, R. C. Burns, *Plant Physiol.* **43**, 1185 (1968).
10. U. K. Laemmli, *Nature (London) New Biol.* **227**, 680 (1970).
11. R. M. Zacharius, T. E. Zell, J. H. Morrison, J. J. Woodlock, *Anal. Biochem.* **30**, 148 (1969).
12. H. Byrne, N. V. Christou, D. P. S. Verma, G. A. Maclachlan, *J. Biol. Chem.* **250**, 1012 (1975); D. P. S. Verma, G. A. Maclachlan, H. Byrne, D. Ewings, *ibid.*, p. 1019.
13. D. W. Cleveland, S. G. Fischer, M. W. Kirschner, U. K. Laemmli, *ibid.* **252**, 1102 (1977).
14. D. P. S. Verma, N. Hunter, A. K. Bal, *Planta* **138**, 107 (1978).
15. M. J. Trinick, *Nature (London)* **244**, 459 (1973).
16. D. P. S. Verma and S. Ball, in *Translation of Synthetic and Natural Polynucleotides*, A. B. Legocki, Ed. (Poznan Agricultural University Press, Poznan, Poland, 1977), p. 211.

17. V. R. Lingappa, J. R. Lingappa, R. Prasad, K. E. Ebner, G. Blobel, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2338 (1978).
18. W. M. Bonner and R. A. Laskey, *Eur. J. Biochem.* **46**, 83 (1974); R. A. Laskey and A. D. Mills, *ibid.* **56**, 335 (1975).
19. We thank Dr. W. Brill, University of Wisconsin, for providing *Rhizobium* strains SM3 to SM5, and Dr. J. Burton, Nitragin Co., Milwaukee, for *Rhizobium* strains 61A76 and 61A24. Supported by grants from the Rockefeller Foundation, National Research Council of Canada, Quebec Ministry of Education, and the Canada Department of Agriculture.

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Automatic Classification of Electroencephalograms: Kullback-Leibler Nearest Neighbor Rules

Abstract. *A prototypic problem in screening of electroencephalograms in the automatic classification of stationary electroencephalogram time series is treated here by the Kullback-Leibler nearest neighbor rule approach. In that problem, the category or state of an individual is classified by comparison of his or her electroencephalogram with those taken from other individuals in the alternative categories. The Kullback-Leibler nearest neighbor classification rules yield a statistically reliable estimate of the smallest possible probability of electroencephalogram misclassification with a relatively small number of labeled sample electroencephalograms. The automatic classification of anesthesia levels L1 and L3, respectively the anesthesia levels insufficient and sufficient for deep surgery, is treated by machine computation on the electroencephalogram alone.*

We have applied the recently developed Kullback-Leibler nearest neighbor (KL-NN) rule approach (*1*) to the problem of automatic classification of stationary electroencephalogram (EEG) time series. In that problem, the category or state of an individual is classified by comparison of his or her EEG with EEG's taken from other individuals. The computation of a Kullback-Leibler (KL) number metric or measure of the difference between two different stationary EEG time series is a key point of our approach. We report here on the automatic classification of anesthesia levels L1 and L3, respectively the anesthesia levels insufficient and sufficient for deep surgery, by machine computations on the EEG alone. Extension of the KL-NN rule approach to distinguish between more than two categories or anesthesia levels does not involve any new concepts.

The anesthesia level EEG data discussed here originated in an experiment at Vancouver General Hospital. In that experiment, 280 epochs of visually screened EEG's that were relatively free of artifact and reflected stationary halothane–nitrous oxide anesthesia level were collected from 20 individuals in surgery. The anesthesia levels, determined by non-EEG criteria, were classified by a single anesthesiologist to eliminate the problem of interrater variability for EEG's. Details of the surgical anesthesia procedures and a review of the status of automatic classification of anesthesia levels by EEG data appear elsewhere (*2*). The data consisted of 64-second recordings of four-channel EEG epoch data (F4-C4, F3-C3, C4-02, and C3-01 in the 10-20 EEG system) analog-frequency modulation recorded through a 0.54- to 30-Hz band-pass filter and then digitally transcribed at 128 samples per second. An examination of the available data suggested that we confine our attention to a two-category classification problem, to classify anesthesia levels L1 and L3, which are, respectively, insufficient and just sufficient for deep surgery. The data selected for analysis were 73 EEG epochs, all the 35 L1 EEG epochs available and 38 L3 EEG epochs (in sets of two to three per individual) from a total of 18 different individuals. The analysis was performed on the first 20-second intervals of each EEG data epoch at a reduced data rate of 128/3 samples per second on $d = 4$ and $d = 2$ EEG data channel data (C4-02 and C3-01).

The implicit conjecture in the EEG population screening problem is that there is sufficient information in the EEG alone to achieve clinically acceptable levels of discrimination between EEG state categories. The credibility of this conjecture is strained by evidence of the broad intersubject EEG variability. The data in Fig. 1, two-channel 20-second anesthesia level L1 and L3 EEG epochs from five subjects, illustrate this broad intersubject EEG variability. The L1 data appear to be relatively homogeneous "fast" EEG's whereas the L3 data include fast, slow regular, and irregular EEG's. The bottom two L3 EEG's (labeled F145, L3, S71 and F170, L3, S73) appear more similar to L1 EEG's than to other L3 tracings. No obvious visual properties distinguish the L1 from L3 EEG's.

A useful statement of the conjecture in the EEG population screening problem is as follows: given labeled EEG samples from two categorical populations, estimate the theoretically best achievable statistical classification performance. The use of a KL-NN classification, in which one subject's EEG is deleted at a time in classification of the labeled EEG sample data base, yields that desired es-

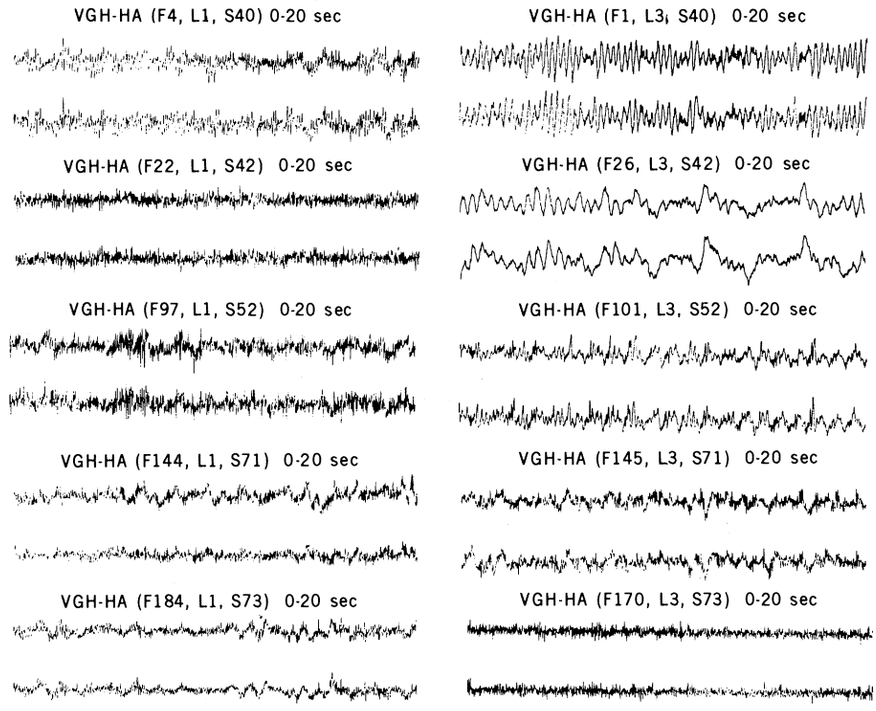

Fig. 1. Twenty-second bipolar (C3-01 or C4-02) epochs of anesthesia levels L1 and L3 from five different individuals.

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