4. L. W. Roberts, G. Rapmund, F. Cardigan, *J. Med. Entomol.* **14**, 89 (1977).
5. J. R. Edwardson, *Bot. Rev.* **36**, 341 (1970); K. D. Laser and N. R. Lersten, *ibid.* **38**, 425 (1972).
6. J. H. Werren, S. W. Skinner, E. L. Charnov, *Nature (London)* **293**, 467 (1981).
7. S. W. Skinner, *Genetics* **109**, 745 (1985); thesis, University of Utah, Salt Lake City (1983); *Science* **215**, 1133 (1982).
8. A. M. Huger, S. W. Skinner, J. H. Werren, *J. Invertebr. Pathol.* **46**, 272 (1985).
9. J. H. Werren, *Science* **208**, 1157 (1980); *Evolution* **37**, 116 (1983).
10. The *psr* trait was first referred to as "daughterless" (Dl) (*6*). However, this identification caused confusion and is now changed to *psr* to reflect its paternal inheritance pattern.
11. The following tests were used to characterize isolates (results for the slow-growth bacterium, which causes the *sk* trait, are indicated in parentheses): (i) sugar fermentative tests: glucose (+), fructose (−), xylose (−), lactose (−), maltose (−), mannose (−), 10 percent lactose (−); (ii) miscellaneous tests: NO_2/N_2 (−); motility (−/+), catalase (+), oxidase (−), Gram stain (negative pleiomorphic rod); (iii) media growth tests (1,5 days, 25°C): TSB (−,+), BHI (−,+), thioglycolate (−,−), TSA (−,+). In addition, the *Proteus* isolates were identified by the Enteric Tec test kit.
12. J. H. Werren, *Neth. J. Zool.* **34**, 151 (1984).
13. The bacterium shows an antibiotic sensitivity characteristic of Gram-negative organisms (polymyxin-, penicillin-, methicillin-, and erythromycin-resistant; gentamicin-, chloramphenicol-, tetracycline-, and sulfathiazole-sensitive). A fatty acid profile determined by M. Sasser (University of Delaware) did not match any in his current library (104 species) but was typologically most similar to that of Enterobacteraceae. R. Gherna (American Type Culture Collection) is further characterizing this species.
14. R. Koch, *Über Bacteriologisches Forschung Verhandlungen 10* (International Medical Congress, 1890) (Hirschwald, Berlin, 1891), vol. 1.
15. J. H. Werren and J. van den Assem, in preparation.
16. J.H.W. thanks S. R. Simms and D. Ohasi for assistance in microbiological identification, D. Roberts and I. Schneider for use of facilities, and H. Schneider and I. Schneider for advice and guidance. S.W.S. thanks E. Patou for use of facilities, J. Phillips for discussions, and J. James for assistance. S.W.S. was supported by NSF grant DE-1381-19206 and NIH grant 5 T32 6MO7131. Helpful comments were provided by R. Gherna, K. Thorpe, B. Martin, J. Phillips, M. Raupp, and C. Reichelderfer.

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Three-Year Incidence of AIDS in Five Cohorts of HTLV-III–Infected Risk Group Members

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The incidence of the acquired immune deficiency syndrome (AIDS) among persons infected with human T-lymphotropic virus type III (HTLV-III) was evaluated prospectively among 725 persons who were at high risk of AIDS and had enrolled before October 1982 in cohort studies of homosexual men, parenteral drug users, and hemophiliacs. A total of 276 (38.1 percent) of the subjects were either HTLV-III seropositive at enrollment or developed HTLV-III antibodies subsequently. AIDS had developed in 28 (10.1 percent) of the seropositive subjects before August 1985. By actuarial survival calculations, the 3-year incidence of AIDS among all HTLV-III seropositive subjects was 34.2 percent in the cohort of homosexual men in Manhattan, New York, and 14.9 percent (range 8.0 to 17.2 percent) in the four other cohorts. Out of 117 subjects followed for a mean of 31 months after documented seroconversion, five (all hemophiliacs) developed AIDS 28 to 62 months after the estimated date of seroconversion, supporting the hypothesis that there is a long latency between acquisition of viral infection and the development of clinical AIDS. This long latency could account for the significantly higher AIDS incidence in the New York cohort compared with other cohorts if the virus entered the New York homosexual population before it entered the populations from which the other cohorts were drawn. However, risk of AIDS development in different populations may also depend on the presence of as yet unidentified cofactors.

HUMAN T-LYMPHOTROPIC VIRUS type III (HTLV-III) is the primary etiologic agent of the acquired immune deficiency syndrome (AIDS) (*1*). Surveys of the number of people with antibodies to the virus have suggested that hundreds of thousands of persons in the United States have been infected with HTLV-III, and as of January 1986 more than 16,400 patients with signs and symptoms meeting the surveillance-definition of AIDS have been reported. Because the interval between HTLV-III infection and the development of AIDS may be lengthy, the long-term prognosis following HTLV-III infection is unknown. In the current analysis, we have evaluated the incidence of AIDS following HTLV-III infection in five cohorts—homosexual men in Manhattan, New York; homosexual men in Washington, DC; homosexual men in Copenhagen and Aarhus, Denmark; hemophilia A patients in Hershey, Pennsylvania; and parenteral drug users in Queens, New York.

All five cohorts have been reported in detail elsewhere (*2–8*). Briefly, the homosexual men in Manhattan and Washington, DC, were invited to enroll as consecutive male patients entering the offices of three primary care physicians during May and June 1982, with follow-up approximately on an annual basis through June 1985 (*2, 3*). Follow-up was 90 percent complete at 2 years and 76 percent complete at 3 years. With 6 months of additional tracing, follow-up was 92 percent complete and revealed that one more member of the Manhattan cohort had developed AIDS. Because the subjects followed for the additional 6 months were not actually seen for the study, and because their inclusion in the final results made a negligible difference (<1 standard error), the additional follow-up data are not included in our analysis.

The homosexual men in Denmark were invited to enroll in the study by means of the mailing list of a national (Danish) organization and open clinic sessions in Copenhagen and Aarhus during December 1981 with follow-up sessions in April 1982, February 1983, and September 1984 (*4, 5*). Follow-up was 82 percent complete at the last date. The hemophilia A patients were all those receiving comprehensive care as of September 1982 at the Milton S. Hershey Medical Center in Hershey, Pennsylvania, with follow-up at the semiannual or annual clinic visits (*6, 7*). Their follow-up is 100 percent complete and virtually current. The parenteral drug users were enrolled in late 1981 as part of a case-control study in Queens comparing immunologic parameters in patients hospitalized with soft-tissue infections with those in outpatient methadone detoxifica-

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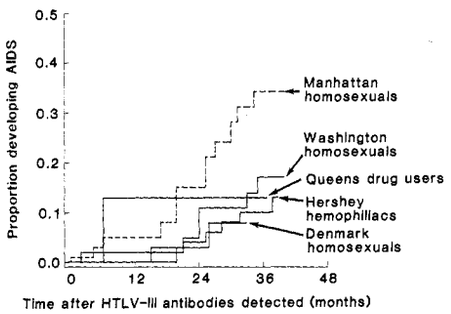

Fig. 1. Actuarial incidence of AIDS among all 276 HTLV-III seropositive study subjects in five different cohorts. Data are computed by the Kaplan-Meier survival technique, with the first seropositive specimen (or September 1982 for hemophiliacs with positive historic specimens, see text) being used as time 0. The cohort of Manhattan homosexual men has a significantly higher incidence of AIDS ($\chi^2 = 10.48$, $P = 0.001$) than the other cohorts combined. Cumulative AIDS incidence in the Queens drug users was 25 percent with an additional case reported at follow-up (see *Note added in proof*).

tion (*8*). Follow-up was obtained through tracing efforts in early 1985, at which time it was 29 percent complete. Informed consent was obtained from all study subjects.

Serum samples that had been stored at −20° to −70°C were tested for HTLV-III antibodies by an enzyme-linked immunosorbent assay (ELISA) with the use of disrupted whole virus (*9*). The current analysis was limited to subjects who were HTLV-III seropositive, with seropositivity being defined as an ELISA ratio ≥ 5.00 or a Western blot demonstrating characteristic bands of HTLV-III proteins (*1, 9*).

All subjects were apparently free of AIDS at enrollment. The subsequent development of AIDS was defined as culture- or biopsy-proved *Pneumocystis carinii* pneumonia, other life-threatening opportunistic infection, Kaposi's sarcoma, or malignant lymphoma. The dates of incident seroconversion were estimated as the midpoint in time between the dates on which the last seronegative and the first seropositive blood samples were obtained. The survival of AIDS-free subjects was calculated by the Kaplan-Meier method with cumulative incidence and standard errors (*10*), and was measured from the estimated date of seroconversion to the date of last evaluation, date of AIDS diagnosis, or date of non-AIDS death. Non-AIDS deaths were regarded as censored observations. Because many seropositive subjects had HTLV-III antibodies at enrollment, a separate Kaplan-Meier analysis of AIDS-free survival was performed to include these prevalent cases with the incident seroconverters. For this separate analysis we used the same parameters except that the starting point was the date of the first seropositive serum sample or, for sera that were collected prior to the initiation of the hemophilia study, the starting point was September 1982 (Fig. 1). The Gehan-Breslow test was used to evaluate differences in the AIDS incidence between the Manhattan and other cohorts (*10*).

Antibodies to HTLV-III were demonstrated in 276 (38.1 percent) of the 725 subjects in the five cohorts. AIDS subsequently developed in 28 (10.1 percent) of the seropositive subjects. Among these cases were nine with Kaposi's sarcoma, two with high-grade malignant lymphoma, 17 with *Pneumocystis* pneumonia, and 12 with other life-threatening opportunistic infections (Table 1). Seven of the nine cases of Kaposi's sarcoma were in the Manhattan cohort, while the other AIDS conditions were distributed across the cohorts more evenly (Table 1). Nineteen (68 percent) of the 28 subjects who developed AIDS are known to have died, which is consistent with national trends. Three additional AIDS cases were not included in these statistical analyses, two (Nos. 2623 and 8005) because they occurred in December 1985, after the censoring date, and one (No. 5001) because he had common variable hypogammaglobulinemia and was negative for antibodies to HTLV-III as well as to common infectious agents (*11*). Pneumonia, not attributable to AIDS, accounted for two additional deaths in the hemophiliacs. One additional drug user died of an unspecified type of pneumonia, and two additional Danish homosexual men died of causes unrelated to AIDS.

Kaplan-Meier survival analysis of all 276 HTLV-III seropositive subjects showed that the 3-year incidence of AIDS was 34.2 percent (±8.0 percent standard error) in the Manhattan cohort, 17.2 percent (±6.4 percent) in the Washington homosexual cohort, 8.0 percent (±5.5 percent) in the Denmark homosexual cohort, 12.5 percent (±11.7 percent) in the Queens drug-user cohort, and 12.8 percent (±4.8 percent) in the Hershey hemophilia cohort (Fig. 1). The incidence of AIDS was significantly higher in the Manhattan homosexual cohort (34.2 percent) than in the other cohorts combined (14.9 percent; $\chi^2 = 10.48$, $P = 0.001$). If Kaposi's sarcoma is excluded, the AIDS incidence still was higher in the Manhattan cohort (25.0 percent opposed to 14.2 percent; $\chi^2 = 4.73$, $P = 0.03$).

A minimum 3-year incidence of AIDS was defined as the number of known AIDS cases divided by the number of all subjects known to be HTLV-III seropositive as of September 1982. ("Minimum" is used here in the sense that those lost to follow-up are assumed to be alive and AIDS-free but could be uncounted AIDS cases.) The minimum 3-year incidence of AIDS was 29.5

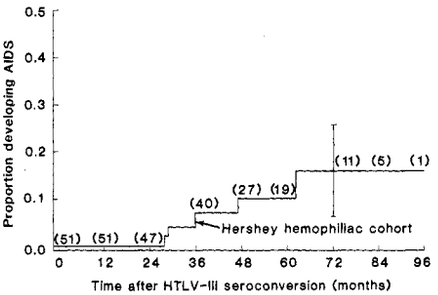

Fig. 2. Actuarial incidence of AIDS among 51 hemophiliacs developing antibodies to HTLV-III. Data are computed by the Kaplan-Meier survival technique, with each individual patient's seroconversion date (the midpoint in time between the last seronegative and first seropositive specimens) being used as time 0 [for actual calendar dates, see (*7*)]. Numbers in parentheses indicate the number of persons still being followed at each 12-month interval after seroconversion. Vertical bars at 72 months indicate ±1 standard error.

percent (13/44) in Manhattan homosexuals, 14.3 percent (6/42) in Washington homosexuals, 7.7 percent (2/26) in Denmark homosexuals, 4.2 percent (1/24) in Queens drug users, and 12.5 percent (5/40) in Hershey hemophiliacs. (These 176 subjects include those with documented HTLV-III antibodies before September 1982. The remaining 100 subjects, including the sixth hemophiliac with AIDS, developed HTLV-III antibodies later than September 1982.)

Incident HTLV-III antibody seroconversion, not restricted to a particular calendar date, was demonstrated in 117 subjects. In 51 hemophiliacs, with a mean and median follow-up of 53 months, five cases of AIDS occurred 28 to 62 months after estimated seroconversion for a cumulative AIDS incidence of 14.9 percent (±6.8 percent) (Fig. 2). Only 11 subjects have been followed for six or more years, which results in a large standard error beyond 72 months (Fig. 2). Incident HTLV-III antibody seroconversion was demonstrated in 66 subjects in the four other cohorts (10 Manhattan homosexuals, 29 Washington homosexuals, 23 Denmark homosexuals, and 4 Queens drug users), with a mean follow-up of only 17 months and a maximum follow-up of only 32 months. No cases of AIDS have occurred among the seroconverting subjects in these other cohorts.

In our initial reports of HTLV-III risk factors among homosexual men in Manhattan and Denmark, 9 to 14 percent of the prevalent seropositive subjects developed AIDS over a 2-year period (*3, 5*). In the present study, actuarial calculations showed that the 3-year incidence of AIDS in our Manhattan cohort was 34.2 percent, and it averaged 14.9 percent in our other cohorts, with a range of 8.0 to 17.2 percent. Why

homosexual men in Manhattan should have such a high rate of AIDS is unclear, and it is only partially explained by the high concentration of Kaposi's sarcoma in this cohort (seven of nine cases). It is possible that many persons were ill when they enrolled in this cohort, although the enrollment procedures carefully avoided selection bias and were identical to those in the Washington cohort (*2*). In addition, most of the AIDS diagnoses in the Manhattan cohort occurred long after the subjects enrolled in the study (Fig. 1). A possible explanation for the high incidence of AIDS in the Manhattan cohort is that they had been seropositive for a longer period of time than had the other cohorts at the time of enrollment. The first cases of AIDS probably developed in homosexual men in New York City, San Francisco, and Los Angeles, and the epidemic of AIDS in homosexual men throughout the United States appears to be 1 year ahead of the epidemic in parenteral drug users and more than 2 years ahead of the epidemic in hemophiliacs (*12*). It also is possible that unknown cofactors in the Manhattan cohort have contributed to their high risk of AIDS, and of Kaposi's sarcoma in particular. Detailed analyses of serologic, demographic, and life-style variables have not yet shown any clear-cut, dominant cofactors (*13*).

The data from our hemophilia cohort and from other studies are starting to reveal the natural history of HTLV-III infection. Hemophiliacs who used commercial factor VIII concentrate underwent mass seroconversion during 1981 and 1982 (*7*), and the epidemic of AIDS in hemophiliacs followed 2 to 3 years later (*14*). The data on our subjects with documented seroconversion, particularly those in the Hershey hemophilia cohort, suggest that AIDS in adults usually develops more than 2 years after HTLV-III infection and that new cases may continue to appear more than 5 years after seroconversion. Cases of transfusion-associated AIDS have developed a median of 27.5 months after the suspected date of HTLV-III infection, although mathematical modeling of these surveillance data suggests that the mean interval between transfusion and AIDS may be closer to 5 years (*15*).

Two other studies have quantified the risk of AIDS among HTLV-III seropositive homosexual men. In one, 4 (16 percent) of 25 homosexual men developed AIDS 1 to 35 months (median 15.5 months) after donating blood that was later implicated in transmitting HTLV-III (*16*). In the other, 2 (6.4 percent) of 31 homosexual men in San Francisco had developed AIDS during a median follow-up period of 61 months (*17*). Neither of these studies used actuarial methods because of uncertainty about the time of seroconversion or a sizable loss to follow-up. The latter problem was particularly evident in the San Francisco study (*17*), in which 183 men (23.3 percent of the cohort) were lost to follow-up. Of our AIDS cases in Manhattan and Washington, 5 (26.3 percent) of 19 occurred outside the original metropolitan area, suggesting that a significant proportion of the 183 men not located in the San Francisco cohort might be uncounted AIDS cases. We have minimized these problems by using careful tracing methods and by explicitly calculating minimum-risk figures that assume all persons lost to follow-up are alive and well. Finally, we have calculated cumulative AIDS incidence by pooling recent (that is, incident) seroconverters, who have a relatively low risk of becoming ill during the first 2 years after seroconversion, with initially seropositve (that is, prevalent) persons, who are farther along in the disease process.

Table 1. Development of AIDS conditions in HTLV-III seropositive members of five cohorts. Abbreviations: KS, Kaposi's sarcoma; Lymphoma, high-grade non-Hodgkin's lymphoma; PCP, *Pneumocystis carinii* pneumonia; other OI, other opportunistic infection indicative of cell-mediated immunodeficiency.

Subject No.	AIDS diagnosis			
	KS	Lymphoma	PCP	Other OI
*Homosexual men in Manhattan, NY (n = 44)**				
2083			x	
2233		x		
2343			x	
2443	x			
2473	x			
2503	x			
2563			x	
2573	x		x	
2623†			x	
2693			x	
2743			x	x
2783	x		x	x
2823	x			
2853	x			x
*Homosexual men in Washington, DC (n = 42)**				
0071		x		x
0231				x
0311			x	
0911				x
0981			x	x
1112	x		x	x
*Homosexual men in Denmark (n = 26)**				
365			x	x
510	x			
*Hemophiliacs in Hershey, PA (n = 40)**				
1007			x	
1046			x	
1066				x
1071‡			x	
1081			x	
5001§				x
5004			x	x
8005†			x	
*Drug users in Queens, NY (n = 24)**				
7006			x	x
6006¶				x

*Number of subjects with HTLV-III antibodies as of September 1982. †These two subjects developed AIDS after the study period (December 1985) and are not included in statistical analyses (see text). ‡This hemophiliac developed HTLV-III antibodies after September 1982. He is included in the Kaplan-Meier survival analyses (Figs. 1 and 2) but not in the minimum incidence calculations [see text and (*11*)]. §This hemophiliac was HTLV-III antibody negative because of hypogammaglobulinemia and is not included in statistical analyses [see text and (*11*)]. ¶This drug user is not included in calculations or in Figs. 1 and 2. See *Note added in proof.*

It would not be surprising if the rate of AIDS continued to increase with duration of seropositivity. There are strong indications that the total number of $OKT4^+$ (helper) lymphocytes progressively declines in HTLV-III seropositive persons (*7, 18*) and that the initial $OKT4^+$ lymphocyte count is strongly associated with the subsequent development of AIDS (*13*). It is possible, however, that an individual's risk of AIDS could be increased or decreased by factors such as size of the HTLV-III inoculum, route of HTLV-III infection, concomitant administration of HTLV-III antibodies or inactivated HTLV-III (for example, by way of factor VIII concentrate), infection with several different strains of HTLV-III, infection with other agents, exposure to numerous allogeneic antigens (for example, semen, blood), use of immunotoxic or mutagenic drugs, or host determinants of immune response. In addition, the spectrum of HTLV-III–related illnesses is expanding to include neurological diseases and perhaps malignancies other than Kaposi's sarcoma and non-Hodgkin's lymphomas, and each of these manifestations may have different risk factors. Although preliminary analyses have not yet revealed unequivocal risk factors (*13*), the contrasts between our cohorts, if not due to time-related factors, provide hope that cofactors susceptible to intervention will be found.

Note added in proof: Reexamination of autopsy specimens from a drug-using subject (No. 6006) who died at 22 months of follow-up of "an unspecified type of pneumonia" (not included as AIDS in this report) revealed disseminated cryptococcosis (an AIDS diagnosis; A. Macher, Armed Forces Institute of Pathology). The 3-year AIDS incidence in the Queens drug users is thus 25.0 percent ± 15.3 percent, which approaches the high AIDS incidence in the Manhattan homosexual men.

REFERENCES AND NOTES

1. M. Popovic *et al.*, *Science* **224**, 497 (1984); R. C. Gallo *et al.*, *ibid.*, p. 500; J. Schüpbach *et al.*, *ibid.*, p. 503; M. G. Sarngadharan *et al.*, *ibid.*, p. 506.
2. J. J. Goedert *et al.*, *Am. J. Epidemiol.* **121**, 629 (1985); J. J. Goedert *et al.*, *ibid.*, p. 637.
3. J. J. Goedert *et al.*, *Lancet* 1985-1, 711 (1985).
4. R. J. Biggar *et al.*, *J. Am. Med. Assoc.* **251**, 1441 (1984).
5. M. Melbye *et al.*, *Brit. Med. J.* **289**, 573 (1984).

6. J. J. Goedert *et al.*, *Blood* 65, 492 (1985).
7. M. E. Eyster *et al.*, *J. Am. Med. Assoc.* 253, 2219 (1985).
8. G. R. Orangio *et al.*, *Ann Surg.* 199, 97 (1984); G. R. Orangio *et al.*, *Am. J. Surg.* 146, 738 (1983); M. Robert-Guroff *et al.*, in preparation.
9. S. H. Weiss *et al.*, *J. Am. Med. Assoc.* 253, 221 (1985).
10. D. G. Thomas, N. Breslow, J. J. Gart, *Computers Biomed. Res.* 10, 373 (1977). We used the Gehan-Breslow test statistic for adjusted trends.
11. Patients with common variable hypogammaglobulinemia are unable to generate antibodies; for details of this case see K. L. Koch *et al.*, *Ann. Intern. Med.* 99, 337 (1983).
12. J. J. Goedert and W. A. Blattner, in *AIDS: Etiology, Diagnosis, Treatment and Prevention*, V. T. DeVita, S. Hellman, S. A. Rosenberg, Eds. (Lippincott, Philadelphia, 1985), p. 1.
13. J. J. Goedert *et al.*, in preparation.
14. Centers for Disease Control, *Morbid. Mortal. Weekly Rep.* 34, 241 (1985).
15. J. W. Curran *et al.*, *N. Engl. J. Med.* 310, 69 (1984); K. J. Lui *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
16. H. W. Jaffe *et al.*, *J. Am. Med. Assoc.* 254, 770 (1985).
17. H. W. Jaffe *et al.*, *Ann. Intern. Med.* 103, 210 (1985).
18. M. Melbye *et al.*, *ibid.*, in press.
19. We thank M. Gail for helpful suggestions; M. G. Sarngadharan, M. Robert-Guroff, C. W. Saxinger, A. Bodner, and S. Alexander for the serological data; M. Hirsh, H. Lindsley, M. Fischl, V. Faber, I. Lorentzen, K. Schmidt, I. B. Margolis, and F. Shamash for clinical follow-up data; and M. Jacobus for manuscript preparation.

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Parvalbumin in Most γ-Aminobutyric Acid–Containing Neurons of the Rat Cerebral Cortex

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γ-Aminobutyric acid (GABA) is one of the major inhibitory neurotransmitters in the central nervous system. In the cerebral cortex, GABA-containing cells represent a subpopulation of interneurons. With semithin frozen sections, it is possible to demonstrate that most GABA neurons in the rat somatosensory cortex contain the calcium-binding protein parvalbumin and that parvalbumin is found virtually only in GABA neurons. Parvalbumin seems to influence the electrical properties and enzymatic machinery to modulate neuronal excitability and activity. The specific role of parvalbumin in GABA-containing cortical cells may be related to controlling the effectiveness of their inhibitory action.

THE CALCIUM-BINDING PROTEIN parvalbumin (PV) is a marker for a subpopulation of neurons (*1*). Calcium-binding proteins are low molecular weight, acidic proteins that buffer Ca^{2+}-ions or trigger the activity of various enzymes upon binding Ca^{2+} (*2*). Their presence may privilege a given neuron for certain Ca^{2+}-dependent processes. Since the cortical mantle displayed a large number of PV-immunoreactive local-circuit neurons, I sought to determine the relationship between PV- and γ-aminobutyric (GABA) interneurons in the rat somatosensory cortex.

Adult male albino rats were perfused transcardially with fixative, and small brain fragments were frozen in liquid nitrogen after imbibition with sucrose. Consecutive semithin (0.5- to 1-μm) sections were cut at −60°C with an ultracryomicrotome and mounted on glass slides (*3*). Adjacent sections were alternately incubated with PV-antiserum and GABA-antiserum. The primary antibody bound to the antigen was detected with the unlabeled antibody method and the peroxidase visualized histochemically (*4*). The methods currently available (legend to Fig. 1) made it possible to eliminate the danger of cross-reactions of the antisera used in immunohistochemistry.

In the 0.5- to 1-μm-thick cryo-sections, the GABA and PV immunolabelings were closely matched (Fig. 2, A and B). Two different interneuronal populations were revealed: the major population (approximately 70 percent) contained both GABA and parvalbumin (GABA-plus-PV neurons), whereas a minor population (approximately 30 percent) had only GABA immunoreactivity (GABA-only neurons). With few exceptions, parvalbumin distributed itself only in GABA cells (Fig. 2). In the sections incubated with the GABA-antiserum (Fig. 2A), the cell body and the nuclei were homogeneously immunostained. The neuropil was abundant with immunoreactive ter-

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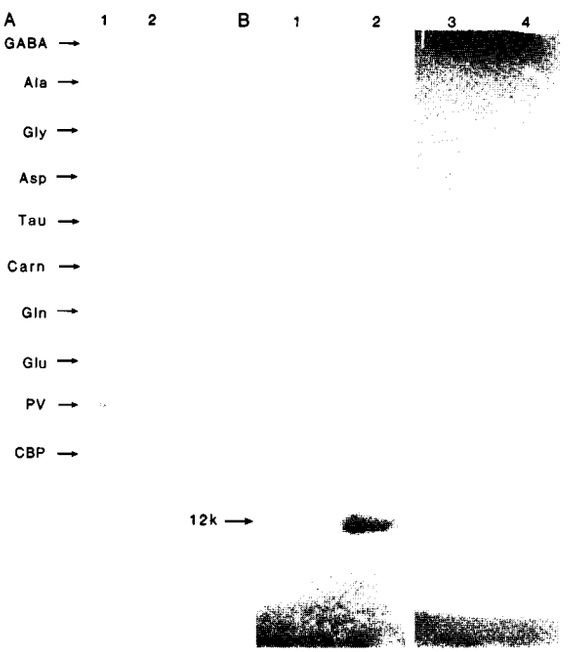

Fig. 1. Specificity controls for the immunoreaction. (A) The specificities of the GABA and PV antisera were tested by spotting possibly crossreacting substances on glutaraldehyde-activated nitrocellulose paper (*5*). A 2-μl portion of a 200-m*M* solution of following amino acids was applied: GABA; Ala, β-alanine; Gly, glycine; Asp, aspartate; Tau, taurine; Carn, carnosine; Gln, glutamine; Glu, glutamate (all from FLUKA, Switzerland). The calcium-binding proteins PV and calbindin (CBP) were applied in concentrations of between 0.01 and 1 mg/ml. In this system, which mimics immunohistochemistry, the PV antiserum reacted only with PV (lane 1), whereas the GABA-antiserum recognized only GABA (lane 2) at concentrations which may be assumed to occur in the tissue. The GABA antiserum, however, reacted with β-alanine at high concentrations of this substance spotted on nitrocellulose. The antisera were also adsorbed with the homologous and heterologous antigen as well as with bovine calmodulin (FLUKA) and β-alanine bound to polyacrylamide beads with glutaraldehyde (*5*).The immunostaining with the PV antiserum was inhibited only by the PV conjugate, while the GABA-immunostaining was inhibited only by GABA. (B) In an alternative specificity test (*15*), the cerebellum of a rat killed with an overdose of anesthetics was homogenized and subjected to sodium dodecyl sulfate–gel electrophoresis, subsequently transferred to a sheet of nitrocellulose paper, treated with glutaraldehyde, and incubated with the PV or the GABA antiserum. Cerebellar extract (10 μg) (lanes 1 and 3) and 10 μg of purified rat muscle parvalbumin (lanes 2 and 4) were run in parallel, blotted to the nitrocellulose paper, incubated with the PV (lanes 1 and 2) or the GABA (lanes 3 and 4) antisera, and reacted with an indirect peroxidase method. The PV antiserum used in this study recognized a protein which displayed the same electrophoretic mobility as purified muscle PV (12K). The GABA antiserum did not cross-react with any cerebellar proteins. Lanes 1 and 2 were left for 15 minutes and lanes 3 and 4 for 1 hour in the peroxidase substrate solution.