7. L. G. Davis, M. D. Dibner, J. F. Battey, in *Basic Methods in Molecular Biology* (Elsevier, New York, 1986), pp. 143–146; M. Noda *et al.*, *Nature* **320**, 188 (1986).
8. In these experiments, mRNAs were loaded onto a sucrose density gradient solution (22 to 5%, w/v) containing 70% formamide (IBI, spectral grade; deionized before use with mixed bed resin), and centrifuged at 260,000*g* for 26 hours at 10°C.
9. G. Grenningloh *et al.*, *Nature* **328**, 215 (1987).
10. T. L. Schwarz *et al.*, *ibid.* **331**, 137 (1988); B. Bossy, M. Ballivet, P. Spieve, *EMBO J.* 7, 611 (1988); B. L. Tempel *et al.*, *Nature* **332**, 837 (1988).
11. S. Konishi, K. Saito, M. Otsuka, *Jpn. J. Pharmacol. Suppl.* **25**, 72P (1975); R. H. Evans, *Br. J. Pharmacol.* **62**, 171 (1978); T. Takahashi, *Proc. R. Soc. London Ser. B* **221**, 103 (1984); C. E. Jahr and K. Yoshioka, *J. Physiol. (London)* **370**, 515 (1986).
12. For the experiments shown in Figs. 2 and 3 and Table 1, we used adult spinal cord fraction 15 concentrated four times relative to that in Fig. 1B so as to facilitate comparison with fraction 6.
13. K. Kusano, R. Miledi, J. Stinnakre, *J. Physiol. (London)* **328**, 143 (1982).
14. C. B. Gundersen, R. Miledi, I. Parker, *Proc. R. Soc. London Ser. B* **221**, 235 (1984); *J. Physiol. (London)* **377**, 40P (1986).
15. A possible explanation for these results is that picrotoxin blocks the Cl^- channels of both types of GlyRs, as well as those of GABA receptors. Blocking effects of picrotoxin on glycine-induced responses were demonstrated in cat cuneate nucleus [R. G. Hill *et al.*, *Br. J. Pharmacol.* **56**, 9 (1976)]. However, our results contrast with those observed in cultured mouse spinal neurons in which picrotoxin selectively suppressed GABA responses without altering the sensitivity to glycine [R. L. Macdonald and J. L. Barker, *Neurology* **28**, 325 (1978)].
16. J. L. Barker, R. L. Macdonald, T. G. Smith, *J. Gen. Physiol.* **70**, 1a (1977); J. L. Barker, in *Neurotransmitter Actions in the Vertebrate Nervous System*, M. A. Rogawski and J. L. Barker, Eds. (Plenum, New York, 1985), pp. 71–100.
17. I. Parker, K. Sumikawa, R. Miledi, *Proc. R. Soc. London Ser. B* **233**, 201 (1988).
18. S. Heinemann *et al.*, in *Molecular Neurobiology*, S. Heinemann and J. Patrick, Eds. (Plenum, New York, 1987), pp. 45–96; M. G. Rosenfeld, S. G. Amara, R. M. Evans, *Science* **225**, 1315 (1984); S.-H. Chen *et al.*, *ibid.* **238**, 363 (1987).
19. We thank M. Nieto-Sampedro, M. Panicker, I. Parker, K. Sumikawa, and E. Wagner for helpful comments on the manuscript. Supported by grant R01-NS23284 from the U.S. Public Health Service. H.A. was supported in part by the Mitsubishi Kasei Institute of Life Sciences, Tokyo.

29 June 1988; accepted 25 August 1988

Acetaldehyde Production and Transfer by the Perfused Human Placental Cotyledon

PETER I. KARL, BARBARA H. J. GORDON, CHARLES S. LIEBER, STANLEY E. FISHER*

Fetal injury associated with maternal ethanol ingestion is a major cause of congenital anomalies and mental retardation. Studies with animals suggest that acetaldehyde, the primary hepatic oxidative metabolite of ethanol, may contribute to fetal damage. It is not known, however, whether acetaldehyde reaches the human fetus, either by placental production or transfer. Studies utilizing the perfused human placental cotyledon show that the human placenta oxidizes ethanol to acetaldehyde, releasing it into the fetal perfusate. Moreover, when acetaldehyde is present in the maternal perfusate, it is transferred to the fetal side, reaching approximately 50 percent of the maternal level. These findings suggest that the human placenta may play a pivotal role in the pathophysiology of ethanol-associated fetal injury.

ETHANOL INGESTION DURING PREGNANCY may result in fetal injury, including congenital anomalies, intrauterine growth retardation, and mental deficiency (*1*). Ethanol-associated fetal damage is a major cause of mental retardation in the Western world (*1*). However, the pathophysiology of ethanol-associated fetal injury is not well understood. To a large degree, the etiology may involve direct toxicity, with ethanol adversely affecting both fetal development and placental function (*2*). However, acetaldehyde (AcH), the major oxidative product of ethanol, has also been implicated in many of the adverse effects of ethanol consumption (*3*). In pregnant animals treated with ethanol, AcH is found in the fetus (*4*), and administration of AcH to pregnant animals can reproduce the fetal damage seen with ethanol exposure (*5*). Moreover, in humans, the degree of fetal injury may be related to the level of AcH in the maternal circulation (*6*), and there is indirect evidence for maternal-to-fetal transfer of AcH (*7*).

In the experiments described here, we attempted to answer two questions: (i) At clinically relevant ethanol levels, does the human placenta oxidize ethanol so that AcH may appear in the fetal circulation? (ii) At AcH levels attainable in maternal blood, does the human placenta transfer AcH to the fetal circulation? Inasmuch as the human fetal liver has a low capacity for both ethanol and AcH elimination (*8*), the placental delivery of AcH could be a major contributor to the pathogenesis of ethanol-associated fetal injury.

To examine the potential for human placental oxidation of ethanol, we perfused the maternal circulation of a single cotyledon in

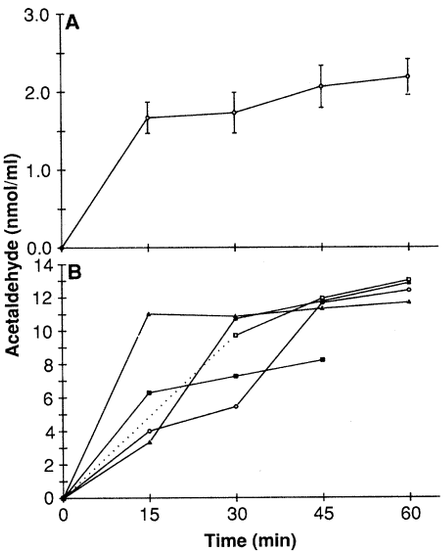

Fig. 1. Acetaldehyde production (**A**) and transfer (**B**) by the perfused cotyledon. Addition of 65 m*M* ethanol to the maternal influx after the equilibration and blank perfusion periods (*14*) resulted in the appearance of AcH on the fetal side (A) ($n = 7$) (mean ± SE). AcH also appeared in the maternal venous efflux (see text). After the perfusion with ethanol alone and a 15-min washout (*14*), both 65 m*M* (300 mg/dl) ethanol and 25 μ*M* AcH were added to the maternal influx and 100 ml of fresh fetal perfusate (recirculated) was analyzed for AcH transfer (B) ($n = 5$) [each transfer pattern is shown; one of the curves did not have a 15-min time point (dotted line)]. The maternal perfusate was nonrecirculated, so that influx concentrations were constant. Perfusate samples (250 μl) were placed in chilled vials containing 250 μl of 1.2*N* perchloric acid and 80 m*M* thiourea, sealed, held on ice for less than 6 hours, and analyzed for AcH and ethanol content by head-space gas chromatography (*9*). Compared to raw perfusate or perfusate with barium hydroxide added, perfusate with perchloric acid–thiourea gave the least spontaneous AcH production. Standards for AcH were prepared from redistilled AcH dissolved in deoxygenated water; we estimated the spontaneous formation of AcH by using fetal-side blank perfusate (*14*), to which varying amounts of ethanol were added. For a given ethanol concentration in the fetal perfusate, a corresponding value for spontaneous AcH formation (determined by linear regression) was subtracted from the total AcH, yielding the net values shown. During maternal perfusion of ethanol alone (A), spontaneous formation accounted for 56.7 ± 3.2% of the total AcH in the fetal perfusate; during perfusion with ethanol and AcH, it accounted for only 11.7 ± 2.0% as a result of the higher AcH levels in the fetal perfusate.

P. I. Karl and S. E. Fisher, Departments of Pediatrics and Research, North Shore University Hospital, Manhasset, NY 11030, and Cornell University Medical College, New York, NY 10021.
B. H. J. Gordon, Nutrition and Food Science Program, Hunter College School of Health Sciences, City University of New York, New York, NY 10010.
C. S. Lieber, Alcohol Research and Treatment Center, Veterans Administration Medical Center, Bronx, NY 10468, and Mt. Sinai School of Medicine, City University of New York, New York, NY 10029.

*To whom correspondence should be addressed at Boas-Marks Biomedical Science Research Center, North Shore University Hospital, 350 Community Drive, Manhasset, NY 11030.

each of seven normal, term placentas for 60 min with 65 m*M* (300 mg/dl) ethanol (a level found in the blood of heavy drinkers). The appearance of AcH and ethanol in the maternal and fetal venous efflux was monitored by head-space gas chromatography (*9*). By 15 min, the concentration of AcH in the recirculated fetal perfusate was 1.67 ± 0.20 μ*M* (mean ± SE), and this concentration remained relatively constant for the next hour (Fig. 1A). Concurrently the ethanol concentration in the fetal perfusate rose steadily, reaching 39.86 ± 3.5 m*M* by 60 min (61.3% of the maternal level). The maternal-to-fetal diffusion of ethanol was slower than that of antipyrine, a nonmetabolized, nonvolatile, flow-dependent diffusion marker, which increased to more than 80% of the maternal concentration by 60 min. The metabolism of ethanol by the placenta (plus evaporation from the aerated fetal reservoir) could have contributed to the seemingly delayed transfer of ethanol. However, our finding is consistent with an in vivo delay in maternal-fetal blood ethanol equilibrium reported in sheep (*10*).

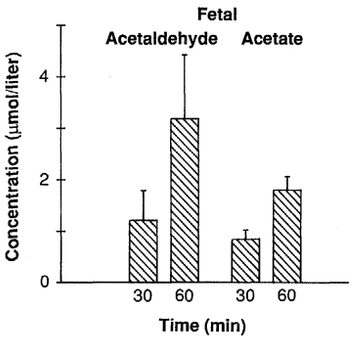

Fig. 2. Acetaldehyde and acetate production as measured with ^{14}C-labeled ethanol. In a cotyledon from each of three placentas, the maternal side was perfused with 25 m*M* (115 mg/dl) [^{14}C]ethanol (41.8 to 62.8 μCi/mmol). The ethanol had been redistilled after borohydride reduction, so that nonvolatile and semicarbazide-trapped contaminants were less than 0.02%. The maternal perfusate was recirculated (100 ml) to conserve substrate. Samples of maternal and fetal perfusate were treated with semicarbazide or potassium hydroxide (KOH) to measure AcH or acetate (the oxidative product of AcH), respectively (*15*). Samples of fetal perfusate obtained at 30 and 60 min were added to equal volumes of 30 m*M* semicarbazide (AcH recovery) or 0.67*N* KOH (acetate recovery) and mixed. After 15 min, 500-μl aliquots were placed on oxidizer sponges, dried overnight at room temperature, and washed three times with ethanol. The residual radioactivity, representing either AcH or acetate, was measured by scintillation counting after oxidation in a Packard 306 oxidizer. For the determination of spontaneous AcH or acetate formation, fetal perfusate was obtained from the blank preparation period (*14*); [^{14}C]ethanol was added to yield varying concentrations, and the blanks were gently agitated at 5-min intervals for 60 min. Portions were then processed as above. The blank value for a given total radioactivity in the fetal perfusate was determined from a linear regression curve. The blanks represent radioactivity due to impurities in the [^{14}C]ethanol and spontaneous formation of AcH or acetate in the perfusate.

Simultaneous with the appearance of AcH in the fetal perfusate, the AcH concentration in the maternal efflux was virtually the same as that in the fetal perfusate. Thus, the continuously nonrecirculated (open) maternal efflux led to a steady state between release of AcH into the fetal perfusate and fetal-to-maternal clearance. As a result, a total of 0.6 ± 0.09 μmol of AcH was cleared via the maternal efflux over 60 min; the recirculated fixed volume (100 ml) of the fetal perfusate contained a total of 0.2 ± 0.03 μmol. From these data, a minimum ethanol oxidation rate can be estimated for the 17.6 ± 1.44 g of perfused cotyledons: 45.6 nmol per hour per gram of tissue. This rate is considerably lower than the in vivo human hepatic rate of 178 μmol per hour per gram (*11*), although the calculation cannot take into account the evaporation of AcH from the fetal reservoir, placental metabolism of AcH, or binding of AcH to tissue proteins (*12*). Nonetheless, placental ethanol oxidation was sufficient to produce measurable levels of AcH in the fetal perfusate. This suggests that human maternal ingestion of ethanol may result in fetal exposure to AcH (via placental metabolism) even if there is no AcH in the maternal blood.

We confirmed the placental production of AcH, as determined by head-space gas chromatography, by perfusing three additional placentas with ^{14}C-labeled ethanol (Fig. 2). In experiments in which the ethanol concentration in the maternal perfusate was reduced to 25 m*M* (115 mg/dl), a concentration found in the blood of the occasional or "social" drinker, both the maternal and fetal perfusates were recirculated, and a time-related accumulation of metabolic products was expected. Indeed, there was a more than twofold rise in AcH and acetate from 30 to 60 min. This experiment confirmed the overall magnitude of AcH production observed in the initial perfusions, yet at a considerably lower ethanol concentration in the maternal perfusate. Moreover, the appearance of acetate provides evidence of not only AcH production but AcH oxidation as well. The latter could have bearing on maternal-to-fetal AcH transfer (*13*).

To this point, our model has reflected an in vivo human circumstance in which there would be no circulating AcH of maternal (hepatic) origin. However, many people, especially alcoholics, have circulating AcH during ethanol ingestion (*9*). Maternal-to-fetal transfer of AcH might augment the AcH produced by the placenta, resulting in even higher AcH levels in the fetal circulation. Accordingly, in five of the seven placentas initially perfused with ethanol alone, we followed the first perfusion phase with a second 60-min period in which the nonrecirculated maternal perfusate contained 65 m*M* (300 mg/dl) ethanol and 25 μ*M* AcH (*14*), an AcH level reported in the blood of alcoholics (*9*). This protocol simulates the in vivo circumstance in which the maternal blood provides a relatively constant supply of AcH over a short period.

Perfusion of the placental cotyledon with ethanol and AcH resulted in maternal-to-fetal transfer of AcH (Fig. 1B). Acetaldehyde transfer was leveling off at 45 min and did not approach equilibrium with the maternal influx concentration of 25 μ*M* AcH, being only 49.8 ± 1.0% of that concentration by 60 min. A similar maternal-to-fetal AcH gradient has been described in animal models of AcH transfer (*6*). Despite the failure to reach AcH equilibrium, AcH continued to disappear from the perfused maternal circulation: the maternal efflux concentration of AcH was 31% lower than the influx, being 17.26 ± 1.13 μ*M* at 60 min. This continued influx-efflux difference was presumably the result of metabolism and protein binding. Therefore, the results from the second phase of the placental perfusions indicate that the presence of AcH in a mother's blood results in partial transfer to the fetal circulation. This transfer load, superimposed on the AcH produced by placental oxidation of ethanol, would present an additional burden to the fetus.

Our data indicate that the human placenta has the potential to produce AcH, which could enter the fetal circulation. Furthermore partial transfer of AcH from maternal to fetal blood may occur. Because AcH is a potent cellular toxin (*3*), its presence in the fetal circulation, whether of placental or maternal origin, could help explain the pathophysiology of ethanol-associated injury. Fetal exposure to AcH might augment the direct fetotoxicity of ethanol and could be a cause of impaired fetal growth and development. Central to this mechanism for fetal injury is the major maternal-fetal interface, the placenta.

REFERENCES AND NOTES

1. S. K. Clarren and D. W. Smith, *N. Engl. J. Med.* **298**, 1063 (1978); H. L. Rosett and L. Weiner, *Annu. Rev. Med.* **36**, 73 (1985).
2. C. L. Randall, W. J. Taylor, D. W. Walker, *Alcoholism (NY)* **1**, 219 (1977); G. F. Chernoff, *Teratology* **15**, 223 (1978); G. I. Henderson, A. M. Hoyumpa, Jr., C. J. McClain, S. Schenker, *Alcoholism (NY)* **3**, 99 (1979); J. Diaz and H. H. Samson, *Science* **208**, 751 (1980); S. E. Fisher, M. Barnicle, R. Steis, I. Holzman, D. H. Van Thiel, *Pediatr. Res.* **15**, 335 (1981); K. K. Sulik, M. C. Johnston, M. A. Webb,

Science **214**, 936 (1981); G. I. Henderson, R. V. Patwardhan, A. M. Hoyumpa, Jr., S. Schenker, *Neurobehav. Toxicol. Teratol.* **3**, 73 (1981); S. E. Fisher *et al.*, *Alcoholism (NY)* **6**, 197 (1982).
3. C. S. Lieber, in *Medical Disorders of Alcoholism*, C. S. Lieber, Ed. (Saunders, Philadelphia, 1982), pp. 43–64; J. P. von Wartburg and R. Buhler, *Lab. Invest.* **50**, 5 (1984); M. F. Sorrell and D. J. Tuma, *Alcoholism (NY)* **9**, 306 (1985).
4. C. L. Randall, W. J. Taylor, B. Tabakoff, D. W. Walker, in *Alcohol and Aldehyde Metabolizing Systems*, R. G. Thurman *et al.*, Eds. (Academic Press, New York, 1977), vol. 3, pp. 659–670; A. B. Mukherjee and G. D. Hodgen, *Science* **218**, 700 (1982); P. M. Blakley and W. J. Scott, Jr., *Toxicol. Appl. Pharmacol.* **72**, 364 (1984); B. H. J. Gordon, E. Baraona, C. S. Lieber, *Alcohol* **2**, 271 (1985).
5. K. S. O'Shea and M. H. Kaufman, *J. Anat.* **128**, 65 (1979); I. Dreosti *et al.*, *Alcoholism (NY)* **5**, 357 (1981); W. S. Webster, D. A. Walsh, S. E. McEwen, A. H. Lipson, *Teratology* **27**, 231 (1983); M. A. Campbell and A. G. Fantel, *Life Sci.* **32**, 1641 (1983).
6. P. V. Veghelyi *et al.*, *Acta Paediatr. Acad. Sci. Hung.* **19**, 171 (1978); P. V. Veghelyi, *Lancet* **ii**, 53 (1983).
7. J. F. Brien, C. W. Loomis, J. Tranmer, M. McGrath, *Am. J. Obstet. Gynecol.* **146**, 181 (1983).
8. C. L. Pikkarainen and N. C. R. Raiha, *Nature* **222**, 563 (1969); C. L. Pikkarainen, *Life Sci.* **10** (suppl.), 1359 (1971).
9. M. A. Korsten *et al.*, *N. Engl. J. Med.* **292**, 386 (1975); this gas chromatographic method is sensitive to AcH concentrations <0.5 μ*M*.
10. L. I. Mann, *Am. J. Obstet. Gynecol.* **122**, 837 (1975).
11. C. S. Lieber and L. M. DeCarli, *J. Pharmacol. Exp. Ther.* **181**, 279 (1972).
12. K. C. Gaines, J. M. Salhany, D. J. Tuma, M. F. Sorrell, *FEBS Lett.* **75**, 115 (1977); F. Nomura and C. S. Lieber, *Biochem. Biophys. Res. Commun.* **100**, 131 (1981); V. J. Stevens *et al.*, *J. Clin. Invest.* **67**, 361 (1981); R. C. San George and H. D. Hoberman, *J. Biol. Chem.* **261**, 6811 (1986); D. J. Tuma and M. F. Sorrell, *Alcohol Alcoholism* **1** (suppl.), 61 (1987).
13. M. Kouri, T. Koivula, M. Koivusalo, *Acta Pharmacol. Toxicol.* **40**, 460 (1977).
14. For each normal, term human placenta, a single cotyledon was dually perfused according to the method of H. Schneider, M. Panigel, and J. Dancis [*Am. J. Obstet. Gynecol.* **114**, 822 (1972)] and H. Schneider and A. Huch [*Contrib. Gynecol. Obstet.* **13**, 40 (1985)]. Briefly, a chorionic arterial (fetal influx) vessel and a venous (fetal efflux) vessel, supplying an intact lobe, were catheterized and perfused with Earle's balanced salt solution containing 3% dextran, molecular weight 77,000 (EBSS), aerated with 95% oxygen and 5% carbon dioxide. The fetally perfused cotyledon was cut from the placenta with generous circumscription and placed on a water-jacketed, temperature-controlled perfusion stage with the maternal decidua facing up. Four metal cannulae pierced the maternal basal plate to provide perfusion of the intervillous space (maternal influx). The perfusate leaving the intervillous space (maternal efflux) was allowed to accumulate to a volume of 3 to 5 ml, which was then pumped out of the perfusion stage. The flow rate in both maternal and fetal circulations was 6.3 ml/min. The perfusion stage, perfusate reservoirs, and all tubing were kept in a temperature-controlled perfusion case at 37°C. Perfusate reservoir volumes were monitored to detect leaks; pressures were below 50 mmHg throughout all successful perfusions. Antipyrine, detected by ultraviolet spectroscopy [B. B. Brodie, J. Axelrod, R. Soberman, B. B. Levy, *J. Biol. Chem.* **179**, 25 (1949)], was used as a diffusion marker for maternal-to-fetal transfer characteristics. The general perfusion protocol was as follows: 30 min of washout or equilibration (EBSS with no substrate; maternal perfusate nonrecirculated or open, fetal open); 30 min of blank perfusate preparation (EBSS with no substrate; maternal open, fetal recirculated or closed); 60 min of perfusion with EBSS plus 65 m*M* (300 mg/dl) ethanol in the maternal influx (maternal open, fetal closed; fresh fetal perfusate); 15 min of washout (EBSS with no substrate; maternal open, fetal open); 60 min of perfusion with EBSS plus 65 m*M* (300 mg/dl) ethanol and 25 μ*M* AcH in maternal influx (maternal open, fetal closed; fresh fetal perfusate).
15. T. Takagi, J. Alderman, J. Gellert, C. S. Lieber, *Biochem. Pharmacol.* **35**, 3601 (1986).
16. We thank the Department of Obstetrics and S. Stross and her delivery room staff at North Shore University Hospital for their cooperation; we thank B. Harvey and A. Tamari for technical assistance. Supported by grants (HD 17375, AA 07284, AA 07275, and AA 03508) from the Department of Health and Human Services; the Veterans Administration, and the March of Dimes Birth Defects Foundation. S.E.F. is the recipient of Research Scientist Development Award AA 00074 from the Alcohol, Drug Abuse, and Mental Health Administration.

17 May 1988; accepted 25 August 1988

Antibodies to Asp-Asp-Glu-Asp Can Inhibit Transport of Nuclear Proteins into the Nucleus

YOSHIHIRO YONEDA, NAOKO IMAMOTO-SONOBE, YOSUKE MATSUOKA, RYO IWAMOTO, YUKIO KIHO,* TSUYOSHI UCHIDA†

The signal sequence of simian virus 40 (SV40) large T-antigen for translocation into the nucleus is composed of positively charged amino acids Lys-Lys-Lys-Arg-Lys. Rabbit antibodies to a synthetic peptide containing the negatively charged amino acid sequence Asp-Asp-Asp-Glu-Asp were obtained. Indirect immunofluorescence of the antigens recognized by the antibody was punctate at the nuclear rim or the nuclear surface, depending on the plane of focus. The antibody blocked transport of nuclear proteins into the nucleus. The antigens recognized by the antibody were predominantly localized to the nuclear pores.

NUCLEAR PROTEINS HAVE SPECIFIC signal sequences that are necessary for their transport from the cytoplasm into the nucleus (*1*). Colloidal gold particles coated with nucleoplasmin and injected into the cytoplasm of *Xenopus* oocytes enter the nucleus through nuclear pores (*2*). Wheat germ agglutinin binds to *O*-linked *N*-acetylglucosamine (GlcNAc) residues of nuclear pore complex proteins and inhibits import of proteins into the nucleus in vivo (*3*) and in vitro (*4*). The nuclear targeting signal of SV40 large T antigen is composed of positively charged amino acids and has the characteristic sequence KKKRK (*1*, *5*). Other nuclear proteins are reported to have similar sequences (*6*). When the SV40 large T-antigen signal sequence is conjugated to nonnuclear proteins with molecular sizes of more than 68 kD (too large to enter the nucleus by passive diffusion) and these are injected into the cytoplasm of cells, these conjugates are transported into the nucleus within 30 min, without cell division (*7*). Thus, the signal sequence of SV40 large T antigen may be the active nuclear transport signal.

"Receptors" that interact with the signal sequence may have a negatively charged region electrostatically complementary to the positively charged signal sequence. The putative signal sequence-binding site might contain either DDDED or EEEDE. We therefore synthesized these peptides (Table 1) and prepared and purified antibodies to them (Fig. 1). The antibodies consisted almost exclusively of immunoglobulin G (IgG) and reacted specifically with the corresponding peptides (Fig. 1, A and B) (*8*), but did not react directly with iodinated nucleoplasmin (Fig. 1C). Almost the same result was obtained with bovine serum albumin (BSA) conjugated to the nuclear targeting sequence of either SV40 large T antigen or polyoma virus large T antigen (SV40 T–BSA or polyoma T–BSA).

We used indirect immunofluorescence to determine the intracellular locations of the antigens recognized by these antibodies. Both kinds of antibodies gave similar results, although 10 to 20 times as much anti-EEEDE as anti-DDDED was required for equal staining. In interphase cells anti-DDDED stained mainly the nucleus, with weak staining of the cytoplasm (Fig. 2A). At higher magnification (Fig. 2, C, D, and E), the immunofluorescence of the nucleus appeared punctate. Depending on the plane of focus, the punctate staining was seen at the nuclear rim (Fig. 2C) or the nuclear surface (Fig. 2, D and E). A punctate pattern was also seen in the cytoplasm close to the

Y. Yoneda, N. Imamoto-Sonobe, Y. Matsuoka, R. Iwamoto, Y. Kiho, Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamada-oka, Suita, Osaka, 565, Japan.
T. Uchida, Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamada-oka, Suita, Osaka, 565, and National Institute for Basic Biology, Myodaiji-cho, Okazaki, Aichi, 444, Japan.

*Present address: Central Research Laboratory, Ishihara Sangyo Kaisha, Ltd., Kusatsu, Shiga, 525, Japan.
†To whom correspondence should be addressed at Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamada-oka, Suita, Osaka, 565, Japan.