The parent compound, cyanamide itself, which is probably atypical of the series, is listed as "irritating and caustic" (*4*).

Our original investigation concerned the metabolism of morpholine (**1**) in human saliva. Whole saliva was collected without stimulation over a period of less than 30 minutes and then incubated with 500 mg of added morpholine per liter for 4 hours at 37°C (*5*). Under these conditions the nitrite naturally present in human saliva (*1*) reacted with morpholine to yield concentrations on the order of a few micrograms of *N*-nitrosomorpholine (NNM) (*2*) per liter of saliva. Analysis of the saliva was carried out by extraction with CH_2Cl_2 followed by gas chromatography and mass spectroscopy (*6*). In addition to the expected NNM (with $m/e = 116$), a peak with a longer retention time was detected with $m/e = 112$ for its molecular ion (M^+). In many cases the concentration of this compound was on the order of several milligrams per liter. Since its mass spectrum also contained a conspicuous ion at M-30 (a frequent characteristic of *N*-nitrosamines), we initially believed that this compound might be *N*-nitrosooxazine, the 2,3,5,6-dehydrogenation product of NNM. This assumption was shown to be incorrect, however, when the compound failed to produce any response from a sensitive, nitrosamine-specific detector (*7*) and, later, when high-resolution mass spectrometry (*8*) indicated that this molecular ion at $m/e = 112.0631$ had a molecular formula of $C_3H_8N_2O$ rather than $C_4H_4N_2O_2$. Formation of analogous products from secondary amines (such as diphenylamine) with no reactive sites other than the amine hydrogen suggested that the unknown compound was formed by the substitution of a [CN] moiety at this position.

An authentic sample of compound **2** was prepared by established procedures (*9*) and was found to have chromatographic retention times (*6*) and mass spectrum indistinguishable from that of the unknown morpholine metabolite. Consequently, we believe that this metabolite was the cyanamide shown in Eq. 1.

1-Cyanomorpholine did not form in saliva that was centrifuged to remove bacteria and other cellular material. It was not detected in any suspensions of several pure strains of nitrate-reducing bacteria when morpholine was present during the course of growth or nitrate reduction (or both). Moreover, the addition of $^{15}NO_2^-$ or $^{15}NO_3^-$ caused no incorporation of ^{15}N into the 1-cyanomorpholine formed in whole saliva. Hence, this reaction seems to represent a novel method of secondary metabolism by salivary microorganisms and is apparently unrelated to concurrent nitrification.

Although cyanamides appear to be unknown as biological products, the occurrence of the cyano group—attached to carbon or sulfur—is well known. Organic cyanides are common constituents of plant materials (*10*) and include β-cyanoalanine, β-aminopropionitrile, and several cyanoglucosides. In microorganisms and animals, these compounds are known to be metabolized, in turn, to thiocyanate (*11*), a common constituent of human saliva.

Several possibilities can therefore be suggested to explain the biotransformation of amines to cyanamides in saliva. For example, it may be caused (i) by the enzymatic transfer of a cyano group from thiocyanate or an organic cyanide to a secondary amine, or (ii) by a nonenzymatic reaction, such as a direct reaction of the secondary amine with thiocyanate. The potential biological activity of these materials and the mechanism of their synthesis remain to be determined.

JOHN S. WISHNOK
STEVEN R. TANNENBAUM
Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge 02139

References and Notes

1. S. R. Tannenbaum, A. J. Sinskey, M. Weisman, W. Bishop, *J. Natl. Cancer Inst.* **53**, 79 (1974).
2. S. R. Tannenbaum *et al.*, in *Analysis and Formation of N-Nitroso Compounds* (International Agency for Research on Cancer, Lyon, in press).
3. Compounds tested include the following secondary amines: dibutylamine, diisobutylamine, diphenylamine, pyrrolidine, and piperidine. All yielded products with an m/e corresponding to the loss of H and the addition of CN.
4. *The Merck Index*, P. G. Stecher, Ed. (Merck, Rahway, N.J., ed. 8, 1968), p. 306.
5. Saliva was collected in sterile test tubes: saliva from a single individual was used for almost all experiments. A total of seven individuals provided saliva. Incubation was conducted aerobically in Erlenmeyer flasks on a shake table. Reactants were added to the saliva in a manner that avoided dilution of more than 5 percent, and the *p*H was checked and adjusted to 7.2, if necessary. Each individual's saliva had activity in forming the cyanamides, and individuals were tested more than once during the course of the experiments. Since the population of individuals tested is both small and homogeneous, we cannot arrive at any conclusion on the generality of this reaction in the overall population.
6. Analyses were carried out on a gas chromatograph–mass spectrometer combination (Varian-Aerograph series 200: Hitachi–Perkin-Elmer RMU-7E) equipped with a precolumn solvent stripping system [J. M. Essigman and P. Issenberg, *J. Food Sci.* **37**, 684 (1972)]. The precolumn was 0.6 cm by 0.3 m stainless steel packed with 20 percent Carbowax 20M on 40/60 Chrom W; the analytical columns were 2 cm by 150 m stainless steel coated with a Carbowax mix (20M and 4000, 1 : 1) or 0.3 cm by 1.8 m stainless steel packed with 4 percent OV-1 on 80/100 Chrom W.
7. D. H. Fine, F. Rufeh, B. Gunther, *Anal. Lett.* **6**, 731 (1973).
8. The high-resolution gas chromatography–mass spectrometry was performed by Dr. Peter Roller of the National Cancer Institute, Bethesda, Md.
9. D. Martin and A. Weise, *Chem. Ber.* **99**, 3367 (1966); W. L. Garbrecht and R. M. Herbst, *J. Org. Chem.* **18**, 1003 (1953); L. F. Fieser and M. Fieser, *Reagents for Organic Synthesis* (Wiley, New York, 1967), vol. 1, p. 174.
10. G. W. Butler and R. W. Bailey, *Chemistry and Biochemistry of Herbage* (Academic Press, New York, 1973), vol. 2, pp. 35 and 457.
11. R. T. Williams, in *Biogenesis of Natural Compounds*, P. Bernfeld, Ed. (Pergamon, London, 1963), p. 589.
12. Supported by PHS contract NO1-CP-33315 from the National Cancer Institute.

10 December 1975; revised 12 January 1976

Specificity of Naturally Occurring Antibody in Normal Gibbon Serum

Abstract. *Gibbon natural antibody examined by immunoelectron microscopy reacted with the entire envelope of type C virus and with areas on the cell surface equivalent to or smaller than the diameter of a virion in gibbon and human culture cells infected with or releasing type C viruses. The antibody activity was absorbed completely by two cell cultures infected with gibbon ape leukemia virus and by the virus itself, and partially by normal gibbon spleen cells and dog thymus-derived cells infected with baboon endogenous type C virus, and fresh white blood cells obtained from a patient with chronic myelogenous leukemia in acute blastic crisis.*

It has become evident that natural antibodies against RNA type C virus-associated cell surface antigens (CSA) and viral envelope antigens (VEA) are present in the serum of healthy animals. The occurrence of humoral antibodies against CSA and VEA as well as the induction of cell-mediated immunity was reported in mice (*1, 2*), and natural antibodies against type C viruses of gibbon lymphosarcoma and granulocytic leukemia were found in 15 out of 133 serums from healthy gibbon apes by immunofluorescence microscopy and neutralization tests (*3*). In earlier studies of gibbon type C VEA by neutralization tests and immunoelectron microscopy xenogeneic antiserum was used (*4*), so that the interpretation of specificity was complicated by the possibility of interfering xenogeneic antibodies other than specific antibodies.

The determination of antigen or antibody specificity of nonhuman primates presents special problems because of their relatedness to humans; RNA-directed DNA polymerase (reverse transcriptase) in cells from human patients with acute myelogenous leukemia (AML) was shown to be antigenically related to the reverse transcriptase of two oncogenic type C viruses from nonhuman primates—the woolly monkey simian sarcoma virus

(SSV) and gibbon ape leukemia virus (GALV) (*5*). By means of immunoelectron microscopy (IEM), we have studied the relation between the specificities of nonhuman primate and human surface antigens through an examination of the reactions of naturally occurring gibbon antibody with the CSA or VEA (or both) of various human and animal cell lines. Indirect IEM makes antigen-antibody reactions visible with the aid of an electron-opaque marker (such as ferritin) chemically conjugated to a second antibody directed against γ-globulin from the species of origin. Therefore, IEM has the great advantage of making it possible to distinguish VEA from CSA at high resolution on an electron microscope (*6*).

Cells used as target cells or for absorption of the test serum are described in the footnote of Table 1. Gibbon ape leukemia virus was harvested from the tissue culture of gibbon lymphosarcoma cells. The culture supernatant was centrifuged for 20 minutes at 16,300*g*. The virus pellet was resuspended in TNE (tris-HCl + NaCl + EDTA) buffer solution, and the resuspended pellet was centrifuged on a discontinuous sucrose gradient (1 ml of 45 percent sucrose and 5 ml of 25 percent sucrose solutions) for 75 minutes at 272,000*g*. Finally, the purified GALV was isolated from the opalescent band at the 25 to 45 percent interface.

IEM was performed with 3×10^6 target cells as described, with the use of ferritin-conjugated goat antibody (IgG) to human immunoglobulins G and M (IgG and IgM) (*6, 7*); the goat antibodies to human IgG and IgM were used since the γ-globulin of primates carries antigenic determinants common to those of human γ-globulin (*8*). The serum containing natural antibodies, which was used in the direct test with different target cells, was obtained from gibbon apes (*3*); it was pooled and stored at −70°C. Before use, the test serum was neutralized with fetal calf serum at a 3 : 1 ratio by incubation at 0° to 4°C (ice-bath) for 45 minutes to remove antibody against fetal calf serum. The above conditions were also used in the absorption tests, except that the undiluted test serum was mixed with an equal volume of packed cells and incubated at 4°C for 45 minutes with periodic agitation; the absorption was repeated with fresh cells (*9*). In the absorption with virus, 0.05 ml of test serum was mixed with 10^{11} GALV particles, incubated for 45 minutes at 4°C, and recovered by ultracentrifugation for 150 minutes at 53,000*g*. All procedures were carried out at 0° to 4°C to avoid the synthesis of new cell surfaces, budding of viruses, pinocytosis, and phagocytosis. The incubation at 4°C also caused adsorption of many virions onto the cell surface, a phenomenon utilized for "synchronization" of virus infection (*10*). At least 50 cells and associated type C viruses were screened by thin section on a Philips 300 electron microscope. Since gibbon ape cell histocompatibility antigens seem to be similar to those of human cells, VEA and VEA-related CSA were the only antigens we analyzed.

When gibbon natural antibody reacted with surface antigens of gibbon lymphosarcoma cells (Table 1), all virions (GALV) were labeled quite densely on the entire envelope, and approximately 65 percent of the cells showed a positive reaction in small areas on the cell surface equivalent to or smaller than the diameter of a virion (Fig. 1A). The reactivity of gibbon serum was completely absorbed with purified GALV, gibbon lymphosarcoma cells, or G-204 cells (which are GALV-infected human rhabdosarcoma A-204 cells). The G-204 cells released at least two different type C virus populations. In one population, 50 percent of the virions were different in terms of VEA from the GALV in the gibbon lymphosarcoma cells (Fig. 1B). The G-204 cells also expressed CSA common to VEA because the small amount of positive CSA (5 percent) was absorbed with purified GALV. In addition, fresh peripheral white blood cells from a patient with CML (HR-158A) and normal gibbon spleen monolayer GSpl significantly removed the activity from the gibbon serum. Since these cells did not release type C virus, the findings suggest that the cells carried CSA common to VEA.

There are two explanations for the release by the G-204 cells of two virus populations carrying different VEA. (i) VEA itself changed during budding from the hu-

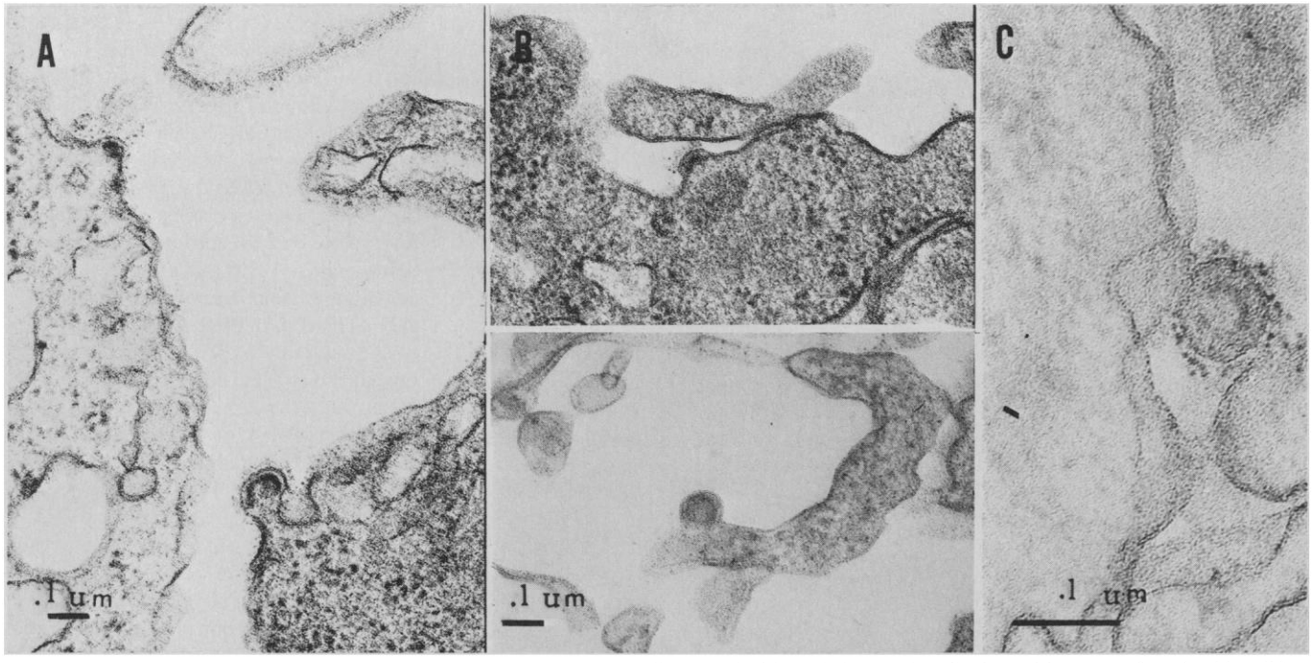

Fig. 1. (A) Type C virus (GALV) released from gibbon lymphosarcoma cells reacted with pooled natural antibody-positive serum from healthy gibbon apes. The entire envelope and areas on the cell surface equivalent to or smaller than the diameter of a virion were labeled. (B) GALV-infected human rhabdosarcoma cells G-204 released two different virus populations; 50 percent of virions reacted positively (upper) and the other 50 percent did not react (lower) with the same serum used in (A). (C) The entire viral envelope but not the cell surface in B-101 cells (human rhabdosarcoma cells A-204 infected with HL-23 virus from cells of a patient with acute myelogenous leukemia) reacted with the gibbon serum.

man cell plasma membrane, as has been reported in other classes of type C virus (*11*), or (ii) the genome of a putative endogenous or exogenous type C virus in human cells was activated by GALV infection. The same possibilities may apply to our other findings: (i) Although the reverse transcriptase of GALV has been shown to be antigenically similar to that of SSV (*5*) and the xenogeneic antiserum against GALV reacted with the envelope of SSV released from the woolly monkey cells (*4*), the NC-37-SSV cells did not remove the activity from the gibbon test serum while this serum reacted weakly with VEA of type C viruses from the A-204-SSV cells. (ii) The uninfected A-204 cells did not react with the gibbon serum; after infection with putative human AML virus (HL-23 virus), 40 percent of the HL-23 viruses in the B-101 cells were labeled with ferritin on the entire envelope (Fig. 1C), although not densely, whereas the cell surface was completely negative. (iii) The gibbon serum did not react with the newly cultured CIH-1(V^-) cells (human embryonic diploid cells), which do not release virus; but it did react with 66 percent of the virions in the late passage CIH-32(V^+) cells, which do release virus (*12*). Although we cannot conclude from these findings whether these "human" type C viruses are actually of human origin, our results do indicate relatedness of putative human type C virus to viruses isolated from nonhuman primates. It should be emphasized that, when type C virus isolated from given cells infected other cells especially of different species, VEA was altered to a great extent, and CSA and VEA of virions released from these infected cells often changed markedly during culture in vitro (*13*). Furthermore, BAB-455K cells (dog thymus-derived cells infected with baboon type C virus) showed a weakly positive reaction with CSA by the direct test while absorption with these cells significantly removed the activity of the test serum. In contrast, B-101 cells (A-204 cells infected with HL-23 virus) released very small numbers of type C viruses that reacted with the test serum in the direct test (two positive out of five particles observed) and showed no reaction with CSA (350 cells were screened); these cells, however, did not remove the activity from the test serum by the absorption test. These seemingly contradictory results indicate that the amount of CSA in the BAB-455K cells used for absorption was large enough to absorb the activity against CSA while the B-101 cells carried no CSA and too few type C viruses to absorb the serum activity against VEA. All other cells tested, including various human malignant cells, did not react with the gibbon serum.

The finding that fresh white cells from myelogenous leukemia patients, HR-158A, and the GSpl cells carried CSA common to VEA of GALV is of interest since these cells did not produce type C virus. This suggests that the viral genome can still express CSA common to VEA in nonproducer cells. A similar phenomenon has been reported in both the murine and the avian systems (*14*). This postulate is supported by the poor production of putative human type C viruses (*15*) and by the presence of intraviral components, reverse transcriptase and p30 (gs antigen), in at least some human and nonhuman primate cells (*5, 16*). Consequently, we may at least

Table 1. Specificity analysis of naturally occurring gibbon ape antibodies against cell surface antigens (CSA) and viral envelope antigens (VEA) of various cells by immunoelectron microscopy. The designations of cells and virus are as follows. GLS, gibbon lymphosarcoma cells (*11*); GALV, type C virus released by GLS which induces myelogenous leukemia in gibbon apes (*15*); GSpl, gibbon normal spleen monolayer; A-204, human rhabdosarcoma cells (from G. Todaro); A204-SSV, woolly monkey sarcoma virus (SSV)–infected A-204 (from R. Ting); G-204, GALV-infected A-204 (from W. Parks); WHE, whole human embryo cells (from R. Ting); NC-37, normal human lymphoid cell culture line (from R. Ting); NC-37-SSV, SSV-infected NC-37 (from R. Ting); MOLT, human acute myelogenous leukemia (AML) culture line (from G. Cannon); HOS, human osteosarcoma culture line (from G. Rhim) (*17*); A-1 Ewing Sa, A-1 Ewing sarcoma culture line (from G. Cannon); 2089, fresh human rhabdosarcoma tissue; 3173, fresh human neurofibrosarcoma tissue; HR-158A, fresh human peripheral chronic myelogenous leukemia cells in acute blastic crisis; BAB-455K, baboon type C virus-infected dog thymus-derived cells (G. Todaro); B-101, A-204 infected with HL-23 virus isolated from human AML (from R. Gallo) (*18*); A7573-ASV, dog thymus-derived cells infected with HL-23 virus (from R. Ting); CIH(V^-); human embryonic diploid cells at early passage, releasing no virus (from S. Panem) (*12*); CIH-32(V^+), human embryonic diploid cells at late passage, releasing type C virus (from S. Panem) (*12*); KSL-6, transplanted AKR spontaneous leukemia; BALB-M-S, Moloney murine sarcoma virus–induced BALB/c primary sarcoma; A-204-FeLV, A-204 infected with feline leukemia virus.

Absorption cell or virus	Target cell	Results (% positive reaction)		Judgment	
		CSA	VEA	Direct test*	Absorption†
None‡	GLS	65	100 (100)§	++	
GLS	GLS	0	0 (100)		Complete
GALV	GLS	0	0 (100)		Complete
None	GSpl	34	No virus	+	
GSpl	GLS	8	10 (100)		Partial
None	A-204	0	No virus	–	
A-204	GLS	60	85 (100)		None
None	A-204-SSV	30	33 (6)	+	
None	G-204	5	50 (12)	+	
G-204	GLS	0	0 (100)		Complete
GALV	G-204	0	0 (10)		Complete
WHE	GLS	62	84 (100)		None
NC-37	GLS	78	90 (100)		None
NC-37-SSV	GLS	66	81 (100)		None
MOLT	GLS	80	80 (100)		None
HOS	GLS	62	100 (100)		None
A-1 Ewing Sa	GLS	80	90 (100)		None
2089	GLS	50	81 (100)		None
3173	GLS	90	90 (100)		None
HR-158A	GLS	20	50 (100)		Partial
None	M7-520	10	0 (10)	±	
None	BAB-455K	4	0 (7)	±	
BAB-455K	GLS	44	65 (100)		Partial
None	B-101	0	40 (5)‖	+	
B-101	GLS	24	96 (100)		None
None	A7573-ASV	20	91 (35)	++	
None	CIH-1(V^-)	0	No virus	–	
None	CIH-32(V^+)	43	66 (6)	++	
KSL-6	GLS	90	80 (100)		None
BALB-M-S	GLS	50	85 (100)		None
None	A-204-FeLV	0	0 (12)	–	

*–, No reaction; ±, weakly positive reaction with CSA only; +, 50 percent or fewer virions showed positive reaction; ++, more than 50 percent virions showed positive reaction. †Complete, the whole activity of test serum was absorbed; partial, > 20 percent direct positive reactivity was removed; none, ≤ 20 percent reactivity was removed. ‡Direct test. §Parentheses indicate the number of virus particles scored. ‖Since the B-101 cells released few type C virions, 350 cells were screened and only 5 accompanying virions were found.

conclude that the CSA of certain human cells is common to VEA of GALV, SSV, and baboon endogenous type C virus.

TADAO AOKI, MARGARET LIU
MARY JANE WALLING, GRACE S. BUSHAR
PHYLLIS B. BRANDCHAFT
Immunology Section, Laboratory of Viral Carcinogenesis, National Cancer Institute, Bethesda, Maryland 20014
THOMAS G. KAWAKAMI
Comparative Oncology Laboratory, University of California, Davis 95616

References and Notes

1. T. Aoki, E. A. Boyse, L. J. Old, *Cancer Res.* **26**, 1415 (1966); M. B. A. Oldstone, T. Aoki, F. J. Dixon, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 134 (1972); M. I. Colnaghi and G. Della Porta, *J. Natl. Cancer Inst.* **50**, 173 (1973); T. Aoki, E. A. Boyse, L. J. Old, E. deHarven, U. Hämmerling, H. A. Wood, *Proc. Natl. Acad. Sci. U.S.A.* **65**, 569 (1970); R. B. Herberman and T. Aoki, *J. Exp. Med.* **136**, 94 (1972); J. N. Ihle, M. Yurconic, M. G. Hanna, *ibid.* **138**, 194 (1973); R. C. Nowinski and S. I. Kaehler, *Science* **185**, 869 (1974).
2. M. Nunn, J. Djeu, D. Lavrin, R. B. Herberman, *Proc. Am. Assoc. Cancer Res.* **14**, 87 (1973); R. Kiessling, E. Klein, H. Wigzell, *Eur. J. Immunol.* **5**, 112 (1975); *ibid.*, p. 117; R. B. Herberman, M. E. Nunn, D. H. Lavrin, *Int. J. Cancer* **16**, 216 (1975); R. B. Herberman, M. E. Nunn, H. T. Holden, D. H. Lavrin, *ibid.*, p. 230; J. M. Zarling, R. C. Nowinski, F. H. Bach, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2780 (1975); F. Sendo, T. Aoki, E. A. Boyse, C. K. Buafo, *J. Natl. Cancer Inst.* **55**, 603 (1975).
3. T. G. Kawakami, P. M. Buckley, T. S. McDowell, A. Depaoli, *Nature (London) New Biol.* **246**, 105 (1973).
4. T. G. Kawakami and P. M. Buckley, *Transplant. Proc.* **6**, 193 (1974).
5. G. J. Todaro and R. C. Gallo, *Nature (London)* **244**, 206 (1973); R. E. Gallagher, G. J. Todaro, R. Smith, D. Livingston, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1309 (19 → H. Mondal, R. Gallagher, R. C. Gallo, *ibid.* **72**, 1194 (1975).
6. G. E. Andres, K. C. Hsu, B. C. Seegal, *Handbook of Experimental Immunology* (Oxford, Blackwell, 1973), p. 34.1.
7. T. Aoki and T. Takahashi, *J. Exp. Med.* **135**, 443 (1972).
8. T. Aoki, unpublished observations; J. Shuster, N. L. Warner, H. H. Fudenberg, *Ann. N.Y. Acad. Sci.* **162**, 195 (1969); F. W. Putnam, *Prog. Immunol.* **2** (1), 25 (1974).
9. The amount of cells used for absorption was almost 100 times more than that of cells used for the direct test, resulting in a tremendous increase in sensitivity.
10. C. Morgan, H. M. Rose, B. Medinio, *J. Virol.* **2**, 507 (1968).
11. S. A. Aaronson, *Nature (London)* **230**, 445 (1971); K. Maruyama, M. F. Miller, S. H. Wagner, L. Domochowski, *Proc. Electron Microsc. Soc. Am.* **32**, 246 (1974).
12. S. Panem, E. V. Prochownik, F. R. Reale, W. H. Kirsten, *Science* **189**, 297 (1975).
13. T. Aoki, R. B. Herberman, M. Lui, *Intervirology* **5**, 31 (1975).
14. S. J. Kennel, B. C. Del Villano, R. L. Levy, R. A. Lerner, *Virology* **55**, 464 (1973); H. Hanafusa, T. Aoki, S. Kawai, T. Miyamoto, R. E. Wilsnack, *ibid.* **56**, 22 (1973).
15. S. P. Synder, D. L. Dungworth, T. G. Kawakami, E. Callaway, D. T-L. Lau, *J. Natl. Cancer Inst.* **51**, 89 (1973).
16. H. Mondal, R. E. Gallagher, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1194 (1975); C. Sherr and G. J. Todaro, *Science* **187**, 855 (1975); M. Strand and J. T. August, *J. Virol.* **14**, 2584 (1974).
17. J. S. Rhim, H. Y. Cho, R. J. Huebner, *Int. J. Cancer* **15**, 23 (1975).
18. R. E. Gallagher and R. C. Gallo, *Science* **187**, 350 (1975).
19. We thank D. T.-L. Lau (San Francisco Medical Center) for providing the gibbon ape serum and the other investigators cited in the legend of Table 1 for providing the cells, and J. L. Thompson for photography. Supported in part by contracts NO1-CP-43429 and NO1-CP-33243 within the Virus Cancer Program of the National Cancer Institute.

21 November 1975; revised 14 January 1976

Phylogeny of DNA Polymerase-β

Abstract. *Analyses of various organisms for DNA polymerase-β activity show that the enzyme is widely distributed in cells from multicellular animals but absent in bacteria, plants, and protozoa. These results suggest that DNA polymerase-β may have evolved with the development of metazoan forms. Further evolutionary changes of the enzyme protein may account for some of the minor differences in properties of the enzyme in various organisms.*

DNA polymerase-β is a low molecular weight ($\leq$ 50,000), *N*-ethylmaleimide–resistant species of DNA-dependent DNA polymerase (*1*). It is widely distributed in higher vertebrate species (*2*). Its presence appears not to be related to the proliferative state of a cell or to any specific function of tissues (*2*). To ascertain whether DNA polymerase-β is some vestigial form of a primitive replicative enzyme or whether it evolved with development of new functions, I have carried out a phylogenetic survey for this enzyme activity in organisms covering a wide range of evolutionary time. The results demonstrate the widespread occurrence of this type of enzyme in multicellular animals. A comparable enzyme was not found in unicellular eukaryotes or in plants.

The operating conditions were established at the outset. Two essential properties defined for DNA polymerase-β are resistance to *N*-ethylmaleimide and low molecular weight ($\leq$ 50,000) (*1*). The *N*-ethylmaleimide resistance of the enzyme is tested by assaying enzyme activity remaining after treatment of the crude extracts (or sucrose gradient fractions) with 10 m*M* *N*-ethylmaleimide at 4°C for 30 minutes. The molecular weight of the enzyme was estimated roughly by centrifugation of the extracts on linear (5 to 20 percent by weight) sucrose gradients in 0.5*M* NaCl, 0.05*M* tris-HCl at *p*H 7.8, 1 m*M* EDTA, and 1 m*M* 2-mercaptoethanol. Centrifugation was carried out for 16 hours at 40,000 rev/min (SW 50.1 rotor; Spinco centrifuge). Two other conditions, established as optimal for mammalian enzymes, were also used to distinguish DNA polymerase-α and -β activities (*2*). The DNA polymerase-β activity was assayed with 0.05*M* Ammediol hydrochloride buffer (*p*H 8.6) and 80 m*M* NaCl; the DNA polymerase-α was assayed with 0.025*M* potassium phosphate (*p*H 7.2) and either no NaCl (for the crude extract) or 40 m*M* NaCl (for the sucrose gradient fraction). The other components in the DNA polymerase reactions were 8 m*M* $MgCl_2$, 0.1 m*M* deoxyadenosine triphosphate, 0.1 m*M* deoxycytidine triphosphate, 0.1 m*M* deoxyguanosine triphosphate, 0.1 m*M* [methyl-^{3}H]deoxythymidine triphosphate, 2 m*M* adenosine triphosphate, and 200 μg of activated calf thymus DNA per milliliter.

In interpreting the results of this survey (Table 1), the following conditions were applied. If an enzyme activity was of low molecular weight and *N*-ethylmaleimide–resistant, it was scored as DNA polymerase-β, although it is obvious that not all such activity would be absolutely identical in organisms over such a wide evolutionary scale. If an activity was of high molecular weight (> 100,000) and *N*-ethylmaleimide–resistant, it was considered outside the realm of eukaryotic enzymes, possibly of prokaryotic origin [like *Escherichia coli*

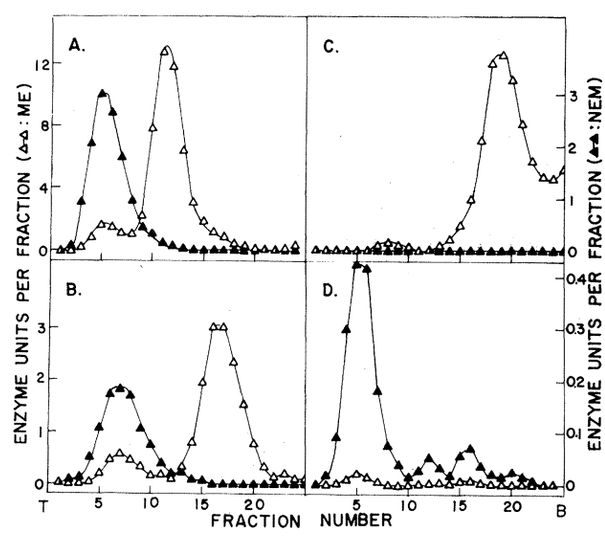

Fig. 1. Sucrose gradient analysis of DNA polymerases in (A) chameleon testes, (B) earthworm gonads, (C) silk gland, and (D) sponge. Abbreviations: ME, enzyme activity remaining after preliminary incubation with 10 m*M* 2-mercaptoethanol; NEM, enzyme activity remaining after preliminary incubation with 10 m*M* *N*-ethylmaleimide.