C and D). Others have observed antigenic similarities between human, calf, and pig rotavirus (*1*).

We conclude from these and other (*1*, *4*, *5*) data that rotavirus occurs in most mammals, but it is generally benign. Our results suggest that rotavirus owes its benignity to its high prevalence which inevitably leads to the neonate encountering the virus while being passively protected (*5*, *8*) by local antibody in the gut provided by the mother's milk. Active immunity, without the burden of overt disease, probably issues from this constant encounter. This is analogous to the sequence of subclinical infection and immunity seen with infantile paralysis (poliomyelitis) in primitive societies (*10*).

Problems arise when the dose of the virus exceeds the capacity of the immune system to contain viral growth. According to our results, this occurs when the highly susceptible piglet (less than 1 week of age) is exposed to large doses of rotavirus while being reared artificially. At this time, the piglet is weaned and no longer protected by antibodies in the milk, and the virus is free to multiply. The disease can be prevented by cleaning the delivery room (thus lowering the dose) and rearing the piglets in a nursery that can be entirely cleaned between groups (thus preventing the buildup of the infecting dose). Symptoms can be ameliorated by promptly feeding antibodies, for example, cows' colostrum.

Most children by 6 years of age have antibody to rotavirus. Rotaviral diarrhea peaks in the winter months in children ½ to 1½ years of age, and is often acquired in infant wards. Nursing infants, in comparison to infants fed artificially, have a much lower incidence of the disease in hospitals (*1*, *2*, *11*). In view of our findings, these data on human infants suggest (i) that the virus is prevalent in the community and the disease exacerbates when dose is increased (because of crowding, decreased ventilation in the winter, continuous use of nurseries), and (ii) that the weaned infant is removed from protective antibody in milk. These circumstances, when coupled with lower levels of sanitation and nutrition, could account for the high number of infant deaths in the Third World. Thus the management practices that precipitate rotaviral diarrhea in piglets may be similar to those used for some infants.

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References and Notes

1. Editorial, *Lancet* **1975-I**, 257 (1975).
2. J. R. Hamilton, D. G. Gall, B. Kerzner, D. G. Butler, P. J. Middleton, *Pediatr. Clin. North Am.* **22**, 747 (1975).
3. R. F. Bishop, A. S. Hewstone, G. P. Davidson, R. R. W. Townley, I. H. Holmes, B. J. Ruck, *J. Clin. Pathol.* **29**, 46 (1976); R. L. Guerrant, M. D. Dickens, R. P. Wenzel, A. Z. Kapikian, *J. Pediatr.* **89**, 885 (1976).
4. G. R. Pearson, J. B. McFerran, W. L. Curran, R. M. McCracken, *Infect. Immun.* **14**, 1332 (1976); G. N. Woode, J. C. Bridger, J. M. Jones, T. H. Flewett, A. S. Bryden, H. A. Davies, G. B. White, *ibid.*, p. 804.
5. J. G. Lecce, M. W. King, R. Mock, *ibid.*, p. 816.
6. J. G. Lecce and G. Matrone, *J. Nutr.* **70**, 13 (1960).
7. Sows were brought to a tiled isolation room 5 days prior to parturition and washed with an iodinated detergent three times per day. No feces accumulated in the room and air was changed ten times per hour.
8. J. G. Lecce, *J. Anim. Sci.* **41**, 659 (1975).
9. Air was sampled at the same time each day with a Microbiological Air Sampler (New Brunswick Scientific). Total counts were made from Trypticase soy agar plates (BBL Products, BioQuest Division), *E. coli* counts from eosin-methylene blue agar plates (Difco), and enterococci from selective enterococcus agar (Pfizer).
10. M. Burnet and D. O. White, *Natural History of Infectious Diseases* (Cambridge Univ. Press, New York, ed. 4, 1972), p. 91.
11. A. S. Bryden, H. A. Davies, R. E. Hadley, T. H. Flewett, *Lancet* **1975-II**, 241 (1975); A. Z. Kapikian, H. W. Kim, R. G. Wyatt, W. L. Cline, J. O. Arrobio, C. D. Brandt, W. J. Rodriguez, D. A. Sack, R. M. Chanock, R. H. Parrott, *N. Engl. J. Med.* **294**, 965 (1976); B. M. Totterdell, I. L. Chrystie, J. E. Banatvala, *Arch. Dis. Child.* **51**, 924 (1976).
12. This report was presented in part at a symposium on Recent Advances in Bacterial and Viral Gastroenteritis, Medical College of Virginia, Richmond, February 1976. Paper No. 5356 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh. We thank Norden Laboratories for antiserum to calf rotavirus and R. G. Wyatt of NIAID for antiserum to human rotavirus. Fluorescent antibody to porcine and bovine gamma globulin came from Cappel Laboratories; fluorescent antibody to rabbit gamma globulin came from Antibodies, Inc.

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Binding of Benzo[*a*]pyrene 7,8-Diol-9,10-Epoxides to DNA, RNA, and Protein of Mouse Skin Occurs with High Stereoselectivity

Abstract. *The formation, stereostructure, and cellular reactions of the 7,8-diol-9,10-epoxide metabolites of the carcinogen benzo[a]pyrene have been examined after topical application of benzo[a]pyrene to the skin of mice. In this known target tissue, polymer adducts from diastereomeric diol epoxides, (+)-(7S, 8R, 9R, 10R) and (+)-(7R, 8S, 9R, 10R), were formed stereospecifically from their corresponding 7,8-dihydrodiols. Both diol epoxides bind with proteins, RNA, and DNA in vivo. For the nucleic acids, binding occurs preferentially at the 2-amino group of guanine in cellular RNA and DNA in vivo. Methods for establishing the structure of the cellular adducts as well as the possible biological implications of their formation are discussed.*

Since the initial studies indicating that metabolites of benzo[*a*]pyrene (BP) 7,8-dihydrodiol are bound to DNA to a much greater extent than any other metabolites of BP (*1*), substantial evidence has accumulated suggesting that the diastereomeric BP 7,8-diol-9,10-epoxides (diol epoxides **1** and **2**) are ultimate carcinogenic metabolites of BP. For example, BP 7,8-oxide is a potent skin carcinogen although weaker than BP, BP 7,8-dihydrodiol is slightly more carcinogenic than BP on mouse skin, and BP 7,8-oxide and BP 7,8-dihydrodiol are noncarcinogenic when the 9,10 double bond of the molecule is hydrogenated (*2*). In addition, both BP 7,8-dihydrodiol and diol epoxide **2** are much more potent car-

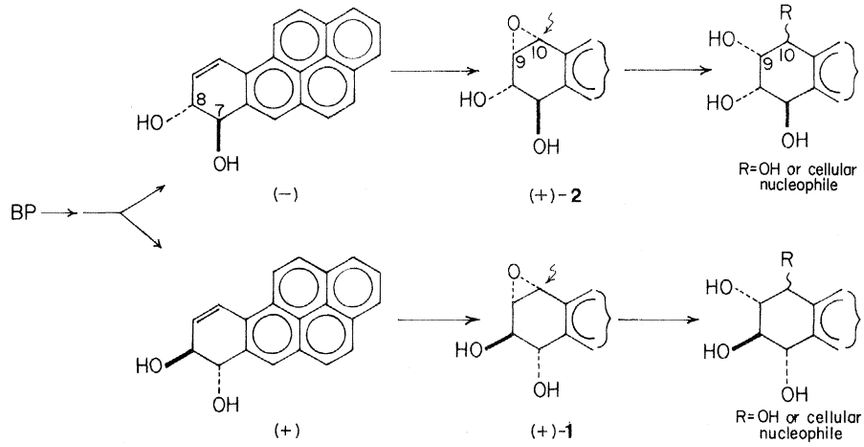

Fig. 1. Metabolic activation of BP in mouse skin. The absolute stereochemistry shown for the BP 7,8-dihydrodiols has been established by exciton chirality studies (*15*, *16*).

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cinogens than BP in the newborn mouse (*3*).

Studies of the metabolism of the enantiomeric (+)- and (−)-BP 7,8-dihydrodiols by mono-oxygenase systems from rat liver indicate that both **1** and **2** are formed from BP 7,8-dihydrodiol, principally **1** from (+)-BP 7,8-dihydrodiol and **2** from (−)-BP 7,8-dihydrodiol (*4, 5*) (see Fig. 1). Since metabolism of BP to diol epoxides has not yet been studied in a known target tissue in vivo, we examined the nature of the bound products which are formed from BP when the hydrocarbon is applied to mouse skin.

Each of 11 female C57BL/6J mice was painted with 100 μg of [^{3}H]BP (1.4 mCi per mouse, Amersham/Searle) in 100 μl of acetone. After 24 hours the mice were killed and the epidermal cells were isolated. After homogenization (*6*), a protein fraction (14 mg, 3×10^6 dpm/mg), an RNA fraction (1.8 mg, 2.8×10^5 dpm/mg), and a DNA fraction (0.9 mg, 6.0×10^4 dpm/mg) were isolated by acetone, sodium acetate, and ethanol precipitations, respectively (*7*). Thus, the bound BP metabolites per milligram of each polymer varied more than 50-fold, with protein having the highest specific activity and total amount of binding and DNA the lowest. Modified RNA and DNA were hydrolyzed chemically and enzymatically to their respective nucleosides (*8, 9*).

Two approaches were taken to establish the presence and the ratio of bound **1** and **2** in the macromolecular fractions isolated from the skin of mice treated with BP. In the first of these approaches, the nucleoside adducts and the protein adducts were treated with acid to release the bound diol epoxides as tetraols. Previous studies had established that most of the binding of these diol epoxides to nucleic acid occurs through *cis* and *trans* addition of the exocyclic 2-amino group of guanine (*9, 10*) and to a lesser extent of the backbone phosphate oxygen (*9*) at C-10 of the diol epoxides. The acid hydrolysis conditions were selected such that diol epoxides bound to the 2-amino group of guanosine would be completely released as tetraols. Since the pairs of tetraols from **1** and **2** which result by *cis* and *trans* addition of water at C-10 of the epoxides are readily separated by high-pressure liquid chromatography (HPLC) (*4, 5*), the stereochemical origin of the tetraols released after acid hydrolysis of the cellular adducts could readily be established (Table 1). Of the total radioactivity bound covalently to protein, RNA, and DNA, 25, 80, and 75 percent, respectively, could be released in tetraols by acid hydrolysis. The nature of the residual radioactivity was not further investigated. The relative stereochemistries of the released tetraols indicated that the covalent products in each of the cellular fractions resulted predominantly from **2**. Interestingly, the amount of tetraols from **1** relative to those from **2** decreased from 39 percent in the protein fraction to only 12 percent in the DNA hydrolysate (Table 1). This decrease in the relative amount of products from **1** in DNA may arise from the greater chemical reactivity of **1** (*11*) and hence its decreased ability to migrate intact from its presumed microsomal site of origin to more remote biological targets.

The second method used for the analysis of the nucleic acid–bound diol epoxides consisted of analysis of the radioactive adducts by HPLC. Since the absolute amount of nucleoside adducts which can be obtained in this experiment in vivo is limited, it was first necessary to

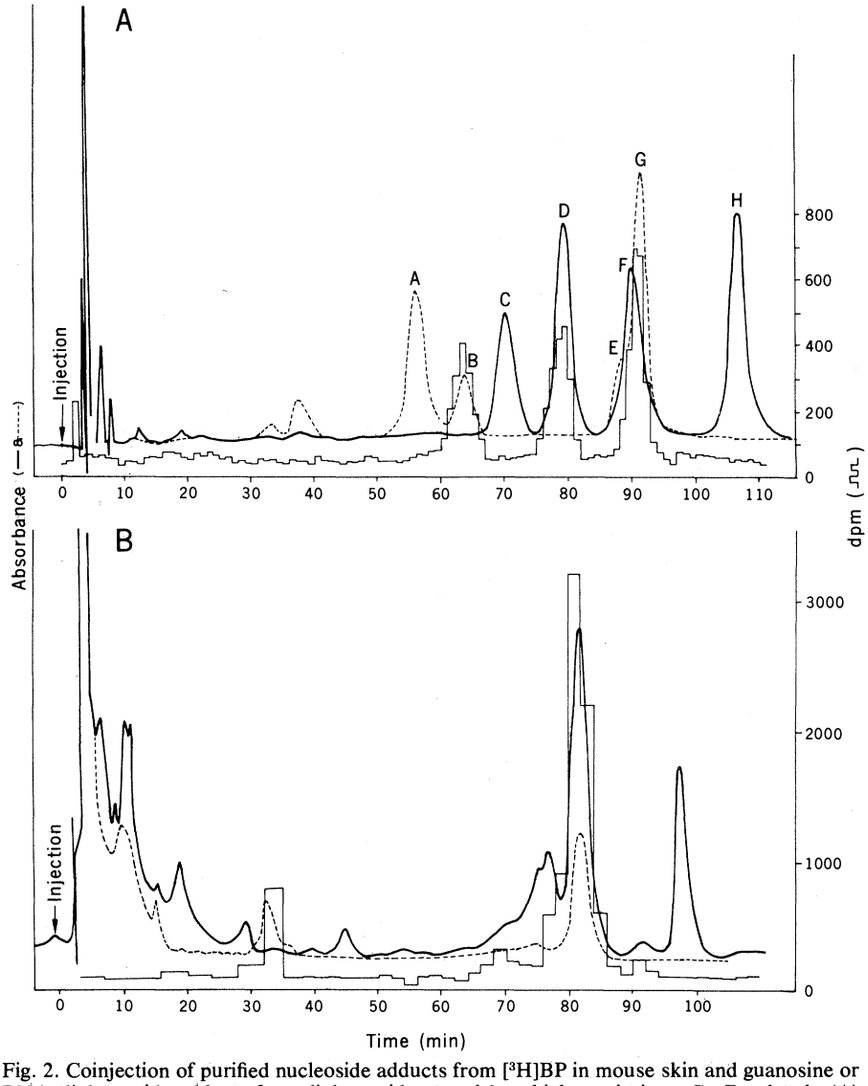

Fig. 2. Coinjection of purified nucleoside adducts from [^{3}H]BP in mouse skin and guanosine or DNA–diol epoxide adducts from diol epoxides **1** and **2** on high-resolution μC_{18}-Bondapak. (A) Guanosine–diol epoxide **1** and **2** adducts in vitro, and in vivo RNA adducts. Elution was monitored at 280 nm for the guanosine adducts produced by treatment of (±)-**1** (—) and (±)-**2** (----) with polyguanylic acid potassium salt. The elution of RNA nucleoside adducts obtained from treatment of mouse skin with tritiated BP was monitored by radioactivity (⎍⎍). (B) 2′-Deoxyguanosine–diol epoxides **1** and **2** adducts and in vivo DNA adducts. Elution was monitored at 344 nm for the nucleoside adducts of calf thymus DNA with (±)-**1** (——) and (±)-**2** (----). The elution of the DNA nucleoside adducts (see text) was monitored by radioactivity (⎍⎍). Both BP tetraols and unreacted nucleosides were first removed by HPLC with a Poragel PN column (3/8 inch by 3 feet, eluted with 85 percent methanol in H_2O). In the hydrolysate of in vivo RNA adducts, before purification with Poragel PN, a substantial fraction (approximately one-third of the radioactivity of the guanosine adducts) of less polar radioactive products was observed in the HPLC retention volume where adenosine–BP diol epoxide adducts appear. In contrast, the in vivo DNA adducts are predominantly those of BP and 2′-deoxyguanosine.

establish the chromatographic mobilities of all the possible diastereomeric guanosine adducts. To do this, (+) and (−) **1** and **2** were separately treated with polyguanylic acid potassium salt under conditions previously described for the racemic material (*9*), and the chromatography mobilities of the resulting nucleoside adducts were determined (Fig. 2A). When in vivo RNA nucleoside adducts were chromatographed with ultraviolet-detectable quantities of authentic marker compounds, the three major radioactive peaks were observed to coelute with guanosine–diol epoxide adducts. Each radioactive peak showed a change in partition coefficient (an indication of ionization) in the region *p*H 9.0 to 11.0 (Fig. 3), providing evidence that they are indeed guanosine adducts (*12*). Furthermore, acid hydrolysis of these in vivo products produced radioactive products coeluting with the tetraols from **1** and **2**. Although the third radioactive product coelutes with a guanosine product from both **1** and **2** (*G* and *F* in Fig. 2A), the distribution of radioactivity in the acid-liberated tetraols (see Table 1) suggests that the overall **1/2** product ratio is approximately 1 to 4. Lack of cochromatography of radioactivity in some of the potential diastereomeric guanosine adducts indicates that both **1** and **2** formed in vivo in mouse skin are nearly optically pure (+) enantiomers (see Fig. 2A).

To obtain appropriate marker compounds for the DNA adducts, racemic **1** and **2** were allowed to react with calf thymus DNA and the resulting modified DNA was hydrolyzed with deoxyribonuclease I, snake venom phosphodiesterase, and bacterial alkaline phosphatase. Essentially a single peak was obtained on HPLC for nucleoside products from **2**, while **1** showed three major peaks (Fig. 2B). Cochromatography of the in vivo DNA nucleoside adducts from [^{3}H]BP, partition versus *p*H analysis (Fig. 3), and acid hydrolysis of the DNA nucleoside adducts to radioactive tetraols (Table 1) confirmed the structure of the major in vivo DNA products as diol epoxides **1** and **2** linked to the 2-amino group of 2′-deoxyguanosine.

Metabolism of BP and BP 7,8-dihydrodiol to the BP 7,8-diol-9,10-epoxide products that bind to RNA and DNA has previously been examined in vitro with cultured mammalian cells (*13*) and with bovine bronchial explants (*14*). For the incubation of BP with bovine bronchial explants, evidence was presented indicating that only (+)-**2** was bound to RNA (*14*, *15*). Although the results reported here establish that this same stereoisomer produces the major RNA

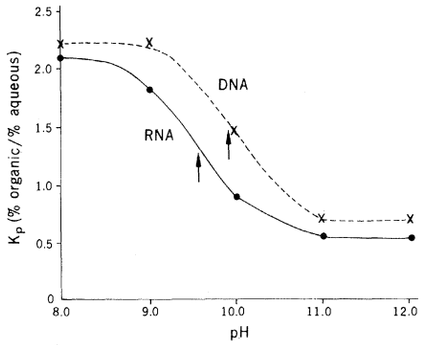

Fig. 3. Change in partition coefficient plotted against *p*H for RNA and DNA nucleoside adducts formed from BP. Portions of tritiated adducts (~ 750 and 1800 dpm per *p*H point for RNA and DNA nucleoside adducts, respectively) were partitioned between equal volumes (0.5 ml) of 20 percent 1-butanol in ethyl acetate and 0.05*M* buffers. The relative amount of radioactive adducts was determined by counting both layers [K_p = counts (cpm) in organic layer/counts (cpm) in aqueous layer] (*12*).

products in vivo in mouse skin treated with BP, an adduct with (+)-**1** is also observed. Nucleoside products from both **1** and **2** were also observed in the analysis of the DNA isolated from cultured cells treated with BP 7,8-dihydrodiol, although the stereochemistry of addition in the products bound to DNA remains unknown (*13*).

As shown above, in both RNA and DNA of mouse skin treated with BP, diol epoxide **2** forms the majority of the covalently bound products. To our knowledge, this study provides the first evidence that diol epoxide **1** adducts are also formed in vivo in a tissue susceptible to carcinogenesis by BP. Furthermore, since (+)-**1** is formed from (+)-BP 7,8-dihydrodiol and (+)-**2** is formed from (−)-BP 7,8-dihydrodiol (*16*), the monooxygenase in mouse skin which produces these 9,10-epoxides (9*R*, 10*R*) is highly stereoselective. This remarkable stereoselectivity parallels that observed for the formation in vitro of **1** and **2** from the optically active BP 7,8-dihydrodiols by rat liver microsomes (*5*). For example, Baird and Diamond (*17*) reported that the ratio of DNA-bound **1** to **2** in cultured hamster embryo cells is strongly dependent on the time at which DNA is analyzed. At early time points **1** adducts predominate. Such an effect may also be important in vivo. Conclusions as to the relative importance of the diastereomeric BP 7,8-diol-9,10-epoxides in the mutagenicity and carcinogenicity of BP cannot be based solely on quantitative measurements of the amounts of these diol epoxides bound to nucleic acid. More complex questions regarding the relative rate of repair and the function of diol epoxide–damaged DNA must also be considered.

Table 1. Percentage of tetraols related to diol epoxides **1** and **2** obtained by acid hydrolysis of the adducts formed from BP in mouse skin. All values are within 1.0 percent. Identification of the tetraols was further confirmed by converting them to their tetraacetyl derivatives and comparing their mobilities in silica gel thin-layer chromatography (Analtech, 2 percent methanol in CH_2Cl_2). More than 80 percent of the radioactivity applied to the plate coeluted with authentic tetraacetate ultraviolet markers. No epimerization between the two sets of tetraols was observed under these acid hydrolysis conditions.

Modified cellular fraction	Tetraols	
	From **1**	From **2**
Protein*	39	61
RNA†	21	79
DNA†	12	88

*Crude protein fraction was treated with 0.5*N* HCl at 85°C for 1.5 hours. †The RNA and DNA fractions after hydrolysis to nucleosides and purification by Poragel PN were hydrolyzed by acid (85°C, 0.5*N* HCl, 0.75 hour).

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References and Notes

1. A. Borgen, H. Darvey, N. Castagnoli, T. T. Crocker, R. E. Rasmussen, I. Y. Wang, *J. Med. Chem.* **16**, 502 (1973); P. Sims, P. L. Grover, A. Swaisland, K. Pal, A. Hewer, *Nature (London)* **252**, 326 (1974); P. Daudel *et al.*, *FEBS Lett.* **57**, 250 (1975).
2. W. Levin, A. W. Wood, H. Yagi, P. Dansette, D. M. Jerina, A. H. Conney, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 243 (1976); W. Levin, A. W. Wood, H. Yagi, D. M. Jerina, A. H. Conney, *ibid.*, p.3867; W. Levin, A. W. Wood, P. G. Wislocki, J. Kapitulunik, H. Yagi, D. M. Jerina, A. H. Conney, *Cancer Res.* **37**, 3356 (1957); W. Levin, A. W. Wood, R. L. Chang, T. J. Slaga, H. Yagi, D. M. Jerina, A. H. Conney, *ibid.*, p. 2721.
3. J. Kapitulunik, W. Levin, A. H. Conney, H. Yagi, D. M. Jerina, *Nature (London)* **266**, 378 (1977).
4. D. R. Thakker, H. Yagi, A. Y. H. Lu, W. Levin, A. H. Conney, D. M. Jerina, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3381 (1976); S. K. Yang, D. W. McCourt, P. P. Roller, H. V. Gelboin, *ibid.*, p. 2594; S. K. Yang, D. W. McCourt, J. C. Leutz, H. V. Gelboin, *Science* **196**, 1199 (1977).
5. D. R. Thakker, H. Yagi, H. Akagi, M. Koreeda, A. Y. H. Lu, W. Levin, A. H. Conney, D. M. Jerina, *Chem. Biol. Interact.* **16**, 281 (1977); D. R. Thakker, H. Yagi, W. Levin, A. Y. H. Lu, A. H. Conney, D. M. Jerina, *J. Biol. Chem.* **252**, 6328 (1977).
6. G. T. Bowden, B. G. Shapas, R. K. Boutwell, *Chem. Biol. Interact.* **8**, 379 (1974).

7. C. C. Irving and R. A. Veazey, *Biochim. Biophys. Acta* **166**, 246 (1968).
8. W. M. Baird, A. Dipple, P. L. Grover, P. Sims, P. Brookes, *Cancer Res.* **33**, 2386 (1973).
9. M. Koreeda, P. D. Moore, H. Yagi, H. J. C. Yeh, D. M. Jerina, *J. Am. Chem. Soc.* **98**, 6720 (1976); P. D. Moore, M. Koreeda, P. G. Wislocki, W. Levin, A. H. Conney, H. Yagi, D. M. Jerina, in *Drug Metabolism Concepts*, D. M. Jerina, Ed. (American Chemical Society, Washington, D.C., 1977), p. 127.
10. A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, I. B. Weinstein, F. A. Beland, R. G. Harvey, H. Kasai, I. Miura, K. Nakanishi, *J. Am. Chem. Soc.* **98**, 5714 (1976).
11. H. Yagi, O. Hernandez, D. M. Jerina, *ibid.* **97**, 6881 (1975); H. Yagi, D. R. Thakker, O. Hernandez, M. Koreeda, D. M. Jerina, *ibid.* **99**, 1604 (1977).
12. P. D. Moore and M. Koreeda, *Biochem. Biophys. Res. Commun.* **73**, 459 (1976).
13. H. W. S. King, M. H. Thompson, E. M. Tarmy, F. A. Beland, R. G. Harvey, P. Brookes, *Chem. Biol. Interact.* **13**, 343 (1976); H. W. S. King, M. R. Osborne, F. A. Beland, R. G. Harvey, P. Brookes, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2679 (1976).
14. I. B. Weinstein, A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, R. G. Harvey, C. Harris, H. Autrup, H. Kasai, K. Nakanishi, *Science* **193**, 592 (1976).
15. K. Nakanishi, H. Kasai, H. Cho, R. G. Harvey, A. M. Jeffrey, K. W. Jennette, I. B. Weinstein, *J. Am. Chem. Soc.* **99**, 260 (1977).
16. H. Yagi, H. Akagi, D. R. Thakker, H. D. Mah, M. Koreeda, D. M. Jerina, *ibid.*, p. 2358.
17. W. M. Baird and L. Diamond, *Biochem. Biophys. Res. Commun.* **77**, 162 (1977).
18. This research was supported in part by National Cancer Institute grant CA18580 (to M.K.).

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Chronologic and Physiologic Age Affect Replicative Life-Span of Fibroblasts from Diabetic, Prediabetic, and Normal Donors

Abstract. *Cultured skin fibroblasts from subjects with clinically apparent diabetes mellitus and from subjects genetically predisposed to diabetes have a replicative life-span that is inversely related to donor age. Fibroblasts from carefully defined normal subjects not predisposed to diabetes fail to show this correlation. The data support the idea that physiologic status of the tissue donor is a more precise determinant of fibroblast replicative lifespan than chronologic age.*

The cultured human fibroblast has a finite replicative lifespan (*1–3*) which is inversely proportional to the age of the donor (*3–7*). Moreover, this negative correlation appears to hold true whether the tissue of origin is lung (*3*), liver (*6*), or skin of various anatomical sites (*4, 5, 7*). Most tissue donors in such studies have, as a rule, been randomly chosen from living subjects and from subjects at postmortem, many of them with overt pathology (*1–3, 5–7*). But other donors, affected by specific inherited disorders of premature and severe aging, give rise to fibroblast strains with significantly decreased replicative lifespans in comparison to age-matched controls (*5, 8*). At several laboratories it has now been demonstrated that diabetes mellitus, a common genetically determined disorder that reduces life expectancy (*9*), also has an adverse, although more subtle, influence on fibroblast growth capacity (*4, 5, 10*). The present results indicate that both clinically apparent diabetics and subjects genetically predisposed to diabetes show the inverse correlation between donor age and replicative lifespan of cultured fibroblasts, whereas carefully selected normal individuals fail to show this phenomenon.

Three groups of subjects, 25 normal controls, 26 diabetics, and 21 "prediabetics" (both parents of each prediabetic had maturity-onset diabetes) volunteered skin biopsies for this study. Fibroblast strains were developed from 4-mm punch biopsies of anterior forearm skin and grown in a humidifed atmosphere (95 percent air, 5 percent CO_2 at 37.0 ± 0.3°C) (*4*). The normal subjects were in excellent health, had a negative family history for diabetes, and showed normal glucose tolerance after repeated testing (*11*). Diabetic subjects were predominantly free of clinical complications and were not affected by other specific genetic syndromes that are frequently associated with diabetes (*5, 8*). Of the 26 diabetics, 24 were on daily insulin therapy, while the other two were regulated on oral hypoglycemic agents. The prediabetics, although they show no clinical evidence of diabetes, presumably carry an increased risk of developing diabetes, although it is now believed that these subjects may only have a 10 to 20 percent risk of developing clinically apparent diabetes by the age of 45 (*11*).

Samples were assigned code numbers at the time of biopsy and handled in random order to avoid systematic bias. All tissue culture operations were then carried out by one of us (E.J.M.) without knowledge of the specific or group identities of each donor. Cells were harvested from explants and subcultured at a 1 : 8 ratio as soon as they attained confluence, three mean population doublings (MPD) being counted each time (*2–7*). The number of cells that became attached to the petri dish after each subculture was not routinely determined. But in random tests on all strains, plating efficiency 6 hours after subculture ranged from 80 to 100 percent at early passage and 50 to 80 percent at late passage; no significant differences were found between the cells of normal subjects, prediabetics, or diabetics at these two passage levels. Thus, while the cumulative number of MPD is clearly an underestimate in all cases (*2–7*), values in the three groups can still be compared with validity.

The onset of senescence in cultures was marked by a slowing of growth so that longer periods were required between subculture. Cultures not confluent by day 7 were given a complete change of medium then and each week thereafter until they became confluent, whereupon they were subcultured. If a cell strain was unable to grow to confluence after 28 days with three changes of medium (on days 7, 14, and 21), it was declared dead and the cumulative number of MPD at the last confluence was desig-

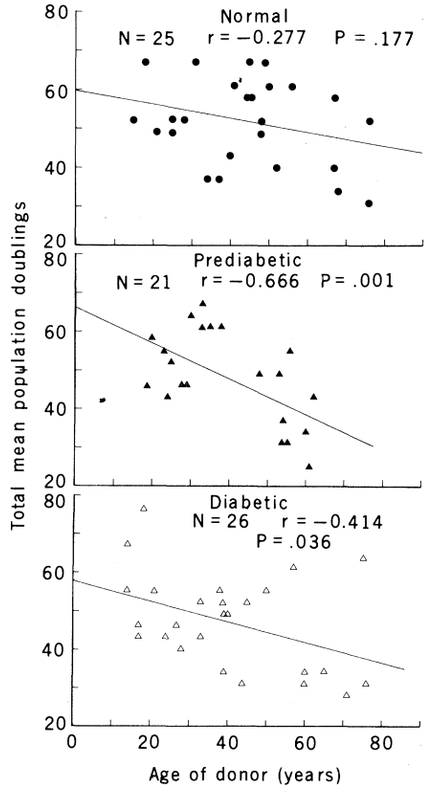

Fig. 1. Correlation between donor age and replicative life-span of cultured fibroblasts (total mean population doublings) in normal, prediabetic, and diabetic individuals. The linear regression equations are: for normals, $y = 59.24 - 0.25\ x$; prediabetics, $y = 66.57 - 0.46\ x$; and diabetics, $y = 57.80 - 0.27\ x$. The mean age (± S.D.) of diabetic patients was 40.15 ± 19.25 (range 14 to 76) years; prediabetics, 40.00 ± 15.06 (15 to 62) years; and normals, 44.28 ± 17.50 (15 to 76) years. The mean weight in the three groups according to Metropolitan Life Insurance tables was between 100 to 108 percent of ideal body weight.

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