References and Notes

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5. The Garden of Eden vent is located at 0°48.3′N, 86°13.4′W; the Rose Garden vent is located at 0°48.9′N, 86°13.3′W; and the Mussel Bed vent is located at 0°48.18′N, 86°04.1′W.
6. To establish the accuracy of Hem-O-Scan usage with hemocyanins, we compared data from *Callianassa californiensis* collected with the Hem-O-Scan to previously published results obtained with a different method [K. Miller and K. E. Van Holde, *Biochemistry* **13**, 1669 (1974)]. Our data showed a P_{50} of 8 mmHg at 16°C, *p*H 8.0, $PCO_2 = 0$, and a P_{50} of 14 mmHg at 25°C, *p*H 8.0, $PCO_2 = 0$; $\Delta H = -10.6$ kcal/mole. The Miller and Van Holde data showed a P_{50} of 7 mmHg at 16°C, *p*H 7.8, $PCO_2 = 0$, and a P_{50} of 12 mmHg at 25°C, *p*H 7.8, $PCO_2 = 0$; $\Delta H = -10.2$ kcal/mole. The value of $\Delta \log P_{50}/\Delta p\text{H}$ from the Hem-O-Scan data was calculated to be −1.06, compared to −1.22 with the Miller and Van Holde method. Our instrument is modified to prevent excessive oxygen leakage in the sample compartment, insulated to prevent condensation at lower temperatures, and equipped with an air pump and control valve for slow oxygen introduction. Dehydration of the sample is prevented by the use of a double Teflon membrane (0.006 mm thick) instead of the copolymer membrane recommended by Aminco for sample containment. In adapting the Hem-O-Scan for use with blood containing hemocyanin, we used interference filters that transmit at 380 and 420 nm. For a more complete description of the Hem-O-Scan see D. A. Powers, H. J. Fyhn, U. E. H. Fyhn, J. P. Martin, R. L. Garlick, S. C. Wood, *Comp. Biochem. Physiol.* **62A**, 67 (1979); G. Lapennas, J. M. Colacino, J. Bonaventura, *Methods Enzymol.*, in press.
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14. This research was carried out on the NSF-funded vessels D.S.R.V. *Alvin*, R.V. *Lulu*, and R.V. *New Horizon*. It was supported by NSF grants OCE78-08852 and OCE78-08933 (to J.J.C.) and OCE78-10458 (to J. F. Grassle). This work was made possible by the physical and intellectual efforts of many people, including the captains and crews of the vessels named above. In particular, we thank J. F. Grassle for serving as chief scientist and R. Ballard for help in locating the vents. We also thank R. Hollis, G. Ellis, K. Smith, A. Arsenault, and S. Witherow for help and encouragement. Special appreciation to S. Horvath of the Institute of Environmental Stress, University of California, Santa Barbara, for the mixing and analysis of the gases, to G. Lapennas, J. Bonaventura, and to C. Bonaventura for advice on the Hem-O-Scan, C. Mangum, P. Adams, A. De Bevoise, and D. Gluck for critical review of the manuscript. We thank T. J. Mickel for maintaining animals on shipboard, advice on the research, and careful review of the manuscript. This report is contribution No. 11 of the Galápagos Rift Biology Expedition.

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Dietary Restriction Retards the Age-Associated Loss of Rat Striatal Dopaminergic Receptors

Abstract. *In male Wistar rats subjected to dietary restriction by alternate days of feeding and fasting the normal age-associated loss of striatal dopamine receptors in the brain was substantially retarded. The mean survival time of the rats on the restricted diet was increased by approximately 40 percent compared to control rats given free access to food. Dopamine receptor concentrations in striata of 24-month-old rats that had been on a restricted diet since weaning were 50 percent higher than those of control animals of the same age, and essentially comparable to 3- to 6-month-old control rats.*

An impaired ability to respond and adapt to various stimuli is associated with increasing chronological age (*1*). Such stimuli include drugs, hormones, neurotransmitters, and various physical and chemical agents. The mechanisms responsible for alterations in response to these stimuli include changes in receptors, cell membranes, nuclei and chromatin, cyclic nucleotide metabolism, and various cellular enzyme systems (*2*). Although there have been many attempts to retard the aging process and its associated deterioration, the only confirmed method of life-span extension in mammals to date involves dietary manipulation (*3–5*). It has been suggested that such intervention may act by modifying disease patterns and concomitant destruction of physiological systems (*3–5*).

Table 1. Effect of age and diet on rat striatal dopamine receptor concentrations and affinities. Concentrations (B_{max}) and affinities (dissociation constant, K_D) were determined from individual Scatchard plots, and values are the means ± standard errors for the numbers of experiments indicated. One to three rats were used for each analysis; the number of rats refers to the total number used in the experiments.

Age (months)	Number of		B_{max} (fmole/mg protein)	K_D (n*M*)
	Experiments	Rats		
		Control rats		
3 to 6	8	18	212 ± 12*	13.1 ± 2.4
12	9	20	160 ± 4*	10.3 ± 1.6
24	7	17	132 ± 8*	12.2 ± 3.1
		Rats maintained on restricted diet		
24	7	14	197 ± 8†	15.0 ± 2.4

*Significantly different from other control groups; $P < .01$ (unpaired *t*-test). †Significantly different from 12- and 24-month-old control groups; $P < .01$.

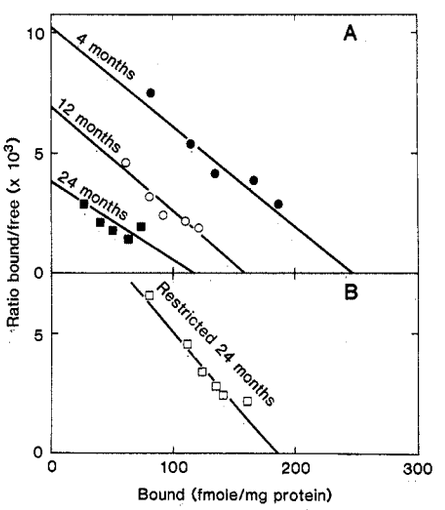

Fig. 1. Scatchard analyses of the binding of ^{3}H-labeled amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) to rat striatal membranes. Rat striatal dopamine receptors were measured by the specific binding of 3.5 n*M* to 17 n*M* ^{3}H-labeled ADTN (New England Nuclear, specific radioactivity 35 Ci/mmole) according to the method of Creese and Snyder (*16*), with unlabeled 10^{-5}*M* (+)-butaclamol being used as competitor. Protein concentrations were determined by the method of Lowry *et al.* (*17*) with bovine serum albumin (Pentex) being used as a standard. The animals used were Wistar rats from the colony of the Gerontology Research Center, NIA, Baltimore. The control rats were given free access to NIH or Laboratory Chow; the rats on the restricted diet had access to the same food but only on alternate days from weaning. Body weights were 250 to 500 g, 550 to 650 g, 500 to 600 g, and 350 to 400 g for the 3- to 6-month-old controls, 12-month-old controls, 24-month-old controls, and 24-month-old experimental rats on the restricted diet, respectively. Data were analyzed by the method of Scatchard (*14*) with the use of linear regression analysis for determining slopes of intercepts. (A) Typical Scatchard analysis of control animals aged 4, 12, and 24 months. (B) Typical Scatchard analysis of 24-month-old rats maintained on the restricted diet. Striata from three rats were pooled for each individual analysis.

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However, the actual mechanism of dietary life-span extension is not understood, and only a few investigators have examined the relation between dietary manipulation and the functional manifestations of senescence (*6–8*). Since degeneration and reduced responsiveness of the striatal dopaminergic system is one of the best documented functional impairments of the aging mammalian brain (*9–13*), we have examined the effects of dietary restriction on the normal age-related loss of dopamine receptors from the rat corpus striatum.

In Fig. 1A the Scatchard analysis (*14*) of ^{3}H-labeled amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene binding to crude striatal membrane preparations shows that there is a progressive decrease in dopamine receptor concentration (abscissa intercept) but not affinity (dissociation constant, K_D, is equal to the negative reciprocal of the slope) as the rats age from 3 to 24 months. Table 1 indicates that this reduction is statistically significant ($P < .01$) and amounts to about 40 percent over the adult life-span.

When male rats are subjected to a restricted diet by being given access to food only every other day, the mean life-span of the Wistar strain used in these experiments is increased from 99 to 138 weeks (*15*). Figure 1B shows that the concentrations of striatal dopamine receptors in the brains of 24-month-old animals maintained on restricted diets from weaning are substantially higher than those of 24-month-old control rats given free access to food everyday. Table 1 contains data from a number of separate analyses of data from rats on restricted diets, and confirms that receptor concentrations in these animals are indeed significantly different from 24-month-old controls ($P < .001$) and essentially comparable to those of 3- to 6-month-old control rats. Binding affinities are equivalent in all groups.

It therefore appears that dietary restriction, in the form of alternate days of feeding and fasting, substantially retards the loss of striatal dopamine receptors that is responsible for altered dopaminergic control of physiological and behavioral functions in senescent rats (*9–12*). The preservation of receptor levels typical of young adults late into the life-span is in concert with the approximately 40 percent increase in mean survival time effected by the present dietary manipulation (*15*). Although prolonged retention of striatal dopamine receptors probably represents a consequence rather than a cause of dietarily increased rat life-span, such findings suggest exciting possibilities, especially if they are applicable to man.

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Differentiation of Respiratory and Abortigenic Isolates of Equine Herpesvirus 1 by Restriction Endonucleases

Abstract. *Viruses classified by immunologic criteria as equine herpesvirus 1 cause respiratory disease and abortion in horses. Restriction endonuclease analyses of the DNA's of viruses from animals with respiratory disease and from aborted fetuses show that the patterns for respiratory viruses, while similar to each other, are entirely different from the patterns for fetal viruses. It is therefore proposed that the DNA restriction endonuclease patterns of fetal and respiratory viruses analyzed in this study be designated as prototypic of equine herpesvirus 1 and 4, respectively.*

Three distinct herpesviruses designated as equine herpesviruses 1, 2, and 3 (EHV1, EHV2, and EHV3) have been isolated from horses (*1*). The viruses classified by their immunologic specificity as EHV1 are the major cause of acute viral upper respiratory disease and of abortion in horses. It has been stated from time to time that viruses isolated from respiratory infections (R) differ from viruses isolated from aborted fetuses (F) with respect to antigenicity, host range in vitro, plaque size, growth rate in vitro and in vivo, and epidemiology (*2–5*). A central question, therefore, was whether the R and F isolates could be differentiated with respect to nucleotide sequence of their DNA's, as reflected by the distribution of restriction endonuclease cleavage sites.

In addition, because of the serious economic losses caused by EHV1, particularly from epizootic abortion (abortion "storms"), several attempts have been made to control such losses by vaccination; one of the three EHV1 viruses licensed for vaccine use was associated with neurologic disease in 486 of 60,000 recipients of the vaccine and was subsequently withdrawn. We showed earlier that no two epidemiologically unrelated herpes simplex viruses are identical with respect to the number and distribution of the restriction endonuclease cleavage sites in their DNA's and that these patterns can be used as fingerprints for tracing the spread of herpes simplex viruses in the population (*6–8*). It was of interest, therefore, to determine whether the viruses classified as EHV1 varied, and whether the DNA fingerprinting technique could be used to differentiate between vaccine and wild-type viruses.

We now report that the R and F viruses are clearly different. Furthermore, although the number of isolates was small, no two epidemiologically unrelated viruses within each of the groups appeared to have identical DNA fragment patterns.

The origin and designation of the EHV1, EHV2, and EHV3 viruses are listed in Table 1. The viruses were grown in equine kidney cell cultures (passages 5 to 7), in equine dermal (EDerm) cell line (*9*), or in Vero cells. Viral DNA was extracted from the cytoplasm or from whole infected cells. The procedures for

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