water-soluble compounds like sucrose and erythritol have permeability coefficients equal to 10^{-8} to 10^{-7} cm sec^{-1} (*6, 9*).

Low but significant cerebrovascular permeabilities explain why opioid peptide uptake by brain might not be measurable by the brain uptake index method (in which brain uptake is measured within 15 seconds after injection), or why plasma concentrations must be maintained for minutes if observable central effects are to be produced (*2, 4, 7*). Figure 1 illustrates the prediction by Eq. 3, as based on data in Table 1, that α-[D-Ala^2,^{14}C-Homoarg^9]endorphin will fill half of the extracellular space of the caudate nucleus in 5.9 minutes, after a step rise in plasma concentration of unbound peptide. In contrast, the brain concentration does not rise above 0.1 percent of the initial plasma concentration of unbound peptide if the peptide is injected as a bolus. Whereas the half-time for brain uptake is equal to 0.693 *V/PA* (Eq. 3), the peptides in Table 1 would enter a 30 percent extracellular brain space with half-times of 3 to 11 minutes.

We have shown that four synthetic analogs of natural opioid peptides have a moderate cerebrovascular permeability that is sufficient to produce significant brain uptake within 3 to 11 minutes, after a step rise in plasma concentration. There may be little uptake after a bolus injection if the peptide disappears rapidly from plasma, or if binding to plasma protein is marked. The findings are consistent with observations that some peptides exert central effects in conscious animals when administered systemically (*3, 7*). A significant cerebrovascular permeability suggests, furthermore, that feedback may operate between circulating peptides that have potential central effects, and brain sites that regulate their release into the circulation (*1*).

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10. With the exception of methionine[^{3}H-Tyr, D-Ala^2]enkephalin amide (New England Nuclear), each peptide was guanidinated and labeled with ^{14}C as follows: peptide (0.5 to 1.2 mg) was added to 200 μl of 0.2*M* *O*-[^{14}C]methylisourea (58 mCi/mmole), adjusted to *p*H 10.5 with NaOH. After storage for 5 days, the reaction mixtures were neutralized with 10 μl of 4*M* ammonium acetate buffer, *p*H 7. The ^{14}C-labeled guanidinated peptides were purified by passage through Biogel-P-2 columns (1 by 17 cm) in 50 percent acetic acid (α- and β-endorphin analogs), or by adsorption to XAD-2 beads, exhaustive washing with water, and elution with 90 percent aqueous methanol (analog of β-lipotropin 61-69). They were then taken to dryness and dissolved in saline for use. Amino acid analysis of the modified peptides showed that 50 percent of the lysine residues had been converted to ^{14}C-homoarginine by the procedure. *O*-[^{14}C]methylisourea was prepared from ^{14}C-labeled barium cyanamid (DHOM Products Ltd., North Hollywood, Calif.) by treatment with methanolic HCl at room temperature for 3 days, with subsequent filtration and removal of solvent. α-[D-Ala^2]-endorphin was supplied by Dr. Nicholas Ling and β-[D-Ala^2]endorphin by Dr. C. H. Li. β-[D-Ala^2]lipotropin 61-69 was prepared from α-[D-Ala^2]endorphin by treatment with trypsin (1/200) at *p*H 8 for 16 hours at 37°C, followed by XAD-2 chromotography as described.
11. Abbreviations: Ala, alanine; Gly, glycine; Homoarg, homoargenine; Met, methionine; Tyr, tyrosine.
12. Data for α-[D-Ala^2,^{14}C-Homoarg]endorphin were also analyzed by a three-constant model, where k_1 and k_2 (per second) represent inward and outward rate constants at the blood-brain barrier, and k_3 represents the rate constant for binding to brain tissue for free peptide. The mean value of k_3 is equal to 1.03×10^{-3} sec^{-1}; L. Sokoloff *et al.*, *J. Neurochem.* **28**, 897 (1977).
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Gene Affecting Superoxide Dismutase Activity Linked to the Histocompatibility Complex in H-2 Congenic Mice

Abstract. *The activity of cyanide-sensitive, Cu-Zn superoxide dismutase (SOD) was studied in liver cytosols from H-2 congenic strains of mice. Higher SOD activity was found in livers of mice having H-2^b/A.BY, B10, and C_3H.SW/ haplotypes than in those of H-2^a, H-2^k and H-2^d haplotypes. Segregation studies supported these correlations. In H-2 recombinant strains of mice, the genes influencing the liver SOD activity occur, as ascertained by mapping techniques, at or near the H-2D region of the major histocompatibility complex.*

Superoxide dismutase (SOD) (E.C. 1.15.1.1) appears to be detectable in all aerobic cells (*1*). This enzyme catalyzes the dismutation of the superoxide anion, O_2^-, in which one electron is transferred to form molecular oxygen, in the sense of the following equation;

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

The work of Fridovich (*2*) suggests that the most important function of SOD is to protect aerobic cells from the oxidoreduction effect of the toxic O_2^- formed during aerobic cell metabolism. Atmospheric oxygen is capable of inducing the synthesis of SOD; molecular oxygen may be toxic to cells displaying an anaerobic metabolism and, in high concentration, to aerobic cells also (*3*).

On the basis of studies on the structure of SOD, Richardson *et al.* (*4*) drew attention to the similarity between the three-dimensional protein structures of the immunoglobulin domain and Cu-Zn SOD subunits.

They proposed that the similarity in the tertiary structures of the two molecules of different functions is indicative of their evolutionary relatedness. Furthermore, the structures of immunoglobulins are similar to those of the major histocompatibility antigens (H-2) in mice (*5*) and it has been proposed that both molecules are the products of genes that were differentiated from a common, primor-

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dial "primitive H-2" gene complex in the course of evolution (*6*).

On the basis of this assumption of an evolutionary connection, and the existence of such antigens on all somatic cells, we have sought a correlation between the allele series and the activity of SOD or a correlation in individual mice of different backcross generations from parental strains with high or low SOD activity. Approximate mapping of the genes responsible for differences in SOD activity was carried out on different H-2 recombinant strains of mice.

All experimental mice were produced in the Animal House of the Institute of Genetics (*7*), and originated from breeding nuclei of inbred strains furnished by P. Ivanyi of the Institute of Experimental Biology and Genetics, Prague.

The lines were maintained by sister-brother mating, and the H-2 phenotype of backcross progenies was checked by hemagglutination tests, with polyvinylpyrrolidone K-60 and 0.1 percent human serum albumin, according to Capkova and Demant (*8*). All animals were male mice kept under standardized conditions, and were 3 to 4 months old at the time of experiment. Twenty hours before the animals were killed, feeding was discontinued but drinking water was not withdrawn.

Although all aerobic cells contain SOD and display H-2 antigens, liver analyses are given here, since they offer high values for SOD, and differences in this organ satisfactorily represent comparisons made for several tissues of the different mouse strains.

The activity of SOD was measured via its interference in the adrenaline to adrenochrome conversion (*9*). The fresh liver was homogenized in a French press and diluted with distilled water (1:100). The slurry was clarified by centrifugation (30 minutes, 13,000*g*). The supernatant was treated with ethanol and chloroform (0.25 and 0.15 by volume, respectively) to exclude the interfering mitochondrial SOD. Then, K_2HPO_4 (300 g/liter) was added and to portions of the ethanol-rich phase two volumes of acetone were added. After centrifugation, the precipitate was lyophilized, and then diluted in 0.5*M* phosphate buffer (*p*H 7.0); the cytosol Cu-Zn SOD activity was then determined by the method of Veisiger and Fridovich (*10*) with slight modification. Protein was determined (*11*), with bovine serum albumin as the standard.

The activity of SOD per milligram of protein was higher in the liver of mice with H-2^b, than those with H-2^a, H-2^k, and H-2^d haplotypes on B10.A and C_3H background (Figs. 1 and 2). These differences were significant ($P < .001$). The activity of SOD of F_1 hybrids resulting from a cross of a high ($C_3H.SW$) and low (C_3H/Di) parental strain was intermediate, being significantly lower ($P < .04$) and slightly higher ($P < .08$) than that of the two parental strains, respectively. Similarly, intermediate SOD activity was detected in kb heterozygotes of the backcrosses and significantly higher values were observed in backcross with bb than with kk phenotypes (Fig. 2).

Since significantly higher SOD activity was found in B10.A/2R/ and B10.A/4R/ than in B10.A/5R/ mice ($P < .01$ and $P < .001$), it can be inferred that genes mapped at or linked to the H-2D region might be responsible for the differences in the SOD activity detected among the H-2 recombinant strains on B10 background.

There are differences in the SOD activity of strains C_3H/Di, B10.BR, and CBA, as well as B10.A and A/Ph, all with the same H-2^k or H-2^a phenotypes, respectively, differing only in their genes other than H-2 (background), but these differences are of low significance ($P < .50$ and $P < .28$) (Figs. 1 and 2). Our results suggest that the liver SOD activity exhibits a well-defined correlation with the H-2 haplotypes of different H-2 congenic strains and a strong association in the individual mice of their backcross progenies.

Although the "regulatory" nature of these genes has only been suggested by our experiments, their approximate linkage at, or near to, the H-2D region on chromosome 17 (linkage group IX) of mice seems clearly indicated. It should be noted that the "structural" genes of SOD-1 and SOD-2 are linked to human chromosomes 21 and 6, respectively, as revealed by somatic cell hybridization (*12–14*).

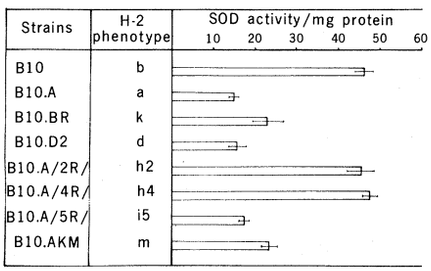

Fig. 1. Association of high and low SOD activity (in liver) with different H-2 phenotypes in H-2 congenic strains and their backcrosses. The length of columns indicates the arithmetic mean of specific SOD activity of at least ten mice of each H-2 congenic strain including 30 of C_3H/Di, 21 of $C_3H.SW$, 29 of /Di × SW/F_1, and 10 to 21 of the different backcrosses. The bars indicate the standard deviation. Six samples from the same liver were used for parallel determinations of specific SOD activity.

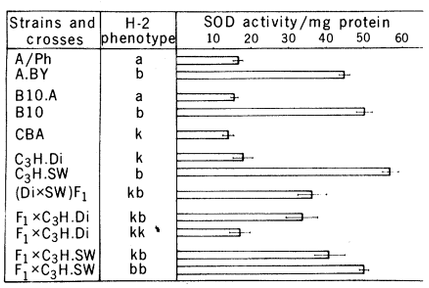

Fig. 2. Liver SOD activity in different H-2 recombinant strains of mice on B10 background. The length of columns indicates the arithmetic mean of ten mice of each strain and the bars the standard deviation. Six samples from the same liver were used for parallel determinations.

Although the "regulatory" nature of these genes has only been suggested by our experiments, their approximate linkage at, or near to, the H-2D region on chromosome 17 (linkage group IX) of mice seems clearly indicated. It should be noted that the "structural" genes of SOD-1 and SOD-2 are linked to human chromosomes 21 and 6, respectively, as revealed by somatic cell hybridization (*12–14*).

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