the second generation. No differences were found between the two groups in weight of the pups at birth (1.5 g) or at 5 days (3.6 g), or in the number of pups per litter (eight to ten).

The fluoride concentrations in the ash of the humeri of mice of the low fluoride group at the conclusion of the experimental period averaged 0.010 percent in the first generation and 0.009 percent in the second generation. The corresponding values for the high fluoride group were 0.78 and 0.77 percent.

The ability of added fluoride to restore normal fertility to mice previously rendered subfertile on a low fluoride intake was also investigated. Weanling female mice were maintained on the low fluoride diet plus deionized water and mated at 8 weeks of age, as before. After 5 or 15 weeks of exposure to males, female mice, of demonstrated impaired fertility (Table 2), were divided into two groups. Half of the animals at each breeding time period were transferred to an intake of 50 ppm of fluoride in the water, while the remaining animals were retained on the low fluoride intake. After 7 days separation from males (*4*), the mice were remated and litter production was assessed over 20 weeks.

Mice retained on the low fluoride intake continued to show impaired reproduction. Only 40 to 50 percent produced four litters during the 20-week recovery period (Table 2). By contrast, mice transferred to the high fluoride intake showed an improvement in litter production. In five instances all animals produced two or three litters and approximately 85 to 90 percent of these animals produced four litters in the 20-week period after transfer to the high fluoride intake. These last values are not significantly less than 100 percent, demonstrating that fertility was restored in this group.

The infertility resulting from a restricted intake of fluoride and the delay in production of the first litter in the second generation of low fluoride mice point clearly to a deficiency state with respect to fluoride. While complete infertility of all mice on the low fluoride intake was not demonstrated, it is possible that all mice in this group would have become infertile if the intake of fluoride could have been more severely restricted.

The failure of previous studies (*1*) to demonstrate a role of fluoride in reproduction may be attributed to the small numbers of animals involved and the short duration of the studies, since in this work the infertility developed slowly in each generation. The delayed production of the first litter in the second generation of the low fluoride group may have been a result of an irregular estrous cycle or the onset of sexual maturity may have been retarded. A similar finding in manganese deficiency has been ascribed to a delayed onset of sexual maturity (*5*).

The basis of the infertility in mice receiving a low fluoride intake is not known. Infertility is a relatively common manifestation of deficiency in trace elements, including deficiencies of copper, zinc, manganese, iodine, and selenium (*6*). Thus it may represent, at least in part, a nonspecific response to the stress of a nutritional deficiency.

This study demonstrates that fluorine satisfies the major criteria for an essential trace element: (i) A deficiency state, characterized by a delayed production of the first litter and a progressive infertility, has been produced in mice on a diet low in fluoride. (ii) The deficiency is prevented and cured by addition of fluoride alone to the diet. (iii) The deficiency correlates well with low tissue (bone) levels of fluoride. Thus, there is good evidence that fluorine is an essential element, at least in the diet of the mouse.

Note added in proof: After this report was sent to the printer we received a copy of a publication by Schwarz and Milne (*7*) in which it was found that addition of 2.5 to 7.5 ppm of fluoride to highly purified amino acid diets (basal fluoride contents, 0.04 to 0.46 ppm) significantly enhanced the growth rates of young mice given these diets and improved the pigmentation of their incisor teeth. The amounts of added fluoride are within the range of the fluoride contents of human diets.

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Coordinated Development of β-Glucuronidase and β-Galactosidase in Mouse Organs

Abstract. *Changing concentrations of β-glucuronidase and β-galactosidase are coordinated during the development of mouse liver, heart, and brain. Although coordinate, the developmental patterns for the two enzymes are under independent control by genetic elements apparently linked to the respective structural genes.*

Mutations affecting developmental changes in enzyme concentration are known for mouse β-glucuronidase (*1*) and corn esterase (*2*). We now report that in mice the developmental pattern for β-glucuronidase is shared by β-galactosidase, and that an analogous developmental mutant of β-galactosidase exists.

β-Glucuronidase and β-galactosidase are easily detectable in liver, brain, and heart of mice of the DBA/2J and C57BL/KsJ strains as early as the 14th day of gestation. In each organ there is a distinctive pattern of changes in enzyme activities until stable adult levels are reached at 40 to 50 days of age (Fig. 1). Parallel development of the two enzymes in liver and heart is apparent. There is some difference in early development of the two enzymes in brain, but after 10 days of age the patterns are quite similar. β-Glucuronidase and β-galactosidase thus appear to share a common developmental program in mice of these strains.

The β-glucuronidase present in liver, brain, and heart of adult mice is coded

for by a single structural gene (*1*); both strains studied here are homozygous for the *G* allele at that locus. Comparisons of thermolability, substrate affinity, and electrophoretic mobility indicate that a single species of enzyme also accounts for the measured β-galactosidase activity of these tissues (*3*). When the electrophoretic mobilities of β-galactosidase and β-glucuronidase from liver, brain, and heart of mice 2 and 50 days old were compared (*4*), we found no evidence of a "fetal" isozyme of either enzyme. The enzyme patterns for each tissue were identical at 2 and 50 days. Apparently, a single structural gene is responsible for the synthesis of each enzyme throughout this developmental period. The coordinate development of the two enzymes thus reflects a coordinated developmental control of two structural genes.

The coordinated expression of these genes differs from coordinate regulation within a prokaryotic operon in two important respects. First, the structural genes for β-glucuronidase and β-galactosidase appear to be unlinked in the mouse (*5*). Second, the levels of β-glucuronidase and β-galactosidase are under independent hormonal control. The β-glucuronidase of mouse kidney is induced by testosterone (*6*). However, we have failed to detect any increase in kidney β-galactosidase activity during the course of a tenfold induction of β-glucuronidase in C57BL/6J mice.

Table 1. β-Glucuronidase and β-galactosidase activities of adult mouse organs. Organs of four male animals 50 days of age were pooled and assayed as described in Fig. 1. Activity is expressed as nanomoles of product produced per hour per milligram of protein.

Strain	β-Glucuronidase	β-Galactosidase	Ratio
	Liver		
DBA/2J	410	67	6.1
C57BL/KsJ	430	80	5.4
	Heart		
DBA/2J	30	18	1.7
C57BL/KsJ	31	16	1.9
	Brain		
DBA/2J	34	52	0.65
C57BL/KsJ	36	54	0.67

Although a nearly constant ratio of β-glucuronidase to β-galactosidase is maintained during the development of each organ, the ratio of activities in different organs ranges from 0.65 in brain to 6.1 in liver (Table 1). This difference in relative enzyme activities could be produced by additional tissue-specific regulation of enzyme synthesis or by tissue-specific enzyme degradation systems. Tissue specificity in rates of enzyme degradation has previously been observed; a mutation that alters the rate of catalase degradation in mouse liver does not affect the rate of catalase degradation in kidney (*7*). In considering mechanisms for coordinate regulation of gene expression in higher organisms, it will be necessary to compare rates of enzyme synthesis and degradation in addition to the final enzyme activities.

Coordinated development of β-glucuronidase and β-galactosidase was not observed in rat liver (*8*); to our knowledge these two enzymes have not been studied previously in developing brain and heart. The development of these enzymes is coordinate in rat kidney (*8*) and rat intestine (*9*). The development of β-glucuronidase in rat liver resembles our results for mouse liver, although the β-galactosidase pattern differs. In light of the genetic observations we now report, the independent development observed in rat liver may reflect the genetic constitution of the particular strain studied, rather than a species difference.

The present observations are significant because they have been made in a system amenable to genetic as well as to biochemical analysis. A hereditary alteration of the β-glucuronidase developmental pattern has been described in C3H mice (*1*). The genetic factor responsible for the altered developmental pattern proved to be linked to the structural gene for β-glucuronidase (*1*). We have found that β-galactosidase development is not affected similarly in C3H mice. However, the developmental pattern of β-galactosidase is altered in livers of C57BL/6J mice. In this case also, the genetic factor responsible for the altered developmental pattern appears to be linked to the structural gene for this enzyme (*5*).

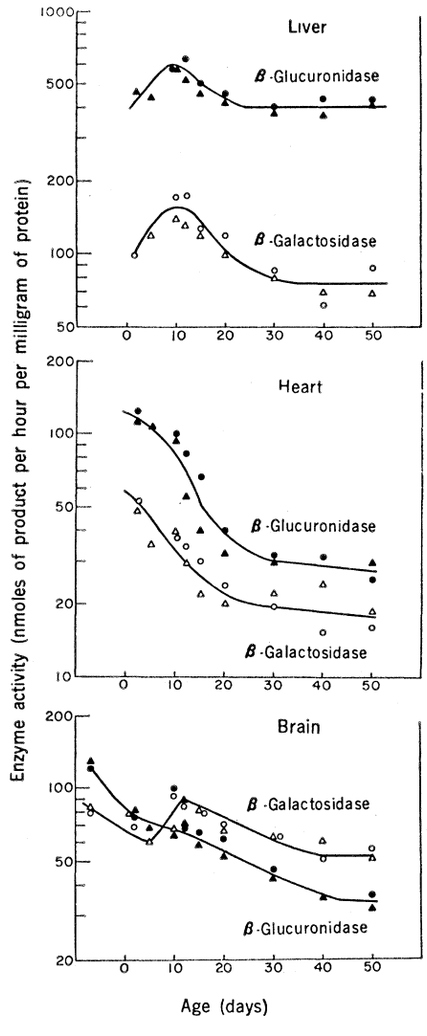

Fig. 1. Development of β-glucuronidase and β-galactosidase in mouse liver, heart, and brain. Mice of various ages were obtained from the production stocks of the Jackson Laboratory, Bar Harbor, Maine. For fetal samples, organs from an entire litter were pooled for each time point; for postnatal samples, organs from four male animals were pooled (*11*). Tissues were homogenized in five to ten volumes of a solution consisting of 0.25*M* sucrose and 0.05*M* tris(hydroxymethyl)aminomethane (tris) at *p*H 7.5 with a Polytron homogenizer (Kinematica GMBH Lucerne) for 30 minutes. β-Galactosidase was assayed for 30 to 60 minutes at 37°C in a mixture containing 0.08*M* sodium citrate buffer at *p*H 3.5, 5m*M* *p*-nitrophenyl-β-D-galactoside (Pierce), and 0.05 to 0.15 ml of tissue homogenate in a final volume of 1.0 ml. β-Glucuronidase was assayed for 30 to 60 minutes at 56°C in a mixture containing 0.14 percent Triton X-100, 0.14*M* acetate buffer at *p*H 4.6, 2 m*M* *p*-nitrophenyl-β-D-glucuronide (Pierce), and 0.05 to 0.15 ml of tissue homogenate in a final volume of 0.5 ml. After incubation the tubes were chilled, 0.2 ml of 30 percent trichloroacetic acid was added (0.5 ml of water was also added to the β-glucuronidase assays), and precipitated protein was removed by centrifugation. The supernatants were made alkaline with 0.2 ml of 5*M* 2-amino-2-methyl-1,3-propanediol (Aldrich), and the absorption of the *p*-nitrophenol released was measured at 415 nm. Enzyme activity is expressed as nanomoles of product formed per hour per milligram of protein (*12*), with the value 14,000 used for the molar extinction coefficient of *p*-nitrophenol. For both reactions product formation was proportional to enzyme concentration and time of incubation; circles, strain DBA/2J; triangles, strain C57BL/KsJ.

Further analysis of such variants should clarify the role of temporal genes (*10*) in determining developmental processes.

The sharing of a common developmental program by two apparently unlinked structural genes implies the existence of additional genetic factors determining the program itself. For this reason it will be of interest to identify mutations simultaneously affecting the development of these two enzymes.

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4. Electrophoretic studies were performed with samples of 105,000*g* supernatant fractions prepared from the tissue homogenates described in Fig. 1. Electrophoresis was carried out at *p*H 8.1 at 300 volts (1 ma per tube) for 60 minutes at 0°C. Gels containing 7.5 percent polyacrylamide were prepared according to the method of J. T. Clarke [*Ann. N.Y. Acad. Sci.* **121**, 428 (1964)], except that the gel buffer contained 29 g of glycine and 12 g of tris per liter. β-Galactosidase activity was visualized with the substrate 4-methylumbelliferyl-β-D-galactoside [L. Fluharty, E. L. Lassila, M. T. Porter, H. Kihara, *Biochem. Med.* **5**, 158 (1971)], and β-glucuronidase activity with the method of Hayashi [M. Hayashi, Y. Nakajima, W. H. Fishman, *J. Histochem. Cytochem.* **12**, 293 (1964)].
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11. The reproducibility of enzyme assays for individual animals was determined for animals 5 days of age and for adults. At both ages, the standard deviation of determinations of β-glucuronidase and β-galactosidase was 8 to 13 percent for liver and heart and 3 percent for brain. Since the variance was quite reproducible and agreed with variance determinations from other experiments, pooled samples were used for the remaining time points. The estimated standard errors of our measurements are ± 5 percent for liver and heart and ± 1.5 percent for brain.
12. Protein was determined by the biuret method [E. Layne, *Methods Enzymol.* **3**, 447 (1957)], with bovine serum albumin as standard.
13. We are indebted to the staff of the Jackson Laboratory for helping to gather and analyze the samples. This work was supported in part by research grants GM 18484 from National Institutes of Health and NP-29B from American Cancer Society.

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Mobility Gaps: A Mechanism for Band Gaps in Melanins

Abstract. *The semiconductor behavior of melanins is reviewed and compared with quantum mechanical models of conduction in amorphous solids. The available data are consistent with extensions of Mott's basic model for amorphous semiconductors, whereas they are inconsistent with crystalline semiconductor models. An investigation of the specific conduction mechanisms operative in melanins in terms of the amorphous model should reveal important aspects of the band structure.*

Melanins are good electron acceptors and have semiconductor properties (*1*), which appear to be important in the midbrain structures (*2*). In relation to these electronic properties, Cotzias *et al.* (*2*) noticed that both naturally occurring and drug-induced dyskinesia occur in species which possess visible melanin in the substantia nigra. Such data suggest that melanins have a more fundamental biological role than that of providing pigmentation or an ultraviolet sunscreen (*3*). For this reason a model of the electronic structure of melanins is of more than academic interest.

An analysis of data on melanins and melanin-containing systems suggests that the electronic properties of melanins can best be explained in terms of a band model for semiconduction in amorphous materials. The electronic properties of melanins have been of considerable experimental interest. Blois (*4*), in addition to measuring other solid-state properties, investigated the possibility that melanins are intrinsic semiconductors. Blois (*5*) has pointed out that inconsistencies exist between the data on pure melanins and interpretations based on crystalline models of semiconductor behavior. Interpretation has, therefore, been ambiguous, even for the most elementary system, purified melanin. A quantum mechanical calculation based on the monomer unit indole-5,6-quinone was presented by Pullman and Pullman (*1*) in an effort to calculate the band structure of melanins.

It is necessary to differentiate between the melanin macromolecule and more complex systems, such as melanin-containing organelles. However, in some systems of greater complexity the electronic properties of melanin may dominate the behavior of the system in a particular situation. Pant and Rosenberg (*6*) found that the photoconductivity through a Fe^{3+}-completed oxidized cholesterol bimolecular membrane reaches a peak as the electron donor concentration is increased, which indicates a band-filling mechanism. The interaction of certain electron-donating drugs, such as chlorpromazine, with melanin has been shown to follow a similar band-filling pattern (*7*). The discussion in this report is restricted to the macromolecular form of melanin unless otherwise noted.

The basis for most current research in the quantum mechanical description of amorphous materials was presented by Mott (*8*), in terms of the exact solution of the one-dimensional random square well model for the single-particle wave functions. Mott used perturbation theory to show that the one-dimensional results should extend to three dimensions.

The solution of the random square well model yields a set of energy levels similar to those of crystals, but with some interesting differences (*9*). For example, in amorphous materials there is an essentially Gaussian density of states (*8*, *10*). The states under the peak are extended, whereas the states under the tails are localized. An extended state is one in which the electron has "essentially" the same probability of being found anywhere in the crystal. Conversely, in a localized state the electron is essentially restricted to a local volume. The existence of tails of localized states is peculiar to the band model of amorphous materials; in crystals the band edges are more sharply defined (*8*). The mobility of electrons in localized states is less than that in extended states, since electrons in localized states must depend on tunneling or phonon-assisted hopping to change their states. Extensions of Mott's model have appeared in the last 2 years (*9*, *11*). The result for amorphous semiconductors is a model of conductivity based on mobility of electrons in localized states rather than on a gap in the density of states, which is characteristic of crystalline solids. The conductivity changes as the highest occupied level is moved through regions of extended and localized states by the action of doping.

Some of the inconsistencies between the melanin data and theories of conduction based on crystalline semiconductors are removed when the amorphous band model is used. The existence