incubated in phloroglucinol–hydrochloric acid (*7*).

After demineralization of this lignite a coherent tissue residue is obtained which retains much of the structural pattern of the original wood. Included in the preserved organic wall substance are substantial amounts of cellulose and lignin or a lignin-like material.

This lignitized wood thus consists of a mineralized phase in which much of the original cell-wall substance is retained in a highly organized state. The mineralization of this specimen may have slowed or arrested the degrading forces active upon the organic substance of the cell wall at an early stage in the deposition of the original plant material. By contrast, in a ground thin section of *Taxodium* also of Tertiary age, there was considerably more degradation of the secondary cell wall (*1*). It appears that the preservation of the organic substance of the cell wall in mineralized woods may depend upon the time at which mineralization begins relative to the degradative changes which occur during the initial stages in the development of a sediment.

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L-Tyrosine-3,5-^{3}H Assay for Tyrosinase Development in Skin of Newborn Hamsters

Abstract. *Tyrosinase in hamster skin, measured by the production of ^{3}HOH from* L*-tyrosine-3,5-^{3}H, increases about sevenfold in specific activity from the 14-day fetus to day 6 of life. Albino hamsters exhibit no activity, but enzyme is present in the ventral (white) areas of the Syrian hamster. 3,4-Dihydroxy-*L*-phenylalanine is required as a cosubstrate.*

The low levels of tyrosinase in skin have been difficult to measure in small samples because there has been no sensitive assay. The enzyme was detected in mouse skin and in fetal guinea pig skin by manometry (*1*) and in the skin of mice, goldfish, reptiles, frogs, and rats (*2*, *3*) by measurement of the incorporation of L-tyrosine-^{14}C into melanin, by the method of Kim and Tchen (*2*). This latter method, although more sensitive than manometry, is inconvenient because it requires incubations of 16 to 24 hours. It may also lead to errors because the reaction rate is probably not constant for that period, and because melanin, the product measured, is far removed from the substrate and is itself of variable composition. The melanin assay has been used by Coleman (*2*) and by Chen and Chavin (*3*) to follow development of tyrosinase in skin of young pigmented mice and rats, respectively.

A sensitive assay (*4*), which measures ^{3}HOH released from L-tyrosine-3,5-^{3}H as a result of the tyrosine hydroxylase activity of tyrosinase, was introduced to study the enzyme in melanoma. Hall and Okazaki (*5*) used this method to demonstrate tyrosinase in the feather tracts of male weaver birds, and more recently it was applied to the assay of tyrosinase in adult rat skin (*6*). The method requires only a 1-hour incubation, during which the reaction rate is constant. It is used to investigate skin tyrosinase changes occurring during late fetal and newborn life in hamsters.

Randomly bred Syrian golden hamsters (Lakeview Hamster Colony, Newfield, New Jersey) have a gestation period of 16 days. In some instances dated pregnant hamsters were killed by ether anesthesia and the fetuses removed on days 14, 15, and 16 of gestation. For newborn animals the day of littering was designated as day 1 of life. Animals were decapitated and, after removal of the tail and limbs at the first joint, the body skin was peeled off and scraped to remove visible fat. It was then frozen for later assay or used immediately. The skin was blotted, weighed, minced, and then homogenized in a threefold volume of 0.02*M* sodium pyrophosphate buffer, *p*H 7.4, in the micro attachment of the VirTis homogenizer. For animals of day 15 of gestation to 4 days of life, two or three skins were pooled for each homogenization, while single skins of older animals were used. Skin from 14-day fetuses was very fragile, and it was not possible to remove it in one piece or to obtain a wet weight. Consequently, skin from 14-day fetuses in several litters was pooled in groups, and each was homogenized. The DNA contents of homogenates were measured (*7*) with calf thymus DNA as standard and the enzyme activity was expressed as micro-

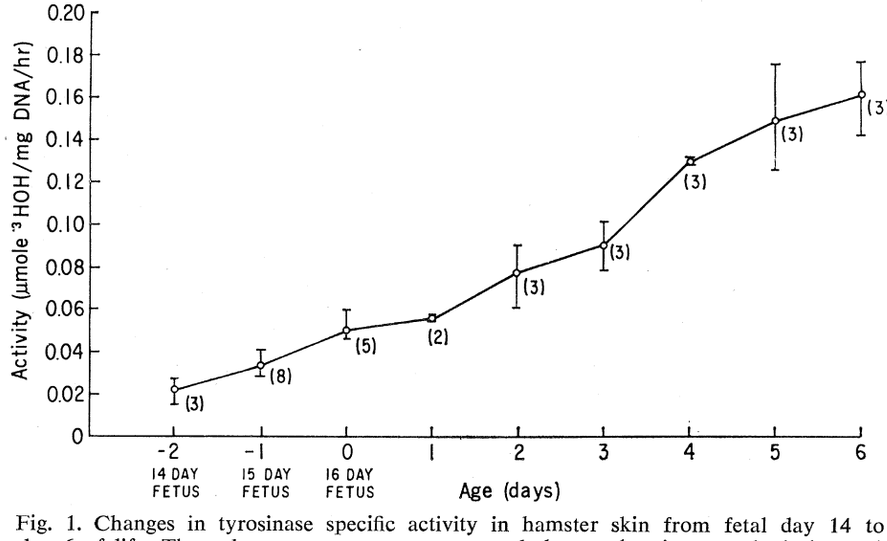

Fig. 1. Changes in tyrosinase specific activity in hamster skin from fetal day 14 to day 6 of life. The values are means ± range, and the number in parenthesis by each value is the number of homogenates for that age.

Table 1. 3,4-Dihydroxy-L-phenylalanine (dopa) requirement and time course of tyrosinase reaction in skin of 5- to 6-day-old hamsters. Each value is the mean of two homogenates and has been corrected for a control, lacking the enzyme, equivalent to 0.015 μmole of ^{3}HOH.

Incubation time (min)	L-Dopa (μmole)	Activity*
60	0	0.018
60	0.05	0.643
60	0.10	0.840
60	0.15	1.00
60	0.20	0.676
30	0.15	0.52
90	0.15	1.69
120	0.15	2.18

* Micromoles of ^{3}HOH per hour per gram of tissue (fresh weight).

Table 2. Comparison of tyrosinase in dorsal and ventral areas of the skin. The areas were separated on the basis of hair pigmentation and homogenized separately.

Age of animal (days)	Activity*	
	Ventral	Dorsal
3	0.106,0.136	0.640,0.660
4	0.138,0.112	0.971,0.770
7	0.144	1.00

* Micromoles of ^{3}HOH per hour per gram of tissue (fresh weight).

moles of ^{3}HOH per hour per milligram of DNA or per gram (wet weight) of skin.

The standard assay mixture contained L-tyrosine-3,5-^{3}H (5 to 7 × 10^6 disintegrations per minute; 1 μmole); 3,4-dihydroxy-L-phenylalanine (L-dopa) (0.15 μmole); sodium phosphate buffer, *p*H 6.8 (25 μmole); and homogenate (0.50 ml) in a total volume of 1.25 ml. The reactions were stopped, ^{3}HOH was isolated, and the radioactivity was counted as described (*8*).

The activity is strictly dependent upon dopa, as with melanoma tyrosinase (*8*), and is proportional to time (Table 1). A similar dependence on dopa has been reported for rat skin tyrosinase (*3, 6*) but apparently not for mouse skin tyrosinase (*2*). The reaction is also proportional to the concentration of enzyme. In a large-scale experiment, dopa-^{3}H was isolated and identified as a product of the reaction by electrophoresis at *p*H 1.9 and chromatography on Dowex 2 (borate) (*9*). Skins from male and female animals aged 1, 2, 4, 6, and 10 days were compared, and no significant sex differences were apparent. The K_m of tyrosine for skin tyrosinase is $2 \times 10^{-4} M$, about the same value found for melanoma tyrosinase (*8*).

Overall there is a seven- to eightfold increase in tyrosinase specific activity and about a threefold increase from day 1 to day 6, with a leveling off at days 5 to 6 (Fig. 1). If the enzyme values are computed on the basis of wet or dry weight of skin, curves of similar shape are obtained. Although not shown in the figure, the specific activity remains close to the maximum until about day 16. By day 22 it falls to the level found for day 1 or 2 and then remains constant at least until day 32. Coleman (*2*) observed a leveling off of increase in specific activity on days 4 to 6 for brown and black mice, but found no activity by day 30. Black rats (*3*) exhibit maximum activity at 5 to 13 days of age and continued low activity at 10 percent of the maximum even on day 69.

The results shown so far are for whole body skin. Although ventral skin (white) clearly has enzyme, it is only about 15 percent that of dorsal skin (pigmented) (Table 2). Experiments with mixtures of dorsal and ventral skins showed that there was no inhibitor in ventral skin.

In contrast to the results for white ventral skin, skin from albino hamsters at 1, 2, 3, or 4 days of age (*10*) had no detectable enzymatic activity, even after a 3-hour incubation, and exhibited no inhibition of tyrosinase from Syrian golden hamster skin. I estimate that activity of 4-day-old albino skin is < 0.02 μmole/hr per gram of wet skin, compared to about 0.6 to 7 μmole/hr per gram of skin from a 4-day-old Syrian golden hamster. Gaudin and Fellman (*6*), on the other hand, report activity in adult albino rat skin at about 10 percent of that in adult hooded rats.

Although the increase in specific activity is only threefold over the first 6 days of life, the total tyrosinase activity per animal body skin increases from about 0.03 μmole/hr to about 0.6 μmole/hr, a 20-fold increase in total enzyme content during this period. An estimate of activity of 0.8 to 1.0 μmole/hr per gram of wet skin for 5- to 6-day-old hamsters may be compared to an activity of 10 to 20 μmole/hr per gram of hamster melanoma (*8*). The maximum specific activity for young black rats by the melanin assay can be calculated from the data of Chen and Chavin (*3*) to be 0.07 μmole/hr per gram of wet skin, about 10 percent of the maximum activity for hamster skin. The difference may be due to both method and species differences. Gaudin and Fellman (*6*) report a maximum activity of only 0.5 to 0.7 nanomole/hr per gram of wet skin for adult hooded rats, with an assay similar to the one used here. However, these workers employed only 0.05 μmole of tyrosine in the incubation, which was considerably lower than saturating amounts.

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10. Adult albino hamsters of this type (also obtained from Lakeview Hamster Colony) have pink eyes, but a small amount of pigment on the tips of the ears.
11. Supported by grant CA-07093 from the National Institutes of Health. I thank L. Chuang for technical assistance.

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Glucose-6-Phosphate Dehydrogenase Deficient Red Cells: Resistance to Infection by Malarial Parasites

Abstract. *Erythrocyte mosaicism occurs in females heterozygous for glucose-6-phosphate dehydrogenase deficiency. In blood from female children with acute* Plasmodium falciparum *malaria the parasite rate was 2 to 80 times higher in normal than in deficient erythrocytes. This may be the mechanism whereby the gene for glucose-6-phosphate dehydrogenase deficiency confers selective advantage against malaria to heterozygous females, and thus may have attained the polymorphic frequency occurring in populations living in areas with endemic malaria.*

Balanced polymorphism is a theoretically well-characterized situation in Mendelian populations (*1, 2*). However, there are very few examples, in which the mechanism has been clarified whereby a heterozygote is at ad-