Experimental Epilepsy in Rabbits: Failure to Detect a Difference in Phenylalanine Metabolism in Convulsant Rabbits.* (30503)

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In the search for the biochemical abnormality which might be associated with the susceptibility to audiogenic seizures in strains of rabbits previously described(1,2), the conversion of phenylalanine to tyrosine by these rabbits was studied. This investigation was carried out because Coleman had reported greatly reduced *in vivo* production of tyrosine in 2 dilute strains of mice susceptible to audiogenic seizures(3).

Materials and methods. All animals came from intensely inbred lines of seizure-susceptible and seizure-resistant rabbits. The L-phenylalanine was obtained from Mann Research Laboratories. In preliminary studies phenylalanine determinations were made by Bio-Science Laboratories; the later phenylalanine and tyrosine determinations were made at Children's Memorial Hospital(4). On the basis of the initial studies, it was hypothesized that phenylalanine metabolism in the rabbit related to coat pigmentation, but not to seizure-susceptibility.

To test this hypothesis 6 nonpigmented and 6 pigmented convulsant rabbits, aged 50-66 days, their period of greatest seizure susceptibility, were matched for age, sex, coat color, and number of times tested with rabbits from seizure-resistant lines. The seizure-susceptible rabbits had each convulsed 85% to 100% of the time in 10 or more tests. All animals were fasted overnight, but were allowed water. Blood samples were taken from the central ear artery immediately prior to, and at 15, 30, 45, 60 and 90 minute intervals following intraperitoneal injection of 100 mg L-phenylalanine/kg body weight.

Results. The phenylalanine and corresponding tyrosine values were pooled for each of the 4 groups, *i.e.,* the 6 nonpigmented and 6 pigmented convulsant rabbits and their respective controls. Two graphs were plotted: the values for the 12 seizure-susceptible *vs* the 12 seizure-resistant rabbits, and the values for the 12 nonpigmented, albino rabbits *vs* the 12 pigmented ones. The results indicate that the phenylalanine hydroxylase system is functioning in each group of rabbits, since tyrosine levels rise in all after phenylalanine loading. No statistically significant difference is demonstrated between the seizure-susceptible and the seizure-resistant animals. However, it is evident that phenylalanine is converted to tyrosine more slowly in the albino rabbits than their pigmented controls. At 30 minutes the phenylalanine blood levels were significantly different ($t = 2.89$, $p = <.01$) as were the tyrosine values ($t = 2.55$, $p = <.025$) in the 2 groups.

Discussion. In studying the metabolism of phenylalanine in 2 dilute strains of mice, the DBA/1J and DBA/2J strains, and in nondilute strains, Coleman(3) observed the enzyme, phenylalanine hydroxylase, to have only 50% of normal activity in mice homozygous for the dilute allele (d) and 14% in those homozygous for the dilute (d^c) allele. He showed that the decreased tyrosine synthesis in the dilute strains was paralleled by the production of an abnormal metabolite of phenylalanine, identified as phenylacetic acid. Coleman also discussed the possible relationship of the constant production of this compound *in vivo* to the audiogenic seizures in the DBA strain and the spontaneous seizures in the dilute lethal strain by pointing out that phenylacetic acid is a potent inhibitor of such enzymes as dihydroxyphenylalanine decarboxylase, 5-hydroxytryptophan decarboxylase and glutamic acid decarboxylase, all of which produce amines, especially serotonin and GABA, considered important in normal cerebral metabolism.

The present investigation showed that, as might be expected, a delay in the disappear-

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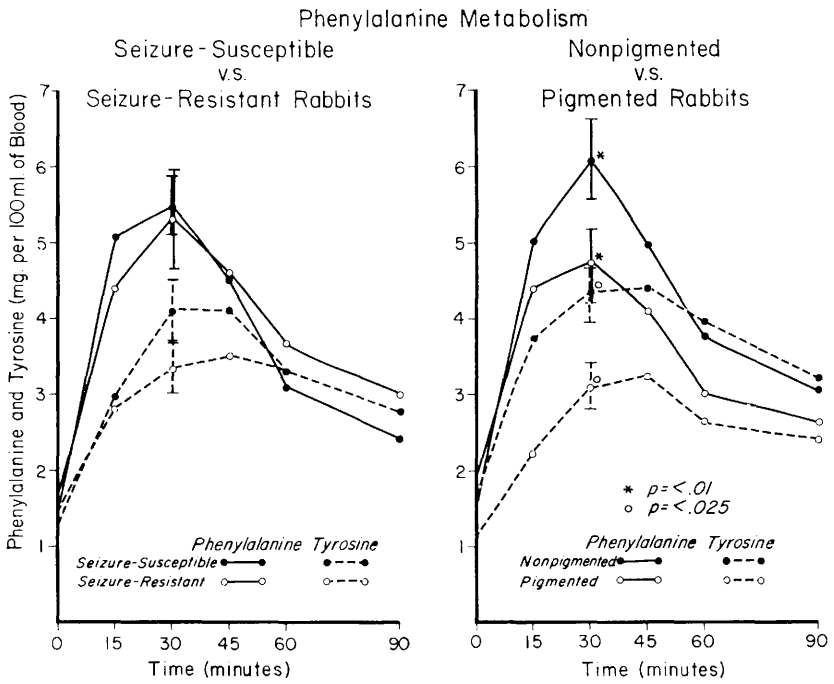

FIG. 1. Phenylalanine loading test in 12 seizure-susceptible and 12 seizure-resistant rabbits, 6 pigmented and 6 nonpigmented animals in each group. Animals were injected intraperitoneally at zero time with 100 mg L-phenylalanine/1 kg body weight. Values represent the averages in each group of 12 rabbits.

ance of phenylalanine from the bloodstream could be related to the degree of coat pigmentation since phenylalanine is a precursor of melanin. However, the metabolism of phenylalanine in these rabbits appeared to be unrelated to their susceptibility to audiogenic seizures.

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Lowered Delayed Skin Reactivity to Fractions of *Mycobacterium bovis* (BCG) in Guinea Pigs Sensitized with Large Doses of BCG. (30504)

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Guinea pigs sensitized with varying doses of heat-killed *Mycobacterium bovis* (BCG) were studied for delayed skin reactivity, complement fixing, and precipitating antibody activity towards 2 fractions of culture filtrates of BCG. A different amount of BCG was needed to induce optimum sensitization toward each fraction. Lesser reactivity to both fractions was observed when the sensitizing dose was greater than optimum. The circu-