hybridization (*18*), the BLV [^{125}I]RNA hybridized only with the DNA's from the tissues of the leukemic cows and from infected cell culture lines. We conclude from these results that the BLV genome is not endogenous to the bovine cellular genome.

These results are consistent with the acquisition of the BLV genome by leukemic cattle as a result of the horizontal transmission of the virus from some other species. The BLV [^{3}H]DNA probe was therefore used to examine the cellular DNA of other species for the presence of BLV-related sequences. Some of these species live side by side with cattle and might thus be the source of BLV. None of the species tested contain nucleic acid sequences related to the BLV genome (Table 1). We conclude that the BLV genome is not highly related to any of the endogenous type C viral genomes of the species tested. Similar results have been obtained by Kettman *et al.* (*6*), and are consistent with immunological studies showing a lack of relationship of the major BLV structural protein to that of other mammalian type C viruses (*7*, *19*).

In summary, we have shown by nucleic acid hybridization that the BLV genome is not endogenous to the cellular genome of normal cattle. The etiologic agent causing lymphosarcomas or leukemia in this species is an infectious virus derived from another as yet unknown species. This is, therefore, one species where eradication of the vectors for virus spread should lead to prevention of the disease.

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α-Methylphenylalanine, a New Inducer of Chronic Hyperphenylalaninemia in Suckling Rats

Abstract. *α-Methylphenylalanine reduces the phenylalanine hydroxylase activity of rat liver by 75 percent. Daily injections of this substance (plus phenylalanine) into rats from the 3rd to 15th day of age had no obvious toxic effects, and maintained a plasma concentration of phenylalanine comparable to that of phenylketonuric subjects.*

The first report of an agent, *p*-chlorophenylalanine, which could effectively diminish the rate of clearance of ingested phenylalanine, appeared in 1966 (*1*). Since then rats treated with *p*-chlorophenylalanine provided the most promising animal models (*2*) for phenylketonuria, the condition of mental deficiency associated with the absence (from genetic causes) of phenylalanine hydroxylase (*3*). A general problem in imitating the consequences of this absence of an enzyme in experimental animals is that the agents used to inhibit that enzyme are not completely specific. *p*-Chlorophenylalanine, for example, decreases the activity of tryptophan hydroxylase in the brain (*4*) as well as that of the phenylalanine hydroxylase in the livers of rats, and thus one might question whether the various cerebral abnormalities observed were caused by the hyperphenylalaninemia itself. The new inducer of hyperphenylalaninemia described here, α-methylphenylalanine, has a different chemical structure, and its side effects, if any, are unlikely to be identical to those of *p*-chlorophenylalanine. The study of rats treated with α-methylphenylalanine is thus important because abnormalities in common with those in rats treated with *p*-chlorophenylalanine are the ones most likely to be specifically associated with hyperphenylalaninemia, the common metabolic effect of the two agents.

The hepatic phenylalanine hydroxylase activity was determined (*5*) in an assay system supplemented with substrate, pteridine cofactor, and dithiothreitol. The activity of liver extracts was not inhibited by the addition of 0.8 to 8 m*M* α-methylphenylalanine in vitro (nor did the extracts catalyze the conversion of this analog to α-methyltyrosine). However, the phenylalanine hydroxylase activity in the livers of adult or immature rats injected with α-methylphenylalanine decreased. Since the purpose was to maintain hyperphenylalaninemia throughout early postnatal life (in the rat the first 15 days), we determined the response to various doses of α-methylphenylalanine on 6-day-old rats—an age when the phenylalanine hydroxylase content is about 60 percent of that in adult liver (*5*); maximal inhibition occurred with 24 μmole/10 g (Fig. 1). Additional experiments indicated that at least 20 hours were required to attain this minimal level. The content of cerebral serotonin (254 and 205 ng/g) and that of 5-hydroxyindoleacetic acid (303 and 324 ng/g) in two of these rats were not different from those in two control rats (249 and 243, and 318 and 342, respectively) (*6*). [In contrast, the

other known inhibitor of phenylalanine hydroxylase, *p*-chlorophenylalanine, decreases the cerebral concentration of serotonin (*4*), presumably because of its inhibition of tryptophan hydroxylase activity (*4*).] Nor did the injection of α-methylphenylalanine alter appreciably the concentrations of tyrosine or of ten amino acids in the plasma, which were determined in the automatic amino acid analyzer (the ten amino acids were threonine, serine, glutamine, proline, glutamic acid, glycine, alanine, valine, isoleucine, and leucine).

The slow clearance of phenylalanine in rats treated with *p*-chlorophenylalanine was evidenced by the increase (10 to 20 times the normal) of phenylalanine in plasma, which prevailed for as long as 2.5 hours after an injection of phenylalanine (*1*). Since our purpose was to test the suitability of α-methylphenylalanine for inducing prolonged hyperphenylalaninemia, we assayed the plasma as late as 12 hours after a "loading" dose of phenylalanine. At this time, as shown in Table 1 (lines 1 to 4), the phenylalanine concentration was still more than ten times elevated; injections of either phenylalanine or α-methylphenylalanine alone had no significant effect. The concentrations of phenylalanine were somewhat higher in the 2-day than in the 1-day experiment. More important, this treatment remained effective in the course of prolonged experiments in which α-methylphenylalanine plus phenylalanine were administered daily to the rats, from the 3rd to the 15th day of age (line 7). These measurements provide a minimal estimate of the extent of hyperphenylalaninemia since the very high concentrations of phenylalanine that prevailed each day up until the 12th hour were not determined nor do we know how long after the 12-hour time point it finally returned to normal. Twice the dose of phenylalanine administered daily with the standard amount of α-methylphenylalanine also appeared to be well tolerated during the first week of life; on day 3 of treatment the concentration of phenylalanine in the plasma (again, 12 hours after the last injection) was 33 times higher than normal (line 8).

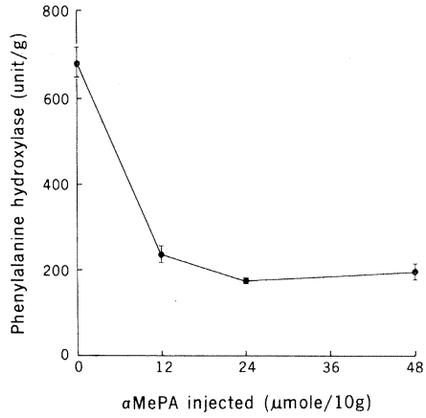

Fig. 1. Phenylalanine hydroxylase activity of rat liver after injection of α-methylphenylalanine. The indicated amounts of α-methylphenylalanine (αMePA), dissolved in 0.1 to 0.2 ml of water, were injected subcutaneously into 6-day-old rats 24 hours before assay. The activities, in units per gram of liver (wet weight), are means (the bars indicate ± standard deviation) of results with three to four animals. A unit is 1 nmole of tyrosine formed per minute.

In rats that have received prolonged treatment with α-methylphenylalanine and phenylalanine (Table 1, line 7), the levels of five enzymes in liver—tyrosine aminotransferase, soluble and mitochondrial aspartate aminotransferase, β-hydroxybutyrate dehydrogenase, and malate NADP (nicotinamide adenine dinucleotide phosphate) dehydrogenase—and seven enzymes in the cerebral hemispheres—glutamate decarboxylase, glutamate dehydrogenase, β-hydroxybutyrate dehydrogenase, malate NADP dehydrogenase, succinate dehydrogenase, and soluble and mitochondrial aspartate aminotransferase—were indistinguishable from those in control rats of the same age. Of the 24 experimental rats, none died or showed any obvious symptoms of toxicity; their body weights (20.1 ± 2.3 g; $N = 12$) were similar to those of the control rats (19.1 ± 1.5 g; $N = 24$). Prolonged treatment with *p*-chlorophenylalanine, on the other hand, was reported to interfere with the growth of developing rats (*9*).

The concentrations of phenylalanine in the plasma of untreated phenylketonuric subjects are 15 to 25 times above normal, and it is during early childhood that this metabolic abnormality is thought to interfere with cerebral development, leading to irreversible mental deficiency (*3*). Thus, rats treated with α-methylphenylalanine during suckling life should constitute a suitable animal model for this human disease. As shown in our study it is possible to maintain 10- to 30-fold elevations in plasma phenylalanine during the better part of each day of a crucial developmental period.

Table 1. The concentration of phenylalanine (PA) in plasma of rats treated with α-methylphenylalanine. The doses (per 10 g of body weight) of subcutaneously administered α-methylphenylalanine (αMePA) were 24 μmole and those of phenylalanine were 26 μmole, except in one case when they were 52 μmole, shown in square brackets. In the experiments of lines 2 and 4, phenylalanine was given once, alone, or after α-methylphenylalanine; simultaneous injections of phenylalanine and α-methylphenylalanine, at 24-hour intervals, began at the age of 4 days (line 5) or at the age of 3 days (lines 7 and 8). The concentration of phenylalanine in plasma was determined by the method of McCaman and Robins (*7*) as modified by Faulkner (*8*); α-methylphenylalanine does not react or interfere with this fluorometric assay. Each value is a mean of closely agreeing duplicate measurements on plasma pooled from two or three animals.

Line	Age (days)	Injections	Plasma phenylalanine (nmole/ml)*
1	6	None	100
2	6	PA	96
3	6	αMePA	97
4	6	αMePA; 12 hours later PA	1134
5	6	αMePA + PA for 2 days	1572
6	15	None	97
7	15	Daily αMePA + PA for 12 days	1053
8	6	Daily αMePA + PA [52] for 3 days	3300

*Twelve hours after the last injection.

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