References and Notes

1. J. S. Huxley, *Problems of Relative Growth* (Methuen, London, 1932).
2. S. Brody, *Bioenergetics and Growth* (Reinhold, New York, 1945).
3. W. R. Stahl, *Science* **137**, 205 (1962).
4. ———, *Advan. Biol. Med. Phys.* **9**, 356 (1963).
5. G. Crile and D. P. Quiring, *Growth* **4**, 291 (1940).
6. A. Hrdlicka, *Am. J. Phys. Anthropol.* **8**, 201 (1925).
7. M. A. Kennard and M. D. Willner, *Endocrinology* **28**, 955, 967, 977 (1941).
8. G. H. Herrmann *et al., Proceedings of the First International Symposium on the Baboon, Nov. 1963* (Southwest Foundation for Research Education, San Antonio, Texas).
9. A. Knezevic, "Autopsy findings obtained at the Oregon Regional Primate Research Center." Supplied to author by courtesy of Drs. A. Knezevic and W. McNulty.
10. M. R. Malinow, *Biology of the Howler Monkey*, in press.
11. The assistance of Dennis Lindgren, Robert W. Coffin, and L. B. Lusted of the Department of Biomathematics and Automatic Data Processing at the Oregon Regional Primate Center, is gratefully acknowledged.
12. D. Sholl, *Proc. Roy. Soc. London, Ser. B.* **135**, 243 (1948).
13. W. R. Stahl, *Physiological Modeling and Performance Analysis*, in preparation.
14. H. J. Jerison, *Science* **121**, 447 (1955).
15. ———, *ibid.* **133**, 1012 (1961).
16. H. Stephan and O. J. Andy, *Am. Zoologist* **4**, 59 (1964).
17. W. R. Stahl, *Proc. 17th Conf. on Engineering in Med. and Biol., Cleveland, Nov. 1964* **6**, 60 (1964); *Proc. 18th Conf. on Engineering in Med. and Biol., Philadelphia, Nov. 1965*, in press.
18. H. Lumer and A. H. Schultz, *Human Biol.* **13**, 283 (1941).
19. E. H. Ashton and S. Zuckerman, *Am. J. Phys. Anthropol.* **9**, 283 (1951).
20. D'A. W. Thompson, *On Growth and Form* (Cambridge Univ. Press, New York, ed. 2, 1959), vol. 1.

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Phenylketonuria: Limit in Capacity of Pre-Weanling Rats To Oxidize β-Phenyllactate and Other α-Hydroxy Acids

Abstract. *In comparison with mature animals, pre-weanling rats have markedly higher and longer lasting serum levels of β-phenyllactate following the intraperitoneal injection of a moderate dose. A corresponding age-dependent difference in the rate of oxidation of β-phenyllactate and other α-hydroxy acids was observed in experiments with homogenates and supernatant fractions of rat kidney.*

The possibility that the oxidation of some long-chain α-hydroxy acids might be significantly limited during an early postnatal stage was noted in the course of studies with phenylalanine-loaded rats (*1*). Pre-weanling rats receiving high doses of L-phenylalanine by repeated intraperitoneal injection deaminated the amino acid and reduced the resulting phenylpyruvate efficiently, but accumulated considerably more phenyllactate than more mature animals did under comparable conditions. The findings suggested the development or augmentation within a brief interval of a key system affecting the relative concentrations and, possibly, the absolute amounts of phenylpyruvate and phenyllactate in the body fluids. The large increase in the ratio of phenylpyruvate to phenyllactate in the urine which occurred between the 18th and 25th days of age, with concomitant reduction of phenyllactate concentrations in serum and brain tissue, suggested an increased capability in conversion of the hydroxy to the keto form. Since, in the case of phenyllactate, this oxidative step intervenes between a metabolic dead-end and access to a main artery of aromatic metabolism (tyrosine formation), the activity of the system would concern phenylalanine metabolism under certain conditions and should be rate-limiting in the metabolism of administered phenyllactate.

Measurements of serum phenyllactate concentrations following intraperitoneal administration of a single small dose (100 mg/kg) of the hydroxy acid to rats at varying developmental stages are shown in Fig. 1. These results clearly demonstrate the comparative inability of the infant rats to deal effectively with injected phenyllactate. For periods of more than 3 hours, relatively high concentrations of the hydroxy acid persisted in the blood of animals less than 18 days of age (30 g with this colony), whereas much lower and rapidly declining levels were observed with older animals treated similarly.

Kidney has been the tissue of choice for studies on the oxidation of α-hydroxy acids. The relative rates of L-phenyllactate oxidation by homogenates and 30,000*g* supernatant fractions of the kidneys from rats of different ages are shown in Fig. 2. At least a ninefold increase was apparent in a comparison of the activities of equivalent fresh weights of tissue from rats progressing from infancy to an adult stage (200 g). Based on the yield of phenylpyruvate per gram of tissue during the 30-minute incubation period, activity increased at an apparently linear rate from an observed low value of 1 μmole in the case of 2- to 3-day-old rats to nearly 10 μmole with fully matured animals. Kidney weight also increased linearly during the same period, but less than a twofold increase in D-amino acid oxidase activity was noted in a similar assay system with D-phenylalanine as substrate.

With the α-hydroxy derivatives of valeric, caproic, and caprylic acids and with *p*-hydroxyphenyllactate, however, age-dependent increases similar in magnitude to that encountered with phenyllactate were indicated (Table 1). A number of aliphatic α-hydroxy acids, indole lactate, indole glycollate, and α-phenyllactate inhibited the con-

Table 1. Oxidation of higher α-hydroxy acids by kidney extracts prepared from rats of differing degrees of maturity. Each value represents the activity during 30 minutes of a 30,000*g* supernatant fraction equivalent to 0.3 g (fresh weight) of tissue. The incubation mixtures consisted of 0.5 ml of buffered extract and 10 μmole of a DL-α-hydroxy acid or approximately 7.5 μmole of an α-keto acid in a total volume of 1.5 ml. Incubation conditions were the same as those described in the legend of Fig. 2. α-Keto acid controls were deproteinized at zero time. The method of Penrose and Quastel (*19*) was used to determine the concentration of α-keto acid.

Addition	α-Keto acid (μmole)			
	26 g*	73 g*	100 g*	270 g*
	Increase in α-keto acid			
DL-α-Hydroxyvalerate	0.3	3.8	4.1	5.0
DL-α-Hydroxycaproate	.6	2.9	3.0	4.6
DL-α-Hydroxycaprylate	< .3	2.0	2.1	4.1
DL-β-Phenyllactate	< .3	1.3	1.5	2.9
DL-*p*-Hydroxy-β-phenyllactate	< .3	0.7	1.0	1.7
	Decrease in α-keto acid			
α-Ketovalerate	0.1	0.2	0.2	0.2
α-Ketocaproate	.3	.6	.4	.4
Phenylpyruvate	.7	.4	.3	.7
p-Hydroxyphenylpyruvate	.7	.8	.7	.7

* Average weight of rat.

version of β-phenyllactate to phenylpyruvate in various in vitro systems or impeded the loss of serum phenyllactate in vivo. Leucine (0.03*M*) was inhibitory with homogenates and slices. Competitive inhibition of phenyllactate oxidation in the soluble extract by α-hydroxycaprylate (octanoate) and by α-hydroxy-γ-methylmercaptobutyrate was demonstrated by the method of Lineweaver and Burk (*2*).

The enzyme systems catalyzing the oxidation of higher α-hydroxy acids in animal tissues have not been the subject of extensive investigation. A number of workers (*3*) have reported on the ability of rat kidney extracts to oxidize α-hydroxy acids to the corresponding keto derivatives. Of numerous tissues of the rat assayed, Iselin and Zeller (*4*) found activity only in kidney, liver, and heart and comparatively low activity with preparations of the latter tissues. Blanchard *et al.* (*5*) selected rat kidney as the best available source for the isolation of a mammalian L-amino acid oxidase. With the resultant purified preparation a number of α-hydroxy acids, including phenyllactic acid, were found to be good substrates (*6*). While this enzyme may be of little importance to amino acid metabolism, further observations in our current investigation, not described in detail here, suggest that in the rat, at least, this flavin-linked oxidase is a prime system for oxidation of L-phenyllactate and possibly other long-chain α-hydroxy acids. This inference is based on (i) incubation requirements for optimum α-hydroxy acid oxidation; (ii) studies with known substrates and inhibitors of the L-amino acid oxidase of Blanchard *et al.* (*6*); (iii) a comparison of in vitro activities and observed changes in blood phenyllactate levels (Figs. 1 and 2) and urine phenyllactate concentrations following injections; and (iv) a similar age-dependency for the oxidation of other α-hydroxy acids by rat kidney extracts (Table 1). More widely distributed enzymes bring about reduction of phenylpyruvate (*7*), but a significant reversibility of these systems under normal conditions has not been demonstrated. Two flavoprotein oxidases, together covering a broad range of L-α-hydroxy acid specificity, have recently been purified almost 1000-fold from renal cortex of hog by Robinson *et al.* (*8*). Hydroxyisocaproate, β-phenyllactate, and *p*-hydroxy-β-phenyllactate were particularly good substrates for the long-chain oxidase which specifically oxidizes many aliphatic and aromatic L-α-hydroxy acids larger than hydroxybutyrate to the corresponding α-keto acids.

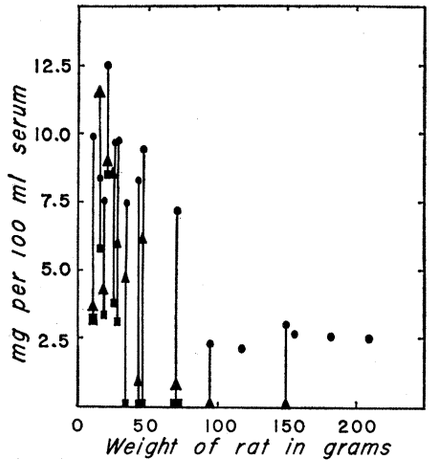

Fig. 1. Serum phenyllactate concentrations at hourly intervals after intraperitoneal injections of L-phenyllactate (100 mg/kg) to rats of varying degrees of development. Symbols: ●, value at 1 hour; ▲, 2 hours; ■, 3 hours. L-Phenyllactic acid was synthesized by the method of Suwa (*17*). Details of the experimental procedures are similar to those reported previously (*1*). In most of the phenyllactate determinations 0.9- by 12.5-cm columns of Dowex 1X2 Ac^- (200 to 400 mesh) were eluted with 25 ml of 0.5*M* sodium acetate (*p*H 6.5) in the mixing bottle and a mixture of 65 ml of 2.0*M* NaOAc (*p*H 6.5) and 25 ml of 95 percent ethanol in the reservoir. The phenyllactic acid peak emerged at about 50 ml.

Long-chain α-hydroxy acids normally play an inconspicuous role in mammalian metabolism. In certain heritable disorders involving amino acids, however, the inactivity of a crucial enzyme blocks the usual catabolic pathway, and an accumulation in tissue of the branched-chain amino acids, or of phenylalanine, tryptophan, or histidine, subjects these molecules to attack at the α-carbon of common configuration. This attack results in formation of α-keto and α-hydroxy acids in large amounts. The distinctive odor imparted to the urines of infants inheriting maple syrup urine disease has been attributed to the presence of α-hydroxybutyric acid (*9*). It is well known that phenylketonurics may excrete β-phenyllactate in gram quantities daily. In a limited survey with specimens from five institutionalized patients beyond childhood with unrestricted phenylalanine intake, serum phenyllactate levels in the range of 1 to 3 mg/100 ml were found (*10*). Studies of enzymes of animal tissues indicate that DOPA decarboxylase (*11*), 5-hydroxytryptophan decarboxylase (*12*), a tryptophan hydroxylating system (*13*), cerebral diglycinase (*14*), and gluconeogenesis in rat kidney (*15*) are inhibited by β-phenyllactate, in some instances (*13, 14*) at concentrations compatible with the observed serum concentrations in phenylketonurics. With tissue culture systems phenyllactate was found to be a phenylalanine antimetabolite (*16*). Interference by metabolic derangement during a sensitive postnatal period in the development of the central nervous system is a current concept in relation to the manifestations of phenylketonuria and the branched-chain amino acid anomaly. The conceivable coexistence and close interaction of a developmental rate limitation and inborn error at a critical stage in neural maturation appear to be worthy of consideration.

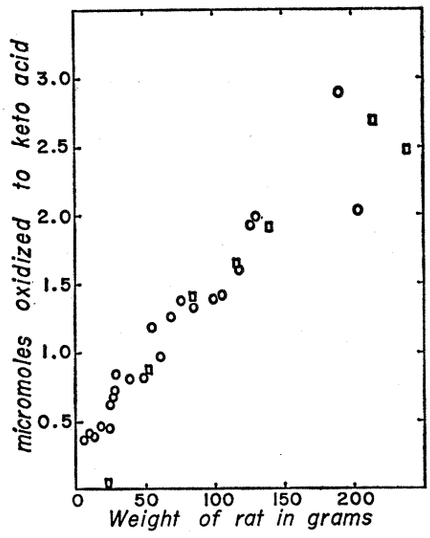

Fig. 2. Relative oxidation rates of L-phenyllactate by homogenates and 30,000*g* supernatant fractions prepared from the kidneys of rats of varying degrees of development. Symbols: ○, whole homogenate; □, 30,000*g* supernatant phase. The kidneys were homogenized in 0.05*M* phosphate buffer, *p*H 7.5 (3.25 ml/g). Each assay mixture consisted of 1.0 ml of homogenate or extract in buffer, 5 μmole of L-phenyllactate, and 20 μmole of α-ketoglutarate in a total volume of 1.5 ml. The mixtures were incubated, with shaking, at 37°C for 30 minutes in closed vessels saturated with oxygen. α-Ketoglutarate was added to reduce the transamination of phenylpyruvate. Phenylpyruvate and phenylalanine were determined in neutralized perchloric acid deproteinized aliquots by the method of La Du *et al.* (*18*).

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References and Notes

1. F. B. Goldstein, *J. Biol. Chem.* **236**, 2656 (1961).
2. H. Lineweaver and D. Burk, *J. Am. Chem. Soc.* **56**, 658 (1934).
3. K. Closs and S. D. Henriksen, *Z. Physiol. Chem.* **254**, 107 (1938); K. Closs and A. Fölling, *ibid.*, p. 254; E. A. Zeller and A. Maritz, *Helv. Physiol. Pharmacol. Acta* **3**, C19 (1945); C. G. Baker, *Arch. Biochem. Biophys.* **41**, 325 (1952).
4. B. Iselin and E. A. Zeller, *Helv. Chim. Acta* **29**, 1508 (1946).
5. M. Blanchard, D. E. Green, V. Nocito, S. Ratner, *J. Biol. Chem.* **155**, 421 (1944).
6. ———, *ibid.* **161**, 583 (1945).
7. A. Meister, *ibid.* **184**, 117 (1950); W. W. Weber, V. Zannoni, B. N. La Du, *Federation Proc.* **23**, 475 (1964); G. E. Skye and K. E. Ebner, *Biochim. Biophys. Acta* **96**, 331 (1965).
8. J. C. Robinson, L. Keay, R. Molinari, I. W. Sizer, *J. Biol. Chem.* **237**, 2001 (1962).
9. J. H. Menkes, *Neurology* **9**, 826 (1959).
10. F. B. Goldstein, unpublished.
11. J. H. Fellman, *Proc. Soc. Exptl. Biol. Med.* **93**, 413 (1956).
12. I. Huang and D. Y-Y. Hsia, *ibid.* **112**, 81 (1963).
13. R. A. Freedland, I. M. Wadzinski, H. A. Waisman, *Biochem. Biophys. Res. Commun.* **6**, 227 (1961).
14. L. L. Uzman, M. K. Rumley, S. van den Noort, *J. Neurochem.* **10**, 795 (1963).
15. H. A. Krebs and P. de Gasquet, *Biochem. J.* **90**, 149 (1964).
16. T. Gustafson and S. Hörstadius, *Exptl. Cell Res.* Suppl. 3, 170 (1955); C. E. Wilde, Jr., *J. Morphol.* **96**, 313 (1955).
17. A. Suwa, *Z. Physiol. Chem.* **72**, 113 (1911).
18. B. N. La Du, R. R. Howell, P. J. Michael, E. K. Sober, *Pediatrics* **31**, 39 (1963).
19. L. Penrose and J. H. Quastel, *Biochem. J.* **31**, 266 (1937).

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Oncogenicity of the Simian Adenoviruses

Abstract. *Five of 17 adenoviruses of rhesus or cynomolgus monkey origin induced tumors in newborn hamsters. The tumors appeared between 42 and 280 days after subcutaneous inoculation and had the general characteristics of lymphomas. The tumors were specific by cross-complement fixation tests. An adenovirus recovered from* Cercopithecus *monkeys appeared to be highly oncogenic; all 23 inoculated hamsters developed tumors within 30 to 40 days.*

The oncogenicity of human adenoviruses for newborn hamsters has been reported (*1, 2*), and we have observed that 6 of 18 simian adenoviruses behave similarly. Five of these were part of the SV (simian virus) series and were of rhesus or cynomolgus monkey origin (*3*). The other, SA7, was isolated from an African green monkey (*4*). All tests were performed with prototype strains.

Small groups of newborn hamsters were inoculated with one of each of the 18 simian adenoviruses, or with one of two known oncogenic agents, adenovirus 12 and SV40. Each animal received 0.05 ml of virus subcutaneously, in the dorsal cervical area. Tumors were obtained in the general area of the inoculation with five of the viruses in the SV series, with SA7 (Table 1), and with the known tumor viruses. On the basis of incidence of tumors and duration of latent periods, SA7 appeared to be the most oncogenic, followed by SV20 and SV38. SA7, furthermore, produced multiple, rapidly growing subcutaneous tumors, while the other viruses produced single masses. What effect, if any, the virus dose had on the degree of oncogenicity was not determined. None of the other viruses produced tumors in these initial tests, although Huebner *et al.* (*5*) previously reported that SV1 (M-1) produced tumors in hamsters. Because of the limitations in our studies—in number of animals inoculated, virus concentrations and periods of observation—the possibility cannot be excluded that other simian adenoviruses are oncogenic.

The capacity of most of these viruses to produce tumors has been confirmed by further studies. Only SV37 and SV38 have failed to produce tumors in repeated tests, but the animals involved have only been under observation for 90 days, and much longer latent periods than this were noted in the original study. A summation of the results of all inoculations to date is presented in Table 2.

The oncogenicity of SA7 was not only readily confirmed for baby hamsters, but was also observed for suckling rats. Tumors were obtained in 3 of 21 inoculated animals. In addition to producing tumors in hamsters, SV20 produced a tumor in one of 15 newborn C3H mice after a 92-day latent period. Tumors produced by SA7, SV20, SV33, SV34, and SV38 were successfully transplanted to weanling hamsters.

Tumors produced by these simian adenoviruses were locally invasive, but not lethal within the experimental period, except for the rapidly growing SA7 tumors, which did kill some hamsters. Metastases generally were not observed, but one animal bearing an SV20-induced tumor and killed for pathologic examination did have a small metastasis to the brain. Histopathologically, the tumors produced by the various viruses except for the SV34 tumor, were similar and more like tumors produced by adenovirus (*2*) than like the fibrosarcomas produced by SV40 virus. The tumors were undifferentiated neoplasms with some characteristics of lymphomas of the reticulum-cell type although, few, if any, reticulum fibers were present. The actual cell of origin was not determined. The SV34 tumor was somewhat more differentiated, with some areas resembling a spindle-cell sarcoma. Electron micrographs of SV34 revealed a second particle, smaller than the adenovirus; in this respect, it was unique among the viruses in the oncogenic group (Fig. 1). Similar particles were seen in other simian adenoviruses which did not produce tumors. Representative fields of the histological sections of tumors produced by SV20, and SV34, and by SV40, for comparison, are shown in Fig. 2.

Attempts to recover or to demonstrate the presence of SV20 in tumors produced by the virus failed. Homogenates were inoculated into susceptible cell cultures, and explanted cultures were prepared from the tumor tissue. After sedimentation procedures, electron microscope examination, which readily showed particles in fluids from SV20-infected tissue culture, failed to

Table 1. Oncogenicity of simian adenoviruses in newborn hamsters. The viruses in the SV series were grown in strain LLC-MK2 cells; and SA7 in BS-C-1 cells. Inoculation of newborn hamsters with 10^6 or more viable cells of either strain failed to produce tumors.

Virus	Inoculations (No.)	Virus dose ($\log_{10}$)	Survivors beyond 30 days/ No. with tumors	Time of tumor detection (days)
SV20	12	6.75	6/3	53–62
SV33	8	6.00	6/1	229
SV34	8	5.50	6/1	110
SV37	7	*	6/1	231
SV38	9	4.83	8/3	154–280
SA7	23	7.50	23/23	31–41

* Not determined.

Table 2. Compilation of oncogenic findings in hamsters with simian adenoviruses, adenovirus 12, and SV40. All animals were inoculated within 24 hours of birth with 0.05 ml of undiluted virus and observed until death or until killed for histopathology.

Virus	Inoculations (No.)	40-day survivors/No. with tumors	Time of tumor detection (days)
SA7	83	47*/46	28–48
SV20	250	141/54	40–211
SV33	31	25/2	82–229
SV34	62	48/3	110–330
SV37	23	20/1	231
SV38	36	14/3	154–280
SV40	75	39/24	121–251
AV12	29	21/3	55–89

* Includes animals killed for histopathology between 30 and 40 days.