drates by *L. bifidus* did not observe significant differences among the sugars we have studied. However, they measured acid production after a 2-week incubation period so that differences in the rate of fermentation such as we observed in 20 to 64 hours were not apparent.

Organisms which grow preferentially on disaccharides vary in the specificity of their requirements. A strain of *L. bulgaricus* studied by Snell, Kitay, and Hoff-Jorgensen(13) used only lactose of the disaccharides but grew on a number of B-galactosides. The strain of *Streptococcus thermophilus* discussed by Wright(1) grew best on lactose and sucrose. *L. bifidus* used sucrose to only a limited extent but metabolized both maltose and lactose readily.

It has been noted by a number of workers that even though an organism used a disaccharide much better than one or both of the constituent monosaccharides there was no accumulation of monosaccharides during the growth period(13-15). Doudoroff *et al.*(15) using dried cell preparations of a mutant of *Escherichia coli* which utilized maltose did observe accumulation of glucose and his suggested mechanism accounts for its formation. However, glucose was never found in more than trace amounts when intact cells were used. *L. bifidus* on the contrary does show accumulation of both glucose and galactose indicating that it does contain an active lactase which converts a considerable portion of the lactose present to glucose and galactose but lacks the enzyme system to make effective use of these sugars.

Summary. 1. *Lactobacillus bifidus* utilizes the disaccharides lactose and maltose, but not sucrose, more readily than the monosaccharides, glucose and galactose. The reverse is true of straight rod variants of *L. bifidus*. Several strains of *L. acidophilus* utilize glucose or lactose equally well or use glucose more readily. 2. During growth of *L. bifidus* on lactose, glucose and galactose accumulated in the medium. The straight rod mutant of *L. bifidus*, on the other hand, utilizes lactose completely.

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Studies on Phenylpyruvic Oligophrenia. Phenylpyruvic Acid Content of Blood. (19998)

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Phenylpyruvic oligophrenia (phenylketonuria) is a form of mental deficiency characterized by the urinary excretion of phenylpyruvic, phenyllactic, phenylacetic acids and phenylalanine(1). Although abnormally high amounts of phenylalanine have been observed

in the blood of these patients(2,3), the presence in the blood of the deaminated phenylacids has never been reported. The purpose of this note is to report data on the blood content of phenylpyruvic acid and to comment briefly on their significance.

Materials and methods. The subjects were 39 phenylketonuric patients ranging in chronological age from 4 to 68 years and in intelligence quotient from 5 to 51. Blood for examination was obtained after 10-12 hours fasting and, during feeding experiments, one, 2 and 6 hours after the ingestion of the compound fed. For the quantitative determination of phenylpyruvic acid, the color reaction with ferric chloride was utilized which was first described by Erlenmeyer(4) and previously applied to the estimation of the acid in the urine(5-7). Some 30 ml of heparinized blood was immediately centrifuged and the plasma separated. Exactly 50 ml (corresponding to 10 ml of plasma) of a 1 to 5 sulfuric acid-tungstate filtrate was prepared in the routine manner, acidified to Congo red and extracted 3 times with 40 ml of ether. The combined ether was dried with anhydrous sodium sulfate and evaporated in vacuum. The residue was dissolved in 5 ml of glycine-sodium chloride-hydrochloric acid buffer of pH 2.2, filtered and to 4 ml of the clear filtrate, 0.5 ml of a freshly prepared 1% aqueous solution of ferric chloride was added. The green color was read in a Coleman junior spectrophotometer at 600 wavelength exactly 2 minutes after mixing. A standard curve was prepared using various amounts (20 to 300 μg) of twice recrystallized phenylpyruvic acid dissolved in 50 ml of water and treated exactly as the blood filtrate. In all readings the reagent blank was used as reference. Under these experimental conditions, samples of blood from 20 non-phenylketonuric healthy individuals of various chronological ages and intelligence quotients, gave values corresponding to .012-.016 mg/100 ml of plasma. Since no evidence could be obtained indicating that this figure represents phenylpyruvic acid normally present in the blood, the value .015 mg was arbitrarily subtracted from all readings. The chemicals used were commercial preparations with the exception of phenylpyruvic and phenyllactic acids which were prepared in the laboratory following standard procedures.

TABLE I. Phenylpyruvic Acid Content of Blood.

Patient	C.A.	I.Q.	Wt deviation, kg	Phenylpyruvic acid, mg %
1. A.	36	7	+ 9	.53
2. B.	68	23	— 5	.40
3. B.J.	45	47	+18	.73
4. C.	8	32	— 2	.63
5. C.J.	6	26	+ 3	.83
6. C.B.	31	37	— 6	.73
7. C.W.	30	7	—12	.66
8. D.	28	30	+ 3	.58
9. D.Al.	23	9	— 7	.36
10. Do.	41	17	— 2	.44
11. D.M.	38	20	—10	.83
12. D.E.	25	31	— 2	.40
13. F.	32	5	+ 7	.83
14. F.A.	4	36	+ 4	.90
15. Gi.	28	13	—10	.31
16. G.	12	36	+ 1	.66
17. G.S.	9	44	+ 2	.70
18. G.E.	33	5	+15	.49
19. H.	32	11	+ 3	.70
20. K.	9	24	— 6	.36
21. K.A.	33	43	— 5	.39
22. K.P.	5	23	— 4	.42
23. K.H.	11	9	—13	.66
24. K.J.	10	10	— 6	.60
25. K.R.	26	6	+12	.44
26. K.W.	37	26	+14	.80
27. L.	52	51	— 2	.53
28. M.	18	21	—10	.63
29. M.M.	35	11	+ 8	.52
30. M.R.	24	31	+ 2	.52
31. N.	27	7	— 6	.60
32. P.	17	6	—14	.36
33. P.J.	10	8	—16	.31
34. Q.	33	13	— 9	.31
35. R.	32	47	+16	1.78
36. S.	4	32	+ 2	.66
37. S.N.	28	20	— 3	.42
38. S.E.	31	32	— 2	.66
39. W.W.	53	42	— 6	.38
		Mean .714	S.D. .248 ± .018	

Results. In Table I, the results of the determination of phenylpyruvic acid in 39 patients are presented. The patients are in alphabetical order; C.A. is chronological age; I.Q. the intelligence quotient; deviation from normal weight was calculated from standard tables taking into consideration age, weight and height of the subject; phenylpyruvic acid values are expressed in mg per 100 ml of plasma. It may be seen that the keto-acid is present in the blood of every patient. The coefficient of correlation for degree of mental deficiency and amount of phenylpyruvic acid present in the blood is +.434 ± .084. If

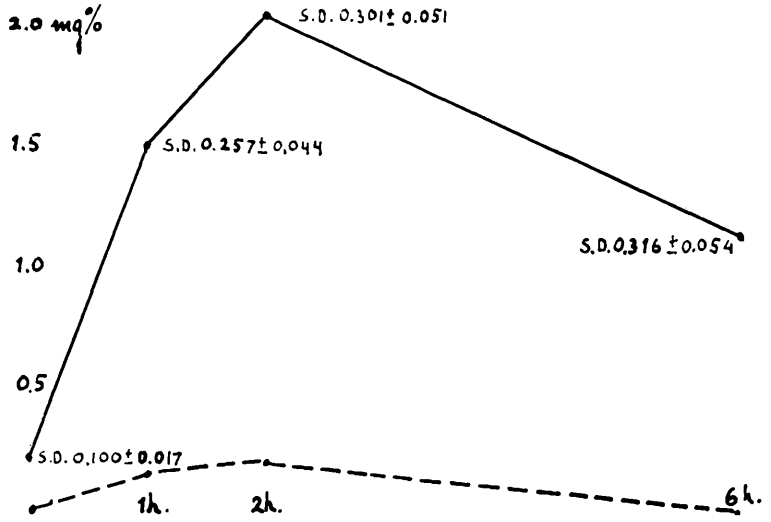

FIG. 1. Phenylketonemic curve following ingestion of DL phenylalanine (10 g) in 8 patients (upper curve, mean values) and 2 controls (lower curve).

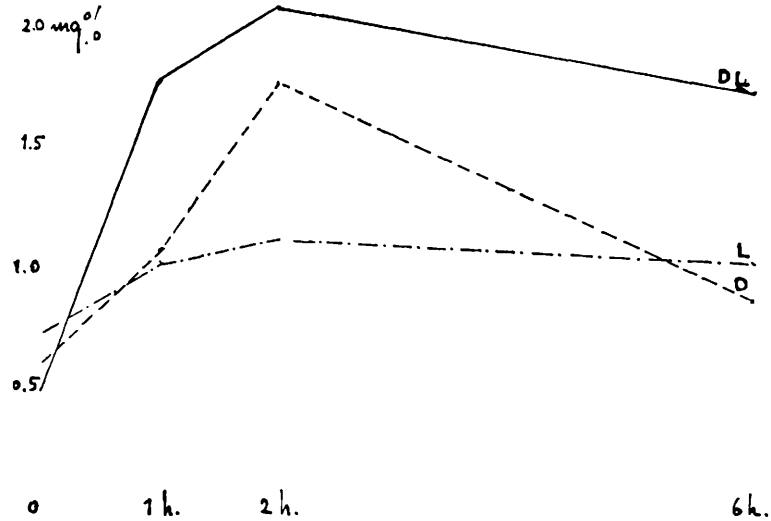

FIG. 2. Phenylketonemic curve following ingestion of DL (10 g), D (5 g) and L phenylalanine (5 g).

phenylketonemia is correlated with nutritional state as measured by the deviation from normal weight, the coefficient is $+.470 \pm .083$.

Eight adult patients whose weight averaged normal value were fed 10 g DL-phenylalanine. In Fig. 1 the phenylketonemic curve is shown. It represents the mean value at the first, second and sixth hour. It may be seen that the increase averaged about 4 times the fasting value. The peak of the increase was at about the second hour but elevated values were still apparent 6 hours after the feeding. In 2 non-phenylketonuric healthy adults of normal weight, a slight increase (.4 mg/100 ml) was observed under the same experimental conditions. During the 6 hours of this experiment, 3 phenylketonuric patients showed a urinary excretion of phenylpyruvic acid approximating 1200 mg in excess of the amount excreted during the same period of time the day prior to the feeding. The 2 normal controls excreted 553 and 675 mg, respectively.

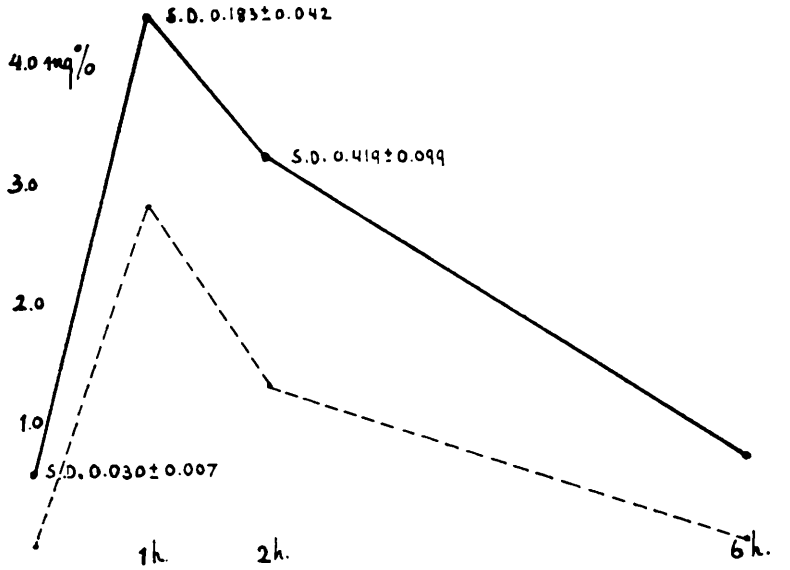

FIG. 3. Phenylketonemic curve following ingestion of phenylpyruvic acid (4.5 g) in 5 patients (upper curve, mean values) and 2 controls (lower curve).

In Fig. 2, the results of feeding the D and L phenylalanine to one patient are seen. It is apparent that the increase of phenylpyruvic acid in the blood is due mostly to the D form although a significant increase is shown also following the feeding of the L form. In this experiment, 10 g of DL and 5 g of L and D forms, respectively, were fed. In a control subject the L form (5 g) produced no appreciable effect while a small increase was observed following feeding of the D form (5 g). This control subject excreted 410 mg keto-acid following D phenylalanine and none following the L form.

In Fig. 3, the results of feeding 4.5 g phenylpyruvic acid to 5 phenylketonurics are shown. In all patients there was a sharp increase of phenylketonemia averaging 7 times the fasting value at the first hour. In 2 controls the type of curve was similar to that of phenylketonurics although the peak of the increase was some 25% lower. It is pertinent to note that in a non-phenylketonuric subject suffering from acute infectious hepatitis, the phenylketonemic curve following the ingestion of 4.5 g phenylpyruvic acid reached 4.6 mg/100 ml after one hour and 2.75 mg after the second hour, the curve being similar to that shown by phenylketonurics.

In 2 patients, 2 g per day of Benemid (p-di-n-propylsulfamylbenzoic acid) were administered for 3 days prior to feeding phenylalanine and phenylpyruvic acid. The resulting phenylketonemic values are seen in Table II. It is apparent that Benemid had the effect of raising significantly the phenylketonemic curve. In phenylketonurics kept on nitrogen constant diet, the ingestion of Benemid resulted in some 50% decrease of urinary excretion of phenylpyruvic acid.

Following the feeding of 5 g of the calcium salt of phenyllactic acid to one patient, there was an increase of phenylketonemia. The following values were observed: at one hour 2.60 mg/100 ml of plasma; at 2 hours 3.22 mg. After 6 hours the pre-feeding level of 0.9 mg was noted. No increase was observed following the ingestion of alanine (10 g), tyrosine (20 g), glycine (20 g), and phenylacetic acid (10 g).

Comments. The results clearly indicate that phenylpyruvic acid is present in the blood of every patient tested and that no appreciable amount may be found in non-phenylketonuric subjects. Phenylketonemia is therefore as characteristic of the disease as phenylketonuria. That the keto acid present in the blood derives from phenylalanine seems established

TABLE II. Phenylketonemic Curve Following Ingestion of Phenylalanine and Phenylpyruvic Acid in 2 Patients Treated with Benemid.

	Hr	Blood phenylpyruvic acid: mg/100 cc plasma	
		Before Benemid	After Benemid
DL phenylalanine (10 g)	0	.60	.96
	1	1.75	2.40
	2	2.40	3.50
	6	1.10	1.20
Phenylpyruvic acid (6 g)	0	.80	1.20
	1	5.05	6.60
	2	4.55	5.10
	6	.90	1.10

by feeding experiments. The major part of the increased amount of phenylpyruvic acid observed in the blood following DL-phenylalanine feeding apparently derives from the D form and only a minor part from the L isomer. In this respect the phenylketonuric behaves differently from the normal subject who showed no phenylpyruvic acid in the blood following ingestion of L-phenylalanine and only slight phenylketonemia following the D form. This observation would lend some support to the hypothesis of Fölling *et al.*(8) that the phenylketonuric organism derives phenylpyruvic acid from D-phenylalanine following conversion of the L into the D isomer. However, the hypothesis is contrary to the evidence brought forward by Prescott *et al.* (9) indicating that all phenylalanine present in the blood of phenylketonurics is of the L form.

Concerning the place of formation of phenylpyruvic acid from phenylalanine, it was previously suggested that most if not all keto-acid excreted in the urine might be formed in the kidneys by deamination of the excess phenylalanine present in the blood(3), but it appears probable from the present findings that this deamination process takes place also in extrarenal tissue. Since it is likely that parahydroxylation of phenylalanine is defective in the disease(10), formation of phenylpyruvic acid would represent an attempt by various tissues to dispose of the accumulating excess of phenylalanine using an alternative metabolic pathway.

Whether a difference exists between the phenylketonuric and the normal organism in the further disposal of phenylpyruvic acid is not clear from the present experiments. The phenylketonemic curve following ingestion of phenylpyruvic acid shows a similar shape in both patients and controls indicating similar mechanisms of breaking down the metabolite. However, the peak is higher in the phenylketonuric than in the normal subject approximating in the former the values seen in disorders of the liver. This would suggest that in phenylketonuria some mechanism by which the normal liver may dispose of the keto-acid is at fault. One may speculate that in normal individuals the liver is able to form phenylalanine from excess phenylpyruvic acid by amination(11), a process which might be defective in phenylketonuria owing to the accumulation of phenylalanine in the body fluids. Other known ways of disposing of the keto-acid such as urinary excretion, transformation into phenyllactic and phenylacetic acid(12), would be common to both normal and phenylketonuric subjects. If this hypothesis is correct, the disturbance of phenylpyruvic acid metabolism would be only partial in character and secondary to the error of phenylalanine metabolism. The importance of the kidney in the disposal of phenylpyruvic acid is indicated by the experiment with Benemid. Inhibition of tubular transfer of phenylpyruvic acid by the drug would easily explain the increased phenylketonemia and the decreased phenylketonuria observed in these experiments.

The correlation between phenylketonemic level and degree of mental defect is of pertinence to the still unsolved problem of the relationship of metabolic disorder to intellectual development(13). If one assumes that a hypothetic toxic action of phenylpyruvic acid is responsible for the retarded mental growth, one would expect a negative coefficient of correlation, *i.e.*, as the I.Q. increases, phenylketonemia should decrease. Since the correlation is positive (+.434), the hypothesis is probably incorrect. The correlation coefficient for degree of nutritional state and phenylketonemia (+.47) suggests that good nutrition and, presumably, high intake of protein is often associated with high phenylketo-

nemia level. Observation of single patients seems to confirm this statistical finding; patient No. 35, for instance, who showed the highest phenylketonemic level, was a voracious eater, while patients No. 15, 33 and 34, who showed low phenylketonemia, were "feeding problems" and consumed limited amounts of food. The observed correlations for I.Q. and phenylketonemia may then depend simply on the fact that in this series, subjects with high I.Q. usually show better eating habits than low I.Q. patients.

Summary. Phenylpyruvic acid was demonstrated in the blood of 39 patients affected with phenylpyruvic oligophrenia. The values ranged from 0.31 to 1.78 mg per 100 ml of plasma. A significant increase of phenylketonemia was observed following ingestion of DL-phenylalanine, the D form causing considerably greater increase than the L isomer. Feeding of phenylpyruvic acid resulted in a sharp increase of phenylketonemia. The significance of these findings is briefly discussed.

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Nature of the Metabolic Products of C^{14}-Cholesterol Excreted in Bile and Feces.* (19999)

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It has been generally considered that cholesterol is eliminated from the body principally as coprosterol, dihydrocholesterol and cholesterol, and that their excretion—or the excretion of their precursors—takes place across the intestinal wall(1-3). Bile has come to be regarded as a relatively minor path for the elimination of cholesterol or of its products by metabolism(4). Recent studies carried out with cholesterol-4-C^{14} have revealed, however, that this view is no longer tenable, for approximately 90% of the C^{14} of administered cholesterol-4-C^{14} is recoverable in bile. Furthermore, the nonsaponifiable fraction, which includes coprosterol and dihydrocholesterol as well as cholesterol, does not amount to more than 25% of the total C^{14} found in either bile or feces (5). Thus, by far the major part of the administered C^{14} is eliminated as saponifiable materials. It seemed likely that these excretion products are bile acids. Supporting evidence for this view is presented here.

Treatment of rats. Male Long-Evans rats, weighing between 250 and 300 g, were lightly anesthetized with ether. One mg of cholesterol-4-C^{14} containing 3 x 10^6 c.p.m. dissolved

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