nuclear cells were observed in the liver, spleen and adrenal glands. These changes were not considered sufficient to cause death and it was believed that the toxin in high concentration was capable of acting on vital centers before significant demonstrable lesions had occurred. On comparing the toxicity of the virus for mice and ferrets, it may be observed that virus with a hemagglutination concentration of 154 units per g of body weight was required to kill 75% of the ferrets, whereas 53 units per g of body weight were required to kill approximately 75% of the mice. Thus it appeared that mice were more susceptible to the toxin than ferrets.

A knowledge of the stability of the virus is of obvious practical importance. The Henles found that toxicity was unaltered for 1 to 3 months at 4°C, decreasing slowly thereafter. In our experiments, the toxicity was very slight after periods of longer than one week at 4°C; this was true whether the virus was preserved unpurified and unconcentrated in allantoic fluid or in the purified and concentrated state in 0.1 M phosphate buffer solution at pH 7.0. However, there was no decrease in toxicity in material stored in allantoic fluid for 3 months at –30°C or at –60°C. On two occasions our stock virus lost its capacity to stimulate production of the toxic factor on inoculation into eggs while it retained its ability to produce the hemagglutination and infectivity factors when it had been stored for periods of 6 months at –60°C. This loss of toxin production was also observed on one occasion on serial passage in the embryonated egg. These findings lend further evidence to the hypothesis that the toxic factor is different from the infective moiety.

Summary. The mouse toxic factor of influenza virus, PR_8 strain, originally reported by the Henles was reinvestigated, and similarities and differences between their observations and ours are reported and discussed. The toxin also was shown to affect ferrets.

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Excretion of Phenylalanine and Derivatives in Phenylpyruvic Oligophrenia. (18108)

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It is established that patients affected with phenylpyruvic oligophrenia excrete in the urine phenylalanine(1,2), phenylpyruvic acid (2-8), and phenyllactic acid(1,2,6). Quantitative determinations of phenylpyruvic acid in a few patients have been published(2,4,5), but only in one patient were data reported on the excretion of these 3 phenyl compounds (2). It is the purpose of this note to present quantitative data on the urinary excretion of phenylalanine, phenylpyruvic and phenyllactic acid in 20 patients, under varying dietary conditions.

Materials and methods. Twenty individuals affected with phenylpyruvic oligophrenia ranging in chronological age from 2 to 42 years and in the intelligence quotient from 5 to 47, were used as experimental subjects. For the determination of free phenylalanine

1. Föling, A., Closs, K., and Gammes, T., *Z. physiol. Chem.*, 1938, v256, 1.

2. Dann, M., Maples, E., and Levine, S. Z., *J. Clin. Invest.*, 1943, v22, 87.

3. Fölling, A., *Z. physiol. Chem.*, 1934, v227, 169.

4. Penrose, L., and Quastel, J. H., *Biochem. J.*, 1937, v31, 266.

5. Jervis, G. A., *J. Biol. Chem.*, 1938, v126, 305.

6. Zeller, E. A., *Helv. Chem. Acta.*, 1943, v26, 1614.

7. Lepow, H., *Monatschr. f. Psychiat. u. Neur.*, 1945, v110, 161.

8. Delay, J., and Pichot, P., Polonowski, M., Desgrez, P., and Delbarre, F., *Sem. Hôp. de Paris*, 1947, v23, 1749.

10 ml of urine were diluted to 50 ml and extracted with chloroform for 24 hours to remove phenylpyruvic and phenyllactic acids. The chloroform was evaporated and phenylalanine was determined on 5, 4, 3, 2, and 1 ml of the urine diluted from 20 to 100 times following the microbiological assay with *Leuconostoc mesenteroides* P-60 described by Dunn *et al.*(9). Five ml of medium was used for each test and the determinations were performed titrimetrically with 0.04 N sodium hydroxide after 5 days incubation period. A standard curve was prepared, using DL-phenylalanine tested for purity.* Calculations were made on the assumption that all urinary phenylalanine is of the L form, as maintained by Fölling *et al.*(1) and confirmed by Borek and Waelsch(10).

For the determination of phenylpyruvic acid, the "direct total hydrazone" method of Friedman and Haugen(11) was used on aliquots of the original urine diluted from 20 to 50 times. A standard curve was prepared using recrystallized phenylpyruvic acid prepared according to Hemmerlé(12). The colorimetric determinations were performed in a Klett photoelectric apparatus using Filter 54.

For the determination of phenyllactic acid, the nitration method of Kappeler-Adler(13), previously reported(14), suitable for this acid, was used in the following manner. To 2-5 ml of urine, 2 ml of a saturated solution of sodium bisulphite was added. Phenylpyruvic acid was thus bound into a chloroform insoluble compound. After 10 minutes the acidified solution was extracted 3 times with 4 ml of chloroform for 30 minutes. The combined chloroform extracts were added to 5 ml of N sulfuric acid and evaporated. Nitration and development of color were performed following the directions of Roche and Michel(15) and using an electric stove as recommended by Albanese(16). Readings were performed in a Klett photoelectric apparatus with Filter 54. A standard curve was prepared using twice recrystallized calcium salt of phenyllactic acid prepared according to the directions of Fischer(17). A blank was run with each determination using the same ingredients but ascorbic acid. Fairly satisfactory recoveries, within 10% error, were obtained upon applying this procedure to a mixture of phenyllactic, phenylpyruvic acid and phenylalanine.

Nitrogen was determined with the macro-Kjeldahl method using a digesting mixture containing selenium.

Results and discussion. Table I shows the results of determinations of phenylalanine, phenylpyruvic acid and phenyllactic acid in 20 patients on normal institutional diet. The values are expressed in mg per g of total urinary nitrogen. This manner of notation was necessarily adopted, since it was impossible in many patients of low mentality to collect complete 24 hour specimens of urine. The urinary output of phenylalanine was found to vary from 50 to 20 mg per g of total nitrogen, phenylpyruvic acid from 170 to 112 mg, and phenyllactic from 91 to 40 mg. No conspicuous differences in the output of phenyl compounds were observed from patient to patient, suggesting that in contrast with other errors of metabolism, only small differences in the degree of metabolic defect occur.

It is significant to note from Table I the lack of correlation between intelligence quotient and amount of urinary phenyl compounds. Were there a causal relationship of

9. Dunn, M. S., Shankman, S., and Camien, M. N., *J. Biol. Chem.*, 1945, v161, 643.

* Furnished by H. M. Chemical Co., 144 North Hayworth Avenue, Los Angeles, Calif.

10. Borek, E., and Waelsch, H., *Fed. Proc.*, 1949, v8, 126.

11. Friedman, T. E., and Haugen, G. E., *J. Biol. Chem.*, 1943, v147, 415.

12. Hemmerlé, R., *Ann. chim. et physiq.*, 1917, v7, 226.

13. Kappeler-Adler, R., *Biochem. Z.*, 1932, v252, 185.

14. Jervis, G. A., Block, R. J., Bolling, D., and Kanze, E., *J. Biol. Chem.*, 1940, v134, 105.

15. Roche, J., and Michel, R., *Bull. Soc. Chem. Biol.*, 1946, v28, 844.

16. Albanese, A. A., *J. Biol. Chem.*, 1944, v155, 291.

17. Fischer, E., and Zempley, G., *Ber. deut. chem. Gesel.*, 1909, v42, 4891.

TABLE I. Urinary Output of Phenyl Compounds on Normal Diet.

Patient	Age	I.Q.	Phenylalanine, mg/g N	Phenylpyruvic a., mg/g N	Phenyllactic a., mg/g N	Total phenyl-compounds, mg/g N
A.	32	7	26	166	66	258
B.	42	46	22	120	68	210
C.W.	26	7	50	145	91	286
C.B.	27	40	25	159	71	255
D.	19	9	34	170	65	269
F.	28	5	35	149	65	289
G.	29	5	33	130	45	208
K.R.	5	24	20	116	52	188
K.A.	30	38	35	114	72	221
K.A.	22	6	20	144	69	233
K.P.	2	23	30	160	42	232
K.W.	34	26	25	150	72	247
M.G.	20	7	20	137	65	222
M.E.	15	35	28	112	48	188
M.R.	26	10	49	139	40	228
M.M.	41	11	35	140	90	265
M.S.	17	12	20	122	51	193
N.	23	7	33	113	61	207
R.	29	47	28	181	90	309
Z.	6	14	25	180	46	251

the metabolic abnormality to the mental defect, as suggested by Fölling *et al.*(18), one would expect that the lower the intelligence quotient the higher the amount of excreted phenyl compounds.

Table II shows the amount of phenyl compounds excreted in 24 hours by one patient on a high and on a low protein diet. It demonstrates the dependence of the daily output of phenyl compounds on the amount of protein ingested. However, that not all phenyl compounds derive from dietary protein was indicated by the observation that two patients still excreted phenylalanine and its keto and hydroxyl acids after 10 days of protein free diet.

The effect of various amino acids on the excretion of phenyl compounds was tested by determining the daily output in patients kept on a nitrogen constant diet following ingestion of some pure amino acid. The following amino acids produced no change: glycine (25 g); glutamic acid (5 g); tyrosine (10 g); alanine (10 g); cystine (10 g). As seen in Table III, the ingestion of DL-phenylalanine (10 g) resulted in an increased excretion of all phenyl compounds. In this experiment, however, a certain amount of the keto acid probably originated from the D phenylalanine. The ingestion of 10 g of phenylpyruvic acid (Table III) resulted in a significant increase in the excretion of both the keto and the hydroxy-acid. In normal

TABLE II. Urinary Output of Phenyl Compounds on High and Low Protein Diet.

Date	N, mg/24 hr	Phenylalanine, mg/24 hr	Phenylpyruvic acid, mg/24 hr	Phenyllactic acid, mg/24 hr
3-29	22.000	440	2289	1080
4-6*	4.010	76	428	208

* Very low protein diet started on 3-30.

TABLE III. Urinary Output of Phenyl Compounds Following Ingestion of Phenylalanine and Phenylpyruvic Acid.

Date	N, mg/24 hr	Phenylalanine, mg/24 hr	Phenylpyruvic acid, mg/24 hr	Phenyllactic acid, mg/24 hr
4-26	15250	323	1640	1060
4-27*	16330	664	5840	1620
3-22	10.20	324	1380	680
3-23†	11.04	348	4200	2000

* Phenylalanine g 10.
† Phenylpyruvic acid g 10.

18. Fölling, A., Mohr, O. L., and Ruud, L., Oligophrenia phenylpyrouvica, Oso, 1945.

TABLE IV. Excretion of Phenylalanine and Phenylpyruvic Acid in the Sweat (mg/100 cc).

Patient	N, mg/100 cc	Phenylalanine, mg/100 cc	Phenylpyruvic acid, mg/100 cc
A.	61	2.3	20
B.	100	3.4	15
C.	57	1.6	19
F.	44	1.6	6
K.P	54	1.4	13
K.A.	56	2.4	56
M.M.	76	2.7	25
M.E.	140	4.4	33
N.	84	3.5	21
R.	152	9.6	47

individuals this amount of phenlypyruvic acid is usually completely metabolized.

From these feeding experiments it would appear that some quantitative changes in the excretion of phenyl compounds should be expected when foods with different phenylalanine content are ingested by the patients. However, since the ratio of phenylalanine to nitrogen in various proteins and foods does not show conspicuous variations(19), the amount of phenyl compounds excreted per g of nitrogen, as expressed in Table I, is probably little affected by the quality of ingested food.

In view of the findings of urinary excretion of phenyl compounds, it was considered of interest to investigate the excretion of these metabolites in sweat. Accordingly, sweat was collected as directed by Hier *et al.* (20) from 10 patients and determinations were performed on suitable dilutions following the procedure described for urine. The patient was bathed before the collection of sweat. Phenyllactic acid was not found in measurable amounts. The data concerning phenylalanine and phenylpyruvic acid are shown in Table IV. The values for the amino acid were somewhat above those reported by Hier *et al.*(20) in the normal individual. Phenylpyruvic acid is not found in the sweat of normal subjects. It would appear, therefore, that a small amount of phenylalanine and its derivative is eliminated by way of sweat.

Summary. Quantitative data are presented on the urinary excretion of phenylalanine, phenylpyruvic acid and phenyllactic acid in twenty patients affected with phenylpyruvic oligophrenia. The values, expressed in mg per g of total nitrogen, show inconspicuous differences from patient to patient kept on similar diets. High protein diet or the ingestion of phenylalanine and phenylpyruvic acid result in an increased daily output of phenyl compounds. Data on sweat excretion of phenyl compounds are also presented.

19. Horn, M. J., Jones, D. B., and Blum, A. E., *J Biol. Chem.*, 1948, v176, 679.

20. Hier, S. W., Cornbleet, T., and Bergeim, O., *J. Biol. Chem.*, 1946, v166, 327.

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Oligophrenia Phenylpyruvica. II. Constancy of the Metabolic Error. (18109)

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In phenylpyruvic oligophrenia mental deficiency is accompanied by faulty phenylalanine metabolism. The metabolic error is manifested by the urinary excretion of large amounts of phenylalanine, phenyllactic acid, and phenylpyruvic acid, and by an elevated phenylalanine concentration in the blood. The genetic analysis strongly suggests that this disease is inherited as an autosomal Mendelian recessive and that; therefore, the mental and metabolic disturbance may be caused by the same gene. The question arises whether both abnormalities, when

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