termined by examining epididymal smears obtained by aspiration. Each of these males has subsequently been bred to 4-month-old virgin females from our stock colony. One sired 40 young when mated with 8 females, the other 25 young when mated with 5 females. Of 50 males on similar diets but without added manganese, other than that found as a contaminant of certain inorganic constituents and natural foods as shown by Orent and McCollum,[3] testicular atrophy and tubular degeneration was marked in 45 males after they had been on the rations from 117 to 833 days.

On the type of diet employed, food consumption averages from 10 to 15 g per adult rat per day. Animals on the above rations were thus consuming about .018, .036, .09, .18, and .36 g of $MnCl_2.H_2O$ per day, or .00449, .00998, .02495, .0499, and .0998 g of manganese. The rations used contained .63 g calcium and .72 g phosphorus per 100 g. Evidently the high level of phosphorus effectively prevented symptoms of toxicity by reducing the amount of absorbable manganese.

It is thus apparent that the conditions in the intestines which influence the absorbability of manganese constitute the controlling factor in determining the level at which it is toxic. From our observations on a small number of rats it also seems clear that when a high intake of this element is provided sexual function is retained in males decidedly longer than is the case in our rats on similar diets without added manganese. This observation is being checked on a larger group of male rats.

10002

Excretion of Homogentisic Acid After Oral Administration of Phenylalanine to Alcaptonuric Subjects.

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That the homogentisic acid excreted in alcaptonuria originates from the aromatic amino acids of the protein molecule, tyrosine and phenylalanine, has been accepted.[1] Further confirmation has

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1 Compare Neubauer, O., *Handbuch der normalen und pathologischen Physiologie*, 1928, **5**, 851, for an excellent review of the literature.

been obtained[2] by the isolation and characterization of homogentisic acid from the urine of healthy white rats fed large amounts of phenylalanine. In most of the studies concerned with the fate of the naturally occurring aromatic amino acids in alcaptonuria, tyrosine has been administered, while experiments with phenylalanine are very limited in number.[1] Since we had available for study 2 alcaptonuric patients in the University Hospital, we have undertaken further quantitative studies of the effect of phenylalanine on the excretion of homogentisic acid in alcaptonuria.

Two patients of middle age, brothers, served as subjects. The older was admitted to the hospital because of a fractured leg and the alcaptonuria was discovered in the course of routine examination of the urine. In neither case did the patient seek medical advice because of the alcaptonuria. Homogentisic acid was determined by

TABLE I.

Effect of Oral Administration of *l*(−)-Phenylalanine on Urinary Excretion of Homogentisic Acid in Alcaptonuria.

Values are expressed as grams excreted per day. Figures in parentheses represent the range of values observed. Patient O.T. served as the subject in series A and B and patient R.T. in series C. The diet of series A and C was the standard hospital diet; that of B, an exclusive milk diet, except as noted.

Day	Homogentisic acid, g	Nitrogen, g	H:N × 100	Notes
		Series A.		
1-8	3.9	10.50	37	Control period
	(2.3-4.8)	(6.90-13.59)	(31-41)	
9	9.1	14.44	63	5 g phenylalanine*
10	13.0	10.99	118	10 " " *
11	4.6	12.12	38	Control day
12	4.8	12.70	38	" "
		Series B.		
1-6†	7.1	14.50	49	Control period
	(6.3-7.6)	(14.26-14.66)	(44-52)	
7	12.1	14.40	84	10 g phenylalanine‡
8	18.0	14.10	128	15 " " ‡
9	9.0	11.24	80	Control day
10	7.2	14.40	50	" "
11-15	5.1	12.40	40	" period§
	(4.4-5.8)	(11.41-13.40)	(36-42)	(hospital diet)
		Series C.		
1-7	3.90	8.90	44	Control period
	(2.8-4.9)	(7.02-10.97)	(43-45)	
8	10.3	9.10	113	10 g phenylalanine‡
9	7.70	8.19	94	10 " " ‡
10-13	3.10	7.60	40	Control period
	(2.3-4.1)	(5.23-9.60)	(39-43)	

*The phenylalanine was given in 2 portions, one at 7 and one at 12.
†The analyses were carried out on the last 3 days of the control period.
‡The phenylalanine was administered in a single dose at breakfast.
§Regular hospital diet.

2 Papageorge, E. T., and Lewis, H. B., *J. Biol. Chem.*, 1938, **123**, 211.

the method of Briggs.[3] The patients received the regular hospital diet without restriction and in one series, a diet of 3 liters of milk daily was fed (Series B). The *l*(−)-phenylalanine was administered as indicated in Table I.

It will be observed that with both patients, the ratio of homogentisic acid to nitrogen (H:N), when the usual hospital diet was fed, ranged from 31 to 42 (Subject O.T.) and 39 to 45 (Subject R.T.), values similar to previously reported values.[1, 4] When, however, the protein of the diet was derived solely from milk (Series B), the H:N ratio was higher, 44-52. This may be assumed to be due to percentages of the aromatic amino acids in the proteins of milk[5] higher than those in the proteins present in the usual mixed diet.

In Series A, 2 doses of 2.5 g each of phenylalanine were fed at 7:30 A. M. and at 12 noon respectively. Two doses of 7.5 g each were similarly ingested on the second experimental day. The conversion to homogentisic acid, as evidenced by its excretion, was practically complete on the day when the smaller doses were fed, but the extra homogentisic acid excreted on the second day, when 15 g of phenylalanine were fed, corresponded to approximately 60% of the phenylalanine. When the same subject was ingesting a milk diet, the administration of 25 g of the amino acid over a period of 2 days (Series B) resulted in extra urinary homogentisic acid, corresponding to about 70% of the theoretical value. Similar results were obtained with patient R.T. This agrees with the observation of Mittelbach[6] that the conversion of the aromatic amino acids to homogentisic acid is more complete when small amounts are fed at short intervals.

It should be noted that the excretion of homogentisic acid derived from the ingested aromatic amino acid was rapid and was, with one exception (Series B, day 9), not prolonged beyond the experimental day. The prompt excretion after the administration of the phenylalanine is also shown in the elimination of homogentisic acid over shorter periods of time on the experimental days. Thus, in Series C, day 8, the H:N ratios were 212, 160, and 58 respectively for the urines collected over 6-, 6-, and 12-hour periods after administration of the phenylalanine. The very high ratio of 212 in the first 6-hour period indicates that the excretion of extra

3 Briggs, A. P., *J. Biol. Chem.*, 1922, **51**, 453.

4 Garrod, A. E., *Inborn Errors of Metabolism*, Second Edition, 1923, 69.

5 Plimmer, R. H. A., and Lowndes, J., *Biochem. J.*, 1937, **31**, 1751.

6 Mittelbach, F., *Deut. Arch. klin. Med.*, 1903, **78**, 161.

homogentisic acid began promptly after the ingestion of phenylalanine and the low ratio of 58 in the third period indicates that the extra excretion of the acid was nearly complete in the first 12 hours. Similar results were obtained in the other series during which the urine was collected over shorter periods of time than 24 hours.

Summary. Oral administration of 10 to 15 g daily of *l*(−)-phenylalanine to two alcaptonuric brothers resulted in a notable increase in the excretion of homogentisic acid in the urine, corresponding to about 70% of the amount theoretically obtained by complete conversion of the phenylalanine fed to homogentisic acid. The excretion of the extra homogentisic acid began promptly and was nearly complete within 12 hours after the phenylalanine was fed.

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A Mechanical Pump for the Drawing of Gas Samples.

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Because of the interest aroused by a device made in this laboratory for the automatic drawing of samples from flowing gas, a brief description is being presented. The apparatus is in use here in conjunction with open-circuit respiratory metabolism determinations, the analyses for CO_2 and O_2 being performed on a Carpenter-Haldane analyzer,[1] but it can obviously be of service in any situation when it is desired to draw a continuous sample of gas over a set period of time.

Benedict and Ritzman[2] have described a method of drawing gas samples by attaching a Lee pump[3] to gearing activated by electromagnets controlled from the gas meter. The present apparatus uses a larger pump of similar construction but with the stopcock built into the cylinder head. The driving power is transmitted by

[1] Carpenter, T. M., in Abderhalden, E., *Handbuch der biologischen Arbeitsmethoden*, Berlin and Vienna, 1933, Abt. IV, Teil 13, 593.

[2] Benedict, F. G., and Ritzman, E. G., *Wissenschaft. Arch. f. Landwirtschaft*, 1931, Abt. B, **5**, 1.

[3] Lee, R. C., *J. Indus. Eng. Chem.*, Anal. Ed., 1933, **5**, 354.