XXXVIII. METABOLIC STUDIES IN PHENYLKETONURIA

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Fölling [1934] discovered that, in certain cases of mental deficiency, phenylpyruvic acid was excreted in the urine. His attention was drawn to the phenomenon by the characteristic colour produced by the addition of ferric chloride solution to the urines of these patients, and he identified the responsible substance as phenylpyruvic acid. Other cases have since been recognized [Penrose, 1935]. The condition is familial: more than one child in a family may be affected but the parents and remaining members of the family are usually normal. Genetically the abnormality appears to be inherited as a single Mendelian recessive character and, in this way, it resembles two other metabolic abnormalities, alcaptonuria and albinism, though it is not so rare as they are. It is proposed to refer to the condition as phenylketonuria.

Proof of the excretion of phenylpyruvic acid in phenylketonurics

100 ml. of urine, giving the typical ferric chloride reaction of phenylketonuria, were filtered and the filtrate treated with 100 ml. of a saturated solution of 2:4-dinitrophenylhydrazine in *N* HCl. There was formed immediately a yellow precipitate which, after standing at room temperature for 1 hour, was collected and washed with water. The precipitate was dissolved in hot alcohol and reprecipitated by the addition of a little more than the same volume of water. (Found (Weiler): C, 52·16; H, 3·61; N, 15·99%. The dinitrophenylhydrazone of phenylpyruvic acid requires C, 52·3; H, 3·49; N, 16·28%.)

The M.P. was 187° (uncorr.) and was not changed by admixture with an authentic specimen of 2:4-dinitrophenylhydrazone of phenylpyruvic acid.

4·2 mg. of the dinitrophenylhydrazone were dissolved in 10 ml. *N*/100 NaOH and titrated with *N*/100 H_2SO_4 [cf. Clift & Cook, 1932]; 8·84 ml. acid were required whence 1·16 ml. *N*/100 acid neutralized 4·2 mg. of the dinitrophenylhydrazone. (Calc. 1·22 ml.)

Methods of estimation of phenylpyruvic acid in the urine of phenylketonurics

The concentration of phenylpyruvic acid in urine can be estimated roughly by comparing the colour obtained after the addition of ferric chloride solution with the colour obtained with a standard solution of the acid; if the urine is made slightly acid and diluted to an appropriate degree the colours can be made to match very closely. This will be referred to as method P.

A more accurate and sensitive and indeed more convenient method depends on the formation of the 2:4-dinitrophenylhydrazone and the solution of this in standard alkali solution. The resulting colour is compared with standard colours

obtained with known amounts of phenylpyruvic acid. This method will be referred to as method Q and the details of this method are as follows.

Reagents required. (A) 0·7 g. of 2:4-dinitrophenylhydrazine is dissolved, with warming, in 250 ml. *N* HCl. The solution is cooled, kept in the dark in a well-stoppered flask and filtered immediately before use.

(B) *N* NaOH solution.

Technique. The suspected urine is filtered, 5 ml. of the filtrate are treated with 5 ml. of the filtered dinitrophenylhydrazine reagent, and the mixture is well shaken. In all cases of phenylketonuria there is formed almost immediately a yellow opalescence or a yellow precipitate. After 30 min. at room temperature, the mixture is again well shaken, and 2 ml. are transferred to a test-tube, to which 4 ml. *N* NaOH are added. There is formed immediately a deep red or reddish brown coloration.[1] The mixture is gently shaken, kept for a few minutes and transferred to a flask containing 24 ml. water. This is immediately compared colorimetrically with standard solutions of phenylpyruvic acid made up in normal urine and treated in the manner described. The colorimetric comparison has been greatly facilitated by the use of a Lovibond Comparator Disc, which has been prepared in conjunction with Messrs The Tintometer, Ltd., of the Colour Laboratory, Milford, Salisbury. The disc gives the colours due to normal urines containing, 0, 20, 40, 50, 60, 70, 80, 90 and 100 mg./100 ml. phenylpyruvic acid after treatment with dinitrophenylhydrazine and NaOH by the above technique. The final reddish brown alkaline solution is poured into a standard cell provided with the comparator and the colour compared with one of the disc colours, using daylight or white light as the source of light. The experimental error lies within $\pm 10\%$. If the colour given by the urine of the phenylketonuric proves to be greater than that corresponding to 100 mg./100 ml. phenylpyruvic acid, the estimation should be repeated using a mixture of 2·5 ml. urine and 2·5 water, to which the dinitrophenylhydrazine reagent is now added. The reading given on the disc is multiplied by two to give the correct estimation of phenylpyruvic acid in the urine. If the final colour is still deeper than that corresponding on the disc to 100 mg./100 ml., further dilutions of the urine should be made.

It is useless to make estimations of phenylpyruvic acid in urines which have become contaminated and have developed an alkaline reaction. Under these conditions the ketonic acid decomposes relatively quickly. Urines should be preserved by the addition of a few crystals of thymol, which does not interfere with the estimation, and should be kept in the ice-chest.

The presence of acetoacetic acid interferes with the estimation and should be tested for in all cases by the usual Rothera test. The addition of glucose seems not to interfere with the estimation, but, so far, no cases of phenylketonuria have been found in which there have been any abnormalities so far as acetone bodies, glucose and protein are concerned.

Typical values of phenylpyruvic acid excretion in phenylketonurics

Patient A, a female imbecile phenylketonuric, age 42. The amount of urine passed in 24 hours was 1090 ml., sp. gr. 1015, *p*H 5·0, urea content 2·02%. The concentration of phenylpyruvic acid in the urine was 126 mg./100 ml. by method Q and 120 mg./100 ml. by method P. The total excretion of phenylpyruvic acid, in this patient, was therefore rather more than 1 g. daily.

[1] A normal urine—containing no phenylpyruvic acid or other ketonic acid—will give under these conditions a red colour which disappears quickly leaving a slightly yellow solution.

With patients of this grade of mental defect it is not always easy to obtain the co-operation necessary for the best experimental conditions. The total quantity of urine passed every 24 hours could not always be obtained and specimens were therefore taken at regular times: (i) 9.30 a.m., (ii) 3.30 p.m., (iii) 6.30 p.m. and (iv) 5.30 a.m. next morning. The mean value of the readings of these four specimens corresponded closely to the readings of the 24 hours' specimen.

A typical set of readings in 1 day is as follows:

9.30 a.m.	... ...	164 mg./100 ml.	phenylpyruvic acid
3.30 p.m.	... ...	120	,, ,,
6.30 p.m.	... ...	176	,, ,,
5.30 a.m.	... ...	98	,, ,,
Mean value	... ...	139	,, ,,

Results with patient A are shown in Table I. In this table it will also be seen that the ratio of phenylpyruvic acid excretion to urea excretion (taking mean values) is approximately constant. About 1 g. phenylpyruvic acid is excreted for every 15 g. urea.

Table I. *Normal diet. Estimation of phenylpyruvic acid in urine by method Q*

Patient	Date	Phenylpyruvic acid mg./100 ml.	Urea %	*p*H	Phenylpyruvic/urea Ratio
		(*a*) 24 hours' specimen			
A	28. xii. 35	126	2·02	5·0	0·063
(*b*) Means of specimens at 9.30 a.m., 3.30 p.m., 6.30 p.m. and 5.30 a.m. next morning					
A	20. xii. 35	139	1·98	5·2	0·070
A	26. iii. 36	136	2·19	5·2	0·062
A	27. iii. 36	115	1·51	6·3	0·076
	Average value	130	1·89	5·7	0·069

Typical figures indicating the range of variation of the phenylpyruvic/urea ratio during the day are shown in Table II.

Table II

Date and time Patient A		Phenylpyruvic acid concentration mg./100 ml.	Urea %	Ratio
26. iii. 36	5.30 a.m.	90	1·59	0·056
	9.30 a.m.	200	2·43	0·080
	3.30 p.m.	120	2·18	0·055
	6.30 p.m.	112	2·00	0·055
27. iii. 36	5.30 a.m.	112	2·16	0·052
	9.30 a.m.	180	2·13	0·085
	3.30 p.m.	60	0·92	0·065
	6.30 p.m.	90	1·32	0·068
28. iii. 36	5.30 a.m.	130	1·65	0·079

Results with patient A using method P are shown in Table III.

In the case of patient B, male phenylketonuric idiot, age 21, the phenylpyruvic acid excretion was distinctly less than in the case of patient A. In this case values of 40–50 mg./100 ml. phenylpyruvic acid in the urine were recorded. The phenylpyruvic/urea ratio was found to be about 0·04.

Table III. *Normal diet. Estimation of phenylpyruvic acid in urine by method P*

Patient	Date	Phenylpyruvic acid mg./100 ml.
	(*a*) 24 hours' specimen	
A	28. xii. 35	120
	(*b*) Means of specimens at 9.30 a.m., 3.30 p.m., 6.30 p.m. and 5.30 a.m. next morning	
A	20. xii. 35	148
A	26. iii. 36	80
A	27. iii. 36	75
A	1. vii. 36	108
A	2. vii. 36	133
	Average value	109

Effects of partial protein starvation on phenylpyruvic acid excretion

If phenylketonurics are unable properly to metabolize a constituent of the protein of the diet, it was thought likely that if the protein intake could be reduced to a minimum, the concentration of phenylpyruvic acid in the urine would be greatly decreased.

This experiment was carried out on patient B. This patient was not sufficiently co-operative to make the collection of urine specimens at definite times possible. Daily specimens were, however, collected and the average daily excretion of phenylpyruvic acid found to be about 50 mg./100 ml. The patient was then placed on a diet containing less than half the usual amount of protein. The phenylpyruvic acid excretion dropped at once, only traces of the ketonic acid being found in the urine during the first 2 days. On the 4th, 5th, 6th and 7th days the concentration rose to a constant level of 35 mg./100 ml. and on the 8th day it rose to 75 mg./100 ml. During this period the patient lost ½ lb. in weight. The conclusion would be that phenylpyruvic acid formation is dependent on the breakdown of a constituent of the dietary protein; when the latter is greatly reduced in amount, endogenous breakdown of tissue proteins would account for the secondary production of phenylpyruvic acid in the urine.

Effects of feeding phenylalanine

Fölling reported that, in phenylketonuria, the excretion of phenylpyruvic acid is increased on addition of phenylalanine to the diet. We have confirmed this observation.

The addition of 1 g. phenylalanine to the diet causes a slight, but not very marked rise in the concentration of phenylpyruvic acid in the urine and also a rise in the phenylpyruvic acid/urea ratio. After a dose of 3 g. the increase of phenylpyruvic acid excretion and of the phenylpyruvic acid/urea ratio is very marked.

Results obtained with patient A after dosages of 3 g. *dl*-phenylalanine, *d*-phenylalanine and *l*-phenylalanine are shown in Table IV. The results indicate a rise of the order 0·03 in the phenylpyruvic acid/urea ratio (i.e. from a normal value of 0·069 (see Table I) to an average value of 0·095 after 3 g. phenylalanine administration). Both optically active forms of phenylalanine seem to be about equally effective in causing extra phenylpyruvic acid excretion in the urine. In the experiments the results of which are recorded in Table IV, 1 g. of the amino-acid was administered to the patient with her breakfast at about 7.30 a.m. and 2 g. were given at about 12.30 p.m.

Table IV. *Normal diet. Means of 4 specimens of urine per day*

Patient	Date	Substance added to diet	Phenylpyruvic acid mg./100 ml. Method Q	Phenylpyruvic acid mg./100 ml. Method P	Urea %	Phenylpyruvic (Q)/urea Ratio
A	3. xii. 35	*dl*-Phenylalanine (3 g.)	114	143	1·17	0·097
A	11. ii. 36	*d*-Phenylalanine (3 g.)	159	180	1·65	0·096
A	14. i. 36	*l*-Phenylalanine (3 g.)	158	147	1·80	0·088
A	29. iv. 36	*l*-Phenylalanine (3 g.)	200	185	1·98	0·101
A	18. xii. 35	Tyrosine (6 g.)	171	151	2·47	0·069

It seems likely from these results that the phenylpyruvic acid excreted in phenylketonuria arises from faulty breakdown of phenylalanine present in the diet. The disturbance in the phenylalanine metabolism cannot be considered complete, however, for calculation shows that in the normal diet of patient A there is a daily intake of 2–2·5 g. phenylalanine with a daily output of 1–1·5 g. phenylpyruvic acid. Nearly half, therefore, of the phenylalanine in the normal diet of the patient seems to be metabolized completely.[1] Moreover, the extra production of phenylpyruvic acid, after administration of an extra 3 g. phenylalanine, is, so far as we can judge, definitely less than what would be expected if the amino-acid were transformed only into the ketonic acid.

Experiments were carried out with patient B to determine the effects of feeding phenylalanine to a phenylketonuric on a low protein diet. Four daily doses of 1 g. *dl*-phenylalanine caused a rise in the phenylpyruvic acid excretion and in the phenylpyruvic/urea ratio. This may be seen in the results given in Table V, where it should be noted that the observations refer only to single daily specimens of urine. The low protein diet had to be stopped because the patient was continuing to lose weight at the rate of 1 lb. a week.

Table V. *Low protein diet. Single daily specimens of urine; average values*

Patient	Date	Substance added to diet	Phenylpyruvic acid mg./100 ml. Method P	Urea %
B	7–11. x. 35	—	96	1·44
B	12–14. x. 35	Tyrosine (2 g. daily; 3 days)	86	1·29
B	15–18. x. 35	—	152	2·73
B	19–22. x. 35	*dl*-Phenylalanine (1 g. daily; 4 days)	218	2·16
B	23–24. x. 35	—	82	1·38

In order to assess properly the effects of feeding phenylalanine to phenylketonurics, similar experiments were carried out on feeble-minded patients who did not exhibit phenylketonuria.

Patient C, feeble-minded female, age 45, normally excreted no phenylpyruvic acid. After a dose of 3 g. *dl*-phenylalanine a mean concentration of about 20 mg./100 ml. phenylpyruvic acid was observed in the urine during the next 24 hours. Administration of 3 g. *d*-phenylalanine had an even more marked effect, a mean concentration of 30 mg./100 ml. phenylpyruvic acid being found in the urine. Administration of 3 g. *l*-phenylalanine, however, gave rise only to traces of phenylpyruvic acid in the urine. It thus appears that normally *l*-phenylalanine is metabolized more completely than the *d*-form, under similar experimental conditions. These results were confirmed by giving patient D, a

[1] Phenylalanine determinations in the urine have, however, not been made.

feeble-minded female, age 30, with no phenylketonuria, 3 g. of *dl*-phenylalanine after which the excretion of 5 mg./100 ml. phenylpyruvic acid was observed. Patient E, a feeble-minded female age 30, developed no phenylketonuria after being fed on 3 g. *l*-phenylalanine. The increase in the phenylpyruvic/urea ratio, after the administration of *dl*- or *d*-phenylalanine to non-phenylketonurics, is not as great as that obtained after similar administration to phenylketonurics (see Table IV). These results are shown in Table VI.

Table VI. *Control experiments. Non-phenylketonuric patients. Normal diet. Means of 4 specimens per day*

Patient	Date	Substance added to diet	Phenylpyruvic acid mg./100 ml. Method Q	Phenylpyruvic acid mg./100 ml. Method P	Urea %	Phenylpyruvic (Q)/urea Ratio
C	12. xi. 35	*dl*-Phenylalanine (3 g.)	—	15	—	—
C	3. xii. 35	*dl*-Phenylalanine (3 g.)	24	22	1·47	0·016
D	14. xi. 35	*dl*-Phenylalanine (3 g.)	—	5	—	—
C	14. i. 36	*d*-Phenylalanine (3 g.)	30	22	1·72	0·017
C	11. ii. 36	*l*-Phenylalanine (3 g.)	0	0	1·29	0·000
E	29. iv. 36	*l*-Phenylalanine (3 g.)	0	0	1·11	0·000
C	18. xii. 35	Tyrosine (6 g.)	? Trace	0	0·80	?

The results of the feeding experiments with phenylalanine lead to the following conclusions:

(1) In phenylketonuria, only part of the phenylalanine ingested appears as phenylpyruvic acid.

(2) The phenylketonuric has almost as great a difficulty in metabolizing *l*-phenylalanine as in metabolizing *d*-phenylalanine. The non-phenylketonuric, on the other hand, apparently metabolizes *l*-phenylalanine with greater ease than either *dl*- or *d*-phenylalanine. Both optically active forms are metabolized less completely in the phenylketonuric than in the normal.

Effects of feeding tyrosine

Administration of tyrosine to phenylketonurics (patients A and B) did not consistently result in any increase in the phenylpyruvic acid excretion. The effect of tyrosine feeding (patient A) in small quantities (2–3 g.) on the rate of phenylpyruvic acid excretion during the subsequent 24 hours was scarcely noticeable. During the next 2 or 3 days, however, there seemed to be a slight increase in the ketonic acid formation. 6 g. of tyrosine given in doses of 2 and 4 g. appeared to cause a small rise in the ketonic acid excretion but the phenylpyruvic/urea ratio was unaltered (Table IV). In the case of patient B, 2 g. tyrosine were given daily for 3 days without noticeable alteration in the ketonic acid production (Table V), but during the 3 days following, the concentration of phenylpyruvic acid appeared to be slightly higher.

It was found impossible to induce any phenylketonuria in control patients by feeding tyrosine, though in patient C after a dose of 6 g. tyrosine there may have been a trace of ketonic acid excreted (Table VI).

The results indicate that in phenylketonuria tyrosine is probably normally metabolized.

Effects of feeding l-*alanine*

Administration of alanine (in doses of 10 g.) to the phenylketonuric (patient A) did not result in an increased excretion of ketonic acids in the urine. The phenylpyruvic/urea ratio before the administration of the alanine was 0·07, and

4 hours after feeding the amino-acid the ratio was 0·06. This result indicates, as may have been expected, that alanine is normally metabolized in the phenylketonuric. The amino-acid produced no ketonuria in the control patient (patient C).

Effects of feeding phenylpyruvic acid

The results, so far, might be explained by assuming that phenylalanine undergoes two modes of breakdown in the body: (i) through phenylpyruvic acid and (ii) possibly through tyrosine. Evidence for the first process comes from the work of Fölling and from our own results. There is also evidence that, *in vitro*, in presence of kidney slices phenylalanine is oxidized to phenylpyruvic acid [Krebs, 1933]. The evidence for the second process comes from the studies of alcaptonuria (Falta), from the work of Embden & Baldes [1913] who reported that perfusion of the liver with *dl*-phenylalanine leads to the production of small amounts of *l*-tyrosine, and from the work of Medes [1932]. Probably the first of these processes takes place more easily than the second. It is not unlikely that phenylpyruvic acid is a normal product of metabolism. The fault in phenylketonuria may lie not in excessive conversion of phenylalanine into phenylpyruvic acid but in the failure to break down phenylpyruvic acid further.

Experiments were therefore carried out to determine whether phenylpyruvic acid is broken down with equal ease in the phenylketonuric and the non-phenylketonuric.

Results of feeding phenylpyruvic acid to two phenylketonurics (patients A and B) and to a non-phenylketonuric (patient C) are shown in Table VII, where the mean results of 4 specimens of urine per day are recorded. Administration of phenylpyruvic acid to a phenylketonuric causes an immediate increase in the

Table VII. *Normal diet. Means of 4 specimens of urine per day*

Patient	Date	Substance added to diet	Phenylpyruvic acid mg./100 ml. Method Q	Method P	Urea %	Phenylpyruvic (Q)/urea Ratio
A	21. iii. 36	Phenylpyruvic acid (1·5 g.)	190	—	2·28	0·083
A	1. iv. 36	,, (3 g.)	198	175	1·67	0·119
C	19. iii. 36	,, (1·5 g.)	12	2	0·78	0·015
C	1. iv. 36	,, (3 g.)	29	21	1·02	0·028
		Normal diet: single daily specimens of urine, means of 3 readings				
B	—	—	38	—	1·56	0·024
B	9. iii. 36	Phenylpyruvic acid (1 g.)	160	—	2·02	0·079

excretion of this acid and a decided rise in the phenylpyruvic/urea ratio. Thus with patient A, a normal ratio of 0·069 (see Table I) is increased to 0·119 after administration of 3 g. of the acid. Administration of phenylpyruvic acid to the non-phenylketonuric causes phenylketonuria, but the rise in the phenylpyruvic/urea ratio (0·00–0·028 with patient C: see Table VII) is not as great as with the phenylketonuric. Curves showing the variation of the phenylpyruvic/urea ratio with time after giving doses of 3 g. phenylpyruvic acid to patients A and C are given in Fig. 1. It will be seen that the increased phenylpyruvic acid excretion is more prolonged in the case of the phenylketonuric than in that of the non-phenylketonuric.

The total excretion of phenylpyruvic acid over 24 hours with and without phenylpyruvic acid administration was determined by collection of 24-hour

specimens of urine from patients. The results are shown in Table VIII and confirm the conclusion that phenylpyruvic acid is metabolized with greater difficulty in the phenylketonuric than in the non-phenylketonuric.

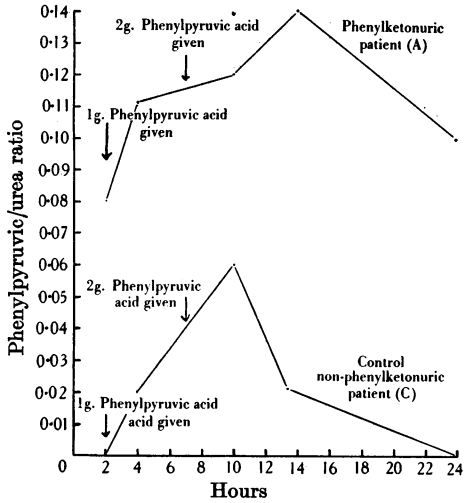

Fig. 1. Effects of phenylpyruvic acid feeding on phenylpyruvic acid excretion in the urine.

Table VIII

Patient	Date	Substance added to diet	Vol. of urine collected in 24 hours ml.	Phenyl-pyruvic acid mg./100 ml.	Amount of phenyl-pyruvic acid excreted in 24 hours mg.
A	21. x. 36	Normal diet	1338	100	1338
A	22. x. 36	1 g. phenylpyruvic acid (given in two doses of 0·5 g.)	1556	110	1712
E (Control)	21. x. 36	Normal diet	—	0	Nil
E	22. x. 36	1 g. phenylpyruvic acid (given in two doses of 0·5 g.)	1049	12	126

Increase in amount of phenylpyruvic acid excreted with patient A = 374 mg.
Increase in amount of phenylpyruvic acid excreted with patient E = 126 mg.

Breakdown of phenylpyruvic acid in the body

The fact that phenylpyruvic acid administration increases phenylpyruvic excretion in the phenylketonuric to a greater extent than in the normal makes it a reasonable view that the metabolic disturbance in phenylketonuria is directly concerned with the rate of breakdown of phenylpyruvic acid.

The normal mode of breakdown in the body of the ketonic acid is at present unknown. It would be attractive to suppose, as in Neubauer's scheme, that normally oxidation to *p*-hydroxyphenylpyruvic acid takes place with subsequent formation of homogentisic acid. If this were so, the conditions of phenylketonuria, tyrosinosis [Medes, 1932] and alcaptonuria would be chemically related in the sense that they represent separate metabolic disturbances in the same line of breakdown of phenylalanine. Another possibility is that the

benzene ring in phenylpyruvic acid may undergo complete rupture as in Dakin's scheme. Whichever view be correct, it seems likely that phenylketonurics are unable to attack the ring structure in phenylpyruvic acid at the same rate as the normal. The facts presented in this paper may be accounted for on this view. The development of phenylketonuria in non-phenylketonurics after administration of *d*- and *dl*-phenylalanine may be simply explained by the more rapid rate of oxidation of the *d*-form than of the *l*-form of the amino-acid to phenylpyruvic acid. Such a difference between the rates of oxidation appears to occur *in vitro* with kidney slices [Krebs, 1933].

Further feeding experiments on phenylketonurics with derivatives of phenylalanine and of phenylpyruvic acid are necessary and these should help to settle the problem of the mode of breakdown of phenylpyruvic acid in the body.

Summary

1. It is confirmed that in phenylketonuria, phenylpyruvic acid is excreted into the urine. In one patient 1–1·5 g. phenylpyruvic acid are excreted in 24 hours; this represents the incomplete metabolism of at least half of the phenylalanine in the daily protein intake.

2. A rapid and reasonably accurate method for the estimation of phenylpyruvic acid in urine is described.

3. Phenylketonuric and control patients have been fed with the following substances: alanine, tyrosine, *dl*-phenylalanine, *d*-phenylalanine, *l*-phenylalanine and phenylpyruvic acid. The effects of these substances on the rate of excretion of phenylpyruvic acid into the urine have been studied.

4. The results of phenylalanine feeding show:

(*a*) that in phenylketonurics, the three forms of the amino-acid lead to increased excretion of phenylpyruvic acid to approximately equal degrees;

(*b*) that in control patients, ingestion of *l*-phenylalanine (in the specified amounts) does not lead to phenylketonuria, whereas ingestion of *d*- or *dl*-phenylalanine produces phenylketonuria to a slight extent;

(*c*) that the extra phenylalanine ingested by phenylketonurics is not all transformed into phenylpyruvic acid; possibly a portion is metabolized completely in a normal way.

5. In phenylketonurics the ratio of phenylpyruvic acid to urea content of the urine, when taken as the mean of several specimens of urine during the day, is approximately constant. This ratio is increased by phenylalanine feeding. It is not increased by tyrosine or alanine feeding. Tyrosine appears to cause a slight increase in the concentration of ketonic acids in the urine of phenylketonurics; it is, on the whole, normally metabolized.

6. Phenylpyruvic acid feeding causes a greater excretion of phenylpyruvic acid in phenylketonurics than in control patients.

7. It is suggested that the metabolic disturbance in phenylketonurics is due largely to a diminished rate of oxidation, or rupture, of the benzene ring in phenylpyruvic acid.

REFERENCES

Clift & Cook (1932). *Biochem. J.* **26**, 1802.
Embden & Baldes (1913). *Biochem. Z.* **55**, 301.
Fölling (1934). *Hoppe-Seyl. Z.* **227**, 169.
Krebs (1933). *Hoppe-Seyl. Z.* **217**, 191.
Medes (1932). *Biochem. J.* **26**, 917.
Penrose (1935). *Lancet*, i, 23; ii, 192.