or are formed by the intestinal flora. In any case there appears little reason to suppose that decarboxylation is a major pathway of lysine metabolism in the mammalian body, although it cannot be completely ruled out, especially in view of the fact that enzymes, capable of decarboxylating specific amino-acids, have been demonstrated in animal tissues (Blaschko, 1942*a*, *b*).

It seems, therefore, most likely that lysine is metabolized by one of the two mechanisms discussed above, which lead either to glutaric acid or to δ-amino-valeric acid; Ringer, Frankel & Jonas (1913) suggested some time ago that the former was an intermediate in lysine metabolism.

SUMMARY

1. The ingestion of large amounts of *l*-lysine hydrochloride by the rat gives rise to a greatly increased excretion of urea, or ammonia if the hydrochloric acid is not neutralized by sodium bicarbonate. It is concluded that both the nitrogen atoms are ultimately converted into urea in the rat. *d*-Lysine is largely excreted unchanged.

2. A method for measuring, with a specific bacterial decarboxylase, the formation or disappearance of lysine in tissue slices is described. It is shown by this method that kidney demethylates ε-methyl-lysine to lysine.

3. The action of *d*-amino-acid oxidase on diamino-acids and their derivatives is investigated. This enzyme oxidizes ε-acetyl- and ε-benzoyl-*d*-lysine, although it is inactive towards lysine. In the case of ornithine the free amino-acid is slightly more rapidly oxidized than the δ-acetyl derivative. The *l*-amino-acid oxidase also attacks ε-acetyl-*l*-lysine slowly and *l*-lysine itself not at all.

4. The intermediary metabolism of lysine is discussed and it is suggested that the first step in the breakdown might consist either of acetylation of the terminal amino group, followed by oxidation of the ε-amino group, or an initial oxidation of the ε-amino group leading either to δ-amino-valeric acid or to glutaric acid.

REFERENCES

Abderhalden, E. & Tetzner, E. (1935). *Hoppe-Seyl. Z.* **232**, 79.
Blaschko, H. (1942*a*). *Biochem. J.* **36**, 571.
—— (1942*b*). *J. Physiol.* **101**, 337.
Borsook, H. & Dubnoff, J. W. (1941). *J. biol. Chem.* **138**, 389.
Butts, J. S. & Simmhuber, R. O. (1941). *J. biol. Chem.* **140**, 597.
Cohen, P. P. (1939). *Biochem. J.* **33**, 1478.
—— (1940). *J. biol. Chem.* **136**, 585.
Conway, E. J. (1933). *Biochem. J.* **27**, 430.
Corley, R. C. (1926). *J. biol. Chem.* **70**, 99.
Dakin, H. D. (1913). *J. biol. Chem.* **14**, 321.
Doty, J. R. & Eaton, A. G. (1937). *J. biol. Chem.* **122**, 139.
—— —— (1939). *J. Nutrit.* **17**, 397.
Feigl, F. (1939). *Qualitative Analysis by Spot Tests*, 2nd ed. New York: Nordmann Publishing Co.
Felix, H. & Zorn, K. (1939). *Hoppe-Seyl. Z.* **258**, 16.
—— & Naka, S. (1940). *Hoppe-Seyl. Z.* **264**, 123.
Gale, E. F. & Epps, H. M. R. (1943). *Nature, Lond.*, **152**, 327.
Green, D. E., Nocito, V. & Ratner, S. (1943). *J. biol. Chem.* **148**, 461.
Handler, P., Bernheim, F. & Klein, J. R. (1941*a*). *J. biol. Chem.* **138**, 203.
—— Bernheim, M. L. C. & Klein, J. R. (1941*b*). *J. biol. Chem.* **138**, 211.
Krebs, H. A. (1933). *Hoppe-Seyl. Z.* **217**, 191.
—— (1935). *Biochem. J.* **29**, 1620.
—— (1939). *Enzymologia*, **7**, 53.
—— & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
Mende, F. (1896). *Ber. dtsch. chem. Ges.* **29**, 2887.
Negelein, E. & Broemel, H. (1939). *Biochem. Z.* **300**, 225.
Neuberger, A. & Sanger, F. (1943). *Biochem. J.* **37**, 515.
—— —— (1944). *Biochem. J.* **38**, 125.
Ratner, S., Weissmann, N. & Schoenheimer, R. (1943). *J. biol. Chem.* **147**, 549.
Ringer, A. I. (1912). *J. biol. Chem.* **12**, 223.
—— Frankel, E. M. & Jonas, L. (1913). *J. biol. Chem.* **14**, 339.
Schoenheimer, R., Ratner, S. & Rittenberg, O. (1939*a*). *J. biol. Chem.* **127**, 333.
—— —— —— (1939*b*). *J. biol. Chem.* **130**, 703.
Sharp, G. O. & Berg, C. P. (1941). *J. biol. Chem.* **141**, 739.
Udransky, L. v. & Baumann, E. (1889). *Hoppe-Seyl. Z.* **13**, 562.
Van Slyke, D. D., Hiller, A. & MacFadyen, B. A. (1941). *J. biol. Chem.* **141**, 681.
Vickery, H. B. & White, A. (1933). *J. biol. Chem.* **103**, 413.
Weissmann, N. & Schoenheimer, R. (1941). *J. biol. Chem.* **140**, 779.

The Availability of ε-Acetyl-*d*-lysine and ε-Methyl-*dl*-lysine for Growth

By A. NEUBERGER and F. SANGER (Benn W. Levy Student), *Biochemical Department, Cambridge, and the National Institute for Medical Research, London*

(*Received* 27 *January* 1944)

It was shown in an earlier paper (Neuberger & Sanger, 1943) that ε-acetyl-*l*-lysine can replace *l*-lysine in the diet, presumably because the ε-acetyl derivative is converted in the body into the free amino-acid. This work has now been extended to ε-acetyl-*d*-lysine. *d*-Lysine itself cannot replace the

natural *l*-isomer (Berg, 1936*a*), probably because it is not attacked by the *d*-amino-acid oxidase (Felix & Naka, 1940), and the series of reactions which consists of oxidation to the keto acid and asymmetric reductive amination to the *l*-amino-acid cannot take place. It was found, however (Neuberger & Sanger, 1944), that, if the terminal amino group is masked as in ε-acetyl or ε-benzoyl-lysine, oxidative deamination by the *d*-amino-acid oxidase occurs. It appears possible, therefore, that ε-acetyl-*d*-lysine might be available for growth, although *d*-lysine itself is not. ε-Acetyl-*d*-lysine can be oxidized presumably to ε-acetamino-α-ketocaproic acid which then might be aminated, like many other amino-acids, to ε-acetyl-*l*-lysine, a compound which is known to replace *l*-lysine in the diet.

The *d*-lysine required for this work has been made by resolution of *dl*-lysine with camphoric acid (Berg, 1936*b*). The difficulty of obtaining *l*-camphoric acid has caused us to investigate other ways of preparation. We describe in this paper two methods which we have found convenient: one makes use of the stereo-chemical specificity of the lysine decarboxylase found in coliform organism (Gale & Epps, 1943); the other, which gives an optically purer product, consists of a Walden inversion occurring on treatment of ε-benzoyl-*l*-lysine with nitrosyl bromide followed by amination.

The results so far obtained with lysine derivatives justify the generalization that compounds which are altered in the α-position are not available for growth on a lysine-deficient diet. Thus α-hydroxy-ε-amino-caproic acid (McGinty, Lewis & Marvel, 1924), α-*N*-methyl- and α-*N*-dimethyl-*dl*-lysine (Gordon, 1939), α-acetyl-*l*-lysine (Neuberger & Sanger, 1943) and *d*-lysine (Berg, 1936*a*) cannot replace *l*-lysine. On the other hand, ε-acetyl-*l*-lysine, the only derivative altered in the terminal amino group, so far investigated, is active on a lysine-deficient diet (Neuberger & Sanger, 1943). It seemed of interest, therefore, to examine the availability of ε-*N*-methyl-lysine for growth. This compound has been obtained by Enger & Steib (1930) in an amorphous form. We have modified their method of preparation and obtained the crystalline monohydrochloride of this amino-acid.

EXPERIMENTAL

Preparation of compounds

Preparation of d-*lysine by the bacterial decarboxylase.* Gale & Epps (1943) have shown that the purified cell-free preparation of lysine decarboxylase obtained from *Bact. cadaveris* is stereochemically specific for *l*-lysine; they have also demonstrated that the reaction goes almost to completion and is not inhibited by the *d*-isomer. This method has been adapted to preparative work. *d*-Lysine was separated from cadaverine by benzoylation (Udransky & Baumann, 1889) and the dibenzoyl-*d*-lysine was then hydrolyzed to the free amino-acid. It was found that the preparations of *d*-lysine thus obtained contained small amounts—usually about 3–4%—of the *l*-isomer; this is probably due not so much to the incompleteness of the enzymic reaction as to a slight racemization during the benzoylation.

An extract of the acetone-dried tissues of the organism was adsorbed on alumina as described by Gale & Epps (1943); for preparative purposes the centrifuged alumina, suspended in phosphate buffer, was used as such. The activity of the enzyme was estimated manometrically and the amount of enzyme suspension used was twice as much as would be required to finish the reaction, with the amount of substrate taken, in 30 min.

To the enzyme alumina suspension (100 ml.) was added 0·25 M-phosphate of pH 6·0 (700 ml.) containing *dl*-lysine hydrochloride (2 g.). The mixture was shaken at 37° for 90 min.; an estimation with fresh enzyme on a sample of the mixture indicated that no *l*-lysine was then left in the solution. The alumina was then filtered off and the filtrate made alkaline to phenolphthalein with $Ba(OH)_2$; the precipitate of $Ba_3(PO_4)_2$ was filtered off and well washed with boiling water. The combined filtrate and washings were made neutral with H_2SO_4, and the $BaSO_4$ was filtered off and washed. The solution was then evaporated to dryness *in vacuo*, the residue was taken up in water, and benzoylation with benzoyl chloride (9·2 g.) and N-NaOH (160 ml.) was then carried out in the usual way. Dibenzoyl-cadaverine, which precipitated during the reaction, was filtered off; the filtrate was then extracted with chloroform and acidified. The precipitate, which consisted of benzoic acid and dibenzoyl-lysine, was filtered off and dried. The dry material was extracted with boiling ligroin and the residue dissolved in warm acetone. On careful addition of water, and cooling, dibenzoyl-*d*-lysine of m.p. 145° was obtained. This was hydrolyzed by boiling with 20% HCl for 8 hr. Benzoic acid was then removed by filtration and extraction with ether, and the aqueous solution concentrated to dryness *in vacuo*. The residue was taken up in ethanol, and addition of pyridine precipitated the monohydrochloride, which was recrystallized from aqueous ethanol. The yield was 45% of the amount of *d*-lysine used. It gave N, 15·3 (calc. 15·3) and $[\alpha]_D$ (5% HCl, $c = 2{\cdot}1$) $= -14{\cdot}7°$. The latter compares with a value of $-15{\cdot}6°$ obtained by Berg (1936*b*); if the optical purity of Berg's preparation is accepted, it follows that *d*-lysine obtained by this method contains about 3% of the *l*-isomer.

Preparation of d-*lysine by Walden inversion.* The action of nitrosylbromide on ε-benzoyl-*l*-lysine leads to a laevorotatory α-bromo-ε-benzamino caproic acid which on treatment with ammonia gives an ε-benzoyl-*d*-lysine having a rotation equal in absolute value but opposite in sign to the starting material. This indicates that a Walden inversion without any racemization has taken place in one of the two reactions. The absolute configuration of the α-bromo-acid is not known; the change of rotation on ionization (Levene & Kuna, 1941) may, however, be taken as evidence that the bromo-acid belongs to the *d*-series and inversion has taken place during the first reaction.

α-Bromo-ε-benzamino-caproic acid. ε-Benzoyl-*l*-lysine (7·5 g.) was dissolved in 2·5 N-H_2SO_4 (60 ml.) containing KBr (12 g.) and the solution was stirred at 0°. $NaNO_2$ (3·15 g.) was then added in small portions over 1 hr. The white crystalline precipitate which had formed during the reaction was filtered off, washed with water and dried.

Yield was 80% of the theoretical; m.p., after recrystallization from 50% aqueous ethanol, 132°; $[\alpha]_D = -32{\cdot}9°$ (70% ethanol; $c = 1{\cdot}9$); $[\alpha]_D = -16{\cdot}4°$ (60% ethanol + 1 mol. NaOH; $c = 1{\cdot}6$). (N, 4·2%; calc. for $C_{13}H_{16}O_3NBr$, 4·4.)

*ε-Benzoyl-*d*-lysine.* *d*-α-Bromo-ε-benzamino-caproic acid (12·3 g.) was dissolved in ammonia of sp. gr. 0·88 (140 ml.), and the solution left for 4 days at room temperature. The crystals which had formed were filtered off, and the mother liquors concentrated to give a further crop of crystals. Total yield was 90% of the theoretical. $[\alpha]_D = -19{\cdot}6°$ (5% HCl; $c = 1{\cdot}76$). The original benzoyl-*l*-lysine gave $[\alpha]_D = +19{\cdot}1°$ (5% HCl; $c = 1{\cdot}95$).

d-*Lysine hydrochloride.* ε-Benzoyl-*d*-lysine was hydrolyzed with 20% HCl for 10 hr., and the hydrolysate worked up as described above. Yield of the monohydrochloride was 85% of the theoretical. N, 15·2; calc., 15·3. $[\alpha]_D = -15{\cdot}7°$ (5% HCl; $c = 2{\cdot}0$).

*ε-Acetyl-*d*-lysine.* This substance was prepared from *d*-lysine sulphate exactly as described for the *l*-compound (Neuberger & Sanger, 1943). It had $[\alpha]_D = -3{\cdot}5°$ ($c = 4{\cdot}5$).

Preparation of ε-N-*methyl*-dl-*lysine.* The preparation of this compound followed on the lines described by Enger & Steib (1930). The procedure of these authors was, however, modified in several of the preparative steps.

ε-Amino-caproic acid obtained from *cyclo*-hexanone oxime (Wallach, 1900) was treated with *p*-toluene sulphonyl chloride as described by Thomas & Görne (1918); the resulting ε-*p*-toluenesulphonyl-amino-caproic acid was methylated with methyl sulphate and alkali in the usual manner. The removal of the toluene sulphonyl group was carried out by reduction with phosphonium iodide and hydriodic acid.

ε-N-benzoyl-methylamino-caproic acid. ε-*p*-Toluene-sulphonyl-methylamino-caproic acid (24 g.) was heated in a pressure bottle with phosphonium iodide (16 g.) and hydriodic acid of sp. gr. 1·94 (120 ml.) at 70° for 3–4 hr. Water was then added, and the crystalline *p*-tolyl-mercaptan was filtered off, and the solution concentrated *in vacuo* to dryness. Excess HI was removed by repeated evaporation *in vacuo*. The oily residue was then taken up in water and treated with excess $Ba(OH)_2$ to remove phosphate. It was found convenient not to isolate the hygroscopic ε-methylamino-caproic acid, but to benzoylate the neutralized aqueous solution directly. Benzoyl chloride (10 g.) and 50% NaOH (12 ml.) were added with shaking in 10 portions over 30 min. The solution was then filtered and acidified. The oil crystallized on seeding, and after recrystallization from aqueous ethanol had m.p. 75°. Yield was 84% of the theoretical from ε-*p*-toluene-sulphonyl-ε-methylamino-caproic acid.

To obtain the initial seed crystals, the oil was extracted into ether and from ether into a solution of K_2CO_3 which was then acidified and again extracted into ether. The ethereal solution was then dried and the ether evaporated. The oily residue was boiled up with a little ligroin and then left in a desiccator. On prolonged standing it slowly crystallized.

ε-N-Benzoyl-ε-N-methyl-dl-*lysine.* The benzoyl methylamino-caproic acid (30 g.) was brominated as described by Enger & Steib (1930), and the oily residue treated with ammonia (sp. gr. 0·88) at room temperature for 4 days. The solution was then filtered and concentrated. ε-*N*-Benzoyl-ε-*N*-methyl-*dl*-lysine was obtained in a 30% yield.

ε-N-Methyl-dl-*lysine picrate.* It was found that hydrolysis by acid removed methyl groups and gave a mixture of methyl lysine and lysine. Alkaline hydrolysis, however, gave a pure product. ε-Benzoyl-ε-*N*-methyl-*dl*-lysine (29·5 g.) was refluxed with 2N-$Ba(OH)_2$ (100 ml.) for 4 hr. The solution was then made acid to Congo red with H_2SO_4, boiled and the $BaSO_4$ filtered off and well washed. Benzoic acid was removed by filtration of the cooled solution and extraction with ether. Excess H_2SO_4 was then exactly removed and picric acid (8 g.) in hot ethanol was added. The picrate was filtered off and twice recrystallized from water. Yield of the recrystallized product was 77% of the theoretical. It had m.p. 229°. (Found: C, 40·3; H, 5·0; N, 18·0. Calc. for $C_{13}H_{19}O_9N_5$: C, 40·1; H, 4·9; N, 18·0%.)

ε-N-Methyl-dl-*lysine hydrochloride.* 10·8 g. of the pure picrate were dissolved in hot water and 10% HCl (50 ml.) was added. On cooling, picric acid crystallized out and was filtered off; the solution was extracted with benzene until colourless. It was then evaporated *in vacuo* to dryness, the oil was taken up in ethanol and a slight excess of pyridine was added. The methyl-lysine hydrochloride crystallized slowly on standing. It was dissolved again in a small amount of water, and ethanol added. The hydrochloride crystallized in well-formed rosettes, containing 1 mol. of water of crystallization. Yield was 84% of the theoretical. This material was free from lysine as estimated by the lysine decarboxylase method. This enzyme does not attack ε-*N*-methyl-*dl*-lysine and is not inhibited by it. From the limits of error of the enzymic method, it is possible to say that it did not contain more than 0·3% of lysine. (Found: C, 39·6; H, 8·9; N, 12·8; Cl, 16·7. Calc. for $C_7H_{17}O_2N_2Cl.H_2O$: C, 39·3; H, 8·9; N, 13·0; Cl, 16·6%.)

Feeding experiments

Young, recently weaned rats, of a piebald stock, weighing about 40 g., were used. Rats from the same litter and the same sex were used as controls in each experiment, since the rates of growth varied considerably between different litters. The basal diet, which contained 18% of gliadin, was the same as that used in earlier experiments (Neuberger & Sanger, 1943). Vitamin supplements were fed separately as described. The diet was fed *ad lib.* The animals were kept for about 5–6 days on the basal diet and vitamins before lysine or the lysine derivatives were added.

RESULTS

Table 1 shows that ε-*N*-acetyl-*d*-lysine does not replace *l*-lysine on a deficient diet. The very slight increase of weight produced is the same as that obtained with the unsupplemented basal diet. Even a dose of 82 mg./day, which is equivalent to 80 mg. of lysine hydrochloride, proved completely ineffective.

ε-*N*-Methyl-lysine, on the other hand, replaces lysine very well (Table 2). Although it is impossible to make any strictly quantitative deductions from weight changes, it appears from the data given that the racemic ε-methyl compound is about as effective as half the equivalent amount of *l*-lysine. It can be assumed that, as in the case of lysine itself, only the *l*-methyl-lysine is utilized for growth, especially since ε-methyl-*dl*-lysine is not attacked by the *d*-amino-acid oxidase (Neuberger & Sanger, 1944). On that basis ε-methyl-*l*-lysine is about as effective as *l*-lysine itself.

Table 1. *Body weights and food consumption of rats receiving basal diets with and without added ε-acetyl-*dl*-lysine and* l*-lysine*

Rat	Duration of exp. (days)	Dietary supplement: Substance	Dietary supplement: Amount added (mg./day)	Weight of rat (g.): Initial	Weight of rat (g.): Final	Weight of rat (g.): Increase (g./day)	Average food consumption (g./day)
(*a*) *Litter H*							
1 ♂	5–19	*l*-Lysine HCl	40	41	58	1·2	6·0
2 ♂	5–19	ε-Acetyl-*d*-lysine	41	42	46	0·3	5·6
3 ♂	5–19	ε-Acetyl-*d*-lysine	41	42	46	0·3	5·7
(*b*) *Litter I*							
5 ♂	5–19	None	—	39	43	0·3	5·3
5 ♂	5–23	None	—	44	51	0·4	5·3
7 ♂	5–23	ε-Acetyl-*d*-lysine	82	46	53	0·4	5·4
8 ♂	5–23	*l*-Lysine HCl	40	44	81	2·0	6·4

Table 2. *Body weights and food consumption of rats receiving basal diets with and without added ε-methyl-*dl*-lysine and* l*-lysine*

Rat	Duration of exp. (days)	Dietary supplement: Substance	Dietary supplement: Amount added (mg./day)	Weight of rat (g.): Initial	Weight of rat (g.): Final	Weight of rat (g.): Increase (g./day)	Average food consumption (g./day)
(*a*) *Litter H*							
1 ♂	5–19	*l*-Lysine HCl	40	41	58	1·2	6·0
5 ♂	5–19	None	—	39	43	0·3	5·3
6 ♂	5–19	ε-Methyl-*dl*-lysine HCl	86	44	64	1·4	6·5
(*b*) *Litter I*							
5 ♂	5–23	None	—	44	51	0·5	5·3
6 ♂	5–23	ε-Methyl-*dl*-lysine HCl	47	48	76	1·55	6·7
8 ♂	5–23	*l*-Lysine HCl	40	44	81	2·05	6·4
(*c*) *Litter K*							
1 ♂	5–19	*l*-Lysine HCl	20	42	56	1·00	6·3
2 ♂	5–19	ε-Methyl-*dl*-lysine HCl	46	44	58	1·00	7·1
3 ♀	5–19	None	—	32	31	0·07	4·3
4 ♀	5–19	*l*-Lysine HCl	40	39	64	1·78	6·9
5 ♀	5–19	ε-Methyl-*dl*-lysine HCl	46	36	55	1·36	6·8
6 ♀	5–19	*l*-Lysine HCl	10	40	47	0·5	5·9
7 ♀	5–19	ε-Methyl-*dl*-lysine HCl	23	39	47	0·6	6·1

DISCUSSION

The results obtained with ε-*N*-acetyl-*d*-lysine show clearly that this compound is not converted by the rat to ε-acetyl-*l*-lysine or to *l*-lysine itself, to any appreciable extent. This is best explained by assuming that deacetylation proceeds at a faster rate than the deamination and reamination reactions, or else that the metabolic decomposition of the ε-acetamino-keto-acid is more rapid than the deamination.

The experiments with ε-methyl-lysine appear to indicate that this compound can be converted into lysine, although the possibility that ε-methyl-lysine is incorporated into proteins as such cannot be completely ruled out. The fact, however, that the conversion of the ε-methyl compound to *l*-lysine can be shown to occur in kidney slices (Neuberger & Sanger, 1944) makes it almost certain that this conversion can take place *in vivo* too and renders the assumption of an incorporation of ε-methyl-lysine into proteins at least unnecessary. The conversion to lysine may take place by several possible mechanisms. The reaction may be similar to that assumed to occur in the conversion of α-methyl-amino-acids to the corresponding amino-acids, i.e. oxidation to a carboxyl compound followed by reamination. Such a mechanism is, however, unlikely. The experiments with isotopic N (Schoenheimer, Ratner & Rittenberg, 1939 *a*, *b*) have shown at least for lysine itself that the removal of both amino groups is an irreversible process and the same will probably apply to ε-methyl-lysine too. It seems more probable that a direct conversion of ε-methyl-lysine to lysine occurs and it may be similar to the demethylation of sarcosine to glycine (Abbott & Lewis, 1939; Bloch & Schoenheimer, 1940). It is also possible that the methyl group is removed by a hydrolytic reaction or by transmethylation. These feeding experiments can, of course, give no direct information as to the reaction mechanism; the fact, however, that ε-methyl-lysine, in contrast to

α-methyl-lysine, is available for growth shows that the terminal amino group can be modified without influencing the growth effect. The bearing of these results on lysine metabolism has been discussed (Neuberger & Sanger, 1944).

SUMMARY

1. The preparation of *d*-lysine by the action of a bacterial decarboxylase on *dl*-lysine, and by Walden inversion from ε-benzoyl-*l*-lysine, is described. An improved method for the preparation of ε-*N*-methyl-*dl*-lysine is also given.

2. It has been found that, unlike the *l*-isomer, ε-*N*-acetyl-*d*-lysine does not replace *l*-lysine in the diet. ε-*N*-Methyl-*dl*-lysine, however, is available for growth and is about as active as *dl*-lysine itself. The different behaviour of α-methyl-lysine is emphasized.

REFERENCES

Abbott, L. O., Jr. & Lewis, H. B. (1939). *J. biol. Chem.* **131**, 479.
Berg, C. P. (1936*a*). *J. Nutrit.* **12**, 671.
—— (1936*b*). *J. biol. Chem.* **115**, 9.
Bloch, K. & Schoenheimer, R. (1940). *J. biol. Chem.* **135**, 99.
Enger, R. & Steib, H. (1930). *Hoppe-Seyl. Z.* **191**, 97.
Felix, K. & Naka, S. (1940). *Hoppe-Seyl. Z.* **264**, 123.
Gale, E. F. & Epps, H. M. R. (1943). *Nature, Lond.*, **152**, 327.
Gordon, W. O. (1939). *J. biol. Chem.* **127**, 487.
Levene, P. A. & Kuna, M. (1941). *J. biol. Chem.* **142**, 391.
McGinty, O. A., Lewis, H. B. & Marvel, C. S. (1924). *J. biol. Chem.* **62**, 75.
Neuberger, A. & Sanger, F. (1943). *Biochem. J.* **37**, 515.
—— —— (1944). *Biochem. J.* **38**, 119.
Schoenheimer, R., Ratner, S. & Rittenberg, D. (1939*a*). *J. biol. Chem.* **127**, 333.
—— —— —— (1939*b*). *J. biol. Chem.* **131**, 479.
Thomas, K. & Görne, M. O. H. (1918). *Hoppe-Seyl. Z.* **104**, 75.
Udransky, L. v. & Baumann, E. (1889). *Hoppe-Seyl. Z.* **13**, 562.
Wallach, O. (1900). *Liebigs Ann.* **312**, 179.