difference between the urinary calcium after the water blank tests, and the urinary calcium after the tests with the peptone powders, shows a probable significance of above 95%. This is not the case for the difference between the water blank tests and the lactose control tests.

Table 2 shows four experiments in which the water blank tests and the experiments with the lactose control powders were done as usual. The peptone powders, however, contained only one-half the amount of peptone and glutamic acid; they were made up to full weight with lactose ('½ peptone powders'). It will be seen that an increase in urinary calcium was also found when the protein derivatives were diminished by one-half.

Serum calcium. The serum calcium of all subjects was determined before the tests. It was normal (with one exception) and varied between 9·8 and 10·6 mg./100 ml. In the case of four subjects it was measured immediately before the peptone powders were taken, and for five hours afterwards at half-hourly intervals; no rise in serum calcium was observed. This was to be expected from the work of McCance & Widdowson (1939) on the regulatory mechanism of the kidney, which exerts its function by preventing the calcium serum level from rising above normal. Thus any calcium filtering from the intestine into the blood stream after the ingestion of the powders would not be allowed to accumulate. Provided the serum calcium level was normal initially, the excess calcium would either be deposited in the tissues or excreted by the kidneys. One of our subjects (L.P.), however, was found to have a hypocalcaemia, the serum calcium being 8 mg./100 ml. In his case the serum calcium rose to normal after the ingestion of the peptone powders, but not when '½ peptone powders' or lactose control powders were given (Fig. 1). This patient was treated with four peptone powders, three times daily, for 17 days. His serum calcium was determined during this period in the morning while he was still fasting. It rose on the first day to 10 mg./100 ml. and remained at this level throughout. When the treatment was stopped, the serum calcium fell slowly and was, after 3 weeks, back at 8 mg./100 ml.

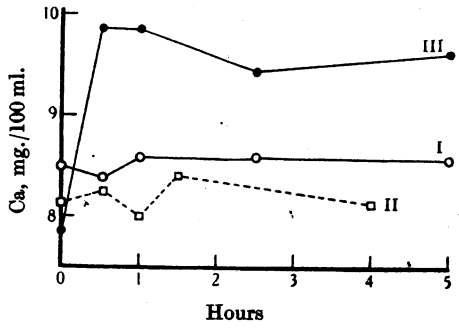

Fig. 1. Serum calcium, mg./100 ml., in an hypocalcaemic subject (L.P. ♂), after ingestion of 750 ml. of distilled water and 279 mg. calcium as $CaHPO_4.2H_2O$. I, 12 lactose control powders; II, 12 '½ peptone powders'; III, 12 peptone powders.

SUMMARY

A calcium peptone powder has been devised which appears suitable for increasing calcium absorption in man.

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The Metabolism of Lysine

By A. NEUBERGER and F. SANGER (Benn W. Levy Student), *Biochemical Laboratory, Cambridge and National Institute for Medical Research, Hampstead, London, N.W.* 3

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In spite of the considerable amount of work which has been done on the biochemistry of lysine, the intermediary metabolism of this important amino-acid still remains obscure. The work recorded here does not give a clear-cut answer to this problem, but the observations reported may, we believe, be helpful in arriving at an ultimate solution.

There is little doubt that at least a part of the

nitrogen of lysine is ultimately excreted in the form of urea. Doty & Eaton (1939) injected lysine into dogs and found a rise of urea content in blood and urine. Weissmann & Schoenheimer (1941) showed that urea, isolated from the urine of rats fed with lysine containing N^{15} in the α-amino group, contained isotopic N. It is not known, however, whether this conversion is quantitative and especially whether both amino groups are thus metabolized. Experiments dealing with the ultimate fate of lysine-N are reported in Part I of this paper.

Experiments on the behaviour of tissue slices and of enzymes to lysine have led to conflicting results. Krebs (1933) has found that incubation of kidney slices with *l*-lysine brings about a disappearance of amino groups, whilst Felix & Naka (1940) report that neither kidney nor liver slices show an increased oxygen uptake in the presence of lysine. One of the main difficulties in the application of the tissue-slice technique to lysine has been the absence of a specific and accurate analytical method. It was found that the production of CO_2 arising from the action of a bacterial decarboxylase on *l*-lysine (Gale & Epps, 1943) can be utilized for an accurate and specific estimation of lysine in complex mixtures. The application of this method to tissue slices is described in Part II; the information obtained about the intermediary metabolism of lysine itself has been rather disappointing. It has, however, been possible to study the conversion of ε-methyllysine to lysine by this method.

Part III deals with the action of *d*-amino-acid-oxidase on *d*-lysine, its derivatives and related compounds. *d*-Lysine itself is not attacked appreciably by this enzyme (Felix & Zorn, 1939; Handler, Bernheim & Klein, 1941*a*). This was thought to be possibly due to the presence of a second basic group in the molecule. Derivatives of lysine and of ornithine, in which the terminal group is masked, were therefore tested.

PART I. THE ULTIMATE FATE OF THE NITROGEN OF *d*- AND *l*-LYSINE IN THE RAT

The aim of the experiments recorded in this part was to trace quantitatively the N of lysine in rats fed with large amounts of this amino-acid and to discover, if possible, any intermediary nitrogenous derivatives excreted in the urine.

Experimental

Male rats of the piebald type were put into metabolism cages and fed a constant amount of the stock laboratory diet, i.e. 15 g. dry material/day. Urines were collected quantitatively, the funnels and bulbs of the cages being washed down with 0·01 N-HCl and the washings combined with the main bulk of the urine. After 10–20 days the daily excretion of N became steady, i.e. the daily variations were less than ±5% of a mean value. The N contents of the different fractions were then estimated daily, as described below, for 5 days. The supplement was then added and mixed with the diet. The estimation of the different fractions was continued for several days until the values were again constant. The differences between the figures in the experimental period and the average figures for the control period, i.e. the 5 days preceding the addition of the supplement, were then calculated. The accuracy of such calculations is not very great. The method tested with *l*-leucine gave a recovery of 105% of excess urea-N.

Total N was estimated by the micro-Kjeldahl method; urea-N and NH_3-N were estimated according to Conway (1933). Non-volatile basic N was estimated as follows: A measured portion of the urine was adjusted to pH 1, and phosphotungstic acid added. After standing at 0° for 18 hr., the precipitate was centrifuged and washed with a 2% phosphotungstic acid solution. The precipitate was then decomposed by the addition of NaOH to pH 11·0. The mixture, which still contained some solid material, was then aerated until all the volatile bases were removed. An N estimation on a portion of this suspension gave a measure of the non-volatile basic N. The sum of urea-N, NH_3-N and non-volatile basic N was deducted from the total N and the difference termed 'Other N'. Lysine added to urine was recovered to 96% in the non-volatile basic fraction.

Results

Table 1 gives the results obtained in a few typical experiments. It was found that when the rats were fed 1 g. *l*-lysine hydrochloride, containing 0·154 g. of N, over 1 or 2 days, there was only 0·10–0·12 g. of extra N in the urine, i.e. 67–78% of the expected value. This may be due to incomplete absorption, since *l*-lysine is not quickly absorbed from the intestine (Doty & Eaton, 1937); or it may be caused by the comparatively slow metabolism of lysine (Doty & Eaton, 1939) and consequent tailing off of the excretion of extra N, which would thus be difficult to estimate. In experiments in which $NaHCO_3$ equivalent to the lysine hydrochloride was added to the diet, the increase in urinary N was confined to the urea fraction. The increase of NH_3-N was, however, considerable if no $NaHCO_3$ was given, and in several experiments amounted to 40% of the total extra N. This increase in NH_3 is presumably due to the fact that *l*-lysine hydrochloride is broken down to CO_2 and urea, leaving free HCl to be excreted as NH_4Cl. In neither case was there any excretion of unchanged lysine, nor of any nitrogenous metabolite other than urea and NH_3.

Feeding *dl*-lysine leads to a smaller increase of urea-N than in corresponding experiments with *l*-lysine and also to a definite increase of the non-volatile basic fraction. This suggests that *d*-lysine is metabolized more slowly than *l*-lysine and is largely excreted unchanged. Recently Ratner, Weissmann & Schoenheimer (1943) have fed labelled *d*-lysine to rats and isolated some unchanged material from the urine. We have also fed 1 g. of

Table 1. *The effect of the ingestion of* l- *and* dl-*lysine with and without* $NaHCO_3$ *on the concentration of various nitrogenous fractions in the urine*

Day	Dietary supplement (*l*-lysine hydrochloride g./day)	$NaHCO_3$	Urinary N (mg.) Total	Urea-N	NH_3-N	Non-volatile basic N	Other N
Rat F							
32	Nil		197	150	9·2	4·9	34
33	Nil		202	149	7·1	5·0	41
34	0·5	Nil	247	183	28·9	6·0	29
35	0·5		243	184	26·4	6·7	26
36	Nil		205	150	13·9	5·6	25
37	Nil		197	155	7·1	5·0	30
Rat E							
30	Nil		185	144	7·1	5·0	29
31	Nil		190	144	9·2	4·2	33
32	0·5		249	190	28·9	6·0	24
33	0·5	Nil	240	182	25·3	6·7	26
34	Nil		191	141	13·8	5·6	31
35	Nil		185	143	7·1	4·9	30
Rat G							
37	Nil	Nil	188	150	8·0	6·0	22
38	Nil	Nil	180	146	7·5	5·2	21
39	1·0	+	278	230	12·0	5·3	29
40	Nil	Nil	189	149	11·7	3·7	23
41	Nil	Nil	190	148	11·7	6·0	24
Rat M							
37	Nil	Nil	195	154	6·5	6·5	30
38	Nil	Nil	196	153	7·1	5·2	31
39	0·5*	+	241	178	7·8	25·1	30
40	0·5*	+	240	178	11·5	22·8	28
41	Nil	Nil	205	158	11·5	6·7	29
42	Nil	Nil	193	153	8·0	5·1	27

* *dl*-Lysine hydrochloride.

d-lysine hydrochloride to an adult rat and isolated 0·55 g. of unchanged amino-acid from the urine, applying a technique of isolation similar to that used by the American authors. It appears, therefore, that *l*-lysine is metabolized fairly well and the only identifiable end-products of its N are urea and ammonia. *d*-Lysine, on the other hand, is metabolized slowly; it shares this property with other amino-acids of the unnatural *d*-variety (Abderhalden & Tetzner, 1935).

PART II. ESTIMATION OF *l*-LYSINE BY THE DECARBOXYLASE METHOD. THE METABOLISM OF LYSINE IN TISSUE SLICES

The preparation and purification of lysine decarboxylase and the measurement of the CO_2 produced have already been described by Gale & Epps (1943). The enzyme is highly specific; apart from the compounds named in that paper, we have found that neither α-*N*-methyl-*dl*-lysine nor ε-*N*-methyl-*dl*-lysine are attacked by the decarboxylase. In order to test the applicability of this method to a complex medium, the lysine content of casein was estimated, by means of the decarboxylase, and found to agree with established values. The method was then applied to tissue slices and the disappearance of lysine under different conditions was studied. The conversion of ε-*N*-methyl-lysine to lysine which we had reason to believe occurs *in vivo* (Neuberger & Sanger, 1944) was then examined *in vitro*.

Experimental

Preparation of the enzyme. In earlier experiments the crude acetone-dried powder of the whole organism was used directly. This has the advantage that it is easy to prepare and that it can be kept for several weeks. It has the disadvantage, however, that it contains some arginine decarboxylase and can, therefore, not be used if the presence of arginine is suspected. In all experiments reported here the enzyme was purified as described by Gale & Epps (1943) by extraction with borate buffer, adsorption on alumina and elution with phosphate buffer. This preparation does not attack arginine, but it retains its full activity only for 1–2 days.

Method of estimation. The sample for estimation, which should contain 1–2 mg. of lysine, is adjusted to pH 5·0 with acetic acid. The solution is then placed in the cup of a Warburg manometer and is made up to 2·5 ml. with 0·1 M-acetate buffer of pH 5·0. The enzyme solution (0·5 ml.) is put in the side-bulb of the manometer; enough enzyme is taken to complete the reaction in 5 min. The manometer is shaken at 37° until equilibrated, the zero reading is taken and the enzyme is tipped in. When the reaction is finished, the CO_2 evolved is calculated from the reading, and hence the amount of *l*-lysine deduced. Duplicates should agree within 2%. The reaction can also be carried out at pH 6·0,

which is the optimal pH for the enzyme. In this case, however, it is necessary to tip in acid at the end of the reaction, since some CO_2 is retained in the solution at pH 6·0.

Estimation of l-lysine in casein. 1·46 g. of light white casein (calculated on an ash- and water-free basis) were hydrolyzed by boiling with 10 ml. 20% HCl for 18 hr. The solution was then concentrated *in vacuo* and excess HCl was removed by repeated evaporation *in vacuo*. The residue was made up with water to 50 ml.; 5 ml. of this solution were neutralized to pH 5·0 with N-NaOH and made up to 10 ml.; 1 ml. was then used for the estimation as described above. All the CO_2 was liberated in the first few minutes. Three estimations gave the following values: 151, 149 and 152 cu.mm. CO_2. From the mean value of 151 a lysine content of 6·7% was calculated for the anhydrous casein. This agrees quite well with the value of 6·25% found by isolation (Vickery & White, 1933), especially if it is taken into account that hydroxy-lysine, of which 0·33% is present in casein (Van Slyke, Hiller & MacFadyen, 1941), is also decarboxylated by the enzyme.

Estimation of l-lysine in the presence of tissue slices. In preliminary experiments it was found that lysine added to a solution which had been incubated with kidney or liver slices could be quantitatively recovered. There was no evidence of liberation of lysine by autolysis of the tissue.

2 ml. of the bicarbonate-Ringer solution (Krebs & Henseleit, 1932), with or without added substrate, were measured into a Warburg manometer cup. In some experiments a phosphate-Ringer solution (Krebs, 1933) was used. The tissue slices, which were cut in the usual way, were washed in a solution of the same composition as the one to be used, allowed to drain and then transferred to the manometer cup. The manometer was then filled with O_2-CO_2 mixture, and incubated at 37° for 1–2 hr., and the oxygen uptake was measured. The slices were then removed, allowed to drain, dried at 105° and weighed. The solution was adjusted to pH 5 by the addition of either 0·5 ml. 0·1 N-acetic acid or 0·5 ml. 3 N-acetate buffer of pH 5. The enzyme was then added and the estimation of lysine carried out as described above. In some cases the manometers were filled with N_2 at this stage. This was necessary with brain slices, which could not be completely removed from the solution and which used up O_2, thus interfering with the estimation of lysine.

The results were expressed in terms of Q_{lysine}. This is defined as the cu.mm. 'lysine CO_2' formed by 1 mg. of dry tissue in 1 hr. By 'lysine CO_2' is meant the volume of CO_2, measured under standard conditions, evolved by the lysine decarboxylase from lysine.

Results

Disappearance of lysine in the presence of tissue slices. A large number of experiments was carried out in which the disappearance of lysine was estimated when slices of different tissues of the rat were incubated with lysine. In some experiments there was a small, but significant loss of lysine in kidney, liver, brain and heart. In other experiments such an effect could, however, not be demonstrated. Negative or variable results were obtained with rats of different ages and in different states of nutrition; thus tissues were used from rats weighing 80, 150 and 300 g. respectively. Also experiments on tissues of animals starved for 24 hr., or fed on a high-protein diet for a few days before being killed, were equally inconclusive. The effect of the following substances on the disappearance of lysine in the presence of kidney and liver slices was tested with negative results: alanine, proline, pyruvic acid, NH_4Cl, α-ketoglutaric acid, citrulline and a casein hydrolysate. The oxygen uptake of tissue slices was slightly increased by lysine in some experiments; the magnitude of this increase was, however, fairly small. There was no decrease in amino-N, as measured by the gas evolved in the Van Slyke apparatus after shaking for 5 min. and after shaking for 30 min., if kidney slices were incubated with lysine for periods up to 2 hr. These variable or negative results have little significance for the general problem of lysine metabolism. It is known both from the work of other authors and from the results reported in Part I of this paper that *l*-lysine is broken down in the body, though more slowly than many other amino-acids, and we can assume that there exist enzymes in certain tissues able to attack this amino-acid. We can only say that under the conditions used in these experiments it has not been possible to demonstrate such a breakdown *in vitro* with certainty. There may have been some disappearance of lysine in some experiments; but in any case the reaction is so slow that it does not lend itself to the isolation of intermediary products.

The formation of l-lysine from ε-N-methyl-lysine in vitro. Table 2 gives the results obtained on incubating ε-*N*-methyl-*dl*-lysine with kidney and liver slices. It can be seen that kidney can convert

Table 2. *The formation of l-lysine from ε-methyl-dl-lysine by kidney and liver*

(The substrate was incubated with the tissue for 2 hr. at 38° in O_2-CO_2, unless otherwise stated, and the lysine estimated as described.)

Exp.	Substrate added (mg. ε-methyl-*dl*-lysine HCl)	Tissue	Lysine-CO_2 (cu.mm.)	Wt. of tissue (mg.)	Q_{lysine}
1	3·7	Kidney	62	15·3	2·0
	3·7	Liver	18	34·8	0·26
	0·0	Kidney	2	13·0	—
2	1·74	Kidney	48	13·4	1·8
	1·74	Kidney, in N_2-CO_2	9	9·1	0·5
3	3·8	Kidney	42	31·0	1·36
	0·0	Kidney	4	31·5	0·13

ε-methyl-lysine to *l*-lysine, whilst the amounts of lysine formed by liver are quite small. The specificity of the enzyme makes it impossbile to ascertain whether the *d*-isomeride is also demethylated. Demethylation is considerably reduced in the absence of oxygen and it is likely therefore that the removal of the methyl group involves an oxidation. Cell-free

extracts of kidney in phosphate or bicarbonate buffer of pH 7 were inactive. The mechanism of this demethylation is obscure. Various attempts to demonstrate the formation of formaldehyde by the bisulphite and fuchsin sulphurous acid tests (Feigl, 1939) were unsuccessful. This enzyme appears to be different in distribution and action from enzymes responsible for demethylations of other compounds. The enzyme which catalyzes the demethylation of sarcosine, and which occurs in the liver, produces formaldehyde (Handler *et al.* 1941*b*). Another enzyme investigated by these authors, which demethylates several other α-methylamino-acids, also occurs chiefly in the liver, and this also applied to the enzyme involved in transmethylations. The finding, however, that demethylation of ε-methyl-lysine can be demonstrated *in vitro* readily explains the availability of ε-methyl-lysine for growth (Neuberger & Sanger, 1944).

PART III. THE ACTION OF *d*-AMINO-ACID OXIDASE ON LYSINE AND LYSINE DERIVATIVES

Experimental

Preparation of the enzyme. Two preparations of the enzyme were used. One was a crude extract of the acetone-dried tissues prepared as described by Krebs (1935). This preparation showed a slow oxygen uptake in the absence of added amino-acid and in some cases appeared to attack *dl*-lysine somewhat. In later experiments the enzyme was purified up to stage A of the method described by Negelein & Broemel (1939). This preparation was completely inactive towards *dl*-lysine and had no blank oxygen uptake.

Measurement of oxygen uptake. The enzyme solution was placed in the main cup of a Warburg manometer and made up to a volume of 2·6 ml. by addition of 0·1 M-pyrophosphate buffer of pH 8·6. About 2×10^{-2} μmol. of the substrate in 0·4 ml. pyrophosphate buffer was put in the side-bulb and 0·2 ml. N-NaOH in the centre cup. The oxygen uptake was then measured in the usual way. The oxygen uptake of the enzyme preparation without substrate was measured separately and subtracted from the experimental readings. In most experiments the oxidation of *dl*-alanine and *dl*-phenylalanine by the enzyme was also measured for comparison.

Preparation of compounds. *d*-Lysine was prepared by inversion of the ε-benzoyl compound (Neuberger & Sanger, 1944), the acetyl-lysines as described (Neuberger & Sanger, 1943); *dl*-pipecolinic acid was obtained by the method of Mende (1896).

dl-δ-Acetyl-ornithine was prepared from ornithine sulphate by the method that was used for the preparation of ε-acetyl-lysine. It was slightly less soluble in water than the lysine compound and could be recrystallized from a small volume of water. It separated in the form of large rod-shaped crystals, m.p. 239–240° (decomp.). (Found: C, 48·2; H, 8·0; N, 15·9. Calc. for $C_7H_{14}N_2O_3$: C, 48·2; H, 8·0; N, 16·9.)

Results

Table 3 shows that *dl*-lysine is not attacked by the *d*-amino-acid oxidase at all or only extremely slowly. Similar results with pure *d*-lysine show that the negative result is not due to an inhibitory effect of the *l*-isomeride; ε-methyl-*dl*-lysine also is not oxidized. However, if the ε-amino group is masked as in ε-acetyl- or ε-benzoyl-lysine, oxidation at a moderate rate can take place. It appears, therefore, that the free terminal amino group inhibits the enzyme, presumably owing to its basic character. Similar experiments with ornithine gave rather surprising results. *dl*-Ornithine is oxidized at a not very fast rate, as already found by Krebs (1939). It appears, therefore, that an amino group in the δ-position inhibits less than an amino group in the ε-position. Moreover, in this case acetylation of the terminal amino group actually decreases the rate of oxidation. It is likely that a free basic group in the ε-position inhibits the formation of an enzyme-substrate complex due to a specific steric effect, probably by repelling another basic group on the surface of the enzyme molecule.

dl-Pipecolinic acid, which stands in the same relationship to lysine as proline does to ornithine, is attacked at a fairly rapid rate. Krebs (1939) also found that proline was more quickly oxidized than ornithine.

An animal *l*-amino-acid oxidase has recently been isolated by Green, Nocito & Ratner (1943). Dr Green has kindly tested some lysine derivatives with this enzyme preparation and informed us that ε-acetyl-*l*-lysine was slowly but definitely oxidized by the kidney preparation, whilst *l*-lysine, *dl*-ornithine and δ-acetyl *dl*-ornithine were not attacked.

Table 3. *The action of* d-*amino-acids oxidase on lysine and ornithine and their derivatives*

Substrate added: Substance	Amount (mg.)	Oxygen taken up (cu.mm.) during: 30 min.	60 min.
(*a*) *Crude enzyme preparation*			
dl-Phenylalanine	2·98	95	135
dl-Lysine HCl	3·54	−1	3
ε-Benzoyl-*dl*-lysine	4·62	10	24
dl-Pipecolinic acid	2·52	50	84
ε-Acetyl-*dl*-lysine	4·60	13	25
(*b*) *Purified enzyme preparation*			
dl-Alanine	3·30	95	179
dl-Lysine HCl	4·00	1	−1
d-Lysine HCl	1·95	1	0
ε-Acetyl-*dl*-lysine	4·10	15	24
ε-Methyl-*dl*-lysine HCl	3·25	2	1
dl-Ornithine HCl	2·00	7	17
ε-Acetyl-*dl*-ornithine	2·20	4	8

GENERAL DISCUSSION

The results of other workers and the experiments reported in this paper show that the N of lysine is ultimately excreted in the form of urea or ammonia. But no intermediary stages in this breakdown have

so far been definitely established. Thus it has been impossible to demonstrate any definite oxidation of lysine by enzymes or tissue slices *in vitro*, nor could intermediary products of lysine metabolism be isolated in living animals. This may be due to the slow rate of the first step of the oxidation, absolutely and also relatively, compared with that of subsequent oxidative reactions, at least in the rat. The intermediary metabolism of lysine must, therefore, be discussed in the light of other more indirect information. We may perhaps summarize the unusual features of the biological behaviour of lysine under four headings:

(1) Lysine shares with other essential amino-acids the property that it cannot be synthesized from compounds of simpler structure normally occurring in the diet; it differs from other essential amino-acids in that even the first step of its oxidation is apparently irreversible, i.e. once a nitrogen atom is lost, the resulting product cannot be reconverted into lysine (Schoenheimer, Ratner & Rittenberg, 1939*a*, *b*).

(2) Lysine is neither glucogenic nor ketogenic. Thus Dakin (1913) showed that lysine does not give rise to sugar or acetone bodies in the phlorrhizin-poisoned dog, nor does it cause either storage of glycogen or ketonuria in the fasting rat (Butts & Sinnhuber, 1941; Sharp & Berg, 1941). Any postulated intermediate must, therefore, satisfy the condition that it is neither glucogenic nor ketogenic. The conversion of lysine into glutamic acid suggested by Borsook & Dubnoff (1941) can therefore be ruled out, at least as a major pathway, since glutamic acid is glucogenic.

(3) Enzymes which oxidize other amino-acids quite readily are inactive towards lysine; neither *d*- nor *l*-lysine are appreciably attacked by the appropriate amino-acid oxidases.

(4) Compounds derived from lysine by modification of the ϵ-amino group may replace lysine in the diet (Neuberger & Sanger, 1943, 1944), whilst derivatives obtained by modification of the α-amino group cannot be converted into lysine.

The unique irreversibility of lysine oxidation has already been discussed by Weissmann & Schoenheimer (1941), and one of the explanations proposed was that lysine is actually oxidized at the α-amino group to the corresponding α-keto acid which cyclizes to a tetrahydropyridine compound, and which cannot be reconverted into lysine. It is, however, very unlikely that lysine is oxidized to an α-keto-acid. It has already been mentioned that *l*-lysine is not attacked by the *l*-amino-acid oxidase (Green, Nocito & Ratner, 1943), nor does it take part in transamination reactions (Cohen, 1939, 1940). We would have to assume that a new, as yet undiscovered, enzyme is responsible for this oxidation. It is more probable that the α-amino group is quite stable in lysine itself and that its oxidation is not the first step in the metabolic breakdown. The stability of the α-amino group is also shown by the behaviour of ϵ-methyl-lysine, which replaces lysine in the diet quantitatively (Neuberger & Sanger, 1944). From this it follows that the rate of oxidation of the α-amino group is very small compared with the rate of demethylation. The structural similarity of ϵ-methyl-lysine and lysine suggests a similar inertness of the α-amino group of lysine itself.

It is, however, possible that oxidation of the α-amino group is preceded by acetylation of the ϵ-amino group. It is shown in this paper that both ϵ-acetyl derivatives of *d*- and *l*-lysine are attacked by the corresponding amino-acid oxidase. Moreover, acetylation of amino groups has been shown to occur in a large number of compounds. The postulated chain of reactions is as follows:

$$H_2N(CH_2)_4CH(NH_2).COOH$$
$$\rightarrow CH_3CONH(CH_2)_4.CH(NH_2).COOH$$
$$\rightarrow CH_3CONH(CH_2)_4.CO.COOH \begin{cases} \nearrow NH_2.(CH_2)_4.COOH \\ \searrow HOOC.(CH_2)_3.COOH \end{cases}$$

The keto acid could be deacetylated and decarboxylated to δ-amino-valeric acid, or oxidized to glutaric acid. As both δ-amino-valeric acid (Corley, 1926) and glutaric acid (Ringer, 1912) are not sugar-forming, and do not give rise to the formation of ketone bodies, they are possible intermediates.

Another possibility is that the first step is oxidation of the terminal amino group by an unknown enzyme leading to α-amino-adipic acid as follows:

$$H_2N(CH_2)_4.CH(NH_2)COOH$$
$$\rightarrow HOOC.(CH_2)_3.CH.(NH_2)COOH$$
$$\rightarrow HOOC(CH_2)_3.CO.COOH$$
$$\rightarrow HOOC.(CH_2)_3.COOH$$

α-Amino-adipic acid would be oxidized, through the keto-acid, to glutaric acid. Such a hypothesis is in accordance with feeding experiments, which indicate that the ϵ-amino is less inert than the α-amino group. Neither mechanism is supported by any direct evidence, but both explain the peculiarities of lysine enumerated above and are not in conflict with any known facts.

Many inborn errors of metabolism are characterized by the excretion of products which are considered to be normal intermediates which cannot be further broken down, because of the pathological metabolism. Although no condition is known which can be considered mainly a disturbance of lysine metabolism, cases of cystinuria have been reported in which considerable amounts of cadaverine and also some putrescine were excreted (Udransky & Baumann, 1889). It has never been established whether these diamines are products of the cellular diamino-acid metabolism of the cystinuric person,

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.

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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*

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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