IX. ON A NEW TYPE OF CHEMICAL CHANGE PRODUCED BY BACTERIA. THE CONVERSION OF HISTIDINE INTO UROCANIC ACID BY BACTERIA OF THE COLI-TYPHOSUS GROUP.

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The investigation of the products of the putrefactive decomposition of proteins, commenced by Nencki, Baumann, Brieger and E. and H. Salkowski, has more recently been continued by Ellinger, Ackermann and others, who have investigated the bacterial decomposition of the amino-acids. These decomposition products, varied in nature as they are, are rendered all the more interesting because of the very marked pharmacological and toxicological action exhibited by some of them, e.g. tyramine (*p*-hydroxyphenylethylamine and histamine (*β*-iminazolylethylamine).

By far the most common of all the chemical changes induced in amino-acids by bacterial action is simple decarboxylation, i.e. the production of an amine from an amino-acid by the loss of CO_2.

$$R . CH_2 . CH(NH_2) . COOH \rightarrow R . CH_2 . CH_2 . NH_2 + CO_2.$$

Ellinger [1900] proved that the bases putrescine and cadaverine described by Brieger owe their origin to the decarboxylation by putrefactive bacteria of ornithine and lysine respectively. By the use of the same bacteria Ackermann [1910, 1] obtained a 42 % yield of histamine (*β*-iminazolylethylamine) from histidine. His medium contained peptone, glucose, salts and histidine. Barger and Walpole [1909] isolated tyramine (*p*-hydroxyphenylethylamine) from putrid horse flesh and showed that it was derived from tyrosine by the loss of CO_2. More recently Sasaki [1914] obtained a 78 % yield of the same base by the action of *B. coli communis* on a medium containing tyrosine, glycerol, and inorganic salts. These are only a few of a number of similar

results. In fact almost all the amines which might be expected to result from the known amino-acids have been obtained by bacterial action.

Of somewhat less interest, since the products are usually non-toxic, is the change brought about by bacteria which is known as "deaminisation," i.e. the production of a saturated acid and ammonia from the amino-acid.

$$R . CH_2 . CH(NH_2) . COOH + H_2 \rightarrow R . CH_2 . CH_2 . COOH + NH_3.$$

Thus "deaminisation" in the usually accepted sense of the word *is accompanied by reduction.* Baumann [1879] found that when tyrosine is digested with putrefying pancreas it splits up into ammonia and *p*-hydroxyphenylpropionic acid. In their investigation of the constitution of tryptophan Hopkins and Cole [1903] subjected this amino-acid to the action of the Rauschbrand bacillus and of *B. coli communis.* In each case the bacteria, when grown *anaerobically* in a medium containing Rochelle salt and gelatin in addition to the amino-acid, produced an acid identical with Nencki's scatole-acetic acid, which was subsequently shown by Ellinger to be indolepropionic acid. In the research referred to above Ackermann [1910, 1] found in addition to histamine, a small amount of β-iminazolylpropionic acid. δ-Aminovalerianic acid, first isolated by E. and H. Salkowski [1883] from putrid fibrin, was shown by Ackermann [1910, 2] to be produced by the partial deaminisation of ornithine.

In all the cases quoted it will be noticed that, in addition to the amino-acid under observation the medium also contains large amounts of other organic substances, the presence and nature of which have probably an influence on the end-products. Thus Ackermann has shown that the bacterial decarboxylation of pure amino-acids is favoured by the presence of peptone in the medium. Mellanby and Twort [1912] also observe that the production of β-iminazolylethylamine from histidine by a bacillus, which they isolated from the alimentary canal, is inhibited if the medium is slightly acid or if it contains glucose.

The gradual breakdown of the side chain in amino-acids containing a cyclic nucleus, as seen in the bacterial decomposition of tyrosine and of tryptophan, also illustrates the marked effect of external conditions on the end-products. Baumann [1879, 1880, 1, 2] found that in the putrefactive decomposition of tyrosine *p*-hydroxyphenylpropionic acid is first formed, which in presence of air breaks down further into *p*-hydroxyphenylacetic acid, *p*-cresol and, to a less extent, into phenol. Hopkins and Cole [1903] found a similar breakdown with tryptophan. Under anaerobic conditions

both the Rauschbrand bacillus and *B. coli* gave an abundant production of indolepropionic acid and only slight traces of indole. Aerobically, however, the putrefactive bacteria, or *B. coli*, gave a good yield of indole and indoleacetic acid, traces of scatole, but no indolepropionic acid.

In commencing a study of the action of the bacteria of the Coli-Typhosus group on different amino-acids it seemed desirable therefore, in order to avoid any possible secondary reactions, not to introduce any organic compounds into the medium in addition to the amino-acid under observation. To this end the amino-acid—in this case, histidine—was dissolved in Ringer's solution (mammalian) and this medium used for inoculation. In such a simple medium the bacteria did not grow well but this difficulty was to some extent overcome by sowing thickly and by long continued incubation.

In the preliminary stages a medium of the following composition was made up:

Pure histidine hydrochloride equivalent to 0·2 % of the free base.

2 cc. per cent. of 0·04 % solution of phenolsulphonephthalein to act as indicator.

Ringer's solution.

Normal soda solution until P_H after boiling is 7·35.

This was tubed, sterilised, and inoculated with 24 hour old cultures (from agar slopes) of (i) *B. coli communis*, (ii) *B. typhosus*, (iii) *B. paratyphosus A*, (iv) *B. paratyphosus B*, (v) *B. enteritidis* (Gaertner), (vi) *B. dysenteriae* (Flexner). Even after ten days' incubation at 37·5° there was no change in the reaction of the tubes containing *B. coli*, *B. typhosus*, and *B. paratyphosus A*, though the other three organisms had produced a marked alkalinity, which became intense after 20 days' incubation. After this time *B. typhosus* showed a slight alkalinity but there was still no change in the reaction of the tubes containing *B. coli* and *B. paratyphosus A*.

In order to prove whether the alkalinity were due to amine production the experiment was repeated on a larger scale using *B. paratyphosus B* as the organism for inoculation. Experimental details are given later in the paper. A substance was isolated from the products of bacterial action, which gave a crystalline picrate. It was found impossible to isolate any β-iminazolylethylamine picrate from this, but it was shown to be the picrate of urocanic acid (β-iminazolylacrylic acid). This would arise from histidine according to the following equation:

$$C_3H_3N_2 . CH_2 . CH(NH_2) . COOH - NH_3 = C_3H_3N_2 . CH = CH . COOH,$$

i.e. *by deaminisation without subsequent reduction* to β-iminazolylpropionic acid. The production of an unsaturated acid from an amino-acid is a type of bacteriological change which so far as is known has not been previously observed.

The history of the occurrence of urocanic acid is very interesting. It was first isolated by Jaffé [1874] in 1874 from the urine of a dog—hence its name: greatly to its owner's chagrin the dog disappeared after some days and was never recaptured. Although Jaffé and others investigated the urine of a number of dogs and men it was not re-discovered until 1898 when Siegfried isolated it once more from a dog's urine [1898]. These are the only instances on record of its isolation from urine. Since it was continually present in both cases in fairly large amounts (2—3 g. per day) it appears that these two dogs presented a rare anomaly of metabolism. In 1912 Hunter [1912] was able to identify a substance which he obtained by prolonged tryptic digestion of caseinogen as urocanic acid, and by direct comparison with a synthetic specimen of β-iminazolylacrylic acid obtained by Barger and Ewins [1911] proved that the two were identical. Inasmuch as Hunter only obtained the acid from one tryptic digest—subsequent repetition of the experiment failing to give him any urocanic acid—he concluded that it had probably arisen from bacterial contamination although he was unable to observe its formation by any of the bacteria which he tried. The results described in this paper seem to bear out this presumption. It is also possible that the two dogs, whose urine contained urocanic acid, may have been suffering from some bacterial infection of this type, though Jaffé states that his dog was apparently perfectly healthy.

It now became of interest to prove whether this type of change is characteristic of *B. paratyphosus B* to the exclusion of the other members of the group and whether it is peculiar to the particular strain which was employed. Two other different strains of *B. paratyphosus B* have been tried and each of them brought about the same change. It was also shown that the property is possessed to a greater or less extent by the other members of the group, i.e. *B. coli*, *B. typhosus*, *B. paratyphosus A*, *B. enteritidis* (Gaertner) and *B. dysenteriae* (Flexner). The reaction is thus not restricted to those members which produce an alkaline reaction. In fact the largest yield of urocanic acid was obtained with *B. paratyphosus A*.

Experimental.

The composition of the medium for inoculation, which was the same throughout, was as follows:

2·7 g. pure histidine monochloride (= 2 g. of free base).
12·9 cc. normal soda, to neutralise the HCl of the histidine monochloride.
1000 cc. Ringer's solution (mammalian).

900 cc. of this medium were introduced into each of six litre flasks, and the whole sterilised by steaming for 45 minutes on three consecutive days. Agar slopes were sown over the whole surface with 24 hour pure cultures in tryptic broth, the bacteria used being (i) *B. coli communis*, (ii) *B. typhosus*, (iii) *B. paratyphosus A*, (iv) *B. paratyphosus B*, (v) *B. enteritidis* (Gaertner), (vi) *B. dysenteriae* (Flexner). After 24 hours' incubation the growth was washed off the agar slopes with sterile Ringer solution, emulsified by agitating with the platinum needle, and introduced into the flasks taking all precautions to prevent contamination. The growth from five agar slopes was added to each flask. The flasks were placed in an incubator at 37·5°, those containing the first three organisms being allowed to remain for 40 days, while the remainder were incubated for 28 days.

The investigation of the products of bacterial action was the same in each case. After sterilisation, the solution was filtered through kieselguhr, neutralised with HCl, and evaporated to dryness on the water bath. The dried residue was ground up, thoroughly extracted with boiling methyl alcohol, the alcoholic extract evaporated to dryness, and the residue taken up with a little water. This solution was then precipitated completely with saturated aqueous picric acid, the precipitated picrate filtered off and recrystallised once from a minimal amount of boiling water. A small amount was reserved for M.P. determination, and the greater part ground up with a little water (5—10 cc.), acidified strongly with conc. HNO_3 (about 2 cc.) and the picric acid completely extracted by shaking with ether. In this way the very slightly soluble nitrate of urocanic acid was obtained which was washed with alcohol and ether, dried, weighed, and converted into the free acid by the addition of one equivalent of pure sodium bicarbonate to a concentrated aqueous solution of the nitrate. The free urocanic acid readily separated out, was filtered off and recrystallised from hot water with the addition of a very small amount of animal charcoal.

In the case of all the six bacteria used, I succeeded in isolating the picrate and nitrate of urocanic acid and also free urocanic acid itself.

Urocanic acid picrate is only slightly soluble in cold water and crystallises well from hot water. When not quite pure it forms rosettes of needles, which on further recrystallisation change to well-defined golden yellow prisms. The M.P. varies considerably with the rate of heating and so for identification the M.P.s of the six different specimens were determined in pairs, M.P. tubes containing two different specimens being attached to the same thermometer. In each case the different specimens melted at 212—215° according to the rate of heating. Barger and Ewins give for the picrate of β-iminazolylacrylic acid, M.P. 213—214°.

In a later experiment from 13·9 g. of pure histidine monochloride submitted to the action of a different strain of *B. paratyphosus B* for 47 days there were isolated 3·4 g. of urocanic acid picrate (M.P. 212—213°).

Analysis: 0·1100 g. gave 18·5 cc. N at 19° and 752 mm. = 19·31 % N.

Calculated for $C_6H_6O_2N_2 . C_6H_3O_7N_3$, N = 19·07 %.

Urocanic acid nitrate. In each case this salt was obtained, which Jaffé and later Hunter consider characteristic of urocanic acid. It forms a white crystalline precipitate which under the microscope appears as "bent sickle-shaped plates whose ends appear to be frayed or eaten away. Frequently many such plates are combined to form cross- or rosette-shaped aggregates" (Jaffé).

The yield of the nitrate varied largely with the particular organism used, which may have been due to a number of factors—unequal inoculation, growth or duration of incubation. From 2·7 g. histidine monochloride (= 2 g. free histidine) in each case the following amounts of urocanic acid nitrate were obtained: *B. coli* 0·72 g.; *B. typhosus* 0·10 g.; *B. paratyphosus A* 1·30 g. (= 58 %); *B. paratyphosus B* 0·37 g.; *B. enteritidis* (Gaertner) 0·13 g.; *B. dysenteriae* (Flexner) 0·64 g.

The M.P., which also varies with the rate of heating, was determined in the same manner as that of the picrate. In each case the two samples melted simultaneously at 195—197° with explosive decomposition.

Barger and Ewins give for a synthetic specimen M.P. 198°.

Urocanic acid. The free acid crystallises readily from hot water. On allowing a hot solution to cool slowly long iridescent needles were obtained, while on cooling more quickly the acid crystallised as a mass of very fine needles. The M.P. of urocanic acid as given by different observers varies within wide limits. Jaffé gave 212—213°, Siegfried 229°, Barger and Ewins 235—236°, Hunter 231—232° (corr.). As Hunter observes, the M.P. varies

greatly with the rate of heating, which may explain the discrepancies. The six different specimens obtained were compared, in pairs, and were found to melt simultaneously at 226—230° according to the rate of heating. All the specimens gave the red coloration with sodium *p*-diazobenzenesulphonate and all reduced alkaline permanganate in the cold—proving the presence of an unsaturated linking.

Analysis:

0·1672 g.; 0·2543 g. CO_2; 0·0862 g. H_2O.

0·4763 g. dried at 110° lost 0·0988 g. H_2O.

0·1139 g. of substance dried at 110° gave 20·3 cc. moist N at 18·5° and 758·4 mm.

	Found	Calculated for $C_6H_6O_2N_2.2H_2O$
C	41·49	41·38
H	5·73	5·75
N	20·57	20·30
H_2O of crystallisation ...	20·74	20·69

SUMMARY.

Histidine (β-iminazolyl-α-aminopropionic acid) is converted into urocanic acid (β-iminazolylacrylic acid), in a medium consisting of Ringer's solution + histidine, by the bacteria of the Coli-Typhosus group, i.e. *B. coli communis*, *B. typhosus*, *B. paratyphosus A*, *B. paratyphosus B*, *B. enteritidis* (Gaertner), *B. dysenteriae* (Flexner). This is the first recorded instance of the bacteriological conversion of an amino-acid into an unsaturated acid.

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