CXIV. THE FATE OF INDOLEPROPIONIC ACID IN THE ANIMAL ORGANISM.

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Indolepropionic acid was first prepared by Nencki [1889] by the action of anaerobic bacteria upon commercial fibrin. He used three different strains of bacteria, namely *B. liquefaciens magnus*, *B. spinosus* and the Rauschbrand bacillus. He obtained the best yield of indolepropionic acid by the use of the Rauschbrand bacillus.

Hopkins and Cole [1903] subjected tryptophan to bacterial decomposition and obtained indole, indoleacetic acid and indolepropionic acid, proving that the amino acid was the precursor of the putrefactive decomposition bodies, indole, skatole, indoleacetic acid and indolepropionic acid. Indolepropionic acid was prepared by these workers by means of the Rauschbrand bacillus and *B. coli* working under anaerobic conditions in an atmosphere of hydrogen or nitrogen.

Ellinger [1904] fed tryptophan to a dog and found that there was an increased output of kynurenic acid in the urine. Some confusion existed for a long time as to the correct constitution of kynurenic acid, the two alternative forms being γ-hydroxy-α-carboxyquinoline and γ-hydroxy-β-carboxyquinoline. Homer [1913, 1914], by carefully recrystallising crude kynurenic acid from 40 % acetic acid, found that the melting-point agreed with that of γ-hydroxy-α-carboxyquinoline as prepared by Camps [1901].

Homer [1915] fed kynurenic acid to a dog but the material was excreted unchanged by the organism. It appears in that animal to be an end product of metabolism and not some intermediate product that has been excreted through being in excess.

Ellinger and Matsuoka [1914] prepared α-methyltryptophan and fed the product to a rabbit. They expected to obtain a methyl derivative of quinoline and to settle thereby in which form kynurenic acid exists. The only result of this experiment was that the α-methyltryptophan was recovered unchanged in the urine.

Barger and Ewins [1917] prepared α-methyltryptophan and fed it to a small dog. No kynurenic acid was found in the urine, the material being excreted in a form that left the indole ring intact.

Ellinger and Matsuoka [1920] prepared indolepyruvic acid and injected it into a rabbit. They obtained kynurenic acid in varying yields up to 11·7 %.

Injections of tryptophan gave yields of kynurenic acid up to 28 %. They also fed quinaldinic acid to rabbits but failed to obtain kynurenic acid as the oxidation product and found instead the quinaldinic acid conjugated with glycine.

Recently Matsuoka and Takemura [1922] have perfused the surviving liver of dogs and claim to have isolated kynurenic acid to the extent of 12 % after the addition of tryptophan and indolepyruvic acid to the perfusing fluid.

Homer [1915] made some spectrographic examinations of the pigment formed from indole compounds and from the urine of dogs fed with various indole compounds. She concluded that indole, skatole, indole-aldehyde, indole-carboxylic acid and indoleacetic acid undergo little change in the animal body but that indolepropionic acid appears to undergo a more deep-seated change in its passage through the animal organism.

Ewins and Laidlaw [1913] have shown that indole-ethylamine injected in the animal organism is excreted as indoleacetic acid conjugated with glycine. Similarly, when indoleacetic acid is injected into the animal organism, it is also excreted as a conjugate of indoleacetic acid and glycine. They have also shown that when indole-ethylamine is perfused through the surviving liver, the amine is oxidised to indoleacetic acid.

Guggenheim and Loeffler [1916] claim to have isolated indole-ethyl alcohol as a product of the perfusion of indole-ethylamine besides the previously isolated indoleacetic acid.

Experimental.

Preparation of Tryptophan and Indolepropionic acid.

The tryptophan required for the preparation of indolepropionic acid was made by the method of Hopkins and Cole [1901] with Onslow's modification [1921]. The product was finally extracted by butyl alcohol, according to Dakin's method [1918, 1920], a specially designed vacuum apparatus being used, in which the butyl alcohol boiled at about 65°.

Following the previous work of Hopkins and Cole [1903] several attempts were made to repeat the preparation of indolepropionic acid by means of pure cultures of *B. chauvei* obtained from the Lister Institute. Two such attempts failed and, as it was necessary to wait a month for the completion of an experiment, much time was lost. Finally twelve different strains of anaerobes were inoculated into small tubes of the media and kept under anaerobic conditions for a month in an incubator. These small tubes were tested after this lapse of time and four of them were found to have traces of ether-soluble derivatives of indole. These four were *B. coli*, *B. chauvei*, *B. sporogenes* and *B. oedemaciens*. Fresh twenty-four hour cultures were made of these bacteria and a five-litre bottle filled with sterilised medium containing 25 g. of tryptophan was inoculated with the whole four freshly growing cultures. The bottle was stoppered with a cork containing tubes for passing nitrogen gas through the bottle and with a mercury seal on the outlet. The air was removed by passing nitrogen through the culture and medium for a period of five hours.

When the bottle was opened at the end of four weeks, it was found to smell strongly of indole derivatives. In order to obtain the indolepropionic acid the solution was acidified with 5 % by volume of sulphuric acid and the indole compounds precipitated with mercuric sulphate-sulphuric acid solution. The precipitate was allowed to stand for twenty-four hours and then filtered off and washed. It was then suspended in warm water, made alkaline with baryta and the mercury compound decomposed with hydrogen sulphide. The solution was filtered from mercury sulphide and the residue washed. The filtrate and washings were united and acidified and extracted in the extraction apparatus with ether for four or five hours. The ether was then removed and the sticky mass of crystals was dissolved in alcohol, reduced in volume on a water-bath and water added. The indolepropionic acid was recrystallised from dilute alcohol after removing pigment by boiling with animal charcoal.

The average yield was about 30 % of the crude tryptophan used. In all, about 30 g. of indolepropionic acid were prepared.

Metabolism experiments with Indolepropionic acid.

Indolepropionic acid was dissolved in sodium bicarbonate solution and injected subcutaneously into a rabbit and the urine collected. When the urine was acidified with strong hydrochloric acid and boiled it developed a cherry-red colour. This colour is different from that obtained on injecting indoleacetic acid, acidifying the urine with hydrochloric acid, adding a drop of ferric chloride and boiling. It is also different from that obtained on heating free indoleacetic acid with hydrochloric acid.

3 g. were injected in the course of three days into four rabbits and the urine collected, care being taken to keep the collected urine in a cool place in presence of a preservative. The collected samples were precipitated with mercuric sulphate reagent by adding it until no further precipitate was obtained. The precipitate was filtered off and washed with distilled water. It was then suspended in distilled water, made alkaline with baryta, and decomposed with hydrogen sulphide. The mercury sulphide was filtered off and the baryta exactly removed by dilute sulphuric acid. The filtered solution was then evaporated to dryness on the water-bath. A red sticky gum was obtained which was dried in a vacuum desiccator over sulphuric acid. The red coloration developed as the evaporation proceeded and could not be prevented except in alkaline solution. It appeared to be a decomposition product of the precursor in the urine derived from the indolepropionic acid. Once the red pigment was formed, it acted very much like an indicator, turning yellow in alkaline solution and red in acid solution. Several attempts to purify this material by recrystallising were without success. The compound was further purified by dissolving in alkali, acidifying and extracting with butyl alcohol. It was then removed from the butyl alcohol by shaking with alkaline solutions of baryta, the baryta removed with dilute sulphuric acid and the solution again evaporated to dryness. The material was still tarry and could not be

got to crystallise from any of the ordinary solvents. This procedure was repeated several times but was not attended with any greater success.

Perfusion experiments were carried out with indolepropionic acid, tryptophan and indole-ethyl alcohol, but the only products that could be isolated were tarry materials soluble in alcohol but very resistant to all attempts at purification by recrystallisation. The liquid obtained at the end of a perfusion of tryptophan gave a positive nitroprusside test, as given by acetoacetic acid.

Absorption Spectra Experiments.

Owing to the difficulty of obtaining satisfactory results by the usual methods, it was evident that some other procedure must be used to obtain information as to the type of compound formed in the metabolism of indolepropionic acid. It was decided to attempt to make use of the quartz ultra-violet spectrophotometer in order to find out whether it would throw any light on the constitution of the compound present in the urine. There was the further inducement that it would be possible to record the absorption spectra of the compound without having to apply heat in its isolation.

In order to make use of this method it was first necessary to consider the possible metabolic products that might be obtained. This could be done by analogy with the known metabolism of similar compounds. Having listed the possible end products of the metabolism of indolepropionic acid, it was then necessary to make a study of these and related compounds as far as obtainable in order to observe the general shape of the absorption spectra curves and the effect of changing the substituting groups.

There appear to be four possible courses that the metabolism of indolepropionic acid could take which are as follows:

I. β-Oxidation of the side chain giving indolecarboxylic acid.

II. The oxidation of the carbon atom in the pyrrole ring giving rise to α-enol or α-keto indole derivatives.

III. A combination of types I and II giving rise to α-enol or α-keto β-carboxyindole.

IV. A splitting of the pyrrole ring and a linking up again with the formation of a quinoline derivative.

Following out this plan the absorption spectra of ten indole and seven quinoline compounds were measured. These divide themselves naturally into three groups.

Group I. *β-Substituted indole derivatives.*

(*a*) Tryptophan, indolepropionic acid and indole-ethyl alcohol.

(*b*) Indole, β-indolecarboxylic acid and β-indole-aldehyde.

Group II. *α- and β-Substituted indole derivatives.*

(*a*) $\alpha\beta$-Dihydroxyindole and α-hydroxy-β-indole-aldehyde.

(*b*) Isatin and sodium indigosulphonate.

Group III. *Quinoline compounds.*

Quinoline, quinaldine, lepidine, quinaldinic acid, quinolylacetaldehyde, 2:6-dimethylquinoline and kynurenic acid.

The spectra of the compounds of groups I and II have been described in a paper on indole compounds [1923, 1], and those of group III in a paper on kynurenic acid and some related quinoline compounds [1923, 2].

The absorption Spectra of the Metabolic Product of Indolepropionic acid.

Indolepropionic acid was injected subcutaneously into four rabbits and the urine collected over a period of twenty-four hours. The amount of indolepropionic acid used was 1 g. The urine was treated as described on p. 909, but the filtrate from the mercury sulphide was acidified and extracted with butyl alcohol. The butyl alcohol was then extracted with weak caustic soda solution and the separated aqueous layer was acidified and re-extracted with butyl alcohol. It was again extracted with dilute alkali and the alkaline solution acidified. This time the aqueous solution was extracted with ether and the ether extract washed with water. The aqueous solution was afterwards again extracted with butyl alcohol. In this manner two fractions were obtained, one soluble in butyl alcohol alone and the other soluble in butyl alcohol and in ether.

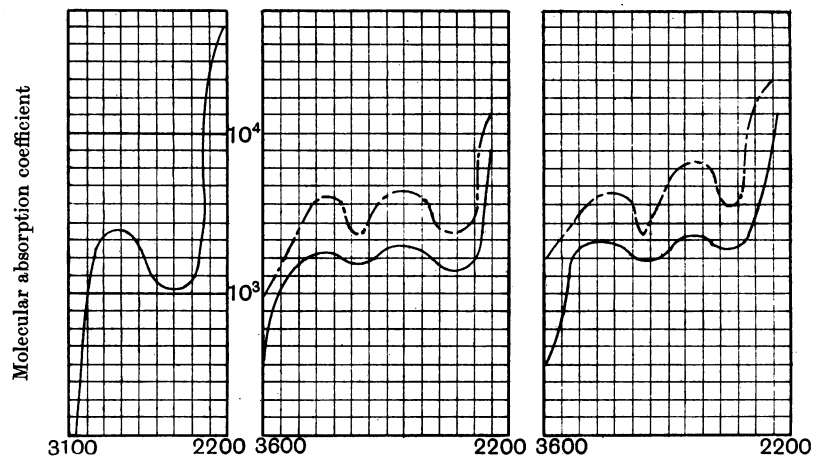
From these two fractions dilutions were made and the absorption spectra of the material measured. In these absorption spectra measurements there are two difficulties to be met with. The first of these is that the solutions are not pure and the second is that the concentration of the material in the solutions is unknown making it difficult to plot the results. However, it is possible to avoid these troubles to a certain extent. If the material to be determined is present in the solution in considerably greater concentration than the impurities, then these impurities will only interfere in the higher concentrations. As for the concentration certain corrections can be introduced based on the position of the peak of the bands and where they should appear in the correct concentrations.

The absorption spectra for indolepropionic acid and its metabolic product are given in Fig. 1. Fig. 1 *b* gives the absorption spectra of the ether-soluble fraction plotted in two ways, the lower uncorrected and the upper corrected for concentration. Fig. 1 *c* gives the absorption spectra curves of the alcohol-soluble fraction, the lower curve being uncorrected and the upper one corrected for concentration.

In order to show that this type of curve was due to some metabolic product of indolepropionic acid and not to impurities from the urine, two other compounds were also studied in the same way. These were indole-ethyl alcohol and indolecarboxylic acid.

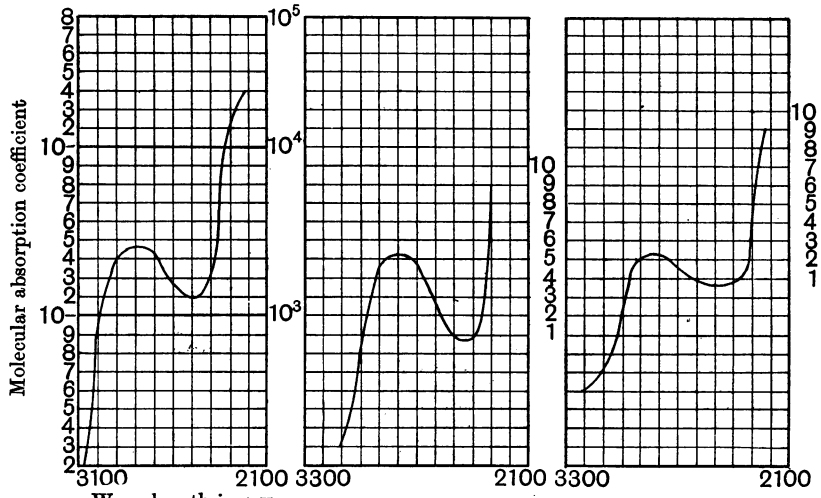
Indole-ethyl alcohol. 1 g. was dissolved in warm water and injected subcutaneously into four rabbits and the urine collected over a period of twenty-four hours. The urine was treated in exactly the same way as in the case of indolepropionic acid and the absorption spectra curves obtained of the two fractions. Further the ether-soluble fraction was identified as containing free indoleacetic acid. This was done in the following way. The ether solution was

treated with light petroleum to precipitate the pigments and tarry material. It was then allowed to stand and the clear solution decanted from the precipitated material. The mixed ethers were then evaporated on the water-bath. White waxy crystals were obtained which melted at 164°. The melting point of indoleacetic acid is given as 165°. Some of the material was dissolved in



(*a*) β-Indolepropionic acid. (*b*) Ether-soluble fraction. (*c*) Butyl alcohol-soluble fraction.

Fig. 1.

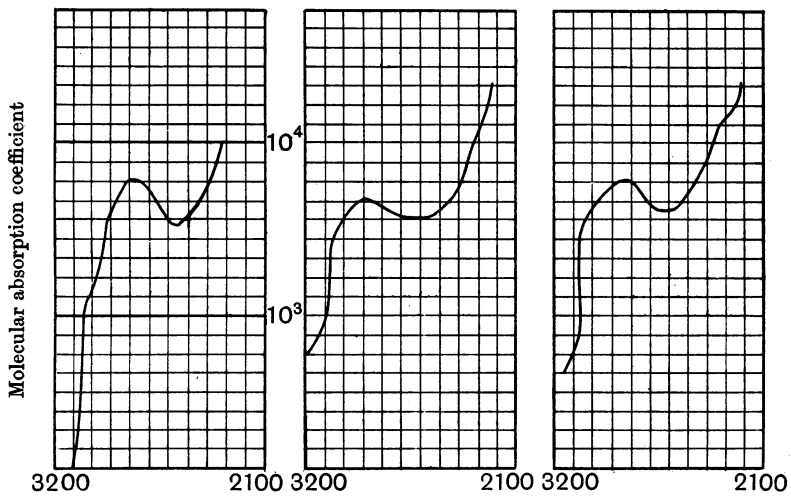


(*a*) β-Indole-ethyl alcohol. (*b*) Ether-soluble fraction. (Indoleacetic acid). (*c*) Butyl alcohol-soluble fraction.

Fig. 2.

water in a test-tube and boiled with strong hydrochloric acid. A red pigment was formed as given by free indoleacetic acid. The original urine also gave a red colour on boiling with strong hydrochloric acid and a drop of ferric chloride solution. The absorption spectra curves are shown in Fig. 2.

Indolecarboxylic acid. About a gram was injected into four rabbits and the urine collected over a period of twenty-four hours. The urine was treated as previously described for indolepropionic acid. Two fractions were again obtained and the absorption spectra measured. The urine on heating with hydrochloric acid gave a purplish colour but different from that given by indolepropionic acid. The absorption curves are given in Fig. 3.



(*a*) β-Indolecarboxylic acid. (*b*) Ether-soluble fraction. (*c*) Butyl alcohol-soluble fraction.

Fig. 3.

Discussion.

On examining the curves given for indole-ethyl alcohol and for indolecarboxylic acid, it can be seen that there has been no marked change in the absorption spectra given by the metabolic products from that given by the original substance. As can be seen by comparing them with the absorption spectra of β-substituted indole compounds they still retain the shape common to these.

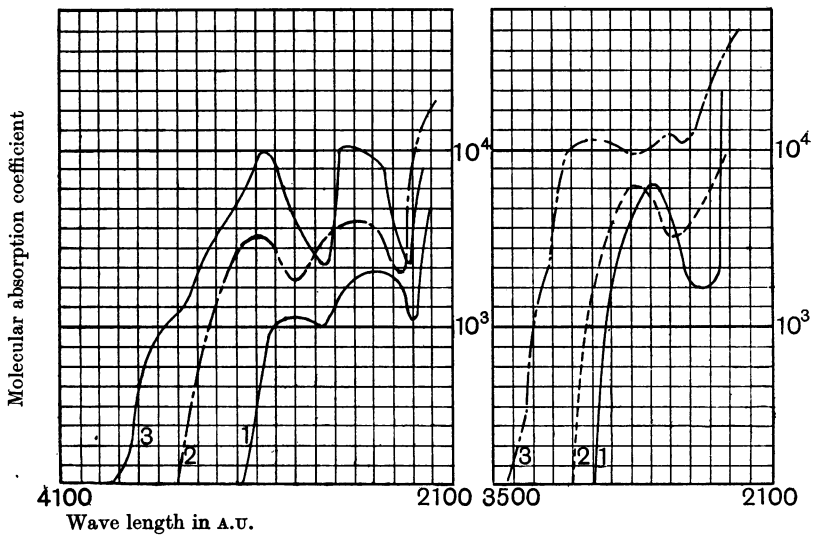
The absorption spectra of indolepropionic acid metabolic products show a marked difference from those of the original substance. The absorption spectra of these substances contain two wide bands, which is not characteristic of β-indole substituted compounds. On comparing the spectra with those given by quinoline compounds, no resemblance can be traced. However, on comparing the absorption spectra of the αβ-substituted compounds there is a noticeable resemblance.

In Fig. 4 the known absorption spectra curves of three β-substituted indole compounds and the absorption curves of two αβ-substituted compounds have been plotted. From these curves the absorption spectra curve of α-hydroxy-β-indolecarboxylic acid has been deduced and plotted. The resemblance

between the curve thus deduced and the curves of the metabolic products of indolepropionic acid is evident and strongly favours the suggestion of the formation of an α-hydroxyindole compound as the metabolic product of indolepropionic acid.

Attempts were made to prepare this compound from α-hydroxy-β-indolealdehyde by oxidation but it appeared to be very unstable as would be expected from the experience with the urinary compound. Several different oxidising reagents were employed but without success. It would appear necessary to stabilise the ring by methylating the hydroxyl group.

The fact that tryptophan and indolepyruvic acid yield kynurenic acid on being injected into the animal organism and the fact that α-methyltryptophan is excreted unchanged, suggest the possibility that the condition of the



(*a*) (1) Dihydroxyindole; (2) α-Hydroxy-β-indole-carboxylic acid (by analogy); (3) α-Hydroxy-β-indole-aldehyde.

(*b*) (1) Indole; (2) Indole-aldehyde; (3) Indolecarboxylic acid.

Fig. 4.

α-carbon atom in the pyrrole ring plays an important part in the metabolism of the indole ring as present in tryptophan. The presence of a methyl group would prevent oxidation at this point.

That the α-carbon atom is chemically reactive is shown from other sources. Hopkins and Cole [1903] oxidised tryptophan with ferric chloride and obtained two bases one of which was shown by Ellinger [1904] to be indole-aldehyde and the other was shown by Perkin and Robinson [1919] to be identical with harman, a derivative of harmine. Perkin and Robinson have prepared this substance by oxidising tryptophan in the presence of acetaldehyde. Under this treatment the end of the aliphatic side chain joined on to the α-carbon atom.

SUMMARY.

Indolepropionic acid in its passage through the animal body appears to undergo oxidation in the α-carbon atom of the pyrrole ring with formation of an α-hydroxyindole compound, which may be in either the enol or keto form.

Indole-ethyl alcohol in its passage through the animal organism undergoes oxidation to indoleacetic acid.

Indolecarboxylic acid, in its passage through the animal body, appears to be unchanged except that it is excreted in a conjugated form. It is not an intermediate step in the metabolism of indolepropionic acid.

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