II. THE FATE OF INDOLETHYLAMINE IN THE ORGANISM.

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It has been shown by several observers that the naturally occurring amino-acids are readily attacked by bacteria, and that among other products the corresponding amines are formed [Winterstein and Kuntz, 1909; Barger and Walpole, 1909; Ackermann, 1910; see Barger, 1911, for other references]. It appears probable that they are produced in the intestine in the course of the normal life of the organism and also in certain pathological conditions. Their fate after absorption is therefore a matter of considerable importance. The amines of this type have in most cases been submitted to physiological investigation [Dale and Dixon, 1909; Ackermann and Kutscher, 1910; Dale and Laidlaw, 1910], and in several cases they have been shown to be active substances. The method by which the body deals with such bases has not been investigated so thoroughly.

Some time ago we showed [Ewins and Laidlaw, 1910] that *p*-hydroxyphenylethylamine was largely converted into *p*-hydroxyphenylacetic acid and excreted as such; that the liver and the plain muscle of the body, with the exception of the plain muscle of the lung vessels, could effect the change: and that successive methylation of the amino-group rendered the base increasingly resistant to destruction in the body. Another example of a change of precisely the same type is met with in the fate of benzylamine, which was shown by Mosso [1890] to be almost quantitatively converted into benzoic acid and excreted as hippuric acid. We undertook the investigation of the mode of destruction of indolethylamine (β-indole-*pr*-3-ethylamine) in order to see whether it was dealt with in a similar manner, and whether its administration would increase the kynurenic acid output in dogs.

The indolethylamine used was made according to the synthesis devised by one of us [Ewins, 1911], which readily gives a good yield of the base.

We had already shown [Ewins and Laidlaw, 1910] that it was possible to obtain this base by the action of putrefactive bacteria on tryptophane; but the method is tedious and troublesome, and the yield very poor.

We have carried out two sets of experiments in the course of our investigation: (1) feeding experiments on dogs, and (2) the perfusion of surviving livers of rabbits and cats[1].

PERFUSION EXPERIMENTS.

The method of perfusion was similar to that already described in our paper dealing with the fate of *p*-hydroxyphenylethylamine [Ewins and Laidlaw, 1910]. Small quantities of indolethylamine were perfused through surviving livers for 3—4 hours. The perfusion fluid gradually acquired the property of giving a fine red colour, when it was mixed with one-third of its volume of strong hydrochloric acid, with the addition of one drop of dilute ferric chloride, and then boiled. The red colour occasionally had a faint blue component if the hydrochloric acid was in excess or if the boiling was prolonged. A cherry red colour also developed if a trace of sodium nitrite was substituted for the ferric chloride in the above tests. Amyl alcohol rapidly extracted the pigment from the solution, when a well-defined absorption band in the green was seen with a spectroscope. The red colour is brighter and purer and the absorption band more intense and better defined when sodium nitrite is used. When the chromogen of the pigment appeared to be fairly abundant in the perfusion fluid (usually about 3–4 hours) the perfusion fluid was collected, and the liver vessels washed through with salt solution. The combined washings and perfusion fluid were rendered faintly acid with acetic acid and boiled. The coagulated proteins were filtered off and the filtrate evaporated to small bulk. The concentrated perfusion fluid was made acid to Congo-red with strong hydrochloric acid and shaken out with ether, which readily and completely extracted the chromogen. The ethereal extracts were combined, washed twice with water and taken to dryness. If the residue were taken up in water and allowed to stand, a small quantity of a crystalline acid was regularly obtained, which when recrystallised from benzene melted at 163–164°. These crystals gave the colour reactions mentioned above with great intensity and readily gave a deep orange-red coloured picrate melting at 174°. These properties taken together are characteristic of indoleacetic acid.

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[1] The animal experiments were performed by P. P. Laidlaw only.

Experiment. 0·25 grm. indolethylamine hydrochloride was perfused through a rabbit's liver for 2·5 hours. Perfusion fluid and washings were worked up as described above when 50 mgm. of indoleacetic acid were obtained. Yield 44 % of theoretical.

The identification was completed by comparison of colour reactions, melting point, and mixed melting point with synthetic β-indole-*pr*-3-acetic acid.

There can be no doubt then that indolethylamine is readily converted by the liver into indoleacetic acid, just as *p*-hydroxyphenylethylamine is converted into *p*-hydroxyphenylacetic acid.

FEEDING EXPERIMENTS.

One half to one gram of the hydrochloride of indolethylamine is well borne by a 7–8 kilo dog. Larger doses are inadmissible for our purpose since vomiting and sometimes purgation ensue.

The urine of the 36 hours following the administration did not give the colour reactions which we have always found in our perfusion experiments, but on adding to a small quantity of urine half its volume of strong hydrochloric acid and a trace of nitric acid and gently warming, a fine purple colour develops, which gradually (unless the mixture is cooled) becomes deep red and then orange and ultimately yellow. The colour reminds one of a very intense indican reaction. It is not, however, due to the development of indigo since at no stage can a blue component be extracted by chloroform.

We found that the chromogen of this colour reaction was an acid, which could be shaken out from the strongly acidified urine with ether or ethyl acetate, but the complete extraction of the chromogen by either of these solvents from acid urine was found to be almost impossible. The acid, however, was found to be much more readily extracted if its solubility in urine was depressed by saturation with ammonium sulphate. In a few experiments with rabbits' urine this preliminary saturation with ammonium sulphate was found to be of considerable assistance, since the procedure caused a large proportion of the hippuric acid to crystallise [cf. Roaf, 1908].

The ether or ethyl acetate extracts were carefully collected, and washed with ammonium sulphate, and then with a small quantity of water. The acid was then extracted from the ether by sodium carbonate solution. The alkaline extract was acidified, saturated with ammonium sulphate and shaken out with ether. The ether extracts were washed with water and taken to dryness. A thick gum resulted from which nothing would crystallise. The gum was extracted with boiling water, in which most of it dissolved, excess of picric acid was added and the whole boiled with a small quantity of charcoal for five minutes. On filtration and prolonged standing the filtrate gradually deposited a deep orange-red coloured picrate. This picrate was recrystallised from water or water with a trace of acetone several times, when it separated in large, orange-red, rhomboidal plates melting at 145°. The crystals gave the colour reaction with great intensity. It appeared to us to be very probable that this acid would prove to be an indoleacetic-glycine complex, comparable with hippuric and phenaceturic acids, but for some time we were unable to obtain sufficient material to prove or disprove our assumption. The yield of picrate of the acid is not good and, as we have pointed out, the feeding experiments are limited to the administration of small quantities.

In one experiment for example, from 0·5 grm. of the hydrochloride of the base we only obtained 0·2 grm. of the picrate or a little over 20 °/₀ of the theoretical maximum yield. In some other experiments the yield was rather better but never more than 30 °/₀.

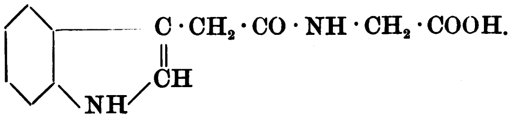
From a series of three experiments we obtained sufficient of the picrate which, although not quite pure, could be analysed:

$$0{\cdot}0986 \text{ grams picrate gave } 0{\cdot}1668\ CO_2 \text{ and } 0{\cdot}0322\ H_2O$$

$$C = 46{\cdot}1 \qquad H = 3{\cdot}6$$

$$C_{12}H_{12}O_3N_2 . C_6H_3O_7N_3 \text{ requires } C = 46{\cdot}8,\ H = 3{\cdot}2 \text{ per cent.}$$

The figures obtained although not accurate were sufficiently so to indicate that the acidic chromogen was an indoleacetic-acid-glycine condensation product, and that the substance in question has the probable constitution denoted by the formula



For this acid we suggest the name *indoleaceturic acid*, in conformity with the nomenclature of this class of bodies to which hippuric and phenaceturic acids belong.

It may also be mentioned that quite recently Ackermann [1912] has shown that among the products of metabolism of nicotinic acid fed to a dog is found a similar glycine derivative, to which he gives the name nicotinuric acid.

Our supposition with regard to the constitution of the urinary chromogen was further confirmed by hydrolysis of the picrate with sodium carbonate.

Experiment. 0·250 grm. of the picrate was dissolved in 80 c.c. of 5 % sodium carbonate solution and hydrolysed on the water bath for one hour. On cooling and acidifying, 0·19 grm. of indoleacetic acid picrate separated out, and from the mother liquor 0·017 grm. more was obtained crystalline. Total 0·207 grm. or 95 % of the theoretical amount if the original picrate had been the suggested indoleacetic-glycine complex.

Since the amount of acid obtainable in the experiments with indolethylamine was so very small, and we have shown by perfusion experiments that indoleacetic acid is probably an intermediate step in the production of indoleaceturic acid from indolethylamine, we carried out a series of feeding experiments with indoleacetic acid in the hope of obtaining larger quantities of the desired acid. Several grams of indoleacetic acid were synthesised by a method which will be published at a later date, and given by mouth to a dog, when a 60 % yield of picrate was obtained, identical in all respects with that obtained from the feeding experiments with the base. This fact enabled us to obtain the picrate in comparatively large quantities, since the indoleacetic acid caused no symptoms. In this way we obtained without trouble 3 grams of indoleaceturic acid picrate from 3 grams of indoleacetic acid. The preparation of the free acid from the picrate could not be carried out in the usual manner (removal of the picric acid from the acidified solution by means of ether) owing to the well-marked acidic properties of the indoleaceturic acid and its solubility in ether. This difficulty was overcome by the use of the base known as nitron (1.4-diphenyl-1.3.5-endanilino-dihydrotriazole) [Busch, 1905] which precipitates picric (as well as nitric) acid as an almost insoluble salt of the base. The experiment was carried out as follows.

1·5 grams of picrate were dissolved in 150 c.c. of hot water and 1 molecular proportion of nitron dissolved in hot dilute alcohol was added. The mixture was thoroughly cooled and the precipitated picrate sucked off at the pump. The nearly colourless filtrate was neutralised with sodium carbonate, two or three drops of acetic acid added and the solution evaporated to dryness. The residue was dissolved in a few (7–8) c.c. of water and made just acid to Congo-red with dilute hydrochloric acid. An oily product separated which soon crystallised in bunches of needle-like prisms. This was filtered off, dried in vacuo over sulphuric acid for a short time and weighed. In this way 0·6 gram of the crystalline acid was obtained. This was twice recrystallised from water and then melted at 94°. The acid so obtained is very slightly soluble in cold water (about 0·2 % at ordinary temperature), almost insoluble in ligroin and benzene, easily soluble in alcohol, ether and ethyl acetate. It crystallises from water with one molecule of water of crystallisation which is only very slowly removed by sulphuric acid in vacuo at 37°.

0·1022 gram acid (m.p. 94°) gave 0·2184 CO_2 and 0·0514 H_2O

$C = 58·2$ $H = 5·6$

Calc. $C_{12}H_{12}O_3N_2 \cdot H_2O$ $C = 57·6$ $H = 5·6$ per cent.

0·1026 gram acid (anhydrous) gave 0·2330 CO_2 and 0·0492 H_2O

$C = 61·9$ $H = 5·3$

Calc. $C_{12}H_{12}O_3N_2$ $C = 62·0$ $H = 5·2$ per cent.

This result fully confirms our supposition that the acid in question is indoleaceturic acid.

It will be observed that we have never obtained anything like a quantitative yield of indoleaceturic acid by giving indolethylamine by mouth. In our best experiments about 30 °/₀ of the base is accounted for in this manner. It is true that this probably represents a much larger quantity of indoleacetic acid, which must be the intermediate product; because the administration of indoleacetic acid has never in our experience given a quantitative yield of indoleaceturic acid. However, making some allowance for this and a little for the imperfect methods used in the isolation of the acids from such a complex mixture as urine, we still have not accounted for the whole of the base.

It appeared to us to be possible that some of the indolethylamine or indoleacetic acid might be converted into kynurenic acid, although none of the specimens of urine that we had examined showed obvious excess of this metabolite. Some experiments were therefore undertaken in which kynurenic acid estimations were made before and after administration of indolethylamine and indoleacetic acid.

A dog was kept on a standard diet of milk and dog biscuit. On this particular diet the kynurenic acid excretion was minimal, and traces only could be detected in 24 hours. The kynurenic acid was estimated by Capaldi's method [1897].

Experiment.

Date	Volume of urine	Kynurenic acid	Remarks
Sept. 23—24	400 c.c.	Trace	—
Sept. 24—25	440 c.c.	Trace	—
Sept. 25—26	600 c.c.	·480 grm.	On morning of 25th 1·6 grm. tryptophane by mouth.
Sept. 30—Oct. 1	350 c.c.	Nil	—
Oct. 1—2	600 c.c.	Trace	On Oct. 1st 1·6 grm. indoleacetic acid by mouth.
Oct. 2—4	850 c.c.	Traces	—
Oct. 4—5	470 c.c.	Traces	On Oct. 4th 1·0 grm. indolethylamine by mouth.

Neither indolethylamine nor indoleacetic acid increases the kynurenic acid output in dogs.

It seems clear that this path of metabolism of indole-derivatives does not aid us in accounting for the quantitative difference between the ingested substances and those excreted.

Samples of urine were examined in various ways for other possible end products but without success, and we suggest that the portion of the base unaccounted for is completely broken up in the body.

OCCURRENCE OF INDOLEACETURIC ACID.

We have some evidence of the occurrence of indoleaceturic acid in normal urine of herbivora. We have found that an acidic, ether-soluble substance, giving the purple colour reaction, occurs in small quantity in the urine of rabbits, but attempts at isolation of the acid or its picrate have always failed. The small amount of the chromogen cannot be separated from the other acid, ether-soluble substances present in all urines.

In examining urine for indican it occasionally happens that a fine purple colour is produced by hydrochloric acid and an oxidising agent but no blue component can be extracted by chloroform. Here, again, it is suggested that the chromogen is indoleaceturic acid, but considerable quantities of urine would be required to isolate the acid and such have not been at our disposal.

Herter [1908] described a case of a young girl who was suffering from some unusual intestinal infection and from whose faeces an unusual organism was isolated. The urine of the patient gave a marked urorosein reaction if the urine were stale. Indoleacetic acid was isolated from this urine and Herter identified it as the chromogen of urorosein originally described by Nencki and Sieber in 1882. The colour, solubilities and other characteristics of the pigment agree very well with those which Nencki and Sieber described as characteristic for urorosein, although other observers have described other substances as the chromogen of the same pigment. It is unfortunate that Herter gives no description of the method of isolation of indoleacetic acid from the urine of his patient. It is quite possible that it was originally present as indoleaceturic acid and that this was hydrolysed to indoleacetic acid in the process of isolation, for the complex acid is readily hydrolysed by weak alkalies such as sodium carbonate. The fact that stale urine gave a colour, while fresh did not, was attributed by Herter to the formation of nitrites. It is quite possible that the indoleaceturic acid was decomposed into indoleacetic acid and glycine, just as hippuric acid is readily split up by bacteria, and that the nitrites, though certainly playing a part, did not furnish the whole explanation.

If it could be demonstrated that indoleaceturic acid was a normal constituent of urine it would by no means follow that its precursor in the body was indolethylamine, for bacteria often produce indoleacetic acid from tryptophane and proteins; in fact it is a more frequent product of bacterial action than indolethylamine.

SUMMARY.

1. Indolethylamine is converted, by the perfused liver, into indoleacetic acid.
2. Indoleacetic acid is excreted in combination with glycine forming indoleaceturic acid.
3. About 30 °/₀ of a given dose of indolethylamine is excreted as indoleaceturic acid in dogs.
4. Neither indolethylamine nor indoleacetic acid affects the kynurenic acid output in dogs.

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