XVII. THE ALLEGED ANTINEURITIC PROPERTIES OF α -HYDROXYPYRIDINE AND ADENINE.

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In the hope of obtaining a compound which would possess antineuritic properties Williams [1916] prepared and tested a series of substances. By adopting this line of research he hoped to throw some light on the chemical identity of the active principle which cures polyneuritis in birds and beri-beri in man. He confined himself to the study of pyridine derivatives mainly because the active fraction obtained from rice polishings is closely associated with nicotinic acid and under certain treatment gives off a pyridine-like odour, whilst the colour reactions given by antineuritic substances with phosphotungstic and phosphomolybdic acids respectively are also characteristic of the hydroxypyridines. He prepared and tested a large series of pyridine derivatives including nicotinic, cinchomeric, quinolinic, 6-hydroxynicotinic and citrazinic acids, glutazine, α -hydroxypyridine, 2:4:6-trihydroxypyridine (and its anhydride) and 2:3:4-trihydroxypyridine. The last three of the above Williams found to be definitely active but the activity disappeared after some time. As there was no evidence of decomposition, Williams ascribed the loss of the antineuritic properties to isomerisation. To substantiate his hypothesis he made a detailed study of one of the active substances— α -hydroxypyridine. He found that there were two crystalline modifications of this substance, needles and granules. By treating polyneuritic birds with the needle variety Williams managed to effect in every case a definite cure or improvement; the granules on the other hand were inactive. The absolute inactivity of the granular modification of α -hydroxypyridine induced him to put forward the further suggestion that the polyneuritic condition is brought about not by the lack of a particular isomer, since isomers are in equilibrium in solution and any biological preference for one

would disturb the equilibrium and thus effect the corresponding conversion, but by a want of a certain type of potential energy supplied by the process of isomerisation of certain substances. In a subsequent investigation Williams in collaboration with Seidell [1916] isolated from yeast an active substance which they considered to be identical with adenine and which lost its antineuritic activity on keeping. This loss of therapeutic potency the authors also ascribed to isomerisation.

If isomerisation is really responsible for the therapeutic activity of antineuritic principles it is of great scientific importance and interest and it was our object to study the matter in detail. We chose α -hydroxypyridine, with which Williams claims to have achieved some success, as our starting point. Although we could confirm most of the facts about the chemistry of that substance we were unable by its use to effect anything which in our opinion could be considered as a cure or even an improvement in the condition of the polyneuritic birds. We have taken great care in following Williams' method in the preparation of the active needles and no doubt exists in our minds that our preparations were identical with those described by Williams. We have also taken great pains in watching our birds and nothing of clinical importance could have missed our notice.

EXPERIMENTAL.

Like Williams we prepared the α -hydroxypyridine by distilling hydroxynicotinic acid according to the method of Pechmann and Baltzer [1891]. The latter was made from the methyl ester of coumalic acid by the method of Pechmann and Welsh [1884]. The coumalic acid was obtained from malic acid by the method described by Pechmann [1891]. Before distillation the hydroxynicotinic acid was decolorised with charcoal and purified by recrystallising it several times from hot 50 % acetic acid. It always gave the characteristic reactions and correct melting point. The α -hydroxypyridine obtained by distilling the hydroxynicotinic acid gave the correct nitrogen figures on determination by the Dumas method.

When hydroxynicotinic acid is distilled the α -hydroxypyridine comes over as syrup which on cooling crystallises in a mixture of needles and non-acicular crystals which Williams classified as granules. In Williams' opinion the amount of moisture present in the process of distillation is a determining factor as to the relative amounts of the two crystalline modifications formed. We found that by excluding moisture entirely needles only were formed.

We therefore heated the hydroxynicotinic acid and the distilling apparatus at 110° for several hours before distilling. The receiver was also fitted with a CaCl_2 tube in order to exclude all traces of moisture. From a recent publication of Williams [1917] which reached us only lately we find that he independently adopted a similar procedure and like ourselves was able to obtain needles only. On cooling, the needles crystallise out from the syrup and finally complete solidification occurs with formation of a dense mass. In parts of the apparatus where the layer of crystals is thin, well-defined needles are discernible even after complete cooling down. The solidified syrup did not show a definite melting point, but melted somewhere between 94° – 98° . On keeping the open apparatus under ordinary laboratory atmospheric conditions a marked change could be observed in the needles in those parts of the distilling flask where their crystalline form was well defined after complete cooling. The needles after about 10 days broke up by transverse fissures and ultimately changed into non-acicular crystals of a granular form. On heating the cooled down syrup at a temperature of 120° for 2–3 hours and then allowing the molten mass to cool slowly in the absence of moisture dissolving it in hot dehydrated benzene and adding dehydrated ligroin to the cold solution a milky turbidity was produced and after a short time a deposit of acicular crystals was formed. Few, if any, granules were obtained by this precipitation, but a preponderance of granular crystals was obtained on concentrating the mother liquor. The needles thus obtained melted at 108° – 110° (uncorrected), whilst the granules melted at 106° – 108° (uncorrected). It is to be remarked that the configuration of the needles obtained by the benzene-ligroin recrystallisation differed in the different preparations obtained by that method. We did not however study the conditions governing these differential configurations. When the freshly distilled α -hydroxypyridine is dissolved in hot water and crystallised from it a mixture of needles and prisms is obtained. It is thus seen that there are at least two distinct crystalline modifications of α -hydroxypyridine, namely needles and non-acicular crystals. It will however be remembered that the melting points of the needles obtained by direct distillation of hydroxynicotinic acid and the needles obtained by the benzene-ligroin crystallisation method are not the same and therefore the two varieties of needles cannot be considered to be identical.

Polyneuritis was induced in our pigeons by forcible feeding with rice, the birds receiving daily 20 g. each morning and evening. The crops of the birds were examined daily and if any hardness was observed the feeding was suspended until the bird became normal again. The time taken by the

birds to develop polyneuritis varied from two to five weeks. All the injections were done intramuscularly in the breast and freshly prepared crops of crystals were always used unless otherwise mentioned. Following the practice of Williams the crystals were always dissolved in a convenient quantity of distilled water immediately before injection. The birds under treatment were kept under very close observation. Varying doses were tried. Needles obtained by direct distillation and needles from benzene and ligroin crystallisation as well as crystals obtained by crystallisation from hot water were tested. The following protocol describes eight experiments.

Expt. 1. 10 mg. of freshly distilled α -hydroxypyridine (needles) were dissolved in 2 cc. of distilled water and injected intramuscularly in pigeon 151 at 4 p.m. The bird showed at the time paralysis of the legs and retracted neck. At 8 p.m. its head was found to be markedly improved, and the condition of improvement was maintained at 11 p.m. Next morning the bird had a relapse. 20 mg. of needles obtained by distillation on the previous day were administered and as the bird showed no improvement a further 0.1 g. of the same substance was injected two hours later. The condition became worse and the bird died during the night.

Expt. 2. Pigeon 219 walked with great difficulty and was entirely unable to use its wings; head normal. It received 50 mg. of needles of freshly distilled α -hydroxypyridine at 12 noon. It was slightly worse at 11 p.m. the same evening, and worse again next morning. On the third day the bird showed a retracted neck. On the fourth day its condition was still worse and finally it was found dead on the morning of the fifth day.

Expt. 3. Pigeon 189 could not fly and walked with difficulty. It received 10 mg. of needles of freshly distilled α -hydroxypyridine at 12 noon. Next day there was no change in condition. On the third day the bird was decidedly worse. On the fourth day its condition was still worse, no retracted neck however was to be observed. It was found dead on the fifth morning.

Expt. 4. Pigeon 229 was paralysed and showed retracted neck. It received 50 mg. of needles obtained by recrystallising the α -hydroxypyridine from benzene with ligroin at 11.30 a.m. At 8 p.m. the same day its condition was worse and it died at 3 p.m. the following day.

Expt. 5. Pigeon 205 only showed incipient symptoms. It received 200 mg. of freshly distilled needles at 11.45 a.m. It became worse during the day and developed a retracted neck. Next day an improvement was observed but it became worse again in the course of the day and was found dead on the morning of the third day after treatment.

Expt. 6. Pigeon 230 showed paralysed limbs. It received 2 mg. of freshly distilled needles at 10 a.m. No change occurred in its condition during the day, but it became gradually but decidedly worse during the next three days and died on the fourth day.

Expt. 7. Pigeon 222 showed paralysed limbs. It received 50 mg. of α -hydroxypyridine recrystallised from hot water at 10.30 a.m. No change in condition occurred during that day. The next day the bird was worse and developed a retracted neck. It became gradually worse during the day and was found dead the next morning.

Expt. 8. Pigeon 149 showed paralysed limbs. It received 10 mg. of needles obtained by recrystallisation from benzene with ligroin at 6 p.m. No improvement was observed at 11 p.m. the same evening and the condition remained unchanged for some time. On the fourth day the bird was worse. 50 mg. of needles of the same crop were injected at 10.30 a.m. on that day. The bird died at 1 o'clock in the afternoon.

It is seen from the above experiments that no cure or improvement in the condition of the birds was effected by the treatment. Some birds lived several days after being treated, but they were types of cases, well known to those who work with polyneuritic birds, which would have behaved similarly if they had received no treatment at all. In many of the above experiments we started our treatment as soon as polyneuritic symptoms manifested themselves in order to give the drug a good chance to act. Some of these cases have turned out to be of the prolonged type where the onset is mild and gradual and lasts several days before terminating fatally, others have proved themselves to be of the intermittent type, in which the well-declared symptoms are strikingly relieved for a few hours before the second onset takes place; after the second attack the birds usually succumb. Expts. 2, 3, 6, and 8 are characteristic of the prolonged type. Expts. 1 and 5 represent the intermittent type. Special attention is to be paid to Expt. 5. That bird turned out to be of the intermittent type. It was treated when the symptoms were not more than of a premonitory character and the dose it received was very high, namely 200 mg. In spite of that, the first attack developed in the usual way and the bird succumbed as is common in cases of this type after the second onset within two days of the manifestation of the early symptoms. A special experiment showed that the injection of this amount of α -hydroxypyridine produced no toxic symptoms in a normal pigeon. In spite of the fact that we used both varieties of needles in various doses we are unable from our experiments

to attribute any antineuritic properties to the needle variety of α -hydroxypyridine.

Reference was made above to the work of Williams and Seidell [1916] in connection with the isomerisation of adenine. They obtained an active substance from autolysed yeast by adsorption which they considered to be adenine and which on keeping became inactive. The inactive adenine on being treated with sodium ethylate in a sealed tube at 100° for four hours, according to the observations of these workers became active. While we were engaged in repeating some of these experiments there appeared a paper by Voegtlin and White [1916] in which a variety of experiments with adenine, all of a negative nature, were described. We have therefore repeated only the crucial experiments of Williams and Seidell, and like Voegtlin and White were unable to confirm their results.

We prepared our adenine from a sample of pure adenine sulphate kindly supplied to us by Mr A. Chaston Chapman. The adenine sulphate which was recrystallised several times was obtained by Mr Chapman by hydrolysis from yeast nucleic acid. The adenine was precipitated from the sulphate by neutralising it exactly with ammonia and passing a stream of CO_2 . The filtered residue was washed with water, alcohol and ether. The adenine gave Kossel's reaction; when heated quickly it melted at 343° (uncorrected) and yielded a picrate consisting of yellow silky needles which charred at 240° . In accordance with Williams and Seidell's directions 50 mg. of adenine were heated at 100° for five hours with 5 cc. of absolute alcohol in which 10 mg. of metallic sodium were dissolved. The orange coloured liquid was then evaporated to dryness on a water-bath and the residue dissolved in distilled water immediately before injection. The following protocol describes five experiments.

Expt. 9. Pigeon 256, which showed well-developed symptoms of polyneuritis with retracted neck, received 10 mg. of untreated adenine; it became worse next day and was found dead on the morning of the third day.

Expt. 10. Pigeon 187 showed paralysed limbs. It received at 4 p.m. the entire residue from 50 mg. of adenine treated as described above. Slight improvement was noticeable on the next day when it again received the residue from 50 mg. of adenine. No change occurred on the third and fourth days; it became worse on the fifth day and remained in the same state during the sixth. On the seventh day it became decidedly worse and was treated with the residue from 50 mg. of adenine. No improvement was observed and the bird died during the night.

Expt. 11. Pigeon 247 showed well-declared symptoms of polyneuritis with retracted neck. It received at 4 p.m. 10 mg. from residue obtained by treating 50 mg. of adenine. The bird was found dead next morning.

Expt. 12. Pigeon 198, which was paralysed but kept its head well forward, received 5 mg. of treated adenine. On the next day it was a little better and received another 5 mg. of the adenine preparation of the previous day in the morning and further 5 mg. of a fresh preparation in the afternoon. On the third day the condition was unchanged and the remaining portion of the 50 mg. of adenine, treated on the previous day, was administered at 10.30 a.m. The bird became worse and died at 2 p.m. the same day.

Expt. 13. Pigeon 217 showed incipient signs of polyneuritis. It received 50 mg. of adenine treated with sodium ethylate. It was better the next day, but had a relapse on the third day and received a further 50 mg. of treated adenine. No improvement ensued and the bird was found dead on the fourth morning.

The above experiments like those performed with α -hydroxypyridine must be regarded as negative in character. In *Expt. 10* the bird remained alive for six days, but the condition of the bird during that time was such that nothing could be definitely ascribed to the action of the administered adenine. The results obtained in the remaining experiments are so distinctly negative that no comment is necessary.

SUMMARY.

1. The authors can confirm the following facts observed by Williams about the nature of α -hydroxypyridine.

(a) α -Hydroxypyridine obtained by distilling hydroxynicotinic acid crystallises in needles and non-acicular crystals.

(b) On keeping the needles of α -hydroxypyridine, thus obtained, exposed under ordinary laboratory atmospheric conditions, they break up into granules in about 10–14 days.

(c) Needles of α -hydroxypyridine are also obtained by heating the freshly distilled substance for several hours at 120° and then adding ligroin to a solution of the cooled substance in benzene.

2. The authors find that the needles obtained from benzene-ligroin differ in their melting point from those obtained by direct distillation of α -hydroxypyridine.

3. Both varieties of needles of α -hydroxypyridine were injected into

polyneuritic pigeons but no cure or amelioration in the condition of the birds was observed. The observations of Williams were therefore not confirmed.

4. Crystals obtained by recrystallising α -hydroxypyridine from water proved also to be inactive.

5. Pure adenine, as well as adenine treated with sodium ethylate in a sealed tube for five hours at 100°, yielded negative results in agreement with Voegtlin and White but in disagreement with Williams and Seidell.

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