CLAIMS have recently appeared in the literature as to the curative properties of certain quinoline and glyoxaline derivatives to pigeons suffering from polyneuritis. Sahashi [1926, 1927] isolated from oryzanin a substance which was identified as 2:6-dihydroxyquinoline; it was claimed that the hydrochloride of this compound was temporarily curative (in doses of 4–15 mg.) to pigeons suffering from polyneuritis produced by rice-feeding. These birds subsequently died. Later in an attempt to synthesise a glyoxaline derivative of the same empirical formula as that of Jansen and Donath's [1927] crystalline compound, Sahashi [1928] prepared 4 (or 5)-glyoxalinemethylethylcarbinol. Injections of about 6 mg. of this substance intramuscularly into the breasts of pigeons were temporarily curative, though the birds invariably died in 7–10 days. These results seemed to be sufficiently striking to warrant confirmation by the methods of testing employed in this laboratory. The substances have accordingly been prepared and tested. In this paper are also recorded certain isolated tests carried out at various times upon glyoxaline and other compounds. None of these compounds possesses the true curative activity similar to that given by the torulin concentrates which we have so repeatedly used.

**Experimental.**

*The pigeon tests.* It is not necessary to give in detail all of the protocols of tests. They have not been numerous, but according to our experience are conclusive: in fact a few birds tested under rigorous conditions are worth considerably more than a number not specially controlled. The standard conditions laid down in a previous paper [Kinnersley, Peters and Reader, 1928] have been followed throughout with the following additional improvement. It has been the custom lately, where special care is required, to give a standard dose of a torulin preparation which is curative for about 3 days, before giving the substance under test. If after this the test substance is not effective, but it is subsequently possible to relieve the symptoms by the use of a second dose of the standard preparation, then the negative answer is decisive. The technique of such an experiment is to give a dose of glucose in water, followed by a dose of a standard preparation in 4 hours if the glucose
and water do not cure. After re-appearance of the symptoms in 3–4 days, a
dose of the test substance is given as soon as possible and before actual head
retraction occurs. If the test dose does not cure in 6 hours, a further dose
of some known curative material can be given. It will be seen from the table
that none of the substances has proved itself to be curative under these
conditions.

Table I. Compounds with alleged antineuritic properties.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bird</th>
<th>Symptoms</th>
<th>Dose of glucose in water</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 (or 5)-Glyoxalinemethylethylcarbinol hydrochloride</td>
<td>Bird 375</td>
<td>12 mg. injected in 1 cc. of fluid. Died</td>
<td>12.30 p.m. No better. Injected 5 mg. compound, and gave 5 cc. water by mouth</td>
<td>6.15 p.m. better</td>
</tr>
<tr>
<td></td>
<td>749</td>
<td>9 mg. injected in 1 cc. of fluid. Died in 6 hours</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>775</td>
<td>5 mg. injected in 0.5 cc. fluid. No improvement in 3 hours, after which the injection of a standard preparation cured the bird</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>963</td>
<td>15 mg. given by mouth. In 3 hours no better; 4 mg. injected. No better in 18 hours; given an injection of a standard torulin preparation which cured within 3 hours</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2:6-Dihydroxyquinoline</td>
<td>Bird 585</td>
<td>March 31st</td>
<td>Symptoms 10.30 a.m.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose of glucose in water</td>
<td>12.30 p.m.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>April 1st</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>April 2nd</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Note. In this case sufficient time was not allowed for the action of the glucose and water on March 31st, thereby giving a temporary improvement.</td>
</tr>
<tr>
<td>136</td>
<td>March 31st</td>
<td>Symptoms still present. 12 mg. compound by mouth</td>
<td>12.30 p.m.</td>
<td>Symptoms still present. 12 mg. compound by mouth</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.0 p.m.</td>
</tr>
</tbody>
</table>

How then is it possible to explain the results of the Japanese worker? It is clear from the protocols of his papers that he has observed some sort of cure in his birds. It is probable that this was one of the varieties of "pseudo-cure" which often occur in these tests. The matter has been dealt with at some length elsewhere [Kinnersley, Peters and Reader, 1928]. The giving of a standard test dose largely eliminates this condition. As an illustration the following case is quoted in detail. The experiment represents an attempt to give one of these compounds to a case of pseudo-avitaminosis in order to imitate the results of the Japanese worker.

Exp. Pigeon June 28th Polished rice diet
July 12th Symptoms of opisthotonus. Given 100 mg. glucose (approx.) in 5 cc. H₂O. This relieved the bird, and it was well for 10 days. Glucose cure
July 22nd Mild symptoms of opisthotonus, not completely developed
5 p.m. The bird had symptoms mildly when handled. 8 mg. 2:6-dihydroxyquinoline injected into the breast muscle in approx. 2 cc. H₂O. This solution was prepared by adding sufficient alkali to dissolve the substance and then bringing the reaction back towards the neutral point with dilute HCl, to a point at which the substance still remained in solution. The pH of injected solution however was more alkaline than 8.0.
July 23rd Morning. Bird showed a very slight improvement, but was still inclined to put its head back when handled
July 24th Bird more normal, but could not perch
July 25th Practically well
July 29th (7th day). Died overnight

If this case is compared with Chart IV (p. 197) of Sahashi’s paper [1926], it will be seen to resemble it closely. Sahashi’s case recovered for 10 days after the first injection, and then was rather ill for 4 days after a subsequent one.

At first sight such an experiment might be considered to be convincing evidence for the curative properties of the substance. However, during the recent hot spell, several birds of this class were observed, all of which tended to give such abnormal results. They could be readily detected by giving initial standard doses. It is not certain what abnormality they represent, but it is quite clear that tests upon such birds give fallacious results. The effect of a true standard extract is to cure a bird quite definitely in the course of a few hours. A subsequent dose given when symptoms appear again has a further definite and rapid action. It is probable that intramuscular injection is complicated by the fact that laceration of the tissues produced by the injection is apt to liberate vitamin into the blood-stream from the site of injury, as it is well known that extracts of tissues of birds with head retraction will often induce cures.

Preparation of chemical substances.

2: 6-Dihydroxyquinoline-4-carboxylic acid and 2: 6-dihydroxyquinoline. The acid was prepared by the method used by Sahashi [1927], namely demethylation of 6-methoxy-2-hydroxyquinoline-4-carboxylic acid [Halberkann, 1921]. 2: 6-Dihydroxyquinoline was obtained by subliming small quantities of the acid (about 0.25 g.) in a test-tube over a free flame. Both substances had the properties recorded by Sahashi, with the exception that the acid did not exhibit the indole (pine-shaving) reaction described by that author.

4 (or 5)-Glyoxalinemethylmethylenecarbino1 was prepared from pure 4 (or 5)-glyoxalinemethylketone (m.p. 80° [Pyman, 1911, 1]) by the method used by Sahashi. The ketone (1.2 g.) dissolved in water (30 cc.) was reduced by 2 % sodium amalgam (200 g.) during 5 hours, a stream of carbon dioxide being passed into the solution throughout the reaction. Finally the aqueous solution was filtered, rendered just acid with hydrochloric acid, and evaporated to dryness under diminished pressure on the water-bath. The solid residue was mixed with saturated sodium carbonate solution, and extracted twice with chloroform. This extract, when dried with sodium sulphate and distilled, yielded glyoxalinemethylmethylenecarbino1 as a colourless oil (1 g.) which did not crystallise and was therefore converted into the picrate by dissolving in hot alcohol, adding a hot alcoholic solution of picric acid (1.7 g.), and cooling. The picrate separated in crystalline condition, and crystallised from 95 % alcohol in yellow prisms, m.p. 156° (corr.). The hydrochloride, obtained from the picrate by the benzene-hydrochloric acid method of Koessler and Hanke
ALLEGED ANTINEURITIC SUBSTANCES

[1918], was a colourless, hygroscopic gum. The chloroplatinate, prepared by mixing hot absolute alcoholic solutions of the hydrochloride and a slight excess of platinic chloride and cooling, crystallised in orange, leaf-shaped plates, m.p. 196° (corr.; decomp.) agreeing with Sahashi’s description.

Other compounds.

Tests upon the following substances\(^1\) have all given negative results: 2:6-dihydroxyquinoline-4-carboxylic acid, 5 mg.; 4-hydroxy-2:6-dimethylpyrimidine [Gabriel, 1885] 10 mg.; 2-hydroxy-4:6-dimethylpyrimidine [Stark, 1909] 10 mg.; 2-thiol-4 (or 5)-aminomethylglyoxaline [Pyman, 1911, 2] 5 mg.; ergothioneine hydrochloride [Tanret, 1909] 0.5 mg.; 2-methylglyoxaline, 12 mg.; glyoxaline-4 (or 5)-formaldehyde, 15 mg.; 4 (or 5)-glyoxaline-4 (or 5)-methyl]-glyoxaline-5 (or 4)-methyl alcohol, 13 mg.; glyoxaline-4 (or 5)-acetic acid, 7.5 mg.; \( dl-a \)-hydroxy-\( \beta \)-glyoxaline-4 (or 5)-propionic acid, 8.5 mg.

Doses have been injected intramuscularly in 2-5 cc. aqueous solution, neutralised as far as possible to \( \text{pH} \) 7.4 prior to injection.

The above tests have been performed on single birds; in some cases two separate injections have been made.

SUMMARY.

Neither 4 or (5)-glyoxalinemethylethylcarbinol hydrochloride nor 2:6-dihydroxyquinoline has antineuritic vitamin properties similar to those possessed by torulin. Certain other compounds containing glyoxaline and pyrimidine rings had no curative properties.

We are indebted to the Medical Research Council for a grant towards expenses, and to Mr H. W. Kinnersley for the preparation of the torulin used in these experiments.

REFERENCES.


\(^{1}\) Compounds 1-5 were prepared by one of us (J. M. G.), compounds 6-8 were kindly supplied by Prof. Pyman, and compounds 9 and 10 by Prof. Barger.