Isolation of a New Alkaloid from Perennial Ryegrass

INDEPENDENT investigations in our respective laboratories have resulted in the isolation from perennial ryegrass (*Lolium perenne* L.) of a new constituent with unusual optical properties. Further study has shown that the material is an alkaloid of empirical formula (hydrochloride) $C_{36}H_{22}O_3N_4(OCH_3)_4.2HCl$. It yields precipitates with all the usual alkaloidal reagents and is soluble in alcohol and chloroform, slightly soluble in acetone, ether and water. Dilute solutions of the base in chloroform are golden yellow, with a green fluorescence which can be detected in ordinary daylight at concentrations of 1 in 5×10^6. The alkaloid is reduced by titanous chloride to a colourless material which can be quantitatively reoxidized by ferricyanide. The name perloline is proposed for this fluorescent alkaloid.

Some 40 gm. of perloline have been prepared in our two laboratories in the course of the past growing season, using the usual methods for chloroform- and alcohol-soluble alkaloids. The key to large-scale preparation lies in growing grass with sufficiently high alkaloid content, and this problem has not been completely solved.

The observed variation in concentration is high, ranging from a trace (about 3 μgm. per gram dry matter) to 1 mgm. per gram. The conditions governing high alkaloid content are obscure, but in general the highest concentrations have been noted at times corresponding with rapid growth.

A simple and rapid method of assay has been devised, depending on the colour of solutions of the base in chloroform. It requires only 2 gm. dry material.

In addition to perloline, several other alkaloids have been found in ryegrass, but data concerning them is at present indefinite.

A full account of the investigations on the preparation, characterization and pharmacology of perloline will appear in the *N.Z. Journal of Science and Technology*.

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Determination of Death in the Larvæ of the Potato Root Eelworm

ONE of the major difficulties in the work of helminthologists has been to differentiate between living and dead nematodes. According to Lapage[1], most of those who have studied the metabolism of these worms have taken their failure to move either as a result of or without the action of a stimulus as an indication that they are not alive.

This difficulty has again arisen in the course of work by me on the biology and control of *Heterodera schachtii*, the eelworm which attacks potato roots and causes a serious diminution in crop in many districts.

First-stage larvæ are liberated by the action of potato root excretion on the cysts of the eelworm which are found in the soil of an infested field. These larvæ then penetrate the potato rootlets with the ultimate formation of new cysts which remain in the soil and thus increase the infestation. In the laboratory the larvæ can easily be obtained in quantity by immersing cysts in potato root excretion.

Normally under suitable conditions most of these larvæ are in active motion, but it frequently happens that they lie quite still for a relatively long period and then, for some undiscovered reason, recommence active movement. They may also show no sign of movement when observed in watch-glasses, but yet be able to penetrate potato rootlets. Thus it is exceedingly difficult to distinguish whether larvæ are in a state of dormancy or whether they are dead. According to Baunacke[2] who made a study of the beet strain of this eelworm, that part of the larval body near the oral end, which is almost completely hyaline in the living larva, becomes granular after death occurs. This tends to impart a uniform opacity to the dead larva. After treatment with certain solutions or after exposure to certain degrees of heat, larvæ of the potato strain of *H. schachtii* have been observed to be in a condition similar to that ascribed by Baunacke to a dead larva. Other larvæ have been seen to show a peculiar distension of the body wall near the higher end of the intestine, while this organ itself had become displaced.

Though it is known that living nematodes may in certain cases have the appearance of being dead, it has been ascertained that larvæ of the potato strain of *H. schachtii* in the above-described conditions could not be induced to form cysts on the roots of potato plants. Such larvæ were considered to be dead.

It was also determined that dead larvæ of both these types could be more clearly distinguished from living larvæ by staining with a solution of 0·025 gm. iodine in 100 c.c. 1 per cent potassium iodide solution. The most satisfactory procedure was to use five drops of this solution in 2 c.c. of a larval suspension. The suspensions employed contained some larvæ in active motion and some considered to be dead. The latter absorbed the iodine and were stained within a few minutes, being best observed after 10–20 minutes. Those larvæ which were moving and thus known to be alive originally became motionless after a few minutes but yet retained the appearance of living larvæ for several hours and did not absorb the stain. They were killed, however, when left in the solution overnight.

The iodine penetrates the dead larvæ through the mouth, and by careful observation a yellowish coloration may be seen starting at this end and gradually permeating the whole body.

Larvæ which exhibit a slight granularity in the upper region of the body, that is, larvæ which according to Baunacke's criterion are newly dead, do not take up the iodine immediately. They do stain, however, after being allowed to stand for 24–48 hours before applying the solution. Work along this line is being conducted in connexion with the thermal death point of the larvæ.

First-stage larvæ which had been kept in potato root excretion solutions for several weeks, and which by using up all their reserve food material had become practically colourless and clear except for a small granular portion near the mouth, absorbed iodine only slightly in this region while the rest of the body remained almost unstained. This is apparently due to the absence of intestinal contents in larvæ which had been in a free state for several weeks.