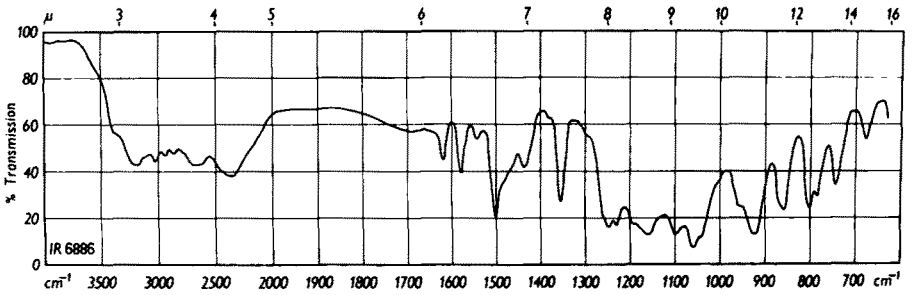

Abb. 3. IR.-Spektrum von Psilocybin in KBr.

Psilocin äusserst zersetzlich ist, konnte es bis jetzt nicht zur Analyse gebracht werden.

Psilocybin zeigt bei peroraler Applikation beim Menschen die gleiche psychotrope Wirkung wie der Pilz. Nach der Einnahme von 4 bis 8 mg *Psilocybin* entsteht nach etwa $^3/_4$ h ein mehrere Stunden andauernder Rauschzustand mit körperlicher Entspannung und ausgesprochenen psychischen Alterationen, der keinerlei Nachwirkungen hinterlässt. Die Symptome variieren individuell und sind zum Teil ähnlich den durch Mezkalin und Lysergsäurediäthylamid (LSD) erzeugten Wirkungen. Damit ist es zum ersten Mal gelungen, aus einem mexikanischen Rauschpilz das wirksame Prinzip in kristallisierter Form zu isolieren und chemisch zu charakterisieren.

Die Analysen wurden im mikroanalytischen Laboratorium SANDOZ (Dr. W. SCHÖNIGER) ausgeführt, die Spektren in der spektralanalytischen Abteilung (Dr. H. G. LEEMANN) aufgenommen. Wir danken Herrn H. TSCHERTER für sehr geschickte, experimentelle Mitarbeit.

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Summary

The active principle of *Psilocybe mexicana* Heim, a mexican mushroom with hallucinogenic properties, has been isolated in crystalline form. The compound has been given the name *Psilocybin;* it possesses indole characteristics and contains phosphorus. A second substance, closely related to *Psilocybin* but found only in traces, has been called *Psilocin.*

PRO EXPERIMENTIS

Solutions of Potato-Root Diffusate of Low Ion Content

There are two reasons why it is desirable to obtain solutions of root diffusates of very low ionic content: both arise from the demonstration that the hatching factor of the potato-root eelworm (*Heterodera rostochiensis* Wollenweber) possesses cardiotonic properties and can be assayed by means of a frog heart preparation[1]. Firstly, for the heart test, the ionic composition of the perfusion fluid must be accurately controlled. Secondly, recent work has shown that cardiac glycosides may influence sodium-potassium transport[2]: if the potato-root eelworm hatching factor works in a similar way, the hatching process should also be sensitive to the ionic background. This we have found to be the case[3]; as will be shown in full elsewhere, hatching can be increased by $CaCl_2$ and, although the position is here not quite so clear, decreased by KCl. The standardisation of solutions by the hatching test or by the heart assay can therefore be completely misleading unless the ionic composition is controlled.

Solutions of diffusate containing the potato-root eelworm hatching factor are usually obtained from potato or tomato plants grown in pots; these are 'leached' at varying times with known volumes of water, the same plants being used repeatedly during the period of their active growth. Some workers put the leachings back through the pots twice more in an endeavour to obtain a stronger solution. Since the plants are raised in a medium containing minerals, and since tap water is usually used, it is clear that the solutions will contain appreciable and variable quantities of salts. We have found, leaching with 100 ml lots of distilled water, that the conductivity of the leachings was equivalent to 0·14% NaCl; and this rose approximately 20% if the leachings were put through the pots twice more. Moreover, these leachings were from plants grown in John Innes seed compost from which the minerals were omitted.

In this laboratory, root diffusates have always been obtained directly from the roots[4]. The plants are lifted, their roots thoroughly washed, and the whole root system then stood in water, usually for 2 h; originally tap water was used, later distilled water. It has always been felt that this method would give a purer product, but, until recently it was not realised that by this method solutions could be obtained virtually ion free. The method has also been used successfully with hosts of other species of *Heterodera.*

In a recent test, the roots of four fully grown plants were thoroughly washed, first in tap water, finally in distilled water. They were immersed, tops still attached, in a litre of ion exchange water equal to triple glass distilled, and placed in a polythene bucket, in a greenhouse. Samples removed at intervals during a 24 h period showed little change from a very low conductivity, the value being equivalent to 0·012% NaCl at the outset,

[1] C. ELLENBY and A. B. GILBERT, Nature *180*, 1105 (1957).

[2] H. J. SCHATZMANN, Helv. physiol. Acta *11*, 346 (1953). – M. WEATHERALL and B. RAYNER, Personal communication. – V. K. JOHNSEN, Personal communication. – J. B. KAHN Jr. and G. H. ACHESON, J. Pharmacol. *115*, 305 (1955).

[3] C. ELLENBY and A. B. GILBERT, Unpublished.

[4] C. ELLENBY, Nature *154*, 363 (1944).

and 0·017% after 24 h; a parallel test gave values of 0·015 and 0·018 respectively. Our best performance to date gave a value after 24 h equivalent to only 0·01% NaCl but we feel that even this can be improved, for, since the plants were raised in the compost mentioned above, we were operating under unfavourable conditions; the peat granules adhere very intimately to the roots and, even after prolonged washing, not all particles are removed. In future we intend raising the plants in vermiculite.

After 24 h, the plants still stand well. Indeed, we have kept plants in this fashion for some days, but there appears to be a decrease in the output of the hatching factor after this period and we have never used such solutions.

For small volumes of solution, filtration through filter paper should be avoided if possible; in a test with 20 ml samples, we found, in some cases, that if all the increase in conductivity were due to salts, this was more than doubled by passage through a Whatman No. 40 acid extracted filter paper. The increases appear to be connected with the nature of the solutions for they were much less with distilled water, our samples of Whatman No. 1 being superior in this respect to the No. 40. The total increase is slight where large volumes of solutions are dealt with; solutions are then filtered through a Büchner funnel after giving the filter paper a preliminary wash with at least 100 ml of high grade distilled water. Resistance glass and polythene bottles are used throughout.

Although the solutions are of very low ionic content, our investigations have shown that, if it were all calcium chloride, its addition to low calcium Ringer would be sufficient, very occasionally, to increase the beat of the frog heart even in the absence of the hatching factor, since the heart is very sensitive to Ca^{++}; the assay would therefore be misleading. However, the 24-h solutions are very strong; they can therefore be diluted until it is quite certain that their ionic contribution to the low calcium Ringer in which they are assayed can safely be ignored. Such an assay showed that the initial solution obtained from the four plants after 24 h was about 10 times as strong as the minimal concentration of Cambridge 'standard solid'[5] hatching factor; the yield from the four plants in 24 h was therefore equivalent to more than 1 g of the Cambridge solid. We have since found that even stronger solutions can be obtained from the small root systems of younger plants; these can be contained in smaller quantities of water.

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Zusammenfassung

Zur quantitativen Bestimmung des Schlüpffaktors der Kartoffelnematode, entweder mit sogenanntem Schlüpftest, oder durch unsere, vor kurzem beschriebene Froschherzmethode, muss die Ionenkonzentration in der Lösung kontrolliert werden. Eine Methode zur Herstellung geeigneter Lösungen wird beschrieben.

[5] C. T. CALAM, H. RAISTRICK, and A. R. TODD, Biochem. J. *45*, 513 (1949).

Obtention de lignées cellulaires en culture de tissus d'invertébrés

Le rôle prépondérant des cultures de tissus dans la recherche médicale, notamment en virologie, est avant tout le résultat de l'obtention de souches de cellules à partir de tissus humains ou de vertébrés, avec possibilité de maintien de celles-ci.

Chez les invertébrés, plusieurs tentatives de cultures de différents tissus ont été faites soit pour l'étude physiologique, soit en vue de travaux de pathologie cellulaire. Dans quelques cas on a noté, à partir d'un explant, l'émigration de fibroblastes ou de l'épithélium; ces développements étaient toujours réduits[1] et ce n'est que rarement que l'on a observé des mitoses ou des divisions amitotiques[2]. Mais les repiquages successifs des cultures en voie de multiplication et la formation de souches cellulaires, critères actuels des cultures de tissus, n'ont pas été réalisés.

Nos expériences ont porté parallèlement sur deux lépidoptères: *Bombyx mori* L., et *Galleria mellonella* L., sur un orthoptère *Blabera fusca* et sur les mollusques: *Helix aspersa* MULLER et *H. pomatia* L. Nous résumons ici ce qui concerne la culture des lignées relatives à *B. mori* et *H. aspersa* les plus anciennement maintenues.

L'enceinte de culture a été choisie de telle manière qu'on associe les procédés de multiplication modernes sur grande surface avec changement de milieu et les repiquages aseptiques. Nous avons abandonné les techniques en goutte pendante, sur lame détachée et sur anneaux, ainsi que les procédés septiques, pratiquement les seuls à être employés jusqu'alors pour les insectes ou pour les mollusques. Les cultures ont été réalisées en tubes roulants, en tubes plats avec lame intérieure en boîtes de Kolle et de Roux et dans des flacons permettant le contrôle au microscope, à l'immersion sans interruption de la culture[3].

Les tissus de *B. mori* ont été cultivés dans un milieu contenant pour 1000 cm³ d'eau bi-distillée, les éléments minéraux suivants: KCl: 3,0 g; $CaCl_2$: 0,5 g; NaH_2PO_4: 1,0 g; $Mg\,SO_4 7H_2O$: 3,5 g; $MgCl_2 6H_2O$: 3,0 g; CO_3NaH jusqu'à pH 6,4. Les glucides étaient représentés par le glucose à raison de 1 g °/°° et la source d'acides aminés par l'hydrolysat de caséine (Vitrum) ayant montré préalablement une certaine polyvalence pour les tissus de vertébrés. Ce produit (0,5 g) a été complété de glutamine (0,1 °/°°). Les facteurs de croissance étant peu connus chez les insectes, un extrait de levure (obtenu par effet de NaCl) à raison de 2 cm³ °/°°, avec choline à 0,002 °/°° a été ajouté. En plus de 200000 U.I. °/°° de pénicilline et de 50 mg °/°° de streptomycine le milieu est additionné d'hémolymphe de *B. mori* (10%) chauffée à 60°C pendant 5 min pour éliminer l'effet de la tyrosinase[4].

A partir des explants de gonades femelles de larves à la fin du 5e âge, l'émigration des cellules type «fibroblastes» s'intensifie à 30°C, après 48 h, avec formation

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