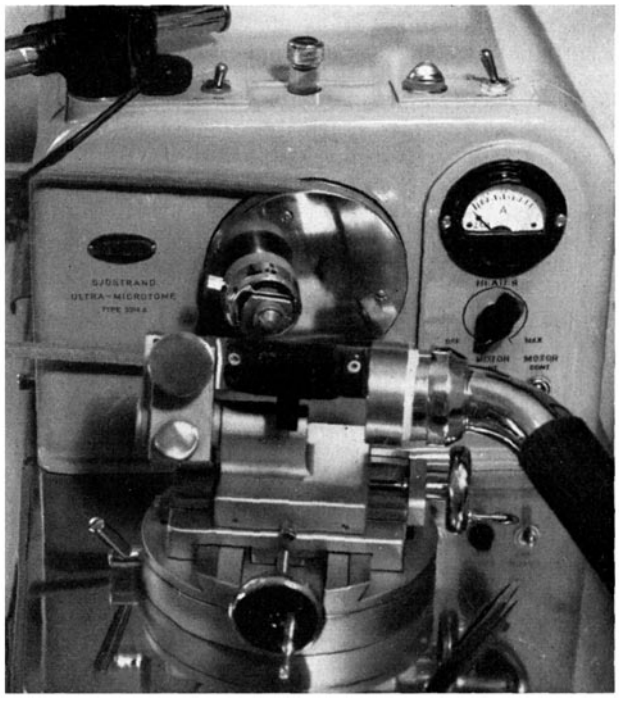
Zusammenfassung

Synthetisches Oxytozin hat auch vasopressorische und antidiuretische Wirkungen, die etwa 1/30 und 1/15 der oxytozischen Aktivität ausmachen. (Hochgereinigte natürliche Oxytozin-Präparate haben nach LAWLER und DU VIGNEAUD kaum solche Eigenschaften.) Die Differenz zwischen synthetischem und hochgereinigtem Oxytozin weist in dieser Hinsicht darauf hin, dass diese nicht identisch wären.

PRO EXPERIMENTIS

Larger Section Areas for Electron Microscopy by Aid of Ultrasonics

Well established section techniques for 200 Å section thickness in electron microscopy (SJÖSTRAND[1], *inter alia* 1954) require a careful treatment of the object block to prepare a small cone for sectioning. In rare cases it is desirable to increase the section area, particularly when very large cells are concerned e.g. the salivary gland cells of *chironomus*. To proceed 50 μ into the tissue structure perpendicular to the level of the first section, about 2500 sections have to be cut, even though only about 10% of such series are actually studied in the electron



microscope. If the ratio of the size of the object of interest – the tissue detail – to the tissue volume is very low, large survey sections seem to be of greater advantage than many small sections into the depth of the tissue. Because section quality rapidly decreases when the section area is increased, an ultrasonic generator was fitted to the knife holder of a SJÖSTRAND microtome (Figure) vibrating the knife in a transversal direction, thus allowing one to section up to 1·5 mm² blockface area without endangering the section quality. Unavoidable oscillations of the knife toward and away from the object block had no visible influence on section smoothness. Obviously the magnitude of such vibration effects do not exceed the practical resolution power of the electron microscope. The figure demonstrates the arrangement at the microtome. On the right side can be seen the handle with the vibration head (Sonostat 812, Siemens-Reiniger, Erlangen), which generates 800 Khz at – for our purpose – 1 watt/cm². The vibration head is held by a brass cup in which contact of the vibrating surface and the cup bottom is effected by a layer of thick oil. The brass cup in turn is tightly screwed on to the knife holder.

The above described arrangement seems to be advantageous even when harder materials are to be sectioned. We are indebted to the Elema Company, Stockholm, for kindly putting the Sonostat at our disposal.

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Institute for Cell Research and Genetics, Karolinska Institutet, Stockholm, February 2, 1955.

Zusammenfassung

Um in besonderen Fällen die Schnittflächengrösse in der Schneidetechnik für die Elektronenhistologie erhöhen zu können, wurde mit Erfolg das Mikrotommesser in seiner Längsachse in Ultraschallschwingungen versetzt.

PRO EXPERIMENTIS

A Modified Petri Dish Method for Rust Infection of Excised Leaves

The use of Petri Dishes for culturing rust fungi on detached leaves has been recommended and attempted by many investigators with varying degree of success. The literature on detached leaf culture in all its aspects has been reviewed by YARWOOD[1]. Depending upon the purpose of investigation numerous variants of the Petri dish method have been suggested. CRAIGIE[2] used the method of suspending the infected plant parts (culms, leaves, twigs, etc.) over the healthy host plants for demonstrating heterothallism in rust fungi. The writer has used with moderate success a modification of CRAIGIE's method by using detached leaves in Petri dishes. He has studied the sex behaviour of rust fungi like *Scopella gentilis* (Syd.) Mund. and Thirum. on *Mimusops hexandra* Roxb. (Sapotaceae) and *Puccinia thwaitesii* Berk. on *Justicia gendarussa* Burm. (Acanthaceae) by this modified Petri dish method. As far as the writer is aware the method of using excised leaves in Petri dishes for studying sex-behaviour of rust fungi has not been attempted so far.

Leaves of the plant of which the rust is to be studied are preferably detached in the afternoon so that the carbohydrate, protein and mineral contents may be higher and the leaves consequently may remain in fresh condition longer (YARWOOD[1]). The rusted leaves are collected earlier for testing the viability of the teliospores

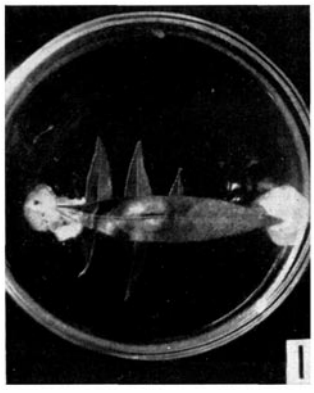
[1] F. S. SJÖSTRAND, Z. wiss. Mikroskop. *62*, 65 (1954).

[1] C. E. YARWOOD, Bot. Rev. *12*, 1 (1946).

[2] J. H. CRAIGIE, Phytopathology *21*, 1013 (1931).

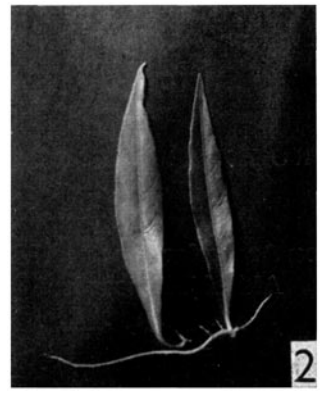
(in case of *Puccinia thwaitesii* the teliospores on maturity germinate within 6 h). The rusted leaves are then soaked in water for 2 to 3 h. To the lids of Petri dishes such infected and water-soaked leaf material is stuck at both ends by means of melted paraffin or any other suitable adhesive in such a way that the telia project downwards (Fig. 1). In the other half of the Petri dish the washed and moist healthy leaves are kept supported by wood blocks or by any other means of support like rubber bands, built-in notches in Petri dishes (CLINTON and MCCORMICK[1]), paraffin-coated circular discs of wire-screen with bent edges (WATERS[2]), etc. The upper surfaces of the healthy leaves should face the lower sides of the stuck infected leaves if the telia are hypophyllous for the basidiospores penetrate directly through the epidermis of the upper leaf surface. Care must be taken that the healthy leaves in the lower half of the dish and the stuck infected material on the lid of the Petri dish do not come in contact with one another.

The healthy leaves are sprayed with an atomizer. The bottom of the Petri dish is just sufficiently covered with distilled water. The stuck infected material is then set in position over the lower half of the dish (Fig. 1). In this method also, like that of CRAIGIE[3], advantage is taken of the forcible discharge of the basidiospores. After 6 to 8 h sporidial shower occurs. During this period the lids can be rotated for obtaining scattered pustules on different regions of the leaf or leaves. If the leaves are longer than the diameter of the Petri dish they can be trimmed to the required length with petioles always intact. Normally after a period of 48 h the stuck infected leaves are removed, the lids cleaned thoroughly and replaced in position. The dishes are then kept near a laboratory window in diffused light. Water in the Petri dishes has to be changed every alternate day, if not daily. The inoculated leaves also should be periodically sprayed with water.



If the leaves selected are very young, infection manifests itself after 6 days of inoculation in case of *Puccinia thwaitesii*. If, however, the leaves are not very young and have already matured, i.e., they have developed a thick cuticle, the infection is slow in appearance. For pustules to appear on such leaves a period of 12 to 15 days is necessary.

When the experiment is in progress an interesting phenomenon may occur. It is the formation of callus at the petioles resulting in rooting of the leaves (Fig. 2). This phenomenon, according to MULLAN[1], is very common in certain members of Acanthaceae including *Justicia gendarussa* – the host of *Puccinia thwaitesii*. In the writer's experiments such rooted leaves lasted up to 60 days. The rust pustules could be maintained for about 42 days.



WATERS[2] found in case of the bean rust (*Uromyces appendiculatus* Fries) that if only sterile distilled water was used for floating the leaves, the pustules became weakened in vigour. If, on the other hand, 5% sucrose solution was used the pustules developed very vigorously, thereby killing the leaves early. However, when the leaves were first kept in distilled water and then in sucrose solution, normal development of the pustules occurred. The writer has used only distilled water. Sucrose solution was found to hasten the contamination of the leaves with fungi and bacteria.

One difference was noted between the pustules of *Puccinia thwaitesii* occurring in nature and those obtained experimentally on excised leaves in Petri dishes. In nature, rust pustules having a diameter of 5 to 20 mm are quite common and in exceptional cases even the entire lamina may get plastered with telia. It may be the result of infection taking place early on very young leaves at which stage they are very susceptible. In Petri dishes, on the other hand, because of chance selection of somewhat mature leaves, probably only limited infection took place. It may also be due to the fact that monosporidial infections are not usually so vigorous in development as are the multisporidial ones. The pustules were just barely visible as minute (2 to 4 mm diameter), raised or depressed spots. On each pustule occasionally only one telium developed. However, the fact that single pustules produced telia shows that the rust is homothallic.

Advantages of the Petri dish Method. (1) Ease in operation, handling, and observation. (2) Requiring minimum amount of material. (3) Possibility of studying sex-behaviour of rust fungi without having recourse to potted plants. (4) Greater accuracy in counting, measuring, and classifying rust pustules in their initial as well as latter stages as simple, compound, mono-, bi-, or multi-sporidial. If necessary, the leaves can be carefully taken out of the Petri dishes and their surfaces examined at higher magnifications under a stereoscopic microscope. (5) Ease and accuracy in keeping track of individual pustules up to the time the experiment lasts.

[1] G. P. CLINTON and F. A. MCCORMICK, Conn. Agric. Expt. Sta. Bulletin *260*, 475 (1924).
[2] C. W. WATERS, Phytopathology *18*, 157 (1928).
[3] J. H. GRAIGIE, loc. cit.

[1] D. P. MULLAN, J. Indian Bot. Soc. *10*, 167 (1931).
[2] C. W. WATERS, Papers Michigan Acad. Sci., Arts and Letters *5*, 163 (1925).

Disadvantages of the Petri dish Method. (1) The survival of the leaves for a time long enough to complete observations on the rust infection is not always assured. (2) The exact environments as obtained in nature for the best development of any given rust fungus are not simulated to the fullest extent.

Acknowledgments.–The writer is indebted to the late Prof. S. L. AJREKAR and Dr. M. J. THIRUMALACHAR for their kind advice. Acknowledgments are also due to Dr. S. P. AGHARKAR, Director, Botany Laboratory, Maharashtra Association for the Cultivation of Science, Poona, for providing laboratory facilities and useful suggestions.

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Botany Laboratory of M.A.C.S., Law College Building, Poona (India), January 25, 1955.

Zusammenfassung

Es wird eine Methode beschrieben, um festzustellen, ob ein Rostpilz homo- oder heterothallisch ist. Hiefür wird der Pilz auf einem geeigneten Medium in einer Petri-Schale gezüchtet. Die Vor- und Nachteile dieser Methode werden erörtert.

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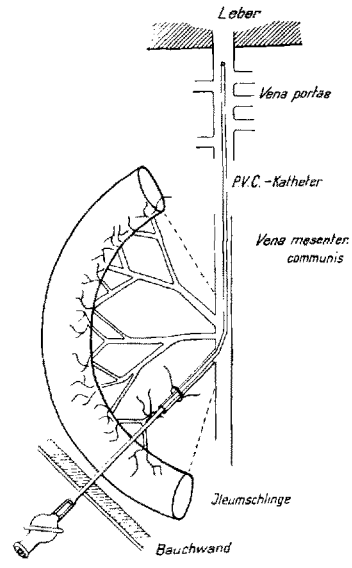
PRO EXPERIMENTIS

Eine Methode zur fortlaufenden Blutentnahme aus der Vena portae von Hunden

Ausser der von DENT und SCHILLING[1] wiederverwendeten Londonkanüle und dem von BESSMANN, MAGNES und WAELSCH[2] durchgeführten Einbinden einer T-Kanüle in die Vena portae von anästhesierten Katzen hat sich vor allem die Methode von DENTON, GERSHOFF und ELVEHJEM[3] zur Gewinnung von Portalblut als sehr geeignet erwiesen. Die Autoren punktierten die Vena portae von Hunden und schoben durch die Punktionsnadel einen silikonüberzogenen «Genflex plastic»-Katheter bis in Lebernähe vor. Das Gefäss wurde durch eine Tabaksbeutelnaht und das Aufnähen von Mesenterium abgedichtet. Aus dem unter der Haut bis in den Nacken der Tiere verlegten Katheter war bis zu 8 Wochen eine Blutentnahme möglich. Als technisch schwierig ist bei der Methode nur das blutungssichere Einbringen des Katheters in die Portalvene anzusehen. Um diese Schwierigkeit zu umgehen, verbesserten wir ein an Ratten erprobtes Verfahren für Versuche an Hunden.

Wir eröffneten in Urethan-Chloralosenarkose (0,75 g Urethan + 55 mg Chloralose/kg i.v.) das Abdomen mit einem Medianschnitt und lagerten die zweitletzte Ileumschlinge vor. In eine Mesenterialvene, die das Blut aus einem höchstens 4 cm langen Darmstück ableitete, wurde ein Polyvinylchlorid (PVC.)-Katheter eingeführt und durch die Vena mesenterica communis bis in die Vena portae vorgeschoben. Der meistverwendete, relativ grosskalibrige PVC.-Schlauch (innerer ⌀ 1 mm, äusserer ⌀ 2 mm) liess sich nach Punktion der Vene mit einer scharfen Injektionskanüle ohne Spritzenansatz durch die Kanüle leicht in das Gefäss einbringen. Zusammen mit der benutzten Mesenterialvene unterbanden wir die begleitende Arterie in beiden Richtungen von der Eingangsstelle, um eine, wenn auch nur vorübergehende, venöse Stauung des betroffenen Darmabschnittes auszuschliessen. Der Katheter wurde damit gleichzeitig am Mesenterium fixiert. Die Lage des PVC.-Schlauches in der Vena portae kontrollierten wir durch Palpation. Zur Blutentnahme wurde das Schlauchende seitlich von der Schnittwunde aus der Bauchhöhle geleitet und mit einem Zweiweghahn für Rekordspritzen armiert. Im Peritonealraum verblieb ausser dem in den Gefässen liegenden PVC.-Schlauch ein etwa 15–20 cm langes Schlauchstück, durch das Zerrungen am Mesenterium bei Darmbewegungen vermieden werden sollten. Am Ende der Operation wurde die Bauchdecke in 3 Schichten geschlossen und zur Infektionsprophylaxe eine



0,25 g-Dosierung Combiotic[1] i.m. injiziert. Die Abbildung gibt die Lage des Katheters schematisch wieder. Wegen der relativ kurzen Versuchsdauer von 2 bis 3 Tagen verzichteten wir auf die subkutane Verlegung des Katheters und befestigten ihn lediglich mit Heftpflaster so, dass der Zweiweghahn auf den Rücken des Tieres zu liegen kam und dort nochmals mit einer besonderen Pflasterlasche abgedeckt werden konnte.

An 4 mit dieser Methode vorbereiteten Hunden im Gewicht von 10 bis 17 kg unternahmen wir insgesamt 8 Verdauungsversuche mit verschiedenen Milchproben, davon 3 an narkotisierten und 5 an wachen Tieren. Die Kontrolle der Verdauungs- bzw. Resorptionsprodukte erfolgte durch stündliche Blutentnahme aus der Vena portae. Nach jeder Blutentnahme wurde der Katheter mit einer verdünnten Thrombocidlösung[1] blutleer gespült. Über die Ergebnisse dieser Versuche wird an anderer Stelle ausführlich berichtet.

Während die Katheter bei allen übrigen Tieren bis zum Schluss der Versuche in beiden Richtungen durchgängig blieben, war bei einem Hund 8 h nach Operationsende keine Blutentnahme mehr möglich, wohl aber noch die

[1] C. E. DENT und J. A. SCHILLING, Biochem. J. *44*, 318 (1949).

[2] S. P. BESSMANN, J. MAGNES, P. SCHWERIN und H. WAELSCH, J. Biol. Chem. *175*, 817 (1948).

[3] A. E. DENTON, S. N. GERSHOFF und C. A. ELVEHJEM, J. Biol. Chem. *204*, 731 (1953).

[1] Der Firma Chas. Pfizer danken wir für Combiotic, der Firma Dr. W. Benend für Thrombocid.