

mycetes producing an opaque precipitate only after 10–14 days. Furthermore, the lecithovitellin reaction is to a certain degree influenced by the composition of the medium. However, the species mentioned above as strongly positive gave consistent results and 'negative' strains were negative under all conditions.

So far the results indicate that egg-yolk agar is a useful diagnostic medium for the characterization of streptomycetes¹⁷.

Zusammenfassung. Eigelb-Agar erwies sich als ein geeignetes Medium für die Charakterisierung von Streptomyceten. Während zahlreiche der 300 geprüften Kulturen auf diesem Medium proteolytische und lipolytische Ak-

tivität zeigten, beschränkte sich eine positive Lecithovitellin-Reaktion auf nur wenige Arten. Soweit bisher festgestellt wurde, waren Angehörige der morphologischen Gruppe «Verticillatus» (Gattung *Streptoverticillium* Baldacci) besonders starke Lecithinase-Produzenten.

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Calcium Alginate: A New Approach in the Artificial Culturing of Insects, Applied to *Spodoptera littoralis* (Boisduval)

Most of the artificial media used as gels for the nutrition of insect larvae, and all those containing an agar carrier, require heating at some stage of their preparation. Avoidance of heating is beneficial in 4 ways: (a) by conserving the organoleptic properties, if not the actual chemical entity of some nutrients; (b) when insect pathogens are to be tested on or produced by the host through incorporation in the larval food (in this case substances which inhibit or destroy these pathogens must be excluded and the larval food renewed often enough); (c) in toxicological studies when toxicants are to be homogeneously distributed through the insect's food and (d) in mass production, by elimination of a time-consuming step.

A novel approach is used here in the preparation of gelled insect media. Advantage is being taken of the reaction between alginic acid and calcium ions to produce, under suitable conditions, an insoluble and irreversible gel at room temperature¹. Using this reaction, we were able to develop media for the Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.), and lately the codling moth, *Carpocapsa pomonella* (L.). The medium used for the continuous rearing of *S. littoralis* will serve as an illustration. While artificial media for rearing this insect have been developed by us², LEVINSON and NAVON³, and by BOR⁴, they all contained agar and required heating.

The larval medium consists of 3 fractions. Fraction A contains 200 cm³ distilled water, 0.7 g triethanolamine, 10.0 g vitamin-free casein, 45.0 g finely ground full-fat soy, 35.0 g *Torula* yeast, 1.3 g methyl-*p*-hydroxybenzoate (Nipagin), 0.7 g sorbic acid and 2.7 g dibasic calcium phosphate. Fraction B consists of 300 cm³ distilled water and 11.0 g sodium alginate (Protanal L, produced by Protan, Drammen, Norway). Fraction C comprises 50 cm³ distilled water, 5.4 g glucono- δ -lactone and 3.32 g ascorbic acid.

The ingredients of A are blended in the order given, with 5 min allocated for dissolving the casein before the remainder is added. After B has been blended separately to a smooth paste, it is transferred to the mixer bowl together with A and both fractions are mixed thoroughly. Finally, fraction C is mixed rapidly with A and B and the mixture allowed to set.

In another diet for *S. littoralis* containing agar², ascorbic acid was included to obviate a possible defic-

iency; it was also one of the nutrients in the agar medium of BOR⁴. It has been shown by LEVINSON and NAVON³ with a semi-synthetic diet that ascorbic acid was indeed essential for *S. littoralis* and that the optimum amount was 0.5% w:w, which is the level used in the present medium. However, glucono- δ -lactone being an ascorbic acid analogue, we made separate tests with this semi-synthetic diet to check whether ascorbic acid could be replaced by its analogue: all the larvae died in various instars. Glucono- δ -lactone thus cannot be substituted for ascorbic acid in the larval nutrition of *S. littoralis*.

Triethanolamine is used in the present medium in lieu of potassium hydroxide in order to dissolve casein and the soy protein as well as to emulsify the lipids.

Thus far, larvae have been bred collectively through 4 serial generations, and 3 individually, with the food changed daily. The average adult yield in individual breedings was 64.0%. At $25 \pm 1^\circ\text{C}$, larval development lasted an average of 21 ± 0.2 days, and the mean pupal weight was 283 ± 12 mg. The sex ratio did not depart significantly from unity in any of these generations; fecundity and fertility also were normal.

Since the approach taken is new and adaptable to the development of similar media for other insects, a somewhat detailed discussion of the principles involved seems warranted.

Alginic acid is obtained from various seaweed species and is a polymer of mannuronic and guluronic acids. Since the proportion of these acids varies according to the seaweed species, alginates of different origin have different properties¹, which may explain why there has been no report of actual alginate gels being used in insect media. While alginic acid is insoluble, its sodium salt is very soluble in water and can enter into a controlled chemical reaction with a calcium salt to produce an insoluble calcium alginate gel at room temperature. This

¹ M. GLICKSMAN, Adv. Food Res. 11, 138 (1962).

² I. MOORE and A. NAVON, Entomophaga 9, 181 (1964).

³ H. Z. LEVINSON and A. NAVON, J. Insect Physiol., in press.

⁴ J. BOR, S. Afr. J. agric. Sci. 9, 535 (1966).

reaction will take place in the presence of an acid-releasing agent such as glucono- δ -lactone¹.

When dissolved in water, glucono- δ -lactone converts into gluconic acid, which is then in equilibrium with its 2 lactones (α and δ)⁵. On the basis of this reaction, glucono- δ -lactone has 3 functions in the medium. (1) It provides the acidity necessary for the slow liberation of calcium ions from the calcium phosphate, and these conditions favour the gradual replacement of sodium by calcium in the alginate. A relatively slow rate of reaction is very important for texture: too rapid a formation of calcium alginate results in a lumpy gel, while too slow a rate of formation gives a very soft gel¹ which the larvae find difficult to manage. (2) Being in a state of equilibrium, glucono- δ -lactone maintains a pH suitable for prolonging the biological activity of ascorbic acid (final pH, approximately 4.7). (3) It potentiates the anticon-taminants (sorbic acid and nipagin).

The calcium concentration also determines the rate of reaction so that the total amount of calcium in the system is important¹; the concentration indicated has been found adequate.

In addition to its nutritional role, ascorbic acid contributes with glucono- δ -lactone to the gelling reaction which, although speeded up, still proceeds at a rate commensurate with proper mixing.

Where agar is included in media for insect mass-production, replacing this component by a sodium

alginate-calcium salt-acid donor system can represent an appreciable saving. As compared with a previous agar medium for the cotton leaf worm², the cost of an equal weight of the present diet is about one half^{6,7}.

The use of a similar system for the artificial rearing of the codling moth and the mass rearing of the Egyptian cotton leaf worm will be described in other communications.

Résumé. La réaction entre l'alginate de soude et les ions de calcium a été utilisée dans la préparation à froid d'un gel nutritif servant à l'élevage artificiel de *Spodoptera littoralis* (Boisduval).

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Division of Entomology, The Volcani Institute of Agricultural Research, Bet Dagan (Israel), 5 August 1968.

⁵ E. S. WEST and W. R. TODD, Textbk. Biochem. 221 (1956).

⁶ We thank Miss H. SILBERSTEIN for her devoted technical assistance and Mr. K. KRISTENSEN of Protan a/s for helpful information and samples of alginate.

⁷ Contribution from The Volcani Institute of Agricultural Research (N.U.I.A.), Bet Dagan, Israel. 1968 Series; No. 1386-E.

PRO LABORATORIO

Dauerüberwachung der fetalen Herzaktionen unter der Geburt mittels Ultraschall¹

Durch die Anwendung von Ultraschall, unter Ausnutzung des Dopplereffektes, wurde die Gynäkologie um eine neue diagnostische Methode bereichert²⁻⁷. Mit diesem Verfahren können neuerdings auch in der Geburtshilfe die kindlichen Herzaktionen kontinuierlich überwacht werden. Dazu wurde von uns ein Gerät entwickelt, das die multiformen Signale des Ultraschall-Pulsdetektors in uniforme Steuerimpulse umwandelt. Diese sind exakt herzsynchron, wobei Störungen eliminiert werden. Mit Hilfe dieser uniformen Signale kann die Impulsfrequenz integriert und der akustischen oder optischen Direktanzeige zugeführt werden. Eine telemetrische Übertragung ist möglich.

Das Gerät bietet den Vorteil, dass es sowohl in der Diagnostik während der Schwangerschaft als auch zur Dauerüberwachung unter der Geburt verwendet werden kann. Die Pulsfrequenz ist sofort ablesbar und zur Dokumentation auf einem Direktschreiber registrierbar. Registrierungsmaximal 10 V an 10 k Ω . Die Eichfrequenzen betragen 60 und 180/min.

Da dieses Gerät nicht auf die Intensität biologischer Schallerscheinungen angewiesen ist, sondern eine eigene Energiequelle besitzt, ist es weit zuverlässiger und weniger störungsanfällig als die üblichen Mikrophongeräte.

Der Messwertaufnehmer hat eine zylindrische Form. Seine Dimensionen sind: 30 mm Durchmesser, Höhe 15 mm, Gewicht ca. 30 g. Der Aufpressdruck beträgt 20 g. Die Befestigung geschieht mittels eines Heftpflasterstreifens auf der Bauchwand. Die geringe Dimension des Messwertaufnehmers belästigt die Patientin unter der

Wehentätigkeit nicht. Die Bewegungen der Patientin haben im allgemeinen keinen Einfluss auf die Messung. Zusätzlich besteht die Möglichkeit der Tokometrie (Figur).

Natürlich wird die Frage nach der Schädlichkeit einer länger dauernden Applikation von diagnostischem Ultraschall auf den menschlichen Fetus gestellt. Nach WOEBER und VELTMANN (Zitat nach KRATOCHWILL⁸) besteht eine biologische Wirkung des Ultraschalls erst von einem gewissen Schwellenwert der Intensität an. Der Schwellenwert liegt bei etwa 0,5 Watt/cm². Die Dauer der Applikation unterhalb dieses Wertes dürfte dabei keine Rolle spielen. Bei den von uns verwendeten Ultraschallgeneratoren wird direkt am Messwertaufnehmer die Ultraschall-

¹ Mit Unterstützung der Deutschen Forschungsgemeinschaft und der Paul-Martini-Stiftung der Medizinisch-pharmazeutischen Studiengesellschaft e.V. Frankfurt a.M.

² J. BABENERD und K. H. MOSLER, Münch. Med. Wschr. 38, 2146 (1968).

³ E. H. BISHOP, Am. J. Obstet. Gynec. 96, 863 (1966).

⁴ D. CALLAGHAN, T. C. ROWLAND JR. und D. E. GOLDMAN, Obstet. Gynec., N.Y. 23, 637 (1964).

⁵ W. L. JOHNSON, H. STEGALL, J. N. HEIN und R. F. RUSHMER, Obstet. Gynec., N.Y. 26, 305 (1965).

⁶ M. G. SMYTH, Proc. Inst. Ultrasonics Med. (1964).

⁷ H. WEVER und H. STOCKHAUSEN, Geburtsh. Frauenheilk. 12, 1209 (1967).

⁸ A. KRATOCHWILL und H. HUSSLEIN, *Ultraschalldiagnostik in Geburtshilfe und Gynäkologie* (Thieme, Stuttgart 1968).