

traps in the same locality were baited with single caged virgin female budworm moths for the duration of the test period. An unbaited control trap captured only 2 male budworm moths during the 9-day field test. It is evident from these data that *n*-octadecanenitrile can compete successfully with single caged virgin female insects in luring male spruce budworm moths. We have also ascertained in field tests that this nitrile and some homologues are comparable in potency with crude exudant material collected from several hundred virgin female moths².

These results leave little doubt that *n*-octadecanenitrile holds a pronounced attraction for the male spruce budworm moth. We plan to carry out more exhaustive bioassays including dilution studies during the next budworm mating season³.

Zusammenfassung. Die besondere Eigenschaft des *n*-Octadekanitrils als Lockstoff für die männliche Motte *Choristoneura fumiferana* (Clem.) wurde festgestellt.

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The Assessment of the Rate of Digestion of Serum Proteins in Mosquito by Gel-Diffusion and Immunoelectrophoresis

The rate of digestion of blood in mosquitoes has been previously assessed by various workers using the ring or the interfacial precipitin reaction (CLEMENTS¹). However, the disadvantage in using the ring test is that it cannot differentiate between various components of the serum, unless antisera especially prepared against these components are used. The ring test, therefore, as it is normally performed, can only indicate the rate of digestion of the whole serum and not of individual fractions. It is possible to overcome this difficulty by the use of relatively newer techniques such as gel-diffusion and immunoelectrophoresis. The aim of this work was, therefore, to find out if different components of the serum are digested by mosquito at the same rate, or whether their rate of breakdown is different using the above 2 techniques. WILLIAMS² had previously used immunoelectrophoresis to analyse the blood meal of *Aedes aegypti* for the same purpose, and had found that the rate of digestion of serum proteins in that of mosquitoes is not uniform.

We have conducted our studies on *Armigeres subalbatus* as this mosquito was readily available in our insectory. The strain was first established in this laboratory by BARR and CHELLAPPAH³, who also describe the rearing techniques. The temperature in the insectory ranged from 28–30°C. The unfed female mosquitoes were fed on a human subject under constant observation, when they had fed to repletion, each of them was carefully transferred to a separate container. They were then removed after varying time intervals and the antigens were prepared. For the preparation of antigens, the fed mosquitoes were dissected and the gut contents were laid on small filter paper strips which were then allowed to dry in a dessicator at 5°C. Before conducting the test, the strips were cut into smaller parts and transferred to a tuberculin syringe containing 0.2 ml of physiological saline. The saline was repeatedly sucked and ejected until the filter paper became almost colourless. This saline extract was used as an antigen. The double diffusion in Agar and immunoelectrophoresis was done on slides using LKB immunoelectrophoresis equipment^{4,6}. The antisera used were obtained commercially⁵ and consisted of Anti-human rabbit serum.

Results of the study showed that in the early stages of digestion at least 2 precipitin bands are seen in the gel-diffusion set-up. The outer band disappears relatively early while the inner band persists for a much longer period. Figure 1 shows that the outer band has started

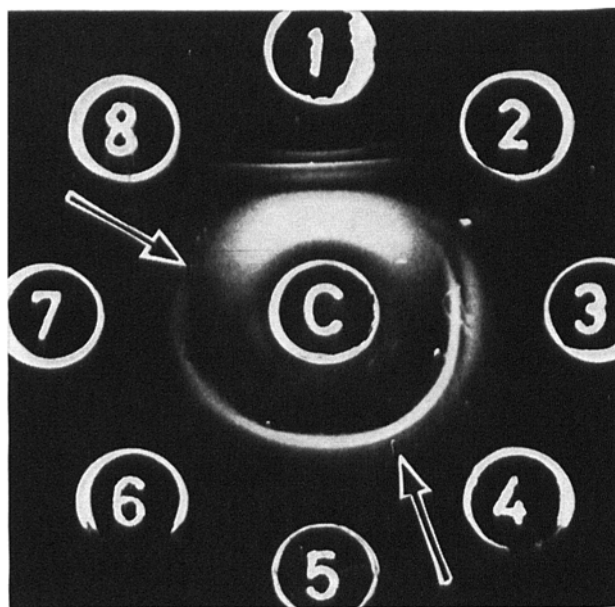


Fig. 1. (C) Rabbit Antihuman serum; (1) Human serum; (2) Antigen, 3 h, (3) 6 h, (4) 12 h, (5) 18 h, (6) 24 h, (7) 48 h, (8) 56 h after intake of blood meal. Arrow between 4 and 5 marks the complete disappearance of the outer band. Arrow between 7 and 8 marks the complete disappearance of the inner band.

¹ A. N. CLEMENTS, *The Physiology of Mosquitoes* (Pergamon Press, Oxford-London 1963).

² C. A. WILLIAMS, *Int. Congr. Zool.* 278 (1956).

³ RALPH A. BARR and W. T. CHELLAPPAH, *Bull. Wild Hlth Org.* 31, 439 (1964).

⁴ LKB-Produkter AB Stockholm (Sweden).

⁵ Mann Research Laboratories, New York (USA).

⁶ The LKB immunoelectrophoresis equipment was bought through a grant from the Wellcome Trust to whom we extend our thanks.

to fade by 12 h and has completely disappeared before 18 h. The inner band has started to fade by 48 h and has completely disappeared before 56 h. It therefore seems that the rate of digestion of serum protein is not uniform. To identify the fraction which persists for a longer period, immunoelectrophoresis was done, using normal human serum on one side and the mosquito antigen obtained after 48 h of digestion on the other (Figure 2). The Antihuman serum in the central trough was the same as used for gel-diffusion. A clear arc of precipitin was obtained with the mosquito antigen whose mobility

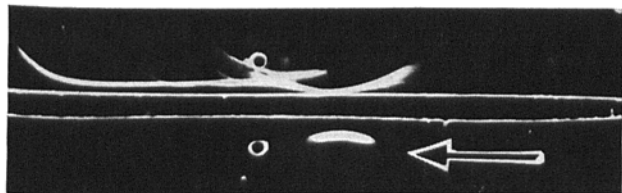


Fig. 2. Normal human serum on the upper side and antigen obtained 48 h after intake of blood meal on the lower side. Arrow marks the precipitin arc produced by the antigen.

corresponded to the Albumin fraction of the human serum. These findings confirm the observations made by WILLIAMS² that the Albumin fraction of the serum persists for a much longer period than the Globulins. In view of these findings, it is felt that antisera produced against the Albumin fraction of the serum could be used with a greater advantage for identification of blood meals in the field.

Zusammenfassung. Die Verdauungsgeschwindigkeit von Serumproteinen im Darm von Mücken (*Armigeres subalbatus*) wurde mit Gel-Diffusion und Immunelektrophorese bestimmt. Es konnten wenigstens 2 Bänder mit Gel-Diffusion in einer Mücke nachgewiesen werden, die menschliches Blut gesaugt hatte. Während das äussere Band nach 12–18 h verschwindet, konnte das innere noch nach 48–56 h als Albuminfraktion des menschlichen Serums bestimmt werden.

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The Effect of D-Mannoheptulose on Islets of Langerhans Cultured in vitro

D-mannoheptulose blocks the release of insulin from the pancreas¹⁻³. It was found⁴ that the β -cells of the islets of Langerhans isolated from the guinea-pig pancreas and cultured in vitro up to 14 days underwent more or less intensive degranulation. Since the number of granules in β -cells is proportional to the amount of extractable insulin in the pancreas⁵, it was supposed that the degranulation of β -cells occurring in vitro could be prevented by mannoheptulose.

Methods. The islets of Langerhans of guinea-pigs were isolated and cultured as before⁴. The tissue culture medium consisted of equal parts of human plasma, human serum, chick embryo extract, Hanks solution and glucosol. Groups of islets were cultured for 7 days at low (about 100 mg%) and at high (about 400 mg%) glucose concentration, without mannoheptulose or in presence of mannoheptulose at 300 mg% concentration. In other experiments, islets were cultured for 1 week at low glucose concentration and during the second week in the 4 media mentioned above. Over 20 islets were cultured in each medium. Mannoheptulose dissolved in glucosol was sterilized by autoclaving. Isotonicity of the medium was secured by proper adjustment of the NaCl concentration. The cultured islets were embedded, sectioned and stained as before⁴.

Results. The morphological appearance of the islets cultured in the absence of mannoheptulose was similar to that described before⁴. β -cells in culture as compared to normal uncultured β -cells were partially degranulated, particularly at high glucose concentration. The islets cultured in the presence of mannoheptulose were well preserved morphologically but the β -cells both at low and high glucose concentration, contrary to expectation, were completely degranulated. This effect of mannoheptulose was observed in one- as well as in two-week cultures.

The blocking action of mannoheptulose on insulin release is firmly established¹⁻³. To explain the complete degranulation of the β -cells cultured in the presence of mannoheptulose, it is necessary to assume that this sugar also interferes with insulin synthesis. Small output of insulin at low glucose concentration is not affected by mannoheptulose³. If mannoheptulose interferes with insulin synthesis, which is compatible with its inhibitory action on glucose phosphorylation⁶, the insulin present in the granules of β -cells at the beginning of culture could be slowly released and the formation of new insulin containing granules would be prevented. It remains to be seen whether prolonged administration of mannoheptulose evokes also degranulation of β -cells in vivo⁷.

Zusammenfassung. β -Zellen der Langerhansschen Inseln vom Meerschweinchen in vitro gezüchtet, unterliegen bei Anwesenheit von Mannoheptulose einer vollkommenen Degranulierung. Es wird die Möglichkeit diskutiert, ob Mannoheptulose nicht nur die Auslösung des Insulins blockiert, sondern auch seine Bildung beeinflusst.

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¹ E. SIMON, R. O. SCOW and S. S. CHERNICK, *Am. J. Physiol.* **201**, 1073 (1961).

² E. SIMON, P. F. KRAICER and M. C. SHELESNYAK, *Endocrinology* **71**, 83 (1962).

³ H. G. COORE, P. J. RANDLE, E. SIMON, P. E. KRAICER and M. C. SHELESNYAK, *Nature* **197**, 1264 (1963).

⁴ S. MOSKALEWSKI, *Gen. comp. Endocr.* **5**, 342 (1965).

⁵ W. S. HARTROFT and S. A. WRENSHALL, *Diabetes* **4**, 1 (1955).

⁶ H. G. COORE and P. J. RANDLE, *Biochem. J.* **91**, 56 (1964).

⁷ The author is indebted to Prof. E. SIMON, Rehovoth (Israel) for his generous gift of mannoheptulose required for this study.