Since in metaphase, however, interchromosomal bridges can be caused or made apparent by hypotonic saline, and since hypotonic fluids have invariably been used to demonstrate them, it remains an open question whether metaphase chromosomes have these connections in living cells.

Résumé. Dans les cultures de cellules du triton, les ponts interchromosomaux peuvent se former dans les figures métaphasiques sous l'influence d'une solution hypotonique. Ce résultat, obtenu au microscope à la lumière ordinaire, pose la question de savoir si les ponts déjà décrits dans les micrographes électroniques sont également dus à l'emploi des solutions hypotoniques.

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Transformation of a Lys-Auxotroph to Prototrophy in *Neisseria catarrhalis*

KINGSBURY[1] demonstrated by hybridization studies that the genus *Neisseria* was heterogenous in nature, forming at least three distinct groups: Group I, *N. meningitidis* and *N. gonorrhoeae;* Group II, *N. perflava, N. subflava, N. sicca* and *N. flava;* Group III, *N. catarrhalis* and *N. caviae*. Drug resistant markers, especially streptomycin resistance, have been used as criteria to determine the genetic relationship(s) between these saprophytic and pathogenic *Neisseria*[2-4]. Nevertheless, it seems apparent to seek further information using a different genetic marker, for example for a nutritional marker. Thus, this investigation developed a minimal medium for *N. catarrhalis*, the production of a nutritional auxotroph, and established competency for intraspecific transformation.

N. *catarrhalis* NC-19 used in this investigation was from the University of Maryland collection. The morphological, cultural and biochemical characteristics of this organism were observed periodically and were found to be typical for this species.

The minimal nutritional requirements for *N. catarrhalis* NC-19 were developed from MARTIN et al.[5] medium to support the growth of *N. perflava* 876. A formula differing from MARTIN's medium, essentially in the inorganic salt composition and amino acid mixture, was established that supported the growth of *N. catarrhalis* NC-19. The amino acid composition consisted of glycine, arginine, proline and glutamic acid whereas the salt solution was composed of NaCl, KCl, $NaHPO_4$, $MgSO_4$ and $CaCl_2$. This medium (MM) propagated a suspension of cells of *N. catarrhalis* similar to that obtained in trypticase soy broth plus 0.35% yeast extract.

Nitrous acid was used successfully by LIE[6] as a mutagen for *N. meningitidis*. A similar procedure was employed in this study. The detection of biochemical deficient mutants was determined by using the velvet replica technique of LEDERBERG and LEDERBERG[7]. Only 2 types of nutritional mutants were obtained: lysine (lys^-) and tryptophan ($trypt^-$) auxotrophs. Only the lys^- mutant was used in this investigation for transformatioal studies. The mutants were maintained on CTA medium and working cultures were grown on TSA slants. No reversions with the lys^- or the $trypt^-$ mutants were detected. The composition of the lysine medium (LM) consisted of the MM + lysine.

The wild-type DNA used for transformational studies in this investigation was extracted by the use of $CH_3(CH_2)_{10}CH_2OSO_3Na$ (5%, w/v) and stored in 2*M* NaCl. No deproteinization steps or RNAse treatment were performed. The concentration of DNA was determined by the method of BURTON[8].

N. catarrhalis auxotrophic mutant cells (lys^-) were prepared for transformation by growing them in trypticase soy broth with yeast extract and 1.5% calf serum for 18 h on a rotary shaker (200 rpm) at 37 °C. To obtain actively dividing cells an aliquot was removed and added to fresh broth and serum and grown for an additional 2 h. The cells were then centrifuged and washed twice in MM and resuspended in 9 ml of same. 1 ml of this suspension was added to 9 ml of MM. 1.5 ml quantities of suspension were then added to each of several tubes containing: DNA and lysine; DNAse (Worthington Biochemical, 1 x crystallized and 0.002*M* $MgSO_4 \cdot 7H_2O$) treated DNA and lysine; and lysine only. Tubes were incubated at 37 °C and shaken for 20 min. Afterwards, 100 µg of DNAse was added to the tubes containing DNA and incubated for 10 min at room temperature. 0.1 ml of appropriate dilutions made in saline were plated out on minimal and lysine agar (1.5% agar). Controls consisted of DNAse treated DNA and recipient cells, and recipient cells only. Plates were incubated for 72 h at 37 °C. The number of transformants was determined by the following

Comparison of cell numbers from intraspecific auxotrophic transformational studies with *N. catarrhalis* NC-19 Lys^- [a]

Experiment No.	DNA donor	E [b]	T [c]	%T
1	NC-19WT	4.5×10^6	5.6×10^5	12.4
2	NC-19WT	8.0×10^6	6.9×10^5	8.6
3	NC-19WT	7.6×10^6	6.6×10^5	8.8
4	NC-19WT	3.2×10^6	3.0×10^5	9.3
5	NC-19WT	7.1×10^6	5.2×10^5	7.3

[a] Transformation system: As described in test. [b] Number of exposed organisms. [c] Number of transformants.

[1] D. T. KINGSBURY, J. Bact. *94*, 870 (1967).
[2] K. BOVRE, Acta path. microbiol. scand. *64*, 229 (1965).
[3] B. W. CATLIN, Science *131*, 608 (1960).
[4] B. W. CATLIN and L. S. CUNNINGHAM, J. gen. Microbiol. *26*, 303 (1961).
[5] W. H. MARTIN and M. J. PELCZAR and P. A. HANSEN, Science *116*, 483 (1952).
[6] S. LIE, Acta path. microbiol. scand. *63*, 615 (1965).
[7] J. LEDERBERG and E. M. LEDERBERG, J. Bact. *63*, 339 (1952).
[8] K. A. BURTON, Biochem. J. *62*, 315 (1956).

formula: %T = transformants counted on minimal agar/ number of exposed cells (colonies) counted on lysine agar X 100.

High level frequencies of transformation of the lysine autotroph to prototrophy ranged from 7–12% (Table). The controls in the experiments performed were always negative[9,10].

Zusammenfassung. Die Kompetenz für intraspezifische Transformation bei *Neisseria catarrhalis* wird nachgewiesen. Als genetischer Marker wird Lys⁻ gewählt und hohe Frequenzen von Protrotophen festgestellt.

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Dietary Sterols: Role in Larval Feeding Behaviour of the Southwestern Corn Borer, *Diatraea grandiosella*

Although the effect of dietary sterols has been examined in many insects, research has focused primarily on sterol indispensability, utilization, and metabolism[1,2]. Studies examining the effect of sterols on feeding behaviour have not kept pace[3,4]. This paucity of behavioural data is unfortunate since some growth effects caused by dietary sterols may have been due to feeding stimulation or deterrency rather than to lesions in sterol absorption or metabolism. For this reason the present study examined the effect of sterols and sterol esters on feeding behaviour of the southwestern corn borer, *Diatraea grandiosella* Dyar. Since nutritional experiments have already shown that the structure of dietary sterols influences the growth rate of this corn borer[5], the present experiments were designed to differentiate behavioural from nutritional responses to sterols.

Methods. A culture of *D. grandiosella* was reared on a meridic diet[6], and newly-hatched first stage larvae were used for the experiments. The control diet was a simplified version of the rearing medium and contained (in g): casein (vitamin-free) 3.3; cellulose powder 2.8; agar (Bacto) 2.6; glucose 1.0; and water 90.3. Sterols (0.18 g) or sterol esters (0.2–0.32 g) were incorporated into the test diets at the expense of water. Each medium was prepared by mixing the boiling agar solution with the dry components and was then dispensed into Petri dishes. Cylinders (13 mm ⌀) cut from the gel weighed about 1.4 g each and served as the feeding stations in the bioassay.

A 24 h bioassay examined what effect the test compounds had on the maintenance of larval feeding rather than on initial attraction or biting responses[7,8]. Plastic Petri dishes (13 × 85 mm) served as the test arenas and contained 2 control and 2 test stations with like stations diametrically opposite to each other and 25 mm from the centre. 50 first stage larvae were placed in the centre of each arena and incubated for 24 h at 30 °C in darkness. Each experiment was replicated 4 times. Following incubation the number of larvae on each test and control station was recorded. About 70% of the larvae were usually found at the feeding stations. The results are presented as the percent of larvae on the control or test stations in relation to the total number feeding, ignoring the few larvae which did not establish during the incubation period. The data were analyzed using Student's *t*-test to determine statistical differences[9]. Preliminary experiments showed that larvae were randomly distributed among feeding stations in 'no-choice' situations and no larval-larval interactions were detected.

Results and Discussion. The results (Table) show that cholesterol, 7-dehydrocholesterol, and ergosterol had neutral effects on larval feeding behaviour. The first two sterols have already been shown to support larval growth while larvae died when ergosterol was the sole dietary sterol[5]. Since all these sterols had a neutral effect on feeding behaviour the larval mortality on the ergosterol diet can now be attributed to an inability to absorb or metabolize ergosterol to cholesterol rather than to any feeding deterrency and starvation. *D. grandiosella* may well lack a C_{24} demethylating enzyme required to convert this sterol to cholesterol.

The Table also shows that the 3 cholesterol esters tested were feeding deterrents. Nutritional experiments have already shown that diets supplemented with these esters resulted in low larval growth rates[10]. It is now apparent that this poor growth resulted from feeding deterrency and a low rate of food consumption rather than to any lesion in the absorption or metabolism of the dietary cholesterol esters. Since the feeding deterrency was obtained with 3 different acid moieties (acetate, myristate, oleate) it appears that a substituted 3 hydroxyl group adversely affects the insect's sensory mechanisms. Similar results were obtained with β-sitosterol acetate. In this case substitution of the hydroxyl group transformed the molecule from a feeding stimulant into one which had a neutral effect on feeding behaviour. It may therefore be concluded that the 3 hydroxyl is one crucial position controlling the effect of sterol molecules on feeding behaviour.

[1] R. B. Clayton, J. Lipid Res. *5*, 3 (1964).

[2] W. E. Robbins, J. N. Kaplanis, J. A. Svoboda and M. J. Thompson, A. Rev. Ent. *16*, 53 (1971).

[3] J. K. Nayar and G. Fraenkel, J. Insect Physiol. *8*, 505 (1962).

[4] T. Ito, K. Kawashima, M. Nakahara, K. Nakanishi and A. Terahara, J. Insect Physiol. *10*, 225 (1964).

[5] G. M. Chippendale and G. P. V. Reddy, J. Insect Physiol. *18*, 305 (1972).

[6] D. Jacob and G. M. Chippendale, Ann. ent. Soc. Am. *64*, 485 (1971).

[7] S. D. Beck, Ann. ent. Soc. Am. *49*, 399 (1956).

[8] S. Gothilf and S. D. Beck, J. Insect Physiol. *13*, 1039 (1967).

[9] R. G. D. Steel and J. H. Torrie, *Principles and Procedures of Statistics* (McGraw Hill, New York 1960).

[10] G. P. V. Reddy and G. M. Chippendale, manuscript in preparation.