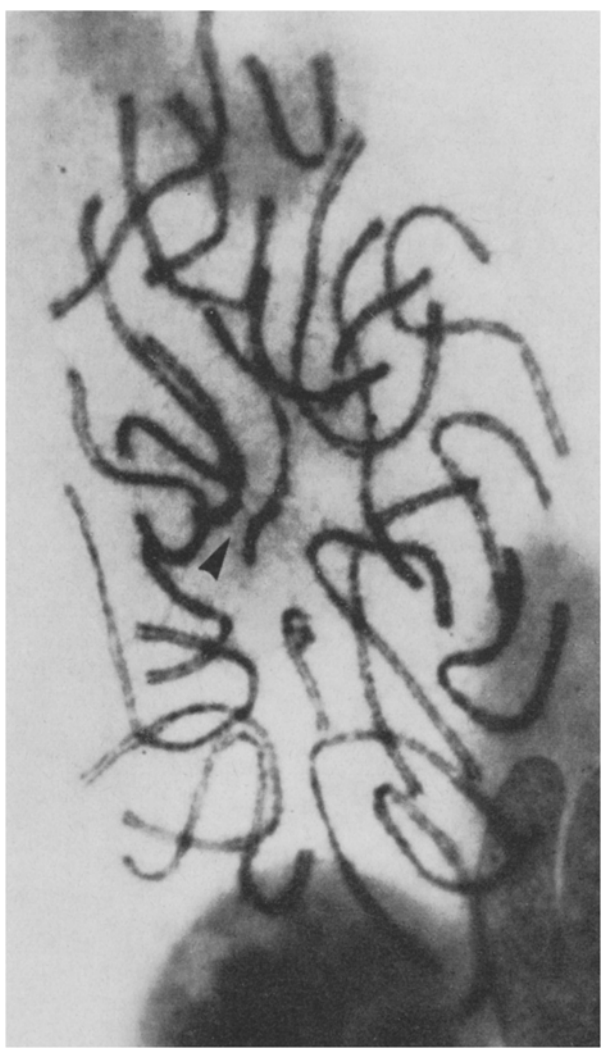
Bridges Between Chromosomes

Fine strands connecting chromosomes have been demonstrated with the electron microscope in material subjected unfixed to treatment with a hypotonic solution and picked up off distilled water[1,2]. The effects of a hypotonic solution on metaphase chromosomes are therefore reported here.

Perichondrial and cardiac fibroblasts of the adult newt *Triturus cristatus* were grown in hanging-drop cultures[3], which were then immersed for 10–12 min in a hyptonic saline, Tyrode's solution from which sodium chloride had been omitted. They were fixed for 10 min in a 3:1 mixture of absolute ethanol and glacial acetic acid, and stained with 1% aqueous gentian violet differentiated by clove oil, and were mounted whole. About 200 mitotic figures were examined with the 2 mm lens (N.A. 1.3).

At all stages of division the chromosomes appeared rough in outline. In places this roughness could be resolved as fine protruding whiskers. In metaphase it was frequently possible to trace the continuity of whiskers to make bridges between chromosomes (Figure).



Unretouched photomicrograph of the central region of a cultivated newt fibroblast in early metaphase, treated by hypotonic saline before fixation. Gentian violet. The pointer indicates interchromosomal bridges. ×3000.

In control preparations, fixed without prior immersion in hypotonic saline, a similar number of mitotic figures was examined. The chromosomes of metaphase and all that part of anaphase before the apparent clumping of chromosomes were smooth in outline; neither whiskers nor bridges could be seen. The absence of brigdes confirmed observations in a range of experiments with several fixatives and stains[4–7], although the point was not made explicit in the publication of those studies.

The bridges may be explained in one of two ways. They may have been always present, but clumped by the hypotonic saline into structures visible with the light microscope. The connections seen by the electron microscope would then correspond to structures present in life. The observed bridges, however, connect not only chromosomes but sister chromatids[8], and their presence in life would thus imply the rupture or dissociation of the constituent chromonemata of chromosomes in early anaphase. On the other hand, both light and electron microscopical findings are explicable if the bridges are produced by the experimental treatment. Since a hypotonic solution disorganizes the spindle[9] and renders the inner cytoplasm fluid (as indicated by Brownian motion)[10], chromosomes may be expected to touch their neighbours before spreading, and their surface may be changed by the abnormal conditions to permit adhesion, so that threads are drawn out during separation. (The 'stickiness' of damaged chromosomes is well known to radiobiologists.)

DuPraw[11] has argued in favour of interchromosomal bridges not only from electron microscopical findings but also from Hoskins's experiments[12], in which material linking chromosomes broke on being pulled in the presence of desoxyribunuclease. DuPraw rejects Hoskins's own explanation, that DNA is a component of spindle fibres, because a chain of chromomes, such as Hoskins drew out of metaphase figures with a needle, could not be linked by a single spindle fibre. The published photograph shows a structure much coarser than a single spindle fibre, and it appears that Hoskins drew out a bundle of experimentally extended spindle material with chromosomes attached at various positions along it, but not necessarily in series on any one fibre.

Interchromosomal bridges have been seen in meiotic prophase by light microscopy after fixation and staining[13] and, in mitosis, with the electron microscope after treatment with hypotonic fluids[14]. In telophase these connections are visible not only in fixed cells[15] but also in life[7,16].

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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.

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