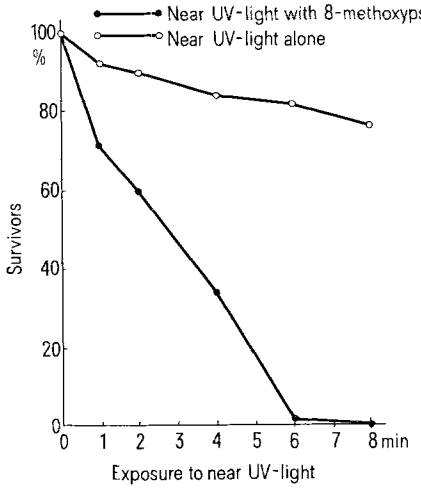
Effect of near UV-light in presence of psoralen on mutations in *Serratia marcescens* (threonine less)

Treatment	Survivors (%)	Auxotrophs (%)	Threonine revertants (%)	Achromogenic variants (%)
Control	100	nil	0.1	1.0
Near UV	100	0.1	0.1	3.9
Psoralen + near UV	0.006	1.6	0.7	13.5

auxotrophy in addition to threonine requirement and 3. colour variations. Reversions were scored by plating out dense suspension of known number of cells on the minimal medium agar. Auxotrophs were detected by (spotting colonies developed on nutrient agar) on minimal and complete agar media. The colour variants were discerned on complete medium agar.

The Figure depicts the pattern of cell inactivation by near UV in the absence and presence of the photosensitizing substance (MOP). It was evident that MOP has a very significant effect on the killing due to near UV. There was practically no cell inactivation even after 10 min of UV-irradiation alone, whereas with MOP cell inactivation followed in somewhat linear proportionality to the duration of irradiation. With 2 min of irradiation in presence of MOP nearly 60% of the cells were surviving, and at 6 min still 1% of survivors were found. After 10 min of irradiation with MOP, 0.006% survivors were scored.

In the Table are given the yield of auxotrophs, revertants and colour variants obtained after irradiation with near UV alone and with near UV in presence of 100 µg/ml MOP. It was seen that the reversion frequency to threonine independance in the case of the culture irradiated in presence of MOP was 4 times that in the culture irradiated without it. The auxotrophs yield was 9 times higher. The parental culture gave a typical pattern of colour variants predominantly purple coloured colonies. Buff coloured and achromogenic colonies were detected wherever MOP was used.



Survival curves of *Serratia marcescens* (threonineless) after treatment with near UV-light in the presence and absence of 8-methoyxpsoralen

The results indicate the advantage of employing near UV in conjunction with a photosensitizing chemical which gave a considerable yield of auxotrophs and colour variants with only moderate killing of the cells. The beneficial role of the near UV in presence of photosensitizing chemical in the development of industrial strains of *Streptomyces* has already been demonstrated by Townsend et al.[2]. Earlier, Alderson and Scott[3] and Scott and Alderson[4] have also shown that irradiation of *Aspergillus nidulans* conidia with near UV-light in presence of 8-methoxypsoralen resulted in high mutant yield with no cistron specificity.

From our own results obtained in the case of a bacterium, and those obtained in the case of *Streptomyces* and fungi by others, it would appear that it is more advantageous to employ near UV in conjunction with the photosensitizing chemical for the development of mutants in industrial research because of the high yield of mutants under conditions of moderate killing.

Zusammenfassung. Zur Erzeugung von Mutanten wurden an Stelle von UV-Licht (Wellenlänge 253–265 nm) längerwellige Strahlen (350–360 nm) in Kombination mit dem photosensitierenden 8-Methoxy-Psoralen verwendet. Mit diesem System (früher an *Aspergillus nidulans* und an *Streptomyces*-Stämmen ausgetestet) ist der mutagene Effekt am Bakterium *Serratia marcescens* geprüft worden.

R. Joseph, M. S. Shanthamma, F. Rehana and K. Nand

Discipline of Microbiology,
Fermentation Technology & Sanitation
Central Food Technological Research Institute,
Mysore–570013 (India),
29 August 1973.

[2] M. E. Townsend, H. M. Wright and D. A. Hopwood, J. appl. Bact. *34*, 799 (1971).
[3] T. Alderson and B. R. Scott, Mutation Res. *9*, 569 (1970).
[4] B. R. Scott and T. Alderson, Mutation Res. *12*, 29 (1971).
[5] Acknowledgment. The authors wish to thank Dr. T. N. Ramachandra Rao for his keen interest and valuable suggestions.

Virus-Like Particles in Human Laryngeal Papilloma. An Ultrastructural Study

In an earlier communication, under light microscope, we have reported the high incidence of papilloma of larynx in Nigerian children and an adeno-virus was suggested as an etiological factor. The clinical manifestations, histological characteristics and treatment of this benign tumour has been discussed by several workers[1–5]. In spite of numerous investigations on the subject, its pathogenesis still remains obscure. However, a viral

etiology has been suggested as the causative agent for papillomas in various animals[6,7]. Recently, virus-like particles were also located in the intranasal[8] and laryngeal papillomas from USA.[9]. The purpose of this communication is to provide electron microscopic evidence in support of the viral etiology of papillomas of larynx in Nigerian children and to determine whether the morphology of the above-noted virus is similar to the one reported previously[8,9].

Material and methods. 5 cases of laryngeal papillomas, 1 male and 4 females, from Nigerian children were examined. Tissue specimens in the above cases were obtained for light and electron microscopy. Light microscopy was performed by routine paraffin embedding and sections were stained by the standard haematoxylin and eosin method.

For electron microscopy, several biopsy specimens from all 5 cases were fixed in 3% glutaraldehyde at pH 7.4 with sodium cacodylate buffer. The tissues were postfixed in 1% osmic acid solution, dehydrated and embedded in Araldite. Ultrathin sections were cut on Porter-Blum microtome, stained with uranyl acetate and Reynold's lead citrate and examined in an Hitachi HS-8 electron microscope.

Results. General architecture of the tumour. Under light microscope, the neoplasm presented the typical morphological features of the laryngeal papilloma. The details of these observations had been reported earlier[1].

Under the electron microscope, the neoplasm was composed of several layers of epithelial cells, separated from one another with dilated intercellular spaces (Figure 1). The cytoplasmic processes at the intercellular junction showed well developed desmosomes (Figure 1). The nuclei of the tumour cells were round or oval in shape with moderately dense and evenly distributed nucleoplasm. Dense bodies of 30–45 nm in diameter were noted in most of the nuclei. These bodies were randomly scattered in the nucleoplasm and were usually surrounded by clear haloes (Figures 1 and 2). Sometimes several such bodies were seen lying close to each other.

Discussion. In the voluminous literature on the papilloma of larynx, a virus infection has been postulated as an etiological factor by ULMAN[10] as early as 1923. This was later confirmed by DMOCHOWSKI et al.[11], and WILLIAMS et al.[9]. The observations reported in this paper provided for the first time evidence of a viral agent being present in the laryngeal papilloma of Nigerian children. The virus

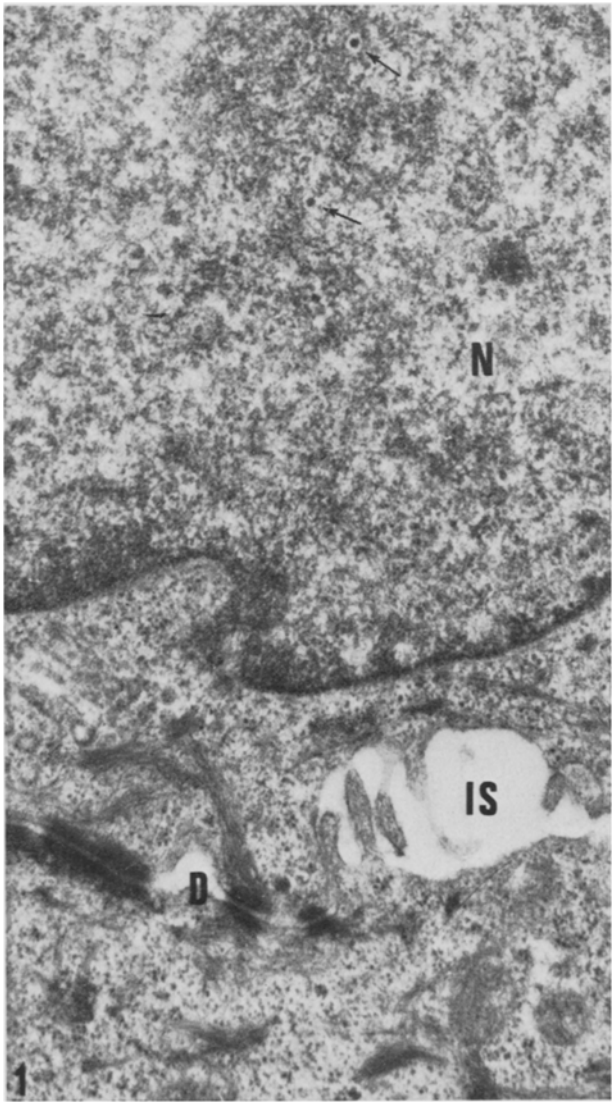


Fig. 1. Epithelial cells from a laryngeal papilloma showing the dilated inter-cellular space (IS), desmosome (D) and nucleus (N). Note the intranuclear-dense particles (↑). ×33,000.

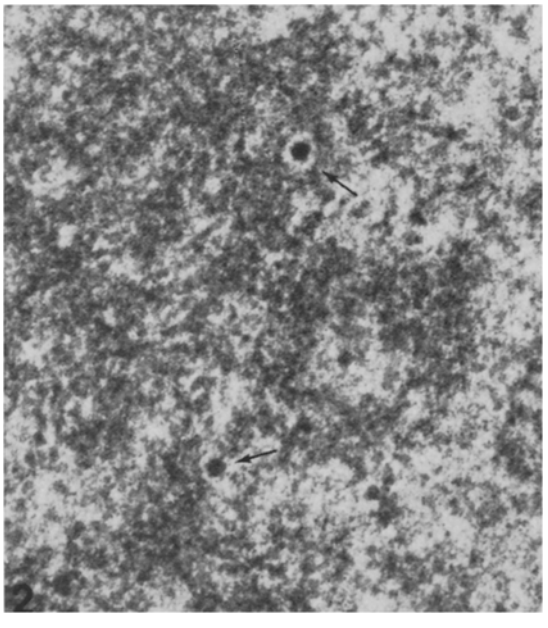


Fig. 2. An enlarged view of a portion of Figure 1 illustrates virus like dense particles in the nucleoplasm. ×66,000.

[1] D. K. MUKHERJEE and M. M. AHMED, Nigerian med. J. *3*, 92 (1973).
[2] H. A. OBERMAN, Am. J. clin. Path. *42*, 245 (1964).
[3] A. M. DEKELBONM, Arch. Otolaryng. *81*, 390 (1965).
[4] P. SCHLAPPI, Pract. Otorhinolaryng. *28*, 365 (1967).
[5] C. W. GROSS, Laryngoscope *80*, 532 (1970).
[6] R. S. STONE, R. E. SHOPE and D. H. MOORE, J. exp. Med. *110*, 543 (1959).
[7] R. E. FULTON, F. W. DOANE and L. W. MACPHERSON, J. Ultrastruct. Res. *30*, 328 (1970).
[8] V. JAHNKE, Ann. Otol. Rhinol. Laryng. *80*, 78 (1971).
[9] F. B. WILLIAMS, E. G. MCCOY and W. A. FOGARTY, Ann. Otol. Rhinol. Laryng. *80*, 51 (1971).

particles were seen as dense bodies surrounded by clear haloes in the nuclei of all specimens examined. The morphology of these virus-like particles is similar to that reported by WILLIAMS et al.[9]. However, HOLINGER et al.[12] failed to demonstrate the presence of viral particles in their studies on human papillomas. This could be due to the loss of the inherent properties of the virus necessary for its identification. The agreement between the present findings and the one reported earlier[9,11] provide strong evidence of virus being the causative factor for the papilloma of larynx.

Zusammenfassung. In Papillomen, welche von nigerianischen Kindern stammten, wurden elektronenmikroskopisch Bildungen, welche als Viruspartikel anzusprechen waren; gefunden. Diese sind denjenigen Viruseinlagerungen vergleichbar, die man im Papillom des Larynx nachweisen kann.

M. M. AHMED and D. K. MUKHERJEE

Department of Anatomy, University of Singapore, Sepody Lines, Singapore 3, and Department of Otolaryngology, Lagos University Teaching Hospital, Lagos (Nigeria), 20 September 1973.

[10] E. V. ULMAN, Acta Oto-laryng. *5*, 317 (1923).
[11] L. DMOCHOWSKI, C. E. GREY and J. A. SYKES, Tex Rep. Biol. Med. *22*, 454 (1964).
[12] P. H. HOLINGER, N. L. SHIPKOWITZ, J. C. HOLPER and M. C. WORLAND; Acta Oto-laryng. *65*, 63 (1968).

Einfluss der Gibberellinsäure auf die Sporulation von *Saccharomyces cerevisiae*

Diploide Hefezellen können in bestimmten Nährmedien, die so zusammengesetzt sind, dass sie einen intensiven Atmungsstoffwechsel der Zellen gewährleisten, sich zu Asci differenzieren (Sporulation). Bei der Ausbildung des Ascus findet gleichzeitig die Meiose statt. Für diese Differenzierung ist eine spezifische RNS- und Proteinsynthese notwendig[1]. Nach KAMISAKA et al.[2] erhöht ein Zusatz von Gibberellinsäure (GS) zum Sporulationsmedium die Sporulationsfähigkeit. Im Gegensatz hierzu steht die Angabe von FOWELL[3], dass unter seinen Versuchsbedingungen GS, selbst in sehr niedrigen Konzentrationen, die Sporulationsfähigkeit vermindert.

Um diesen Widerspruch zu klären, wurde die Wirkung der GS unter verschiedenen Sporulationsbedingungen geprüft. Zellen eines diploiden, aus Bäckerhefe isolierten Stammes W wurden in Vollmedium bis zur frühen stationären Phase vorkultiviert und dann a) 18 h in Praesporulationsmedium nach PONTEFRACT und MILLER[4], abgeändert nach CROES[5] bei 33 °C im Schüttelbad inkubiert, gewaschen und in dem Sporulationsmedium nach MCCLARY et al.[6] weitergeschüttelt (= Standardbedingungen), oder b) 24 h in YHG-Praesporulationsmedium nach KAMISAKA et al.[2] ungeschüttelt bei 30° C inkubiert, gewaschen und in SG-Sporulationsmedium nach KAMISAKA et al.[2] bei 28 °C geschüttelt. Den Sporulationsmedien wurde GS in verschiedenen Konzentrationen von 0–400 mg/l zugesetzt. Bei beiden Methoden erfolgte die Auszählung der Asci nach 48 h. Die in der Tabelle angegebenen Ascushäufigkeiten sind Mittelwerte aus 3 Versuchen mit jeweils 2 Wiederholungen.

Die Sporulationshäufigkeit liegt bei Standardbedingungen in allen Proben mit GS-Zusatz unter dem Kontrollwert. In der Probe mit 1 mg/l GS ist der Unterschied signifikant auf dem 5%-Niveau. Diese Ergebnisse entsprechen somit der Beobachtung von FOWELL. Unter KAMISAKA-Bedingungen ist eine Zunahme der Sporulationshäufigkeit mit steigender Gibberellinkonzentration offensichtlich. (Signifikanz ab 200 mg/l GS). Nach KAMISAKA et al.[2] ist allerdings die optimale Konzentration schon bei 100 mg/l GS erreicht, nach unseren Versuchen erst bei 800 mg/l mit 46,65% Asci. Weitere Versuche ergaben, dass sich dieser ausgeprägte GS-Effekt völlig aufheben lässt, wenn das KAMISAKA-Sporulationsmedium mit dem Praesporulationsmedium der Standardbedingungen kombiniert wird.

Figur 1 zeigt, dass die GS nicht nur die Endhäufigkeit der Asci steigert, sondern auch die Ascusbildung beschleunigt.

Die fördernde Wirkung der GS scheint mit der Synthese der die Sporulation regulierenden RNS assoziiert zu sein[7]. Nach ZIEMONS[8] ist unter Standardbedingungen die für die Sporulation notwendige RNS-Synthese 7 h vor der sichtbaren Differenzierung zum Ascus abgeschlossen. Um zu prüfen, zu welcher Zeit die GS ihre Wirkung in der Sporulations-Kultur entfaltet, wurden folgende Versuche durchgeführt: a) Die GS (800 mg/l) wurde zu verschiedenen Zeiten nach Ansetzen der Kultur dem SG-Sporulationsmedium zugegeben (Figur 2).

Je später die GS-Zugabe erfolgte, desto geringer war die Ascushäufigkeit. Bei Zugabe in der 15. Stunde wurde annähernd der Wert erreicht, den man auch ohne GS im SG-Sporulationsmedium erhält. b) GS wurde gleich zu

[1] K. KADOWAKI und H. O. HALVORSON, J. Bact. *105*, 826 (1971).
[2] S. KAMISAKA, Y. MASUDA und N. YANAGASHIMA, Physiologia plant. *20*, 90 (1967).
[3] R. R. FOWELL, *The Yeasts* (Academic Press, London 1969), vol. 1, chapt. 7.
[4] R. D. PONTEFRACT und J. J. MILLER, Can. J. Microbiol. *8*, 573 (1962).
[5] A. F. CROES, Planta *76*, 209 (1967).
[6] D. O. MCCLARY, W. L. NULTY and U. R. MILLER, J. Bact. *78*, 362 (1959).
[7] S. KAMISAKA, Y. MASUDA and N. YANAGASHIMA, Physiologia plant. *20*, 98 (1967).
[8] E. ZIEMONS, Diss. Köln (1972).

Ascushäufigkeiten (%) bei 2 verschiedenen Kulturbedingungen und unterschiedlichen Konzentrationen von GS im Sporulationsmedium

Bedingungen / (GS mg/l)	0	1	5	10	50	100	200	400
a)	49,30	34,60	40,20	43,30	42,16	37,50	35,90	43,80
b)	14,20	18,30	17,73	15,40	21,00	22,70	28,20	34,10