

adrenalectomized infected mice observed on 6th day after infection (Figure 3) are characterized by the damage of dark reticular epithelial cells (DREC) and focal thymolysis. Moreover cortical thymocytes show a great difference in the intensity of staining (Figure 3). The population of intensely stained thymocytes is less numerous. At a later period of virus infection (10 days, Figure 4), the cortical layer of thymocytes tends to disappear. Focal thymolyses are more frequent. At the same time a number of mitoses may be observed in the thymus (Figure 4).

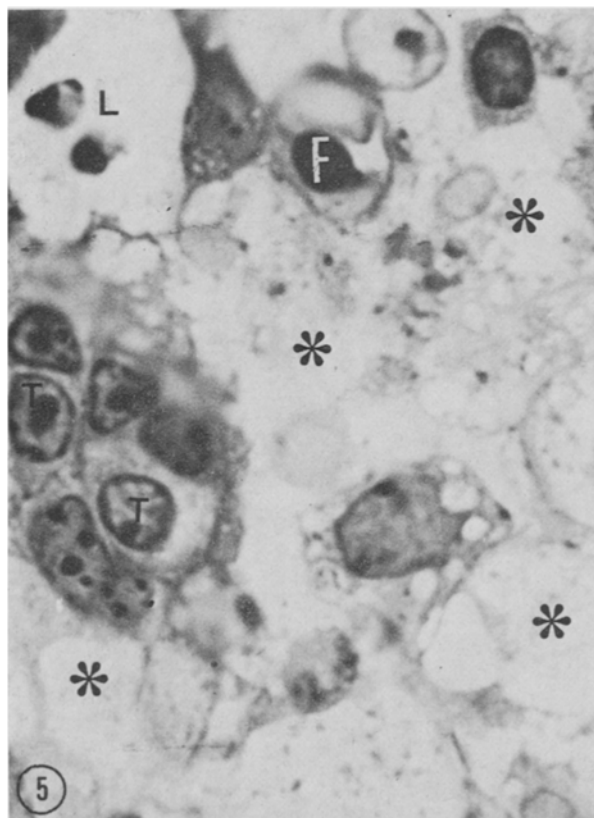


Fig. 5. Thymus of intact infected mouse collected on 10th day after virus infection. Large confluent zones of lysed cells are marked by asterisks. A small islet of fairly well preserved thymocytes (T) is seen on the left of the micrograph. Phagocytized pyknotic nucleus of the thymocyte is marked 'F'. At 'L' a light reticular epithelial cell containing 2 huge lysosomes. $\times 1,200$.

Virus infection of intact mice rapidly causes a complete disappearance of the cortex of the thymus. In the medulla DREC are heavily damaged and a focal thymolysis is a frequent phenomenon. On 10th day after virus inoculation thymuses show large confluent zones of lysed cells (Figure 5). Only small islets of practically intact cells may be observed (Figure 5). We did not observe any mitotic divisions during the whole period of virus infection of intact mice.

Sham operated infected mice behave in all respects as intact infected mice.

Discussion. PR8 virus infection provokes the thymus involution in mice². We now report the morphological alterations responsible for the involution, which is more clearly expressed in intact mice than in adrenalectomized mice. A model of indirect effects of PR8 virus infection on thymus is offered by adrenalectomized mice. DREC damage is common to both experimental groups of animals. Focal thymolysis is best seen in adrenalectomized mice. We were also able to distinguish, on the basis of the intensity of staining with toluidine blue, two cell types in the population of thymocytes in adrenalectomized infected mice. It is reasonable to advance a hypothesis that less intensely stained thymocytes, not present in intact infected mice, probably correspond to the corticosteroid-sensitive population of thymocytes in the classification of RAFF and CANTOR⁵. For a better functional definition of the less intensely stained thymocytes more experiments are needed. Thymuses of adrenalectomized mice show a tendency to recover at later experimental periods as shown by the presence of mitotic divisions.

Riassunto. Le alterazioni morfologiche del timo nel corso dell'infezione con virus PR8 sono più evidenti nei topi normali che nei topi adrenalectomizzati, i quali mostrano una differenziazione dei timociti in due classi sulla base dell'intensità di colorazione con bleu di toluidina.

E. GARACI, R. CALIÒ and W. DJACZENKO

*Institute of Microbiology, Chieti University,
I-66100 Chieti (Italy); and
Institute of Microbiology, Rome University,
I-00100 Roma (Italy), 8 October 1973.*

⁵ M. C. RAFF and H. CANTOR, in *Progress in Immunology* (Ed. B. AMOS; Academic Press, New York 1971), p. 83.

Induced Mutations in *Serratia marcescens* by Near UV-Light in Presence of Psoralen

Ultraviolet light of wave length ranging 253–265 nm results in a rapid inactivation of cells of micro-organisms and consequently yields a low percentage of mutants among survivors. The present study was aimed to find out the effect of near UV-light (NUV) of 350–360 nm wave length on the mutagenesis in *Serratia marcescens* (threonine less mutant). A photosensitizing chemical, 8-methoxy psoralen (MOP) was used to increase the effect of NUV, since at this wave length, irradiation alone was ineffective in cell inactivation (Figure).

Treatment of cells with MOP was done before near UV-irradiation. 8-methoxy psoralen (Manaderm, Jeffrey Manners Co. Ltd., India) was dissolved in absolute ethyl

alcohol to give 1 mg/ml solution. 1 ml of MOP solution was added to 9 ml of the cell suspension of *Serratia marcescens*(thre^o) grown in DAVIS¹ minimal medium. The cell suspension was left for 1/2 h, in dark for uptake of MOP. The cell suspensions treated with or without (MOP) were subjected to near UV-irradiation for different intervals of time in an open Petridish at a distance of 15 cm from a 125 W UV-lamp (Philips Model 57236 E 170 HPW, Holland). Mutations were scored for 1. reversions to threonine independence; 2. forward mutations to

¹ B. D. DAVIS and E. S. MINGIOLI, *J. Bact.* 60, 17 (1950).

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 independence 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.

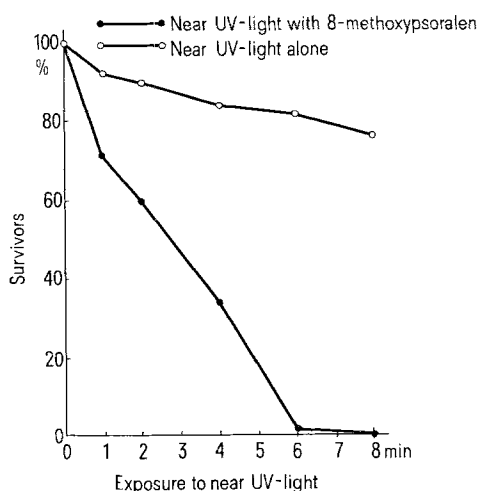
The results indicate the advantage of employing near UV in conjunction with a photosensitizing chemical which gave a considerable yield of auxotrophs and colour variants with only moderate killing of the cells. The beneficial role of the near UV in presence of photosensitizing chemical in the development of industrial strains of *Streptomyces* has already been demonstrated by TOWNSEND et al.² Earlier, ALDERSON and SCOTT³ and SCOTT and ALDERSON⁴ 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),
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Survival curves of *Serratia marcescens* (threonineless) after treatment with near UV-light in the presence and absence of 8-methoxypsoralen

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 manifesta-

tions, histological characteristics and treatment of this benign tumour has been discussed by several workers¹⁻⁵. In spite of numerous investigations on the subject, its pathogenesis still remains obscure. However, a viral