Einwirkung von *Fusarium solani* auf verschiedene Steroide

Eingesetzte Substanz (0,025% in Azeton oder Methanol zur Kultur gegeben)	Produkt nach Inkubation von Tagen		
	1	2	7
Progesteron (I)	III	III	«Oxyd. prod. 2»
11-Desoxy-corticosteron (II)	III	III	«Oxyd. prod. 2»
Δ^4-Androsten-3,17-dion (IV)	III	III	«Oxyd. prod. 2»
$\Delta^{1,4}$-Androstadien-3,17-dion (III)	III	III	«Oxyd. prod. 2»
Pregnenolon (V)	III + VI	III + 2 weitere Substanzen	«Oxyd. prod. 2» + 2 weitere Substanzen
Dehydro-isoandrosteron (VI)	III + 1 weitere Substanz	III + 2 weitere Substanzen	«Oxyd. prod. 2» + 2 weitere Substanzen
Allopregnan-3,20-dion (VIII)	X	X	III
3β-Azetoxy-allopregnan-20-on (IX)	X	X	III
Androstan-3,17-dion (X)	X	X	III
REICHSTEIN's Substanz S	S + 1 weitere Substanz	S + 1 weitere Substanz	2 unbekannte Substanzen
Cortison (Compound E)	E + 1 weitere Substanz	E + 1 weitere Substanz	E + 1 weitere Substanz

letztere, bisher noch verhältnismässig kostbare Substanz auf einem neuen, äusserst einfachen Wege gewonnen, der zweifelsohne technische Anwendung finden wird.

Im folgenden sei ein typischer Ansatz mit Progesteron beschrieben:

Die Nährlösung, enthaltend pro Liter Leitungswasser 15 g Pepton, 6 cm³ Maisquellwasser und 50 mg Rohglukose, wurde durch Zugabe von 2-n. Natronlauge auf pH 6,5 gebracht.

4 l dieser Nährlösung wurden gleichmässig in 13 Erlenmeyer-Kolben à 1 l verteilt, nach dem Sterilisieren mit Fusarium solani beimpft und bei 25° auf einer rotierenden Maschine geschüttelt (100 U./min). Nach 48 h gab man zu den gut entwickelten Kulturen unter sterilen Bedingungen in gleichen Anteilen eine Lösung von 1,0 g Progesteron in 45 cm³ Azeton zu, schüttelte noch weitere 48 h und filtrierte dann vom Myzel ab. Das Kulturfiltrat (pH 7,9) wurde erschöpfend mit frisch destilliertem Methylenchlorid ausgezogen, der Extrakt mit 0,1-n. Salzsäure, 1%ige Natriumhydrogenkarbonatlösung und Wasser gewaschen, getrocknet und eingedampft. Der teilweise kristalline Rückstand (1,14 g) wurde an einer Säule von 30 g Aluminiumoxyd chromatographiert und letztere jeweils 3–4mal mit je 0,1 Liter Petroläther-Benzol-Gemisch (2:3), Benzol, Äther und Azeton eluiert.

Die papierchromatographische Untersuchung[1] der einzelnen Fraktionen zeigte, dass mit den beiden ersten Elutionsmitteln 84% des Gesamtgewichtes an reiner Substanz erhalten wurde, die sich als etwas weniger polar als Desoxy-corticosteron erwies und mit Phosphorsäure im UV.[2] eine ganz schwache, dunkelblaue Fluoreszenz zeigte. Aus wenig Azeton mit Petroläther umkristallisiert erhielt man rhombische Platten, die bei 145–146° schmolzen; Mischprobe mit $\Delta^{1,4}$-Androstadien-3,17-dion ebenso. $[\alpha]_D^{23} = +110° \pm 3°$ (c = 1,060 in Chloroform). $C_{19}H_{24}O_2$ (284,38): Ber. C 80,24 H 8,51%; Gef. C. 80,32 H 8,54%. UV.-Spektrum: λmax. = 243 mμ; log ε = 4,21. E. VISCHER und A. WETTSTEIN

Forschungslaboratorien der Ciba Aktiengesellschaft, Basel, Pharmazeutische Abteilung, den 10. September 1953.

[1] R. NEHER und A. WETTSTEIN, Helv. chim. Acta *35*, 276 (1952). – System Propylenglykol-Toluol s. R. B. BURTON, A. ZAFFARONI und E. H. KEUTMANN, J. Biol. Chem. *188*, 763 (1951); System A und B_3 s. J. E. BUSH, Biochem. J. *50*, 370 (1952).

[2] R. NEHER und A. WETTSTEIN, Helv. chim. Acta *34*, 2278 (1951).

Summary

Certain fungi, especially of the genus *Fusarium*, are capable of rapidly degrading the side chain of 20-keto-pregnenes to the corresponding 17-ketones and, simultaneously, of dehydrogenating ring *A*. Thus $\Delta^{1,4}$-3,17-diketo-androstadiene is formed almost quantitatively from Δ^4-3,20-diketo-pregnenes and from Δ^4-3,17-diketo-androstene, and in lower yields from the analogous Δ^5-3β-hydroxy compounds. The side chain of saturated 3-keto- or 3β-hydroxy-allopregnane-20-ones is also degraded quickly, but dehydrogenation in ring *A* proceeds more slowly. 17α-hydroxy-groups prevent the degradation of the side chain.

By means of this microbiological process in combination with INHOFFEN's aromatisation reaction, progesterone can be transformed in only two steps into estrone.

Induction of Mutations in Bacteriophage T 3 by Ultra-Violet Light

It has been shown that mutations[1] in a temperate bacteriophage λ can be induced by ultraviolet (UV) irradiation of the phage *and* of the bacteria in which they shall multiply. It seemed interesting to see if the same method applied to a typically virulent phage produced the same results. T 3 was chosen because of its relatively high resistance[2] to UV irradiation, for fairly large doses are necessary to produce mutations. The mutations looked for were of the host range type[3] (T 3 h). The phages were irradiated, in buffer, with a dose of UV leaving a surviving fraction of $9 \cdot 10^{-6}$. They were plated as a control, on a mixture of two strains of *Escherichia Coli*: B and B/3α (a very weak B/3) and on the same mixture of bacteria having received a dose of UV (in buffer) six times smaller than that given to phages.

As in the case of the temperate phage λ, a reactivation by UV'd bacteria of the UV inactivated phages took place: when the same number of UV'd T 3 particles

[1] J. J. WEIGLE, Proc. Nat. Acad. Sci. *39*, 628 (1953).

[2] R. DULBECCO, Radiations and Biologie, Oakridge (in press).

[3] D. FRASER and R. DULBECCO, Cold Spring Harbor Symposium 1953.

were plated, for 100 plaques on non-irradiated bacteria, 600 plaques appeared on irradiated bacteria.

The control plaques showed that the stock of T 3 used contained two *h* mutants among 12000 h^+ particles. These *h* mutants give, on mixed B + B/3*a* indicator, plaques that are clear right from the beginning of their growth. Among the phages reactivated by UV'd bacteria, there were 61 *h* mutants among 30000 h^+ particles, a proportion twelve times larger than that of the control.

The plaques of *h* mutants observed on irradiated bacteria were not sectored. Some of these plaques were picked, when very young, and analyzed. Each plaque was found to be pure within the accuracy of our analysis (1%) but different plaques were due to *h* mutants differing their plaque morphology.

In addition to these *h* mutations, others were observed showing diverse plaque morphologies, the increase over the control being again of the order of 10.

We conclude that mutations in virulent bacteriophage can be induced by ultraviolet irradiation of the phages and of the bacteria in which they shall multiply. Thus the production of mutations by ultraviolet irradiation is not restricted to temperate, inducible bacteriophage.

We wish to express our thanks to Miss L. HUBER for her assistance during these investigations.

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Department of Biophysics, University of Geneva, August 1, 1953.

Résumé

Il est possible d'induire des mutations chez le bactériophage virulent T3. Il faut pour cela irradier avec des rayons ultraviolets *et* les bactériophages *et* les bactéries dans lesquelles ils se multiplieront. Les mutations dont on a mesuré la fréquence concernaient le spectre d'action des phages (T3h), mais on en a observé d'autres agissant sur la morphologie des plaques.

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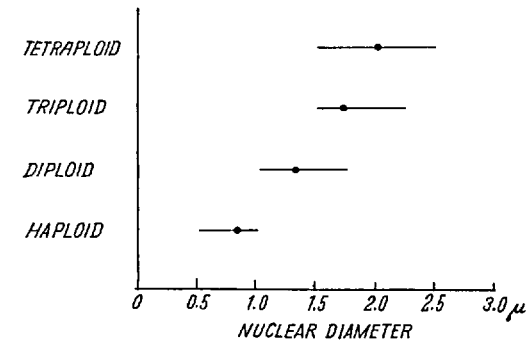
Interphase Nuclei and Cell Sizes in a Polyploid Series of Saccharomyces

Variations in cell dimensions and frequently in nuclear areas correlated with degrees of ploidy are well known cytological phenomena; they have been reported often in plants though their occurrence in higher animals is less common[1]. Similar information concerning the fungal interphase nucleus is lacking, owing mainly to the recentness of intensive genetical interest in the fungi. Undoubted polyploid yeast stocks recovered through genetical procedures have been reported, however, by ROMAN *et al.*[2] and by LINDEGREN and LINDEGREN[3]. These stocks were not investigated cytologically. On the other hand, even cytological claims[4] (unsupported by any genetical analyses) of the existence of an extensive haploid to hexaploid series of yeast provide no information regarding size differences, if any, among the interphase nuclei of these cells. Indeed, had such measurements been made the true dimensions of the nuclei would not have been accurately assessable, since chemical fixatives, such as those hitherto used in yeast cytology, yield appreciably shrunken preparations.

The clones of a polyploid series (kindly supplied by Dr. C. C. LINDEGREN) we now consider, are of single cell origins. Their haploid, diploid, triploid, and tetraploid constitutions have been adequately established by the LINDEGRENS with the use of 6 to 11 genetical markers. The present report concerns cytological preparations of these clones obtained with the ALTMANN-GERSH[1] technique of freezing and drying, of which the superiority over conventional chemical fixatives is universally acknowledged. Advantages of the method consist principally in that shrinkage is absent or is negligible; diffusion currents within the cell are eliminated; and morphological and cytochemical preservation of the fixed cell approximates the living state as closely as is possible with known fixing agents.



Cells for freezing-drying were harvested from fermenting cultures as well as from vigorously aerated liquid medium and frozen in isopentane cooled to – 155° C in liquid nitrogen. Dehydration in vacuum at – 30°C was followed by immersion of the cells in chilled absolute alcohol. Celloidin sections of the fixed material were cut such that their thicknesses only slightly exceeded the longer dimensions of cells of each degree of ploidy, thus ensuring the inclusion of whole as well as sectioned cells. The conventional FEULGEN methods ($HClO_4$ or HCl hydrolysis) as well as the RAFALKO[2] modification were used with or without precipitation of the DNA as the lanthanum salt. Adequately differentiated hematoxylin-pyronin preparations were also obtained. These preparations are characterized by excellent uniformity of staining with the nucleus being very clearly differentiated from the cytoplasm.

The interphase nucleus is defined here as that single, spherical or near-spherical, extra-vacuolar area showing a positive FEULGEN reaction. I have been unable to corroborate LINDEGREN's "nuclear vacuole" view[3], the polymorphic structures identified by him as chromosomes exhibiting, in my experience, neither a FEULGEN positive reaction nor increases in numbers consistent with ploidy, nor even an invariable presence in dividing cells stained with toluidine blue in fixed, or in fresh wet

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