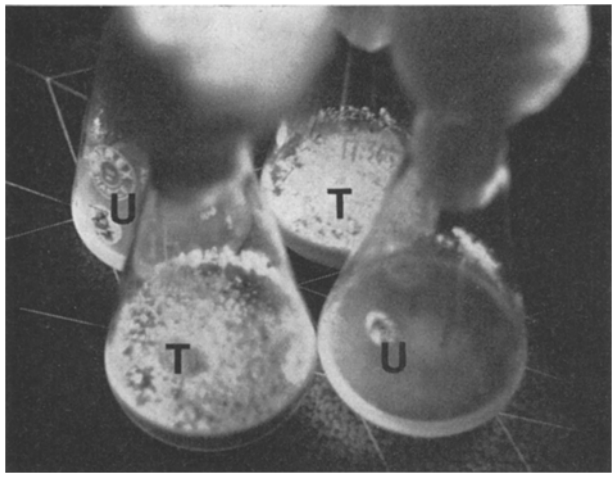
L'intérêt de l'effet de la thymine, c'est qu'elle exerce une forte action conidiogène sans accroissement préalable de la croissance. Cette conidiation ne contribuant que quelques mg supplémentaires de poids sec final (il en a été tenu compte dans les résultats) couvre l'entière surface du fin mycélium porteur; elle se réalise aux extrémités d'hyphes aériens très courts (Figure), ce qui doit correspondre à une économie de croissance si on la compare à celle du mutant sur sérine optimale, intervenant sur une couronne de longs et touffus hyphes aériens.

L'inactivité de l'uracile suggère que la thymine exerce un effet morphogène spécifique par la voie d'une synthèse accrue de l'ADN. Celle-ci ne peut toutefois s'exercer par incorporation directe (après conversion en D-thymidine) car *Neurospora* est dépourvu de thymidine kinase[7]. L'on peut donc penser que la thymine offerte est re-circuitée par déméthylation[8], et activité subséquente de la polynucléotide méthyltransférase[9].



Cultures du mutant sérine-1 de *Neurospora crassa* photographiées au 7ème jour de leur croissance, à 25 °C, sur milieu minimal WM liquide enrichi de thymine (T) $10^{-2}M$ ou d'uracile (U) $10^{-2}M$. Observer la surface entièrement conidiée sur thymine et les rares amas conidiens latéraux sur uracile.

Summary. Thymine, but not uracil, induces conidiation without supplementary growth, while suboptimal serine increases mycelial mass only, in the leaky serine-1 mutant of *N. crassa* grown aconidially in liquid minimal medium.

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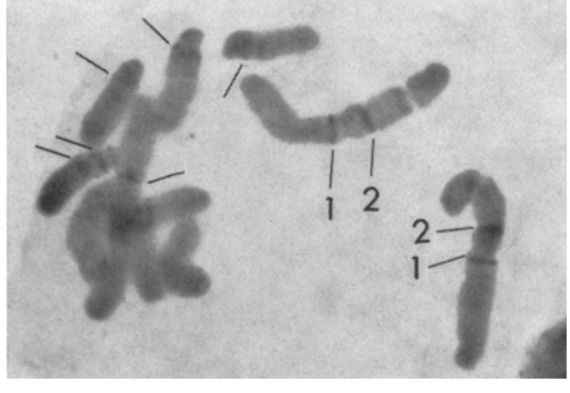
Visualization of Metaphase Heterochromatin in *Vicia faba* by the Denaturation-Renaturation Giemsa Staining Method

In *Vicia faba* specific regions of metaphase chromosomes are differentially stained by various methods[1–6]. These regions are considered to correspond to chromocenters in interphase nucleus and are called heterochromatic segments (H segment)[1,2]. Differential staining of heterochromatic and euchromatic segments produced by various methods is supposed to reflect the chemical differentiation along the length of metaphase chromosomes. Most of the H segments in *Vicia faba*, which are revealed by cold treatment[1–3] or treatment with HCl-acetic acid[3–5], appear as non- or pale-staining segments (–H segment), but the H segment adjacent immediately to the nucleolus organizing stalk of the M chromosome[2], which does not respond to cold treatment or treatment with HCl-acetic acid, becomes discernible as more darkly stained segment (+H segment) after treatment with trichloracetic acid followed by treatment with HCl-acetic acid[6]. The visualization of +H segment by the specific treatment suggests that detection of some other chromosome regions which behave like H segment, i.e. further unveiling of chemical differences along chromosomes, could be possible by using new techniques which produce differential staining of specific metaphase chromosome regions.



Metaphase chromosome complement stained with the denaturation-renaturation Giemsa staining method showing the +H bands indicated by arrows. Number 1 and 2 denote the No. 1 and No. 2 site of the –H segments of M chromosome.

It has been shown that, after various treatments which supposedly denature and anneal chromosomal DNA in cytological preparations, Giemsa staining preferentially stains centromeric heterochromatin and/or produces specific banding patterns in mammalian metaphase chromosomes[7–15]. We attempted to see whether the known H segments in *Vicia faba* metaphase chromosomes are differentially stained by the denaturation-renaturation Giemsa staining method and whether the specific chromosome regions which behave like H segment are newly found by this method.

Main root-tips of *Vicia faba* (Nagasaya Soramane) were excised and fixed for 1 h in ethanol-acetic acid (3:1) after pretreatment with 0.05% colchicine for 2 h. They were briefly rinsed in running tap-water and macerated for 2 h at 20°C in the enzyme solution which contained 5% macerozyme 4S and 5% cellulase 'Onozuka' 4S (both from Kinki Yakult Co.) and was adjusted to pH 5.5 with

2N HCl. The macerated root-tips were rinsed in running tap-water for 5 min, placed in a drop of 45% acetic acid on a slide, and then squashed under a coverslip. The coverslip was removed after freezing on dry ice and the slide was placed in running tap-water for 5–10 min, then air dried. Dried slides were placed in 0.01N NaOH at room temperature for 30 min and washed in running tap-water. They were then incubated in 6 × SSC (SSC:0.15*M* NaCl-0.015*M* sodium citrate) at 70°C overnight. After incubation the slides were rinsed in running tap-water and then stained in buffered Giemsa solution (Merck's Giemsa Lösung, 1 ml, to 50 ml of phosphate buffer pH 6.8) for 1 h. Cells detached from a slide during the above operation were few.

The present method brought about differential staining of metaphase chromosomes in *Vicia faba*. Metaphase chromosomes swelled slightly and specific regions of a chromosome were stained more darkly than the remainder of the chromosome (Figure). In the M chromosome the two densely stained bands were consistently and prominently observed. They corresponded to the –H segments at No. 1 and No. 2 site[3–5] which were revealed by cold or HCl-acetic acid treatment (Table). The –H segment at No. 3 site[3–5] of the M chromosome and those at the proximal region to centromere of the long arm of the S chromosome also appeared as densely stained bands but the former was not always visible. Centromeric regions of the chromosomes and both regions adjacent to the nucleolus organizing stalk of the M chromosome (one of which is distinguished as the +H segment after the treatment with TCA followed by treatment with HCl-acetic acid[6]) were occasionally observed as densely stained regions.

The results obtained showed that the present method differentially stains the known H segments as +H, and suggested that the centromeric regions and the region adjacent to the nucleolus organizing stalk possess H segment-like properties.

It has been believed that NaOH treatment and hot SSC incubation, which presumably denature and anneal chromosomal DNA, are a prerequisite for differential staining properties of the Giemsa stain and thus the differentially stained Giemsa positive segments represent the segment consisting of rapidly annealing, highly repetitive DNA[8–16]. The –H segments in *Vicia faba*, which are revealed by cold treatment, correspond to the strongly fluorescent regions induced by quinacrine mustard (QM)[17,18]. QM is supposed to bind specifically to guanine-rich regions of DNA[19]. Therefore, the Giemsa positive segments in *Vicia faba* might reflect at least differences resided in DNA itself. However, we have found that, though results were less reproducible, the differential staining patterns were obtained by the modified methods in which either NaOH treatment or SSC incubation step was omitted from the present method, and even by Giemsa staining alone when its staining period was short such as 10 min. The Giemsa stain following the treatment with 1N HCl at 60°C for 10 min did not produce the differential staining. The treatment with 1N HCl presumably effects denaturation of DNA performing fixation and hydrolysis simultaneously[20–22]. These findings do not favour the interpretation that the Giemsa positive segments in *Vicia faba* reflect differences in DNA. Since there have been some reports[23–25] suggesting that the differential Giemsa staining reflects differential patterns of DNA-protein association along the length of the chromosome, the Giemsa positive segments in *Vicia faba* may represent a difference in patterns of DNA-protein association[26,27].

Relationship between the positions of the 2 Giemsa positive bands which were always prominent on M chromosome and the –H segments at No. 1 and No. 2 site on M chromosome

Treatment	No. 1 site	No. 2 site
HCl-acetic acid	8.6[a] ± 1.8[b]	3.9 ± 1.0
Cold	8.7 ± 1.4	3.5 ± 0.5
Denat.-renat. Giemsa	8.8 ± 1.9	3.7 ± 0.9

[a] Mean ratio of 50 chromosomes. Ratio; length of the chromosome arm carrying the given site/length from the centromere to the given site.
[b] Standard deviation.

Zusammenfassung. Die Denaturierungs- und Renaturierungs-Giemsafärbungsmethode auf die Metaphasenchromosomen von *Vicia faba* angewandt ergab heterochromatische Chromosomensegmente, die sich nach Kältebehandlung oder HCl-Essigsäure Behandlung negativ heterochromatisch verhalten und sich als die stärker Giemsa-gefärbten Segmente erweisen.

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