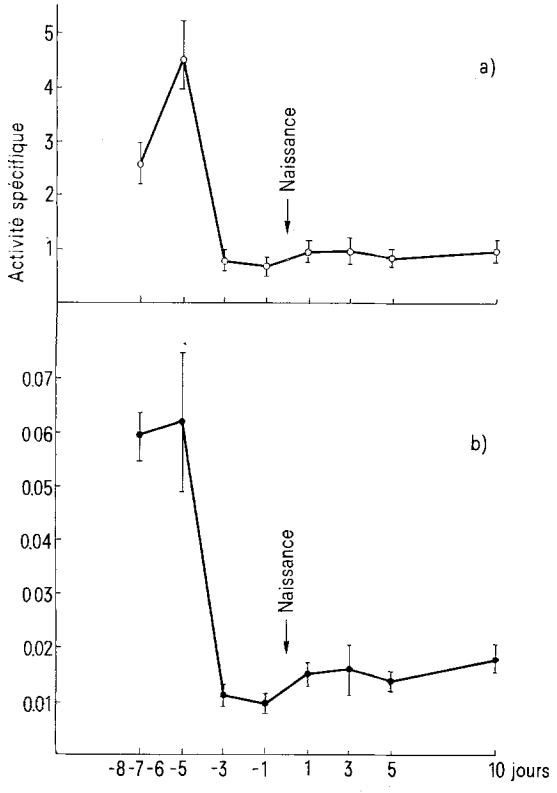

Fig. 2. Evolution de l'activité spécifique de la succinocytochrome-c-réductase dans les hémisphères cérébraux du rat du 15e jour de la gestation jusqu'à 10 jours de vie. a) μM de cytochrome-c réduit/min/g de tissu frais. b) μM de cytochrome-c réduit /min/mgde protéines.

POTTER[10] a fait l'étude du développement de la SCR et de la COX sur les hémisphères cérébraux du rat à partir du 19e jour de la gestation et jusqu'à 30 jours de vie post-partum. Ces enzymes présentaient une faible activité jusqu'à 10 jours de vie, puis augmentaient ensuite parallèlement.

Le fait que les activités de la SCR et de la COX du cerveau augmentent à partir du 55e jour de gestation chez le porc, du 41e jour chez le cobaye et à 10 jours de vie post-partum chez le rat, (dates correspondant à la maturation de l'organe pour les espèces considérées), amène GREENGARD[11] à conclure que l'activité des enzymes respiratoires est liée à la maturation fonctionnelle.

L'étude que nous avons effectuée in vitro sur l'évolution de la COX et de la SCR nous a conduit aux constatations suivantes: La COX, faible chez le foetus, s'élève légèrement après la naissance. Par contre l'activité de la SCR est très élevée entre le 15e et le 17e jour de la gestation, période qui, selon ALTMAN[12], correspond au maximum de multiplication des neurones et de synthèse protéique. L'existence d'une activité de la SCR durant la vie foetale laisse supposer que celle-ci n'est pas simplement liée avec la maturation fonctionnelle, mais est en rapport avec la multiplication cellulaire.

Les résultats obtenus indiquent une dissociation entre les activités de la COX et de la SCR. Nous avons été amenés à faire deux suppositions: soit la SCR existe sous deux formes, foetale et adulte ou bien il existe une chaîne respiratoire foetale possédant des propriétés différentes. Afin d'élucider ce problème, nous envisageons de faire une étude appronfondie de la SCR chez le rat durant la vie foetale.

Summary. The evolution of cytochrome c-oxidase and succinocytochrome c-reductase specific activities in rat brain before and after birth indicates that enzymic development could be related not only to functional maturation but also to the cellular multiplication.

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Autoconjugates in *Tetrahymena*

Studies on sexual reproduction and the genetics of ciliates demonstrated the existence of selfers in several species and mating types. Some information and much speculation on *Tetrahymena* selfers has been forthcoming in recent years. However, little is known of the occurrence of selfers during normal conjugation, or whether selfers occur within one or both mating types of a sexual reproduction.

Triads occur in low percentage during normal conjugation. This study deals with selfers occurring during conjugation (e.g. autoconjugates) and the origin of triads and their connection with autoconjugates.

Materials and methods. Mating types I (WH_6) and III (WH_{52}) of syngen I (obtained from the American Type Culture Collection) of *Tetrahymena pyriformis* were grown axenically according to the method described by ELLIOTT and HAYES[1] at 25 °C (± 1 °C). The organisms were removed to starvation medium by 3 successive washings followed by short centrifugations (1600 RPM for 1–3 min). The starvation medium was composed of 0.005 *M* tricine (Sigma) buffer, pH 7.4. Cell concentrations in starvation medium were adjusted to 100 Klett units (filter 54). Cell counts in the cultures during experiments varied between 1–10.10^6 cells/ml. The experiments were performed at 30 °C.

The mating types were mixed immediately after the beginning of starvation. In each experiment one mating type was grown with tritiated thymidine. 20 μC/ml of thymidine-6-H^3 (27 Ci/mmol, Amersham, England) was added 2 days before the cells were used in the experiments. Another portion of the same amount of triated thymidine was added 12 h before the experiments started. This treatment caused all the cells in the culture to be labelled significantly in their macronuclear DNA.

[1] A. M. ELLIOTT and R. E. HAYES, Biol. Bull *105*, 269 (1953).

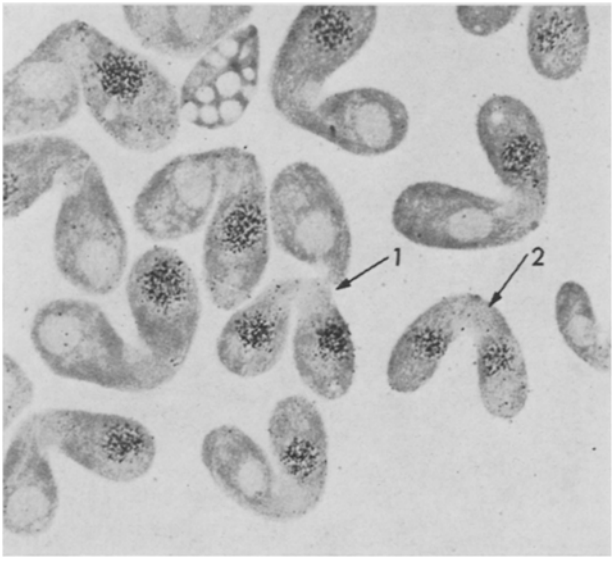
The labelled culture was washed three times before mixing with the second mating type. Each wash was followed by a short centrifugation. Radioactivity was assayed by radioautography. Drops containing cells were placed on subbed slides, air dried, exposed to alcohol-acetic acid fixative (3:1) for 10 min, extracted for 5 min in ice cold 5% TCA, rinsed for 5 min in 70% and 100% alcohol and washed for 1 min in acetone. The slides were dipped in a water-diluted (1:1) K_5 (Ilford) radioautographic emulsion, dried and stored in light-proof boxes for 5–10 days.

Radioautographs were developed in Kodak D-19 developer for 3 min and fixed for about 5 min. Development and fixation were done at 20°C. Developed slides were stained with toluidine blue (0.5% W/V) for 1–5 sec.

Results. In some experiments, macronuclear deoxyribonucleic acid (DNA) of mating type WH_{52} was prelabelled with tritiated thymidine and later mixed with an equal amount of non-labelled WH_6 mating type. Conversely, in other experiments, mating type WH_6 was prelabelled with tritiated thymidine and later mixed with an equal amount of non-labelled WH_{52} mating type. The prelabelled strain was always examined by radioautography and in all the experiments 100% of the pre-labelled cels were found to be labelled in their macronuclear DNA.

Thousands of conjugates were examined under the microscope. About 60 autoconjugates were recorded (Figure) of which both conjugates always belonged to mating type WH_{52}[2]. No autoconjugates, of which both conjugates belonged to mating type WH_6, were ever recorded.

Approximately the same number of triads were studied (Figure). In all the triads, 2 cells out of 3 belonged to WH_{52} mating type. Only in one triad, did 2 cells belong to WH_6 mating type. In another single triad all 3 cells belonged to mating type WH_{52}. Each of the mating types cultures was maintained under starvation separately for several days and examined periodically for selfers. Selfers were frequently recorded in mating type WH_{52}. Their appearance, however, varied considerably. Sometimes selfers appeared 5–6 h after the beginning of starvation and sometimes after 3–4 days from the beginning of starvation. Sometimes no selfers at all could be found. In mating type WH_6 selfers were never recorded.



Radioautogram of conjugating *Tetrahymena pyriformis*. Mating type III (WH_{52}) of variety I was prelabelled with tritiated thymidine during exponential growth. Before removed to starvation medium and mixed with mating-type I (WH_6) (non-labelled), the radioactive medium was removed by three successive washings. 1. A triad consisting of 2 organisms belonging to WH_{52} mating type and 1 organism belonging to WH_6 mating-type. 2. An autoconjugate. The 2 conjugates belong to mating-type WH_{52}. All other conjugates seen on the radioautogram are normal. The preparation was stained with 0.5% Toluidine Blue for 1 sec. ×650.

Discussion. ELLIOTT and HAYES[1] isolated 127 *Tetrahymena* clones at Woods Hole (WH), Massachusetts, where mating types I, II and III ($WH_{6,14,52}$), respectively, were established. Only 3, out of the 127 clones, turned out to be selfers[3]. Most probably, mating types I, II and III did not contain selfers.

The appearance of selfers in mating type III (WH_{52}) in our experiments could perhaps be a result of cultures retained over a longer period of time, as suggested by SONNEBORN[4]. This speculation is doubtful because the mating types of variety I were kept in our laboratory for only a few months. However, these mating types were not examined for selfers on their arrival. The tendency to mate within the clone could also be a sign of senility as a consequence of the appearance of amicronucleated cells, as suggested by ELLIOTT[3]. In general our mating type WH_{52} cells contained their micronuclei.

The method of ELLIOTT and HAYES[1] to rule out the possibility of selfing (named autoconjugates in this study) would possibly not detect the small number of autoconjugates. In their study ELLIOTT and HAYES mixed a small number of cells (15–20) of one mating type with a great many (500 or more) of the opposite mating type.

The main point of this study was to demonstrate the rare occurrence of autoconjugation during sexual reproduction of these organisms. This phenomenon was clearly shown with radioautography. Triads' appearance in the conjugating cultures is closely related to the tendency of selfing of one mating type. In control experiments of which mating types I (WH_6) and II (WH_{14}) were used, hardly any triads seemed to appear. A further step might perhaps demonstrate that the appearance of triads in a conjugating population of Tetrahymena is a sign of the existence of selfers and autoconjugates in one or both of the mating types[5].

Zusammenfassung. Autoradiographische Untersuchung der Autoconjugation während der normalen geschlechtlichen Fortpflanzung von *Tetrahymena*, wobei einer der beiden Paarungstypen mit ^3H-Thymidin markiert wurde (Markierung aller Macronuclei). Nach Mischung beider Paarungstypen fand sich neben sexueller Conjugation auch Autoconjugation von *Tetrahymena*, stets vom Paarungstyp WH_6.

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[4] T. M. SONNEBORN, Am. Ass. Adv. Sci., Washington, D.C. (1957), p. 155.
[5] I should like to acknowledge the valuable technical assistance of Mrs. PIRCHIA YAFFE and Miss SIMCHA URIELI.