This surface seems to show some unique biochemical characteristics which may be of functional significance.
More recently we have studied human lymphocytes stimulated in vitro with phytohaemagglutinin[9]. Again there is a considerable nett synthesis of plasma membrane: after 3 days, the average blast cell has more than 3 times the surface of the unstimulated lymphocyte. Unfortunately, we do not have any morphometric data on the characteristics of daughter lymphocytes in this model.

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Inhibition of fading in fluorescence microscopy of fixed cells

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Summary. Following Hirschfeld et al., dithionite was added to mounting media to inhibit fluorescence fading. Excellent response is reported for fluorescein, acridine orange, 33258 Hoechst, acriflavine, berberine (and ethidium bromide), but not for quinacrine.

The sensitivity advantage of fluorescence techniques is severely diminished by photochemical fading of the fluorescence, a process accelerated by intense excitation[2-6]. Fortunately, the once remote hope of slowing these processes by mounting the sample with a chemical fade retardant has been fulfilled[7]. The prescription of Hirschfeld et al.[7] is to mount cells stained with ethidium bromide in 0.01 M phosphate buffer at pH 7.2, containing 0.02 M sodium dithionite ($Na_2S_2O_4$).
Here, the effect of dithionite was tested on several other fluorochromes of importance. As the sample material was irrelevant to the case, we chose the inner cuticles from mature bulbs of onion. Only fixed samples were tested, since application to live cells involved the problem of their tolerance to 0.02 M dithionite. A compromise suitable for live cells or their constituents is not excluded, though.
The samples were fixed for 15 min in 3:1 ethanol-acetic acid, then stored in cold ethanol. After being brought to water in a graded ethanol series, the samples were stained for 10 min in 10^{-5} M fluorochrome solutions in 0.01 M phosphate buffer at pH 5.5. As an imitation to the labeling of antibodies by fluorescein conjugates, fluorescein was adsorbed to onion cuticle and buffered at pH 7.5. Rinsing and mounting was done either in buffer or, alternatively, in buffer containing 0.02–0.10 M of $Na_2S_2O_4$ (Merck). Coverslips were sealed with nail polish.
The microscope was Leitz Ortholux-II Fluorescence (Ploem)/Interference-contrast hybrid. A Nikon camera setup cast a real image onto a screen, from the center of which emerged a fibre optics light guide to a Hamamatsu R928 photo-multiplier, installed in a PAR Model 1140A Quantum photometer. A high pressure 200 W Hg bulb emitted the excitation beam, from which the Hg-line exciting the most intense fluorescence in each respective dye was selected by filters. In this microscope of incident illumination, the intensity Io of excitation increases as the square of the objective magnification. With our present set of filters and with $\times 100$ objective, Io at which nearly all our experiments were done was too intense for the needs of fluorescence microscopy. Switching to $\times 40$ objective lowered Io by $(40/100)^2 = 0.16$.
The figure shows representative fading curves, i.e., plots of fluorescence intensity vs time. With the exception of quina-

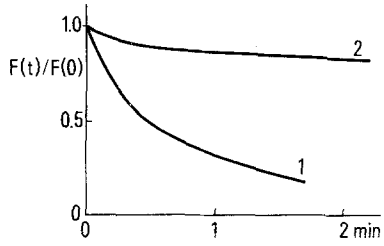
Fluorescence intensity (normalized) after 2 min of excitation by Hg line chosen for maximum fluorescence (conditions as in the figure)

Fluorochrome	F (2 min) / F(0) Buffer	$+Na_2S_2O_4$
Fluorescein[a]	0.67	1.00
Acridine orange	0.55	1.00
33258 Hoechst[b]	0.87	1.00
Acriflavine	0.22	0.90
Ethidium-Br[7]	0.27	0.87
Berberine	0.92	1.00
Quinacrine · 2 HCl	0.64	0.65
Rhodamine 3G[c]	0.54	0.62
ANS[11]	<0.1	<0.1
Quercetin-Al^{3+}[d]	(0.30)	(0.88)

[a] Adsorbed to onion skin and buffered at pH 7.5. [b] Courtesy of Dr H. Loewe, Farbwerke Hoechst, Frankfurt a. M. 80 (FRG). [c] Covalently bound to nuclei by Schiff-SO_2 reaction. [d] Excitation intensity $\times 0.16$ of that used for all other samples.

crine, the addition of dithionite did not change the observed intensity. Fading is accelerated by increasing excitation intensity Io, and as this is not specified, the figure and its like[2-6] are qualitative (Io-independent dimensionless parameters can be derived from the curves[8]). In the table, the fade retarding effect of $Na_2S_2O_4$ is reported for several fluorochromes.

The results are most encouraging for fluorescein, the mainstay of immunofluorescence, and for several cationic dyes of central importance to cytogenetics. With fluorescein and acridine orange, the intensity of fluorescence actually *increased* in time before assuming a slow downward trend ~ 5 min later. Quinacrine hydrochloride lost much of its fluorescence with dithionite, while the fading rate remained the same. This agrees with known properties of this dye[9].



Fluorescence intensity emerging from a nucleus of onion cell stained in 10^{-5} M acriflavine, placed in fluorescence microscope and excited at 405 nm with 200-W Hg-lamp by incident illumination through × 100 objective. 1: mounted in 0.01 M phosphate buffer, pH 5.5, 2: same buffer, containing 0.02 M $Na_2S_2O_4$. Absolute initial intensities were nearly equal for the 2 samples.

Rhodamine 3GO was covalently bound to DNA by the SO_2-Schiff reaction[10], also meant to imitate rhodamine-labeled antibodies. ANS, the probe of lipid-water boundaries[11] was not protected from fading. Quercetin resembles morin[12] in being a phenolic natural product which binds to a trivalent metal cation and the complex is a nuclear stain. It did not endure Io at × 100 magnification, but with Io reduced to 0.16 initial value, dithionite was definitely effective as fade retardant. Dithionite could not be substituted by sodium thiosulfate, sodium azide or propyl gallate.

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Contribution of germ-line cells to formation of the nurse chamber in egg follicles of nonpaedogenetic gall midges (Diptera, Cecidomyiidae)[1]

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Summary. One of the nuclei present in the syncytial nurse chamber in nonpaedogenetic gall midges contains lamellae characteristic only of the germ-line cells. This finding indicates that the nurse chamber in bisexually reproducing gall midges, as in the case of paedogenetic species, derives from both germ-line and somatic cells.

The egg follicle in a polytrophic insect ovary consists of an oocyte and nurse cells surrounded by a simple follicular epithelium. In typical cases, the oocyte and all nurse cells associated with it, are germinal in origin and they are joined together by a system of intercellular bridges. The oocyte-nurse cell complex is produced by a number of consecutive and synchronous divisions of an oogonial cell, frequently referred to as a cystoblast, and the cytoplasmic interconnections between all members of the complex are interpreted to arise as a result of incomplete cytokinesis[2]. Since the number of synchronous divisions which a cystoblast undergoes is species-specific, the number of nurse cells associated with the oocyte is also constant and characteristic of each species.

Although the ovaries of gall midges are polytrophic in structure, the process of formation of their egg follicles seems, however, to be essentially different from that in other polytrophic ovarioles. The cecidomyiid egg follicle contains a syncytial, multinuclear nurse chamber instead of a group of nurse cells, and the number of nuclei in nurse chambers varies considerably within each species. It has been suggested that nurse nuclei in egg follicles of Cecidomyiidae are of somatic origin and result from fusion of a variable number of mesodermal cells lying adjacent to the oocyte[3-6]. However, in paedogenetic gall midges, such as Heteropeza and Miastor, one of the nurse nuclei differs distinctly in size and morphology from the remaining nuclei found in the same nurse chamber, and it has been suggested that this nucleus is germinal in origin[7-9]. Decisive evidence in support of the view that the nurse chamber in paedogenetic gall midges derives from both germ-line and mesodermal cells has recently been obtained in developmental investigation[10], and also in ultrastructural studies[11].

In nonpaedogenetic gall midges belonging to the subfamily Cecidomyiinae, there has as yet been no observation indicating that the nurse chambers in their egg follicles contain descendants of germ cells. It has been assumed, therefore, that the nurse chamber in these gall midges is exclusively of somatic origin[6], although according to other authors it is composed exclusively of the germ-line derivatives[12]. The object of the present ultrastructural study was to clarify the origin of the nurse chambers in bisexually reproducing species of gall midges.

Material and methods. Investigations were carried out on the larval and early pupal ovaries of the following gall midges: *Boucheella artemisiae, Mayetiola poae, Mikiola fagi*