the failure to extrude Na^+ and to maintain a high internal K^+ concentration, would inhibit the conduction of nervous impulses. It is worthwhile to record that it was also found in some preliminary experiments *in vivo*, that rat brain lost K^+ and gained Na^+ and water after treatment with ultrasound. These results on the changes in permeability are also of interest in the treatment of Menières' disease where it is thought that there is a failure to maintain the ionic balance of the vestibular fluids. It is noteworthy that an effect on a biochemical process has been found in the absence of significant structural changes in the muscle. This supports the suggestion that "biochemical lesions" precede the histological changes observed in tissues some days or months after treatment with ultrasound[8].

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PN 1269

A flow chamber for the differential microfluorimeter of CHANCE and LEGALLAIS. Preliminary work with glass-grown ascites cells

Microfluorimetric studies[1,2] have revealed a close relationship between the blue fluorescence of mitochondria in living cells and their pyridine nucleotide content. Coverslip preparations afforded only limited possibilities of biochemical investigation in the microfluorimeter, since the cells were confined in a closed environment which could not be modified once the preparation was sealed. This necessitated the adaptation of a flow chamber for the microfluorimeter, using for biological material glass-grown cells as suggested by CHANCE[3].

The difficulties met with in the use of the microfluorimeter for work with a flow chamber were largely optical, since it was not possible to focus the short working distance darkfield condenser of the microfluorimeter through even a thin layer of

medium. Focusing and light intensity were therefore improved by replacing the Bausch and Lomb cardioid condenser of the microfluorimeter with a Cooke darkfield condenser, the adjustable aperture of which was set at 0.75. It was further necessary to reduce the depth of the flow chamber in order to limit the thickness of the layer of medium. The required minimum depth of the chamber was obtained by the use of a "sandwich-type" flow chamber[4], which was especially adapted for use with the microfluorimeter. The details of the design, which were worked out by Mr. V. LEGALLAIS, of this laboratory, on the basis of a model obtained from Dr. L. F. JAFFE, were as follows: the side of the flow chamber (Fig. 1) facing the condenser of the microfluorimeter was made of a No. 2, 48 × 60-mm Thomas Red Label coverslip with inlet and outlet orifices. The depth of the chamber was limited by the thickness of a nylon separator (0.2 mm). The side of the flow chamber facing the objective of the microfluorimeter was made of a No. 2, 48 × 60-mm Thomas Red Label coverslip on which tissue culture hyperdiploid ascites cells (EL2 cells) were grown adhering to glass.

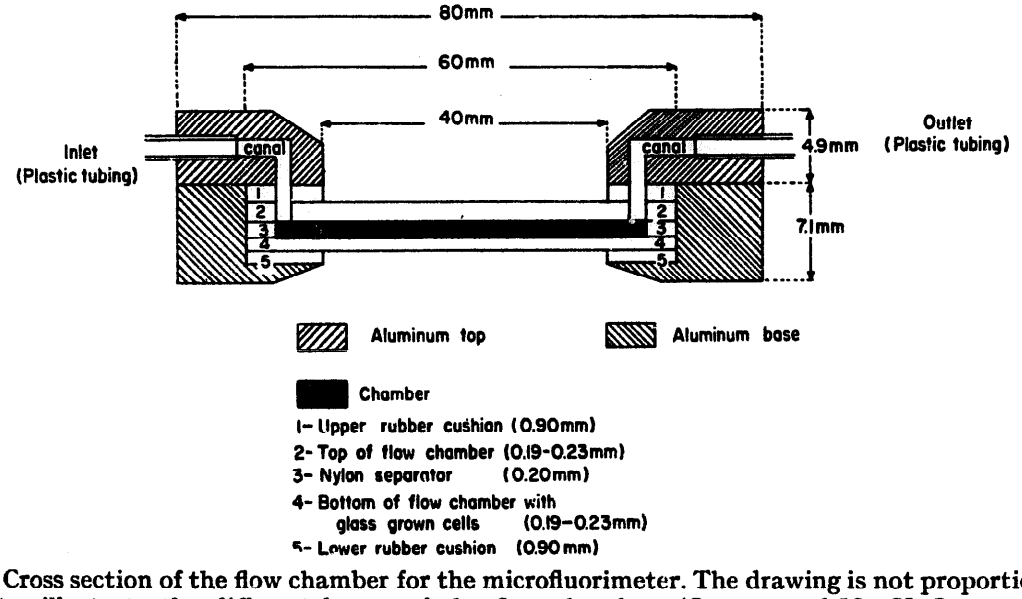

Fig. 1. Cross section of the flow chamber for the microfluorimeter. The drawing is not proportional to better illustrate the different layers of the flow chamber. (Courtesy of Mr. V. LEGALLAIS, Johnson Foundation.)

Fresh medium (Krebs Ringer bicarbonate buffer: 70, distilled water: 30, 0.5% glutathione and 0.5% albumin) was circulated through the system with gentle suction, using a 10-ml syringe. The thickness of the nylon separator was found to be critical. When it was reduced to much below 200 μ most of the glass-grown cells were washed off; but with a 200-μ-thick nylon spacer, only loosely adherent cells were removed. Most of the morphologically healthy cells did not show the slightest displacement, even after prolonged suction. This allowed fluorometric determinations to be made on the same cell after successive changes in the environmental conditions.

The incubation of glass-grown EL2 cells in a stationary medium for a prolonged period resulted in an increase in fluorescence which may be explained by a reduction of pyridine nucleotide, due to lowering of the oxygen tension of the medium by the respiratory activity of the cells. With 10–15 glass-grown EL2 cells per oil-immersion field, the fluorescence reached a maximum after 15–30 min. Then suction of about 10 ml of fresh medium through the system was sufficient to induce a return to the

low fluorescent state. Under the anaerobic conditions there was a very close correlation between cytological detail and intracellular distribution of fluorescence, regions of high mitochondrial density corresponding to regions of maximum fluorescence. With a single Corning CS 7-60 filter in the path of the exciting ultraviolet irradiation, the primary photo-current obtained from regions of high mitochondrial density in anaerobiosis was in the range of $1.0 \cdot 10^{-16}$ A or over. The signal-to-noise ratio was over 20:1 for a primary photo-current of $1.0 \cdot 10^{-16}$ A. In regions of high mitochondrial density, fluorescence in aerobiosis was below 40 % of the anaerobic value.

Addition of 2–10 mM glucose (Fig. 2) to an aerobic preparation of glass-grown ascites cells resulted in a increase of fluorescence to about 60–100 % of the anaerobic value. A further rise in fluorescence was observed when sufficient time was allowed for the preparation to reach anaerobiosis. It was not possible to detect in the microfluorimeter the initial transient phase of oxidation described by CHANCE AND HESS[5] for suspensions of ascites cells. This is understandable in view of the duration of the transient phase (less than 50 sec), since after glucose-containing medium was circulated through the system, time was needed to refocus the cell under observation,

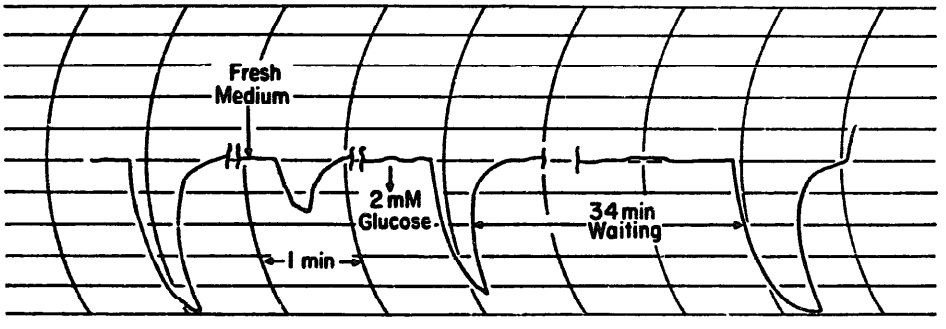

Fig. 2. Microfluorimetric recording of the fluorescence changes, in a high-mitochondrial-density region (3 μ in diameter) of an glass-grown ascites cell, during an experiment with glucose in the flow chamber. The time scale proceeds from left to right, and increase in fluorescence is indicated as a downward deflection. The results in here are representative of 25 other similar experiments. The interval between two horizontal lines corresponds to a primary photo-current of about $0.4 \cdot 10^{-16}$ A. The initial peak of fluorescence was obtained after incubation for 30 min in a stationary medium (anaerobiosis). Suction of fresh medium (aerobiosis) resulted in decreased fluorescence. 2 mM glucose induced an increase of fluorescence. After waiting for several minutes, there was an additional increase of fluorescence, probably due to onset of anaerobiosis. (Experiment EK154).

to shift aside the tungsten lamp of the microfluorimeter and to recenter the mercury arc lamp.

Addition of 5.5 mM Amytal resulted in a sharp increase of mitochondrial fluorescence to about 70% of the anaerobic value. During a subsequent 30-min period, in the presence of Amytal, there was a further increase of fluorescence, which was possibly due to the onset of anaerobiosis[6].

Thus, it appears that with the system described here, it is possible to observe in single cells some of the effects of substrates and drugs, that have been observed by CHANCE and others *in vivo*[6] or in cell suspensions[5] and tissue slices[6].

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Light-induced changes of α-tocopherylquinone in spinach chloroplasts

The presence of both α-tocopherol and α-tocopherylquinone in chloroplasts from spinach (*Spinacea oleracea*) and lilac (*Syringa vulgaris*)[1,2] implies that these compounds may function as a redox system in photosynthetic electron transport. In order to investigate this possibility, we have studied the effect of light and certain electron acceptors on the steady-state levels of α-tocopherol and α-tocopherylquinone in spinach chloroplasts. We have not yet found significant changes in the levels of α-tocopherol, but the level of quinone undergoes significant changes.

In order to study these changes, chloroplasts prepared by the method of JAGENDORF AND AVRON[3] are extracted with 80 % acetone after exposure to the experimental conditions. The lipids from the acetone extract are then chromatographed on thin layers of silica gel G using 1 % ethyl ethyl ether in chloroform for development. The region of the chromatogram containing α-tocopherylquinone is scraped off and eluted with ethanol. The change in absorbance of this solution at 262 mμ when treated with potassium borohydride shows the amount of quinone present. A similar procedure has been used to determine changes in plastoquinone A plus B after they are eluted from a different portion of the chromatogram[4]. In order to determine the recovery of total tocopherylquinone, the reduced form (presumed to be the hydroquinone) is converted to quinone by heating a sample of the lipid in isooctane to 60° and bubbling air through the solution for 2 min. This treatment will convert all hydroquinone to quinone, but does not oxidize α-tocopherol to a significant extent.

TABLE I

α-TOCOPHERYLQUINONE IN CHLOROPLASTS

Reaction mixture: 60 mg chlorophyll in fresh chloroplasts, 0.05 M Tris–HCl (pH 8.0), 0.01 M NaCl. Total volume 200 ml in a 2000-ml erlenmeyer flask. Light intensity 1600 ft candles from a 250-W Tungsten bulb at the bottom of the flask shining through 5 cm of water. 7.5 μmoles NADP added as indicated.

Treatment	*α-Tocopherylquinone (no oxidation) (mg)*	*α-Tocopherylquinone (oxidized extract) (mg)*	*Plastoquinones A + B (no oxidation) (mg)*
5 min in the dark	0.025	0.050	1.6
5 min in the light	0.056	0.048	2.2
5 min in the light + NADP	0.015	0.083	1.2
5 min in the light + NADP followed by 5 min in the dark	0.040	0.048	1.5