The results obtained agree with the data reported in the literature using other methods[13,15,16]. The absence of Hex A, responsible for Tay-Sachs disease, and the severe dificiency of both A and B isoenzymes in Sandhoff disease has been clearly demonstrated in our patients. The reduced percentage of Hex A, less than 45%, is the hall-mark of Tay-Sachs carriers[10]; in our obligate heterozygotes the A isoenzyme percentage agreed well with the above value. Lowden et al.[15] stated that the detection of Sandhoff carriers requires the concomitant presence of low Hex total activity and reduced Hex B percentage. Our findings in leukocytes of obligate Sandhoff heterozygotes support these criteria.

In conclusion, by chromatofocusing coupled with automated assay it has been possible to obtain good, rapid and reliable separations of leukocyte hexosaminidase isoenzymes. This technique can be usefully employed for the diagnosis of GM_2 gangliosidosis; the identification of Tay-Sachs and Sandhoff heterozygotes was also possible, but the utilization of the method for this purpose require further confirmation on a larger number of subjects.

In this study leukocytes were chosen, since they represent the best source of enzyme, and the differences in Hex activity and isoenzyme pattern between controls, heterozygotes and GM_2 patients are clearly evident. For the application of these techniques in a large screening program it would be important to establish whether or not more easily available sources, such as tears and serum, could be used.

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An improved flow-through chamber for time-lapse film analysis of oogenesis and embryogenesis[1]

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Summary. A simple flow-through chamber for time-lapse film analysis of developing organisms has been constructed. The medium is replaced efficiently and uniformly in the chamber which is, therefore, ideally suited for studying the effect of various drugs on development.
Key words. Time-lapse film; flow-through chamber; oogenesis; embryogenesis; insects.

Time-lapse film analysis has been used as a tool to study embryogenesis and oogenesis in a number of insects including *Drosophila*[2,3]. For long-term film studies the design of the flow-through chamber is of importance if cultivation artifacts are to be avoided. For example, when closing the chamber, hydrostatic pressure may build up due to capillary forces in the inlet and outlet connections, and during the experiment uneven flow in the chamber or insufficient replacement of culture medium may lead to local oxygen depletion or local heating of the medium by the microscope lamp. The design of our film chamber minimizes these potential artifacts. We are particularly interested in being able to replace the culture medium fast and efficiently by a test solution (for example culture medium containing various inhibitors) whose effect on normal development can then be studied in time-lapse films. The design of the chamber is illustrated in figures 1 and 2. The two hatched parts are made of plexiglass. If sterile conditions are essential, the chamber can be washed in 0.1% diethyl pyrocarbonate[4] or exposed to UV, and the medium is pumped through a sterilizing micropore filter before entering the chamber.

The main advantages of the chamber are:

1. The distance between slide and coverslip can be varied by screwing the lid until the desired distance is obtained and the object is immobilized between slide and coverslip.
2. Trapped air bubbles anywhere in the chamber can be removed by a vent. By turning the lid the vent opening in the chamber is moved to the position of the bubble, the vent is opened and the air bubble removed.
3. When closing the chamber lid little pressure builds up inside the chamber. Multiple inlet and outlet openings (fig.3a: position of inlet openings indicated by colored medium) give a large cross-sectional area and hence capillary forces are minimized.
4. Due to the large number of inlet and outlet openings the culture medium streams fast and rather evenly through the chamber. Replacement of water with trypan blue-colored water in the chamber illustrates this point (fig.3a–d). If the object to be studied is placed in a central position between inlet and outlet openings any drugs to be tested reach the object within 60 s using a moderate flow-rate of 0.37 ml/min (fig.3). Small concentration differences in the chamber equilibrate within a few minutes.

The chamber has only two movable parts and is easy to use. When closing the chamber displacement of eggs or follicles can be prevented by coating the slide with polylysine. To obtain a constant flow-rate a motor-driven 50 ml syringe (Unita I, Braun Melsungen) has been used rather than a peristaltic pump. The film chamber described above has been employed successfully in analyzing the effects of various drugs on cytoplasmic streaming in vitellogenic follicles of *Drosophila*.

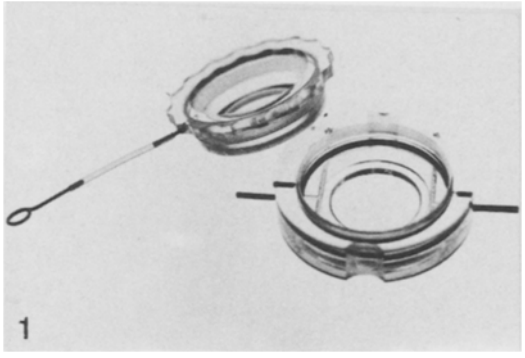

Figure 1. Photograph of the chamber (lid opened).

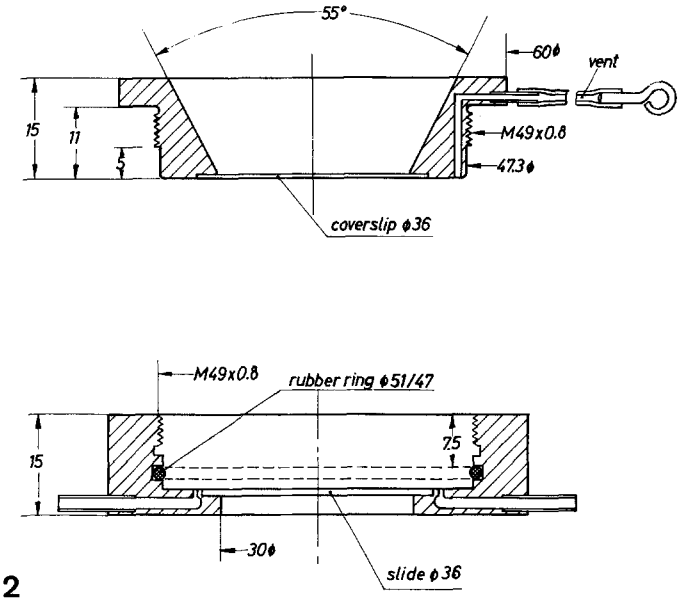

Figure 2. Drawing of the chamber viewed from the side. Dimensions given in mm.

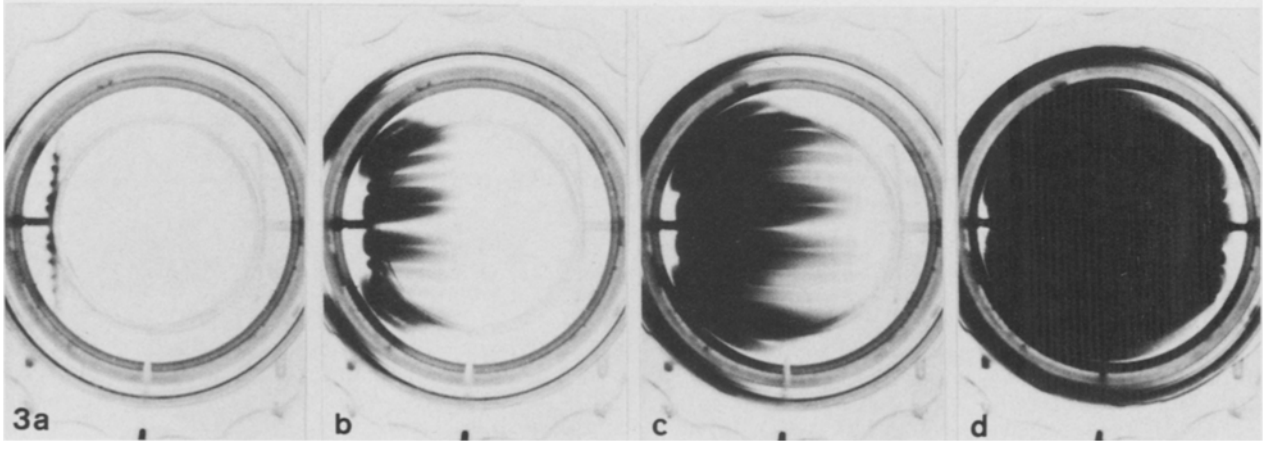

Figure 3. Kinetics of medium replacement (water replaced by trypan blue) a: 0 s (start), b: 45 s, c: 90 s, d: 180 s. Flow rate: 0.37 ml/min.

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Announcements

Italy

2nd meeting of the European neuroendocrine association (E.N.E.A.)

Milan, 15–17 October 1985

The scientific program will include a symposium on 'Hypophysiotropic peptides in basic and clinical neuroendocrinology', round tables, oral communications and posters.
Further information by: Dr Eugenio E. Müller, Department of Pharmacology, School of Medicine, University of Milan, Via Vanvitelli 32, I-20129 Milano, Italy.

The Netherlands

14th international summer school of brain research on 'Aging of the brain and senile dementia'

Amsterdam, 26–30 August 1985

For information please contact: Mrs W. Chen-Pelt, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam ZO, The Netherlands.