Heating of the contents of the ampoule has to be avoided. After 30 min of exposure to the combined freezing media at room temperature, the ampoule is inserted into a 'biological freezer'[7] and cooled at a rate of 1 °C/min. 3 h later the ampoule is transferred briefly into a dry ice-acetone bath and there attached to an aluminum holder which has been cooled in advance, in order to avoid warming up of the ampoule. Finally the aluminum holder is transferred into a liquid nitrogen tank for storage.

For thawing, the ampoule holder is put directly into a 37 °C water bath. It is recommended to use a face mask for this purpose, since ampoules which have not been sealed properly can explode. Immediately after thawing, the ampoule is opened and the contents is poured into a 1 ml glass depression slide. Usually, at least 10 of the ovaries are recovered and injected directly into genetically marked (*e mwh*)[8] female host larvae of the middle third instar (80 h after egg deposition, at 25 °C). There is no need to wash the ovaries prior to injection since the freezing medium is not toxic. After metamorphosis the host fly can be examined for the presence of the donor ovary. An additional ovary of normal size is found in 60% of the host females, in 20% the additional ovary is reduced in size, and in another 20% it is absent.

By mating the host females to genetically marked (*e mwh*) males, it can be shown that the donor ovary establishes contact with a host oviduct in slightly fewer than $^2/_3$ of the cases, which would be expected if the donor ovaries were to attach at random. The donor ovary produces on the average only 20% offspring as compared to the host ovary, but the donor offspring is viable and fertile. In the Table the data for a series of typical experiments are listed. The reduced number of offspring is also observed when control ovaries, which have not been frozen, are transplanted, indicating that it is the transplantation rather than the freezing technique which reduces the number of offspring. It may be advantageous to use mutants lacking ovaries as hosts in order to reduce competition between the implanted and the host ovaries.

The production of viable and fertile offspring by the ovary which has been frozen, thawed and transplanted allows us to use this method for storing mutant stocks. How long the ovaries can be stored in liquid nitrogen without damage, remains to be seen. A detailed report of this work and its possible application to the freezing of testis will be published later[2].

Zusammenfassung. Eine Methode zum Gefrieren lebender Ovarien von *Drosophila melanogaster*-Larven wird beschrieben. Diese Gefrierkonservierung ist geeignet, Mutantenstämme aufzubewahren.

W. Brüschweiler[9,10] and W. Gehring[10]

Department of Anatomy, Yale University, New Haven (Connecticut 06510, USA), 25 July 1972.

[7] 'Biological Freezer' BF-6, Union Carbide Comp.

[8] D. Lindsley and E. Grell, *Genetic variations of Drosophila melanogaster* (Carnegie Inst. Wash. Publ. No. 627, 1968).

[9] Present address. Zoological Institute, University of Zürich, Künstlergasse 16, CH-8006 Zürich, Switzerland.

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A Simplified Silver Impregnation Method

The methods of Nauta and Gygax[1] and Fink and Heimer[2] for the silver impregnation of anterograde degeneration have been a great advance in the research of the central nervous system. The technical processes involved in these methods, however, require a lot of time. Besides, we have proved that at times they do not give good results in brains which have been kept in formaldehyde for a long time.

Silver impregnation methods in general give variable results[3]. This is due to the large number of physical and chemical factors involved[4]. However, some data must be taken into consideration: a) According to Jacobson[5], sensitivity to the impregnation increases if potassium permanganate is left out in the pretreatment. b) Auto-oxidation phenomena are produced in tissues which have been kept a long time in formaldehyde, and hence it is innecessary to use potassium permanganate[6,7]. c) The silver impregnation methods can be further simplified by omitting the initial reaction of the tissues with aqueous silver[8].

Bearing in mind these data, and hoping to find a simplified method which can also be used for brains kept for a long time in formaldehyde, we carried out several tests, and arrived at the following method:

1. Distilled water. 2. 2'5% uranyl nitrate, 5 min. 3. Distilled water. 4. Ammoniacal silver: 30 ml of 2.5% silver nitrate, 1–1.5 ml of concentrated ammonium hydroxide, 1.8 ml of 2.5% sodium hydroxide, 15–20 min. 5. Reducer: 800 ml of distilled water, 90 ml of 100% ethyl alcohol, 27 ml of 1% citric acid, 27 ml of 10% formalin, briefly. 6. Reducer: the same reducer, briefly. 7. Distilled water. 8. 1% sodium thiosulfate, 2 min 9. Distilled water.

By using 0.025% potassium permanganate and 1% hydroquinone, either independently or both at the same time, the pretreatment has been shortened. We have observed, in accordance with Nauta and Gygax[1], that suppresion is produced when potassium permanganate is used (Figure 1). When used on its own, hydroquinone-oxalic acid seems to increase both the number of fibres impregnated and the intensity of the impregnation. Since hydroquinone-oxalic acid produces reduction, it is sufficient to use it alone in the pretreatment when the auto-oxidation phenomena are prolonged. In such cases, leaving out permangante and hydroquinone-oxalic acid gives a good result (Figure 2). Otherwise, it is necessary to use either potassium permanganate and hydroquinone-oxalic acid together in adequate proportions, or potassium permanganate alone.

As suggested by Eager[8], we obtained better results by using uranyl nitrate in the pretreatment. It also seems

[1] W. J. H. Nauta and P. A. Gygax, Stain Tech. *29*, 91 (1954).

[2] R. P. Fink and L. Heimer, Brain Res. *4*, 369 (1967).

[3] F. Walberg, Brain Res. *31*, 47 (1971).

[4] L. Heimer and R. Ekholm, Experientia *23*, 237 (1967).

[5] S. Jacobsen, Stain Tech. *38*, 275 (1963).

[6] R. P. Eager and R. J. Barnett, Anat. Rec. *148*, 368 (1964).

[7] R. P. Eager and R. J. Barnett, J. comp. Neurol. *126*, 487 (1966).

[8] R. P. Eager, Brain Res. *22*, 137 (1970).

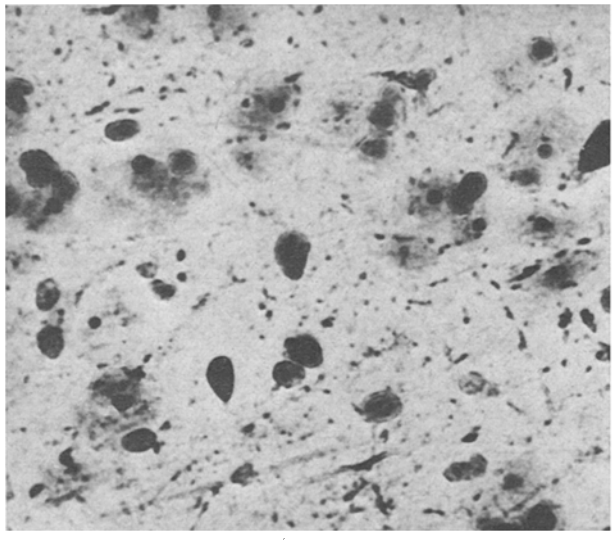

Fig. 1. Cat G-560.10 months fixation. Cortical lesion. Pretreatment: 0.025% potassium permanganate, 5 min; 2.5% uranyl nitrate, 5 min; no hydroquinone-oxalic acid.

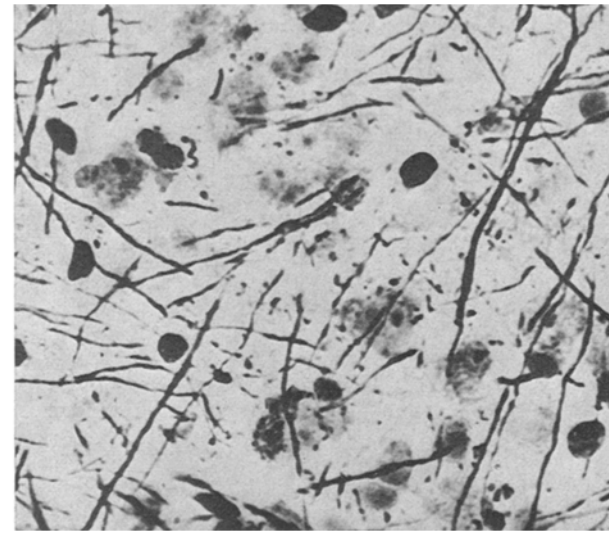

Fig. 2. The same animal as in Figure 1. Pretreatment: 2.5% uranyl nitrate, 5 min; no permanganate, no hydroquinone-oxalic acid.

that, in agreement with the same author[8], the deposition of silver does not depend on an initial reaction of the tissues with aqueous silver. The results improve when alcohol is eliminated from the composition of ammoniacal silver.

Resumen. Método sencillo de impregnación argéntica para degeneración walleriana. Buenos resultados en cerebros guardados mucho tiempo en formol. Se discute la acción del permanganato y la hidroquinona-oxálico como tratamiento previo. Se omite reacción con plata acuosa y se elimina el alcohol de la plata amoniacal.

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CONGRESSUS

Switzerland
8th EUCHEM Conference on Stereochemistry

at Bürgenstock, near Lucerne, 29 April–5 May 1973.

Inquiries and applications (no special forms are required) should be addressed before January 15, 1973 to the Chairman: Prof. R. H. Martin, Département de Chimie Organique, Université Libre de Bruxelles, 50, Avenue F. D. Roosevelt, B–1050 Bruxelles (Belgique).

Israel
1st International Congress for Bacteriology

in Jerusalem, 2–7 September 1973.

This will be the first international congress of the newly formed Bacteriology Section of the International Association of Microbiological Societies.
Further information about the congress may be obtained from the Congress Secretariat, P.O. Box 16271, Tel Aviv, Israel.

ACTUALITAS

International Cell Research Organization (ICRO)

1. *Training Courses.* One of the main activities of ICRO is the organization of training courses on topics of high novelty and on modern techniques in cellular and molecular biology: Principles and techniques of tissue and organ culture; Genetics and Physiology of Bacterial viruses; Energy transducing systems on the sub-cellular level; Methods in mammalian cytogenetics; Membrane Biophysics; DNA-RNA Hybridization; Biogenesis of Mitochondria; Embryology and Epigenetics; Interaction between Animal Viruses and host cells, application of computers to experimental work in biology and chemistry; Methods in molecular biology, etc. The courses generally last 3–5 weeks, and include 16–20 young participants (sometimes more). The ICRO courses are fully international, both the teaching staff and the participants coming from the largest possible number of countries.

2. *The Problem of Developing Countries.* Most of the past ICRO courses have been organizing in European countries – east and west – but the demand from developing countries is increasing steadily. ICRO activities in developing countries may tend to give preference to topics of potential economic usefulness, such as applied microbiology, microbial protein production, fermentation industries, soil microbiology, plant genetics, etc.

Inquiries for more information should be addressed to: Dr. Adam Kepes, International Cell Research Organization, c/o Unesco – AVS, Place de Fontenoy, 75 Paris 7e, France.