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Instituut voor Toepassing van Atoomenergie in de Landbouw - ITAL

STUDY OF THE INCORPORATION OF URIDINE-^{3}H IN NEURONS BY VARIOUS AUTORADIOGRAPHIC METHODS

by

M. SENSENBRENNER*, E. WITTENDORP** and R. RECHENMANN**

*CNRS, Strasbourg (France)
**ITAL, Wageningen (Netherlands)

1971

Association No. 076-69-1 BIAN

Paper presented at
the VIIth International Colloquium
on Corpuscular Photography and Visual Solid Detectors
Barcelona (Spain) - July 1970

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The sections were covered with a layer of nuclear emulsion (Gevaert Nuc. 7.15 or Ilford K5). After drying by temperature gradient, the plates were exposed for 1 to 4 days as required. After activation with gold, the autoradiographs were developed in amidol or ferrous oxalate for suitable periods giving the best signal/noise ratio.

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KEYWORDS

URIDINE
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IN VITRO
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CHICKEN
INCUBATION
NUCLEAR EMULSIONS
GOLD
BIOCHEMICAL REACTION KINETICS
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C O N T E N T S *)

*) Manuscript received on March 19, 1971

STUDY OF THE INCORPORATION OF URIDINE-^{3}H IN NEURONS BY VARIOUS AUTORADIOGRAPHIC METHODS*

M. SENSENBRENNER**
Centre de Neurochimie du CNRS, Strasbourg (France)
and
E. WITTENDORP and R. RECHENMANN
Association Euratom-ITAL, Wageningen (Netherlands)

A. INTRODUCTION

In a previous work (1), Sensenbrenner and Mandel studied by autoradiography the incorporation of a labelled precursor of RNA in cerebral hemisphere, nerve and glial cells during the course of differentiation, in the chick embryo. Judes and Jacob (2) analysed the biosynthesis of the various types of RNA's during this differentiation by incubating fragments of cerebral hemispheres in vitro. The development of high-resolution and high-efficiency ionographic processes (3,4) led us to resume the autoradiographic study of uridine incorporation in chick embryo brain neurons.

B. COMPARATIVE AUTORADIOGRAPHIC STUDY

A comparative preliminary study was made of the various possibilities offered by commercial nuclear emulsions in conjunction with various methods of development.

Three emulsions which are commonly used in autoradiography were chosen: Ilford K5, Gevaert NUC 7.15 and Kodak AR 10 stripping film. Development was made in amidol or ferrous oxalate (with sodium sulphite added), with or without gold activation. Previous studies of the development process have shown that gold activation of the latent image, after exposure and before development, considerably increased the response of nuclear emulsions, without a significant increase in fog (4,5).

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In addition, graphs of optical density as a function of development time with or without previous gold activation, obtained by a method previously described (4,5), dictated the optimum developing time (6). Gold activation results in a remarkable increase of the signal/noise ratio, the number of developed grains ('signal') being strongly increased, and the fog ('noise') remaining practically constant.

The following results were achieved:

1) the detection of very small quantities of radioelements which cannot be detected by the usual autoradiographic methods, even when forced development is employed.

2) a significant reduction in exposure time.

3) the possibility of obtaining virtually fog-free autoradiographs, if the photographic factors described (4,5) are carefully observed.

Systematic experiments described in this work have demonstrated that for this biological study, the combination of Gevaert NUC 7.15 and a ferrous oxalate developer with added sodium sulphite (7) is the most favourable. Results obtained using the stripping film (AR 10), even after gold activation, were not as satisfactory as those obtained with the emulsions K5 and NUC 7.15.

C. MATERIAL AND METHODS

1. Biological material.

Cerebral hemispheres of 14 day-old chick embryos were cut into small pieces of about 1 mm^3 and incubated in an atmosphere of 95-5 CO_2 in a slightly modified Eagle's medium (2), containing uridine-5-^3H (specific activity 10 Ci/mmole) in doses of 10 or 50 µCi/ml. After 30 min and one hour of incorporation, the fragments were fixed in Carnoy solution. Following dehydration and embedding in cytoparaffin, serial sections (5 µ thick) were prepared for autoradiography.

2. Autoradiography

A layer of nuclear emulsion (15 μm ± 2 μm thick) was poured onto the preparations (for details see Table 1). After drying by temperature gradient (8), the plates were exposed at +5°C in a lead castle (8) for periods varying from 1 to 4 days. After 20 min gold treatment and 10 min washing in double distilled water, the preparations were developed, either for 30 min in amidol or for 30 or 75 min in ferrous oxalate, and then fixed and washed. During all these procedures the photographic baths were kept at 15°C ± 0.2°C (8).

Table I

Emulsion	K5 - L4	NUC 7.15
Emulsion/water dilution	1:1	3:7
Melting time	10 min	10 min
Melting temperature	40°C	37°C
Drying: temperature gradient	15°C	15°C

Some preparations (AR 10 and NUC 7.15) were stained with a toluidine blue solution at 0.1 %. N.B.: the K5 emulsion does not lend itself to this staining method.

D. RESULTS

The autoradiographs showed the normally expected localization of the tritium incorporated in the RNA's within the nerve and glial cells. After 30 min of incubation, without intensification a fairly high concentration of grains was present in the nucleus, but there was no evidence of labelling in the cytoplasm. Only after one hour of incubation a few grains appeared in the cytoplasm. On the other hand, after activated development, radioactivity was revealed in the cytoplasm within 30 min (Fig. 1), even with the low dose of tritiated uridine used (10 μCi/ml). After one hour's incorporation, the amount of radioactivity became appreciable (Fig. 2).

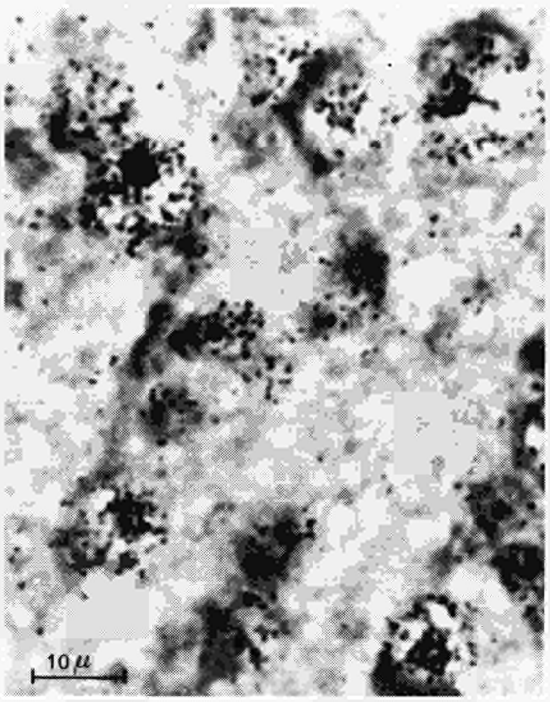

Fig. 1

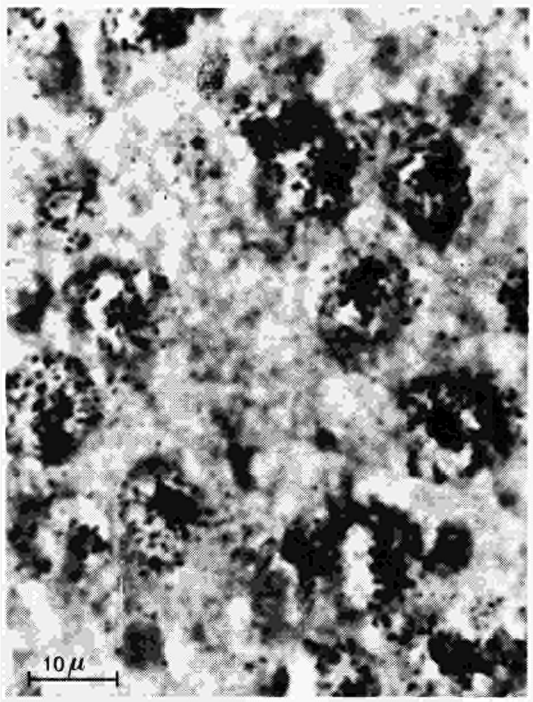

Fig. 2

E. DISCUSSION

It was demonstrated that with gold intensified nuclear emulsions there was an increase in the quantity and quality of biological information which could be obtained.

The results obtained by conventional autoradiographic methods, namely the presence of labelled cytoplasmic RNA after one hour of incorporation, agree with other work on the incorporation of tritiated uridine in neurons (9). On the other hand, the passage of RNA's newly synthesized in the nucleus into the cytoplasm was detectable after 30 min of incorporation when using gold activation. These results are highly significant because the amount of fog present in our preparations was negligible.

In conclusion, the work shows the value of using optimum-yield autoradiographic methods in biology. Experiments designed to show the presence of labelled RNA in the cytoplasm at even earlier stages are in progress.

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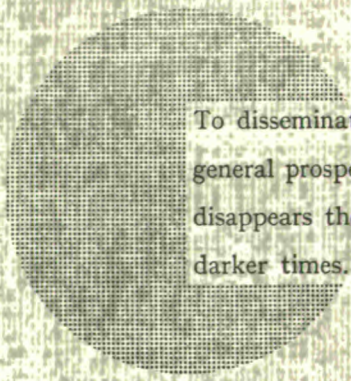
DISCUSSION

BRAUN - Pourquoi la préparation de vos autoradiogrammes requière-t-elle les précautions spéciales que vous avez citées?

WITTENDORP - Dans le but d'obtenir des autoradiogrammes reproductibles et pratiquement sans voile, le séchage doit se faire d'une façon aussi homogène et reproductible que possible. La vitesse du séchage (qui dépend du gradient de température) doit être choisie soigneusement pour éviter la formation de grains spontanément développables, due probablement à la contraction de la gélatine.



To disseminate knowledge is to disseminate prosperity — I mean general prosperity and not individual riches — and with prosperity disappears the greater part of the evil which is our heritage from darker times.

Alfred Nobel

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