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Instituut voor Toepassing van Atoomenergie in de Landbouw - ITAL

PROGRESS IN PROCESSING THE CORPUSCULAR LATENT IMAGE

Prospects for Micro-Autoradiography

by

R. V. RECHENMANN

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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Homogeneous activation of the emulsion layers was obtained by treatment with a gold solution before development in amidol or other chemical developers.

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The results show that :

a) the increase in "fog" is generally negligible ;
b) the development rate is considerably increased, mainly at short development times ;
c) even after long immersion in the developer, grain density remains higher in activated emulsions.

Furthermore it also appears that :

d) the activation effect is more important for less sensitive emulsions ;
e) the activation effect is directly related to the average energy of the β-spectra emitted by the isotopes used in these experiments.

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KEYWORDS

NUCLEAR EMULSIONS
PHOTOGRAPHIC FILM
ELECTRON BEAMS
ALPHA BEAMS
LATENT IMAGE
DEVELOPING AGENTS
GOLD
PARTICLE TRACKS
RADIOGRAPHY
RADIOAUTOGRAPHY

CONTENTS

PROGRESS IN PROCESSING THE CORPUSCULAR LATENT IMAGE

Prospects for Micro-autoradiography *)

R.V. Rechenmann

A. Introduction

The use of nuclear emulsions in corpuscular physics, medicine and mineralogy depends upon the quantity and quality of information obtained by this method.

Much progress has been made at the fabrication stage, finally resulting in the production of ionographic emulsions which are highly sensitive, stable and characterised by a low chemical fog level.

In addition, particularly in corpuscular physics, investigators have sought to increase the efficiency of these emulsions, either by hypersensitizing (e.g., triethanolamine (1,2)) or by intensifying the latent image (3.4). Most of these procedures, however, have been of little practical use due to many inherent disadvantages, such as non-reproducibility (5), prohibitive increase of fog, poor light penetration in the case of latensification by post-exposure (6) and results which often vary from one emulsion to another.

As regards to autoradiographic applications, and particularly in biology, the photographic processes are usually disregarded in favour of other problems related to these methods.

The quantities of radioelements to be detected by microautoradiographic methods are frequently only slightly higher than the background; furthermore, observation under the optical or electron

*) Manuscript received on March 30, 1971

microscope requires a high number of developed grains especially at the origin of the electron trajectories. In the application of nuclear emulsions to biology and medicine it is thus essential to develop ionographic methods to the highest degree of perfection.

A description will be given of a treatment of ionographic emulsions which makes it possible to increase their efficiency, sometimes spectacularly, without increasing the chemical fog.

The results and application of this procedure to the sensitive nuclear layers after exposure to charged particles will be discussed as well as the improvements thus attained to autoradiographic methods. An example of an application to biology is also given.

B. Theoretical Considerations and Basic Hypotheses

It is now generally admitted that the latent image is formed by silver aggregates of different sizes, distributed mainly near the AgBr-Gelatine interface (7,8,9,10,11). Some of these germs would be too inactive to make a microcrystal developable, even by strong developers. This hypothesis of a latent 'sub-image', proposed by Webb and Evans (12), has been subsequently considered by many authors (13,14,15,16,17,18,19).

With nuclear emulsions, which have a low reciprocity failure at short exposure times, there might be a particularly high probability of creating a sub-image. This should be the case especially for electrons, where the mean energy loss per grain is relatively low, corresponding to a greater probability of production of sub-germs. The electrons are of particular interest in biology and medicine, where the radioactive isotopes employed are in most cases β-emitters. The hypothesis of this author is that after the passage of a charged particle, the latent image formed in the microcrystal consists partly of sub-image specks. This 'sub-image' is <u>stable</u> and not developable, even by a strong, solvent developer and <u>even for developing</u>

times corresponding to the maximum of the density curves. If this hypothesis is correct a substantial increase in photographic efficiency should result from an intensification of the sub-germs which permits them to cross the threshold of developability*.

C. Activation of the Corpuscular Latent Image

As early as 1915 Lüppo-Cramer published the results of latensification experiments with hydrogen peroxide (22). In 1945 Sheppard, Vanselow and Quirk (23) proposed methods of intensifying the latent image, i.e., chemical or physical processes following exposure, and before development, whereby the probability of developing microcrystals having a latent image could be increased. Numerous results concerning the same subject have since been published. Among others, James et al. (24,25) used low-intensity light, mercury, gold, metabisulphite and perborate as latensification agents. They found that intensification results in an increase of the kinetics at the beginning of development, but does not increase the number of grains developed if the immersion time in the developer is prolonged. It will herein be described that this last finding is not valid when the latent image is activated with gold in the usual ionographic emulsions (see also Fig. 1).

In 1952 Zuber (4) and Grenishin (3) latensified nuclear emulsions exposed to electrons by post-exposure to low-intensity actinic light, working on the hypothesis of a corpuscular latent 'sub-image'. Schmitt et al. (6) studied the influence of the wavelength of the post-exposure and found a continuity between the latensification and the Herschel effect.

Unfortunately the action of low-intensity actinic light on the

*At the present stage of this study no conclusion can be drawn as to the sizes or levels of the sub-germs (see 12,18,20,21).

corpuscular latent image has only a limited practical interest for investigators using nuclear emulsions, due to the fact that it always results in an increase of fog, which is particularly pronounced when it is desired to develop fully the sensitive layers exposed to low-ionizing radiations (particles at the minimum of ionization or electrons). Furthermore, since the light is stopped by the first layers of AgBr grains, it is impossible, using this procedure, to obtain a deep and uniform action over the entire volume subjected to treatment (6). This mode of intensification could be considered only for ionographic layers destined for observation under the electron microscope, where the thickness is usually less than one micron.

On the other hand, gold-intensification combined with a forced chemical development is a simple and highly reproducible procedure which permits a homogeneous action throughout the emulsion. The mode of operation will be described in the following chapters, together with some experimental results confirming the working hypothesis.

D. Experimental Results and First Interpretations

Nuclear plates were subjected to various types of actinic radiations: light flash ($2 \cdot 10^{-8}$ sec), β-particles emitted by ^{3}H, ^{14}C, ^{45}Ca and ^{32}P sources, and α-particles of ^{210}Po.

The time between exposure and activation was kept to within four hours; preliminary tests had shown that within this limit the evolution of the sub-image is smaller than the experimental error.

After exposure a series of plates was immersed for 20 minutes in double-distilled water, and another series in a gold solution prepared according to a formula similar to that given by James (16). Experiments have shown that after 20 min the increase in density due to prolonged activation could be neglected (Fig. 2). After washing, the two sets of plates were treated with a chemical developer for periods of time ranging between 1 min and 4 h (Amidol, ID19, ID19+Hypo, solution of ferro-oxalate with added Na_2SO_3, superficial pyrocatechine developer).

The emulsions were then immersed in a stop-bath, fixed, carefully washed and dried at controlled speed (26). All these baths were maintained at $15^o \pm 0.2^oC$. The global optical densities were measured with a Baldwin densitometer. The curves plotted from these measurements are similar to the graphs resulting from a grain count, as verification tests have shown. Furthermore, the reproducibility of the results was found to be excellent, the curves obtained with identical experiments being perfectly superimposed within the measurement errors.

Whatever the type of emulsion activated or the type of irradiation used, and whatever the chemical developer (with the exception of non-solvent ferro-oxalate), the gold solution always has the following effects:

(a) there is a considerable increase in the kinetics at the beginning of development.

(b) a substantial and sometimes spectacular increase in the number of grains developed, is also obtained.

These two points are immediately interpretable: either by the deposition of gold on the silver specks constituting the latent image, or by replacement of this silver by the gold, or possibly by a combination of these two mechanisms (16,27,28), the electron levels of the germs are always lowered, and hence the probability of development of the microcrystals is increased (29,30). Similarly gold activation partly or entirely transforms the sub-images into centres of development. A series of curves giving the density in function of the development time in a ferro-oxalate* developer confirms this interpretation (Fig. 3). The threshold potential of developability of the activated grains is considerably lower (in absolute value). The intensification, given by the ratio D_a/D_i (density of the activated plates/density without activation), diminishes if the redox potential increases (in absolute value) (Fig. 4), i.e., when the developer becomes less discriminating.

(c) the intensification varies with the type of radiation used (Fig. 5). It is the strongest when a light flash 2.10^{-8} sec. is used; hence corresponding to the highest production of sub-germs. As could be expected, it is the weakest in the case of α-particles, where the energy release per grain is relatively high. It will be seen below that the method even does distinguish between the mean energies of continuous β spectra (Section D, III).

(d) the chemical fog is remarkable stable. The stabilizing influence of gold on the fog is a known phenomenon. The transformation of Ag_2S sensitivity specks into gold sulfide is the mechanism most frequently suggested (31,32); the fogging action of the gold baths would therefore apply only to the sensitivity centres obtained by reduction. It appears that in our experiments the two phenomena counterbalance each other.
With prolonged developments even inversion of the fog was occasionally observed after three hours.

We wish to emphasize that the proposed activation method does not produce any noticeable increase in fog during long development times, provided that strict precautions are taken in treating the

*The ferro-oxalate developer was usable only after the addition of sodium sulphite; otherwise a sharp increase in fog was observed on the intensified areas. The sulphite appears to act here as a stabilizer.

sensitive layers at the various stages. For example, if the technique is applied to emulsions in 'gel' form, the drying rate after pouring must be carefully controlled. The manipulations have to be performed in near complete darkness, since activation increases the sensitivity to light usually considered inactinic.

Apart from these observations, which are valid for all the activation studies given in this publication, there will briefly be described herein some specific effects related to the sensitivity of emulsions, the grain size, the energy of the β particles and the action of gold on alpha particle tracks.

I. Variations in the efficiency of activation with the sensitivity of the emulsions

Ilford K5 and K2 plates with grains of identical mean diameters were exposed to a ^{14}C source, activated and developed in amidol. From the curves in Fig. 6 and 9b it will be seen that the intensification is much greater in the K2 emulsion, which is the less sensitive. These results agree with the observations of Grenishin (3), but contradict his assertion that the germs produced in a hypersensitive emulsion cannot be latensified.

According to our curves for development kinetics, the sub germs/germs ratio is greater in the K2, as more grains are 'activated' there.

II. Intensification effect and grainsize

Due to the fact that the average energy lost per microcrystal by an ionizing particle is a function of the grain diameter, the probability of creation of sub-germs increases for smaller AgBr grains. The intensification due to activation is indeed more marked with ultrafine grain emulsions (Fig. 7, 8), where the increase in sensitivity may attain some several hundreds of %. Gold-intensification is thus of direct value for emulsions destined for observation under the electron microscope (26).

III. Variation of the intensification effect with the average energy of spectra of β particles

In as much as the mean energy deposited in a microcrystal by an electron is a decreasing function of the particle's energy, the sub-germs/germs ratio increases with the energy of the electrons*. The experimental results obtained demonstrate that:

1. in the short development time region (Cabannes-Hoffman effect), the shape of the curves is different for the three β-emitting isotopes considered (Fig. 9).
2. for an immersion time in the developer of over 60 min, the activation effect increases with the mean energy of the β spectra (Table I).

Isotope	development time (min) 60	75	90	105	120	
^{3}H	1.44	1.39	1.35	1.33	1.29	D_a/D_i
^{14}C	1.50	1.44	1.41	1.39	1.38	
^{45}Ca	1.66	1.52	1.47	1.44	1.43	
^{32}P	1.80	1.74	1.68	1.63	1.56	

Table I

IV. Alpha particles

If the emulsions exposed to alpha particles of ^{210}Po are intensified, the following facts are noted:

1. despite the relatively high loss of energy per grain (about 25

*Only the range of energies lower than those corresponding to the minimum of ionization is concerned. All of the mean energies of the β-spectra emitted by the isotopes currently used in autoradiography are, indeed, in this region.

keV for alphas of 5 MeV and grains of 0.2 μm), the activation results in a substantial increase in density, even with very sensitive emulsions such as K5 (Fig. 10)*.

2. as in the case of the electrons, the intensification is more marked if the emulsions are less sensitive (Fig. 11).
3. the effect of the gold treatment is particularly marked in ultrafine grain ionographic media (Fig. 12).

As can be seen on Fig. 13 and 14, representing electron micrographs of alpha tracks in Nuc 3.07 and L4 emulsions, the activation results in a reduction of the number and length of gaps (Fig. 13b and 14b).

E. Interest for Autoradiography

This process is applicable to all the autoradiographic methods, whether they be stripping-film, track-autoradiography or electron-microscopic autoradiography. Two examples will be given below of the application of activated development which demonstrate that it opens new perspectives to investigators using ionographic emulsions in biological studies (33,34,35).

1. Two successive serial sections of an oat leaf containing incorporated ^{45}Ca were mounted on different slides. A thickness of 20 ± 2 μm of K5 emulsion was then poured on the preparations. The plates were exposed for 48 hours. One of the preparations was given a forced development with amidol (Fig. 15a), while the other was subjected to activated treatment (Fig. 15b).

   The observable difference in the number of grains developed corresponds to the increase in efficiency obtained by activation.

2. The microcrystals crossed by a particle at the beginning of its path will have a higher sub-specks/specks ratio than at the end.

*Further research on this point is in progress.

The intensification will therefore be more marked at the beginning. The track-autoradiography, the resolution of which depends on the exact location of the penetration of ionizing particles in the sensitive layer, will thus have an optimum resolving power after intensification. The microphotographies in Fig. 16 and 17 are a highly enlarged representation of a section of an oat leaf containing incorporated ^{45}Ca. Activated track-autoradiography has permitted unambiguous localization of the ^{45}Ca in the chloroplasts 'in situ'.

Other advantages of the gold activation include the following:

(a) the possibility of obtaining <u>preparations which are practically fog free</u> (Fig. 16, 17) even after a forced development.

(b) the enormous increase in efficiency with emulsions of the Lippmann type, suggesting that in the near future it may be possible to use currently ionographic media with microcrystals of 100-300 Å diameter in electron microscopic autoradiography.

(c) the possibility of detecting very low specific activities, or an important reduction of the exposure times.

F. Conclusions

The studies described in this paper have been made in connection with the development of autoradiographic methods of high efficiency and resolution for immediate application to biological problems. This work on the improvement of the response of ionographic emulsions has led to the formulation of simple and reproducible techniques directly applicable in practice.

We wish to emphasize that the effects mentioned are remarkably constant and can probably be extrapolated to nuclear emulsions and to particles which have not been mentioned in this paper. We have in mind particularly nuclear or cosmic applications, where gold-activation will certainly be of interest, especially in connection with particles at the minimum of ionization.

In addition, the whole of the experiments described in this study confirms the hypothesis of a stable corpuscular latent sub-image created in ionographic emulsions by ionizing particles and by short-light exposures.

Lastly, these results constitute a step towards the development of an optimum developing process for ultra-fine AgBr grains, which is indispensable for the observation of emulsions under the electron microscope.

Acknowledgements

This study on the development of the corpuscular latent image forms part of a research on ionographic methods carried out in our laboratory by Miss E. Wittendorp and Mr. J. Oortwijn.

The biological studies mentioned in this paper were conducted in collaboration with Dr. A. Ringoet.

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Discussion

DEMERS - Ces résultats sont remarquables. C'est la première fois que je vois un traitement entre l'irradiation et le développement présentant un tel pouvoir discriminant entre les traces et le voile, et variable d'ailleurs selon le pouvoir ionisant des traces.

1) Quelle est la solution employée?

2) Quelle est l'épaisseur?

En effet, que ce soit pour les émulsions Montréal, ou pour les autres émulsions (référence Voyvodic), la qualité (rapport densité de la trace/voile) est beaucoup meilleure pour les couches minces.

RECHENMANN - 1) Nous avons utilisé la solution préparée par James (voir Réf. 24). 2) La méthode d'activation a été appliquée à des couches ayant des épaisseurs comprises entre 0,1 µm et 100 µm. Nous espérons pouvoir l'utiliser pour des couches sensibles épaisses (600 µm par exemple) employées pour la détection de particules de haute énergie, en collaboration avec le groupe de Strasbourg.

NICOLAE - Les résultats obtenus dans ce travail semblent très intéressants pour leurs applications. Comment s'expliquer le fait que les sous-germes d'images sont activés, renforcés, mais pas les germes latents de voile?

RECHENMANN - Nous supposons que les centres de sensibilité du type Ag_2S sont transformés en sulfure d'or, moins susceptibles de rendre des grains développables (voir travaux de Basset et Dickinson - voir Réf. 32 - et de Faelens et Tavernier - voir Réf. 31). Au contraire, les centres voilants obtenus par réduction sont probablement activés; les deux effets semblent s'équilibrer, sauf dans certains cas, où des développements prolongés au-delà de deux heures résultent, soit en un léger accroissement du voile chimique, soit en une diminution de ce voile après intensification, suivant les cas (émulsions, révélateurs etc.)

CASANOVA - Pouvez-vous me dire si les émulsions sont sensibilisées à l'or par le fabricant?

RECHENMANN - Il est fort probable que la plupart des émulsions nucléaires ont été soumises à une présensibilisation à l'or, comme semble d'ailleurs l'indiquer le fading peu accusé des émulsions nucléaires commerciales récentes.

BASTIN - Concerne les électrons de faible énergie et la variation de l'effet avec l'énergie de la particule incidente.

RECHENMANN - L'Activation augmente la réponse des émulsions même pour des particules très ionisantes, comme les électrons émis par le tritium ou les particules α.

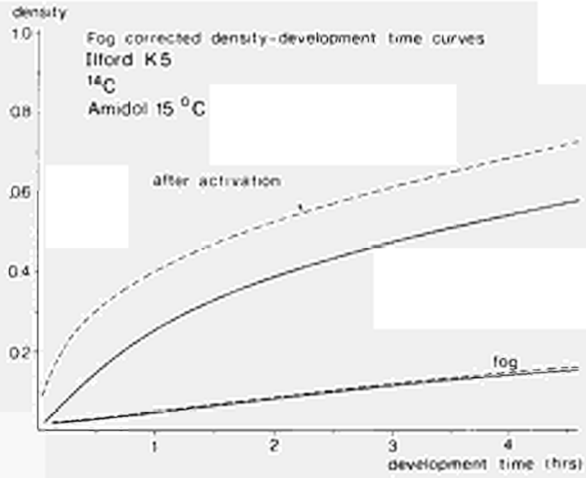


Fig. 1

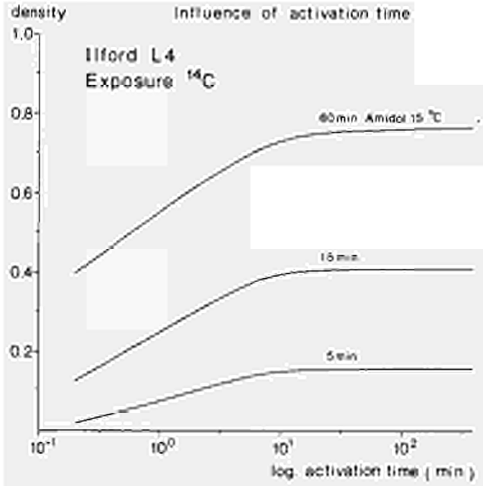


Fig. 2

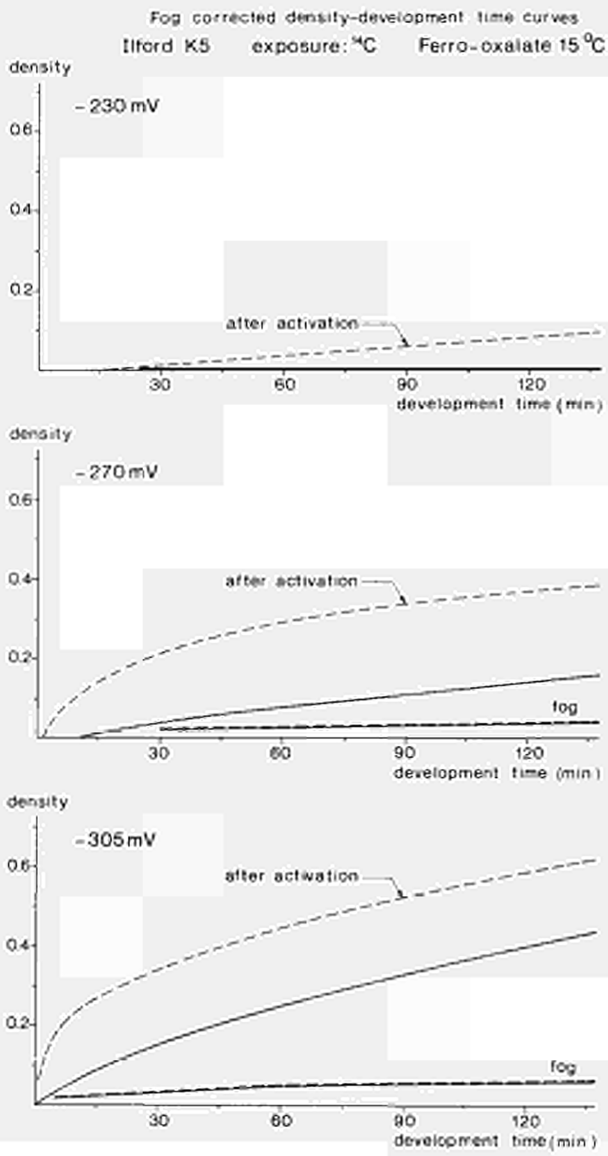


Fig. 3

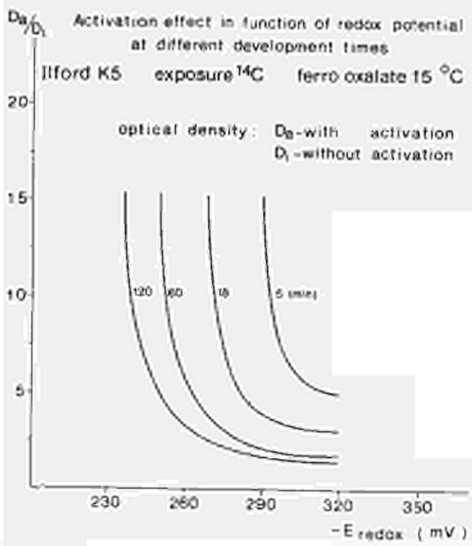
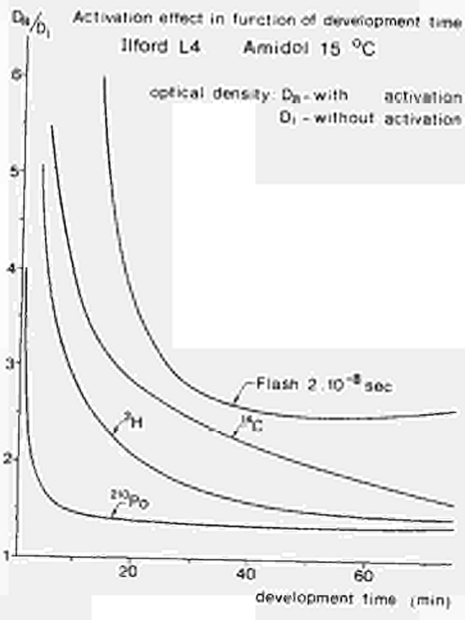


Fig. 4



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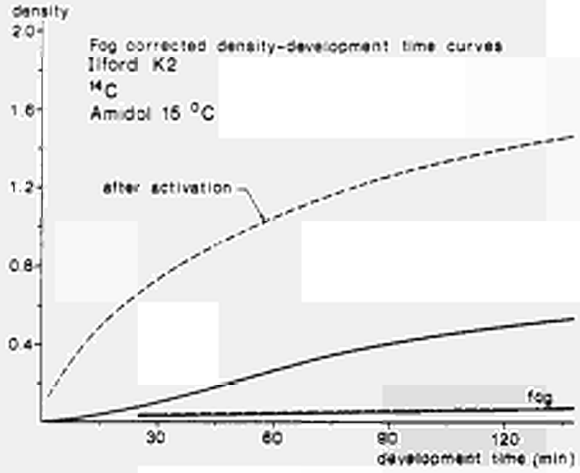

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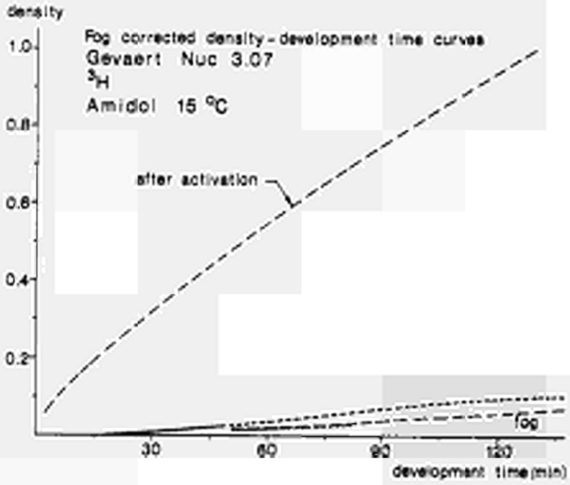

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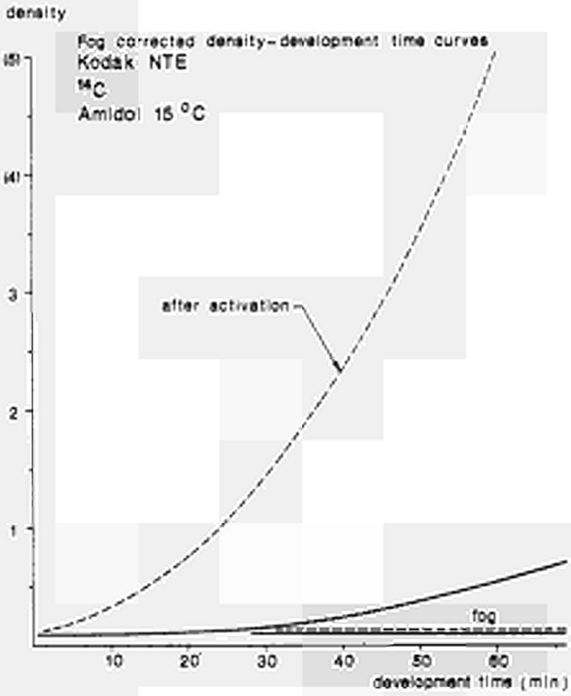

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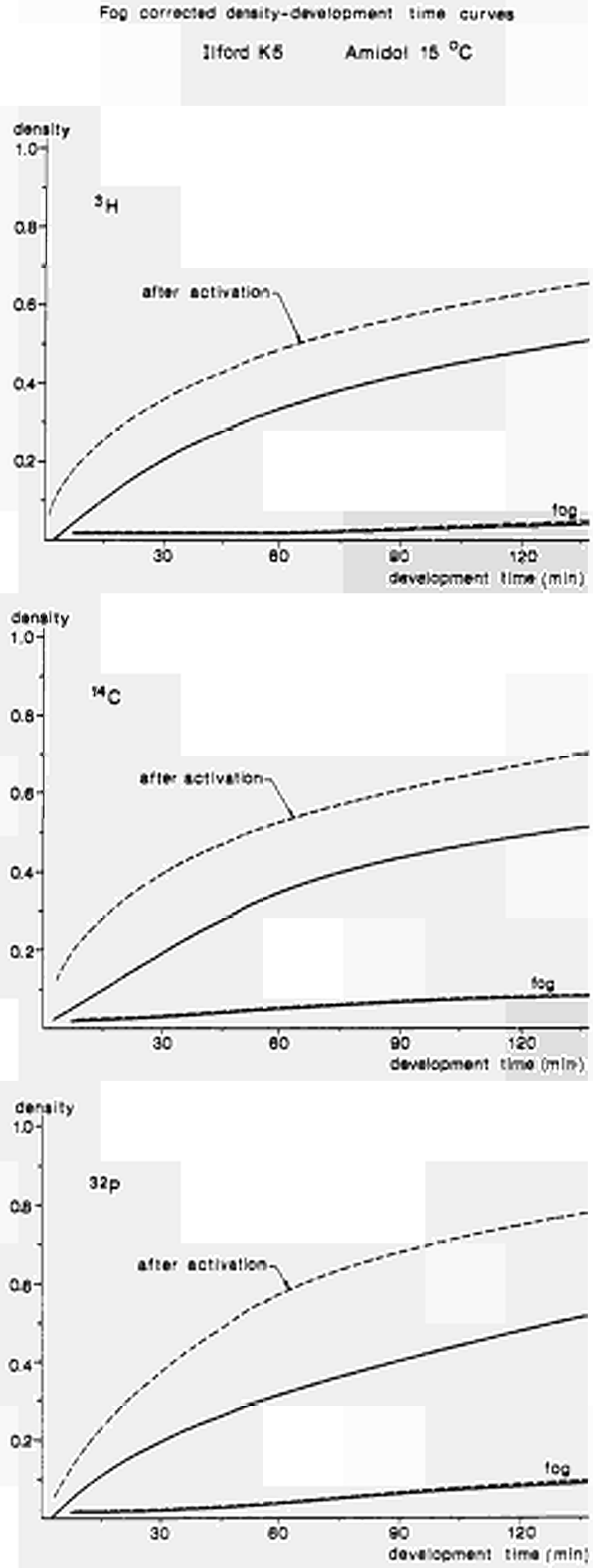

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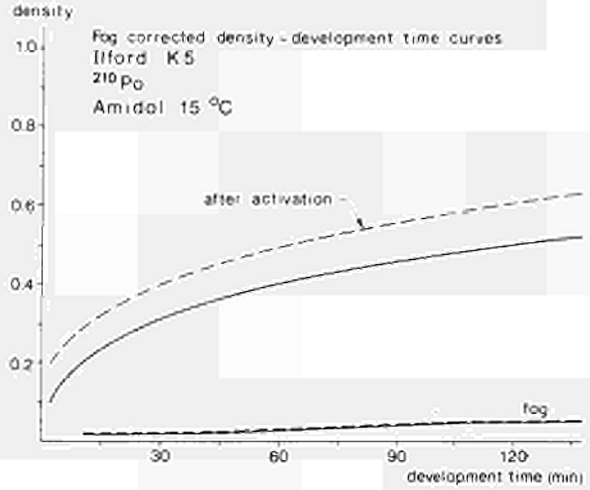

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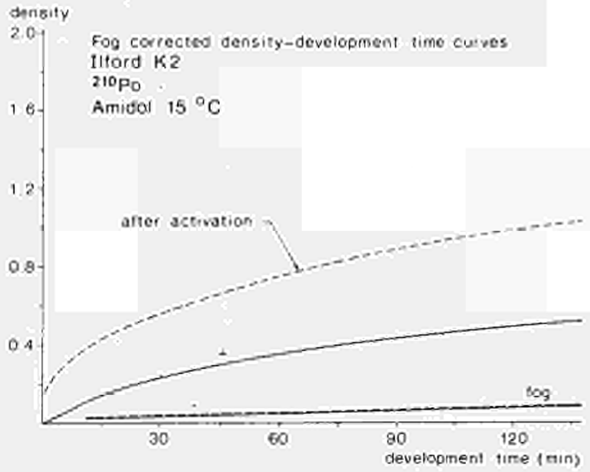

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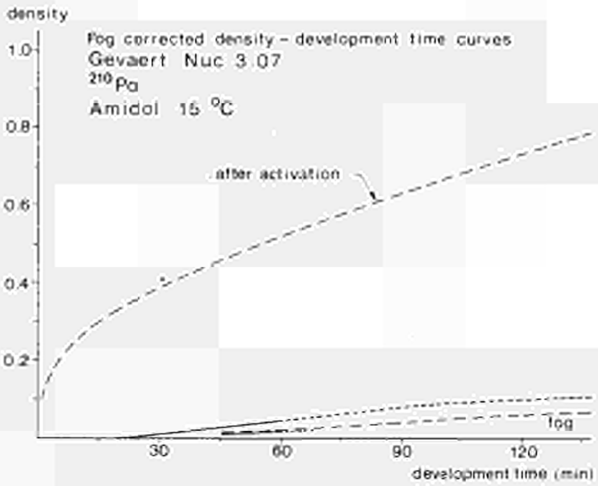

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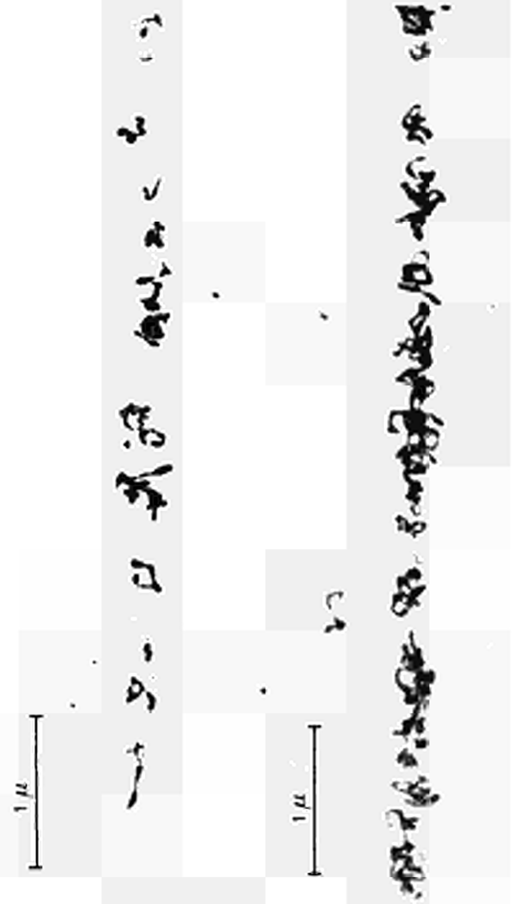

Fig. 13

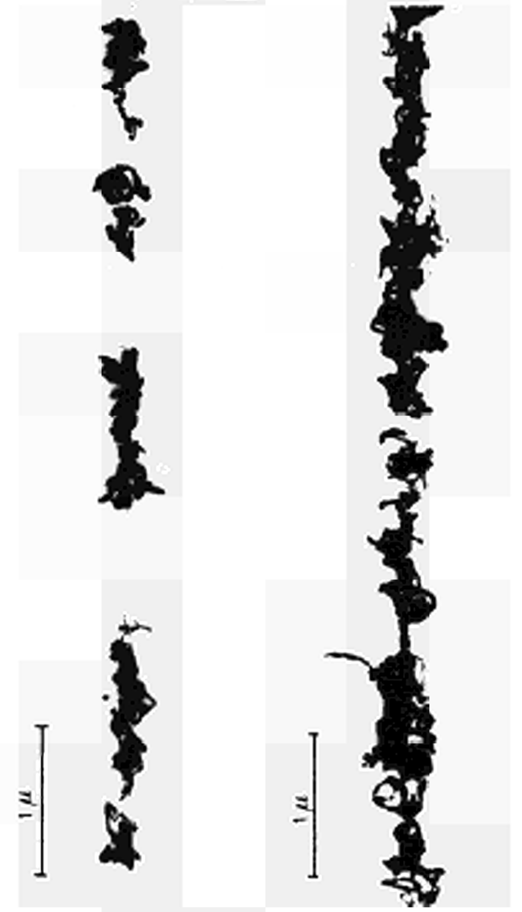

Fig. 14

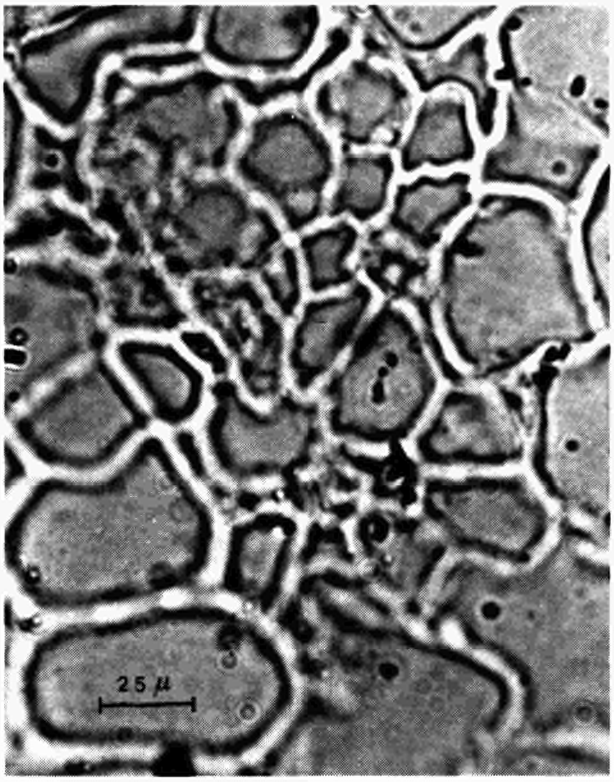

Fig. 15 a

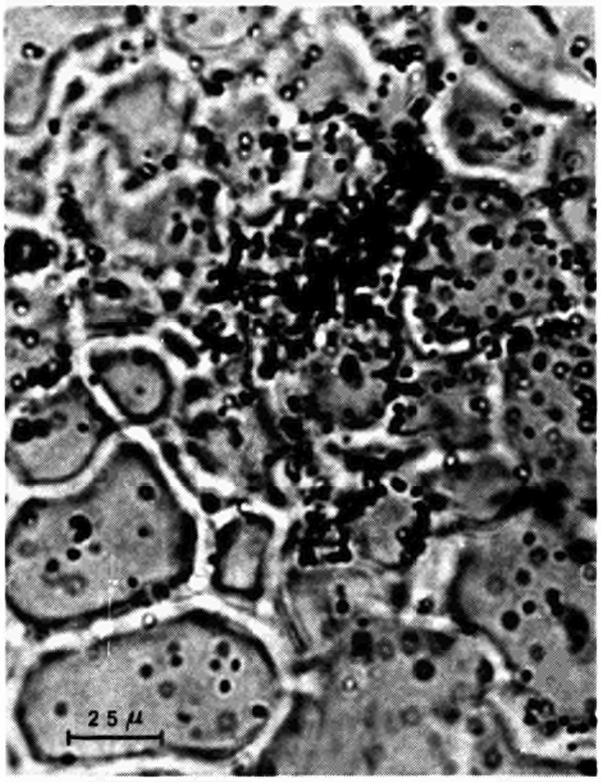

Fig. 15 b

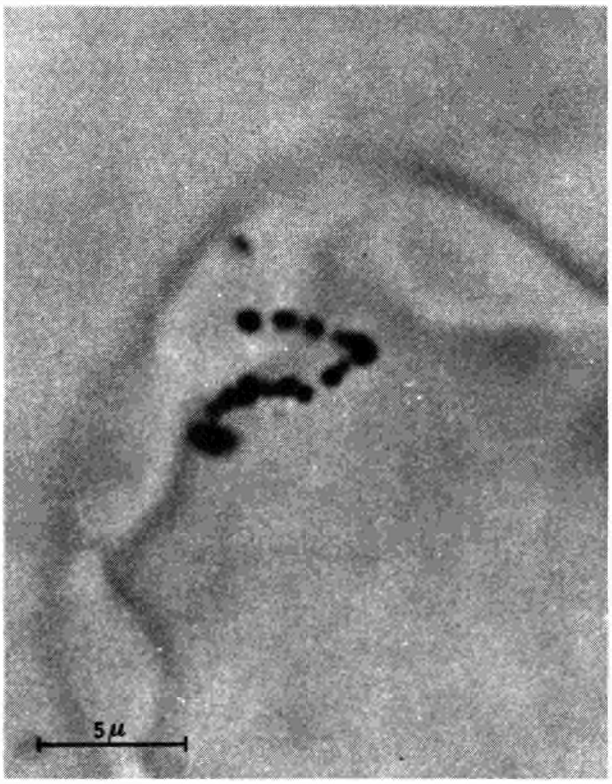

Fig. 16

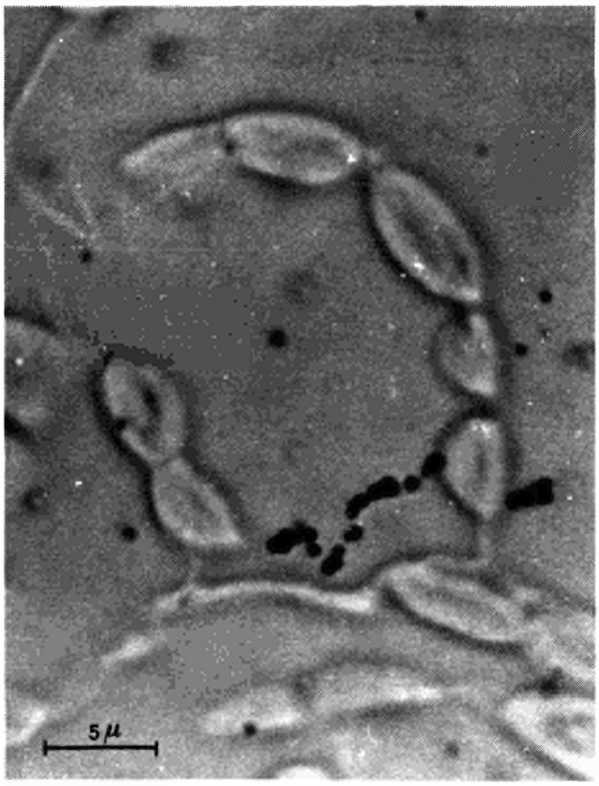

Fig. 17

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Alfred Nobel

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