Investigations of Different Types of AgBr-Layers for Use in Electron Microscope Autoradiography.

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ABSTRACT

On account of the low grain size three commercial emulsions GEVAERT NUC 307, ILFORD L4 and KODAK NTE have been investigated to assess their qualities for electron microscope microautoradiography. Grain size distribution curves were determined and a developer suitable for microautoradiography was selected after having tested different types of developers.

In order to investigate the sensitivities of the three emulsions monolayer preparations were irradiated in the electron microscope using an energy of 5.7 keV, corresponding to the mean β-energy of Tritium. After exposure the specimens were developed but left unfixed. The sensitivity may then be determined, using the ratio of developed to the total number of grains. For the formation of one latent image the ILFORD L4 emulsion must be hit on the average by 1-1.4 electrons per AgBr-grain; the corresponding figures for GEVAERT NUC 307 and KODAK NTE are 2-3 and 4-5, respectively.

The problem of resolution of point and plane sources of radioactivity is discussed.

In biological and medical research nuclear track emulsions are often used for autoradiographical demonstration of radionuclides in biological materials; for example localisation of labelled tracer molecules in cells, substructures of cells and other small particles. With the presently available techniques the differences in resolution between light and electron microscope autoradiography (microautoradiography) are relatively small. Nevertheless, we concentrate on microautoradiography, as in the future sensitive emulsions with smaller grai . size, or methods such as the use of solid state track detectors may increase a toradiographic resolution. This would permit one to take advantage of the hig.ı resolving power of the electron microscope in this system.

In our experiments we have used a technique modified after Caro et al.[1]:

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The emulsion is applied with a copper wire loop to the electron microscope grids which have been coated with an ultrathin « sandwich » of formvar, carbon, tissue and carbon, in that order (see Fig. 5). The limiting factors of the resolving power are, therefore:

1) Thickness of specimen;
2) Close contact of specimen and emulsion;
3) Energy and range of the radionuclide;
4) Method of developing;
5) Grain size and sensitivity of the AgBr-emulsion.

In the present study, the last two points have been investigated using three different commercially available emulsions with small grain size.

Fig. 1 shows the grain size distribution measured for the emulsions GEVAERT NUC 307 (.04 μ), ILFORD L4 (.11 μ) and KODAK NTE (.04 μ). The grain size

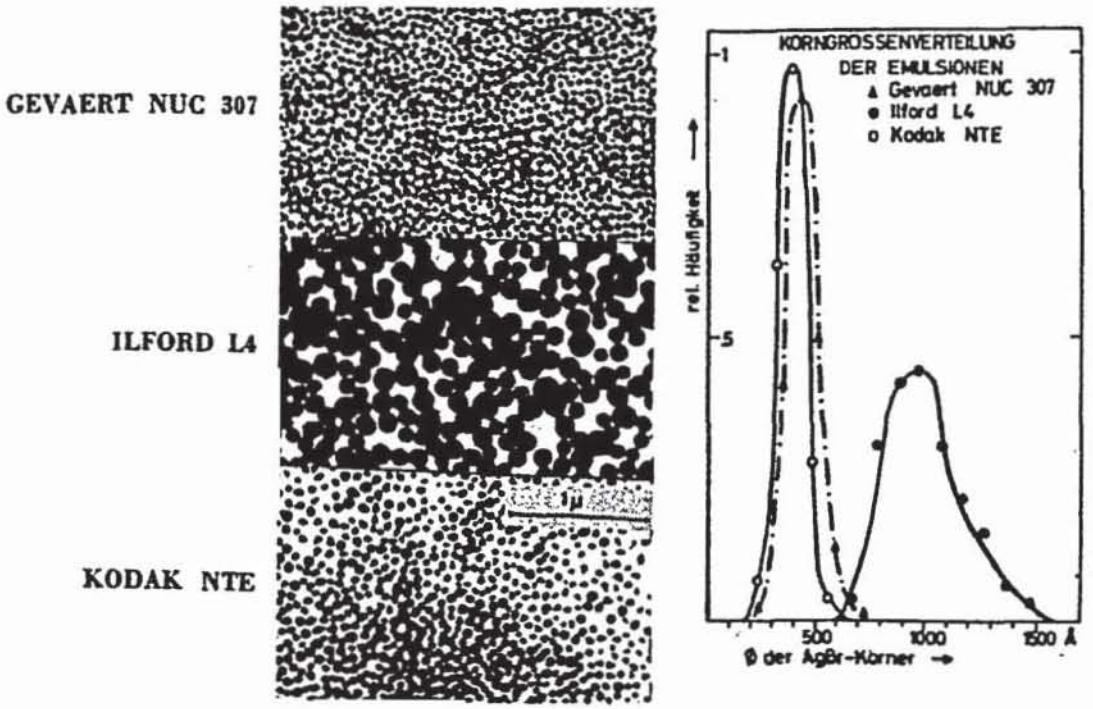

Fig. 1

distribution curves of all three emulsions show half-width values of about 20 % of the mean. Whereas the ILFORD L4 and KODAK NTE emulsions were found to have the grain size stated by the manufacturers, the value found for the GEVAERT NUC 307 emulsion was about 40 % lower than that stated. There may be variations of grain size between different batches of this emulsion and it is, therefore, recommended to check the grain size before use.

A developer suitable for microautoradiography should produce typically shaped silver filaments which are as small as possible. Fig. 2 demonstrates for the emulsion

ILFORD L4 (developed 1 minute) the types of silver filaments produced by three different developers.

Physical development as used by Caro [1] and other authors was not used because of the likelihood of artefacts, a particularly serious problem in electron microscopy. MICRODOL X fine grain developer produced rather small silver filaments of typical shape in all three emulsions, the fine end of a filament coinciding with the locus of the original latent image in the AgBr grain. This developer was chosen for all subsequent experiments.

FIG. 2

In order to test the sensitivity of the three emulsions in question, monolayer preparations have been irradiated in the electron microscope and measurements of electron intensity have been made. The energy of the electron beam was maintained at 5.7 keV, corresponding to the mean β-energy of Tritium, the radionuclide most frequently used in biological tracer studies. After irradiation in the electron microscope the specimens were developed in MICRODOL X but left unfixed. This procedure permits the determination of the ratio of developed to the total number of grains. Fig. 3 shows the result of a typical experiment with *ILFORD L4* emulsion and exposures to 0, 1, 2 and 20 electrons per AgBr grain. Due to the short developing time of 1 minute not all latent images do grow out completely, as it takes longer to develop the internal latent image than those on the surface of the grain. The points, however, where silver filaments are just starting to grow out from the grains are clearly distinguishable.

If we assume that electrons hit AgBr grains according to a Poisson distribution, we can calculate the number of grains which are hit by k and more electrons when the mean exposure is N electrons per grain. In Fig. 4 the percentage of developed AgBr grains is given on the ordinate, the number of electrons necessary for the formation of a latent image can be read from the abscissa.

Our experiments show that for the formation of one silver filament an AgBr

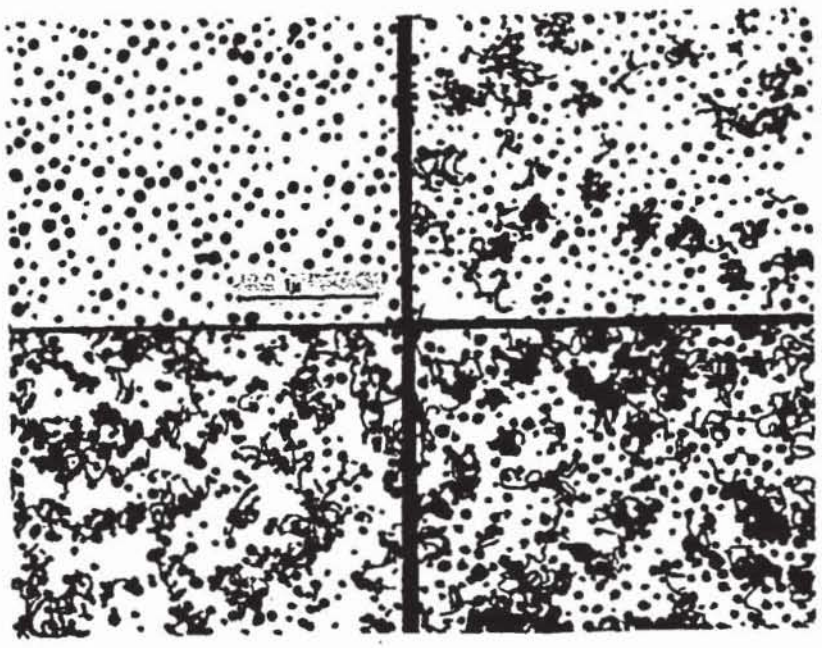

FIG. 3

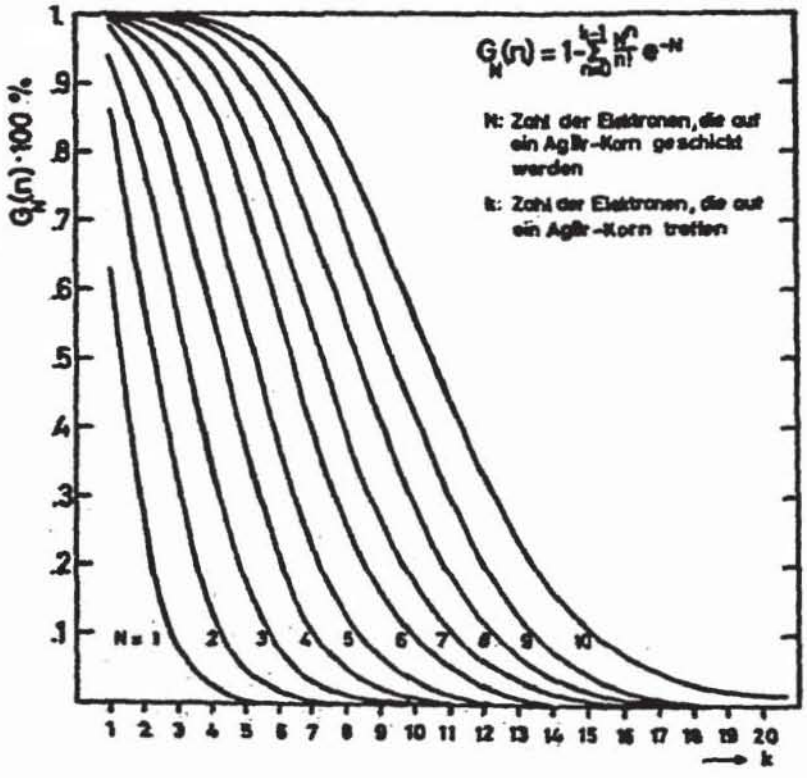

FIG. 4

grain of the ILFORD L4 emulsion must be hit on the average by 1-1.4 electrons. The corresponding figures for the GEVAERT NUC 307 and KODAK NTE emulsions are 2-3 and 4-5, respectively. From the energy loss in silver bromide for 5.7 keV

electrons it can be estimated that the ILFORD L4 and GEVAERT NUC 307 emulsions absorb about 800 eV for the formation of a latent image as compared to 1300 eV for the KODAK NTE emulsion.

In sections of biological material we are often dealing with plane sources of radioactivity. In this case, the differences in sensitivity between the emulsions are not large enough to be important. What may be more critical, particularly where the localization of label in specific cell components is being studied, is the degree of resolution.

The resolution may be visualized as a sphere with the diameter of the AgBr grain as its radius, the center of the sphere being the latent image. For MICRODOL X developed grains the thin end of the filament corresponds to the latent image and hence to the center of the sphere. If, as in the case of the ILFORD L4 emulsion, only one electron is required for the formation of a latent image in one grain, it will with great certainty have come from the sphere. For emulsions, however, which require several electrons for the formation of a latent image, the proba-

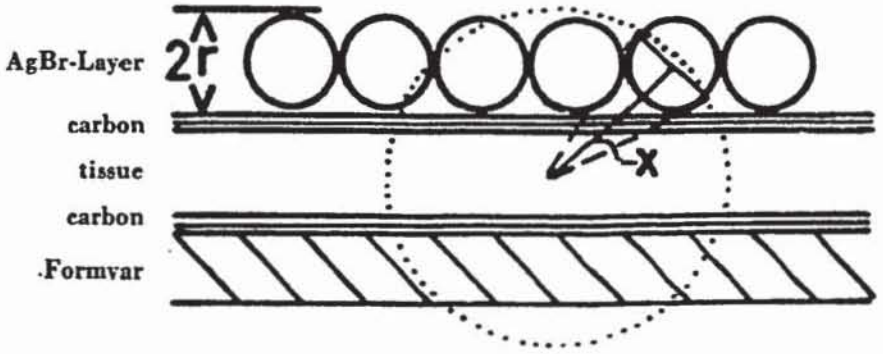

FIG. 5

bility for the formation of a latent image must be taken into account. This is demonstrated in Fig. 5. The probability (P_s) for latent image formation is a function of the number of electrons (i), necessary for the production of one latent image, and of the probability for one AgBr grain to be hit by one electron. Thus,

$$P_s = \left(\frac{r^2}{4x^2}\right)^i$$

Normally one would assume that resolution goes up with decreasing grain size. As, however, the degree of resolution is proportional to the probability of latent image formation (P_s) the advantage of emulsions with smaller grains is lost because the smaller the grains are the greater will be the number of electrons (i) required for latent image formation. Regarding point sources this means that with emulsions requiring several electrons for latent image formation very little labelling will be obtained. In the case of line sources, as for instance membranes, the « electron collecting area » becomes so extended that the advantages of low grain size are eliminated, as was mentioned above. Hence, giving a similar reso-

lution as the GEVAERT NUC 307 emulsion, the ILDFORD L4 emulsion is preferred, because with a considerably shorter autoradiographic exposure time, results are of comparable quality.

In Fig. 6 the degree of resolution which can be obtained using ILFORD L4 emulsion and MICRODOL X developer is demonstrated in a microautoradiogram of a cell from the transplantable « Marshall » rat carcinoma. ^{3}H-thymidine has been incorporated into the DNA of this cell « in vitro » (^{3}H-thymidine concentration in the medium 20 μc/ml, incubation time 90 minutes, exposure time 15 days, magnification 11.000 x). The resolution is shown by circles around two silver filaments).

FIG. 6

It may be worth mentioning that another approach to produce suitable photosensitive silver bromide layers with very small grain size has been vacuum evaporation of metallic silver and subsequent exposure to bromium vapour, a technique which has in principle been described by Silk et al. in 1961 [2]. However, our results with this method indicate that although very small crystals of AgBr are indeed obtainable, the sensitivity of the grains is extremely low by comparison with commercially available autoradiographic emulsions in gel form.

Future advances in microautoradiography will depend on the development of emulsions with lower grain sizes, but such improvement must not be at the expense of sensitivity.

On the other hand it seems to be of interest to investigate the possibilities of the use of solid-state track detectors for high resolution microautoradiography.

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