AUTORADIOGRAPHY WITH A POSITION SENSITIVE COUNTER

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1. Introduction

The position sensitive counter was first used to measure very high energy electrons and protons [1] and has recently been adapted to detect X-ray radiation for diffraction studies [2, 3]. We report here what we believe to be the first application of a position sensitive counter to biological studies involving the localisation of radioactively labelled material.

The position sensitive counter, like all proportional counters, contains a cell holding a gas mixture and a fine wire. A thin window is usually found on one side of the cell; this window in our counter is aluminized and serves as a cathode, while the central wire is the detector anode. The application of a high voltage between the two electrodes allows for the collection of the charges produced by ionizing radiation in the gas mixture. In our system, a determination of difference in the rise time of the gas discharge signal after its arrival at the two ends of the proportional counter wire yields the position of the discharge along the wire. Thus, scanning is done electronically and does not require any mechanical displacement of the system. Although we discuss here only electrophoresis, applications to chromatography and sedimentation experiments should be quite feasible. The counter permits a 10 000-fold reduction in exposure time compared to conventional film techniques for analysing ^{14}C-labelled autoradiograms. Bands separated by 3 mm can be resolved and an energy discrimination for double label experiments of 20% is possible.

2. Methods

In addition to the counter, several units of electronic equipment are required for these experiments. Two linear amplifiers, which amplify the signal at each end of the wire, are connected to a crossover detector to determine the differences in the rise time of the signals. This difference in time is then converted to a difference in amplitude by another amplifier and these differences are stored and displayed by a multichannel analyser. The details of the electronics are given in ref. [3] and [1].

The counter employed for these experiments contained a gas detector cell measuring 250 mm by 25 mm and was 10 mm thick. A continuous flow of gas, 90% argon and 10% methane, filled the detector which was maintained at 1350 V. Electrophoretograms were laid on the cell's 25 μ-thick aluminized mylar window.

After the experiments with the counter the electrophoretogram paper was cut into small fragments which were then analyzed in a liquid scintillation counter. The resulting curve was in good agreement with that shown in fig. 1b.

3. Results and discussion

We have applied the linear-position sensitive counter to the simple measurement of the distribution of radioactivity on a one-dimensional paper electrophoretogram. Hitherto, autoradiograms have generally been analysed by exposure to photographic emulsions

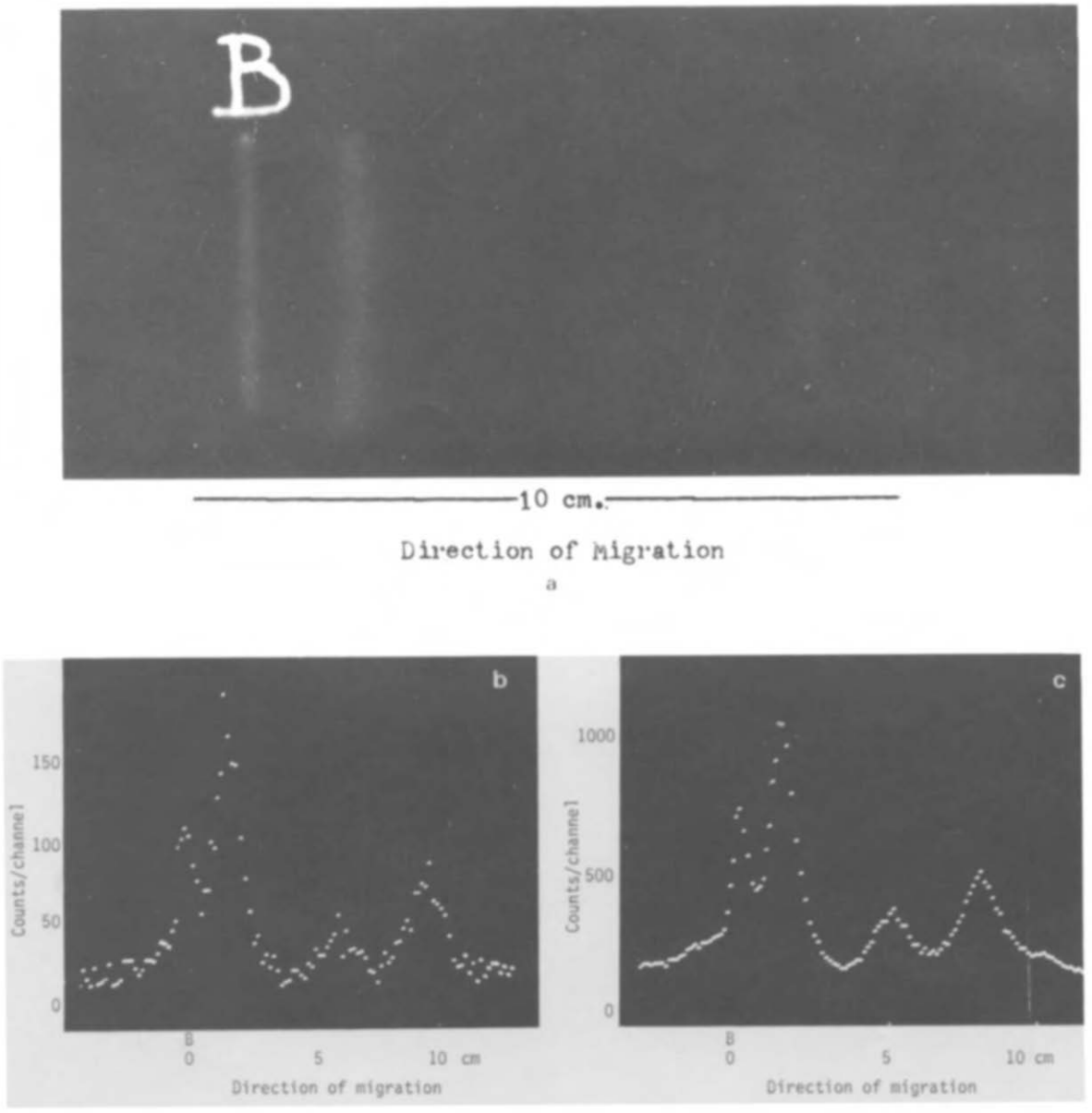

Fig. 1. A paper electrophoretogram of [^{14}C] carboxymethylated aspartokinase I-homoserine dehydrogenase I of *E. coli* after trypsin digestion. Further details can be found in ref. [4]. B marks the original of the experiment and shows a band which did not migrate. One can estimate diffusion spreading and the background from the region to the left of B. The total number of counts was 40 000/min: a) A photograph of a film exposed to the bands for 15 days. Only two bands reproduced well in the photograph but three others could be discerned on the original film; b) The contents of the multichannel analyzer after a 100 sec exposure to the electrophoretogram which was laid on the counter's mylar window. Five distinct peaks are visible; c) A 10 min count of the electrophoretogram as in 1b. The statistics are improved but the general form of the pattern is unchanged.

or scanned with a simple proportional counter covered by a fine slit. The great advantage of the position sensitive counter is its 100–10 000 times enhanced sensitivity with regard to film for the detection of electrons from ^{32}P or ^{14}C. Thus, instead of exposing and waiting for two or three weeks to have a film with several barely visible bands, as was the case for the electrophoretogram of fig. 1 a, only one min was required to clearly see the bands and note their position (see fig. 1b). Furthermore, no scanning is required as the data is directly obtained as intensity vs distance, and the area in each peak is determined as accurately as one chooses the corresponding counting time. Double-labelled experiments which are almost impossible with film are simple with the counter; like all gas-proportional counters the energy resolution or discrimination is about 20%. If a scanning counter had to be used to obtain the curves presented in fig. 1b or 1c, which contain 170 points in the direction of migration, approximately 170 measurements, one at each position would be required for the same precision. Thus the experiment of fig. 1c would require 28 hr instead of 10 min. The resolution of the present experiments was limited to about 3 mm by the relatively large chamber thickness (1 cm) and by variations from planarity of the support. Thinner counter chambers with mechanically straight supports should allow a resolution of approximately 1 mm. Furthermore, a counter with a series of parallel wires to measure autoradiograms in two dimensions is under construction.

The experiments present a new, fast, and very efficient manner of detecting the distribution and transport of radioactively labelled markers. Although the linear counter may well be adapted to measure the weak electrons from tritium by using very thin entrance windows, many experiments could just as well be done with ^{14}C or another label. Several other uses can easily be imagined; for example, with ^{32}P wet samples could be scanned and this would allow for the analysis of electrophoresis in the course of an experiment, or sedimentation gradient bands could be scanned with a suitable thin window container without collection.

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