CORRECTION FOR AUTORADIOGRAPHIC IMAGE SPREAD: A COMPARISON BETWEEN TWO METHODS

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ABSTRACT

A set of LM autoradiographic images of ^{125}I-insulin attached to the insulin receptors of free rat hepatocytes have been analysed by two methods incorporating correction for image spread effects. Results obtained by an unrestricted analysis based on the construction and use of crossfire matrices are compared with those obtained using an alternative method more recently proposed for populations of isolated radioactive sources of well-defined shape. In the latter approach, the cell profiles were modelled as radioactive discs with superimposed labelled annuli. Both methods indicate significant peripheral labelling, in accordance with current views on the behaviour of insulin receptors, but quantitative estimates of the ratio of interior to peripheral radioactive concentrations are somewhat disparate. Some possible explanations for this are suggested.

Keywords: Autoradiography, crossfire, image spread, insulin receptors.

INTRODUCTION

Correction for the effects of image spread is essential for the meaningful interpretation of high resolution autoradiographs, particularly at the EM level, but also, in some circumstances, at the LM level. Methods have previously been described (Blackett and Parry, 1973; Downs and Williams, 1978) for the analysis of complex autoradiographic specimens via the construction and use of crossfire matrices. More recently (Downs and Williams, 1984), an alternative method was suggested whereby images derived from populations of isolated radioactive sources of simple, well defined shapes can be analysed by the construction of appropriate summed image spread functions. This latter approach has been implemented in the analysis of a set of LM autoradiographic images of free rat hepatocytes and the results compared with those obtained using a crossfire matrix approach.

MATERIALS AND METHODS

Rat hepatocytes were isolated and kept in suspension culture at 37°C. The cells were labelled by the addition to the medium, for 30 min., of 5×10^{-10} M insulin labelled with ^{125}I. They were fixed in glutaraldehyde, dehydrated, embedded in Araldite and sectioned at 0.6 μm. LM autoradiographs were prepared with a monolayer of Ilford L4 emulsion and photomicrographs made at a calibrated final magnification of 4000x (Fig. 1).
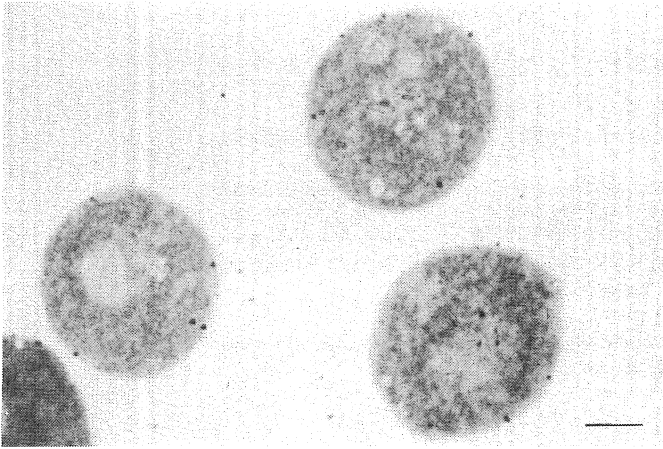
Fig. 1. LM autoradiograph of free rat hepatocytes labelled with ^{125}I-insulin.
Bar = 5 μm (20 HD units).

The set of 42 prints was analysed by each of the two methods. Based on previous line source experiments with the same autoradiographic system (Williams et al., 1983), an HD value of 0.25 μm was used, together with the idealized point source image spread function of Salpeter et al.(1969) (see Downs and Williams, 1984).

(i)Analysis using summed grain distributions:

With very few exceptions, the cell profiles showed a high degree of circularity (mean axial ratio 1.08), with radii in the range 8-39 HD units. In order to investigate differential labelling between the cell surface and the interior, the profiles were modelled as combinations of discoid sources and narrow peripheral annular regions and analysed as described in Downs and Williams (1984). The width of the annular region was chosen to be 2 HD units. Cut-off distances for grains were chosen to include, theoretically, at least 90% of the grains (2-4 HD, depending on profile radius). A total of 148 profiles were included, giving an effective number of 141 after elimination of sectors of insufficiently separated profiles. Grain distances (from profile centres) were recorded in histogram classes of width 2 HD. Expected relative grain numbers in these classes were computed using the measured profile size distribution and assuming (a) uniformly labelled discs and (b) uniformly labelled annuli. Appropriately weighted combinations provided expected grain numbers for various assumed annulus to disc ratios of radioactive concentration. This ratio (k) was varied to obtain the best fit of the observed and expected grain numbers on the basis of the chi^2 test.

(ii) Analysis using crossfire matrices :

The method of Downs and Williams (1978) was employed with three source items; cell interior (I), cell peripheral region (P) and embedding medium (E). The peripheral region was defined to correspond, as far as possible, to the annular source region used in (i). It was delineated by a fluffing out procedure using a 4 HD diameter circle, but including only that part of the junctional region lying within the cell. A total of 1105 grains and 5536 notional disintegrations were recorded.

10.04

RESULTS

Histograms of observed and expected grain numbers obtained by method (i) are presented in Figs. 2 – 4. (Classes were pooled as indicated.) The best fit (chi^2 = 14.8, with 9 d.f.; p ≈ 0.1) was achieved with an annulus to disc concentration ratio of 6:1 (Fig. 2). Figs. 3 and 4 show the distributions to be expected if the radioactivity was either uniformly distributed throughout the cells (Fig. 3; chi^2 = 125, 9 d.f.), or confined exclusively to the peripheral regions (Fig. 4; chi^2 = 871, 9 d.f.). Both of these latter hypotheses are clearly rejected.
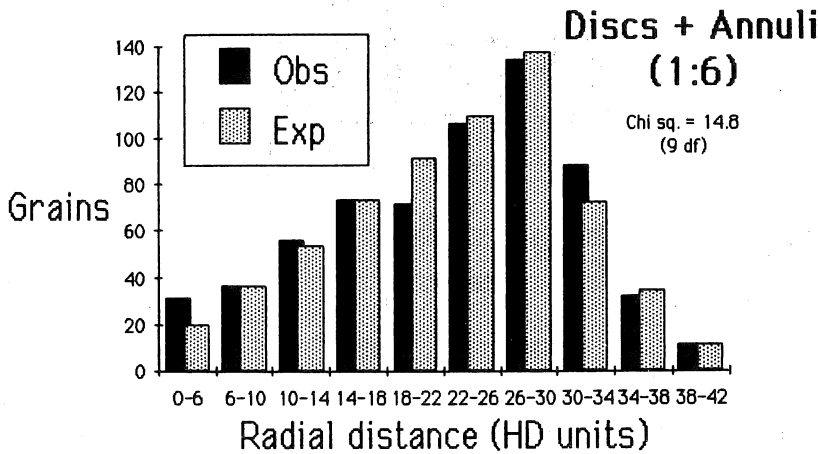

Fig. 2. Observed and expected grain distributions for a model based on a combination of discs and annuli with radioactive concentration ratio 1:6 (method (i); k = 6).
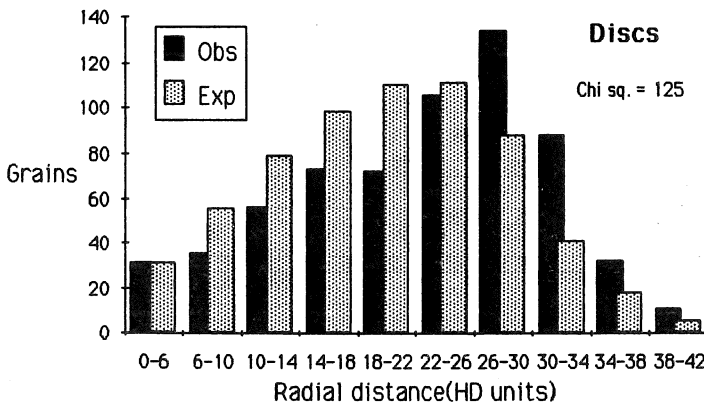

Fig. 3. Observed and expected grain distributions based on modelling by uniformly-labelled discs (method (i); k = ∞).
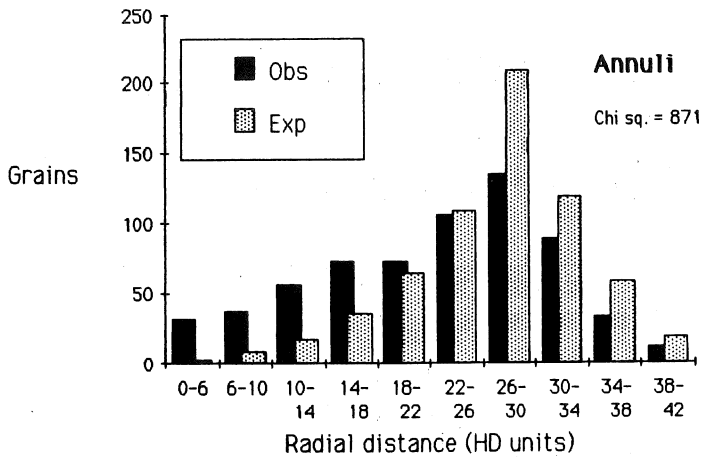
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Fig. 4. Observed and expected grain distributions based on modelling by uniformly–labelled annuli (method(i); k = 0).

Results of the crossfire matrix analysis are summarized in Table 1. They indicate a peripheral to interior concentration ratio of 18:1.

Table 1. Summary of the crossfire matrix analysis

The crossfire matrix:			Area values:	Radioactive	Relative
Items	I	P	E	(points)	disintegrations:	concentration:
I	.873	.046	.081	844	163	0.19
P	.250	.495	.255	216	752	3.48
E	.015	.015	.970	4476	190	0.04
Observed grain numbers:						
	333	383	389		1105	

DISCUSSION

Both analyses clearly support the general conclusion that the radioactive concentration is significantly higher in the peripheral regions than in the interior regions of the cells, in accordance with current views on the behaviour of insulin receptors. The quantitative estimates of the concentration ratio, k, are however rather disparate. Several reasons can be proposed for this.

10.06

Comparison of the two methods requires that equivalent items be chosen in each approach. Hence, although tests suggested that the interior labelling was not entirely homogeneous, the crossfire matrix analysis was not fully pursued, as would normally be the case, by further subdivision of items. In the superimposition method, perturbations of the boundaries of the (generally circular) cell profiles would be expected to result in an underestimation of k. Whilst such perturbations have little effect on the image spread from the interior regions (modelled as discs), the grain distribution, as a function of radial distance, will be more widely spread for a narrow annulus than for an exactly circular (unperturbed) annulus of the same width, with a correspondingly lower peak. Given that much of the radioactivity resides in the peripheral regions, modelling of these regions by narrow circular annuli will result in an observed histogram with a lower peak and a wider spread than it should have, thus giving rise to an underestimation of k. The use of 2 HD-wide annuli appears, in this instance, to have produced such an effect. (The effect was, indeed, even more marked in a pilot analysis based on 1 HD annuli.) The cell profiles studied here were in no way remarkable with regard to the slight crenellations of their outlines. The use of a somewhat wider annular region is thus to be generally recommended in analyses of this type.

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