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High efficiency gene transfer into mammalian cells

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[Plate 1]

We have generalized the protocol of gene transfer, greatly increasing the variety of cells that can be used as recipients of foreign genes. Our approach has been to use a transient assay system that allows rapid screening of expression of foreign DNA. When the initial steps of gene transfer have been optimized with the transient system, these defined conditions are used to yield efficient stable transformation. We have seen that primate cells, including human cells, can be used in gene transfer experiments at levels sensitive enough to allow detection of single copy gene function. Recently we have also used this approach successfully with undifferentiated embryonic cells.

One of the most important developments in recent years in the field of molecular biology has been the advent of techniques that allow the re-introduction of cloned genetic sequences into mammalian cells in culture. The pioneering experiments done by Wigler *et al.* (1977) used solely mouse L cells and depended on the expression of an introduced *Herpes simplex* virus thymidine kinase gene. Therefore, recipient cells were required to be mutant cells lacking endogenous expression of the cellular isozyme for thymidine kinase (Pellicer *et al.* 1978).

More recently it has become desirable to use a wide variety of cell types as recipients of the cloned genes, particularly to study proper regulation of a gene in a homogeneous background. Because many initial attempts at introducing foreign DNA into cells other than mouse L cells had been unproductive, the idea arose that only a few cell types were competent to express cloned DNA. However, it is possible that these first negative results could have been a result of lack of sensitivity in the detection of foreign DNA, or that modification in the basic techniques might be required for the expression of plasmid DNA in various cell types.

We set out to devise a sensitive assay system which could be used to monitor expression of foreign DNA shortly after uptake by the cells in culture. With such a system, expression of DNA can be detected transiently; that is, while the plasmid DNA is in a non-integrated state. In such experiments, thousands of DNA templates are being transcribed. This approach has two distinct advantages over previous experiments, which allowed the detection of foreign DNA only after it was integrated into the host cell chromatin. First, transient expression can be monitored within 48 h of DNA uptake, while stable expression may require 3–4 weeks. Second, since thousands of templates are transcribed, higher levels of RNA homologous to the cloned gene are present.

The system we discuss is based on the expression of a bacterial gene, that encoding chloramphenicol acetyltransferase (CAT) (Shaw 1975), in mammalian cells. This bacterial enzyme has been well characterized and sensitive assays exist. By cloning this bacterial gene under eukaryotic control signals we have constructed a unique plasmid, pSV2cat, which allows the expression of a bacterial gene in mammalian cells (Gorman *et al.* 1982*b*).

We shall briefly discuss two applications of the transient assay system: the quantitative analysis of promoter strength, which has led to the development of expression vectors that transform mammalian cells at very efficient levels, and the use of this system to define optimum transfection protocols for cells previously thought to be difficult to transfect, namely undifferentiated teratocarcinoma cells.

One of the first uses of the CAT assay system has been the analysis of putative promoter sequences. By subcloning these control sequences juxtaposed to the CAT gene the relative levels of transcription from various promoters can be compared by the assay. A comparison of various promoter activities in different cell types is listed in table 1. In addition to the above work, other recent reports have used this assay system to study enhancer elements (for review see Khoury & Gruss 1983; Laimons *et al.* 1982; Weiher *et al.* 1983; Xu *et al.* 1983; Gorman *et al.* 1983*a*; Scholer & Gruss 1984).

TABLE 1. TRANSIENT LEVELS OF PROMOTER ACTIVITY

(Data are expressed as percentage chloramphenicol acetylated when the assay is performed as described by Gorman *et al.* (1983*a*). The pColcat and pTKcat vectors are described in Gorman *et al.* (1984) and pSV0cat in Gorman *et al.* (1982*b*).)

cell type	CV-1	L	CHO
DNA			
pRSVcat	39	38	12
pSV2cat	21	36	46
pColcat	4	3	5
pTKcat	5	4	4
pSV0cat	0	0	0

Based on the varying levels of transient expression from the CAT plasmids we postulated that perhaps the efficiency of stable transformation could be correlated with promoter activity in the transient system. To test this hypothesis we constructed a series of expression plasmids using the Rouse sarcoma virus (RSV) long terminal repeat (LTR) as the promoter (Yamamoto *et al.* 1980). Details of these constructions are given in Gorman *et al.* (1983*b*). The results did indeed show that the levels of expression of the dominant selectable gene (in our case either of the *Eschericha coli* genes *gpt* (Mulligan & Berg 1981) or *neo* (Southern & Berg 1982)) did correlate with efficient stable transformation (table 2). Thus, by first analysing the activity of various promoters in a variety of cell types using transient expression, we have been able to choose the vector which will yield the most efficient stable expression in each cell type (Gorman *et al.* 1983*b*).

TABLE 2. HIGH STABLE TRANSFORMATION EFFICIENCIES

(Stable transformation efficiencies were obtained by counting the number of colonies present after selection with gpt or G418 selective media. Cells were transfected with either pSV2 DNA or RSV marker DNA and split into selective media 48 h later at a density of 1×10. The results with the addition of butyrate show the increase in number of stable colonies when the transfected cells are treated with butyrate for 12 h after the removal of the DNA; for details see Gorman *et al.* (1983*a*).)

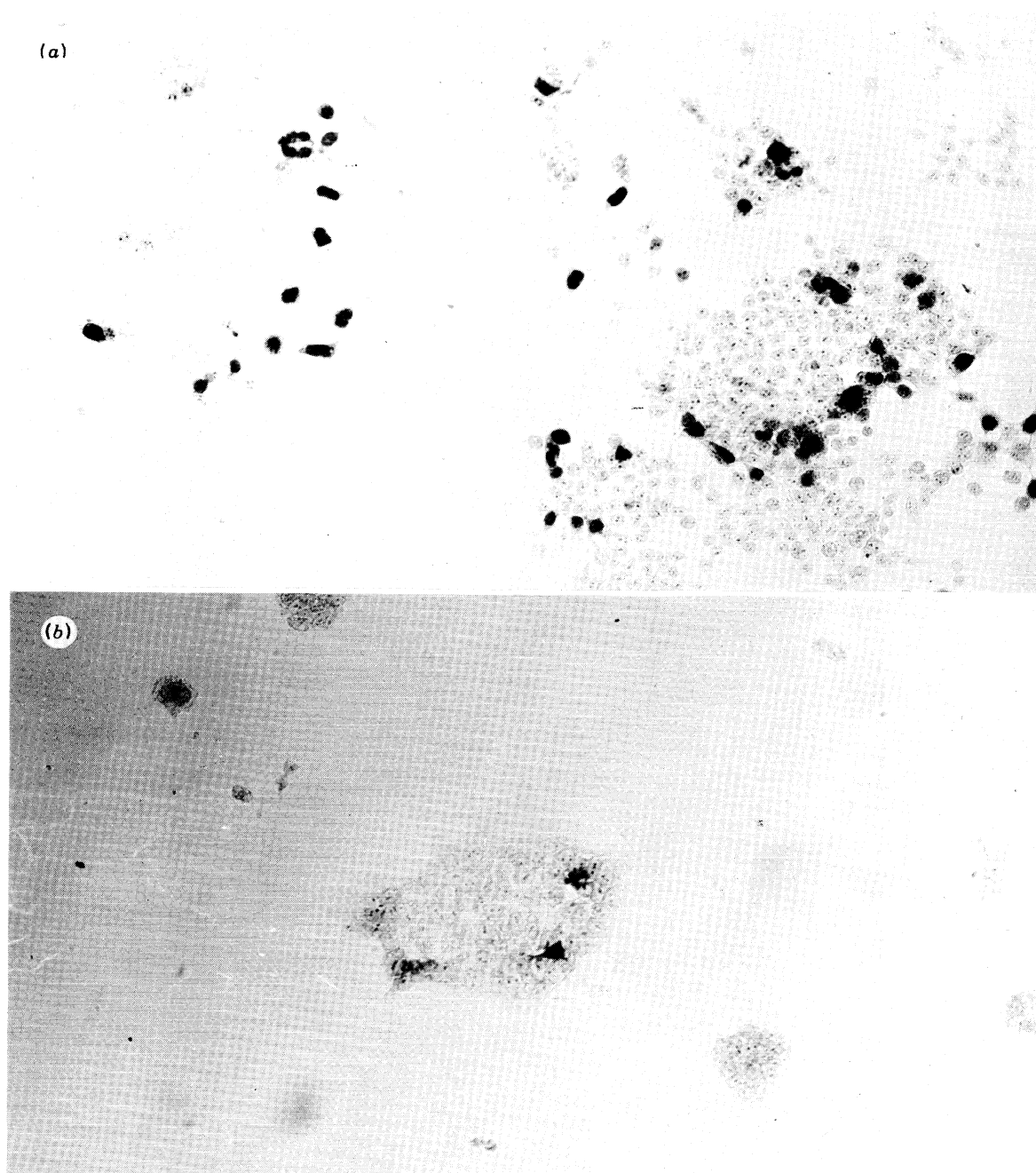| cell type	pSV2	RSV	+butyrate
CV-1	6×10^{-3}	5×10^{-2}	1.5×10^{-1}
Hela	3×10^{-4}	2×10^{-3}	4×10^{-2}
NIH3T3	2×10^{-4}	9×10^{-4}	3×10^{-3}
CHO	1×10^{-3}	9×10^{-4}	6×10^{-2}

FIGURE 1. Immunocytochemical detection of CAT expression in teratocarcinoma cells with the CAT-1 monoclonal antibody and of large T-antigen by antibody staining. (*a*) P19 cells transfected with SV40 DNA and stained PAb419 (Harlow *et al.* 1981). (*b*) F9 cells transfected with pRSVcat and stained with CAT-1.

Second, we have used the expression of the CAT gene to define optimum conditions for transfection of undifferentiated teratocarcinoma cells (Gorman & Lane 1984). In addition to the assay described above we have found the use of a monoclonal antibody to CAT to be extremely useful in these experiments. Using this CAT-1 antibody (Gorman & Lane 1984), with a sensitive method of peroxidase staining (Lane & Lane 1981) we have been able to define parameters that allow more cells within a population to express the plasmic DNA. Figure 1, plate 1, shows F9 cells and P19 cells which have been transfected with pRSVcat (Gorman *et al.* 1982*a*) and stained with the CAT-1 antibody. There is no background with this staining method as CAT-positive cells are clearly distinguishable from the cells that are not expressing the foreign DNA. Using these techniques we have standardized the transfection of undifferentiated teratocarcinoma cells at a transfection efficiency of 2%, comparable with results we have obtained with NIH3T3 cells (Gorman & Lane 1984).

In testing the sensitivity of the staining assay we were surprised to see that the pSV2cat vector gave the same efficiency of transfection as pRSVcat (Gorman *et al.* 1982*a*), since it had been believed that the SV40 early region promoter was non-functional in teratocarcinoma cells. To distinguish between the possibilities that the SV40 virus promoter might be non-transcribed in these cells or that its early gene products are not processed correctly, we transfected cells with SV40 DNA and monitored expression of large T-antigen by staining with a monoclonal antibody against T. Two surprising results were seen from this experiment. First, the apparent transfection efficiency is very different when we use the SV40 large T-antigen as a marker rather than the CAT gene. This very high level of detection of cells expressing T has also been observed by Banerji *et al.* (1981) when comparing the number of cells in a population which stain for beta-globulin and large T after a co-transfection. When we conducted a similar experiment staining for large T and CAT simultaneously, we observed that all CAT-positive cells are also positive for T expression; however, there are cells present that only stained with the T antibody. This latter category is approximately 20% of the population. We are very interested in the possibility that this high level of expression is indeed T specific and not simply due to a difference in the sensitivity of the antibodies used to detect our two markers. However, we can rule out any *trans*-acting function of T increasing the number of cells capable of expressing foreign DNA after co-transfection of plasmids since co-transfection with T does not increase the number of cells expressing a second marker. We are currently investigating the possibility that there may be some *cis*-acting function of the large T-antigen gene.

Second, we were impressed that the teratocarcinoma cells did express T at a high level. The antibody used in this experiment is specific for the N-terminus of T. We are pursuing experiments to determine whether the T-antigen made in these cells is full length and correctly processed. The finding that these viral promoters do function efficiently in these cells is important. Vectors using viral control sequences can indeed be used as marker plasmids for transfection of these teratocarcinoma cells much as we have described for cells in monolayer culture, thereby yielding highly efficient expression of foreign genes in teratocarcinoma cells.

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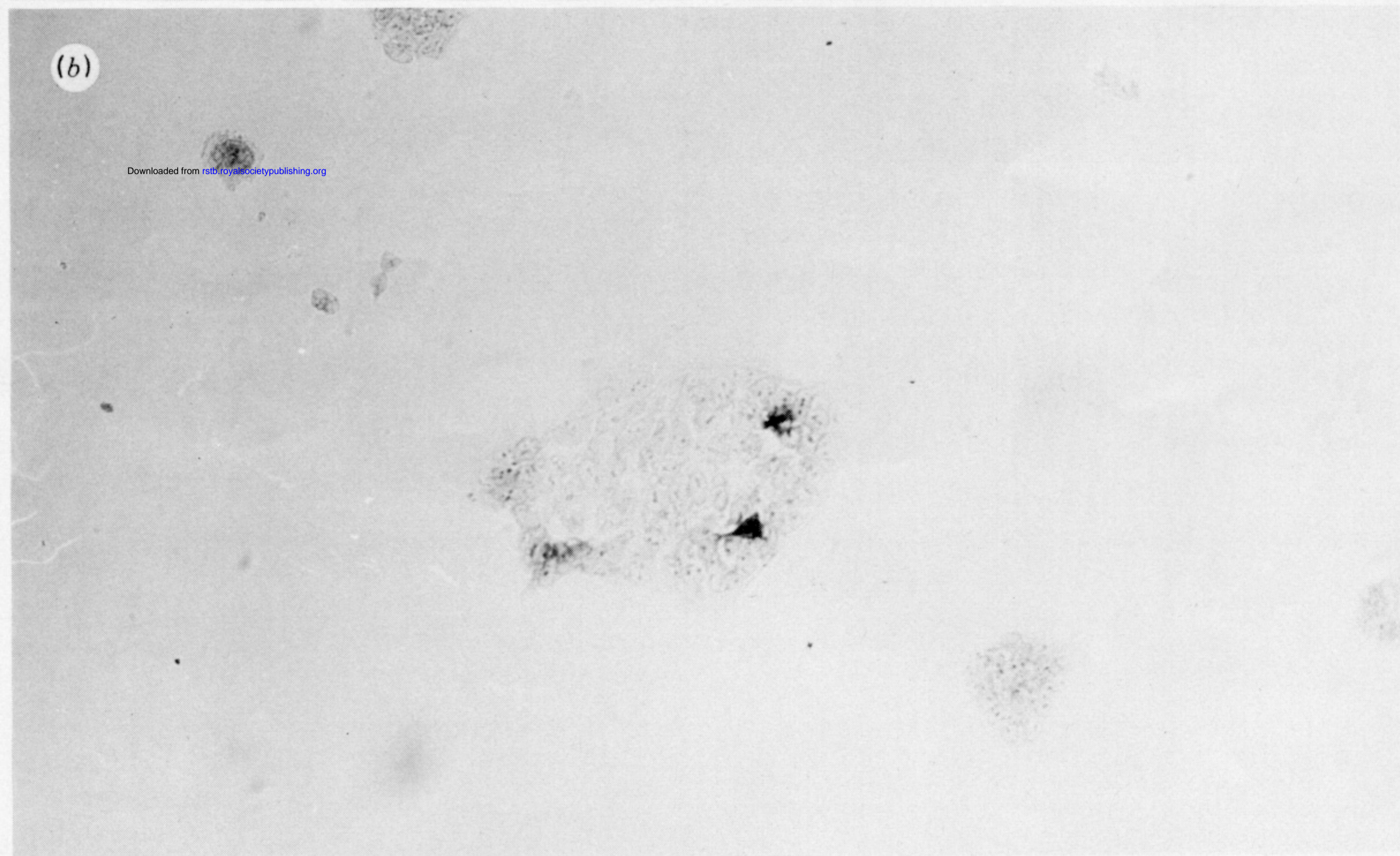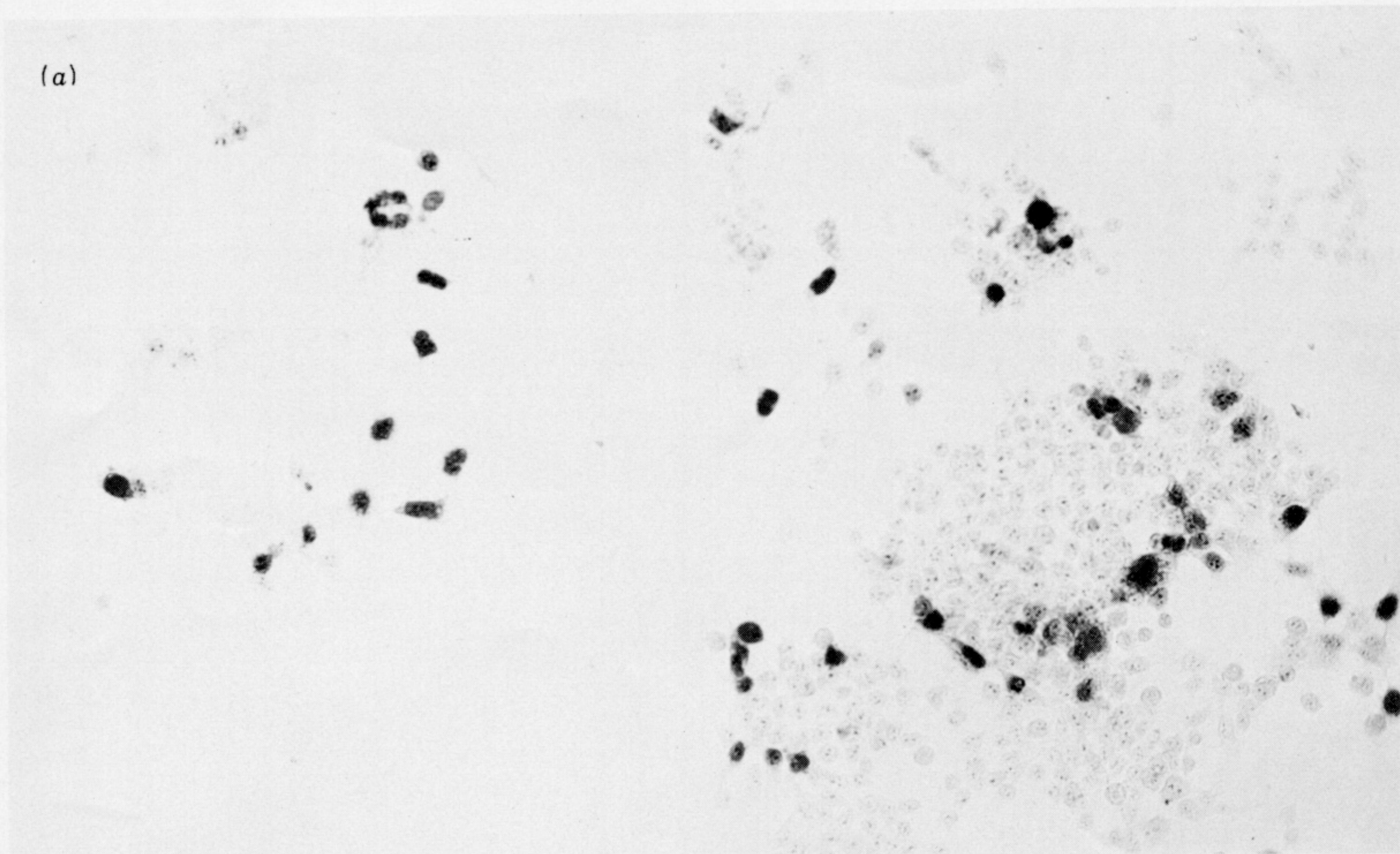
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