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Phil. Trans. R. Soc. Lond. B 1985 **312**, 91-100
doi: 10.1098/rstb.1985.0180

Phil. Trans. R. Soc. Lond. B **312**, 91–100 (1985)
Printed in Great Britain

Interspecific cell markers and lineage in mammals

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[Plates 1 and 2]

Study of cell lineage in the mammalian embryo has relied heavily on the use of chimeras to follow the fate of genetically marked cells in later development. Such studies have often been limited by the types of genetic markers available; there are very few markers that allow analysis of the spatial distribution of individual cells at all stages of development. We have developed a marker system that is based on the identification of cells of *Mus musculus* origin in *M. musculus–M. caroli* chimeras by *in situ* DNA–DNA hybridization using a cloned probe to *M. musculus* satellite DNA. This provides the first ubiquitous *in situ* cell marker system for mammalian chimeras. We have recently refined the system by the use of biotin-labelled probes and detection of hybridization by streptavidin–peroxidase binding. This increases both the speed and the resolution of the assay. We have used the marker for cell lineage analysis in both embryonic and adult chimeras and results from analysis of the derivatives of early cell lineages in later development and study of coherent growth versus cell mixing in the postimplantation embryo are presented. The importance of understanding embryonic cell lineages as a prelude to molecular studies is emphasized.

Lineage marking in mammals: the problems

Cell lineage analysis in mammalian embryogenesis has not produced the same kind of detailed information available for lineage development of some other species, most notably the leech (Weisblat & Blair 1984; Zackson 1984) and *Caenorhabditis elegans* (Sulston *et al.* 1983). In these species it has proved possible to trace the fate of single defined cells in the intact embryo into later development and sometimes into the adult organism. Lineage in such systems is usually very rigid and other experimental manipulations have shown that cell fate in the intact embryo often reflects the heritable commitment of that cell at an early stage of development, particularly in the nematode system (Kimble 1981). Experimental analysis to date of mammalian embryos, particularly those of the mouse, suggests that development is much more labile and that it is not possible to define the lineage of individual cells throughout later development. However, strictly speaking, the same kind of lineage analysis that has been performed in other species has not been carried out in the mouse, for a variety of reasons.

The first reason is that it is not possible to identify specific individual cells in the early embryo and follow their lineage, because of the absence of any polarity in the cleavage stage mouse embryo by which to assign cells an unequivocal location. This problem in itself need not totally impede lineage analysis since rigid patterns of lineage allocation, if they existed, would still be discernible even if cells were marked at random and followed through development. The other problem is more serious. To date, none of the methods used to follow directly cell lineage in the intact embryo has proved applicable to mammalian development. Direct visual observation can only yield very short-term information and the lineage tracer systems used in other species

(Weisblat *et al.* 1978, 1980; Heasman *et al.* 1984) have not so far proved suitable for longer-term study in mammals. Injection of horseradish peroxidase has been used to follow cell fate from cleavage stages through to the blastocyst (Balakier & Pederson 1982) and for a few cell divisions in the endoderm layer of the postimplantation egg cylinder (K. Lawson, personal communication). However, no-one has reported that such markers can be followed from preimplantation into postimplantation development, presumably because the enormous growth that takes place just after implantation (Snow 1977) will cause rapid dilution of such extrinsic marker molecules. It is possible that marker systems of sufficient sensitivity will be developed in the future to allow this kind of direct lineage analysis in the mouse, but in the meantime a different kind of less direct approach has been used.

Genetic chimeras, in which genetically marked cells from one embryo are introduced into another embryo at an early stage of development, have been the most widely used means of investigating cell fate and cell commitment in the mammalian embryo (McLaren 1976). A variety of markers, such as coat pigmentation (Tarkowski 1964; Mintz 1967), chromosomal markers (Ford *et al.* 1975), and, most commonly, electrophoretic enzyme variants (Kidder 1981) have been used to follow the fate of genetically marked cells in chimeras. By definition, of course, cell fate in chimeras cannot be assumed to be completely analogous to cell fate in the intact embryo, since development has been disturbed by the addition of the marked cells of interest. It seems safe to assume however that the information obtained is a reasonably valid reflection of fate in the intact embryo, provided cells are returned as closely as possible to their normal environment. It is possible to reconstruct normal development fairly closely in many instances, for example, in reconstituted blastocysts (Gardner *et al.* 1973). Chimeras can be used to study cell potential without any such caveats, and such studies have provided very detailed information on the timing of cell commitment in the mammalian embryo (Rossant 1984).

Having established that chimeras can be used to study cell lineage, provided that various limitations are acknowledged, the remaining problem is to find a genetic marker system that will allow the kind of detailed spatial analysis of the distribution of marked cells in development required for full lineage analysis. Most of the markers used in analysis of mammalian chimeras are either limited in their use to certain tissues or stages of development or are only detectable after destruction of the tissue of interest. The ideal lineage marker is one that is ubiquitous, cell-autonomous, selectively neutral and detectable *in situ* in intact tissues and histological sections (McLaren 1976). In mammals, such a marker system has been hard to come by. Several enzyme activity variants have proved useful in certain limited applications (Condamine *et al.* 1971; Dewey *et al.* 1976), and recently a null enzyme variant for *Mod-1* has been described (Gardner 1984), which can be used for histochemical detection of chimerism in the extra-embryonic endoderm. It will be of interest to discover whether this marker can be used in other embryonic tissues. Serological variation at the major histocompatibility complex has also been used as a marker system (Ponder *et al.* 1983) but cannot be applied to early embryos where H-2 expression is virtually absent (Ozato *et al.* 1985). None of the interstrain mouse genetic marker systems has therefore proved to be a completely ubiquitous and infallible marker for chimera analysis.

Interspecific chimeras: a solution?

A different kind of approach has been taken to the problem of *in situ* markers in avian chimeras, and that is the use of interspecific chimeras. In particular, differences between chick

and quail are sufficient to allow nuclei of the two species to be distinguished by fairly simple staining of histological sections (Le Douarin & McLaren 1984). This approach of exploiting the larger genetic differences between than within species has also been used to generate *in situ* markers in mammals. The first interspecific marker system was developed by Gardner & Johnson using species-specific antisera to distinguish cells of rat and mouse in sections of chimeras constructed by injection of rat inner cell mass cells into mouse blastocysts (Gardner & Johnson 1973). This marker was used to confirm the lineage derivation of various tissues in the postimplantation embryo and it provided preliminary evidence of limited cell mixing in the early postimplantation embryo (Gardner & Johnson 1975). Since that time it has been shown that rat and mouse cells can be distinguished very clearly by staining with Hoechst dye (Cunha & Vanderslice 1984) and by *in situ* hybridization with species-specific repetitive DNA (Muller *et al.* 1982). Thus, there are several relatively simple ways of distinguishing rat and mouse cells. However, the marker system has not been used extensively for lineage analysis in embryonic chimeras because of questions about the relevance of development in such chimeras to development in the normal embryo. When rat–mouse chimeras were allowed to go to term in the mouse, there was very little evidence of persisting rat cells (Gardner & Johnson 1975), suggesting that rat cells were selected out by some mechanism as development proceeds. In the face of such strong selection, it is difficult to use this system for detailed analysis of clonal development.

We have taken an alternative approach using two more closely related species of rodent, *Mus musculus*, the laboratory mouse, and *M. caroli*, a wild species of mouse from SE Asia. In collaboration with Dr Verne Chapman's laboratory, we have shown that it is possible to make viable interspecific chimeras between the two species by either inner cell mass injection or embryo aggregation (Rossant & Frels 1980). These chimeras resemble intraspecific chimeras in all respects including mosaicism of the germ line (Rossant & Chapman 1983). We performed a detailed analysis of internal tissue mosaicism to determine whether there was any evidence for selection against one or other species type in these animals and the only deviations we found were in the liver and kidney of adult chimeras, where *M. musculus* cells showed a statistically significant predominance. Such skewing was not observed in any other tissue and was not evident in neonatal chimeras, suggesting that the marker system is selectively neutral at least until birth. The skewing observed in adult chimeras is no more extreme than observed for some intraspecific chimeras (Rossant & Chapman 1983), and in the absence of a congenic, ubiquitous, *in situ* marker system within the one species, it seemed justifiable to proceed with developing an *in situ* marker based on *M. musculus–M. caroli* chimeras. Such a marker was developed by *in situ* hybridization with a cloned probe to *M. musculus* satellite DNA (Siracusa *et al.* 1983). This sequence hybridized to both cell spreads and tissue sections of *M. musculus*, but showed little or no hybridization to nuclei of *M. caroli* (Rossant *et al.* 1983*a*). This is the first really ubiquitous cell marker system available in mammalian chimeras and it can be exploited for a variety of cell lineage studies in both embryogenesis and the development of the adult mouse. We have used it to determine the host origin of infiltrating cells in chemically induced tumours (unpublished work) and to confirm the clonal origin of haemopoietic colonies in the neonatal mouse liver. Other groups interested in neural cell lineages are also using the system. However, our main interest in the system is its potential use in facilitating analysis of cell lineage in embryogenesis.

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Interspecific cell marker: the methodology

In situ hybridization, particularly for detection of mRNA expression, has become a very popular technique in the last year or so. There are almost as many variations in methodology, however, as there are experimenters using the technique. For this reason, it is perhaps worth describing some of the variables we have tested in our system and emphasizing which points of protocol are essential for success. We have considerably improved our technique since first publishing the marker system. The improvements have made the system both more sensitive and more reproducible, such that we now consider that it is easy to use by any competent histologist and is also sensitive enough to identify single cells and allow analysis of serial sections.

The main constraint in use of *in situ* hybridization as a cell marker is that it is necessary to compromise between preservation of tissue for good morphology and denaturation of the DNA in the sections for good hybridization. We have found that fixation of small pieces of tissue in acetic acid:ethanol (1:3) for 4–24 h followed by embedding in low melting point, alcohol-miscible, ester wax (BDH 1960) provides reasonable morphology and allows good localization of the *in situ*-hybridized probe. We have used cryostat sections but have found localization to be poor; other fixatives such as PLG and standard paraffin wax embedding can be used but, in our hands, are not so reproducibly successful. Over-fixation of tissue can apparently interfere with the availability of the DNA for hybridization and lack of penetration of fixative into large tissue pieces can also affect success. For larger pieces of tissue, perfusion of the mouse with fixative would seem to be advisable.

Sections of 5 μm thickness are floated on to poly-L-lysine-coated slides and dried. After removal of wax, the DNA on the slides is denatured by immersing the slides for 4 min in 70% (by volume) formamide in 2 × SSC at 70 °C. Slides are then dehydrated and air-dried. We have tried various denaturation techniques including heat, alkaline and acid treatment, but this brief treatment seems to be most effective in denaturation while preserving tissue integrity and minimizing DNA loss. We perform the hybridization step in the absence of formamide, however, since we have found that prolonged incubation in the presence of this chemical tends to destroy tissue morphology. The entire plasmid containing the cloned sequence to *M. musculus* satellite DNA is nick-translated in the presence of biotin-16-UTP (Enzo Biochem) according to the manufacturer's instructions, denatured in the presence of sonicated herring sperm DNA and the hybridization mix of 3 μg ml^{-1} biotinylated probe, 250 μg ml^{-1} herring sperm DNA, 4 × SSC and 10% (by mass) dextran sulphate, is added to the slides. After hybridization overnight at 60 °C, washing is carried out at fairly high stringency to remove any cross-hybridizing sequences. Hybridization of the biotinylated probe is detected by conjugation to a streptavidin-biotinylated horseradish peroxidase complex, followed by detection of peroxidase activity using diaminobenzidine tetrahydrochloride (figure 1, plate 1). Details of the probe and the protocol can be obtained on request.

The use of a biotinylated probe in this system has many advantages over radioactively labelled probes. First, the resolution is much finer; hybridization is clearly localized to patches within the cell nucleus and background hybridization is minimal. Second, analysis is much faster, because the period of exposure of the autoradiographs is eliminated. Third, nick-translated biotinylated probes are stable much longer than those incorporating radioactivity. The ease of use of the biotin system has made it possible to contemplate much more detailed analysis of *M. musculus–M. caroli* chimeras than was considered feasible with radioactive probes.

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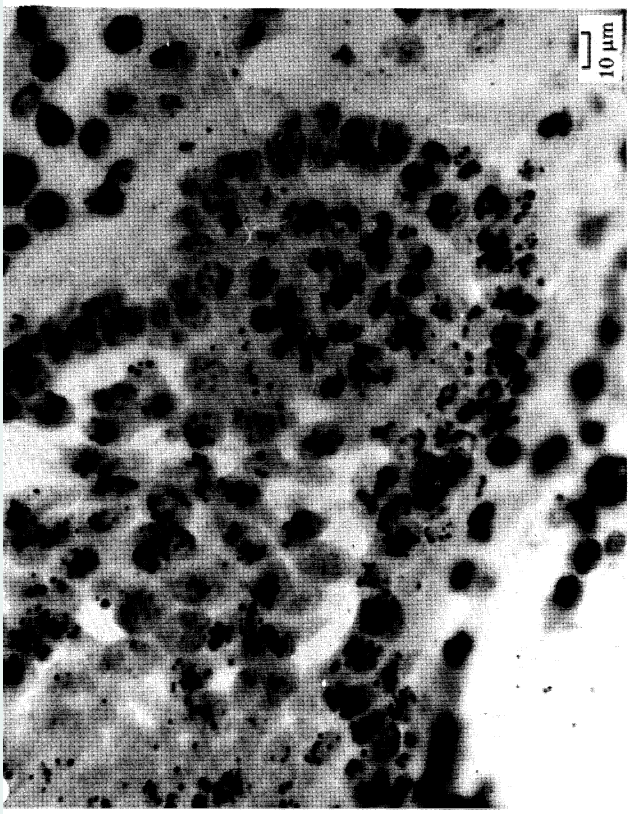

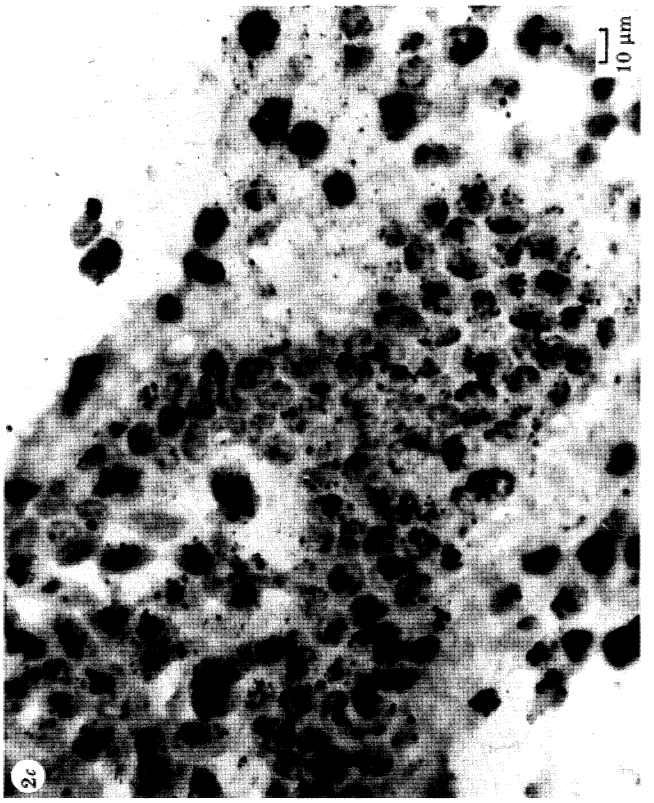

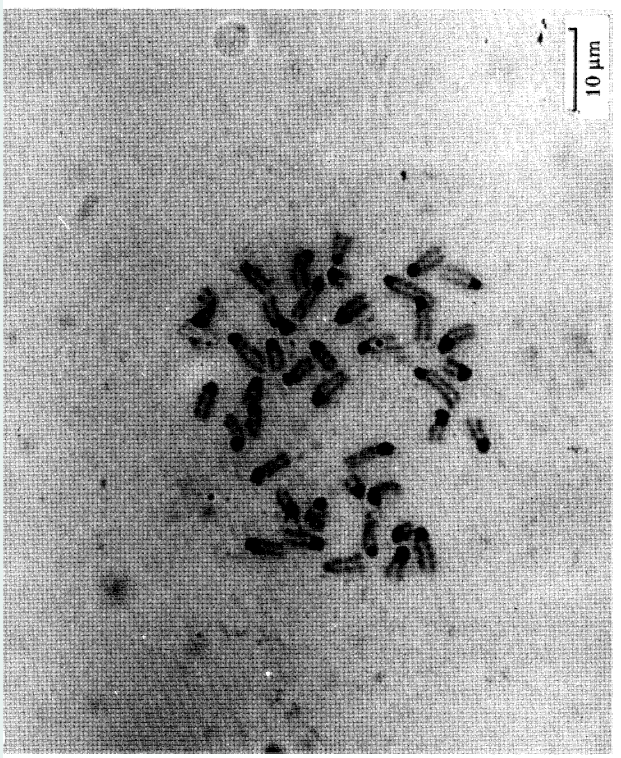

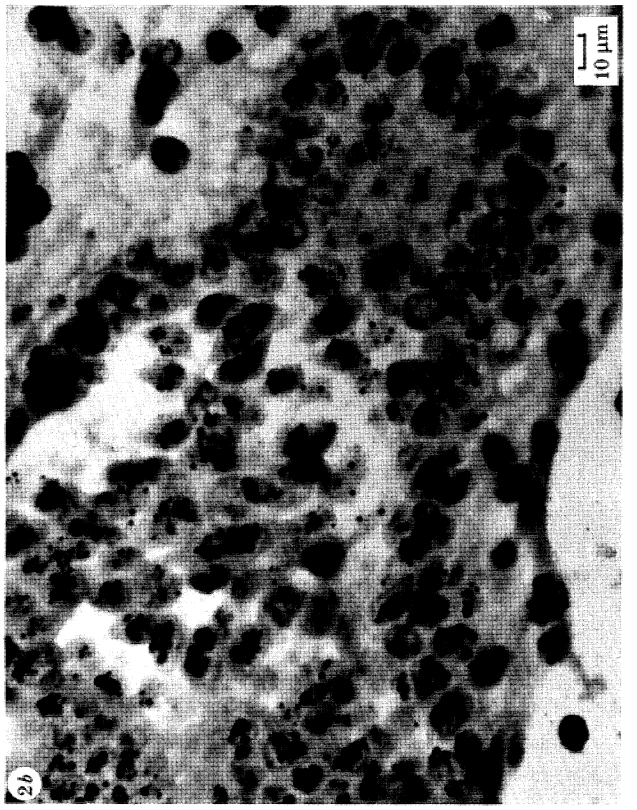

FIGURE 1. *In situ* hybridization of biotinylated probe to *M. musculus* satellite DNA on chromosomes of *M. musculus*, showing expected centromeric localization of the sequence. Dark staining of the centromeres indicates hybridization as detected by peroxidase activity.

FIGURE 2. A series of three consecutive sections of a 6.5 day chimera derived by injection of a *M. caroli* inner cell mass into a *M. musculus* blastocyst. *M. musculus* cells are identified by dark staining granules indicative of hybridization of the satellite DNA probe. A fairly coherent group of unlabelled *M. caroli* cells can be identified in both endoderm and ectoderm layers.

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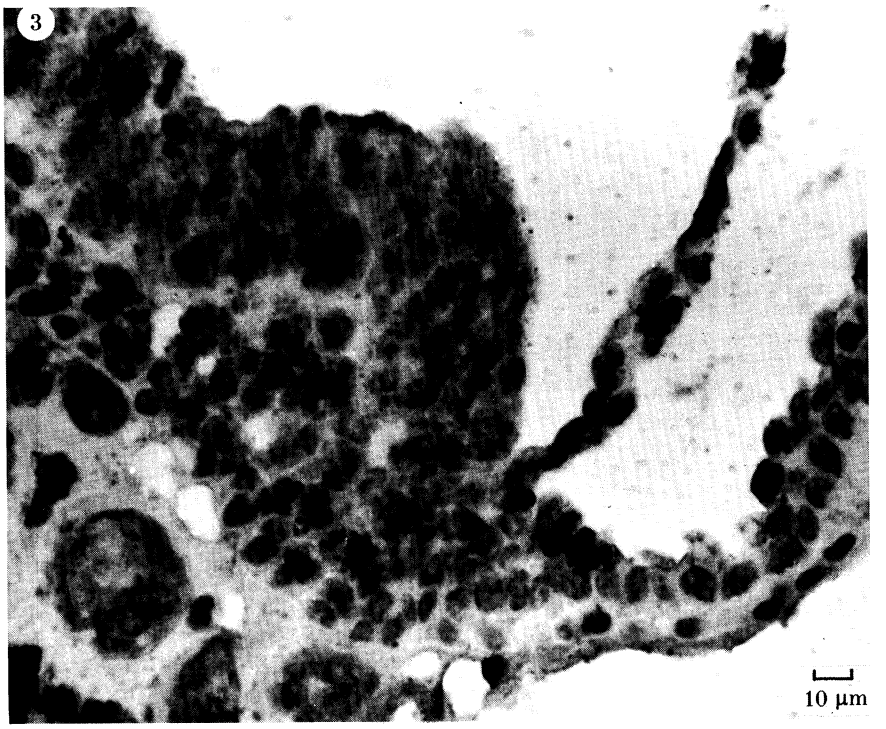

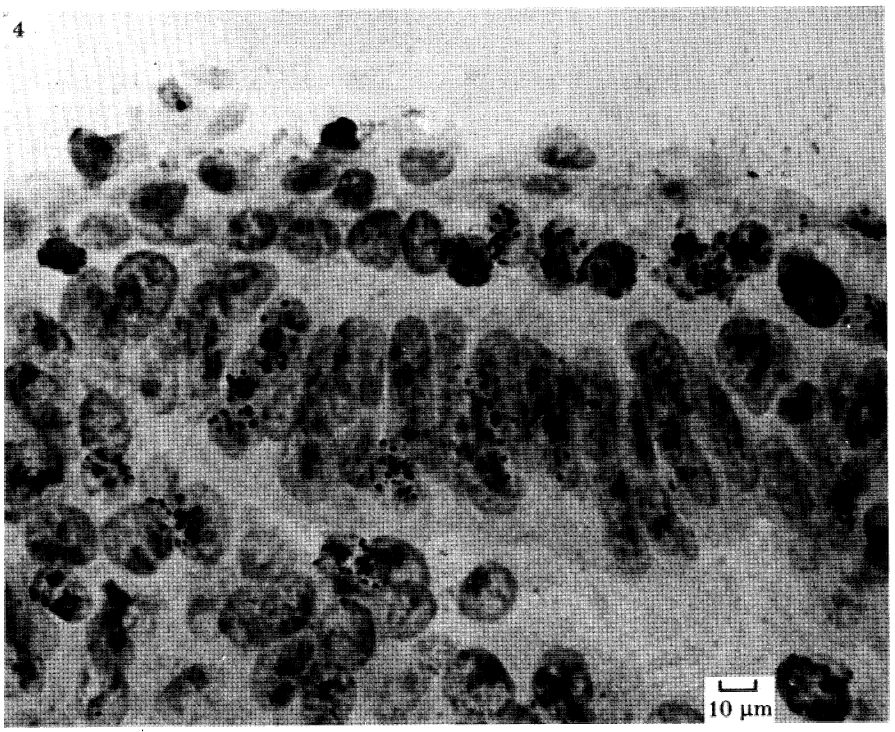

FIGURE 3. Section of a 7.5 day chimera derived by injection of a *M. musculus* inner cell mass into a *M. caroli* blastocyst. Labelled and unlabelled cells are extensively intermingled in both ectoderm and mesoderm layers.

FIGURE 4. Section of a different 7.5 day chimera of the same type as above, showing a portion of the visceral endoderm layer where labelled and unlabelled cells occur in fairly large coherent groups.

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To date, we have only used this approach to detect highly repeated satellite DNA sequences. However, with improvements in the sensitivity of detection of incorporated biotin, such as the use of alkaline phosphatase instead of horseradish peroxidase, it should prove possible to use this same kind of approach to distinguish cells which differ in sequences with much lower repeat frequencies. For example, some transgenic mouse stocks have very large numbers of inserted sequences which might be detectable by *in situ* hybridization. This would provide the possibility of developing a congenic *in situ* marker system within one mouse species, which would overcome any problems of selection associated with the use of different species or strains of mice in chimeras. In the meantime, however, we have begun to use the *M. musculus–M. caroli* system to investigate not only the fate of early cell lineages in the mouse embryo but also the question of how cells become distributed and mixed during postimplantation development. We have also recently obtained a clone of *M. caroli* DNA which appears, on preliminary screening, to be a highly repeated sequence not represented in the *M. musculus* genome (J. Rossant and G. Fraser, unpublished). This clone may provide us with a reverse marker system which would further widen the utility of our marker.

An interspecific cell marker: the results

We have used reconstituted blastocysts in conjunction with the *in situ* marker system to determine the boundaries of the inner cell mass and trophectoderm lineages in later development. Reconstituted blastocysts in which the inner cell mass was *M. caroli* and the trophectoderm was *M. musculus* were transferred back to the *M. musculus* uterus and analysed at 7.5 days of development (Rossant *et al.* 1983*a*). It was found that all of the embryonic ectoderm and overlying endoderm were of *M. caroli* inner cell mass origin, whereas the ectoplacental cone, trophoblast giant cells and extraembryonic ectoderm were entirely of *M. musculus* trophectoderm origin. This study confirmed directly for the first time the cell lineage derivations that had been predicted by using less direct markers (Gardner *et al.* 1973; Papaioannou 1982) and allowed us to show that pure lineage derivatives could be isolated for molecular analysis at this stage of development (Chapman *et al.* 1984).

After 7.5 days of development it was known that the trophectoderm derivatives, both ectoplacental cone and extraembryonic ectoderm, contributed to the structure of the mature chorioallantoic placenta (Enders 1965) but the exact extent of the trophectoderm contribution was uncertain. Various studies have used placental tissues as representative of the trophectoderm lineage (see Rossant & Croy 1985) but none has directly tested the validity of this assumption. By using a combination of embryo transfer and reconstituted blastocysts and both isozymal and *in situ* markers, we were able to show that a majority of the placenta was indeed trophectoderm in origin, but that inner cell mass and maternal tissues made up measurable proportions of the structure (Rossant & Croy 1985). The *in situ* interspecific marker was used to show that the inner cell mass contributions were confined to the labyrinthine portion of the placenta and infiltrated this tissue as cords of mesenchyme, foetal blood capillaries and endodermal sinuses. The remainder of the so-called trophoblast structure of the labyrinth was indeed of trophectoderm origin. However, we have been unable to date to isolate pure trophoblast cells from the placenta and consider that use of placenta as representative of trophoblast should be undertaken with caution.

The *in situ* marker system has, therefore, delineated very clearly the boundaries of the

trophectoderm and inner cell mass lineages throughout development. Similar information is available on the primitive ectoderm and primitive endoderm lineages from both isozymal (Gardner & Rossant 1979; Gardner 1982) and *in situ* marker studies (Gardner 1984). Less information is available on lineage allocation within the primitive ectoderm lineage after implantation. Heterotopic grafting studies on embryos in culture have indicated that cells are not committed to definite axial structures by the primitive streak stage (Beddington 1982), although orthotopic grafts often proceed to form the structures expected for the position of the graft (Beddington 1981). This suggests that cell allocation and commitment in the post-implantation lineage is not very rigid, but further information is required to determine whether there are distinct 'compartment' boundaries during normal development. Although we hope to address this directly in the postimplantation embryo by using our *in situ* marker system, some indirect information can be obtained from studying the patterns of allocation and mixing of cells in chimeras made at the blastocyst stage of development. Previous studies have shown that when chimeras are made by aggregating embryos at the eight-cell stage or by injecting whole inner cell masses into the blastocyst, there is initially very little mixing between the host and donor cell populations (Garner & McLaren 1974; Gardner & Johnson 1975). However, examination of adult mouse chimeras has revealed fine grained mosaicism in nearly all tissues examined (McLaren 1976). Clearly coherent clonal growth is not a feature of mouse development and extensive cell mixing must take place, probably at various stages of development.

We have previously reported that there is little cell mixing apparent in the early stages of postimplantation development of *M. musculus–M. caroli* chimeras (Rossant *et al.* 1983*a*), and we have recently performed a further series of injections of entire inner cell masses either of *M. caroli* origin into *M. musculus* blastocysts or vice versa and begun analysis at various days of development. At 6.5 days in either series we can trace fairly discrete groups of ectoderm and endoderm cells of one species type or the other. For example in figure 2, a series of serial sections of a 6.5 day chimera is shown in which a small but fairly discrete group of unlabelled *M. caroli* cells can be traced through the sections in both ectoderm and endoderm layers. In contrast, preliminary analysis of embryos one day later after primitive streak formation has begun, reveals evidence for considerable cell mixing within the embryonic region of both types of chimeras. An example of this is shown in figure 3, plate 2, where the progeny of an injected *M. musculus* inner cell mass are seen to be widely distributed throughout the ectoderm and mesoderm layers of the foetus. Cell mixing in the mesoderm layer is perhaps not too surprising since these cells have arisen by migration through the primitive streak. What is more surprising is the apparent mixing of cells within the ectoderm layer, which is presumed to grow as a fairly coherent columnar epithelial sheet. The endoderm layer, which also grows as an epithelial sheet, does not show so much cell mixing; there are fairly large coherent groups of cells of one genotype or the other, as shown in two dimensions in figure 4, and as also apparent in preliminary three-dimensional analysis.

It is not yet clear when and how this mixing takes place in the ectoderm layer and more detailed analysis is clearly required to confirm this finding. In particular, it should be noted that these experiments have been performed by using whole inner cell mass injections, which clearly interferes with the normal process of cell allocation in the embryo. We know that size regulation has to occur early in the postimplantation development of embryos with double size inner cell masses (Buehr & McLaren 1974) and it is possible that this process might contribute to the cell mixing that is seen. We consider this unlikely since size regulation probably occurs

by a generalized reduction in cell division rate, rather than selective cell death (Lewis & Rossant 1982). However, this can only be excluded by repeating the studies by using single cell injections where there is minimal interference with normal development. Such studies are underway and will be much aided by the development of the reverse marker system for detecting *M. caroli* cells directly.

Examination of a limited sample of later chimeras around 9.5 days of development has indicated that there may be periods of clonal growth in certain tissues following the general cell mixing that occurs at primitive streak formation. Neural tube tissue in the region of the brain at 9.5 days showed evidence of renewed clonal growth while the adjacent mesenchyme tissues showed apparently random cell mixing (Rossant *et al.* 1983 *b*). Since examination of adult brain tissue revealed fine-grained mosaicism in most areas (Rossant *et al.* 1983 *a* and unpublished), this suggests that there may be one or more later periods of cell mixing within this tissue. Clearly the development of the mammalian embryo involves a complex succession of periods of coherent growth and intervals of cell mixing and it will be virtually impossible to sort out all the events that take place during the development of given adult structures. However, we can obtain more information about the first breakdown of coherent growth and try and relate this to what is known about cell commitment in the postimplantation embryo. Evidence for extensive cell mixing at the primitive streak stage suggests that cells are not strictly allocated or committed to definitive structures at this stage, unless the cell mixing is actually a process of sorting out of precommitted precursors. This seems highly unlikely in view of all other evidence for lability of lineage in the mouse embryo and so all the evidence suggests that lineage allocation to distinct axial structures in the mouse embryo is a late event.

Cell lineages in mammalian embryos: conclusions

From the kinds of chimera study described here, combined with a variety of other experimental approaches to mammalian development, we can begin to gain an idea of the overall picture of the mode of development in the mouse embryo. What we see is a very labile system, with a great deal of regulative capacity, in which fate maps in the intact embryo cannot be drawn with great precision. Indeed, a system in which the only lineages that have been clearly defined and their progenitors identified are the two specialized extraembryonic lineages established in the preimplantation embryo. It is also a system in which cell potential often exceeds cell fate. A system, then, that looks very different from the invariant cell lineages of many invertebrate species. The lack of reproducible cell lineage within the developing embryo seems, however, to be a fairly general feature of vertebrate development. A recent study on cell lineage in the Zebrafish embryo has found evidence for variable cell lineage in the intact embryo and extensive cell mixing during development (Kimmel & Law 1985). Other vertebrate species, like *Xenopus*, show fairly coherent lineage development in the intact embryo suggestive of compartment-like boundaries (Hirose & Jacobson 1979; Jacobson 1983). However, cell grafting experiments have shown that cell potential often exceeds cell fate in these lineages, (Gimlich & Cooke 1983; Heasman *et al.* 1984) so that here too commitment to lineage is a relatively late event in embryogenesis. It appears as though there is a range of options available for regulating early process of embryonic development, from the situation in species like *C. elegans*, where lineage is rigid and often reflects heritable commitment, through species like

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Xenopus, where lineage is fairly rigid but commitment is more labile, to mammals where lineage is not fixed in the early embryo and cell commitment is also very labile.

What does all this imply for the genetic control of development? The two species in which the genetic control of development is best defined are *Drosophila* and *C. elegans*, where compartments and lineages have been shown to be under genetic control (Greenwald *et al.* 1983; Gehring 1985). In mammals, we have no direct information on genes that control lineage development, or even that they necessarily exist. Perhaps that is not surprising when we realize that we do not actually have very much information on cell lineages *per se*, especially in the pluripotent primitive ectoderm pathway giving rise to the definitive foetus. Improved *in situ* markers, such as the one described here, combined with cell grafting experiments in postimplantation embryos and injection of defined postimplantation cells into the blastocyst will hopefully extend our knowledge of such lineages. The importance of such studies is emphasized by the recent discovery that there are mammalian genes containing sequences homologous to the homoeo boxes in *Drosophila* (McGinnis *et al.* 1984). The possibility that such genes may function in an analogous manner to homoeotic genes in controlling lineage development has generated much excitement (Gehring 1985). However, such conserved genes would be unlikely to control the formation of uniquely mammalian lineages, such as the extraembryonic lineages, but would be more likely to act within the embryonic lineage itself, where we are currently woefully ignorant of lineage development.

One is left, therefore, with a paradox: the lineages we understand most about in the mammalian embryo, the trophectoderm and the primitive endoderm, are those that have no counterpart in other systems. It is valid to use the development of these lineages as a model for lineage commitment in general? Although these lineages have some special properties relating to their specialized role in interacting with the maternal environment, the processes of forming the trophectoderm and primitive endoderm lineages are very similar to the establishment of polyclones (Morata & Lawrence 1976) or equivalence groups (Sulston *et al.* 1983) in other systems. Groups of progenitor cells are committed by cell–cell interactions to a particular set of options in development, but single cells within each lineage are not necessarily committed to a given structure. Indeed, the combination of early identification of progenitors, ease of manipulation of single cells before and after cell commitment and the delineation of distinct derivatives in later embryogenesis makes the establishment of these lineages an attractive model system for studying the mechanisms of lineage commitment in general.

The author's own work described here was supported by operating grants from the Natural Sciences and Engineering Research Council and the Medical Research Council of Canada, and an E. W. R. Steacie Memorial Fellowship from N.S.E.R.C.

References

Balakier, H. & Pedersen, R. A. 1982 Allocation of cells to inner cell mass and trophectoderm lineages in preimplantation mouse embryos. *Devl. Biol.* **90**, 352–362.

Beddington, R. S. P. 1981 An autoradiographic analysis of the potency of embryonic ectoderm in the 8th day postimplantation mouse embryo. *J. Embryol. exp. Morph.* **64**, 87–104.

Beddington, R. S. P. 1982 An autoradiographic analysis of tissue potency in different regions of the embryonic ectoderm during gastrulation in the mouse. *J. Embryol. exp. Morph.* **69**, 265–285.

Buehr, M. & McLaren, A. 1974 Size regulation in chimaeric mouse embryos. *J. Embryol. exp. Morph.* **31**, 229–234.

Chapman, V., Forrester, L., Sanford, J. & Rossant, J. 1984 Cell lineage specific undermethylation of mouse repetitive DNA. *Nature, Lond.* **307**, 284–286.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Condamine, H., Custer, R. P. & Mintz, B. 1971 Pure-strain and genetically mosaic liver tumors histochemically identified with the β-glucuronidase marker in allophenic mice. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2032–2036.
Cunha, G. R. & Vanderslice, K. D. 1984 Identification in histological sections of species origin of cells from mouse, rat, and human. *Stain Technol.* **59**, 7–12.
Dewey, M. J., Gervais, A. G. & Mintz, B. 1976 Brain and ganglion development from two genotypic classes of cells in allophenic mice. *Devl. Biol.* **50**, 68–81.
Enders, A. C. 1965 A comparative study of the fine structure of the trophoblast in several haemochorial placentas. *J. Anat.* **116**, 29-67.
Ford, C. E., Evans, E. P. & Gardner, R. L. 1975 Marker chromosome analysis of two mouse chimaeras. *J. Embryol. exp. Morph.* **33**, 447–457.
Garner, W. & McLaren, A. 1974 Cell distribution in chimaeric mouse embryos before implantation. *J. Embryol. exp. Morph.* **32**, 495-503.
Gardner, R. L.,Papaioannou, V. E. & Barton, S. C. 1973 Origin of the ectoplacental cone and secondary giant cells in mouse blastocysts reconstituted from isolated trophoblast and inner cell mass. *J. Embryol. exp. Morph.* **30**, 561-572.
Gardner, R. L. 1982 Investigation of cell lineage and differentiation in the extraembryonic endoderm of the mouse embryo. *J. Embryol. exp. Morph.* **68**, 175–198.
Gardner, R. L. 1984 An *in situ* cell marker for clonal analysis of development of the extraembryonic endoderm in the mouse. *J. Embryol. exp. Morph.* **80**, 251–288.
Gardner, R. L. & Johnson, M. H. 1973 Investigation of early mammalian development using interspecific chimaeras between rat and mouse. *Nature, new Biol.* **246**, 86–89.
Gardner, R. L. & Johnson, M. H. 1975 Investigation of cellular interaction and deployment in the early mammalian embryo using interspecific chimaeras between the rat and mouse. In *Cell patterning* (Ciba Foundation Symp. 29), pp. 183–200. Amsterdam: Elsevier.
Gardner, R. L. & Rossant, J. 1979 Investigation of the fate of 4.5 day post-coitum mouse inner cell mass cells by blastocyst injection. *J. Embryol. exp. Morph.* **52**, 141–152.
Gehring, W. J. 1985 The homeo box: a key to the understanding of development? *Cell* **40**, 3–5.
Gimlich, R. L. & Cooke, J. 1983 Cell lineage and the induction of second nervous systems in amphibian development. *Nature, Lond.* **306**, 471–473.
Greenwald, I. S., Sternberg, P. W. & Horwitz, H. R. 1983 The *lin-12* locus specifies cell fates in *Caenorhabditis elegans*. *Cell* **34**, 435–444.
Heasman, J., Wylie, C. C., Hansen, P. & Smith, J. C. 1984 Fates and states of determination of single vegetal pole blastomeres of *X. laevis*. *Cell* **37**, 185–194.
Hirose, G. & Jacobson, M. 1979 Clonal organization of the central nervous system of the frog. I. Clones stemming from individual blastomeres of the 16-cell and earlier stages. *Devl. Biol.* **71**, 191–202.
Jacobson, M. 1983 Clonal organization of the central nervous system of the frog. III. Clones stemming from individual blastomeres of the 128-, 256- and 512-cell stages. *J. Neurosci.* **3**, 1019–1038.
Kidder, G. M. 1981 The analysis of mammalian development using allelic isozyme variants. *Devl. Genet.* **2**, 385–410.
Kimble, J. 1981 Lineage alteration after ablations of cells in the somatic gonad of *Caenorhabditis elegans*. *Devl. Biol.* **87**, 286–300.
Kimmel, C. B. & Law, R. D. 1985 Cell lineage of Zebrafish blastomeres. III. Clonal analysis of the blastula and gastrula stages. *Devl. Biol.* **107**, 94–101.
Le Douarin, N. & McLaren, A. 1984 *Chimeras in developmental biology*. New York: Academic Press.
Lewis, N. E. & Rossant, J. 1982 Mechanism of size regulation in mouse embryo aggregates. *J. Embryol. exp. Morph.* **72**, 169–181.
McGinnis, W., Hart, C. P., Gehring, W. H. & Ruddle, F. H. 1984 Molecular cloning and chromosome mapping of a mouse DNA sequence homologous to homeotic genes of *Drosophila*. *Cell* **38**, 675–680.
McLaren, A. 1976 *Mammalian chimaeras*. Cambridge University Press.
Mintz, B. 1967 Genetic control of mammalian pigmentary differentiation. I. Clonal origin of melanocytes. *Proc. natn. Acad. Sci. U.S.A.* **58**, 344-351.
Morata, G. & Lawrence, P. A. 1976 Homoeotic genes, compartments and cell lineages in *Drosophila*. *Nature, Lond.* **265**, 211–216.
Muller, U., Singh, L., Grund, S. & Jones, K. W. 1982 Ovarian cells participate in the formation of tubular structures in mouse/rat heterosexual gonadal cocultures: a direct demonstration by *in situ* hybridization. *Differentiation* **22**, 138–148.
Ozato, K., Wan, Y.-J. & Orrison, B. M. 1985 Mouse major histocompatibility Class I gene expression begins at mid-somite stage and is inducible in earlier-stage embryos by interferon. *Proc. natn. Acad. Sci. U.S.A.* **82**, 2427–2431.
Papaioannou, V. E. 1982 Lineage analysis of inner cell mass and trophectoderm using microsurgically reconstituted mouse blastocysts. *J. Embryol. exp. Morph.* **68**, 199–209.
Ponder, B. A. J., Wilkinson, M. M. & Wood, M. 1983 H-2 antigens as markers of cellular genotype in chimaeric mice. *J. Embryol. exp. Morph.* **76**, 83–93.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Rossant, J. 1984 Somatic cell lineages in mammalian chimeras. In *Chimeras in developmental biology* (ed. N. Le Douarin & A. McLaren), pp. 89–109. New York: Academic Press.

Rossant, J. & Chapman, V. E. 1983 Somatic and germ-line mosaicism in inter-specific chimaeras between *M. musculus* and *M. caroli*. *J. Embryol. exp. Morph.* **73**, 193–205.

Rossant, J. & Croy, B. A. 1985 Genetic identification of tissue of origin of cellular populations within the mouse placenta. *J. Embryol. exp. Morph.* (In the press.)

Rossant, J. & Frels, W. I. 1980 Interspecific chimeras in mammals; successful production of live chimeras between *Mus musculus* and *M. caroli*. *Science, Wash.* **208**, 419–421.

Rossant, J., Vijh, K. M., Siracusa, L. D. & Chapman, V. M. 1983*a* Identification of embryonic cell lineages in histological sections of *M. musculus–M. caroli* chimaeras. *J. Embryol. exp. Morph.* **73**, 179–191.

Rossant, J., Croy, B. A., Clark, D. A. & Chapman, V. M. 1983*b* Interspecific hybrids and chimeras in mice. *J. exp. Zool.* **228**, 223–233.

Siracusa, L. D., Chapman, V. M., Bennett, K. L., Hastie, N. D., Pietras, D. F. & Rossant, J. 1983 Use of repetitive DNA sequences to distinguish *Mus musculus* and *Mus caroli* cells by *in situ* hybridization. *J. Embryol. exp. Morph.* **73**, 163–178.

Snow, M. H. L. 1977 Gastrulation in the mouse: growth and regionalization of the epiblast. *J. Embryol. exp. Morph.* **42**, 293–303.

Sulston, J. E., Schierenberg, E., White, J. G. & Thomson, J. N. 1983 The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Devl. Biol.* **100**, 64–119.

Tarkowski, A. K. 1964 Patterns of pigmentation in experimentally produced mouse chimaeras. *J. Embryol. exp. Morph.* **12**, 575–585.

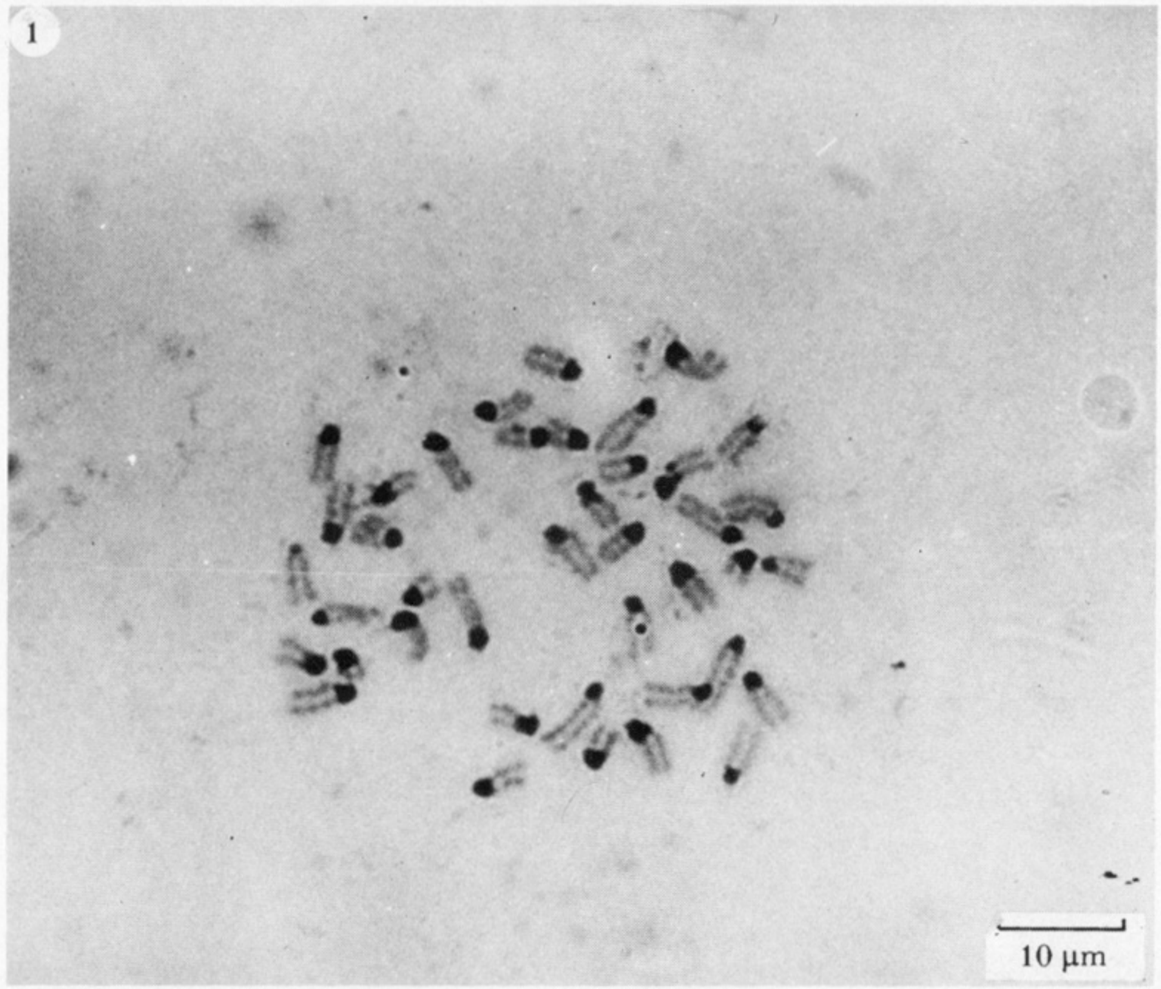
Weisblat, D. A. & Blair, S. S. 1984 Developmental interdeterminacy in embryos of the leech *Helobdella triserialis*. *Devl. Biol.* **101**, 326–335.

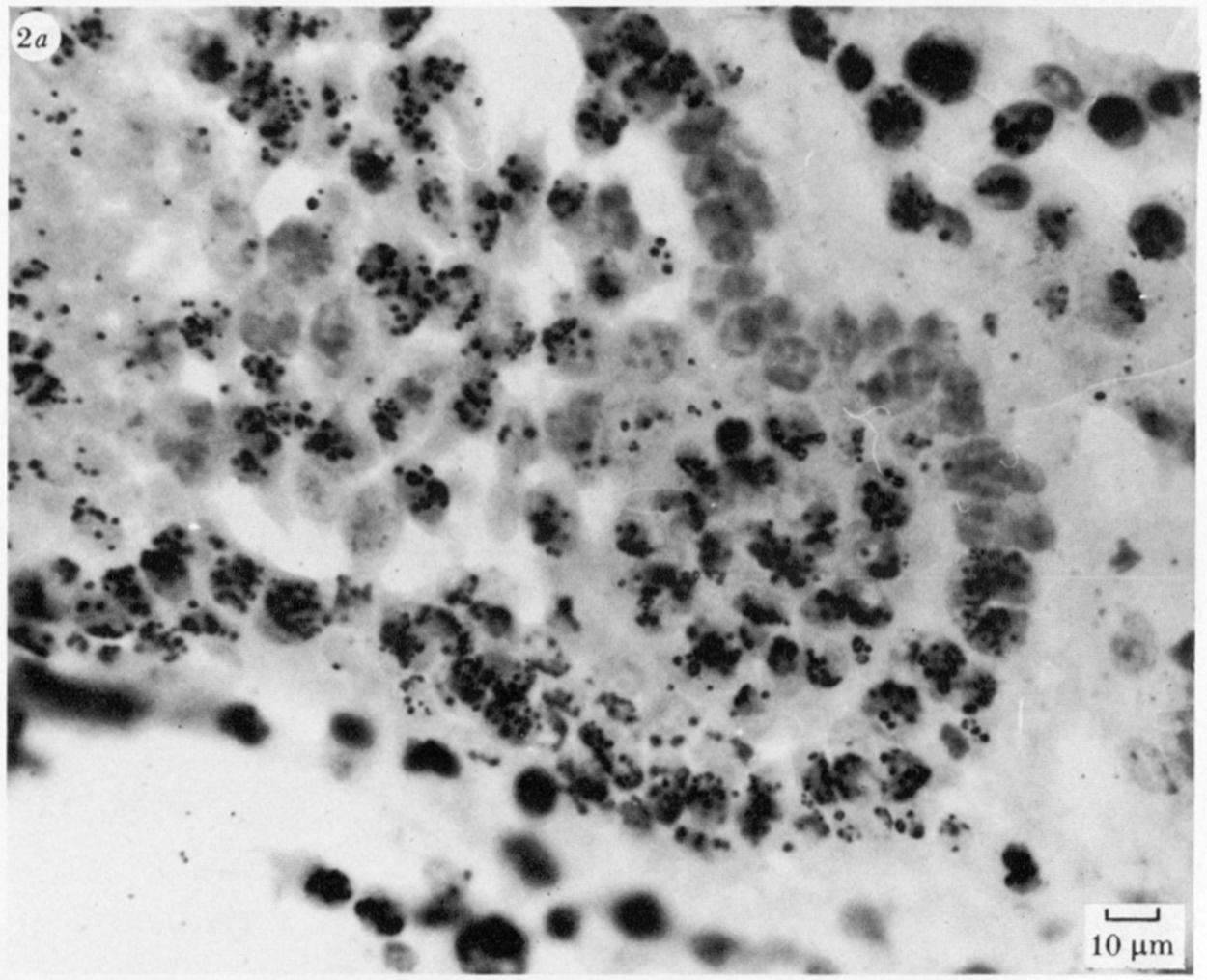
Weisblat, D. A., Sawyer, R. T. & Stent, G. S. 1978 Cell lineage analysis by intracellular injection of a tracer enzyme. *Science, Wash.* **202**, 1295–1298.

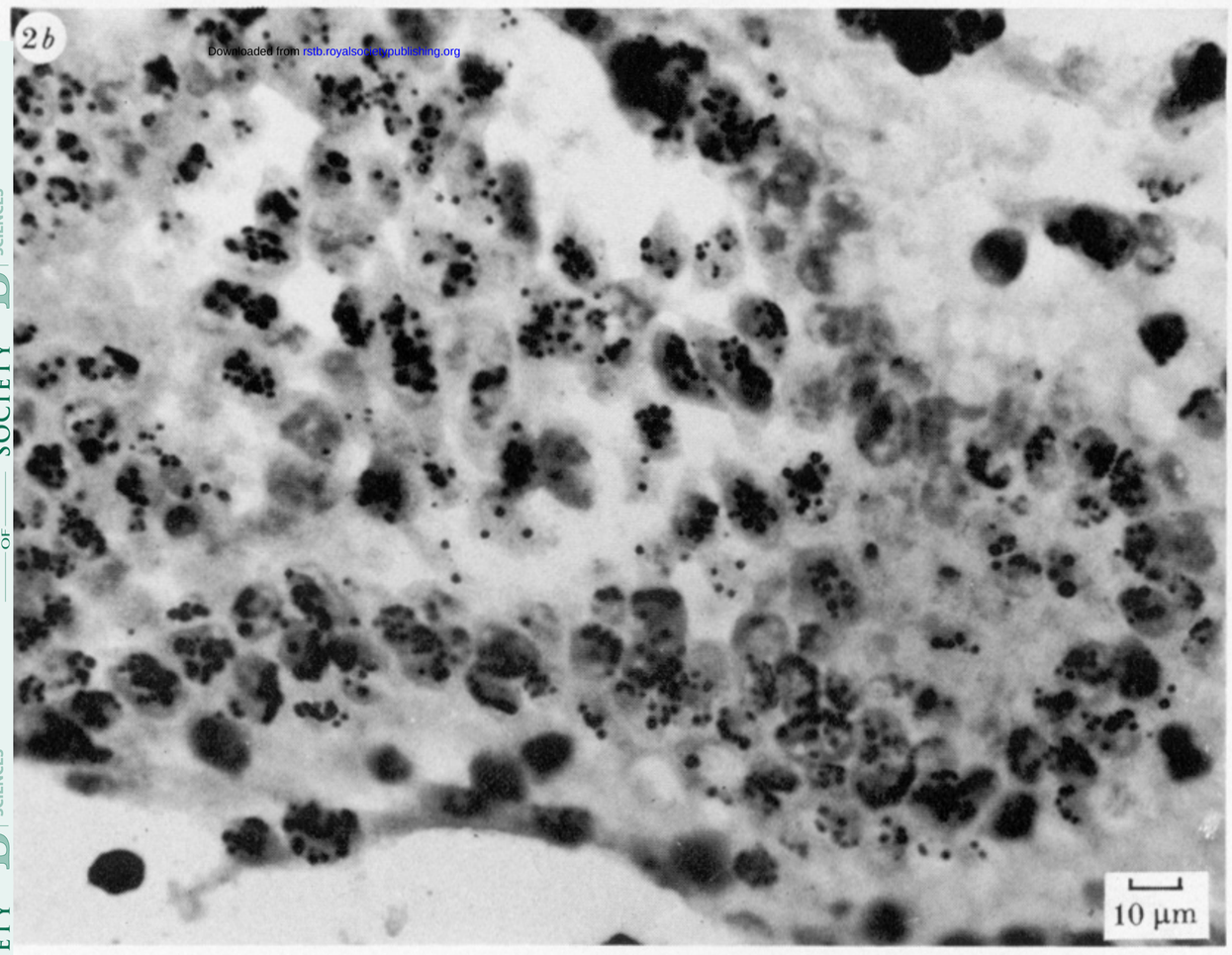
Weisblat, D. A., Zackson, S. L., Blair, S. S. & Young, J. D. 1980 Cell lineage analysis by intracellular injection of fluorescent tracers. *Science, Wash.* **209**, 1538-1541.

Zackson, S. L. 1984 Cell lineage, cell–cell interaction and segment formation in the ectoderm of a glossiphoniid leech embryo. *Devl. Biol.* **104**, 143–160.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES







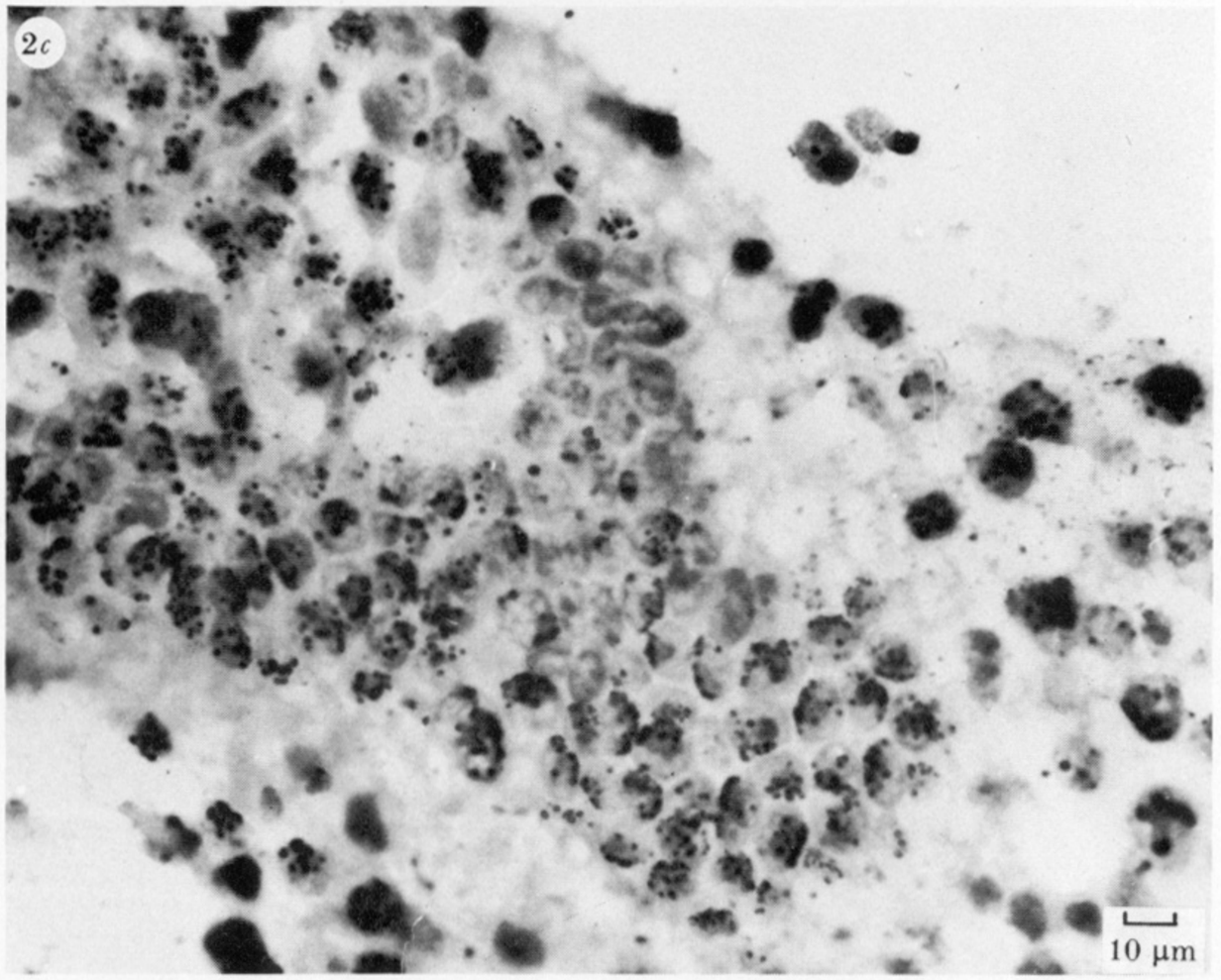


FIGURE 1. *In situ* hybridization of biotinylated probe to *M. musculus* satellite DNA on chromosomes of *M. musculus*, showing expected centromeric localization of the sequence. Dark staining of the centromeres indicates hybridization as detected by peroxidase activity.

FIGURE 2. A series of three consecutive sections of a 6.5 day chimera derived by injection of a *M. caroli* inner cell mass into a *M. musculus* blastocyst. *M. musculus* cells are identified by dark staining granules indicative of hybridization of the satellite DNA probe. A fairly coherent group of unlabelled *M. caroli* cells can be identified in both endoderm and ectoderm layers.

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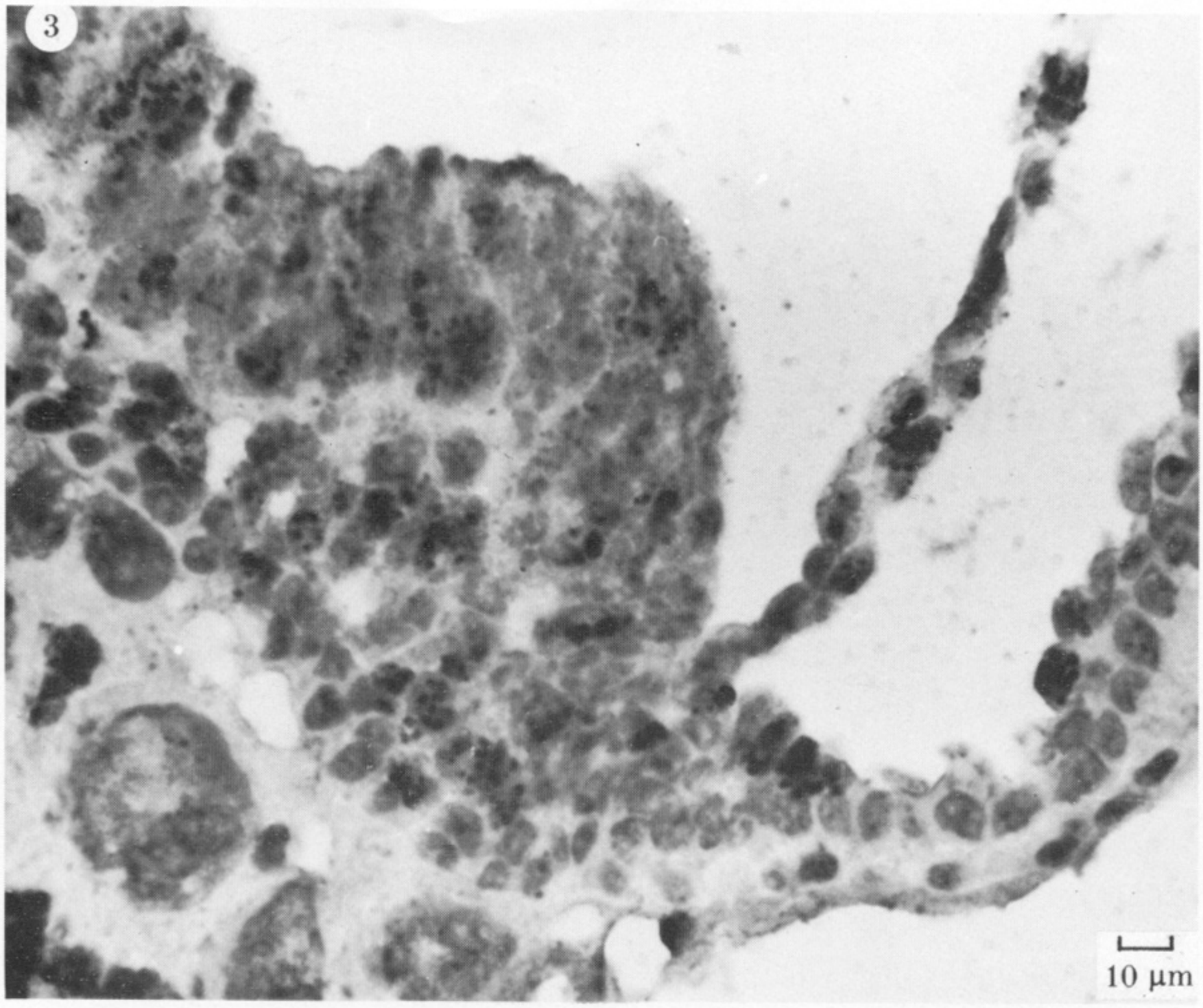

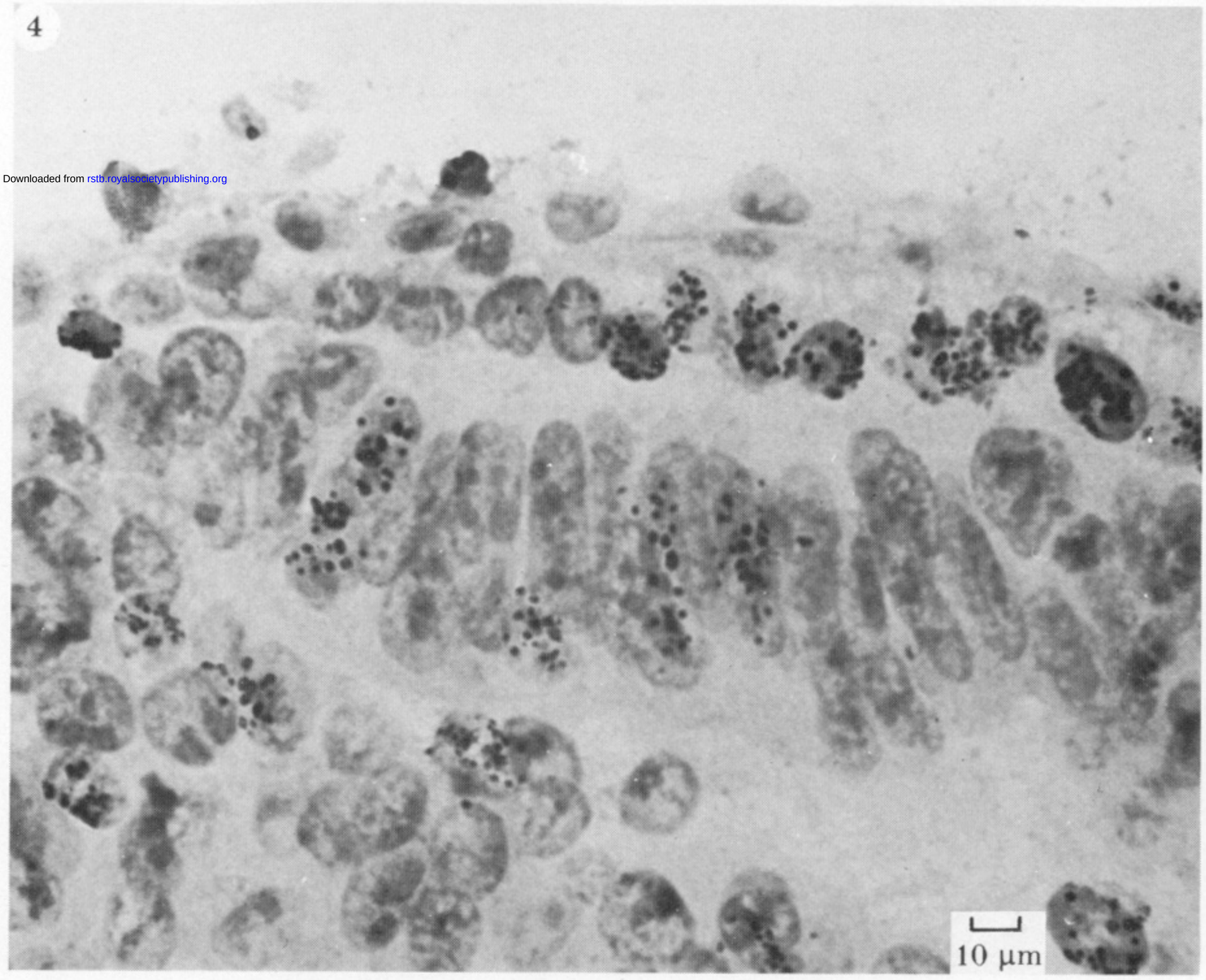

FIGURE 3. Section of a 7.5 day chimera derived by injection of a *M. musculus* inner cell mass into a *M. caroli* blastocyst. Labelled and unlabelled cells are extensively intermingled in both ectoderm and mesoderm layers.

FIGURE 4. Section of a different 7.5 day chimera of the same type as above, showing a portion of the visceral endoderm layer where labelled and unlabelled cells occur in fairly large coherent groups.