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Development of Rat Dorsal Root Ganglion Neurones

Studies of Cell Birthdays and Changes in Mean Cell Diameter*

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Summary. Pulse labelling with tritiated thymidine was used to determine the time of the final division of the neuroblasts which subsequently form rat lumbar dorsal root ganglion neurones. The final division occurred during a 4 day period, the maximum frequency being on day 12 of gestation. Separation of the ganglion cells into large light neurones and small dark neurones showed that the large light neurones were formed earlier than the small dark neurones. In both cases the final divisions occurred over a period of 3–4 days, but the peak rate of formation of large neurones was on day 12, and that of the small neurones was on day 13.

Low power electron micrographs were used to measure mean cell diameter throughout development from day 11 of gestation until a postnatal age of 225 days. A marked increase in cell diameter occurred on day 15–15.5, about 3 days after the final cell divisions of the majority of the cells. The rate of growth increased just before birth, but no increase in mean cell diameter was found between day 21 of gestation and the third day postnatal. The growth was again rapid after this period until a plateau in cell diameter was reached about 33 days after birth.

Key words: Spinal ganglia (Rat) — Cell division — Autoradiography — Neurone morphogenesis — Light and electron microscopy.

Introduction

Various aspects of the *in vivo* development of dorsal root ganglion (DRG) neurones have been investigated in several mammalian species. The morphological changes in the neuroblasts of immature neurones have been studied using light microscopic methods (Angulo, 1951; Sobkowicz *et al.*, 1973) and with the electron microscope (Tennyson, 1965), whilst the histochemical development in these cells has also been described (Kalina and Wolman, 1970; Sarrat, 1970). However the relationship between these structural and histochemical changes and the timing of the birthdays (that is, the day of the last division of the neuroblast) of the DRG neurones has not yet been established. We have therefore undertaken to compare the timing of cell birthdays of DRG neurones in the rat with the increases in mean cell diameter during development since size gives an indication of the state of maturation of cells and provides a point of quantitative comparison between cells of different ages.

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Several authors including those mentioned above, have distinguished two or more neuronal types in the adult on the basis of their appearance using the light microscope (Hatai, 1902; Preto Parvis, 1954), the electron microscope (Hess, 1955; Yamadori, 1970) or histochemical methods (Kalina and Wolman, 1970). We have identified two major cell types, large light and small dark neurones, and have determined their birthdays autoradiographically.

Cell birthdays were ascertained using the autoradiographic technique of pulse labelling with tritiated thymidine (^{3}HTdR). This technique has been widely used for such studies in the CNS and is well documented (Miale and Sidman, 1961; Angevine, 1965; Pierce and Sweet, 1967; Rakic and Sidman, 1968). The parallel study on the changes in mean cell diameter through foetal and postnatal life was carried out using electron microscopic techniques.

Materials and Methods

Throughout these experiments, Porton strain rats were used. The times of mating were recorded by taking vaginal smears each morning. The day on which sperm were found was designated day 0 of gestation. Since the majority of rats in this medical school mate near dawn, the time of mating is assumed to be accurate to within 6 hours.

Autoradiography. Seven pregnant rats were given a single intraperitoneal injection of 5 μc/gm body weight tritiated thymidine (^{3}HTdR, Amersham, specific activity >40 c/mM), in isotonic saline. The injections were given at 10.5, 11.5, 12.5, 13.5, 15.5, 17.5 and 19.5 days of gestation. The time at which the rats gave birth was noted to within ± 6 hours and the dorsal root ganglia (DRG) were dissected from the lumbar region (L_3–L_6) of the 33 days old offspring. Control tissue was obtained from 33 day old offspring of uninjected mothers. The ganglia were placed in Carnoys fixative at 0°C and paraffin embedded.

Autoradiographs were prepared by dip-coating dewaxed 5 μm thick sections of the tissue on glass slides in a 1:1 dilution of Ilford K2 emulsion at 43°C. A hydraulically controlled device was used to withdraw the slides from the emulsion at an even rate and thus provide emulsion coats of fairly uniform thickness. The emulsion was slowly dried as described by Rogers (1973) and autoradiographs were exposed for 42 days at 1°C in an atmosphere of CO_2; they were developed in D19B developer and fixed in 25% hypo. Ehrlich's haematoxylin was used to post-stain the tissue.

Grain Counts: An inverted Nikon microscope with a X90 oil immersion objective was used for grain counting. Grains overlying neuronal nuclei were counted visually within an area defined by an annulus of light (approximately the diameter of the larger nuclear sections) projected onto the cell being counted and centred over the nucleus. Any cell section containing no nucleus was ommitted from the count. Background grains were counted through the same annulus either over the nuclei of control cells or over areas of emulsion alone around the experimental tissue. Thus the background and experimental areas counted were exactly the same size.

A minimum of 1400 neurones were counted for each injection time with the exception of the tissue labelled at 10.5 days, when only 750 cells were counted. The frequency distributions of grains over all ganglia on any one slide were averaged. That is, the numbers of cells in each grain frequency bin were added and divided by the number of cells (100–300) counted on each slide. The numbers in each bin were expressed as percentages and for each litter the percentage grain distributions from a total of 4–9 slides were combined to give final percentages for the frequency distributions of grain counts.

Differential cell count: a graticule was projected onto the cells being counted. Neurones which were less than 17 μm diameter were counted as small neurones (see Fig. 1) and those with diameters greater than 23 μm were counted as large neurones. The grain counts per nucleus for each cell type were recorded, having been counted as above within an area the approximate size of the annulus of light. The total numbers of grains over satellite cell nuclei were counted but there the annulus was not used since the cells were ovoid and also because

several cells could be contained within the annulus. About 400 cells from each cell type and for each injection time were counted in this manner.

Electron Microscopy. The reasons for using electron microscopy rather than light microscopy for this work are the lack of shrinkage of tissue processed for electron microscopy, the increased resolution at low magnification offered by electron microscopy (see Fig. 1) and the range in magnifications needed. (200×–12000×).

DRG from about 50 rats of varying ages (from 11 days of gestation up to 225 days postnatal) were studied by electron microscopy. The normal length of gestation is 22–23 days. Pregnant rats were killed and the uterine horns complete with foetuses were removed and placed in warm Krebs' solution. L_3–L_6 DRG from several embryos were placed in 2.5% glutaraldehyde in cacodylate buffer for 2 hours. Following post-fixation in 1% osmium the tissue was dehydrated and embedded in Epon 812. When postnatal rats were used, the day on which they were born was designated day 0 postnatal. The postnatal tissue was obtained after anaesthetising the rats with nembutal (approx. 50 mg/kg i.p.). A laminectomy was performed and the L_3–L_6 ganglia were quickly removed and processed as described above. Thin sections of ganglia were picked up on formvar coated single slot grids and stained with uranyl acetate followed by lead citrate. These grids enabled the whole ganglion to be photographed at low power by the Phillips EM 300.

Neuronal cell diameters were measured on electron micrographs using a Zeiss Particle Size Analyser. The average ratio of the largest to the smallest diameter of a neuronal section has been shown to be less than 2 in cats and rabbits by Pannese *et al.* (1972) who on this basis assumed neurones to be spherical. We have made the same assumption for the more mature neurons in our study. However, electron micrographs show that this assumption is not valid for foetal tissue 15.5 days and younger. Only cell sections showing nuclei were measured since it was found that the nucleus of the mature DRG cell is in approximately the centre of a roughly spherical cell (see Pannese *et al.*, 1972 and Fig. 1A) and in the more mature neurones this method of selection should reduce the number of cell sections with diameters much smaller than the maximum cell diameter being counted. Histograms were obtained from the Particle Size Analyser and from this data the mean diameters, standard deviations and standard errors were calculated and used to plot the graph of mean diameter against age shown in Fig. 5. For each age 104–262 (mean 160) neurone diameters were measured.

Results

Before discussing the results in detail some general comments will be made on the autoradiographic method and some terms defined.

Autoradiographic Data

Grain Counts. The counting method used yielded grain frequency distributions over experimental tissue which were not Poisson in nature. If a nucleus with tritium randomly distributed throughout is sectioned, different sections will have different volumes and surface areas. This means that the random distribution is not being examined in fixed intervals and the distributions of radioactivity in the different sections cannot be in the form of a Poisson distribution and therefore neither can the grain counts. The method of analysis of grain counts utilising the mathematics of a Poisson distribution described by England *et al.* (1973) and Rogers (1973) is therefore not applicable. We have used the simpler method of applying arbitrary limits of labelling intensity to our results (see below).

Pulse labelling experiments can only label cells which are in the S phase of the cell cycle at the time of the pulse. Since neither the ratio time in S: cell cycle time, nor the exact availability time of ^{3}HTdR is known for this tissue it is not realistic to treat the results as quantitative. However, if all the data are treated

identically and the correct controls are included it is possible to compare accurately the incorporation into DNA of ^{3}HTdR given at different times. Since the results are comparative, arbitrary limits of which grain counts can be considered positive (heavily of lightly labelled) or negative can therefore be decided by the investigator. The precedents for this are well established (Angevine, 1965; Rakic and Sidman, 1968; Haas *et al.*, 1970; Rakic, 1973).

All cells in this experiment will fall into one of three categories:

1. Not significantly labelled.
2. Lightly labelled } significantly labelled.
3. Heavily labelled }

Significant Labelling: silver grains over a nucleus can be caused by background or by the radioactive isotope in the nucleus, or a combination of the two. If there is a probability of $<.05$ that all the grains are part of the background, the cell will be called significantly labelled. The percentage of background areas over emulsion alone with 2 or more, 3 or more grains etc. was found for each injection time. It was found that the lowest grain count which had a probability of $<.05$ of being entirely due to background was 4 grains per nucleus for injection times 10.5 and 12.5 days (batch 2 of autoradiographs) and 3 grains per nucleus for all the other times (batch 1) and these grain counts were therefore necessary for a cell to be considered labelled.

Heavy and Light Labelling: significantly labelled cells will be split into 2 groups, heavily and lightly labelled. In batch 1 heavily labelled cells are those with $\geqslant 7$ grains per nucleus. This number was used since six is the highest number of grains recorded over any neuronal nucleus in a group of cells where no very heavy labelling occurs, (that is, in the tissue labelled on days 17 and 19). Lightly labelled cells are therefore those with 3–6 grains. In batch 2, cells with 8 grains will be called heavily labelled since one more grain was necessary for significant labelling than in batch 1, and the effect of background is a simple additive one. Cells with 4–7 grains will be called lightly labelled.

Heavy labelling will be taken to indicate that a cell was nearing or in its final division at the time ^{3}HTdR was available. However, cells appear to have been lightly labelled by more than one process (see below).

Cell Birthdays. An example of an autoradiograph is shown in Fig. 1 B in which two cells can be seen to be heavily labelled. One is a large neurone, the other a small neurone (see below). The tissue was from a 33 day postnatal rat given ^{3}HTdR on the 13th day of gestation. Changes in the percentage of significantly

Fig. 1 A—D. Lumbar dorsal root ganglion cells of 33 day old rats. (A) Low power electron micrograph, magnification $\times 750$. Small, electron dense neurones (marked *S*) can be compared with the larger lighter neurones (marked *L*). (B) Autoradiograph. The tissue was exposed to ^{3}HTdR on the 13th day of gestation. The shrinkage is due to the Carnoy's fixative. Magnification $\times 750$. In the differential count, cells smaller than the small bar (17 μm) were counted as small, cells larger than the long bar (23 μm) were counted as large. (C) Shows cytoplasm of a large light cell at $\times 12000$. The cell was approx. 40 μm in diameter. (D) Shows cytoplasm of a small dark cell approx. 16 μm in diameter. Magnification $\times 12000$. For description of the features of C and D see text

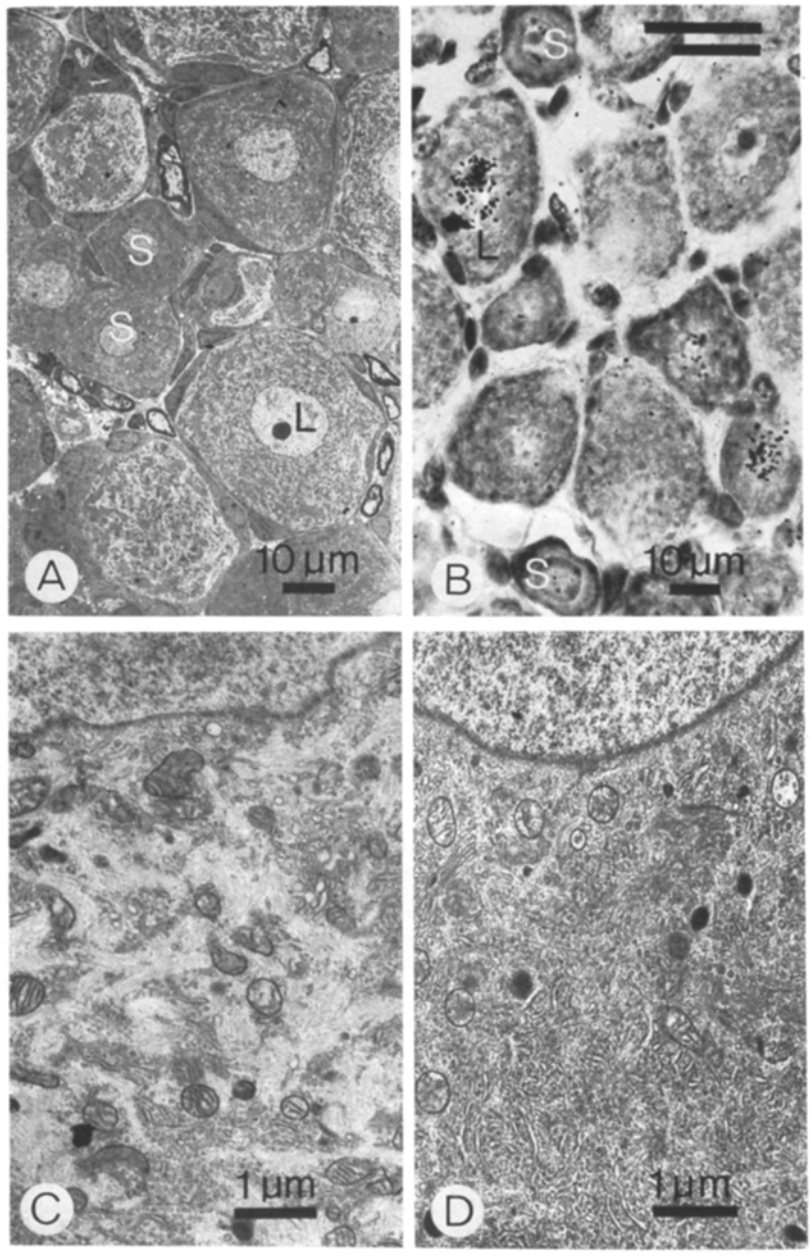

Fig. 1

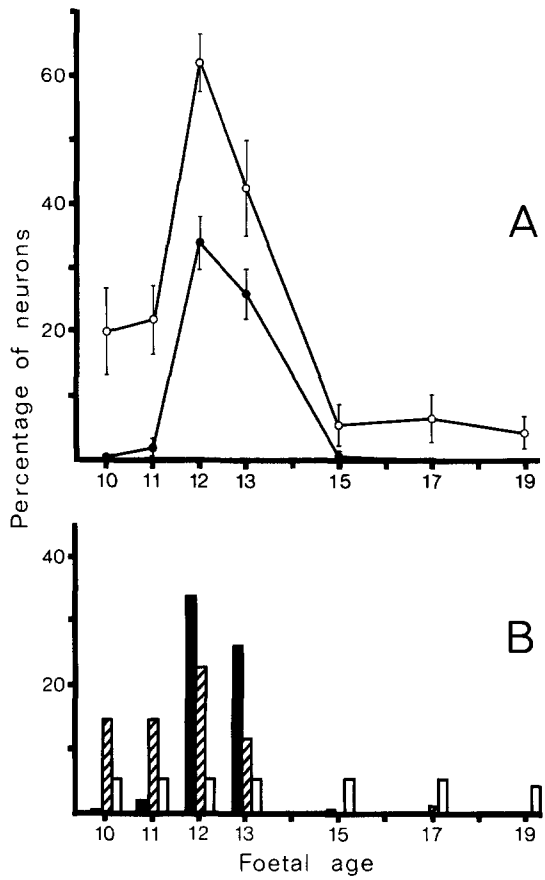

Fig. 2A and B. Main Study-timing of last division in DRG neuroblasts. (A) Graphs of the percentages of significantly (heavily plus lightly labelled neurones, shown by open circles) and heavily labelled neurones (filled circles) plotted against the day of gestation on which the ^{3}HTdR was administered. For each gestational age the means of percentages from 4–9 slides were found, and in each case the vertical line represents ± 1 S.D. For an explanation of significant and heavy labelling see text. (B) This histogram is derived from Graph A. It shows percentages of heavily labelled neurones (black columns), and lightly labelled neurones (cross-hatched and open columns). The open columns show the percentage (approx 5%) of cells which have probably incorporated ^{3}HTdR at some time other than during S. The cross-hatched columns represent the remainder of the lightly labelled cells which were labelled during S of a cell division several generations prior to their final division. Abscissa: The day of gestation on which ^{3}HTdR was administered

and heavily labelled neurones with foetal age at the time of the pulse label are shown in Fig. 2A. It is clear from these graphs that few cells were in or near their final cell division on days 10 and 11 (as indicated by the lower graphs of heavily labelled cells); the greatest number of cell birthdays was on day 12; slightly fewer birthdays occurred on day 13; and by day 15 very nearly all cell division appeared to have stopped. The distance between the two graphs represents the number of lightly labelled cells. About 20% or more of the neuroblasts were lightly labelled by injections on days 10, 11 and 12. This number was reduced

on day 13, and was only about 5% on days 15, 17 and 19. Since final cell divisions occurred mainly on days 12 and 13, it is reasonable to suppose that the majority of lightly labelled cells up to day 13 were caused by cell divisions subsequent to the pulse label, causing dilution of the label. However 5% of cells lightly labelled on days 17 and 19 cannot be explained in this manner since the cell birthdays were completed by this time. Two possible explanations for this light labelling are: 1. A small population of neurones continued to divide until at least a few days after day 19. However if the label was incorporated during cell division it might be expected that cells would be more heavily labelled at the later age of day 19 than on day 17. Fig. 2 shows that this is not the case. 2. Synthesis of DNA outside S (e.g. DNA repair) occurred. Whether or not this phenomenon normally occurs is a matter of some controversy. Pelc (1963) describes light labelling of cells which he suggests results from DNA repair. Rasmussen and Painter (1966) have found DNA synthesis in non-dividing cells irradiated with ultraviolet light. It may be that during development more DNA repair is necessary than in adult tissue. Whichever is the case, 1 or 2, it is possible that the same number of cells are involved in the process throughout.

The percentage of cells involved in each labelling process, assuming that in every case 5% of the cells are lightly labelled by one of the above two processes, is shown in the histogram of Fig. 2B. The black columns show the cells in their final divisions, and the cross hatched columns show the cells labelled several generations prior to their final cell divisions.

The Differential Cell Count. The evidence for the existence of two types of DRG neurone was referred to above and will be given in greater detail in the discussion. The ultrastructural differences between the 2 cell types can be seen in the higher power electron micrographs in Fig. 1C and D. The large light neurone in C with a cell diameter of about 40 μm has cytoplasm with clumps of mitochondria rough endoplasmic reticulum and free ribosomes interspersed with patches of neurotubules. The small dark neurone in D, which had a diameter of approximately 16 μm, has a much more dense distribution of mitochondria, rough endoplasmic reticulum, free ribosomes and neurotubules. It is clear from Fig. 1 that the Carnoy's-fixed neurones (Fig. 1B) are shrunken as compared to the neurones in the low power electron micrograph (Fig. 1A). All the dark (electron dense) neurones in Fig. 1A would therefore have fallen inside the size range counted as small if fixed in Carnoy's solution. Hatai (1902) gave the maximum diameter of small cells in lumbar DRG of rats of a similar age and size to ours, as approx. 23 μm. We counted cells larger than 23 μm as large cells, and those smaller than 17 μm as small cells on the basis of the appearance and sizes of these two cell types in our tissue culture sections. These figures therefore correspond fairly well with Hatai's. In order to give some idea of the relative proportions of neuronal sections in the two size categories used, a count of 743 cells in 8 different sections was made. 46.3% (S.D. $\pm 7.2\%$) of the cells had diameters of <17 μm; 23.6% (S.D. $\pm 4.26\%$) had diameters of >23 μm and 30% (S.D. $\pm 4\%$) of the cells fell between these two size ranges and would therefore not be included in autoradiographic counts.

Fig. 3 shows that when large and small neurones are counted separately it becomes apparent that the large light neurones are formed some time earlier

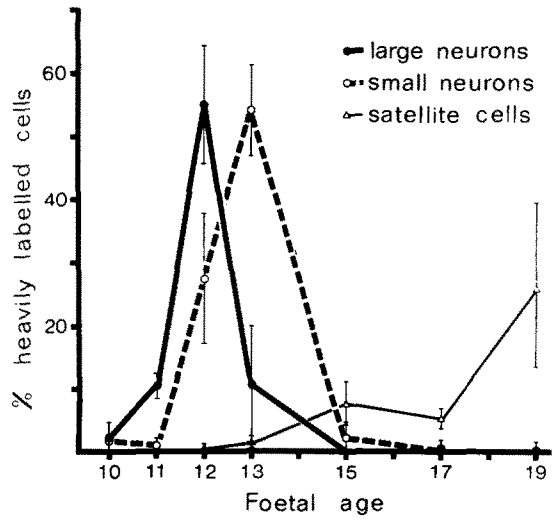

Fig. 3. Timing of last division in DRG neuroblasts. The percentages of heavily labelled DRG cells from 33 day rats are plotted against foetal age at the time of ^{3}HTdR administration. For dimensions of large and small neurones see Fig. 1

than the small dark neurones. The day on which the maximum number of cell birthdays occurs is day 12 for the large cells and day 13 for the small cells. By day 13 most large cells have ceased dividing. The upward slope of the graph is rather steeper for large than for small cells, and this probably indicated a greater rate of cell formation for the larger cells. The graph of heavily labelled satellite cells must be interpreted rather differently from the neurone graphs. This is because i) dividing non-neuronal cells can be more heavily labelled than dividing neuronal cells by the same dose of ^{3}HTdR (Sidman, 1970); and ii) it is known that glia can continue to divide in the adult (Smart and Leblond, 1961) and therefore the term "cell birthday" cannot be applied here. Suffice it to say that the label taken up by the glia becomes less diluted by subsequent divisions the later the label was administered.

Mean Cell Diameters and Electron Microscopy

In both the 11 and 12 day embryos no discrete groups of cells resembling DRGs could be seen, although it was possible to see neural crest cells of 8–12 μm diameter occupying the site of the presumptive dorsal root ganglion. Some of these cells may have been sympathetic cells but if so they could not be distinguished from DRG cells. In the 13 day embryo, however, clearly defined DRGs were easily located and the cells were photographed (Fig. 4A). The cellular outlines were

Fig. 4A—D. Low power electron micrographs of embryonic rat lumbar DRG at various stages of development. Magnification 3000× throughout. (A) 13 days embryo DRG. Note the rounded nuclei and clumped chromatin. (B) 14 days embryo DRG. The cells are more elongated than in A. (C) 15.5 days embryo DRG. (D) 22 days embryo DRG. There is considerable increase in cell size compared with C, and the cells have a more rounded appearance characteristic of an adult DRG

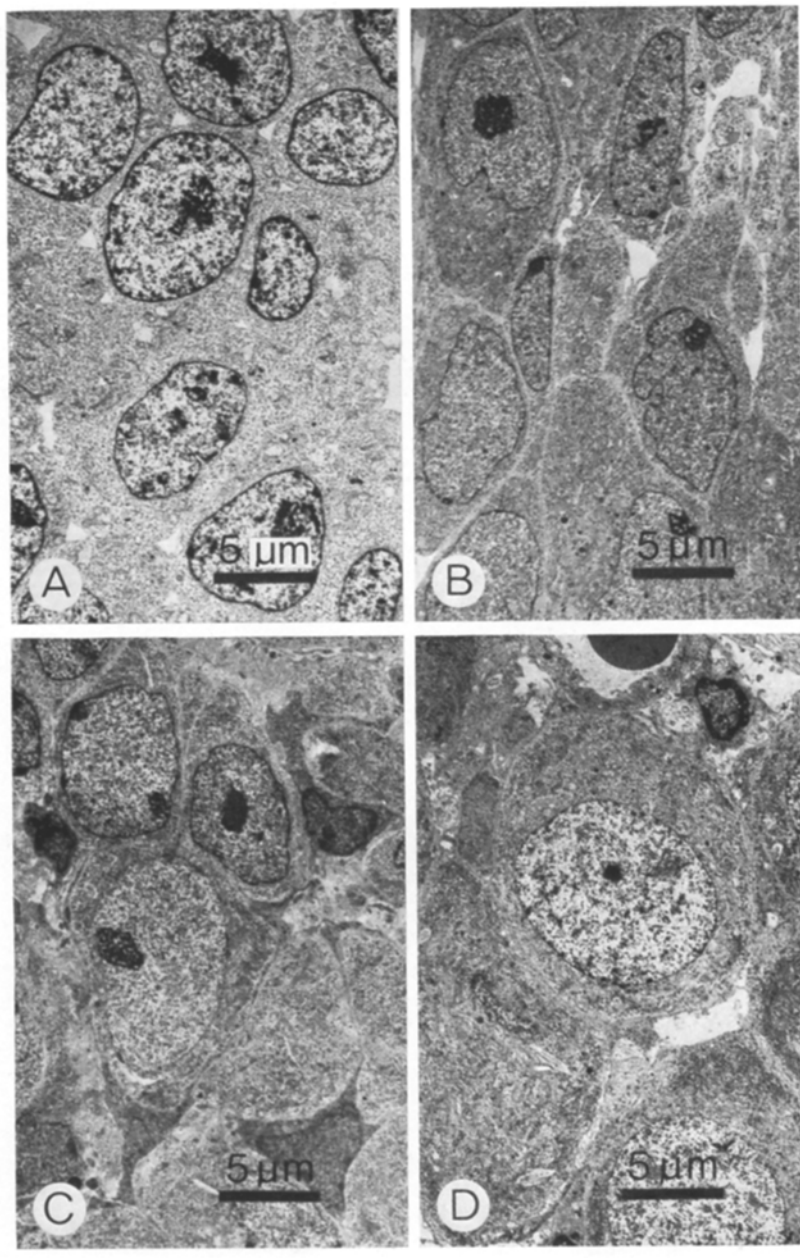

Fig. 4

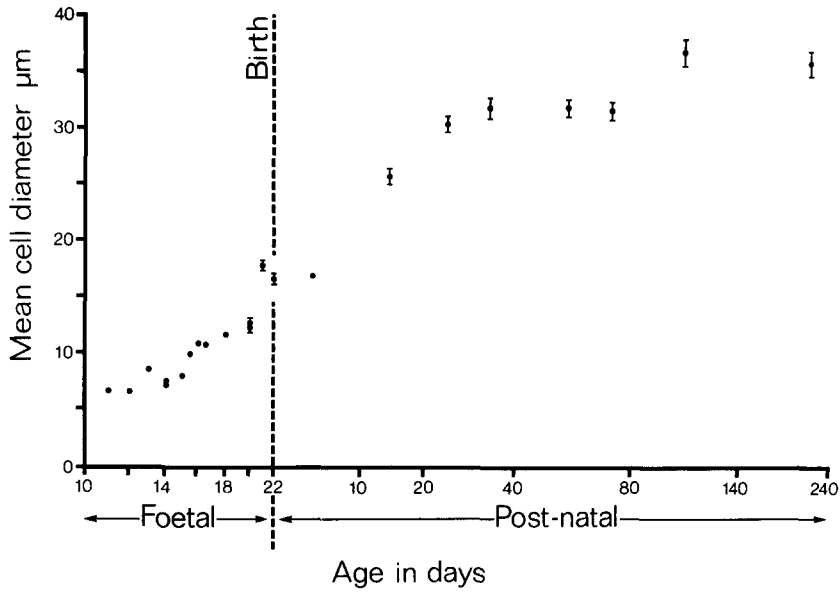

Fig. 5. Graph of mean neurone diameter against age of rats in days. For the method of obtaining data see Methods

difficult to distinguish at this age, and in some sections many mitotic figures were visible. The increase in cell diameter between days 12 and 13 of foetal life (see Fig. 5) was highly significant. This apparently sharp increase was probably due to an actual increase in mean volume of the cell body, since the majority of future large cells had stopped dividing by day 13 (see Fig. 3). The decrease in mean cell diameter between days 13 and 14 (shown in Fig. 5) was also highly significant. This decrease can in part be explained by the electron micrographs A and B in Fig. 4. At 13 days many cells were still dividing and the cells and nuclei appeared rounded up. By 14 days, however, a marked change in shape of neuronal sections was visible; the nuclei were much less rounded, some appearing elongated, and the cells resembled the bipolar neuroblasts of Tennyson, 1965, (see her Fig. 2). It may therefore be the case that this change in shape (which is probably related to outgrowth of processes) in itself led to a measured decrease in mean neuronal section diameter, while the neuronal volume may not have been falling but was more probably increasing. The increases in mean cell diameter between days 14–14.5 and 14.5–15.5 were both highly significant; however that between 14.5–15 days was particularly marked (see Fig. 5). At this time the number of cell birthdays was decreasing and by 15 days had reached a rate of less than 2% of the maximum rate reached at 12 days (see Fig. 2). A period of 2–3 days elapsed between the peak occurrence of cell birthdays on day 12 (Fig. 2A) and this sudden increase in mean cell diameter at 14.5–15 days. From day 15 to day 20 a steady increase in cell diameter occurred, and by birth the DRG neurones had a mean cell section diameter of more than twice that of the migrating neuroblasts of days 11 and 12. An electron micrograph of 22 day (just prenatal) DRG neurones is shown in

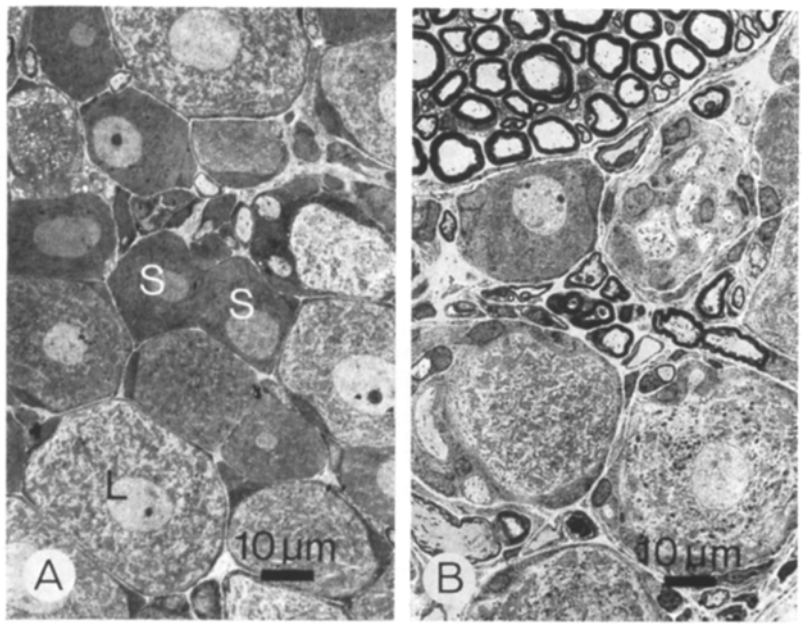

Fig. 6A and B. Low power electron micrographs of rat lumbar DRG. (A) 14 day postnatal DRG, ×750. Small dark cells (marked *S*) and large light cells (marked *L*) can clearly be seen. (B) 225 day postnatal DRG. ×750. Myelinated fibres can be seen and a sheath of satellite cell cytoplasm completely surrounds the neurones

Fig. 4D. These neurones were beginning to resemble adult DRG neurones in that the nuclei and cell bodies appeared nearly circular in cross section. However, the ratio, cell section diameter: nuclear section diameter is very low compared with the more mature neurones shown in Figs. 1A, 6A and B. This ratio is considerably increased 14 days after birth (Fig. 6A) and at this age electron dense neurones can easily be distinguished from the larger light cells. Perineural glia can be seen at 14 days (Fig. 6A) but the satellite cell sheath is much more complete by 33 days postnatal, Fig. 1A. Cell diameter increased steeply after birth up to 24 days postnatal but by 33 days reached a plateau. A highly significant increase in mean diameter was found between 73 and 100 days, but no increase was found between 100 and 225 days postnatal. Fig. 6B shows the large 225 day DRG neurones; light and dark neurones are again easily distinguished.

Discussion

The autoradiographic experiment showed that neurones in rat lumbar DRGs stop dividing over a period of about 4 days, that is 11–15 days of gestation, the greatest rate of neurone formation being on days 12 and 13. A particularly marked

increase in mean cell diameter between 15 and 15.5 days would seem to indicate that a rapid growth of cells began about 3 days after the final cell division. The rate of growth increased just before birth, but no increase in mean cell diameter occurred between day 21 of gestation and day 3 postnatal. After day 3 the growth was again rapid until 33 days, after which the rate of growth was very much decreased, and a plateau in cell diameter appeared to have been reached.

The value of the measurement of mean cell diameter is that it can provide a baseline against which to measure neurone development in culture (see Sobkowicz *et al.*, 1973), or abnormal development in vivo. The time course of the growth gives some indication of the time course of maturation in these cells. Similar measurements have been made by other workers. For instance Sobkowicz *et al.* (1973) measured the change in cell volume with age. The majority of their measurements, however, were made soon after birth, and little data on prenatal growth was given. Our measurements, if treated as diameters of spheres fit well with their volume measurements.

The differential cell counts in the autoradiographic experiments were based on the fact that at least two different types of nerve cell in dorsal root ganglia (DRG) have been described by several workers. Since we are interested in mammalian DRG this discussion will be limited to work on mammalian species. As early as 1902, Hatai described large and small neurones having different structural characteristics in the DRG of the cat. Two different sizes of neurone have also been described in cat DRG by Preto Parvis (1954). The large neurones appeared early and prevailed in the cervical, lumbar and coccygeal regions, whereas small neurones appeared later. Preto Parvis suggested that the large neurones may be somatic afferent neurones and the small ones may be splanchnic afferent neurones. Ultrastructural differences between large and small DRG neurones have also been described by Hess (1955) in the guinea pig and Yamadori (1970) in the rat. Compared with the large neurones, the smaller ones are darker (the cytoplasm is more osmophillic and therefore more electron dense) with evenly distributed rough endoplasmic reticulum (E.R.) or Nissl substance, less filamentous cytoplasm but with more compact organelles. The larger cells were lighter (less osmophillic) and had clumped rough E.R. Kalina and Wolman (1970) described histochemical differences between the two groups of DRG neurones. They found that whereas the discrepancy between the neuronal size in the two groups increased between 1 and 10 days post partum, in general the histochemical differences did not become apparent until between 10 and 20 days post partum and increased with age up to about 50 days. Although in general two groups of neurones–large (light) and small (dark) neurones are described, in some cases the sub-classification has been taken further. Parfianovicz *et al.* (1971) found three types of pseudo-unipolar cell comprising 93% of the neurones of lumbar DRG of adult cat and rabbit. A fourth type of cell (approximately 7% of the total) with very darkly silver-staining cytoplasm and an angular appearance (also see Bacsich and Wyburn, 1953) is multi-polar and they suggest probably autonomic. The existence of autonomic cells forming 7% of the cell population (Parfianovicz *et al.*, 1971) was not taken into account during differential counting and they would have been included in the group with small cells. Such a small number would be unlikely to bias the results markedly.

In our autoradiographs neurones in the large size range had a clumped appearance in their rather less electron dense cytoplasm which fits with the observation that large light neurones have clumped rough E.R. The small neurones had a much more even distribution of their contents. It also appears that there is a different time course of production for the two cell types, the rate of increase in percentage of heavily labelled cells being higher for large than small cells.

The different shape of the time course of production of the large and small neurones, the different time of the peak occurrence of birthdays, and the morphological difference apparent both in the light and electron micrographs at 33 days indicate that the two cell types we have counted are, as in other species, different from one another and not merely representatives of two stages in the life cycle of a homogeneous cell population. In other neural systems studied autoradiographically large neurones have often been found to arise before small neurones, e.g. in the epithalamus of the mouse (Angevine, 1970), and in the mesencephalic nucleus of the Vth nerve in the chick (Rogers and Cowan, 1973). In this nucleus the large (lateral) neurones arise about 6 hours earlier than the small neurones. This nucleus is in many ways comparable to DRGs (Rogers and Cowan, 1973). It appears then, that the DRG is a further example of a neural system in which the large neurones are formed before the smaller ones.

The results presented in this paper will now be put into the context of findings of other workers on the development of rat DRG. It is generally accepted that DRG are of neural crest origin. Cells from the neural crest migrate ventrally and on day 11 of gestation the DNA content of these cells begins to increase steeply and the ganglia are said by some workers to be recognisable (Sobkowicz *et al.*, 1973) although we did not find discrete ganglia until the 13th day. Angulo (1951) describes the ganglia very late on day 11 as being clusters of cells near the ventral region of the neural crest. At this time there is much cell division amongst the DRG neuroblasts as shown by our results and also Sobkowicz *et al.* (1973) described mitotic figures. Our results also show that some large DRG neurones stop dividing on day 11. The greatest occurrence of cell birthdays amongst the large DRG neurones is on day 12 and many small neurones are also formed. This fits with the observation of Vaughn and Grieshaber (1973) that many DRG cells at this age possess nuclear and somal characteristics of neurones but that others appear undifferentiated and large mitotic cells are frequently seen within the ganglia. In cervical DRG neurofibrils are just appearing at this time in some cells with short processes (Angulo, 1951) although Windle and Baxter (1936) found that the first sensory fibres reach the spinal cord at 12.5 days. The last cell division of most of the small DRG neurones is on day 13, but the number of large neurones being formed is considerably fewer than on day 12. At this time an increase in mean cell section diameter was noted and many mitotic figures could be seen in our low power electron micrographs. Angulo (1951) found that by the end of day 13 the peripheral processes have grown into the ventral root to form mixed spinal nerves, the motor parts of which reach the base of the forelimb. In a study of chemodifferentiation in developing rat DRG, Sarrat (1970) found that alkaline and acid phosphatase, glucose-6-phosphate dehydrogenase and NADH diaphorase could be demonstrated in neuroblast cytoplasm in the DRGs for the first time on day 13. Thus chemodifferentiation is noticeable in DRG cells about

24 hours after the majority of them are formed. Although day 14 was not examined autoradiographically, it seems likely from the 13 and 15 day autoradiographic results that all the large cells had stopped dividing by 14 days, but that some small DRG cells were still being formed. The decrease in mean cell section diameter is thought (see results section) to be due to change in cell shape—i.e. elongation, and not to a decrease in cell volume. At 14.5 days collaterals of primary afferent fibres are seen in the dorsal half of the intermediate zone of the spinal cord (Vaughn and Grieshaber, 1973). All large DRG neurones have ceased to divide by day 15, and the last very few small neurones are formed at about this time. On days 15–16 a noticeable increase in mean cell diameter can be seen in the DRG. It appears from experiments by Narayanan *et al.* (1971) that cutaneous stimulation of the hind limb on day 17 sometimes elicits a reflex action (about 30% positive responses). By day 20 nearly 100% positive responses occur. This suggests that in these 3 days more nerve fibres finish growing and form synapses, this is also the kind of lag found from the beginning to the end of the cell birthdays in the DRGs.

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