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Structure and Expression of Differentiation Antigens on Functional Subclasses of Primary Sensory Neurons

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Structure and expression of differentiation antigens on functional subclasses of primary sensory neurons

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[Plates 1–4]

Subpopulations of dorsal root ganglion neurons can be distinguished on the basis of their peripheral receptive properties, spinal terminal arbors and neuropeptide content. We have used monoclonal antibodies (MAbs) to define antigenic determinants on functional populations of DRG neurons projecting to the superficial dorsal horn of the spinal cord. Three MAbs recognize defined carbohydrate epitopes associated with lacto- and globo-series glycolipids that constitute the stage-specific embryonic antigens (SSEAs) 1, 3 and 4. SSEA-3 and SSEA-4 are present in the cytoplasm of about 10% of DRG neurons in adult rat. These neurons are distinct from those that contain substance P, somatostatin or the fluoride-resistant acid phosphatase enzyme, FRAP. SSEA-1 is present in a small percentage of DRG neurons. SSEAs are present on the surface of DRG neurons maintained in dissociated cell culture: 6% are SSEA-1^+, 7% are SSEA-3^+ and 10–15% are SSEA-4^+. MAbs LD2, KH10, TC6 and TD10 identify epitopes expressed coincidently in 25% of small DRG neurons that project to lamina II of the dorsal horn. All somatostatin- but less than 1% of substance P-immunoreactive DRG neurons express these antigens. MAb LA4 labels a distinct population of small DRG neurons that also projects to lamina II. There is extensive overlap between $LA4^+$ neurons and those that contain FRAP. Antigens recognized by these MAbs are expressed on the surface of 10–20% of DRG neurons in culture. Preliminary biochemical studies suggest that these antigens may be glycolipids. Molecules bearing carbohydrate differentiation antigens may be involved in the development and specification of sensory connections in the dorsal horn of the spinal cord.

1. Introduction

Anatomical and physiological studies over the last decade have provided a detailed description of the functional diversity of primary sensory neurons that transmit cutaneous sensory information to the spinal cord (Iggo 1973; Perl 1983). More than a dozen classes of cutaneous sensory afferents can be identified by their peripheral receptive properties and sensory terminal morphology (Willis & Coggeshall 1978). Reconstruction of the central projections of single afferent fibres after intra-axonal injection of HRP has revealed that functionally distinct classes of cutaneous sensory afferents exhibit stereotyped and restricted patterns of axon collateral arborization in the dorsal horn (Brown 1981). It is now clear that the majority of the central terminals of nociceptive cutaneous afferents are confined to the superficial dorsal horn (laminae I and II) (Rethelyi 1977; Light & Perl 1979; Ribiero Da Silva & Coimbra 1982), while the terminals of low threshold mechanoreceptive afferents are found in deeper regions (laminae III–IV) (Brown *et al.* 1977; Semba *et al.* 1983; Ralston *et al.* 1984).

Analysis of the morphological features of the cell bodies of primary sensory neurons in the dorsal root ganglion (DRG) has not resulted in a clear correlation with sensory fibre modality.

However, several classes of DRG neurons can be distinguished on the basis of cell body diameter and by ultrastructural features of the neuronal cytoplasm (Lawson *et al.* 1974; Rambourg *et al.* 1983). Separate populations of DRG neurons, which project to the superficial dorsal horn, contain the neurotransmitters substance P and somatostatin and a fluoride-resistant acid phosphatase enzyme (FRAP) (Hokfelt *et al.* 1976; Nagy & Hunt 1982; Dodd *et al.* 1983). Other populations of DRG neurons can be classified by the presence of several other neuropeptides, isoenzymes, cytoskeletal proteins and immunoglobulin binding sites (Dodd *et al.* 1983).

Physiological studies have provided evidence for a differential sensitivity of primary sensory neurons to transmitters, toxins and inflammatory mediators (Fjällbrant & Iggo 1961; Werz & Macdonald 1982; Baccaglini & Hogan 1983). However, the molecular nature of the sensory membrane receptors for these agents has not been determined. Molecules on the surface of DRG neurons are also likely to be important in the restriction of sensory terminal arbors and in the specification of appropriate sensory connections during development. The identification of surface antigens that distinguish functional classes of DRG neurons represents a first step in determining the nature of such molecules.

2. Carbohydrate differentiation antigens

Studies on several cell types have demonstrated that cell surface antigenic variation during development and differentiation can occur by modifications in the length and branching patterns of oligosaccharide side chains associated with glycoproteins and glycolipids (Feizi 1981; Hakomori 1981). One striking example, during erythrocyte development, is the transition from unbranched (i) to branched (I) lactosaminyl oligosaccharides that can be detected by human monoclonal autoantibodies (Feizi *et al.* 1979; Kapadia *et al.* 1981). The major blood group ABH antigens also possess carbohydrate determinants that undergo regulation during the development of erythrocytes and other cell types (Szulman 1980). Oligosaccharide sequences associated with blood group determinants can be expressed as developmentally regulated cell surface molecules on pre- and peri-implantation mouse embryos (Solter & Knowles 1979). These sequences include epitopes of the Forssman antigen and globoside (Willison *et al.* 1982), and the stage-specific embryonic antigens SSEA-1, SSEA-3 and SSEA-4 (Solter & Knowles 1978; Gooi *et al.* 1981; Kannagi *et al.* 1982, 1983*b*). SSEA-1 is an α1–3 fucosylated carbohydrate sequence associated with blood group i antigens whereas SSEA-3 and SSEA-4 are overlapping carbohydrate epitopes on a unique globo-series glycolipid (GL7) (Kannagi *et al.* 1983*b*) (table 1).

Subpopulations of DRG neurons can be distinguished by the presence of surface carbohydrate sequences associated with the gangliosides GD3 and GT1b and the neutral glycolipid, globoside (Raff *et al.* 1979; Fields 1983). Variations in cell surface carbohydrate expression could therefore reflect or contribute to the differentiation of DRG neurons. We have used monoclonal antibodies that recognize defined carbohydrate epitopes associated with blood group antigens and embryonic stage-specific differentiation antigens, to determine whether these sequences are expressed by functional subpopulations of DRG neurons.

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TABLE 1. CARBOHYDRATE STRUCTURES OF BLOOD GROUP AND STAGE-SPECIFIC EMBRYONIC DIFFERENTIATION ANTIGENS

	ABH blood group antigens
A	GalNAcα1—3Galβ | 1,2 Fucα
B	Galα1—3Galβ | 1,2 Fucα
H (type 2)	Fucα | 1,2 Galβ1—4GlcNAc
	globoseries antigens
SSEA-3	GalNAcβ1—3Galα—4Gal
SSEA-4	NeuAcα2—3Galβ1—3GalNAc
Forssman	GalNAcα1—3GalNAcβ1—3Galα1
Globoside	GalNAcβ1—3Galα1—4Galβ1—4Glcβ—1Cer
	Lewis and other fucosylated antigens
Le^a	Galβ1—3GlcNAc | 1,4 Fucα
X (SSEA–1)	Galβ1—4GlcNAc | 1,3 Fucα
Le^b	Galβ1—3GlcNAc | 1,2 | 1,4 Fucα Fucα
Y	Galβ1—4GlcNAc | 1,2 | 1,3 Fucα Fucα

3. ABH BLOOD GROUP ANTIGEN EXPRESSION BY DRG NEURONS

The expression of ABH blood group carbohydrate epitopes (Szulman 1980; Watkins 1980) in DRG neurons was examined by indirect immunofluorescence on fresh-frozen sections of adult rat DRG and spinal cord. Monoclonal antibodies directed against A and H carbohydrate epitopes (table 1) do not label sensory neurons or other cellular elements in the DRG. All DRG neurons, however, express an antigen recognized by monoclonal anti-B antibodies (figure 1*a*, plate 1). In sections of DRG, the antigen appears to be restricted to neurons and is not detected on other cell types. The surface membrane of all DRG neurons grown in dissociated cell culture is labelled by monoclonal anti-B antibodies with no labelling of other cell types (figure 1*b*, *c*) whereas the A and H carbohydrate determinants are not expressed on the surface of neurons or non-neuronal cells (figure 1*d*, *e*). Monoclonal anti-B antibodies can therefore be used as a selective surface marker for the identification and manipulation of DRG neurons in cell culture.

4. GLOBOSERIES CARBOHYDRATE EPITOPES ON DRG NEURONS

Several groups have identified carbohydrate epitopes on globoseries glycolipids as stage-specific embryonic antigens in murine development (Shevinsky *et al.* 1982; Willison *et al.* 1982; Kannagi *et al.* 1983*a*, *b*). Many of the globoseries glycolipids so far characterized also play a

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role as alloantigens in the P blood group system (Naiki & Marcus 1974). We used antisera directed against globoside and monoclonal antibodies that recognize the Forssman antigen and SSEA-3 and SSEA-4 to detect the presence of globoseries carbohydrate sequences in DRG neurons.

Monoclonal antibody (MAb) M1/22.25.8, directed against a Forssman carbohydrate epitope (Willison *et al.* 1982), labels the plasma membrane of all DRG neurons and many cellular elements in the spinal cord, indicating that this determinant is not expressed selectively by DRG neurons. Many DRG neurons and spinal cord cells are also labelled by anti-globoside antisera. About 60% of DRG neurons grown in culture appear to express cell surface carbohydrate determinants with globoside specificity (figure 2, plate 2). These results are in agreement with the observations of Raff *et al.* (1979). Monoclonal antibodies directed against SSEA-3 and SSEA-4, however, revealed a much more selective distribution of antigen. Incubation of DRG sections with anti-SSEA-3 or with anti-SSEA-4 resulted in the labelling of a subpopulation of intermediate and large diameter (30–60 μm) DRG neurons, with no detectable staining of non-neuronal elements in the ganglion (figure 3*a*, plate 3). Intense SSEA-3 and SSEA-4 immunoreactivity is present in the cytoplasm and in association with the plasma membrane of 10% of all DRG neurons. The SSEA-3 and SSEA-4 epitopes are also present in subpopulations of DRG neurons in other mammalian species (mouse, cat, guinea pig, squirrel monkey).

Since the SSEA-3 and SSEA-4 epitopes are expressed on the same globoseries glycolipid (GL7) on human embryonal carcinoma cells (Kannagi *et al.* 1983*b*), we determined the relationship between SSEA-3$^+$ and SSEA-4$^+$ DRG neurons. Most immunoreactive DRG neurons are SSEA-3$^+$ or SSEA-4$^+$. However, some small neurons are only SSEA-3$^+$ and many large-diameter neurons are only SSEA-4$^+$. DRG neurons that are SSEA-3$^+$ and/or SSEA-4$^+$ are distinct from those populations that contain substance P, somatostatin and FRAP (Dodd *et al.* 1984).

Within the central nervous system, neuronal cell bodies are unlabelled and the SSEA-3 and SSEA-4 epitopes are restricted to regions that contain the terminals of primary afferent fibres. In the spinal cord, a few labelled fibres and terminals are present in lamina I (figure 3*b*) whereas the tract of Lissauer and lamina II are devoid of immunoreactive fibres. A dense plexus of immunoreactive fibres is present in lamina III and in the medial region of lamina IV (figure 3*b*). SSEA-3$^+$ and SSEA-4$^+$ fibres are also present within sensory fibres in the dorsal columns that terminate in the gracile nucleus and the external cuneate nucleus. No immunoreactive fibres are observed on the lesioned side of the spinal cord in animals that have been unilaterally deafferented by section of the dorsal roots. All SSEA-3$^+$ and SSEA-4$^+$ fibres within the dorsal horn of the spinal cord are, therefore, probably derived from sensory neurons in DRG.

The restricted laminar distribution of SSEA-3 and SSEA-4 immunoreactive afferent terminals indicates that these carbohydrate epitopes define the cell bodies and central processes of functional subpopulations of DRG neurons. The absence of labelled terminals in lamina II suggests that unmyelinated primary afferents do not express the SSEA-3 and SSEA-4 epitopes. Immunoreactive afferent fibres in lamina I may represent high threshold mechanoreceptors that are known to terminate in this region (Light & Perl 1979; Rethelyi *et al.* 1982). SSEA-3$^+$ and SSEA-4$^+$ terminals in laminae III and IV are probably low threshold mechanoreceptors some of which may have peripheral terminals associated with hair follicles (Brown *et al.* 1977; Semba *et al.* 1983; Ralston *et al.* 1984). A detailed correlation of SSEA antigen expression with sensory fibre modality is currently in progress.

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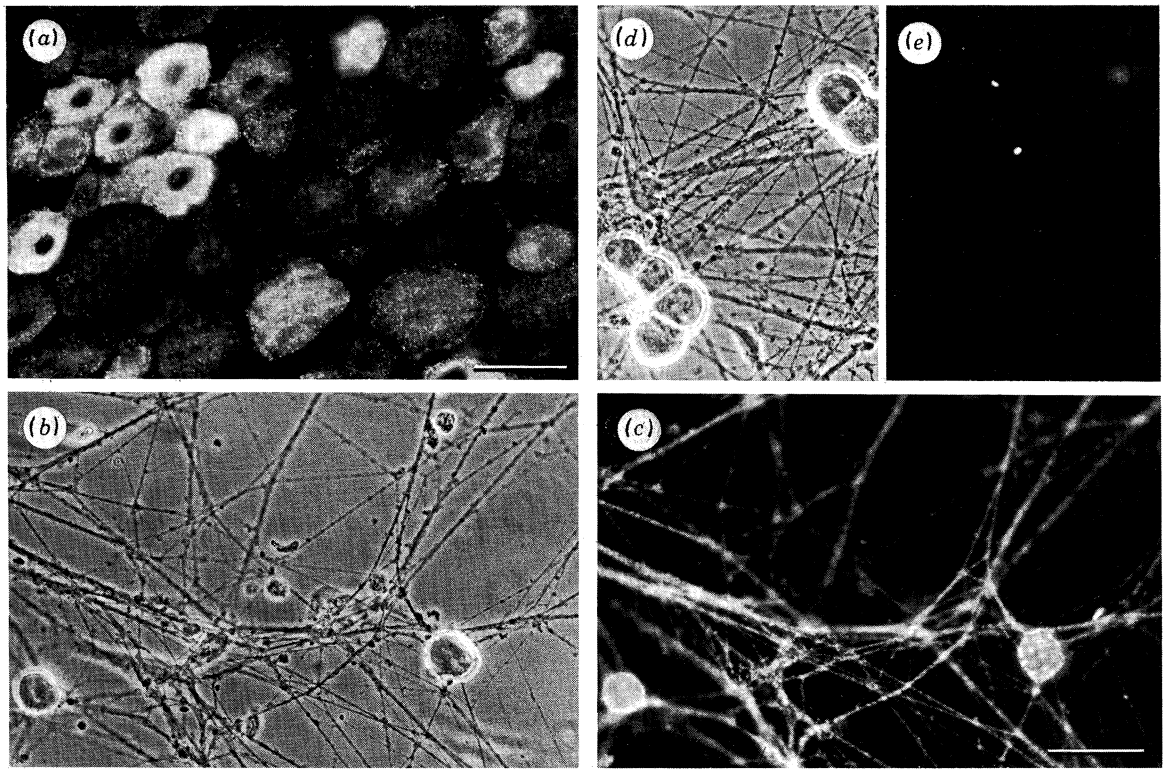

FIGURE 1. Expression of blood group B carbohydrate epitope by DRG neurons. (*a*) Cryostat section (10 μm) of adult rat DRG labelled with a MAb directed against the blood group B carbohydrate epitope. The antigen is visualized, in this and all subsequent figures, by indirect immunofluorescence histochemistry using FITC-labelled second antibodies (Dodd *et al.* 1984). All DRG neurons express the B carbohydrate epitope, with a greater intensity of immunoreactivity in small diameter neurons. The antigen cannot be detected in non-neuronal elements within DRG. (*b*, *c*) The blood group B epitope is expressed, selectively, on the surface of all DRG neurons in culture. (*b*) Phase contrast micrograph showing neuronal processes and the cell bodies of two cultured DRG neurons, obtained from neonatal rats. (*c*) Fluorescence micrograph of the same field after labelling with a monoclonal anti-B antibody. The B carbohydrate epitope is expressed on the surface of all DRG cell bodies and processes but cannot be detected on non-neuronal cells. (*d*, *e*) The blood group A epitope is absent from the surface of DRG neurons. (*d*) Phase contrast micrograph of cultured DRG neurons. (*e*) Fluorescence micrograph of the same field after incubation with a monoclonal anti-A antibody. Scale bars, 30 μm.

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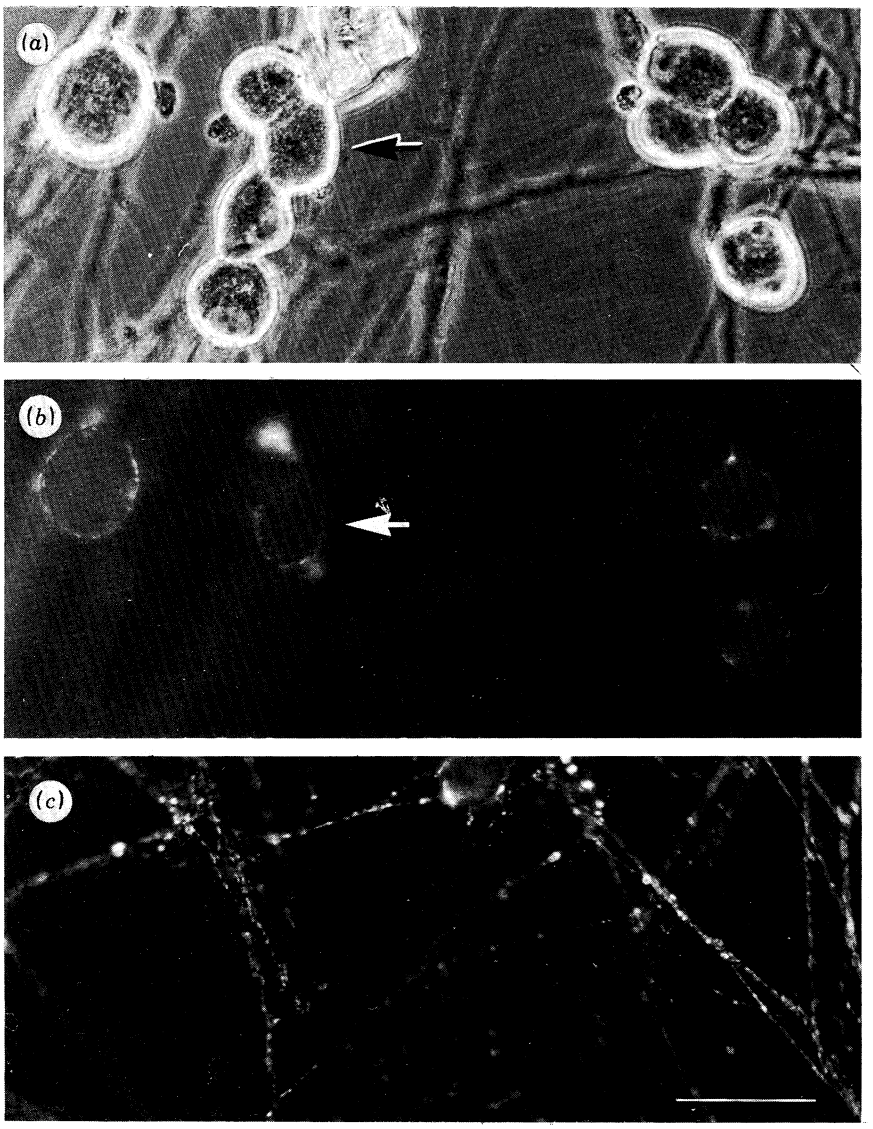

FIGURE 2. Globoside immunoreactivity on the surface of a subpopulation of DRG neurons in culture. (*a*) Phase contrast micrograph showing the cell bodies and processes of nine DRG neurons. (*b*) Fluorescence micrograph of the same field in which six of the nine neurons are labelled with a serum anti-globoside antibody. (*c*) Fluorescence micrograph of a different field, showing intense immunoreactivity on the processes of DRG neurons. Scale bar, 20 μm.

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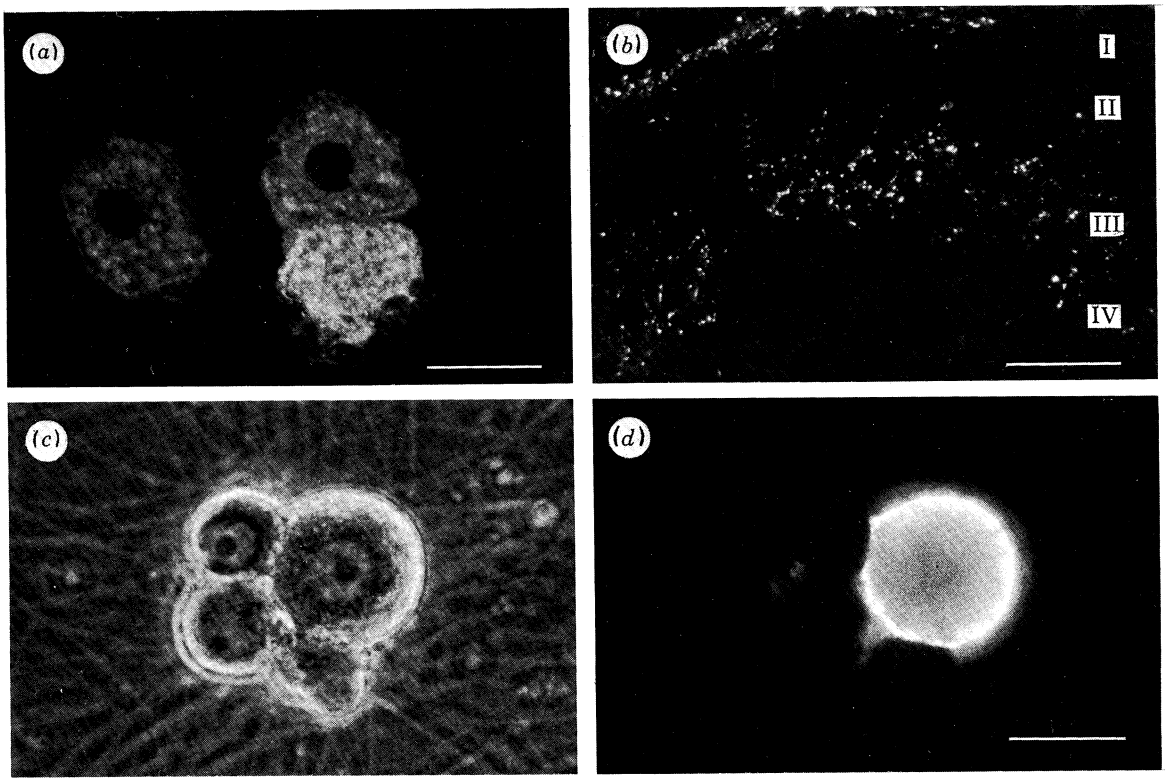

FIGURE 3. Expression of SSEA-3 and SSEA-4 epitopes by a subpopulation of DRG neurons. (*a*) Fluorescence micrograph of a 10 μm cryostat section of adult rat DRG showing three large-diameter DRG neurons that express the SSEA-4 carbohydrate epitope, detected with a monoclonal anti-SSEA-4 antibody. Scale bar, 30 μm. (*b*) Fluorescence micrograph of a 10 μm cryostat section of rat dorsal horn showing SSEA-3$^+$ fibres and terminals in lamina I and III. Roman numerals refer to the laminae of Rexed. Scale bar, 100 μm. (*c*) Phase contrast micrograph showing the cell bodies of four DRG neurons in culture. (*d*) Fluorescence micrograph of the same field in which only one neuron expresses the SSEA-4 antigen on its surface. Scale bar, 15 μm.

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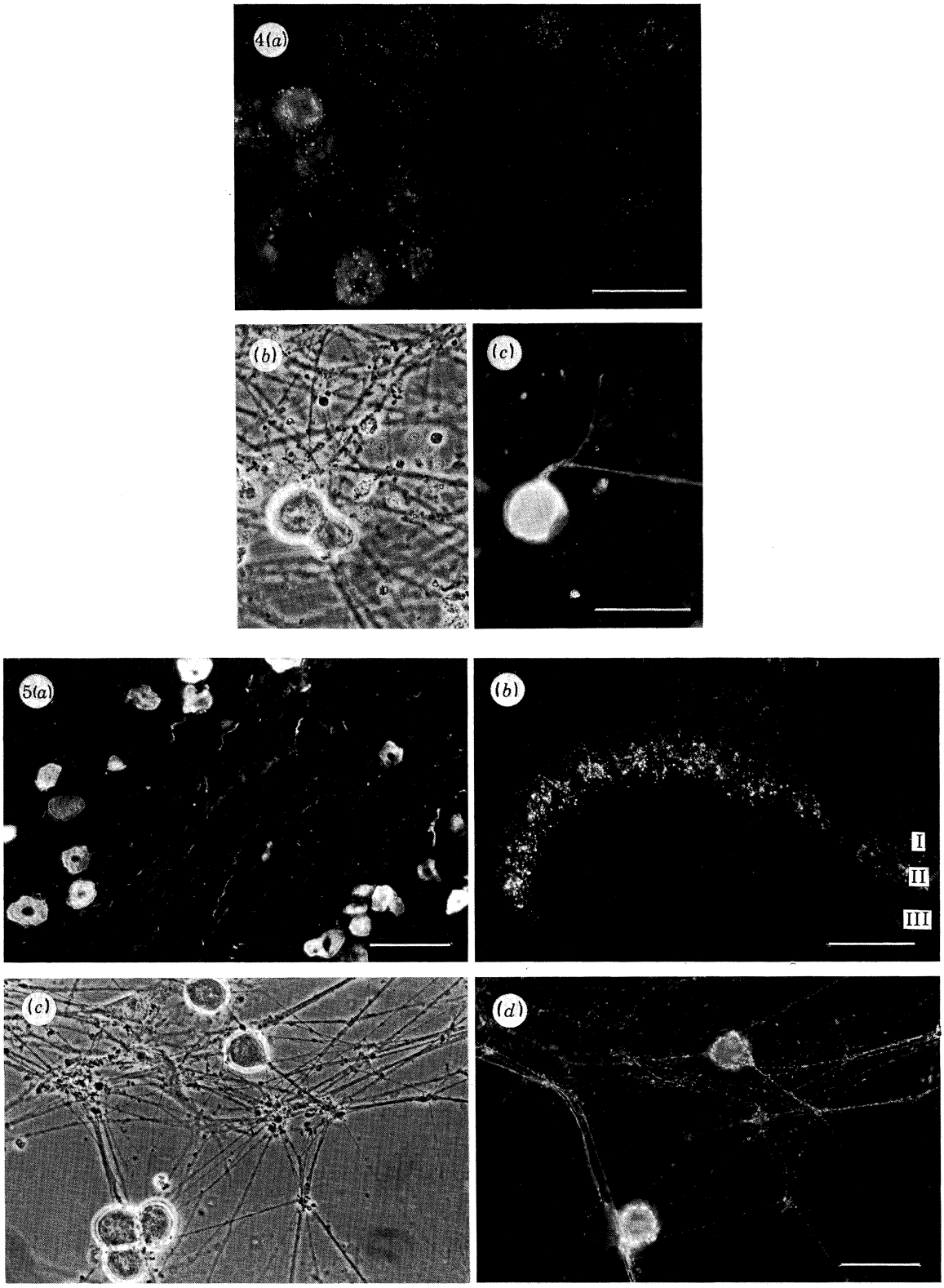

FIGURES 4 AND 5. For description see opposite.

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The SSEA-3 and SSEA-4 carbohydrate sequences are also expressed on the surface of DRG neurons (figure 3*c*, *d*). Approximately 7% of neonatal rat DRG neurons maintained in dissociated cell culture are SSEA-3$^+$ and 10–15% are SSEA-4$^+$ (Dodd *et al.* 1984). The relationship between DRG neurons expressing SSEA-3 and SSEA-4 epitopes is maintained in culture. Over 80% of SSEA-3$^+$ DRG neurons are SSEA-4$^+$ and, conversely, about 40% of SSEA-4$^+$ neurons are SSEA-3$^+$. Since about 60% of DRG neurons can be labelled with antisera to the purified globoside glycolipid, it seems possible that other classes of DRG neurons may express globoseries carbohydrate epitopes that are not recognized by anti-SSEA-3 and anti-SSEA-4. The identification of several new globoseries sequences on erythrocytes has been reported (Kundu *et al.* 1983). SSEA carbohydrate epitopes are associated with glycolipids and glycoproteins on murine embryos (Shevinsky *et al.* 1982; Kannagi *et al.* 1983). Preliminary biochemical studies on the nature of the molecules bearing SSEA-3 and SSEA-4 epitopes on DRG neurons suggests that both antigens are glycolipids (Dodd *et al.* 1984).

5. Expression of Lewis and other fucosylated blood group antigens by DRG neurons

We examined the expression of Lewis blood group antigens by DRG neurons with monoclonal antibodies directed against carbohydrate epitopes associated with the Le^a, X (SSEA-1 specificity), Le^b and Y determinants. Le^a is expressed in the cytoplasm of a subpopulation of small-diameter DRG neurons (figure 4*a*, plate 4) but not on the surface of cultured DRG neurons. Anti-SSEA-1, which is directed against the X epitope (Gooi *et al.* 1981; Kannagi *et al.* 1982), labels about 1% of DRG neurons. The few SSEA-1$^+$ DRG neurons are not SSEA-4$^+$. In the spinal cord, the SSEA-1 epitope is present as an intense, diffuse band within the superficial dorsal horn and at lower density in deeper laminae (figure 6). Dorsal rhizotomy did not noticeably reduce the intensity of SSEA-1 immunoreactivity in the superficial dorsal horn, indicating that the epitope is present on spinal cord cells in addition to DRG neurons. Studies on spinal cord cells in culture have demonstrated that the SSEA-1 epitope is absent from dorsal horn neurons and appears to be expressed on the surface of astrocytes (Dodd *et al.* 1984).

Few DRG neurons in adult rats are SSEA-1$^+$. However, the surface expression of lacto-series carbohydrates associated with the SSEA-1 epitope undergoes marked changes during preimplantation development of murine embryos (Solter & Knowles 1978). During differentiation

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Description of plate 4

Figure 4. Expression of Lewis blood group antigens by subpopulations of DRG neurons. (*a*) Fluorescence micrograph of a 10 μm cryostat section of adult rat DRG showing a subpopulation of small DRG neurons that express the Le^a carbohydrate epitope within small granules in the neuronal cytoplasm. Scale bar, 30 μm. (*b*) Phase contrast micrograph of the cell bodies of two DRG neurons in culture. (*c*) Fluorescence micrograph of the field shown in (*b*) in which one of the neurons expresses the SSEA-1 (X) carbohydrate epitope. Scale bar, 30 μm.

Figure 5. Localization of SNAC antigens in subpopulations of DRG neurons. (*a*) Fluorescence micrograph of a 10 μm cryostat section of adult rat DRG showing the localization of the antigen recognized by MAb KH10 in a subpopulation of small DRG neurons and in neuronal processes running through the ganglion. Scale bar, 40 μm. (*b*) Fluorescence micrograph of a 10 μm cryostat section of rat dorsal horn showing KH10$^+$ fibres and terminals within lamina IIo and scattered fibres within the tract of Lissauer. Scale bar, 100 μm. (*c*). Phase contrast micrograph of five DRG neurons in culture. (*d*) Fluorescence micrograph of the same field shown in (*b*) in which the surface of only two neurons is labelled with MAb LA4. Processes originating from these neurons are also intensely labelled. Scale bar, 30 μm.

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of human embryonal carcinoma cells there is also a transition from the SSEA-1$^-$ to the SSEA-1$^+$ phenotype (Andrews *et al.* 1982) suggesting that surface expression of the SSEA-1 epitope by DRG neurons may be subject to modulations during development or differentiation. In fact, 6% of DRG neurons in culture are SSEA-1$^+$ and represent a population that is largely separate from SSEA-4$^+$ neurons (figure 4*b*, *c*) (Dodd *et al.* 1984). The closely related Le^b and Y epitopes are not detected in the cytoplasm or on the surface of DRG neurons.

6. Novel antigens on DRG neurons

The demonstration of carbohydrate determinants associated with the ABH, P and Lewis blood group antigens (Watkins 1980) on early murine embryos and DRG neurons, raises the possibility that there may be other antigens shared by these cell types. Immunization with embryonal carcinoma cells and other transformed cell lines has been particularly successful in generating monoclonal antibodies against carbohydrate sequences on stage-specific embryonic antigens (Solter & Knowles 1978; Randle 1982; Blaineau *et al.* 1983; Kannagi *et al.* 1983*b*). We have, therefore, raised monoclonal antibodies against transformed cell lines derived from primary cells that are known to express blood group specificities (Szulman 1980; Rouger *et al.* 1981) in order to identify novel developmentally regulated antigens on subsets of DRG neurons. Several monoclonal antibodies generated by immunization with the rat pancreatic acinar line AR4-2J (Jessup & Hay 1980) label the surface of acinar cells and subpopulations of DRG neurons (Dodd & Jessell 1984).

Four monoclonal antibodies (KH10, LD2, TC6 and TD10) that recognize sensory neuron-acinar cell (SNAC) antigens, are expressed coincidently in a subpopulation of small and intermediate-diameter DRG neurons. Approximately 25% of DRG neurons exhibit intense immunoreactivity in cell bodies and processes (figure 5*a*, plate 4). These antigens are not detectable in sympathetic or parasympathetic neurons, or in neurons that originate in the CNS. Dual colour immunofluorescence labelling revealed that there is no overlap between SNAC$^+$ and SSEA-4$^+$ populations of DRG neurons. In the spinal cord, immunoreactive fibres and terminals are localized within lamina IIo with scattered fibres in the tract of Lissauer and occasionally in lamina I (figures 5*b* and 6). No immunoreactivity is detectable in the superficial dorsal horn after dorsal root section, indicating that labelled fibres and terminals originate from neurons in the DRG. Small-diameter DRG neurons that project to the superficial dorsal horn are known to contain substance P and somatostatin (Hokfelt *et al.* 1976). All somatostatin-

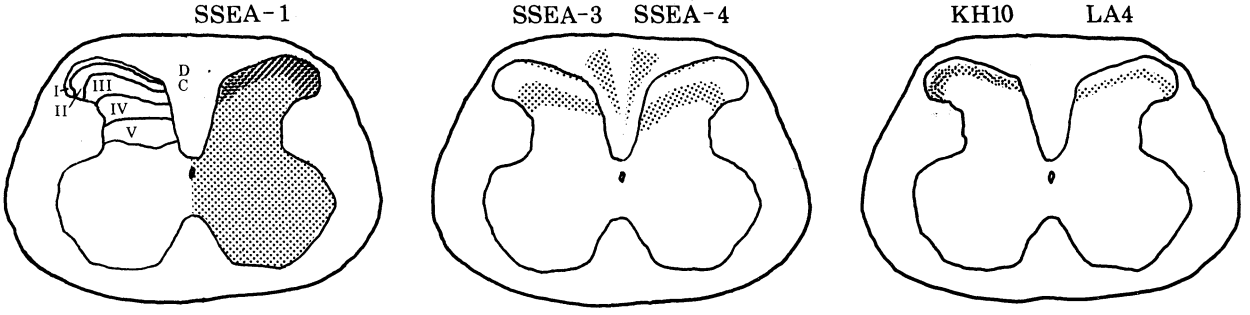

Figure 6. Localization of differentiation antigens in rat spinal cord. Summary diagram showing the distribution, in coronal sections of rat spinal cord, of antigens recognized by monoclonal antibodies directed agaist SSEA and SNAC antigens. Roman numerals indicate laminae of Rexed in the dorsal horn, DC indicates dorsal columns.

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immunoreactive, but virtually no substance P-immunoreactive, DRG neurons express the antigens recognized by MAbs KH10 and LD2 (Dodd & Jessell 1984). Approximately 60% of SNAC$^+$ DRG neurons do not contain somatostatin-immunoreactivity suggesting that other peptides may be present in SNAC$^+$ neurons.

Another SNAC antigen, recognized by MAb LA4, is present in a separate subpopulation of small and intermediate diameter DRG neurons which represents about 25–30% of all DRG neurons. Primary afferent terminals labelled by MAb LA4 are also restricted to lamina II in the dorsal horn (figure 6) (Dodd & Jessell 1984). The subset of DRG neurons labelled by LA4 exhibits extensive overlap with DRG neurons that express immunoglobulin Fcγ 2b binding sites (Dodd *et al.* 1983) and FRAP (Nagy & Hunt 1982; Dodd *et al.* 1983). The location of SNAC$^+$ primary afferent terminals in lamina II of the dorsal horn suggests that the SNAC antigens may be restricted to DRG neurons with unmyelinated processes (Rethelyi 1977).

SNAC antigens are also expressed on the surface of DRG neurons grown in culture. Approximately 10% of DRG neurons can be labelled with MAbs KH10 and LD2 and 15–20% of DRG neurons express the LA4 antigen (figure 5*c*, *d*). We have also detected SNAC antigens on the surface of the murine embryonal carcinoma line PC-13 (Bernstein *et al.* 1973) which suggests that these antigens may be regulated during murine embryogenesis. The distribution of SNAC antigens in DRG neurons is similar, or identical, to that of the 2C5 antigen recognized by a monoclonal antibody generated against PC-13 embryonal carcinoma cells that also reacts with pre-implantation murine embryos (Coakham *et al.* 1982; Randle 1982). Unlike the SNAC antigens, however, the 2C5 antigen is not expressed on the surface of AR4-2J cells or on cultured DRG neurons (J. Dodd & T. M. Jessell, unpublished observations). The anti-SNAC monoclonals and 2C5 may, therefore, recognize distinct epitopes on a family of structurally related differentiation antigens that vary in their cytoplasmic and cell surface expression. The identification, with MAbs, of distinct carbohydrate epitopes associated with globoseries and fucosylated lacto-series oligosaccharide differentiation antigens provides a precedent for this possibility. No SNAC$^+$ immunoreactive neurons can be observed in DRG sections after treatment with methanol or periodate suggesting that the SNAC antigens may also be glycolipids.

7. The role of differentiation antigens on DRG neurons

Several groups have raised monoclonal antibodies in an attempt to identify novel surface antigens on sensory neurons. All DRG neurons appear to express an uncharacterized antigen, labelled by monoclonal antibody 38/D7 (Vulliamy *et al.* 1981), whereas small- and large-diameter sensory neurons have been reported to segregate the protein antigens NSP-4 and NSP-5, respectively (Rougon *et al.* 1983, 1984). The functions of these antigens, which are also expressed by other classes of central and peripheral neurons, are unknown at present.

Our findings indicate that DRG neurons can be identified by monoclonal antibodies that recognize differentiation antigens, many or all of which have carbohydrate epitopes. The distribution of immunoreactivity in DRG and the dorsal horn, indicates that there is a restriction of these antigens to functionally distinct classes of DRG neurons (figure 6). SSEA epitopes appear to be expressed by myelinated, and predominantly low threshold sensory afferents, whereas the SNAC antigens seem to be associated with unmyelinated sensory fibres. Neurons expressing SSEA and SNAC antigens account for over half the total population of

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sensory neurons in the DRG. The results obtained with MAbs directed against other blood group carbohyrate determinants suggest that it may be possible to identify the remaining functional classes of DRG neurons by the selective expression of cytoplasmic and cell surface carbohydrate epitopes.

In preliminary experiments, we have been able to detect SSEA and SNAC antigens on DRG neurons from 15–16-day rat embryos, soon after final mitotic division has occurred (Lawson *et al.* 1974) and before the formation of the majority of afferent synapses in the spinal cord (Vaughan & Grieshaber 1973; Smith 1983). Anti-SSEA and anti-SNAC antibodies may, therefore, provide specific probes with which to examine the early development and differentiation of defined functional classes of DRG neurons. These antibodies will also be useful in combination with fluorescence-activated cell sorting techniques for the isolation and maintenance of functional subpopulations of DRG neurons in cell culture. Electrophysiological studies to determine the nature and role of synaptic transmitters released from specific populations of DRG neurons (Dodd *et al.* 1983; Jahr & Jessell 1983) should then become possible.

The role of differentiation antigens on DRG neurons will be more difficult to determine. The identification of these antigens on murine embryos and embryonal carcinoma cells has not been accompanied by an immediate understanding of their biological function in early embryogenesis (Solter & Knowles 1979; Feizi 1981). However, the carbohydrate nature of many of these antigenic determinants suggests a number of possible functions that can be tested experimentally. Carbohydrate sequences on glycolipids and other glycoconjugates have been implicated in the intercellular adhesion and differentiation of teratocarcinoma stem cells (Grabel *et al.* 1983; Shur 1983), intercellular recognition associated with the homing of lymphocytes (Stoolman & Rosen 1983) and neuronal adhesion in embryonic development (Edelman *et al.* 1983; Rutishauser 1983).

The molecular events that contribute to the formation of appropriate sensory connections in the dorsal horn of the spinal cord remain to be determined. Studies on organotypic cultures of DRG and spinal cord have demonstrated that DRG neurons retain a dorsal preference in the innervation of spinal cord explants (Crain & Peterson 1982; Smallheiser *et al.* 1982). Although the specificity of sensory connections *in vitro* remains to be determined, it may be possible to modify afferent input with synthetic carbohydrate sequences (Baker 1983). It will be important, in future, to determine whether molecules bearing SSEA and SNAC determinants play any role in the development and specification of sensory connections in the dorsal horn of the spinal cord.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

References

Andrews, P. W., Goodfellow, P. N., Shevinsky, L. H., Bronson, D. L. & Knowles, B. B. 1982 Cell-surface antigens of a clonal human embryonal carcinoma cell line; morphological and antigenic differentiation in culture. *Int. J. Cancer* **29**, 523–531.

Baccaglini, P. I. & Hogan, P. G. 1983 Some rat sensory neurons in culture express characteristics of differentiated pain sensory cells. *Proc. natn. Acad. Sci. U.S.A.* **80**, 594–598.

Baker, R. E. 1983 Effects of gangliosides on the development of selective afferent connections within fetal mouse spinal cord explants. *Neurosci. Lett.* **41**, 81–84.

Bernstine, E. G., Hooper, M. L., Grandchamp, S. & Ephrussi, B. 1973 Alkaline phosphatase activity in mouse teratoma. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3899–3903.

Blaineau, C., Le Pendu, J., Arnaud, D., Connan, F. & Avner, P. 1983 The glycosidic antigen recognised by a novel monoclonal antibody, 75.12 is developmentally regulated on mouse embryonal carcinoma cells. *EMBO J.* **2**, 2217–2222.

Brown, A. G. 1981 *Organization in the spinal cord.* Berlin and New York: Springer Verlag. (238 pages.)

Brown, A. G., Rose, P. K. & Snow, P. J. 1977 The morphology of hair follicle afferent fibre collaterals in the spinal cord of the cat. *J. Physiol., Lond.* **272**, 779–797.

Coakham, H. B., Garson, J. A., Harper, A. A., Harper, E. I., Lawson, S. N. & Randle, B. J. 1982 Monoclonal antibody 2C5; a new marker for a subset of small neurons in the rat dorsal root ganglion. *J. Physiol., Lond.* **332**, 60*P*.

Crain, S. M. & Peterson, E. R. 1982 Selective innervation of target regions within fetal mouse spinal cord and medulla explants by isolated dorsal root ganglia in organotypic co-cultures. *Devl Brain Res.* **2**, 342–362.

Dodd, J. & Jessell, T. M. 1984 Surface antigens on functional subsets of primary sensory neurons. *Soc. Neurosci. Abstr.*, p. 992.

Dodd, J., Jahr, C. E., Hamilton, P. N., Heath, M. J. S., Matthew, W. D. & Jessell, T. M. 1983 *Cold Spring Harb. Symp. quant. Biol.* **48**, 685–695.

Dodd, J. Solter, D. & Jessell, T. M. 1984 Monoclonal antibodies against carbohydrate differentiation antigens identify subsets of primary sensory neurons. *Nature. Lond.* (In the press.)

Edelman, G. M., Hoffman, S., Chuong, C. M., Thiery, J. P., Brackenbury, R., Gallin, W. J., Grumet, M., Greenberg, M. E., Hemperly, J. J., Cohen, C. & Cunningham, B. A. 1983 Structure and modulation of neural cell adhesion molecules in early and late embryogenesis. *Cold Spring Harb. Symp. quant. Biol.* **48**, 515–526.

Feizi, T. 1981 Carbohydrate differentiation antigens. *Trends biochem. Sci.* **11**, 333–335.

Feizi, T., Childs, R. A., Watanabe, K. & Hakomori, S. 1979 Three types of blood group I specificity among monoclonal anti-I autoantibodies revealed by analogs of a branched erythrocyte glycolipid. *J. exp. Med.* **149**, 975–980.

Fields, K. 1983 Monoclonal antibodies binding to subsets of rat neurons in cell cultures. *J. Neurochem.* **41**, S147.

Fjällbrant, N. & Iggo, A. 1961 The effects of histamine, 5-hydroxytryptamine and acetylcholine on cutaneous afferent fibres. *J. Physiol., Lond.* **156**, 578–590.

Gooi, H. C., Feizi, T., Kapadia, A., Knowles, B. B., Solter, D. & Evans, M. J. 1981 Stage specific embryonic antigen involves α1-3 fucosylated type 2 blood group chains. *Nature, Lond.* **292**, 156–158.

Grabel, L. B., Singer, M. S., Rosen, S. D. & Martin, G. R. 1983 The role of carbohydrates in the intercellular adhesion and differentiation of teratocarcinoma stem cells. *Cold Spring Harb. Conf. Cell Proliferation* **10**, 145–161.

Hakomori, S. I. 1981 Glycosphingolipids in cellular interaction, differentiation and oncogenesis. *A. Rev. Biochem.* **50**, 733–764.

Hokfelt, T., Elde, R., Johansson, A., Luft, R., Nilsson, G. & Arimura, A. 1976 Immunohistochemical evidence for separate populations of somatostatin-containing and substance P-containing primary afferent neurons in the rat. *Neuroscience* **1**, 131–136.

Iggo, A. (ed.) 1973 *Handbook of sensory physiology*, volume 2 (*Somatosensory system*). Berlin and New York: Springer.

Jahr, C. E. & Jessell, T. M. 1983 ATP excites a subpopulation of rat dorsal horn neurons. *Nature, Lond.* **304**, 730–733.

Jessup, N. W. & Hay, R. J. 1980 Charateristics of two rat pancreatic exocrine cell lines derived from transplantable tumors. *In vitro* **16**, 212.

Kannagi, R., Nudelman, E., Levery, S. B. & Hakomori, S. I. 1982 A series of human erythrocyte glycosphingolipids reacting to the monoclonal antibody directed to a developmentally regulated antigen, SSEA-1. *J. biol. Chem.* **257**, 14865–14874.

Kannagi, R., Levery, S. B., Ishigami, F., Hakomori, S. I. Shevinsky, L. H., Knowles, B. B. & Solter, D. 1983*a* New globoseries glycosphingolipids in human teratocarcinoma reactive with the monoclonal antibody directed to a developmentally regulated antigen, stage-specific antigen-3. *J. biol. Chem.* **258**, 8934–8942.

Kannagi, R., Cochran, N. A., Ishigami, F., Hakomori, S. I., Andrews, P. W., Knowles, B. B. & Solter, D. 1983*b* Stage-specific embryonic antigens (SSEA-3 and -4) are epitopes of a unique globoseries ganglioside isolated from human teratocarcinoma cells. *EMBO J.* **2**, 2355–2361.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Kapadia, A., Feizi, T. & Evans, M. J. 1981 Changes in the expression and polarization of blood group I and i antigens in post-implantation embryos and teratocarcinomas of mouse associated with cell differentiation. *Expl Cell Res.* **131**, 185–195.

Kundu, S. K., Samuelsson, B. E., Pascher, I. & Marcus, D. M. 1983 New gangliosides from human erythrocytes. *J. biol. Chem.* **258**, 13857–13866.

Lawson, S. N., Caddy, K. W. T. & Biscoe, T. J. 1974 Development of rat dorsal root ganglion neurons. Studies of cell birthdays and changes in mean cell diameter. *Cell Tiss. Res.* **153**, 399–413.

Light, A. R. & Perl, E. R. 1979 Spinal termination of functionally identified primary afferent fibres with slowly conducting myelinated fibres. *J. comp. Neurol.* **186**, 133–150.

Nagy, J. I. and Hunt, S. P 1982 Fluoride-resistant acid phosphatase containing neurons in dorsal root ganglia are separate from those containing substance P or somatostatin. *Neuroscience* **7**, 89–97.

Naiki, M. & Marcus, D. M. 1974 Human erythrocyte P and p^k blood group antigens: identification as glysosphingolipids. *Biochem. biophys. Res. Commun.* **60**, 115–121.

Perl, E. R. 1983 Characterization of nociceptors and their activation of neurons in the superficial dorsal horn: first steps for the sensation of pain. *Adv. Pain. Res. Ther.* **6**, 23–51.

Raff, M. R., Fields, K. L., Hakomori, S. I., Pruss, R. M. & Winter. J. 1979 Cell-type specific markers for distinguishing and studying neurons and the major classes of glial cells in culture. *Brain Res.* **174**, 283–308.

Ralston, H. J., Light, A. R., Ralston, D. D. & Perl, E. R. 1984 Morphology and synaptic relationships of physiologically identified low-threshold dorsal root axons stained with intra-axonal horseradish-peroxidase in the cat and monkey. *J. Neurophysiol.* **51**, 777–792.

Rambourg, A., Clermont, Y. & Beaudet, A. 1983 Ultrastructural features of six types of neurons in rat dorsal root ganglia. *J. Neurocytol.* **12**, 47–66.

Randle, B. J. 1982 Cosegregation of monoclonal antibody reactivity and cell behaviour in the mouse preimplantation embryo. *J. Embryol. exp. Morph.* **70**, 261–278.

Rethelyi, M. 1977 Preterminal and terminal arborizations in the substantia gelatinosa of cat's spinal cord. *J. comp. Neurol.* **172**, 511–528.

Rethelyi, M., Light, A. R. & Perl, E. R. 1982 Synaptic complexes fomed by functionally defined primary afferent units with fine myelinated fibres. *J. comp. Neurol.* **207**, 381–393.

Ribiero Da Silva, A. & Coimbra, A. 1982 Two types of synaptic glomeruli and their distribution in laminae I–III of the rat spinal cord. *J. comp. Neurol.* **209**, 176–186.

Rouger, P., Goossens, D., Gane, P. & Salmon, C. 1981 Antigens common to blood cells and tissues – from red blood cell antigens to tissue antigens. In *Blood group antigens*, pp. 101–109.

Rougon, G., Hirsch, M. R., Hirn, M., Guenet, J. L. & Goridis, C. 1983 Monoclonal antibody to neural cell surface protein: identification of a glycoprotein family of restricted cellular localization. *Neuroscience* **10**, 511–520.

Rougon, G., Hirn, M., Hirsch, M. R., Guenet, J. L. & Goridis, C. 1984 Identification and immunolocalization by monoclonal antibody of NSP-5, a surface polypeptide of neural cells. *J. Neurochem.* (Submitted.)

Rutishauser, U. 1983 Molecular and biological properties of a cell adhesion molecule. *Cold Spring Harb. Symp. quant. Biol.* **48**, 501–514.

Semba, K., Masarachia, P., Melamed, S., Jacquin, M., Harris, S., Yang, G. & Egger, M. D. 1983 An electronmicroscopic study of primary afferent terminals from slowly adapting Type 1 receptors in the cat. *J. comp. Neurol.* **221**, 466–481.

Shevinsky, L. H., Knowles, B. B., Damjanov, I. & Solter, D. 1982 Monoclonal antibody to murine embryos defines a stage-specific embryonic antigen expressed on mouse embryos and human teratocarcinoma cells. *Cell* **30**, 697–705.

Shur, B. D. 1983 The role of cell-surface galactosyltransferase in embryonal carcinoma cell adhesion. *Cold Spring Harb. Conf. Cell Proliferation* **10**, 185–195.

Smallheiser, N. R., Peterson, E. R. & Crain, S. M. 1982 Specific neuritic pathways and arborizations formed by fetal mouse dorsal root ganglion cells within organized spinal cord explants in culture: a peroxidase labelling study. *Devl Brain Res.* **2**, 383–395.

Smith, C. S. 1983 The development and post-natal organization of primary afferent projections to the rat thoracic spinal cord. *J. comp. Neurol.* **220**, 29–43.

Solter, D. & Knowles, B. B. 1978 Monoclonal antibody defining a stage-specific embryonic antigen (SSEA-1). *Proc. natn. Acad. Sci. U.S.A.* **75**, 5565–5569.

Solter, D. & Knowles, B. B. 1979 Developmental stage specific antigens during mouse embryogenesis. *Curr. Top. dev. Biol.* **13**, 139–165.

Stoolman, L. M. & Rosen, S. D. 1983 Possible role for cell surface carbohydrate binding molecules in lymphocyte recirculation. *J. Cell Biol.* **96**, 722–729.

Szulman, A. E. 1980 The ABH blood groups and development. *Curr. Top. dev. Biol.* **14**, 127–145.

Vaughan, J. W. & Grishaber, J. A. 1973 A morphological investigation of an early reflex pathway in developing rat spinal cord. *J. comp. Neurol.* **148**, 177–210.

Vulliamy, T., Rattray, S. & Mirsky, R. 1981 Cell surface antigen distinguishes sensory and autonomic peripheral neurons from central neurons. *Nature, Lond.* **291**, 418–420.

Watkins, W. M. 1980 Biochemistry and genetics of the ABO, Lewis, and P blood group systems. *Adv. hum. Genet.* **10**, 1–136.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Werz, M. A. & Macdonald, R. L. 1982 Heterogeneous sensitivity of cultured dorsal root ganglion neurons to opioid peptides selective for mu and delta opiate receptors. *Nature, Lond.* **290**, 730–733.
Willis, W. D. & Coggeshall, R. E. 1978 *Sensory mechanisms in the spinal cord.* London: Plenum Press. (485 pages.)
Willison, K. R., Karol, R. A., Suzuki, A., Kundu, S. K. & Marcus, D. M. 1982 Neutral glycolipid antigens as developmental markers of mouse teratocarcinoma and early embryos: an immunologic and chemical analysis. *J. Immun.* **129**, 603–611.

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FIGURE 1. Expression of blood group B carbohydrate epitope by DRG neurons. (*a*) Cryostat section (10 μm) of adult rat DRG labelled with a MAb directed against the blood group B carbohydrate epitope. The antigen is visualized, in this and all subsequent figures, by indirect immunofluorescence histochemistry using FITC-labelled second antibodies (Dodd *et al.* 1984). All DRG neurons express the B carbohydrate epitope, with a greater intensity of immunoreactivity in small diameter neurons. The antigen cannot be detected in non-neuronal elements within DRG. (*b*, *c*) The blood group B epitope is expressed, selectively, on the surface of all DRG neurons in culture. (*b*) Phase contrast micrograph showing neuronal processes and the cell bodies of two cultured DRG neurons, obtained from neonatal rats. (*c*) Fluorescence micrograph of the same field after labelling with a monoclonal anti-B antibody. The B carbohydrate epitope is expressed on the surface of all DRG cell bodies and processes but cannot be detected on non-neuronal cells. (*d*, *e*) The blood group A epitope is absent from the surface of DRG neurons. (*d*) Phase contrast micrograph of cultured DRG neurons. (*e*) Fluorescence micrograph of the same field after incubation with a monoclonal anti-A antibody. Scale bars, 30 μm.

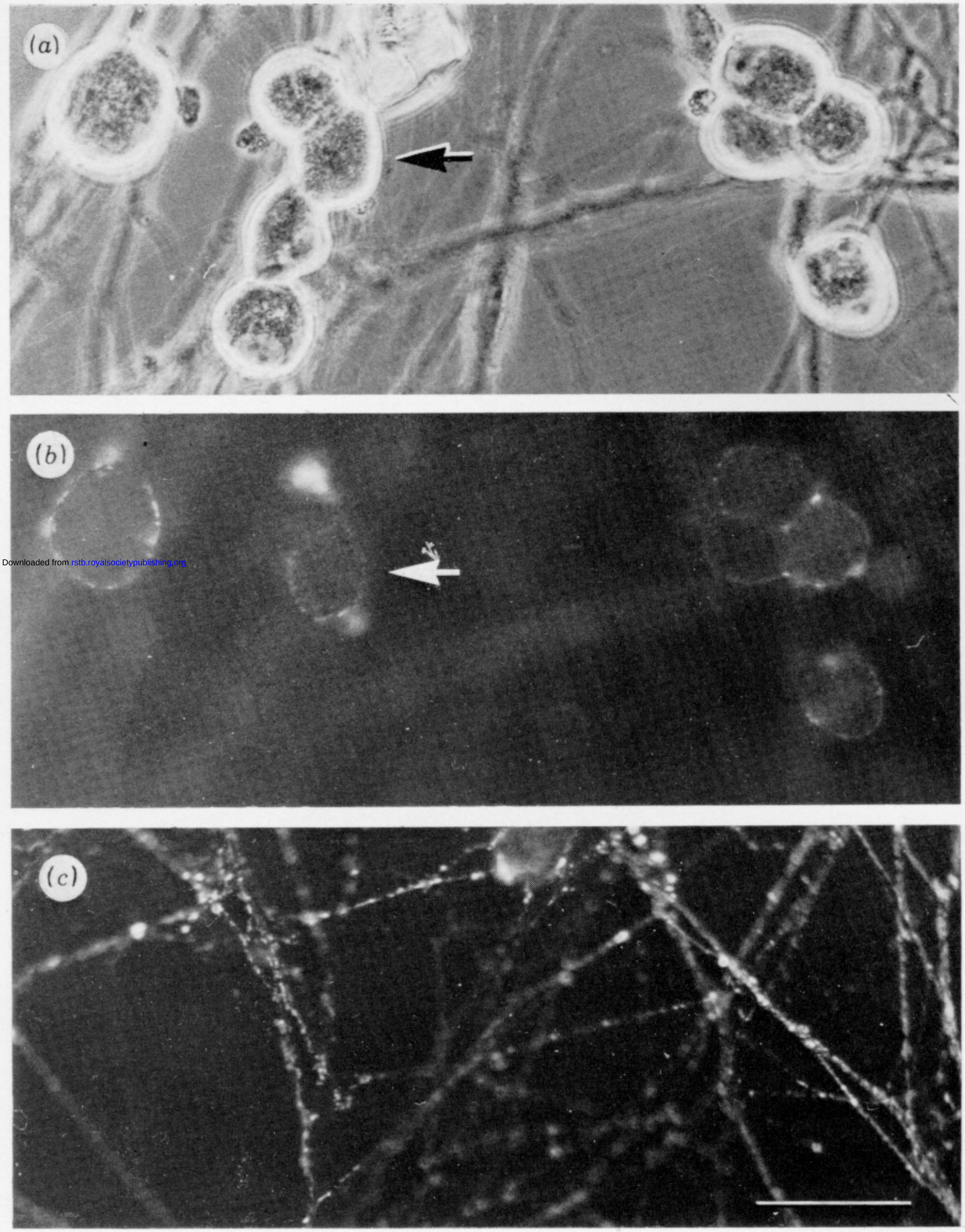

FIGURE 2. Globoside immunoreactivity on the surface of a subpopulation of DRG neurons in culture. (*a*) Phase contrast micrograph showing the cell bodies and processes of nine DRG neurons. (*b*) Fluorescence micrograph of the same field in which six of the nine neurons are labelled with a serum anti-globoside antibody. (*c*) Fluorescence micrograph of a different field, showing intense immunoreactivity on the processes of DRG neurons. Scale bar, 20 μm.

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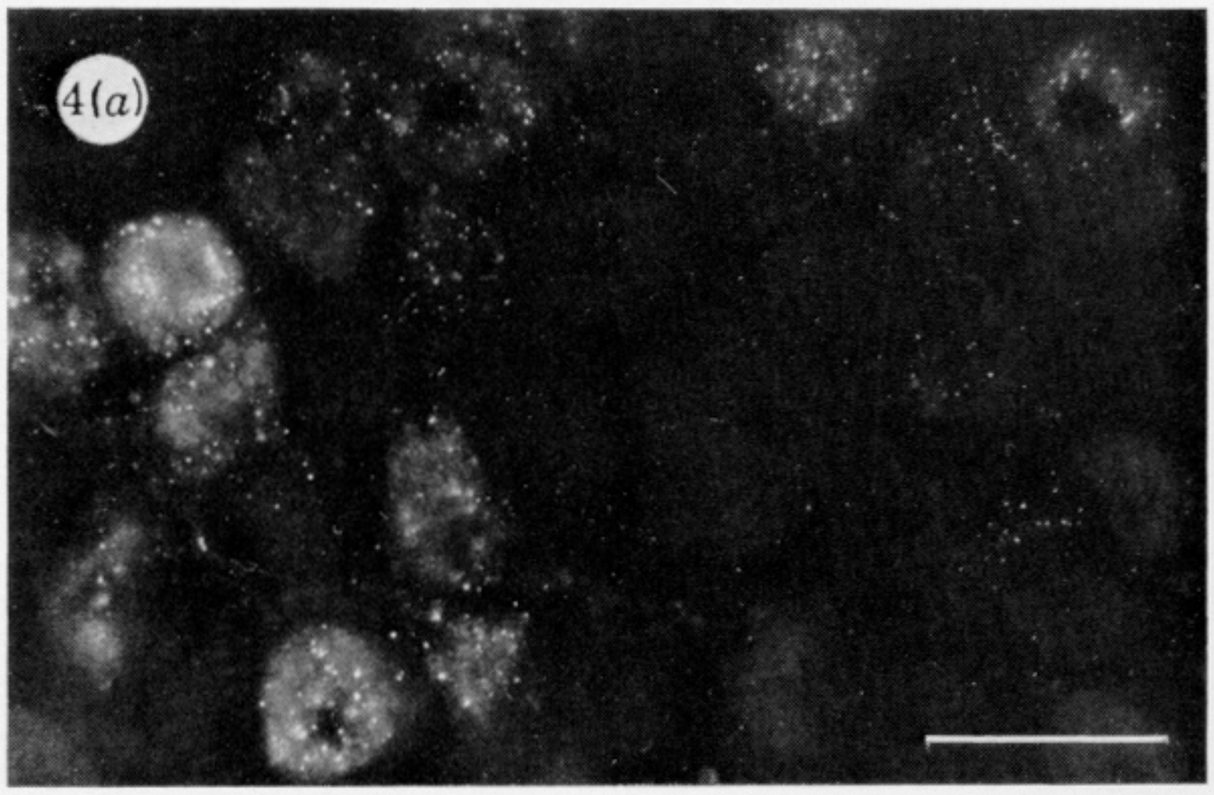
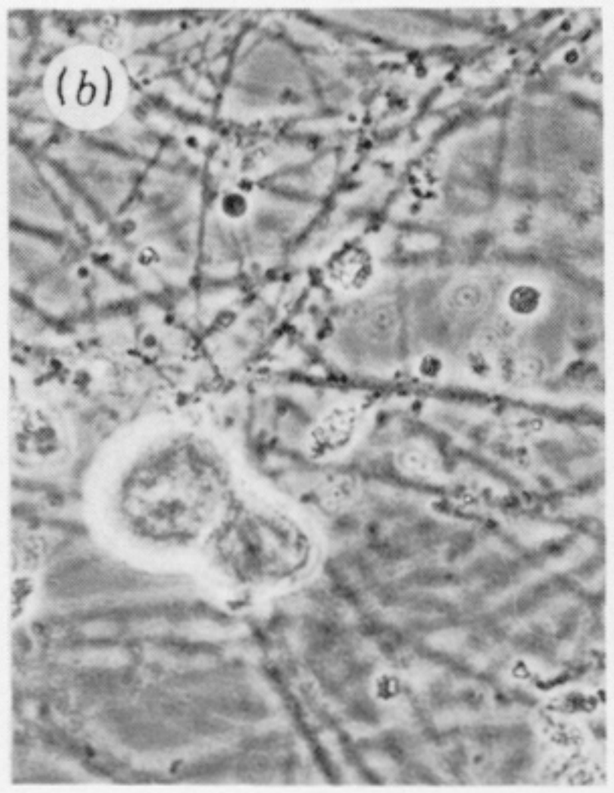
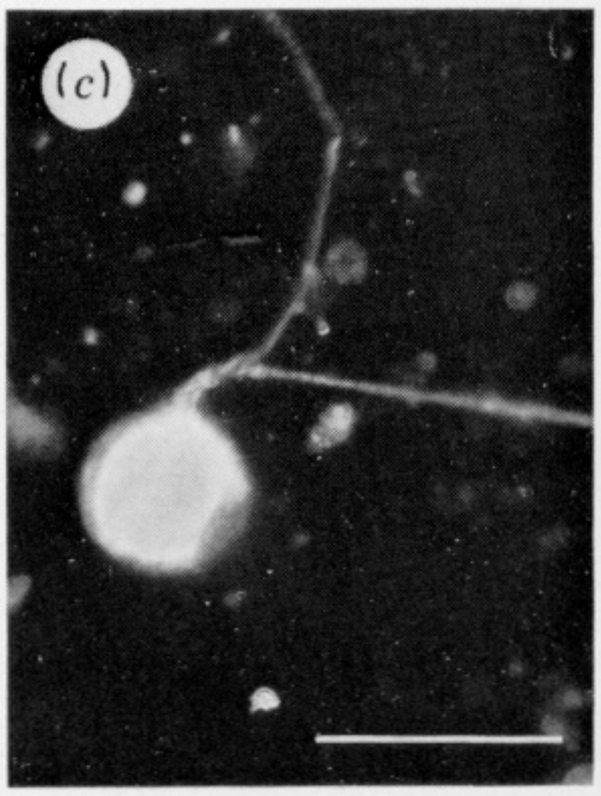
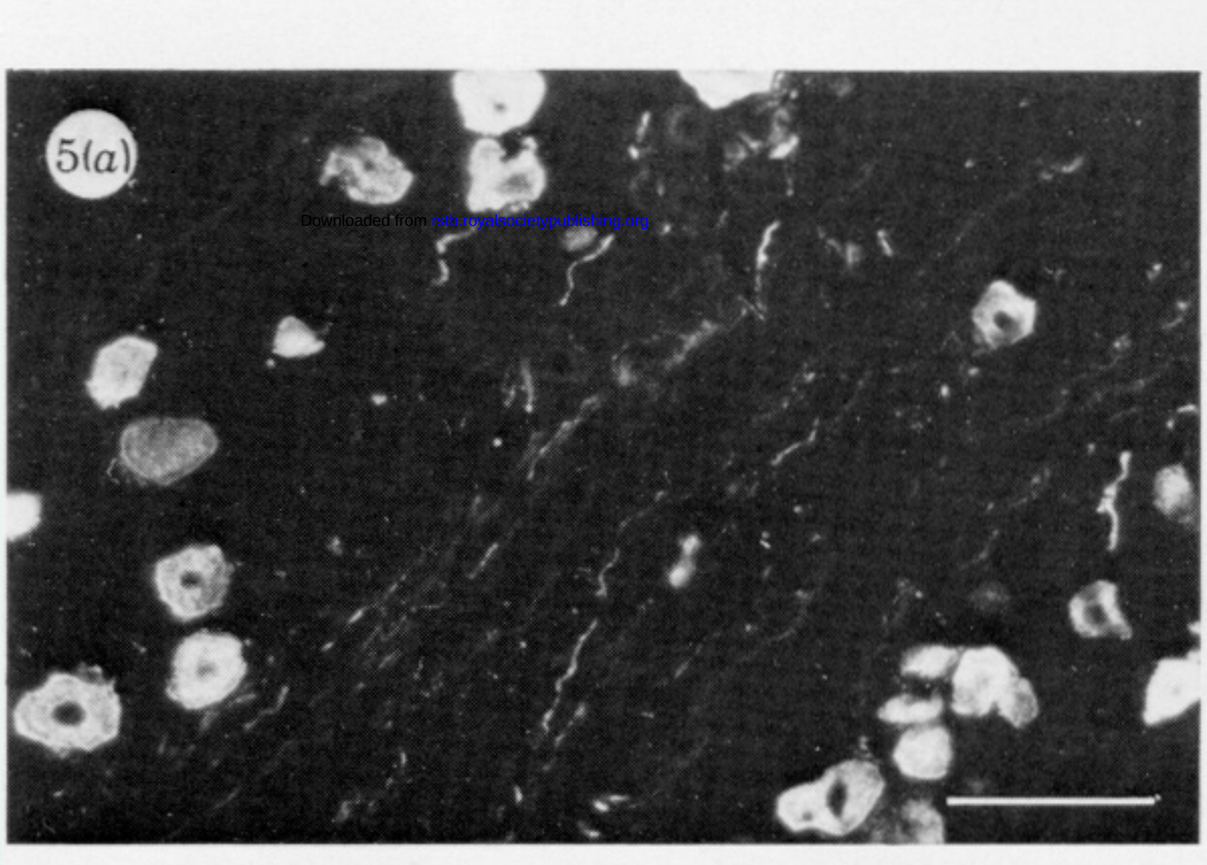
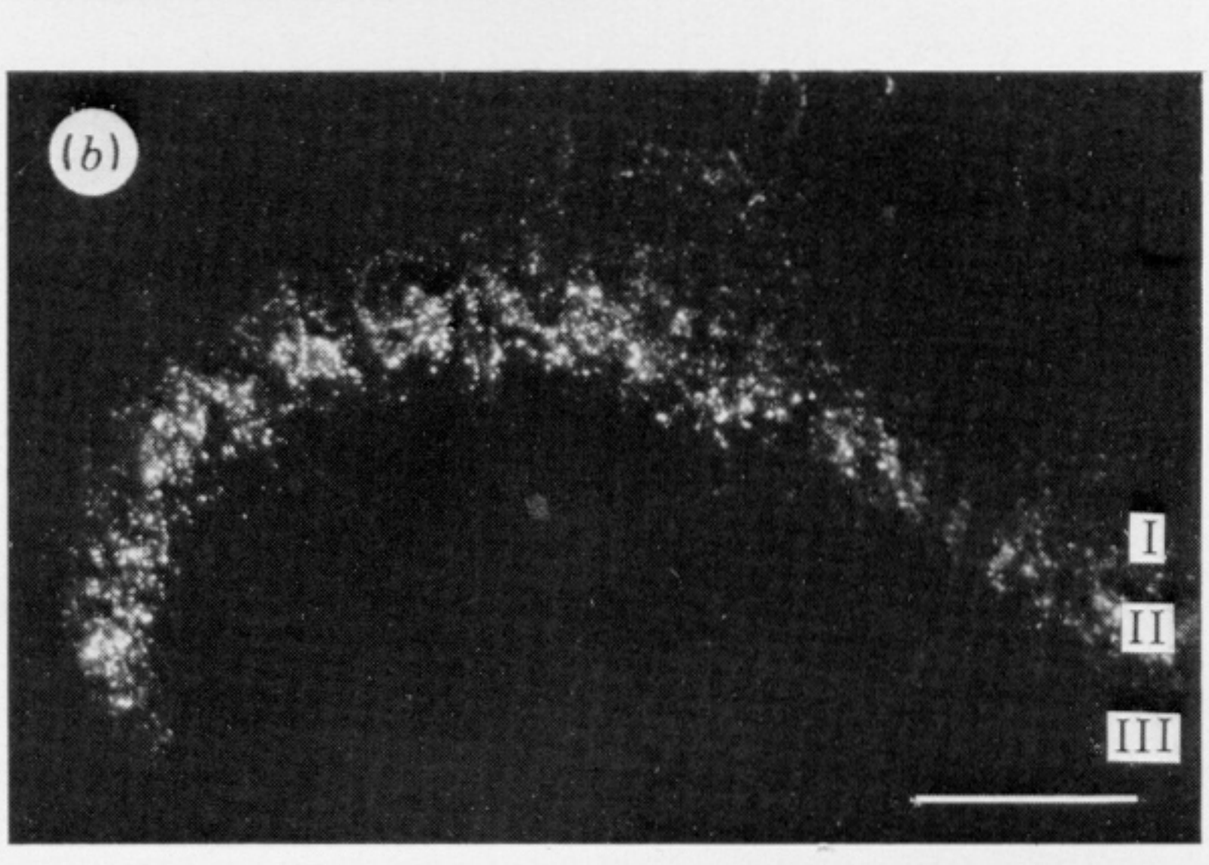
FIGURE 3. Expression of SSEA-3 and SSEA-4 epitopes by a subpopulation of DRG neurons. (*a*) Fluorescence micrograph of a 10 μm cryostat section of adult rat DRG showing three large-diameter DRG neurons that express the SSEA-4 carbohydrate epitope, detected with a monoclonal anti-SSEA-4 antibody. Scale bar, 30 μm. (*b*) Fluorescence micrograph of a 10 μm cryostat section of rat dorsal horn showing SSEA-3$^+$ fibres and terminals in lamina I and III. Roman numerals refer to the laminae of Rexed. Scale bar, 100 μm. (*c*) Phase contrast micrograph showing the cell bodies of four DRG neurons in culture. (*d*) Fluorescence micrograph of the same field in which only one neuron expresses the SSEA-4 antigen on its surface. Scale bar, 15 μm.

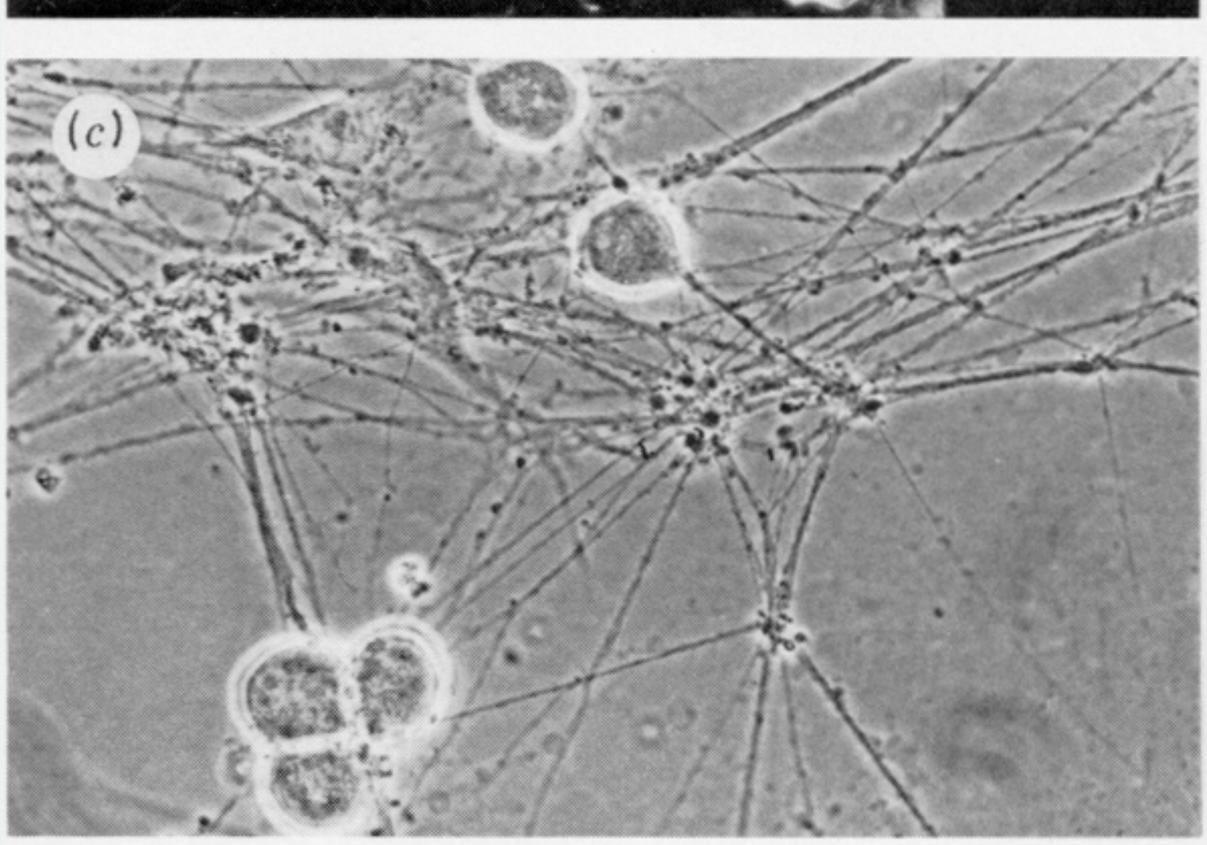


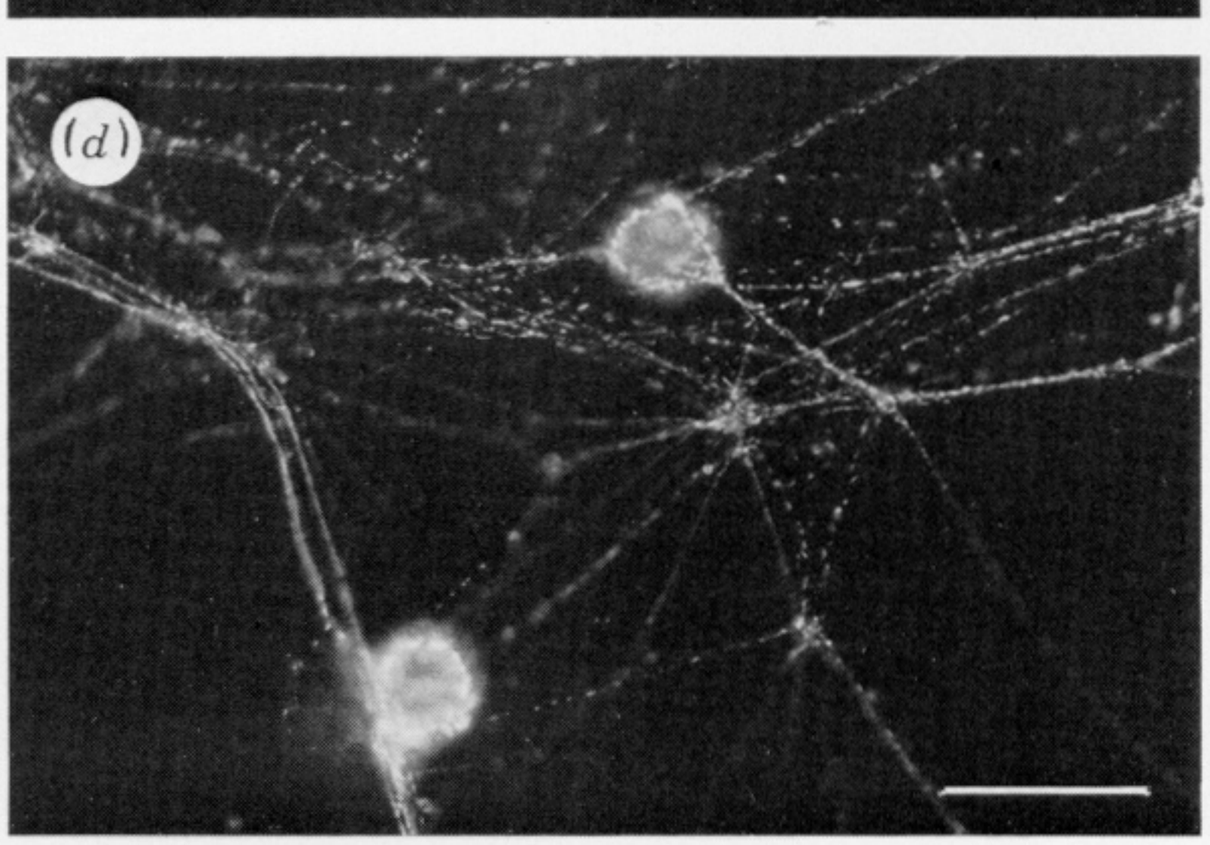












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FIGURES 4 AND 5. For description see opposite.