index representing the relation of cpm of the experiment (+template) to the control (−template) samples. At an index ⩾ 3 the reaction was considered positive.
The statistical analysis of the results was performed according to Student's t-test.
Results and discussion. The treatment of the animals with sodium selenite 7 days before the virus inoculation and during the whole experiment resulted in inhibition of splenomegaly up to 39.7% (table 1). The effect observed was independent of the sex of the animals. The treatment of non-inoculated mice with sodium selenite in drinking water did not influence the spleen weight. In some experiments any major direct virocidal action of the trace element was excluded (see table 2). The results summarized in table 3 show that sodium selenite treatment for different periods after the virus inoculation had a tendency to depress splenomegaly, though less markedly and, within the various series, non-significantly.
In order to obtain additional information on the mode of action of selenite we studied in vitro the influence of Na_2SeO_3 on the reverse transcriptase activity of BLV, another type C oncornavirus which was available. The results summarized in table 4 show a drastic depression of RNA-dependent DNA-polymerase activity. The mechanism of this effect is not clear.
Billard[10] and Oxford[11] show that selenocystine and selenocystamin inhibit the RNA-dependent RNA-polymerase of influenza A_1, A_2 and B viruses. The blocking of the enzyme is reversible. Its activity is restored by adding dithiothreitol or mercaptoethanol, which shows that the RNA-dependent RNA-polymerase is sensitive to sulfhydryl reagents. Selenium is able to displace sulphur from the sulfhydryl groups with formation of selenohydryl groups[12]. This fact may be involved in the effect observed by us.
We suppose that the sodium selenite inhibition of the splenomegaly induced in BALB/c mice with MLV-R is connected with the influence of the trace element on the reverse transcriptase activity of the virus. Possibly, the sodium selenite prevents the integration of the virus genome with the cellular DNA.
The suppressive effect of selenite on the reverse transcriptase activity may well account for its inhibition of carcinogenesis induced by different chemical agents.

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Localization of cholesterol in the Golgi apparatus of cardiac muscle cells

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Summary. Filipin (a polyene) interacts with cholesterol in membranes, producing distinctive deformations that can be detected by freeze-fracture. The distribution of filipin-induced deformations in the Golgi apparatus of cardiac myocytes suggests a role for this organelle in the transformation of cholesterol-poor membrane to cholesterol-rich membrane.

Cholesterol is an important constituent of cellular membranes, exercising a critical influence on their fluidity and permeability[2-4]. In muscle cells, cholesterol depletion of the plasma membrane leads to a significant increase in calcium and sodium influxes during depolarization[5], and the ATPase activity of sarcoplasmic reticulum (SR) is strongly inhibited by interaction of cholesterol with the calcium pump protein[6,7]. Although plasma membranes have a high cholesterol content compared with intracellular membranes[3,8-11], the mechanisms by which this difference is established and maintained are uncertain[11]. The polyene antibiotic, filipin, interacts specifically with cholesterol and related 3-β-hydroxysterols[12,13], producing distinctive deformations in membranes[14]. These deformations can be visualized by freeze-fracture electron microscopy[15,16], enabling direct localization of cholesterol in the membrane plane[17]. Using this approach in rabbit and rat cardiac muscle cells, I report here evidence for a specific distribution of cholesterol within Golgi apparatus membranes. The distal (mature) face appears cholesterol-rich, the proximal (forming) face cholesterol-poor, and the intercalary (mid-region) cisternae intermediate in cholesterol content. From these results, a Golgi apparatus function in enrichment of membrane cholesterol – as part of the process by which membranes are transformed from endoplasmic reticulum-like to plasma membrane-like – is proposed.
Materials and methods. Adult rabbits (New Zealand strain) and rats (BD IX strain) were killed by dislocation of the neck. Rabbit hearts were perfused by the Langendorff technique with Krebs-Henseleit buffer (5 min) followed by 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (5 min)[18]. Rat hearts were prepared similarly using a simple perfusion apparatus. Small (0.5 mm^3) samples of fixed left ventricle wall were immersed in the same fixative containing 100 µg filipin cm^{-3} for 3 h or 22 h in light-proof containers at 22 °C. Filipin was initially dissolved in dimethylsulphoxide (DMSO) before addition to the fixative solution; the final concentration of DMSO was 1%. Control tissue was treated in parallel with glutaraldehyde containing 1% DMSO, and with the fixative alone. Specimens were rinsed in cacodylate buffer and processed for freeze-fracture and thin-section[18] electron microscopy. For freeze-fracture, the tissue blocks were infiltrated with cacodylatebuffered 25% glycerol, frozen in melting propane,

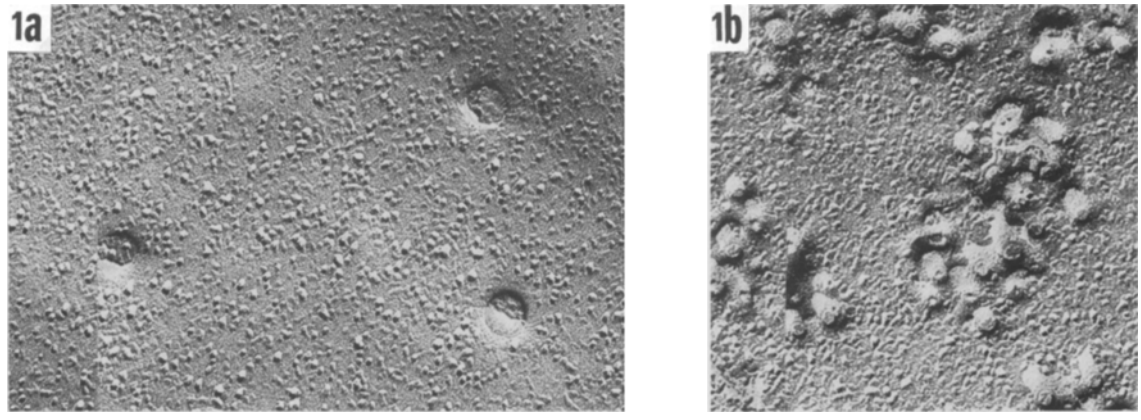
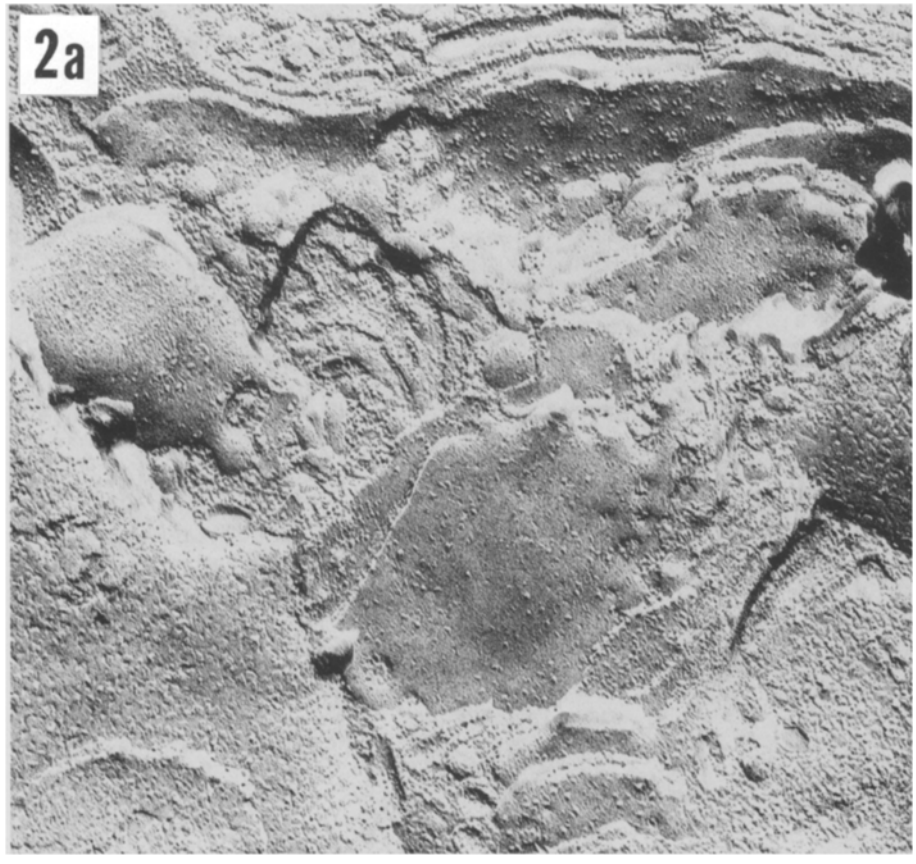


Figure 1. Freeze-fracture replicas of rabbit cardiac muscle plasma membrane, P-face[20]. *a* shows a DMSO control and *b* a filipin-treated specimen. DMSO controls appeared identical to specimens fixed without DMSO; thus (a) illustrates the typical structural features of normal plasma membrane. Intramembrane particles (8 nm diameter) are abundant, and 3 caveolar invaginations (70 nm diameter) are also present in this field. Filipin induces numerous 25 nm-diameter deformations, which are seen predominantly as protrusions in (b) and which usually occur in clusters. Intervening portions of the membrane remain unaffected. × 133,600.

and fractured and replicated in a Bullivant type II apparatus[19] or a Balzers BAF 400T unit. Replicas and sections were examined using a Philips EM 301 electron microscope.

Results. The effect of filipin on the plasma membrane is illustrated in figure 1. Numerous 25 nm-diameter deformations are induced by the agent though substantial areas of the membrane remain unaffected. Unlike smooth muscle cells[21], no preferential association of the deformations with caveolae is evident. The response of the SR appeared less marked than that of the plasma membrane as also reported in frog skeletal muscle[22]. Mitochondrial and nuclear membranes remained completely resistant to treatment. Golgi apparatus membranes were affected, however, but in a specific non-uniform manner (fig. 2). The forming face (adjacent to the nucleus) was always devoid of deformations, whereas the mature face (directed towards the plasma membrane) displayed them in abundance. Intercalary membranes often showed patches of deformations which were more extensive towards the mature face. This pattern was found in all Golgi dictyosomes whose polarity could be established. The results were similar in both species and are based on observation of 13 replicas of rabbit hearts and 7 of rat. In the rabbit, > 80 plasma membranes, the SR of > 50



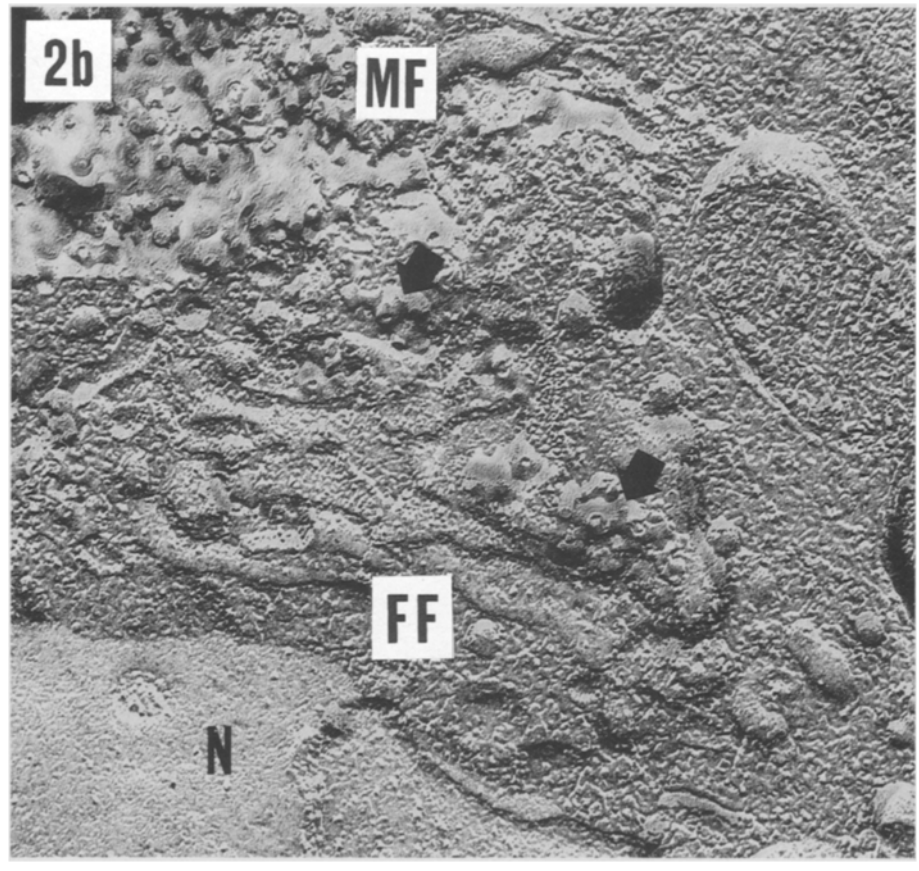


Figure 2. Freeze-fractured Golgi dictyosomes from rabbit cardiac muscle cells. *a* The normal appearance of the Golgi membranes as seen in a control which received neither DMSO nor filipin treatment. *b* In cells exposed to filipin, the cisternal membranes at the Golgi forming face (FF) are unaffected, but those at the mature face (MF) reveal numerous deformations. Some intercalary membranes show an intermediate response (arrows). The polarity of the Golgi is determined by the position of the nucleus (N); the forming face is adjacent to the nucleus and the mature face furthest from it. The nuclear membranes are unaffected by filipin. *a* ×86,600; *b* ×83,400.

cells, and the membranes of > 80 mitochondria, 25 nuclei and 17 Golgi apparatus were examined. Variation in treatment time (3 h vs 22 h) did not affect the results. Thin-section studies confirmed that cell preservation was satisfactory after filipin treatment.

Discussion. Cholesterol is the principal sterol of mammalian cell membranes[4] and so a positive response to filipin in rabbit and rat myocardium should mark the presence of a cholesterol-rich membrane or membrane domain. That it does so is confirmed by the specific and reproducible responses given by the different membrane systems. Biochemical analysis has consistently shown low cholesterol levels in isolated nuclear and mitochondrial membranes, high levels in plasma membranes and intermediate levels in smooth membrane systems (i.e. smooth endoplasmic reticulum or SR, and Golgi apparatus)[3,8–11]. It is therefore valid to interpret the response reported here in the Golgi apparatus as showing a transition from cholesterol-poor membrane at the forming face to cholesterol-rich membrane at the mature face.

How differences in the cholesterol content of membranes arise is not clearly understood[11]. Mammalian cells can synthesize their own cholesterol in the smooth membrane systems but this mechanism is suppressed in the presence of an exogenous supply[23]. Cholesterol is directly exchangeable between the plasma membrane and serum lipoprotein[24], and may also be taken into the cell by endocytosis of low density lipoprotein[25]. Furthermore, the proportion of unsaturated fatty acids in a membrane may influence its propensity to interact with cholesterol[3].

Membrane conversion is a principal function of the Golgi apparatus[11,26]. Although this organelle has some synthetic capacity (notably for carbohydrates and lipids), most membrane lipids and intrinsic proteins are synthesized in the endoplasmic reticulum, and delivered to the Golgi apparatus as endoplasmic reticulum-derived or outer nuclear membrane-derived vesicles[26]. Following fusion of these vesicles at the forming face, the membrane undergoes progressive transformation through the intercalary cisternae, emerging at the mature face as membrane that structurally resembles plasma membrane and which may ultimately fuse with it[11,26].

The present report establishes that cholesterol content rises in concert with this process. It is uncertain whether this is achieved by synthesis of cholesterol within the Golgi apparatus itself or by incorporation of cholesterol transported from elsewhere (i.e. from another site of synthesis or an external source). It is also unknown what role, if any, alterations in lipid saturation play in facilitating cholesterol incorporation into Golgi membranes. The present results do, however, demonstrate that the membrane flow-differentiation model of biogenesis[11,26], derived from studies of other membrane components, is also applicable to cholesterol. Golgi apparatus function may therefore contribute to the maintenance of high cholesterol levels in the plasma membrane. That these conclusions apply to more than one cell type is demonstrated in a recent article by Orci et al.[27], which appeared after the present report was submitted for publication.

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Neurons with dual axons in the substantia gelatinosa (SG) of the adult cat lumbosacral spinal cord[1]

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Summary. A small percentage of SG neurons possessing two separate and complete axons were observed in the lumbosacral spinal cord of the adult cat. Since they are found in small numbers and are structurally similar to single axon SG cells, dual axon cells may represent a developmental aberrancy rather than a functionally distinct cell type.

The concept that a neuron gives rise to one and only one axon is a maxime strongly established by Ramón y Cajal[3] and other classic anatomists. Modern anatomists, however, have periodically found exceptions to the 1-axon axiom. Several investigators have demonstrated interneurons with multiple somatic or dendritic appendages in the form of spines and beaded axon-like processes which have been shown ultrastructurally in several instances to be presynaptic processes (see Beal and Cooper[4] for references). The present report demonstrates a unique spinal neuron which generates 2 major, separate and complete axons. Although similar cells have been reported in the caudal trigeminal nucleus[5], this is the first report of dual axon cells in the SG of the spinal cord.

The present study is part of an extensive Golgi analysis of the morphological characteristics of neurons located in the SG, lamina II of Rexed[6], in the lumbosacral spinal cord of the adult cat. Spinal cord tissue was obtained from 15 sexually mature young adult cats weighing 1.5–2.0 kg. Each was processed according to a modified rapid Golgi procedure[7] and sectioned at 100–150 µm in the sagittal and transverse planes. Drawings of SG neurons were made with the aid of a light microscope with 100X objective and drawing tube attachment.

In the present analysis a small percentage of Ramón y Cajal's[8] 'central' cells were found to have 2 separate axons. The axons originate from the cell body or from dendrites at various distances from the cell body, either from the same pole or from opposite poles of the cell. The axons branch repeatedly and generate an extensive plexus in the vicinity of the cell body. The 2 axonal arbors display considerable overlap and have branching patterns similar to those produced by single axon cells. The axon collaterals are thin and characterized by numerous small 'boutons en passant' (fig.). Aside from an additional axon these cells have no distinctive structural features to separate them from other 'central' SG neurons. In fact, in the present study, single and dual axon cells have been found which are nearly identical with respect to cell size, shape, position, dendritic pattern and specializations.

The significance of neurons with dual axons is puzzling. One possibility is that each of the 2 axons has a different destination. In chick embryos of 10 days incubation, Ramón y Cajal[8] described SG neurons in the spinal cord with axons which left the gelatinous substance and entered the overlying white matter at 2 different and remote locations. These axons, however, did not originate from separate portions of the neuron, but rather were derived from a single stem fiber which branched near the cell body of origin into 2 distinct fibers with separate destinations. These cells were referred to as the 'cells of the combined axis-cylinder'. That such cells exist in the adult has been shown in several areas of the nervous system by Hayes and Rustioni[9] who reported dual projections of single neurons after double labeling of cells utilizing the retrograde transport of horseradish peroxidase (HRP) and (^{3}H) apo-HRP. Even though dual axon cells of the present study have collaterals in the vicinity of the cell body, the terminal ramifications of these fibers cannot be followed and may go on to separate and remote locations. One of the axons could conceivably project to brain stem centers, since some SG neurons of the spinal cord have been shown to project to higher levels[10,11]. The dual axon cells of the present study, then, could be a simple variation of the 'cells of the