Table 1 Quantitative analysis of the response of the smooth muscle plasma membrane to filipin and tomatin

	Filipin (single treatment)		Filipin/tomatin (double treatment)		Tomatin (single treatment)
Band regions	91±6(30)	—NS—	96±10/3±1(12)	—S—	28±4(30)
	S		S S		S
Inter-bands	37±4(30)	—NS—	25± 6/40±4(12)	—S—	66±3(30)
Total plasma membrane	62±4(30)	—NS—	64±16/26±4(12)	—S—	58±4(30)

Comparison of the response to each agent after double treatment (central column) with that observed after the corresponding single treatments (filipin, left column; tomatin, right column) in: (1) the caveolar band regions, (2) the inter-band zones and (3) the total plasma membrane. The response to filipin was assessed by determining the numerical density of filipin deformations per μm^2, and that to tomatin by measuring the percentage area of membrane corrugated by the agent. (The tomatin effect cannot be expressed as a numerical density because of the irregular size of the deformations induced by this agent.) Values are the mean ±s.e.m. Statistical analysis was carried out using Student's t-test. S indicates significant differences ($P<0.001$); NS, not significant. Numbers in parentheses show numbers of cells analysed (taken from six animals for each experimental treatment). Note that there is no significant difference between the effect of filipin in samples treated with filipin alone and those treated with filipin followed by tomatin. The response to tomatin, however, does show a reduction in samples receiving the double treatment compared with those exposed only to the saponin. This reduction is marked in the bands but slight in the inter-bands, reflecting the relative quantities of cholesterol in each region bound by the previous exposure to filipin. The data shown come from a representative series of experiments in which exposure to tomatin was for 5 h, and that to filipin was for 22 h. These periods were optimal; longer tomatin treatments produced disruption of some membranes and shorter treatments with filipin left deeper cell layers unpenetrated by the agent. Analysis of cells remaining undisrupted following 22 h treatment with tomatin, and of those cells affected after 3 or 5 h exposure to filipin, gave a similar response pattern.

act as a physical constraint on deformation of the bands by tomatin.

The effect observed with tomatin indicates that either the inter-bands are cholesterol-rich or that lateral translocation of cholesterol from band to inter-band occurs during exposure to tomatin. To distinguish between these possibilities we carried out double-labelling experiments, treating first with filipin to bind the cholesterol present in the bands, and then with tomatin to discover whether the inter-bands remained sensitive to the saponin. This they did, as can be seen from Fig. 1*e* and Table 1, giving a response similar to that obtained with tomatin alone.

As both filipin and tomatin interact specifically with 3-β-hydroxysterols[7,9], the predominant species of which is cholesterol in mammalian cellular membranes[10], our results cannot be explained by differences in specificity of action between the two agents. It also seems unlikely that differences in sensitivity are involved, as filipin is slightly more sensitive to low cholesterol concentrations than is tomatin[7]. Our findings therefore strongly suggest, contrary to prevailing opinion[1], that the distribution of cholesterol in the smooth muscle cell plasma membrane is in fact homogeneous, at least at the level of resolution afforded by freeze-fracture cytochemistry. As the resistance of the inter-band membrane to filipin cannot be attributed to a dearth of cholesterol, it must be considered, from the cytochemical point of view, a false-negative result. The factors responsible for this resistance remain uncertain; closely-packed intramembrane particles—which may have an inhibitory effect on filipin action[11]—are evidently not involved because the inter-band regions are sparsely covered in intramembrane particles compared with the bands[3–6]. However, recent evidence suggests that dense membrane-associated (that is, peripheral) protein components may also influence the effects of filipin[12], and a prominent structural feature of the smooth muscle plasma membrane is the presence of dense myofilament-insertion plaques attached to the cytoplasmic surface of the inter-bands[2,6]. If these plaques form a mechanical scaffold, rendering the membrane too rigid to be easily deformed, then the occurrence of caveolae in discrete zones and the preferential formation of filipin deformations in these zones could have a common explanation.

Filipin is now widely used as a sterol probe in freeze-fracture cytochemistry on the assumption that the deformations induced faithfully reflect the planar distribution of membrane sterols. Our present findings demonstrate that this assumption is not always valid. Negative filipin results may, however, be verifiable using saponins.

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Corrigendum

Correction to 'Geomagnetic secular variation as a precursor of climatic change' by V. Courtillot, J. L. Le Mouël, J. Ducruix & A. Cazenave: It has recently been brought to our attention that there was an error of sign on the ordinate axis of Fig. 1 of our paper[1]. This should read $d(\Delta T)/dt$ and not $d(\Delta T)/dt$. This error was made only in the figure and the discussion in the text is based on the correct sign. Thus our conclusions remain unaltered.

The error unfortunately also occurred in two earlier papers[2,3]. The original data are found in Morrison's[4] Fig. 4. $d(\Delta T)/dt$, termed the excess length of day, is actually measured in ms per SI day. It may be clearer, as stated in the legend to Fig. 1 of the paper by Le Mouël *et al.*[2] to express $-d(\Delta T)/dt$ as the fractional increase in the Earth's rotation rate $\Delta\Omega/\Omega = m$. This requires multiplication by 1.157×10^{-8}. Thus, for instance, m decreases from about 0 to about -3.5×10^{-8} beween 1930 and 1970.

We take the opportunity of this correction to mention work in progress on the correlation between the fractional increase in the Earth's rotation rate and the westward drift of the geomagnetic field. This suggests that the rotation lags the field by 15 rather than 10 years. Thus, the forecasted acceleration in rotation following the 1970 secular acceleration jerk might not occur before 1985 and the increase in average global temperature before 1995–2000.

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