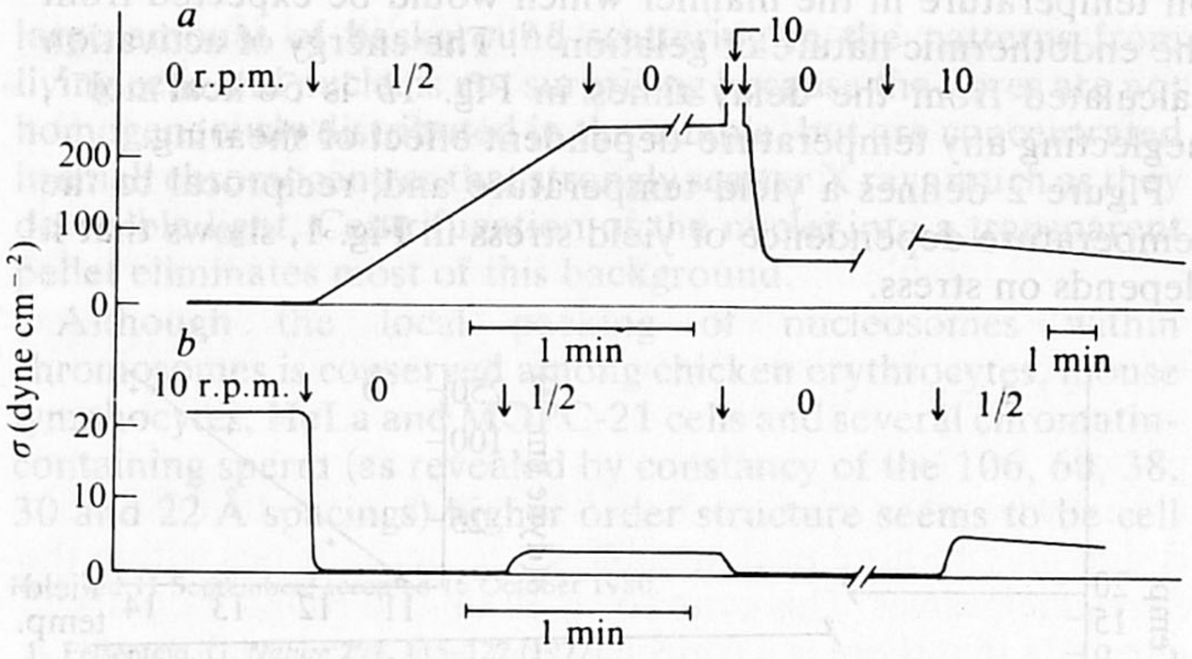

Fig. 4 Well sheared gels are viscous. *a* shows, as in Fig. 1, the stressing after annealing of a gel of 13.5 mM deoxyhaemoglobin S at 20 °C; full scale stress was held. Then the gel was sheared at 10 r.p.m. for 10 s (during which stress was off scale), resulting in partial relaxation to an asymptotic stress of 64 dyn cm^{-2}, indicating viscoplastic behaviour. Fifteen minutes later (during 8 of which the gel was sheared at 10 r.p.m.) the gel showed a time-dependent thixotropic decay in viscosity under continuing shear at 10 r.p.m. After prolonged shear for 120 min at 10 r.p.m., the viscosity reached a final constant value, shown at the start of *b*. Stopping the viscometer then resulted in rapid relaxation to zero stress. Under stressing at 0.5 r.p.m. the gel failed to hold any significant stress, a constant level reflecting viscosity only being attained. On stopping the motor, relaxation was again complete. After 30 min of annealing under no shear, the final part of *b* shows that rotation of the motor at 0.5 r.p.m. resulted in a peak in stress higher than that in the previous stressing, followed by a fall to a level dictated by viscosity, also higher than previously, indicating that some reannealing occurred.

In Fig. 3 shearing was continued after the delay period into the stage of fibre growth[8] to different levels of viscosity. When shearing was stopped after viscosity had reached about 50 cP or more little or no relaxation occurred and the gels held large stresses, showing that the rheological behaviour was essentially solid-like rather than viscous.

By contrast, Fig. 4 shows that well sheared gels held no measurable stresses and were therefore purely viscous. Also, (apparent) viscosity decreased with time under shear and, after a period of no shear, reannealing occurred, demonstrating the presence of viscous thixotropy. Also in Fig. 4, gels subject to brief shear were viscoplastic, manifesting viscous decay to a non-zero asymptotic stress.

These results show that unsheared gels of haemoglobin S are solid-like, well sheared gels are purely viscous, and intermediate, viscoplastic conditions also occur. Also, thixotropy exists in the sense of conversion between solid-like and viscous behaviour as well as in the more usual sense of shear and time dependence of viscosity.

This observation of solid-like behaviour with a plastic yield stress provides justification for the designation 'gel', applied previously even though the system was generally described as 'highly viscous'.

The existence of solid-like properties may have a bearing on pathogenesis in sickle cell disease. An erythrocyte containing a purely viscous interior would deform to an extent dependent on the time integral of stress and thus might eventually pass the microvasculature. On the other hand, a solid-like erythrocyte could not deform unless a critical yield stress were attained. Because shearing history governs the solid-like or viscous nature of the gel, it may also govern the pathogenic potential of each erythrocyte. Whether shear is a favourable or unfavourable factor, however, depends on its effect on gel rheology and also on the fact that it accelerates the delay stage of gelation[3,4].

Generally, these observations show that gel rheology is highly modifiable even in the absence of alteration of the thermodynamics and kinetics of gelation, and thus that pathogenesis in this highly variable disease may be influenced by the highly variable rheology of gels.

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A variant surface glycoprotein of *Trypanosoma brucei* synthesized with a C-terminal hydrophobic 'tail' absent from purified glycoprotein

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Sequential expression of variant surface glycoproteins (VSGs) enables the parasitic protozoan *Trypanosoma brucei* to evade the immune response of its mammalian hosts[1,2]. Studies of several VSGs, which have been isolated as soluble molecules following disruption of cells in the absence of detergent, have indicated extensive amino acid diversity[3–5] and the absence of a hydrophobic segment which might serve to anchor the carboxy terminus to the membrane[5]. The carboxy-terminal tryptic peptides of six VSGs have recently been characterized and shown to be glycosylated[6]. Three of these VSGs terminated with a glycosylated aspartate or asparagine residue (Asx), suggesting that the VSG was cleaved following synthesis and glycosylation and before characterization. We present here nucleotide sequence data which suggest that the primary translation product of one VSG gene contains a hydrophobic tail at the carboxy terminus which is not found on the isolated, mature glycoprotein[6]. The data also predict that the glycosylated residue is aspartic acid rather than the anticipated asparagine.

We have recently reported the synthesis and molecular cloning into the plasmid pBR322 of complementary DNA (cDNA) molecules corresponding to the VSGs of four variants of a single clone of *T. brucei*[7]. As extensive amino acid sequence data on one of these VSGs (VSG 117) already existed (G. Allen, personal communication), we have selected the corresponding recombinants (T-cV117 · 1–8) for further study. As purified VSG 117 terminates with a glycosylated Asx[6], suggesting post-glycosylation proteolytic cleavage of a VSG precursor, we decided first to determine the nucleotide sequence corresponding to the 3′ end of the mRNA, coding for the carboxy terminus of the protein.

A physical map of the cDNA insert which extended furthest in the 3′ direction (T–cV117 · 8) and the protein region it covers is given in Fig. 1. Also shown are the regions where useful nucleotide sequence information was obtained. Figure 2 presents a representative sequencing gel corresponding to the carboxy-terminal portion of the protein. The complete nucleotide sequence of this region and the implied amino acid sequence are

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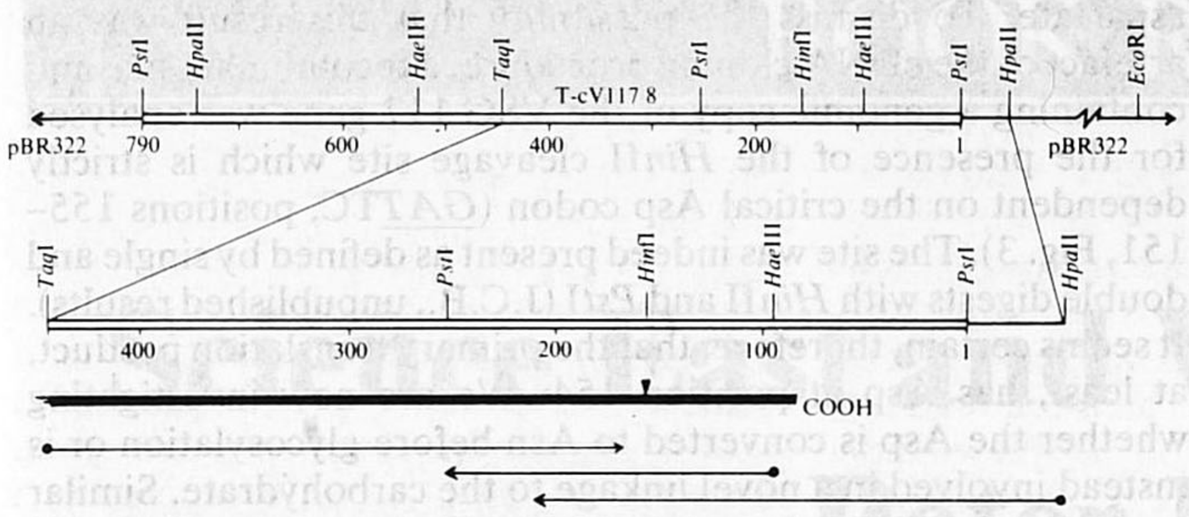

Fig. 1 Physical map of T-cV117.8 showing restriction endonuclease cutting sites. Distances are in base pairs from the end of the insert nearest the *Eco*RI site of pBR322. These data were obtained by standard mapping procedures and subsequently confirmed by the DNA sequence. Beneath the map are shown the region of the insert which codes for the putative primary translation product and the carboxy terminus of the mature VSG (▼). Below this are shown the sites which were [5′-^{32}P]-end-labelled (●) and the regions where useful sequence information was obtained (→).

given in Fig. 3. The fact that there is neither a poly(A) stretch before the dC tail in Fig. 3 nor the sequence (AATAAA) usually found about 25 nucleotides upstream of the site of poly(A) addition[8] suggests that T-cV117 · 8 does not extend to the 3′ end of the VSG 117 mRNA. This may be an artefact of the process by which these recombinants were constructed or selected[7]. The inferred amino acid sequence shown in Fig. 3 is in complete

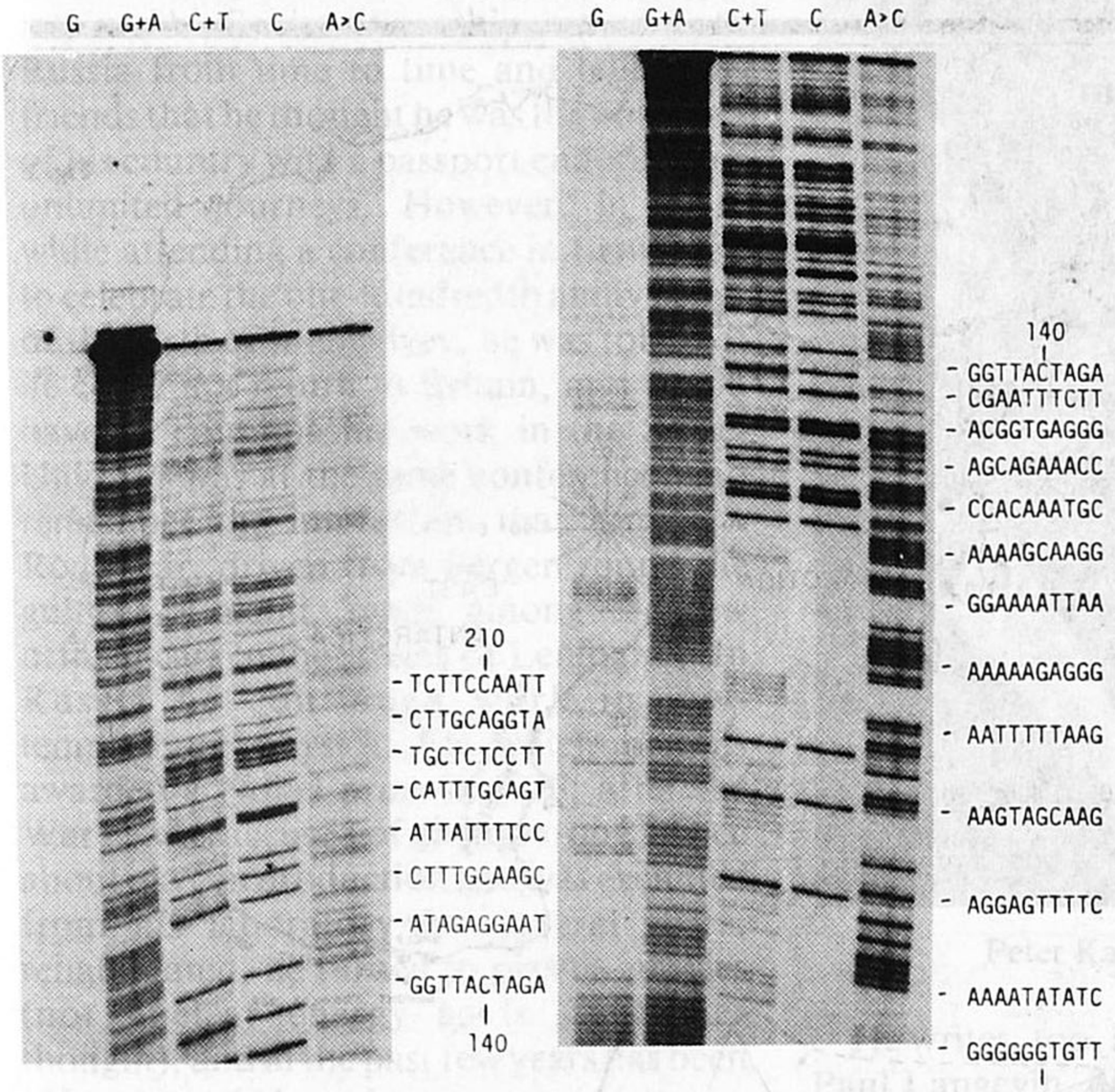

Fig. 2 Autoradiograph of a representative sequencing gel corresponding to the carboxy terminus of VSG 117. A *Hpa*II fragment of DNA from T-cV117.8 was [5′-^{32}P]-end-labelled with polynucleotide kinase, recut with *Taq*I and purified on a 7% polyacrylamide gel essentially as described[14] except that the eluted fragment was further purified by binding to a 1-ml column of diethylaminoethyl-cellulose followed by elution in 0.5 M NaCl. Base-specific modifications and cleavage were as described[14], with minor alterations as recently recommended by the authors (A. Maxam and W. Gilbert, personal communication). Analysis was on a thin (0.4-mm) 8% polyacrylamide gel in 7 M urea run at high voltage (1.6–2.0 kV) to reduce secondary structure effects[15]. Aliquots of each sample were loaded on to different tracks of the same gel at 0 (*a*) and 120 min (*b*), the gel being run for a total of 270 min. After cooling, the gel was fixed by immersing in 10% (v/v) acetic acid for 20 min, dried under vacuum with heat and autoradiographed at −70 °C in a Kodak cassette containing a Dupont Cronex Xtra-life intensifying screen. The nucleotide sequence corresponding to the C-terminal region of the protein is given to one side. This sequence is of the noncoding strand, the nucleotides being numbered as in Fig. 1.

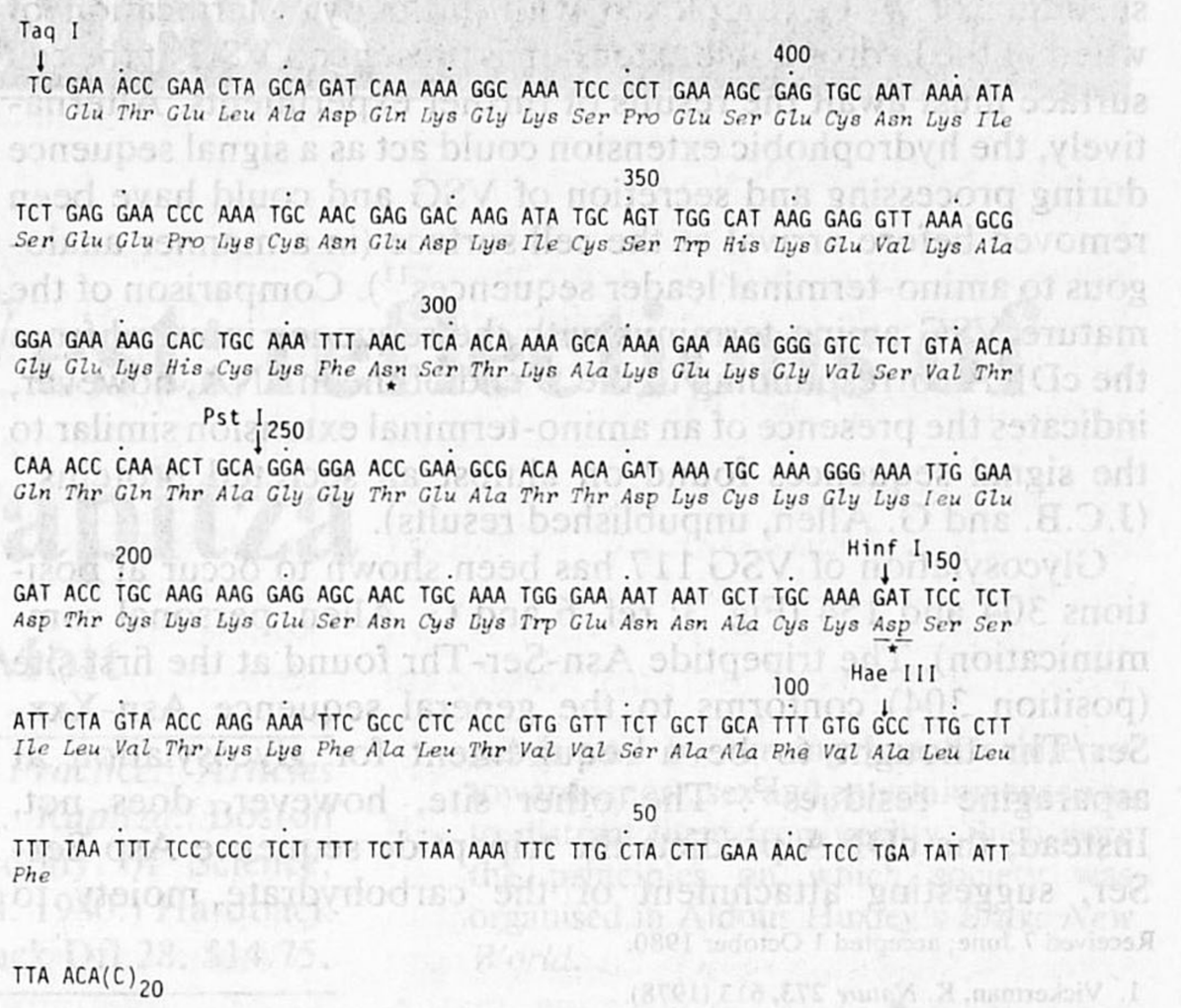

Fig. 3 Summary of nucleotide and protein sequence data. The nucleotide sequence of the coding strand between positions 445 and 1 (see Fig. 1) is given together with the implied amino acid sequence. Only one reading frame is open. The carboxy-terminal residue of the mature, isolated glycoprotein is underlined (see text). Glycosylated residues are marked with asterisks. $(C)_{20}$ indicates the dC-tail used to anneal the cDNA insert to the dG-tailed plasmid.

agreement with independently derived data from the purified glycoprotein (ref. 6 and G. Allen, personal communication) except that protein sequencing indicated the carboxy terminus to be exclusively glycosylated Asx at position 154, whereas the nucleotide sequence shows 23 sense codons beyond this position before a stop codon is reached. It seems, therefore, that at some time after translation of the polypeptide and before characterization of the VSG, a highly specific proteolytic cleavage has occurred 23 amino acids in from the carboxy terminus.

Although we have not directly examined the carboxy-terminal sequence of the primary mRNA translation product, it is a reasonable assumption that it contains the extension predicted by the DNA sequence. This would be consistent with our previous observation[7] that 117 VSG mRNA translated *in vitro* yields a polypeptide which seems to be slightly larger on SDS-polyacrylamide gels than mature VSG isolated from trypanosomes, despite glycosylation of the latter which should increase its apparent molecular weight. The amino acid sequence towards the end of the predicted extension is extremely hydrophobic. We have been unable to define the role of the hydrophobic extension and the time at which it is cleaved. The fact that all six VSGs studied terminate with a glycosylated amino acid[6] suggests cleavage may be a specific event common to VSG processing in *T.brucei*.

Hydrophobic carboxy termini have been reported in surface proteins of influenza virus[9] and *Bacillus stearothermophilus*[10] and may generally serve to anchor membrane-bound cell-surface proteins[11]. Observations that carboxy-terminal glycopeptides of trypanosome VSGs carry an immunologically cross-reacting group[6], which on living cells is not accessible to antibody[12], are consistent with the carboxy terminus being in close proximity to the surface membrane. If present in the VSG molecules forming the surface coat of living trypanosomes, the hydrophobic extension could serve a membrane-binding function. However, no precedent exists for the rapid and specific cleavage which would be required to explain the observation that mature VSG is released on cell disruption, even when several inhibitors of proteolysis are present (G.A.M.C., unpublished observations). The existence of such a mechanism for specific cleavage and release of VSG from the cell surface suggests a role in antigenic variation, possibly in allowing

shedding of VSG complexed with antibody. Clarification of whether the hydrophobic extension is present on VSG at the cell surface must await the results of further experiments. Alternatively, the hydrophobic extension could act as a signal sequence during processing and secretion of VSG and could have been removed before arrival at the cell surface (in a manner analogous to amino-terminal leader sequences[11]). Comparison of the mature VSG amino terminus with the sequence inferred from the cDNA corresponding to the 5′ end of the mRNA, however, indicates the presence of an amino-terminal extension similar to the signal sequences found on almost all secreted proteins[11] (J.C.B. and G. Allen, unpublished results).

Glycosylation of VSG 117 has been shown to occur at positions 304 and 154 (Fig. 3; ref. 6 and G. Allen, personal communication). The tripeptide Asn-Ser-Thr found at the first site (position 304) conforms to the general sequence Asn-Xxx-Ser/Thr thought to be a requirement for glycosylation at asparagine residues[13]. The other site, however, does not. Instead, the cDNA predicts the tripeptide sequence Asp-Ser-Ser, suggesting attachment of the carbohydrate moiety to aspartate. To dismiss the possibility that this result was an artefact of the cDNA cloning procedure, a recombinant plasmid containing a genomic copy of the VSG 117 gene was analysed for the presence of the *Hin*fI cleavage site which is strictly dependent on the critical Asp codon (*GATTC*, positions 155–151, Fig. 3). The site was indeed present as defined by single and double digests with *Hin*fI and *Pst*I (J.C.B., unpublished results). It seems certain, therefore, that the primary translation product, at least, has Asp at position 154. We are now investigating whether the Asp is converted to Asn before glycosylation or is instead involved in a novel linkage to the carbohydrate. Similar analyses of cDNAs for other VSGs (now underway) should indicate whether the hydrophobic extension and glycosylated aspartic acid are universal features of the surface glycoproteins of *T.brucei*.

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Erratum

Figures 1, 2 and 3 were omitted from the letter 'Lower Cretaceous sediment from the East Antarctic continental shelf' by E. W. Domack *et al. Nature* **287,** 625–626.

Fig. 3

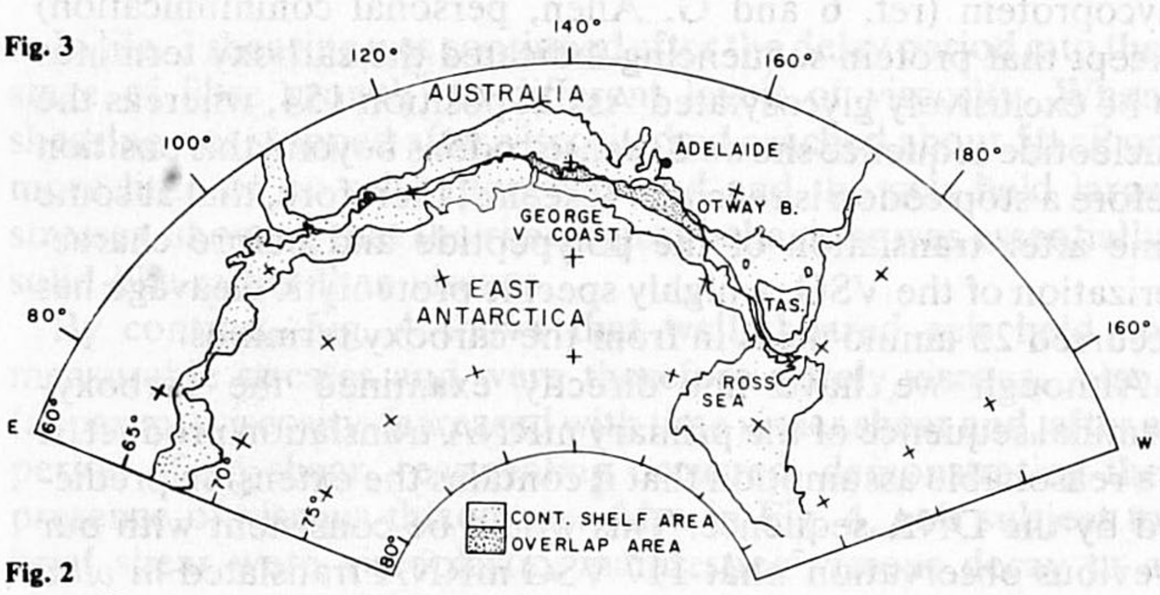

Fig. 2

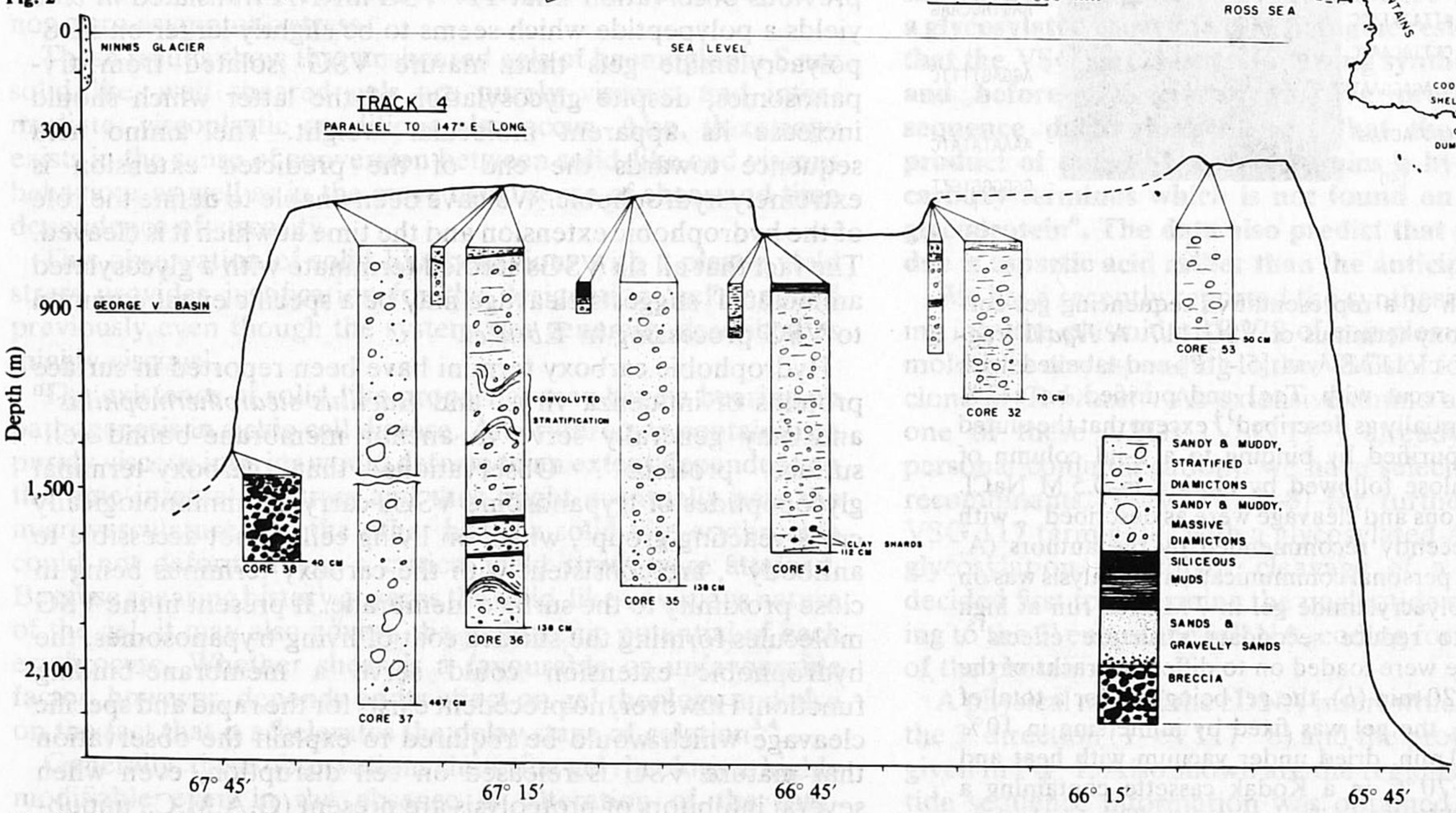

Fig. 1

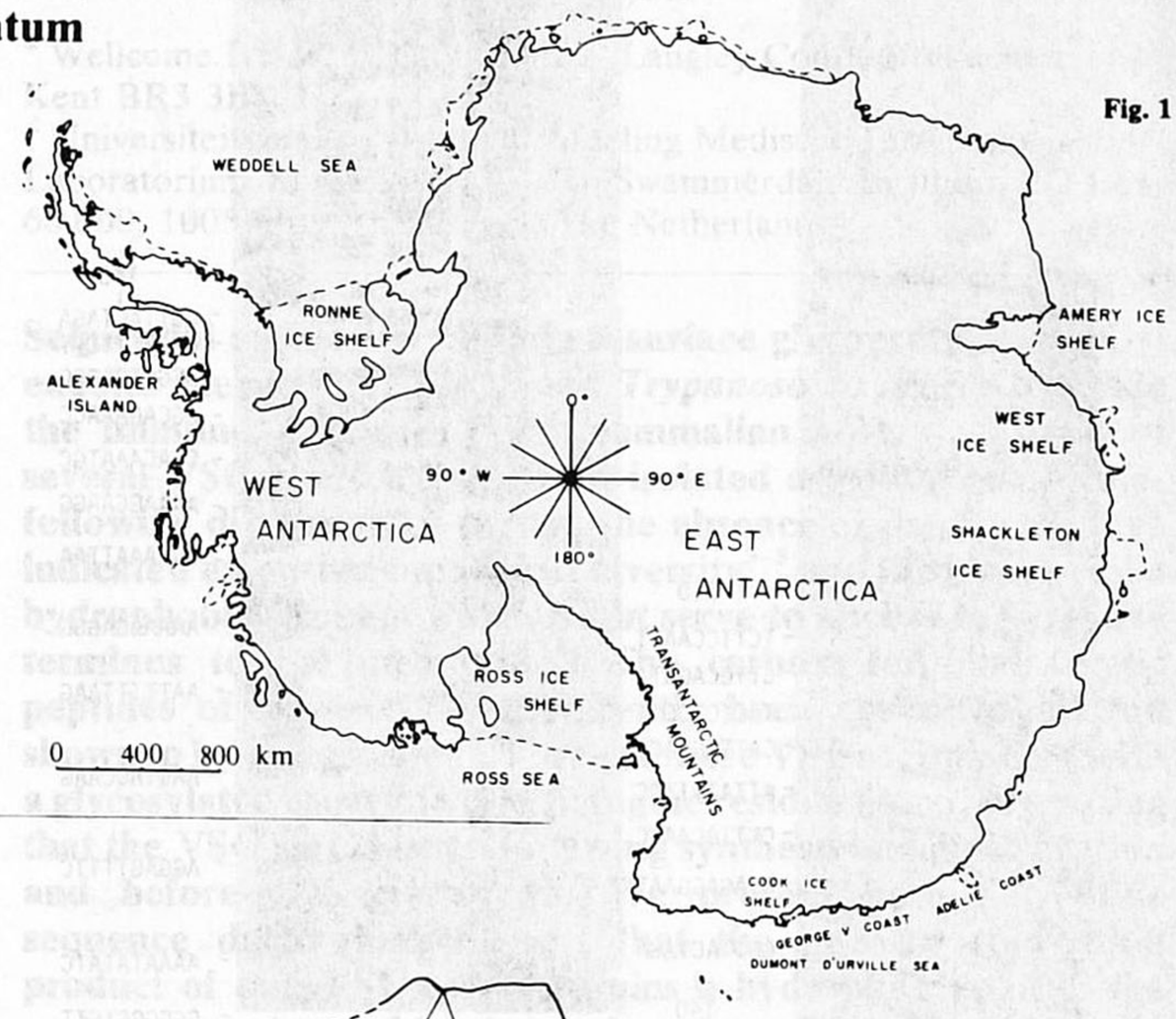

Fig. 1 Map of the Antarctic continent showing the locations of regions mentioned in the text.
Fig. 2 Profile of the continental shelf off the George V coast showing the relationship of core 38 to other sediments of the shelf (after ref. 7, Fig. 24).
Fig. 3 Morphological reconstruction of Australia and East Antarctica showing the positions of localities mentioned in the text (after ref. 9, Fig. 3).