However, no significant binding of proteins labeled with $^{35}SO_4$ (that is, <1% of the sample radioactivity) was obtained from either the proteoglycan-containing fraction itself (before or after heparitinase treatment) or from the two preceding fractions from the ion-exchange column. Moreover, recent studies indicate that the size of the ABPP is 110 to 135 kD (*9*), rather than the 65 kD that correspond to the HSPG core protein proposed by Schubert *et al.*

Although the ABPP may be secreted by PC12 cells, we believe there is as yet insufficient experimental support for the conclusion that it is a HSPG core protein.

Note added in proof: Schubert *et al.* have recently published a paper (*14*) in which they have significantly revised several of their original conclusions and confirmed a number of the points made above. These include the following. (i) Two tyrosine-sulfated proteins which bind to heparin and are detected by an antiserum to residues 175 to 186 of the predicted ABPP sequence (which they name the GID antigen) have the same molecular size range (115 to 140 kD) as the PC12 cell HSPGs (110 to 135 kD) and are found to copurify with HSPG when examined by SDS-PAGE. (ii) The protein sequenced in their purified HSPG preparation may be the GID antigen. Although these two components have now been separated by ion exchange chromatography on DEAE-cellulose (*14*), as evidence of homogeneity it was previously stated that "all of the sulfate-labeled material migrates as a single peak with heparan sulfate on a DEAE column" (*1*). (iii) The GID antigen is not secreted by the F3 mutant line of PC12 cells, which lacks a cell-surface HSPG (*10*), but secretes the same HSPGs as the parent cell line (*4*).

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   Media from cells labeled with $^{35}SO_4$ were clarified by centrifugation and made to a concentration of 6*M* urea, 50 m*M* sodium acetate, 0.15*M* NaCl, 5 m*M* *N*-ethylmaleimide, and 0.2% Triton X-100, at *p*H 6.0, to which was added 5 m*M* of unlabeled sodium sulfate carrier. After several exchanges with the same solution on a Centriprep-30 membrane (Amicon), the medium (10-ml original volume) was made to 0.1*M* NaCl/0.5% CHAPS and applied to a 0.9 by 8 cm column of DEAE-Sephacel equilibrated with 50 m*M* sodium acetate (*p*H 6.0), containing 6*M* urea, 0.1*M* NaCl, and 0.5% CHAPS. Proteoglycans were eluted with the same buffer containing 0.06*M* NaCl after elution with 0.1*M* and 0.31*M* NaCl.
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Response: Although we were aware of the work of Gowda *et al.* on PC12, I do not believe that the properties of the PC12 clone that we were dealing with were the same as the properties of the clone they have used. Different PC12 clones have different properties. Since we were interested in sequencing a neuronal heparan sulfate proteoglycan (HSPG), our intention was to find a clone among our many nerve cell lines that secreted only one type of proteoglycan. The implication of the first part of the comment by Gowda *et al.* is that we did not distinguish heparan sulfate from chondroitin sulfate proteoglycan; the second part concerns some unpublished experiments that Gowda *et al.* believe are contradictory to our results. They conclude that it is possible that the precursor protein is a protein that copurified with the HSPG. This possibility was stated explicitly on page 225 of our report. However, all of the available data are consistent with the identity of the PC12 HSPG core protein, defined by us and by Matthew *et al.* (Gowda *et al.*'s reference 7), with the amyloid β-protein precursor (ABPP).

The following are my answers to specific comments.

1) We outlined in the text and in reference 14 of our original manuscript the results showing that only HSPG is secreted. The data were deleted from the text of the manuscript at the request of *Science* to conserve space. Most of these data have recently been published (*1*).

2) We have looked at the proteoglycans of the F3 variant in great detail. Although the F3 cells clearly secrete sulfated proteoglycans, some of which migrate in the position of HSPG on a DEAE column, the proteoglycan with the core protein of Matthew *et al.* was not present or was greatly reduced in amount [as initially shown by Inestrosa *et al.* (reference 10 in Gowda *et al.*)]. The sulfated proteoglycan that migrates in the position of HSPG on DEAE columns has very different characteristics from those of a typical HSPG. It is relatively insensitive to heparitinase and nitrous acid degradation and has a different size from that of the PC12 proteoglycan. The F3 variant does not secrete the antigen defined by the anti-ABPP antiserum used in our experiment, nor does it secrete a different but related form of the ABPP that is detected by a different antiserum that unambiguously (by means of comparison of cells transfected with ABPP gene and parent) reacts with the precursor molecule (*1*). We agree with Matthew *et al.* (reference 7 of Gowda *et al.*) that the PC12 HSPG is about 200,000 daltons. We obtained this value on both sizing columns in guanidine and as heparitinase-sensitive material on SDS gels. The 110- to 135-kD PC12 protein that Gowda *et al.* say is an HSPG on SDS gels appears in fact to be a tyrosine-sulfated form of ABPP which lacks sugar sulfate. This form of ABPP does not coelute on DEAE or sizing columns with PC12 HSPG. Data for these points are presented in (*1*).

3) The transfer systems for Western blots vary from laboratory to laboratory. That was undoubtedly why Matthew *et al.* (Gowda *et al.*'s reference 7) stained whole gels rather than transferred material. In the original version of our text, we stated that silver-stained patterns of secreted protein did not change with heparitinase digestion, suggesting, but not totally ruling out, that proteases were not present. We certainly considered this possibility. Furthermore, proteases alone could not have explained the experimental results, and heparitinase did not degrade the core of the 115- and 140-kD forms of ABPP (*1*).

4) It is implied by Gowda *et al.* that the PC12 cell lines tested were obtained from us

by means of the Reichardt laboratory. Our cells were given to that laboratory about 10 years ago and may bear little resemblance to our original clone.

5) It may be that Gowda *et al.* did not precipitate the protein with their antipeptide sera because of the nature of antibodies to peptides that are derived from larger proteins. Many (at least 15) antipeptide antisera from several laboratories, when tested in cells transfected with the ABPP protein, do not react with the processed precursor molecule. Antipeptide sera usually do not immunoprecipitate proteins well.

6) The size of ABPP (110 to 135 kD) cited by Gowda *et al.* is for the glycosylated molecule, not the unprocessed protein.

The comments and unpublished data of Gowda *et al.*, even if correct (I believe many are not), do not rule out the possibility that one form of the ABPP protein contains heparan sulfate sugar. One would need to completely sequence the PC12 proteoglycan core (Matthew *et al.*'s antigen) and show that it is not the amyloid precursor. Finally, it should be pointed out that, to our knowledge, no attempt was made by Gowda *et al.* to obtain the PC12 line or sulfate-labeled supernatant directly from us.

Note added in proof: The note added in proof by Gowda *et al.* does not represent either the data or the conclusions of our recent paper (*1*) correctly. (i) None of our original conclusions was revised. As stated in (*1*), they were strengthened by the observation that the PC12 HSPG and the lower molecular weight form of ABPP do not copurify on the ion exchange columns used in our initial purification. [figure 1 of (*1*)]. That the same "core" protein may exist as both a proteoglycan and a classical glycoprotein in a clonal cell line has been previously demonstrated. (ii) The GID antigen does *not* have the same molecular weight as the HSPG. The HSPG has a molecular weight centered around 200,000 daltons on gels [figure 2, lane 1, of (*1*)] and sizing columns [see above and reference 7 of (*1*)]. (iii) GID and HSPG do not copurify [see figure 1 of (*1*) and above]. (iv) All of the *detectable* sulfate-labeled material (except the flow-through fractions 10 to 15) purifies on DEAE as a single symmetrical peak around fraction 40 [figure 1 of (*1*)]. Most of the GID antigen (which contains only 0.07% of the total sulfate) elutes well before the HSPG peak on a flat part of the sulfate elution curve (fraction 33). (v) The observation that the F3 variant secretes neither the Matthew HSPG nor the GID antigen argues in favor of the relationship between the two proteins, and that is why these data were included in (*1*). Finally, the proteoglycans secreted by PC12 and F3 are not identical (see above).

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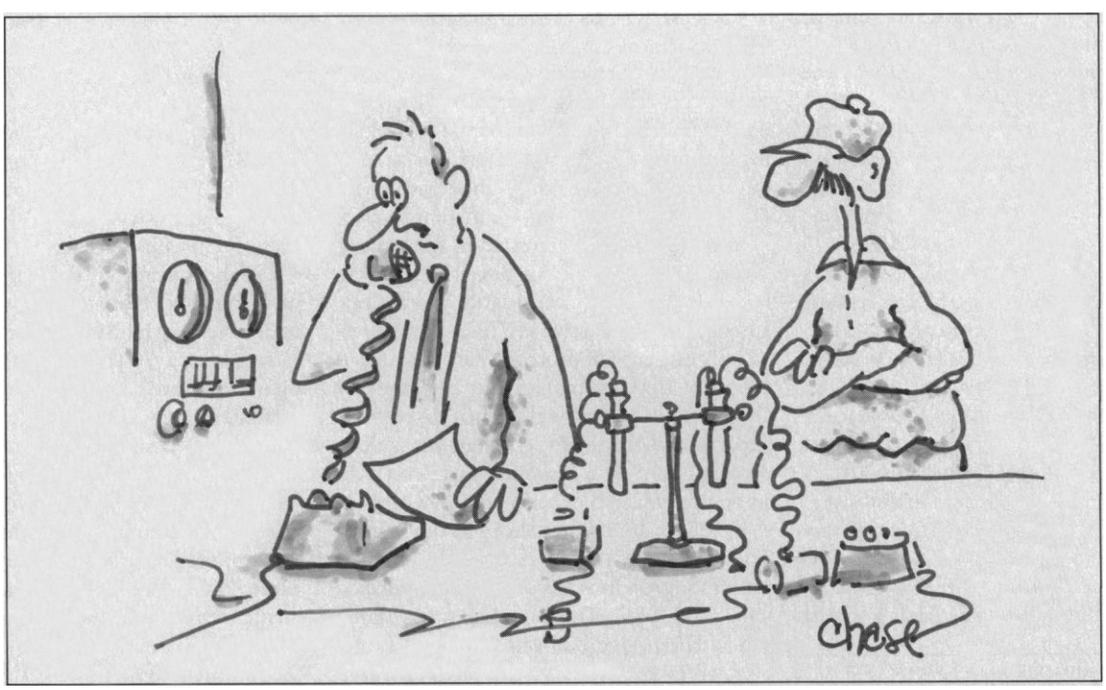

"Our experiment was confirmed by the University janitor...claims he's been doing fusion in the main boiler for sixteen years!"