ously, marine incursions moved up major river valleys, across the Alpine fault, and deposited estuarine silts with marine bivalves in the 250-km-long region between Haast and Kaniere (*8*). Former islands and peninsulas northwest of the fault were notched by sea-level highstands; close terrace spacings indicate much slower uplift than for the Southern Alps.

We believe the available data show that the accordant steps are marine terrace time lines that can be used for inferring uplift and erosion rates. Alternative hypotheses for the origin of accordant bedrock benches with rounded quartz pebbles fail simple tests. It is apparent that moas did not violently thrash rocks in the gizzards and later deposit them selectively on flat topographic steps. Neither glaciers nor streams can account for (i) flat shore-parallel benches more than 5 km long or (ii) the presence of rounded quartz pebbles on flat summits along the main divide of the Southern Alps. Gradual spatial changes in lithospheric properties and stress fields are reflected by changes in uplift rates along the Alpine fault. The temporal doubling of inferred uplift rates at about $135 \times 10^3$ years ago may be the result of a structural realignment brought about by progressively larger convergence between the Pacific and Australian plates.

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# Guanylate Cyclase and the Adrenal Natriuretic Factor Receptor

We read with great interest the paper "Coexistence of guanylate cyclase and atrial natriuretic factor [ANF] receptor in a 180-kD protein" by A. K. Paul *et al.* (*1*). However, several aspects of the data and discussion require comment.

First, the details of the purification were unclear. There is no description of the percent recovery of enzyme and binding activity or of protein. Also, it is unclear whether these preparations represent a single peak fraction off the final chromatographic step or are actually representative of the majority of cyclase and receptor molecules in these cells. It would seem important that, in a report concerned with the purification of an enzyme, these data be included.

In the legend to figure 1, the authors state that 0.12 μg of purified guanylate cyclase was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). However, this amount of protein was well-visualized with Coomassie-blue staining. It is commonly accepted that the lower limit of sensitivity for detection of protein on gels with Coomassie blue with routine use is about 0.5 μg. Paul *et al.* report significantly greater sensitivity in their staining. To detect lower amounts of protein, a sensitive silver stain is required, with sensitivities down to 1 ng of protein (*2*). Thus, Coomassie-blue staining of denaturing gels is not adequate proof of purity of a protein preparation. The authors iodinated their purified guanylate cyclase preparations for isoelectric focusing. It would have been of interest if they had performed SDS-PAGE on these preparations to assess their purity.

In addition, important experiments central to the authors' hypothesis were not mentioned. For example, if the 180-kD protein contains both guanylate cyclase and ANF receptor binding activity, then it should be specifically labeled with $^{125}$I-labeled ANF when this peptide is cross-linked to these preparations, as demonstrated in previous studies (*3*). Also, if the cyclase and receptor are the same molecule, then they should coprecipitate, with identical recoveries, when incubated with specific antibody directed at either activity in an immunoprecipitation assay. Paul *et al.* report having used antibodies for Western blot analysis that specifically inhibit particulate guanylate cyclase, yet they do not report the effects of these antibodies on enzyme and receptor binding activities in immunoprecipitation assays. Nor do they indicate how these antibodies were prepared. Are these monoclonal or polyclonal antibodies? What was the source of antigen, and how much antigen was used for immunization? These details would facilitate analysis and interpretation of the data.

Paul *et al.* state, "Only partial purification of any mammalian particulate guanylate cyclase has been achieved to date" and cite an earlier report (*4*). However, the copurification of particulate guanylate cyclase and the ANF receptor to apparent homogeneity from rat lung was published (*3*) 1 year before the appearance of the report of Paul *et al.* The authors do not mention this earlier report in their initial discussion and say in the latter part of their discussion only that these preparations were "highly purified," even though the earlier work provided a rigorous biochemical demonstration of the apparent homogeneity of the protein preparations and the identity of the cyclase-receptor complex (*3*).

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*Response*: Waldman *et al.* refer to three concerns: (i) lack of detail in the description of the method used to purify the enzyme; (ii) less-than-rigorous documentation of the enzyme's purity; and (iii) lack of acknowledgment of an earlier study in which the authors say they completely purified the enzyme (*1*).

Because of space restrictions, only essential features of the purification of the enzyme were provided in our report. Details that could be excluded without compromising accuracy were deleted. We will be glad to provide these details to any investigator upon request. In the legend to figure 2 of our reference 2, the specific activities of atrial natriuretic factor (ANF) binding and guanylate cyclase at specified purification steps were from single peak-activity fractions.

We did not state that a single protein band, as evidenced by the Coomasie-blue staining of the denatured gel, is absolute

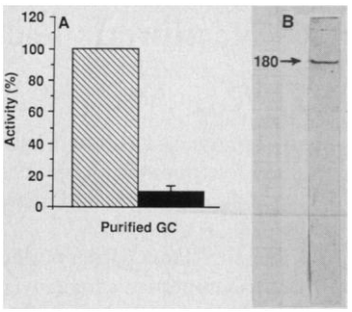


**Fig. 1.** (**A**) Inhibition of purified membrane guanylate cyclase activity by antibody to the 180-kD protein. The enzyme was preincubated with antibody in ice for 1 hour and then assayed for guanylate cyclase activity (*5*). Hatched bar, without antisera; solid bar, with antisera. (**B**) Western blot analysis of guanosine triphosphate–affinity purified fraction depicting one immunogenic band migrating at 180 kD.

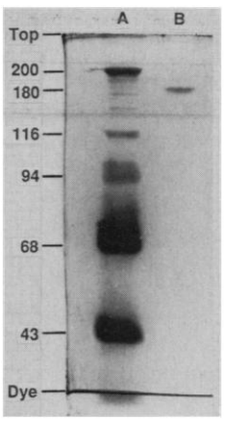


**Fig. 2.** Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of purified membrane guanylate cyclase (GC). Purified enzyme preparation (0.5 μg of protein) was analyzed by 7.5% SDS-PAGE and silver-stained as described in (*6*). (Lane A) Molecular weight markers; myosin (200,000, β-galactosidase (116,000), phosphorylase B (94,000), bovine serum albumin (68,000), and ovalbumin (43,000); (lane B) purified membrane guanylate cyclase.

proof of the enzyme's purity. The gel-staining results, however, should be considered together with the following evidence. (i) Western blot analysis of the crude enzyme reveals a single band made by the 180-kD antibody to guanylate cyclase although the protein staining shows multiple proteins (the antibody is specific to the hormone-dependent guanylate cyclase epitope, since the antibody blocks the ANF-dependent guanylate cyclase activity in crude membranes and up to 90% of the activity of the pure enzyme); (ii) only a single peak of radioactivity superimposed over the enzyme activity peak is observed from isoelectric focusing studies; and (iii) the enzyme binds ANF stoichiometrically.

Figures showing the Western blot and antibody inhibition of guanylate-cyclase activity were omitted from the original manuscript. These figures (Fig. 1, A and B) are now included. A silver-stained gel of the enzyme (Fig. 2) reveals a single band.

Cross-linking and antibody precipitation experiments are obviously important, but at the time were beyond the scope of our studies.

The rabbit antisera to 180-kD guanylate cyclase were raised by immunizing rabbits with purified 180-kD membrane guanylate cyclase emulsified in complete Freunds adjuvant. This technique is essentially similar to what we used in (*3*).

In our report (*2*), we made the following statement with regard to our finding that the 180-kD protein has both guanylate cyclase and ANF receptor activities.

Although the antibody to the 180-kD guanylate cyclase blocks guanylate cyclase activity, it does not inhibit the binding of ANF to the protein. This indicates that either the antibody is solely against the guanylate cyclase epitope of the protein, or that there are two tightly coupled 180-kD proteins which are inseparable by the present techniques.

We properly referenced Waldman *et al.*'s work (*1*) in our statement, "During the course of our investigations, Kuno *et al.* (*16*) showed that in a highly purified rat lung preparation, ANF receptor and guanylate cyclase are copurified." Reference 16 in our report is (*1*) in this response. Waldman *et al.* apparently object to the description of their lung guanylate cyclase as "highly purified" instead of homogeneous. However, examination of their gel picture, figure 2 of (*1*), reveals at least 13 minor proteins, including the major 120-kD protein, in the lung enzyme. Most significant, the lung enzyme bound only 14.5% of ANF at the noted theoretical value. Furthermore the authors state in another publication (*4*), reflecting on the purity of their enzyme

With further modification of this purification scheme for rat lung membranes, the enzyme has been purified about 15,000-fold with a specific activity of 19 μmol/mg/min (*206*). These preparations contain one major protein band (about 95% pure) after SDS gel electrophoresis (*206*).

The quoted reference (*206*) is reference 16 in our original report and (*1*) in this response.

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