nuclides are introduced into the ocean in large quantities they may be used to study local water circulation. Use of an *in situ* detector greatly simplifies the procedure and many more analyses can be made than would be possible if extensive radiochemical separations were required.

M. GRANT GROSS
CLIFFORD A. BARNES
Department of Oceanography, University of Washington, Seattle
GORDON K. RIEL
U.S. Naval Ordnance Laboratory, White Oak, Silver Spring, Maryland

References and Notes

1. Environmental Studies and Evaluation Group, *Evaluation of Radiological Conditions in the Vicinity of Hanford for 1963, Publ. HW-80991* (Hanford Atomic Products Operation, Richland, Wash., 1964).
2. C. Osterberg, J. Pattullo, W. Pearcy, *Limnol. Oceanog.* **9**, 246 (1964).
3. F. F. Koczy and J. N. Rosholt, in *Nuclear Radiation in Geophysics*, H. Israel and A. Krevs, Eds. (Academic Press, New York, 1962), p. 18.
4. J. Z. Holland, *Federation Proc.* **22**, 1390 (1963).
5. G. K. Riel, *Electronics* **36(10)**, 56 (1963).
6. The data were analyzed by a spectrum-stripping technique in which the total number of counts in the photopeak of each radionuclide was corrected by subtracting the counts due to Compton scattering associated with photopeaks of higher energy. The concentration of each radionuclide was calculated after determining the efficiency of the detector by measuring its response in 10^5 liters of various solutions containing known concentrations of Cr^{51}, Cs^{137}, La^{140}, and K^{40}. The efficiency for Zr^{95}-Nb^{95} and Zn^{65} was estimated by interpolation on a plot of detector efficiency as a function of the photofraction of the crystal, divided by the square of the mean free path in water of the gamma photon. The data, computed as picocuries per liter for each radionuclide, are considered significant if the activity was greater than two standard deviations estimated from the counting statistics. Zirconium-95 and Nb^{95} were ubiquitous in the surface waters, so spectra obtained in the upwelled coastal waters were arbitrarily taken as background. Subsequent analysis of the spectra used to obtain the correction for the Compton effect indicates that these waters contained Zr^{95}-Nb^{95} at 3 to 4 pc/liter.
7. T. F. Budinger, L. K. Coachman, C. A. Barnes, "Columbia River effluent in the northeast Pacific Ocean, 1961, 1962: Selected aspects of physical oceanography," Dept. of Oceanography, Univ. of Washington, Seattle, Tech. Rept. 99 (February 1964).
8. We thank A. H. Seymour and D. E. Engstom, Laboratory of Radiation Biology, and R. Pedrick, U.S. Naval Oceanographic Office, for assistance. Dick Duffy, Department of Nuclear Engineering, University of Maryland, assisted in the design and construction of the *in situ* gamma-ray detector. Studies conducted in a cooperative program with the Laboratory of Radiation Biology, University of Washington. Supported by AEC contract AT(45-1)-1725 and by ONR contracts Nonr-477(10) and 477(37), project NR 083 012. Contribution No. 344, Department of Oceanography, University of Washington.

6 July 1965

Immunoglobulin Structure:
Partial Amino Acid Sequence of a Bence Jones Protein

Abstract. *Sequence analysis and ordering of the soluble tryptic peptides of one Bence Jones protein and comparison with partial sequence data for another have revealed many structural differences in the half of the molecule with the terminal amino group, but only one structural difference in the half of the molecule having the terminal carboxyl group. Somatic chromosomal rearrangements may effect such changes and account for variability in antibody structure.*

Because Bence Jones proteins are the L-chains of the myeloma globulin from the same patient and are related to normal L-chains (*1*), analysis of the amino acid sequence of Bence Jones proteins facilitates study of the structure of normal human immunoglobulins (*2, 3*). There are two wholly different types of L-chains that correspond to the two antigenic types of Bence Jones proteins (type I and type II); these differentiate each of the structure of normal human immunoglobulins (γG, γA, and γM) into the two corresponding antigenic types. The two types of L-chains differ in terminal amino groups, peptide maps, and composition of their tryptic peptides (*4*); hence, they differ greatly in primary structure. Furthermore, within each antigenic type the Bence Jones proteins of individual patients differ in primary structure in multiple positions rather than in just one, as in the abnormal hemoglobins.

From comparison of peptide maps and partial sequence studies (*3*), we have concluded that the NH_2-terminal portion of type I Bence Jones proteins is subject to variation whereas the COOH-terminal octapeptide is an invariant part of the structure of type I L-chains. We now report the amino acid sequence (*5*) of about three-fourths of an individual's type I Bence Jones protein (specimen Ag) including the consecutive sequence of 118 residues in the COOH-terminal half of the molecule. Figure 1 presents a comparison of our data with the partial amino acid sequence of another individual's type I Bence Jones protein (specimen Roy) and with the tryptic peptide composition of a third specimen (Cu), both of which have recently been reported (*6*). From these data we deduce that Bence Jones proteins—and presumably normal L-chains—differ individually in primary structure in a manner heretofore unknown for proteins with analogous function in the same species. With the exception of a minor homologous interchange, the COOH-terminal portion of the molecule is apparently identical in the two specimens (Ag and Roy) for which partial sequence is known and is probably the same for the third protein (Cu) for which only the order and composition of the tryptic peptides is known. On the other hand, the NH_2-terminal portion has many replacements involving the exchange of both homologous and nonhomologous amino acids. Although most of these interchanges appear randomly distributed, some are clustered in a tetrapeptide sequence (designated the "switch peptide") that is located just before the exact middle of the polypeptide chain where the sequence becomes almost invariant.

The procedures for the isolation of the tryptic peptides of the type I Bence Jones protein Ag and for their sequence determination have been described (*3*). The sequence of the larger tryptic peptides was established by comparison of the fragments resulting from peptic and chymotryptic digestion of these peptides. The ordering of the tryptic peptides is based on two kinds of evidence: (i) on the overlaps we have established independently from the amino acid composition of peptides obtained from a separate chymotryptic digest of the intact carboxymethylated protein, and (ii) on the deductions drawn by Hilschmann and Craig (*6*) who compared their results with our data (*2*). Furthermore, just as they have used identity or similarity in the amino acid composition to order our tryptic peptides relative to theirs, we have tentatively assigned amino acids whose position was not determined by one laboratory to the position determined by the other. If the sequence of a peptide is not known in any of the three proteins, the amino acids comprising it are written in the order of their elution from the column of the amino acid analyzer. For comparative purposes the polypeptide chain is assumed to contain 212 amino acids, and the numbering system proposed by Hilschmann and Craig is adopted. In this way we have constructed a chart comparing the tentative sequence of three Bence Jones proteins of type I.

The test of our assumption lies in the conflict or agreement in position where sequences are known in both laboratories. Although there are many sites of agreement, only one small area of conflict has been found (positions 173 to 176) (*7*).

Detailed discussion is restricted to the two proteins Ag and Roy, for which much sequence data is available. Of the assumed 212 positions in the chain, 96 have been reported for Roy (*6*), 148 are given herein for Ag, and a total of 164 different positions are known in one case or the other. Among the 80 positions known in both cases, there are 72 sites of agreement and five known sites of interchange. There are also at least two probable sites of interchange where the sequence is known in only one protein. All five known interchanges involve homologous amino acids (Ileu and Leu at position 46; Leu and Val at 102; Glu and Asp at 103; Ileu and Phe at 104; Val and Leu at 189). There are two instances where the peptide composition suggests that interchanges have probably occurred which involve nonhomologous amino acids, though the sequence is not yet certain (Asp and Lys at 53; Gln and Gly at 98). Additional interchanges undoubtedly occur in the "core" region where the tryptic peptides are insoluble and the sequences are not yet available for specimen Ag (positions 19–

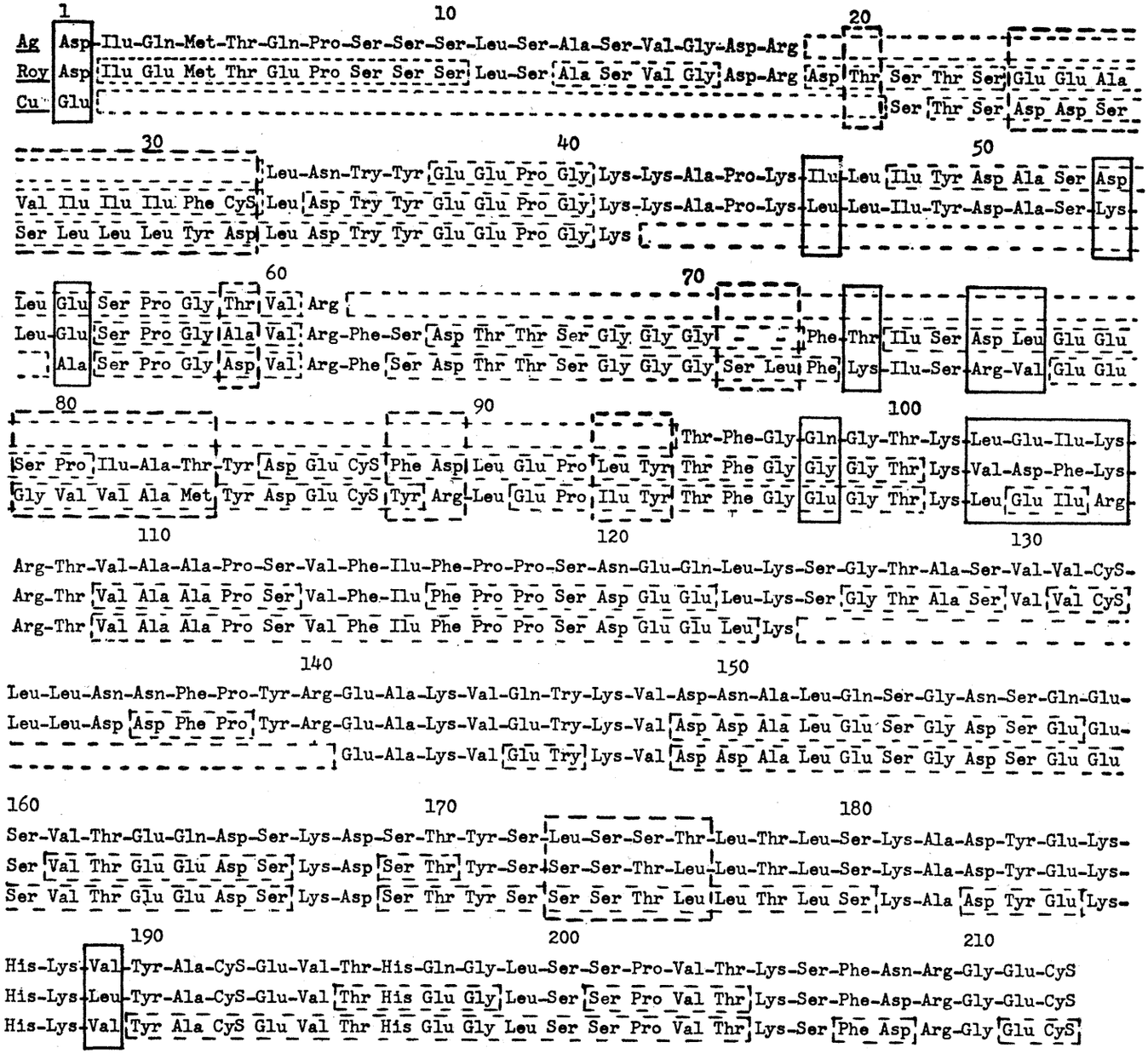

Fig. 1. Comparison of the known partial amino acid sequence and the putative sequence of three Bence Jones proteins of antigenic type I. Known sequences are indicated by joining together the abbreviations for the amino acids by dashes [for example, Asp-Ileu-Gln- (*5*)]. Areas of undetermined sequence are enclosed by a horizontal dashed line. In cases of known composition the amino acid residues are placed in the order of their elution from the column of the analyzer if the sequence is unknown for any protein; however, if the sequence is known for one protein, the residues enclosed by a dashed line (undetermined sequence) are ordered to correspond with the known sequence. Regions of probable homologous or nonhomologous interchanges are emphasized by enclosure in a box with a solid line. Regions of possible interchange are enclosed with a vertical dashed box. Data for specimens Roy and Cu are derived from the report by Hilschmann and Craig (*6*). The latter did not report which glutamic and aspartic acid residues are present as the amides.

32 and 62–94), though most of the chymotryptic peptides have been analyzed.

In view of the frequent occurrence of interchanges in the NH_2-terminal portion, the remarkable feature of the COOH-terminal portion is the probable identity in sequence of the last 106 amino acids except for the Val-Leu interchange at position 189, which was independently observed by Hilschmann and Craig (*6*) for the two proteins they studied (*7*). This agreement begins at position 106 after the "switch peptide," in which four homologous interchanges occur in the three proteins (*8*). Although the switch point involves two basic amino acids, it has no other unique features.

The conclusions reached by comparison of the sequence data for Ag and Roy are supported by the limited data available for the third protein, Cu. Although positions at only 37 residues can be assumed from end-group data on the tryptic peptides, these include four definite homologous interchanges with either Ag or Roy (positions 1, 102, 105, 189) as well as two nonhomologous interchanges (55 and 72). In agreement with that of Hilschmann and Craig (*6*), our comparative data suggest that there are many other interchanges, both homologous and nonhomologous, in the NH_2-terminal half of this protein. Some of these involve the replacement by arginine and lysine in specimens Cu and Ag of amino acids present in Roy for which trypsin lacks specificity; this, of course, leads to different tryptic peptides. Although sequence data are not available for the Cu protein, our data for Ag support the hypothesis of Hilschmann and Craig (*6*) that its entire sequence from 106 to 212 is like that of Roy.

The possibility of widespread rearrangement in amino acid sequence has obvious significance for antibody structure since Bence Jones proteins are abnormal products of a tumor of cells (plasmocytes) that have the normal function of antibody synthesis. We have other results that indicate that at least four different NH_2-terminal tryptic peptides may be obtained from different type I Bence Jones proteins and L-chains. This appears to exclude the mechanism of chromosomal rearrangement through a single event of unequal but homologous crossover ascribed to Lepore-type hemoglobins, since only two different NH_2-terminal sequences could arise by such a mechanism.

The multiple structural differences reported here for Bence Jones proteins are incompatible with the concept of single point mutations now accepted for the abnormal hemoglobins. We favor the hypothesis of somatic chromosomal rearrangements in the genes controlling antibody structure. In Smithies' hypothesis of γ-globulin variability (*10*), he postulates that the γ-globulin genes contain local inverted duplications of base sequence that permit intragenic crossing over. One possible consequence of duplications within a gene is the expression as repeated amino acid sequences, but the number of these does not seem significant in our data (*11*). Complete analysis of amino acid sequence of a number of Bence Jones proteins of each antigenic type will be needed for an experimental test of the Smithies hypothesis and of other theories of γ-globulin variability.

KOITI TITANI, EDWARD WHITLEY, JR.
LAUREL AVOGARDO, FRANK W. PUTNAM
Department of Biochemistry,
University of Florida College of Medicine, Gainesville

References and Notes

1. G. M. Edelman and J. A. Gally, *J. Exp. Med.* **116**, 207 (1962); S. Cohen, *Biochem. J.* **89**, 334 (1963); F. W. Putnam, *Biochim. Biophys. Acta* **63**, 539 (1962).
2. F. W. Putnam, C. W. Easley, J. W. Helling, *Biochim. Biophys. Acta* **78**, 231 (1963).
3. K. Titani and F. W. Putnam, *Science* **147**, 1304 (1965).
4. F. W. Putnam and C. W. Easley, *J. Biol. Chem.* **240**, 1626 (1965).
5. Abbreviations for amino acid residues: Lys, lysine; His, histidine; Arg, arginine; Asp, aspartic acid; Asn, asparagine; Thr, threonine; Ser, serine; Glu, glutamic acid; Gln, glutamine; Pro, proline; Gly, glycine; Ala, alanine; Val, valine; Met, methionine; Ileu, isoleucine; Leu, leucine; Tyr, tyrosine; Phe, phenylalanine; Try, tryptophan; CyS, half-cystine. In Fig. 1 Ilu is used instead of Ileu for typographic reasons.
6. N. Hilschmann and L. C. Craig, *Proc. Nat. Acad. Sci. U.S.* **53**, 1403 (1965).
7. Through independent experiments we have verified the sequence Leu-Ser-Ser-Thr at positions 173 to 176 which is reported in a different order by Hilschmann and Craig (*6*). Our sequence is in accord with that expected from the specificity of chymotrypsin and the composition of the chymotryptic peptides in this region.
8. The sequences Val-Val-CyS-Leu at positions 130 to 133 and Tyr-Ala-CyS-Glu at positions 190 to 193 accord with the sequence reported by Milstein (*9*) for the peptic peptide containing the disulfide bridge present in all type I L-chains and Bence Jones proteins he examined.
9. C. Milstein, *J. Mol. Biol.* **9**, 836 (1964).
10. O. Smithies, *Nature* **199**, 1231 (1963).
11. In our data the dipeptide sequences Lys-Val and Pro-Ser recur three times and Leu-Ser four times.
12. We thank Caroline W. Easley for peptide maps and consultation. Supported by grants CA-02803 and H-02966 from NIH. Reprint requests should be addressed to the authors at the Division of Biological Sciences, Indiana University, Bloomington.

2 August 1965

Liquid Waves by Computer

Abstract. *A numerical, fluid-dynamics technique for high-speed computers is described and illustrated. It applies to the solution of problems dealing with incompressible viscous fluids and involving nonsteady motions in several dimensions in space. The ability to handle free-surface boundary conditions allows waves to be studied through all phases of breaking and splashing, as well as a number of related phenomena.*

We have developed a new technique for numerically solving problems in fluid dynamics. It is particularly applicable to studies of waves and of other phenomena that are associated with the motion of an incompressible fluid with a free surface. Examples are the flow of water from a broken dam, the generation of water waves by an explosion, the formation of breakers on a beach, and the splash of a jet of liquid hitting a plate.

The application illustrated in Fig. 1 is to the surge of water under a sluice gate. The initial frame (top left) shows the water at rest immediately after the gate has quickly opened; the deep reservoir (left) is subjected to a surface pressure in addition to that produced by gravity and the shallow pond (right) is initially quiescent. Subsequent frames show the formation of a *backward* breaker, in which the flow is partly smooth, partly irregular.

The problem was scaled to give unit density to the water. The downward gravitational acceleration was also of unit magnitude, while the scale of distance is determined by the initial height of the reservoir behind the sluice gate, 2.9 distance units. In these dimensions, the applied surface pressure was 2.5, the coefficient of kinematic viscosity was 0.01, and the times of the six frames are $t = 0, 1.0, 1.5, 2.0, 2.5,$ and 2.73.

The elements of fluid are represented in the calculations by marker particles. Determination of the trajectories of the particles is based on a finite-difference approximation to the full, nonlinear, Navier-Stokes equation for a viscous, incompressible fluid. The finite-difference equations are related to a Eulerian mesh of cells not shown in the figure. The cells cover the entire region of interest, amounting to 1500 in this case.

The computing method has been designed for use with a high-speed computer. The present program is run on