for obtaining peptide maps (see Fig. 1). The amino acid compositions of these peptides for proteins HS 92 and HS 5 agree with the sequences in Fig. 2 within experimental error (maximum deviation ± 0.2 residue per mole). The peptide compositions and amino-terminal residues are identical in all three proteins except for the arginine-lysine interchange at position 190. The two CL-lys regions appeared to be identical to their CL-arg counterparts except for the interchange at position 190 (*13*).

A lambda common gene duplication early in immunoglobulin evolution seems unlikely in that the CL-lys regions have diverged from their CL-arg counterparts by only a single amino acid interchange. Rather, this virtual identity of sequences in CL-lys and CL-arg regions suggests a genetic model of the lambda common region with an ambiguous codon at position 190 or a model of recent gene duplication followed by a single mutational event.

LEROY HOOD
DANIEL EIN

National Cancer Institute,
Bethesda, Maryland 20014

References and Notes

1. S. Cohen and C. Milstein, *Nature* **214**, 449 (1967); E. Lennox and M. Cohn, *Ann. Rev. Biochem.* **36**, 365 (1967).
2. F. W. Putnam, K. Titani, M. Wikler, T. Shinoda, *Cold Spring Harbor Symp. Quant. Biol.* **32**, 9 (1967).
3. E. Appella and D. Ein, *Proc. Nat. Acad. Sci. U.S.* **57**, 1449 (1967).
4. D. Ein, *ibid.* **60**, 982 (1968).
5. The beta and delta hemoglobin genes, presumably derived from a common ancestral gene, differ from one another by at least 10 codons (that is, amino acid residues) out of 146.
6. C. Milstein, J. B. Clegg, J. M. Jarvis, *Nature* **214**, 270, 1967.
7. M. Raftery and R. Cole, *J. Biol. Chem.* **241**, 3457, 1966.
8. A. M. Katz, W. J. Dreyer, C. B. Anfinsen, *ibid.* **234**, 2897 (1959).
9. C. Baglioni, *Biochim. Biophys. Acta* **48**, 392, 1961.
10. W. J. Dreyer and E. Bynum, *Meth. Enzymol.* **11**, 32, 1967.
11. W. R. Gray, *ibid.*, p. 139; dansyl is an acronym for 1-dimethylaminonaphthalene-5-sulfonyl chloride.
12. J. C. Bennett, L. Hood, W. J. Dreyer, M. Potter, *J. Mol. Biol.* **12**, 81 (1965).
13. It should be pointed out that since these proteins have not been completely sequenced we cannot say unequivocally that the peptide sequences are identical to protein Sh. Certain amide differences could not be detected by the methods used (Fig. 2). Neither could one detect complementary double amino acid changes within a single peptide. For example, in peptide T1 the alanine at position 208 may have mutated to serine, and in the same peptide the serine at position 213 may have changed to alanine. The likelihood of such an event appears remote, however, since it requires two independent mutations within a single tryptic peptide.
14. The amino acid analyses were carried out by M. Miller. We thank J. Williams for technical assistance and Dr. J. L. Fahey for reviewing the manuscript.

1 August 1968

Specificity of Ribonuclease Ch from Chalaropsis Species

Abstract. *A ribonuclease is produced by the fungus* Chalaropsis *sp. that appears to have an absolute specificity for 3′-guanylic acid residues in ribonucleic acid.*

In the course of isolating a staphylolytic enzyme from the fungus *Chalaropsis* sp. (*1*), it was discovered that a ribonuclease was also produced by this organism (*2*, *3*). This ribonuclease, which will be designated ribonuclease Ch, appears to have an absolute specificity for 3′-guanylic acid (3′-GMP) residues in ribonucleic acid.

The ribonuclease Ch used to establish the specificity was prepared by column chromatography on Amberlite CG-50 (*3*). Yeast RNA, purified according to Frisch-Niggemeyer and Reddi (*4*), was used as substrate. After incubation with ribonuclease Ch, cyclic phosphates were hydrolyzed with dilute HCl at 4°C and monophosphate groups were removed with ribonuclease-free alkaline phosphatase (Worthington). Samples were then hydrolyzed with 0.3*N* KOH at 37°C to give mixtures of 2′- and 3′- nucleotides and nucleosides. The specificity is indicated by the nucleosides that are produced.

Results of two-dimensional paper chromatography of a hydrolyzate of RNA by ribonuclease Ch are shown in Fig. 1. The products are the three nucleotides 2′- and 3′- adenylic acid, cytidylic acid, and uridylic acid (2′- and 3′- AMP, CMP, and UMP), and one nucleoside, guanosine. These results indicate that ribonuclease Ch cleaves RNA at 3′-GMP residues. Column chromatography, according to the procedure of Blattner and Erickson (*5*), fully corroborated these results (*6*). Guanosine was obtained from thin layer chromatograms for identification pur-

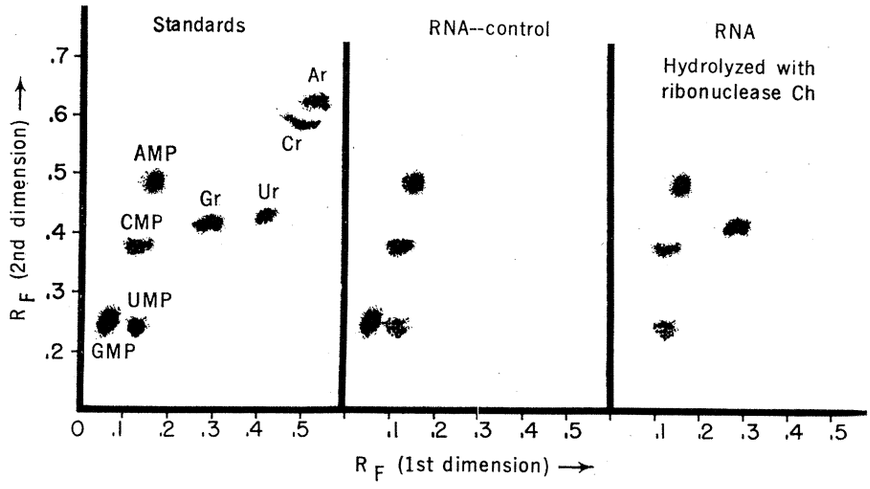

Fig. 1. Two-dimensional chromatography of RNA which has been hydrolyzed with ribonuclease Ch. RNA (50 mg) was dissolved in 2.5 ml of 0.02*M* phosphate buffer, *p*H 7.0, and 0.5 ml was removed as control. Ribonuclease Ch (10 μg protein in 5 μl buffer) was added and the sample was incubated at 37°C. Aliquots of 0.5 ml were removed at 10 minutes, 60 minutes, and 16 hours. Reactions were terminated by adding 50 μl of 1*N* HCl. Cyclic phosphates were hydrolyzed by holding the samples at the acid *p*H for 16 hours at 4°C. Tris-HCl buffer (0.5 ml of 0.4*M*, *p*H 7.9) containing 20 μg of ribonuclease-free alkaline phosphatase was added to the samples, which were then incubated at 37°C for 16 hours. KOH was added to 0.3*N* (60 μl of 5*N* KOH) and the samples were incubated 24 hours at 37°C. The *p*H of the samples was adjusted to 4.5 with 3*N* $HClO_4$, and $KClO_4$ was removed by centrifugation. Sample volumes were adjusted to 1 ml, and 20 μl aliquots were chromatographed in a descending fashion for 16 hours on Whatman No. 1 paper. The first-dimension solvent was isopropanol, concentrated NH_4OH, water (7 : 1 : 2, by volume) and the second dimension solvent was isobutyric acid, concentrated NH_4OH, water (66 : 1 : 33, by volume). The solvents were adapted from Wyatt (*11*). Contact prints of ultraviolet absorbing compounds were made with Ilford R4-1P photographic paper and a shortwave ultraviolet lamp. Images were reversed by conventional photography. Only that portion of each chromatogram which contained ultraviolet-absorbing compounds is shown. The nucleotides GMP, UMP, CMP, and AMP are the 2′- and 3′-derivatives, and Gr, Ur, Cr, and Ar represent the corresponding ribosides, guanosine, uridine, cytidine, and adenosine, respectively. The 10- and 60-minute samples were identical to the 16-hour sample shown.

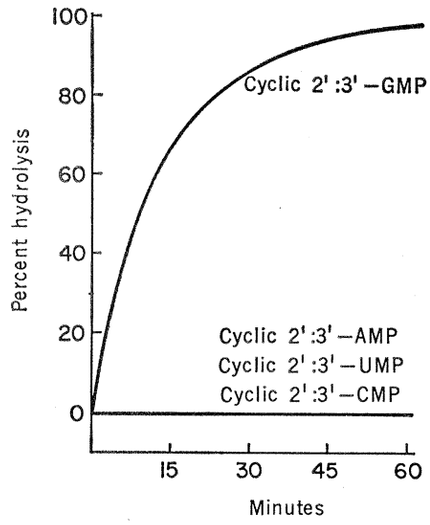

Fig. 2. Hydrolysis of cyclic 2′:3′-nucleotides by ribonuclease Ch. Cyclic 2′:3′-AMP, UMP, CMP, and GMP were obtained from Schwarz BioResearch as their barium salts. Barium was removed with an equivalent quantity of sodium sulfate, and cyclic nucleotides were diluted to 0.002*M*. One-milliliter samples were titrated in a Radiometer Titrigraph TTT-1, and 0.005*N* NaOH was used to maintain a *p*H of 7.0. The reactions were started by adding 50 μg of ribonuclease Ch protein. After alkali consumption had ceased, the samples were chromatographed in isopropanol, saturated ammonium sulfate, water (2 : 79 : 19, by volume) (*12*). In the case of cyclic 2′:3′-GMP, there was quantitative conversion to 3′-GMP. The other cyclic 2′:3′-nucleotides were unchanged.

poses. Eastman thin layer chromatograms, and silica gel without fluorescent indicator (K 301R2), were activated 30 minutes at 110°C, and aliquots of the enzymatic digest were streaked along a line 1 cm from the bottom. Separation was accomplished with the first-dimension solvent named in the legend to Fig. 1. The fastest-moving band, which corresponded to authentic guanosine, was eluted with 0.25*M* ammonium formate, *p*H 4.1. Its ultraviolet absorption spectrum was identical to that of authentic guanosine.

Results in Fig. 1 and those from column chromatography indicate an absolute specificity of ribonuclease Ch for 3′-GMP residues. In both cases the results for the longest incubation period were identical with those obtained with an incubation period of 10 minutes. Thus, with an amount of ribonuclease Ch sufficient to give maximum hydrolysis in 10 minutes, no further hydrolysis of nucleotide bonds occurred in 16 hours.

Ribonucleases from other sources hydrolyze RNA through the intermediate cyclic 2′:3′-nucleotides (*7*). Ribonuclease Ch presumably acts in the same manner because cyclic 2′:3′-GMP is hydrolyzed to 3′-GMP (Fig. 2). These data also indicate an absolute specificity for guanosine, because the other cyclic 2′:3′-nucleotides were unaffected by ribonuclease Ch.

Evidence was sought for the presumed intermediate, cyclic 2′:3′-GMP. For this purpose, polyguanylic acid, (Miles Laboratory) was used as substrate, and the reaction was monitored by alkali consumption in a Radiometer Titrigraph TTT-1. At intervals, aliquots were removed and chromatographed in the system in the legend to Fig. 2. In the first 5 to 15 minutes of hydrolysis there were small spots corresponding to cyclic 2′:3′-GMP, but in longer periods of time the only spots observed were those corresponding to 3′-GMP. Cyclic 2′:3′-GMP does not accumulate, which indicates that the rate-limiting reactions are those concerned with the hydrolysis of the 3′-5′ linkages in polyguanylic acid.

Ribonuclease Ch thus has the same specificity as ribonuclease T_1 (*8*), N_1 (*9*), and U_1 (*10*). Ribonuclease T_1 has proven to be of great value in structural studies of nucleic acids, and ribonuclease Ch may also have similar applications. It will also be of interest from the standpoint of comparative enzyme structure.

JOHN H. HASH
SUSAN ELSEVIER
Department of Microbiology, Vanderbilt University School of Medicine, Nashville, Tennessee 37203

References and Notes

1. J. H. Hash, *Arch. Biochem. Biophys.* **102**, 379 (1963).
2. ——— and J. P. Robinson, *Fed. Proc.* **25**, 742 (1966).
3. J. H. Hash and M. V. Rothlauf, *J. Biol. Chem.* **242**, 5586 (1967).
4. W. Frisch-Niggemeyer and K. K. Reddi, *Biochim. Biophys. Acta* **26**, 40 (1957).
5. F. R. Blattner and H. P. Erickson, *Anal. Biochem.* **18**, 220 (1967).
6. Details are in preparation for publication.
7. C. B. Anfinsen and F. H. White, in *The Enzymes*, P. D. Boyer, H. Lardy, K. Myrback, Eds. (Academic Press, New York, 1961), vol. 5, p. 95.
8. T. Uchida and F. Egami, in *Procedures in Nucleic Acid Research*, G. L. Cantoni and D. R. Davies, Eds. (Harper and Row, New York, 1966), p. 3.
9. N. Takai, T. Uchida, F. Egami, *Biochim. Biophys. Acta* **128**, 218 (1966).
10. T. Arima, T. Uchida, F. Egami, *Biochem. J.* **106**, 609 (1968).
11. G. R. Wyatt, in *The Nucleic Acids*, E. Chargaff, and J. D. Davidson, Eds. (Academic Press, New York, 1961), vol. 1, p. 243.
12. K. Sato-Asano, *J. Biochem.* (*Tokyo*) **46**, 31 (1959).
13. Supported by grant PHS No. AI 06712. J.H.H. was the recipient of career development award AI 38631.

21 August 1968

Serum Copper Alteration after Ingestion of an Oral Contraceptive

Abstract. *Changes in the concentration of copper in the serum after administration of an oral contraceptive were determined with atomic absorption spectrophotometry. Statistically significant* ($P = .001$) *increases were observed in all volunteers.*

Concentrations of heavy metals and trace elements in serums of women taking the oral contraceptives have not been well studied. We now report the use of the atomic absorption spectrophotometer to determine changes in total serum copper before and 1 month after administration of an oral contraceptive.

Fourteen healthy female subjects, more than 6 weeks after parturition were tested before ingestion of 10 mg of norethynodrel with mestranol daily for 21 days; hence they served as their own controls. Venous blood was again obtained after this cycle. All specimens were obtained from fasting individuals and were run in duplicate.

Prior to treatment, the mean concentration of copper in the serum was 142 μg per 100 ml with a range of 104 to 168 μg per 100 ml, and the standard error of the mean was 12.0 μg per 100 ml, a range consistent with value in the serums of women of childbearing age. After one cycle of administration of oral contraceptive the mean rose to 241 μg per 100 ml with a standard of the mean of 7.3 and a range of 184 to 296 μg per 100 ml. The *t* value was 6.607, and *P* was .001.

These observations demonstrate a marked increase in the concentrations of serum copper after ingestion of an oral contraceptive for only 21 days. The change is statistically significant. The long-term effects of this alteration in serum copper content remain to be determined. The mechanism of action of this increase is unknown but may represent a variation in the plasma proteins that bind the various metals. These findings may be significant for two reasons. (i) They may help to explain the changes demonstrated in normal pregnancy, and (ii) they may point to a potential long-term hazard.

JAMES A. O'LEARY
WILLIAM N. SPELLACY
Department of Obstetrics and Gynecology, University of Miami School of Medicine, Miami, Florida

16 August 1968