Table 2. Schematic representation of the types of A protein in the double mutant, double suppressor strain. X is the amino acid in the A-11 A protein that replaces a wild-type amino acid, Y is the amino acid in the A-36 A protein that replaces a wild-type amino acid, and *M* and *N* are the amino acids replacing X and Y, respectively, as a result of suppressor gene action.

A-11-36 CRM protein	---X--------Y-----
Altered protein due to action of the su_{11} gene (A-36 CRM)	---*M*-------Y----
Altered protein due to action of the su_{36} gene (A-11 CRM)	----X-------*N*----
Altered protein due to action of the su_{11} and su_{36} genes (active protein)	--*M*-------*N*-----

than the ratio for either the A-11 su_{11} or A-36 su_{36} strains (Table 1). Since it had been shown that the A protein which was active in reaction 3 in the A-11 su_{11} and A-36 su_{36} strains was similar to the wild-type A protein (*2*), it was assumed that the A protein active in reaction 3 in the A-11-36 su_{11}, su_{36} strain was also similar to the wild-type A protein in its enzymatic properties (that is, an InGP → Tryp/In → Tryp activity ratio of 40 percent). If this assumption is correct, then the InGP → Tryp/In → Tryp activity ratio of the A proteins of suppressed mutants can be multiplied by 2.5 to give the ratio of *su*-A protein to A-CRM protein. That is, if a ratio of 40 percent were obtained it would indicate that all the A-protein molecules had wild-type activity. If an activity ratio of 10 percent were obtained it would indicate that only 25 percent of the A-protein molecules had wild-type activity. On this basis, 8 percent of the A-protein molecules in the A-36 su_{36} strain has wild-type activity, while 2.3 percent in the A-11 su_{11} strain and 0.22 percent in the A-11-36 su_{11}, su_{36} strain had wild-type activity. It was apparent that the value obtained from the experimental data (0.22 percent) was very similar to the value which would have been predicted on the basis of independent actions of the two suppressor genes—that is, 8 percent × 2.3 percent. Therefore, it was concluded that the two suppressor genes studied had independent and nonadditive actions in restoring InGP → Tryp activity.

Since it has been established that these particular suppressor genes produce changes in the primary structure of the A protein (*2*), the independent action of the two suppressor genes in the A-11-36 su_{11}, su_{36} strain could be interpreted in the following way: The su_{36} gene altered the primary structure of the A protein at one particular site of 8 percent of the A-protein molecules, while the su_{11} gene altered the primary structure at some other site in 2.3 percent of the A-protein molecules. On this basis then, at least four different A proteins could be formed in the A-11-36 su_{11}, su_{36} strain (Table 2)—that is, the A-11-36 CRM protein (the bulk of the A protein), the A-11 CRM protein (8 percent), the A-36 CRM protein (2.3 percent), and an *su*-A protein (0.22 percent). Although it was not determined if all four types of A protein were present, the finding of only low amounts of InGP → Tryp activity suggested that these two suppressor genes did affect different sites in the A protein.

At present, the mechanism of allele-specific suppression can be most reasonably explained as "mistakes" in the incorporation of amino acids into protein due to an alteration in the specificity of any one of the cell components which participate in protein synthesis (*2*, *7*, *8*). This cell component could be DNA, messenger RNA, ribosomes, transfer RNA, or amino acyl RNA synthetases (*8*, *9*), since it is conceivable that mutational alteration of any one of these components could lead to a mistake in amino acid incorporation. However, it remains to be determined which of these components are responsible for the independent, nonadditive effects observed in this study.

STUART BRODY*
CHARLES YANOFSKY
Department of Biological Sciences,
Stanford University,
Stanford, California

References and Notes

1. I. Crawford and C. Yanofsky, *Proc. Natl. Acad. Sci. U.S.* **45**, 1280 (1959); M. Allen and C. Yanofsky, *Genetics* **48**, 1065 (1963).
2. S. Brody and C. Yanofsky, *Proc. Natl. Acad. Sci. U.S.* **50**, 9 (1963).
3. I. Crawford and C. Yanofsky, *ibid.* **44**, 1161 (1958).
4. C. Yanofsky and I. Crawford, *ibid.* **45**, 1016 (1959).
5. B. D. Maling and C. Yanofsky, *ibid.* **47**, 551 (1961).
6. E. Lennox, *Virology* **1**, 190 (1955).
7. S. Benzer and S. Champe, *Proc. Natl. Acad. Sci. U.S.* **47**, 1025 (1961); A. Garen and O. Siddiqi, *ibid.* **48**, 1121 (1962).
8. S. Brody and C. Yanofsky, in preparation.
9. L. Gorini and E. Kataja, *Proc. Natl. Acad. Sci. U.S.* **51**, 487 (1964).
10. Supported by PHS and NSF grants.
* Present address: Rockefeller Institute, New York, N.Y. 10021.

14 May 1964

Differences in Complement Titers of Serums from Spaced Bleedings in Guinea Pigs

Abstract. *Complement titers of serum samples collected 24 hours apart from an untreated guinea pig differed greatly. The mean of the differences between the titers at the beginning and 24 hours later for each of 11 animals was significantly different from zero (.01<p<.02).*

It has generally been accepted that the complement activity of a "normal" individual, animal or human, is relatively stable. We present evidence that this assumption may be without foundation.

In preliminary experiments we found that the antihypertensive drug 1-hydrazinophthalazine hydrochloride (hydralazine) (*1*) inactivated about 90 percent of the hemolytic activity of guinea pig complement in vitro. We then studied the complement activity of the serums of guinea pigs which had received an intraperitoneal injection of hydralazine. A serum sample was taken about 24 hours prior to the injection of the hydralazine. A second blood sample was obtained 15 minutes after the drug was injected. The titers of the two serums are designated T_1 and T_2, respectively, and are expressed as "percentage change," $100 \times (T_1 - T_2)/T_1$.

In several of the control animals which had received nonsterile 0.85 percent NaCl solution intraperitoneally we observed changes of 25 to 40 percent. In other experiments we found similar,

Table 1. Complement titers (C′), in 50 percent hemolytic units per milliliter of serum, of bloods drawn at 0 hours (T_1) and 24 hours (T_2); body weight at 0 hours; number of punctures (P); and number of bleedings (B).

C′ units		Wt. (g)	0 hr		24 hr	
T_1*	T_2*		P	B	P	B
122.25	173.16	380	1	1	1	1
139.68	210.06	270	3	1	1	1
179.10	231.12	370	2	1	8	2
196.21	258.84	350	1	1	6	3
220.91	275.48	450	4	2	1	1
223.05	237.15	410	1	1	1	1
247.52	309.63	430	5	2	2	1
253.59	281.42	415	1	1	2	1
283.55	313.41	440	3	2	1	1
287.91	243.90	405	1	1	7	2
291.54	271.00	420	1	1	1	1

* For computational purposes only. Not intended to denote five significant figures.

but less striking, percentage changes when pyrogen-free saline was injected. However, the insertion through the abdominal wall of a sterile syringe needle alone gave rise to changes as great as 44 percent. Finally, we found that the cardiac bleeding procedure itself led to "relatively large" percentage changes. Consequently, we designed an experiment to confirm the last observations.

Four-week-old male, albino, guinea pigs were obtained (*2*) and maintained on Purina Guinea Pig Chow, alfalfa, and water. Lettuce, as a source of vitamin C, was given several times daily. After 1 month the animals were bled by cardiac puncture. Exactly 24 hours later the guinea pigs were bled again. The number of punctures (number of times the chest was entered) and the number of bleedings required to obtain 1 to 1.5 ml of blood were recorded per animal. The serums were stored at −60°C until titrated. All assays for complement (*3*) were performed on the same day with the same reagents, the method being modified by using (i) veronal-buffered saline containing 0.0005*M* Mg^{++} and 0.00015*M* Ca^{++} as diluent, (ii) sheep erythrocytes preserved in modified Alsever's solution, and (iii) a 45-minute incubation period at 32°C. For guinea pig complement, hemolysis is greater at 32°C than at 37°C and the reaction is essentially complete after 45 minutes (*4*). The titers were estimated from the logarithmic form of the von Krogh equation (*5*) by the method of least squares, and not by the graphic "free-hand" method. The results are presented in Table 1.

The percentage changes in complement titers ranged from approximately −50 to 15. Nine of the changes were negative and two were positive. The loss of blood was roughly 5 to 8 percent of an animal's total blood volume.

We estimated our experimental error by repeatedly testing, simultaneously, a pool of guinea pig serum. Five tubes were assayed against sheep erythrocytes which had been preserved in modified Alsever's solution (A) and five against erythrocytes from defibrinated sheep blood (D). The coefficients of variation were 2.3 percent for A and 5.3 percent for D.

The mean of the differences between the pairs T_1 and T_2 of each animal is significantly different from zero ($t = -2.9574$; $.01<p<.02$). This significant difference is the basis for our statement that an individual's complement activity may not be relatively stable.

Because of the small size of the sample we attach little importance to the significant ($p = .02$) correlation coefficient for (i) T_1 and T_2 and (ii) T_1 and body weight at 0 hours.

We have been unable, as yet, to uncover any reason for the variations between the T_1 and T_2 values. We assumed that the unassessable stresses arising from the restraint of the animals during the cardiac bleeding procedure and the stresses connected with the process of collecting the blood itself were contributory. However, we failed to demonstrate, by ranking methods, any correlation between (i) T_1 and P_1 (number of punctures at 0 hours), (ii) T_1 and B_1 (number of bleedings at 0 hours), (iii) T_2 and P_2 (number of punctures at 24 hours), (iv) T_2 and B_2 (number of bleedings at 24 hours), (v) T_2 and $P_1 + P_2$, and (vi) T_2 and $B_1 + B_2$.

We began to test the ideas that neurologic responses or humoral factors or both come into play during and between the bleeding procedures. We cannot conclude from preliminary experiments that etherization of the animals prior to bleeding lessened the response or, in other experiments, that the intraperitoneal injection of histamine accentuated the response.

In view of the phenomenon described we believe that the inferences based on changes in complement reported for various human diseases, infectious or otherwise, must be reviewed. Currently, hypotheses are being formulated suggesting that autoimmune mechanisms may possibly play an etiologic role in certain diseases (*6*). The changes in complement activity are reported to be related to exacerbation and remission of the disease in question. We are not yet convinced that changes in complement values necessarily reflect changes in a disease process. Until proved otherwise we must consider the possibility that such changes in titer are not unusual.

We believe that many of the shortcomings in the studies of complement in vivo arise because attention has not always been paid to experimental design, to statistical analysis of the results, to the determination of complement titers by procedures more precise than the graphic "free-hand" method, and to attempts to compare titers obtained on different days employing different sheep erythrocytes. The validity of the last practice is open to question in the absence of experimental evidence that different batches of sheep erythrocytes react similarly.

L. J. BRENNER
E. E. ECKER
Department of Pathology Research, St. Luke's Hospital, and *Department of Pathology, School of Medicine, Western Reserve University, Cleveland, Ohio*

References and Notes

1. Apresoline. Ciba Pharmaceutical Co., Summit, N.J.
2. A. W. Starrett, Akron, Ohio.
3. E. E. Ecker and C. W. Hiatt, *Am. J. Clin. Pathol.* **19**, 141 (1949).
4. M. A. Leon, *Proc. Soc. Exptl. Biol. Med.* **91**, 150 (1956).
5. M. von Krogh, *J. Infect. Dis.* **19**, 452 (1916).
6. W. L. Nastuk, O. J. Plescia, K. E. Osserman, *Proc. Soc. Exptl. Biol. Med.* **105**, 177 (1960).
7. Supported by grants 2003 and 2107 from the Cleveland Area Heart Society, the G. N. Stewart Memorial Fund of the Rogoff Foundation, and the Harry K. and Emma R. Fox Charitable Foundation. We thank A. J. Plummer, Ciba Pharmaceutical Co., for supplying Apresoline; and R. Dominguez, Department of Pathology Research, St. Luke's Hospital, and Dr. Z. Govindarajulu, Department of Mathematics, Case Institute of Technology, for assistance in the statistical analysis.

14 May 1964

Poliovirus: Growth in Non-Nucleate Cytoplasm

Abstract. *Cytoplasmic fragments were produced by micromanipulation of cells from a human amnion cell line cultured on coverslips. The cultures were infected with type 1 (Mahoney) poliovirus, and incubated for 7 hours with tritiated uridine (H^3U). Fluorescent antibody to the poliovirus indicated antigenic sites in a number of non-nucleate fragments. By autoradiography the incorporation of H^3U was demonstrated at some of the same sites. The occurrence of poliovirus antigen at the same site as induced synthesis of RNA in non-nucleate cytoplasm of mammalian cells indicates that poliovirus infection and growth occurred independently of immediate contribution from the nucleus.*

Certain picornaviruses have been shown to develop in cells in which nuclear RNA synthesis was inhibited by treatment with actinomycin D prior to infection (*1, 2*). Even without actinomycin D, the infectious process was accompanied by suppression of nuclear RNA synthesis (*2*), indicating a virus-induced inhibition of this nuclear function. While growth of these picornaviruses occurs under conditions which stop normal synthesis of nuclear RNA,