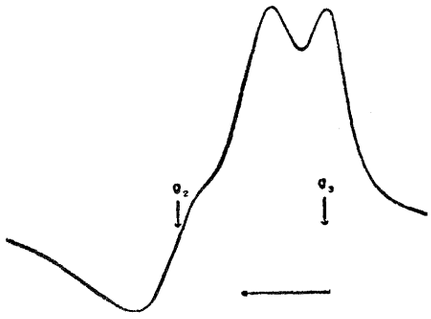

Fig. 2. Electron spin resonance of pure ferri-Hb M Saskatoon (Kurume). Magnetic field modulation is 12 gauss and microwave power attenuation is 10 db. Horizontal arrow represents direction of magnetic field. This material was examined at a hemoglobin concentration of $7.6 \times 10^{-3}M$ in $0.1M$ potassium phosphate buffer at *p*H 7.0 with 100 times the sensitivity of the output of ESR spectrometer.

considered a kind of doublet, because the peak of Hb M Iwate signal is shifted to the lower magnetic field than ferri-Hb A and a slight shoulder is at the higher side.

We have discussed the origin (*12*) and the theoretical basis (*13*) of the doublet signal in studying the chromatographically isolated ferri-Hb M Iwate and M Boston (Osaka). This doubtlet signal was attributed to splitting of a degenerated *g*-value of 6.0. The estimated values of g_2 and g_3 of Hb M variants calculated by the method of Kneubühl (*14*) were $g_2 = 5.80$ and $g_3 = 6.21$ in Hb M Iwate; $g_2 = 5.72$ and $g_3 = 6.26$ in Hb M Boston (Osaka); and $g_2 = 5.35$ and $g_3 = 6.53$ in Hb M Saskatoon (Kurume) respectively. Hence Hb M Iwate, M Boston (Osaka), and M Saskatoon (Kurume) can be easily and accurately identified by the ESR method.

In Hb M Iwate and M Boston (Osaka) ESR signals of both fresh blood and pure ferri-Hb M (*15*) were similar. However Hb M Saskatoon (Kurume) showed a doublet while the pure ferri-Hb M showed a triplet signal (Fig. 2). The signal between the doublet had the *g*-value of 6.0 as ferri-Hb A. This signal diminished by the addition of sodium azide at the concentration required for the formation of azide complex with the α-chain of ferri-Hb M Saskatoon (Kurume). It also diminished, like that of ferri-Hb A, above the temperature of liquid-nitrogen though the doublet signal showed no remarkable change (*16*). Therefore this signal is attributed to the heme, linked with the α-chain of Hb M Saskatoon (Kurume). In ferri-Hb M Iwate and M Boston (Osaka), the ESR signal due to heme linked with the β-chain should also have been revealed, but in fact it was not; and in this way the ESR of blood and of ferri-Hb M closely resembled each other. The absence of signals could result from the narrowness of the ESR signal of ferri-Hb M Iwate and M Boston (Osaka), especially that of abnormal heme of these hemoglobins: that is, when the absorption band is narrow, the peak height of ESR markedly increases, and the effect of the heme of β-chain becomes negligibly small. The recorded signal height of ferri-Hb M Iwate was about ten times larger than that of ferri-Hb A, and the intensity of ferri-Hb M Boston (Osaka) was about 20 times larger than that of ferri-Hb A (*12*).

Mention should be made of the ESR signal of Hb M Milwaukee-I, which was not available to us. The structure of this Hb M is represented as $\alpha_2{}^{A}\beta_2{}^{67Glu}$ and has similar physicochemical properties to Hb M Saskatoon (*17*). Therefore it is suspected that the shape of the Hb M Milwaukee-I signal might be similar to that of Hb M Saskatoon.

Furthermore it is expected that the ESR study of Hb M variants whose primary structures remain uncertain might furnish clues to their structures.

AKIRA HAYASHI
AKIRA SHIMIZU
YUICHI YAMAMURA
Third Department of Internal Medicine, Osaka University Medical School, Osaka, Japan

HIROSHI WATARI
Department of Physicochemical Physiology, Osaka University Medical School

References and Notes

1. H. Hörlein and G. Weber, *Deutsch. Med. Wochensch.* **73**, 476 (1948).
2. K. Singer, *Am. J. Med.* **18**, 633 (1955).
3. K. Betke, in *Haemoglobin Colloquium*, H. Lehman and K. Betke, Eds. (Thieme Verlag, Stuttgart, 1961), p. 39.
4. P. S. Gerald and M. L. Efron, *Proc. Nat. Acad. Sci. U.S.* **47**, 1758 (1961).
5. S. Shibata, T. Miyaji, I. Iuchi, A. Tamura, *Acta Haematol. Jap.* **27**, 13 (1964); A. Shimizu, A. Tsugita, A. Hayashi, Y. Yamamura, *Biochim. Biophys. Acta* **107**, 270 (1965).
6. A. Shimizu, A. Hayashi, Y. Yamamura, A. Tsugita, K. Kitayama, *Biochim. Biophys. Acta* **97**, 472 (1965).
7. S. Shibata, T. Miyaji, I. Iuchi, S. Ueda, I. Takeda, N. Kimura, S. Kodama, *Acta Haematol. Jap.* **25**, 690 (1962).
8. R. T. Jones, R. D. Coleman, P. Heller, *Fed. Proc.* **23**, 173 (1964).
9. A. M. Josephson, H. G. Weinstein, V. J. Yakulis, L. Singer, P. Heller, *J. Lab. Clin. Med.* **59**, 918 (1962).
10. V. O. Tönz, H. A. Simon, W. Hasselfeld, *Schweiz. Med. Wochensch.* **92**, 1311 (1962).
11. K. Betke, E. Gröschner, K. Bock, *Nature* **188**, 864 (1960).
12. A. Hayashi, A. Shimizu, Y. Yamamura, H. Watari, *Biochim. Biophys. Acta* **102**, 626 (1965).
13. H. Watari, Kun-Joo Hwang, K. Kimura, K. Murase, *ibid.*, in press.
14. F. Kneubühl, *J. Chem. Phys.* **33**, 1074 (1960).
15. Pure ferrihemoglobins M Iwate, M Boston (Osaka) and M Saskatoon (Kurume) were isolated chromatographically with CG-50 and oxidized with potassium ferricyanide.
16. A. Hayashi, A. Shimizu, T. Suzuki, Y. Yamamura, H. Watari, in preparation.
17. A. V. Pisciotta, S. N. Ebbe, J. E. Hinz, *J. Lab. Clin. Med.* **54**, 73 (1959).
18. We thank the late A. Tamura for the blood specimen of patient with Hb M Iwate, and N. Kimura for the specimen with Hb M Saskatoon (Kurume).

7 February 1966

Bacteriophage T5 Chromosome Fractionation: Genetic Specificity of a DNA Fragment

Abstract. *The bacteriophage T5 DNA fragments retained by a population of blended early complexes, formed under conditions of limited viral DNA transfer to host cells, appear to include only one of six tested cistrons. When complexes harboring wild-type fragments are infected with appropriate amber mutants, recombination occurs but apparently is not needed for productive infection.*

When *Escherichia coli* is infected with bacteriophage T5 at high bacterial concentration (5×10^9 cells/ml), transfer of the phage DNA molecule to the host cell effectively stops after about 8 percent has been transferred (*1, 2*). Such arrested complexes are called FST (first-step transfer) complexes, and the transferred section of phage DNA, which is cut off and remains with the complexes upon vigorous stirring in a blendor, is called the FST section. Blended FST complexes are unable to form colonies and, except for rare survivors of blending, do not produce phage; but about 50 percent of the complexes can be superinfected (secondarily infected) productively with T5 (*3*). When unblended complexes are diluted (for example, to 10^8 cells per milliliter), the rest of the phage DNA is transferred after a short lag (*1*). Circumstantial evidence suggested that the FST section might contain structural genes for at least two proteins: one involved in breakdown of the host DNA (*4*), and the second needed to complete the transfer of the phage DNA molecule (*3*). We now report results of experiments that indicate that the FST section indeed carries at least

one phage gene (cistron) and not certain others.

We used amber (*am*) mutants (*5*), which grow on *E. coli* strain CR63 (here called CR), but not on strain B or F. The wild-type (*am*+) grows on all these strains. Conventional complementation and recombination tests showed that the 13 mutants chosen for study represent six cistrons (Table 1), four of which have been identified with temperature-sensitive (*ts*) mutants (*6*). We were not aware at the outset that *am*9 was a double mutant. The four *ts*-marked cistrons embrace over half of the known *ts* map (*6*). Of particular interest is the fact that *am*15, chosen to represent its cistron, gave maximum or near-maximum recombination (average value, 39 percent) with each of six scattered *ts* tester mutants. This result, suggesting that *am*15 lay outside the known map and perhaps within the small FST section, encouraged us to undertake the present study (*7*).

To analyze the genetic composition of the FST section, we prepared blended FST complexes of strain F infected with *am*+ phage, superinfected them with one or another amber mutant, and plated the complexes on CR (for total yielders irrespective of output genotype) and on F (for yielders of *am*+ recombinants). A positive result in such a "blendor-rescue" experiment requires that the FST fragments include the *am*+ (wild) allele of the superinfecting mutant. Suitable differentials among the amber mutants would indicate that the fragments do not contain all of the respective *am*+ alleles in equal abundance.

There were two series of assays (Table 2). In the FST series, blended FST complexes were prepared as described (*2*, *3*) with about five *am*+ phage particles per cell. In the control series, this primary infection was omitted. After blending and centrifuging in the cold, the cells of both series were brought to 5×10^8 cells per milliliter in cold nutrient broth, mixed with about 6 to 7 mutant particles per cell, and held for 15 minutes at 0°C. They were then diluted to 5×10^7 cells per milliliter with broth at 37.5°C, incubated for 15 minutes, chilled, centrifuged, and finally suspended in broth at room temperature (*8*). Suitable dilutions were plated, before lysis, on F or CR. Each mutant phage was tested at least twice, with concordant results. Where a mutant mixture was used as the second phage, the mutant ratio was 1:1, and the total input was as before. A heat-stable (*st*) mutant was used as FST donor in these experiments but the usual wild-type also serves (Table 3).

The results (Table 2) were positive with *am*2, *am*5, and *am*15 (all in one cistron) and negative with the remaining mutants, including *am*9, which we had assigned to the *am*2 cistron. We surmised that *am*9 might be a double mutant, with one mutation inside, and the second outside, the FST section. By crossing *am*9 to wild-type, we indeed succeeded in isolating reciprocal single-mutant recombinants (*am*9-1 and *am*9-2) in high yield; and, in blendor-rescue experiments, we obtained a positive result (Table 2) only with the recombinant (*am*9-2) which failed to complement the *am*2 group. Thus, the blendor procedure anticipated conventional genetic analysis in suggesting the mutational complexity of *am*9.

The positive results are well above the background contributed by *am*+ blendor survivors (usually about 1 percent of the complexes), revertants (Table 2, column 2), and leak or desorption (column 3). With *am*9-2 and *am*15, the CR assay value (total yielders) equals that found with wild-type as superinfecting phage, that is, complementation by the FST fragments seems to have maximum efficiency. The F assay (recombinant yielders) gives about half this value, suggesting that FST fragments can relieve a mutant defect without recombining. Both values for *am*2 and *am*5 in Table 2 are lower than those for *am*9-2 and *am*15. In parallel tests, however, only the CR values are significantly lower. We do not yet understand this difference among the mutants (*9*).

Because of a depressor effect of superinfection, the negative results (low values) in both the F and the CR assays (Table 2) are always lower than the uncorrected background, which varies slightly from day to day. We cannot, therefore, calculate a true zero rigorously. In the F assays, however, where the background consists almost entirely of *am*+ blendor survivors, the 3 or 4 low values in a given experiment appear constant within a small error. Since various mutants are involved, we conclude that the average of the low values is the effective zero and that none of the relevant mutants shows a significant enhancement. We extend this conclusion to the corresponding CR assays, since these usually exceed the F assays

Table 1. The amber mutants used to analyze the genetic composition of FST fragments. Mutants were isolated from stocks of the wild-type grown in the presence of 5-bromodeoxyuridine, those in a given cistron being isolated from different stocks. Recombination has not yet been observed between *am*6 and *am*8, or *am*2 and *am*5. With these possible exceptions, mutants in the same cistron appear to occupy different sites.

Cistron	Mutants
*ts*5E	*am*1, *am*6, *am*8, *am*10
*ts*5	*am*7, *am*30
*ts*6	*am*24, *am*29
*ts*11	*am*26
*am*2	*am*2, *am*5, *am*9 (9-2 site), *am*15
*am*9-1	*am*9 (9-1 site)

Table 2. Blendor-rescue experiments with T5*am*+ *st* as FST donor and individual amber mutants, mutant mixtures, or wild-type (*am*+ *st*+) as superinfecting phage. The prefix *am* is omitted. All plaque-count assays (columns 2 to 5) are expressed as percentage of the input bacteria, measured as colony-formers. In the same terms, the blendor survivors (blended FST complexes capable of producing a plaque when plated as such on strain F) amounted to 0.9, 1.1, and 1.8 percent, respectively, in experiments 1, 2, and 3. Before blending, the corresponding values were about 90 percent.

Second phage	Control series, plated on		FST series, plated on	
	F (%)	CR (%)	F (%)	CR (%)
	Experiment 1			
1	10^{-3}	1.3	0.5	1.1
2	2×10^{-3}	0.05	11	15
6	5×10^{-4}	1.3	0.5	1.1
7	6×10^{-4}	0.4	.5	0.7
30	2×10^{-3}	1.0	.6	.8
1 + 7			35	39
6 + 30			27	31
	Experiment 2			
5	4×10^{-3}	0.2	9.3	13
8	2×10^{-4}	.9	0.4	0.7
10	4×10^{-4}	2.2	.4	1.6
24	8×10^{-4}	0.4	.4	0.7
29	10^{-3}	.2	.4	.5
8 + 24			18	20
10 + 29			19	21
	Experiment 3			
Wild	71	68	41	41
9	2×10^{-4}	0.05	0.9	1.1
9-1	10^{-3}	.2	1.0	1.1
9-2	2×10^{-3}	.05	21	44
15	4×10^{-4}	.1	20	39
26	7×10^{-4}	6.7	0.9	1.3
9-1 + 26			23	25

Table 3. Reciprocal (order-reversal) blendor-rescue experiments between *am*15 and other amber mutants or wild-type (*am*+ *st*+). Only the FST series was assayed, and values are reported as in Table 2.

FST donor	Second phage	FST series, plated on	
		F (%)	CR (%)
Wild	*am*15	15	32
*am*7	*am*15	14	28
*am*8	*am*15	15	26
*am*15	wild	36	29
*am*15	*am*7	1.2	1.3
*am*15	*am*8	1.2	1.6

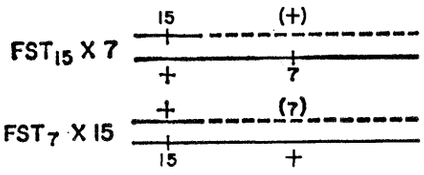

Fig. 1. Hypothetical genetic inputs in reciprocal blendor-rescue experiments (crosses) with *am*7 and *am*15. In each cross, the DNA molecule (or genome) of the FST donor is depicted as the upper line, the interrupted segment showing the portion deleted by blending; the lower line depicts the intact genome of the superinfecting phage. The upper cross is expected to be unproductive in strain F since the combined genetic inputs lack the wild (+) allele of *am*7. The reciprocal cross is expected to supply all the wild alleles needed for productive infection.

by a small amount related to the CR control assays. That the negative results do not have a trivial cause is shown by the fact that superinfection with a mixture of two "negative" mutants is productive (Table 2). The mutants paired for these controls were always from different cistrons and therefore produce progeny efficiently in conventional mixed infections in strain F.

The genetic specificity exhibited by the blended FST complexes supports the conclusion that the retained FST fragments include the *am*2 cistron but not the five others tested, that is, the population of FST fragments seems genetically homogeneous. If so, an FST mutant like *am*15 should not, when used as FST donor, be able to complement or to produce am^+ recombinants with any superinfecting amber mutant that is defective outside the FST section. The reciprocal experiment should, however, give a positive result, provided that the alternate mutant does not harbor also an FST mutation that makes it inactive as FST donor for *am*15. The results of such "order-reversal" experiments (Table 3) fully confirm the expectations (Fig. 1).

Strictly speaking, a blendor-rescue experiment tests the genetic makeup only of the FST donor's FST section, not that of the superinfecting phage. So far as they go, the combined data of Tables 2 and 3 do, however, support the assumption that wild-type and mutant chromosomes have the same gene sequence.

Analysis of the plaques produced on F by blended FST complexes superinfected with *am*15 (Table 2) has confirmed the division of the genome by blending and the genetic homogeneity of the retained fragments. As already noted, the FST donor had the genotype am^+ *st*. Twelve plaques from platings on F were sampled and scored for the am^+ and *st* markers of the FST donor. Ten plaques contained am^+ phage in high frequency, but no *st* phage in the samples tested. Both markers of the FST donor were recovered in high frequency from the remaining two plaques, which probably represent blendor survivors. A conventional cross between *am*15 and the *st* mutant in strain F gave about 40 percent recombinants and no sign of selection against the *st* marker. It appears that the *st* marker, which is associated with a 7-percent decrease in DNA mass (*10*), is an additional marker lying outside the FST section.

The apparent genetic homogeneity of a population of FST fragments is in agreement with inferences derived from the physiology of invasion (*3*, *4*), with the apparent molecular homogeneity of FST fragments (*2*), with evidence of sequential homogeneity of a population of unbroken T5 DNA molecules (*11*), and with the fact that the linkage map based on *ts* mutants appears to be linear, not circular (*6*).

The map needs further study. As already noted, *am*15 has given maximum or near-maximum recombination in conventional crosses with a wide selection of mutants. So has the *st* mutant that was used here as FST donor. Our hunch is that this relatively free recombination is correlated with breaks in the individual strands of the phage DNA molecule (*12*). It remains to be seen, however, whether linkage can be demonstrated among all the mutants and, if so, whether the resulting map will still be linear (*13*).

YVONNE THÉRY LANNI
FRANK LANNI
MARY JUDITH TEVETHIA*
Department of Microbiology,
Emory University,
Atlanta, Georgia 30322

References and Notes

1. Y. T. Lanni, *Virology* **10**, 514 (1960).
2. D. J. McCorquodale and Y. T. Lanni, *J. Mol. Biol.* **10**, 10 (1964); Y. T. Lanni, D. J. McCorquodale, C. M. Wilson, *ibid.* **10**, 19 (1964).
3. Y. T. Lanni, *Proc. Nat. Acad. Sci. U.S.* **53**, 969 (1965).
4. ——— and D. J. McCorquodale, *Virology* **19**, 72 (1963).
5. R. H. Epstein, A. Bolle, C. M. Steinberg, E. Kellenberger, E. Boy de la Tour, R. Chevalley, R. S. Edgar, M. Susman, G. H. Denhardt, A. Lielausis, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 375 (1965).
6. W. D. Fattig and F. Lanni, *Genetics* **51**, 157 (1965).
7. We have tried such experiments with other types of mutants. The previous failures with *ts* mutants evidently were due to the use of markers lying outside the FST section.
8. Successful results do not require close specification of times, temperatures, or phage inputs. The routine procedure, allowing superinfection in the cold, gives a somewhat reduced positive result. This procedure was adopted to allow synchronous superinfection with a mixture of mutants, as one of the controls.
9. Mutants *am*2 and *am*5 are strongly *ts* (in CR), whereas *am*9-2 is moderately *ts*, and *am*15 has no obvious *ts* character. The available evidence, although not decisive, suggests that the *ts* and *am* characters of *am*2 and *am*5 are pleiotropic. Incubation of assay plates at 28°C instead of the usual 37°C had a negligible effect. The differences in the CR assays might reflect differences either in map position within the cistron or in properties of the hypothetical polypeptide products. We incline to the second alternative.
10. I. Rubenstein, *Biophys. J.* **5** (Suppl.), 96 (1965).
11. C. A. Thomas, Jr., and I. Rubenstein, *Biophys. J.* **4**, 93 (1964).
12. P. F. Davison, D. Freifelder, B. W. Holloway, *J. Mol. Biol.* **8**, 1 (1964); M. M. Rhoades, Jr., J. Abelson, T. C. Pinkerton, C. A. Thomas, Jr., *Biophys. J.* **5** (Suppl.), 96 (1965); J. Abelson, thesis, Johns Hopkins University (1965).
13. Publication No. 704 from the Division of Basic Health Sciences; supported in part by PHS grant AI-00857. We thank Miss Diane Wiley and Mrs. Shirley Latimer for technical assistance, Dr. Ling Chu for her continuing interest, and Dr. R. S. Edgar and J. G. Van Dyke for samples of *E. coli* CR63 and a T4 amber mutant, which served as a useful guide. Van Dyke collaborated in some experiments with *ts* mutants. One of us (M.J.T.) was supported (as M. J. Robinson) by a PHS fellowship (1-F2-AI-25,289-01).
* Present address: Section of Molecular Biology, University of Texas M. D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston.

25 February 1966

Cyanide Intoxication: Protection with Oxygen

Abstract. *Prophylactic protection against cyanide intoxication in mice can be enhanced by the administration of oxygen, especially when it is used in combination with the conventional cyanide antidotes, sodium nitrite and sodium thiosulfate. The LD_{50} values were compared in groups of mice premedicated with sodium thiosulfate or sodium nitrite, or both, in air and in oxygen. These results indicate that oxygen alone provides only minimal protection. Although oxygen enhances the protective effect of sodium thiosulfate to a minor degree, it does not enhance the protection of sodium nitrite at all; and yet, it potentiates the effectiveness of a combination of these two antagonists against cyanide intoxication.*

The predominant toxic effect of cyanide appears to be the inhibition of the respiratory enzyme, cytochrome oxidase (*1*). In cyanide intoxication oxygen transport and oxygen tension are usually adequate, and only the cellular utilization of oxygen is diminished. This would mean that there is an ade-