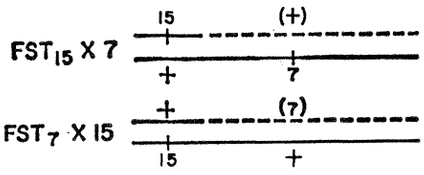

Fig. 1. Hypothetical genetic inputs in reciprocal blendor-rescue experiments (crosses) with *am*7 and *am*15. In each cross, the DNA molecule (or genome) of the FST donor is depicted as the upper line, the interrupted segment showing the portion deleted by blending; the lower line depicts the intact 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

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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.
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* Present address: Section of Molecular Biology, University of Texas M. D. Anderson Hospital and Tumor Institute, Texas Medical Center, Houston.

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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-

quate supply of oxygen to the tissue; hence the administration of oxygen is generally believed to serve no useful purpose. The commonly accepted treatment of cyanide poisoning includes the use of sodium nitrite and sodium thiosulfate (*2*, *3*). Sodium nitrite is employed to form methemoglobin (*4*) which effectively binds cyanide; and sodium thiosulfate (*5*) is utilized as a substrate for the enzyme, rhodanese, which detoxifies cyanide by converting it to thiocyanate. This report demonstrates that an increase in oxygen tension not only produces a protective effect against cyanide intoxication but also strikingly potentiates the efficacy of other antagonists.

Swiss-Webster male mice were distributed randomly into experimental groups, and the effects of various treatments on the lethality of cyanide were assessed. The LD_{50} (lethal dose, 50 percent of population) values, which were based on 24-hour mortality, were determined in five or more groups that contained ten mice each. Respective slopes of the dose-response curves for each experiment employing a cyanide antagonist, or antagonists, were found to be not significantly different from that for cyanide alone. The LD_{50} values of these experiments were statistically analyzed according to the method of Litchfield and Wilcoxon (*6*), and the confidence limits of the LD_{50} values are expressed as 19/20 probability ($P = .05$).

Doses and routes of administration of the antagonists are given in Table 1. Oxygen, sodium nitrite, and sodium thiosulfate were administered 60, 45, and 15 minutes, respectively, prior to the administration of potassium cyanide. A gas mixture containing 95 percent oxygen and 5 percent carbon dioxide was flushed into the chamber for the next 24 hours at a rate sufficient to maintain the oxygen tension above 700 mm-Hg.

The LD_{50} values of potassium cyanide, with and without antagonists, were obtained in air and in oxygen (Table 1). Administration of oxygen alone (experiment 2) produced very minimal protection against potassium cyanide intoxication. Although sodium nitrite was effective in protecting against cyanide poisoning (experiment 3), the administration of oxygen in combination with sodium nitrite (experiment 4) gave no additional protection. Sodium thiosulfate (experiment 5) was more efficacious than sodium nitrite when it was administered alone; and, more important, the protective effect of sodium thiosulfate, in contrast to sodium nitrite, was enhanced when it was given in combination with oxygen (experiment 6). When sodium nitrite and sodium thiosulfate were combined (experiment 7), the protective effect against cyanide poisoning was additive rather than synergistic, as reported by Chen and Rose in dogs (*3*). The best prophylactic protection was obtained when both sodium nitrite and sodium thiosulfate were administered in combination with oxygen (experiment 8). In comparing the last two experiments (7 and 8), the administration of oxygen strikingly enhanced the antagonism of potassium cyanide poisoning by approximately 20 mg/kg, or 2.4 LD_{50} doses, of potassium cyanide.

Table 1. Effect of oxygen, sodium nitrite, sodium thiosulfate, alone or in combination, on the LD_{50} of potassium cyanide in mice. Potassium cyanide (8 to 75 mg/kg) and sodium nitrite (100 mg/kg) were administered subcutaneously, and sodium thiosulfate (1.0 g/kg), intraperitoneally.

Exp.	Treatment before KCN	Slope function (limits, $P = .05$)	LD_{50} (mg/kg) (limits, $P = .05$)*
1	Control (air)	1.27 (1.03 to 1.56)	8.50 (7.73 to 9.44)
2	O_2	1.28 (1.02 to 1.70)	11.3 (10.3 to 12.4)†
3	$NaNO_2$	1.13 (1.06 to 1.21)	21.2 (19.9 to 22.6)
4	$NaNO_2 + O_2$	1.10 (1.02 to 1.20)	22.5 (21.4 to 23.6)‡
5	$Na_2S_2O_3$	1.33 (0.98 to 1.70)	34.6 (30.2 to 39.1)
6	$Na_2S_2O_3 + O_2$	1.15 (1.06 to 1.24)	42.2 (40.0 to 44.5)†
7	$NaNO_2 + Na_2S_2O_3$	1.12 (1.03 to 1.26)	53.5 (51.2 to 55.6)
8	$NaNO_2 + Na_2S_2O_3 + O_2$	1.12 (0.96 to 1.31)	73.0 (69.8 to 76.8)†

* Each LD_{50} value was obtained from five graded doses of potassium cyanide administered to five groups of mice containing ten mice each. † Significantly different when compared with experiments 1, 5, and 7, respectively. ‡ No significant difference when compared with experiment 3.

The generally recommended treatment of cyanide poisoning includes the use of amyl nitrite, sodium nitrite, and sodium thiosulfate (*3*). Although there appears to be no rational basis for employing oxygen in antagonizing cyanide intoxication, oxygen has been advocated in the past. In those studies which employed oxygen alone, the experimental designs were incomplete, and the results obtained were not very convincing; more important, none of these studies with oxygen was evaluated in combination with the other known cyanide antagonists. Gordh and Norberg (*7*) and Ivanov (*8*) reported that oxygen under atmospheric and hyperbaric conditions, respectively, was beneficial in cyanide poisoning. Cope (*9*) indicated that oxygen could reverse the EKG tracing that is produced by cyanide.

Antagonism of cyanide intoxication with oxygen has important implications with respect to the treatment of cyanide poisoning. Although oxygen alone has relatively minimal effects when compared with the conventional cyanide antagonists, its efficacy is clearly demonstrated when it is used with a combination of both sodium nitrite and sodium thiosulfate.

Experimental laboratory findings cannot always be applied to clinical situations, but these studies suggest the feasibility of employing oxygen as an antagonist in combination with sodium nitrite and sodium thiosulfate in cyanide poisoning. Since there seems to be no hazard in using oxygen in this manner and since the procedure could be life-saving, its adoption as a routine measure appears justified. Further studies are needed to ascertain the optimum oxygen tension for treatment under hyperbaric conditions and to investigate the biochemical mechanism or mechanisms by which oxygen enhances the protective effects against cyanide intoxication.

JAMES L. WAY
STANLEY L. GIBBON
MAUREEN SHEEHY
Department of Pharmacology,
Marquette University,
Milwaukee, Wisconsin 53233

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