pages 170 and 171), and because the type of ♂ used transfers the Sm^s gene to only a very small percentage of the cells. Under these conditions, enzyme is formed in the mated population with a time course and in amounts showing that the synthesis can be due only to zygotes having received the z^+ factor. Figure 2 shows the

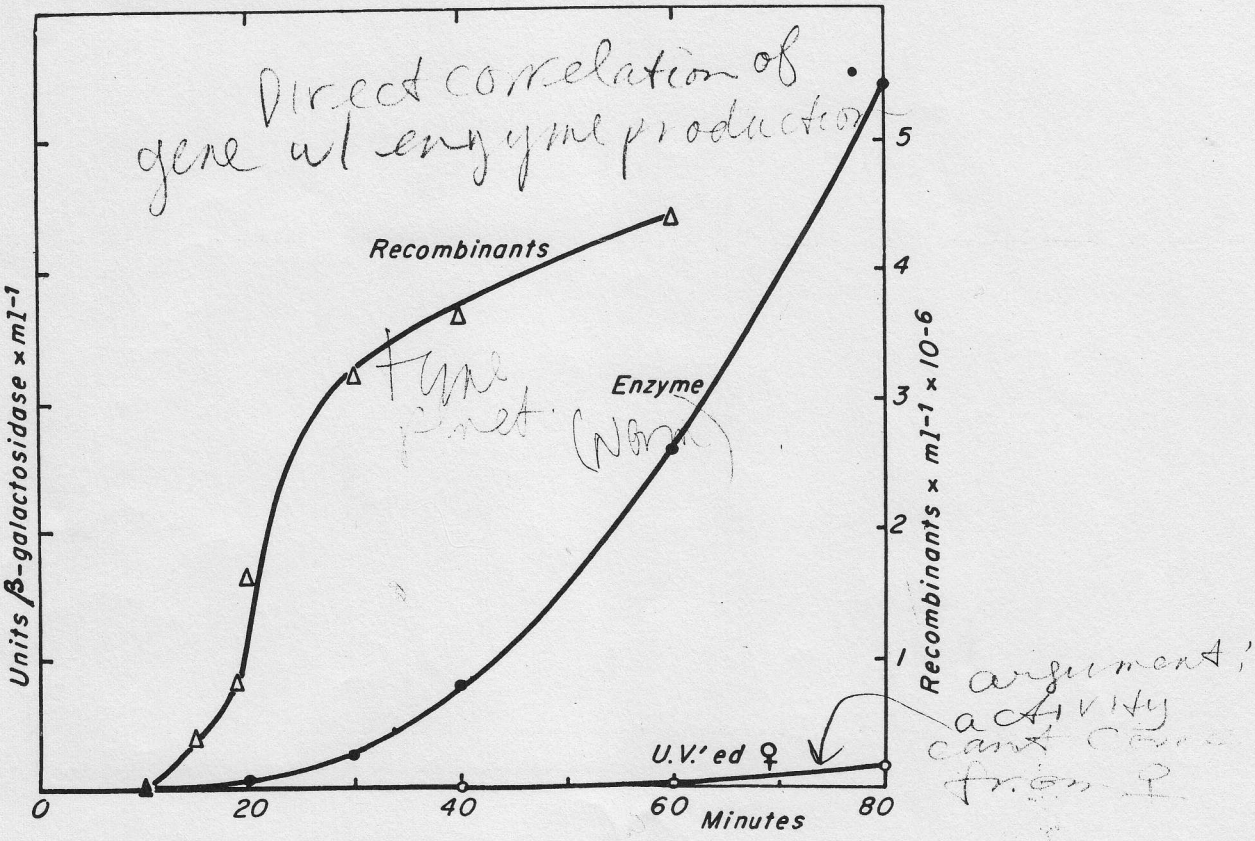

FIG. 2. Enzyme formation and appearance of recombinants in mating A.
Mating in presence of streptomycin (1 mg/ml.) and IPTG (10^{-3}M). A control with u.v.-treated ♀ cells (0·01 % survival) is shown. Recombinants (z^+Sm^r) selected by plating on Sm-lactose agar after blending (separate experiment with the same ♂ culture).

kinetics of galactosidase accumulation, compared with the appearance of z^+Sm^r recombinants, determined on aliquots of the same population (cf. Methods). The latter curve corresponds, as shown by Wollman & Jacob (1955), to the distribution of times of penetration of z^+ genes in the zygote population. It will be remarked that enzyme synthesis commences just within a few minutes after the first z^+ genes enter into zygotes. Assuming that the number of zygotes having received a z^+ gene is 4 to 5 times the number of recovered z^+Sm^r recombinants, and taking into account the fact that normal cells are on the average trinucleate (i.e., have three z^+ genes), the rate of enzyme synthesis per injected z^+ appears nearly normal.

This rapid expression of the z^+ factor poses the problem whether cytoplasmic constituents are injected from the ♂ into the zygote. This already appeared unlikely from the previous observations of Jacob & Wollman (1956). We reasoned that if there occurred any significant cytoplasmic mixing, such a mixing should allow the

♂ cells to feed the ♀ cells with any small metabolites which the ♂ had and the ♀ lacked. This condition is obtained in the following mating:

♂ z^+Sm^s *maltose*$^+$ × ♀ z^-Sm^r *maltose*$^-$

if it is performed in presence of maltose as sole carbon source, using a ♂ which virtually does not inject the *maltose*$^+$ gene. It results in a very strong inhibition of enzyme synthesis (and recombinant formation) showing that the ♂ cannot effectively

TABLE 1

Enzyme formation in nutritionally deficient zygotes

Deficiency	Rate of enzyme formation †			Mean % inhibition of recombinant formation
	Control	Deficient	Mean % inhibition	
Carbon source ‡	1·6	0·4	73	75
	0·66	0·20		
Arginine §	0·28	0·02	96	65
	0·36	0·01		

† Units of enzyme × hr^{-1}.

‡ ♂ z^+Sm^s *maltose*$^+$ × ♀ z^-Sm^r *maltose*$^-$ mated in presence of inducer and Sm with glycerol plus maltose (control) or maltose as sole carbon source.

§ ♂ z^+Sm^s Arg^+ × ♀ z^-Sm^r Arg^- mated in presence of inducer and Sm with and without arginine (10 μg/ml.).

feed the ♀. An even stronger effect is observed when the ♀ requires arginine, the ♂ not, and mating takes place in absence of arginine (again on condition that the Ar^+ gene is not injected by the ♂) (Table 1). These observations indicate that even small molecules do not readily pass from the ♂ into the ♀ cell during conjugation.†

It therefore appears that cytoplasmic fusion or mixing does not occur to an extent which might allow cross-feeding. That the contribution of the ♂ is exclusively genetic, and does not involve cytoplasmic constituents of a nature, or in amounts, significant for our purposes, is however only proved by the results of the opposite matings, which we shall consider in the next section.

(b) *Expression and interaction of the alleles of the z and i factors*

We should first consider which of the alleles of the *z* factors are dominant, and whether they all belong to a single cistron. Experiments of the type described above (mating A) were performed with each of the eight z^- mutants, used as ♀ cells, receiving a z^+ from the ♂. Enzyme was synthesized to similar extents in all cases, showing that the z^- mutants in question were all recessive. Each of the mutants was also mated (as ♂) to a z^- ♀. No enzyme was synthesized by any of these double recessive heterozygotes where the mutations were in the *trans* position

† However such leakage may occur when the concentration of a compound is exceptionally high in the ♂. This happens when a ♂ with the constitution $z^-i^-y^+$ is used in the presence of lactose. The constitutive permease then may concentrate lactose up to 20 % of the cells' dry-weight (Cohen & Monod, 1957). Adequate tests have shown that this lactose does flow from the ♂ into a permease-less ♀ during conjugation.

$$\frac{z_\alpha^+ z_\beta^-}{z_\alpha^- z_\beta^+}$$

showing that all the (tested) z^- mutants belong to the same cistron as defined by Benzer (1957).

The next and most critical problem is whether the z and i factors also belong to the same unit of function (gene or cistron) or not. Let us recall that cells with the constitution z^+i^+ synthesize enzyme in presence of inducer only, while z^+i^- cells synthesize enzyme without induction, and z^-i^+ or z^-i^- cells do not synthesize enzyme under any condition. The extremely close linkage of z and i mutations suggests that they may belong to the same unit. If this were so, they would not be able to interact through the cytoplasm, but could act together only when in *cis* position within the same genetic unit. The heterozygote, z^+i^+/z^-i^- would then be expected not to synthesize galactosidase constitutively.

In order to test this expectation, the following mating:

$$\text{♂}\ z^+i^+ \times \text{♀}\ z^-i^- \qquad \text{(B)}$$

was performed *in absence of inducer*. The ♂ cannot synthesize enzyme, because they are i^+. The ♀ cannot because they are z^-. The zygotes, however, do synthesize enzyme (Fig. 3): during the first hour following mating the synthesis is, if anything, even more rapid and vigorous than when both parents are i^+ and inducer is used, as in mating (A).

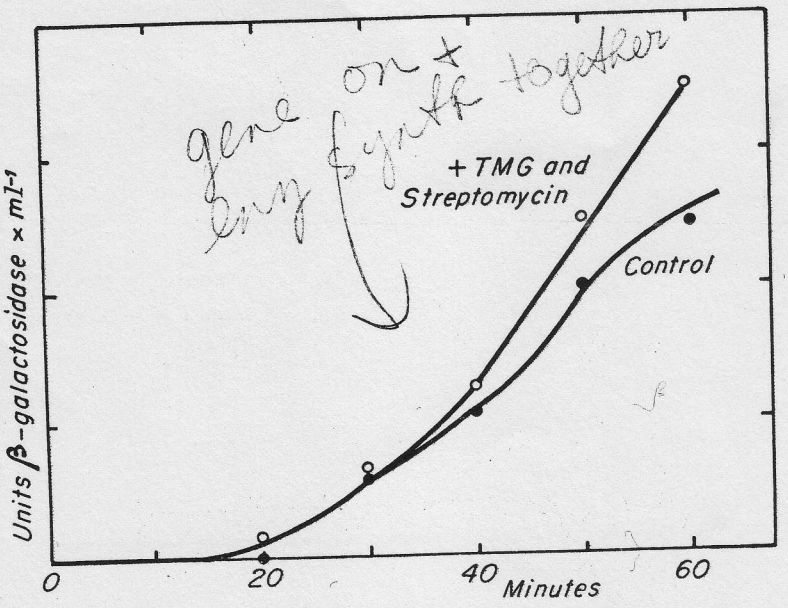

FIG. 3. Enzyme formation during first hour in mating B.
Mating under usual conditions. To an aliquot streptomycin (0·8 mg/ml.) was added at 20 minutes, and TMG at 25 minutes, to allow comparison of synthesis with and without inducer.

Such a mating therefore allows immediate and complete interaction of the z^+ from the ♂ with the i^- from the ♀. The possibility that the interaction depends upon actual recombination yielding z^+i^- in *cis* configuration is excluded because: (a) the synthesis begins virtually immediately after injection whereas genetic recombination is known (Jacob & Wollman, 1958) not to occur until 60 to 90 min after injection; (b) the factors z and i are so closely linked that recombination is an exceedingly rare

event (less than 10^{-4} of the zygotes) while the rate of enzyme synthesis is of an order indicating that most or all of the zygotes participate.

The possibility should also be considered that, rather than taking place through the cytoplasm, the interaction requires actual *pairing* of the homologous chromosome segments. This is excluded by the fact that the following mating:

$$z^-i^+ \times z^+i^-$$

when performed in the *absence* of inducer, yields no trace of enzyme, at any time after mixing, although conjugation and chromosome injection occur normally as shown by adequate controls involving other markers. The zygotes obtained in matings B and C are genetically identical, except that the wild type alleles (z^+i^+) are in relative excess (about 3 to 1) in (B), while the mutant alleles are in similar excess in (C). This quantitative difference cannot account for the absolute contrast of the results of the reciprocal matings, one allowing vigorous constitutive synthesis, the other none at all. This can only be attributed to the fact that the cytoplasm of the zygote is entirely furnished by the ♀ cell, with no significant contribution from the ♂. Therefore the $i^- \rightarrow z^+$ interaction must be considered to take place through the cytoplasm.

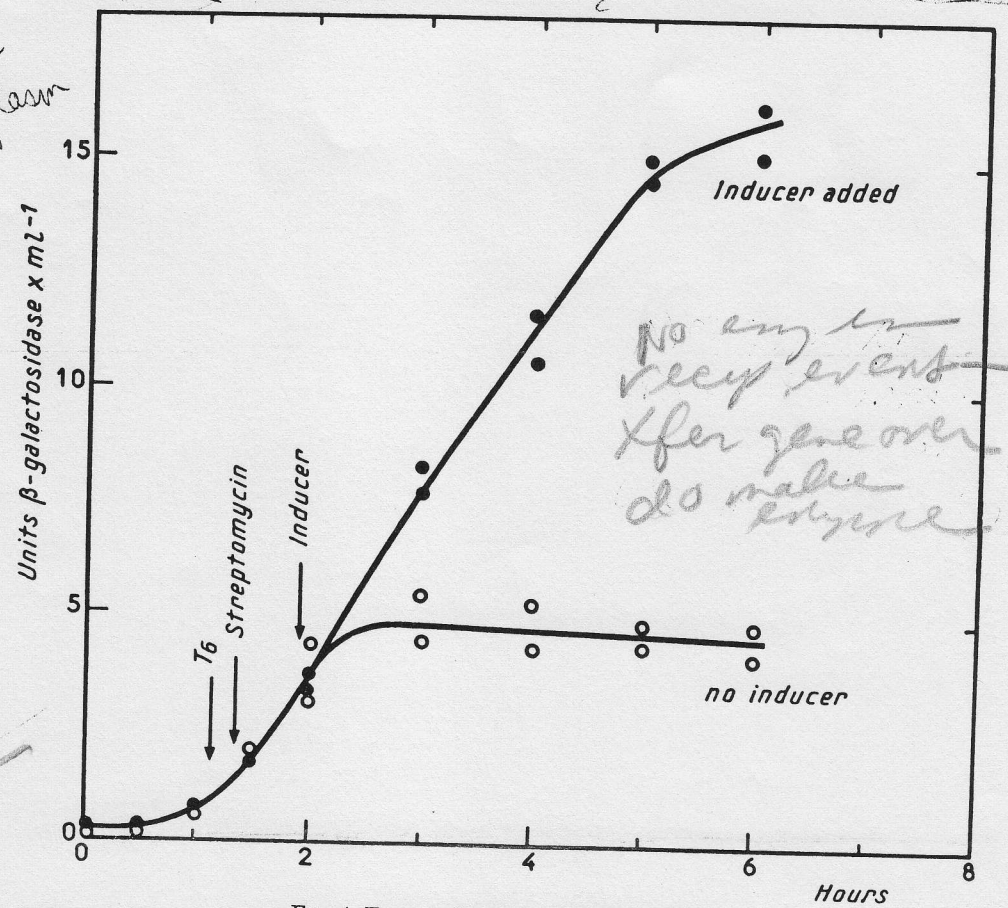

FIG. 4. Enzyme formation in mating D.
Mating performed under usual conditions in quadruplicate in absence of inducer. At times indicated, a suspension of phage T6 (20ϕ/B final concentration) and streptomycin (1 mg/ml.) were added to all of the cultures and TMG (2×10^{-3}M) was added to two of them (black circles) while the other two (white circles) received no addition.

This result may also be expressed by saying that the *i* factor sends out a cytoplasmic message which is picked up by the *z* gene, or gene products. Postulating, as we must, that this message is borne by a specific compound synthesized under the control of the *i* gene, we may further assume that one of the alleles of the *i* gene provokes the synthesis of the message, while the other one is inactive in this respect. If these assumptions are adequate, one of the alleles should be absolutely dominant over the other, but the dominance should become expressed only gradually when the cytoplasm of the zygotes came from the recessive parent, while it should be expressed immediately when the cytoplasm came from the dominant parent.

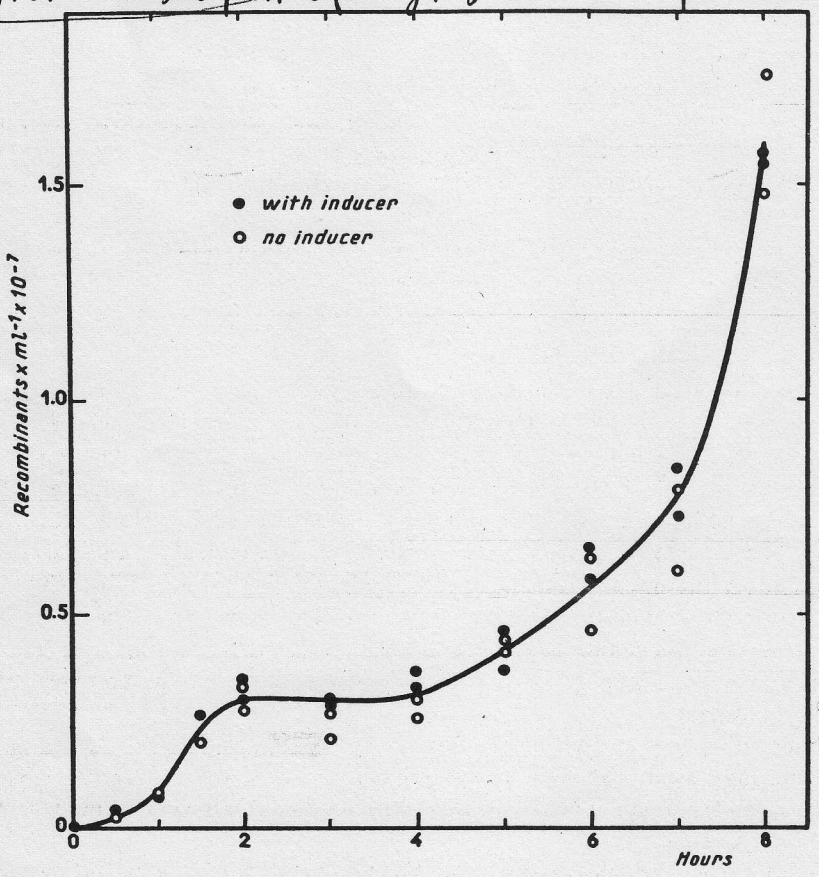

FIG. 5. Recombinant appearance in mating D.

Formation of z^+Sm^r recombinants tested by plating aliquots of the four cultures used in the experiment above (Fig. 4) on lactose-Sm agar. Portions of the culture were diluted 1000-fold and shaken vigorously at 100 minutes to prevent further mating. The increase up to the second hour is due to increasing numbers of zygotes. The increase after the fourth hour is due to *multiplication* of segregants (Wollman, Jacob & Hayes, 1956).

The fact that in matings of type (C) no enzyme is synthesized, even several hours after mating, means that the constitutive (i^-) allele from the ♂ is never expressed. This suggests that the dominant allele is the inducible (i^+). If so, the i^+ should eventually become expressed in matings of type (B)—i.e., the zygotes, initially constitutive (since their cytoplasm comes from the i^- parent), should eventually become inducible. To test this prediction, the following mating was performed: