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The Genetic Control of Transient Repression

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1. Disagreement exists concerning the role of the lactose regulating genes (i and o) in the control of transient repression of β-galactosidase synthesis in Escherichia coli. Palmer & Moses (1967, 1968) reported that certain mutations in these genes abolished transient repression. Perlman & Pastan (1968), and Tyler & Magasanik (1969), failed to confirm these observations. 2. Differences in experimental techniques in the various studies have been investigated and found not to be responsible for the discrepancies, nor are the discrepancies due to variation in the mutant bacterial strains. 3. Transient repression, normally absent in a strain carrying mutant regulator and operator genes, was not elicited by prolonged growth in rich medium. 4. Formal tests of the effect of growth history on transient repression in mutant and wild-type strains showed that transient repression was a function of the genotype, not of growth history. These tests confirmed the role of the regulator and operator genes in this type of repression. 5. A strain carrying a deletion of the lac promoter gene which extends into the i gene failed to show transient repression.

The addition of glucose to exponential cultures of wild-type Escherichia coli in glycerol medium causes a severe transient repression of β-galactosidase synthesis (Paigen, 1966; Moses & Prevost, 1966). The role of the regulator (i) and operator (o) genes in the control of this phenomenon was studied by Palmer & Moses (1967, 1968). They reported that such repression was abolished in full operator constitutive (o^c) strains, and in those carrying a suppressible amber mutation (i^{-sus}) or certain temperature sensitive lesions (i^{TL}) of the regulator gene. They concluded that transient repression requires the interaction of repressor with operator.

Later studies by Perlman & Pastan (1968), and by Tyler & Magasanik (1969), failed to support some of the observations on which the mechanism advanced by Palmer & Moses (1968) was based. The matter seemed of sufficient importance to warrant investigation of these discrepancies.

We have therefore repeated the principal experiments of all three groups, using techniques described in their reports. We have also compared strain o^c_{67} obtained from Perlman and Pastan with the same strain as used in this laboratory. None of these experiments succeeded in resolving the discrepancies. In our hands, all the mutant strains continue to behave as Palmer and Moses reported.

Yudkin (1969) has found that in some strains the intensity of transient repression is influenced by recent growth history. Such an effect may be the cause of the discrepant experimental results. We have tested this possibility by constructing diploid strains from newly re-isolated recipients, and maintaining these in strictly parallel culture until they were tested for transient repression. Our findings with such diploids have confirmed that regulator and operator gene function is required for transient repression.

EXPERIMENTAL

Bacterial strains. The genetic constitutions and sources of *E. coli* strains are as follows: o_{67}^{c} (*lac* $i^{-}o_{67}^{c}z^{+}y^{+}$; a recent description of this strain is given by Davies & Jacob, 1968), from Dr. E. Steers; o_{67}^{c}(P) (nominally identical with strain o_{67}^{c}), from Dr. E. Steers, via Dr. R. L. Perlman; 112-12-A-84 (F^{-} *lac* $i^{-sus}o^{+}z^{+}y^{+}$ *his*$^{-}$ *cys*$^{-}$ *gal*$^{-}$ *su*$^{-}$ Sm^{S}), from Dr. B. Müller-Hill; RV/F'o_{67}^{c} (*lac* $i^{+}o^{+}z^{-}$ *thr*$^{-}$ *leu*$^{-}$ Sm^{S}/F' *lac* $i^{-}o_{67}^{c}z^{+}y^{+}$), from Dr. E. Steers; RV (F^{-} lac^{del} Sm^{S}) and RV/F'MS 37 (lac^{del} Sm^{S}/F' *lac* $i^{+}o^{+}z^{-}y_{MS37}^{del}$), both from Dr. M. H. Malamy via Dr. M. D. Yudkin; AB 1157 (F^{-} *lac* $i^{+}o^{+}z^{+}y^{-}$ *thr*$^{-}$ *leu*$^{-}$ pro_{B}^{-} *his*$^{-}$ *arg*$^{-}$ Sm^{R}), from Dr. A. J. Clark; CA 8001 (HfrH *lac* $i_{LI}^{+}p_{LI}^{-}o^{+}z^{+}y^{+}Sm^{S}$), from Dr. J. R. Beckwith; *Salmonella* strain TR 132 ($ade_{67}^{-}pro_{A46}^{-}ile_{405}^{-}gal_{601}^{-}Sm^{R}$ azaserine^{R}/*E. coli* F' *lac* $i^{+}o^{+}z^{+}y^{+}pro_{A,B}^{+}$) was obtained from Dr. J. R. Roth, and was used to construct the diploid AB 1157/F' $pro_{A,B}^{+}$ lac^{+}.

Growth and β-galactosidase induction. Growth conditions in liquid medium followed the techniques described by Moses & Prevost (1966). An additional medium, lactose-tryptone broth, contained lactose (2 g./l.), NaCl (5 g./l.) and Bacto-Tryptone (8 g./l.) (Difco Laboratories, Detroit, Michigan). β-Galactosidase synthesis was induced with 0.5 mM IPTG* when necessary. All experiments were performed at 37°.

Enzyme measurements. These were performed as described by Palmer & Moses (1968). In some experiments toluene treatment was omitted and replaced

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*Abbreviations: IPTG, isopropyl-β-D-thio-galactopyranoside; BCIG, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside.

by the use of hexadecyltrimethylammonium bromide in the assay medium (Tyler & Magasanik, 1969). The latter used 0.1 mg./ml. final concentration of this detergent; we have found 0.022 mg./ml. to be more satisfactory.

Construction of diploids. (a) i^{-sus}/i^{+}. A streptomycin-resistant mutant of 112-12-A-84 was obtained by mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine (Adelberg, Mandel & Chen, 1965), followed by selection on minimal medium containing streptomycin (135 µg./ml.), histidine and cysteine. This strain is designated 112-12-A-84 Sm^{R}.

Young exponential cultures of strains 112-12-A-84 Sm^{R} and RV/F'MS 37 in glycerol minimal medium containing histidine and cysteine were mixed in the proportional of 1:5 (v/v) and incubated at 37° for 2 hr. with very gentle shaking. Suitable dilutions were plated onto glycerol-minimal agar containing histidine, cysteine, streptomycin (135 µg./ml.) and BCIG (40 µg./ml.) (Davies & Jacob, 1968). Parallel incubations and platings were performed with each parent strain separately.

After about 36 hr. at 37° all colonies from unmated 112-12-A-84 Sm^{R} were blue, and almost all from the mated strains were white. Strain RV/F'MS 37 failed to grow on these plates. Several blue and white colonies were picked and re-isolated by streaking on similar plates. Single blue and white colonies were then picked into lactose-minimal medium containing histidine, cysteine and streptomycin; all were found to be lac^{+}. Each was subcultured into glycerol-minimal medium: blue colonies produced β-galactosidase in the absence of inducer; white colonies required inducer for enzyme synthesis. On repeated plating on glycerol-BCIG plates, blue colonies always yielded only blue colonies,

while an occasional blue colony was obtained from white ones. Blue colonies were thus the original parent strain 112-12-A-84-$\underline{Sm}^R$ and the white colonies were 112-12-A-84-$\underline{Sm}^R$/F'MS 37.

For measurement of transient repression, overnight cultures in glycerol minimal medium were inoculated from the lactose-minimal cultures into which the colonies had been picked from plates. These were diluted and used the next day.

(b) $\underline{lac^{del}/F'o^c_{67}}$ and $\underline{lac^{del}/F'lac^+}$. A streptomycin-resistant mutant of strain RV was obtained as above, and designated RV$\underline{Sm}^R$.

Strains RV$\underline{Sm}^R$/F'$\underline{o^c_{67}}$ and RV$\underline{Sm}^R$/F'$\underline{pro^+_{A,B}lac^+}$ were constructed by mating RV$\underline{Sm}^R$ with RV/F'$\underline{o^c_{67}}$ and AB1157/F'$\underline{pro^+_{A,B}lac^+}$, respectively, using selection on lactose-minimal plates containing streptomycin (135 µg./ml.). Diploid colonies were picked and purified by two successive streakings on similar plates. Pure isolates were finally picked into lactose-minimal medium containing streptomycin and were checked for inducibility. As expected, RV$\underline{Sm}^R$/F'$\underline{o^c_{67}}$ was constitutive and RV$\underline{Sm}^R$/F'$\underline{pro^+_{A,B}lac^+}$ was inducible.

Cultures for measurement of transient repression were taken from the lactose-minimal culture into which pure isolates had been picked. They were inoculated into glycerol-minimal medium for overnight growth, and used the following day after dilution.

Chemicals. Streptomycin and IPTG were obtained from Calbiochem, Los Angeles, California; BCIG was from Cyclo Chemical Corp., Los Angeles, California.

RESULTS

Variations in experimental technique. In testing for transient repression Palmer & Moses (1967, 1968) used lower concentrations of both glycerol and glucose (22 mM and 10 mM, respectively) than either Tyler & Magasanik (1969) (43 mM and 22 mM), or Perlman & Pastan (1968) (54 mM and 25 mM). In unmasking the enzyme, Tyler & Magasanik (1969) employed hexadecyltrimethylammonium bromide (0.1 mg./ml.) in the assay solution, while the other two groups used toluene treatment before adding substrate. Finally, while Palmer & Moses (1967, 1968) added 1 vol. of M-glucose solution to 100 vol. of cells in glycerol medium, both of the other groups split the culture and incubated portions in different media. We have examined each of these variables to determine whether the transient effect is dependent on any of them.

Increasing both the glycerol and glucose concentrations (to 44 mM and 25 mM, respectively), in an experiment with strain o^{c}_{67} which was otherwise performed exactly as Palmer & Moses (1967, 1968) described, gave a result identical with that reported previously (Fig. 1): no transient repression was observed. Using this strain, Tyler & Magasanik (1969) reported transient repression which lasted for 15% and 86% of one mass doubling time. Fig. 1 shows the cell concentrations corresponding to these periods. The use of hexadecyltrimethylammonium bromide (0.1 mg./ml.) in the assay system instead of toluene also failed to demonstrate transient repression (Fig. 2A). In the same experiment, portions of the culture were rapidly transferred into two other vessels, one empty and the other containing sufficient M-glucose solution to give

a final glucose concentration of 25 mM. Transfers were completed within 30 sec. No transient repression was observed (Fig. 2B). We are thus unable to elicit transient repression of β-galactosidase with glucose in strain o^c_{67}.

Possibility of strain variation. All three groups reporting the behaviour of strain o^c_{67} originally obtained this mutant from Dr. E. Steers. It was possible that prolonged culture in the various laboratories had resulted in sub-varieties with differing physiological properties. A culture of strain o^c_{67} was therefore obtained from Dr. R. L. Perlman and designated o^c_{67}(P). This strain was maintained for a short period in glycerol-minimal medium and then tested for transient repression by our standard procedure. No such repression was observed (Fig. 3).

Effect of previous growth history. Yudkin (1969) has reported that the appearance of transient repression in *E. coli* strains 3000, 3000L8 and 300U is influenced by their recent growth history. Maintenance of these strains for months in glycerol-minimal medium weakened or abolished transient repression. Transient repression was restored by growing the cells through seven subcultures in glucose-peptone medium.

In this laboratory, working strains are routinely maintained in minimal media. Both of the other groups maintain their stocks in rich media and transfer them to minimal media the night before an experiment (personal communications from Drs. R. L. Perlman & B. Tyler). We therefore maintained strain o^c_{67}(P), taken directly from the slant supplied by Dr. R. L. Perlman, for 45 subcultures in lactose-

tryptone broth. Each subculture permitted about 10 mass doublings. The cells were then transferred to glycerol-minimal medium for overnight growth and tested for transient repression by our standard procedure. None was observed (Fig. 4).

Behaviour of diploids in parallel culture. A more rigorous test both of the effect of growth history and of the role of the regulator genes was provided by the construction of appropriate strains from a common parent, and their maintenance in strictly parallel cultures. An earlier study (Palmer & Moses, 1968) showed that insertion of F'lac^+ into the i^{-sus} strain 112-12-A-84 simultaneously restored inducibility and transient repression. That study did not employ parallel culture conditions. Furthermore, the i^{-sus}/F'lac^+ diploid carried two functional z and y genes and this could be held to influence the repression pattern.

The episome F'MS37 was inserted into 112-12-A-84Sm^R and the resulting i^{-sus}/i^+ diploid compared with the haploid female parent. Both strains were grown in the presence of 0.5 mM IPTG for this experiment, though in subsequent experiments it has been found that the repression pattern of the constitutive parent is unaffected by inducer. Fig. 5 shows that permanent repression was absent in both strains, but that transient repression, in agreement with our earlier findings, depended upon the presence of a functional i-gene product.

In an analogous experiment, the episomes F'$pro^+_{A,B}lac^+$ and F'o^c_{67} were inserted into a RVSm^R, a strain carrying a total lac deletion. The diploids were isolated, and maintained and tested in parallel culture. Transient repression was observed only in the strain carrying functional i and o genes (Fig. 6).

Behaviour of strain CA8001. Silverstone et al. (1969) reported that strains carrying a partial deletion of the lac promoter region (L1) were insensitive to catabolite repression. We have confirmed that under our standard conditions strain CA8001 exhibits neither transient nor permanent repression (Fig. 7).

DISCUSSION

The three groups who have studied transient repression in lac regulatory mutants have failed to agree about the behaviour of such strains. Comparison of the experimental techniques, and of one of the bacterial strains, has not resolved the experimental disagreements; personal discussion between members of the groups has thrown no further light on the discrepancies.

Extensive studies with our experimental procedure has confirmed that transient repression depends upon the presence of functional i and o genes, as shown previously by Palmer & Moses (1968). Transient repression elicited by glucose is abolished in the L1 promoter deletion mutant. It may not be irrelevant to note that the L1 deletion extends into the i gene (Miller et al., 1968).

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CAPTIONS FOR FIGURES

Fig. 1. Absence of transient repression in strain o_{67}^{c}. Cells grown in minimal medium plus glycerol (44 mM); glucose (25 mM) added at arrow. Cells treated with toluene for determination of enzyme activity. Mass doubling times before and after glucose addition were 77 min. and 58 min., respectively. The arrows at the top of the graph show the bacterial protein concn. corresponding to 15% and 86% increases in cell mass after the addition of glucose; for the significance of this, see text.

Fig. 2. Absence of transient repression in strain o_{67}^{c}. (a) Repeat of experiment shown in Fig. 1, but with enzyme activity measured in the presence of hexadecyltrimethylammonium bromide instead of toluene. This culture was not removed from the original flask. Glucose (25 mM) added at arrow. (b) Same experiment, in which portions of the culture were transferred from the parent vessel into two other flasks, one empty and the other containing M-glucose to give a final concn. of 25 mM. Transfer time 30 sec.; transfer was carried out at the time that sampling started in part (a). Mass doubling times in glycerol and glycerol plus glucose were 70 min. and 56 min., respectively. The arrows at the top of the graphs show the bacterial protein concn. corresponding to 15% and 86% increases in cell mass after the addition of glucose. In (b): ● , glycerol; o , glycerol plus glucose.

Fig. 3. Absence of transient repression in strain o_{67}^{c}(P). Experimental details as for Fig. 2 (a) except glycerol concn. 22 mM and glucose concn. 10 mM. Mass doubling times before and after glucose were 90 min. and 60 min., respectively.

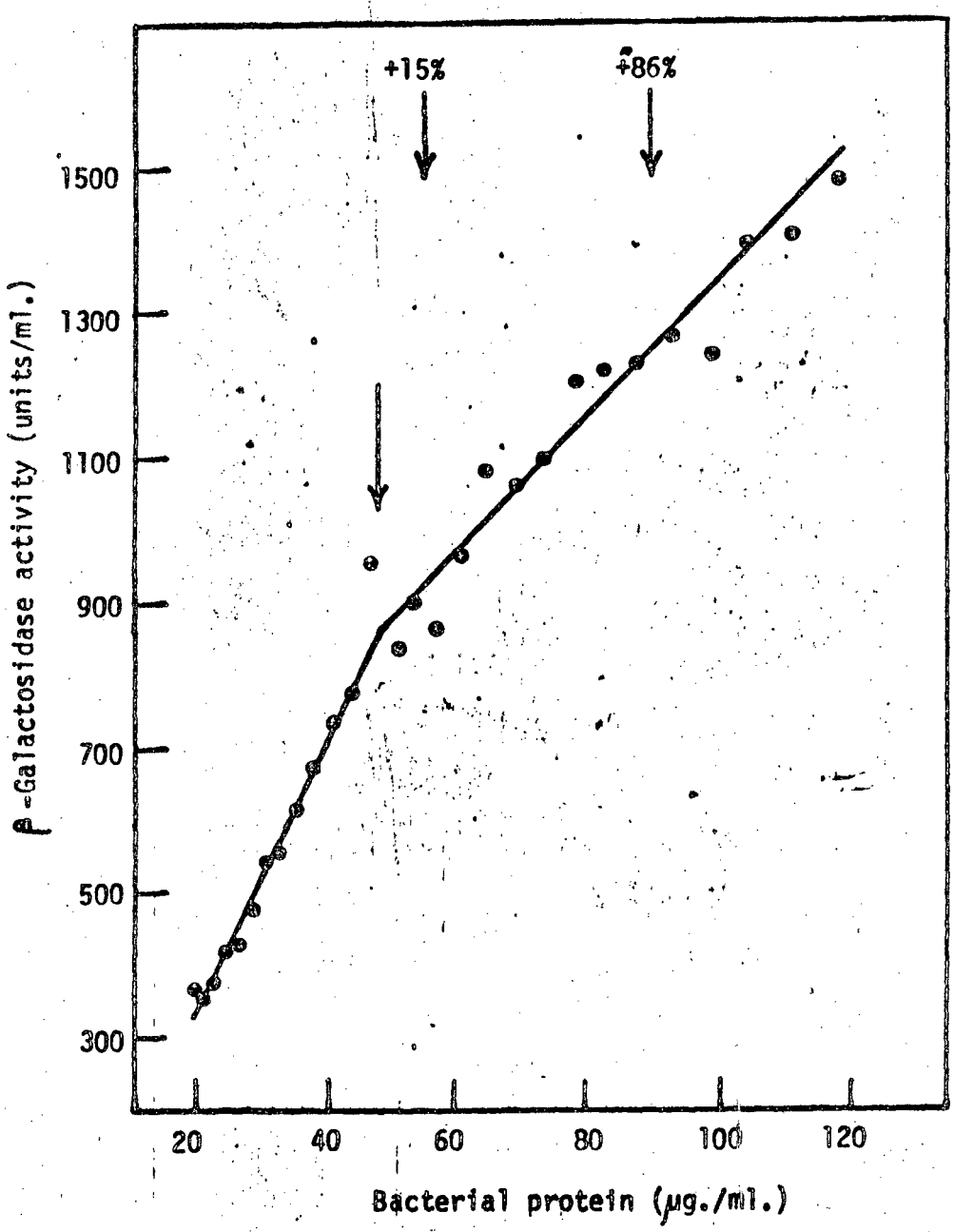
Fig. 4. Absence of transient repression in strain o_{67}^{c}(P) after 450 doublings in lactose-tryptone medium, followed by 10 doublings in glycerol-minimal medium. Experimental details as for Fig. 3. Mass doubling times before and after addition of glucose were 90 min. and 62 min., respectively.

Fig. 5. Transient repression in strains 112-12-A-84 Sm^{R} and 112-12-A-84 Sm^{R}/F'MS 37, maintained in parallel cultures. Experimental details as for Fig. 3. Mass doubling times before and after addition of glucose were 70 min. and 60 min., respectively. Transient repression in (b) lasted about 50 min. Both cultures contained 0.5 mM-IPTG. (a), 112-12-A-84 Sm^{R}; (b), 112-12-A-84 Sm^{R}/F'MS 37.

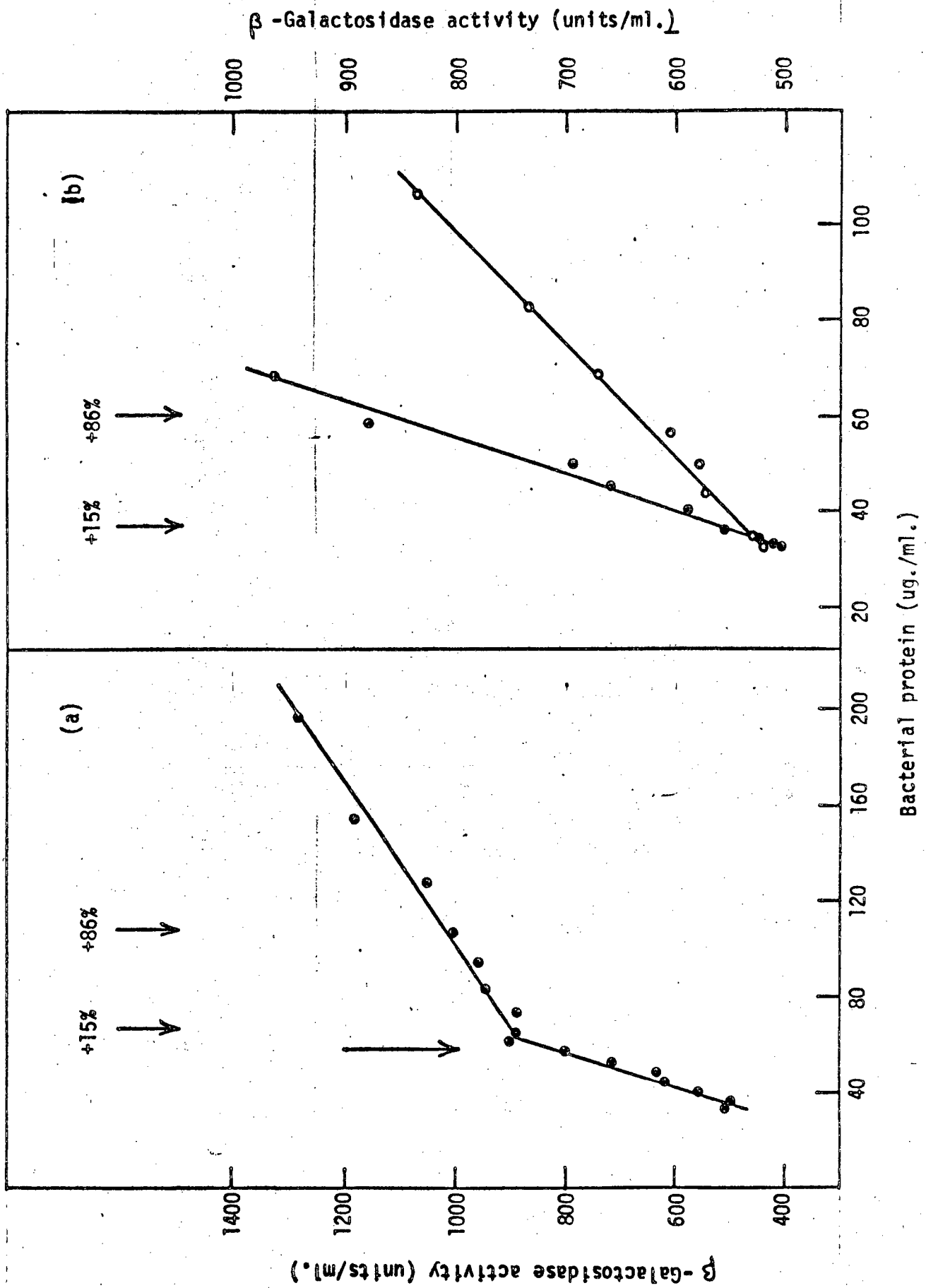
Fig. 6. Transient repression in strains RV Sm^{R}/F' $pro^{+}lac^{+}$ and RV Sm^{R}/F'o_{67}^{c} maintained in parallel cultures. Experimental details as for Fig. 3. Mass doubling times before and after addition of glucose: (a) RV Sm^{R}/F' $pro^{+}lac^{+}$ (induced with 0.5 mM-IPTG), 78 min. and 64 min., respectively; transient repression lasted about 60 min.; (b) RV Sm^{R}/F' o_{67}^{c}, 71 min. and 61 min., respectively.

Fig. 7. Effect of glucose on β-galactosidase synthesis in strain CA8001 induced with 0.5 mM-IPTG. Experimental details as for Fig. 3. Mass doubling times before and after addition of glucose were 102 min. and 70 min., respectively.

Moses & Sharp. Fig. 1.



Moses & Sharp. Fig. 2.



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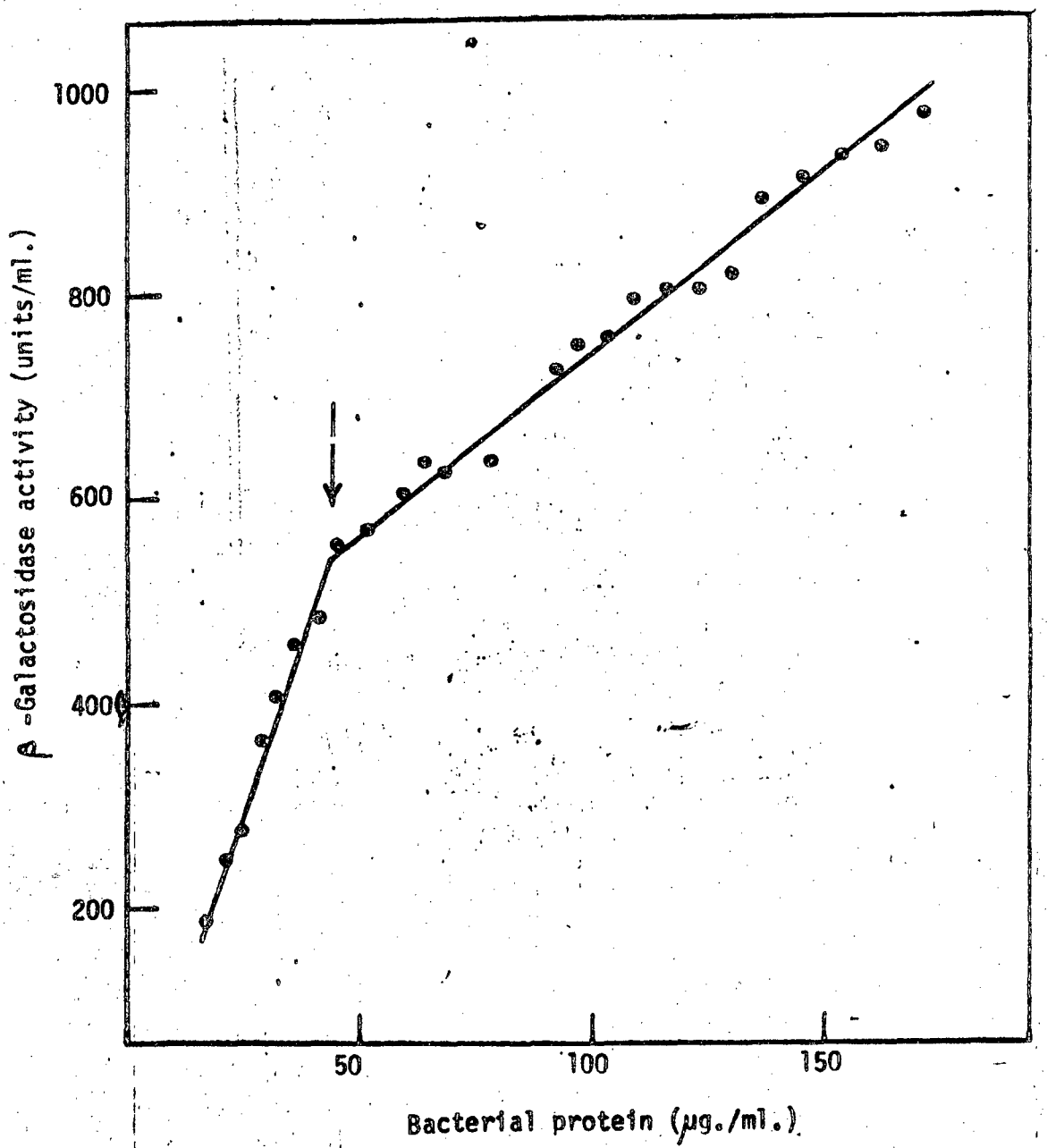

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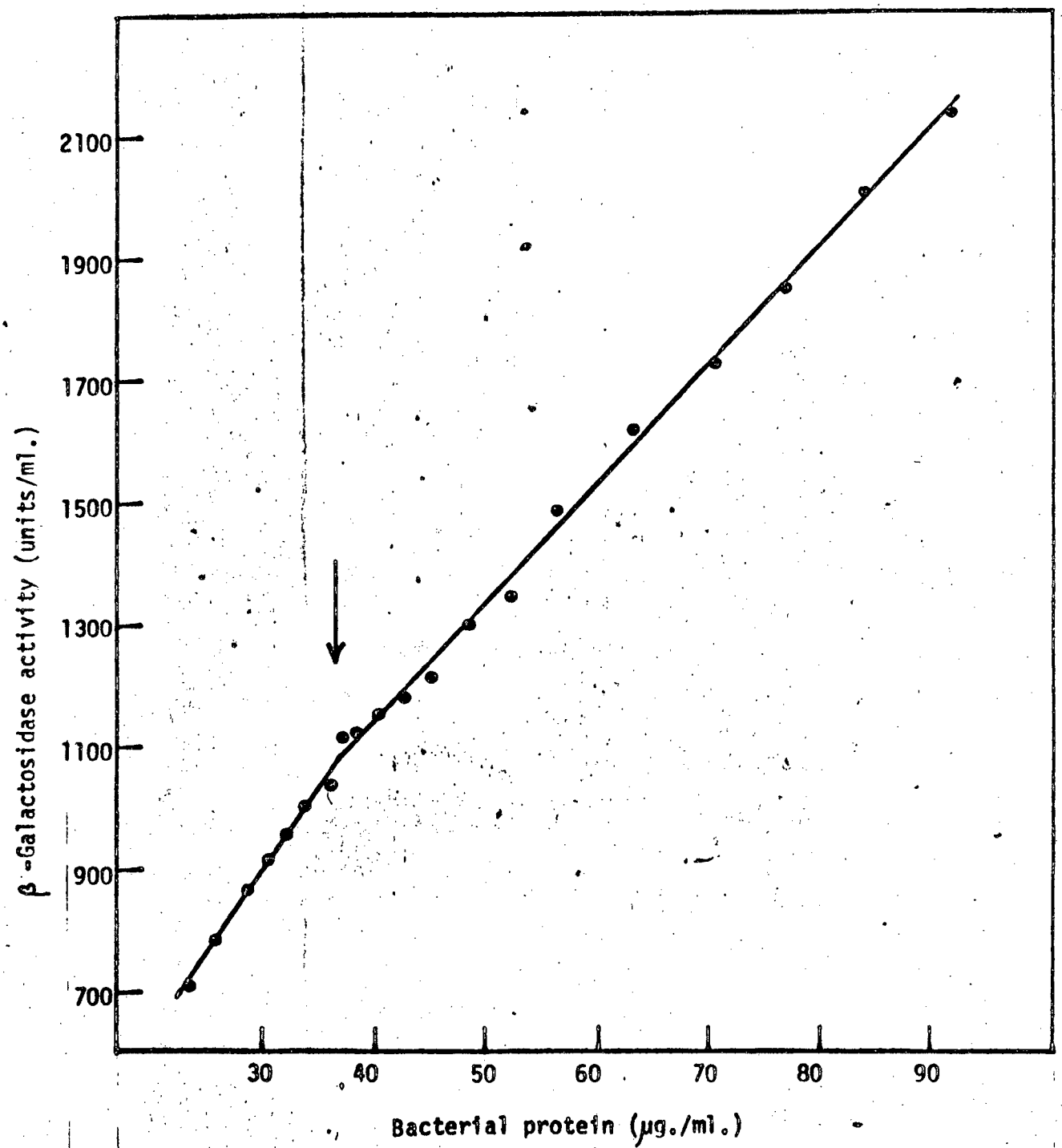
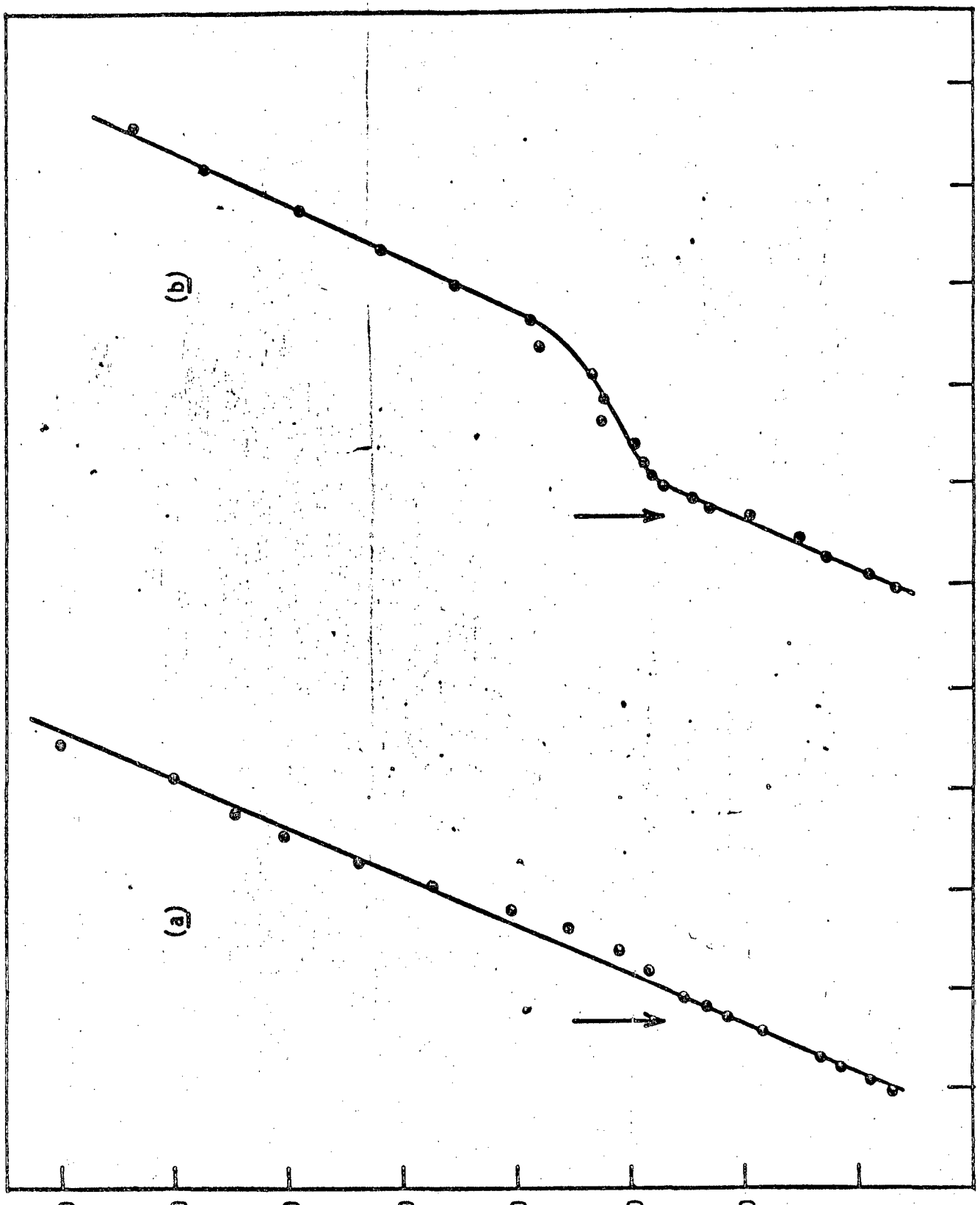
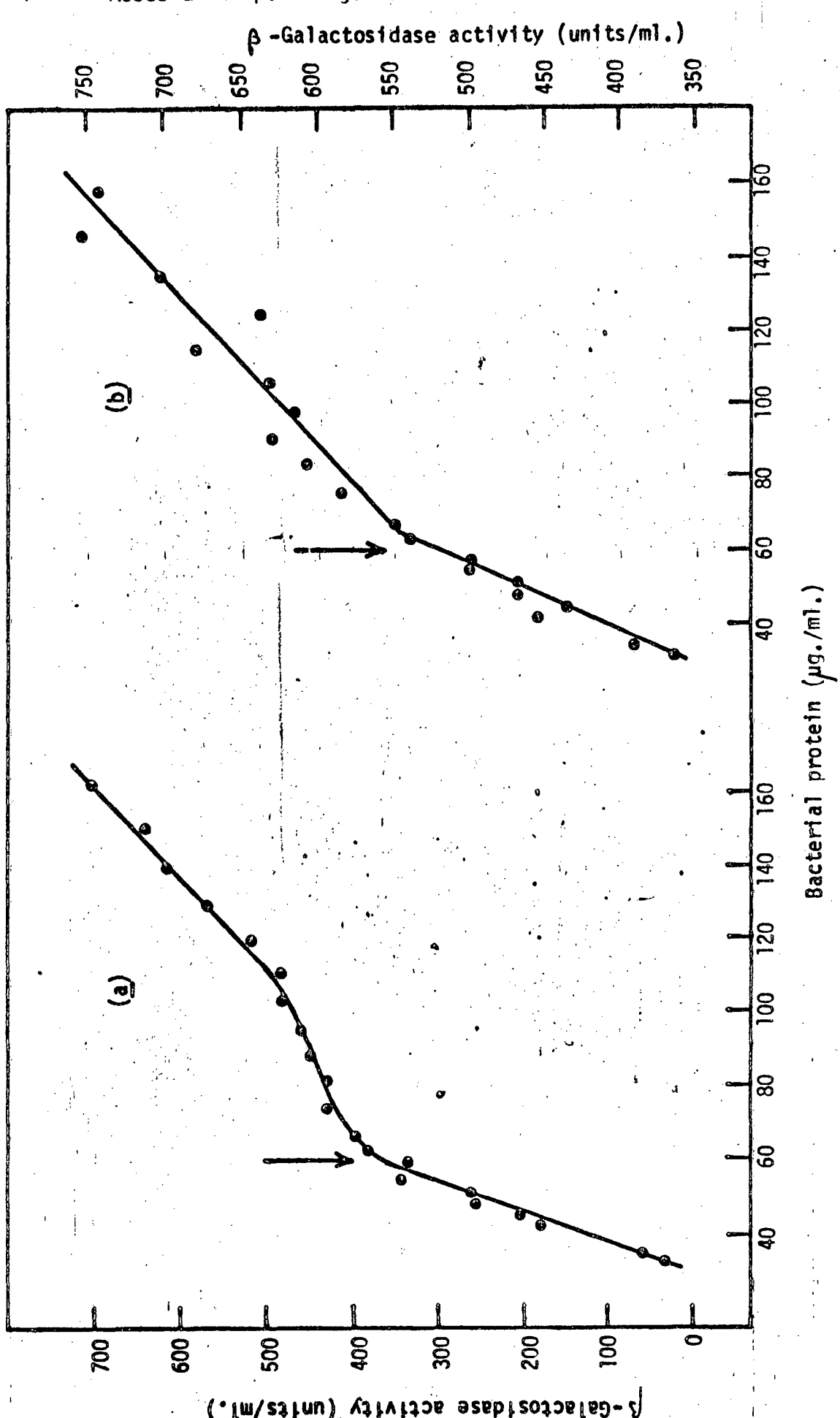
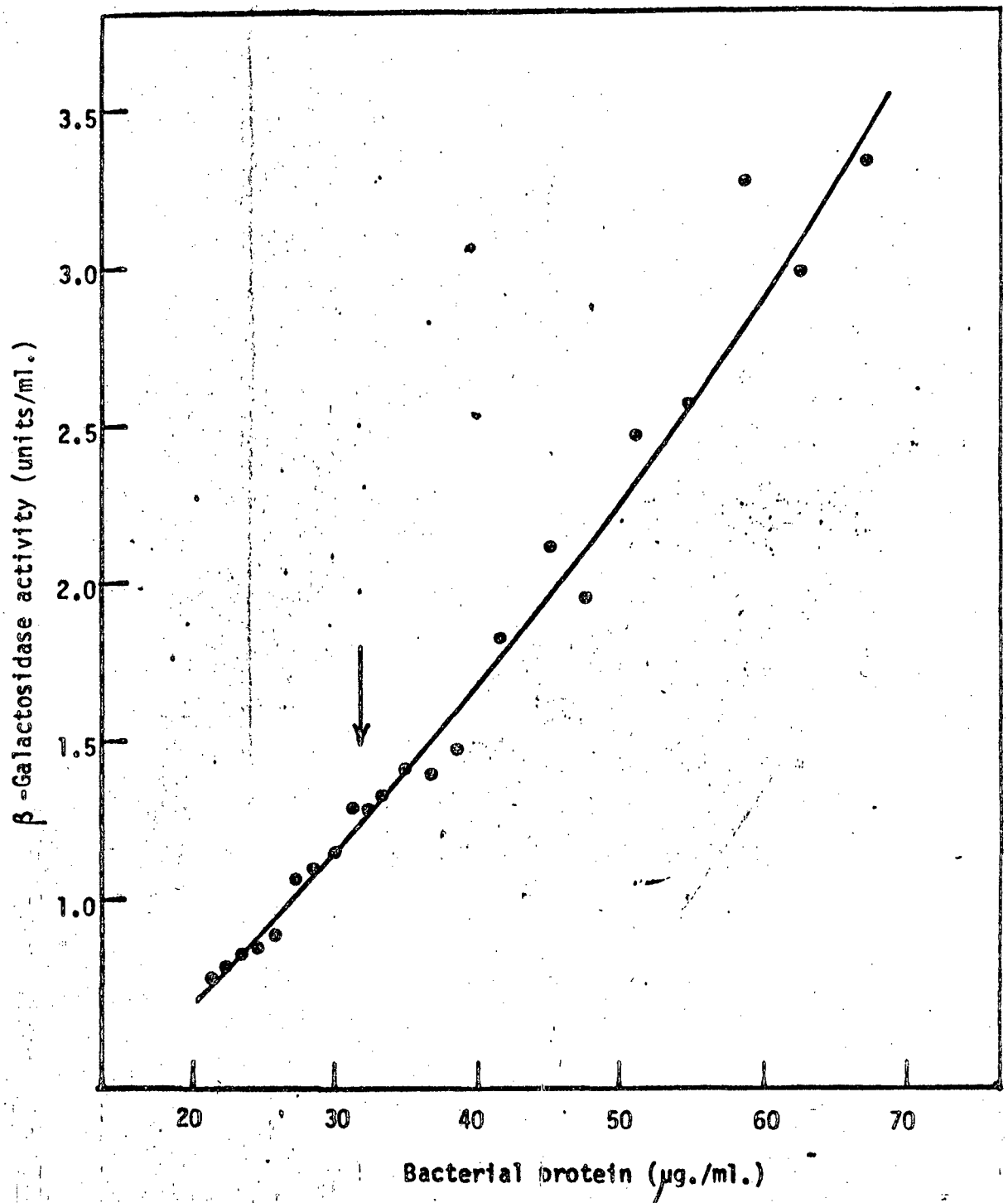


Moses & Sharp. - Fig. 5.




β-Galactosidase activity (units/ml.)
750
700
650
600
550
500
450
400
350
(b)
(a)
β-Galactosidase activity (units/ml.)
700
600
500
400
300
200
100
0
Bacterial protein (μg./ml.)
40
60
80
100
120
140
160

Moses & Sharp. Fig. 7.



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