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CATABOLITE REPRESSION OF β-GALACTOSIDASE SYNTHESIS IN ESCHERICHIA COLI

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CATABOLITE REPRESSION OF β-GALACTOSIDASE SYNTHESIS IN *ESCHERICHIA COLI*

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Catabolite Repression of β-Galactosidase Synthesis in *Escherichia coli*

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1. Repression by glucose of β-galactosidase synthesis is spontaneously reversible in all strains of *E. coli* examined long before the glucose has all been consumed. The extent of recovery and the time necessary for reversal differ among various strains. Other inducible enzymes show similar effects. 2. This transient effect of glucose repression is observed in constitutive (i^-) and permeaseless (y^-) cells as well as in the corresponding i^+ and y^+ strains. 3. Repression is exerted by several rapidly metabolizable substrates (galactose, ribose, and ribonucleosides) but not by non- or poorly-metabolized compounds (2-deoxyglucose, 2-deoxyribose, phenyl-thio-β-galactoside, and 2-deoxyribonucleosides). 4. The transient repression with glucose is observed in inducible cells supplied with a powerful inducer of β-galactosidase synthesis (e.g. isopropyl-thio-β-galactoside) but not with a weak inducer (lactose); in the latter instance glucose repression is permanent. Diauxic growth on glucose plus lactose can be abolished by including isopropyl-thio-β-galactoside in the medium. 5. In some strains phosphate starvation increases catabolite repression; in others it relieves it. Adenine starvation in an adenine-requiring mutant also relieves catabolite repression by glycerol but

not by glucose. Restoration of phosphate or adenine to cells starved of these nutrients causes a pronounced temporary repression. Alkaline phosphatase synthesis is not affected by these nutritional factors. 6. During periods of transient repression of induced enzyme synthesis the differential rate of RNA synthesis, measured by labelled uracil incorporation in 2 min. pulses, shows a temporary rise. 7. The differential rate of uracil incorporation into RNA falls during exponential growth of batch cultures of E. coli. This is equally true for uracil-requiring and non-requiring strains. The fall in the rate of incorporation has been shown to be due to a real fall in the rate of RNA synthesis. The significance of the changes in the rate of RNA synthesis are discussed. 8. A partial model of catabolite repression is presented with suggestions for determining the chemical identification of the catabolite corepressor itself.

The inducible synthesis of a variety of bacterial catabolic enzymes has long been known to be influenced by the nutritional state of the cells. Growth of cells in media containing very rapidly metabolized sources of carbon results in the relative repression of the synthesis of enzymes such as β-galactosidase and L-tryptophanase, even though the appropriate inducers are also present. Repression of this type is responsible for the diauxic form of growth (Monod, 1945) observed, for example, when cells of Escherichia coli are supplied with a mixture of glucose and lactose; many examples of such diauxic growth patterns are known.

It gradually came to be realized that this repression, called originally the "glucose effect", was by no means confined to glucose. Repression was found under a wide variety of conditions in which, as a general rule, carbohydrate consumption (i.e. catabolism) exceeded the cells' requirements for biosynthetic purposes (Neidhardt, 1960). It was thus observed with many rapidly metabolized substrates, or under conditions in which protein synthesis was partially reduced by the presence of inhibitors or by nutritional deficiencies. Magasanik (1963) therefore proposed a more general title of "catabolite repression" for the phenomenon.

Kinetically, it has almost universally been accepted that the repression continues as long as the rapidly metabolized carbohydrate is present and the metabolic imbalance remains (see, for example, the kinetic graphs in McFall & Mandelstam, 1963). However, Roozi & Cowie (1961) reported a case in which spontaneous recovery from repression occurred, even though the glucose or other carbohydrate had not been exhausted. Although these authors did not pursue this observation, it seemed to us that a transitory effect of this type, rather than a terminal or permanent repression, offered more hope of success in studying the metabolic origins of the glucose inhibition. The present communication reports on a detailed investigation of transient repression and its relation to other physiologic phenomena.

MATERIALS AND METHODS

Organisms. The following strains of *E. coli* were used; their relevant genetic characteristics are noted: C 600-1 ($i^+y^-z^+r_1^-$) (from A. B. Pardee); AB 1105 ($i^+y^-z^+gal^-r_1^-his^-met^-$) (C. Willson); ML 3 ($i^+y^-z^+$) and JC 14 ($i^+y^-z^+ade^-met^-$) (both from A. J. Clark); ML 30 ($i^+y^+z^+$), 300 U

($i^+y^-z^+$) and 230 U ($i^-y^-z^+$) (all from J. Monod); CR 34 ($thr^-leu^-met^-thy^-U^-B_1^-$) (D. M. Freifelder); Cavalli ($i^+y^+z^+met^-thy^-$) (Aleen Simmons). Two y^+ revertants (AB 1105-1 and AB 1105-2) were isolated from AB 1105 by selection on lactose agar. An alkaline phosphatase constitutive mutant (JC 14-2) was isolated from JC 14 by the method of Torriani & Rothman (1961).

Culture conditions. Cells were grown at 37° in M63 medium (Pardee & Prestidge, 1961) containing glycerol or succinate (0.022 M), and supplemented with specific nutrients as necessary. Vigorous aeration was provided by teflon-coated bar magnets rapidly rotated by magnetic stirrer motors below the flasks. The maltose medium of Boezi & Cowie (1961) has also been used for the ML strains as noted in the text.

Enzyme induction and assay. Inducers for β-galactosidase, L-tryptophanase and D-serine deaminase were isopropyl-thio-β-D-galactopyranoside (IPTG) (5×10^{-4} M), L-tryptophan (2.5×10^{-3} M) and D-serine (2.9×10^{-3} M), respectively. Samples (0.2 ml.) of cultures for β-galactosidase and alkaline phosphatase assay were mixed with 20 µl. of chloramphenicol solution (1 mg./ml.). Later 1 drop of toluene was added and after vigorous agitation for 20 sec. with a Vortex Mixer the enzyme was measured by the rate of hydrolysis of o-nitrophenyl-β-D-galactoside for β-galactosidase (Kepes, 1963), or p-nitrophenylphosphate (at pH 8.0) for alkaline phosphatase. Samples (also 0.2 ml.) for L-tryptophanase and D-serine deaminase were assayed as described by Pardee & Prestidge (1961). One unit of enzyme activity is defined for each enzyme as 1 nmole substrate hydrolyzed/min. at 37°.

Growth was determined by the extinction at 650 mμ in a 1 cm. cuvette, using a Beckman DK-2 double-beam spectrophotometer. Extinction was directly proportional to total protein under all conditions studied (cf. Fig. 21); at extinction of 1.0, 1 ml. of culture contained 225 μg. protein.

Chemical determinations. Samples of suitable vol. for total protein and RNA were mixed with sufficient ice-cold trichloracetic acid to give a final concn. of 5% (w/v). RNA was extracted by heating the cells in trichloracetic acid to 90° for 15 min., and was determined by the method of Schneider (1957). The residue was washed free from trichloracetic acid, dried and dissolved in N-NaOH; protein was determined as described by Lowry, Rosebrough, Farr & Randall (1951).

Residual glucose in the medium was determined, after removal of the cells, by the Glucostat enzyme method (Worthington Biochemical Corp., Freehold, N. J.).

Labelling with radioactive precursors. Pulse-labelling experiments were performed by mixing 0.5 ml. of cell suspension with 10 μl. of an aqueous solution containing L-[^{14}C]phenylalanine (0.25 μc.; specific activity, 1 μC./μmole) plus [^{3}H]uracil (2 μC.; specific activity, 2600 μC./μmole). After 2 min. at 37°, 2 ml. of ice-cold 6.25% (w/v) trichloracetic acid was added, and the suspension kept at 0° for at least 30 min. The precipitate was then filtered through a pre-wetted millipore membrane filter (0.45 μ pore size), washed repeatedly with cold trichloracetic acid followed by water, and after being sucked roughly dry the whole filter was dissolved with agitation in scintillation solution (Kinard, 1957). This solution was gelled by the addition of Cab-O-Sil Thixotropic Gel Powder (Packard Instrument Co., Inc., Downers Grove,

Illinois, U.S.A.), maintaining the precipitate in suspension. Samples were counted in a Packard Tri-Carb Scintillation Counter using an external standard. Occasionally L-[$^{14}CH_3$]methionine has been used as the labelled amino acid.

In experiments requiring measurement of radioactivity in the supernatant medium, the cells were removed by filtration through a dry millipore membrane filter as above, and measured volumes of filtrate counted in the scintillation counter.

Chemicals and radiochemicals. Isopropyl-thio-β-D-galactopyranoside, o-nitrophenyl-β-D-galactopyranoside, p-nitrophenylphosphate, 2-deoxyglucose, 2-deoxyribose, D-serine and L-tryptophan were obtained from Calbiochem, Los Angeles, Calif.; chloramphenicol was from Parke, Davis & Co., Detroit, Mich.; phenyl-thio-β-D-galactopyranoside was a gift from Dr. C. Willson. Other chemicals were standard commercial products.

L-[^{14}C]-Phenylalanine, L-[$^{14}CH_3$]methionine and [^{3}H]uracil were obtained from New England Nuclear Corp., Boston, Mass.; D-[^{14}C]glucose was prepared by the method of Putman & Hassid (1952).

RESULTS

Transient repression of β-galactosidase synthesis by glucose. The effect on the inducible synthesis of β-galactosidase of adding glucose (10^{-2} M) has been investigated in seven inducible strains of E. coli. In each case, as reported by Doezi & Cowie (1961), glucose was only temporarily effective in repressing enzyme synthesis. In a period which varied widely between different strains (Fig. 1), the differential rate

[INSERT FIG. 1 NEAR HERE]

of enzyme synthesis increased after an initial repression, and in some cases nearly reached the rate found in the absence of glucose. The length

of the repression period varied from about 4 min. with strains ML 3 and ML 30 (Figs. 1 and 8) to 150 min. with AB 1105 (Fig. 20). Paigen (1964) in a brief note has also described mutants exhibiting the property of transient repression.

The carbon content of the medium was almost doubled by the addition of glucose. Fig. 2 shows that the glucose concentration did not fall

[INSERT FIG. 2 NEAR HERE]

significantly until long after the end of the transient repression.

The relation between the time at which glucose was supplied to the cells, relative to the time of adding the inducer, may be predicted from the existence of the transient. The experimental results are shown in Fig. 3. Glucose added during β-galactosidase synthesis resulted in a

[INSERT FIG. 3 NEAR HERE]

transient repression which lasted in that experiment about 22 min. (Fig. 3C). Adding glucose together with inducer delayed the attainment of a constant differential rate of enzyme synthesis from the characteristic 3 min. (Pardee & Prestidge, 1961) to 27 min. (Fig. 3B). When glucose was added 40 min. before inducer only the usual 3 min. delay was observed (Fig. 3A). These results suggest that metabolic rearrangements occur when glucose is added to the cells, and that it is this readjustment, rather than glucose metabolism *per se*, which is responsible for the greatest degree of inhibition of enzyme synthesis. The final steady rate of enzyme synthesis in the presence of glucose, while lower than in its absence, was invariably two or more times greater than the rate during maximum inhibition.

The glucose transient during growth on carbon sources other than glycerol. Fig. 1 has already shown the glucose transient with strain ML 3 growing on glycerol-minimal media. A similar response to glucose was shown

when ML 3 grew on succinate or maltose (Fig. 4). There was thus no

[INSERT FIG. 4 NEAR HERE]

special effect of glucose on glycerol-grown cells.

Elimination of the diauxic growth pattern. A further prediction of the transient response is that cells supplied with glucose and lactose should no longer grow in a diauxic manner if IPTG is also present in the medium. This was indeed found to be the case (Fig. 5), and may probably be explained in the following manner. Competition between catabolite

[INSERT FIG. 5 NEAR HERE]

repression exerted by glucose and β-galactosidase induction has been demonstrated by Clark & Marr (1964). IPTG is known to be a more powerful inducer than lactose for the enzymes of the lactose operon; i.e. higher levels of these enzymes are induced by equal concentrations of IPTG and lactose (Jacob & Monod, 1961). Glucose repression thus becomes more effective with a weak inducer than with a strong one, and IPTG seems to be strong enough to prevent most of the glucose inhibition, while lactose is not. At the end of the glucose phase of growth, cells grown with IPTG have sufficient enzyme to permit immediate utilization of lactose. Those without IPTG must start to synthesize enzyme in the absence of an available carbon source. This can only be done slowly, so that the resumption of growth on lactose is delayed.

Effects of glucose and the entry of inducer into the cell. A possible explanation for the glucose transient would reside in the mechanism of glucose transport into the cell. It was suggested to us by Dr. A. L. Koch (personal communication) that glucose and IPTG might be transported into and out of the cell by the same non-specific carrier system. Addition

of glucose to the medium would result in the sudden flooding of glucose into the cell, with the liberation on the inside of many available free carrier molecules. These might then eject from the cell a large number of inducer molecules, thereby slowing the rate of induced enzyme synthesis. After a time it might be supposed that the intermediary pools of glucose metabolism would become saturated, and this would result in a fall in the rate of glucose entry. Some inducer molecules would then be able to regain entry and induced enzyme synthesis would to some extent be restored.

In support of this theory we must note that Kepes (1960) did observe a lowering of the internal inducer concentration of cryptic cells in the presence of inducer. Unfortunately, Kepes' results are open to criticism. Inducer uptake was measured by the accumulation in the filterable cell fraction of radioactivity from labelled inducer (methyl-thio-galactoside). It was not shown that this radioactivity was inside the cells, as distinct from being adsorbed on the surface; above all, there was no way of measuring the effective inducer concentration at the induction site. A dense, non-growing cell suspension was employed (just how dense is not clear from the paper) in which the cells were poisoned with chloramphenicol. Some doubt would arise in applying his findings to a viable and growing population.

In the present studies we have investigated the ability of a number of compounds other than glucose to cause transient repression. Substances tested have included galactose, ribose, uridine, adenosine, cytidine and guanosine. All of these are metabolized by the cells and all produce transient repression. However, 2-deoxyglucose, 2-deoxyribose, and 2-deoxyribonucleosides are not rapidly metabolized and produced no

transient. Deoxyribose is degraded by E. coli to ethanol, acetate and carbon dioxide (Bernstein, Fossitt & Sweet, 1958). None of these substances would be expected to cause severe catabolite repression. 2-Deoxyglucose is known to be phosphorylated in E. coli (Gershanovich, 1962), and/inhibit growth in high concentration (Schick, Landon & Tschudy, 1958). It must therefore enter the cells, but we have found that it has no effect on β-galactosidase synthesis (Fig. 6). Cohn & Horibata (1959) have also shown that 2-deoxyglucose did not preferentially

[INSERT FIG. 6 NEAR HERE]

inhibit β-galactosidase synthesis under conditions in which glucose abolished it almost entirely.

A more rigorous test of the expulsion theory would consist of supplying the cells with a compound similar in structure to the inducer but not itself active in promoting enzyme induction. With IPTG as inducer a convenient compound to test is phenyl-thio-β-galactoside which has no inducing capacity (Jacob & Monod, 1961). Phenyl-thio-galactoside, at a concentration of 10^{-3} M compared with 5×10^{-4} M for IPTG in the same culture, produced no detectable repression in either strain ML 3 or 300 U (Fig. 6). Demonstration of the penetration of phenyl-thio-galactoside was measured by adding it to a 45% (v/v) suspension of cells, and determining its concentration in the supernatant, after removal of the cells, by its extinction at 245 mμ. The absence of effect of phenyl-thio-galactoside directly contradicts the findings of Herzenberg (1959), who, in the same strain of cells, found inhibition of 20-40% of β-galactosidase synthesis but reported no kinetic experiments of this inhibition. From these experiments we may exclude expulsion of inducer as a factor in transient repression.

Effect of mutation at the regulator gene locus. The effects of glucose on the synthesis of β-galactosidase were compared in inducible (i^+) and constitutive (i^-) strains. While it is, for technical reasons, more difficult to obtain scatter-free kinetic curves with a constitutive than with an inducible strain, the results demonstrate no significant difference between the glucose transients with the two strains (Fig. 7).

[INSERT FIG. 7 NEAR HERE]

Effect of mutation at the permease gene locus. Comparison of two strains isogenic except for the galactoside permease gene (y) showed no difference between y^+ and y^-. Strains ML 30 and ML 3 are compared in Fig. 8; similar comparisons were made between strains AB 1105 and AB 1105-1 or AB 1105-2. This again excludes entry of inducer as a factor in repression, except in the sense that glucose repression of the lactose operon would

[INSERT FIG. 8 NEAR HERE]

presumably inhibit the synthesis of galactoside permease as well as β-galactosidase itself. Such an effect on permease was not studied in detail, and would in any case be of no consequence in the y^- mutants.

Elimination of the i and y genes as being controlling factors in producing the transient shifted attention to aspects of glucose metabolism itself.

Nutritional deficiencies and catabolite repression (i) Phosphate. Clark & Marr (1964) reported that starvation of cells for sulphate, an essential inorganic nutrient, resulted in catabolite repression of β-galactosidase, while McFall & Magasanik (1962) observed a similar effect with phosphate starvation. We have studied the effects of phosphate starvation on two strains of *E. coli* and found variable effects depending on the strain used.

Fig. 9 shows the findings with AB 1105, a strain more sensitive than most to glucose repression (Fig. 1). During growth on glycerol in the presence of adequate phosphate (Fig. 9, section 1) the differential rate of enzyme synthesis was high (2300 enzyme units/ml. culture/extinction

[INSERT FIG. 9 NEAR HERE]

of culture at 650 mμ); simultaneous addition of glucose and IPTG resulted in a period of 65 min. of very slow enzyme production, and then the rate of synthesis increased to 350 enzyme units/ml. culture/extinction. When the phosphate was exhausted, growth slowed down from a doubling time of 84 min. to one of 660 min. At this point alkaline phosphatase synthesis was derepressed, indicating that the concentration of inorganic phosphate had fallen to less than 10^{-5} M (Torriani, 1960). During phosphate starvation induction with IPTG resulted in a very low rate of enzyme synthesis (190 units/ml. culture/extinction) which was depressed still further by glucose to 46 units/ml. culture/extinction (Fig. 9, section 2). Addition of 10^{-2} M-inorganic phosphate to phosphate-starved cells supplied 20 min. earlier with IPTG resulted in a rapid increase in the rate of enzyme synthesis, which reached 840 units/ml. culture/extinction in 23 min. The presence of glucose, however, prevented this rise, and the rate of enzyme synthesis with IPTG plus glucose was still only 70 units/ml. culture/extinction 100 min. after the addition of phosphate (Fig. 9, section 3). Following phosphate addition to starved cells, the growth rate increased, and exponential growth with a doubling time of 107 min. was achieved in about 80 min. (see Fig. 10 for a similar experiment). IPTG added 20 min. after phosphate rapidly induced enzyme synthesis which achieved a value of 860 units/ml. culture/extinction. In the presence of glucose this

was much reduced, and 120 min. after the addition of IPTG (equal to 140 min. after the replacement of phosphate) the rate with glucose present was still only 135 units/ml. culture/extinction (Fig. 9, section 4). These results confirm the observations of Clark & Marr (1964) that essential inorganic ion deprivation may enhance catabolite repression.

Restoration of the pre-starvation state of metabolism permitting enzyme synthesis was rapidly achieved when phosphate was supplied to the cells, provided glucose was not also present. The lower post-starvation differential rate of enzyme synthesis may be correlated with a lower growth rate after starvation until the end of the experiment. We may conclude that this relatively high degree of catabolite repression, even in the absence of glucose, observed in the post-starvation period, coupled with an alteration of glucose metabolism at that time, resulted in the intense repression in the presence of glucose shown in sections 3 and 4 of Fig. 9.

Glucose utilization, measured by the disappearance of [^{14}C]glucose from the medium, was determined at various stages during growth on limiting phosphate (Fig. 10). It was found that the rate of glucose

[INSERT FIG. 10 NEAR HERE]

utilization paralleled the rate of growth rather closely throughout the experiment, showing that the enhanced repression exerted by glucose during phosphate starvation was due not to a relative increase in glucose consumption compared with growth, but rather to a rearrangement of internal metabolism during this period.

The situation in strain JC 14, an adenine and methionine auxotroph, was quite different (Fig. 11). In this organism phosphate starvation relieved catabolite repression due to glycerol in the medium, since the

[INSERT FIG. 11 NEAR HERE]

rate of enzyme synthesis increased from 1650 enzyme units/ml. culture/extinction in the presence of phosphate, to 5300 enzyme units/ml. culture/extinction in its absence. Replacement of phosphate produced a rapid growth response, while β-galactosidase showed a transitory period of severe repression followed by recovery, some 33 min. after phosphate was added, to a synthesis rate of 2135 units/ml. culture/extinction.

The respiration of the cells on the glycerol used as growth substrate was studied manometrically during the various states of phosphate nutrition. Periodically, duplicate 2 ml. samples of the growing culture were introduced into Warburg flasks, one of which contained sufficient chloramphenicol (50 μg./ml.) to prevent further enzyme synthesis. Oxygen consumption at 37° was measured after absorption of respiratory carbon dioxide with potassium hydroxide. Initially, respiration was identical in both flasks of each pair, indicating that chloramphenicol had no effect on the rate of oxygen uptake. In time, of course, cells without chloramphenicol increased in quantity, and the oxygen uptake in those flasks increased in rate. From the results shown in Table 1, it can be seen that the

[INSERT TABLE 1 NEAR HERE]

specific rate of respiration was much reduced during the period of phosphate starvation. This is presumably due to a shortage of available phosphate for intermediary compounds required in the respiratory processes, and suggests that such compounds are also required for the maintenance

of catabolite repression. Addition of inorganic phosphate (4×10^{-3} M) caused an immediate increase in the rate of oxygen absorption even in the presence of chloramphenicol.

Phosphate starvation produces quite different responses with regard to catabolite repression in the two strains JC 14 and MB 1105. One must conclude that the balance of metabolism in the two strains differs considerably as far as phosphate dependence is concerned.

(ii) Adenine. The derepressing effect on JC 14 of phosphate starvation, having suggested an involvement with phosphorylation processes, prompted us to study in another way an impairment of phosphorylating activity. Since most phosphorylations require the participation of ATP we have made use of the adenine requirement of JC 14 to try to deplete the intracellular availability of ATP.

The effects of adenine starvation in cells supplied with glycerol were strongly reminiscent of those of phosphate starvation (Fig. 11). The differential induced rate of β-galactosidase synthesis increased from 1480 units/ml. culture/extinction in the presence of adenine to 6500 units/ml. culture/extinction when it limited growth. Growth itself was strongly inhibited (Table 1). On restoring the supply of adenine, growth was resumed, and after a period of intense repression lasting 45 min. the rate of β-galactosidase formation recovered to a value of 2210 units/ml. culture/extinction (Fig. 11). Respiration was even more strongly inhibited by adenine starvation than by phosphate deficiency (Table 1), but also recovered immediately when adenine was supplied.

The inhibition of β-galactosidase synthesis by adding adenine to adenine-starved cells appeared to be due to catabolite repression, since alkaline phosphatase synthesis showed a constant differential rate through

the whole course of adenine starvation and restoration (Fig. 12). In

[INSERT FIG. 12 NEAR HERE]

this experiment repression of β-galactosidase synthesis lasted about 25 min. McFall & Magasanik (1960) have shown that alkaline phosphatase is not subject to catabolite repression by carbon compounds.

Strain JC 14 growing with all the required nutrients showed a typical repression transient when glucose was added to induced cells. When starved of adenine these cells were strongly and permanently (more than 150 min.) repressed by glucose (Fig. 13).

[INSERT FIG. 13 NEAR HERE]

(iii) Thymine. Cohen & Barner (1955) as well as McFall & Magasanik (1962) have shown that enzyme induction can take place in thymine-deficient cells. The latter authors found that after prolonged thymine deficiency catabolite repression developed following a fall in the rate of protein synthesis.

The recovery from glucose repression in thy⁻ cells has now been studied in cultures with and without thymine. A strain of E. coli with requirements for both thymine and methionine was used. The cells were grown on glycerol minimal medium supplemented with both essential nutrients. β-Galactosidase was induced in part of the culture with IPTG, and in two parallel flasks glucose was added either with inducer, or 20 min. later (Fig. 14). In the first case enzyme synthesis was delayed (cf. Fig. 3B), a constant differential rate of synthesis being

[INSERT FIG. 14 NEAR HERE]

reached some 40 min. later than in the parallel flask without glucose. In the second case, a typical transient repression, lasting 25 min., of β-galactosidase synthesis was observed.

The rest of the cells were centrifuged, washed twice and resuspended for 2 hr. in the same medium without methionine. This treatment permitted the completion of replication of DNA already in progress, but prevented initiation of further replication (Maaløe & Hanawalt, 1961). The cells were washed again, resuspended in medium containing methionine but now devoid of thymine, and were distributed between two flasks. After 5 min. of incubation, IPTG with or without glucose (10^{-2} M) was added, and growth (by extinction), total protein and β-galactosidase activity were followed in each case. No growth occurred for 20 min. in the absence of thymine. It then began slowly, becoming exponential by about 45 min. and remaining so for over 60 min. when the experiment was terminated. However, the doubling time was 97 min. compared with 74 min. in medium with thymine and methionine. Glucose did not increase the growth rate. Total protein was always directly proportional to extinction. β-Galactosidase synthesis in the absence of glucose started 35 min. after IPTG was added, but the differential rate of synthesis did not become constant for a further 35 min. In the presence of glucose enzyme synthesis started later, 45 min. after the addition of IPTG, and the differential rate became approximately constant 60 min. later still (Fig. 14).

Both before and during thymine starvation glucose repressed the final constant differential rate of enzyme synthesis by 10-20%. It should be noted that during thymine starvation β-galactosidase in the absence of glucose was synthesized about 1.6 times more rapidly than when thymine was present.

Catabolite repression and the rate of synthesis of RNA. The rate of synthesis of RNA has been investigated in a number of physiological

situations leading to catabolite repression. Sypherd & Strauss (1963) have already shown an inverse relationship between RNA synthesis and repression during partial inhibition of protein synthesis by chloramphenicol.

Simultaneous pulse-labelling for 2 min. at 37° with a mixture of [^{3}H]uracil and L-[^{14}C]phenylalanine (or L[$^{14}CH_3$]methionine) has been used to measure the differential rate of RNA synthesis--i.e. the rate of RNA synthesis versus the rate of protein synthesis. We have invariably found experimentally that the rate of incorporation of [^{14}C]phenylalanine into trichloracetic acid-insoluble material is directly proportional to the extinction of the bacterial culture, so that the incorporation of [^{3}H]uracil versus extinction of the culture may be used as a measure of the differential rate of RNA synthesis. The validity of pulse-labelling as a measure of RNA synthesis will be discussed later.

RNA synthesis has been measured in adenine-requiring cells during adenine starvation, and following the restoration of adenine to starved cells (Fig. 15), in an experiment similar to those reported in Figs. 11 and 13. During adenine starvation the rate of synthesis was very low,

[INSERT FIG. 15 NEAR HERE]

consistent with a deficiency of adenine for ATP which is required both for energy and as a precursor of RNA. On addition of adenine, the differential rate of uracil incorporation increased some 18-fold within 2 min. and declined thereafter, reaching an approximately steady-state level about 35 min. later; this steady-state rate was about 9 times greater than the rate during adenine starvation. Measurement of the differential rate of β-galactosidase synthesis in the same experiment

showed that repression was greatest during the period of maximum RNA synthesis, and enzyme synthesis recovered to a new constant rate at almost exactly the time that RNA synthesis had declined to a steady-state level. In this experiment the steady differential rate of β-galactosidase synthesis after adenine addition was 25% of the rate during adenine starvation. We conclude that the rearrangement of metabolism following adenine addition which resulted in enhanced RNA synthesis was also responsible for repression of enzyme formation. The addition of glucose to adenine-starved cells (Fig. 15) totally repressed β-galactosidase synthesis (cf. Fig. 13) but had no effect on RNA synthesis.

It was of interest to study also the behaviour of RNA synthesis during the typical transient repression of β-galactosidase synthesis in exponentially growing cells caused by glucose. When this was attempted, however, a new phenomenon was observed which must first be described. Although exponential growth on an extinction basis (and hence on a total protein basis) was observed to continue uninterruptedly in the culture for more than four generations, the differential rate of RNA synthesis rose some 30% from the beginning of the experiment to a peak value two generations later; in the next two generations it fell by 97% from the maximum value (Fig. 16). The effects of glucose on the rate of RNA

[INSERT FIG. 16 NEAR HERE]

synthesis must therefore be considered superimposed on this rising and falling pattern normally occurring in the absence of glucose.

RNA synthesis during the glucose transient in four strains of E. coli showing transients of different durations was examined in exponentially growing cells (Figs. 17-20). Addition of glucose to each

strain growing on glycerol increased the growth rate (cell divisions/hr.) by 20-30%. The differential rate of RNA synthesis showed an immediate upward response, later declining towards the control values. There was a definite correlation between the transient effects of glucose on RNA synthesis and on β-galactosidase synthesis. With strains JC 14 (Fig. 17)

[INSERT FIG. 17 NEAR HERE]

and C 600-1 (Fig. 18), β-galactosidase recovery began when the rate of

[INSERT FIG. 18 NEAR HERE]

RNA synthesis was well past its peak. Strain ML 30 represents a special case since the glucose repression transient was very short (4-5 min.) and this strain was obviously much less sensitive than the other three to glucose repression. Genetically the ML series of strains is different from the other three, which are all derivatives of K-12. Nevertheless it can be seen from Fig. 19 that the peak of RNA synthesis was reached in a

[INSERT FIG. 19 NEAR HERE]

short time (not more than 5 min.) and the rate declined rapidly thereafter. In strain AB 1105, one showing a prolonged glucose transient for enzyme synthesis, with relatively poor recovery, it should be noted (Fig. 20)

[INSERT FIG. 20 NEAR HERE]

that the differential rate of RNA synthesis never declined to the control value. The rate of β-galactosidase synthesis recovered to its greatest extent at just about the time that RNA synthesis approached the control level most closely. The relatively high continuing rate of RNA synthesis might be related to the low rate of β-galactosidase synthesis attained following the period of repression.

Occasionally strain ML 30, growing on glucose-free maltose, showed

no increase in growth rate upon the addition of glucose. In such cases there was also neither transient repression of ß-galactosidase synthesis nor transient stimulation of RNA synthesis.

Validity of pulse-labelling with [^{3}H]uracil as a measure of the rate of RNA synthesis. Pulse-labelling of bacterial cultures with labelled uracil has been widely used as a measure of the rate of RNA synthesis. From the paper of Levinthal, Keynan & Higa (1962) it can be estimated that pulsing for 2 min. with uracil results in 50-60% of the radioactivity being incorporated into an unstable RNA fraction, the remainder residing in more stable species. The unstable fraction has often been equated with messenger RNA (Levinthal et al., 1962), but it must be recognized that while all unstable RNA may have a messenger function, not all messenger RNA is necessarily unstable (Moses & Calvin, 1965a, b; Yudkin, 1965).

In the present studies we have observed unequivocal changes in the specific rates of [^{3}H]uracil incorporation both as a result of continued growth, and following glucose addition. Other interpretations, apart from changes in the rate of RNA synthesis, may be evoked to explain these findings. The organisms used were not uracil-requiring, and we have assumed that when supplied with external uracil they have used this in preference to endogenous uracil. McCarthy & Britten (1962) have obtained evidence in support of this assumption, but they did not extend their observations to include cultures at different stages of development. Thus, a possible explanation of the changes in uracil incorporation might be related to an alteration of the balance of the use of external versus endogenous uracil to make RNA. This has been examined by studying the uptake of [^{3}H]uracil in a uracil-requiring mutant, in

which only external uracil was available to the cell. Such an experiment is reported in Fig. 21.

[INSERT FIG. 21 NEAR HERE]

Two parallel cultures of U^- cells were grown under identical conditions in medium containing unlabelled uracil (50 µg./ml.). To one culture a small quantity of high specific activity [^{3}H]uracil was added. By periodic measurements of residual radioactivity in the supernatant medium of the flask containing [^{3}H]uracil the overall rate of utilization of uracil was determined; this was found to be directly proportional to growth as measured by extinction. Hence, when samples of the second culture were pulse-labelled with [^{3}H]uracil, the specific activity of the labelled uracil at each stage could be calculated since the quantity of unlabelled uracil remaining in the medium was known. It was found that, like the U^+ strains, the uracil-requiring cells showed a declining differential rate of uracil incorporation into RNA as the culture became older; exponential growth was maintained through the whole course of this experiment. Glucose temporarily stimulated incorporation.

The use of uracil-requiring cells, however, poses a new difficulty. Although, as shown by McCarthy & Britten (1962), U^+ cells incorporate external uracil directly into RNA without equilibration with the internal uracil pool, this may not be the case with U^- cells. In the latter the declining rate of external [^{3}H]uracil incorporation into RNA might have been the result of an increasing internal pool with which external uracil equilibrated, thus effectively lowering the specific activity of the labelled precursor. This aspect of the problem has been examined by measuring the effective intracellular pool of uracil by the method

described by McCarthy & Britten (1962).

The method essentially is to study the kinetics of uracil incorporation into RNA as a function of time. If no equilibration with a pre-existing pool is necessary, uracil incorporation would be expected to be essentially linear (for short exposures to the labelled substrate), with the slope of incorporated uracil passing through the origin. Equilibration with an unlabelled pool would result in an increasing rate of uracil incorporation which would finally become maximal when the specific radioactivity of the pool was in equilibrium with that of the incoming precursor. Since the U^- mutant depended for growth on uracil in the medium only a very small proportion of the total external uracil was incorporated into RNA in the course of a few sec., and the problems of removing the enormous excess of label in order to count the incorporated ^3H proved insuperable at very low cell concentrations. It was thus not possible to study uracil pool sizes over the whole range of growth shown in Fig. 21. Satisfactory results were obtained, however, by the middle of the growth period, when the extinction had already reached values of 0.27 and more. Kinetic curves obtained at extinction values of 0.27 and 0.51, by which stage the differential rate of RNA synthesis had fallen nearly to a minimum value (Fig. 21), failed to reveal any pool equilibration (Fig. 22). A change in the size of an equili-

[INSERT FIG. 22 NEAR HERE]

brating unlabelled pool may therefore be excluded as an explanation for the results of Fig. 21, and we may conclude with confidence that in both U^+ and U^- cells the fall in the differential rate of uracil incorporation reflected a real fall in the differential rate of RNA synthesis.

Chemical determinations demonstrated that the total RNA of the cells was directly proportional to extinction and to total protein throughout the whole course of exponential growth, for extinction values at least as high as 1.9 (Fig. 23). The significance of these findings in relation to RNA turnover will be discussed below.

[INSERT FIG. 23 NEAR HERE]

Effect of glucose in the absence of a source of nitrogen. Both Clark & Marr (1964) and Nakada & Magasanik (1964) have shown that catabolite repression is very severe in cells supplied with carbohydrate but devoid of a source of nitrogen. In view of our observations of the comparatively great resistance of strains ML 3 and ML 30 to repression by glucose in growing cells, it was of interest to investigate the behaviour of these strains in a condition of nitrogen starvation.

Exponentially growing cells were harvested, washed and resuspended in 0.1 M-phosphate buffer, pH 7.2, containing 0.125 M-NaCl. After 20 min. IPTG was added, followed 30 min. later by glucose to a concentration of 10^{-2} M. Measurement of β-galactosidase activity (Fig. 24) showed that

[INSERT FIG. 24 NEAR HERE]

the addition of glucose resulted in an increase in the rate of enzyme synthesis. A slight increase in optical density was also observed following glucose addition.

Catabolite repression by glucose of other enzymes. Glucose repression of β-galactosidase synthesis in ML 30 was compared with effects on L-tryptophanase and D-serine deaminase (Fig. 25). Both these other enzymes showed spontaneous recovery from glucose repression, though the repression

[INSERT FIG. 25 NEAR HERE]

lasted longer than with β-galactosidase. The recovery times for the

three enzymes were 4 min., 18 min. and 54 min. for β-galactosidase, serine deaminase and tryptophanase, respectively.

DISCUSSION

The nature of catabolite repression by glucose. Although catabolite repression of the lactose enzymes in *E. coli* has been extensively studied for many years a great deal of the mechanism by which the repression is exerted remains unclear. Nakada & Magasanik (1964) have published very convincing evidence that the repression prevents the transcription of β-galactosidase messenger RNA, rather than the translation of that messenger molecule into protein. With two other aspects of catabolite repression we are on much less certain ground: the identity of the catabolite corepressor itself, and the regulatory site at which it acts.

The realization that catabolite repression is to a large extent a transient phenomenon, with spontaneous recovery taking place even in the continued presence of the carbohydrate giving rise to the catabolite corepressor, has enabled a fresh approach to be made in studying this question. A transient repression implies a temporary metabolic rearrangement when the cells are provided with a rich carbon source following a poor one, and evidence of several consequences of the rearrangement, and the establishment of a new steady state of metabolism, is provided in this communication. In view of our observation that spontaneous recovery occurs when galactose is the repressing sugar we would need to contest the view of McFall & Mandelstam (1963) that a derivative of this sugar is the corepressor.

The existence of the transient in cells with non-functional regulator and permease genes (i.e. i^- and y^-) suggests at first sight that neither of these genes is involved with catabolite repression. Indeed, Loomis &

Magasanik (1964) claim to have excluded entirely the regulator gene as the place where the corepressor exerts its action. In fact, however, their results are less conclusive than that. They showed in conjugation experiments that catabolite repression was not dependent on the regulator gene product being present in sufficiently high concentration to confer inducibility, but they did not ensure the total absence of this product. We thus cannot exclude the possibility that catabolite repression is powerful enough to activate repression by the regulator gene product even when the latter is present in too low a concentration to be effective by itself. Loomis & Magasanik have already been justly criticized along these lines by Clarke & Kramar (1964). The present observations have similarly shown that the transient repression is not directly connected with the normal functional ability of the regulator gene product to confer inducibility, but the i gene product may yet have a role in catabolite repression in this connection.

Whatever the site of corepressor action, it should be noted that competition between the inducer and corepressor, described by Clark & Marr (1964), has been confirmed in the present studies. Partial recovery from glucose repression was manifest when IPTG was the inducer, but not with lactose. This supports the contention that following the usual period of intense repression when glucose is added, which may be ascribed to a high concentration of corepressor, recovery follows when the corepressor concentration falls to a new steady-state value after the metabolic rearrangements are complete. The new corepressor concentration may be too low to antagonize completely such a powerful inducer as IPTG, and yet be quite sufficient to counteract the weaker inducing properties of lactose.

Consideration of many of the results we have obtained, particularly those resulting from phosphate and adenine starvation in strain JC 14, suggests that the catabolite corepressor may not be derived from the carbon skeleton of glucose or other external repressing agent. The repression may depend, rather, on changes in the energy status of the cells resulting from the sudden onset of rapid metabolism. Mandelstam (1961) showed that repression by some substances was relieved by low concentrations of dinitrophenol, an uncoupler of oxidative phosphorylation. Those repressions which were relieved were all exerted by substances which are metabolized aerobically only; glucose and fructose, which can also be fermented, continued to repress in the presence of dinitrophenol. This may indicate that the catabolite corepressor is closely dependent on ATP, since aerobically-formed ATP is sensitive to inhibition by dinitrophenol, while ATP formed at the substrate level, as in fermentation, is not. In some organisms it has been shown that metabolic inhibition with uncouplers has very little effect on the pool sizes of most intermediary compounds produced from glucose. In baker's yeast, for instance, the greatest reductions of pools by dinitrophenol were of those substances requiring ATP for their synthesis: uridine-diphosphoglucose, other nucleotides and derivatives, and trehalose, the common disaccharide in yeast. By contrast, the pool sizes of sugar phosphates and of organic and amino acids were hardly affected (Moses & Smith, 1960). In another instance, the effect of azide on the metabolism of a filamentous fungus, a similar situation was found. In the latter case the disaccharide was maltose, and was similarly reduced in amount (Moses, 1959). Thus changes under the influence of uncouplers in the

concentrations of common catabolic intermediates derived from the carbon skeleton of glucose metabolism cannot explain the relief of catabolite repression by dinitrophenol described by Mandelstam (1961).

Catabolite repression may in some cases be relieved by anaerobiosis (Cohn & Horibata, 1959; Dobrogosz, 1965). This again suggests that the corepressor is dependent on ATP, since the availability of ATP in the cell might be expected to fall considerably during anaerobiosis. These earlier observations fit in with the results reported in the present communication, particularly those with strain JC 14: relief of repression by glycerol during adenine and phosphate starvation (possibly resulting from a fall in the supply of ATP), and intense repression when these nutrients are restored, perhaps as a result of a rapid increase in the availability of ATP.

It seems likely, therefore, that the catabolite corepressor is a substance whose concentration depends sensitively on the availability of ATP. It could conceivably be ATP itself, or a compound of the "phosphogen" type, bearing a relation to ATP analogous to that of creatine and creatine phosphate in muscle. A mechanism for its action would then be as follows: the entry of glucose or other rapidly metabolizable substance into metabolism would suddenly increase the availability of ATP. The actual concentration of ATP may not in fact rise very much before a change in concentration of the corepressor is triggered, and this might prevent a further change in ATP concentration. The rise in concentration of the corepressor would stop the overall process of messenger RNA transcription for a sensitive category of operons which would include the lactose enzymes. After a certain period, the altered concentrations of these several compounds would revert to a greater or

lesser extent towards the initial level, and a new steady state be achieved. This reversion would now permit some messenger RNA synthesis for the sensitive operons; how much RNA were made would depend on the extent of reversions and the sensitivities of the various operons.

It is well known that the introduction of luxury quantities of carbohydrates and other nutrients to cells previously maintained on limited supplies results in a complex series of transient phenomena affecting the pool sizes of metabolic intermediates of carbohydrate metabolism as well as those of ATP and ADP (Calvin & Massini, 1952; Wilson & Calvin, 1955; Lonberg-Holm, 1962). These transients eventually disappear, resulting in a new steady-state concentration of metabolites. An explanation based on such transients of ATP or functionally related substances might be invoked to explain the recovery from catabolite repression which we have observed to occur spontaneously in every strain of E. coli we have examined.

The temporary increase in the differential rate of RNA synthesis which follows the introduction of glucose is unlikely to be directly responsible for the inhibition of induced enzyme synthesis, even though the two processes are of about the same duration. Rather we might suppose that both are direct consequences of the metabolic readjustments. A programme of investigation is currently being carried out in this laboratory to study directly the pool sizes of phosphorylated and other intermediates during transient catabolite repression, with the aim of correlating the kinetic behaviour of one or more of these compounds with that shown by inducible enzyme synthesis.

It is not clear at this juncture how closely related is repression by glucose of the three catabolic enzymes studied in this communication. The same metabolic product may or may not be responsible for repression for each of the enzymes; we also do not know whether there is a different regulatory site for each enzyme at which the corepressor operates. The three enzymes studied recover from repression at different times and to different extents. These observations may be tentatively explained in the following way. If the corepressors for each enzyme are identical, or at least metabolically closely related, while the sensitivity of the regulatory site differs in each case, we may have a situation like that illustrated in Fig. 26. Addition of glucose results in a rapid increase

[INSERT FIG. 26 NEAR HERE]

in corepressor concentration, which slowly falls to a new steady-state level somewhat higher than that before glucose was introduced. The sensitivity to corepressor concentration would be greatest for L-tryptophanase, intermediate for D-serine deaminase and least for β-galactosidase. The rapid rise in corepressor concentration would repress the synthesis of each enzyme almost simultaneously, while the slow fall in concentration would permit relief of repression first for β-galactosidase, next for D-serine deaminase and last for L-tryptophanase (cf. Fig. 25). The extent of the fall in corepressor concentration would determine the degree of recovery of each enzyme. Since the corepressor concentration would be related to the magnitude of the metabolic disturbance, which in turn would be reflected in the increase in the differential rate of RNA synthesis, we might note that in strains where RNA synthesis returned most closely to its original rate, enzyme synthesis recovered to the greatest extent

(Figs. 17 and 19). In strain AB 1105 the extent of recovery of enzyme synthesis was least and the rate of RNA synthesis remained significantly elevated for a prolonged period (Fig. 20).

A number of examples have been reported in the literature of resistance to catabolite repression, at least as exerted by glucose. Some authors have failed to recognize a difference between resistance to catabolite repression generally and resistance to repression exerted by glucose specifically. This point has been fully discussed by Neidhardt (1960). In a recent paper by Loomis & Magasanik (1965) the authors claim to have isolated a mutant of E. coli specifically resistant to catabolite repression of β-galactosidase. Close examination of their results, however, reveals that in their mutant resistance was not restricted to β-galactosidase, although it was most pronounced for this enzyme: galactokinase also showed increased resistance. Furthermore, while β-galactosidase was resistant to repression by glucose and glycerol, considerable sensitivity still remained with respect to gluconate and lactate. An alternative explanation of their observations is available in the mechanism shown in Fig. 26: an altered glucose metabolism (e.g. affecting the formation of gluconic acid: cf. Neidhardt, 1960) might fail to increase the effective concentration of corepressor sufficiently to cause any inhibition of β-galactosidase synthesis. There may nevertheless be sufficient corepressor to cause some repression of galactokinase synthesis, and total repression of the syntheses of maltase and L-tryptophanase, two other enzymes studied by Loomis & Magasanik (1965).

RNA synthesis as a function of cell population density. The fall in the differential rate of RNA synthesis as a function of increasing

cell density appears to be a real phenomenon. Explanations based on changes in the rates of utilization of external versus endogenous uracil have been excluded. We might consider three possible causes for this effect: the exhaustion of a nutritional factor in the medium, the excretion of an inhibitory factor into the medium, or the results of increased frequency of collision between cells as the population density increased. These have not yet been studied in detail, but it is worth noting that in spite of rapid stirring of the cultures, no dissolved oxygen was measureable in the culture with an oxygen electrode once the extinction exceeded about 0.5. Partial anaerobiosis might therefore have been a factor, although exponential growth was maintained. As far as cell density is concerned, Rao & Bhargava (1965) have found with resting cells of E. coli that the specific rates of uptake from the medium of arginine and glucose decreased with increasing bacterial concentration.

From the data of the experiments reported in Figs. 21 and 23 it is possible to calculate the rate of RNA synthesis compared with the RNA pre-existing in the population. We have found the total RNA-ribose in E. coli to be 17 µg./ml. at an extinction of 1.0. Using the averages of the base ratios for E. coli RNA published by Elson & Chargaff (1955) and Belozersky & Spirin (1958), in which uracil constitutes 20% of the total bases on a molar ratio, the uridine-ribose content would be 3.4 µg./ml., corresponding to 2.5 µg./ml. of uracil. (The total uracil content of the cells measured by the uptake of [^{3}H]uracil from the medium by U^{-} cells was 9.3 µg./ml. at an extinction of 1.0; thus 27% of the total uracil was precipitable by trichloracetic acid.) The cells were growing

exponentially with a doubling time of 70 min. Thus in 2 min. the cells increased in mass by 2%. Since the total RNA content per cell remained constant during exponential growth (Fig. 23), the amount of [^{3}H]uracil incorporated into RNA during a 2 min. period needed to be at least 2% of the pre-existing RNA-uracil, simply to account for the net RNA synthesis during this time. The data presented in Table 2 show that

[INSERT TABLE 2 NEAR HERE]

during the early stages of growth the [^{3}H]uracil incorporated in 2 min. was actually about 12% of the pre-existing RNA-uracil, while at extinctions of greater than 0.75 this had fallen to 2.5%.

From these values we might conclude that in dilute cultures over 80% of the uracil incorporated in 2 min. was located in a labile form of RNA which did not contribute permanently to the total RNA. Towards the end of the growth period only 20% of the incorporated uracil was found in unstable RNA.

Evidence has accumulated that messenger RNA, usually considered to be the most important component of labile RNA, is not necessarily all unstable (Moses & Calvin, 1965b). It has been suggested that in bacteria unstable messenger RNA is associated particularly with operons under the repressible control of regulator genes, while more stable messenger functions may be characteristic of those structural genes which are not regulated in this way (Moses & Calvin, 1965a; Yudkin, 1965). A consequence of the change of RNA stability with growth may be that the induction or derepression of some enzymes dependent on labile messenger RNA may become progressively more difficult as the population density increases during exponential growth. Preliminary results from this laboratory have suggested

that this is indeed the case with L-tryptophanase, and phenomena of this sort have also been observed with various enzymes in several species of bacteria by Pinsky & Stokes (1952) and by Nurmikko, Soini, Terho & Kantonen (1964).

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Running title: "Catabolite Repression in Escherichia coli"

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CAPTIONS FOR FIGURES

Fig. 1. Transient glucose repression of β-galactosidase synthesis in three strains of E. coli. In each case IPTG (5×10^{-4} M) was added (↓) followed 15 or 20 min. later by glucose (10^{-2} M) (↑). Differential rate of β-galactosidase plotted. Mass doubling times before and after glucose addition, and length of transient repression, respectively, for each strain as follows (min.): A, C 600-1: 104, 81, 26; B, AB 1105: 77, 57, >45; C, ML 3: 67, 52, 4.

Fig. 2. Kinetics of transient repression of β-galactosidase synthesis and utilization of glucose in exponentially-growing cells of strain C 600-1. IPTG (5×10^{-4} M) added at 0 time, glucose (10^{-2} M) added 16 min. later. Transient repression started at first arrow when glucose was added; maximum degree of recovery was completed at second arrow after 55 min. A, β-galactosidase activity; B, concn. of glucose in the medium.

Fig. 3. Glucose (10^{-2} M) and IPTG (5×10^{-4} M) added at various times to Cavalli strain. A, glucose added 40 min. before IPTG (arrow shows IPTG addition); B, glucose and IPTG added simultaneously at arrow; C, IPTG added at ↓, glucose added 20 min. later at ↑.

Fig. 4. Transient glucose repression of β-galactosidase synthesis in succinate- and maltose-grown cells of ML 3. IPTG added at ↓; glucose added at ↑. A, succinate-grown cells; B, maltose-grown cells.

Fig. 5. Abolition of diauxie. Cavalli strain cells grown under following conditions: A, glucose + lactose + IPTG; B, glucose + lactose; C, glucose alone.

Fig. 6. Effect of non-metabolizable and metabolizable substances on β-galactosidase synthesis in ML 3. Exponentially growing cells supplied with IPTG (5×10^{-4} M) at time 0. Following additions made 15 min. later: A, phenyl-thio-β-D-galactoside (10^{-3} M); B, 2-deoxy-glucose (10^{-3} M); C, glucose (10^{-3} M).

Fig. 7. Effect of mutation at the regulator gene on transient repression. Glucose added to exponentially growing cultures of strains 230 U (i^-) or 300 U (i^+), induced with IPTG, as shown by arrows. A, 230 U; B, 300 U.

Fig. 8. Effect of mutation at the galactoside permease gene on transient repression. Glucose added to exponentially growing induced cultures of strains ML 30 (y^+) or ML 3 (y^-) as shown by arrows. A, ML 30; B, ML 3.

Fig. 9. Inorganic phosphate nutrition, and the effects of glucose on β-galactosidase synthesis in strain AB 1105. Section 1: adequate phosphate. IPTG added at ↓. A, no glucose; B, with glucose. Section 2: during phosphate starvation. IPTG added at ↓. A, no glucose; B, with glucose. Section 3: addition of phosphate (↑) to phosphate-starved cells 20 min. after IPTG added (↓). A, no glucose; B, with glucose. Section 4: IPTG (↓) added to phosphate-starved cells 20 min. after restoration of inorganic phosphate. A, no glucose; B, with glucose.

Fig. 10. Utilization of glucose during phosphate starvation in AB 1105. Glucose added at ↓; inorganic phosphate added at ↑. A, glucose concn. in medium; B, growth by extinction.

Fig. 11. Synthesis of β-galactosidase in JC 14 before, during and after starvation for adenine or phosphate. Section 1: limiting adenine; section 2: limiting phosphate. A, before exhaustion of limiting nutrient; IPTG added at ↓; B, during exhaustion of limiting nutrient; IPTG added at ↓; limiting nutrient replaced 30 min. later at ↑.

Fig. 12. Synthesis of β-galactosidase and alkaline phosphatase during and after adenine starvation in JC 14. IPTG added at ↓; adenine added at ↑. A, β-galactosidase; B, alkaline phosphatase.

Fig. 13. Glucose repression of β-galactosidase synthesis as a function of adenine nutrition in JC 14. IPTG added at ↓; glucose added at ↑. A, with adequate adenine (transient repression lasted 24 min.); B, during adenine starvation (repression lasted more than 144 min.).

Fig. 14. Glucose repression of β-galactosidase synthesis as a function of thymine nutrition in Cavalli strain. Section 1: adequate thymine; section 2: during thymine starvation. A, IPTG added at ↓, glucose added at ↑; B, IPTG and glucose added at ↓; C, IPTG added at ↓; D, IPTG and glucose added at ↓.

Fig. 15. Differential rates of uracil incorporation into RNA and of β-galactosidase synthesis in adenine-starved cells of JC 14, before and after addition of adenine or glucose. Section 1: addition of IPTG (↓) and adenine (↑); section 2: addition of IPTG (↓) or glucose (↑). A, [^{3}H]uracil/[^{14}CH$_3$]methionine incorporated in 2 min. pulses; B, β-galactosidase.

Fig. 16. Differential rate of uracil incorporation into RNA and growth by extinction of JC 14. A, absorbance at 650 mμ; B, [^{3}H]uracil incorporated/[^{14}C]phenylalanine incorporated in 2 min. pulses.

Fig. 17. Effect of glucose on differential rates of uracil incorporation into RNA, and of β-galactosidase synthesis, in exponentially growing cells of JC 14. IPTG (↓) and glucose (↑) added as indicated. A, β-galactosidase; B, [^{3}H]uracil/[^{14}CH$_3$]methionine incorporated in 2 min. pulses.

Fig. 18. Effect of glucose on differential rates of uracil incorporation into RNA, and of β-galactosidase synthesis, in exponentially growing cells of C 600-1. IPTG (↓) and glucose (↑) added as indicated. A, β-galactosidase and glucose; B, β-galactosidase with no glucose; C, [^{3}H]uracil/[^{14}C]phenylalanine incorporated in 2 min. pulses + glucose; D, as C with no glucose added.

Fig. 19. Effect of glucose on differential rates of uracil incorporation into RNA, and of β-galactosidase synthesis, in exponentially growing cells of ML 3. IPTG (↓) and glucose (↑) added as indicated. A, β-galactosidase and glucose; B, β-galactosidase with no glucose; C, [^{3}H]uracil/[^{14}C]phenylalanine incorporated in 2 min. pulses + glucose; D, as C with no glucose added.

Fig. 20. Effect of glucose on differential rates of uracil incorporation into RNA, and of β-galactosidase synthesis, in exponentially growing cells of AB 1105. IPTG (↓) and glucose (↑) added as indicated. A, β-galactosidase with glucose; B, β-galactosidase with no glucose;

(Continued)

Fig. 20 (Continued)

C, [^{3}H]uracil/[^{14}C]phenylalanine incorporated in 2 min. pulses + glucose; D, as C with no glucose added.

Fig. 21. Effect of glucose on the differential rate of uracil incorporation into RNA as a function of exponential growth in uracil-requiring cells (CR 34). A, glucose added at arrow; B, no glucose added.

Fig. 22. Kinetics of uracil incorporation into RNA during exponential growth of a uracil-requiring strain (CR 34). Extinction of culture: A, 0.272; B, 0.513.

Fig. 23. Relation of extinction to total protein and to total RNA in C 600-1. A, total protein; B, total RNA (measured as ribose). Extinction measured in 1 cm. cuvette at 650 mμ.

Fig. 24. Effect of glucose on β-galactosidase synthesis in starved cells of ML 30. Cells starved in phosphate buffer, pH 7.2. Glucose added at arrow.

Fig. 25. Transient repression by glucose of the synthesis of three inducible enzymes in ML 30. Inducers (↓) and glucose (10^{-2} M) (↑) added as shown. A, D-serine deaminase (repression lasted 18 min.); B, β-galactosidase (repression lasted 4 min.); C, L-tryptophanase (repression lasted 60-80 min.).

Fig. 26. Proposed model for transient changes in corepressor concentration when glucose is added (↑). Corepressor concn. necessary to repress various enzymes as follows: A, lactose enzymes; B, galactose enzymes and serine deaminase; C, tryptophanase. Possible deviation from standard kinetic behaviour in mutant isolated by Loomis & Magasanik (1965) shown by dashed line (see text).

Table 1. Effect of adenine and phosphate starvation on the rates of growth, respiration and β-galactosidase synthesis in the adenine-requiring strain JC 14

Cells were grown with limiting quantities of adenine or inorganic phosphate. Samples of the cultures were removed before the essential nutrient was exhausted, during starvation, and following restoration of the nutrient. In each case IPTG was added to part of the sample and the subsequent differential rate of β-galactosidase synthesis was measured. The remainder of the sample was treated with chloramphenicol (50 μg./ml.) and the respiration measured by conventional manometric techniques. Growth was measured by the extinction at 650 mμ in a 1 cm. cuvette.

	β-Galactosidase synthesis (units/extinction)	Oxygen uptake (μl./hr./extinction)	Growth (generations/hr.)
Adenine supply:			
adequate	1480	167	0.70
exhausted	6500	39	0.10
restored	2210	193	0.79
Phosphate supply:			
adequate	1650	164	0.97
exhausted	5300	99	0.08
restored	2140	229	0.86

Table 2. Rate of RNA synthesis compared with total RNA content as a function of growth measured by extinction

Strain CR 34 (uracil-requiring). Rate of uracil incorporated into RNA in 2 min. measured at intervals and expressed as percentage of calculated pre-existing RNA-uracil. Total RNA content of culture directly proportional to extinction: 1 ml. of culture of extinction 1.0 contained 2.5 µg./ml. of RNA-uracil (Fig. 23).

Extinction	Pre-existing RNA-uracil (µg./ml.)	Uracil incorporated into RNA/2 min. (µg./ml.)	Uracil incorporated in 2 min. as % of pre-existing RNA-uracil
0.046	0.115	0.0119	10.3
0.056	0.140	0.0193	13.8
0.068	0.170	0.0167	9.8
0.091	0.228	0.0153	6.7
0.126	0.315	0.0172	5.5
0.165	0.413	0.0194	4.7
0.227	0.568	0.0225	4.0
0.301	0.753	0.0293	3.9
0.409	1.023	0.0329	3.2
0.578	1.445	0.0426	2.9
0.750	1.875	0.0447	2.4
1.028	2.570	0.0644	2.5

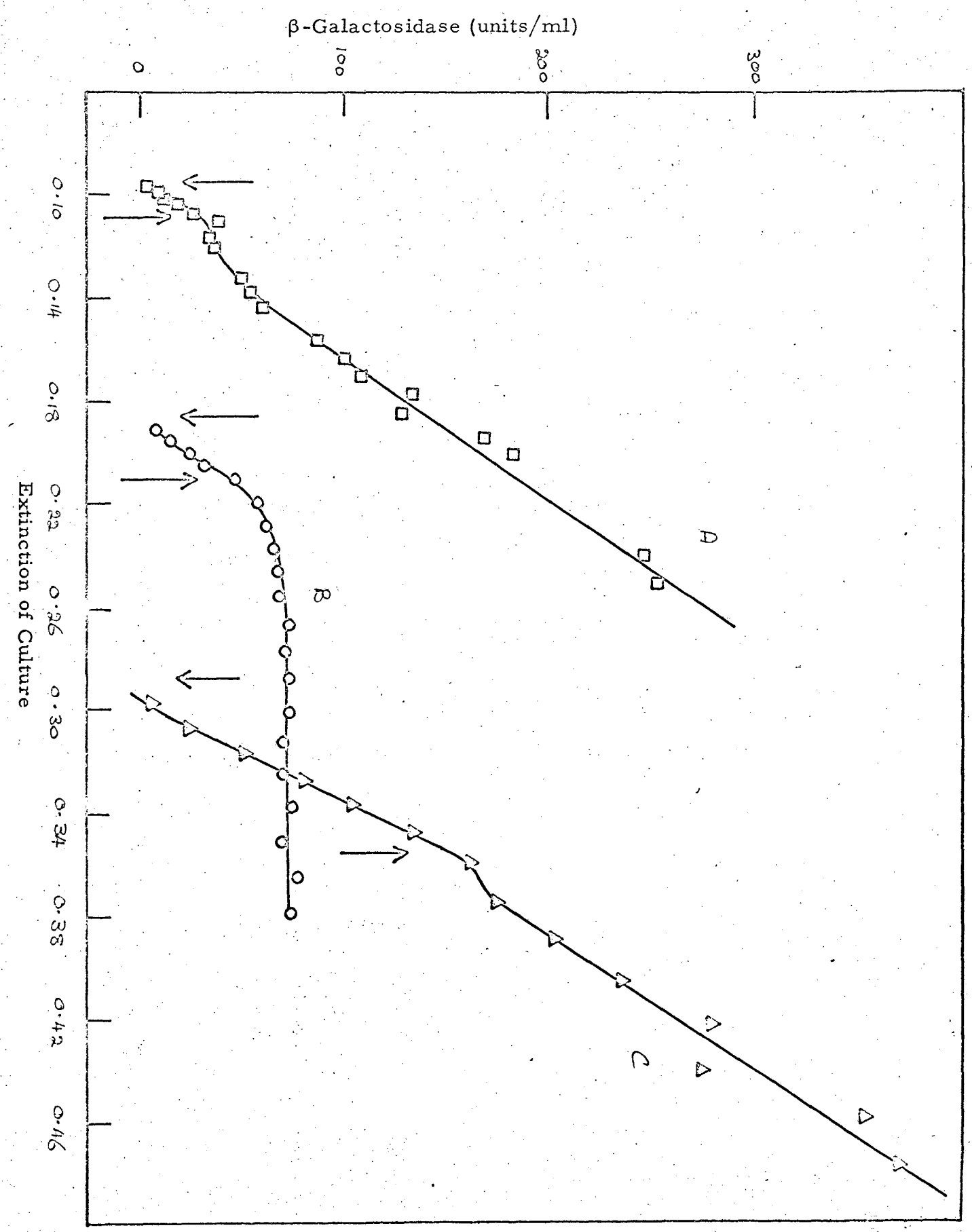
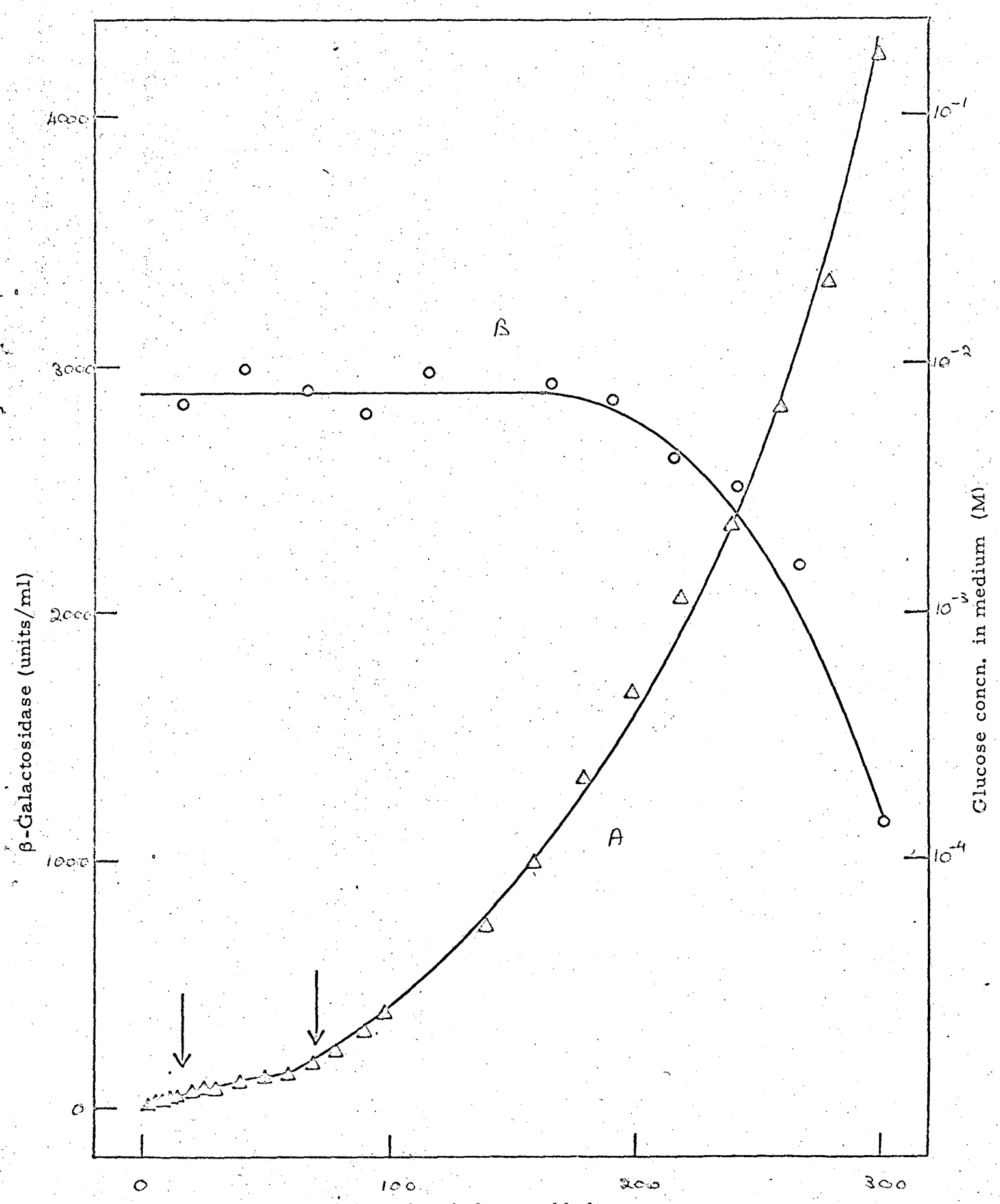

β-Galactosidase (units/ml)
0
100
200
300
0.10
0.14
0.18
0.22
0.26
0.30
0.34
0.38
0.42
0.46
Extinction of Culture
A
B
C

Fig. 1



Min. after inducer added

Fig. 2

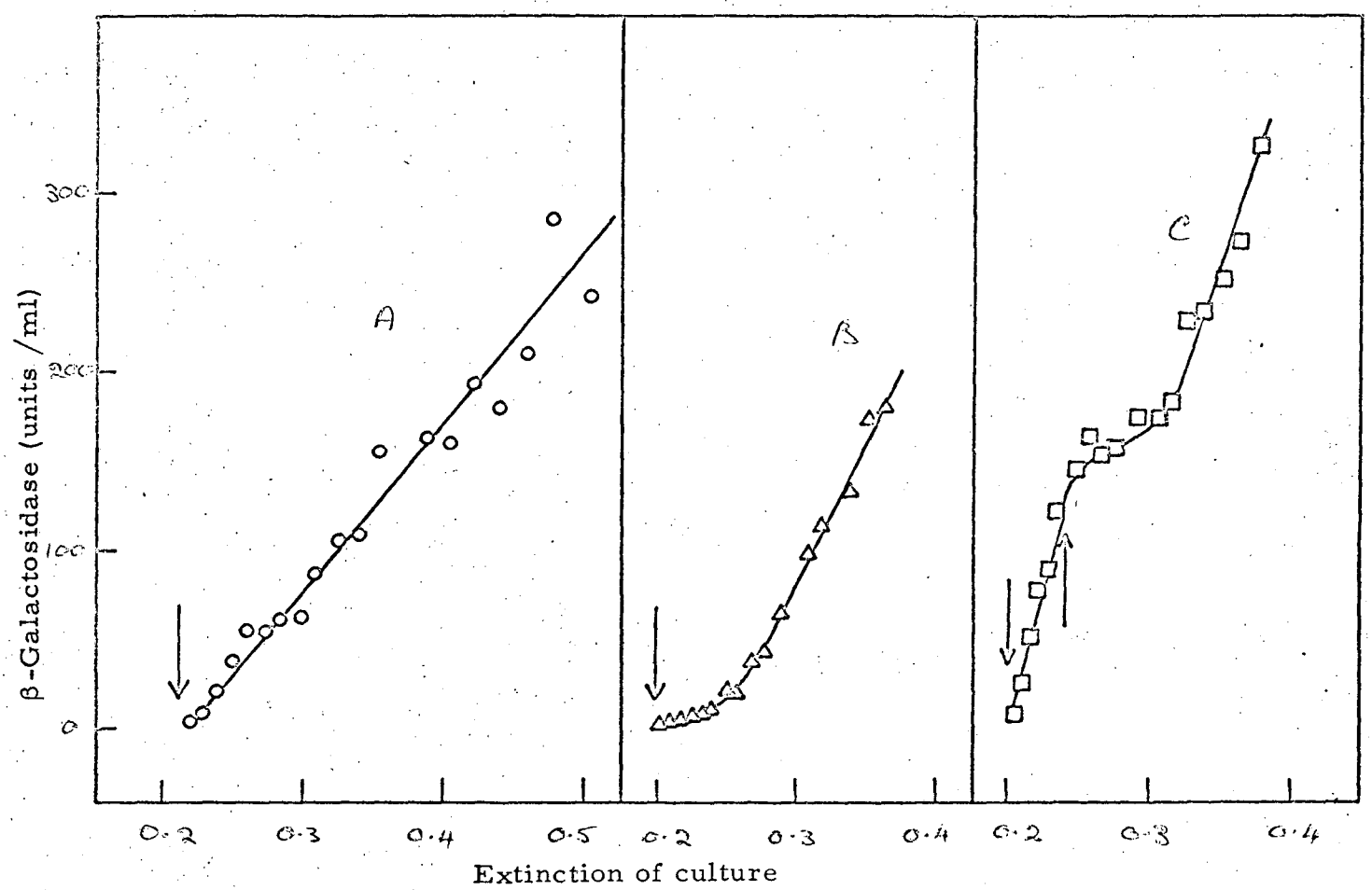
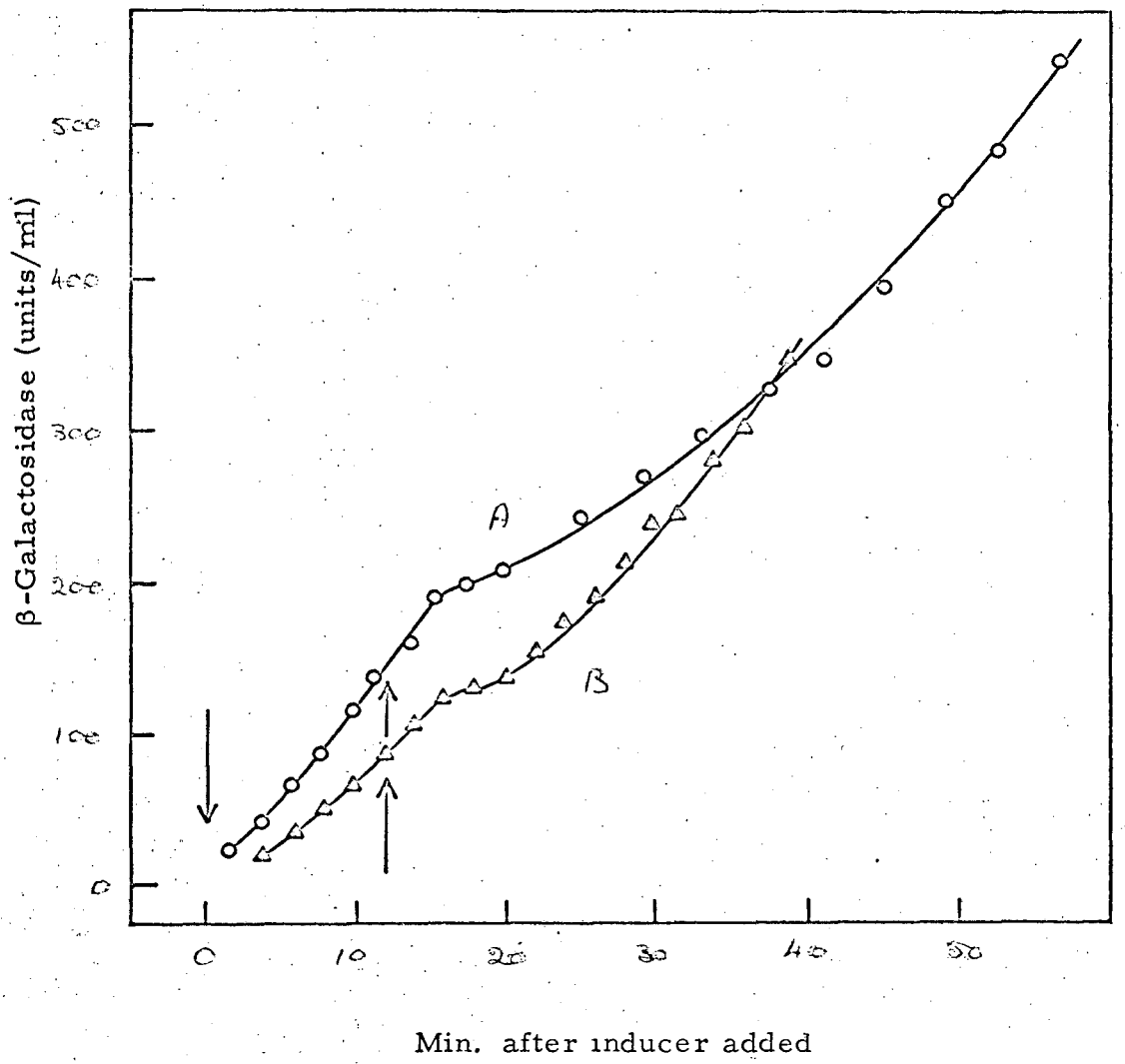

β-Galactosidase (units /ml)
300
200
100
0
A
B
C
0·2
0·3
0·4
0·5
0·2
0·3
0·4
0·2
0·3
0·4
Extinction of culture

Fig. 3


β-Galactosidase (units/ml)
500
400
300
200
100
0
A
B
0
10
20
30
40
50
Min. after inducer added

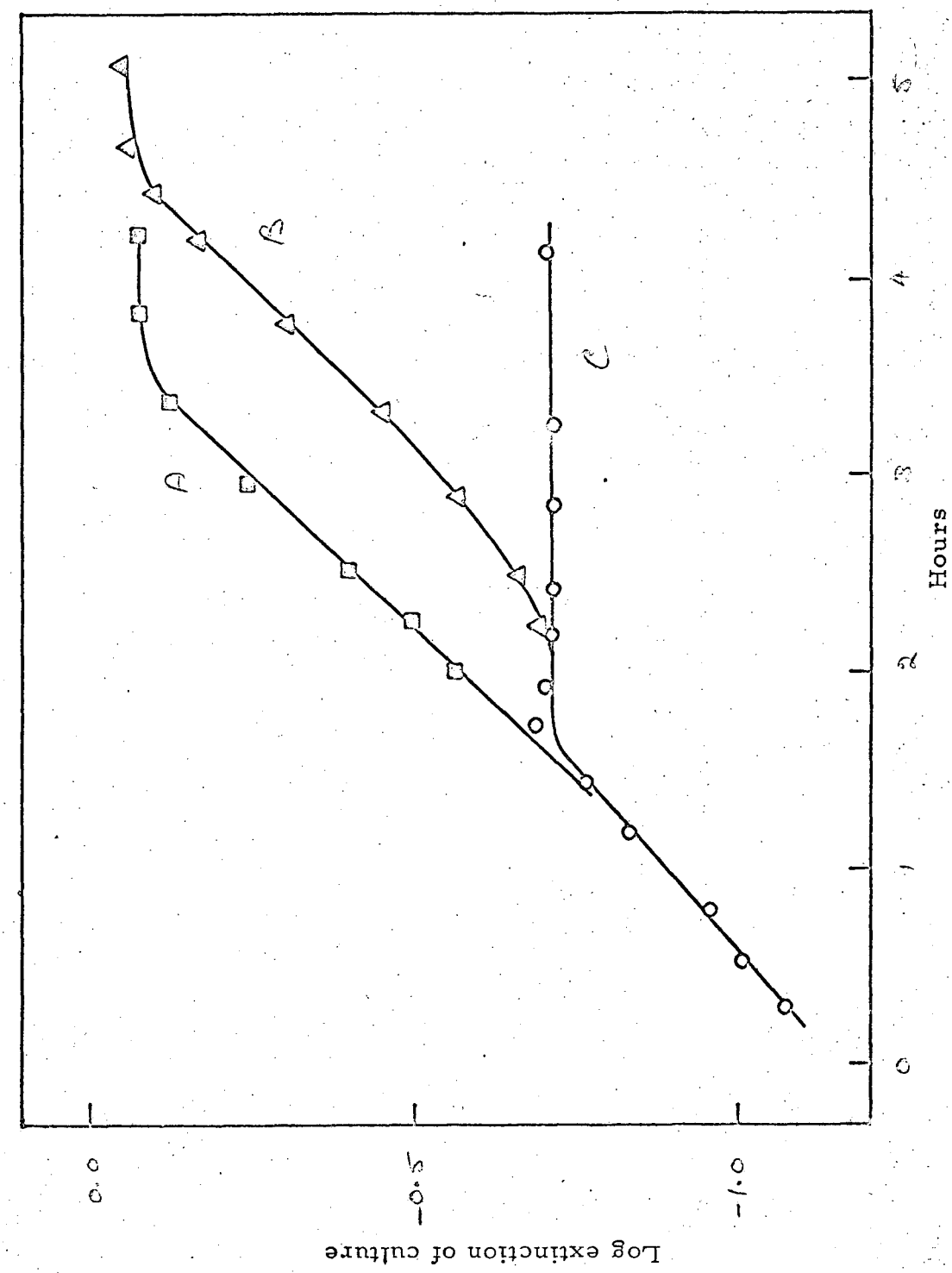


Fig. 5

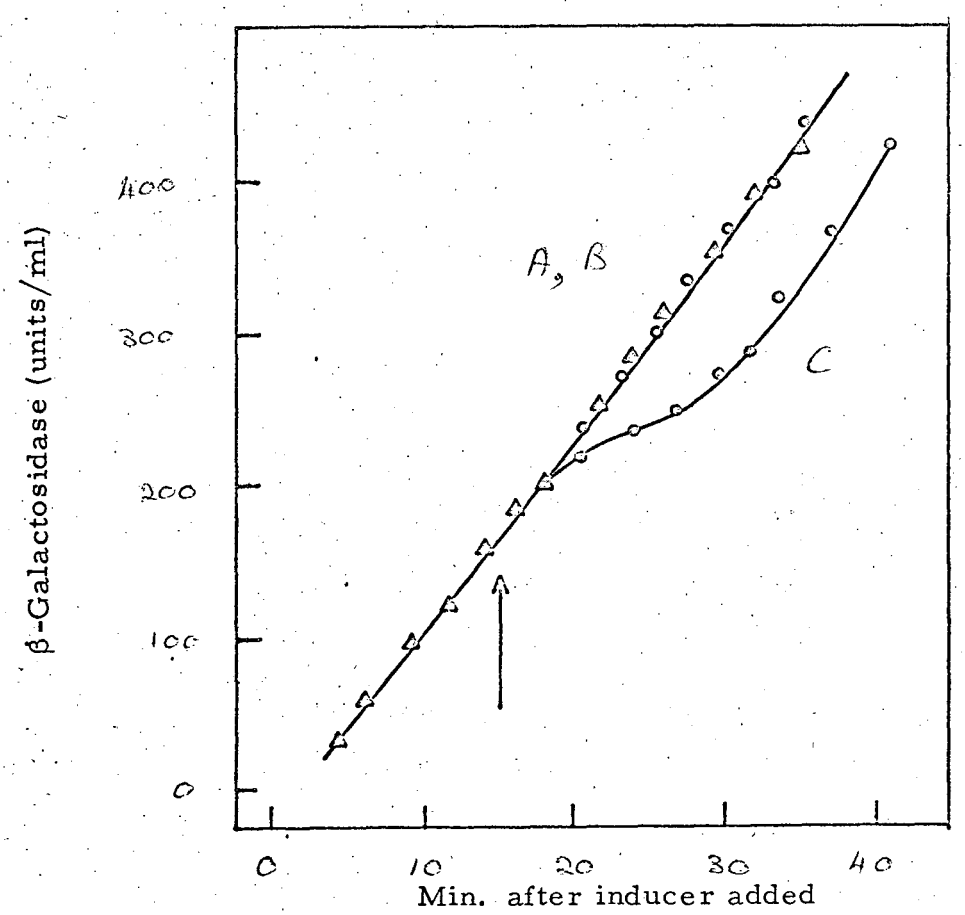

β-Galactosidase (units/ml)
400
300
200
100
0
A, B
C
0
10
20
30
40
Min. after inducer added

Fig. 6

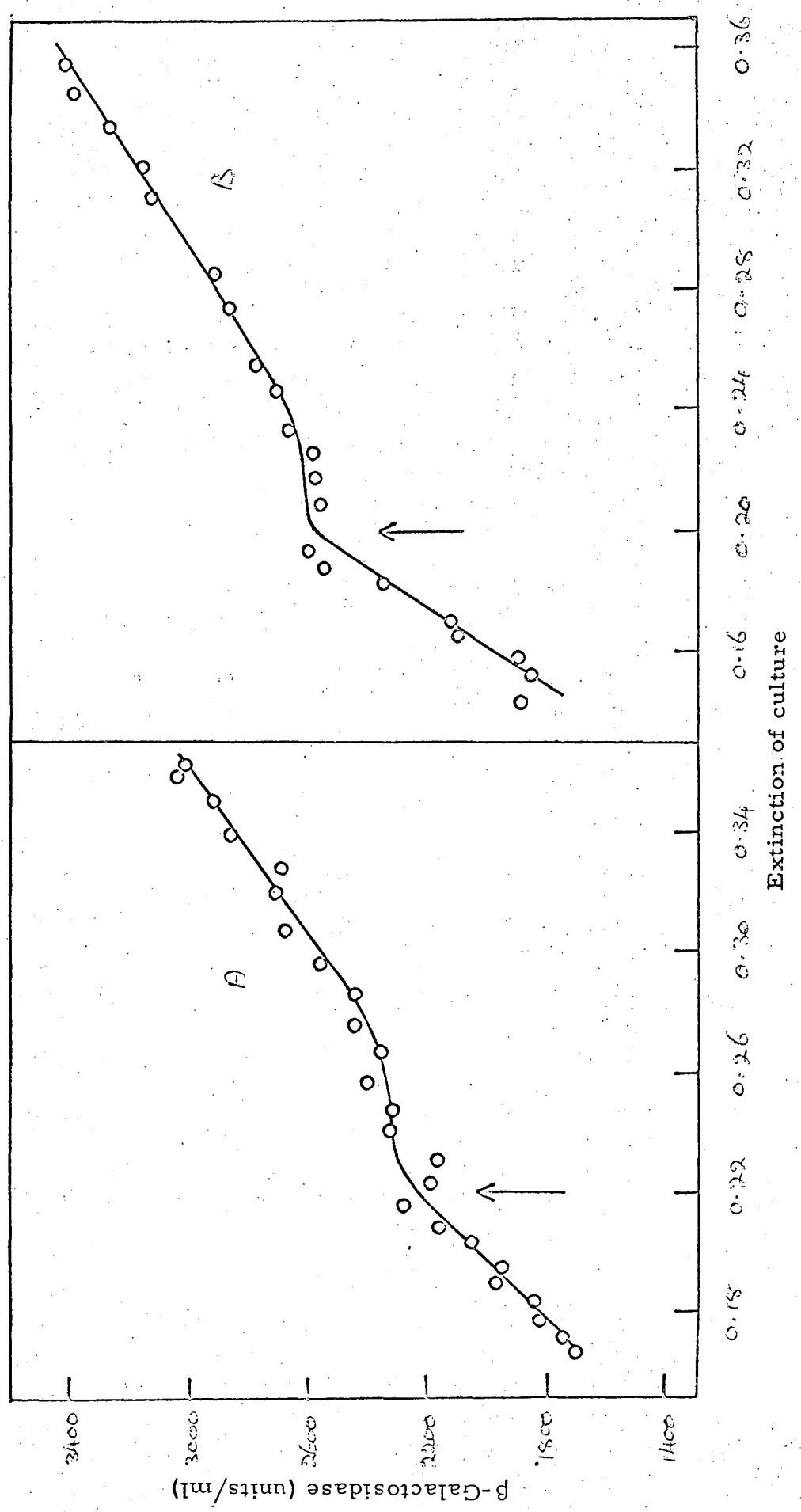

A
B
0·18
0·22
0·26
0·30
0·34
0·16
0·20
0·24
0·28
0·32
0·36
Extinction of culture
1400
1800
2200
2600
3000
3400
β-Galactosidase (units/ml)

Fig. 7

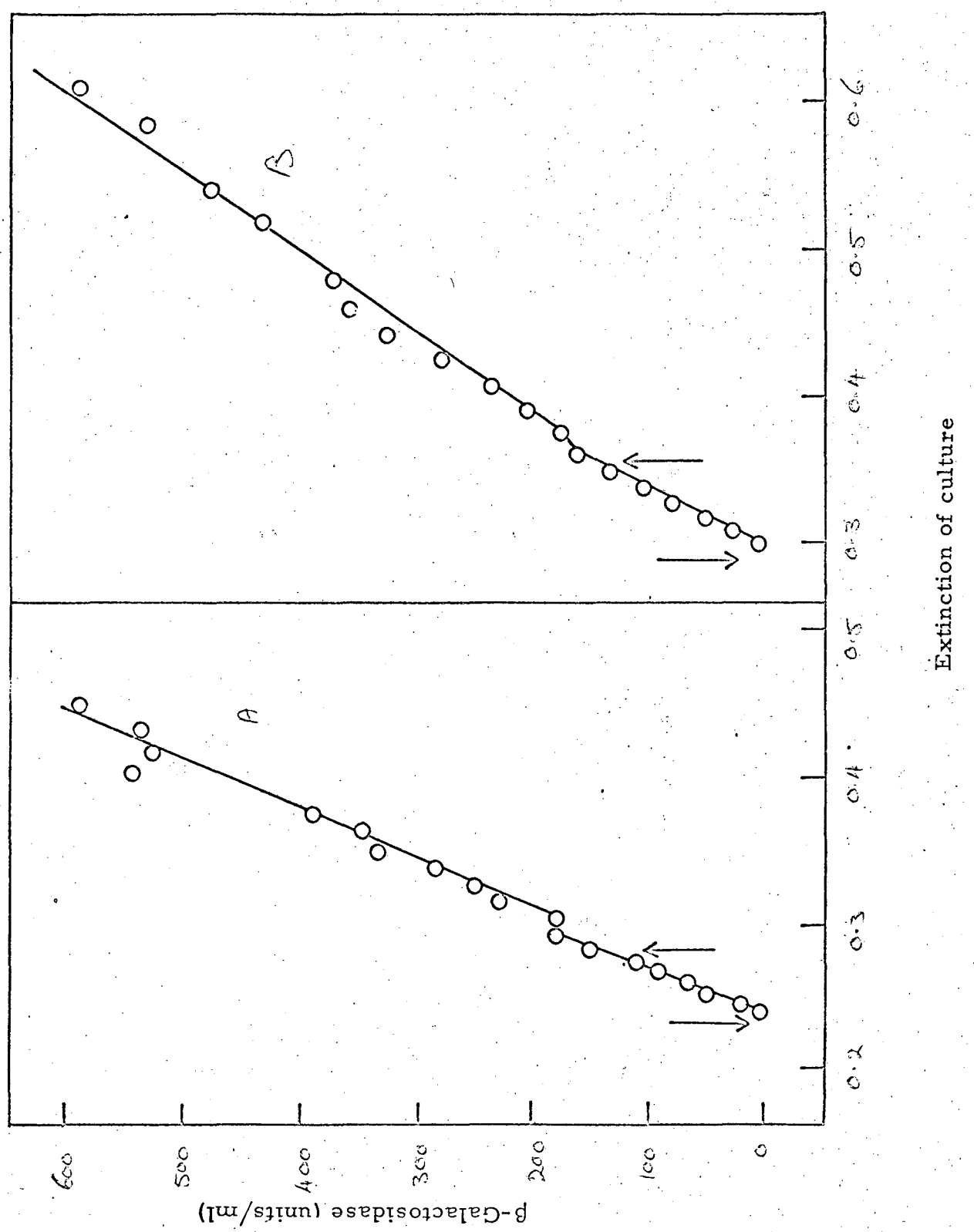

β-Galactosidase (units/ml)
600
500
400
300
200
100
0
0·2
0·3
0·4
0·5
0·3
0·4
0·5
0·6
A
B
Extinction of culture

Fig. 8

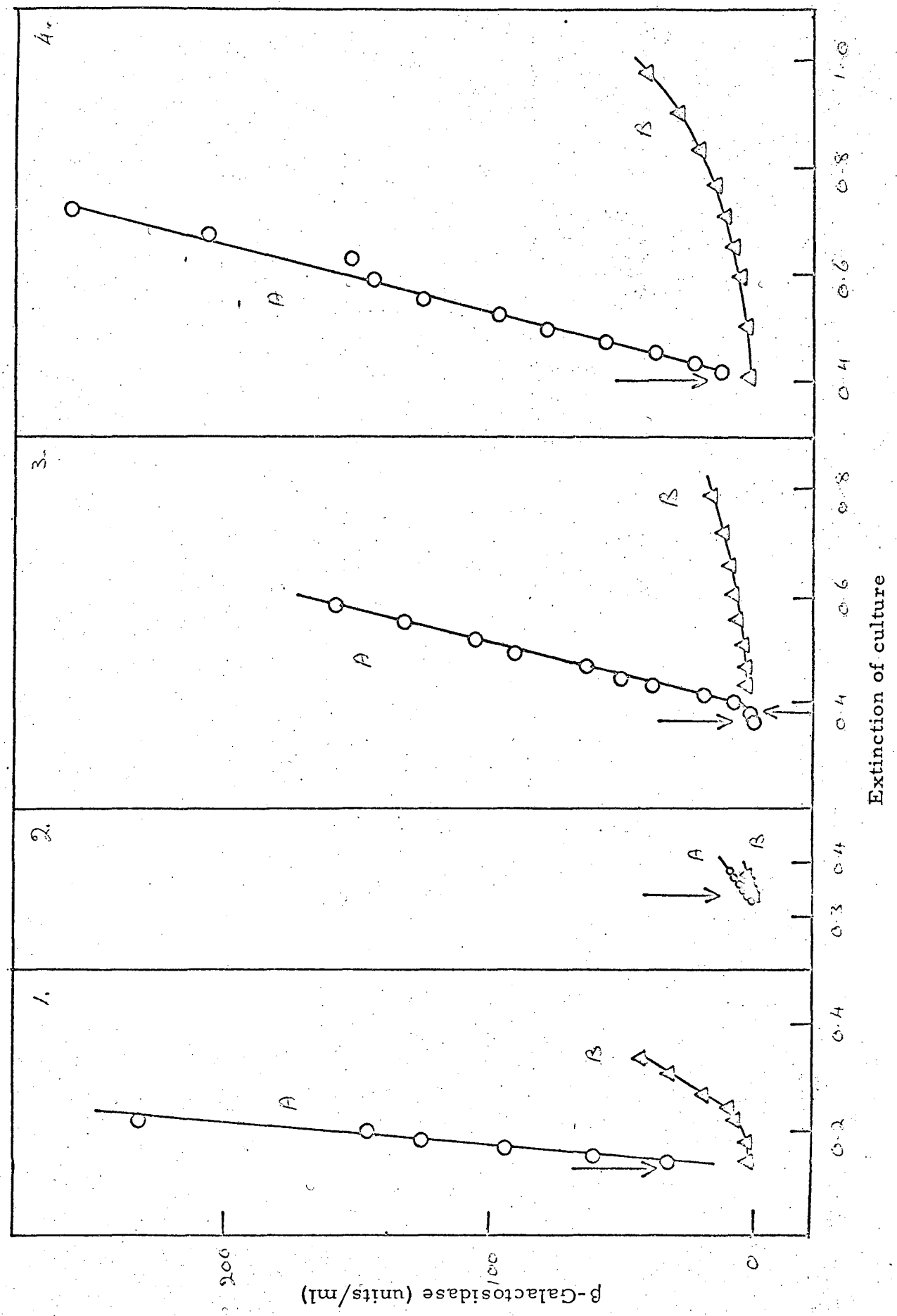

1.
2.
3.
4.
A
B
0·2
0·3
0·4
0·6
0·8
1·0
Extinction of culture
0
100
200
β-Galactosidase (units/ml)

Fig. 9

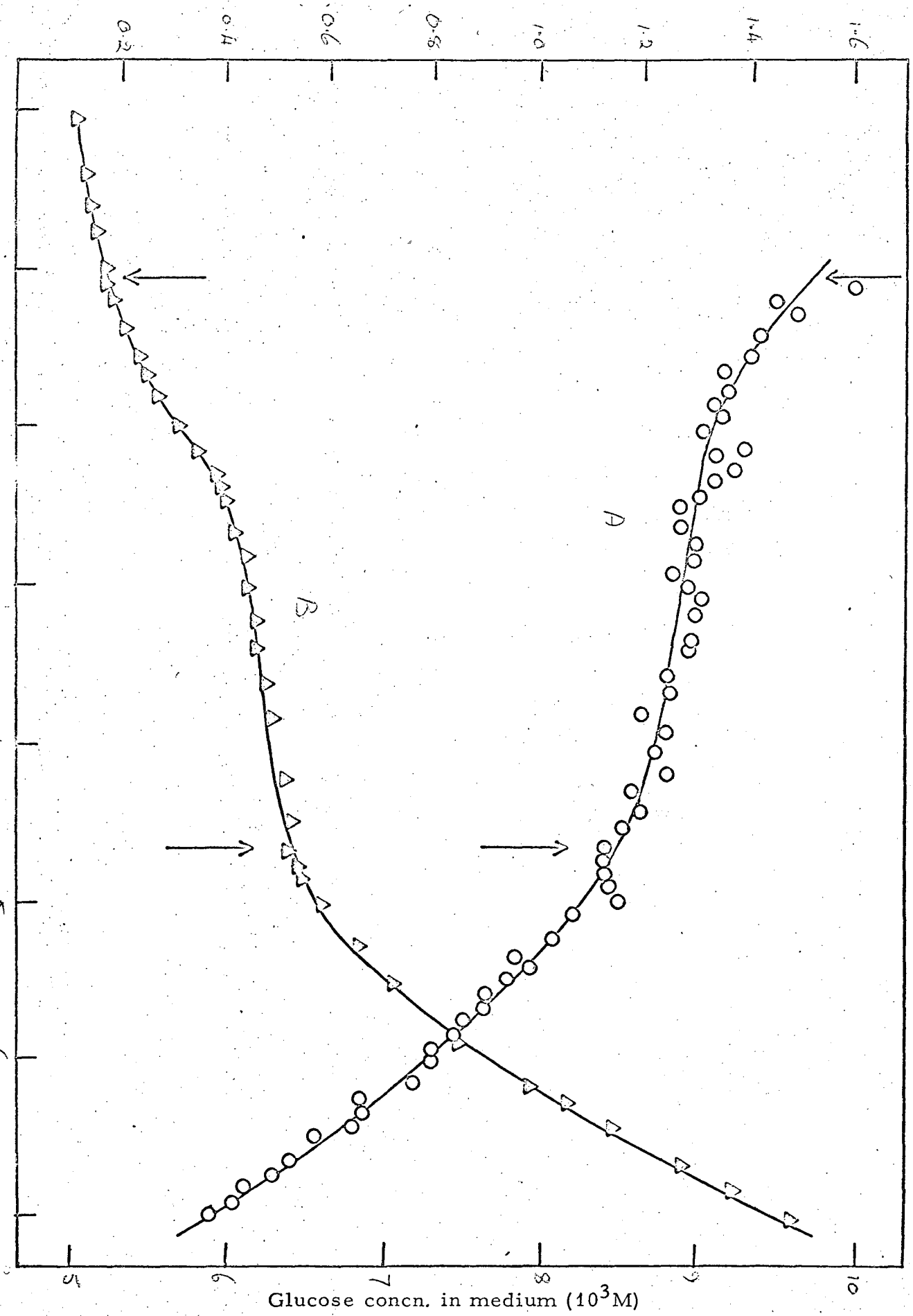

Extinction of culture
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
A
B
0
1
2
3
4
5
6
7
Hours
5
6
7
8
9
10
Glucose concn. in medium (10^3M)

FIG. 10

Fig. 10

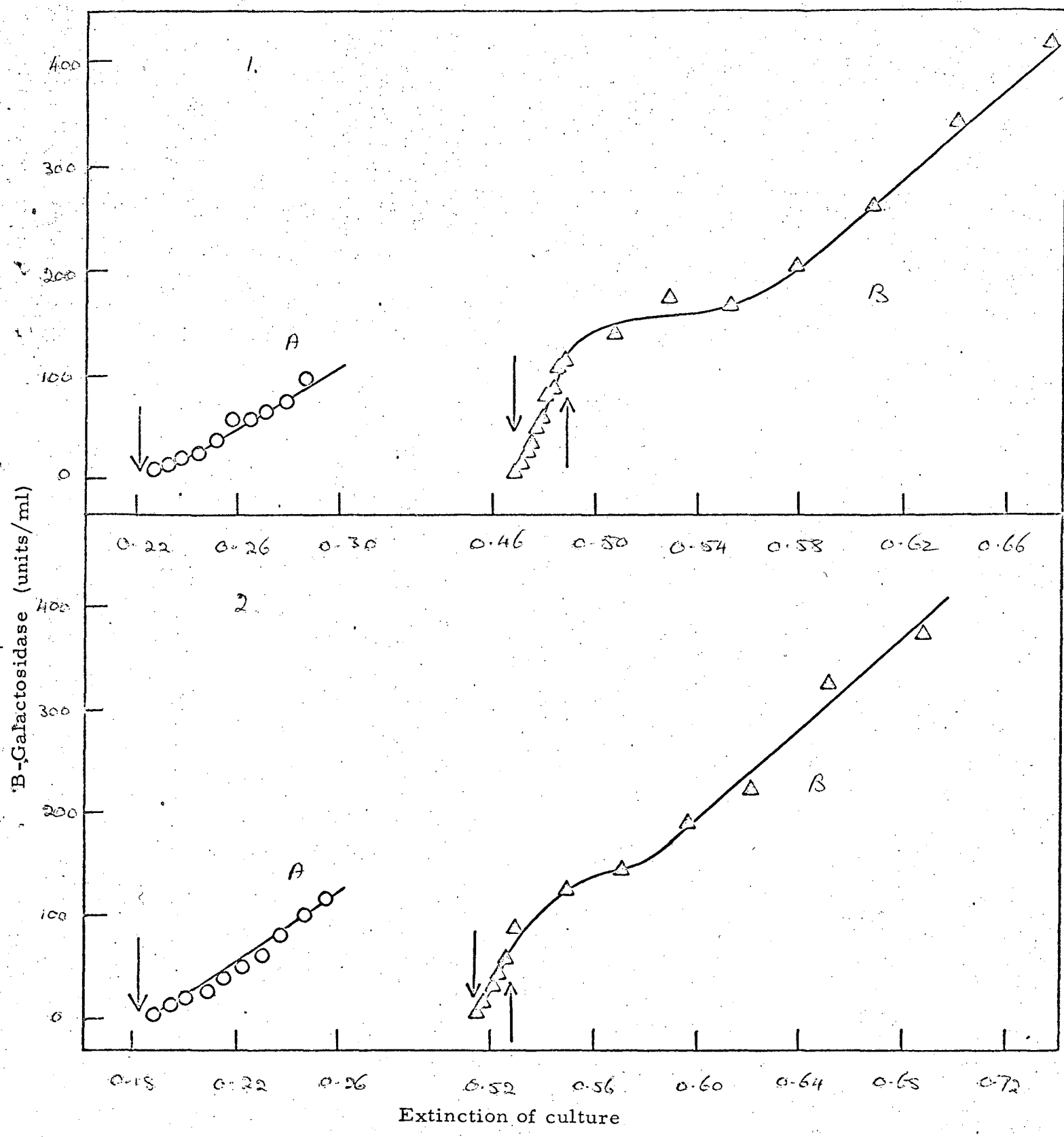

1.
2.
A
B
400
300
200
100
0
0.22
0.26
0.30
0.46
0.50
0.54
0.58
0.62
0.66
0.18
0.22
0.26
0.52
0.56
0.60
0.64
0.68
0.72
B-Galactosidase (units/ml)
Extinction of culture

Fig. 11

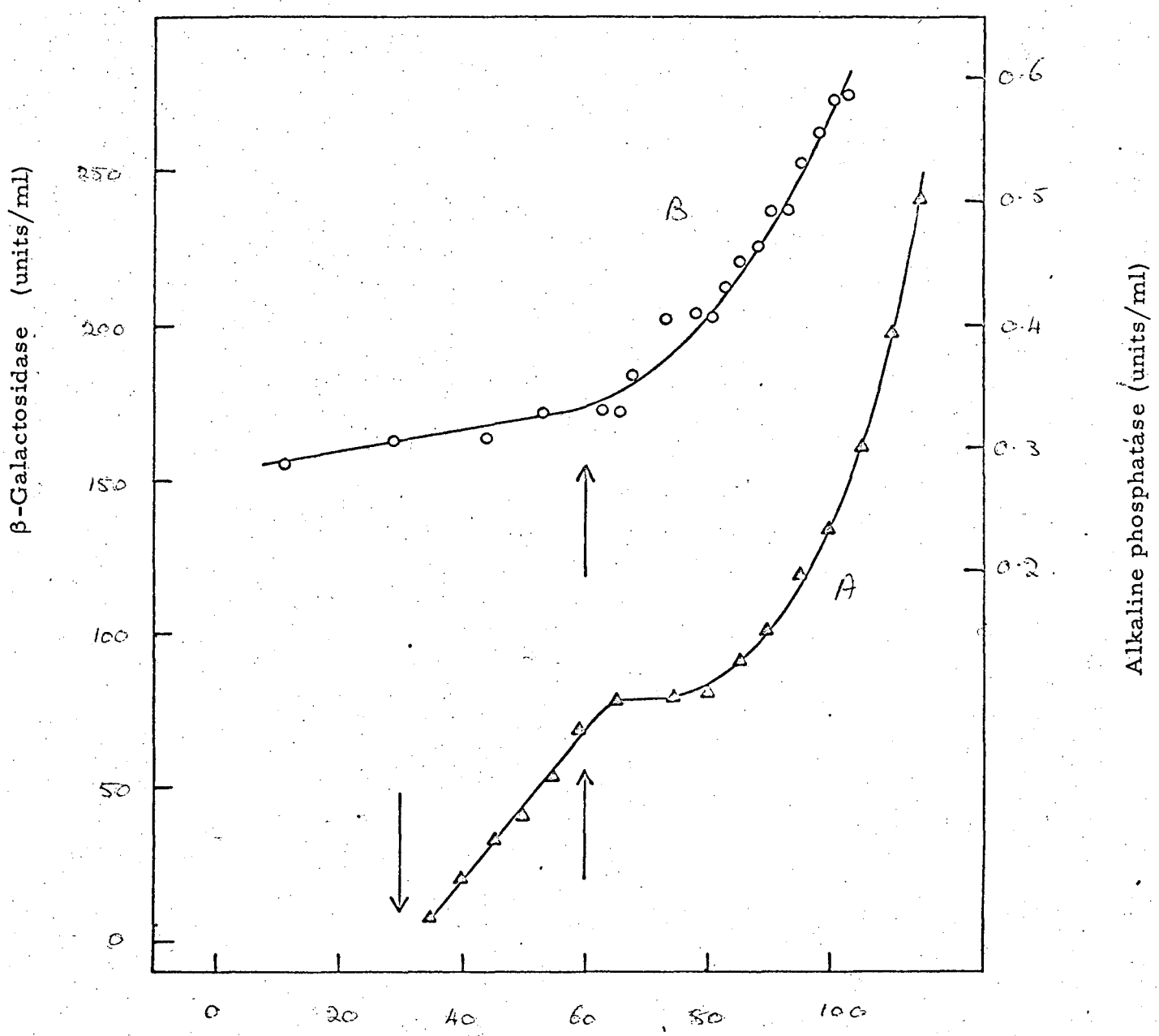

β-Galactosidase (units/ml)
250
200
150
100
50
0
0.6
0.5
0.4
0.3
0.2
Alkaline phosphatase (units/ml)
B
A
0
20
40
60
80
100
Min.

Fig. 12

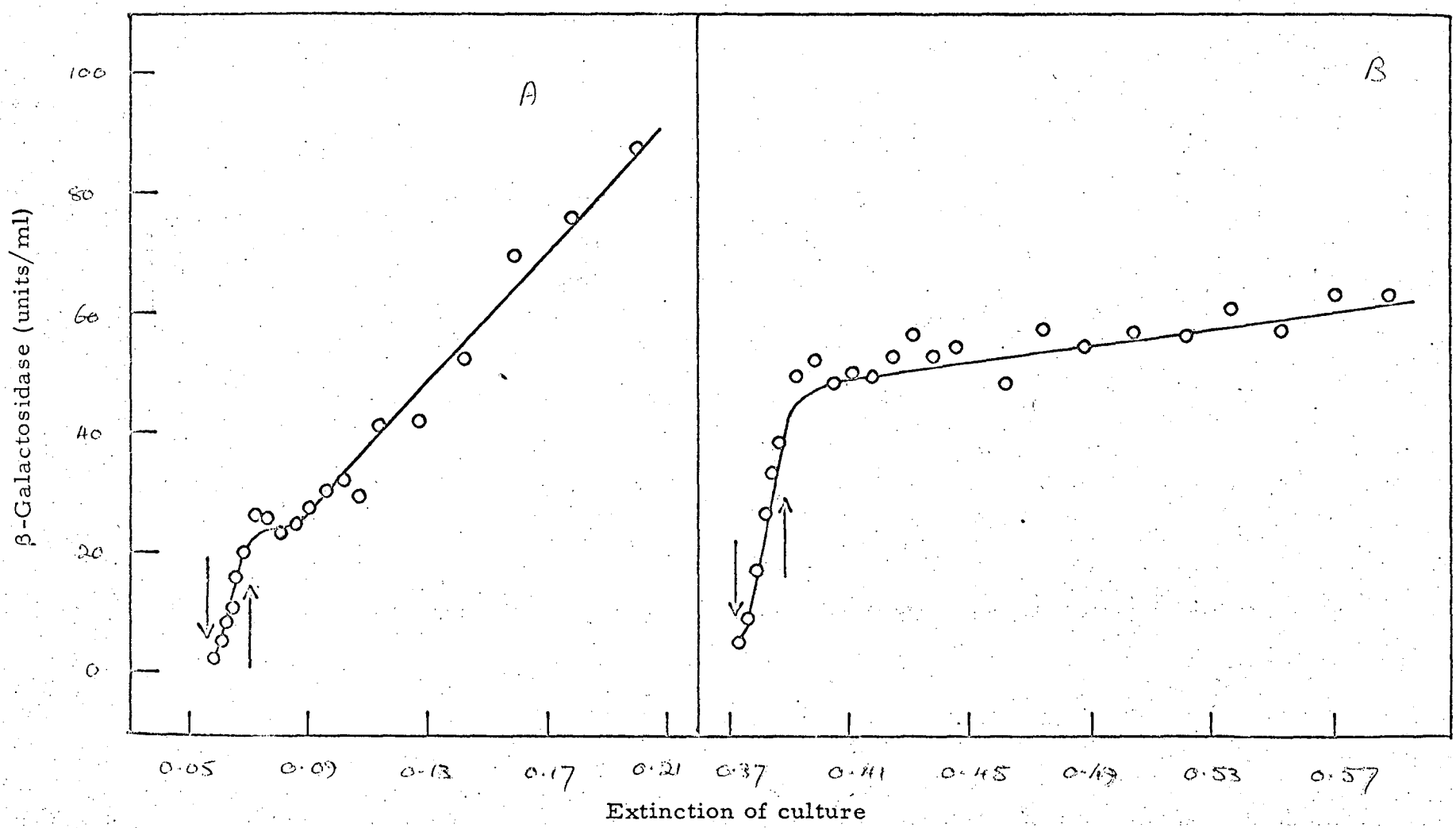

A
B
β-Galactosidase (units/ml)
100
80
60
40
20
0
0·05
0·09
0·13
0·17
0·21
0·37
0·41
0·45
0·49
0·53
0·57
Extinction of culture

Fig. 13

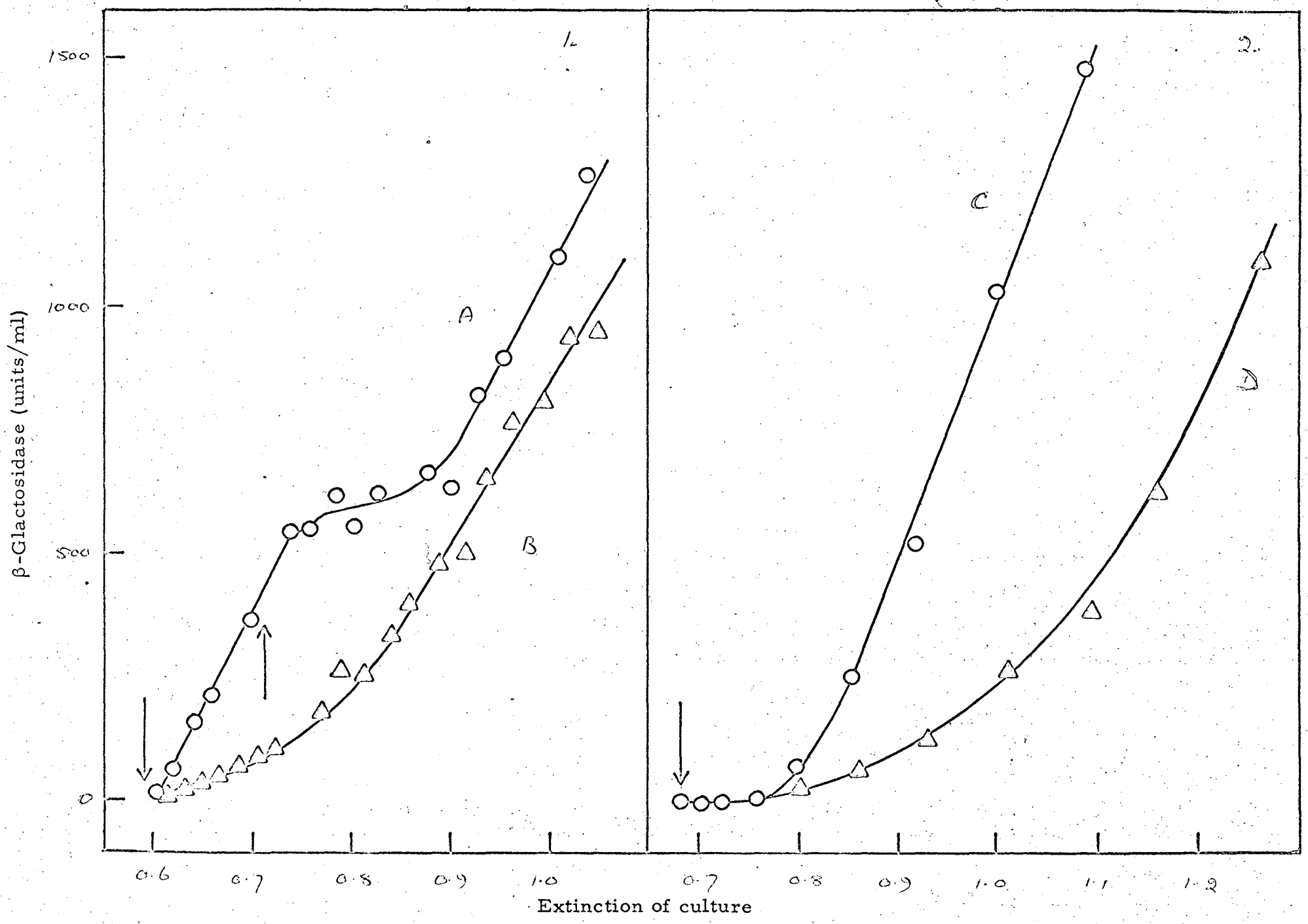

1.
2.
A
B
C
D
1500
1000
500
0
β-Glactosidase (units/ml)
0.6
0.7
0.8
0.9
1.0
0.7
0.8
0.9
1.0
1.1
1.2
Extinction of culture

Fig. 14

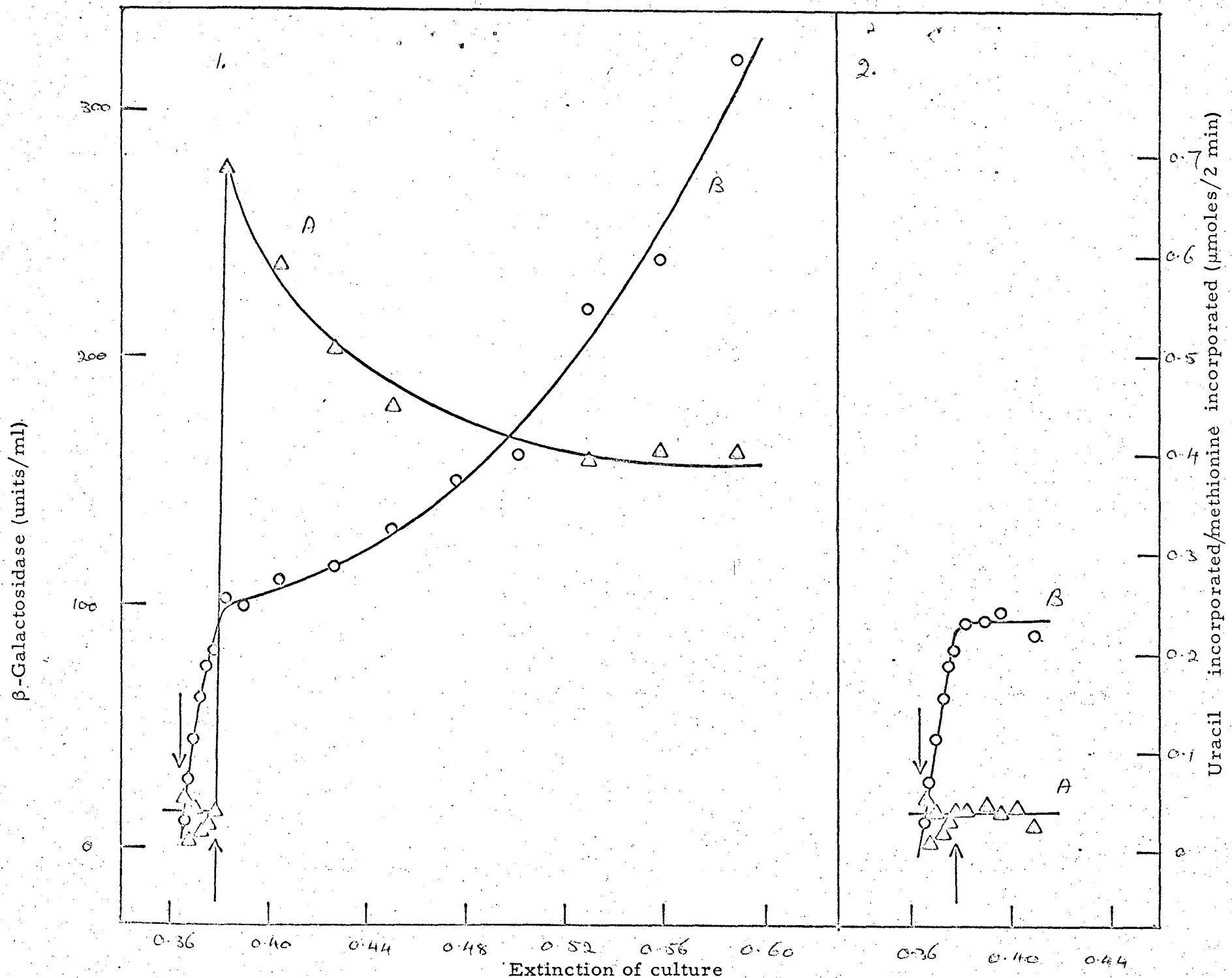

1.
2.
A
B
β-Galactosidase (units/ml)
300
200
100
0
0·36
0·40
0·44
0·48
0·52
0·56
0·60
Extinction of culture
Uracil incorporated/methionine incorporated (μmoles/2 min)
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0

Fig. 15

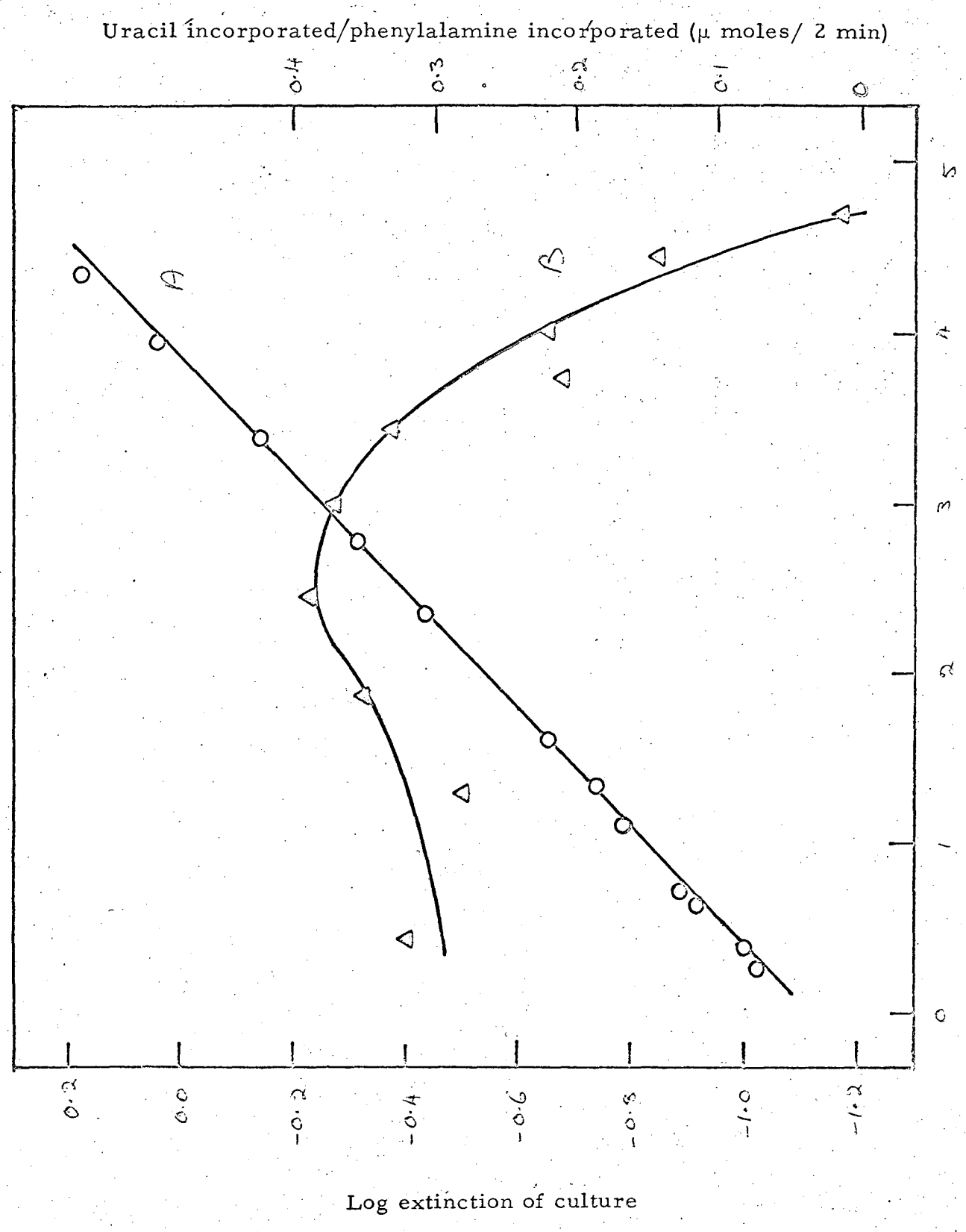
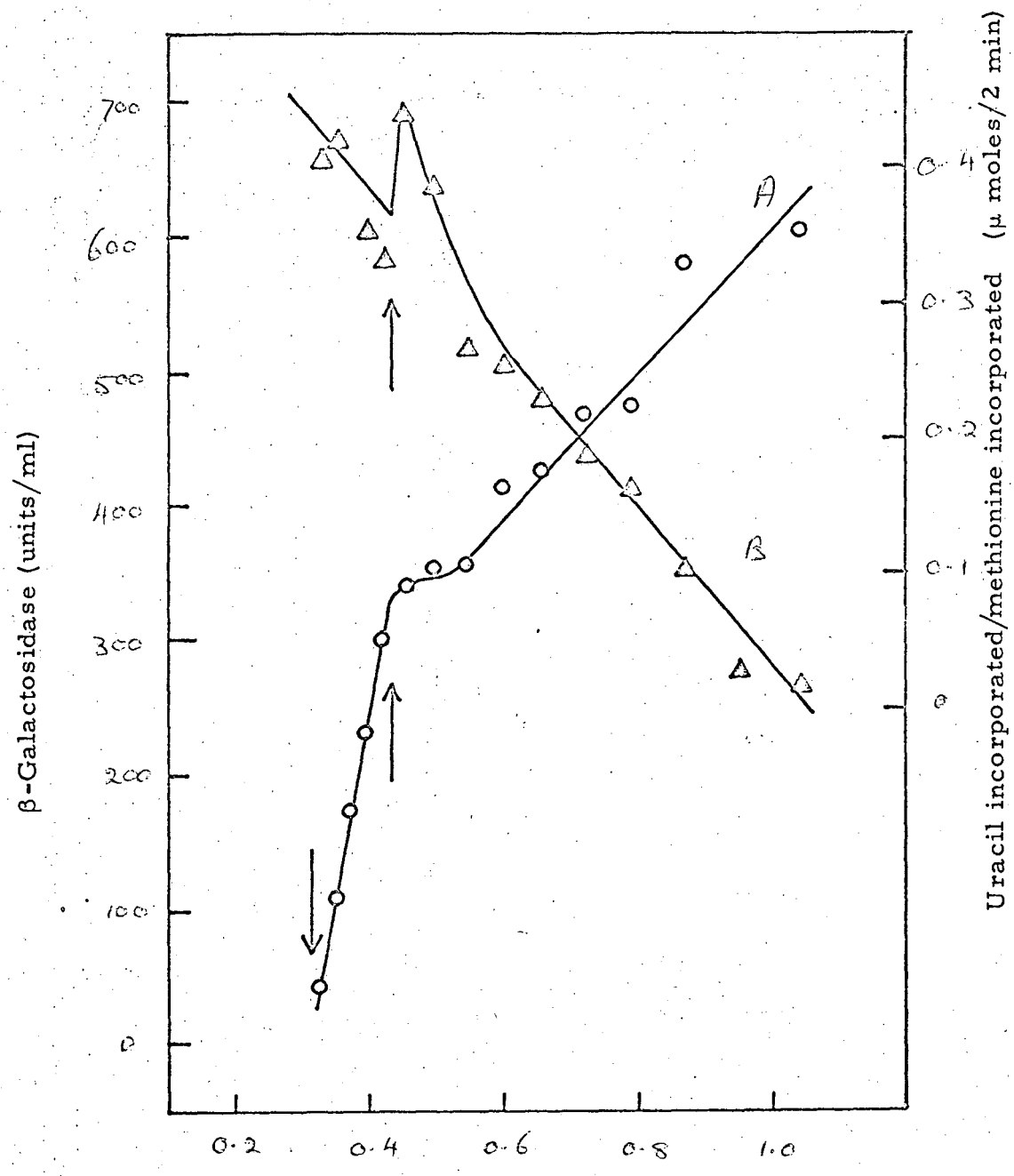

Uracil incorporated/phenylalamine incorporated (μ moles/ 2 min)
0.4
0.3
0.2
0.1
0
A
B
0
1
2
3
4
5
Hours
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
Log extinction of culture

Fig. 16



Extinction of culture

Fig. 17

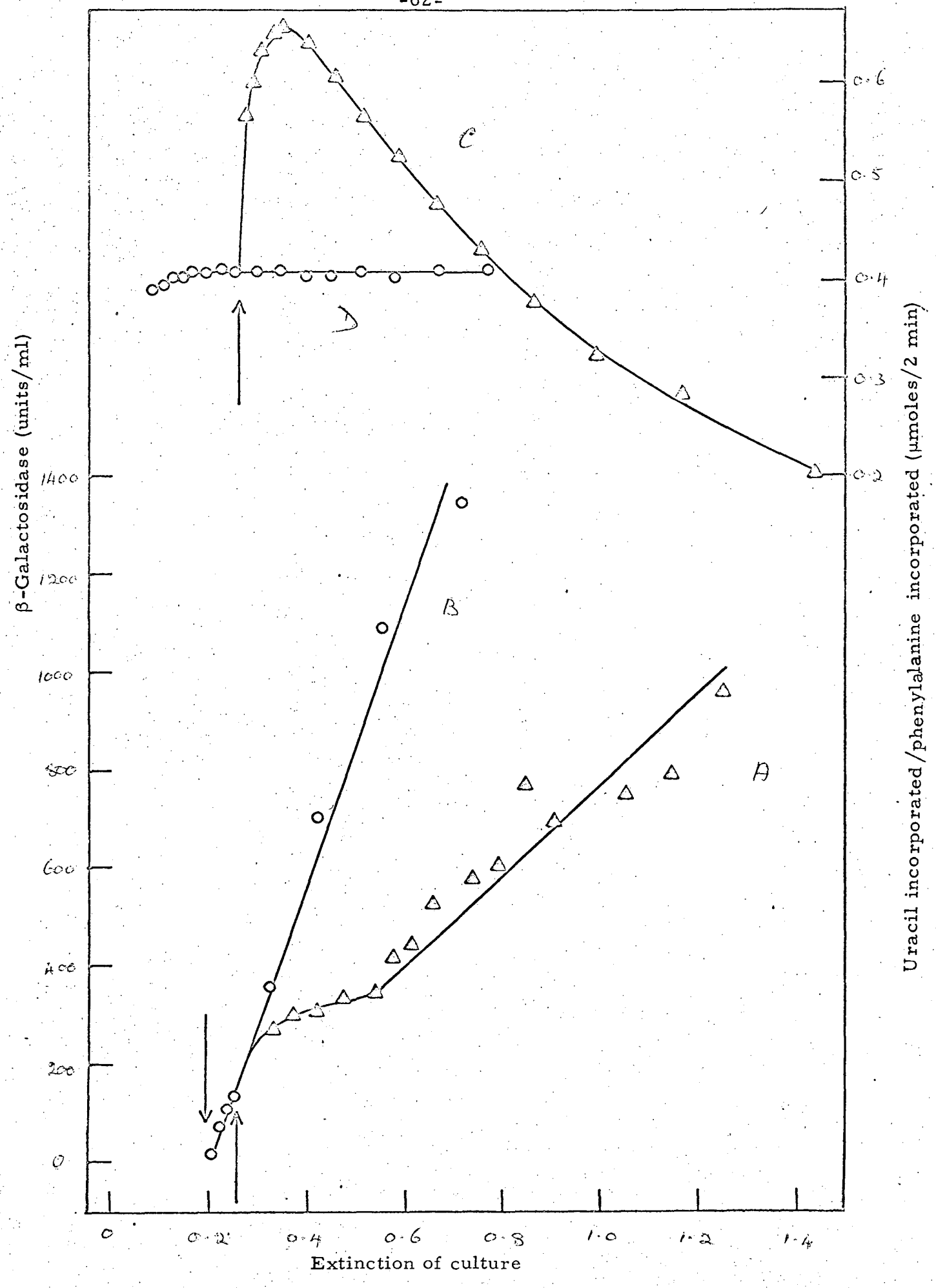

β-Galactosidase (units/ml)
Uracil incorporated/phenylalanine incorporated (μmoles/2 min)
Extinction of culture
0
200
400
600
800
1000
1200
1400
0·2
0·3
0·4
0·5
0·6
0·8
1·0
1·2
1·4
A
B
C
D

Fig. 18

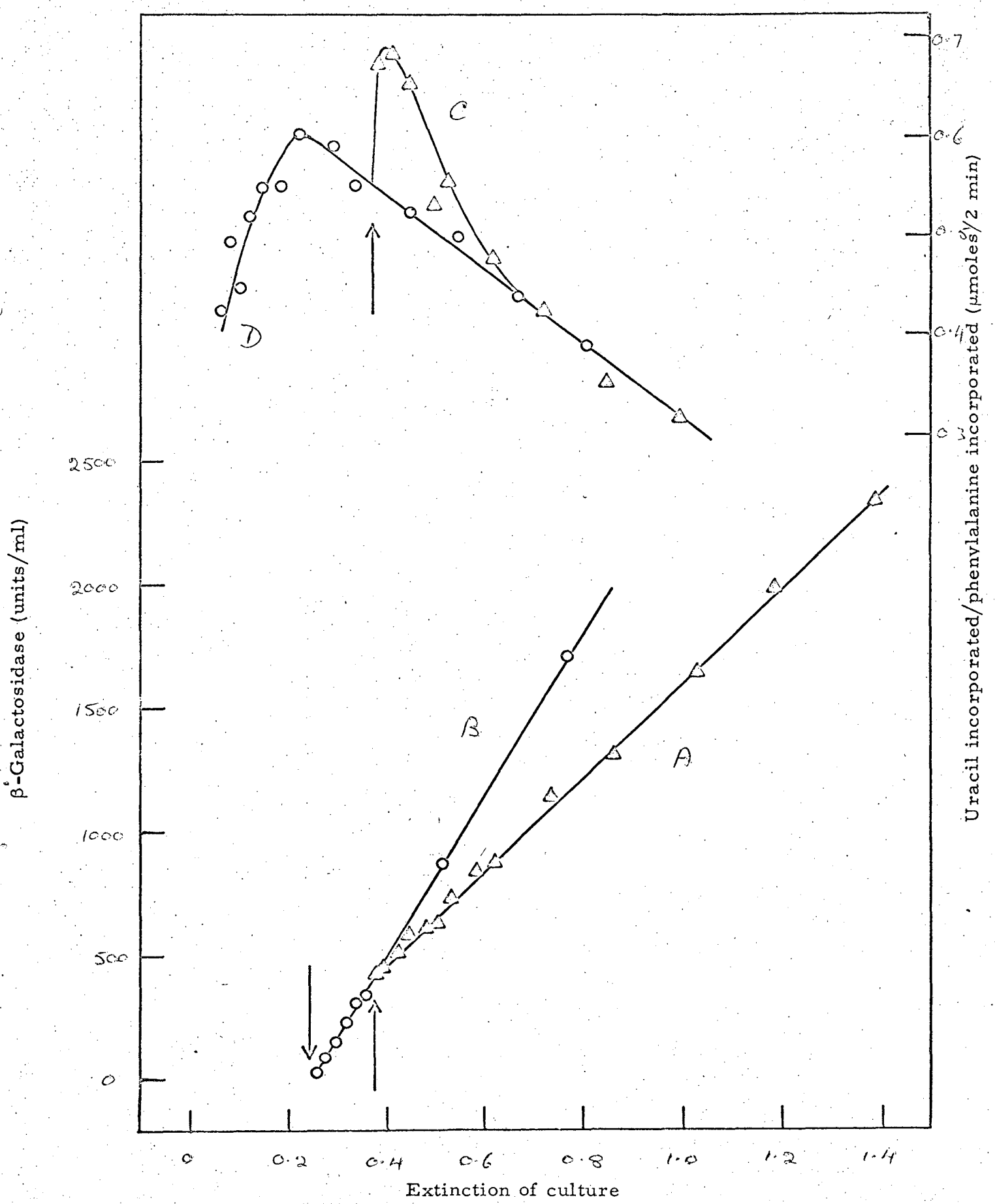

β-Galactosidase (units/ml)
2500
2000
1500
1000
500
0
Uracil incorporated/phenylalanine incorporated (μmoles/2 min)
0·7
0·6
0·4
0·3
C
D
B
A
0
0·2
0·4
0·6
0·8
1·0
1·2
1·4
Extinction of culture

Fig. 19

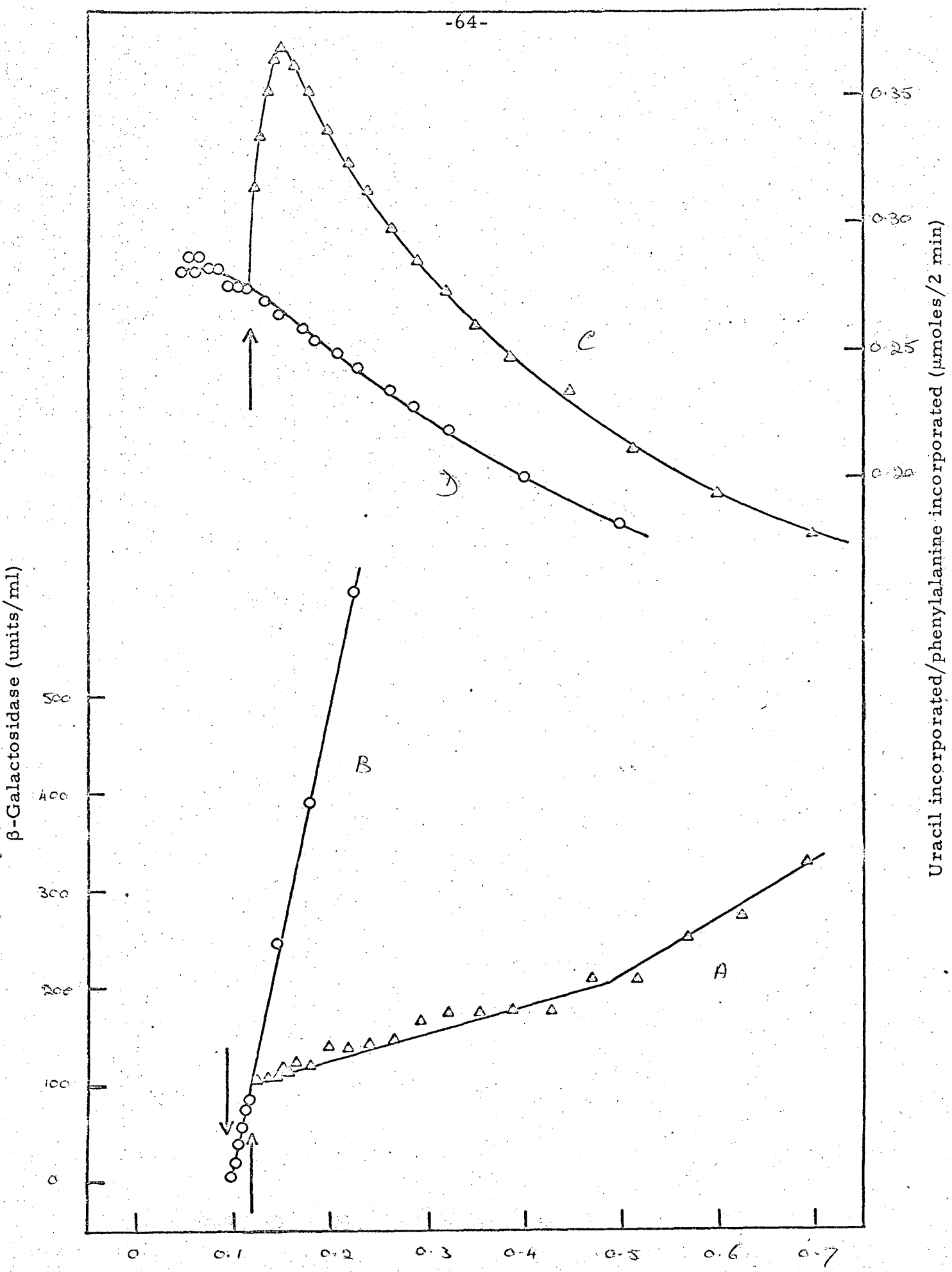

β-Galactosidase (units/ml)
Uracil incorporated/phenylalanine incorporated (μmoles/2 min)
Extinction of culture
A
B
C
D
0
100
200
300
400
500
0.20
0.25
0.30
0.35
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7

Fig. 20

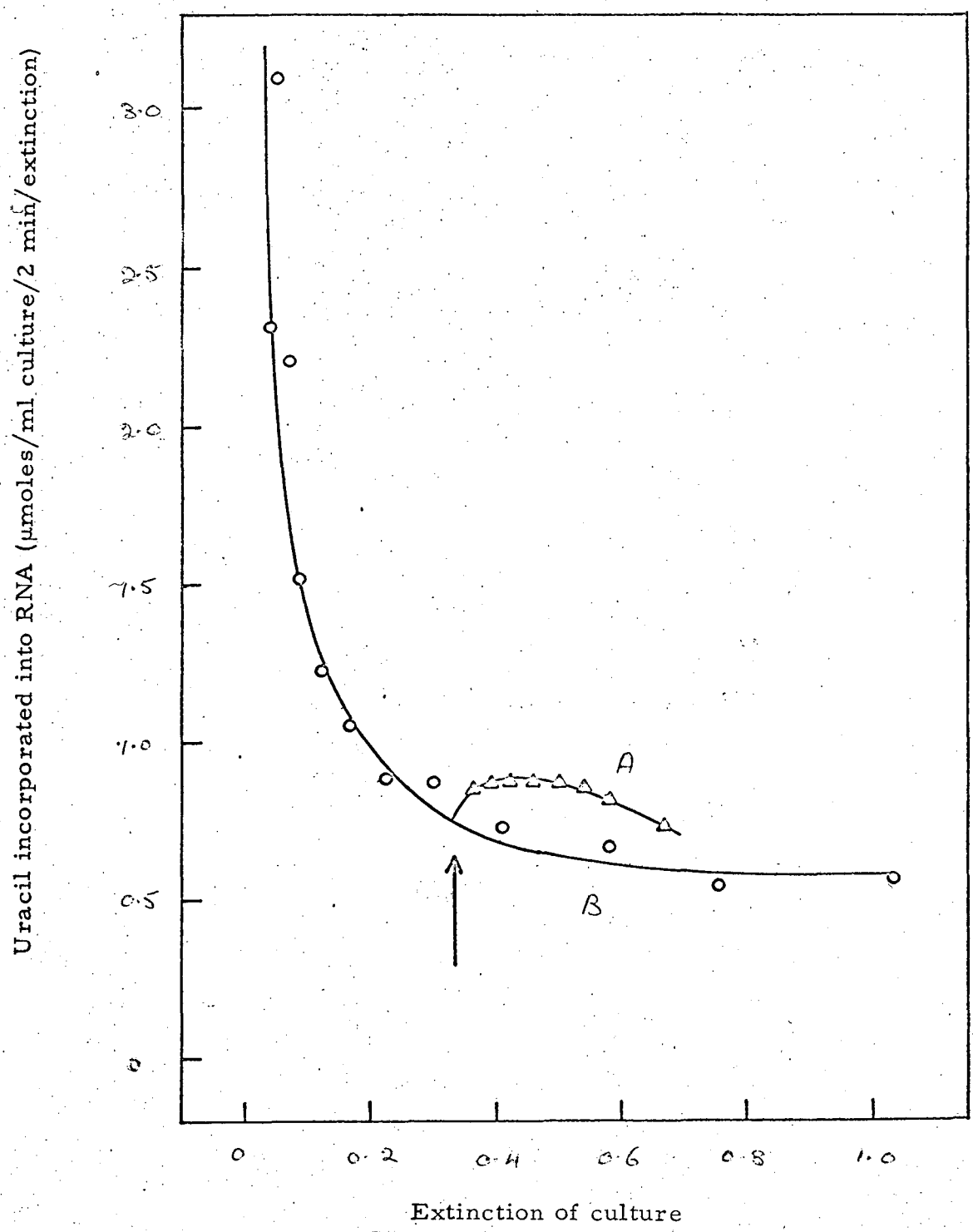

Uracil incorporated into RNA (μmoles/ml culture/2 min/extinction)
3.0
2.5
2.0
1.5
1.0
0.5
0
A
B
0
0.2
0.4
0.6
0.8
1.0
Extinction of culture

Fig. 21

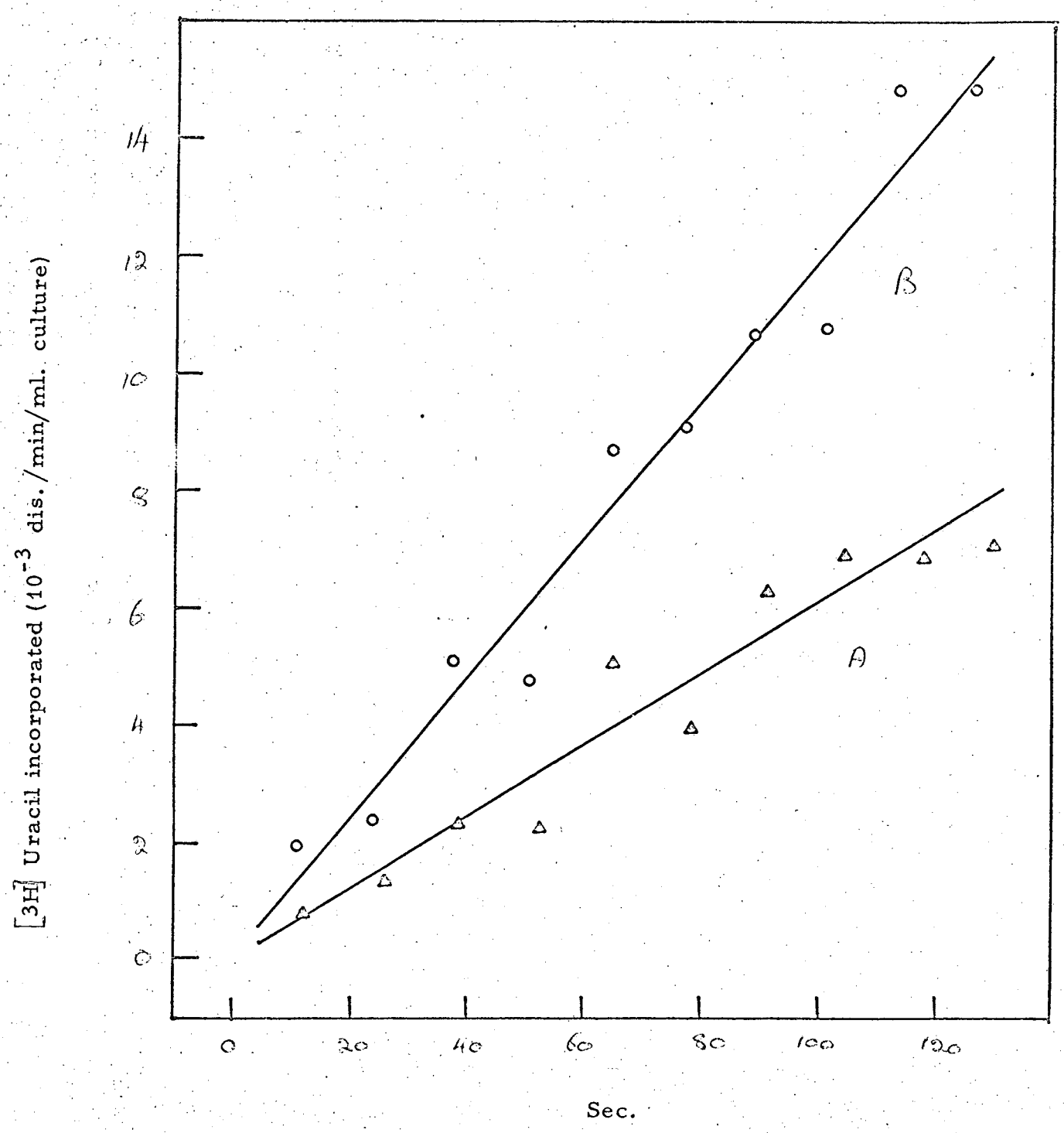

[3H] Uracil incorporated (10^{-3} dis./min/ml. culture)
14
12
10
8
6
4
2
0
B
A
0
20
40
60
80
100
120
Sec.

Fig. 22

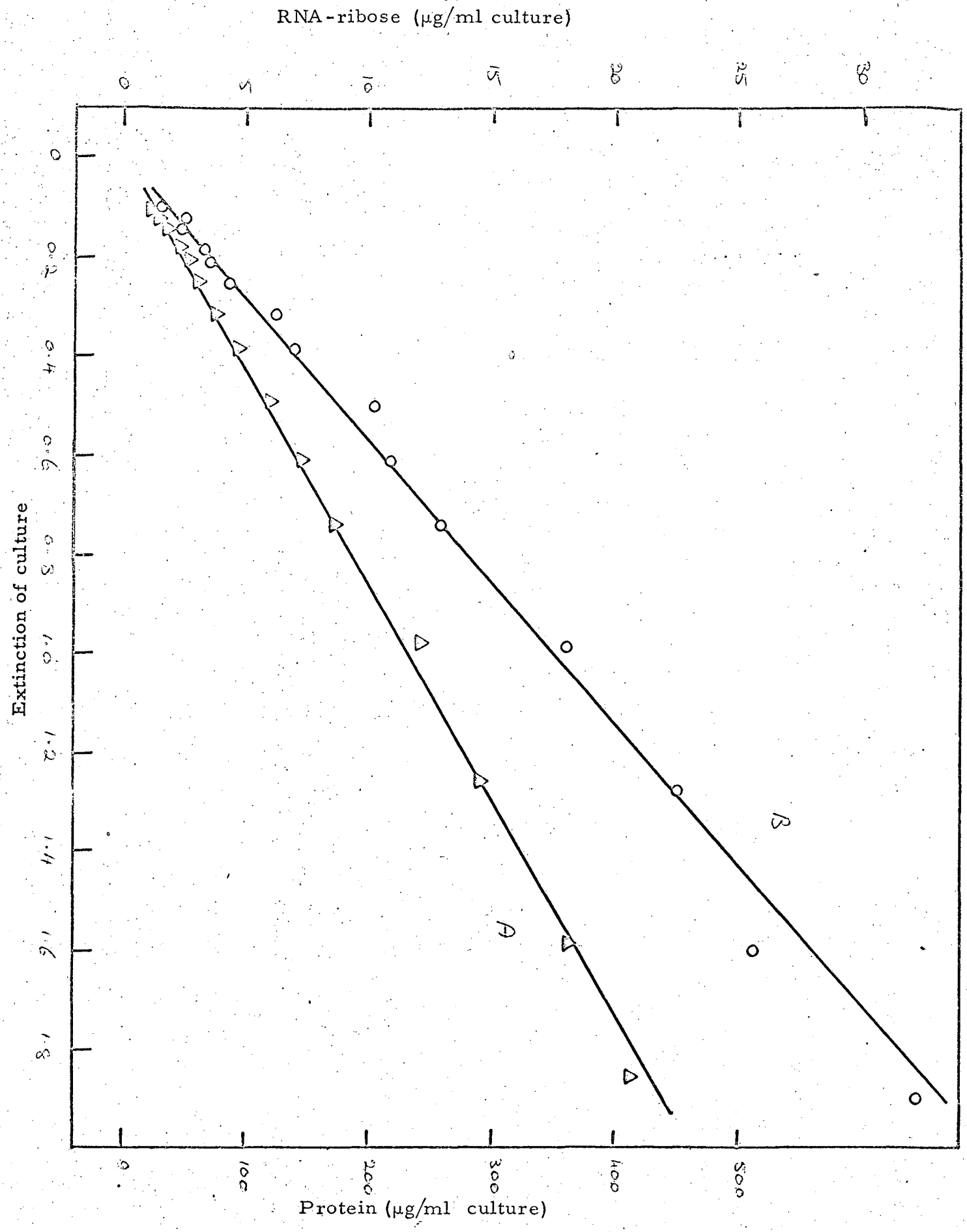
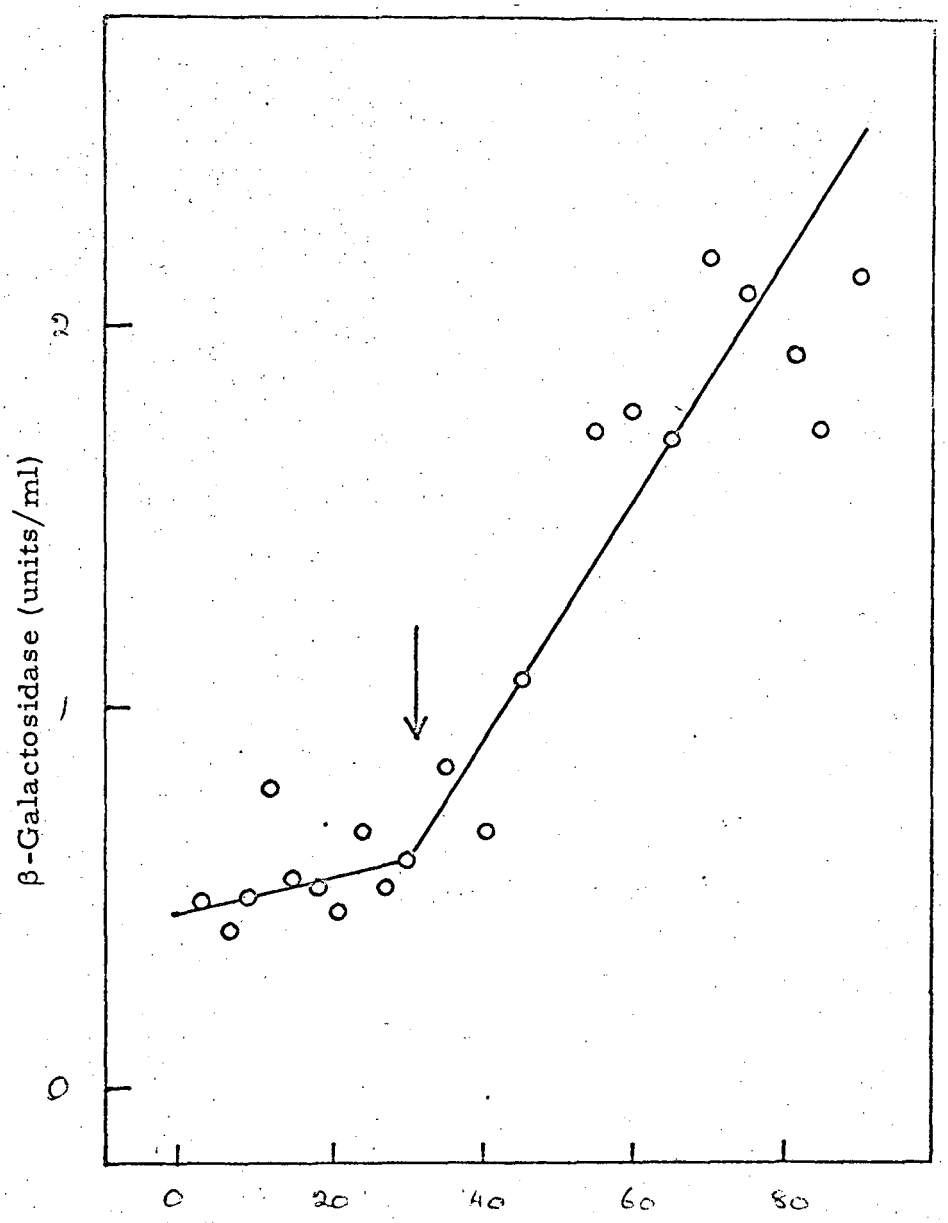

RNA-ribose (μg/ml culture)
0
5
10
15
20
25
30
Extinction of culture
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
B
A
0
100
200
300
400
500
Protein (μg/ml culture)

Fig. 23



Min. after inducer added

Fig. 24

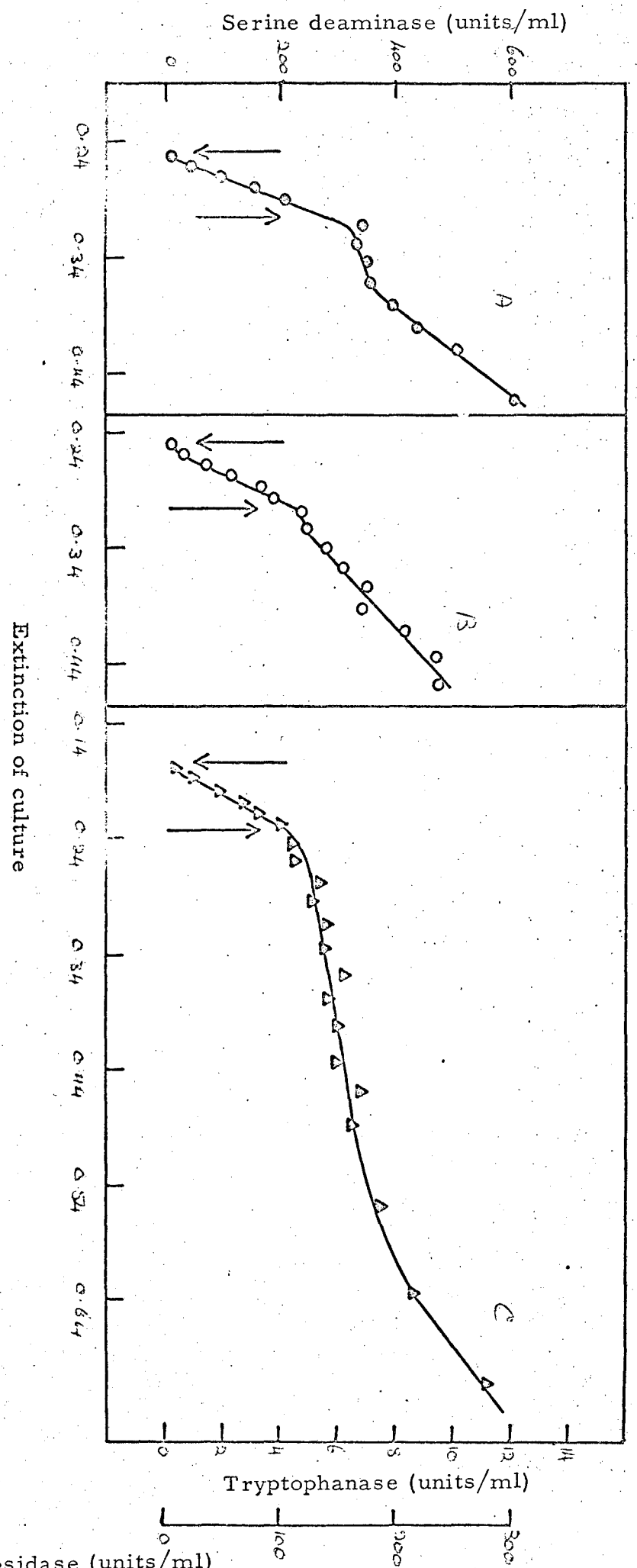
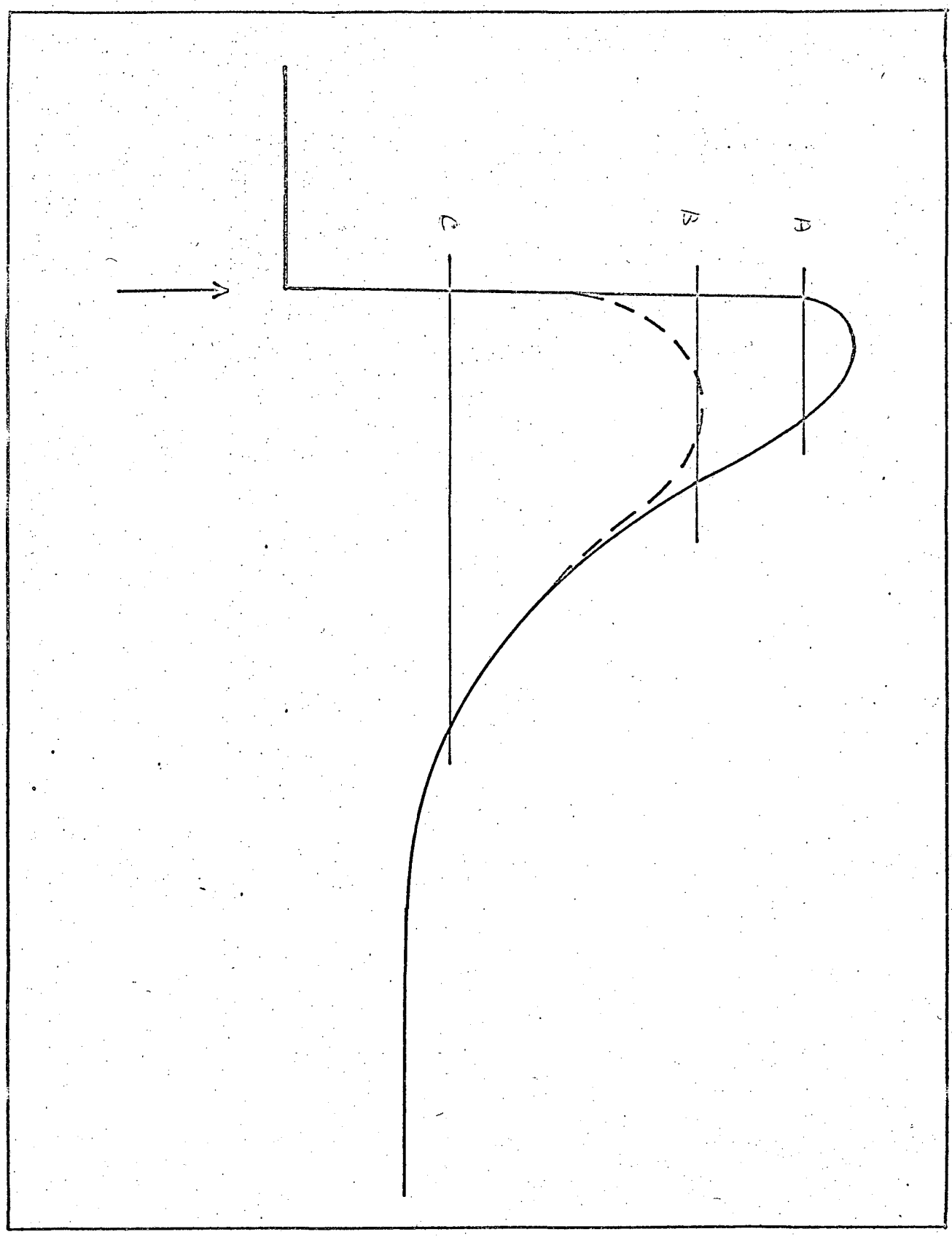

Serine deaminase (units/ml)
0
200
400
600
A
B
C
Extinction of culture
0.24
0.34
0.44
0.14
0.64
Tryptophanase (units/ml)
0
2
4
6
8
10
12
14
β-Galactosidase (units/ml)
0
100
200
300

Fig. 25

Effective concn. of catabolite corepressor



Time

Fig. 26