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MOLECULAR REGULATION AND ITS POSSIBLE
EVOLUTIONARY SIGNIFICANCE

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V. Moses and M. Calvin

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Molecular Regulation and Its Possible Evolutionary Significance

V. MOSES AND M. CALVIN

Lawrence Radiation Laboratory and Department of Chemistry
University of California, Berkeley, California

The model for the control of enzyme biosynthesis proposed by Jacob and Monod (16), which depends on repression and derepression of genetic expression, supposes in the case of inducible enzymes that the presence of a specific effector (or inducer) is required to initiate enzyme production. It is envisaged that only in the presence of inducer is transcription of the appropriate cistron of DNA carried out, with the formation of a corresponding RNA molecule. This so-called "messenger" RNA (mRNA) then carries the informational message for the synthesis of the specific protein to the ribosomes, where translation of the nucleic acid informational sequence to a polypeptide structure occurs. Much of the chemical and genetic evidence in support of this theory has been reviewed by Jacob and Monod (16). Recent additional pieces of evidence have been provided by measurement of the levels of specific mRNA in inducible cells, before and after induction with a specific effector, and in the corresponding constitutive strain (1,2,11).

The attainment within three minutes of a constant rate of specific enzyme synthesis following introduction of the inducer (18,27) indicates that mRNA production starts very soon after the inducer is added. Similarly, studies by Kepcs (18) have shown that enzyme synthesis ceases, also in about three minutes, after inducer is removed from the system or diluted to a concentration

so low that it is no longer effective. This has led to the conclusion that, at least for the β -galactosidase system of Escherichia coli, mRNA is both rapidly synthesized and rapidly destroyed. Removal of the inducer results in almost instant cessation of mRNA synthesis and the mRNA already existing at that time decays with a half-life of about one minute (18). Maintenance of enzyme synthesis thus requires continuous production of mRNA and this, in turn, provides a very sensitive response to changes in the environment, since the removal of an inducing substance from the vicinity of the cells brings to a rapid halt the biosynthesis of the corresponding inducible enzymes.

Similar results for the formation and decay of mRNA in the β -galactosidase system of E. coli were obtained by Nakada and Magasanik (25), who obtained a value of 2.5 minutes for the half-life of mRNA. In a study of protein synthesis in Bacillus subtilis and its relation to mRNA formation, Levinthal, Keynan and Higa (20) found that the incorporation of [14 C]valine into protein is rapidly inhibited shortly after the administration of actinomycin D. This antibiotic prevents the DNA-dependent synthesis of RNA (15). Levinthal et al. (20) calculated a value of 2 minutes for the half-life of mRNA, and other authors have also assumed or concluded that mRNA's decay with short half-lives (10,23).

The widespread occurrence of genetic repression-depression phenomena, and their obvious potential importance as prime regulators of genetic expression and cellular activity, has led to suggestions that the whole enzymic apparatus of a cell may be subject to this sort of genetic regulation. Enzymes would be produced in response to the presence of environmental or intracellular inducers and repressors, and the production of each protein might be envisaged as under a genetic regulating control of the type postulated for β -galactosidase in E. coli (26). There may thus be no fundamental genetic differences

between inducible and constitutive enzymes in the sense originally intended by Karstrom (17), but the apparent constancy of constitutive enzymes may be the consequence of an unvarying balance of repressing and derepressing factors at a genetic regulatory site. A short life for their mRNA's would then be an important element in the exertion of fine control on the biosynthesis of these phenotypically constitutive enzymes. The alternative possibility, recognized by Pardee and Beckwith (26), was that certain enzymes appear to be constitutive because of the absence of a control mechanism involving inducers and repressors. The rate of synthesis of such an enzyme would then be constant in relation to the synthesis of all other constitutive proteins in the cell, the actual rate being controlled, presumably, by some inherent feature of the coding of the structural gene (19). Were this second possibility to be the case one might question the value of short-lived mRNA for constitutive enzymes, since the fine control of protein biosynthesis which may be achieved with unstable mRNA for inducible enzyme synthesis is not relevant in the case of constitutive enzyme formation.

It has already been recognized that all mRNA in all organisms cannot be so unstable as to have a half-life of only one or two minutes. Spencer and Harris (30) have shown that the biosynthesis and regulation of biosynthesis of some proteins may continue in cells of Acetabularia crenulata many days after removal of the nucleus. This certainly suggests a very great stability for the mRNA's involved. However, it must be borne in mind that the algal cells contain a chloroplast, and it might be this organelle, rather than the nucleus, which in Acetabularia controls the biosynthesis of the enzymes studied; chloroplasts of some plants have been shown to contain DNA (4). No such reservation need be made in the case of the continuance of protein synthesis for

several hours after the enucleation of various animals cells (9,28,29). In the case of E. coli, too, it has been suggested that not all mRNA is as short-lived as that for β -galactosidase (7). The induced synthesis of β -galactosidase in E. coli is preferentially inhibited by streptomycin compared with the total synthesis of protein. It was inferred from the data obtained that the synthesis of all proteins requiring the continuous formation of labile mRNA will be inhibited by streptomycin under appropriate circumstances, while the residual incorporation of labelled amino acids into the protein which continues in the presence of streptomycin may depend on a relatively stable mRNA (7).

The variation in stability of different mRNA's in the same organism may thus be of significance as a means of investigating the basic mechanism of phenotypic expression, with important consequences, for example, in the field of cellular differentiation. We have recently obtained evidence which may be interpreted as supporting the concept of varying lability for different mRNA's, and we will consider the significance of this data from an evolutionary point of view.

Three strains of E. coli have been used: C600-1 ($i^+y^-z^+$) (obtained from Dr. A. B. Pardee); 300U ($i^+y^-z^+$) and 230U ($i^-y^-z^+$) (from Dr. J. Monod). The organisms were all cultured on M63 medium, an inorganic salts medium containing glycerol and thiamine (27), at 37° in air. The inducers used for β -galactosidase were isopropylthio- β -D-galactopyranoside (IPTG) (usually at 5×10^{-4} M) and methylthio- β -D-galactopyranoside (TMG) (10^{-3} M). Samples of cell suspension for enzyme assay were removed from the culture flasks, violently agitated with toluene, and assayed for enzyme activity as described by Kepes (18). Units of enzyme activity will be expressed as μ moles of o-nitrophenyl- β -D-galactoside hydrolyzed/min./ml. of cell suspension at 37° .

Inoculation of the bacterium into the M63 medium results in a logarithmic growth pattern as measured by increase of optical density at 650 m μ (Fig. 1). The doubling time during this phase of growth varies on different occasions between about 65 and 85 minutes. Eventually growth stops and the optical density remains constant. If after an hour or more of being in the stationary phase of growth a portion of the culture is rapidly added to several volumes of fresh culture medium, logarithmic growth starts again with no detectable lag, the first sample for optical density measurement being taken about 30 seconds after dilution to allow for thorough mixing. Growth in this medium stops as a result of exhaustion of the glycerol used as carbon and nitrogen source (Fig. 2); a very great excess of ammonium nitrogen is present.

Addition of an inducer of β -galactosidase to E. coli growing logarithmically typically results in the sudden onset of the appearance of enzyme activity some 2.5 to 3 minutes later (Fig. 3) (5,25,27). The time required for the differential rate of enzyme synthesis (rate of enzyme synthesis vs. rate of growth measured by optical density (24)) to increase from virtually zero to a constant value is very short, and the constant rate is always achieved within 3 minutes after the addition of inducer. However, if a culture in the stationary phase of growth is diluted into fresh medium already containing inducer (as in Fig. 1), a somewhat different kinetic course of enzyme synthesis is observed (Fig. 4). Although as during normal logarithmic growth, the differential rate of enzyme synthesis begins to increase about 3 minutes after the first contact with inducer, the rate continues to increase for a considerable period and does not become constant until about 15 minutes have elapsed. Some variation is observed in the length of this period. Growth, however, is logarithmic from the time of dilution (Fig. 1). This finding suggests that an impairment of the enzyme induction and synthesis process has developed during the period of carbohydrate starvation. Using Jacob and Monod's theory of induction (16) we might suppose

that since the initial appearance of the enzyme is not delayed there has been no interference with the initiation of DNA-dependent mRNA synthesis. On the other hand, since the steady-state differential rate of synthesis takes so long to become constant it might be concluded that essential components necessary for the overall process of enzyme protein synthesis have become depleted in the absence of a source of carbon and energy and that these are only slowly replaced. Such components might be amino acids for protein synthesis or ribonucleotides for RNA formation. It should be noted that dilution of a culture already growing logarithmically into fresh medium containing inducer results in the characteristic 3 minute period to achieve a constant rate of synthesis (Fig. 5); this dilution per se is not responsible for the delay.

No absolute block to β -galactosidase synthesis develops during starvation which must first be overcome on the resumption of logarithmic growth before enzyme induction can be effected. Such a block might be envisaged as a consequence of catabolite repression (21) developing during starvation. However, the conditions of this starvation are completely reversed from those giving rise to catabolite repression; the latter effect is observed in conditions of carbohydrate surplus and nitrogen deficiency. Furthermore, β -galactosidase may be induced, albeit very slowly, during the starvation period itself (Fig. 6). Although the induction mechanism can be brought weakly into action during starvation, doing so does not potentiate enzyme synthesis during a subsequent resumption of growth in the continued presence of inducer. This is true even if the inducer is added in the previous logarithmic phase of growth, hours before dilution takes place. Fig. 7 illustrates an experiment in which stationary state cells were diluted into fresh medium. The first contact with inducer in the upper curve was about 4 hours before dilution, 2.5 hours before the end of the earlier logarithmic phase. In the lower curve inducer was introduced at the time of dilution; in both cases the concentration of inducer was maintained

constant at all times after it was first added to the cells. If no inducer was present before dilution, the differential rate of synthesis became constant about 14 minutes after dilution; when inducer was present before dilution, a constant rate of synthesis was reached about 18 minutes after dilution. From this we may conclude that decay of part of the enzyme synthesis mechanism takes place during starvation, and this would be consistent with the decay of labile mRNA when precursors and energy for its maintenance were not available.

Freter and Ozawa (8) have shown that it is possible to obtain a second crop of bacteria by reinoculation into medium which had previously supported bacterial growth to stationary phase. The first crop of bacteria is removed by filtration or centrifugation and these manipulations result in considerable aeration of the liquid. In this newly aerated condition the medium will support growth of a dilute culture which did not occur in the first very dense stationary culture. We have investigated the effects on induction of diluting a stationary culture into millipore-filtered exhausted medium, the inducer being introduced at the time of dilution. Growth was indeed resumed at a very rapid rate with no lag (Fig. 8), the doubling time being only 24 minutes compared with 53 minutes for a control sample diluted into fresh medium. The rapid growth continued, however, for 15 minutes only, the doubling time then suddenly increasing to 362 minutes. The differential rate of enzyme synthesis became constant in exhausted medium (Fig. 9 curve B) in about the same time (16 minutes) as in the control (Fig. 9, curve A) (14 minutes). The delay of development of a constant rate of enzyme synthesis following dilution from a stationary state into fresh medium is therefore not the result of the need to overcome the effects of accumulation of an inhibitor in the medium as a result of growth.

Direct attempts to overcome a delay resulting from precursor deficiency by providing such precursors in the fresh medium at the time of dilution and introduction of the inducer were not effective in accelerating the attainment of a constant rate of synthesis. Neither the provision of enzymic hydrolysate of casein (200 $\mu\text{g}/\text{ml}.$) (Fig. 10), nor of a mixture of 50 $\mu\text{g}/\text{ml}.$ each of the 5'-diphosphates of adenosine, cytidine, guanosine and uridine (Fig. 11), influenced the period required to achieve a constant differential rate of synthesis. While it is known that E. coli will concentrate amino acids from the medium (6) it is probable that nucleoside diphosphates are unable to penetrate through the cell membranes. Attempts were made to circumvent the barrier to nucleotides by supplying a mixture of 128 $\mu\text{g}/\text{ml}.$ each of adenosine, cytidine, guanosine and uridine as free nucleosides. The result was rather unexpected (Fig. 12): in the presence of the nucleosides it took even longer (20 minutes) to reach a steady rate of synthesis than in the control (14 minutes) and the rate was only 20% of the control rate. This effect of the mixed nucleosides was probably related to some sort of catabolite repression and is being separately investigated. It must also be remembered that the free nucleosides are not normal metabolic intermediates in the biosynthesis of nucleotides.

As efforts to reduce the delay for constant enzyme synthesis were not successful the approach was reversed and attempts were made to increase it by interfering with RNA synthesis. The inhibitor of choice of RNA synthesis, actinomycin D, is not active in E. coli (15) and it was therefore decided to use 6-azauracil, shown by Habermann (12) to inhibit RNA synthesis by being converted to 6-azauridine-5'-phosphate and blocking orotidylic acid decarboxylase, an enzyme in the biosynthetic pathway of pyrimidine ribotides. 6-Azauracil (25 $\mu\text{g}/\text{ml}.$), added to a stationary culture 30 minutes before dilution, did not

affect the immediate logarithmic increase of optical density of the culture after dilution into fresh medium still containing the same concentration of the inhibitor. However, about 44 minutes after dilution the doubling time suddenly increased from 56 to 160 minutes (Fig. 13). The differential rate of β -galactosidase synthesis in the presence of 6-azauracil was almost identical with the control for the first 27 minutes after dilution, the control rate becoming constant after 18 minutes. Thereafter in the period between 27 and 30 minutes the differential rate in the presence of 6-azauracil fell by 90% (Fig. 14). The relatively long time necessary for 6-azauracil to exert its effect precluded any inhibition of the initial increase in the rate of β -galactosidase synthesis. When it did become effective it showed itself much more inhibitory to the synthesis of the inducible enzyme than it did towards growth in general, as illustrated by the large fall in the rate of differential enzyme synthesis. Addition of 6-azauracil to a logarithmically growing population similarly inhibited growth in about 30 minutes (Fig. 15) and enzyme synthesis a few minutes earlier (Fig. 16).

These data permit a number of conclusions to be reached concerning the response of the bacterial cell to a sudden transition from a stationary state of growth into fresh medium. The addition of inducer to a logarithmically growing population results in a steady differential rate of enzyme synthesis being reached within 3 minutes (Fig. 3). This rate is maintained until growth stops due to exhaustion of glycerol in the medium. In this condition of starvation mRNA specific for β -galactosidase is not synthesized since addition of the inducer during/stationary phase does not result in the immediate synthesis of enzyme when dilution takes place and the carbon and energy source again becomes plentiful. Pre-existing β -galactosidase mRNA breaks down during this stage, a

conclusion reached because the continued presence of an inducer from a previous period of enzyme synthesis also fails to ensure the synthesis of more enzyme immediately following dilution (Fig. 7). During starvation, then, mRNA is not formed and a labile mRNA decomposes.

Yet, with no lag, the optical density of the culture begins to increase logarithmically as soon as dilution occurs. We may thus pose two vital questions: (a) is the lag in the attainment of a steady differential rate of enzyme synthesis related to the inducibility of β -galactosidase (i.e. the control of its synthesis by a regulator gene)?; (b) what, in terms of various growth parameters, does a logarithmic increase immediately after dilution in the optical density of the culture signify?

If increase in optical density is related to growth, at least as far as protein synthesis is concerned, the mRNA's required to synthesize such protein must have survived from the previous growing period as, judging by the one for β -galactosidase, mRNA is not readily produced during starvation or immediately after dilution into fresh medium. Further, if β -galactosidase mRNA is unstable and must continuously be resynthesized to maintain enzyme production, while other proteins depend on stable mRNA's, the greater sensitivity to 6-azauracil of the β -galactosidase synthesis compared with growth becomes clear. 6-Azauracil eventually stops the formation of all mRNA. As the one for β -galactosidase synthesis is unstable, the synthesis of this protein rapidly stops. But other proteins, dependent on longer-lived mRNA's, are able to continue to be produced at a decreasing rate in the absence of mRNA transcription from DNA.

In the event that the above remarks on the instability of β -galactosidase mRNA and its lack of formation during starvation and immediately after dilution owing to a deficiency of precursors are correct, one would not expect to alter kinetics depending on mRNA stability by mutation of the regulator gene of the lac operon. Thus a corresponding i^- strain of E. coli would also be

show
expected to/a differential lag of β -galactosidase synthesis when it is diluted from stationary phase. This is indeed the case. There is no lag before the optical density of a constitutive culture, diluted from stationary phase into fresh medium, begins to increase logarithmically, but β -galactosidase formation shows the same sort of lag as in the inducible strain (Fig. 17). In this experiment the differential rate of synthesis was not constant until 42 minutes after dilution though there was no lag in optical density increase. 6-Aza-uracil has an effect in the constitutive strain similar to that with the inducible: a zero rate of differential enzyme synthesis after 30 minutes (Fig. 17). In a logarithmically growing culture, too, 6-azauracil affects the constitutive strain as it affects the inducible: growth slows down (Fig. 18) and enzyme synthesis stops (Fig. 19) in about 30 minutes. It should be noted that the scatter of the experimental points is invariably greater in the constitutive experiments as the proportional increase of enzyme activity for each minute of incubation is very much less than in the inducible strain shortly after induction is initiated.

An investigation was made of several aspects of growth following dilution in the presence and absence of 6-azauracil. In the first of these experiments (Fig. 20), the increase with time of the optical density of the culture was compared with the numbers of cells/ml. and with the total volume of cellular material/ml. of suspension. Cell number was measured in a suitably diluted aliquot of the culture with a Coulter particle counter (22). The size distribution of each population was obtained with a Coulter size distribution plotter, and the total volume of cells in the sample calculated in arbitrary units as the sum of the products of each channel number and the number of particles in that channel. An average cell volume was also calculated from

these data. The results in Fig. 20 show that in the absence of 6-azauracil, optical density increased logarithmically from the time of dilution as before, cell number showed no increase for 34 minutes and then increased logarithmically, while the total cell volume did not change for 19 minutes, when an exponential increase started. In the presence of 6-azauracil, the optical density increased rapidly for 30 minutes and then slowed down. There were low rates of increase of cell number and cell volume compared with the control, with delays of about 44 and 18 minutes, respectively. Thus the exponential increase in optical density which starts immediately after dilution is directly related neither to the number of cells nor to the total volume of cells. This suggests that the optical density rise may result from an increase in the refractive index of the cells derived from an elevated internal level of certain macromolecules.

A second experiment was thus concerned with the levels of total DNA, RNA and protein, again with and without 6-azauracil. For measurement of these substances, aliquots of the suspension were removed at intervals after resumption of growth into chilled trichloroacetic acid to give a final concentration of 6% trichloroacetic acid. Protein, DNA and RNA were determined as described by Berrahand Konetzka (3). Since samples were collected initially at 2 minute intervals and very little increase was to be expected from one sample to the next, all volumetric measurements throughout this experiment were checked gravimetrically by using tared tubes, etc. The findings are presented in Fig. 21. As carefully as we can determine from the experimental data, there is in the control series indeed no lag in the increase in protein (14), while a lag of 5.5 minutes was found for RNA and one of about 27 minutes for DNA. In the presence of 6-azauracil the initial lags for RNA and DNA were about the same as in the control, but production of these substances slowed down or stopped after 20-25 minutes. Protein increased very rapidly, and with no lag, for about 5 minutes, and then slowed down.

The precise times reported in this experiment are certainly subject to appreciable error, but we see no way at this time of improving the accuracy of these experimental readings. It seemed desirable in addition to investigate the rate of formation of a particular identifiable protein, preferably a constitutive one, following dilution. Malic dehydrogenase was chosen and this was determined by incubating toluene-treated cells with oxalacetate and NADPH_2 at 37° in buffer at pH 7.4 and following the fall in optical density at 340 μ with a Gilford Model 2000 Multiple Sample Absorbance Recorder. As with other measurements of the kinetics of formation of constitutive substances, considerable experimental scatter was found. Nevertheless the results shown in Fig. 22 demonstrate fairly convincingly that there is no lag in the increase in activity of this enzyme when the cells are placed in fresh medium. The line drawn in Fig. 22 was calculated by the least squares method to be the best fit for the points represented by solid circles; the open triangle points were excluded from the calculation.

All the evidence points, then, to the lag in β -galactosidase synthesis being due to a corresponding lag in the production of mRNA as a consequence of depletion of the nucleotide pools during starvation. Kepes (18) has divided the sequence of events between introduction of the inducer and appearance of active enzyme into six stages and we may consider the β -galactosidase lag in these terms. Stages 1 and 2 concern the penetration of the inducer into the cell and its interaction with "repressor." These events cannot be responsible for the lag since the constitutive strain, requiring no inducer, demonstrates the lag as markedly as does the inducible strain. Stages 5 and 6 refer to the synthesis of polypeptide using the information carried by mRNA and the establishment of secondary, tertiary and quaternary structural characteristics associated with the enzymic activity of the protein. The lag cannot be ascribed to these phenomena since other proteins, including

malic dehydrogenase, are synthesized without lag, and no shortage of amino acids seems to result from starvation. We are thus left with stages 3 and 4: the transcription of DNA into mRNA and the transfer of the message to the ribosomes. The latter stage seems difficult to reconcile with the lag because there appears to be no hurdle to the transfer of messages for other proteins to the ribosomes. Stage 3, then, remains the most likely origin of the lag, and this interpretation is supported by the lag in total RNA formation found experimentally (Fig. 21) and by the greater sensitivity of inducible enzyme formation compared with growth to agents interfering with RNA production. 6-Azauracil, as shown in the present study and by Habermann (12), inhibits the production of an inducible enzyme earlier and more severely than growth. The action of actinomycin D, as reported in the literature is also in this direction. In Bacillus subtilis the inducible formation of histidase is completely inhibited at 10 $\mu\text{g/ml}$. (13), while at 0.2 μM actinomycin RNA synthesis is inhibited 90-95% and protein synthesis only 50-75%, and the base composition of the RNA produced did not resemble the normal base composition of B. subtilis RNA or DNA (15). The point of the latter observation was not lost on the authors. They remarked (15) that "the observation that RNA synthesis can be inhibited in vivo with only partial inhibition of protein synthesis.....raises some questions concerning the nature of "messenger" RNA, which, according to Jacob and Monod (16) should possess a short half-life. A possible explanation is that not all "messenger" RNA molecules are short-lived but that some may act catalytically for a long time after the synthesis of new messenger is interrupted." We conclude that if the lag for inducible enzyme formation is to be ascribed to a lag in RNA production, including mRNA, and not associated with the induction process itself, then the absence of lag for other proteins reflects the continued presence of functional mRNA for

these proteins remaining from the previous growth period. The latter mRNA is therefore not labile.

The evolutionary consequences of differential mRNA stability is intriguing if we consider that mRNA for inducible enzymes is labile and that for constitutive enzymes is stable. The high rates of formation and decay of inducible messenger gives the cell a means of responding very sensitively to environmental changes by adapting its content of enzymes. This may well have become superfluous for more basic enzymes which need always to be present in about the same quantities, irrespective of the external environment. Such enzymes would most satisfactorily be truly constitutive because they would always be made whenever the cell was growing. Their mRNA's would similarly always need to be present, and no advantage would accrue to a cell which retained an inducible-type regulatory mechanism which was always switched on during growth. Rather this might be expected to be a disadvantage, resulting as it would in the continuous expenditure of energy to produce mRNA which was just as rapidly decomposed. We might expect that in evolutionary time such a disadvantage would have been selected against, and inducible control and labile mRNA for constitutive proteins to have died out in strains exposed to evolutionary pressure. The intracellular balance of constitutive enzymes might now be controlled in the manner proposed by Pardee and Beckwith (26). In the laboratory, where regulatory control can be removed by genetic mutation, we might still expect artificial constitutive proteins with short-lived mRNA, and this indeed appears to be the case with i^- mutants of the lac operon in E. coli.

This leads to further questions concerning the differences in structure between stable and labile mRNA which permits one type to be destroyed but not

the other. This, of course, is bound up further with the reasons for the apparent stability of the other forms of RNA which do not have a messenger function. Indeed the explanations of stability differences may/not so much ^{be} in the differences of structure of the molecules themselves but perhaps in the different environments within the overall cell structure in which they normally occur, and the varying spectrum of hydrolytic and other enzymes to which they are exposed. One way in which the difference between the short-lived and the long-lived molecules might be determined would be in their degree of association with particular organelles, especially the ribosomes. Thus, those types of mRNA/^{which} for some reason remain intimately a part of the ribosomes would be expected to resist the intracellular RNase much more than the free-living messenger molecules. The transition, then, from short to long life would involve the degree of association with the ribosomal protein and lipid.

If in evolutionary terms a change from inducible regulation to constitutive production of a protein, or vice versa, eventually results in a change in stability of the corresponding mRNA, we might expect that on occasion the cell has had to pay a price for an advantage gained. An alteration in stability ^{ultimately} would be expected/to be dependent on a change in base sequence. This change might have no consequence other than a change in stability control, either directly or indirectly through the binding with ribosomes. On the other hand, the change might result sometimes in the protein also having a slightly altered structure, an effect which in some cases might lead to enhanced activity of the protein and in other instances to diminished effectiveness. Evolutionary pressure in such a situation might lead to a whole series of compromises,

from a sacrifice of the preferential means of control of biosynthesis, through the retention of mRNA with atypical stability for the type of biosynthetic control being used, to the production of a protein molecule which does not possess the most effective structure for the catalytic or other function which it performs. The testing of such ideas must await the development of more refined techniques for measuring the levels of many specific mRNA's, for determining nucleic acid structure and for understanding how structure and function are intimately related in the nucleic acids and proteins.

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Moses and Calvin: Figure Captions (1)

Fig. 1. Growth of E. coli C600-1 in M63 medium at 37°. Optical density measured at 650 m μ in a Cary Model 14 spectrophotometer. At about 4 hr. a portion of the stationary culture was diluted fivefold into fresh M63 medium.

Fig. 2. Growth of E. coli C600-1. At the time indicated by the arrow addition of glycerol (curve A) or ammonium sulphate (curve B) was added to the stationary culture.

Fig. 3. Induction of β -galactosidase activity in logarithmically growing E. coli C600-1 with TMG added as indicated by the arrow.

Fig. 4. Induction of β -galactosidase activity in E. coli 300U following dilution of a stationary culture into fresh medium containing IPTG. Absorbance of culture immediately after dilution indicated by the arrow.

Fig. 5. Induction of β -galactosidase activity in E. coli 300U following dilution of an exponentially growing culture into fresh medium containing IPTG.

Fig. 6. Induction of β -galactosidase activity in E. coli C600-1 with IPTG: A, in exponentially growing culture; B, in stationary culture.

Fig. 7. Induction of β -galactosidase activity in E. coli C600-1 with IPTG following dilution from stationary phase into fresh medium: A, IPTG added 4 hr. before dilution; B, IPTG added at time of dilution.

Fig. 8. Growth of E. coli C600-1 when diluted from stationary phase into fresh medium (A) or exhausted medium (B).

Fig. 9. Induction of β -galactosidase activity in the same experiment as shown in Fig. 8; in both cases IPTG added at time of dilution. A, fresh medium; B, exhausted medium. Arrows indicate absorbance of culture immediately after dilution.

Fig. 10. Effect of casein hydrolysate on induction of β -galactosidase activity in E. coli C600-1 following dilution from stationary phase into fresh medium containing IPTG: A, control; B, plus casein hydrolysate (200 μ g/ml.).

Moses and Calvin: Figure Captions (2)

Fig. 11. Effect of four ribonucleotides on induction of β -galactosidase activity in E. coli C600-1 following dilution from stationary phase into fresh medium containing IPTG: A, control; B, plus 50 μ g/ml. each of ADP, CDP, GDP and UDP.

Fig. 12. Effect of four ribonucleosides on induction of β -galactosidase activity in E. coli C600-1 following dilution from stationary phase into fresh medium containing IPTG: A, control; B, plus 128 μ g/ml. each of adenosine, cytidine, guanosine and uridine.

Fig. 13. Effect of 6-azauracil on growth of E. coli C600-1 following dilution from stationary phase to fresh medium: A, control. B, plus 6-azauracil (25 μ g/ml.). Azauracil added to B and water added to A as indicated by solid arrows: dilutions indicated by dashed arrows.

Fig. 14. Induction of β -galactosidase activity in the same experiment as shown in Fig. 13; in both cases IPTG added at time of dilution. A, control; B, plus 6-azauracil.

Fig. 15. Effect of 6-azauracil on exponentially growing cells of E. coli C600-1. A, control; B, 6-azauracil (25 μ g/ml.) added at arrow.

Fig. 16. Induction of β -galactosidase activity in the same experiment as shown in Fig. 15; IPTG added at arrow. A, control; B, 6-azauracil, added 2 min. before IPTG.

Fig. 17. Activity of β -galactosidase in E. coli 230U (i⁻ constitutive) following dilution from stationary phase into fresh medium. A, control; B, plus 6-azauracil (25 μ g/ml.) added 30 min. before dilution.

Fig. 18. Effect of 6-azauracil on growth of E. coli 230U. A, control; B, 6-azauracil (25 μ g/ml.) added at arrow.

Moses and Calvin: Figure Captions (3)

Fig. 19 β -Galactosidase activity in the same experiment as in Fig. 18.

A, control; B, 6-azauracil (25 μ g/ml.) added at arrow.

Fig. 20. Effect of 6-azauracil on cell enlargement and division following dilution of a stationary culture of E. coli C600-1 into fresh medium.

Series A, control; series B, 6-azauracil (25 μ g/ml.) added 30 min. before dilution.

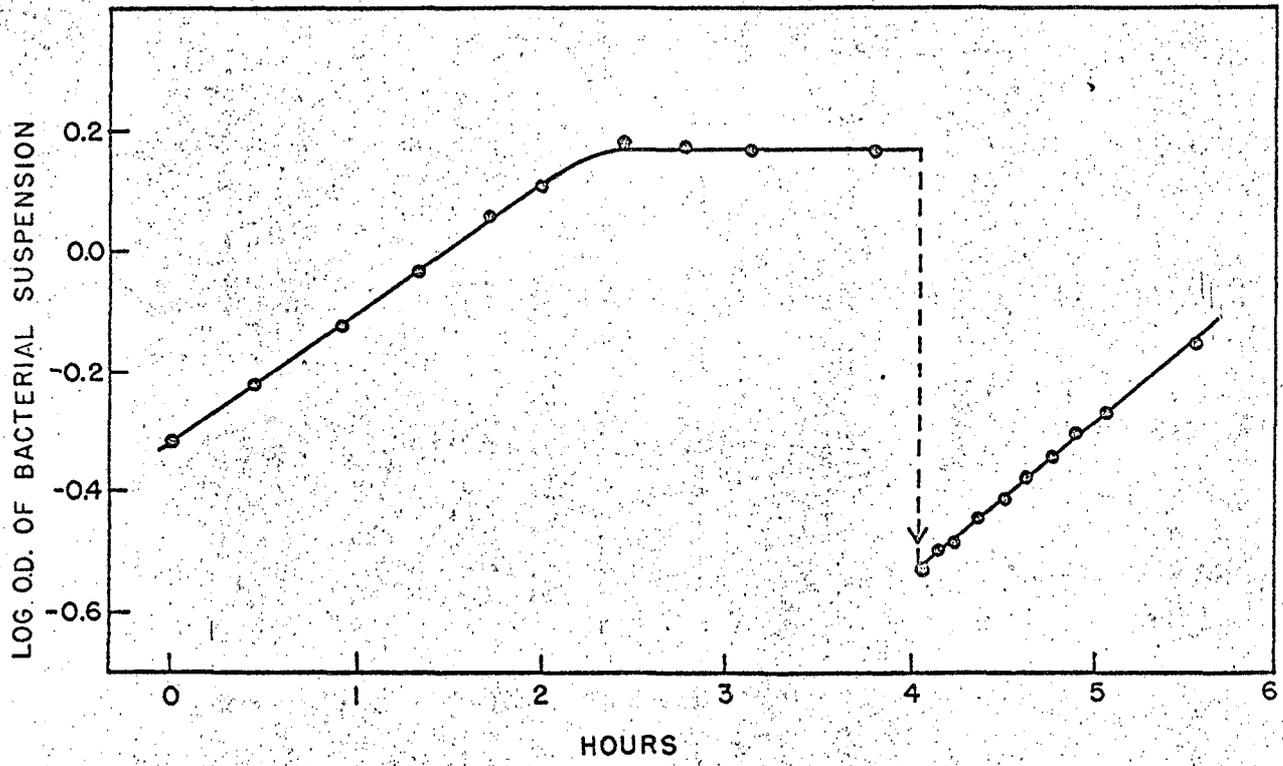
Fig. 21. Effect of 6-azauracil on synthesis of protein, RNA and DNA following dilution of a stationary culture of E. coli C600-1 into fresh medium.

Series A, control; series B, 6-azauracil (25 μ g/ml.) added 30 min. before dilution.

Fig. 22. Activity of malic dehydrogenase in E. coli C600-1 following dilution of a stationary culture into fresh medium. Open triangle points excluded from calculation of best fitting straight line.

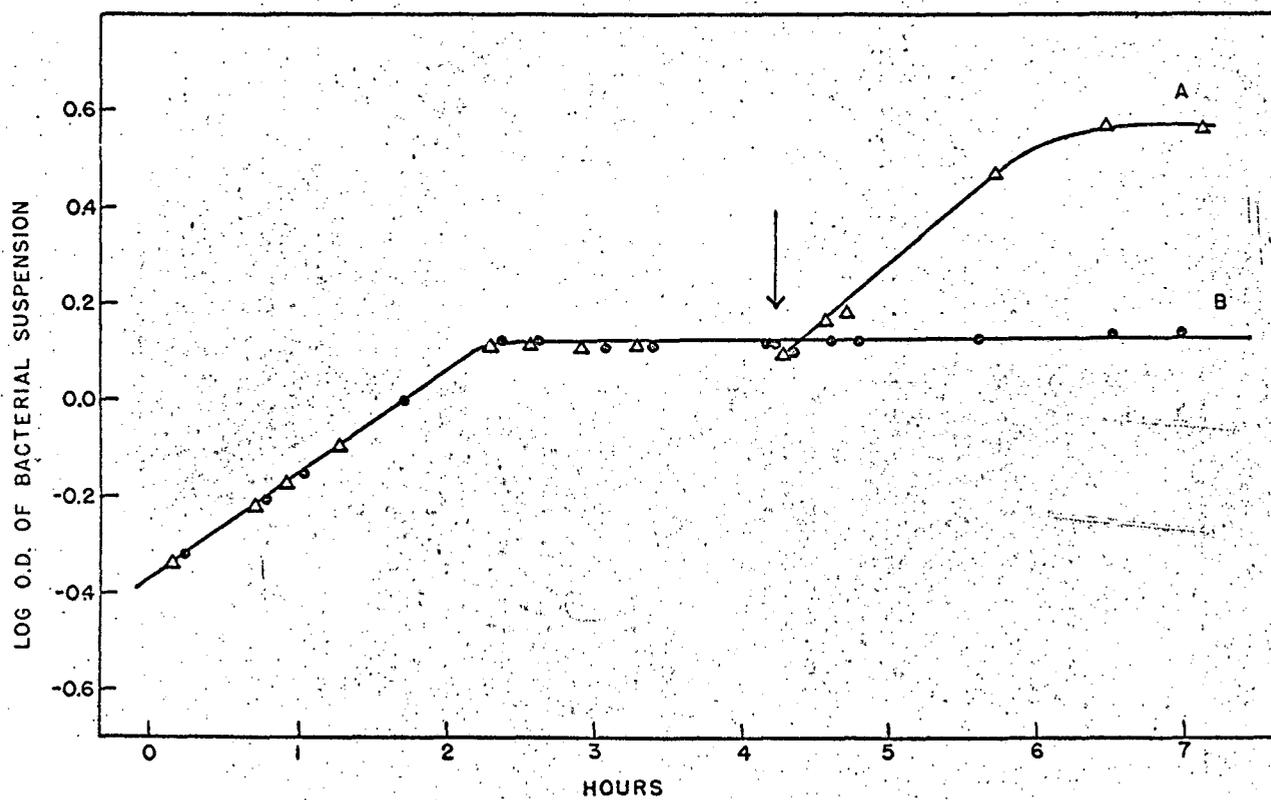


FIG. 1.



MUB-3980

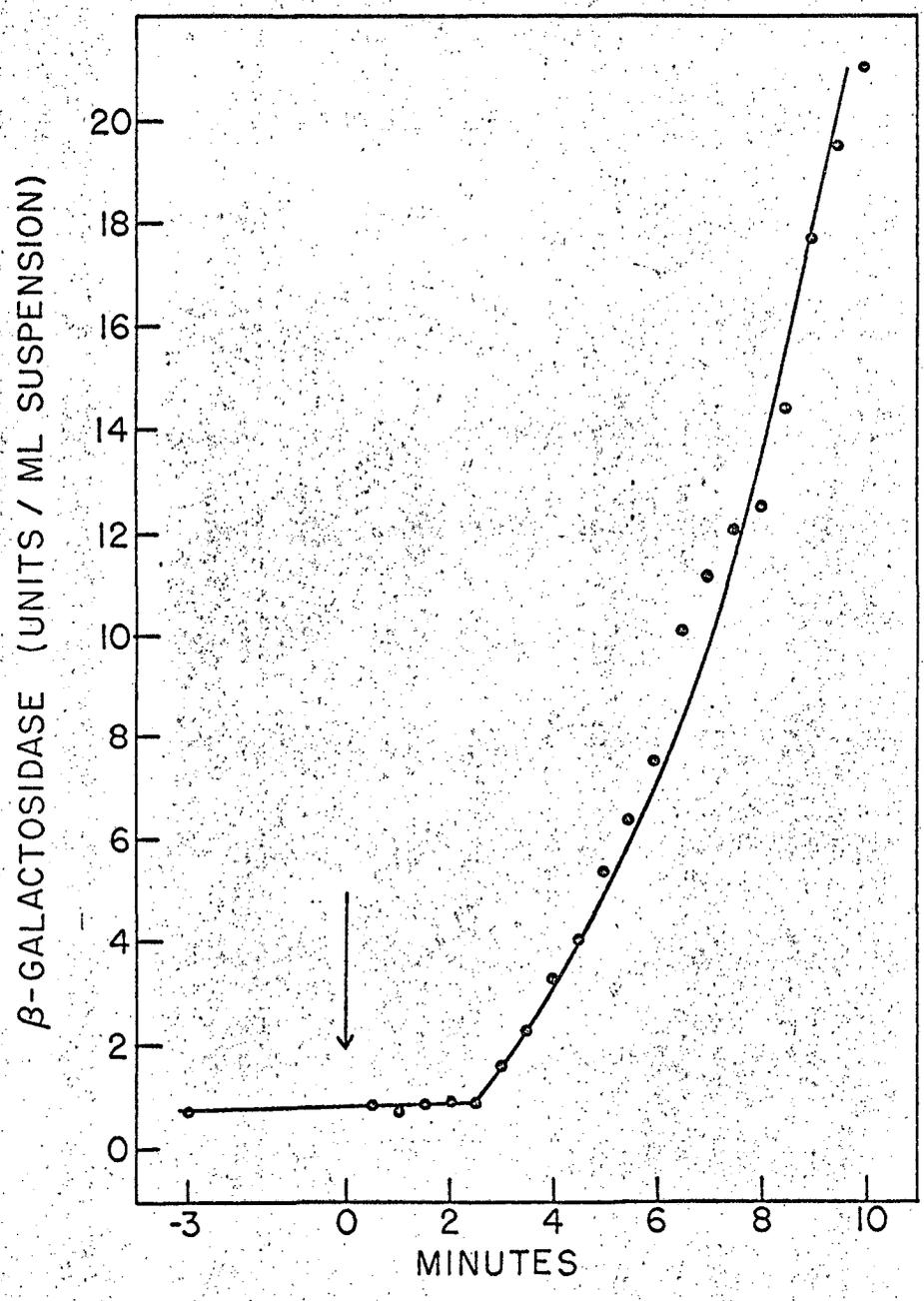
FIG. 2.



MUB-3981

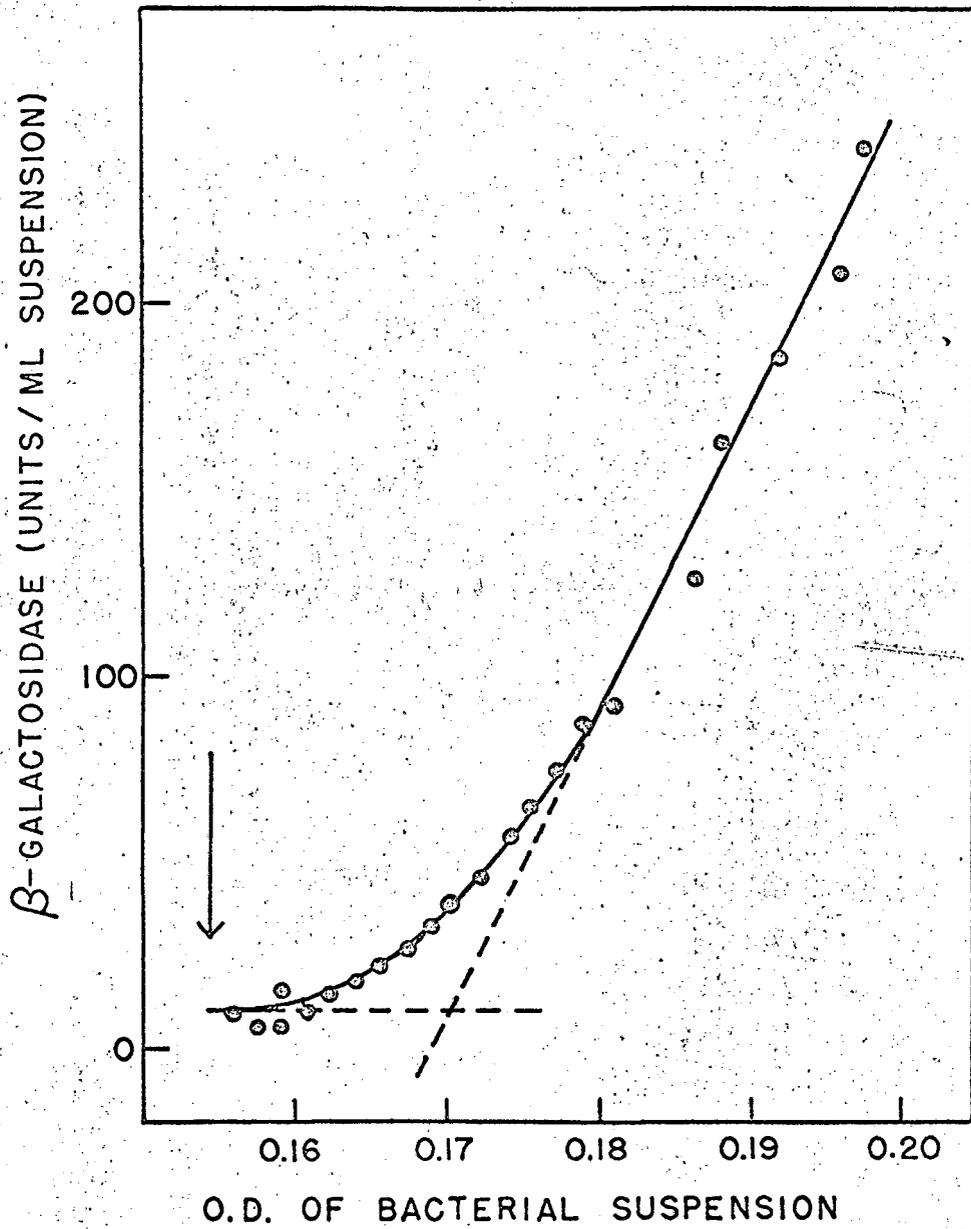


FIG. 3



MUB-3979

FIG-4



MUB-3982

FIG. 5

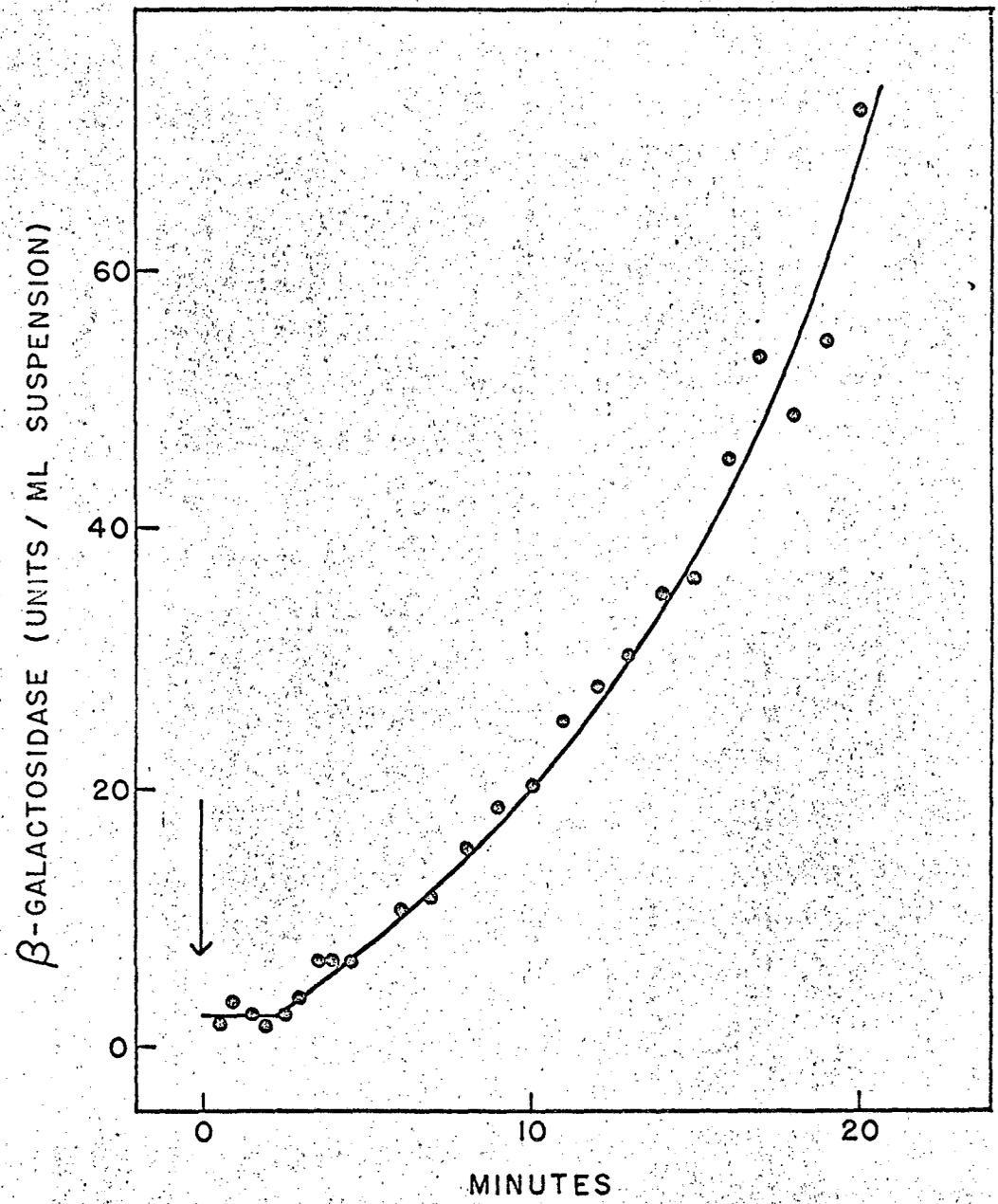
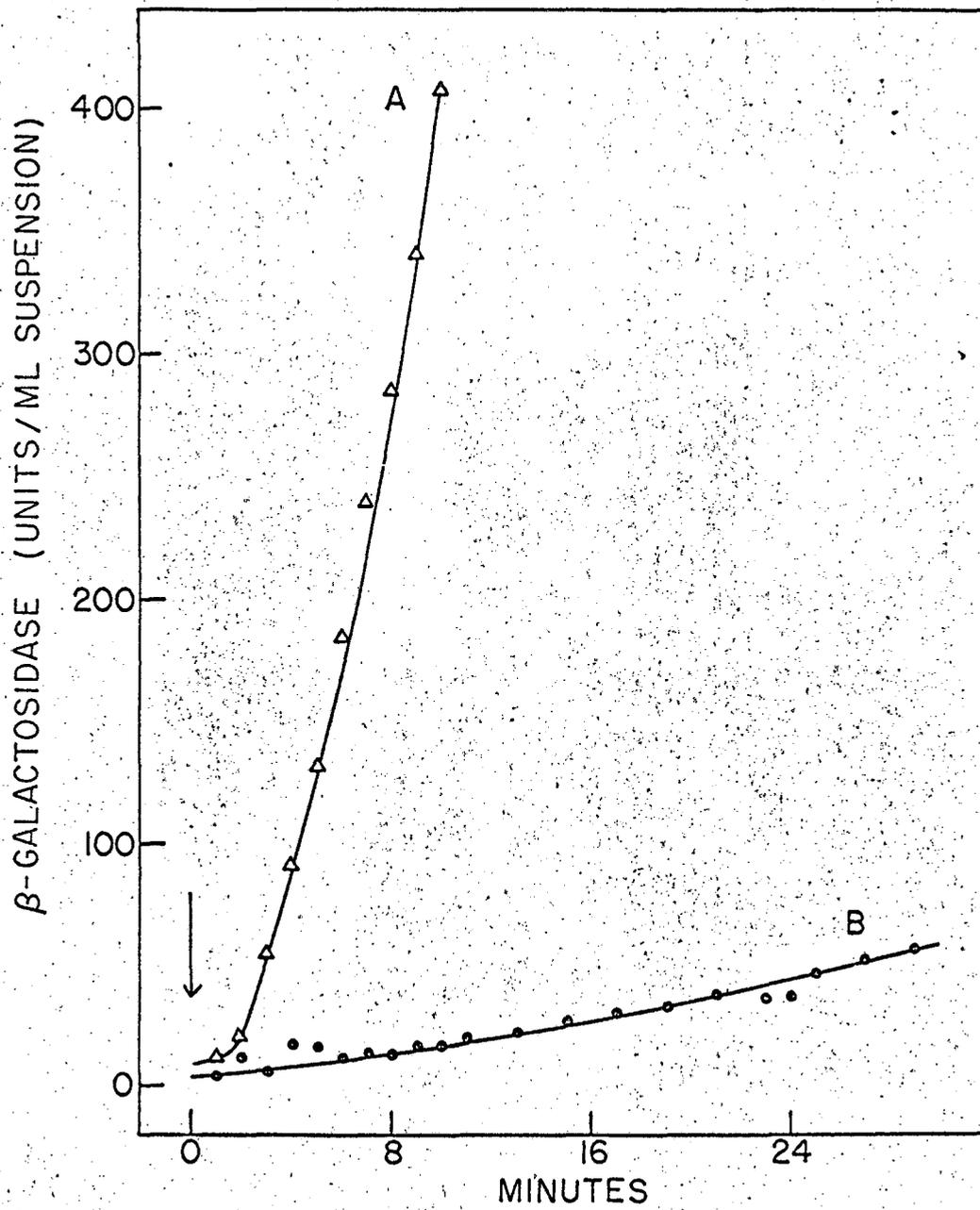


FIG. 6



MUB-3984



FIG. 7

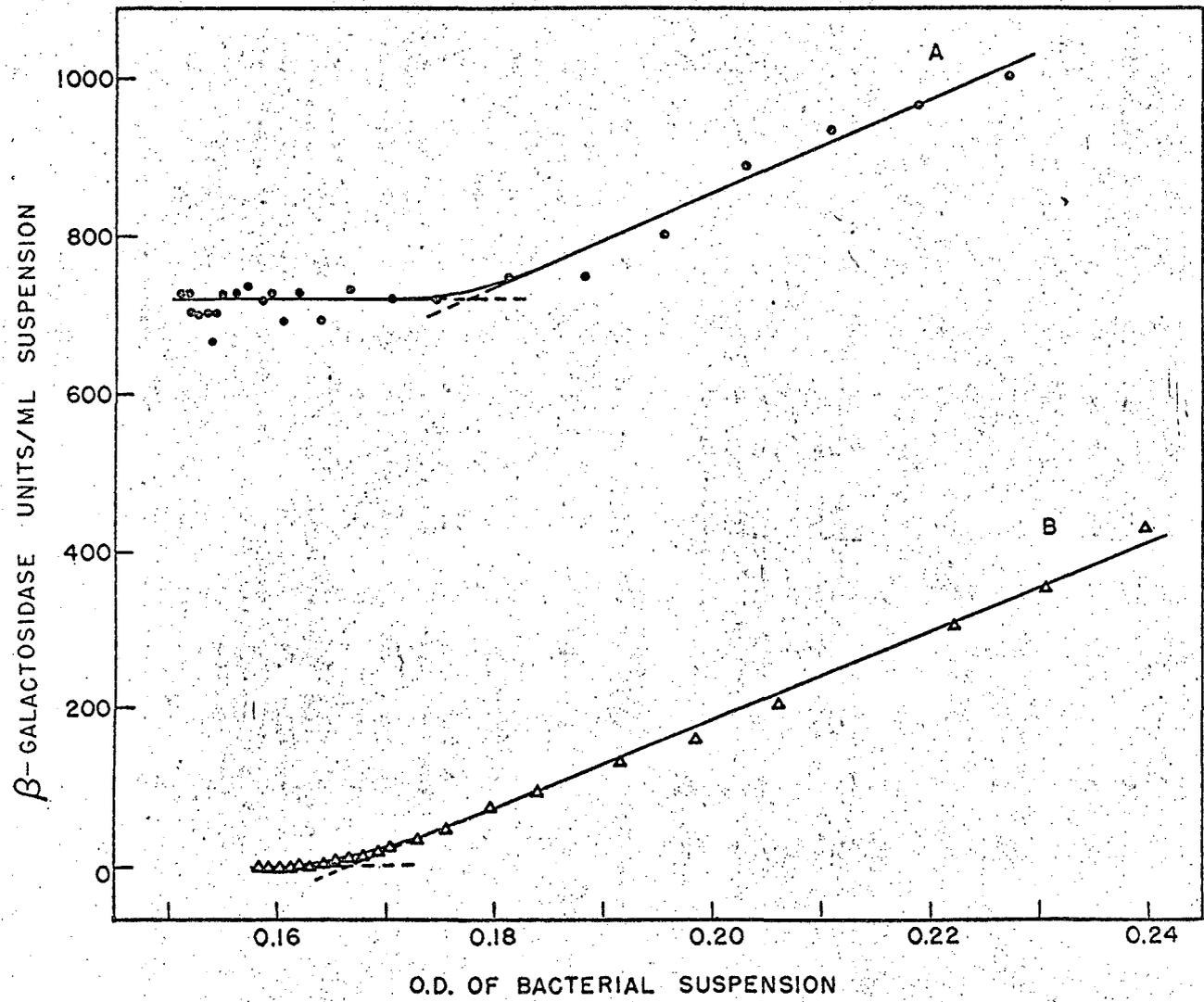


FIG-8

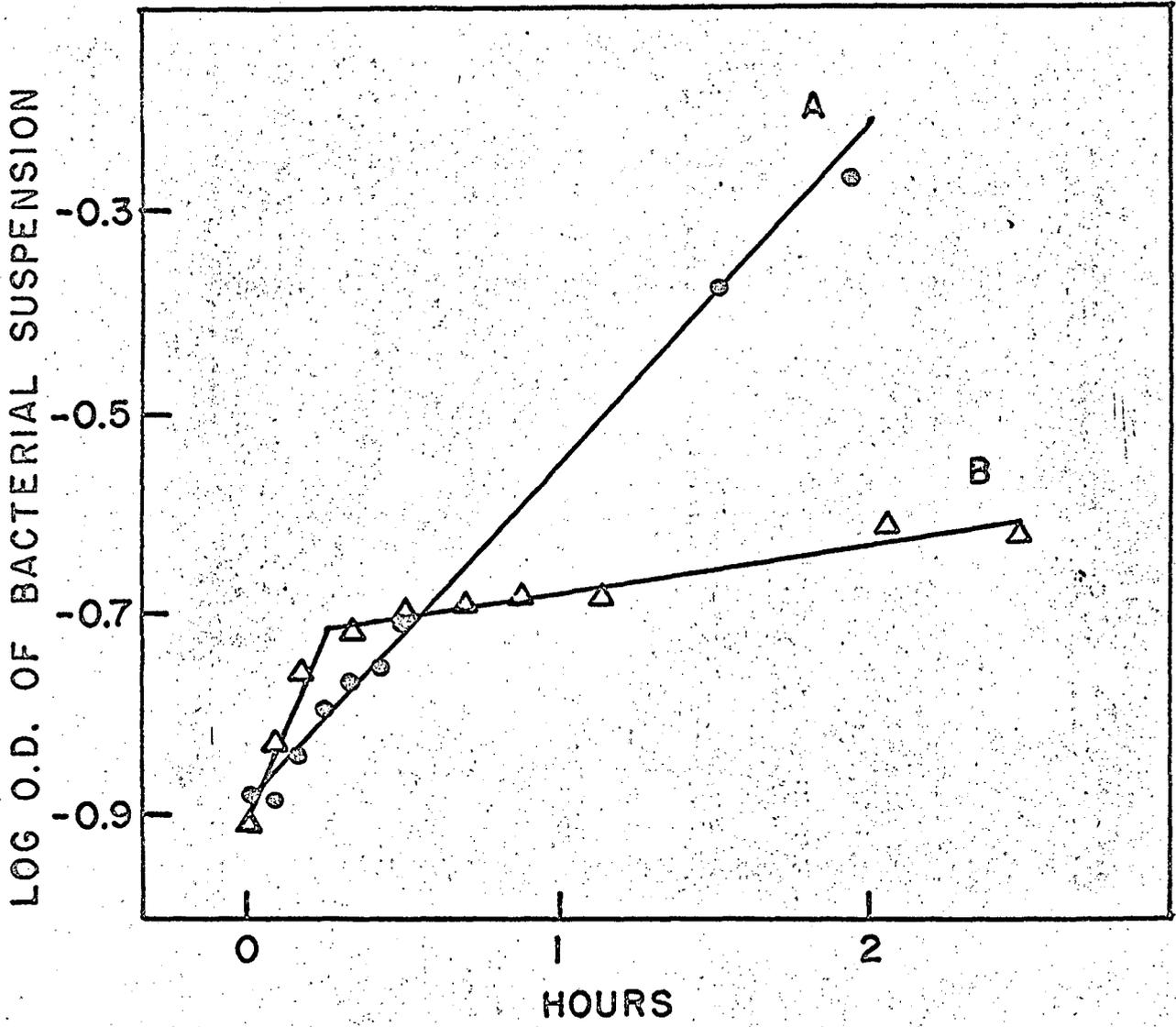
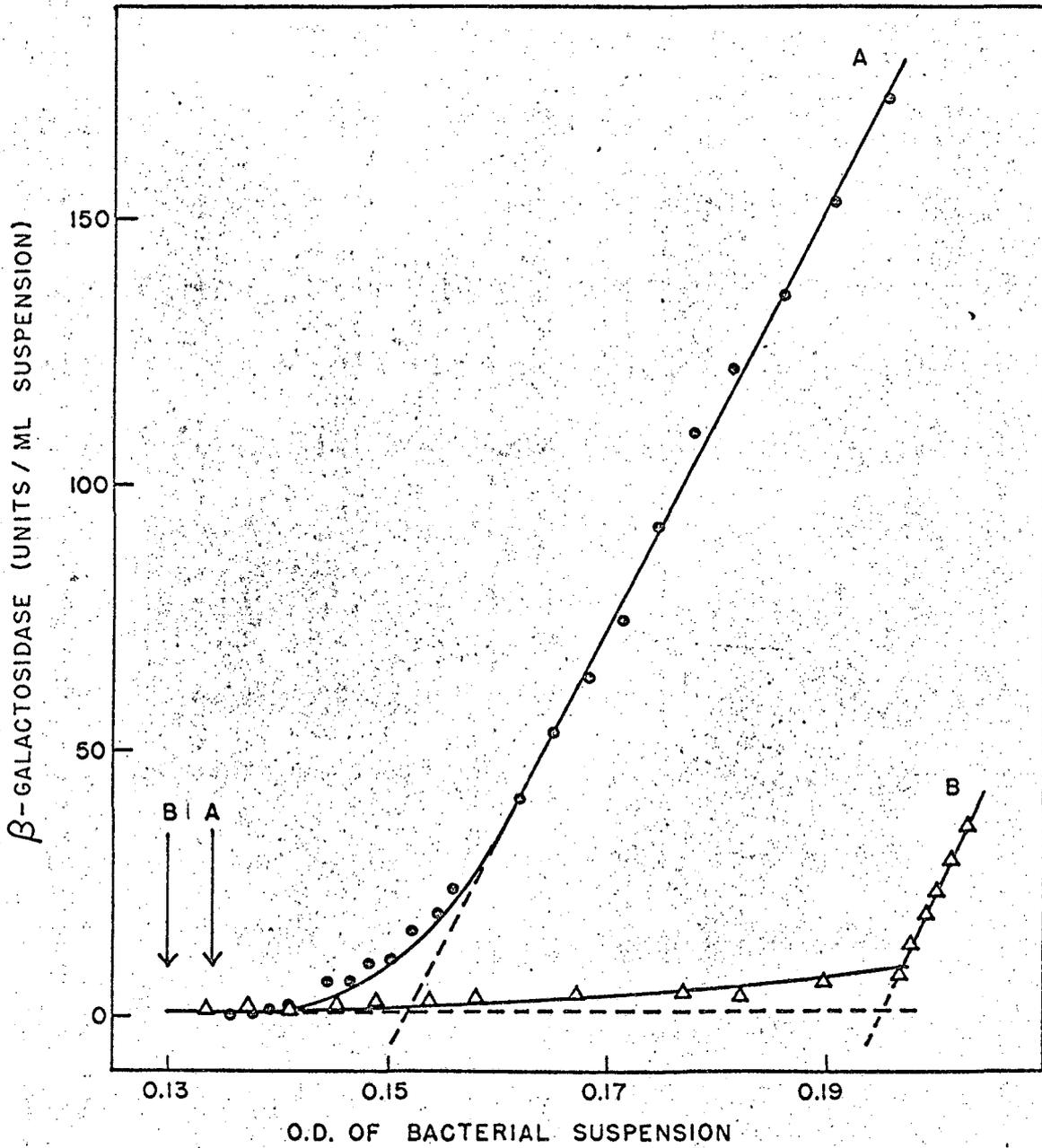


FIG. 9



MUB-3989

FIG. 10

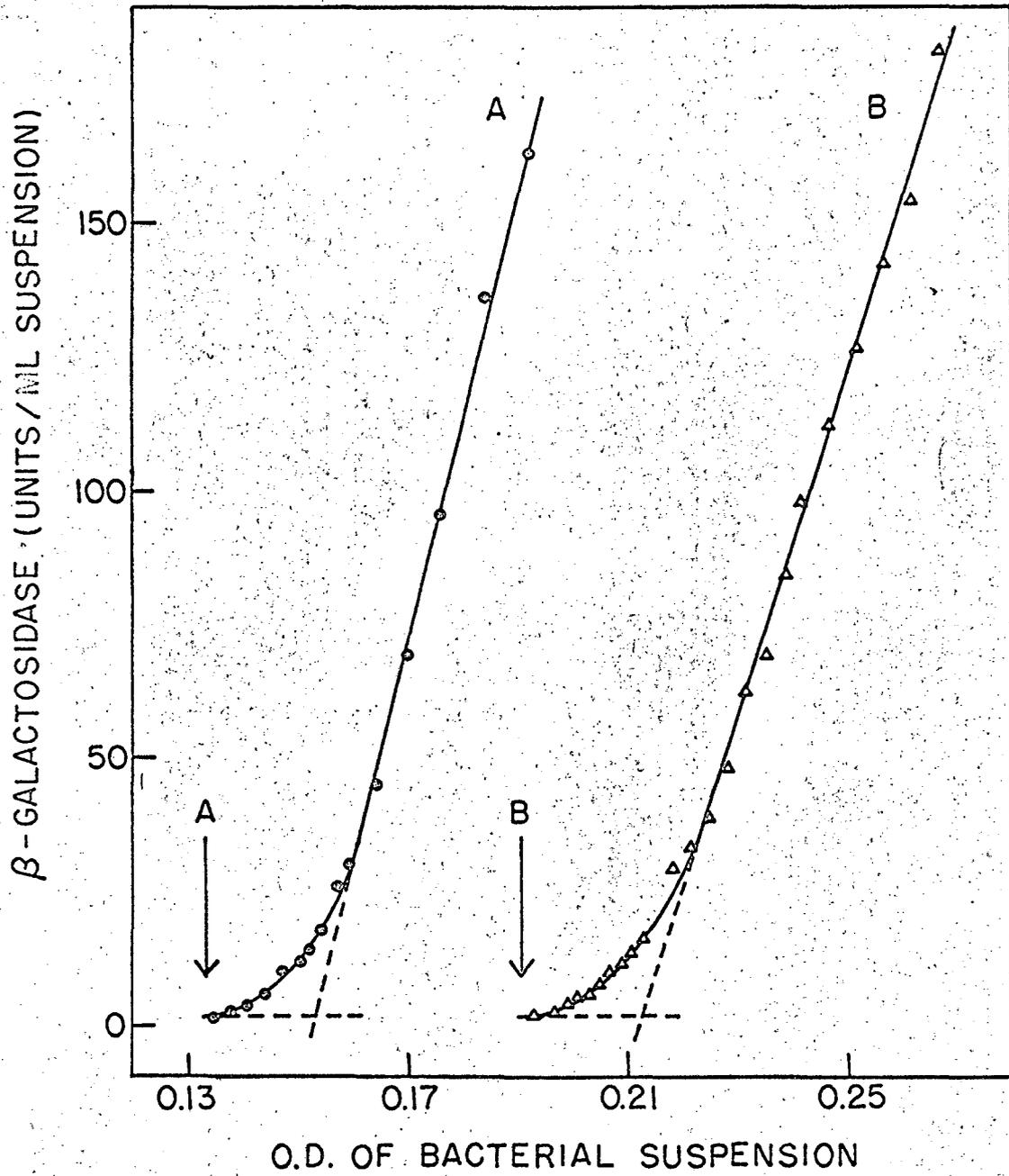




FIG. 11

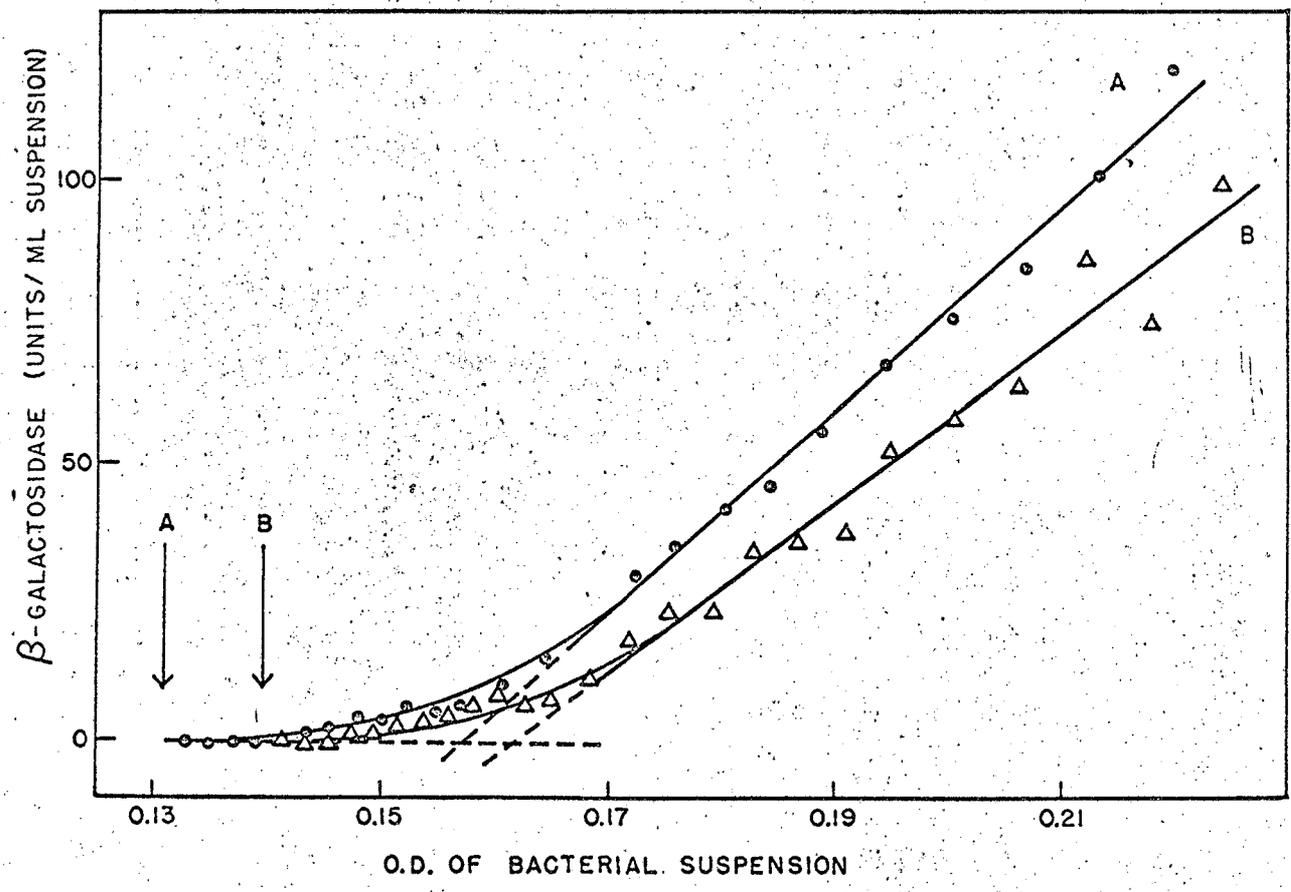


FIG. 14

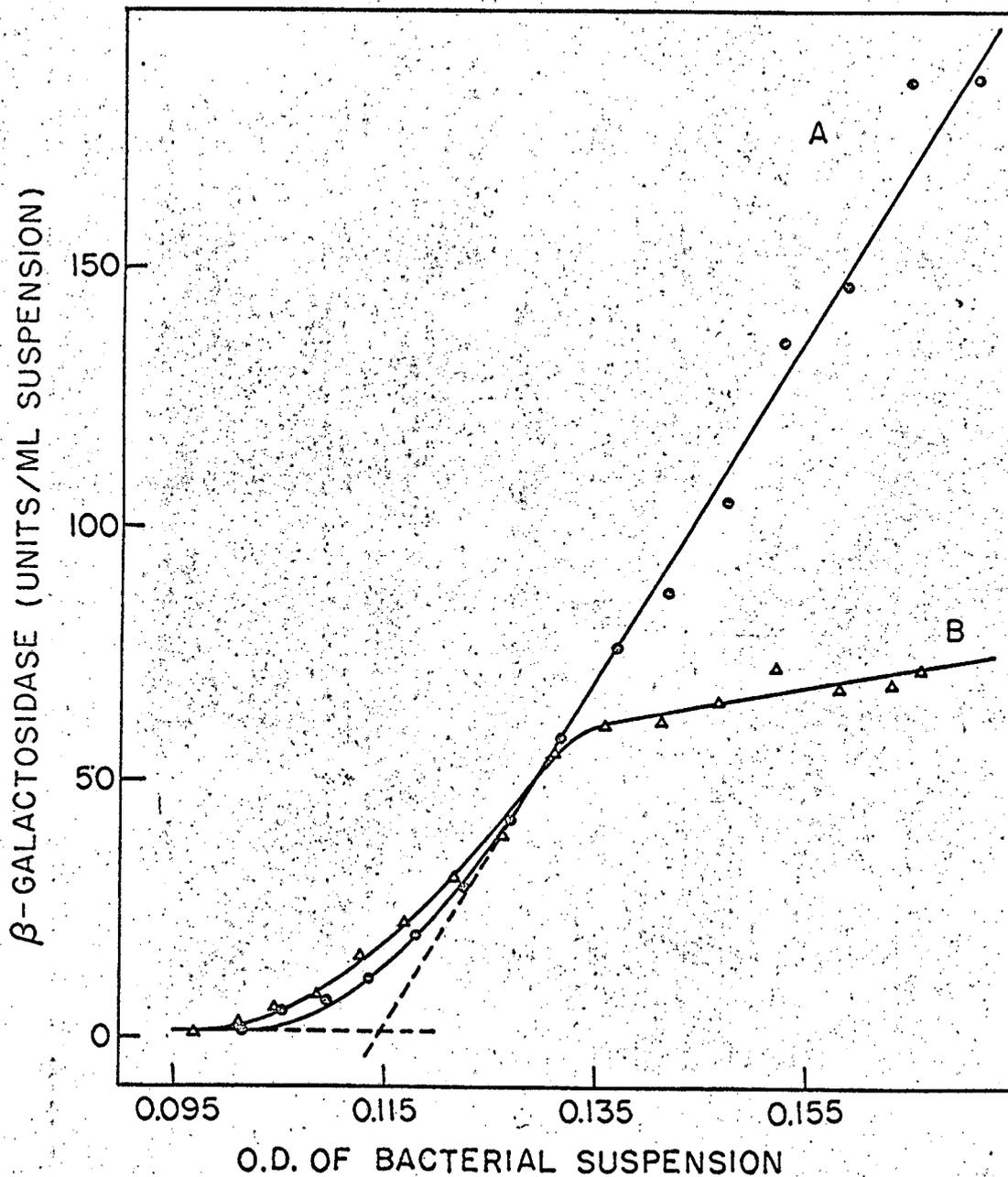
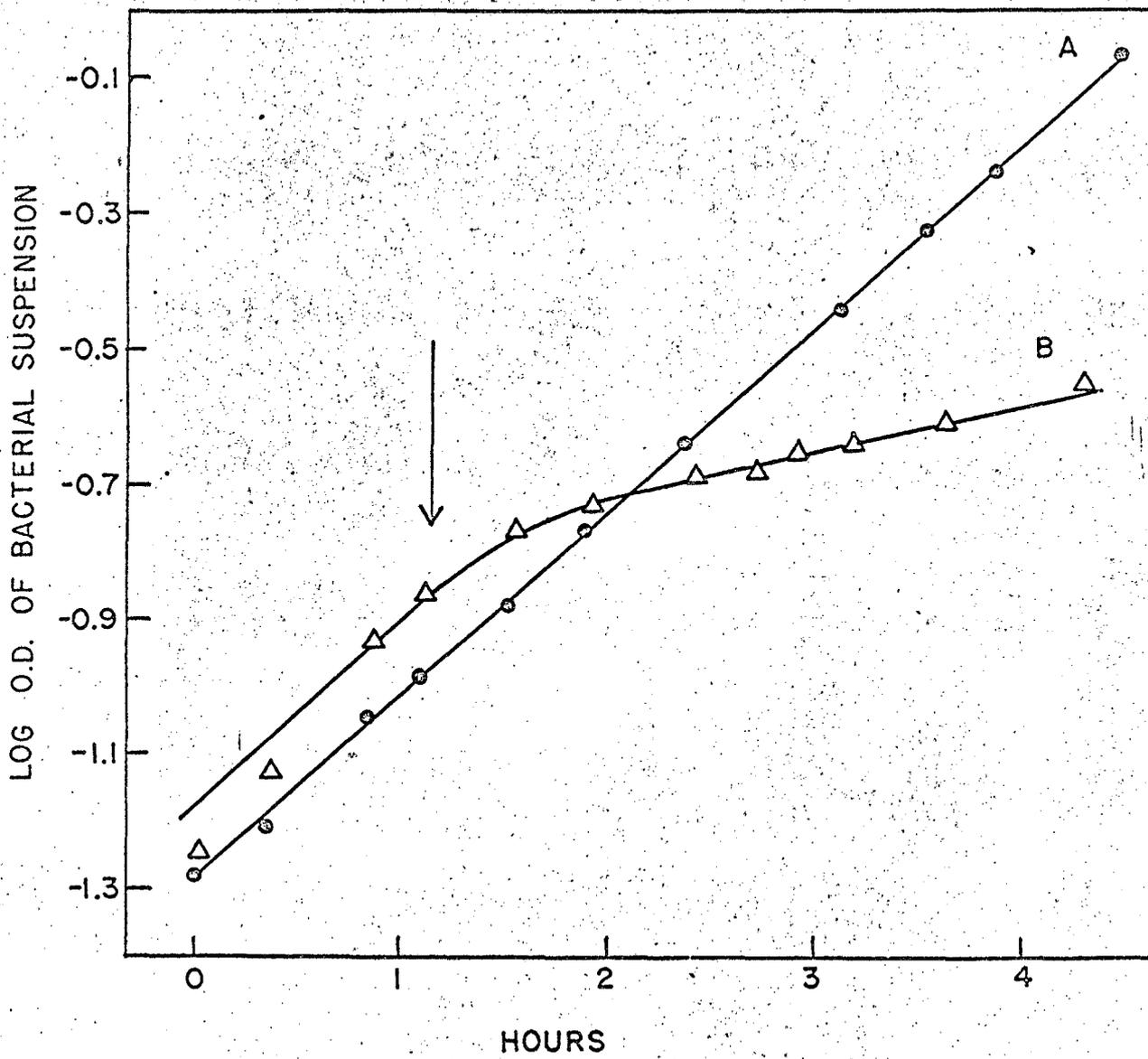
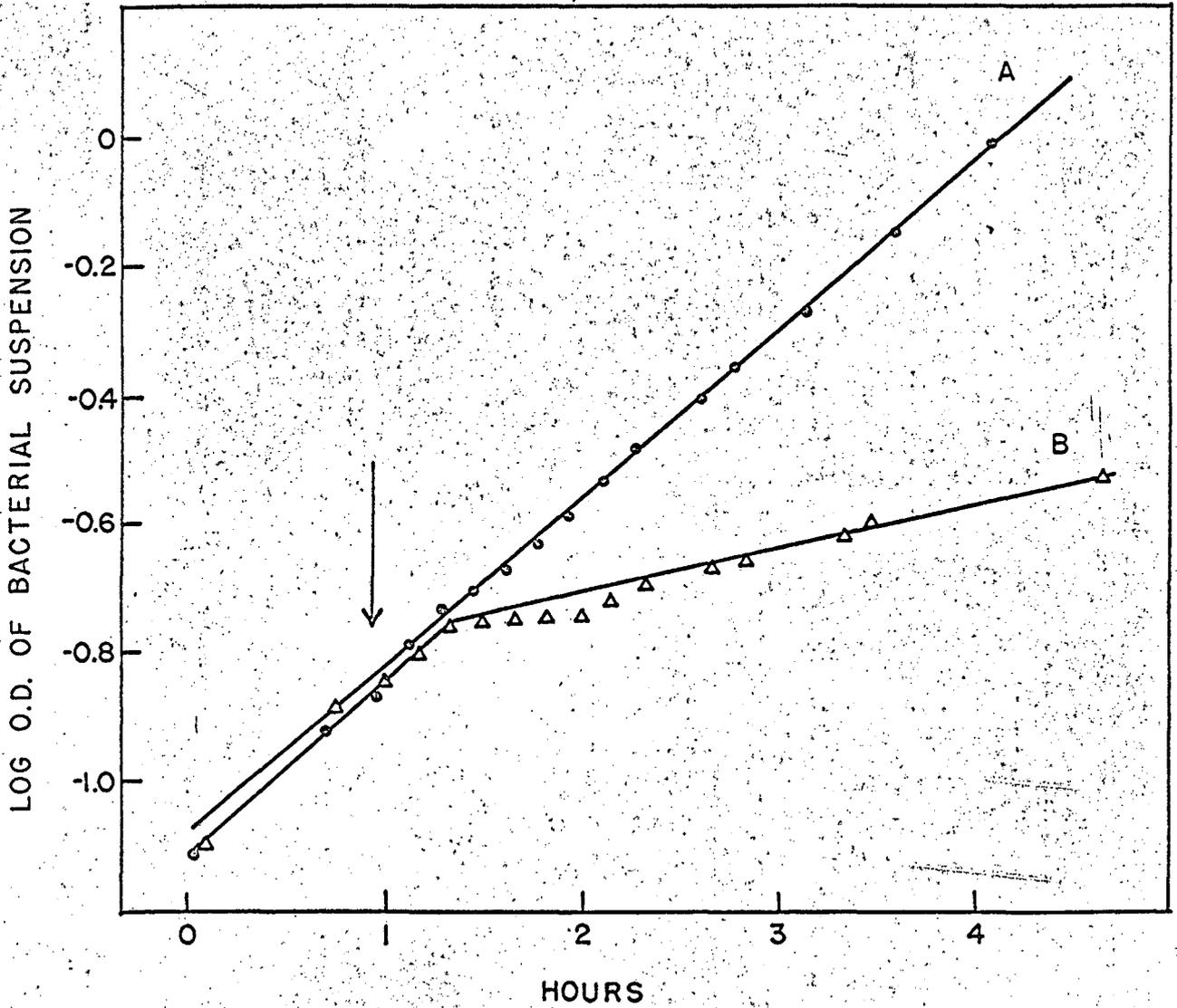


FIG. 15



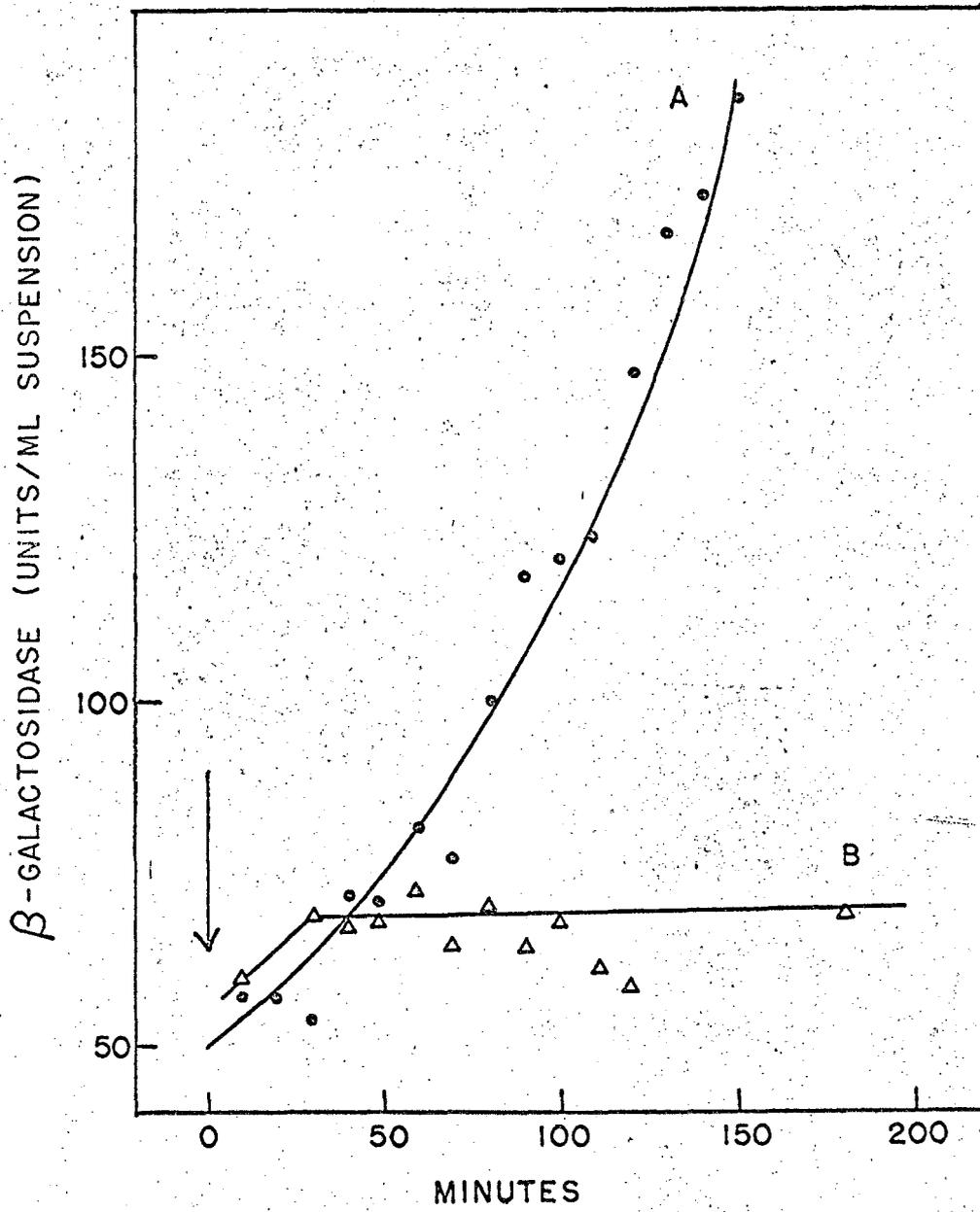
MUB-3992

FIG. 18



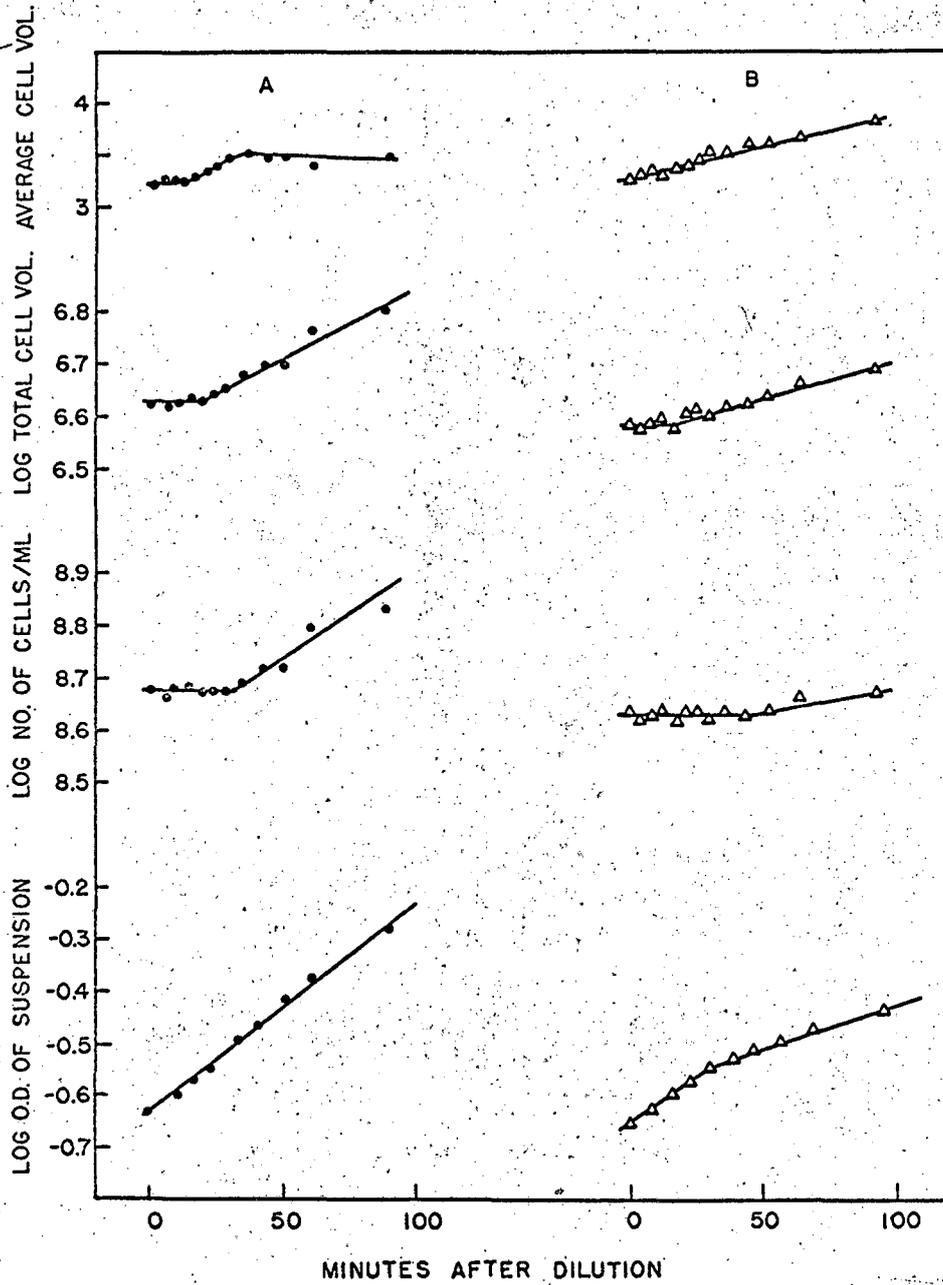
MUB-3995

FIG-19



MUB-3996

FIG. 20



MUB-3998



FIG. 21

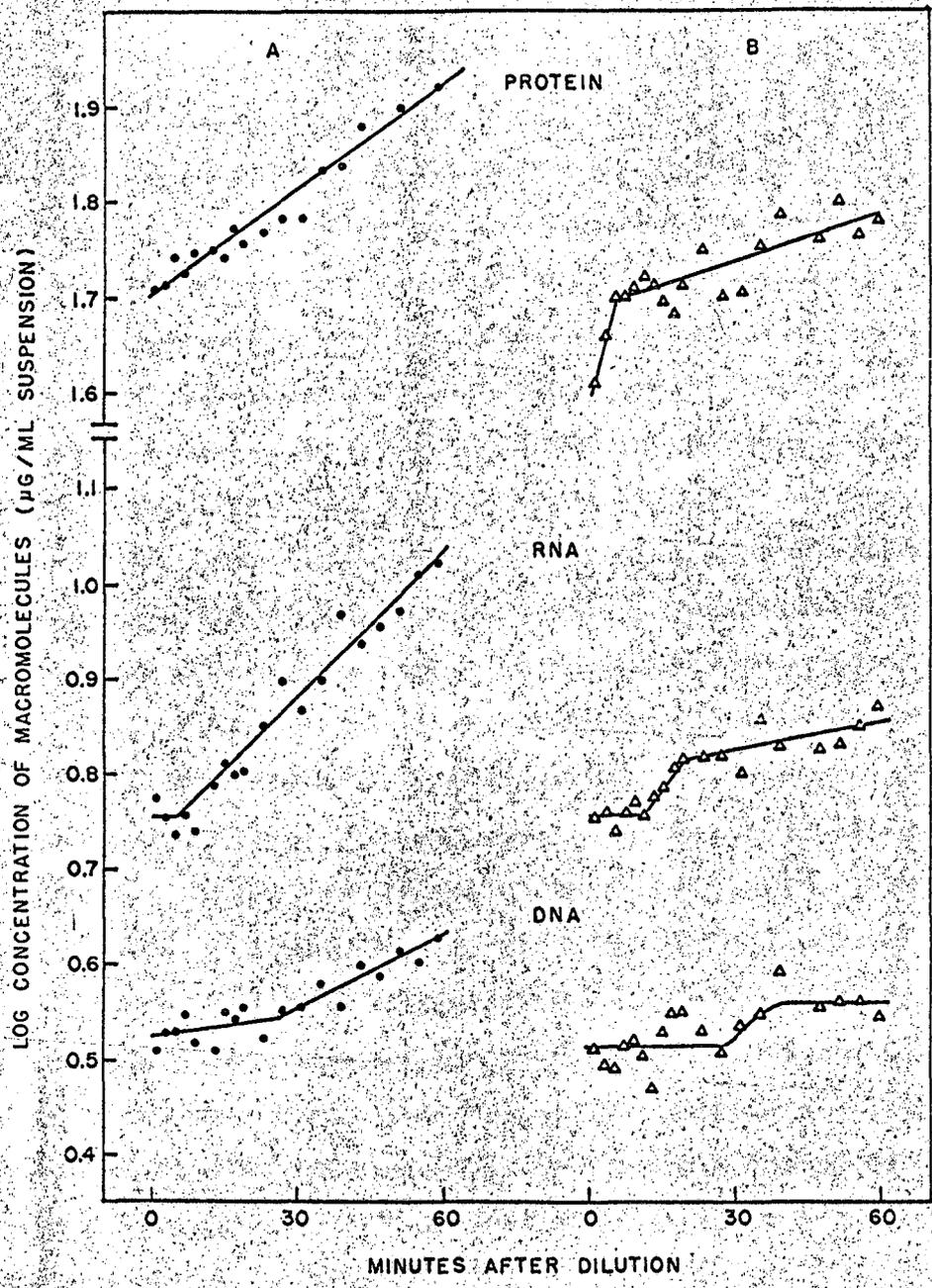
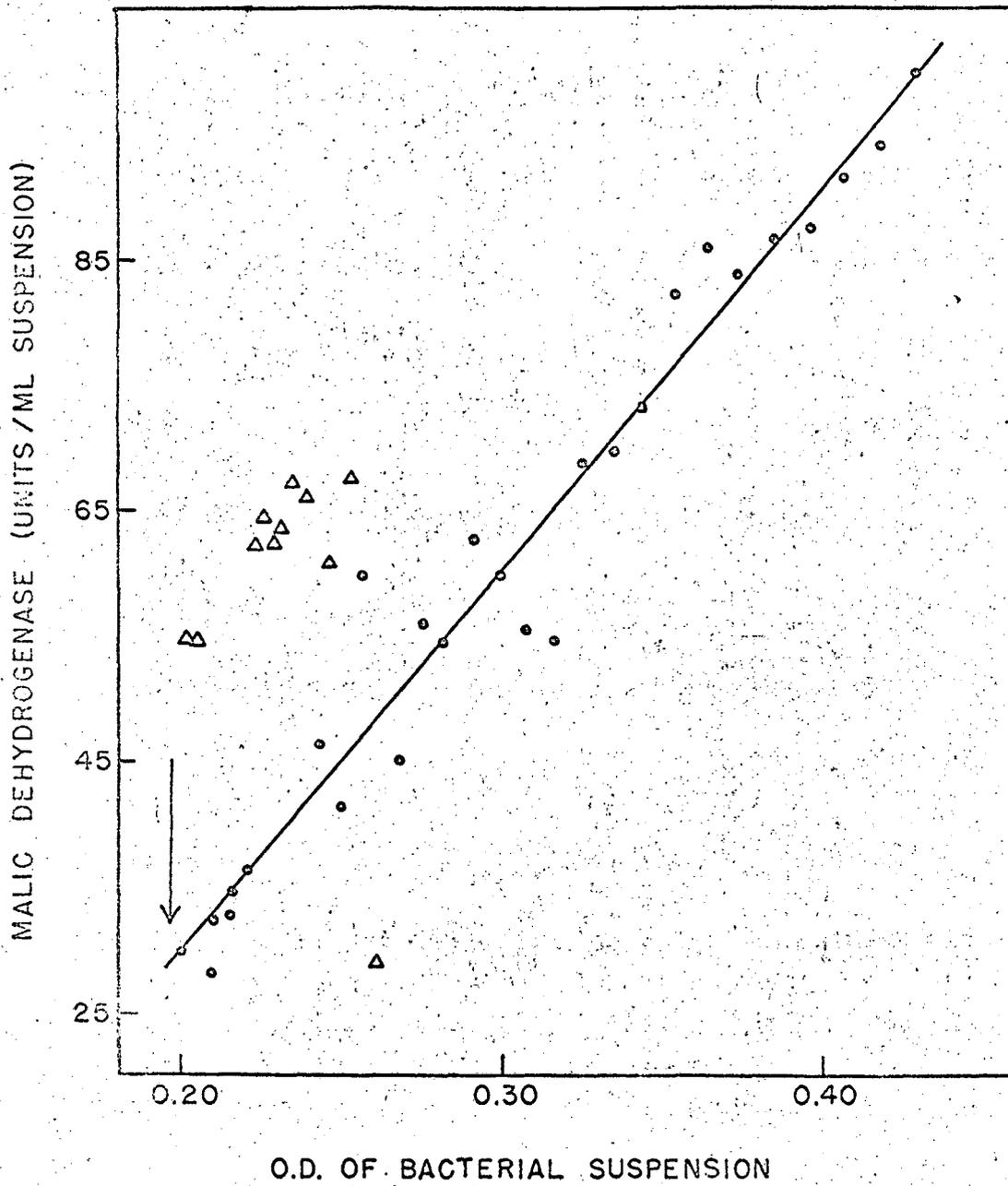


FIG. 22



MUB-4000

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