Submitted to Biochemical Journal

UCRL-19499
Preprint

c. 2

ADENOSINE 3', 5'-(CYCLIC)-MONOPHOSPHATE AND CATABOLITE REPRESSION IN *ESCHERICHIA COLI*

RECEIVED
LAWRENCE
RADIATION LABORATORY

MAR 23 1970

LIBRARY AND
DOCUMENTS SECTION

V. Moses and Pamela B. Sharp

January 22, 1970

AEC Contract No. W-7405-eng-48

TWO-WEEK LOAN COPY

This is a Library Circulating Copy which may be borrowed for two weeks. For a personal retention copy, call Tech. Info. Division, Ext. 5545

UCRL-19499

LAWRENCE RADIATION LABORATORY
UNIVERSITY of CALIFORNIA BERKELEY

48

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

Adenosine 3',5'-(Cyclic)-Monophosphate and Catabolite Repression in *Escherichia coli*

BY V. MOSES AND PAMELA B. SHARP

Laboratory of Chemical Biodynamics and Lawrence Radiation Laboratory, University of California, Berkeley, California, U.S.A. 94720

1. Both permanent and transient catabolite repression of β-galactosidase synthesis in *E. coli* are abolished by 5 mM-3',5'-(cyclic)-AMP when elicited by glucose, but not when caused by a mixture of glucose, glucose 6-phosphate, gluconate and casamino acids. 2. Glucose uptake is slightly increased by 3',5'-(cyclic)-AMP. 3. No significant effects of the nucleotide were found on the synthesis of protein and RNA, either in exponential growth on one substrate, or during a growth shift from glycerol to glycerol plus glucose. 4. Marked changes in the soluble protein profiles of cells growing in glycerol and glucose were caused by the presence of 3',5'-(cyclic)-AMP. 5. Measurements of $^{14}CO_2$ release from specifically-labelled glucose showed that 3',5'-(cyclic)-AMP greatly stimulated glycolytic activity while having a minor depressing effect on the metabolic flow through the pentose phosphate cycle. 6. The levels of several metabolic intermediates, particularly of fructose 1,6-diphosphate, were greatly affected by the presence of 3',5'-(cyclic)-AMP. 7. Several metabolites partially relieved glucose repression of β-galactosidase synthesis in EDTA-treated cells; three out of five of these metabolites reversed more effectively

than did 3',5'-(cyclic)-AMP. 8. The evidence for and against a direct role for 3',5'-(cyclic)-AMP is discussed. It is concluded that the evidence for indirect action is at least as strong as that for direct action.

Recent reports (Perlman & Pastan, 1968a,b; Pastan & Perlman, 1968; Jacquet & Kepes, 1969; Ullmann & Monod, 1968) have shown that catabolite repression of β-galactosidase synthesis in Escherichia coli is prevented by the presence of 1-5 mM-3',5'-(cyclic)-AMP.* It was suggested that relief of repression resulted from a direct involvement of 3',5'-(cyclic)-AMP on either the transcription or the translation of the lactose genes.

Goldenbaum & Dobrogosz (1968) found that repression by glucose was indeed blocked, but that glucose 6-phosphate and mannitol remained effective repressing substrates in the presence of 1 mM-3',5'-(cyclic)-AMP. They concluded that the action of the latter was on the formation of glucose 6-phosphate from glucose, and not directly on the synthesis of β-galactosidase per se. K. Paigen & B. Williams (personal communication) suggested that 3',5'-(cyclic)-AMP may either be the effector for catabolite repression, or may affect an enzyme involved in effector mechanism.

We have studied the effect of 3',5'-(cyclic)-AMP on several aspects of the physiology of E. coli with a view to resolving the question of its

*Abbreviations: IPTG, isopropyl-β-D-thio-galactopyranoside; 3',5'-(cyclic)-AMP, adenosine 3',5'-(cyclic)-monophosphate; ONPG, o-nitrophenyl-galactopyranoside; PRPP, 5-phosphorylribose 1-pyrophosphate.

direct or indirect action. Our results have indicated that 3',5'-(cyclic)-AMP has widespread effects on carbohydrate metabolism in this bacterium. It remains a possibility that 3',5'-(cyclic)-AMP acts directly in catabolite repression, but there is ample reason for concluding that this is probably not the case, and that relief of repression results from secondary effects on intermediary cell metabolism.

EXPERIMENTAL

Bacterial strains. The following bacterial strains were used in this study; their relevant genotypes and sources are indicated: 300 U ($i^+o^+z^+y^-$) from J. Monod; CA 8001 ($i^{\pm}_{L1}p^-_{L1}o^+z^+y^+$) and XA 7010 ($F^-i^{del}o^{del}z^{del}_{M41}y^+$) from J. R. Beckwith. An additional strain (VP 19) was constructed by inserting the episome F' $pro^+_{A,B}lac^+$ from AB 1157/F' $pro^+_{A,B}lac^+$ (Moses & Sharp, 1970) into strain XA 7010, using selection on lactose-minimal medium.

Growth conditions. Medium 63, supplemented with the desired carbon source (0.2% w/v) was used as the standard minimal medium. Cultures were shaken at 37°, and growth followed by measuring E_{650} (Moses & Prevost, 1966).

EDTA-treated cells. Cells growing exponentially in minimal medium were collected on a membrane filter, washed, and resuspended in 0.12 M-tris-HCl buffer, pH 7.6; the temperature was maintained at 37° throughout these manipulations. EDTA (1 mM) was then added; 1 min. later the cells were diluted with 9 vol. of medium containing the desired carbon source (Jacquet & Kepes, 1969).

Enzyme induction and assay. The synthesis of β-galactosidase was induced with 0.5 or 1 mM-IPTG. Samples for measurement of the enzyme were handled as described by Palmer & Moses (1968), modified by the replacement of toluene treatment by hexadecyltrimethylammonium bromide (22 μg./ml.) during the enzyme assay (Tyler & Magasanik, 1969; Moses & Sharp, 1970).

Protein and RNA synthesis. These were measured by the accumulation of trichloroacetic acid-precipitable radioactivity following the addition of L-[G-^{14}C]phenylalanine and [^{3}H]uracil to the cultures. Preparation of the samples for radioactivity measurements used the techniques described by Moses & Prevost (1966).

Glucose uptake. This was measured radiochemically by the removal of ^{14}C from the medium following the addition of [G-^{14}C]glucose to the culture (Prevost & Moses, 1967).

Labelling of intermediary metabolites. Cells growing in low-phosphate medium (Prevost & Moses, 1967) were labelled by incubation with $KH_2{}^{32}PO_4$. Samples of the culture were taken into 4 vol. of methanol 10, 25, 40 and 60 min. after the introduction of $^{32}P_i$. Portions of the cell suspension in methanol were analyzed by paper chromatography (Prevost & Moses, 1967), and radioactivity determined with an automatic chromatogram spot counter (Moses & Lonberg-Holm, 1963).

Release of respiratory CO_2. The release of $^{14}CO_2$ from cells supplied with [1-^{14}C]glucose or [6-^{14}C]glucose was measured by a simplified adaptation of the radiorespirometric method (Wang et al., 1958). Cells were grown in medium 63 containing glucose, and adjusted with HCl to

pH 6.2. Samples (10 ml.) of the cultures during exponential growth were placed in 25 ml. Erlenmeyer flasks which were agitated on a reciprocal shaker at 37°. The flasks were flushed with air at about 20 ml./min./flask. The stream of effluent air was led through a fine tube into a bubbler tube (1.2 cm. internal diam.) containing 5 ml. NCS Solubilizing Reagent Model 190620 for absorption of CO_2. After addition of labelled glucose to the cultures, $^{14}CO_2$ was collected for 15 min. intervals from each flask. Portions (4 ml.) of the NCS Reagent containing $^{14}CO_2$ were mixed with 14 ml. of a toluene-based scintillation fluid, and ^{14}C was measured in a Packard Tri-Carb Scintillation Counter, using external standardization. A preliminary experiment showed that a second trap in series with the first was superfluous; at least 99.9% of the $^{14}CO_2$ in the effluent stream was trapped in the first tube.

Soluble protein profiles. Soluble protein profiles of cells growing in various media were determined by electrophoresis on polyacrylamide gels (Moses & Wild, 1969). Typically, 20 ml. of cell culture, containing about 90 µg. bacterial protein/ml., was mixed with 1 ml. of chloramphenicol (1 mg./ml.) and chilled to 0°. The cells were harvested by centrifugation and washed in a buffer free from divalent metal cations. They were lysed with EDTA and lysozyme as described by Godson (1967); the final vol. of the lysed cell preparation was 0.14 ml. Cell debris, ribosomes, etc. were removed by centrifugation for 2 hr. at about 45000 x g. Solid sucrose and bromthymol blue were added to each protein solution; aliquots (10-25 µl.) were then subjected to electrophoresis in slabs of polyacrylamide gel. The protein bands were subsequently stained with Amido black 10B. After removal of excess stain electrophoretically,

the colour intensities of the bands were measured with a Gel-scan scanner connected to a Servogor recorder (Gelman Instrument Co., Ann Arbor, Michigan).

Chemicals and radiochemicals. Labelled materials were obtained from New England Nuclear Corp., Boston, Mass. NCS Solubilizing Reagent was from Nuclear-Chicago Corp., Del Plaines, Ill. Other chemicals were from the following sources: Lysozyme, PRPP, fructose 1,6-diphosphate, 3',5'-(cyclic)-AMP, ONPG, IPTG, glucose 6-phosphate, NADP, NADPH, NAD and NADH were from Calbiochem., Los Angeles, Calif.; ribose 5-phosphate was from Sigma Chemical Co., St. Louis, Mo.; chloramphenicol was from Parke, Davis & Co., Detroit, Mich.; hexadecyltrimethylammonium bromide was from Matheson, Coleman & Bell, Cincinnati, Ohio; cyanogum-41 was from E. C. Apparatus Corp., Philadelphia, Pa.; Amido black 10B was from E. Merck A.G., Darmstadt, Germany.

RESULTS

Effect of 3',5'-(cyclic)-AMP on β-galactosidase synthesis. β-Galactosidase synthesis in wild-type cells growing in glycerol-minimal medium was stimulated by 5 mM-3',5'-(cyclic)-AMP (Fig. 1). Neither transient nor permanent repression was elicited by the addition of 10 mM-glucose.

If more severe repression was caused by the simultaneous addition of several repressing components, the protection afforded by 3',5'-(cyclic)-AMP was incomplete, in confirmation of the results reported by Goldenbaum & Dobrogosz (1968). Supplementing the medium with glucose (10 mM) plus glucose 6-phosphate (10 mM) plus Na gluconate (10 mM) plus

casein hydrolyzate (1 mg./ml.) resulted in a degree of repression in wild-type cells greater than could be overcome by 5 mM-3',5'-(cyclic)-AMP (Fig. 2). It should be noted, nevertheless, that in the presence of 3',5'-(cyclic)-AMP, repression was slow to develop. It was only just beginning to become significant by the time recovery from transient repression was observed in the absence of the nucleotide. Transient repression was thus completely abolished in the presence of 3',5'-(cyclic)-AMP, though permanent repression was nearly as pronounced as in its absence.

Repression by the mixed substrates was less marked in the presence of 20 mM-3',5'-(cyclic)-AMP. The relative differential rates of β-galactosidase synthesis in glycerol medium were as follows: no additions, 1.00; plus mixed substrates, 0.18; plus mixed substrates with 5 mM-3',5'-(cyclic)-AMP, 0.27; plus mixed substrates with 20 mM-3',5'-(cyclic)-AMP, 0.66.

Silverstone et al. (1969) found that strain CA 8001, carrying the L1 deletion of the lac promoter gene, was unaffected by 3',5'-(cyclic)-AMP, and was not repressed by glucose 6-phosphate (concn. not stated). We have found the behaviour of this strain to be anomalous. In some experiments, the rate of β-galactosidase synthesis was increased by 3',5'-(cyclic)-AMP in glycerol-grown cells. Further, the induced rate of β-galactosidase synthesis was increased about sixfold by the addition of the mixed substrates described above; this increase was less pronounced in the presence of 3',5'-(cyclic)-AMP (Fig. 3). The growth characteristics of strain CA 8001 are typical both in glycerol-minimal medium and in the highly supplemented medium. We do not understand

the behaviour of this strain, and are unable to assess the role of the promoter gene in catabolite repression as it has been proposed by Silverstone et al. (1969).

Effect of 3',5'-(cyclic)-AMP on glucose uptake. The amount of glucose removed from the medium per unit increase of cell mass was increased about 15% by the presence of the nucleotide (Fig. 4).

Effect of 3',5'-(cyclic)-AMP on cell growth and on the synthesis of RNA and protein. No consistently significant effects of the nucleotide have been found on either the mass doubling times, or on the rates of incorporation of L-[G-^{14}C]phenylalanine or [^{3}H]uracil into the macromolecules (Fig. 5).

Effect of 3',5'-(cyclic)-AMP on soluble protein profiles. de Crombugghe, Perlman, Varmus & Pastan (1969) have found that the synthesis of several inducible enzymes is increased by 3',5'-(cyclic)-AMP. An expected consequence of this is that the relative proportions of various proteins in growing cells would be altered by the presence of the nucleotide.

The soluble protein profiles of cells growing in glycerol, glucose, or glucose plus glycerol, with and without 3',5'-(cyclic)-AMP, were investigated. From the profiles shown in Fig. 6 it is clear that the growth substrate was responsible for many characteristics of the pattern; similar observations have been made by Moses & Wild (1969). Consistent changes in the patterns resulted from the presence of 3',5'-(cyclic)-AMP in the growth medium. Prominent among these were the enhancement of peaks M and P in all growth media tested, and of peak Y in glucose-containing media; peak AG was reduced by the presence of 3',5'-(cyclic)-AMP

in glycerol-minimal medium. The effects on the balance of protein synthesis exerted by 3',5'-(cyclic)-AMP were thus sufficiently distinct to be observed even in these complex profiles.

These changes in the proportions of soluble proteins would be expected to result in a redistribution in the balance of metabolic activities. The detailed consequences of this cannot at the moment be assessed, but it is likely to cause changes in the intracellular concentrations of a number of intermediary metabolites, some of which may be effector molecules. We conclude that the actions of 3',5'-(cyclic)-AMP are widespread, and that caution must be exercised before ascribing any of its effects to direct interaction.

Effect of 3',5'-(cyclic)-AMP on carbon dioxide release from labelled glucose. It is known that 3',5'-(cyclic)-AMP activates phosphofructokinase in some animal tissues (Stone & Mansour, 1967). If this also occurs in *E. coli*, metabolism of glucose through glycolysis would probably be favoured in comparison with oxidation via the pentose phosphate cycle. The specific rates of release of $^{14}CO_2$ from [1-^{14}C]glucose and [6-^{14}C]glucose may be used to study the relative utilization of the two pathways since the Entner-Doudoroff pathway does not operate in *E. coli* (Wang *et al.*, 1958).

Strain VP19 was grown exponentially in glucose-minimal medium adjusted to pH 6.2. Of four parallel cultures, two received 5 mM-3',5'-(cyclic)-AMP 90 min. before the addition of labelled glucose; no addition was made to the other pair. At the start of the experimental period one flask from each pair was supplied with 4 μC of [1-^{14}C]glucose and the other with a similar quantity of [6-^{14}C]glucose. The resulting

specific radioactivity was 0.46 μC/ mole glucose in each case. Respiratory $^{14}CO_2$ was trapped for successive 15 min. intervals over a total period of 3 hr. Growth, measured by E_{650}, was followed throughout in parallel cultures; cultures with 3',5'-(cyclic)-AMP grew more slowly than those without (0.74 doublings/hr. and 0.83 doublings/hr., respectively).

Fig. 7 shows that early in the experiment 3',5'-(cyclic)-AMP markedly stimulated the release of $^{14}CO_2$ from [6-^{14}C]glucose, and, to a lesser extent, reduced its rate of evolution from [1-^{14}C]glucose. Both effects weakened with time; some 4.5 hr. after the introduction of the nucleotide, the pattern of respiratory CO_2 release in the cultures with 3',5'-(cyclic)-AMP was approaching that of the untreated ones. In the absence of the nucleotide, the proportion of CO_2 released as a result of glycolytic activity increased with increasing cell density in the culture. This probably reflected an increasing limitation of O_2 availability as the culture became more dense.

The effect of 3',5'-(cyclic)-AMP on glucose dissimilation was mostly to increase the flow through glycolysis, though some decrease in metabolism through the pentose phosphate pathway was also observed. The total release of $^{14}CO_2$ was higher in the presence of 3',5'-(cyclic)-AMP, a result consistent with the finding that the net consumption of glucose was also increased by this substance (Fig. 4). The presence of the nucleotide in cultures of growing cells thus appeared to shift the balance of glucose metabolism away from the pentose phosphate cycle and towards glycolysis. Such a result is not unexpected in view of its known action in stimulating phosphofructokinase.

Effect of 3',5'-(cyclic)-AMP on levels of phosphorylated intermediary metabolites. The levels of a number of phosphorylated intermediates were measured at various periods after the introduction of $KH_2{}^{32}PO_4$ to cells grown in low phosphate medium containing glycerol or glucose, with and without 3',5'-(cyclic)-AMP. Table 1 records the levels of incorporated ^{32}P in 18 compounds after 1 hr. of labelling. The results are presented as d.p.m., and are to be used only to compare the relative degrees of labelling among metabolites.

The labelling of several substances varies with the carbon source; UTP + UDP, UDP-glucose, ATP, glucose 6-phosphate, α-glycerol phosphate and an unidentified mononucleotide were the most prominent of these. The extent of labelling of some substances also varied as a result of the presence of 3',5'-(cyclic)-AMP. Among these, label in UTP + UDP and glucose 6-phosphate was reduced by the nucleotide, while that in fructose 1,6-diphosphate and in two unidentified phosphorylated compounds was dramatically increased.

The effect of 3',5'-(cyclic)-AMP on ^{32}P incorporation into fructose 1,6-diphosphate is consistent with its presumed stimulatory action on phosphofructokinase, and with its effect on $^{14}CO_2$ release from [6-^{14}C] glucose. To judge both from the release of $^{14}CO_2$ from [1-^{14}C]glucose, and the extent of ^{32}P incorporation into PRPP (Table 1), its effect directly on the activity of the pentose phosphate cycle was relatively minor.

Ability of several intermediary metabolites to reverse catabolite repression. Jacquet & Kepes (1969) showed that cells shocked with 1 mM-EDTA for 1 min. and the returned to growth medium remained sensitive to

catabolite repression by glucose, and to its reversal by 3',5'-(cyclic)-AMP. EDTA-treated cells are believed (Leive, 1965) to be permeable to substances which are not normally able to enter them. The ability of a number of metabolites to elicit or reverse catabolite repression was investigated in such cells.

Strain 300U, maintained for 18 subcultures in glucose-nutrient broth medium, was used for these experiments. Cells were grown for several generations in glycerol-minimal medium, treated with EDTA, diluted into minimal medium containing either glycerol or glucose (see Experimental section) and incubated at 37°.

IPTG (1 mM) was added, and 3 min. later four 2 ml. aliquots were simultaneously removed from the culture and immediately mixed with 2 ml. of the same medium containing IPTG (1 mM), the test substance at a concentration of 10 mM, plus L-[G-^{14}C]phenylalanine and [^{3}H]uracil. Samples for measurements of enzyme activity and acid-precipitable radioactivity were taken at intervals during the following 20 min.

None of the test substances altered the rates of incorporation of the labelled precursors for the period of the experiments. Table 2 shows that in glycerol medium only glucose caused significant repression of β-galactosidase synthesis. All other compounds were either without effect, or increased enzyme synthesis. When two metabolites were present simultaneously, their effects were additive. In glucose medium, repression was alleviated by all six compounds which were tested; 3',5'-(cyclic)-AMP was not the most effective antagonist of glucose repression.

DISCUSSION

A direct or indirect role for 3',5'-(cyclic)-AMP. de Crombrugghe et al. (1969) have presented the arguments supporting a direct role for 3',5'-(cyclic)-AMP in the synthesis of inducible enzymes. Summarized briefly, these are: (a) in certain mutants of the phosphoenolpyruvate-phosphotransferase system unable to grow on glucose, or to accumulate glucose or α-methyl glucoside, both substances repress β-galactosidase synthesis, and this repression is overcome by 3',5'-(cyclic)-AMP (Pastan & Perlman, 1969). (b) At concn. of 3',5'-(cyclic)-AMP which effectively reverse catabolite repression by glucose, the uptake of glucose is little affected (Pastan & Perlman, 1969). (c) Chambers & Zubay (1969) have reported that 3',5'-(cyclic)-AMP stimulates β-galactosidase synthesis in a cell-free system. (d) High concn. of the nucleotide reverse repression exerted by glucose 6-phosphate, and by glucose plus gluconate, as well as by glucose alone (Perlman, de Crombrugghe & Pastan, 1969).

In view of the observations we report in this communication, a number of comments are appropriate: (a) The levels of many metabolic intermediates are affected by the growth substrate (Prevost & Moses, 1967), and by the presence of 3',5'-(cyclic)-AMP, as we have shown in this communication. Before mutants of the phosphoenolpyruvate-phosphotransferase system can be fully understood in terms of catabolite repression responses, it is important to know more about them. This applies particularly to their levels of intermediary metabolites, and how these may be altered by the presence in the medium of glucose and α-methyl glucoside, even if the latter are not metabolized. It is

further necessary to know how completely blocked is the metabolism of these substances. Even with this information, the possibility would remain that the local concn. of metabolites at the sites of interaction between effector molecules and their targets are significantly different from those in the cell as a whole. (b) While we confirm that glucose uptake is not markedly affected by 3',5'-(cyclic)-AMP, we have found that the oxidation pattern is greatly altered. (c) The evidence given by Chambers & Zubay (1969) is perhaps the most suggestive of all in support of a direct role for 3',5'-(cyclic)-AMP. However, their cell-free system contains very many components, some of which may themselves have an indirect part in regulation. It is difficult to be sure, even in their system, that the nucleotide is acting directly as an effector. (d) We have found that 3',5'-(cyclic)-AMP is unable to overcome repression produced simultaneously by several substrates.

We should also consider the evidence against a direct role for 3',5'-(cyclic)-AMP. A very considerable shift in the flow of glucose carbon is caused by its presence. The Embden-Meyerhof pathway assumes a much greater importance compared with the pentose phosphate cycle. This is reflected both in the levels of metabolic intermediates, and in the pattern of release of $^{14}CO_2$ from specifically-labelled glucose. Both may readily be understood from the known ability of 3',5'-(cyclic)-AMP to activate phosphofructokinase. We have been able to observe some of the consequences of this activation, and have little doubt that there are others which have escaped our attention. It should further be noted that earlier studies (Prevost & Moses, 1967) suggested that pentose phosphate cycle activity was necessary at least for transient repression. One of the main consequences of treatment with 3',5'-(cyclic)-AMP

is a reduction in the relative importance of the pentose phosphate cycle as a metabolic pathway. The inhibitory effects of high concn. of 3',5'-(cyclic)-AMP on RNA synthesis (de Crombrugghe et al., 1959) may be due to a reduction in the availability of pentose precursors for nucleotide synthesis as a result of impaired pentose phosphate cycle activity.

The changes in metabolite concn. are likely to arise concurrently from two sources. The direct activation of phosphofructokinase, and perhaps other enzymes, will rapidly change metabolite levels. The regulation of many enzymes probably depends on such metabolites, and, in time, the protein composition of the cell will undergo an appreciable rearrangement. One might expect that this, in turn, will have further effects on metabolite levels, until a new equilibrium is reached. Our studies on the soluble protein profiles have shown that marked changes in the protein composition result from the presence of 3',5'-(cyclic)-AMP.

Finally, we must mention two more significant findings: 3',5'-(cyclic)-AMP has a restricted ability to reverse catabolite repression, and it is not the only substance which can do so. Of five other phosphorylated intermediates tested for their ability to relieve glucose repression in EDTA-treated cells, three were at least as effective as 3',5'-(cyclic)-AMP. They are, however, more difficult to study than the latter because of the difficulty with which some of them enter cells not previously rendered permeable with EDTA. No specific conclusion can readily be drawn from such a result unless it is that the metabolic interrelations are complex and require further investigation.

This work was sponsored by the United States Atomic Energy Commission. We wish to express our thanks to those who kindly supplied us with bacterial strains.

REFERENCES

Chambers, D. A. & Zubay, G. (1969). Proc. natn. Acad. Sci. U.S.A., 63, 118.

de Crombrugghe, B., Perlman, R. L., Varmus, H. E. & Pastan, I. (1969). J. biol. Chem. 224, 5828.

Godson, G. N. (1967). Methods in Enzymol. 12, 503.

Goldenbaum, P. E. & Dobrogosz, W. J. (1968). Biochem. Biophys. Res. Commun. 33, 828.

Jacquet, M. & Kepes, A. (1969). Biochem. biophys. Res. Commun. 36, 84.

Leive, L. (1965). Biochem. biophys. Res. Commun. 18, 13.

Moses, V. & Prevost, C. (1966). Biochem. J. 100, 336.

Moses, V. & Lonberg-Holm (1963). Analyt. Biochem. 5, 11.

Moses, V. & Sharp, P. B. (1970). Biochem. J., in the press.

Moses, V. & Wild, D. G. (1969). Folia Microbiol. 14, 305.

Palmer, J. & Moses, V. (1968). Biochem. J. 106, 339.

Pastan, I. & Perlman, R. L. (1968). Proc. natn. Acad. Sci. U.S.A. 61, 1336.

Pastan, I. & Perlman, R. L. (1969). J. biol. Chem. 244, 5836.

Perlman, R. L., de Crombrugghe, B. & Pastan, I. (1969). Nature Lond., 223, 810.

Perlman, R. L. & Pastan, I. (1968a). Biochem. biophys. Res. Commun. 30, 656.

Perlman, R. L. & Pastan, I. (1968b). J. biol. Chem. 243, 5420.

Prevost, C. & Moses, V. (1967). Biochem. J. 103, 349.

Silverstone, A. E., Magasanik, B., Reznikoff, W. S., Miller, J. H. & Beckwith, J. R. (1969). Nature Lond., 221, 1012.

Stone, D. B. & Mansour, T. E. (1967). Mol. Pharmacol. 3, 161.

Tyler, B. & Magasanik, B. (1969). J. Bact. 97, 550.

Ullmann, A. & Monod, J. (1968). FEBS Lett. 2, 57.

Sang, C. H., Stern, I., Gilmour, C. M., Klungsoyr, S., Reed, D. J., Bialy, J. J., Christensen, B. E. & Cheldelin, V. H. (1958). J. Bact. 76, 207.

CAPTIONS FOR FIGURES

Fig. 1. Effect of 3',5'-(cyclic)-AMP on glucose repression of β-galactosidase synthesis in strain VP 19 growing in glycerol-minimal medium and induced with 0.5 mM-IPTG. Glucose (10 mM) was added at arrows. Mass doubling times before and after glucose addition were 90 min. and 79 min., respectively, in each case. In the control, transient repression lasted for 35 min. ● , control; o , plus 5 mM-3',5'-(cyclic)-AMP.

Fig. 2. Effect of 3',5'-(cyclic)-AMP on repression by several substrates of β-galactosidase synthesis in strain VP 19 growing in glycerol-minimal medium and induced with 1 mM-IPTG. The arrow indicates the simultaneous addition of glucose (10 mM), glucose 6-phosphate (10 mM), Na gluconate (10 mM) and casamino acids (1 mg./ml.). Mass doubling times before and after additions were 92 min. and 47 min., respectively,

Fig. 2 (cont.)

in each case. In the control, transient repression lasted for 17 min. ● , control; o , plus 3',5'-(cyclic)-AMP.

Fig. 3. Effect of 3',5'-(cyclic)-AMP on stimulation of β-galactosidase synthesis in strain CA 8001 growing in glycerol-minimal medium and induced with 1 mM-IPTG. Supplementation as for Fig. 2. Mass doubling times before and after additions: control, 98 min. and 37 min., respectively; plus 3',5'-(cyclic)-AMP, 94 min. and 48 min., respectively. ● , control; o , plus 5 mM-3',5'-(cyclic)-AMP.

Fig. 4. Effect of 3',5'-(cyclic)-AMP on glucose uptake in strain VP 19. Cells in glucose-minimal medium received [G-^{14}C]glucose, and measurements were made of ^{14}C in the medium at intervals thereafter. ● , control; o , plus 5 mM-3',5'-(cyclic)-AMP.

Fig. 5. Effect of 3',5'-(cyclic)-AMP on macromolecule synthesis during a growth shift. Strain VP 19, in glycerol-minimal medium, was supplied with L-[G-^{14}C]phenylalanine and [^{3}H]uracil. Samples were taken at intervals for determination of trichloroacetic acid-precipitable radioactivity. Glucose (10 mM) was added at the arrow. (a) [^{3}H]uracil incorporation; (b) L-[G-^{14}C]phenylalanine incorporation. ● , control; o , plus 5 mM-3',5'-(cyclic)-AMP.

Fig. 6. Soluble protein profiles of strain VP 19 in minimal media supplemented as follows: (a) glycerol; (b) glycerol plus 5 mM-3',5'-(cyclic)-AMP; (c) glucose; (d) glucose plus 5 mM-3',5'-(cyclic)-

Fig. 6 (cont.)

AMP; (e) glycerol plus glucose; (f) glycerol plus glucose plus 5 mM-3',5'-(cyclic)-AMP.

Fig. 7. Effect of 3',5'-(cyclic)-AMP on release of $^{14}CO_2$ from labelled glucose. Strain VP 19, growing in glucose-minimal medium, received [1-^{14}C]glucose or [6-^{14}C]glucose. $^{14}CO_2$ was trapped for a series of 15 min. intervals, while growth was measured by E_{650}. (a) control; (b) plus 5 mM-3',5'-(cyclic)-AMP. ●○, $^{14}CO_2$ from [1-^{14}C]glucose; ▲△, $^{14}CO_2$ from [6-^{14}C]glucose.

Running Title:

3',5'-(cyclic)-AMP and Catabolite Repression

Table 1. Incorporation of $^{32}P_i$ into intermediary metabolites

Strain VP 19 was grown exponentially in low-phosphate (0.75 mM-P_i) minimal medium containing glycerol or glucose, with or without 5 mM-3',5'-(cyclic)-AMP. $KH_2{}^{32}PO_4$ was added to give a specific radioactivity of 400 μC./μmole. The labelling in intermediary metabolites was determined after 1 hr. incubation at 37°, using paper chromatography. Values are recorded as 10^{-3} x d.p.m./μg. of bacterial protein.

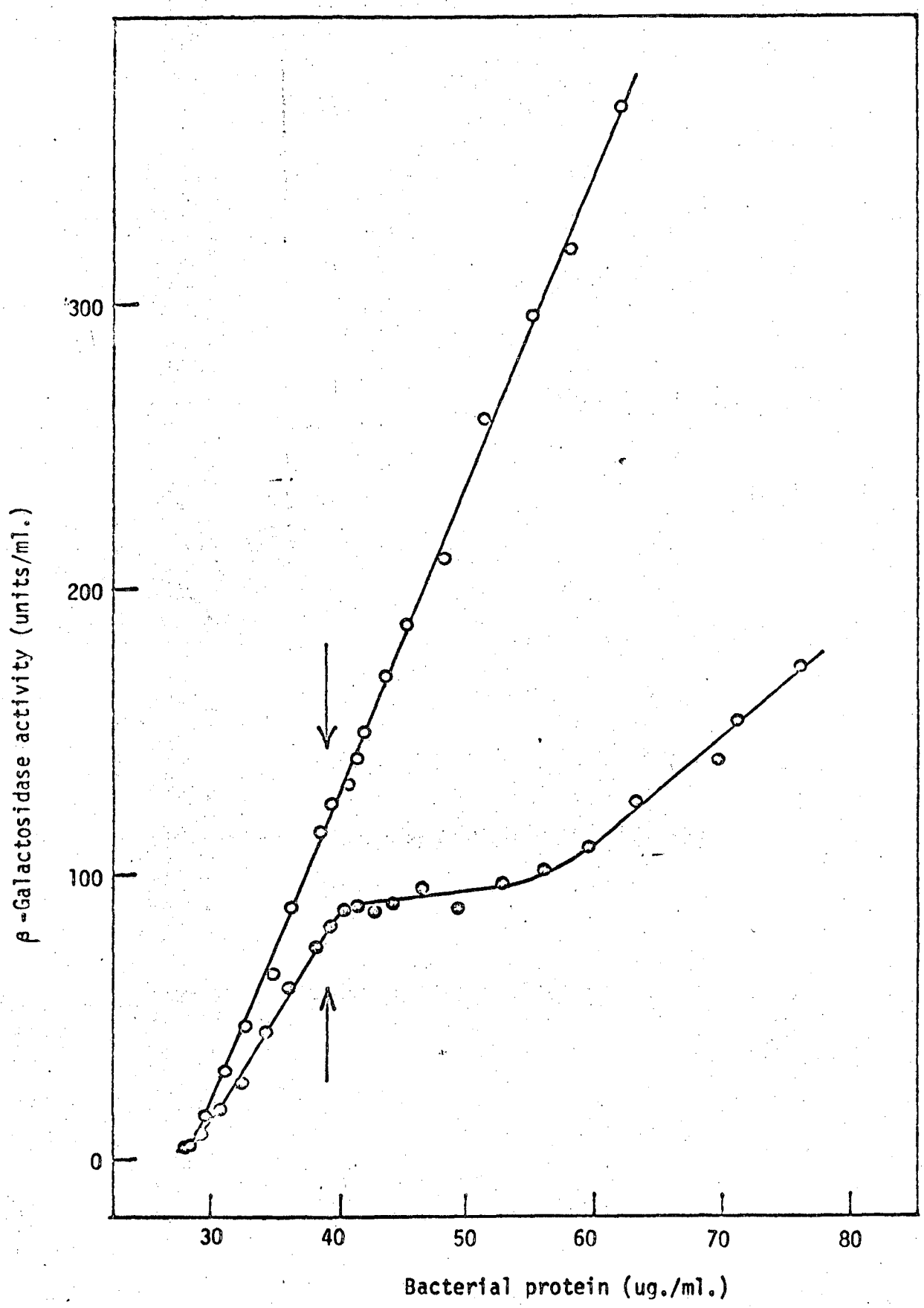
Medium	glycerol	glycerol + 3',5'-(cyclic)-AMP	glucose	glucose + 3',5'-(cyclic)-AMP
UTP + UDP	104	40	128	64
UDP-glucose	48	52	62	74
ATP	42	54	84	80
ADP	22	22	25	22
PRPP	4.8	4.8	5.6	6.0
Glucose 6-phosphate	16	5.2	30	5.2
Fructose 6-phosphate	16	16	20	20
Fructose 1,6-diphosphate	0	74	0	96
α-Glycerophosphate	80	100	28	32
Dihydroxyacetone phosphate	8.0	6.8	8.0	8.0
NADP + NADPH	10	15	13	15
Unidentified sugar phosphate	12	8.8	20	19
Unidentified compound 1	18	18	18	18
Unidentified compound 2	0	12	0	152
Unidentified compound 3	0	9.6	0	9.6
Unidentified compound 4*	11	11	11	11
Unidentified compound 5*	28	43	46	52
Unidentified compound 6*	16	16	16	16

*These are probably nucleoside monophosphates.

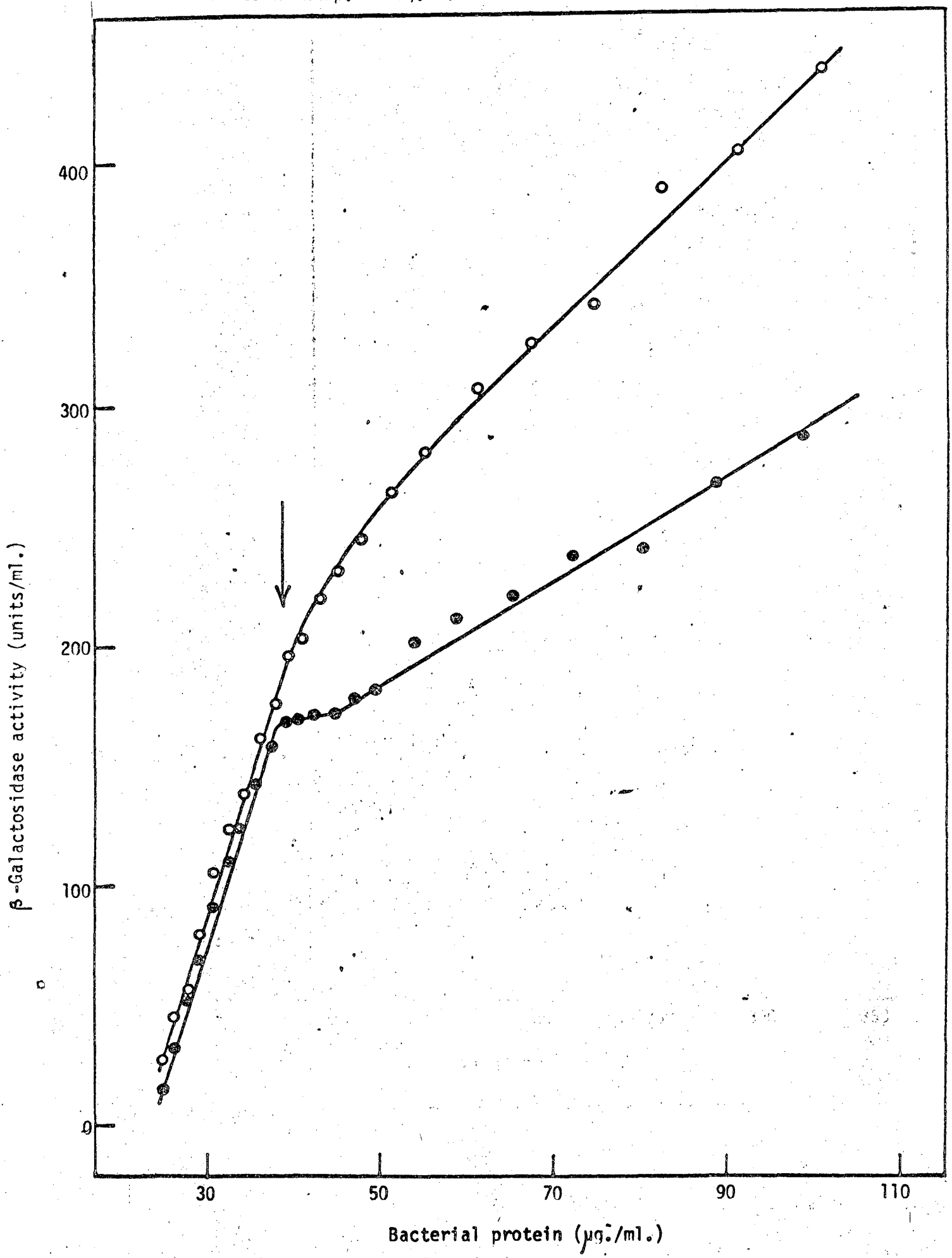
Table 2. Effects of intermediary metabolites on the induced synthesis of β-galactosidase in EDTA-treated cells

Strain 300U, grown in glycerol-minimal medium, was treated with EDTA as described in the Experimental section. The cells were resuspended in minimal medium containing either glycerol (22 mM) or glucose (5 mM) as indicated. IPTG (1 mM) was added; 3 min. later aliquots of the culture were mixed with an equal vol. of the same medium containing the test substances (10 mM). Samples for β-galactosidase determination were removed periodically during the next 18 min. Results are expressed as enzyme synthesized in 18. min. compared with the control value for that experiment.

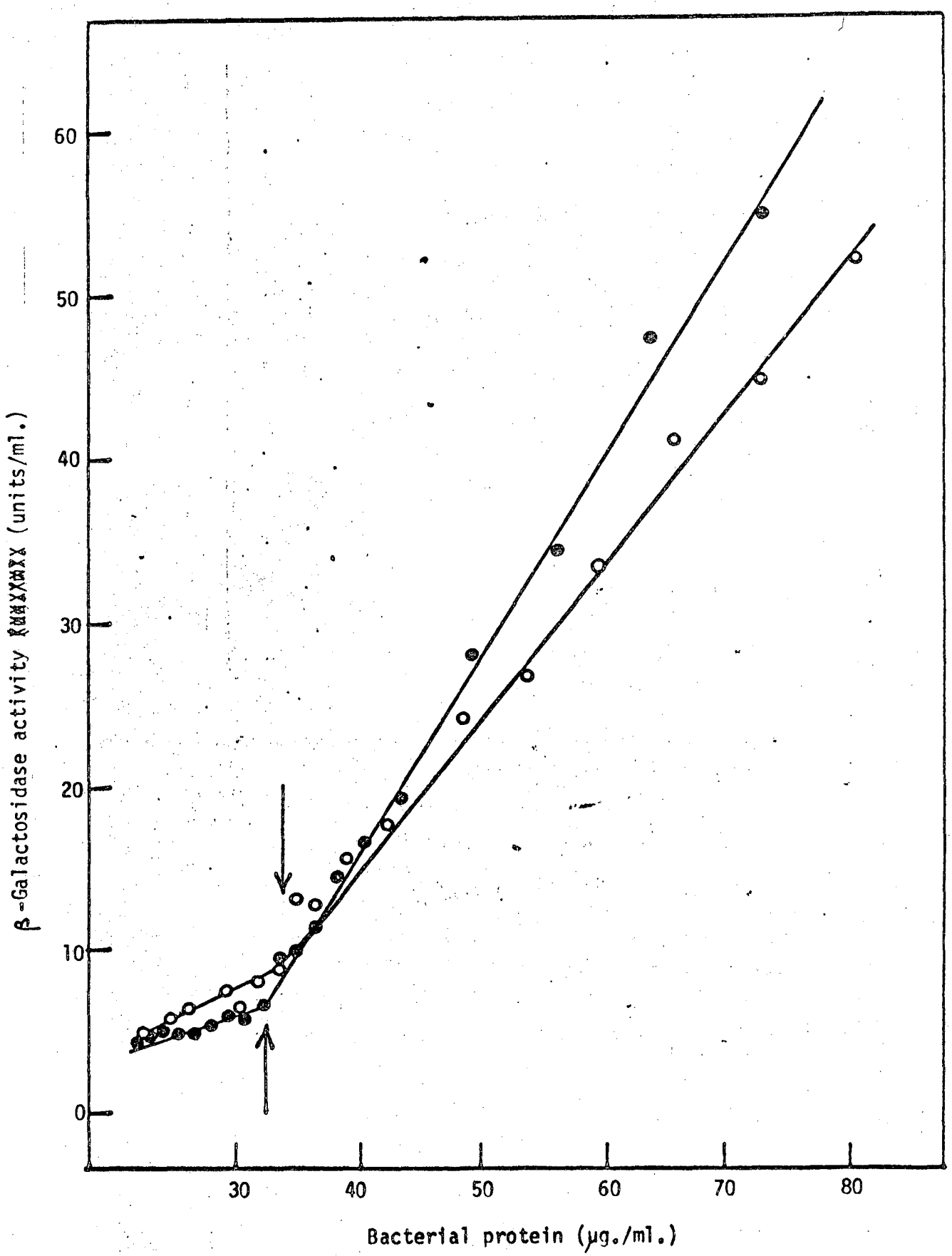
Expt.	Carbohydrate in suspension medium	Metabolite	β-Galactosidase synthesized in 18 min.
1.	Glycerol	None	100
		3',5'-(cyclic)-AMP	980
		fructose 1,6-diphosphate	180
		3',5'-(cyclic)-AMP + fructose 1,6-diphosphate	1110
2.	Glycerol	None	100
		3',5'-(cyclic)-AMP	224
		PRPP	344
		3',5'-(cyclic)-AMP + PRPP	615
3.	Glycerol	None	100
		glucose	10
		fructose 1,6-diphosphate	90
		PRPP	123
4.	Glycerol	None	100
		glucose	18
		$NADP^+$	96
		NADPH	111
5.	Glucose	None	100
		3',5'-(cyclic)-AMP	170
		fructose 1,6-diphosphate	217
		PRPP	136
6.	Glucose	None	100
		$NADP^+$	222
		NADPH	134
		ribose 5-phosphate	197

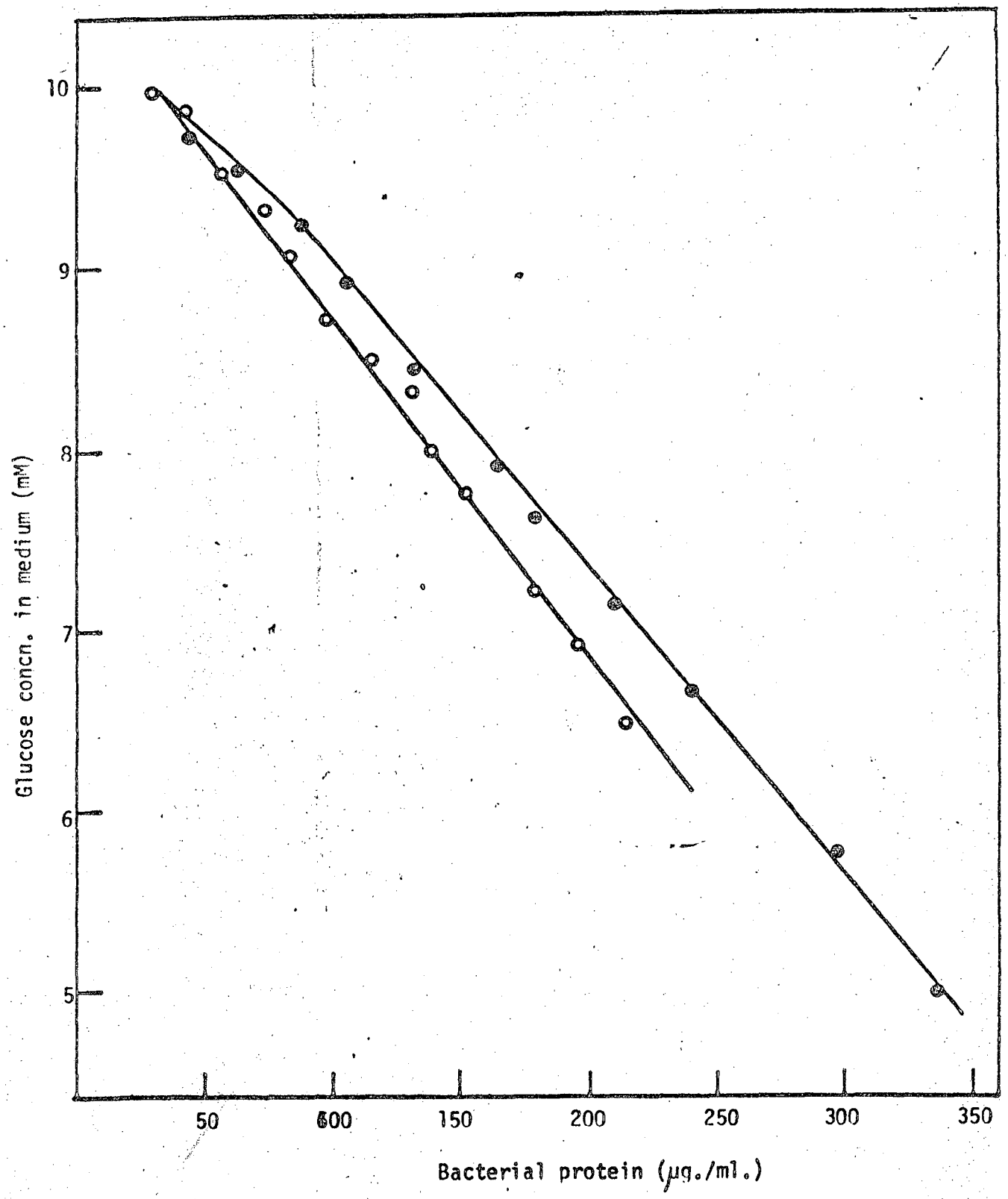

300
200
100
0
β-Galactosidase activity (units/ml.)
30
40
50
60
70
80
Bacterial protein (ug./ml.)

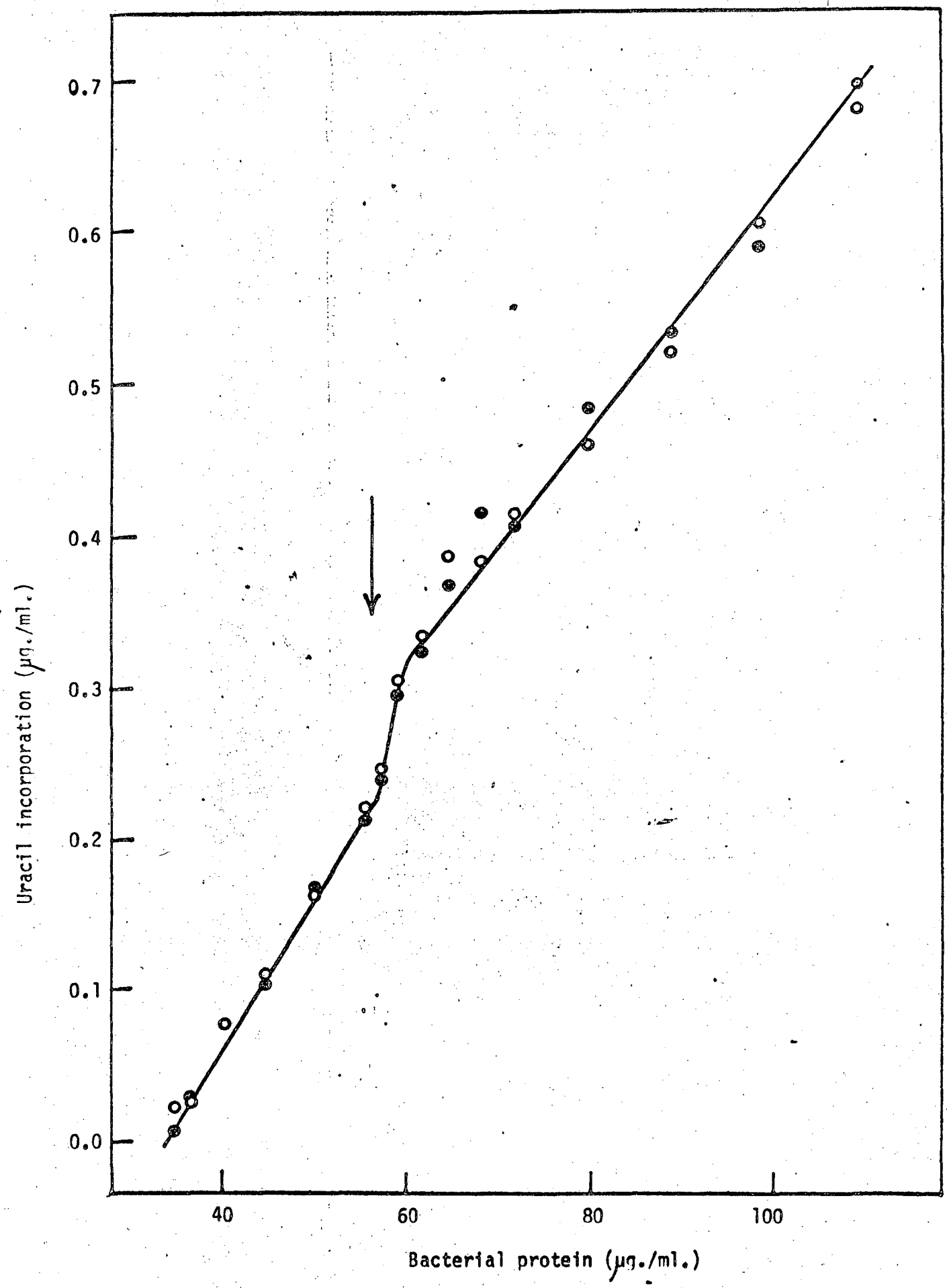
Moses & Sharp. Fig. 2.

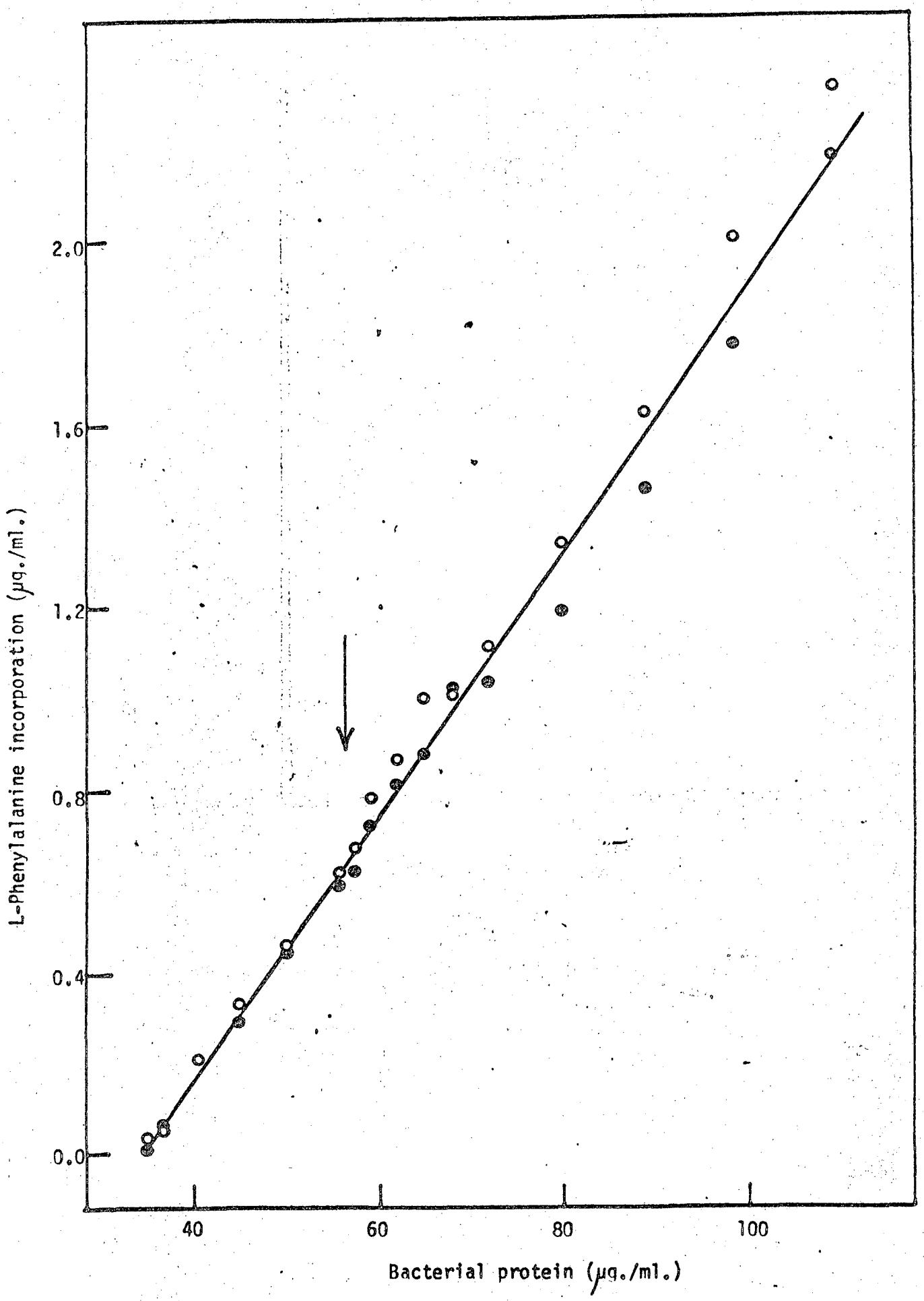


Moses & Sharp. Fig. 3.

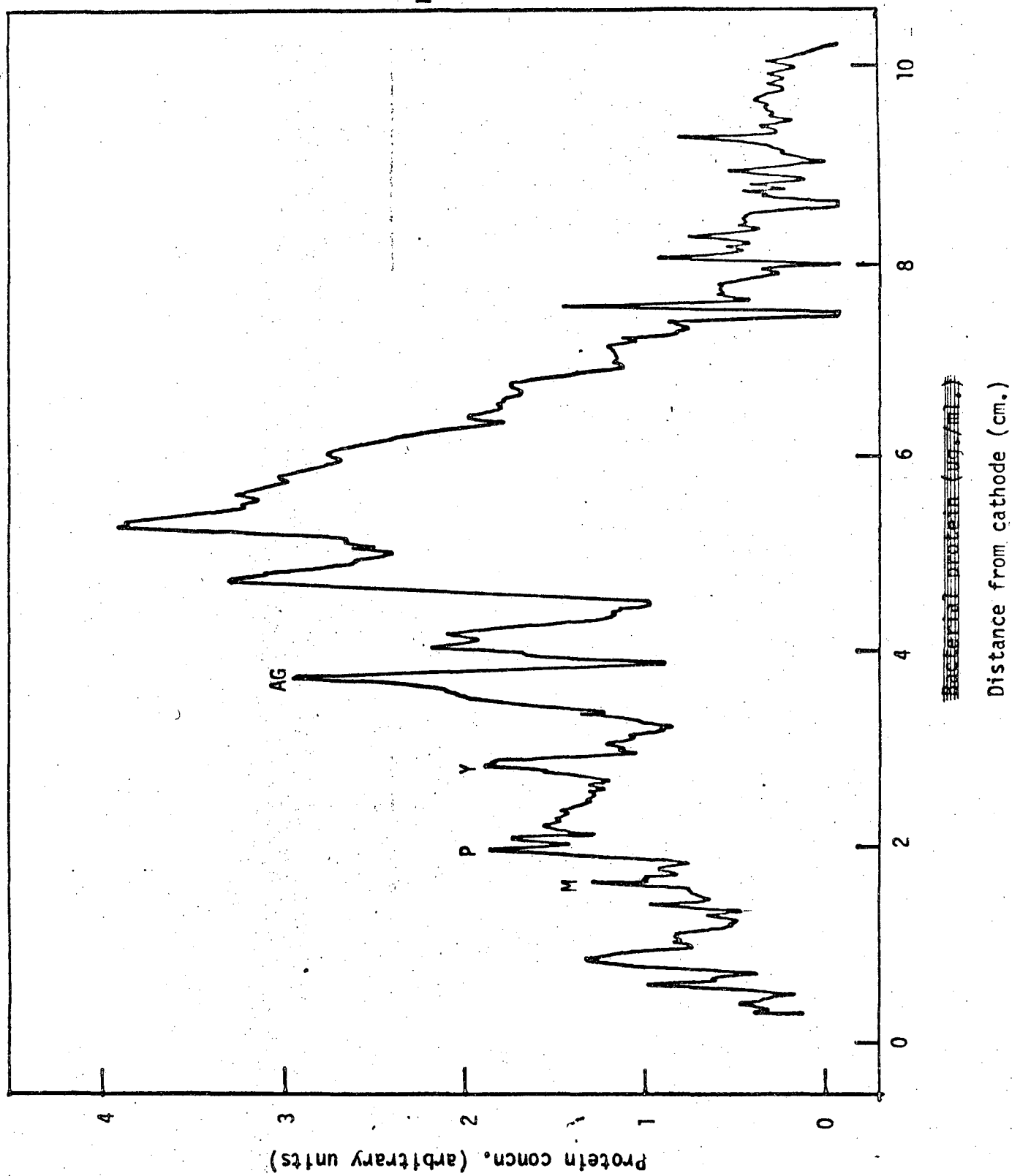



10
9
8
7
6
5
Glucose concn. in medium (mM)
50
100
150
200
250
300
350
Bacterial protein (µg./ml.)

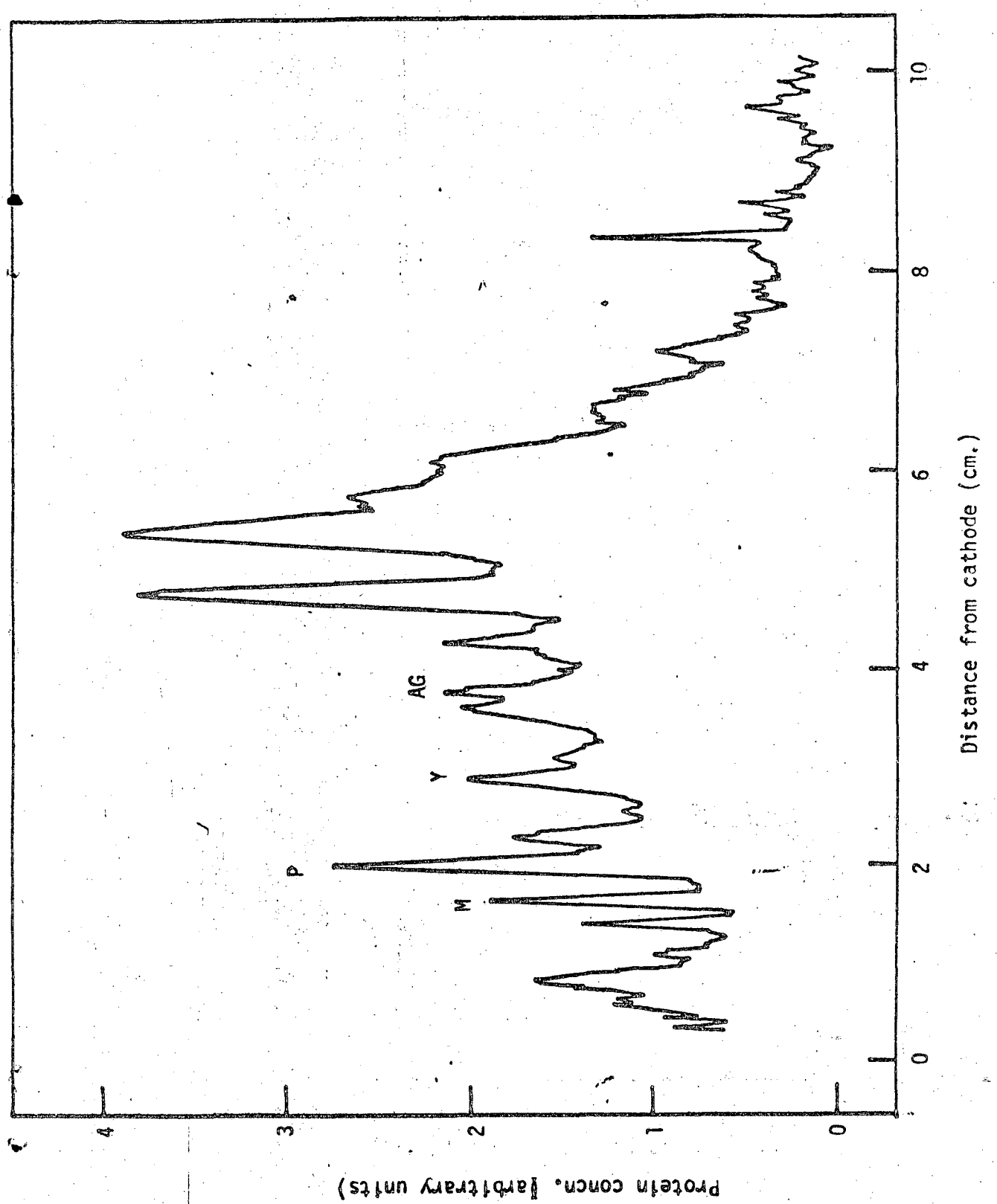

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Uracil incorporation (µg./ml.)
40
60
80
100
Bacterial protein (µg./ml.)


2.0
1.6
1.2
0.8
0.4
0.0
L-Phenylalanine incorporation (µg./ml.)
40
60
80
100
Bacterial protein (µg./ml.)

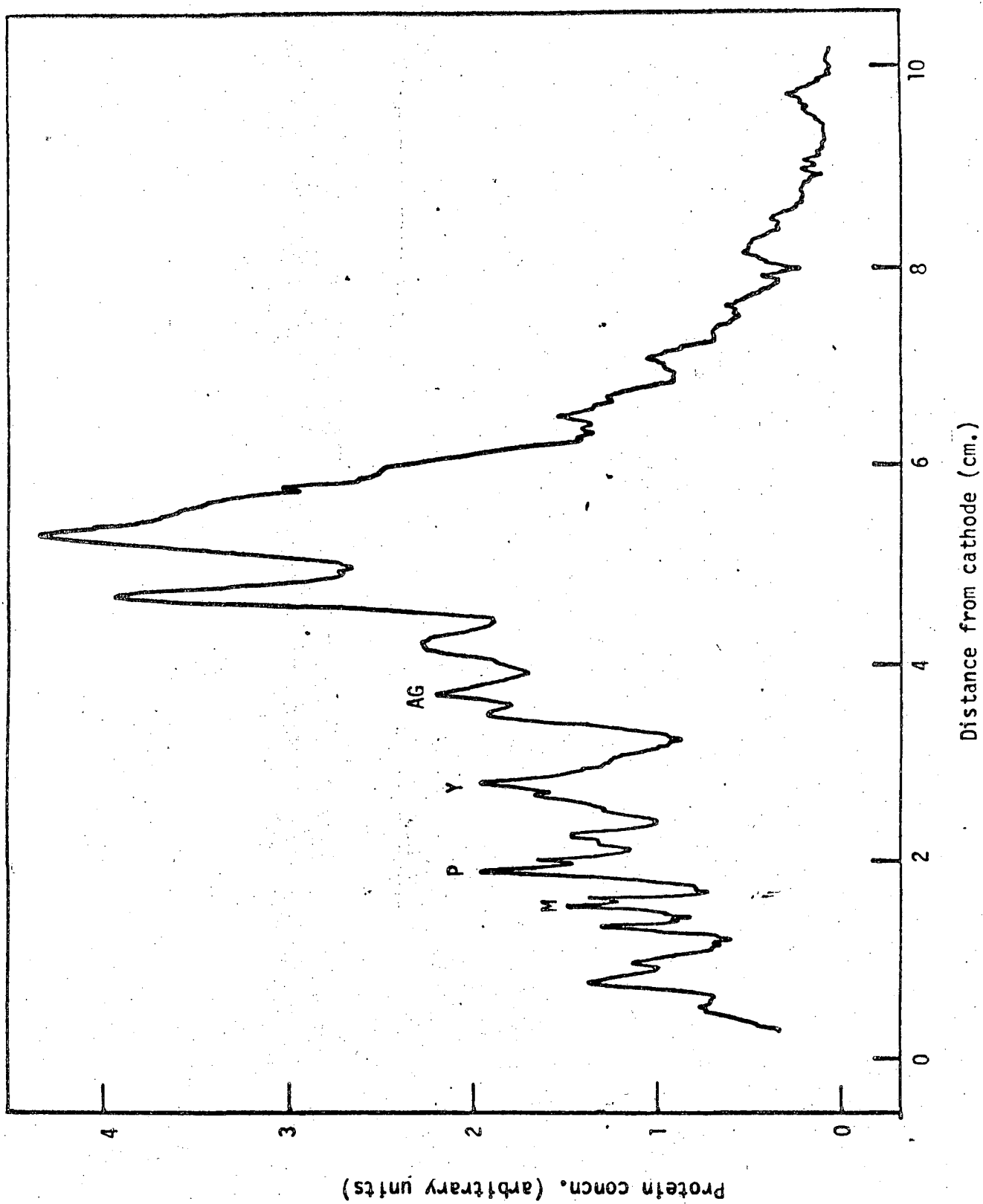
Moses & Sharp. Fig. 6(a).



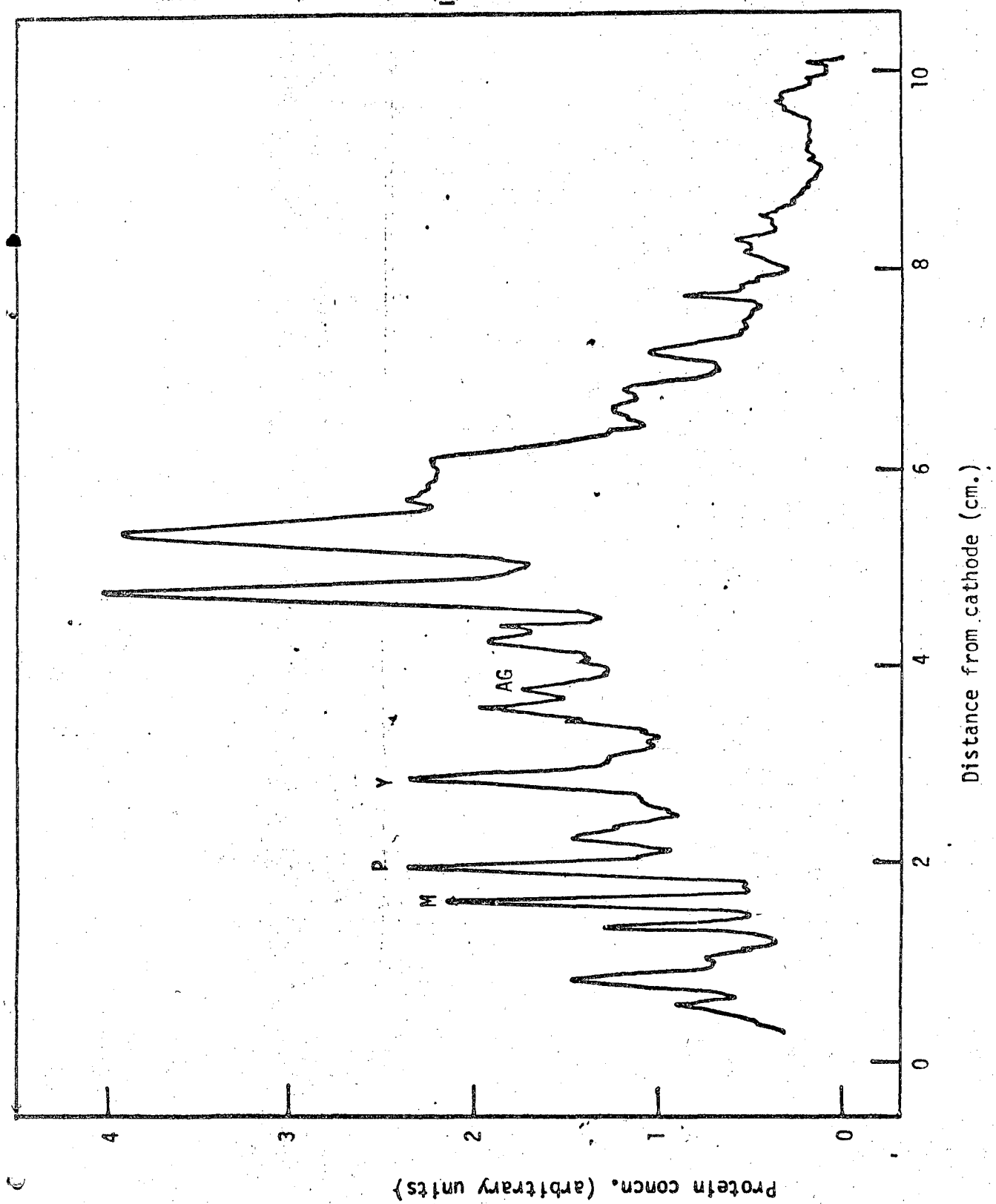
Moses & Sharp. Fig. 6(b).



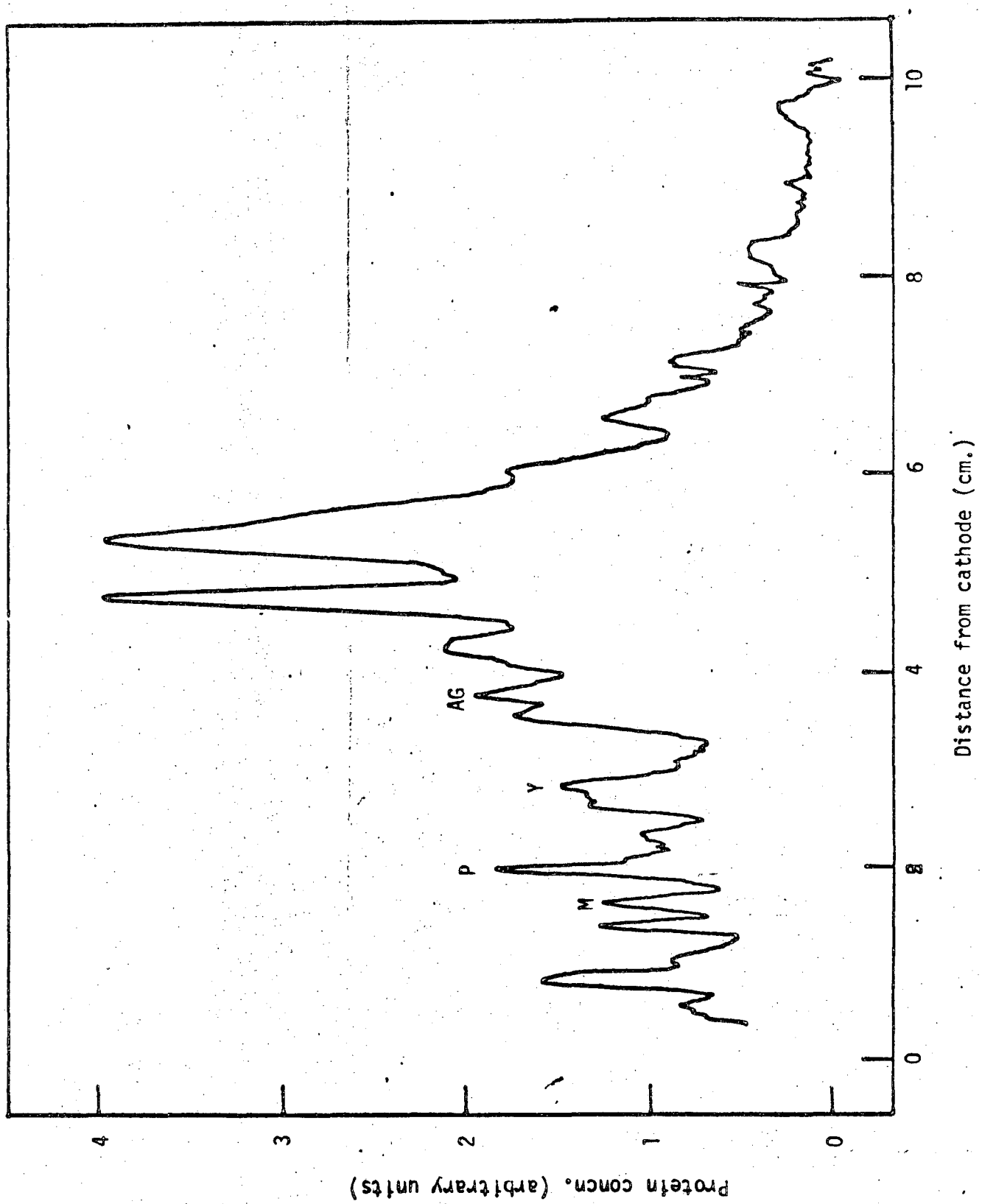
Moses & Sharp. Fig. 6(c).

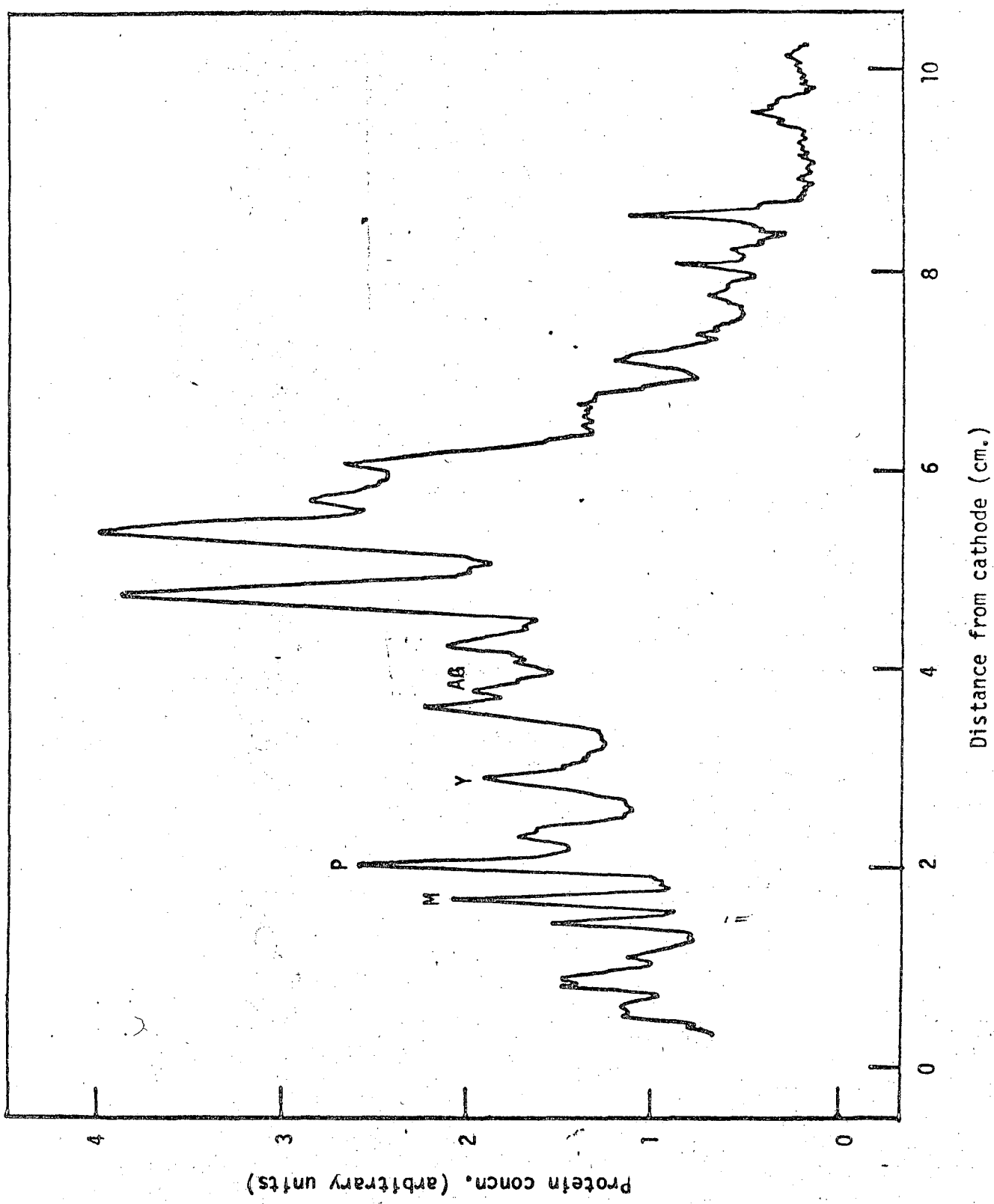


Moses & Sharp. Fig. 6(d).

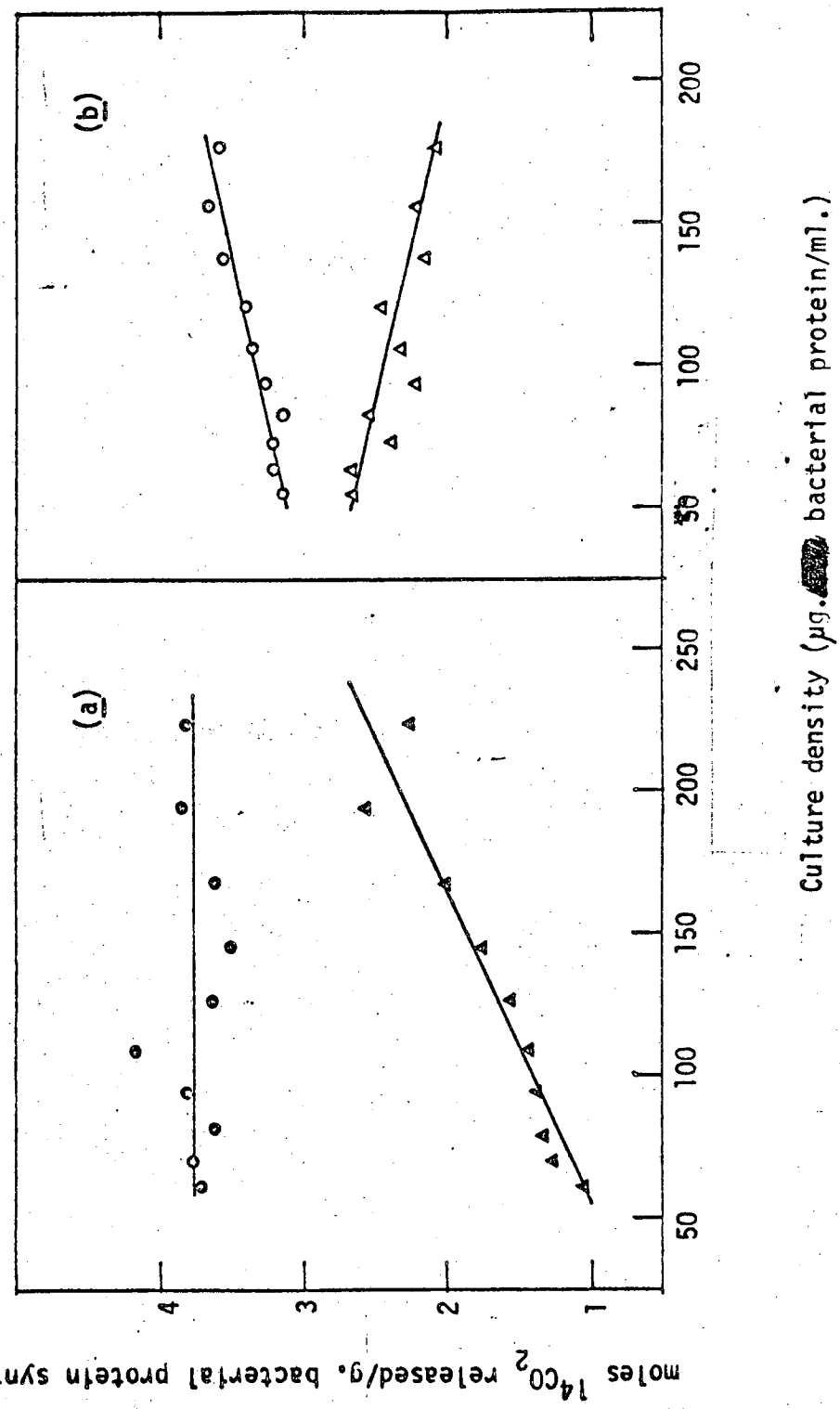


Moses & Sharp. Fig. 6(e)




Protein concn. (arbitrary units)
0
1
2
3
4
Distance from cathode (cm.)
0
2
4
6
8
10
P
M
Y
Aß

Moses & Sharp. Fig. 7.



LEGAL NOTICE

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or

B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.

TECHNICAL INFORMATION DIVISION
LAWRENCE RADIATION LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720