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TRANSPORT BY THE LACTOSE PERMEASE OF *ESCHERICHIA COLI*

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SUMMARY

Fluxes of galactosides into and out of *Escherichia coli* were measured under various conditions of temperature and pH. The effects of glucose on entry and exit of lactose, methylthio-galactoside, and *o*-nitrophenyl-galactoside were explored.

It was found that glucose and α-methylglucoside inhibited entry only when both the lactose and glucose permeases were functional, and only in cultures which had been recently exposed to glucose. The induction of the lactose permease could occur in the absence of glucose and still produce glucoside-sensitive entry if the cells were exposed to glucose immediately before or after the induction of the lactose operon.

Exit of methylthio-galactoside was sensitive to stimulation by glucose, but exit of lactose was not. Several rapidly-metabolized sugars could stimulate the exit of methylthio-galactoside, but the non-metabolizable α-methylglucoside could not.

It is concluded that the inhibition of galactoside entry by glucosides is due to an interaction between internal glucosides (or glucoside phosphates) and the lactose permease. The sensitivity of the permease units to glucosides is due to the physiological state of the membrane in which they are embedded. It is also concluded that the mechanism of glucose stimulation of exit is different from the mechanism of glucoside inhibition of entry, and that stimulation of exit is independent of the lactose permease.

INTRODUCTION

Although the lactose permease was one of the first bacterial transport systems to be recognized[1], its mode of action has yet to be satisfactorily explained. There have been two approaches to the study of this system: the isolation and characterization of the protein product of the y-gene of the lactose operon[2-4], and the investigation of transport kinetics in various mutant strains of _E. coli_[5-9]. This paper is the result of the latter approach.

Kepes[5] and Koch[6,7] proposed models in which all galactosides crossed the membrane only when complexed with small, non-specific "carriers". The protein product of the y-gene was located on the outer surface of the membrane and catalyzed the formation of carrier-substrate complexes. Glucose inhibition of entry and stimulation of exit were thought to be mediated by the carriers, which carried glucose as well as well as galactosides.

Fox and co-workers[2-4] have postulated only that the y-gene product (the M protein) binds galactosides on one side of the membrane and releases them on the other. No details of the actual transport were suggested, and no explanation for the effects of glucose was offered.

In this study, the artificial substrate oNPG has been used to study the transport of a hydrolyzable substrate into the cells, and the non-hydrolyzable substrate TMG has been used to study accumulation and exit functions of the permease. A complication of this practice is that both of these substrates cross the permeability barrier in the absence of permease, and such passive diffusion appears to account for a substantial proportion of the overall transport observed in the presence of permease[10]. A number of experiments were designed to compare the effects of various experimental conditions on transport by permease-positive and permease-negative bacteria.

The major studies of the lactose permease[5-8] were completed before the discovery of the phosphotransferase transport systems for glucose and other carbohydrates[11-13]. It now seems most unlikely that there are any components common to the glucose and lactose permeases, and alternatives must be sought to replace Koch's hypothesis. The present study is devoted to the glucose inhibition of the lactose permease, and to some of the physical parameters of galactoside transport. A mechanism is proposed for the effect of glucose on permease-mediated entry of galactosides; it is suggested that the activity of the permease, and its sensitivity to inhibition by glucose, depends upon the precise state of the membrane into which it is integrated.

The composition of the membrane is presumed to change as a consequence of growth conditions, and hence physiological state, of the cell.

MATERIALS AND METHODS

Bacterial strains

The strains of bacteria used are listed in Table I. Five additional mutant strains were used. These are derivatives of strain XA 7010/F' pro lac, mutagenized either with N-methyl-N'-nitro-N-nitrosoguanidine[14] or with ethyl methane sulfonate[15]. The mutants were selected for their ability to produce β-galactosidase in the presence of glucose, with lactose as the inducer. Mutagenized cultures were spread on minimal agar containing glucose (1%), lactose (0.2%) and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (40 μg/ml)[16]. Colonies containing appreciable quantities of β-galactosidase activity appeared blue after 36 hours at 37°. Those containing only basal level amounts of the enzyme were white. Blue colonies were then selected for inability to grow on glucose minimal medium, and concurrent ability to grow on maltose. The five strains were designated E7, E38, E45, N52, and N78; the letters indicate the mutagen used.

Growth conditions

Cultures were grown on minimal salts Medium 63 (ref. 17) containing a carbohydrate at 2g/liter, and supplemented with 2.3 μM

Abbreviations: oNPG, o-nitrophenyl-β-D-galactoside; TMG, methylthio-β-D-galactoside; IPTG, isopropylthio-β-D-galactoside; αMG, α-methylglucoside; CAP, chloramphenicol.

thiamine. For cultures of strain Cavalli, the medium was also supplemented with 0.33 mM methionine, and 16 μM thymine. When the bacteria were to be induced for the lactose operon, 0.10 mM IPTG was also present in the medium. All strains were grown aerobically with shaking at 37°.

Assays of oNPG hydrolysis

The bacteria to be assayed were harvested at 3000 x g, and resuspended in M 63 containing no carbon source (M 63 NC). The bacteria were added to oNPG (3.3 mM) in M 63 NC. At intervals, 1.2 ml aliquots were withdrawn into 0.8 ml 1 M Na_2CO_3 solution, which stopped the reaction. The bacterial debris was later removed by centrifugation, and the absorption of the supernatant was read at 420 nm.

The assays were sometimes conducted in 0.05 M potassium-Tris-maleate buffers made up to the desired pH values[18] instead of in M 63 NC.

Radioactive entry or exit experiments

The accumulation or exit of radioactive sugar was measured as in Koch[6], except that the samples (usually 1 ml) of bacteria were pipetted onto a prewetted Millipore filter (47 mm diameter, 1.2 μ pore size filters were usually employed). The bacteria were washed two or three times with 4 ml of M 63 NC at 0° and sucked dry. The filter was immediately dissolved in 18 ml dioxane scintillation fluid (Packard Fluor II formula), and radioactivity in the cells measured with a Packard Tri-Carb scintillation counter using external standardization of counting efficiency.

Lactose hydrolysis assays

Lactose hydrolysis was measured in the same way as oNPG hydrolysis, except that 2 ml aliquots of the reaction mixture were withdrawn and plunged into boiling water for 2 min. to stop the reaction. After the bacterial debris was removed, 1 ml samples of the supernatant were tested for galactose with Galactostat reagents (Worthington Biochemical Corp., Freehold, New Jersey). Suitable blanks were used to account for the slight reactivity of lactose in this system.

Chemicals and Radiochemicals

The oNPG and non-radioactive TMG were purchased from Calbiochem, Los Angeles, Calif.; α-methylglucoside was from Eastman Chemicals, Rochester, New York; and IPTG was from Cyclo Chemical Corp., Los Angeles, Calif.; radioactive TMG and glucose were purchased from New England Nuclear Corp., Boston, Mass; radioactive lactose was bought from Amersham/Searle, Des Plaines, Illinois. These products were used without further purification.

All other chemicals were ordinary laboratory grades, and were used without purification.

RESULTS

Temperature dependence of transport processes

There is a correlation between the temperature dependence of a flux of galactoside and the participation of a permease in that flux (Table II). The entry of both oNPG (measured by the rate of hydrolysis *in vivo*)* and lactose into ML 3 is less dependent on the temperature

*The rate of hydrolysis of oNPG by whole cells is much lower than the rate in broken cells (Table III), so the rate-limiting step for hydrolysis *in vivo* must be transport into the cells[6].

than the entry of these substrates into ML 30. Since ML 3 differs from ML 30 only in lacking the lactose permease, one may conclude that non-mediated transport, or diffusion, is less sensitive to the temperature than is permease-mediated transport.

The accumulation of TMG by ML 30 cells is even more dependent on the temperature than is oNPG hydrolysis. Similarly, the accumulation of lactose in the z^-y^+ strain XA 21 is more dependent on temperature than is oNPG hydrolysis in ML 30. The observed rate of lactose or TMG accumulation should not reflect any significant non-mediated entry, because non-mediated entry cannot produce a net flux against the concentration gradient. The rate of hydrolysis of oNPG, on the other hand, reflects both mediated and non-mediated entry. Thus, finding that the observed temperature dependence of oNPG hydrolysis is intermediate between those of mediated and non-mediated entry is consistent with the bimodal entry of oNPG.

The temperature dependence of lactose exit is low, indicating that lactose exit is not appreciably facilitated by the lactose permease. This agrees with the observation of Winkler and Wilson[10] that the lactose permease has a very low affinity for internal substrate. However, the high temperature dependence of TMG exit is not consistent with the pattern so far established. The inconsistency might be due to the partial mediation of TMG exit by a permease other than the lactose permease. Such a permease would not transport lactose.

Energy of activation of transport processes

The kinetics of oNPG hydrolysis in y^+ cells can be fitted by the classical Michaelis-Menten equations. Kinetic experiments at several

temperatures showed that changes in the rate of hydrolysis are due to changes in the value of V_{max}. The value of K_m remains approximately constant, while the value of V_{max} increases by a factor of twenty between 0° and 37°. The increase in V_{max} is exponential, and a fairly good fit of the data is obtained with the Arrhenius equation:

$$V_{max} = Ae^{-\Delta E^{\ddagger}/RT}$$

The value of $\Delta E^{\ddagger}/RT$ can be calculated from a plot of V_{max} vs. T^{-1} (Fig. 1). The values obtained from two such experiments were 24 Kcal/mole degree (strain ML 30) and 21 Kcal/mole degree (strain Cavalli). Although these values are subject to variation depending on the physiological condition of the cultures used, they indicate approximately the order of magnitude of the activation energy for permease-mediated transport. For comparison, the energy of activation required for the three-fold increase in hydrolysis rate between 0° and 20° in ML 3 is 8.8 Kcal/mole degree.

Enhancement of galactoside transport by a functional permease

The cell membrane of *E. coli* is slightly permeable to galactosides, even when it does not contain any galactoside permeases (Table III). Since the specific rate of entry for ML 3 was more than half the specific rate for ML 30 (Table III), the rate of non-mediated entry into ML 30 must be assumed to be large.* The y-deletion strain, RV/MS 1054, showed an anomalously high specific rate of entry of oNPG, although tests (*e.g.*

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*The effect of the non-mediated component on Michaelis-Menten kinetics is to increase the apparent value of K_m. Since V_{max} is not affected by non-mediated transport, the temperature-dependence study using V_{max} is valid for permease-mediated transport.

failure to grow on melibiose at 42°) showed that the culture was a pure y^- strain. (However, this strain is able to grow slowly on lactose medium which has been supplemented with IPTG. This is not typical of y^- strains.)

The membrane of RV/MS 1054 is much less permeable to lactose than it is to oNPG (Table III). (In the y^+ strain XA 7010/F' pro lac the rates of entry of oNPG and lactose were approximately equal.) A comparable experiment with ML 30 and ML 3 suggests that the rate of lactose entry in ML 3 is about 5% of the rate in ML 30. In one experiment, the rate of lactose entry was 0.0393 μmole/min/mg dry cells at 20° for ML 30, and 0.0015 μmole/min/mg dry cells for ML 3.

The rate of exit of TMG is affected by the presence of lactose permease to about the same degree as the rate of entry of oNPG. Exit half-times for cultures of ML 3 were 1.5 to 3 times longer than half-times for parallel cultures of ML 30 (Table IV).

The rate of lactose exit from a y^- strain was not measured. The rate of lactose exit from a z^-y^+ strain (XA 21) is lower than the rate of TMG exit from the same strain. In one typical experiment the half-time of exit at 20° was 43 minutes for lactose and 6.4 minutes for TMG. This suggests either that TMG diffuses through the membrane much more easily than lactose, or that there is a TMG exitase, as postulated earlier (see above).

Effects of pH and NaN_3 on oNPG hydrolysis

The rates of oNPG hydrolysis *in vivo* by y^+ and y^- strains at various pH values are shown in Fig. 2. The curve of rate vs. pH for the y^- strains ML 3 and RV/MS 1054 shows the combined effects of

internal pH and general membrane permeability on hydrolysis. The curve for ML 30 is also influenced by the permease activity. When the pH curve for ML 30 is normalized by subtracting the corresponding ML 3 rate from each point (Fig. 3), the optimum pH for transport is shown to be 6.0. The optimum pH is the resultant of the pH effects on both the permease protein and proton gradient across the membrane.

Mitchell[19] has proposed that the energy for active transport is derived from the electrochemical gradient of protons across the membrane, and there is some evidence[9] which suggests that this is true in the case of the lactose permease. He has also suggested that sodium azide, an inhibitor of active galactoside transport, destroys the impermeability of the membrane to protons, and in this way removes the energy source for active transport. In the Mitchell model, the proton concentration must be higher outside the cell membrane to achieve the active transport of sugars inward[19]. Thus a slightly acid external medium should, according to this model, be optimal for inward transport.

As shown in Fig. 3, permease-mediated transport is almost completely abolished by NaN_3 at pH 5.2. This might be due to the reduced internal pH caused by free movement of protons into the cell in the presence of NaN_3. However, this seems unlikely, as NaN_3 had no significant effect on the hydrolysis of oNPG in the y^- strain RV/MS 1054 at low pH values (Fig. 4). Thus, the great effect of NaN_3 on hydrolysis at low pH is due either to an interaction between the NaN_3 and the permease, or to the loss of the proton gradient across the membrane, or to both.

Michaelis-Menten kinetics and the effects of pH and NaN_3 on hydrolysis

Kinetic studies of oNPG hydrolysis show that the value of V_{max} is little affected by the pH of the medium (Fig. 5). The differences in

hydrolysis rates are due to the effect of pH on the binding constant K_m. (As mentioned above, the value of K_m reflects both binding of substrate to the permease and the contribution of non-mediated entry to the total entry.) At pH 7, where the effect of NaN_3 on hydrolysis is relatively small, only the value of K_m is affected by the poison (Fig. 6). At pH 5.2, 10^{-3} M NaN_3 abolishes permease-mediated transport, and the kinetics of poisoned transport show direct proportionality between external concentration of substrate and rate of its hydrolysis (Fig. 6).

Effect of pH on the exit of TMG

The rate of exit of TMG from ML 3 is not sensitive to the pH of the external medium (Table V), which suggests that there is no general increase in membrane permeability to galactosides with increasing pH. The exit from the permease-positive strains ML 30 and XA 7010/F' pro lac does increase with pH. The external proton concentration will be higher in an acid external medium and lower in a basic external medium; we assume that the internal pH of the cells remains constant. If, as mentioned earlier, galactoside transport is favored in the direction from lower to higher proton concentration, then entry should be favored in slightly acid media, and exit should be favored in slightly basic media. This is, in fact, observed in the permease-positive cells (Table V and Fig. 3).

Glucose inhibition of oNPG hydrolysis *in vivo*

The inhibition of oNPG hydrolysis by carbohydrates is limited to galactosides and glucosides[6]. (But see Holmes, Sheinin and Crocker[8], who found inhibition of entry by fructose, etc.) Inhibition by galactosides is competitive, in that it affects the observed value of

K_m but not V_{max}. Glucoside inhibition, though non-competitive[6], is stereospecific. It is limited in our experience to glucose, αMG, 2-deoxyglucose, and maltose. (However, it is possible that the inhibition observed in the presence of maltose results from excess glucose being released when maltose is hydrolyzed inside the cells faster than the resulting glucose can be utilized.) Of the glucosides, αMG was the most effective inhibitor. Other compounds which were tested included L-arabinose, fructose, mannose, ribose and glycerol, but none of these inhibited oNPG hydrolysis.

The inhibition of oNPG hydrolysis was not due to inhibition of β-galactosidase itself. Crude preparations of the enzyme were made by sonically disrupting cells of ML 30, and these displayed no inhibition of oNPG hydrolysis by αMG. In addition, such inhibition was not observed in hydrolysis by living cells of eight different permease-negative strains.* Four different y^+ strains* were tested for inhibition of hydrolysis, and all of them were sensitive to αMG when grown previously on glucose.

Temperature dependence of inhibition of entry by αMG

Inhibition excited by αMG is most severe at 37°, falling with decreasing temperature, to disappear at 0°. This phenomenon can be most easily visualized by comparing the Arrhenius plots of V_{max} values measured in the presence and absence of αMG (Fig. 7). It is apparent that αMG interferes with or abolishes that part of the transport mechanism which has a high energy of activation.

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*The y^- strains tested were ML 3, 230 U, RV/MS 1054, AB 1105, GR 101, JC 14 and C600 λ^-. The y^+ strains were ML 30, Cavalli, XA 7010/F' pro lac, and "wild-type" from J. Evans.

The accumulation of TMG is also inhibited by glucose at 20°, but not at 0°. Fig. 8 shows the kinetics of TMG uptake at 0° and 20° in strain XA 7010/F' pro lac; similar results were obtained with strain ML 30. Lactose hydrolysis *in vivo* shows the same temperature dependence of inhibition by αMG in strain ML 30, leading to the conclusion that the permease is not sensitive to glucosides at 0°.

Temperature dependence of inhibition of hydrolysis by TMG

By contrast to the case for inhibition by αMG, TMG inhibition of hydrolysis is completely independent of temperature. This is to be expected, since TMG is a competitive inhibitor of lactose entry. Since the value of K_m for oNPG entry does not change with temperature, one would expect the affinity of the permease for TMG also to remain constant.

Induction of glucoside sensitivity in oNPG entry

Cultures which have grown on glycerol plus IPTG may show sensitivity to inhibition of oNPG entry (as determined by hydrolysis *in vivo*) by glucoside, but the degree of inhibition in such cells is less than that in cells grown on glucose plus IPTG. In ML 30, growth on glycerol, fructose, arabinose, mannose or ribose typically fails to produce sensitivity to glucosides; growth on glucose or maltose always produces sensitivity.

The time required for cells growing in glucose medium to develop sensitivity to glucoside inhibition of oNPG entry is about 90 minutes, or rather more than one mass doubling time. Sensitivity will not develop in the presence of chloramphenicol; protein synthesis is therefore required (Table VI).

Although the entry of oNPG into y^- strains is not inhibited by glucosides, the synthesis of the y-gene product need not occur in glucose medium for sensitivity to glucose to be present. Bacteria grown in glycerol medium plus IPTG were washed and transferred to glucose medium without IPTG. Within two hours, oNPG hydrolysis became sensitive to inhibition by glucose (Table VII).

Conversely, when uninduced glucose-grown cells were washed and exposed to IPTG in a medium lacking a carbon source, the hydrolysis of oNPG which gradually developed as a result of the synthesis of the lactose enzymes was sensitive to αMG inhibition, and remained so for at least two hours (Table VII). When similar cells were exposed to IPTG in a glycerol medium, sensitivity to αMG was lost more rapidly. Since growth could occur in the presence of glycerol, but not in the absence of a carbon source, it is concluded that some aspect of the growth process is necessary to remove sensitivity of the permease to αMG.

Role of the glucose permease in αMG inhibition of oNPG hydrolysis.

Although the common carrier hypothesis[5-7] is probably incorrect, it seemed possible that an active glucose permease is necessary to bring the glucoside inside the cell before it can inhibit the lactose permease. A series of five glucose-negative, lactose-positive mutants derived from XA 7010/F' pro lac were used to study this hypothesis. Each mutant was tested for the effect of αMG on oNPG hydrolysis in maltose-grown cells, and for the rates of entry of [^{14}C]glucose. The results are presented in Table VIII. In two strains, oNPG hydrolysis

in vivo was insensitive to αMG inhibition, and in those strains glucose accumulation was only 10% as fast as it was in the parent.

Effect of glucose on exit of labelled TMG

The presence of glucose in the external medium causes a marked increase in the rate of exit of TMG. Several other metabolizable sugars are also able to increase the rate of exit (Table IX). αMG has no effect on exit. This glycoside has been shown to enter the cells via the glucose permease, but it is accumulated as the phosphate and not further metabolized[20,21]. Stimulation of TMG exit is thus dependent on metabolism of the sugars, rather than on their transport.

The concentration of glucose required to speed the exit of TMG is small (Table X). It is not possible to determine the inhibitor constant exactly because the glucose is continuously being removed from the medium by the bacteria, and, in the concentration range of 10^{-4} to 10^{-5} M, the change of concentration during the experiment is significant.

Cultures grown on a variety of sugars lose their accumulated TMG more rapidly in the presence of glucose. In general, those sugars which speed exit also induce sensitivity to glucose. Glycerol and succinate are not effective either as agents for speeding exit themselves or for inducing susceptibility to glucose speeding of exit (Table XI)[6].

A culture of ML 30 develops sensitivity to glucose in about 2 hours of growth on glucose. The presence of chloramphenicol prevents development of sensitivity, so either new and specific proteins must be made, or general growth is required for sensitivity to develop (Table XII).

In maltose-grown cells of the glucose-permease negative strains N52 and N78, neither glucose nor maltose speeds exit of TMG. In the parent strain XA 7010/F' pro lac, glucose shows the typical stimulatory effect on TMG exit.

Exit of lactose

The exit of lactose accumulated by the z^-y^+ strain XA 21 was compared with TMG exit from the same strain. The rate of lactose exit was much lower than that of TMG, with a half-life of 35 minutes for lactose and 12.5 minutes for TMG. In addition, the exit of lactose was either unaffected or slowed by the presence of glucose, although the exit of TMG was stimulated by glucose (Table XIII).

DISCUSSION

Temperature dependence and independent carriers

Koch[6] observed that the temperature dependence of a diffusion process is considerably less than that observed for oNPG transport into ML 3, or for the exit of TMG from any strain. For this reason he concluded that the non-mediated transport of galactosides must depend on a non-specific carrier within the membrane, rather than occuring by simple diffusion across the membrane. However, a simple diffusion process is not excluded by the high temperature dependence of non-mediated transport. We know that galactosides are not very soluble in lipids, and it follows that a molecule passing from the aqueous external medium into the membrane must experience a large positive change in chemical potential. On passing from the membrane

back into an aqueous phase inside the cell, the chemical potential of the molecule will return to a lower level. This series of changes is mathematically analogous to a chemical reaction with a high energy of activation.

In a chemical reaction, the temperature dependence depends on the magnitude of the energy of activation. For diffusion across the membrane, the temperature dependence is determined by the difference between the chemical potential of the galactoside inside and outside the membrane.

Non-specific membrane permeability to oNPG

The rate of oNPG hydrolysis by y^- strains has been reported to be much lower than the rate of hydrolysis by y^+ strains[6]. We have not been able to confirm this report in the permease-negative strains which have been studied. From the data presented in this paper, one may conclude that about 20-30% of the hydrolysis observed in ML 30 is due to non-mediated entry of oNPG. (The proportion of lactose entry due to non-specific entry is lower, being about 5%.) Thus the inhibition of oNPG entry by as much as 70% with αMG or NaN_3 reflects complete inhibition of the permease-mediated entry.

These observations may explain why the accumulation (but not the passive entry) of TMG is completely inhibited by a concentration of NaN_3 which inhibits the rate of oNPG hydrolysis by only 50%. The rate of TMG entry is slowed to the point where non-specific (or TMG exitase-mediated) exit is just as fast as entry, and thus there is no accumulation. Permease-mediated oNPG entry is reduced by the same amount

as the TMG entry, but the large non-specific component of oNPG entry is unaffected. It is not necessary in this case to suppose that the energy-yielding part of the permease mechanism has been uncoupled from the carrier part of the mechanism.

Temperature dependence of inhibition by αMG

The failure of αMG to inhibit hydrolysis at 0° is probably due to a lack of sensitivity of the permease to αMG at that temperature. There can be no doubt that the glucoside is transported into the cell at 0°, since glucose enters at a rate sufficient to stimulate exit of TMG even at 0° (Table IV). Further, since oNPG hydrolysis at 0° can be inhibited by TMG, one concludes that the permease itself functions normally at 0°.

The sensitivity of the permease to glucosides may be determined by the lipid matrix into which it fits in the cell membrane. (This conclusion is suggested by the provisional nature of the sensitivity which depends on the carbon source in which the cells were grown.) It is not unreasonable to suppose that the exact configuration of the membrane lipid is partly determined by temperature, and it is entirely possible that the glucoside-sensitive configuration of the permease protein is lost when the lipids in the membrane assume their low-temperature configurations.

Relation of the permease protein to the membrane

Fox[4] has shown that unsaturated fatty acid must be present during the induction of the lactose operon in order to produce functional permease units. Although M (i.e. permease) protein is made in the

absence of the fatty acid, no increase in the transport of galactosides is observed in the absence of unsaturated fatty acid. Fox[4] suggests two hypotheses to explain his observations: either new sites must be manufactured in the membrane to accomodate the M protein as it is synthesized, or functional M protein is a lipoprotein which must be bound to the correct fatty acid as it is synthesized[4].

All of the strains used in this study were competent to synthesize all their necessary fatty acids. The studies of induction of permease sensitive to glucose inhibition of oNPG hydrolysis in vivo suggest that glucose metabolism creates a physiological condition which persists after the glucose is removed. This condition, which may be a configuration of membrane lipids, is sufficient to create glucose-sensitive permease protein. We have no evidence to determine whether or not new permease protein can be incorporated into previously synthesized membrane. If the permease can be incorporated into old membrane, however, it seems likely that membrane synthesized during glucose metabolism determines a glucose-sensitive configuration of the M protein incorporated into it.

Effects of glucose and αMG on entry and exit

The effects of glucose and αMG on entry and exit of galactosides are so different that two separate processes must be involved. First, the specificities of the two processes are different: entry is sensitive only to glucose, αMG, 2-deoxyglucose, and maltose; while exit is sensitive to a number of metabolizable sugars, but not to αMG. Second, inhibition of entry does not occur at 0°, but the stimulation of exit is not affected by temperature. Third, it is possible to induce

sensitivity to glucose in only one process at a time. Entry can be made sensitive by growth on glycerol in the presence of αMG, while exit cannot. Exit is made sensitive to glucose by growth on fructose, etc., but oNPG entry is not. Lastly, the inhibition of entry by glucosides occurs only in y^+ strains, while the speeding of exit occurs in both y^+ and y^- strains.

Exit of TMG

The high temperature dependence of TMG exit suggests that this process is not entirely passive, but the difference in exit rate between y^+ and y^- cells is so small that the lactose permease cannot play a very large part in the exit of TMG. The very slow rate of lactose exit, and the insensitivity of lactose exit to stimulation by glucose, suggest that exit of lactose and exit of TMG do not occur entirely by the same pathway. The TMG II permease described by Rotman, Ganesan and Guzman[22] might be responsible for the difference between exit of lactose and that of TMG, as the TMG II permease does not transport lactose.

CONCLUSIONS

Entry

We conclude that the entry of oNPG into y^+ cells is bimodal, with an appreciable contribution of passive diffusion to the total. Lactose entry is largely unimodal, because passive diffusion of lactose is much slower than diffusion of oNPG.

The inhibition of galactoside entry by glucose is due to an interaction between internal glucose and the lactose permease. Since glucose and glucosides are phosphorylated in the process of entry[21],

glucoside-6-phosphates are probably responsible for the inhibition. The sensitivity of the permease to inhibition by glucosides depends both on the growth medium and the temperature. Pre-formed permease units can become sensitive to glucosides after exposure to glucose, and permease units synthesized in the absence of glucose are sensitive to glucoside inhibition if the cells have recently been exposed to that sugar. We conclude that the sensitivity of the permease units to glucosides is due to the physiological state of the membrane in which they are embedded.

Exit

TMG probably leaves the cells via three pathways: the hypothetical TMG exitase (which may be the same as the TMG II permease[22]), the lactose permease, and by passive diffusion. Glucose stimulation of exit probably is due to interaction between glucose and the TMG exitase.

The mechanism of glucose stimulation of exit is entirely separate from the mechanism of glucoside inhibition of entry. The stimulation of exit applies only to TMG and not to lactose, and the presence or absence of the lactose permease has no effect on the stimulation of TMG exit. The stimulation of exit can be effected by several rapidly-metabolized sugars, but not by non-metabolizable glucosides. In contrast, the lactose permease must be present for inhibition of galactoside entry by glucosides, and only glucosides inhibit entry, though non-metabolizable glucosides are as effective as those that are metabolized.

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CAPTIONS TO FIGURES

Fig. 1. Arrhenius plot of the temperature dependence of V_{max} for oNPG hydrolysis *in vivo* in ML 30. Data for oNPG hydrolysis *in vivo* at several temperatures was plotted in Lineweaver-Burk plots. The values of V_{max} from these plots were used in this figure.

Fig. 2. oNPG hydrolysis *in vivo* in 0.05 potassium tris maleate buffers as a function of pH. (o) ML 30 (y^+); (Δ) ML 3 (y^-); (●) RV/MS 1054 (y^{del}). Assays at 37°.

Fig. 3. Calculated activity of the lactose permease as a function of pH, together with the effect of 1 mM NaN_3. Rates of oNPG hydrolysis were determined at different pH values for parallel cultures of ML 30 and ML 3. The difference between the two sets of values is plotted against pH; this measures only the permease-mediated entry of oNPG. (o) control; (●) NaN_3.

Fig. 4. The effect of 1 mM NaN_3 on oNPG hydrolysis in RV/MS 1054 as a function of pH. Assays at 37°. (o) control; (●) NaN_3.

Fig. 5. Lineweaver-Burk plots for oNPG hydrolysis *in vivo* in 0.05 M potassium tris maleate buffers at three pH values. ML 30 assayed at 28°. (o) pH 8.6; (●) pH 5.2; (Δ) pH 6.8.

Fig. 6. oNPG hydrolysis *in vivo* in ML 30 in 0.05 M potassium tris maleate at 37°, pH 5.2 and 7.0, with and without 1 mM NaN_3. Lineweaver-Burk plots. (o) control; (●) NaN_3.

CAPTIONS TO FIGURES (continued)

Fig. 7. Effect of 1 mM αMG on Arrhenius plot of ln V_{max} for oNPG hydrolysis *in vivo* in ML 30 grown on glucose + IPTG. (o) control; (Δ) αMG.

Fig. 8. Effect of 1 mM αMG on accumulation of TMG in ML 30 grown on glucose + IPTG at 20° (A) and 0° (B). (o) control; (●) αMG.

TABLE I

STRAINS OF *E. COLI* USED

Name	Lactose operon	Origin
ML 30	$i^+z^+y^+$	J. Monod
ML 3	$i^+z^+y^-$	A. J. Clark
Cavalli	$i^+z^+y^+$	A. Simmons
230U	$i^-z^+y^-$	J. Monod
RV/MS 1054	$lac^{del}/i^+z^+y^{del}$	M. Malamy
XA 7010/F' pro lac	$i^{del}z^{del}_{M41}y^+/pro^+_{A,B}lac\ i^+z^+y^+$	V. Moses
AB 1105	$i^+z^+y^-$	E. A. Adelberg
GR 101	$i^+z^+y^-$	M. Riley
JC 14	$i^+z^+y^-$	A. J. Clark
WI 4	$i^+z^+y^-$	A. Novick
C600 λ^-	$i^+z^+y^-$	C. Wilson
"wild"	$i^+z^+y^+$	J. Evans
XA 21	$i^-z^{del}_{M15}y^+$	J. G. Scaife

TABLE II

TEMPERATURE DEPENDENCE OF TRANSPORT PROCESSES

The cultures used were all grown on glucose medium and induced with IPTG. The rates of transport processes were measured at 20° and 0° as described in Materials and Methods. The rate at 20° for each process was divided by the rate at 0° in the same culture of bacteria.

Process	Strain (y-gene indicated)	Rate at 20° / Rate at 0°
oNPG hydrolysis *in vivo*	ML 30 (y^+)	13
oNPG hydrolysis *in vivo*	ML 3 (y^-)	3.2
[^{14}C]lactose entry	ML 30 (y^+)	14
" " "	ML 3 (y^-)	4.3, 3.4
" " "	XA 21 (y^+)	24
[^{14}C]TMG exit	ML 30 (y^+)	19, 16, 17.5
" " "	ML 3 (y^-)	10, 10, 14.5
" " "	XA 21 (y^+)	10.8
[^{14}C]lactose exit	XA 21 (y^+)	2.9
[^{14}C]TMG accumulation	ML 30 (y^+)	54

TABLE III

EFFECT OF PERMEASE ON RATE OF SUBSTRATE ENTRY

Strain	y-gene	Rate of oNPG entry*	Rate of lactose entry**
ML 30	wild	2.69×10^{-2}	
ML 3	point mutation	1.40×10^{-2}	
XA 7010/F' pro lac	wild	2.75×10^{-2}	3.72×10^{-2}
RV/MS 1054	deletion	2.24×10^{-2}	7.87×10^{-3}

* Rate of oNPG hydrolysis in whole cells divided by the rate of hydrolysis in sonicated cells from the same culture.

** Rate of [^{14}C]lactose entry in μmole/min. divided by rate of hydrolysis (μmole/min.) in corresponding sonicated cells.

TABLE IV

EFFECT OF INHIBITORS OF THE LACTOSE PERMEASE ON EXIT OF TMG FROM ML 30 AND ML 3 AT 23° AND 0°

The bacteria were grown on glucose + IPTG; the concentrations of inhibitors were 1 mM in the exit media for the experiments at 0°, and 2 mM for those at 23°.

Strain	Sugar in exit medium	Half-time of exit (min) at 0°	Half-time of exit (min) at 23°	Half-time as % of control at 0°	Half-time as % of control at 23°
ML 30	None	120	4.5	100	100
	Galactose	73	2.2	61	49
	TMG	61	3.3	51	73
	Lactose	58	1.9	48	42
ML 3	None	--	7.2	--	100
	TMG	--	7.9	--	110
	Lactose	--	8.4	--	117
	Glucose	--	4.2	--	58

TABLE V

EFFECT OF pH ON TMG EXIT AT 23°

Exit media were 0.05 M potassium-Tris-maleate buffers

pH of exit medium	Exit half-times, in minutes				
	Strain ML 30			Strain XA 7010/F' pro lac	Strain ML 3
	Control	mM NaN_3	mM NaCl		
5.2	8.2	12.4	7.4		7.9
5.3	---	---	---	19.2	
6.0	---	---	---	22.4	
6.4	8.7	7.4	9.3	---	7.7
6.8	---	---	---	16.0	---
7.6	7.6	5.2	6.3	17.0	7.7
8.0	---	---	---	11.3	
8.5	4.4	3.7	3.6	---	7.9
8.6	---	---	---	7.7	---

TABLE VI

GROWTH CONDITIONS AND SUSCEPTIBILITY TO GLUCOSE INHIBITION OF HYDROLYSIS

In vivo hydrolysis of oNPG becomes sensitive to severe inhibition by αMG only when the permease is synthesized in the presence of glucose or αMG. The first three sections of this table are results obtained with overnight cultures of ML 30 grown in M63 + glycerol + IPTG. One culture was poured into 300 ml of M63 + glucose; the sample for section #1 was removed into chloramphenicol (CAP) (2 ml of 1 mg/ml CAP for an 18 ml sample of culture) after 20 seconds. The samples listed in section #3 were later withdrawn in the same way. The samples were spun down within a few minutes of their withdrawals, then resuspended in M63 NC and assayed for oNPG hydrolysis with and without 1.5 mM glucose at 37°.

Exposure to glucose	Rate in glucose as % of control
1. Control: glucose and CAP added simultaneously; culture chilled at once.	70, 100
2. Glucose added with CAP; culture incubated two hours at 37° before chilling.	109
3. Glucose added x min before CAP; culture chilled immediately after the addition of CAP	
x = 30 min.	100, 124
x = 60 min.	69
x = 90 min	44
x = 120 min.	51
4. Glucose-grown, uninduced culture harvested and resuspended in M63 NC with IPTG for two hours at 37°.	82

TABLE VII

GROWTH CONDITIONS PRODUCING SENSITIVITY OF oNPG HYDROLYSIS TO αMG

1. Bacteria grown and induced with IPTG in the absence of glucose

Incubation medium after induction	Hydrolysis rate of control	Hydrolysis rate in αMG
Glucose	100	29.4
Glycerol	69.5	67.8

2. Bacteria grown in glucose, then induced in NC or glycerol media

Sample time after addition of IPTG	Control (NC)	αMG (NC)	αMG as % of control	Control (glyc)	αMG	αMG (glyc)
10'	1.00	0.125	12.5	1.00	0.183	18.3%
20'	1.13	0.127	11.2	1.25	0.200	16.0
40'	0.744	0.113	15.2	1.67	0.375	22.5
120'	0.620	0.113	18.2	8.00	5.25	65.6

TABLE VIII

SENSITIVITY OF GLUCOSE-NEGATIVE MUTANTS TO αMG INHIBITION OF oNPG HYDROLYSIS *IN VIVO*

Strain	Specific glucose uptake	oNPG hydrolysis *in vivo* $\frac{\text{rate + αMG}}{\text{rate - αMG}}$
XA 7010/F' pro lac	1.00	0.25
E 7	0.83	0.32
E 38	0.50	0.32
E 45	0.50	0.25
N 52	0.091	1.01
N 78	0.110	1.08

TABLE IX

SUGARS OTHER THAN GLUCOSE WHICH INCREASE THE EXIT RATE OF TMG

ML 30, grown on several carbohydrates + IPTG, was tested for sensitivity. All the experiments were done at 23°.

Sugar in growth medium	Sugar in exit medium	Half-time of exit as % of control	Half-time (min)
Lactose	Lactose	14	0.9
Lactose	Galactose	25	1.6
Galactose	Galactose	40	3.4
Glucose	Maltose	105	6.0
Maltose	Maltose	62	3.7
Glucose	Fructose	90	5.2
Fructose	Fructose	56	3.1
Glucose	Mannose	75	4.3
Mannose	Mannose	89	2.4
Glucose	Arabinose	92	5.3
Arabinose	Arabinose	69	4.0
Glucose	Glucose-6-P	107	6.6
Glucose	Ribose	84 50	5.1 2.6
Ribose	Ribose	34	2.7

TABLE X

CONCENTRATION OF GLUCOSE REQUIRED TO CAUSE INCREASE IN EXIT RATE OF TMG

ML 30 grown in glucose + IPTG. Exits at 23°

Conc. of glucose in exit medium	Half-life of exit (min)	Exit half-life as % of control
None	6.04	100
10^{-6} M	5.96	99
10^{-5} M	4.98	83
10^{-4} M	3.31	55
10^{-3} M	3.05	51

TABLE XI

CONDITIONS INDUCING TMG EXIT SENSITIVE TO GLUCOSE

Bacteria were grown on the sugar listed + IPTG except where otherwise stated. Exits at 23°.

Strain	Carbohydrate in growth medium	Half-time of exit (min)	(% control)
ML 30	Glycerol	3.3	94
"	Glycerol + αMG	3.6	100
"	Glucose	2.32	45
		2.25	52
		3.1	50
		2.8	67
"	Lactose	3.8	90
		3.4	54
"	Galactose	9.0	107
"	Maltose	3.4	65
"	Ribose	3.7	48
"	Fructose	3.1	56
"	Arabinose	5.1	88
"	Mannose	2.7	71
"	Na gluconate	3.8	99
		3.9	69
"	Glucose (no IPTG)	2.1	34
Cavalli	Glucose	1.6	57
ML 3	Glucose	4.2	59
230 U	Glucose	1.5	33

TABLE XII

KINETICS OF DEVELOPMENT OF SENSITIVITY TO GLUCOSE FOR TMG EXIT

ML 30 was grown overnight in 100 ml of M 63 + glycerol + IPTG. At the beginning of the experiment the culture was poured into 500 ml of M 63 + glucose. Samples were withdrawn into CAP as before, centrifuged and loaded with TMG. The rates of exit were then measured as described in the Methods section.

Length of exposure to glucose	Half-time of exit without glucose (min)	Half-time of exit with glucose (min)	(% control)
20 sec	4.04	4.07	101
5 min	4.46	4.27	96
15 min	3.87	3.77	98
30 min	5.46	3.28	60
45 min	4.47	4.45	101
60 min	5.65	4.98	88
90 min	9.51	5.78	61
120 min	12.78	5.67	44

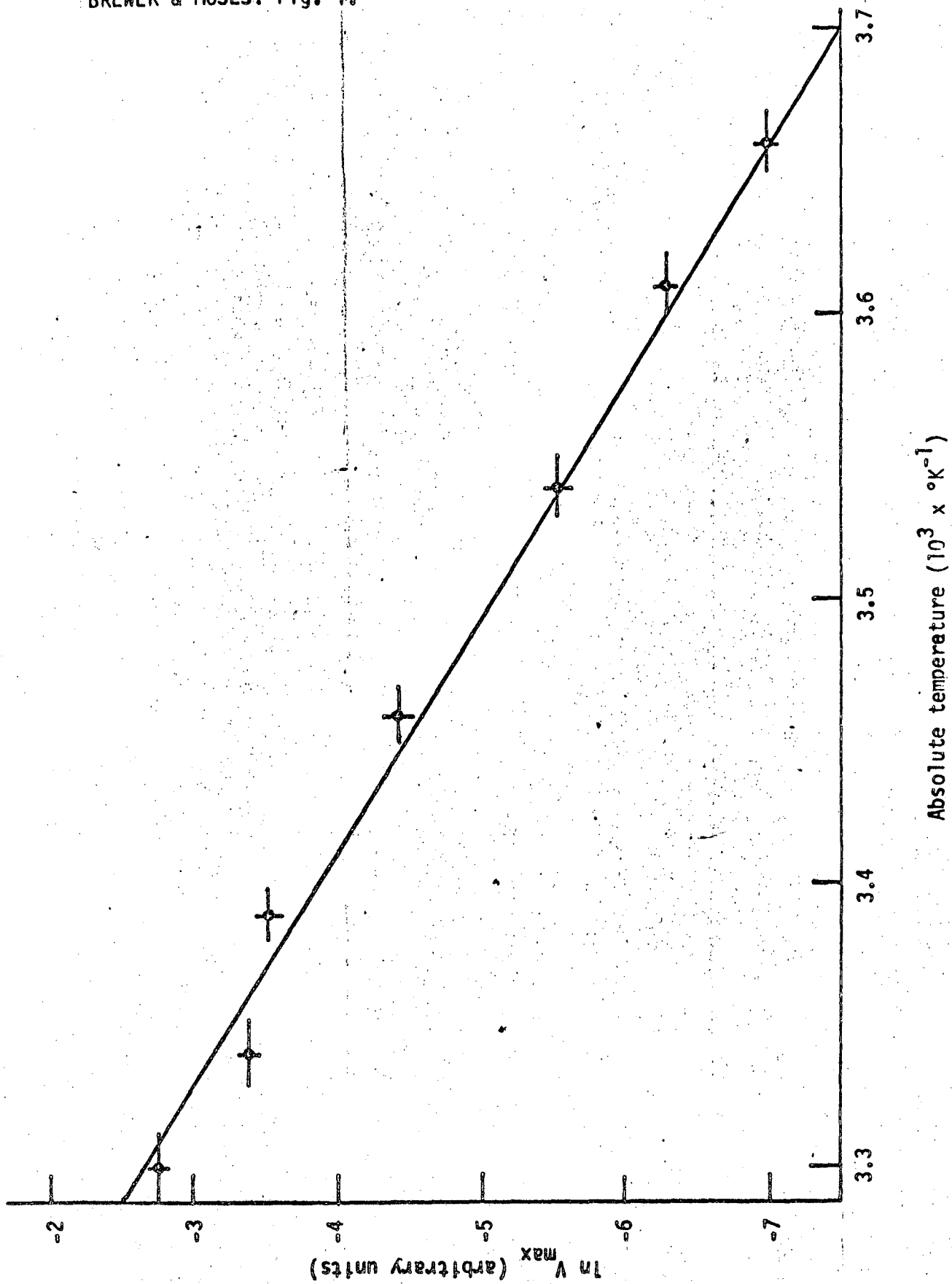
TABLE XIII

EXIT OF TMG AND LACTOSE IN STRAIN XA 21

The exits were at 23°, as described in Materials and Methods

Expt. No.	Substrate	Half-time for exit (control) (min)	Half-time for exit (+ glucose, 10^{-3} M)
1	TMG	6.4 min	4.3 min
2	Lactose	42.5 min	58 min
3	Lactose	36 min	33 min
4	Lactose	36 min	35 min

BREWER & MOSES: Fig. 1.



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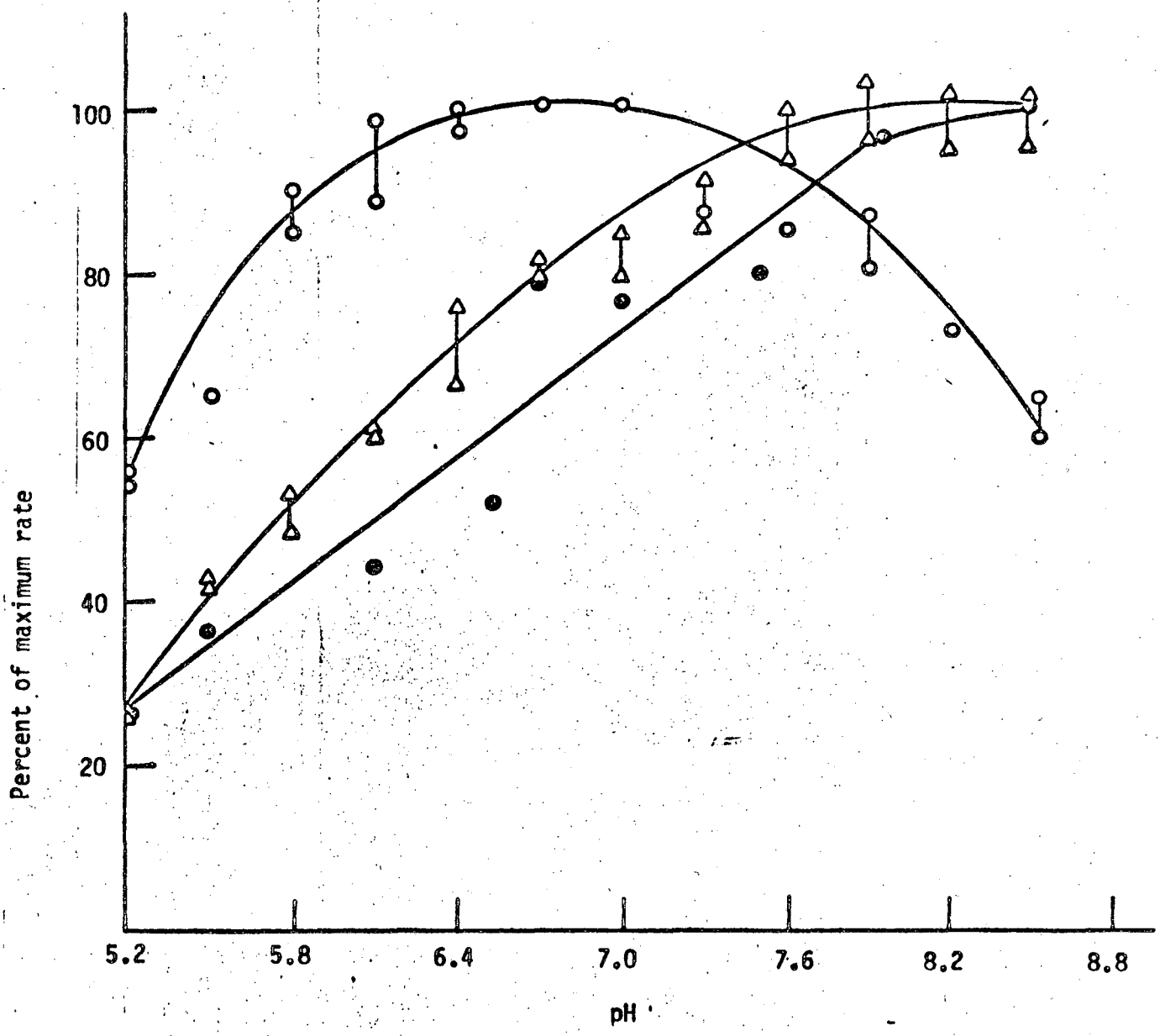

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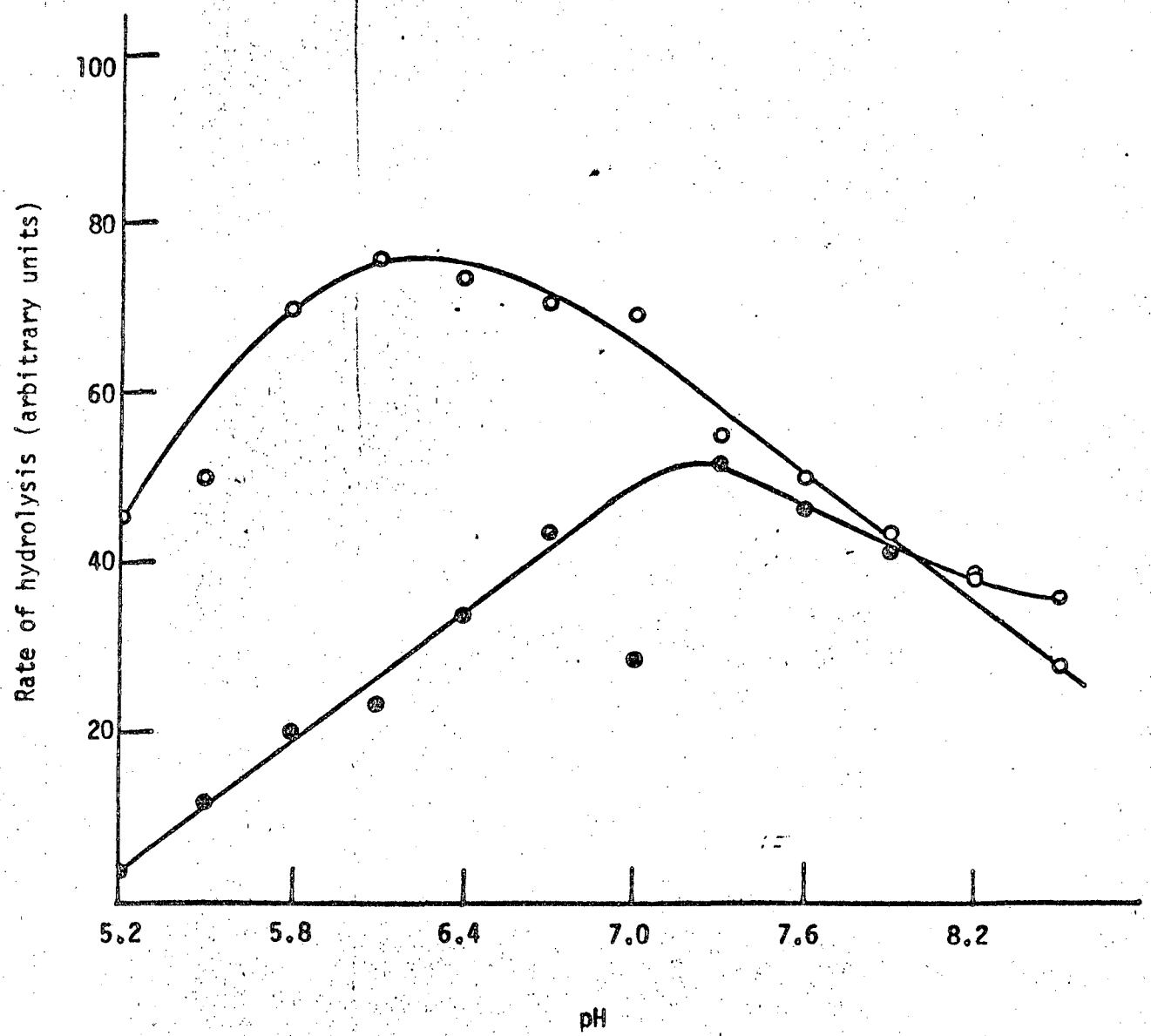

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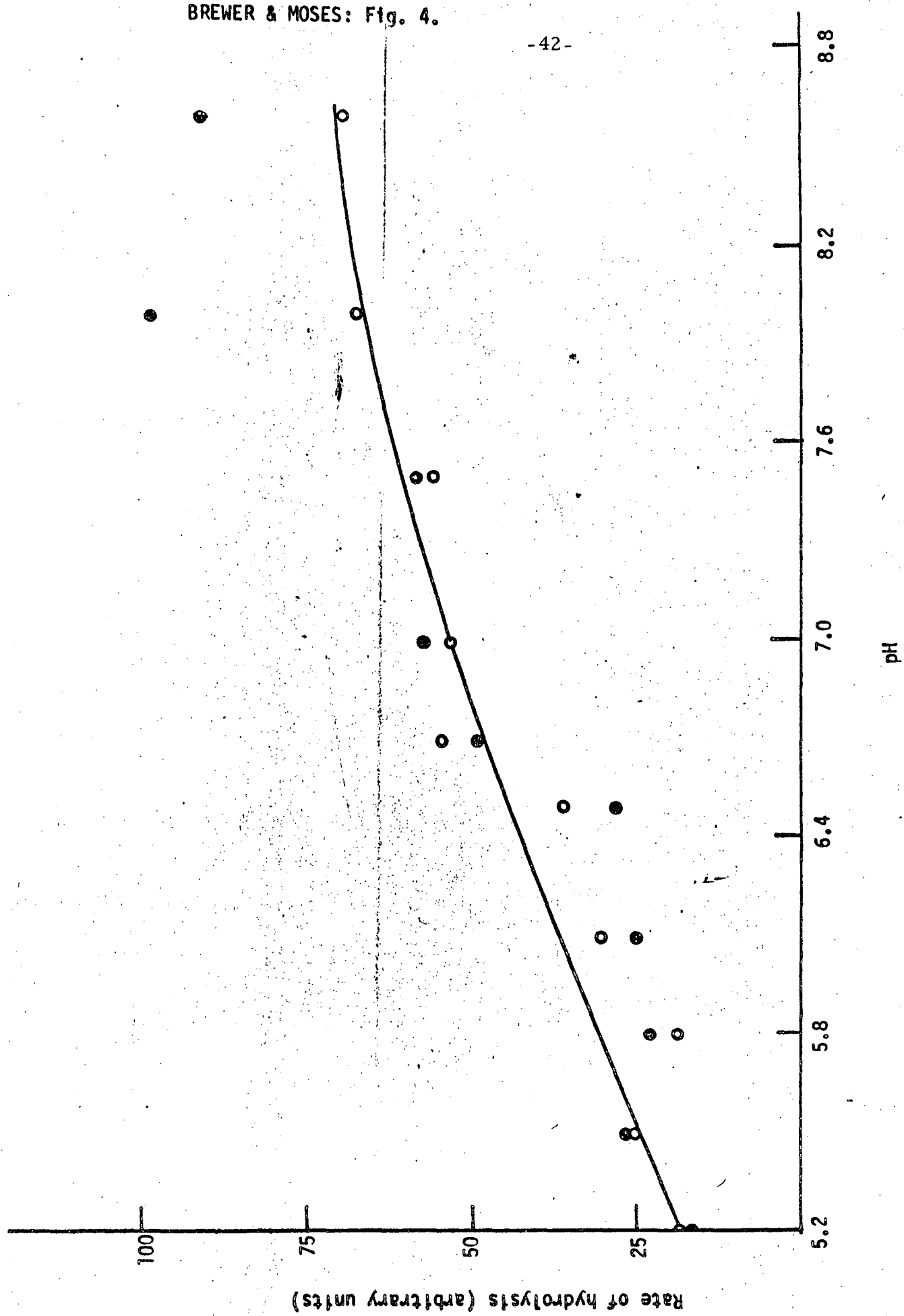
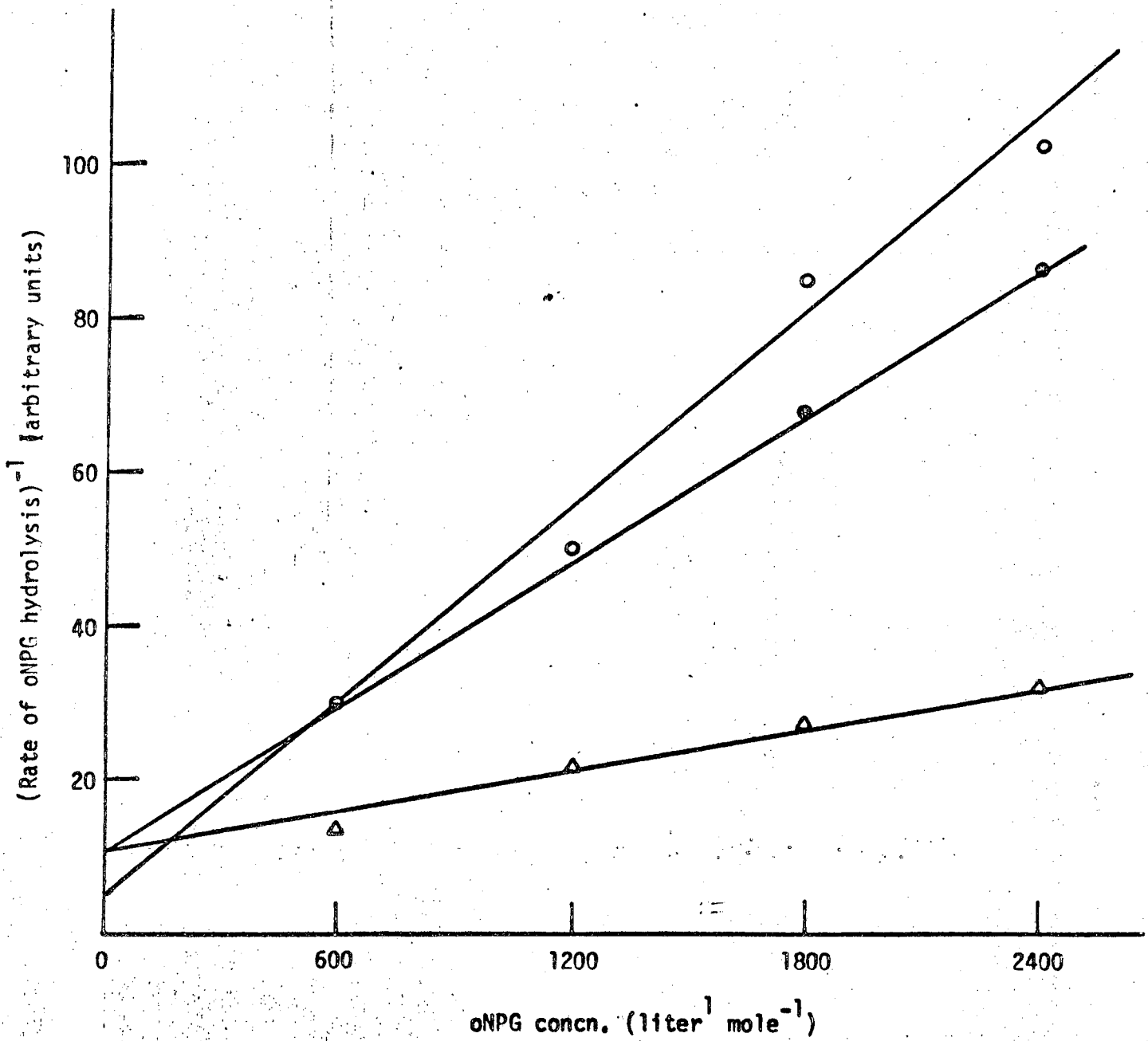
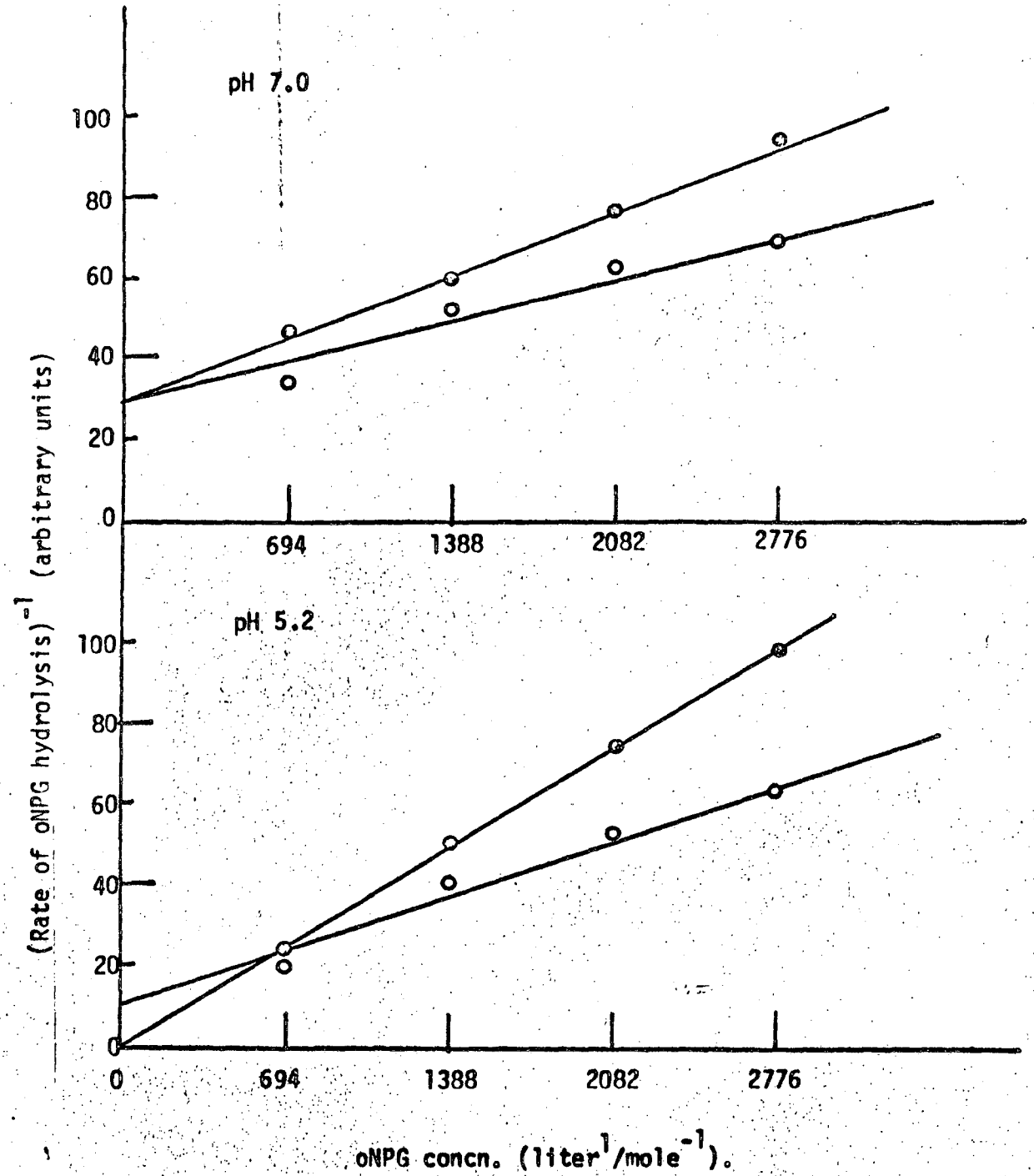
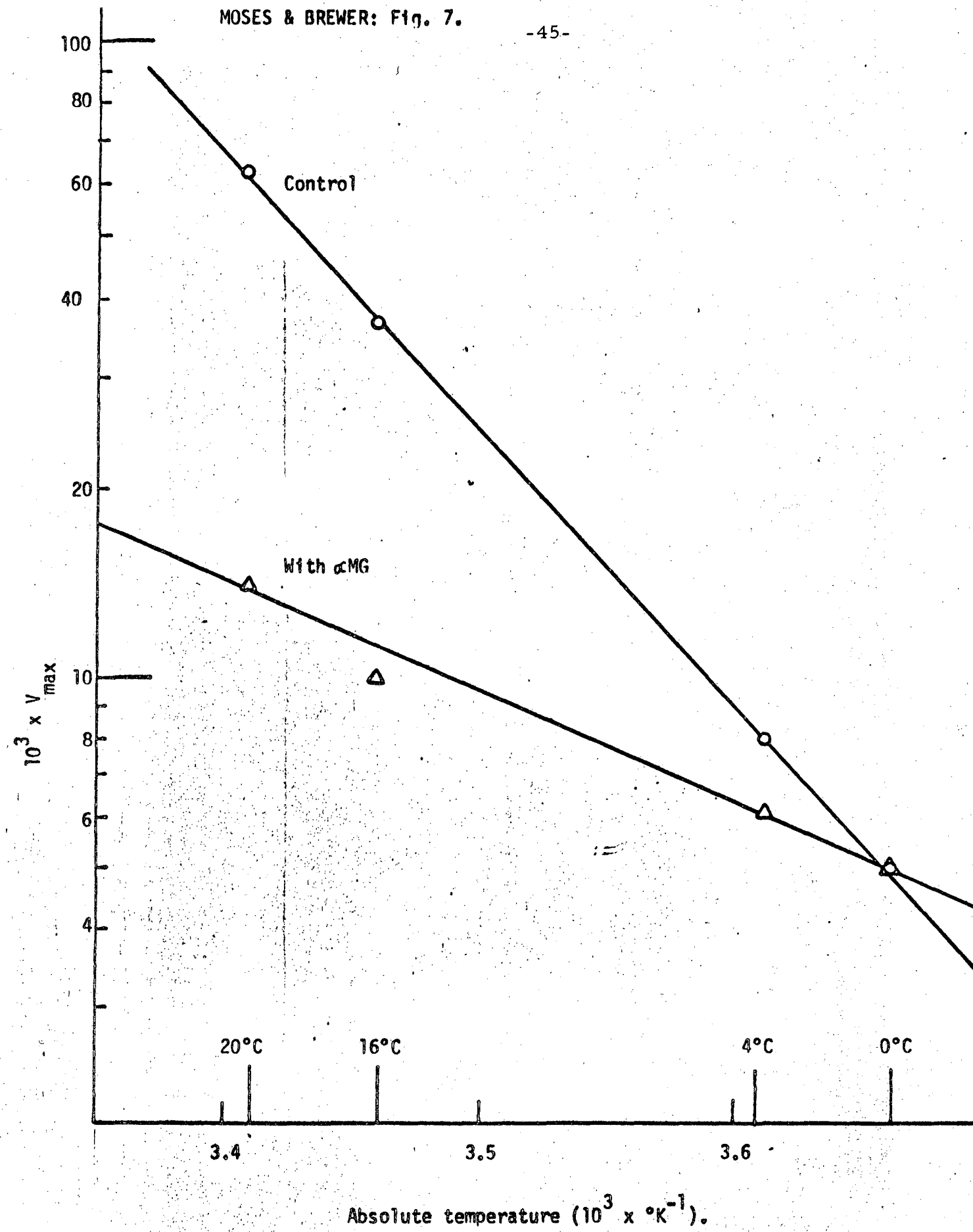
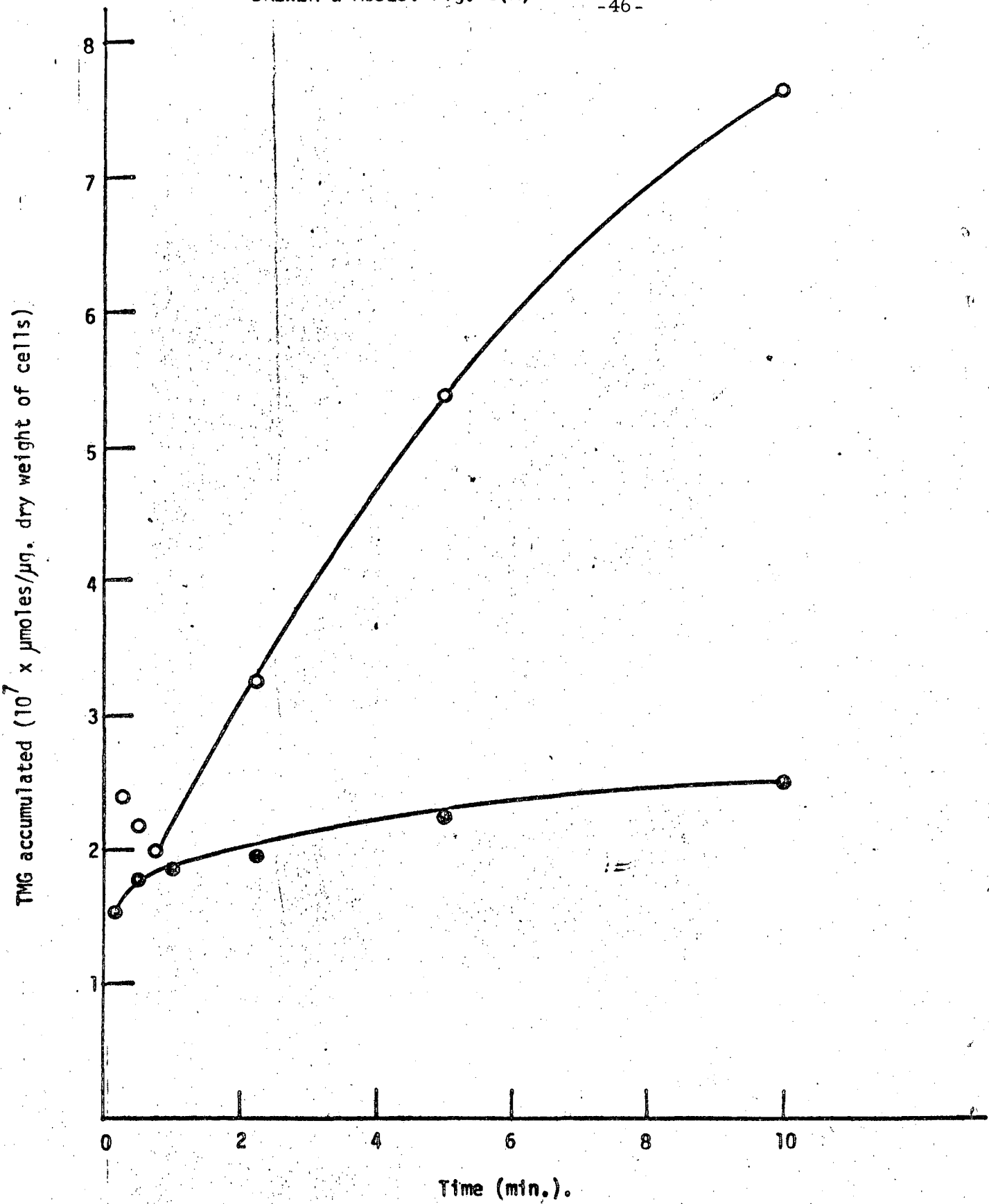



100
80
60
40
20
(Rate of oNPG hydrolysis)$^{-1}$ (arbitrary units)
0
600
1200
1800
2400
oNPG concn. (liter^{1} mole^{-1})

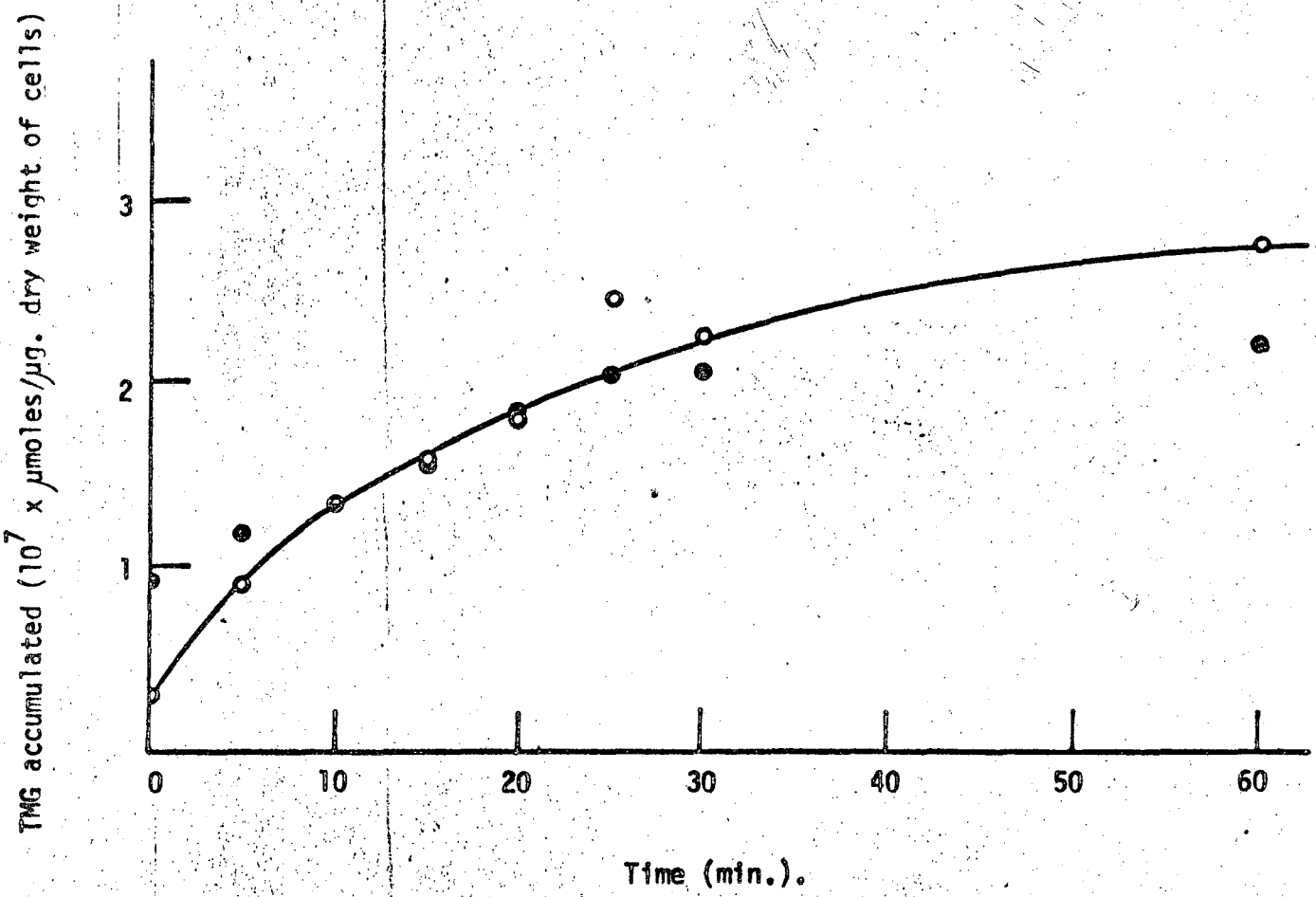

pH 7.0
100
80
60
40
20
0
694
1388
2082
2776
pH 5.2
100
80
60
40
20
0
0
694
1388
2082
2776
(Rate of oNPG hydrolysis)$^{-1}$ (arbitrary units)
oNPG concn. (liter^{1}/mole^{-1}).


100
80
60
40
20
10
8
6
4
$10^3 \times V_{max}$
Control
With αMG
20°C
16°C
4°C
0°C
3.4
3.5
3.6
Absolute temperature ($10^3 \times °K^{-1}$).

BREWER & MOSES: Fig. 8(A)



BREWER & MOSES: Fig. 8(B).



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