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Studies in Biochemical Adaptation. Some Aspects of Galactozymase Production by Yeast in Relation to the 'Mass Action' Theory of Enzyme Adaptation

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The experiments to be described were designed to test some of the predictions of the extended 'mass action' theory (Mandelstam, 1952). The enzyme system chosen for study was that involved in the fermentation of galactose by yeast cells. Several enzymes are involved in this reaction, at least two of which are adaptive, so that it is not perhaps the ideal enzyme system for such a study (Stanier, 1951). It has, however, been the subject of so much work by such investigators as Stephenson & Yudkin (1936) and Spiegelman (1946), that it was nevertheless chosen for this work. For simplicity, we shall speak as if one enzyme, galactozymase, is involved.

The predictions selected to be tested were:

(1) The rate of adaptation in the presence of a given amount of galactose.

(2) The effect of substrate concentration on the rate of adaptation.

(3) The effect of substrate concentration on the amount of enzyme formed.

(4) The relative amounts of two adaptive enzymes (galactozymase and maltozymase) formed in the presence of varying amounts of both substrates.

The detailed theoretical treatment for each of these situations is given by Mandelstam (1952). A simpler diagram is given here (Fig. 1) to facilitate the necessary reference to the theory.

A general description will first be given of the techniques used. A summary of the expectations from the theory for each of the selected situations, the experiments designed to test them, and the results obtained will then be presented in order.

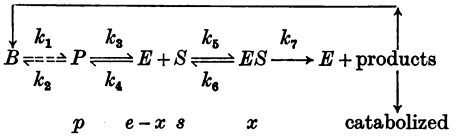

Fig. 1. Simplified diagram illustrating mass action theory of enzyme adaptation. *B*, pool of building blocks; *P*, enzyme precursor; *E*, enzyme; *S*, substrate. Concentrations are denoted by small letters. k_1, k_2, etc., are velocity constants. Broken arrows indicate that a number of reactions may be involved.

METHODS

The organism used was a pure strain of *Saccharomyces cerevisiae* (N.C.T.C. No. 815). It was maintained on slopes, the medium containing bacto-peptone 2%, yeastrel 3%, glucose 4%, agar 1·5%. The pH was adjusted to 6·0 and the organisms grown at 29°. Cultures for the experiments were made in a synthetic medium containing 50 g. glucose, 4·7 g. $(NH_4)_2SO_4$, 750 mg. KH_2PO_4, 100 mg. $MgSO_4$, 6·6 mg. inositol, 3 mg. nicotinic acid, 3 mg. calcium (+)-pantothenate, 150 μg. aneurin, 900 μg. riboflavin, 240 μg. pyridoxin, 6 μg. biotin and water to 1 l. The pH was adjusted to 6·0 and the organisms were grown at 29° for 40–48 hr. The cultures were centrifuged and the cells washed twice in saline and suspended in galactose containing phosphate buffer ($1·67 \times 10^{-2}$ M, pH 6·0). The suspensions were made up so that 1 ml. contained about 30 mg. dry weight of cells.

Enzyme activity was measured anaerobically in the Warburg apparatus at 38° with 3 ml. samples and expressed as μl. CO_2 evolved in 10 min. Cell counts were made in a standard blood count chamber. Dry weights of cells were determined by washing 3 ml. samples of suspension three times with distilled water by centrifugation, and drying to constant weight at 110°.

Since all samples of galactose purchased were found to contain small quantities of material fermentable by unadapted yeast cells, the galactose was purified by the method of Stephenson & Yudkin (1936).

EXPERIMENTAL

Rate of adaptation in the presence of a given concentration of substrate

From the theory, two situations are likely to occur in practice, though a third may arise in certain circumstances.

(*a*) Linear rate of enzyme adaptation. This should occur if the amount of precursor, or of building blocks, is comparatively large, and the products of enzyme action are not used for making more precursor.

(*b*) Exponentially increasing rate of enzyme adaptation. This should occur if the products of enzyme action are 'fed back' to the pool of building blocks and are used for making more precursor.

Experiments. A culture of yeast was grown in 2 l. of inorganic medium containing 5% glucose for 40–48 hr. At the end of this time, the cells were centrifuged and washed twice by suspending in saline and centrifuging. They were then suspended in 50 ml. of 5% galactose in phosphate buffer ($1{\cdot}67 \times 10^{-2}$ M, pH 6·0) and incubated at 29°. After about 15 hr., 3 ml. portions were placed at intervals in Warburg flasks and the evolution of CO_2 measured. Counts and determinations of dry weight were made at the beginning and end of the period of adaptation.

Results. Altogether fifteen experiments of this nature were carried out.

In six experiments, the rate of production of enzyme was linear (Fig. 2). In each case, there was no detectable increase in the number of cells or in their dry weight. In nine experiments, the rate of production of enzyme increased exponentially (Fig. 3). Of these, seven showed a significant increase in the number of cells. The increase in weight of cells was always proportionately less, as the cells were much smaller. In three of the seven instances there was a significant increase in the dry weight of the cells. The fact that there was no such increase in the remaining experiments showing an exponential rate of adaptation can be explained along the lines suggested by Mandelstam (1952).

Conclusions. The theory predicts that the rate at which adaptation occurs is likely to be either linear or exponential. Both of these forms of adaptation curves have been obtained, although we have not yet found the experimental factors which determine which form the curve will take. In particular, a linear rate of enzyme adaptation should not occur with increase in cell weight and we have found this to be the case.

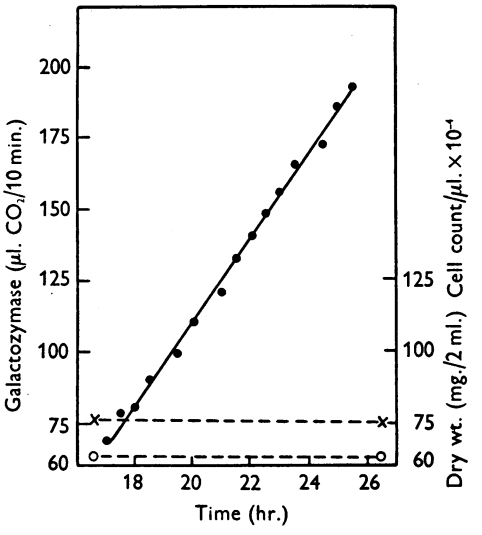

Fig. 2. Rate of production of galactozymase. Yeast cells grown in glucose medium and suspended in buffer containing 5% galactose. Rate of enzyme production constant, ●—●; cell count constant, ×---×; weight of cells constant, O---O.

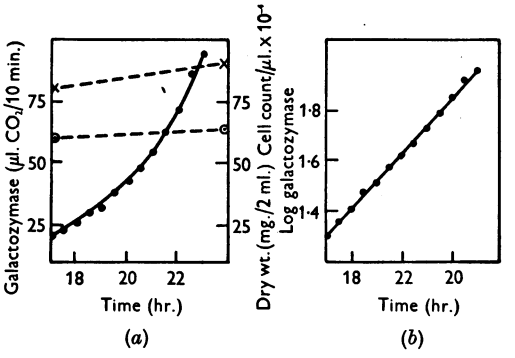

Fig. 3. Rate of production of galactozymase. Yeast cells grown in glucose medium and suspended in buffer containing 5% galactose. (*a*) Rate of enzyme production continuously increasing, ●—●; cell count increasing, ×---×; weight of cells increasing, O---O. (*b*) Same experimental data plotted to show that increase of enzyme is logarithmic.

Effect of substrate concentration on rate of adaptation

The theory requires that the initial rate of enzyme adaptation should be independent of substrate concentration over a wide range.

Experiments. A culture was grown for 40 hr. in the inorganic medium with 5% glucose, washed twice and suspended in saline. Samples (5 ml.) of suspension were added

to flasks containing 10 ml. of phosphate buffer with galactose so as to give concentrations of 3–8 % of the sugar. This range was chosen to ensure, at the lower level, that the maximal rate of enzyme production would be maintained for a sufficient time (see Mandelstam, 1952) and, at the higher level, that the cells were not damaged. The flasks were left at 29° and some of the suspension was removed at intervals for determination of galactozymase in the Warburg apparatus.

Results. The amount of enzyme formed after 22 hr. was approximately the same at all concentrations of galactose above 3 % (Fig. 4).

Fig. 4. Rate of production of galactozymase with varying concentrations of galactose. Yeast cells grown in glucose medium and suspended in buffer containing varying concentrations of galactose. Constant amount of enzyme formed after 22 hr. adaptation with galactose from 3 to 8%.

Conclusions. As predicted, the rate of adaptation is initially independent of the concentration of the substrate. Later the total amount of enzyme formed increases with increasing concentration of the substrate (see below).

The effect of substrate concentration on amount of enzyme formed

The theory forecasts that the final amount of enzyme formed will depend on the substrate concentration. The quantitative relationship between the amount of enzyme and substrate concentration depends on whether or not there is an ample supply of building blocks.

(*a*) Building blocks unrestricted. If e_a is the equilibrium concentration of enzyme produced in response to substrate concentration s_a, then the concentration of enzyme produced in response to another concentration of substrate s_b is given by

$$e_b = e_a\left(\frac{s_b + K_m}{s_a + K_m}\right), \qquad (1)$$

where K_m is the Michaelis constant.

This implies a linear relationship between the substrate concentration and the amount of enzyme formed.

(*b*) Building blocks restricted. The corresponding equation is

$$e_b = e_a\left(\frac{K - \dfrac{s_a}{s_a + K_m}}{K - \dfrac{s_b}{s_b + K_m}}\right), \qquad (2)$$

where K is a constant.

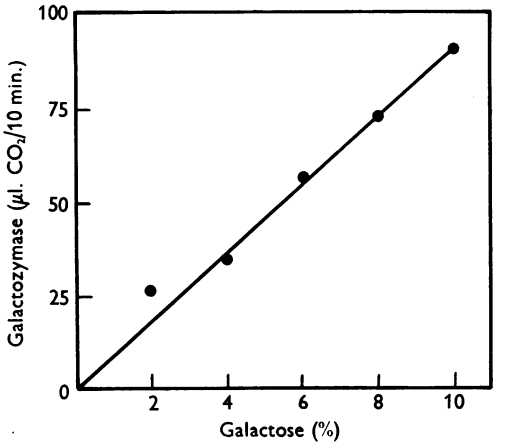

Fig. 5. Effect of galactose concentration on amount of galactozymase formed. No restriction of 'building blocks'. Yeast cells grown in media containing varying amounts of galactose. Galactozymase estimated in washed cells after 36 hr. of growth. Points give experimental results; line calculated from theory (Equation 1), using experimental value found with 10 % galactose.

(*a*) *Building blocks unrestricted*

Experiments. These were of two types.

Method 1. The culture was grown in the inorganic medium with varying amounts of galactose, from 2 to 10 %. The amount of the vitamin solution was reduced to one-third of the usual amount, with the object of limiting the growth of the cells but, it was hoped, not limiting the amount of galactozymase formed. After 36 hr., the cells were washed by centrifugation in the usual way, suspended in phosphate buffer and samples removed for the estimation of the enzyme. Counts and dry weight were also determined at the end of the growth period.

Results. In two experiments, there was equal growth in all concentrations of galactose. The amount of enzyme formed was in these instances found to be related to substrate concentration by a straight line passing almost through the origin (Fig. 5). The points in the figure refer to experimental results; the line represents the theoretical relationship and is calculated from Equation (1) and the experimental value at 10 % galactose. The value for K_m was 0·0038 M (see below).

In five other experiments, however, there was more growth in the solutions containing more galactose. Adaptation was still occurring in these at the end of the period of growth. It was consequently not possible to obtain reproducible results by this method, and a second type of experiment was adopted to achieve the necessary conditions to test this point.

Method 2. The cells were grown in the inorganic medium containing 5% glucose. They were centrifuged and washed as usual and then suspended in phosphate buffer at 29° containing varying amounts of galactose from 2 to 8%, together with 0·1% $(NH_4)_2SO_4$. At intervals, samples were removed for determination of galactozymase activity. The small amount of growth which occurred in these conditions was the same at all concentrations of galactose.

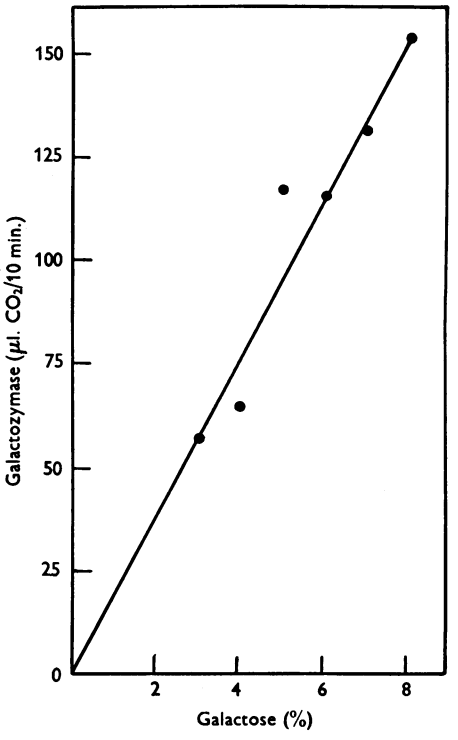

Fig. 6. Effect of galactose concentration on amount of galactozymase formed. No restriction of 'building blocks'. Yeast cells grown in glucose medium and suspended in buffer containing varying amounts of galactose, together with 0·1% $(NH_4)_2SO_4$. Points give experimental results; line calculated from theory (Equation 1) using experimental value found with 8% galactose.

Results. As in previous experiments, the rate of adaptation was initially independent of substrate concentrations. After 48 hr. of adaptation, however, equilibrium was established, resulting in a linear relationship between the amount of enzyme formed and the substrate concentration (Fig. 6). The line was calculated from Equation (1), using the experimental value at 8% galactose.

(*b*) *Building blocks restricted*

Experiments. Equation (2) involves K_m and K, both of which it is necessary to determine in order to calculate the relationship between the amount of enzyme and substrate concentration.

(i) *Determination of* K_m. Galactose-adapted cells were suspended in buffer solution containing galactose up to 0·024 M and CO_2 production measured at 38°. Four determinations were made and the mean value of K_m was 0·0038 M. Since K_m was determined in intact cells this value may be too high. However, in relation to the sugar concentrations used to produce adaptation (0·1–0·5 M) any such error in the determination is negligible.

(ii) *Determination of* K. The yeast was grown in the glucose medium, washed and placed in two flasks containing phosphate buffer with 2% (0·111 M) and 5% (0·278 M) galactose. At 12 hr. intervals the cells were centrifuged and resuspended in fresh solution to keep the sugar concentration constant. After 38–40 hr. the amount of enzyme produced in each of the solutions was measured. From these values (e_a and e_b), and knowing s_a and s_b, K was calculated from the equation

$$K=\frac{1}{e_b-e_a}\left(\frac{e_b s_b}{s_b+K_m}-\frac{e_a s_a}{s_a+K_m}\right). \qquad (3)$$

In one experiment e_a and e_b were respectively 17 and 35 for $s_a=0{\cdot}111$ M and $s_b=0{\cdot}278$ M. Substituting these values in (3)

$$K=\frac{1}{35-17}\left(\frac{35\times 0{\cdot}278}{0{\cdot}278+0{\cdot}004}-\frac{17\times 0{\cdot}111}{0{\cdot}111+0{\cdot}004}\right)$$
$$=1{\cdot}005.$$

The mean value of K obtained from 10 separate determinations was 1·006 ($\sigma=0{\cdot}001$).

(iii) *Determination of amounts of enzyme formed with varying concentrations of substrate: building blocks restricted.* A washed suspension was made from yeast grown in the glucose medium. Samples were set up with phosphate buffer containing 2–8% galactose and, after 40–48 hr., the amount of enzyme was measured. In earlier experiments the cells were transferred to fresh phosphate-galactose solution every 12 hr. Later experiments showed, however, that this was not necessary.

Results. The results of one experiment are shown in Fig. 7. These were further data obtained in the experiment shown in Fig. 4 and shown again in Fig. 7*A* as the degree of adaptation after 22 hr. Values at 30 and 48 hr. demonstrate that little further adaptation occurred with 3% galactose, but progressively increasing adaptation occurred with higher amounts of sugar. The shape of these two curves is similar, though the absolute values increased between 30 and 48 hr. Results of another experiment are shown in Fig. 8. Again, the points give the experimental results and the curve was calculated as in Fig. 7*B* and *C*.

It is of interest to compare Figs. 6 and 8, since the results were obtained from the same suspension of cells. The amount of enzyme formed in the absence

of ammonium sulphate is little more than half of the amount formed in the presence of the salt.

Conclusion. The effect of restricting the supply of building blocks is twofold. The first is to reduce the total amount of enzyme formed. The second is to alter the relationship between substrate concentration and amount of enzyme formed from a straight

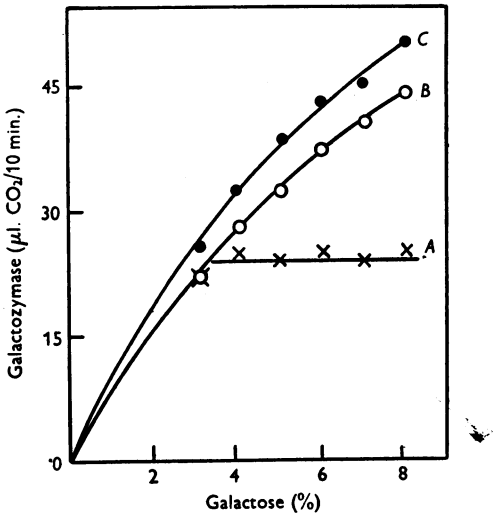

Fig. 7. Effect of galactose concentration on amount of galactozymase formed. 'Building blocks' restricted. Yeast cells grown in glucose medium and suspended in buffer containing varying amounts of galactose. Curve *A*, 22 hr. adaptation. Initial rate of enzyme production is independent of substrate concentration (see Fig. 4). Curves *B* and *C*, 30 and 48 hr. adaptation. Amount of enzyme formed proportional to substrate concentration; points represent experimental results, curves calculated from theory (Equation 2), using experimental values found with 8% galactose.

line to one in which diminishing amounts of enzyme are produced by progressive increments of substrate. These findings are qualitatively and quantitatively in accordance with the theoretical predictions.

The relative amounts of galactozymase and maltozymase formed in the presence of both substrates

Let us consider the situation in which cells are suspended in a solution containing two 'adaptive' substrates and in which the respective enzymes are in equilibrium with a common restricted pool of building material and hence in equilibrium with each other. Both enzymes will be produced, probably at a different rate. That which is produced faster will tend to take a greater share of building material and so restrict this for the other enzyme. This second enzyme should, therefore, at this stage increase slowly or not at all. If the substrates are not replenished, the first enzyme will reach a maximum, either because it has come into equilibrium with the existing concentration of substrate or because the substrate is completely exhausted. In either case, because of the continued fall in substrate concentration or its absence, there will be a decrease in the amount of the enzyme, so liberating material for the building of the second enzyme. Thus the second enzyme should now increase at the expense of the first. Apart therefore from the

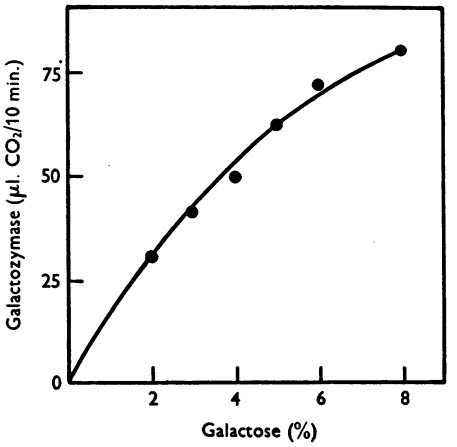

Fig. 8. Effect of galactose concentration on amount of galactozymase formed. 'Building blocks' restricted. Yeast cells grown in glucose medium and suspended in buffer containing varying amounts of galactose. Effect of presence or absence of building blocks shown by comparing Figs. 6 and 8, since same suspension of cells used in both experiments. Points give experimental results; curve calculated from theory (Equation 2) using experimental value found with 8% galactose.

earliest stages of simultaneous adaptation, there will be a continuously changing ratio of the two enzyme concentrations.

If the concentrations of the substrates are held constant, the ratio of the enzymes will tend towards a constant value given by

$$\frac{e_1}{e_2}=\frac{K_{e_1}}{K_{e_2}}\frac{K_{m_2}}{K_{m_1}}\left(\frac{s_1+K_{m_1}}{s_2+K_{m_2}}\right), \qquad (4)$$

where e_1 and e_2 are the concentrations of the two enzymes with Michaelis constants of K_{m_1} and K_{m_2}, s_1 and s_2 are the substrate concentrations and K_{e_1} and K_{e_2} are the equilibrium constants for the reactions $B \rightleftharpoons E_1$ and $B \rightleftharpoons E_2$.

The conditions under which this equation is strictly valid are in general unobtainable since, in the derivation of the equation, it is assumed that both enzymes are in an equilibrium state simultaneously. The equation can, therefore, in most instances, have only semi-quantitative application. It is nevertheless of interest to compare the theoretical prediction

with the experimental results. For this it is necessary to know K_{m_1}, K_{m_2}, K_{e_1} and K_{e_2}.

The Michaelis constant for galactozymase has already been determined, $K_{m_1} = 0{\cdot}0038$ M. For maltozymase, K_{m_2} was determined in the same way and its value is 0·0028 M.

K_e can be obtained from K (Equation 3), since $K_e = K - 1$. For galactozymase, K was found to be 1·006, whence $K_{e_1} = 0{\cdot}006$. For maltozymase, $K_{e_2} = 0{\cdot}003$. Both of these values, and especially that for maltozymase, can only be taken as rough approximations, since they depend on the fourth significant figure in the calculated value of K. Thus, absolute values for e_1/e_2 can be obtained only very approximately. However, it should be possible to calculate changes in the value of e_1/e_2 with changes in s_1 and s_2 with some degree of accuracy. When s_1 and s_2 are varied, the variation of e_1/e_2 is independent of the constant term $(K_{e_1} K_{m_2})/(K_{e_2} K_{m_1})$, and e_1/e_2 is directly proportional to $(s_1 + K_{m_1})/(s_2 + K_{m_2})$.

Experiment 1. This experiment was designed to study the fluctuation of the two enzymes in solutions in which the substrates were not renewed. Cells grown in the glucose medium were washed and placed in two flasks with different concentrations of galactose and maltose. The first contained galactose 1·4% (0·078 M) and maltose 3·6% (0·100 M); the second contained galactose 3·6% (0·200 M) and maltose 1·4% (0·039 M).

Results. Fig. 9 shows how the amounts of the two enzymes change between 10 and 38 hr. adaptation. Other noteworthy features are the more rapid formation of galactozymase in both solutions, the retardation of maltozymase formation when its relative concentration was lower, i.e. in the second solution, and the rise in maltozymase in both solutions associated with the fall of galactozymase. Similar results have been obtained by Spiegelman & Dunn (1947).

Experiment 2. Cells grown in glucose medium were washed and suspended in two flasks containing buffer solutions with the following amounts of galactose and maltose: (1) galactose 1·4% (0·078 M), maltose 3·6% (0·100 M); (2) galactose 3·6% (0·200 M), maltose 1·4% (0·039 M).

The suspensions were kept at 29° and the cells transferred to fresh solutions every 12 hr. At 40 hr., samples were removed and washed and the activity of the enzymes determined.

Results. A relative increase in concentration of galactose to maltose of about sixfold (s_1/s_2 from 0·78 to 5·1) should give about the same relative increase in the ratio of the enzymes (Table 1). In fact, the relative increase in the enzymes was about twofold.

This degree of discrepancy was not unexpected since, as we have seen, theoretical values can only be achieved in conditions where both enzymes are in equilibrium with their substrates at about the same time.

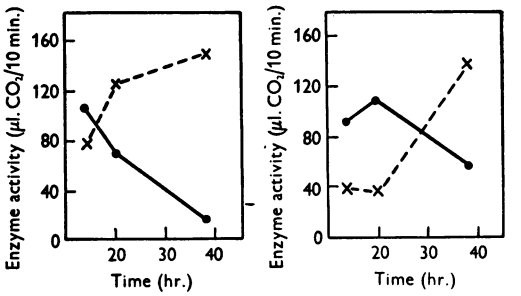

Fig. 9. Simultaneous production of galactozymase and maltozymase; competition in enzyme formation. ●—●, Galactozymase; × --- ×, maltozymase. Yeast cells grown in glucose medium and suspended in solutions containing (*a*) galactose 0·078 M, maltose 0·100 M; (*b*) galactose 0·200 M, maltose 0·039 M. Solutions not renewed during adaptation.

Experiment 3. This experiment was carried out in a similar way to Exp. 2, but with two differences designed to give conditions nearer to those necessary for simultaneous equilibrium of the two enzymes (see Mandelstam, 1952). Firstly, the concentrations of the substrates were lower: (1) galactose 1·6% (0·089 M), maltose 0·53% (0·015 M); (2) galactose 0·53% (0·030 M), maltose 1·6% (0·0445 M). Secondly, the cells were suspended in twice the usual volume of sugar solutions, so that the concentration of cells was about 15 mg. dry wt./ml.

Results. As expected, agreement between theoretical and experimental values was better than in the previous experiments (Table 1). The calculated

Table 1. *Effect of variation in substrate concentrations on formation of galactozymase and maltozymase*

(Enzyme concentration expressed as μl. CO_2 liberated in 10 min.)

Exp. no.	Suspension	Substrate concn. (M) Galactose s_1	Maltose s_2	s_1/s_2	Enzyme concn. Galactozymase e_1	Maltozymase e_2	e_1/e_2	R_1/R_2 Found	Calculated
2	1	0·200	0·039	5·1	115	25	4·6 (R_1)	2·1	5·9
	2	0·078	0·100	0·78	87	40	2·2 (R_2)		
3	1	0·089	0·015	5·9	84	34	2·5 (R_1)	6·2	7·1
	2	0·030	0·044	0·67	44	109	0·4 (R_2)		

increase in the ratio of e_1/e_2 was about sevenfold; the increase found was about sixfold.

Experiment 4. In this experiment, the conditions were as in Exp. 3, except that the concentration of maltose was held constant and that of galactose increased. Four flasks were set up, each with maltose 0·53% (0·015M) and with galactose 0·53% (0·030M), 1·06% (0·059M), 1·6% (0·089M) and 2·13% (0·119M); the enzymes were measured after 44 hr. of adaptation.

Results. The results are given as the points in Fig. 10, and the straight line represents the theoretical results, calculated from Equation (4), and from the experimental value found with galactose at 0·030M.

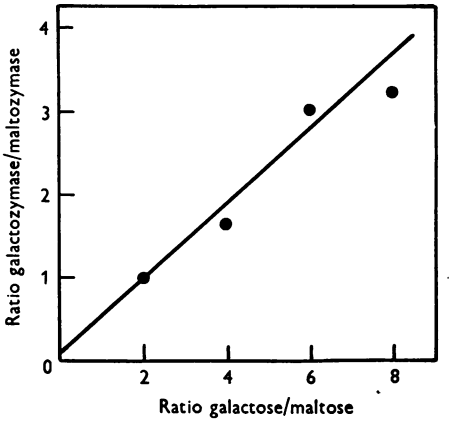

Fig. 10. Effect of relative concentrations of galactose and maltose on production of the two enzymes. Yeast grown in glucose medium and suspended in solutions containing the two sugars; the solutions were renewed every 12 hr. Maltose held constant (0·015M) and galactose increasing (0·030, 0·059, 0·089 and 0·119M) produces linear increase in the ratio of the two enzymes. Points give experimental results; line calculated from theory (Equation 4), using value found with galactose 0·030M.

Conclusion. In the introduction to this section, it was explained that quantitative predictions from the theory concerning simultaneous adaptation to two substrates are more difficult to verify than other predictions in which only one enzyme is involved. Nevertheless, the experimental results which we have obtained conform well to the predictions made.

DISCUSSION

The experiments have confirmed all the predictions from the theory which have been tested. We may now refer to investigations reported by other workers which support our own findings. Firstly, we have shown that the rate of adaptation is sometimes linear, but is sometimes of an exponentially increasing type. Both types of curve are to be expected theoretically, the former when there is no increase in material to the pool of building blocks and the latter when there is such an increase. Knox (1951) has also demonstrated two types of curve with the production of tetrathionase by *Salmonella paratyphi* B. With tetrathionate alone, the rate of production of the enzyme was linear. With the addition of mannitol or glucose, there was an increasing rate of production of the enzyme, though it is not clear whether the increase was exponential or not.

We ourselves have shown that a linear rate of adaptation occurs with hepatic arginase of adult rats, where we can assume negligible increase of material in the pool of building blocks (Mandelstam & Yudkin, 1952).

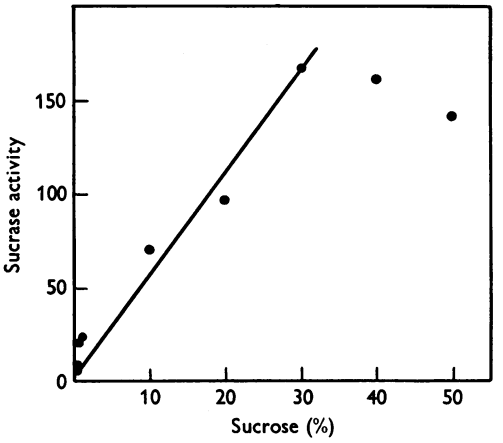

Fig. 11. Effect of sucrose concentration on production of sucrase by *Penicillium glaucum*. Points give experimental results found by Kertész (1928); line calculated from theory (Equation 1), using experimental value found with sucrose 30%. The lower amounts of enzyme found with concentrations above 30% are attributable to the injurious effect of these high amounts of sugar upon the organisms.

Secondly, in conditions where varying amounts of substrate did not affect the growth of the culture, the amount of enzyme produced was found by us to be directly proportional to the concentration of substrate. An experiment reported by Kertész (1928) produced similar results. He grew cultures of *Penicillium glaucum* in media containing from 0·01 to 50% of sucrose, and after 12 days estimated the amount of sucrase formed. Kertész says that considerable growth had occurred after 8 days, so that it is likely that little further growth was occurring after 12 days. We should then expect, as in our own experiments, that the amount of sucrase formed would increase linearly with increase in sucrose. We have plotted in Fig. 11 the results of Kertész, together with the theoretical curve calculated from Equation 1, using his value of sucrase for 30% sucrose. Kertész concluded that there was a linear relationship between the amount of enzyme formed

and concentration of sucrose up to 30 % sugar, above which the high concentrations of sucrose have an adverse effect upon the organism. The figure shows that the theoretical curve agrees quite well with the experimental values up to 30 % sugar.

Thirdly, the experiments concerning simultaneous adaptation to two substrates recall the work of Monod (1945). In experiments with *Escherichia coli*, he has studied the rate of growth in the presence of the 'constitutive' substrate glucose and one of a series of 'adaptive' substrates such as xylose. It may be taken that the rate of growth is proportional to the amount of the two enzymes present. With similar concentrations of the two sugars, growth proceeded until the glucose was exhausted and then there was a pause in the growth whilst adaptation to xylose was taking place. Monod showed that this pause decreased progressively with an increase in the ratio of xylose to glucose. When this ratio was 1000 to 1, growth proceeded without a pause, indicating that in these conditions adaptation to xylose was occurring whilst glucose was still present. Thus, adaptation can be induced in the continuing presence of a 'constitutive' substrate by making the relative concentration of 'adaptive' substrate sufficiently high.

These results are in conformity with the 'mass action' theory. Monod, like ourselves, assumes that the distinction between a constitutive enzyme and an adaptive enzyme is quantitative rather than qualitative and depends upon whether the equilibrium between precursor and enzyme lies more toward the latter or the former. The theory then predicts that adaptation in the presence of both a 'constitutive' and an 'adaptive' substrate will occur, as Monod has shown, when the concentration of the 'adaptive' substrate is relatively very high. Adaptation in the presence of two 'adaptive' substrates, on the other hand, will occur, as we have shown, when the two concentrations are of the same order.

SUMMARY

1. Predictions made from the 'mass action' theory of enzyme adaptation have been tested in experiments on the adaptation of yeast cells to the fermentation of galactose.

2. The rate of adaptation in a given concentration of galactose was found to be either linear or exponentially increasing. In no instance was a linear rate of adaptation found when there was an increase in cell mass during adaptation.

3. The initial rate of adaptation was independent of the concentration of galactose over the tested range of 3–8 %.

4. When there was no restriction of material for the building of the enzyme, the amount of galactozymase formed was linearly related to the concentration of galactose. When there was such a restriction, there was a decreasing increment of enzyme with increasing concentration and the total amount of enzyme formed was less.

5. The relative amounts of galactozymase and maltozymase formed in the presence of both sugars are determined by the relative amounts of galactose and maltose present.

6. These results, and some others reported in the literature, are quantitatively in agreement with the predictions made from the theory.

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