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D. C. H. McBrien and V. Moses

July 19, 1967

Compartmentation of the Metabolism of Lactose, Galactose and Glucose in *Escherichia coli*

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SUMMARY

Compartmentation phenomena have been studied in the course of the simultaneous metabolism of glucose, galactose and lactose by cells of *Escherichia coli* which were induced for either the *lac* operon, the *gal* operon, both or neither. Metabolic patterns were investigated in each phenotype by incubating parallel identical cultures with the three sugars in equal chemical concentration but labelled differently with ^{14}C. The four labelled substrates were glucose, galactose, and lactose labelled either exclusively in the glucose moiety or exclusively in the galactose moiety.

The metabolites from free glucose in the medium equilibrated with those from free galactose in the medium, but did not equilibrate with metabolic products derived from glucose generated endogenously by the hydrolysis of lactose. Similarly, metabolic products derived from galactose formed in the hydrolysis of lactose equilibrated with those from glucose from the same source, but not with metabolic intermediates formed from either free glucose or free galactose in the medium. Other

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interpretations of these results, not involving metabolic compartmentation, have been considered and found inadequate to account for the observed results. Some of the implications of compartmentation in bacterial cells are discussed.

INTRODUCTION

Subcellular compartmentation of pools of metabolic intermediates is known to be a widespread phenomenon (Moses, 1966), and it is probable that compartmentation plays a role in the control of biochemical reactions *in vivo*. Most evidence for metabolic compartmentation so far obtained has come from studies on cells which contain observable barriers to the free diffusion of solutes. The present communication describes an investigation made upon an organism which contains no visible internal membranes--*Escherichia coli*.

Compartmentation is not necessarily a morphological phenomenon; the free diffusion of metabolic intermediates may be inhibited in other ways. At a molecular level, for example, one might envisage compounds bound to the surface of an integrated enzyme sequence, with intermediates along the pathway never being released to equilibrate with their respective pools. Similarly, if the position at which a particular compound is formed and released in the cell is sufficiently distant from the location of its intracellular pool it may undergo reaction before reaching the intracellular pool, thus preventing the latter from achieving true equilibrium with the compound at all its locations in the cell.

Experimentally, the study of compartmentation in living cells presents certain problems arising from the difficulty of distinguishing between

several reservoirs of a substance without fractionating the cell and thereby causing its death. Those attempts which have been made to investigate compartmentation *in vivo* have relied upon studies of the metabolic behaviour of certain compounds. In some investigations metabolic data is best interpreted by supposing that certain substances exist in two or more non-equilibrating reservoirs. Moses & Lonberg-Holm (1966) extended this approach and sought to expose metabolic compartmentation by designing experiments in which certain metabolites would be generated intracellularly in a number of ways, and would be recognizable as a result of radioactive labelling. This method has been used in the present study.

The area of metabolism chosen for study was that involving the hydrolysis of lactose to glucose and galactose, the subsequent phosphorylation of the monosaccharides, the isomerization of galactose derivatives to glucose derivatives, and the further metabolism of the latter in the cell. *E. coli*, when grown in appropriate media, is able to metabolize lactose, glucose and galactose. During lactose metabolism, the monosaccharides are presumably released within the cell, while metabolism of the monosaccharides supplied directly from the medium must involve also the entry of the substances into the cell either as free sugars or as derivatives. The question we sought to answer was this: is glucose (or galactose) produced inside the cell by the hydrolysis of lactose distinct metabolically from glucose (or galactose) presented to the outside of the cell? Or put slightly differently: is the metabolic fate of glucose (or galactose) in any way dependent on its origin?

The problem may be studied by using a radioactive labelling technique. If cells are supplied simultaneously with glucose, galactose and lactose as substrates one might study the fate of the various substrates by parallel experiments differing only in the location of the radioactive marker. In the present study it was necessary to use four parallel reaction vessels, each identical chemically, and containing all three sugars. The labelling pattern was then varied so that the four flasks were supplied with labelled glucose, labelled galactose, lactose labelled in the glucose moiety and lactose labelled in the galactose moiety, respectively. It was then possible to study, for example, the fate of glucose derived by the hydrolysis of lactose in cells which were simultaneously metabolizing glucose from the medium, and to compare this with the fate of glucose from the medium in a parallel culture of cells with an identical history which were also metabolizing glucose derived from lactose. Similar comparisons were also made for galactose, and between glucose and galactose.

Some of the enzymes of lactose and galactose metabolism are inducible and their intracellular levels may be greatly altered by growth in the presence of the appropriate inducers. Glucose metabolism, on the other hand, appears to be phenotypically constitutive in _E. coli_. We have included in our studies a comparison of lactose, glucose and galactose metabolism in cells induced either for the lactose enzymes, or for the galactose enzymes, or both, or neither.

MATERIALS AND METHODS

Organism and growth conditions. *E. coli* Cavalli strain (*lac*$^+$ *gal*$^+$ *met*$^-$ *thy*$^-$) was obtained from Aleen Simmonds. Stock cultures were maintained on M63 minimal medium (Pardee & Prestidge, 1961) supplemented with glycerol (0.2% w/v) and specific growth factors. Cells were grown in liquid medium with stirring at 37°. Full induction of the *lac* operon was achieved by growing the cells for many generations in the presence of 0.5 mM-isopropyl-β-D-thiogalactoside (IPTG). The *gal* operon was induced by growth in the presence of 5 mM-D-fucose. When IPTG and fucose are present together at these concentrations there is no appreciable interaction of either inducer upon the inductive effect of the other (McBrien & Moses, 1966).

Labelled substrates. [G-^{14}C]-D-Glucose (107 μC./μmole) was prepared using the method of Putman & Hassid (1952). [G-^{14}C]-D-Galactose (143 μC./μmole) was prepared according to Bean, Putman, Trucco & Hassid (1953) with minor modifications. It was found necessary to illuminate the alga used for the photosynthetic incorporation of $^{14}CO_2$ into glycerol-galactoside at a much lower light intensity (11,000 lux) than was previously recommended. Galactose was separated and purified in the manner described by Abraham & Hassid (1957). We are grateful to Dr. S. Abraham for assistance in isolating the galactose.

[(G-^{14}C)glucose]Lactose was prepared with lactose synthetase isolated from unpasteurized cow's milk (kindly provided by Challenge Dairies, Berkeley, Calif.) using the method of Babad & Hassid (1964). [(G-^{14}C)-galactose]Lactose was synthesized with a tissue preparation from the mammary gland of a lactating rat (Bartley, Abraham & Chaikoff, 1956). In the preparation of both forms of labelled lactose the labelled hexoses described above were used undiluted by unlabelled sugars, and the products

which were isolated and purified by preparative paper chromatography were assumed to have the same specific radioactivities as the parent hexoses. A small sample of each of the labelled lactoses was used to determine the proportion of ^{14}C activity which had been incorporated into the desired moiety. The labelled lactose was hydrolysed with a crude preparation of β-galactosidase obtained from a lac-constitutive strain of E. coli and the glucose and galactose produced were separated by paper chromatography in ethyl acetate : pyridine : acetic acid : water (5 : 5 : 2 : 3, by vol.). The sugars were located by radioautography using known radioactive markers on guide strips, the spots excised and the radioactivity measured with opposed end-window Geiger-Müller tubes. In each case at least 97% of the activity was found in the desired moiety.

Labelling experiments. The experimental procedure was adapted from that described by Moses & Lonberg-Holm (1966). Cells growing in exponential phase in glycerol-minimal medium were harvested at a concn. of approx. 225 μg. bacterial protein/ml. Growth and protein synthesis were halted prior to harvesting by the addition of chloramphenicol (100 μg./ml.). The cells were washed and resuspended in 0.01 M-phosphate buffer, pH 7.1, containing chloramphenicol (25 μg./ml.). To 20 ml. of this bacterial suspension was added 0.4 ml. of a solution containing 0.7% (w/v) each of lactose, glucose and galactose: after mixing, this gave a concn. of 0.76 mM for the monosaccharides and 0.40 mM for lactose. Four samples of bacterial suspension, each of 2.0 ml., were transferred to 20 ml. flat-bottomed glass vials (as used for scintillation counting) and placed in a water bath at 37°. The contents of the vials were

stirred vigorously using small polythene-covered magnets over magnetic stirrer motors operating at maximum speed. Fifteen min. after adding the mixed sugars to the bacterial cells the labelled sugars were introduced as indicated in Table 1. There was no significant contribution to the total sugar content by the addition of the labelled material.

[Insert Table 1 near here]

During the following 45 min. after adding the labelled sugars fifteen samples of approx. 30 µl. each were withdrawn from each vial at known times. Each sample was mixed immediately with 0.4 ml. of ethanol (90% v/v) in pre-weighed stoppered tubes. The tubes were subsequently reweighed to determine the sample size actually taken. The contents of each tube were chromatographed *in toto* using two-dimensional paper chromatography on Ederol No. 202 paper (J. C. Binzer G.m.b.H., Hatzfeld/Eder, Germany). The solvents were: in the first dimension 90% phenol : water : glacial acetic acid : 0.5 M-K_2EDTA (420 : 80 : 5 : 1, by vol.), and in the second dimension butan-1-ol : propionic acid : water (20 : 9 : 11, by vol.). Radioactive materials on the chromatograms were located by radioautography, and spots so located were excised and counted using the automatic apparatus described by Moses & Lonberg-Holm (1963). Spots selected for identification were eluted and cochromatographed with known unlabelled markers. Dicarboxylic acids were located using bromocresol green reagent (Lugg & Overell, 1948), amino acids with ninhydrin and sugars with $AgNO_3$ (Smith, 1960).

The experiment was performed four times with cells in different states of induction for the *lac* and *gal* operons. In cells in which

neither of these operons were induced the amount of radioactivity incorporated into products from labelled galactose or labelled lactose was so low that no useful information could be obtained. This experiment will not therefore be discussed further. The arrangements for the other three experiments are noted in Table 2.

[Insert Table 2 near here]

RESULTS

The amount of utilization of the labelled substrates during the period of 45 min. after their addition to the cells is shown in Table 3.

[Insert Table 3 near here]

It can be seen that in cells in which the lactose operon was induced lactose was rapidly hydrolysed to hexose, a large proportion of which accumulated, presumably because subsequent steps in its metabolism were rate limiting. In Table 3 the residual hexose formed from labelled lactose is counted as unused substrate.

Table 3 shows that in both experiments in which the galactose operon was induced (1 and 2) the galactose moiety formed from the hydrolysis of lactose, in cells containing both basal and induced levels of β-galactosidase, was used to a greater extent than the glucose moiety simultaneously produced. However, the utilization of the externally supplied hexoses does not show the same consistency--in the first experiment galactose being used more rapidly than glucose and in the second experiment the reverse being true.

With both the lactose and galactose operons induced (Experiment 1) about 78% of all the hexose used was galactose, 37% of the total being

galactose derived from lactose. When the main source of galactose was curtailed (Experiment 2) by failing to induce the lactose operon, the total consumption of carbohydrate did not fall, and indeed it increased to some extent. The lack of galactose from lactose was entirely compensated by the additional metabolism of glucose from the medium; the metabolism of galactose from the medium did not increase. This suggests that the pathway leading into metabolism from galactose in the medium was saturated in both experiments by galactose from this source. The other pathway leading into metabolism from galactose derived from lactose was not accessible to galactose in the medium. However, at a later stage in metabolism probably the second of these pathways could be ntered by glucose from the medium, since the pathway was now largely unoccupied by metabolites from the galactose moiety of lactose. Glucose from the medium could not enter this pathway, however, when it was occupied by galactose metabolites.

This pathway from lactose-galactose was more readily accessible to the glucose moiety of lactose than to free glucose when it was not occupied by galactose metabolites. In Experiment 3 elimination of both galactose pathways by failure to induce the galactose operon resulted in the greater utilization of lactose-glucose, since this was now available, rather than glucose from the medium. Thus we may conclude that when all the relevant operons were induced the carbohydrate of choice was galactose derived from the hydrolysis of lactose. When it was available, glucose derived from lactose was also used in preference to free glucose in the external environment, although the latter could be used when there was sufficient need. These considerations imply that

the pools formed inside the cell from externally supplied and internally produced hexose do not mix to any large extent.

Inspection of the radioautographs of the chromatograms produced in these experiments prompted the selection for comparisons of the spots corresponding to citric, succinic and glutamic acids, because they were found on most of the chromatograms from each of the experiments and contained readily measureable ^{14}C activity. For the sake of simplicity data obtained from other spots, labelled to a smaller extent (with a correspondingly greater scatter of results) and spots which were not subsequently identified will not be presented. Figs. 1-3 show the activities of the three selected compounds from all the papers on which their activity was measureable. The results from a single, four vessel, experiment are shown in each Figure.

[Insert Figs. 1-3 near here]

In considering the behaviour of various compounds shown in Figs. 1-3 it should be borne in mind that quantitative comparisons between experiments performed on different days and with different batches of cells must always be made with caution. One is more confident in comparing parallel portions of the same culture since these certainly had a common history until a few minutes before the experiment started, and considerable effort was expended to maintain chemically identical conditions in each subculture after division of the parent stock.

Examination of Figs. 1-3 shows that there were considerable differences between the relative pool sizes in different vessels of the same experiment. Before these can be discussed in detail one must be quite sure that they reflect aspects of metabolic organization and not merely

trivial differences which might by chance have arisen among the four parallel vessels and which alone might account for the metabolic phenomena we have observed. Within each experiment it is known that the cells and their chemical environment were the same in each vessel, differing only in the nature of the labelled substrate added. The only other possible variation we can conceive between the experimental conditions in each vessel is the level of aeration. As stated earlier, aeration was achieved by using miniature magnetic stirrers made of stainless steel, sheathed in polythene, and rotated by stirrer motors with the rheostat speed control set at its maximum setting (100 on the dial). The following experiment was designed to determine how critical the speed of stirring was for the rate of respiration of the cells.

Warburg flasks with no centre wells were each charged with 0.25 ml. of 20% (w/v) sodium hydroxide placed in the side-arms. A suspension of cells in 0.01 M-phosphate buffer (pH 7.1) containing chloramphenicol (25 μg./ml.) was prepared in exactly the same way as for the labelling experiments. The suspension contained 194 μg. bacterial protein/ml. The cells had been grown in the absence of inducers. The external diameter of the Warburg flasks was approximately 30 mm. and of the vials used in the labelling experiments approximately 23 mm. However, the latter were made of thinner glass and the difference between the internal diameters of the two vessels was probably less than between their external diameters.

Three samples of 2.0, 1.0 and 0.5 ml. of the cell suspension, each diluted to 2.0 ml. with buffer, were placed in the cups of the Warburg flasks. The miniature magnetic stirrers were added, followed by 0.04 ml.

of a solution containing 0.7% (w/v) each of glucose, galactose and lactose. The flasks were attached to their manometers, immersed in a water bath at 37°C, and stirring commenced with the rheostat speed controls set at 85-90 on the dial. This gave a speed somewhat less than that used in the labelling experiments when it was possible to operate the stirrers at maximum speed. After 10 min. equilibration, measurements of oxygen consumption were commenced. For the first 60 min. the rates of oxygen consumption in each flask were constant, the values (μl. O_2/min./μg. bacterial protein) being 4.38, 4.25 and 4.51 for flasks containing 2.0 ml., 1.0 ml. and 0.5 ml. of original cell suspension, respectively.

Since the rate of respiration was proportional to the volume of cell suspension used over a fourfold range, it must have been maximal. After 50 min. incubation the rate of stirring was decreased by turning down the speed controls to 60. The speed of stirring was not proportional to the potentiometer setting and it was observed that the stirrers appeared to slow down to much less than 2/3 of the original rate. As measured in the next 50 min. the respiration rates were 1.65, 3.92 and 2.89 μl. O_2/min./μg. bacterial protein for the three flasks, respectively. The rate had thus decreased in three vessels to different extents. However, in the labelling experiments the stirrers were always operated at maximum speed. It must also be borne in mind that the total volume in each vessel during a labelling experiment decreased continuously as samples were withdrawn. It is therefore clear from these results that differences in aeration were not sufficient to account for the differences observed between the labelling patterns in different vessels.

DISCUSSION

If true steady-state conditions had been achieved in the labelling experiments each metabolic pool would gradually have been filled with ^{14}C until equilibrium with the outside solution was achieved; the activity would then have attained a constant maximum value. From Figs. 1-3 it can be seen that an approximation to these conditions was achieved in most cases. At the end of 45 min. the rate of rise of radioactivity in each pool tended to decrease. In a few cases the level of activity in the glutamate pool began to show a fall after reaching a maximum value, and in some cases no plateau of activity was attained. However, the data presented in Fig. 1 indicate that the size of the succinic acid pool through which passed the products of metabolism of the glucose and galactose derived from the hydrolysis of lactose was much larger, in comparison to the size of the pool of glutamate, than that which received the products of metabolism of exogeneous glucose and galactose. Fig. 3 indicates that this same effect, although less pronounced, also holds true for exogenous and endogenous glucose in cells which have a different complement of induced enzymes.

In addition to determinations of the relative sizes of pools, observations on the rates at which pools fill with activity may be helpful in determining whether more than one pool of each compound exists in the cells. For example, in Fig. 1 it can be seen that the pool of glutamate derived from either moiety of lactose began to decrease in activity about 20 min. after the addition of label to the vessels, at which time the activity of the pool of glutamate derived from exogenous glucose or galactose was still increasing. Similarly, from Fig. 3 it will be seen

that the size of the labelled pool of citric acid from external glucose became equal to, and subsequently greater than, the pool of labelled glutamate 36 min. after the addition of the labelled material to the cells. However, the pool of labelled citrate derived from endogenously produced glucose did not exceed the size of the labelled glutamate pool within the period of the experiment. If all the labelled substrates fed into a common pathway--there being only one pool of any intermediate in each cell--as shown in Fig. 4, then the pools of the three acids would behave identically in each vessel regardless of the nature of the labelled substrate. Since in any one of the labelling experiments the cells and their chemical environment were ostensibly identical, some explanation must be sought for the differences shown by the behaviour of the labelled pools depending on the identity of the substrate which was labelled. [Insert Fig. 4 near here]

Although metabolic compartmentation is able to account satisfactorily for the results of these experiments, two other possible interpretations must also be considered: lack of homogeneity in the bacterial population itself, and different environmental conditions in the several incubation vessels comprising each experiment.

The culture used in the present experiments was not synchronous, and it could be argued that cells in different stages of the growth cycle might have different enzyme complements (and thus might themselves act as separate compartments). There is considerable evidence that certain enzymes in Bacillus subtilis, E. coli and yeast are produced discontinuously during the growth cycle of individual cells (Masters, Kuempel & Pardee, 1964; Kuempel, Masters & Pardee, 1965; Halvorson, Gorman, Tauro, Epstein

& LaBerge, 1964; Masters & Donachie, 1966) although, taken as a whole, protein synthesis is a continuous process (Schaechter, Williamson, Hood & Koch, 1962). The enzymes exhibiting discontinuous (or 'stepwise') synthesis are those whose synthesis is partially or wholly induced or derepressed. Kuempel et al. (1964) proposed a model to explain this stepwise synthesis which involves a cyclic variation in the level of repressor during one generation period. Halvorson et al. (1964) suggested a similar explanation. They envisaged that transcription is switched on and off sequentially along the DNA molecule during the growth cycle. Thus, if inducers and repressors act by stimulating the rate of messenger RNA synthesis they must only function at given periods of the growth cycle. Halvorson et al. (1964) found that the addition of inducer to cells synthesizing enzyme under a repressed regime (e.g., at a basal rate) did not alter the timing of the induced stepwise synthesis of the enzyme. Evidence contrary to this view has been presented by Masters et al. (1964) and Masters & Donachie (1966), who found that the synthesis of β-galactosidase under conditions of severe repression did not exhibit stepwise formation but was continuous at a rate which was proportional to the gene dosage (i.e., when the appropriate section of DNA was replicated the rate of synthesis also doubled).

No information is available on the timing of synthesis of enzymes on the central pathways of metabolism. Fig. 4 shows the early stages in the metabolism of the sugars used in the present experiments. With the exception of those controlled by the added inducers the enzymes mediating the metabolism of these sugars are found in all normally functioning cells. These enzymes are probably subject to control

mechanisms other than those which act solely at the genetic level, and the presence of any 'cytoplasmic' control over the enzymic activity of already synthesized protein would tend to counteract the in vivo effects of sudden increases in enzyme content caused by stepwise synthesis if this were to occur. For this reason, and because stepwise synthesis has never been demonstrated for these enzymes, it is not considered likely that cells in different stages of their life cycle in the cultures used in the present experiments acted as separate compartments due to differences in enzyme content.

Another explanation for the present observations which must be considered is the possibility that the differences in the relative pool sizes observed in different incubation vessels during a single experiment were caused by variation in the immediate environment of the cells. Aeration is the only environmental factor which can reasonably be considered as a source of variation, since it is difficult to appreciate how other local differences would have arisen.

Temperature, mixing, population and medium were undoubtedly identical in each case. Each incubation vessel received a different labelled substrate, but these represented minute chemical quantities of material and we are unable to ascribe our results to differences resulting from unforeseen characteristics of the tracer preparations. Variations in the pool sizes of citric, glutamic and succinic acids have been observed as the result of the growth of E. coli under anaerobic conditions. In such circumstances the tricarboxylic acid pathway acts not as a cycle but as two pathways branching at the point of entry of acetyl-CoA (Amarasingham & Davis, 1965). However, the operation of the anaerobic mode of the

tricarboxylic acid pathway requires that the formation of α-ketoglutarate dehydrogenase be repressed (Amarasingham & Davis, 1965) and a specific enzyme for the formation of succinate from fumarate (fumarate reductase) be induced (Hirsch, Raminsky, Davis & Lin, 1963). The cultures in the incubation vessels in the present experiments were non-growing due to the presence of chloramphenicol. Thus the presence or absence of any enzymes caused by anaerobiosis during the period of growth of the cultures would be observed in each of the incubation vessels to the same extent and so cannot be used to explain differences between them. Variations in the aeration of individual vessels might be expected to cause variations in the ratios of ATP/ADP, $NADH/NAD^+$, etc., which would be reflected in the activities of a number of enzymes. However, the respiratory studies reported above indicate that the cells in the labelling experiments were respiring at a constant and maximum rate, since the specific rate of respiration did not increase when the concentration of cells was decreased fourfold. In view of these results the authors do not consider that there were any differences between the levels of aeration in different vessels during a single labelling experiment large enough to account for the considerably different metabolic patterns obtained.

The most probable explanation for the observations is that the products of metabolism of glucose and galactose, derived from either internal or external sources, feed into pools of citric, succinic and glutamic acid which are at least partially separate. It is, perhaps, surprising that the cells can maintain functional separation of the metabolic intermediates from different sources through so many enzymic stages, although the ability to do so is plainly an advantage from the

point of view of metabolic control. This might be possible, however, if the enzymes on the pathways involved were very closely coordinated. The results presented above, particularly those in Fig. 1, suggest that among the metabolic reactions we have studied compartmentation plays a role only in distinguishing between hexoses produced within the cell and those presented to the exterior surface. Within each of these two categories the evidence suggests that glucose phosphate produced by epimerization of galactose phosphate equilibrates fully with glucose phosphate formed directly from free glucose. On the other hand, glucose phosphate derived from either of the hexoses in the medium is metabolically distinct from glucose phosphate derived from either of the hexoses produced by the hydrolysis of lactose.

Compartmentation of glycolytic metabolism in E. coli has been suggested by Eisenberg & Dobrogosz (1967) as a possible explanation for the fact that cells simultaneously oxidizing glucose and gluconate oxidise the former exclusively via glycolysis and the latter exclusively via an inducible Entner-Doudoroff pathway. Pollock (1966) has suggested that micro-organisms might coordinate their metabolic activity by incorporation of enzymes into an organized structural framework. The growing amount of information on multi-enzyme complexes on the central pathways of metabolism tends to support the view that metabolic compartments exist in bacteria. It has been known for some time from other systems that the enzymes responsible for the oxidative decarboxylation of pyruvate and α-ketoglutarate exist in such particulate complexes (Reed & Cox, 1966). There is also growing evidence for protein-protein interactions occurring much closer to the beginning of the glycolytic

pathway. For instance, the activity of aldolase has been shown to be enhanced by glycerophosphate dehydrogenase (Baranowski & Niederland, 1949) and by glyceraldehyde-3-phosphate dehydrogenase and triose phosphate isomerase (Gulyi, 1960; Gulyi, Dvnorikova, Fedorchenko & Pechenova, 1962; Sereda, 1963; Kwon & Olcott, 1965). All these effects were ascribed to protein-protein interactions and not to effects due to the removal of end products. The effects were specific, and proteins not functionally related to the enzyme under study had no effect. These enzymes, which came from a variety of higher animal sources, are found in what is normally regarded as the soluble fraction of the cell, but this evidence suggests that there is in the living cell a high degree of cytoplasmic organization of enzymes functionally related to one another in metabolic sequences. If, in such complexes, an enzyme preferentially accepts its substrate from another enzyme in the complex, rather than from a 'soluble' pool, the situation would be one of compartmentation as described in the Introduction. The concept of metabolic compartmentation in bacteria has rarely been explicitly stated in the past but it is an important one with a significant bearing on the understanding of mechanisms of biological control.

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Table 1. Labelled substrates added to the four parallel vessels of each experiment

Cells suspended in 0.01 M-phosphate buffer (pH 7.1) containing chloramphenicol (25 μg./ml.) received 0.76 mM-glucose, 0.76 mM-galactose and 0.40 mM-lactose. Four parallel portions of the suspension (each of 2 ml.) were incubated at 37° with stirring. Fifteen min. later labelled sugars were added as indicated below.

Vessel	Substrate	Vol. of substrate solution (μl.)	Amount of labelled substrate added	
			μC	μmoles
A	[G-^{14}C]glucose	40	8.04	0.075
B	[G-^{14}C]galactose	40	6.52	0.046
C	[(G-^{14}C)glucose]lactose	30	7.08	0.066
D	[(G-^{14}C)galactose]lactose	40	7.80	0.055

Table 2. Phenotypes of the bacteria used in the labelling experiments

Cells were grown in glycerol-minimal medium with or without IPTG (0.5 mM) to induce the lactose enzymes or D-fucose (5 mM) to induce the galactose enzymes. The cells in exponential growth received chloramphenicol (100 μg./ml.) immediately before harvesting and were then washed and resuspended in 0.01 M-phosphate buffer (pH 7.1) containing chloramphenicol (25 μg./ml.) for the labelling experiments. As a result of the presence of chloramphenicol no enzyme induction could take place during the subsequent metabolism with a mixture of glucose, galactose and lactose. Two ml. of cell suspension were used in each reaction vessel.

Experiment	*lac* operon	*gal* operon	Cell concentration (μg. bacterial protein/ml.)
1	induced	induced	198
2	not induced	induced	246
3	induced	not induced	244

Table 3. Utilization of labelled substrates

Cells of the phenotypes shown in Table 2 were allowed to metabolize a mixture of glucose, galactose and lactose in four parallel incubation vessels. Each vessel contained ^{14}C in a different substrate: in free glucose, free galactose, the glucose moiety of lactose or the galactose moiety of lactose. This Table presents data on the hydrolysis of lactose and on the utilization of hexoses both from free sugars in the medium and from those produced by lactose hydrolysis, all after 45 min. incubation at 37°.

Phenotype*	lac^+ gal^+	lac^- gal^+	lac^+ gal^-
Extent of lactose hydrolysis (% of total)			
based on [(G-^{14}C)glucose]lactose	98	9	81
based on [(G-^{14}C)galactose]lactose	96	17	85
Lactose remaining as free hexose (% of total)			
based on [(G-^{14}C)glucose]lactose	87	2	19
based on [(G-^{14}C)galactose]lactose	37	4	92
Glucose from lactose metabolized (μmoles/mg. bacterial protein)	0.214	0.111	0.842
Free glucose metabolized (μmoles/mg. bacterial protein)	0.371	2.175	0.417
Total glucose metabolized (μmoles/mg. bacterial protein)	0.585	2.286	1.259
Galactose from lactose metabolized (μmoles/mg. bacterial protein)	0.996	0.203	0
Free galactose metabolized (μmoles/mg. bacterial protein)	1.078	0.817	0.153
Total galactose metabolized (μmoles/mg. bacterial protein)	2.074	1.020	0.153
Total hexose metabolized (μmoles/mg. bacterial protein)	2.659	3.306	1.412

* + indicates enzymes induced
- indicates enzymes not induced

Captions for Figures

Fig. 1. Formation of glutamate, citrate and succinate in cells induced for both the lac and gal operons, and supplied with a mixture of glucose, galactose and lactose labelled in various ways. A, [G-^{14}C]glucose; B, [G-^{14}C]galactose; C, [(G-^{14}C)glucose]lactose; D, [(G-^{14}C)galactose]lactose. o, glutamate; ●, citrate; ▲, succinate.

Fig. 2. Formation of glutamate, citrate and succinate in cells induced for the gal operon only, and supplied with a mixture of glucose, galactose and lactose labelled in various ways. A, [G-^{14}C]glucose; B, [G-^{14}C]galactose; C, [(G-^{14}C)glucose]lactose. No activity in these compounds was obtained with [(G-^{14}C)galactose]lactose. o, glutamate; ●, citrate; ▲, succinate.

Fig. 3. Formation of glutamate, citrate and succinate in cells induced for the lac operon only, and supplied with a mixture of glucose, galactose and lactose labelled in various ways. A, [G-^{14}C]glucose; B, [(G-^{14}C)glucose]lactose. No activity in these compounds was obtained with [G-^{14}C] galactose or [(G-^{14}C)galactose]lactose. o, glutamate; ●, citrate; ▲, succinate.

Fig. 4. Initial metabolic interrelations of lactose, galactose and glucose, showing inducible enzymes and some inducers.

Running title: "Metabolic compartmentation in E. coli"

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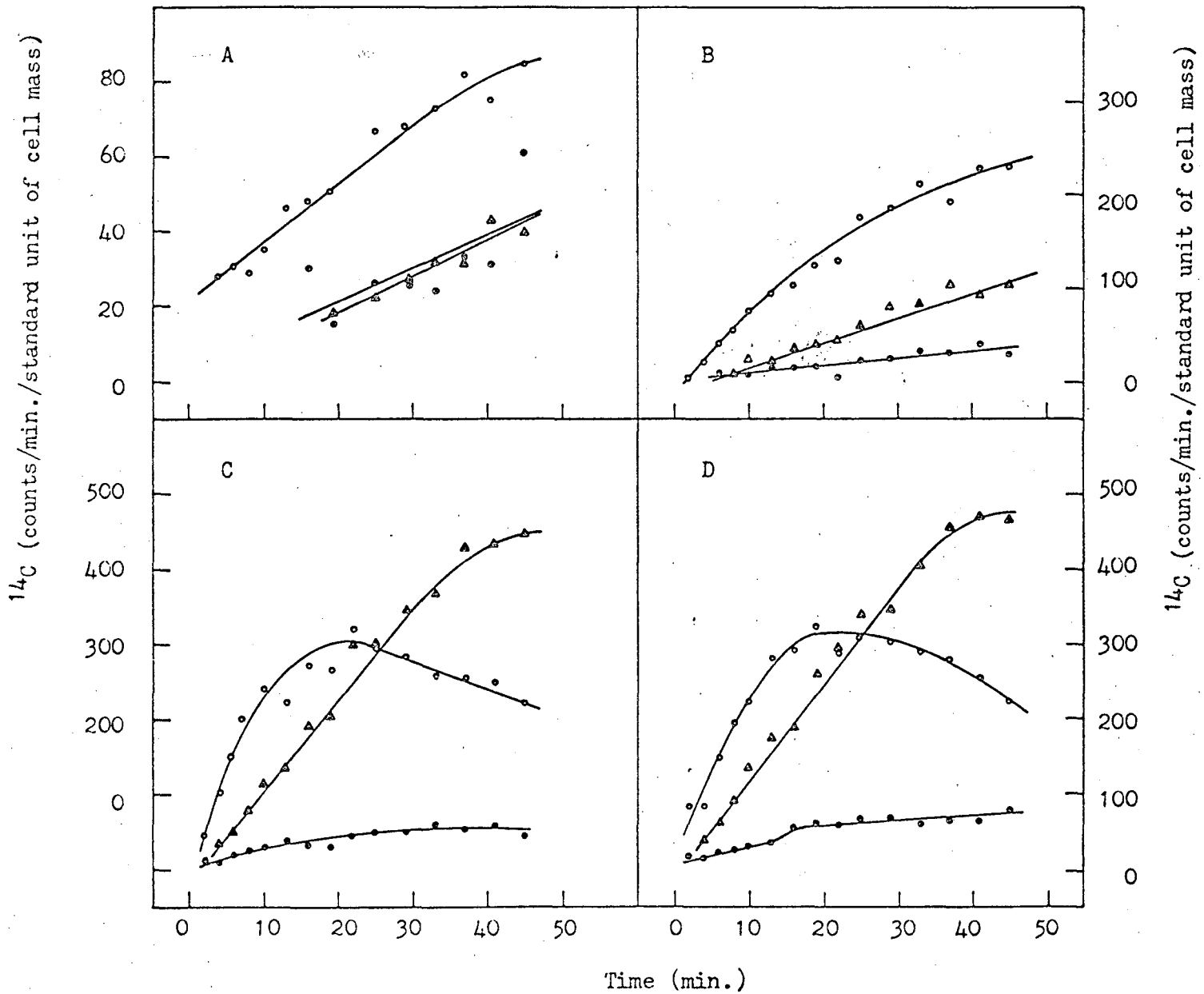
A
B
C
D
^{14}C (counts/min./standard unit of cell mass)
^{14}C (counts/min./standard unit of cell mass)
0
20
40
60
80
0
100
200
300
0
200
300
400
500
0
100
200
300
400
500
0
10
20
30
40
50
Time (min.)

Fig. 1

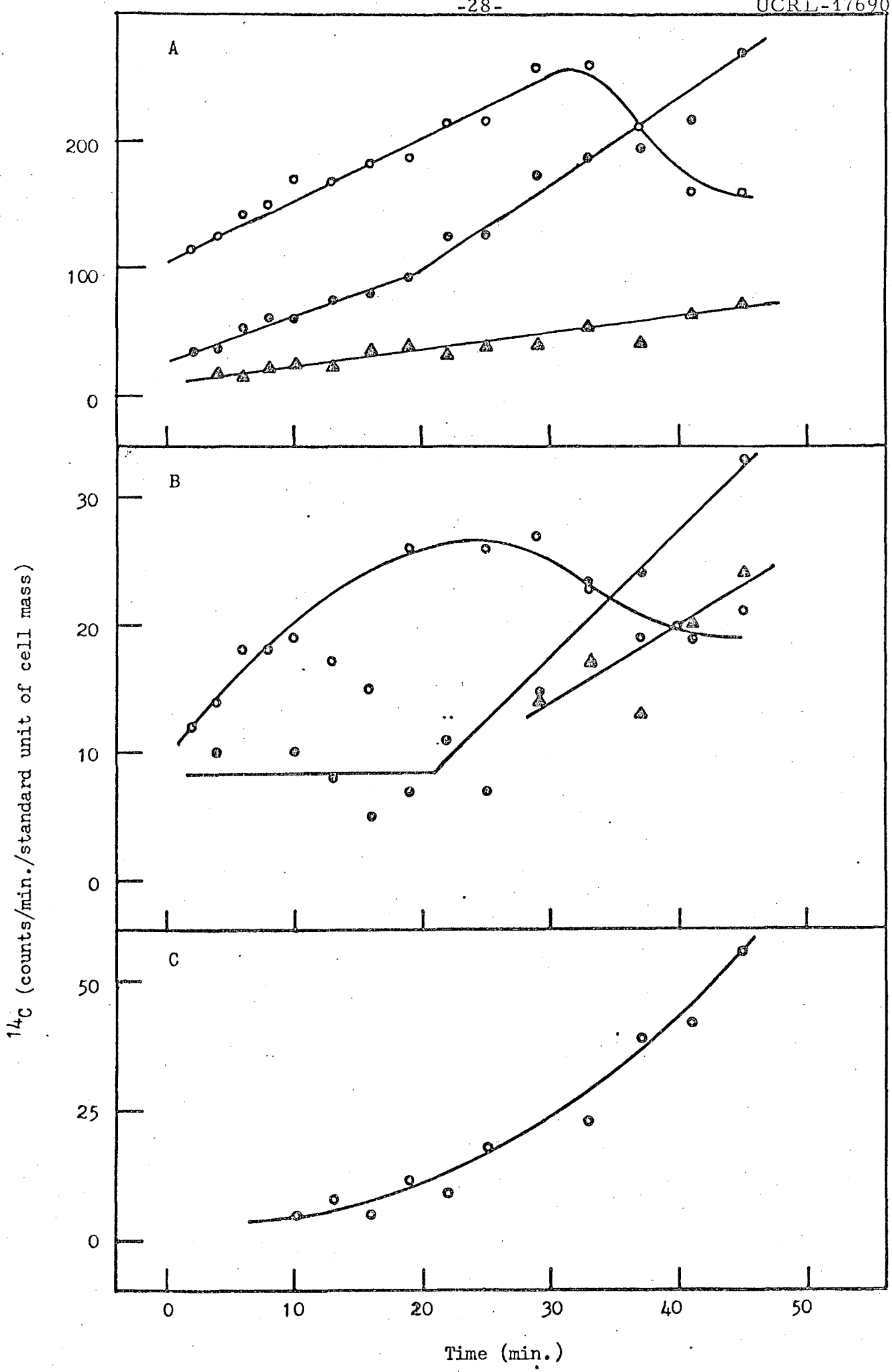
A
B
C
200
100
0
30
20
10
0
50
25
0
0
10
20
30
40
50
^{14}C (counts/min./standard unit of cell mass)
Time (min.)

Fig. 2

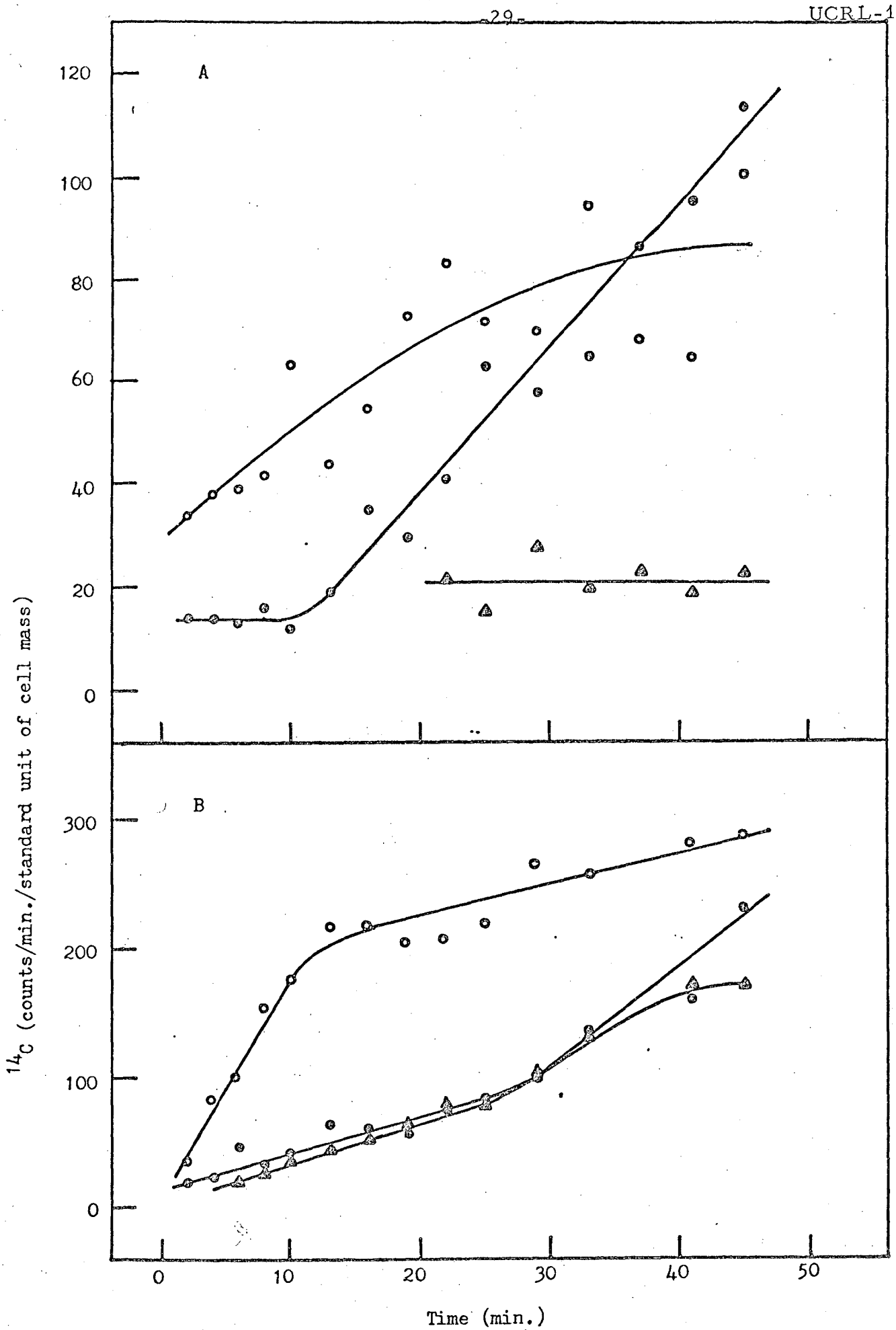
A
B
120
100
80
60
40
20
0
300
200
100
0
0
10
20
30
40
50
Time (min.)
^{14}C (counts/min./standard unit of cell mass)

Fig. 3