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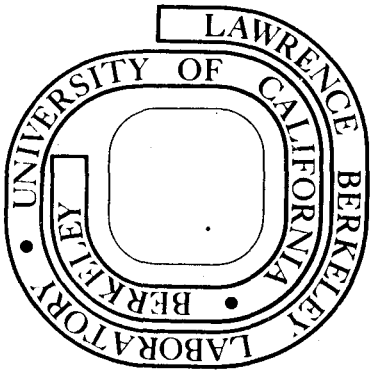

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2-Deoxyglucose Compared with an Uncoupler of Oxidative Phosphorylation: Effects on Glucose Metabolism and Adenylate Ratios in Chick Cells in Culture*

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SUMMARY

Chick embryo fibroblast cells in tissue culture were grown under carefully controlled conditions in the presence of [^{14}C]-glucose and [^{32}P]-inorganic phosphate for a time period sufficient to establish a steady-state of labeling for all intermediary metabolites. Perturbation of the steady-state metabolism by either 2-deoxyglucose (2-DG) or carbonyl cyanide m-chlorophenylhydrazone (CCCP), followed by kinetic analysis of the metabolite pools, showed that both of these agents induced rapid fluctuations in most steady-state pools. The uncoupler, CCCP, drops the energy charge which stimulates glycolysis and brings levels of metabolites back toward control values. The addition of 2-DG causes both inhibition of glucose uptake and a very rapid utilization of ATP to give 2-DG-6-phosphate, with consequent inhibition of conversion of glucose-6-phosphate to fructose-6-phosphate. The combined effect of these inhibitions is to reduce glycolytic flux and energy charge nearly to zero during the first 30 sec. In spite of continued severely restricted uptake of substrate and the destruction of more than 80% of the total adenylate pool, the energy charge recovers to intermediate levels, and many metabolite pool levels also recover.

INTRODUCTION

An important determinant in variations of metabolism in both normal and virus-transformed chick cells in tissue culture appears to be the rate of glucose uptake (1). Recent measurements of glycolytic pool sizes during steady-state metabolism of these cells, for example, showed a clear correlation between these pool sizes and rate of glucose uptake (1,2).

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The techniques used for kinetic steady-state studies in these cells lend themselves to the investigation of rapid metabolic transients. Such transient changes follow perturbation of the steady-state by introduction of chemicals or sudden changes in other physiological factors. As one such perturbation, glucose uptake could be suddenly affected by the introduction of a chemical agent with a specific action on glucose transport into the cell. Glucose uptake could also be affected by reducing the general availability of chemical energy in the form of ATP.

The glucose analogue, 2-deoxyglucose (2-DG), is taken up by animal cells, and is converted to 2-deoxyglucose 6-phosphate (2-DG6P) by hexokinase. Neither this compound nor 2-DG is further metabolized to any appreciable extent (3,4). The transport of 2-DG has been shown to be competitive with glucose transport, although its affinity for the binding site is much lower (5,6,7). In the presence of high concentration of 2-DG, its rapid phosphorylation depletes the cell's supply of ATP (8,9), and there is evidence that 2-DG6P which accumulates in the cell, noncompetitively inhibits the transport of glucose (10). Thus, 2-DG was chosen as an agent which specifically affects glucose uptake by direct competition as well as by indirect effects resulting from changes in the "energy charge": $\left(\frac{ATP + 1/2\ ADP}{ATP+ADP+AMP}\right)$ (11).

An uncoupler of oxidative phosphorylation, carbonyl cyanide m-chlorophenylhydrazone (CCCP), was employed in parallel experiments to provide information about general metabolic effects in chick cells of lowered adenylate ratios in the absence of a specific block on glucose uptake.

MATERIALS AND METHODS

Growth of Cell Cultures--Primary cultures were prepared from 10-day old C/O or C/B type SPF chick embryos (12) free of resistance-inducing factor essentially as described (13,14). Briefly, after decapitation and evisceration, the embryos were minced, washed with tris-saline buffer and digested with 0.25% trypsin. After 15 min the suspended cells were poured into a "stop bath" containing 2/3 cold medium 199 (Gibco) and 1/3 calf serum. This process was repeated twice. The single cells were then plated in 100 mm culture dishes at 8×10^6 cells per plate in medium 199. Two percent tryptose phosphate broth (Gibco), 1% calf serum, and 1% chicken serum (Microbiological Associates, Inc.) were added to the medium. Fungizone was eliminated entirely, as harmful side effects have been observed in this laboratory. Secondary cultures were prepared 4 days after the primary seeding by trypsinization of primary cultures and were seeded at the desired cell concentration in 35-mm petri dishes. The concentration of glucose and calf serum was doubled (11 mM and 2% respectively) at the time of secondary seedings. Each "sample" referred to below was one such petri dish culture.

Steady-state Labeling of Cells--Thirty-two hours after secondary seeding the medium of secondary cell cultures was replaced by one which contained 5.5 mM [^{14}C]-glucose (specific activity 10.2 Ci/mole), 1.25 mM inorganic [^{32}P]-phosphate (specific activity 1 Ci/mole), and the cells were allowed to metabolize for at least 15 hr under the usual growth conditions (tissue culture incubators, 5% CO_2 in air, 39°C). The cells were then transferred to our steady-state apparatus (1). At 1 hr and again just before each experiment, the growth medium was replaced by one which contained 1/10 the original glucose concentration (0.5 mM), with the specific

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radioactivities kept constant. Fifteen minutes after the last medium change, either 2-DG (20 mM) or CCCP (10 μM) was added to perturb the steady-state unless otherwise indicated. Each experiment included a series of kinetic points (1, 5, 15, and 30 min) in order to establish the levels of steady-state metabolite pools, as well as kinetic points (30 sec, 1, 2, 5, 15, and 30 min) taken after the perturbation, for measurement of the fluctuations in these pools.

For experiments in which the rate of entry of [^{14}C]-glucose carbon into metabolite pools was measured, the radioactive medium was added to the cells at 0 time and then samples were taken at various times as previously described (1).

Analytical Methods--Immediately before the cells were killed, the medium was removed and the culture was rapidly washed with cold Hanks' buffer containing unlabeled glucose. The cells were killed by addition of cold 80% methanol (less than 15 sec after removal of medium). The dead cells were then scraped from the dish with a rubber policeman and disrupted by sonic oscillation, after which an aliquot portion was applied to filter paper for analysis by two-dimensional paper chromatography (15). The samples were first developed with phenol-water-acetic acid (84:16:1) for either 24 or 48 hr. After the paper was dried, it was turned 90° and developed with butanol-water-propionic acid (50:28:22) for another 24 or 48 hr. After the paper was again dried, the locations of the labeled metabolites were detected by radioautography and the content of ^{14}C in each was determined.

Protein concentrations were determined on aliquots of each sample by the method of Lowry (16). ^{14}C-Labeled components of the medium were isolated by column chromatography on a Bio-gel P-2 (200-400 mesh) column (1.5 x 120 cm).

Chemicals--The [^{14}C]-glucose (306 Ci/mole) was purchased from Amersham, and the ^{32}P as $H_3{}^{32}PO_4$ (carrier free) in H_2O from New England Nuclear. 2-Deoxy-D-glucose (A grade) and carbonyl cyanide m-chlorophenylhydrazone (CCCP) were purchased from Calbiochem.

RESULTS

Effects of 2-Deoxyglucose on Labeled Metabolites in Cells Previously Grown on ^{14}C-glucose and ^{32}P-labeled Pi--The glucose concentration of the growth medium in these experiments was low (0.5 mM); therefore when a high concentration of 2-DG (20 mM) was added, the transport and phosphorylation of 2-DG was rapid (Fig. 1). This phosphorylation resulted in an immediate decrease in the ATP pool concurrent with a rise in the ADP (Fig. 2) and AMP (Fig. 3) pools. The suddenly elevated AMP pool as compared to the changes in the ADP pool suggests that these cells have an extremely active adenylate kinase enzyme. Adenylate kinase has been shown to be active in other mammalian cells (8).

Within 30 sec after the addition of 2-DG, the cell utilized approximately 95% of its ATP pool. While some of the ^{32}P thus released from ATP can be accounted for as 2-DG-6 ^{32}P, smaller amounts could be accounted for in increased phosphorylation of glucose to give hexose monophosphates. There is a large initial increase in the internal ^{32}Pi pool (Fig. 4, Table I). Thus, it appears that some activity resulting in conversion of ATP terminal phosphate to Pi has been stimulated during the first 30 sec after 2-DG addition. This might be a hexose phosphatase, ATPase, or some other activity involving a more indirect conversion. After the initial rise in Pi, its level drops rapidly and remains low for the duration of the experiment.

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The addition of 2-DG caused a very rapid decrease in the energy charge of the cells (Figs. 1, 2 and 3, Table I). This drop in energy charge of the cells was partially adjusted by the destruction of AMP and ADP 1 min after the addition of 2-DG. There was no evidence from the paper chromatograms of the appearance of a new intracellular pool derived from the adenosine portion of these molecules. A previous report has shown that inosine and hypoxanthine are released into the medium in the presence of 2-DG (9). While these products were not specifically identified in the column chromatograms of the culture medium (see Methods), there was an increased radioactivity in a fraction which could not be attributed to glucose or lactate. It is therefore possible that the breakdown product of AMP was rapidly released into the medium.

Some of the cells' response to these changes in adenylate ratios can be seen in the kinetic fluctuations in other metabolite pools. For example, the pools of glutamate, aspartate, citrate, and malate (Fig. 5) change concurrently with the decrease in the energy charge of the cells. One of the most notable changes after addition of 2-DG occurs in the glutamate pool within the first minute. The large amount of ^{14}C lost from the glutamate pool is balanced by the ^{14}C increase in the aspartate pool. Simultaneously, there are decreases in the citrate and malate pools.

The metabolite pools of the glycolytic sequence all decrease very rapidly in the first minute after the addition of 2-DG, probably due to both the decrease in glucose transport and the drop in the energy charge of the cell. However, the metabolite pools tend to recover to varying degrees 1 min after the addition of 2-DG, with the largest increase in pentose monophosphate and the smallest in lactate. The recovery of these

pools occurs concurrently with the partial rebound in the energy charge caused by the drop in the AMP and ADP pools. The levels of ^{14}C-glycogen in samples before and after 2-DG were measured (1) and found to be unchanged.

After other glycolytic pools have reached new steady-state levels, the hexose monophosphate concentration continues to rise. Analysis of this mixture shows the increase to be almost completely due to an increase in glucose-6-phosphate and not fructose-6-phosphate (Table II). This indicates that the interconversion of glucose-6-phosphate and fructose-6-phosphate, normally rapid and reversible, is inhibited by the accumulation of 2-DG6P.

The high 2-DG/glucose ratio used in these experiments was chosen to produce maximal perturbation of the system. When 2-DG concentration is lowered to 10 mM and glucose concentration raised to 1 mM, the energy charge changes in a similar way but at a slower rate (Fig. 7). Thus the fluctuations in other metabolite pools are small and more difficult to interpret.

Effects of Carbonyl Cyanide m-Chlorophenylhydrazone (CCCP)--When CCCP, an uncoupler of phosphorylation, is added to the cells, one can see by the rise in the ADP pool (Fig. 2) that oxidative phosphorylation has been blocked at the final step in the phosphorylation process. The initial rapid drop and the later slow decay of the ATP pool (Fig. 1) and rise in the AMP pool (Fig. 3) are due to the consumption of ATP in energy coupled reactions without replacement via oxidative phosphorylation. The rise in the inorganic phosphate pool (Fig. 4) is also a reflection of the blocked oxidative phosphorylation system. While the initial fast rise in Pi parallels that observed with 2-DG addition, the subsequent continued rise in Pi after CCCP addition is in strong contrast to the drop and subsequent low level of Pi after 1 min with 2-DG.

The uncoupling of phosphorylation results initially in an increased citric acid cycle and electron transport activity, as indicated by the fluctuation of the glutamate, aspartate, citrate, and malate pools (Fig. 5). These changes are similar to those observed when the energy charge was lowered by the phosphorylation of 2-DG.

The glycolytic pools, except for fructose-1,6-diphosphate, increased when CCCP was added to the cells (Fig. 6). The fructose-1,6-diphosphate pool decreases during the first minute, but later increases. The immediate rise in the pool of lactate indicates that glycolysis is greatly accelerated.

Effect of 2-Deoxy-D-glucose on the Appearance of ^{14}C and ^{32}P in Metabolite Pools: Labeling from Zero Time--When 2-DG and [^{14}C]-glucose were added together, at zero time to cells which were growing on unlabeled 0.5 mM glucose, the initial rate of ^{14}C appearance in various metabolic pools decreased (Fig. 8). The level of labeled fructose-1,6-diphosphate, and other metabolites derived from it, always stays lower in the 2-DG treated cells than in the control. While the labeling of hexose monophosphate pools in untreated cells stopped rising (indicating a steady-state), the labeling of hexose monophosphate pools in cells treated with 2-DG continued to rise (Fig. 8). This again indicates that the conversion of glucose-6-phosphate to fructose-6-phosphate is inhibited as 2-DG6P accumulates in the cells.

The intracellular Pi pool is labeled more slowly with ^{32}P in the cells treated with 2-DG (Fig. 9). This indicates that either the rate of entry of ^{32}Pi is lower, or the total intracellular pool of Pi is rapidly decreased in the presence of 2-DG. The results with 2-DG addition to already labeled cells, Fig. 4, showed that the pool of Pi rapidly decreased.

DISCUSSION

The various transient changes in pools of metabolites accompanying the addition of an uncoupler of phosphorylation (CCCP) are for the most part expected, based on well known regulatory mechanisms of intermediary metabolism (17). As the energy charge and the level of ATP fall, there is a stimulation of metabolism, particularly glycolysis to provide glycolytic phosphorylation, leading to a large increase in lactate. These changes provide a useful pattern for comparison with transients induced by 2-DG addition.

Since in both cases (CCCP and 2-DG addition) there is a rapid initial drop in energy charge, some of the metabolic transient changes are similar. Thus, the citric acid cycle responds to the low energy charge by a surge of activity. The glutamate pool may serve as the substrate for citric acid cycle activity in order to produce electron flow for the production of ATP via oxidative phosphorylation. Although the complete cycle is not able to accelerate sufficiently due to a limited supply of acetyl CoA, a partial cycle may function via the glutamate-aspartate transaminase enzyme and conversion of α-ketoglutarate to oxalacetate plus CO_2. As the energy charge of the cells partially recovers after the first minute, these pools return nearly to normal levels. Later, with CCCP addition, this uncoupler continues to "pull" the cycle so that the level of citrate drops and remains low.

A basic difference between the action of 2-DG and that of CCCP is in their effect on glucose transport. The addition of a high concentration of 2-DG essentially blocks the uptake of glucose while CCCP causes an increased uptake and utilization of glucose. When the energy supply is depleted by addition of 2-DG, glycolysis cannot compensate as an energy source as well

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as it does in the case of CCCP addition. Thus, the rate and extent of energy stress is much greater on addition of 2-DG, and this appears to cause marked differences in certain metabolite pools.

The AMP pool is elevated to a greater extent and the total adenylates (Table I) are rapidly depleted in the case of 2-DG addition. This seems to indicate activation of adenylate kinase and AMP deaminase activities to a much greater extent than in the uncoupled system. The Pi level remains high in the presence of CCCP but is very low after 30 sec in 2-DG. The decreased uptake of Pi, and continued low level of Pi after 1 min following 2-DG addition may be related to the decreased uptake of glucose or the decreased rate of glycolysis (18).

Since Pi has been shown to be an inhibitor of AMP deaminase (19), the higher level of Pi could partially explain the slower destruction of adenylates in the presence of CCCP even though substantial AMP is produced. It is noteworthy that despite the large destruction of adenylates in the case of 2-DG addition, the energy charge is restored to about as high a level (0.6) as in the c se of CCCP addition, which produced only minor adenylate destruction (Table I).

Another effect of 2-DG addition appears to be an at least partial inhibition of the interconversion of glucose-6-phosphate and fructose-6-phosphate (Table II). The <u>in vitro</u> inhibition of hexose phosphate isomerase by 2-DG6P has been reported (20). Apparently this partial block to glycolysis is bypassed to some extent by the pentose phosphate shunt. Thus, after the first 30 sec following addition of 2-DG, there is a recovery of some of the pools of glycolytic intermediates from the low levels reached just after 2-DG addition. This recovery is greatest for pentose monophosphate and

least for lactate. This might be expected if the inhibition of glycolysis (due both to inhibition of glucose uptake and glucose-6-phosphate conversion to fructose-6-phosphate) is partially relieved by conversion of some glucose-6-phosphate to lactate via the pentose phosphate cycle. The slow but steady increase in glucose-6-phosphate, together with the failure of the glycolytic pools to recover completely to control levels suggests that the pentose phosphate cycle is also limited in some way, perhaps by the availability of acceptors of electrons from NADPH produced by the pentose phosphate cycle.

The metabolic changes produced by 2-DG and by CCCP might be used to some extent to examine proposed mechanisms for regulation of glycolysis, for example, by energy charge (21), by inorganic phosphate level (22), and by ADP level (23). Thus, the intracellular lactate pool can be taken as a rough measure of glycolytic flux, and can be compared with levels of ADP, Pi and energy charge.

With CCCP addition, there is correspondence consistent with each mechanism. The increased glycolytic flux indicated by the elevated level of lactate correlates with increased ADP level, increased Pi level, and with lower energy charge.

With 2-DG addition, interpretation is made more difficult because the primary cause of decreased glycolytic flux is diminished glucose uptake. This diminished glucose uptake clearly is not a consequence of decreased energy charge, since with CCCP glycolytic flux increased. This is consistent with earlier studies showing that glucose uptake in chick cells (6,7) is by facilitated diffusion, not active transport. Although glycolytic flux is much lower after 2-DG addition, partial recovery of glycolysis seen in the lactate and fructose-1,6-diphosphate pools after 1 min (Fig. 6) appears to be in response to the operation of control mechanisms. The initial

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recovery (after 1 min) might be attributed to either the 1 min peak in ADP, the 1 min peak in Pi, or the drastically lowered energy charge. The sustained partial recovery after 2 min is most easily attributable to the continued low energy charge, since the levels of ADP and Pi are below the control levels after this time with 2-DG.

The effects of the two inhibitors thus operate through very different mechanisms: CCCP acting as an uncoupler produces the expected results on glycolysis and tricarboxylic acid cycle. High levels of 2-DG, added to cells metabolizing low levels of glucose, inhibits entry of glucose into the cell and interconversion of glucose-6-phosphate and fructose-6-phosphate. While in both cases the energy charge is lowered, the metabolic causes and consequences of this lowered energy charge are quite dissimilar. Perhaps the most striking result of the 2-DG addition is the tendency of the energy charge to recover to at least intermediate levels in spite of massive destruction of adenylates, very low level of inorganic phosphate, and a severely restricted uptake of substrate. Almost as remarkable is the return nearly to control levels of many measured metabolic pools, even though the metabolic flux must be greatly reduced. This demonstrates the impressive ability of the regulatory system of the cell to recover under extreme conditions.

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TABLE I

Change in Energy Charge and Total Adenylate Pool after 2-DG and CCCP Addition

	2-DG			CCCP		
	E.C.	Total Adenylate	Pi	E.C.	Total Adenylate	Pi
Control	0.91	106.5	35	0.91	106.5	35
30"	0.28	59.6	63.5	0.71	110.1	61
1'	0.24	79.0	58.1	0.64	111.6	66
2'	0.59	64.9	14.5	0.75	117.2	52
5'	0.50	37.7	9.2	0.75	104.4	64
15'	0.60	20.7	7.9	0.66	88.5	86
30'	0.62	19.9	11.3	0.54	69.3	103

These data represent the same steady-state experiment described in Fig. 1. The total adenylate pool (ATP+ADP+AMP) and energy charge ($\frac{ATP + 1/2\ ADP}{ATP+ADP+AMP}$) were calculated from values shown in Figs. 1, 2, and 3. Phosphate values are the same as shown in Fig. 4.

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TABLE II

Pools of Glucose-6-phosphate and Fructose-6-phosphate in Control and 2-DG Treated Cells

Addition	Time	G6P (nmoles ^{14}C/mg protein)	F6P (nmoles ^{14}C/mg protein
Control		0.98	0.78
2-DG	30"	0.50	0.09
"	1'	0.70	0.12
"	2'	1.41	0.60
"	5'	1.61	0.64
"	15'	3.58	1.18
"	30'	4.66	1.06

These pool levels were obtained from the 48 hr chromatographic separation of the HMP pool shown in Fig. 6. The experimental detail is as described in Fig. 1.

FIGURE CAPTIONS

FIG. 1. Effects of 20 mM 2-DG and of 10 μM CCCP on the steady-state pool of ATP. The medium of cells used in this experiment was changed to that containing [^{14}C]-glucose (46 Ci/mole) and $H_3{}^{32}PO_4$ (1 Ci/mole) 15, 1-1/2, and 0 hr to insure complete labeling before measurement of pools. The glucose concentration of the medium was 0.5 mM at "0" time and the specific activities were constant throughout. Cell samples were taken after the last medium change to establish steady-state pools, and again after the additions. Fifteen min after the last medium change either 20 mM 2-DG or 10 μM CCCP was added directly to the plates as indicated by the arrow. ATP was quantitated by its ^{14}C content (nmoles ^{14}C/mg protein); and 2-DG 6-P by its ^{32}P content (nCi ^{32}P)mg protein). o, steady-state pool; Δ, pool after addition of 20 mM 2-DG; □, pool after addition of 10 μM CCCP; - - - - o, pool of 2-DG 6-P which accumulates in the cell.

FIG. 2. Effects of 20 mM 2-DG and of 10 μM CCCP on the steady-state pool of ADP. Measurement of the ADP pool was from the same experiment described in Fig. 1. The arrow indicates the direct addition of 20 mM 2-DG or 10 μM CCCP. o, steady-state pool; Δ, pool after addition of 20 mM 2-DG; □, pool after addition of 10 μM CCCP.

FIG. 3. Effects of 20 mM 2-DG and of 10 μM CCCP on the steady-state pool of AMP. Measurement of the AMP pool was from the same experiment described in Fig. 1. The arrow indicates the direct addition of 20 mM 2-DG or 10 μM CCCP. o, steady-state pool; Δ, pool after addition of 20 mM 2-DG; □, pool after addition of 10 μM CCCP.

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FIG. 4. Effect of 20 mM 2-DG and 10 μM CCCP on the steady-state pool of inorganic phosphate. Measurement of the Pi pool was from the same experiment described in Fig. 1. The arrow indicates the direct addition of 20 mM 2-DG or 10 μM CCCP. o, steady-state pool; Δ, pool after addition of 20 mM 2-DG; □, pool after addition of 10 μM CCCP.

FIG. 5. Effect of 20 mM 2-DG and 10 μM CCCP on the steady-state pools of amino acids and TCA intermediates. These pools were measured from the same experiment described in Fig. 1. The arrow indicates the direct addition of 20 mM 2-DG or 10 μM CCCP. o, steady-state pools; Δ, pools after the addition of 20 mM 2-DG; □, pools after the addition of 10 μM CCCP. The significance of the final difference in the malate pool is not clear. However, this pool is very small (the final difference between CCCP and 2-DG treated pools is in fact less than the final difference in other pools) and the possibility for variation is greater.

FIG. 6. Effect of 20 mM 2-DG and 10 μM CCCP on the steady-state glycolytic pools. These pools were measured from the same experiment described in Fig. 1. The arrow indicates the direct addition of 20 mM 2-DG or 10 μM CCCP. o, steady-state pools; Δ, pools after addition of 20 mM 2-DG; □, pools after addition of 10 μM CCCP.

FIG. 7. Effects of 10 mM 2-DG on the steady-state adenylate and Pi pools. The cells used in this experiment were treated as described in Fig. 1, except that the glucose concentration was 1 mM at "0" time. The arrow indicates the time at which 10 mM 2-DG was added directly to the plates. ATP, ADP and AMP were quantitated by ^{14}C content (nmoles ^{14}C/mg protein); and Pi and 2-DG 6-P by ^{32}P content (nCi ^{32}P/mg protein). o,

steady-state pools; Δ, pools after addition of 10 mM 2-DG; - - - - o, accumulation of 2-DP 6-P in the cell.

FIG. 8. The rate of labeling of glycolytic and amino acid pools in the presence and absence of 20 mM 2-deoxyglucose. After equilibration of the cells in the "steady-state apparatus" in the presence of 0.5 mM unlabeled glucose, the medium was changed to that which contained 0.5 mM [^{14}C]-glucose (46 Ci/mole) at "0" time with or without 20 mM 2-DG. o, absence of 2-DG; Δ, presence of 2-DG.

FIG. 9. Labeling of the inorganic phosphate pool in the presence and absence of 20 mM 2-deoxyglucose. The experimental conditions were the same as in Fig. 8 except that $H_3{}^{32}PO_4$ (1 Ci/mole) was added at "0" time. o, absence of 2-DG; Δ, presence of 20 mM 2-DG.

0 0 0 0 4 1 0 2 0 2 9

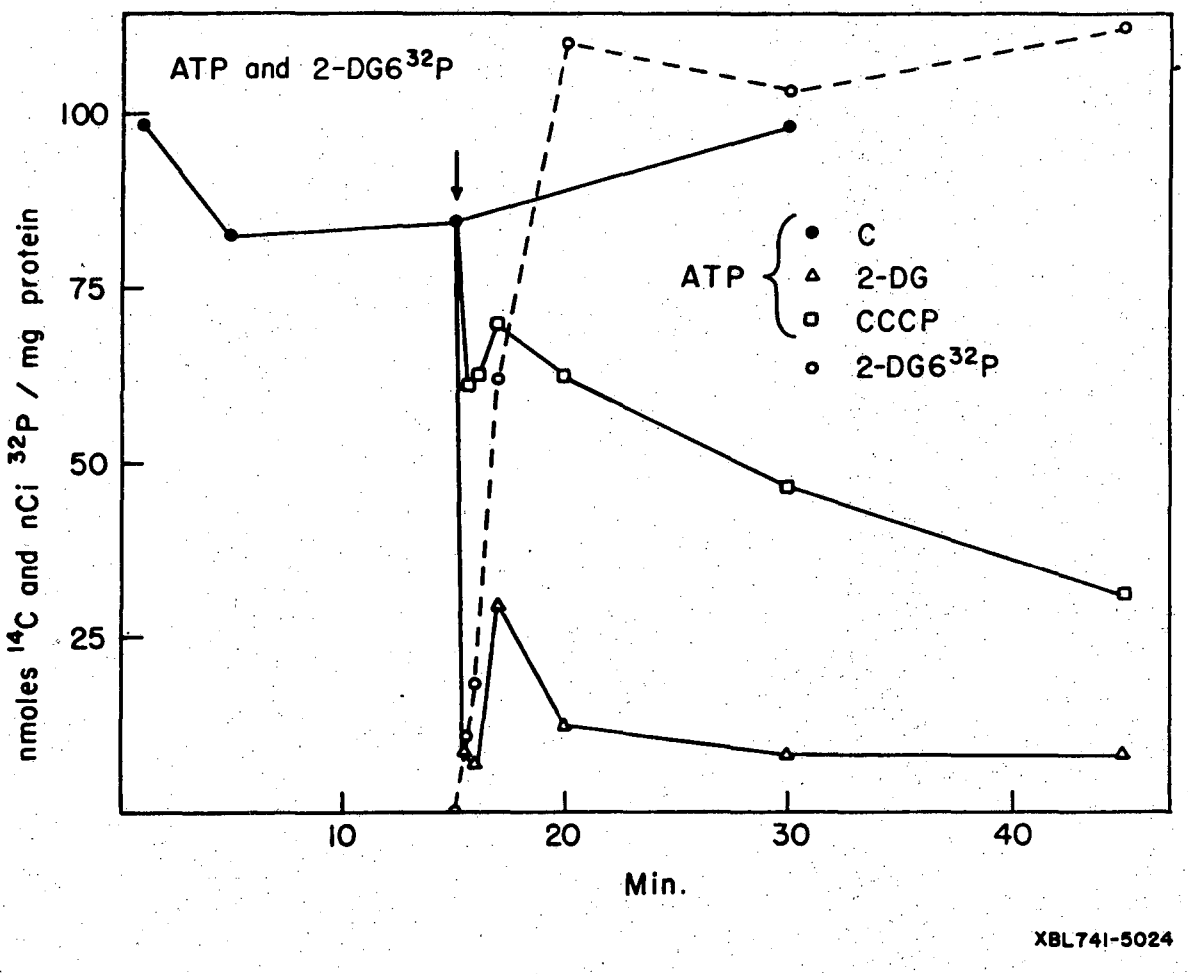
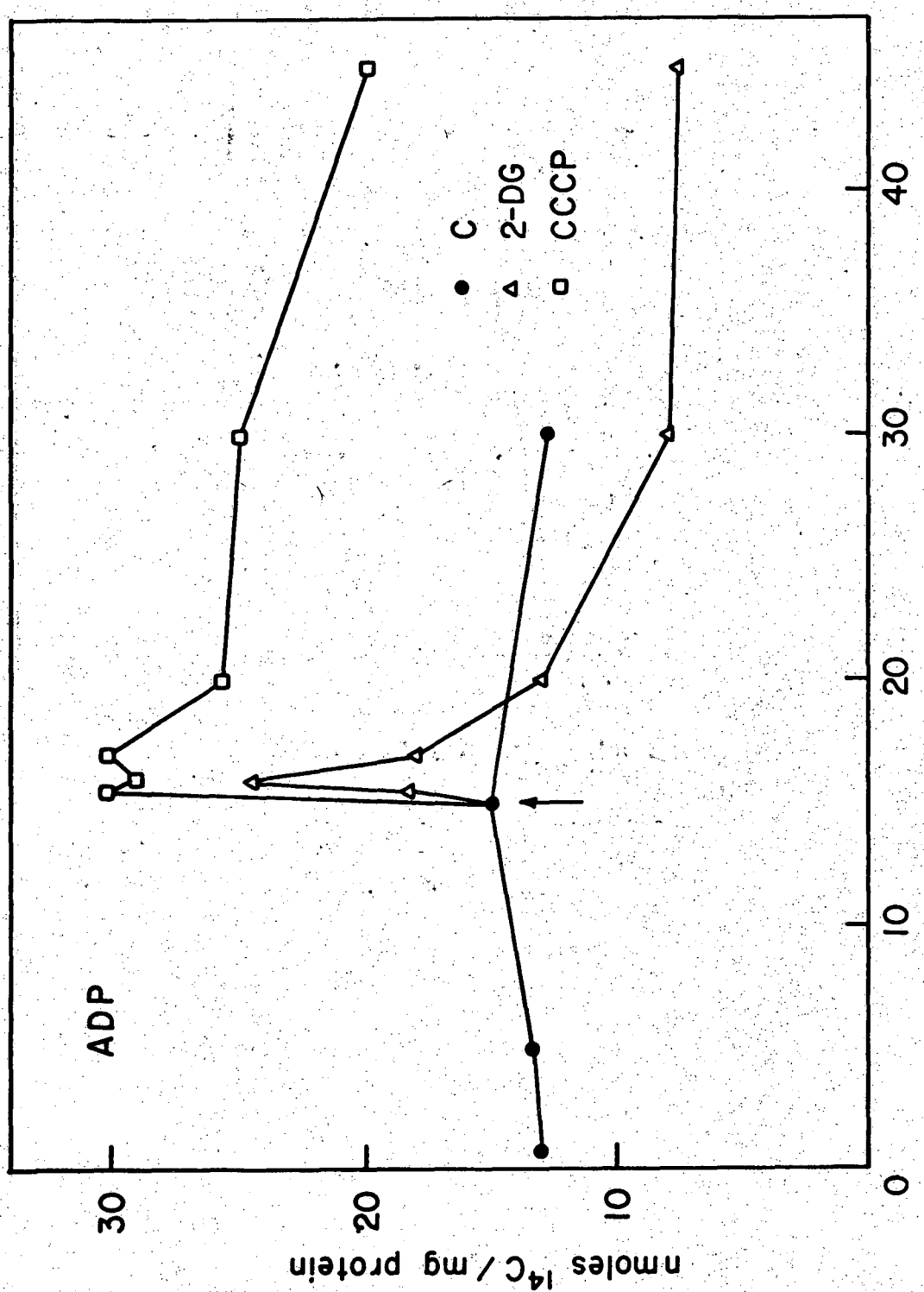


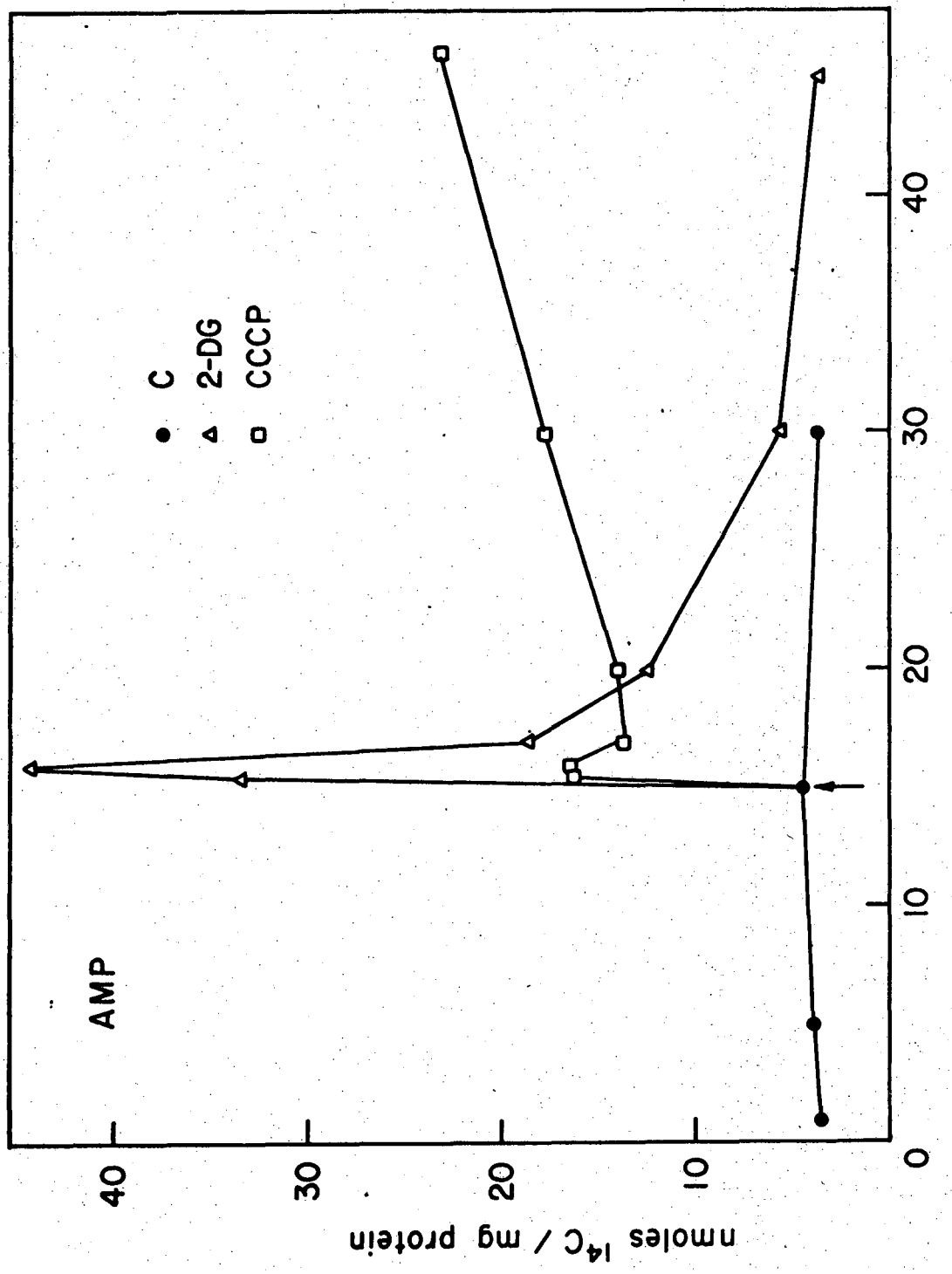
Fig. 1.



XBL741-5022

Fig. 2.

0 0 0 0 4 1 0 2 0 3 0



XBL 741-5023

Fig. 3.

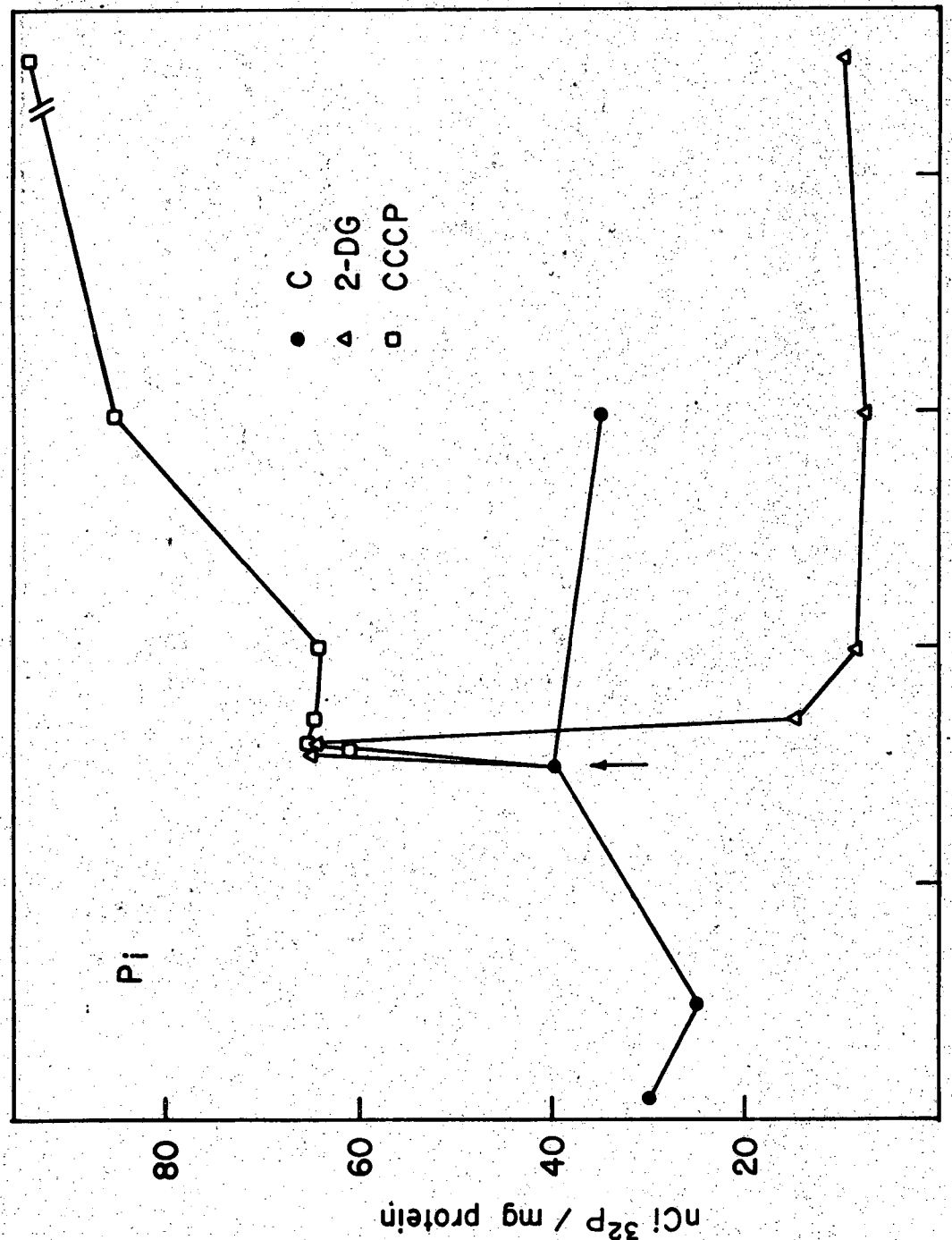

nCi 32P / mg protein
80
60
40
20
Pi
C
2-DG
CCCP
10
20
30
40
Min.
XBL741-5025

Fig. 4.

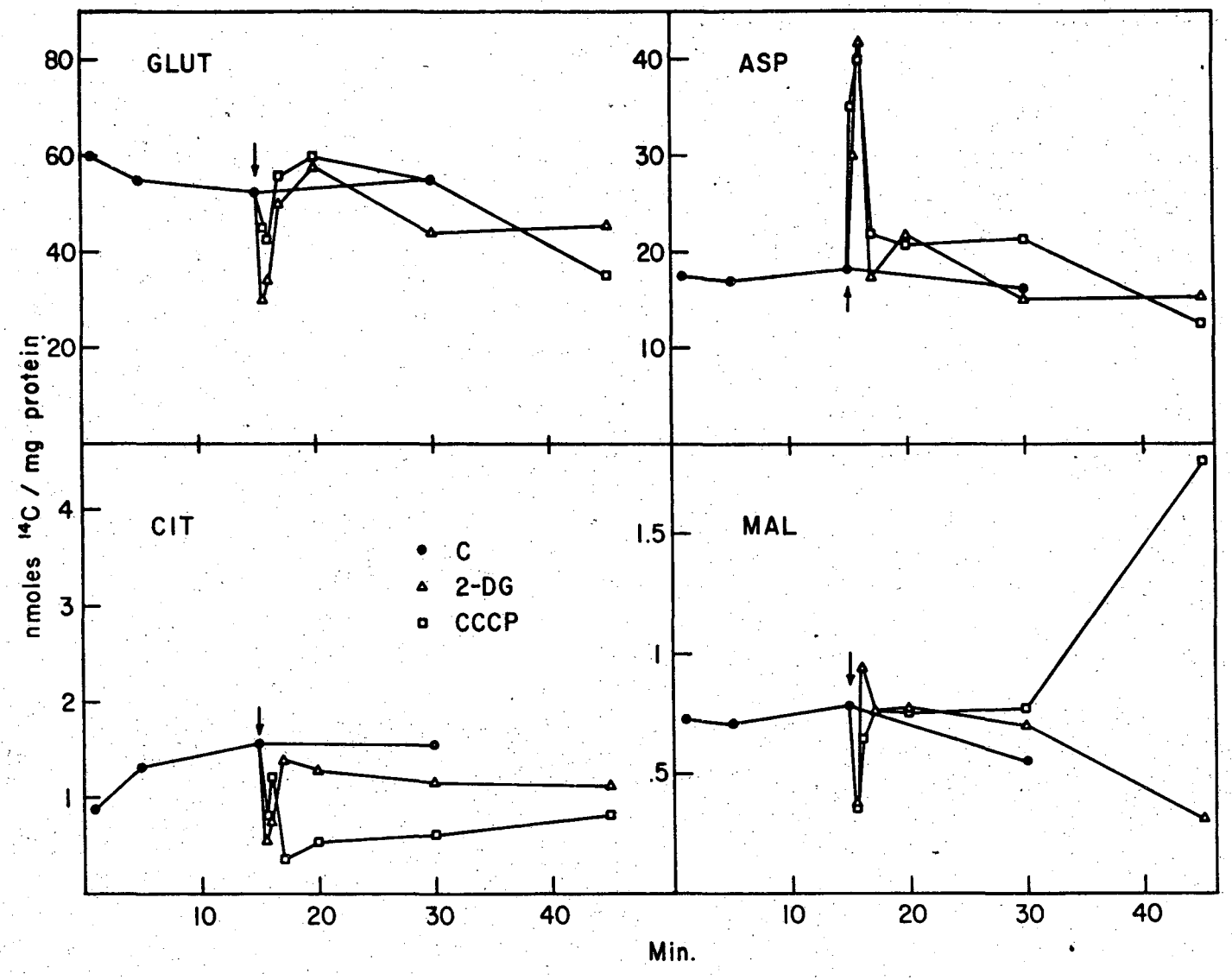


Fig. 5.

0 0 0 4 1 0 2 0 3 1

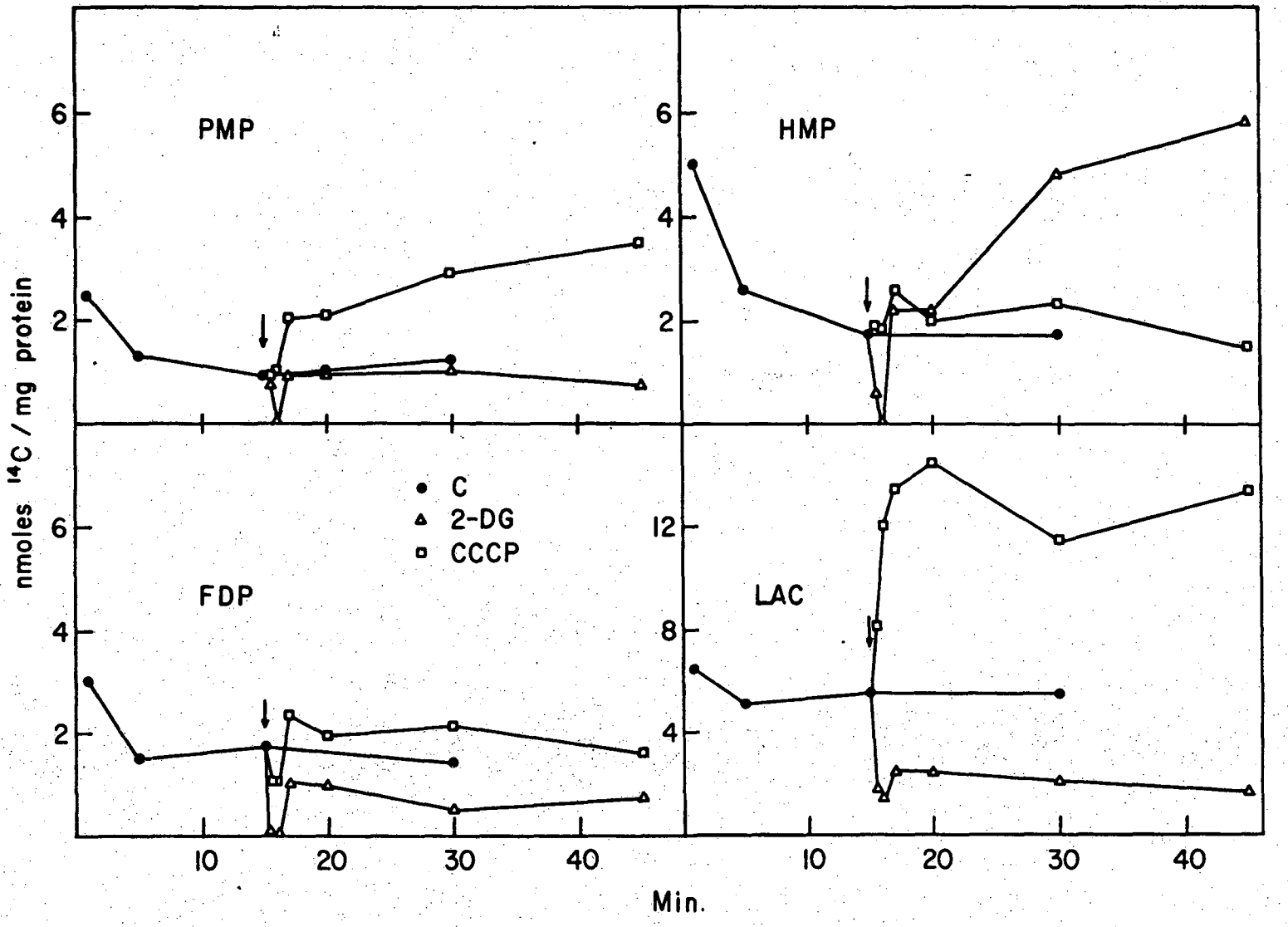

PMP
HMP
FDP
LAC
C
2-DG
CCCP
nmoles ^{14}C / mg protein
Min.
XBL741-5029

Fig. 6.

0 0 0 0 4 1 0 2 0 3 2

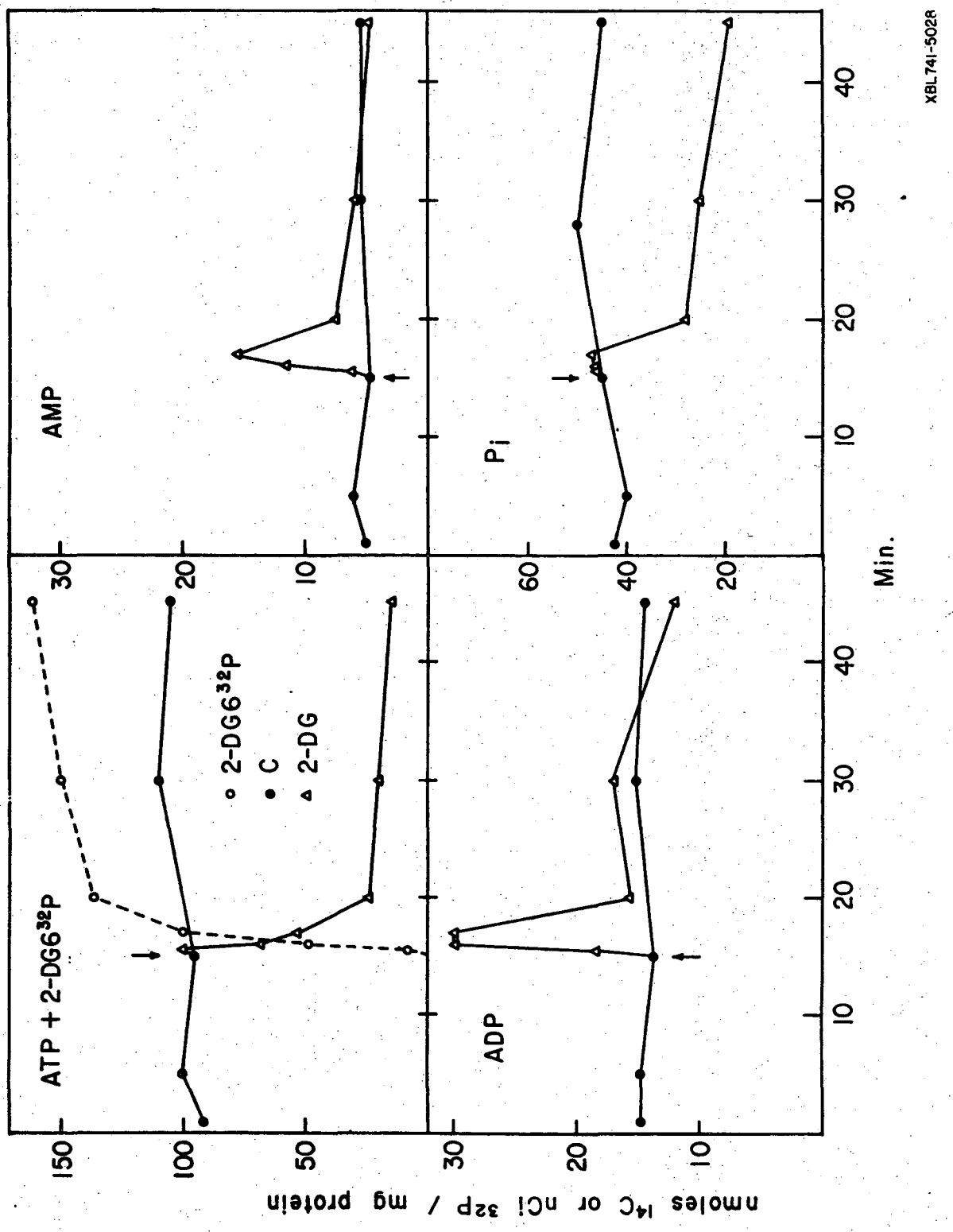


Fig. 7.

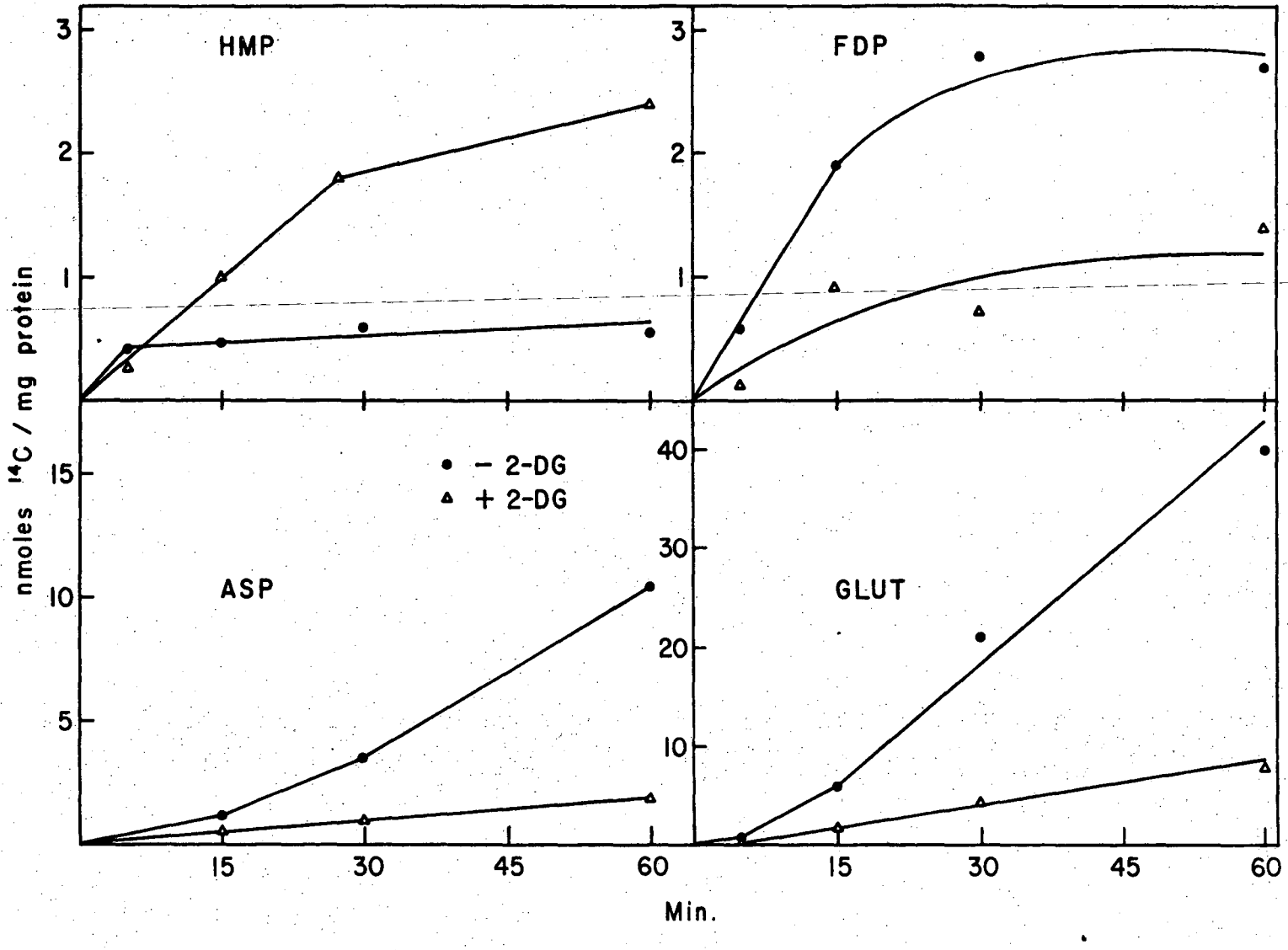

HMP
FDP
ASP
GLUT
• – 2-DG
▵ + 2-DG
nmoles ^{14}C / mg protein
Min.
XBL 741-5027

Fig. 8.

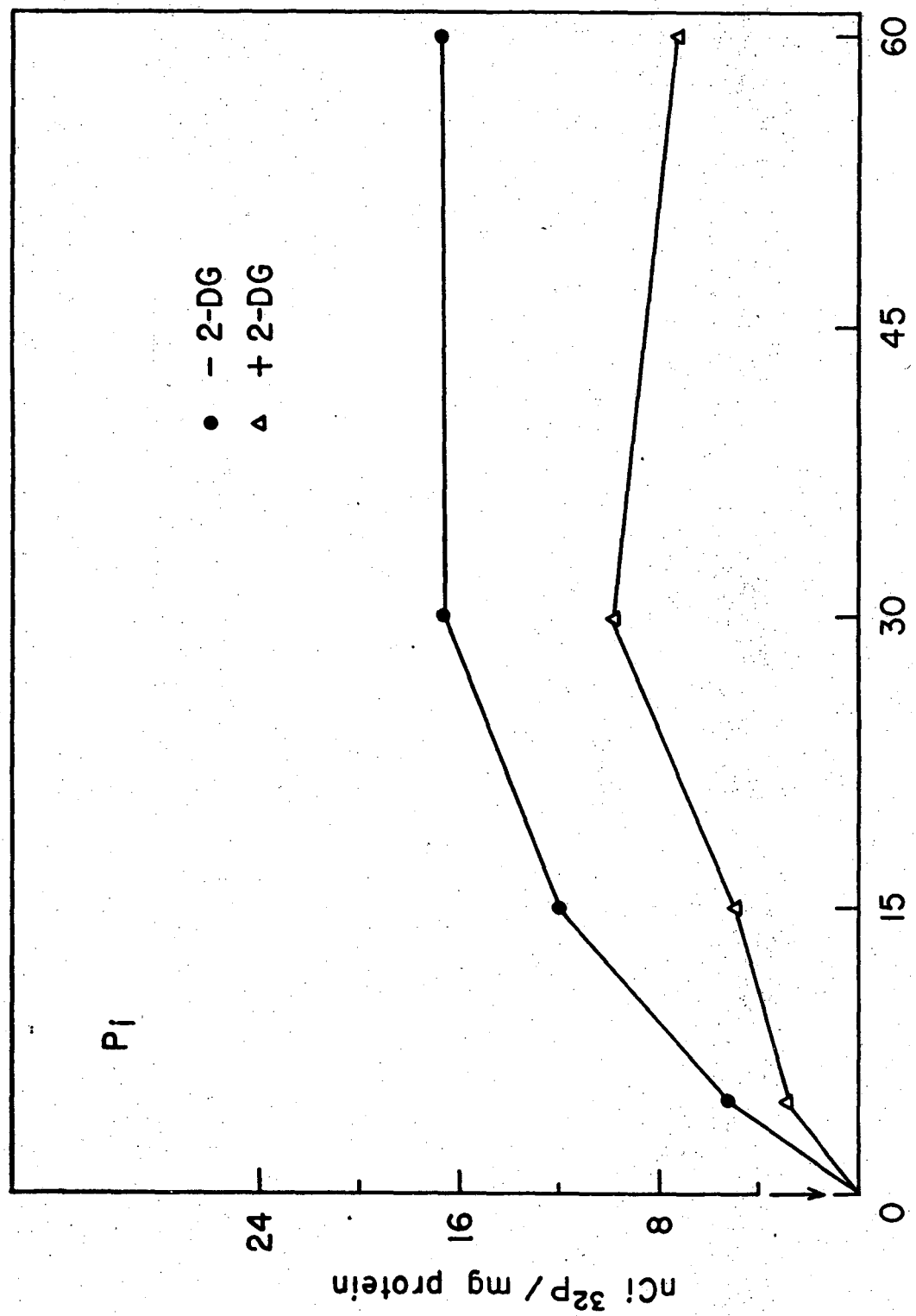

nCi 32P / mg protein
24
16
8
0
15
30
45
60
Min.
Pi
- 2-DG
+ 2-DG
XBL741-5021

Fig. 9.

0 0 0 0 4 1 0 2 0 3 3

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