THE EFFECT OF VIRUS INFECTION ON PYRUVATE METABOLISM*

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INTRODUCTION

The metabolic alterations which occur in a cell as a result of virus infection are at present poorly understood. With virulent T bacteriophages infecting cells of *E. coli* B, some systems involved in normal growth such as ribonucleic acid synthesis and adaptive enzyme formation are largely prevented[1,2,3**].

The effect of phage infection on enzyme activities of the host cells has also been investigated[3,4]. Reduced oxidative ability as a result of phage infection with the following substrates was observed: pyruvate, succinate, acetate, alanine, and glycine. In cell-free extracts[4] from infected and uninfected cells little difference in the activities of a number of enzymes could be detected.

We have observed[5] an accumulation of pyruvate when *Escherichia coli* cells are infected with phage T_7 or T_2 in the presence of *dl*-lactate or *l*-serine. This suggested that infected cells were inhibited in their ability to metabolize pyruvate, a compound which plays a key role in the metabolism of normal cells. The nature of this inhibition is the subject of the present investigation.

METHODS

The strains of *E. coli* used were obtained from A. D. HERSHEY and are designated "B" and "R-2". For the studies with coliphages T_2r^+ and T_2H (the latter obtained from A. D. HERSHEY), the medium used for the growth of the host cells was a glucose-synthetic medium modified from SZULMAJSTER *et al.*[6***]. For studies with the T_7 phage, the cells were grown in Difco nutrient broth or in a 1% tryptone plus 0.5% yeast extract medium. All growth experiments were conducted in shallow layers in Erlenmeyer flasks on a reciprocal shaker at 37°C. Phage titers were determined by the plaque technique[6a]. Concentrations of bacterial cells were assayed by plating for colonies[6a] and by turbidity measurements in a Klett-Summerson colorimeter with a 660 mμ filter.

The phage stocks of T_7 and T_2 were either in the form of crude lysates prepared in the glucose-synthetic medium or concentrates prepared by centrifugation at 20,000 × *g* for 45 minutes and resuspending the sediments in 0.001 *M* potassium phosphate buffer, pH 7.0 containing 0.5% NaCl.

Ultraviolet-irradiated T_7 phage was prepared by exposing lysates prepared in synthetic

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** JOKLIK[3] reported that the adaptive formation of formic hydrogenlyase was accelerated by phage infection. Studies in this laboratory (SPIZIZEN AND GEST, unpublished), however, have shown that with T_2 infection the adaptive synthesis of formic hydrogenlyase was inhibited as with with other adaptive enzymes. Stimulation of formic hydrogenlyase adaptation has occurred in some of our experiments when the culture was incompletely infected. The uninfected cells formed enzyme more rapidly due to the stimulating effect of materials liberated from infected cells.

*** Na_2HPO_4 4.54 g; KH_2PO_4 1.08 g; $(NH_4)_2SO_4$ 0.8 g; $MgSO_4 \cdot 7H_2O$ 0.08 g; $CaCl_2$ 0.002 g; $FeSO_4 \cdot 7H_2O$ 0.001 g. To 1 liter with H_2O (pH 7.4) glucose added (aseptically) to 0.5%.

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medium (centrifuging to remove debris) to a 15 watt GE germicidal ultraviolet lamp. Shallow layers not more than 2 mm in depth were irradiated for 1 minute from a distance of 30 inches. The titers of lysates containing $2 \cdot 10^{11}$ plaque-forming units per ml were thereby reduced to 10^8 viable particles per ml. Such preparations were capable of adsorbing as shown by the fact that 95% of an *E. coli* suspension in nutrient broth containing $3 \cdot 10^8$ cells per ml were killed in 10 minutes at 37°C by a 1:10 dilution of irradiated virus preparation.

Young, aerobically grown *E. coli* cells (3–4 hours) were employed for phage infection. With phage T_2H, concentrations of $2.0 \cdot 10^9$ virus particles per ml were mixed with cells (grown in the synthetic medium and diluted with fresh medium) at a density of $4 \cdot 10^8$ cells per ml (Klett colorimeter reading of 40 with a 660 mμ filter). For T_7 infection, cells grown for 3–4 hours in nutrient broth or tryptone-yeast extract medium were diluted with fresh medium to a density of $2 \cdot 10^8$ bacteria per ml and mixed with $1 \cdot 10^9$ phage per ml. The conditions stated were found to effect at least 99% infection of the cells in 10 minutes at 37°C determined by plating for "infective centers" or plaques after the addition of specific antiserum to inactivate "free" phage.

In most of the experiments, cells infected for 10 minutes were centrifuged at 2,500 × *g* for 3 minutes in a Servall centrifuge and then resuspended in 1/10 the original volume of test solutions. Test solutions contained 50 μmoles of the compound (K pyruvate, *l*-serine, *dl*-lactate, etc.) in synthetic medium (without glucose). Uninfected cell suspensions were similarly treated. One ml volumes were dispensed into tubes (13 mm inside diameter) and shaken in a water bath at 37°C (open tubes). For the preparation of cell-free extracts, 10 to 20 liter volumes of cells were grown with vigorous aeration for 3 to 4 hours at 37°C, then diluted appropriately for the type of viral infection (as described previously). The diluted cell suspensions were infected at 37°C for 10 minutes, then poured over chipped ice for rapid cooling and centrifuged in a refrigerated Sharples centrifuge. The harvested cells were disrupted by grinding the paste in a chilled mortar in the cold with Alumina A-301 (2.5 times the wet weight of cells). The ground mass was extracted with 1.5 ml of 0.01 *M* potassium phosphate buffer pH 7.0 for each gram of cell paste used, and the alumina removed by centrifugation in a refrigerated Servall centrifuge at 9,000 × *g* for 15 minutes. The supernatant was recentrifuged for 45 minutes at 21,500 × *g* to provide the cell-free extracts used in these studies. Tests carried out with extracts were performed as in the intact cell experiments, namely with a final volume of 1 ml in open tubes and shaken at 37° C. K pyruvate was used as 50 μmoles per ml in synthetic medium (without glucose).

Chemical

Pyruvate was determined by the "direct" method of FRIEDEMANN AND HAUGEN[7] in which total hydrazones are measured. This assay was frequently checked with the "extraction method" in which specific estimations of pyruvic and dicarboxylic keto acids are made.

Lactic acid was determined by the method of BARKER AND SUMMERSON[8].

Protein was determined by the method of LOWRY *et al.*[9].

EXPERIMENTAL RESULTS

1. *Accumulation of pyruvate by infected cells*

E. coli, R-2 cells grown aerobically in synthetic medium with 0.2% *dl*-lactate as carbon source were infected with T_2H as described under METHODS. When the infected cells were centrifuged and resuspended in 1/10 volume of synthetic medium containing 50 μmoles of sodium lactate, pyruvate was found to accumulate. A total of 6 μmoles of pyruvate was formed from D-lactate in 60 minutes, 2.2 μ*M* from *dl*-lactate, whereas none accumulated in a control suspension of uninfected cells. Even larger amounts of pyruvate accumulated when virus-infected cells were suspended in synthetic medium containing 50 μmoles of *l*-serine, T_7 infection resulting in the accumulation of more pyruvate than T_2H (Fig. 1). Pyruvate did not accumulate when the substrate was D-serine. The curves in Fig. 1 show the rates of accumulation of pyruvate from *l*-serine with $2 \cdot 10^9$ cells per ml. T_7 infection resulted in the formation of 33.5 μmoles of pyruvate after 60 minutes; with ultraviolet-inactivated T_7 (which does not produce lysis of the cells) 16 μmoles of pyruvate accumulated and with T_2H, 9 μmoles. No accumulation of pyruvate occurred with uninfected cells or with cells to which antiserum-inactivated phage was added. Similarly, pyruvate did not accumulate from

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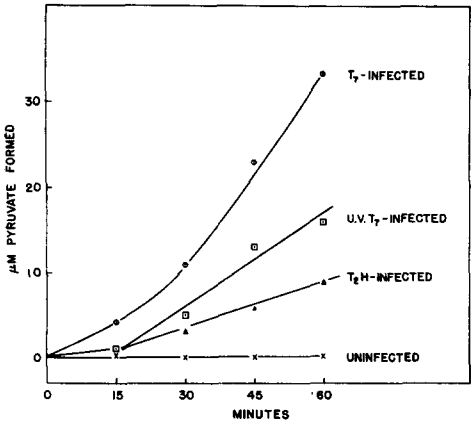

Fig. 1. Accumulation of pyruvate from *l*-serine (50 μmoles/ml) in *E. coli*, R-2. (Bacteria grown aerobically in nutrient broth, diluted to $2 \cdot 10^8$ cells per ml with broth and infected with 10^9 phage particles per ml for 10 minutes, 37°C. Suspensions then centrifuged and resuspended to $2 \cdot 10^9$ cells per ml in 50 μmoles/ml *l*-serine in synthetic medium. Uninfected cells treated similarly. Shaken in open tubes at 37°C).

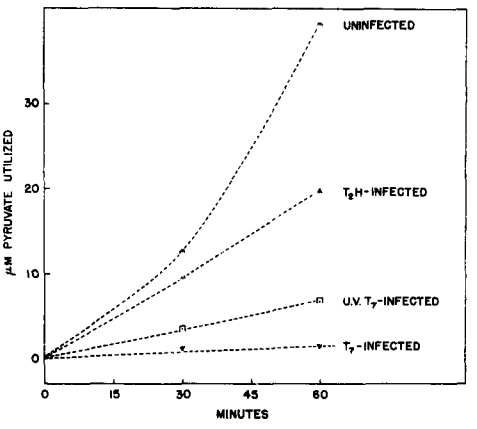

Fig. 2. Utilization of pyruvate by *E. coli*, R-2. (Bacteria grown aerobically in nutrient broth, diluted to $2 \cdot 10^8$ cells per ml with broth and infected with 10^9 phage particles per ml for 10 minutes, 37°C. Suspensions then centrifuged and resuspended to $2 \cdot 10^9$ cells per ml in 50 μmoles per ml of K pyruvate in synthetic medium. Uninfected cells treated similarly. Shaken in open tubes at 37°C).

lactate or *l*-serine when the cells were infected in the presence of low or high salt concentrations which inhibit adsorption of T_2 to cells, or in the absence of necessary cofactors for T_7 adsorption*.

2. *Rate of pyruvate utilization by infected cells*

When *E. coli* cultures were infected with T_7 for 10 minutes at 37°C, and suspended in synthetic medium containing K pyruvate, they were found to be incapable of utilizing pyruvate either when placed under aerobic conditions (open tubes, shaken at 37°C) or when placed in Warburg manometer cups under nitrogen at 37°C. T_2H-infected suspensions, however, were found to utilize pyruvate but at a reduced rate, as compared with uninfected cells. These results are illustrated in Fig. 2. Thus, whereas uninfected cells utilized 37 μmoles of pyruvate in 60 minutes and T_7-infected cells less than 2.5 μmoles, T_2H-infected cells used 17.3 μmoles in the same period. This reduced rate of pyruvate utilization with T_2 infection could be demonstrated using different cell concentrations. This is seen in Table I, in which the specific activities (*i.e.*, μmoles pyruvate utilized in 60 minutes per 10^8 cells) of infected and uninfected cells are compared for a range of cell concentrations. Between 5 and $40 \cdot 10^8$ cells ml the specific activity of uninfected cells was 1.3 and higher, but that of infected cells was 0.5 to 0.7.

Table II shows data which indicates that T_2H-infected cells when subsequently infected with T_7 still continue to utilize pyruvate at the rate characteristic for T_2H infection. The reverse was also observed, namely, the complete inhibition of pyruvate utilization caused by T_7 was still maintained even when T_2H was subsequently added. Although multiplication of the second phage was "excluded" by the presence of the

* Studies to be reported elsewhere indicate that factors present in certain casein digests are required for irreversible adsorption.

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TABLE I

PYRUVATE UTILIZATION BY *E. coli* R-2 CELLS

E. coli, R-2 cultures grown in glucose-synthetic medium in aerated flasks at 37° C for 4 hours. Infected at $4 \cdot 10^8$ bacteria per ml with $20 \cdot 10^8$ T_2H per ml, 10 minutes, 37° C. Chilled, centrifuged and resuspended in synthetic medium containing 50 μmoles/ml K pyruvate at cell concentrations indicated (one ml quantities in open tubes, shaken, 37° C).

Cell concn. $\times 10^8$/*ml*	*Specific activity**	
	Uninfected	*T_2H-infected*
40	—	0.53
30	—	0.65
20	>1.3	0.70
10	1.75	0.58
5	1.32	0.72
2.5	1.08	0

* μmoles pyruvate utilized in 60 min per 10^8 cells.

TABLE II

INTERFERENCE OF T_2H AND T_7 IN PYRUVATE BLOCK

*Cells** ($2 \cdot 10^8$/*ml*)	*Pyruvate utilization*	
	μmoles/ml 60 min	*Specific activity***
1 Uninfected	25.0	1.25
2 T_2H-infected "only"	13.3	0.67
3 T_7-infected "only"	1.8	0.09
4 T_2H-infected + T_7-infected	9.1	0.46
5 T_7-infected + T_2H-infected	0	0

* *E. coli* cells grown in nutrient broth and diluted to $2 \cdot 10^8$/ml with broth. No. 2 and No. 3 cells at $2 \cdot 10^8$/ml in contact with phage (using phage:cell ratio of 5:1) for 10 min at 37° C. No. 4 and No. 5, 10 min with first phage followed by 10 min at 37° C with second phage. After phage contact periods, cells were centrifuged and resuspended in 1/10 volume of 50 μmoles/ml K pyruvate in synthetic medium.

** μmoles pyruvate utilized in 60 min per 10^8 cells.

first phage used for infection (DELBRUCK AND LURIA[10]), adsorption of both phage types still occurred on the cells under these conditions. Thus the effect on pyruvate utilization was characteristic of the phage undergoing replication.

A large number of compounds (cofactors, etc.) were tested for their ability to prevent the inhibitory effects of T_7 and T_2 on pyruvate utilization. Relief of the inhibition was not achieved in tests with the following compounds: thiamine, thiamine pyrophosphate, diphosphopyridine nucleotide, coenzyme A, flavin adenine dinucleotide, casein hydrolysate, yeast extract, ribose and desoxyribonucleic acids, nucleotides and nucleosides, fumarate, succinate, malate, formate, etc. In these tests the supplements were present both during the adsorption period and/or after infection.

3. *Leakage of cell constituents from infected cells*

PUCK AND LEE[11,12] have shown that materials escape from the host cells as a direct result of virus T_2 penetration. We have found that the supernatant obtained from T_2H-infected cells within the first 5 to 10 minutes required for adsorption (with multiplicity of 5 phage particles to 1 cell) contains substances which stimulate pyruvate

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TABLE III

STIMULATION OF PYRUVATE UTILIZATION IN *E. coli* EXTRACT BY SUPERNATE OF T_2H-INFECTED CELLS

Extracts in a final volume of 1 ml containing 50 μmoles K pyruvate

Extract (2.1 mg protein)	*μmoles pyruvate utilized 60 min*	*Specific activity*
No addition	9.2	4.4
+ Supernate* (0.3 ml)	12.6	6.0
+ DPN (50 γ), CoA (50 γ)	18.3	8.7

* Cells at $4 \cdot 10^8$/ml in synthetic medium + $20 \cdot 10^8$ T_2H/ml for 10 min at 37°C, centrifuged, and supernate tested.

TABLE IV

PYRUVATE UTILIZATION BY EXTRACT OF *E. coli* R-2, T_2H-INFECTED

(Alumina ground, dialysed 4 hours, 4°C, against 100 volumes of water). Extracts in a final volume of 1 ml containing 50 μmoles K pyruvate.

Extract (4.1 mg protein)	*μmoles pyruvate utilized 60 min*	*Specific activity*
No addition	0	0
+ DPN (50 γ), CoA (50 γ)	4.4	1.07
+ TPP (1 mg)	5.8	1.41
+ DPN (50 γ), CoA (50 γ), TPP (100 γ)	11.7	2.85
+ TPP (1 mg) + heated supernate* (0.3 ml)	10.6	2.59

* Cells at $4 \cdot 10^8$/ml in synthetic medium + $20 \cdot 10^8$ T_2H/ml, for 10 min at 37°C, centrifuged. Supernatant was then heated for 5 min at 100°C.

utilization by diluted cell-free extracts of *E. coli* R-2 (Table III). Supernates from infected cells incubated for longer periods of time, from cells rapidly lysed by high phage multiplicities ("lysis from without") and from uninfected cells were inactive in this respect. We have found that stimulation of pyruvate utilization by diluted extracts could be obtained by the addition of DPN* and CoA*. Thus it appeared that these cofactors might be liberated from infected cells during viral adsorption.

To test this point further an extract of T_2H-infected *E. coli* was prepared by alumina grinding and then this extract was dialyzed at 4°C against 100 volumes of water for 4 hours. This type of extract required in addition to DPN and CoA, cocarboxylase (TPP**) for maximal activity on pyruvate. It was found that the addition of a supernatant from infected cells and TPP produced rapid pyruvate utilization (Table IV). The activity in the supernate was stable to heating for 5 minutes at 100°C and in fact, unless the supernate were heated, its ability to activate rapidly disppeared, presumably because of enzymic destruction of the stimulating cofactors. Cells lysed "from without" by high multiplicities of phage gave little or no stimulation of pyruvate utilization with extracts, presumably because of the rapid destruction of such factors by enzymes also liberated.

* DPN = diphosphopyridine nucleotide
CoA = coenzyme A
** TPP = thiamine pyrophosphate (cocarboxylase)

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TABLE V

PYRUVATE UTILIZATION BY ALUMINA GROUND EXTRACTS OF *E. coli* R-2 (SYNTHETIC MEDIUM GROWN)

Extracts in a final volume of 1 ml containing 50 μmoles K pyruvate

Extract (mg protein)	*Pyruvate utilization per hour (μmoles)*		*Specific activity*	
	Unfortified	*+ DPN (50γ),CoA (50γ)*	*Unfortified*	*+ DPN, CoA*
4.2	> 25.0	> 25.0	> 6	> 7
3.15	22.0	> 25.0	7.05	> 7.9
2.1	9.2	18.3	4.4	8.7
1.05	0	5.8	0	5.52

TABLE VI

PYRUVATE UTILIZATION BY ALUMINA GROUND EXTRACTS OF *E. coli* R-2 (GROWN IN SYNTHETIC MEDIUM)

Extracts in a final volume of 1 ml containing 50 μmoles K pyruvate

Extract type	*Extract (mg protein)*	*Specific activity*	
		Unfortified	*+ DPN (50γ) + CoA (50γ)*
Uninfected	1.25	0	3.13
Uninfected	2.5	2.12	7.75
Uninfected	3.75	4.75	6.7
Uninfected	5.0	4.82	—
Infected (T_2H)	1.2	0	1.60
Infected (T_2H)	2.4	0	2.00
Infected (T_2H)	3.6	1.25	2.88
Infected (T_2H)	4.8	2.23	3.04
Infected (T_2H)	6.0	2.86	3.24

4. *Pyruvate utilization by extracts from uninfected cells*

Table V summarizes data from a typical experiment in which the ability to utilize pyruvate by various dilutions of extracts from uninfected *E. coli* R-2 cells grown in synthetic medium was studied. It can be seen that with extracts containing 3.15 mg protein per ml and higher, the specific activities of the unfortified preparations were 7 or more. With 2.1 mg of protein in the extract, the specific activity dropped to 4.4; with 1 mg of protein the activity was entirely absent. This dilution effect could be partly overcome by the addition of DPN and CoA, giving specific activities of 8.7 or more even with 2.1 mg of protein in the extract. With a concentration of 1 mg of protein and a specific activity of 0, the addition of DPN and CoA produced an activity of 5.52. TPP had little or no effect on these extracts. In contrast with these results, alumina extracts of *E.* coli R-2 cells grown in nutrient broth medium could not be stimulated by the addition of DPN and CoA (see Table VII).

5. *Activities of extracts from T_2H-infected cells*

Extracts were prepared from T_2H-infected *E. coli*, R-2 cells which were grown in glucose-synthetic medium and also from uninfected cells grown under similar conditions and their activities on pyruvate compared (Table VI). Although DPN and CoA stimulated pyruvate utilization in both kinds of extracts, the specific activity attained was lower in the infected system over a wide range of protein concentrations. These results and the supernatant experiments of section 3 suggest that other components

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TABLE VII

PYRUVATE UTILIZATION BY EXTRACTS OF BROTH-GROWN *E. coli* R-2

Extracts in a final volume of 1 ml containing 50 μmoles K pyruvate

Extract type	*Extract (mg protein)*	*Specific activity*		
		Unfortified	*+ DPN, CoA*	*+ DPN, CoA, TPP*
Uninfected	4.0	6.06	—	—
Uninfected	3.0	5.7	4.9	—
Uninfected	2.0	2.79	3.0	3.2
Uninfected	1.0	0	0	1.1
T_7-infected	6.0	0.72	2.1	3.1
T_7-infected	4.0	0.38	2.3	3.3

TABLE VIII

EFFECT OF COCARBOXYLASE ON INFECTED AND UNINFECTED EXTRACTS

(Extracts incubated 30 min 37°C before addition of 1 mg/ml TPP and pyruvate)

Extracts in a final volume of 1 ml containing 50 μmoles K pyruvate

Extract type	*Extract (mg protein)*	*Specific activity*		
		no TPP	*+ TPP*	*TPP present during preincubation*
Uninfected	6.2	3.4	3.5	—
Uninfected	4.7	2.95	3.6	3.85
Infected (T_2H)	5.4	1.20	2.2	3.84
Infected (T_2H)	4.1	1.25	2.42	3.4

in addition to DPN and CoA are missing from infected cells. Extracts from T_7-infected cells grown in broth showed even lower specific activities (Table VII) as compared with those from uninfected cells. Such extracts were stimulated by the addition of DPN, CoA and TPP unlike those from uninfected cells but not to levels exhibited by the uninfected extract.

6. The effect of cocarboxylase on extracts from T_2H-infected cells

It has been observed that although crude extracts from synthetic medium-grown uninfected cells were little affected by added TPP, comparable extracts from infected cells were markedly stimulated in their ability to utilize pyruvate. These differences in response to TPP could often be accentuated by a preincubation period in the absence of added TPP (Table VIII). In these experiments, preincubation of the extracts for 30 minutes before the addition of pyruvate reduced the specific activities of extracts from infected cells to 1.2 in the absence of added TPP, whereas extracts from normal cells retained their activity at levels of 2.95 or higher. Addition of cocarboxylase to the inactivated extracts from infected cells partially restored their acitivity. Also, the inactivation of such extracts could be prevented by the presence of added TPP (1 mg/ml). This suggests the presence in extracts from infected cells of some factor which inactivates TPP. This possibility is being further investigated.

7. Manometric study of pyruvate utilization

By the use of conventional Warburg manometric techniques, the oxygen consumption and CO_2 production resulting from aerobic pyruvate metabolism by T_2H-infected and non-infected cells and extracts were studied (Table IX). The data show that somewhat more than one-half mole of O_2 was taken up and 1.0 mole of CO_2 was produced for each

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TABLE IX

PYRUVATE OXIDATION BY *E. coli* R-2

Warburg manometric studies, aerobic. Reaction terminated at 60 minutes with sulfuric acid

*System**	*Specific activity***	*μmoles O_2 uptake 60 min*	*μmoles CO_2 production 60 min*	O_2/CO_2	*O_2/pyruvate utilized*
Uninfected cells ($4 \cdot 10^9$)	0.73	18.0	31.9	0.57	0.62
T_2H-infected cells ($4 \cdot 10^9$)	0.37	11.0	18.7	0.59	0.75
Uninfected extract (2.5 mg)	7.02	3.98	10.8	0.37	0.23
Uninfected extract (2.5 mg) + DPN, CoA (50 γ each)	8.05	5.92	13.7	0.43	0.30
T_2H-infected extract (3.6 mg)	4.17	0.8	4.4	0.18	0.054
T_2H-infected extract (3.6 mg) + DPN, CoA (50 γ each)	7.4	2.5	7.1	0.35	0.094

* Total volume per cup, 2 ml; consisting of synthetic medium, 50 μmoles K pyruvate and cells or extract as indicated.

** For cells: μmoles pyruvate utilized per 10^8 cells in 60 min. For extracts: μmoles pyruvate utilized per mg protein in 60 min.

mole of pyruvate used by whole cells, whether infected or not. Lactate determined colorimetrically[8] was not formed. Thus, a direct oxidation of pyruvate occurred, and although infection decreased the rate of oxidation, the ratios of O_2 to CO_2 were essentially similar in infected and non-infected systems.

With extracts, the O_2/CO_2 and O_2/pyruvate ratios were considerably lower than observed with whole cells. Addition of DPN and CoA increased the O_2 consumption in extracts from both infected and uninfected cells. Little or no lactate was found to accumulate in the extracts even when DPN and CoA were added. Since the ratio of O_2 consumption/pyruvate utilization was considerably lower in extracts from infected cells as compared with extracts from normal cells it seems possible that there may be qualitative differences in the pathways of pyruvate metabolism in these two types of extracts.

DISCUSSION

These studies show that as a results of phage infection a marked alteration occurs in the ability of the host cells to metabolize pyruvate. T_7 virus almost completely inhibits pyruvate utilization, while infection with T_2 reduces the rate of pyruvate oxidation appreciably. These effects occur under conditions where new phage synthesis takes place. It is known (EVANS[13]) that T_7 is synthesized mainly from pre-existing cell components present before infection, whereas exogenous sources are utilized for synthesis of T_2. It seems possible that the differences observed between the two virus systems with respect to the effect on pyruvate metabolism may be a reflection of the energy and carbon requirements for synthesis in these two instances.

Once one type of phage infection has been established, subsequent adsorption of another phage type does not influence the characteristic modification of pyruvate metabolism. This would indicate that the effect of virus infection on pyruvate oxidation involves intracellular metabolic mechanisms.

Since pyruvate accumulates when infected cells are placed in contact with *l*-serine or lactate it appears that altered permeability is not the limiting factor causing the change in rate of pyruvate utilization observed. This is supported by the finding that the specific activities of extracts from infected cells were lower than those from normal cells.

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Previous work[11,12] indicating that viral penetration into cells creates temporary openings through which cell contents escape is confirmed and extended by the present studies. We have shown that heat stable factors required for pyruvate metabolism are released by cells during the first 10 minutes of infection with T_2. The present experiments indicate that these factors include DPN and CoA, but no direct assays have been made to determine the quantities of these coenzymes released. The loss of soluble cellular contents is probably also reflected in the lowered specific activities of extracts of infected cells as compared with those from normal cells.

It has also been shown in these experiments that the pyruvate metabolism of undialysed extracts from T_2-infected cells can be greatly stimulated by cocarboxylase, particularly after a short incubation in the absence of added TPP. In contrast, extracts from uninfected cells do not show this sensitive response to TPP. This suggests that the extract from infected cells contains systems inactivating TPP, which might further contribute to the lowered ability of the infected systems to utilize pyruvate. Experiments designed to investigate the nature of the inactivation of TPP are now in progress.

The loss of cofactors and other materials from cells as a result of phage infection must undoubtedly have effects on other metabolic systems. Other enzyme reactions requiring diffusible cofactors at critical concentrations might well be suppressed in this way.

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SUMMARY

E. coli cells infected with T_2 or T_7 virus accumulate pyruvate during the metabolism of *l*-serine or lactate. This was shown to be due to a markedly reduced ability of virus-infected cells to metabolize pyruvate as compared with uninfected cells. When cells are first infected with T_2 and then with T_7, pyruvate continues to be utilized at the reduced rate characteristic of T_2 infection, indicating that the effect of virus is on intracellular control mechanisms for pyruvate metabolism.

During viral adsorption and penetration, materials "leak" from the infected cells which have the ability to stimulate pyruvate metabolism in diluted cell-free extracts. In addition, cocarboxylase appears to be more labile in extracts from T_2-infected cells as compared with extracts from normal cells. These facts may account for the reduced rate of oxidation of pyruvate in phage-infected cells. The present results are discussed in relation to other known metabolic effects of virus infection.

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