Intermediary Metabolite Levels in *Escherichia coli*

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SUMMARY

The intra- and extracellular concentrations of a number of phosphorylated intermediary metabolites in *Escherichia coli* were measured radiochemically during growth in low phosphate (0.75 mM) media containing [^{32}P]P₁. Distribution studies showed that most of the monophosphate metabolites were present in the supernatant medium outside the bacteria. This was much less marked for the di- and triphosphates, and was not due to bacterium lysis. Adenosine 3',5'-cyclic monophosphate was present solely in the medium under all conditions tested. 3',5'-Cyclic-AMP added to the medium was only partly effective in reversing repression of β -galactosidase synthesis by gluconate. This was not due to degradation of the nucleotide.

In another series of strains, chemical determinations were made of the intra- and extracellular concentrations of fructose 1,6-diphosphate and triose phosphate under varying conditions of growth in high phosphate (0.1 M) media; while most of the fructose 1,6-diphosphate remained inside the bacteria, triose phosphate was present largely in the medium. In all of these studies there was no correlation between the severity of catabolite repression and the concentrations of metabolites either inside the bacteria, or in the medium.

INTRODUCTION

Studies of the soluble-protein profiles of *Escherichia coli* have shown marked differences in phenotype as a function of growth in different media (Moses & Wild, 1969; Moses & Sharp, 1970). The relationship of such phenotypic differences to the cellular levels of intermediary metabolites appeared to be of interest, and preliminary experiments, using short-term labelling of bacteria with [^{32}P]P₁, have already been presented; these included the effects of adding adenosine 3',5'-cyclic monophosphate to the medium (Moses & Sharp, 1970).

We have extended these measurements of metabolite levels to bacteria grown in minimal medium supplemented with one of five carbohydrates. In each case, we have determined the intra- and extracellular concentrations of a number of substances, using, for technical reasons, long-term labelling of the bacteria with ^{32}P , and thereby restricting our observations to phosphorylated compounds. Makman & Sutherland (1965) reported that the intracellular concentration of 3',5'-cyclic-AMP varies under different physiological conditions, and that the presence of glucose in the medium prompts the export of the nucleotide from the cells. One of the objects of the present study was to clarify the significance of this export phenomenon by determining the distribution between the bacteria and the medium of a wider range of intermediates, and with a variety of growth substrates.

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METHODS

Bacterial strains. *Escherichia coli* strains 3000, LA12G and B were from Dr E. Steers, Dr W. F. Loomis, Jun., and Dr M. R. Lunt, respectively. Strain VP19 has previously been described (Moses & Sharp, 1970).

Growth conditions and media. The standard minimal medium was M63 (Pardee & Prestidge, 1961) supplemented with thiamine (1 mg/l), and containing the appropriate carbon source at 0.8 mg carbon/ml. For experiments using [^{32}P]P_i, a low-phosphate (0.75 mM-P_i) minimal medium (LP medium) was employed (Prevost & Moses, 1967) containing carbon sources as desired. Bacteria in liquid culture were grown as described by Moses & Prevost (1966).

Induction and assay of β -galactosidase. These were performed by previously established methods (Palmer & Moses, 1968). One enzyme unit is defined as that quantity giving rise to 1 nmol of product/min at 37 °C.

Intermediary metabolite concentrations. (a) *Sugar phosphates and nucleotides.* Bacteria were grown through several subcultures in LP medium containing the desired carbon source. [^{32}P]bacteria from an overnight culture were diluted into fresh LP medium containing [^{32}P]P_i (0.625 mCi/ μ mol) to give a concentration of about 11 μ g bacterial protein/ml. Samples (2 ml) were vigorously shaken at 37 °C in air in stoppered tubes (15 $\times$ 125 mm), held at an angle to promote maximum agitation. Growth was followed at 650 nm with a Spectronic 20 spectrophotometer (Bausch & Lomb, Rochester, New York, U.S.A.) masked to permit reading 2 ml volume of liquid in the tubes. When the bacterium density reached 115 μ g bacterial protein/ml, 1 ml samples of the suspension were mixed with 4 ml ethanol. In some experiments, samples of the bacterium-free supernatant were also obtained by centrifugation of 0.4 ml of the suspension at room temperature for 5 min at 15,000 g (max) in a Microfuge Model 152A (Beckman Instruments, Inc., Spinco Division, Palo Alto, California, U.S.A.). This instrument reaches full speed, and comes to rest, within 5 s of being switched on or off. Measured portions of the ethanolic bacterium suspension, and of the supernatant medium, were chromatographed *in toto* on sheets of Ederol no. 202 paper (J. C. Binzer, G.M.B.H., Hatzfeld/Eder, Germany), with the solvent system described by Crowley, Moses & Ullrich (1963). Chromatograms were over-run to permit maximum separation of phosphorylated compounds. The labelled components were located on the chromatograms by autoradiography. The spots were excised and the radioactivity measured automatically at known counting efficiency (Moses & Lonberg-Holm, 1963), or by immersion of the pieces of paper in toluene-based scintillation fluid, followed by counting in a Tri-Carb Liquid Scintillation Spectrometer Model 3375 (Packard Instrument Co., Inc., Downers Grove, Illinois, U.S.A.). Corrections were made for efficiency and for the decay of ^{32}P . The concentrations of phosphorylated compounds were then calculated in terms of μ mol P/g bacterial protein. For spot identifications, authentic marker compounds were chromatographed on parallel chromatograms with the same labelled bacterium extract.

(b) *Fructose 1,6-diphosphate and triose phosphate.* Bacteria in M63 medium containing the desired carbon source were grown exponentially to a measured density of 150 to 200 μ g bacterial protein/ml. Samples (6 ml) of the suspension were mixed with 0.66 ml of 50% (w/v) trichloroacetic acid. Simultaneously, portions of the culture were filtered through a pad consisting (top to bottom) of one Whatman glass-fibre disc GF/B, one Whatman glass-fibre disc GF/C, and one Millipore filter disc (0.45 μ m pore size). The Millipore filter ensured removal of the bacteria, but rapidly clogged unless the glass-fibre discs were present as prefilters. Portions of the filtrate (6 ml) were mixed with 0.66 ml of 50% (w/v) trichloroacetic acid. After 1 h at room temperature (and after centrifugation for the whole-suspension

Table 1. *Growth and enzyme synthesis in strain VP 19*

The bacteria were induced with 0.5 mM-IPTG during growth in the media listed. β -Galactosidase as units/mg protein; growth rates as doublings/h

Carbon source	M63 medium		Low P ₁ medium	
	β -Galactosidase	Growth rate	β -Galactosidase	Growth rate
Acetate	11.8	0.59	12.8	0.35
N-Acetylglucosamine	6.7	0.92	—	—
Fructose	10.8	0.73	—	—
Galactose	5.1	0.85	—	—
Gluconate	6.2	0.74	3.4	1.05
Glucose	3.9	0.94	3.6	1.05
Glucose-6-phosphate	1.6	1.09	—	—
Glucose + tryptone	1.0	1.62	—	—
Glycerol	11.9	0.67	8.5	0.86
Lactate	11.8	0.59	—	—
Lactose	5.3	0.81	—	—
Maltose	7.1	0.81	—	—
Ribose	12.1	0.58	10.2	0.71
Succinate	10.4	0.72	—	—

samples), the clear solutions were extracted five times with ether to remove trichloroacetic acid, and residual ether was removed with a stream of air. The concentrations of fructose 1,6-diphosphate and triose phosphate in the solutions were measured by the method of Bücher & Hohorst (1965). The concentration of the metabolites within the bacteria was calculated from the difference in concentration between the whole suspension and the filtered medium.

Chemicals and radiochemicals. Carrier-free [³²P]P₁ was obtained from International Chemical and Nuclear Corporation, Irvine, California U.S.A. [8-¹⁴C]Adenosine-3',5'-cyclic monophosphate was purchased from New England Nuclear Corporation, Boston, Massachusetts, U.S.A.; 3',5'-cyclic-AMP from Calbiochem, Los Angeles, California, U.S.A.; dihydroxyacetone phosphate, glyceraldehyde 3-phosphate and NADP⁺ from Sigma Chemical Co., St Louis, Missouri, U.S.A.; and isopropyl-thio- β -D-galactoside (IPTG) from Cyclo Chemical Corporation, Los Angeles, California, U.S.A.

Enzymes for the assay of fructose 1,6-diphosphate and triose phosphate, rabbit muscle ketose 1-phosphate aldehyde lyase (EC. 4.1.2.7), yeast D-glyceraldehyde 3-phosphate: NAD oxidoreductase (EC. 1.2.1.12), and rabbit muscle D-glyceraldehyde 3-phosphate ketol-isomerase (EC. 5.3.1.1), were purchased from Boehringer Mannheim Corporation, San Francisco, California, U.S.A.

RESULTS

Intermediary metabolite concentrations. The rates of growth, and of induced β -galactosidase synthesis, in strain VP19 growing in a variety of media are shown in Table 1.

The intra- and extracellular concentrations of a number of phosphorylated intermediates in this strain, growing exponentially in LP medium on each of five carbon sources, are given in Table 2. The experimental data were obtained as counts of ³²P/min/ml of cell suspension of known *E*₆₅₀. The specific radioactivity of the ³²P was known. The intra- and extracellular concentrations were then calculated by means of the following assumptions: (i) at *E*₆₅₀ = 1.0, 1 ml of cell suspension contains 225 μ g bacterial protein (Moses & Prevost, 1966); (ii) protein constitutes 56% (w/v) of the bacterial dry wt (Roberts *et al.* 1955); (iii) the density

Table 2. *Intra- and extracellular concentrations of phosphorylated intermediates in strain VP19 grown in LP medium containing [³²P]P_i*

The analytical procedures are described in Methods. Separate determinations were made of the total and extracellular concentrations, from which the intracellular concentrations were calculated on the basis of the assumptions stated in the text. Metabolite concentrations are expressed in terms of P.

Carbon source ... Cell concentration at time of sampling (μ g protein/ml) No. of doublings in final incubation before sampling ...	Acetate 46	Glucosate 95	Glucose 53	Glycerol 59	Ribose 56
	3.2	3.9	3.7	3.7	3.6
	Metabolite in external medium (%)	Metabolite in external medium (%)	Metabolite in external medium (%)	Metabolite in external medium (%)	Metabolite in external medium (%)
	Extracellular concen- tration (μ M)	Extracellular concen- tration (μ M)	Extracellular concen- tration (μ M)	Extracellular concen- tration (μ M)	Extracellular concen- tration (μ M)
	Intracellular concen- tration (μ M)	Intracellular concen- tration (μ M)	Intracellular concen- tration (μ M)	Intracellular concen- tration (μ M)	Intracellular concen- tration (μ M)
ADP*	1000	1300	1200	1300	1300
ATP*	2400	3600	3600	3100	3100
CMP*	260	900	840	1100	640
3',5'-cyclic-AMP*	260	69	0	0	0
Dihydroxyacetone phosphate*	460	460	370	380	400
Disaccharide phosphate†	980	1300	900	1400	50
Fructose 6-phosphate*	16	1300	1100	800	770
Fructose 1,6-diphosphate*	3600	12000	8800	8000	8900
+UTP*†					
Glucose 6-phosphate*	2600	1200	1100	2300	3200
6-Phosphogluconic acid*	0	700	240	0	57
3-Phosphoglyceric acid*	320	1100	750	1100	970
Phosphoglycollic acid*	0	110	160	120	190
Ribose 5-phosphate*	46	280	110	87	90
Uridine diphosphoglucose*	630	1000	810	920	720
Unidentified 1	600	700	620	700	700
Unidentified 2	610	560	610	690	500

* Identity checked by co-chromatography with authentic marker in this work. † Identity inferred from other chromatographic studies.

‡ Separation usually incomplete.

Table 3. *Sedimentation of strain VP19*

Exponentially growing cultures in LP medium, or M63, plus gluconate, were sedimented by centrifugation for 5 min at 37 °C in the Microfuge. Samples of the total suspension, and of the supernatant medium, were used for measurement of visible bacteria, and for total protein by the method of Lowry, Rosebrough, Farr & Randall (1951). Total protein could not be measured in this way in M63; there appeared to be interference by the high concentration of K⁺.

	VP19
Percentage of bacteria sedimented	
(a) from LP medium + gluconate	98.5
(b) from M63 + gluconate	98.1
Percentage of total protein sedimented	
(a) from LP medium + gluconate	97.5

Table 4. *Intra- and extracellular concentrations of fructose 1,6-diphosphate and triose phosphate in strain 3000*

For bacteria during exponential growth in the media indicated, separate determinations were made of the total and extracellular concentrations, from which intracellular concentrations were calculated on the basis of the assumptions defined in the text. N-starved bacteria were prepared by washing and suspending bacteria from an exponential culture in M63 without NH₄Cl. After 15 min at 37 °C, the bacteria were washed again and incubated for 45 min at 37 °C in M63 devoid of both NH₄Cl and the carbon source. A carbon source (0.2%, w/v) and 0.5 mM-IPTG were then added. During the following 30 min at 37 °C samples were taken periodically for β -galactosidase assays, after which further samples were removed for determination of the intra- and extracellular metabolite concn. Relative rates of β -galactosidase synthesis for the exponential cultures are expressed in arbitrary units/unit of bacterial protein synthesized. For N-starved cells, enzyme synthesis is reported as relative activity after 30 min induction/unit of bacterial protein present. Metabolite concentrations are expressed in terms of P.

Carbon source	Fructose 1,6-diphosphate			Triose phosphate			Relative rate of β -galactosidase synthesis	Bacterium concentration at time of sampling (μ g protein/ml)	No. of doublings (or min) in final incubation before sampling
	Intracellular concentration (μ M)	Extracellular concentration (μ M)	Metabolite in external medium (%)	Intracellular concentration (μ M)	Extracellular concentration (μ M)	Metabolite in external medium (%)			
Exponential bacteria									Doublings
Glucose	3900	0.0	0	830	1.5	57	52	220	2.0
Glucose + casein hydrolysate	3800	0.98	14	220	2.3	86	35	247	1.9
Glycerol	4700	0.44	6	2400	3.1	46	100	236	3.3
Glycerol + casein hydrolysate	5200	0.14	2	3000	2.5	38	101	215	3.0
Succinate	240	0.0	0	500	3.7	80	100	268	1.8
N-starved bacteria (after growth in succinate)									Min
None	92	0.0	0	380	0.14	20	100	223	40
Glucose	3400	0.0	0	1200	2.7	55	0	223	40
Glycerol	6000	0.0	0	2200	3.7	53	0	223	40
Succinate	1100	0.0	0	900	2.1	62	0	223	40

Table 5. *Intra- and extracellular concentrations of fructose 1,6-diphosphate and triose phosphate in strain B*

	Experimental details as in Table 2								
	Fructose 1,6-diphosphate			Triose phosphate					
Carbon source	Intracellular concentration (μM)	Extracellular concentration (μM)	Metabolite in external medium (%)	Intracellular concentration (μM)	Extracellular concentration (μM)	Metabolite in external medium (%)	Relative rate of β -galactosidase synthesis	Bacterium concentration at time of sampling (μg protein/ml)	No. of doublings (or min) in final incubation before sampling
Exponential bacteria									Doublings
Glucose	7700	0.13	1	1700	1.9	45	36	215	2.4
Glucose + casein hydrolysate	5100	0.14	2	770	2.8	71	23	225	2.0
Glycerol	4700	0.44	6	2300	1.8	34	100	233	2.4
Glycerol + casein hydrolysate	4300	0.0	0	1100	3.8	71	73	211	2.3
Lactate	690	0.0	0	0	1.4	100	100	241	2.2
N-starved bacteria (after growth in lactate)									min
None	420	0.0	0	830	0.0	0	100	193	40
Glucose	3700	0.0	0	1600	0.67	26	7	193	40
Glycerol	720	0.0	0	0	0.27	100	174	193	40
Lactate	0	0.0	—	310	0.0	0	22	193	40

of living bacterial cells is 1.1 (Ruffilli, 1933); (iv) the dry wt of bacterial cells is 20% of the wet wt (Porter, 1946); (v) the whole of the aqueous phase of the bacteria is accessible to the metabolites. Replication of this experiment showed that for most compounds the concentration was reproducible to within about $\pm 10\%$.

The appearance of large amounts of monophosphate metabolites in the medium seems to have been due to a genuine leakage from the bacteria. Had it been due to bacterial lysis, or a failure to obtain samples of the supernatant medium free from bacteria, the diphosphates and triphosphates should have been similarly distributed between bacteria and medium. Table 3 shows that 5 min of centrifugation in the Microfuge was sufficient to sediment more than 97% both of the visible bacteria and of the total protein.

Leakage of the monophosphates into the medium was not the result of growth in LP medium, or in the presence of [^{32}P]P_i. This medium was necessary for measurements using [^{32}P]P_i in order to achieve a suitably high specific radioactivity in the metabolic products, while at the same time avoiding levels of radiation hazardous both to the bacteria and to the experimenters. Chemical determinations of the intra- and extracellular concentrations of fructose 1,6-diphosphate and triose phosphate, in bacteria growing in high P_i (0.1 M) medium, showed again the leakage of the monophosphates but not of the diphosphate (Tables 4 to 6).

3',5'-Cyclic-AMP. The data in Table 2 indicate that 3',5'-cyclic-AMP was present entirely in the medium with all growth substrates tested. We therefore investigated the uptake of

Table 6. *Intra- and extracellular concentration of fructose 1,6-diphosphate and triose phosphate in strain LA12G*

Experimental details as in Table 2									
Carbon source	Fructose 1,6-diphosphate			Triose phosphate			Relative rate of β -galactosidase synthesis	Bacterium concentration at time of sampling (μ g protein/ml)	No. of doublings (or min) in final incubation before sampling
	Intracellular concentration (μ M)	Extracellular concentration (μ M)	Metabolite in external medium (%)	Intracellular concentration (μ M)	Extracellular concentration (μ M)	Metabolite in external medium (%)			
Exponential bacteria									
Glucose	3800	2.0	22	720	3.1	70	63	277	2.3
Glucose + casein hydrolysate	2400	2.3	35	770	1.6	54	55	264	2.1
Glycerol	2900	0.0	0	1900	4.5	59	100	254	2.2
Glycerol + casein hydrolysate	2000	0.71	16	1400	1.8	42	55	275	2.6
Lactate	0	0.0	—	0	1.5	100	100	228	1.8
N-starved cells (after growth in lactate)									
None	430	0.0	0	0	0.14	100	100	151	40
Glucose	5200	2.4	31	1300	3.2	71	4	151	40
Glycerol	1600	0.0	0	0	0.0	—	0	151	40
Lactate	130	0.0	0	0	0.28	100	4	151	40

Table 7. *Effect of 3',5'-cyclic-AMP on growth and β -galactosidase synthesis*

Strain VP19 was grown exponentially in LP medium containing ribose or gluconate. β -Galactosidase was induced with 0.5 mM-IPTG. The rates of growth and of β -galactosidase synthesis were measured in the presence of two concentrations of 3',5'-cyclic-AMP.

Carbon source	Concentration of 3',5'-cyclic-AMP (M)	Growth rate (doublings/h)	β -Galactosidase synthesis (units/mg bacterial protein)
Ribose	0	0.69	8500
Ribose	10^{-5}	0.68	8600
Ribose	10^{-3}	0.65	9100
Gluconate	0	0.93	3200
Gluconate	10^{-5}	0.93	3000
Gluconate	10^{-3}	0.71	4500

the nucleotide, its further metabolism, and its effect on growth and β -galactosidase synthesis. At a concentration of 1 mM, neither growth nor the rate of enzyme synthesis was affected in ribose medium, while the growth rate and catabolite repression were reduced in bacteria growing in gluconate medium (Table 7). In both media, at 10^{-5} M, and 10^{-3} M, added [$8\text{-}^{14}\text{C}$]3',5'-cyclic-AMP was recovered entirely from the medium (100.5% in ribose, and 99.0% in gluconate). No soluble metabolic products were observed chromatographically, and the incorporation of ^{14}C into insoluble material corresponded to rates of nucleotide metabolism of 0.40 and 0.075 nmol/mg bacterial protein synthesized, for bacteria in ribose

and glutonate, respectively. Other studies, with *Escherichia coli* strain 3000, have confirmed these results, and showed that with 1 mM 3',5'-cyclic-AMP added to the medium, the intracellular concentration was but slightly higher than that in the medium (V. Moses & J. Mowbray, unpublished work). Since, in the present experiments, intact bacteria constituted about 0.1% of the total volume of the suspension, these results are consistent with only the observations that, within experimental error, essentially all of the 3',5'-cyclic-AMP formed endogenously leaks to the medium.

DISCUSSION

In measurements of the distribution of metabolites between the bacteria and the medium, it was found with all media that a considerable fraction of the monophosphorylated substances appeared in the medium. Nevertheless, in almost every case the concentration remained considerably higher inside the bacteria. Since the bacteria constituted only about 0.1% of the total bulk of the suspension, the intracellular concentration would exceed the extracellular unless more than 99.9% of the substance was outside the bacteria. Our techniques were not sufficiently precise to permit such evaluations. We have not distinguished experimentally between metabolites actually within the bacteria and those, if there are any, absorbed on to the surface membrane or wall. This distinction does not greatly affect the conclusions if one considers simply two general pools of metabolites, one directly associated with the bacteria, and the other in the medium.

Several trivial explanations might account for the presence of these compounds in the medium: a sizeable proportion of the bacteria in the culture lysed and released their contents; the bacteria were not completely removed from the medium before the latter was sampled; leakage was promoted by growth in low phosphate medium, or by the presence of ^{32}P . Lysis would be expected to release not only monophosphates, but also diphosphates, triphosphates and protein. It has been found (V. Moses, unpublished work), that *Escherichia coli* will not use fructose 1,6-diphosphate and ATP as sole sources of carbon unless the P_i concentration is low enough (less than 0.025 mM) to permit derepression of alkaline phosphatase; monophosphates, such as glucose 6-phosphate, are utilized as carbon sources in the presence of P_i . There is, as a result, no reason to believe that had di- and triphosphates, as well as monophosphates, been released, they would have been preferentially utilized and thus not been observed in the extracellular medium. Failure to remove bacteria from the medium would similarly not specifically have favoured the presence of the monophosphates in the supernatant medium. It is clear from Table 2, however, that the monophosphates were preferentially released, and from Table 3 that both bacteria and cellular protein were adequately sedimented. Leakage appeared not to be the result specifically of growth in low phosphate medium, or in the presence of ^{32}P , since it was also observed in limited studies in high phosphate medium in the absence of radioactivity (Tables 4 to 6). The leakage of some metabolites to the medium thus appears to be a genuine aspect of cell physiology.

We have not, to date, made kinetic measurements of the rate of leakage. The results reported in Table 2 were obtained with cultures at similar densities, although the bacteria were growing at different rates. The bacteria were thus resident in the incubation media for varying periods, and the accumulation of extracellular metabolites may have proceeded at different rates. There was, however, no clear pattern of greater accumulation in the more slowly growing cultures.

Correlation has not been obtained between the degree of catabolite repression and the concentration, either inside or outside the bacteria, of the metabolites studied. Of particular interest are the observations that in all carbon sources, 3',5'-cyclic-AMP was entirely, or

almost entirely, restricted to the medium. This nucleotide has been implicated as one of the essential factors in the synthesis of inducible enzymes subject to catabolite repression (de Crombrughe, Perlman, Varmus & Pastan, 1969).

Makman & Sutherland (1965) reported that the intracellular concentration of 3',5'-cyclic-AMP declined in the presence of glucose, and that this decline was a consequence of its excretion into the medium when glucose was present. From their report has arisen the hypothesis that the severity of catabolite repression is linked to the intracellular level of 3',5'-cyclic-AMP. Close examination of the published results leads one to doubt their validity. The criticisms of their work may be summarized as follows: (a) bacteria for analysis were harvested by centrifugation at room temperature for 15 min; they were not washed. No estimate could be made of changes in 3',5'-cyclic-AMP concentration during this procedure, nor of how much might have been contained in the interstitial water; (b) they used non-growing bacteria in buffer containing a carbon source, but no nitrogen. It is not clear to us how to relate growing and nitrogen-starved bacteria; (c) the intracellular concentration of 3',5'-cyclic-AMP during growth was reported to rise when glucose in the medium was exhausted. However, the total growth observed was less than one doubling, and the evidence actually presented suggests that the concentration of the nucleotide began to rise when nearly 50% of the initial glucose remained. The residual glucose at that time was quite sufficient to support maximal exponential growth (Monod, 1942); (d) the measured amount of 3',5'-cyclic-AMP appearing in the medium when glucose was supplied to bacteria in buffer was 1.5 to 7.5 times the measured quantity lost from the bacteria; (e) it was reported that considerable amounts of other ribose-containing nucleotides appeared in the medium, but these were not identified; (f) although Makman & Sutherland (1965) used Crooke's strain of *Escherichia coli*, while we have used a derivative of K12, we have thought it useful to compare their data for the intracellular concentration of 3',5'-cyclic-AMP with bacteria in different carbon sources with our own data on growth rates and differential rates of β -galactosidase synthesis in those same carbon sources (Table 1). We have not found correlation.

The distribution of phosphorylated esters between the bacteria and the medium appears to be a controlled process, since not all substances leak in the same proportion. We suggest that the controlled leakage may have a homeostatic function for the culture as a whole, in which substances, possibly including 3',5'-cyclic-AMP, play a role in regulating the growth and physiology of the culture in a manner analogous to that of hormones in animal systems. It is not without interest that Okabayashi, Ide & Yoshimoto (1963) and Okabayashi, Yoshimoto & Ide (1963) have observed the presence of 3',5'-cyclic-AMP in the medium of *Brevibacterium liquefaciens* cultures.

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