species of micro-organisms. Pardee & Prestidge (1958) have reported that canavanine is not capable of supporting growth of an arginine-requiring mutant of *Escherichia coli*. This is, perhaps, because some strains of *E. coli* are reported to decarboxylate canavanine to yield an amine containing an *O*-guanidyl group (presumably, $H_2N \cdot C(:NH) \cdot NH \cdot O \cdot [CH_2]_3 \cdot NH_2$) (Hagihara, 1956). Furthermore, Kihara, Prescott & Snell (1955) have shown that canavanine can be degraded to homoserine + guanidine by some strains of *Strep. faecalis* and that these strains are therefore resistant to canavanine. Other actions of bacteria on canavanine are summarized by Kalyankar, Ikawa & Snell (1958).

As canavanine is so similar in structure to arginine and active in so many enzyme systems which are normally active on that amino acid (see Kihara *et al.* 1955; Meister, 1957) it is not, perhaps, surprising that it should be incorporated into the protein fraction of organisms. The results reported in this paper give no indication whether canavanine replaces arginine residues in the protein. If such replacement does occur, however, the marked difference between the pH value of the isoelectric point of the two amino acids (arginine, 10·7; canavanine 7·9; Spector, 1956) gives rise to the hope that the 'analogous' proteins may be separated readily by physicochemical methods.

SUMMARY

1. The presence of canavanine was detected in the protein + cell wall fraction of *Staphylococcus aureus* 524 grown in its presence.

2. No canavanine could be detected in cell-wall preparations from cells grown in the presence of arginine under similar conditions.

3. The canavanine was found to be incorporated in the protein + cell wall fraction in such a way that the amino group of the amino acid was not free to react with 1-fluoro-2:4-dinitrobenzene.

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Permeability of *Escherichia coli* to Ribose and Ribose Nucleotides

By L. V. EGGLESTON and H. A. KREBS

Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Oxford

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Lutwak-Mann (1936) and Stephenson & Trim (1938) have shown that washed suspensions of *Escherichia coli* are able to degrade adenine nucleotides to form hypoxanthine, inorganic phosphate, ammonia and ribose. Stephenson & Trim also found that ribose did not accumulate but was fermented with the liberation of a gas, and that the rate of gas production from adenosine was much greater than that from free ribose.

In previous experiments on the effect of nucleotides on various enzyme reactions in *E. coli* (Eggleston, 1957, 1958; Krebs, Jensen & Eggleston, 1958),

it was noted that one of seven strains of *E. coli* (National Collection of Industrial Bacteria 8571; see Knivett, 1954), when grown on glucose, decomposes anaerobically adenosine 5′-phosphate with the liberation of gas. The gas production proved to be due to the fermentation of the ribose moiety of adenosine 5′-phosphate. Free ribose and ribose 5′-phosphate were not fermented, as was the case with the organism studied by Stephenson & Trim. Ribose and ribose 5′-phosphate were, however, decomposed when the cells were grown in the presence of ribose. This paper is concerned with an investigation of the factors which affect the fermentation of ribose and related substances. The results indicate that the penetration of ribose and ribose 5′-phosphate into the cells is controlled by a mechanism which possesses all the criteria of a permease (Cohen & Monod, 1957).

EXPERIMENTAL

Materials

Nucleotides and nucleosides. Adenosine triphosphate (ATP), sodium salt, and adenosine diphosphate (ADP), barium salt, were obtained from Schwarz Laboratories Inc., N.Y., U.S.A. The chromatographic technique of Krebs & Hems (1953) showed that contamination with other phosphates was less than 5% (in terms of P). Adenosine 5′-phosphate was obtained from Zellstoff-fabrik Waldhof, Wiesbaden, Germany; yeast adenylic acid, guanylic acid, uridylic acid and cytidylic acid, inosine and guanosine from L. Light and Co. Ltd. Adenosine was supplied by Genatosan Ltd., Loughborough, Leics. Flavin mononucleotide was a free gift from Sigma Chemical Co., Mo., U.S.A.

Other reagents. Adenine, hypoxanthine, and D(−)-ribose were supplied by L. Light and Co. Ltd.; ribose 5-phosphate from Sigma Chemical Co. was found to give a single spot when examined by the paper-chromatographic technique of Eggleston (1954), and the concentrations of solutions of this compound were checked by ribose determination. A HCl-hydrolysate of casein was prepared according to Barton-Wright (1952); the HCl was removed by evaporation *in vacuo*.

Buffers. 2-Amino-2-hydroxymethylpropane-1:3-diol (tris)–maleate buffer, pH 6·8, 0·5 M-potassium salt, was made according to Gomori (1955). Phosphate buffer, pH 6·8, M-sodium salt, was made by mixing equal quantities of M-Na_2HPO_4 and M-NaH_2PO_4 solutions. The pH of these buffers at the concentrations used in the experiments was checked with a glass electrode and a Pye pH meter.

Micro-organisms. E. coli strains 8571, F, 86, 8114, 4, T and M/48, were described previously (Eggleston, 1957).

Methods

Suspensions of *E. coli* were grown semi-anaerobically in round flat-bottomed 1 l. flasks filled to the neck and washed as previously described (Eggleston, 1957), except that in some experiments (where stated) the liquid growth medium contained 1% (w/v) of D(−)-ribose instead of the usual 2% (w/v) of glucose. The washed cells were stored at 0° as suspensions in water (about 30 mg. dry wt./ml.).

Whole cells (8–15 mg. dry wt.) were incubated in Warburg vessels in a total volume of 2 ml. This included 0·2 ml. of 0·5 M-tris–potassium maleate buffer, pH 6·8, or variable quantities of M-sodium phosphate buffer, pH 6·8, and 0·1 ml. of 0·1 M-$MgCl_2$ in the main compartment and substrate (usually 0·1 ml. of 0·05 M) in the side bulb. The gas space was filled with N_2 and the temperature of incubation was 25°. The gas evolved on addition of the substrate usually consisted of CO_2 and H_2. In order to measure the proportions of these two gases two vessels (of nearly identical volume) were used, the centre well of one of which contained 0·2 ml. of 2 N-NaOH, freshly prepared, soaked into a pleated filter paper to absorb CO_2. After deduction of the H_2 evolved (in mm.), CO_2 evolution in the vessel without NaOH was calculated with the correction for CO_2 retention at pH 6·8 according to Johnson (1951).

At the end of the incubation period the cup contents were deproteinized by the addition of 0·5 ml. of 30% (w/v) trichloroacetic acid, followed by centrifuging. The clear supernatant solutions were stored at −15° until required for analysis. Paper chromatography was carried out on 50 µl. samples according to Krebs & Hems (1953). When chromatograms were to be examined in u.v. light phosphate buffer was used in place of tris–maleate buffer as the latter absorbed in the u.v. Inorganic phosphate was determined in the trichloroacetic acid extracts by the method of Berenblum & Chain (1938) as modified by Bartley (1953). Ammonia determinations were carried out by distillation in Conway units followed by nesslerization. Ribose was determined by the colorimetric method of Mejbaum (1939), acetic acid by the method of Serlin & Cotzias (1955) and ethanol by the method of Williams, Linn & Zak (1958), except that in place of Conway units the distillation vessels described by Bartley (1953) were used. Succinic acid was determined according to Krebs (1937) and malic acid according to Hummel (1949).

RESULTS

Degradation of nucleotides by whole cells

Washed cells of *E. coli* 8571, when incubated anaerobically at pH 6·8 and at 25°, evolved gas from ATP, ADP, adenosine 5′-phosphate, adenosine

Table 1. *Comparison of the rate of fermentation of various ribose-containing compounds by whole cells of* Escherichia coli 8571

The system contained: 12·4 mg. dry wt. of whole cells; 0·05 M-tris–potassium maleate buffer, pH 6·8; 5 mM-$MgCl_2$; substrates 2·5 mM; final vol. was 2 ml. Gas phase N_2. Temp. 25°.

Substrate	$CO_2 + H_2$ liberated in the first hr. (µmoles)
None	0·51
Ribose	0·80
Ribose 5-phosphate	0·71
ATP	2·21
ADP	3·10
Adenosine 5′-phosphate	6·92
Adenosine	7·05
Inosine	8·08

Table 2. *Degradation of various nucleotides and their derivatives by whole cells of* Escherichia coli 8571

The system contained 16 mg. dry wt. of whole cells; other details were as given in Table 1. After adding the substrate (5 μmoles) from the side arm, the manometers were read until gas evolution from the nucleosides had ceased (90 min.).

Expt. no.	Substrate	$CO_2 + H_2$ liberated (μmoles)	NH_3 formed (μmoles)	Inorganic phosphate formed (μmoles)
1	None	1·92	1·58	2·18
	Adenine	1·88	3·04	—
	Yeast adenosine monophosphate	9·20	5·54	7·03
	Inosine	10·36	1·27	—
	Adenosine	10·52	5·80	—
	Adenosine 5′-phosphate	10·40	5·70	7·68
	Guanosine	11·15	3·04	—
2	None	1·16	1·58	2·18
	Flavin mononucleotide	1·03	2·17	3·08
	Hypoxanthine	1·69	1·58	2·32
	Guanylic acid	6·70	2·66	5·70
	Uridylic acid	7·28	1·20	5·42
	Cytidylic acid	7·68	4·60	5·40
	Inosine	9·15	1·27	2·10

and inosine, but not from ribose and ribose 5-phosphate (Table 1). CO_2 and H_2 were usually formed in equal proportions, though in some experiments CO_2 prevailed in the proportions of 3:2. The fermentation rate was highest with inosine, slightly less high with adenosine and adenosine 5′-phosphate and still lower with ADP and ATP. Gas evolution usually ceased just before 2 μmoles/μmole of substrate added was reached.

The gas evolution from other nucleotides, nucleosides and related substrates is shown in Table 2. Yeast adenylic acid, guanylic acid, uridylic acid, cytidylic acid and guanosine were all fermented, whereas no gas was formed from purines and flavin mononucleotide. Thus fermentable compounds were those in which a ribose molecule was in combination with either a purine or pyrimidine base. Phosphate-containing substrates all liberated inorganic phosphate. There was a release of approximately 1 mol.prop. of NH_3 from adenosine 5-phosphate and adenosine and of less from adenine, guanosine, guanylic acid and cytidylic acid.

After cessation of the gas evolution from the nucleosides no ribose could be detected in the suspensions. When free ribose or ribose 5-phosphate was added their concentrations remained unchanged on incubation. Hypoxanthine was identified by paper chromatography as an end product of inosine and adenine nucleotide degradation. When ATP was the added substrate chromatography showed the intermediate formation of ADP; no adenosine 5′-phosphate or adenosine were detectable but hypoxanthine appeared together with some NH_3 and inorganic phosphate, and the total ribose content decreased approximately in proportion to the gas produced; e.g. during incubation for 70 min. 4·45 μmoles of gas were liberated and 2·21 μmoles of the total ribose disappeared.

There was a major deficit in the balance sheet of the fermentation of the ribose. Thus on incubation with inosine, the carbon of the ribose moiety was recovered as follows: 15–19 % as CO_2, 15–25 % as acetic acid, 15–20 % as ethanol, 10–15 % as succinic acid, 1·5–2·0 % as malic acid; 19–44 % remained unaccounted for. No lactic acid (method of Barker & Summerson, 1941) could be detected. A search for other phosphorylated end products (Krebs & Hems, 1953) or for carboxylic acids (Buch, Montgomery & Porter, 1952) by paper chromatography was unsuccessful.

The rate of degradation of nucleosides, as measured by gas production, was not increased by the addition of inorganic phosphate (0·005–0·05 M). Higher concentrations of phosphate (0·1 M) were slightly inhibitory, and arsenate (0·025 M) completely inhibited gas production from inosine. Omission of K^+ and Mg^{2+} ions did not affect the rates of gas production from nucleosides. Iodoacetate (0·01 M) caused complete inhibition by gas production.

Cells of *E. coli* which have been frozen and thawed repeatedly are known to have an increased permeability to amino acids and nucleotides (Eggleston, 1957). This treatment did not alter their permeability to free ribose or ribose 5-phosphate, but the rates of fermentation of nucleotides and nucleosides were increased by about 60 %.

No appreciable gas production could be detected when whole cells of other *E. coli* strains (F, 4, 8114, 86, M/48 and T) were incubated with adenosine 5′-phosphate. ATP, ADP, adenosine, guanylic acid, uridylic acid and cytidylic acid also gave negative results with strains F and 4.

Fermentation of ribose, ribose 5-phosphate and inosine in cell-free preparations

As the differences in the fermentability of nucleosides on the one hand and of free ribose and ribose 5-phosphate on the other could be due to permeability barriers, the fermentation of these substances was examined in disrupted cells. Disruption of cell suspensions by shaking with Ballotini beads (Mickle, 1948) and centrifuging gave clear extracts incapable of fermenting nucleoside; suspensions of acetone–ether-dried cells (Gale & Epps, 1944) were also inactive. Subjection of a cell suspension (310 mg. dry wt.) in water (10 ml.) to supersonic vibration (at 25 kcyc./sec., generated by a 600 w Mullard magnetostrictor oscillator at 3·5 A) for 3 min. followed by centrifuging gave a clear supernatant with no nucleoside-fermenting activities, and a small sediment with a trace of fermenting power. Combination of sediment and supernatant gave a suspension capable of fermenting inosine, ribose 5-phosphate and free ribose (Table 3). The rate of gas production from inosine in this preparation was about 20 % of that found in whole cells. There was a lag period of over 1 hr. before the fermentation of free ribose began, but incubation of whole cells with ribose under the same conditions failed to lead to an appreciable gas production.

Table 3. *Fermentation of ribose, ribose 5-phosphate and inosine in a cell-free preparation obtained by supersonic vibration*

The sediment and supernatant extracts were prepared and combined as described in the text; each cup contained an amount of enzyme preparation equivalent to about 30 mg. dry wt. of cells; other details were as given in Table 1.

	$CO_2 + H_2$ liberated (μmoles)			
Time (hr.)	No substrate	Ribose	Ribose 5-phosphate	Inosine
1	1·29	1·29	4·02	2·16
2	1·92	2·30	8·36	4·82
3	2·23	4·03	9·38	7·72
4	2·61	6·88	10·05	10·52

Addition of phosphate (0·01–0·05 M) had no effect on the rate or fermentation of inosine by the combined sediment and supernatant fractions.

Effects of cetyltrimethylammonium bromide and lyzosyme on cell permeability

Cetavlon (cetyltrimethylammonium bromide) has been used previously (Eggleston, 1957) to increase the permeability of *E. coli* cells to amino acids and nucleotides. Repaske (1958) has recently described conditions for the partial lysis of *E. coli* cells by a mixture of tris-HCl buffer, ethylenediaminetetra-acetic acid (EDTA) and lysozyme (from egg white). Table 4 shows the effect of these reagents on the permeability, as indicated by the fermentability of inosine, ribose 5-phosphate and ribose. Ribose was not fermented in the presence of cetyltrimethylammonium bromide, but ribose 5-phosphate generated gas at a greater rate than did inosine; the fermentation of the latter substance was inhibited by Cetavlon by about 50 % during the first hour. In other experiments cells were pre-incubated for 50 min. with tris-HCl buffer (0·033 M, pH 8·2), EDTA (1·33 mM) and lysozyme (0·4 mg; The Armour Laboratories), in a volume of 3 ml., before addition of 1 ml. from the side bulb containing 0·4 ml. of 0·5 M-tris–potassium maleate buffer, pH 6·8, substrate and water. The final pH on mixing was 7·0. Some lysis of cells occurred (as shown by density measurements in a Beckman spectrophotometer at 660 mμ) and the cell suspension became slimy. Again free ribose was not fermented, and the rate of gas production from ribose 5-phosphate was faster than that from inosine during the first half hour. A feature of the cells treated with cetyltrimethylammonium bromide or lysozyme was that the liberation of gas from ribose 5-phosphate ceased when it had reached about half the volume of that obtained from inosine. In contrast, in cell-free suspension similar volumes of gas were evolved from the two components though at different rates (see Table 3).

Table 4. *Effects of cetyltrimethylammonium bromide and lysozyme on the fermentation of ribose, ribose 5-phosphate and inosine by intact cells*

Cells: 12·4 mg. dry wt. Other conditions were as given in Table 1. Cetyltrimethylammonium bromide (CTAB), where added, was mM in final concentration. Pretreatment with lysozyme was as described in the text. The data refer to μmoles of $CO_2 + H_2$ liberated after addition of substrate.

	$CO_2 + H_2$ liberated (μmoles)						
	Cells with no further treatment		Cells + CTAB		Cells preincubated with lysozyme		
Substrate / Time (hr.)...	1	2	1	2	0·5	1	2
None	0·11	0·11	0·83	0·93	1·50	1·74	2·14
Ribose	0·26	0·29	1·18	1·29	1·67	1·99	2·64
Ribose 5-phosphate	0·20	0·36	4·33	4·35	3·48	5·45	5·54
Inosine	5·80	9·74	2·82	5·05	2·86	5·63	10·20

Various other substances were tested for their ability to facilitate the fermentability of ribose in whole cells. ATP, adenosine 5′-phosphate, inosine, hypoxanthine, glucose, inorganic phosphate, lysine and acid-hydrolysed casein had no effect.

Table 5. *Fermentation of ribose and ribose-containing compounds by intact cells grown in a ribose medium*

Whole cells: 8·2 mg. dry wt. Other conditions were as given in Table 1.

Substrate	$CO_2 + H_2$ liberated after addition of substrate (μmoles)		
Time (min.)...	30	60	90
None	0·71	0·85	1·32
ATP	1·72	2·46	3·62
Ribose 5-phosphate	3·35	5·80	5·95
Guanylic acid	4·47	5·85	6·39
Uridylic acid	2·68	5·22	6·92
Cytidylic acid	2·43	4·95	7·28
AMP-5′	3·26	6·96	7·86
Inosine	3·28	6·88	8·70
Ribose	3·60	8·45	9·11

Fermentation of ribose by cells grown in a medium containing ribose

When the glucose of the culture medium was replaced by ribose, cells were obtained which were capable of fermenting ribose readily (Table 5). Ribose reacted at a faster rate than ribose 5-phosphate or inosine and the total yield of gas from ribose 5-phosphate was again lower than that obtained in cell-free suspensions. As with cells grown on glucose, gas production from ATP was relatively slow. It was more rapid from adenylic acid, guanylic acid, uridylic acid, and cytidylic acid, inosine and ribose 5-phosphate, but all these substances reacted less rapidly than free ribose. Acetic acid and ethanol were identified among the end products. The proportions of the various products formed were not substantially different from those found with cells grown in the presence of glucose.

Induction of the fermentation of ribose in washed cells grown in the presence of glucose

Washed cells grown in the standard medium and incapable of fermenting free ribose acquired the capacity to ferment ribose if kept in ribose-

Table 6. *Development of the ability to ferment ribose in cells grown in a glucose medium*

Washed cells of *E. coli* 8571 (13–15 mg. dry wt.) were incubated in O_2 or N_2 at 25° for 5·5 hr. in pairs of Warburg vessels. The medium (2·8 or 3·0 ml.) contained KCl (0·01 M), $MgCl_2$ (3 mM), buffer, pH 6·8 (0·033 M), and the following concentrations of substrates (where added): ribose 0·013 M; ATP 1·6 mM; NH_4Cl 2 mM; HCl-hydrolysed casein (casein hydrol.) 1% (w/v); glucose 0·013 M; inosine 0·013 M. At the end of this 'pretreatment' period the gas space in the vessels was filled with N_2 and after further equilibration for 25 min. 0·2 ml. of 0·1 M-ribose was added from the side arm of one of each pair. Results are expressed as μmoles of gas evolved in 1 hr. from the time of adding the second amount of ribose.

Expt. no.	Conditions for the 5·5 hr. 'pretreatment' period: Gas phase	Buffer	Substrate	Gas evolved after the 'pretreatment' period (μmoles): Without extra ribose	With extra ribose
1	O_2	Phosphate	Ribose	6·56	10·6
	O_2	Phosphate	Ribose + ATP	11·7	17·7
	O_2	Phosphate	Ribose + NH_4Cl	7·95	13·7
	O_2	Phosphate	Ribose + NH_4Cl + ATP	12·0	18·4
	O_2	Phosphate	Ribose + casein hydrol.	1·18	8·66
	O_2	Phosphate	Ribose + glucose	7·68	11·1
	N_2	Phosphate	Ribose + ATP	1·38	1·51
2	O_2	Tris–maleate	None	0	0
	O_2	Phosphate	None	0	0
	O_2	Tris–maleate	Ribose + ATP	5·36	12·3
	O_2	Phosphate	Ribose + ATP	9·06	17·0
	O_2	Phosphate	Inosine + ATP	0·71	2·59
	O_2	Phosphate	ATP	0	2·12
	O_2	Phosphate	ATP + glucose + casein hydrol.	9·82	11·3
3	O_2	Phosphate	Ribose	4·67	9·52
	O_2	Phosphate	Ribose + NH_4Cl (2 mM)	7·68	15·2
	O_2	Phosphate	Ribose + NH_4Cl (4 mM)	7·45	14·9
	N_2	Phosphate	Ribose + NH_4Cl (2 mM)	1·30	1·35
4	O_2	Phosphate	Ribose + NH_4Cl	8·30	14·7
	O_2	Phosphate	Ribose + NH_4Cl + chloramphenicol (10 μg./ml.)	—	1·05
	O_2	Phosphate	Ribose + NH_4Cl + chloramphenicol (20 μg./ml.)	—	0·54
	O_2	Phosphate	Ribose + NH_4Cl + chloramphenicol (40 μg./ml.)	—	0·11
	O_2	Phosphate	Ribose + NH_4Cl + chloramphenicol (60 μg./ml.)	—	0

containing media under the following conditions: the washed cells were shaken for 5·5 hr. at 25° in pairs of Warburg vessels in the medium described under Methods, containing KCl, $MgCl_2$ and a buffer of pH 6·8. In addition, ribose (0·4 ml.; 0·1 M) and other substances listed in Table 6 were present in a final volume of 3 ml. The gas space contained O_2. At the end of this 'pretreatment' period the O_2 was replaced by N_2 and after an equilibration period of 25 min. extra ribose (0·2 ml.; 0·1 M) was added from the side arm of one of each pair of vessels. An evolution of gas indicated that the cells had acquired the ability to ferment the added ribose. The control vessel to which no extra ribose was added measured the gas evolution from the residual ribose and from other substances, e.g. amino acids.

The presence of O_2 during the pretreatment was found to be essential for the development of the fermentability of ribose under the conditions stated, although the cells had been grown anaerobically and can acquire the ability to ferment added ribose if grown in a ribose-containing medium anaerobically under other conditions. The amount of cell material (dry wt.) did not increase during the incubation, indicating that the ability to ferment ribose was acquired without growth. As shown in Table 6 the addition of a source of nitrogen such as NH_4Cl, casein hydrolysate or ATP (which undergoes deamination and dephosphorylation) enhanced the development of the ability to ferment ribose. The effects of NH_4Cl and ATP were not additive but phosphate caused some small increases. When ribose was omitted from the pretreatment medium the cells remained unable to ferment added ribose and inosine did not replace ribose. Glucose caused an inhibition. The time curve of the rate of ribose fermentation after different periods of pretreatment shows that the maximum is reached in about 5–6 hr. (Fig. 1). At this stage the rate of ribose fermentation was about the same as in cells grown on ribose.

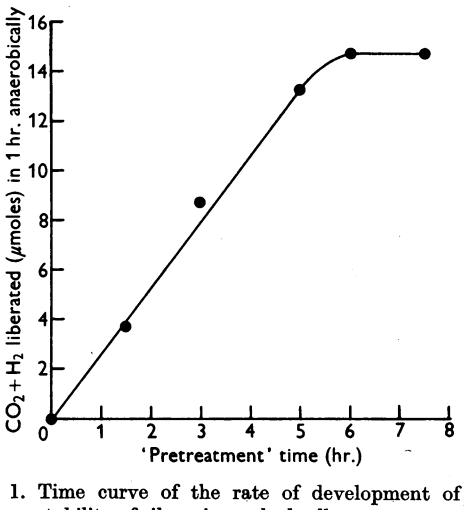

Fig. 1. Time curve of the rate of development of the fermentability of ribose in washed cells grown anaerobically in a glucose-containing medium. The cells were pretreated by shaking for various times at 25° with ribose and NH_4Cl in an atmosphere of O_2, as described in the text.

Chloramphenicol (chloromycetin) which is known to inhibit protein synthesis, prevents the development of the ability to ferment free ribose (Table 6, Expt. 4). Concentrations as low as 10 μg./ml. were sufficient to cause an inhibition of 93 %. The ribose-fermenting enzymes themselves were not inhibited by chloramphenicol concentrations of 60 μg./ml., as shown by the fact that inosine was fermented at the full rate in the presence of this concentration of chloramphenicol.

DISCUSSION

Ribose permease. The main findings of this investigation can be satisfactorily accounted for by the assumption that the penetration of ribose into the cells requires a 'permease' (Cohen & Monod, 1957) and that the formation of this permease depends on the presence of ribose in the medium. Different mechanisms must be responsible for the uptake of ribose and ribose 5-phosphate on the one hand and ribosides and ribotides on the other. Whether the penetration of ribotides and ribosides is also mediated by a permease or by simple diffusion remains to be investigated.

It is noteworthy that the fermentation of ribose 5-phosphate, but not of ribose, is very much enhanced by the addition of cetyltrimethylammonium bromide or lysozyme. The effects of these agents are likely to be due to a modification in the structure of the cell walls and they indicate that the barrier which prevents the fermentation of added ribose 5-phosphate may be overcome in at least two different ways, either by structural modifications or by a specific protein acting in an unknown fashion as a permease.

Strain differences. Although many strains of *E. coli* are known to possess enzymes required for degradation of nucleotides and nucleosides, the ability of washed cells to form gas from these compounds was found in one strain only of seven strains examined.

This strain also has other unusual properties not shared by the other six: (*a*) its ornithine decarboxylase is greatly accelerated by the addition of adenosine 5′-phosphate at the optimum pH (4·5), in contrast with the ornithine decarboxylase of other *E. coli* strains, which are accelerated by adenosine 5′-phosphate only at or near pH 7·0 (Eggleston, 1957); (*b*) it contains a 'factor' which

accelerates the phosphorolysis of citrulline by mammalian liver (Krebs, Eggleston & Knivett, 1955; Krebs *et al.* 1958).

Related observations. In a brief note Kopper (1956) reported that washed cells of *E. coli*, *Proteus vulgaris* and twelve other un-named species can reduce tetrazolium salts in the presence of various nucleosides but cannot do so on addition of free ribose or ribose phosphates. It is likely that this behaviour is related to the phenomena described in this paper.

SUMMARY

1. Washed intact cells of *Escherichia coli* strain N.C.I.B. 8571, grown semi-anaerobically in a glucose medium, are able to degrade anaerobically D-ribose added in the form of purine and pyrimidine nucleotides or nucleosides. The products formed include carbon dioxide, hydrogen, ethanol, acetic acid and succinic acid.

2. Free ribose and ribose 5-phosphate are not degraded by intact cells grown in a glucose medium, but are fermented by disintegrated cell material obtained by supersonic vibration.

3. Intact cells grown in a medium containing ribose instead of glucose rapidly ferment ribose and 5-phosphate, as well as the ribose moiety of purine and pyrimidine nucleotides.

4. Washed cells grown in a glucose medium acquire the ability to ferment ribose if they are incubated for a few hours in the presence of oxygen, ribose and a source of nitrogen.

5. The observations are in accord with the assumption that the penetration of ribose and ribose 5-phosphate into the cell is mediated by a specific permease.

6. Washed cells also acquire the ability to ferment ribose 5-phosphate (but not ribose) when treated with cetyltrimethylammonium bromide or lysozyme plus ethylenediaminetetra-acetic acid. These agents presumably cause structural modifications in the cell wall.

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Cholinesterase, Succinic Dehydrogenase, Nucleic Acids, Esterase and Glutathione Reductase in Sub-cellular Fractions from Rat Brain

By W. N. ALDRIDGE and M. K. JOHNSON
Unit for Research in Toxicology, Medical Research Council Laboratories, Carshalton, Surrey

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Differential centrifuging of homogenates of tissues in sucrose solution has indicated, particularly for liver, that enzymes are localized in particles of various sizes as well as in the soluble fraction. Fractions from liver homogenates have been extensively studied and various enzymes may now be used as markers so that the distribution of other enzymes may be compared with them (de Duve, Pressman, Gianetto, Wattiaux & Applemans, 1955).

Initially in this study our aim was to determine the distribution of cholinesterase in nervous tissue, but experiments showed that although a large proportion of the cholinesterase could be sedimented with the microsomal fraction there was also a