CCXXVI. THE METABOLISM OF ADENINE COMPOUNDS BY *BACT. COLI*

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WITH A MICRO-METHOD FOR THE ESTIMATION OF RIBOSE

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(Received 30 August 1938)

THE importance of nucleic acid derivatives in cell metabolism is becoming increasingly apparent; one may instance the function of muscle adenylic acid in fermentation and its presence as a constituent of coenzymes I and II; the pyrimidine ring occurs in cocarboxylase and uracil has been shown by Richardson [1936] to be essential for the anaerobic growth of *Staph. aureus*. In the metabolism of bacteria it has recently been shown that muscle adenylic acid prevents the loss of activity of *dl*-serine deaminase from a strain of *Bact. coli* [Gale & Stephenson, 1938], whilst adenosine behaves as a coenzyme for aspartase II prepared from the same organism [Gale, 1938].

As a preliminary to studying the mode of action of adenine compounds as coenzymes in the metabolism of bacteria the present study was undertaken in order to elucidate the changes which they themselves undergo in the presence of the enzymes of the organism. Previous studies on various bacteria (including *Bact. coli*) have shown that adenine and guanine are present as nucleotides, nucleosides and free bases in the acid-soluble extracts of the cells and that adenine compounds are more than ten times in excess of guanine compounds. On autolysis of the cells nucleic acid nitrogen decreases and nucleotides, nucleosides and free bases increase [Mesrobian, 1936].

It has also been shown that in the case of *Bact. coli* there exist within the bacterial cell enzymes decomposing adenine compounds and that muscle adenylic acid is dephosphorylated and deaminated by various organisms of the Bacteriaceae. Hypoxanthine was identified as the end product of the action of *Bact. coli* on adenine, adenosine, inosine and inosinic acid, and adenylyl pyrophosphate was shown to be a cell constituent of the same organism [Lutwak-Mann, 1936]. It now seems probable that the acid-soluble purine derivatives found in the cell originate from the nucleic acid. It has been shown that yeast decomposes its own nucleic acid autolytically and that the adenylic acid thus formed (adenosine-3-phosphoric acid) is rapidly dephosphorylated giving adenosine; the latter can be phosphorylated in its turn by the enzymes of the same organism giving adenosine-5-phosphoric acid ("muscle adenylic acid") and also the di- and tri-phosphates functioning in fermentation [Ostern *et al.* 1937; 1938].

In the present paper the same strains of *Bact. coli* as those employed in previous work were used; three types of decomposition were studied: (1) the dephosphorylation of muscle adenylic acid; (2) the deamination of adenine, adenosine and adenylic acid; (3) the removal of ribose from adenosine and adenylic acid.

METHODS

The organisms were grown in flasks of tryptic caseinogen digest broth for 12–14 hr. and centrifuged and washed with water in the usual way. The strength of the bacterial suspension was estimated by the photoelectric turbidimeter [Clifton *et al.* 1935]. The inorganic phosphate was estimated by the method of Fiske & Subbarow [1925], ammonia by the method of Parnas, and ribose by the method described in the appendix. The rates of dephosphorylation, deamination and liberation of ribose are generally expressed as $\mu\text{l.}$ ammonia, phosphate or ribose per mg. dry wt. bacteria per hr. ($\mu\text{l.} = \frac{\text{mol. wt. in mg.}}{22,400}$). Deamination is also expressed in Q_N ($\mu\text{g. nitrogen/mg. bacteria/hr.}$). The following stock solutions were used. Phosphate buffer pH 7.7 3M/450. Adenine 0.602 mg./ml. ($3 \times 33 \mu\text{l./ml.}$). Adenosine 1.15 mg./ml. ($3 \times 33 \mu\text{l./ml.}$). Muscle adenylic acid (adenosine-5-phosphoric acid) neutralized with NaOH 1.54 mg. ml. ($3 \times 33 \mu\text{l./ml.}$). *d*-Ribose 0.670 mg./ml. ($3 \times 33 \mu\text{l./ml.}$). Bacterial suspensions 3–15 mg. dry wt./ml. as convenient.

The decomposition of adenylic acid

Adenylic acid is dephosphorylated and deaminated almost simultaneously, but in all cases so far tried the former process appears to precede the latter.

Exp. 1. 5 ml. each of stock phosphate, adenylic acid ($2 \times \text{stock}$) and bacterial suspension (3 mg./ml.) were incubated at 37° ; 2 ml. and 1 ml. for the estimation of

Fig. 1.

Fig. 2.

Fig. 1. The dephosphorylation and deamination of muscle adenylic acid by *Bact. coli* I.

Fig. 2. Effect of pH on the deamination of adenosine by *Bact. coli* I.

ammonia and phosphate respectively were withdrawn at intervals; the results are shown in Fig. 1. The rate of deamination in this experiment corresponds to

$Q_N=24$. The rate of these reactions varies only slightly with pH increasing by 12% between pH 6.5 and 8.0 after which it falls off. From the pH curve for the deamination of adenosine (Fig. 2) it appears that the deamination of adenylic acid is controlled by the dephosphorylation and what is actually being measured is the effect of pH on dephosphorylation.

The action of inhibitors

The action of various inhibitors was tested in the hope of separating the dephosphorylation from the deamination. The results are given in Table I.

Table I

Inhibitor	% inhibition	
	Dephosphory- lation	Deamination
1. Phenol 1%	0	100
2. KCN $M/500$	100	100
3. KCN $M/1000$	100	100
4. NaF $M/50$	60	66
5. Iodoacetate $M/300$	0	0
6. Toluene (saturated sol.)	33	90
7. Chloroform (saturated sol.)	100	100

In the cases of 1, 6 and 7 the bacteria were exposed to the poison for 30 min. at room temperature and then centrifuged and washed before use; 2, 3, 4 and 5 were present during the reaction.

The only useful inhibitor found was 1% phenol which effects a clean separation of the two mechanisms; the phenol-treated and control organisms are washed twice with distilled water before use.

Exp. 2. 4 ml. each of adenylic acid phosphate and phenol-treated bacteria (6 mg./ml.) were incubated for 30 min. at 37° ; 2 ml. were then withdrawn for P and 1 ml. for NH_3 estimations. The remainder was then divided into two 4 ml. samples *A* and *B*. To *A* was added 0.26 ml. fresh bacterial suspension 15 mg./ml. so that the preparation contained 1 mg./ml. fresh bacteria; to *B* 0.26 ml. water was added. After 1 hr. further incubation the phosphate and ammonia estimations were repeated; the usual blank estimations were done (see Table II). Phenol treatment has no effect on dephosphorylation; when rates were measured with phenol-treated and untreated bacteria superimposable curves were obtained.

Table II

Time in min.		NH_3 -N $\mu l./2$ ml.	Phosphate P $\mu l./2$ ml.
30	(Phenol-treated organism)	<8	47.6
90	(Phenol-treated organism)	<8	47.2
90	(Phenol-treated + fresh organism)	46.4	47.2

Complete deamination and dephosphorylation corresponds to 66 $\mu l./2$ ml.

The decomposition of adenosine

The adenosine used was obtained from Georg Henning (Berlin) and gave the following figures on analysis:

C 45.11%, H 4.90%, N 25.4% (calculated C 45.10%, H 4.86%, N 26.2%).

Deamination. Adenosine is deaminated more rapidly than adenylic acid, especially by strain I, the difference being less marked in strain II (see Table III).

Table III

	Strain I	Strain II
Average Q_N adenylic acid	20	13
„ adenosine	104	24

Phenol treatment completely inhibits deamination by both strains.

The effect of *pH* is much more marked in the case of adenosine than in that of adenylic acid (see Fig. 2), the optimum being at *pH* 7.75. These facts are consistent with the view that adenosine is the compound actually deaminated.

Removal of ribose

The method used for the estimation of free ribose is described in the appendix. Having there shown that 10 μ g. (1.5 μ l.) ribose can be accurately estimated after the removal of the bacteria it became possible to study the liberation of this pentose from adenosine.

Exp. 3. 1 ml. each of stock adenosine phosphate buffer and bacterial suspension (3 mg./ml.); 1 ml. of this mixture contains 33 μ l. (221 μ g.) ribose in the form of adenosine. Control exp. in which adenosine is replaced by water. Incubation at 37°.

1 ml. was withdrawn and deproteinized with 3 ml. 0.2 *N* HCl and 2 ml. of the filtrate used for the estimation of ribose, this is equivalent to 111 μ g. ribose which requires 2.00 ml. ceric sulphate (Table IV).

Table IV

	Time in min.	Ceric sulphate ml.
Control	0	0.36
Exp. A	0	0.54
„	60	0.50
Exp. B	0	0.52
„	60	0.55

From the results in Table IV it seems that either no ribose is liberated in the experimental period or it is immediately decomposed to substances which do not reduce ferricyanide; Exps. 4 and 5 show that the latter alternative is correct.

Exp. 4. Six test tubes each containing 0.5 ml. stock adenosine, 0.5 ml. water and 3.0 ml. 0.2 *N* HCl were heated in a boiling water bath; at various intervals a tube was removed in which the total free ribose was estimated in the usual way; the results are given in Fig. 3. From this it is seen that 90% of the theoretical amount of ribose is liberated from adenosine and can be estimated after 2 hr. acid hydrolysis. In order to raise the yield of ribose, stronger acid and longer hydrolysis were employed without effect. From the analytical figures for the adenosine used it seems unlikely that this deficit is due to impurity,

Table V

Time in min.	Ribose added μ g.	Ribose estimated μ g.	Furfuraldehyde test
0	100	99	0
30	100	93	0
60	100	97	(+)
120	100	104	(+)
180	100	98	(+)

though a control in which 100 μ g. ribose were heated under the same conditions shows a higher recovery of ribose and a slight furfuraldehyde reaction by the aniline acetate test (Table V).

Fig. 3. Hydrolysis of adenosine by 0.2 N HCl at 100°.

We next tried to determine whether the ribose is actually split off from the adenosine as a result of the bacterial action.

Exp. 5. Equal vols. of stock adenosine, phosphate buffer (3M/450 at pH 7.7) and bacterial suspension 3 mg./ml. were incubated at 37° (1) aerobically, (2) anaerobically; 2 ml. samples were removed at intervals and 6 ml. 0.2 N HCl added, shaken with kieselguhr and filtered; the ribose was estimated in the filtrate after 2 hr. acid hydrolysis (A); 2 ml. of the filtrate without acid hydrolysis were used as control (B). $A - B (=C)$ is the ribose present as adenosine.

If C_0 , C_{15} and C_{30} etc. are the quantities of ribose found after the action of the bacteria for 0, 15 and 30 min. $C_0 - C_{15}$ is the amount of ribose split off from adenosine in 15 min. as the result of bacterial action.

Table VI

Time min.	μg. ribose removed/mg. bacteria	
	Aerobically	Anaerobically
0	0	0
15	42	45
30	76	76
60	182	177

Total available ribose = 182 μ g. (90 % of theoretical, see Exp. 4).

Table VI shows that in 60 min. 100 % of the ribose of adenosine has disappeared yet no ribose can be detected in the culture fluid at any period as shown in Exp. 3. This implies that ribose must be destroyed as quickly as it is liberated, so a comparison was made of the rate of destruction of free ribose and that of ribose combined in adenosine.

Exp. 6. 2 ml. stock buffer and 2 ml. bacterial suspension (3 mg./ml.) were incubated (a) with 2 ml. stock adenosine, (b) 2 ml. stock ribose.

2 ml. samples from (a) and (b) were removed at intervals and the ribose in each estimated in (a) after acid hydrolysis (as in *Exp. 5*) in (b) direct. From these results the rate of decomposition of ribose (a) as adenosine, (b) as free ribose were determined both for organisms grown on broth and on glucose broth. The results appear in Table VII.

Table VII

Rate of fermentation of ribose $\mu\text{g.}/\text{mg. bacteria}/\text{hr.}$	Free	As adenosine
Organism grown on plain broth	15	198
„ „ glucose „	20	204
Both inhibited 100% by treatment of organism with 1% phenol.		

The rate of disappearance of ribose in combination as adenosine being so much greater than when free, confirmation of the phenomenon was sought by the manometric measurement of the fermentation. The course of the fermentation was followed by measuring the total gas evolved in bicarbonate buffer, which may be CO_2 or H_2 , and also gas liberated by fermentation acid. The experiments were done in Warburg manometers.

Exp. 7. Each manometer contained 3 ml. total liquid.

0.2 ml. *M*/50 substrate (side bulb),
1 ml. bicarbonate buffer *M*/13,
1 ml. bacterial suspension (3 mg./ml.),
0.8 ml. water.

Fig. 4. Total gas produced in bicarbonate buffer from adenosine, inosine and free ribose (*Bact. coli* I)

The usual controls were performed. Gas production from the following substrates was measured: adenosine, inosine, *d*-ribose and hypoxanthine; the results are given in Fig. 4. From this it is seen that the rate of fermentation of free

ribose is about 0.1 that of ribose combined in adenosine and in inosine. (The rate of fermentation of adenosine is the same in the presence and absence of phosphate.) The results of Exp. 6 are therefore corroborated and the disappearances of ribose in the free state and in adenosine are seen to be due to fermentation.

The deamination of adenine

Adenine is very slowly deaminated (Q_N 3-5); this rate is increased two- to six-fold by the presence of adenosine or inosine.

Exp. 8. Solutions were made up as in Table VIII; 2 ml. from each solution were removed at intervals and the NH_3 -N estimated; the results are given in Table IX.

Table VIII

	ml.	ml.	ml.	ml.	ml.
Bacterial susp. 3 mg./ml.	3	3	2	3	2
Adenine stock	3	3	0	3	0
Adenosine stock	0	0.3	0.2	0.6	0.4
Water	1.5	1.2	2.8	0.9	2.6
Buffer 3 <i>M</i> /225	1.5	1.5	1.0	1.5	1.0
Total vol. ml.	9	9	6	9	6
No. of exp.	1	2	2a	3	3a

Table IX. NH_3 -N

Exp. no. ...	1		2		2a		2-2a		3		3a		3-3a	
Time min.	mg.	μ l.	mg.	μ l.	mg.	μ l.	mg.	μ l.	mg.	μ l.	mg.	μ l.	mg.	μ l.
0	<0.005	<8	<0.005	<8	<0.005	<8	0	0	0.005	8	<0.005	<8	0	0
30	<0.005	<8	0.0279	44.5	0.005	8	0.0274	36.5	0.035	56	0.010	16	0.025	40
90	0.007	11	0.036	55	0.005	8	0.031	47	0.0457	72	0.040	16	0.0357	56

Table X

	ml.	ml.	ml.	ml.	ml.	ml.	ml.
Bacterial susp. 3 mg./ml.	1	1	1	1	1	1	1
Adenine stock sol.	1	1	1	1	1	1	1
Adenosine $\frac{\text{stock}}{10}$ sol.	0	0.5	0.25	0.05	0	0	0
Inosine $\frac{\text{stock}}{10}$ sol.	0	0	0	0	0.5	0.25	0.05
Water	0.5	0	0.25	0.45	0	0.25	0.45
Buffer 3 <i>M</i> /225	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total vol.	3	3	3	3	3	3	3
NH_3 -N in 35 min.							
mg./2 ml.	0.005	0.0178	0.0141	0.0124	0.0176	0.0139	0.0114
μ l./2 ml.	8	28.5	22.6	19.8	28.2	22.2	18.3

Table XI

Concentration = x (33 μ l./ml.)

x Adenine	x Adenosine	x Inosine	Q_N Adenine
1	0	0	4.2
1	0.005	0	10.6
1	0	0.005	9.7
1	0.025	0	12.0
1	0	0.025	12.0
1	0.05	0	15.0
1	0	0.05	15.0
1	0.1	0	25.0
1	0.2	0	27.0

Effect of adenosine and inosine on the rate of deamination of adenine by Bact. coli I. Adenylic acid also has a catalytic effect on the deamination of adenine but slightly smaller than that of adenosine (Fig. 5); whilst the effect of ribose is comparatively insignificant, a concentration equivalent to that of adenine merely doubling the rate of deamination.

The effects of lower concentrations of adenosine and of inosine on the rate of deamination of adenine were then tested; the results are given in Table X, and the combined results from Tables IX and X shown in Table XI.

Fig. 5.

Fig. 6.

Fig. 5. The effect of adenosine and adenylic acid (3.3 μ l./ml.) on the deamination of adenine (33 μ l./ml.).

Fig. 6. Effect of adenosine (3.3 μ l./ml.) on deamination of adenine (99 μ l./ml.).

The catalytic effect of adenosine falls off with time, owing probably to its decomposition by the liberation of ribose; if fresh adenosine is added the initial rate is again restored (Fig. 6). The decreasing catalytic effect due to concentration shown in Table XI is partly accounted for in this way.

It is premature to discuss the nature of the catalytic effect of adenosine on the deamination of adenine. It is conceivable that the NH_2 group of adenine may be transferred to inosine which is then rapidly deaminated. The maximum rate of deamination of adenine in presence of adenosine is still less than that of adenosine, so evidence thus afforded is not inconsistent with this view. On the other hand Gale [1938] has recently found the rate of deamination of aspartic acid by aspartase II from the same organism increased by the addition of adenosine far beyond the rate of deamination of adenosine alone; in this case therefore the above hypothesis cannot hold and further work is necessary to arrive at the mechanism involved.

Lutwak-Mann [1936] showed that hypoxanthine was formed as end product of the action of *Bact. coli* on adenine, adenosine and adenylic acid. We have confirmed this.

Isolation of hypoxanthine from the deamination of adenine

The hypoxanthine was isolated as a silver nitrate compound [Bruhns, 1890]; 200 mg. adenosine, 100 ml. *M*/50 phosphate at pH 7.7 and 5 ml. bacterial suspension containing 100 mg. bacteria were incubated aerobically at 37°; after 3 hr. 2 ml. adenosine solution (66 μ l./ml.) were added; the course of the reaction

was followed by NH_3 estimations; fresh adenosine was added at intervals of $1\frac{1}{2}$ hr. till $700\mu\text{l.}$ had been added. At the end of 24 hr. deamination had stopped having reached 70% completion. 2 ml. $10N$ HCl was added, the whole shaken with kieselguhr and filtered; the clear filtrate was evaporated *in vacuo* to about 30 ml. and treated with excess of sodium picrate to remove the unchanged adenine, the adenine picrate was filtered on a Büchner funnel and washed. The filtrate and washings were then treated with excess ammonia and completely precipitated with silver nitrate. The precipitate (consisting of silver chloride, phosphate and a silver hypoxanthine derivative) was filtered on a Büchner funnel, washed with hot water and the residue boiled with 50 ml. HNO_3 sp. gr. 1.1; the acid was decanted off and the precipitate filtered whilst hot and re-extracted several times with HNO_3 . 0.25 g. AgNO_3 in HNO_3 was added to the filtrate which was left 24 hr. The precipitate was filtered off and dried at 100° for 30 min. and again taken up and reprecipitated with AgNO_3 and dried. The analysis was as follows:

	Found %	Calculated %
C	20.52	19.68
H	1.65	1.31
N	22.8	22.95
Ag	34.78	35.49

Hypoxanthine can also be detected and estimated by the use of xanthine oxidase.

Exp. 9. 3 ml. adenylic acid stock $\times 2$. 3 ml. phosphate $3M/450$ pH 7.7. 3 ml. bacterial suspension 3 mg./ml. 2 ml. samples were taken at intervals and 1 ml. N HCl added and the whole shaken with kieselguhr and filtered. 1.5 ml. filtrate, 0.5 ml. N NaOH and 1 ml. $M/5$ phosphate pH 7.0 were put in a Warburg manometer vessel and 0.5 ml. of a 20% neutralized xanthine oxidase solution [Dixon & Kodama, 1926] in the side bulb. $1\mu\text{g.}$ hypoxanthine in presence of the xanthine oxidase preparation took up $0.24\mu\text{l. O}_2$. With all substrates tested

Fig. 7. Production of hypoxanthine from adenylic acid by *Bact. coli* I.

(adenylic acid, adenine and adenosine) about 70% appeared as hypoxanthine (see Fig. 7). The bacterial suspension had a negligible xanthine oxidase activity ($Q_0 = 1.5$).

APPENDIX

A MICRO-METHOD FOR THE ESTIMATION OF RIBOSE IN
THE PRESENCE OF BACTERIAL SUSPENSIONS

By A. R. TRIM

Ribose may be estimated by a modification of the ceric sulphate titration method for reducing sugars previously worked out for blood sugar estimation by Giragossintz *et al.* [1936]. By this method the sugar is allowed to reduce ferricyanide to ferrocyanide at an alkaline reaction and then the ferrocyanide is re-oxidized with standard ceric sulphate after making strongly acid with concentrated H_2SO_4 . Xylene cyanol FF is used as an indicator. The sugar does not reduce its equivalent of ferricyanide according to the stoichiometric relation represented by the equation

but the extent of reduction of the ferricyanide varies somewhat with the conditions of the experiment; this being the case the estimations must be made under standard conditions and a calibration curve constructed. The advantage of this method is that only the ceric sulphate need be accurately standardized; once standardized the stock solution may be kept for months without deteriorating. Provided that they are in excess, the other reagents need not be used in precise quantities, and changes during storage, so important in certain other methods, are without effect.

Before proceeding with the estimation it is necessary to remove the bacteria; trichloroacetic acid cannot be used for this purpose as during the subsequent procedure it produces substances which reduce ferricyanide. HCl is free from this disadvantage and in concentrations of 0.2 *N* to 2.0 *N* followed by filtration through kieselguhr gives a clear filtrate which on treatment with trichloroacetic gives no further precipitate.

The ribose used was supplied by Dr Georg Henning of Berlin and gave the following constants:

M.P. 85–87°; rotation $[\alpha]_D^{18} = 219$, kindly determined by Dr D. J. Bell. The analysis by Dr G. Weiler gave the following results:

C%	H%	
39.87	6.67	Found
40.0	6.58	Calculated
	Ash none.	

PROCEDURE

1 ml. bacterial suspension containing 10–250 μg . ribose was taken and 3 ml. 0.2 *N* HCl added, shaken with kieselguhr and filtered on a pleated filter paper; 2 ml. of filtrate, 2 ml. of 0.8 % potassium ferricyanide and 2 ml. of 15 % sodium carbonate were heated on a boiling water bath for 5–10 min., cooled thoroughly and one-third to one-half the total vol. 50 % H_2SO_4 added; the end point of the final titration requires this large excess of acid. To the cold acid solution a few drops of 0.2 % xylene cyanol FF were added and the solution titrated against standard ceric sulphate in *N* H_2SO_4 ; in these experiments 0.002 *N* ceric sulphate was used.

It was found that the reduction of the ceric sulphate for any one batch of bacteria was constant and remained unchanged when the suspension was

incubated. It was therefore possible to correct for the reduction due to the reagents and bacteria by running blank controls for each set of experiments.

A curve showing the relation between $\mu\text{g.}$ ribose and ml. standard ceric sulphate was constructed over the range 0–250 $\mu\text{g.}$ ribose at intervals of 50 $\mu\text{g.}$ using 0.002 N ceric sulphate. The curve showed a linear relation in which 1 $\mu\text{g.}$ ribose was equivalent to 0.018 ml. ceric sulphate. The concentration of the ceric sulphate is quite arbitrary, so that the amount of ribose is given by the equation:

$$x = \frac{V \times N}{0.18 \times 0.002}.$$

Where $x = \mu\text{g.}$ ribose, $N = \text{normality of the ceric sulphate}$ and $V = \text{ml. ceric sulphate used}$. Similar results were obtained over the range 0–100 $\mu\text{g.}$ ribose at 10 $\mu\text{g.}$ intervals. Table XII shows a typical set of results:

Table XII

Vol. of solution ml.	Ribose $\mu\text{g.}$	ml. ceric sulphate		ml. ceric sulphate equivalent to ribose		ml. ceric sulphate $\mu\text{g.}$ ribose
		A	B	A	B	
2	0	0.47	—	—	—	—
2	0	0.47	—	—	—	—
2	0	—	0.62	—	—	—
2	0	—	0.63	—	—	—
2	50	1.31	1.46	0.86	0.84	0.0172
2	100	2.21	2.36	1.75	1.74	0.0175
2	150	3.08	3.23	2.61	2.61	0.0174
2	200	4.12	4.27	3.65	3.65	0.0183
2	250	4.90	5.05	4.43	4.43	0.0177

In both A and B bacteria were originally present and were removed before the estimations were made. In A the original solution contained 1 mg. of bacteria per ml. and in B 2 mg. per ml.

SUMMARY

1. Muscle adenylic acid is deaminated and dephosphorylated by *Bact. coli* (two strains). Dephosphorylation appears to precede deamination.

2. Deamination is completely inhibited by treatment of the washed suspension of *Bact. coli* with 1% phenol; this treatment has no effect on dephosphorylation.

3. Deamination of adenosine is faster than that of adenylic acid (Q_N about 100 and 20 respectively with strain I) that of adenine is the slowest of the three (Q_N about 5 using strain I).

4. Adenosine increases the rate of deamination of adenine 6–7 times.

5. Adenosine (and inosine) lose ribose when acted on by *Bact. coli*. This ribose can be shown to disappear from its combination in adenosine but cannot be detected in the free state. This disappearance is shown to be due to fermentation.

6. The rate of fermentation of ribose in adenosine (or inosine) is about 10 times that in the free state.

7. The activity of strain II was less than that of strain I but the order of the activities was the same.

8. A micro-method for the estimation of ribose is appended.

Our thanks are due to Prof. Sir Frederick Hopkins for his interest in our work, and to Mr S. W. Cole for advice on a number of points in connexion with the estimation of ribose.

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