4. ATP and AMP inhibit gluconeogenesis and accelerate the disappearance of carbohydrate in the absence of lactate.

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The Autodegradation of ^{32}P-Labelled Ribosomes from *Escherichia coli*

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Two features of a RNA in bacteria have led to its wide acceptance as a messenger of information between DNA and sites of protein synthesis. This 'messenger RNA', which has been the subject of several recent reviews (Jacob & Monod, 1961; Burma, 1962; Canellakis, 1962; Grunberg-Manago, 1962; Volkin, 1963), has a base composition similar to concomitantly synthesized DNA and exhibits a

rapid metabolic turnover that is attributed to alternating degradation and synthesis.

Newly synthesized RNA in the nuclei of HeLa cells has been shown to be unstable and to be degraded readily into nucleoside 5′-phosphates or nucleoside 5′-diphosphates (Harris, Fisher, Rodgers Spencer & Watts, 1963; Harris, 1963). Similarly, the unstable 'messenger RNA' in bacterial systems has been shown to break down into nucleoside 5′-phosphates (Cohen, Barner & Lichtenstein, 1961; Barondes & Nirenberg, 1962; Sekiguchi & Cohen, 1963). Ribosomal RNA, however, also breaks down into these nucleotides during the autodegradation of ribosomes (Wade, 1961; Wade & Lovett, 1961; Imada, Nakao & Ogata, 1962), and it is possible that some of the observations on 'unstable RNA' or 'messenger RNA' may be attributable to the behaviour of ribosomal RNA. This possibility has been examined by following the liberation of nucleotides during the autodegradation of labelled ribosomes from *Escherichia coli*.

MATERIALS AND METHODS

Cultural conditions. Escherichia coli, American type B, was grown either in a defined medium containing glucose (2%, w/v), glutamate (0·01 M), $(NH_4)_2SO_4$ (0·05 M), orthophosphate (4 mM) and essential salts, or in one of two tryptone media: TG3/W (Wade & Lovett, 1961), or TG4/W, prepared as TG3/W but with the omission of orthophosphate. The media TG3/W and TG4/W contained orthophosphate in concentrations of about 17 mM and 2 mM respectively.

The unlabelled ribosomes were prepared from bacteria grown in TG3/W (12 l.) by continuous culture (Wade, 1961). The harvested bacteria (division time 40 min.) were cooled rapidly and washed in a solution of NaCl (0·145 M) and $MgCl_2$ (5 mM) at pH 6·5 at 2–4°.

The ^{32}P-labelled ribosomes were prepared from bacteria grown in 12 l. of tryptone medium by using a closed system (Herbert, 1961). The bacteria were harvested 10 min. after the addition of [^{32}P]orthophosphate (10 mC) just before the end of the exponential phase of growth, passed through cooling coils into an aspirator at 2–4° and treated with a mixture of NaCl (50 mM), $MgCl_2$ (10 mM) and Na_2HPO_4 (10 mM) to increase flocculation. After about 3 hr. the thick bacterial suspension at the bottom of the aspirator was removed and centrifuged. The bacteria were washed as above and stored at −20°.

For the alkali degradation of ^{32}P-labelled RNA, the bacteria were grown aerobically in 1 l. of defined medium or TG3/W medium. During the exponential phase of growth 1 mC of carrier-free [^{32}P]orthophosphate was added. The culture was harvested 15 min. later into a steel container which was cooled to 2–4° with acetone–solid CO_2 and immediately treated with $HClO_4$ (final concn. 5%, w/v). The bacteria were sedimented and washed in 0·1 N-$HClO_4$ (10 vol.) at 2°.

Alkaline degradation of ^{32}P-labelled bacteria. Acid-extracted bacteria were suspended in 0·3 N-KOH to give a final RNA concentration of about 0·5%, incubated for 18–22 hr. at 37°, adjusted to pH 3–4 with 72% (w/v) $HClO_4$ and centrifuged. A 0·1 ml. volume of the supernatant was applied to the starting line of an ionophoresis paper and the nucleotides were separated as described below.

Preparation of ribosomes. The bacteria were disrupted in a press (Hughes, 1951) and a suspension (8–10% wet wt.) of the product in a solution of NaCl (0·145 M) and $MgCl_2$ (5 mM) at pH 6·5 was centrifuged at 25000*g* for 1 hr. in the no. 30 rotor of a Spinco model L centrifuge. The supernatant was centrifuged at 85000*g* for 7·5 hr. to sediment the ribosomes; the deposit was washed once in the same salt solution and finally made up to give a concentration ten times that of ribosomes in the initial suspension of disrupted bacteria (Wade & Lovett, 1961).

Ribosome autodegradation. The ribosomes were subjected to one of the two conditions reported by Wade (1961). The ribosome preparation (about twice the concentration of ribosomes in the original suspension of disrupted bacteria) was incubated at 37° with either 0·1 M-tris buffer, pH 7·5, containing EDTA (0·01 M) and $MgCl_2$ (1 mM) (*V*-route), or 0·45 M-tris buffer, pH 8·5, containing sodium orthophosphate (10 mM) and $MgCl_2$ (1 mM) (*M*-route). Samples (0·05 ml.) were taken in duplicate at intervals up to 60 min. (*V*-route) or 6 hr. (*M*-route) to determine the extent of RNA degradation (Wade, 1961).

At other intervals larger samples were taken and delivered into 72% $HClO_4$ at 4°, to give a final concentration of 0·2 N-$HClO_4$, to recover the nucleotides produced. The volumes taken for this purpose were decreased during the course of the processes so that the quantities of nucleotides yielded at different stages were similar.

Ion-exchange fractionation. The nucleotides produced by the *M*-route conditions were adsorbed at pH 6 on to 40 ml. (wet vol.) of Dowex 1 (X2; formate form; 200–400 mesh) resin in a glass column (1 cm. diam.), and eluted with 0·06 M-ammonium formate flowing at 1·7 ml./min. and adjusted with HCl to pH 5 and to progressively lower pH values at the rate of 2·5 pH units/l. of eluent (Wade & Lovett, 1961). The nucleotides liberated by the *V*-route were fractionated on 20 ml. of the same resin with 0·04 M-ammonium formate flowing at 0·8 ml./min. and adjusted with HCl to pH 4·5 and to progressively lower pH values at the rate of 3·5 pH units/l. of eluent.

The nucleoside 5′-phosphates from the *M*-route degradation were further purified by adsorption on to Norit (Wade & Lovett, 1961) and paper ionophoresis to eliminate traces of orthophosphate and nucleoside 5′-diphosphates.

Paper ionophoresis. The separation was carried out at pH 3·5 (Wade & Lovett, 1961) with 600 V for either 80 min. for the products of alkaline hydrolysis or 3 hr. for the nucleoside 5′-phosphates separated initially by ion-exchange fractionation.

The paper was examined under 253·7 mμ light from a Chromatalite (Hanovia Ltd., Slough, Bucks.). The bands of nucleotides were cut out and extracted with 0·1 N-$HClO_4$. The concentration of each nucleotide was calculated from the extinctions at 260 or 280 mμ.

Specific radioactivities of nucleotides. These were calculated from the radioactivity and the nucleotide concentrations determined from the ultraviolet absorption of either ion-exchange fractions (products of the *V*-route autodegradation) or acid extracts of fractions from paper ionophoresis (products of alkali hydrolysis or *M*-route autodegradation).

RESULTS

Ribosomes from Escherichia coli *grown in low-phosphate medium* (*TG*4/*W*). The mean specific radioactivity of ribosomal nucleotide from bacteria grown under these conditions was about 80 counts/min./μm-mole of nucleotide.

When incubated under *V*-route conditions, there was a rapid liberation of nucleotides from the ribosomes which was complete in 30 min. (Fig. 1*a*: *V*-route). The specific radioactivities of the nucleotides were determined at different times during the process. That the products were all nucleoside 2′,3′-cyclic phosphates, or other derivatives in which the 2′- or 3′-position of the nucleoside ribose was blocked, was confirmed by the absence of periodate reaction (Dixon & Lipkin, 1954).

Unlike the ribosomes from *E. coli* N.C.T.C. 1100, which produce mainly nucleoside 2′,3′-cyclic phosphates with traces of nucleoside 3′-phosphates (Wade, 1961), the ribosomes from *E. coli* American type B under similar conditions produce nucleoside 3′-phosphates in at least as high a concentration as the cyclic phosphates. The close proximity of small quantities of nucleoside 2′-phosphates and oligonucleotides to some of these nucleotides in the resin column effluent prevented the specific radioactivities of both nucleoside 3′-phosphates and nucleoside 2′,3′-cyclic phosphates from being determined in every instance, but, where it was possible, the specific radioactivities of both isomers were determined and the means recorded in the results (Fig. 1*b*: *V*-route). To avoid unnecessary repetition, the products of the *V*-route degradation are described below as nucleoside 3′-phosphates. The specific radioactivity of each nucleotide remained constant throughout the process, but, whereas the specific radioactivities of cytidine 3′-phosphate, adenosine 3′-phosphate and uridine 3′-phosphate were similar, that of guanosine 3′-phosphate was significantly lower (Fig. 1*b*: *V*-route).

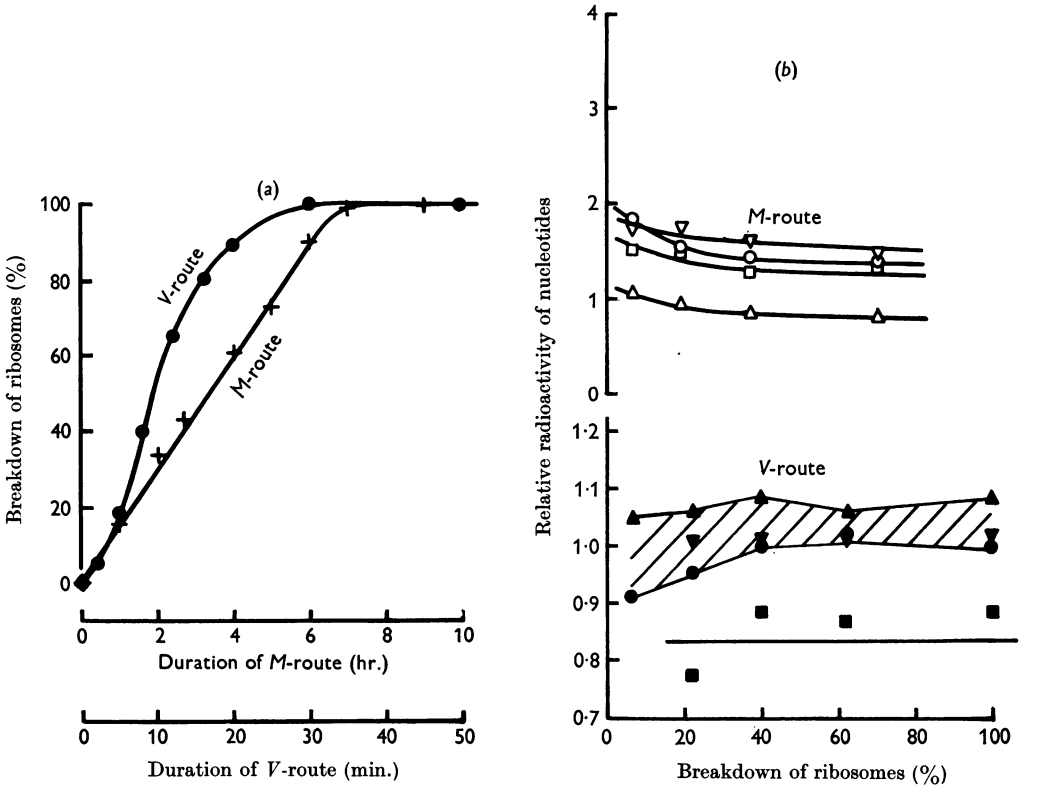

Fig. 1. Autodegradation of ribosomes labelled, *in vivo*, from bacteria grown in low-phosphate (TG4/W) medium. (*a*) The progress of RNA breakdown; (*b*) the relative specific radioactivities of the liberated nucleotides. The ribosomes (8 mg. dry wt./ml.) were incubated at 37° with either a solution of $MgCl_2$ (1 mM) and sodium orthophosphate (10 mM) at pH 8·5 (*M*-route), or a solution of $MgCl_2$ (1 mM) and EDTA (10 mM) at pH 7·5 (*V*-route). ▲, Cytidine 3′-phosphate; △, cytidine 5′-phosphate; ▼, uridine 3′-phosphate; ▽, uridine 5′-phosphate; ●, adenosine 3′-phosphate; ○, adenosine 5′-phosphate; ■, guanosine 3′-phosphate; □, guanosine 5′-phosphate.

When the ribosomes were incubated under the *M*-route conditions, nucleoside 5′-phosphates were liberated at a constant rate (Fig. 1*a*: *M*-route). The incubation was carried out in the presence of unlabelled orthophosphate to diminish the chance of contaminating these nucleotides with highly radioactive orthophosphate and nucleoside 5′-diphosphates. Even so, a further purification by paper ionophoresis was necessary. As the degradation of ribosomal RNA proceeded there was a significant fall in the specific radioactivity of each nucleotide liberated (Fig. 1*b*: *M*-route), and the specific radioactivities of individual nucleotides were significantly different.

Ribosomes from Escherichia coli *grown in normal phosphate medium* (*TG*3/*W*). The mean specific radioactivity of ribosomal nucleotide in these bacteria was about 1·2 counts/min./μm-mole. The rates of ribosome degradation by the *V*-route and the *M*-route (Fig. 2*a*) resembled those in the previous experiment (Fig. 1*a*).

As before, the specific radioactivities of cytidine, adenosine and uridine nucleotides liberated by the *V*-route were similar and consistently higher than those of guanosine nucleotides (Fig. 2*b*: *V*-route), and the specific radioactivities of all the nucleotides remained constant with time. The specific radioactivities of the nucleotides liberated by the *M*-route fell more sharply than before and there were wider differences between individual nucleotides (Fig. 2*b*: *M*-route). In common with the previous experiment (Fig. 1*b*: *M*-route), cytidine 5′-phosphate had the lowest specific radioactivity.

Exchange of [^{32}P]*orthophosphate with ribosomal phosphate.* The possibility that the differential labelling of nucleotides in ribosomal RNA (Figs. 1*a* and 2*a*) is partly due to a direct exchange with [^{32}P]orthophosphate was examined by exposing unlabelled ribosomes in a solution of sodium chloride (0·145 M) and magnesium chloride (2 mM) to carrier-free [^{32}P]orthophosphate for 10 days at 2–4°. The ribosomes were then autodegraded by the *M*-route and the specific radioactivities of the liberated nucleoside 5′-phosphates were determined.

As in the experiments *in vivo* (Figs. 1*b* and 2*b*: *M*-route) nucleotides liberated early in the process had a higher specific radioactivity than those liberated at the end (Fig. 3). On this occasion, however, more than 70 % of the radioactivity associated with guanosine 5′-phosphate, uridine 5′-phosphate and cytidine 5′-phosphate was liberated by the time 12 % of the ribosomal RNA had been degraded. In addition, the pattern of labelling was different, guanosine 5′-phosphate and uridine 5′-phosphate having the highest specific radioactivities.

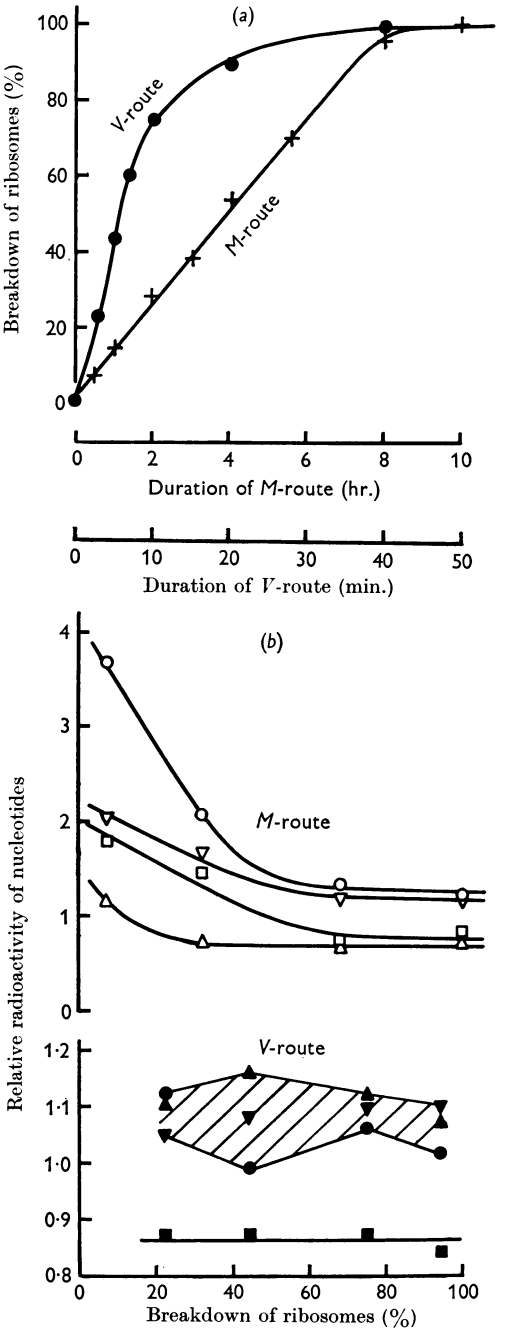

Fig. 2. Autodegradation of ribosomes labelled, *in vivo*, from bacteria grown in normal (TG 3/W) medium. (*a*) The progress of RNA breakdown; (*b*) the relative specific radioactivities of the liberated nucleotides. The ribosomes (8 mg. dry wt./ml.) were incubated at 37° with either a solution of $MgCl_2$ (1 mM) and sodium orthophosphate (10 mM) at pH 8·5 (*M*-route) or a solution of $MgCl_2$ (1 mM) and EDTA (10 mM) at pH 7·5 (*V*-route). See Fig. 1 for nucleotide symbols.

Degradation of ^{32}P-*labelled* Escherichia coli *ribonucleic acid by alkali.* The finding that persistently lower specific radioactivities of guanosine nucleotides were released from ribosomes by treatment with EDTA (Figs. 1*b* and 2*b*: *V*-route) was re-examined with direct alkaline hydrolysis of unfractionated labelled bacteria.

E. coli were exposed to [^{32}P]orthophosphate for 15 min. during exponential growth in both a complex medium (TG3/W) and in a synthetic glucose–salts medium. The cells were recovered, washed, acid-extracted and hydrolysed with alkali.

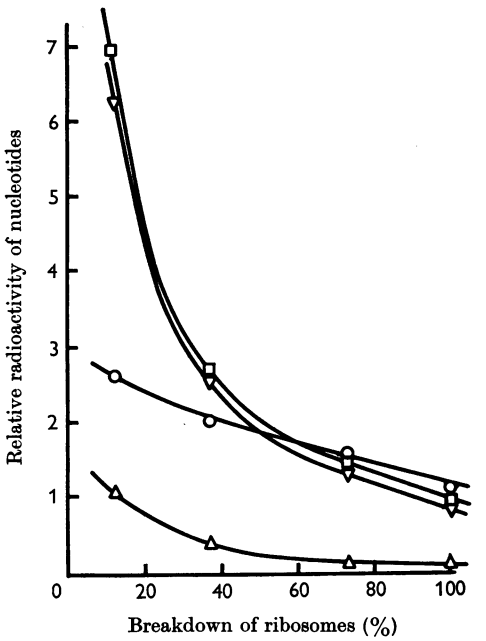

Fig. 3. Autodegradation of ribosomes labelled, *in vitro*. Unlabelled ribosomes were exposed to [^{32}P]orthophosphate in a solution of NaCl (0·145 M) and $MgCl_2$ (2 mM) for 10 days at 2–4°, then incubated at 37° with a solution of $MgCl_2$ (1 mM) and sodium orthophosphate (10 mM) at pH 8·5. See Fig. 1 for nucleotide symbols.

The expected series of nucleoside 2′(3′)-phosphates was observed by ultraviolet light, but radioautography revealed additional radioactive substances. The latter, fortunately, separated from the nucleotides and did not therefore interfere with their recovery. The results (Table 1) confirmed the low specific radioactivities of the guanosine phosphates obtained from the autodegradation of ^{32}P-labelled ribosomes.

DISCUSSION

In normal growing cells of *E. coli*, 'messenger RNA' represents only 1 % of the total RNA (Bolton & McCarthy, 1962). Since the cells were exposed to [^{32}P]orthophosphate for one-quarter of their generation time and the delay in its entering ribosomal RNA is probably less than one-fortieth of the generation time (McCarthy, Britten & Roberts, 1962), it is likely that about 20 % of the ribosomal RNA will have received labelled precursors during this period. The pattern of labelling obtained by much shorter periods of exposure and attributed to 'messenger RNA' would therefore be obscured. The results of Midgely & McCarthy (1962) support this contention.

The uniform rate at which nucleoside 5′-phosphates are liberated from ribosomes (Figs. 1*b* and 2*b*: *M*-route) reflects an exogenous attack which is common among enzymes that degrade nucleic acids into nucleoside 5′-phosphates or their derivatives (Hilmoe, 1959; Weiss, 1960; Lehman, 1960; Williams, Shan-ching & Laskowski, 1961; Reddi, 1961). There is no evidence from these graphs of the existence of a fraction of the RNA which is particularly susceptible to degradation. The very different specific radioactivities of the individual nucleoside 5′-phosphates, compared with nucleoside 3′-phosphates produced by the *V*-route (Figs. 1*b* and 2*b*: *V*-route), have also been observed with yeast (Ycas & Vincent, 1960) and tobacco-mosaic virus (Staehelin, 1958), and conform to the general view that the natural precursors of ribonucleic acids are derivatives of nucleoside 5′-phosphates.

Table 1. *Specific radioactivities of nucleotides liberated from* Escherichia coli *by alkaline hydrolysis*

Exponentially growing *E. coli* were exposed to [^{32}P]orthophosphate for 15 min. The bacteria were acid-extracted and treated with 0·3 N-KOH to hydrolyse RNA into nucleoside 2′(3′)-phosphates. Details are given in the text.

	Base ratio				Specific radioactivities of nucleoside 2′(3′)-phosphates			
Medium	Cytosine	Uracil	Adenine	Guanine	Cytosine	Uracil	Adenine	Guanine
Tryptone	22·6	20·6	24·2	32·7	23·0	24·1	22·3	21·8
	22·4	20·5	24·5	32·6	22·9	24·8	22·2	21·2
Defined	22·6	19·7	24·5	33·1	17·4	18·8	17·2	15·0
	22·6	20·2	24·6	32·6	17·7	18·6	17·2	14·9

The higher and dissimilar specific radioactivities of the nucleoside 5′-phosphates liberated early in the process suggest a tendency for those nucleotides last incorporated to be the first released (Adler, Lehman, Bessman, Simms & Kornberg, 1958; Lehman, 1960). It is unlikely that an exceptionally unstable RNA, e.g. messenger RNA or eosomes (Roberts, Britten & McCarthy, 1963), is responsible, since nucleotides with high specific radioactivity are not liberated during the early stages of ribonuclease attack (Figs. 1*b* and 2*b*: *V*-route). It is known that messenger RNA is particularly susceptible to ribonuclease (Gros *et al.* 1961; Riseborough, Tissières & Watson, 1962).

The absence of a convergence of the specific-radioactivity curves in Fig. 2 (*b*) (*M*-route) to a common value at zero breakdown is probably due to the relatively slow equilibration of [^{32}P]orthophosphate with pool phosphate and the dissimilar delays in incorporation into RNA to which the individual bases are subjected (Roberts *et al.* 1963). More specifically, the extensive delays in cytosine incorporation are reflected in the low specific radioactivity of cytidine 5′-phosphate in the nucleotide pool (Volkin, 1962) as well as in ribosomal RNA (Figs. 1*b* and 2*b*: *M*-route). The exceptionally high metabolic activity of adenosine phosphates, through commitments in other processes, may account for the high specific radioactivity of adenosine 5′-phosphate liberated from ribosomes (Figs. 1*b* and 2*b*: *M*-route) and from the RNA in rat-liver ribonucleoprotein (Shigeura & Chargaff, 1958).

The less-pronounced fall in the specific radioactivities with bacteria grown in a low-phosphate medium (Fig. 1*b*: *M*-route) may reflect a more pronounced general turnover of ribosomal nucleotide when the bacteria become starved of this element, in the manner demonstrated with nitrogen-starved *E. coli* (Goldstein, Goldstein & Brown, 1958; Mandelstam & Halvorson, 1960).

The very different pattern of labelling obtained by exposing the ribosomes to [^{32}P]orthophosphate *in vitro* (Fig. 3) does not reflect the base compositions of RNA or DNA. The rapid liberation of the label during autodegradation suggests that it may be confined to the terminal nucleotide. This is supported by the similar high specific radioactivities of guanosine 5′-phosphate and uridine 5′-phosphate, suggesting that these nucleotides occupy the opposite ends of paired strands of ribosomal RNA (Hall & Doty, 1959; Helmkamp & Ts'o, 1961; Bonner, 1961). Extrapolation of the logarithmic plots of the data in Fig. 3 shows that the specific radioactivities of adenosine 5′-phosphate and cytidine 5′-phosphate are also similar at the beginning of the process, about one-sixth those of the other nucleotides. The endogenous phosphatase in ribosomes (Spahr & Hollingworth, 1961; Cowie, Spiegelman, Roberts & Duerksen, 1961) cannot be solely responsible since the phosphodiesterase attacks the nucleoside end of the polynucleotide, not the free phosphate end. A more probable explanation is that the terminal nucleoside 5′-phosphate at the nucleoside end is only loosely bound to the rest of the polynucleotide, allowing its phosphate to exchange with free [^{32}P]orthophosphate.

The less rapid decline in the specific radioactivity of the adenosine 5′-phosphate, compared with the other nucleotides (Fig. 3), suggests that in this instance the phosphate of non-terminal nucleotides is also capable of exchange.

The initial acceleration in the release of nucleoside 2′(3′)-phosphates (Figs. 1*a* and 2*a*: *V*-route) reflects the endogenous character of the ribonuclease. The generally more uniform specific radioactivities of these nucleotides, compared with those liberated by the *M*-route, result from the shift of the phosphate from the nucleoside 5′-phosphate precursor to the adjacent nucleoside. The persistently lower specific radioactivity of guanosine phosphates from both the *V*-route degradation of ribosomes (Figs. 1*b* and 2*b*: *V*-route) and the alkaline degradation of whole cells (Table 1), however, is less easily explained.

It has been observed with briefly labelled yeast RNA (Ycas & Vincent, 1960) and tissue RNA (Davidson & Smellie, 1952; Boulanger & Montreuil, 1952; Hokin & Hokin, 1954) that guanosine 2′(3′)-phosphate with low specific radioactivity is released by alkali from RNA labelled *in vivo*. Ycas & Vincent (1960) attribute their observations to the labelling of 'messenger RNA'; however, in the present instance it is more likely to be due to a tendency for cytidine 5′-phosphate and guanosine 5′-phosphate, both of which have low specific radioactivities (Figs. 1*b* and 2*b*: *M*-route), to take up positions next to guanosine 5′-phosphate, in the way demonstrated with soluble RNA (McCully & Cantoni, 1962). If this is correct, the base composition of newly formed RNA in normally growing cells, deduced from the incorporation of [^{32}P]-orthophosphate for much shorter periods of exposure, must be unreliable. In *E. coli* the low specific radioactivity of the guanosine 2′(3′)-phosphate would bring the apparent base composition of newly formed RNA much closer to that of DNA, and may account for the reported association of 'messenger RNA' with all the ribosome components (Taki, Kondo & Osawa, 1962) and a membrane fraction (Suit, 1963) of *E. coli*.

These results cast some doubt upon the apparent instability of newly formed ('messenger') RNA which has been based on the incorporation and release of radioactive tracers. The well-known

exogenous character of phosphodiesterases and the association of such an enzyme with ribosomes permits the much simpler explanation that the end of the polynucleotide last synthesized is the first to be degraded. Even the incorporation of [^{32}P]-orthophosphate into ribosomes *in vitro* has produced a labelled RNA the behaviour of which, had the incorporation been performed *in vivo*, would have been attributed to newly formed unstable RNA.

There can be little doubt that RNA with a base composition resembling DNA is formed under certain circumstances (Bolton & McCarthy, 1962), and that this RNA, and certain polynucleotides synthesized *in vitro*, have the ability to direct protein synthesis. It remains to be proved, however, that this RNA has a vital function in normal growing bacteria and does not accumulate only when the normal pathways of nucleic acid synthesis are subjected to various stresses like those that result from phage invasion or nutritional deprivation. The subjection of growing bacteria to such treatments results in an abrupt, although in some instances temporary, halt in RNA synthesis with at least a short uninterrupted period of DNA synthesis. It is possible that only under these peculiar circumstances a DNA-like RNA accumulates.

SUMMARY

1. Exponentially growing cells of *Escherichia coli* were exposed to [^{32}P]orthophosphate to label 20–30 % of the ribosomal RNA. The ribosomes were autodegraded into nucleoside 3′-phosphates (*V*-route) and nucleoside 5′-phosphates (*M*-route).

2. The specific radioactivities of adenosine 3′-phosphate, uridine 3′-phosphate and cytidine 3′-phosphate liberated by the *V*-route were similar at different times during the process and remained constant; the specific radioactivity of guanosine 3′-phosphate was significantly lower.

3. The specific radioactivities of nucleoside 5′-phosphates liberated by the *M*-route process were dissimilar and diminished during the course of the process. This decrease was less pronounced with bacteria grown in a low-phosphate medium, suggesting a more radical incorporation into the ribosomal phosphate.

4. The pattern of the incorporation of [^{32}P]-orthophosphate into ribosomes *in vitro* suggested its confinement to terminal nucleotides.

5. The results suggest the possibility that the instability of newly formed RNA attributed to 'messenger RNA' may be due to the autodegradation of ribosomal RNA.

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Pyruvate Oxidation and the Permeability of Housefly Sarcosomes

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It has been shown by Van den Bergh & Slater (1962) that pyruvate is probably the most important physiological substrate for respiration and energy production in housefly flight-muscle mitochondria (sarcosomes). Isolated sarcosomes rapidly oxidize added pyruvate (Gregg, Heisler & Remmert, 1960), and this oxidation is accompanied by a rapid esterification of P_i and is under the control of the ADP and P_i concentrations. Evidence was presented that the low respiratory rates found with most substrates (e.g. the Krebs-cycle intermediates and glutamate) are caused by permeability barriers for these substrates in the sarcosomes.

In the present study the oxidation of pyruvate by isolated housefly sarcosomes was further investigated. During this work additional evidence was found in support of the suggested permeability barrier in the sarcosomes and information was obtained on the pathway of glutamate oxidation in housefly sarcosomes.

The work reported in the present paper was part of a Ph.D. Thesis, which was published in December 1962 (Van den Bergh, 1962).

METHODS AND MATERIALS

Isolation of sarcosomes. Sarcosomes were isolated from the thoracic muscle of the housefly, *Musca domestica* L., as described by Van den Bergh & Slater (1962). The medium used for the isolation was 0·154 M-KCl–1 mM-EDTA, pH 7·4.

Measurement of respiration and oxidative phosphorylation. The methods have been described by Van den Bergh & Slater (1962). The standard reaction medium used in all experiments contained a mixture of KCl (15 mM), EDTA (2 mM), $MgCl_2$ (5 mM) and tris (50 mM), adjusted to pH 7·5 by the addition of HCl, potassium phosphate buffer, pH 7·5 (30 mM), ADP (1 mM), glucose (30 mM) and hexokinase (150–180 Cori units). Unless otherwise stated, the respiratory substrate for the sarcosomes was 20 mM-pyruvate with or without 10 mM-L-malate. The gas phase in all experiments was air. In some experiments, the oxygen uptake was measured with an oxygen polarograph (Oxygraph, manufactured by Gilson Medical Electronics, Middleton, Wis., U.S.A.). The reaction medium in these polarographic measurements was the same as in the manometric experiments, but the reaction volume was 2·0 ml.

Qualitative demonstration of transaminase activity. The amino acid and the oxo acid (20 μmoles of each) were incubated in a medium containing 50 mM-potassium phosphate buffer, pH 7·5, 50 μg. of pyridoxal phosphate and 1 μg. of antimycin. After the addition of the enzyme preparation the volume was 1 ml. The reaction was run, with occasional shaking, for 45 min. at room temperature and was stopped by heating the reaction mixture for 2 min. at 100°. The protein was removed by centrifuging for 5 min. at 3000*g* and the deproteinized reaction mixture was analysed for its amino acid content by paper chromatography.

Analytical procedures. Protein determinations were made by the biuret method as described by Cleland & Slater (1953), with crystalline egg albumin as a standard.

Pyruvate was determined spectrophotometrically with NADH and lactate dehydrogenase by measuring the decrease of extinction at 340 mμ. For the determination of the sum of oxaloacetate and pyruvate, malate dehydrogenase was added to this system.

Succinate was determined manometrically as described by Borst (1962).

Glutamate was determined manometrically with glutamate decarboxylase (Gale, 1945). The reaction medium contained 150 mM-sodium acetate buffer, pH 5·0, and up to 5 μmoles of glutamate; 4 mg. of glutamate decarboxylase, dissolved in 0·1 ml. of 0·1 M-sodium acetate buffer, pH 5·0, was placed in the side arm. The final volume was 1·1 ml., the gas phase air and the temperature 25°. After temperature equilibration the enzyme was added to the main compartment of the manometer flask and readings were taken until a constant level was reached (10–20 min.).

α-Oxoglutarate was determined by the spectrophotometric method of Slater & Holton (1953).

Citrate and isocitrate were determined spectrophotometrically by conversion into α-oxoglutarate (Ochoa, 1948). To the reaction medium in the test cuvette (1·90 ml.) con-