The Cause of Loss of Viability of Airborne *Escherichia coli* K 12

By C. S. COX*

Microbiological Research Establishment, Porton Down, Salisbury, Wiltshire

(Accepted for publication 8 March 1969)

SUMMARY

The uptake of uracil, pyruvate, methionine and oxygen and the breakdown of RNA and protein have been examined in *Escherichia coli* K 12 HfrC after storage as an aerosol for 15 min. in nitrogen at 80 % and 40 % relative humidity (RH). At 80 % RH there was greatest loss of viability and almost complete breakdown of RNA together with severe inhibition of energy production. At 40 % RH all these effects were mitigated.

INTRODUCTION

Microbial survival has been reviewed by Anderson & Cox (1967). The aerosol survival of *Escherichia coli* K 12 has been discussed in detail by Cox (1968*b*). At high RH values in atmospheres devoid of oxygen, critical minima occur in the survival versus RH curves (Cox, 1966). These minima may be caused by failure of RNA synthesis, of protein synthesis or of energy production (Cox, 1968*b*). This paper describes further work on the cause of death in *E. coli* K 12 HfrC aerosols.

METHODS

Growth of organisms. *Escherichia coli* K 12 HfrC was grown in a 2 % tryptone medium (pH 7.2) for 16 hr at 37° in a shaken flask. The bacteria were centrifuged and resuspended in glass-distilled water to 10^{11} bacteria/ml. before spraying.

Apparatus. The aerosol was produced by means of a 3-jet collision spray (Collison, 1935) operated with nitrogen (> 99.9 %). It was mixed with more nitrogen of controlled temperature ($26.5^\circ \pm 0.2$) and RH, and flowed into a 75 l. rotating drum (Goldberg, Watkins, Boerke & Chatigny, 1958). The 3-jet spray was used because high densities of bacteria were required in some of the experiments. Control of RH was not as good as with the 1-jet spray (Cox, 1968*a*) but neither was it so critical with the present organism (Cox, 1968*b*). Aerosol samples after storage for 15 min. were collected in a Porton 'raised' impinger (May & Harper, 1957) containing 10 ml. of collecting fluid, and the bacterial concentration estimated optically. Phosphate buffer (Cox, 1966) or minimal growth medium were collecting fluids. The minimal growth medium contained 1.0 % (w/v) glycerol; 0.5 % ammonium citrate; 1.0 % $K_2HPO_4 \cdot 3H_2O$; 0.05 % $MgSO_4 \cdot 7H_2O$; 0.05 % NaCl; 0.005 % ferric ammonium citrate adjusted to pH 7.0; with NaOH.

Uptake of labelled substrates. Suspensions (2.5×10^8 bacteria/ml.) either collected from the aerosol or made up from non-aerosolized controls, both in minimal growth

* Present address: Fort Detrick, Frederick, Maryland, U.S.A.

medium, were incubated at 37° in separate shaken flasks containing 0.025 µC/ml. of one of the following substrates: [¹⁴C]uracil (for labelling RNA), Na[¹⁴C]pyruvate (for determining the general metabolic rate of the bacterial cell), [¹⁴C]thymidine (for labelling DNA) or [³⁵S]methionine (for labelling protein) (The Radiochemical Centre, Amersham, Bucks). Samples taken at intervals were filtered through a Millipore filter, which was subsequently well washed with ice-cold 5 % trichloroacetic acid; the filtrates combined with the washings were assayed for radioactivity at room temperature by a coincidence scintillation technique (Anderson & Smith, 1965). The amount of each substrate incorporated into the bacteria was then estimated by the decrease in the amount of radioactivity in the corresponding filtrate (Benbough, 1967).

Breakdown of RNA or protein. Bacteria containing labelled RNA or protein were grown by adding 0.05 MC of ¹⁴C-uracil or 0.1 MC. of ³⁵S-methionine to 100 ml. of the tryptone medium at the time of inoculation with *Escherichia coli* K12 HfrC. After aerosolization and collection into minimal growth medium, the amount of labelled uracil or methionine released by the bacteria on incubation was followed by a procedure similar to that detailed above.

Oxygen uptake. The aerosol was collected into phosphate buffer (Cox, 1966) to give 5×10^8 bacteria/ml. Oxygen uptake at 37° was determined by the Warburg technique with tryptone medium in the flask, bacteria in the side-arm and concentrated sodium hydroxide in the centre-well, and compared with the uptake from non-aerosolized bacteria.

RESULTS

RNA metabolism. Survival was zero after 15 min. in aerosol at 80 % RH and a small initial uptake of uracil was followed by zero uptake; at 40 % RH the survival was 18 % and there was a much greater uptake of uracil (Fig. 1). Hence at 80 % RH RNA synthesis was drastically reduced, while, in accordance with the levels of survival, at 40 % RH RNA synthesis was greater. At 80 % RH considerable breakdown of pre-labelled RNA occurred (Fig. 2); at 40 % RH, where survival was higher, the amount of RNA breakdown was less.

Protein metabolism. Figure 3 indicates that the uptake of pyruvate was only slightly inhibited by aerosolization at 40 % RH, while at 80 % RH the uptake was less, but still quite appreciable. The uptake of methionine was considerably inhibited at both RHs (Fig. 4). At 80 % RH release of [³⁵S]labelled protein was slight while at 40 % RH

Fig. 1. [¹⁴C]uracil uptake by *Escherichia coli* K12 HfrC. ×, Control unaerosolized bacteria; ⊙, bacteria stored in nitrogen for 15 min. at 80 % relative humidity; □, bacteria stored in nitrogen for 15 min. at 40 % relative humidity.

Fig. 2. Breakdown of prelabelled [¹⁴C]RNA by *Escherichia coli* K12 HfrC. ×, Control unaerosolized bacteria; ⊙, bacteria stored in nitrogen for 15 min. at 80 % relative humidity; □, bacteria stored in nitrogen for 15 min. at 40 % relative humidity.

Fig. 3. Sodium [¹⁴C]pyruvate uptake by *Escherichia coli* K12 HfrC. ×, Control unaerosolized bacteria; ⊙, bacteria stored in nitrogen for 15 min. at 80 % relative humidity; □, bacteria stored in nitrogen for 15 min. at 40 % relative humidity.

Fig. 4. [³⁵S]methionine uptake by *Escherichia coli* K12 HfrC. ×, Control unaerosolized bacteria; ⊙, bacteria stored in nitrogen for 15 min. at 80 % relative humidity; □, bacteria stored in nitrogen for 15 min. at 40 % relative humidity.

Fig. 5. Oxygen uptake by *Escherichia coli* K12 HfrC. ×, Control unaerosolized bacteria; ⊙, bacteria stored in nitrogen for 15 min. at 80 % relative humidity; □, bacteria stored in nitrogen for 15 min. at 40 % relative humidity.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

it was extremely small and very comparable to that from an unsprayed control. Loss of viability therefore did not arise from loss of protein.

Oxygen uptake. A severe inhibition of oxygen uptake occurred for bacteria collected from the aerosol at 80 % RH, while at 40 % RH the degree of inhibition was less, but still significant (Fig. 5).

DISCUSSION

Aerosol loss of viability was greatest in the region of 80 % RH and least in the region of 40 % RH (Cox, 1968*b*) and these values were therefore chosen for the present studies.

Most of the RNA, as measured by the release of radioactivity, was broken down following aerosolization at 80 % RH. The failure of the bacteria to take up oxygen following recovery from the aerosol meant that no energy was available for resynthesis of RNA, and inevitably led to death of the organism. At 40 % RH RNA breakdown was incomplete, and oxygen uptake, although sub-normal, was still evident.

An unstressed bacterium contains RNA complexed with protein and also ribonuclease, which normally does not break down the ribosomal RNA. However, if in the aerosol the RNA-protein complex dissociates then the RNA can be attacked and broken down by the enzyme.

Water movement in the region of 80 % RH might cause a dissociation of the RNA-protein complex; Rich & Watson (1954) have suggested that RNA undergoes structural changes depending upon RH. Previous evidence reviewed by Cox (1968*b*) pointed to damage on rehydration following collection from the aerosol; on this basis rehydration caused dissociation of the RNA-protein complex, with the other changes following. The higher survival at 40 % RH is only to be expected considering the all-round improvement in metabolism; the main puzzle is why rehydration from 40 % RH should be so much less damaging than rehydration from 80 % RH.

The author thanks Mr I. H. Silver for his interest in this work and thanks Mr C. M. Saunders for technical assistance.

REFERENCES

- ANDERSON, J. D. & COX, C. S. (1967). Microbial survival. *Symp. Soc. gen. Microbiol.* **17**, 203.
ANDERSON, J. D. & SMITH, H. (1965). The metabolism of erythritol by *Brucella abortus*. *J. gen. Microbiol.* **38**, 109.
BENBOUGH, J. E. (1967). Death mechanisms in airborne *Escherichia coli*. *J. gen. Microbiol.* **47**, 325.
COLLISON, W. E. (1935). *Inhalation Therapy Technique*. London, William Heinemann (Medical Books).
COX, C. S. (1966). The survival of *Escherichia coli* atomized into air and into nitrogen from distilled water and from solutions of protecting agents, as a function of relative humidity. *J. gen. Microbiol.* **43**, 383.
COX, C. S. (1968*a*). The aerosol survival of *Escherichia coli* B in nitrogen, argon and helium atmospheres and the influence of relative humidity. *J. gen. Microbiol.* **50**, 139.
COX, C. S. (1968*b*). The aerosol survival and cause of death of *Escherichia coli* K12. *J. gen. Microbiol.* **54**, 169.
GOLDBERG, L. J., WATKINS, H. M. S., BOERKE, E. E. & CHATIGNY, M. A. (1958). The use of a rotating drum for the study of aerosols over extended periods of time. *Am. J. Hyg.* **68**, 85.
MAY, K. R. & HARPER, G. J. (1957). The efficiency of various liquid impinger samplers in bacterial aerosols. *Br. J. ind. Med.* **14**, 287.
RICH, A. & WATSON, J. D. (1954). Some relations between RNA and DNA. *Proc. natn. Acad. Sci. U.S.A.* **40**, 759.