191. *The Uptake of Alkali Metals by Bacteria.*

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By means of radio-active tracers the uptake of potassium, rubidium, and sodium ions by a strain of *Bact. lactis aerogenes* growing on a single synthetic medium has been investigated.

The heaviest growth is supported by potassium, but the cells can also take up rubidium and they can accumulate potassium and rubidium in the ratio 5 : 2 from a medium containing roughly equal concentrations of these two ions. They are, however, unable to take up detectable amounts of sodium from a mixture of sodium and rubidium. The accumulation of rubidium in the bacteria under the conditions of the test amounted to about 60% of the total present. Previous work has shown the corresponding figure for potassium to be 97%.

That the ion which actually finds its way into the cells is really rubidium was verified by measuring the radio-active decay constant of bacteria grown in presence of active rubidium and subsequently dried.

It has previously been shown (Danby, Eddy, and Hinshelwood, *Proc. Roy. Soc.*, *B*, 1950, **136**, 544) that, as might be expected from much previous work on cells of different kinds, the presence of potassium is essential for the growth of *Bact. lactis aerogenes* in various synthetic media containing simple carbon and nitrogen sources. (References to the work of Conway and O'Malley, Leibowitz and Kupermintz, Pulver and Verzar, and others are given in the publication cited.) With the available chemical reagents an amount of potassium sufficient to support a small bacterial population (60 millions/ml.) was always present but further additions of potassium chloride increased this population linearly. Of the other alkali ions, rubidium alone seemed capable of filling the rôle of potassium in the cell, additions of rubidium chloride raising the population, though less effectively than did corresponding amounts of potassium chloride.

A detailed study of the relations between the bacterial population and the initial hydrion and potassium concentrations in glucose–ammonium sulphate media showed that hydrion antagonises the action of potassium. The facts were confirmed by measurements of the uptake by the cells of the radio-active isotope ^{42}K. In the range where the population was dependent on the potassium concentration the bacteria could take up 97% of the potassium available in the medium. This could be washed out of the cells by acid.

In the present work these observations have been confirmed and extended. In the first place, proof was desired that rubidium did in fact enter the cells and that the growth had not been due to impurities of potassium. ^{86}Rb was therefore added to the medium. From measurements of the activity of the final culture before and after centrifugation and from the activity of the separated bacteria, it was shown that radioactivity was present in the cells. The magnitude of the decay constant, moreover, confirmed that this was in fact due to rubidium. In the second place, comparisons were made of the uptake of the three ions sodium, potassium, and rubidium by use of the appropriate isotopes. The result showed that rubidium can be taken up in competition with potassium but less readily, while sodium is not taken up at all from a mixture of sodium and rubidium.

Methods.—The methods for the cultivation of the bacteria were those previously described (reference above), a standard strain of *Bact. lactis aerogenes* being maintained by serial subculture at 40·0° in a medium containing glucose, ammonium sulphate, magnesium sulphate, and an ammonium phosphate buffer. For the experiments to be described suitable additions of the appropriate alkali metals were made to the medium as required. The changes in cell population were determined by a turbidimetric technique calibrated with suspensions of known count.

The radio-active alkali metals were obtained by neutron irradiation of the carbonates at A.E.R.E., Harwell. The weighed samples of active materials were neutralised with the calculated quantity of acid and standard solutions made up. From activity measurements on these standards and the known half-lives of the radio-elements the relation between the observed rate of count of a sample at any time and the concentration of the corresponding alkali metal could be obtained.

The activity measurements were made with a liquid-type Geiger counter of about 10-ml. capacity. The electronic counting equipment followed conventional lines and was built in the laboratory. The recovery time of each Geiger tube was measured with a calibrated oscilloscope and a paralysis–time circuit in the amplifier set to a value some 10% greater, the appropriate corrections being applied to the observed rates of count.

The problem arose of determining the activity of a bacterial suspension which had been separated from an active supernatant liquid. Since it is impossible to remove all adhering fluid by centrifugation, and washing of the cells is not permissible, a method similar to the method of residues in phase-rule studies was adopted. For example, 10 ml. of a suspension were centrifuged and 9 ml. of the cell-free supernatant liquid taken for test. The remaining 1 ml. was left with the cells. From a knowledge of the activity of this concentrated suspension and of the supernatant liquid itself the activity of the cells themselves is calculable.

The similarity between the half-lives of ^{24}Na (14·8 hours) and ^{42}K (12·4 hours), and the fact that they are both γ-emitters, makes it difficult to analyse a mixture of the two either by measurement of the decay curves or by absorption measurements. But each component in a mixture of ^{86}Rb and ^{24}Na, or of ^{86}Rb and ^{42}K, can be readily estimated, since ^{86}Rb is a simple β-emitter and has a half-life of 19·5 days. Accordingly, the form of the composite decay curves was used to determine the amounts of the individual ions taken up by cells grown on the one hand in mixtures of rubidium and sodium and on the other in mixtures of rubidium and potassium.

There is, in such experiments, the possibility that one of the various alkali ions, once they reach low concentrations in the medium, might be selectively taken up by the cells. It is even more likely that one or the other might suffer displacement by hydrion if the medium were allowed to become acid. To ensure that neither of these factors came into play conditions were so chosen that the maximum bacterial population which the medium could support was limited by the glucose concentration. At the value chosen there were always appreciable amounts of alkali ion remaining outside the cells, and the pH changed little from its initial value of 7·10.

Experimental.

(1) *Growth with Rubidium only.*—Tubes containing medium with the appropriate amounts of added active rubidium were inoculated and at the suitable stage of growth the contents were centrifuged, samples of 10 ml. being used. 9 Ml. of the supernatant liquid were drawn off, the remaining 1 ml. being left with the total amount of cell material. The samples were tested in a liquid counter, the original culture and the separated cells after dilution in a known ratio so as to obtain a convenient counting rate (4000—10,000 counts/minute), and the supernatant liquid after being made up to a standard volume with water. From the various counts and dilution factors the activity in cells and (supernatant liquid was calculated. Two independent values for the activity of the cells are obtainable, one from (separated cells with 1 ml. of liquid) and (supernatant liquid), the other from (total culture) and (supernatant liquid). The results are given in Table I (see next section).

The bacteria from one of the samples were washed and dried in a small aluminium dish, and the rate of count was determined at intervals over a period long enough to give the decay constant. The decay was compared with that of standard ^{86}Rb samples prepared from the stock solution directly.

(2) *Growth with Rubidium and Sodium.*—A culture medium was made up to contain approximately 10 g.-ions of active Rb and 5 g.-ions of active Na per l. The general procedure was then as described for the experiments with rubidium alone. The activities of total cultures, supernatant liquids, and separated cells were all measured (*a*) as soon as possible after growth and (*b*) after 5 days, by which time only the rubidium contributes appreciably to the total activity (^{24}Na and ^{42}K fall to about 10^{-3} of the initial activity in 6 days). By extrapolating back the readings from (*b*) one can obtain the activity due to Rb at the time of (*a*) and so the activity due to the ^{24}Na at that time. Both the activities are then extrapolated to that time at which the growth of the culture is deemed to have terminated. Factors for conversion of activity to concentration were obtained by measurements on the standard solutions and referred in each case to zero time. Results are given in Table II.

(3) *Growth with Rubidium and Potassium.*—Procedure was similar to that described under (2). The calculations are somewhat more elaborate since, in contrast with the previous case, the bacteria prove to contain both metals. The contribution from each to the activity of each kind of sample has to be worked out for various times. Extrapolation to zero time in the usual way is then possible. The results are given in Table III.

Results.

(1) *Rubidium.*—The measurements recorded in Table I are values corrected for the various dilution factors. The different horizontal lines refer to various cultures sampled when growth

had proceeded to a bacterial population which is expressed in the first column as a fraction of what the particular medium could have supported had time been allowed.

It is evident that the cells take up as much as 60% of the total rubidium supplied in the medium, but that after growth has ceased they give it up again. Previous experiments with potassium showed that the loss is due to the formation of acid in the medium by the continued fermentation of the glucose.

TABLE I.

Uptake of rubidium.

Medium contained : inactive potassium, $1{\cdot}1 \times 10^{-5}$ *g.-ions/l. ; radio-active rubidium,* $9{\cdot}9 \times 10^{-5}$ *g.-ions/l. ;* M/40*-ammonium ion (buffer constituent).*

Growth ceased at bacterial population of 240 *millions/ml.*

Bacterial population, % of max.	Activity of whole culture.	Activity of supernatant liquid.	Activity of cells + 1 ml. of liquid.	% of total Rb taken up by cells, direct.	by difference.
75	21·2	10·5	10·7	45·7	50·5
79	20·4	10·2	10·6	46·8	50·2
85	21·3	10·3	—	—	51·6
94	20·3	8·9	12·0	54·8	55·6
98	21·1	8·8	12·3	53·9	58·4
100	20·6	8·3	12·4	56·2	59·7
12 hrs. later	22·1	25·0	1·8	0	0
12 hrs. later	22·7	25·2	1·7	0	0

(All activities are given as counts/min. $\times 10^{-4}$.)

TABLE (II).

Growth in presence of rubidium and sodium.

Medium also contained : inactive potassium, $1{\cdot}1 \times 10^{-5}$ *g.-ions/ml. ;* M/40*-ammonium ion (buffer constituent).*

Growth was restricted to about 100 *millions/ml. by limitation of glucose, otherwise* 200 *millions/ml. would have been reached.*

Alkali-metal concns., gm.-ions/l. $\times 10^5$.				Alkali metal in cells (expressed as an equivalent concn. in the medium).			
Whole culture,		Supernatant liquid,		Direct measurement,		By difference,	
Rb.	Na.	Rb.	Na.	Rb.	Na.	Rb.	Na.
13·0	4·9	10·5	5·5	2·1	0	2·5	−0·6
12·6	6·3	10·4	6·3	—	—	2·2	0·0
11·9	6·5	10·0	7·9	1·7	0	1·9	(−1·4)

TABLE III.

Growth in presence of rubidium and potassium.

Medium also contained : inactive potassium, $1{\cdot}1 \times 10^{-5}$ *g.-ions/l. ;* M/40*-ammonium ion (buffer constituent).*

Growth was restricted to 200 *millions/ml. by limitation of glucose, otherwise* 400 *millions/ml. would have been reached.*

Alkali-metal concns. g.-ions/l. $\times 10^5$.				Alkali metal in cells (expressed as equivalent concn. in medium).			
Whole culture,		Supernatant liquid,		Direct measurement,		By difference,	
Rb.	K.	Rb.	K.	K.	K.	Rb.	K.
9·9	10·9	6·1	2·9	2·7	5·6	3·8	8·0
8·9	13·8	5·1	2·9	—	—	3·8	10·9

The decay constant corresponding to a half-life of 19·9 days makes the identification of the alkali metal in the cells with rubidium quite certain.

(2) *Rubidium and Sodium.*—Here growth was limited by exhaustion of the glucose, the concentration of this being kept low to prevent fermentation at the end of growth. Consequently the total uptake of alkali is proportionately less than in (1). No sodium could be found in the cells in the direct test. The amount found by the difference method was either zero or, as a result of manipulative losses, negative (Table II).

(3) *Rubidium and Potassium.*—Here again growth was deliberately controlled by limitation of glucose supply so that rubidium and potassium should complete on approximately equal terms throughout. The total uptake is therefore far less than it could be. The amount of

rubidium consumed is approximately 40% of the potassium. The actual form of the radioactive-decay curves confirmed the presence of the two metals with their respective half-lives (Table III).

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