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Uptake of Potassium Tellurite by a Sensitive Strain of *Escherichia coli*

By P. D. COOPER and A. V. FEW
Wright-Fleming Institute of Microbiology, St Mary's Hospital Medical School, Paddington, W. 2

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As early as 1913 Schurmann & Hajos added potassium tellurite to a differential medium for the isolation of the diphtheria bacillus, and this use of potassium tellurite has now become familiar. Cavazutti (1921) and Joachimoglu (1920, 1922) found that in general Gram-positive organisms were less affected by tellurite than Gram-negative, and that the activity of related compounds decreased in the order $TeO_3^{2-} > SeO_3^{2-} > TeO_4^{2-} > SeO_4^{2-}$. Fleming (1932, 1942) demonstrated the value of potassium tellurite when used with penicillin and gentian violet for the isolation of certain bacteria from badly contaminated sources. Penicillin and potassium tellurite were both active at high dilution on entirely different bacterial species so that, with a few exceptions, bacteria which were penicillin-sensitive were tellurite-resistant and vice versa. Strains of *Escherichia coli* sensitive to tellurite could rapidly acquire resistance by serial subcultivation in increasing concentrations of the salt (Fleming & Young, 1940) and the antigenic and fermentation characteristics of both sensitive and naturally resistant strains of *Esch. coli* appeared to be identical. There was also some evidence of a very high absorption of tellurite by actively growing bacteria. Growth on tellurite agar yields black colonies, and granules can be seen inside the cells which are presumably composed of elementary tellurium (Hewitt, 1951).

In the present communication, as an approach to the means by which potassium tellurite (K_2TeO_3) inhibits bacterial growth, the mechanism of assimilation of potassium tellurite by bacterial suspensions is studied by the quantitative determination of the uptake using ^{127}Te incorporated into potassium tellurite as radioactive tracer.

Although the tellurium was added to the bacterial suspensions as the alkaline salt K_2TeO_3, the true substrate of the assimilation process at pH 5·5 may be TeO_2, H_2TeO_3 or $HTeO_3^-$. The pK_a values of the two dissociation stages of tellurous acid were found to be 8–9 and > 10, so that only about 0·1 % of H_2TeO_3 is ionized at pH 5·5. Whatever the nature of this substrate, however, or of the final chemical form in which Te accumulates in the cells, uptakes and concentrations have been expressed throughout in terms of K_2TeO_3.

METHODS

Preparation of radioactive potassium tellurite

Acidic residues, containing radioactive Te obtained in the preparation of $^{131}I_2$, were supplied by the Atomic Energy Research Establishment, Harwell, with an activity of approximately 5 mc. in 100 ml. Oxalate, sulphate and chromic ions were also present, and elementary Te was slowly precipitated from the acid solution by the addition of 10 g. of $Na_2S_2O_5$ to 50 ml. of residues. After 48 hr. the precipitation appeared to be complete and the Te was separated and washed five times with distilled water. The moist Te was dissolved in a slight excess of 50 % (v/v) HNO_3, and the solution was cleared by centrifuging and diluted with distilled water to 6 ml. On addition of 20 ml. of ethanol a flocculent white precipitate of TeO_2 appeared. After standing overnight this was washed three times with distilled water in the centrifuge and dried for 24 hr. *in vacuo* over P_2O_5. The product was weighed, and the theoretical amount of 1 % (w/v) KOH solution added to form K_2TeO_3. The solution was finally diluted to a concentration of 1000 μg. K_2TeO_3/ml.

The overall yields of K_2TeO_3 were 100–150 mg., with an initial specific activity of about 0·5 μc./mg. and a radioactive recovery of 2 %. The half life of this solution corresponded exactly with that of ^{127}Te (90 days).

The concentration of the radioactive K_2TeO_3 solution was then confirmed by the colorimetric method of Shakhov (1945) and by isotope dilution with a standard 0·1% (w/v) K_2TeO_3 solution.

These determinations showed that the saturation concentration of K_2TeO_3 at 18° and pH 5·5 was 120–150 μg./ml. Accordingly, no experiments were conducted at a higher concentration than 100 μg./ml.

As a sample of commercial potassium tellurite was found to contain more than 30% excess of the theoretical quantity of TeO_2, the standard solution of K_2TeO_3 used above was prepared from dried TeO_2 obtained from the commercial salt by precipitation with acid.

Preparation of bacterial suspensions

One of the organisms most sensitive to tellurite among ten strains tested was found to be *Esch. coli* D. 433, from the National Collection of Type Cultures, and this has been used throughout these experiments. Saline suspensions were prepared from a 16 hr. agar slope, and used for inoculation of 250 ml. of the semi-synthetic medium described below. After incubation in Roux bottles at 37° for 16–18 hr., the cultures were harvested and washed three times with distilled water at 4°, yielding approx. 60 mg. dry wt. of bacteria/250 ml. medium.

1 l. of the medium contained 1 g. KH_2PO_4, 0·7 g. $MgSO_4.7H_2O$, 1 g. NaCl, 4 g. $(NH_4)_2HPO_4$, 0·5 g. trisodium citrate, and 0·5 mg. $FeCl_3$, together with 100 ml. of a neutralized acid casein hydrolysate containing 14·2 mg. N/ml. and 9·1 mg. PO_4^{3-}/ml., prepared by the method of Mueller (1939). NaOH solution was added to pH 6 and the medium was autoclaved in 250 ml. screw-capped bottles. The pH was unchanged by sterilization and growth.

Measurement of uptake

The procedure was similar to that previously used in this laboratory for the determination of the uptake of radioactive penicillin by bacteria (see Rowley, Cooper, Roberts & Smith, 1950). The washed bacterial suspensions, at approximately the required concentration, were aerated for 1 hr., as this procedure was found to increase tellurite uptake. A 0·1 ml. sample was diluted to 10 ml. for assay of bacterial dry wt./ml. on a calibrated Hilger absorptiometer. Samples (1 ml.) in centrifuge tubes were then incubated with buffer solution for 15 min. before addition of K_2TeO_3 and further buffer up to 5 ml. After a given time at 37° the tubes were cooled in ice-water, centrifuged rapidly (8 min. at 3500 *g*) and washed three times in distilled water after which the final supernatant solutions were entirely free of radioactivity. Care was taken not to remove bacteria with the supernatant solution. The washed bacteria were transferred to calibrated tubes and made up to 2 ml. with distilled water, and two 0·25 ml. samples were allowed to dry on planchettes for radioactive assay. As no attached radioactivity was found to be lost by the washing process, the uptake could then be calculated in terms of μg. K_2TeO_3/mg. bacterial dry wt., knowing the specific activity of the K_2TeO_3 and the initial bacterial concentration.

Phosphate buffer (0·03 M) at the optimum pH (5·5) and temperature (37°) was employed, unless otherwise stated. Preliminary experiments were made to ensure that the total amount of tellurite removed from the solution did not decrease its concentration by more than 3%, which was regarded as an insignificant decrease as its effect was well within experimental error. This generally involved the use of bacterial concentrations <2 mg./ml. with tellurite concentrations of 40 μg./ml. and a reaction time of 45 min.

Radioactive assay

This was performed on the same apparatus used previously (Rowley *et al.* 1950). Total counting rates were generally of the order of 100/min., and duplicate planchettes were each counted for two 10 min. periods at different times during the day. The probable counting error is approx. $\pm 2{\cdot}0\%$ for each sample. A background count (approx. 14/min.) was taken for 60–80 min. at midday and the value obtained was subtracted from the average total count for each sample. A uranium standard planchette was counted daily at three different voltages to determine plateau stability, together with a Te standard three times during the day. With Te no self-absorption correction was found to be necessary with the layer thicknesses used. Allowance was made for radioactive decay when calculating the weight of K_2TeO_3 bound by the cells.

The total error associated with the bacterial uptakes of radioactive Te was found to be approximately $\pm 10\%$, composed of the counting error ($\pm 2\%$), the absorptiometer error of $\pm 3\%$ and the sampling error.

RESULTS

Non-removal of ^{127}Te *from* Escherichia coli *by washing with distilled water.* The amount of ^{127}Te attached to the organisms after incubation with radioactive K_2TeO_3, but before the organisms were washed, was calculated from the difference between radioactive assays of the bacterial suspension of known bacterial concentration and the supernatant solution after centrifuging. The supernatant solution was discarded and the bacteria resuspended to the same concentration as initially. The suspension was then re-assayed, centrifuged, and the supernatant re-assayed. This was repeated several times and the uptakes were found to be constant within experimental error.

The effect of temperature variation. The uptake of K_2TeO_3 by *Esch. coli* was found to be dependent upon the temperature at which the reaction between the bacterial suspensions and K_2TeO_3 occurred. Fig. 1 shows that the uptake was maximal at about 37°, falling off at higher or lower temperatures.

The activity of the bacteria in suspensions at different pH, *and in different buffers.* Fig. 2 shows the effect of variation of pH in the suspending medium on the uptake of K_2TeO_3.

The activity was maximal at pH 5·5 whether the sample was aerated beforehand or not, although aeration markedly increased the uptake by the organisms. A mixture of 0·03 M-acetate and 0·03 M-phosphate buffers was employed to ensure pH stability over the range 4·7–6·5. The use of 0·03 M-acetate, phosphate and succinate buffers gave approximately the same uptake at the optimum pH, but citrate buffer lowered the uptake by about

75 %. Sodium citrate was also effective in preventing the precipitation of TeO_2 from K_2TeO_3 between pH 3 and 7 and it seems likely that a complex is formed between these salts.

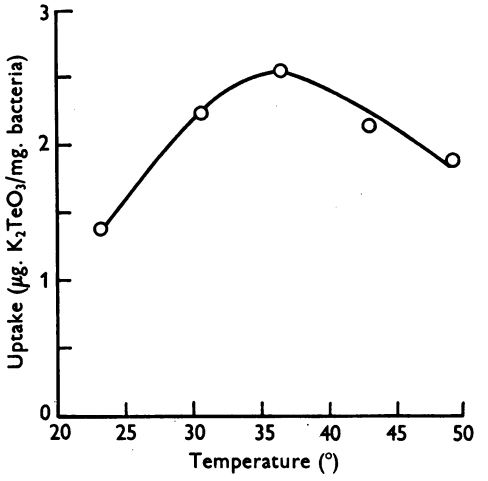

Fig. 1. Uptake of K_2TeO_3 on *Esch. coli* after incubation for 45 min. at different temperatures in 0·03 M-phosphate (pH 5·5) containing 40 μg./ml. K_2TeO_3.

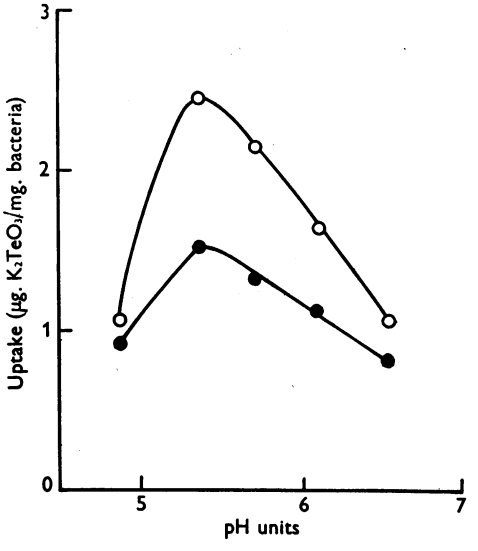

Fig. 2. Variation of uptake of K_2TeO_3 on *Esch. coli* with pH of the suspending medium. Organisms were incubated for 45 min. at 37° in a mixture of 0·03 M-phosphate and 0·03 M-acetate containing 40 μg./ml. K_2TeO_3. ○—○, one sample of bacteria aerated for 1 hr. at 18° before incubation; ●—●, the residue allowed to stand in contact with air at 18° concurrently.

In general, therefore, when experiments were performed at pH 5·5, 0·03 M-phosphate buffer was employed as this seemed without effect on the uptake.

Other factors affecting the activity of the system responsible for tellurite assimilation. The exposure of the bacterial suspensions to low pH for 1 hr. at 37° before adjusting to pH 5·5 caused a considerable decrease in the power of the organisms to assimilate K_2TeO_3 (Fig. 3). No loss of activity occurred at

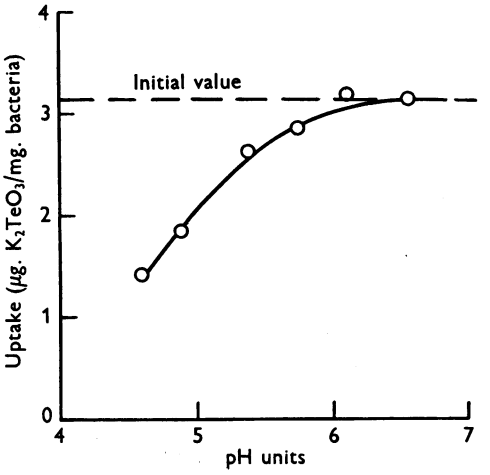

Fig. 3. Effect of pH on the stability of the system in *Esch. coli* responsible for tellurite uptake. Organisms were suspended in a mixture of 0·03 M-phosphate and 0·03 M-acetate for 1 hr. at 37°, centrifuged rapidly and resuspended in warm 0·03 M-phosphate at pH 5·5. K_2TeO_3 was then added to 40 μg./ml. and the uptake was assayed after a further 45 min. at 37°.

pH 6–7 and only about 10 % at the working pH of 5·5. When the activity of the organisms was determined after different times of incubation at pH 5·5 there appeared to be a slight loss after several hours, but during the uptake period of 45 min. usually employed the loss was negligible (Table 1).

Table 1. *Rate of inactivation at* pH 5·5 *of the system responsible for the uptake of* K_2TeO_3 *by* Esch. coli

(K_2TeO_3 was added to a concentration of 40 μg./ml. after varying times of incubation at pH 5·5, and the radioactivity on the bacteria was assayed after a further 45 min. at 37°.)

Time (hr.)	Uptake (μg./mg.)
0	3·3
1	3·3
2·25	3·1
3·5	2·9
4·75	2·3
22	1·5

As bacteria contain easily oxidizable substances which interfere with processes such as methylene-blue reduction (Quastel & Whetham, 1925) it seemed advisable to determine the effect of removal of these substances before the addition of K_2TeO_3,

since this substance is also easily reduced. Accordingly, air was rapidly bubbled through the bacterial suspensions for 1 hr. at room temperature before commencing the experiment. In contrast with the effect on the rate at which methylene blue was reduced by bacteria, aeration was found to increase the tellurite uptake whilst treatment with nitrogen decreased it in relation to a suspension left in contact with air (Table 2). Treatment with nitrogen for 1 hr. after aeration gave uptake values which were identical with those of the aerated organisms.

Table 2. *Effect of aeration on the uptake of* K_2TeO_3

(Portions of an *Esch. coli* suspension at pH 5·5 were simultaneously stood in air, bubbled with air and bubbled with N_2 respectively for 1 hr. at room temperature. Half of the aerated sample was then bubbled with N_2 for a further hour, K_2TeO_3 was added to 40 μg./ml. to all four samples and the uptake was measured after a further 45 min. at 37° during which time the N_2 treatment was continued.)

Treatment	Uptake (μg./mg.)
N_2 bubbled	1·6
Stood in air	2·3
Air bubbled	2·8
Air bubbled for 1 hr. then N_2 for 1 hr.	2·7

The effect of the inclusion of glucose in the growth medium on the ability of Esch. coli *to assimilate tellurite.* Glucose was at first included in the growth medium in order to increase the yield of *Esch. coli*, but it was found that the resultant organisms had a lowered capacity to take up K_2TeO_3. Fig. 4 shows that as little as 0·05 % glucose markedly inhibited the formation of the tellurite-absorbing system so that the uptake was lowered whether the organisms were aerated before incubation with K_2TeO_3 or not. At this low glucose concentration the pH of the growth medium was scarcely altered after growth of the organisms so that the inhibition was not due to decomposition caused by fermentation acids before harvesting.

The further decrease in activity at higher glucose concentrations may be due to decomposition caused by acidity.

The effect of variation of the bacterial concentration on the uptake. When K_2TeO_3 at 40 μg./ml. was incubated for 45 min. at 37° with varying concentrations of *Esch. coli*, the uptake per mg. dry weight was found to be independent of the bacterial concentration, when this was less than 2 mg./ml. The uptake decreased at higher values, no doubt owing to the removal of a significant fraction of the K_2TeO_3 during uptake.

Variation of uptake with time of contact with tellurite. The amount of K_2TeO_3 attached to *Esch. coli* increased rapidly with time of incubation at 37°, reaching a maximum at 3–4 hr. and decreasing to a very low value after 22 hr. (Fig. 5). The rate of uptake was much less at 1°.

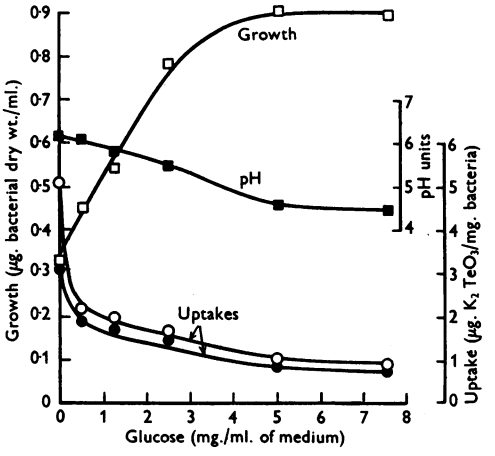

Fig. 4. Effect of inclusion of glucose in the growth medium on the ability of *Esch. coli* to take up K_2TeO_3, after being washed free from the medium. Organisms were incubated at 37° for 45 min. in 0·03 M-phosphate (pH 5·5) containing 40 μg./ml. K_2TeO_3. □—□, bacterial growth in the medium after 16 hr. incubation; ■—■, pH after growth (initial value 6·0); ○—○, uptake after prior aeration for 1 hr. at 18°; ●—●, uptake after standing for 1 hr. at 18° without aeration.

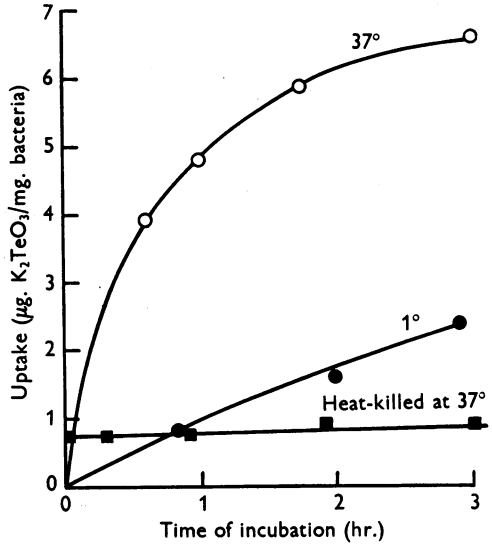

Fig. 5. Rate of uptake of K_2TeO_3 on aerated samples of *Esch. coli* in 0·03 M-phosphate containing 40 μg./ml. K_2TeO_3. ○—○, live organisms at 37°; ●—●, live organisms at 1°; ■—■, heat-killed organisms at 37°.

Variation of rate of uptake with concentration of tellurite. The amount of K_2TeO_3 attached to *Esch. coli* increased with the concentration of K_2TeO_3 in

solution in a manner shown in Fig. 6. When the reciprocal of the rate of uptake at 37° was plotted against the reciprocal of the tellurite concentration, following the procedure of Lineweaver & Burk (1934) for enzyme systems, a straight line resulted. Thus the relationship between the rate of uptake and the tellurite concentration was the same as that between the rate of an enzyme-catalysed reaction and its substrate concentration. The Michaelis constant (K_m) was calculated from this straight line by the method of Lineweaver & Burk.

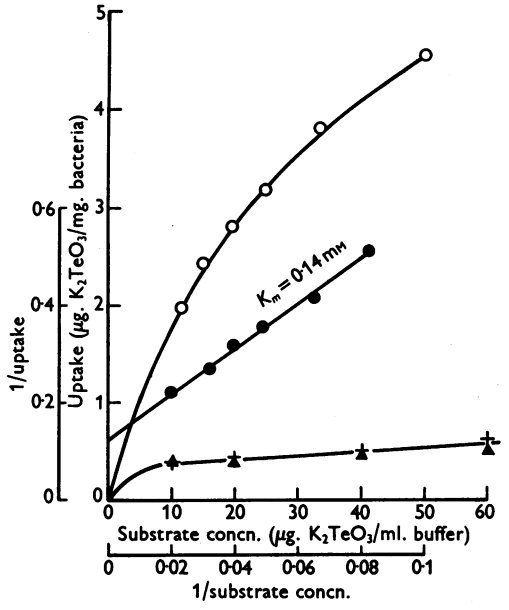

Fig. 6. Variation of uptake of K_2TeO_3 on *Esch. coli* with tellurite concentration. Organisms were incubated at 37° for 45 min. in 0·03 M-phosphate buffer (pH 5·5) containing K_2TeO_3. ▲—▲, heat-killed organisms unaerated; +—+, heat-killed organisms aerated for 1 hr.; ○—○, live organisms aerated for 1 hr.; ●—●, plot of reciprocal uptake against reciprocal tellurite concn. for the experiment using live organisms.

Table 3 shows that such experimental values of the Michaelis constant obtained varied between rather wide limits, but within these limits no difference was noticed whether the organisms were previously (*a*) grown in glucose, (*b*) unaerated, (*c*) aerated or (*d*) aerated and subsequently bubbled with nitrogen. Procedures (*a*) and (*b*) significantly decreased the activity of the organisms compared with the procedures (*c*) and (*d*).

The solubility of K_2TeO_3 at pH 5·5 was too low to allow the K_m to be derived by the alternative method of saturation of enzyme with substrate.

Effect of heat treatment. Immersion of a suspension of *Esch. coli* in a boiling-water bath for 20 min., followed by rapid cooling, altered the rate of uptake by the organisms (Fig. 5). A small amount of tellurite was taken up very rapidly, but after this no further uptake occurred. The amount of tellurite absorbed was also much less dependent upon concentration of the salt (Fig. 6), and the site of attachment appeared nearly saturated at quite low tellurite concentrations. Prior aeration had no effect.

Table 3. *Values for the Michaelis constant of the system responsible for* K_2TeO_3 *uptake on* Esch. coli

K_m (mM)			
Aerated organisms	Unaerated organisms	Aerated and bubbled with N_2	0·05% Glucose added to the growth medium, aerated
0·25	0·23	0·25	0·39
0·094	0·67	—	0·16
0·14	—	—	—
0·14	—	—	—
0·47	—	—	—

DISCUSSION

It has been shown above that potassium tellurite is rapidly assimilated by *Esch. coli* in amounts up to 1% of its dry weight and calculation shows that the concentration in the bacteria can reach 20 times the concentration in the medium. There is thus some mechanism allowing active transfer from the medium into the cells, and it appears very likely that this mechanism is associated with an enzyme or a group of enzymes. However, as Slater (1949) has pointed out, the fact that application of the Lineweaver & Burk equation gives a straight line is not conclusive evidence of an enzymic reaction, nor does this fact show whether or not an enzymic uptake has superimposed upon it an uptake of constant amount similar, for example, to that fixed by heat-killed organisms.

If the organisms were first killed by heat the uptake was radically altered, being much lower and much less dependent on K_2TeO_3 concentration, pH, time and temperature. The uptake curves in this case suggest a rapid chemical combination with the bacteria which is completed at relatively low tellurite concentrations.

The lowered activity when the organisms are grown with glucose suggests that the formation of the enzyme is inhibited in a similar manner to that reported by Epps & Gale (1942) for the formation of several enzymes in *Esch. coli* including certain deaminases and dehydrogenases. In these cases, as with the tellurite absorbing system, only a small part of the inhibition was due to the low pH in the medium induced by glucose.

As passage of nitrogen through the suspensions does not increase activity, the increase caused by aeration is unlikely to be due to the removal of volatile inhibitors. The activation is thus presumably due to the chemical action of oxygen and is not neutralized by the subsequent passage of nitrogen.

It is interesting that this aeration of the suspension before addition of K_2TeO_3 (a process employed by Quastel & Whetham (1925) to remove reducing substances before addition of methylene blue in Thunberg experiments) actually increased the rate of uptake of tellurite. The uptake of K_2TeO_3, which appears to involve a reduction to tellurium, may be similar to the reduction of methylene blue by bacteria observed in the absence of substrate in the Thunberg estimation. Both reactions use up hydrogen donors which are still present in the bacteria after washing and aeration, and one cause of the stimulation of tellurite uptake by oxygen may be due to the inhibition by this gas of alternative processes which utilize and remove the intracellular 'H' donors. This is similar to a suggestion made by Stephenson (1949) as an explanation of the Pasteur effect where yeast fermentation is repressed by oxygen in favour of complete oxidation of the substrates to carbon dioxide.

SUMMARY

1. By incorporating ^{127}Te into potassium tellurite (K_2TeO_3), the amount of the salt absorbed by a buffered suspension of a tellurite-sensitive strain of *Escherichia coli* could be calculated from a measure of the radioactivity remaining on the washed organisms. No radioactivity was lost by washing.

2. The system responsible for tellurite uptake was unstable at low pH but did not deteriorate greatly over several hours at pH 5·5–7.

3. The uptake of tellurite by the organisms increased with time, and was most rapid at pH 5·5 and 37°. The uptake had the properties of an enzyme action.

4. Growth of *Esch. coli* in a medium containing 0·05% glucose decreased the activity of the organisms, while the pH of the medium was scarcely affected.

5. Heating to 100° for 20 min. destroyed the enzymic system. The low uptake then obtained corresponded to a rapid combination with the cells which were saturated at fairly low tellurite concentrations.

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