Active Transport of Magnesium Across the Yeast Cell Membrane

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(*Received* 28 *May* 1957)

In the preceding paper an account has been given of the general relations of the cation carrier in the yeast cell wall with different ions and conditions of transport.

In this the effects of various factors on the active transport of magnesium are described.

EXPERIMENTAL

Analysis of magnesium, potassium and sodium. The methods used were the same as those described in the preceding paper (Conway & Duggan, 1958).

Procedure. Fresh baker's yeast (supplied by the Cork Yeast Co.), was twice washed. A portion (1 g.) of the yeast washed and packed by centrifuging was suspended in 20 vol. of a medium containing 5% (w/v) of glucose and 200 mM-magnesium acetate (or chloride) and fermented for 2 hr. or longer. Before the analytical determinations all samples were washed twice in tap water after centrifuging.

RESULTS

Uptake of magnesium by the cation carrier

Fig. 1, curve *A*, shows the rate at which Mg is taken up by the yeast cell from a suspension, the suspending fluid containing (at zero time) 0·2 M-$MgCl_2$ and 5% of glucose. The suspension was maintained at approx. pH 6·3 (determined by the Beckman glass-electrode apparatus) by the addition from time to time of small volumes of 0·1 N-NaOH, the amount added being recorded.

In Fig. 1, curve *B*, the rate of uptake is shown when magnesium acetate (0·2 M) is used instead of the chloride, the condition being the same but without addition of NaOH solution, the suspension remaining in the region of pH 6·5–6·0. The difference between the two curves after about 4 hr. may possibly arise in part from the additions throughout of small volumes of 0·1 N-NaOH to the magnesium chloride suspension, the Na^+ ions competing appreciably for the carrier.

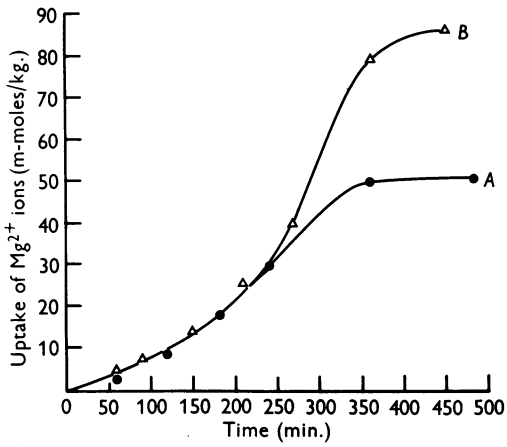

Fig. 1. Rate of uptake of magnesium from 0·2 M-$MgCl_2$ (curve *A*) and 0·2 M-magnesium acetate (curve *B*); 1:20 suspension (w/v), room temperature (average 18°).

Effect on uptake of magnesium of varying the external concentration of magnesium

Results are shown in Fig. 2. It will be seen that the uptake of Mg^{2+} ions as magnesium acetate increases with the concentration of Mg up to a level of about 300 mM. This contrasts with K^+ ions, for which a maximum uptake is reached at about 2 mM in the external fluid (Conway & Duggan, 1958), the carrier being more readily saturated with K than Mg.

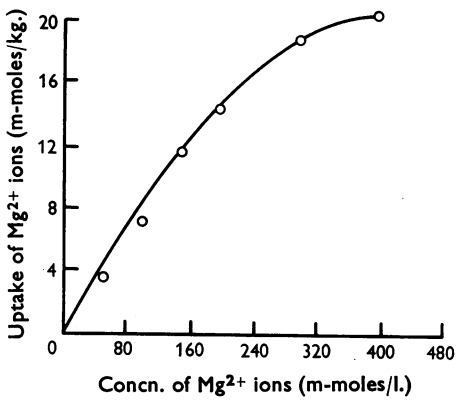

Fig. 2. Effect on uptake of magnesium of varying the external Mg^{2+} ion concentration. Magnesium was present as the acetate and the period of fermentation was 2 hr.

Effect of pH *on the uptake of magnesium*

In Fig. 3 the effect of changing the pH in the suspending fluid is shown. The general conditions were the same as for Fig. 1, there being a 1:20 (w/v) suspension of yeast, the external fluid containing 5% glucose and 0·2M-$MgCl_2$. The uptake was determined over 2 hr., the pH being maintained by the addition of 0·1N-NaOH.

A similar curve (up to about pH 6) was obtained with magnesium acetate, the pH being adjusted below 6 by addition of 0·1N-HCl.

In conjunction with the determination of the effect of pH on transport of Mg, that on uptake of K was also investigated. The uptake of K from a solution containing 10 m-equiv./l. as the citrate, and 5% glucose, was determined from the amount of K remaining in the supernatant fluid after fermentation for 5 min. Two parts by volume of the suspending fluid were used to one part by weight of yeast and the pH was brought to different levels by the addition of N-HCl solution before fermentation. Experiments were also carried out with 50 m-equiv. of K/l. and allowing fermentation for 15 min.

Fig. 4 shows the effect of pH on uptake of Mg and K.

Competition between magnesium and other inorganic cations for the cation carrier

K^+ ions inhibit the active transport of Mg^{2+} ions with an efficiency comparable with that of cyanide on cytochrome oxidase. This is illustrated in Fig. 5, where the uptake of Mg after 2 hr. from a 1:20 (w/v) suspension with 0·2M-magnesium acetate and 5% glucose is shown, the concentration of K in the suspending fluid being varied (curve *A*). While suspension in larger volumes of fluid would be advisable for comparisons (since there is a definite lowering of the external concentrations at lower levels of K^+ ion owing to uptake of K^+ ions), the results are considered sufficiently exact for the present purposes.

Inhibition of the uptake of Mg by 50% occurs when the K^+ ion concentration is 0·58 mM; this shows that the relative affinities of K^+ and Mg^{2+} ions (expressing Mg concentration as milli-equivalents) are approx. 700:1. A curious feature of the curve in Fig. 5 is that it descends more slowly from the 50% inhibition level than would be expected theoretically on the basis of the Michaelis–Menten kinetics; this may be due possibly to a small residual amount of absorption of Mg^{2+} ions by the second mechanism of uptake of Mg^{2+} ions operating at about pH 3·5–4·5, which is not inhibited by K^+ ions (Rothstein, 1954, 1955). The effect is not observed with $MgCl_2$ instead of magnesium acetate.

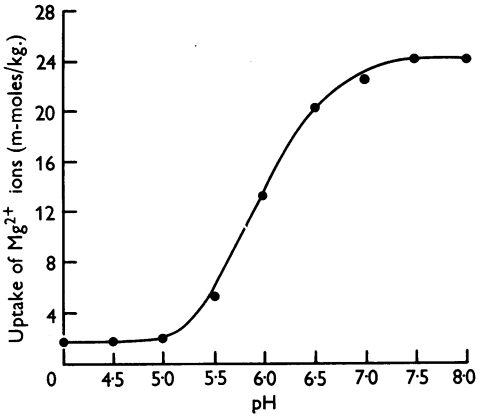

Fig. 3. Effect of pH on the uptake of magnesium from $MgCl_2$; suspending medium contained 0·2M-$MgCl_2$ and 5% glucose. The period of fermentation was 2 hr.

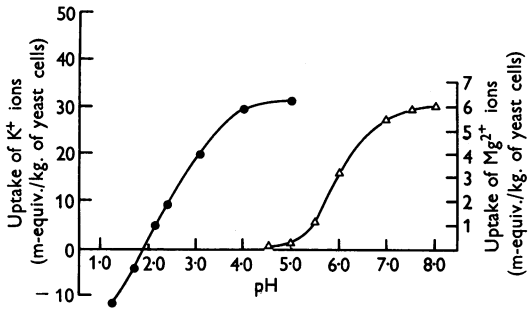

Fig. 4. Effect of pH on the uptake of potassium and magnesium. The uptake of both is expressed as m-equiv./kg./15 min. Potassium and magnesium were present as the chlorides.

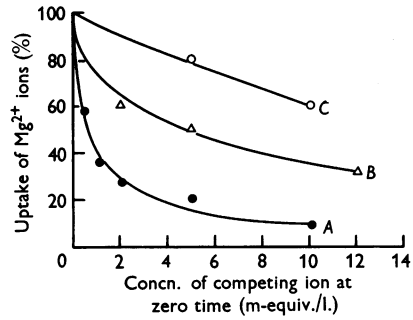

Fig. 5. Effect of K^+ ions (curve *A*) on the uptake of Mg^{2+} ions from magnesium acetate (1:20 suspension, w/v). Uptake of magnesium is plotted as a percentage of the total occurring in the absence of K^+ ion. Effect of Rb^+ and Cs^+ ions (curves *B* and *C*) on uptake of magnesium. Fermentation occurred for 2 hr. at room temperature.

Double reciprocal curves and inhibition of uptake of magnesium

To study such curves, it was decided to select Rb rather than K, because its rate of uptake is only about one-third of that of K and this allows more accurate determinations. The conditions were similar to those of Fig. 5 (for K competition) except that the suspending fluid was in the ratio to cells of 100:1 (v/w), and time of fermentation was only 30 min. The results of plotting $1/v$ against $1/S$ (v being the uptake of Mg/min., and S the external concentration of Mg) are shown in Fig. 6. The lower line is for uptake of Mg with magnesium acetate only in the external fluid. The upper line refers to similar conditions with 3 mM-RbCl present as well. Owing perhaps to the large amounts of the suspending fluid and the short period of fermentation, the pH was depressed only a little below 7; thus the uptake of Mg expressed as m-moles/kg. is higher than in the general uptake curve for Mg at about pH 6·3 (Fig. 1). From the double reciprocal graph (Fig. 6) it may be concluded that Rb^+ ion is competing for the same active carrier as the Mg^+ ion, since the two chief characteristics of competitive inhibition, increase of slope and no increase of intercept, are apparent. With non-competitive inhibition the line with Rb could be expected to cut the ordinate at the level 3·3.

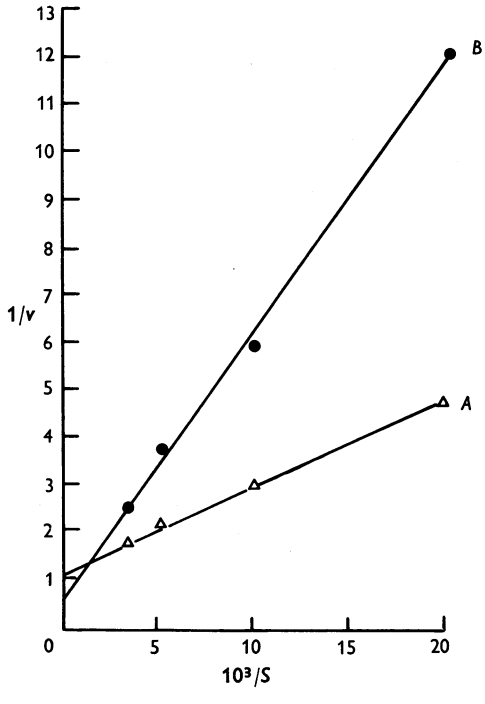

Fig. 6. Double reciprocal curve, showing interference of Rb^+ ions with uptake of magnesium; v is the uptake of magnesium/min. and S the external concentration of magnesium present as the acetate. The upper line (*B*) represents the uptake in the presence of 3 mM-RbCl.

Ions exchanging with or accompanying magnesium across the cell wall

During the large uptake of Mg over 7·5 hr. by a 1:20 (w/v) suspension in 5% glucose and 0·2 M-$MgCl_2$ maintained at pH 6, no uptake of Cl^- ions occurred and there was no appreciable loss of K^+ ions. It may be concluded therefore that Mg^{2+} ions exchanged for H^+ ions. Evidence for this exchange was obtained as follows. Washed, centrifuged yeast (10 g.) was suspended in a fluid containing 0·1 M-$MgCl_2$ and 5% glucose to a total volume of 200 ml.; allowing 3 ml. for the interspace, plus outer region for free diffusion (Conway & Downey, 1950), in the 10 g. of yeast, the total external fluid volume to be considered was 197 ml. This suspension was immediately transferred to a 1 l. beaker, and O_2 bubbled through. The pH, measured with a Beckman glass electrode, was maintained at 6·3 by adding 0·1 M-2-amino-2-hydroxymethylpropane-1:3-diol (tris) buffer. The fermentation was maintained for about 4 hr., when samples were taken and centrifuged at once. A volume (20 ml.) of the suspending fluid was gassed with O_2 for about 20 min. to free it of CO_2, a pH of 6·7 being then recorded, then titrated to pH 2·15 with N-HCl. The mixture was then titrated back with CO_2-free N-NaOH; the mixture was covered with a layer of paraffin after a pH of 6 was reached. The tris base was standardized with N-HCl, being titrated to pH 6·7.

Fig. 7 shows the back-titration. The base added neutralized the organic acid formed (to pH 6·3)

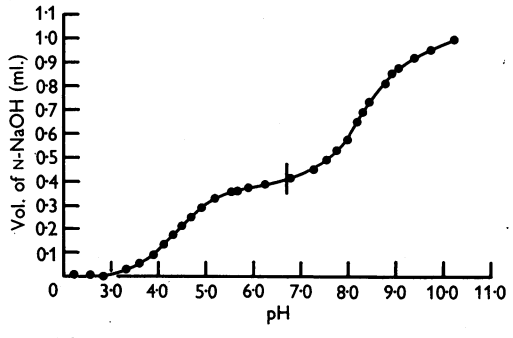

Fig. 7. Electrometric titration curve for 20 ml. of supernatant fluid, after fermentation and uptake of Mg for 4 hr., in the presence of tris buffer as described in the text. The 20 ml. was first titrated to pH 2·15 with N-HCl. In the curve the free H^+ ion concentration has been subtracted.

and any H^+ ions excreted in exchange for Mg^{2+} ions; any CO_2 had been eliminated by gassing with O_2.

The organic acid excreted could be determined from the titration data: in the titration with HCl, the H^+ ions form free organic acid and increase the H^+ ions in the solution. Subtraction of the latter from the total gives the organic acid; titration of organic acid anions was complete at approx. pH 3.

For the experiment here recorded (a second one gave similar results) the calculations gave 631 m-equiv. of base added/kg. of yeast, and 541 m-equiv. of organic acid. Thus 90 m-equiv. of base was required for neutralizing the free H^+ ions excreted. The Mg absorbed over the 4 hr. was 84 m-equiv. (The Mg in the yeast at zero time was determined from a sample of a similar suspension made up without glucose.) These values are sufficiently close to allow the conclusion that the Mg^{2+} ions absorbed exchanged for H^+ ions.

Effect on the accumulated magnesium of suspending the yeast in 0·1 M-KCl *or in tap water*

When Na has been accumulated by fermentation in the presence of a high concentration of Na^+ ions, it is actively excreted on subsequently suspending the yeast in water, and two to three times as fast as when suspended in 0·1 M-KCl (Conway, Ryan & Carton, 1954). Magnesium, however, is not appreciably excreted into either tap water or 0·1 M-KCl: a magnesium-rich yeast was produced by fermenting for 7·5 hr. in 5% glucose containing 0·2 M-magnesium sulphate. Portions of the twice-washed yeast were then suspended in tap water and in 0·1 M-KCl for 90 min. at room temperature, while shaking in air. The results are shown in Table 1.

Effects of various inhibitors and of anoxia on uptake of magnesium during fermentation

Various inhibitors, as their Na salts, were introduced into the suspending fluid before fermentation. An equivalent amount of Na (as NaCl) was used in the control experiment. The concentration of inhibitor outside the yeast cells was usually 2 mM.

To produce anoxia, fermentation was carried out in a small conical flask fitted with a rubber stopper having a capillary outlet. The suspending fluid practically filled the whole flask. The yeast was first allowed to ferment for about 45 min., the magnesium acetate was then added through a tap in the stopper and fermentation was allowed to proceed anaerobically.

Table 2 summarizes the effect of cyanide, anoxia and azide, marked inhibition being produced by each over a 2 hr. period.

The action of 2:4-dinitrophenol is shown in Table 3. When magnesium acetate, propionate, butyrate or valerate is used as the magnesium salt in the external solution, practically no inhibition is caused by 2:4-dinitrophenol, but when the external magnesium salt is chloride or sulphate, an inhibi-

Table 1. *Effect on accumulated magnesium of resuspending the magnesium-rich yeast in tap water and* 0·1 M-*potassium chloride*

In Expt. 3*a*, the resuspending fluids contained also 5% glucose. All values are expressed in m-moles/kg.

No. of expt.	Concn. of Mg at zero time	Concn. of Mg after reimmersion for 90 min. in 0·1 M-KCl	Concn. of Mg after reimmersion in water
1	136·6	120·0	120·0
2	103·3	100·0	103·3
3	85·7	—	83·3
3*a*	85·7	81·2	89·5

Table 2. *Effect of anoxia, azide and cyanide on the uptake of magnesium from* 0·2 M-*magnesium acetate*

Inhibitor	Uptake of Mg^{2+} ion in the presence of O_2 (m-moles/kg.)	Uptake of Mg^{2+} ion in the presence of inhibitor (m-moles/kg.)	Inhibition (%)
Anoxia	7·73	0·67	89
Azide (2 mM)	13·35	1·87	87
Cyanide (2 mM)	7·58	2·16	77

Table 3. *Effect of* 2:4-*dinitrophenol on the uptake of magnesium from the acetate, propionate, valerate, chloride and sulphate over a* 2 *hr. fermentation*

The inhibitor strength was 2 mM.

Anion (0·2 M)	Uptake of Mg in control (m-moles/kg.)	Uptake of Mg with 2 mM-2:4-dinitrophenol (m-moles/kg.)	Inhibition (%)
Acetate	5·0	5·0	0
Acetate	5·85	6·3	−7
Acetate	6·7	6·7	0
Acetate	11·7	10·0	15
Acetate	8·4	8·4	0
Propionate	10·7	10·0	8
Propionate	8·4	9·2	−10
Valerate	7·1	5·0	30
Average	8·0	7·6	4·5
Chloride	13·75	0·0	100
Chloride	8·4	3·35	60
Chloride	6·65	1·67	75
Sulphate	10·8	2·5	77
Average	9·9	1·88	78

tion of about 80 % was obtained. With magnesium chloride and sulphate, the pH was maintained by small additions of 0·1 N-NaOH.

DISCUSSION

Large amounts of magnesium can be taken up by the cation carrier, there being a marked sensitivity to the pH between 5·5 and 7·0. The uptake may be expected to involve an exchange for H^+ ions, as has been shown for the uptake of K^+ ions (Conway & O'Malley, 1946). It has been shown here by similar methods that enough H^+ ions are excreted to account for the uptake of Mg^{2+} ions.

The uptake of magnesium is extremely sensitive to the presence of K^+ ions and also to the presence of H^+ ions. A potassium concentration of even 0·58 mM reduces the uptake of magnesium by 50 %. This inhibition by potassium is a striking example of how an inorganic cation can inhibit an active process when present in concentrations of a similar order to that of cyanide when used for the inhibition of uptakes of oxygen. It would appear that the effect can be explained only by the displacement of magnesium from the carrier. It also shows that this process of uptake of magnesium has no physiological significance, since it occurs only when magnesium is present alone as the external cation and in high concentration.

The active uptake of magnesium by the cation carrier is characterized by the fact that it requires oxygen to be further transported into the cell and in this it is markedly different from the active transport of potassium; but since the primary carrier of the two ions is the same, this must be due to the inability of the carrier when carrying magnesium to transport electrons to whatever acceptor it uses in the anaerobic transport of K^+ ions.

Of special interest is the observation that whereas the uptake of magnesium present as the acetate is inhibited by cyanide, it is independent of the presence of 2:4-dinitrophenol; this characteristic is also shared with magnesium propionate, butyrate and valerate. On the other hand, when magnesium is present as the chloride or sulphate it is inhibited by 2:4-dinitrophenol (in 2 mM concentration). It therefore seems likely that the active transport of magnesium from magnesium acetate solution during fermentation is of the kind expected from the redox mechanism (Conway, 1951, 1953, 1955) rather than from a mechanism involving the active intervention of substances with energy-rich phosphate. Also, the fact that potassium has over 400 times the affinity for the carrier as magnesium indicates that the active carrier group is not part of a polyphosphate carrier.

The evidence shows that Mg^{2+} and K^+ ions are taken up by the same active carrier (in the region of pH 6–7), but oxygen is necessary for the uptake of magnesium and relatively little for the transport of potassium, and that whereas 2:4-dinitrophenol inhibits the uptake of potassium, it has practically no effect on the uptake of magnesium from magnesium acetate. The following explanation of these facts may be given provisionally in the light of the redox-pump theory. The complex of the carrier with potassium can transfer electrons to an acceptor, the existence of which at a suitable redox potential is maintained by the action of adenosine triphosphate, and in this way oxygen as electron acceptor is not necessary. The uptake of potassium will therefore be susceptible to 2:4-dinitrophenol, and but little to cyanide or anoxia. The complex of the carrier with magnesium cannot, apparently, transfer electrons to the same acceptor available for the potassium complex, but only along the cytochrome system to oxygen, and so the transport is susceptible to cyanide and anoxia, but not to dinitrophenol.

In the region of pH 3·5–4·5 magnesium is actively taken up in the presence of phosphate and potassium has no inhibitory effect. This system has been investigated by Rothstein (1954, 1955). This carrier would also appear to be that investigated by Schmidt, Hecht & Tannhauser (1949).

SUMMARY

1. Magnesium ions can be actively transported in large amounts across the yeast-cell membrane by the physiological carrier of K^+ ions. This occurs only when the Mg^{2+} ion is the only inorganic cation present in the external fluid in appreciable concentration.

2. The uptake of magnesium in this way from 0·2 M concentration is 50 % inhibited by 0·58 mM-K^+ ion and is inhibited by other inorganic cations in varying degrees.

3. The strongest inhibitor of the uptake of magnesium is H^+ ion; below pH 5·5 magnesium is not taken up by the same mechanism in appreciable amounts.

4. The uptake of Mg^{2+} ions is inhibited by anoxia or by cyanide (2 mM) and azide (2 mM). When present as the acetate, propionate butyrate or valerate it is not inhibited by 2:4-dinitrophenol (2 mM) but it is inhibited by this when present as the chloride or sulphate.

5. Even when large amounts of Mg^{2+} ions are taken up, practically none come out on reimmersion in water or even in 0·1 M-potassium chloride during fermentation.

Our thanks are due to the Medical Research Council of Ireland and to the Rockefeller Foundation for grants-in-aid.

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The Synthesis of Tritiovaline and its Incorporation into Rat-Visceral Proteins

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(*Received* 12 *September* 1957)

There are certain investigations in which it is an advantage to label an amino acid with a radioactive isotope other than ^{14}C. Tritium can be employed as a tracer where double labelling is required and in detailed investigations of metabolic pathways (cf. Arnstein & Crawhall, 1957). It is of value when the synthesis of the corresponding ^{14}C compound is difficult (Pearlman, 1957), and it has great economical advantages in the biosynthetic preparation of labelled proteins where the efficiency of incorporation is low (Done & Payne, 1956).

The difference in mass between the isotopes of hydrogen gives rise to a greater 'isotope effect' than occurs with isotopes of an element of higher atomic number (Eidinoff, 1953). We thought that this effect might not be appreciable if the tritium label were attached to the amino acid molecule at a site remote from that at which enzyme action occurs. To test this hypothesis we have prepared [β-^{3}H]valine and have compared its rate of incorporation into rat-visceral proteins with that of [γ-^{14}C]valine. A preliminary account of some of this work has been published (Crawhall & Smyth, 1955).

EXPERIMENTAL

Radioactivity measurements

Thin-window Geiger counter. This consisted of an Ecko EHM2S Geiger–Müller tube coupled to a scaler and E.H.T. unit (Panax Ltd., Type 100C). A poly[^{14}C]methyl methacrylate disk (1 μc/g. supplied by the Radiochemical Centre, Amersham, Bucks) was mounted in a stainless-steel planchet (internal diameter 1·51 cm.). This had an activity of 1053 counts/min. (S.D. 1%) with a background count of 12/min.

Windowless flow-type counter. This instrument, described by Banks, Blow & Francis (1956*a*), was coupled to a scaler and E.H.T. unit (Panax Ltd., Type 100C). The same poly-[^{14}C]methyl methacrylate standard gave 3362 counts/min. (S.D. 1%) with a background of 15/min. The method of counting solid tritium samples was described by Banks, Crawhall & Smyth (1956*b*). It was necessary to use a tritium-labelled compound as a standard for tritium counting, as the windowless counter can show a greater daily variation of sensitivity to tritium emissions than to ^{14}C emissions. Certain compounds when mixed with graphite and mounted in planchets showed a greater decrease in count rate over a period of 3 months than could be accounted for by the natural rate of decay of the isotope. The 2:4-dinitrophenylhydrazone of [1:2-^{3}H]*iso*butyraldehyde, and [α-^{3}H]cystine (Arnstein & Crawhall, 1957) had a reproducible count rate over a period of 2 years and were used as standards. At the time of the experiments reported in this paper the standard deviation in count rate of the tritium standards was 7%. This is a maximum value and has subsequently been reduced with different anode wires and is now only 2%. All comparative counts are the mean of three determinations made on the same day (S.D. 2%).

DL-[$\alpha\beta$-^{3}H]*Valine*

Adams platinum oxide catalyst (100 mg.) was suspended in dry tetrahydrofuran (10 ml.) and reduced with hydrogen. 2-Phenyl-4-*iso*propylideneoxazol-5-one (1·0 g.) was then added and the apparatus (see Fig. 1) evacuated. The seal of an ampoule containing tritium (100 mc) was broken and the tritium transferred to the reaction vessel by using the gas burette as a Töpler pump. Inactive hydrogen was then added and mechanical stirring of the solution commenced. More hydrogen was added at intervals to maintain atmospheric pressure in the reaction vessel, and after 2 hr., when 120 ml. (theoretically 112 ml.) of hydrogen had been absorbed, no further absorption was observed. The catalyst was removed by centrifuging, and the solvent by distillation *in vacuo* at room temperature. 6N-Hydrochloric acid (50 ml.) was added to the residue and the solution heated under reflux for 24 hr. The solvent was removed by distillation *in vacuo*, water (10 ml.) was added and the solution again distilled *in vacuo* to remove excess of