The Dissimilation of Glucose and Gluconate by *Acetobacter xylinum*

2. PATHWAY EVALUATION

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As shown by White & Wang (1964), *A. xylinum*, ATCC 10821, oxidizes glucose and gluconate to pyruvate and carbon dioxide by two pathways, the Entner–Doudoroff system and the pentose phosphate cycle. A fraction of the triose phosphate formed from these reactions recombines via aldolase to yield hexose phosphate which is metabolized further via the pentose phosphate cycle and the Entner–Doudoroff pathway.

CALCULATIONS AND RESULTS

Certain assumptions have been made in order to evaluate these pathways quantitatively by using the observed distribution or specific activity of ^{14}C in alanine and pyruvate derived from specifically ^{14}C -labelled glucose. The assumptions are as follows: (a) the Entner–Doudoroff system yields pyruvate from C-1–C-3 of glucose and triose phosphate from C-4–C-6; (b) C-1 of glucose appears in C-1 of pyruvate via the Entner–Doudoroff pathway; (c) the pentose phosphate cycle produces 3 mol.prop. of carbon dioxide, 1 mol.prop. of triose phosphate and 2 mol.prop. of fructose 6-phosphate from 3 mol.prop. of glucose 6-phosphate; recycling of hexose phosphate via the pentose phosphate cycle redistributes C-1, C-2 and C-3 of glucose; triose phosphate arises only from C-4, C-5 and C-6 of glucose (Katz & Wood, 1960); (d) triose phosphate arising from the pentose phosphate cycle and the Entner–Doudoroff reactions enters a common pool, a fraction of which recombines to form hexose phosphate; the remaining fraction is converted into pyruvate glycolytically; (e) glucose 6-phosphate and fructose 6-phosphate are not converted into triose phosphate glycolytically; (f) C-6 of glucose is randomized into C-1 of hexose phosphate via triose recombination; (g) hexose phosphate synthesized by triose recombination and the pentose phosphate cycle enters the pentose phosphate-cycle and Entner–Doudoroff pathways in the same proportion as phosphorylated substrate glucose; (h) pyruvate is in rapid equilibrium with alanine, and fructose 6-phosphate is readily isomerized to glucose 6-phosphate; (i) there are no

reactions randomizing the labelling pattern in pyruvate and alanine once formed; (j) drainage of pentose units for synthetic processes is negligible. An additional and important assumption is that the formation of gluconate and glucose 6-phosphate from glucose and the formation of 6-phosphogluconate from glucose 6-phosphate and gluconate are fast processes. In other words, the pools of glucose 6-phosphate and 6-phosphogluconate are considered as isotopically equivalent. That this may be the actual case is supported by the fact that cells contain very active glucose 6-phosphate-dehydrogenase and gluconate-kinase enzymes, and rapidly form gluconate from glucose by 'direct' oxidation and via glucose 6-phosphate dehydrogenase (Gromet, Schramm & Hestrin, 1957). The reaction leading from glucose 6-phosphate to 6-phosphogluconate is irreversible. For simplification of pathway estimation, drainage of hexose units into cellulose or oxogluconates is assumed to be irreversible, as is likely to be the situation since they accumulate in the medium during glucose metabolism.

Case A: Drainage of glucose 6-phosphate into cellulose or oxogluconate occurs from a pool of initial (unrecycled) hexose phosphate. The model shown in Scheme 1 was used as an approximation of the pathways described. The pentose phosphate-cycle, Entner–Doudoroff and triose-recombination reactions are assumed to interact over the range 0–100% from a common pool of glucose 6-phosphate and 6-phosphogluconate. The fructose 6-phosphate formed by the pentose phosphate cycle will re-enter the glucose 6-phosphate pool, and all the triose phosphate will form a common pool of which 0–100% will recombine to yield glucose 6-phosphate, the remainder becoming pyruvate. The end products after complete reaction (infinite recycling) are carbon dioxide and pyruvate exclusive of the fraction engaged in synthesis by non-triose pathways.

An extension of the method of Dawes & Holms (1958, 1959) has been applied to calculate the relative amounts of each pathway. The present method treats the recycling of both hexose phosphate and triose phosphate. By starting with the simplest case (only the pentose phosphate cycle), it is seen that for each amount (x) of substrate glucose

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6-phosphate entering a cycle, $\frac{1}{3}x$ of pyruvate and x of carbon dioxide will be formed. Of the newly formed glucose 6-phosphate, $\frac{2}{3}x$ re-enters the pool to react in a second cycle which, in turn, will produce more carbon dioxide, pyruvate and glucose 6-phosphate. Thus the overall equation for this case becomes:

where the numbers refer to the carbon positions in the original glucose 6-phosphate molecules.

In the first cycle, $\frac{1}{3}xC_{(1)}C_{(5)}C_{(6)}$ pyruvate is formed; in the second cycle an amount x of the $\frac{2}{3}x$ of glucose 6-phosphate formed by the first cycle will re-enter the pentose phosphate cycle to give $(\frac{1}{3}x)(\frac{2}{3}x)$ of pyruvate and $(\frac{2}{3}x)^2$ of glucose for a third cycle to produce $(\frac{1}{3}x)(\frac{2}{3}x)^2$ of pyruvate etc. Therefore the amount of pyruvate formed is a series:

$$\left\{ \frac{1}{3}x + \frac{1}{3}x\left(\frac{2}{3}x\right) + \frac{1}{3}x\left(\frac{2}{3}x\right)^2 + \frac{1}{3}x\left(\frac{2}{3}x\right)^3 + \dots + \frac{1}{3}x\left(\frac{2}{3}x\right)^n \right\}_{n \rightarrow \infty}^{n=0}$$

As a series of the type:

$$\{1 + a^2 + a^4 + a^6 + \dots + a^{2n}\}_{n \rightarrow \infty}^{n=0} \quad (1)$$

can be approximated by $1/(1-a^2)$, factoring out $\frac{1}{3}x$ gives:

$$\frac{1}{3}x \left\{ 1 + \left(\frac{2}{3}x\right) + \left(\frac{2}{3}x\right)^2 + \left(\frac{2}{3}x\right)^3 + \dots + \left(\frac{2}{3}x\right)^n \right\}_{n \rightarrow \infty}^{n=0}$$

and a further grouping of these terms:

$$\frac{1}{3}x \left[\left\{ 1 + \left(\frac{2}{3}x\right)^2 + \left(\frac{2}{3}x\right)^4 + \dots + \left(\frac{2}{3}x\right)^{2n} \right\}_{n \rightarrow \infty}^{n=0} \right. \\ \left. + \left\{ \frac{2}{3}x + \left(\frac{2}{3}x\right)^3 + \left(\frac{2}{3}x\right)^5 + \dots + \left(\frac{2}{3}x\right)^{2n-1} \right\}_{n \rightarrow \infty}^{n=0} \right]$$

Factoring the second series once more:

$$\frac{1}{3}x \left[\left\{ 1 + \left(\frac{2}{3}x\right)^2 + \left(\frac{2}{3}x\right)^4 + \dots + \left(\frac{2}{3}x\right)^{2n} \right\}_{n \rightarrow \infty}^{n=0} \right. \\ \left. + \frac{2}{3}x \left\{ 1 + \left(\frac{2}{3}x\right)^2 + \left(\frac{2}{3}x\right)^4 + \dots + \left(\frac{2}{3}x\right)^{2n} \right\}_{n \rightarrow \infty}^{n=0} \right]$$

Thus:

$$\frac{1}{3}x(1 + \frac{2}{3}x) \left\{ 1 + \left(\frac{2}{3}x\right)^2 + \left(\frac{2}{3}x\right)^4 + \dots + \left(\frac{2}{3}x\right)^{2n} \right\}_{n \rightarrow \infty}^{n=0}$$

Using the above approximation:

$$\frac{1}{3}x(1 + \frac{2}{3}x) \frac{1}{1 - (\frac{2}{3}x)^2} \quad \text{or} \quad \frac{1}{3}x(1 + \frac{2}{3}x) \frac{1}{(1 + \frac{2}{3}x)(1 - \frac{2}{3}x)}$$

which becomes: $\frac{1}{3}x \left(\frac{1}{1 - \frac{2}{3}x} \right)$

as the amount of pyruvate formed when fraction x of the glucose 6-phosphate enters the pentose phosphate cycle and no Entner-Doudoroff or triose-recombination pathways operate.

As the Entner-Doudoroff pathway is introduced, it is assumed that a fraction $(1-x)$ of the glucose 6-phosphate pool not entering the pentose phosphate cycle with fraction x will enter the Entner-Doudoroff pathway, giving the overall equation:

Of interest is the labelling pattern in pyruvate derived from $[^{14}\text{C}]$ glucose. Several types of labelled glucose 6-phosphate molecules are formed on each recycling. Pyruvate arising from the Entner-Doudoroff pathway has the distribution of C atoms shown in Scheme 2, identical with that in C-1, C-2 and C-3 of the glucose 6-phosphate resynthesized via the pentose phosphate cycle. Since $\text{C}_{(1)}\text{C}_{(2)}\text{C}_{(3)}\text{C}_{(4)}\text{C}_{(5)}\text{C}_{(6)}$ glucose 6-phosphate appears only in the initial pool, the amount of $\text{C}_{(1)}\text{C}_{(2)}\text{C}_{(3)}$ formed is $(1-x)$.

As a factor, x , of $\text{C}_{(1)}\text{C}_{(2)}\text{C}_{(3)}\text{C}_{(4)}\text{C}_{(5)}\text{C}_{(6)}$ glucose 6-phosphate is recycled, $\frac{1}{3}x$ of $\text{C}_{(2)}\text{C}_{(3)}\text{C}_{(4)}\text{C}_{(5)}\text{C}_{(6)}$ glucose 6-phosphate and $\frac{1}{3}x$ of $\text{C}_{(2)}\text{C}_{(3)}\text{C}_{(4)}\text{C}_{(5)}\text{C}_{(6)}$ glucose 6-phosphate are formed, $(1-x)$ of which proceeds to pyruvate. The $\text{C}_{(2)}\text{C}_{(3)}\text{C}_{(4)}\text{C}_{(5)}\text{C}_{(6)}$ glucose 6-phosphate and $\text{C}_{(2)}\text{C}_{(3)}\text{C}_{(4)}\text{C}_{(5)}\text{C}_{(6)}$ glucose 6-phosphate combinations appear in the third cycle again. Also, an extension of the scheme shows

Scheme 1. Model of glucose and gluconate metabolism in *Acetobacter xylinum*.

their reappearance in the fifth, seventh, ninth, etc. cycles. Therefore:

$$\begin{aligned} C_{(2)}C_{(3)}C_{(3)} \\ \text{or} \\ C_{(2)}C_{(3)}C_{(2)} \end{aligned} = (1-x) \left\{ \frac{1}{3}x + \left(\frac{1}{3}x\right)^3 + \left(\frac{1}{3}x\right)^5 + \dots \right\}$$

From the same line of reasoning, $C_{(3)}C_{(2)}C_{(3)}C_{(4)}C_{(5)}C_{(6)}$ glucose 6-phosphate and $C_{(3)}C_{(2)}C_{(2)}C_{(4)}C_{(5)}C_{(6)}$ glucose 6-phosphate appear in the second, fourth, sixth, eighth, etc. cycles. Hence:

$$\begin{aligned} C_{(3)}C_{(2)}C_{(3)} \\ \text{or} \\ C_{(3)}C_{(2)}C_{(2)} \end{aligned} = (1-x) \left\{ \left(\frac{1}{3}x\right)^2 + \left(\frac{1}{3}x\right)^4 + \left(\frac{1}{3}x\right)^6 + \dots \right\}$$

Scheme 2 shows that $C_{(3)}C_{(3)}C_{(3)}C_{(4)}C_{(5)}C_{(6)}$ glucose 6-phosphate first appears in the second cycle and $\frac{2}{3}x$ part of each amount in every cycle thereafter. As the pattern is repetitive, the situation is as above starting in the fourth, sixth and eighth cycles. Thus the formula is

$$\begin{aligned} C_{(3)}C_{(3)}C_{(3)} = (1-x) \{ & [2\left(\frac{1}{3}x\right)^2 + 4\left(\frac{1}{3}x\right)^3 + 8\left(\frac{1}{3}x\right)^4 + \dots] \\ & + \{2\left(\frac{1}{3}x\right)^4 + 4\left(\frac{1}{3}x\right)^5 + \dots\} \\ & + \{2\left(\frac{1}{3}x\right)^6 + 4\left(\frac{1}{3}x\right)^7 + \dots\} + \dots \end{aligned}$$

Similarly, $C_{(2)}C_{(2)}C_{(2)}C_{(4)}C_{(5)}C_{(6)}$ glucose 6-phosphate appears first in the third cycle and then with repetition of pattern in the fifth, seventh, ninth, etc. cycles. Thus:

$$\begin{aligned} C_{(2)}C_{(2)}C_{(2)} = (1-x) \{ & [2\left(\frac{1}{3}x\right)^3 + 4\left(\frac{1}{3}x\right)^4 + \dots] \\ & + \{2\left(\frac{1}{3}x\right)^5 + 4\left(\frac{1}{3}x\right)^6 + \dots\} + \dots \end{aligned}$$

$C_{(4)}C_{(5)}C_{(6)}$ pyruvate is obtained from each completed cycle, the amount being $\frac{1}{3}x$ of the fraction of glucose 6-phosphate present and $(1-x)$ via the splitting of 6-phosphogluconate in the Entner-Doudoroff pathway. Consequently, the sum of $[(\frac{1}{3}x) + 1-x]$ multiplied by the sum of all glucose 6-phosphate molecules, initial and re-formed, gives the expression:

$$\begin{aligned} C_{(4)}C_{(5)}C_{(6)} = (\frac{1}{3}x + 1-x) \{ & 1 + 2\left(\frac{1}{3}x\right) \\ & + 4\left(\frac{1}{3}x\right)^2 + 8\left(\frac{1}{3}x\right)^3 + \dots \} \end{aligned}$$

By algebraic manipulation, series of the type (1) (see above) are factored out and approximated. The above expressions then become:

$$\left. \begin{aligned} C_{(1)}C_{(2)}C_{(3)} &= 1-x \\ C_{(2)}C_{(3)}C_{(3)} \text{ and } C_{(2)}C_{(3)}C_{(2)} &= \frac{(1-x)(\frac{1}{3}x)}{1-(\frac{1}{3}x)^2} \\ C_{(3)}C_{(2)}C_{(3)} \text{ and } C_{(3)}C_{(2)}C_{(2)} &= \frac{(1-x)(\frac{1}{3}x)^2}{1-(\frac{1}{3}x)^2} \\ C_{(3)}C_{(3)}C_{(3)} &= \frac{2(1-x)(\frac{1}{3}x)^2}{\{1-(\frac{1}{3}x)^2\}(1-\frac{2}{3}x)} \\ C_{(2)}C_{(2)}C_{(2)} &= \frac{2(1-x)(\frac{1}{3}x)^3}{\{1-(\frac{1}{3}x)^2\}(1-\frac{2}{3}x)} \\ C_{(4)}C_{(5)}C_{(6)} &= \frac{\frac{1}{3}x + 1-x}{1-\frac{2}{3}x} \end{aligned} \right\} (2)$$

where the fraction of 1 mole of glucose 6-phosphate entering the pentose phosphate cycle is x .

As triose recombination is introduced, none of the preceding expressions for pyruvate is affected except $C_{(4)}C_{(5)}C_{(6)}$. If y is the fraction of $C_{(4)}C_{(5)}C_{(6)}$ that recombines to yield glucose 6-phosphate of the type $C_{(6)}C_{(5)}C_{(4)}C_{(4)}C_{(5)}C_{(6)}$ (which by recycling will produce pyruvate molecules of the type $C_{(6)}C_{(5)}C_{(4)}$, $C_{(5)}C_{(4)}C_{(5)}$ etc.), then $C_{(6)}C_{(5)}C_{(4)}C_{(4)}C_{(5)}C_{(6)}$ glucose 6-phosphate can be considered analogous to $C_{(1)}C_{(2)}C_{(3)}C_{(4)}C_{(5)}C_{(6)}$ glucose 6-phosphate and hence $C_{(6)}C_{(5)}C_{(4)}$ to $C_{(1)}C_{(2)}C_{(3)}$, $C_{(5)}C_{(4)}C_{(5)}$ to $C_{(2)}C_{(3)}C_{(2)}$, etc., except that the amount does not equal 1 like that for $C_{(1)}C_{(2)}C_{(3)}C_{(4)}C_{(5)}C_{(6)}$ glucose 6-phosphate. The amount, a , of

$C_{(6)}C_{(5)}C_{(4)}C_{(4)}C_{(5)}C_{(6)}$ glucose 6-phosphate

formed from recycling of $C_{(1)}C_{(2)}C_{(3)}C_{(4)}C_{(5)}C_{(6)}$ glucose 6-phosphate would be:

$$a = \frac{1}{2}y(\frac{1}{3}x + 1-x) \{ 1 + 2\left(\frac{1}{3}x\right) + 4\left(\frac{1}{3}x\right)^2 + 8\left(\frac{1}{3}x\right)^3 + \dots \}$$

i.e. the amount of $C_{(4)}C_{(5)}C_{(6)}$ formed times the fraction, y , that recombines times 0.5, since two molecules of triose recombine. With recycling, we obtain a^2 more $C_{(6)}C_{(5)}C_{(4)}C_{(4)}C_{(5)}C_{(6)}$ glucose 6-phosphate and then a^3 , a^4 , a^5 , etc., so that the total amount produced is:

$$a + a^2 + a^3 + a^4 + \dots$$

or, after factoring and approximating the series, $a/(1+a)$, and by resubstituting and approximating the $f(x)$ series:

$$\frac{\frac{1}{2}y(\frac{1}{3}x + 1-x)}{1-\frac{2}{3}x - \frac{1}{2}y(\frac{1}{3}x + 1-x)} = \frac{\frac{1}{2}y}{1-\frac{1}{2}y}$$

This fraction multiplied by the various expressions (2) analogous to those for pyruvate molecules derived from C-1, C-2 and C-3 of glucose 6-phosphate will give expressions for pyruvate molecules derived from C-4, C-5 and C-6 of glucose 6-phosphate as follows:

$$\left. \begin{aligned} C_{(6)}C_{(5)}C_{(4)} &= \frac{(1-x)(\frac{1}{2}y)(\frac{1}{3}x + 1-x)}{1-\frac{2}{3}x - \frac{1}{2}y(\frac{1}{3}x + 1-x)} \\ C_{(5)}C_{(4)}C_{(4)} \text{ and } C_{(5)}C_{(4)}C_{(5)} &= \frac{(1-x)(\frac{1}{3}x)}{1-(\frac{1}{3}x)^2} (\frac{1}{2}y) \frac{(\frac{1}{3}x + 1-x)}{1-\frac{2}{3}x - \frac{1}{2}y(\frac{1}{3}x + 1-x)} \\ C_{(4)}C_{(5)}C_{(4)} \text{ and } C_{(4)}C_{(5)}C_{(5)} &= \frac{(1-x)(\frac{2}{3}x)^2}{1-(\frac{1}{3}x)^2} (\frac{1}{2}y) \frac{(\frac{1}{3}x + 1-x)}{1-\frac{2}{3}x - \frac{1}{2}y(\frac{1}{3}x + 1-x)} \\ C_{(4)}C_{(4)}C_{(4)} &= \frac{(1-x)2(\frac{1}{3}x)^2}{(1-\frac{2}{3}x)\{1-(\frac{1}{3}x)^2\}} (\frac{1}{2}y) \frac{(\frac{1}{3}x + 1-x)}{1-\frac{2}{3}x - \frac{1}{2}y(\frac{1}{3}x + 1-x)} \\ C_{(5)}C_{(5)}C_{(5)} &= \frac{(1-x)2(\frac{1}{3}x)^3}{(1-\frac{2}{3}x)\{1-(\frac{1}{3}x)^2\}} (\frac{1}{2}y) \frac{(\frac{1}{3}x + 1-x)}{1-\frac{2}{3}x - \frac{1}{2}y(\frac{1}{3}x + 1-x)} \end{aligned} \right\} (3)$$

For $C_{(4)}C_{(5)}C_{(6)}$ pyruvate an amount:

$$(1-y) \left(\frac{1}{3}x + 1 - x \right) \left\{ 1 + \frac{2}{3}x + 4\left(\frac{1}{3}x\right)^2 + \dots \right\}$$

is formed by recycling $C_{(1)}C_{(2)}C_{(3)}C_{(4)}C_{(5)}C_{(6)}$ glucose 6-phosphate (defined as fraction 1). As a fraction of $C_{(6)}C_{(5)}C_{(4)}C_{(4)}C_{(5)}C_{(6)}$ glucose 6-phosphate which equals

$$a + a^2 + a^3 + \dots 1$$

gives rise to further $C_{(4)}C_{(5)}C_{(6)}$ pyruvate, the expression for $C_{(4)}C_{(5)}C_{(6)}$ pyruvate becomes:

$$C_{(4)}C_{(5)}C_{(6)} = (1-y) \left(\frac{1}{3}x + 1 - x \right) \left\{ 1 + \frac{2}{3}x + 4\left(\frac{1}{3}x\right)^2 + \dots \right\} \times (1 + a + a^2 + a^3 + \dots)$$

or
$$(1-y) \frac{2}{y} (a + a^2 + a^3 + \dots)$$

and by simplification:

$$C_{(4)}C_{(5)}C_{(6)} = \frac{(1-y) \left(\frac{1}{3}x + 1 - x \right)}{1 - \frac{2}{3}x - \frac{1}{2}y \left(\frac{1}{3}x + 1 - x \right)} \quad \text{or} \quad \frac{2(1-y)}{2-y}$$

It is now possible to calculate the percentage distribution of label in pyruvate derived from specifically ^{14}C -labelled glucose and, in addition, the molar specific activity of pyruvate relative to substrate glucose as a function of various percentages of the pentose phosphate-cycle, Entner-Doudoroff and triose-recombination pathways.

To find the percentage distribution of label in pyruvate, the expressions (2) with label from the

carbon position of interest are added, multiplied by 100 and divided by the sum of the expressions of all pyruvate molecules having label. The expressions in the denominator are multiplied by the number of atoms in the molecule that carry label. For example, to obtain the percentage of total label at C-2 of pyruvate arising from $[2-^{14}\text{C}]$ glucose, the expressions (2) for the pyruvate molecules are substituted into the following equation:

$$\frac{C_{(1)}C_{(2)}C_{(3)} + 2C_{(3)}C_{(2)}C_{(3)} + C_{(2)}C_{(2)}C_{(2)}}{C_{(1)}C_{(2)}C_{(3)} + 3C_{(3)}C_{(2)}C_{(3)} + 3C_{(2)}C_{(3)}C_{(3)} + 3C_{(2)}C_{(2)}C_{(2)}}$$

As the expressions for patterns $C_{(3)}C_{(2)}C_{(3)}$ and $C_{(3)}C_{(2)}C_{(2)}$ are identical, it is expressed as $2C_{(3)}C_{(2)}C_{(3)}$ in the numerator. The same explanation applies to $3C_{(3)}C_{(2)}C_{(3)}$ and $3C_{(2)}C_{(3)}C_{(3)}$ in the denominator.

To find the percentage of total pyruvate molecules labelled (molar specific activity relative to substrate glucose) the expressions of all pyruvate molecules having label from the carbon position of interest are added and again multiplied by the number of atoms carrying label as in the case above, then divided by the sum of all expressions.

Sets of theoretical curves (Figs. 1a, 1b and 1c) are obtained which show the apparent percentage of total pyruvate molecules labelled from glucose having ^{14}C at C-1, C-2 or C-6, and as a function

Scheme 2. Distribution of C-1, C-2 and C-3 from glucose 6-phosphate in pyruvate arising from the Entner-Doudoroff pathway. (C-4, C-5 and C-6 are omitted from the Scheme.)

of various percentage contributions of the pentose phosphate cycle and of triose recombination. Figs. 1(d), 1(e) and 1(f) show the percentage distribution of ^{14}C in pyruvate derived from specifically ^{14}C -labelled glucose molecules as a function of the percentage participation of the pentose phosphate-cycle and of triose-recombination pathways.

The percentage of glucose 6-phosphate molecules metabolized via the pentose phosphate-cycle and Entner–Doudoroff pathways can be estimated from the percentage of total pyruvate molecules labelled at a certain fraction of triose recombination. However, the method requires a high degree of accuracy in the determination of specific activity of pyruvate or alanine. Further, the slopes of the curves for ^{14}C -labelled pyruvate derived from $[2\text{-}^{14}\text{C}]\text{glucose}$ and $[6\text{-}^{14}\text{C}]\text{glucose}$ (Figs. 1b and 1c) show that a small error in the measurement of the specific activity would result in a correspondingly large error in the estimation of the pentose phosphate cycle, particularly at low values of pentose phosphate-cycle opera-

tion. The situation improves with $[6\text{-}^{14}\text{C}]\text{glucose}$ as the amount of triose recombination approaches 100 %. Results found with $[1\text{-}^{14}\text{C}]\text{glucose}$ will provide a more reliable estimate of the contribution of the pentose phosphate cycle (Fig. 1a). As an example, the percentage of total pyruvate molecules labelled from $[1\text{-}^{14}\text{C}]\text{glucose}$ in Expt. IV of White & Wang (1964) was 27 % after correction for endogenous dilution as calculated from results with $[\text{U-}^{14}\text{C}]\text{glucose}$. This value corresponds to a 61 % contribution by the pentose phosphate cycle when 83 % of the triose phosphate recombines (see Table 1).

A second approach uses the ratio of percentage distribution of label from two carbon positions in pyruvate or alanine. Two sets of experimental labelling results are required. One set is needed from the triose-recombination-independent percentage distribution of label in pyruvate derived from $[2\text{-}^{14}\text{C}]\text{glucose}$ or $[3\text{-}^{14}\text{C}]\text{glucose}$ and the other from a triose-recombination-dependent percentage distribution of label from, for instance, $[6\text{-}^{14}\text{C}]\text{glucose}$. A ratio of the first determines the percentage of

Fig. 1. (a)–(c) Theoretical curves relating the apparent percentage of total pyruvate molecules labelled from $[1\text{-}^{14}\text{C}]\text{glucose}$ (a), $[2\text{-}^{14}\text{C}]\text{glucose}$ (b) and $[6\text{-}^{14}\text{C}]\text{glucose}$ (c) to the fraction of initial glucose 6-phosphate entering the pentose phosphate cycle at the following percentages of triose recombination (y): A, $y = 0.0$; B, $y = 0.4$; C, $y = 0.6$; D, $y = 0.8$; E, $y = 1.0$. (d)–(f) Percentage distributions of ^{14}C in pyruvate derived from $[1\text{-}^{14}\text{C}]\text{glucose}$ (d), $[2\text{-}^{14}\text{C}]\text{glucose}$ (e) and $[6\text{-}^{14}\text{C}]\text{glucose}$ (f) as a function of the percentage participation of the pentose phosphate cycle. In (d) and (e), C-1, C-2 and C-3 refer respectively to C-1, C-2 and C-3 of pyruvate. In (f), all the curves refer to C-1 of pyruvate, but for the following different fractions of triose recombining: A, 0.0; B, 0.2; C, 0.4; D, 0.6; E, 0.8; F, 1.0.

glucose 6-phosphate molecules metabolized via the pentose phosphate cycle. A ratio from the second, from the values for the pentose phosphate cycle found from the first ratio, gives the amount of triose recombination.

Thus the ratio of the percentage label at C-1 to that at C-2 of pyruvate derived from [2-¹⁴C]glucose is:

$$\frac{a_1}{a_2} = \frac{\frac{2(1-x)\frac{1}{3}x}{1-(\frac{1}{3}x)^2} + \frac{2(1-x)(\frac{1}{3}x)^3}{\{1-(\frac{1}{3}x)^2\}(1-\frac{2}{3}x)}}{(1-x) + \frac{2(1-x)(\frac{1}{3}x)^2}{1-(\frac{1}{3}x)^2} + \frac{2(1-x)(\frac{1}{3}x)^3}{\{1-(\frac{1}{3}x)^2\}(1-\frac{2}{3}x)}}$$

The denominators of the percentage distributions of label cancel themselves. This equation simplifies to:

$$a_1/a_2 = \frac{2}{3}x$$

or, solving for unknown x :

$$x = \frac{3}{2}(a_1/a_2)$$

Similarly, x could be solved for by using ratios of other carbon positions.

The ratio of the percentage label at C-1 (b_1) to that at C-2 (b_3) of pyruvate from [6-¹⁴C]glucose is:

$$\frac{b_1}{b_3} = \frac{(1-x)(\frac{1}{3}y)(\frac{1}{3}x+1-x)}{(1-y)(\frac{1}{3}x+1-x)} = \frac{(1-x)\frac{1}{3}y}{(1-y)}$$

Solving for y and substituting $b_3 = (100 - b_1)$, the following equation for triose recombination is given:

$$y = \frac{2b_1}{2b_1 + (1-x)(100 - b_1)}$$

A third method of pathway evaluation utilizes the theoretical curves for percentage distribution of

label in pyruvate derived from specifically labelled glucose (Figs. 1d, 1e and 1f). The theoretical values for the pentose phosphate-cycle, Entner-Doudoroff and triose-recombination pathways, i.e., x , $(1-x)$ and y , were calculated as mentioned above. Use of the labelling results on pyruvate from [2-¹⁴C]glucose is probably the most accurate for determining the contribution of the pentose phosphate cycle. C-2 of pyruvate has the bulk of the label. C-1 and C-3 contain less total label and therefore are affected more by experimental error. Hence the use of ratios of activity in carbon positions tends to be inaccurate.

The ratio method is more desirable for the determination of percentage of triose recombination, since it provides an exact value rather than a value interpolated from the theoretical curves in Fig. 1(f).

Table 1 gives the calculated percentages of glucose 6-phosphate molecules entering the pentose phosphate-cycle and Entner-Doudoroff pathways and the percentage of triose molecules recombining as calculated from results in Expts. I-IV of White & Wang (1964). Of the total glucose 6-phosphate entering the pentose phosphate-cycle plus Entner-Doudoroff pathways in younger (20-24 hr.) cells, approx. 58% was metabolized via the pentose phosphate cycle and 42% via the Entner-Doudoroff pathway. In older (27 hr.) cells, the Entner-Doudoroff pathway appeared more predominant, with 20% of the glucose 6-phosphate entering the pentose phosphate cycle and 80% the Entner-Doudoroff pathway. With gluconate [Expt. III of White & Wang (1964)], 90% of the glucose 6-phosphate, i.e. 6-phosphogluconate, metabolized via the

Table 1. *Evaluation of pathways of glucose metabolism in Acetobacter xylinum when non-triose reactions involve the initial (unrecycled) pool of glucose 6-phosphate*

All cells were grown on glucose. Details of Expts. I-IV are given by White & Wang (1964). For the percentage of the fraction of glucose 6-phosphate molecules metabolized via the pentose phosphate-cycle and Entner-Doudoroff pathways, the non-triose fraction (cellulose, 2-oxogluconate, 5-oxogluconate) was drained off from the initial non-recycled pool of hexose phosphate. For the percentage contribution of triose recombination, the values in parentheses are those calculated when the percentage of label in C-2 is added to the percentage of label in C-1 of pyruvate or alanine derived from 6-¹⁴C-labelled substrate.

Expt. no.	Substrate	Intermediate degraded	Age of cells (hr.)	Percentage of fraction of glucose 6-phosphate metabolized via pentose phosphate-cycle plus Entner-Doudoroff pathways		Percentage contribution of triose recombination (y)	Percentage formation of triose via Embden-Meyerhof-Parnas pathway (100 - y)
				Via pentose phosphate cycle	Via Entner-Doudoroff pathway		
I	[2- ¹⁴ C]Glucose	Alanine	20	58	42	84 (87)	16
II	[2- ¹⁴ C]Glucose	Alanine	27	18	82	81 (85)	19
III	[2- ¹⁴ C]Gluconate	Alanine	27	8	92	92 (94)	8
IV	[2- ¹⁴ C]Glucose	Pyruvate	24	54	46	83 (87)	17

pentose phosphate-cycle plus Entner-Doudoroff routes entered the Entner-Doudoroff system. The amount of triose phosphate undergoing recombination was fairly constant even though the percentage of glucose 6-phosphate molecules metabolized via the pentose phosphate-cycle and Entner-Doudoroff pathways varied. Approx. 10% more triose recombined when gluconate was substrate. Here, 84% of the total ^{14}C activity was located at C-1 of alanine derived from $[6\text{-}^{14}\text{C}]\text{gluconate}$. If the participation of triose recombination were 100%, all the activity would be at C-1 (Fig. 1f).

Case B: Drainage of glucose 6-phosphate into non-triose pathways occurs from a pool of unrecycled, recycled and recombined molecules. The preceding expressions apply when there is an initial drainage of glucose 6-phosphate molecules into non-triose pathways, i.e. before recycling via the pentose phosphate cycle or triose recombination occurs. In Case A, the pentose phosphate-cycle and Entner-Doudoroff pathways are expressed as a percentage of the fraction of glucose 6-phosphate entering these routes. Thus, for example, if initial drainage via non-triose pathways is 20 mol.prop. of glucose 6-phosphate from each original 100 mol.prop. of glucose 6-phosphate, the fraction of initial glucose 6-phosphate dissimilated via the pentose phosphate cycle is $0.8 \times$ the calculated percentage contribution of the pentose phosphate cycle.

Most probably, in *A. xylinum*, drainage of hexose phosphate into cellulose and oxogluconates is continuous from a pool containing unrecycled, recycled and recombined glucose 6-phosphate molecules. That is, a specific fraction of these glucose 6-phosphate molecules is removed continuously by non-triose pathways. Schramm, Gromet & Hestrin (1957) found marked variation in the percentage of total glucose converted into cellulose, 2-oxogluconate and 5-oxogluconate among different batches of cells. The values with cells incubated for 4–5 hr. with glucose ranged from 20% to 72%, averaging 49%. When it is assumed that a constant percentage of hexose phosphate drainage occurs at each cycle, then a fraction denoted as z will be free to react further, and:

$$1 - \frac{\text{percentage participation of non-triose pathways}}{100} = z$$

Thus the expressions presented in Case A will simply be modified by z . Any $(1-x)$ becomes $(1-x)z$ since only the fraction z of glucose 6-phosphate not drained off will enter the Entner-Doudoroff pathway. Similarly, it is found that z appears with every x since drainage leaves only the z fraction to enter the pentose phosphate cycle for recycling (y is not concerned with z as it represents only the amount of triose phosphate recombined).

The fraction of non-triose pathways after recombination is accounted for by the xz portion of the following expressions:

$$\begin{aligned} C_{(1)}C_{(2)}C_{(3)} &= (1-x)z \\ C_{(2)}C_{(3)}C_{(3)} \quad \text{and} \quad C_{(2)}C_{(3)}C_{(2)} &= \frac{(1-x)z(\frac{1}{3}xz)}{1 - (\frac{1}{3}xz)^2} \\ C_{(3)}C_{(2)}C_{(3)} \quad \text{and} \quad C_{(3)}C_{(2)}C_{(2)} &= \frac{(1-x)z(\frac{1}{3}xz)^2}{1 - (\frac{1}{3}xz)^2} \\ C_{(3)}C_{(3)}C_{(3)} &= \frac{2(1-x)z(\frac{1}{3}xz)^2}{\{1 - (\frac{1}{3}xz)^2\}(1 - \frac{2}{3}xz)} \\ C_{(2)}C_{(2)}C_{(2)} &= \frac{2(1-x)z(\frac{1}{3}xz)^3}{\{1 - (\frac{1}{3}xz)^2\}(1 - \frac{2}{3}xz)} \\ C_{(6)}C_{(5)}C_{(4)} &= (1-x)z \left[\frac{\frac{1}{2}y(\frac{1}{3}xz + (1-x)z)}{1 - \frac{2}{3}xz - \frac{1}{2}y(\frac{1}{3}xz + (1-x)z)} \right] \\ C_{(5)}C_{(4)}C_{(4)} \quad \text{and} \quad C_{(5)}C_{(4)}C_{(5)} &= \frac{(1-x)z(\frac{1}{3}xz)}{1 - (\frac{1}{3}xz)^2} \left[\frac{\frac{1}{2}y(\frac{1}{3}xz + (1-x)z)}{1 - \frac{2}{3}xz - \frac{1}{2}y(\frac{1}{3}xz + (1-x)z)} \right] \\ C_{(4)}C_{(5)}C_{(4)} \quad \text{and} \quad C_{(4)}C_{(5)}C_{(5)} &= \frac{(1-x)z(\frac{1}{3}xz)^2}{1 - (\frac{1}{3}xz)^2} \left[\frac{\frac{1}{2}y(\frac{1}{3}xz + (1-x)z)}{1 - \frac{2}{3}xz - \frac{1}{2}y(\frac{1}{3}xz + (1-x)z)} \right] \\ C_{(4)}C_{(4)}C_{(4)} &= \frac{2(1-x)z(\frac{1}{3}xz)^2}{\{1 - (\frac{1}{3}xz)^2\}(1 - \frac{2}{3}xz)} \left[\frac{\frac{1}{2}y(\frac{1}{3}xz + (1-x)z)}{1 - \frac{2}{3}xz - \frac{1}{2}y(\frac{1}{3}xz + (1-x)z)} \right] \\ C_{(5)}C_{(5)}C_{(5)} &= \frac{2(1-x)z(\frac{1}{3}xz)^3}{\{1 - (\frac{1}{3}xz)^2\}(1 - \frac{2}{3}xz)} \left[\frac{\frac{1}{2}y(\frac{1}{3}xz + (1-x)z)}{1 - \frac{2}{3}xz - \frac{1}{2}y(\frac{1}{3}xz + (1-x)z)} \right] \\ C_{(4)}C_{(5)}C_{(6)} &= \frac{(1-y)\{\frac{1}{3}xz + (1-x)z\}}{1 - \frac{2}{3}xz - \frac{1}{2}y(\frac{1}{3}xz + (1-x)z)} \end{aligned}$$

The method of calculation of percentage distribution of label in pyruvate or the percentage of total pyruvate molecules labelled is as in Case A, except that values of z require specification. The series of curves presented in Fig. 2 show the percentage distribution of label in C-2 of pyruvate derived from $[2\text{-}^{14}\text{C}]\text{glucose}$ for different values of z . At 62% of label in C-2 [average experimental value from Expts. I and IV of White & Wang (1964)], the values for the percentage contribution of the pentose phosphate cycle are found for different percentage participations of non-triose pathways as given in Table 2.

The values for the percentage participation of non-triose pathways are the percentages of drain-off of all glucose 6-phosphate molecules, unrecycled, recycled and recombined. To express the percentage participation of non-triose pathways as percentage drainage of only the initially introduced glucose 6-phosphate molecules it is necessary to derive an expression for total non-triose-pathway drainage.

This can be accomplished by adding all the expressions in Case B except that for $C_{(4)}C_{(5)}C_{(6)}$ after the substitution of $(1-z)$ for all values of $(1-x)z$. The latter is done since, instead of the fraction $(1-x)z$ of the glucose 6-phosphate (unrecycled, recycled and recombined) going via the Entner-Doudoroff pathway the fraction $(1-z)$ of the same glucose 6-phosphate which is drained off is required. Simplification after the addition of the expressions gives the total number of glucose 6-phosphate molecules engaged in non-triose pathways as:

$$\frac{1-z}{1-\frac{2}{3}xz} \left[1 + \frac{\frac{1}{2}y\{\frac{2}{3}xz + (1-x)z\}}{1-\frac{2}{3}xz - \frac{1}{2}y\{\frac{2}{3}xz + (1-x)z\}} \right]$$

Fig. 2. Percentage distribution of ^{14}C in C-2 of pyruvate derived from $[2\text{-}^{14}\text{C}]\text{glucose}$ for different fractions of glucose 6-phosphate metabolized via the pentose phosphate cycle and different fractions of glucose 6-phosphate drained off via non-triose pathways [z , defined as: $1 - (\text{percentage participation of non-triose pathways}/100)$]. A, $z = 0.2$; B, $z = 0.4$; C, $z = 0.6$; D, $z = 0.8$; E, $z = 0.9$; F, $z = 1.0$.

As the initial amount of glucose 6-phosphate is always considered to be 1.0, the above equation multiplied by 100 gives the percentage of glucose 6-phosphate molecules entering non-triose pathways. Column (VI) in Table 2 gives these values. To express the pentose phosphate-cycle pathway as a percentage of the initial amount of glucose 6-phosphate rather than as a percentage of the fraction of glucose 6-phosphate molecules entering the pentose phosphate-cycle and Entner-Doudoroff pathways, column (III) of Table 2 is recalculated as explained above; at each cycle: (percentage contribution of pentose phosphate cycle) $\times$ (1 - non-triose-pathway fraction). The results are shown in column (VII). However, the percentage label drainage shown in column (II) is used as it is the initial non-triose-pathway drainage. On this basis, the percentage contribution of the pentose phosphate-cycle pathway does not change within the error of determining the number from the graph and becomes independent of non-triose-pathway reactions. Conversely, the percentage label distribution in C-2 of pyruvate also stays within the limits of experimental error. As the contribution of the pentose phosphate cycle decreases and that of the Entner-Doudoroff pathway increases, non-triose pathways have less effect on the amount of label at C-2. Calculations when $z = 0.8$, corresponding to a 26% contribution of non-triose pathways, and for various values of y showed only a 3% decrease in the percentage label at C-1 of pyruvate derived from $[6\text{-}^{14}\text{C}]\text{glucose}$. Thus less label is found at C-1 as the participation of non-triose pathways increases.

The percentage of triose recombination [column (V) of Table 2] can be calculated as in Case A by the modified ratio formula:

$$y = \frac{2b_1}{(1-x)z(100-b_1) + 2b_1}$$

Table 2. *Evaluation of pathways of glucose metabolism in Acetobacter xylinum when non-triose reactions involve unrecycled, recycled and recombined glucose 6-phosphate molecules*

$[2\text{-}^{14}\text{C}]\text{Glucose}$ was used for the evaluation. $z = 1 - (\text{percentage participation of non-triose pathways}/100)$.

z (I)	Percentage contribution of non-triose pathways at each cycle (II)	Percentage contribution of pentose phosphate cycle at each cycle (III)	Percentage contribution of Entner- Doudoroff pathway (IV)	Percentage contribution of triose recombination (V)	Percentage contribution (exp.) of non-triose pathways (VI)	Percentage contribution of pentose phosphate cycle per initial fraction of glucose 6-phosphate (VII)
0.6	40	99-100	0-1	99	80-100*	60
0.8	20	73	27	91	48	58
0.9	10	63	37	86	25	57
0.95	5	60	40	85	13	57
1.0	0	58	42	84	0	58

* A small change in the percentage contribution of the pentose phosphate cycle gives a large change in the percentage of non-triose pathway drainage. When $z = 0.6$, it is improbable that C-2 has 62% of the total activity.

DISCUSSION

The assumptions and the model proposed for glucose breakdown in *A. xylinum* are probably minimal, the situation being more involved if extensive drainage of pentose into assimilatory reactions occurs. Pentose utilization is, however, usually considered as minor or nil compared with such products as glucose 6-phosphate, triose phosphate or pyruvate. Non-equilibration of intermediate pools would also influence the quantitative determination of pathways. One criticism of the present study is that a decrease in the initial glucose or gluconate concentration during metabolism could cause a shift in the percentage of glucose 6-phosphate molecules entering the pentose phosphate-cycle or Entner-Doudoroff pathways. Such a shift might be reflected in the labelling patterns in pyruvate or alanine. Only in Expt. I of White & Wang (1964), when the incorporation period was 6 hr., might this apply. Dawes & Holms (1958) found that the percentage contribution of pathways in *Sarcina lutea* varied little over a wide range of glucose concentrations.

Alterations in the distribution of label in pyruvate or alanine may occur through resynthesis of hexose phosphate from pyruvate ('reverse' glycolysis) or via carbon dioxide fixation. Schramm *et al.* (1957), however, showed conclusively that pyruvate is not converted into cellulose by glucose-cultured cells. Though the present work (White & Wang, 1964) indicated little, if any, fixation of carbon dioxide into pyruvate in a non-static air-swept system, carbon dioxide fixation would affect the pathway calculations by all methods described. Dilution by endogenous unlabelled pyruvate should only affect pathway estimation based on the molar specific activity of glucose relative to substrate glucose but not that based on labelling-distribution ratios or percentage distribution of ^{14}C in pyruvate.

The contribution of the phosphoketolase pathway discovered by Schramm & Racker (1957) in a cellulose-less mutant of *A. xylinum* has been considered by White & Wang (1964). Experimental findings suggested that it was non-functional in the intact cellulose-forming cells used in the present study. One additional argument against an extensive phosphoketolase pathway is that C-6 of glucose or gluconate enters C-1 of fructose 6-phosphate via triose recombination and thence C-2 of acetate via phosphoketolase. Little ^{14}C from C-6 could thus be expected in C-1 of pyruvate (which is not the case). It is admitted that more elaborate (and probably difficult) experiments would be required to discount completely the participation of this alternative route for glucose dissimilation. On the basis of the present results it seems justifiable

to conclude that it has a very minor role under the experimental conditions used.

The methods of pathway evaluation used in the present investigation can be extended to include other systems such as glycolysis. Degradation and specific-activity determination of cellulose and a triose intermediate arising from suitably ^{14}C -labelled glucose might allow a direct estimation of non-triose and pentose phosphate-cycle pathways in *A. xylinum* (Katz & Wood, 1960). In contrast with liver tissue, where the ^{14}C pattern in glycogen provides little indication of the pentose phosphate cycle (owing to strong glycogen synthesis), the distribution of ^{14}C in cellulose from *A. xylinum* should permit evaluation of the pentose phosphate cycle since the polymer molecules come from a pool containing a large proportion of recycled hexose phosphate molecules. The extent of randomization of ^{14}C in C-1, C-2 and C-3 of glucose 6-phosphate is proportional only to the fraction of the total metabolism of [^{14}C]glucose proceeding by the pentose phosphate cycle. The curves for a 0% participation by triose recombination (Figs. 1a, 1b and 1c) may be used for the case of glycolysis in conjunction with the pentose phosphate cycle when the hexose phosphate re-formed via the pentose phosphate cycle enters these pathways in the same proportion as substrate glucose (Dawes & Holms, 1958).

Pathway evaluation by the use of $^{14}\text{CO}_2$ yields or ratios of ^{14}C in triose intermediates (Katz & Wood, 1960) would be greatly complicated by triose recombination. Recombination of triose to form hexose has usually been ignored in both theoretical and experimental studies of quantitative glucose dissimilation. There is some justification for this in animal tissues but not, apparently, in plant tissue (Shibko & Edelman, 1958) or in certain micro-organisms (Lewis, Blumenthal, Weinrach & Weinhouse, 1955; Segal & Topper, 1957). With few exceptions (Casselton & Syrett, 1962), no attempt has been made to correct the specific activity or radiochemical yields of carbon dioxide or some isolated intermediate for the effect of triose recombination. The use of C-6:C-1 (carbon dioxide) ratios to evaluate glucose metabolism has numerous pitfalls of which triose recombination has been the least recognized. Randomization of label between C-1 and C-6 of hexose phosphate would seriously influence a C-6:C-1 (carbon dioxide) ratio, making the value for maximum contribution of the pentose phosphate cycle too low. Studies of glucose metabolism in *Chlorella* illustrate this point (Casselton & Syrett, 1962).

The more active pentose phosphate cycle in younger cells is in agreement with a metabolic stage in which the cell utilizes pentose for biosynthetic purposes as contrasted with mature cells

where pentose requirement may be decreased. Vardanis & Hochster (1961) found that the ratio of glucose 6-phosphate-dehydrogenase to 6-phosphogluconate-dehydrogenase activity in *Agrobacterium tumefaciens* was 8:1, a factor favouring the Entner-Doudoroff pathway over the pentose phosphate cycle. A similar situation may exist in older-growth-phase cells of *A. xylinum*.

SUMMARY

1. A mathematical model is presented for the metabolism of specifically ^{14}C -labelled glucose by the cellulose bacterium, *Acetobacter xylinum*.

2. The analysis treats the case of the pentose phosphate cycle in conjunction with the Entner-Doudoroff system and glucose resynthesis from triose phosphate generated by both pathways. In addition, triose phosphate and fructose 1,6-diphosphate but not fructose 6-phosphate can be converted glycolytically into pyruvate.

3. Recycling of glucose 6-phosphate via the pentose phosphate cycle and drainage of hexose phosphate into cellulose and oxogluconates is taken into account.

4. Sets of theoretical curves are obtained which show the molar specific activity of pyruvate molecules (relative to substrate glucose) arising from [^{14}C]glucose labelled at C-1, C-2 or C-6 as a function of various percentage participations of the pentose phosphate-cycle pathway and triose recombination. Theoretical curves are given for the percentage distribution of label in pyruvate derived

from specifically ^{14}C -labelled glucose as a function of different pathway percentages.

5. The pentose phosphate cycle appears to be more active in young cells. About 80–90% of the triose phosphate formed via the pentose phosphate-cycle and Entner-Doudoroff pathways appears to recombine via fructose 1,6-diphosphate to yield hexose monophosphate.

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Adenosine Triphosphate-Nicotinamide Mononucleotide Adenylyltransferase of Pig-Liver Nuclei

EXTRACTION AND PURIFICATION OF THE ENZYME

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In previous work (Atkinson, Jackson & Morton, 1961) a partially purified preparation of ATP-NMN adenylyltransferase (EC 2.7.7.1) was used to determine the influence of pH and NMN concen-

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tration on the rate of formation of NAD. For further work on the specificity of this enzyme, a preparation of higher specific activity, and free of possible interfering enzymes, was needed.

The present paper describes the large-scale preparation of pig-liver nuclei, the preliminary removal of inactive protein, and the extraction of enzyme

Bioch. 1964, 90