Conversion of inositol to CO_2 by rat-kidney preparations*

We have found that when several kinds of rat tissues (kidney, liver, pancreas and diaphragm) were supplied with [$^{14}C_6$]*myo*-inositol prepared photosynthetically using the pea plant[1], only kidney caused a disappearance of the substrate and an appearance of $^{14}CO_2$. Evidence obtained by various workers employing rat-kidney preparations would suggest the following sequence as a reasonable explanation of the observed reaction.

myo-inositol $\xrightarrow{a}$ L-glucuronate

myo-inositol $\xrightarrow{b}$ D-glucuronate $\xrightarrow{c}$ L-gulonate $\xrightarrow{d}$ [3-keto-L-gulonate] $\longrightarrow$ L-xylulose + CO_2

CHARALAMPOUS and his co-workers[2,3] have demonstrated that soluble extracts of rat kidney catalyze steps a and b. The overall reaction, D-glucuronate $\longrightarrow$ CO_2, has been shown to occur with both soluble preparations and homogenates of this tissue[4]. The reduction of L-gulonate in step d has been established using a soluble system and L-xylulose has been definitely identified as the product[5]. The L-xylulose in an intact system may be presumed to undergo conversion to D-xylulose-5-phosphate, and thence to hexose phosphate via the conventional pentose phosphate pathway. However, with the kidney preparations used it is unlikely, on the basis of the work of RABINOWITZ AND SALI[4], that significant formation of CO_2 occurs following the last step shown.

If the proposed sequence is correct, the introduction of sufficiently large pools of unlabeled intermediates should reduce the rate of output of $^{14}CO_2$ from [$^{14}C_6$]*myo*-inositol. The data in Table I shows that the addition of D-glucuronate and D-glucuronolactone virtually eliminated the $^{14}CO_2$ out put from labeled inositol catalyzed by the

TABLE I

THE EFFECT OF UNLABELED INTERMEDIATES ON THE FORMATION OF $^{14}CO_2$ FROM $^{14}C_6$-*myo*-INOSITOL BY RAT-KIDNEY PREPARATIONS

Each Warburg vessel contained the following: 1.0 ml of kidney preparation, the compound being tested (neutralized, if necessary) at a final concentration of 0.025 *M*, approx. 5 μg [$^{14}C_6$]*myo*-inositol (26,000 counts/min) water to give a final reaction vol. of 1.7 ml and 0.2 ml 20% KOH in center well. The reaction temperature was 30°; the time 2.5 h. Samples were counted as $BaCO_3$ and were corrected for self-absorption. The kidney homogenate was obtained by grinding each gram of tissue with 4 ml of cold 0.2 *M* phosphate buffer, pH 7.5, in a Potter–Elvehjem homogenizer for 2 min. The supernatant was obtained by centrifuging the homogenate for 1 h at 56,000 × *g*.

System	*Additions*	*Counts/min in recovered CO_2*
Homogenate	None	1545
	D-glucuronate	1225
	D-glucuronolactone	1311
	L-gulonolactone	1325
Supernatant	None	860
	D-glucuronate	0
	D-glucuronolactone	0
	L-gulonolactone	185

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supernatant from rat kidney, in agreement with the postulated sequence (b $\longrightarrow$ c $\longrightarrow$ d), but these additions had only a slight effect on the enzyme system in the homogenate. Gulonolactone when added to the supernatant caused a very marked reduction in $^{14}CO_2$ production but did not eliminate it entirely; in the homogenate it had little effect. It thus appears that inositol is metabolized to CO_2 in the two systems by different pathways. It is to be noted that in this case the amount of $^{14}CO_2$ produced by the homogenate exceeded that yielded by the supernatant. Glucose and *myo*-inosose-2 did not reduce $^{14}CO_2$ productions in either case, excluding the participation of these compounds as free intermediates. [$^{14}C_6$]D-glucuronolactone was converted to $^{14}CO_2$ in the homogenate and supernatant at equal rates, proving that this span of the sequence is equally operative in both cases.

A further difference between the supernatant and homogenate was indicated by the observation that arsenite ($5.9 \cdot 10^{-3}$ *M*) caused an 81 % inhibition in the production of $^{14}CO_2$ from inositol by the homogenate but did not affect the supernatant under similar reaction conditions.

Not only do these results indicate that inositol is converted to CO_2 in the homogenate by a pathway differing from that in the supernatant but they also imply that step b is suppressed by the particulate matter in the homogenate. It was indeed found that the enzymic conversion of inositol to glucuronic acid was inhibited by 41 % when the particulate matter was added to the supernatant reaction mixture which was 0.067 *M* with respect to inositol (Table II). The method employed for the determination of the glucuronic acid did not distinguish between the isomers.

TABLE II

INHIBITION BY RAT-KIDNEY PARTICULATE MATTER OF THE CONVERSION OF *myo*-INOSITOL TO GLUCURONIC ACID

The reaction mixture contained 0.5 ml 0.02 *M* inositol, 0.5 ml of either or both the particulate and supernatant fractions, and additional buffer when necessary to give a final vol. of 1.5 ml. Controls for slight endogenous formation of chromogenic material were obtained by replacing the inositol solution with water. The reaction was carried out at 37° for 230 min.

Kidney fraction		*Glucuronic acid formed*
Supernatant ml	*Particulate* ml	*μmoles*
0.5	—	1.82
—	0.5	0.05
0.5	0.5	1.07

The distribution of label in urinary glucose and glycogen-derived glucose as found by POSTERNAK *et al.*[6], who used [2-^{2}H]*myo*-inositol and ANDERSON AND COOTS[7] who used [2-^{14}C]*myo*-inositol is in harmony with the operation of the sequence and the subsequent channeling of the product into the conventional pentose phosphate and glycolytic pathways. While it seems reasonable to conclude that the sequence shown (b $\longrightarrow$ d) does not represent the pathway by which CO_2 may arise from inositol in rat-kidney homogenate, the significance of the alternate metabolism in the intact animal must await further study. In this connection, COOTS AND ANDERSON have pointed out to us, in a private communication, that they have observed a rapid conversion of labeled inositol to CO_2, *in vivo*, but only a slow conversion to glucose in confirmation

of the findings of others[8,9]. This, and the fact that glucose so formed from [2-^{14}C]*myo*-inositol is always found to be labeled equally in the 1 and 6 position rather than predominately in the 6 position they regard as support for an alternate pathway.

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Observations on the catalyzed hydrolysis of *p*-nitrophenyl acetate by peptides of histidine and methylhistidine

The implication of imidazole groups as the sites of action of some hydrolytic enzymes has prompted several investigations of the catalytic hydrolysis of esters by simple imidazole compounds[1-3]. The rates are appreciable and suggest a possible function for the histidine and other imidazole compounds which occur in some tissues in relatively large amounts. For example, the skeletal muscle of the tuna may contain as much as 50 μmoles histidine and 40 μmoles anserine per g wet tissue[4]. The relative catalytic effectiveness of carnosine and anserine have not yet been determined. In the present study, these and also 1-methylhistidine, histidylhistidine, and β-aspartylhistidine, have been compared to histidine with respect to their ability to accelerate the hydrolysis of *p*-nitrophenyl acetate (NPA) at pH 6.2.

The imidazole compounds were of commercial origin except for the β-aspartylhistidine which was from V. VIGNEAUD. The technique used was essentially that of BRECHER AND BALLS[3]. Results are shown in Table I. With two exceptions, the order of effectiveness is that expected from the general theory of basic catalysis; *viz.*, the log of the rate of hydrolysis is proportional to the pK value of the imidazole group. The values fall on a straight line (not shown) except for those of β-aspartylhistidine and histidylhistidine. In view of the unknown effects of the various polar groups involved[1], it is not possible as yet to account for these discrepancies.

Information was also obtained on the inhibition of the catalytic effect of histidine and 1-methylhistidine by Cu^{++}, Zn^{++}, Ni^{++}, Co^{++}, and Mn^{++}. These experiments were done at pH 7.15 in 0.05 M veronal buffer. When metal salt and imidazole were in equimolar proportion (0.0003 M), Cu^{++} effectively inhibited both histidine and 1-methylhistidine (94 %, 91 % inhibition). Zn^{++} and Ni^{++} were more effective as inhibitors for histidine (94 %, 100 %) than for 1-methylhistidine (22 %, 41 %), Co^{++}