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.

The authors are indebted to Dr. N. E. ARTZ of the Corn Products Refining Co., who kindly made available the radioactive glucuronolactone used in these experiments.

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Received September 18th, 1958

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^{++}

TABLE I

EFFECT OF IMIDAZOLE COMPOUNDS ON RATE OF HYDROLYSIS OF *p*-NITROPHENYL ACETATE

3 μmoles imidazole compound, 25 μmoles *p*-nitrophenyl acetate in 0.2 ml acetone, 0.067 *M* phosphate buffer, pH 6.2, total vol., 10 ml. Nitrophenol liberation calculated from linear rate of increase of absorbance at 402 mμ, at 30°.

Imidazole compound	*pK (Im)**	*Rate of liberation of nitrophenol* μ*moles/l/min*	*Relative rate***
None	—	1.0	—
Histidylhistidine	5.60, 6.80	6.0	2.1
Histidine	6.00	3.3	1.0
1-Methylhistidine	6.48	5.8	2.0
Carnosine	6.83	7.3	2.7
β-Aspartylhistidine	6.93	3.4	1.0
Anserine	7.04	9.3	3.6

* p*K* values from COHN AND EDSALL[6], 25°. The p*K* of the imidazole group of β-aspartylhistidine was determined by GREENSTEIN AND KLEMPERER[7], 38°.

** Compared to histidine after subtraction of the blank.

inhibited only histidine (92 %), and Mn^{++} had no effect on either. Co^{++} also had no effect on carnosine or anserine.

KOLTUN AND GURD[5] have reported that the Zn^{++} and Cu^{++} complexes of imidazole were ineffective catalysts for NPA hydrolysis. These observations therefore suggest that copper forms poorly dissociable complexes with the imidazole group of both histidine and 1-methylhistidine, that the complexes of Zn^{++} and Ni^{++} with the histidine imidazole group are stronger than with that of 1-methylhistidine, and that Co^{++} forms a complex with the histidine imidazole group but not with that of 1-methylhistidine, carnosine, or anserine. The cobalt complexes of histidine combine readily and reversibly with oxygen[8–10]. However, this complication was apparently not involved in the present case since the inhibition was demonstrable also in an anaerobic system. By other methods, LI *et al.*[11] have recently shown that the Cu^{++} and Ni^{++} complexes of 1-methylhistidine are only slightly less stable than are those of histidine.

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Received August 11th, 1958