the amount of $^{14}CO_2$ produced from [γ-^{14}C]aspartic acid is very small compared with the incorporation of $^{14}CO_2$; the incorporation therefore involves a net fixation of CO_2.

This conclusion is further supported by observations on the interrelations between CO_2 and aspartic acid in the growth of this organism (Fig. 1). When a defined medium[7] was used, with or without aeration, there was no difference in the growth rate when aspartic acid was omitted from the medium. When precautions were taken to exclude CO_2 from the culture medium, and a stream of CO_2-free air was passed through the culture, the growth rate and the final cell density in the absence of aspartic acid were virtually zero, indicating that fixation of CO_2 is the main pathway of synthesis of aspartic acid in this organism under these conditions.

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[1] J. T. PARK AND R. HANCOCK, *J. Gen. Microbiol.*, 22 (1960) 249.
[2] H. A. LARDY, *J. Biol. Chem.*, 179 (1949) 721.
[3] V. H. LYNCH AND M. CALVIN, *J. Bacteriol.*, 63 (1952) 525.
[4] J. C. MACDONALD, *Can. J. Microbiol.*, 4 (1958) 335.
[5] W. R. MARTIN AND C. F. NIVEN, *J. Bacteriol.*, 79 (1960) 295.
[6] L. V. CRAWFORD, *Biochem. J.*, 68 (1958) 221.
[7] R. HANCOCK, *Biochim. Biophys. Acta*, 28 (1958) 402.

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The catalytic effect of glutamic acid derivatives in urea synthesis

From the experiments of JONES *et al.*[1] it seems most probable that the first step in the synthesis of urea in mammalian liver leads to the formation of carbamyl phosphate. This step is followed by a reaction of carbamyl phosphate with ornithine to form citrulline. The earlier work, emanating from the laboratory of COHEN, has demonstrated convincingly that the first step requires the presence of a cofactor with catalytic properties which was identified as acetylglutamic acid[2], though related derivatives of this compound also exerted catalytic activities[3]. The enzymes responsible for this reaction[4] (see below) were recently extracted from frog liver[5,6]:

$$2\ ATP + NH_3 + CO_2 \xrightarrow{\text{acetylglutamic acid}} 2\ ADP + \text{carbamyl phosphate} + H_3PO_4 \quad (1)$$

It can be assumed that initially acetylglutamic acid is involved in a reaction with one or more of the substrates. From this the question arises which of the three substrates would react first and which of the molecular groups of the cofactor would take part in this reaction. As a working hypothesis it was thought that the carboxyl groups of acetylglutamic acid would in the first place react with either NH_3 or with ATP whereby acid amides and phosphoric acid anhydrides respectively would be formed. It was therefore decided to synthesize various amide and anhydride derivatives of acetylglutamic acid and of related compounds and to test such compounds for their catalytic activities in the above reaction.

The references given below refer to known methods used for the synthesis of

Abbreviations: ATP, ADP, adenosine tri- and diphosphate.

the following compounds: N-acetylglutamic acid[7,8]; N-carbamylglutamic acid[9]; N-carbamylglutamine[10]; N-carbobenzoxyglutamic acid[11]; L-isoglutamine and N-carbobenzoxy-L-isoglutamine[12]; 3-acetylhydantoin-5-propionic acid lactam[13].

Three compounds were synthesized as follows:

N-acetylglutamine. A suspension of L-glutamine in pyridine was treated with acetyl chloride under cooling and shaking. To the resulting mixture ether was added, the precipitate formed was taken up in weak alkaline solution and the pyridine was removed by distillation. The oily residue was taken up in ethanol, ether was added until an oil separated which was discarded; more ether was added to the remaining solution until an amorphous precipitate could finally be separated; a solution of the precipitate gave no ninhydrin reaction. M.p., 185–190°.

N-acetylisoglutamine. This was prepared from isoglutamine by treatment with acetic anhydride. The acidified mixture was evaporated to dryness, the residue was taken up in ethanol and reprecipitated with ethyl acetate. Both the precipitate and the supernatant contained acetylisoglutamine in about equal quantities as ascertained by paper chromatography. The presence of traces of N_1N'-diacetylisoglutamine in the preparation was revealed by preparing its hydroxamic acid derivative[14] and by applying the chlorination test[15,16].

α- and γ-N-acetylglutamyl phosphate. Acetylglutamic acid was treated with hot acetic anhydride until completely dissolved. After removal of the latter and of the acetic acid by distillation, the residue was stirred in the cold with KH_2PO_4 and pyridine until the substance was completely dissolved. By adding a solution of 4 *N* LiOH the lithium salt of the phosphorylated compound was formed, inorganic phosphate was removed by precipitation with ethanol in the cold and the final product was obtained by further treatment with ethanol. By estimating quantitatively the hydroxamic acids[14] prepared from the components of the preparation and the heat-labile phosphate[17], it was found that the final product contained a mixture of the α- and γ-phosphates as well as some unchanged acetylglutamic acid which could not be separated.

Hydantoin-5-propionic acid was a gift of Dr. H. WAELSCH, Columbia University, New York.

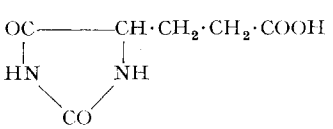
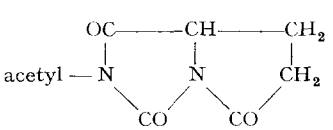
From Table I it will be seen that none of the amide or anhydride derivatives of glutamic acid was able to replace acetylglutamic acid as a catalyst in reaction (1) and they cannot therefore be regarded as intermediary compounds in the synthesis of carbamyl phosphate. Only a very slight catalytic activity could be observed with compounds 6 and 8 in which one of the carboxyl groups of acetylglutamate was substituted with ammonia. Carbamylglutamate, which is known to act similarly to acetylglutamate[3], became inactive when its γ-carboxyl group had been substituted with ammonia (compound 9). The activity of the mixture of 1- and 5-phosphorylated acetylglutamate (compounds 11a and 11b) was considerably less than that of the phosphate-free catalyst and was obviously due to the presence of "free" acetylglutamate (see preparation). The acyl group of the catalyst acid appears to be essential, since compounds 3, 5 and 7 were inactive, nor can it be replaced by the carbobenzoxy group (compounds 4 and 10). More drastic alterations of the structure of the catalyst through ring formation (compounds 12 and 13) showed the same negative result.

The possibility of the primary formation of phosphoric acid and amide derivatives during the catalytic action of acetylglutamic acid had also been envisaged by other

TABLE I

THE CATALYTIC EFFECT OF GLUTAMIC ACID DERIVATIVES ON THE SYNTHESIS OF CITRULLINE

Composition of incubation mixture (quantities in μmoles): ATP, 4; 3-phosphoglycerate, 30; L-ornithine, 20; NH_4Cl, 30; $NaHCO_3$, 100; $MgCl_2$, 20; phosphate buffer, pH 7.4, 20; glutamic acid derivative, 10. Liver enzyme, 1 ml; muscle enzyme (for regeneration of ATP), 0.4 ml; total volume, 2 ml. Incubation time, 40 min at 37°. After incubation the mixture was deproteinised by adding 5 ml 0.5 *M* $HClO_4$ and the citrulline estimated according to ARCHIBALD[23]. The citrulline synthesis was considered a measure of the catalytic activities of the test substances. The liver enzyme was prepared by extracting 1 g ox liver acetone powder with 10 ml water. 0.3% rabbit muscle extract was prepared according to RATNER AND PAPPAS[24].

No. of compound			*Citrulline synthesis*
			%
1	$HOOC{\cdot}CH(NH){\cdot}CH_2{\cdot}CH_2{\cdot}COOH$ (acetyl)	N-acetyl-L-glutamic acid	100
2	$HOOC{\cdot}CH(NH){\cdot}CH_2{\cdot}CH_2{\cdot}COOH$ (carbamyl)	N-carbamyl-L-glutamic acid	35
3	$HOOC{\cdot}CH(NH_2){\cdot}CH_2{\cdot}CH_2{\cdot}COOH$	L-glutamic acid	0
4	$HOOC{\cdot}CH(NH){\cdot}CH_2{\cdot}CH_2{\cdot}COOH$ (carbobenzoxy)	N-carbobenzoxy-L-glutamic acid	1
5	$HOOC{\cdot}CH(NH_2){\cdot}CH_2{\cdot}CH_2{\cdot}CONH_2$	L-glutamine	0
6	$HOOC{\cdot}CH(NH){\cdot}CH_2{\cdot}CH_2{\cdot}CONH_2$ (acetyl)	N-acetyl-L-glutamine	7
7	$H_2NOC{\cdot}CH(NH_2){\cdot}CH_2{\cdot}CH_2{\cdot}COOH$	L-isoglutamine	0
8	$H_2NOC{\cdot}CH(NH){\cdot}CH_2{\cdot}CH_2{\cdot}COOH$ (acetyl)	N-acetyl-L-isoglutamine	1
9	$HOOC{\cdot}CH(NH){\cdot}CH_2{\cdot}CH_2{\cdot}CONH_2$ (carbamyl)	N-carbamyl-L-glutamine	
10	$H_2NOC{\cdot}CH(NH){\cdot}CH_2{\cdot}CH_2{\cdot}COOH$ (carbobenzoxy)	N-carbobenzoxy-L-isoglutamine	1
11a	$^-O_3P{-}OC{\cdot}CH(NH){\cdot}CH_2{\cdot}CH_2{\cdot}COOH$ (acetyl)	mixture of: 1- and 5-N-acetyl-L-glutamyl phosphate	62
b	$HOOC{\cdot}CH(NH){\cdot}CH_2{\cdot}CH_2{\cdot}CO{-}PO_3^-$ (acetyl)		
12		hydantoin-5-propionic acid	1
13		3-acetylhydantoin-5-propionic acid lactam	0

workers[18,19]. More recently, METZENBERG *et al.*[5,20] denied this possibility and further concluded that ammonia did not take part in the first phase of the catalysis, a view also shared by JONES[21].

Thus, free carboxyl groups are prerequisite for the catalytic action of acetylglutamic acid; further, since the carbon-bonded hydrogen atoms do not take part in the catalysis[22], the reactivity of acylglutamic acid seems to be associated with its substituted amino group.

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[1] M. E. JONES, L. SPECTOR AND F. LIPMANN, *J. Am. Chem. Soc.*, 77 (1955) 819.
[2] L. M. HALL, R. L. METZENBERG AND P. P. COHEN, *J. Biol. Chem.*, 230 (1958) 1013.
[3] S. GRISOLIA AND P. P. COHEN, *J. Biol. Chem.*, 204 (1953) 753.
[4] R. L. METZENBERG, L. M. HALL, M. MARSHALL AND P. P. COHEN, *J. Biol. Chem.*, 229 (1957) 1019.
[5] R. L. METZENBERG, M. MARSHALL AND P. P. COHEN, *J. Biol. Chem.*, 233 (1958) 1560.
[6] M. MARSHALL, R. L. METZENBERG AND P. P. COHEN, *J. Biol. Chem.*, 233 (1958) 102.
[7] P. KARRER, K. ESCHER AND A. WIDMER, *Helv. Chim. Acta*, 9 (1926) 301.
[8] B. H. NICOLET, *J. Am. Chem. Soc.*, 52 (1930) 1192.
[9] H. A. KREBS, L. V. EGGLETON AND V. A. KNIVETT, *Biochem. J.*, 59 (1955) 185.
[10] P. P. COHEN AND S. GRISOLIA, *J. Biol. Chem.*, 182 (1950) 747.
[11] S. GOLDSCHMIDT AND C. JUTZ, *Ber.*, 86 (1953) 1120.
[12] T. WIELAND AND H. WEIDENMÜLLER, *Ann. Chem., Liebigs*, 597 (1955) 111.
[13] D. L. SZABO AND J. V. KARABINOS, *J. Am. Chem. Soc.*, 66 (1944) 650.
[14] F. LIPMANN AND L. C. TUTTLE, *J. Biol. Chem.*, 159 (1945) 21.
[15] H. N. RYDEN AND P. W. G. SMITH, *Nature*, 169 (1952) 922.
[16] T. WIELAND, *Angew. Chem.*, 66 (1954) 787.
[17] C. H. FISKE AND Y. SUBBAROW, *J. Biol. Chem.*, 66 (1925) 375.
[18] S. GRISOLIA, in W. D. MCELROY AND AND B. GLASS, *Phosphorus Metabolism*, Vol. I Baltimore, 1951, p. 619.
[19] S. GRISOLIA AND J. C. TOWNE, *Biochim. Biophys. Acta*, 25 (1957) 224.
[20] R. L. METZENBERG, M. MARSHALL, P. P. COHEN AND W. G. MILLER, *J. Biol. Chem.*, 234 (1959) 1534.
[21] M. E. JONES, *Federation Proc.*, 18 (1959) 255.
[22] S. GRISOLIA, R. H. BURRIS AND P. P. COHEN, *J. Biol. Chem.*, 210 (1954) 761.
[23] R. M. ARCHIBALD, *J. Biol. Chem.*, 156 (1944) 121.
[24] S. RATNER AND A. PAPPAS, *J. Biol. Chem.*, 179 (1949) 1183.

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Hexose monophosphate pathway in pituitary tissue

Few studies have been made on glucose catabolism in the pituitary. Rat pituitary is characterized by a high endogenous respiration, which is unaffected by the presence of glucose or succinate in the medium[1,2]. The concentration of the enzymes of the tricarboxylic acid cycle is significantly smaller than in liver[2].

An active hexose monophosphate pathway has been demonstrated in the adrenals[3], in the testis[4], and in the thyroid[5], and the hypothesis has been suggested that this might be a common property of endocrine tissues. The present study presents evidence for an active hexose monophosphate pathway in beef pituitary.

The experimental methods were the same as those previously reported. The