# ORNITHINE-CATALYZED UREA FORMATION IN LIVER HOMOGENATE*

*Sirs:*

Heretofore attempts to obtain synthesis of urea from ammonia (and carbon dioxide) in cell-free extracts have been unsuccessful. We have found the reaction to proceed in guinea pig liver homogenate. The following is the reaction mixture which has given the highest yields obtained so far: L-ornithine (0.00075 M), ammonia (0.0025 M), L-glutamate (0.01 M), oxalacetate (0.005 M), ATP (0.00025 M), and 0.33 gm. of homogenized liver in a final volume of 3.5 ml. The following are typical increases in urea over the blank observed in 1 hour, in micrograms: ornithine + ammonia 0, glutamate + oxalacetate 0, glutamate + oxalacetate + ornithine 49, glutamate + oxalacetate + ammonia 105, glutamate + oxalacetate + ornithine + ammonia 317, glutamate + ocalacetate + ornithine + ammonia (without ATP) 150.

The buffer solution consisted of 90 parts of the phosphate-saline solution of Cohen and Hayano[1] and 10 parts of 0.13 per cent sodium bicarbonate. The liver was homogenized by the method of Potter and Elvehjem.[2] The reaction was carried out at 38° and pH 7.5 under 5 per cent carbon dioxide and either 95 per cent oxygen or nitrogen.

Urea was determined by two methods: the xanthydrol method of Engel and Engel[3] or the colorimetric diacetyl monoxime method of Barker[4] before and after digestion with urease. The two methods gave similar results.

Addition of pyruvate (0.01 to 0.005 M) to the above reaction mixture did not give higher yields of urea. The yield was smaller when oxalacetic acid was omitted, and greater when fumarate (0.05 M) was substituted for it. Glutamine was as effective, but not more so than equivalent concentrations of glutamate and ammonia.

Urea formation in any of the above reaction mixtures was completely inhibited by anaerobiosis and by 0.0036 M sodium arsenate.

Analyses of the absorption curves of the color obtained with diacetyl monoxime after digestion with urease indicated that citrulline and other chromogenic material were formed during the reaction. By omitting ATP from the reaction mixture less urea was formed and the total residual

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[1] Cohen, P. P., and Hayano, M., *J. Biol. Chem.*, **166**, 251 (1946).

[2] Potter, V. R., and Elvehjem, C. A., *J. Biol. Chem.*, **114**, 495 (1936).

[3] Engel, M. G., and Engel, F. L., *J. Biol. Chem.*, **167**, 535 (1947).

[4] Barker, S. B., *J. Biol. Chem.*, **152**, 453 (1944).

chromogenic material after urease digestion (designated as "citrulline" for the time being) was doubled. Expressed in terms of citrulline standards, ornithine plus ammonia gave at 38° per 0.33 gm. of liver per hour, an increase in "citrulline" of 80 $\gamma$; ornithine plus ammonia plus glutamate 560 $\gamma$; glutamate with either ornithine or ammonia omitted 200 $\gamma$ or less. Pyruvate was as effective and oxalacetate less effective than glutamate.

Anaerobiosis or 0.0036 M sodium arsenate inhibited the formation of "citrulline." Anaerobiosis also inhibited the conversion of citrulline to urea (the reaction mixture contained added glutamate and ATP). On the other hand 0.0036 M sodium arsenate did not inhibit urea formation from citrulline. The conversion of citrulline to arginine in rat kidney slices is completely inhibited by 0.0036 M arsenate.[5] It is difficult to reconcile the two observations with the view that conversion of citrulline to arginine is the obligatory next step in the path to formation of urea. It would appear that in guinea pig liver homogenate citrulline may give urea by another path as well as via its immediate conversion to arginine.

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[5] Borsook, H., and Dubnoff, J. W., *J. Biol. Chem.*, **141**, 717 (1941).