their strains did not fix nitrogen aerobically. This is, of course, unlike the strain employed by Jensen[6], which fixed nitrogen equally well aerobically or anaerobically. One might also mention that Pirt[4] shows that in the presence of fixed nitrogen maximum conversion of carbohydrate to cell material occurs aerobically. Whereas Pengra and Wilson[5] employed a mineral salts and sucrose medium, Jensen[6] found a small amount of yeast extract or agar necessary. We were also unable to show nitrogen fixation unless whey or agar was present. This appears to suggest that a growth factor is required for aerobic nitrogen fixation by some strains of the genus *Aerobacter*.

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[1] Wix, P., and Woodbine, M., *Dairy Sci. Abst.*, **20**, 539 (1958).
[2] Pfeffer, M., M.S. thesis, University of Vermont (1958).
[3] Blanchard, G. C., M.S. thesis, University of Vermont (1958).
[4] Pirt, S. J., *J. Gen. Microbiol.*, **16**, 59 (1957).
[5] Pengra, R. M., and Wilson, P. W., *J. Bact.*, **75**, 21 (1958).
[6] Jensen, V., *Physiol. Plantarum.*, **9**, 130 (1956).

Amino-Acid Metabolism in Wool Roots

Follicle material referred to as wool roots can be prepared in quantity from fresh sheep-skin by Ellis's method[1]. Each wool root consists of a bulb region, a pre-keratinization region and some fully keratinized fibre. Frequently, the inner root sheath is found to be attached to the wool root. Previous studies of homogenates of wool roots revealed the presence of several enzymes[2,3], some of which have been localized in hair follicles by histochemical methods[4,5]. Very little investigation of enzymes concerned with protein metabolism in the hair follicle has been made, despite the fact that protein synthesis is the major activity of this tissue.

The demonstration of free α-amino- and α-oxo-acids in wool roots led Ellis, Gillespie and Lindley[2] to suggest that enzymes could be present which participate in the tricarboxylic acid cycle as well as in transamination reactions. In the present work transaminase activity has been demonstrated. In addition it has been found that another enzyme commonly found in tissues actively engaged in protein synthesis is present, namely, transpeptidase[6,7].

A homogenate was prepared by macerating 5 gm. wool roots with 50 ml. water in a Waring blendor, filtering through coarse 'Terylene' cloth, centrifuging at 2,000*g* and dialysing the supernatant against 0·02 *M* phosphate buffer *p*H 7·4 overnight at +2° C. The supernatant was more satisfactory to use than the original homogenate because of the difficulty of removing the endogenous amino acids from the latter.

The systems used were :

(*a*) For transamination : 0·7 ml. reaction mixture containing 0·5 ml. extract or 0·02 *M* phosphate buffer *p*H 7·4, 5 μgm. pyridoxal phosphate, 20 μmoles α-oxo-acid and 20 μmoles L-amino-acid ; incubated for 2 hr. at 37° C.

(*b*) For transpeptidation : 0·6 ml. reaction mixture containing 0·5 ml. extract or 0·02 *M* phosphate buffer *p*H 7·4, 20 μmoles amino-acid (or water), and 2 μmoles neutralized glutathione ; incubated for 30 min. at 30° C.

(*c*) For citrulline iminase : (1) 1·0 ml. reaction mixture containing 0·5 ml. extract, 5 μgm. pyridoxal phosphate, 0·02 *M* potassium dihydrogen phosphate, 15 μmoles L-citrulline, 20 μmoles magnesium chloride, 5 μmoles neutralized adenosine triphosphate, and 2 μmoles ammonium chloride ; incubated for 30 min. at 37° C. ; (2) as (1) with 0·05 *M* borate instead of potassium dihydrogen phosphate and 15 μmoles L-arginine instead of L-citrulline and without ammonium chloride.

Following incubation, the reactions were stopped by the addition of 1 ml. warm ethanol ; in (*b*) the ethanol contained 2·2 μmoles N-ethyl maleimide. The clear supernatants were dried *in vacuo* and then applied to Whatman No. 1 paper ; samples (*a*) and (*c*) were developed in 80 per cent aqueous phenol, and (*b*) in 80 per cent aqueous *n*-propanol for 24 and 240 hr. respectively.

So far it has been possible to demonstrate the formation of alanine from pyruvic and aspartic acids, and glutamic acid from α-oxoglutaric acid and alanine or citrulline. It is of interest that citrulline, which occurs in the free state in wool roots[8] as well as combined in the inner root sheath[9], participates in transamination.

It was not possible to obtain evidence of citrulline iminase activity[10]. This enzyme catalyses the reversible conversion of citrulline to arginine and has been suggested as a possible constituent of hair follicles[11].

Evidence for the presence of γ-glutamyl transferase activity[6,7] in wool root homogenates was found by the demonstration of the formation of γ-glutamyl glycine from glutathione and glycine. It is perhaps significant that Haurowitz *et al.*[12] have recently provided evidence for the occurrence of γ-glutamyl residues in wool and the present finding of transpeptidase activity could have relevance to their formation. In addition to transpeptidation, the chromatograms indicated that wool roots probably also possess peptidase and/or protease activity, although cysteinyl–glycinase[13] activity was weak or absent. This enzyme is usually found in conjunction with transpeptidase activity.

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[1] Ellis, W. J., *Nature*, **162**, 957 (1948).
[2] Ellis, W. J., Gillespie, J. M., and Lindley, H., *Nature*, **165**, 545 (1950).
[3] Gillespie, J. M., *Aust. J. Biol. Sci.*, **9**, 448 (1956).
[4] Rogers, G. E., *Quart. J. Micro. Sci.*, **94**, 253 (1953).
[5] Montagna, W., "Structure and Function of Skin" (Academic Press, New York, 1956).
[6] Hanes, C. S., Hird, F. J. R., and Isherwood, F. A., *Biochem. J.*, **51**, 25 (1952).
[7] Hird, F. J. R., and Springell, P. H., *Biochem. J.*, **56**, 417 (1954).
[8] Rogers, G. E. (unpublished experiments).
[9] Rogers, G. E., *Biochim. Biophys. Acta*, **29**, 33 (1958).
[10] Szörenyi, E., Elödi, P., and Deutsch, T., *Acta Physiol. Acad. Sci. Hung.*, **5**, 337 (1954).
[11] Rogers, G. E., and Simmonds, D. H., *Nature*, **182**, 186 (1958).
[12] Haurowitz, F., *et al.*, *J. Biol. Chem.*, **224**, 827 (1957).
[13] Binkley, F., *Exp. Cell Res.*, Supp. 2, 145 (Academic Press, New York, 1952).