

It has been shown<sup>6</sup> that the wool proteins most readily extracted by thioglycollate, including the new protein, originate in the orthocortex while those which are difficult to extract, including keratine 2, originate in the paracortex. The similarity between these two proteins suggests that they may have a similar function in the architecture of the two segments. They account for 30 to 40 % of the fibre and have a relatively low sulphur content. Much of the sulphur is concentrated in the small fraction precipitating at pH 2.9 and this may constitute part of the high-sulphur interfibrillar matrix<sup>7</sup>.

J. M. GILLESPIE

Biochemistry Unit, Wool Textile Research Laboratories, C.S.I.R.O.,  
Parkville, Victoria (Australia)

<sup>1</sup> J. M. GILLESPIE AND F. G. LENNOX, *Biochim. Biophys. Acta*, 12 (1953) 481.

<sup>2</sup> J. M. GILLESPIE AND F. G. LENNOX, *Australian J. Biol. Sci.*, 8 (1955) 97.

<sup>3</sup> J. M. GILLESPIE, *Proc. Int. Wool Text. Res. Conf. Aust.* 1955, B (1956) 35.

<sup>4</sup> J. M. GILLESPIE, *Australian J. Biol. Sci.*, 10 (1957) 105.

<sup>5</sup> D. H. SIMMONDS, *Australian J. Biol. Sci.*, 7 (1954) 98.

<sup>6</sup> R. D. B. FRASER AND G. E. ROGERS, *Biochim. Biophys. Acta*, 12 (1953) 484.

<sup>7</sup> E. H. MERCER, *Proc. Int. Wool Text. Res. Conf. Aust.* 1955, F (1956) 215.

Received September 17th, 1957

## Incorporation of hydropyrimidine derivatives in ribonucleic acid with liver preparations

The biosynthesis of pyrimidine nucleotides, via orotate, has been clarified mainly by KORNBERG and co-workers<sup>1</sup>. Evidence was drawn principally from bacterial preparations, although animal tissues seem capable of using the same pathway<sup>2</sup>. CANELLAKIS<sup>3,4</sup> found that uracil is incorporated into mammalian RNA<sup>5</sup> but there is some disagreement on the relative importance of pyrimidine precursors of RNA<sup>1,4</sup>.

The interconversion of pyrimidines and carbamyl-aminoacids via hydropyrimidines in animal tissues has been presented previously<sup>5,6</sup>. We are evaluating the relative contributions of the orotate and the hydropyrimidine pathways to RNA synthesis in the tissues of several animal species; this note presents evidence that the intermediates related to the hydropyrimidine pathway are incorporated into RNA. The relative activities shown in Tables I and II seem to exclude the mediation of orotate. This is supported by experiments showing that there is no direct interconversion between orotate and the hydropyrimidine derivatives. These enzymic mechanisms are under investigation and several of the isolated enzymic systems are being characterized.

TABLE I  
INCORPORATION OF OROTATE AND CARBAMYL- $\beta$ -ALANINE INTO RNA  
BY LIVER FRACTIONS OF SEVERAL SPECIES

Preparation	Precursor		Preparation	Precursor	
	Orotate	C- $\beta$ -alanine		Orotate	C- $\beta$ -alanine
Dog, Sup. F	19.1	3.9	Chicken, FI	4.5	2.4
Rat, Sup. F	4.8	3.2	Chicken, FII	3.8	0.4
Pigeon, Sup. F	22.0	3.9	Chicken, FIHI	33.6	1.9
Chicken, Sup. F	11.5	4.2	Chicken, FIV	4.1	7.7

The incubations contained the following in 3 ml: 1 mg muscle preparation<sup>7</sup>, enzyme preparation (about 100 mg protein containing RNA, total RNA made up to 6 mg with added RNA),

\* The following abbreviations are used in this paper; RNA, ribose nucleic acid; Sup. F, supernatant fraction, FI, II, III and IV, Fractions I, II, III and IV; C- $\beta$ -alanine, carbamyl- $\beta$ -alanine; C $\beta$ ARP, carbamyl- $\beta$ -alanine ribotide; HUMP, hydrouridine-5'-phosphate; UMP, uridine-5'-monophosphate; C $\beta$ AR, carbamyl- $\beta$ -alanine riboside.

15  $\mu$ moles  $\text{MgSO}_4$ , and the following as potassium salts at pH 7.6: ribose-5-phosphate, 6  $\mu$ moles; phosphate, 60  $\mu$ moles; adenosine triphosphate, 3  $\mu$ moles; 3-D-phosphoglycerate, 15  $\mu$ moles.

Sup. F was the high-speed (25,000 g) supernatant fraction of 25% liver-0.25  $M$  sucrose homogenates. The chicken liver fractions were obtained as follows: Sup. F was made 0.01  $M$  with potassium phosphate buffer pH 7.4, and 0.30, 0.45, 0.65 and 1.30 volumes (referred to original volume) of acetone ( $-20^\circ$ ) were added stepwise (maintained at  $-5^\circ$ ) and the precipitates collected and taken in water to give F I, II, III, and IV respectively. RNA was recovered from the trichloroacetic acid (TCA) precipitates of the reaction mixture, after repeated washing with 0.3  $M$  TCA, by the method of PAIN AND BUTLER<sup>6</sup>. RNA was measured by the orcinol reaction or by its ultraviolet light absorption. RNA samples were plated in thin layers (self-absorption correction, 10% or less) on copper planchets and counted to a probable error of 4%. Figures in the table are counts/min/mg RNA.

TABLE II  
RELATIVE INCORPORATION OF PRESUMED PYRIMIDINE PRECURSORS

Precursor	counts/min/mg RNA	Precursor	counts/min/mg <sup>a</sup> RNA
Orotate**	46	UMP**	516
C- $\beta$ -alanine*	9.6	Uracil***	1.5
Hydrouracil*	12.6	Uridine****	3.5
C $\beta$ ARP**	125	C $\beta$ AR****	3.9
HUMP**	148	Hydrouridine****	2.7

The components and conditions were as described in the legend of Table I. Fraction III and IV were combined and used in the experiments of the Table. \*, \*\*, \*\*\*, \*\*\*\*, S.A. 1, 0.9, 0.5 and 0.3  $\mu\text{C}/\mu\text{mole}$ , respectively.

\* Uncorrected figures. The Geiger tube used gives about 3% of the actual S.A.

We wish to thank Drs. A. KORNBERG and E. S. CANELLAKIS for samples of carbamyl- $\beta$ -alanine and orotate. Supported by grants from the National Institutes of Health. One of us (S.G.) is an Established Investigator of the American Heart Association.

McIlwain Laboratories, University of Kansas Medical Center,  
Kansas City, Kan. (U.S.A.)

LEWIS C. MOKRASCH  
SANTIAGO GRISOLIA

<sup>1</sup> A. KORNBERG, in W. D. McELROY AND B. GLASS, *The Chemical Basis of Heredity*, Johns Hopkins Press, Baltimore, 1956.

<sup>2</sup> R. WU AND D. W. WILSON, *J. Biol. Chem.*, 223 (1956) 195.

<sup>3</sup> E. S. CANELLAKIS, *Biochim. Biophys. Acta*, 23 (1957) 217.

<sup>4</sup> E. S. CANELLAKIS, *J. Biol. Chem.*, 227 (1957) 701.

<sup>5</sup> D. P. WALLACH AND S. GRISOLIA, *J. Biol. Chem.*, 226 (1957) 277.

<sup>6</sup> S. GRISOLIA AND S. S. CARDOSO, *Biochim. Biophys. Acta*, 25 (1957) 430.

<sup>7</sup> S. RATNER, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. II, Academic Press, Inc., New York, 1955, p. 356.

<sup>8</sup> R. H. PAIN AND J. A. BUTLER, *Biochem. J.*, 66 (1957) 299.

Received October 5th, 1957

## The enzymic breakdown of monophosphoinositide by phospholipase B preparations

Recent work has shown that phospholipase B preparations prepared from *Penicillium notatum* will attack lecithin providing certain activating lipids obtainable from liver are added to the system in small quantities<sup>1</sup>. These lipids have been isolated and identified as monophosphoinositide (phosphatidyl inositol) and a polyglycerol phospholipid<sup>2</sup>. While studying the mechanism of this