

The relatively high alanine, glycine, proline and total apolar amino acid contents suggests the possible presence of a major amount of a fibrous type of protein with perhaps a β -pleated structure. Non polar amino acids usually achieve greater quantitative significance in structural proteins, particularly those of the silk fibroin, collagen, elastin, resilin group. This is partly because of the small size of the side chains of glycine and alanine and partly because of the interaction properties of the apolar side chains. Globular proteins or α keratin type structural proteins in general have lower apolar amino acid contents than the group of structural proteins already mentioned. A high content of proline will also limit the degree of α helix formation possible in a protein. The apolar amino acids are usually held to be glycine, alanine, proline, valine, leucine, isoleucine and phenylalanine. Thus wing scales, thorax hairs and tail hairs had total apolar contents of 635, 639 and 601 residues per 1000 total residues, respectively. These might be compared with values of 750, 660, 572, 547, 413 and 385 for lepidopteran β -silk fibroins (average value), resilin, invertebrate collagens (average value), feather (β -keratin), wool (α -keratin) and fibrinogen (globular and fibrous α -helical regions)^{10,11}.

A high alanine content found in the cuticle of the cricket *Anabrus simplex* has been suggested to be responsible for the hardness of the cuticle¹². High levels of glycine and alanine have been noted in the cuticular proteins of *Calliophora erythrocephala*⁹ while high concentrations of proline were detected in the water and urea soluble fractions of *Agrianome spinicollis* cuticle⁸. β -alanine is now recognized as frequently occurring in cuticular protein where it seems often to be present as the preponderant N-terminal amino acid of puparial proteins¹³.

The glucosamine contents of the scales and other structures are also given in the Table. If the glucosamine is assumed to be totally derived from chitin then these values would agree in general with figures quoted elsewhere for cuticle chitin-protein ratios estimated by other methods¹⁴. The tail hairs gave a typical α -chitin X-ray diffraction pattern.

Thus the scales and hairs from this species of moth are composed of chitin and protein the latter constituent having a composition suggestive of a fibrous structure. The amino acid and hexosamine composition of the scales and hairs seems to be essentially similar to the rest of the cuticle of the wings and body.

Résumé. Les écailles du papillon *Xylophasia monoglypha* sont constituées par de la protéine accompagnée de chitine. La protéine a une composition ressemblant à celle des protéines «fibreuses».

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¹⁰ S. HUNT, *Biochim. biophys. Acta*, 207, 347 (1970).

¹¹ R. F. STEINER, *The Chemical Foundations of Molecular Biology* (Van Nostrand, Princeton 1965), p. 168.

¹² B. W. DE HASS, L. H. JOHNSON, J. H. PEPPER, E. HASTINGS and G. L. BAKER, *Physiol. Zool.* 30, 121 (1957).

¹³ R. P. BODNARYK and L. LEVENBOOK, *Comp. Biochem. Physiol.* 30, 909 (1969).

¹⁴ A. G. RICHARDS, *The Integument of Arthropods* (University of Minnesota Press 1951), p. 109.

Persistent Circadian Rhythmicity of Protein Synthesis in the Liver of Starved Rats¹

The rhythmic behavior of hepatic tyrosine transaminase activity² and polysome profiles³ is abolished upon protein or food deprivation. Yet, fluctuations in pituitary gland content of growth hormone⁴, rat serum urea and sodium levels⁵ and hepatic content of glycogen⁶ are unaffected by lack of food. We have previously observed an increased uptake of ³H-leucine into liver protein midway through the dark period⁷, and considered this was due to the cyclic postprandial influx of amino acids into the liver². Consequently, the present study was done to see whether removal of food would abolish the expected rhythmic incorporation of ³H-leucine into rat liver protein.

Male Sprague-Dawley rats were kept in a controlled lighting regimen of 12 h light and 12 h dark for 7 days prior to the experiments. Lights were on at 06.00 h and off at 18.00 h, with Purina Rat Chow and water given ad libitum during the week of adaptation. Beginning at 06.00 h, and at subsequent 2 h intervals, each rat was given an i.v. injection of 5 μ C/g body weight of L-³H-4,5-leucine (58.0 C/mM speciactivity, Schwarz BioResearch). When 06.00 h arrived, all food was removed from the cages. Each rat was dispatched 20 min after the radioisotope injection, and post-mitochondrial supernatant fractions were prepared from sucrose homogenates of their liver as described previously^{8,9}. Only the left median lobe was analyzed since it is known this lobe receives its portal blood primarily from the small intestine⁸. The supernatant fluid

was fractionated into portions soluble and insoluble in 10% trichloroacetic acid-0.5% sodium tungstate (TCA-T)⁸. Blood serum was also recovered and treated with TCA-T. Radioactivity in the liver and serum samples was estimated with liquid scintillation spectrometry¹⁰. In order to reveal general trends, the data were plotted as 6-hour moving averages¹¹. Calculated standard errors of triplicate determinations were small (5 to 7%) and are omitted from the histogram for clarity.

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² R. J. WURTMAN, in *Mammalian Protein Metabolism* (Ed. H. N. MUNRO; Academic Press, New York 1970), vol. 4, p. 445.

³ B. FISHMAN, R. J. WURTMAN and H. N. MUNRO, *Proc. natn. Acad. Sci. USA* 64, 677 (1969).

⁴ M. S. BAHORSKY and L. L. BERNARDIS, *Experientia* 25, 755 (1969).

⁵ M. S. BAHORSKY and L. L. BERNARDIS, *Experientia* 23, 634 (1967).

⁶ E. HAUS and F. HALBERG, *Experientia* 22, 113 (1966).

⁷ A. V. LEBOUTON and S. D. HANDLER, *Fedn. Europ. Biochem. Soc. Letters* 10, 78 (1970).

⁸ A. V. LEBOUTON and T. E. HOFFMAN, *Proc. Soc. exp. Biol. Med.* 132, 15 (1969).

⁹ A. V. LEBOUTON, *Analyt. Biochem.* 20, 550 (1967).

¹⁰ A. V. LEBOUTON, *Biochem. J.* 106, 503 (1968).

¹¹ J. P. GUILFORD, *Fundamental Statistics* (McGraw-Hill, New York 1956).

As expected, the TCA-T insoluble radioactivity in the liver began to decrease in the dark period. Then surprisingly, it rose to a high value at 14.00 h and then reversed by decreasing to amounts nearly equal to those seen during the light period, prior to food removal. Hepatic soluble radioactivity was in phase with the insoluble values, except after the lights went back on, when it seemed to exhibit a slight reciprocal relationship. Soluble radioactivity in serum was considerably out of phase with the hepatic insoluble fraction, while serum insoluble radioactivity was generally in phase with that of liver.

The increase in incorporation of ^3H -leucine into hepatic protein seen here cannot simply be explained as the result of an increased specific radioactivity of the intracellular leucine pool. If this were so, the TCA-T insoluble radioactivity should have continued to increase almost indefinitely, instead of abruptly decreasing as it did at 04.00 h. In addition, it is well known that essential amino acids such as leucine are conserved during periods of starvation,

which would have a diluting effect on radioactivity. Such a diluting effect was not observed here. Even so, this interpretation is subject to revision since we do not have data on the actual specific radioactivity of the hepatic intracellular leucine pool.

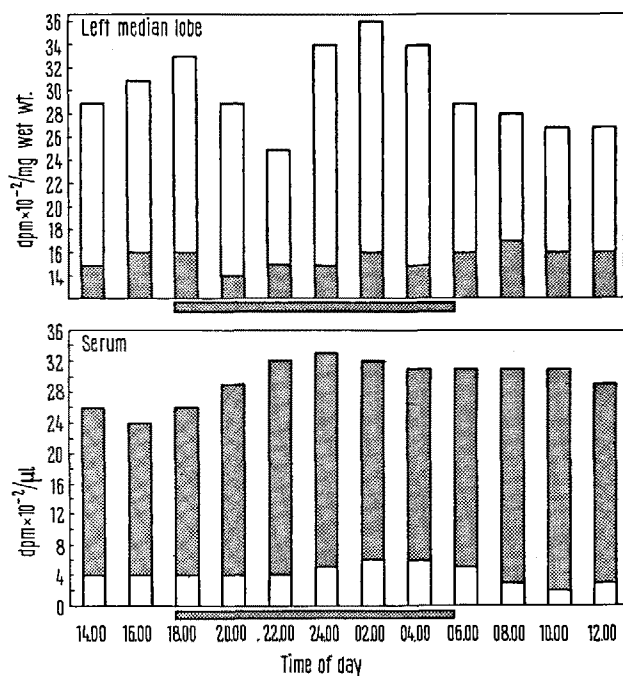
The slight increase in insoluble hepatic radioactivity seen at the end of the light phase and continuing early in the dark phase could be a reflection of the synthesis of proteins such as tyrosine transaminase, which is highest in activity at 20.00 h². If so, and with respect to the second and largest rise in radioactivity of protein seen here, an uncoupling of biphasic or even multiphasic hepatic protein synthesis as a result of food deprivation is indicated. In other words, feeding might entrain different cyclic aspects of hepatic protein synthesis, with the result that one may appear to act as a primary synchronizer for the other. In fact, if the stressful effects of starvation were not so adversely directed towards protein synthesis³, it would be interesting to see whether hepatic protein synthesis would as indicated above, continue to be biphasic with something other than a 24 h cycle. Under these conditions, liver protein synthesis would truly be 'circadian' in nature and the ingestion of food could be considered the primary synchronizer or 'Zeitgeber'.

Obviously other interrelated factors such as uncoupling of primary and secondary synchronizers¹², endocrine effects¹³, and variable half-life of mRNA species¹⁴ are concerned with this phenomenon. In any case, the fact that a cyclic incorporation of ^3H -leucine into hepatic protein still occurs in fasted animals does suggest that general hepatic protein synthesis may be subject to the influence of the 'Biological Clock'¹⁵.

Zusammenfassung. Die Aufnahme von ^3H -Leucin in das Lebereiweiss wurde während der Dunkelphase ihres Tag-Nachtzyklus bei fastenden Ratten untersucht. Die übliche rhythmische Aufnahme von ^3H -Leucin wurde durch die Fastenperiode nicht verhindert, obwohl die Menge des aufgenommenen ^3H -Leucin zwei zeitlich verschiedene Maxima aufwies.

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Radioactivity in rat serum and liver during a 24-h period. Values are 20 min after ^3H -leucine injection. Open block is the trichloroacetic acid-sodium tungstate (TCA-T) insoluble fraction, solid block is the TCA-T soluble fraction. Radioactivity was estimated by liquid scintillation spectrometry. Dimensions of the abscissa for the left median lobe start above the baseline to conserve space. Lights were off from 18.00 h to 06.00 h.

¹² J. W. HASTINGS, *Ann. Rev. Microbiol.* 13, 297 (1959).

¹³ F. T. KENNEY, in *Mammalian Protein Metabolism* (Ed. H. N. MUNRO; Academic Press, New York 1970), vol. 4, p. 131.

¹⁴ S. H. WILSON and M. B. HOAGLAND, *Biochem. J.* 103, 556 (1967).

¹⁵ E. BÜNNING, *The Physiological Clock* (Springer-Verlag, Heidelberg 1964).

Angiotensin Tachyphylaxis and Vascular Angiotensinase Activity

It has long been known that smooth muscle preparations frequently become refractory to repetitive stimulation¹. When the loss of responsiveness is nonspecific, in the sense that responses are lost to all stimuli, it seems likely that this reflects an abnormality of either the contractile process itself or in the metabolic pathways which support contraction. More puzzling is a phenomenon which has been called specific desensitization, or tachy-

phylaxis¹. In this situation the contractile process is intact, and the loss of response involves only the agent to which the tissue has been exposed. While this poorly understood phenomenon has been described for a large number of agents and systems, much of the recent interest has focused on angiotensin tachyphylaxis, presumably because of the potential physiological importance of reactivity of the renal vasculature to angiotensin^{2,3}