vate-kinase 15  $\mu g$ ) ne modifie d'ailleurs pas la cinétique d'incorporation.

Le dithiotréitol à des concentrations comprises entre  $10^{-3}$  et  $10^{-6}M$  entraîne une inhibition de l'activité enzymatique voisine de 35%. Enfin la puromycine active la transglycosylation alors que le cycloheximide l'inhibe de manière significative (Figure 2). Ce résultat est important: il montre que, pour la mannosyl-transférase microsomique splénique, il n'est pas nécessaire que le polypeptide soit encore fixé aux polysomes pour que la transglycosylation ait lieu; au contraire le système est beaucoup plus efficace lorsque le polypeptide, détaché des polysomes par la puromycine, est à l'état libre dans le milieu. Quant au cycloheximide, il inhibe le système en le privant vraisemblablement des molécules protéiniques endogènes acceptrices du mannose  $^{14}$ C.

Summary. The splenic cellular microsomic mannosyltransferase is inhibited by detergent (Triton X 100 and Cemulsol), deoxycholate, dithiotreitol and cyclcheximide, but activated by nucleoside-triphosphates (ATP and GTP) and analogous ( $\beta$   $\gamma$ -methylene-ATP or GTP); the enzyme is also activated by puromycine. This is good evidence for the sensitivity of the enzyme to any alteration of subcellular architecture at the microsomal level, and for the possibility of good transglycosylation when polypeptide is detached of ribosomes.

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## Composition of Scales from the Moth Xylophasia monoglypha

Although there have been several detailed accounts of the ultrastructure of lepidopteran scales with some studies of molecular orientation 1-4 information is still sparse regarding the chemical composition of the macromolecules in these specialized cuticular projections. Such chemical studies as there are have been largely confined to tests for chitin based on solubility of scales in alkali or response to the chitosan test 1, 2, 5.

RICHARDS<sup>5</sup> examined scales from a wide range of lepidopteran species demonstrating chitin to be present in most cases but absent in some. When first formed, scales seemed to be largely protein and had chitin added later. The elaborately organized iridescent scales of Morpho cypris apparently contained no chitin 1. Picken 2 noted that the earliest scale rudiments in the moth Ephestia sericarium contained more orientated protein than chitin while in the mature scales the chitin fraction increased considerably. These observations point to the architecture of the mature scale being a function of the fibrillar protein organization in the scale rudiment. Amino acid analyses of scales might therefore be expected to reflect the presence of structural protein. The present paper gives amino acid and glucosamine analyses for the scales and hairs of the moth Xylophasia monoglypha.

Scales were hydrolysed for  $24-48\,\mathrm{h}$  with  $6\,N$  HCl under nitrogen at  $105\,^\circ\mathrm{C}$ . Aliquots of hydrolysate containing  $0.03-0.05\,\mathrm{mg}$  material were taken for amino acid and amino sugar analysis in a Locarte amino acid analyser. Corrections were made to the computed analyses for hydrolytic losses of serine, threonine, tyrosine and glucosamine.

The amino acid compositions of the forewing scales, thorax hairs and tail tuft hairs are given in the Table together with analyses of scale and vein-free cuticle from the forewing and hair-free abdominal cuticle.

It has been demonstrated on several occasions that at the macromolecular level the arthropod cuticle consists of a complex association of proteins with the polysaccharide chitin, stabilized to a greater or lesser extent by 'tanning's. It has also been shown that several different protein fractions can be obtained by selective extraction of cuticle and more recently that these fractions, each of which has a different amino acid composition, are not themselves homogeneous but contain mixtures of proteins 7-9. The pattern of protein and amino acid composition in cuticle alters markedly during development some of the changes being attributable to sclerotisiza-

tion<sup>8,9</sup>. While it was prohibitively difficult to obtain sufficient quantities of scales for meaningful selective extraction of proteins, the present results indicate that their overall amino acid compositions do not differ too radically from that of the cuticle with which they have a common cellular origin and that their constituent proteins are therefore probably similar. This is particularly clear in the case of the wing cuticle and scales.

Amino acid and glucosamine composition of scales hairs and cuticle from  $Xylophasia\ monoglypha$ 

Residue	Forewing scales	Thorax hairs	Tail hairs	Forewing cuticle	Abdominal cuticle
Aspartic acid	64	68	57	57	89
Threonine	36	36	31	34	60
Serine	66	70	56	82	83
Glutamic acid	77	69	69	72	106
Proline	57	101	66	74	69
Glycine	203	180	158	176	110
Alanine	145	103	94	140	126
Valine	66	73	119	82	58
Isoleucine	70	57	61	58	79
Leucine	82	80	129	79	15
Tyrosine	38	26	14	26	31
Phenylalanine	12	7	12	18	27
β-Alanine	pres.	pres.	pres.	pres.	pres.
Histidine	27	69	82	51	48
Lysine	24	39	28	34	57
Arginine	33	22	24	24	42
Glucosamine	342	182	616	234	341

Amino acids in residues per 1000 total residues. Glucosamine in residues per 1000 amino acid residues.

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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  $\beta$ -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  $\alpha$  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 a 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  $\beta$ -silk fibroins (average value), resilin, invertebrate collagens (average value), feather ( $\beta$ -keratin), wool ( $\alpha$ -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 cuticle  $^{12}$ . 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 cuticle  $^{8}$ .  $\beta$ -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 proteins  $^{13}$ .

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 methods  $^{14}.$  The tail hairs gave a typical  $\alpha\text{-chitin X-ray}$  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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## Persistent Circadian Rhythmicity of Protein Synthesis in the Liver of Starved Rats<sup>1</sup>

The rhythmic behavior of hepatic tyrosine transaminase activity <sup>2</sup> and polysome profiles <sup>3</sup> is abolished upon protein or food deprivation. Yet, fluctuations in pituitary gland content of growth hormone <sup>4</sup>, rat serum urea and sodium levels <sup>5</sup> and hepatic content of glycogen <sup>6</sup> are unaffected by lack of food. We have previously observed an increased uptake of <sup>3</sup>H-leucine into liver protein midway through the dark period <sup>7</sup>, and considered this was due to the cylic postprandial influx of amino acids into the liver <sup>2</sup>. Consequently, the present study was done to see whether removal of food would abolish the expected rhythmic incorporation of <sup>3</sup>H-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 μC/g body weight of L-3H-4,5-leucine (58.0 C/m M 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 intestine 8. The supernatant fluid

was fractioned into portions soluble and insoluble in 10% trichloroacetic acid-0.5% sodium tungstate (TCA-T)<sup>8</sup>. Blood serum was also recovered and treated with TCA-T. Radioactivity in the liver and serum samples was estimated with liquid scintillation spectrometry <sup>10</sup>. In order to reveal general trends, the data were plotted as 6-hour moving averages <sup>11</sup>. Calculated standard errors of triplicate determinations were small (5 to 7%) and are omitted from the histogram for clarity.

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