capacity was barely measurable. That histidine was destroyed during photo-oxidation was demonstrated by thin-layer chromatography of acid hydrolysates and by estimation of histidine with bacterial histidine decarboxylase.

These results indicate that the binding of copper to protein in *M. trunculus* haemocyanin involves imidazole groups, although the possibility that other amino acid residues also participate cannot be completely excluded.

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The Effect of Isolation Conditions on the Polyamine Content of Rat-Liver Microsomes

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Recently evidence has accumulated to suggest that polyamines are associated with cellular RNA and its biosynthesis. Raina & Telaranta (1967) have reported the effect of various ions on the polyamine content of the microsomes isolated in a sucrose medium, and of the ribosomes isolated from them by disruption with deoxycholate. In the present work similar experiments have been carried out using microsomes and ribosomes isolated in an ionic medium.

When microsomes are isolated in an ionic medium (Zamecnik & Keller, 1954) and given a single wash in the same medium they contain approximately 15% of the total spermidine and 40% of the total spermine present in the homogenate. When sucrose medium (Hogeboom, 1955) is used a substantially higher proportion of both polyamines are present in the microsome fraction. The effect of dialysing the microsomes isolated in ionic medium against four changes of NaCl solutions of various concentrations was tested. When the concentration of NaCl was increased above 0·1M all the spermidine was removed, and increasing to 0·5M removed all the spermine. When NaCl was replaced by $MgCl_2$ the corresponding concentrations were 0·05M and 0·07M. Thus Mg^{2+} ions are more effective than Na^+ ions at removing polyamines, the effect appears to depend partly on the ionic strength of the cation.

The ability of microsomes isolated in ionic medium to exchange their polyamines with those of other cell fractions was tested. Liver microsomes were isolated from rats injected with [1,4-$^{14}C_2$]-putrescine. Unlabelled microsomes were isolated in a parallel experiment. The unlabelled microsomes were mixed with labelled nuclei, mitochondria, and cell sap and a homogenate reconstituted. The microsomes were then re-isolated. The specific activities of spermidine and spermine in the original labelled microsomes and the re-isolated microsomes showed that extensive exchange had occurred. When ribosomes were isolated according to Von der Decken & Campbell (1962) using deoxycholate and Lubrol W they contained less than 20% of the polyamines present in the microsome fraction.

It is concluded that the amounts of polyamines present in isolated microsomes depends largely on the isolation procedure and it is possible to prepare ribosomes which are active in protein synthesis and which contain only a small proportion of the total cellular polyamines.

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Removal of Ammonia by Formation of Amino Acids in Rat Liver

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When liver of fed rats was severed from the blood circulation (and the urea cycle ceased to function) the alanine content increased from 0·87 ± 0·64 μmoles/g. wet. wt (mean value 6 rats ± S.D.) at 0 min. to 1·53 ± 0·93 after 2 min. ischaemia and to 2·26 ± 0·60 after 5 min. The tissue content of ammonia, aspartate and glutamate did not alter significantly during this period. Inhibition of alanine aminotransferase by preinjection with L-cycloserine (Otto, 1965) prevented the accumulation of alanine. In these experiments rat liver was freeze-clamped, powdered and extracted with perchloric acid as described by Williamson, Lund & Krebs (1967) and the metabolites were estimated by enzymic methods (Williamson, Lopez-Vieira & Walker, 1967). There was also a rapid increase in the concentration of alanine when fed rats were given an intraperitoneal injection of 2·5 m-moles of NH_4Cl/kg. body wt. and their livers were analysed

at various intervals. The concentration of alanine rose from $1\cdot07 \pm 0\cdot30$ μmoles/g. wet. wt. (mean value 5 rats $\pm$S.D.) at 0min. to $3\cdot24 \pm 0\cdot82$ μmoles at 5min. and that of aspartate from $1\cdot05 \pm 0\cdot17$ μmoles/g. wet. wt. at 0min. to $3\cdot11 \pm 0\cdot46$ at 5min. The levels returned to normal 25min. after injection. When the capacity of the urea cycle was increased by preinjection of L-arginine (Greenstein, Winitz, Gullino, Birnbaum & Otey, 1956) the rise in liver alanine and aspartate was smaller and more transient, returning to normal within 10min. The glutamine concentration did not change appreciably after injection of NH_4Cl.

The results can be explained by the assumption that ammonia primarily reacts to form glutamate, and that alanine and aspartate are formed by transamination. The removal of ammonia by these reactions occurs when the urea cycle is not functioning, as in ischaemia or when the cycle is overloaded experimentally after ammonia injection.

The ammonia concentration of the freeze-clamped (i.e. living) liver from fed rats was 0·5–0·7mM which is more than 50–100 times higher than that of the blood plasma. The liver thus maintains a steep ammonia concentration gradient against the plasma. The formation of alanine (and less so of aspartate) appears to play a role in keeping the tissue concentration of ammonia near-constant.

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The Thiol Sequences and Sub-units of Light Meromyosin Fraction 1

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We have recently concluded that there are at least 22 unique thiol sequences in rabbit skeletal myosin (Weeds & Hartley, 1967). Since there are 44 half-cystine residues per mole (mol.wt. 530000), the molecule would appear to contain two identical sub-units. To support this conclusion I have studied the thiol sequences of light meromyosin fraction 1 (LMM Fr. 1), which is a helical fragment from the 'tail' of the myosin molecule and has a mol. wt. of between 135000 (Holtzer, Lowey & Schuster, 1961) and 146000 (Young, Himmelfarb & Harrington, 1964).

The LMM Fr. 1 was prepared according to Lowey & Cohen (1962) and labelled with [^{35}S]cystamine by disulphide-thiol interchange at pH 9·0, first in 0·5M-KCl, 10^{-3}M-EDTA, 0·02M-tris-Cl for 24hr. at 0°, and then adjusted to 5M-guanidine hydrochloride for 24hr. at 20°, using a 100-fold molar excess of reagent. After exhaustive dialysis at acid pH, the recovery of protein was quantitative, as judged by extinction at 280mμ. The sedimentation coefficient of the labelled protein in 0·5M-KCl at neutral pH was similar to that of the unreacted LMM Fr. 1 at the same concentration, suggesting that dissociation into protomyosin had not occurred. The half-cystamine incorporation was 6·4 groups per mole, and amino acid analysis of the performic oxidised protein showed 6·7 cysteic acid residues per 140000g.

The labelled protein was digested with trypsin and the cystamine labelled petides were fractionated on Zeo-Karb 225 resin followed by paper ionophoresis at pH 6·5, giving 3 radioactive peptides in approximately equal yields. The diagonal electrophoretic procedure of Brown & Hartley (1966) was then used to purify the cysteic acid peptides from contaminants. Sequence analysis showed that T1 was a mixture of two peptides:

T1a: Thr-Asn-Ala-Ala-Cya. and T1b: Ser-Asn-Ala-Ala-Cya.

in approximately 3:2 molar ratio.

Peptide T2 had the sequence: Cya-Ala-Ser-Leu-Glu-Lys.

Peptide T3 was: His-Asp-Cya-Asp-Leu-Leu-Arg.

In a parallel experiment with cystine-exchanged LMM Fr. 1 a larger variety of peptide T1 was isolated: Thr- or Ser,Asn,Ala,Ala,Cya,Ala,Ala, Leu,Asp-Lys-Lys, which corresponds to peptide 13 of Weeds & Hartley (1967). Clearly trypsin has split at the cystamine residue.

Hence LMM Fr. 1 contains three unique thiol sequences as expected from a two sub-unit structure. The variation between peptides T1a and T1b may reflect the presence of homologous genes, since this observation has been consistently noted with several preparations from different rabbits.

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