[1] S. V. PERRY, *Biochem. J.*, 74 (1960) 94.
[2] S. V. PERRY AND G. D. WOODWARD, unpublished results (1960).
[3] J. BRAHMS, *J. Am. Chem. Soc.*, 81 (1959) 4997.
[4] A. G. SZENT-GYÖRGYI, *Arch. Biochem. Biophys.*, 42 (1953) 305.
[5] S. V. PERRY AND M. ZYDOWO, *Biochem. J.*, 72 (1959) 682.
[6] W. R. MIDDLEBROOK, Abstr. 4th Int. Congr. Biochem., Vienna, (1958) p. 84.
[7] S. F. VELICK, *Biochim. Biophys. Acta*, 20 (1956) 228.
[8] G. SCHAPIRA, G. BROUN, J-C. DREYFUS AND J. KRUH, *Compt. rend. soc. biol.*, 160 (1956) 944.
[9] A. HOLTZER AND J. MARSHALL, Comm. Gordon Conf. Muscle Cell., Meriden, N.H., 1958.

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On the transfer of labeled amino acids from "S-RNA" to purified ribonucleoprotein particles from rat liver microsomes

The incorporation of labeled amino acids into protein by RNP from rat-liver microsomes is enhanced by an enzyme preparation (S-protein[1]) from the soluble liver fraction[2]. The effect of S-protein is increased by GSH or MEA.

The S-protein preparation used in the present experiments corresponded to the purified "S_{50}-protein" of RENDI *et al.*[2], and it had been treated with charcoal for 1 h at 4°. The absence of ribonucleotides in the preparation was ascertained in the following way. S-protein was prepared from rats, treated with 2 mC of [^{32}P]phosphate for 18 h. Carrier RNA was added to the preparation, and ribonucleotides were isolated by chromatography after hydrolysis. No radioactivity was observed in the nucleotide fractions. The activity of the preparations in amino-acid-dependent isotope exchange between [^{32}P]pyrophosphate and ATP (PP–ATP exchange) was slightly variable, but usually small. As a rule no significant formation of [^{14}C]L-leucylhydroxamate could be demonstrated by paper chromatography[3] after incubation of [^{14}C]L-leucine with ATP and NH_2OH in the presence of S-protein.

RNP were prepared by treating mitochondria-free, rat-liver homogenates (in 0.25 *M* sucrose, 0.5 *M* KCl, 0.01 *M* $MgCl_2$ and 0.035 *M* Tris buffer, pH 7.8) with 0.5 % Lubrol W and 1 % sodium deoxycholate, followed by centrifugation through a layer of medium with higher density[2,4]. The incorporation of labeled amino acids into protein by these particles in the presence of S-protein, MEA, ATP, GTP, PEP and pyruvate kinase was amino-acid specific. The incorporation was not inhibited by the presence of other, unlabeled amino acids in a 10-fold excess. Since the S-protein preparations used in these experiments had a quite low activity of amino acid activation, the possibility was considered that certain amounts of amino-acid-activating enzymes were still present in the particles. By use of prolonged incubation periods (60 min) and incubation samples containing 5–7 mg of protein an amino-acid-dependent PP-ATP exchange, amounting to 3.7 %/mg protein, was actually observed in particles of this kind.

Abbreviations: RNP, ribonucleoprotein particles; RNA, ribonucleic acid; ATP, GTP, triphosphates of adenosine and guanosine, respectively; PEP, phosphoenol pyruvate; GSH, reduced glutathione; MEA, 2-mercaptoethylamine; Tris, tris(hydroxymethyl)aminomethane; PP, [^{32}P]pyrophosphate.

In order to get RNP of higher purity, the particles were homogenized with the same combination of detergents and medium as before, and centrifuged a second time by layering. By this treatment the RNA/protein ratio of the particles increased from 0.42 to 0.54. The amino-acid-dependent PP–ATP exchange, measured by a 60-min period, decreased to 1.9 %/mg protein. The incorporation of [^{14}C]L-leucine into protein by the particles in the absence of soluble enzymes decreased by the washing from 90 counts/min/cm^2 to 16 counts/min/cm^2. In the case of the washed particles only a slight enhancement of the incorporation was obtained by the addition of S-protein, even in the presence of MEA or GSH.

When a preparation of a pH-5 enzyme fraction, prelabeled with [^{14}C]L-leucine[5], was incubated with the washed particles in the presence of ATP, GTP, PEP and pyruvate kinase, an incorporation of isotope into protein occurred. The incorporation was not significantly stimulated by MEA or GSH, but an increase by about 20 % was observed, when S-protein was added in combination with one of these reducing agents. When S-RNA, prelabeled with [^{14}C]L-leucine[5] or with a mixture of [^{14}C]-labeled amino acids, was incubated with washed RNP in a system containing ATP, GTP, PEP and pyruvate kinase, no significant isotope incorporation was observed, and no stimulation was obtained with MEA or GSH. In the presence of the pH-5 enzyme fraction, however, the labeled S-RNA-amino acids were readily transferred to the particles and incorporated into protein, even in the presence of a large excess of the corresponding non-labeled amino acids (Table I). The incorporation was stimulated by GSH or MEA (10^{-2} *M*). The pH-5 precipitable fraction could be replaced by S-protein, and also in this case a marked increase in the incorporation was observed in the presence of GSH or MEA. The pH optimum of this reaction was 7.8–8.0. The activity of the S-protein is of a particular interest, since this preparation had a much lower activity of amino-acid-dependent PP–ATP exchange than the pH-5 precipitable fraction.

When washed pH-5 enzyme fractions were preincubated for 45 min at 35° and reprecipitated at pH 5.1, their activity in the transfer systems diminished. At the

TABLE I

TRANSFER OF [^{14}C]-L-LEUCINE FROM S-RNA TO PURIFIED RNP

The incubation tubes contained in 1.5 ml of 0.015 *M* KCl, 0.01 *M* $MgCl_2$, and 0.035 *M* Tris, pH 7.8: 1 μmole ATP, 10 μmoles PEP, 15 μg pyruvate kinase, 0.2 μmole GTP, 0.2 μmole unlabeled L-leucine, 80 μg S-RNA-[^{14}C]-L-leucine, RNP corresponding to 5 mg protein, and the indicated amounts of enzyme preparations and GSH. After 30 min at 35° the reaction was stopped by 2 ml 10 % trichloroacetic acid. The samples were extracted with hot trichloroacetic acid, ethanol and ether and the radioactivity determined by a thin-mica end-window counter.

Enzyme preparation added	*mg protein*	*PP–ATP exchange**	*Transfer of [^{14}C]-L-leucine to RNP; total counts*	
			without GSH	*GSH, 10^{-2} M*
None	—	—	9	10
pH-5 enzyme fraction	2.5	17.7	67	88
S-protein	4.2	5.6	16	48
S-protein from pH-5 fraction	0.9	19.8	34	42

* Exchange was measured by incubating the indicated amounts of the enzyme preparations for 12 min with 6 μmoles ATP, 6 μmoles $MgCl_2$, 4 μmoles [^{32}P]pyrophosphate and 60 μmoles KF[3].

same time a significant, but less pronounced, decrease of their PP–ATP exchange activity was observed. Proteins, non-precipitable at pH 5.1, appeared during the incubation, and from the incubation mixture a nucleotide-free enzyme preparation could be obtained by the same method, which was used for the preparation of the S-protein. The transfer activity per mg protein was considerably higher in this S-protein from the incubated pH-5 fraction than in the S-protein obtained from the pH-5 supernatant[2]. At the same time, however, a higher activity of PP–ATP exchange was observed (Table I).

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The Wenner-Gren Institute for Experimental Biology, University of Stockholm (Sweden)

A. VON DER DECKEN
T. HULTIN

[1] H. SACHS, *J. Biol. Chem.*, 228 (1957) 23.
[2] R. RENDI AND T. HULTIN, *Exptl. Cell Research*, 19 (1960) 253.
[3] M. B. HOAGLAND, E. B. KELLER AND P. C. ZAMECNIK, *J. Biol. Chem.*, 218 (1956) 345.
[4] A. VON DER DECKEN AND T. HULTIN, *Exptl. Cell Research*, 15 (1958) 254.
[5] M. B. HOAGLAND, M. L. STEPHENSON, J. F. SCOTT, L. I. HECHT AND P. C. ZAMECNIK, *J. Biol. Chem.*, 231 (1958) 241.

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A comment on the pH-dependent dissociation of haemoglobins

It is known that normal adult human haemoglobin (Hb-A) dissociates reversibly both at low pH (5–5.5)[1] and high pH (11–11.2)[2] into units having half the molecular weight of the parent molecule, and evidence has been adduced which indicates that the dissociation is asymmetric with respect to the four peptide chains ($\alpha_2\beta_2$) in the molecule[3,4].

These observations have a bearing on the question of the different resistance of Hb-F (foetal haemoglobin) and Hb-A to denaturation by alkali. Under selected conditions[5] (pH about 12.8) the reaction follows apparent first-order kinetics with $t_{1/2}$ values (*i.e.* time for 50 % conversion to alkaline haematin) at 20° of 11 sec and 1030 sec for Hb-A and Hb-F respectively. A mixture of the two species thus gives a first-order reaction plot consisting of two linear components with widely differing slopes, joined by an intermediate curved region.

Hb-F—if its behaviour is comparable with that of Hb-A—must have dissociated into half-molecules, α_2^A and γ_2^F, well below this pH, and Hb-A into α_2^A and β_2^A; the α-chains are reported to be common to both species[6,7]. Since Hb-A is wholly labile to alkali, both the α_2^A and β_2^A chains must be rapidly denatured. It seems therefore to follow that the alkali resistance of Hb-F is a property of the γ_2^F units. Furthermore, if it indeed consists of equal proportions of alkali-labile and alkali-resistant chains, there should be a marked change in slope in its denaturation-rate plot, corresponding to the presence of a resistant portion amounting to 50 % of the total Hb-F, irrespective of

Abbreviations: Hb-A, normal adult human haemoglobin; Hb-F, human foetal haemoglobin.