TABLE II

THE COFACTOR REQUIREMENTS FOR THE TRANSFER REACTION

Incubation mixture contained, in a final volume of 1.0 ml per tube, 2.5 mg RNP; s-RNA–[^{14}C]leucine 180 counts/min (A_{260} = 1.6); 0.55 mg enzyme fraction; 5 μmoles $MgCl_2$; 30 μmoles KCl; 50 μmoles Tris buffer, pH 7.6. After incubation for 15 min at 37°, the reaction was stopped by 0.5 *N* $HClO_4$ and counts in protein were determined according to the method described in Table I. ATP, GTP, UTP, and phosphocreatine were supplied by the Sigma Chemical Co.

Incubation conditions	*Activity transferred (counts/min)*
Incubation mixture	0
+ 0.3 μmole GTP	97
+ 0.3 μmole ATP	6
+ 0.3 μmole CTP	7
+ 0.3 μmole UTP	5
+ GTP, ATP, CTP, UTP, each 0.3 μmole	104
+ 0.3 μmole GTP, 10 μmoles phosphocreatine, 0.05 mg creatine kinase	102
+ 0.3 μmole GTP, 0.3 μmole ATP, 10 μmoles phosphocreatine, 0.05 mg creatine kinase	98
+ 0.3 μmole GTP, cell dialyzate	75

Further purification and characterization of the transferring enzyme are in progress.

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Isolation of free acid-soluble nucleotide peptides from normal rat liver

It has been shown by WEINSTEIN *et al.*[1] that normal rat liver contains nucleotides of adenine, cytosine and uracil associated with amino acids or peptides. WILKEN AND HANSEN[2] also identified two adenine nucleotide peptides from bovine liver.

During our investigation of nucleotide peptides from normal rat liver we have been able to isolate three different peptides associated with an adenine–free acid-soluble nucleotide.

25 Wistar rats fed on stock laboratory diet have been utilised for this purpose. The animals, divided in groups A, B and C, were killed by exsanguination, the livers

Abbreviations: ADP, adenosine 5′-diphosphate; UDPAG, uridine 5′-diphosphate acetyl glucosamine; UDPG, uridine 5′-diphosphate glucose; DPN, diphosphopyridine nucleotide; UMP, uridine 5′-monophosphate; CMP, cytidine 5′-monophosphate; and TMP, thymidine 5′-monophosphate.

quickly removed and extracted by the cold-$HClO_4$ method previously described by ONDARZA[3,4]. The extracts were applied to columns of Dowex 1, chloride form (20 × 1.2 cm) according to the method of COHN[5], the nucleotides eluted with 0.01 *N* HCl in a gradient concentration of NaCl, and recovered from the eluates by charcoal adsorption and elution as described by SMITH AND MILLS[6].

Between the compounds ADP and UDPAG eluted with the solvents 0.01 *N* HCl–0.01 *M* NaCl and 0.01 *N* HCl–0.03 *M* NaCl appeared from rat-liver group A two ultraviolet-absorbing spots, named compounds I and II, and another two spots from groups B and C corresponding to compounds I and III (see Table I).

TABLE I

Nucleotide peptide	R_{UDPG}*	R_{DPN}*
Compound I	0.41	0.64
Compound II	0.27	0.40
Compound III	0.26	0.40

* Obtained by descending paper chromatography with the ethanol–ammonium acetate solvent of PALADINI AND LELOIR[7].

The above separated nucleotides were eluted with water after washing the paper with absolute alcohol, and the following analysis carried out, mainly with compound III.

(a) 0.2 μmole of compounds I, II and III were hydrolysed with 6 *N* HCl (110° for 18 h) in a sealed tube, evaporated until free of acid and then chromatographed bidimensionally with the solvents butanol–acetic acid–water (4:1:5) and phenol–water (75:25). The chromatograms after reaction with the ninhydrin reagent in isopropanol are shown in Fig. 1.

(b) Hydrolysis of 0.3 μmole of compound III with 2 *N* HCl (100° for 1 h), followed by evaporation to dryness and separation by paper chromatography for bases by the method of WYATT[8], gives a visible spot with the u.v. lamp. The R_F value of this spot is shown in Table II along the values for the standards.

The spot from compound III after elution with 0.1 *N* HCl gives a maximum absorption at 262 mμ; at pH 9, this is at 267 mμ.

(c) Estimations of base by absorption at 262 mμ at pH 2 (assuming a value of 11,500 for the molar extinction coefficient), total phosphorus[9] and sugar by the orcinol

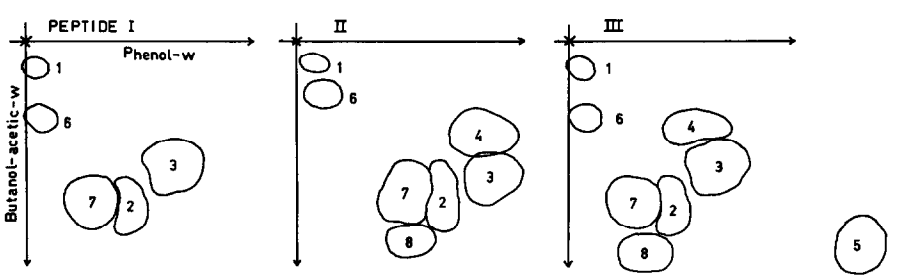

Fig. 1. Amino acid separation by paper chromatography with butanol–acetic acid–water and phenol–water (PARTRIDGE[10]). The ninhydrin-positive spots are: 1, cysteic acid; 2, glutamic acid; 3, glycine; 4, taurine; 5, alanine; 6, a pink spot (probably amino acid); 7, a yellow spot (a furfural derivative of the sugar nucleotide); 8, an unidentified amino acid. Standards of amino acids were run by paper chromatography under the same conditions for the unknowns.

method[11] using *d*-ribose as standard, give the ratios: base/sugar/phosphorus = 1:0.99:0.96.

TABLE II

Bases and nucleotides	*RF in HCl–Isopropanol*[8]
Guanine	0.35
Hypoxanthine	0.40
Adenine	0.55
Compound III	0.55
CMP	0.61
UMP	0.68
TMP	0.80

It is concluded that the material isolated is an adenine ribonucleoside-monophosphate peptide. The peptide, as far as we know, has not previously been described in this form.

It is possible that these nucleotide peptides are involved in protein synthesis, as has been postulated by HOAGLAND[12] for active amino acids. If this is the case, these peptides must be in an active form when bound to a nucleotide, and should be considered as "activated" peptides.

The sequence of the amino acids and the type of union between the nucleotide and the peptide is under investigation in this laboratory.

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The amino acid sequence of peptide B of co-fibrin

Evidence obtained from peptide fragments identified in pepsin digests of desulfated Peptide B is presented here in support of a proposed complete amino acid sequence for this peptide.

Peptide B is one of the two large acidic peptides released from the N-terminal portion of bovine fibrinogen through the hydrolytic action of bovine thrombin[1]. The complete amino acid sequence of the other acidic peptide, Peptide A, has been