TABLE I

THE SULFHYDRYL CONTENT OF DIFFERENT HUMAN HAEMOGLOBINS

(The values are given as half-cystine residues per mole haemoglobin, mol. wt. = 68,000)

	Chemical method			*Amperometric titration method*					
					with $HgCl_2$ after different denaturation times				
	*n**	*Found*	*Assumed*	*with $AgNO_3$*	*0'*	*2'*	*6'*	*10'*	*60'*
Hb-A	15	8.28 ± 0.17**	8	7.6	5.4	6.2	6.7	7.6	7.6
Hb-B	8	8.30 ± 0.18	8	8.2	7.0			6.8	7.2
Hb-C	15	8.38 ± 0.17	8	7.7	6.0	6.5	6.1		6.2
Hb-F	15	5.67 ± 0.15	6	4.0	4.8			3.6	2.8
Hb from cord blood 91% Hb-F				4.8					

* n = number of analyses.
** Standard deviations of the means.

amount was slowly increased and reached the same value found with the $AgNO_3$ titration. Studying the haemoglobins B and C different numbers of titratable –SH groups were established, which were not influenced by the alkali denaturation procedure. The results with foetal haemoglobin agree quite well with those obtained with the $AgNO_3$ titration. After longer periods of alkali denaturation, however, the amount of titratable –SH groups decreased. The differences between the results obtained with the $HgCl_2$ and $AgNO_3$ titrations may be explained by a steric handicap of the mutual –SH groups. This hindrance may be decreased (Hb-A), not altered (Hb-B and Hb-C), or increased (Hb-F) during the denaturation with alkali.

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Effects of monovalent cations on the incorporation of amino acids into protein*

It has been demonstrated rather clearly that magnesium ions promote the incorporation of amino acids into protein in a variety of experimental systems[1–5], while other divalent cations are generally inhibitory[5,6]. In contrast, little information is available on the effects of monovalent cations. Several observations, however, support the possibility that monovalent cations influence protein synthesis. Thus, STEWARD AND PRESTON[7] showed that potassium ions increase the rate of protein synthesis by potato slices. CANNON *et al.*[8] noted that utilization of amino acid mixtures for protein synthesis by starved rats depends on the simultaneous administration of potassium

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ions. LANDMAN AND SPIEGELMAN[9] have reported that β-galactosidase synthesis in protoplasts of *Bacillus megaterium* is promoted by potassium ions, but not sodium ions. It is, therefore, of considerable interest to determine whether monovalent cations, especially potassium, exert a direct effect on the incorporation of amino acids into protein.

It has been shown[5,10] that a convenient system for the measurement of amino acid incorporation into protein can be derived from cell-free extracts of rapidly-growing pea roots. In this system, incorporation of any amino acid into protein is enhanced by adenosine triphosphate, magnesium ions, and by a mixture of the other amino acids commonly found in protein[5]. Incorporation is inhibited by adenosine diphosphate, various amino acid analogues, and by ribonuclease[5,10]. In the present experiments, 3-day-old pea roots were washed in 5% sodium hypochlorite, and ground at 1° C with an equal weight of 0.5 *M* sucrose-0.01 *M* tris-(hydroxymethyl)-aminomethane of pH 7.5. Cellular debris was removed by centrifugation for 15 min at 1500 × *g*, and the active material was sedimented at 40,000 × *g* for 60 min. The sediment was suspended in one-tenth volume of 0.5 *M* sucrose-0.01 *M* tris-(hydroxymethyl)-aminomethane (pH 7.5), and incubated for one hour at 38° C with: 0.01 *M* glutamate-2-^{14}C, 0.001 *M* of each of 16 amino acids[5], 0.01 *M* $MgCl_2$, 0.01 *M* adenosine triphosphate, and 0.01 *M* of the chlorides of each of the monovalent cations listed in Table I. Assay for radioactive glutamate incorporated into protein was performed as described previously[5,10].

TABLE I

EFFECT OF MONOVALENT CATIONS ON GLUTAMATE INCORPORATION INTO PROTEIN

Ion added to system	*μmoles glutamate incorporated per protein per h*
None	1.57
Potassium	2.38
Sodium	1.50
Lithium	1.48
Ammonium	1.45
Rubidium	0.75

Table I presents the effects of a series of monovalent cations on the incorporation of glutamate-2-^{14}C into the protein of the particulate preparation. It is apparent that when sodium, ammonium, or lithium ions are the only monovalent cations present, slight inhibitions of amino acid incorporation occur. In contrast, potassium ions markedly enhance incorporation, while rubidium ions are strongly inhibitory.

These results indicate that certain monovalent cations exert marked effects on amino acid incorporation into protein. The mechanism by which potassium promotes and rubidium inhibits such incorporation is as yet unknown, but it is noteworthy that potassium ions are necessary for the biosynthesis of the peptidic linkages of glutathione[11,12] and pantothenic acid[13]. It may be that the promotion of amino acid incorporation into protein by potassium ions is due, at least in part, to a necessity of this ion for the formation of the peptide bonds in the protein molecule.

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