structures presented do not represent a membrane component and that they are intracellular structures that have been bound by the membrane during haemolysis and the subsequent washings.

Work is in progress regarding the purification and characterisation of this extractable material. Preliminary results indicate it is of protein composition and may represent up to 5 % of the protein of the *intact* ghost. Two preparations of ox erythrocyte ghosts have revealed a very similar release of material when fragmented by either of the treatments described (see Fig. 4). The significance of the results presented cannot be fully discussed at the present stage of the investigation. But it would appear that, as the material is so readily released not only by sodium dodecyl sulphate but by the mild treatment of water dialysis, it is unlikely that an artifact is being created.

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The activation by leucine of ouabain-sensitive ATPase of HeLa cells

Cell membrane ATPase which is Mg^{2+} dependent and (Na^+-K^+) activated is believed to be the carrier enzyme for the active transport of Na^+ and K^+ at cell surface (*cf.* ref. 1). It has been suggested that this kind of ATPase contributes to amino acid transport in ascites tumor cells[2] and L cells[3] on the basis of the findings that glycine uptake in tumor cells was greatly decreased by ouabain, as in the case of cation transport, and that certain Na^+ concentrations in the medium are capable of accelerating the inward transport of α-aminoisobutyric acid in L cells. The authors have noted that the penetration of glycine and leucine into HeLa cells is greatly inhibited by 10^{-5} M ouabain, while on the other hand, HeLa cells have been fractionated into specifically (Na^+-K^+)-activated and also ouabain-sensitive ATPase. In the present experiments, two ATPase preparations from HeLa cells were examined for activation of the enzyme activity with amino acids.

HeLa cells were cultivated in the glass flask as monolayers with medium consisting of 90 % (v/v) Hanks balanced salt solution, 10 % (v/v) inactivated bovine serum and 0.4 % (w/v) lactalbumin hydrolysate (NBC).

Enzyme preparation I was obtained and assayed for ATPase activity by the following procedures; cells were collected as a suspension in 0.25 M sucrose, this suspension was centrifuged at 1000 rev./min, and aliquots ($4 \cdot 10^6$–$6 \cdot 10^6$ cells each) of the precipitate were incubated for 30 min at 38° in 1.0 ml of medium containing 2.5 mM ATP–Tris, 0.015 M Tris, and 1 mM $MgCl_2$ (pH 7.4) for each. Preparation II corresponds to the 0.8 M NaI-insoluble fraction of NAKAO *et al.*[4]; cells were harvested with water, centrifuged at 1000 rev./min, aliquots (approx. 2 mg protein each) of the precipitate were dissolved, in an ice bath, in 2 ml of 4.8 M NaI containing 6 mM ATP, 0.6 mM EDTA, 0.012 M $MgCl_2$, 6 mM cysteine and 0.09 M Tris (pH 8.0); these solutions were diluted with water to 0.8 M NaI and then centrifuged at 20000 $\times g$ to obtain faintly yellow precipitates. These insoluble fractions were submitted to assay under the conditions mentioned above, except that the buffers of Tris and 2-amino-2-methyl-1,3-propandiol, 0.015 M each, were pH 8.4. To stop the enzyme reaction, 3.0 ml of 30 % trichloroacetic acid were added to each sample, and the chilled mixture was centrifuged to divide the solution, an aliquot of which was used for the estimation, by the method of Fiske and SubbaRow, of P_i released from protein whose content was determined by the method of Lowry *et al.*

The effect of amino acids on ATPase activity of Prep. I is summarized in Table I. The results show that leucine significantly activated ($P < 0.01$) ATPase both at 20 mM and 10 mM, and slightly activated the enzyme at 2 mM. Arginine and aspartic acid somewhat increased at 20 mM ATPase activity. All other amino acids examined here, however, were found to be without effect or to depress slightly the activity. Glycine is one of the amino acids that are accumulated markedly within HeLa cells[5], and the rate of inward glycine transport has been observed to be rather larger than that for leucine (unpublished results). The lack of parallelism between ATPase-activating ability and permeability across cell membrane for glycine and leucine has not yet been explained. Activation in Prep. II by 20 mM leucine does not reach the level of activation by 140 mequiv/l Na^+ *plus* 20 mequiv/l K^+ (Table II), but is significant ($P < 0.01$) compared to the control as well as the control *plus* Na^+ only. A point to

TABLE I

ACTIVATION BY AMINO ACIDS OF ATPase OF PREP. I

Each value indicates the mean of the results for 3–5 samples ± S.E.

Amino acid conen. (mM)	*ATPase activity (μmoles P_i per h per mg protein)*						
	Leucine	*Aspartic acid*	*Glycine*	*Arginine*	*Glutamine*	*Phenyl-alanine*	*Isoleucine*
0	2.09 ± 0.07	2.36 ± 0.13	2.36 ± 0.13	2.40 ± 0.14	2.40 ± 0.14	2.40 ± 0.14	2.40 ± 0.14
20	2.73 ± 0.09	2.53 ± 0.04	2.23 ± 0.10	2.63 ± 0.07	2.41 ± 0.04	2.32 ± 0.05	2.34 ± 0.03
10	2.45 ± 0.07						
2	2.31 ± 0.06	2.45 ± 0.12	2.40 ± 0.02				

	Methionine	*Lysine*	*Proline*	*Histidine*	*Glutamic acid*
0	2.33 ± 0.18	2.33 ± 0.18	2.33 ± 0.18	2.33 ± 0.18	2.33 ± 0.18
20	2.45 ± 0.20	2.46 ± 0.15	2.37 ± 0.20	2.18 ± 0.07	2.57 ± 0.13

be studied in order to clarify the amino acid specificity in ATPase activation is the possibility that some factor in addition to amino acid is required. The action of 10^{-5} M ouabain on leucine activation of ATPase of Prep. II is shown in Table III. Ouabain did not significantly inhibit the (Na^+-K^+)-independent ATPase (leucine = 0, ouabain = 10^{-5} M), but suppressed ($P < 0.05$) leucine activation. The results strongly suggest that leucine accelerates ouabain-sensitive, *i.e.* (Na^+-K^+)-activated, ATPase, or in other

TABLE II

ACTIVATION BY Na^+ *plus* K^+ AND LEUCINE OF PREP. II ATPase

Each value indicates the mean of results from 5 samples ± S.E.

Addition			*ATPase activity* (*μmoles P_i per h per mg protein*)
Na^+ (*mequiv/l*)	K^+ (*mequiv/l*)	*Leucine* (*mM*)	
0	0	0	1.03 ± 0.05
140	20	0	2.43 ± 0.15
0	0	20	1.82 ± 0.08
140	0	0	1.12 ± 0.16

TABLE III

ACTIVATION BY LEUCINE AND INHIBITION BY OUABAIN OF PREP. II ATPase

Each value indicates the mean of results from 3–4 samples ± S.E.

Addition		*ATPase activity* (*μmole P_i per h per mg protein*)
Leucine (*mM*)	*Ouabain* (*M*)	
0	0	0.453 ± 0.065
0	10^{-5}	0.430 ± 0.041
20	0	0.551 ± 0.032
20	10^{-5}	0.419 ± 0.026

words, that leucine substitutes, at least to some extent, for cations in ATPase activation. According to OXENDER AND CHRISTENSEN[6] and WHEELER AND CHRISTENSEN[7], leucine transport in Ehrlich ascites cells is Na^+ independent, in contrast to glycine and alanine transport. In rabbit reticulocytes, glycine and alanine are dependent on Na^+ concentration in the medium for their active transport, while, in the erythrocytes, transport of those amino acids as well as leucine and valine is independent of Na^+. These facts are considered to suggest that, in some cell systems, the amino acid-carrying system for each amino acid or amino acid group could differ from those of the others in its dependence on cations.

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