despite the obvious degeneration of white matter in globoid leucodystrophy, other transferases were within normal limits in brain from a globoid-leucodystrophy patient in previous studies (e.g. UDP glucose-glucosyltransferase) (Austin *et al.* 1963).

An enzymic disorder of sulphate conjugation has not hitherto been reported. The expected result *in vivo* of a cerebroside sulphotransferase activity deficiency would be a deficiency of sulphatides. Because sulphatides are normally most concentrated in myelin, a deficiency of sulphatides could interfere with the proper development of myelin in this human disease.

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Hydroxylamine and a ³²P-Labelled Intermediate in Sodium-plus-Potassium Ion-Activated Adenosine Triphosphatase

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An acid-stable ³²P-labelled phosphoprotein complex can be recovered after incubation of membrane preparations exhibiting (Na⁺ + K⁺)-activated ATPase* activity with [γ -³²P]ATP (Charnock & Post, 1963a; Gibbs, Roddy & Titus, 1965; Rodnight, Hems & Lavin, 1966). Formation of this complex is stimulated by Na⁺ but not K⁺; when both ions are present the steady-state concentration of ³²P-labelled phosphoprotein complex is greatly decreased whereas the hydrolysis of [γ -³²P]ATP is enhanced. Most investigators have concluded that the ³²P-labelled phosphoprotein complex recovered in these experiments is an acyl-phosphate-enzyme derivative that functions as an intermediate (E ~ P) during the hydrolysis of ATP by (Na⁺ + K⁺)-activated ATPase (Charnock, Rosenthal & Post, 1963; Nagano *et al.* 1965; Bader, Sen & Post, 1966).

However, this concept has been challenged by Schoner, Kramer & Seubert (1966), who reported

that the Na⁺-stimulated formation of ³²P-labelled phosphoprotein complex was greatly decreased when the reaction was conducted in the presence of hydroxylamine, but that hydroxylamine did not affect the rate of hydrolysis of ATP by this enzyme in the presence of Na⁺ and K⁺. From these findings these workers concluded that the ³²P-labelled phosphoprotein complex was not an intermediate in the hydrolysis of ATP by (Na⁺ + K⁺)-activated ATPase.

The present experiments are a further examination of the interaction of hydroxylamine with (Na⁺ + K⁺)-activated ATPase.

Materials and methods. Preparation of guinea-pig kidney microsomal (Na⁺ + K⁺)-activated ATPase and the assay conditions for the colorimetric determination of ATP hydrolysis by liberation of inorganic phosphate have been described previously (Charnock & Post, 1963b).

The ³²P-labelling experiments were carried out

* Abbreviation: ATPase, adenosine triphosphatase.

with 1 ml. of enzyme suspension in 1mM-tris-HCl buffer, pH 7.5, containing about 0.4 mg. of protein N/ml. (Lowry, Rosebrough, Farr & Randall, 1951). The enzyme was added last to a reaction mixture that contained in a final volume of 5 ml. carrier-free [γ - ^{32}P]ATP (5 μM) (an aqueous solution of the tetra-ammonium salt brought to pH 7.5 by the addition of solid tris; specific activity 1050 mc/m-mole; The Radiochemical Centre, Amersham, Bucks.), MgCl_2 (5 μM), H_3PO_4 -tris buffer, pH 7.5 (30 mM), and where required Na^+ (20 mM) and K^+ (0.5 mM) as the chloride salts, ouabain (0.1 mM) (Sigma Chemical Co., St Louis, Mo., U.S.A.) and freshly prepared hydroxylamine at the concentrations indicated in the text. After 10 min. temperature equilibration, incubation was carried out for 10 sec. at 37° and the reaction stopped by the addition of sufficient ice-cold 1% (w/v) trichloroacetic acid solution to bring the pH to 2.5; usually

10–20 ml. was required. The precipitated phosphoprotein complex was collected and washed by centrifuging four times with 10 ml. portions of ice-cold tris-buffered 1% (w/v) trichloroacetic acid, pH 2.5, containing carrier disodium ATP (0.1 mM) (Sigma Chemical Co.). All centrifugings were performed at 0°. The inorganic phosphate ^{32}P content of the first acidified reaction mixture was determined after charcoal absorption of nucleotide phosphate ^{32}P by the method of Crane & Lipmann (1953) with an Ecko well-type (N597) crystal scintillator. The washed ^{32}P -labelled phosphoprotein precipitate was dissolved in Lowry's alkaline-copper reagent and samples were taken for protein N determination (Lowry *et al.* 1951). Radioactivity (^{32}P) was determined with an end-window Geiger-Müller tube (Ecko). Sufficient counts were collected to reduce the random error of counting to less than 1%. Hydroxylamine solutions were prepared by passing fresh solutions of hydroxylamine hydrochloride (L.R. grade; May and Baker Ltd., Dagenham, Essex) down a column of Dowex 1 exchange resin (OH⁻ form). The concentration of chloride-free hydroxylamine (Schales & Schales, 1941) was determined by the bromate procedure described by Vogel (1961).

Table 1. *Effect of hydroxylamine, ouabain and K^+ on the incorporation of ^{32}P into a phosphoprotein intermediate of ($\text{Na}^+ + \text{K}^+$)-activated ATPase.*

A 1 ml. sample of enzyme suspension was incubated in 30 mM-phosphate buffer, pH 7.5, with 5 μM -magnesium [γ - ^{32}P]ATP (carrier-free) for 10 sec. at 37°, with Na^+ (20 mM), K^+ (0.5 mM), ouabain (0.1 mM) and hydroxylamine (160 mM) where shown. Experimental conditions are described in text. ^{32}P -labelled phosphoprotein is given as counts/sec. of ^{32}P /mg. of protein N that remain in the washed acid-stable protein precipitate. [^{32}P]Phosphate formed refers to that portion of the 'total radioactivity' of the acidified reaction system after incubation that is non-adsorbable on activated charcoal.

Expt.	Additions	^{32}P - labelled phospho- protein	[^{32}P]- Phosphate formed (%)
(i)	None	360	8
	Na^+	2240	15
	$\text{Na}^+ + \text{ouabain}$	850	9
	$\text{Na}^+ + \text{hydroxylamine}$	510	31
	$\text{Na}^+ + \text{hydroxylamine} + \text{ouabain}$	860	14
(ii)	None	350	8
	Na^+	1430	13
	$\text{Na}^+ + \text{ouabain}$	940	9
	$\text{Na}^+ + \text{hydroxylamine}$	470	24
	$\text{Na}^+ + \text{hydroxylamine} + \text{ouabain}$	800	14
(iii)	None	490	15
	Na^+	4120	38
	$\text{Na}^+ + \text{ouabain}$	1890	17
	$\text{Na}^+ + \text{hydroxylamine}$	730	61
	$\text{Na}^+ + \text{hydroxylamine} + \text{ouabain}$	1160	49
	$\text{Na}^+ + \text{K}^+$	374	44
	$\text{Na}^+ + \text{K}^+ + \text{ouabain}$	980	39

Results and discussion. The results of three labelling experiments are given in Table 1, where all experiments show that, on the addition of Na^+ (20 mM), there is a considerable increase (more than fourfold) in the amount of ^{32}P -labelled phosphoprotein complex formed over that in the control situation (5 μM - Mg^{2+} only). This increase in ^{32}P labelling is accompanied by a rise in the amount of ^{32}P liberated from [γ - ^{32}P]ATP. The addition of ouabain (0.1 mM) to the Na^+ -containing reaction mixture decreased both the amount of ^{32}P released from [γ - ^{32}P]ATP and the amount of ^{32}P -labelled phosphoprotein complex formed. On the other hand, when hydroxylamine (0.16 M) was added in the presence of Na^+ there was a marked increase in the amount of ^{32}P released from [γ - ^{32}P]ATP whereas the amount of ^{32}P -labelled phosphoprotein complex was decreased to a level similar to that found in the absence of both Na^+ and hydroxylamine. This finding is in agreement with that reported by Schoner *et al.* (1966). However, the addition of ouabain (0.1 mM) to a system containing both Na^+ and hydroxylamine inhibited the additional ^{32}P released by the action of hydroxylamine and increased the amount of ^{32}P -labelled phosphoprotein complex to levels clearly greater than that obtained in the controls (twofold). In Expt. (iii) the effect of substituting 0.5 mM- K^+ is contrasted with the action of hydroxylamine. It is clear that both hydroxylamine and K^+ decrease the Na^+ -stimulated incorporation of ^{32}P into phosphoprotein complex, though increasing the

amount of ^{32}P liberated from $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. Hydroxylamine (0.16M) is more effective than 0.5mM- K^+ in this regard. This effect of K^+ has been reported many times previously (e.g. Charnock & Post, 1963a; Whittam, Wheeler & Blake, 1964; Post, Sen & Rosenthal, 1965; Gibbs *et al.* 1965) and is generally thought to represent a greatly increased rate of dephosphorylation of the phosphoprotein intermediate under the influence of K^+ .

The presence of 0.1mM-ouabain in addition to either K^+ or hydroxylamine again decreases the liberation of ^{32}P from $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ and increases the amount of ^{32}P -labelled phosphoprotein complex formed. This finding supports the view that hydroxylamine functions as a cation and can replace K^+ in this system.

To examine this further, hydroxylamine (0.36M) was substituted for K^+ (20mM) in three assays of ATPase activity with a standard chemical assay system for the liberation of inorganic phosphate from non-radioactive ATP (2mM) in the presence of 80mM- Na^+ (Charnock & Post, 1963b). In these experiments the presence of K^+ increased the phosphate released from 53 to 277 μmoles of phosphate/mg. of protein N/hr. whereas addition of hydroxylamine increased the release of phosphate to 93 μmoles of phosphate/mg. of protein N/hr. (values are the means of three experiments, assays in duplicate). In every experiment addition of 0.1mM-ouabain decreased the liberation of phosphate to that found with Na^+ alone.

Thus hydroxylamine appears to function as a K^+ -like cation in the reaction mechanism of $(\text{Na}^+ + \text{K}^+)\text{-activated ATPase}$. The conclusion of Schoner *et al.* (1966) that the ^{32}P -labelled phospho-

protein complex formed ($\text{E} \sim \text{P}$) from $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ cannot be an intermediate in the overall reaction mechanism of this enzyme is therefore no longer valid. Whether hydroxylamine acts directly as a cation, or indirectly by the liberation of NH_4^+ ions, is not resolved by these experiments.

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Effects of Serum Proteins *in vitro* on the Synthesis of Rapidly Labelled Ribonucleic Acid and on the Polysome Pattern of Yoshida Ascites-Hepatoma Cells

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Normal rabbit serum globulins strongly stimulate the incorporation *in vitro* of radioactive glycine into protein of Yoshida ascites-hepatoma cells (Marsilii & Chiarugi, 1965) and this stimulation is abolished by actinomycin D pretreatment of tumour-bearing rats (Marsilii & Chiarugi, 1966).

The present experiments were carried out to study the effects of normal rabbit globulins *in vitro* on the synthesis of rapidly labelled RNA and on the polysome pattern of Yoshida ascites-hepatoma cells.

Material and methods. Yoshida ascites hepatoma