In the course of an extensive investigation of urinary and serum proteins in multiple myeloma[2] proteins were isolated from the urine of eleven patients by precipitation with ammonium sulfate, dissolved in water and dialyzed free of ammonium sulfate against water. This precipitation, dissolution and dialysis was repeated 2 to 3 times to purify the protein, and finally the protein solution was dialyzed against 0.02 *M* KCl.

The protein solutions thus obtained were salted out with a 3.5 molar potassium phosphate buffer, pH 6.5. The amount of protein remaining in solution was estimated by micro-Kjeldahl determination of protein nitrogen in the filtrates. The so-called "derived" salting-out curves obtained in each case are assembled in Fig. 1.

The great disparity of the diagrams is seen at a glance. Some proteins are fairly homogeneous for the greater part (nos. IV, V, VII, IX), others are markedly heterogeneous. The solubilities also vary distinctly. As to the degree of homogeneity, it is not possible to draw a distinction between the group of proteins with Bence-Jones characteristics (in our case, nos. II, IV, VII, XI and part of no. VI) and those lacking the property of coagulating at 50 to 55° C and redissolving upon further heating to 100° C. In both groups of proteins we find examples of fairly homogeneous and of markedly heterogeneous composition. The same applies to the solubility of the main component of each protein. It cannot be said that there is always one Bence-Jones protein, characterized by a certain solubility.

Thus, the sensitive salting-out analysis, which gives such detailed pictures of the composition of protein mixtures, serves to emphasize the diversity and complexity of the proteins excreted in urine in multiple myeloma.

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The formation of nucleoside triphosphate from inosine diphosphate in yeast

Recent work has demonstrated the existence of several enzymes catalysing the phosphorylation of nucleoside mono- and diphosphates[1-4]. Two of these, the adenyl kinase of KALCKAR[1], and the uridyl kinase of LIEBERMAN *et al.*[3], catalyse the dismutation of two molecules of nucleoside diphosphate to one molecule of nucleoside monophosphate plus one molecule of nucleoside triphosphate. In this communication there is described evidence for the existence in yeast extracts of an enzyme catalysing a similar dismutation of two molecules of inosine diphosphate.

The sources of the enzyme were commercial samples of baker's and brewer's yeast*. The yeast was rapidly chilled in solid carbon dioxide and thawed, and then extracted for 5 minutes at 60° C with 2 volumes of *N*/10 HCl. The preparation was centrifuged at 20,000 *g* for 10 minutes and brought to pH 6. This preparation (the yeast enzyme) was stable for two to three days at 0° C, but occasionally lost activity more rapidly. Nucleoside phosphates present in the extract were removed by treatment with Dowex-1.

The assay system chosen to trap nucleoside triphosphate was phosphofructokinase, which has been shown by LING AND LARDY[5] to react with ATP, ITP and UTP at almost equal rates, a finding which was confirmed here. Fructose-1:6-diphosphate was estimated spectrophotometrically by measuring the oxidation of DPNH following conversion to triose phosphate according to the method of RACKER[6]. The complete series of reactions is:

2 IDP $\longrightarrow$ ITP + IMP (1)
glucose-6-phosphate $\longrightarrow$ fructose-6-phosphate (2)
fructose-6-phosphate + ITP $\longrightarrow$ fructose-1:6-diphosphate + IDP (3)
fructose-1:6-diphosphate $\longrightarrow$ dihydroxyacetone phosphate + 3-phosphoglyceraldehyde (4)
3-phosphoglyceraldehyde $\longrightarrow$ dihydroxyacetone phosphate (5)
dihydroxyacetone phosphate + DPNH $\longrightarrow$ glycerol-3-phosphate + DPN (6)

DPNH free from nucleotide impurities was prepared by the method of OHLMEYER[7]. Table I gives the results of a typical experiment demonstrating the necessity for the simultaneous presence

* Among yeasts from which active enzyme preparations were obtained were those from the Compressed Yeast Co., of Sidney; Nycander & Co., Melbourne; and the Richmond Brewing Co., Melbourne. In several other brewer's yeasts tested the enzyme could not be demonstrated. Grateful acknowledgement is made to all companies which generously supplied samples of their cultures.

TABLE I

DEPENDENCE OF DPNH OXIDATION ON THE SIMULTANEOUS PRESENCE OF YEAST ENZYME, GLUCOSE-6-PHOSPHATE AND IDP

The volume of the reaction mixtures was 1.0 ml. Each contained Tris buffer pH 7.5 (50 μM), Mg (20 μM), glutathione (1 μM), and 1 mg protein containing phosphohexose isomerase, phosphofructokinase, and aldolase. Where indicated the following additions were made: glucose-6-phosphate (2 μM), IDP (3 μM), UDP (2 μM), ADP (1.5 μM) and yeast enzyme (2 mg). At the end of a 40 minute incubation period the reaction was stopped with 25 μl perchloric acid. After 5 minutes the mixtures were neutralised with potassium hydroxide and centrifuged. 100 μl aliquots were then analysed for triose phosphate by addition of aldolase, isomerase, α-glycerophosphate dehydrogenase and DPNH. In order to eliminate the effects of residual triphosphate in the system, pairs of tubes were read against each other.

Reaction mixture containing	*read against*	*Reaction mixture containing*	*Excess DPNH oxidised (μM)*
IDP + Yeast enzyme		IDP + Yeast enzyme + glucose-6-phosphate	0.3
Glucose-6-phosphate + yeast enzyme		Glucose-6-phosphate + yeast enzyme + IDP	0.25
Glucose-6-phosphate + yeast enzyme		Glucose-6-phosphate + yeast enzyme + UDP	0
Glucose-6-phosphate + yeast enzyme		Glucose-6-phosphate + yeast enzyme + ADP	1.5
Glucose-6-phosphate + IDP		Glucose-6-phosphate + IDP + yeast enzyme	0.29
Yeast enzyme		Yeast enzyme + IDP	0
Yeast enzyme		Yeast enzyme + glucose-6-phosphate	0

of yeast enzyme, glucose-6-phosphate and IDP for the oxidation of DPNH. This oxidation is taken as evidence for the formation of fructose-1:6-diphosphate by the phosphorylation of fructose-6-phosphate by ITP arising from IDP, and thus for the dismutation of IDP. Under the same conditions UDP was completely inactive, while ADP exhibited great activity. It has not yet been determined whether it is yeast adenyl kinase which exhibits the inosyl kinase activity, or whether there are two enzymes. However, a number of batches of extract have shown great adenyl kinase activity with negligible inosyl kinase. Adenyl kinase is also much more stable. Hence it is likely that the two reactions have different active centres.

Work on the identification of the products of the transphosphorylation as ITP and IMP is in progress. The marked instability of the enzyme has so far prevented its far-reaching purification. A brief survey failed to demonstrate it in a number of animal tissues.

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Further observations on the effects of ribonuclease on living root-tip cells

It has been recently shown by KAUFMANN AND DAS[1] that ribonuclease produces various mitotic disturbances in growing onion roots, indicating that the enzyme penenetrates the living cells. Ribonuclease, when acting on living root-tip cells, also produces a considerable inhibition of the incorporation of labelled amino acids, without exerting any measurable effect on the oxygen consumption (BRACHET[2]). These observations provide further evidence for the hypothesis that ribonucleic acid plays an important role in protein synthesis (CASPERSSON[3], BRACHET[4]).

More recent experiments have shown that crystalline ribonuclease (Armour, 1 mg/ml) strongly inhibits the growth rate (measured with a cathetometer every 30 minutes) of living onion roots: the inhibition of the increase in length (means of 15 experiments) was 35% during the first hour, 77% during the 2nd hour and 88% during the 3rd hour of the ribonuclease action. These values are in good quantitative agreement with the inhibitions usually found for the incorporation of labelled