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

glycine or phenylalanine into the proteins of the growing roots. Oxidized ribonuclease, which has little or no enzymic activity, usually did not inhibit growth; inhibitions, when observed, were delayed and of a quantitatively much lower order.

Protein synthesis in growing roots has been followed by measuring the tyrosine content (Folin's method) in roots whose growth rate was simultaneously observed with a cathetometer focussed on a fine glass capillary inserted into the root, 6–10 mm from the tip: a net synthesis of proteins, corresponding to an average of 2–3% per hour (*cf.* ROBINSON AND BROWN[5]) occurred during a 24 hours growth of 10–20 mm. Inhibition of growth by ribonuclease thus presumably means that the enzyme actually inhibits *net protein synthesis* in living cells.

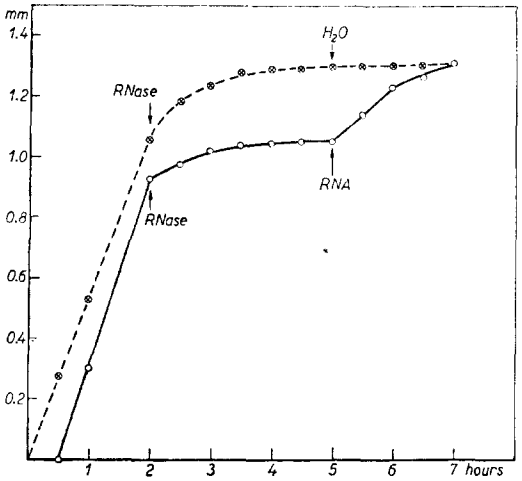

Fig. 1. Growth rate of onion roots. RNase: addition of Armour ribonuclease 1 mg/ml H_2O: washed with distilled water. RNA: addition of ribonucleic acid (1%). Experiment performed on the same onion, cut into two parts. All solutions were aerated.

It is interesting to note that the inhibition of growth produced by ribonuclease in the living onion roots is partially and temporarily overcome by addition of yeast ribonucleic acid (Schwarz; purified on column). As shown in Fig. 1, a 3 hours treatment of the roots with ribonuclease usually irreversibly inhibits growth when the onion is transferred to distilled water; however, if the onion is put in 1% ribonucleic acid instead, a resumption of growth is found in most experiments. The inhibition of growth in roots treated with ribonuclease for 3 hours, and subsequently treated with 1% ribonucleic acid is, on the average (7 experiments), only 50%, whereas it is 90% when roots, from the same onions, are placed in distilled water.

Ribonucleic acid (1%) seems to exert a slight and transitory stimulating effect (30% during the first hour) on the growth of intact, untreated onion roots; later on, an inhibition sets in progressively, in accordance with HUSKINS'[6] and KODANI'S[7] findings that ribonucleic acid produces mitotic abnormalities in onion roots.

Treatment of living onion roots with ribonuclease, even for as long as 20 hours, does not modify appreciably the free amino acids/proteins ratio; this finding seems to rule out the possibility that ribonuclease acts by releasing a proteolytic enzyme in the living cells. Such a possibility was to be considered in view of the recent results of LUNDBLAD AND HULTIN[8], who found that ribonuclease considerably enhances the proteolytic activity of lyophilized sea urchin eggs.

Finally, it is worth mentioning that ribonuclease (the oxidized enzyme used as a control being again completely inactive) inhibits the incorporation of labelled phenylalanine into the proteins to a much greater extent (in the case of short treatments) than the penetration of the same marked amino acid into the free amino acid fraction. Table I gives the results of a typical experiment and shows that the primary action of ribonuclease is less on the actual penetration of the amino acid inside the cell than on the synthesis or turnover of the proteins.

TABLE I

Duration of action of ribonuclease	*Inhibition of penetration in the free amino acids fraction (in %)*	*Inhibition of incorporation in proteins (in %)*
30 min	0	12
60 min	0	32
90 min	11	44
2 h	19	60
3 h	36	65
6 h	71	80
20 h	74	84

In conclusion, the experimental evidence so far obtained is in favour of the view that the integrity of ribonucleic acid is essential for protein synthesis in living cells. However, the possibility that ribonuclease, acting as a basic protein and not as a specific enzyme, combines with ribonucleic acid withoutbreaking it down to a large extent cannot be excluded: the beneficial effects of yeast ribonucleic acid on the growth of the ribonuclease-treated roots could then be explained on the basis of a competition between the native ribonucleic acid and the added yeast ribonucleic acid for ribonuclease.

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Preparation of a sodium salt of 3-phosphoryl-D-glyceric acid

Soluble salts of 2-phosphoryl-D-glyceric acid and 3-phosphoryl-D-glyceric acid, free of heavy metal ions, were needed in studies of phosphoglycerate mutase now in progress in this laboratory. The synthesis of a sodium salt of 2-phosphoryl-D-glyceric acid has been described by BALLOU[1]. A sodium salt of 3-phosphoryl-D-glyceric acid may be prepared from the purified barium salt by the following procedure.

Procedure

3-Phosphoryl-D-glyceric acid (3-PGA) was isolated as the barium salt from a yeast fermentation mixture[2]. The barium salt was recrystallized three times before conversion to the sodium salt*.

9.5 g of $Ba_3PGA \cdot 2H_2O$ was shaken with 200 ml of a 1:1 slurry of Dowex 50 (Na^+) for 2 hours. The mixture was filtered and the resin was washed twice with 25 ml portions of water. Total volume of filtrate and washings was 150 ml. The solution, at pH 7, was concentrated *in vacuo* to 30–40 ml and filtered if not clear. Absolute methanol was added to 100 volumes and then *n*-hexane was added to appearance of the white salt and/or appearance of a second phase. The mixture was stored at room temperature to complete precipitation of the sodium salt. The salt was removed by centrifugation and washed with acetone. After drying in a vacuum oven at 55°, the stable white powder amounted to 4.5 g. Yield = 75% as Na_3PGA. The compound lost no weight on drying over P_2O_5 at 78° for eight hours.

Element analysis	*Calculated for* $C_3H_5O_7PNa_2$	*Found*
% C	15.68	15.48**
% P	13.48	13.10**
% Na	20.00	19.87

$[\alpha]_{20}^{D} = -735° \pm 8°$ (−0.82°, 1 d.cm tube, c = 1.11 mg as the free acid) in 1/3 volume of 25% (w/v) $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$***.

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* Mr. LEWIS PIZER, a graduate student of this department, has prepared the sodium salt from the barium salt of phosphoglyceric acid as supplied by Schwarz Laboratories Inc., New York, without further purification. Yield = 37%.

** Minimum value.

*** The high rotation of 3-PGA in molybdate solution is well known[3]. Customarily such measurements have been made in 1/3 volume of 25% $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (8% molybdate in the final mixture) at neutral pH. The writer[2] has observed, however, that neither concentration of molybdate nor pH are critical. The same rotation was observed in mixtures containing from 2.5 to 12.5% molybdate and at pH values from 7 to 4. The rotation is lowered in alkaline solutions.