Zinc in Aspermic Human Semen

Normal human seminal plasma has been shown to contain a higher concentration of zinc than any tissue except sperm[1]. The sperm contain a very high concentration of zinc, but their removal from semen by centrifugation causes no significant reduction in the zinc concentration of the fluid because of their small relative volume. No previous opportunity had occurred for analysis of naturally aspermic semen, but recently a specimen of this nature was received. It had not been collected in specially cleaned glassware, but any contamination due to this fact could not have added more than a few μgm./ml. to the zinc content.

The centrifuged deposit from the whole specimen was examined by phase microscopy and by the staining method of Isenberg[2] and found to contain a few epithelial cells, but no sperm. The volume of the semen was 5 ml.

Zinc was estimated in 1 ml. of the plasma by the method of Vallee and Gibson[3]. The dry weight was 7·6 gm./100 gm. and the zinc concentration 234 μgm./ml. This result did not differ significantly from normal: four specimens of normal human seminal plasma contained 134 ± 59 (S.D.) μgm. zinc/ml.

It is known that dietary zinc deficiency will cause a reduction of androgenic hormone production and aspermia in rats[4]. The wide distribution of zinc in Nature makes dietary zinc deficiency in man very improbable, and the normal volume and the high zinc content of this semen suggest normal prostatic function. The donor was clinically normal, with no evidence of androgenic insufficiency.

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Reticulocyte Enzymes and Protein Synthesis

The hæmoglobin of red cells appears to be synthesized during the early stages of maturation, and its formation is more or less complete by the time the cells leave the bone marrow. Although reticulocytes have practically their full complement of hæmoglobin, evidence from amino-acid incorporation studies[1] suggests that these cells, unlike mature erythrocytes, still have protein-synthesizing capacity. In view of this, it was felt that it would be of interest to see if certain enzymes were at a high level of activity in reticulocytes; Table 1 summarizes the results of a preliminary investigation of the level of activity in the two types of cell for a number of enzymes which might be involved in some way in a protein-synthesizing mechanism.

In addition to the enzymes shown, attempts were made to detect glutamine synthetase and enzymes synthesizing glutathione. However, under the conditions of assay which were tried, such activities were not detected in either erythrocytes or reticulocytes.

The phosphatases listed in Table 1 were investigated in view of the possibility that their *in vivo* function may be concerned with the transfer of phosphate bond energy utilized in the linking of amino-acids. The reticulocyte preparations were similar to those used in the amino-acid incorporation studies of Borsook[1], that is, preparations of rabbit blood containing 70–90 per cent reticulocytes, obtained following reticulocytosis induced by phenylhydrazine. The assays were carried out on whole hæmolysates obtained by freezing and thawing the washed cells, and activities were expressed per milligram of protein nitrogen.

Table 1

Enzymatic activity	*p*H	Activator	Approximate increase in activity in reticulocyte preparations
Cathepsin	3·2	none	6-fold
Cathepsin	7·5	0·001 *M* Zn^{++}	no increase
Glycylglycine dipeptidase	7·8	0·001 *M* Co^{++}	8-fold
Leucine aminopeptidase	7·8	0·001 *M* Mn^{++}	2-fold
Tripeptidase	7·1	none	2- to 3-fold
Liberation of inorganic phosphate from adenosine tri-, di- and mono-phosphate	7·5	0·001 *M* Mg^{++}	2- to 3-fold
Acid phosphatase	6·0	none	3- to 5-fold
Alkaline inorganic pyrophosphatase	7·5	0·025 *M* Mg^{++}	8-fold
Acid inorganic pyrophosphatase	5·2	none	12-fold

During the transition from reticulocyte to erythrocyte, much of the cellular protein, other than hæmoglobin, disappears, and it might be expected that, relative to total protein nitrogen, a generally higher level of enzymatic activity might be found in the former type of cell. This may account for the fact that most of the enzymes studied showed some increase in activity in reticulocytes.

However, from Table 1 it is apparent that some enzymes are increased to a greater extent than the others: namely, cathepsin active at *p*H 3·2, one particular peptidase (glycylglycine dipeptidase), and inorganic pyrophosphatase.

The possibility that cathepsins have a synthetic function has long been considered, and there appears to be some correlation between the rate of growth, or of protein synthesis, and the level of catheptic activity in various tissues[2,3]. On the other hand, recent reports that cathepsins are mitochondrial enzymes[4] are not in accord with a protein-synthesizing function, as the latter process appears to be associated with microsomal particles. It has been suggested[3] that catheptic activity is concerned with the provision of free amino-acids for utilization in protein formation, rather than with the synthetic mechanism itself.

The observation of a specific increase in dipeptidase activity is of interest in relation to recent reports by Binkley[5], who, in the course of attempts to purify cysteinyl glycinase from swine kidney, obtained a soluble fraction which possessed a very high non-specific dipeptidase activity and which was apparently non-protein in nature. This material exhibited some of the properties of a polynucleotide, and in view of this, it was suggested that *in vivo* the activity might be related to protein biosynthesis.

Among the phosphatases studied, the largest increases were found in the case of the inorganic pyrophosphatases. This observation may have a bearing on results obtained by Hoagland and Keller[6], who have formulated the following scheme for the carboxyl activation of amino-acids (detected in their experiments by hydroxamate formation):