Degradation of Nucleotide Pyrophosphate Compounds by Human Seminal Plasma Enzymes.* (26102)

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The observation has been made that phosphate is relased from a variety of purine and pyrimidine containing nucleotide pyrophosphate compounds by combined action of nucleotide pyrophosphatase activities(1) and 5-nucleotidase(2) found in bovine seminal plasma. The present report indicates the presence of similar activities in human seminal plasma.

Materials and methods. Substrates were purchased from Sigma Chemical Co., St. Louis, Mo., except for yeast cytidylic acid, which was a Nutritional Biochemicals preparation. Pooled frozen human semen samples, 5 or more per pool, were obtained from the Duke Medical Center Fertility Clinic. The pooled samples were thawed and centrifuged at 10,000 $\times$ g for 15 minutes at 5° to remove sperm. The crude human seminal plasma was then fractionated by the short procedure used by Heppel and Hilmoe to prepare 5-nucleotidase from bovine seminal plasma(2), followed by precipitation with ammonium sulfate. The 0 to 67 and 67 to 80% saturated ammonium sulfate precipitates were dissolved in minimum amounts of water, and frozen until used. Phosphate was determined by the method of Gomori(3). Phosphate release is recorded as the difference between that amount found in zero time (unincubated) and final time (incubated at 37°) supernatants of 5% trichloracetic acid deproteinized, 0.2 ml reaction mixtures. Spectrophotometric activity measurements, using O-carboxyphenylphosphate and Ca(bis-pNO_2 phenyl phosphate)$_2$ as substrates, were carried out at room temperature according to procedures described by Hofstee(4) and Privat de Garilhe and Laskowski(5), respectively. Reaction mixtures contained 0.08 M glycine, pH 8.9, 10^{-2} M $MgCl_2$, enzyme, and substrate as indicated.

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Results. The 2 ammonium sulfate fractions obtained were assayed at several concentration levels for 5-nucleotidase activity. Roughly two-thirds of total activity was found in the 0-67% ammonium sulfate fraction, which was then used for the experiments summarized in Table I. In addition to the substrates shown, crude seminal plasma and both enzyme fractions were assayed for activity against the phosphomonoesterase substrate, O-carboxyphenylphosphate and the phosphodiesterase substrate Ca(bis-pNO_2 phenyl phosphate)$_2$. Crude seminal plasma samples slowly hydrolyzed both substrates, but neither of the 2 ammonium sulfate fractions exhibited appreciable activity.

Discussion. The observations reported indicate that the "short" procedure of Heppel and Hilmoe(2) allows the preparation, from human seminal plasma, of 5-nucleotidase relatively free of "alkaline" phosphomonoesterase. The preparation also contains nucleotide pyrophosphate degrading activities, presumably nucleotide pyrophosphatases. It is interesting to note that the substrate spec-

TABLE I. Orthophosphate Release from Nucleotides by Human Seminal Plasma Enzymes.

Additions, μmoles		Phosphate released, μmoles
30 min. incubation		
Cytidine-5-phosphate	.24	.20
Uridine-5-phosphate	.25	.17
Adenosine-5-phosphate	.25	.15
Guanosine-5-phosphate	.25	.10
Yeast cytidylic acid	.25	.01
Glucose-1-phosphate	.20	.02
Phosphoryl-choline	.20	.02
Flavine mononucleotide	.20	.02
Inorganic pyrophosphate	.20	.00
60 min. incubation		
Cytidine diphosphate choline	.20	.14
Uridine diphosphate glucose	.34	.19
Diphosphopyridine nucleotide	.20	.22
Reduced diphosphopyridine nucleotide	.4	.30
Flavine adenine dinucleotide	.2	.24

trum exhibited by the human seminal plasma enzyme preparation is similar to that of the bovine seminal plasma enzyme preparation made by the same procedure(1).

Summary. Enzymes prepared from human seminal plasma were found to release phosphate from the 5-mononucleotides, cytidylic, uridylic, adenylic and guanylic acids. The preparations were not, or only slightly, active in hydrolyzing yeast cytidylic acid, glucose-l-phosphate, phosphorylcholine, flavine mononucleotide, and O-carboxyphenyl phosphate. Phosphate was also released from cytidine diphosphatecholine, uridine diphosphate glucose, oxidized and reduced diphosphopyridine nucleotide, and flavine adenine dinucleotide.

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Cholic Acid in Guinea Pig Bile.* (26103)

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During the investigation of formation of gall bladder precipitates produced by parenteral administration of a bacterial polysaccharide, we found that 3α, 7α, 12α-trihydroxycholanic (cholic) acid was not only present in guinea pig bile, but was the major bile acid. Further study disclosed that 3α, 7α, 12α-trihydroxycholanic acid was present in adult but not in immature guinea pigs, thereby offering an explanation for the previously reported absence(1,2) of this bile acid.

Methods. Non-inbred, albino and varicolored guinea pigs were obtained from commercial suppliers in Oregon, Utah, and Missouri. Eighteen adult guinea pigs of either sex, approximately 5 months of age and weighing 500-700 g, were fed with Purina rabbit chow and fresh green vegetables, carrots and apples *ad libitum*. Bile fistulae were performed on 6 animals from this group. Bile from the gall bladders of the remaining 12 animals was pooled in 2 samples. Seventy-two immature guinea pigs of either sex, 6-8 weeks of age, and weighing 250-350 g were divided into 3 equal groups. Each group of 24 animals was kept for 2 weeks on one of the following diets: a) Vit. C deficient diet of Rinehart and Mettier(3), rich in other vitamins, fats, and minerals; b) Purina rabbit chow heated for one hour at 100°C; c) Purina rabbit chow with green vegetables, carrots and apples as supplied to the group of adult guinea pigs. Bile from gall bladders was pooled in 3 samples per each dietary group. All gall bladders were aspirated by syringe within one minute after application of a neck clamp.

The bile fistulae were performed under ether anesthesia. One polyethylene catheter was inserted into the gall bladder and another one into the proximally ligated common bile duct. Both catheters were passed under the skin to the back where their free ends were pulled through a cutaneous wound and connected to allow enterohepatic cycling. To avoid operative and postoperative abnormalities, collection of the first bile samples was not begun until 48 hours after the surgical procedure. A volume of normal bile equal to that diverted was supplied through the duodenal fistulae. These samples obtained from the bile fistulae were not pooled but processed separately.

Except for small aliquots, the bile samples

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