INVESTIGATIONS ON PGM ACTIVITY AND ELECTROPHORETIC PATTERNS FROM EJACULATES AND SEMINAL STAINS

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PGM activity from semen samples was measured in vitro from the supernatant (i.e. seminal plasma) and from the sediment (i.e. spermatozoa) using equal volumes of both. The activity from the sediment was also tested after a 4 times freezing and thawing procedure and after treatment with various organic solvents in an attempt to perforate or dissolve the membrane. These solvents included benzene, toluene, xylene, chloroform, carbon tetrachloride, acetone and phenol. From the results of these experiments it was observed that in comparison to the purely mechanical method of membrane disruption, i.e. freezing and thawing, there was a further significant increase in the measured PGM activity after treatment with toluene and benzene, but only in the range of 50 to 100 %. Furthermore activity was found in both the plasma and the sediment, and the maximum activity for the sediment was achieved using benzene (fig. 1). The variation in activity between the sediment and plasma ranged from 1:0,5 to 1:4.
The case history of the individual semen samples was not made available to us so that the precise storage details and the possible presence of any abnormalities were not known. Some of these unknown factors would also be encountered in routine casework samples. The proportion of spermatozoa in a semen sample also varies enormously but is normally in the range of 1 % of the total volume, with an average 100 million spermatozoa per ml plasma (4). The percentage of the total PGM activity contributed by the

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spermatozoa would therefore be small in comparison to the plasma. In the second stage, methods of improving the quality and quantity of activity found in bands after isoelectric focusing were investigated. It is not always the case, but strong activity is often observed with such distortion of the bands that it is not possible to identify the phenotype. In these cases systematic dilution of the extract in aqua dest or different buffer solutions resulted only in a weakening of complete absence of bands. One of the reasons for this distortion could be a high ion concentration in these samples causing an imbalance in the pH structure of an isoelectric focusing plate. With this in mind semen samples and stain extracts were dialysed using dialysis tubes, Centrisart^{R}1, produced by Sartorius, as follows:
200 µl semen or a 1 cm x 1 cm piece of dried seminal stain from the same sample was placed in 2 ml of aqua dest. After freezing and thawing, the liquid was placed in the outer tube (fig. 2). The inner tube with the attached membrane was then inserted over the sample and centrifuged at 2000 g, after allowing 5 minutes for the membrane to be uniformly wetted. The water is forced through the membrane leaving only a concentrated sediment between the inner and outer tubes. This sediment was removed and applied to the gel. After this treatment it was observed that the bands showed weaker activity than the untreated samples but were not distorted and a phenotype could be determined.

The results of the experiments were not completely satisfactory because of the decrease in activity and would not therefore be suitable for weak samples, but the procedure has been successfully used for samples which before

treatment had strong activity but pronounced deformation of bands, making a clear identification impossible. It is hoped to improve the procedure sufficiently for use with the majority of samples.

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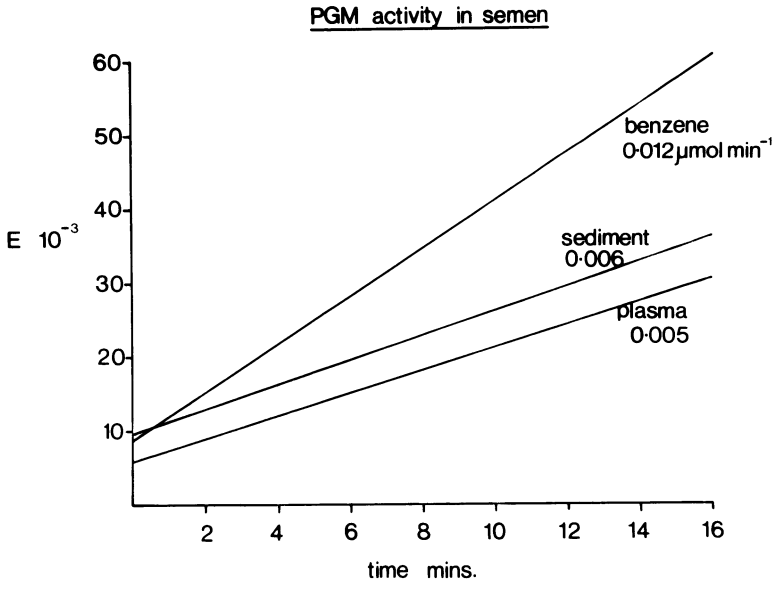

Fig. 1: Comparison of PGM activity in plasma, in spermatozoa after freezing/thawing and after benzene extraction

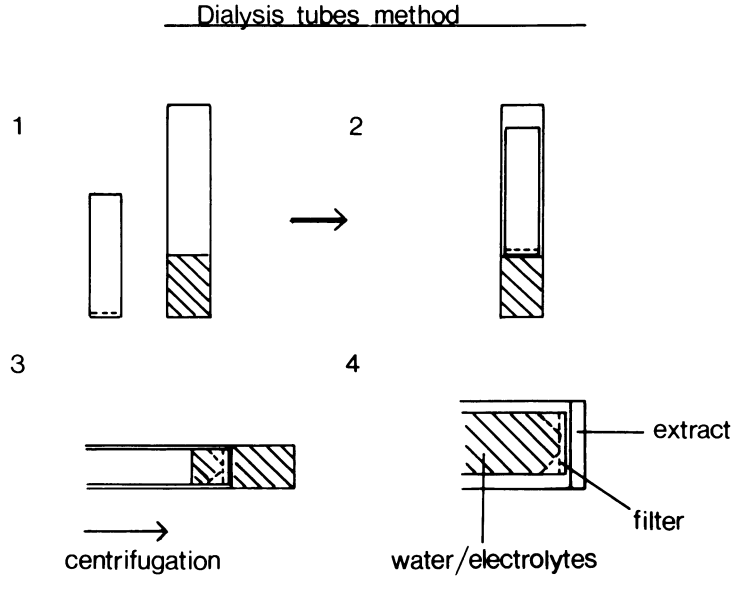

Fig. 2: Diagrammatic representation of procedure for dialysis of semen samples