The Presence of Specific Proteins, in the Absence of many Serum Proteins, in the Rat Seminiferous Tubule Fluid

Seminiferous tubules of the testis secrete fluid which carries the spermatozoa out of the testis and into the epididymis. Resent studies have shown that this fluid is unique in many respects, due to the existence of a barrier mechanism in the walls of the seminiferous tubules, which prevents the entry of various endogenous and administered substances[1]. Recent studies on rete testis fluid proteins, based mainly on the ram fluid obtained by the catheter implantation technique[2], suggest that all the individual serum proteins are present in the rete testis fluid, but in much lower concentrations. JOHNSON and SETCHELL[3] found a specific protein in ram rete testis fluid. This protein migrated between α_1 and α_2 globulin in cellulose acetate electrophoresis. The existence of a protein smaller than albumin, with a molecular weight between 20,000 and 30,000 has also been reported[4]. Since cellulose acetate electrophoresis provides only a relative low level resolution, it was deemed desirable to investigate the composition of testicular fluids using a step gradient polyacrylamide gel system.

Material and methods. Fluids collected from the rat seminiferous tubules and from the rete testis were subjected to electrophoretic separation using a step gradient, flat bed acrylamide gel system recomended by the manufacturer of the system[5]. This system utilizes a 0.75*M* *Tris*-sulfate pH 9.0 separating gel buffer with the concentration of acrylamide at the origin being 4.5% and increasing in steps to 6%, 8%, and 12%. Both upper and lower buffer tanks were filled with 0.065*M* pH 9.0 *Tris*-borate buffer. Samples ranging in size from 0.5 to 1.0 µl were absorbed onto cellulose acetate paper strips measuring 2×3 mm. The strips containing the samples were placed on the surface of the 4.5% gel and a 12% acrylamide gel cap was pressed against the upper surface of sample containing strips. The gel cap is made up of the same composition as the 12% acrylamide portion of the running gel, except that the buffer concentration is one-half of the running gel. Constant power for the electrophoretic separation is provided by means of a pulsed constant power supply in which the initial pulse rate is 75 pulses/sec. The pulse rate is increased 75 pulses/sec at 5 min intervals until 300 pulses/min is reached. The capacitance is set at 1.0 µ-farad and the voltage at 280 volts. The total elapsed time for the run is 45 min. The gel is removed and fixed in 12% trichloro-acetic acid for 30 min. After rinsing the gel slab 5 or 6 times in tap water, it is placed in a 0.20% aqueous solution of Coomassie Blue for 1 h at 37 °C. The gel is then rinsed in several changes of 10% acetic acid until no more dye is being extracted.

The rete testis fluid was collected by ligating the efferent ducts and puncturing the dilated rete testis 12, 24 or 48 h later, from 4, 8 and 6 animals, respectively. Samples of free-flow primary secretion of the seminiferous tubules were obtained from 8 animals under pentobarbitone anaesthesia through an incision in the tunica albuginea, where one of the protruding seminiferous tubules was micropunctured with a glass capillary with an outer tip diameter of 20–30 µm. 2 to 4 samples of tubular fluid, taken from the same testis, 0.2–0.5 µl each, were poooled to make 1 sample for electrophoresis. For comparison, samples of rat serum and intratesticular lymph were studied. The latter was obtained with a glass capillary placed between unperforated seminiferous tubules.

Results and discussion. Serum and intratesticular lymph proved to have a highly similar pattern of protein bands except for the slight predominance of fast moving proteins in the lymph (Figure 2). A total of 21 separate bands were identifiable in the serum (Figures 1 and 3), although some of them (bands 1, 3, 8, 14) were weak and not always seen. The proteins of the tubule fluid revealed a total of 23 bands (band 4 only occasional and weak). 9 bands, all weakly stained, were identical electrophoretically with serum proteins. Band 27 was a strong, intense band both in serum and in rete testis fluid, but in tubule fluid it was a weakly stained doublet (27a and 27b in Figure 3). 2 distinct groups were typical of tubule fluid. One of them consisted of 6 bands, 3 of which were remarkably strong, moving in front of the fastest moving albumin fraction of serum (bands 29 to 34). These were thought to be equivalent to the small protein found in the ram rete testis fluid[4]. Another characteristic group of bands showed intermediate mobility. They appeared as 3 thin but strong bands (bands 17, 18 and 19). The rest of the non-serum protein bands were weaker and mainly slow moving.

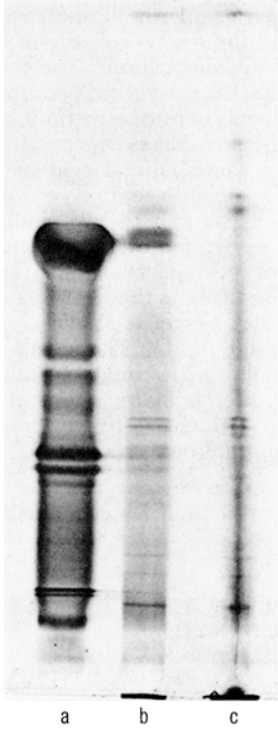

Fig. 1. Electrophoretic protein pattern of a) rat serum, b) rete testis fluid and c) seminiferous tubule fluid on the same acrylamide gel plate.

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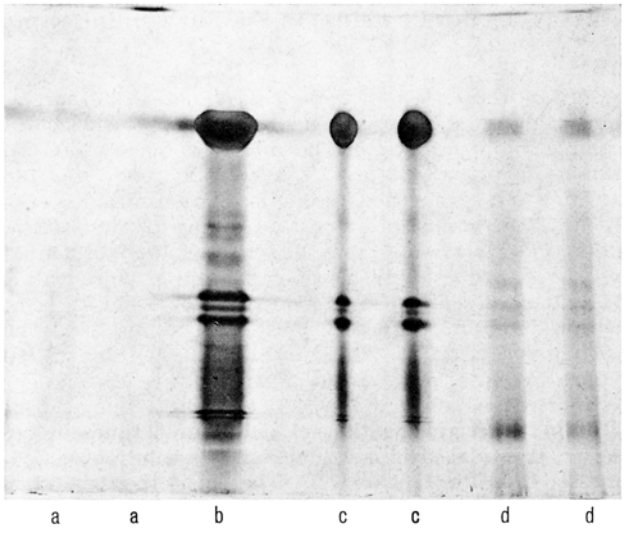

Fig. 2. Comparison of protein pattern of a) rete testis fluid 48 h after efferent duct ligature, b) serum, c) intratesticular lymph, d) rete testis fluid 24 h after efferent duct ligature. Protein staining in rete testis fluid after 48 h ligature is very weak.

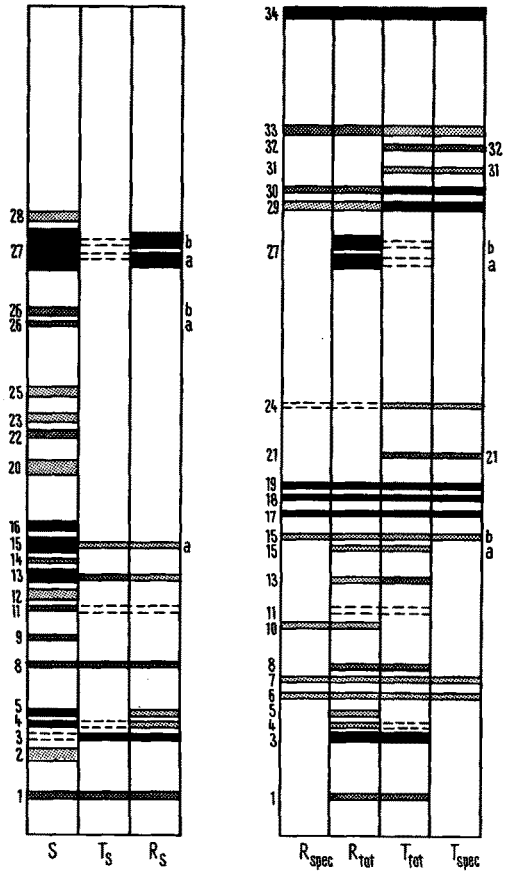

Fig. 3. Diagrammatic presentation of the protein bands seen in step gradient acrylamide gel electrophoresis and the numbering used in the text. S, serum; T_s, serum protein bands seen in the seminiferous tubule fluid; R_s, serum protein bands seen in the rete testis fluid; R_{spec}, protein bands specific to the rete testis fluid (e.g., those not seen in the serum); R_{tot}, total pattern of the rete testis fluid; T_{tot}, total protein band pattern of the seminiferous tubule fluid; T_{spec}, bands specific to the tubule fluid (e.g., not seen in the serum). Very weak or inconstant bands are drawn with dotted lines.

Proteins from rete testis fluid appear to be a mixture of the proteins from the seminiferous tubule fluid and some serum proteins. Except for one inconstant band (No.10), all the other rete fluid bands were visible either in the tubule fluid or in serum. There were some weak bands (No. 21, 31 and 32) which were found in tubule fluid but never in rete testis fluid. All the serum bands visible in the tubule fluid were seen also in rete testis fluid, but bands 27a and 27b especially were constantly much stronger in the latter. The serum band No. 5 was seen in rete testis fluid although not in tubule fluid.

The electrophoretic pattern of the fluid was virtually identical after 12 and 24 h ligature. Removal of spermatozoa by centrifugation did not influence the pattern, either. At 48 h, the intensity of protein staining was markedly decreased (Figure 2).

The use of micropuncture technique together with high resolution electrophoresis has made it possible to demonstrate the originality of the protein constituents of seminiferous tubule fluid and the very small contribution of plasma proteins to the milieu of the tubules. In contrast to the considerable amounts of serum protein in rete testis fluid, there were very small amounts of serum protein in tubule fluid, although the same, fast-moving serum bands were seen in both fluids. Although a difference in the serum contribution to the constituents of these fluids is more quantitative than qualitative, this observation emphasizes the fact that samples of rete testis fluid cannot be used as representatives of the composition of the primary secretion of the seminiferous tubules. This is already suggested by the observations of TUCK et al.[6] on the differences in the ionic composition of the seminiferous tubule fluid versus rete testis fluid in the rat. Various other observations, such as better permeability to dyes of the rete testis[7] and the involvement of the rete testis before the rest of the organ in experimental allergic orchitis[8], suggest that rete testis is the weak point of the testicular barrier system, or may even be outside it. The presence of numerous microvilli and cytoplasmic vesicles in the cells lining the rete is consistent with the concept of fluid transport in this region[9]. The evidence of resorption, and especially the early disapperance of a few specific tubular protein bands in rete testis fluid, is highly interesting as regards the possible existence of 'inhibing' in this fluid[1,10]. There is some indirect evidence to suggest that such a feedback of information on the intensity of spermatogenesis exists and that it would most logically happen through resorption of the testicular fluid[10,11].

The pattern of proteins was remarkably uniform in different samples of tubule fluid. This is not incompatible with the view that Sertoli cells are responsible for secretion of the tubule fluid[12]. These cells are the only ones within the epithelium which might be related to transport functions[13]. The interesting questions of whether protein pattern is at all influenced by different stages of the cycle of the seminiferous epithelium, or whether rete

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testis fluid is able to contaminate the tubular protein pattern due to reflux, as suggested recently by TUCK et al.[14] on the basis of ionic concentrations of the fluids, will need further experimentation.

Zusammenfassung: Die Flüssigkeit in den Hodenkanälchen enthielt mehrere Proteine, die im Serum oder in der Hodenlymphe nicht nachweislich waren. Nur von einigen der Serumproteine waren in der Flüssigkeit der Hodenkanälchen schwache elektrophoretische Bänder zu sehen.

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La localisation extra-embryonnaire des cellules germinales chez l'embryon de Lézard vivipare (*Lacerta vivipara* Jacquin)

Des recherches descriptives ont conduit à situer les gonocytes du Lézard vivipare dans un croissant extra-embryonnaire postérieur qui embrasse directement l'extrémité caudale de l'embryon[1-3]. Cette localisation a été en partie confirmée expérimentalement[4] par ablation chirurgicale du croissant extra-embryonnaire, supposé contenir les gonocytes, chez des embryons de 6 paires de somites (stade 19 de la table de développement de ce Lézard[5]). Le nombre de gonocytes contenu dans les crêtes génitales des embryons opérés était significativement inférieur à celui des témoins mais il restait en moyenne de l'ordre de 20. Ce résultat pouvait tenir au fait que des gonocytes avaient déjà pénétré dans les tissus embryonnaires au moment de l'intervention chirurgicale. Nous avons donc renouvelé l'expérience d'une part à des stades de développement plus précoces pour vérifier cette interprétation, d'autre part à un stade plus âgé pour préciser à quelle période les cellules germinales ont envahi l'embryon.

Matériel et méthodes. Les femelles gestantes ont été sacrifiées par décapitation dans les 2 à 3 jours suivant la capture. Immédiatement après le sacrifice, les utérus sont disséqués et placés dans du liquide physiologique de Tyrode stérile. Les oeufs sont extraits des utérus et mis en attente dans un nouveau bain de Tyrode stérile. Chaque œuf est ensuite déposé dans une coupelle dont le fond de paraffine est creusé d'une petite loge; cette coupelle est remplie de Tyrode. L'œuf, débarrassé de ses membranes coquillière et vitelline à l'aide de pinces fines est immobilisé dans la petite loge, l'embryon face à l'opérateur. Le territoire extra-embryonnaire supposé contenir les gonocytes, d'après l'étude histologique d'embryons normaux (Figures 1 et 2), est découpé à l'aide de ciseaux Pascheff-Wolff et extirpé avec des pinces fines. Le vitellus de l'œuf est assez consistant pour ne pas s'écouler en l'absence des membranes et l'embryon est donc laissé en place sur son

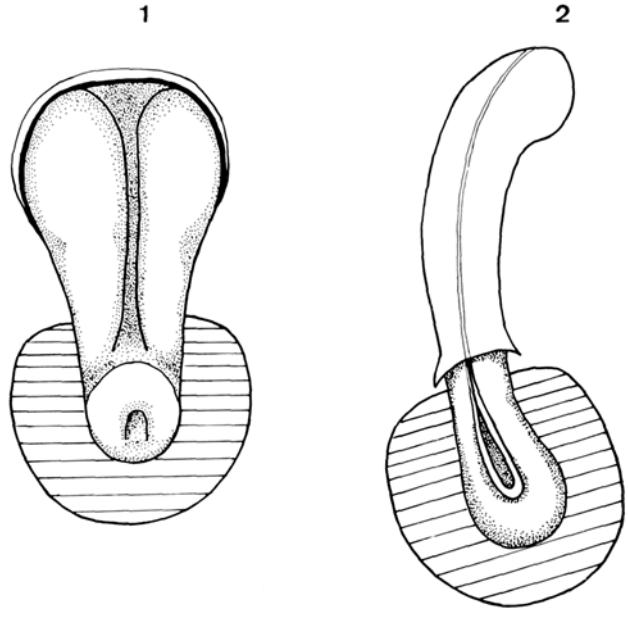

Fig. 1. Représentation schématique d'un embryon au stade 15.
Fig. 2. Représentation schématique d'un embryon au stade 20. Pour les figures 1 et 2 la zone extra-embryonnaire supprimée est indiquée en hachures.

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	Opérés		Témoins	
Stade de mise en culture	15 et 17	20	15 et 17	20
Nombre d'embryons cultivés	13	11	9	7
Nombre d'embryons fixés au stade 30–31	7	8	7	6
Nombre moyen de gonocytes au stade 30–31	11	97	82	101