Nature of Some Components of Epididymis and Ductus Deferens Secretion in Mouse

A particular secretion was described in human epididymis and ductus deferens as early as 1924[1,2]. The nature of this secretion was investigated by histochemical tests from which it was concluded that it was a lipoprotein[3,4]. Vacuolae were formed in epithelium cell nuclei and subsequently released into the lumen of the gland. A possible trophic function was postulated.

More recent investigations[5,6] established the presence of holocrine secretory cells in rat epididymis, a conclusion which could be extended to mouse and chinese hamster[5,6]. These PAS positive cells are characterized by their high acid phosphatase and aliesterase activity[5,6] and may disintegrate in situ or discharge in the lumen, but no conclusion could be drawn about the functional importance of each component as well as the exact location where it is released.

In recent investigations on several differences among mouse strains for various secretions of the male genital tract, we drew the conclusion that there was a regular transfer of RNA molecules from the epithelial cells into the lumen of the epididymal canal in which RNA-containing bodies could be regularly observed. This might be connected with the holocrine cells or with more specific nuclear or nucleolar components. The RNA nature of some components is the subject of the present investigation.

Material and methods. Males (3–4 months; 28 g) of C_{57}BL strain were preliminary tested for fertility. For histochemical tests the organs were fixed (Zenker-formol) and embedded in paraffin. Sections were stained with a methyl-green pyronin solution. Some slides were treated at increased times with a Kunitz's ribonuclease solution[3].

For chemical identification, the content of epididymis and ductus deferens was gently squeezed out into a 0.14 *M* NaCl solution avoiding too much damage to the epithelia. The solution extracted was centrifuged (5000 rpm/30 min at 0 °C) twice. The supernatant was verified microscopically to be cell-free (spermatozoa and epithelium debris). RNA was also identified by spectroscopy after phenol extraction[4] from the supernatant. To investigate the kinetics of these molecules, two RNA precursors were injected i.p. (0.5 ml) 5-^{3}H-uridine (specific activity: 10 Ci/m*M*) or ^{3}H-orotic acid (specific activity: 16 Ci/m*M*) (25 μCi per animal in both cases). Animals were sacrificed at different times during a 18-day period for excretion measurement.

After the same saline extraction (in 1 ml solution) and centrifugation as indicated above, the radioactivity was measured by liquid scintillation (Packard Tricarb counter). The scintillation medium was composed of 60 g naphtalene, 3.5 g PPO, and 25 mg POPOP dissolved in 500 ml dioxane.

Results. After the histochemical test, pyronine-stained bodies were repeatedly observed in the lumen of epididy-

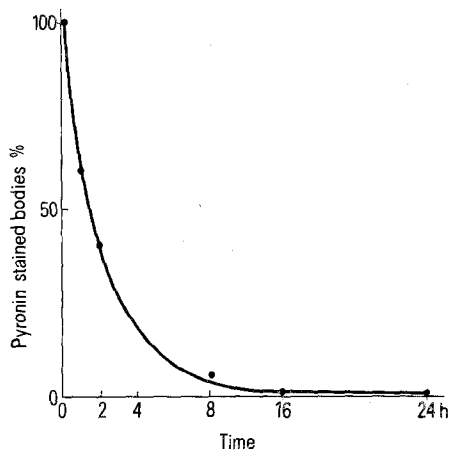

Fig. 1. Decrease of the number of pyronin-stained bodies observed in epididymis sections (10 μm) after increased times of action of RNAase (counts over 100 tubules).

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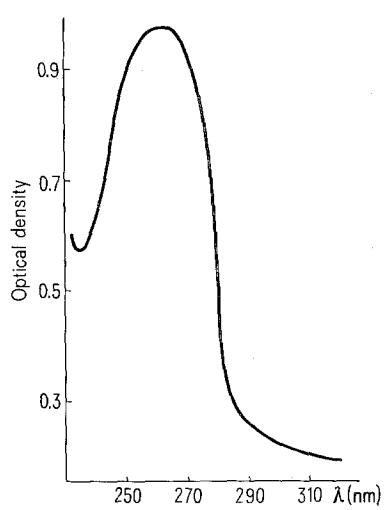

Fig. 2. Spectrum obtained after phenol extraction of epididymis supernatant.

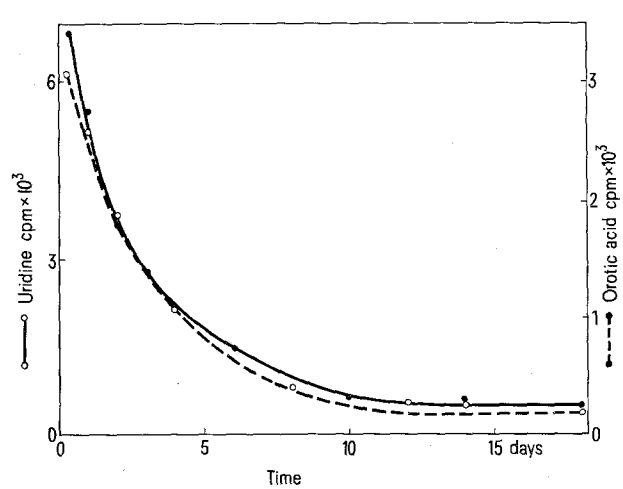

Fig. 3. Release of radioactivity of epididymis after ^{3}H-uridine and ^{3}H-orotic acid (25 μCi/animal in counts per min).

mis as well as the ductus deferens. Some figures clearly showed their epithelial origin (to be reported in detail in another context).

Since their RNA or RNP nature was strongly suspected, some slides were submitted to a RNAase solution at increased times. The number of pyronine-stained bodies was counted over a certain number of tubules. Figure 1 shows the decrease of number per tubule expressed as a percentage of the number counted before digestion. After 24 h, no pyronin-body could be observed. These histochemical data, which are only a first approach due to the variation of size of the stained corpuscules were chemically verified.

After phenol extraction[4], a typical RNA spectrum was obtained (Figure 2). After ^{3}H-uridine or ^{3}H-orotic acid, the major part of the radioactivity was located in the supernatant. The release of radioactivity from epididymis tubules and ductus deferens was investigated during a 18-day period. The curves of release are very comparable in both cases (^{3}H-uridine or ^{3}H-orotic acid) (Figure 3).

Since the decrease is exponential, it can mean that few or no radioactivity can be transferred from the organ cells to the gland lumen during a long period of time, or that the amount of secretion is not sufficient to compensate the decrease of activity due to the movement of the sperm fluid to the end segments of the sexual tract.

Discussion and conclusion. The present experiments have demonstrated by 3 different methods *i.e.* histochemistry, spectrophotometry, and liquid scintillation spectrometry, the occurrence of a RNA-containing compound in the lumen of epididymal canals and ductus deferens. This component is of quite different chemical nature from those previously described[1,2,5,6].

Since parts of these molecules at least are resistant to the histochemical techniques of fixation and staining, it can be deduced that they belong to a stable kind. It does not exclude that this resistant RNA may be the only one appearing in the secretion and that the supernatant could be partly contamined by diffusible RNA from the epithelial cells.

Whether the RNA compounds are part of the holocrine cells, secretion of other cells, or both, cannot be presently ascertained.

New experiments designed to elucidate the kinetics of the secretion in various segments of the sexual tract are in progress.

Résumé. Chez la souris C_{57}BL, on a démontré par voie histochimique (test de BRACHET) et par spectrophotométrie, qu'une des composantes de la sécrétion de l'épididyme et du canal déférent consiste en RNA. Son excrétion a été mesurée à la suite d'injections d'uridine ^{3}H et d'acide orotique ^{3}H.

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Lipids of Guinea-Pig Alveolar Macrophages

Earlier reports from our laboratory indicated changes in lipid contents during the development of silicosis[1]. Macrophages are unique among cells, being capable of phagocytizing silica and other dusts[2]. Little data are available at present on the various constituent lipids like Ubiquinone (Coenzyme Q) of alveolar macrophages. There is increased incorporation of labelled building blocks into lipids during the process of phagocytosis[3,4]. It will be interesting to know lipid composition of the cells capable of phagocytosis in resting stage. Availability of small number of macrophages for extraction may be one of the limiting factors for characterizing and analyzing various lipid constituents. In the present study we have collected macrophages at various intervals which were freeze-dried and kept in cold at −20 °C until pooled samples were processed.

Isolation of alveolar macrophages. Adult guinea-pig (average weight 500 g) of ITRC colony maintained on standard laboratory diet were used in the present studies. Macrophages were isolated by the modified technique of MAXWELL et al.[5]. Salient features of modification are as follows. The entire trachea and lungs were separated out en bloc and care was taken to avoid puncturing the lungs. The excised organ was washed free from blood in a HANKS[6] balance salt solution. With the aid of a syringe, balance salt solution, about 10–15 ml at a time, was injected via trachea into lungs. The point of insertion of the needle into trachea was held firmly to avoid leakage. The fluid was allowed to run out of the lung keeping it in vertical position and slowly back by sucking into the syringe. The process was repeated a few times. The cells were then centifuged, washed and resuspended in balance salt solution. Total and differential counts were made. Most of the batches contained more than 95% macrophages. Sometimes there was a little contamination of blood, then the entire batch was discarded. Washed cells were then freeze-dried and stored at −20 °C until lipids were extracted.

Freeze-dried cells were extracted with various solvents like *n* hexane, ether and alcohol-acetone mixture in a soxhlet.

Total cholesterol and esterified cholesterol was estimated by the method of WOOTTON[7]. Phospholipids were estimated as a total *P* in the lipids by the method of KING and WOOTTON[7] and reported as lecithin equivalent. Lipids were chromatographed on silicic acid or alumina

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