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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column according to BISHOP, PANDYA and KING[8]. Diglycerides, triglycerides, hydrocarbons and free fatty acids were estimated by the procedures of ELSBACH[9].

Table I gives the gross composition of various lipids. Total lipids were about 7.8% of dry wt. of the cells out of which phospholipids were the major constituents (55–58%). Amount of phospholipids in total lipids of human leukocyte[10] of undifferentiated morphological type is 10% less compared with that of alveolar macrophages, while that of rabbit polymorphonuclear leukocytes[9] phospholipids is slightly more (about 3%) than that of macrophage phospholipids. Free cholesterol content was about 7% of the total lipid content and a similar amount of esterified cholesterol was also present. Results indicate that the composition of alveolar macrophage lipids differ from that of lung lipids[11].

Table I. Lipids of alveolar macrophages

	Dry wt. of cells (%)
Total lipids	7.84 ± 2.6
Phospholipids	54.98 ± 3.10
Neutral lipids	
Cholesterol, free	6.53 ± 1.3
Cholesterol, esterified	7.96 ± 1.5
Triglycerides and free fatty acids	21.18 ± 2.4
Hydro carbons	2.25 ± 0.5
Diglycerides and others	6.39 ± 1.1

The lipids were fractionated by alumina or silica column chromatography[8]. The 4% and 6% ether in light petroleum fraction gave a typical sprectrum of ubiquinone with a peak at 275 nm (in ethanol). On reduction with sodium borohydride, 275 nm peak disappeared and a new peak at 291 nm was emerged. It is of significance to note that about 0.32 μm moles/g dry wt. of ubiquinone was present in the guinea-pig alveolar macrophages (Table II). This indicates that alveolar macrophages may have a well developed oxidative system. In the cells which have well defined mitochondria like leukocytes and alveolar macrophages, presence of ubiquinone is expected but has not been detected or isolated so far. The present report of separation of ubiquinone from alveolar macrophages reinforces the above view-point. The erythrocytes do not contain detectable amount of ubiquinone[12]. Isoprenologue of ubiquinone was identified by thin layer chromatography as described by PANDYA et al.[13]. It was found to contain ubiquinone-10. IR–spectrum of petroleum fraction of the silicic acid column chromatography indicates absorption maxima for the presence of long chain monohydric isoprenoid alcohol (like dolichol or solanesol).

Zusammenfassung. Lipidanalyse von Meerschweinchen-Makrophagen.

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Table II. Ubiquinone (coenzyme Q) content of guinea Pig alveolar macrophages

Dry wt. of tissue (mg)	Method of extraction	Ubiquinone (μmoles/g dry wt.)
1798	*n* hexane	0.31
1144	*n* hexane	0.38
1550	methanol	0.27
953	methanol	0.33

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Nitrogen Pretreatment Period Required for Complete Elimination of the Oxygen Effect in X-rayed *Drosophila* embryos

With nearly all biological test systems with which radiobiological experiments are performed, an oxygen effect is observed. By 'oxygen effect' we mean that irradiation of a test system with a given dose in the presence of oxygen is more efficient than the same dose given in nitrogen, with respect to the amount of damage induced. The maximum enhancement of the radiation response due to oxygen is called the oxygen enhancement ratio (OER). The OER is calculated as the ratio 'effect in the presence of oxygen/effect in the absence of oxygen'.

The anoxic condition is usually achieved by treatment of the cells or organisms with nitrogen. For the complete elimination of the oxygen effect and in consequence for the determination of the OER the length of the pretreatment period with nitrogen needed for a particular test system is critical. For *Drosophila* embryos a pretreatment period of 1 min has been claimed to be sufficient (ULRICH[1], WÜRGLER[2], FINSINGER[3], MATTER[4]). Experimental evidence on which this statement is based has never been published.

We describe here experimental data indicating that as short a time as 15 sec is sufficient to remove all oxygen from the radiosensitive structures of the cells. For our experiments we used 15±5 min old eggs which were

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