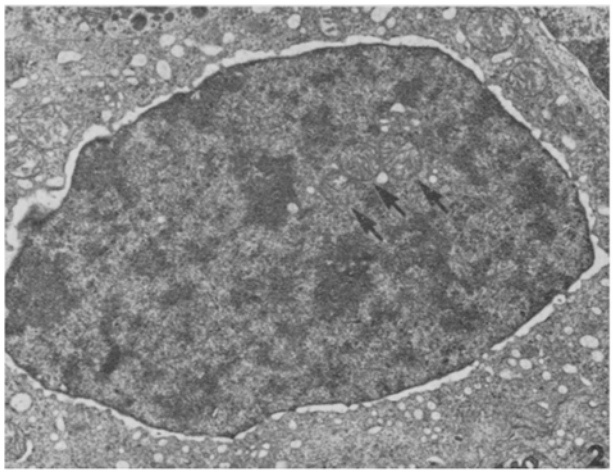

Fig. 2. Cells of the same tumor. A nucleus contains several mitochondria (arrows) and vesicles. The perinuclear space is markedly dilated and the chromatin is not yet reduced to a marginal zone of the nucleus showing that this cell is at early interphase. In the cytoplasm mitochondria and numerous vesicles, the same found in the nucleus, are scattered. ×12,790.

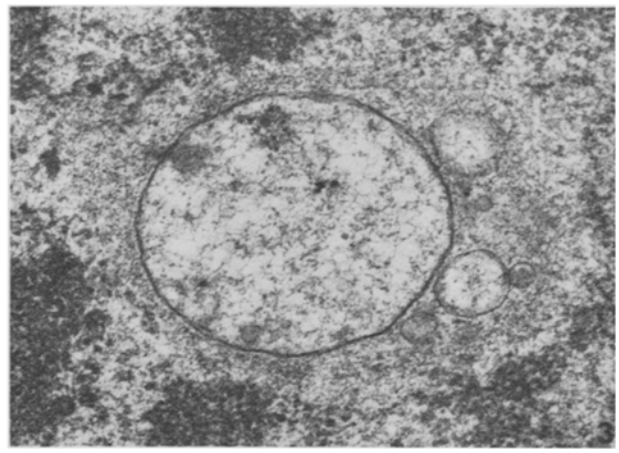

Fig. 3. A swollen and altered intranuclear mitochondria with the single thicker membrane and matrix areas of lesser density, containing prominent fine filamentous components and opaque aggregates. Such a mitochondrion is always separated by a halo area of medium density from the nucleoplasm. ×59,680.

ation, or inclusion within the nuclear envelope at ana-telophase may have been induced. We found these structures mostly in the cells at early interphase. Furthermore, an evidence of seizing of the mitochondria by a rapid and abnormal fusion of the numerous, comparatively large vesicles, in which some of them were adhering to the coalescent chromosomal masses and mitochondria, during the course of the reformation of the nuclear envelope[8] at ana-telophase, was obtained (Figure 1). We, therefore, emphasize the importance of the seizing at ana-telophase as the most likely mechanism. Observation of sequential alterations of the intranuclear mitochondria shows that mitochondria can survive for several hours, and finally go into disintegration, in the abnormal condition of the nucleus, in the resting cell stage. The features of the altered mitochondria closely resemble those of yeast mitochondria under anaerobic conditions and of mitochondria from rapidly growing root tip of the broad bean, *Vicia faba*[2]. It is especially interesting to note that in these cells the mitochondria have prominent DNA filaments. The presence of the intranuclear mitochondria and their possession of a genetic material, DNA, may influence some of the properties of cancer cells[9].

Zusammenfassung. In Kernen transplantierter Tumorzellen, die aus adrenocortikalem Carcinom des syrischen Goldhamsters stammen, wurden häufig Mitochondrien und kleine Bläschen festgestellt. Diese Strukturen wurden in Anatelophase von Kernen umgeben und innerhalb eines Zellenzyklus werden die eingekreisten Mitochondrien allmählich im Kern aufgelöst.

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In Vitro Fertilization of the Mongolian Gerbil Egg

Heated bovine follicular fluid (BFF) is reported[1-3] to endow epididymal spermatozoa from mice and hamsters with the capacity to penetrate homologous eggs in vitro. This success prompted an attempt to achieve in vitro capacitation and fertilization for Mongolian gerbil gametes in the presence of BFF; unusually high irradiation resistance, exhibited even by the early zygote, makes this rodent a subject of particular experimental interest[4,5].

Mature female Mongolian Gerbils were superovulated by injection of 20–40 IU PMS (Equinex, Ayerst) and 48–52 h later 10 IU HCG (APL, Ayerst). Eggs were recovered from the oviduct 13–15 h after HCG injection in the manner described by YANAGIMACHI and CHANG[6]. A sperm suspension was prepared by cutting the cauda epididymis and pressing out the spermatozoa in a watch-glass, containing 0.5 ml of Tyrode's solution; there were $15–20 \times 10^6$ sperm cells/ml in this suspension. BFF was collected by means of a sterile syringe from large follicles in ovaries from freshly slaughtered cows. The fluid was centrifuged at 2,500 rpm ($800 \times g$) for 15 min and stored at −4 °C. Before incubation BFF was heated as described

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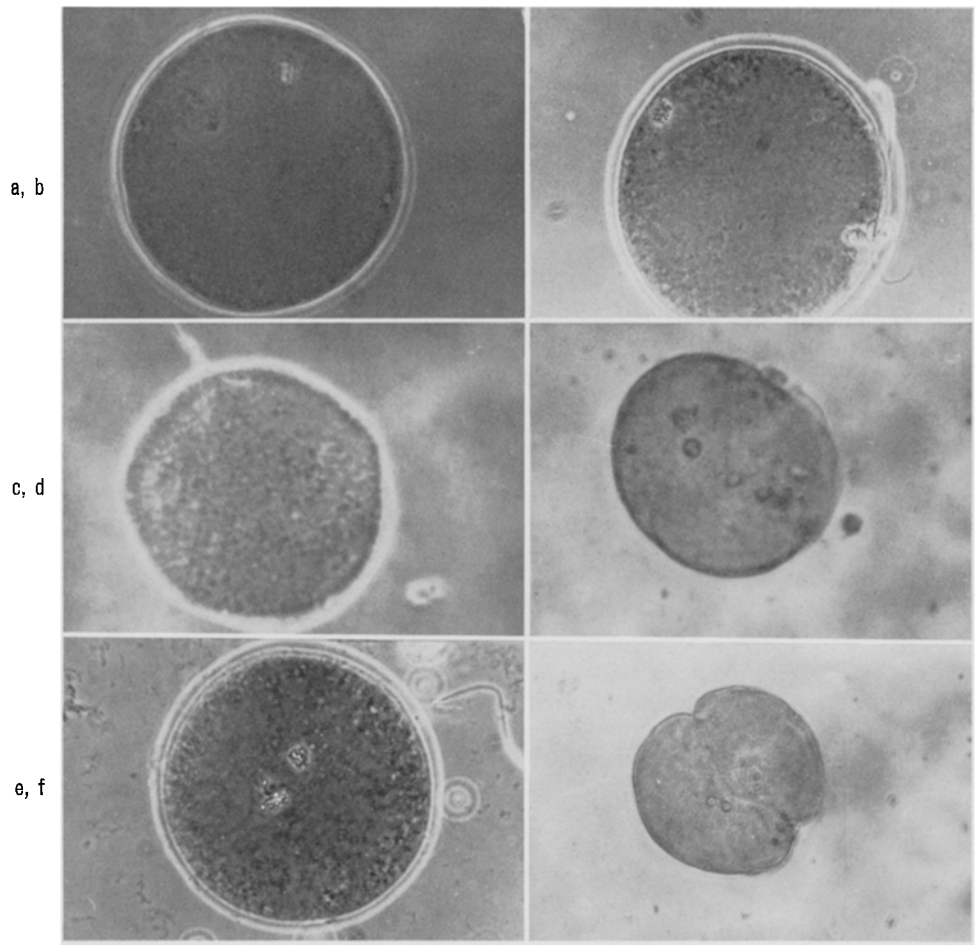

Different stages of in vitro fertilized Mongolian gerbil eggs. a) Unfertilized egg; approximately × 400. b) Sperm entering the zona pellucida; approx. × 400. c) Spermatozoon in perivitelline space; approx. × 320. d) Emission of the second polar body; approx × 320. e) Two sets of chromatin present in the egg; approx. ×400. f) Firstcleavage stage; approx. ×320.

Fertilization of Mongolian gerbil eggs in the presence of bovine follicular fluid

Time after insemination (h)	Culture media	Eggs		Stage of penetrated eggs (%)		
		Total No. examined	Penetrated (%)	Z.p. penetrated	PVM penetrated	PN
0–3	BFF+					
	Tyr.	35	29	90	0	10
	Tyr.	12	0	0	0	0
4–6	BFF+					
	Tyr.	35	54	37	53	10
	Tyr.	10	40	50	50	0
7–9	BFF+					
	Tyr.	36	14	60	20	20
	Tyr.	28	4	3	0	0
Total	BFF+					
	Tyr.	106	32[a]			
	Tyr.	50	10			

[a] Statistical analysis significant $0.01 > P > 0.005$; $\chi^2 = 7.69$.

by YANAGIMACHI[3]. 10–50 µl heated BFF was used to incubate freshly collected eggs. An equal volume of the sperm suspension was added to this preparation. Tyrode's solution replaced BFF in the control medium. The fluid containing eggs and spermatozoa was covered with heavy mineral oil (Squibb) and incubated at 37 °C in an atmosphere of 5% CO_2 in air. Incubation lasted for 1–9 h. After incubation the eggs were recovered, mounted on a wax-spot-slide, fixed in buffered formalin and stained with lacmoid[7,8]. Eggs considered to be penetrated had spermatozoa in the zona pellucida, the vitellus, or had formed 2 pronuclei.

A total of 156 Mongolian Gerbil eggs were examined in this experiment. The Figure shows various stages of penetration and fertilization found among ova penetrated by spermatozoa in the present in vitro system. An unpenetrated egg is shown in Figure a, and penetration of the zona pellucida by a spermatozoon can be seen in Figure b. The vitellus has been penetrated and the second polar body extruded in the ova shown in Figure c and d, respectively. Figure e shows an egg with 2 sets of chromatin and a cleaved egg can be observed in Figure f.

Eggs incubated in BFF + Tyrode's showed a penetration rate of 32% (Table). Whereas incubation in Tyrode's alone was associated with a 10% penetration rate. Clearly, BFF has enhanced capacitation of epididymal spermatozoa of the Mongolian gerbil under in vitro conditions. The best penetration rate in BFF + Tyrode's was after 4–6 h incubation; 54% of the eggs were penetrated. Significant levels of ova penetration by spermatozoa were achieved in Tyrode's alone. This suggests that sperm capacitation can also occur in certain inorganic solvents in vitro. Eggs examined in the first 2 h of incubation revealed that no spermatozoa had become attached or had penetrated the zona pellucida in either Tyrode's + BFF or Tyrode's alone. However, as shown in the Table, after 3 h incubation 29% of the eggs in BFF + Tyrode's had been penetrated; 90% of the penetrated eggs had spermatozoa in the zona pellucida, and the remainder had advanced to the pronuclear stage. In contrast, it may be seen that in Tyrode's solution alone no eggs were penetrated at this time. This suggests that epididymal spermatozoa of the Mongolian gerbil require about 2–3 h to capacitate in vitro in the presence of BFF. In Tyrode's solution alone this process seems to take longer. YANAGIMACHI[3] reported that hamster spermatozoa capacitate in 2–4 h with BFF. Between 4–6 h of incubation in BFF + Tyrode's, half the penetrated eggs had spermatozoa in the perivitelline space or in the vitellus and about 10% of them had formed pronuclei. In Tyrode's medium, no pronuclear eggs could be found and this is consistant with a slower rate of spermatozoa capacitation in this medium. The percentage of eggs in the pronuclear stage doubles between 6–9 h of incubation in BFF + Tyrode's.

According to MARSTON and CHANG[9], in vivo penetration of the Mongolian gerbil egg begins approximately 1 h after ovulation. The first pronuclear eggs were found 2–3 h after ovulation, that is 1–2 h after fertilization. This agrees reasonably with the time course described for BFF + Tyrode's medium. Between 20–23 h after ovulation the first cleavage occurs in vivo and this accounts for the fact that only 1 cleaved egg was found out of 39 penetrated eggs in this study, where incubation did not extend beyond 9 h. Possibly longer incubation periods would yield more cleaved eggs, since it appears cleavage is possible in these eggs under in vitro conditions.

Zusammenfassung. Ova von mongolischen Wüstenmäusen *Meriones unguiculatus* (Gerbillinae) wurden mit Spermatozoa aus der Epididymis in Follikelflüssigkeit von Kühen zusammengebracht. Es konnte gezeigt werden, dass die Spermatozoen während der Inkubation in diesem Medium die Fähigkeit erlangen, in die Ova einzudringen.

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Further Studies on Endothelial Cells of Vertebrates and the Problem of Endothelial Granules

The vascular endothelium of common laboratory animals has been the object of several fine-structural studies[1–5], but less attention has been given to the cytoplasmic granules of endothelial cells[6–8]; little work was devoted to comparative studies in this field[9]. The fine structure of endothelial cells of 17 species was described by us in a former paper[9]. In that study it was shown that endothelial granules are widely but not uniformly distributed among vertebrates. Differences were found when the number of granules in the cytoplasm of endothelial cells of lower vertebrates was compared with that of higher vertebrates. These bodies were scarce and even absent in the endothelium of the latter.

In the present extension of our observations to the bat, seal and penguin, we were interested in knowing whether the special adaptive characters represented by these species are accompanied by modifications of the presence and frequency of endothelial granules. Observation on vertebrates which, thanks to their specialization and adaptation, live in an environment uncommon for most species of their class, is of particular interest in this line of research.

The fine structure of the aorta endothelial cells of bat, seal and penguin is described in Figures 1 to 3. Adult Weddel seals (*Leptonichotes weddelli*) and '*adelie*' penguins (*Pygoscelis adelie*) of both sexes were used. These animals were caught at the Base Esperanza (Hope bay) rockery (lat. 63° 23′ S., long. 59° 59′ W.) in the Antarctic peninsula.

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