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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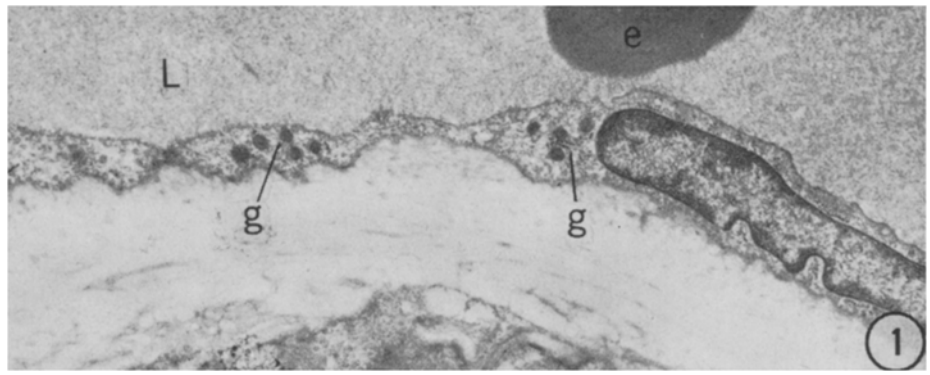

Fig. 1. Endothelium of bat aorta. The thin layer of cytoplasm presents scarce dense bodies of spherical or ovoid form as well as vacuoles of variable size. Vacuoles protuding into the lumen of the vessel were occasionally observed. e, erythrocyte; g, cytoplasmic granules; L, lumen. ×10,000.

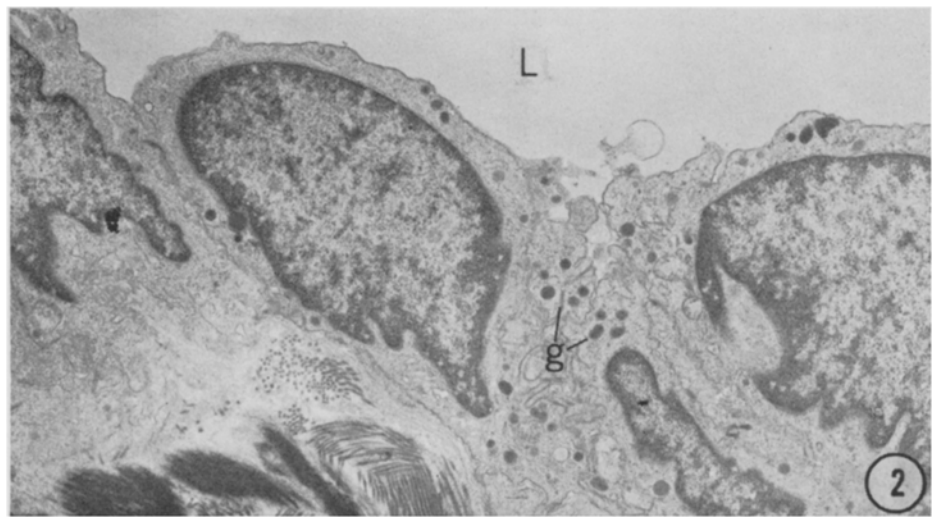

Fig. 2. Endothelium of Weddell seal aorta. The cytoplasm has a poorly developed endoplasmic reticulum and abundant vesicles. Scarce spherical or avoid granules are distributed in the cytoplasm (0.2–0.4 ∅). They are dense and surrounded by a membrane. g, cytoplasmic granules; L, lumen. ×10,000.

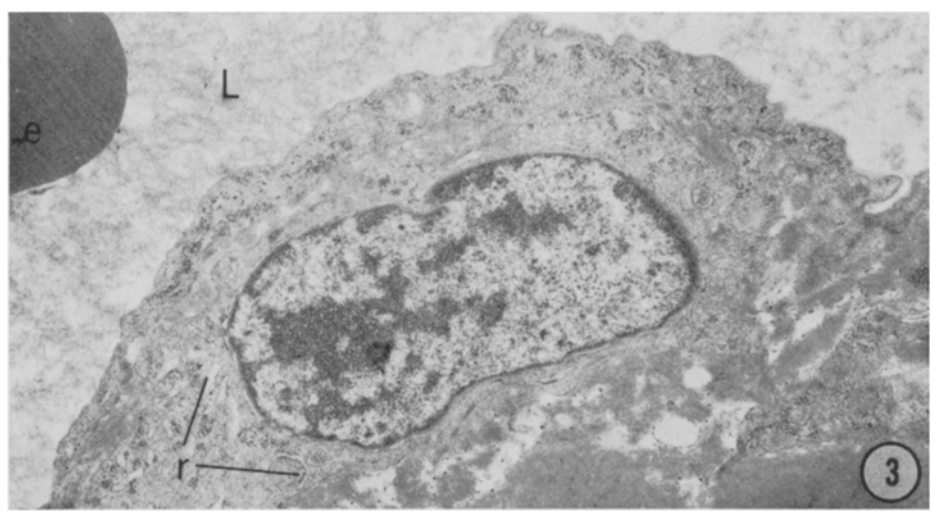

Fig. 3. Endothelium of penguin aorta. In the cytoplasm of these endothelial cells no dense granules were observed. The granular reticulum is well developed. The cytoplasmic vacuoles are abundant. As is the case for bat and seal, the segments of the vessel studied were abdominal. e ,erythrocyte; L, lumen; r, endoplasmic reticulum. ×10,000.

Presence of endothelial granules in the aorta of vertebrates

Class	No. of species studied	No. of species in which the granules are absent and percentage	Estimation of granules frequency in cytoplasm	Authors
Birds	4	3 (75%)	low	SANTOLAYA and BERTINI[9]
Mammals	10	1 (10%)	low	WEIBEL and PALADE[6]; LEMEUNIER, BURRI and WEIBEL[10] SANTOLAYA and BERTINI[9]
Reptiles, Amphibians, Fishes	11	0 (0%)	high	SANTOLAYA and BERTINI[9], STEINSIEPE and WEIBEL[8]

The data were prepared by pooling the results of present and previous observations made by us and other authors

The bats (*Myotis chiloensis*) were captured in the proximity of the city.

The species studied present, inspite of their peculiar evolutionary characteristics, a picture similar to that found by us or other authors in most species of the same class, as regards the presence and frequency of endothelial granules. In fact these bodies are absent in penguin, and they are found in a low number in bat and seal. The endothelial granules are absent as well in birds other than penguin, such as chick (*Gallus domesticus*) and pigeon (*Columba livia*). These 3 birds differ widely with respect to environment, flight and feeding habits; one of them underwent cross breeding and selection by man. Nevertheless, these species have in common the lack of such granules. The results of our former and present observations on endothelial granules (abdominal aorta) together with those of other authors, are shown in the Table. It appears that none of the 11 cold-blooded species gave a picture similar to that found in birds or mammals. Only one of the 10 mammals studied did not present endothelial granules. These structures were absent in 3 of the 4 birds studied.

Certainly more species must be investigated to reach a reliable conclusion; however it seems at present that endothelial granules tend to disappear in birds, their frequency is much lower in mammals than in reptiles, amphibians and fish. It is therefore tempting to consider the granules as a fine-structural aspect of evolution in vertebrates. Anyway, the data of the Table strongly suggest that important changes happened in endothelial cells through the evolutionary steps that produced warm-blooded animals[10,11].

Resumen. Se describe la ultraestructura de células endoteliales de aorta abdominal de murciélago, foca y pinguino y se discuten los resultados en función de estudios precedentes nuestros y de otros autores sobre esas células en diferentes especies. La presencia, o ausencia, y frequencia de los gránulos citoplasmáticos en las células endoteliales en las especies estudiadas parecería la expressión de un proceso evolutivo a nivel de clases.

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Release of a Cytotoxic Factor by Murine Spleen Cells in the Presence of a Tumour Allograft

The immune response is a complex phenomenon involving cellular differentiation which results in a serological immunity and in a cell-mediated immunity which play a part in numerous defence mechanisms of the organism and, in particular, in graft rejection.

During the past few years many reports have indicated the importance of soluble mediators, especially lymphotoxins, in cell-based immunity. These lymphotoxins are liberated by the secondary stimulation in vitro of lymphoid cells sensitized against molecular antigens such as tuberculin or B.G.G.[1-5] or against cellular antigens[6].

Using double compartmented diffusion chambers we have demonstrated that the spleen cells of mice which have rejected a tumour-allograft release a large amount of a soluble factor when re-exposed to the antigen. This soluble factor is cytotoxic[7] and non-specific.

On the other hand, it is known that a tumour allograft may be accepted either temporarily or permanently. In this situation it is relevant to ask whether production of the cytotoxic factor is continued, reduced or completely suppressed. In order to study this problem we have used a specific tumour from one strain of mice which, when grafted into a different strain, takes successfully in a certain number of cases.

Material and Method. The tumour concerned is a rhabdomyosarcoma induced in C3H mice by methylcholanthrene and used between the 63rd and 72nd generation

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