These results should be considered with respect to the present state of knowledge about substances inhibiting proliferation and differentiation of CFC-c. It is known that inhibitors of CSF biosynthesis and chalones of granulopoiesis are produced by mature granulocytes and have been found only in these cells[1]. Up to now, in serum, only the presence of an unspecific lipoprotein inhibitor of CSF has been generally confirmed[5–7].

The experimental system used here precludes any inhibiting action at the level of CSF biosynthesis, and any inhibitors found cannot be described as lactoferrin from granulocytes. The fact of the disappearance of peak I in the serum after chloroform extraction, and its molecular weight (200,000), confirmed the lipoprotein structure of this inhibitory activity[8]. In this situation, I suggest that the inhibitory activity of peaks II and III is due to low molecular weight substances (13,000; 7000) which can act as a polypeptide chalones[2,9,10].

The action of these polypeptides on a GM colony may be unspecific, and we should also take into consideration the fact that human serum was tested on mouse target bone marrow cells and species-differences may be responsible for some of the phenomena. Therefore, the most probable explanation is the releasing of these polypeptides from mature granulocytes into the bloodstream, as specific inhibitors of granulopoiesis.

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Surface topography of granulosa cells accompanied by fragmented oocytes

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Summary. Scanning electron microscopy of granulosa cells (GC) and granulosa cell-like structures (GCLS) revealed that both had lacy foldings, or plicae, on the surface and were identical. The plicae did not always cover the entire surface of GC or GCLS. Both structures were interconnected by multivalent processes.

Granulosa cells surrounding an oocyte extend processes through the zona pellucida to the oocyte, forming gap junctions[2], and via this connection granulosa cells and oocyte continue to interact during their growth and maturation[3–5]. However, when an oocyte shows fragmentation, which is defined as a sign of degeneration[6] leading to elimination, granulosa cells and the processes exhibit concomitant morphological changes. They become larger in size and no longer have a uniformly round shape. The cytoplasm contains more coarse granules and vacuole formation is prominent. Often extremely thick processes of granulosa cells (up to several microns) are observed in photomicroscopy. Granulosa cells often invade the perivitelline space, and on the surface of fragmented oocytes a structure similar to a granulosa cell is observed. Shinohara and Matsuda[7] suggested the possibility that granulosa cells outside the zona pellucida transfer their cytoplasm into the tips of the processes and thus develop the granulosa cell-like structure and ultimately change their position and more into the perivitelline space.

Using scanning electron microscopy, the present research describes the surface morphology of granulosa cells and thickened processes accompanied by fragmented oocytes, which have been described as above on the basis of light microscopy.

Materials and methods. Female Wistar strain rats, aged between 8 and 12 weeks, were used. They were kept in an air-conditioned room at 24 °C and exposed to light from 07.00 h to 19.00 h. Only animals showing regular 4- or 5-day sexual periodicity were supplied for the experiment, without regard to estrus cycles. Bilateral ovaries were extirpated under ether anesthesia and the surrounding connective tissues and blood were removed on a clean filter paper. In a plastic dish containing cold 0.9% NaCl the ovaries were randomly punctured in order to liberate the ovarian oocytes. Under a stereoscopic microscope, fragmented oocytes were selectively collected by a mouth-controlled micropipet, and fixed in 2.0% glutaraldehyde in 0.05 M Sorensen's phosphate buffer solution (SPBS), pH 7.4, for 2 h. After a brief rinse in 0.15 M SPBS, the zona pellucida of the oocytes was removed in 0.15 M SPBS containing 0.1% trypsin, at 25 °C, without separation of the granulosa cells from the oocytes. Usually 3–6 min of the enzymic treatment was sufficient for obtaining zona-free and granulosa cell-attached oocytes. Rinsing again, the oocytes were attached to a coverslip coated with 0.2% poly-L-lysine[8]. Following dehydration in a graded series of ethanol-distilled water solutions from 30 to 100%, the specimen was infiltrated with isoamyl acetate. The oocytes were critically point-dried in liquid-gaseous CO_2, and gold sputter-coated about 300 Å in thickness. A scanning electron microscope (Super 3A: ISI-Akashi) was used for observation of the surface topography.

Results. Granulosa cells (GC) outside the zona pellucida, and granulosa cell like structures (GCLS) in the perivitelline space had a common surface architecture; lacy foldings, or coarse plicae, against a relatively smooth background surface. Occasionally, GCLS in the perivitelline

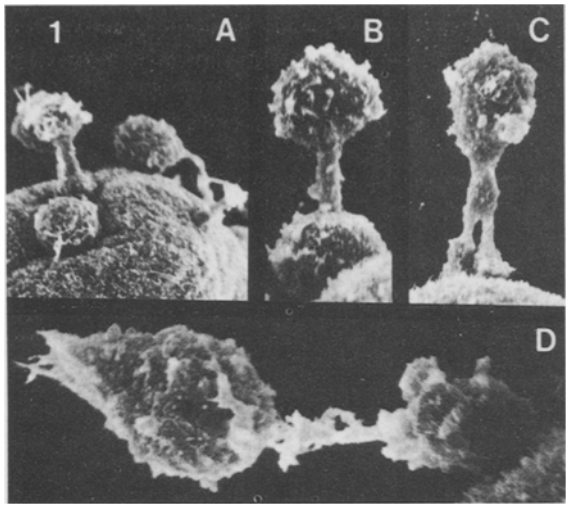

Figure 1. Four different types of GC and GCLS. All had, more or less, plicae on the surface. A: A GC which broked if it was intruding into the oocyte surface (×1800). B: A GC and GCLS were connected by a thickened process (×2200). C: A process bifurcated at the midpoint between a GC and GCLS (×2100). D: A GC with 'flaming' configuration (×4100).

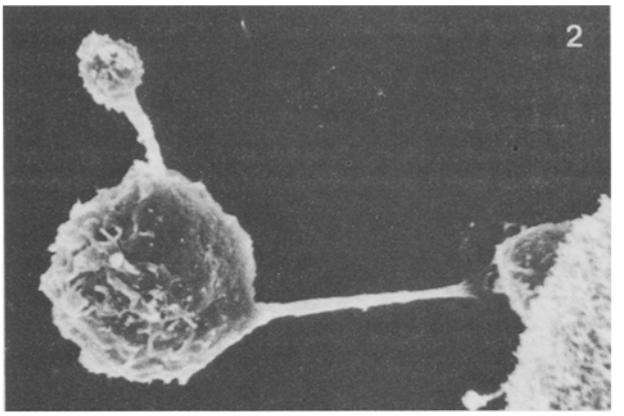

Figure 2. A GC with multivalent process. In SEM, multivalent processes, which are not observable in photomicroscopy, were not unusual. It is hardly possible to discriminate GC from GCLS only by the surfaces. Note that the plicae and plicae-free surfaces from which the processes extend coexist on the surface (×4200).

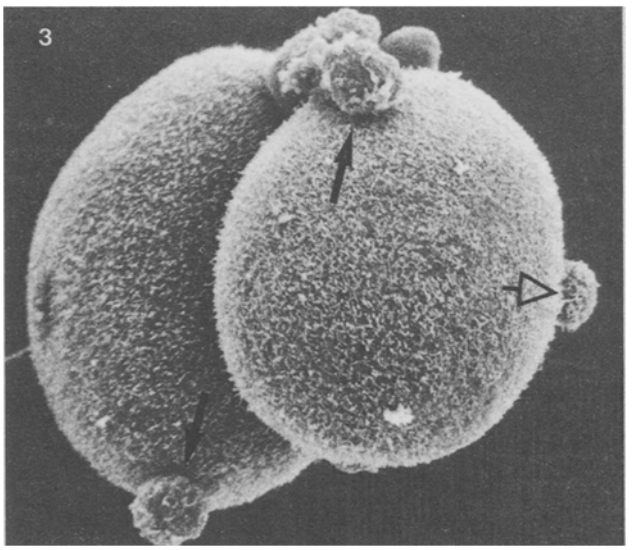

Figure 3. A fragmented oocyte. It is not difficult to distinguish a GC in the perivitelline space from a small fragmented oocyte of about the same size. The cells indicated by closed arrows are granulosa cells and that indicated by an open arrow is a small fragment, judging from the surface morphology, plicae and microvilli (×2236).

space looked as if they were intruding into an oocyte fragment (fig. 1). Preservation of a long and thread-like process of a GC during dehydration was very difficult, but, as is shown in figure 2, it was not unusual for a GC to have multivalent processes. In other words, observation of 3 interconnected GC and/or GCLS was not infrequent. Plicae on the GC surface disappeared at the region from which a process extended, and those on GCLS showed a clear contrast to microvilli covering the surfaces of fragmented oocytes.

Discussions. Granulosa cells clinging to a fragmented oocyte are considered to be degenerating, and indeed, vacuole formation, cytoplasmic condensation and other signs of degeneration were usually observed. However, these signs do not necessarily mean that the cells are functionally deceased. They alter in cell shape and surface architecture and form GCLS. As for the plicae on the surface, the regions from which thickened GC processes extended often had fewer plicae, or were entirely free of them. Surface structures in oocytes[9,10] as well as in other cells[11] change remarkably by reorganization of peripheral membrane components such as actin, myosin and tubulin together with cytokinesis. Degeneration of GC, therefore, may include a process of reorganization of these components in the rooting region of the GC process.

Intercellular communication in cultured granulosa cells obtained from immature rats was evidenced by the presence of gap junctions between the cells[12]. It is interesting, in vivo, that the granulosa cell processes of mature rats can be multivalent. In addition to 'vertical' communications between an oocyte and the accompanying GC, 'horizontal' communications among granulosa cells via the process were considered to exist, although it is not clear whether they form gap junctions, as was found in immature rats, or not.

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