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The Role of Temperature in the Crystallization of Ribosomes in Chick Embryos

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The relationship between ribosome crystallization and cell degeneration has been studied in chick embryos at various temperatures, and new methods of inducing ribosome microcrystals are described. A model is discussed that reinterprets the role of low temperatures in these phenomena and provides a unitary explanation of the various cases in which the occurrence of ribosome crystallization in chick embryos has been reported.

Key words: ribosomes, crystallization, hypothermia, chick embryos, degeneration, cell suffering

Since the discovery by Byers [1, 2] that cooling produces the formation of ribosome microcrystals in chick embryos, the incubation of embryonated eggs at low temperatures has become the most widely used method of inducing ribosome crystallization.

The microcrystals disappear when the cells are rewarmed at 38°C, and it has therefore been assumed that low temperatures play a critical role in the phenomenon. This conclusion has been strengthened by the discovery that ribosome microcrystals of the same symmetry group occur also in lizard oocytes [3] because it has been recognized [4] that even in these systems the phenomenon is critically dependent upon hypothermic conditions.

In a number of cases identical ribosome crystals were reported to occur spontaneously at 38°C [5–10], but these findings were seen as exceptions, because crystallization occurred only in the dying cells of a few specialized tissues. These reports, therefore, did not seem to challenge the assumption that low temperatures play a critical role in ribosome crystallization, and a discrepancy appears to exist between the cases in which the crystals are induced by cooling and those in which they are induced by degeneration processes [6]. The starting point of this study was the supicion that this discrepancy could, after all, be resolved.

The possibility exists, in fact, that in the hypothermic treatments it is not the physical effect of temperature that plays a critical role but the fact that low temperatures induce cell suffering and this, in turn, promotes physiologic responses that lead to crystallization as they do in the degeneration cases. To test this possibility experiments on ribosome crystallization were devised in which the respective roles of cooling and cell suffering could be clearly evaluated.

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METHODS

Electron Microscopy

Tissues samples were fixed in 0.1 M sodium cacodylate buffer (pH 7.6) containing 2.5% glutaraldehyde for 6–8 h at 4°C, rinsed in buffer for 12–16 at 4°C, postfixed in 1% osmium tetroxide at room temperature for 1 h, dehydrated in an acetone dilution series, and finally embedded in Epon. Sections were cut on a Reichert ultramicrotome, stained with uranyl acetate and lead citrate at room temperature, and observed under a Siemens Elmiskop 1A electron microscope.

The morphologic analysis was standardized by removing prefixed parts (usually the tail and the limbs) from the embryos, by examining sections from at least two different parts of each organ, and by scanning sections that covered at least 50 holes of 300-mesh grids per case. When necessary, the study was made nearly quantitative by adopting the following procedure.

The square region of the hole of a 300-mesh grid was assumed as a unitary area of which serial photographs were taken at magnifications 5,000, their enlarged prints being composed together to give a 1-m^2 picture of each area. The pictures were then used to count the percentage of cells where microcrystals appear, the average number of microcrystals per cell, and the average dimensions of the microcrystals.

RESULTS

Four groups of experiments were performed and each of them will be described separately in the following sections.

Hypothermic Treatments at 20°C

The first aim of the study was to find a range of subphysiologic temperatures at which ribosome crystallization is not induced in untreated chick embryos for long periods of time. This was determined by removing from the incubator 4-day-old embryonated eggs and by simply keeping them at room temperature (which varied between 18°C and 22°C) for any period of time between one and five days. Samples were removed and prepared for electron microscopy every 24 h but no trace of ribosome crystallization was ever found in any tissue. This provided a convenient reference system because if treatments were to be found that induced ribosome crystallization in all tissues at room temperature, the conclusion could be drawn that temperature had not played the crucial role in the crystallization process.

Degeneration Treatments at 20°C

Cell degeneration was induced at ∿20°C, without opening the eggs, in two ways. In one case 4-day-old embryonated eggs were immersed in water and kept there at room temperature for 24, 48, 60, and 100 h (water treatment). In the other case eggs were transferred from the incubator to a freezer at −80°C for 30 min, after which they were removed and left at room temperature for the same periods of time as in the previous case (shock treatment).

In sharp contrast with the complete absence of ribosome crystals in the controls, both treatments produce extensive crystallization not only in the cytoplasms (Figs. 1 and 2) but also in the nuclei (Figs. 3 and 4) and the phenomenon was not limited to specific cells but occurred in all examined tissues.

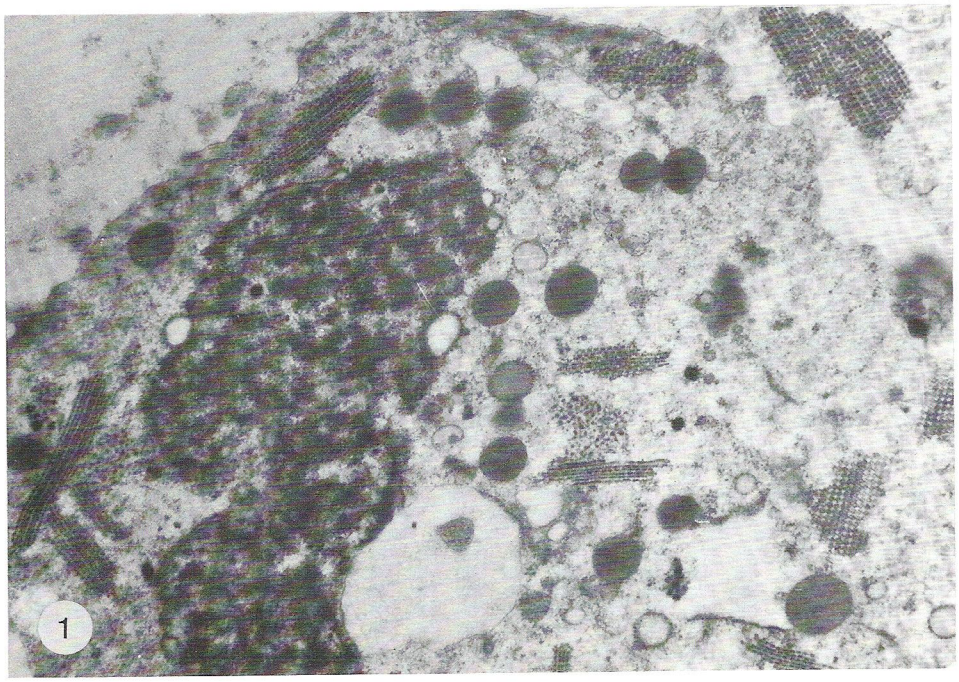

Fig. 1. Ribosome crystallization in the cytoplasm of chick embryo cells after 48 h of water treatment at ∿20°C. ×10,800.

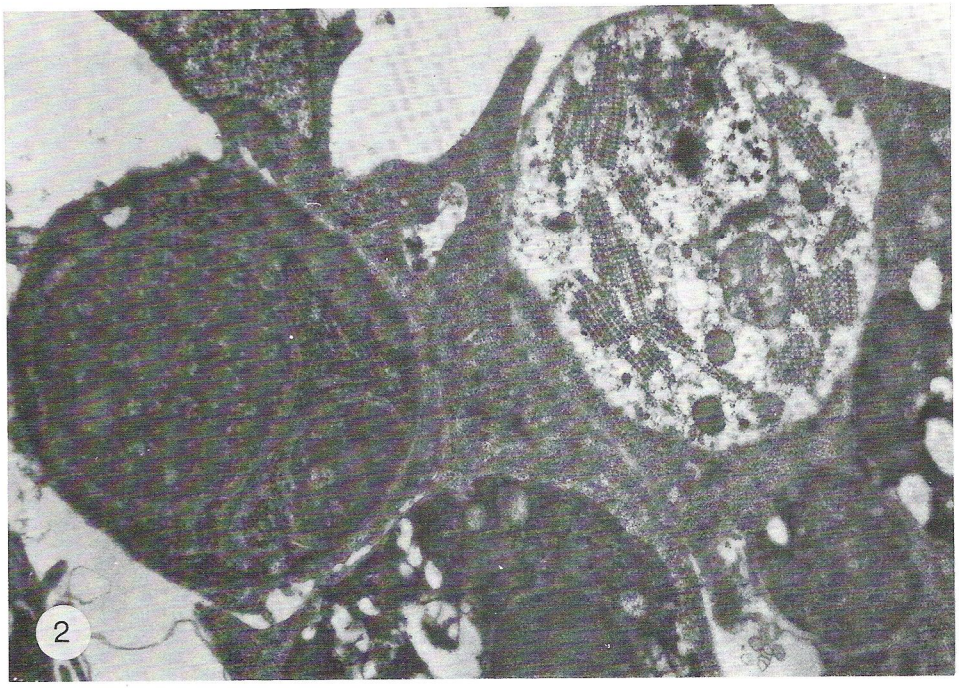

Fig. 2. Ribosome crystallization in the cytoplasm of chick embryo cells after 48 h of shock treatment at ∿20°C. × 10,800.

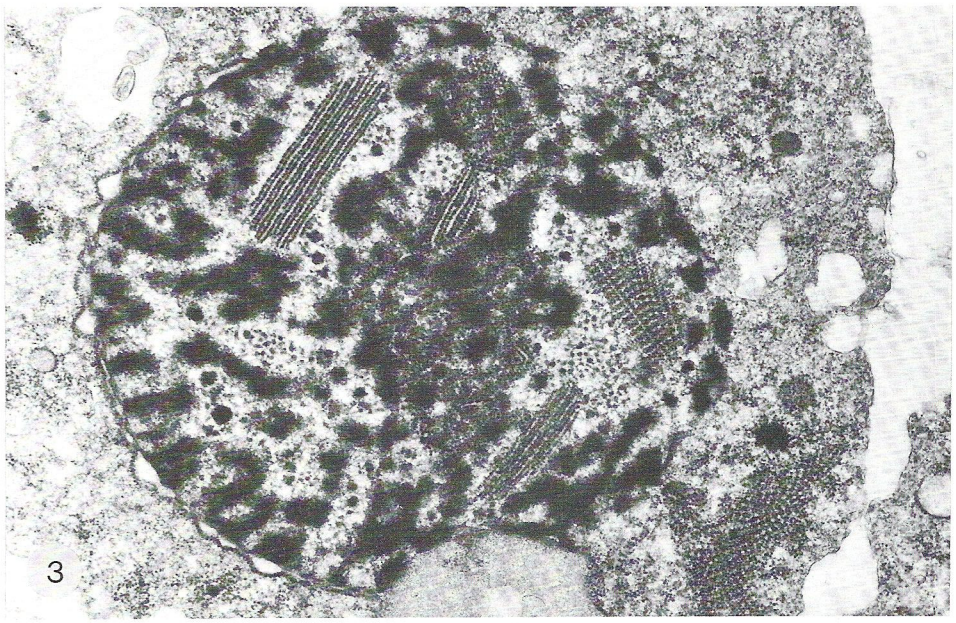

Fig. 3. Ribosome crystallization in nuclei of chick embryos after 60 h of water treatment at ∿20°C. × 19,800.

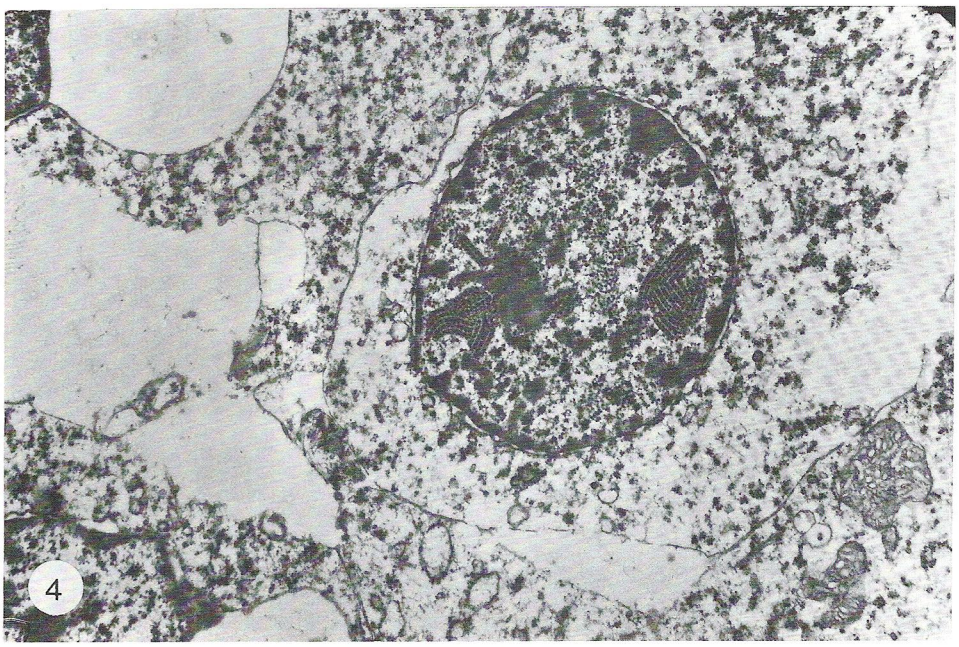

Fig. 4. Ribosome crystallization in nuclei of chick embryos after 60 h of shock treatment at ∿20°C. × 10,800.

The treatments appeared to differ in that numerous crystals were observed even after 24 h of shock treatment, while the water treatment produced similar results only after 48 h or more. In both cases, however, the number of cytoplasmic and nuclear crystals increased with time, despite the fact that the progressive effects of degeneration were often so devastating as to induce a complete disintegration of the cell structures (Fig. 4).

Until now the generalized formation of ribosome microcrystals in *all* tissues of chick embryos was obtained only by hypothermic treatments at 4°C or 10°C [1, 2], but the above results show that cell degeneration at 20°C provides a highly efficient alternative method of inducing crystallization.

Degeneration Treatments at 4°C

The previous results have indicated that cell degeneration at subphysiologic temperatures, and cooling at 4°C, are treatments that induce extensive ribosome cyrstallization in all tissues of chick embryos, and it was of interest, therefore, to see what happens when both are applied simultaneously. This was done by using either the water treatment or the shock treatment at 4°C for the same periods of time as were used in the previous cases at room temperatures.

The results indicated that the combination of either treatment with cooling at 4°C does not increase the formation of ribosome crystals but produces instead a net decrease of it in all examined tissues: The number of cells that contain crystals and the average number of crystals per cell correspond to decreases of 30% to 60% in respect to the corresponding cases at 20°C.

Degeneration Treatments at 38°C

When either water treatments or shock treatments were performed at 38°C, for times that ranged from a few to several hours, no sign of ribosome crystallization was ever found in any cell of all the examined tissues.

DISCUSSION

The main conclusion of the present study is that cell suffering and degeneration at 20°C are highly effective ways of inducing ribosome crystallization in chick embryos. Furthermore, their effect cannot be attributed to the temperature because in untreated embryos crystallization is totally absent. This leads us to reconsider the role of temperature even in the classic hypothermic experiments described by Byers, and the hypothesis proposed here is that intracellular ribosome crystallization is produced in all the reported cases by a physiologic response to cell suffering.

The basic experimental result to which attention is drawn is the fact that when ribosome crystallization is studied as a function of the temperature, the patterns obtained in degenerating tissues and in healthy ones are opposite: In the first case ribosome crystallization is greater at 20°C than at 4°C, while in the second case the reverse is true. These contrasting behaviors would be inexplicable if it were assumed that subphysiologic temperatures merely have the physical role of favoring the aggregation of ribosomes through their kinematic effects, whereas the hypothesis proposed provides a natural explanation.

According to this hypothesis, in fact, ribosome crystallization in degenerating systems is greater at 20°C than at 4°C because the effects of degeneration are more pronounced at higher temperatures, while in healthy embryos the reverse is true because in these systems the lower the temperature the greater the degree of cell suffering that stimulates crystallization.

Further results that help clarify the role of temperature come from the degeneration experiments at 38°C. Since ribosome crystallization increases in degenerating tissues when the temperature of the treatment rises from 4°C to 20°C, one may be tempted to conclude that the same treatments at 38°C should produce an even more pronounced increase in crystallization, but the evidence shows that this is not the case. The results of this study confirm instead the previous suggestion made by Mottet and Hammar [10] and O'Connor and Wyttenbach [7] that at 38°C ribosome crystals are not normally formed in dying cells and occur only in a restricted group of specialized tissues that are undergoing programmed processes of cell necrosis. Therefore, temperature does not have a linear or monotonic influence on ribosome crystallization even within the class of cells that are degenerating, and its effects appear, once again, to have not a kinematic but a physiologic basis.

A possible explanation for the results observed was proposed in a previous report [11], when it was assumed that ribosomes are crystallizable only in a transient period of their life cycle, and at 38°C they do not normally form crystals because they do not remain in this intermediate state long enough.

Whatever the true explanation, this study concludes that the whole body of experimental results on ribosome crystallization can scarcely be interpreted by attributing a merely kinematic role to temperature. Temperature appears, instead, to be important from a physiologic viewpoint as a factor influencing the subcellular responses to a wide variety of conditions that produce cell suffering by altering, arresting, or damaging the normal development of the embryos and that are considered the real cause of ribosome crystallization in all the cases reported.

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