mucosa did not undergo a deciduous reaction and the embryo failed to develop trophoblastic giant cells in lead-treated mice. Nevertheless, as long as our knowledge of the complicated interrelations between mother and embryo remains fragmentary, it is difficult to decide whether the hormonal changes reflect only the non-implantation of a insufficiently developed embryo or are the cause of non-implantation. Indeed, the embryo, although developed to a normal blastocyst at the time of implantation[3], is somewhat smaller after lead treatment and its early divisions had been retarded[4]. More studies on the activation of the embryo and on the possible reversal of the damage to implantation by hormonal treatment will be needed to decide this question. Nevertheless, we presently favour the principal role of the mother in the early effects of lead on pregnancy, because those few lead-treated mothers capable of implanting embryos have a normal litter size, and because the hormonal changes described arise already on day 5 when the activation of the embryos is just beginning.

Effect of actinomycin D on the quail oocyte nucleolus during meiotic prophase I

A. M. Vagner-Capodano, M. H. Pinna-Delgrossi and A. Stahl[1]

Laboratoire d'Histologie et Embryologie II, Faculté de Médecine, 27, boulevard Jean-Moulin, F-13385 Marseille Cédex 4 (France), 18 March 1977

Summary. Actinomycine D alters profoundly the distribution of the nucleolar constituents in the quail oocyte at prophase I of meiosis. As a consequence of nucleolar segregation, the normally existing relations between the nucleolus fibrillar centers and the microchromosomes are ruptured. The relations between the fibrillar center and the dense fibrills which surround it remain intact, suggesting that they constitute together a functional unit.

The quail oocyte during prophase I of meiosis is a very suitable material for studying the nucleolar organizers and the stages of nucleolar synthesis. Active ribosomal RNA synthesis takes place during late pachytene and the onset of diplotene[2]. Ultrastructural study of the oocyte nucleus at these stages demonstrates that the euchromatic portion of the microchromosomes containing the ribosomal cistrons penetrates into the fibrillar center of the nucleolus[3]. This constant relation suggests that the fibrillar center may be the site of the nucleolar organizer, as Goessens[4,5] has postulated.

1 With the technical assistance of Mrs A. Calisti.
2 M. Hartung and A. Stahl, Experientia *32*, 96 (1976).
3 C. Mirre and A. Stahl, J. Ultrastruct. Res. *56*, 186 (1976).
4 G. Goessens, Etude ultrastructurale des nucléoles au cours du cycle cellulaire. Thèse de Doctorat en Sciences, Université de Liège 1975.
5 G. Goessens, Exp. Cell Res. *100*, 88 (1976).

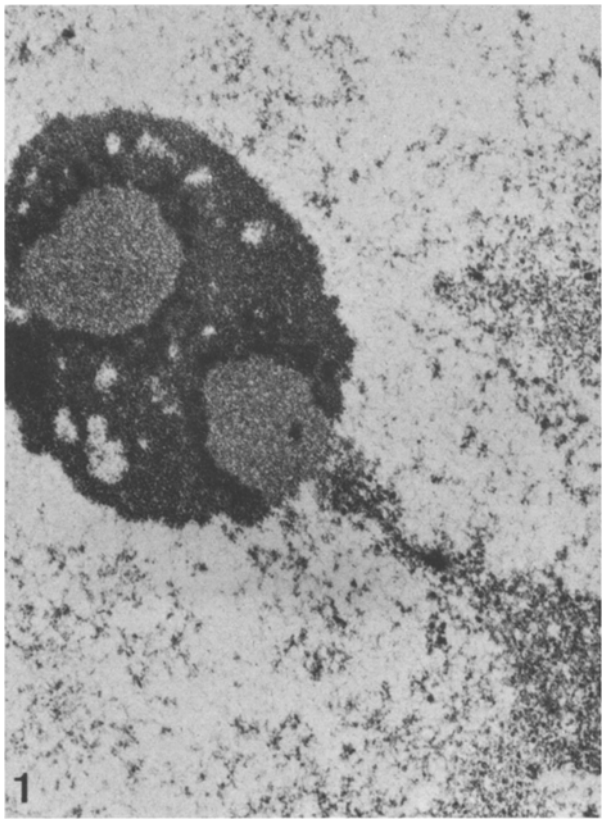

Fig. 1. Quail oocyte nucleolus at diplotene following 1 h treatment with actinomycin D (25 µg/ml). Connections between microchromosome and fibrillar center remain intact.

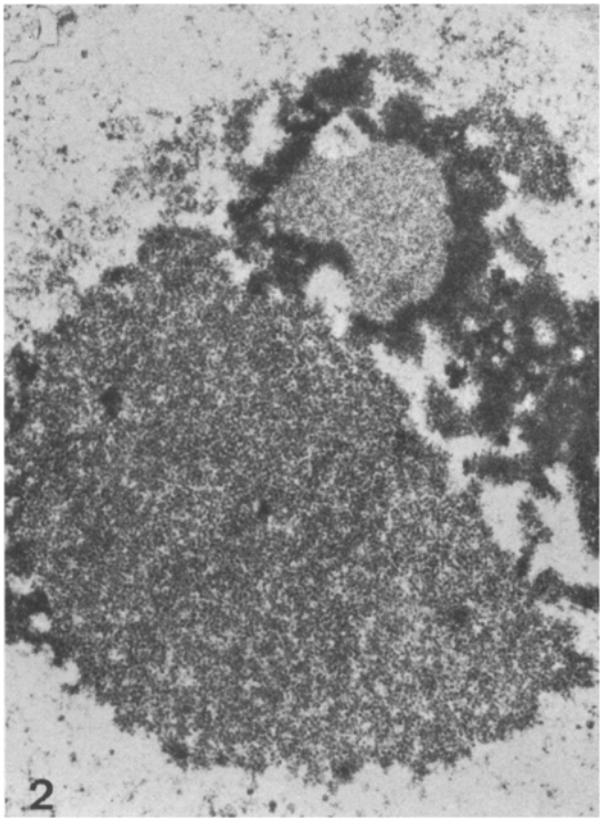

Fig. 2. After treatment of the quail oocyte with actinomycin D (25 µg/ml) for 2 h, segregation of fibrillar and granular constituents of the nucleolus is observed. Connection between the fibrillar center and microchromosomes is no longer visible.

Many agents are capable of profoundly altering the distribution of the nucleolar constituents, such as actinomycin D which inhibits the transcription of DNA by binding to the DNA helix, as a result of highly specific interaction. The drug blocks the synthesis of 45S RNA[6], without interfering with the further processing of 45S RNA transcribed prior to the block[7]. The morphological result of the action of actinomycin D is separation and redistribution of nucleolar components, described as 'nucleolar segregation'[8]. This study was undertaken to verify whether the connections between the nucleolar chromosome and the fibrillar center persisted following structural alteration of the nucleolus by actinomycin D.

Material and methods. Fragments of quail ovaries, obtained 6–12 h after hatching, were incubated for 1, 2 and 3 h in Eagle's solution containing actinomycin D (25 µg/ml). Control fragments were incubated in the same medium without actinomycin D. Ovarian specimens were fixed in 3% glutaraldehyde in 0.2 M phosphate buffer for 15 min and post-fixed in osmic acid for 20 min. Following dehydration in a graded series of acetones, the fragments were embedded in Epon. Ultrathin sections were cut on a diamond knife and then contrasted with uranyl acetate and lead citrate. Preparations were examined in a Siemens Elmiskop 101 electron microscope at 80 kV.

Observations and discussion. After treatment for 1 h with actinomycin D only the beginning of segregation can be noted: A confluence of the fibrillar components of the nucleolonema which migrate toward the fibrillar center is observed. These components are thus seen added to the dense fibrillar component which normally surrounds the fibrillar center. The connections between the fibrillar center and the chromosome remain intact at this stage. Indeed, a microchromosome, which enters in contact with the fibrillar center, can be seen emerging from a chromocenter (figure 1). Deoxyribonucleoprotein fibres emanating from the euchromatic portion of the microchromosome penetrate into the fibrillar center.

After treatment with actinomycin D for 2 h, segregation is far advanced in most nucleoli (figure 2). The granular constituent forms a large mass. The fibrillar center remains surrounded by a dense fibrillar component whose external border presents an irregular contour. In certain zones, this dense fibrillar component is in continuity with fibrillar strands which still present a reticular appearance (figure 2). Such an aspect manifestly corresponds to the fibrillar constituent of the nucleolonema whose granules have separated. At this stage, no connection between the fibrillar center and the microchromosomes can be observed.

Following actinomycin D for 3 h, rearrangement of the nucleolar constituents is complete (figure 3). A very large fibrillar center can be observed. It must be noted that it remains surrounded by a dense fibrillar component. The

6 R. P. Perry, Exp. Cell Res. *29*, 400 (1963).
7 R. P. Perry, Nat. Cancer Inst. Monogr. *23*, 527 (1966).
8 W. Bernhard, C. Frayssinet, C. Lafarge and E. Le Breton, C. r. Acad. Sci. *216*, 1785 (1965).

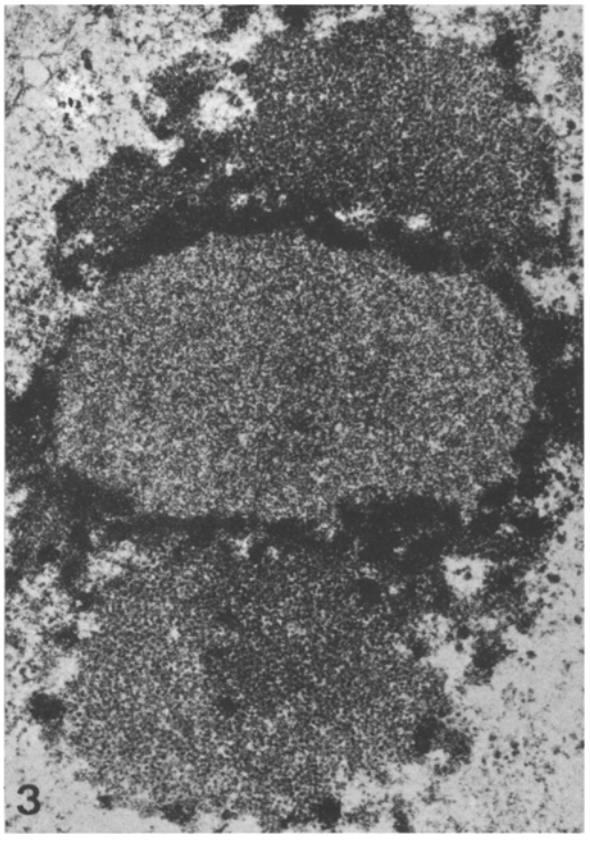

Fig. 3. Following 3 h actinomycin D (25 µg/ml) treatment of the quail oocyte nucleolus at diplotene, segregation of nucleolar constituents is complete. The fibrillar center is hypertrophied.

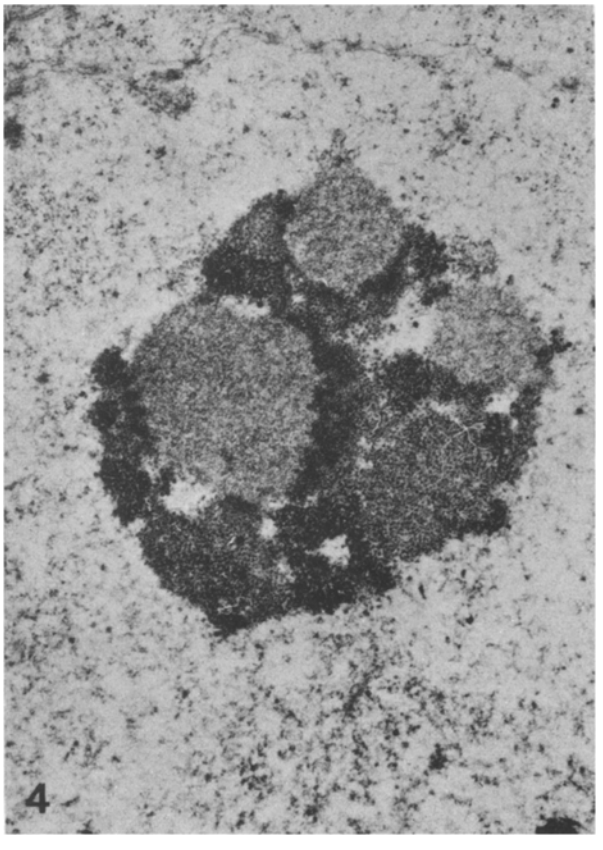

Fig. 4. In the quail oocyte after 3 h actinomycin D (25 µg/ml) several fibrillar centers and segregated granular masses are visible. No relation between fibrillar center and chromosomal fibres can be detected.

fibrillar center does not present any connection with a chromosomal structure. The rupture of the normally existing relations with the nucleolar chromosome is particularly evident when the fibrillar centers are situated at the periphery of the rearranged nucleolar mass (figure 4). Indeed, in the normal state, penetration of a microchromosome into the fibrillar center is always observed in this case[3].

Hypertrophy of the fibrillar center after treatment with actinomycin D recalls that described by Bassleer et al.[9] using daunomycin. In the case where the fibrillar center resulting from nucleolar rearrangement is both single and voluminous, a process of fusion of 2 centers cannot be excluded. Such fusion is obviously not involved when 2 or 3 large fibrillar centers can be observed.

The rearrangement of nucleolar structure results from displacement of its constituents with respect to one another. Such displacement could possibly explain the disappearance of the connections between the fibrillar center and the chromosomal fibres. Rupture of these relations is undoubtedly facilitated by alterations of the deoxyribonucleoprotein fibres under the action of the drug. It is remarkable to note that reorganization of the nucleolus leaves intact the relations between the fibrillar center and the dense fibrils which normally surround it. This observation suggests that the fibrillar center and its dense fibrillar crown may constitute a functional unit.

9 R. Bassleer, G. Goessens, A. Lepoint, C. Desaive and C. Kinet-Denoel, Beitr. Path. *150*, 261 (1973).

Haemolysis of dog erythrocytes by sorbose in vitro

A. Kistler and P. Keller[1]

Biological Pharmaceutical Research Department, F. Hoffmann-La Roche & Co., Ltd, CH-4002 Basle (Switzerland), 20 April 1977

Summary. In vitro, L-sorbose induces in dog erythrocytes haemolysis which is dependent upon time of incubation, temperature, pH and the concentration of sorbose. The in vitro susceptibility to sorbose-induced haemolysis is different in various species.

In the companion paper[2], we reported on the susceptibility to haemolysis which was observed after sorbose administration in dogs but not in rats. In this study we have investigated in vitro whether L-sorbose acts directly on erythrocytes, and if there are species differences in the susceptibility of erythrocytes to sorbose.

Materials and methods. Swiss Beagle dogs, rabbits and cats and outbred stocks of Fü-albino SPF rats and mice were obtained from the Institute of Biological and Medical Research (Füllinsdorf BL). Further, blood from a standardbred horse and from a Swiss Simmental cow was obtained from the School of Veterinary Medicine, University of Berne, and human blood was made available from one of the investigators.

Blood samples were collected in heparinized tubes and the red blood cells washed 2–3 times with physiological NaCl solution. The red blood cells were incubated in Hanks balanced salt solution (without phenol red, supplemented with 60 µg/ml penicillin and 100 µg/ml streptomycin) in cell culture dishes (Falcon, 5.5 cm, final volume 5 ml) in a H_2O-saturated 5% CO_2-air atmosphere. In some experiments the bicarbonate-buffer in the Hanks solution was replaced by 25 mM Hepes-buffer, pH 7.4. L(–)sorbose or D(+)glucose was added as indicated in the text. After incubation, the medium together with the cells were centrifuged and the haemoglobin (Hb) concentration was determined in the supernatant as described[2]. The Hb release into the medium was expressed as percent of the Hb content of the erythrocytes added to the medium.

Results. The incubation of dog erythrocytes (containing 10–14 mg Hb) at 34 °C in presence of L(–)sorbose (5.6–

1 The authors are greatly indebted to Prof. A. Studer, Prof. K. F. Gey and Dr H. Hummler for critical reading of the manuscript. The technical assistance of Mrs E. Stöckli and Mrs C. Villien and of Mr P. Back and Mr V. Loechleiter is gratefully acknowledged.

2 P. Keller and A. Kistler, Experientia *33*, 1380 (1977).

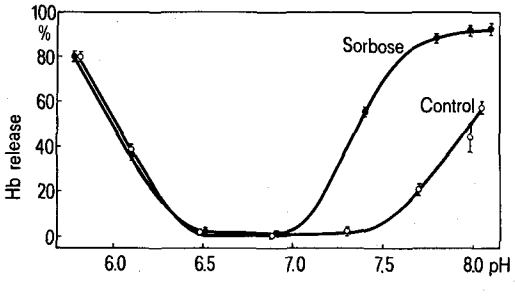

Fig. 1. Effect of pH on the haemolytic effect of sorbose in dog erythrocytes. Dog erythrocytes (containing 10.7 mg Hb) were incubated with or without sorbose (17 mM) in Hanks solution of different pH (range 5.0–9.0). Incubation was performed at 34 °C for 23 h and the pH determined in the medium. Results are expressed as percent of Hb release into the medium. Means of 4 determinations ± SD.

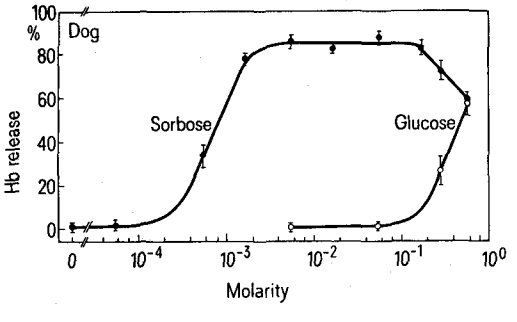

Fig. 2. Dependence of the haemolytic effect on sorbose concentration in dog erythrocytes (containing 13.6 mg Hb). 48-h-incubation in Hanks solution at 34 °C in the absence and presence of sorbose or glucose. The glucose concentrations were not corrected for the glucose content of 5 mM in the Hanks solution. Results are expressed as percent Hb release into the medium ± SD.