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Autonomous cortical activity in mouse eggs controlled by a cytoplasmic clock

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SUMMARY

Mouse eggs of Swiss albino origin, both parthenogenetic and fertilized, were bisected into nucleate (NHs) and anucleate halves (AHs) and observed *in vitro* (semicontinuous observations) for up to 40 h for possible manifestations of cortical activity. Three experimental groups were studied: (1) Non-fertilized eggs activated 17 h after administration of hCG with a heat-shock and bisected 4 h later. (2) Non-fertilized eggs first bisected, and the resulting sister halves activated 17 h after administration of hCG with ethyl alcohol. (3) *In vivo* fertilized eggs bisected 27 h after administration of hCG into an AH and a binucleate half. Parthenogenetic eggs (intact, zona-free, and incompletely bisected), and fertilized eggs collected 17, 20, and 27 h after administration of hCG were also studied.

In the middle of the first cell cycle the cell surface in all types of cells studied changed from smooth to slightly undulate. In nucleate cells the surface deformations lasted for several hours and disappeared shortly before the first mitosis. In contrast, in AHs the indentations of the cell surface deepened, and developed into manifold furrows, thus leading to fragmentation. However, in 20 % of AHs fragmentation was partially or completely reversed. The incidence and the intensity of fragmentation were lower, and its reversibility was more common in AHs carrying the 2nd polar body. We suggest that the interphase nucleus, i.e. the pronucleus in whole eggs and NHs, and the 2nd polar body nucleus (if 2nd polary body is attached to an AH) exerts a moderating effect on cortical activity. However, the initiation of cortical activity is nucleus-independent, as shown by the behaviour of AHs separated before activation. We believe that the observed phenomena reflect autonomous cortical activity which is regulated by a cytoplasmic clock.

INTRODUCTION

Numerous embryological studies on animal eggs or anucleate egg fragments have shown that various cytoplasmic and/or cortical activities are under the control of a cytoplasmic clock. To be considered as controlled by the cytoplasmic clock, the particular activity has to fulfil two conditions. First it has to be initiated independently from the nuclear activity thus showing an autonomous character. Second it has to reappear at definite time intervals thus displaying a periodic character. Although the biochemical basis of cytoplasmic clocks remains obscure, the autonomous and periodic cytoplasmic and/or cortical activities are

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real developmental phenomena which appear to play an important role in embryogenesis. Satoh (1982) has recently proposed that cytoplasmic clocks may determine the time of initiation of morphogenetic events during early embryonic development.

The autonomy of a cytoplasmic and/or cortical activity can be experimentally proved by showing that it occurs in the absence of the nucleus, i.e. in anucleate eggs halves. This experimental approach together with observations on intact eggs has been extensively used by many embryologists working with amphibian and invertebrate eggs.

The observed autonomous and periodic cytoplasmic and cortical activities include:

1. Changes in the egg shape, for example formation of the polar lobe in some molluscs (reviewed by Conrad & Rappaport, 1981) and rounding-up/relaxation movements of ascidian and amphibian eggs or egg fragments (Bell, 1962; Sawai, 1979; Hara, Tydeman & Kirschner, 1980; Sakai & Kubota, 1981).

2. Various surface phenomena, like wrinkling of *Tubifex* egg fragments (Shimizu, 1981), surface contraction wave in amphibian eggs and egg fragments (Hara *et al.* 1980; Sakai & Kubota, 1981; Yoneda, Kobayakawa, Kubota & Sakai, 1982), cyclic thickening of the hyaline layer in activated sea urchin eggs (Kojima, 1960*a*), and formation of cytoplasmic protrusions in refertilized sea urchin eggs (Kojima, 1962).

3. Cyclic changes in the tension of the cell surface of sea urchin eggs and egg fragments (Yoneda & Dan, 1972; Yoneda, Ikeda & Washitani, 1978).

4. Cyclic changes in the physiological properties of the egg cytoplasm, for example: the ability to induce germinal vesicle breakdown and chromosome condensation following injection into oocytes (amphibians: Wasserman & Smith, 1978), stage-limited formation of sperm asters in refertilized sea urchin eggs, and cyclic formation of clear cytoplasmic spots in sea urchin eggs treated with some chemical agents or hypertonicity (Kojima, 1960*b*, 1962).

It is not clear whether various manifestations of the autonomous cytoplasmic activity which can co-occur in eggs of a given species are controlled and timed by one common mechanism and, if so, how they are interrelated. The recent work by Kojima (1980) suggests that cyclic changes in the cytoplasm and the cortex can occur independently.

Autonomous cytoplasmic and/or cortical activity in mammalian eggs has not been so far described. This study which is based on *in vitro* observations of intact mouse eggs, both fertilized and parthenogenetic, and of anucleate and nucleate egg halves, provides evidence for the existence of such an activity and suggests that it may be regulated by a cytoplasmic clock.

MATERIALS AND METHODS

Swiss albino randomly bred mice were used throughout this study. Ovulation

was induced by intraperitoneal injection of pregnant mares' serum gonadotrophin (PMSG) and human chorionic gonadotrophin (hCG) (5–10 i.u. of each) given 48 h apart. For artificial activation eggs were harvested from unmated females 16–16.30 h after administration of hCG. Fertilized eggs were obtained 17, 20 and 27 h after hCG from females which had been caged with F_1 (CBA × C57B1/10) males immediately after the second injection and which had mated during the next 12 h.

Eggs were released from ampullae into phosphate-buffered saline (PBS) or medium M2 (medium 16 (Whittingham, 1971) buffered with HEPES (Fulton & Whittingham, 1978)) containing 200–400 i.u. of hyaluronidase per ml. After the cells of cumulus oophorus had been dispersed, the zona pellucida was partly digested with 0·5 % pronase in PBS, and then finally removed by vigorous pipetting in PBS or M2.

Artificial activation

Eggs were subjected to the activating agent 17 h after hCG. Two techniques of activation were employed: 1. heat shock for eggs bisected after activation (Experiment A), and 2. ethyl alcohol for egg halves produced before parthenogenetic treatment (Experiment B). In the first case dissected oviducts with ovulated eggs were subjected for 5 min to a heat shock of 44·5–45·0 °C according to the procedure described by Komar (1973). In the second case pairs of zona-free sister egg halves were placed for 5–6 min in 7–8 % ethyl alcohol in Whitten's medium (Whitten, 1971) and thoroughly washed afterwards (technique developed by Cuthbertson (Cuthbertson, Whittingham & Cobbold, 1981; Cuthbertson, 1983)), and inspired by observations of Dyban & Khozhai (1980)). The reason for using two different methods of activation was purely technical: the heat-shock technique which was used in the main (and chronologically first) part of the work (Experiment A) appeared to be unsuitable for activation of zona-free egg halves in Experiment B.

Bisection of eggs

Eggs were bisected into nucleate and anucleate halves with a glass needle on the surface of 1 % agar according to the technique of Tarkowski (1977). Non-fertilized eggs were treated in two ways: 1. activated with a heat shock, cultured for 4 h, selected for the presence of the 2nd polar body and clearly visible pronucleus, and then bisected. 2. bisected at metaphase II and then activated with ethyl alcohol. Bisection of activated eggs with the 2nd polar body and one pronucleus produces as a rule a nucleate and an anucleate half. In eggs cut in half in metaphase II, the spindle is displaced as a whole to one half, and, as has been previously demonstrated (Tarkowski, 1980), both halves can initiate development following activation by sperm.

The reliability of anucleation was ascertained by examination of selected pairs

of egg halves in haematoxylin-stained whole mounts (Tarkowski & Wròblewska, 1967).

Fertilized eggs collected 27 h after hCG were bisected immediately after dispersion of the follicle cells and removal of the zona pellucida. At this stage of development the two pronuclei were situated close together in the centre of the egg, and as a result of cutting a binucleate and an anucleate halves were usually produced. Pairs of sister halves in which the pronuclei were separated each to one half were discarded.

Only those pairs of egg halves were used for further study in which both partners were of equal or nearly equal size.

Control eggs

Three types of control eggs were used: 1. intact eggs, 2. zona-free eggs, and 3. incompletely bisected zona-free eggs (when the halves remain connected by a cytoplasmic bridge they soon re-unite into one egg-cell). Incomplete bisection was performed to assess the traumatic effect of the cutting operation on the further performance of egg halves.

Culture of egg halves and control eggs

Cells were cultured in drops of Whitten's medium in siliconized-glass Petri dishes or plastic tissue-culture dishes, under liquid paraffin. The cultures were kept at 37 °C in the atmosphere of 5 % CO_2 in air.

Design of the work and time-schedule of observations

Three types of experiments were carried out.

Experiment A. Egg halves produced 4 h after activation were inspected every hour between 5 and 28 h (variant I) or only between 14 and 28 h (variant II), and all halves were examined once again 40 h after activation.

Experiment B. Egg halves activated after bisection were first observed 4 h after activation, then every hour between 17 and 28 h and once again 40 h after activation.

Experiment C. Egg halves produced from fertilized eggs 27 h after hCG were observed every hour between 29 and 36 h after hCG and once again at 48 h. Whole, fertilized eggs collected 17, 20 and 27 h after hCG were observed for 10 h at one hour intervals.

The cells were observed under the inverted microscope. Schematic drawings were made of all eggs and egg halves throughout the period of observations and photomicrographs were taken of selected cells.

RESULTS

Experiment A: Eggs bisected after parthenogenetic activation

226 pairs of nucleate and anucleate halves and 83 incompletely bisected eggs

were studied in two variant experiments (variant I: 74 pairs, variant II: 152 pairs and 83 control eggs).

Nucleate halves (NHs) and control eggs

NHs underwent the first cleavage division with a lower frequency than the incompletely bisected eggs (61·1 % *versus* 85·5 %, Table 1). However, among the cleaving NHs and control eggs similar proportion underwent normal division with the formation of two blastomeres of equal size (Table 1). In the remaining cells the division was irregular: the two blastomeres were usually of unequal size and were accompanied by cytoplasmic globules, variable in size and number. As shown in Fig. 1, the rate of the first cleavage division was only slightly slower in NHs than in whole eggs: 50 % of cells of both types studied according to variant II completed division between 21 and 22 h after activation. A slightly slower rate of cleavage in NHs in variant I was most probably due to cooling of cultures as a result of every hour inspection over a 28 h period.

In all NHs a transitory change in the cell shape was observed. This change comprised the appearance of small evaginations and invaginations which altered the cell surface from smooth to slightly undulate (Figs 3B, 19B). Eleven NHs were used to establish the timing of these phenomena. Surface deformations in this group began between 8 and 13 h after activation (mean time: 10 h 21 min), lasted for 6 to 11 h (mean time: 8 h 33 min) and as a rule disappeared at the time of the breakdown of the pronucleus. From this moment until the onset of division NHs remained smooth and spherical (Figs 5B, 21B).

The control eggs, both incompletely bisected (without zona) and unoperated (with the zona intact) displayed the same surface changes. As in NHs the deformations disappeared with the onset of mitosis (Figs 10–17).

Anucleate halves (AHs)

When the activated egg is bisected 4 h after activation the pronucleus is located

Table 1. *First cleavage division of NHs and incompletely bisected eggs and fragmentation of AHs* (eggs bisected after activation)*

Type of cells	Total number	Cleaved or fragmented: total (%)	Cleaved or fragmented: regular cleavage (%)	$\frac{\text{Regularly cleaved}}{\text{Total cleaved}} \times 100$
NHs	226	138 (61·1)	99 (43·8)	71·7
AHs	202	202 (100·0)	n.a.	n.a.
incompletely bisected eggs	83	71 (85·5)	48 (57·8)	67·6

* only AHs without 2nd polar body.
n.a. – not applicable.

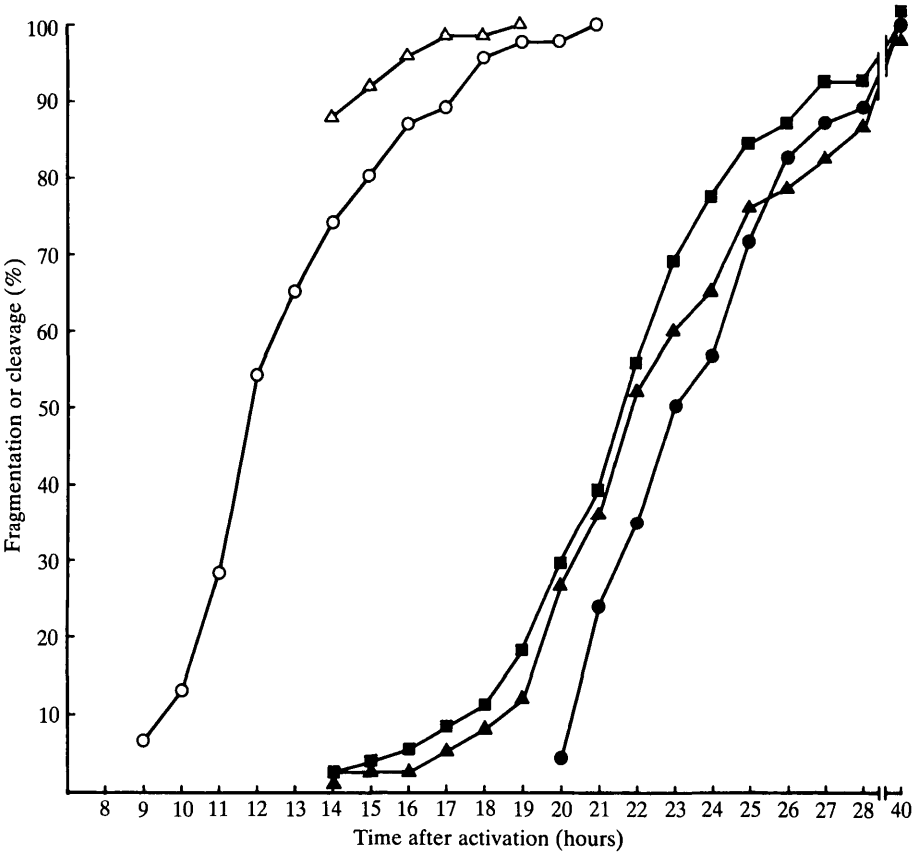


Fig. 1. The rate of fragmentation in anucleate egg halves (○△) and of cleavage division in sister nucleate halves (●▲) produced from eggs bisected after parthenogenetic activation (Experiment A).
○● sister pairs studied according to variant I
△▲ sister pairs studied according to variant II
■ control eggs (incompletely bisected, zona-free) from variant II.

close to the 2nd polar body and as a result of cutting the nucleate partner usually inherits the polar body (202 out of 226 halves). Since the presence of the 2nd polar body affects the behaviour of anucleate halves, the group of 24 AHs with the 2nd polary body will be dealt separately at the end of this heading.

AHs which regained spherical shape (Fig. 2A), by about 30 min after the cutting operation, started to display deformations of the cell surface after the next few hours similar to those observed in NHs and control eggs (Figs 3A, 4A). Surface deformations were observed as early as 8 h after activation, and by the 16 h the process has begun in all AHs (mean time: 11 h 23 min). The further course of events was, however, different from that observed in nucleate cells. The invaginations of the cell surface gradually deepened and developed into furrows which partitioned anucleate egg halves into cytoplasmic fragments, variable in size and number (Fig. 5A). We call this phenomenon fragmentation, but

judging from the fact that in some AHs the process is reversible (see below), partitioning of the cytoplasm must be originally incomplete and the fragments must be internally joined together by cytoplasmic bridges. Because surface deformations intensify gradually and fragmentation represents the final phase of a continuous process, the distinction between these two states is to some extent subjective. However, two successive observations taken 1 h apart allowed each anucleate half to be classified correctly. The interval between the beginning of surface deformations of AHs and fragmentation varied between 1 and 8 h, the mean time interval being 1 h 43 min.

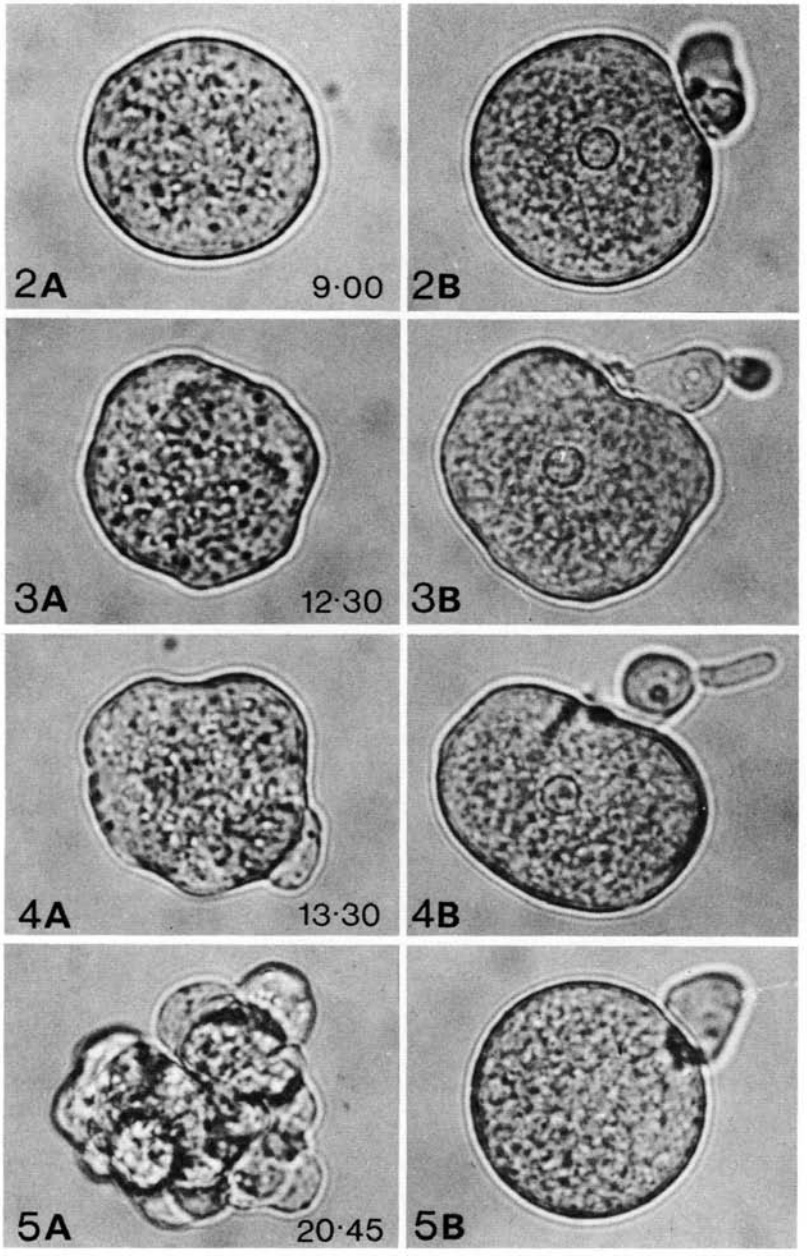
All 202 AHs without the 2nd polar body underwent fragmentation irrespective of whether or not the nucleate counterpart cleaved (Table 1). 121 AHs whose sister nucleate halves cleaved, fragmented between 9 and 21 h after activation with fragmentation of 50 % of halves at 12 h and the mean time 13 h 6 min. In all pairs of sister halves fragmentation of an AH preceded the cleavage division of a NH by several hours, the minimal time interval being 4 h. The time interval between the commencement of fragmentation in AHs and cleavage in NHs estimated on the basis of 50 % points of these two events was 11 h (Fig. 1).

The fragmented AHs initially looked like aggregates of tightly packed cytoplasmic globules (Fig. 5A). By the end of a 40 h culture period, 97 out of 121 AHs were partly disaggregated into individual globules and often showed degenerative changes in the cytoplasm. However, in 21 cases the reduction in the number of fragments (down to between two and six) (Fig. 9A) and in three cases the complete reversion to a single cytoplast of spherical shape has occurred (Table 2). Partial or complete reversion of fragmentation of AHs took place between 20 and 40 h after activation, and either preceded or followed the time of cleavage of their nucleate counterparts (variation between −4 and +7 in relation to the time of cleavage).

Table 2. *Effect of the 2nd polar body on the behaviour of AHs* (eggs bisected after activation)*

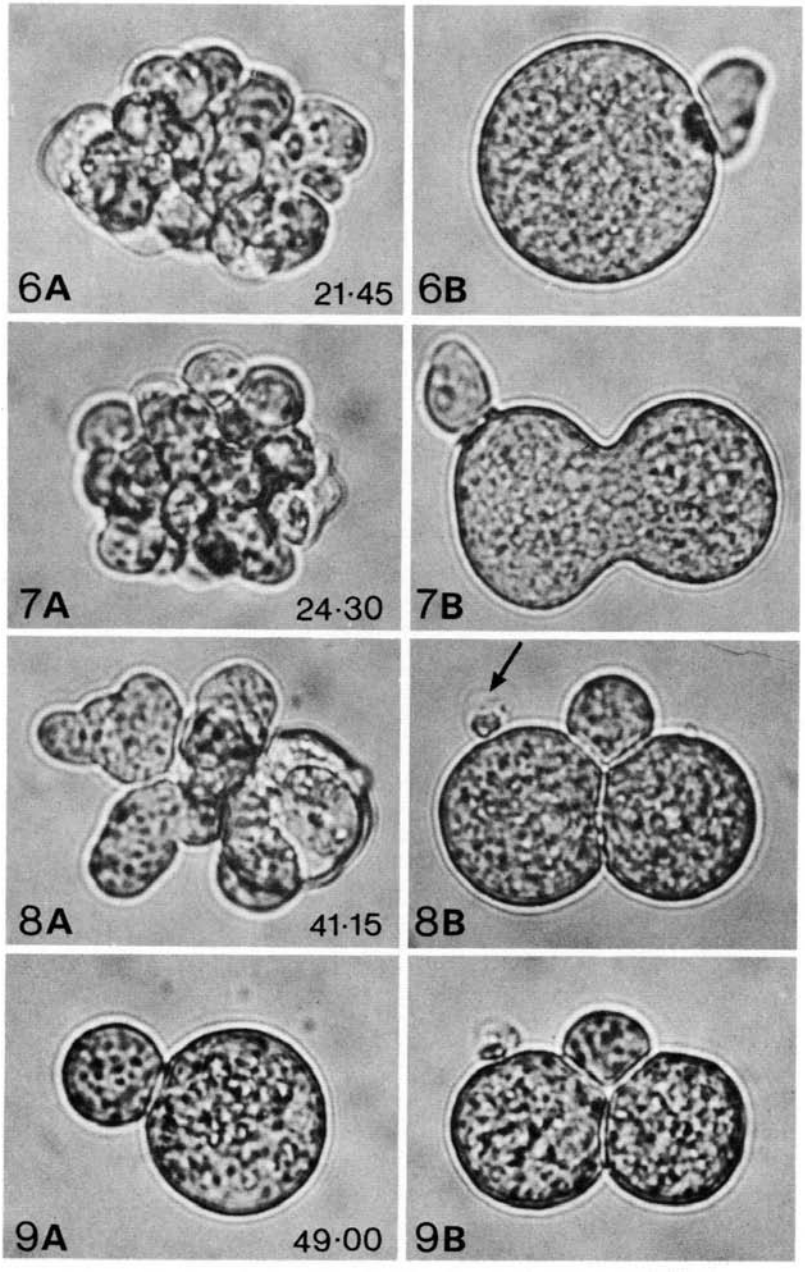
Type of AHs	Total number	Non-fragmented	Frag-mented	Reversed fragmentation		Time of fragmentation in 50 % of halves
				complete	incomplete	
with 2nd polar body	17	5	12	9	1	20–21 h
without 2nd polar body	121	0	121	3	21	11–12 h

* out of 24 AHs with the 2nd polar body only those anucleated halves are tabulated whose sister NHs have divided.

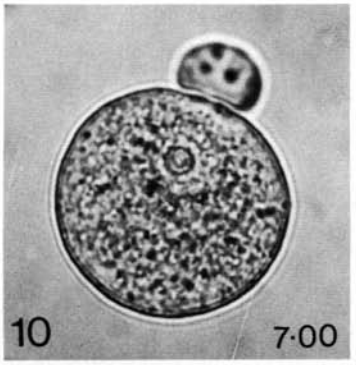


Figs 2–9. Cortical activity in anucleate (column A) and nucleate (column B) sister egg halves produced after parthenogenetic activation. Second polar body attached to the nucleate half (NH). Numerals indicate the time (hours, mins) after activation.

Initially both egg halves were spherical and had a smooth surface (Figs 2A, 2B), but 12 h 30 min after activation their surface became undulate (Figs 3A, 3B). In the anucleate half (AH) indentations deepened and developed into manifold furrows



thus leading to fragmentation (Fig. 5A). The NH regained a spherical shape at the time of pronucleus breakdown prior to the first cleavage division (Fig. 5B). Cleavage of the NH took place long after the AH had fragmented (Fig. 7B); during cleavage a small anucleate fragment was produced and 2nd polar body degnerated (arrow) (Figs 8B, 9B). By 49 h after activation most of the furrows in the AH have disappeared with partial reversion of fragmentation to just two fragments (Figs. 8A, 9A). ×530.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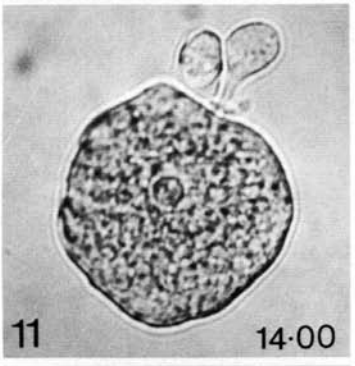
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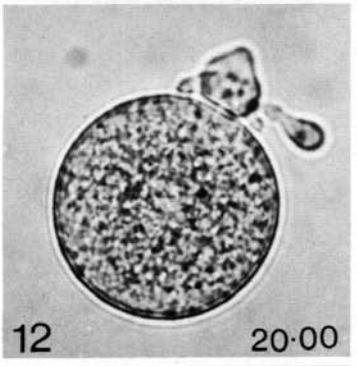
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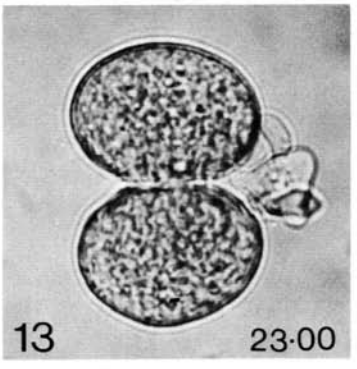
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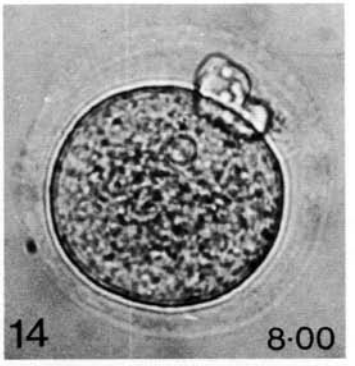
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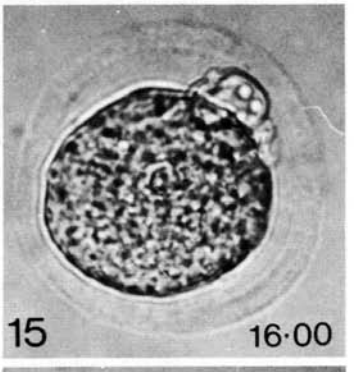
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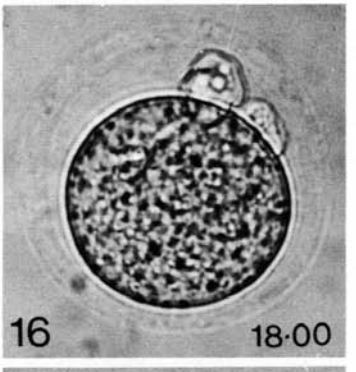
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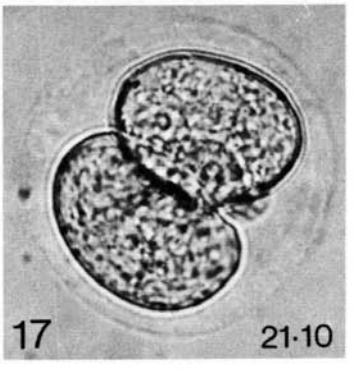

17
21·10

Anucleate halves with the 2nd polar body

Out of 24 AHs with the 2nd polar body, 17 were selected because their nucleate counterparts had cleaved (Table 2). AHs of this particular type either maintained a spherical shape throughout the whole period of observation (3 cases), or formed a temporary invagination at the place of contact with the 2nd polar body (2 cases), or underwent fragmentation (12 cases) (Figs 18–25). Fragmentation was often (7 cases) initiated by formation of the indentations in the proximity of the 2nd polar body, soon followed by formation of additional furrows in other regions of the egg half. Fragmentation of AHs with the 2nd polar body was considerably delayed as compared to AHs lacking the polar body (50 % points: 20–21 h after activation *versus* 11–12 h after activation). In 9 out of 12 AHs which had fragmented, fragmentation was fully reversed (Fig. 25A, Table 2).

Experiment B: Eggs bisected before parthenogenetic activation

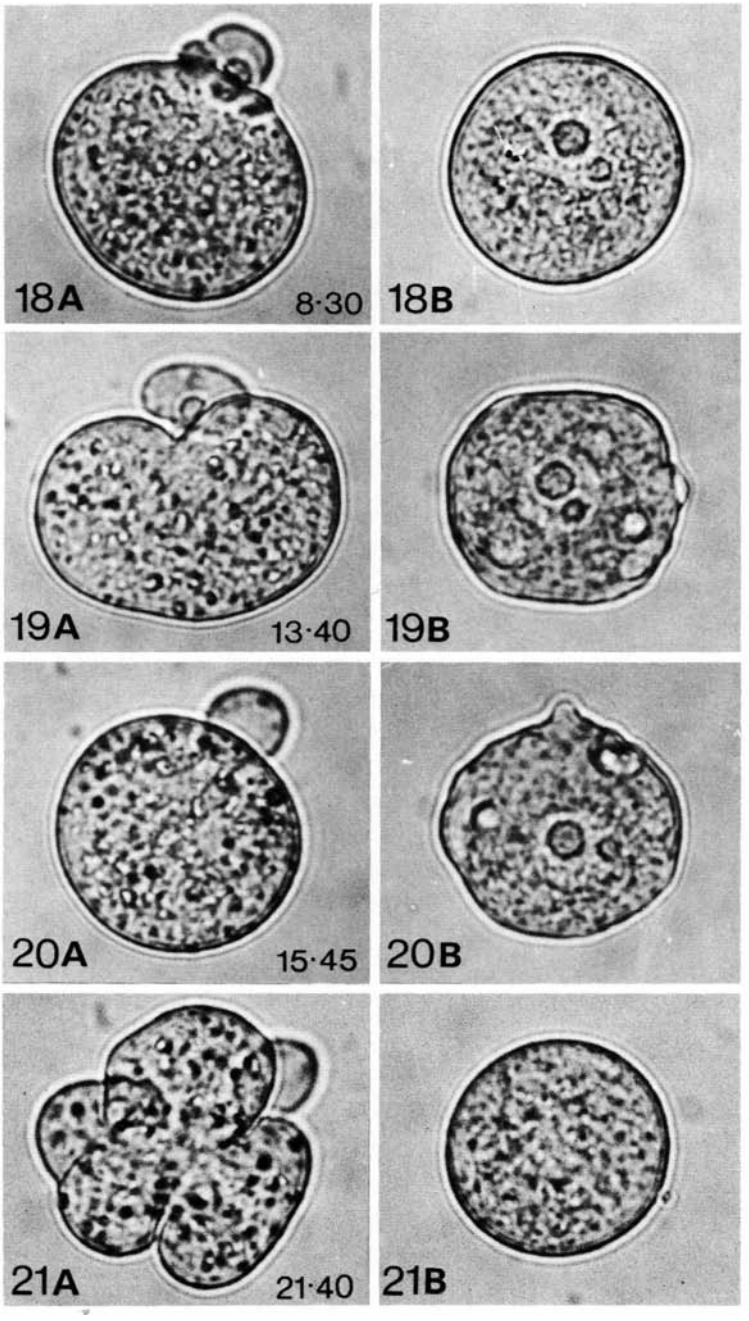
The aim of this experiment was to find out whether cell surface deformations and fragmentation of anucleate halves can occur in the absence of any signal from the pronucleus.

98 pairs of sister egg halves and 131 control eggs (non-operated, zona-free) were activated and scored for signs of activation after 4 h of culture. Only NHs and eggs with a pronucleus (pronuclei) were considered as activated. 79·6 % of NHs underwent activation and the majority of them (71/78) reacted by extrusion of the 2nd polar body and formation of one pronucleus. The overall incidence of activation was slightly lower in NHs than in control eggs (79·6 % *versus* 88·5 %). The rate of spontaneous activation in a group of 253 control eggs was 2·7 %).

In the case of anucleate egg halves it was not possible during the first few hours after application of the parthenogenetic agent to determine whether or not they had been activated. We considered those halves as activated which after several hours of culture underwent surface deformations and fragmentation. Using this criterion 56·1 % of AHs were classified as activated (Table 3). This figure is definitely below those for NHs and control eggs. Because sister halves were always subjected to the solution of ethyl alcohol together and for the same period of time, one could expect the same response in both partners, i.e. either positive

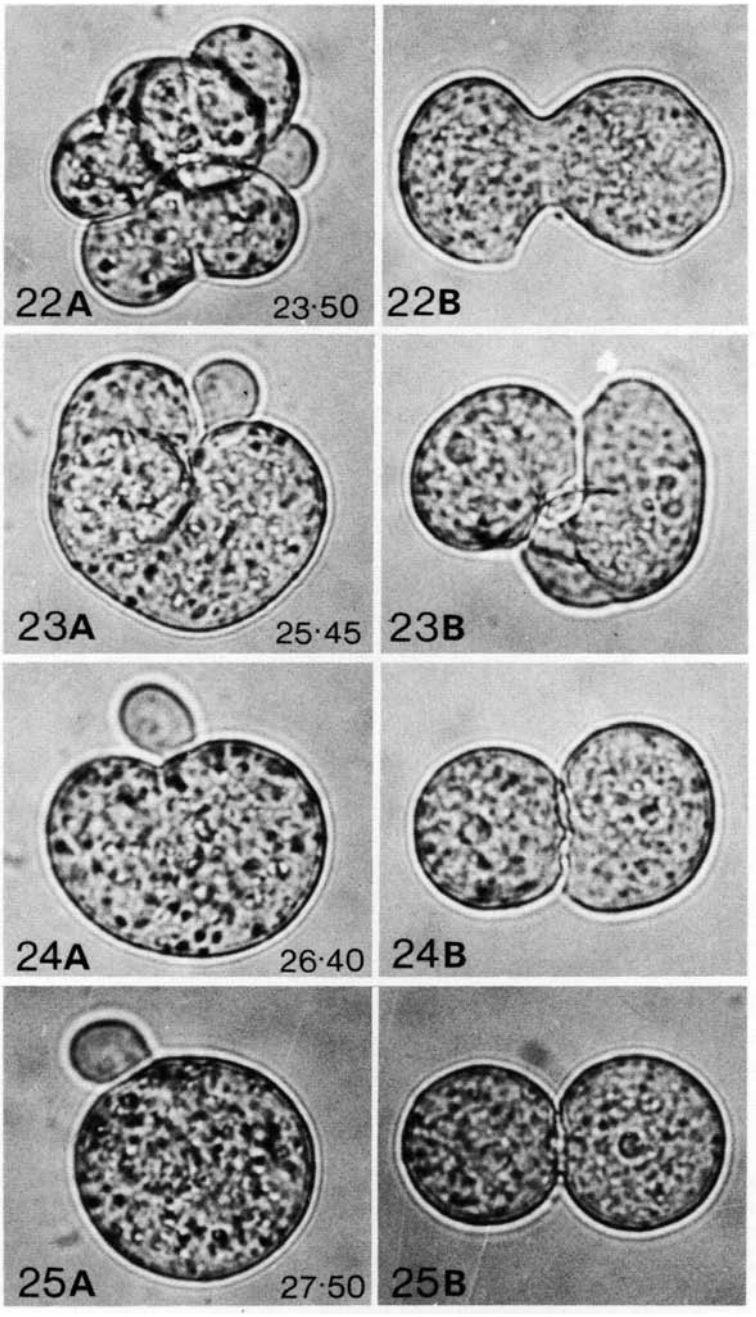
Figs 10–17. Changes of the cell surface in parthenogenetic eggs with the zona pellucida removed (Figs 10–13) and with the zona intact (Figs 14–17). Numerals indicate the time (hours, mins) after activation. Abbreviations as in Figs 2–9.

Initially spherical eggs (Figs 10, 14) underwent transient surface deformations (Figs 11, 15), and regained a spherical shape (Figs 12, 16) shortly before the first cleavage division (Figs 13, 17). Note that in Figs 12 and 16 the pronucleus is no longer visible. ×400.



Figs 18–25. Cortical activity in anucleate (column A) and nucleate (column B) sister egg halves produced after parthenogenetic activation. Second polar body attached to the AH. Numerals indicate the time (hours, mins) after activation. Abbreviations as in Figs 2–9.

For about 9 h after separation both sister halves remained unchanged (Figs 18A, 18B). 13 h 40 min after activation undulations appeared in the NH, and an indentation



was temporary formed underneath 2nd polar body in the AH (Figs 19A, 20A). At the time of pronucleus breakdown in the NH (Fig. 21B), the AH underwent fragmentation (Fig. 21A) which was, however, transient (Figs 22A–24A) and has been completely reversed (Fig. 25A). The second polary body has affected the initiation and duration of fragmentation in the AH (cf. the pair of halves shown in Figs 2–9). The NH had cleaved before reversion of fragmentation of the AH was completed (Figs 22–25). ×530.

Table 3. *Reaction of sister egg halves to ethyl alcohol**

Type of egg half		Nucleate: no. (%) activated	Nucleate: no. (%) non-activated	Total
Anucleate no. (%)	activated	46 (46·9)	9 (9·2)	55§ (56·1)
	non-activated	32 (32·6)	11 (11·2)	43 (43·9)
Total		78 (79·6)	20 (20·4)	98 (100·0)

* χ^2 test does not reveal correlation between activation of NHs and AHs ($\chi^2 = 0{\cdot}758$, $0{\cdot}5 > P > 0{\cdot}25$).
§ for criteria of activation – see text.

or negative. Since this was not the case (Table 3) one has to conclude that either AHs are more resistant to activation, or the criterion of fragmentation underestimates the incidence of activation.

At 17 h after activation (first observation), 80 % of AHs were already fragmented, while only 16 % of NHs and control eggs completed the cleavage division. By 26–28 h after activation cleavage or fragmentation have been practically completed in all three types of cells (Fig. 26). As in Experiment A, AHs started fragmentation earlier than their nucleate counterparts began cleavage. However, because of the time schedule of observations, the interval between these two events could not be estimated.

In this experiment the incidence of the first cleavage division of NHs and control eggs was lower than in Experiment A, and irregular cleavages were more common (cf. Tables 1 and 4). The difference could have been due to the different type of parthenogenetic activation (heat shock *versus* ethyl alcohol) or to some unidentified differences in culture conditions (the two experiments were separated in time by several months).

The results of this experiment provide conclusive evidence that surface deformations and fragmentation of AHs are not programmed or stimulated by the pronucleus and, therefore, represent autonomous cortical activity.

Experiment C: Eggs bisected after fertilization and intact fertilized eggs

The aim of this experiment was twofold: first to prove that surface deformations and fragmentation of anucleate egg halves are not side effects of artificial activation, and second to estimate the time of appearance of surface deformations in intact eggs under physiological conditions.

Egg halves

The experimental design differed from that in Experiments A and B in that bisection was carried out shortly before the first cleavage division, i.e. at the end

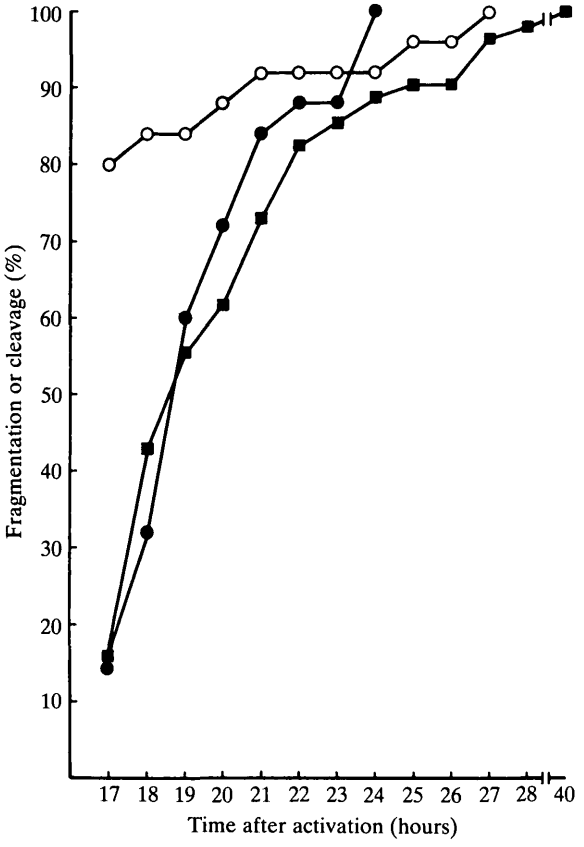


Fig. 26. The rate of fragmentation in anucleate halves (○), and of cleavage division in nucleate sister halves (●) activated after separation (Experiment B). ■ control eggs (unoperated, zona-free).

Table 4. *First cleavage division of NHs and control eggs and fragmentation of AHs (eggs bisected before activation)**

Type of cells	Total number	Cleaved or fragmented: total (%)	Cleaved or fragmented: regular cleavage (%)	$\frac{\text{Regularly cleaved}}{\text{Total cleaved}} \times 100$
NHs	71	35 (49·3)	24 (33·8)	68·6
AHs	71	45 (63·4)	n.a.	n.a.
zona-free eggs	91	63 (69·2)	44 (48·3)	69·8

* only those pairs are tabulated in which NH extruded the 2nd polar body and formed one pronucleus.

n.a. – not applicable.

rather than at the beginning of the cell cycle. The reason for this was that the efficiency of producing anucleate halves was low until the second half of the cell cycle, when the two pronuclei occupied the centre of the egg and could be displaced together to one egg half.

In the case of eggs fertilized *in vivo* their age after activation can be calculated only indirectly. Ovulation in the mouse occurs 11–14 h after administration of hCG (Edwards & Gates, 1959). Assuming that matings take place before or at the beginning of ovulation, then the age of eggs after activation is the age after hCG minus 11–14 h.

In 60 out of 83 pairs of egg halves both cleavage of NHs and fragmentation of AHs was completed by 36 h after hCG (22–25 h after activation), and only these pairs were used to compare the rate and timing of cleavage and fragmentation (Table 5). In this group of egg halves 2nd polar body was attached as a rule to a NH. Undulation of the cell surface appeared simultaneously in NHs and AHs with the mean time 29 h 35 min after hCG ($15\frac{1}{2}$–$18\frac{1}{2}$ h after activation). In AHs it was soon followed by formation of many furrows and fragmentation. In NHs the surface deformations were maintained until the breakdown of pronuclei, when the cells resumed a spherical shape and smooth surface. In 50 out of 60 pairs, fragmentation preceded the first cleavage division of NHs by 1–5 h, in 5 it followed cleavage, and in 5 fragmentation and cleavage were synchronous. The time interval between these two events in 50 % of egg halves was about 2 h (data not shown), and the mean time difference was 1 h 42 min (Table 5). Taking into account that in intact fertilized eggs surface deformations begin 21 h after hCG (see below), it follows that at the time of bisection (27 h after hCG) this process must have been under way for about 6 h. The burst of fragmentation in AHs observed shortly after bisection is in agreement with the results of Experiments A and B, namely that in the absence of a pronucleus surface deformations rapidly evolve into fragmentation.

In another group of 24 pairs of egg halves the 2nd polar body was attached to an AH. As in the case of AHs produced from parthenogenetic eggs (Experiment A), in the presence of the 2nd polar body anucleate halves fragmented more

Table 5. *Rate of the first cleavage division in NHs and of fragmentation in AHs (fertilized eggs bisected 27 h after hCG)*§

Type of egg halves	No. of egg halves	No. of egg halves divided or fragmented at subsequent hours after hCG								Mean time of cleavage or fragmentation (h)
		29	30	31	32	33	34	35	36	
NHs	60	1	1	2	8	9	19	33	60	34·47
AHs	60	1	1	3	22	37	52	59	60	33·05

§ only those pairs are tabulated in which both cleavage and fragmentation occurred by 36 h.

rarely, and the reversibility of fragmentation was more common than in the absence of the polar body. Out of 24 AHs with the 2nd polar body, 17 fragmented (70·8 %) but in 6 of them fragmentation was partially or completely reversed. In AHs without the 2nd polar body (previous group) the corresponding figures were 72 out of 83 (86·7 %), and 1 out of 72.

Intact fertilized eggs

Eggs collected 27 h after hCG and examined immediately after recovery displayed a slightly undulate surface. During subsequent manipulations the surface deformations were not evident, but they reappeared in culture after ca 1 h. Eggs harvested 17 and 20 h after hCG had at the time of recovery a smooth surface and the undulations appeared in culture after ca 4 and $1\frac{1}{2}$ h respectively, i.e. at the same time after hCG. We conclude from this observations that under physiological conditions the deformations of the egg surface appear about 21 h after hCG (7–10 h after activation), i.e. slightly earlier than in parthenogenetic eggs and nucleate egg halves cultured *in vitro* from the time of activation (cf. Experiment A).

DISCUSSION

We believe that our experiments provide evidence for the existence of an autonomous cortical activity in the mouse egg.

In the middle of the first cell cycle the surface of the mouse egg cells and egg halves changes from smooth to slightly undulate. In whole eggs and nucleate egg halves surface deformations last until mitosis, when the cells resume a spherical shape and smooth surface. The appearance and disappearance of these deformations must reflect changes in the tension of the plasma membrane. We have shown that the observed surface events represent a developmental phenomenon and are not side effects of artificial activation, culture *in vitro* or removal of the zona pellucida.

The crucial piece of evidence that surface deformations reflect autonomous cortical activity is provided by the observation that they occur also in anucleate egg halves. Moreover, the process starts synchronously in both types of egg halves. Cortical activity is not programmed by the pronucleus because it is initiated also in anucleate halves which had been separated before parthenogenetic activation. On this basis we postulate that egg activation triggers in the cytoplasm and/or cortex a clock mechanism which is nucleus independent.

While the appearance of cortical activity is autonomous, its intensity and duration appear to be moderated by the interphase nucleus. Three pieces of evidence support this conjecture. 1. In contrast to nucleate cells in which the undulated cell surface is maintained until the transition from interphase to mitosis, in anucleate egg halves surface deformations rapidly evolve into manifold furrows which result in fragmentation. 2. The anucleate halves carrying

the 2nd polar body do not fragment at all or start fragmentation with a considerable delay in comparison to AHs lacking the polar body. Also the intensity of furrowing is much lower in the former than in the latter egg halves. We assign this effect to the moderating activity of the second polar body's nucleus exerted through the cytoplasmic bridge connecting the two cells. It is probable that the longer this contact is maintained, the more distinct is the effect. 3. In the overwhelming majority of sister egg halves produced from fertilized eggs about 6 h *after* the appearance of surface deformations, fragmentation in AHs starts soon after their separation from the interphase nucleus and precedes cleavage in nuclear counterparts.

Because furrowing in anucleate egg halves like cytokinesis in nucleate cells is suppressed in the presence of cytochalasin B (Waksmundzka, unpublished observations), it follows that in both events cortical microfilaments are involved. However, in contrast to cytokinesis, fragmentation is characterized by formation of multiple furrows and random partitioning of the cytoplasm. It is conceivable that in the absence of the mitotic spindle the microfilaments cannot be properly organized and that several rather than one contractile ring are formed. We conclude that fragmentation reflects acquisition by the cytoplasm of the ability to form furrows, which in normal development is one of the prerequisites of cytokinesis. In nucleate cells this ability is suppressed by the nucleus and therefore cannot manifest itself until mitosis.

The major piece of evidence that fragmentation of anucleate egg halves represents a physiological rather than a degenerative phenomenon is its reversibility. In many AHs the number of furrows was substantially reduced so that the egg halves were finally composed of a few rather than numerous globules. In some anucleate halves the reversion was complete and the cytoplasts resumed a spherical shape and smooth surface. Our belief that furrowing of AHs is a temporary and transitory phase and that fragmentation is a fully reversible phenomenon, finds support in the observation that in F_1 eggs which develop *in vitro* much better than Swiss eggs, fragmentation was completely reversed in over 60 % of anucleate egg halves (Waksmundzka, unpublished observations).

Our observations on mouse eggs and anucleate egg halves support the hypothesis of a 'cytoplasmic clock' formulated on the basis of earlier experiments on invertebrate and amphibian eggs and anucleate egg fragments (for references see Introduction; for review see Satoh, 1982). However, what distinguishes the mouse egg from the eggs of other animals studied so far in this respect, is the timing of cortical activity during the cell cycle. In invertebrate and amphibian eggs the periods of cytplasmic and/or cortical activity most often coincide with successive cleavages. This is not the case with the mouse egg in which surface deformations appear half way through the first cell cycle, and in which fragmentation of anucleate egg halves precedes cytokinesis of their nuclear counterparts by several hours. This difference in the timing of cortical activity could be attributed to the differences in the total length of the first cell cycle (sea urchins:

several minutes, amphibians: 1–3 h; mouse: several hours (in the present experiments about 20 h)) or, more specifically, in the duration of G_2 phase which in the first cell cycle of mouse eggs is particularly long, lasting in some strains 5–6 h, or possibly even longer (cf. Molls, Zamboglou & Streffer, 1983). One may speculate that in the evolution of placental mammals the G_2 phase has been secondarily extended and that this evolutionary new feature has not modified the timing of cortical activity which proceeds at its ancient pace. Consequently, the cortical and nuclear cycles have been desynchronized.

Experiments which are in progress have shown that in anucleate halves of F_1 mouse eggs a second wave of surface deformations appears after the reversion of fragmentation. The results of these pilot experiments suggest the periodic character of the observed cortical activity and strongly support the existence of a cytoplasmic clock in the mammalian egg.

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