Abnormal oogenesis and embryogenesis resulting from centrifuging Drosophila melanogaster females

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SUMMARY

Females of Drosophila melanogaster were centrifuged at 2400 g and 4200 g for 3 h in a number of different orientations. The oocytes in various stages of vitellogenesis become separated into three layers; a centrifugal yolk layer, a central cytoplasmic layer, and a centripetal lipid layer. The direction of layering is related to the orientation of the female. The process of recovery of the ovaries was followed and the development of the eggs laid analysed. Many of the eggs laid over the subsequent 3 days die very early in development and fail to produce any differentiated structures. Some hatch into normal larvae and others produce defective embryos. The most common defect being a misarrangement of the segmentation. The differences in the kinds of experimentally inducible pattern aberrations in Drosophila and other diptera is discussed.

INTRODUCTION

Embryologists have for many years centrifuged the uncleaved eggs of various animal species to displace their cytoplasmic components and observed the effects of this treatment on the subsequent embryogenesis (Conklin, 1931; Overton & Raab, 1967). Information was gained about the pattern-forming mechanisms present in the early embryo. Since the Drosophila embryo becomes mosaic very early in development (Chan & Gehring, 1971; Illmensee, personal communication) it seems appropriate to use centrifugation to discover what information is laid down in the oocyte by the mother during oogenesis.

It was predicted by Bownes (1973) that centrifuging Drosophila oocytes would lead to the production of 'double abdomen' embryos where the head and thorax are replaced by a second abdomen arranged in mirror image symmetry to the first. The general rationale for this was that centrifugation and u.v. irradiation of the eggs of lower diptera (Yajima, 1960, 1964; Kalthoff, 1971, 1976) produces double abdomen embryos, yet these techniques in Drosophila lead to defective and missing parts of the embryo (Howland, 1941; Bownes & Kalthoff 1974; Bownes & Sander, 1976). The bicaudal mutation of Drosophila (Bull, 1966) is a maternal effect mutation and consequently it was thought that if polarity

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reversals could be induced in *Drosophila* embryos, the time for this would be during oogenesis (Bownes, 1973).

Since the oocytes of *Drosophila* are arranged in ovarioles running parallel to the anterior/posterior axis of the adult (King, 1970) with the anterior of the oocyte facing the head of the fly, orienting the fly in the centrifuge orients the oocytes in a similar way. The aligned oocytes are also arranged in increasing age in the ovarioles with the most advanced nearest the tip of the abdomen. The time course of oogenesis is well known (King, 1970); therefore, by analysing the ovaries we can observe the effects of centrifugation on the various stages of oogenesis and observe the way in which these oocytes, when laid in sequence, develop.

Double abdomens were not observed after centrifuging oocytes. However, both the abnormal embryos laid by centrifuged females and the way in which the ovaries of the females recover from centrifugation provide some clues to the mechanism of development and pattern formation in *Drosophila*.

**MATERIALS AND METHODS**

*Preparation of females*

Newly hatched females and males were collected over 24 h, mated and placed in bottles of well yeasted Lewis medium for 4 days at 25 °C. The females are by this time producing lots of eggs. They are etherized and arranged for centrifugation.

*Arrangements of flies for centrifugation*

Small centrifuge tubes in which one female could just fit vertically were used for centrifuging towards the anterior and towards the posterior (see Fig. 1a, b). Small pieces of tygon tubing which could just hold a female were used for centrifuging in other directions. Larger centrifuge tubes were packed with Blu-tac (Bostic) such that the pieces of tygon tubing sat on them at a 45 ° angle or horizontally. Thus flies were centrifuged at a 45 ° angle towards the anterior or posterior (Fig. 1c, d) or sideways (Fig. 1e). Small pieces of cotton wool were inserted at both ends of the tygon tubing after the flies were inserted. Control flies were placed in tubes for an equal length of time to centrifuged flies.

For all the experiments flies were centrifuged in a Sorvall RC5 using an HS4 rotor. Flies were centrifuged at approximately 2400 or 4200 g for 3 h. At these speeds up to 60 % of the flies may die, usually due to ruptured abdomens. How many die varies with the orientation of the flies, most dying in arrangement 1a and least in 1e, (Fig. 1). We are therefore at 4200 g using the maximum speed the flies can withstand under our conditions of centrifugation.

*Analysis of ovaries*

Ovaries were dissected at various times after centrifugation. The ovarioles were teased apart in Gehring's Ringer solution, then mounted in Ringer's
Centrifugation of Drosophila females

Fig. 1. The five orientations used for the centrifugation of females.

and observed using Nomarski interference optics. This shows the arrangement of the oocytes and any layering of the cytoplasm quite clearly. The nuclei of the nurse cells and follicles are visible and distinct. To further check the nuclei some ovaries were stained with Feulgen and analysed.

Collection of eggs

After centrifugation females were placed in bottles and their eggs collected on yeasted agar plates for the next 3 days.

Some eggs were dechorionated manually by rolling them on Scotch tape and their development was followed under Volatolef oil (Lehmann & Voss).

Other eggs were dechorionated in 3% sodium hypochlorite for 5 min and kept on agar plates for 24 h at 25 °C.

Analysis of embryos

At the time when most larvae have hatched the embryos were further classified into: (1) normal or hatched larvae; (2) abnormal embryos or larvae; (3) dead eggs with no sign of differentiated structure. Abnormal embryos were further analysed and classified using Zeiss Nomarski interference optics. Some of the embryos were mounted in Gurr's water mounting medium both to preserve them and to make analysis of the abnormal segmentation easier.

RESULTS

Normal oogenesis

Oogenesis has been described in detail by King (1970). We have used his stages to describe our results. Stages 7–14 of his series are shown in Fig. 2, since those are the stages relevant to our results. Yolk formation commences at stage 8 and the eggs are ready to be laid at stage 14.

Appearance of ovaries immediately after centrifugation

Oocytes at stages 10–14 of vitellogenesis generally became stratified into three layers: a centrifugal layer which is an accumulation of yolk; a centripetal layer of lipid droplets; and a central layer of transparent cytoplasm (Fig. 3). This
type of stratification was also observed by King, Bentley & Aggarwal (1966).
In an earlier stage of vitellogenesis (stage 9) only a slight separation into two
layers could usually be observed. No clear cytoplasmic layer could be detected.
Stratification was most apparent and most complete in stages 10–13. Not all
stage-14 oocytes became separated into three layers, some appeared to be normal
and in others the layers were indistinct.

The direction of stratification was related to the direction of centrifugation.
Flies were centrifuged in the five orientations shown in Fig. 1. Throughout the
results they will be referred to as 1a–1e to identify the orientation. However,
not all oocytes had exactly the same pattern of layering. Due to the curved shape of the ovaries different oocytes had slightly different orientations. Also tilting of follicles in the ovary, or of eggs in the oviduct during centrifugation may account for some of the variations in layering. Within one ovary there was a distribution of patterns (Fig. 4) of layering and consequently an overlap in the direction of the layers was observed between the different orientations of the females.

Depending upon the direction of the centrifugation there was some displacement of oocyte contents. At stage 10, when centrifugation was towards the anterior pole (1 b) or at a 45° angle towards the anterior (1 d) the oocyte contents spread out amongst the nurse cells (Fig. 5). In no case did the contents of the oocyte leak out of the egg chamber. Quite often at stage 10, when the nurse cells are heavily laden with lipids, the nurse cells would become separated into two layers. This was especially apparent after sideways centrifugation (1 e) when all the lipid yolk lays to the same side of all the nurse cells (Fig. 6). With centrifugation towards the head (1 b) and at a 45° angle (1 c and 1 d) the nurse cells at stages 12 and 13 were often displaced and found to one side of the oocyte rather than at the anterior (Fig. 7). This was clearest in the Feulgen-stained preparations.

Changes in the ovaries over the 30-h period after centrifugation

(a) Controls. To be sure that any changes observed were due to the centrifugation and not to the fact that the flies were etherized, confined in tubes and starved for 3 h, control flies were placed in the tubes for 3 h. The ovaries were then dissected at regular intervals during the next 30 h. At all times the ovaries
Fig. 3. Stage 12. Oocyte after sideways centrifugation. There is a centrifugal yolk layer, a central cytoplasmic layer and a centripetal lipid layer.

Fig. 4. Stage 14. Oocytes dissected from a female centrifuged towards the abdomen. Some are unlayered and one is layered sideways instead of vertically as expected and as seen in the other two oocytes.

Fig. 5. Material from oocyte has been pushed amongst the nurse cells. By observing the contents at different focal levels the material amongst the nurse cells can be seen to be connected to the oocyte by a narrow channel.

Fig. 6. The contents of the nurse cells can be seen to be displaced by centrifugation.

Fig. 7. The nurse cells of this stage-13 oocyte are lying lateral to instead of anterior to the oocyte.

Fig. 8. A decaying stage-10 egg chamber.
Fig. 9. Oocytes up to stage 9 are present. There are none in stages 11–13. There are still some stage-14 oocytes to be laid.

Fig. 10. There are no oocytes beyond stage 10 in this ovary.

appear large and healthy. All stages of oogenesis were always present and appeared normal.

(b) Experiments. The pattern of change in the ovary was followed using females centrifuged in all five directions. Ovaries were dissected every 2 h during the 30 h period after centrifugation. Some were observed directly and others were Feulgen stained and then observed. A minimum of three females were observed by each technique for every 2 h period and each direction of centrifugation. For these experiments the speed of centrifugation was 4200 g. Less frequent dissections were also made after centrifugation at 3500 g and similar results were observed. Although the stratification patterns in the oocytes was different after the various orientations during centrifugation the mechanism of recovery of the ovary was similar in all cases. From 2 h onwards some decaying egg chambers were observed. By their size and position one could deduce they were at stages 8, 9 and 10A (Fig. 8). Not all females showed decaying chambers and not all of the chambers at that stage within a female decayed. Decaying chambers were observed for 24 h. Each female seemed to go through the ‘recovery’ process at a different rate. Sometimes the ovaries appeared quite normal by 24 h, yet in other cases there were still some layered stage-14 oocytes present after 30 h.

All females observed fit into the following general pattern. Oocytes which were in the various stages of vitellogenesis during centrifugation either decay or proceed to stage 14 and are laid. Oocytes at stages 1–7 during centrifugation are delayed in their development. They do not proceed into vitellogenesis for several hours. This is recognized by ovaries having egg chambers up to stage 7, for example, then none from stage 8 to 13, then lots at stage 14. Subsequently all the ovarioles commence vitellogenesis again and females are observed
with oocytes from stages 1-8 and stage 14 present; stages 1-9 and stage 14 present; and in some instances stage 1-10 and stage 14 present (Fig. 9). There is then a period when the dissected ovaries are very small and contain no later stages at all. Some were observed with nothing beyond stage 9 or 10 (Fig. 10). From this point the ovaries gradually regain their normal appearance and later egg stages from 11 to 14 are observed. Ovaries were found with stages missing but stage 14 still present from 10 to 30 h. Small ovaries with no late stages were observed from 12 to 30 h. The first normal ovary with eggs at all stages was observed at 18 h. Since all the females were the same age and had come from the same environment there is no obvious explanation of why they should proceed to recovering normal oogenesis at such different rates. Because it is not possible to follow the recovery process of oocytes in an individual female we cannot tell if there is any reorganization of the layered oocyte contents after centrifugation, but before the mature eggs are laid.

**Fate of eggs laid by centrifuged females**

Females at all five orientations were centrifuged at approximately 2400 g and 4200 g. At 2400 g some double centrifugations were performed. Flies were spun for 3 h in one direction then were reoriented and spun for 3 h in the opposite direction. This was done with the flies in orientation 1a followed by 1b, and 1c followed by 1d.

For most experiments eggs were collected for 24-h periods over 3 days following centrifugation. In two experiments eggs were collected every 2 h over the first 30 h after centrifuging to see if there was any particular time when abnormal eggs were deposited or if there was any sequence in the types of abnormal eggs laid. Abnormal eggs of all types were laid during the whole of this period. Consequently all the data have been pooled in 24-h periods.

Eggs were classified into (1) normal larvae, (2) abnormal embryos, and (3) undifferentiated eggs which had either failed to develop entirely or had died very early in development and had produced no differentiated tissues. The mean number of eggs produced per female was also calculated to see if centrifugation interfered with egg production. The results can be seen in Tables 1a–c.

The average number of eggs laid per female per day is either slightly reduced or not affected after centrifugation at 2400 g, except in the case of the double centrifugations, in which there was a considerable reduction in egg laying. At 4200 g, however, egg production was considerably reduced when compared to the controls. Centrifugation at high speeds does, therefore, affect egg production in females as well as the development of the eggs which are laid.

The number of abnormal eggs laid is generally greatest on the first day after centrifugation and falls off on the second and third days. Over all 3 days more abnormal embryos are produced after centrifugation at 4200 g than at 2400 g. As can be seen from Table 1, there are few exceptions to this general pattern.
### Table 1. Fate of eggs laid by centrifuged females

<table>
<thead>
<tr>
<th>Orientation of females</th>
<th>Period of egg collection in hours</th>
<th>Eggs dead (%)</th>
<th>Eggs abnormal (%)</th>
<th>Normal larvae (%)</th>
<th>Total eggs laid</th>
<th>Eggs laid per female</th>
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<tr>
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<td>(b) Centrifugation at 2400 g</td>
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<td>1d, followed by 1b</td>
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<td>(c) Centrifugation at 4200 g</td>
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<td>6-3</td>
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<td>15-4</td>
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Fig. 11. (a) Class 1. Abnormal embryo. This shows partial deletion of rows of chaetae (Ch) and abnormal fusions of the segment.

(b) Class 1. Abnormal fusions of segments and a case where half an abdominal segment is linked to half a thoracic segment (×).

(c) Class 1. Rows of chaetae radiate out from one point near the posterior of the embryo.

(d) Class 2. There is a patch of undifferentiated tissue at the posterior and extended Malpighian tubules (MT). The segments are crowded into the anterior half of the egg.

(e) Class 3. There is yolk at the anterior of the embryo. Segments are crowded into the posterior two-thirds of the egg. Abnormal parts of the mouthhook apparatus are present (MH).

(f) Class 6. Half an embryo has formed along one side of the egg. There is only one mouthhook. To the other side is a mass of undifferentiated tissue.
The greatest increase in abnormal embryos with increasing speed came from those flies which were oriented vertically (1a and 1b).

Large numbers of eggs failed to develop on all 3 days subsequent to centrifugation. Even when the ovaries appear normal once more, there must still be some defects in the organization of the eggs, such that normal development is prevented or not even initiated. It is also possible that centrifugation affects the stored sperm in some way and prevents some eggs from being fertilized normally.

Most of the eggs which were laid were of normal size and shape. However, after all directions of centrifugation a few eggs were laid (approximately 1%) which were half or two-thirds the size of a normal egg. These failed to develop except in one case where an abnormal embryo was observed. They were seen on all 3 days subsequent to centrifugation. Centrifugation also seems to have affected the amount of yolk deposited in the eggs, but these eggs are presumably deficient in various factors needed to initiate development.

**Analysis of abnormal embryos developing from eggs laid by centrifuged females**

Embryos which developed abnormally were classified according to the following scheme:

**Class 1: Abnormal segmentation.** Embryos developed with abnormal segments. This could be clearly seen by the rows of chaetae which mark the segmental boundaries on the ventral side of the embryos.

Whole or partial rows of chaetae were missing; rows of chaetae were fused into one another (Fig. 11a, b); large patches of chaetae were observed not organized into rows; sometimes small ‘scars’ were observed with chaetae radiating from that point; the rows of chaetae could be disoriented and run at angles or anterior/posterior in the egg instead of transversely (Fig. 11e). In some embryos one half of an abdominal segment was joined to half of a thoracic segment. Also sometimes two or three segments could be present and normally organized on one half of the embryo and totally absent in the same region of the other half. This often gave the embryo a very abnormal shape. Some embryos in this class had abnormal head and mouthpart formation, although most had a normal head and mouthparts. Some hatched into larvae whilst others, which were more drastically disorganized, failed to develop tracheae and the internal tissues began to decay.

**Class 2: Anterior development.** The posterior of all the embryos in this class consisted of a mass of yolk and disorganized gut tissue. Sometimes Malpighian tubules were observed in the posterior mass. The mouthparts of these embryos were well formed. Next to the mouthparts were the thoracic segments and varying numbers of abdominal segments either normally or abnormally arranged. Often all eight abdominal segments were at least partially present, but crowded towards the anterior of the egg. In some cases there were even spiracles present, the most posterior part of the normal embryo, about two-thirds
from the anterior, thus showing an almost complete embryo crowded into the anterior region of the egg (Fig. 11d). When the embryos were stretched out, however, most of the segments were of normal size.

Class 3: Posterior development. The embryos in this class had yolk and undifferentiated tissue masses at the anterior of the embryo. The spiracles were present at the posterior with various numbers of abdominal segments, either normally or abnormally organized. Sometimes abnormal head formation and even abnormal mouthparts were observed about one-third from the anterior of the embryo, showing an almost complete embryo crowded towards the posterior of the egg (Fig. 11e). As in class 2 the segments were generally of normal size.

Class 4: Decaying embryos. These embryos had a normal shape and were surrounded by cuticle. Malpighean tubules were usually present. Tracheae did not form and often the segmented chaetae were very faint or absent. When they were present they were normally organized. However, the internal tissues were decayed and it was not possible to observe clearly defined gut tubes as in the normal larva.

Class 5: Abnormal head. Embryos in this class had abnormal head involution and abnormally formed mouthparts. Segmentation was normal.

Class 6: Non-specific defects. The embryos in this class were not abnormal in any particular region of the embryo. Most consisted of contracting gut masses with no real organization. A few embryos were also put in this class where a partially formed embryo was present on one side of the egg, occupying the middle two-thirds of the anterior/posterior axis (Fig. 11f).

Since there were no distinct differences in the classes of abnormal embryos laid on each of the 3 days the results are pooled for the whole 72 h. The results are shown in Table 2. These results were compared to the distribution of the abnormal embryos found in the controls using a chi-squared test. This showed that there was no significant increase at the 0.05 probability level in the number of abnormal embryos developing as class 3, 5 or 6. In the case of class 4, only one orientation was significantly higher, and that was 1d (45° head down) after centrifugation at 2400 g. This was not, however, found after centrifugation at 4200 g. In all experiments there was a significant increase in the embryos in class 1. In class 2 there was a significant increase after centrifugations 1e and 1c at both speeds and 1a at the higher speed.

These results show that redistributing the oocyte contents interferes with the organization of the segment pattern, no matter how they are oriented. Fusions, deletions and misarrangements of the rows of thoracic and abdominal chaetae were observed in all regions of the embryo. Affected segments ranged from the first to the last abdominal segment. There was no restriction of affected segments to one particular region of the embryo after any orientation for centrifugation. Class-2 embryos, however, where the anterior is formed and segments are sometimes crowded towards the anterior leaving the posterior as a mass of
Table 2. Classification of abnormal embryos

<table>
<thead>
<tr>
<th>Orientation of flies</th>
<th>Speed of centrifugation</th>
<th>Class – number in class (percentage in parentheses)</th>
<th>Total number abnormal embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>2400 g</td>
<td>0 (3 10) 3 (13 3) 4 (10) 7 (23 3) 13 (43 4)</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>4200 g</td>
<td>16 (23 9)* 17 (25 4) 11 (16 4) 12 (17 9) 7 (10 4) 4 (6 0)</td>
<td>67</td>
</tr>
<tr>
<td>1b</td>
<td>2400 g</td>
<td>25 (37 9)* 8 (12 1) 15 (22 7) 6 (9 1) 7 (10 6) 5 (7 6)</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>4200 g</td>
<td>21 (33 9)* 18 (29 0) 11 (17 8) 2 (3 2) 3 (4 8) 7 (11 3)</td>
<td>62</td>
</tr>
<tr>
<td>1c</td>
<td>2400 g</td>
<td>12 (30 8)* 18 (46 1)* 1 (2 6) 2 (5 1) 4 (10 3) 2 (5 1)</td>
<td>39</td>
</tr>
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<td></td>
<td>4200 g</td>
<td>12 (19 7)* 38 (62 3)* 6 (9 9) 3 (4 9) 1 (1 6) 1 (1 6)</td>
<td>61</td>
</tr>
<tr>
<td>1d</td>
<td>2400 g</td>
<td>29 (25 5)* 13 (11 4) 14 (12 3) 15 (44 7)* 4 (3 5) 3 (2 6)</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>4200 g</td>
<td>11 (28 9)* 8 (21 1) 11 (28 9) 2 (5 3) 3 (7 9) 3 (7 9)</td>
<td>38</td>
</tr>
<tr>
<td>1e</td>
<td>2400 g</td>
<td>46 (39 3)* 37 (31 6)* 10 (8 6) 8 (6 8) 7 (6 0) 9 (7 7)</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>4200 g</td>
<td>32 (36 0)* 29 (32 6)* 12 (13 5) 10 (11 2) 4 (4 5) 2 (2 2)</td>
<td>89</td>
</tr>
<tr>
<td>1a/1b</td>
<td>2400 g</td>
<td>4 (25 0)* 7 (43 8) 2 (12 5) 0 0 3 (18 7)</td>
<td>16</td>
</tr>
<tr>
<td>1c/1d</td>
<td>2400 g</td>
<td>8 (24 2)* 8 (24 2) 2 (6 1) 5 (15 2) 9 (27 3) 1 (3 0)</td>
<td>33</td>
</tr>
</tbody>
</table>

* A significantly greater number (P = < 0.05) of the abnormal embryos were in this class than in the controls.
undifferentiated tissue and yolk, form with a significantly increased frequency only after centrifugation towards the side, 45° towards the posterior or (at higher speeds only) directly towards the posterior. No examples of bicaudal or bicephalic embryos were observed.

Following the development of individual eggs

Since there were some differences in the frequency of occurrence of various classes of embryonic defects after centrifuging in different orientations, we wanted to see if there was any correlation between how an individual egg was layered and how it developed. The eggs used for this study were the first eggs laid by females centrifuged in positions 1a, 1b and 1e at 4200 g. The followed-up eggs are not included in Tables 1c and 2. They are very fragile and many break during the procedures to prepare them for analysis, in particular the heavily layered eggs. These eggs are not, therefore, a true representation of the normal distribution into classes since one particular type of egg may be more fragile than others.

The development of 492 eggs was followed. Of these 40% had the contents redistributed at the initiation of development. Some of the layered eggs showed the three distinct layers as the oocytes, in others only the lipid layer was clearly visible and the cytoplasmic and yolk layers had become intermingled. When the blastoderm forms the surface cells appear clear, as usual, and the layering can still be seen in the acellular cytoplasmic region of the egg. Of the eggs which were followed 32.5% died without differentiation, 19.9% developed abnormally and 47.6% developed into normal larvae. Of the eggs which died very early in development or failed to begin development 41.3% came from layered eggs. This layering was in all the directions possible. Of the normal larvae, 27.4% came from layered eggs; these too were layered in all directions. The abnormal embryos fell into all the classes and 68.4% developed from layered eggs. The 59 embryos in class 1 developed from eggs layered in all directions. Three of the 13 embryos in class 2 developed from unlayered eggs, the rest came from eggs layered such that there was lipid either directly at the anterior or at a 45° angle at the anterior of the egg. The other embryos – seven in class 3, eight in class 4, one in class 5, and ten in class 6 – mostly came from unlayered eggs but the few layered ones did not fit any special pattern. In the case of class 3 most of the embryos already had defects at the anterior at the blastoderm stage. Most of the eggs which failed to differentiate any structures failed to ever begin development.

From these results we can see that some normal embryos can hatch from layered eggs, and abnormal embryos and dead eggs can result from unlayered eggs. It is important to note that the fact that an egg has no layers when it is laid does not necessarily mean that there were not layers in the oocyte, which had subsequently become redistributed to their original position. They also show that having the components layered in any direction can cause defective
segmentation, yet embryos with the segmentation crowded into the anterior of the egg occurred only when the yolk was pushed to the posterior of the egg and the lipid to the anterior.

Adults hatching from eggs laid by centrifuged females

Some of the larvae which hatched from centrifuged oocytes were allowed to form adults. They were mostly normal in morphology. Some had abnormal abdomens with fusions and deletions of segments as might have been predicted from the abnormalities in the embryo (Bownes, 1976a, b). The only other defect was that occasionally the male genitalia failed to evert correctly. Because the embryos were of more interest, we did not do a detailed analysis of all the adults; anyway, this would have been a biased sample as most of the embryos with defective segments were mounted for analysis and could not, therefore, form adults. Generally, the adults we did look at were similar to those observed by Howland (1941) after centrifuging Drosophila eggs.

DISCUSSION

These results show clearly that redistributing the contents of oocytes during their development produces pattern defects during embryogenesis. They also show that the factors involved in establishing the body segmentation pattern are placed in the oocyte during oogenesis. Our results cannot, however, show that any determinants are laid in pre-localized positions during oogenesis. The most frequent defect after centrifugation in any direction was in the establishment of the normal segmentation which appears along the anterior/posterior axis of the embryo.

Sander (1975) and Kalthoff (1976) have proposed that there are anterior and posterior factors which gradually interact during early insect embryogenesis to establish the body segmentation pattern. It is possible that centrifugation of the oocytes displaces such factors and thus leads to aberrant pattern formation. We have clear examples where the egg was stratified laterally and abnormal segments developed. This suggests that there may also be some laterally distributed component needed to specify the anterior/posterior segmentation pattern in Drosophila embryos. Alternatively the abnormal egg architecture resulting from lateral centrifugation may interfere with the propagation of signals from the anterior or posterior, or with the interpretation of such signals into the correct developmental pathways.

Centrifugation of other dipteran eggs (Yajima, 1960; Overton & Raab, 1967; Kalthoff, Hanel & Zissler, 1976) leads to the production of double abdomen embryos. Yet in Drosophila these results are not obtained either with eggs (Howland, 1941) or with the developing oocytes. This is not just the case with centrifugation; u.v. irradiation, and puncturing the anterior of the embryo may also produce double abdomens in Smittia but not in Drosophila.
It seems that there must be some substantial difference in the organization of the *Drosophila* egg and other closely related dipteran eggs. Yet we know that the bicaudal genotype (Bull, 1966) produces double abdomens and is active during oogenesis, so the basic pattern specifying mechanism is probably similar in all these insects.

Kalthoff et al. (1976) have recently localized an anterior determinant in the cytoplasm at the anterior of *Smittia* eggs. In a series of very elegant experiments they were able to show that the factors are not bound to the oolemma but are probably ribosomes or subribosomal RNP particles present in the cytoplasm. It is possible that the difference between *Drosophila* and *Smittia* is that these factors become very rapidly membrane or cortex bound in *Drosophila* and are not therefore so easily displaced as in *Smittia*. Gross damage to particular regions of *Drosophila* eggs or oocytes would therefore result in defective development rather than the reprogramming of an alternative developmental pathway.

I would like to thank Sarah Roberts for her excellent technical assistance. This research was supported by the Science Research Council.

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**Centrifugation of Drosophila females**


(Received 11 October 1976)