Contribution to the Study of Germ-cells in the Anura

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WITH TWO PLATES

INTRODUCTION

The problems of the germ-line of cells are of long standing in animal biology, but of these two surpass the rest in importance. At what stage in the life-cycle do the primordial germ-cells make their appearance, and do these gonocytes give rise to all, some, or none of the definitive sex-cells?

Since Nussbaum (1880, 1884) first discussed the continuity of the germ-cells from one generation to the next, study in this field of embryology has resulted in a measure of agreement that the primordial germ-cells make their appearance in the endoderm or mesoderm early in development.

The subject has been extensively reviewed in recent years by Bounoure (1939), Dantschakoff (1941), Everett (1945), Nieuwkoop (1946), and Johnston (1951). In the invertebrates, the origin of the gonocytes during cleavage is well established for some species (e.g. Parascaris), but, for the vertebrates, only Bounoure (1934) makes a claim for the formation of the primordial germ-cells as early as the blastula stage. His papers report germinal localizations in the cytoplasm of the fertilized egg of Rana temporaria which eventually become included in the primordial germ-cells. Apart from a report of a similar plasm in the early egg of Xenopus laevis by Nieuwkoop (1956 a, b), the claim of Bounoure occupies an isolated position, in spite of its obvious importance.

The work reported in this paper represents in the first instance a test of the validity of Bounoure's observations, but the author was interested also in examining the fertilized but uncleaved egg in greater detail, and in investigating stages earlier than those described by him. The extension of the cytological techniques involved to the eggs and embryos of other Anuran species seemed of importance in establishing the general status of Anuran germinal cytoplasm.

MATERIALS AND METHODS

The material employed in these investigations consisted of the eggs, embryos, and larvae of R. temporaria, R. esculenta, Bufo bufo and X. laevis. Eggs of the first three species were obtained from ponds in Surrey, from animals that had

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been kept in the laboratory for some time, and from pairs in amplexus brought into the laboratory to spawn. *Xenopus* eggs were obtained by the usual method of gonadotrophic hormone injection.

Most sections were cut at 5 and 7½ μ and stained according to the procedure outlined by Bounoure (1934). Dehydration with butanol gave better results than ordinary laboratory ethanol, and it is most important not to post-chrome the material for longer than 50 hours at 45°C if sections with reasonably intact yolk-endoderm are to be obtained. The adherence of yolk to the slide is facilitated by dipping the dewaxed sections in a weak solution of celloidin prior to staining.

Some sections were tested for their pentosenucleic acid (PNA) content using the method of Brachet (1940) as modified by Darlington & La Cour (1947).

**RESULTS**

The results are set out separately for each of the four species, but for purposes of comparison Table 1 shows the number of eggs and embryos (but not larvae) examined for each species, and the number positive for localizations of germinal cytoplasm.

<table>
<thead>
<tr>
<th>Description of stage</th>
<th>R. temporaria</th>
<th>B. bufo</th>
<th>R. esculenta</th>
<th>X. laevis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfertilized</td>
<td>7(–)</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Fertilized</td>
<td>35(29)</td>
<td>.</td>
<td>.</td>
<td>10(7)</td>
</tr>
<tr>
<td>Cleavage</td>
<td>31(28)</td>
<td>13(12)</td>
<td>.</td>
<td>19(16)</td>
</tr>
<tr>
<td>Blastulae</td>
<td>20(17)</td>
<td>7(7)</td>
<td>.</td>
<td>16(12)</td>
</tr>
<tr>
<td>Gastrulae</td>
<td>25(22)</td>
<td>6(6)</td>
<td>12(–)</td>
<td>9(9)</td>
</tr>
<tr>
<td>Neurulae</td>
<td>21(17)</td>
<td>2(2)</td>
<td>13(–)</td>
<td>13(10)</td>
</tr>
<tr>
<td>Tailbud</td>
<td>7(4)</td>
<td>4(4)</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

In the case of *Bufo, Xenopus*, and *R. esculenta* the results are set out in a regular order of developmental stages, but not in the case of *R. temporaria*. For, since the blastula of this species is the most convenient stage for the clearest observation of germinal cytoplasm, it was thought best to deal with this first.

**A. R. temporaria**

1. **From the blastula stage to tadpoles of length 11–12 mm.**

The germinal cytoplasm of Bounoure may be found most easily in sections cut from early to mid blastulae where it is contained within cells sited between the floor of the blastocoel and the vegetal pole. It is stained a distinct purple and is always seen lying peripherally in the cells that contain it in an area free of yolk inclusions. The number of cells that possess the plasm varies between 5 and 7, each cell usually containing a single islet whose maximum area is about three
times that of the nucleus. During mitosis, the germinal plasm usually, but not always, becomes confined to one only of the daughter-cells.

In late blastulae some cells may retain the plasm in the position as described above, but others clearly show the germ-plasm enveloping the nucleus as a ‘cap’. A few cells may actually demonstrate the intracellular migration of the germinal cytoplasm from the cell periphery to the juxtanuclear position (Plate 1, fig. A). By the time gastrulation is really under way, every cell possessing germinal plasm exhibits it in the nuclear position.

During gastrulation the cells with germ-plasm, which now number between 11 and 23, are moved by the morphogenetic movements from a superficial position in the floor of the blastocoel to the region of the mid-endoderm of the young neurula. From this time forward, the plasm progressively loses its affinity for the stains employed until, by the time the larva is 12 mm. in length, the plasm no longer takes up stain.

No significant change in the number of plasm-bearing cells is to be observed during neurulation, and, indeed, no mitoses were ever seen in them during this period.

At hatching (larvae of about 8 mm.) the number remains the same, but in the intervening period the plasm-bearing cells have moved from their deep endodermal position, around the archenteron (which by this time has been reduced to a slit) on both sides, to the dorsal crest of the endoderm. Sections showing all stages of this migration have been obtained. The large and clear nucleus, with its two nucleoli and corona of deep-red germ-plasm, is clearly seen in each of the plasm-bearing cells of the endodermal crest (Plate 1, fig. B). The position of these cells is precisely the same as that described for the primordial germ-cells of other Anuran tadpoles at this stage by Kuschakewitsch (1910), Humphrey (1925), Cheng (1932), and others.

In the 10-mm. tadpole the cells with germ-plasm are to be found in a median ridge at the top of the dorsal mesentery; the outlines of the cells are more clearly seen if thionin or light green stains are used instead of methylene azur. The length of this ridge and the number of germ-cells in it for six larvae are given in Table 2.

<table>
<thead>
<tr>
<th>Larval length (mm.)</th>
<th>Germ-cell. no.</th>
<th>Length of ridge (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.4</td>
<td>31</td>
<td>550</td>
</tr>
<tr>
<td>10.0</td>
<td>47</td>
<td>600</td>
</tr>
<tr>
<td>10.2</td>
<td>41</td>
<td>480</td>
</tr>
<tr>
<td>10.0</td>
<td>33</td>
<td>700</td>
</tr>
<tr>
<td>10.0</td>
<td>...</td>
<td>600</td>
</tr>
<tr>
<td>10.2</td>
<td>43</td>
<td>550</td>
</tr>
</tbody>
</table>

Stages demonstrating a ‘migration’ of the cells up the dorsal mesentery from the dorsal endodermal crest were never seen; as the endoderm becomes with-
drawn from the region of the aorta, the ridge of cells becomes left behind during the formation of the mesentery. The median position adopted by the cells described was exactly the same as that described for the primordial germ-cells of this species at this stage by Bouin (1901), and there seems little doubt in consequence that the cells that contain germinal cytoplasm (now, however, only weakly staining) are indeed primordial germ-cells. Their cytological characteristics (such as the clarity of the nucleus, the possession of yolk platelets, &c.), apart from the germ-plasm itself, are consistent with this view.

In 11-mm. tadpoles the primordial germ-cells come to lie in paired gonadal rudiments, except anteriorly where the ridge is still median. At this time the germ-plasm no longer stains, but the abundance of juxtanuclear mitochondria, and the persistence of yolk platelets (which in other kinds of cell are almost always reduced in size and number, or, in the case of the somitic mesoderm and blood, used up) do not permit of any other interpretation but that the same cell-type is being dealt with. Between the primordial germ-cells, and also around them, lie cells with elongated, densely-staining nuclei. It was difficult to be sure that any of these peritoneal cells were not transforming into primordial germ-cells, but equally there was no indication that they were doing so. Germ-cell counts and ridge lengths for six tadpoles of 11–12 mm. overall length (snout to anus 4 mm.) are given in Table 3.

### Table 3

<table>
<thead>
<tr>
<th>Ridge length in μ</th>
<th>570</th>
<th>507</th>
<th>650</th>
<th>559</th>
<th>415</th>
<th>600</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germ-cell no. in left ridge</td>
<td>30</td>
<td>26</td>
<td>29</td>
<td>21</td>
<td>29</td>
<td>24</td>
</tr>
<tr>
<td>Germ-cell no. in right ridge</td>
<td>29</td>
<td>25</td>
<td>23</td>
<td>22</td>
<td>36</td>
<td>20</td>
</tr>
<tr>
<td>Germ-cell no. in median position</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>8</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Total germ-cell number</td>
<td>63</td>
<td>53</td>
<td>57</td>
<td>51</td>
<td>66</td>
<td>48</td>
</tr>
</tbody>
</table>

The above observations confirm in almost every detail Bounoure's own findings, and show that certain cells of the blastula stage are directly ancestral to the primordial germ-cells, whether or not these latter cells actually give rise to definitive sex cells.

2. From the blastula stage to the ovarian oocyte

In cleavage stages, the islets of germ-plasm are found in the vegetal macromeres closely applied to the vertical cleavage cell-walls (see Plate 1, fig. E for an example from another species).

Now when batches of fertilized but uncleaved eggs came to be examined, islets of germinal cytoplasm could sometimes be found among the yolk platelets
in the neighbourhood of the vegetal pole, sometimes not. This remained true when a fresh batch of eggs was prepared; in two samples totalling 12 eggs only 5 had positive examples of plasm. It seemed reasonable at the time to suppose that perhaps some of the eggs in the uncleaved samples were unfertilized, and, working on this assumption, material was prepared from a batch of eggs obtained by artificial fertilization.

The examination of sections of a sample of 5 eggs fixed immediately prior to the first cleavage (the first furrow was present only as a wrinkle at the animal pole) revealed islets of germinal cytoplasm lying in a region concentrated at the vegetal pole. Each islet measured about 10 \( \mu \) and was surrounded by conspicuous mitochondria (see Plate 2, figs. A, D for examples of other species).

By contrast, sections cut from a sample of unfertilized eggs (fixed from the same batch later artificially fertilized) did not exhibit any germ-plasm. All 7 of these eggs were spherical in contrast to the oval shape of the eggs comprising the sample referred to in the previous paragraph, a point confirming the observation of Selman & Waddington (1955), that fertilized eggs become elongated in the animal–vegetal axis before the first cleavage.

Samples of fertilized eggs fixed 46 minutes, 1 hour 36 minutes, and 2 hours prior to cleavage all showed germ-plasm in sections, although the islets were smaller (7–5 \( \mu \)) and more widely scattered over the periphery of the vegetal hemisphere the nearer fertilization the sample had been fixed. A sample of eggs fixed 15 minutes after fertilization did not exhibit any islets of germinal plasm in sections.

The most interesting sample studied had been fixed at 2 hours 26 minutes prior to cleavage (i.e. about 35 minutes after fertilization). The germ-plasm was seen in the process of formation in the vegetal cortical region of all 8 eggs in the sample. The cortex itself stains blue with the methylene azur, and the islets of plasm were seen as pale purple areas with mitochondrial associations occupying a very superficial position (Plate 1, fig. D). The sites of formation were widely spread over the vegetal hemisphere.

Sections of yolk-free oocytes from the winter ovary of a frog are found to be free of any localizations of cytoplasm that may be interpreted as germinal. The same is true of ovarian yolky oocytes.

3. Parthenogenetic blastulae

As the appearance of the islets of germinal cytoplasm is so closely associated with fertilization, it is of some interest to know whether this appearance is dependent or independent of sperm entry. Eggs were taken from a ripe female frog and stimulated to develop parthenogenetically by pricking with a tungsten needle to which were adherent traces of frog's blood, i.e. after the manner of Bataillon (1910 a, b; 1913).

A small sample of 6 normal-looking parthenogenetic blastulae showed the presence of germinal cytoplasm in cells below the blastocoel floor. Thus the
appearance of germ-plasm in the fertilized egg seems to be independent of sperm entry and, in fact, is part of the cortical reaction that follows the fertilization process.

4. **Pentosenucleic acids (PNA) and germinal cytoplasm**

   (a) *Ovary.* In the yolk-free oocytes the whole cytoplasm stains pink, and the nucleoli (of which up to 25 per section may be seen) a distinct red with methyl green-pyronin. In the yolky oocytes only the cytoplasm in the immediate vicinity of the nucleus stains pink, although red granules are also to be found in the cortical plasm.

   When sections are treated with ribonuclease, the areas mentioned no longer stain pink. These results are in accord with those of Brachet (1940) and indicate the presence of PNA in nucleoli and cytoplasm.

   (b) *Fertilized material.* Sections of 5 μ thickness were cut from a sample of 7 fertilized but uncleaved eggs fixed just before cleavage and mounted so that alternate sections appeared on each of two slides. For any one egg, one slide was treated with ribonuclease and then both slides were taken through the stain together. All untreated slides exhibit pink islets among the yolk platelets of the vegetal region, and these were indistinguishable in character from islets of germinal cytoplasm stained according to the methods of Bounoure. In treated slides, no part of the sections is stained pink and areas corresponding to those stained pink in the control slides are clear. Here, then, is an indication that germ-plasm is associated with a PNA component.

   In blastula material, pink-staining areas were found in some of the endodermal cells. Unfortunately their outlines were indistinct and it was not possible to make an accurate estimate of the number of such areas. In ribonuclease-treated sections these areas are clear. An association of germ-plasm with PNA is again indicated, but not on such strong grounds as before.

   When six gastrulae were sectioned the stain did not reveal pink areas in any cells of any section.

**B. B. bufo**

Islets of germinal plasm similar to those found in *R. temporaria* are to be found scattered among the yolk platelets in the vegetal pole region of eggs fixed prior to and during the first cleavage. At the 32-cell stage some of the vegetal macromeres show examples of germ-plasm in the position already seen in *R. temporaria;* however, the clarity of the plasm is very striking at this stage and the plasm is often surrounded by black pigment granules. When a cell adjacent to the developing blastocoel contains germinal plasm a narrow protuberance may sometimes be seen projecting from it into the lumen. This protuberance contains germ-plasm together with some yolk and marked pigment aggregations, and it seems reasonable to believe that some extrusion process, unknown for the eggs of *R. temporaria, is under way.*
In a sample of 7 mid-late blastulae germinal plasm was found with ease in sections cut from every one. The average number of plasm-bearing cells at this stage is 17. In 5 of the blastulae globules of germinal cytoplasm, yolk, and pigment were found lying free in the blastocoel (Plate 2, fig. B); the biggest of these were 65 μ in diameter. The areas of germ-plasm within the cells of the blastocoel floor measured about 35 μ in diameter.

Gastrulae and neurulae possess examples of juxtanuclear germinal plasm in cells lying deep in the endoderm. Here the plasm has lost its striking purple appearance and stains mauve; another feature is the density of the mitochondrial population around the nuclei of the plasm-bearing cells (Plate 2, fig. C). In four tail-bud stages examined, primordial germ-cells were seen between the mid-endoderm and the base of the archenteron. The fugitive staining of the germ-plasm made counting difficult in these embryos, but the numbers of ‘positive’ gonocytes in each were 11, 17, 17, and 14.

In 6 tadpoles of length 10-11 mm. the primordial germ-cells were found at the top of the dorsal mesentery and in the germ-ridges. The nuclei of these cells stain pale blue with the methylene azur, and are often bilobed. One or two red nucleoli may be present together with a circumnuclear cloud of mitochondria.

These results show that the history of the primordial germ-cells in *B. bufo* is essentially the same as that in *R. temporaria*, the main differences being the peculiar extrusion of germinal plasm into the blastocoel and the large mitochondrial populations in the germ-cells.

**C. *R. esculenta***

Because of the difficulty experienced in trying to get freshly deposited spawn and the lateness of the season, only late stages of development were available for study. This was particularly unfortunate in view of the discovery that the blastula stage is the best stage in which to distinguish the germinal cytoplasm for the first time.

*(a) Tadpole stages.* Primordial germ-cells possessing the characteristics described by earlier workers in this field are easily found in sections cut from tadpoles of 12 mm. total length (Cambar & Marrott, stage 38). At this stage the cells are sited in the gonadal ridges in which the primary gonadal cavity has already made its appearance. The ridges are 350–450 μ in length and begin about 3 mm. posterior to the snout. The nuclei stain pale-red, are large and often possess two nucleoli, and are surrounded by mitochondria and yolk platelets (which by this time have disappeared from other tissues). The germ-cells are very numerous (Plate 2, fig. F).

Sections from all of 9 tadpoles fixed at Cambar & Marrott stage 30 demonstrated the process of the separation of the primordial germ-cells from the dorsal crest of the endoderm (Plate 2, fig. E), and in 7 tadpoles of stage 25 the gonocytes were seen in the dorsal crest of the endoderm. In these the average ridge length is 570 μ and the germ-cell number 26. The red corona, which is so charac-
teristic of the gonocytes of \textit{R. temporaria} at this stage (Plate 1, fig. B), was never seen.

(b) Neurulae and gastrulae. Of 13 neurulae examined no section showed any cell that could be confidently assigned to the germ-line. No germinal cytoplasm, as seen in \textit{B. bufo} or \textit{R. temporaria}, was to be seen, and the nuclear size and clarity varied widely from cell to cell.

The earliest stages examined consisted of 12 gastrulae at various stages of blastopore formation. No germinal cytoplasm was seen in any section cut from this material.

D. \textit{X. laevis}

(a) Cleavage. A study of 29 eggs demonstrated the presence and similar position as in \textit{R. temporaria} of islets of germinal plasm (Plate 2, fig. D).

(b) Embryonic stages. At stage 8 (\textit{Xenopus} normal table, Nieuwkoop & Faber, 1956) the germ-plasm is confined to cells lying in the floor of the blastocoel, in which cells it consistently adopts a peripheral position. The areas are somewhat larger than in eggs of the common frog. Cases of the envelopment of the nucleus by the germ-plasm are found in stage 9.

In gastrula stages (11 and 12), the plasm is always investing the nuclei of cells containing it. From this time on the plasm assumes a slate-grey colour in sections stained with Altmann and methylene azur stains.

In neurulae the gonocytes are found deeply embedded in the endoderm, but they are sited much nearer each other than in \textit{Bufo} or \textit{Rana}. The germ-plasm is very conspicuous and occupies a large part of the cells that contain it; the yolk platelets in these cells are somewhat smaller than in cells that are not gonocytes.

(c) Tadpole stages. At the stage when the mouth is breaking through (stage 40), the primordial germ-cells are lying in the dorsal endoderm or to one side of the archenteric slit. The nuclei of these cells are large and associated with conspicuous mitochondria, but germinal cytoplasm fails to stain. Thus, by comparison with tadpoles of the common frog and toad, the primordial germ-cells of \textit{Xenopus} are not so clearly demarcated.

In the latest tadpole stages examined (44–47), the gonocytes were either median or on each side of the dorsal mesentery in its upper attachment. Again, the appearance of these cells is not so impressive as in the other species, including \textit{R. esculenta}, where the yolk platelets are large and numerous compared with those of the South African Clawed Toad.

DISCUSSION

The histological observations made on eggs and embryos of \textit{R. temporaria} in this study substantiate Bounoure’s descriptions of the germinal cytoplasm and its history in almost every respect. As this cytoplasm is the only specific germinal element of the cytoplasm reported for any vertebrate, as far as the author is
aware, the simple confirmation of Bounoure's original findings is, perhaps, the most important outcome of this contribution.

Almost the same sequence of events leads up to the establishment of the primordial germ-cells in *X. laevis* and *B. bufo*, and it might be thought that the germ-plasm is a characteristic of all Anuran eggs were it not for our experience with *R. esculenta*. It is most unfortunate that these observations had to be carried out on such a small amount of material fixed later than when most of the interesting events in the history of the germ-cells in the other species were occurring. An examination of the early history of the primordial germ-cells in this species needs to be performed, although it may be that the germinal element in the cytoplasm of the gonocyte is not so localized as to be visually demonstrable.

The primordial germ-cells make their definitive appearance in late blastula stages, but cells of a germinal peculiarity extend back as far in development as the fertilized egg. Thus, while Nussbaum's theory of the continuity of the germ-cells remains still arguable, it appears no longer necessary to debate the origin of the gonocytes as being in the endoderm or mesoderm, but to view these cells as being specific elements whose history happens to coincide with the history of one or other of these germ-layers.

The appearance of the germinal cytoplasm shortly after fertilization, and its gradual loss of staining affinity following gastrulation, parallels the origin and disappearance of those 'Keimbahn determinants' that have been reported in certain invertebrates (see Hegner, 1914; Bounoure, 1939).

But have we any reason for believing the germ-plasm of the frog to be a germ-cell determinant? In the opinion of the author, if the germ-plasm is not an active determinant then it is closely associated with some invisible substance that is, in the same way that the determination of the germ-cells in *Parascaris* is effected by a difference in the cytoplasm near the vegetal pole (Boveri, 1910; Hogue, 1910). An incidental point to note is the polar position of the germ-plasm in the eggs of such invertebrates as nematodes and insects, as well as in frogs, shortly after fertilization.

From fertilization to the late blastula stage the plasm-bearing cells do not differ cytologically from other cells (with the exception, of course, of the germ-plasm itself). But when the plasm becomes juxtanuclear (as in Plate 1, fig. C), the cells that contain it become mitotically inhibited and retain their embryonic character (e.g. yolk platelets) while other cells are becoming increasingly specialized. While the early appearance of the germinal cytoplasm does not seem to have much significance therefore, the early establishment of the gonocytes might have a functional significance in 'protecting' the unspecialized nature of these cells from the influences that mediate determination and differentiation. While this is only a suggestion that does not receive much support from the work reported here, it is difficult to disregard the fact that the definitive gonocytes are formed just before gastrulation commences, that is, precisely before that period when so many other cell groups will be determined to develop in specific ways.
At this time also the loss of staining affinity of the germ-plasm, and the drastic reduction in its PNA content, point to important changes taking place within the gonocytes themselves.

Another striking feature of germinal cytoplasm is its constant association with large numbers of spherical mitochondria (Plate 1, fig. F). These become the more conspicuous as development proceeds, for the mitochondria of somatic cells become smaller and more difficult to see, except in the liver and intestinal wall. In neurula stages it is the mitochondrial population that first betrays the presence of gonocytes in sections (Plate 2, fig. C), and is thus of interest in that a similar characteristic has been reported for the primordial germ-cells of guinea-pig (Rubaschin, 1910) and chick (Tscheschin, 1910).

At their first appearance the islets of germinal plasm are widely spread over the surface of the vegetal hemisphere. Their subsequent concentration at the vegetal pole and ascent of the cell-walls of early cleavage are almost certainly a visible manifestation of that polar ingestion described by Schechtmann (1934). In other words, we are dealing with a passive migration. When the first four macromeres are formed, each contains more than one islet of plasm and it is probably a matter of chance, during the formation of the early blastula, how many islets come to lie in any one cell. The intracellular movement of the plasm in late blastula stages is by some unknown protoplasmic rearrangement. The displacement of the gonocytes from the superficial blastocoel floor to the deep endoderm by the morphogenetic movements of gastrulation may again be described as passive. On the other hand, the ascent of the gonocytes from their deep endodermal position to the germ-ridges cannot be ascribed to any known morphogenetic event, and this ascent is perhaps brought about by amoeboid movement of the gonocytes themselves. However, no preparation in this present investigation afforded any evidence on this latter point, and the author thus cannot agree with the observation of Bounoure that the primordial germ-cells do move in an amoeboid fashion.

The final migration of the primordial germ-cells from the dorsal crest of the endoderm to the germ-ridges is largely due to the withdrawal of endodermal material from the axial structures. This explains why ‘strays’ are sometimes discovered left behind in the gut-wall, an observation made in this study and by previous authors as well.

**SUMMARY**

1. Shortly after fertilization of the egg of *R. temporaria* there arise, near the vegetal pole, small islets of specifically stainable cytoplasm that eventually become included in cells lying in the blastocoel floor. From this time forward the special cytoplasm becomes increasingly difficult to identify with the cytological techniques used, but not until it is possible to confirm that the cells in question are primordial germ-cells. Thus the findings of Bounoure (1934) for this species are confirmed and extended.
2. This germinal cytoplasm has also been found in the eggs and embryos of *X. laevis* and *B. bufo*, but, rather surprisingly, not in *R. esculenta*.

3. The germ-plasm is, at first, rich in pentosenucleic acid (PNA) but loses this property shortly after an intracellular change in its position which occurs in the earliest stages of gastrulation.

4. Examination of parthenogenetically activated eggs reveals that germinal cytoplasm can be formed in the absence of sperm.

**ACKNOWLEDGEMENTS**

I should like to thank Dr. D. R. Newth for his advice and criticism throughout the course of this study, Professor P. B. Medawar, F.R.S., in whose Department the author was privileged to work, Mr. W. Brackenbury for the photographs, and the Department of Scientific and Industrial Research for financial support.

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EXPLANATION OF PLATES

PLATE 1

Fig. A. Migrating germinal cytoplasm (dark bow) in cell of late blastula of R. temporaria. Note the copious mitochondria between the plasm and the oval nucleus. ×680.

Fig. B. Primordial germ-cell of R. temporaria at crest of endoderm. Note the large, pale nucleus containing two nucleoli (black dots), and the corona of germ-plasm (black ring). ×630.

Fig. C. Gonocyte in the blastocoel floor of a mid-gastrula of R. temporaria. Compare the pale nucleus and the dark ring of germ-plasm which surrounds it (at the bottom of the photo) with the smaller dense nucleus lacking plasm in the somatic cell shown at top of picture. ×400.

Fig. D. Formation of an islet of germinal cytoplasm (framed area) from cortical material, in R. temporaria. ×770.

Fig. E. Germ-plasm (dark area) lying apposed to cell-wall (the vertical line) of macromere of 32-cell stage in X. laevis. ×560.

Fig. F. The same islet as in fig. E but at magnification ×1,500. Note the granularity of the plasm and the absence of yolk from it.

PLATE 2

Fig. A. Two islets of germinal cytoplasm in the vegetal region of the fertilized egg of B. bufo. The plasm areas are the two dark blotches. ×630.

Fig. B. Part of the blastocoel (the clear area) and blastocoel floor of B. bufo to show an islet of germ-plasm (at boundary of yolky material) and two globules of germ-plasm, pigment, and a little yolk lying free in the cavity. ×630.

Fig. C. Part of the floor of the blastocoel, containing two gonocytes (gc), from a young gastrula of B. bufo. Note the large numbers of mitochondria (small dots). ×630.

Fig. D. The extreme vegetal pole of a two-cell stage of X. laevis showing the islets of germ-plasm (dark) among the yolk platelets. One islet is already adjacent to the first cleavage plane. ×560.

Fig. E. Three primordial germ-cells (pgc) at the top of the dorsal mesentery in a tadpole of R. esculenta (the area with profuse yolk platelets is part of the endoderm, the oblique white area
A. W. BLACKLER

Plate 2
is the coelom, and the germ-cells are lying at the end of the chain of cells—the dorsal mesentery—that crosses the coelom. Note the sharpness of the nuclear membrane and the presence of two nucleoli in two of the cells. The nuclei are pale compared with the nuclei of surrounding cells. ×450.

Fig. F. The germ ridges of a 12-mm. tadpole of *R. esculenta* with enclosed primordial germ-cells. Note the presence of two prominent nucleoli in the nuclei of some of the germ-cells. ×450.

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