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The Technique of Rabbit Blastoderm Culture

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THE TECHNIQUE OF RABBIT BLASTODERM CULTURE

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I. INTRODUCTION.

Brachet (1912) was the first to put forward the idea of culturing the mammalian blastoderm " in vitro " and managed to obtain some growth and differentiation of the rabbit blastoderm on homologous plasma clots. Not until thirteen years later did Maximov (1925) attempt to develop the idea of Brachet. In this year he published a lengthy work on the detailed changes that whole and part rabbit blastoderms undergo in tissue culture. There was another lapse of interest in this problem for eight years until Waddington and Waterman (1933) successfully cultivated rabbit blastoderms on clots from chick plasma and embryo extract. Waddington (1934) was mainly interested in the experimental morphogenesis of the rabbit blastoderm and obtained neural plate induction by the transplantation of chick primitive streaks into rabbit blastoderms which were subsequently cultured. He followed up this work (Waddington, 1937) by obtaining neural plate induction with rabbit primitive streak. Waterman (1933), interested in the culture of rabbit blastoderms on media other than the classical plasma-embryo extract clot, tried out a number of bacteriological media with little success.

Parallel with this work on the culture of the rabbit blastoderm, work on the culture of the early chick, rat and guinea pig blastoderm and early embryo was being carried out, all utilizing blood plasma or serum as the basic medium.

Thus, while it was shown that a number of vertebrate blastoderms and early embryos could be grown " in vitro " and withstand certain experimental procedures, no light was shed on the nutritive requirements during this critical stage of development, owing to the variable and unknown composition of the plasma, until Spratt (1947b) showed that the early chick blastoderm could be grown on yolk and albumen extract, saline, agar substrata. In the same year he showed (Spratt 1947c) that the hitherto strict aseptic technique could be abandoned if fresh egg albumen was present, due to the bacteriolytic action of the latter. In the following year Spratt (1948) showed that the chick embryo can be grown on a wholly synthetic medium consisting of a Ringer's-agar-glucose clot.

Seeing that Spratt had obtained such excellent results in the case of the chick, it was decided to attempt to apply his method to the rabbit blastoderm and at the same time to work out a standardized technique for the explantation of the early blastoderm.

II. TECHNIQUE.

The technique of rabbit blastoderm culture has never been adequately described and for this reason the details of the methods employed will be given. Except where otherwise stated, strict aseptic precautions have been taken.

A. Media—

The two saline solutions have been Tyrodes and Ringer's with the following composition :—

(i) *Tyrodes.*

Sodium chloride	4.00 gms.
Potassium chloride	0.10 gms.
Calcium chloride	0.10 gms.

* This work was carried out while on the staff of the Zoology Department, The University of Melbourne.

Magnesium chloride ($MgCl_2.6H_2O$)	0.05 gms.
Sodium acid phosphate ($NaH_2PO_4.H_2O$)	0.025 gms.
Sodium bicarbonate ($NaHCO_3$)	0.50 gms.
Glucose	0.50 gms,
Water	500 c.c.

(ii) *Ringer's.*

Sodium chloride	4.50 gms.
Potassium chloride	0.21 gms.
Calcium chloride	0.12 gms.
Water	500 c.c.

Tyrodes is sterilized by filtration through a Seitz bacteriological filter and Ringer's by autoclaving.

In the preparation of Spratt's minimal medium phosphate and bicarbonate buffers are added separately.

The composition of the phosphate buffer is :—

$Na_2HPO_4.12H_2O$	1.45 gms.
KH_2PO_4	0.26 gms.
Water	500 c.c.

and of the bicarbonate buffer :—

$NaHCO_3$	5.5 gms.
Water	500 c.c.

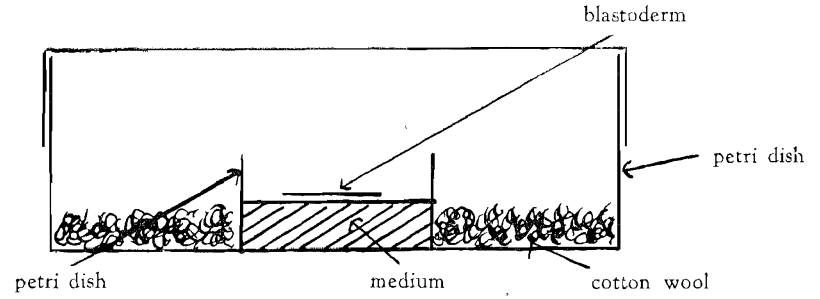
The phosphate buffer is sterilized by autoclaving and the bicarbonate by filtration.

B. Preparation of clots—

The culture chamber most often used in this type of work consists of a watch glass inside a petri dish packed with moist cotton wool. However, it has been found that if a small petri dish is substituted for the watch glass, greater stability is obtained (see Fig. 1).

FIG. 1.

BLASTODERM CULTURE CHAMBER.



(*a*) *Agar-egg albumen clot.*

In the preparation of agar-egg albumen clots, the following procedure has been adopted :—

1. Wash the surface of a fresh hen's egg with 90% alcohol.
2. Break the egg into an egg separator resting on a 250 c.c. beaker.
3. Pour the white into a 250 c.c. flask containing 50 c.c. saline.
4. Stopper and shake vigorously for 1 minute.

5. Add 0.15 gms. of agar to 30 c.c. of saline in 250 c.c. flask. Boil.
6. Suck up 20 c.c. of mixture in (4) with 20 c.c. pipette and add to (5) after cooling to 105° F. If the saline contains $NaHCO_3$, saturate with CO_2.
7. Pour 2 c.c. of (6) into each culture chamber.
8. Moisten cotton wool with sterile water.

(*b*) *Buffered-Ringer's-agar-glucose clot* (Spratt's Minimal).

In the preparation of Buffered-Ringer's-agar-glucose clot the following procedure has been adopted :

1. Boil up the following mixture—
   (*a*) Ringer's solution ... 36 c.c.
   (*b*) Agar ... ... 0.15 gms.
   (*c*) Glucose ... ... 0.34 gms.
   and cool to 40° C.
2. Add 2 c.c. each of the phosphate and bicarbonate buffer.
3. Saturate with CO_2.
4. Place 2 c.c. in each culture chamber.
5. Moisten cotton wool with sterile water.

C. Explantation of blastoderms—

In the explantation of rabbit blastoderms the following procedure has been adopted :—

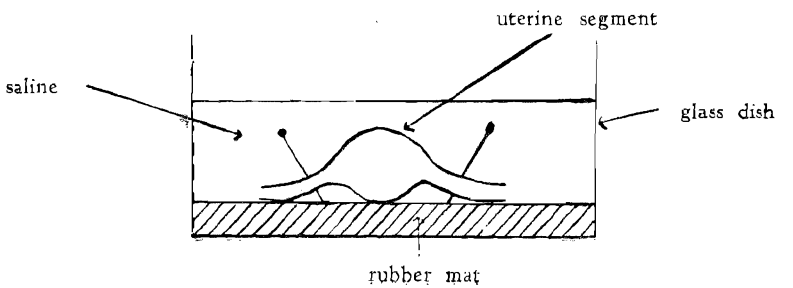
1. Kill the rabbit by a blow on the neck, skin and tie down on an operating table.
2. Sponge abdomen with antiseptic (*e.g.*, Zepharin).
3. Open abdomen.
4. Remove sections of uteri containing blastocysts, and place in a dish under saline.
5. Pin sections down in the operating dish (see figs. 11a, b).
6. Cover with saline.
7. Place a few drops of saline on clots with a pipette.
8. Dissect out blastoderms (see fig. 11c).
9. Transfer to clots with a section lifter.

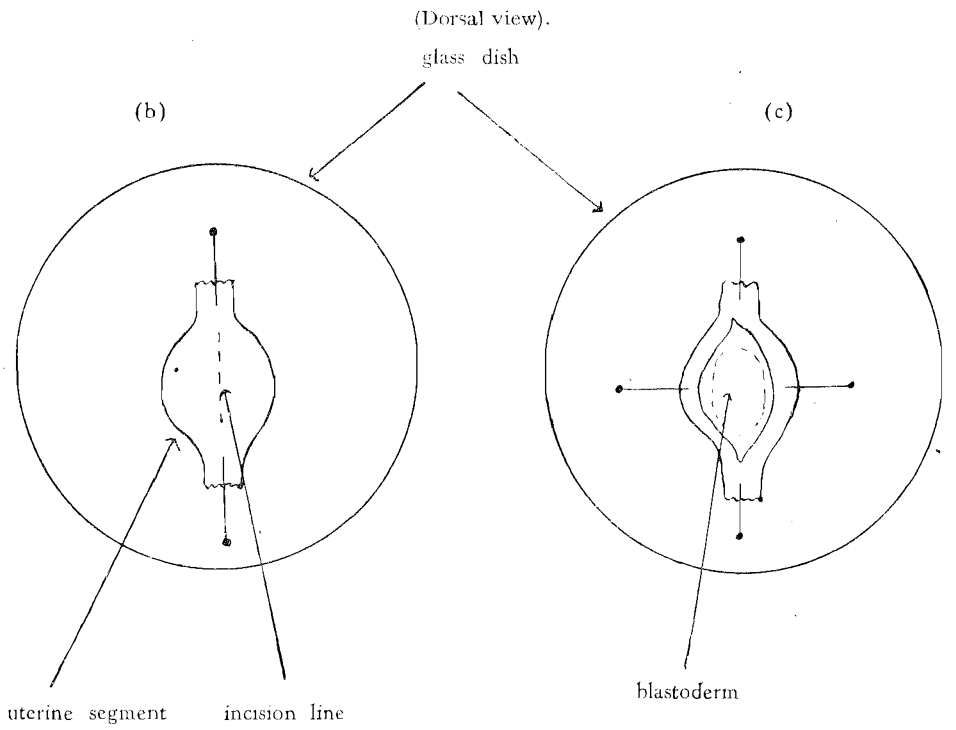
FIG. II.

RABBIT BLASTODERM EXPLANTATION TECHNIQUE.

(Lateral view).

(a)





10. Arrange with glass needles.

11. Suck off surplus saline.

The ages of the blastoderms have been reckoned from the observed time of copulation. Pregnancy has been diagnosed by palpation (Suitor 1946) immediately before killing.

D. Incubation and recording of results—

The cultures have been incubated at 100° F. and removed from the incubator for observation and description at 0 hours, 12 hours and 24 hours.

III. RESULTS.

Twenty-three rabbit blastoderms at approximately eight days have been cultured. Details of the media used and the results obtained are set out in Table I.

IV. DISCUSSION.

Series A and B have given negative results, but in series C heart beat has maintained for 24 hours and in one case (culture C I) for 144 hours. In culture D 3, a neural tube and three pairs of somites have been formed.

Series E, in which Spratt's minimal medium was tried, has given negative results.

It is the opinion of the author who has also cultured chick blastoderms under identical conditions, that the rabbit blastoderm is far more difficult to manipulate without serious damage and that it is far less viable in culture.

However, it is suggested that this method utilizing an agar clot may be developed to throw important light on the nutritive requirements of the rabbit blastoderm as Spratt (1947b, and 1948) has done with the chick and also in working out the morphogenetic movements as has been done in the case of the chick by a number of workers and more recently by Spratt (1947a) using carbon particles.

TABLE I.

RABBIT BLASTODERM CULTURES.

Series	No.	Age	Medium	Results		
				0 Hours	12 Hours	24 Hours
A	1	8d. 0h.	Ringer's-agar-egg albumen	primitive streak	primitive streak	dead
	2	,,	,,	,,	,,	,,
	3	,,	,, (Non-sterile)	,,	,,	,,
	4	,,	,,	,,	,,	,,
	5	,,	,,	,,	,,	,,
	6	,,	,,	,,	,,	,,
B	1	8d. 3h.	,,	6 somites	6 somites	dead
	2	,,	,,	,,	,,	,,
	3	,,	,,	,,	,,	,,
	4	,,	,,	,,	,,	,,
	5	,,	,,	,,	,,	,,
C	1	8d. 20h.	Tyrodes-agar-egg albumen	11 somites, heart beating	13 somites, heart beating	heart beating
	2	,,	,,	,, ,,	11 ,, ,,	,,
	3	,,	,,	,, ,,	14 ,, ,,	,,
	4	,,	,,	,, ,,	14 ,, ,,	,,
	5	,,	,,			
D	1	8d. ½h.	,,	primitive streak	primitive streak	primitive streak
	2	,,	,,	,,	,,	,,
	3	,,	,,	,,	,,	Neural tube and 3 prs. of somites
	4	,,	,,	,,	,,	primitive streak
E	1	8d.	Buffered Ringer's-agar-Glucose	,,	,,	,,
	2	,,		,,	,,	,,
	3	,,		,,	,,	,,

V. CONCLUSIONS.

It is concluded that the 8-day rabbit blastoderm may be maintained and may undergo some growth and differentiation on a Tyrodes-albumen-agar clot under aseptic conditions.

VI. SUMMARY.

The details of rabbit blastoderm culture are described using a variety of new media and the results of some preliminary experiments on the culture of the 8-day rabbit blastoderm are described and discussed.

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