Total Replacement of the Yolk of Chick Embryos

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Isolation of the avian embryo from its normal food material is an essential step in a fundamental study of embryonic nutrition. With present methods, however, it has not been possible to grow embryos in culture for much longer than one day. In 1932 Waddington described methods of growing explanted embryos on serum clots. Several years later Spratt (1947, 1952) devised simplified media for embryos cultured on agar with which he studied various aspects of morphogenesis and carbohydrate needs of the young embryo. New (1955) has recently reported another technique for growing explanted embryos which allows development to proceed to about 60 hours, 40 hours of which are in vitro.

A number of workers have injected various nutrients, antimetabolites, drugs, poisons, and hormones, directly into the yolk sac or other areas of the egg (e.g. Landauer, 1954). This type of study has been very informative, but its scope has been limited by the fact that only limited amounts of material can be injected, and their concentrations may not be uniform in the different parts of the relatively large yolk mass.

The technique described below permits the total replacement of the original egg yolk by yolk material from other eggs without disturbing the normal position of the embryo or its normal growth. This technique was developed as the first necessary step in an attempt to replace the yolk with an artificial medium. If the researcher could manipulate all the nutrients available to the embryo, he could then study directly those egg components which are affected little by the diet of the hen—components such as lipoproteins and their constituent amino acids, phospholipids, sterols, and fatty acids. Other studies which might utilize embryos grown on a defined medium include: the interactions of virus and host; neoplasms; mechanisms of hormone action; and enzyme induction. The exchange of yolk material between one embryo and another should also prove useful in physiology and in genetic studies of such problems as maternal effects, heterosis, and mechanisms of lethal gene action.

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METHODS

The success of the present technique depends primarily upon the formation of a coagulum of albumen which binds the vitelline membrane to the shell membranes. This operation is performed with an electrosurgical unit ('Blendtome', manufactured by the Birtcher Corp.) that combines cutting and coagulation currents. A connexion is made between the yolk and the outside of the shell, allowing either total removal of the yolk by flushing with Ringer's solution, or partial removal by suction. A maximum of approximately 5 ml. of yolk can be removed by direct suction using a syringe; attempts to remove larger volumes result in collapse of the vitelline membrane. When yolk is removed in this way a supplementary hole must be opened into the albumen to allow for change in volume, otherwise air will be drawn into the yolk around the needle.

Fertile eggs from White Leghorn hens on stock diet are incubated for about 2½ days (stages 15–17 of Hamburger & Hamilton, 1951). Sterile technique is used hereafter. The shell is wiped with 70 per cent. ethanol, a hole 3 mm. in diameter is drilled through the shell but not through the shell membrane, and the hole is cleaned with 70 per cent. ethanol. The hole is located on the side of the egg, at the equator or slightly above. The drilled eggs are returned to the incubator where they are kept with the hole downward for a few minutes to orient the embryo away from the hole. Next the egg is supported with the large end upward in a metal cup containing a few drops of an electrolyte, to assure a good electrical contact, and the cup is placed on the indifferent plate of the electrosurgical unit. A straight, sharp, 20-mm. electrode (Birtcher No. 795–B) is inserted quickly through the hole and into the yolk, and the current is turned on for 5–6 seconds (Text-fig. 1, coagulation step). Then, while the current is maintained, the needle is drawn outward in a spiral motion, over a period of about 4 more seconds, until the point is about 5 mm. inside the egg. The current is then turned off and the needle is withdrawn slowly, carrying with it small amounts of coagulated yolk. (The current used is for maximum coagulation (setting 7) and partial cutting (setting 4).) By this method a direct connexion is established between the inside of the yolk and the outside of the shell through a tunnel surrounded by the coagulum.

The egg is next supported in a horizontal position with the hole downward (Text-fig. 1, flushing step) and a hypodermic needle (16 gauge) is used to clear the tunnel of coagulated yolk and albumen fragments. Now a hypodermic needle (20 gauge), clamped firmly and attached to tubing and a reservoir of Ringer's solution containing erythromycin (0.1 mg./ml.) and streptomycin (1.0 mg./ml.), is inserted about 15 mm. into the hole and a pressure of 25–35 cm. of water is used to flush out the yolk. The egg, which is moved occasionally in order to keep the yolk flowing, is kept warm by a heat lamp. Flushing is completed in 5–15 minutes if the preparation is satisfactory. After all the yolk has been flushed out, as determined by clarity of effluent and direct observation before the candling
light, the egg is placed hole upward, and the donor yolk, which contains the same antibiotic concentration as the Ringer's solution, is injected through a 16-gauge needle into the yolk cavity (Text-fig. 1, replacement step), thus displacing the Ringer's solution. When the desired amount of yolk (usually 15 ml.) has been injected, the hole is sealed with paraffin and a piece of plastic adhesive tape, and the egg is returned to the incubator.

RESULTS

Normal growth and development have been observed in embryos after more than 95 per cent. of the original yolk was flushed out with Ringer's solution, and the flushing solution in turn replaced by yolk from other eggs. A number of
embryos that were 2½ to 3 days old at the time of replacement have lived until time of hatching, and one actually hatched.

Estimation of the completeness of yolk removal was made by sacrificing embryos just before yolk replacement and determining the carotenoid content of the total egg contents. This was done by extracting exhaustively with acetone, filtering, diluting, and determining the absorbance of the extract at 445 mμ, in comparison to intact eggs. Most of the yolk that was found after flushing was part of the coagulum surrounding the tunnel connecting the yolk cavity with the exterior. This material was denatured and probably would not contribute a significant amount of nutrients to the embryo.

Embryos of various ages up to 5 days have been subjected to the technique, but in none older than 3½ days or younger than 2 days have we yet obtained a successful replacement. In the older embryos the coagulum was not strong enough to withstand the flushing procedure. In unincubated eggs or for the first 2 days of incubation, the embryo is not tightly attached to the vitelline membrane, and is easily washed free during flushing.

Attaching the yolk to the shell membrane is accomplished with little mortality, and can be performed before incubation, or at any time up to 3 days of age. The coagulum is quite strong, and may be used repeatedly as an entrance into the yolk sac. If desired, the yolk can be cut free from the shell after sealing with paraffin by running an L-shaped knife blade around the inside of the hole. The small gas bubbles which are formed in both the yolk and the albumen during coagulation are apparently not harmful to the embryo.

In the course of developing the present technique we used some modifications in which the albumen was removed from a partially opened egg by suction with a syringe. This step could be done with the present method, after the yolk was replaced and the hole sealed, thus permitting replacement of all nutrients available to the embryo.

Table 1

Numbers of surviving embryos after total replacement of yolk by yolk from three different sources. The first column of figures represents the number of 3-day-old embryos that survived the operation and were incubated.* The remaining columns show the numbers of these embryos that were alive on the days indicated.

<table>
<thead>
<tr>
<th>Kind of replacement yolk</th>
<th>Embryo age in days</th>
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<tbody>
<tr>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Unincubated infertile</td>
<td>.</td>
</tr>
<tr>
<td>Unincubated fertile</td>
<td>.</td>
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<tr>
<td>From 5-day embryos</td>
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<tr>
<td>Totals</td>
<td>.</td>
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* 38 out of 74 survived the operation. † One of these hatched.
The data presented in Table 1 show how one group of embryos prepared during a 2-day period survived subsequent days. Failures during the operation were caused by unsatisfactory coagulation, which could readily be detected by the presence of albumen in the flushed yolk, or by separation of the embryo from the vitelline membrane, which could be observed by candling. These failures did not entail much loss of effort. In the periods after the operation the greatest losses were those resulting from separation of the extra-embryonic circulation from the vitelline membrane. Most of these failures were detected during the first 24 hours. Embryos that died after the 24-hour period appeared to be normal except for haemorrhagic areas in the embryo itself or in its external circulation.

From the data of Table 1 and others obtained previously, it appears that the chances of survival for more than 24 hours are greater when the donor yolk comes from embryos than when it comes from unincubated or infertile eggs. We believe that survival after this initial period is not much affected by the source of yolk.

SUMMARY

A technique is described in which a coagulum of albumen binds the vitelline membrane to the shell membranes of 3-day chicken embryos, thus allowing the yolk to be flushed out with Ringer's solution. Donor yolk is then injected into the yolk cavity, where it displaces the Ringer's solution. Normal development occurred, including the hatching process, when more than 95 per cent. of the original yolk was replaced by other yolk.

REFERENCES


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