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Culture of Tubal Mouse Ova

Tubal mouse ova at the eight-cell stage developed consistently to blastulæ, when cultured in the egg white – saline mixture used by Hammond[1] for the actual collection of ova. This fluid was freshly prepared each day and kept in tightly stoppered vessels. Unlike that of Hammond, the *p*H was about 7·0; but this rapidly rose to 7·8 during measurement with a glass electrode or when exposed in shallow vessels, and the change was attributed to loss of carbon dioxide contained in the egg white. Since ova failed to differentiate at a *p*H greater than 7·7, Krebs–Ringer bicarbonate[2] (*p*H 7·4), with 10 γ/ml. penicillin and streptomycin, was adopted as the standard saline medium. With the addition of 1 per cent fresh thin egg white, this proved an excellent culture medium for the ova, but no growth was obtained in the Krebs–Ringer bicarbonate alone.

The essential factor or factors in egg white were shown to be non-dialysable, but an attempt to identify them with one or more of the protein fractions of egg white was unsuccessful. However, normal growth was obtained with Krebs–Ringer bicarbonate plus crystalline bovine albumin (Armour and Armour) in concentrations ranging from 0·03 to 6 per cent, and this preparation was used in all subsequent observations.

Mice were killed on the third day after mating and the ova obtained by inserting a 28-gauge hypodermic needle at the uterine end of the oviducts, and flushing them out into a cavity slide with about 0·5 ml. of the medium. The ova were then collected in about 0·3 μml. of fluid, with as little tubal debris as possible, in a mechanically controlled pipette. They were transferred to a second cavity slide containing 1 ml. of medium, the contents agitated, and the ova again collected with a fresh pipette and transferred to an agglutination tube containing 1 ml. of fluid. This was tightly stoppered and incubated in a water bath at 37° C. After 48 hr. the contents were tipped on to a cavity slide and examined.

Using this technique, only three out of 148 ova failed to develop into blastulæ when cultured in 0·4 per cent bovine albumin in batches of 10–14, and 86 out of 87 developed when the concentration was 0·1 per cent. These blastulæ appeared normal and produced characteristic growth when inserted under the kidney capsule of mice.

It is unlikely that tubal cells or secretions transferred to the culture tubes with the ova played any part in growth, since development continued even if the washing procedure described above was repeated ten times.

The mode of action of the albumin is not clear. It did not appear to function by preventing extraction of any component of the ova, since they failed to grow in Krebs–Ringer bicarbonate containing 200 ova/ml. which had been ruptured by alternate freezing and thawing. However, it did alter the physical properties of the medium and greatly facilitated the manipulation of the ova with a pipette, as those in Krebs–Ringer bicarbonate alone adhered to the glass. So far it has been shown that the omission of calcium, magnesium, potassium or glucose from the Krebs–Ringer bicarbonate prevented growth, and development was delayed without phosphate. Growth occurred only between *p*H 6·9 and 7·7 and continued when the molarity was reduced to 0·09.

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Microbiology of Silage

Although several investigators have studied the micro-organisms present in silage, the various types of organisms and the number of each found in different qualities of silage are rarely given. Preliminary results of a study of the micro-organisms present in grass silage made in four different ways may therefore be of interest.

The silage was made in glass tubes in a manner similar to that described by Allen *et al.*[1], 40 gm. of the grass mixture—perennial ryegrass and white clover—being mown and after treatment being packed into tubes 6 in. × 1 in., care being taken that all the implements used had been previously sterilized. Each tube was immediately closed with an air-tight stopper, and stored at ordinary laboratory temperature for seven weeks, after which the tubes were opened and their contents examined for colour and smell as well as bacteriologically.

Four contrasting treatments were adopted as follows: (*A*) the grass was packed without chopping and without the addition of any water or preservative; (*B*) the grass was chopped into inch lengths but packed without the addition of any water or preservative; (*C*) the grass was left unchopped but sprinkled with sterile water before packing; (*D*) the grass was left unchopped but was well mixed with 0·2 gm. of sodium metabisulphite in powdered form before being packed into the tube.

The silage from treatment (*A*), though made without the addition of any preservative, was still green and had kept well. A slight aroma of a pleasant nature had developed; otherwise the condition of the grass appeared almost identical with what was originally put in. In fact, it was considered the best-preserved of all the four methods. In treatment (*B*) the silage was fairly similar in appearance to that in treatment (*A*), but it had a slightly objectionable smell. In treatment (*C*), the silage turned slightly brown after a few days and when opened it smelt strongly of butyric acid; it was deemed the poorest of all the four silages. In treatment (*D*) the grass had kept well and had a pleasant aroma, but it had developed an unnatural shade of green—a bright