planktonic foraminiferal zones of Blow[1], to European stages, and to East Indian Letter Stage classification of the Tertiary (Clarke and Blow[3]). The hard, dark brown marl bed (Lower unit) which contains *Spiroclypeus ranjanae*, *M. complanata*, *M. bantamensis*, etc., is referable to zone P. 21 on the basis of *M. complanata*, while the soft, yellow marl bed (Upper unit) containing *M. bantamensis*, *S. ranjanae* and *Miogypsina gunteri* can be considered close to znoe P. 22 on the basis of *M. gunteri* and *M. bantamensis*. The zones P. 21 and P. 22 refer to the latter part of Bormidian stage of Late Oligocene. The lower and upper units can, therefore, be dated as Late Oligocene (Bormidian). In East Indian Letter Stage classification of Tertiary, the above rock units are also comparable with the latter part of "Lower" Te (Te 1–4).

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A NEW AND SIMPLE TECHNIQUE FOR AMNIOTIC INOCULATION OF CHICK EMBRYO

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ABSTRACT

A new and simple technique of amniotic inoculation of chick embryo without the aid of transilluminated egg candler at the time of inoculation is described. The chief advantage of this technique is that it can be done in light and only a hole has to be made for inoculation whereas the earlier techniques of amniotic inoculation described have to be done in darkness or a flap of egg shell has to be removed before inoculation which are cumbersome and inconvenient. This new technique is as simple as the allantoic method of egg inoculation and hence it may be used for virological work. Amniotic route of chick embryo inoculation is commonly employed for isolation of influenza types A, B, C and mumps viruses and for passage of influenza isolates until they get adapted. Allantoic route of chick embryo inoculation is employed for isolation and antigen preparation of influenza types A and B. However, it is not suitable for isolation and antigen preparation of influenza type C which has to be done only by amniotic inoculation.

INTRODUCTION

THE method of cultivating many important viruses in embryonated hens' eggs has widened the scope of virological investigations and research which otherwise had to be carried out mainly in animals. There are four major routes of chick embryo inoculation allantoic, amniotic, yolk sac and chorioallantoic. In addition, intravenous, intraembryonic, intracerebral or intraocular methods of chick embryo inoculation (Rhodes and Van Rooyen[1]) are also used.

Amniotic route of inoculation is commonly used for primary isolation of influenza and mumps viruses and for the passage of influenza isolates until they become egg adapted and for production of influenza C antigens. By this route the virus inoculum is brought directly in contact with the embryo and its developing alimentary and respiratory tracts.

There are two methods of amniotic inoculations described earlier and are employed routinely. One is the open method in which a flap of shell is flipped off at the air sac end of the chicke mbryo and the shell membrane is swabbed with sterile liquid paraffin, the egg is held close to the transilluminated candler and the virus is inoculated. The inoculation by this method can also be done without the aid of transilluminated candler (Grist *et al.*[2]). In the second

method, the egg is held over transilluminated candler and inoculated by means of tuberculin syringe fitted with 23 gauge needle 1 3/4" long. The needle is aimed towards the shadow of the embryo; 0·1–0·2 ml of inoculum is deposited in the amniotic cavity. The needle is then withdrawn (Lennette and Schmidt[3]). The transilluminated candler is required for the second method at the time of virus inoculation which has to be done in darkness.

Materials and Methods

The new technique of amnictic inoculation described here is done in light as transilluminated egg candler is not required at the time of inoculation for visualizing the embryo. The steps are:

1. Nine to eleven days' old embryonated eggs are taken from the egg incubator.
2. They are kept at room temperature (27° C–30° C) for 2–3 hours before inoculation. This step is essential as it lessens the active movement and stabilizes the embryo during inoculation.
3. Eggs are candled and the air sac boundaries are marked.
4. The embryo is visualized while candling and a circular or oblong area is marked on the side of the embryo measuring approximately one inch long and one inch wide taking the shadow of the embryo (eyes seen as a dark mass) as the centre point of this area. The upper boundary of this oblong area is formed by the arc of the air sac margin.
5. The egg shell is swabbed with denatured spirit.
6. A hole is made with the help of a push pin wiped with denatured spirit just above the air sac margin at the centre of the arc which forms the upper boundary of the oblong area as shown in Fig. 1. For making the hole, half of the push pin is inserted in the direction of the air sac taking care not to puncture the allantoic or amniotic cavities by going vertically downwards.
7. The egg is placed horizontally with a slight upward tilt of air sac end to make the hole accessible for inoculation. The oblong area is placed facing downwards and resting on the portable egg candler (transillumination not required) or egg holder.
8. A tuberculin syringe attached with 23 gauge 1" long needle filled with a dye such as methylene blue if it is for practice and demonstration, or virus inoculum if it is for virus inoculation is taken.
9. The egg is held by the left hand to stabilize it and from the right hand the needle is inoculated into the hole, holding the syringe approximately at 25° angle as shown in Fig. 2.

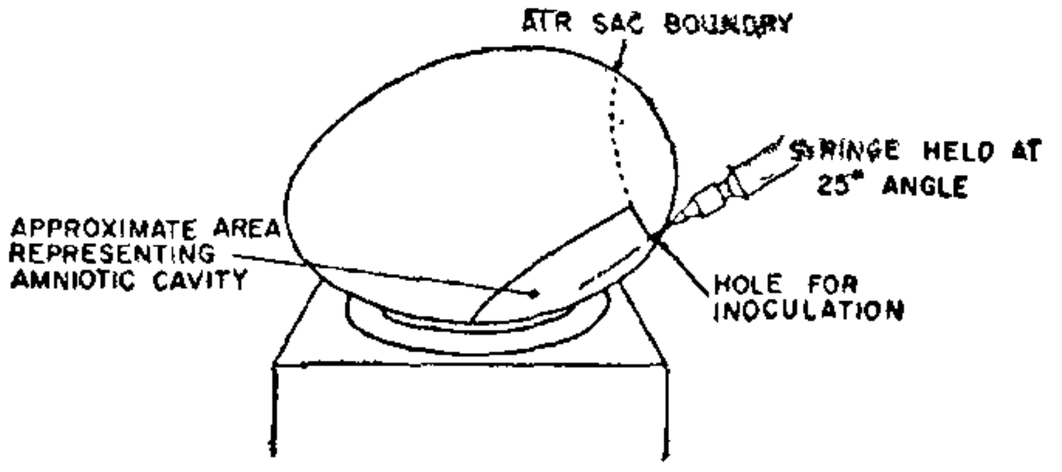

Fig. 2

10. 0·1 or 0·2 ml of inoculum is deposited and the needle is withdrawn.
11. The hole is sealed with adhesive cellophane tape and the eggs are incubated at appropriate temperature. The entire batch of eggs used for inoculation can be sealed at a stretch after completion of the inoculation.

The success rate of inoculation by this new technique of amniotic inoculation is 70–90%.

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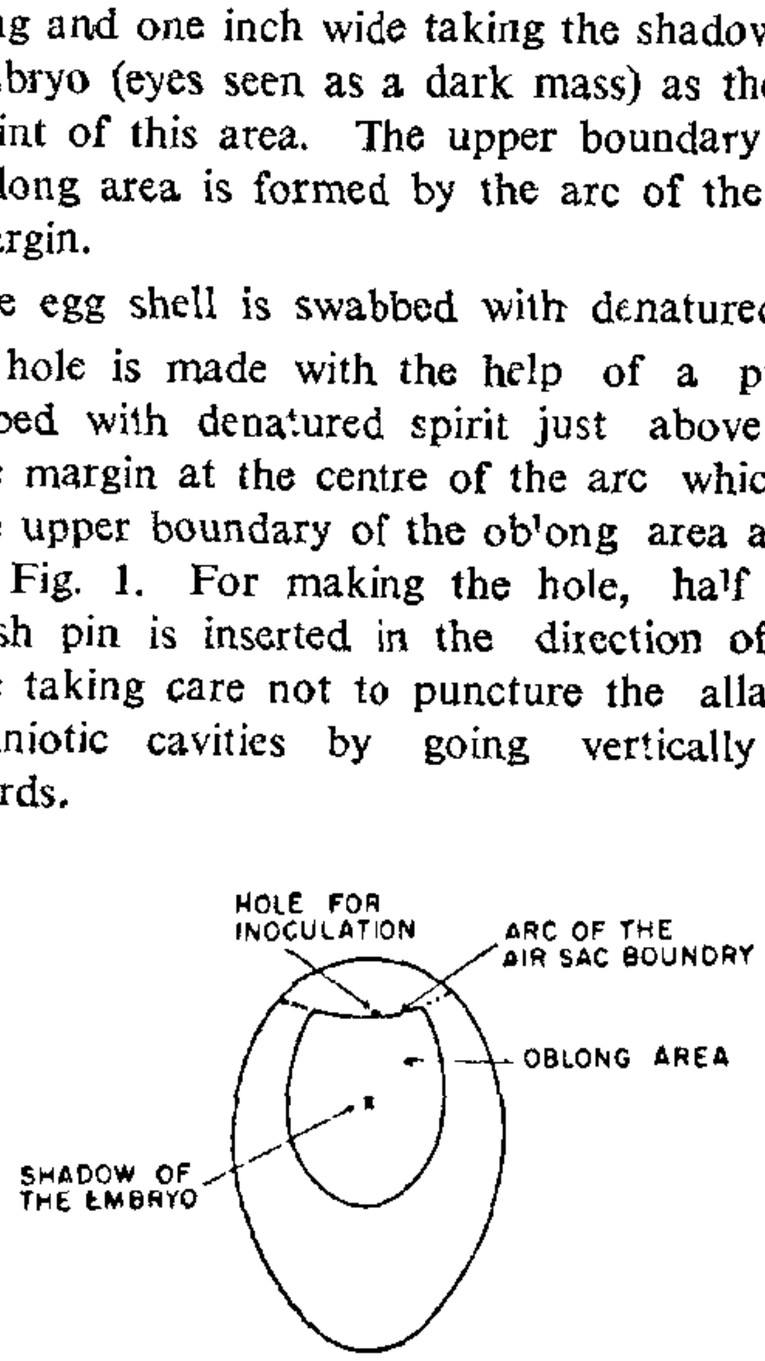

Fig. 1

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