state of rehydration is the same as the initial state of hydration, the surface ultra-structures of the ghosts used in these experiments contain about 33 per cent lipoprotein with associated hæmoglobin and about 67 per cent of water. It should be noticed that the thickness of the ultra-structure of these ghosts is probably greater than that of ghosts prepared by some other methods, for example, the method used by Waugh and Schmitt[1] in connexion with their leptoscopic measurements, or the method used to give the ghosts employed in Hillier and Hoffman's[7] electron microscope observations. It is not only possible but likely that the surface ultra-structure is made up of layers or regions, some more resistant to washing and probably less hydrated than others; the figure for the degree of hydration given by these experiments is therefore one for the average hydration of the whole surface ultra-structure of the ghosts as prepared by the method described.

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Observation of *in vitro* Fertilization in the Rabbit

In 1950[1] I showed that it is possible to obtain spermatic penetration of the pellucid membrane and the cytoplasm of oocytes of the female rabbit cultivated in fragments of Fallopian tubes to which spermatozoa taken from the vagina were added. This result was discussed in a symposium of the Ciba Foundation in 1952 on Mammalian Germ Cells[2,3].

A microcinematographic study of fertilization obtained *in vivo* followed by *in vitro* culture made possible the identification of a very characteristic aspect which can be used as a test for the first stage of fertilization of the oocyte of the female rabbit. It is the presence of mobile spermatozoa which are visible in the perivitelline space; the spermatozoa are included between the pellucid membrane and the vitelline membrane of the ovular cytoplasm that has shrunk after the penetration of the fertilizing spermatozoon, which in this position cannot be seen by direct examination. The presence of spermatozoa in the perivitelline space shows that the pellucid membrane has been penetrated by a fertilizing spermatozoon. Retraction of ovular cytoplasm is not always characteristic of fertilization[4].

A microcinematographic study has been made of *in vitro* fertilization. The technique is as follows: (*a*) After coitus with a sterile male (spermatic ducts tied), tubal oocytes are taken from the female rabbit (11 hr. after coitus). They are drawn into a pipette about 1 mm. in diameter, which has been filled beforehand with paraffin oil; the oocytes are placed in a watchglass in oil. (*b*) Another female rabbit is put with a normal male and the horn of the uterus is tapped 10 hr. after normal coitus; in this way it is easy to take 0·25 c.c. of uterine secretion containing a few thousand spermatozoa per cu. mm. (*c*) A few cubic millimetres of this secretion containing mobile spermatozoa is added to the oocytes. (*d*) The whole is cultivated under oil at 36·5°–37° C. in air or nitrogen.

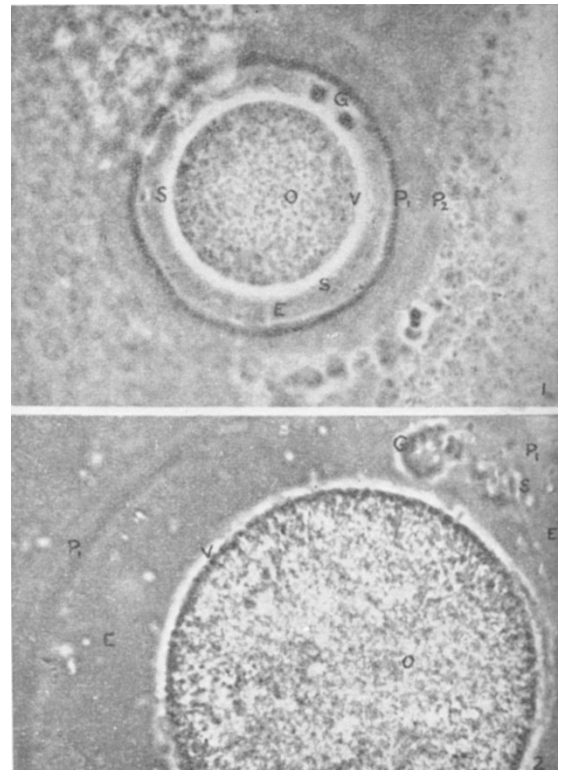

Fig. 1. Oocyte fertilized *in vivo* and cultivated *in vitro* in female rabbit serum. P_1, P_2, pellucid membrane; *G*, polar bodies; *V*, vitelline membrane; *E*, between *V* and P_1, perivitelline space; *S*, spermatozoa; *O*, retracted ovular cytoplasm

Fig. 2. Oocytes fertilized *in vitro* (tubal oocyte cultivated in the uterine secretion containing spermatozoa). P_1, pellucid membrane; *G*, polar body; *V*, vitelline membrane; *E*, perivitelline space; *S*, spermatozoon near a polar body; *O*, retracted ovular cytoplasm

It has been possible to record by microcinematography the arrival of a spermatozoon on the pellucid membrane, the retraction of the ovular cytoplasm and the differentiation of two lighter central zones corresponding to the pronuclei; it is also possible to observe the presence of spermatozoa in the perivitelline space. Under these conditions, segmentation of the ovum was not obtained. About 30 per cent of tubal oocytes are thus 'fertilizable' in the utero-tubal secretion containing spermatozoa[5].

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