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This Week's Citation Classic®

New D A T. A new technique for the cultivation of the chick embryo *in vitro*.
J. Embryol. Exp. Morphol. 3:326-31, 1955.
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It was found that early chick embryos could be grown *in vitro* on pieces of vitelline membrane stretched across a glass ring. The method was simple and reliable and would support embryonic development as far as the formation of a functional blood circulation. [The *SCI*® indicates that this paper has been cited in over 310 publications.]

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This technique was born out of frustration and luck. As a research student in the early 1950s at University College, London, I had embarked on a developmental study of the early chick embryo (blastoderm) that involved grafting precisely located groups of radiolabelled cells from one embryo to another. The techniques were difficult and laborious and were only possible on embryos *in vitro*. Unfortunately, the *in vitro* methods then available—growing the blastoderms on plasma or agar clots—proved totally inadequate for the project. After several months of trying in vain to extract information from stunted, distorted, or dead embryos, it became clear that a change of direction was needed.

It seemed that a major defect of the clot techniques was that the blastoderm failed to expand over the clot surface, with the result that growth of the embryo was unnaturally restricted. In the egg the blastoderm rapidly extends over the vitelline membrane surrounding the yolk; I wondered if pieces of vitelline membrane could be used *in vitro*. Luckily, the properties of the vitelline membrane proved more helpful than one might have dared to hope. A few trials showed that (1) the membrane was strong enough for large pieces to be pulled intact off the yolk, (2) it could be supported in a culture dish simply by wrapping the edges round a glass ring, and (3) the membrane slowly contracted during incubation so that initial wrinkles were pulled flat. Such ring-supported membrane provided an excellent substrate for explanted blastoderms and, as a bonus, it turned out that a little albumen from the same egg was all that was needed as a nutrient medium.

On sending a short account of the method to press, I innocently called it "A new technique..." and only later became aware from amused colleagues of the pun on my own name; perhaps it is just as well that the publication date was nowhere near April 1st! The technique rapidly became popular among embryologists and after more than 30 years still seems to be useful.[1,2] Its simplicity and reliability have been obvious attractions, but I think another reason it has been so frequently cited is that it allows the embryo to grow under conditions sufficiently similar to those in the egg thus providing a useful model of development *in vivo*.

The method has found applications in a wide variety of studies, including those on normal and teratogenic development of the embryo, embryonic and extra-embryonic metabolism, fluid movements within the egg, cell adhesion and migration, the early development of the embryonic membranes, and the incubation requirements of eggs. Its success made me acutely aware of the dependence of embryology on good *in vitro* techniques and provided the impetus for writing a book on culture methods for vertebrate embryos.[3] It also led to a long-lasting interest in devising improved methods for culturing mammalian embryos.[4]

Finally, I am very grateful to my research supervisor from 35 years ago, Michael Abercrombie, who encouraged me to follow up what at the time seemed a rather wild idea.

1. Khaner O, Mitrani E & Eyal-Giladi H. Developmental potencies of area opaca and marginal zone areas of early chick blastoderms. *J. Embryol. Exp. Morphol.* 89:235-41, 1985.
2. Ooi V E C, Sanders E J & Bellairs R. The contribution of the primitive streak to the somites in the avian embryo. *J. Embryol. Exp. Morphol.* 92:193-206, 1986.
3. New D A T. *The culture of vertebrate embryos*. London: Logos Press, 1966. 245 p. (Cited 115 times.)
4. ————. Whole-embryo culture and the study of mammalian embryos during organogenesis. *Biol. Rev. Cambridge Phil. Soc.* 53:81-122, 1978. (Cited 170 times.)

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