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Review

The use of the chick embryo chorioallantoic membrane as experimental model to study virus growth and to test the clonal selection hypothesis. The contribution of Sir Mac Farlane Burnet

Domenico Ribatti[a,b,*]

[a] Department of Basic Medical Sciences, Neurosciences and Sensory Organs, University of Bari Medical School, Bari, Italy
[b] National Cancer Institute "Giovanni Paolo II", Bari, Italy

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ABSTRACT

Sir Mac Farlane Burnet was the most honored of all Australian scientists. In 1960, Burnet shared the Nobel Prize for Medicine with Peter Medawar of Britain for the discovery of acquired immunological tolerance. He developed techniques for growing influenza viruses in the chorioallantoic membrane of the chick embryo. This became a standard laboratory practice. He continued to work with chick embryos long after the use of cell cultures had become general. His virology research resulted in significant discoveries concerning the nature and replication of viruses and their interaction with the immune system.

1. Introduction

Sir Macfarlane Burnet (1899–1985) has been one of the twentieth century's outstanding biologists. In the years 1941–1956, Burnet focused his effort on the elaboration of a theory of antibody synthesis, the clonal selection theory, capable of bridging the gap between physiological findings such as the kinetics of antibody production, self-tolerance and immunological memory on the one hand, and the newest ideas on synthesis of proteins, on the other [1]. For 25 years from the 1930s Burnet's research was devoted to the study of viruses, principally influenza. In 1960, the Nobel Prize in Physiology or Medicine was awarded jointly to Macfarlane Burnet and Britain's Peter Medawar 'for discovery of acquired immunological tolerance'.

During his experimental work, Burnet regularly used the chick embryo chorioallantoic membrane (CAM) assay to study virus and tumor gowth and their immunological aspects.

The CAM, is an extraembryonic adnex formed by the fusion of the chorion and the allantois. When this has happened, the CAM consists of three layers, ectoderm (from the chorion), mesoderm (fused somatic mesoderm from the chorion and splanchnic mesoderm from the allantois) and endoderm (from the allantois) (Fig. 1). The CAM has a rich vascular system that develops within the mesodermal layer and is served by paired allantoic (umbilical) arteries and paired allantoic (umbilical) veins. The pattern of the blood vessels in the allantois and chorioallantois is the subject of many studies [2].

The CAM has long been a favored system for the study of tumor angiogenesis and metastasis, because at this stage the chick's immunocompetence system is not fully developed and the conditions for rejection have not been established [3]. In fact, immunocompetence in birds develops only after hatching [4]. Other studies using the tumor cell/CAM model have focused on the invasion of the chorionic epithelium and the blood vessels by tumor cells, i.e., the metastatic potency of tumors. The cells invade the chorionic epithelium and the mesenchymal connective tissue below, where they are found in the form of a dense bed of blood vessels, which is a target for intravasation. Finally, the CAM is used to study inhibition of the induction of angiogenesis by tumor cells by anti-angiogenic drugs.

CAM assay may be used also in virology. Viruses that form pocks on the CAM can be assayed by counting the number of pocks formed on the inoculated CAM and each pock arises from a single virus particle. The CAM assay was widely used during the smallpox eradication campaign; lesion material obtained from smallpox patients was inoculated onto the CAM and following inspection of pock morphology, the causative agent could be identified.

2. Burnet's approach in the use of the CAM in the study of viruses

During his career, Macfarlane Burnet studied the viruses that cause polio and influenza, among others, and helped develop a flu vaccine in Australia using a technique that employed fertilized chicken eggs.

* Corresponding author at: Department of Basic Medical Sciences, Neurosciences and Sensory Organs, University of Bari Medical School, Policlinico–Piazza G. Cesare, 11, 70124 Bari, Italy.
E-mail address: domenico.ribatti@uniba.it.

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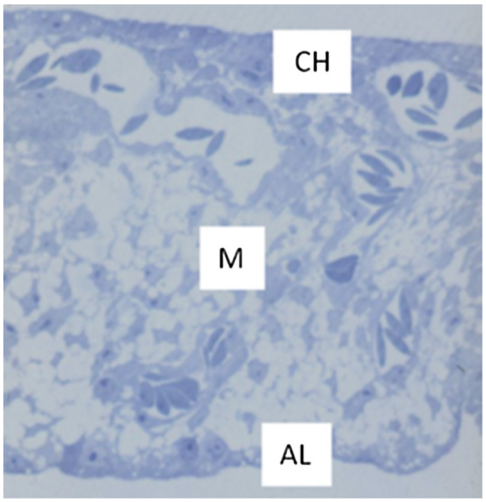

Fig. 1. A semithin section of the CAM,showing the chorionic epithelium (CH),the intermediate vascularized mesenchyme (M), and the deep allantoic epithelium (AL). (Reproduced from [17]).

Burnet had decided that main topic of research at the Hall Institute in Melbourne would be methods of growing viruses on the CAM. Burnet adopted the basic technique developed by Ernest Goodpasture in 1931 [5]. These Authors reported that the virus of fowl-pox could be grown on the chorioallantois of the developing chick, and that proliferative lesions containing typical inclusion bodies were produced.

As Burnet pointed out: “I knew of Goodpasture’s work with fowlpox and felt that here was a likely way of handling my canary virus. My wife has a favorite story of how I come home one day, borrowed an egg from her larder and a nail file, and tried to open an egg by the Gooodpasture method. I took a long, long time, but fortunately there was a young dentist working on tooth sections in the lab and our first advance in technique was to make a triangular opening in the egg shell with a dental drill. The important thing was that the canary virus grew well on the chorioallantoic membrane. In the course, I found that it was very closely related to fowl pox, and one of its claims to fame is that it was one of the first animal viruses to be photographed by the use of Barnard’s method of ultraviolet photography.” [6].

Burnet developed a technique that involved ‘dropping’ the CAMs of embryonated hen's eggs, then using these flat surfaces as biological substrates to grow virus colonies at limiting dilution. This method involved drilling most of the way through the shell at two points, one at the end over the air sac and one on the side, then pricking holes through the lining membrane with a flamed needle. The next step was to apply a negative pressure with a rubber bulb at the pole, so that the air sac was evacuated and the allantois dropped away to create an egg membrane ‘monolayer’ below the second hole in the shell (Figs. 2 and 3). After suitable incubation, the CAM was cut from the egg and placed in a Petri dish for counting. The CAMs could also be fixed, stained and mounted

Fig. 3. Burnet is shown drilling eggs in preparation for CAM experiments (Reproduced from University of Melbourne Archive).

Table 1
Viruses studied by Burnet using the CAM assay.

Laryngotracheitis virus [19]
Flow plague virus [20]
Newcastle disease virus [20]
Vesicular stomatitis virus [21]
Influenza virus [22]
Psittacosis virus [23]
Lopingill virus [24]
Ectromelia virus [25]

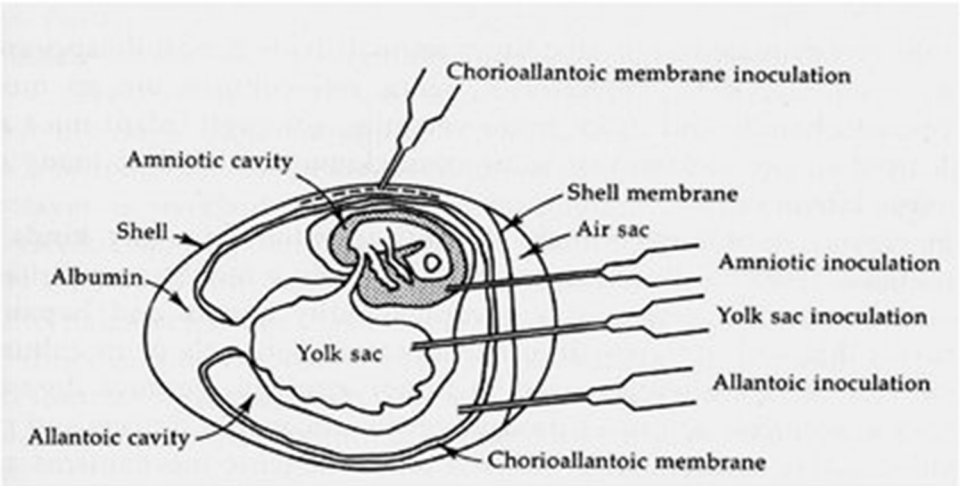

Fig. 2. The diagram illustrates variants of the chick embryo inoculation techniques that were used extensively in the laboratory of Sir FM Burnet (Reproduced from [18]).