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Popular Article

Unintentional bursectomy of chick by Bruce Glick accidentally exposed bursal function grabbed wide reputation while Pioneer of bursa Hieronymus Fabricius Italian anatomist remained under mist

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Unique organ of avian

While dissecting young birds we might have detected a chestnut-size, sac-like organ located at the terminal portion of hind gut, dorsal to the rectum, anterior to the sacrum communicating with the posterior portion of the cloaca by a short duct, known as bursa of Fabricius. It is arguably true that birds and mammals are evolved from reptiles yet this unique organ never been detected in any other animal species including reptilian ancestors. Scientific community is highly indebted to chicken species that has provided a unique and precious model or else we could have failed to study basic immunology what we have explored today. In this regard unlike mammal avian immunology is a fascinating subject for several unusual features like devoid of encapsulated lymph nodes (Nochi *et al*., 2018) and development of B lymphocytes in bursa instead of bone marrow which will be discussed in the later part of our discussion. In recent time we have gathered enough information about bursa and its role in humoral immunity in avian species. Within our knowledge in all most all reputed text book of immunology hardly any of these text books have mentioned about Hieronymus Fabricius, the great teacher an anatomist of Italian origin

Hieronymus Fabricius 1533-1619

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who first detected and described avian bursa in 1621. According to his name the organ is named as bursa of Fabricius; whereas the contribution of Bruce Glick is highly cited in scientific journals for unfolding bursal function. Hieronymus Fabricius or Girolamo Fabrizio or by his Latin name Fabricius ab Aquapendente was born in 1533 at Aquapendente, Italy (Smith *et al*., 2004). He was graduated in medicine from the University of Padua, Italy subsequently in 1565 he joined as a professor of anatomy and surgery in the parent university and completed a long tenure till 1613. The manuscript entitled "De Formatione Ovi et Pulli" (On the Formation of the Egg and of the Chick) is the first publication of Fabricius having description about bursa was published posthumously in 1961. Besides human cadaver, Fabricius carried out dissections on several animal species. His most important work was published with titled 'De formatu foetu'(the formed fetus), in this publication there are beautiful hand drawing diagrams about the anatomy of fetuses of dogs, cats, mice, rabbits, goats, guinea pigs, sheep, cows, horses, birds, sharks, and humans (Adelmann 1942). In his published document it has been mentioned that the bursa is a porous sac and having an opening from anus to uterus, similarly another opening from uterus to the bursal sac is there. According to Fabricius avian bursa is only found in hen and the sac like organ is meant for storing semen delivered from the rooster during mating; ultimately his observation was later proved to be highly erroneous (Hilary 1621). The exact role of bursa could not be established and remained as an enigma for several years. Earlier report has shown that avian bursa, grows rapidly till the birds attain 4 months of age then its size decrease and starts atrophy (Joly 1913). It has been observed that once bursa and thymus began to involute, the testes increased rapidly in size (Riddle 1928). Others believed that bursa might be playing contributory role in growth and sexual development and act as an endocrine organ was a debatable issue (Woodward 1931). Subsequently more detail about bursa and its physiological role was assembled through histological data from various research labs. Accumulated information reveled bursa is comprised of densely populated dividing lymphocytes arraigned in follicles separated by pseudostratified columnar epithelial cells giving structural resemblance with typical lymphoid organ as seen in thymus so nicknamed as "cloacal thymus" (Calhoun 1933). As per finding of Calhoun 1933; it is logical to speculate that bursa might be playing major role in immune system to protect against infection. Bruce Glick during his Ph.D research work at Ohio state University USA using specific avian breeds such as White Leghorns and Rhode Island Reds, observed that avian bursa attains maximum size within 4 to 5 weeks and 9 to 10 weeks of age respectively in those breeds, subsequently bursal growth was ceased resulting

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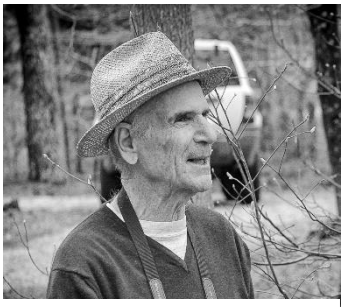
regression of bursal tissues (Glick 1955). If the physiological function of bursa is exclusively devoted to provide protection against infection concomitantly there is regression of bursa with the advancement of age of the birds (Glick 1955), then how the adult birds are protected with bursal remnant was a perplexing issue. In continuity, an interesting point draws our attention that unlike thymus, anatomically avian bursa is located at the terminal part of small intestine connected through a duct with cecum, that is enriched with gut microbiota; why so and what is the benefit of microbial contact is unclear. Apparently, question arise in mind why an organ alleged to be lymphoid in nature needs association of microbes, and whether the relation of microbes with bursa is essential for the development of bursal components? Recent findings have established that gut microbiota is associated with the development of bursal B cells in young chickens. Similarly antibiotic treatment of chicken at its early life that deprive microbial growth in gut causes aberration in bursal development and much more (Cheng *et al*., 2023). Whether animal or man foetal development takes place under sterile environment without any contact with gut microflora, but newborns acquire microbes from food and other environmental source after birth not before that (Bäckhed *et al.*,2015). The effect of gut microbes to shape the early immune-system is so vast that those aspects have been intentionally omitted from this manuscript, and interested readers may find it elsewhere (Cheng *et al*., 2023). During mid 1900s most of the fundamental aspects of immunity was already been disclosed by the researchers, and it was confirmed that lymph glands and lymphocytes plays central role in immunoglobulin synthesis (Raffle 1953), yet all these findings were not substantial enough to precisely advocate in favour of bursa to confirm its immunological role. Accumulated information gathered from several working groups have now established that during late embryogenesis, bursa grow rapidly to attain maximum size at 10-12 weeks of age, subsequently with sexual maturity bursal growth is restricted and enter the phase of regression (Ciriaco *et al,* 2003). An exceptional finding defining the exact role of bursa was surfaced out accidentally by Bruce Glick and one of the graduate students, Timothy Chang from Poultry Science Department at Ohio State University, USA. Their finding was published in Poultry Science journal, once got rejection from the editor of Science (Glick *et al*., 1956). Further detail is described in later part of this manuscript with schematic diagram designed by one of the co-authors SKB (Fig-1).



Bruce Glick

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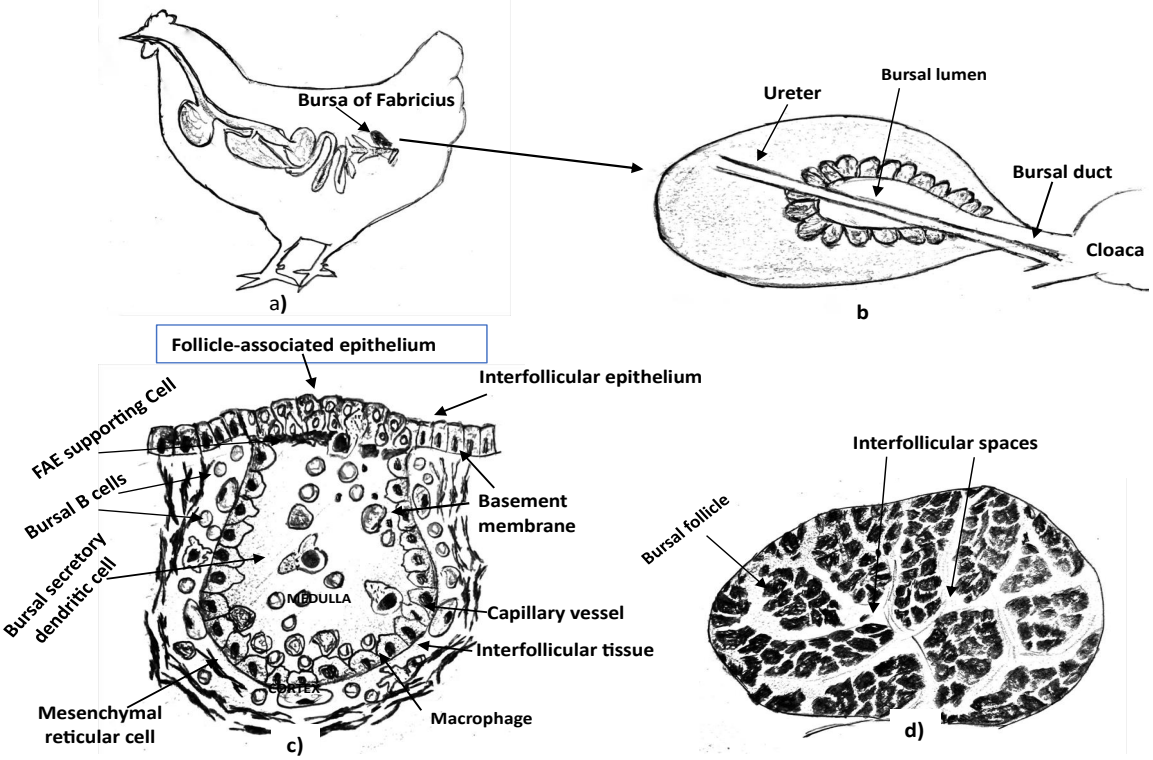

Fig-1. a, b, c, and d: The Schematic diagram of Bursa of Fabricius and its structure; a) Location of Bursa within body of the poultry; b) Various anatomical parts of Bursa; c) Diagram of histological structure of Bursal Follicle; d) Diagram of Follicular arrangements and interfollicular spaces

Finding fundamental: incidental or accidental?

It was the year 1954 a graduate student named Timothy Scott Chang from Poultry Science Department at Ohio State University, USA, while working with Bruce Glick there was a requirement of poultry birds to raise antibody against O antigen of Salmonella Typhimurium for under graduate students' class room demonstration. In that experiment enough birds were not available at that point of time, however the left over surplus six months old female birds already bursectomized (BSX) at 12 days age from an experiment of Bruce Glick was available to Chang. Without any option Chang incorporated those BSX birds to raise the antibody along with normal birds to complete the task. Forty-eight hours heat inactivated Salmonella Typhimurium broth culture was given to those birds through intravenous route at periodical interval for 20 days period. After one week post immunization the birds were bled to conduct plate agglutination test to check the antibody level against Salmonella O antigen. To their surprise six birds after post immunization succumbed early and only three survived. Interestingly during postmortem, it was observed that all the dead birds were devoid of bursa and the antibody against salmonella in the blood samples collected before death was negative. Those birds which produced antibody was normal birds having intact bursa. The results were not so convincing to Glick, so in their subsequent experiment they

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used White Leghorn and Rhode Island Red chicken breed either having intact bursa or surgically removed bursa to raise the antibody, to confirm their earlier finding. The results were similar like that of accidental finding observed by Timothy. Due to the repeatability of their results, they ultimately published their findings in poultry science journal once rejected by the editor of Science journal (Glick 1956). As on 14th January 2024 this research publication (Glick 1956) has been cited 1061 times as per google scholar website. This report drew attraction by many other research groups. Back-to-back to this publication another report in which sheep RBC and Salmonella Typhimurium antigen was used by Chang also confirmed the significance of the bursa of Fabricius in antibody formation (Chang *et al*., 1957). Using a different approach Glick was able to prove that crude extract of bursal tissue derived from 4-week-old New Hampshire birds when infused to birds already bursectomized at 2 weeks of age can enhance the antibody titre against sheep RBC. Although it was a new finding but it could not confirm whether the bursal cells or cell extract was responsible to restore the function of bursa (Glick 1958).

from dark to land mark discovery

In this context it is pertinent to elaborate the work of another reputed worker Max Dale Cooper a pathbreaking physician specialization in paediatrics simultaneously clinical immunologist, who identified two distinct classes of lymphocytes with different origins, now known as T cells and B cells. It was the year 1963 once Max Dale Cooper joined the laboratory of Robert Good at the University of Minnesota in the year 1963, Cooper took serious interest in the scientific reports already published in Poultry Science journal in 1956 by Bruce Glick (Glick 1956).In one of the interview conducted by Brien Williams on 6th May 2012, during 99th annual [ed. 96th annual] meeting of the American Association of Immunologists at the Sheraton Boston Hotel in Boston, Massachusetts, Cooper has mentioned that during 1960s, Poultry Science Journal was not the choice for many medical immunologist but a few group like Cooper`s lab and Noel Warner group from Australia took interest on Glick`s finding published in Poultry Science Journal (Cooper 2012). An independent group working from a distantly located laboratory at Wisconsin demonstrated that injecting testosterone a male hormone to birds can halt bursal development along with antibody deficiency, it was noticed by Noel Warner at Australia, so Warner and his colleague also treated the chicks with testosterone and observed that some of the treated chicks was unable to produce antibody and a few also failed to reject skin graft with damaged thymus (Warner *et al.*, 1962). With this finding a dissociation concept of immune-response was unfolded, describing humoral immune-

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response is governed by bursa and cell mediated immune-response is controlled by thymic tissues proposed by Jankovic`s lab (Jankovic and Isvaneski 1962). Conceptually it was acceptable for avian species at that pint of time but not substantially convincing for mammalian species those lacking bursa. While investigating the small intestine of mammalian species like rabbit (Orycotolagus cuniculus) the terminal part of ileum at the ileo-coecal valve an ampulla (outpouching of the lower gut) known as sacculus rotundus is unique to rabbit and partly act as homologue to chicken bursa. Early excision of sacculus rotundus in neonates cause impaired antibody repertoire in rabbit (Archer et al 1963; Perey and Good 1968). The ileal Peyer's patch (PP), of sheep and cattle act as a site of B-cell development (Yasuda *et al*., 2006). Extensive works conducted by Reynolds from Basel Institute for Immunology, Switzerland, has shown that there is ample evidence to support the role PP of sheep as a primary lymphoid organ like that of bursa of Fabricius in birds (Reynolds and Morris 1983). Further this group has advocated for the existence of two distinct types of Peyer's patches in sheep (Reynolds *et al.*, 1985). The head kidney, analogous to the mammalian adrenal gland of bony fish act as a bone marrow equivalent, responsible for production of B-cells (Soulliere and Dixon2017). All these findings give an indication towards evolutionary versatility in the development of primary lymphoid organs in vertebrate phylogeny.

Exploring further on avian immune system a remarkable finding was published by Cooper group in Nature, 9th Jan 1965; in that publication they have described an experimental model of agammaglobulinemia, and have defined the thymic and bursal systems in the chicken. In their experiment neonatal bursectomy and thymectomy on day of hatching followed by sublethal irradiation to destroy the dividing lymphocytes in peripheral circulation made the bird to be non-responsive to antigenic stimulation and such birds failed to produce antibody against bovine serum albumin and Brucella abortus antigen. It is one of the influential findings to show that bursa is the organ where B cell development takes place and antibody formation depend upon thymus derived lymphocyte (Cooper *et al*., 1965). Existence of bursa equivalent organ in mammal that produce B cell was difficult to trace and most of the speculation was moving around intestine but due to lack of concrete evidence all efforts were moving in blind directions (Cooper 2010). In the year 1974 Martin Raff and John Owen from University college of London extended their collaboration to Cooper and initiated in-vitro culture of mouse foetal liver cell harvested from 14 days embryo. Within 4 to 7 days of incubation differentiation of foetal liver cells to B cells was observed by them (Owen *et al*., 1974). Simultaneously Gustav Nossal working independently from University of

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Geneva, Switzerland was also able to establish the differentiation of B cells from bone marrow cells grown under laboratory condition (Osmond and Nossal 1974). Their findings ultimately brought evidence in favour of foetal liver cells as source of B lymphocytes during embryogenesis till bone marrow take up the responsibility for steady supply of B cells in adult life (Owen *et al*., 1974; Osmond and Nossal 1974). Till 1960 the role of bursa for antibody production was known to be obligatory however subsequent research findings from 1970 onwards has shown that bursal independent immunoglobulin from non-bursal site also operative in avian species. (Lerner *et al*.,1971; Fitzimmons *et al*., 1973). Several workers have reported that in-ovo bursectomy prior to 72 hours of incubation invite a situation where the appearance of B cells and immunoglobulin was evidenced in hatch out chicken but no antibody response in those birds. Presently we know more about the complexity of immunoglobulin gene diversity yet avoiding those complexity in this manuscript simply we want to emphasize that the immunoglobulin gene rearrangement occurs in non-bursal site but the gene conversion demands bursal microenvironment for the diversification of the immune repertoire (Masteller *et al*., 1997).

from illusion to conclusion: In 2018 Bruce Glick became the recipient of Golden goose award and detail can be seen from the website as "The Goose Gland: Discoveries in Immunology" (https://www.goldengooseaward.org/01awardees/goose-gland-immunology). Today we have felt the impact of goose gland discovery by which we are now moved from neonatal to in-ovo vaccination, of poultry birds. It is a scientific trick to prick the developing embryo with vaccine agent for mass immunization is astonishingly unbelievable scientific development. No way this is possible for human or primate embryos to be primed in this manner. But as time and science moved forward so we are fearless to achieve peerless. Presently we know that before hatching the immune system of avian species is already competent enough having enough number of mature B cells in their peripheral circulation expressing membrane immunoglobulin on its surface released after proper schooling from developing bursa. Those B cells within embryo can recognise and respond against foreign antigen to induce protective immune response to the new born chick; this is the contribution of Bruce Glick.

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