

The Bursa of Fabricius isolated from intestinal flow in chicken at hatching: The surgical technique

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Summary. A proposed operational technique is described whereby the intestinal flow from the Bursa of Fabricius in chickens at hatching is excluded.

Many authors have been anxious not only to define the maturative events in the bursal lymphoid follicles but also to characterize the factors and the essential conditions for the maturation of the follicle itself. Among the stimuli capable of favouring this maturation, the role exerted by the intestinal flow seems to assume particular significance¹. In order to check whether the role of the latter is a significant inducer of lymphopoiesis and bursal immune response, it was considered important to exclude the intestinal flow from the Bursa of Fabricius. For this purpose we have built a modified experimental model which had been tried out by Mueller in 1962 'with no success'².

The operation consists in tying and cutting the connecting duct between the Bursa of Fabricius and the cloaca with a minimal lesion of blood vessels. Moreover, an inert and sterile diaphragm was interposed between the Bursa of Fabricius and the cloaca in order to prevent the 2 organs being in contact with one another.

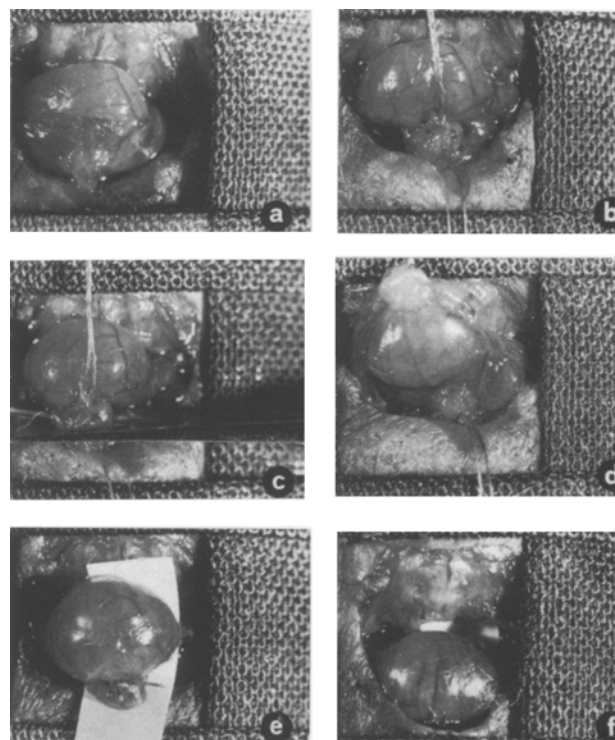
Before the detailed description of the surgical technique, it seems worthwhile pointing out certain anatomical and histological observations regarding the burso-cloacal region. Vascular connections and the localization of bursal lymphoid follicles allow us to obtain the complete isolation of the lymphoid component from the contents of the intestinal flow with no substantial alteration of the blood flow. In a histological examination the bursal duct does not contain lymphatic follicles; therefore by tying and cutting the duct at the same point as that of its outlet in the cloaca, we are sure to obtain the isolation of the whole bursal lymphoid component from the intestinal flow. Anatomical examination of the burso-cloacal region makes it evident that the major supply of blood is from the large terminal branches of the internal pudendae arteries. At the same point as that of the ureteral outlet into the Urodeo, the branches are directed down towards the cloaca, and up towards the lateral wall of the Bursa of Fabricius. Next, these arteries give rise to a vascular stalk situated laterally on each side of the Bursa of Fabricius³. During the surgical operation these 2 branches are saved including the satellite veins, so that blood supply of the Bursa of Fabricius is sufficient although the small collateral branches of the internal pudendae arteries and veins are removed.

To put the surgical technique into practice we utilize a Zeiss Stereoscopic microscope with coaxial light incorporated. The animals are anaesthetized with local injections of 0.25 cm³ of a solution containing lidocaine 2%. Suture stitches are given with Ethicon 0.7 mounted needles or 0.4 catgut. The experimental animals used are 20 chickens of the Hubbard strain obtained on the day of hatching from a commercial hatchery.

The surgical operation is carried out during the first few hours after hatching. The result of the operation is checked 15 days after hatching and based on direct macroscopic observation and the histological examination of the Bursa of Fabricius.

The surgical technique (figure): a horizontal cut of 5 mm is made just above the upper lip of the anus. After divaricating the skin we proceed to dissect the tissues overlying the Bursa of Fabricius, taking care to keep the point of the lancet turned upward in order not to cut the Bursa of

Fabricius. The latter can be seen when the connective layer which separates the s.c. tissue from the Bursa of Fabricius is reached. Now we can see the dorsal surface at the point of the duct which connects it to its outlet in the cloaca. As the ureters, the internal pudendae arteries and the veins followed by the burso-cloacal branches of the pelvic nerves are uncovered, we proceed from the point where the ureters form an angle before their outlet reaches the cloaca. Making an incision from each side, we liberate a small part of the bursal duct at the point of the dividing-line between the Urodeo and the Proctodeo. Then we lift the bursal duct and slide 2 Ethicon 0.7 threads under it. The 1st thread is tied near the beginning of the duct from the Bursa of Fabricius; the 2nd thread is as far away as possible and is brought posteriorly as far as the upper surface of the cloaca.



The surgical technique whereby the intestinal flow from the Bursa of Fabricius in chickens at hatching is excluded. The operative area is limited to the zone between the upper edge of the anus and the base of the tail in chickens at hatching. The Bursa of Fabricius is clearly seen thanks to a horizontal cut in the skin and the s.c. connective tissue; the bursal duct is isolated (a) 2 0.7 Ethicon threads are put between the bursal duct and the cloaca. The threads are then knotted: one near the ureters and the other close to the outlet of the duct in the Proctodeo (b), a cut is made between the 2 threads (c), the surfaces of the cut are separated in order to ensure that the bursal duct is completely severed (d), afterwards the bursa is gently removed from the cloaca, leaving the lateral vascular stalks in tact and a diaphragm made of inert material is slid into the space between the 2 organs (e) and is left placed between the two (f). $\times 3.5$.

We then cut the duct between the 2 threads, which are carefully raised. At this point the Bursa of Fabricius is isolated from direct contact with the cloacal materials, and is gently lifted in such a way that, though the connections with the lateral vascular stalks are integrated, it is also possible to enter the space between the 2 organs. In this space an inert diaphragm (Silastic, 5 mm thick obtained from Dow Corning Corporation USA) is introduced to ensure that the separation of the 2 organs is a lasting one. Finally the edges of the cut on the skin are sutured.

This experimental model is obtained with minimal surgical damage; it allows us to effect a complete and sure interruption of the intestinal flow between the Bursa of Fabricius and the cloaca. Furthermore the blood circulation is practically not modified since the operation does not involve the large vessels of the Bursa of Fabricius. They remain topographically outside the point of operation. The survival rate of the chickens operated on at hatching is very high (85%).

After the operation the animals feed normally and in a short time they acquire normal vitality. On the 15th day after the operation the Bursa of Fabricius takes on a spherical shape and it becomes slightly larger in size than the Bursa of Fabricius in control animals. This fact is explained by the accumulation of a viscous PAS positive and sterile liquid in the Bursa itself. It is not possible to see the residual duct of the Bursa applied to the cloaca. Histologically the pattern of the Bursa of Fabricius does not differ from that in unoperated controls.

- 1 J.H. Thompson and M.D. Cooper, *Transplantation* 11, 71 (1971).
- 2 A.P. Müller, H.R. Wolfe, R.K. Meyer and R.L. Aspinall, *J. Immun.* 88, 354 (1962).
- 3 V. Pintea, Gh.M. Constantinescu and C. Radu, *Acta vet. hung.* 17, 263 (1967).

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