CHAPTER 2

PRELIMINARY OBSERVATIONS ON THE APPEARANCE OF LYMPHOCYTOPOIETIC NODULES IN THE LIVER AND CAECA OF DEVELOPING PIGEONS

The lymphocytopoietic nodules in the pigeon liver (Pilo, 1967 & 1970; Chapter 1) are reported so far from the adult pigeons only. There is no relevant information that shows when such lymphocytopoietic activity commences in the liver of pigeon. In fact, one could only speculate that the nodules found in the pigeon liver are lymphocytopoietic in function. It is then necessary to obtain enough information by which one could state that the function of nodules is lymphocyte production as well as the time when nodules appear in the liver.

In the previous chapter, it is suggested that the pigeon liver with its lymphocytopoietic nodules could function like lymph nodes of mammals. The lymph nodes in mammals usually depend on the thymus for the lymphocytopoietic activity. In birds, both thymus and bursa of Fabricius are in a position to influence the lymphocytopoiesis in other organs such as spleen, liver and caeca. Intestinal caeca of pigeon is known to have

lymphoid follicles (Marshall, 1960). The present investigation was carried out in the liver, spleen and caeca of developing pigeons inorder to obtain informations regarding the time of appearance of nodules in the liver and a possible relationship or parallelism in the activities of these organs with other lymphoid tissues like thymus and bursa of Fabricious.

MATERIAL AND METHODS

Young ones of the pigeon, in the post-hatching (developing) period were collected for the studies. These young ones were of parent pigeons reared in large aviary. Out of a clutch of two that hatched the first was only utilized.

Young ones were taken at regular intervals upto one month. Two month old as well as adult pigeons at different seasons were also taken for the study. It is observed that when one month old the pigeom are able to fly and hence could be treated as juveniles. All these birds were decapitated under mild anesthesia and thymus, bursa of Fabricipus, spleen, liver and caeca were removed for the present studies. The weights of thymus, bursa and spleen were recorded. For histological studies liver was fixed in calcium formol and frozen sections

were stained with haematoxylin and mounted in glycerine jelly. The nodular count per unit area was determined from such sections only. Caeca was fixed in formol saline and paraffin sections were stained with haematoxylin-eosin as well as with Jenmer-Giemsa stains.

RESULTS

Nodular count in the liver:

Lymphocytopoietic nodules in the liver of growing pigeons appeared only in the post hatching period. However, small lymphocytic aggregations could be detected in the liver during late embryonic stages. First day after hatching an appreciable number of nodules (7 per field) were seen in the liver. But this number decreased by 5th day only to increase again to a considerable extent between 7th (13 per field) and 8th day (Table 1 & Fig. 15). Thereafter again reduction in their number was observed. Towards the juvenile stage the nodule number increased again but in the adults that varied according to the state of moulting and reproduction. During moulting period the nodule number was very high compared to that in the liver of an adult bird during non moulting or non reproductive phase (Table I).

TABLE I

Weight of spleen and nodular count in the liver of growing pigeons

Age	Body weight in g	Spleen weight in g	Nodular count in the liver per unit area
1 day prior to hatching	11	0.008	...
1 day after hatching	17	0.01	7
2 days	25	0.01	7
3 days	40	...	6
4 days	45	0.06	5
5 days	57	0.05	4
7 days	90	0.18	13
8 days	120	0.16	10
10 days	160	0.15	9
11 days	170	...	3
13 days	180	0.11	2
14 days	190	0.10	4
15 days	167	0.11	5
21 days	170	0.07	1
25 days	173	0.05	1
30 days	175	0.14	4
2 months	250	0.325	6
Adult (Non-breeding and moulting)	304	0.567	5
Adult (beginning of moulting period)	350	0.581	8
Adult (moulting period)	361	0.623	12

TABLE II

Weight of Thymus, Bursa of Fabricius, spleen and nodular count in the liver of growing pigeons

Age	Body weight in g	Thymus weight in g	Bursa weight in g	Spleen weight in g	Nodules in the liver
1 day prior to hatching	11	0.005	....	0.008	...
1 day after hatching	17	0.019	0.027	0.01	7
5 days	57	0.127	0.115	0.05	4
10 days	160	0.343	0.1863	0.15	9
15 days	167	0.422	0.1497	0.11	5
20 days	170	0.261	0.220	0.11	1
30 days	175	0.454	0.2303	0.14	4
2 months	250	0.217	0.019	0.325	6
Adult (Non-breeding and moulting period)	304	0.064	....	0.567	5
Adult (beginning of moulting period)	350	0.155	....	0.581	8
Adult (moulting period)	361	0,370	....	0.623	12

Weight of spleen:

Spleen weight was found to vary along with the nodular count in the liver in the growing young ones pigeon. Maximum weight noted in the young ones was between 7th and 10th day after hatching (Table I and Fig. 15). The spleen weight in 24 days old pigeons decreased considerably only to increase again during later period of growth. In the adult birds the weight of the spleen was found to be highly variable according to the seasons of physiological state of bird such as moulting or reproductive periods. Thus in adult birds the spleen increased in weight during moulting period just as the number of nodules in the liver of adult pigeons.

Changes in the caeca:

Until 4th day after hatching the caeca of growing pigeons resembles the rectum to which it is attached in the pigeons. But by 6th day lymphocytes appeared in the regions between tunica propria and submucosa. By 10th or 24th day, nodules were found to develop in the caeca and in adults many such lymphocytopoietic nodules could be~~en~~ seen in the caeca (Figs. 1 - 14).

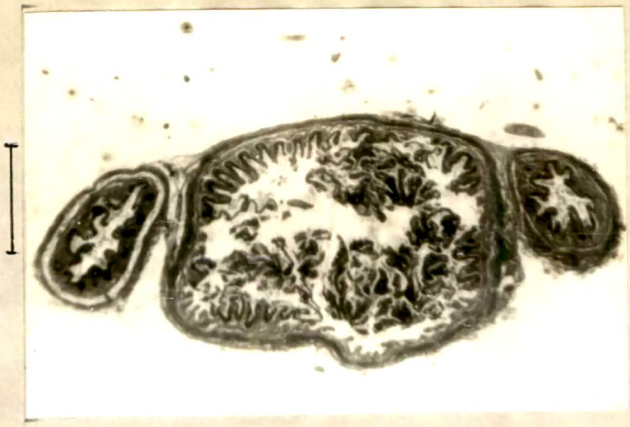
(Chapter 2: Figs. 1 to 4. Photomicrographs of the C.S. of intestinal caeca of developing pigeons. Haematoxylin-eosin stained)

Fig. 1. Caeca of 1 day old pigeon along with the intestine.

Fig. 2. Caeca of 1 day old pigeon. Higher magnification of Fig. 1. Note the absence of lymphocytes in the region between the tunica propria and submucosa. The histological picture is similar to that of intestine.

Fig. 3. Caeca of 4 days old pigeon along with the intestine.

Fig. 4. Caeca of 4 days old pigeon. Even at this stage the lymphocytes are absent in the caeca.



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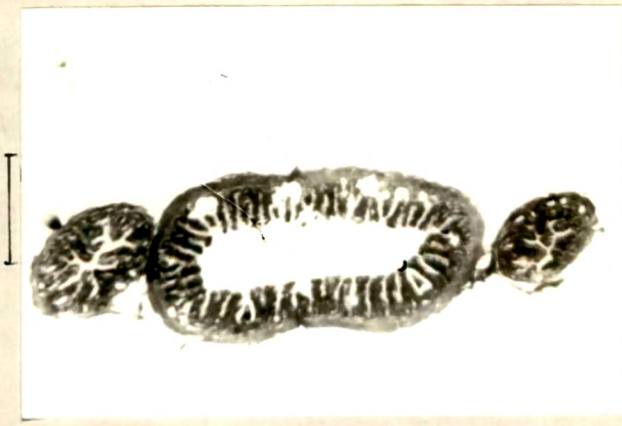

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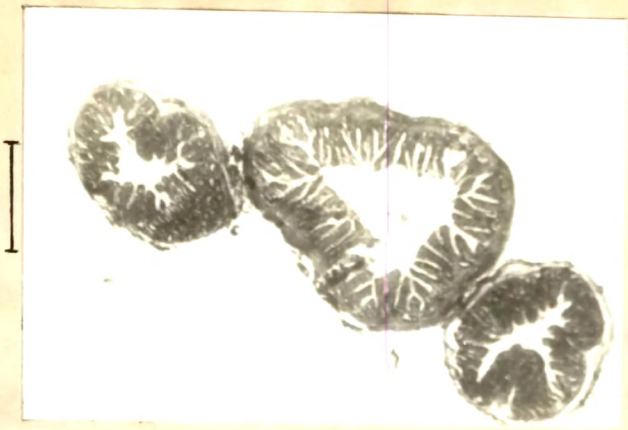
(Chapter 2: Figs. 5 to 8. Photomicrographs of the C.S. of intestinal caeca of developing pigeons. Haematoxylin-eosin stained)

Fig. 5. Caeca of 6 days old pigeon.

Fig. 6. Caeca of 6 days old pigeon. Large number of lymphocytes are present (arrow) in the region between tunica propria and submucosa.

Fig. 7. Caeca of 10 days old pigeon.

Fig. 8. Caeca of 10 days old pigeon. Note the increased number of lymphocytes that displaced both tunica propria and submucosa.



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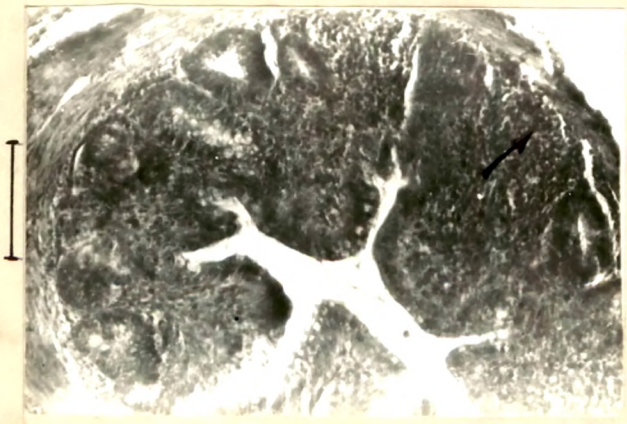

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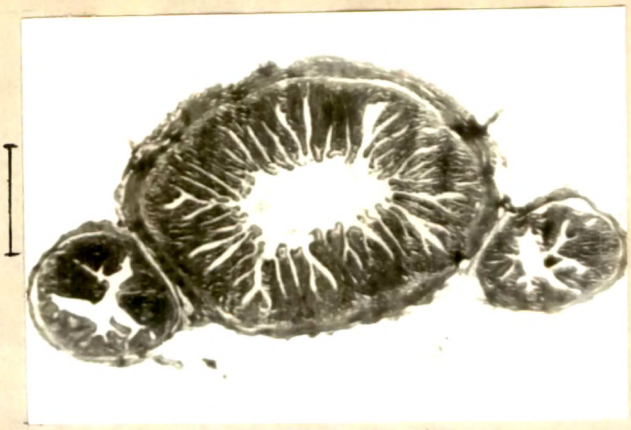
(Chapter 2: Figs. 9 to 12. Photomicrographs of the C.S. of intestinal caeca of developing pigeons. Haematoxylin-eosin stained)

Fig. 9. Caeca of 24 days old pigeon along with intestine.

Fig. 10. Caeca of 24 days old pigeon. Submucosa is completely obliterated by the lymphocytes. The appearance of villi is due to the folds of mucous membrane.

Fig. 11. Caeca of 1 month old pigeon. The caeca becomes highly enlarged.

Fig. 12. Caeca of 1 month old pigeon. Whole region is filled up with lymphocytes.



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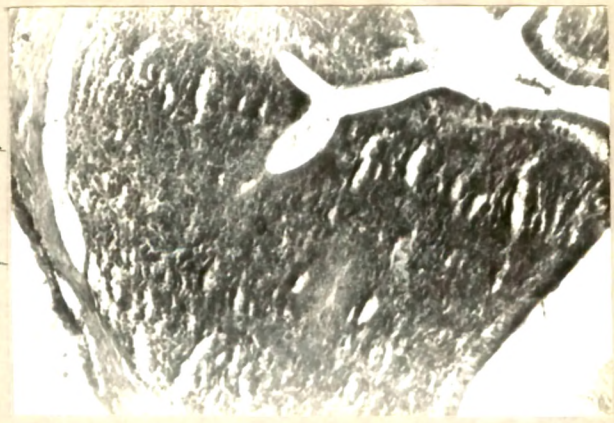

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(Chapter 2: Figs. 13 & 14. Photomicrographs of the C.S. of intestinal caeca of developing pigeons. Haematoxylin-eosin stained)

Fig. 13. Caeca of adult pigeon. Note the size of the caeca.

Fig. 14. Caeca of adult pigeon. The nodules are seen in large number in the caeca of adult birds. In the photomicrograph one such nodule (N) is seen.

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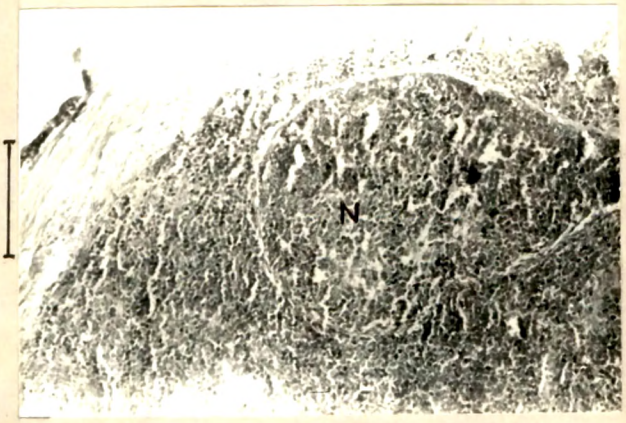

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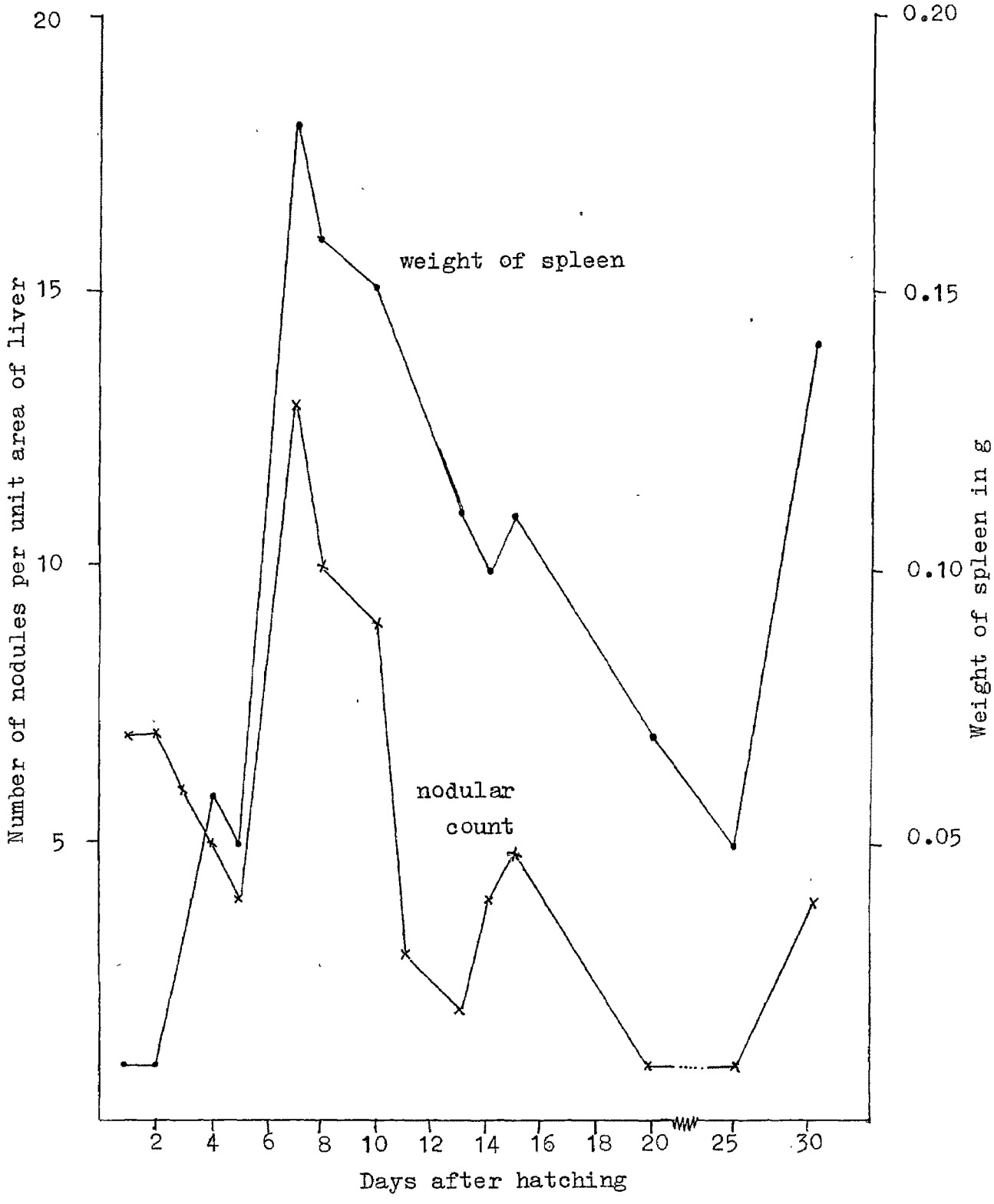
Fig. 15. Changes in the weight of spleen and the number of nodules in the liver of growing pigeons.



Bursa of fabricius:

Bursa of Fabricius ~~right~~ increased in weight upto one month and in the later months its weight decreased to a lowest as regression of the organ took place at this period. In adult, mature birds, bursa became almost nonexistant (Table II).

Thymus:

Thymus was found to increase in weight upto 1 month. In birds of 2 to 3 months old, thymus weight, decreased considerably. In adult birds the thymus weight was highly variable. The thymus of moulting birds was found to become enlarged and hence a weight increase was noticed (Table II).

DISCUSSION

From the data of this preliminary study, it could be reasonably suggested that liver gets the blast cells of lymphocytic series from the early embryonic period itself and thus have very little dependance on the stem cells from bursa or thymus. Since, along with the variations in the weight of thymus and spleen a corresponding variation ~~is~~ was seen in the number of nodules

in the liver during certain seasons it could be stated that in this bird, liver acts almost like a lymphoid tissue. Thus any demand for lymphocyte production, phagocytosis or the antibody production might get reflected on all these lymphoid tissues especially the spleen and liver.

The thymus is known to play some role during moulting. This strange relationship has already been noticed in ring-necked pheasants by Anderson (1970). It is unlikely that some humoral substance is elaborated by thymus for the moulting processes since a parallel increase in the weight of spleen and the number of nodules in the liver was also seen. This aspect of the study is inconclusive since a large number of samples were not examined for fear of degressing from the present aspect of the problem.

All one could say is that perhaps lymphocytes are required in large number during moulting and this demand is reflected in lymphocytopoietic activity in thymus, spleen and liver. Perhaps the lymphocytes may be required near feather follicles for phagocytic activity that may be necessary for discarding the old feather remains. It is interesting to note here that lymphocytes could transform into macrophages and collagen producing fibroblasts (Petrakis, 1961). Perhaps these lymphocytes

are necessary for the formation of connective tissue at the time of feather growth or moulting. Whatever may be the reason, the fact that lymphocytes are necessary during the moulting or feather growth in young ones is evident from the data; the thymus, spleen and number of nodules all showed increased activity between 7 to 10 days after hatching as well as during moulting. Anderson (1970), though of the opinion that the thymus activity during moulting may be having a survival value at periods when the bird is "at their annual low ebb in body weight and stress resistance", also agrees that during moulting an increased supply of lymphoid cells is necessary.

Coming back to the present interest in the study, it could be then stated that liver in this bird is a lymphocytopoietic centre just as bone marrow is the haematopoietic site. Since liver is an embryonic haematopoietic centre, the continuation of such activity throughout the life span is not a strange phenomenon. Due to this, the lymphocytopoietic function of the liver also needs not depend on the supply of cells from either bursa or thymus as it is seen that the nodules in the liver of pigeon develop right from hatching period and not late during growth period unlike in caeca where the lymphocytes appear only by 6th day. Though well defined

nodules could be seen in the liver just by the time of hatching, it is likely that lymphocytes or the stem cells are already there from early stage of embryonic development.

In conclusion, it could be said that the liver in pigeon, in all probability, acts as a lymphoid tissue. This is not stray incident where such nodules are seen in the liver of adult animals. The passerines Sturnus roseus also have such nodules in their liver (George and Naik, 1963) and frogs (Rana tigrina) too have them in their liver (Chapter 5).