CHAPTER 3

INCREASED LYMPHOCYTOPOIESIS IN THE LIVER OF PIGEON FOLLOWING HAEMORRHAGE, SPLENECTOMY AND INJECTION OF CARBON TETRACHLORIDE

Concerning the presence of lymphocytopoietic nodules in the liver of pigeon it was stated that these structures in the liver are not only important for the production of lymphocytes (Chapter 1; Pilo, 1967; 1970) but also in phagocytic and immunological reactions, supplementing the function of lymphoid tissue like spleen or act as a substitute for the lymph nodes (Chapter 4) which are absent in most of the birds (Jordan, 1936; 1939; Romanoff, 1960).

The present investigation was undertaken with a view to add other experimental evidences in support and confirmation of the contention that number of lymphocytopoietic nodules per unit area would vary according to the demand for lymphocytopoiesis.

MATERIAL AND METHODS

Healthy adult pigeons (Columba livia) were selected for experiments. In one set of pigeons (Nos. 34), a sublethal amount (6 ml) of blood was withdrawn using

glass syringe from each bird. Following the sublethal haemorrhage, pigeons were killed three at a time at regular intervals of 1, 2, 3, 4, 6, 8, 10, 15, 20, 25, and 30 days. For histological studies liver and bone marrow from long bones like, femur were removed. Smear preparations of bone marrow were stained with Jenner-Giemsa and frozen sections from 10 % neutral formalin fixed liver were stained with haematoxylin-eosin as well as with Jenner-Giemsa for the nodular count per unit area (per 3.5x objective field).

In the second experiment splenectomy was carried out on 36 ether anaesthetized birds. From some birds only a part of spleen was removed. After the operation abdominal wall and skin were separately sutured with fine nylon thread. At regular intervals viz., days 6, 8, 12, 18, 30 and 40, five birds each time were sacrificed by decapitation under mild anaesthesia. From these, liver was quickly removed and fixed in 10% neutral formalin and processed for nodular count as described before.

To a third set of 25 pigeons carbon tetrachloride was administered. Each bird received 0.2 ml per 100 g body weight per day for one week. So treated birds were sacrificed at regular intervals (taking five per each interval) viz., days 6, 12, 18, 30 and 40. The liver from each of these birds was processed as described before for nodular count.

RESULTS AND DISCUSSION

The results are tabulated in the Table I, II and III. It is shown that the number of lymphocytopoietic nodules in the liver of pigeon varies with season (Pilo, 1970). In the season during which the experiments were carried out, the normal pigeon liver contained about 8 to 12 nodules per unit area. Following experimental haemorrhage the number of lymphocytopoietic nodules in the liver increased. The maximum number of nodules (38) were seen by about 10th day after haemorrhage (Table I), a period when leucocytes count was increased in the bone marrow. However, no lymphocytopoiesis was observed in the bone marrow. This observation agrees with the report of Jordan (1939) that the bone marrow of active fliers like pigeon is not lymphocytopoietic. Lymphocytes production was then mainly a function of spleen and liver as evidenced from the fact that both, the weight of spleen as well as number of nodules in the pigeon liver increased simultaneously (Table I).

In the second set of experiments in which splenectomy was carried out, the nodular count increased in the liver by 30th day in the total splenectomized birds, whereas no change in nodular number from the normal

TABLE I

Nodular count in the liver, leucocyte count in the bone marrow and the weight of spleen in the pigeons after the subletbal haemorrhage

Days after haemorrhage	Average body weight g	Nodular count in liver	Leucocyte count in the bone marrow *	Weight of the spleen g
Normal	335	8-12 (a)	231*	0.6825 (b)
1	325	12	220	0.5285
2	330	12	234	0.5362
3	340	14	256	0.5862
4	320	19	273	0.7241
6	330	21	276	1.06
8	342	22	289	1.253
10	330	38	330	1.522
15	341	20	320	0.8488
20	345	13	324	0.6265
25	337	13	183	0.654
30	329	12	202	0.589

(a) Normal nodular count in the given season (December-April)

(b) Normal spleen weight in the given season (December-April)

(*) Number of leucocytes per 1000 cells in the differential count

TABLE II

Nodular count in the liver and the weight increase of the regenerating spleen after partial and complete splenectomy

Days after splen-ectomy	Average body weight	Nodular count in liver		Weight increase of the spleen after partial splenectomy	
		WITH COMPLETE SPLENECTOMY	WITH PARTIAL SPLENECTOMY	WEIGHT REMOVED	WEIGHT AT KILLING
Normal	343	13 (a)	--	0.7378 (b)	
6	338	25	14	0.6528	0.2679
12	345	37	15	0.6569	0.3945
18	345	38	12	0.6058	0.7398
30	325	40	13	0.5872	0.6913
40	341	17	12	0.6256	0.7012

(a) Normal nodular count in the given season (December-April)

(b) Normal spleen weight in the given season (December-April)

TABLE III

Nodular count in the liver and spleen weight after the injection of carbon tetrachloride (0.2 ml/100 g body weight)

Days after injection	Average body wt.	Nodular count in the liver	Weight of spleen (g)
Normal	350	12 (a)	0.518 (b)
6	343	10	0.536
12	329	40	0.604
18	325	25	0.529
30	352	14	0.586
40	349	15	0.631

(a) Normal nodular count in the given season (December-April)

(b) Normal spleen weight in the given season (December-April)

one was noticed in the partially splenectomized birds (Table II). When splenectomy was total the increase in nodular count in the liver was three to four fold compared to the number of nodules in the unoperated birds. Unlike after haemorrhage, here the highest number was observed by about 30th day only. This could be perhaps due to the fact that the demand on lymphocytopoiesis may not be acute. Since in the splenectomized birds the number of nodules per unit area was very high it could be reasoned that the birds liver had taken over the burden of spleen function of lymphocytopoiesis. The nodular count in the birds with the partial splenectomy did not increase (Table II) as the remaining part of the spleen readily regenerated (Table III) and hence the liver might not have been called for the additional lymphocytopoietic activity.

In the last set of experiments, carbon tetrachloride caused focal necrosis in the liver. The nodular count in the liver increased tremendously by about 12 days. This sudden increase in the number of nodules could be for the production of large number of lymphocytes which could participate in the phagocytic activity necessary to remove damaged cells. Phagocytic activity of lymphocytes from the nodules in the pigeon liver has also been showed (Chapter 4). This observation shows that, the

demand of lymphocytes for phagocytic activity, is also one of the factors that may enhance the production of more lymphocytes (by increasing the number of nodules) in the pigeon liver. Though changes in the weights of spleen were noticed when carbon tetrachloride was injected, the variation remained within the normal range of its weights in the season during which the experiments were conducted.