

LEUCOCYTIC CHANGES AFTER SPLENECTOMY IN DOGS*

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Our earlier study of Erythrocytic changes after splenectomy in dogs (17) has already been reported. The mechanism of splenic function still remains obscure. The present study is to find the effects of the removal of normal spleen in the same animal with respect to leucocytes and lymph-nodes and to assess the mechanism of its function.

MATERIALS AND METHODS

Stray mongrel dogs with average weight of 13.5 kg. were chosen for the experiments. The dogs were operated under chloralose anaesthesia (85 to 100 mg/kg body weight) after usual pre-operative precautions and control collections of blood.

The spleen was removed under strict aseptic conditions after careful and complete ligation of the splenic vessels.

Usual post-operative aseptic and antiseptic precautions and procedures were observed. The samples of blood were collected from the tibial, saphenous or cubital veins immediately after the operation and on 2nd, 3rd, 5th, 7th, 10th, 15th, 20th, 30th, 45th, 60th and 90th day. The blood samples thus collected were subjected to the usual methods of count, microscopic and morphological examinations.

On the 7th, 30th (after the leucocytosis started showing definite regression) and 60th day a mesentric lymph node was taken out per abdomen for histological examination. Frequent nodal examinations were avoided to save interference of further operative trauma.

RESULTS

The spleen was removed in a total of eleven dogs. The results are given as below:

1. Changes in leucocytes — as per table and figs 1 and 2.
2. Changes in lymph nodes — fig. 3,4 and 5.
 - (a) No change till 7th day.
 - (b) Progressive increase in size and density, most marked on 60th day.
 - (c) Cellular hyperplasia of the cortex, germ centre almost disappears.
 - (d) Increased vascularity of the medulla resulting in large blood sinusoids.

Mean values of the eleven experiments (Dogs)

Days	W.B.C. per C mm	Differential W.B.C. Count												
		Neutrophils				Eosinophils				Lymphocytes		Monocytes		
		P	%	P	Abs.	P	%	P	%	P	Abs.	P	%	P
Pre	9877		57.4		5650		1.3		38.3		3813		3.0	
—Op														
S.E.	814		0.9		266		0.7		1.7		221		1.0	
1st	16805	<0.01	68.5	<0.01	11767	<0.01	1.0	>0.05	27.5	<0.01	4457	>0.05	3.0	>0.05
S.E.	1768		2.4		1980		0.1		1.4		378		0.6	
2nd	24905	<0.01	71.2	<0.01	18472	<0.01	1.4	>0.05	24.4	<0.01	5500	>0.05	3.0	>0.05
S.E.	3248		3.1		2140		0.6		2.6		818		1.0	
3rd	26168	<0.01	75.3	<0.01	20226	<0.01	0.5	>0.05	21.1	<0.01	5402	>0.05	3.6	>0.05
S.E.	4222		2.7		3258		0.4		3.5		1369		0.7	
5th	32664	<0.01	78.3	<0.01	25531	<0.01	0.4	>0.05	18.3	<0.01	6112	<0.05	3.0	>0.05
S.E.	2265		22.3		1550		0.4		2.2		727		0.7	
7th	35691	<0.01	79.4	<0.01	28659	<0.01	0.6	>0.05	17.6	<0.01	6117	<0.05	2.4	>0.05
S.E.	4787		2.4		4158		0.3		2.8		919		0.4	
10th	39691	<0.01	76.3	<0.01	30824	<0.01	0.4	>0.05	20.1	<0.01	7071	<0.05	3.2	>0.05
S.E.	5789		1.4		4624		0.2		1.5		1245		0.8	
15th	32482	<0.01	76.6	<0.01	24976	<0.01	0.9	>0.05	18.5	<0.01	5991	<0.05	4.0	>0.05
S.E.	3902		1.7		3094		0.3		1.6		796		0.8	
20th	33032	<0.01	79.8	<0.01	26672	<0.01	0.6	>0.05	16.8	<0.01	5437	>0.05	2.8	>0.05
S.E.	4793		1.6		4241		0.2		1.2		785		0.8	
30th	27455	<0.01	77.1	<0.01	21992	<0.01	0.7	>0.05	19.2	<0.01	5407	>0.05	3.0	>0.05
S.E.	4017		2.4		3141		0.3		2.2		997		0.9	
45th	21264	<0.01	73.0	<0.01	15492	<0.01	1.4	>0.05	21.4	<0.01	4547	>0.05	4.2	>0.05
S.E.	2062		1.9		115		0.4		1.7		536		1.2	
60th	18614	<0.01	71.2	<0.01	13240	<0.01	1.3	>0.05	23.0	<0.01	4235	>0.05	4.5	>0.05
S.E.	1077		2.2		709		0.4		1.5		411		0.8	
90th	14841	<0.01	65.8	<0.01	9609	<0.01	1.4	>0.05	28.0	<0.01	4089	>0.05	4.8	>0.05
S.E.	1179		1.5		672		0.5		1.3		264		0.7	

S.E. = Standard Error
P Evaluated by 't' test

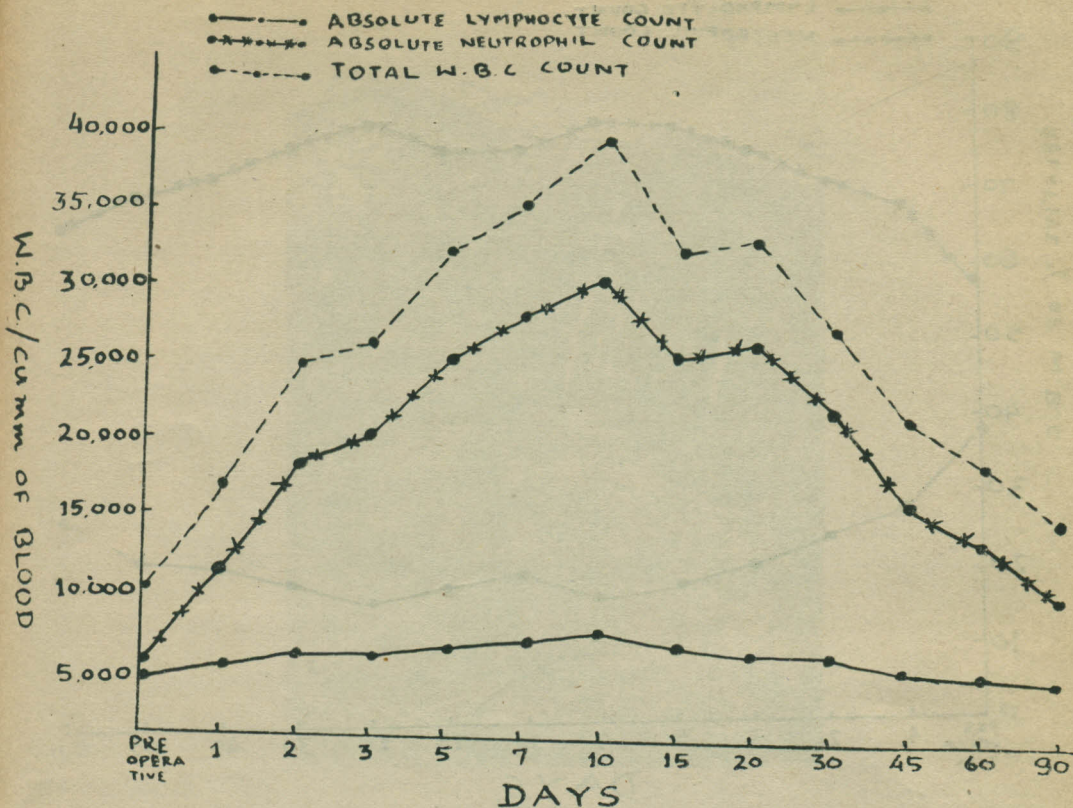


Fig. 1

DISCUSSION

The white cell count in the present set of experiments registered a definite, consistent and marked increase as compared to preoperative levels. The mean preoperative count of 9877 ± 814 cells/cmm of blood increased to 16805 ± 1768 cells/cmm on the first day and to a maximum of 39691 ± 5789 cells/cmm on the 10th day. The count then gradually declined to 14841 ± 1179 cells/cmm on the 90th day of splenectomy. The increase in the leucocyte count above the preoperative level was found to be statistically significant throughout ($P < 0.01$). Out of all the white cells the increase was most manifest in the neutrophils. The neutrophil count rose up to the 20th day, beyond which there was a tendency towards gradual regression. The absolute counts of both neutrophils and lymphocytes increased but the increase in the former was by far the greater—so much so that when percentage levels were compared it denoted marked neutrophilia mainly at the expense of lymphocyte percentage (the major component) and eosinophil percentage to lesser extent. Hence although lymphocytes increased in absolute number (statistically insignificant throughout, ($P > 0.05$), fig. 1 and 2), still percentage values decreased. Hence, on calculating and comparing percentages, neutrophils demonstrated a statistically significant increase ($P < 0.01$), whereas lymphocytes a

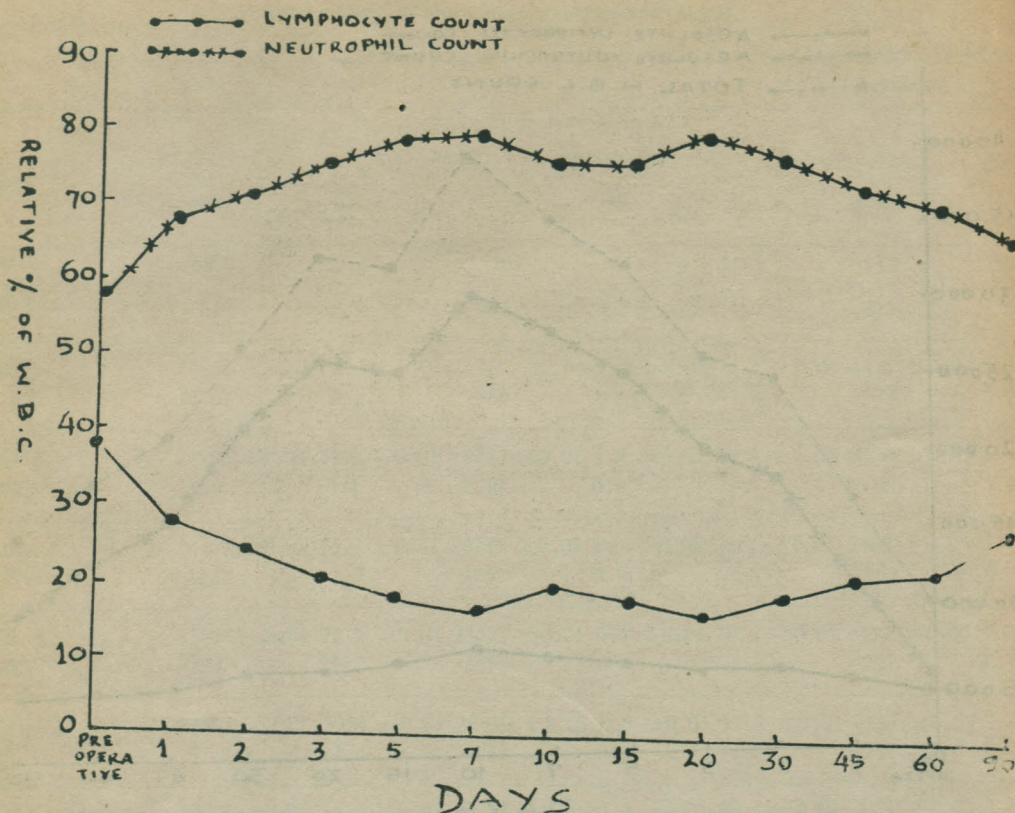


Fig. 2

significant decrease ($P < 0.01$). The eosinophils being the negligible component, although showed increased absolute count and decreased percentage, were statistically insignificant throughout ($P > 0.05$). Monocytes remained almost unchanged, and changes in relative and absolute counts were insignificant throughout ($P > 0.05$). Occasional immature cells were seen particularly when the leucocytosis was severe.

A macroscopic and microscopic study of nodes was carried out as a representative of R.E.S. The first nodes removed on the 7th day were almost normal, fig.3. The subsequent nodes were taken out on 30th day of operation because:

- (1) to save undue operative trauma involved in more frequent removal of nodes,
- (2) it was the method adopted by previous workers.
- (3) and when the leucocytosis showed definite regression. Nodes taken out on the 30th day of operation were found to have progressively increased in size. The cortex showed a cellular hyperplasia. The vascularity of the medulla too had increased (fig. 4)

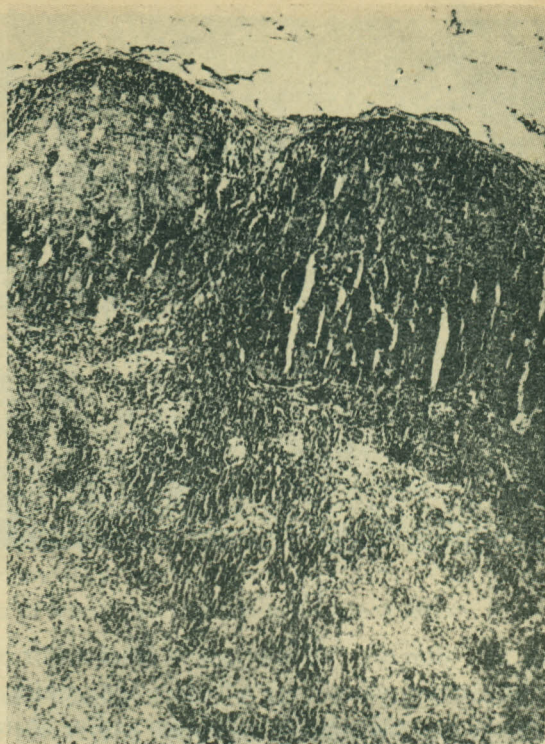


Fig. 3

On the 60th day the changes were same but much too marked (fig.5). The overall rate of progress of changes gave an impression as if the changes started near about 10th/15th day.

A similar rise in leucocyte count after splenectomy was observed by Frank (4), Ask-Upmark (1), Wiseman and Doan (18,19), Singer Miller and Dameshek (16), Palmer *et al.* (13), Learmonth *et al.* (10) and many others (2,3,6,7,8,9,11,12,15). The only disparity being with regard to its severity and duration after the operation.

However, a few workers, like Gibson (5), observed leucocytosis only in one of his three dogs, while the other two exhibited no change, while some workers found only mild and transient leucocytosis.

As regards lymph nodes Rodney Maingot (14) observed hyperplasia similar to our findings.

Frank, Wiseman and Doan (4,18) suggested that spleen normally destroys white blood cells which therefore, increase after the removal of the spleen. Ask-Upmark and Singer, Miller and Dameshek (1,16) concluded that the leucocytosis after splenectomy was much more

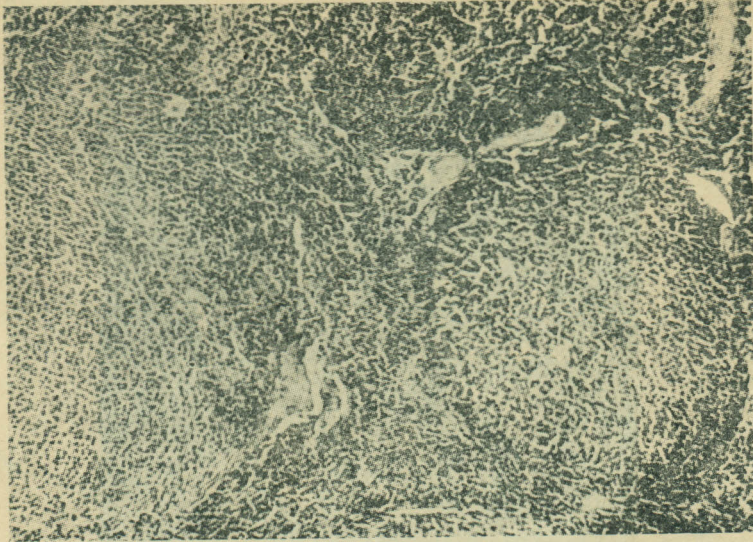


Fig. 4

marked both in severity and duration in contrast to other major operations and were of the opinion that spleen probably exerts a restraining influence upon white cell number. According to Palmer *et al.* (13) spleen controls the rate of production and/or liberation of leucocytes from the bone marrow, because there is maximum effect over the granulocytes. But they do not exclude the possibility of a destructive action of spleen on the white blood cells.

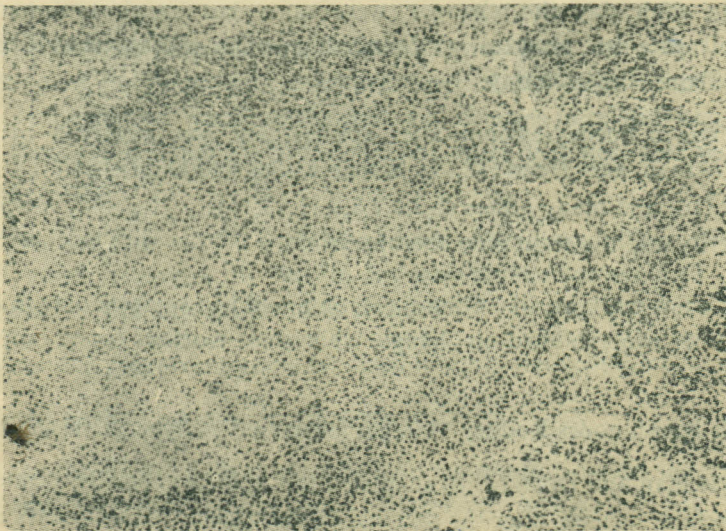


Fig. 5

The present observations demonstrated a very severe leucocytosis with insignificant number of precursors. Neutrophil Polymorphs increased out of proportion and most of them were polylobed. These findings indicate an increase in the mature cells i.e. less of destruction. The fact why only neutrophils increased out of proportions can be explained in two ways:

- The normal spleen restrains the release of mature cells from the bone-marrow — the granulocytes, of which neutrophils form the major most component.
- Spleen is the only normal grave-yard of neutrophils whereas lymphocytes and monocytes are destroyed by whole of the R.E.S., including spleen. On removal of spleen, therefore, although all the leucocytes increase, maximum rise is in neutrophils.

Scarcity of immature cells is an evidence which does not favour a comparable increase in the rate of production of leucocytes from the bone marrow. The gradual remittance of leucocytes is due to the various compensatory mechanisms coming into force - R.E.S. gradually taking up the burden of work of spleen.

The cellular hyperplasia with increased vascularity of the lymph nodes seems to be an indication of these compensatory mechanisms in R.E.S. The extent of hyperplasia being correlated with the degree of leucocytic regression — the changes in nodes starting only after about 10 to 20 days.

Hence our collective evidence is more in favour of normal spleen having mainly a destructive influence over the leucocytes. Another possible effect of spleen over the bone marrow, if any, could be restraint over liberation/release of mature cells. Because of the absence of immature cells, effect over production seems to be remote possibility.

A further probe in the project may clinch the issue by doing counts after six months and a year to demonstrate whether the increase was permanent or not. But unfortunately it could not be done because of certain practical difficulties.

SUMMARY

The spleen was removed under aseptic precautions in dogs and the animals were observed for ninety days. Venous blood was collected pre-operatively, immediately after the operation and then on the 2nd, 3rd, 5th, 7th, 10th, 15th, 20th, 30th, 45th, 60th and 90th days and a total, differential and an absolute count of the leucocytes was done. A marked leucocytosis, particularly manifest in neutrophils was observed. Experimental evidence is in favour of destruction of the leucocytes by the normal spleen. A comparative increase in the production and/or liberation of the leucocytes from the bone marrow has been discussed.

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