Mean values and standard deviations of the percent drop of the platelet count in respect to the basal number for every group of experiments (5 rats for each case reported, 6 rats for the control).

Group of experiments	Variation of the basal platelet count after ADP			
	2 min	4 min	15 min	30 min
A) Control	-44.70 ± 2.60	-21.95 ± 5.14	-14.17 ± 5.86	-9.42 ± 6.38
B) Exclusion of the splenic circulation	-33.09 ± 5.34 $t = 3.94 > 2.262$	-27.03 ± 1.48	-19.22 ± 10.48	-8.43 ± 3.70
C) Exclusion of the renal circulation	-29.73 ± 6.44 $t = 4.30 > 2.262$	-26.51 ± 5.07	-13.33 ± 6.50	-6.57 ± 2.21
D) Exclusion of a limb circulation	-31.77 ± 3.53 $t = 5.89 > 2.262$	-22.25 ± 5.59	-10.85 ± 3.93	-5.25 ± 5.52
E) Exclusion of the cerebral circulation	-30.35 ± 2.81 $t = 7.85 > 2.262$	-22.17 ± 7.17	-5.66 ± 2.43	-1.85 ± 2.04

The t-values are reported for p 0.05 of the data measured at the maximum of the effect of ADP (2 min) in each group of experiments in respect to the control.

trapping of the platelet aggregates by the renal and the hind-leg microvasculature, besides the well known pulmonary district[1,2]. The degree of the maximum drop of the platelet count observed 2 min after the beginning of the ADP injection, was similar in all the operated animals in spite of the different blood flow, length, rheological and metabolic characteristics of the renal, splenic, cerebral and of hind-limb circulations which we excluded respectively. Consequently the contribution of each circulatory district to the trapping of the platelets is not valuable in our experimental conditions; however, we suggest that the exclusions leave out a part of the total trapping endothelium, while some contemporaneously occurring haemodynamic changes may affect the pool of the platelets trapped elsewere. For example we observed a marked increase in the blood pressure following the exclusion of the cerebral circulation and a less marked increase following the renal exclusion. Moreover, the enhanced sympathetic activity occurring after the exclusion of the cerebral circulation might be responsible also for the increased velocity and extent in the recovery of the platelet count observed in this case. We suggest that the spleen, which is known to sequester platelets[10] and release haematic cells in such circumstances[11,12] was obviously induced to squeeze out and put in circulation the sequestered platelets.

Summary. The effect of the ADP infusion on the basal platelet count was studied in controls and in rats submitted to the exclusion of the following circulatory districts: splenic, renal, cerebral and of a hind-limb. After these exclusions the ADP-induced thrombocytopenia was less marked than the controls.

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Change in Levels of Cholesterol and Free Fatty Acids of Lymphoid Cells During Tumor Growth

It is well known that the structural lipid of mammalian cell membrane consists primarily of phospholipid and cholesterol in fixed proportions specific for species and cell types[1-4]. Studies on the lipids from normal lymphocytes and leukemic cells indicated marked decrease of cholesterol in leukemic cells as compared to normal lymphocytes[5,6]. However, little information is available about the lipid composition of lymphocytes from animals with carcinoma, except leukemia. Previously we reported high levels of cytotoxic free fatty acids in the splenic lymphoid cells from guinea-pigs[7]. The present study demonstrates marked change in levels of cholesterol and free fatty acids of lymphoid cells from different tissues of mice following the growth of Ehrlich's ascites carcinoma.

Adult female mice of *dd*N strain, weighing 24–26 g, were used throughout. The mice were inoculated i.p. with Ehrlich's ascitic tumor cells (5×10^6 cells/mouse). At intervals of 5 and 10 days after tumor implantation, the animals were killed by cervical dislocation. The thymus, spleen and lymph nodes (cervical and mesenteric lymph nodes), all of these tissues were removed and pooled from 100 individual mice, which had been fed with diet and given water ad libitum. Suspensions of lymphoid cells were prepared as follows: the pooled tissues were cut into small pieces, suspended in phosphate-buffered saline (pH 7.2)[8] and filtered through gauze. Then, the cell suspensions were centrifuged for 10 min at

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Lipid composition of lymphoid cells from thymus, spleen, cervical lymph node and mesenteric lymph node of normal and tumor-bearing mice

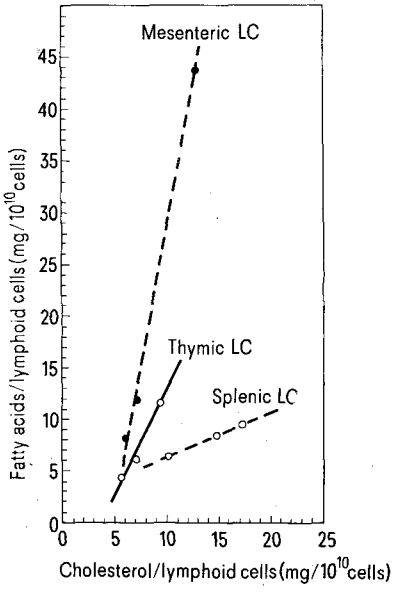
Tissues	Days after implantation	Tissue weight (g/100 mice)	Lipid/lymphoid cells (mg/10^{10} cells)[a]					Cholesterol
			Total lipids	Phospholipids	Neutral lipids			Phospholipids (molar ratio)[b]
					Cholesterol	Fatty acids	Triglycerides	
Thymus	Normal	9.8 ± 0.5	44.2 ± 1.6	23.3 ± 1.3	5.6 ± 0.5	4.6 ± 0.4	7.9 ± 0.8	0.48
	5	7.4 ± 0.4	57.5 ± 1.4	30.0 ± 1.4	6.8 ± 0.4	6.1 ± 0.4	9.0 ± 1.5	0.45
	10	3.5 ± 0.1	74.7 ± 4.1	34.7 ± 2.7	9.3 ± 0.6	11.6 ± 0.4	8.8 ± 0.6	0.54
Spleen	Normal	11.8 ± 0.2	56.5 ± 1.7	29.5 ± 2.4	10.1 ± 0.5	6.5 ± 0.5	7.6 ± 1.7	0.69
	5	21.4 ± 0.4	65.5 ± 1.8	35.8 ± 1.9	14.4 ± 0.6	8.6 ± 0.3	3.6 ± 0.2	0.81
	10	9.9 ± 0.2	85.8 ± 5.5	46.5 ± 3.6	17.0 ± 0.6	9.5 ± 0.3	7.6 ± 0.7	0.73
Cervical lymph node	Normal	10.0 ± 0.7	73.3 ± 1.7	30.7 ± 0.8	6.9 ± 0.7	7.5 ± 0.6	22.9 ± 1.8	0.45
	5	8.0 ± 0.6	85.4 ± 2.9	35.0 ± 2.3	9.2 ± 1.0	11.1 ± 1.0	23.3 ± 1.8	0.53
	10	4.5 ± 0.4	97.8 ± 3.5	38.5 ± 1.4	15.2 ± 1.0	16.4 ± 0.6	16.0 ± 1.0	0.79
Mesenteric lymph node	Normal[c]	11.0 ± 0.5	107.1 ± 4.3	30.4 ± 2.2	12.7 ± 0.4	43.8 ± 3.1	13.1 ± 1.2	0.84
	5	13.1 ± 0.2	60.2 ± 3.8	29.9 ± 1.7	7.0 ± 0.6	11.8 ± 1.2	7.9 ± 1.1	0.47
	10	8.1 ± 0.2	55.4 ± 2.0	28.9 ± 1.2	6.0 ± 0.5	8.1 ± 0.4	7.2 ± 0.7	0.42

[a] Each value is given as mean ± SE of 6 pools from 100 individual mice. [b] The phospholipid molecular weight was assumed to be 775. [c] Lipid contents (mg/10^{10} cells) of mesenteric lymphoid cells from normal mice deprived of diet for 48 h before the experiments were as follows: total lipids, 101.9; phospholipids, 32.7; cholesterol, 16.5; fatty acids, 37.4; triglycerides, 8.5.

200 *g*, followed by repeated washings. Red cells contaminating in splenic cells were lysed by treatment with 0.83% NH_4Cl[9]. Morphologically, 92–97% of leukocytes in the cell suspensions were lymphocytes, the remainder principally reticular cells. Extraction, fractionation and quantitation of lipid components of the lymphoid cells, were all performed by the method described previously[7].

As can be seen in the Table, there was only a slight difference in the phospholipid contents among lymphoid cells from 4 different tissues of normal mice. The cholesterol levels of lymphoid cells from normal animals differed considerably with tissues, ranging between 5.6 mg/10^{10} cells for thymus and 12.7 mg/10^{10} cells for mesenteric lymph node. The cholesterol to phospholipid (C/P) molar ratio for normal mice was 0.84 in the mesenteric lymphoid cells and 0.69 in the splenic lymphoid cells, whereas the C/P molar ratio in the thymic and cervical lymphoid cells was less than 0.50. Following tumor inoculation, the cholesterol contents in lymphoid cells, except the mesenteric lymphoid cells, increased progressively, with a concomitant increase in the phospholipids. The increases of cholesterol in the lymphoid cells were about 66% for thymus, 68% for spleen and 120% for cervical lymph node, respectively, at 10 days after tumor implantation ($p < 0.01$). On the other hand, the phospholipid contents in the thymic and splenic lymphoid cells from tumor-bearing mice exceeded by about 49% and 58% the values for normal animals on the 10th day ($p < 0.01$). But the increase of phospholipids in the cervical lymphoid cells was slightly (about 25% increase at 10 days; $p < 0.05$). In contrast to the other 3 lymphoid cells, the mesenteric lymphoid cells from tumor-bearing mice contained lower levels of cholesterol than those for normal animals (about 53% decrease at 10 days, $p < 0.01$). As a result of these progressive changes in the quantities of cholesterol and phospholipids, the C/P molar ratio in the cervical lymphoid cells increased from 0.45 to 0.79 during the 10-day period of tumor growth, whereas the C/P molar ratio in the mesenteric lymphoid cells decreased from 0.84 to 0.42. However, changes in the C/P molar ratio for thymic and splenic lymphoid cells were less striking.

The results presented in the Table also indicate that the quantities of free fatty acids in lymphoid cells changed significantly during the tumor growth, as the cholesterol did. After implantation of tumor cells, the fatty acid levels of the mesenteric lymphoid cells were markedly reduced being at 10 days about $^1/_5$ of the normal levels ($p < 0.01$). Concerining this, there was no significant difference in the quantities of fatty acids between mesenteric lymphoid cells from normal mice fed ad libitum and normal animals deprived of diet for 48 h before the experiments (43.8 mg/10^{10} cells and 37.4 mg/10^{10} cells, $p < 0.1$). On the other hand, the fatty acid



Linear relationship between the quantities of cholesterol and free fatty acids in lymphoid cells. The contents of these lipids in lymphoid cells are given in the Table. LC, lymphoid cells.

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contents in the other 3 lymphoid cells were increased by about 153% for thymus, 46% for spleen and 118% for cervical lymph node, respectively, during the 10-day period of tumor growth ($p < 0.01$).

From the results described above, the following conclusions can be drawn. 1. In mice, marked changes occur in levels of cholesterol and free fatty acids of lymphoid cells following the growth of Ehrlich's ascites carcinoma. 2. The change of lipid composition in the mesenteric lymphoid cells is quite different from that in the other lymphoid cells. 3. Values of the cholesterol to phospholipid molar ratio also change during the tumor growth. Recently, INBAR and SHINITZKY[6] indicated that increase of cholesterol in lymphocytes results in increase of the viscosity of membrane lipid layer relating to the physiological function of lymphocyte membrane. Thus it is assumed that changes in cholesterol levels of lymphoid cells during the tumor growth may reflect changes in the properties of lymphoid cell membrane. In this connection, it is interesting to note that there is an accurately linear relationship between the quantities of cholesterol, and free fatty acids within each of lymphoidcells from thymus, spleen and mesenteric lymph node of mice (Figure)[10].

Summary. Growth of Ehrlich's ascitic carcinoma in mice resulted in increase of free cholesterol and free fatty acids in lymphoid cells from thymus, spleen and cervical lymph node, but decrease of these lipids in the cells from mesenteric lymph node.

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Depression of Humoral and Cell-Mediated Immune Responses by Coxsackieviruses in Mice

Viruses belonging to various groups have been shown to impair the immunological responsiveness of the host, but attention has mainly been focused on viruses having little or no clinical relevance in humans[1-3].

Patients simultaneously infected with polioviruses and group A coxsackieviruses are more severely affected by paralytic poliomyelitis than patients infected with polioviruses alone[4-7]. This potentiation of the pathological effects of polioviruses has found experimental support in monkeys[8]. Furthermore, cases of association between *Pneumocystis carinii* pneumonia and coxsackievirus B infection have been described[9]. These observations suggest that also coxsackieviruses might depress the immune functions of the host.

To test this possibility, we studied the immunological reactivity of adult mice infected with all members of group B or with selected members of group A coxsackieviruses under conditions of multiple antigenic stimulation, which were thought better to mimic what may happen in natural infections. The antibody responses against two unrelated antigens, poliovirus 1 and sheep red blood cells (SRBC), and a cell-mediated reaction, contact sensitivity to 4-ethoxymethylene-2-phenyl oxazolone (oxazolone), were used as monitors.

Materials and methods. Female Swiss mice aged 12–18 weeks were used throughout and assigned to the different experimental groups at random. Coxsackieviruses B and

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Table I. Effect of coxsackievirus infection on the circulating antibody response of mice to different antigens

Infecting coxsackievirus	No. of mice	Circulating antibody to			
		Sheep red cells[a]			Homologous coxsackievirus[a]
		lysins	agglutinins	Poliovirus[b]	
A-13	7	463	565	7	18
A-15	7	287[c]	282[c]	3[c]	11
A-18	7	623	688	6	7
B-1	7	ND[d]	ND	0[c]	304
B-2	7	839	927	2[c]	10
B-3	12	175[c]	225[c]	3[c]	27
B-4	12	180[c]	202[c]	5[c]	26
B-5	8	608	662	6	25
B-6	12	191[c]	228[c]	9	20
Controls	36	564	612	32	ND

[a] Geometric mean of the reversal of the highest dilution giving neutralization. Significance of the differences assessed by the *t*-test. [b] Number of mice with neutralizing antibody detectable in serum diluted 1:5. Significance of the differences assessed by the χ^2 test. [c] The difference with the controls is significant at $p < 0.05$. [d] ND, not done.