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.

Table II. Effect of coxsackievirus infection on the splenic antibody-forming cell response of mice to sheep red cells

Infecting coxsackievirus	No. of mice	Antibody-forming cells/spleen	
		Geometric mean	95% Confidence limits
A-13	6	90,600	46,200–117,400
A-15	7	63,400[a]	38,400–104,500
A-18	6	122,200	52,000–287,100
B-1	6	108,200	35,000–334,200
B-2	6	168,700	86,900–327,300
B-3	10	46,900[a]	16,700–131,700
B-4	12	65,400	28,500–150,000
B-5	6	144,600	79,600–262,400
B-6	10	53,200[a]	24,300–116,400
Controls	29	107,600	81,900–141,300

[a] The difference with the controls is significant at $p < 0.05$ (t-test or Mann-Whitney U-test).

poliovirus 1 were grown on HeLa cells and coxsackieviruses A on KB cells in serum-free medium and titrated as previously described[10,11]. Direct haemolytic antibody-forming cells to SRBC were assayed by the method of Jerne, as previously described[12]. Contact sensitivity was established by applying 0.2 ml of 2% oxazolone (BDH Chemicals Ltd, Poole, England) in absolute ethanol to the skin of the abdomen, and quantitated by challenging the ears with 1% oxazolone in olive oil and measuring the increase of ear thickness 24 h later, as previously described[13]. Neutralizing antibodies were tested against 100 TCD_{50} (tissue culture infections dose 50) of virus[10,11] and haemoagglutinins and haemolysins against 1% SRBC.

The design of the experiments was as follows: day 0: groups of mice were infected by i.v. inoculation of 10^4 TCD_{50} of coxsackievirus in 0.2 ml. 1 control group received extracts of HeLa or KB cells diluted as in the preparation of the virus inocula, and 2 control groups were left untreated. Day 1: infected and control groups were immunized i.p. with 3×10^6 TCD_{50} of poliovirus 1 in 0.5 ml. Day 5: all groups except 1 untreated control group to be used as base line for aspecific inflammatory response to challenge, were painted with oxazolone. Day 8: all animals were i.v. immunized with 1×10^8 SRBC in 0.2 ml. Day 10: all groups were tested for sensitivity to oxazolone. Day 12: all mice were bled and their spleens individually tested for antibody-forming cells. The sera were frozen to be individually assayed for antibodies to poliovirus 1, to the homologous coxsackievirus and to SRBC in the subsequent days.

Results. The results obtained in various experiments are pooled in Tables I, II, and III, where the data relative to the controls receiving cell extracts or left untreated are given together, since they showed no significant differences in immunological reactivity.

The antibody response to SRBC was significantly depressed in mice infected with coxsackievirus B-3, B-4, B-6 and A-15. This depression was evident at the level of both the numbers of haemolytic antibody-forming cells in the spleen and of titres of circulating agglutinins and lysins. The antibody response elicited by poliovirus 1, which does not replicate in mice, was generally low, but was significantly reduced by preinfection with coxsackievirus B-1, B-2, B-3, B-4 and A-15. Contact sensitivity to oxazolone was decreased by coxsackievirus B-3 infection only.

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Table III. Effect of coxsackievirus infection on the contact sensitivity response of mice to oxazolone

Infecting coxsackievirus	No. of mice	Contact sensitivity[a]	
		Geometric mean	95% Confidence limits
A-15	7	10.00	5.54–17.95
A-18	7	10.72	6.56–17.50
B-1	7	8.71	4.66–16.29
B-2	7	10.23	6.05–17.30
B-3	11	4.93[b]	3.95– 6.17
B-4	12	8.59	6.12–12.05
B-5	8	7.83	5.15–11.91
B-6	8	8.07	6.14–10.62
Controls			
Sensitized	36	10.52	8.79–12.59
Unsensitized	14	1.16	0.75–1.79

[a] Increase of ear thickness at 24 h after challenge in units of 10^{-3} cm. [b] The difference with the controls is significant at $p < 0.05$ (t-test)

All the groups of mice produced antibody towards the homologous coxsackievirus used for infection. There was no correlation between the extent of this response and depression of immune response towards heterologous antigens.

Discussion. Coxsackieviruses display a prominent age-dependent pathogenicity in mice, adults being relatively resistant. Nevertheless, infection of adult mice with 6 out of the 9 serotypes examined exerted a significant depression of the immune reactivity of the host to unrelated antigens. Coxsackieviruses A-15, B-1, B-2, B-4 and B-6 depressed the antibody response to either SRBC or poliovirus 1 or both and coxsackievirus B-3 depressed the cell-mediated contact sensitivity to oxazolone, as well as the humoral responses.

The data collected so far do not give much insight on the mechanism of the immunodepression observed. The lack of correlation between occurrence of immunodepression and extent of antibody response to the coxsackievirus used for infection seems to exclude immunological competition and suggests a less aspecific mode of action. Coxsackieviruses have been detected in the spleen[14–17] and in lymph nodes[14] of adult mice for several days after infection, but it is not known whether the presence of virus results from local replication or from drainage of other organs. In one study[17], no microscopical lesions suggestive of virus replication were seen in the spleen; in another study[18], extensive degeneration of the lymphoid elements of the thymus were observed but were attributed to intercurrent reovirus infection. Further studies that might clarify the mechanism of immunodepression are underway in this laboratory.

Whatever the mechanism, young mice, which are more sensitive to coxsackieviruses, should conceivably be more severely immunodepressed than adult mice. In this context it should also be considered that the process of immunological maturation with ageing can be more drastically impaired by viral infections than fully established immunological reactivity[12].

An impairment of immune functions might occur also in the course of human coxsackievirus infections and so explain the clinical observations referred to in the introduction. Moreover, it might help to explain the poor serological responses to live poliovirus vaccines in children excreting enteroviruses[19], a phenomenon that so far has not been satisfactorily understood[20]. Coxsackieviruses have been implicated in the aetiology of congenital heart malformations[21]. The present findings suggest that infection of the embryo with these viruses might also be responsible for congenital immunological defects, in analogy to what can happen in intrauterine rubella infection[22].

Summary. Adult mice infected with coxsackieviruses A-15, B-1, B-2, B-4 and B-6 showed depressed antibody responses to unrelated antigens; mice infected with coxsackievirus B-3 developed reduced humoral and cell-mediated immune responses. These findings might have clinical and epidemiological implications.

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Antigenic Heterogeneity among Monoclonal IgM: Observations on Guinea-Pig Sera

Antigenically distinct subclasses of immunoglobulins have been delineated for IgG[1,2] and IgA[3–5], and found to depend on structural differences. The situation regarding IgM is, however, still unclear. Antigenic differences have been reported by several investigators[6–10], but no correlation between the groups found in different laboratories has emerged. In the present study, antigenic subgroups were sought, utilizing monoclonal components from human sera and antisera raised in guinea-pigs, since observations with α_2-macroglobulin antisera had indicated guinea-pigs to be more sensitive to antigenic differences in primates, than are rabbits[11].

Material and methods. Sera from 54 patients with electrophoretically detected IgM bands were used, which had been kept frozen at −20 °C for periods up to 5 years. M-components were isolated by preparative electrophoresis in agarose gel, followed by gel filtration on Sephadex G 200. Immunoelectrophoresis and Ouchterlony double diffusion was performed in 1% agarose on 25 × 75 mm glass slides. Guinea-pigs in groups of 3 were immunized by s.c. injection with 50 µg of antigen in complete Freund's adjuvant at weekly intervals. In all, 9 antigens were used for immunization. The animals were sacrificed 4 weeks after the start, and exsanguinated by heart puncture. The yield was up to 12 ml of blood. Absorptions were performed at 37 °C for 1 h and 4 °C for 15 h. 2 animals in each of 2 groups were lost during the immunization and thus left no antiserum.

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