Serial subcultures on nutrient agar were obtained from 6 primary broth cultures. 5 strains could be indefinitely propagated and were recognized as belonging to the family Micrococcaceae[10]: further characterization of such microorganisms is actually in progress. The 6th bacterial strain ceased to grow following the 3rd transfer; it showed structures looking like L-forms and coccoidal forms of various size with budding rodlets giving origin to a considerable number of small bacillary forms, some of which appeared to increase in size and revert to the coccal phase (Figures 3 and 4); such aspects were in full agreement with the description of *Mycococcus* (Krassilnikov) given by PEASE[11].

In order to realize all the precautions needed to avoid the possibility of contamination, only a limited number of cases has been kept under examination: nevertheless the results here described appear to be significant and may help to explain why the microorganisms which are the object of our research show a large diffusion in the circulating blood of adult subjects and do not give rise to an efficient immunological reactivity. Such results may be provisionally interpreted on the basis of the assumption that the bacteria, probably in the stage of minimal reproductive units of the unstable L-phase, may reach the foetus through the placental circulation.

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High Levels of Free Fatty Acids and their Esters in Lymphoid Cells Resistant to Cortisone or Cyclophosphamide

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Summary. The lymphoid cells from thymus, slpeen or mesenteric lymph node of mice treated with hydrocortisone or cyclophosphamide contained the significantly high levels of free fatty acids, triglycerides and cholesterol esters as compared to the corresponding cells from untreated animals.

It has been known for a long time that corticosteroids are lympholytic and immunosuppressive[1-5]. However, there are remarkable differences in susceptibility to corticosteroids among various species: mice, rats and rabbits being far more sensitive than guinea-pigs, monkeys and man[6]. In mice, systemic administration of corticosteroids results in rapid atrophy of thymus, spleen and lymph nodes, and pronounced decrease of lymphocytes in these tissues[4,5,7,8]. The remaining cells in mouse thymus after cortisone treatment are known as cortisone-resistant thymic cells which are very efficient in the cell-mediated immune reactions, in that the cortisone-resistant splenic and lymph node lymphocytes are also involved[9-15]. Cyclophosphamide, an immunosuppressive agent, also has been reported to deplete the lymphocytes present in thymus and other lymphoid tissues of mice[1,2,5,16]. However, little information is available about the cellular components of lymphocytes resistant to corticosteroids or cyclophosphamide in animals. In the present experiments, we examined the lipid composition of lymphoid cells from thymus, spleen and mesenteric lymph node of mice treated with hydrocortisone or cyclophosphamide. This paper reports a significant difference in lipid composition of lymphoid cells between drug-treated mice and untreated animals.

Adult female mice of *dd*N strain, weighing 24–26 g, were used throughout. The thymus, spleen and mesenteric lymph node were obtained either from mice treated with hydrocortisone, mice treated with cyclophosphamide or from untreated animals (normal mice). The first group of mice was injected i.p. with 12.5 mg of hydrocortisone acetate (Schering AG, Germany) per 100 g of body weight and killed by cervical dislocation 2 days later[10,11]. The second group of animals received the i.p. injection of 7.5 mg of cyclophosphamide (Asta Werke AG, Germany) per mouse 3 days before the experiments[16]. To obtain a sufficient amount of lymphoid cells, the tissues were removed and pooled from 50–150 individual mice which had been fed with diet and given water ad libitum. The pooled tissues were cut into small pieces, suspended in phosphate-buffered saline (pH 7.2)[17] and filtered through gauze. A small portion of the cell suspension was used for cell counting. The remaining cell suspension was treated with 0.83% NH_4Cl, followed by repeated washings[18]. Extraction, fractionation and quantitation of lipid components of the lymphoid cells were performed by the method described previously[19].

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Table I. Numbers of leukocytes and yields of lymphoid cells from thymus, spleen and mesenteric lymph node of normal mice and animals treated either with hydrocortisone (HC) or with cyclophosphamide (CP) [a]

Tissues	Agents used	Tissue weights (mg/mouse)	No. of leukocytes in tissues ($\times 10^5$ cells/tissue) [b]		Yields of lymphoid cells from tissues ($\times 10^5$ cells/tissue) [b]	
Thymus	Normal	101 ± 2	595 ± 28	(100.0%)	494 ± 44	(100.0%)
	HC [c]	47 ± 1	32 ± 3	(5.4%)	26 ± 3	(5.3%)
	CP [d]	50 ± 1	96 ± 4	(16.2%)	91 ± 4	(18.4%)
Spleen	Normal	118 ± 2	541 ± 51	(100.0%)	308 ± 37	(100.0%)
	HC	38 ± 1	45 ± 3	(8.3%)	37 ± 4	(11.9%)
	CP	46 ± 3	63 ± 6	(11.6%)	52 ± 6	(16.8%)
Mesenteric lymph node	Normal	109 ± 8	192 ± 9	(100.0%)	119 ± 20	(100.0%)
	HC	34 ± 3	12 ± 1	(6.3%)	9 ± 1	(7.6%)
	CP	39 ± 2	15 ± 1	(7.8%)	11 ± 1	(9.0%)

[a] All mice were fed with diet and given water ad libitum before the experiments. [b] Each value is given as mean ± SE of 7 experiments. [c] Mice were injected i.p. with 12.5 mg of hydrocortisone acetate per 100 g of body weight and killed 2 days later. [d] Animals received the i.p. injection of 7.5 mg of cyclophosphamide per mouse 3 days before the experiments.

As can be seen in Table I, treatment of mice with hydrocortisone (cortisone) or cyclophosphamide resulted in marked decrease of leukocytes in all the 3 tissues examined. After cortisone treatment, the leukocyte numbers in thymus, spleen and mesenteric lymph node of mice reduced pronouncedly, being 5.4%, 8.3% and 6.3% of the normal cell numbers, respectively ($p < 0.01$)[10–13]. On the other hand, the leukocyte counts in thymus, spleen and lymph node from cyclophosphamide-treated mice were 16.2%, 11.6% and 7.8% of that for the normal animals, respectively ($p < 0.01$)[16]. Accordingly, the yields of lymphoid cells from tissues of drug-treated mice were also decreased significantly (about 5–18% of the normal cell yields, $p < 0.01$).

Table II shows the lipid composition of lymphoid cells from thymus, spleen and mesenteric lymph node of normal mice and animals treated with cortisone or cyclophosphamide. Comparison of lipid composition of lymphoid cells among three groups of mice indicates that, within each of the lymphoid tissues, there was a significant difference in the quantities of free fatty acids, triglycerides and cholesterol esters in lymphoid cells between normal mice and animals treated with cortisone or cyclophosphamide: whereas the difference in the contents of phospholipids and free cholesterol of lymphoid cells between normal mice and drug-treated animals was less striking than that for the other 3 lipid fractions. The quantities of fatty acids, triglycerides and cholesterol esters were about 2–7 times higher in the lymphoid cells from the drug-treated mice than in the cells from normal animals ($p < 0.01$). On the other hand, there was only a slight difference in the lipid composition of lymphoid cells between cortisone-treated mice and cyclophosphamide-treated animals within the respective tissues, except the triglycerides and cholesterol esters in the thymic lymphoid cells. Thus, it is apparent that in mice the lipid composition of the splenic or mesenteric lymphoid cells resistant to cortisone is quite similar to that of the corresponding cells resistant to cyclophosphamide, since the lymphoid cells obtained from mice treated either with

Table II. Lipid composition of lymphoid cells from thymus, spleen and mesenteric lymph node of normal mice and animals treated either with hydrocortisone (HC) or with cyclophosphamide (CP) [a]

Tissues	Agents used	Lipid/lymphoid cells (mg/10^{10} cells) [b]					Cholesterol
		Phospholipids	Neutral lipids				Phospholipid (molar ratio) [c]
			Cholesterol	Fatty acids	Triglycerides	Cholesterol esters	
Thymus	Normal	22.2 ± 0.7	6.7 ± 0.5	5.9 ± 0.6	6.9 ± 0.8	2.8 ± 0.6	0.60
	HC	36.8 ± 4.2	11.9 ± 0.1	21.5 ± 1.3	36.9 ± 6.6	20.9 ± 3.1	0.65
	CP	27.1 ± 1.5	9.1 ± 0.6	15.4 ± 2.1	15.2 ± 1.6	7.8 ± 1.2	0.67
Spleen	Normal	30.8 ± 2.4	10.4 ± 0.5	6.7 ± 0.3	6.3 ± 1.4	3.6 ± 0.4	0.67
	HC	49.1 ± 5.4	10.6 ± 1.0	15.0 ± 1.7	37.2 ± 4.2	8.8 ± 0.8	0.43
	CP	54.6 ± 2.9	12.0 ± 1.2	17.2 ± 1.1	40.8 ± 7.4	10.8 ± 1.0	0.44
M.L.N. [d]	Normal	31.5 ± 2.2	12.6 ± 0.4	32.9 ± 1.3	13.8 ± 1.2	7.2 ± 0.6	0.80
	HC	47.4 ± 3.3	22.5 ± 1.8	86.2 ± 3.6	38.5 ± 5.5	12.3 ± 2.1	0.95
	CP	41.2 ± 4.6	17.6 ± 1.7	61.1 ± 4.5	37.9 ± 4.6	13.4 ± 1.2	0.86

[a] All mice were fed with diet and given water ad libitum before the experiments. [b] Each value is given as mean ± SE of 7 experiments. [c] The phospholipid molecular weight was assumed to be 775. [d] M.L.N.: mesenteric lymph node. 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: phospholipids, 32.6 ± 1.4; cholesterol, 13.9 ± 0.6; fatty acids, 36.8 ± 2.7; triglycerides, 10.2 ± 0.8; cholesterol esters, 6.1 ± 1.0; cholesterol to phospholipid molar ratio, 0.85 (number of experiments = 7).

cortisone or with cyclophosphamide are considered to be resistant to the drug. In addition, it is noticeable that there was no significant difference in the cholesterol to phospholipid molar ratio for lymphoid cells between 2 groups of the drug-treated mice within each of the tissues examined[18].

The present results clearly demonstrate that the lymphoid cells from thymus, spleen or mesenteric lymph node of cortisone-treated mice contained significantly high levels of free fatty acids, triglycerides and cholesterol esters as compared to the corresponding cells from normal animals. As described above, cortisone destroyed almost all the thymic leukocytes of mice, resulting in the pronounced decrease of lymphoid cell yields (about 5% of the normal cell yields). This striking cell destruction by cortisone indicates that the lipid composition of the cortisone-sensitive lymphoid cells in mouse thymus is similar to that of the thymic lymphoid cells from normal animals. Thus it appears that in mouse thymus the contents of free fatty acids and their esters are considerably higher in the cortisone-resistant lymphoid cells than in the cortisone-sensitive lymphoid cells. Similarly, the cortisone-resistant lymphoid cells in spleen or mesenteric lymph node of mice appears to contain the markedly high levels of the neutral lipids as compared to the cortisone-sensitive corresponding cells. It has been reported that the cortisone-resistant lymphocytes in mouse thymus are immunocompetent lymphocytes, in which the cortisone-resistant splenic and lymph node lymphocytes are also involved[4,5,9–15]; whereas the cortisone-sensitive lymphocytes in thymus and other lymphoid tissues of mice have been shown to be immunologically incompetent. It is assumed, therefore, that the lipid composition of immunocompetent lymphocytes differs significantly from that of immunologically incompetent lymphocytes within the respective lymphoid tissues of mice.

The present results also indicate that in mice the high levels of free fatty acids and their esters were found in the cyclophosphamide-resistant lymphoid cells, of which the proportion in lymphoid tissues was less than 20%, and the lipid composition of cyclophosphamide-resistant lymphoid cells from spleen or lymph node was very similar to that of the cortisone-resistant corresponding cells. Histological studies on mouse lymphoid tissues revealed that the lymphocytes present in thymus medulla and in the thymus-dependent areas of spleen and lymph nodes are resistant to corticosteroids as well as to cyclophosphamide[5,7,8,16]. These histological findings indicate that the population of cortisone-resistant lymphocytes appears to be similar to that of cyclophosphamide-resistant lymphocytes within the respective tissues of mice. Therefore it is conceivable that the lipid composition of cortisone-resistant lymphoid cells in spleen or mesenteric lymph node of mice resembles that of cyclophosphamide-resistant corresponding cells. The reason that a considerable difference was found in lipid composition of lymphoid cells between normal mice and animals treated with cortisone or cyclophosphamide within every one of the tissues examined, is unknown at the present time. However, the present results suggest that the lymphoid cells resistant to immunosuppressive agents differ markedly from the drug-sensitive lymphoid cells in the significantly high levels of free fatty acids and their esters within the respective lymphoid tissues of mice.

Distribution of the A Blood-Group Activity in Porcine Serum[1]

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Summary. The A blood-group activity of pig serum is bound to a lipid, in some cases also to a nonlipid fraction. The major lipidic A activity (roughly 50%) is carried with the HDL class, while the VLDL and LDL classes contain roughly 25% each.

In various mammalian species, certain blood-group active substances are known to occur primarily in blood plasma as soluble constituents and to be secondarily transferred from the plasma onto the erythrocyte membrane. The Lewis substance of man[2], the J substance of cattle[3], the R substance of sheep[4], and the A substance of pig[5] are examples of those kinds of blood-group substances. It has been established in previous papers that the J blood-group activity of cattle[6] as well as the A blood-group activity of pig[7] can be found in both the total lipid and in a lipid-free preparation of the respective serum. The A system – the first of the 15 porcine blood-group systems detected – comprizes 2 factors, the first of which, A, is dominant, the second, 0, is recessive[8]. 2 types of A positive cells are usually discriminated, Ac and Ap. Since Ac cells give strong reactions, and Ap cells weak reactions, it was suggested[9] to adopt another, more reasonable nomenclature, i.e. simply A instead of Ac, and Aw (w = weak) instead of Ap. The sera of all (Ac) positive pigs contain a soluble A substance giving high inhibition titers. Aw (Ap) sera, however, have either low inhibition titers or none at all.

The distribution of bovine J activity on lipoprotein and protein fractions of serum has been investigated in more detail recently[10]. One-third of the total J activity of bovine serum was found in the total lipids, two-thirds in the lipid-free residue precipitated by lipid extraction. Furthermore, one-third of the lipidic J substance was found in the very low density lipoproteins (VLDL), two-thirds in the low density lipoproteins (LDL), while the high density lipoprotein fraction (HDL) of bovine serum is free of J activity. All non-lipidic J activity is present

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