mit unbehandelten, mit Äther getöteten Milben. Das Resultat war nicht befriedigend, da die Tiere bei stärkeren Vergrösserungen Risse in der Hautstruktur («finger prints») aufwiesen (siehe Figur 10) und sich dazu noch relativ stark aufluden, was das Fotografieren erschwerte.

Später wurden die folgenden Fixierungsmethoden ausprobiert: 1. Die Tiere wurden in 70% Alkohol während 12 h fixiert. Resultat: die Milben waren stark geschrumpft und somit unbrauchbar.

2. Die Tiere wurden während 20 h mit 2% Glutaraldehyd in 0,1 mol Natriumphosphat-Puffer (pH 7,4) fixiert, danach mit 0,1 mol Natriumphosphat-Puffer gewaschen, für weitere 20 min in 4% OsO_4-Lösung nachfixiert, wiederum mit Natriumphosphat-Puffer gewaschen und anschliessend in einer aufsteigenden Alkoholreihe jeweils 5 min entwässert. Resultat: die Exemplare waren wiederum mehr oder weniger geschrumpft und wiesen bei stärkeren Vergrösserungen Risse zwischen den «finger prints» auf.

3. Die Tiere wurden während 2 h mit 4% OsO_4-Lösung bei 5 °C bedampft, nachher mit Aqua dest. abgespült und in 50% Alkohol entwässert. Resultat: die Tiere waren auch hier stark geschrumpft und somit nicht brauchbar.

4. Die Tiere wurden in 4% Formaldehydlösung während 4 h bei 5 °C fixiert, danach mit 0,1 mol Natriumphosphat-Puffer abgespült und anschliessend in einer aufsteigenden Alkoholreihe entwässert. Resultat: die Milben waren z. T. geschrumpft und zeigten Aufladungserscheinungen, doch konnte man einige brauchbare Exemplare finden.

5. Die Tirere wurden während 12 h mit 4% OsO_4-Lösung bei 5 °C bedampft. Resultat: mit dieser Methode wurden die besten Resultate erzielt; die meisten der abgebildeten Fotos stammen aus dieser Reihe.

Versuche mit der «critical-point-Trocknung» sind im Gange. Bei allen obenerwähnten Methoden wurden die Milben, die in Zuchtgläsern aufbewahrt waren, für 3–5 min bei −20 °C gehalten. Solange die Tiere noch in der Kältestarre waren, wurden sie in eine Petrischale resp. in ein Tuch gebracht. Bei den Methoden 1–4 wurden die Tiere in ein feines Baumwolltuch eingeschlagen, um den Transport von einer Flüssigkeit zur andern zu erleichtern.

Alle Tiere wurden nach der jeweiligen Fixation luftgetrocknet und auf doppelklebende Fotoecken aufgeklebt. Bedampft wurde mit Kohle und Gold. Die Aufnahmen wurden auf dem «Stereoscan» Mark II A der Firma Cambridge hergestellt.

Morphologie von Weibchen und Männchen. a) Das Weibchen (Figuren 1–7). Die Übersichtsaufnahmen (Figuren 1 und 7) zeigen die Ventral- resp. Dorsalansicht. Auf der Ventralseite befinden sich Genital- und Analregion (Figuren 2 und 3). Die Cheliceren sind von dieser Seite besser sichtbar (Figur 4). Dorsal liegen das Supracoxalhaar (Figur 5), welches vermutlich als Sinnesorgan fungiert, und die «Öldrüsen» (Figur 6), die eventuell für die Imprägnierung des Milbenkörpers verantwortlich sind.

b) Das Männchen (Figuren 8–10). Die Genitalien sind durch zwei Klappen überdeckt (Figur 9). Die Analregion ist mit zwei Saugnäpfen versehen (Figur 10), die zusammen mit dem stark entwickelten 3. Beinpaar, als Klammerorgane bei der Kopulation dienen. In allen Abbildungen wird die eigenartige Hautstruktur eindrücklich sichtbar.

Summary. Various methods of fixation of *Dermatophagoides pteronyssinus* for the REM were tested. OsO_4 vapor yielded the best results. The morphology of male and female mites is described, based on REM exposures.

Y. MUMCUOGLU[10], L. HENNING[11] and R. GUGGENHEIM[11]

Naturhistorisches Museum Basel, Augustinergasse 2, CH–4000 Basel (Schweiz); und Geologisch-paläontologisches Institut der Universität, Labor für Raster-Elektronenmikroskopie, Bernoullistrasse 32, CH–4000 Basel (Schweiz), 4. Juli 1973.

[10] Naturhistorisches Museum Basel.

[11] Geol. Paläontol. Inst. Universität Basel, Labor für Raster-Elektronenmikroskopie.

High Levels of Free Fatty Acids in Lymphoid Cells, with Special Reference to their Cytotoxicity

Immune and non-immune lymphoid cells have recently been shown to be of prime importance in certain tissue-damaging immune reactions, in the various allograft phenomena, and in some forms of tumor rejection[1,2]. However, the mechanisms involved in the lymphocyte-mediated tissue injury are still incompletely understood except that close contact between lymphoid cells and target cells is believed to be a principal requirement for killing the latter. We have examined the levels and composition of lipid extracts of the lymphoid cells from spleens and lymph nodes of guinea-pigs, in relation to their cytotoxicity against Ehrlich ascites tumor cells in mice. This paper reports the occurrence of markedly high quantities of cytotoxic free fatty acids in the lymphoid cells.

Outbred guinea-pigs of Hartley strain, weighing 500–700 g, were used in the experiments. The guinea-pigs were killed by exsanguination. To obtain sufficient amounts of lymph node cells, sensitization of the animals was induced by footpad injection of Ehrlich ascites tumor cells (about 10^6 cells) in Freund's complete adjuvant. The inguinal lymph nodes were removed 1–2 weeks after sensitization. The spleens were obtained from both normal and sensitized animals. Suspensions of lymphoid cells from spleens and lymph nodes were prepared by cutting the tissues into small pieces, pressing them through a stainless steel sieve into approx. 30-fold volumes (v/w) of Eagle's minimum essential medium under sterile techniques, and dispersing the cells by shaking. The resulting suspension was filtered through 3 layers of gauze, and centrifuged for 2 min at $70 \times g$. The supernatant fluids were transferred to fresh tubes and centrifuged for 10 min at $200 \times g$. The cells sedimented were washed thrice with saline and the final pellets used for lipid extraction. Red cells contaminating in splenic cells were lysed by treatment of the sediment with 0.83% NH_4Cl[3]. Approx. 3–5 g (wet weight) of splenic lymphoid cells and 2 g of lymph node cells from 60 guinea-pigs were used for each lipid extraction. Lipids were also obtained from other tissues, i.e., liver, heart, lung, and kidney. The

[1] P. PERLMANN and G. HOLM, Adv. Immun. *11*, 117 (1969).

[2] K. E. HELLSTRÖM and I. HELLSTRÖM, Adv. Cancer Res. *12*, 167 (1969).

[3] J. C. DANIELS and W. O. WEIGLE, J. Immun. *101*, 1223 (1968).

Table I. Lipid content of lymphoid cells, liver, heart, lung and kidney of normal and sensitized guinea-pigs

Guinea-pigs	Tissues	Lipid/dry tissue (mg/g) [a]					
		Total lipids	Neutral lipids Sterols	Fatty acids	Triglycerides	Sterol esters	Phospholipids
Normal	Lymphoid cells from spleen (5) [b]	122.12 ± 8.73	22.93 ± 5.06	21.25 ± 0.63	10.55 ± 2.45	9.57 ± 0.84	52.60 ± 3.64
	Liver (4)	103.06 ± 8.77	9.99 ± 0.27	2.49 ± 0.19	9.01 ± 2.67	1.06 ± 0.67	76.31 ± 1.47
	Heart (4)	122.81 ± 7.07	10.10 ± 0.93	3.37 ± 0.35	36.50 ± 6.03	1.25 ± 0.34	70.42 ± 5.17
	Lung (4)	144.60 ± 8.48	17.20 ± 1.41	3.68 ± 0.38	49.14 ± 9.03	2.32 ± 0.26	69.86 ± 8.18
	Kidney (4)	141.60 ± 9.55	15.38 ± 0.45	3.11 ± 0.28	54.52 ± 3.91	1.14 ± 0.07	67.39 ± 6.00
Sensitized	Lymphoid cells from spleen (3)	145.12 ± 4.03	28.91 ± 3.27	20.71 ± 0.79	10.70 ± 2.97	11.35 ± 2.39	71.83 ± 6.02
	Lymphoid cells from lymph node (6)	127.71 ± 4.93	17.12 ± 1.38	22.28 ± 0.71	18.59 ± 3.40	12.23 ± 2.62	57.14 ± 5.01
	Liver (3)	111.10 ± 1.32	10.95 ± 0.83	2.73 ± 0.03	11.12 ± 0.38	1.72 ± 0.32	84.58 ± 1.98
	Heart (3)	117.97 ± 12.27	9.81 ± 0.94	3.82 ± 0.03	33.93 ± 6.05	1.94 ± 0.54	68.79 ± 0.42
	Lung (3)	139.33 ± 19.11	17.79 ± 0.26	4.24 ± 0.20	40.25 ± 9.25	1.60 ± 0.60	76.86 ± 1.94
	Kidney (3)	159.12 ± 11.83	15.41 ± 1.34	3.92 ± 0.23	55.05 ± 2.65	1.15 ± 0.74	83.36 ± 8.38

[a] Each value is given as mean ± S.E. [b] Figures in parenthesis indicate number of experiment.

tissues from 2 guinea-pigs were lightly homogenized in a small amount of saline and then used for lipid extraction. Total lipids of the cells and tissues were extracted with chloroform-methanol (2:1) by the method of FOLCH et al.[4]. The lipids were separated into neutral lipid classes and phospholipids on a silica gel H plate by two-dimensional thin-layer chromatography using ethylene chloride-methanol (98:2) (solvent I) and *n*-hexane-diethyl ether-acetic acid (70:30:2) (solvent II) as a solvent system[5]. Identification of individual lipids was achieved by spraying with phosphomolybdate and antimony trichloride and comparing Rf values with known lipid standards. Lipid quantitation was performed by the AMENTA's dichromate reduction procedure[6] after development of total lipids with solvent II on silica gel lanes. For biological testing, lipids were isolated by preparative one-dimensional chromatography with solvent II. Cytotoxicity of the lipids was assessed by the in-vitro antitumor effect against Ehrlich ascites tumor cells of *dd*N mice as described previously[7]: admixture of lipids (2 mg/ml cell suspension) and tumor cell suspension (2×10^7 cells/ml), after incubation at 37 °C for 90 min, was implanted into mice. Control mice received the same dose of tumor cell suspension incubated without admixture of lipids. The survival time of mice was observed for a period of 60 days.

The lipid class composition of lymphoid cells, liver, heart, lung and kidney from normal and sensitized guinea-pigs is shown in Table I. The quantity of total lipids differed considerably with tissues, ranging between 103 mg per 1 g of dry tissue for liver and 159 mg for kidney. Total lipids from lymphoid cells, kidney, lung, and heart were composed of approx. 50% phospholipids, but liver contained a higher proportion (approx. 80%). The lipid classes found in the neutral lipid fraction of all these tissues were sterols, free fatty acids, triglycerides and sterol esters. Traces of mono- and diglycerides were occasionally spotted on the plate. The most pronounced differences in lipid class composition were found in the neutral lipid fraction between lymphoid cells and other tissues, in that the lymphoid cells from both spleens and lymph nodes contained the high quantities of fatty acids and sterol esters; approx. 21 mg/g fatty acids and 11 mg/g sterol esters in lymphoid cells, as compared to 2–4 mg/g and 1–3 mg/g in the other tissues. There was no significant difference in these lipid quantities between lymphoid cells from spleens and lymph nodes or from normal and sensitized guinea-pigs. These increases in fatty acids and sterol esters were reflected by a concomitant decrease in the triglycerides; approx. 11 and 19 mg/g for lymphoid

[4] J. FOLCH, M. LEES and G. H. SLOAN STANLEY, J. biol. Chem. *226*, 497 (1957).
[5] S. KIGOSHI, Experientia *29*, 375 (1973).
[6] J. S. AMENTA, J. Lipid Res. *5*, 270 (1964).
[7] S. KIGOSHI, Experientia *27*, 976 (1971).

Table II. In vitro cytotoxicity of lipids extracted from lymphoid cells and liver of normal and sensitized guinea-pigs

Guinea-pigs	Tissues	Number of survivors at 60 days					
		Sterols	Fatty acids	Triglycerides	Sterol esters	Phospholipids	Cell control
Normal	Lymphoid cells from spleen	3/10	8/10	0/10	2/10	0/10	0/10
	Liver	0/10	10/10	0/10	1/10	0/10	0/10
Sensitized	Lymphoid cells from spleen	2/10	9/10	0/10	0/10	0/10	0/10
	Lymphoid cells from lymph node	4/10	10/10	0/10	2/10	0/10	0/10
	Liver	0/10	10/10	0/10	0/10	0/10	0/10

cells from spleens and lymph nodes, respectively, as compared to 30–60 mg for heart, lung and kidney. Liver differed from these tissues in the low contents of both fatty acids and triglycerides. Table II shows a representative of cytotoxicity tests in which the neutral lipid classes and the phospholipids from lymphoid cells and the livers were examined for their cytotoxic activity against the tumor cells as described above. It can be seen that all, or nearly all, groups of the mice receiving the tumor cells preincubated with fatty acids, irrespective of the original tissues, at a concentration of 2 mg per ml of cell suspension failed to develop tumors at the end of the 60-day period, while control mice invariably died from ascitic tumors in less than 20 days. In contrast to this, all other lipid classes were practically ineffective to suppress the growth of tumor cells. From the foregoing it may be concluded that lymphoid cells are sharply distinguishable from other mammalian cells in the exceptionally high contents of the cytotoxic free fatty acids.

Although fatty acids and their esters from various sources[5,8–13] have long been known to be highly cytotoxic to mammalian cells including tumor cells, OKUDAIRA et al.[14] were the first to suggest the possibility of fatty acids isolated from lymph node extract as a cytotoxic factor of lymphoid cells. Very recently, TURNELL et al.[15] have presented evidence that accumulation of free fatty acids is involved in corticosteroid-induced lymphocytolysis. The present results, demonstrating the occurrence of high levels of cytotoxic fatty acids concomitant with the increase of sterol esters in lymphoid cells, point to characteristic features of fatty acid metabolism in lymphoid cells, presumably underlying the physiological function of lymphoid cells as a surveillance mechanism.

Résumé. On a trouvé, chez le cobaye, que la teneur en acides gras des cellules lymphoïdes isolées de la rate et des ganglions est 5–6 fois plus abondante que celle d'autres tissus. Ces acides sont fortement cytotoxiques pour la cellule cancéreuse d'Ehrlich.

S. KIGOSHI and R. ITO

Department of Pharmacology, School of Medicine, and Department of Pharmacology, Cancer Research Institute, Kanazawa University, Kanazawa (Japan), 20 June 1973.

[8] W. NAKAHARA, J. exp. Med. *40*, 363 (1924).
[9] L. R. BENNETT and F. E. CONNON, J. natn. Cancer Inst. *19*, 999 (1957).
[10] G. F. TOWNSEND, J. F. MORGAN and B. HAZLETT, Nature, Lond. *183*, 1270 (1959).
[11] S. SENO and M. YAMAMOTO, Acta med. Okayama *19*, 59 (1965).
[12] S. M. MILEU, I. POTOP, R. HOLBAN-PETRESCU, V. BOERU, E. GHINEA and C. TASCA, Neoplasia *16*, 473 (1969).
[13] A. KATO, K. ANDO, G. TAMURA and K. ARIMA, Cancer Res. *31*, 501 (1971).
[14] H. OKUDAIRA, T. KATAOKA, H. OKADA, R. FURUSE-IRIE, S. KAWACHI, S. NOJIMA and K. NISHIOKA, J. Biocchem., Tokyo *68*, 379 (1970).
[15] R. W. TURNELL, L. H. CLARKE and A. F. BURTON, Cancer Res. *33*, 203 (1973).

A Comparison of the Effects of Several Diuretics and Sulfhydryl Reagents on the in vitro Clotting Time of Rat, Guinea-Pig and Human Blood

In a recent communication GADD et al.[1] demonstrated that the organomercurial compounds meralluride, mersalyl and *p*-chloromercuribenzoic acid (PCMB), all capable of reacting with protein sulfhydryl groups, inhibit platelet aggregation induced by adenosine diphosphate (ADP), while ethacrynic acid, a non-mercurial diuretic but also capable of reacting with protein sulfhydryl groups, had no effect. Conversely methyl-mercuric chloride, an organomercurial sulfhydryl reagent, caused aggregation in the absence of exogenous ADP. Because of the central role occupied by platelets in blood coagulation, thrombosis formation and the hemostatic mechanism[2], the effect of these compounds on the clotting time of recalcified whole blood from rat, guinea pig and human was investigated. The results of this study are reported in the present communication.

Materials and methods. Blood was collected from Wistar strain rats and Syrian Random strain guinea pigs by cardiac puncture and from human male volunteers by venipuncture into siliconized glass tubes containing 3.8% sodium citrate. Final ratio of blood/citrate was 9:1.

The silicone clotting time was determined according to the method of CONSTANTINE et al.[3]. The reaction was started by the addition of 0.2 ml of 2.8×10^{-2} *M* $CaCl_2$ (final concentration 9.5×10^{-3} *M*) to siliconized test tubes (10×75 mm) containing 0.2 ml citrated blood and 0.2 ml of the test compound prepared in modified tyrodes solution (Ca^{++}, Mg^{++}-free). Final volume was 0.6 ml. Mixing was achieved by inversion of the test tubes every 30 sec. Clotting time was noted when the blood was unable to flow upon inversion of the test tube[4].

Compounds were obtained from the following sources: Methylmercuric chloride (Alpha Inorganics), *p*-chloromercuribenzoic acid (Mann), mersalyl (Sigma), ethacrynic acid (Merck Sharp and Dohme), meralluride (Lakeside Lab.) and mercuric chloride (Mallinckrodt Chemicals).

Results and discussion. The results of this study are shown in the Table. In contrast to the shortening of the silicone clotting time of rat and guinea pig blood, methylmercuric chloride prolonged the clotting time in all human subjects. In the case of B. St. only a 40% increase was observed as opposed to the greater than 500% increase for all other volunteers. The mercurial diuretic, meralluride prolonged the clotting time in 2 of 4 human subjects, but had no effect on blood from either rat or guinea-pig. A greater than 5-fold increase in the clotting time of blood from all three species was observed in the presence of the other mercurial diuretic, mersalyl. In addition to lengthening the clotting time, mersalyl also caused hemolysis of erythrocytes from rat and guinea-pig blood but had no effect on any of the 4 human blood samples. In contrast to the effects of the 2 mercurial diuretics, the non-mercurial diuretic agent, ethacrynic acid, caused a slight acceleration in the clotting time in 3 out of 4 of the human subjects but had no effect on either of the 2 animal species. The sulfhydryl reagents,

[1] R. E. A. GADD, S. CLAYMAN and D. HÉBERT, Experientia *27*, 1339 (1971).
[2] J. F. MUSTARD and M. A. PACKHAM, Pharmac. Rev. *22*, 97 (1970).
[3] J. W. CONSTANTINE, I. M. PURCELL and M. GOTTHELF, J. Pharmac. exp. Ther. *176*, 76 (1971).
[4] TH. SCHOENDORF, J. WILKENING and E. E, CLIFFTON, J. Med. *1*, 117 (1970).