not differ significantly with respect to the weight (fig. 1, $\chi^2 = 3.83$, ndf = 5) and the duration of feeding (fig. 2, $\chi^2 = 5.94$, ndf = 3). On the other hand, on reinfestation, we observed significant differences (weight of ticks: $\chi^2 = 12.91$, ndf = 4; duration of feeding, $\chi^2 = 14.17$, ndf = 4). This last result confirms the inhibition of resistance by the antihistamine treatment.

The effect of the antihistaminic is also seen on the means of the weights of engorged ticks and the duration of the blood meal (table 2). The assertion made above that the antihistaminic decreased the effects of resistance is also valid in this case. As seen in table 2 the weights of ticks engorged on rabbits in the treated group during a primary and a subsequent infestation differed less than those of ticks engorged on the control rabbits. The mean duration of feeding of ticks on the treated groups did not differ on primary infestation or reinfestation, whereas the figures were distinctly different on control animals.

Discussion and conclusions. The effects of mepyramine described in this study indicate that histamine may play an important role in the expression of the resistance acquired by rabbits to *I. ricinus.*

Histological examination of the skin of infested animals has shown degranulation of mast cells and basophils, particularly during a reinfestation[6]. Further, using a degranulation test[3], we have demonstrated progressive sensitization of circulating basophils to tick salivary antigens. Thus, during a reinfestation of rabbits by ♀♀ *I. ricinus,* one observes 2 types of local anaphylaxis; a type I hypersensitivity and a cutaneous basophil hypersensitivity. The antihistaminic blocks the action of histamine liberated as a result of these 2 reactions by binding to histamine receptors and therefore inhibiting the H_1 effects of histamine in a specific manner[7]. Consequently, mepyramine does not have an antagonistic action on all the effects of histamine. In the present study the drug would have inhibited the increase in vascular permeability resulting from the local anaphylactic reaction. Thus, the assumption can be made that both the humoral factors such as antibody and complement which are important for the expression of resistance (demonstrated in a preliminary way by the transfer of immune serum[4,5]) as well as cellular factors (eosinophils, etc.) do not appear with the same intensity as protective agents in the feeding lesion of the tick.

The importance of type I hypersensitivity in the establishment of resistance has been shown in other parasitic systems, notably in cattle parasitized by *Boophilus microplus*[8]. Histamine liberated naturally under these conditions could induce an unstable attachment of larvae, which then became more vulnerable to grooming by the host[9]. Detachment of larvae of this species has been reported to occur after the injection of histamine under the site of tick attachment[10].

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Different methotrexate effects in cultured normal and leukaemic human leukocytes[1]

Danuta Rożynkowa, Janina Stępień and Zofia Rupniewska[2]

Laboratory of Human Genetics and Haematology Clinic, Medical School Lublin, Jaczewskiego 8, PL-20-090 Lublin (Poland), 24 August 1981

Summary. Evidence is presented that the use of 2 different culture media, with or without methionine, can help to distinguish between normal and leukaemic methotrexate (MTX)-sensitive cells derived from chronic myeloid leukaemia patients. During the blast crisis MTX assays may help directly in the diagnostics of lymphoid involvement.

First experiments performed by Gunz[3] 30 years ago showed the decrease in proliferative activity by acute leukaemic cells grown in vitro after the addition of aminopterin to the culture. As yet selective toxicity of antifolates has not been demonstrated for leukocytes from patients with chronic myelocytic leukaemia (CML). This phenomenon might have its basis in subnormal rates of methionine synthetase activity (EC 2.1.1.13) in CML cells (Bloss and Sauer[4] and Peytreman et al.[5]). Under conditions in vitro some protection against methotrexate (MTX) may be offered in the form of 5-methyl-tetrahydrofolic acid, which under normal conditions balances the intracellular tetrahydrofolate deficiency due to dihydrofolate reductase inhibition by MTX.

Material and methods. Leukaemic leukocytes obtained from the bone marrow and peripheral blood of CML patients were investigated in short-term suspension culture. Normal proliferating bone marrow cells were used as a control. The material was enriched in proliferating granulocyte precursors by the buffy coat technique and the dextran procedure[6]. Methotrexate (Lederle) was made up in physiological saline and added to 10-ml leukocyte cultures (cell density 1×10^6 per ml) to the final concentration 10^{-5} M for 48 h. The uptake of this high dose of MTX by normal and leukaemic leukocytes was expected to be similar i.e. by diffusion as shown by Kessel and al.[7] and by Hoffbrand et al.[8]. The effect of MTX was studied in 2 culture media: the 1st medium was Eagle's MEM 59 containing methionine 15 mg per l and folic acid 2.3×10 M^{-6}; the 2nd medium was Eagle's MEM 59 deprived of methionine and supplemented with homocystine (Fluka) 15 mg/l, 5-methyltetrahydrofolate 1×10 M^{-5} and vitamin B_{12} 4 mg/l. Inactivated calf serum was added to final 20% of culture medium volume. The serum was previously dialyzed against 30 vol. of normal saline with 3 changes at 4 °C for 48 h. Mitotic activity was studied by morphological examination of stained air-dried cell preparations after previous incubation with Colcemide (Ciba) 0.2 µg/ml for 2 h. Tritiated thymidine uptake into cell nuclei was measured over 2-h periods of exposure in separate cultures and examined autoradiographically. The final external concentration of ^{3}H-thymidine was 1.0 µCi in 1 ml incubation fluid.

Results and discussion. Incubation with medium 1 and medium 2 caused a markedly increased number of cells incorporating tritiated thymidine, i.e. entering the S phase,

Table 1. Mean percentages of the relative increase in ^{3}H-thymidine labeling index of cells incubated for 48 h in media supplemented with methotrexate 10^{-5} M (100% applies to control cultures i.e. without MTX). For explanation of media see text. Individual results were obtained on the basis of 4 trials

	Number of subjects examined	Medium 1 (complete) Range	Mean	SDM*	Medium 2 (modified) Range	Mean	SDM
A Normal bone marrow	6	114–263	168	51.8	109–517	250	145.7
B Chronic myeloid leukaemia cells							
1. Bone marrow	5	190–932	319	286.0	106–525	295	142.0
2. Peripheral blood	6	115–466	292	117.8	117–664	264	184.0
3. MTX resistant patient (marrow)	1		800	40.5		423	25.0

p-Value for the difference between results A:B.2. Medium 1 = 0.05 (Student's t-test). * SD of the mean.

Table 2. Mean percentages for mitotic activity found in cells incubated for 48 hours in media supplemented with methotrexate 10^{-5} M. For differences between the two media see explanation in text

	Number of subjects examined	Medium 1 (complete) Range	Mean	SDM	Medium 2 (modified) Range	Mean	SDM
A Normal bone marrow	8	0.25–2.0	0.65	0.59	0.38–1.50	1.12	0.12
B Chronic myeloid leukemia cells							
1. Bone marrow	7	0–1.01	0.29	0.32	0–0.45	0.19	0.18
2. Peripheral blood	9	0–0.20	0.044	0.07	0–0.60	0.066	0.19
3. MTX resistant patient (marrow)	1		1.25	0.05		1.80	0.08

p-Value for the difference between A:B.2. (Medium 1) < 0.02. Between A:B.1. (Medium 2) < 0.01; between A:B.2. (Medium) < 0.01.

Table 3. Proliferative activity of CML blastic crisis cells after incubation with MTX in vitro

Culture No.	Haematologic and clinical course	Mitotic activity per 100 cells after 48 h MTX/ml				^{3}HTdR labeling index per 100 cells after 48 h MTX/ml			
		0	0.5 µg	5 µg	50 µg	0	0.5 µg	5 µg	50 µg
44/80	Myeloid, Ph'chromosome	0.14	0.2	0.17	0.1	1.9	2.6	2.0	2.0
69/80	Lymphoid, 2 Ph'chromosomes and aneuploidy	0.1	0	0	0	3.9	6.2	5.3	3.3

above the values obtained for cells incubated without MTX. This presumably occurred through an increase in the 'salvage' pathway of thymidilate for DNA synthesis. Normal and leukaemic cells appear to profit from similar thymidine rescue pathways. However, results from the same experiments, reported in table 2, seem to suggest that normal bone marrow cells had a metabolic response after 5-methyltetrahydrofolate treatment different from that of CML cells; the supressing effect of MTX on the mitotic activity could be prevented in all normal bone marrow cells by incubation in medium 2, whereas leukaemic cells remained sensitive to MTX, i.e. were not protected by 5-methyltetrahydrofolate in medium 2. Resistance to MTX was shown by only one CML patient. The inverse relationship between a high thymidine labeling index and decreased or blocked mitotic activity also suggests a prolongation of the S-phase, being more pronounced in leukaemic than in normal myeloid cells.

A similar metabolic defect was reported by Frei et al.[9] in metastatic epithelioma in contrast to normal bone marrow cells protected effectively by leukovorin (5-formyl-tetrahydrofolate). Cells of a human lymphoid SKL-1 line were also protected against MTX by the use of methyltetrahydrofolate (Sauer and Jaenicke[10]). It seems therefore that 5-methyltetrahydrofolate must act as an in vitro rescue agent to exploit differences between normal bone marrow cells and chronic leukaemia cells in their sensitivity to the antipurine effect of MTX. Proper interpretation of these findings must take into account 1. possible differences in the activity of folate binding protein and 2. possible differences in methionine synthetase activity, including B_{12} deficiency, indicated by Sauer and Wilmanns[11].

It must be added that during a blast crisis of CML the MTX medium alone allows us to distinguish between sensitive (lymphoid) and resistant (myeloid origin) cells. Two examples are given in table 3. Thus assays for MTX cytotoxicity, in addition, may be important in the diagnosis of lymphoid blast crisis in CML.

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