(Figure 1) These particles ranged from 0.5 to 1.5 μm in diameter and several inclusions were found in some cells. They reacted positively with methylene blue (blue), toluidine blue (blue), Nile blue sulphate (blue), and Prussian blue. Negative reactions were obtained with other dyes. The neutrophile granulocytes exhibited a very marked positive alkaline phosphatase reaction.

Ultrastructural observations revealed the presence of medium and large lymphoid cells with undulated cytoplasmic membrane and irregular nuclear surface. Nuclear pockets and nuclear inclusions were also observed (Figures 2 and 3). The cytoplasm contained mitochondria and the rough endoplasmic reticulum was poorly developed. Few lysosomes could be seen in the cytoplasm.

Phagocytic vacuoles containing electron-dense material of different consistency were regularly found in the lymphoid cells. A unit membrane surrounding the phagocytic vacuoles was always present. The material included in the vacuoles had an electron-dense appearance in unstained as well as in stained sections. High voltage electron microscopy of the unstained material revealed characteristic ferritin granules (Figures 4 and 5).

The supposition that the cytoplasmic inclusions described here are composed of serum-binding protein, most probably hemosiderin, as suggested from cytochemical evidence by KOSZEWSKI[1], was borne out by the electron micrographs presented in this article. KOSZEWSKI[1] and KOSZEWSKI et al.[11] in their previous studies have demonstrated the appearance of similar inclusions in man and animals treated with saccharated iron oxide compounds. In the case described here, iron therapy was omitted but a few blood transfusions were administered. Furthermore, KOSZEWSKI et al.[11] described the phagocytic activity of non-malignant lymphocytes. The present study showed that the same phenomenon could be brought about by the malignant lymphoid cells. Another hypothesis related to the presence of hemosiderin in lymphoid cells should be considered. It is still not known whether the lymphocytes serve as cells of origin of the blood corpuscules[11,12]. In patients with a malignant lymphoid disease treated with various antileukemia drugs, there is a possibility of the appearance of erythroid cells in the peripheral blood that are otherwise morphologically similar to the peripheral lymphocytes. It is suggested that further studies, as well as retrospective observations on this phenomenon, should be performed in order to clarify the underlaying mechanism.

Zusammenfassung. In den lymphoiden Zellen eines an chronischer lymphatischer Leukämie und Lymphosarcoma leidenden Patienten wurden cytoplasmische, Haemosiderin enthaltende und von Einzelmembranen umgebene Einschlusskörper gefunden. Es ist ungewiss, ob diese Erscheinung erhöhter phagocytischer Aktivität oder dem Auftreten anomaler peripherer erythroider Zellen im peripheren Blut zuzuschreiben ist.

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District Laboratory, Health Insurance Institution Kupat Holim, Rehovot 76-100 (Israel); The Hebrew University of Jerusalem, Rehovot Campus, and Harzfield Chronic Diseases Hospital, Gedera (Israel), 30 October 1974.

[11] B. J. KOSZEWSKI, C. W. EMERICK and D. R. DICUS, Blood *12*, 559 (1957).

[12] J. ALEKSANDROWICZ, H. GEARTNER and J. URBANCZYK, in *Nuclear Hematology* (Ed. E. SZIRMAI; Academic Press, New York and London 1965), p. 193.

Suppression of Adjuvant Disease by Bacillus CALMETTE-GUÉRIN (BCG)

Modifications of the host immune mechanism produced by BCG are thought to be important in the response of patients with various malignancies after treatment with that agent[1-3]. We describe here the suppressive effect of BCG in a non-malignant experimental model, adjuvant disease in the rat[4].

This is a polysystemic syndrome of incompletely defined pathogenesis probably involving immune responses to one or several antigens[5-9]. It was induced in inbred male adult Wistar-Furth rats as described[10] by intradermal injection of adjuvant mixture into the left hind paw. The arthritis, which appears beginning at about day 10, is a major feature of the syndrome, and was scored on a four point scale (0–3) based on the degree of involvement in each of the limbs (exclusive of the adjuvant-injected hind paw)[10]. Rats were divided into 8 groups of 10, as shown in the Table. BCG was given according to 1 of 3 schedules to the appropriate groups. Each injection consisted of 25 mg BCG (BCG-S frais, Institute Pasteur, Paris, France) in 1.0 ml sterile 0.9% w/v NaCl in water given i.p.

Significant suppression of the disease by all BCG treatment schedules was observed, as shown in the Figure. In Figure A, it is shown that pretreatment with BCG significantly suppressed the arthritis relative to that of the adjuvant-injected controls ($p < 0.02$ on day 22), and also delayed its onset. BCG pretreatment followed by twice weekly BCG for 50 days completely prevented the disease until day 88. At that time, 3 of 10 rats developed mild, transient disease lasting only a few days. BCG therapy given after the adjuvant injection (Figure B), either before appearance of the arthritis or during the acute phase, significantly lessened the disease relative to that of the adjuvant-injected control group ($p < 0.02$ on day 14 and 19 respectively). It should be noted, however, that the arthritis in the post-adjuvant, BCG-treated groups progressed following the cessation of therapy to equal that of the non-treated adjuvant-injected control rats. ^{51}Cr-labelled thoracic duct (TD)

[1] R. C. BAST JR., B. ZBAR, T. BORSOS and H. J. RAPP, New Engl. J. Med. *290*, 1413 (1974).

[2] R. C. BAST JR., B. ZBAR, T. BORSOS and H. J. RAPP, New Engl. J. Med. *290*, 1458 (1974).

[3] Natn. Cancer Inst. Monogr., USA *39*, 139 (1973).

[4] C. M. PEARSON and F. D. WOOD, Arthritis Rheum. *2*, 440 (1959).

[5] J. T. SHARP, B. H. WAKSMAN, C. M. PEARSON and S. MADOFF, Arthritis Rheum. *4*, 169 (1961).

[6] B. H. WAKSMAN, C. M. PEARSON and J. T. SHARP, J. Immun. *85*, 403 (1960).

[7] I. GERY and B. H. WAKSMAN, Int. Arch. Allergy *31*, 57 (1967).

[8] M. A. KAPUSTA and J. MENDELSON, Arthritis Rheum. *12*, 463 (1969).

[9] F. QUAGLIATA and J. M. PHILLIPS-QUAGLIATA, Cell. Immun. *3*, 78 (1972).

[10] F. QUAGLIATA, P. M. SANDERS and D. L. GARDNER, Ann. rheum. Dis. *28*, 163 (1969).

Experimental design

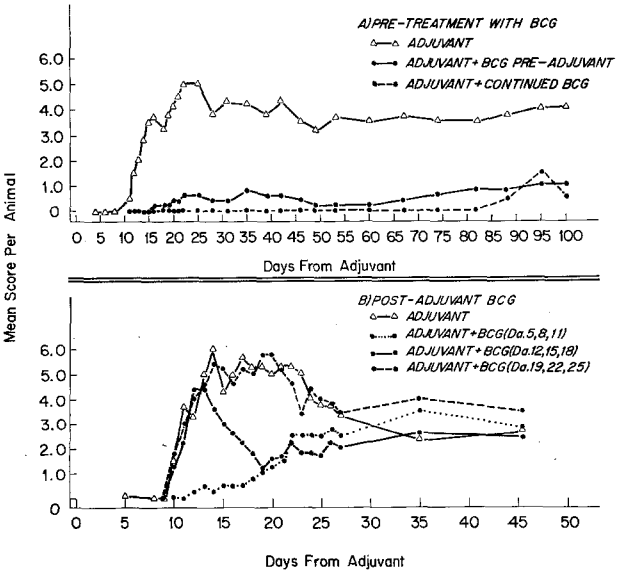
Group No.	No. of rats	Day of adjuvant injection	Days of BCG injections
1	10	0	—
2	10		−6, −3, 0
3	10	0	−6, −3, 0
4	10	0	−6, −3, 0, 3, 7, 10, 14, 17, 21, 24, 28, 31, 35, 38, 42, 45, 50
5 (a)	10	0	5, 8, 11
(b)	10	0	12, 15, 18
(c)	10	0	19, 22, 25
6	20	—	—

lymphocytes from normal or BCG-treated donors were injected i.v. into normal, BCG-treated, adjuvant-injected or BCG-treated + adjuvant-injected recipients. When they were killed, 5 days later, it was found that the administration of BCG to either donors or recipients correlated with higher ^{51}Cr counts in the thymus ($p < 0.001$), lower counts in the bone marrow ($p < 0.001$) and no difference in counts in blood, lungs, liver or spleen when compared to normal recipients of normal TD cells[11].

Suppression of adjuvant disease has been observed in experiments in which the adjuvant-injected rats were pretreated from the neonatal period with *M. tuberculosis*[6]. It has also been reported that sensitization of adult rats with *M. tuberculosis* or its cell wall derivatives given prior to injection of an adjuvant mixture containing either *M. tuberculosis* or *Nocardia* can suppress the diasease[7].

It is possible that the suppression which we observed and that which results from other pretreatment schedules[6,7,12–15] may be mediated by antigenic competition[7,12,15] tolerance[6] or immunological paralysis[6,7], as has been suggested. However, the marked improvement of arthritis occurring within 4 h of a single BCG injection given during the acute phase of the disease is not consistent with the known time-dependent effects of antigenic competition[16,17] or of tolerance[18]. In addition, rats with disease suppressed by BCG had bone marrow-derived (B) blood lymphocyte percentages which were high initially, but fell below normal by day 10; PHA responsiveness was inversely correlated with the B-cell values[11]. These changes in lymphocyte subpopulations and activities may indicate increased helper cell activity[19], decreased suppressor T-cell function[20], or a combination of the two.

The phenomena reported here probably occurred because of the effect of BCG on the immune response, as BCG has been shown to influence both afferent and efferent immune mechanisms[19,21–32]. It is conceivable that the overall suppressive effect of BCG which we have noted may be due to a direct BCG-induced lymphocyte membrane alteration[31] or to the interference of BCG with macrophage-thymus-antigen interactions[31,32]. This is suggested by the experiments mentioned above[11] which showed increased homing of TD lymphocytes from BCG-treated donors to the thymi of normal syngeneic recipients and by the increased homing of normal TD lymphocytes to the thymi of BCG-treated recipients[33].



Suppression of adjuvant disease by different BCG treatment schedules: A) = before, and B) = after, adjuvant injection.

[11] R. I. L. SUTHERLAND, M. A. SPADARO, V. J. W. LAWRENCE and F. QUAGLIATA, in preparation.
[12] F. QUAGLIATA, J. M. PHILLIPS-QUAGLIATA and G. L. FLOERSHEIM, Cell. Immun. *3*, 198 (1972).
[13] K. ISAKOVIC and B. H. WAKSMAN, Proc. Soc. exp. Biol. Med. *119*, 676 (1965).
[14] C. M. PEARSON and F. D. WOOD, Arthritis Rheum. *7*, 337 (1964).
[15] F. QUAGLIATA and A. TARANTA, Ann. rheum. Dis. *31*, 500 (1972).
[16] N. I. BRODY and G. W. SISKIND, Immunology *22*, 75 (1972).
[17] J. RADOVICH and D. W. TALMAGE, Science *158*, 512 (1967).
[18] J. M. PHILLIPS-QUAGLIATA, Cell. Immun. *4*, 116 (1972).
[19] P. ALEXANDER, Natn. Cancer Inst. Monogr., USA *39*, 127 (1973).
[20] R. K. GERSHON, Contemp. Top. Immunobiol. *3*, 1 (1974).
[21] J. G. HOWARD, G. BIOZZI, G. N. HALPERN, C. STIFFEL and D. MOUTON, Br. J. exp. Path. *40*, 281 (1959).
[22] R. V. BLANDEN, M. J. LEFFORD and G. B. MACKANESS, J. exp. Med. *129*, 1101 (1969).
[23] D. O. ADAMS, Am. J. Path. *75*, 17 (1974).
[24] R. C. FRANSON and M. WAITE, J. Cell Biol. *56*, 621 (1973).
[25] P. BARAM, L. SOLTYSIK and W. CONDOULIS, Lab. Animal Sci. *21*, 727 (1971).
[26] K. YAMAMOTO and Y. TAKAHASHI, Nature New Biol. *233*, 261 (1971).
[27] M. S. MITCHELL, D. KIRKPATRICK, M. B. MOKYR and I. GERY, Nature New Biol. *243*, 216 (1973).
[28] T. E. MILLER, G. B. MACKANESS and P. H. LAGRANGE, J. Natn. Cancer Inst. *51*, 1669 (1973).
[29] J. B. HIBBS, JR., Science *184*, 468 (1974).
[30] S. R. ROSENTHAL, Natn. Cancer Inst. Monogr., USA *39*, 91 (1973).
[31] E. KOLSCH, Experientia *24*, 951 (1968).
[32] F. MODABBER, S. MONKAWA and A. H. COONS, Science *170*, 1102 (1970).
[33] This work was supported by NIH Grant No. AM 01431.

Résumé. Par l'administration du vaccin BCG nous avons observé la réduction des symptômes provoqués chez le rat par l'injection intradermique de l'adjuvant complet de Freund (maladie ou arthrite d'adjuvant). Cet effet peut être attribué à une action directe du BCG sur certaines populations de lymphocytes.

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Foetal Red Cell Macrocytosis Induced by Pyrimethamine; its Teratogenic Role

Pyrimethamine (2,4-diamino-5-p-chlorphenyl-6-ethyl-pyrimidine), administered to pregnant rats, induces a variety of malformations, including cleft palate, brachygnathia and limb defects: syndactylia, oligodactylia and phocomelia[1–4]. Its biochemical effects are known[5]: it inhibits the activity of the dihydrofolate reductase and thus the formation of tetrahydrofolate. This active form of folic acid mediates the transfer of 'one-carbon units' in a series of essential metabolic processes. The megaloblastic anaemia-provoking effect of folate antagonists, e.g. pyrimethamine, via alterations in the cell division is deducible from these key-functions of folic acid[6].

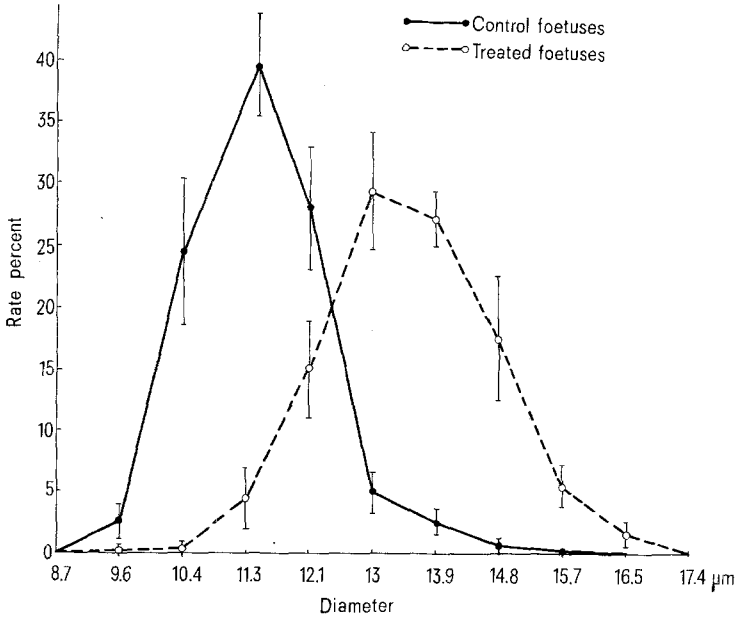
However, the actual teratogenic process of pyrimethamine and other folate antagonists still remains unknown. In this study, we tried to test whether a) pyrimethamine could provoke red cell macrocytosis in the foetus as it does in the adult, and b) whether this induced macrocytosis could cause thrombosis resulting in limb amputations. An analogous process has been described in a rabbit strain[7] (brachydactylia strain), in which the foetal primordial red cells are especially large and numerous and can give rise spontaneously, between the days 15 and 16 of gestation, to thrombosis, oedema, haemorrhages and necrosis of the foetal extremities in the litter.

For studying the drug's influence on the blood, day 14 of gestation was chosen, since the nucleated primordial cells are still numerous at that stage. Other treated foetuses were examined on day 16, in order to determine whether limb haemorrhages were present. Their presence would show that the amputations are not due to a developmental failure of limb buds, but to a necrotic process. In addition, some treated foetuses were examined after birth for the presence of amputations or deformities.

Blood study in 14-day-old foetuses. 3 pregnant Sherman rats were given daily 6 mg pyrimethamine in 2 ml isotonic saline i.p. on days 10 to 13. The foetuses were removed on day 14, blood smears were made and stained by the panoptic method. The diameter of 200 nucleated primordial cells was determined for each foetus (19 treated foetuses from 4 mothers and 16 control foetuses from 4 mothers). A very obvious macrocytosis was observed (Figure): Blood cells having a diameter $\geqslant 12\ \mu m$: approx. 37% in control foetuses and approx. 94% in treated foetuses.

Observations in 16-day-old foetuses. 2 pregnant rats were given daily 6 mg pyrimethamine from day 12 to 15 of gestation; 23 living but growth-retarded foetuses were examined: all presented severe haemorrhages of the 4 limbs and haemorrhagic areas on the snout.

[1] J. B. Thiersch, Proc. Soc. exp. Biol. Med. *85*, 571 (1954).
[2] J. B. Thiersch, Proc. int. Congr. Chemother. *3*, 367 (1964).
[3] A. P. Dyban and I. M. Akimova, Akush. Ginek. *41*, 21 (1965).
[4] M. Krähe, Arch. Gynäk. *202*, 104 (1965).
[5] G. E. Sullivan and E. Takács, Teratology *4*, 205 (1971).
[6] L. Hamilton, F. S. Philips, D. A. Clarke, S. S. Sternberg and G. H. Hitchings, Blood *9*, 1062 (1954).
[7] C. Petter, J. Bourbon, J. P. Maltier and A. Jost, C.r. Acad. Sci., Paris *277*, 801 (1973).



Distribution of nucleated primordial cell diameters in rat foetus treated by pyrimethamine and in controls (day 14 of gestation). Mean ± confidence interval for $p = 0.05$.