Results from the cytogenetical analysis of mice germ cells after chronic γ- or neutron-irradiation with and without Adeturon protection

Group	No. animals	No. analyzed cells	Cells with translocations		
			R IV	C IV	Total (% ± SE)
γ-Rays	10	2,000	9	18	1.35 ± 0.15
Adeturon + γ-rays	10	2,000	4	8	0.60 ± 0.10
Neutrons	7	1,740	9	13	1.17 ± 0.32
Adeturon + neutrons	4	1,200	4	—	0.32 ± 0.17
Control[a]	10	2,000	—	—	0.00

[a] 5 mice C57BL and 5 mice H.

diation drugs show a decrease in their ability to protect somatic as well as germ cells. Our present findings indicated that Adeturon may still be protective when administered daily to mice receiving chronic γ-exposure. Presumably, the compound reduces radiation damage to germ cells by altering the general reactivity of organism as well as by favoring repair processes. Supportive evidence for this assumption is provided by studies where Adeturon, given as pretreatment + treatment, has been observed to protect the hemopoietic system, speeding up recovery during the postradiation period[14,15].

The present study gives further evidence of the ability of Adeturon to protect germ cells from chronic neutron exposure. Biological response to high-LET radiation is generally considered to be subject to little, if any, dose-rate effect or modification by protectants acting through oxygen-effect-related mechanisms[16,17]. However, from a more critical review of the relevant literature, it appears that some reservation is needed in such a generalization, since experimental results are often diverging or incomplete. It should be pointed out that, until disproved in experiments with dose rates sufficiently low to allow repair to take place, there is no a-priori ground to rule out the possibility of occurrence of repair processes in the case of high-LET radiation exposures. Furthermore, positive evidence has been obtained that S-containing protectants may be effective against neutron-induced damage by mechanisms differing from those that underlie protection from low-LET radiation[16,18].

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Unstable L-Forms of Micrococci in Human Foetal Blood

G. G. Tedeschi, D. Amici and I. Santarelli

Istituto di Fisiologia Generale dell'Università, I–62032 Camerino (Italy), 2 February 1976.

Summary. In human foetal blood the presence of Micrococcaceae in the unstable L-form, probably taking origin from the placental transmission of minimal reproductive units, has been recognized by means of microscopic and cultural methods.

An incorporation of ^{14}C- and ^{3}H-labelled nucleosides and amino acids in suspensions of erythrocytes[1] and platelets[2] from normal human subjects has been detected and considered to be attributable to metabolic processes of bacterial L-forms. The ubiquitous symptomless presence of unstable L-forms of Micrococcaceae in the circulating blood has been described[3–5] and further confirmed, following other authors'[6] and our own observations. This presence appears to be correlated to a state of immunological tolerance (manuscript in preparation). Previous researches[7] have demonstrated the placental transmission of *Haemobartonella muris*, a microorganism which, from the point of view of other authors and our-

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selves, may be considered as a mycoplasma or as the stable L-form of a bacterium [8, 9].

Some observations (unpublished results) concerning rates and kinetics of incorporation of 2-^{14}C-thymidine in platelet-rich plasma from human foetal blood led us to investigate the possible presence of bacterial L-forms in the blood of human foetuses.

Materials and methods. Smears were prepared with blood obtained at birth under strictly sterile conditions from the umbilical cord of 30 normal human foetuses. 12 blood specimens were mixed with ACD (1:10) and centrifuged 20 min at 225 *g*: the supernatants were utilized for the incubation assays. The cultural and microscopic examination methods were those used previously [5].

Results and discussion. Structures which could be interpreted as immature microbial forms were often recognizable following careful examination of the blood smears. They were free or connected to the erythrocytes and platelets and looked like minute spheres or rods, irregularly shaped, isolated or in groups, often embedded in a matrix of more or less basophil mucous material (Figures 1 and 2). They showed various degrees of staining affinity with Giemsa, basic fucsin, Dienes' strain and, following acridine orange staining, yellow-green, orange or reddish fluorescence. Various numbers of apparently fully developed coccoidal forms were also detectable in some smears.

Following 10 days incubation of 5 from 12 platelet-rich plasma specimens taken under examination from this point of view, the presence of structures free in the medium or connected to platelets undergoing a process of lysis could be recognized by means of centrifugation at 2000 *g* and microscopical examination of the sediments: such structures could easily be distinguished from platelet granules and mitochondria on the basis of their size, morphology and staining affinity, and could be interpreted as bacterial forms in different stages of evolution and degeneration. In the other 7 cases, the bacterial growth was macroscopically recognizable between the 2nd and 5th day of incubation on the basis of the increasing turbidity followed by sedimentation. Coccoidal forms in various stages of evolution free in suspension or adhering to amorphous material were present.

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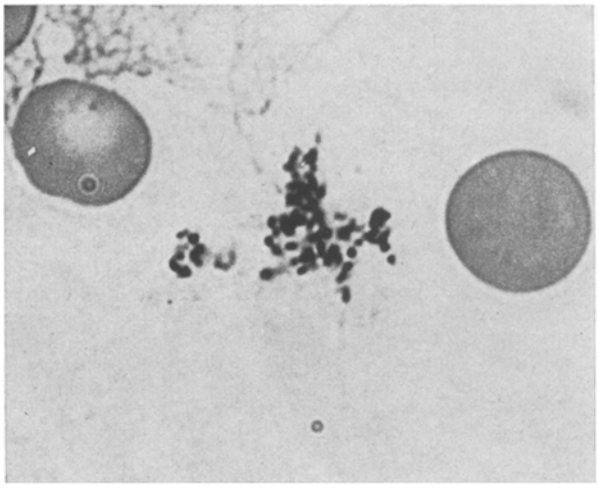

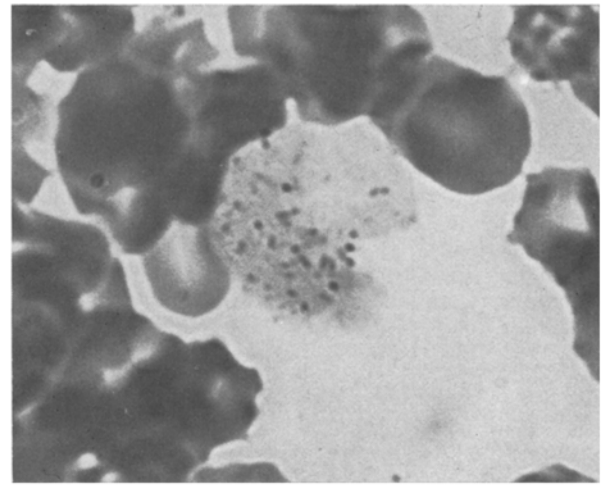

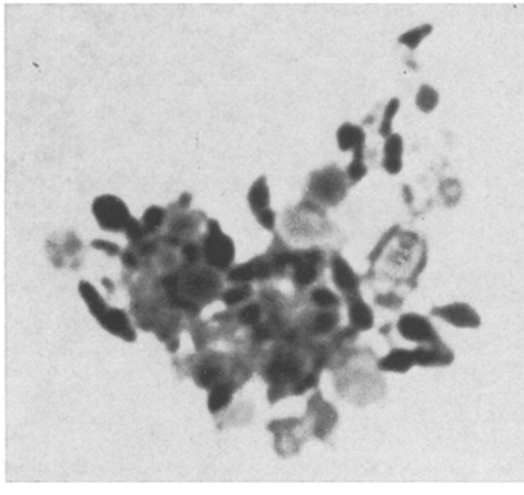

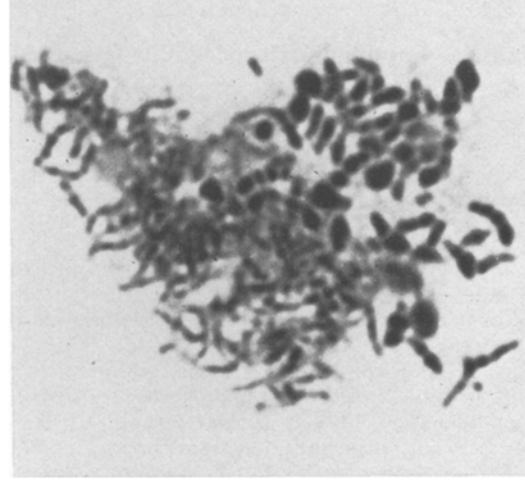

Figs. 1 and 2. Smears of blood from the umbilical cord. Immature microbial forms free or embedded in a matrix of mucous material may often be recognized. Giemsa, × 2,000.

Figs. 3 and 4. Microbial forms looking like *Mycococcus* (Krassilnikov) grown in culture from a specimen of blood from the umbilical cord. Basic fucsin, × 3,000.

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

S. KIGOSHI and M. AKIYAMA

Department of Pharmacology, School of Medicine; and Department of Pharmacology Cancer Research Institute, Kanazawa University, 13-1 Takara-machi, Kanazawa 920 (Japan), 16 January 1976.

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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