Suppression of DNA Synthesis in Phytohaemagglutinin Stimulated Lymphocytes by Prednisone Treatment

Successful chemotherapy of leukemia is dependent on a clear understanding of the degree of action of the anti-leukemic drugs. The present preliminary communication deals with the extent to which prednisone therapy depresses DNA synthesis in phytohemagglutinin (PHA) stimulated blast cells in leukemic children.

Materials and Methods. Peripheral blood leucocyte cultures were set up[1] from 2 children with acute lymphocytic leukemia. Both were in clinical relapse at the time of investigation; one being newly diagnosed and untreated, the other having relapsed after 6 months on prednisone therapy (2 mg/kg body weight). Both these patients are now deceased.

Cultures were incubated for 68 h after which tritiated thymidine (1 μc/ml, specific activity 1.9 C/mM) was added. For the 2 h continuous labelling experiment, colchicine (0.1 ml/ml of a 0.3% solution) was added at the same time as the tritiated thymidine; for the 4 h continuous labelling experiment, colchicine was added 2 h before termination of the cultures. Auto-radiographic treatment of the cells then followed the method described by YUNIS[2].

The percentage of labelled mitoses was calculated and the number of grains of each of 50 metaphases was counted. This was done from the cultures of both the treated and untreated patients for the 2 and 4 h experiments.

Results. 2-h labelling: The grains per metaphase cell in the untreated case varied from 90–152 (S.D. ± 35.7). Percentage of labelled mitoses (labelling index) was 80%. No labelling was obtained from the cultures of the treated case (Table). 4-h labelling: The grains per metaphase cell from the untreated case varied from 200–460 (S.D. ± 40.8) and the labelling index was 86.6%. In the treated case the grain count varied from 70–192 (S.D. ± 29.2) and the labelling index was 80.3%.

Discussion and conclusions. Anti-leukemic therapy is usually aimed at destroying the neoplastic leukemic blast cells, or sufficiently suppressing its metabolic activities as to render their existence short-lived. The circulating leucocytes also inevitably became affected, resulting in a slower rate of DNA synthesis and mitotic activity. This is compatible with the results obtained in the present experiments in which prednisone treatment has caused a significant ($P < 0.001$) depression in the DNA synthesis in apparently normal transformed 'blastoid' cells. Since the cells from both patients are transformed blast cells, the difference in cellular DNA activity observed in the treated and untreated child is due to the prednisone therapy and not to any difference in metabolic activities of the cells. No significant difference had been observed in the labelling indices, hence it is evident that blastoid transformation and DNA synthesis are not completely blocked, but that DNA synthesis continues at a slower rate with a corresponding increase in the generative cycle of the cell.

Details of grain counts and labelling indices

Labelling time	Range	Untreated			Range	Treated		
		Mean	S.D.	Labelled mitosis		Mean	S.D.	Labelled mitosis
2 h	90–152	64.5	± 35.7	80%		No labelling		
4 h	200–460	248.6	± 40.8	86.6%	70–192	128.7	± 29.2	80.3%

Résumé. Recherches autoradiographiques faites sur des cultures de sang périphérique de deux enfants leucémiques, l'un traité et l'autre non traité au prednisone. Un abaissement significatif de la synthèse DNA s'est produit après 68 h dans la culture du cas traité.

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Suppression of Experimental Allergic Encephalomyelitis by Vitamin A

The experimental allergic encephalomyelitis (EAE) is an autoimmune process which may be readily induced in various animals by inoculating a homogenate of central nervous system tissue, emulsified in Freund's complete adjuvant. It is assumed that tissue damage in EAE appears as a result of a delayed hypersensitivity reaction between sensitized cells and the encephalitogenic antigen from the nervous tissue[1] but the intimate mechanism of the delayed hypersensitivity is unknown.

An increase in the catheptic and acid phosphatase activity in the brain of animals with EAE has been found[2,3]. These enzymes are secluded in lysosomes and for this reason we found it tempting to investigate the participation of lysosomes in the pathogenesis of EAE. Large amounts of vitamin A release the lysosomal enzymes into the cell sap and the serum[4]. Therefore we studied the influence of high doses of vitamin A upon EAE in guinea-pigs.

Three groups of guinea-pigs, of both sexes, weighing 450–530 g were experimented upon. The animals were observed for 3 weeks before the beginning of the experiment. The first group (A) contained 13 animals, the

second (B) 10 animals and the third (C) 5 animals; the latter 2 groups were controls. The animals of the former 2 groups were injected intradermally, in the 4 pads, with 0.2 ml of encephalitogenic emulsion, which consisted of equal parts of rabbit brain homogenate and Freund's complete adjuvant (5 mg B.K., H 37 Rv strain, heat-killed, per ml of adjuvant). Starting on the day of inoculation of encephalitogenic emulsion (day 0), the animals were inoculated as follows: the animals of the group A were injected 3 times a week with 1 ml of vitamin A in oil solution (300,000 U/ml), i.m. during the first 2 weeks and s.c. in the third week. The animals of group B were injected in the same way, only with oleum heliantis, the solvent of vitamin A. The animals of group C, controls, were injected only with vitamin A, 3 times a week, i.m., without being inoculated with encephalitogenic emulsion. In brief, each animal was injected 9 times with vitamin A (2,700,000 U) or with oleum heliantis. Because of the toxicity of the vitamin A administered in high doses, only the presence of definite paralysis, paresis or histological lesions were accepted as an unequivocal evidence of the disease. The animals with obvious signs of disease were killed the day of the onset of the symptoms, while the others, including all the animals of group C, were killed on the twenty-first day after inoculation with encephalitogenic emulsion. Brains of all animals were fixed in 10% formalin, embedded in paraffin and the sections stained with hematoxylin and eosin. Sections of 3 levels in brain were examined to find out the histological features of EAE.

The animals of group C exhibited signs of hypervitaminosis about 2 weeks after the beginning of the treatment; the fur was dry and rough, and the eyelids showed keratinization.

These animals became apathetic, anorexic, and lost weight; they seldom had disturbances in walking, and the bones of the cranium were very thin. Histological studies of the brain showed hyperemia of the meningeal and cerebral veins, meningeal edema and edema of the nervous matter.

Group	With lesions of EAE with symptoms	without symptoms	Without lesions or symptoms of EAE
Treated with vitamin A	2	1	10
Injected with solvent oil	9	1	0

The data obtained (Table) suggest that high doses of vitamin A inhibit EAE in guinea-pigs; the results are statistically significant ($\chi^2 = 13$). The effect of high doses of vitamin A in EAE may be due to its action upon lysosomal membranes, producing depletion of the lysosomes and therefore suppressing the delayed hypersensitivity[5]. The hydrolytic enzymes from the lysosomes might play a role in unmasking the encephalitogenic antigen from the myelin structure, that having a role in self-perpetuation of the disease. In experimental autoimmune thyroiditis in guinea-pigs, inoculated with large amounts of vitamin A, a decrease of the cellular infiltrates in thyroid has lately been shown[6].

Our results suggest a lysosome participation in the pathogenesis of EAE in guinea-pigs; the question arises which are the cells whose lysosomes play a role in the mechanism of the disease[7].

Résumé. L'encéphalomyélite allergique expérimentale n'apparaît pas chez les cobayes auxquels on a injecté de fortes doses de vitamine A.

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Suppression of Immunization to Tuberculosis and Diphtheria by an Extract of Lymphoid Tissue

ACTH and cortisone given in small doses tend to increase resistance to infection[1]. It has been established that these hormones have a dramatic favorable effect in allergic states and in the 'collagen' diseases. ACTH and cortisone cause a decrease and dissolution of lymphocytes with release of lymphocytic cellular constituents[2-3]. It is possible that the favorable effect of ACTH and cortisone in hypersensitivity and 'collagen' diseases is mediated by some of the components of the lymphoid tissue.

It may be possible to isolate a substance or substances from lymphoid tissue which has the therapeutic properties of the corticosteroids and hopefully, without the unfavorable side effects of these hormones. Thus, lymphoid tissue might represent a target organ in a hypothalamic anterior pituitary-adrenal cortical-lymphopoietic system, which would be responsible for an organism's reaction to stress. In such a system, the cellular components of lymphoid tissue may exercise a feedback effect on the anterior pituitary-adrenal cortical-endocrine axis. In addition, the lymphocyte components may possibly be re-

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