levels attained did not change in the following 5 to 6 months. Moreover, in a patient with PH made iron-deficient with removal of further 1.4 g of iron after iron overload was already treated, serum transferrin rose to very high levels, such as are seen in iron-deficient subjects without congenital disorders of iron metabolism[9,10]. Serum transferrin was also reduced in 2 out of 12 relatives, and only in these 2 cases body iron stores were already increased. Venesection therapy removed the iron overload and serum transferrin become normal. Rise of serum transferrin both in controles propositi and in relatives are clearly related to the removal of iron overload and not to venesection per se, since steady levels of transferrin were maintained for months after last subtraction of blood. Among the relatives studied by others[2] and with reduced serum transferrin levels the amount of body stored iron was not evaluated, and effect of venesection not investigated.

Moreover, the responsiveness to oestrogens was not lost in our cases with still untreated PM which supports our hypothesis that in this disease synthesis of transferrin is not congenitally impaired. The smaller rise of transferrin here observed in comparison to that obtained with the same treatment in subjects without iron overload might be due to stored iron itself.

On the basis of the existence of an inverse relationship between body iron stores and serum transferrin[1,3,8,9,11,12], and also considering that serum transferrin may be lower in secondary than in primary haemochromatosis[7], the hypothesis of BLANC and VANNOTTI[2] remains unproved. In our opinion the reduced serum transferrin levels most often observed in PH are due to the already increased body iron stores and eventually to liver cirrhosis.

Riassunto. Nella siderocromatosi primitiva (SP) (propositi e consanguinei ipersiderotici) la transferrinemia (STr) era ridotta. La salassoterapia accrebbe e normalizzò stabilmente la STr. Nei consanguinei non siderotici la STr era normale. Un proposito divenne anemico sideropenico e mostrò iper-STr. In 2 propositi non ancora salassati, gli estrogeni orali (per un mese) accrebbero la STr come in maschi senza SP. La ipo-STr della SP non sembra dovuta al calo congenito di sintesi ma al sovraccarico marziale.

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The Effect of Common Antibiotics on Lymphocyte Transformation

The immunosuppressive effects of several anti-neoplastic antibiotics are well established[1]. The finding that chloramphenicol also has a significant immunosuppressive action[2] has stirred interest in the possibility of immunosuppressive effects induced by more commonly used antibiotics[3].

The decrease in resistance to infections from certain organisms such as *Candida albicans*, sometimes associated with long-term tetracycline treatment, occasioned our interest in further investigating the question of possible immunosuppression associated with commonly used antibiotics. In this study we have investigated the in vitro effects of therapeutic concentrations of tetracycline, penicillin and erythromycin on lymphoblastic responses to phytohemagglutin (PHA). Aspirin was used as a measure of the test system because of its known ability to suppress lymphocyte transformation in therapeutic concentrations in vitro[4].

Materials and methods. Whole blood, aseptically collected from 26 healthy volunteers, was added to the culture medium, RPMI 1640 supplemented with 10% Agamma Calf Serum and 1% L-glutamine (BBL Co., Cokeyville, Md.), in an amount to give a concentration of 2×10^5 lymphocytes per ml. 250 µg PHA (Difco, Detroit, Mich.) was added to each 4 ml culture. 16 µg tetracycline (Achromycin-IV, Lederle), erythromycin (Erythromycin Lactobionate-IV, Abbott), and penicillin (Buffered Sodium Penicillin G-IV, Squibb) in 0.1 ml sterile water, and 0.8 mg acetylsalicyclic acid dissolved in 0.2 ml sterile water were added to triplicate culture sets. After 4 days incubation at 37 °C with 5% CO_2, lymphocyte blastogenesis was measured by adding 0.2 ml ^{14}C-thymidine (0.6 µCi) to each culture. Harvesting of lymphocytes to measure thymidine incorporation was performed 24 h later by serial centrifugations at 1600 rpm for 15 min with decantations of the supernatant. 4 ml of 3% glacial acetic acid was added to each tube to lyse the red cells. The cells were washed with 4 ml isotonic saline followed by 1.0 ml 0.1 *N* NaOH to lyse white cells. Finally 4.5 ml of 6.7% trichloracetic acid were added to each tube to form acid insoluble precipitates with released nuclear material on overnight standing. The precipitates were bleached with 3.0 ml cold methanol, solubilized in 0.5 ml Packard Solulene, washed into a scintillation vial (15 ml of 4% Packard Permafluor in scintillation grade toluene), and counted for 5 min in a liquid scintillation spectrometer.

Results. The values for percent suppression of thymidine uptake, calculated by dividing the average cpm of an antibiotic containing culture set by the control set, are plotted on the graph. 24 tetracycline – 14 erythromycin – and 14 penicillin – containing culture sets, all at a concentration of 4 µg/ml, showed no suppression. Composite values were 101%, 99% and 103% respectively.

The potential effectiveness of the assay method was demonstrated by finding that concentrations of acetylsalicylic acid, analogous to post-treatment levels in humans, suppressed thymidine incorporation by 29% in 10 cultures at a 20 mg/100 ml concentration ($p < 0.02$) and by 65% in 4 additional culture sets at 30 mg/100 ml, values similar to those found by OPELZ et al.[4]. The Achromycin preparation contained ascorbic acid as a stabilizer (1250 mg ascorbic acid per 500 mg tetracycline). Studies with ascorbic acid alone (Ascorbic Acid Injection, Upjohn) in concentrations equal to that in the tetracycline containing cultures, 10 µg/ml, as well as 300 µg/ml, failed to demonstrate any effect on PHA responsiveness.

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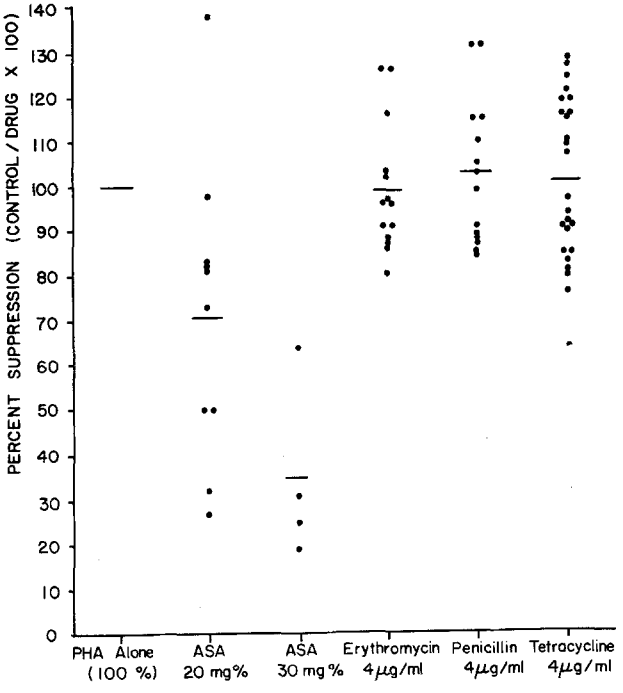
Discussion. Many antibiotics such as chloramphenicol, streptomycin, gentamicin, tetracycline and rifampicin act by interfering with various steps of protein synthesis, translation, transcription or RNA function within bacteria[2,5–8]. Evidence is strong that anti-neoplastic antibiotics as well as chloramphenicol exert an influence on host cell protein synthesis and subsequent immunologic responsiveness[1,2]. Rifampicin has been shown to suppress PHA responsiveness of human lymphocytes in culture[9] and there is a possibility that it may have immunosuppressive effects in vivo[10]. The PHA - stimulated lymphocyte culture system is a widely used measure of one aspect of immunologic competence. Within hours of incubation with PHA, increased RNA and protein synthesis begins in the lymphocyte, followed in about 24 h by increased DNA synthesis[11]. The system thus offers a broad range of synthetic steps for the study of potential inhibitory effects. The long-term use of tetracycline for conditions such as acne and hidradentitis suppurativa, as well as its use for presumptive, though empirical, anti-inflammatory effects in rosacea and certain forms of panniculitis made its investigation for possible immunosuppressive activity seem reasonable.

The concentration of tetracycline used approximates that found in human blood samples after an oral dosage of 500 mg q.i.d.[12]. The erythromycin and penicillin concentrations utilized are also similar to the higher blood levels achieved by standard dosage administration of these antibiotics. The findings in this experiment imply that protein, RNA and DNA synthesis in lymphocytes are not impaired by the antibiotics tested in therapeutic concentrations, since PHA-induced transformation is dependent on all of these metabolic processes. No studies of the metabolites of these antibiotics were investigated.



The effects of antibiotics and acetylsalicylate upon PHA-induced blastogenesis of human lymphocytes. Erythromycin, penicillin and tetracycline failed to demonstrate any inhibitory effect.

Zusammenfassung. Therapeutische Konzentrationen von Penicillin, Erythromycin und Tetracycline wurden Lymphozytenkulturen zugefügt um mögliche Ähnlichkeiten zu der immunsuppressiven Wirkung antineoplastischer Antibiotika zu bestimmen. Eine Hemmung der PHA-induzierten Blastogenese konnte nicht beobachtet werden.

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Mitogenic Responses of Thymus Cell Subpopulations

The central role of the thymus gland in the immune system has been well documethod[1]. This organ provides the peripheral lymphoid system with a heterogenous population of immunologically competent cells (T cells) which function in humoral immunity[2,3] and are paramount in the cellular immune response[4,5]. T cells and their progenitors (thymocytes) exhibit distinctive responses to specific plant mitogens. Thymocytes exhibit marked DNA synthesis when stimulated with concanavalin A (Con A) while phytohemagglutinin (PHA) induces only a slight stimulation[6]. However, T cells within the spleen respond almost equally to both Con A and PHA[7]. The present study investigates the mitogenic responses of subpopulations of murine thymus cells separated on albumin discontinuous gradients to the thymus dependent mitogens Con A, PHA, and the thymus independent mitogen *E. coli* lipopolysaccharide (LPS).

Materials and methods. Thymus glands were removed aseptically from 6–10-week-old male and female C57Bl/6

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