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ANTICONVULSANT DRUGS IN IMMUNOSUPPRESSION AND CARCINOGENESIS

by

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SUMMARY

Criteria of normal immunological responsiveness were established for a control population of 177 subjects. B-cell function was assessed by quantitating serum concentrations of the immunoglobulins G, A, and M, primary antibody responses to Salmonella typhi and secondary antibody responses to Tetanus toxoid. In addition, sera were tested for the presence of antinuclear antibody, and complement (C_3) levels were determined. Indices of T-cell function included delayed hypersensitivity reactivity to intradermal and contact antigens, circulating lymphocyte counts, phytohaemagglutinin (PHA) - induced lymphocyte blastogenesis, and PHA-induced deoxyribonucleic acid (DNA) synthesis in the presence of autologous serum and foetal calf serum. DNA synthesis was also measured in the leukocytes of freshly drawn blood.

Conditions were established for the routine measurement of lymphocyte transformation in cultures of peripheral blood. Under these conditions lymphocyte DNA synthesis was related directly to the incorporation of tritiated thymidine into cell DNA. Comparisons were made between the measurement of lymphocyte transformation by morphological criteria, and the incorporation of radio-labelled thymidine into acid-precipitable DNA. There was a positive correlation between lymphocyte blastogenesis assessed by the microscopic examination of stained smears, and that measured by electronic pulse-height analysis of nuclear volume changes. Neither parameter could be correlated with PHA-induced DNA synthesis. Nuclear volume

analysis showed that transformation was maximal on different days of culture in different subjects, in contrast to PHA-induced DNA synthesis, which was maximal on the same day in different subjects. The microscopic assessment of blastogenesis is a subjective measurement, based on counting a small number of cells. It was concluded that the most practicable method for the measurement of lymphocyte transformation in blood cell cultures was by the incorporation of tritiated thymidine into DNA.

PHA-induced DNA synthesis was measured routinely in blood cell cultures. These were technically superior to lymphocyte enriched cultures because of the small volume of blood required and the lack of initial cell manipulation (some methods of cell separation are known to cause detectable impairment of lymphocyte function (Ling, 1968)). DNA synthesis and nuclear volume changes were measured in PHA-stimulated cultures containing lymphocytes of greater than 98% purity, or whole blood. In each case, there was no consistent relationship between the results of blood and lymphocyte cultures. This may have been due to technical factors, for example it is probable that at least part of a subpopulation of lymphocytes was removed during the lymphocyte separation procedure. Stimulation of lymphocyte DNA synthesis by other blood components has been reported, but only with blood component - lymphocyte ratios much smaller than those found in the peripheral blood of subjects used in this study. The curves obtained by varying serum concentrations, lymphocyte numbers, pH, and PHA concentrations, in

stimulated blood cell cultures were similar to those reported for lymphocyte-rich cultures. Mean DNA synthesis remained constant in a group of subjects over a 3 week period, although the results of individual subjects varied, in agreement with reported results in lymphocyte-rich cultures.

Immunological function was measured in two groups of patients on long-term therapy with the anticonvulsant drug, phenytoin sodium. At least one immunological defect was found in 60% of 63 general hospital patients (70% of female and 53% of male patients) compared with 7 of 15 patients treated with another anticonvulsant, carbamazepine, and 7 of 87 control subjects. One or more deficiencies were present in 38% of 52 intellectually retarded (I.R.) patients taking phenytoin, compared with 2 of 15 I.R. barbiturate-treated patients, and 3 of 45 I.R. control subjects. Eleven patients were studied before and at least two months after the commencement of phenytoin therapy. Mean IgA, measured in 10 patients, was significantly depressed (by 31%) after the commencement of therapy. Failure to make antibody to Salmonella typhi, and to manifest delayed hypersensitivity reactivity, was also observed after therapy had commenced.

Analysis of the data indicated that a number of factors influence the effect of phenytoin on immunological responsiveness. IgG concentrations were depressed in the I.R. patients, whereas IgA and IgM were depressed in the general hospital group. Antibody responses to Salmonella typhi, delayed hypersensitivity reactivity,

and lymphocyte responses to PHA were depressed in both groups of patients. These differences were not due to serum folic acid deficiency, but differences in phenytoin dosage and nutrition may have been factors. Analysis of the hospital series showed a sex influence on serum levels of IgA and IgM, and DNA synthesis (mean serum phenytoin levels being the same in males and females). IgA was depressed to a significantly greater extent in females than males, IgM was low only in males. PHA-induced DNA synthesis in autologous serum, and DNA synthesis in circulating leukocytes, were depressed in females, not males. Depression of PHA-induced synthesis in foetal calf serum was not significant, suggesting that a serum factor, probably phenytoin itself, was responsible for the depression of DNA synthesis observed in females. Antinuclear antibody was found in only 3% of the I.R. patients, and in none of the hospital patients treated with phenytoin.

Studies in vitro confirmed that phenytoin directly inhibits DNA synthesis in human lymphocytes. DNA synthesis was depressed by 50% in cultures containing phenytoin concentrations within the therapeutic range of 10-20 ug/ml. Depression of DNA synthesis by phenytoin varied inversely with the concentration of serum in cultures, suggesting that only the unbound form of phenytoin depresses DNA synthesis. Therapeutic concentrations of phenytoin caused a relatively small depression of DNA synthesis in the presence of high serum concentrations. Despite the effect of protein binding, this depression was significant in vivo, as DNA synthesis in

circulating leukocytes was impaired. In addition, depression of DNA synthesis in 10% autologous serum was significant, even though the maximum phenytoin concentration was not greater than 2 ug/ml. Higher concentrations of phenytoin depressed lymphocyte blastogenesis, RNA synthesis, protein synthesis, and cell counts in vitro. Kinetic studies suggested that phenytoin affected the early stages of lymphocyte activation in particular, causing a maximal depression of DNA synthesis when added within 8 hours of the initiation of culture.

Both T-cell and B-cell function, but especially T-cell function, were depressed in a patient (F.K.) who developed Hodgkin's disease 3 years after the commencement of phenytoin therapy. These tests were performed 10 months after the patient had received radiotherapy to a thoracic mantle field. In vitro, phenytoin induced a significant increase in DNA synthesis in lymphocytes from patient F.K., in contrast to the findings in control subjects. This response suggested an anamnestic response of lymphocytes to specific antigen.

It is suggested that immunosuppression is related to the role of phenytoin in carcinogenesis. Immunodeficiencies in phenytoin-treated subjects were similar to those in a study of 38 patients with untreated lymphoma. Deficiencies were present in 3 of 8 patients with Stage I Hodgkin's disease. The stimulation of lymphocyte DNA synthesis by phenytoin in patient F.K. supports the hypothesis of Kruger, that phenytoin-induced lymphoma is due to the combination of chronic antigenic stimulation and partial immunosuppression.