VALIERI had proposed that both D-DPe and D-RPe were multiunit enzymes. KEIR[19] also suggested a multiunit structure for D-DPe according to his studies of Landschutz ascites tumor D-DPe.

In view of these observations it appears safe to say that CT-DPe showing ss-DNA preference might be an artifact of isolation, particularly since the presence of ss-DNA has so far not been demonstrated in living cells. In other words the isolated enzyme is probably the enzymatically active subunit of the so far not isolated 'native form' of the enzyme[20]. It is quite likely that during the purification of the enzyme a crucial subunit, the function of which is to hold enzymatically active subunits together in a certain spatial conformation, is being lost. The early work on *E. coli* D-RPe is a case in point; the σ factor had eluded the researchers for a long time. Q_β replicase was also shown to consist of 3 host and 1 viral specific polypeptide chains with at least one of the host subunits shown to be essential for enzymatic activity[21]. Hence, interpretation of results based on a property that can easily change spontaneously or can be manipulated at will should be made with great caution. It should not be too surprising if the enzyme(s) found in oncogenic RNA viruses will turn out to be of host origin being modified by viral coded factors.

Zusammenfassung. DNS-Polymerase aus Kalbsthymus (in 50%-Glycerin-haltigem Puffer gelöst) zeigt bei Kühlaufbewahrung Alterung mit Änderung ihrer Template-Präferenz von einstrangiger zu doppelstrangiger DNS.

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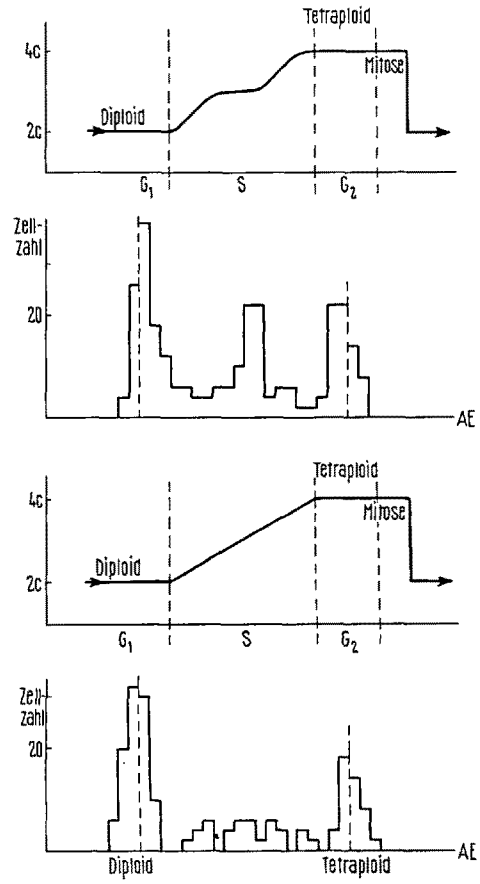
Cytophotometric Studies of the Course of the S Phase in PHA Stimulated Lymphocytes

Studies made by various authors have shown that there are obvious differences in the course of the S phase in different kinds of cells. The present authors, by cytophotometric measurements and by a determination of mitoserates, made an attempt to study the situation with respect to lymphocytes in a tissue culture subsequent to PHA stimulation. 2 ml of a leukocyte suspension (800,000/ml) were incubated at 37 °C in Eagle's medium containing 20% of autologous plasma after addition of 0.2 ml of PAH-M to 10 ml of culture medium. For one thing, the mitoserates were determined within 72 and 76 h after the short-time (15 min) action thereon of colchicine by counting 5000 cells per culture (totaling 40,000 cells); and, for another, they were measured 76 h after preparation and subsequent to a prolonged (4-h) treatment with colchicine. Cytophotometry was performed with a recording cytophotometer[1] (which was designed by the authors) using the scanning method at 575 nm. The formalin-fixed streaks were stained by Feulgen's method making use of a 35-min cold hydrolysis[2]. A total of 800 cell nuclei were measured. The determination of mitoserates made 72 and 76 h after short-time action thereon of colchicine gave similar values (the mean values being 20.45‰ and 25.5‰, respectively), while, in those cases in which colchicine was allowed to act thereon for 4 h, the mitoses were found to have doubled, the mean values being of the order of 40.5‰. Our karyophotometric results have been plotted in the form of karyograms, showing the frequency as a function of the relative DNA content in arbitrary units (AE). Here, we usually found a distinct intermediary peak in addition to the high peak of diploid cells and the low peak of tetraploid cells. SANDRITTER[3] has given a scheme for proliferating tissue (lower half of the Figure), showing how the length of the G_1 phase corresponds to the level of the diploid peak in the karyogram and how the level of the tetraploid peak corresponds to the duration of G_2. The intermediate values correspond to the S phase which, in this scheme, is assumed to show a continuous increase. Our studies have shown that, so far as PHA-stimulated lymphocytes are concerned, the situation is obviously quite different (upper half of the Figure). The high peak between diploid and tetraploid values is explained by assuming a decrease in the rate of synthesis of DNA in the middle of the S phase. This has been shown diagrammatically in the



Relation between the course of DNA synthesis and DNA karyogram. Lower part: proliferating tissue with continuous S phase (according to SANDRITTER[3]). Upper part: Discontinuous S phase in PHA stimulated lymphocytes in tissue culture.

upper part of the Figure. Accordingly, the PHA stimulated lymphocytes may be considered to show a behavior similar to that which has been described by KLEVECZ[4] for a body of diploid cells obtained from Chinese hamsters. In a body of murine fibroblasts (L cells), a different rate of DNA synthesis in the S phase was also found; here, however, the delay was found to be at the very start of the S phase[5-8]. The authors believe that eu- and heterochromatin show a different behavior here. It should also be considered that the synthesis of DNA, on the one hand, and the synthesis of RNA and protein, on the other hand, show a reciprocal relation[4,9]; this may necessitate a transient slowing down of DNA synthesis in the S phase, in order to make increased synthesis of both RNA and protein possible. Anyway, our studies have shown that it is necessary for different bodies of cells to be examined for their different behavior in the S phase[10].

Zusammenfassung. Karyologische und zytophotometrische Untersuchungen an PHA-stimulierten menschlichen Lymphozyten ergaben nach 76 h im DNS-Karyogramm einen weiteren Gipfel zwischen diploiden und tetraploiden Werten. Daraus wird geschlossen, dass bei Lymphozyten in der Kultur die DNS-Synthese in der S-Phase diskontinuierlich verläuft.

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Hematopoietic Alterations Produced by Long-Term Treatment with Phytohemagglutinin

It is known that the lymphoreticular system of the mouse undergoes profound cellular modifications in response to a single dose of phytohemagglutinin (PHA)[1-5]. The characteristic changes are depletion of mature lymphocytes and the appearance of numerous large undifferentiated cells in the spleen, lymph nodes, and thymus. Lymphopenia, neutrophilia and an abnormal number of lymphoblastoid cells are seen in peripheral blood[3-4].

It is not known, however, if repeated administration of PHA for a long period of time would produce permanent cell changes similar to those described earlier. This study was undertaken to find out whether the long-term treatment with PHA produces a leukemoid reaction in C3H and Swiss inbred strains of mice.

The administration of PHA (Bactophytohemagglutinin-P, Difco, Detroit, Mich.) began when the mice were 2 months old, and continued twice weekly for 16 (Swiss) and 24 (C3H) weeks at the dose of 5 mg/100 g of body w. i.p. Control animals were injected with heat-inactivated PHA[2] at identical intervals and doses. Groups of 10 mice were sacrificed on the 1st week of treatment and every 2 weeks thereafter to the end of the experiments. 50 mice of each strain served as untreated controls. A total of 320 mice were studied. Peripheral blood values, marrow (one femur), and splenic cell counts were assessed by standard techniques. Histologic sections were stained with hematoxylin-eosine. Cytologic examinations were also performed in smears stained with Giemsa.

Mice (C3H) given PHA or inactivated PHA developed splenomegaly (+40%) and hypertrophy of lymph nodes. The splenomegaly was mainly caused by a hyperplasia of splenic follicles and proliferation of hematopoietic foci, among which myelopoietic cells were predominant. The number of nucleated splenic cells was between 185×10^6 and 252×10^6. Untreated control mice had 150 ± 20.6 (S.D.) $\times 10^6$ splenic cells. Thymic atrophy was only observed in C3H mice throughout the study (Control thymus $0.10 \pm 0.01\%$ body wt.; Experimental 0.044 to 0.067% body wt.). Treatment with heat inactivated PHA did not change the thymus weight. No alterations of erythrocyte values and platelet counts were found in C3H mice. Control, given inactivated PHA, and experimental animals of this strain had leucocytosis (14,800 to 24,600/mm³; Normal = $9,200 \pm 950$/mm³) due to absolute lymphocytosis and neutrophilia. The number of immature cells in peripheral blood was within normal limits (0.2% of the total leucocyte count). Myelopoietic cells were predominant in the marrow. The number of nucleated marrow cells in the right femur was similar to that of untreated control and ranged from 7.23×10^6 to 9.40×10^6 regardless of the treatment.

Similar alterations were observed in the spleen of Swiss mice under chronic treatment with PHA. The spleen cell number was 201×10^6 in untreated control animals. Following PHA or inactivated PHA their number rose to a maximum of 332.1×10^6 cells on the 6th week of the treatment. Normal erythrocyte ($9.61 \pm 0.16 \times 10^6$/mm³) and platelet ($1.11 \pm 0.01 \times 10^6$/mm³) counts were found from the 1st to the 10th week of PHA administration. Anemia as indicated by erythrocyte counts of 6.71 to 7.14×10^6/mm³, with normal reticulocyte counts (4.8 to 5.8%) was observed from the 12th to the 16th week of the treatment with PHA. The administration of inactivated PHA did not cause anemia in Swiss mice. The normal leucocyte count was $24,800 \pm 1,230$/mm³. Following either PHA or inactivated PHA the total leucocyte counts were between 24,700/mm³ and 33,800/mm³. Lymphocytosis,

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