Electron Microscopical Evidence for Innervation of Chief Cells in Human Parathyroid Gland

The innervation of the parathyroid glands in dogs and cats was described by RAYBUCK[1] at the light microscopic level. He found nerve endings in intimate relationship with the cell membrane of the chief cells. Many electron microscopists have mentioned unmyelinated nerve fibers in the perivascular spaces of parathyroid glands in various species, including man[2-11], but the termination of nerve fibers remained unclear. Nerve endings at endocrine parathyroid cells were not observed[5,7,8].

In our study (parathyroids of 3 patients with secondary hyperparathyroidism and 3 patients without impairment of calcium metabolism), we often found unmyelinated nerve bundels joining the blood vessels. Generally there is a partial unfolding of axons within the free interstitial spaces without contact with epithelial cells, fibroblastoid cells, or blood vessel cells. The mode of innervation found in arterioles is similar to that found in other organs[12].

Additionally we often found an approach of preterminal nerve fibres to the basement membranes of parathyroid chief cells. The preterminal axons are rich in synaptic vesicles and neurosecretory granules. Sometimes a close direct contact of unfolded axons with chief cells can be demonstrated (Figure). This represents the morphological appearance of a neuroepithelial synapse. The intersynaptic space measures 150 Å.

This mode of innervation in blood vessels, interstitial tissue and especially neuroepithelial synapses with chief cells, occurs in both normal and secondary hyperplastic parathyroid glands. Although most work indicates that parathyroid activity is predominantly regulated by plasma calcium level[10,13] and calcium concentration in tissue culture medium[14], the demonstrated innervation of parathyroid chief cells might have some significance for the regulation of endocrine parathyroid activity and endocrine cell mechanisms. The findings are of special interest, as HODGES and GOULD[15] found evidence for partial nervous control of avian ultimobranchial body, the other endocrine organ involved in calcium metabolism.

Zusammenfassung. Erstmalig werden ultrastrukturell neuroepitheliale Synapsen vegetativer Nerven an den Hauptzellen von Nebenschilddrüsen (Mensch) nachgewiesen. Eine nervöse Beeinflussung der Parathormonsynthese und -sekretion ist daher möglich.

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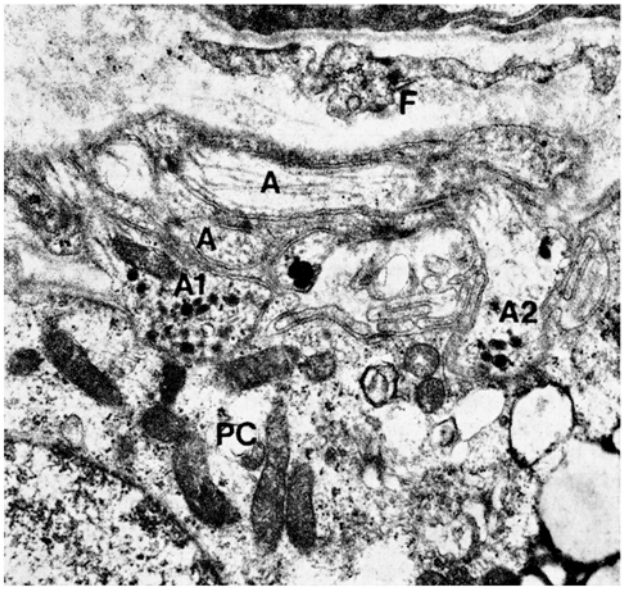

Parathyroid chief cell (PC) with adjacent axon bundle. Two axons (A1, A2) are in synaptic contact with the chief cell. F = fibroblastoid cell. ×19,000.

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Template Preference of Polymerases and its Relevance to Oncogenic RNA Virus Replication[1]

Recent reports of BALTIMORE[2], TEMIN and MIZUTANI[3], SPIEGELMAN et al.[4,5], ROKUTANDA et al.[6] have caused great excitement because it was thought that the viral polymerases represented unique enzymes found only in oncogenic RNA viruses and that they were contrary to the central dogma of molecular biology, namely, they were using RNA to synthesize DNA. According to these reports there seemed to be two enzymatic activites in these virions: one capable of synthesizing DNA on an RNA template and one that could use the DNA-RNA duplex formed by the former as a template to synthesize a double stranded DNA product. The presence of DNA in these virions has also been reported[7]. Some of the recent investigations show differences exhibited by these enzymes in their preference toward denatured and native DNA template[8,9]. The cause of this excitement was the view that DNA polymerase can only use DNA as template and will only produce a DNA-like product. Consequently an enzyme using RNA or RNA-DNA hybrid as template was considered unique for oncogenic RNA viruses. However,

this view had to be abandoned when it was demonstrated that non-oncogenic viruses, such as Disno- and Monkey Foamy viruses contain similar enzymes. Later the presence of similar enzymes were demonstrated in normal animal tissues[10]. Furthermore, whether there really are 2 different enzymes or one enzyme showing different template specificities due to the presence of virus coded factors and whether these activities are exhibited by viral coded enzyme(s) or by host enzyme(s) as well as the function of viral DNA remain obscure at the present.

Here we describe some of our observations which indicate that template preference of an enzyme can change spontaneously or can be manipulated at will. The enzyme in question is calf thymus DNA polymerase (CT-DPe) which has been studied rather exensively during the past decade and its most prominent characteristic was reported to be its absolute preference for single stranded DNA (ss-DNA) template[11,12].

Experimental. The enzyme, dissolved in 50% glycerol containing 0.01 *M* phosphate buffer pH 7.0, was kindly furnished by Dr. J. J. FURTH of the Department of Pathology, School of Medicine, University of Pennsylvania. Normal and labeled deoxynucleoside-triphosphates were obtained from Schwarz-Mann, Orangeburgeburg, N.Y., all other chemicals were analytical reagent grade. Assay conditions are given under Tables.

Results. The enzyme when it was first obtained (Summer of 1969) showed a definite preference for ss-DNA, however, incorporation of ^{3}H-TTP amounting up to 20% of ss-DNA was also obtained while using double stranded DNA (ds-DNA) as template (Table I, A). The same enzyme, about 1 year after it was obtained, showed an unexpected change in template preference (Table I, B). During this period the enzyme was kept at –17 °C without undergoing treatment.

To find out whether this change could be reversed by certain treatments, aliquots of the enzyme were treated with urea and EDTA. Treatment with 7 *M* urea caused a reversion back to ss-DNA preference but over 95% of enzyme activity was lost during this treatment. On the other hand dialysis against $1 \times 10^{-3} M$ EDTA for 48 h has definitely caused a change of template preference in favor of ss-DNA (Table II).

Experiments performed on a commercially available *M. luteus* DPe also show that it is possible to affect the template preference of this enzyme. It is possible to shift the preference of the enzyme toward more efficient use of ss-DNA by dialyzing it against EDTA and then to bring it back to ds-DNA preference by keeping the dialyzed enzyme in the presence of 1 *M* sucrose, 1 *M* NaCl or 50% glycerol. This enzyme can also utilize yeast RNA as a template[13].

Discussion. In 1962 BERG et al.[14] have demonstrated that a mere change from Mg^{++} in the assay solution, was sufficient for the incorporation of ribonucleotides onto the growing chains of DNA. A low molecular weight glycopeptide that we have isolated from Ehrlich ascites fluid of mice, which we believe is the initiator of DNA replication[15], stimulates the incorporation of RNA precursors by crude as well as partially purified DNA dependent RNA polymerase (D-RPe). The same factor can also induce CT-DPe to utilize ss-DNA 1.5-2 fold better[16]. These observations indicate a rather close relation between DNA and RNA syntheses by *E. coli* DNA dependent DNA polymerase (D-DPe).

In 1964 LEE HUANG and CAVALIERI[17,18] have isolated an enzyme from *E. coli*, which was shown to synthesize a double stranded polymer, one strand of which was ribo- and the other strand deoxyribonucleotide polymer. Moreover, this enzyme, which was named hybrid polymerase, has been shown to synthesize DNA like molecules on a ribonucleotide template. It was also demonstrated that ageing and treatments known to dissociate protein subunits caused a decrease of hybrid activity with a concommitant increase of D-DPe and D-RPe activities. CA-

Table I. Incorporation of ^{3}H-TTP by fresh and aged CT-DPe using native and denatured DNA templates

	cpm/30 min/100 γ protein	
1969	ssDNA	dsDNA
A) Experiment 1	9,245	1,860
Experiment 2	5,631	1,507
1970	ssDNA	dsDNA
B) Experiment 1	6,725	7,890
Experiment 2	5,700	7,640

The assay solution contained, in a final volume of 0.5 ml. 0.02 ml of 1 *M* glycine buffer (ph 8.0), 0.01 ml of 0.05 *M* $MgCl_2$, 0.01 ml of 0.1 *M* β mercaptoethanol, 0.05 ml of 0.001 *M* deoxynucleoside-triphosphates, 0.05 ml of ^{3}H-TTP (10^7 cpm/ml), 0.05 ml of 0.5 mg/ml DNA, 0.01 ml of enzyme (100 γ). The mixture was incubated at 37 °C for 30 min, was cooled in ice, 0.2 ml of the mixture was applied onto filter paper discs, dried in warm air.
Paper discs were treated twice with ice cold 10% TCA, then washed twice with 5% ice cold TCA, twice with 95 ETOH for 10 min and once with ethyl ether, dried and counted in a Tricarb scintillation spectrometer using thixotropic gel.

Table II. Incorporation of ^{3}H-TTP by aged CT-DPe after dialysis against EDTA

	cpm/30 min/100 γ protein	
	ssDNA	dsDNA
Experiment 1	3,490	2,005
Experiment 2	833	622

The enzyme was dialyzed for 48 h against 0.05 *M tris* (pH 7.7), $5.10^{-3} M$ β mercapto ethanol and $1 \times 10^{-3} M$ EDTA. 0.01 ml of enzyme solution was then assayed as given under Table I.

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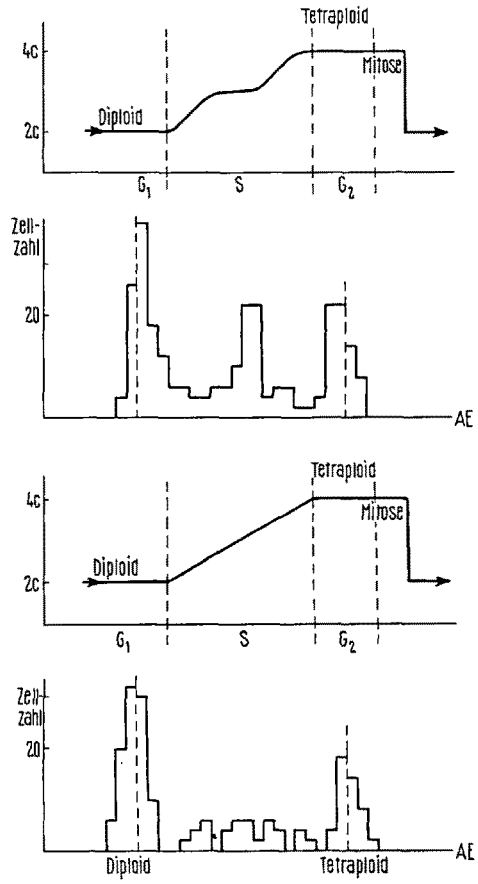
Cytophotometric Studies of the Course of the S Phase in PHA Stimulated Lymphocytes

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 $20.45^0/_{00}$ and $25.5^0/_{00}$, 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 $40.5^0/_{00}$. 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.