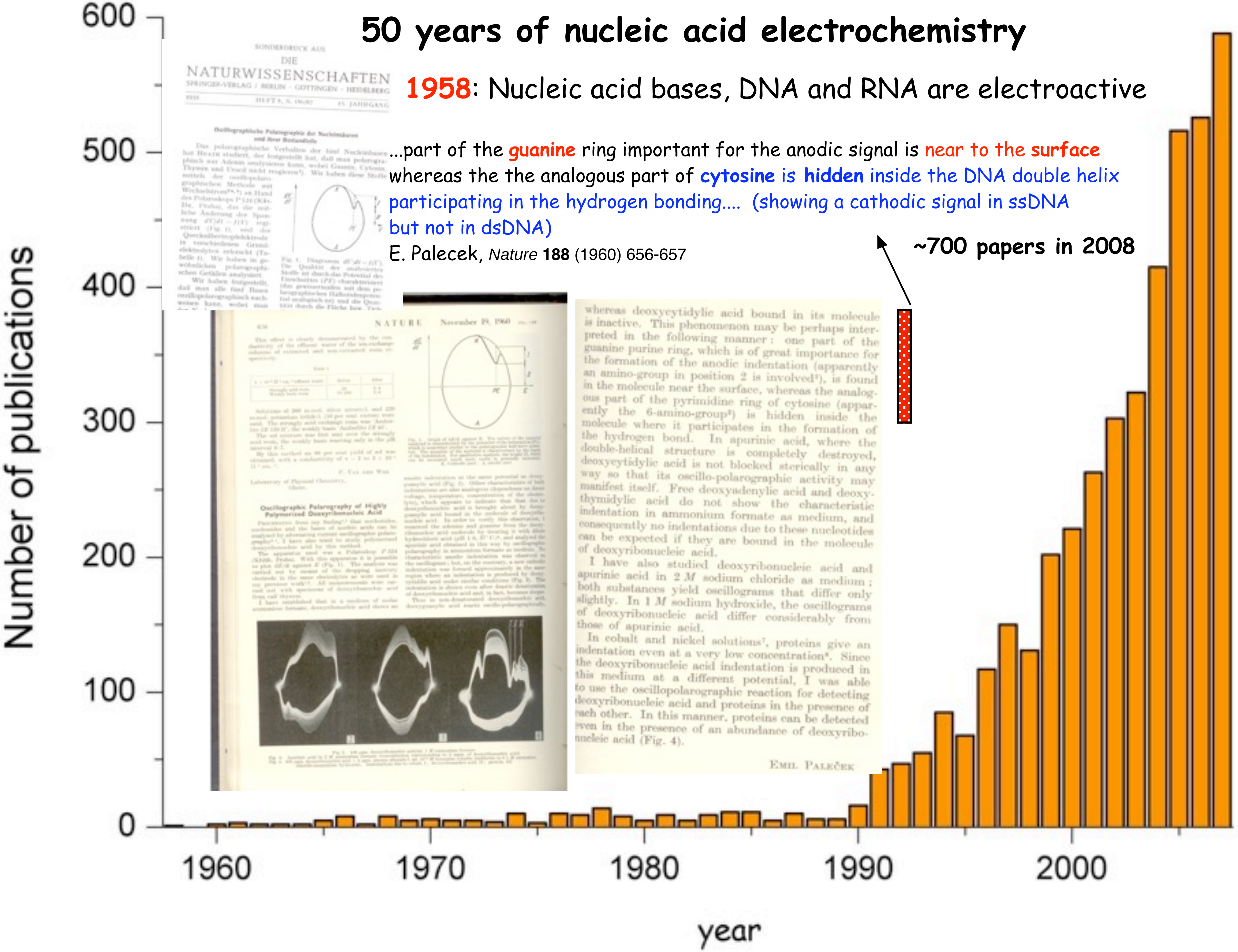
50 years of nucleic acid electrochemistry



1958: Nucleic acid bases, DNA and RNA are electroactive

...part of the **guanine** ring important for the anodic signal is near to the **surface** whereas the the analogous part of **cytosine** is **hidden** inside the DNA double helix participating in the hydrogen bonding.... (showing a cathodic signal in ssDNA but not in dsDNA)

E. Palecek, *Nature* **188** (1960) 656-657

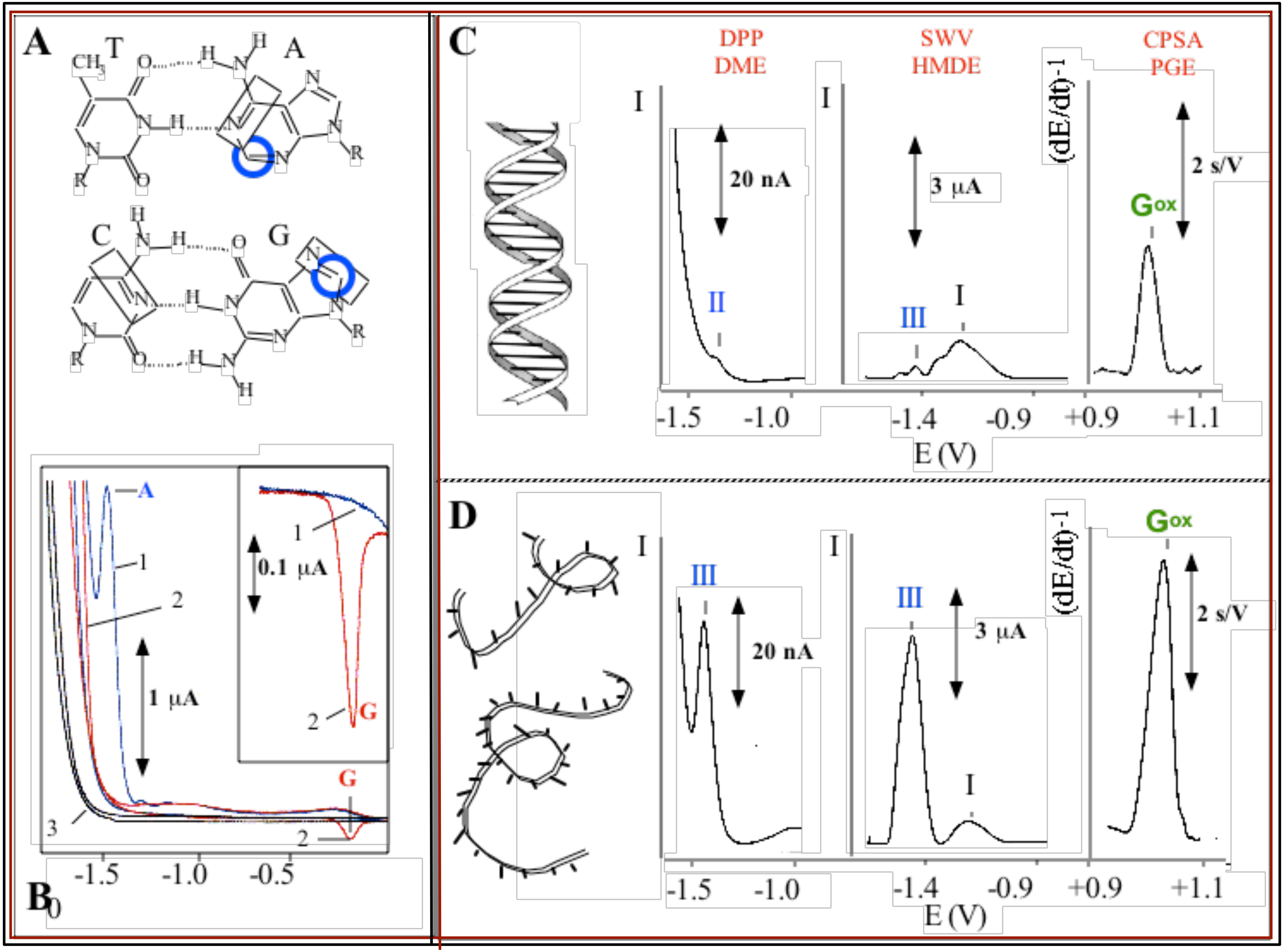
whereas deoxycytidylic acid bound in its molecule is inactive. This phenomenon may be perhaps interpreted in the following manner: one part of the guanine purine ring, which is of great importance for the formation of the anodic indentation (apparently an amino-group in position 2 is involved[3]), is found in the molecule near the surface, whereas the analogous part of the pyrimidine ring of cytosine (apparently the 6-amino-group[3]) is hidden inside the molecule where it participates in the formation of the hydrogen bond. In apurinic acid, where the double-helical structure is completely destroyed, deoxycytidylic acid is not blocked sterically in any way so that its oscillo-polarographic activity may manifest itself. Free deoxyadenylic acid and deoxythymidylic acid do not show the characteristic indentation in ammonium formate as medium, and consequently no indentations due to these nucleotides can be expected if they are bound in the molecule of deoxyribonucleic acid.

I have also studied deoxyribonucleic acid and apurinic acid in 2 *M* sodium chloride as medium; both substances yield oscillograms that differ only slightly. In 1 *M* sodium hydroxide, the oscillograms of deoxyribonucleic acid differ considerably from those of apurinic acid.

In cobalt and nickel solutions[7], proteins give an indentation even at a very low concentration[8]. Since the deoxyribonucleic acid indentation is produced in this medium at a different potential, I was able to use the oscillopolarographic reaction for detecting deoxyribonucleic acid and proteins in the presence of each other. In this manner, proteins can be detected even in the presence of an abundance of deoxyribonucleic acid (Fig. 4).

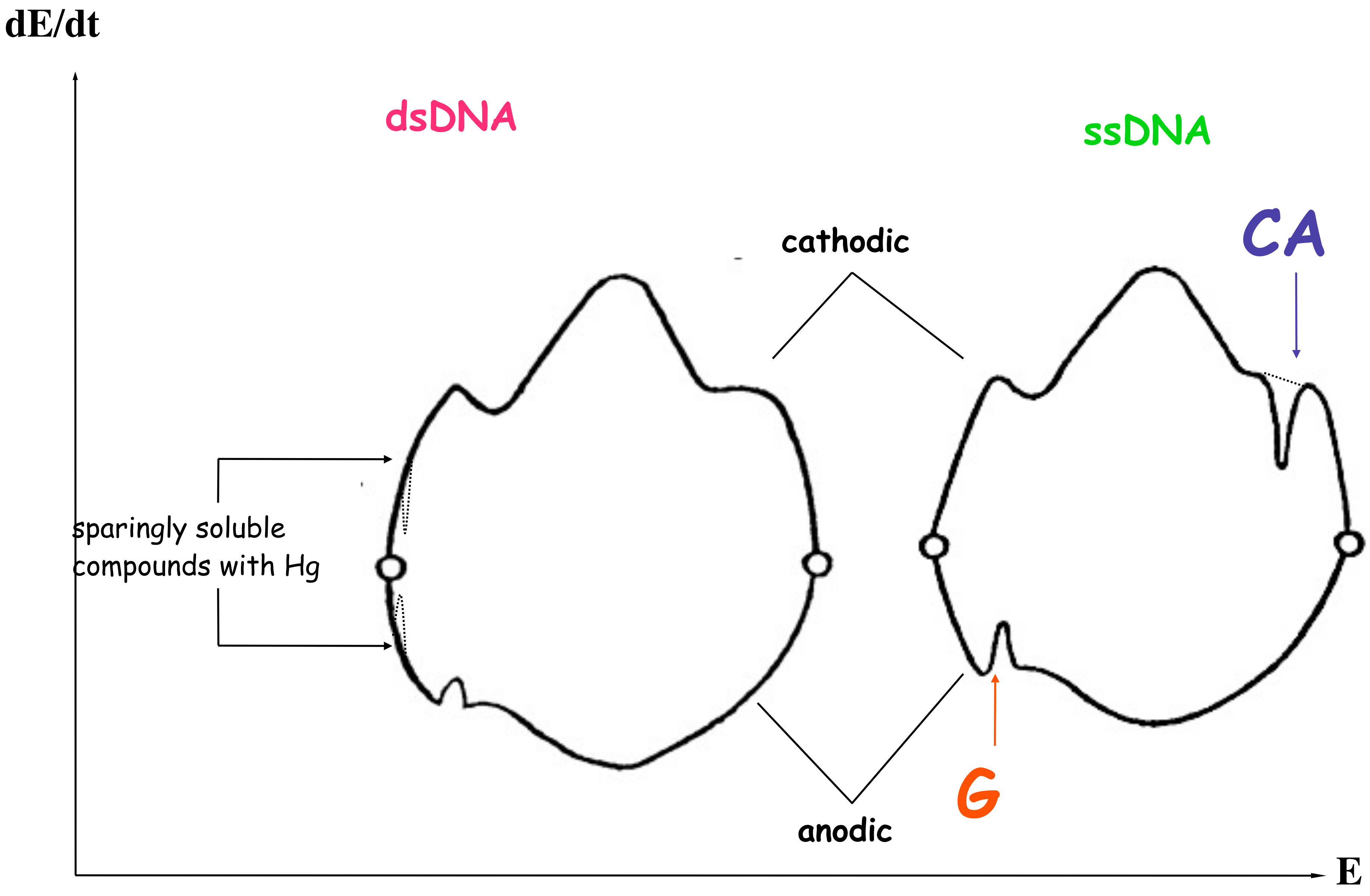
Emil Paleček

E. Palecek, Fifty years of nucleic acid electrochemistry, **Electroanalysis 2009**, **21,** 239-251.


A
CH3
T
O
H
N
H
A
N
N
N
H
N
R
N
R
O
C
H
N
H
O
G
N
N
N
H
N
N
R
N
R
O
H
N
H
B
-A
1
2
1 μA
0.1 μA
1
2
G
G
3
2
-1.5
-1.0
-0.5
0
C
DPP
DME
SWV
HMDE
CPSA
PGE
I
I
(dE/dt)-1
20 nA
3 μA
2 s/V
II
III
I
Gox
-1.5
-1.0
-1.4
-0.9
+0.9
+1.1
E (V)
D
I
I
(dE/dt)-1
III
III
I
20 nA
3 μA
2 s/V
Gox
-1.5
-1.0
-1.4
-0.9
+0.9
+1.1
E (V)

OSCILLOGRAPHIC POLAROGRAPHY

At controlled alternating current (constant current chronopotentiometry)



LITERATURE in 1958: Adenine is polarographically reducible at strongly acid pH while other NA bases as well as DNA are inactive

J.N.Davidson and E.Chargraff: *The Nucleic Acids*, Vol. 1, Academic Press, New York 1955

Palecek E.: Oszillographiche *Polarographie der Nucleinsauren und ihrer Bestandteile*; **Naturwiss**. 45 (**1958**), 186
Palecek E.: Oscillographic *polarography of highly polymerized deoxyribonucleic acid*; **Nature** 188 (**1960**), 656

J. Heyrovsky invented
POLAROGRAPHY in 1922.
After 37 years he was awarded
a Nobel Prize

In difference to most of the electrochemists I met in the 1960's and 1970's, J Heyrovsky was interested in nucleic acids and he greatly stimulated my polarographic studies of DNA

Nobel Prizes 1959


J Heyrovsky S Ochoa A Kornberg

J. Heyrovsky

D.c. polarography vs. oscillopolarography (OP)

Why d.c. polarography was rather poor in DNA analysis?

(a) no DNA accumulation at the electrode
(b) DNA adsorption at negatively charged DME (~-1.4V) compared to open current potential in OP

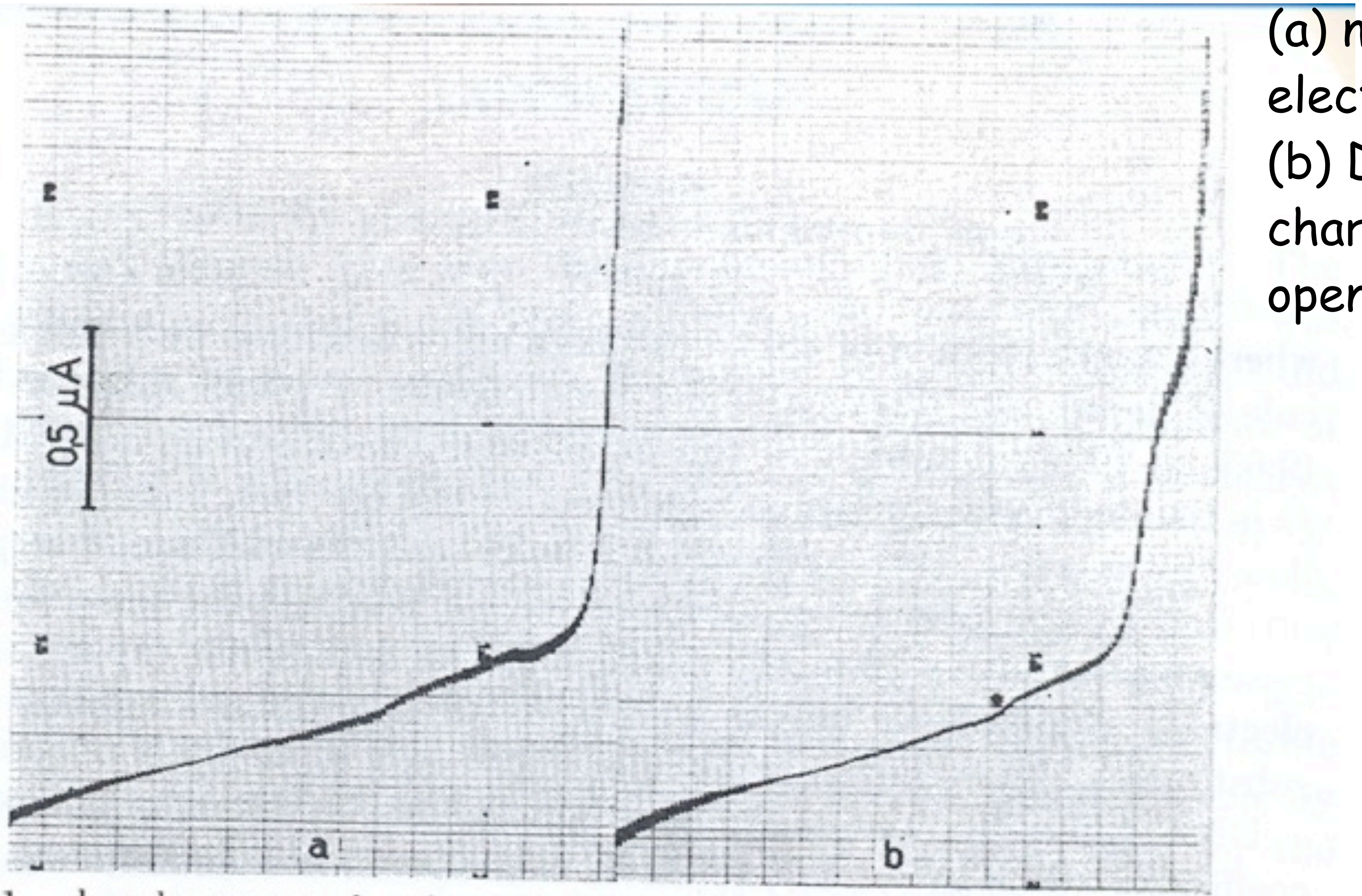


Fig. 1. dc polarograms of native and denatured calf thymus DNA: (*a*) native DNA at a concentration of 500 μg/ml in 0.5*M* ammonium formate with 0.1*M* sodium phosphate (pH 7.0); (*b*) denatured DNA at a concentration of 500 μg/ml in 0.5*M* ammonium formate with 0.1*M* sodium phosphate (pH 7.0). DNA was denatured by heat at the concentration of 666 μg/ml in 0.007*M* NaCl with 0.7 m*M* citrate. Both curves start at 0.0 V, 100 mV/scale unit, capillary I, saturated calomel electrode.

In 1960 when I published my NATURE paper on electrochemistry of DNA I obtained invitations from 3 emminent US scientists:
J. Marmur - Harvard Univ.
L. Grossman - Brandeis Univ.
J. Fresco - Princeton Univ.
To work in their laboratories as a postdoc

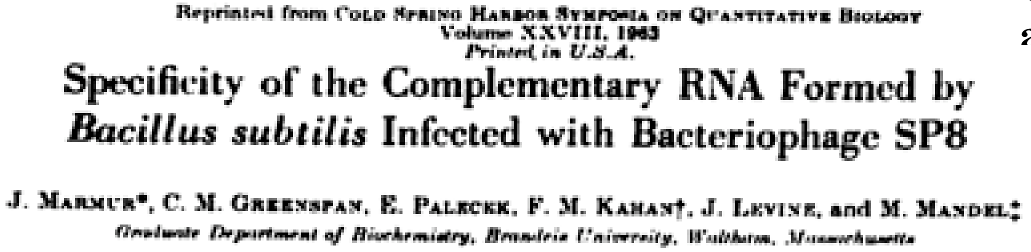
In 1960 new techniques were sought to study DNA Denaturation and Renaturation. To those working with DNA Oscillographic Polarography (OP) appeared as a very attractive tool. Invented by J. Heyrovsky, it was fast and simple, showing large differences between the signals of native and denatured DNA. The instrument for OP was produced only in Czechoslovakia.

I accepted the invitation by Julius Marmur but for more than two years I was not allowed to leave Czechoslovakia. In the meantime JM moved from Harvard to Brandeis Univ. By the end of November 1962 I finally got my exit visa and with Heyrovsky Letter of Reccommendation in my pocket I went to the plane just 24 hours before expiration of my US visa. Before my departure I sent my OP instrument by air to Boston. It arrived after 9 months completely broken. Instead of OP I had to use ultracentrifuges and microbiological methods.

Julius Marmur discovered DNA Renaturation/Hybridization and proposed (in JMB) a new method of DNA isolation which was widely applied. His paper was quoted > 9000x.

J M at the 40th Anniversary of the Discovery of the DNA Double Helix

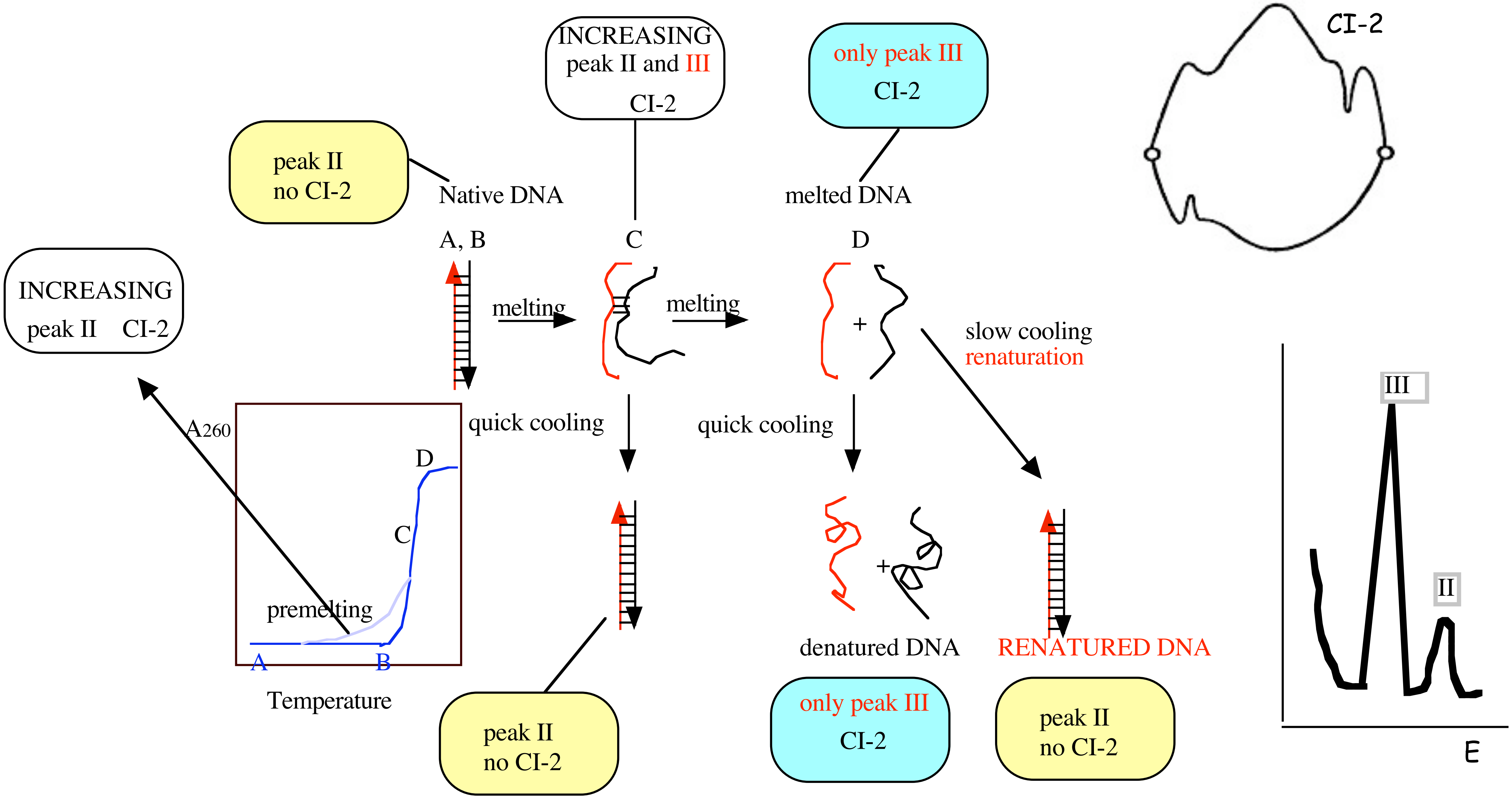
At the end of my stay at Brandeis I did some OP experiments which I finished in Brno and published in J. Mol. Biol. in 1965 and 1966.



Reprinted from Cold Spring Harbor Symposia on Quantitative Biology
Volume XXVIII, 1963
Printed in U.S.A.

Specificity of the Complementary RNA Formed by *Bacillus subtilis* Infected with Bacteriophage SP8

J. Marmur*, C. M. Greenspan, E. Palecek, F. M. Kahan†, J. Levine, and M. Mandel‡
Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts



INCREASING
peak II and III
CI-2

only peak III
CI-2

peak II
no CI-2

Native DNA

melted DNA

INCREASING
peak II CI-2

melting

melting

slow cooling
renaturation

quick cooling

quick cooling

premelting

denatured DNA

RENATURED DNA

peak II
no CI-2

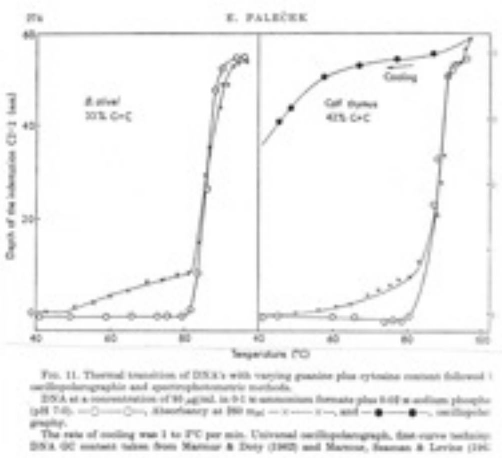
only peak III
CI-2

peak II
no CI-2

DNA Premelting and Polymorphy of the DNA Double Helix

Before my departure to the US I observed Changes in the polarographic behavior of DNA far below the denaturation temperature. These changes were later called DNA Premelting

J. Mol. Biol.
20 (1966) 263-281



POLAROGRAPHIC BEHAVIOR OF dsDNA
At roomand premeltig temperaturse depended on DNA nucleotide SEQUENCE

B. sublilis and B. brevis DNAs have the same G+C content and different nucleotide sequence

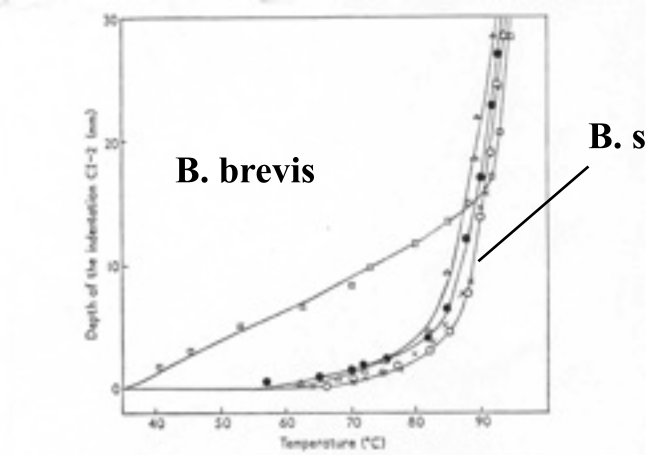
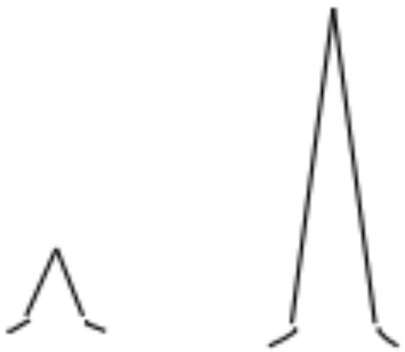


FIG. 12. Thermal transition of DNA's isolated from bacteria of the genus *Bacillus*. DNA at a concentration of 100 µg/ml. in 0·23 M-ammonium formate plus 0·023 M-sodium phosphate (pH 7·0).

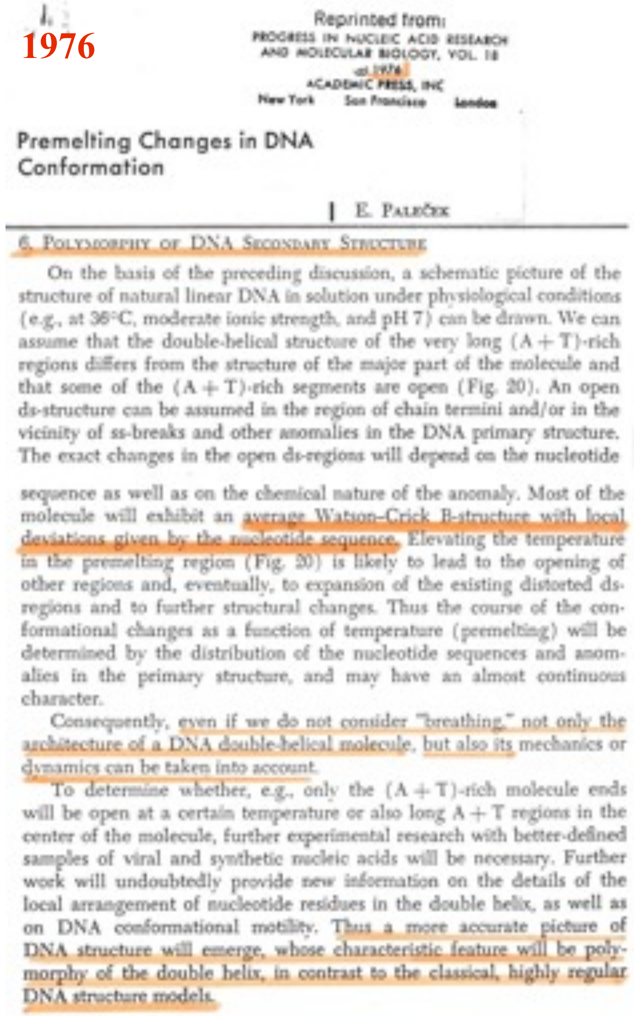
—●——●—, *B. subtilis* 168; —×——×—, *B. natto*; —○——○—, *B. subtilis* var. *niger*; —△——△—, *B. subtilis* var. *aterrimus*; —□——□—, *B. brevis* (ATCC 9999).

F 334 polaroscope, dropping mercury electrode polarized with repeated cycles of a.c. The measurements were carried out in the laboratory of Prof. J. Marmur, Department of Biochemistry, Brandeis University, Waltham, Mass., U.S.A.



poly(dA)·poly(dT)   poly d(A-T)·d(A-T)

Meeting F. Crick in Copenhagen and Arhus, 1977 (B. Clark)



1976

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PROGRESS IN NUCLEIC ACID RESEARCH
AND MOLECULAR BIOLOGY, VOL. 18
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ACADEMIC PRESS, INC
New York San Francisco London

Premelting Changes in DNA Conformation

E. PALEČEK

6. POLYMORPHY OF DNA SECONDARY STRUCTURE

On the basis of the preceding discussion, a schematic picture of the structure of natural linear DNA in solution under physiological conditions (e.g., at 36°C, moderate ionic strength, and pH 7) can be drawn. We can assume that the double-helical structure of the very long (A + T)-rich regions differs from the structure of the major part of the molecule and that some of the (A + T)-rich segments are open (Fig. 20). An open ds-structure can be assumed in the region of chain termini and/or in the vicinity of ss-breaks and other anomalies in the DNA primary structure. The exact changes in the open ds-regions will depend on the nucleotide sequence as well as on the chemical nature of the anomaly. Most of the molecule will exhibit an average Watson–Crick B-structure with local deviations given by the nucleotide sequence. Elevating the temperature in the premelting region (Fig. 20) is likely to lead to the opening of other regions and, eventually, to expansion of the existing distorted ds-regions and to further structural changes. Thus the course of the conformational changes as a function of temperature (premelting) will be determined by the distribution of the nucleotide sequences and anomalies in the primary structure, and may have an almost continuous character.

Consequently, even if we do not consider "breathing," not only the architecture of a DNA double-helical molecule, but also its mechanics or dynamics can be taken into account.

To determine whether, e.g., only the (A + T)-rich molecule ends will be open at a certain temperature or also long A + T regions in the center of the molecule, further experimental research with better-defined samples of viral and synthetic nucleic acids will be necessary. Further work will undoubtedly provide new information on the details of the local arrangement of nucleotide residues in the double helix, as well as on DNA conformational motility. Thus a more accurate picture of DNA structure will emerge, whose characteristic feature will be polymorphy of the double helix, in contrast to the classical, highly regular DNA structure models.

December 3, 1976

Professor Emil Palecek
Institute of Biophysics
Czechoslovak Academy of Sciences
Brno 12, Kralovopolska 135
Czechoslovakia

Dear Professor Palecek,

I do apologise for taking so long to reply to your letter of September 29 and the very interesting review you sent with it. Unfortunately I myself will not be able to attend the Symposium you plan for September, 1977 and my Cambridge colleague Aaron Klug tells me that he too is unable to be present. Had you considered the possibility of asking Dr. Hank Sobell? He has just published in PNAS an account of the other (base-paired) kink and has ideas about premelting conformations. I have no idea whether he would be able to come but should you wish to invite him his address is: Department of Chemistry, The University of Rochester, River Station, Rochester, New York 14627.

Yours sincerely,

Francis Crick

F. H. C. Crick
Ferkauf Foundation Visiting Professor

FHCC:lt

What the people said

Before 1980
No doubt that this electrochemistry must produce artifacts because we know well that the DNA double helix has a unique structure INDEPENDENT of the nucleotide SEQUENCE

After 1980
Is not it strange that such an obscure technique can recognize POLYMORPHY OF THE DNA DOUBLE HELIX?

RENATURATION OF RNA AS DETECTED BY DPP
Time dependence

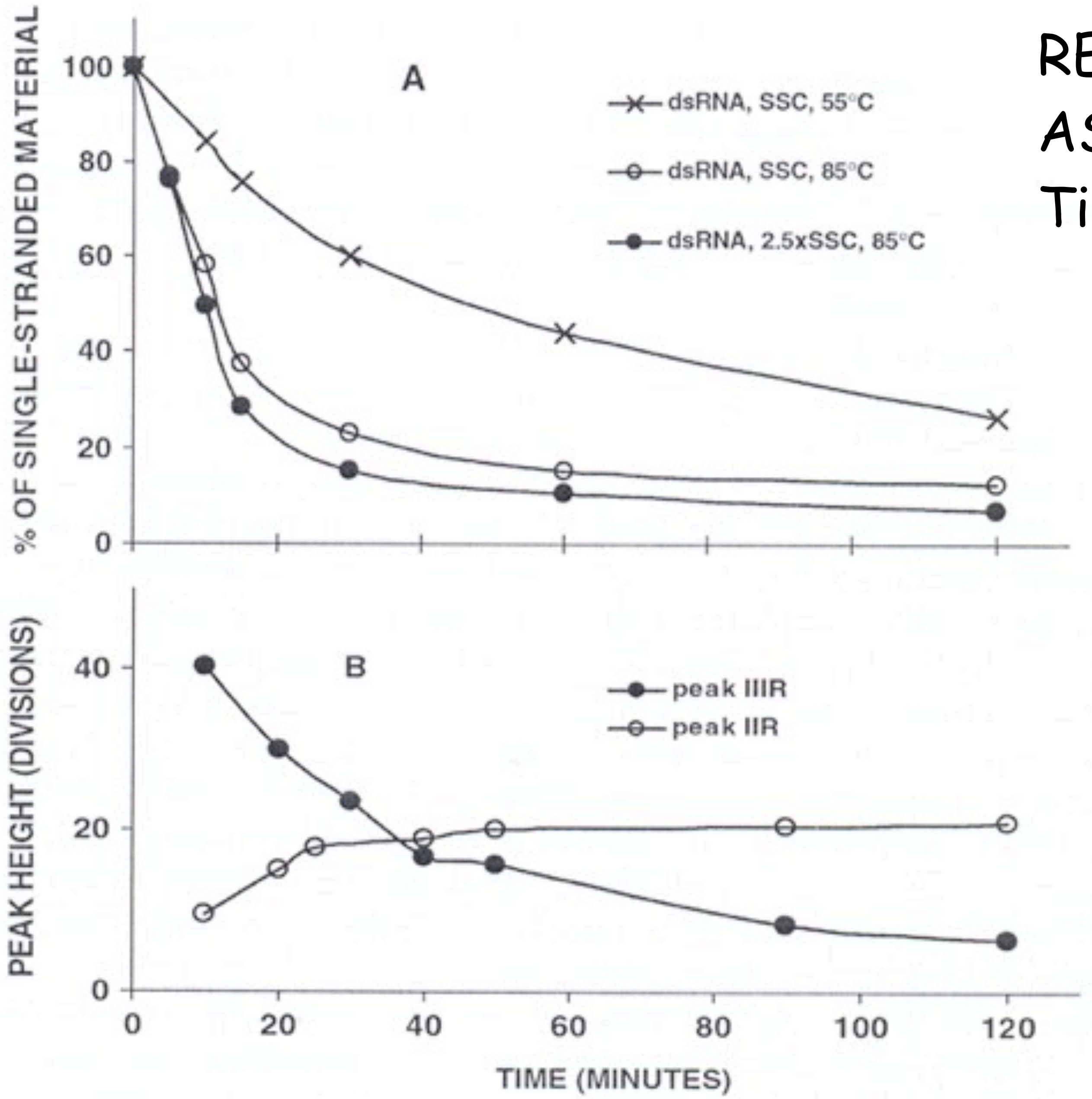


Fig. 10. Time-course of renaturation of phage f2 dsRNA. (A) Thermally denatured ssRNA was incubated (●—●) at 85°C in 2.5 × sodium saline citrate (SSC) or (o—o) at 85°C in SSC, and (x—x) at 55°C. Samples were withdrawn in time intervals given in the graph and quickly cooled. DPP measurements were performed at room temperature at a RNA concentration of 3.2 μg/mL in 0.3 M ammonium formate with 0.2 M sodium acetate, pH 5.6; PAR 174. (B) (o—o) peak IIR. (●—●) peak IIIR. ssRNA (108 μg/mL) in 0.01 × SSC was heated for 6 min at 100°C. Then it was placed into a thermostated polarographic vessel with the same volume of 0.6 M ammonium formate with 0.2 M sodium phosphate, pH 7, preheated to 58°C. The pulse polarograms were measured at 58°C in times given in the graph. Southern–Harwell A 3100, amplifier sensitivity 1/8. Adapted from Palecek and Doskocil (1974). Copyright 1974, with permission from Academic Press.

Firsts in Electrochemistry of Nucleic Acids during the initial three decades

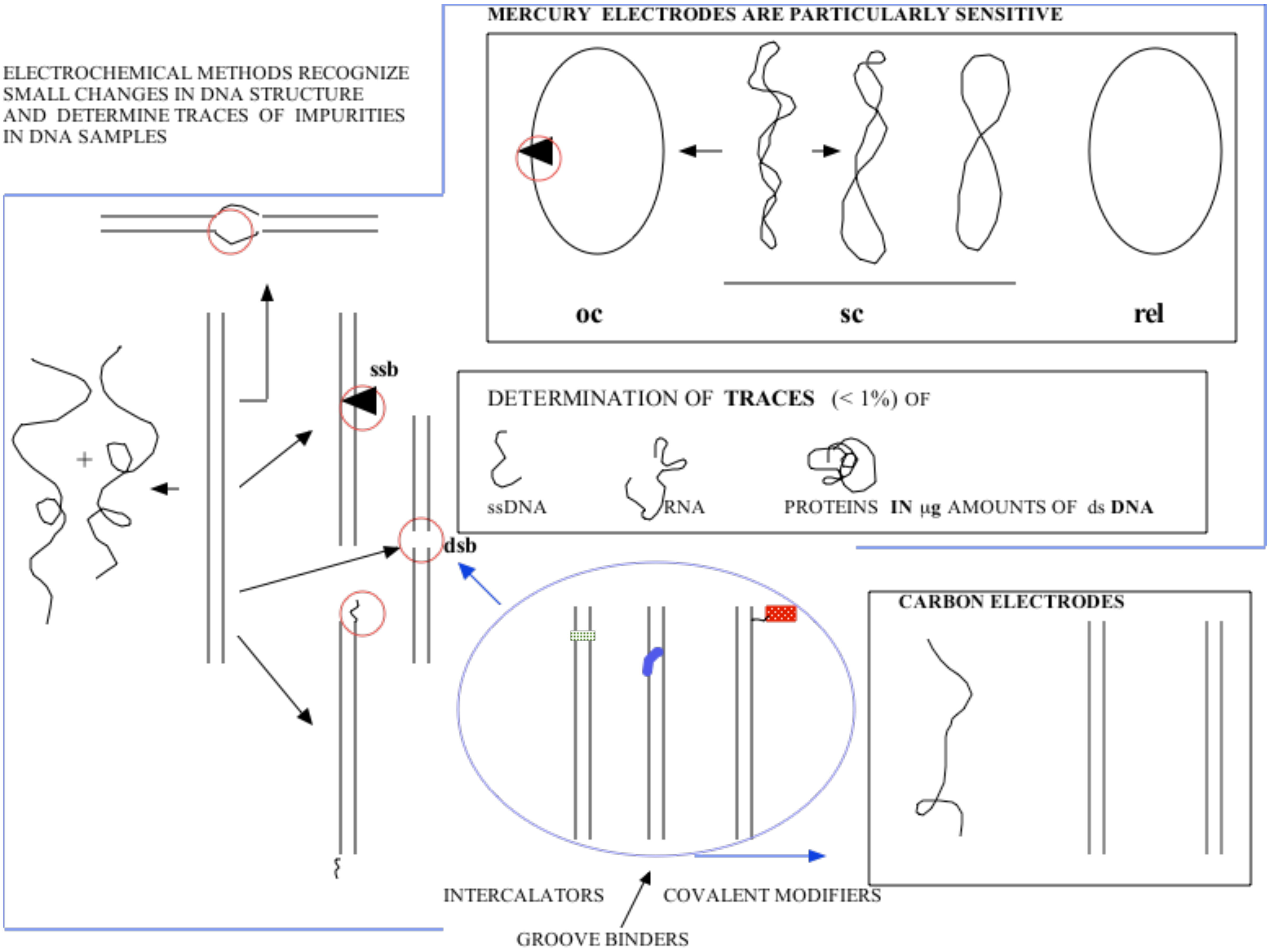
1958 DNA and RNA and all free bases are electrotractive
1960-61 assignment of DNA electrochemical signals to bases, relation between the **DNA structure** and electrochemical responses
1961 adsorption (ac impedance) studies of DNA (IR Miller, Rehovot)
1962-66 DNA premelting, denaturation, renaturation/hybridization detected electrochemically, traces of single stranded DNA determined in native dsDNA. Nucleotide sequence affects dsDNA responses
1965 Association of bases at the electrode surface (V. Vetterl)
1966 application of pulse polarography to DNA studies
1967 detection of DNA damage
1967-68 Weak interactions of low m.w. compounds with DNA (P.J. Hilsson, M.J. Simons, Harrow, UK and H. Berg, Jena)
1974 DNA is unwound at the electrode surface under certain conditions (EP and H.W. Nürnberg, Jülich, independently)
1976 Evidence for polymorphy of the DNA double-helical structure

For two decades only mercury electrodes were used in NA electrochemistry

1978 Solid (carbon) electrodes introduced in nucleic acid research (V. Brabec and G. Dryhurst, Norman)
1980 Determination of bases at nanomolar concentrations by cathodic stripping
1981-83 Electroactive markers covalently bound to DNA
1986-88 DNA-modified electrodes

Results obtained at: IBP, Brno or elsewhere (author's name is given); the results which have been utilized in the DNA sensor development are in **blue**

ELECTROCHEMICAL METHODS RECOGNIZE SMALL CHANGES IN DNA STRUCTURE AND DETERMINE TRACES OF IMPURITIES IN DNA SAMPLES



MERCURY ELECTRODES ARE PARTICULARLY SENSITIVE

DETERMINATION OF **TRACES** (< 1%) OF

PROTEINS **IN** μg AMOUNTS OF ds **DNA**

CARBON ELECTRODES

INTERCALATORS

COVALENT MODIFIERS

GROOVE BINDERS

DNA unwinding at negatively charged surfaces

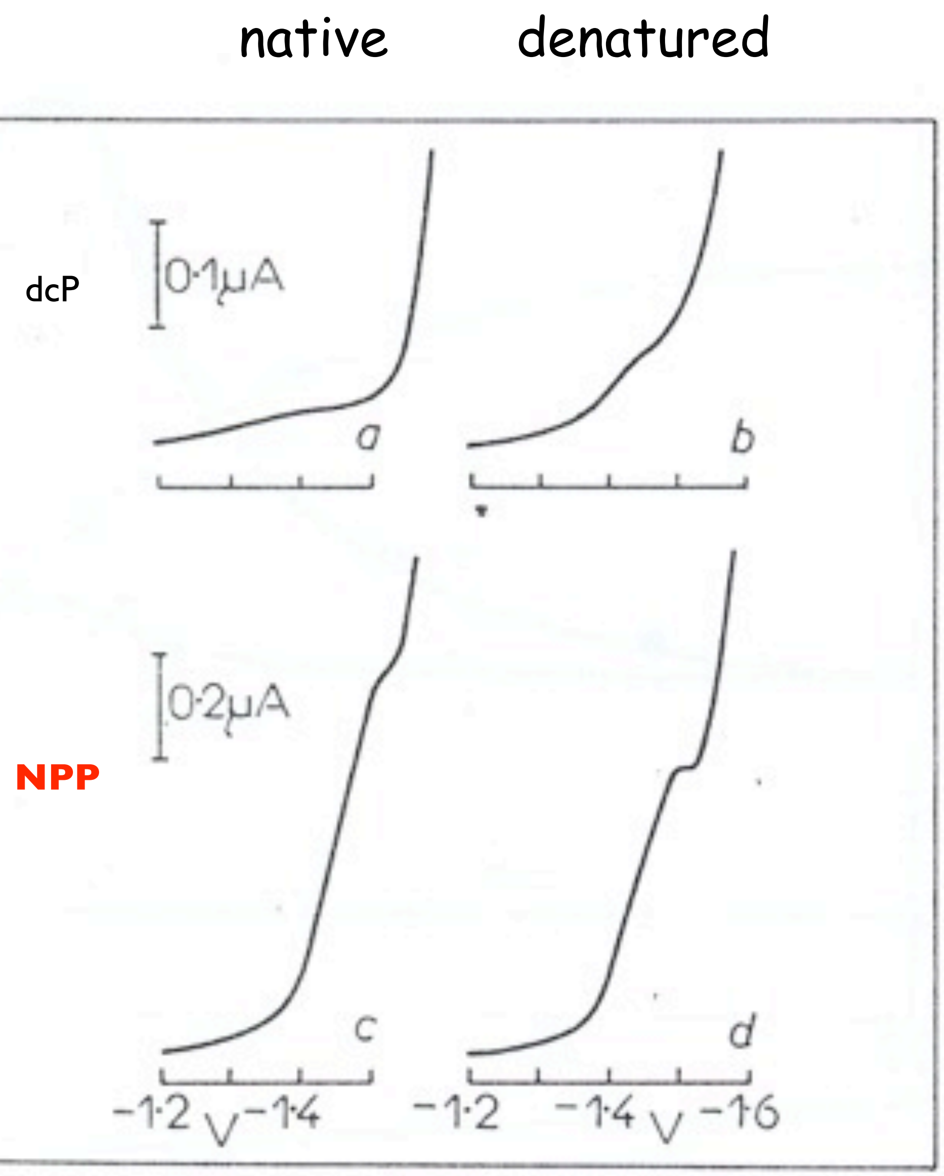


FIG. 1

Polarograms of Native and Denatured DNA

Upper curves: current-sampled d.c. polarography; lower curves: normal pulse polarography. *a*, *c* native DNA 500 μg/ml; *b*, *d* denatured DNA 50 μg/ml. 0·6M ammonium formate with 0·1M sodium phosphate pH 6·8. PAR 174.

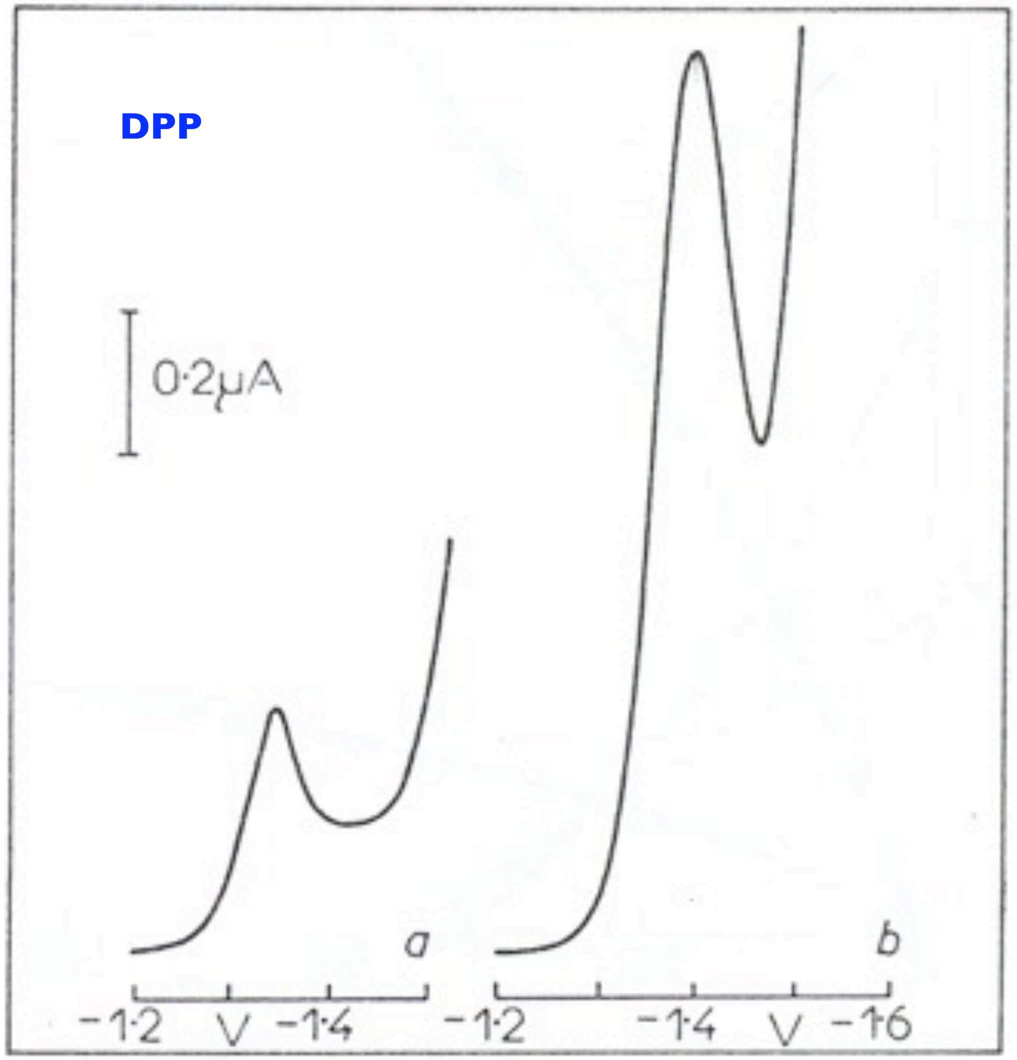


FIG. 2

Derivative Pulse Polarograms of Native and Denatured DNA

a Native DNA 500 μg/ml; *b* denatured DNA 50 μg/ml. Other conditions as in Fig. 1.

1974

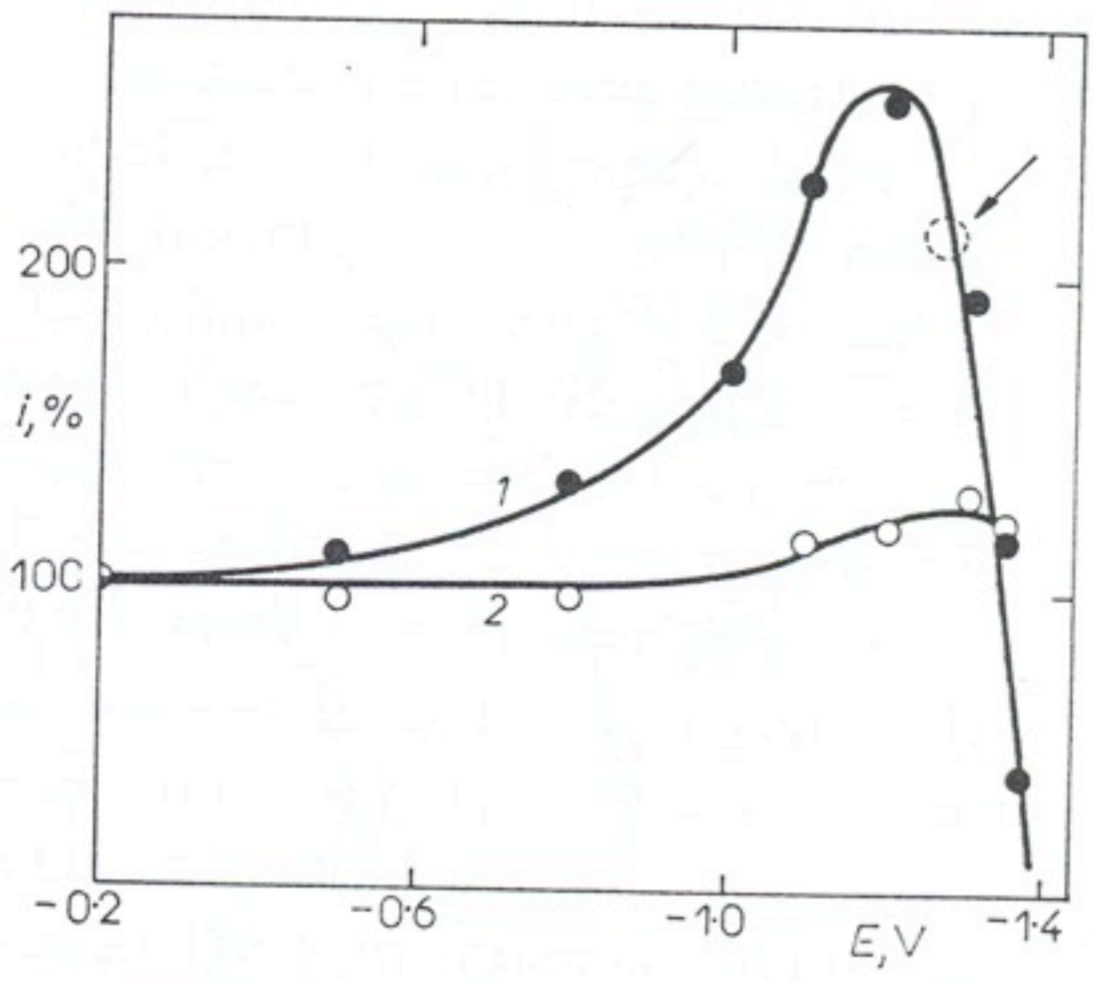
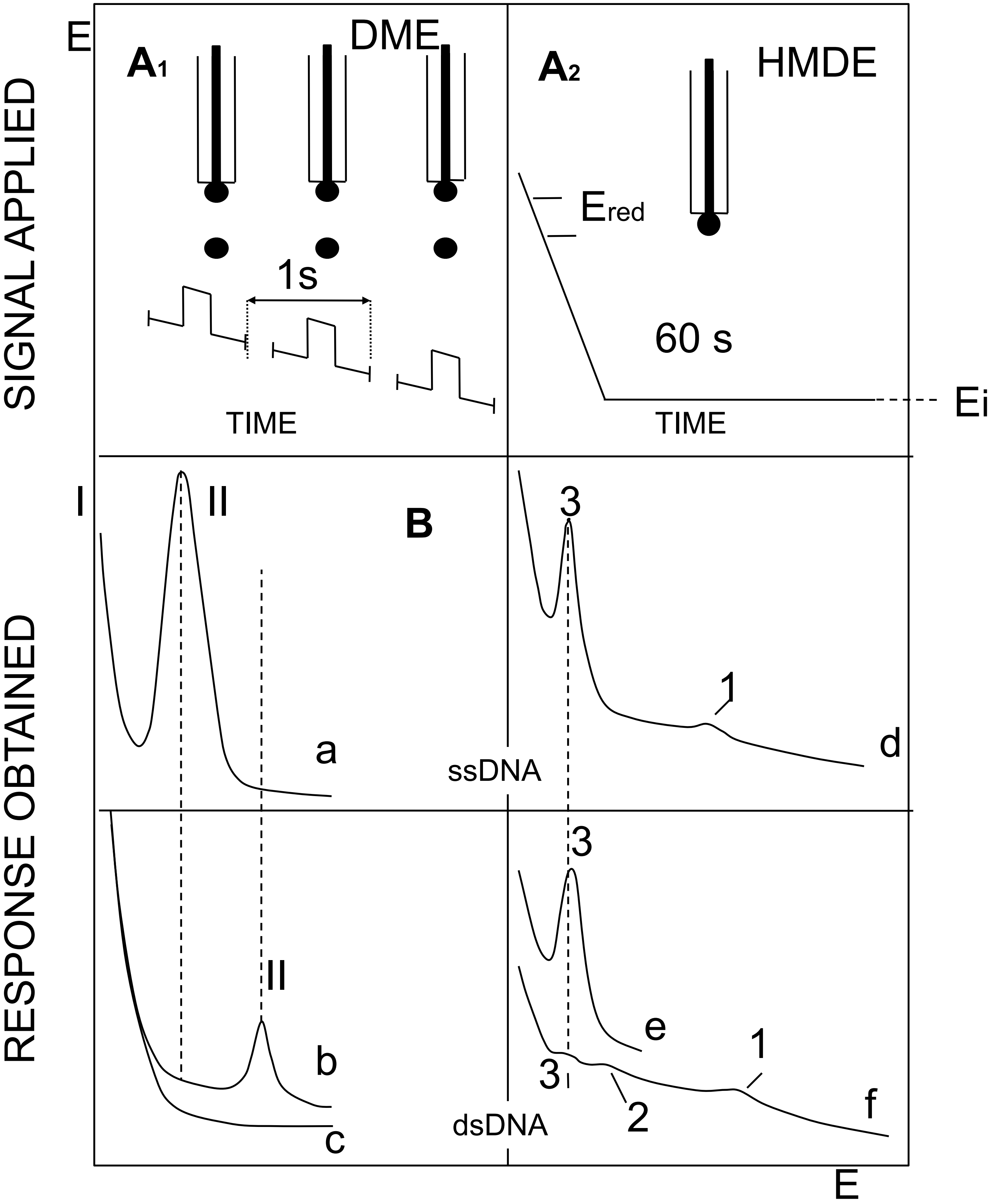


FIG. 4

Dependence of the Normal Pulse-Polarographic Wave Height of DNA on Starting Potential

1 Native DNA 500 µg/ml; *2* denatured DNA 50 µg/ml. The wave heights of native and denatured DNA at a starting potential of −0·2 V were taken as 100%. Scan range 1·5 V, other conditions as in Fig. 1.

In native DNA its NPP responses depended on the initial potential, Ei


SIGNAL APPLIED
RESPONSE OBTAINED
E
A1
DME
1s
TIME
A2
HMDE
Ered
60 s
Ei
TIME
I
II
B
a
ssDNA
3
1
d
3
II
b
c
e
3
2
1
f
dsDNA
E

Effect of pH on DNA unwinding

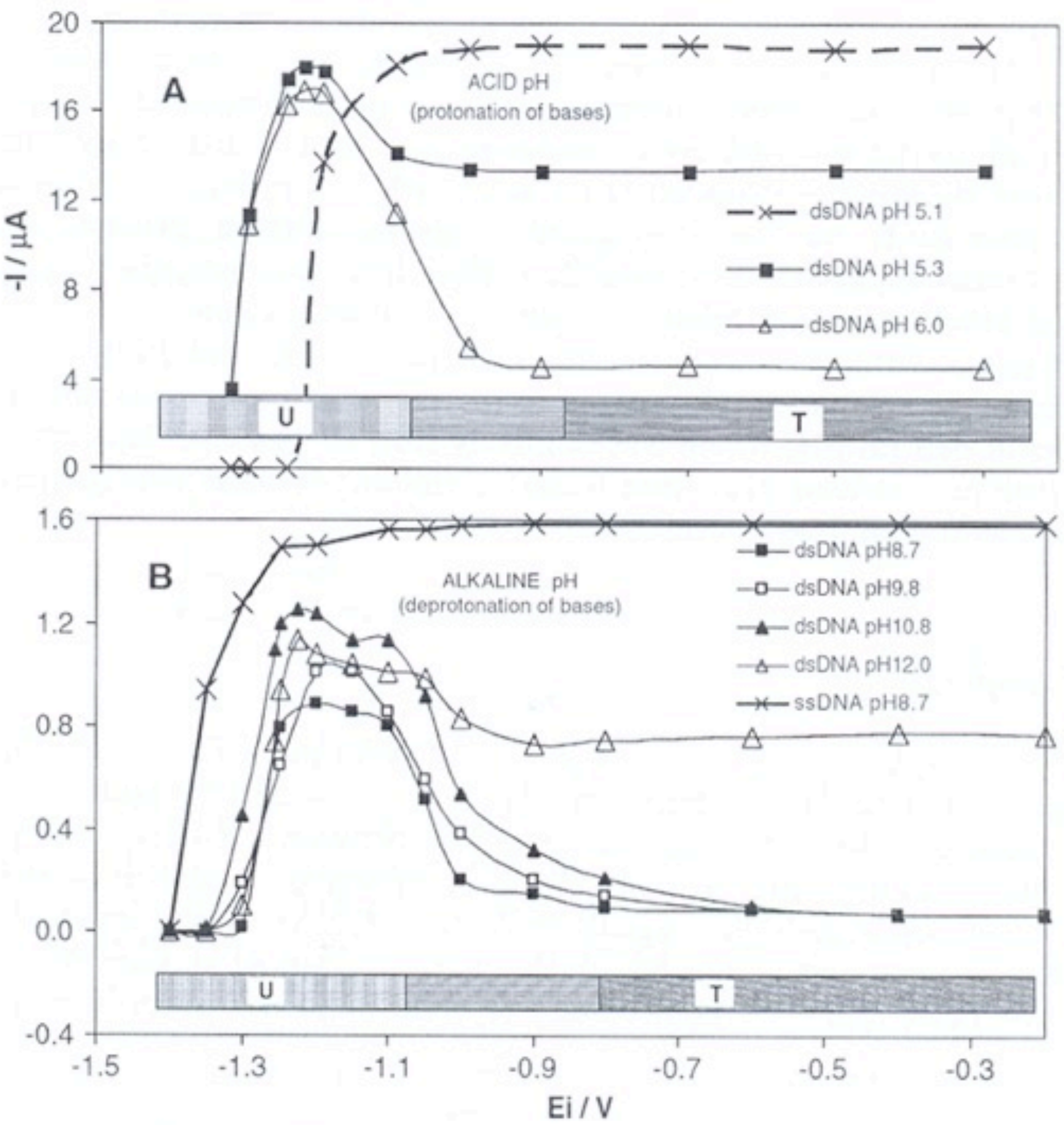


Fig. 17. Dependence of the height of the DNA voltammetric peak 3 on initial potential E_i (A) at acid pHs. dsDNA at concentration of 420 µg/mL: △—△, pH 6.0; ■—■, pH 5.3; x—x, pH 5.1. The graphical indication of the region T and U is valid only for the curve of dsDNA at pH 6.0. (B) at alkaline pH's. dsDNA: ■—■, pH 8.7; □—□, pH 9.8; ▲—▲, pH 10.8; △—△, pH 12.0. ssDNA: x—x, pH 8.7. PAR 174, DME, LSV, scan rate 5 V/s, waiting time 60 s. Potentials were measured against SCE. Adapted from Brabec and Palecek (1976b) and Palecek (1983). Copyright 1976, with permission from John Wiley and Sons Ltd.

Effect of nucleotide sequence on DNA unwinding

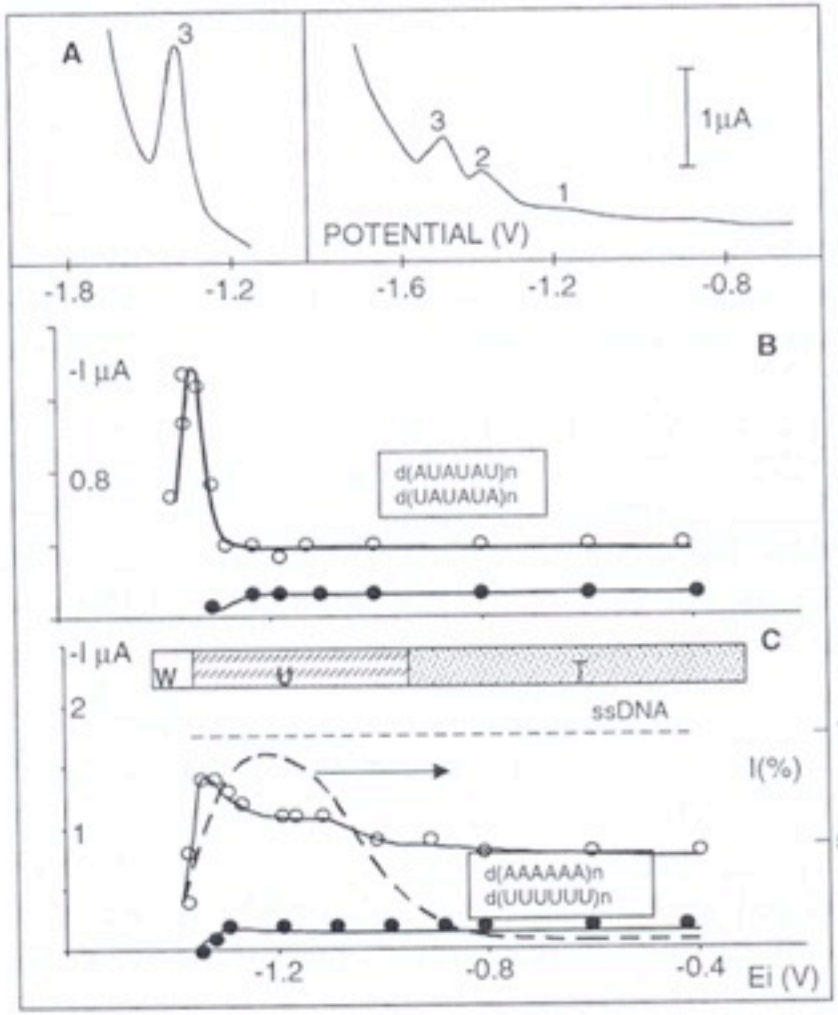
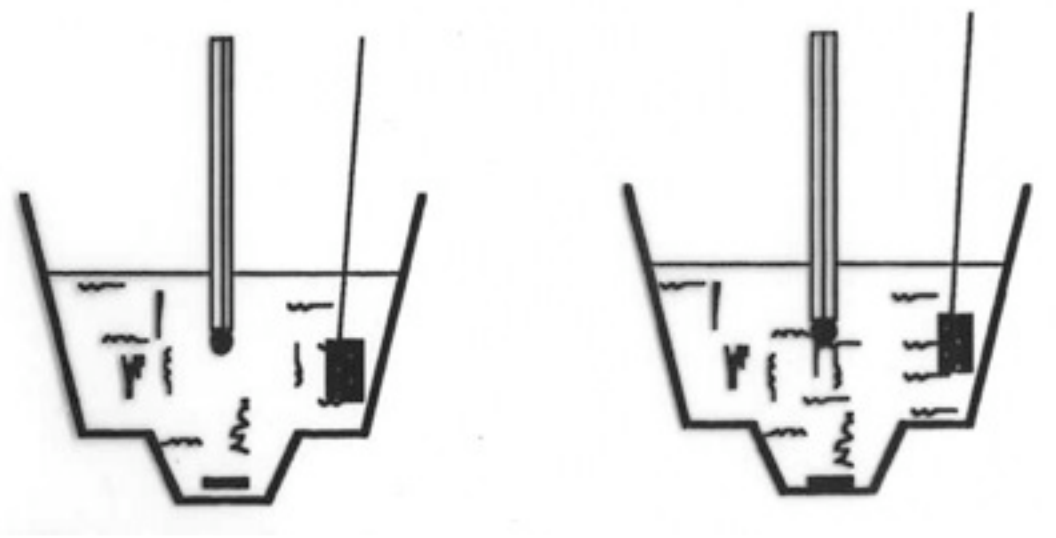


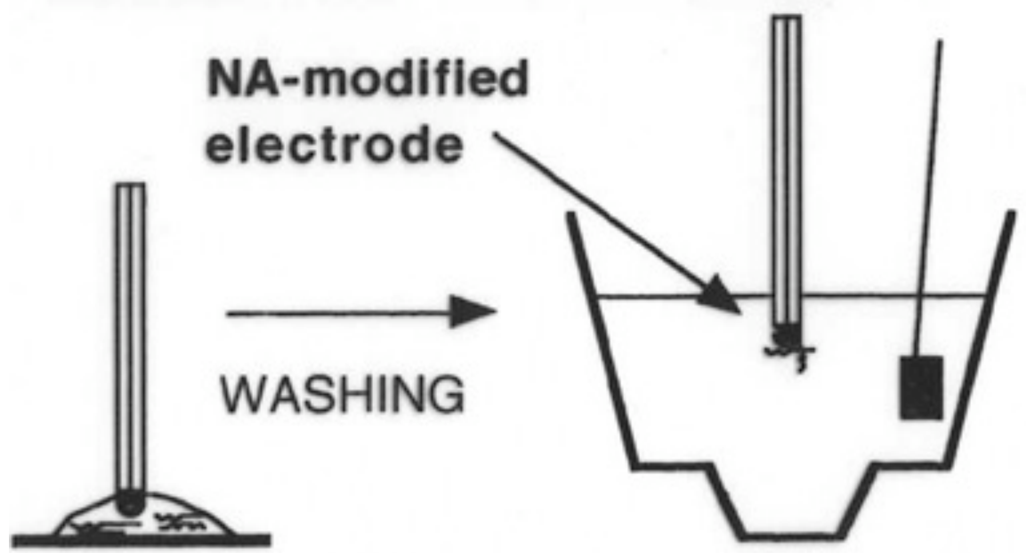
Fig. 16. Dependence of the voltammetric behavior of biosynthetic polynucleotides with different nucleotide sequences on the initial potential (E_i). (A): voltammetric peaks of poly (dA–dU) · poly (dA–dU). $E_i = -0.6$ V (left), $E_i = -1.35$ V (right); (B): ●—● , peak 2; ○—○, peak 3; (C): poly (rA) · poly (rU), ●—●, peak 2; ○—○, peak 3; - - - -, calf thymus DNA (data extracted from Palecek and Kwee (1979), peak height expressed in percents of the height of peak of thermally denatured DNA. DNA at a concentration of 100 μg/mL, concentration of other polynucleotides was 5×10^{-5} M (related to phosphorus content). Background electrolyte: 0.3 M ammonium formate with 0.05 M sodium phosphate (pH 6.9). HMDE, scan rate 0.5 V/s, waiting time 60 s. U is the potential region in which relatively slow opening of the DNA double helix occurs, involving an appreciable part of the molecule (provided the time of DNA interaction with the electrode is sufficiently long). T is the potential region where fast opening of the DNA double helix takes place; it is limited to several percents of the molecule in the vicinity of certain anomalies in the DNA primary structure (e.g. single-strand breaks). W is the potential region where no changes in the DNA conformation were detected. Potentials were measured against SCE. Reproduced from Jelen and Palecek (1985). Copyright 1985, with permission from the Slovak Academy of Sciences.

ADSORPTIVE STRIPPING



NA is in the electrolytic cell and accumulates at the electrode surface during waiting

ADSORPTIVE TRANSFER STRIPPING



NA is attached to the electrode from a small drop of solution (3-10 μl)

NA is at the electrode but the electrolytic cell contains only blank electrolyte

In 1986 we proposed **Adsorptive Transfer Stripping Voltammetry (AdTSV)** based on easy preparation of DNA-modified electrodes
AdTSV has many advatages over conventional voltammetry of NAs:
1) Volumes of the analyte can be reduced to few microliters
2) NAs can be immobilized at the electrode surface from media not suitable for the voltammetric analysis
3) Low m.w. compounds (interfering with conventional electrochemical analysis of NAs) can be washed away
4) Interactions of NAs immobilized at the surface with proteins and other substances in solution and influence of the surface charge on NA properties and interactions can be studied, etc.

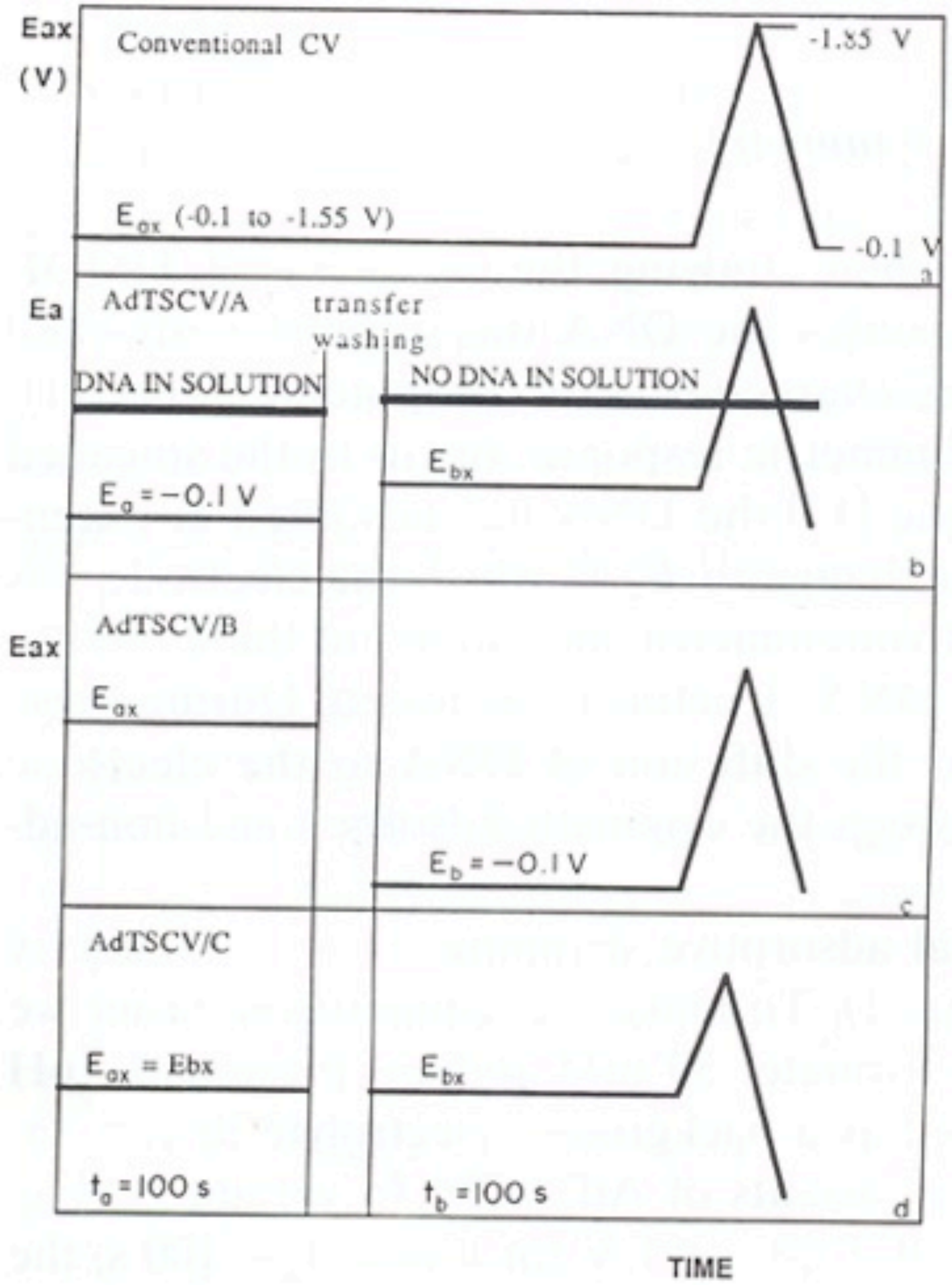


Fig. 1. Schematic diagram of HMDE polarization in (a) conventional (adsorptive stripping) CV and (b–d) variants A, B and C of AdTSCV. (b) AdTSCV variant A: the HMDE charged to a potential $E = -0.1$ V was immersed in a DNA solution for a time $t = 100$ s, the electrode was then washed and transferred to the background electrolyte (0.3 M ammonium formate with 50 mM sodium phosphate, pH 6.9 not containing DNA, medium 0). A potential E (varying in the range between -0.1 V and -1.55 V) was then applied to the HMDE for $t = 100$ s followed by a triangular voltage sweep in the cathodic direction from E to -1.85 V and back in the anodic direction to -0.1 V. (c) AdTSCV variant B: this variant differs from variant A in that DNA is adsorbed at potentials E (varying between -0.1 V and -1.55 V) and kept in medium 0 at $E = -0.1$ V. (d) AdTSCV variant C: in contrast to variant B both potentials E_{ax} and E_{bx} were variable but they were always the same in a given experiment. This variant thus resembles conventional CV (a) where the HMDE was kept for $t_a = 200$ s at the potential E_{ax} followed by CV measurements during which the electrode was immersed in the DNA solution.

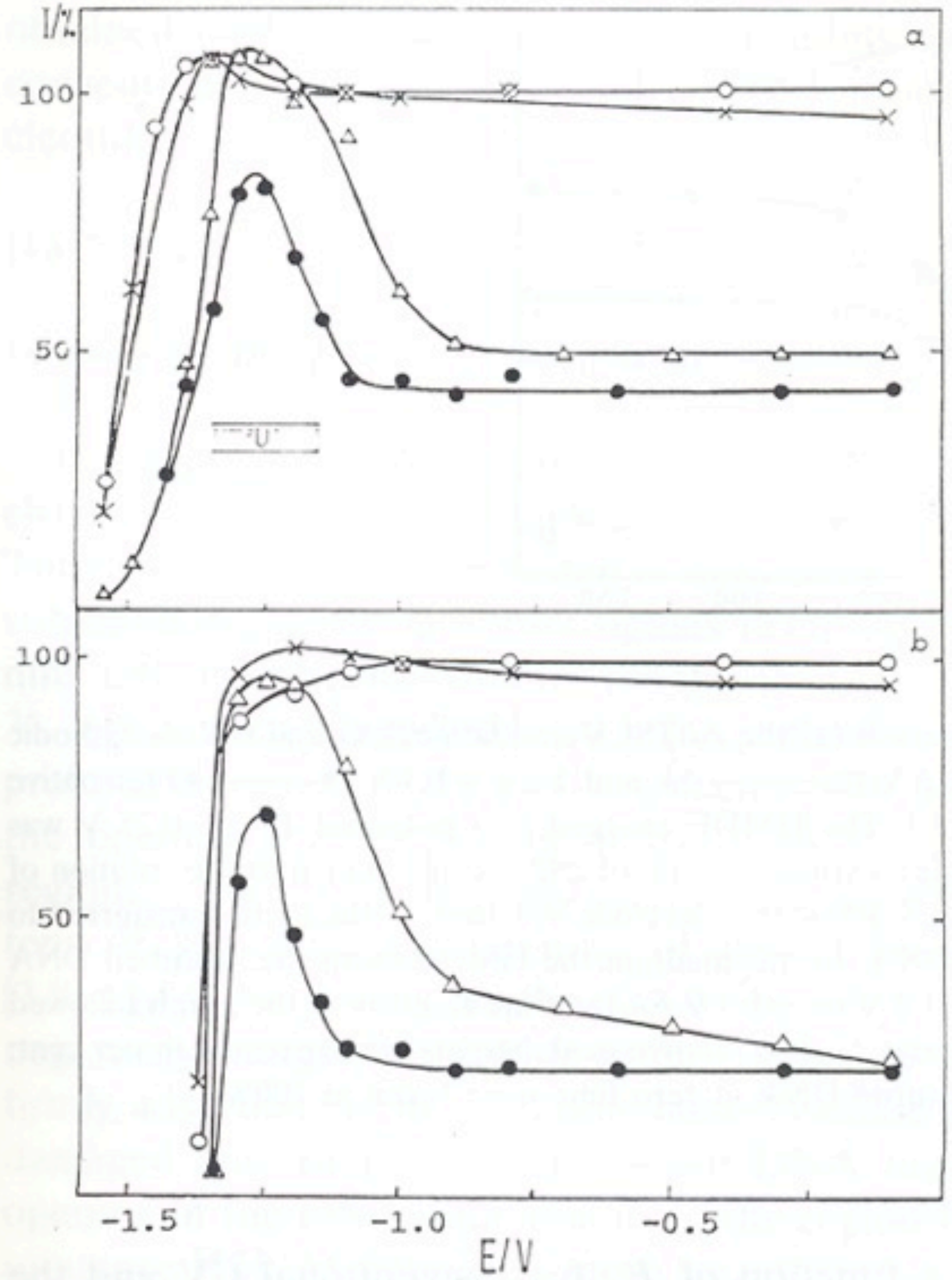
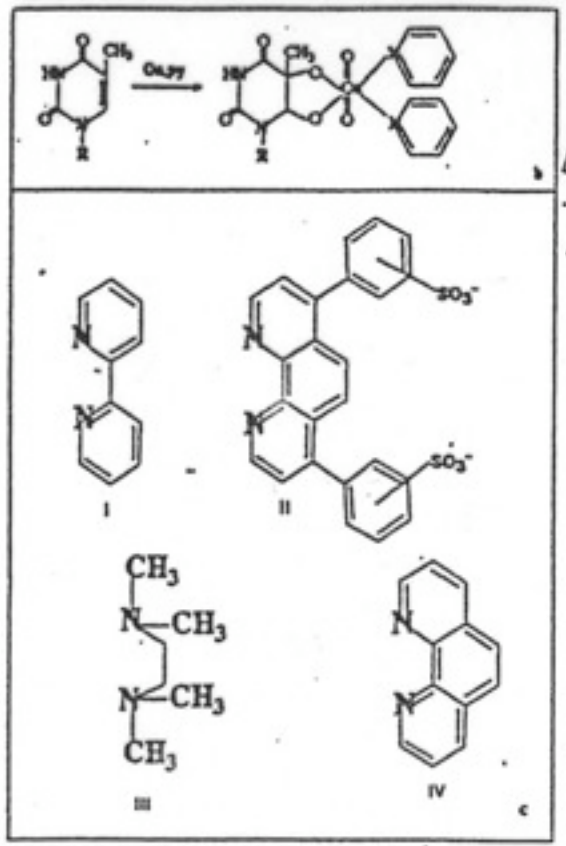
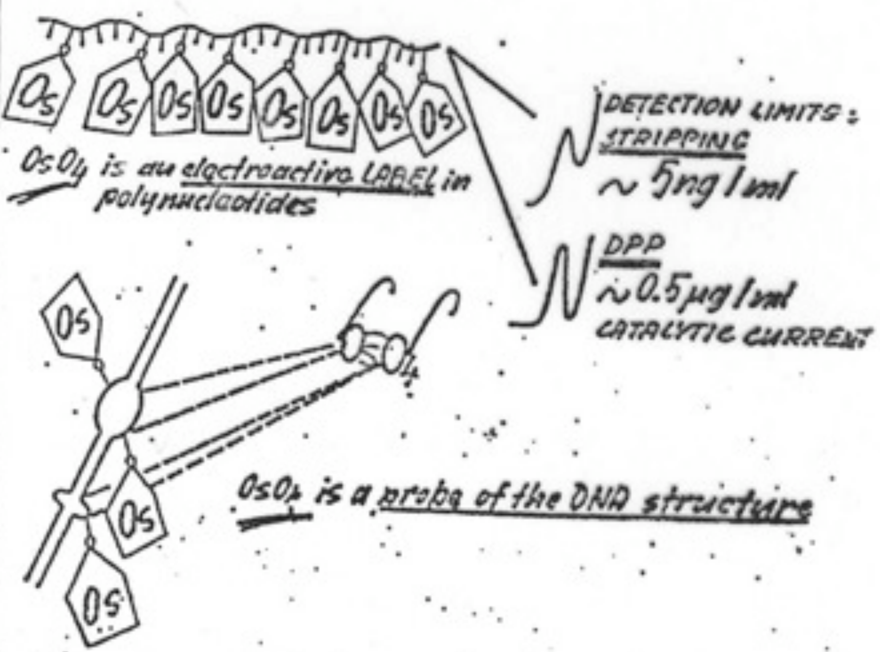


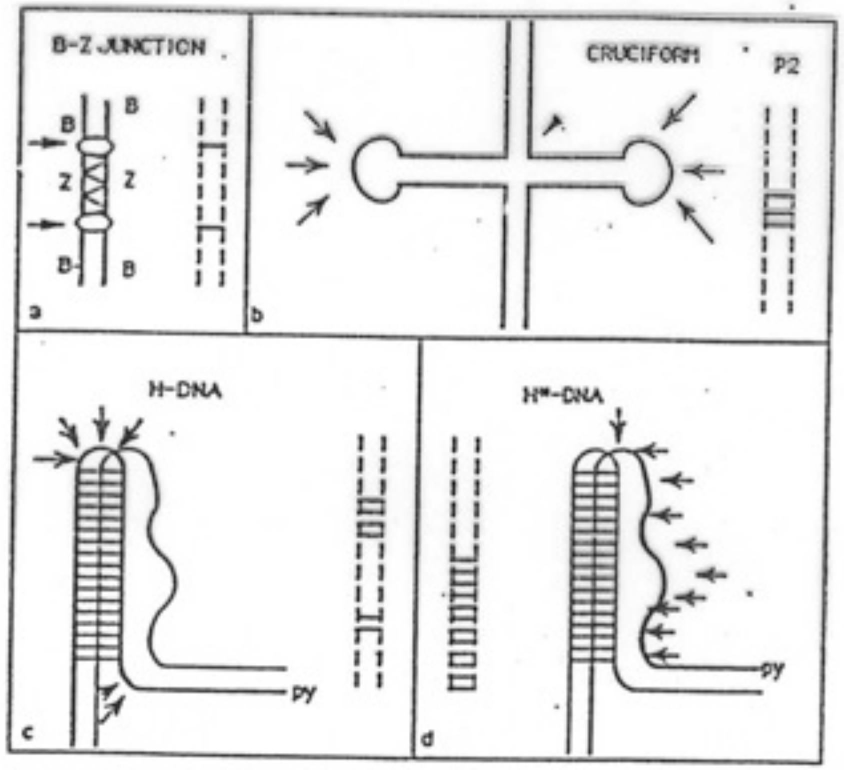
Fig. 5. The dependence of the relative peak heights of (a) the anodic peak G and (b) the cathodic peak AC of native (△——△, ●——●) and denatured (×——×, ○——○) DNA on the HMDE potential obtained by conventional CV (△——△, ○——○) and by AdTSCV variant A (●——●, ×——×) (for details see Figs. 1 and 2). The relative peak heights are expressed in per cent; the height of the peak of thermally denatured DNA obtained by conventional CV at $E_a = -0.1$ V was taken as 100% Region U is shown for AdTSCV variant A.

Probing of DNA structure with osmium tetroxide complexes

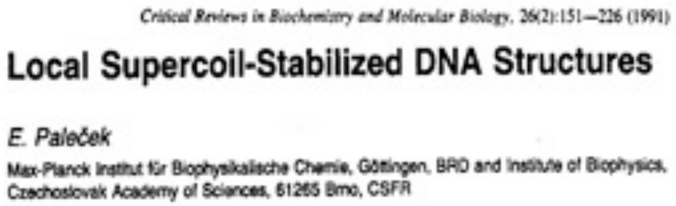




We developed methods of chemical probing of the DNA structure based on osmium tetroxide complexes (Os,L). Some of the Os,L complexes react with single-stranded DNA but not with the double-stranded B-DNA.

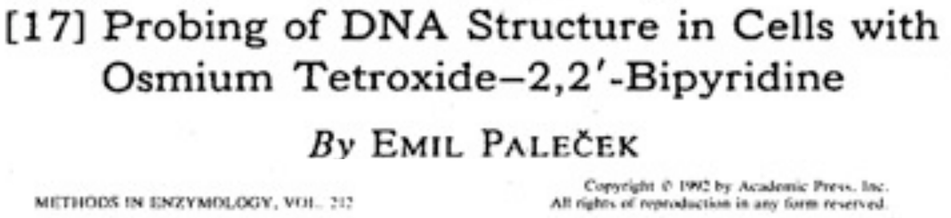


In the beginning of the 1980‘s Os,L complexes were the first electroactive labels covalently bound to DNA. These complexes produced catalytic signals at Hg electrodes allowing determination of DNA at subnanomolar concentrations


Critical Reviews in Biochemistry and Molecular Biology, 26(2):151—226 (1991)

Local Supercoil-Stabilized DNA Structures

E. Paleček
Max-Planck Institut für Biophysikalische Chemie, Göttingen, BRD and Institute of Biophysics, Czechoslovak Academy of Sciences, 61265 Brno, CSFR


[17] Probing of DNA Structure in Cells with Osmium Tetroxide–2,2′-Bipyridine

By Emil Paleček

METHODS IN ENZYMOLOGY, VOL. 212

These methods yielded information about the distorted and single-stranded regions in the DNA double helix at single-nucleotide resolution. DNA probed both in vitro and directly in cells.

peak G

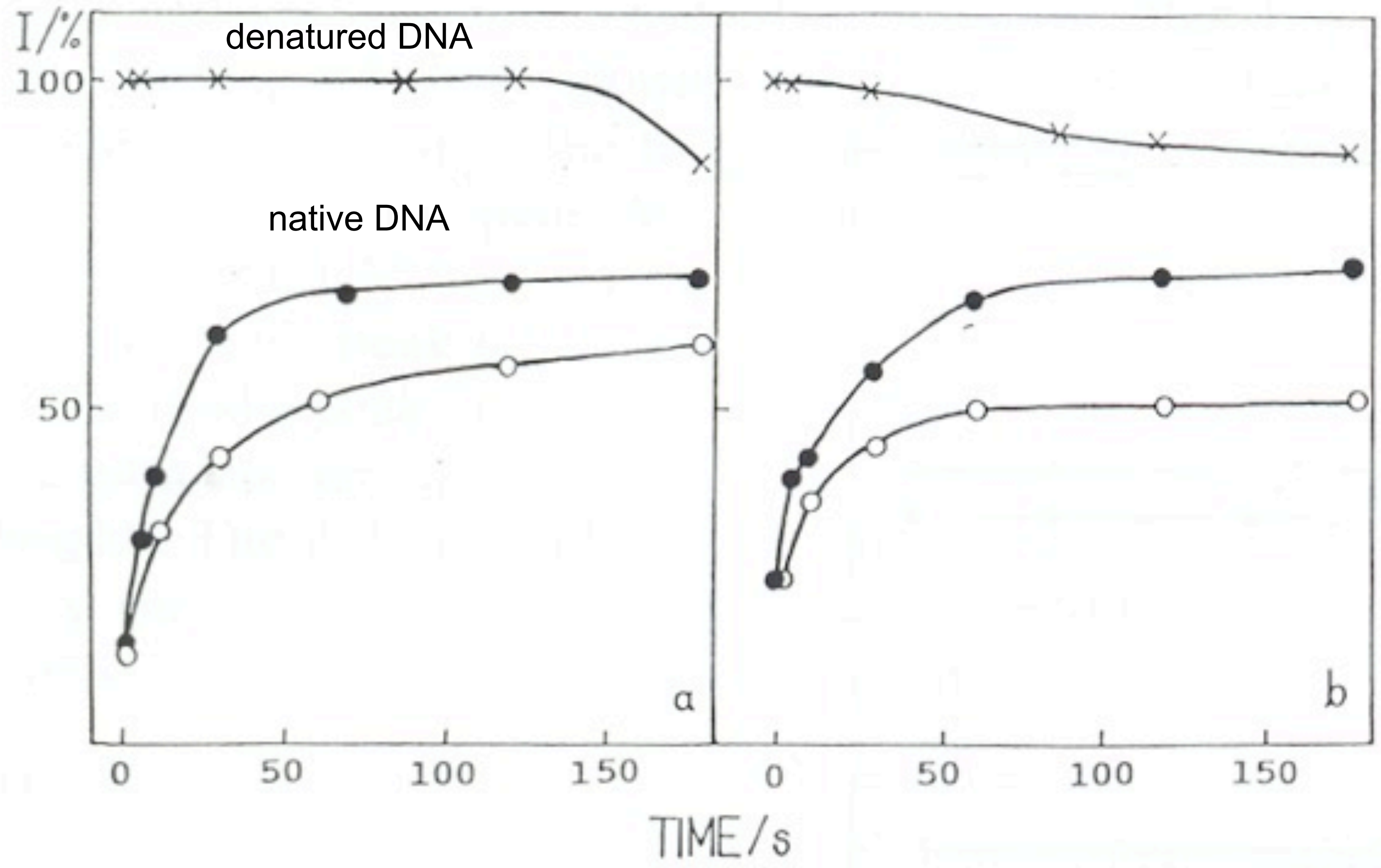


Fig. 6. The dependence of the relative heights of (a) the AdTSCV anodic peak G and (b) the cathodic peak AC on time t_b at potentials $E_b = -1.2$ V (○——○), and $E_b = -1.3$ V (●——●) for native DNA and for denatured DNA (×——×). The HMDE charged to a potential $E_a = -0.25$ V was immersed into the solution of native DNA (at a concentration of 292 μg ml^{-1}) or into the solution of denatured DNA (140 μg ml^{-1}) for a time $t_b = 100$ s; the electrode was then washed and transferred to the background electrolyte not containing DNA. In this medium the HMDE (with the adsorbed DNA layer) was exposed to the potentials $E_b = -1.2$ V or -1.3 V for the time t_b given in the graph followed by CV measurement (for details see Figs. 1 and 2). The relative peak heights are expressed in per cent; the heights of peaks AC and G of the denatured DNA at zero time were taken as 100%.

Scheme 1

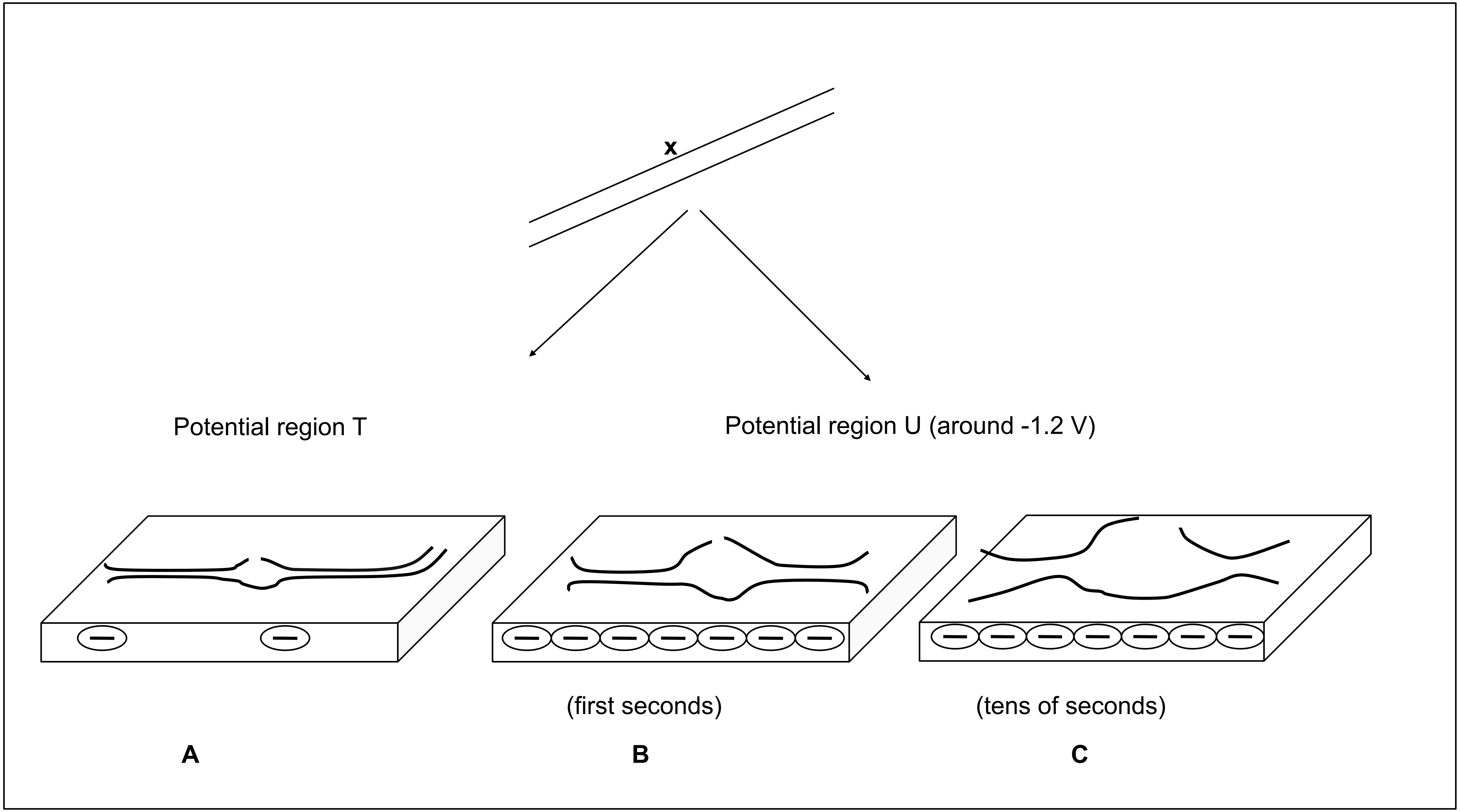
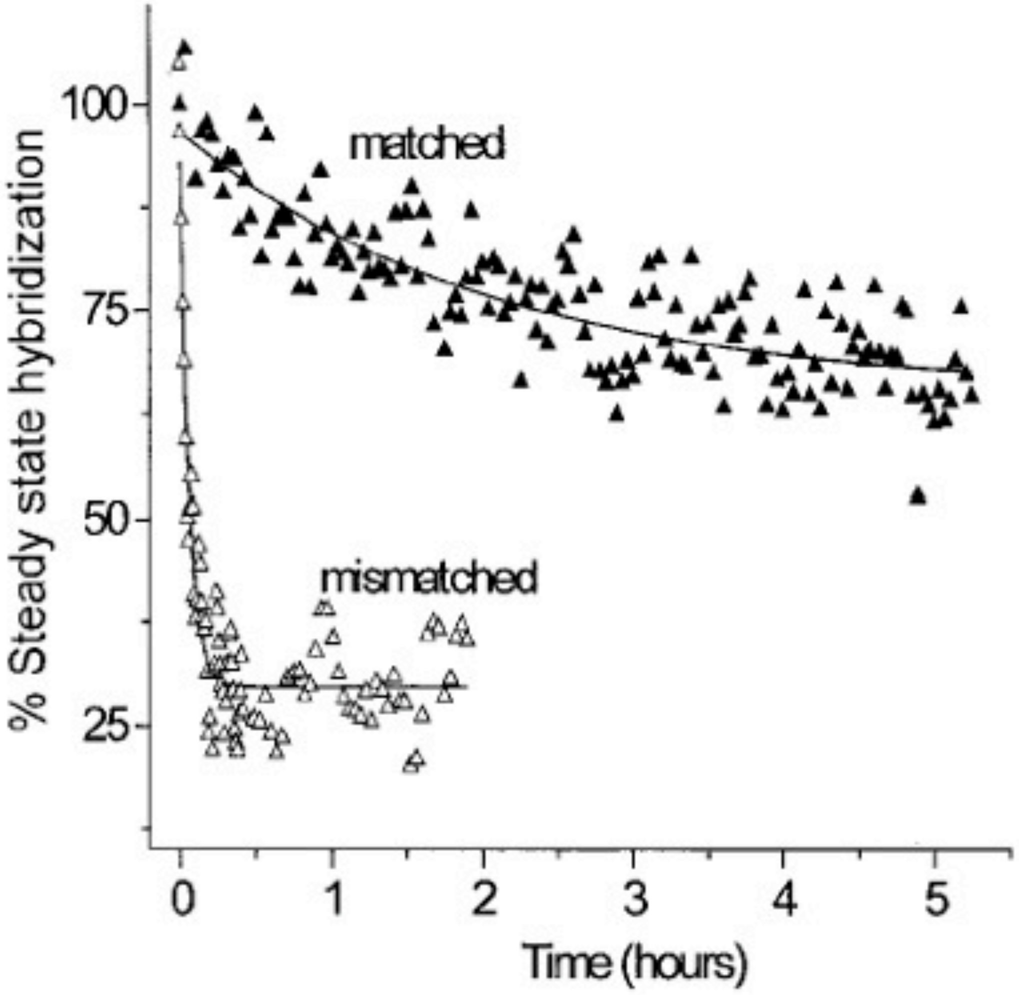

x
Potential region T
Potential region U (around -1.2 V)
(first seconds)
(tens of seconds)
A
B
C

Figure 19

DNA unwinding at negatively charged Au surfaces was recently observed by R. Georgiadis et al. and applied in DNA sensors



Heaton RJ, Peterson AW, Georgiadis RM, PNAS 98 (2001) 3701

IFFY stories

On this day 50 years ago, Watson and Crick published their double-helix theory. But, what if...

By Steve Mirsky (2003)

“I am now astonished that I began work on the triple helix structure, rather than on the double helix,” wrote Linus Pauling in the April 26, 1974 issue of Nature.

In February 1953, Pauling proposed a triple helix structure for DNA in the Proceedings of the National Academy of Sciences (PNAS). He had been working with only a few blurry X-ray crystallographic images from the 1930s and one from 1947.

If history´s helix had turned slightly differently, however, perhaps the following timeline might be more than mere musing...

August 15, 1952: Linus Pauling (finally allowed to travel to England by a US State Department that thinks the words “chemist” and “communist” are too close for comfort) visits King´s College London and sees Rosalind Franklin´s X-ray crystallographs. He immediately rules out a triple helical structure for DNA and concentrates on determining the nature of what is undoubtedly a double helix.

February 1953: Pauling and Corey describes the DNA double helix structure in PNAS .....

84 *CHEMISTRY: PAULING AND COREY* PROC. N. A. S.

A PROPOSED STRUCTURE FOR THE NUCLEIC ACIDS

By Linus Pauling and Robert B. Corey

Gates and Crellin Laboratories of Chemistry,* California Institute of Technology

Communicated December 31, 1952

92 *CHEMISTRY: PAULING AND COREY* PROC. N. A. S.

which are involved in ester linkages. This distortion of the phosphate group from the regular tetrahedral configuration is not supported by direct experimental evidence; unfortunately no precise structure determinations have been made of any phosphate di-esters. The distortion, which corresponds to a larger amount of double bond character for the inner oxygen atoms than for the oxygen atoms involved in the ester linkages, is a reason-

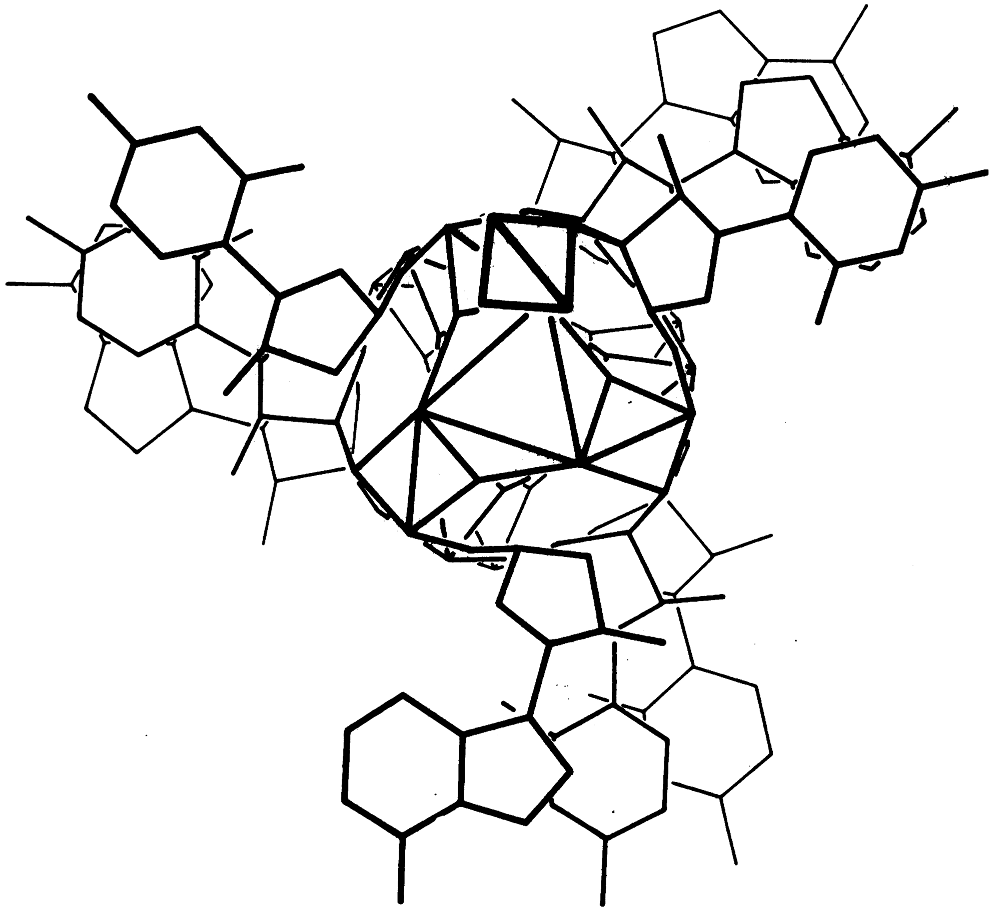


FIGURE 6

Plan of the nucleic acid structure, showing several nucleotide residues.

Triple helix

with bases on the outside and sugar-phosphate backbone in the interior of the molecule

My IFFY story:

If L. PAULING had in his lab an oscillopolarograph in 1952 he would never proposed this structure. Polarography clearly showed that bases must be hidden in the interior of native DNA molecule and become accessible when DNA is denatured

SUMMARY

Electroactivity of nucleic acids was discovered about 50 years ago
Reduction of bases at Hg electrodes is particularly sensitive to changes in DNA structure. The course of DNA and RNA denaturation and renaturation can be easily traced by electrochemical methods

At present electrochemistry of nucleic acids is a booming field, particularly because it is expected that **sensors for DNA hybridization** and for **DNA damage** will become important tools in biomedicine and other regions of practical life in the 21st century

DNA-modified electrodes can be easily prepared;
microL volumes of DNA are sufficient of its analysis but miniaturization of electrodes decreases these volumes to nL.
Sensitivity of the analysis has greatly increased in recent years.

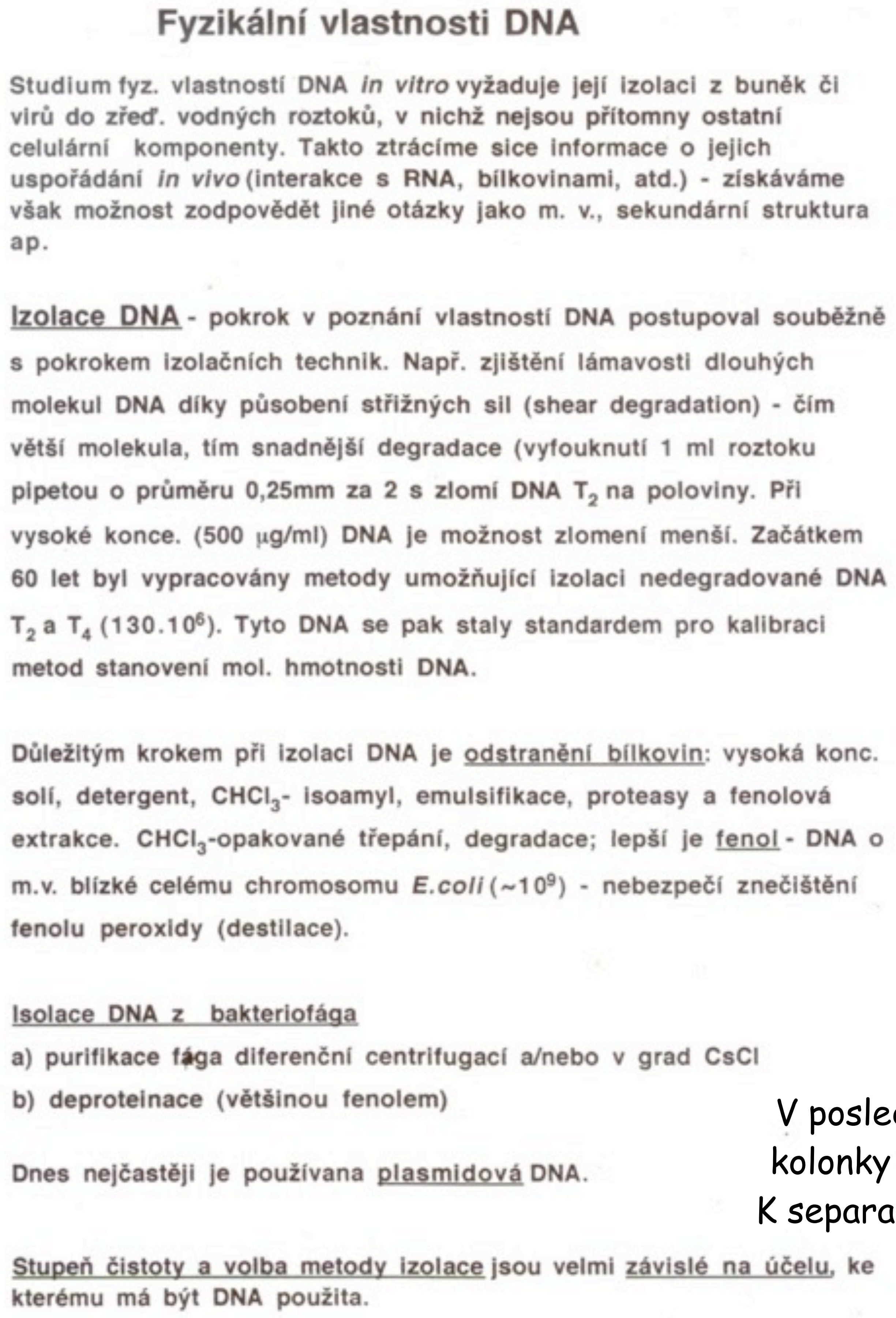
Chemie, struktura a interakce nukleových kyselin

2008-09 3.EP/6. PŘEDNÁŠKA 22.10.08

Fyzikální vlastnosti a izolace DNA

Denaturace, renaturace a hybridizace DNA

Biosyntetické polynukleotidy



Fyzikální vlastnosti DNA

Studium fyz. vlastností DNA *in vitro* vyžaduje její izolaci z buněk či virů do zřeď. vodných roztoků, v nichž nejsou přítomny ostatní celulární komponenty. Takto ztrácíme sice informace o jejich uspořádání *in vivo* (interakce s RNA, bílkovinami, atd.) - získáváme však možnost zodpovědět jiné otázky jako m. v., sekundární struktura ap.

Izolace DNA - pokrok v poznání vlastností DNA postupoval souběžně s pokrokem izolačních technik. Např. zjištění lámavosti dlouhých molekul DNA díky působení střižných sil (shear degradation) - čím větší molekula, tím snadnější degradace (vyfouknutí 1 ml roztoku pipetou o průměru 0,25mm za 2 s zlomí DNA T_2 na poloviny. Při vysoké konce. (500 μg/ml) DNA je možnost zlomení menší. Začátkem 60 let byl vypracovány metody umožňující izolaci nedegradované DNA T_2 a T_4 (130.10^6). Tyto DNA se pak staly standardem pro kalibraci metod stanovení mol. hmotnosti DNA.

Důležitým krokem při izolaci DNA je odstranění bílkovin: vysoká konc. solí, detergent, $CHCl_3$- isoamyl, emulsifikace, proteasy a fenolová extrakce. $CHCl_3$-opakované třepání, degradace; lepší je fenol - DNA o m.v. blízké celému chromosomu *E.coli* ($\sim 10^9$) - nebezpečí znečištění fenolu peroxidy (destilace).

Isolace DNA z bakteriofága

a) purifikace fága diferenční centrifugací a/nebo v grad CsCl
b) deproteinace (většinou fenolem)

Dnes nejčastěji je používana plasmidová DNA.

Stupeň čistoty a volba metody izolace jsou velmi závislé na účelu, ke kterému má být DNA použita.

V posledních letech jsou k dispozici komerčně dostupné kolonky využívající imobilizaci DNA na pevném podkladu. K separaci DNA jsou rovněž používány magnetické kuličky (magnetic beads)