The identification of hydroxymethyluracil in DNA of *Trypanosoma brucei*

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ABSTRACT

We have previously reported the detection of two unusual nucleotides, pdJ and pdV, in the DNA of *Trypanosoma brucei* (Gommers-Ampt et al., 1991). pdJ was found to be a novel nucleotide and is possibly involved in the regulation of variant specific surface antigen gene expression in trypanosomes. Recent evidence suggests that V could be a precursor of J, making V a key compound in the study of the biosynthesis and function of J. We have therefore determined the structure of V and here we present proof that V is HOMeU. The identity is based on a detailed comparison of dV(p) with authentic HOMeU(p), showing: I) co-migration in three different liquid chromatography analyses II) identical UV absorbance characteristics III) identical behavior in acetyl-pentafluorobenzyl derivatization and subsequent Gas chromatography/Mass spectrometry (GC/MS). The GC/MS technique has not been used before to analyse HOMeU purified from biological material. Because of its high sensitivity, it may also be useful for the detection of the low amounts of HOMeU resulting from oxidative damage of DNA.

INTRODUCTION

The thymine analogue 5-hydroxymethyluracil (HOMeU) is found in the DNA of many organisms. It is a natural component in the DNA of both dinoflagellates (2) and some *Bacillus subtilis* bacteriophages (3), in which it replaces between 12% and 68% (dinoflagellates) or even up to 100% (*B.subtilis* phages) of thymine. *Bacillus subtilis* bacteriophage SP10 and *Pseudomonas acidovorans* bacteriophage ϕ W14 contain HOMeU in their DNA as an intermediate in the synthesis of the thymine analogues α -glutamylthymine and putrescinythymine respectively (reviewed in ref. 4). In both SP10 and ϕ W14 the HOMeU is

incorporated into the DNA from a HOMeUTP precursor, and subsequently converted in the unusual T analogues. Finally, HOMeU is formed in any DNA as a product of oxidative attack on the methyl group of thymine. This oxidative attack is primarily mediated by highly reactive oxygen radicals such as the hydroxyl radicals produced by ionizing radiation (5).

In a previous paper we reported the detection of small amounts of an unusual nucleotide pdV in the DNA of the African trypanosome *Trypanosoma brucei* (1). This pdV co-migrated with hydroxymethyldeoxyuridine 5'-monophosphate (pHOMeU) in 2-dimensional thin layer chromatograms (1). We have hypothesized that V is an intermediate in the synthesis or degradation of another minor modified base in trypanosome DNA, called J, since J also appeared to be a pyrimidine and levels of pdV were higher in the bloodstream form life cycle stage, to which pdJ is restricted, than in the insect stage (1). The DNA base J is novel and may be involved in the regulation of variant specific surface antigen gene expression in trypanosomes (6,7). Recently we have found that J is a sugar derivative of HOMeU (J.G-A and P.B. unpublished results). Hence, if V is HOMeU, HOMeU in DNA could indeed be the precursor of J at the polynucleotide level, as it also is in the case of the thymine analogues α -glutamylthymine and putrescinythymine in bacteriophage DNAs (4). The synthesis of J could then be analogous to the synthesis of glucosylated HOMeC in the T even phages of *E.coli*, in which a glucosyl transferase converts HOMeC in the sugar derivative of HOMeC at the polynucleotide level (8). We have therefore analysed V in more detail, as the identification of its structure may help in the study of the biosynthesis and function of J. This paper describes the purification of dV(p) and HOMeU(p) from total DNA of bloodstream form *T.brucei* and bacteriophage H1 respectively, and the comparison of their behavior in three different liquid chromatography columns, UV absorbance spectroscopy and a Gas chromatography/Mass spectrometry (GC/MS) technique made very sensitive by a novel derivatization procedure.

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MATERIALS AND METHODS

Trypanosomes

The trypanosomes used belong to strain 427 of *T. brucei brucei*. A variant which expresses the VSG gene 1.8 and is a relapse of variant 221a was used. Trypanosomes were grown in Sprague Dawley rats until high parasitemias developed. They were harvested by cardiac puncture and separated from blood cells by DEAE cellulose chromatography according to Lanham et al. (9).

Isolation of DNA

Total trypanosome DNA was isolated as described by Bernards et al. (10) and resuspended in 2 mM Tris-HCl, pH 7.6. *Bacillus subtilis* bacteriophage H1 DNA, in which thymine is completely replaced by hydroxymethyluracil, was isolated as described by Arwert and Venema (11).

Enzymatic hydrolysis of DNA

Total genomic trypanosome and bacteriophage H1 DNA were hydrolysed to deoxyribonucleoside 3'-monophosphates (dNp's) with micrococcal nuclease and spleen phosphodiesterase as described (1). After the incubation the reaction mixture was heated at 100°C for 5 minutes and then centrifuged to remove precipitated material which would interfere with subsequent purification steps.

Deoxyribonucleosides and deoxyribonucleotides

Trypanosome dVp and bacteriophage H1 HOMedUp, both isolated from the MonoQ anion exchange column (see below) were dephosphorylated to obtain deoxyribonucleosides (dN's). For this the lyophilized nucleotides were dissolved in water and 70 pmoles of each were incubated with 2 U nuclease PI (Boehringer) per pmole dNp in 18 mM sodium acetate, pH 7.2, containing 0.4 mM ZnCl₂ in a total volume of 100 µl, for 1 h at 37°C. The reaction mixture was then heated for 5 minutes at 100°C, centrifuged, and the supernatant used for reversed phase HPLC. Reference deoxyribonucleosides hydroxymethyl-deoxyuridine (HOMedU), deoxyguanosine (dG), deoxyadenosine (dA), deoxythymidine (dT) and deoxycytidine (dC) and deoxyribonucleoside 3'-monophosphates dGp, dAp, dTp, dCp were obtained from Sigma. As HOMedU was not commercially available, we prepared ³²P labeled HOMedUMP, to determine its retention time in the first two columns (see below). For this, bacteriophage H1 DNA was ³²P-postlabeled and the resulting labeled nucleotides were separated on TLC sheets as described (1; 0.3 M ammonium formate, pH 3.5, for the first dimension and 0.5 M lithium chloride for the second dimension). The pHOMedU containing spot was excised from the sheet and washed three times with methanol. pHOMedU was eluted from the sheet with 500 µl of 1 M ammonium formate, pH 3.5. After brief centrifugation the supernatant was collected and dried *in vacuo*.

Chemicals

Acetic anhydride was from BDH (Poole, UK). Acetic anhydride-d₆ (98.5 atom% D) and 4-(N,N-dimethylamino)pyridine (99%) were obtained from Janssen (Beerse, Belgium). Pentafluorobenzyl bromide was from Pierce (Rockford, Illinois, USA). Silica gel 60, triethylamine, toluene, acetonitrile, dichloromethane, ethylacetate, ammonia, acetic acid, formic acid, methanol and lithium chloride were purchased from Merck (Darmstadt, Germany) and methane (99.995% purity) from

Hoekloos, Schiedam, the Netherlands. All organic solvents and chemicals were of analytical grade unless otherwise stated.

Liquid chromatography

Nucleotides, derived from hydrolysis of 100 µg total genomic DNA, were separated on a Supelco LC-18-S reversed phase High Performance Liquid Chromatography (HPLC) column. Elution was in 40 mM ammonium acetate, pH 5.6, with a linear gradient of 0 to 13.5% acetonitrile from 30 to 60 minutes. The flow rate was 0.8 ml min⁻¹. Fractions containing dVp (from the trypanosome hydrolysate) or dHOMedUp (from the H1 hydrolysate) were concentrated by lyophilization and dissolved in 8 mM ammonium formate, pH 3.5. Each of them was chromatographed on a MonoQ anion exchange column (type HR 5/5, Pharmacia), using a buffer of ammonium formate pH 3.5, with a linear gradient of 8 mM to 45 mM in 40 minutes, and a flow rate of 1 ml min⁻¹. Collected fractions containing either dVp or HOMedUp were lyophilized to remove the elution buffer and to concentrate the nucleotide. Subsequent dephosphorylation was done as described above. The resulting nucleosides were purified with reversed phase HPLC, using a Supelco LC-18-S column. Elution was in a mixture of ammonium acetate pH 5.6 and acetonitrile, with a linear gradient of 39.2 mM resp. 1% to 30 mM resp. 12.5% in 20 minutes to 8 mM resp. 40% at 40 minutes. The flow rate was 1 ml min⁻¹. The nucleoside containing fractions were lyophilized, dissolved in H₂O and used for derivatization and Gas chromatography/Mass spectrometry (GC/MS) as described below. A blank control sample for GC/MS was prepared as follows: from a blank run of the first, reversed phase, column fractions corresponding to the retention time of HOMedUp were collected, lyophilized and applied to the second, anion exchange, column. Fractions with an equivalent retention time as HOMedUp were collected, lyophilized and treated with nuclease PI as described for dVp and HOMedUp. The reaction mixture was used for the third, reversed phase, column and fractions eluting off at the retention time of HOMedU were collected, lyophilized and used for derivatization and GC/MS analysis. With all three columns a photodiode array detector (Waters 990) was used to continuously monitor the UV absorbance spectrum of the eluted peaks.

Identification of dVp and HOMedUp containing fractions

The elution time of dVp was determined by ³²P-postlabeling analysis. For this 7 µl of each 0.8 ml collected fraction was dried *in vacuo* to remove the solvent. The deoxyribonucleoside 3'-monophosphates (dNp's) were labeled with [γ-³²P] ATP and subsequently converted to deoxyribonucleoside 5'-monophosphates (pdN's) as described (1). The labeled nucleotides were separated in two dimensions on polyethyleneimine (PEI) cellulose thin layer sheets as described (1). The migration properties were compared with those of pdV in fig.2B of Gommers-Ampt et al. (1). Identification of HOMedUp was based on its characteristic UV absorbance spectrum (12).

Derivatization of the nucleosides

The deoxynucleoside (8 pmoles) was acetylated and pentafluorobenzylated by using a modification of the method described by Teixeira et al. (13) and will be described in detail elsewhere (A.J.R.T., J.H.G-A, G.v.d.W., G.Westra and A.de Jong, manuscript in preparation). Briefly, the nucleoside was acetylated by treatment with 65 µl of a 0.05 M solution of dimethylaminopyridine in acetonitrile, 155 µl of acetonitrile and

30 μ l of 10% acetic anhydride (v/v) for 60 minutes at 80°C. The excess reagent was then hydrolysed and the derivative was extracted with dichloromethane after the addition of 100 μ l of a solution of dT-Ac₂(d₆) and HOMeU-Ac₃(d₉) (5 ng/ml of each) in acetonitrile. The deutoacetyl analogues, used as internal controls for subsequent pentafluorobenzyl derivatization, were prepared as described above using deutoacetic acid anhydride. To the acetylated nucleosides 100 μ l of a 2% solution (v/v) of pentafluorobenzyl bromide in toluene and 10 μ l of triethylamine were added and the mixture was heated at 54°C for 3.5 hours. The acetylated and pentafluorobenzylated nucleoside was then purified using a silica solid phase extraction column and 1% of the final solution was used for GC/ECNICI-SIM-MS analysis.

Gas chromatography/mass spectrometry

Gas chromatography/Electron capture negative ion chemical ionization-selected ion monitoring-mass spectrometry (GC/ECNICI-SIM-MS) analyses were carried out on a VG70S two sector mass spectrometer equipped with a Hewlett Packard gas chromatograph. Chemical ionization was performed using methane (99.995% purity) as reagent gas at a pressure optimized to obtain maximum sensitivity. The mass spectrometer was operated at an ion source temperature of 290°C and with a filament emission of 500 μ A. The energy of the primary electrons was 70 eV. Data were acquired in the selective ion monitoring

mode, at m/z 325.1036 and m/z 331.1412 for the acetyl-PFB and deutoacetyl-PFB derivatives respectively, with a settle time of 50 ms per channel and at a resolution of 3000 (5% valley definition). Chromatographic separations were performed on a 12 m $\times$ 0.25 mm i.d., 0.12 μ m film thickness, CPSil5CB (Chrompack, Middelburg, The Netherlands) fused-silica capillary column coupled to 60 cm of 0.15 mm i.d. deactivated fused silica tubing. The outlet end was directly introduced into the ion source of the mass spectrometer through a heated transfer line kept at 290°C. Helium was used as the carrier gas at an average linear velocity of 30.6 cm/sec. Samples were introduced using an all-glass solid injector with the injection port kept at 280°C. The oven temperature was kept at 250°C for 1 minute, then increased with 15°C/min until 290°C, and finally held at 290°C for 10 minutes. For each analysis, 1 μ l of the 100 μ l of derivatized material was used, which is equivalent to 80 fmoles of nucleoside.

RESULTS

We have previously shown comigration of ³²P-postlabeled pdV with pHOMeU in two different 2-dimensional Thin Layer Chromatography (TLC) systems. Their calculated R_f values, as well as those of pdG, pdA, pdT and pdC are presented in Table 1 (³²pdN). To analyse the possible identity of V and HOMeU in more detail, we purified the corresponding nucleosides from

Table 1. Chromatographic properties of (p)dV(p) and marker nucleosides/nucleotides.

	G	A	T	C	HOMeU	V
³² pdN:						
TLC						
unmodified cellulose						
solvent D1	0.32	0.62	0.42	0.48	0.29	0.29
solvent D2	0.46	0.35	0.54	0.72	0.65	0.65
PEI cellulose						
solvent D1	0.10	0.42	0.35	0.63	0.34	0.34
solvent D2	0.21	0.11	0.43	0.45	0.56	0.56
dNp:						
Reversed phase	13	36	14	7	8	8
Anion exchange	25	17	22	6	17	17
dN:						
Reversed phase	17–19	23–25	17–19	10–12	12–14	13
Derivatised dN:						
Gas Chromatography	n.d.	n.d.	2.50	n.d.	3.39	3.39

Data derived from thin layer chromatography (TLC) are presented as R_f values, calculated as *elution distance of the solute/ elution distance of the mobile phase*. The composition of the solvents has been described (1). Data obtained from liquid and gas chromatography experiments are presented as retention time (minutes). Experimental conditions are described in materials and methods. n.d.: not determined. PEI: polyethyleneimine. dN: deoxyribonucleoside. pdN: deoxyribonucleoside 5'-monophosphate. dNp: deoxyribonucleoside 3'-monophosphate.

Table 2. Spectral characteristics of dV(p) and marker nucleosides/nucleotides.

dN(p):	G	A	T	C	HOMeU	V
pH 5.6:						
$\lambda_{\max}$ (nm)	252	260	267	271	263	263
A ₂₅₀ /A ₂₆₀	1.15	0.80	0.66	0.82	0.70	0.70
A ₂₈₀ /A ₂₆₀	0.68	0.14	0.64	0.97	0.52	0.52
pH 3.5:						
$\lambda_{\max}$ (nm)	252	258	267	278	263	263
A ₂₅₀ /A ₂₆₀	1.14	0.85	0.68	0.56	0.73	0.73
A ₂₈₀ /A ₂₆₀	0.68	0.18	0.65	1.73	0.53	0.52

Data were obtained by measuring UV absorbance spectra of deoxyribonucleoside 3'-monophosphates (dNp's) eluting from the anion exchange column at pH 3.5 and of deoxyribonucleosides (dN's) from the reversed phase column at pH 5.6.

Figure 1. Structure of hydroxymethyldeoxyuridine (HOMedU) and its acetyl-pentafluorobenzyl derivative (HOMedU-Ac₃-PFB). In deuterated acetyl-PFB derivatives, each of R₁, R₂ and R₃ of HOMedU and both R₁ and R₂ of dT will be COCD₃ instead of COCH₃, resulting in an increase of 3 a.m.u. per acetyl group.

Figure 2. GC/MS of acetyl-PFB derivatized HOMedU, dV and blank, together with an internal positive control. The upper three panels present ECNICI selected ion recordings at m/z 325.1036, corresponding to $[(M-PFB-58)]^-$ of HOMedU-Ac₃-PFB ($t = 3:39$ min), dV-Ac₃-PFB ($t = 3:39$ min) and a blank of the LC isolation procedure. The small peak eluting at 2:50 min is due to contaminating dT, detected as $[(M-PFB)]^-$ of dT-Ac₂-PFB, resulting from the derivatization procedure. The lower panel presents ECNICI selected ion recordings at m/z 331.1412, showing a representative deuterioacetyl positive control. After acetylation of the samples, dT-Ac₂(d_6) and HOMedU-Ac₃(d_9) were added to each of them as an internal control for subsequent PFB derivatization. Upon NICI, loss of PFB from dT-Ac₂(d_6)-PFB resulted in a peak (m/z 331.1412) eluting at 2:49 min, and loss of PFB and rearrangement of one Ac(d_3) group from HOMedU-Ac₃(d_9)-PFB in a peak (m/z 331.1412) at 3:37 min. The intensities of the second (dV) and the third (blank) panel were normalized to that of the upper (HOMedU) panel.

genomic DNA of *T.brucei* and *B.subtilis* phage H1 respectively, and compared their: I) chromatographic behavior in 3 different liquid chromatography (LC) columns II) UV absorbance properties III) Gas Chromatographic/ mass spectrometric (GC/MS) behavior.

Purification of the nucleosides dV and HOMedU

To purify both nucleosides we used 3 LC columns in sequence, as described in materials and methods. Total genomic DNA of both *T.brucei* and bacteriophage H1, the latter containing

hydroxymethyluracil instead of thymine, was enzymically hydrolysed and the resulting deoxyribonucleoside 3'-monophosphates (dNp's) were separated on a reversed phase column. The resulting retention times of the nucleotides are presented in Table 1 (dNp, reversed phase). Elution times of dNp's derived from the genomic digests were similar to those of the corresponding marker nucleotides dGp, dAp, dTp, dCp (Sigma) and ³²pHOMedU (purified from TLC). The retention time of dVp, determined by ³²P-postlabeling of collected fractions since the low level of dVp was hidden in the tail of dCp, was similar to that of HOMedUp. Fractions containing either dVp or HOMedUp (derived from the *T.brucei* or H1 hydrolysate respectively) were collected and resolved from dCp using a MonoQ anion exchange column. Retention times of marker nucleotides were determined and are shown in Table 1 (dNp, anion exchange). dVp was now visible as a separate peak and eluted off with a retention time similar to that of HOMedUp. Its identity was confirmed by ³²P-postlabeling analysis and 2D-TLC. Since the applied GC/MS method required nucleosides rather than nucleotides, both dVp and HOMedUp, isolated from the MonoQ column, were dephosphorylated (see Materials and Methods). The resulting dV and HOMedU were purified from the released phosphate and from salts present in the incubation buffer on a reversed phase column. Both dV and HOMedU eluted with a similar retention time as a marker nucleoside HOMedU (Sigma). Their retention times, together with those of marker nucleosides dG, dA, dT, dC (Sigma) are shown in Table 1 (dN, reversed phase). The variation in retention times of the marker nucleosides is possibly due to a delayed start of the gradient, resulting in a shift of the complete profile. The yield of dV and HOMedU was 65 pmoles, isolated from 400 µg of *T.brucei* and about 0.7 µg of H1 DNA respectively.

Spectral characteristics

The UV absorbance spectrum of each eluted nucleotide was measured with a diode array detector. Spectra of dGp, dAp, dTp and dCp derived from both genomic digests were identical to those of the analogous marker nucleotides (Sigma). The spectrum of dV(p) was identical to that of HOMedU(p) derived from phage H1 at both pH 3.5 and 5.6, as well as to that of HOMedU obtained from Sigma, measured only at pH 5.6. In Table 2 the spectral characteristics are presented as wavelength with maximal absorbance (λ_{max}) and the ratio's A_{250}/A_{260} and A_{280}/A_{260} .

Gas chromatography/mass spectrometry (GC/MS)

Analysis of nucleosides by GC/MS requires chemical modification of the native compound to increase its volatility and thermostability. In earlier work Teixeira et al. (13) showed that acetylation followed by pentafluorobenzoylation yields derivatives suitable for gas chromatography for some nucleosides, like dT and HOMedU. Moreover, the presence of the electrophoric pentafluorobenzyl group makes these derivatives suitable for electron capture negative ion chemical ionization (ECNICI; 13), which is known to be a very sensitive GC/MS technique. Preparation of such a derivative of HOMedU results in a tri-acetylated and mono-pentafluorobenzylated compound, HOMedU-Ac₃-PFB (Figure 1). MS analysis of the latter under ECNICI conditions gives a spectrum containing an intense diagnostic peak at m/z 325.1036 ($[(M-PFB-58)]^-$), due to loss of the PFB (R₄) moiety and rearrangement of one acetyl (R₃) group with the loss of CH₂CO₂. The derivatized dT, dT-Ac₂-PFB, is also detected at m/z 325 ($[(M-PFB)]^-$), due to loss of PFB during

the ionization. Using the selected ion monitoring (SIM) mode at m/z 325, as low as a few fmoles of the derivatized HOMeU and dT can be detected. The deuterioacetyl derivatives, present in each sample as an internal control for PFB derivatization and to calculate the relative retention time, can be detected in the SIM mode at m/z 331.1412 (see legend of Figure 1).

Derivatives were made of column purified dV, HOMeU and a blank fraction (see Materials and Methods), each sample containing the deuterioacetyl derivatives of both dT and HOMeU as internal controls. Results of the subsequent analyses of the acetyl-PFB derivatives by GC/ECNICI-MS are shown in Figure 2. The upper three panels (m/z 325.1036) show the profiles of derivatized HOMeU (H1), dV (*T. brucei*) and blank. The lower panel (m/z 331.1412) shows the internal control for HOMeU and was identical in the other two samples (not shown). The slight difference in retention times between the deuterioacetyl derivatives versus the corresponding not deuterated derivatives is a phenomenon commonly observed in gas chromatography. The upper two panels in Figure 2 strongly suggest that trypanosomal dV and bacteriophage H1 HOMeU are the same compound based on mass selectivity and relative retention time. Moreover, these profiles are identical to that of HOMeU obtained from Sigma, that we derivatized as described above (result not shown). The blank panel shows that HOMeU was not present as a contaminant on the LC columns used to isolate dV and HOMeU, as the internal control (m/z 331.1412) was positive but no HOMeU was detected with m/z 325.1036. Trace amounts of dT, eluting at 2:50 min., were present in all samples, including a derivatization without any added material.

CONCLUSIONS

The analyses summarized in Tables 1 and 2 and Figure 2 show that the modified DNA nucleoside dV from *T. brucei* is identical to HOMeU. We have previously shown that bloodstream form trypanosomes contain more pdV than insect form trypanosomes. This has raised the possibility that low levels of pdV in both life-cycle stages are the result of DNA damage, and that levels of pdV are increased in bloodstream form DNA because V is related to J, a novel DNA base only present in the bloodstream form stage and associated with transcriptional inactivation. Our present finding that V is HOMeU supports this hypothesis. Low levels of pdV can be the result of DNA damage, as HOMeU is a known product of oxidative DNA damage, and found in small amounts in many DNAs. Since J is a sugar derivative of HOMeU (J.G.-A and P.B., unpublished data), the finding that V is HOMeU makes V indeed a good candidate precursor of J. The HOMeU in the DNA could serve as a substrate for the synthesis of J, analogous to hydroxymethylcytosine (HOMeC) in the DNA of the T even phages of *E. coli*, which is converted into the sugar derivative of HOMeC by a phage specific glucosyl transferase (8). It will therefore be of interest to test whether trypanosomes can be forced to incorporate exogenous HOMeU into their DNA and whether this HOMeU is converted into J. Both unicellular eukaryotes and cells from higher eukaryotes incorporate HOMeU into their DNA when they are grown in medium containing HOMeU. Mammalian cells remove the HOMeU again from their DNA with the enzyme HOMeU-DNA glycosylase (14), but unicellular eukaryotes appear to lack this enzyme (15). When *Tetrahymena* was grown in a medium containing HOMeU, up to 30% of dT in the DNA was replaced by HOMeU without apparent ill effects (16). We have therefore started experiments to test the effect of

exogenous HOMeU on both insect form and bloodstream form trypanosomes. The results presented in this paper may thus help to unravel the biosynthesis and function of J.

Our results also demonstrate that the novel derivatization procedure in combination with GC/MS is very suitable for the detection of low levels of HOMeU in biological material. This may be useful since the presence of HOMeU is used as a marker of exposure of DNA to ionizing radiation (5).

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REFERENCES

- Gommers-Ampt,J., Lutgerink,J. and Borst,P. (1991) Nucleic Acids Res., 19, 1745–1751.
- Rae,P.M.M. (1976) Science, 194, 1062–1064.
- Kallen,R.G., Simon,M. and Marmur,J. (1962) J.Mol.Biol., 5, 248–250.
- Warren,R.A.J. (1980) Ann.Rev.Microbiol. 34, 137–158.
- Frenkel,K., Cummings,A., Solomon,J., Cadet,J., Sreiner,J.J. and Teebor,G.W. (1985) Biochemistry, 24, 4527–4533.
- Bernards,A., Van Harten-Loosbroek,N. and Borst,P. (1984) Nucleic Acids Res., 12, 4153–4170.
- Pays,E., Delauw,M.F., Laurent,M. and Steinert,M. (1984) Nucleic Acids Res., 12, 5235–5247.
- Kornberg,S.R., Zimmerman,S.B. and Kornberg,A. (1961) J.Biol.Chem., 236, 1487–1493.
- Lanham,S.M. and Godfrey,D.G. (1970) Exptl.Parasitol., 28, 521–534.
- Bernards,A., Van der Ploeg,L.H.T., Carlos,A., Frasch,C., Borst,P., Boothroyd,J.C., Coleman,S. and Cross,G.A.M. (1981) Cell, 27, 497–505.
- Arwert,F. and Venema,G. (1974) Molec. gen. Genet. 128, 55–72.
- Hall,R.H. (1971) The modified nucleosides in nucleic acids, Columbia University Press, New York, London.
- Teixeira,A.J.R., Van de Werken,G., Stavenhuter,J.F.C., Westra,J.G., Van der Greef,J. and De Jong,A.P.J.M. (1992) Biol. Mass Spectrom., 21, 441.
- Boorstein,R.J., Chiu,L.-N. and Teebor,G.W. (1992) Mol.Cell.Biol., 12, 5536–5540.
- Boorstein,R.J., Levy,D.D. and Teebor,G.W. (1987) Mut.Res., 183, 257–263.
- Rae,P.M.M. and Steele,R.E. (1978) BioSystems, 10, 37–53.