The discrepancy between these two ratios may be partially accounted for by the general differences in technique employed in the two iodination methods.

The large reproducible difference between the two analytical methods for plasmalogen in rat liver is unexplained. RAPPORT AND LERNER also found that rat liver gave lower molar ratios of iodine to p-nitrophenyl-hydrazone than the other rat tissues studied. We believe this observation has significance in regard to plasmalogen metabolism and is under further investigation.

This work was supported by U.S.P.H.S. Research Grant B1006(C4) and Interdisciplinary Grant 2M-6418(C2) N.I.M.H.

Department of Medicine, Division of Neurology and WILLIAM T. NORTON Department of Biochemistry, Albert Einstein College of Medicine of Yeshiva University, New York, N.Y. (U.S.A.)

```
<sup>1</sup> M. M. RAPPORT AND B. LERNER, Biochim. Biophys. Acta, 33 (1959) 319.
```

Received August 6th, 1959

Biochim. Biophys. Acta, 38 (1960) 340-342

## Thermal denaturation of deoxyribosenucleic acid isolated from a thermophile

In the course of an investigation of the thermal stability of highly polymerized deoxyribosenucleic acids, isolated from various microorganisms, it was found that the denaturation of the DNA was related to the overall mole percent composition of guanine plus cytosine in the sample. It was thought of interest to study the heat denaturation of polymerized DNA isolated from a thermophile grown at an elevated temperature. Bacillus stearothermophilus was grown in the basal medium of BAKER et al.2 supplemented with 0.5% acid-hydrolyzed casein in shallow players at 55° for 16 h (cells mostly in the vegetative state). The cells were harvested, washed, lyophilized and send to us by Dr. BAKER<sup>2</sup>. The guanine plus cytosine content of the DNA of B. stearothermophilus has been found to average  $46.7\%^{2,3}$ . The denaturation of Escherichia coli (K-12) DNA, which has a base composition (50 % guanine plus cytosine) similar to that of B. stearothermophilus, was studied simultaneously for comparison. DNA was isolated from both organisms by first disrupting the vegetative cells with sodium lauryl sulfate followed by deproteinization with chloroform and isoamyl alcohol\*. The sedimentation coefficient,  $S_{20, w}$ , measured at infinite dilution was found to be 18.3 for B. stearothermophilus and 33.4 for E. coli. Thermal denaturation of the DNA was studied by determining the absorbance-temperature profile n a thermostated Beckman Spectrophotometer Model DU at 260 mµ. DNA dissolved in 0.15 M NaCl plus 0.015 M sodium citrate, pH 7, was placed in glass-stoppered

<sup>&</sup>lt;sup>2</sup> J. Folch, M. Lees and G. H. Sloane-Stanley, J. Biol. Chem., 226 (1957) 497.

<sup>&</sup>lt;sup>3</sup> J. B. Wittenberg, S. R. Korey and F. H. Swenson, *J. Biol. Chem.*, 219 (1956) 39. <sup>4</sup> C. H. Fiske and Y. Subbarow, *J. Biol. Chem.*, 66 (1925) 375.

Abbreviation: DNA, deoxyribosenucleic acid.

<sup>\*</sup> A detailed description of this method will be published shortly.

cuvettes and the absorbance recorded at each successive elevated temperature after the solutions reached temperature equilibrium.

The absorbance-temperature profiles are shown in Fig. 1. It is apparent from the narrow temperature range in which the absorbance rises that the DNA's isolated from both B. stearothermophilus and E. coli are native  $^{4,5}$ . The temperature at the midpoint of the absorbance rise,  $T_m$ , is  $87.5^{\circ}$  for B. stearothermophilus and  $90^{\circ}$  for E. coli. The value of the  $T_m$  for B. stearothermophilus predicted from the relationship between the base composition and  $T_{m^1}$  is in very close agreement, namely 88°. The

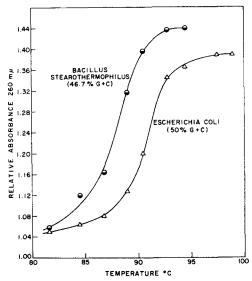


Fig. 1. Thermal denaturation of B. stearothermophilus and E. coli (K-12) DNA in 0.15 M NaCl + 0.015 M Na citrate. The relative absorbance (the absorbance of the heated sample, corrected for thermal expansion, divided by the absorbance of the native sample at 25°) is plotted against the temperature of the sample after it has reached equilibrium.

lower molecular weight of the DNA from the thermophile could not be expected to have a significant influence on its  $T_m$  since it has been shown that the sonic degradation of calf thymus and Diplococcus pneumonia DNA's to molecular weights of the order of 600,000  $(S_{20,w}$  about 9) has very little influence on their  $T_m$ .

Thus the thermal stability of the DNA from B. stearothermophilus is not unusual: the high temperature adaptation of this bacterium appears to arise from its proteins, some of which exhibit high heat resistance<sup>7,8</sup>.

Department of Chemistry, Harvard University, Cambridge, Mass. (U.S.A.) J. MARMUR

```
<sup>1</sup> J. MARMUR AND P. DOTY, Nature, 183 (1959) 1427.
```

Received July 22nd, 1959

<sup>&</sup>lt;sup>2</sup> H. Baker, H. Sobotka and S. H. Hutner, J. Gen. Microbiol., 9 (1953) 485.

<sup>&</sup>lt;sup>3</sup> A. L. Opper, personal communication.

J. Shack, J. Biol. Chem., 233 (1958) 677.
 P. Doty, H. Boedtker, J. R. Fresco, R. Haselkorn and M. Litt, Proc. Natl. Acad. Sci. U.S., 45 (1959) 482.

Gaussian J. Marmur and P. Doty, unpublished.

<sup>&</sup>lt;sup>7</sup> M. B. Allen, Bacteriol. Rev., 17 (1953) 125.

<sup>8</sup> H. KOFFLER, G. E. MALLETT AND J. AYDE, Proc. Natl. Acad. Sci. U.S. 43 (1957) 464.