# THE COMPLETE RETENTION OF TRANSFORMING ACTIVITY AFTER REVERSAL OF THE INTERACTION OF DNA WITH MERCURIC ION*

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It has been reported that the addition of mercuric ion markedly decreases the intrinsic viscosity of calf thymus DNA (Katz, 1952) and causes a shift in the ultraviolet absorption spectrum to longer wavelengths (Thomas, 1954). These effects occur even at high salt ($NaClO_4$) concentrations, with concentrations of mercuric ion equivalent to the concentration of DNA bases. The DNA-mercury (II) complexes can be decomposed by the addition of an excess of a good complexing agent for mercury (II), such as chloride, cyanide, or cysteine. If one starts with "native" DNA, with a high intrinsic viscosity and a large hypochromicity, the material recovered after the cycle of mercuration and demercuration has the same physical properties as the initial material and is by these criteria undenatured. However, the decrease in viscosity on adding the mercury (II) indicates that there has been some change in the shape or state of aggregation of the molecules upon formation of the complex. The object of the present experiments was to ascertain whether pneumococcal transforming DNA retains its biological activity after this cycle of mercuration and demercuration.

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A detailed chemical study of the DNA–Hg(II) complexes will be reported separately (Yamane and Davidson, in press). Some spectrophotometric results bearing on the formulae of the complexes should be mentioned here however. The spectral effects of adding $HgCl_2$ [$Hg(ClO_4)_2$ can be used also] to E. coli DNA is shown in Fig. 1a. Let r be the ratio of

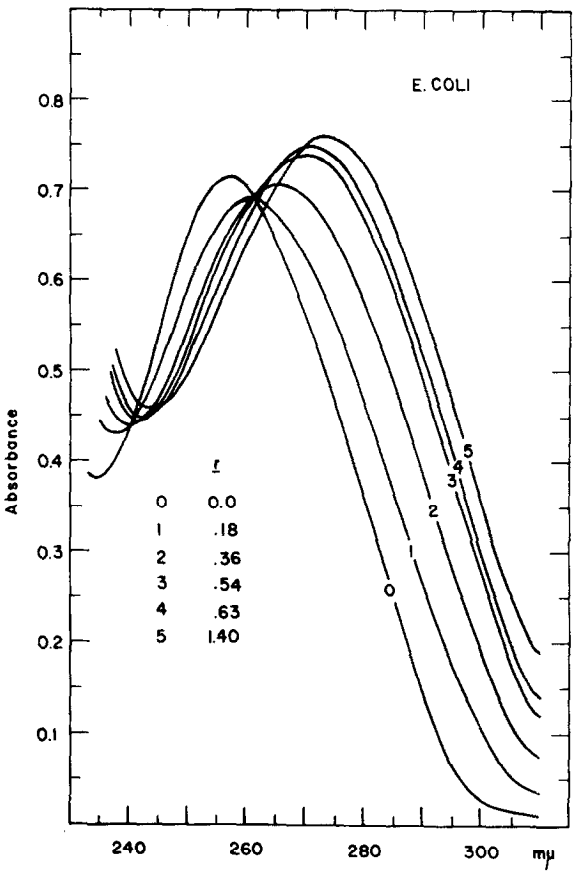


Figure 1a. Effect of $HgCl_2$ on the ultraviolet absorption spectra of DNA of E. coli. Spectra of DNA in 0.10 F $NaClO_4$, pH 5.60, with increasing r; r = moles of $HgCl_2$/moles of P.

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Hg(II) ions to DNA bases. Upon addition of $HgCl_2$ for $0 \leq r \leq 0.5$, there is a depression of the maximum at 258 mμ, an isosbestic point at 262.5 mμ, and the development of a new maximum at 268 mμ. On increasing the $HgCl_2$ concentration above $r \cong 0.5$, the wavelength 262.5 mμ ceases

to be an isosbestic point; there is a new isosbestic point at about 270 mμ and a maximum at about 275 mμ. Thus, there are two complexes: one for $0 \leq r \leq 0.5$, that is, one Hg(II) per two bases; and a second for $r > 0.5$. It is probable but not certain that upon complete formation of the second complex there is one Hg(II) bound per base. Similar spectral results and a similar interpretation were first reported by Thomas (op. cit.) for calf thymus DNA; Yamane and Davidson (op. cit.) have observed them for a number of DNA's of different GC:AT ratio. These spectral shifts and the other chemical studies indicate that the mercuric ion is interacting with the purine and pyrimidine bases rather than with the phosphate groups.

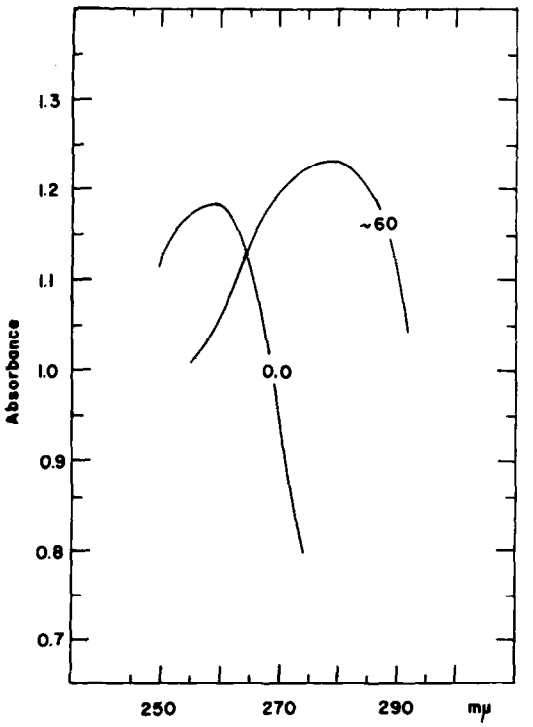


Figure 1b. Ultraviolet absorption spectra of non-mercurated and mercurated transforming DNA; r, ca. 60.

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Transforming DNA from a streptomycin-, optochin-, and bryamycin-resistant strain of D. pneumoniae was prepared by the Marmur technique

(Marmur, 1959). The DNA samples, at a concentration of 45 $\gamma$/ml, were made up as follows: The test sample was dissolved in 0.1 F $NaClO_4$, 0.01 F $HgCl_2$ (giving a ratio of added $HgCl_2$ to bases of ca. 60), and 0.001 F phosphate buffer at pH 6.8. Ultra-violet absorption spectra (Fig. 1b) indicate that the DNA has been fully complexed to form the "second complex". The control sample was identical, except that no $HgCl_2$ was added. The two samples were dialyzed for 6 hours against 3 changes of 0.01 F cysteine, 0.1 F NaCl, and then for 15 hours against 4 changes of 0.1 F NaCl, all at 4°C.

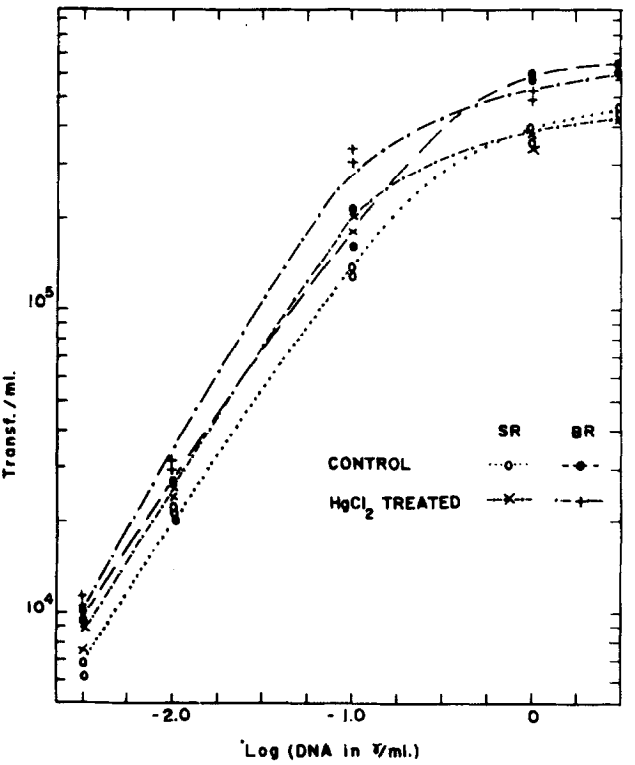


Figure 2. Comparison of titration curves obtained with $HgCl_2$-treated and control samples of transforming DNA containing the streptomycin- and bryamycin-resistance factors.

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Competent cells for transformation were prepared by transfer of a logarithmically-growing culture from a complete medium into a charcoal-adsorbed medium containing albumin. At the onset of competence, trans-

forming DNA was added to such a culture and incubated at 37°C for 20 minutes. These reaction cultures were then plated onto blood agar at appropriate dilutions and incubated at 37°C for two hours to develop the resistant phenotype. The plates were then overlayered with antibiotic-containing agar, incubated overnight, and counted.

Figure 2 represents an assay for activity in transformation to streptomycin- and bryamycin-resistance in the test and control samples. It is clear that no transforming activity is lost in the complete cycle of mercuration and demercuration of transforming principle. Taken together with the physical reversibility this indicates that, if the base pairing and integrity of the DNA molecule are ever lost in the transition caused by interaction with mercuric ion, they are completely recovered on removal of mercuric ion. We did not endeavor to test the transforming activity of the DNA-Hg(II) complex itself since the test organisms and other component of the transformation system contain many strong complexers of Hg(II), making the interpretation of any results ambiguous.

Thus, the mercuration of DNA may be a useful tool for the study and fractionation of DNA preparations since it is completely non-destructive.

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