THE HEAT OF DENATURATION OF DNA

Sirs:

Measurements of the changes in enthalpy accompanying the acid denaturation[1] of sodium desoxyribosenucleic acid (DNA) have been made by a previously described technique.[2] The DNA employed, (isolated from salmon testes by the method B of Simmons[3]) had a weight-average molecular weight[4] of 6.3×10^6. Determinations of the enthalpy changes on mixing solutions of DNA in 0.1 *M* NaCl with HCl in 0.1 *M* NaCl were made at 5, 25 and 40°. The results, shown in Fig. 1 for 25°, are qualitatively similar at all three temperatures: the entire enthalpy change occurs in the narrow *p*H range associated with the macromolecular configuration change.

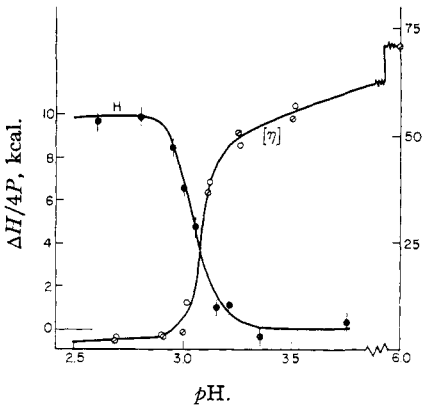

Fig. 1.—The apparent heat content and intrinsic viscosity of DNA as a function of *p*H at 25° and $\Gamma/2 = 0.1$: *M* ●: heat absorbed (per 4 moles nucleotide phosphorus) when solutions of DNA at *p*H 6 are mixed with HCl solutions to give the indicated final *p*H values; ○, ⊖: intrinsic viscosity (zero gradient) at the indicated *p*H.

The calorimetric data at *p*H values above and below the denaturation region are consistent with the view that the heats of ionization of the bases in both native and denatured DNA are close to zero. Independent evidence[1b,c] supports this conclusion with respect to denatured DNA. If we assume negligibly small heats of ionization, and enthalpy changes proportional to the extent of denaturation, then we may, with the aid of titration curves at each temperature, obtain the dependence of the extent of denaturation on the extent of proton binding. This is given in Fig. 2. It is seen that as many as two-thirds of the possible protons may be bound by the DNA molecule without causing any enthalpy change and without causing denaturation.

This fact is of interest in connection with the helical structure[5] of DNA. It is to be expected that heat should be absorbed when a strong hydrogen bond is broken. If hydrogen bonds are responsible for maintaining the helical structure, then it would appear that protonation of the donor group in a hydrogen bond does not necessarily break the bond. Thus, the denaturation of DNA which takes place when a sufficient number of protons is bound, cannot be considered as evidence for the existence of hydrogen bonds between the base pairs in the native molecule.

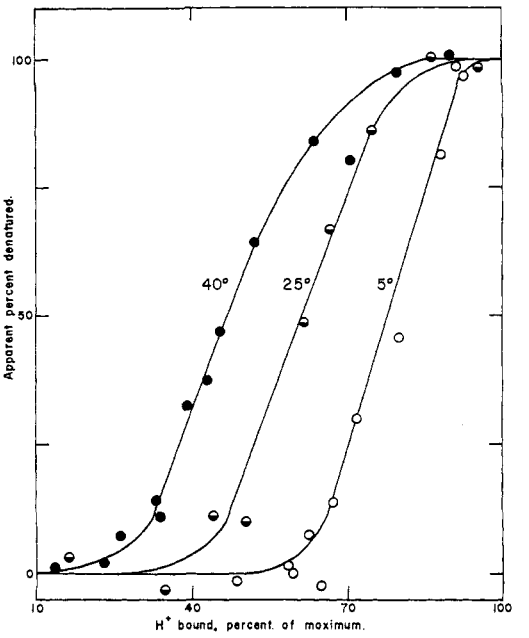

Fig. 2.—The extent of denaturation of DNA as a function of the temperature and the extent of proton addition to the bases. The extent of denaturation is computed on the assumption that the enthalpy change is proportional to the extent of denaturation.

(1) For recent investigations see (a) P. Doty, *J. Cell. Comp. Physiol.*, **49**, (Suppl. 1), 27 (1957); (b) L. F. Cavalieri and B. H. Rosenberg, THIS JOURNAL, **79**, 5352 (1957); (c) R. A. Cox and A. R. Peacocke, *J. Polymer Sci.*, **23**, 764 (1957); (d) A. R. Mathieson and S. Matty, *ibid.*, **23**, 747 (1957); (e) H. K. Schachman, *J. Cell. Comp. Physiol.*, **49**, (Suppl. 1), 71 (1957).

(2) A. Buzzell and J. M. Sturtevant, THIS JOURNAL, **73**, 2454 (1951).

(3) N. S. Simmons, A.E.C. Report, U.C.L.A., 184 (1952), and private communication.

(4) See E. P. Geiduschek, *J. Polymer Sci.*, in press, for additional properties.

(5) J. D. Watson and F. H. C. Crick, *Nature*, **171**, 737 (1953).

(6) Contribution No. 1479. This research was aided by grants from the National Science Foundation and the United States Public Health Service.

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KANAMYCIN. III. KANAMYCIN B

Sir:

Kanamycin fermentations yield a second antibiotic, designated kanamycin B,[1] similar in properties to kanamycin.

Kanamycin B has been isolated in pure form by countercurrent distribution of the salicylidene derivatives of the crude antibiotic mixture, using a methanol–water–chloroform–benzene (5:4:2:1) system, and by chromatography of the crude antibiotic on columns of Amberlite XE-64(NH_4^+)[2]

(1) M. J. Cron, D. L. Johnson, F. M. Palermiti, Y. Perron, H. D. Taylor, D. F. Whitehead and I. R. Hooper, THIS JOURNAL, **80**, 752 (1958).

(2) A product of the Rohm & Haas Co.