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STUDIES ON THE SECONDARY STRUCTURE OF NUCLEIC ACIDS BY MEANS OF OPTICAL ROTATION*

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THE success of employing optical rotation and rotatory dispersion methods for studying secondary structures of proteins and polypeptides has been well demonstrated in the literature,[1-3] as well as in reports given in this conference. Recently this technique also has been applied profitably to a study of the secondary structure of nucleic acids.[4-7] By the use of this method we have been able to obtain certain interesting information about nucleic acids *in vitro*, for it was found that the organic solvents formamide and dimethyl sulfoxide have profound effects on the secondary structure of DNA and RNA.‡ At times, it was necessary to supplement optical rotation data by measurements of ultraviolet absorption of these solutions or by analyses of the analytical ultracentrifuge.

The specific optical rotation, $[\alpha]$, of a solution containing equimolar amounts of deoxyribonucleotides and ribonucleotides is low (slightly levorotatory, depending on the isomers of the nucleotides used) with respect to that of the individual nucleotides, and it is essentially independent of temperature between 25° and 90°. On the other hand, the specific rotation of either the biosynthetic polynucleotides or the naturally occurring nucleic acids is strongly dextrorotatory (see Fig. 1 for pea microsomal RNA) and is greatly influenced by temperature. The decrease of dextrorotatory optical rotation of these nucleic acids has been shown to be related to the rupture of intramolecular hydrogen bonds and the transformation of helical conformation to random conformation[4-7] reminiscent of that of the polypeptides.[1,3]

The specific optical rotation of calf thymus DNA as a function of temperature is given in Fig. 2A, the results showing close agreement with those published by the Harvard group.[5] The specific rotation is only weakly dependent on temperature§ until near 80° in this particular buffer, then it drops abruptly within a narrow zone of temperature referred to as the "melting temperature" or "temperature of transition". The DNA, once so heated, gives a rotation vs. temperature curve different from

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‡ Abbreviations: DNA = deoxyribonucleic acid; RNA = ribonucleic acid.

§ The small increase of optical rotatory power before the transition temperature was also observed before.[5] No clear explanation has been suggested.

[1] E. R. Blout in *Optical Rotatory Dispersion* (Edited by C. Djerassi) p. 238. McGraw-Hill, New York (1959).
[2] J. T. Yang and P. Doty, *J. Amer. Chem. Soc.* **79**, 761 (1957).
[3] E. R. Blout, P. Doty and J. T. Yang, *J. Amer. Chem. Soc.* **79**, 749 (1957).
[4] P. Doty, H. Boedtker, J. R. Fresco, B. D. Hall and R. Haselkorn, *Ann. N. Y. Acad. Sci.* **81**, 693 (1959).
[5] P. Doty, H. Boedtker, J. R. Fresco, R. Haselkorn and M. Litt, *Proc. Natl. Acad. Sci.* **45**, 482 (1959).
[6] J. R. Fresco, *Trans. N. Y. Acad. Sci.* Ser. II, **21**, 653 (1959).
[7] J. R. Fresco, *Fed. Proc.* **18**, 904 (1959).

that of non-heated DNA, indicating irreversibility in the process of loss of helical conformation. The once-heated DNA exhibits cyclic reproducibility of rotation vs. temperature curves between 25° and 90°, with the absence of any narrow zone of transition temperature. A comparable result was obtained from the measurement of the temperature dependence of optical density for the DNA solution (hypochromic effect) as shown in Fig. 2B. An increase of optical density of nucleic acid solutions had also been related to the loss of hydrogen bonding and helical structure. The once-heated

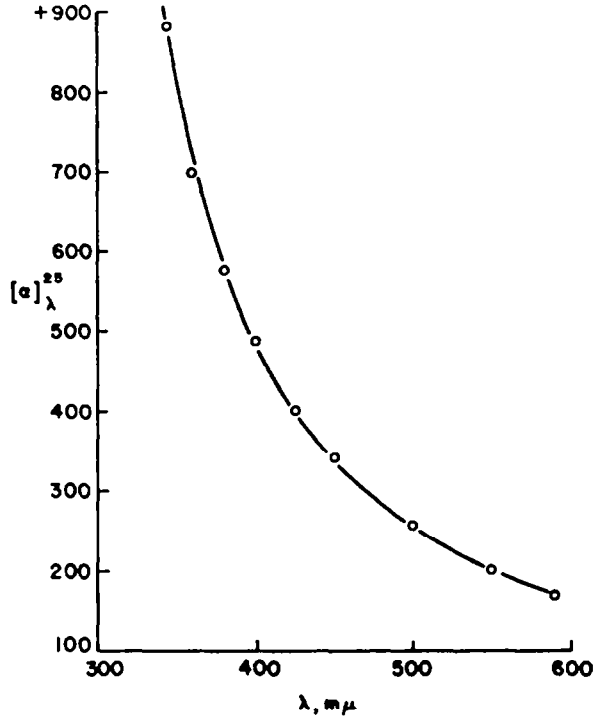

FIG. 1. Optical rotatory dispersion of pea microsomal RNA.

DNA again gave reproducible optical density vs. temperature curves cyclically, without a sharp transition zone of temperature, in contrast to the behavior of the unheated DNA. In this communication, any DNA will be referred to as denatured if it gives the rotation or optical density characteristics of that of the heated DNA (above 90° for 15 min).

In contrast to the DNA pattern, both the specific rotation vs. temperature and optical density vs. temperature curves of ribosomal RNA are smooth, without a narrow zone of transition temperature (Figs. 3A, B). Furthermore, they are essentially reversible, with only slight changes in magnitude and general shape of the curves.* In other words, although the RNA at 25° is likely to have a higher degree of hydrogen bonding and helical conformation than the RNA at 90°, the heated and unheated materials cannot be distinguished from each other by the dependence of their optical rotation or optical density on temperature; another measurement is required to identify the material with a history of heat treatment.

* Heating RNA in the buffer used for these measurements caused a drop of about 5% in the specific optical rotation after 20 min at 95°.

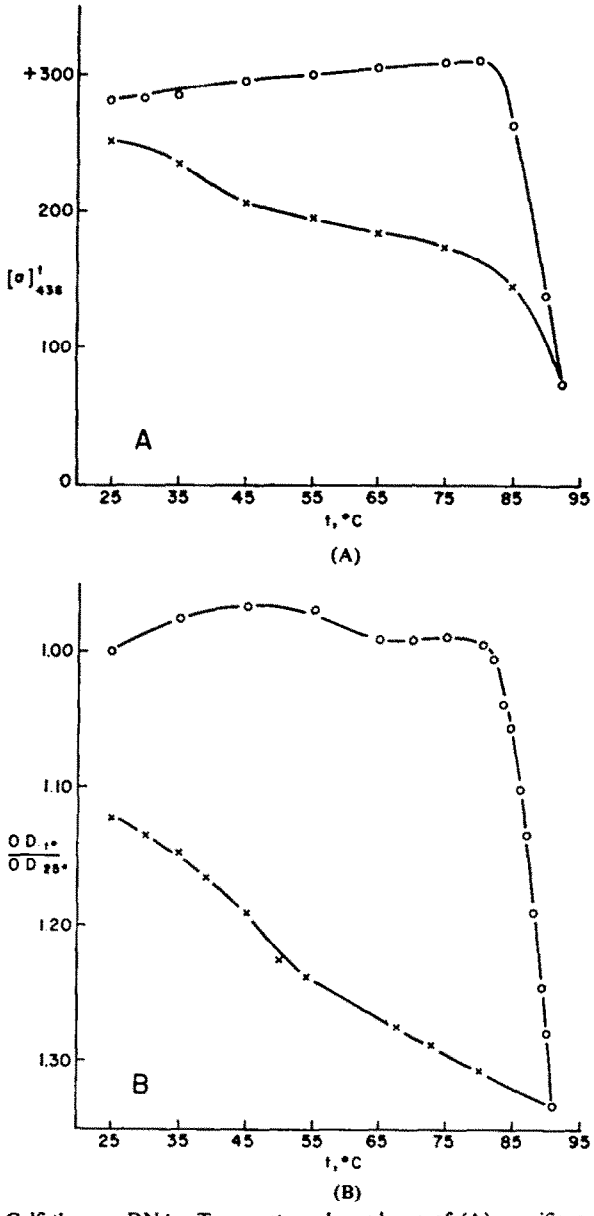

Fig. 2. Calf thymus DNA. Temperature dependence of (A) specific optical rotation, and (B) optical density at 260 mμ.

Upon examination in the ultracentrifuge, the pea microsomal RNA consists of two components with sedimentation coefficients of 27–28S and 17–18S (Fig. 4A).[8] After heat treatment at 85–95° for 10–15 minutes, it exhibits only a broad boundary of about 8–11S (Fig. 4B). Therefore, by analyses of ultracentrifuge, it is possible to detect RNA which has been once heated to a point where it has lost its intramolecular hydrogen bonding and original helical structure.

[8] P. O. P. Ts'o and R. Squires, *Fed. Proc.* **18**, 341 (1959).

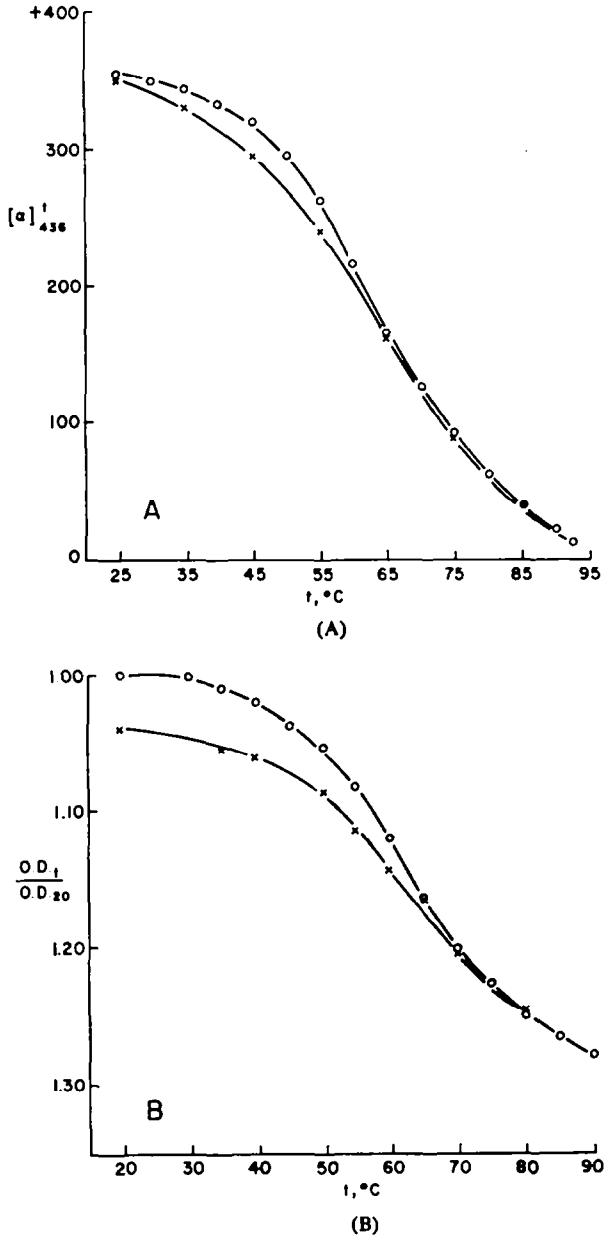

FIG. 3. Pea microsomal RNA. Temperature dependence of (A) specific optical rotation, and (B) optical density at 260 mμ.

The specific rotation vs. temperature curves given by DNA and RNA in formamide, dimethyl sulfoxide, and buffer are shown in Figs. 5A, B. The specific rotations of either DNA or RNA in these organic solvents are very low, or even negative, and essentially independent of temperature. These results suggest the loss of helical conformation of these nucleic acids while in solution in these organic solvents. The uncertainty

of other solvent effects on the optical rotation does exist, however, since free nucleotides in formamide or dimethyl sulfoxide tend to give more negative values than those obtained in aqueous solution.

After DNA and RNA had been dissolved in the two indicated organic solvents at room temperature, and without any heating, they were recovered by precipitation procedures and redissolved in aqueous buffer. It was apparent that the graphs of specific optical rotation vs. temperature and optical density vs. temperature from this recovered DNA (Figs. 6A, B) have the same characteristics as those of heat-denatured

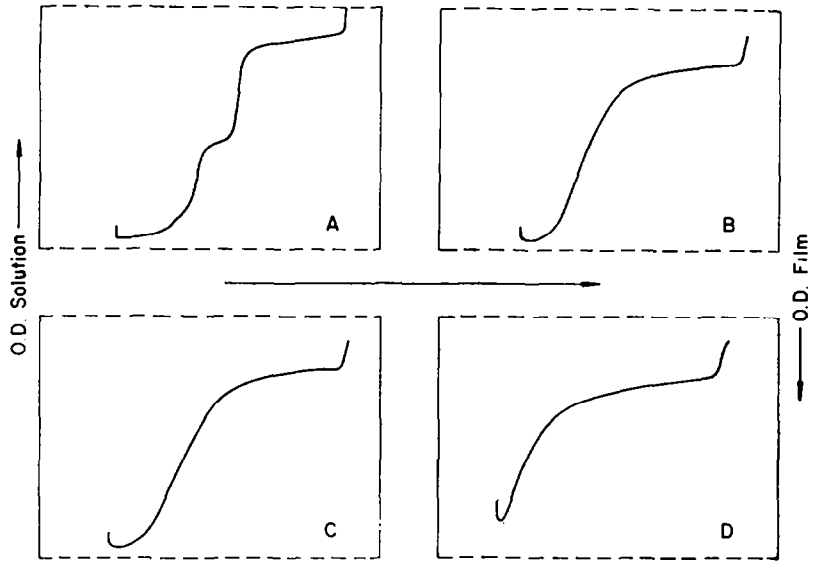

FIG. 4. Sedimentation patterns of pea microsomal RNA. (A) native, (B) heat denatured, (C) recovered from formamide, (D) recovered from dimethyl sulfoxide.

DNA, even though the DNA had never been heated higher than about 25° while in any solution. The data, therefore, does indicate that DNA in solutions of formamide and dimethyl sulfoxide at low temperatures loses most of its helical conformation just as if it had been in aqueous buffer at 90°.

Again in contrast to the behavior of DNA, the graphs of specific rotation vs. temperature (Fig. 7) and optical density vs. temperature for the recovered RNA are very similar to that of the original RNA. This is not surprising since the curves of heated and unheated RNA also show no variation. Ultracentrifuge analysis, however, shows the recovered RNA with a broad boundary with an average sedimentation coefficient of 8–10S (Fig. 4C, D). Thus the ultracentrifuge pattern of the RNA recovered from the organic solvents is similar to that of the heated RNA (Fig. 4B) but vastly different from that of the native, unheated RNA (Fig. 4A). These results again suggest that RNA in solution of these two organic solvents at room temperature loses most of its ordered secondary structure.

Titration of buffer solutions of the nucleic acids with formamide or dimethyl sulfoxide at a given temperature was followed by means of specific rotation measurements. DNA gave a sharp transition zone at 58 volume per cent (28 mole per cent*)

* Deviations in volume from ideality of up to 3% were not considered in making calculations of specific rotations.

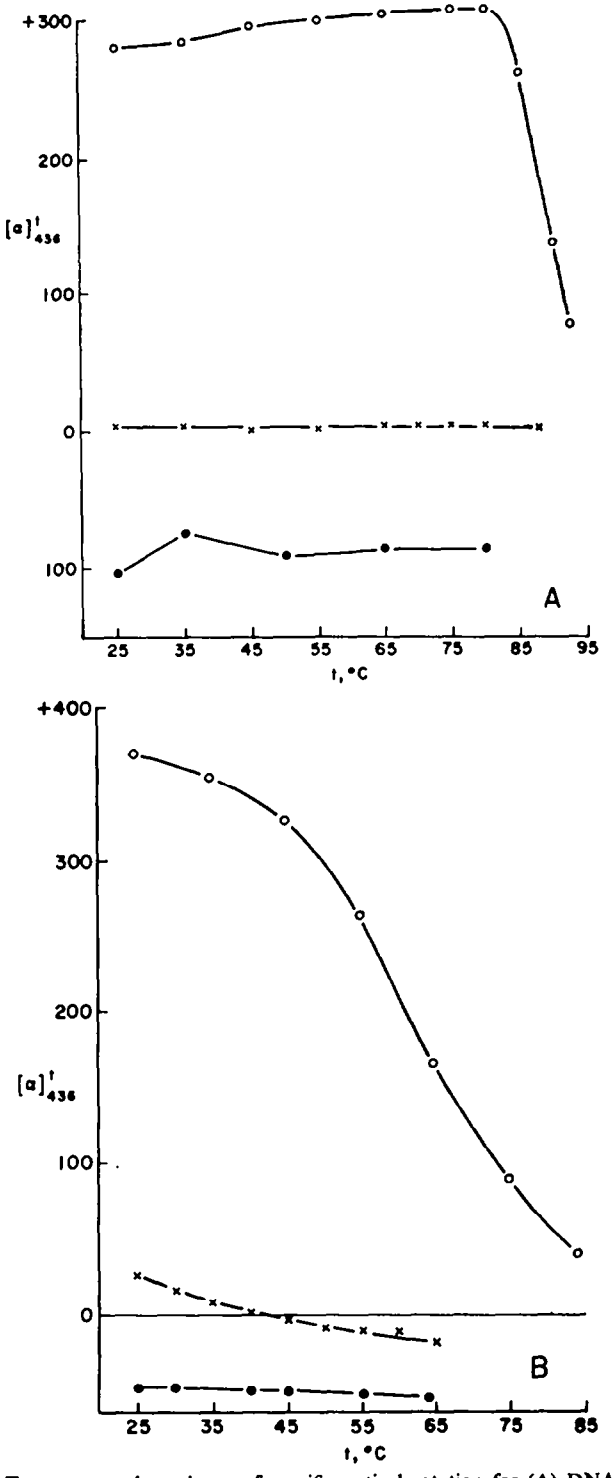

FIG. 5. Temperature dependence of specific optical rotation for (A) DNA, and (B) RNA; -- ×—×—, in formamide; —●—●—, in dimethyl sulfoxide; —○—○—, in buffer.

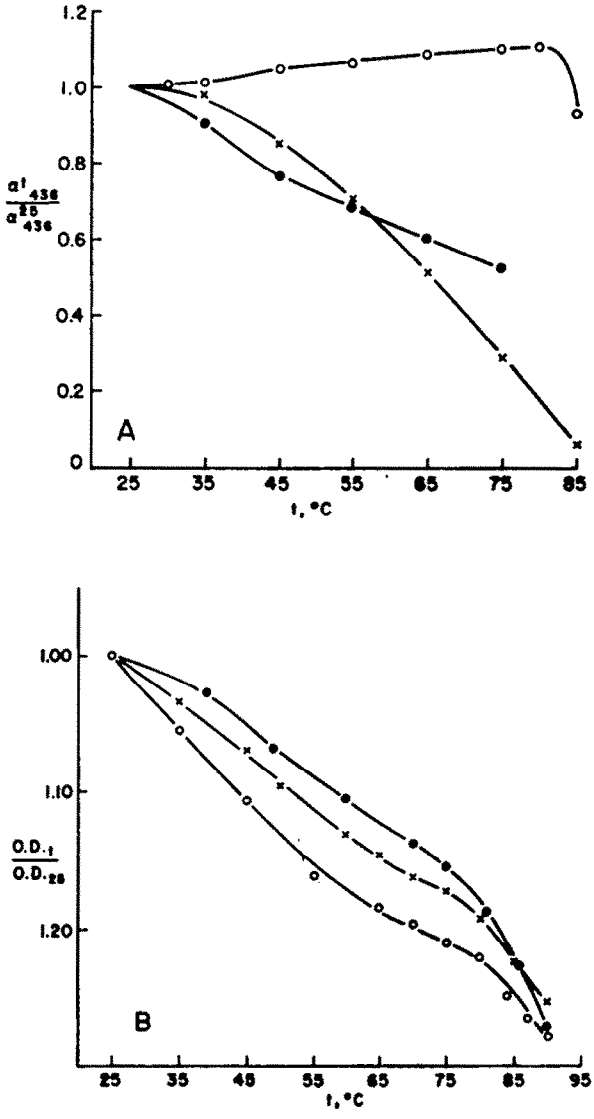

Fig. 6. Temperature dependence of (A) specific optical rotation and (B) optical density for calf thymus DNA. —○—○—, native in (A), heat denatured, in (B); —×—×—, formamide denatured; —●—●—, dimethyl sulfoxide denatured.

dimethyl sulfoxide and 70 volume per cent (51 mole per cent*) formamide (Fig. 8). RNA, on the other hand, gave a curve (Fig. 9) which, with appropriate coordinate scale, could be superimposed on that of the typical rotation vs. temperature curve. It should be mentioned that the DNA solution in an organic-aqueous mixture having the same composition as that for the transition zone exhibited a decrease of specific rotation with time which finally reached an equilibrium value. The kinetics of the denaturation process could then be studied.

The system of nucleic acid solutions in mixtures of buffer and formamide or

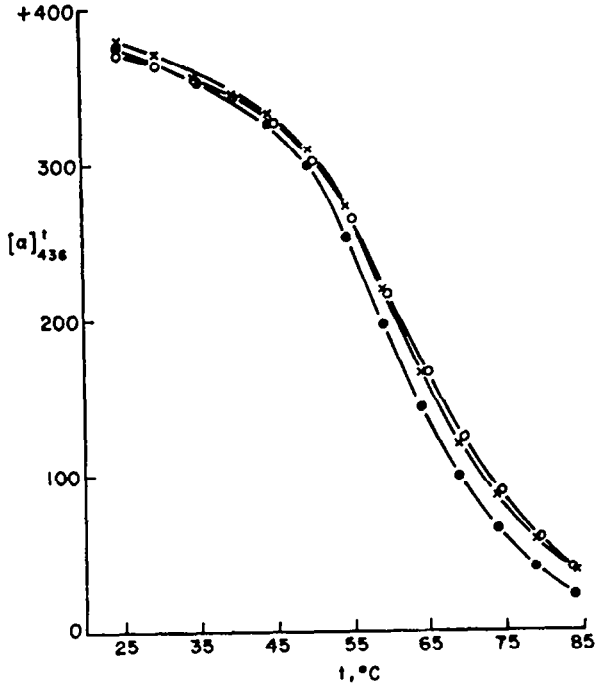

FIG. 7. Specific optical rotation vs. temperature for RNA denatured by: —○—○—, heat; —×—×—, formamide; —●—●—, dimethyl sulfoxide.

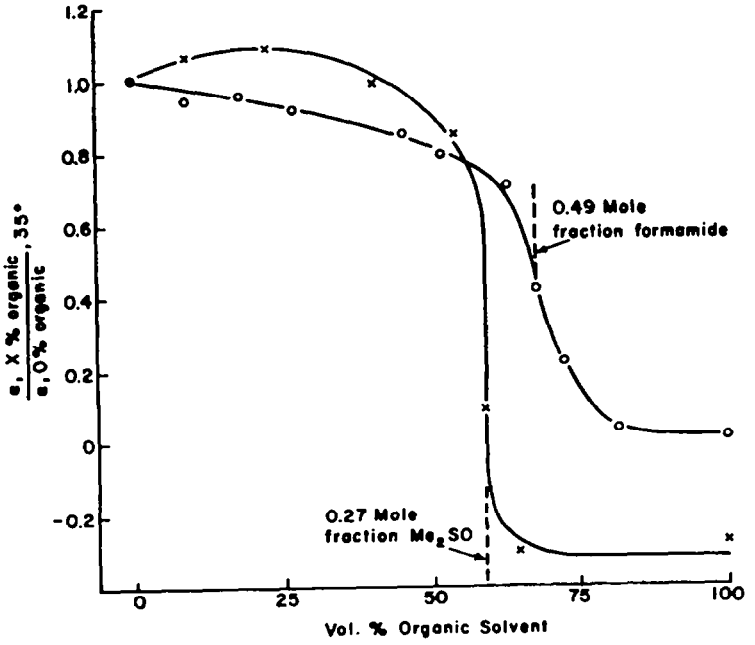

FIG. 8. Titration of DNA in buffer with: —○—○—, formamide, and —×—×—, dimethylsulfoxide.

dimethyl sulfoxide is reminiscent of that of the synthetic polypeptides such as poly-γ-benzyl glutamate in chloroform-dichloroacetic acid.[1,8] The helix-coil transformation in the case of the synthetic polypeptides is reversible, but that of the nucleic acids is not. Thus, a process of denaturation for both DNA and RNA is available at room temperature. In this process, the danger of unwanted chemical or enzymatic hydrolyses accompanying the rupture of hydrogen bonds is likely to be avoided. Information of the physical chemical properties of nucleic acids in these solvents (such as molecular weight) will be of great interest.

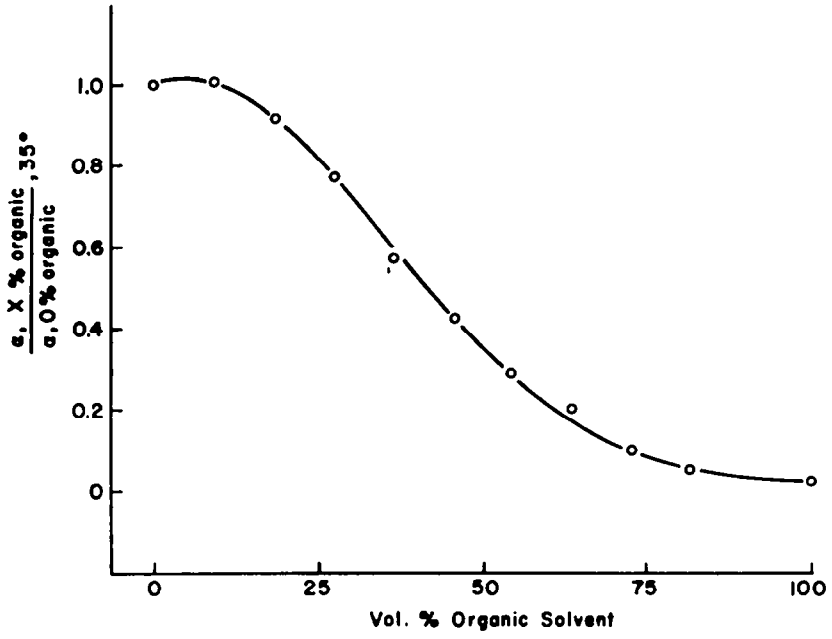

Fig. 9. Titration of RNA in buffer with formamide.

EXPERIMENTAL

Materials and reagents. All of the DNA used for the described experiments was calf thymus DNA purchased from Nutritional Biochemical Corporation, and some of its properties have previously been described.[9] Aqueous solutions of about 0·1% were prepared by mixing solvent and DNA in a slowly rotating flask for two days at room temp. Formamide solutions were prepared similarly, but the rate of solution was considerably higher. Dimethyl sulfoxide failed to show any significant effect on DNA after several days, even when the temp was raised to about 40°; solvation was effected by the addition of 20% by volume of water and rotating the mixture for 12 hr. Most of this water was removed by partial distillation at reduced pressure to about $\frac{1}{4}$ original volume. DNA could be precipitated from formamide solution by the addition of 2 volumes of 100% ethanol and $\frac{1}{4}$ volume absolute ethyl ether at 0°; or the formamide could be replaced directly by buffer solution by 3 dialyses over a period of 5 days in 20-fold amounts of buffer at 0°. The addition of 4 volumes of benzene precipitated DNA from dimethyl sulfoxide. Measured physical constants in buffer solution were $[\alpha]_D^{25}$ +135°, and $[\alpha]_{436}^{25}$ +260°.

Pea microsomal RNA was prepared according to the method described previously.[9] Immediately after preparation it was quick-frozen in buffer solution and stored at −50° until needed. Solutions in the 2 organic solvents could be prepared directly from the precipitated RNA (isolated from buffer solution by the addition of 3 volumes of cold ethanol and washing with absolute ethanol). Isolation from formamide solution was carried out by precipitation with 2 volumes of buffer and 6 volumes of

[9] W. F. Dove, F. A. Wallace and N. Davison, *Biochem. Biophys. Res. Com.* **1**, 312 (1959).

ethanol at ice temp. Last traces of solvent could be removed by repeated washing with absolute ethanol or ethyl ether; or by redissolving the precipitate in buffer and separating it a second time by alcohol dilution. The addition of 2 parts benzene and 2 parts ethanol (ethyl ether can be substituted for benzene) brought about precipitation of RNA from dimethyl sulfoxide. Measured physical constants were $[\alpha]_D^{25}$ +170°, and $[\alpha]_{436}^{25}$ +370°.

The organic solvents were 99% formamide (Eastman White Label), and practical grade dimethyl sulfoxide (Eastman) with a m.p. of about 18°. The aqueous solvent was an 0·1 M acetate buffer at pH 5·5 containing 0·1 M sodium chloride and 0·001 M magnesium chloride.

Instrumentation and methods of analysis. Measurements of optical rotations were made on a Rudolph Model 200S polarimeter with oscillating polarizer and xenon and mercury arc lamps. The polarimeter tubes were of unitized glass construction with water jacket, center-fill device, and quartz windows. The windows were sealed to the optically ground tube ends with an epoxy resin, Epocast 502. Although some problem of strain birefringence may have been inherent in the system, it was possible to reproduce zero readings at various temperatures with pure solvents in the polarimeter tubes. In most of the measurements it was possible to duplicate results on the instrument to better than ±2%.

Optical density measurements were made with a Beckman DK-2 ultraviolet spectrophotometer fitted with a modified temperature control device. Quartz cells were fitted with 20 mm immersion, standard taper thermometers for direct reading of solution temperatures. The cell compartment cover was adapted to pass the calibrated portion of the thermometer.

Analysis for the concentrations of nucleic acids was made indirectly by the colorimetric phosphate determination described by Allen[10], employing a Beckman DU spectrophotometer. Direct perchloric acid digestions were carried out only on the aqueous and formamide solutions. The thymus DNA was calculated to contain 9·30% phosphorus[11] and the microsamal RNA 9·09%.[12]

Analytical ultracentrifugation was performed in the Model E ultracentrifige, Spinco Division, Beckman Inc., with phase-plate schlieren optics and with ultraviolet absorption optics. The instrument was provided with a temperature control system. The absorption patterns were taken on commercial film with a 20 second exposure time on solutions of $O.D._{260}$ of 1·0 in 12 mm Kel-F cells. The photometric records were within the linear range of the characteristic curve of the film as shown by the trace of exponential aperture[13] in the counter balance cell in each run. The films were traced with a Double-Beam Recording Microdensitometer, Joyce Lobel Co., Newcastle upon Tyne, England. The direction of sedimentation of all the patterns is from left to right.

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[10] R. J. L. Allen, *Biochem. J.* **34**, 858 (1940).
[11] E. Chargoff in *Nucleic Acids* (Edited by Chargoff and Davidson) Vol. II, p. 335. Academic Press, New York (1955).
[12] J. W. Wallace and P. O. P. Ts'o, unpublished data.
[13] E. Robkin, M. Meselson and J. Vinograd, *J. Amer. Chem. Soc.* **81**, 1305 (1958).