of Berns and Thomas[1] with *H. influenzae*, *S. marcescens*, and *E. coli*, although the size of the DNA is somewhat different. Whether this DNA-protein assembly is the equivalent of the chromosome of genetics or of the nuclear body of cytology, and what its precise relation is to the linear structure seen by Cairns[17] by autoradiography are interesting questions whose answers are not yet certain.

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*THE USE OF HOT PHENOL IN PREPARING DNA**

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Extraction with phenol has been widely used to remove proteins when preparing DNA, and hot phenol seems to be especially effective, as described, for example, in a preceding paper.[1] However, since phenol also lowers the melting point of DNA, it is necessary to avoid too high a temperature. This note reports experiments on the effect of various concentrations of phenol on DNA solutions, and proposes a method for efficient and safe extraction of such solutions with phenol.

Materials and Methods.—These were mainly as described in the preceding paper.[1]

Buffers were either phosphate or borate, approximately 0.01 M, with 0.001 M EDTA and enough added NaCl to bring the total sodium concentration to 0.195 M. Calf thymus DNA with an intrinsic viscosity of 83 dl/gm was purchased from Nutritional Biochemicals Company. DNA solutions containing phenol were prepared by mixing a small quantity of DNA solution with a large quantity of phenol solution to avoid temporary large excesses of phenol. For the melting experiments a rotor with a shear stress of 0.007 dynes/cm^2 (about 35 sec/rev in water) was used in the viscometer.

Results.—The effect of phenol on DNA was observed through measurement of the viscosity of the solutions as a function of temperature, since viscosity is sensitive to the intactness of the DNA helix. It was first established that the pH of the solution had no material effect on the DNA melting point in the range of pH from 7 to 10. At the higher pH it was possible to dissolve large quantities of the otherwise sparingly soluble phenol because of its weak acidity. The effect of different concentrations of phenol on the melting point of the DNA was then studied.

Results are shown in Figure 1. Increasing amounts of phenol lower the midmelting point from 85 to 55°C. The peculiar phenomenon that sets in above 16.5 per cent phenol is precipitation of the DNA; this was proved by centrifuging the very fine precipitate out of the solution and identifying it. At 18 per cent phenol the precipitate redissolves on heating to 40°C. Another kind of precipitate, apparently aggregated denatured DNA, sometimes forms when concentrated solutions are heated well above the melting point and recooled. However, the viscosity of the solution is at least 95 per cent recovered on recooling if the solution is heated only to about the midmelting temperature, to the points marked on the figure with horizontal arrows. The viscosity is 50 per cent recovered on recooling even if the heating is to the higher temperatures marked with the vertical arrows.

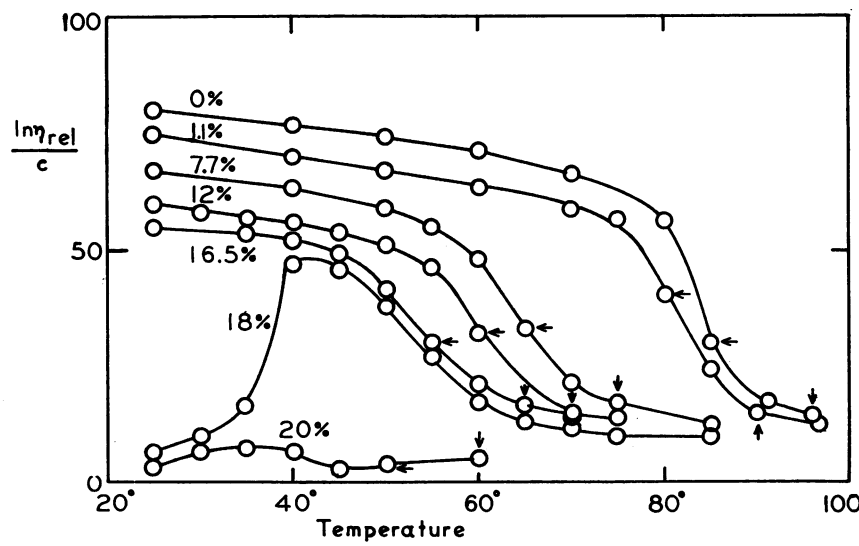

FIG. 1.—Viscosity of DNA solutions in water-phenol-salt mixtures as a function of temperature. The ordinate is the natural logarithm of the ratio of the viscosities of the solution and of solvent all divided by the concentration of DNA in grams per deciliter. The numbers on the curves are the weight-% phenol. The pH was 9.0–9.5 by glass electrode; the DNA concentration was between 100 and 400 μg/ml. The arrows are explained in the text.

Method of purification of DNA: Although we showed in the previous paper[1] that the hot phenol extraction leads to excellent results in the purification of DNA, it is obvious that the uncontrolled addition of too much phenol to a hot solution could lead to the denaturation of the DNA. To avoid this danger we have lately adopted the following protocol.

The DNA-containing material is suspended in a "1-molar" pH 7 buffer (NaCl, 1 *M*; EDTA, 0.001 *M*; NaH_2PO_4, 0.002 *M*; Na_2HPO_4, 0.006 *M*) and warmed to 55°C in a glass centrifuge tube in a water bath. An equal volume of buffer-saturated phenol is then added dropwise and the mixture allowed to stand at 55°C for about 5 min. At this temperature the phenol phase has practically the same density as the salt solution, so that phenol drops remain suspended throughout the solution and all parts of the solution come in close contact with the phenol without the need of shaking. The solution is then chilled and centrifuged cold to remove the suspension of fine reprecipitated phenol and denatured protein, if any. The phenol treatment may be repeated as many times as desired. Finally, the supernatant is transferred with a wide-mouthed pipette and the dissolved phenol is dialyzed away.

The solubility of phenol in the "1-molar" buffer at 60°C was found to be somewhat less than 6 per cent. The curves of the accompanying figure show that DNA is safely below the irreversible melting point at this temperature and phenol concentration at 0.195 *M* salt, and even more so at 55°C in the presence of 1 *M* salt, which latter has a stabilizing effect on DNA.[2, 3] Therefore, no critical control of the temperature or of the amount of phenol is required, and rough handling, e.g., shaking, is also unnecessary.

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*OVER-ALL SYNTHESIS OF ISOLEUCINE BY MEMBRANE FRACTIONS OF SALMONELLA TYPHIMURIUM**

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The properties of each of four enzymes—condensing enzyme, reductoisomerase, dihydroxy acid dehydratase, and transaminase—required for the conversion of pyruvate to valine or of pyruvate plus α-ketobutyrate to isoleucine have been characterized by a number of investigators for a variety of different organisms ranging from bacteria and fungi to higher plants. However, it has been demonstrated recently that these four enzymes are associated with the mitochondrial fractions of *Neurospora crassa;* these fractions contain a functional complex capable of converting substrate through the several enzymatic reactions to end product.[1-3]

Following this finding, it was of interest to investigate the possibility of subcellu-