concentrated nucleoprotein of the intact nucleus. This is, in fact, observed since thymus homogenates heated for 2 minutes at 70°C do not give rise to high viscosity solutions when extracted with strong saline.

SUMMARY

Heating nucleoprotein extracts causes a decrease in viscosity that is proportional to the time of heating. Linear Arrhenius plots that yield an activation energy of 20 kcal/mole are obtained. The rate is concentration dependent (rate $\propto C^{1.7}$). The viscosity decrease is interpreted in terms of protein-DNA bonds in linear aggregates of protein and nucleic acid.

We are indebted to Dr. Norman G. Anderson for continued advice and encouragement, and for the facilities used in these studies.

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IV. EVIDENCE FOR THE EXISTENCE OF DNA-PROTEIN STRANDS IN INTACT NUCLEI

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PREVIOUS studies [4, 5] suggest that protein and deoxyribonucleic acid (DNA) are organized in the nucleus in such a manner as to yield on extrac-

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tion with 1 *M* NaCl a solution containing long strands of DNA interlinked by protein. It has been suggested that these nucleoprotein strands are the fundamental units of chromosome structure [1]. An alternate proposal, however, is that the strands are reassociation artifacts produced when DNA and protein

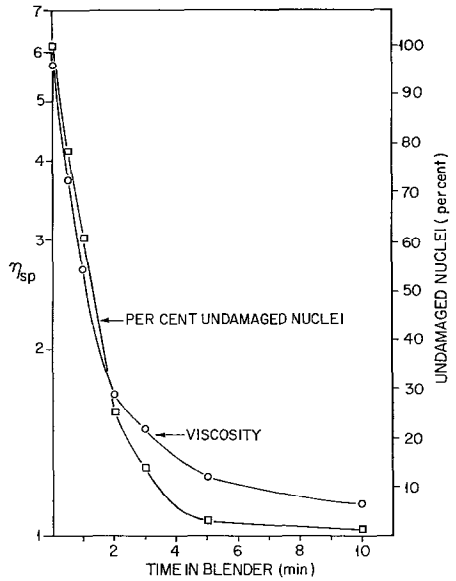


Fig. 1.—Effect of blending thymus homogenates in 0.14 *M* NaCl on the viscosity of subsequently prepared 1 *M* NaCl extracts and on the number of intact nuclei or cells remaining.

are extracted from the nucleus [6]. This view probably originated in the suggestion that nucleohistone, first dissociated in strong salt and then precipitated or reassociated by lowering the ionic strength, may not regain the original configuration or molecular arrangement [3]. However, the strands examined in the present study have not been reassociated by decreasing the ionic strength. To think of the strands as being produced during the extraction requires a number of assumptions concerning the original disposition and properties of the nuclear consituents. These assumptions are that (1) the protein and DNA involved in strand formation are spatially separated in the nucleus, (2) are released by strong salt, and (3) that the protein is able to find and cross-link the DNA molecules in solution.

The experiments described here that distinguish between these possibilities are based on the observation that the nucleoprotein of nuclei isolated in 0.14 *M* NaCl behaves as a gel [2] and does not dissolve even when extensively deformed or broken. If the multimolecular strands observed in solution in 1 *M* NaCl preexist in the nucleus, it would appear that they are cross-linked by

bonds readily broken by strong salt. If such a multistrand gel is extensively deformed, the long strands would very likely be broken before one strand could move over or past another. It would, in fact, be expected that the gelwork would be broken down into short cross-linked bundles of strands. It seems, therefore, that the two possibilities of strand origin may be distinguished by merely deforming the intact nuclei in a high shearing field and observing whether such shearing affects the viscosity of extracts subsequently prepared in 1 *M* NaCl.

EXPERIMENTAL

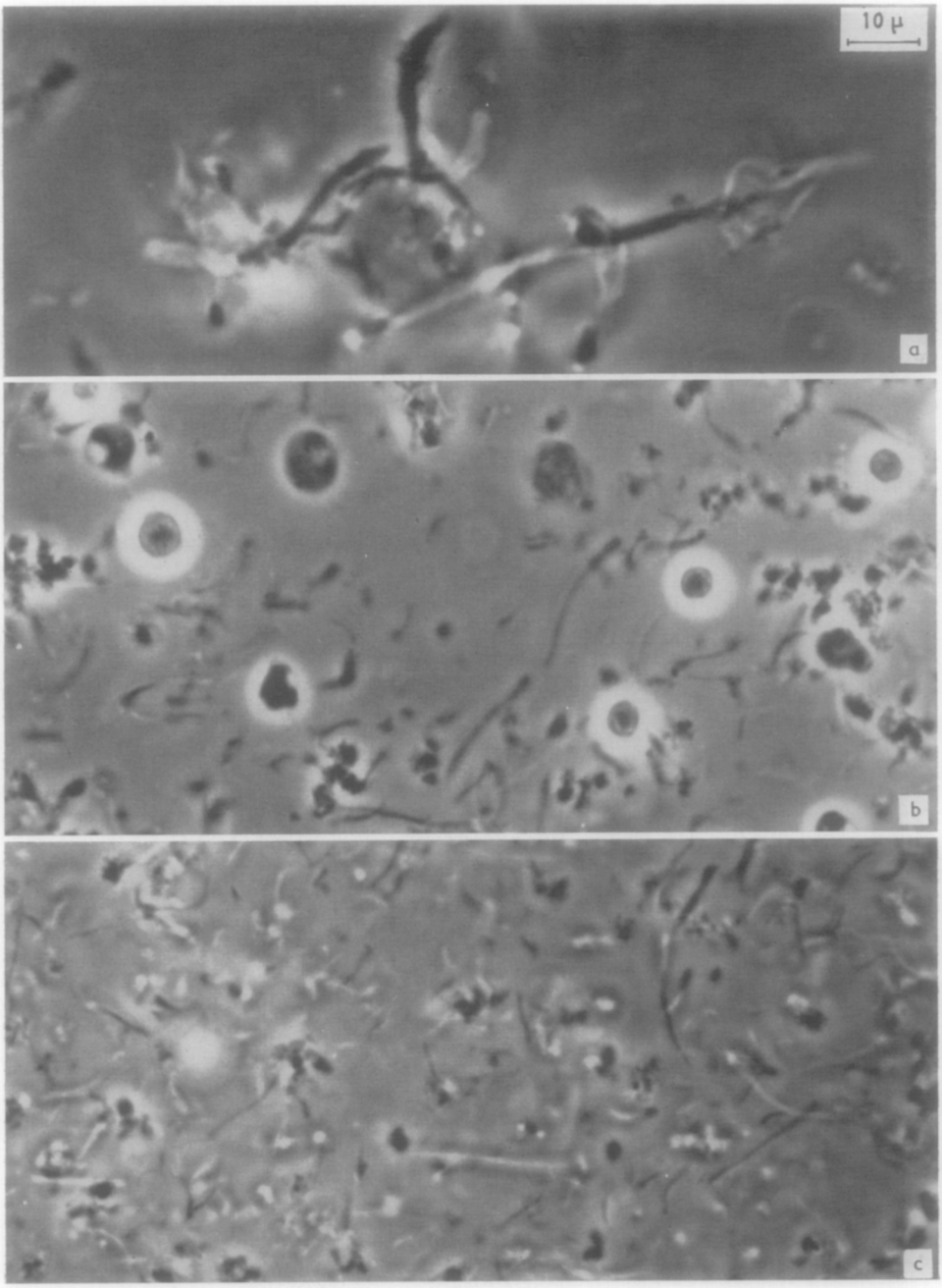
Rat thymus homogenates were prepared by the method previously described [4] to contain 0.455 per cent wet weight of tissue. The supension (ca 15 μg/ml of DNA) was then placed in a cold Waring Blendor and blended for varying periods of time at full speed. In some experiments the glass blending vessel was placed in a -4°C bath at intervals to keep the suspension temperature from rising above 6°C. Aliquots were removed at intervals for counting the numbers of intact nuclei in a hemocytometer cell, for photomicrography, and for extraction with strong salt in preparation for viscometry. The extracts were allowed to stand at room temperature for approximately 18 hours before use.

The results presented in Fig. 1, which are representative of the experimental findings, show that the viscosity-producing potentiality of the thymus suspension is directly related to the number of intact nuclei over a wide range. The viscosity is plotted on a logarithmic scale since previous studies show that the log of the viscosity is proportional to nucleoprotein concentration [4]. The shredded or "pulled out" nuclei were only slightly effective as shown by the fact that, after the nuclei were blended for longer periods of time, the viscosity continued to fall although almost all the nuclei had been damaged. Microscopic observation showed that the nuclei were either very extensively deformed or remained spherical (Fig. 2). Continued blending caused the thick nucleoprotein bundle to be pulled out into thinner shreds, which were apparently broken into shorter pieces. As the shreds became smaller the viscosity-producing capability decreased to a very low value. No difference in the viscosity-decreasing effect of shearing was noted when the entire 10-minute treatment was run continuously or when it was interrupted at intervals to cool the suspension.

Extracts of sheared nuclei examined at various times after extraction showed no decrease in viscosity suggestive of enzymatic degradation. The viscosity in each instance rose to a plateau during the extraction period, as described for intact nuclei [4].

DISCUSSION AND SUMMARY

The marked decrease in viscosity-producing potential of nuclei deformed by shearing strongly supports the concept that the high anomalous viscosity observed in fresh 1 *M* NaCl extracts is caused by preexisting linear DNA-protein aggregates or strands. The possibility that the viscosity decrease is


10 μ
a
b
c

caused by release of degradative enzymes from ruptured cytoplasmic particles is excluded by the observation that the viscosity produced by sheared preparations did not reach a high value initially or decrease with time.

The shearing nuclei were first elongated very considerably without apparent loss of mass, as observed in the phase contrast microscope. The absence of intermediate elliptical nuclei suggests that the nuclei resist deformation until a certain critical shearing force is reached, i.e., they exhibit a yield value. The bonds broken when this yield value is exceeded seem to be those that maintain the integrity of the nucleofibrils extractable with 1 *M* NaCl.

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Fig. 2*a*.—Thymus nucleus and nuclear shreds after blending 1 minute. Oil immersion, dark medium phase contrast.

Fig. 2*b*.—Effect of blending 2 minutes. High dry objective.

Fig. 2*c*.—Effect of blending for 5 minutes. Shreds appear much thinner than with less blending.