BRIEF NOTES

A Stabilizing Factor for Cytoplasmic Nucleoproteins.* BY MARY L. PETERMANN AND MARY G. HAMILTON. (*From the Sloan-Kettering Institute for Cancer Research and the Sloan-Kettering Division of Cornell University Medical College, New York.*)‡

The macromolecular nucleoproteins of rat liver cytoplasm give rise to a number of ultracentrifugal boundaries with sedimentation rates between 107 and 27 S (1). These are probably the same structures which appear as spherical particles 100 to 150 A in diameter in electron micrographs of thin sections of whole tissue (2). They contain about 45 per cent PNA (3). Since the uptake of labelled amino acids is most rapid in cell fractions containing these nucleoproteins (4), their isolation and characterization are of great importance to the elucidation of protein synthesis. Unfortunately, they are extremely unstable, and their instability increases with purification. One of the factors which influence stability is dialyzable; dialysis of resuspended nucleoprotein pellets (from mouse spleen) for only 3 hours results in a 25 per cent loss of the units which give the characteristic ultracentrifuge patterns (3). It has now been found that dialysates of rat liver cytoplasm have a stabilizing effect on these nucleoproteins, as described below.

All procedures except the ultracentrifugal analyses were carried out in the cold. The pulp from perfused livers of adult male Wistar rats (1) was homogenized in a Waring blendor at a speed which would not break the nuclei (5), in 4 to 6 volumes of 0.88 M sucrose (6), distilled water, or veronal-chloride buffer. Unbroken cells, nuclei, and mitochondria were removed by centrifuging at 20,000 *g* for 30 minutes when sucrose was used or for 15 minutes in water or buffer. The supernatant was dialyzed in 50 ml. lots against an equal volume of 0.79 M sucrose, distilled water, or buffer, for 16 hours at 5°C., on a rocking platform. These preparations will be referred to as sucrose-SF, water-SF, and buffer-SF. The buffer contained 0.05 M sodium chloride, 0.05 M sodium diethylbarbiturate, and 0.01 M diethylbarbituric acid, and had a pH of 8.6 and an ionic strength of 0.10.

For the nucleoprotein preparations mitochondria supernatant was prepared in 6 volumes of 0.88 M sucrose as described above, shell-frozen in dry ice and alcohol, and stored in a deep-freeze cabinet. As needed, a portion was thawed quickly by immersing the bottle in water at 37°, transferred to an ice bath, and titrated to pH 8.3 with 0.05 N NaOH in 0.79 M sucrose. One-tenth volume of 5 per cent sodium desoxycholate made up in 0.79 M sucrose at pH 8.3 was added to disrupt the microsome structure (4,7), and the nucleoproteins were sedimented at 105,000 *g* for 3 hours.

Ultracentrifugal analysis was carried

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out as described elsewhere (8). In these experiments the sedimentation rate of the principal boundary, B, varied between 27 and 65 S, depending on the protein concentration, the amount of sucrose present, etc. The sedimentation rates of the other components varied with that of B, so that fairly constant ratios were maintained; A′ moved about 1.8 times as fast as B, A 1.5, C 0.8, D 0.7, and E 0.6 times as fast. The amount of each component was estimated from the area under the boundary (8). No correction was made for the percentage of cells not broken during the homogenization. Since it now appears that component A consists of two particles of B in association (9), the sum of A and B has been taken to represent the total amount of B present.

Experiment I.—The effect of sucrose-SF on nucleoprotein stability during one cycle of sedimentation was first determined. Nucleoprotein pellets were prepared as described above. Three pellets, representing 33 ml. of mitochondria supernatant, were resuspended in 0.05 M $NaHCO_3$–0.05 M NaCl, pH 8.3, to a final volume of 4.5 ml. (the control, 1, in Table I); 4 pellets in 45 ml. of sucrose-SF, 2; and 4 pellets in 45 ml. of plain 0.79 M sucrose, 3. Both sucrose

TABLE I

The Effect of Stabilizing Factor on the Sedimentation Properties of the Cytoplasmic Nucleoproteins

Experiment No.		Treatment	Ultracentrifugal analysis				
			A′	A + B	C	D	E
			mg. per gm. of liver pulp				
I	1	Control	0.38	1.83	0.25	0	0.60
	2	Resedimented in sucrose-SF	0.23	2.05	0.35	0	0.50
	3	Resedimented in sucrose	0.07	0.84	0	0	0.94
II	1	Control	0.14	1.39	0.32	0	0.72
	2	Resedimented in water-SF + sucrose	0.15	1.78	0.25	0	0.46
	3	Resedimented in lyophil SF + sucrose	0.05	1.64	0.21	0	0.36
	4	Resedimented in sucrose	0.04	0.84	0.10	0	0.54
III	1	Control	0.28	2.20	0.39	0	0.77
	2	Dialyzed against buffer-SF	0	2.22	0.34	0	0.67
	3	Dialyzed against buffer	0	2.20	0	0.68	0
IV	1	Control	0.58	2.97	0.40	0.10	0.25
	2	Dialyzed against buffer-SF*	0.04	2.49	0.63	0.32	0.32
	3	Sample from ascending boundary‡	0	1.44	0	0.13	0.61
V	1	Control	0.28	1.78	0.56	0.14	0.44
	2	Dialyzed against buffer-SF*	0	1.94	0.34	0	0.40
	3	Sample from ascending boundary§	0	0.92	0.13	0	0.31

* Values corrected for dilution during dialysis.

‡ Mobility of principal boundaries = −9.1 (A) and −7.9 (D) × 10^{-5}cm.^{2}/volt/sec.

§ " " " " = −9.6 (A) and −7.7 (D) " " " " "

solutions were resedimented for 3 hours at 105,000 *g* and three pellets of each were resuspended in 4.2 ml. of the bicarbonate-chloride. All three solutions were examined in the analytical ultracentrifuge. The nucleoproteins resedimented in plain sucrose showed a considerable loss of B and C, while in sucrose-SF recovery of both B and C was excellent.

Experiment II.—It was desirable to know whether the SF could be prepared in distilled water, and whether it could be lyophilized. Water-SF was therefore prepared by dialyzing against 50 ml. of water for 3 hours, then changing the water, and dialyzing for 16 hours. Fifty ml. of the first dialysate and 100 ml. of the second were lyophilized, and the concentrate made up into 40 ml. of 0.79 M sucrose, 3. The remainder of the first dialysate was also adjusted to 0.79 M, by the addition of solid sucrose, 2. Nucleoprotein pellets were resuspended in 0.7 ml. of 0.79 M sucrose per gm. of liver, and shell-frozen in 5 ml. portions. One portion, the control, 1, was adjusted to 0.05 M NaCl–0.05 M $NaHCO_3$ by the addition of one-fourth volume of concentrated buffer, and analyzed immediately in the ultracentrifuge. Three other portions were each diluted to 45 ml. with 0.79 M sucrose containing fresh, 2, or lyophilized, 3, SF, or plain sucrose, 4. These three preparations were resedimented, resuspended, and analyzed as in Experiment I. From Table I it may be seen that water-SF is just as effective as sucrose-SF, and that the SF is not destroyed by lyophilizing.

Experiment III.—The ability of buffer-SF to preserve the nucleoproteins during overnight dialysis was next determined. Six nucleoprotein pellets were resuspended in veronal-chloride buffer (final volume, 6 ml.). One sample, 1, was analyzed immediately in the ultracentrifuge. Another, of 2 ml., was dialyzed overnight against buffer-SF, 2, and a third, also 2 ml., against plain buffer, 3 (see Table I). Overnight dialysis against the veronal-chloride buffer damaged rat liver nucleoproteins much less than mouse spleen nucleoproteins, which are completely destroyed (3); component B appeared unchanged. Addition of SF to the buffer resulted in the preservation of most of the components C and E also.

Experiment IV.—The electrophoretic analysis of the nucleoproteins in the presence of buffer-SF was next undertaken. The mitochondria supernatant from 60 gm. of rat liver was treated with desoxycholate as described above and centrifuged at 90,000 *g* for 4 ½ hours. The pellets were resuspended in buffer (final volume 14 ml.). A control sample, 1, was analyzed in the ultracentrifuge and the remaining 13 ml. dialyzed overnight against 150 ml. of buffer-SF. The volume increased to 16.5 ml. Most of the turbidity was removed by centrifuging at 20,000 *g* for 1 hour. The supernatant was analyzed in the ultracentrifuge, 2, and by electrophoresis. The ascending limb of the cell and both top sections were filled with the dialysate (buffer-SF) and the electrode vessels were filled with plain buffer. After a potential gradient of 5.4 volts/cm. had been applied for 90 minutes, the boundaries were photographed. A sample from the large sharp fast boundary on the ascending side was removed and analyzed in the ultracentrifuge, 3.

Experiment V.—This was similar to Experiment IV except that 0.3 per cent desoxycholate was used.

When these nucleoprotein preparations were subjected to electrophoretic analysis in buffer-SF, the ascending boundaries remained sharp, and nucleoprotein re-

covered from the ascending side still retained its characteristic behavior in the ultracentrifuge (Experiments IV and V). In previous studies, in which plain buffer was used, the ascending boundaries spread badly (3), and attempts to recover unchanged nucleoprotein after electrophoretic separation were unsuccessful.

In many of the ultracentrifugal analyses made on nucleoproteins treated with desoxycholate changes in the C and E boundaries were seen, and component D, which is ordinarily present only in trace amounts, was increased. Desoxycholate therefore appears to be harmful to these components, although no effect on B has been noticed.

Although the crude SF preparations described here must be mixtures of many substances, they are proving extremely useful for the preservation of the nucleoproteins, and do not interfere with either ultracentrifugal or electrophoretic analyses.

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