Cytoplasmic Ribonucleoprotein Components of the Novikoff Hepatoma

By EDWARD L. KUFF, M.D., and ROBERT F. ZEIGEL, Ph.D.

(From the Laboratories of Biochemistry and Biology, National Cancer Institute, Public Health Service, United States Department of Health, Education and Welfare, Bethesda)

PLATES 248 TO 250

(Received for publication, December 11, 1959)

ABSTRACT

Prominent nucleoprotein sedimentation boundaries were demonstrable in cytoplasmic extracts of Novikoff hepatoma. Fractionation of the homogenates by differential centrifugation or a density gradient method revealed that 65 to 75 per cent of the cytoplasmic ribonucleic acid was present in the form of free ribonucleoprotein particles. After purification by differential centrifugation in dilute buffer, the particles contained 37 per cent RNA, very little lipid, and no demonstrable membrane material. Ultracentrifugal boundaries corresponding to those seen in the original extracts were present, the main component having an $s_{20,w}$ of 81 S. Upon exposure to chelating agents, the particles dissociated through an intermediate component with sedimentation rate of 56 S to a final stage in which 46 and 28 S subunits were present in a weight ratio of 2:1. ATP and pyrophosphate were equally effective in causing dissociation. ADP was considerably less effective.

Treatment of the purified particles with deoxycholate removed one-third of the protein and significantly altered the ultracentrifugal pattern. The particles now dissociated directly to the 46 and 28 S subunit when exposed to chelating agents.

Upon electron microscopy, the 81 S particle appeared as an oblate spheroid 24 mμ in diameter. The 46 and 28 S subunits also appeared spheroidal.

Cytoplasmic ribonucleoprotein particles in the size range of 10 to 20 mμ appear both in association with the membranes of the endoplasmic reticulum (the combination often being designated as "ergastoplasm") and as apparently free entities dispersed singly or in small groups throughout the cell sap (1). Within mammalian tissues, an extensive ergastoplasm has been found to be characteristic of cells that are specialized for the synthesis of material for secretion, whereas free particles are particularly abundant in proliferating cells (1, 2). However, there is as yet very little information as to how the specific functions of the nucleoprotein particles might be related to the free or membrane-bound condition (3, 4).

Tumor tissues provide a favorable source of material for use in approaching this question. Elevated concentrations of free nucleoprotein particles have been repeatedly demonstrated in tumor cells by electron microscopy (5–7) and in isolated tumor microsome fractions by combined ultracentrifugal and chemical analysis (8–10). Furthermore, in many types of tumors, the Novikoff transplantable rat hepatoma being one example (5, 11), the cells possess a very sparse endoplasmic reticulum to which only a few of the cytoplasmic nucleoprotein particles are attached. It might be anticipated that membrane-free particle fractions containing the bulk of the cytoplasmic ribonucleic acid could be prepared from such tissues without the help of detergents (12–14) or strong salt solutions (15) and under the same mild conditions that have been employed in the isolation of ribonucleoprotein components from plant and bacterial sources (16–19). Investigation of the fractions obtained in this manner might, in turn, be expected to yield useful information relative to the function of the free particles in mammalian cells.

As an initial step in this direction, the present study was concerned with the isolation and physical characterization of the free cytoplasmic nucleoprotein particles of the Novikoff hepatoma. Investigations of a somewhat similar nature, but employing the Jensen's rat sarcoma, were reported briefly by Petermann and Hamilton (20) while this work was in progress.

Materials and Methods

A. Preparation of Homogenates.–A subline of the Novikoff hepatoma (11)[1] was maintained by alternate subcutaneous and intraperitoneal passage in male Holtzman rats weighing about 150 gm. Solid intraperitoneal tumors harvested on the 5th day after implantation showed few or no hemorrhagic areas (although considerable bloody ascites was usually present) and were used in the present experiments. The excised tumors were rinsed in cold 0.25 M sucrose until they were pale pink in color and then homogenized for 3 or 4 minutes in 2 or 3 volumes of the same medium. Cell breakage, estimated to be 60 to 70 per cent after this treatment, was completed by sedimenting the residual whole cells at low speed, resuspending them in a small volume of fresh medium, and homogenizing for an additional minute. Homogenates of normal rat liver were prepared in the same manner.

B. Fractionation on Homogenates by Differential Centrifugation.–In certain experiments (see Table I), washed nuclear (N_w) and mitochondrial (Mt_w) fractions were isolated by a previously described method (21), slightly modified in the case of the tumor in that the mitochondria were sedimented initially at 11,000 rather than at 5,000 *g*. More commonly, the nuclei and mitochondria were isolated and washed as a single fraction, *NMt*. In the case of both hepatoma and normal liver, microsomal (*M*) and postmicrosomal (*PM*) fractions were isolated from the combined mitochondrial supernatant fluids by successive centrifugations of 17 and 120 minutes, respectively, at 105,000 *g* in the No. 40 rotor of a Spinco model L ultracentrifuge. All procedures were carried out between 0 and 4°C.

C. Chemical Analyses.–Total nitrogen was measured by nesslerization of acid digests (22). Ribonucleic acid phosphorus (RNA-P) was determined by the orcinol method after extraction by the procedure of Schneider (23), or alternatively, by measurement of e_{260} in 70°C. perchloric acid extracts of acid-washed and lipid-extracted material (13). Both assays were calibrated by analysis of a sample of RNA isolated from tumor ribonucleoprotein. This preparation had a weight N:P ratio of 1.67; a molar purine-bound ribose:P ratio of 0.49 in 90°C. TCA extracts; and an $e_{260}^{1\text{cm.}}$ of 0.331 per μg. P in 70°C. PCA extracts. Approximate calculations of the RNA and protein composition of nucleoprotein preparations were made from the determined values of RNA-P and total nitrogen by using the N:P ratio given above and assuming that (*a*) protein equaled 6.25 × non-RNA nitrogen, and (*b*) RNA equaled 11 × RNA-P. In some instances, protein was determined directly by a biuret method (24), using bovine serum albumin as the reference standard.

Acid digests of lipid fractions obtained during the Schneider extraction procedure were analyzed for phosphorus (lipid P) by the method of Fiske and Subbarow (25). On occasion, the lipids were extracted directly from lyophilized nucleoprotein samples, weighed, and analyzed for cholesterol (26) and deoxycholate, the latter by a micro-adaptation of the method of Szalkowski and Mader (27) which permitted the detection of as little as 5 to 10 μg.

D. Analytical Ultracentrifugation.–A Spinco model E ultracentrifuge equipped with ultraviolet absorption system and phase plate schlieren optics was employed. Cells with 12 mm. fluid column were used. The exposure time in absorption runs was 10 seconds, using a General Electric Co. H3FE light source. Film densities were scanned and plotted by means of a Spinco model R analytrol equipped with microanalyzer attachment and optical density cam. A calibration series of absorption runs showed that film densities were linearly proportional to the optical densities of adenosinediphosphate solutions up to an e_{260} of 0.9. Nucleoprotein solutions to be studied by the absorption technique were accordingly diluted to give an initial e_{260} of 0.8 to 0.9.

In calculations of the sedimentation constants ($s_{20,w}$), the adiabatic cooling of the rotor at 37,020 R.P.M. was taken to be 0.4° C. (28). Analytical runs were carried out at 20–23°C. unless otherwise stated.

E. Viscosity Determinations.–Measurements were made at 20.00 ± .02°C. An Ostwald viscosimeter with a shear gradient of about 350 seconds^{-1} was employed.

F. Dry Weights.–The samples were lyophilized at −15 to −20°C. and then heated at 105°C. *in vacuo* over P_2O_5 until constant weight was attained.

G. Electron Microscopy.–Small drops of dilute nucleoprotein solutions in distilled water or 0.4 mM $MgCl_2$ were placed on the formvar-coated specimen grids and allowed to come nearly to dryness. The grids were then rinsed briefly in glass-redistilled water and dried. Some preparations were singly or doubly shadowed[2] with platinum at an angle of 11°. In most instances, polystyrene latex spheres, 88 or 340 mμ in diameter, were added to the nucleoprotein solutions before plating in order to provide an internal measure of the shadow angle.

Pellets of nucleoprotein were fixed for 30 minutes at 0°C. in 1 per cent osmium tetroxide containing 5 mM sodium phosphate, pH 7.5, and 0.5 mM magnesium chloride. After washing and dehydration, representative portions of the pellets were embedded in a 1:3 mixture of methyl and *n*-butyl methacrylate. The temperature was kept at 0°C. until final polymerization of the plastic. Electron micrographs were taken with an RCA model EMU-2C microscope.

H. Reagents.–Adenosinetriphosphate (ATP) and adenosinediphosphate (ADP) were obtained from Sigma

[1] Obtained from Dr. Mary Maver of the National Cancer Institute.

[2] The shadowing was kindly performed by Mr. B. J. Lloyd of the National Cancer Institute.

Chemical Co., St. Louis, in the form of the disodium salts. Ethylenediamine-tetraacetic acid (EDTA) was also obtained as the disodium salt, designated sequestrene Na_2, from Geigy Industrial Chemicals, New York. Sodium deoxycholate (DOC) was a product of Difco Laboratories.

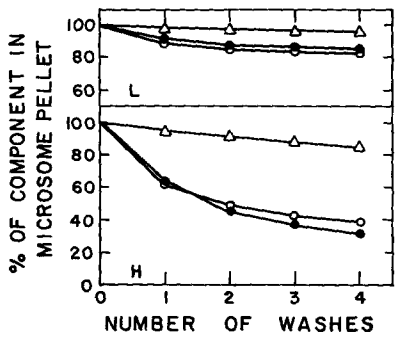
RESULTS

1. Observations on Crude Tumor Extracts:

The extracts remaining after removal of the nuclei and mitochondria from 30 per cent tumor homogenates had a pH of 7.0 and contained approximately 2.5 mg. of RNA and 18 mg. of protein per ml. Direct examination of the extracts in the analytical ultracentrifuge (Fig. 1), revealed a number of moving boundaries with sedimentation rates within the range previously ascribed to the cytoplasmic ribonucleoproteins of tumor cells (8–10, 15). Since subsequent experiments (see below) showed that these boundaries did in fact represent ribonucleoprotein material, the main component, with a sedimentation rate ($s_{20,w}$) of approximately 70 S, has been designated as the *b* component in conformance with the system of nomenclature employed by Petermann and coworkers (9, 10, 14). Other components (Fig. 1) and their sedimentation rates relative to that of *b* were as follows: *a'*, 1.86; *a*, 1.46; *c*, 0.84; *e*, 0.63, and *f*, about 0.4. There was considerable variation in the relative heights of the *b* and *c* peaks in different preparations; however, *b* was consistently the major component. Storage of the extracts at 2°C. for 24 hours produced no noticeable change in the sedimentation patterns. Incubation at 37°C. for 30 minutes led principally to marked reduction in the areas of the *a* and *a'* components. After freezing and thawing, a precipitate was present and all peaks were greatly reduced in size.

2. The Proportion of Free and Membrane-Bound Cytoplasmic Ribonucleoprotein:

Centrifugation of the crude tumor extracts (mitochondrial supernatant fluids) for 17 minutes at 105,000 *g* (29) produced opaque, cream-colored pellets which contained 80 to 90 per cent of the lipid P and somewhat less than half of the RNA-P of the extracts. A subsequent centrifugation of the extracts for 90 minutes at the same speed resulted in the formation of pink, hyaline sediments which were very low in lipid P but contained most of the remaining RNA. Since



TEXT-FIG. 1. The effect of repeated washing upon the microsome fractions of liver (*L*) and Novikoff hepatoma (*H*). The microsome pellets were obtained by centrifugation at 105,000 *g* for 17 minutes and were resedimented under the same conditions after each wash. The washing fluid was 0.25 M sucrose containing 5 mM sodium phosphate, pH 7.0, and 0.5 mM magnesium chloride. The quantity of each component in the original fraction was taken as 100 per cent. Symbols: △, lipid P; ●, RNA P; ○, total N.

lipid P has been shown to be a constituent of the membrane elements of microsomes (1, 12), it was inferred from the above results that membrane material was confined chiefly to the first fraction (for which the designation "microsomes" (*M*) was retained). Upon ultracentrifugal analysis, the second, or postmicrosome (*PM*) fraction was found to consist almost entirely of discrete nucleoprotein components corresponding to those seen in the original extracts and of more slowly moving material with an average sedimentation rate of about 3 S (Fig. 2).

Similar boundaries were also observed in the resuspended microsome fractions, however, indicating that the separation of free and bound ribonucleoprotein was not complete. Text-fig. 1 illustrates the results of an experiment in which hepatoma microsomes were washed repeatedly in an effort to remove the free particles. A corresponding microsome fraction from normal liver, which was subjected to the same procedure, attained a constant composition after only small losses of RNA-P and total nitrogen. In the case of the hepatoma, an extensive extraction of these two biochemical components in the initial washes was apparently superimposed upon a rather steady breakdown of the membrane-containing elements of the fraction (note the release of lipid P). The material removed in the first wash was found to be essentially identical with the *PM* fractions in ultracentrifugal

TABLE I

The Distribution of Total Nitrogen, Ribonucleic Acid, and Lipid Phosphorus in Subcellular Fractions of Novikoff Hepatoma and Normal Rat Liver

All values are per gram wet weight of tissue. Whole homogenates are designated *H*. The normal liver was obtained from a male Holtzman rat weighing about 150 gm.

	Total nitrogen		RNA-P				Lipid P		
		Fraction of *H*		Fraction of *H*	Per mg. N	Per μg. Lipid P		Fraction of *H*	Per mg. N
	mg.	*per cent*	*μg.*	*per cent*	*μg.*	*μg.*	*μg.*	*per cent*	*μg.*
Hepatoma									
H	18.7	(100)	936	(100)	50.1	2.25	416	(100)	22.2
N_{w2}	6.27	33.5	128	13.7	20.4	.81	158	38.0	25.2
Mt_w	.91	4.9	21.3	2.28	23.5	.28	75.6	18.2	83.4
M_w*	1.99	10.6	214	22.9	108	1.66	129	31.0	64.8
PM	2.77	14.8	426	45.5	154	21.9	19.4	4.66	7.0
S	6.08	32.5	84.0	8.88	13.8	4.42	19.0	4.57	3.12
Liver									
H	28.6	(100)	672	(100)	23.5	.52	1,270	(100)	44.4
M_w*	5.01	17.5	294	43.8	58.7	.49	602	47.4	120
PM	.772	2.70	44.8	6.67	86.3	1.58	28.4	2.24	36.8

* Washed once with 0.25 M sucrose containing 5 mM sodium phosphate, pH 7.0, and 0.5 mM magnesium chloride (see text).

behavior and chemical composition and clearly consisted primarily of free nucleoprotein particles.

TABLE II

The Effect of Added Magnesium Ion in the Homogenization Medium upon the Ribonucleic Acid Content and Sedimentation Behavior of Postmicrosome Fractions from Novikoff Hepatoma

For analytical ultracentrifugation, the postmicrosome fractions were resuspended in a medium consisting of 5 mM sodium phosphate, pH 7.5, 0.5 mM magnesium chloride, and 50 mM sodium chloride. See text for further details.

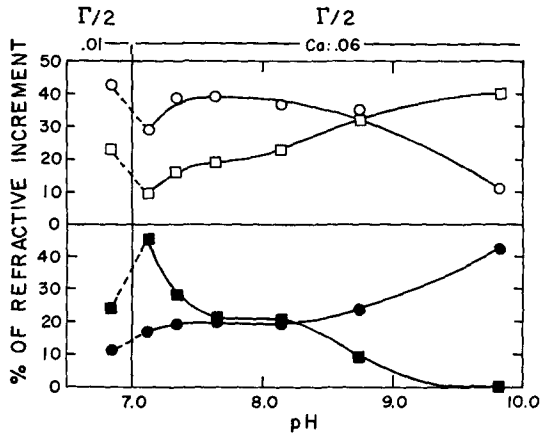
$MgCl_2$ added to homogenization medium (mM)	RNA-P		Area under schlieren pattern					
	Per gm. of tissue	Recovery	Units* per gm. of tissue	Recovery	Per cent of recovered area as component			
					a' + a	b	c	e
	μg.	*per cent*		*per cent*				
0	382	(100)	196	(100)	6	54	23	18
.5	379	99	201	103	10	47	23	20
1.0	310	81	163	83	21	38	18	22
2.5	224	59	100	51	14	52	13	21

* Arbitrary units.

The second wash fluid also contained free particles, but in much smaller quantity and relatively heavily contaminated with lipid P-containing components. Therefore, in all complete fractionation studies (such as that shown in Table I), the tumor microsomes were washed only once. The wash fluid was combined with the original microsomal supernatant fluid prior to the isolation of *PM*, and the RNA content of the latter fraction was taken as an approximate measure of the quantity of free nucleoprotein particles in the homogenates.

The tumor data presented in Table I were accordingly interpreted as showing that at least 67 per cent of the sedimentable cytoplasmic RNA and 45 per cent of the total cellular RNA was in the form of free nucleoprotein particles. The corresponding values for normal rat liver, also derived from data shown in Table I, were 13 and 7 per cent, respectively.[3] These figures appear to be

[3] In confirmation of the results of Petermann *et al.* (30) we have observed *b*, *c*, and *e* nucleoprotein boundaries in liver postmicrosome fractions resuspended in phosphate-Mg buffer. Density fractionation and electron microscopy of these fractions showed that they were composed of a mixture of smooth surfaced vesicles and free nucleoprotein particles.



TEXT-FIG. 2. The effect of pH and ionic strength upon the proportions of the ultracentrifugal components in hepatoma postmicrosome fractions. The ordinate refers to the areas under the schlieren patterns represented by the components. The symbols refer to the following components: ○, *b*; □, *c* to *e*; ■, *a'*, *a*, and heterogeneous material faster than *b*; ●, "soluble" material, average 3 or 4 S. The solvent was 5 mM sodium phosphate and 0.5 mM magnesium chloride containing sodium chloride to give the designated ionic strengths.

consistent with the relative proportions of free particles observed in electron micrographs of the two types of tissue (1, 5, 11, 12).

Following the report of Petermann and Hamilton that most of the cytoplasmic RNP was present as free particles in extracts of Jensen's sarcoma (20), the density fractionation method employed by these authors (14, 20) was applied to the hepatoma. Supernatant fluids remaining after the removal of nuclei and mitochondria were adjusted to pH 8.0 at 4°C. with dilute sodium hydroxide. Twenty ml. aliquots were layered over 5 ml. portions of 0.35 and 2 M sucrose (both containing 5 mM sodium phosphate, pH 7.5, 0.5 mM $MgCl_2$) in tubes of the Spinco SW-25 rotor. The tubes were centrifuged for 20 hours at 22,500 R.P.M. (58,300 *g*), and decelerated slowly. Two fractions were collected; (*a*) a pink, very turbid band at the interface between the 0.35 and 2.0 M sucrose solutions; and (*b*) the 2 M sucrose layer itself plus a small colorless sediment. Of the total lipid P represented by the two fractions together, over 90 per cent remained at the interface. On the other hand, 76 and 74 per cent of the RNA-P was found in the 2 M sucrose layer in two experiments. Electron microscopy revealed that this fraction consisted primarily of dense ribonucleoprotein particles and was only minimally contaminated with membranous elements.

Thus, it could be shown by two separate methods of fractionation that between 65 and 75 per cent of the sedimentable RNA in cytoplasmic extracts of hepatoma was in the form of free nucleoprotein particles.

3. Properties of Crude Ribonucleoprotein Fractions:

Buffers containing phosphate and magnesium ions have been frequently employed as a suspension medium for the study of ribonucleoprotein particles. *PM* fractions were accordingly resuspended by homogenization in 5 mM sodium phosphate, pH 7.5, containing varying concentrations of magnesium chloride. Identical ultracentrifugal patterns were obtained in the presence of 0.25 and 0.5 mM magnesium chloride. However, reduction of the magnesium chloride concentration to 0.125 mM resulted in an increase in the proportion of more slowly moving components. Unless otherwise stated, the "phosphate-Mg buffer" to which we will subsequently refer contained 5 mM sodium-phosphate and 0.5 mM magnesium chloride.

In order to determine whether dissociation of the nucleoprotein particles might occur during disruption of the cells in a medium lacking in divalent cation, aliquots of a tumor mince were homogenized in 0.25 M sucrose to which magnesium chloride had been added in the concentrations shown in Table II. The *PM* fractions isolated from each homogenate were examined in the ultracentrifuge and the total and fractional areas of the various components (other than the 3 S

material) were estimated by planimetry of the patterns. The quantity of RNA-P in the *PM* fractions was also determined. The results are presented in Table II. The yield of RNA diminished sharply above 0.5 mM magnesium chloride, and this could be correlated in part with the fact that all of the cells could not be disrupted at higher magnesium concentrations. A closely corresponding decrease in the total area under the peaks was observed. Inspection of the absolute and relative areas represented by the individual components reveals little or no increase in the *c* and *e* peaks at the expense of the more rapidly moving components as the concentration of added magnesium was decreased to zero.

The ultracentrifugal behavior of crude fractions as a function of pH and ionic strength of the suspending medium was also investigated (Text-fig. 2). At or near neutrality (pH 7.0 ± 0.2), the addition of as little as 0.05 M sodium or potassium chloride to the phosphate-Mg buffer (*i.e.*, an increase in ionic strength from 0.01 to 0.06) resulted in a marked decrease in the areas of the *b*, *c*, and *e* components, an increase in the 3 S (soluble) component, and the appearance of heterogeneous material accompanying and preceding the *a* boundary. This phenomenon was more marked at 0.1 M salt concentrations (not shown) where it was accompanied by a noticeable increase in the turbidity of the suspensions. At a constant ionic strength of about 0.06, the microaggregation of the particles was reversed as the pH was increased from 7.1 to 7.6. Between pH 7.6 and 8.1, relatively little change in the patterns occurred. Above this range however, there was a progressive increase in the more slowly-moving components at the expense of *b* and *a*. A slow-moving pink material, presumably hemoglobin, became dissociated from the *b* boundary at pH 7.6.

4. Purification and Properties of Ribonucleoprotein Particles:

Crude nucleoprotein fractions were resuspended in phosphate-Mg buffer, pH 7.9–8.0, and centrifuged for 2 hours at 105,000 *g*. The clear slightly pink supernatant fluids were removed together with any loosely sedimented material. The firm hyaline pellets were resuspended in fresh medium and centrifuged for 15 minutes at 25,000 *g*. The very small, rather tan sediments were discarded, and the supernatant fluid constituted the washed nucleoprotein. The cycle of high and low speed centrifugation was repeated to obtain the final twice-washed preparation (RNP).

TABLE III

The Recovery of Ribonucleic Acid and Total Nitrogen during Purification of Novikoff Hepatoma Ribonucleoprotein Fractions by Differential Centrifugation

PM was a postmicrosome fraction similar to that shown in Table I. The dense fraction consisted of material which passed into the 2 M sucrose layer during the density fractionation of mitochondrial supernatant fluids. The quantity of material in these fractions upon initial isolation is taken as 100 per cent.

	Recovery of RNA-P	Recovery of total nitrogen	RNA-P per mg. total nitrogen
	per cent	*per cent*	*μg.*
PM	(100)	(100)	159
Washed × 1	90	76	185
Washed × 2	88	68	212
Dense fraction	(100)	(100)	213
Washed × 1	68	—	—
Washed × 2	60	61	211

The data presented in Table III indicate that a ratio of about 210 μg. RNA-P per mg. total N was the limiting value which could be reached by this type of purification procedure. This figure, which corresponds to an RNA content of 37 per cent, was approached but not exceeded in seven different preparations.

The recovery of RNA-P was typically very good during washing of the *PM* fractions (Table III). This was not true, however, in the case of material originally isolated by density fractionation, for reasons which are at present obscure. The ratio of RNA-P to lipid P varied between 18 and 22 in the crude preparations, corresponding to a phospholipid content (expressed as lecithin) of 4 to 5 per cent. This value showed relatively little change during washing.

Nucleoprotein solutions obtained by the above methods were slightly turbid, appeared pale orange by transmitted light, and showed a blue scattering effect. Some analytical properties are shown in Table IV (the deoxycholate-treated material will be considered below). The total lipid content was low, and electron microscopy of

TABLE IV

The Composition and Optical Properties of Purified Novikoff Hepatoma Ribonucleoprotein Preparations before and after Treatment with Deoxycholate

For purposes of comparison, some properties of ribonucleoprotein particles isolated from liver microsomes by deoxycholate treatment (14) have been included.

	RNA-P per mg. total N	Protein per mg. RNA		Cholesterol per mg. RNA	Dry weight as lipid§	$\bar{V}$ (22°)	Optical properties‖				
		Calculated*	Determined‡				max.	min.	$\frac{e_{260}}{e_{280}}$	$\frac{e_{260}}{e_{235}}$	e(P)¶ 260 mμ
	μg.	mg.	mg.	μg.	per cent		mμ	mμ			
RNP	211	1.73	1.63	<2	<4	0.69	259	237	1.84	1.45	9,250
RNP (DOC)	272	1.13	1.10	Absent	<2	0.67	259	235	1.90	1.69	9,030
Liver RNP (DOC)	254	1.29	—	Absent**	About 6 per cent phospholipid**	0.67**	259	236	1.87	1.59	9,530

* From ratio of RNA-P to total nitrogen.

‡ Protein determined by biuret method.

§ Chloroform-soluble lipids, by weight.

‖ At pH 7.5, in 5 mM sodium phosphate-.5 mM magnesium chloride.

¶ Molar absorptivity, based on 1 gram-atom of nucleoprotein RNA-P per liter.

** Values taken from Petermann and Hamilton (14).

pelletized samples revealed little or no contamination with membranous elements (Fig. 13).

The sedimentation pattern shown in Fig. 3 was characteristic of washed preparations resuspended in solutions of low ionic strength. By reference to Fig. 1, it is seen that the major losses incurred during the isolation procedure appear to have affected the components at either end of the spectrum of sedimentation rates. Comparison of the schlieren pattern with that obtained from UV absorption runs (Text-fig. 3) indicated that the various components contained roughly the same proportion of RNA to total mass. Consistent with this view was the fact that the relative sedimentation rates of the components were not significantly altered upon substitution of deuterium oxide for half of the water in the solvent medium.

The sedimentation rates of the *b* and *c* peaks showed strong concentration dependence. Detailed analysis of this relationship was hindered by the fact that addition of sodium or potassium salts in quantities sufficient to overcome completely primary charge effects in more concentrated RNP solutions resulted in micro-aggregation of the particles (Fig. 4). The magnitude of the effect is indicated, however, by the observed increase in $s_{20,w}$ of the *b* component from 74 to 81 S as the total RNP concentration was reduced from 0.4 to 0.01 per cent in phosphate-Mg buffer, pH 7.5, containing 0.05 M sodium chloride. Under the same conditions, the sedimentation rates of the *c* and *e* components increased from 60 to 64 S and from 43 to 45 S, respectively.

5. The Action of Deoxycholate Upon RNP Particles:

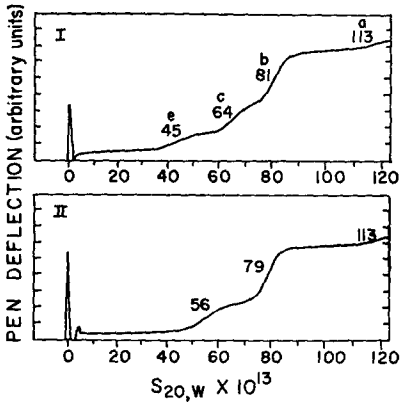
RNP fractions were treated at 4°C. with 0.5 per cent sodium deoxycholate, pH 8.0, in the presence of phosphate-Mg buffer of the same pH. The weight ratio of protein to deoxycholate in the mixtures was approximately 4:3 (13). The solutions were centrifuged for 2 hours at 105,000 *g*. Completely colorless hyaline pellets were obtained. Approximately 23 per cent of the RNA and 42 per cent of the protein remained in the supernatant fluid. Ultracentrifugal analysis of the latter by the UV absorption technique revealed a small boundary with $s_{20,w}$ between 5 and 6 S, plus a considerable amount of faster moving, apparently heterogeneous, material with rates up to 30 S.

The pellet material was washed twice in phosphate-Mg buffer, pH 7.9, with little further loss of RNA or protein. Some properties of the washed nucleoprotein, designated RNP(DOC), are shown in Table IV. In four different preparations, the ratio μg. RNA-P per mg. total nitrogen ranged from 272 to 289, and averaged 280. This corre-

sponds to an RNA content of 49 per cent. A similar value (48 per cent) was obtained when protein was measured directly by the biuret method.

No deoxyribonucleic acid was detected when the hot TCA extract from 10 mg. of RNP(DOC) was tested by the Dische method (23). No DOC was found in the lipid extract of 25 mg. of the particles.

Treatment with DOC resulted in marked changes in the sedimentation patterns, affecting particularly the more slowly moving boundaries (Fig. 5). The *c* component was no longer observed and *e* was greatly reduced in size. A small peak with $s_{20,w}$ of 56 S at infinite dilution was now present. Small *a* and *a'* boundaries were still present. At least 80 per cent of the total area under the schlieren patterns was represented by the *b* component, whereas prior to DOC treatment, the corresponding figure had ranged between 40 and 55 per cent. The slow boundaries (56 and 45 S) represented about 15 per cent of the area of the schlieren patterns but accounted for nearly 25 per cent of the absorption in the UV (Text-fig. 3). Whether the smaller particles had a disproportionately high RNA content or whether some dissociation of the main component occurred upon dilution for the UV runs is not known.



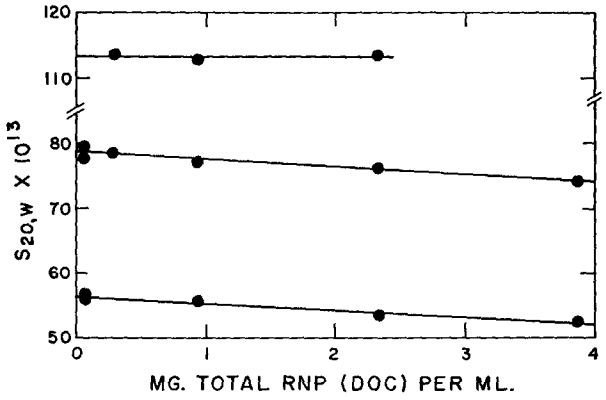
TEXT-FIG. 3. Ultracentrifugal behavior of RNP (I) and RNP(DOC) (II) as determined by ultraviolet absorption method. The charts represent analytrol plots of exposures taken 13 minutes after reaching a speed of 37,020 R.P.M. The plots have been corrected for variations in "base-line" absorption and the distances from the menisci (sharp spikes) recalculated in terms of sedimentation rates. The solvent consisted of phosphate-Mg buffer, pH 7.5, containing 0.05 M sodium chloride. Schlieren patterns of the corresponding preparations are shown in Figs. 3 and 5.

DOC-treated particles were not aggregated by 0.1 M sodium or potassium chloride in the pH range 7–8. The concentration dependence of the sedimentation rates of the three principal components is shown in Text-fig. 4. Extrapolated to infinite dilution, these rates were 113, 79, and 56 S, respectively. The intrinsic viscosity, measured on a single preparation in phosphate-Mg buffer containing 0.05 M sodium chloride, was 0.098 dl./gm.

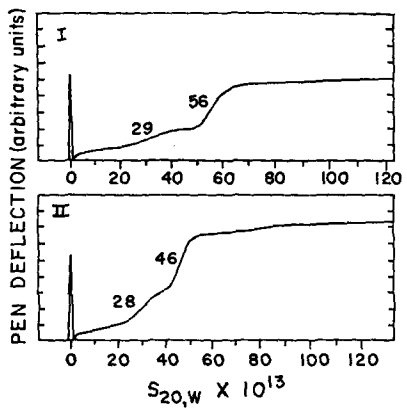
Nucleoprotein particles obtained by DOC treatment of a washed microsome fraction, M_w, were similar in ultracentrifugal behavior and RNA content to RNP(DOC) prepared from the *PM* fractions.

6. The Effects of Chelating Agents:

Nucleoprotein solutions in phosphate-Mg buffer, pH 7.5, were mixed with solutions of chelating agents at the same pH and allowed to stand in the



TEXT-FIG. 4. The relationship between total concentration of RNP(DOC) and the sedimentation rates of the various ultracentrifugal components in the fraction (See Fig. 5). The 79 S material represents the main component. The sedimentation rates at the lowest concentration were determined from ultraviolet absorption runs. The solvent was phosphate-Mg buffer, pH 7.5, containing 0.1 M sodium chloride.



TEXT-FIG. 5. Ultracentrifugal behavior of dissociation products of RNP (I) and RNP(DOC) (II) as determined by ultraviolet absorption method. RNP at a concentration of 4 mg. per ml. was exposed to 2 mM sodium pyrophosphate and then diluted one-hundred-fold in phosphate-Mg buffer, pH 7.5, containing 0.05 M sodium chloride. RNP(DOC) was diluted directly into 5 mM sodium phosphate, pH 7.5, containing 0.05 M sodium chloride.

cold for 15 to 30 minutes. The mixtures were brought to room temperature just before ultracentrifugal analysis. The pH was measured and, if necessary, readjusted to 7.5 with a small volume of dilute sodium hydroxide. In presenting the results, it will be convenient to specify the ratio

$$\frac{\text{Moles chelating agent in excess of initial free Mg ion}}{\text{Moles RNA-P in nucleoprotein}}$$

as a measure of the relative amounts of these components in the mixtures. This ratio will be designated as R.

(*a*) Exposure of RNP(DOC) preparations to EDTA at R equal to or greater than 0.5 produced the pattern shown in Fig. 8, in which 63 per cent of the total refractive increment (average of three experiments) moved as a single boundary with a sedimentation rate of 43 to 44 S. An average of 29 per cent of the area was represented by a peak with $s_{20,w}$ of 26 S, and the remainder, about 8 per cent, by more slowly sedimenting material. The two discrete components had sedimentation rates of 46 and 28 S when the nucleoprotein was diluted to about 0.01 per cent in 0.05 M sodium chloride containing 1 mM EDTA or 5 mM sodium phosphate at pH 7.5 (Text-fig. 5). In three such UV runs, the ratio of the absorptions represented by the 46 and 28 S components was close to 2:1. Patterns essentially identical with that shown in Fig. 8 were obtained by treating the particles with sodium pyrophosphate at R equal 1 or by exposing them to 0.2 M sodium bicarbonate for several hours at 0°C. The same subunits were demonstrated after EDTA- or pyrophosphate-induced dissociation of the nucleoproteins prepared by DOC treatment of washed microsomes.

Conversion of the 79 S particle to the 46 and 28 S subunits appeared to procede directly, that is, without the formation of components of intermediate sedimentation rate. This is clearly illustrated in Figs. 6 and 7, taken from runs in which pyrophosphate and ATP were present at R's of 0.35 and 0.55, respectively. A small amount of material sedimenting between the 79 and 46 S components is seen in Fig. 6; this probably represented 56 S material originally present in the preparation (see Fig. 5). Even before dissociation of the 79 S component was complete, however, this small boundary had disappeared (Fig. 7).

(*b*) RNP preparations that had not been exposed to DOC also dissociated to 46 and 28 S components upon treatment with an *excess* of EDTA (Fig. 9). However, exposure of the particles to milder chelating conditions, as provided by EDTA at R of about 0.2 or by pyrophosphate or ATP at R between 0.3 and 1, resulted in dissociation to an intermediate component which was found to have a sedimentation rate of 56 S in UV absorption runs (Text-fig. 5). Figs. 10 and 11 show two stages in this transition, induced by 2 mM (R equal 0.35) and 4 mM (R equal 0.85) ATP, respectively. ADP was much less effective in altering the particles: for example, no change at all was observed at 2 mM ADP, and at 4 mM dissociation was just beginning (Fig. 12). The simultaneous addition of 1 molar equivalent of magnesium ion prevented the action of ATP.

Complete conversion to the 56 S material was accompanied by the appearance of a boundary with $s_{20,w}$ of about 30 S (Fig. 11 and Text-fig. 5), as well as by the liberation of considerable amounts of more slowly moving material. The latter was apparently poor or lacking in RNA, as indicated by a comparison of the regions near the meniscus in the schlieren (Fig. 11) and UV absorption (Text-fig. 5) patterns.

7. Electron Microscopy:

DOC-treated preparations were fairly homogeneous in appearance and will be considered first. Fig. 14 shows the type of pattern occasionally

formed during drying of dilute RNP(DOC) solutions. The diameters of the individual unshadowed particles averaged about 15 mμ, *i.e.*, nearly the same as the particles visualized in thin sections of fixed tissue (11). On the other hand, the distance between the centers of adjacent clearly resolved particles (see arrow) was 21 to 22 mμ. This corresponded with the average diameter of 22 mμ obtained for shadowed particles (Fig. 15) after correction for the thickness of the shadowing material (see legend, Fig. 15). It would appear that the edges of the particles, which probably presented a relatively short pathway to the electron beam compared to the more central regions, were simply not observed in the unshadowed state. The height of the particles in the shadowed preparations was estimated at 18 mμ. Oblate spheroids of the above dimensions, composed of material with a partial specific volume of 0.67 (Table IV), would have a molecular weight of 4.1 million. A few smaller particles ranging in apparent diameter down to about 15 mμ were also present.

RNP preparations which had not been exposed to DOC presented a more heterogeneous appearance, as expected from their ultracentrifugal behavior. The most numerous class of particles had a diameter of about 24 mμ and an estimated height of 18 mμ (Figs. 16 and 17). Assuming a partial specific volume of 0.69 (Table IV), a molecular weight of 4.6 million may be calculated for these spheroids, which are considered to represent ultracentrifugal component *b*. A considerable number of smaller particles were present, probably corresponding to the *c* and *e* components. It was not practicable to analyze their size distribution in detail. The smallest again had a diameter of about 15 mμ. Ferritin, which has been found in liver postmicrosomal fractions (29) and ribonucleoprotein preparations (14), was not observed in the tumor material.

Several attempts were made to visualize the 46 and 28 S subunits resulting from the dissociation of the particles. Difficulties were experienced because of the very strong tendency of the dissociated preparations to aggregate upon drying. Fig. 18 shows a field taken from one of the preparations. Particles of two general size classes were observed. If the contribution of the platinum to the apparent diameter was taken to be 4 mμ, as was the case in other similarly shadowed preparations, the smaller and less numerous particles could be shown to be spheres between 10 and 11 mμ in size. The larger particles seemed to range in diameter from 18 to 24 mμ (corrected for the shadowing material), with an average of about 21 mμ, or only slightly less than that of the original 79 S component. These particles were greatly flattened however, having an estimated height of 11 to 13 mμ.

DISCUSSION

It is clear that within the cells of the Novikoff hepatoma, only a small minority of the cytoplasmic ribonucleoprotein particles are associated with the rather sparse membranes of the endoplasmic reticulum at a given moment. Nothing is known about the mechanism[4] or duration of this association, however; and the possibility must be entertained that all of the particles pass through a membrane-bound phase as they function in protein synthesis. Particles prepared by DOC treatment from the microsome and postmicrosome fractions were apparently identical (20, and this study). The significance of this observation is obscured, however, by the finding that DOC exerted marked effects upon the properties of the free particles previously isolated in phosphate-Mg buffer.

DOC treatment of these particles resulted in (*a*) abolition of the *c* boundary, (*b*) alteration in the pattern of dissociation of the particles in response to chelating agents, and (*c*) removal of a significant proportion of the protein. The latter effect has also been observed upon DOC treatment of Jensen's sarcoma ribonucleoprotein particles (20). The question arose whether the proteins removed by the detergent represent chiefly material that had been adsorbed non-specifically from the soluble phase during preparation of the homogenates. In order to test this possibility, an RNP-(DOC) preparation was resuspended in tumor supernatant fluid and then reisolated and washed as in the usual preparation of the RNP fractions. As initially reisolated, the pellets were pink and contained 224 μg. of RNA-P per mg. total N. After two washes, the preparation was again colorless and the above ratio had increased to 266, a value similar to that observed in the original RNP(DOC). Apparently adsorption of soluble protein upon the particles did occur but was largely reversed by the washing procedure. It seems un-

[4] It is of some interest in this regard that hepatoma postmicrosomal nucleoprotein was not adsorbed upon liver microsomes when mixtures of the appropriate fractions were made in the presence of liver supernantant fluid.

likely, then, that the marked changes typically induced by DOC treatment reflected simply the removal of fortuitously adsorbed protein.

On the other hand, the persistence of the over-all macromolecular form and of the 46 and 28 S subunits within the DOC-treated preparations suggests that the material removed by the detergent had not been involved as an integral structural unit within the original particles. Exposure to anionic detergents inhibits or abolishes the amino acid–incorporating capacities of ribonucleoprotein fractions from Ehrlich ascites carcinoma (15) and pea seedlings (31), although Korner has recently reported (32) that particles prepared from liver microsomes by DOC treatment retain this ability, at least in part. The material removed from the hepatoma ribonucleoproteins by detergent treatment would appear to be of considerable interest, since it may include auxiliary equipment related to the functions of the particles in protein synthesis.

In their physical and chemical properties and in their response to chelating agents and pH changes, the hepatoma nucleoprotein particles exhibited many points of resemblance to ribonucleoproteins obtained from other sources (16–19, 33–36). Detailed comparisons might be profitably postponed until the tumor particles have been characterized more fully. However, it may be pointed out that the data thus far obtained are fully consistent with the generally observed picture of the *b* component as a spheroidal particle strongly hydrated in solution (note the high intrinsic viscosity), with a molecular weight between 4 and 5 million, and composed of nucleoprotein subunits cemented together by divalent cations, probably magnesium. There appeared to be a number of small differences between the DOC-treated particles of hepatoma and those of normal liver (14, 37), with regard to both the analytical properties of the whole fractions (Table IV) and the sedimentation rates of the *b* component (79 S for hepatoma *vs* 83 S for liver (37)). The significance of these differences is as yet unknown, however, and it might be more to the point to emphasize the over-all similarity of the two types of preparations.

For the readers' convenience, the sedimentation rates of the various hepatoma ribonucleoprotein components as they appear in the tumor extracts and in different stages of the preparative procedures have been summarized in Table V.

The fact that nucleoprotein boundaries could be directly observed in the tumor extracts made it possible to follow the subsequent operations with regard to the question of gross alterations in the particle population. At all stages in the isolation and washing procedure and at all magnesium concentrations up to those which induced obvious aggregation of the particles, the *b* or 81 S boundary was the largest single component. This was also the case in hepatomas harvested on the 3rd, rather than the 5th day after transplantation. The *c* or 64 S component seemed to be present in only small amounts in extracts of the Novikoff hepatoma, although it has been found to occur in relatively

TABLE V

Sedimentation Rates ($s_{20,w}$) of Novikoff Hepatoma Ribonucleoprotein Components and Their Dissociation Products

Italicized values were obtained from ultraviolet absorption runs (nucleoprotein concentration about 0.01 per cent) or by extrapolations to infinite dilution. Dissociation was induced by EDTA, ATP, and pyrophosphate. Blank spaces indicate that the component was not observed.

Letter designation	Tumor extracts and crude ribonucleoprotein fractions	Purified ribonucleoproteins, RNP		Deoxycholate-treated ribonucleoproteins, RNP(DOC)	
		Initially present	Dissociation products	Initially present	Dissociation products
a'	130	135	—	—	—
a	102–110	*113*	—	*113*	—
b	70–75	*81*	—	*79*	—
c	55–60	*64*	—	—	—
d	—	—	*56*	*56*	—
e	40–44	*45*	*46*	45	*46*
f	28	—	*29*	—	*28*

high concentration in resuspended microsome fractions of regenerating liver (9) and azo dye–induced hepatomas (9, 10). It may be noted, however, that the latter observations were made at pH 8.6 (9, 10), where some conversion of the *a* and *b* components to more slowly moving material might be expected to have occurred (37, and this study). The proportion of *c* component usually increased relative to that of *b* during the preparative procedures (compare Figs. 1 and 3). Because of the difficulty in locating the position of the base-line in patterns obtained from the extracts, it was impossible to determine whether this increase occurred at the expense of *b*. The *c* component did not appear as an intermediate when dissociation of the isolated particles was induced by chelating agents, the *b* and *c* components being simultaneously converted to the 56 S material under these circumstances (Figs. 10 to 12). Although it was thus apparent that the 56 S subunit was common to both the *b* and *c* particles, it is not yet clear whether the latter differed from one another in molecular weight or in some configurational property. A boundary corresponding to the 56 S component was not observed in the crude tumor extracts. The 46 and 28 S subunits were apparently present, however, the former in appreciable amounts.

It has been postulated that the *a* component represents a dimer composed of two *b* particles (14, 18). If this is the case, the rather prominent *a* boundaries which were consistently observed in the tumor extracts suggests that such dimer formation may occur extensively within the cells. The proportion of *a* component decreased only slightly during the isolation procedure, and some of the pairs of particles visualized in electron micrographs of the fractions may have represented this material.

The proportions of the different ribonucleoprotein components within yeast and bacteria have been shown to vary in response to changes in both the ionic composition of the medium (38) and the growth phase of the cells (39). The differential effect of ATP and ADP in causing dissociation of the isolated tumor particles suggests a mechanism by which the physical state of the particles might be linked to the processes of energy production (or utilization) within the cells. Within the isolated systems observed in the present experiments, dissociation of the *a*, *b*, and *c* components to discrete subunits was initiated at ATP concentrations comparable to those found in certain types of tumor cells (40), while ADP was much less effective. Since magnesium ion prevented this dissociation, the *in vivo* response of the particles to the phosphorylation of ADP (or the release of pyrophosphate) within their immediate vicinity would of course be expected to depend upon the ionic environment in which such an event might occur. The operation of such a mechanism would be favored by the competitive binding of magnesium ions to soluble proteins and other particulate structures of the cell (41, 42) and by an intimate spatial relationship between the nucleoprotein particles and the ATP-generating enzymes of the glycolytic system.

References

1. Palade, G. E., *in* Microsomal Particles and Protein Synthesis, Washington, D. C., Washington Academy of Sciences, 1958, 36.
2. Haguenau, F., *Internat. Rev. Cytol.*, 1958, **7**, 425.
3. Loftfield, R. B., *Progr. Biophysics and Biophysic. Chem.*, 1957, **8**, 347.
4. Siekevitz, P., and Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 1.
5. Howatson, A. F., and Ham, A. W., *Cancer Research*, 1955, **15**, 62.
6. Dalton, A. J., and Felix, M. D., *Annals New York Acad. Sc.*, 1956, **63**, 1117.
7. Bernhard, W., *Cancer Research*, 1958, **18**, 491.
8. Kahler, H., and Bryan, W. R., *J. Nat. Cancer Inst.*, 1943, **4**, 37.
9. Petermann, M. L., *Texas Rep. Biol. and Med.*, 1954, **12**, 921.
10. Petermann, M. L., Mizen, N. A., and Hamilton, M. G., *Cancer Research*, 1956, **16**, 620.
11. Novikoff, A. B., *Cancer Research*, 1957, **17**, 1010.
12. Palade, G. E., and Siekevitz, P., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 171.
13. Littlefield, J. W., Keller, E. B., Gross, J., and Zamecnik, P. C., *J. Biol. Chem.*, 1955, **217**, 111.
14. Petermann, M. L., and Hamilton, M. G., *J. Biol. Chem.*, 1957, **224**, 725.
15. Littlefield, J. W., and Keller, E. B., *J. Biol. Chem.*, 1957, **224**, 13.
16. Chao, F.-C., and Schachman, H. K., *Arch Biochem. and Biophysics*, 1956, **61**, 220.
17. Ts'o, P. O. P., Bonner, J., and Vinograd, J., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 451.
18. Tissiéres, A., and Watson, J. D., *Nature*, 1958, **182**, 778.
19. Gillchriest, W. C., and Bock, R. M., *in* Microsomal Particles and Protein Synthesis, Washington, D. C., Washington Academy of Sciences, 1958, 1.
20. Petermann, M. L., and Hamilton, M. G., *Fed. Proc.*, 1959, **18**, 302.

21. Schneider, W. C., and Hogeboom, G. H., *J. Biol. Chem.*, 1950, **183,** 123.
22. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., Manometric Techniques and Tissue Metabolism, Minneapolis, Burgess Publishing Co., 1949, 191.
23. Schneider, W. C., *in* Methods in Enzymology, New York, Academic Press, Inc., 1957, **3,** 680.
24. Gornall, A. G., Bardawill, C. J., and David, M. M., *J. Biol. Chem.*, 1949, **177,** 751.
25. Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66,** 375.
26. Stadtman, T. C., in Methods in Enzymology, New York, Academic Press, Inc., 1957, **3,** 392.
27. Szalkowski, C. R., and Mader, W. J., *Anal. Chem.*, 1952, **24,** 1602.
28. Waugh, D. F., and Yphantis, D. A., *Rev. Scient. Instr.*, 1952, **23,** 609.
29. Kuff, E. L., and Dalton, A. J., *J. Ultrastruct. Research*, 1957, **1,** 62.
30. Petermann, M. L., Hamilton, M. G., Balis, M. E., Samarth, K., and Pecora, P., *in* Microsomal Particles and Protein Synthesis, Washington, D. C., Washington Academy of Sciences, 1958, 70.
31. Webster, G. C., *J. Biol. Chem.*, 1957, **229,** 535.
32. Korner, A., *Biochim. et Biophysica Acta*, 1959, **35,** 554.
33. Chao, F. -C., *Arch. Biochem. and Biophysics*, 1957, **70,** 426.
34. Ts'o, P. O., Bonner, J., and Vinograd, J., *Biochim. et Biophysica Acta*, 1958, **30,** 570.
35. Dintzis, H. M., Borsook, H., and Vinograd, J., *in* Microsomal Particles and Protein Synthesis, Washington, D. C., Washington Academy of Sciences, 1958, 95.
36. Bolton, R. T., Hoyar, B. H., and Ritter, D. B., *in* Microsomal Particles and Protein Synthesis, Washington, D. C., Washington Academy of Sciences, 1958, 18.
37. Hamilton, M. G., and Petermann, M. L., *J. Biol. Chem.*, 1959, **234,** 1441.
38. Bowen, T. J., Dagley, S., and Sykes, J., *Biochem. J.*, 1959, **72,** 419.
39. Ashikawa, J. K., *in* Microsomal Particles and Protein Synthesis, Washington, D. C., Washington Academy of Sciences, 1958, 76.
40. LePage, G. A., *Cancer Research*, 1948, **8,** 193.
41. Griswold, R. L., and Pace, N., *Exp. Cell Research*, 1956, **11,** 362.
42. Thiers, R. E., and Vallee, B. L., *J. Biol. Chem.*, 1957, **226,** 911.

Explanation of Plates

Plate 248

Figs. 1 to 12 represent ultracentrifuge patterns of Novikoff hepatoma ribonucleoproteins. Except where noted, the photographs were taken 13 minutes after reaching 37,020 r.p.m. and the solvent consisted of 5 mM sodium phosphate (pH 7.5) and 0.5 mM magnesium chloride with additions as specified. The total nucleoprotein concentration was between 4 and 6 mg. per ml. in all runs. The direction of sedimentation is towards the right.

Fig. 1. Crude tumor extract (mitochondrial supernatant fluid) showing the nucleoprotein peaks, lettered *a'*, *a*, *b*, *c*, *e*, and *f*. A large protein and sucrose boundary extends out of the picture near the meniscus. The solvent was 0.25 M sucrose, and the total protein concentration was 18 mg. per ml. Taken 13 minutes after reaching 52,640 r.p.m., at a rotor temperature of 1.5°C.

Fig. 2. Crude nucleoprotein fraction (*PM*) freshly resuspended in 0.05 M sodium bicarbonate, pH 8.

Fig. 3. Purified nucleoprotein fraction (RNP).

Fig. 4. An RNP fraction similar to that shown in Fig. 3, but in solvent containing 0.1 M sodium chloride. Note the decrease in prominence of the *c* and *e* peaks and the appearance of heterogeneous material in the region just ahead of the *b* boundary.

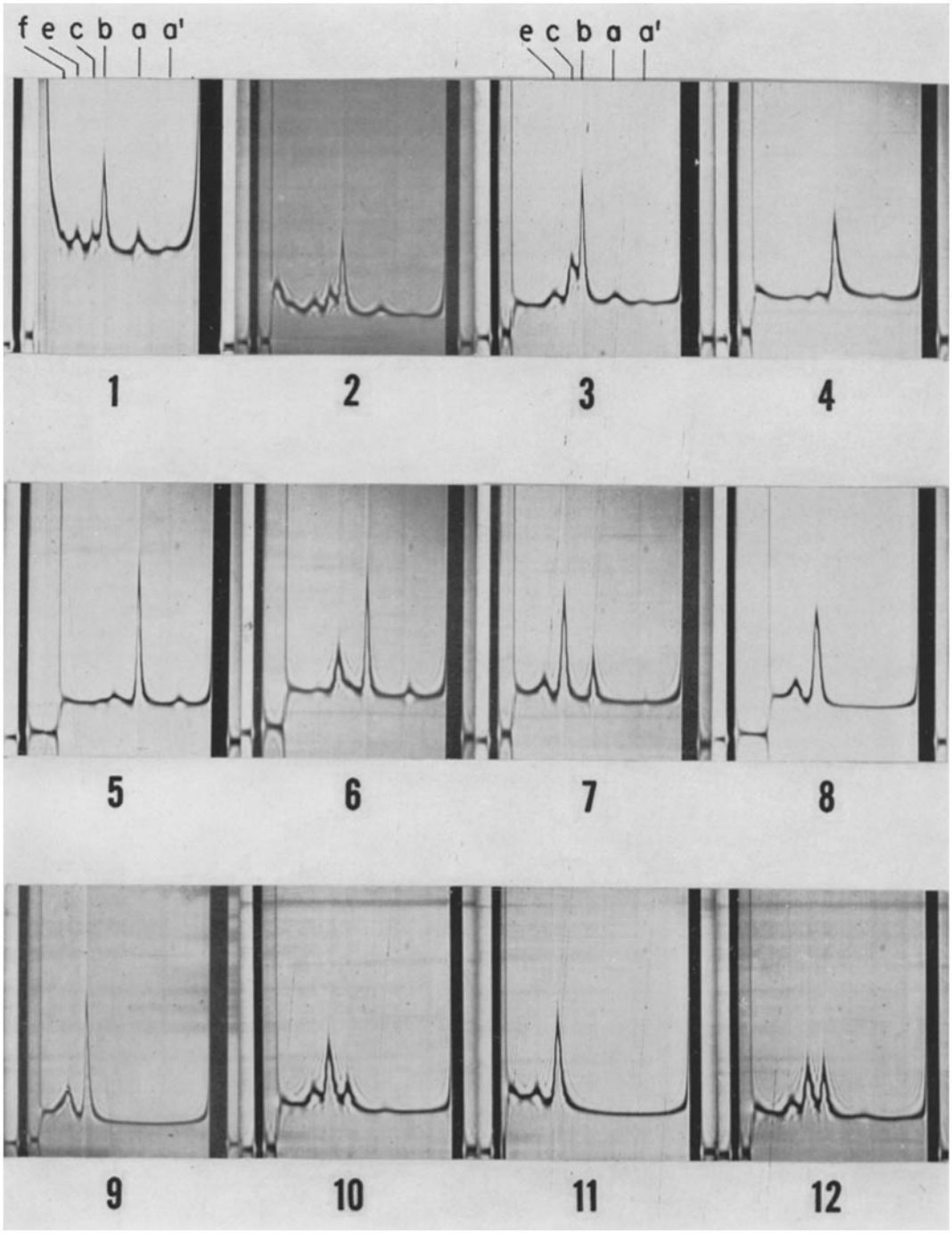
Fig. 5. Deoxycholate-treated nucleoprotein, RNP(DOC). The solvent contained 0.1 M sodium chloride.

Figs. 6 to 8. RNP(DOC), showing stages in the conversion of the 79 S component to smaller units in the presence of chelating agents. The solvent contained 0.1 M sodium chloride in each case, and 2 mM sodium pyrophosphate, 3 mM ATP, and 4 mM EDTA in Figs. 6, 7, and 8, respectively. The sedimentation rates of the two components in Fig. 8 were 45 and 28 S.

Fig. 9. RNP in solvent containing 0.05 M sodium chloride and 10 mM EDTA. The sedimentation rates of the two discrete components were 43 and 25 S.

Figs. 10 and 11. RNP, showing stages in the dissociation of the particles induced by 2 and 4 mM ATP, respectively. The solvent contained 0.05 M sodium chloride; essentially the same patterns were obtained in the presence of 0.1 M sodium chloride. The sedimentation rate of the main component in Fig. 11 was 52 S.

Fig. 12. RNP in solvent containing 0.05 M sodium chloride and 4 mM ADP. Note that dissociation is less than that seen in Fig. 10.



(Kuff and Zeigel: Ribonucleoproteins of Novikoff hepatoma)

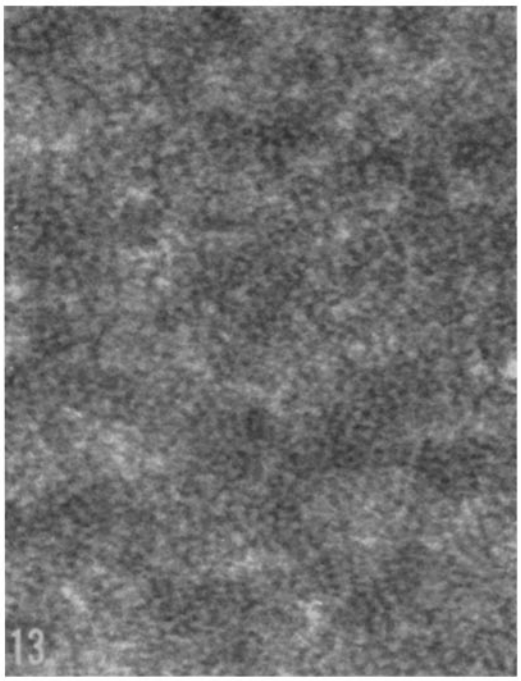
PLATE 249

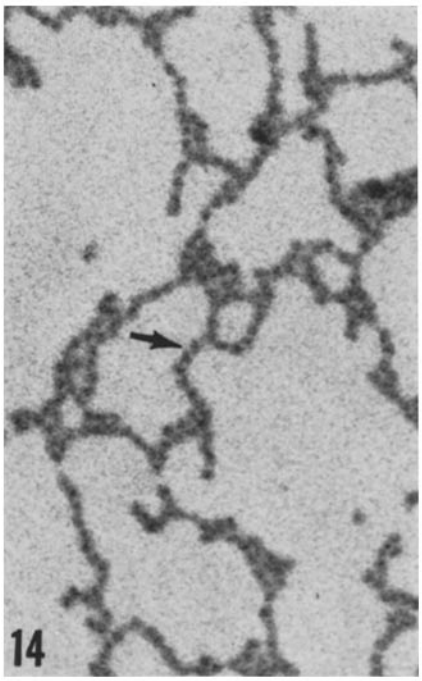
FIGS. 13 to 18 are electron micrographs of Novikoff hepatoma ribonucleoprotein preparations, all shown at a magnification of × 90,000. Except for Fig. 13, the preparations were plated directly on formvar films.

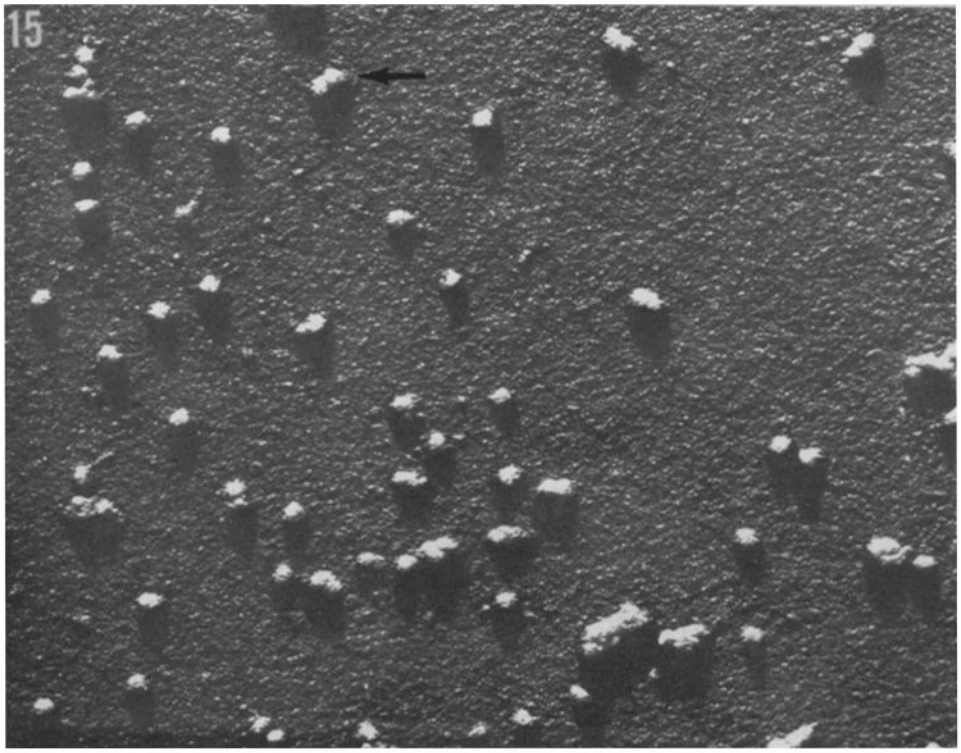
FIG. 13. Thin section through a fixed pellet of purified nucleoprotein, RNP. This field was representative of the entire pellet, in which no membranous elements were seen. The section was exposed to 0.5 per cent uranyl nitrate for 12 hours in order to enhance contrast of the particles. The size of the particles was not altered by this treatment.

FIG. 14. Deoxycholate-treated nucleoprotein, RNP(DOC), unfixed, unshadowed, and unstained. The arrow indicates a region where the particles were clearly resolved. The distance between the centers of the particles was 21 to 22 mμ.

FIG. 15. RNP(DOC), singly shadowed. The arrow indicates one of several pairs of particles which appear in the field. The distance between the centers of such adjacent particles, which may be taken to represent 1 particle diameter, was 22 mμ. On the other hand, an average diameter of 26 to 28 mμ was observed for the singly dispersed particles. The shadowing platinum thus apparently contributed 4 to 6 mμ to the diameter of these particles.







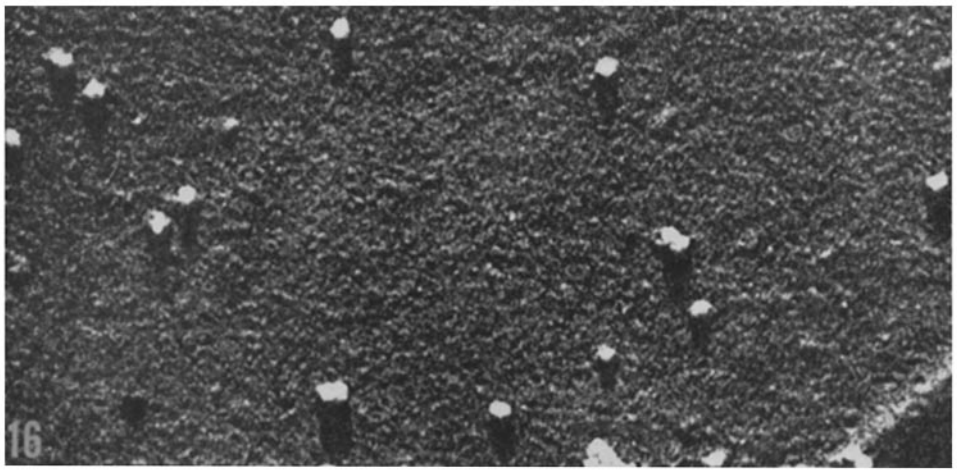
(Kuff and Zeigel: Ribonucleoproteins of Novikoff hepatoma)

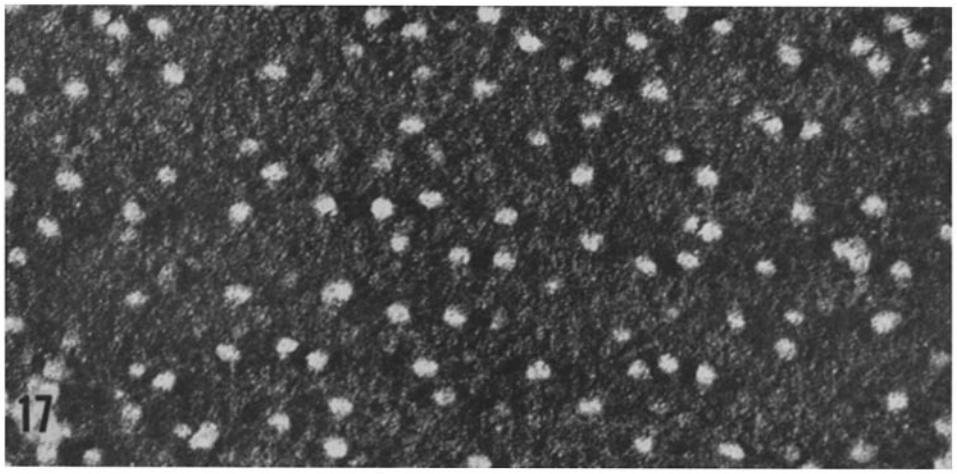
PLATE 250

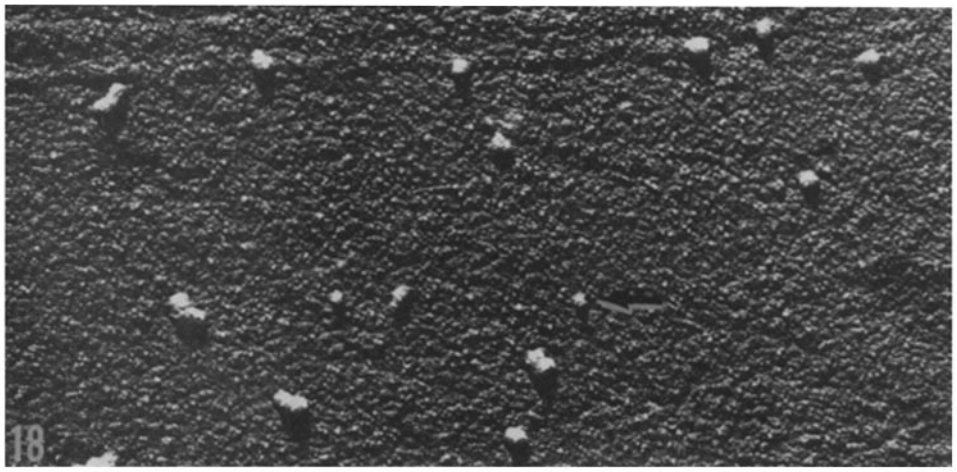
FIG. 16. Purified nucleoprotein, RNP, singly shadowed. The particles are similar in appearance to those seen in Fig. 15, but are more heterogeneous with respect to size. An extensive series of measurements gave an average diameter of 24 mμ for the largest particles, after correcting for the contribution of the shadowing material.

FIG. 17. RNP, doubly shadowed. The heterogeneity in the sizes of the particles is more clearly demonstrated here.

FIG. 18. RNP(DOC) which had been treated with 0.01 M EDTA to induce dissociation into subunits with sedimentation rates of 28 and 45 S. The subunits were sedimented at 105,000 *g* for 3 hours and resuspended in 5 mM sodium phosphate, pH 7.5, before plating on the grid. Particles of two general size classes may be seen. The larger particles had approximately the same diameter as those seen in the original RNP(DOC) (Fig. 15); however, they were considerably more flattened judging by their shadow lengths. The smaller particles (arrow) appeared to be spheres.







(Kuff and Zeigel: Ribonucleoproteins of Novikoff hepatoma)