The comments on the papers of Chatterjee, Chatterjee, Ghosh, Ghosh & Guha (1960) and Chatterjee, Kar, Ghosh & Guha (1960) were added after the present paper had been submitted for publication.

REFERENCES

- Chatterjee, I. B., Chatterjee, G. C., Ghosh, N. C., Ghosh, J. J. & Guha, B. C. (1960). *Biochem. J.* **74**, 193.
- Chatterjee, I. B., Kar, N. C., Ghosh, N. C. & Guha, B. C. (1960). *Arch. Biochem. Biophys.* **86**, 154.
- Chen, Y. T., Isherwood, F. A. & Mapson, L. W. (1953). *Biochem. J.* **55**, 821.
- Grollman, A. P. & Lehninger, A. L. (1957). *Arch. Biochem. Biophys.* **69**, 458.
- Harris, L. J. & Olliver, M. (1942). *Biochem. J.* **36**, 155.
- Hassan, M. & Lehninger, A. L. (1956). *J. biol. Chem.* **223**, 123.
- Hauschka, T. S., Toennies, G. & Swain, A. P. (1945). *Science*, **101**, 383.
- Haynes, L. J. (1948). *Quart. Rev. chem. Soc., Lond.*, **2**, 46.
- Heaton, T. B. (1926). *J. Path. Bact.* **29**, 293.
- Hestrin, S. (1949). *J. biol. Chem.* **180**, 249.
- Hogeboom, G. H., Schneider, W. C. & Pallade, G. E. (1948). *J. biol. Chem.* **172**, 619.
- Hogeboom, G. H., Schneider, W. C. & Striebich, M. J. (1952). *J. biol. Chem.* **196**, 111.
- Isherwood, F. A. (1953). *Proc. Nutr. Soc.* **12**, 335.
- Isherwood, F. A., Chen, Y. T. & Mapson, L. W. (1954). *Biochem. J.* **56**, 1.
- Isherwood, F. A. & Hanes, C. S. (1953). *Biochem. J.* **55**, 824.
- Isherwood, F. A. & Jermyn, M. A. (1951). *Biochem. J.* **48**, 515.
- Ishikawa, S. & Noguchi, K. (1957). *J. Biochem., Tokyo*, **44**, 465.
- Keilin, D. & Hartree, E. F. (1938). *Proc. Roy. Soc. B*, **124**, 397.
- Laird, A. K., Nygaard, O., Ris, H. & Barton, A. D. (1953). *Exp. Cell Res.* **5**, 147.
- Lowry, O. H., Roseburgh, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
- Mapson, L. W. (1943). *J. Soc. chem. Ind., Lond.*, **62**, 223.
- Mapson, L. W. & Breslow, E. (1958). *Biochem. J.* **68**, 395.
- Mapson, L. W., Isherwood, F. A. & Chen, Y. T. (1954). *Biochem. J.* **56**, 21.
- Medawar, P. B., Robinson, G. M. & Robinson, R. (1943). *Nature, Lond.*, **151**, 195.
- Novikoff, A. B., Podber, E., Ryan, J. & Noe, E. (1953). *J. Histochem. Cytochem.* **1**, 27.
- Peterson, E. A. & Sober, H. A. (1956). *J. Amer. chem. Soc.* **78**, 751.
- Potter, V. R. & Elvehjem, C. A. (1936). *J. biol. Chem.* **114**, 495.
- Roe, J. H. & Kuether, C. A. (1943). *J. biol. Chem.* **147**, 399.
- Schneider, W. C. (1948). *J. biol. Chem.* **176**, 259.
- Schneider, W. C. & Hogeboom, G. H. (1952). *J. biol. Chem.* **195**, 161.
- Smith, F. G. (1952). *Plant Physiol.* **27**, 736.
- Smythe, C. V. & King, C. G. (1942). *J. biol. Chem.* **142**, 529.
- Strittmatter, P. & Velick, S. F. (1956). *J. biol. Chem.* **221**, 253.
- Touster, O., Hutcheson, R. M. & Rice, L. (1955). *J. biol. Chem.* **215**, 677.
- Warburg, O. & Christian, W. (1941). *Biochem. Z.* **310**, 384.
- Winkelman, J. & Lehninger, A. L. (1958). *J. biol. Chem.* **233**, 794.

Biochem. J. (1960) **76**, 171

A Comparison of the Nucleic Acids of Rat Liver and Novikoff Hepatoma

BY E. H. CREASER AND J. H. SPENCER

McGill-Montreal General Hospital Research Institute, 3619 University Street, Montreal, Canada

(Received 28 September 1959)

Several workers have shown that qualitative and quantitative differences occur between the ribonucleoproteins present in the various fractions obtained by differential centrifuging of homogenates of normal and neoplastic tissue. Fiala, Sproul, Blutinger & Fiala (1955) showed that several mouse tumours differed from normal mouse tissue in that the cancer cells contained less mitochondria and microsomes than did normal cells, but that the content of 'non-sedimentable' (after 18 000 g for 2 hr.) ribonucleic acid was much higher in the tumours. Petermann, Mizen & Hamilton (1956) compared the concentration of ribonucleoprotein particles in rat liver and in hepatoma by means of ultracentrifugal analysis.

They observed that 'component B', which was associated with the endoplasmic reticulum and corresponded to the ribonucleoprotein particles described by Palade (1955), predominated in normal liver, spleen and pancreas. In tumours and rapidly growing cells such as regenerating liver, components C and E constituted the major part of the cytoplasmic ribonucleic acid. These ribonucleoprotein components were found to be free in the cytoplasm (Palade, 1955; Howatson & Ham, 1955) and correspond to the ribonucleoprotein found in the postmicrosomal fractions of Palade & Siekevitz (1956), and, in part, to the 'non-sedimentable' ribonucleic acid observed by Fiala *et al.* (1955). Comparison of the base ratios (molar proportions

of adenine, guanine, cytosine and uracil) of the ribonucleic acid present in various centrifugal fractions of rat liver and induced hepatoma homogenates by de Lamirande, Allard & Cantero (1955*a, b*) indicated that significant differences occurred between these normal and cancerous cells. They found that the guanine content of the ribonucleic acid was increased in all cell fractions of the tumour relative to liver and also that the differences in base ratios observed between different centrifugal fractions of rat liver were more uniform in the hepatoma.

We have sought to extend these observations by chromatographic analysis of the various nucleic acid-containing cell fractions on ECTEOLA-cellulose. This anion-exchanger, developed by Peterson & Sober (1956), has been used by Bendich (1956), Bendich, Fresco, Rosenkranz & Beiser (1955) and by Bradley & Rich (1956) for the chromatography of purified deoxyribo- and ribo-nucleic acid. ECTEOLA-cellulose has also been found useful in the purification of plant (Commoner, Lippincott, Shearer, Richmann & Wu, 1956), bacterial (Creaser & Taussig, 1957) and animal (Hoyer *et al.* 1958) viruses, and in the chromatography of bacterial nucleoproteins (Taussig & Creaser, 1959). The present investigation was carried out to examine the potentialities of ECTEOLA-cellulose for the chromatographic separation of nucleoproteins from mammalian tissues. A preliminary account of part of this work has appeared elsewhere (Spencer & Creaser, 1959).

MATERIALS AND METHODS

Tissues. Normal rat liver was obtained from 100–150 g. male Wistar albino rats, this type of animal also being used as host for the growth of the Novikoff hepatoma. The hepatoma was obtained from Dr F. Martel of Laval University and was maintained by weekly intraperitoneal transplantation. For the experiments described here the tumour was used after 6–7 days' growth. After excision from the animal the tissue was homogenized in 4 vol. of 0.25 M-sucrose containing 1.8 mM- CaCl_2 in a Potter-Elvehjem homogenizer fitted with a Teflon pestle.

Tissue fractionation. Homogenates of normal liver and Novikoff hepatoma were fractionated by the standard centrifuging procedures of Hogeboom & Schneider (1955) and Palade & Siekevitz (1956). The entire operation was carried out at 0–5° with an International PR2 and a Spinco model L centrifuge. Nuclei were removed from the homogenate by an initial centrifuging at 700 g for 10 min. and were purified by repeated suspension in 15 ml. of 0.25 M-sucrose containing 0.18 mM- CaCl_2 , layering this suspension on 30 ml. of 0.34 M-sucrose with the same CaCl_2 content and centrifuging at 700 g for 10 min. This procedure was repeated until a clear supernatant was obtained; normally four times sufficed. The homogenate was next centrifuged at 5000 g for 15 min. to remove mitochondria and the resultant supernatant was subjected to 59 000 g

for 1 hr. This sedimented microsomal fraction was either used for chromatography directly or was treated with 0.5% sodium deoxycholate (Palade & Siekevitz, 1956) to separate the ribonucleoprotein particles from the bulk of the lipoprotein constituents. The microsome supernatant and the suspension of microsome fraction in sodium deoxycholate were centrifuged for 3 hr. at 144 000 g to sediment the postmicrosome fraction and the microsomal ribonucleoprotein particles respectively. The material not sedimented from the original tissue homogenate by these forces was not further fractionated and is referred to below as the 144 000 g supernatant.

Chromatography of nucleoproteins and nucleic acids. ECTEOLA-cellulose was prepared by the method of Peterson & Sober (1956) from Solka-Flok cellulose powder (Brown Forest Products, Montreal), sieved to the requisite size. For most purposes 325-mesh cellulose was used as the starting material; ECTEOLA-cellulose was also prepared from 200-mesh cellulose, both types of exchanger containing approx. 0.4 m-equiv. of nitrogen/g. After use the ECTEOLA-cellulose was regenerated by successive passage of N-NaOH, m- KH_2PO_4 and water to neutrality, and was dried in air after washing with ethanol.

For use in chromatography a suitable quantity, normally 2.5 g., of the exchanger was suspended in 150 ml. of a buffer solution containing mM- CaCl_2 and mM- Na_2HPO_4 adjusted to pH 6.8 (CaCl_2 - Na_2HPO_4 buffer). Centrifugally obtained tissue fractions were absorbed on ECTEOLA-cellulose as follows: the 144 000 g supernatant was added directly to the slurry of exchanger; the sedimented post-microsome fraction and microsomal nucleoprotein fractions were suspended in 50 ml. of CaCl_2 - Na_2HPO_4 buffer before mixing with the ECTEOLA-cellulose suspension: the nuclei were first disintegrated by homogenization at full speed in a Virtis 45 homogenizer (Fisher Scientific Co.) in a dilute buffer containing 75 μM - CaCl_2 and 5 mM- Na_2HPO_4 adjusted to pH 6.8. After the absorption stage the ECTEOLA-cellulose was filtered off through Whatman no. 54 paper and was resuspended in 200 ml. of CaCl_2 - Na_2HPO_4 buffer to remove non-absorbed material and again filtered. The exchanger was then suspended in a small volume of CaCl_2 - Na_2HPO_4 buffer and made into a 5 cm. $\times$ 2.5 cm. column, which was eluted with NaCl solution in the buffer. This was done either manually by the passage of portions of solutions of NaCl of increasing concentration under an air pressure of 5–10 lb./in.², or automatically by using an increasing NaCl concentration gradient under a 6 ft. hydrostatic head. The rate of flow of eluate was normally 2–10 ml./min.

Estimations. Total nucleic acid was estimated spectrophotometrically in a Beckman model DU spectrophotometer at 260 m μ with an extinction coefficient of 25 for 0.1% nucleic acid. Deoxyribonucleic acid (DNA) was measured by the method of Ceriotti (1952) and ribonucleic acid (RNA) was estimated by measurement of the 260 m μ absorption of the acid-soluble products after digestion with N-KOH for 18 hr. (Schmidt & Thannhauser, 1945). Protein was measured by the method of Lowry, Rosenbrough, Farr & Randall (1951). Base ratios of RNA were determined after hydrolysis of ethanol-precipitated samples in N-HCl for 1 hr. at 100° (Vischer & Chargaff, 1948), followed by chromatography in *tert.*-butanol (700 ml.)-constant-boiling HCl (132 ml.)-water (to 1 l.) (Smith & Markham, 1950), or two-dimensionally in propan-2-ol

(680 ml.)–11.6N-HCl (176 ml.)–water (to 1 l.) (Wyatt, 1951) and propan-2-ol (700 ml.)–water (to 1 l.) with NH_3 in the vapour phase (Markham & Smith, 1952) on Whatman no. 3 MM paper. Ultraviolet-absorbing spots were eluted and estimated as described by Wyatt (1955).

RESULTS

It was found that the nucleic acid and protein present in centrifugally prepared cell fractions of rat liver and Novikoff hepatoma were readily absorbed by ECTEOLA-cellulose. Normally 2.5 g. of exchanger sufficed to absorb quantitatively samples of cell fractions containing up to 50 mg. of nucleic acid. The columns of ECTEOLA-cellulose, prepared as described above, were eluted with portions of NaCl of increasing concentration in CaCl_2 – Na_2HPO_4 buffer. The volume of these portions was normally 10 ml., this being chosen so as to exceed the 'dead space' of the column (the 'dead space' for a 2.5 g. column was 7 ml.). This method of operation is essentially used for batches and has been shown previously (Creaser & Taussig, 1957; Taussig & Creaser, 1959) to be appropriate for the chromatography of nucleoproteins on ECTEOLA-cellulose.

Preliminary experiments showed that in order to obtain reproducible chromatographic patterns it was essential to use a suitable concentration of calcium or magnesium in the eluting solutions (cf. Chao, 1957). In the experiments described here a calcium concentration of mM was used; if the

calcium was omitted or its concentration reduced to below half this value the eluted material failed to resolve and usually appeared as one broad peak over the range 0.2–0.3M-NaCl.

'Supernatant' ribonucleic acid. Hoagland, Stephenson, Scott, Hecht & Zamecnik (1958) and Goldthwait (1958) found that radioactive leucine was bound to RNA by a cell fraction obtained as the supernatant after sedimentation of tissue homogenates at 105 000 g for 2 hr. This RNA, after extraction with phenol by the method of Kirby (1956), was chromatographed on ECTEOLA-cellulose and was eluted as two fractions, one by 0.15–0.20M-NaCl and the other by M-NaOH. In the present experiments it was found that the RNA remaining in the supernatant after centrifuging at 144 000 g for 3 hr. chromatographed on ECTEOLA-cellulose in such a manner that over 90% was eluted over the range 0.10–0.25M-NaCl. Fig. 1 illustrates such a pattern and it can be seen that the eluted RNA is heterogeneous. Identical patterns were observed whether the source of 'supernatant' RNA was rat liver or Novikoff hepatoma.

Fraser, Shimizu & Gutfreund (1959) studied the binding of activated radioactive amino acids by 'cell-sap' or 'supernatant' RNA. Samples of the RNA prepared by centrifuging and chromatography on ECTEOLA-cellulose from both rat liver and Novikoff hepatoma were tested by Dr M. J. Fraser and were found to have the same activity as acceptors of activated glutamine and leucine. The base ratios of the major components of these two RNA samples were not significantly different.

Post-microsomal ribonucleic acid. Chromatography of the postmicrosome fractions of Novikoff-hepatoma and rat-liver homogenates gave patterns which were markedly different, as shown in Figs. 2 and 3. The elution pattern for rat liver (Fig. 2) showed a wide variety of nucleic acid components of differing concentrations. The nucleic acid peaks corresponded to protein peaks over the range 0.18–1.0M-NaCl, the ratio protein:nucleic acid (w/w) being from 6.5:1 to 2.8:1. Over the range 0.01–0.18M the correspondence of nucleic acid to protein peaks was less marked, excess of protein being present over this part of the elution pattern, varying from 17:1 to 2:1. Of the nucleic acid 7.4% was not eluted from the column by NaCl but was released by N-NaOH.

Chromatography of the postmicrosome fraction from Novikoff hepatoma (Fig. 3) showed that it contained one major nucleic acid component and several minor ones. The minor components were eluted over the range 0.170–1.00M-NaCl and corresponded, with the exception of the 0.215M peak, exactly with protein peaks, the ratios protein:nucleic acid being from 2.9:1 to 1.6:1. In the

Fig. 1. Part of the elution pattern of the 144 000 g supernatant from rat liver. Material from 120 g. of liver was absorbed on 2.5 g. of exchanger and eluted with 10 ml. portions of NaCl soln. (in CaCl_2 – Na_2HPO_4 buffer) of the concentration shown. O, Nucleic acid; ▲, protein.

Fig. 2. Chromatography of the postmicrosome fraction from rat liver. ECTEOLA-cellulose (2.5 g.) was used to absorb an amount of the fraction containing 30 mg. of nucleic acid. The fraction was prepared centrifugally and the exchanger was eluted, as described in the text, with 10 ml. portions of NaCl soln. (in CaCl_2 - Na_2HPO_4 buffer) of the concentrations shown. $\circ$, Nucleic acid; $\blacktriangle$, protein. In this and subsequent elution patterns the scales on the abscissae are discontinuous at either end.

Fig. 3. Chromatography of the postmicrosome fraction from Novikoff hepatoma. Conditions were as described for Fig. 2. $\circ$, Nucleic acid; $\blacktriangle$, protein.

Fig. 4. Chromatography of the microsomal ribonucleoprotein (deoxycholate-insoluble) fraction from rat liver. Conditions were as described for Fig. 2. ○, Nucleic acid; ▲, protein.

Fig. 5. Chromatography of microsomal ribonucleoprotein (deoxycholate-insoluble) fraction from Novikoff hepatoma. Conditions were as described for Fig. 2. ○, Nucleic acid; ▲, protein.

earlier part of the pattern the major component (eluted by 0.125M-NaCl) had an apparent 3.2:1 excess of protein over nucleic acid. However, in other experiments where the amount of protein eluted by these low concentrations of NaCl was reduced and there was a true correlation between the protein and nucleic acid patterns, the ratio was 1.7 mg. of protein to 1 mg. of RNA. The major component comprised 50–70% of the RNA of the fraction whereas 12% was eluted only by NaOH.

Microsomal ribonucleoprotein. Chromatography of microsomal nucleoproteins was greatly facilitated by treating the centrifugally obtained microsome fraction with sodium deoxycholate and sedimentation of the ribonucleoprotein particles (Palade, 1955). The same nucleic acid-elution pattern was obtained from the chromatography of either 'microsome fraction' or deoxycholate-treated and sedimented ribonucleoprotein. However, the elution pattern from microsomes was complicated by the presence of an excess of protein, not associated with RNA, and the rate of flow of the column was drastically reduced, possibly due to blocking of the ECTEOLA-cellulose by lipoprotein.

Fig. 4 shows the chromatography of microsomal ribonucleoprotein from rat liver on ECTEOLA-cellulose. There was a close correlation throughout between the protein and nucleic acid-elution patterns. The ratio protein:nucleic acid was within the limits 1.8:1 and 0.9:1. The major component was eluted by 0.125M-NaCl and comprised 28–35% of the total RNA of the fraction. Some 11% of the nucleic acid of the fraction was released from the column only by NaOH. Fig. 5 shows the elution pattern obtained by chromatography of Novikoff-hepatoma microsomal ribonucleoprotein. In comparison with the rat-liver pattern, the amount of the major component was reduced in the hepatoma to 10% of the total ribonucleoprotein. Both the rat-liver and the Novikoff-hepatoma patterns showed a considerable heterogeneity of ribonucleoproteins.

Differences between chromatographically separated ribonucleoproteins. Bradley & Rich (1956) stated that fractions of RNA eluted from ECTEOLA-cellulose by high salt concentrations had a higher mean sedimentation coefficient than those eluted by low salt concentrations. Bendich (1956) found that there were base ratio differences between chromatographically distinct DNA fractions. Tausig & Creaser (1959) showed a correlation between the protein:nucleic acid ratio of bacterial nucleoproteins and the concentration of NaCl necessary to elute them from ECTEOLA-cellulose. In the present experiments we have tried to correlate the base ratios and protein:RNA ratios of nucleoprotein peaks with the concentration of NaCl which elutes them from the exchanger. Fig. 6 shows the

results of such an experiment with Novikoff-hepatoma microsomal ribonucleoprotein. It can be seen that there is considerable variation in the base ratios of different fractions. There is a slight trend towards increase in the guanine and cytosine content and a decrease in uracil in successive fractions. There is a marked correlation between the adenine content of a given ribonucleoprotein peak and the concentration of NaCl by which it is eluted. A similar although less-marked effect was found in the chromatography of the ribonucleoproteins from rat-liver-microsomal ribonucleoprotein and with both normal and tumour postmicrosomal fractions. In addition there was evidence of a decrease in the protein:nucleic acid ratio of successively eluted fractions (see Fig. 4). Further evidence that the protein constituent was of importance in nucleoprotein chromatography was obtained when the ribonucleoproteins eluted by 0.125M-NaCl from ECTEOLA-cellulose were degraded. This material derived either from rat microsomes or Novikoff-hepatoma postmicrosomes was treated with 5 mM-ethylenediaminetetra-acetic acid at 0° for 10 sec. and then precipitated with 2 vol. of cold ethanol. The nucleic acid and protein were quantitatively precipitated by this treatment and the precipitate

Fig. 6. Relationship between base ratios of microsomal ribonucleoproteins and the concentration of NaCl which elutes them from ECTEOLA-cellulose. Base ratios were determined by hydrolysis and chromatography as described in the text. A, Adenine; G, guanine; C, cytosine; U, uracil. The lines were derived from the experimental points by the method of least squares.

was then suspended in $\text{CaCl}_2\text{-Na}_2\text{HPO}_4$ buffer and rechromatographed on ECTEOLA-cellulose. The elution pattern obtained showed that whereas the bulk of the protein was eluted over the range 0.12–0.18M-NaCl, only some 10% of the RNA was so eluted. The rest of the RNA was not eluted from the column by saturated NaCl but was released on passage of NaOH.

Nucleic acid components of cell nuclei. It was found that it was not feasible to chromatograph nuclear extracts on 325-mesh ECTEOLA-cellulose because the high viscosity of eluted DNA blocked the flow through the column. However, it was possible to use 200-mesh ECTEOLA-cellulose for this purpose as it has a much higher flow rate, although the chromatographic resolution is not as good as that obtained with 325-mesh. Chromatography of extracts of the nuclear fraction of rat liver and of Novikoff hepatoma showed a striking difference in their nucleoprotein components (Fig. 7). The rat-liver nucleoprotein was eluted in two groups, the first centred at 0.6M-NaCl, the second over the range 1.0 to 1.5M-NaCl. The first group had a protein:nucleic acid ratio of 5:1 and a DNA:RNA ratio of 4.5:1; the second group had protein:nucleic acid and DNA:RNA ratios of 4:1

and 5:1 respectively. The elution pattern obtained from Novikoff-hepatoma-nuclear extract showed essentially a single peak at 0.4M-NaCl which contained over 90% of the DNA, RNA and protein originally present in the nucleus. When this complex material was degraded by ethylenediamine-tetra-acetic acid and ethanol as described above, and rechromatographed, nucleic acid was eluted over the range 0.2–1.6M-NaCl as a series of imperfectly resolved peaks.

DISCUSSION

Comparison of the chromatographic patterns obtained from centrifugally prepared cellular fractions from rat liver and Novikoff hepatoma has shown that significant differences exist between these two tissues in the nucleoprotein composition of the microsomal, postmicrosomal and nuclear fractions. Whether or not these differences are characteristic of tumour cells or would be shown by non-cancerous, rapidly proliferating tissues is a question which can be elucidated only by study of other types of tissues. The results we obtained are in agreement with those of Petermann *et al.* (1956) if the assumption is made that 'component B'

Fig. 7. Chromatography of nuclear extracts of rat liver and Novikoff hepatoma. ECTEOLA-cellulose (2.5 g.) was used to absorb an amount of the extract containing 40 mg. of nucleic acid. The extract was prepared as described in the text. The exchanger was eluted with an increasing NaCl concentration gradient, such that successive 10 ml. fractions increased in concentration by mM-NaCl. For clarity only every fifth fraction has been plotted. O, Rat-liver nucleic acid; Δ , Novikoff-hepatoma nucleic acid.

corresponds to the ribonucleoprotein peak eluted by 0.125M-sodium chloride in chromatograms of the microsome fraction and that 'component C' is represented by the major component present in the postmicrosome fraction. Differences in base ratios and in chromatographic behaviour on ECTEOLA-cellulose suggest that the cytoplasmic ribonucleoproteins of both rat liver and Novikoff hepatoma have a high degree of heterogeneity.

SUMMARY

1. Chromatography of the nucleoproteins and nucleic acids present in centrifugally prepared cell fractions of rat liver and Novikoff hepatoma on ECTEOLA-anion-exchange-cellulose has been described.

2. Cytoplasmic ribonucleic acid which was not sedimentable by 144 000 g for 3 hr. has been shown to be identical in the two tissues on the criteria of base ratios, chromatographic behaviour and ability to accept activated amino acids.

3. The ribonucleic acid present in the post-microsome fraction chromatographed as several ribonucleoprotein peaks; the major component of this fraction found in preparations from the hepatoma was absent from rat liver. The microsome ribonucleoprotein was also chromatographically heterogeneous; in this fraction the major component was much reduced in the hepatoma as compared with that in normal liver.

4. The chromatographic patterns obtained from extracts of nuclei of the two tissues were completely different.

5. The available data suggests that separation of nucleoproteins on columns of ECTEOLA-cellulose results from differences in base ratios, particularly the adenine content of the various fractions, and is also influenced by the protein content of the nucleoprotein.

We are grateful to Professor J. H. Quastel, F.R.S., for his interest in this work. We also wish to thank the Department of Pharmacology, McGill University, for the use of their Spinco ultracentrifuge. Financial support for this work was provided by the Rockefeller Foundation and the Montreal Cancer Research Society.

REFERENCES

- Bendich, A. (1956). *Cold Spr. Harb. Symp. quant. Biol.* **21**, 31.
- Bendich, A., Fresco, J. R., Rosenkranz, H. S. & Beiser, S. M. (1955). *J. Amer. chem. Soc.* **77**, 3671.
- Bradley, D. F. & Rich, A. (1956). *J. Amer. chem. Soc.* **87**, 5898.
- Cerioti, G. (1952). *J. biol. Chem.* **198**, 297.
- Chao, F. C. (1957). *Arch. Biochem. Biophys.* **70**, 426.
- Commoner, B., Lippincott, J. A., Shearer, G. B., Richmann, E. E. & Wu, J. H. (1956). *Nature, Lond.*, **178**, 767.
- Creaser, E. H. & Taussig, A. (1957). *Virology*, **4**, 200.
- de Lamirande, G., Allard, C. & Cantero, A. (1955a). *Cancer Res.* **15**, 329.
- de Lamirande, G., Allard, C. & Cantero, A. (1955b). *J. biol. Chem.* **214**, 519.
- Fiala, S., Sproul, E. E., Blutinger, M. E. & Fiala, A. E. (1955). *J. Histochem. Cytochem.* **3**, 212.
- Fraser, M. J., Shimizu, N. & Gutfreund, H. (1959). *Biochem. J.* **72**, 141.
- Goldthwait, D. A. (1958). *Biochim. biophys. Acta*, **30**, 643.
- Hoagland, M. B., Stephenson, M. L., Scott, J. F., Hecht, L. I. & Zamecnik, P. C. (1958). *J. biol. Chem.* **231**, 241.
- Hogeboom, G. H. & Schneider, W. C. (1955). In *The Nucleic Acids*, vol. 2, p. 199. Ed. by Chargaff, E. & Davidson, J. N. New York: Academic Press Inc.
- Howatson, A. F. & Ham, A. W. (1955). *Cancer Res.* **15**, 62.
- Hoyer, B. H., Bolton, E. T., Ormsbee, R. A., LeBouvier, G., Ritter, D. B. & Larson, C. L. (1958). *Science*, **127**, 859.
- Kirby, K. S. (1956). *Biochem. J.* **64**, 405.
- Lowry, O. H., Rosenbrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
- Markham, R. & Smith, J. D. (1952). *Biochem. J.* **52**, 552.
- Palade, G. E. (1955). *J. biophys. biochem. Cytol.* **1**, 59.
- Palade, G. E. & Siekevitz, P. (1956). *J. biophys. biochem. Cytol.* **2**, 171.
- Petermann, M. L., Mizen, N. A. & Hamilton, M. G. (1956). *Cancer Res.* **16**, 620.
- Peterson, E. A. & Sober, H. A. (1956). *J. Amer. chem. Soc.* **78**, 751.
- Schmidt, G. & Thannhauser, S. J. (1945). *J. biol. Chem.* **161**, 83.
- Smith, J. D. & Markham, R. (1950). *Biochem. J.* **46**, 509.
- Spencer, J. H. & Creaser, E. H. (1959). *Proc. Canad. Fed. biol. Soc.* **2**, 62.
- Taussig, A. & Creaser, E. H. (1959). *Arch. Biochem. Biophys.* **83**, 436.
- Vischer, E. & Chargaff, E. (1948). *J. biol. Chem.* **176**, 715.
- Wyatt, G. R. (1951). *Biochem. J.* **48**, 584.
- Wyatt, G. R. (1955). In *The Nucleic Acids*, vol. 1, p. 243. Ed. by Chargaff, E. & Davidson, J. N. New York: Academic Press Inc.