Differences in Nuclear Ribonucleic Acid of Human Neoplastic and Normal Tissue* (33903)

JOSEPH G. ROCHE[1], WERNER ROSENAU, AND MELVIN L. GOLDBERG
(Introduced by Henry D. Moon)

Department of Pathology, University of California School of Medicine, San Francisco, 94122

The essence of the neoplastic process is a failure of regulation of cellular growth, differentiation, and integration into the tissues of the organism; the mechanism of these aberrations is not yet understood. This failure of normal regulatory functions focuses attention on possible alterations of the nucleic acids in neoplastic tissue—a problem we approached by electrophoretic comparison of RNA patterns of neoplastic and normal human tissues on acrylamide gels.

Materials and Methods. Normal and neoplastic human tissues from adult patients, identified in Table I, were obtained in the operating room and processed immediately after transsection of the blood supply, avoiding areas of necrosis. Each tissue was processed separately; in addition, in two instances (Table I, 3c and 5c), an equal mixture (2.5–2.5 g) of normal and neoplastic tissue was carried through the entire procedure to determine if either type of tissue contained factors (*e.g.*, nucleases) which might affect the extraction of RNA. Usually 5 g of tissue was used for extraction (range, 1.5–5 g).

We extracted nuclear and cytoplasmic RNA according to the phenol-sodium dodecyl sulfate method of Peacock and Dingman (1, 2) but with the following modifications: (i) their solution A was supplemented with 5 mg/ml of washed bentonite (3); (ii) after the first phenol extraction at 20°, the interface was re-extracted at 66° for 6 min and combined with the first extract. Following the phenol extraction, the nuclear extract was reprecipitated three times with ethanol and digested with DNAse (Worthington, RNAse-free). We removed the DNAse by phenol extraction at 4° for 10 min and reprecipitated the RNA with ethanol. Some duplicate nuclear extracts were precipitated with ethanol and treated with 100 μg/ml of RNAse (Worthington, DNAse- and protease-free) at 37° for 30 min.

Acrylamide and methylene bis-acrylamide were purified according to Loening's method (4). The preparation and loading of gels were similar to the procedure described by Bishop and his associates (5). The 3.5% gels were prepared by mixing 8.75 ml of an aqueous stock solution of 10% acrylamide, 0.5% bis-acrylamide with 7.70 ml of water and 8.33 ml of electrophoretic buffer (0.12 *M* Tris, 0.06 *M* sodium acetate, 0.003 *M* sodium ED TA, adjusted with acetic acid to pH 7.2). Before casting the gels, 0.02 ml of *N, N, N' N'*-tetramethylenediamine and 0.2 ml of fresh aqueous 10% ammonium persulfate were added and the solution was gently swirled. It was then transferred to glass tubes with internal diameters of 0.6 cm and the gel length was adjusted to 5.0 cm. After polymerization, the gels were pre-run for 20 min at 5 mA/-tube at room temperature with the above electrophoretic buffer diluted to one-third strength. The gels were freshly prepared for each analysis. Our usual sample contained 20 μg of RNA in a 20-μl loading volume in the diluted buffer with 10% sucrose (RNAse-free); in addition, duplicate analysis of some normal and neoplastic RNA preparations with fivefold increases in volume and/or concentration was carried out. The samples were electrophoresed at 5 mA/tube for 45 or 60 min at room temperature. Then the tubes were placed in ice water and the gels were removed and stained with methylene blue (1). The excess stain was removed by tap-water rinses.

* Supported by Public Health Service Grant G 15934-01 and the Cancer Research Fund of the University of California.

[1] Recipient Public Health Service Training Grant GM 00349-09.

TABLE I. Tissues and Findings.

Specimen[a]	Material	Additional bands with RNA from tumor tissue
1a	Normal renal cortex and medulla	—[b]
1b	Renal cell carcinoma (5-cm diam), clear cell pattern	5
2a	Normal renal cortex and medulla	—[b]
2b	Renal cell tumor (3-cm diam), clear cell pattern (not clearly classified as benign or malignant by histologic criteria.)	6
3a	Normal renal cortex and medulla	—[b]
3b	Renal cell carcinoma (7-cm diam), mixed clear cell and anaplastic pattern	4
3c	Mixture of tumor and normal tissue	Composite of 3a, b
4a	Normal colonic mucosa	—[b]
4b	Adenocarcinoma of colon	4
5a	Normal ileal mucosa	—[b]
5b	Metastatic melanoma involving ileum	4
5c	Mixture of tumor and normal tissue	Composite of 5a, b

[a] Specimens with the same Arabic number are from the same patient.

[b] The nuclear RNA of all normal tissues displayed almost identical patterns.

Results. The nuclear RNA patterns of three human kidneys from which RNA was extracted and electrophoretically examined at different times were virtually indistinguishable in the number and position of bands, indicating that the method was reproducible. However, when we compared the nuclear RNA pattern of the normal renal tissue to that of three renal neoplasms, in all instances we observed striking differences. These differences consisted of the presence of four to six additional distinct bands occurring in the upper fourth of the gel with preparations of nuclear RNA from renal neoplasms (Table I, Fig. 1). These bands were not seen in nuclear RNA preparations of normal tissue, nor were they seen when duplicate samples were pretreated with RNAse. Comparison between the renal cell carcinomas and renal tissue is of particular interest because renal cell carcinomas are derived from renal tubules which make up the bulk of kidney tissue. Therefore this tissue serves as a good control.

Analysis of the nuclear RNA from an adenocarcinoma of the colon revealed the presence of four additional bands again in the upper fourth of the gel—bands which were not present in gels with nuclear RNA of normal colonic mucosa from the same patient (Table I). Nuclear RNA from a metastatic malignant melanoma also displayed a pattern very similar to that of the other neoplasms, possessing four additional bands (Table I).

Electrophoresis of nuclear RNA extracted from an equal mixture of normal and neoplastic tissue (Table I, 3c and 5c) revealed a composite pattern of normal (3a, 5a) and neoplastic (3b, 5b) nuclear RNA with no bands lost or gained.

The nuclear RNA from three samples of normal renal tissue, one sample of normal colonic mucosa, and one sample of normal ileal mucosa exhibited very similar patterns. In the upper fourth of the gel with neoplastic and normal RNA, we occasionally noted a single band which stained a different color, was resistant to RNAse and DNAse treatment, and probably was glycogen. Only variable and usually minor differences were seen in cytoplasmic RNA of normal and neoplastic tissue.

Discussion. These experiments clearly demonstrated definite and consistent differences between the nuclear RNA of normal and neoplastic tissue obtained from five patients and consecutively examined. Two possible explanations are that the neoplastic tis-

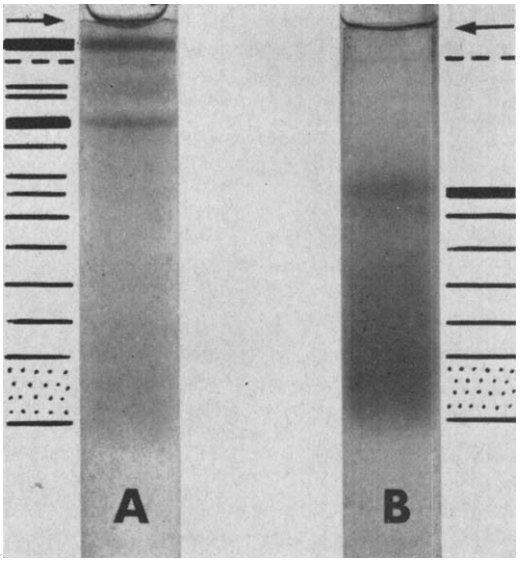

FIG. 1. The nuclear RNA pattern from a renal tumor (A) shows six additional bands of RNA in the upper part of the gel (solid lines) which are not present with nuclear RNA from normal renal tissue (B); The other bands are similar in position, although of different intensity; (broken lines), indicate material other than RNA (see text); (stippled areas) identify zones of poor resolution; the bands are drawn in because of difficulty in recording them photographically; arrow indicates origin; (A) = tissue 2b; (B) = tissue 2a.

sue contains an unidentified oncogenic virus coding for some or all of the additional nuclear RNA, or that in neoplasia, normally inactive genes are activated. These two explanations are not mutually exclusive. From RNA–DNA hybridization experiments, it is known (6, 7) that in some animal tumor cell lines, oncogenic viruses may code for some of the tumor-cell RNA; it also appears that in some neoplasms, activation of normally inactive genes occurs, as evidenced by occasional hormone production by nonendocrine tumors (8). These two possible explanations could be examined experimentally by the isolation of the additional RNA from neoplasms and hybridization of this RNA with the DNA of normal and neoplastic tissue. Hybridization with neoplastic DNA and failure to hybridize with normal DNA would suggest the existence of new genetic material in the tumor tissue (*e.g.*, a virus or a mutation), while similar degrees of hybridization with normal and neoplastic DNA would point to activation of normally inactive genes.

We attempted to minimize the possibility that differences in RNA patterns of normal and neoplastic tissues are artifacts. Normal and neoplastic tissues may possess different nuclease activities which could result in some RNA breakdown of varying degree during the isolation procedure. Therefore equal mixtures of normal renal tissue and renal cell carcinoma, as well as normal ileal mucosa and melanoma, were also carried through the entire extraction procedure. These mixtures revealed composite RNA patterns of normal and neoplastic tissue with no bands lost (or gained), thus ruling against a differential degradation of RNA in the course of the isolation process. Furthermore, the activity of nucleases was minimized by the use of inhibitors and low temperature. The nucleic acid preparations were stirred and pipetted gently to avoid shearing of the RNA. Another consideration is that during the isolation of nuclei, there is a possible preferential loss of RNA in normal tissue; however, this seems unlikely because it would have to involve the selective loss of heavier RNA. We also considered the possibility that the RNA differences in our nuclear preparations were due to cytoplasmic contamination; however, there were only similar, minute amounts of cytoplasm adherent to nuclei in both types of preparation. Unsuccessful attempts were made to free nuclei of small, remaining cytoplasmic tags by centrifugation through discontinuous sucrose gradients, by Behren's method with organic solvents (9), and by removal with detergents. The first two approaches failed to remove all cytoplasmic tags; the latter led to disruption of many nuclei over a wide range of concentration of detergents. It is possible that RNA differences between normal and neoplastic tissue are quantitative and not qualitative, so that the species of RNA we have detected only in neoplastic tissue occur in such small quantities as to escape recognition in normal tissue. Mizuno and his associates (10) described a quantitative increase in nuclear RNA of a Morris hepatoma as compared to nuclear

RNA from rat liver; almost twice as much RNA/nucleus was recovered from the rat hepatoma. However, in connection with the last two considerations—the role of cytoplasmic contamination and possible quantitative differences—it is noteworthy that even five-fold increases in the amount of nuclear RNA from normal tissue applied to the gel did not reveal additional bands on electrophoresis. It is unlikely that the additional RNA is only an expression of rapid cellular proliferation, since these renal tumors differed markedly in mitotic activity. Tumor 2b was essentially devoid of mitosis, while tumor 3b was a rapidly growing mass teeming with mitoses. Furthermore, small-intestinal mucosa, also used as a control tissue, exhibits rapid cellular proliferation, but its nuclear RNA did not contain the additional bands.

The 3.5% gel used here does not allow analysis of RNA heavier than about 28–30 S (1, 4); thus we did not study possible differences in higher molecular-weight RNA. The small molecular-weight RNA was not resolved at this concentration of the gel, and migrated as a fast, large, diffuse band.

As yet, little is known about RNA differences between normal and neoplastic tissue in experimental animals; furthermore, this area of investigation in human neoplasia has been virtually untouched. Studies of some virus-transformed cell lines (6, 7) from experimental animals have disclosed the presence of RNA capable of hybridizing with virus DNA and apparently coded for by the virus. Drews and his associates (11), using RNA–DNA hybridization in studies of rat liver tissue and rat hepatomas, observed that the nuclei of these hepatomas lacked some hybridizable RNA species found in the nuclei of normal liver tissue. Their finding by another technique differs from our results in that they noted the absence of certain RNA species rather than the presence of additional RNA in malignant tissue. Kidson and Kirby (12, 13) applied counter-current techniques to the study of RNA in neoplasia and described differences in the RNA of neoplastic and normal tissue from chemically induced or transplanted murine tumors. Others, however, have raised doubts concerning the use of the counter-current technique for characterization of RNA because of their failure to obtain reproducible results (14).

Our experiments demonstrated definite and consistent differences between the nuclear RNA from normal and neoplastic tissue. Further studies of such dissimilarities may help to elucidate the nature of the neoplastic process and serve to initiate new approaches to the diagnosis of neoplasia.

Summary. We observed definite and consistent differences between RNA isolated from neoplastic and normal human tissue by electrophoresis on polyacrylamide gels. The major differences were in the nuclear RNA preparations; here the preparations from neoplastic tissue contained several fractions not found in those from normal tissue. Only minor differences were detected in the RNA patterns of cytoplasmic preparations.

1. Peacock, A. C. and Dingman, C. W., Biochemistry **6**, 1818 (1967).

2. Dingman, C. W. and Peacock, A. C., Biochemistry **7**, 659 (1968).

3. Fraenkel-Conrat, H., Singer, B., and Tsugita, A., Virology **14**, 54 (1961).

4. Loening, U. E., Biochem. J. **102**, 251 (1967).

5. Bishop, D. H. L., Claybrook, J. R., and Spiegelman, S., J. Mol. Biol. **26**, 373 (1967).

6. Benjamin, T. L., J. Mol. Biol. **16**, 359 (1966).

7. Fuginaga, K. and Green, M., Proc. Natl. Acad. Sci. U. S. **55**, 1567 (1966).

8. Liddle, G. W., Givens, J. R., Nicholson, W. E., and Island, D. P., Cancer Res. **25**, 1057 (1965).

9. Siebert, G. *in* "Biochemisches Taschenbuch" (H. M. Rauen, ed.), p. 541. Springer, Berlin (1964).

10. Mizuno, N. S., Hof, H., and Collin, M. V., Biochim. Biophys. Acta **166**, 656 (1968).

11. Drews, J., Brawerman, G., and Morris, H. P., European J. Biochem. **3**, 284 (1968).

12. Kidson, C. and Kirby, K. S., Cancer Res. **24**, 1604 (1964).

13. Kidson, C. and Kirby, K. S., Cancer Res. **25**, 472 (1965).

14. Jackson, C. D. and Sells, B. H., Biochim. Biophys. Acta **155**, 417 (1968).

Received Jan. 13, 1969. P.S.E.B.M., 1969, Vol. 131.