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A Chromatographic Comparison of the Nucleic Acids from Isologous Newborn, Adult, and Neoplastic Thymus*

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SUMMARY

The total tissue nucleic acids (DNA and RNA) have been isolated from normal and neoplastic mouse thymus glands and chromatographically fractionated. Significant differences in the chromatographic distribution of the nucleic acids from normal adult thymus and x-radiation-induced thymic lymphosarcoma were found. Chromatographic patterns intermediate between normal and tumor were found in the thymuses of irradiated animals prior to gross tumor formation. Since changes in the RNA fraction accounted for the major differences between the samples studied, RNA was then isolated from newborn, adult, and neoplastic thymus glands and chromatographically compared. The RNA distribution patterns for normal adult and tumor-containing thymus were significantly different, but the patterns for the RNA from the thymuses of newborn animals were very similar to those for the tumors. Since mitotic proliferation and immature cell forms are plentiful in rapidly growing newborn thymus, but not in adult thymus, this result suggests that the altered chromatographic pattern of RNA from tumor-containing thymus glands may be a manifestation of rapid growth and/or cell immaturity, rather than an intrinsic property of the neoplastic state.

After several sublethal doses of whole-body x-radiation, strain C57BL mice develop thymic lymphosarcomas in high incidence. Although the DNA content per thymic cell stays constant after irradiation, the RNA content increases as much as threefold and remains elevated until tumor formation occurs (28). It has been suggested that this sustained elevation of RNA content of the thymic cells may be related in some fundamental way to the genesis of this experimental lymphoid tumor.

It was of interest to determine whether there were also qualitative changes in the tumor nucleic acids. In a pilot study, the mixed nucleic acids (DNA and RNA) from normal adult, early tumor and/or pretumor, and neoplastic thymuses were isolated and fractionated by ion-exchange chromatography. Differences in pattern were observed which on analysis of specific peaks were not ascribable to DNA. Accordingly, a second investigation was then conducted with RNA extracted from the above tissues as well as from newborn thymus. These data, reported briefly elsewhere (23, 24), are presented here in full and correlated with the histologic features of the respective tissues.

* This investigation was supported by grant C-2896 from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.

The following abbreviations are employed: DNA for deoxyribonucleic acid; RNA for ribonucleic acid.

Received for publication April 4, 1961.

MATERIALS AND METHODS

Isologous normal and tumor tissues.—Strain C57BL mice, which develop spontaneous lymphoid tumors very infrequently, may be induced to develop such tumors in high yield 4–10 months after suitable doses of total-body x-radiation (9). Irradiation was given in four doses of 168 r each at intervals of 7 days, beginning when the animals were 33 ± 3 days of age. Physical factors were: 9 ma., 0.25 mm. Cu + 1.0 mm. Al added filter, 30 cm. target-mouse distance, output 32 r.p.m. HVL-0.39 mm. Cu. The lymphocytic or lymphoblastic sarcomas thus induced were harvested at autopsy from their usual site of origin, the thymus. Normal thymus glands from adult and/or newborn strain C57BL mice were used as control tissues.

Chromatography of the nucleic acids.—The method developed for chromatography of the nucleic acids on columns of Cato-2 (a diethyl amino ethyl

derivative of starch) has been described previously (25). For the fractionation of 1–2 mg. of nucleic acid, columns 0.8 cm. in diameter, containing about 10 cc. of packed Cato-2 (passing through 1/32 in. perforated screen), have been used. Both linear gradient and discontinuous elutions have been used with increasing concentrations of NaCl and alkaline pH. Two-ml. fractions were collected at a controlled flow rate of 0.4 ml/min. It has previously been demonstrated that biologically active ribonucleic acids can apparently be recovered intact from columns of Cato-2 under the elution conditions used in this study (22).

The isolation of nucleic acids.—(*a*) Since it was not known whether the DNA or the RNA would be the material of interest, it seemed best initially to isolate the mixed thymic nucleic acids. The method developed for the nearly quantitative isolation of the total thymic nucleic acids is a modification of that of Crestfield, Smith, and Allen (3).

Fresh thymic tissue (0.5 gm. or less) was forced through 80-mesh screen with the aid of 2 ml. of cold Locke solution. To this was added 40 ml. of hot (96° C.) 2 per cent sodium lauryl sulfate in 0.025 M phosphate buffer (pH 7.4); the mixture was stirred while immersed in a boiling water bath for 2 minutes, and then cooled to room temperature in an ice bath. The nucleic acids and proteins were precipitated with 2 volumes of cold 95 per cent ethanol and collected by centrifugation. The precipitate was homogenized until uniformly dispersed in a solution of iced 63 per cent ethanol containing 0.1 M NaCl, collected by centrifugation, and then reconstituted to its original volume with the detergent solution. The mixture was stirred for 2 hours at room temperature to ensure dissociation of the nucleoprotein complexes. An equal volume of 1 M KCl was added, the mixture shaken intermittently for about 30 minutes, and the insoluble detergent and proteins removed by centrifugation. The supernatant was filtered by gravity, and the nucleic acids, containing only a trace of protein (6), were recovered by overnight precipitation with alcohol.

The analytical results for several nucleic acid isolations are given in Table 1. The percentage composition data confirm previous results on the ratio of DNA to RNA in the mouse thymus (28). The average recovery in each case was better than 85 per cent and was considered to be sufficiently quantitative to allow meaningful comparisons between the several tissues under study.

(*b*) The method of Kirby (11) was used for the isolation of RNA.

RESULTS

Chromatographic comparison of the total tissue nucleic acids from isologous normal, early tumor and/or pretumor, and gross tumor thymuses.—The sham-irradiated normal control sample of nucleic acids (No. 2) was prepared from animals handled in the same way as the irradiated animals, except that the x-ray tube was not turned on. The nucleic acids were isolated from the thymuses of ten animals (104 days old) whose average thymic weight was 47 mg. The "early tumor and/or pretumor" sample (No. 8) was prepared from seven irradiated animals (104 days old) whose average thymic weight was 33 mg. Other studies have indicated that, on microscopic examination, such thymuses would almost invariably contain either preneoplastic alterations or early lymphoid tumors *in situ* (10). Three animals originally in this group were not used because of enlarged thymuses (about 80 mg. each), which indicated the possibility of gross tumor formation. The gross tumor sample (No. 11) was prepared from the thymic tumors of two animals of the same age (153 days); the tumors weighed 190 and 370 mg., respectively. Table 1 gives the recovery data for the isolated nucleic

TABLE 1

THE ISOLATION AND COMPOSITION OF THE TOTAL NUCLEIC ACIDS OF MOUSE THYMUS

Source of thymic sample	Per cent recovery		Composition
	Total nucleic acids	DNA	Per cent DNA
Normal controls:			
1	89	93	82
2	87	87	82
3	87	85	83
4	89	90	83
5	92	92	86
6	86	91	82
	Av. 88±2	90±2	83±1
Early tumor and/or pre-tumor:			
7	85	77	71
8	85	85	81
9	80	82	78
10	88	96	78
	Av.: 85±2	85±6	77±3
Gross tumor:			
11	87	93	63
12	88	94	64
	Av.: 88±1	94±1	64±1

acids and their DNA and RNA content. The chromatographic comparison of these samples is shown in Chart 1.

Since both the chromatographic method (25) and the method used for the isolation of the nucleic acids gave reproducible results, the differences in the chromatographic profiles probably reflect real and significant differences in these three samples of nucleic acids. There is a marked difference between the profiles for the normal and "early tumor and/or pretumor" samples, yet the analyses show that the ratio of DNA to RNA for the two samples is nearly identical. These results suggest that irradiation has caused an alteration in the nucleic-acid pattern long before gross tumor formation becomes apparent, but do not indicate whether this change is due to RNA, DNA, or both.

When the peaks were assayed for their DNA content (2), only minor differences between the normal and tumor samples were observed. It was

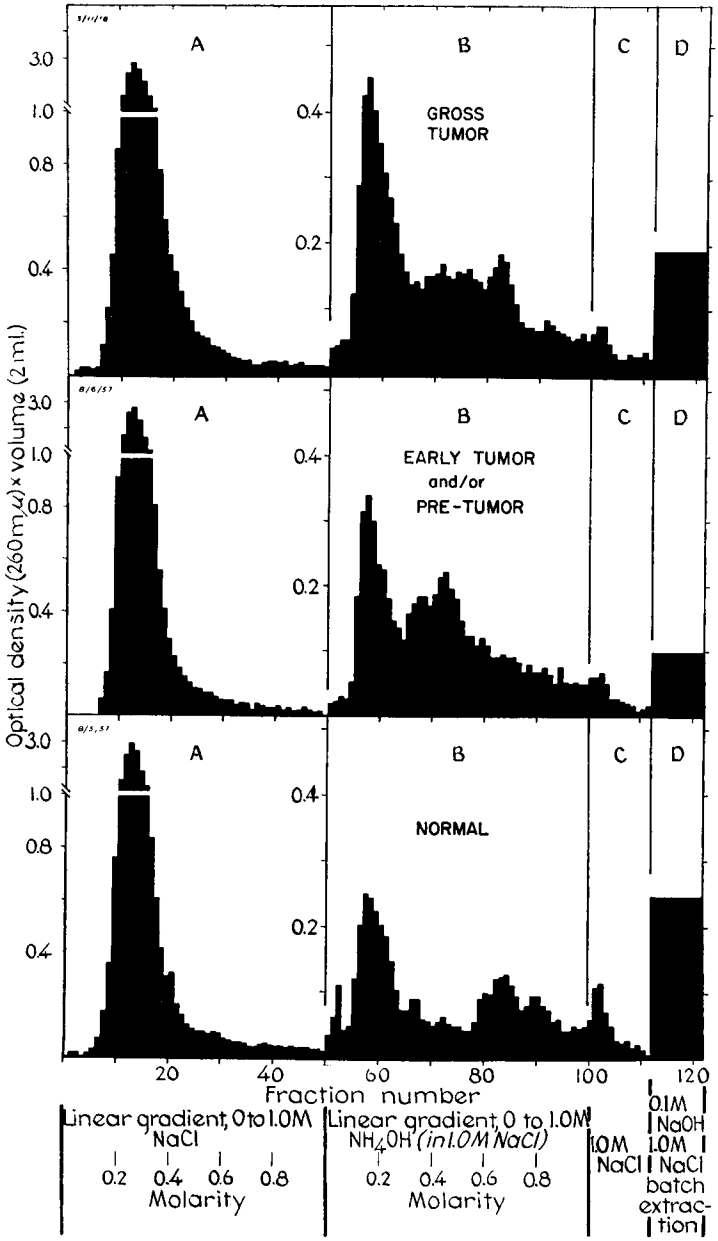

CHART 1.—Chromatographic fractionation of the total nucleic acids (RNA and DNA) from mouse thymus. The data are normalized to equal cell equivalents.

concluded that the rather marked differences found for the total nucleic acids must reflect alterations in the RNA fractions. We proceeded, therefore, to study purified RNA samples from normal and neoplastic thymus tissue.

Comparison of normal and thymic tumor RNA.—The RNA was fractionated on columns of Cato-2 by discontinuous elution. The results given in Table 2 indicate the percentage of the total sample eluted in each of the chromatographic peaks. The results labeled normal are for three separate isolations of RNA from the thymus glands of non-irradiated mice, fractionated on three separate columns. The animals averaged 28, 29, and 56 days old, respectively. The data for the tumors represent the column fractionation of two separate preparations of tumor RNA. The animals averaged 180 and 186 days old, respectively. The reproducibility of the replicate experiments was highly satisfactory.

TABLE 2

CHROMATOGRAPHIC FRACTIONATION OF MOUSE THYMUS RNA

Results expressed as per cent recovery

Chromatographic fractions eluted with:	Normal adult			Tumor		Newborn	
	28 days	29 days	56 days	180 days	186 days	1.7 days	1.3 days
0.125 M NaCl	14	12	11	9	6	9	1, 1*
0.150 M NaCl	3	2	4	3	1	3	2, 1
0.200 M NaCl	11	9	13	9	10	8	6, 5
0.800 M NaCl	19	21	17	15	17	13	10, 12
Sum:	47	44	44	36	34	32	19, 19
1.0 M NaCl 1.0 M NH_4OH	50	50	53	63	65	67	76, 79
1.0 M NaCl 0.1 M NaOH	3	5	3	1	0	1	6, 3

* Values given are for analyses of duplicate columns on the same sample of nucleic acid.

We have previously found that most of the soluble RNA is eluted from columns of Cato-2 by neutral salt (22). Alkaline salt solutions are required, however, to effect the release of the bulk of the microsomal (22), mitochondrial, and nuclear RNA. It would appear, therefore, that the results given in Table 2 reflect an alteration primarily in the particulate fractions of the cells; any increase in the amount of soluble RNA per cell is overshadowed by a larger percentage increase in particulate RNA. This has been confirmed by RNA analyses on the subcellular fractions of normal and neoplastic thymus cells obtained by ultracentrifugation in 0.25 M sucrose. In the tumor the amount of microsomal RNA was increased to 175 per cent relative to that for normal tissue, while the soluble RNA increased only to 140 per cent. These results are similar to those found by Hultin and von der Decken for regenerating liver (8, 27), in which microsomal RNA increased to about 150 per cent while the soluble RNA content increased only slightly over that for normal liver.

The two sets of results are consistent with our present knowledge concerning the relationship and importance of the soluble RNA and the microsomes in cytoplasmic protein synthesis (7). The alterations we find may therefore simply represent possible reorientation of the thymic cells to an increased rate of protein production.

The RNA patterns for thymuses from newborn mice.—We then considered the possibility that the patterns found for the tumors, although markedly different from adult isologous tissue, might be just a reflection of extremely rapid growth. We therefore determined the patterns for RNA isolated from the thymuses of newborn mice. One experiment gave results almost identical with those for tumor RNA (Table 2). A second experiment gave results which differed even more from the normal adult picture than did the tumor data. Also given in the last column are the values for a duplicate column on the same sample of nucleic acid; no peak differed by more than 3 per cent between the two columns. There was a slight age difference in the starting material for the two preparations of newborn thymus RNA. In the first case, 45 per cent of the animals were 1 day old, 43 per cent were 2 days old, and 12 per cent were 3 days old for an average age of 1.7 days. In the second case, 70 per cent were 1 day old, and 30 per cent were 2

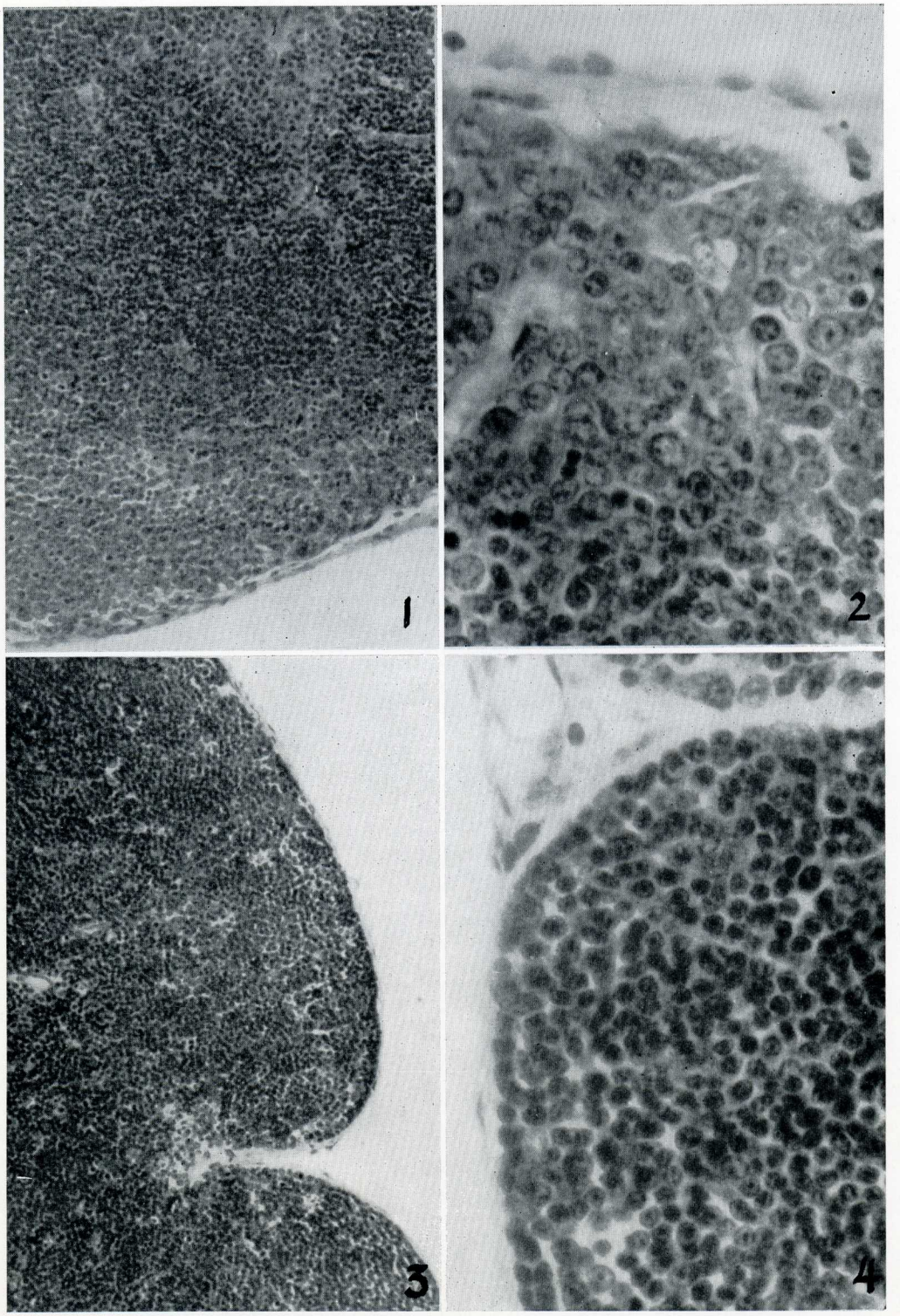
1
2
3
4

FIG. 1. —One-day-old mouse thymus. H.&E., ×125.
FIG. 2. —One-day-old mouse thymus. H.&E., ×500.
FIG. 3.—Thirty-day-old mouse thymus. H.&E., ×125.
FIG. 4. —Thirty-day-old mouse thymus. H.&E., ×500.

days old for an average age of 1.3 days. The average thymic weight for the first group was about 12 per cent greater than that for the second group.

Thymic weight increases over sevenfold between 1 and 10 days, with only a 1.3-fold increase between 10 and 20 days. It is thus not unlikely that thymic growth rate decreases enough even during the first 3 days of life to account for the differences observed in these two experiments.

Histology of the normal and tumor tissue.—The normal and neoplastic thymic tissues were compared histologically to ascertain whether there were changes which could be correlated with the biochemical results. Normal C57BL mice were sacrificed at 1, 10, 20, 30, and 40 days of age. The thymus glands were removed, weighed, and processed for histologic examination.

The 1-day-old thymus (Figs. 1 and 2) differs strikingly from the adult pattern in having a rather sharply defined zone, lying immediately under the capsule, which comprises about one-third to one-half of the entire cortex and is made up almost exclusively of very large, immature lymphocytes, with abundant mitotic figures. This is rather easily demarcated from the inner cortical zone, which shows a more normal distribution, with an abundance of small-sized lymphocytes and fewer immature forms. The medulla of the newborn thymus resembles that of the adult. In terms of volume, it might be estimated that about half of the cortical lymphocytes of the newborn thymus are of the very large, immature type.

By 10 days of age, the zone of immature lymphocytes has become appreciably narrower, but is still discernible around most of the periphery of the cortex, though in some sectors the normal cortical pattern emerges to the capsular surface. By 20 days, the zone of immature lymphocytes is only one or two cells thick and is clearly discontinuous around the rim of the cortex; by 30 and 40 days, no such zone can be made out, and the normal adult pattern is seen (Figs. 3 and 4). By this time the proportion of immature, large forms would seem to have fallen to the levels described by Sainte-Marie and Leblond (21).

The cytologic characteristics of the immature lymphocytes in the outer cortex of the newborn thymus closely resemble those noted in thymus regenerating after acute involution caused by such agents as irradiation or hydrocortisone. These cells are also similar to the very large, immature cells seen in the early stages of evolution of lymphomas in the thymus. The frank lymphomas tend ultimately to become populated by an even larger cell that has more plentiful cytoplasm, more prominent nucleoli, and perhaps other cytologic differences as well; but at least during the early phases of evolution of these tumors, and persistently in many frank and disseminated tumors, the cell type which is seen with great monotony throughout the entire tumor is closely similar to the normal immature large lymphocyte (lymphoblast) seen in abundance in the newborn thymus.

There seems to be a correlation between the biochemical results and the histological results. The cell types prevalent in the newborn and tumor tissue are characteristic of rapidly proliferating undifferentiated lymphoid tissues. The ribonucleic acid patterns of these tissues are similar and yet different from those of adult tissue. It would appear, therefore, that we are seeing chromatographic patterns for ribonucleic acid which are characteristic of rapid growth and/or cellular immaturity rather than an intrinsic property of the neoplastic state.

DISCUSSION

Many comparative studies of the DNA of normal and tumor tissues have been published. These investigations have included (*a*) base composition of the DNA (5, 14, 15); (*b*) DNA content per cell (28); (*c*) physical measurements on the isolated DNA including viscosity (13), sedimentation constants (13, 18), and thermal denaturation temperatures (13); and (*d*) chromatographic studies (5, 14).

Most of these investigations have failed to reveal detectable differences between the DNA of normal and that of tumor tissues. Our data tend to confirm this view. Detailed study of the DNA moiety was postponed, however, when we found that the RNA patterns were apparently grossly altered in tumor tissue.

The problem of possible alterations in the RNA of tumor tissues has received much less attention. Little or no difference from normal has been found in the base composition of the whole cell RNA (12, 15), although there is some disagreement as to whether there is (4) or is not (12) a significant difference in the base composition of the RNA from various tissue subfractions. An increase in the RNA content per cell has been reported for tumors (20, 28). Petermann and co-workers (16, 17) have found that the ultracentrifugal distribution of ribonucleoprotein particles from regenerating liver differed from that of normal liver but was similar to that found for an azo dye-induced tumor. In regenerating liver the amount of microsomal RNA increased to about 150 per cent, while the soluble RNA was only slightly increased over

that for normal liver (8, 27). It has also been reported that malignant cells tend to lose their organized lamellar ergastoplasm and that most of the basophilic granules (ribonucleoprotein particles) are then found scattered in the cytoplasm (1).

Our results confirm the prior finding of an increase in the RNA content per thymic cell in the tumor as compared with that in the normal adult tissue. This increase in RNA is not an over-all increase but preferentially involves certain RNA fractions. The chromatographic distribution of the RNA isolated from thymic tumor tissue is markedly different from that from the normal adult thymus. This implies the production of RNA species necessary for a specific function. The degree of departure of the RNA from the normal adult picture would appear to be more a reflection of rate of growth than of an intrinsic property of the neoplastic state because rapidly growing normal tissue (newborn thymus) shows almost identical results. The biochemical similarities between isologous tumor and newborn tissue are confirmed by histological similarities. These data thus point up the pitfalls inherent in the use of adult, nongrowing tissues as control material for comparison with tumor tissue in biochemical investigations.

ACKNOWLEDGMENTS

We wish to acknowledge the valuable technical assistance of Miss Anna I. Eglite.

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