# CHEMICAL CHANGES IN THE LUNGS OF TUMOR-BEARING RATS*

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It has previously been shown in this laboratory that there occur significant changes in the nucleic acid concentration in certain tissues of tumor-bearing rats[1-4]. In one of these studies, it was found that the presence of the Walker 256 tumor in rats caused an increase in the concentration of desoxyribonucleic acid (DNA) in the host lung[3]. In the present experiments, this effect of the Walker 256 tumor has been investigated in some detail. In addition to DNA, ribonucleic acid (RNA), protein nitrogen (PN), cholesterol, and potassium have been determined in the lung. Furthermore, the effect of a second rat tumor, the Murphy-Sturm lymphosarcoma, on the nucleic acid levels of the host lung has been investigated.

## EXPERIMENTAL

Male rats** were implanted with either the Walker 256 tumor or the Murphy-Sturm lymphosarcoma at about 4 to 6 weeks of age***. Host animals were then killed in groups, at 3 days to 21 days after transplantation. Comparable groups of control animals were killed concurrently. About one half of the animals were killed by lethal doses of sodium amytal, which appeared to result in more uniform wet lung weight than that found in animals killed by decapitation.

The lungs were quickly removed and washed briefly in cold saline. Wet weights and sections for histological examination were obtained at this time, after which the lungs were frozen until analyzed. At such time, they were individually homogenized and aliquots were taken for dry weight determinations at 105°C, the dried samples then being used for potassium determinations. Other aliquots of each homogenate were treated according to the SCHNEIDER procedure for the separation of phosphorus compounds[5]. The alcohol and the alcohol-ether extracts from each tissue sample were taken as the cholesterol-containing fraction. The residue remaining after extraction of the nucleic acids by hot 5% trichloroacetic acid was washed with alcohol and alcohol-ether, dried *in vacuo*, and taken as the protein fraction.

Potassium was determined by ashing the dried homogenate samples in platinum crucibles in a muffle furnace at 700°C for 15 hours, dissolving the ash in 0.05 ml of 1 *N* hydrochloric acid, diluting with water, and comparing with standard solutions by means of flame photometry, using the Beckman Model DU apparatus.

Total and free cholesterol were determined by the method described by SCHOENHEIMER AND SPERRY[6], as modified by SPERRY AND WEBB[7]. DNA was estimated by the method of STUMPF[8] and the RNA by that of VON EULER AND HAHN[9]. Protein nitrogen (PN) was determined by a micro-Kjeldahl procedure, after digestion of the dried protein residues.

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## RESULTS AND DISCUSSION

The results, grouped according to relative tumor size, are presented in Tables I and II. Since the data for groups of control animals killed at different times during each experiment did not differ from each other, they have been incorporated into single values in the tables. Values for free cholesterol in all cases were only slightly less than the total cholesterol values, so that no pattern could be discerned for the ester values determined by difference. Accordingly, only the total cholesterol values are reported.

It can be seen from Tables I and II that during the growth of either tumor the dry weight concentration of host lung DNA increases markedly. Concurrently, the RNA concentration remains relatively constant, as is shown for the lymphosarcoma hosts in Table II. Since RNA, PN, cholesterol, and potassium concentrations in the case of the Walker-256 hosts also remained constant, only the ratios of these components to DNA are given in Table I.

TABLE I

CHANGES IN THE COMPOSITION OF THE LUNG OF RATS BEARING THE WALKER-256 TUMOR

| *Number of animals* | *Tumor size % of body weight* | *DNA mg/g dry wt* | *RNA / DNA* | *PN / DNA* | *Cholesterol / DNA* | *K / DNA* | *RNA / PN* |
|---|---|---|---|---|---|---|---|
| 64 | Control | 29 ± 0.7** | 0.66 | 2.1 | 0.83 | 0.44 | 0.32 |
| 31 | 1.6 ± 0.2** | 30 ± 1.2 | 0.63 | 2.0 | 0.80 | — | 0.32 |
| 16 | 5.5 ± 0.3 | 34 ± 1.4 | 0.62 | 1.9 | 0.71 | 0.37 | 0.33 |
| 23 | 7.9 ± 0.2 | 36 ± 1.6 | 0.56 | 1.7 | — | 0.35 | 0.32 |
| 21 | 13.0 ± 0.3 | 39 ± 1.6 | 0.54 | 1.6 | 0.62 | 0.35 | 0.34 |
| 14 | 17.0 ± 0.4 | 43 ± 2.2 | 0.51 | 1.4 | — | 0.31 | 0.35 |
| 24 | 25.0 ± 0.9 | 44 ± 1.5 | 0.50 | 1.4 | 0.50 | — | 0.37 |
| 4 | 43.0 ± 0.9 | 52 ± 1.0 | 0.38 | 1.2 | 0.40 | — | 0.31 |

* Refers to the number of lungs individually analyzed for DNA, RNA, and PN; the corresponding values for the cholesterol and potassium determinations are about one-third of these.
** Mean ± standard error.

TABLE II

NUCLEIC ACID CONTENT OF THE LUNG OF RATS BEARING MURPHY-STURM LYMPHOSARCOMA

| *Days after transplantation* | *No. of animals* | *DNA mg/g dry wt* | *RNA mg/g dry wt* | *RNA / DNA* |
|---|---|---|---|---|
| Controls | 29 | 27.9 ± 0.7* | 19.7 ± 0.5* | 0.70 |
| 7 days | 16 | 35.3 ± 1.1 | 19.9 ± 0.8 | 0.56 |
| 9 days | 9 | 38.3 ± 1.6 | 20.9 ± 0.8 | 0.54 |
| 11 days | 16 | 41.1 ± 2.3 | 22.0 ± 1.6 | 0.53 |
| 13 days | 7 | 43.7 ± 1.9 | 19.8 ± 0.5 | 0.45 |
| 17 days | 21 | 51.5 ± 2.3 | 19.9 ± 0.8 | 0.39 |

* Mean ± standard error.

If we assume a constant amount of DNA per normal somatic diploid nucleus, as suggested by the work of BOIVIN *et al.*[10], VENDRELY AND VENDRELY[11], and MIRSKY AND RIS[12], the decreasing ratios to DNA of each of the other constituents could be interpreted to mean that the host lung cell is being depleted of the latter substances during the progressive growth of the tumor. A possibility to be considered is that the

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nutritional demands of the tumor may be responsible, although this is unlikely in view of the fact that the effects are obtained with such relatively small tumors and that the RNA/PN ratio remains constant. Moreover, when the data are expressed as total amounts per organ, normalized for body weight, as in Figs. 1 and 2, it can be seen that no overall losses occur. Rather, there are, if anything, slight increases in the RNA, PN, potassium and cholesterol, which almost exactly parallel the slight increase in lung weight. The pronounced increase in DNA, however, is still present. The increase in lung weight has been observed in previous experiments by RODRIGUEZ AND CERECEDO[13], where it was shown to return to normal during later stages of tumor growth.

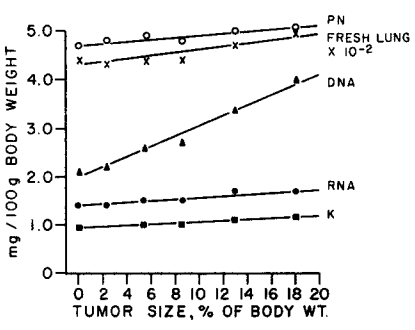


Fig. 1. Effect of the Walker-256 tumor on various constituents of the host lung.

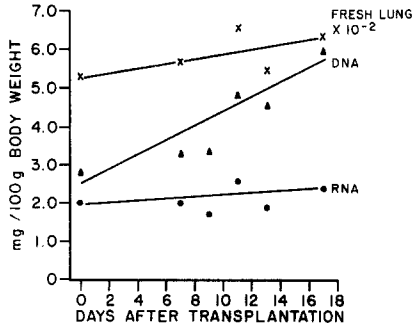


Fig. 2. Effect of the Murphy-Sturm lymphosarcoma on size and nucleic acid content of the host lung.

At present, the result could be explained by either of two alternative possibilities. One is that the presence of the tumor induces an increase in cell number without a corresponding increase in organ size, and, consequently, a decrease in cell mass and in the predominantly extranuclear constituents per cell. The other alternative is that an increase in the degree of ploidy of the average cell is being induced.

Histological examination of fixed sections* showed that the average nuclear area, as percent of the total tissue area (excluding interstices), was constant, and was the same for control lungs as for lungs from rats bearing the Walker tumor. Such evidence, while far from being decisive, does not support the hypothesis that the effects observed are due to an increase in cell number, since an increase in the relative area covered by nuclei would then be expected, assuming that daughter nuclei are of the same size as the parent structures. It is expected that this question will be answered by DNA determinations made on suspensions of known nuclei content. This work is now in progress, and the results will be reported at a later date.

Whatever is the nature of the lung response to the subcutaneous tumor, it may be assumed that it is mediated by an agent produced by the tumor and transmitted to the lung via the circulation. Experiments designed to investigate this possibility by studying the effects of tumors on the *in vitro* incorporation of nucleic acid precursors by lung slices are now in progress.

* Carried out by VINCENT S. PALLADINO, M.D.

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SUMMARY

The nucleic acids were determined in the lungs of rats bearing the Walker 256 tumor, and of rats bearing the Murphy-Sturm lymphosarcoma.

Significant increases in the desoxyribonucleic acid (DNA) concentration during the growth of the tumors were observed. In contrast, the ribonucleic acid, protein nitrogen, potassium, and cholesterol did not show any change.

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# THE NUCLEOTIDE COMPOSITION OF THE TOTAL RIBONUCLEIC ACID IN SUBCELLULAR FRACTIONS OF RESTING AND REGENERATING RAT LIVER*

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The majority of the activity in the field of ribonucleic acid (RNA) chemistry has been concentrated on yeast ribonucleic acid, since this material is easily accessible, and relatively simple to prepare. In recent years there has been renewed interest in the ribonucleic acid of animal cells. Various workers have analyzed such ribonucleic acid for its constituent purine and pyrimidine bases, and some have fractionated the cell into its subcellular components and then analyzed the ribonucleic acids from these[1-4]. The results obtained by different workers have shown considerable variation, depending on the method of analysis used, and primarily on the type of preparation

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