TABLE III. Influence of Source and Age of Tumor or Mean Specific Activity of Tumor Protein (5 μc Glycine-2-C^{14} in 10 μmoles of Glycine Injected 24 Hours before Autopsy).

Source of tumor	Age of tumor transplant (days)	No. of tumors	Mean specific activity of tumor protein (cpm/mg)
Sprague-Dawley	Spont.*	7	65.7 ± 4.8†
Upjohn	247	6	59.5 ± 3.7
"	240	5	48.5 ± 5.0
Sprague-Dawley	174	7	46.8 ± 5.0
Upjohn	290	6	62.0 ± 6.3
"	296	6	66.4 ± 3.3
"	332	10	52.9 ± 1.8
Sprague-Dawley	56	7	64.9 ± 5.9

* Spontaneous.
† ± standard error.

maximum variation from 46.8 ± 5.0 to 65.7 ± 4.8.

The results reported in all 3 Tables are on the basis of autopsies being performed 24 hours after administration of the glycine-2-C^{14}. When this interval was decreased to 6 hours similar data in magnitude and variation were obtained.

Conclusions. A preliminary method has been described for assessing the inhibitory action of compounds on a transplantable rat mammary fibroadenoma. Sufficient data have been obtained to indicate that this can be a useful procedure if the end-point of tumor growth is the glycine-2-C^{14} uptake in the tumor proteins. Crude tumor weight was inadequate on the basis of high variation within groups and between groups.

Reproducible inhibition was obtained with both 2α-methyl-17β-hydroxy-5α-androstan-3-one and testosterone propionate and the former compound appears to be the more active.

Summary. The uptake of glycine-2-C^{14} into the proteins of a rat mammary fibroadenoma has been studied as a possible bioassay procedure for detecting useful antitumor steroids and other substances for further studies in humans. A preliminary method has been evolved consisting in transplantation of the tumor to 140 ± 15 g female rats and after 35 days injecting the rats once daily for 14 days with the test compounds. On the last day of treatment the rats received one injection of glycine-2-C^{14}. Twenty-four hours after the last injection of the test compound and the glycine-2-C^{14} injection, the tumors are removed, weighed and C^{14} specific activity of the tumor protein determined. The tumor was inhibited by testosterone propionate but more regularly and more intensely by equal weights of 2α-methyl-17β-hydroxy-5α-androstan-3-one, a steroid.

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Influence of Age and N, N′-Diethylene-N″-Phenethylphosphoramide (PEDP) Upon Nucleic Acid and Ribonuclease Activity in Tumor Tissue.* (27898)

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A recent report by Allison *et al.*(1) indicates that the development of the Walker 256 carcinosarcoma transplanted into the rat is maximum when ribonuclease activity in the

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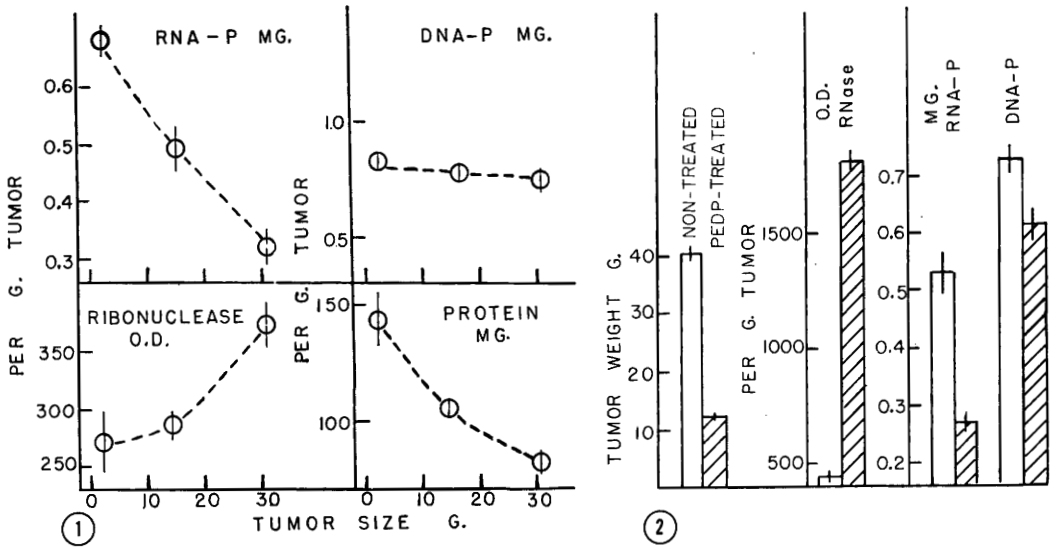

FIG. 1. Ribonuclease activity (optical density difference –O.D.) and milligrams (mg) of ribonucleic acid phosphorus (RNA-P), deoxyribonucleic acid phosphorus and protein per gram of tumor tissue at various tumor sizes. Each point is the mean of 8 animals with center perpendicular line representing standard error of mean.

FIG. 2. Tumor weight and ribonuclease activity (RNase –O.D.), ribonucleic acid phosphorus (RNA-P) and deoxyribonucleic acid phosphorus (DNA-P) per gram of tumor tissue for N, N′-diethylene-N″-phenethylphosphoramide (PEDP) treated (bars with slanted lines) and non-treated (open bars) tumor tissue. Each point is the mean of 10 animals with center perpendicular line representing standard error of mean.

tumor tissue is minimum. In general, ribonuclease activity in rapidly growing tumors is low(2-4) as compared to normal tissues. Zigman and Allison(4) have shown also that administration of a phosphoramide caused a decrease in size of the Walker tumor and an increase in ribonuclease activity. Since transplanted tumors decrease in rate of growth after they reach a certain size(5), the purpose of the investigation was to correlate the size of the tumor and chemotherapeutic effects of a phosphoramide upon ribonuclease activity and the nucleic acid and protein concentration of tumor tissue.

Experimental. Male Wistar rats of approximately 150 g were transplanted with Walker 256 carcinosarcoma as described previously(4). Twenty-four animals were fed 18% of casein(6) and 8 rats were sacrificed at 5, 7, and 15 days after transplantation of the tumor. Another group of 10 tumor-bearing animals were injected intraperitoneally with 7 mg/kg of body weight/week of N, N′-diethylene - N″ - Phenethylphosphoramide (PEDP) at 7 and 14 days after transplantation and were sacrificed on day 15.

The tumor tissue was homogenized in distilled water and the resulting suspension was used to determine ribonuclease activity at pH 7.4 by the spectrophotometric method of Brody(7), nucleic acid phosphorus concentration by a modified procedure of Schmidt and Thannhauser(8) and total protein by micro Kjeldahl assay.

Results. The data in Fig. 1 demonstrate that as the tumor increases in size and approaches maximum development, ribonuclease activity per gram of tumor also increases. The deoxyribonucleic acid phosphorus (DNA-P) per gram of tumor, on the other hand, was essentially constant and independent of the size of the tumor. Thus, ribonuclease activity per mg of (DNA-P) also increased with growth of the tumor. The slower growing, larger tumors also had a markedly decreased ribonucleic acid phosphorus (RNA-P) and protein concentration. Therefore, as the tumors increased in size, there was a decrease in RNA/DNA ratio and in amount of protein per mg of DNA-P. These results support the concept that rate of growth of the tumor is correlated with a reduction in

ribonuclease activity. Similar changes in RNA and ribonuclease were noted in aging apple leaves(9).

When tumor-bearing rats were treated with PEDP there was a marked reduction in size of the tumor as compared to non-treated controls (Fig. 2). The smaller tumors in the animals treated with drugs had a higher ribonuclease activity and a lower RNA-P content than the controls. The DNA-P per mg of tissue was also decreased in treated tumors which suggested a smaller number of cells per gram of tumor tissue. If ribonuclease activity was calculated relative to DNA-P concentration, the values were: $29.5 \pm 1.3 \times 10^2$ and $6.05 \pm 0.39 \times 10^2$ units per mg DNA-P, respectively, for treated and non-treated tumors. Similarly, the RNA/DNA ratio for DEPA-treated tumors was 0.37 ± 0.04 while the non-treated 0.74 ± 0.06. Slower growing tumors, therefore, had an increased ribonuclease activity which also could be correlated with a low RNA concentration per cell.

Protein concentration per mg DNA-P was increased from 150 ± 12 to 224 ± 45 in PEDP-treated tumors. Previous work(10) has demonstrated a marked effect of ethylenephosphoramides upon cell nuclei, producing bizarre, polymorphic, giant cells with distorted nuclear and cytoplasmic configuration. These facts might explain the increased protein/DNA ratio and decreased DNA concentration found in these tumors.

Summary. As the Walker 256 tumor increased in size and approached maximum development, the ribonuclease activity per gram of tumor or per mg of deoxyribonucleic acid phosphorus (DNA-P) also increased. The slower growing, larger tumor had a markedly decreased ribonucleic acid phosphorus (RNA-P) and protein concentration. Treating the tumor-bearing rat with N, N′-diethylene-N″-phenethylphosphoramide (PEDP) resulted in reduced rate of growth of the tumor, increased ribonuclease activity, and decreased RNA-P.

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Preparation of Chromosomes for Light Microscopy Utilizing an Agar Fixation Procedure. (27899)

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Determination of chromosome morphology and number is becoming increasingly important in both clinical practice and as a research aid. Some laboratories routinely monitor their cell cultures and follow the chromosome number as a check on the homogeneity of the cell population. Determination of chromosome number and morphology also provides a means for study of chromosomal change and stability under the action of various chemical and physical agents. In order that such studies can be carried out efficiently, a rapid precise means is needed for monitoring the chromosomal constitution of cell lines.

Many methods are now available for preparing chromosomes for examination; however these are quite time consuming(1,2,3).