phane. Unless such mice were eliminated the results were discordant. In order to minimize the number showing such spontaneous diarrhea it was found helpful to cage the animals together in groups of 6 for about a week so that they were not under the stress of being subjected to a new social order immediately before the assay was performed. Because of the possibility of spontaneous diarrhea a control group receiving no drug and no hydroxytryptophane is recommended. This spontaneous diarrhea was not due to salmonellosis or other infections but seemed to occur among a few individuals even though they were in good health.

Summary. An assay for the potency of antimetabolites of serotonin has been described. This assay was rapid and easy to perform and the results were readily reproducible. The recommended method was an *in vivo* one which measured the effects on the intestinal tract. It involved determination of the potency of a compound in causing protection against diarrhea when a large dose of the serotonin-precursor, 5 - hydroxytryptophane, was injected.

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Nucleic Acids and Their Derivatives in Alcoholic Extracts of Rat Tumors and Normal Muscle.* (24047)

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Our object has been to study the chemistry of a highly selected fraction of tumor tissue *i.e.*, an alcoholic extract, because such a tumor extract appeared to have unique biological properties when injected into living tumors, and, as we showed, it also elicited immunological responses of interest in tumor immunology. Earlier studies showed that there were marked differences between substances extracted with alcohol from tumors and from host muscle tissue. For example, in tumor extracts inorganic phosphorus, glucose, lactic acid and ribonucleic acid (RNA) were low, whereas the deoxyribonucleic acid (DNA) was 3-fold high(1). Differences were found also between amino acid composition (2), as well as between protein contents(3) of tumor extracts, and similar extracts made from muscle. The immunology and chemistry of these proteins were studied(3). The present study is limited to investigation of DNA and RNA components which are different in the 2 types of extracts.

Materials and methods. Three sarcomas (Aptekman sarcoma No. 6 and Lewis sarcomas Nos. 7 and 10) originally induced in albino-inbred rats† by injections of methylcholanthrene and then carried on by transplantation, were used. These tumors were of the late generation spindle-cell type following induction by hydrocarbons, described by

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Lewis(4). Two batches of extracts were also made from a lymphoma‡ No. 8 found by Lewis(5). It had arisen spontaneously in the submaxillary gland of a female breeder of the Lewis inbred strain of rats. *Extracts* were made as previously described(2) in the cold with final concentration of 80% alcohol. They were clarified by filtration after concentration to 30% alcohol at 30°C and standing at −15°C. Since no adequate control is available for tumor tissue, the host tissue, muscle, was chosen for comparison. Similar extracts were, therefore, made from muscle of these same rats and from normal rats, and rats whose tumors had been made to slough off following injection of tumor extracts and which were then shown to be resistant to further challenge with tumor fragments. *Analyses* of the extracts were made for content of ash free solids, RNA or ribosides by orcinol reaction of Drury(6) modified by Miller, Golder and Miller(7), and DNA or deoxyribosides by the diphenylamine reaction of Dische(8), modified for quantitative use by Seibert(9). Sensitivity in the latter method was increased, as suggested by Patterson and Dackerman (10), by heating 24 hours at 37°C instead of boiling 15 minutes. Deoxyribosides were also determined by the tryptophane perchloric acid method, described by Cohen(11) and adapted for use by Seibert, Pfaff and Seibert(12). *Ultraviolet absorption* measurements were made in Beckman spectrophotometer, model DU, on solutions and paper strips which had been subjected to electrophoresis. The strips were passed through attachment designed by Tennent for the Beckman spectrophotometer and described by Block, Durrum and Zweig (13). *Electrophoresis* experiments were carried out in paper strip model apparatus. Four half-inch strips were run simultaneously for 2 hours using phosphate buffer pH 7.38, 0.1 μ. The strips were dried in oven at 90°C for 15 minutes and then studied in 3 different ways. Some were measured in the Beckman spectrophotometer to locate position of ultraviolet absorbing components. Others were sprayed with a modified Dische (cysteine: H_2SO_4) reagent(14) for detecting presence of DNA or deoxyribonucleosides as a pink to rose color. Other strips were sprayed with 2% orcinol in 2 N-HCl, as recommended by Bidwell, Krotkov and Reed(15). A blue color appeared in presence of ribosides (guanosine and adenosine) and a faint one for RNA. *Microbiological assay* for total free deoxyribosides in tumor extracts was also made by the method‡ recommended by Hoff-Jørgenson (16), using the bacterium, *Thermobacterium acidophilus R 26,* which is highly dependent upon presence of these substances. The medium was that recommended by Toennies, Usdin, and Phillips(17), and pure thymidine served as standard. Quantities of extracts, determined by preliminary trial to have a content of deoxyribosides equivalent to 0.01 to 0.5 μg of thymidine/ml final medium, were mixed with equal volume of double strength basal medium and then inoculated with 0.05 ml of a suspension of the organism in its log phase of growth(18). The inoculated sample was then incubated at 37°C for 18-24 hours and turbidity determined in the Klett colorimeter, using λ 660 filter. Comparisons were made, as recommended by Toennies and Gallant(19), with results obtained on a solution of basal medium containing pure thymidine as standard. Analyses for pyrimidine deoxyribosides were also made on aliquots of the extracts which had been adjusted to pH 2.0 and then hydrolyzed in boiling water bath for 5 minutes to destroy purine deoxyribosides, as shown by Hoff-Jørgensen(16). *Thymine* and/or thymidine was also determined by means of the bacterium, *E. coli T*(−) mutant, dependent upon these substances for growth. The medium was double strength basal medium described by MacLoed(20) and Roepke, Libby and Small(21), and method of assay was similar to that described above.

Results. Spectral absorption. Total nucleic acid content of extracts was obtained by adding mg/ml of RNA, determined by orcinol reaction, and mg/ml of DNA, found by diphenylamine reaction. Specific density/mg of nucleic acid was obtained by dividing ultra-

‡ We are deeply grateful to Miss Travaglini, Lankenau Research Institute, for demonstrating the significance of these methods, for supplying cultures and assistance and advice in technics.

TABLE I. Specific Density Related to Nucleic Acid Content of Extracts.

Extracts	No. of extracts studied	Nucleic acid calculated as RNA (orcinol) mg/ml	Nucleic acid calculated as DNA (diphenylamine) mg/ml	Specific density per mg of total nucleic acid
Tumor*	7	2.1	.11	27.5 (20.9–35.7)†
Muscles (normal)	4	3.7	.04	15.8 (15.4–16.2)
" (tumor-bearing)	5	4.0	.06	17.7 (15.4–19.8)
" (immune)	3	3.8	.04	16.8 (15.9–17.3)
Dialysates of				
Tumor		2.1	.11	35.9
Muscle		10.0	.18	22.5
DNA				19.5
RNA				20.4
Guanosine				42.6
Adenosine				46.0
Uridine				43.0
Deoxyguanosine				50.0
Deoxyadenosine				58.0
Thymidine				44.3

* Results on extracts from sarcomas No. 6 (2), No. 7 (1), No. 10 (2) and lymphoma No. 8 (2) were averaged.

† Range in parentheses.

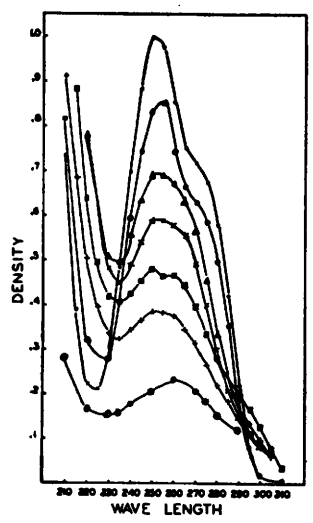
violet absorption density/ml at maximum wave length (λ 2500 or λ 2600) by content of total RNA plus DNA/ml. Table I shows that the specific density of 7 tumor extracts (20.9 to 35.7) was consistently higher than that for 12 extracts of muscle tissue (15.1 to 19.8), and also higher than for pure DNA or RNA (19.5 and 20.4). Moreover, the highly-absorbing substance was dialyzable.

Since ribosides and deoxyribosides have very high specific absorptions with maxima at the same wave length (λ 2500) as tumor extracts, and since the absorption for guanosine and deoxyguanosine show a bulge to the right (at λ 2600 to λ 2700), similar to that for tumor extracts (Fig. 1 a), the possibility of their presence in tumor extracts was considered. In contrast, muscle extracts, as well as adenosine, deoxyadenosine, thymidine and uridine gave single homogeneous curves (Fig. 1 b).

Electrophoresis. Four paper strips, on each of which was placed 4-10 μl of tumor extract, after subjection to electrophoresis for 2 hours, were studied as follows, (1) examination in duplicate for the position of ultraviolet-absorbing components in the special adapter for the Beckman spectrophotometer, (2) for position of blue-staining components with orcinol spray reagent, which would indicate RNA or ribosides, and (3) for position of pink-staining components with the cysteine: H_2SO_4 spray reagent for detection of presence of DNA or deoxyribosides. Pure nucleic acids, ribosides, deoxyribosides, and muscle extracts were studied in similar manner.

Pure DNA and RNA migrated about 35 mm toward the cathode, as shown by ultraviolet absorption at λ 2600 and also by the fact that a blue spot in the same position resulted from the orcinol spray in the case of RNA, and a pink spot in the case of DNA. By the same technics pure guanosine or adenosine migrated about 35 mm toward the anode, and were identified as blue-staining components with the orcinol spray, and as ultra-violet-absorbing at λ 2500 to λ 2600. Pure deoxyguanosine and deoxyadenosine also migrated about 35 mm toward the anode, but were identified as pink-staining components with cysteine: H_2SO_4 spray, and as ultraviolet-absorbing at λ 2500 to λ 2600.

Tumor extracts showed an ultraviolet-absorbing (λ 2550) component about 30 mm toward the anode, and at the same position a component stained pink with cysteine: H_2SO_4 spray, indicating presence of deoxyribosides. No blue staining component was found. Dialysate of the tumor extract gave similar results.



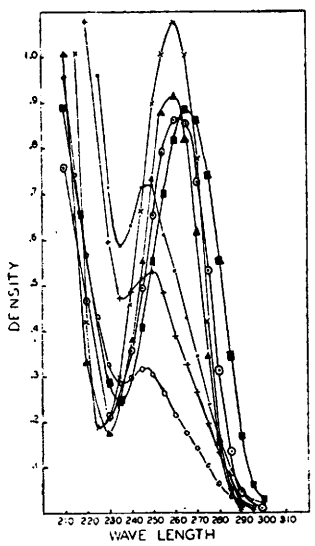


FIG. 1 a (above). Curves of spectral density at different wave lengths from top to bottom. Deoxyguanosine (●——●); guanosine (○——○); tumor extract (▲——▲); tumor extract (×——×); tumor extract (■——■); dialysate of tumor extract (+——+); DNA (⊙——⊙).

FIG. 1 b (below). Curves of spectral density at different wave lengths from top to bottom. Deoxyadenosine (×——×); adenosine (▲——▲); thymidine (■——■); uridine (⊙——⊙); normal muscle extract (●——●); normal muscle extract (+——+); dialysate of normal muscle extract (○——○).

Muscle extracts, on the other hand, showed an ultraviolet-absorbing component (λ 2550) about 30 mm toward the anode, and a blue-staining component at this position, indicating presence of ribosides. Another weakly-staining blue component appeared about 30 mm toward the cathode, probably RNA. No pink-staining material was present, except a trace toward the anode in the dialysate of the muscle extract.

Bioassay analyses. Studies of purine and pyrimidine deoxyribosides by bioassay methods revealed the fact that the ratio of these 2 types of deoxyribosides was not the same in tumor extracts as in normal muscle extracts. For example, the average ratio of purine to pyrimidine deoxyribosides in 5 tumor extracts was 1:1.8 whereas in 3 normal muscle extracts it was 1:3.0 (Table II).

That ratios on lymphoma extracts were closer to those on muscle tissue than were those on sarcoma extracts is consistent with results obtained by Miller(3), who found a protein component only in lymphoma extracts similar to one found in normal muscle extract, and suggested it was due to incorporation of some normal muscle tissue during excision of the widely dispersed lymphomas.

Chemical analyses by diphenylamine, which detects DNA and purine deoxyribosides, and by tryptophane perchloric acid method, which detects in addition pyrimidine deoxyribosides, ascorbic acid and fructose, supported to some extent the difference in ratios observed by the bioassay method. If the difference in the results calculated as deoxyguanosine by these 2 methods (Table III) is considered to be due to pyrimidine deoxyribosides, then the ratio of purine deoxyriboside to pyrimidine deoxyriboside in tumor extracts is 1:5.5 and in muscle extracts 1:10.04. Thus there was relatively about twice as much pyrimidine deoxyribosides in muscle extracts as in tumor extracts, as had been found by bioassay method. However, that all values by these methods were considerably higher than by bioassay methods indicates their lower degree of specificity and that such components as free deoxyribose, ascorbic acid, etc. may be included.

Summary. Spectral absorption analyses on alcoholic extracts of tumors revealed that they had a higher specific density/mg of nucleic acid than did similar extracts of normal muscle or than pure DNA or RNA. This high specific density, as well as type of ab-

TABLE II. Concentration of Deoxyribosides in Tumor and Normal Muscle Extracts (μg/mg Ash-free Solids).

Extracts from rat	Deoxyribosides			Thymine or thymidine	Ratios, purine to pyrimidine deoxyribosides
	Total	Pyrimidine	Purine		
Sarcoma No. 6	1.37	.73	.64	.23	
" No. 7	1.50	.92	.59	.27	
" No. 10	1.66	1.00	.65	.34	
Avg	1.51	.88	.63	.28	1:1.4
Lymphoma No. 8	4.58	2.85	1.68	2.05	
" No. 8	3.19	2.40	.79	1.80	
Avg	3.89	2.63	1.24	1.96	1:2.1
Normal muscle	.65	.50	.16	.10	
" "	.68	.50	.18	.11	
" "	.58	.43	.15	.06	
Avg	.64	.47	.16	.09	1:3.0

TABLE III. Content of Deoxyribosides in Tumor and Muscle Extracts by Chemical Analyses.

Extract	No. of extracts	Deoxyguanosine by— Tryptophane perchloric acid method, μ/mg	Diphenylamine method, μ/mg	Pyrimidine deoxyribosides by difference, μ/mg	Ratio, purine to pyrimidine deoxyriboside
Tumor*	7	14.14	2.17	11.97	1: 5.5
Muscle (normal)	4	8.06	.73	7.33	1:10.04

* Results on sarcomas No. 6 (2), No. 7 (1), No. 10 (2) and lymphoma No. 8 (2) were averaged.

sorption curve, suggested the presence of guanosine or deoxyguanosine. Paper electrophoresis experiments, followed by study of paper strips for the position of ultraviolet-absorbing components, and for presence of ribosides by an orcinol spray, or of deoxyribosides by cysteine: H_2SO_4 spray, led to the conclusion that tumor extracts contained chiefly deoxyribosides, while muscle extract contained ribosides. The small amount of deoxyribosides in normal muscle extracts in contrast to tumor extracts was supported by chemical analyses and by highly specific bioassay methods. Moreover, by these methods the relative amounts of purine deoxyribosides and pyrimidine deoxyribosides in tumor extracts differed from those in normal muscle extracts, suggesting that different nucleic acid derivatives exist in tumor and the host muscle tissue.

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Comparison of New Chemical Method of Determining Isonicotinoyl Hydrazide in Serum with Microbiological Assay. (24048)

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No satisfactory chemical method of determining isoniazid (INH) in blood of patients is as yet available, due to the insensitivity of present colorimetric methods. Extraction with organic solvents as proposed by Kelly and Poet(1) is difficult because of an unfavorable distribution coefficient between water and solvent. 1-chloro-2, 4-dinitrobenzene has been used by Ballard and Scott(2) for pharmaceutical assays of INH, and 1-fluoro-2, 4-dinitrobenzene by Yamaguchi(3) for determination of INH in rabbit urine for quantities far beyond the doses used clinically. No method for determination of INH in blood was mentioned. The present paper describes a spectrophotometric method for determination of INH in blood using 1-fluoro-2, 4-dinitrobenzene. Preliminary tests in aqueous medium showed that the color intensity was influenced by pH; therefore, serum deproteinization methods using either alkali or acid were not applicable.

Methods. Aliquots of 2 cc INH free serum or plasma were mixed with 1 cc absolute alcohol containing graded quantities of INH, 7 cc alcohol was added, the mixture shaken for 5 minutes in a mechanical shaker, centrifuged and 5 cc of clear supernatant (representing 1 cc serum) was used for colorimetry. After addition of 0.5 cc 0.4% alcoholic solution of reagent and approximately 0.1 g borax, the mixture was shaken, heated in screw cap-sealed tube 5 minutes at 80° and decanted from the excess borax for colorimetry. Concentrations of 0.0, 2.5, 5 and 10 μg INH in these samples (= μg/cc serum) were read at wave lengths from 450 to 520 mμ using the 0.0 μg/cc concentration as the blank. Fig. 1 shows the optical densities at various wave lengths on common coordinate paper. Higher degrees of absorption were observed at lower wave lengths, but readings, conforming to the Beer-Lambert Law were found at 500 mμ, which was used for subsequent work.

Results. Line 1, Fig. 2 shows the relation between concentration/cc serum and optical transmission by this method for quantities up to 10 μg per ml of INH. The points were averages of 10 determinations which showed very small deviations. At low INH content of the serum, the readings became less accurate. Experiments were carried out to use a double quantity of serum (4 cc) to be deproteinized with alcohol. In this case the ratio had to be 4 : 6, giving 60% alcohol dilution. Reading in this case had to be done at a temperature >60°. Line 2, Fig. 2 shows the readings for double quantities of serum and INH but is plotted for μg/cc serum. The curves obtained in 60% and 80% alcohol give

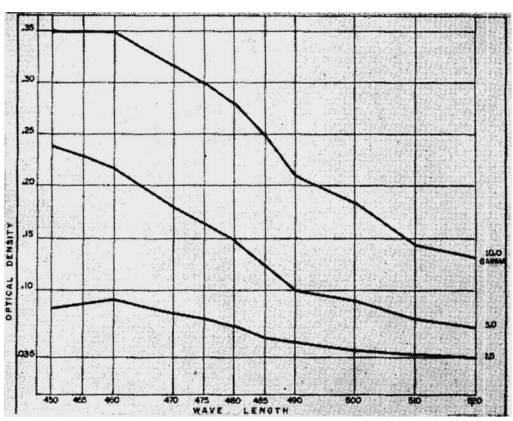

FIG. 1. Absorption spectra for 3 concentrations (μg/ml).