Studies with Psicofuranine in the Tumor-bearing Rat

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SUMMARY

The effects of the antitumor agent, psicofuranine, have been investigated in rats with subcutaneously transplanted Walker 256 adenocarcinomas. Administration of the drug at 500 mg/kg/day for 1 week caused a marked regression and a sharp drop in utilization of phosphate-P^{32} uptake in the tumors. Longer treatment also produced significant decreases in phosphate uptake by host tissues. It was possible to demonstrate the presence of small amounts of psicofuranine phosphates in the tissues by the use of psicofuranine labeled with tritium. No more than traces of the drug could have been incorporated into the nucleic acids. The incorporation of radioactive glycine-2-C^{14} into nucleic acid purines and protein was inhibited in the tumor tissue by the drug. In contrast, the liver showed increases in the incorporation of glycine-2-C^{14} into the adenine nucleotides and the adenine of the nucleic acids. Much of the antineoplastic activity of the drug may result from the inhibition of the conversion of xanthylic to guanylic acids as was found to be the case in bacterial systems. Other possibilities also are discussed.

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The marked inhibitory action of psicofuranine (6-amino-9-D-psicofuranosyl-purine) (5, 16, 23) against transplantable tumors of the rat was described by Evans and Gray (7). Wallach and Thomas (20) demonstrated that 60–80 per cent of the administered drug was recovered unchanged in the urine of dogs within 24 hours after injection, and no metabolites of the nucleoside were obtained. Hanka (11) found that the *in vitro* antibacterial action of psicofuranine against *Staphylococcus aureus* could be reversed completely by low levels of guanine, guanosine, or guanylic acid and by higher levels of adenosine, inosine, and certain of their derivatives. Since these effects might be mediated via drug-induced changes in nucleotide metabolism, we have investigated this possibility in tumor-bearing rats treated with psicofuranine.

MATERIALS AND METHODS

Sprague-Dawley rats weighing 100–140 gm. and bearing subcutaneously transplanted Walker 256 adenocarcinomas were used in all the experiments (7) and were generously supplied by Dr. J. S. Evans of these laboratories. Blood levels of psicofuranine were determined after an intraperitoneal injection of 250 mg/kg as a solution or suspension in 0.25 per cent Methocel (methylcellulose, The Dow Chemical Co., Midland, Michigan) or alternately in 25 per cent dimethylacetamide (DMA). Duplicate animals were sacrificed at various time intervals after administration of the drug, and blood was obtained by decapitation after chloroform anesthesia. The blood samples were allowed to clot at room temperature, and the serum was collected by centrifugation. One-half-ml. aliquots were analyzed for psicofuranine content by the diphenylamine method of Forist *et al.* (9). Values were corrected for low blanks obtained with blood from uninjected animals when DMA was used as vehicle. Methocel was found to give a positive reaction with the diphenylamine reagent. Thus, when Methocel was used as vehicle, values were corrected for the appreciable blank values obtained with Methocel-injected rats. Methocel was apparent in sera by 1.5 hours after injection and increased linearly thereafter to a concentration equivalent to 70 μg/ml of psicofuranine at 6 hours.

Radioactivity was determined in a Packard Tri-Card Scintillation Spectrometer. The counting solution consisted of 10 ml. of 70 per cent redistilled toluene and 30 per cent ethanol with 0.4 per cent 2,5-diphenyloxazole and 0.01 per cent 1,4-di-(2-[5-phenyloxazolyl]) benzene. Samples which contained protein were kept in solution by the use

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of 1 ml. of 10 per cent hydroxide of hyamine 10-X (Packard Instrument Company, LaGrange, Illinois). Determinations of P^{32} and C^{14} were corrected for quenching, when necessary, by the use of internal standards. Tritium samples were counted with and without internal standards and were corrected to total disintegrations/minute.

P^{32} experiment.—The animals in the treated group received 250 mg/kg of psicofuranine (25 mg/ml in 0.25 per cent Methocel) twice daily beginning 4 days after tumor implantation. The control animals received an equivalent volume of vehicle. A sterile solution of $Na_2HP^{32}O_4$ (1 mc/kg,

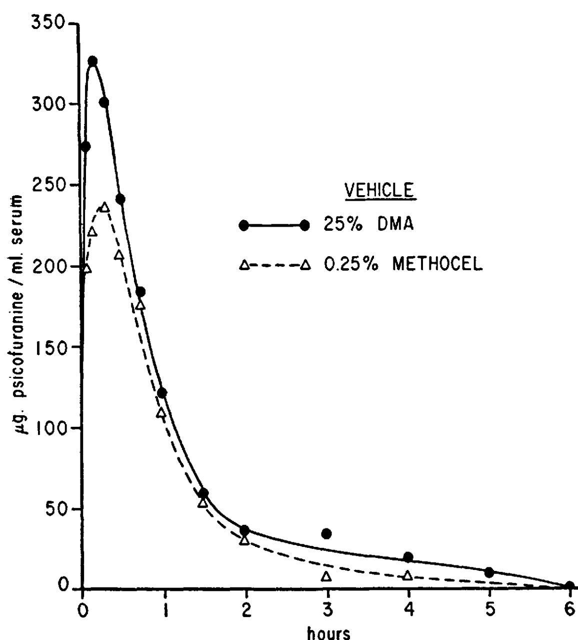

CHART 1.—Serum levels of psicofuranine following a single intraperitoneal dose of 250 mg/kg. Each point represents the average of two animals.

Abbott Laboratories, North Chicago, Illinois) was injected 5, 7, or 10 days after implantation just prior to the usual dose of psicofuranine. One animal for each time period was sacrificed 3 hours after the isotope injection. Excised organs were rinsed in saline, blotted, quick-frozen in a dry ice-alcohol bath, and stored at −30° C. until fractionated.

The frozen tissues were homogenized twice in 2 volumes of cold, 1 N perchloric acid in a glass homogenizer. The combined extracts (acid-soluble fraction) were neutralized with KOH, and the salt was removed by centrifugation. The residue was extracted 3 times with 5 volumes of boiling alcohol: ether (3:1) followed by an ether wash. The tissue powder was dried *in vacuo*, weighed, and dissolved by heating in 0.4 N KOH at 100° C. for 1 hour, or by incubation in 1 N KOH at 37° C. overnight. The resulting alkaline digest was neutralized with perchloric acid, and potassium perchlorate was removed by centrifugation.

Tritium-labeled psicofuranine experiment.—Eleven days after tumor implantation a group of four rats received 160 mg/kg of tritium-labeled psicofuranine as a solution of 16 mg/ml in 0.25 per cent Methocel. The radioactive psicofuranine, specific activity 2.54 mc/gm, was prepared by Dr. R. C. Thomas of these laboratories by the Wilzbach procedure (20, 21). Six hours after injection the animals were killed by decapitation. The average wet weight of the tumors was 3.8 gm. The pooled tissues were handled as described for the phosphate experiment, with the exception that cold-acid extraction was omitted, and instead the tissues were homogenized in 4 volumes of 80 per cent ethanol and washed in ½ volume of 80 per cent ethanol. Lipides and proteins were removed from the ethanol extracts by the extraction procedure of Folch (8). The remainder of the procedure was unchanged.

Glycine-2-C^{14} experiment.—Psicofuranine (250 mg/kg) was injected into rats bearing Walker adenocarcinomas 7 days after implantation. The drug was suspended in 25 per cent DMA to give 50 mg/ml. Control rats received injections of the vehicle. Glycine-2-C^{14} (Volk Radio Chemical Company, Chicago, Illinois) was injected intraperitoneally 30 minutes after the injection of psicofuranine. Groups of three control and treated animals were sacrificed 30 or 120 minutes following the isotope injection. Pooled tissues were extracted with cold acid as described for the P^{32} experiment, except that the Lourdes Multimixer was used, and a total of five extractions were made. The residues were extracted 2 times with boiling alcohol:ether and dried. The dried residues were ground in a mortar and hydrolyzed in 1 N HCl for 1 hour at 100° C. to extract purine bases from the total nucleic acids of the tissue (18).

Chromatography.—Nucleotides from the cold acid extracts and ethanol extracts were separated by chromatography on Dowex-1 (formate) by linear gradient elution with formic acid solutions or with increasing concentrations of ammonium formate at pH 4.9 (12). The nucleotides from ribonucleic acids were separated on Dowex-1 (formate) according to the stepwise solvent system of Cohn and Volkin (3). Adenine and guanine were separated on columns of Dowex-50 (2). Radioactive impurities, followed by guanine, were eluted with 0.5 N HCl. Adenine was eluted with 1 N HCl. The adenine and guanine peaks were lyophilized and the solids taken up in a small amount of 0.1 N HCl and applied as streaks to Whatman No. 1 paper. They then were chromatographed in a

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phosphate-isoamyl alcohol solvent (1). The bases were eluted with 0.1 N HCl, and their specific activity was determined by radioactivity determination and ultraviolet absorption. They were re-applied to Whatman No. 1 paper, and adenine was chromatographed with butanol-acetic acid (15). Guanine was chromatographed with isopropanol-hydrochloric acid (22). The bases once more were eluted and were considered free of radioactive contaminants, since the specific activities agreed closely before and after the final paper chromatography.

RESULTS

The blood levels of psicofuranine observed in rats under the conditions used in these experiments are shown in Chart 1.

Incorporation of radioactive phosphate into nucleotides and nucleic acids was followed in various tissues of the rat and in the Walker adenocarcinoma to determine the extent of alteration of normal metabolism brought about by psicofuranine. The tissue distribution of P^{32} is given in Table 1. The tumors of the treated group had begun to regress by the 5th or 6th day, and this was strongly reflected in the isotope level found in the protein-nucleic acid fractions. Decreases in radioactivity in the acid-soluble fractions probably reflect the changes in morphology of the tumors, since the regressing tumors were firm and whitish with much less blood than the invasive ones of the controls. Uptake into the spleen decreased after 4 days' drug therapy and showed a marked reduction by 6 days (7 and 10 days after implantation).

Uptake by the liver in treated animals was decreased somewhat by 10 days after implanta-

TABLE 1

THE DISTRIBUTION OF RADIOACTIVE PHOSPHORUS IN THE TISSUES OF RATS BEARING WALKER TUMORS IN THE PRESENCE AND ABSENCE OF PSICOFURANINE*

Tissue	Days after implantation	Radioactivity					
		Acid-soluble†		Alcohol-ether†		Alkaline digest‡	
		Control	Treated	Control	Treated	Control	Treated
Walker tumor	5	4.8×10^5	$>3.2\times10^5$	0.7×10^5	$>0.6\times10^5$	1.52×10^3	1.96×10^3
	7	10.3 "	6.3 "	2.1 "	1.3 "	6.58 "	2.96 "
	10	9.7 "	1.7 "	0.7 "	1.1 "	2.57 "	0.7 "
Liver	5	13.5 "	14.3 "	—	—	0.64 "	0.74 "
	7	10.9 "	8.8 "	5.7 "	4.6 "	0.86 "	0.84 "
	10	11.4 "	9.0 "	7.9 "	4.6 "	1.53 "	0.97 "
Spleen	5	6.3 "	6.4 "	1.7 "	2.4 "	3.03 "	3.3 "
	7	6.4 "	7.6 "	1.4 "	1.2 "	5.98 "	3.64 "
	10	8.4 "	9.4 "	1.9 "	1.2 "	5.26 "	1.41 "
Kidney	5	8.6 "	8.2 "	4.4 "	3.2 "	0.46 "	0.43 "
	7	6.7 "	6.9 "	3.1 "	2.2 "	0.42 "	0.42 "
	10	7.2 "	7.4 "	4.9 "	3.1 "	0.43 "	0.26 "
Heart	5	6.1 "	5.6 "	2.4 "	2.7 "	0.23 "	0.28 "
	7	5.9 "	5.8 "	0.9 "	1.2 "	0.17 "	0.2 "
	10	6.9 "	5.3 "	0.8 "	1.1 "	0.14 "	0.2 "
Lung	5	3.8 "	4.0 "	2.7 "	2.6 "	0.41 "	0.48 "
	7	4.2 "	3.5 "	2.3 "	1.4 "	0.39 "	0.35 "
	10	4.1 "	3.9 "	1.9 "	1.2 "	0.37 "	0.32 "
Brain	5	0.7 "	0.5 "	0.1 "	0.2 "	0.05 "	0.04 "
	7	0.5 "	0.5 "	0.1 "	0.1 "	0.04 "	0.04 "
	10	0.3 "	0.5 "	0.1 "	0.2 "	0.03 "	0.02 "
Serum	5	1.75×10^6	2.04×10^6 counts/min/ml				
	7	2.78 "	2.22 " " " "				
	10	2.22 "	2.12 " " " "				

* The control animals received no drug. Treated animals received 500 mg/kg/day intraperitoneally in a divided dose beginning 3 days after tumor implantation. The animals were sacrificed 3 hours after an injection of $P^{32}O_4^{\equiv}$ on the days indicated.

† Values expressed as counts/min/gm wet weight.

‡ Values expressed as counts/min/mg dry weight after alcohol-ether extraction.

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tion, while the other tissues examined (kidney, heart, lung, and brain) showed little or no effect of the drug on phosphate levels.

The acid-soluble components from several of the tissues were separated by column chromatography and examined for evidence of new phosphate peaks or for changes in specific activity of the normal nucleotides of the cell. No new phosphates could be separated from normal cell constituents from either liver of the tumors, nor were there marked, reproducible changes in specific activity or total concentration or those nucleotides appearing in highest concentrations.

The nucleotides of the livers and tumors from the hydrolyzed ribonucleic acids of control and treated animals were separated by column chromatography, and their specific activities were estimated. Uptake into each of the nucleotides was depressed. Values found for the livers 10 days after implantation were: cytidylic acid, 69 per cent; adenylic acid, 86 per cent; uridylic acid, 55 per cent; and guanylic acid, 64 per cent of the controls. Values for the tumors 7 days after implantation were 40–60 per cent of the controls. No new nucleotides which might have resulted from incorporation of psicofuranine into RNA could be demonstrated. Small peaks eluted very near adenylic or cytidylic acids would not have been detected, however. The deoxyribonucleic acids from the alkaline digests of the 7th-day tumor transplants were precipitated, washed, and counted. The radioactivity was low for both samples, the treated one being 35 per cent of the control.

Tritium-labeled psicofuranine was administered to rats to determine if phosphorylated derivatives of the drug were found in the animal and whether there was appreciable incorporation of the drug into nucleic acids. The tissues were extracted with 80 per cent alcohol to avoid breakdown of psicofuranine, which is known to be very labile toward acids (16, 10). Table 2 shows the tissue levels of radioactivity from tritium-labeled psicofuranine. Ten per cent of the initial dose still was retained by the tissues 6 hours after administration of the drug. The polar components of the ethanol fractions then were chromatographed on columns of Dowex-1. An example of this chromatography is shown in Chart 2 for the extract of the tumors (19 gm. wet wt.). A radioactive peak appeared which presumably represented the phosphorylated drug. The amount of material was extremely small and not separated completely from other ultraviolet-ab-

TABLE 2

THE DISTRIBUTION OF RADIOACTIVITY FROM TRITIUM-LABELED PSICOFURANINE IN THE TISSUES OF RATS BEARING WALKER TUMORS*

Tissue	Radioactivity (disintegrations/min/gm wet weight)			Per cent of total dose
	Alcohol extract	Alcohol-ether	Alkaline digest	
Walker tumor	3.39×10^5	0.39×10^5	84	2.0
Liver	9.01 "	1.73 "	604	7.0
Kidney	2.25 "	0.50 "	219	0.2
Spleen	7.51 "	1.47 "	422	1.1
Intestine	4.42 "	0.75 "	148	1.8
Total:				12.1

* Tritium-labeled psicofuranine was injected intraperitoneally in rats 11 days after tumor implantation. The rats were sacrificed 6 hours later. Samples were prepared from pooled tissues of four animals. The average radioactivity in the serum at 6 hours was 12.4×10^4 d/min/ml.

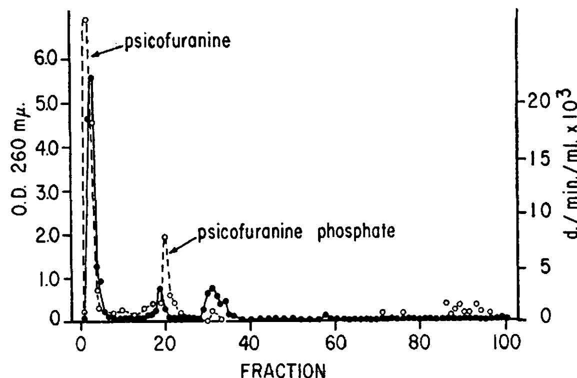

CHART 2.—Detection of psicofuranine phosphate in an alcoholic extract of tumor tissue. Extract was prepared from pooled tumors of four animals. The compounds were eluted from Dowex-1 with a linear gradient elution beginning with water and proceeding to 1 N ammonium formate (pH 4.9) at fraction 74. (●—●, optical density; ○—○, radioactivity.)

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sorbing materials. Subsequent co-chromatography of this pooled peak with authentic psicofuranine phosphate[1] showed it to elute precisely with the synthetic compound. The alkaline digests were investigated to determine whether the radioactivity found there represented incorporated drug. Chromatography of these fractions showed the appearance of radioactive polar compounds, including one component which eluted in the diphosphate area. However, extensive washing of the dried cell residue with cold acid before digestion with alkali removed 80–90 per cent of the radioactivity, indicating that the alcohol and alcohol:ether extractions had been inadequate to remove all the free nucleotides from the residue. Thus, incorporation into nucleic acid, if it occurred at all, was extremely slight and must represent less than 0.01 per cent of the drug found in tissue. This would correspond to less than 0.0002 per cent replacement of the adenylic acid of the nucleic acids.

The influence of psicofuranine on the synthesis of proteins and purines was followed by the incorporation of glycine-2-C^{14} in the rat. The kinetics of the incorporation of glycine into various rat tissues have been studied and have shown that guanine nucleotides were labeled very rapidly, followed closely by the adenine nucleotides (6, 19)

[1] Dr. W. Schroeder, these laboratories, personal communication.

At later times these nucleotides were incorporated into the nucleic acids. Table 3 shows the level of radioactivity in the serum and the incorporation of glycine-2-C^{14} in the livers and tumors of the control and treated rats. At these short time intervals the incorporation of radioactivity was almost entirely into the protein of the tissue, and the level in nucleic acid was very low. Incorporation of glycine-2-C^{14} into tumor proteins was depressed in the treated animals in experiment 1. This effect was not so marked in experiment 2, in which a higher level of glycine was injected. The incorporation into liver protein was somewhat increased in the animals treated with psicofuranine. However, serum radioactivity also was higher in the treated group. The pooled acid-soluble nucleotides from the control and treated livers from experiment 2 were separated on Dowex-1 columns (Chart 3). The various nucleotides were identified by their ultraviolet spectra, order of elution (12), and comparison with the elution of known nucleotides on companion columns. The total concentration of the combined adenine nucleotides was similar in the livers from control and treated animals. However, there was an increase in radioactivity in the adenine nucleotides from the treated extract (see also Table 4). The peak labeled "Ad" in Chart 3 presumably contained adenine and adenosine. This peak was much higher in the extract from the treated animals, but was not

TABLE 3

THE INCORPORATION OF GLYCINE-2-C^{14} INTO LIVERS AND WALKER TUMORS OF THE RAT IN THE PRESENCE AND ABSENCE OF PSICOFURANINE*

TISSUE	TIME† (MIN.)	ALKALINE DIGEST (COUNTS/MIN/MG DRY WEIGHT)			
		Experiment 1		Experiment 2	
		Control	Treated	Control	Treated
Walker tumor	30	1.09×10^3	0.50×10^3		
	120	2.80×10^3	1.40×10^3	4.98×10^3	4.51×10^3
Liver	30	2.00×10^3	3.19×10^3		
	120	3.00×10^3	3.22×10^3	5.76×10^3	6.36×10^3
Serum‡	30	1.12×10^5	1.30×10^5		
	120	2.13×10^5	2.80×10^5	8.46×10^5	9.09×10^5

* Rats were given injections intraperitoneally of 250 mg/kg of psicofuranine on the 7th day after implantation with Walker tumors. Glycine-2-C^{14} was injected 30 minutes later (Exp. 1, 20 mg/kg, 400 μc/kg; Exp. 2, 67 mg/kg, 1000 μc/kg). Average serum level of psicofuranine at 30 minutes was 120 μg/ml and at 120 minutes was 52 μg/ml (Exp. 1) and 35 μg/ml (Exp. 2). Samples were prepared from pooled tissues of three animals.

† Animals were sacrificed 30 and 120 minutes after isotope injection.

‡ Counts/min/ml serum (not an alkaline digest).

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appreciably radioactive (peak 1 eluted slightly ahead of Ad) and did not contain psicose. The concentrations of guanosine monophosphate (GMP) (estimated from orcinol analysis) and guanosine triphosphate (GTP) also were similar in the two extracts. Guanosine diphosphate (GDP) was not estimated, since it was not separated from the uracil-containing component (U-2) which eluted with it. Radioactive peaks 1–5 were not associated with measurable optical densities. These components were presumed to be low molecular weight compounds containing glycine and perhaps were intermediates in purine biosynthesis. Component 1 was greatly increased in the extract from treated livers. Component 5 gave only end absorption, gave a ninhydrin reaction, and chromatographed with glycine on paper following acid hydrolysis. Glutathione elutes in this area. There were no components in sufficient concentration to give a positive Bratton-Marshall test for aryl amines. There was an increase in the U-2 component in the extract from the treated livers. This area has been shown to contain uridine diphosphate-sugar components (12, 14). The other uracil- and cytosine-containing peaks were essentially unchanged.

The total nucleic acids of the livers and tumors were hydrolyzed, and the specific activities of guanine and adenine were determined. Table 4 shows the results of experiment 1. Psicofuranine treatment markedly inhibited the incorporation of

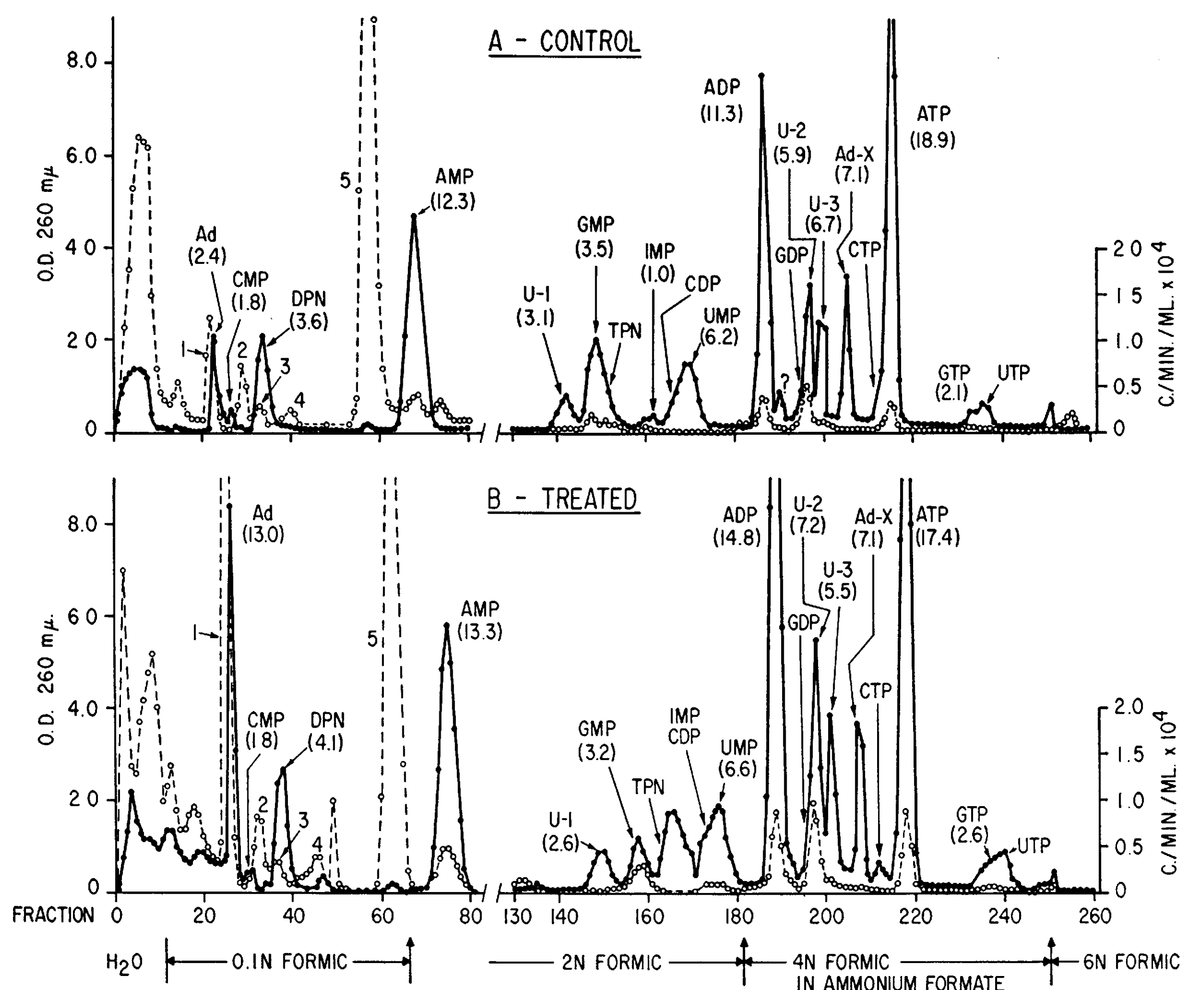

CHART 3.—The separation of acid-soluble nucleotides from the livers of control and treated rats given glycine-2-C^{14} (Experiment 2). Sample was prepared from pooled livers of three animals. Figures in parentheses represent total micromoles in the peak calculated from the optical density readings or orcinol determinations. The Dowex-1 columns were eluted with linear gradients proceeding to the final normalities shown. (●—●, optical density; ○—○, radioactivity.) Abbreviations used were: AMP, ADP, ATP, adenosine mono-, di-, and triphosphate, respectively; CMP, CDP, CTP, cytidine mono-, di-, and triphosphate; GMP, GDP, GTP, guanosine mono-, di-, and triphosphate; UMP, UTP, U-1, U-2, U-3, uridine mono- and triphosphate and uridine-containing components 1, 2, and 3; IMP, inosine monophosphate; DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; Ad, adenine and adenosine; Ad-X, adenine-containing compounds.

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glycine-2-C^{14} into nucleic-acid adenine and guanine of the tumor tissue. In contrast, the incorporation into adenine of the liver of the treated animals was increased. There was some depression of uptake into liver guanine. Table 4 also shows the results obtained in a 2-hour experiment (experiment 2) using a higher level of glycine-2-C^{14}. The specific activities of the acid-soluble adenine and guanine were determined in this experiment. There was a depression of glycine incorporation into the adenine and guanine nucleotides of the tumor and a marked increase into the adenine of the treated liver. These changes brought about by drug treatment were reflected closely in the specific activities found in the adenine and guanine of the nucleic acids. This observation suggested that there was no differential effect of the drug on the incorporation of either of these bases into the nucleic acids.

TABLE 4

THE INCORPORATION OF GLYCINE-2-C^{14} INTO ADENINE-AND GUANINE-CONTAINING NUCLEOTIDES AND NUCLEIC ACIDS*

FRACTION	TIME (MIN.)	ISOLATED PURINE (COUNTS/MIN/μMOLES)			
		Adenine		Guanine	
		Control	Treated	Control	Treated
Experiment 1:					
Total nucleic acid†					
Walker tumor	30	61	39	417	166
	120	706	187	1215	438
Liver	30	19	147	178	115
	120	112	313	325	224
Experiment 2:					
Total nucleic acid†					
Walker tumor	120	2.62×10^3	1.54×10^3	4.92×10^3	4.12×10^3
Liver	120	357	609	522	501
Acid-soluble‡					
Walker tumor	120	2.30×10^4	1.03×10^4	2.22×10^4	1.88×10^4
Liver	120	3.54×10^3	9.98×10^3	5.09×10^3	6.25×10^3

* See footnote, Table 3.

† Purines were hydrolyzed from the total nucleic acids and purified by column and paper chromatography (see "Methods").

‡ Tumor adenine and guanine were determined from the acid hydrolysate of the total acid-soluble fraction. Liver adenine and guanine were determined from the AMP and GMP peaks eluted from Dowex-1 (Chart 3) after acid hydrolysis and further purification (see "Methods").

DISCUSSION

Continuous therapy with high levels of psicofuranine for as long as 1 week did not drastically alter the pattern of incorporation of radioactive phosphate into most organs of the rat. During this same period the Walker 256 adenocarcinoma showed marked regression and nearly ceased to incorporate P^{32} into both ribonucleic acid and deoxyribonucleic acid. The spleen and, to a lesser extent, the liver also showed decreases in total uptake. Phosphorylated derivatives of psicofuranine did not accumulate in the tissues, even with daily treatment, and could be detected only by the use of a highly radioactive drug.

Many of the nucleic acid antagonists have been shown to exert their antineoplastic activity by being incorporated into nucleic acids or by inhibition at the nucleotide level in the cell (for a recent review, see Mandel [13]). This cannot be asserted for psicofuranine, since appreciable levels of the drug were not found in the nucleic acids of the tissues, and only small amounts of phosphorylated derivatives were indicated. Moreover, the antibacterial properties of the drug can be explained adequately by the strong inhibition of xanthylic acid aminase (11,17). Xanthosine accumulated, and incorporation of radioactive precursors into nucleic acid guanine was depressed (17) in a manner analogous to the tumor data reported here. Phosphorylated derivatives of the drug were not detected.[2]

Radioactive psicofuranine was found to penetrate tissues rapidly and was not hydrolyzed ap-

[2] Dr. L. Slechta, these laboratories, personal communication.

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preciably to adenine and psicose (also see [20]). Only low levels of psicofuranine phosphate were detected in the tissues, suggesting that the normal metabolic enzymes did not phosphorylate the nucleotide readily. Additional experiments with chemically synthesized phosphate derivatives of psicofuranine have shown these compounds to be relatively resistant to the action of various phosphatase enzymes (unpublished data). Thus, phosphorylated derivatives of psicofuranine may prove to have entirely different modes of action from the parent compound provided they can gain access to the cell.

The depression of incorporation of glycine-2-C^{14} into tumor proteins could have resulted from decreased cofactor levels in the cell which are required for protein synthesis (guanosine triphosphate), or from other unknown inhibitory activities of the drug. No such depression was observed in the liver, perhaps owing to higher reserves of nucleotides in this organ than in the rapidly growing tumor tissue. Incorporation of glycine into nucleic acid guanine and adenine was depressed in the tumor as it was in the bacterial studies (17). A marked increase in the specific activity of the liver adenine compounds was observed in the treated animals. Darrow *et al.* (4) recently have shown that a similar increase in the specific activity of the acid-soluble adenine of liver resulted when mice were given formate-C^{14} or glycine-C^{14} following high dosages of amethopterin. The liver showed the greatest increase of any organ measured (up to sixfold). Fasted animals showed similar increases, but with less reproducibility. These authors pointed out that a wide variety of hepatotoxic compounds brought about similar results. Increases in the specific activity of adenine could have resulted from an increased rate of *de novo* synthesis or from a decrease in the glycine pool in the livers of the treated animals. The latter possibility did not appear to be the case in our experiments, since component 5 (Chart 3) of the acid-soluble extracts was identified tentatively as glutathione, and the specific activity of this peptide from the control extract was nearly identical to that found in the treated extract. Also, we have been able to demonstrate increases in the specific activity of acid-soluble adenine and decreases in guanine in the presence of 100 μg/ml psicofuranine *in vitro* employing minced liver.[3] Under these conditions the concentration of exogenous glycine-2-C^{14} did not change appreciably during the experiment. The somewhat different results obtained with glycine-2-C^{14} uptake and P^{32} uptake into adenine compounds of the liver might reflect an alteration in the proportion of preformed purines used for nucleic acid synthesis. Also, in the P^{32} experiments the drug was given chronically, while in the glycine-2-C^{14} experiments the animals received a single injection of the drug prior to administration of the glycine-2-C^{14}.

It is possible that inhibition both of the Walker 256 adenocarcinoma and of bacterial growth could be explained by inhibition of xanthylic acid aminase. Blocking this enzyme would result in decreased incorporation of glycine-2-C^{14} into guanine of the nucleic acids and decreased levels of guanine-containing nucleotides and cofactors in the cells. It must be remembered, however, that the presence of even low levels of the phosphate derivatives of psicofuranine in the tissue might exert a profound influence on cellular metabolism. Further, the effect of psicofuranine on deoxyribonucleic acid biosynthesis has not, as yet, been investigated adequately in either bacteria or animals. Finally, the observed effects of the drug on liver metabolism would seem unusual if the inhibition of this single enzyme accounted completely for the action of psicofuranine.

[3] W. E. Magee and L. Slechta, unpublished.

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