and 4 were found. The same four substances were likewise found in organs and excreta taken from intact mice and rats injected 48 hours previously with *radio-iodide,* or in other words, after a time interval sufficient for endogenous production of labeled thyroxine in the thyroid and its subsequent distribution in the body. These findings suggest that thyroxine is metabolized to yield at least the two compounds Nos. 1 and 4 as well as iodide.

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In vitro Inhibition of Radiophosphate Uptake and Growth of a Neurotropic Virus by 5-Chlorouridine.* (18596)

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It has previously been reported(1-3) that infection of minced 1-day-old mouse brain with Theiler's GD VII strain of mouse encephalomyelitis virus increases the incorporation of $P^{32}O_4$ into the phospholipide and total protein-bound phosphate fractions. The increased turnover in the total protein-bound phosphate fraction was due primarily to the increased turnover of the ribonucleic acid fraction, the slower turnover of desoxyribonucleic acid phosphorus being unaffected by virus propagation(1). These experiments suggested that virus propagation was intimately associated with ribonucleic acid metabolism, and raised the question of what effects a nucleoside antagonist might have on virus propagation and nucleic acid metabolism. Previous studies have shown that 8-azaguanine had little effect on the growth of Theiler's virus in minced 1-day-old brain or on $P^{32}O_4$ metabolism of this system(3). Benzimidazole, however, inhibited virus growth and $P^{32}O_4$ uptake(3). 5-Chlorouridine has been shown to be a potent uridine antagonist in Neurospora(4). Preliminary experiments showed that 5-chlorouridine inhibited the propagation of Theiler's GD VII virus and led to the present experiments. In these experiments we have investigated the effects of 5-chlorouridine and of uridine, alone and in combination, on the uptake of $P^{32}O_4$ by the acid-insoluble fractions of minced 1-day-old mouse brain and on the propagation of Theiler's GD VII virus in this tissue.

Methods. The methods employed have been described in previous papers(1-3). Minced brain tissue, 40 to 60 mg, aseptically removed from 1-day-old mice was added to sterile 50 ml Erlenmeyer flasks containing 2.5 ml of Simms' solution containing 1 microcurie of $P^{32}O_4$. Uridine and 5-chlorouridine§ were added in concentrations of 3 mg per ml and 1 mg per ml of Simms' respectively. The pH was adjusted to 9 with dilute NaOH. The preparations were inoculated with virus or control supernatants, and brought to a final volume of 3 ml with Simms' solution. The flasks were closed with rubber stoppers and incubated 24 hours without shaking at 35°C in an atmosphere of air. At the termination

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of the incubation period, all preparations were tested for sterility and the virus titer determined as previously described(1,3,5). Studies were made of the uptake of radioactive orthophosphate into the organic acid-soluble, phospholipid (LP), and the various protein-bound phosphate fractions by procedures previously described(1). As the turnover and the distribution of phosphorus in the organic acid-soluble fractions of the present experiments were not significantly different from values previously reported(1), and neither of the compounds employed in this report exhibited any significant effect on these values, the data for this fraction have been omitted in this report. The protein-bound phosphorus was fractionated as follows:[5] The trichloroacetic acid-insoluble fraction after removal of lipides with ethanol-ether and chloroform-methanol was treated with 5 ml of 1N NaOH (phosphate-free). After a sample of the clear solution was removed for the determination of total protein-bound phosphorus, the solution was acidified with 0.1 volumes each of concentrated HCl and 50% trichloroacetic acid to precipitate desoxyribonucleic acid (DNA) and residual protein (RP). The acid-supernatant contained ribonucleic acid (RNA) and inorganic phosphate (PIP), presumably liberated from phosphoprotein during treatment. Contaminating inorganic phosphate will also be found in this fraction. The PIP fraction was separated from the RNA by the method of Delory(6). The DNA was extracted from the RP by two 15 minute treatments with 5 ml of 5% trichloroacetic acid at 90°C. This method corresponds closely to that developed independently by Furst *et al.*(7). Previous experiments(1) have shown the nucleic acid phosphorus values obtained in this manner were in good agreement with those calculated from pentose and desoxypentose determinations. The chemical analysis for phosphorus was carried out by a modification of the method of Fiske and Subbarow(8) in which ascorbate was used as a reducing agent and a heating period was employed for color development and stabilization. Radioactive samples were prepared in ¼-oz. tin ointment dishes and counted with a thin mica-window Geiger-Muller counter tube. A minimum of 3,000 counts per sample was taken and all counts were at least 3 times background.

Experimental. Table I gives data for the uptake of $P^{32}O_4$ in a 24-hour incubation period into the LP, RNA, DNA, RP, and PIP phosphate fractions of noninfected and virus-infected minced 1-day-old mouse brain as influenced by the presence of uridine and 5-chlorouridine in concentrations of 3 mg and 1 mg per ml of Simms' respectively. Values are expressed as relative specific activity, *i.e.*, the specific activity of the fraction relative to the specific activity of the inorganic phosphate fraction. It can be seen from Table I the presence of virus stimulated the uptake of $P^{32}O_4$ into the LP and RNA fractions as previously reported. The turnover of the other fractions was unaffected by the presence of virus.

Addition of 5-chlorouridine not only eliminated the virus effect on the RNA fraction but reduced the uptake below the values for the noninfected, control tissue. Curiously the stimulating effect of virus on the LP fraction was still present. No effects were noted on any other fractions. It is of interest to note that 5-chlorouridine had no effect on any of the fractions of the control tissue. Along with the inhibition of RNA turnover, virus propagation is also inhibited by 5-chlorouridine as evidenced by a reduction in titer from 50,000 to 1,000. In other experiments virus titers were determined after flasks had been incubated for 72 hours. The titers were 100,000 for control tissue with or without uridine, 50,000 with 5-chlorouridine and 100,000 with uridine plus 5-chlorouridine.

These observations suggest that RNA metabolism associated with virus propagation is more sensitive to 5-chlorouridine than is the

5. Pearson, H. E., *J. Immunol.*, 1950, v64, 447.

[5] In a previous publication(1) this portion of the fractionation procedure was erroneously reported due to the deletion of a sentence.

6. Delory, G., *Biochem. J.*, 1938, v32, 1161.

7. Furst, S. S., Roll, P. M., and Brown, G. B., *J. Biol. Chem.*, 1950, v183, 251.

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TABLE I. Effects of 5-Chlorouridine and Uridine on $P^{32}O_4$ Uptake by Acid-Insoluble Fractions of Minced 1-day-old Mouse Brain and on Propagation of Theiler's GD VII Virus. (Three independent series of experiments).

Fraction		Relative specific activity*			
		No addition	5-Chloro-uridine	Uridine	5-Chloro-uridine + uridine
LP	No virus avg	3.02	3.17	3.05	2.87
	Range	2.8-3.2	3.1-3.3	3.0-3.2	2.8-2.9
	Virus avg	3.87	3.9	3.88	3.89
	Range	3.6-4.1	3.8-4.1	3.8-4.0	3.8-4.0
RNA	No virus avg	2.78	2.42	2.51	2.63
	Range	2.6-3.0	2.2-2.8	2.4-2.6	2.6-2.7
	Virus avg	4.07	1.9	4.15	2.96
	Range	3.6-4.6	1.8-2.0	4.0-4.3	2.9-3.0
DNA	No virus avg	.64	.43	.57	.59
	Range	0.5-0.8	0.4-0.5	0.5-0.6	0.5-0.7
	Virus avg	.66	.55	.62	.66
	Range	0.5-0.9	0.3-0.7	0.6-0.7	0.6-0.7
PIP	No virus avg	4.7	4.31	4.14	4.14
	Range	4.3-5.5	4.2-4.9	4.0-4.3	4.0-4.3
	Virus avg	4.16	4.22	4.76	4.13
	Range	4.1-4.3	4.0-4.4	4.6-4.9	4.1-4.2
RP	No virus avg	.93	.95	1.16	1.1
	Range	0.7-1.3	0.7-1.1	1.1-1.2	0.9-1.3
	Virus avg	1.12	1.09	1.08	1.05
	Range	0.8-1.4	0.9-1.5	1.0-1.1	1.0-1.1
Virus titer†		50000	1000	50000	5000

$$\text{* Relative specific activity} = \frac{\text{counts/}\gamma\text{ P in fraction}}{\text{counts/}\gamma\text{ P in inorganic fraction}} \times 100.$$

† Virus titer is expressed as the highest dilution of the supernatant solution that would kill at least half of a group of 7 mice after intracerebral injection of each with 0.03 ml.

normal metabolism of RNA. Uridine by itself had no measurable effect on virus propagation or on the uptake of $P^{32}O_4$ into any of the tissue fractions of either the control or infected tissue. Uridine, however, partially overcame the inhibiting action of 5-chlorouridine on virus propagation and on the turnover of $P^{32}O_4$ in the RNA fraction of the virus-infected tissue.

In Table II the data for the distribution of phosphorus in the various tissue fractions from the experiments in Table I are given. As previously reported(1) the presence of virus increases the content of RNA and decreases the content of DNA. Addition of 5-chlorouridine inhibits this virus effect and reduces the RNA content to that of the noninfected tissue. The DNA content of the control and infected tissue may possibly increase slightly in the presence of 5-chlorouridine, although the magnitude of this effect is too small to establish this with certainty. Uridine appears to have no effect on the distribution of phosphorus in any of the fractions of the control or virus-infected tissues. Uridine in the presence of 5-chlorouridine partially restores the virus effect on the RNA content as shown by a rise from 15.8 to 18.3 and partially reverses the virus inhibiting action of 5-chlorouridine.

Discussion. It has been shown that propagation of Theiler's GD VII virus in minced 1-day-old mouse brain is associated with both an increased turnover and an increased net amount of RNA. The finding that virus propagation is inhibited when these effects of virus propagation are prevented suggests that RNA plays an intimate role in the propagation of Theiler's GD VII virus in minced 1-day-old mouse brain. Since 5-chlorouridine inhibits these virus effects on the RNA fraction, while having no effects on any other tissue fractions examined, possibly 5-chlorouridine is acting as a specific inhibitor

TABLE II. Effects of 5-Chlorouridine and Uridine on the Distribution of Phosphorus in the Acid-Insoluble Fractions of Minced One-day-old Mouse Brain. (μg of P per 100 mg of tissue).

Fraction		No addition	5-Chloro-uridine	Uridine	5-Chloro-uridine + uridine
LP	No virus avg*	35.7	34.4	38.5	38.3
	Range	35-36	34-35	36-40	38-39
	Virus avg	35.3	36.6	38.2	33.2
	Range	34-37	34-39	36-40	32-35
RNA	No virus avg	16.0	16.0	16.4	15.2
	Range	15-17	15-17	16-17	15-16
	Virus avg	19.9	15.8	20.2	18.3
	Range	19-21	15-17	20-21	18-19
DNA	No virus avg	12.4	14.5	13.0	12.5
	Range	11-13	13-15	12-14	12-13
	Virus avg	10.8	12.3	10.6	10.3
	Range	10-11	11-14	10-11	9-11
PIP	No virus avg	6.2	5.2	6.3	6.5
	Range	4-7	5-6	6-7	5-7
	Virus avg	5.9	6.1	4.8	5.8
	Range	5-7	5-7	4-5	5-6
RP	No virus avg	3.2	2.9	2.6	2.3
	Range	2-4	2-4	2-3	2-3
	Virus avg	3.7	2.7	2.5	2.6
	Range	3-6	2-3	2-3	2-3

* Avg of 3 experiments.

of RNA metabolism associated with virus propagation in this system.

Summary. 5-Chlorouridine inhibits the propagation of Theiler's GD VII virus and the uptake of $P^{32}O_4$ by the ribonucleic acid fraction of minced, 1-day-old mouse brain but had no effects on other tissue fractions studied. Uridine partially reverses the inhibiting effects of 5-chlorouridine on virus propagation and on the uptake of $P^{32}O_4$ by the ribonucleic acid fraction. Virus propagation has been shown to be associated both with an increased turnover and increased net amount of ribonucleic acid phosphorus.

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A Substance Occasionally Found as a Major Ninhydrin-Reacting Component of Urine.*† (18597)

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A ninhydrin-reacting substance has been observed frequently on 2-dimensional paper chromatograms prepared from the urines of many subjects, which appears not to match any of the 60 ninhydrin-reacting substances given in the "map of spots" by Dent(1), most of which were available for direct comparison, as well as a number of additional compounds available for checking. This unidentified compound falls between alanine and proline

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