Conversion of Radioactive Orotic Acid into Pyrimidine Nucleotides of Nucleic Acid by Slices of Rat Liver.* (18141)

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It has been demonstrated that slices of rat liver have caused the *in vitro* incorporation of radioactive orotic acid into the pyrimidine nucleotides of nucleic acid and not into the purines.

Three milligrams of orotic acid (labeled in the 4 position) were incubated with about 6 g of slices of rat liver for 5 hours at 37°C in a Krebs saline-phosphate buffer of pH 7.4. The solution was saturated with a 95% O_2: 5% CO_2 mixture at the beginning and nothing was used to diminish bacterial action. The slices were homogenized after preliminary washing and the homogenate precipitated with 10% trichloroacetic acid. The extraction of lipids was then carried out with a 3:1 alcohol and ether solution. The nucleic acid was extracted from the protein with hot 10% NaCl and precipitated with alcohol. All of the dried impure nucleic acid (35 mg containing both r.n.a. and d.n.a.) was hydrolyzed with 0.75 cc of N HCl in a boiling water bath for one hour. This hydrolyzed the purine nucleotides and left the pyrimidine nucleotides unchanged. 0.04-0.06 cc were placed on each of 4 filter paper strips 12 cm wide and run with ascending columns of tertiary butyl alcohol (70% made 0.8 N with HCl, 30% water)(1). After drying, the papers were viewed with an ultraviolet Mineralite. The separated bands on paper were eluted with 0.01 N HCl, and the solutions read with a Beckman ultraviolet spectrophotometer. The maximum absorptions and the ratios of densities (278-262 mμ for cytidylic and uridylic acids; 248-262 for guanine and adenine) were determined in order to identify and quantitate the bases. The separate solutions were evaporated on plates and radioactivity determined with a windowless counter.

TABLE I. Specific Activity of Pyrimidine Nucleotides from Slices of Rat Liver After Incubation with Radioactive Orotic Acid.

Substance	Wt, γ	Counts per min. corrected for background: Actual	Per mg base
	Paper		
Uridylic acid	544	37	182
Space		1	
Cytidylic acid	674	14	56
Adenine	358	0	0
Guanine	320	0	0
	Resin		
Uridylic acid	1850	153	229
Cytidylic acid	2740	100	103

Approximately three-fourths of the hydrolysate from the above experiment containing purine bases and pyrimidine nucleotides was adjusted to pH 8 and the guanine precipitate removed by centrifugation. Except for slight modifications, a procedure described by Cohn(2) was then used to separate the remaining components in the supernatant of the hydrolysate on a resin column (Dowex I 400 mesh). The purine bases were removed by thorough washing with water. The cytidylic acid was eluted with 0.002 N HCl, identified and measured with the Beckman spectrophotometer, the solution evaporated and the residue counted. The uridylic acid was eluted with 0.01 N HCl and treated in a similar manner. The data are given in the accompanying table. Another experiment yielded data which confirm this one.

It is apparent that no radioactivity appeared in the purines while highly significant amounts appeared in the pyrimidine nucleo-

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tides. Because of the nature of the precursor it is thought that most of the radioactivity was in the pyrimidine ring rather than in the carbons of the sugar.

The radioactivity data obtained from the resin are thought to be better than those from the paper because there was considerable residue after evaporating solutions from the paper with resulting self absorption. A negligible amount of residue was left after evaporating solutions from the resin.

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Effects of Vitamin B_{12} on Thiouracil Action in Rats.*† (18142)

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Vit. B_{12} has been demonstrated to counteract the deleterious actions of several substances administered in toxic quantities to rats. Thus, it has been shown to overcome the retardation of growth which follows administration of toxic amounts of thyroid powder or thyroprotein(1-4), diethylstilbestrol(4) and lactose(5). In addition, Popper *et al.*(6) have reported that vitamin B_{12} can prevent the acute hepatic injury which results from administering carbon tetrachloride to rats.

Thiouracil has been shown to cause a direct depression of thyroid hormone synthesis, thereby stimulating increased production of thyrotrophic hormone by the anterior pituitary with subsequent thyroid hypertrophy (7-8). The inhibition of thyroid function usually results in reductions in growth rate and food intake, although these may also be mediated in part through non-thyroid mechanisms(9). Would vit. B_{12} counteract any of these actions of thiouracil?

Procedure. Immature female rats of the Carworth strain, divided into 8 uniform groups of 10 each, were fed the following basal ration for 30 days: yellow corn meal, 35%; ground wheat, 25%; linseed oil meal, 10%; whole milk powder, 20%; alfalfa leaf meal, 6%; brewers yeast, 3%; and table salt, 1%. The following substances were added to the basal ration of each group of rats: 1, controls; 2, 40 μg of vit. B_{12}§ per kg; 3, 80 μg vit. B_{12} per kg; 4, 0.1% thiouracil; 5, 0.1% thiouracil and 40 μg vit. B_{12} per kg; 6, 0.1% thiouracil and 80 μg of vit. B_{12} per kg; 7, 0.1% thiouracil, and 40 μg of vit. B_{12} per kg during last 10 of 30 days; 8, 0.1% thiouracil, and 80 μg of vit. B_{12} per kg during last 10 of 30 days. Four hours prior to sacrifice, each rat was injected intraperitoneally with a tracer dose of radio-

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§ Crystalline vitamin B_{12} in the form of a triturate of NaCl was kindly furnished by Dr. D. F. Green of the Veterinary Division, Merck and Co., Inc., Rahway, N. J.