in considerations of the target theory, insofar as they involve what appears to be the direct action of ionizing radiations. To date some consideration has been given to temperature (*4, 5*), but apparently no one has considered vacuum-dried material *vs.* freeze-dried material from the viewpoint of direct effects of ionizing radiations.

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Studies on Uracil Utilization in Normal and Acetaminofluorene-treated Rats[1]

Robert J. Rutman, A. Cantarow, Karl E. Paschkis, and Betty Allanoff[2, 3]

Department of Biochemistry* and *Division of Endocrine and Cancer Research, Jefferson Medical College, Philadelphia, Pennsylvania

Although there have been several recent reports of studies of uracil metabolism in lower organisms (*1, 2*), it has been generally assumed, on the basis of isotopic N^{15} studies (*3, 4*) that higher organisms cannot utilize exogenous pyrimidines for nucleic acid synthesis. Data reported recently from this laboratory (*5*), however, indicating that uracil reverses the inhibitory effect of thiouracil on the development of hepatoma in acetaminofluorene (AAF)-treated rats, suggested that the metabolism of uracil in this species may be altered under these circumstances.

Experiments were undertaken to test the possibility that uracil serves as a nutritional factor for tumor tissues. The preliminary results of these experiments demonstrate that, in contrast to normal liver, AAF-induced hepatoma tissue incorporates exogenous C^{14}-uracil into its nucleic acids (*6*) and, further, that the general metabolism of this pyrimidine is slightly accelerated in the tumor-bearing animal.

In a typical experiment, reported here, 20 mg 2-C^{14}-uracil,[4] containing 4×10^6 cpm, was injected intraperitoneally in the test animals (Wistar strain male rats, 300–350 g), normal and tumor-bearing (Table 1). The purity of the labeled compound was established by butanol-H_2O chromatography on paper (*7*); the eluted spot corresponded in R_F to recorded values and contained 95% of the anticipated radioactivity. The animals were sacrificed 3 hr after injection, respiratory CO_2 and total urine being collected during the experimental period. The liver (or hepatoma) was immediately homogenized in cold trichloracetic, and the nucleic acid was obtained from the lipid-free nucleoprotein by the Schneider procedure (*8*). The nucleic acids were freed of TCA by a combination of boiling and ether extraction, and assayed by direct plating. After assay the crude nucleic acids were hydrolyzed in HCl for 1 hr and subjected to ion exchange chromatography on a Dowex 1 formate column (*9*). The acid-soluble activity of the liver was assayed directly after ether extraction of the precipitant. Radioactivity of plasma and urine was determined on dried untreated aliquots of these materials; the respiratory C^{14} was assayed as $BaCO_3$. After extraction of the nucleic acids, the proteins were dissolved in 2 *N* KOH, reprecipitated and assayed by direct plating after solvent dehydration.

The gross distribution of radioactivty and that exhibited by liver components are shown in Table 1. Rapid catabolism of the uracil and excretion of the isotope occur in both normal and tumor-bearing rats, but are somewhat accelerated in the latter. The activity of the ureido-carbon in both normal and tumor-bearing animals requires further evaluation in relation to the formulation of urea as the major end product of pyrimidine metabolism (*10*).

In contrast to the relative inertness of uracil in normal rats, these data reveal considerable incorporation of the isotope into the hepatoma nucleic acids, paralleled by an increased isotope content in the acid-soluble constituents. The levels of isotope in the purified proteins suggest that the recycling of C^{14} may be due

TABLE 1

DISTRIBUTION OF URACIL C^{14} RADIOACTIVITY

	Normal rat*	Tumor-bearing rat†
	(% of dose)	
Respiratory CO_2	23	46
Urinary C^{14}	25	33
Total liver	0.1	2.2
Plasma	0.1	0.05
(extracellular water)	12	5‡
	Normal liver	*Hepatoma*
Acid-soluble§ (cpm)	3200	75,000
Crude nucleic acids (cpm/mg)	5.0	40
Protein (cpm/mg)	2.0	3.0

* Stock diet.

† 0.03% AAF incorporated in the stock diet; animal sacrificed after 10 months; frank hepatoma present.

‡ Based on 55% of body weight, assuming equilibration at the isotope level of the plasma.

§ C^{14} content/whole liver supernatant.

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[4] Synthesis of the C^{14}-uracil was carried out by Charles Miller, of Sharp & Dohme, North Wales, Pa., using C^{14}-urea supplied by Technical Associates, Glendale, Calif., under authorization by the AEC. We wish to express our grateful thanks to Dr. Miller and to Lemuel Wright, of Sharp & Dohme, for this assistance. We also wish to express thanks to Howard E. Skipper, of the Southern Research Institute, whose gift of labeled uracil made initial experiments possible.

to CO_2 fixation. This incorporation, conceivably by direct CO_2 fixation (*11*) or via pyrimidine precursors such as aspartic acid (*12*) and lactic acid (*13*), may be adequate to explain the isotope content of the nucleic acids of the normal liver but not of the hepatoma.

The conclusion that the uracil has entered into nucleotide formation is supported by the results of ion exchange fractionation. The Dowex I formate forecuts, containing the impure purine bases, exhibited activities of *ca.* 10–15 cpm/mg of base, whereas the activity of the main cytidylic and uridylic acid fractions was 170 and 230 cpm/mg of base, respectively. The absence of uptake into the purine bases and the approximate correspondence between observed pyrimidine nucleotide isotope content and that expected (*ca.* 250 cpm/mg base) on the basis of nucleic acid specific activity suggest a reasonably direct utilization of the uracil. The significance of the radioactivity present in other fractions awaits further purification.

On the basis of the detailed experiment reported, as well as of a number of preliminary tests, it appears probable that AAF carcinogenesis involves an alteration of the pattern of nucleic acid metabolism of the liver, one aspect of which involves the utilization of preformed uracil. Although the basic significance of this observation remains to be established, it appears likely that this metabolic alteration underlies the striking ability of thiouracil to inhibit hepatoma formation by AAF. The work of Kidder *et al.* (*14*) indicates that another nucleic acid antagonist, 8-azaguanine, depends for its inhibitory action (in *Tetrahymena geleii*) on the physiological state of the test organism.

Studies are in progress to determine whether the altered metabolism observed in the hepatoma is shared by other growing systems such as regenerating liver and embryonic tissues. The rapid catabolism of uracil has also indicated the desirability of investigating possible exchange reactions around the ureido carbon.

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Comments and Communications

Development of Strains of Albino Mice with Predictable Susceptibilities to Audiogenic Seizures[1]

LABORATORY mice have some advantages over rats for the study of audiogenic seizures. The level of incidence of seizures is highly uniform within specific strains (*1–5*), so that animals with known susceptibilities can be selected for experiments. The incidence of otitis media, which may be a complicating factor in rats (*6–8*), is very low (*9, 10*). The types of seizures and stages in their genesis have been described (*4, 11, 12*). Apparatus has been developed for the control of the stimulus situation (*4, 13*), so that duplication of this factor is assured. And, finally, mice require much less space for rearing than do rats.

A serious objection to the use of mice as test animals in these studies, however, has been that they die in clonic-tonic seizures (*1, 3–5, 10*). To overcome this difficulty and, at the same time, to reduce the variability in response of individual mice at specific ages (*4*), we have had in progress for over two years a program of selection and progeny-testing of mice for specific susceptibilities to audiogenic seizures. The strains thus produced have been developed from an originally random albino stock and are variously inbred and outbred. All records of seizures are available from the start of the selection, because no mice have been saved for breeding without routine testing. As these strains may prove of value to other research workers interested in this aspect of rodent physiology and psychology, the characteristics of the strains that have been produced are briefly described herein. Breeding pairs of any of these strains of mice will be supplied to interested individuals for testing on request, within the limits of our resources.

Mice of the first strain, when tested daily from 15 to 50 days of age, have a very high incidence (90–100%) of clonic-tonic seizures, but they rarely die in the seizures. In the original stock there was one death for every 1.5 clonic-tonic seizures. At present there is only one death in approximately 250 seizures. This figure is by no means the maximum, because the testing of mice for breeding is routinely discontinued at

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