Table 1. Influence of single and repeated doses of certain amino acids on uptake of 5-HTP by rat brain in vivo. Each treatment group contained at least six rats. For quantities of amino acids and 5-HTP administered, see text. Studies involving separation of indoles by column chromatography indicated that more than 80 percent of the total radioactivity in the brain could be accounted for by unchanged 5-HTP-1-C^{14}.

Amino acid administered before 5-HTP	Brain level of 5-HTP (%)	
	Parenteral administration of amino acid	Dietary administration of amino acid
None	100	100
L-Leucine	65	70
L-Tryptophan	50	
D-Tryptophan	97	
L-Phenylalanine	42	56
D-Phenylalanine	85	
L-Tyrosine		59
L-Phenylalanine plus L-tyrosine	57	56

blood at the same rate as it does in the normal rat, but after 8 minutes there is a striking retardation in the uptake of 5-HTP by the brain of the phenylketonuric rat.

It has been demonstrated in other laboratories (*4–6*), and also by us (*7*), that animals made phenylketonuric by appropriate diets have high circulating levels of phenylalanine and tyrosine. It is reasonable to conclude, therefore, that the high blood levels of the appropriate amino acids in our experiments excluded 5-HTP from the brain. Our data strongly suggest that this exclusion is attributable to competition with 5-HTP for the normal transport mechanism in brain. In view of the demonstration of high blood levels of phenylalanine in individuals with phenylpyruvic oligophrenia, it appears reasonable to expect that a similar mechanism of interference may operate in this disease with a consequent reduction of serotonin in cerebral cells.

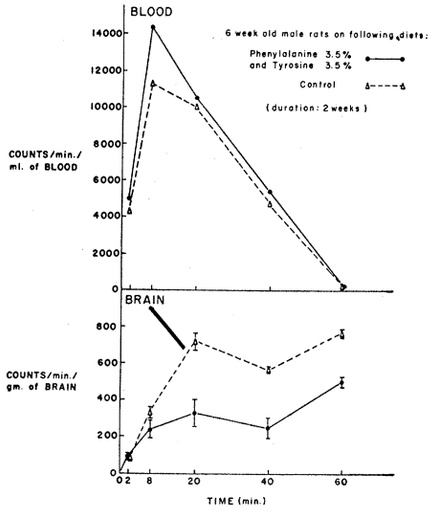

Fig. 1. Influence of chronic (2 weeks) feeding of phenylalanine and tyrosine on rate of appearance of 5-HTP-1-C^{14} in blood and brain after intraperitoneal administration of 2.5 μmole of the labeled compound.

While these experiments have not ruled out the possibility that the inhibition of tryptophan hydroxylase and of 5-HTP decarboxylase may contribute to the lowered levels of serotonin in the brain of the phenylketonuric rat, they do, by direct measurement, indicate that inhibition of the uptake of 5-HTP by the brain of such an animal is an important, if not primary, mechanism of the defect in serotonin metabolism. It is of some interest that the active transport of 5-HTP is not altered by α-methyldihydroxyphenylalanine (*8*), an inhibitor of 5-HTP decarboxylase. This would suggest that the uptake of 5-HTP by brain is independent of the activity of 5-HTP decarboxylase within the brain.

Christensen *et al.* (*10*), working with Ehrlich ascites tumor cells, first suggested that competitions may exist among amino acids for the carrier mechanism responsible for their uptake. Our data support this idea and suggest the general concept that abnormally high levels of one or more amino acids may result in a disturbance of the normal active transport of other amino acids across various anatomic barriers, such as the perivascular glial membrane, gastrointestinal mucosa, and renal tubular epithelium. Udenfriend (*11*) has recently proposed a similar concept, with respect to brain, based upon studies of the uptake of tyrosine by that organ.

Means of reversing the indole-disturbance reported above, within the framework of this general hypothesis, are now under consideration in our laboratory. Preliminary experiments (*7*) with young rats on phenylketogenic diets (described above), performing in a water maze, demonstrated that these animals were less proficient than controls, in their greater number of errors and slower performance. When blood levels of phenylalanine and tyrosine were lowered by supplementing the phenylketogenic diet with 5 percent tryptophan, presumably by competitive inhibition of the transport of phenylalanine across the intestinal mucosa, the biochemical and behavioral disturbances were reversed. The addition of 7 percent glucose to the phenylketogenic diet, while not appreciably lowering amino acid levels in the blood, showed an inconsistent and less striking but nevertheless significant improvement in both the biochemical and behavioral defects. This response to glucose supplementation is apparently through a different mechanism than that proposed for tryptophan (*12*).

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12. This work has been aided in part by USPHS grant B-940 and in part by the United Fund of Bristol.

4 May 1962

Chemical Effect of Ionizing Radiation on Cytosine

Abstract. The formation of uracil and the breakdown of the pyrimidine ring were observed when an aqueous solution of cytosine was irradiated. These two effects were investigated with the aid of radioactive tracer techniques and ultraviolet absorption studies.

In a further study of the radiation chemistry of nucleic acid constituents (*1, 2*) we have examined the chemical effect of ionizing radiation on cytosine.

In the nucleic acids, there are three pyrimidine bases—cytosine, uracil, and thymine. Uracil is found only in RNA, while thymine occurs only in DNA. Cytosine, however, is a constituent of both DNA and RNA and is the only aminopyrimidine there. In view of the observed deamination of adenine (*1*),

the chemical effect of ionizing radiation on this pyrimidine is, therefore, of special interest in a study of the radiation chemistry of nucleic acid constituents.

Several workers have reported the formation of hydroxyhydroperoxides from pyrimidines under the influence of radiation. Sinsheimer and Hastings (*3*) showed that the action of uv light on uracil in aqueous solution produces a well-defined photo-product which was thought to be 4-dihydro-5-hydroxyuracil. Scholes, Ward, and Weiss (*4*) have described the formation of hydroxyhydroperoxides when aqueous solutions of uracil and 1,3-dimethyluracil were irradiated with Co^{60} γ-rays or 200-kv x-rays in the presence of air. In the absence of oxygen, however, dimethyluracil gave 4-dihydro-5-hydroxy-1,3-dimethyluracil and the corresponding 4,5-glycol. Recently, Ekert and Monier (*5*) also demonstrated the formation of pyrimidine glycols when a solution of cytosine was irradiated with x-rays in the absence of oxygen.

The principal chemical effects of ionizing radiation on the purines adenine and guanine are 8-hydroxylation and the breakdown of the imidazole ring system (*6*). A small, but significant, amount of deamination was evident (*1*). Deamination and ring opening have now been observed when a 0.1 percent solution of the pyrimidine cytosine is irradiated at 10^6 to 20×10^6 rads in the absence of oxygen.

The breakdown of the pyrimidine ring. Cytosine-2-C^{14} of specific activity 45.6 μc/mg was supplied by Schwarz BioResearch, Inc., Mount Vernon, New York. Two hundred and fifty microliters of a 0.1 percent solution of cytosine in water were sealed under vacuum after dissolved oxygen was expelled by the bubbling of nitrogen through the solution. A 1.5-kc Co^{60} source was used for the irradiation. The intensity of radiation, determined by Fricke ferrous sulfate dosimetry (*7*), was 5×10^6 rad/hr. The solutions were irradiated at total doses from 10^6 to 20×10^6 rads.

Table 1. Gamma-radiation decomposition of 0.1 percent aqueous cytosine-2-C^{14}.

Exp. No.	Dose (10^6 rad)	Loss of volatile radioactivity (%)	Cytosine destroyed (%)	Uracil found (%)
Control	0	0.0	0.0	0.01
1	1	0.0	9.8	2.7
2	2	0.0	10.2	3.7
3	5	0.0	39.0	0.2
4	10	0.0	53.5	0.05
5	20	8.5	86.5	0.05

To estimate the amount of radioactivity lost on irradiation, probably volatile water-insoluble carbon-14 compounds, 25 μl of each of the irradiated solutions were diluted to 5 ml. Aliquots (100 μl) of this solution were measured by liquid scintillation counting using an internal standard of C^{14}-labeled toluene (*8*).

The residual cytosine was separated from the radiation decomposition products by two-dimensional paper chromatography, using Whatman No. 4 paper washed with oxalic acid. Propanol–ammonia–water (*9*) was used as a solvent in one direction, and butanol–propionic acid–water in the other (*10*). Twenty-five microliters of the irradiated solutions were spotted on each chromatogram. The distribution of radioactivity on the chromatogram was recorded by autoradiography with x-ray film. The amount of residual cytosine was estimated by eluting the cytosine spots with 0.1 percent formic acid and counting aliquots of the eluent. The results are shown in Table 1.

The effect of irradiation on the ultraviolet absorption of a 0.1 percent solution of cytosine is shown in Fig. 1. The measurements were made with a Cary recording spectrophotometer (model 11) at a *p*H of 7. The optical density at 268 mμ decreases with increasing radiation. This, however, is only an approximate indication of the breakdown of the pyrimidine ring as some of the radiation products may absorb at this wavelength. It is also evident that some disintegration products absorbing at a wavelength greater than 290 mμ are formed.

The results shown in Table 1 indicate that cytosine is less sensitive to ionizing radiation than the purines adenine and guanine. At a dose level of 20×10^6 rads, adenine was completely destroyed (*2*), and the loss of radioactivity as volatile material amounted to 46.8 percent. With cytosine, however, the loss of radioactivity, as volatile compounds, was only 8.5 percent, and 13.5 percent of the original material remained intact.

Deamination of cytosine. The deamination of adenine by ionizing radiation has already been reported (*1*). In our present investigation, the conversion of cytosine to uracil has been observed.

The irradiation products of cytosine-2-C^{14} were cochromatographed with nonradioactive uracil as the carrier. The uracil was detected in the chromatogram with the aid of shadowgrams (*11*). These were prepared by laying the chromatogram over Kodagraph contact photographic paper (14 by 17 inches) and shining an ultraviolet source (G.E. Hanovia lamp, 115 watts) over it for 10 seconds from a distance of 1 foot. The ultraviolet absorbing areas appeared as well-defined white spots on a dark background. The shadowgram was then matched with the autoradiograph of the same chromatogram. There was darkening of the x-ray film, corresponding to the uracil area of the shadow-

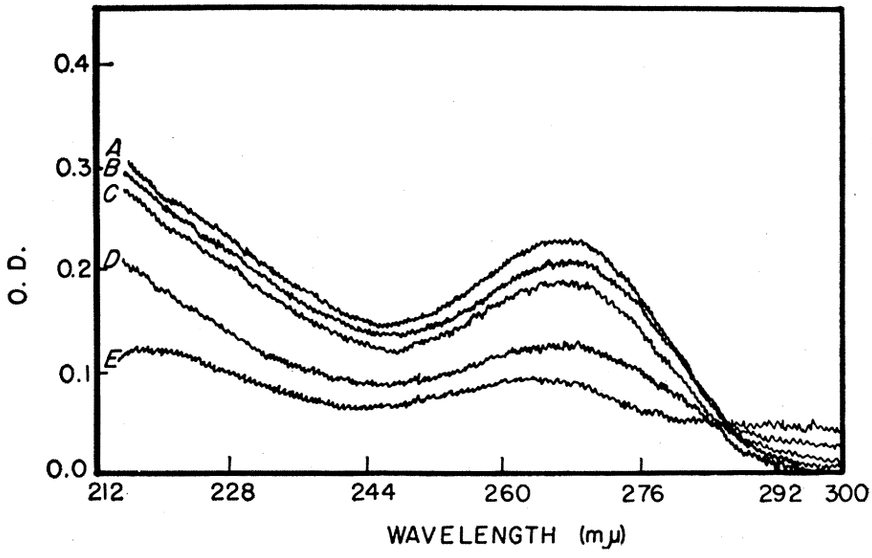

Fig. 1. Ultraviolet spectrum of irradiated cytosine at *p*H 7. *A*, unirradiated sample; *B*, 1×10^6 rads; *C*, 2×10^6 rads; *D*, 5×10^6 rads; and *E*, 20×10^6 rads. O.D., optical density.

gram. This coincidence was obtained with five different solvent systems: butanol–propionic acid–water (*10*); propanol–ammonia–water (*9*); butanol–formic acid–water (*11*); *n*-butanol (*12*); and water at *p*H 10 (*13*). The radioactivity could have come only from the cytosine, establishing the conversion of cytosine to uracil. The maximum yield of uracil (Table 1) is 3.7 percent at 2×10^6 rads. This is significantly larger than the maximum yield of hypoxanthine (1.9 percent at 5×10^6 rads) observed in the deamination of adenine (*1*).

The biological significance of the deamination of purines and pyrimidines has been widely recognized (*14*). Gierer and Mundry (*15*) have shown that nitrous acid treatment of tobacco mosaic virus RNA, or intact tobacco mosaic virus, actually results in the formation of mutants. The alteration of the amino-acid sequence in the protein of the mutant virus has indeed been strikingly demonstrated (*16*).

As cytosine occurs both in DNA and RNA, the conversion of cytosine to uracil may have more biological implications than the deamination of adenine. This change would give rise to an unnatural base in DNA and, if replication occurs, a cytosine-guanine base pair would ultimately be replaced by an adenine-thymine pair. With RNA, it is conceivable that the altered nucleic acid undergoes identical replication. In either case, an altered base would presumably lead to a change in protein composition (*17*, *18*).

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26 March 1962

Molecular Changes in Deoxyribonucleic Acid Induced by X-rays

Abstract. A chromatographic study of molecular changes in DNA after in vitro exposure to x-rays revealed little effect on the DNA macromolecule when either intact red cells of frog or DNA isolated from these cells was exposed to 1000 r. Increasing the dose to 10,000 r resulted in a significant shift from gradients of lower molecular weight to the higher gradients. These data suggest that cross-linking of DNA occurred at the higher radiation level.

Several lines of investigation have shown that the physical and chemical properties of deoxyribonucleic acid (DNA) are profoundly altered by radiation. Main chain scission (depolymerization) of the DNA molecule has been reported as an important action subsequent to ionizing radiation (*1*). Intra- and intermolecular cross-linking of DNA has been proposed as another basic effect of radiation. Alexander and Stacey (*2*) exposed sperm heads to ionizing radiation and observed cross-linking of the DNA. Recently these results were confirmed and extended (*3*). Borenfreund *et al.* (*4*) have reported both aggregation and degradation of DNA molecules from human leukocytes on exposure to tritium.

Our study (*5*) was directed at understanding more about the basic changes occurring in the DNA molecule in an isolated cell type on in vitro exposure to ionizing radiation. The standard procedure of ECTEOLA (*6*) anion exchange fractionation analysis was used as a sensitive indicator of physicochemical changes in the DNA macromolecule. Evidence is presented which suggests the possibility that cellular DNA may increase in molecular weight when it is exposed to ionizing radiation.

Blood was obtained by ventricular puncture of curarized bullfrogs (*Rana catesbiana*). In order to eliminate many of the errors in the isolation and fractionation of DNA, simultaneous experiments were performed under exactly the same conditions. Each experiment consisted of two aliquots of cells, a control sample and an x-irradiated sample with all preparatory and analytical procedures accomplished at 4°C. Blood samples or DNA isolates were exposed to ambient temperatures only during the radiation procedure. X-irradiation was performed at room temperature with either blood cells or the DNA solution layered 3 to 5 mm deep in a thin plastic dish. Samples were irradiated in an 85 kv (peak) x-ray unit with an "Aeromax" T12 tube at 10 ma. This unit gives a relatively "soft" beam with a half value layer of 1 mm Al. All doses were precalibrated with a Victoreen roentgen meter (*7*).

After x-irradiation of the experimental sample, paired experimental and control samples were centrifuged at low speed (115*g*) for 15 minutes. Buffy coat and plasma layers were carefully removed by pipetting. The packed erythrocytes (approximately 3 cm^3) were then transferred into vessels containing 50 ml of distilled water which caused hemolysis and resulted in the formation of a thick red gel. Precipitation and resolution of the deoxyribonucleoprotein, by the technique described by Mirsky (*8*), was repeated three times and yielded a clear white viscous precipitate free from discoloration by hemoglobin.

Protein was removed from the deoxyribonucleoprotein by chloroform-octanol treatment (*9*), which yielded a protein-free DNA solution as determined by the Weichselbaum biuret test (*10*). The fractionation pattern obtained depended in part on the length of treatment. In two early experiments at 1000 r (Fig. 1, *A* and *B*) an increased amount of DNA of lower molecular weight (gradient 2) was obtained after 22 hours of treatment. Subsequently, it was found that approximately 8 hours of treatment gave a more uniform and reproducible product. In all subsequent experiments deproteinization was completed within 5 to 8 hours.

After deproteinization and centrifugation (1020*g*), DNA was precipitated