quantitatively by the method described above. For a usual Beckman cell an amount readily obtained from a chromatographic strip (0.25 to 0.50 mg) is adequate for a reliable determination (*5*).

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Deamination of Adenine by Ionizing Radiation

Abstract. A small amount of hypoxanthine is formed when a solution of adenine is irradiated. This has been detected by using C^{14}-labeled adenine and the techniques of paper chromatography and liquid-scintillation counting. The biological significance of this conversion is suggested.

Deamination takes place quite readily when an aminopurine or aminopyrimidine is hydrolyzed with acid (*1*). Ten-percent hydrochloric acid converts cytosine into uracil (*2*). Nitrous acid deaminates adenosine to inosine (*3*) and guanosine to xanthosine (*4*). A similar conversion takes place enzymatically. Intestinal deaminases, for example, convert adenine to hypoxanthine, adenosine to inosine, and adenylic acid to inosinic acid (*5*). The ultraviolet irradiation of adenine solutions has been reported to give trace quantities of hypoxanthine (*6*).

Previous workers had concluded that deamination of this type probably did not take place under the influence of ionizing radiation (*7*). In our studies of the radiation decomposition of nucleotides and related compounds, however, we have found that adenine is converted into hypoxanthine. The yield is small, but significant.

About 250 μl of a 0.1 percent solution of adenine-2-C^{14} (specific activity, 1.3 mc/mmole) were sealed in a vacuum (*8*). To expel any dissolved oxygen, a steady stream of N_2 was bubbled through the solution for about 10 min before the sample was sealed.

A 1.5 kc cobalt-60 source was used for the irradiation. The dosage was calculated by means of the Fricke ferrous sulfate dosimeter. The radiation intensity was 5×10^6 rad/hr.

The irradiation products were analyzed by paper chromatography, with Whatman No. 4 paper and propanol–ammonia–water and butanol–propionic acid–water as solvents (*9*). Twenty-five microliters of the irradiated solutions of adenine were spotted on paper together with 10 μl of 0.1 percent inactive hypoxanthine as carrier. The ultraviolet-absorbing areas were carefully marked out. Autoradiography with x-ray films showed darkening of the film corresponding to the hypoxanthine spot. The radioactivity could have come only from the adenine, showing the conversion of adenine to hypoxanthine. The chromatography of the control was done at the same time as that of the irradiated samples. Thus, bacterial deamination could have been, at most, a minor effect.

In one experiment, the hypoxanthine spot was eluted with 0.1 percent formic acid and rechromatographed with fresh carrier hypoxanthine, with butanol–water (*10*) and isobutyric acid–ammonia–ethylene diaminetetraacetic acid (*11*) as solvents. The radioactivity was concentrated on the hypoxanthine spot, which confirmed the formation of radioactive hypoxanthine.

The hypoxanthine spots were eluted with 0.1 percent formic acid and counted with a liquid scintillator having an internal standard of C^{14}-labeled toluene. The same technique was used for the estimation of residual adenine. The results of the experiments are shown in Table 1.

The biological importance of a deamination of this type may be very great. If it would be legitimate to extrapolate from a high dose level to a low dose level, we may have a possible clue to the origin of a radiation mutation. When the ribonucleic acid of tobacco mosaic virus was heated with HNO_2, the amino bases were converted to the hydroxy bases without splitting of the nucleotide chain. Deamination of a large number of nucleotides resulted in the inactivation of the molecule (*12*). More gentle treatment by controlled action of HNO_2 gave a maximum number of mutations when an average of 1 deamination resulted per 6000 nucleotides (*13*).

Table 1. Formation of hypoxanthine from adenine by ionizing radiation. In all experiments the initial activity was 4.1×10^5 disintegrations per minute (dpm).

Expt.	Dose (10^6 rad)	Residual adenine (%)	Hypoxanthine	
			Act. (dpm)	Amt. (%)
Control	0	100	330	0.08
1	1	87.7	3602	0.88
2	2	75.4	3807	0.98
3	5	60.1	7840	1.9
4	10	44.4	3839	0.9

Because hypoxanthine, xanthine, and uracil normally exist in the keto form (*14*), the deamination of adenine to hypoxanthine will result in the disruption of the hydrogen bonding between adenine and thymine, suggested by the Watson and Crick structure of deoxyribonucleic acid (*15*). From theoretical considerations Lavalle (*16*) has pointed out that deamination of adenine would result in the replacement of an adenine-thymine base pair by a guanine-cytosine pair, altering the molecular code of heredity (*17*).

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