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Thermal Protection of Choline Chloride from Decomposition by Ionizing Radiation

Changes produced by ionizing radiations in target materials—for example, polymerization of vinyl monomers (*1*) and inactivation of enzymes (*2*)—are in general enhanced by temperature elevation. It appears, however, that choline chloride, which at room temperature is one of the most radiosensitive organic solids known (*3*), becomes markedly radiation-resistant at 150°C.

Studies have been carried out at our laboratory (*4*) on the effects of ionizing radiation on nerve tissue constituents, including the cholinesterase system (*5*). Choline, a substance essential to nerve conduction, has been shown (*3*) to decompose by a free radical chain mechanism to trimethylamine and acetaldehyde when it is subjected to ionizing radiation as the pure crystalline chloride. When exposed at room temperature to 2-Mev electrons, Co^{60} γ-rays, or C^{14} β-rays, the *G* values—that is, the number of molecules decomposed per 100 ev—were 20, 175, and 1250 respectively; at −196°C the compound was stable.

In an attempt to determine the energy of activation of the radiation decomposition of choline chloride, the crystalline compound was exposed to Co^{60} γ-rays at room temperature, 50°C, and 150°C. At each temperature, three Pyrex ampules of twice recrystallized choline chloride, dried under vacuum and nitrogen at 110°C for 2 hours and vacuum sealed, were exposed to the radiation for varying periods of time. Three Co^{60} sources were used, two of which (delivering 232,000 and 792,000 rep/hr) were maintained at room temperature; the third (572,000 rep/hr) had a normal operating temperature of 50°C and was equipped with a furnace for higher temperatures. The percentage of remaining choline in each of the irradiated samples and its control was determined by the reineckate method (*6*). The *G* values, which are listed in Table 1, were then calculated for each run from the obtained semilogarithmic relationship between the percentage of remaining choline and the radiation dose in rep.

Decreasing the radiation dose rate or increasing the temperature from 20° to 50°C resulted in higher yields of decomposition. However, at 150°, regardless of the radiation exposure (4.6 to 13.7×10^6 rep) only 9 to 13 percent of the choline decomposed. The nonirradiated control, which was also kept at 150°C, did not change in appearance. The irradiated samples, however, became brown, and a small amount of insoluble material formed.

In view of these changes, it was necessary to determine whether more than one compound was responsible for the very high choline recovery as indicated by the reineckate analyses. Cholinemethyl-C^{14} chloride was synthesized (*3*) and recrystallized twice. The product had a specific activity of 66.5 mμc/mg of choline chloride (*7*) (calculated, 62.5 mμc/mg). It was shown to be chromatographically pure (Whatman No. 1 paper and 4/1/1 *n*-butanol, concentrated HCl, and water, followed by autoradiography). The labeled choline was irradiated at 150°C in the same manner as the nonlabeled material. Once again, some brown and insoluble substances were formed.

Analysis of the soluble material by the reineckate procedure indicated that the choline recoveries following exposure to 1.4, 2.8, and 3.7×10^7 rep were 94, 94, and 93 percent, respectively (*8*). Solutions of the sample which had been irradiated for 64.5 hours at 150°C, and of the labeled control, which also had been held at 150°C for the same length of time, were chromatographed for 23 hours. Included on the same chromatogram were samples of C^{14}-labeled trimethylamine and nonheated, nonirradiated, labeled choline. The heated and nonheated control samples showed a single spot only. The irradiated sample activity was predominantly at the same R_f as the choline controls with only a faint trace (about 1 percent) at a slightly higher R_f. Apparently, therefore, no C^{14}-labeled compound other than choline had contributed to the color developed in the analytic procedure.

It might be assumed that the brown, irradiation-induced materials had acted as inhibitors of the free radical chain degradation of the remaining choline. If this were the case, one would expect that this type of inhibition should have been evident not only at 150°C but also, to some degree, at lower temperatures.

Lemmon (*3*) has suggested that the spatial arrangement of atoms of crystalline choline chloride may play an important role in the free radical chain degradation. One might speculate that thermal excitation at 150°C (in contrast to that at lower temperatures or to the excitation due to ionizing radiation per se) can disturb the arrangement sufficiently to prevent the chain reaction. Further studies, therefore, may help to determine the relationship of crystalline structure to free radical chain reactions in solids, as well as to establish the use of elevated temperatures to protect some labile materials during irradiation.

Table 1. Effect of temperature and dose rate on Co^{60} γ-ray decomposition of choline.

Co^{60} source (rep/hr)	Temp. of irradiation (°C)	Dose causing 50% decompn. (reps)	G values
792,000	18–20	2.9×10^7	143
232,000	20–25	1.0×10^7	415
572,000	50	0.8×10^7	520
572,000	150	*	

* Regardless of the dose—that is, from 0.5 to 3.7×10^7 rep.—approximately only 10 percent of the choline decomposed.

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8. Since the furnace in this Co^{60} source required approximately 15 minutes to either heat up or cool down from 150°C after the addition or removal of a sample, it is possible that the observed small choline losses were initiated in these periods.

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Segregation of Plasmagenes and the Determination Problem

Investigations on the willow-herb (*Epilobium*) have shown that the intra-individual segregation of plasmagenes is a basic character of cytoplasmic inheritance (*1*). During vegetative cell divisions the plasmagenes may be distributed accidentally. They may, however, enter more or less exclusively one of the daughter cells as well. In such a way differences of cells and characteristic patterns arise within the plant. Besides the cytoplasmic segregation occurring in