ference in staining quality, as compared with control preparations. This type of change is sometimes associated with decreased robustness of cells under unfavorable culture conditions.

When the Schmidt-Ruppin virus was concentrated by ultracentrifugation so that 2×10^6 focus-forming units (FFU) of virus were added to the culture containing approximately 2.25×10^6 cells, there was mitotic inhibition to a degree that prevented chromosome analysis. On two occasions serial titrations of the leukocyte culture fluids after virus addition revealed no evidence of virus multiplication.

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References and Notes

1. C. G. Ahlström, N. Jonsson, N. Forsby, *Trans. Scand. Congr. Pathol. Microbiol. 13th, Turku*, 127 (1962).
2. J. S. Munroe and W. F. Windle, *Science* **140**, 1415 (1963).
3. W. W. Nichols, *Hereditas* **50**, 53 (1963).
4. P. C. Nowell, *Cancer Res.* **20**, 462 (1960).
5. M. A. Bender and D. M. Prescott, *Exptl. Cell Res.* **27**, 221 (1962).
6. B. A. Kihlman, W. W. Nichols, A. Levan, *Hereditas* **50**, 139 (1963).
7. P. S. Moorhead, P. C. Nowell, W. J. Mellman, D. M. Battips, D. A. Hungerford, *Exptl. Cell Res.* **20**, 613 (1960).
8. V. Groupe, V. Dunkel, R. A. Manaker, *J. Bacteriol.* **74**, 409 (1957).
9. H. M. Temin and H. Rubin, *Virology* **6**, 669 (1959); H. Rubin, *ibid.* **10**, 29 (1960).
10. G. Östergren and T. Wakonig, *Botan. Notiser*, p. 357 (1954).
11. This idea that the weak points are broken during the spiralization movements on chromosome contraction is an unpublished interpretation by Östergren.
12. We thank M. Peluse for technical assistance, and Mary Federico for help with the photography. Supported by grants CA-03845, 06415, and 4953 and research career development award RC-17-64 from NIH, and by the Swedish Cancer Society and the Swedish Medical and Natural Sciences Research Council.

9 July 1964

Radiation-Chemical Oxidation of Peptides in the Solid State

Abstract. *Gamma-ray irradiation of polypeptides as highly dispersed fluffs under oxygen leads to chemical degradation of the peptide bond with the remarkably high oxygen consumption of about one molecule per 2 ev of absorbed energy. A radical chain mechanism appears to be involved, and there is evidence that excited states of the polypeptide aggregate undergo chemical quenching by molecular oxygen.*

The radiation-induced destruction of amino acids in solid proteins has been described by Alexander and Hamilton (*1*) and by Bowes and Moss (*2*) as not being significantly greater under oxygen than in a vacuum. However, neither of these studies was undertaken to determine optimum conditions for radiation-chemical reaction in a heterogeneous peptide-oxygen system; in the one study the dose rate was confined to the relatively high value of about 5×10^{21} ev g^{-1} min^{-1}, and in the other the solids were irradiated in a nondispersed form. We have, therefore, re-examined this question by irradiating —as highly dispersed fluffs (*3*)—a polypeptide, gelatin, and a polyamino acid, poly-D,L-alanine, in a vacuum and in an oxygen atmosphere under γ-rays at the relatively low dose rate of 1×10^{16} ev g^{-1} min^{-1}.

The very striking results are the remarkably high G values (see Table 1) for oxygen uptake and for oxidative degradation of both gelatin and poly-D,L-alanine. Chemical analysis of the irradiated peptides after a 3-hour period of hydrolysis in 2N hydrochloric acid established that ammonia and α-ketoacid or acids were principal degradation products. The quantitative procedures were those developed in our previous studies of the radiolytic oxidation of polypeptides in aqueous solution (*4*). Oxygen uptake and carbon dioxide production were followed by mass spectrometry. The observed $G(-O_2)$ values correspond to an energy requirement of only 1.5 and 2.0 ev per molecule of oxygen removed by gelatin and poly-D-L-alanine, respectively.

The magnitude of these G values prompted us to give particular attention to the quantitative basis of such reactions. Dose is determined by extrapolation on the basis of the ratio of the electron densities of the solids and the Fricke dosimeter (*5*). The validity of the extrapolation for the low-density solids and for the geometries used here was established experimentally in a series of control experiments in which the density and the volume of the irradiated samples were varied from about 0.025 to 0.6 g/cm^3 and from 1 to 80 cm^3, respectively. The possibility that not all of the observed chemical change arises as a result of the direct action of the radiation on the polypeptides was also examined in some detail. The fact that the degradation yields in oxygen are independent of pressure from 650 mm down to 20 mm as shown in Table 2 indicates that energy absorption by oxygen is not a major contributing factor. At 20 mm pressure, the oxygen in the gas phase plus that absorbed on the solid peptide represents less than 1 percent of the mass of the total sample (*6*). The possibility that part of the energy absorbed by the pyrex cell is transferred to the peptide through excited states of oxygen is negated by the observed pressure independence and by the observation that addition of a glass-fiber plug above the peptide sample does not result in any change in the degradation yields. There is also the question whether or not the amide and carbonyl yields given in Table 1 include some contributions from "dark reactions" induced in the hydrolysis. Both peroxides and carbonyl compounds, for example, are known to degrade amino acids by way of the Strecker reaction, with the formation of ammonia under certain con-

Table 1. Product yields in the γ-radiolysis of gelatin and poly-D,L-alanine in the solid state. *A*, irradiated in a vacuum and then exposed to oxygen prior to chemical manipulation. *B*, irradiated in oxygen at a pressure of 50 mm-Hg. The dose was 1.44×10^{20} ev/g. G = molecules per 100 ev absorbed energy.

	G-values			
	$>C=O$	NH_3	CO_2	$-O_2$
	A. Evacuated			
Gelatin	2.3 ± 0.3	8.1 ± 0.5	0.63 ± 0.03	
Poly-D,L-alanine	4.8 ± .3	5.6 ± .3	.41 ± .01	
	B. Oxygen atmosphere			
Gelatin	17.9 ± 0.9	42.1 ± 1.8	15.7 ± 3.0	72.7 ± 0.5
Poly-D,L-alanine	24.3 ± .7	30.9 ± 1.7	33.8 ± .5	52.3 ± .9

Table 2. Effect of oxygen pressure on product yields in the γ-radiolysis of gelatin. The dose was 1.44×10^{20} ev/g. G is the number of molecules per 100 ev absorbed energy.

Pressure (mm-Hg)	G-value		
	>C=O	NH_3	$-O_2$
20	17.9 ± 0.9	42.1 ± 1.8	70.8
50	18.8 ± 0.9	42.5 ± 2.0	72.4 ± 0.5
600	16.4 ± 1.0	40.0 ± 2.5	

ditions (*7*). We found, however, in a series of controls that added hydrogen peroxide, organic peroxides, and carbonyl compounds (in amounts corresponding to G of about 40) do not lead to oxidation of the peptide chain under the conditions of acid hydrolysis employed. We also found that treatment of the irradiated polypeptide with 0.1N Na_2SO_3 for the purpose of destroying peroxides prior to hydrolysis does not lead to any diminution in the yields of product. These findings are in accord with earlier observations (*4*) on the negative role of dark reactions in the chemical determination of radiation-induced oxidation in aqueous peptide systems.

Through the use of molecular oxygen and other radical scavengers it has been established that the energy required to produce a chemically detectable radical pair in the γ-radiolysis of organic substances in a condensed phase at room temperature is ordinarily greater than about 15 ev (*8*). It is apparent then that the removal of oxygen at an energy expenditure of only 1.5 to 2.0 ev per molecule as observed implies that a radical chain oxidation is involved or that low-lying excited states are produced, and that such states are chemically quenched by molecular oxygen. The production of ammonia and carbonyl products in the radiolysis of gelatin and polyalanine under oxygen can be formulated as a chain oxidation in which the C—H bond in the α position to the peptide group represents the locus of chain propagation. The preliminary stages of the reaction involve the radiation-induced step (*9*).

$$RCONHCHR_2 \xrightarrow{\sim\sim\sim} RCO\dot{N}H + \dot{C}HR_2 \quad (1)$$

followed immediately by the hydrogen abstraction reactions

$$RCO\dot{N}H + RCONHCHR_2 \longrightarrow RCONH_2 + RCONH\dot{C}R_2 \quad (2)$$

$$CHR_2 + RCONHCHR_2 \longrightarrow CH_2R_2 + RCONH\dot{C}R_2 \quad (3)$$

where $RCONH\dot{C}R_2$ corresponds to the stable long-lived radical species detected in irradiated peptides at room temperature by electron spin resonance methods (*10*). Reactions 2 and 3 are characteristic of the common susceptibility of peptides to radical attack at the α-carbon position (*4*):

$$R' + RCONHCHR_2 \longrightarrow R'H + RCONH\dot{C}R_2 \quad (4)$$

The peptide radicals react with oxygen to form peroxy radicals by way of

$$O_2 + RCONH\dot{C}R_2 \longrightarrow RCONHC(O_2)R_2 \quad (5)$$

or

$$O_2 + RCONH\dot{C}R_2 \longrightarrow RCON{=}CR_2 + HO_2 \quad (6)$$

and further reaction of the radical products of reactions 5 and 6 with the labile C—H bond of the peptide molecule would then lead to chain propagation and, after dissolution, to formation of ammonia and carbonyl products.

$$RCONHC(OOH)R_2 + 2H_2O \longrightarrow RCOOH + NH_3 + R_2CO + H_2O_2 \quad (7)$$

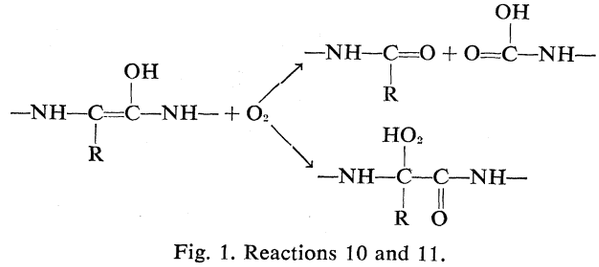
$$RCON{=}CR_2 + 2H_2O \longrightarrow RCOOH + NH_3 + R_2CO \quad (8)$$

The terminating step of the oxidation chain would be of the type

$$RCONHC(\dot{O}_2)R_2 + HO_2 \longrightarrow RCONHC(OOH)R_2 + O_2 \quad (9)$$

From the G values for ammonia and carbonyl given in Table 1, Expt. A, it appears that the stable long-lived $RCONH\dot{C}R_2$ radicals in gelatin and poly-D,L-alanine after radiolysis in a vacuum do not initiate chain oxidation during the exposure to oxygen after irradiation. On the basis of the radical chain mechanism discussed here, the explanation seems to lie in the fact that the instantaneous concentration of peroxy radicals produced on exposure of the already irradiated peptides to oxygen under condition A is very much higher than under condition B, in which the peptide radicals are produced in the presence of oxygen over an irradiation period of some 40 hours. Hence under condition A the peroxy radicals are preferentially removed by way of the radical-radical interactions that are operative as the chain-terminating step under condition B.

While a radical chain mechanism does appear to explain satisfactorily the yields of ammonia and carbonyl function observed in the radiolysis of polypeptide fluffs under oxygen, we have been unable on the basis of a chain mechanism to account for the production of carbon dioxide as a major degradation product. In fact, the very preferential effect (Table 1) of an oxygen atmosphere on yields of carbon dioxide suggests that the formation of this product occurs by some other mechanism. It has already been proposed (*11*) that delocalization of the π electrons of the extended hydrogen bond system of polypeptides gives rise to excited states of the peptide aggregate and that these excited levels are 1.5 to 3 ev above the ground state. Mason (*12*) treats π-electron delocalization in peptides as a triplet-state excitation which is assumed to involve enolization of the peptide bond, that is, —C(OH)=N— or —C(R)=C(OH)—NH— or both. Such states would not lead to net chemical change in the radiolysis of peptides in a vacuum. However, in the presence of molecular oxygen we envisage a chemical quenching of the triplet excited state according to reactions 10 and 11 (Fig. 1), where carbon dioxide is derived from reaction 10 by way of HOOC—NH— $\longrightarrow$ CO_2+NH_2—, and where reaction 11 provides an alternate path for production of ammonia and carbonyl products. Reaction of excited states by way of 10 and 11 would be expected to have a much lower temperature coefficient than the chain propagating step 4 (*13*). Further studies of the effects of temperature on product yields should provide a basis for determining the relative importance of radicals and excited



(10)

(11)

Fig. 1. Reactions 10 and 11.

states in the radiation-induced oxidation of solid peptides which on the basis of the present work appears to have interesting implications both in radiation chemistry and in radiation biology (*14*).

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References and Notes

1. P. Alexander and L. D. G. Hamilton, *Radiation Res.* **13**, 214 (1960).
2. J. H. Bowes and J. A. Moss, *ibid.* **16**, 211 (1962).
3. One percent solutions of gelatin (Eastman, lime processed) and poly-D,L-alanine (Yeda, M. W. 2000) in water and glacial formic acid, respectively, were dialyzed against distilled water, quick-frozen in liquid nitrogen, and vacuum-dried at about —5°C.
4. W. M. Garrison, M. E. Jayko, W. Bennett, *Radiation Res.* **16**, 483 (1962).
5. C. J. Hochanadel and J. A. Ghormley, *J. Chem. Phys.* **21**, 880 (1953).
6. S. W. Benson and D. A. Ellis, *J. Am. Chem. Soc.* **70**, 3563 (1948).
7. A. Schonberg and R. Moubacher, *Chem. Rev.* **50**, 261 (1952).
8. A. J. Swallow, *Radiation Chemistry of Organic Compounds* (Pergamon, London, 1960).
9. W. M. Garrison and B. M. Weeks, *Radiation Res.* **17**, 341 (1962).
10. R. C. Drew and W. Gordy, *ibid.* **18**, 552 (1963); T. Henriksen, T. Sanner, A. Pihl, *ibid.*, p. 147.
11. A. Szent-Györgi, *Nature* **148**, 157 (1941); M. G. Evans and J. Gergely, *Biochim. Biophys. Acta* **3**, 188 (1949); N. Riehl, *Naturwiss.* **43**, 145 (1956).
12. R. Mason *Discussions Faraday Soc.* **7**, 129 (1959).
13. See, for exam → W. Albert Noyes, Jr., *Radiation Res. Suppl.* **1**, 164 (1959).
14. High values for oxygen uptake under γ-radiolysis have also been observed with other highly dispersed macromolecular structures. Of particular interest is the finding that solid DNA obtained on freeze-drying a 1 percent solution gives $G(-O_2)$ about 40. Oxygen uptake by DNA is remarkably sensitive to the degree of dispersion of the solids; material prepared by freeze-drying a several-percent solution showed $G(-O_2) < 10$.
15. Work performed under the auspices of the U.S. Atomic Energy Commission.

4 May 1964

Chromosome Aberrations: Their Role in the Etiology of Murine Leukemia

Abstract. *If the association between chromosome aberrations and leukemia is a causal one, the aberrations should be present before the appearance of tumor. In a virus-induced murine leukemia in which the pre- and early leukemic stages were defined, aneuploidy was observed only during the later stages of the disease. This suggests that the chromosome alteration results from, rather than initiates, the neoplastic transformation.*

Since the speculations of Boveri in 1914 (*1*) there has been abundant demonstration of the association between chromosome alteration and neoplasia. Chromosome aberrations in solid and ascites tumors in vitro and in vivo, in a variety of species including man, have been extensively reviewed (*2*). The suitability of the leukemias as a model system stimulated numerous studies on chromosome abnormalities or their lack in human leukemia as well as those induced in animals by viral, chemical, and physical agents. Despite these efforts, reports to date do not conclusively establish a causal relationship between chromosome alterations and neoplasia. Proof for a causal hypothesis in opposition to chromosome alterations which occur as a result of neoplastic transformation would require the presence of these alterations during the earliest pre-neoplastic stage, before the histologic appearance of tumor (*3*).

The virus-induced murine leukemias offer a convenient model system for a test of the "causal" hypothesis. The system provides a transition from the normal to the neoplastic state under controlled conditions with a reproducible interval between inoculation and the appearance of histologically recognizable tumor. Recently we described a potent murine leukemia virus which induces lymphoid leukemia in 80 to 100 percent of the inoculated animals within 50 to 70 days (*4*). This thymic lymphoma is a rapidly proliferating, undifferentiated tumor histologically similar to the other murine lymphomas. The development of this thymic lymphoma is not preceded by an early erythroblastic splenic response as in Friend (*5*) and Rauscher leukemia (*6*). The increased incidence of secondary chromosome constrictions which we observed (*7*) during the early splenic phase of Friend and Rauscher leukemia was not observed in the disease initiated by our agent.

The appearance of thymic, and subsequently generalized lymphoma in the system reported here, was accompanied by aneuploidy with a predominantly hyperdiploid mode (*8*) similar to that observed during the later, lymphomatous phase of Rauscher leukemia. No characteristic "marker" chromosomes were observed in the leukemia induced by our agent.

During a detailed study of the pathogenesis of this leukemia (*9*), tumor evolution occurred in only one of the two paired thymus glands. The appearance of histologically detectable, proliferating tumor was consistently preceded by unilateral depletion of thymic lymphocytes and by an intermediate preleukemic histological alteration which we termed "lymphoma *in situ*." It was thus possible in this system to study the incidence of chromosomal aberrations at various stages of neoplastic transformation in tissue whose ultimate neoplastic fate had been predetermined.

Newborn (less than 24 hours old) Swiss HaICR mice were inoculated with 0.05 ml of the leukemia virus stock (*9*). At regular intervals thereafter beginning at day 22 the animals were injected with colchicine (*10*). The mice were autopsied and examined thoroughly according to the procedure previously described (*9*, *11*). The right and left thymus were removed for studies of the chromosomes (*12*). In this way, chromosome characteristics could be directly correlated with the stage of leukemogenesis in specific animals. The chromosomes were prepared directly, without being cultured (*7*).

In accord with observations in previous studies of the pathogenesis of this and other virus-induced leukemias (see *9*, *11*), the mice were divided into four groups depending on the stage of the leukemogenic process (Fig. 1).

1) No gross or microscopic pathology: This group included inoculated animals which showed no pathologic changes at the time they were killed.

2) Unilateral thymus depletion: Mice in which one thymus (right or left) showed more than a 30 percent loss of weight relative to the other thymus. The weight loss was reflected by the histologic evidence of unilateral lymphocyte depletion. No evidence of lymphoma or abnormal cell proliferation was observed in this group. Those depleted thymuses which histologically suggested lymphoma but did not fulfill the other criteria for malignancy were also included in this group.

3) Unilateral lymphoma: Animals in which overt lymphoma could be detected in one thymus. In addition to increased cell size, nuclear staining,