vity. The effect of oxygen may be due to: (a) a participation of O_2 in X-ray induced chemical reactions (if so, indicating an indirect effect of the radiation) or (b) an alteration of the metabolic rate *per se*. Probably both effects must be considered. Dormant seeds are much less sensitive to a change in the oxygen pressure, probably due to the very small and slow alteration of the metabolic rate. Other possibilities which have to be considered are however: a slow penetration of oxygen into or out of the dry seeds, or the absence of participation of oxygen in the chemical reactions induced in dormant seeds.

Seeds are 20–30 times more sensitive to fast neutrons than to X-rays, when equal doses are given. The difference between the radiation sensitivities to neutrons of dormant and germinating seeds is 2–3 times less than the corresponding difference in the case of X-rays. Hydrogen sulphide does not seem to protect against radiation damage caused by fast neutrons (compare Figs. 1 and 2 which show the effects of X-rays and neutrons, respectively, on the growth rate of hydrogen sulphide treated plants). Since, further, the oxygen pressure exerts a much smaller, if any, influence on the neutron sensitivity of germinating seeds, a fundamentally different mechanism of action of neutrons compared to that of X-rays has to be supposed, *i.e.* in the case of neutron irradiation the direct action of the radiation dominates. Such direct effects cannot be influenced by chemical and physiological factors in the same way as part of the X-ray effects. The difference in behaviour is probably related to the ionization densities produced in tissue (neutrons and X-rays give about 700 and 100 ion pairs per μ respectively). The field appearance of the damage caused by the two kinds of radiation is different, too: in the case of X-rays, plants not killed at a very early stage of development, survive as a rule, eliminating cells with severely damaged nuclei (chromosomes).

In the case of neutrons, on the other hand, plants often germinate and grow normally for the first few days but then suddenly die. The elimination of chromosome damaged cells is much less marked. This difference leads to a much higher sterilizing and mutagenic action of the neutrons, determined in an analysis of the mature plants.

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Attempted Successive Applications of the Edman Degradation to Insulin

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The Fraenkel-Conrats¹ converted insulin to its phenylthiocarbamide derivative, and effected the removal of the N-terminal amino acids as the hydantoins by heating in 0.6 to 1.2 *N* hydrochloric acid at 75°. This adaptation of the Edman degradation² yielded the hydantoins of glycine, phenylalanine and alanine. Subsequent reapplications of the procedure were reported to release unincreased quantities of thiohydantoins, indicating that probably the vicinal peptide links only had been broken. A further adaptation of the procedure by Ottesen and Wollenberger³, in which the acidity was reduced to 0.1 *N*, permitted the systematic degradation of hexapeptide, with, however, one extra peptide link being split during the second round. The modified procedure has now been applied to insulin. As a result of the first application, the number of free amino

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Degr. No.	Dissociat. Groups Reacted	Hydantoin Extracted	Amino Acids Expected	Amino Acids Found			
	M/12000 g	M/12000 g		Much	Moderate	Small	Trace
1	4.85	3.9	Glycine, Phenylalanine	Glycine	Phenylalanine		
2	14*	(6.2) **	Isoleucine, Valine	Isoleucine or Leucine, Valine	Glycine		
3	9.0	6.2	Valine, Aspartic acid	Valine, Aspartic acid		Glycine, Alanine	Leucine or Isoleucine
4	7.3	6.1	Glutamic acid	Glutamic acid		Glycine, Alanine, Leucine or Isoleucine	Aspartic acid, Valine
5	9.4	7.3	Glutamic acid	Glutamic acid, Alanine	Glycine, Valine, Leucine or Isoleucine	Aspartic acid	

* Including 6 moles of ammonia.

** Analysis unsuccessful. This value taken from a parallel experiment.

groups was approximately doubled, and several amide links were split. Glycine and phenylalanine, but not alanine, were obtained in the first degradation; the expected products appeared through 4 more degradations, but extra amino acids gradually became prominent and numerous. Further diminution of the acidity to pH 2 resulted in excessively slow degradation.

The procedure was applied to solutions of insulin in guanidine chloride solution¹ and followed essentially according to Ottesen and Wollenberger³. The extent of reaction with phenyl isothiocyanate at pH 8 was taken as the sum of the hydrogen ions released during the reaction plus the loss of groups dissociating between pH 5.6 and 8.0, as shown by titration before and after the reaction. Extractions of hydantoin by ether were carried out at about pH 3; during degradations 3 and 5, the extractions were repeated at pH 8.3. Inappreciable amounts of additional hydantoin were obtained at the higher pH. Pure

histidine yielded its hydantoin at pH 8. The observed extra degradation products suggested that the extra cleavages involved some of the amino groups of hydroxyamino acids, but the products could not be simply explained.

The results are summarized in the Table.

Except for the first 2 degradations, each stage was marked by a regular alternation in the position of the titration curve of the protein material indicating the orderly progress of the degradation procedure. The conditions of the cleavage cannot be considered satisfactory yet, however, for larger peptides.

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