

seen. Our results are summarized in Table 1. The errors given for  $S_{20}^{\circ}$  are the calculated standard deviations of the mean of each preparation.

It is seen that  $S_{20}^{\circ}$  varies both between the different species and between the two preparation methods for the same species. In spite of their lower iron content, the preparations according to Loftfield and Bonnichsen give lower  $S_{20}^{\circ}$  values than those according to Paléus and Neilands. This indicates that the former preparations might contain impurities with slightly lower sedimentation constant than cytochrome c itself. The two components cannot be resolved but should give rise to a higher apparent diffusion constant which, however, could not be detected in the ultracentrifuge. The high value of  $V_p$  for salmon cytochrome c according to Loftfield and Bonnichsen might also be due to this impurity.

In only two cases we got sedimentation constants comparable to those of Atlas *et al.*<sup>3</sup>, but one of them might have been modified by partly denaturation.

Our value for beef cytochrome c is 10 % lower than the cited value of Pedersen. This is in accord with the known difference of 5 to 10 % between the oil turbin and the Spinco centrifuge<sup>7,9</sup>.

The calculated frictional ratios also agree quite well with the older value 1.29 of beef cytochrome but not with the data of Atlas *et al.* close to 1.0. The rather high values of  $f/f_0$  for horse cytochrome c might reflect that the diffusion constant of  $9.5 \times 10^{-7} \text{ cm}^2 \times \text{s}^{-1}$  is somewhat too low.

1. Pedersen, K. O. quoted by Paul, K. G. in Sumner, J. B. and Myrbäck, K. *The Enzymes*, Vol. II, Part I, Academic Press Inc., New York, 1951, p. 375.
2. Theorell, H. and Åkeson, Å. *J. Am. Chem. Soc.* **63** (1941) 1804.
3. Atlas, S. M., Farber, E. and Stern, K. G. *122 Meeting of the Am. Chem. Soc.* Atlantic City, Sept. 1952, p. 23c.
4. Edsall, J. T. in Neurath, H. and Bailey, K. *The Proteins I*, Academic Press Inc., New York 1952, p. 634.
5. Paléus, S. and Neilands, J. B. *Acta Chem. Scand.* **4** (1950) 1024.
6. Margoliash, E. *Nature* **170** (1952) 1014.
7. Shulman, S. *Arch. Biochem. Biophys.* **44** (1953) 230.
8. Loftfield, R. B. and Bonnichsen, R., quoted by Boeri, E. and Tosi, L. *Arch. Biochem. Biophys.* **52** (1954) 83.

*Acta Chem. Scand.* **9** (1955) No. 3

9. Pedersen, K. O. in *Les Proteines, Rapports et Discussions, 9eme conseil de chimie, Institut International de Chimie Solvay, 1953, p. 1.*

Received March 11, 1955.

## Effects of X-rays and Water Content on Sugars in Barley Seeds

L. EHRENBORG and M. JAARMA

*Institute of Organic Chemistry and Biochemistry, University, Stockholm, Sweden*

An investigation of the influence of ionizing radiations on biochemical systems demonstrated that the relative concentrations of simple sugars in growing *Vicia faba* plants were strongly affected by chronic  $\gamma$ -irradiation<sup>1</sup>. When resting (*i. e.*, ripe) barley seeds are irradiated, their radiosensitivity was found to decrease with increasing water content<sup>2</sup>. Since the concentration of reducing sugars has been shown<sup>3,4</sup> to increase with increasing moisture content, we found it important to investigate possible relations between sugar content and radiation sensitivity. Under certain conditions glucose may act as a protective agent<sup>5</sup>.

*Methods.* Seeds of the two-rowed barley strain, Bonus, were treated for 6 days with streaming air of the relative humidities 0 % and 90 %. This treatment gave samples containing 7.5 % and 17 %  $\text{H}_2\text{O}$ , respectively. The seeds were irradiated with 175 kV unfiltered X-rays at an intensity of 4 000 r min.<sup>-1</sup>. Analysis of reducing sugars was made 15 hours after irradiation, and again after 2—4 weeks' storage at a constant water content and at 20° C.

For the analysis the material was ground twice in a Wiley mill, using first a 20-mesh, then a 40-mesh sieve. The grist was boiled for 3 min. in 80% ethanol, and thereafter extracted, with the same solvent, for 3 hours in a micro-Soxhlet apparatus. (Using 96 % ethanol instead, only about two thirds of the sugars were extracted by the same procedure.) After deproteinizing<sup>6</sup>, reducing sugars were titrated, with an accuracy of 2 %, according to Somogyi<sup>7</sup>. The maximum variation between individual extractions amounted to 5 %.

*Results.* It was demonstrated (Table 1) that in the unirradiated material the in-

Table 1. Content of reducing sugars (mg glucose/g dry weight) after irradiation and storage at different water contents.

Water content %	Analysis after	Seeds irradiated with, r		
		0	$5 \times 10^5$	$10 \times 10^5$
7.5	15 hours	1.46	1.53	1.46
	4 weeks	1.51	1.61	1.64
17	15 hours	1.15	1.33	1.21
	4 weeks	1.92	2.21	2.05
17*	2 weeks	1.98	2.55	—

\* Experiment not simultaneous with those above.

crease of the water content first caused a fall in reducing sugars, possibly a consequence of the increased respiration<sup>3,4</sup>. During 2–4 weeks storage, at unchanged water content, the value increases, possibly becoming slightly higher at the higher water content.

Irradiation with  $5 \times 10^5$  r increases the content of reducing sugars, especially at the higher water content. ( $10 \times 10^5$  r gives a somewhat lesser chemical change. After treatment with this high dose, a fraction of the seeds do not germinate; and death of seeds might therefore be responsible for any disturbed values.) As this increase is already observed immediately after irradiation, it is probably not an effect of a changed metabolic rate (e. g., decrease of respiration rate<sup>9</sup>). It might be ascribed to a radiation induced disintegration of, e. g., starch. If this is so, intermediates originating from cellular constituents other than carbohydrates and water, obviously play a role, as the disintegration of starch, irradiated at 8–23 % H<sub>2</sub>O, was only measurable as viscosity decrease.

It can be concluded that irradiation causes a greater chemical change in sugars when the seeds have a higher moisture content, and consequently are protected against biological damage. The significance of this result, for the interpretation of the mechanism of action of radiations, will be discussed elsewhere<sup>10</sup>.

1. Jaarma, M. *Suomen Kemistilehti B* **28** (1955) 40.
2. Lefort, M. and Ehrenberg, L. *Arkiv Botan.* [2] **3** (1955) 121; Ehrenberg, L. *Botan. Notiser* **106** (1955) 184.

3. Kiesel, A. and Gordienko, K. *Bull. soc. naturalistes Moscou. Sect. biol. Sér. 2* **45** (1937) 339.
4. Swanson, C. O. *Cereal Chem.* **12** (1935) 89.
5. Dale, W. M. *Brit. J. Radiol. Suppl.* **1** (1947) 46.
6. Somogyi, M. *J. Biol. Chem.* **160** (1945) 69.
7. Somogyi, M. *J. Biol. Chem.* **160** (1945) 61.
8. Bailey, C. H. *Plant Physiol.* **15** (1940) 257.
9. Mikaelson, K. and Halvorsen, H. *Physiol. Plantarum* **6** (1953) 873.
10. Ehrenberg, L. *Svensk Kem. Tidsskr.* **67** (1955) 207.

Received March 16, 1955.

## The States <sup>1</sup>I of Trivalent Praseodymium and Thulium

CHR. KLIXBULL JØRGENSEN

Chemistry Department A, Technical University of Denmark, Copenhagen, Denmark

The identification by Spedding<sup>1</sup> of the states of Pr<sup>3+</sup> has for many years been assumed to be complete. But the proposal of Ellis<sup>2</sup> that the band in the orange is due to a transition to <sup>1</sup>D<sub>2</sub>, rather than to <sup>1</sup>I<sub>6</sub>, was recently supported by the crystal field studies of Hellwege<sup>3</sup>. The Condon-Shortley theory<sup>4</sup> was applied to this f<sup>3</sup>-system by Trefftz<sup>5</sup>. This author calculated the electrostatic interaction parameters  $F_2$ ,  $F_4$ , and  $F_6$  for a hydrogen-like 4f-wave function with an effective charge  $Z_0 = 9.48$  (this seems very reasonable, because the ionization energy of Ce<sup>3+</sup> corresponds to  $Z_0 = 6.56$ ). However, the value of  $F_6$  calculated by Trefftz is 64 times too small due to a misinterpretation of Condon and Shortley's decimal point as a multiplication operator. This led Trefftz to accept Spedding's very small value of  $F_6$ .

In the author's opinion<sup>6</sup>,  $F_6$  is not necessarily negligible<sup>7-9</sup>. The position of <sup>1</sup>D<sub>2</sub>, relatively to <sup>3</sup>P<sub>2</sub>, can easily be accounted for by the set of ratios<sup>6</sup>  $F_4 = 0.2 F_2$ , and  $F_6 =$

\* Note added in proof: Judd<sup>15</sup> has recently drawn the same conclusion from Eu<sup>3+</sup>. The present author must here correct the highest value among the three diagonal elements of energy of the <sup>5</sup>D-states to  $9E^1 - 11E^3$  rather than  $9E^1 + \frac{143}{7} E^3$ , given in Ref. 8, p. 19.