the yield behaviour of certain cereals[10]. In view of this, plants obtained from the large and small seeds of *C. ciliaris* were raised during July 1976 in field plots (gross plot size was 5.25 M × 4.50 M where space between the plants was 75 cm) with 3 levels of nitrogen (0, 20, 40 kg N/ha applied as ammonium sulphate and with a dressing of 20 kg P/ha as superphosphate in all the plots) in loamy sand soil of Central Research Farm of this Institute at Jodhpur which contains about 0.15% of organic carbon, 8 kg/ha of available P_2O_5 and 180 kg/ha of available K_2O. A factorial design was adopted with 5 replications of each treatment. The growth of this grass is normally restricted to between June-July and September-October under the rainfed conditions of the Indian arid zone.

During the 2nd year (i.e. 1977) the plots received similar fertilizer treatment, as mentioned above, at the beginning of the monsoon showers in July and the dry matter production was assessed from a single cut, close to the ground level, during October in both the year 1976 (rainfall: 639.7 mm) and 1977 (rainfall: 353.1 mm).

Table 2. Effect of parent seed size on the dry matter production (q/ha) in *C. ciliaris*

Treatments (nitrogen)		1976 Small	Large	Mean	1977 Small	Large	Mean
0 kg/ha		2.23	3.87	3.05	9.24	11.73	10.49
20 kg/ha		4.60	6.91	5.76	22.22	21.51	21.87
40 kg/ha		5.04	7.69	6.37	25.07	26.58	25.83
Mean		3.95	6.41	5.18	18.84	19.94	19.39
Seed size	± SEM		0.46			0.14	
CD	5%		1.36			NS	
	1%		1.85			NS	
Nitrogen dose	± SEM		0.56			0.19	
CD	5%		1.65			0.56	
	1%		2.24			0.76	

Table 2 indicates that the dry forage production during the 1st year of establishment (i.e. 1976) was significantly higher in plants obtained from the large seeds as compared to that of the small seeds. Increasing level of nitrogen increased the dry matter production in both the cases, but the yields from the plants raised from large seeds were consistently higher at all levels of nitrogen treatment. The interaction between seed size and nitrogen treatment was not significant. In general the dry matter yield was relatively higher in the 2nd year of the growth (1977). However, the effect of seed size on dry matter production was not significant during this year, although the nitrogen levels had a significant effect.

It seems therefore that the higher food reserve of the large seeds has a sustained effect on the maintenance of a higher growth of plants only during the 1st year of their establishment. It is tentatively concluded that the advantages provided by the large seeds, such as higher germination, greater seedling vigour and larger dry matter production in the 1st year of establishment, provide ample scope for improving forage production in the arid areas.

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Isoelectric focusing study of radiation damage to dry conalbumin[1]

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Summary. The effect of γ-rays on iron-free conalbumin was studied by isoelectric focusing technique. The results obtained can be interpreted on the basis of radiation-induced conformational changes of the macromolecules through a cleavage of disulphide bridges. Treatments with reagents acting on disulphide bridges lead to isoelectric focusing patterns that confirm this hypothesis.

The separation and the identification of chemically modified molecules after X- or γ-irradiation of proteins was performed in recent years, using the most advanced methods of analytical biochemistry. In particular, proteins irradiated in aqueous solution were investigated, but considerable interest was also devoted to the study of irradiated dry enzymes after the works of Jung and Schüssler[2,3] and Haskill and Hunt[4-6] on ribonuclease. Up to now however, no general picture can be outlined for clarifying all the phenomena involved in the chemical modification of irradiated dry enzymes leading to their biological inactivation. Several theories are at present debated, including damage of amino acids in the active site, conformational changes, and the intervention of oxygen leading to the formation of peroxide groupings[7].

In the present note, we report the results of a preliminary study of the radiation damage to dry iron-free conalbumin, performed by the isoelectric focusing technique on column, a method based on the separation of proteins according to differences in their isoelectric points. Thin-layer isoelectric focusing was used in radiation biochemistry, first by Delincée and Radola[8,9] in their study on irradiated horseradish peroxidase. The isoelectric focusing technique has the advantage of giving information only on amino acids bearing charges and, indirectly, on conformational changes causing a different exposure to the solvent of the charged residues. Free radical formation on dry chicken egg conalbumin was studied in the past in our laboratory[10]. The present work was performed with the aim of obtaining some inferences on charge modification of such a protein, taking advantage by a convenient pH value of its isoelectric focusing patterns.

Chicken egg conalbumin (iron free) and dithioerythritol were obtained from Sigma Chemical Co. St. Louis, USA, 'Ampholine' carrier ampholites (pH range 5-8) from LKB, Bromma, Sweden. All other chemicals were of analytical

grade. Irradiation was performed with a Co^{60} γ-rays source (18,000 Curie, Gammabeam 650 of Atomic Energy of Canada Ltd). The isoelectric focusing analysis was performed on a 110 ml column apparatus equipped with a Uvicord II 8300 and a chopper bar recorder 6520 (LKB, Bromma, Sweden). The column temperature was maintained at 7 °C during the runs, and 50 fractions were collected. After some preliminary trials, we obtained the best results by introducing in the column 10–15 mg of protein.

Figure 1 shows the isoelectric focusing pattern of native iron-free conalbumin before and after γ-irradiation at 10 Mrad. A good agreement with the data previously reported[11] should be noticed. In the irradiated protein, 2 main characteristics are evident: the smoothing of the peaks and their shift to higher pH values. This last effect can be discussed and tentatively explained starting from 2 different hypothesis:

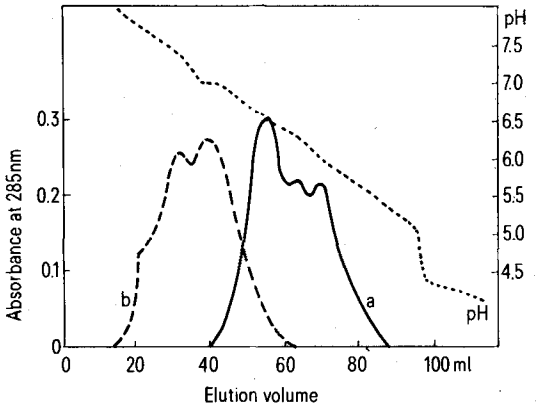

Fig. 1. Isoelectric focusing pattern in the pH range 5–8. *a*, native chicken egg conalbumin. *b*, γ-irradiated conalbumin at 10 Mrad. In both cases, 2 solutions of 15 mg of protein in tri-distilled water were prepared, the first containing 1.5 mg of carrier ampholite and the other 2.5 g of sucrose. The density gradient was produced by introducing the 2 solutions into the column through a gradient mixer (LKB, Stocholm, Sweden). The cathode solution (12 g sucrose plus 14 ml of 1% H_2SO_4 in water) was put into the central electrode compartment. The anode solution (10 ml of 8% NaOH in water) was layered on the top of the gradient. After about 48 h of focusing at 6 V/cm at a temperature of 7 °C, the content of the column was eluted and monitored at 285 nm.

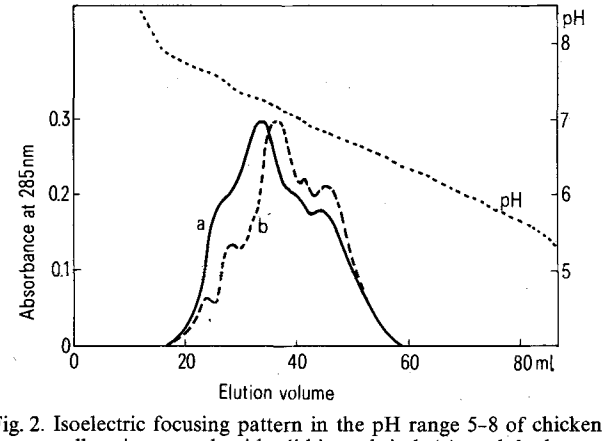

Fig. 2. Isoelectric focusing pattern in the pH range 5–8 of chicken egg conalbumin treated with dithioerythritol (a) and hydrogen peroxide (b). In the 1st case, 15 mg of protein in 50 cm^3 of H_2O were treated with dithioerythritol (final concentration 10^{-3} M). The solution was introduced in the column after 2 h at 4 °C. In the 2nd case, a solution of 15 mg of protein in 5 cm^3 of H_2O (concentration 37.5 µM) was incubated with 50 µl H_2O_2 10 M (final concentration 100 mM). After 24 h at 4 °C, we added 5 λ of catalase 10^{-6} M (final concentration 10^{-9} M) in order to eliminate the hydrogen peroxide. A spectrophotometric control (with a Cary 15) did not show any change of the UV-spectrum of the protein. The volume of the solution was made 50 cm^3 and introduced in the column (see figure 1).

1. Breakage of charged side groups of amino acids. This effect was observed on RNase[3] by amino acid analysis. In such an enzyme a decrease of the arginine, tyrosine, glutamic acid and principally of cysteine content was observed. It is important to notice that the results obtained with a particular protein cannot be considered generally valid for other proteins. So far as our experiment is concerned, we have to consider that the eventual breakage of the side group of tyrosines (pK = 10.07; 20 res/76,000 g[12], would lead to a shift towards more acidic values of the pH. Moreover, conalbumin does not contain free thiol groups. Taking everything into account, we think that this mechanism is negligible in the interpretation of the damage on conalbumin.

2. Breakage of disulphide bridges. This effect was observed in several proteins as a consequence of the preferential localization of the radiation damage on disulphide groups. In this case, the loss of stability of the native macromolecular conformation causes the exposition to the solvent of titrable groups that are buried in the native state.

In order to confirm the latter hypothesis, we performed some isoelectric focalizations on iron-free conalbumin chemically modified in 2 ways. In a 1st experiment we treated the protein with dithyoerythritol (Clealand's reagent) that reduced disulphide bridges to SH, and in a 2nd experiment we used hydrogen peroxide, whose effect should be the same due to the absence of cysteine in the protein molecule[13]. With respect to the structural modifications related to eventual changes of the isoelectric pattern, the 2 reagents should act in a similar way. Figure 2 shows the 2 patterns: in both cases a shift towards more basic pH values was observed, although the details of the isoelectric focusing profiles cannot be explained at the present time. Based on these experimental findings, we can conclude that the main mechanism of radiation damage in conalbumin lies on the breakage of disulphide bridges and, as a consequence, some basic groups, buried in the interior of the molecule, become available to the titration. Such mechanism is supported by the results of Myers and Church[14] on the inactivation of stromal enzymes through both X-rays and chemical reduction. The physical step of such an effect is the preferential localization of the radiation excitation on disulphide groups, as demonstrated by the formation of sulphur free-radicals by EPR technique.

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