Discussion. Assay of an enzyme's activity in situ provides a better understanding than assay in vitro, where the conditions may be altered during its extraction. For in vivo studies the permeability barrier causes serious difficulties. Permeabilization of membranes with organic solvents has been successfully done by several workers[15,18]. Choudary and Rao[9] used a mixture of toluene and ethanol to permeabilize yeast cells and studied NR-activity in situ. But in comparison to these solvents, n-propanol gave more NR-activity in free-living *Rhizobium* cultures. Studies with tungstate and molybdate further confirmed the in situ activity observed. Inhibitory effects of tungstate on NR-synthesis and on its catalysing capacity have been reported for *Chlorella*[19] and higher plants[20] respectively. These findings suggest that NR present in bacteria behaves in a way similar to that of higher plants. The results with amino acids showed that the de novo synthesis of NR in nitrogen-free medium was affected by them, as shown by others in different micro-organisms[21].

From the present observations it may be concluded that nitrate present in the soil can be reduced to NH_3 by rhizobial NR and nitrite reductase (NiR). Thus in legume root nodules, besides nitrogenase, NR in an additional enzyme that provides an alternative route for the formation of NH_4, as shown in free-living *Rhizobium* cultures in the present study.

1 Acknowledgments: M.H.S. is grateful to the authorities of CSIR, New Delhi and Miss Anjali Mathur to the authorities of SCST, Lucknow (U.P.) for financial assistance.
2 To whom reprint requests should be addressed.
3 E. Itagaki, T. Fujita and R. Sato, J. Biochem. *53*, 389 (1963).
4 L. Beevers and R.H. Hageman, A. Rev. Pl. Physiol. *20*, 495 (1969).
5 R.M. Daniel and C.A. Appleby, Biochim. biophys. Acta *275*, 347 (1972).
6 J.R. Manhart and P.P. Wong, Pl. Physiol. suppl. *61*, 3 (1978).
7 R.A. Ludwig, J. Bact. *135*, 114 (1978).
8 R.W. Jackson and J.A. DeMoss, J. Bact. *90*, 1420 (1965).
9 V.P. Choudary and G.R. Rao, Can. J. Microbiol. *22*, 35 (1976).
10 E.K. Allen and O.N. Allen, Bact. Rev. *14*, 273 (1950).
11 R.H. Hageman and D.P. Hucklesby, in: Methods in enzymology, vol. 23, p. 491. Ed. A. San Pietro. Academic Press, London 1971.
12 E.W. Yemm and E.C. Cocking, Analyst *80*, 209 (1955).
13 C.M. Johnson and A. Ulrich, Analyt. Chem. *22*, 1526 (1950).
14 L.D. Doneen, Pl. Physiol. *7*, 717 (1932).
15 D. Mukerji and I. Morris, Z. Pflanzenphysiol. *90*, 95 (1978).
16 R.C. Srivastava, D. Mukerji and S.N. Mathur, Ann. appl. Biol. *96*, 235 (1980).
17 J.W. Radin, Pl. Physiol. *55*, 178 (1975).
18 D. Rhodes and G.R. Stewart, Planta *18*, 133 (1974).
19 I.P. Solomonson and A.M. Spehar, Nature *265*, 373 (1977).
20 M. Aslam and A. Oaks, Pl. Physiol. *57*, 572 (1976).
21 M.G. Guerrero, J.M. Vega and M. Losada, A. Rev. Pl. Physiol. *32*, 169 (1981).

Acid phosphatase activity of small intestine of mice after exposure to different doses of gamma rays

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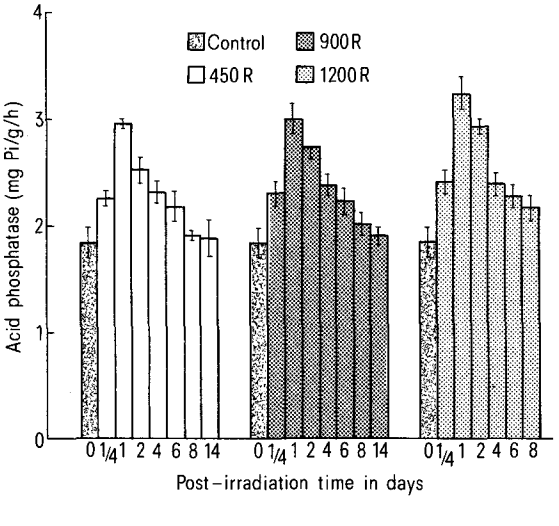
Summary. Effect of whole-body radiation at 3 different dose levels on the activity of acid phosphatase was studied in the small intestine of Swiss albino mice. In all the 3 exposure groups the enzyme activity increased significantly at 24 h after irradiation; the time at which the maximum histological damage was seen. With the beginning of recovery the enzyme tended to decrease and gradually approached control values.

Acid phosphatase is a hydrolytic enzyme localized in lysosomes[1]. Increase in acid hydrolases after irradiation seems to be characteristic of tissue damage by irradiation and has been reported for thymus[2], spleen[3], liver[4] and adrenal glands[5]. A similar post-irradiation increase in the acid phosphatase content of the small intestine has been demonstrated by histochemical studies[6-11]. In the present work an attempt has been made to study the post-irradiation changes in the activity of acid phosphatase in the small intestine of mice by biochemical methods.

Material and methods. Young adult male albino mice of 8-10 weeks weighing 24 g on the average were selected from an inbred colony for all the experiments. The animals were exposed to 450, 900 or 1200 R of whole body gamma radiation from a ^{60}Co source at the rate of 25 R/min and were sacrificed by cervical dislocation at 6 h, 1, 2, 4, 6, 8 and 14 days after irradiation. Exteriorized small intestine from duodenum to ileocoecal junction was immediately removed without the adhering mensenteric blood vessels and fat, slit longitudinally, rinsed thoroughly in ice cold 0.9% sodium chloride solution, minced into small fragments in a chilled petri-dish and homogenized in distilled water. The acid phosphatase activity in this homogenate was estimated by the method of Fiske and Subbarow[12]. Reading were taken on a Klett Summerson colorimeter and the activity of the enzyme was expressed as mg Pi/g/h. Statistical analysis was done using Student's t-test.

Results and discussion. Post-irradiation changes in the activity of the enzyme are presented in the histogram. After all the 3 doses an increase in the activity of the enzyme was evident during the early intervals. The peak values were obtained at 1 day after exposure when the activity in all the



Variations in the activity of intestinal acid phosphatase (mg Pi/g/h) of Swiss albino mice after exposure to 450, 900 and 1200 R of gamma rays* (mean value ± SE)

Dose of irradiation	Autopsy intervals 6 h	1 day	2 days	4 days	6 days	8 days	14 days
450 R	2.280 ± 0.07	2.966 ± 0.03	2.527 ± 0.12	2.328 ± 0.10	2.185 ± 0.14	1.918 ± 0.04	1.89 ± 0.17
	$p < 0.05$	$p < 0.001$	$p < 0.01$	$p < 0.05$	NS	NS	NS
900 R	2.321 ± 0.11	3.009 ± 0.134	2.737 ± 0.06	2.378 ± 0.08	2.230 ± 0.12	2.016 ± 0.08	1.905 ± 0.08
	$p < 0.05$	$p < 0.001$	$p < 0.001$	$p < 0.05$	NS	NS	NS
1200 R	2.416 ± 0.11	3.240 ± 0.14	2.935 ± 0.058	2.408 ± 0.098	2.271 ± 0.10	2.165 ± 0.11	*
	$p < 0.01$	$p < 0.001$	$p < 0.001$	$p < 0.01$	$p < 0.05$	NS	

* The activity of intestinal acid phosphatase (mg Pi/g/h) in sham-irradiated Swiss albino mice is 1.842 ± 0.14. ** Animals do not survive. p-Values calculated by Student's t-test.

3 groups was significantly higher than normal ($p < 0.001$ in all 3 groups). After day 1 the activity showed a gradual decrease and the control value was approached at day 6 in mice exposed to 450 and 900 R and at day 8 in mice exposed to 1200 R.

Lysosomal hydrolases are thought to contribute to the degradation of damaged cells and hence to facilitate their replacement by normal tissue[13]. The intracellular digestion of damaged material by lysosomes could be prompted by irradiation damage to subcellular structures[14]. The post-irradiation increase in acid phosphatase activity noted in the present investigation could be attributed to similar radiation-induced changes in lysosomal activity. The higher the dose, the greater will be the tissue damage, and more of the damaged cells will have to be removed; this may be reflected in an increase in the activity of acid phosphatase. The maximum radiation damage with all the 3 doses was observed at 1 day[15]. After this interval animals exposed to 1200 R showed greater damage than those exposed to 900 and 450 R. Biochemical studies after the same interval showed a higher activity in mice of the former exposure group in comparison to the latter ones. The activity of enzyme remained elevated for a longer period in mice exposed to 1200 R as the radiolesions produced by this dose were more severe and persisted for a longer time; consequently, more time was required for the degradation and removal of damaged cells.

Several mechanisms have been suggested for the release of hydrolases from lysosomes. In the present case an increase in acid phosphatase after irradiation could be due to peroxidation of the lysosomal membrane leading to membrane breakdown[16], or increase in permeability of lysosomal membranes[17], or both. In all 3 groups the small intestine showed the first signs of histological recovery on day 2[15]. With the beginning of recovery the activity of the enzyme started declining, and it gradually approached control values as the histological recovery progressed towards normal, thereby indicating that the major role of the enzyme is during the damage rather than in the recovery process.

1 A.B. Novikoff and E. Essner, Am. J. Med. *29*, 102 (1960).
2 Y. Rahman, Proc. Soc. exp. Biol. Med. *109*, 378 (1962).
3 S.L. Snyder and S.K. Eklund, Radiat. Res. *69*, 306 (1977).
4 V.C. Shah and P.K. Gadhia, J. Radiat. Res. *20*, 322 (1979).
5 J. Jonek, E. Stoklosa and J. Konecki, Strahlentherapie *132*, 226 (1964).
6 J. Jonek, S. Kasmider and J. Kaiser, Int. J. Radiat. Biol. *7*, 411 (1963).
7 H.M. Spiro and A.G. Pears, J. Path. Bact. *88*, 55 (1964).
8 J. Hugon and M. Borgers, J. Histochem. Cytochem. *13*, 524 (1965).
9 J. Hugon and M. Borgers, Lab. Invest. *15*, 1928 (1966).
10 J. Hugon, Jr. Maisin and M. Borgers, Radiat. Res. *25*, 489 (1965).
11 M. Noamann, M.K. Hamdy and W.O. Caster, Bull. Georgia Acad. Sci. *26*, 10 (1968).
12 C.M. Fiske and Y. Subbarow, J. biol. Chem. *66*, 375 (1925).
13 C. De Duve, Lysosomes. Eds A.V.S. de Reuck and M.P. Gameron. Churchill Ltd, London 1963.
14 D.M. McFadyn and D.G. Baker, Gastrointestinal injury. Ed. M.F. Sullivan. Excerpta Medica Foundation, Amsterdam 1968.
15 K. Nandchahal, Ph.D. thesis, University of Rajasthan, Jaipur 1978.
16 E.D. Wills and A.E. Wilkson, Biochem. J. *99*, 657 (1966).
17 A.A. Alkman and E.D. Wills, Radiat. Res. *57*, 416 (1974).

Characterization of a thermosensitive protein from human milk whey

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Summary. A human milk whey protein, which aggregates at room temperature and resolubilizes when cooled, was purified by chromatography on hydroxyapatite. The present study demonstrated that the thermosensitive protein is a non-phosphorylated form of β-casein.

Reports have been made of the isolation of a new protein, which polymerizes at body temperature, from human[2,3] and bovine milk wheys[4]. The protein from human milk whey was called galactothermin and that from bovine whey pyroglobulin. Molecular weights ranging from 14,000 to 30,000 daltons were assigned to the crude material obtained by centrifugation from human milk whey. In the present note we report some data concerning galactothermin.

Materials and methods. The crude material was obtained from 5 individual human milk samples as indicated by