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.

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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

Amino acid composition of the human thermosensitive protein purified on hydroxyapatite

Amino acid	Thermosensitive protein residues/mole	Human β-casein[10] residues/mole
Asp	12.3	11
Thr	9.0	9
Ser	9.8	9
Glu	39.5	39
Pro	33.8	39
Gly	4.6	3
Ala	8.2	7
Val	16.0	19
Met	3.9	3
Cys	0	0
Ile	11.4	13
Leu	23.2	26
Tyr	6.9	7
Phe	6.4	5
His	7.9	5
Lys	6.0	11
Arg	4.8	3
Trp	n.d.	1

Schade and Reinhardt[3]. Samples (15 mg) of thermoprecipitable protein were chromatographed on a 5 × 1 cm hydroxyapatite column eluted with: (a) 5 mM KH_2PO_4, 0.2 M KCl, 4.5 M urea, 2 mM mercaptoethanol buffer and (b), the same buffer as (a) but with 250 mM KH_2PO_4. The pH was 6.8 and a linear gradient from (a) to (b) was applied starting at fraction 5, using a Pharmacia gradient mixer GM-1, containing 100 ml of each buffer per chamber. The flow rate was 10 ml/h[5]. Inhibition of the hemagglutination test was performed using sheep red blood cells covered with human Na-caseinate. Rabbit anti-human Na-caseinate was used as antibody. The samples to be tested were dissolved in 0.9% NaCl (initial concentration 0.1 mg/ml). Serial dilutions (1:2) of the samples were performed starting with 2.5 µg of material. SDS-polyacrylamide gel electrophoresis was performed according to Weber and Osborn[6]. The phosphorous content was determined after mineralization (4 h) at 500 °C according to Fiske and Subbarow[7]. The amino acids were determined on a Beckman Multichrom B Autoanalyzer after a 24-h hydrolysis at 110 °C in 6 M HCl. Automated Edman degradation was carried out in a Beckman Sequencer 890C by the 1 M quadrol double cleavage method. The phenylthiohydantoin amino acids were identified by TLC[8] or by high performance liquid chromatography[9].

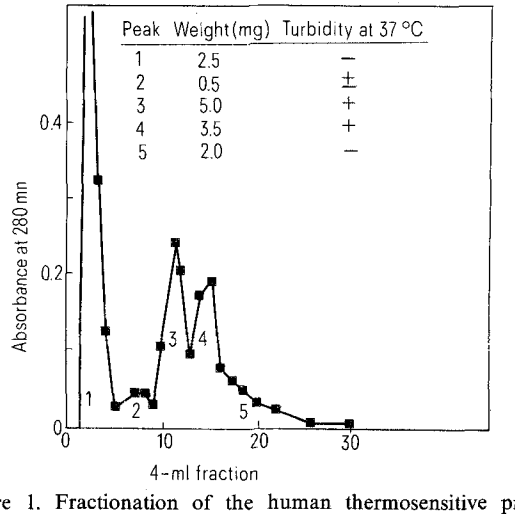

Figure 1. Fractionation of the human thermosensitive protein (15 mg) on hydroxyapatite.

Results and discussion. A good an reproducible fractionation of human thermosensitive protein was obtained on hydroxyapatite (fig. 1).

The antigenic response of the purified thermosensitive protein against the human caseinate antiserum was at least as strong as that obtained with human Na-caseinate. In fact the thermosensitive protein and the Na-caseinate showed a positive response in the passive hemagglutination test at concentrations of 3×10^{-7} and 2.5×10^{-7} mg/ml, respectively. The purified thermosensitive protein behaved homogenously when submitted to SDS-polyacrylamide gel electrophoresis (single band in the β-casein zone, fig. 2) and to the dansylation technique; a unique N-terminal amino acid, arginine, was characterized for the protein contained in peaks 3 and 4 (fig. 2). Its amino acid composition was established (table). It was devoid of cyst(e)ine and contained high amounts of proline and glutamic acid, a common feature of β-caseins. Despite some analytical differences, the regression coefficient (r^2) of the amino acid compositions of the purified thermosensitive protein and human β-casein was 0.96. The phosphorous content of human β-caseins was situated between 1.3 and 1.6%: no phosphorous could be characterized in the thermoprecipitable protein. The sequence of its 1st 11 residues was determined as Arg-Glu-Thr-Ile-Glu-Ser-Leu-Ser-Ser-Ser-Glu and was identical to the N-terminal sequence of human β-casein[10].

The acid precipitation of human caseins is usually incomplete: a fraction of these proteins, defined as 'acid-non-coagulable type'[11] remains in the supernatant after centrifugation in the cold or at room temperature. The thermosensitive protein of human milk whey was found to be essentially free of phosphorous, but it must be emphasised that human β-casein is present in multiphosphorylated forms having 0 to 5 phosphate groups per molecule with the same amino acid composition[10].

The results of the present study showed that the thermosensitive protein from human milk whey (galactothermin) is a non-phosphorylated form of the 'acid-non-coagulable' β-casein.

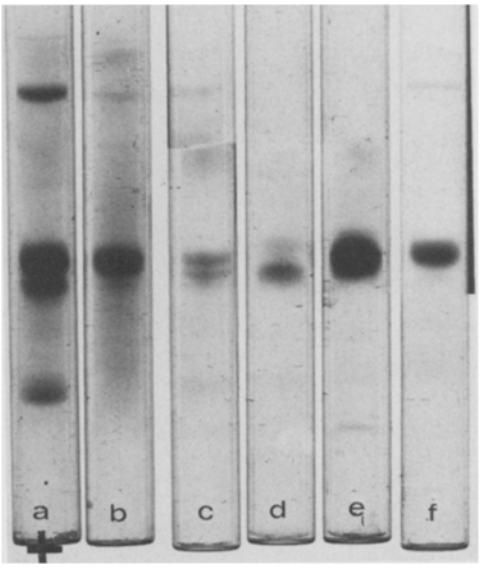

Figure 2. SDS-polyacrylamide gel electrophoresis of a) human Na-caseinate, b) thermosensitive protein, c) peak 1, d) peak 2, e) peak 3 and f) peak 4 obtained after filtration on hydroxyapatite.

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A light microscopic study of denervation atrophy in serial sections[1]

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Summary. Type I and type II muscle fibers from rat extensor digitorum longus muscle were sequentially followed in serial sections after various time intervals following denervation. The type II fibers exhibited the greatest degree of atrophy as well as showing a systematic diameter variability along its length.

In studies of experimental denervation and in various neuromuscular disorders, such as the spinal muscular atrophies, many authors have reported regional differences in fiber sizes, both in the same cross-section as well as in the longitudinal axis of the muscle[2,3]. Such regional differences may lead to problems in sampling error. A basic premise underlying these studies has been that of assuming both diameter and structural homogeneity throughout the length of a given muscle fiber. It has been recently shown that systematic diameter and morphological variability may occur along the length of certain fiber populations in normal and dystrophic extraocular muscle[4,5] as well as in dystrophic peripheral muscle[6]. In these studies, fibers were followed and their identities were retained in serial sections. In the present investigation, a serial sectioning method[4] was used to analyze by light microscopy rat extensor digitorum longus (EDL) muscle which had been denervated for various time intervals in order to sequentially follow the course of atrophy in type I and type II muscle fibers.

Materials and methods. 20 male Wistar rats with an average b.wt of 200 g were used in this study. The right hind limb of 15 rats were surgically denervated under ether anesthesia by excision of a 0.5 cm segment of the sciatic nerve just proximal to the bifurcation of the tibial and peroneal nerves. The cut end of the proximal portion of the nerve was capped with Silastic tubing to prevent subsequent reinnervation. At 14, 21, and 28 days following surgery, a group of 5 rats was sacrificed and the EDL muscles were fixed in situ by dripping 4% glutaraldehyde onto the muscles for 10–15 min. The muscles were then removed whole, fixed overnight in 4% glutaraldehyde and processed by standard electron microscope methods and embedded whole in Epon 812. 5 nonoperated animals served as controls and were sacrificed at the beginning of the experiment. The experimental and control muscles were transversely sectioned at 15 µm by steel knife on a sliding microtome. Such sections were viewed by phase contrast microscopy, the osmium postfixation providing adequate stain to differentiate type I and type II muscle fibers[7]. By phase contrast, the type I fibers stain more intense than the type II fibers. The type I fibers also exhibit larger and more abundant mitochondria, evidenced as granulation, than the type II muscle fibers. From each muscle, several fascicles containing at least 100 muscle fibers were followed throughout their length by the taking of frequent photomicrographs at closely spaced intervals. The diameters of the muscle fibers (both type I and type II) were measured in the muscle's proximal, middle, and distal thirds, according to Brooke and Engel[8]. The fiber diameters were expressed as means ± SD. The significance of the differences between the various regions within each group and time interval was determined by Student's t-test. All p-values greater than 0.01 were considered to signal nonsignificance.

Results. The results of this study are summarized in the table. It is apparent from the table that there is no significant difference for the control muscles in the mean fiber diameters of type I and type II fibers in the various regions examined. 14 days following denervation, both type I and type II fibers exhibited diameter decreases of approximately 25% ($p < 0.001$) and 42% ($p < 0.001$), respectively, as compared to the control. The mean diameters of both fiber populations were not significantly different in the 3 regions examined. After 21 and 28 days post-denervation, the type II muscle fibers exhibited extensive atrophy (approximately 70%) and significant diameter variability (table). These fibers were largest in their middle third and highly atrophic both proximally ($p < 0.001$) and distally

Means and SD (in µm) of diameters of type I and type II muscle fibers from control, 14, 21, and 28 days post-denervated rat extensor digitorum longus muscles. Measurements were made in cross-sections from the proximal, middle, and distal thirds of the muscle's length

	No. of rats	Proximal	Middle	Distal
Control	5			
Type I		27.6 ± 1.5	28.4 ± 2.7	27.8 ± 2.4
Type II		37.2 ± 5.4	36.2 ± 5.2	36.4 ± 6.1
14 days post-denervation	5			
Type I		21.0 ± 2.5	21.1 ± 3.0	19.9 ± 2.0
Type II		20.1 ± 3.3	20.4 ± 2.8	20.2 ± 3.2
21 days post-denervation	5			
Type I		21.8 ± 2.6	21.5 ± 2.3	22.9 ± 3.9
Type II		10.3 ± 3.5	13.1 ± 2.6	9.9 ± 2.9
28 days post-denervation	5			
Type I		25.6 ± 3.1	22.4 ± 4.1	22.8 ± 3.5
Type II		9.6 ± 3.6	16.0 ± 3.0	9.8 ± 3.1