Lysozyme levels in tissues of iron-deficient rats[1]

Adria Rothman Sherman and Ira Wolinsky[2]

Nutrition Program, Division of Biological Health, College of Human Development, The Pennsylvania State University, University Park (Pennsylvania 16802, USA), 3 August 1977

Summary. Rats were fed either diets sufficient (300 ppm) or insufficient (5 ppm) in iron for 10 weeks. The iron-deficient animals had lowered hemoglobin and hematocrit levels and higher levels of kidney lysozyme activity than did control animals. There were no significant changes in serum and spleen lysozyme activity levels.

Lysozyme is a bacteriolytic enzyme discovered by Sir A. Fleming in 1922[3]. Since that time it has been shown to be widely distributed in vertebrate tissues[4,5] and its levels to be modified in a wide range of pathological conditions[6–8] and nutritional states[9–11]. During an investigation of iron nutriture in rats it was of interest to monitor tissue lysozyme levels in iron-deficient and control animals.

Materials and methods. In these studies albino, female, Sprague Dawley rats weighing an average of 68 g, were used. They were fed, ad libitum, either an iron-deficient diet (5 ppm iron) or an iron-sufficient diet (300 ppm iron) and glass-distilled water for 6 weeks, then meal-fed diets during 12.00–13.00 h daily for 4 weeks. The diet composition has been described previously[12]. After the 10 weeks on the diets they were fed, fasted for 1 h and chloroform anaesthetized. Sera were collected by cardiac puncture, spleens and kidneys were removed in the cold and frozen until analyzed. For analysis, 300–400 mg of the right kidney and 100–200 mg of spleen were homogenized in 5 ml (spleen) or 10 ml (kidney) of cold 0.066 M potassium phosphate buffer, pH 6.24, and centrifuged at 100,000 × g for 1 h at 2 °C. After centrifugation, the supernatant fluids were assayed for lysozyme activity by monitoring the lysis of cell walls of Micrococcus lysodeikticus, crystalline egg white lysozyme serving as a standard[13]. Lysozyme rate was expressed as

$$\text{units/mg protein} = \frac{\triangle \text{A } 450/\text{min}}{0.001 \times \text{mg protein/reaction mixture}}.$$

Serum was used directly for analysis in our assay system. Protein was assayed using biuret reagent[14] with bovine serum albumin as standard. For spleen and kidney, protein was expressed as a blot-dry weight of tissue. Hemoglobin was determined by the method of Crosby[15] and micro-hematocrits as described by Winthrobe[16]. Statistical significance between means was detected by Student's t-tests[17].

Results. Body weights, hemoglobin, and hematocrit levels of the animals after 10 weeks on the experimental diets are shown in table 1. The rats fed 5-ppm iron weighed significantly less than those fed the control 300-ppm iron diet. Iron-deficiency anemia, characterized by significantly lowered hemoglobin and hematocrit levels, was found in the group fed 5-ppm iron.

Table 1. Body weights, hemoglobin, and hematocrit levels in rats after 10 weeks on experimental diets

Dietary treatment	Body weight (g)	Hemoglobin (g/dl)	Hematocrit (%)
5 ppm Iron	184 ± 7	7.1 ± 0.4	32.7 ± 0.6
300 ppm Iron	220 ± 10 $p < 0.01$	16.9 ± 0.3 $p < 0.001$	51.8 ± 0.8 $p < 0.001$

The data are expressed as means ± SE of 8 animals.

Tissue weights, protein content, and lysozyme levels are given in table 2. In both iron-deficient and control animals lysozyme activity was found to be highest in kidney, spleen was intermediate in activity, and serum was the lowest. The iron-deficient rats had sera with protein and lysozyme activities which were not significantly different from controls. No significant differences were found in the total protein or lysozyme activity of spleens from iron-deficient and control animals. The iron-deficient rats were observed to have enlarged spleens with no apparent effect on protein or lysozyme contents. In kidney, iron-deficient rats had significantly higher levels of lysozyme activity than did control animals ($p < 0.001$). This increase in lysozyme activity in iron-deficiency was accompanied by a decrease in total kidney weight and no significant difference in protein content of the kidney.

Discussion. Despite intensive investigation over the years, the biological functions of lysozyme in mammalian tissues, aside from a probable antibacterial role, have not yet been established. The wide distribution of the enzyme has led to several hypotheses for roles other than antibacterial ones[18,19]. Enzymatic and nonenzymatic actions on polysaccharides, glycoproteins, peptidoglycans[18], and glycolipids[19] have been proposed. A general role in the regulation of membrane-dependent cell function has also been suggested[19]. Still other functions dependent on the cationic properties of the protein have been proposed.

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2 To whom reprint requests should be addressed.

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Table 2. Tissue weights, tissue protein contents and lysozyme levels

Dietary treatment	Serum Protein content (mg/ml)	Lysozyme (units/ mg protein)	Spleen Total tissue wt (g)	Protein content (mg/g)	Lysozyme (units/ mg protein)	Kidney Total tissue wt. (g)	Protein content (mg/g)	Lysozyme (units/ mg protein)
5 ppm Iron	74.2 ± 3.1	0.34 ± 0.03	0.38 ± 0.05	112.1 ± 10.4	4.83 ± 0.63	0.75 ± 0.02	76.1 ± 1.1	42.85 ± 2.34
300 ppm Iron	91.3 ± 7.3 NS	0.30 ± 0.02 NS	0.36 ± 0.03 $p < 0.005$	137.5 ± 7.9 NS	5.25 ± 0.33 NS	0.97 ± 0.03 $p < 0.001$	83.2 ± 3.7 NS	31.65 ± 1.36 $p < 0.001$

Data are expressed as means ± SE of 7 animals. NS, not significant.

The hitherto unreported effect of iron-deficiency on increasing the level of lysozyme activity in the kidney is clearly documented in this study. However, the exact biological significance of these findings is unknown at this time. It has been established that the kidney plays an important role in the regulation of plasma lysozyme levels. This regulation is believed to occur by way of the lysozyme protein becoming entrapped in the proximal tubules and degraded there[20]. The increased levels of lysozyme observed in the kidneys of iron-deficient rats may, then, reflect a homeostatic mechanism to maintain normal serum levels. Perhaps, the iron-deficient animal is producing more lysozyme which is being degraded in the kidney. The resulting pattern of serum and spleen lysozyme levels is therefore the same in iron-deficient and control animals. One possible explanation may be that the iron-deficient organism, with a lower immunological capacity[21], requires more lysozyme for its antibacterial functions. The increased kidney levels may be a reflection of a greater lysozyme turnover required for this antibacterial role.

Generally, the interrelationships between nutritional status and lysozyme action merits further study. The effect of iron-deficiency tissue lysozyme levels is particularly pertinent since iron deficiency anemia is a common nutrition problem[21].

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Large regional and strain differences in rat brain sialic acid and 2-deoxyribose

R. L. Engen and W. R. Klemm

Department of Anatomy, Pharmacology and Physiology, College of Veterinary Medicine, Iowa State University, Ames (Iowa 50010), and Department of Biology, Texas A and M University, College Station (Texas 77843, USA), 4 July 1977

Summary. Sialic acid, a very polar component of glycolipids and glycoproteins that is exposed on membrane surfaces, was observed in rat brain to vary in the descending order: forebrain, midbrain, cerebellum and medulla. Levels of 2-deoxyribose were also differentially distributed, with about 3.5 times as much in the cerebellum and nearly equal amounts elsewhere. Similar results were obtained with another genetic strain, but clear quantitative differences were evident for both chemicals.

Both lipid-bound (gangliosidic) and protein-bound sialic acid have been known to be associated with cell membranes[1,2], with a significant amount occurring on mitochondrial membranes[3]. SA's highly reactive polar characteristic of the carboxyl group and its exposed position on the membrane surface make it a possible reaction site (receptor) for cationic groups of many drugs and hormones[4]. Since much of the research has dealt with the total brain SA-analyses, it seemed necessary to study the SA-distribution in different areas of the brain. If SA-differences occurred, such data could have relevance in focusing the search for potential mechanisms of action of certain psychoactive drugs and neurohormones.

Methods. Rats were killed by decapitation, and the brains were removed and cut into 4 sections within 2 min; the sections were then frozen in liquid nitrogen. The sections were identified as: 1. forebrain (cut was immediately behind optic chiasma and extended through the hippocampus); 2. midbrain (caudal border of inferior colliculi); 3. medulla; 4. cerebellum.

Rat brains were obtained from young adult rats (250–300 g) of both sexes from different litters. An initial set of experiments was performed on 6 rats of the Wistar strain, and replication experiments were performed on 6 Sprague-Dawley rats.

Tissue content of total sialic acid was determined by a continuous-flow colorimetric method[5], which basically combined the 2 established procedures, by Warren[6] and by Delmotte[7], with a 2-channel Auto Analyzer. In this

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