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Serum Sulfhydryl Group Levels in Experimental Chronic Inflammation*

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Serum sulfhydryl group concentration and protein fraction were determined in adjuvant arthritic rats, during six weeks. Significantly reduced sulfhydryl levels were found in arthritic rats from day 3 and these values remained decreased throughout the study period. Good correlation exists between serum sulfhydryl alterations and degree of inflammation measured as hind paw swelling, as well as between the former and changes in serum protein fractions.

Key words: Sulfhydryl groups. Adjuvant arthritis, Chronic inflammation.

Adjuvant arthritis is an experimental pathology induced in rats by subplantar injection of Freund's complete adjuvant, which is, heat-killed mycobacteria dispersed in mineral oil. Adjuvant arthritis has a first phase (0-10th day) in which a local inflammation only appears in the injected hind paw and a second phase with generalized signs of inflammation (20). This experimental pathology shares many features of human rheumatoid arthritis and therefore is one of the most widely used models for studying anti-inflammatory drugs (24).

Patients with rheumatoid arthritis show low serum sulfhydryl (SH) levels (7, 22). Decrease in serum SH concentration has also been found in patients with some lymphoproliferative diseases like multiple myeloma and macroglobulinemia (12).

It has not been established if the reduction in sulfhydryl levels is a primary aetiological event or another consequence of the overall inflammatory process. Thus, endogenous reducing agents, such as glutathione, were used for peroxide detoxification in some body tissues (6). This antioxidant effect could be of primary importance when tissues are invaded by a large number of metabolically activated inflammatory cells, that release oxygen free radicals during phagocytosis, producing tissue damage, tissue degradation and autoantibody generation (25).

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The present study was designed to find the time-course of serum sulfhydryl levels in rats during the first phase with local inflammation and in the second phase when generalized arthritis has developed. Simultaneously paw swelling and serum protein alterations were studied and compared to serum SH level evolution.

Materials and Methods

Female Sprague-Dawley rats, weighing 150-200 g, were used.

Arthritis was induced by a subplantar injection (0.1 ml) in the right hind-paw of heat-killed *Mycobaterium butyricum* (Difco Lab.) suspended in mineral oil (5 mg/ml). Control studies were carried out in rats receiving 0.1 ml of mineral oil under the same conditions.

The severity of adjuvant arthritis was quantified by the volume increase of left-hind-paw (ml), which was measured using a mercury plethysmometer (Ugo Basile, Milan). On day 14, those rats whose left-hind-paw volume increase exceeded the average left-hind-paw volume increase of the control animals plus two standard deviations, were selected as arthritic rats. This degree of swelling was exhibited by 71% of animals.

Blood samples were obtained on days 0, 3, 6 and 9, and in selected animals weekly between 14 and 42 days after induction. After ether anaesthesia, blood was collected from rats by heart puncture and serum was obtained by centrifugation.

Serum SH levels were determined according to a previously described method (9). This method is based on the exchange reaction between serum protein sulfhydryl groups and 5,5-dithio-bis-2-nitrobenzoic acid (DTNB). Only fresh serum samples were used to avoid loss of activity due to storage (15). A volume of 1.5 ml of a 1/13 dilution of rat serum in 0.1 M phosphate buffer, pH 7.4, was mixed with 0.3 ml of 2 mM DTNB solution (Fluka). The reaction was allowed to proceed at 37°C until equilibrium was reached (10-15 min) (4). The concentration of the yellow final solution was measured spectrophotometrically at 440 nm (Spectronic 100, Bausch & Lomb). Each serum dilution (1.5 ml) added to 0.3 ml of phosphate buffer was used as blanks.

Known concentrations of reduced glutathione (Merck) were reacted with DTNB solution in order to calibrate absorbance at 440 nm with molar SH concentrations. From this, the protein SH group levels with DTNB was expressed as μmol per litre of serum.

The separation and determination of serum protein was done electrophoretically on cellulose acetate. After staining with Ponceau red, the relative distribution of proteins was measured by a Digiscan densitometer. The percentage of albumin, α, β and γ proteins were obtained and the albumin/globulins ratio was also calculated.

Statistical analysis was performed by means of an ANOVA of linear models (13). This was carried out by a MZ-80 B Sharp computer and the following variation causes were considered: arthritis (induced animals versus control animals), time (42 days) and the interaction between arthritis and time.

Results

Time course of injected (right) and uninjected (left) hind-paw volume in control and arthritic rats is represented in figure 1. As it can be seen the arthritic rats show a significant right-hind-paw volume increase (estimated at 4.27 ml, f.l.: 3.79 ~ 4.76) when compared to control group (estimated at 0.24 ml, f.l.: —0.32 ~ 0.81; $p < 10^{-9}$). Left-hind-paw of arthritic animals increased their

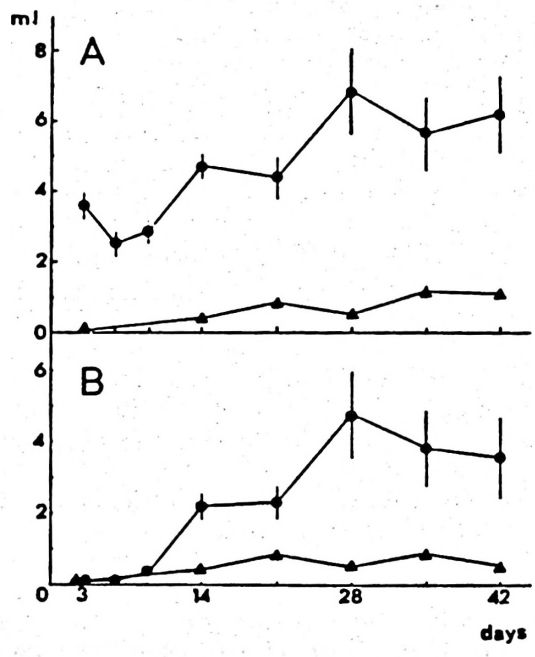

Fig. 1. *Hind-paw swelling of injected (A) and uninjected (B) paw during study period.*
Mean volume increase (ml mean ± S.E.M.) of adjuvant arthritic rats (●) and control rats (▲). Each point represents the means of 8 animals.

volume from day 14 and remained elevated during the study period. The difference between arthritic and control left-hind-paw volumes was estimated on average as 2.42 ml (f.l.: 1.73 ~ 3.12) on days 14-42 ($p < 10^{-9}$).

Adjuvant arthritis produced a decrease in serum SH concentrations when com-

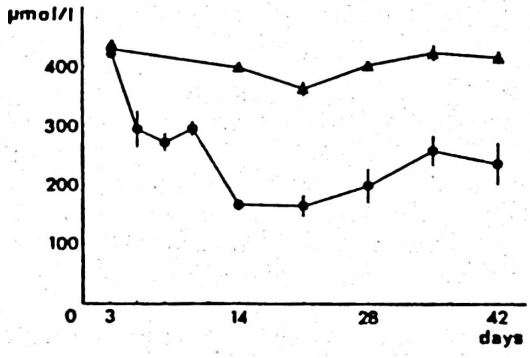

Fig. 2. *Total serum sulfhydryl concentrations (µmol/l) in adjuvant arthritic rats (●) and control rats (▲).*
Each point represents the means of 8 animals (Means ± S.E.M.).

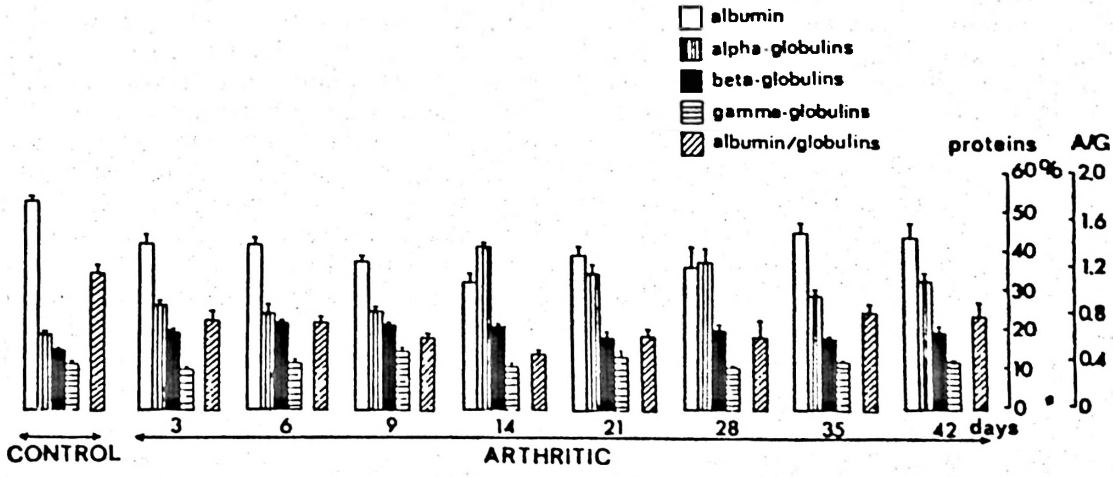

Fig. 3. *Serum electrophoretic pattern in control and arthritic rats.*
Control group consisted of 56 animals and each arthritic group consisted of 8 animals. The vertical bar represents S.E.M.

Table I: *Correlation coefficients between studied parameters.* Degrees of freedom are 78 and $p < 0.001$ in all cases.

	Uninjected hind-paw volume	SH	Albumin	Alpha-globulins	Beta-globulins
Injected hind-paw volume	0.9029	—0.7818	—0.7671	0.6807	0.6350
Uninjected hind-paw volume		—0.7051	—0.7298	0.6320	0.5186
Sulfhydryl groups			0.8930	—0.8191	—0.7581
Albumin levels				—0.8531	—0.7700
α-globulin concentration					0.5267

pared to control animals (fig. 2). This reduction was statistically significant from day 3 and was estimated at 175.4 μmol/l (f.l. 153.2 ~ 197.5) during the study. A significant correlation between serum sulfhydryl concentration and systemic inflammation measured as left-hind-paw volume increase was found (table I).

From day 3 after induction arthritic animals showed significant changes in serum protein concentration (figure 3). In arthritic rats a decreased albumin level ($p < 10^{-8}$) and an increase in α-globulin ($p < 10^{-9}$) and β-globulin ($p < 10^{-8}$) fractions were observed throughout the period studied. There is a significant linear correlation between electrophoretic pattern changes and systemic inflammation and also between the former and serum sulfhydryl concentrations (table I).

Discussion

Chronic inflammation induced by adjuvant, produces decreased serum SH levels and changes in the concentration of the albumin, alpha and beta globulin fractions. The present results obtained during a period of 42 days are in agreement with other data obtained at different times of the arthritic process (2, 4, 16). The alterations observed in adjuvant arthritis are similar to the variations detected in the human rheumatoid arthritic process (1, 7, 26). Alterations in serum proteins have been correlated with unspecific inflammatory processes (20) in which as a consequence of an increase in acute phase protein synthesis, a decrease in albumin synthesis can occur (18).

Both SH levels and protein serum concentrations were significantly modified in an early stage of the process (day 3) prior to the development of the generalized arthritic lesions. How these alterations are produced and what significance they may have are not known, but these modifications are not found in experimental acute inflammation models (4).

Protein SH groups play an important role in a number of biological processes such as protein conformation and binding, blood clotting, and cell division (17), phenomena which are markedly influenced by inflammatory states (19, 27). The role of free radicals in inflammatory is well known (3), and endogenous sulfhydryl groups could act against them, trying to neutralize their harmful effects. This fact could result in a diminished SH serum activity (14).

Decrease in serum SH levels could be a reflection of the reduced albumin serum concentrations. Albumin contains 1 SH/mole and for its high concentration has the major responsability of the sulfhydryl-disulfide exchange reaction with DTNB (7). However, a mean albumin level reduction of 31% and a mean SH concentration decrease of 44%, has been observed; it seems that an additional

factor responsible for SH concentration reduction is involved.

Heat-aggregation of IgG is partially thiol dependent (10) and may be inhibited by SH-containing antirheumatic drugs such as aurothiomalate and D-penicillamine (11) or endogenous substances such as cysteine and glutathion (23). In chronic inflammation the lack of SH groups could lead to a IgG aggregation with the subsequent exposure of new antigenic sites which involves rheumatoid factor production. This rheumatoid factor is an autoantibody present in rheumatoid arthritis (21) as well as in adjuvant arthritis (5).

The depression of serum sulfhydryl concentration correlated well with the severity of adjuvant arthritis. In human rheumatoid arthritis, some authors have seen that SH levels usually return to normal ranges with clinical improvement after anti-inflammatory drug treatment (8, 12). Serum sulfhydryl measurement is a fast and useful determination that can easily be done in the screening of anti-inflammatory drugs. Serum sulfhydryl levels constitute a good marker of arthritis pathology as well as a useful index of chronic inflammation evolution.

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Resumen

Durante un período de seis semanas se han determinado la concentración sérica de grupos sulfhidrilo y las fracciones proteicas en animales a los cuales se indujo la artritis por adyuvante. Los resultados muestran, a partir del día 3, una disminución en los niveles de grupos sulfhidrilo, reducción que se mantiene durante todo el período estudiado. Se ha encontrado una buena correlación entre las alteraciones de los grupos sulfhidrilo y el grado de inflamación, determinado mediante el incremento de volumen de las extremidades posteriores, y, también, entre las primeras y los cambios detectados en las fracciones séricas proteicas.

Palabras clave: Grupos sulfhidrilo, Artritis adyuvante, Inflamación crónica.

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