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RESEARCH ON THE ELECTROPHORETIC SERUM PROTEIN PATTERN & BLOOD SERUM
MUCOPROTEINS DURING GENERALIZED MUSCULAR EFFORT

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Published experimental data from experiments carried out in dogs (11) have shown a correlation of the blood protein pattern with the total mucoproteins and active Donnaggio mucoproteins, with the condition of fatigue induced by generalized muscular effort.

The study of the electrophoretic fractions during the course of physical effort in men has given equivocal results: R. De.Lanne et al. (2) found during intense effort on the bicycle exerciser, an increase of the alpha-2 globulins; I. Nitescu et al. (9) following an effort of moderate intensity for a prolonged time, an increase especially of the gamma globulins; whereas M. Gaglio and R. Mineo (3); and P. Lotti et al. (6) following prolonged effort, found an increase of the alpha-2 and beta globulin fractions.

From our comparative studies regarding the expenditure of energy and the serum protein electrophoresis patterns in effort of average intensity carried on through to fatigue, we found a proportional change of all electrophoretic fractions, but especially of the alpha-globulin group, which are in particular related to effort (10); the hyper-(alpha-2)-globulinemia seems to be especially pronounced when the body was relatively untrained to physical exercise (12).

Following the course of the protein electrophoretic fractions in the blood serum in conditions of measured physical effort, we were able to determine the protein pattern correlating it in biological tests to the intensity of muscular work expended, and by the comparative analysis of active Donaggio mucoproteins, to evaluate the degree of fatigue reached.

Methods of investigation. The studies were carried out in 20 dogs, adult animals, weighing between eight and sixteen kilograms, who were made to expend an effort by running on a conveyor belt (60-70 minutes). Blood was taken from the external saphenous vein, sedimented with anticoagulant; a specimen was taken at rest, before effort, in order to establish a base condition, and at the end of the effort; analyses were carried out with the serum separated by centrifugation, after coagulation, non-hemolyzed. Determinations both during rest and after effort included the following: total proteins, refractometric analysis with a Pulfrich-Reiss; fractions of proteins by electrophoresis in a device for horizontal electrophoresis in Michaelis buffer of pH 8.6, an ionizing force 0.1 on Wathmann 1 filter paper, with a duration of migration of nine hours and an electrical tension of 280 volts; with staining by means of bromphenol blue; the quantitative evaluation was carried out by direct photometry of the electrophoresis patterns; with evaluation of the extinction represented in the curves expressed percentually with fractions on planimetry; the active mucoproteins responsible for the phenomena of obstacle according to Donaggio, were determined with methylene blue B extra.

Results: Statistical mathematical treatments of the values observed have shown the following:

Total proteins. Value during rest ranged between 58.1 and 67.4 grams per thousand, the average being 61.8 grams per thousand with the standard deviation sigma .32; and during the efforts the values ranged between 59.5 72.3, average being 64.6, standard deviation sigma 3.8 (fig. 1 - left side).



Fig. 1. Left: total proteins (Gm/L); right: active Donaggio mucoproteins in UD/ml during effort, compared with values at rest.

Albumins: The value during rest ranged between 28.6 and 35.2, average 31.6 and the standard deviation sigma 1.9; during effort between 26.9 and 36.1, average 30.6 and the standard deviation sigma 2.5 (fig. 2, left side).

Total globulins: Value during rest ranged between 27.2 and 33.9; the average being 30.2 and the standard deviation sigma 1.6; while with effort they range

between 30.0 and 39.9, average 34.1 standard deviation sigma 2.4 (fig. 2 - right hand side). Ratio albumins/globulins. During rest, this was considerably above 1%; the values range from 0.97 and 1.14%, the average was 1.04; with effort they were mostly under 1%; the values ranging between 0.77 and 1.02%, the average relationship being 0.91%. Alpha-1-globulins: Values during rest ranged between 0.82 and 2.15 grams per thousand; the average being 1.25 and 2.74 average 1.94 and standard deviation sigma 0.46 (fig. 3 - left). Alpha-2-globulins: Values at rest ranged between 1.73 and

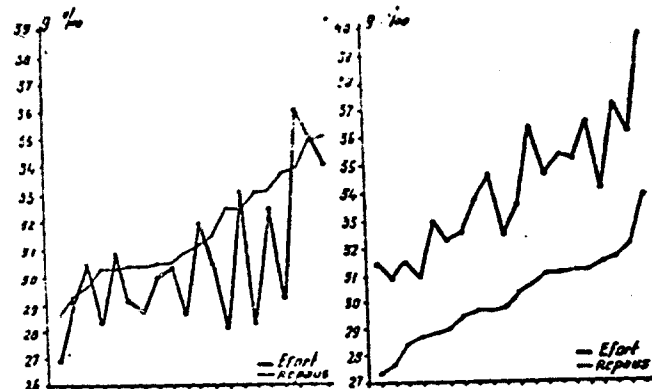


Fig.2. Left: albumins (Gm/L); right: total globulins (Gm/L) during effort, compared with values at rest.

3.16; the average being 2.35 and the standard deviation sigma 0.47; and with effort they ranged between 4.27 and 9.80, average 6.35 and the standard deviation sigma 1.38 (fig. 3 - right side). Alpha-3-globulin values at rest were between 4.24 and 5.57, average 4.89 and standard deviation sigma 0.81; while with effort they ranged between 4.02 and 6.17, average 5.22 and

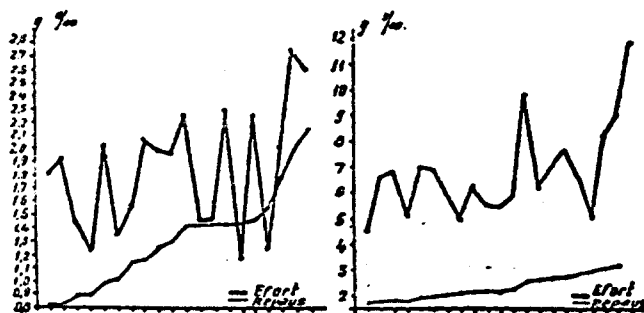


Fig.3. Left: alpha-1-globulins (Gm/L); right: alpha-2-globulins (Gm/L) during effort, compared with values at rest.

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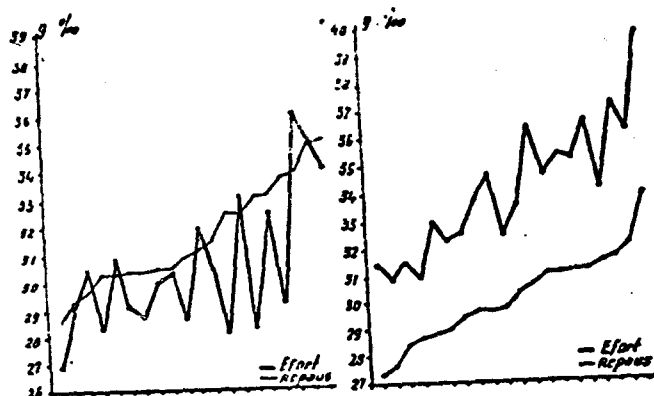


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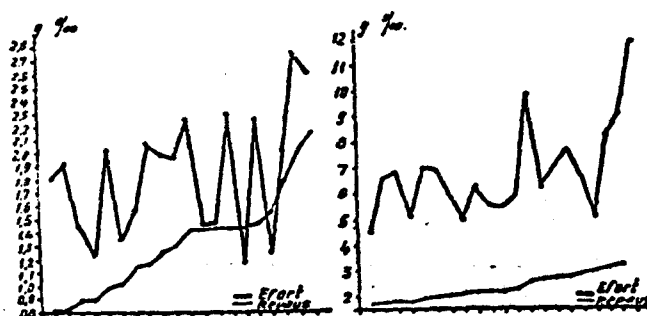


Fig.3. Left: alpha-1-globulins (Gm/L); right: alpha-2-globulins (Gm/L) during effort, compared with values at rest.

standard deviation sigma 0.59. Total Alpha Globulins. Values at rest ranged between 6.86 and 10.07, the average being 8.61 and standard deviation sigma 9.85, and with effort they ranged between 10.75 and 17.57, average 13.72 and standard deviation sigma 1.80. Total beta globulins. Values at rest ranged between 7.4 and 9.4, average 8.0 and standard deviation sigma 0.6; with effort they ranged between 6.0 and 9.1 with average 7.5 and standard deviation sigma 1.0. Total gamma globulins. Values at rest between 11.14 and 15.1, average 13.6, standard deviation sigma equal to 1.0; with effort they ranged between 10.6 and 14.9, average 12.9, standard deviation sigma 1.0. Active Donaggio mucoproteins. Values at rest ranged between 4.92 and 9.83 UD/ml; the average was 7.01 UD/ml, standard deviation sigma 1.53; with effort they ranged between 10.22 and 16.96 UD/ml, the average being 13.95 UD/ml, standard deviation sigma being 2.52 (fig. 1 - right side).

Discussion. Inspection of the various reports devoted to evaluation of paper-electrophoretic protein fractions from the blood serum of dogs reveals that the data obtained are not very easily comparable between authors, and that the physiological ranges fluctuate between very wide limits. This observation makes it mandatory to undertake a control study for the determination of normal values before starting any experimental studies, so that the base values may first be known by repeated tests under various conditions. Following this principle M. Mosinger et al. (7) felt that it was possible to limit the errors to a minimum depending on the natural variation which occurs in dogs. Working in this manner, in a constant environment and with values established in dogs exposed to effort, the results may have a value independent from any inherent technical errors of the study.

Comparing our results with regard to different electrophoretic fractions, with data obtained by work with normal values as reported by various authors who have worked under similar conditions, we find that the average values reported by J. A. Herman (5), can be interpreted quite differently in view of the values reported by W. Boguth (1). Analyzing our results in this way we identify seven globulin fractions in the serum of dogs for which the electrophoresis patterns were reproduced by P. Groulade and J. Groulade (4), and M. Mosinger et al. (8) who have not distinguished further between five globulin fractions. We may comment that in our technique of staining the electrophoresis pattern on paper we utilize a bromphenol blue, whereas the results reported in the literature were obtained with starch black. These aspects do not permit a rigorous comparison of the results obtained by these authors and similarly with the patterns of serum proteins electrophoresis of human serum, obtained in different conditions and showing different fractions, with the number of fractions presented by the authors.

In general, the fractions alpha-3, beta-1, gamma-1, present rather considerable variations at the time when these constituents are quite small and do not exceed 10% of the average value; because of this fact it is difficult to obtain good reproducibility of data and it is essential to carry out experiments with individual animals, comparing the results obtained with rest with dogs following generalized muscular efforts, rather than with a

norm in dogs at rest.

In conditions of physical effort, it is generally observed that there is a modification with regard to the percentual composition of all of the electrophoretic fractions, but especially in a constant manner, of the group of alpha fractions. These variations in the sense of an increase of concentration, following physical effort, with comparison to the values obtained during rest, are especially present in globulins alpha-1, and most pronouncedly among alpha-2-globulins, which show very clear differences; whereas the albumin tends to decrease and the beta and gamma globulins show relatively insignificant variations.

Correspondingly we see that the concentration of active Donaggio mucoprotein in the blood serum becomes an important value, which moves in the sense of globulin fractions of the alpha group, showing a relationship to their behavior in this sense.

These patterns of active Donaggio mucoproteins and of the electrophoretic fractions of the gamma globulins during the course of generalized muscular effort suggest a theory according to which the protein pattern may represent a biological test revealing the intensity of the muscular effort expended; and by comparative analysis of the mucoprotein pattern, it may be possible to deduce the degree of fatigue experienced.