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Influence of Age and Stress from Physical Activity on the Redistribution of Lymphocytes

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The influence of age and stress on the redistribution of lymphocytes between the intra- and extra-vascular spaces has been studied. Young (12 ± 4 weeks) and old (68 ± 6 weeks) mice were made to swim until exhaustion, with and without a previous period of training. Lymphocyte concentration in the blood and in the peritoneal cavity, was evaluated as well as the weight of spleen and thymus in these animals. In parallel, and as an indicator of the level of stress, quantification was made of the serum corticosterone levels and weight as well as the protein, DNA, and RNA content of the adrenal glands. Results indicate that, after being subjected to stress, the young mice present no changes in lymphocyte number in blood or peritoneal cavity. Old mice, however, after acute physical activity, present a greater lymphocyte content in the intravascular compartment, as well as a fall in the peritoneal lymphocyte concentration and spleen weight. The data could indicate that age modifies the response to stress, with an augmentation of lymphocytes in the intravascular compartment at the expense of the extravascular one.

Key words: Age, Physical activity-stress, Lymphocytes.

There is a generalized belief that an individual's immunological capacity decreases with increasing age. In consequence, a greater susceptibility of old individuals to contracting opportunist infections, that in many cases turn out to be incurable, is attributed to this diminished capacity of the defensive response. Nevertheless, the immune response may also be found to be affected by different external factors such as stress, environment, etc. (7, 12).

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The lymphocyte is the main cell in the immune response development. It is generally accepted that the lymphocyte number, fundamentally the Ts, is smaller in old age (9) probably due to an involution of the thymus, although there is currently much controversy on this point (17, 19, 20). It is also known that stress from physical activity provokes changes in lymphocyte blood number (10), probably due to a redistribution between the extra- and intra-vascular spaces induced by the release of different hormones (1).

In the present work a comparative evaluation of the influence of stress from extenuating physical activity on the number and redistribution of the lymphocytes in young and old individuals has been attempted. For obvious reasons, numerical and functional changes in human lymphocytes after exercise stress in tissue compartments other than the circulation have not been elucidated. This work was thus performed on the BALB/c strain of mice, as they represent a good model for this type of study. The number of lymphocytes in blood and peritoneal cavity was quantified, as well as the weight of spleen and thymus as they represent the sites of important lymphocyte reservoirs, and, as suggested by GISLER *et al.* (6), the weight of immunocompetent organs is directly related to the number of cells which they contain. In parallel, and as a measure of the degree of stress to which these animals are subjected, we measured the weight and content in proteins and nucleic acids of the adrenal glands, as well as corticosterone serum levels .

Materials and Methods

Animals.– Male BALB/c mice *(Mus musculus)* (IFFA CREDO) both young (12 ± 4 weeks old) and old (68 ± 6 weeks old) were used. They were maintained at a constant temperature (22 ± 2 °C) on a 12 h light/dark cycle and fed with Sander Mus and water *ad libitum*. The mice were examined, and only those without signs of malignancy or other pathological manifestations were used in the experiments.

Physical stress.– Acute Physical Activity (APA) : Mice were put into individual tanks containing 7 litres of water (at 25 ± 2 °C), and were kept swimming continuously until exhaustion. Mean duration of exercise was 30 ± 5 minutes.

Acute Physical Activity after Training (APAT): Mice were exposed to the same physical activity (swimming until exhaustion), but after a training program of 25 min/day continuous swimming for 1 month.

Immediately following the acute stress, animals were sacrificed by decapitation. Control animals were maintained under the same laboratory conditions but not subjected to the physical stress.

Weight.– Body weight was measured in each group, and weights of spleen, thymus and adrenals were determined both absolutely and as a ratio of the total body weight (relatively).

Lymphocyte-counting.– Lymphocytes were counted in both blood and the peritoneal cavity. After killing the animals by decapitation, 2.5 ml of blood was collected. Then, for each animal, the abdomen was cleansed with 70 % ethanol, the abdominal skin was carefully dissected without opening the peritoneum, and 4 ml of Hank's solution (Sigma) adjusted to pH 7.4 was injected i. p. Cells (macrophages and lymphocytes) were removed, with recovery of 90-95 % of the injected volume. Lymphocyte counts in both blood and peritoneal cavity were performed using standard methods. Cell viability was never less than 95 % as deter-

mined by the trypan blue (Sigma) (0.4 %) exclusion assay.

Serum corticosterone assay.– Blood was centrifuged at 330 g to obtain serum. Serum corticosterone levels were measured by using a commercial radioimmunoassay kit (Cambridge Medical Technology Corporation, Billerica, MA, USA)

Evaluation of nucleic acids (DNA and RNA) in adrenal glands.– The technique described by SCHNEIDER (15) was followed, by carrying out a low temperature extraction of the compounds soluble in acids (trichloracetic acid) and in alcohol (ethanol 96 %), for their subsequent determination via colorimetry.

Evaluation of total proteins in adrenal glands.– After homogenization of the adrenal glands, the total protein content was evaluated using a Biuret and Folin-Ciocalteu commercial kit (Sigma).

Statistical analysis.– The results are expressed as the means ± SD of the number of experiments indicated in the corresponding tables and figures. In the statistical study, the normality of samples was confirmed (normality test). Student's *t* test for unpaired samples was used for comparison between parametric samples, $p < 0.05$ being the minimum significance level.

Results

The number of lymphocytes (cell/mm^3) found in the blood and peritonal cavity of young and old mice who had undergone APA or APAT is shown in figure 1. One observes a significant ($p < 0.05$) rise in the number of lymphocytes in blood in old mice after physical activity until exhaustion. The number of peritoneal lymphocytes of old mice have significantly greater

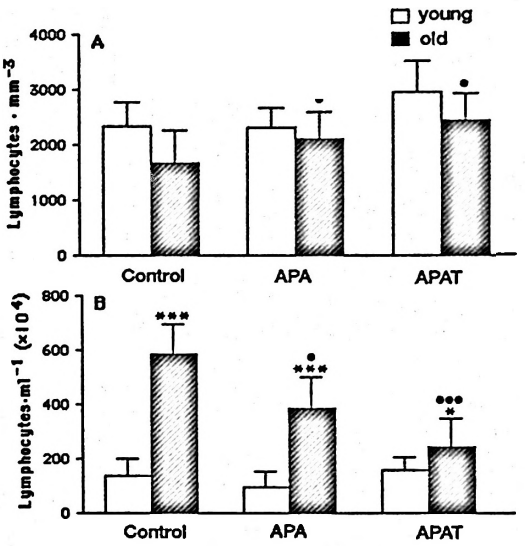

Fig. 1.- *Changes in the number of blood (A) and peritoneal (B) lymphocytes in young and old mice which had undergone Acute Physical Activity (APA) or Acute Physical Activity after Training (APAT).* Each column represents the mean ± S.D. of 10 experiments performed in duplicate. •$p < 0.05$ •••$p < 0.001$ significant differences from their controls. *$p < 0.05$ ***$p < 0.001$ significant differences between young and old mice.

values than the young mice, although there is a drop after the old animals are subjected to stress from physical activity.

Table I shows that old mice have heavier spleens and adrenal glands than the young mice. As it was to be expected however, the thymus was not as heavy in the old animals as in the young. Performing the acute physical activity, with or without a previous period of training, provokes changes in the old animals, there being a drop in the weight of the spleen and a rise in that of the adrenal glands. Table II shows the DNA content of the adrenal glands and indicates that the old animals present a lower DNA content whether the results are expressed as absolute or relative values. The physical activity performed after a previous period of training provokes an increase in the

Table I. *Absolute (mg) or relative (mg/100 g b. w.) weights of spleen, thymus and adrenals in young and old mice subjected to stress from physical activity.*
Each value represents the mean ± S.D. of 10 experiments performed in duplicate. * $p < 0.05$, significant differences from their controls. •$p < 0.05$ significant differences between young and old mice.

Organs		Young mice			Old mice		
		Controls	APA	APAT	Controls	APA	APAT
Spleen	Absolute	89.7 ± 26	110 ± 21*	97.0 ± 30	131 ± 30•	110 ± 18*	100 ± 20*
	Relative	350 ± 91	380 ± 60	328 ± 66	476 ± 82•	404 ± 46*	375 ± 65*
Thymus	Absolute	32 ± 8	31 ± 4	27± 8	17.5 ± 4•	18 ± 5•	20.6 ± 6•
	Relative	130 ± 43	106 ± 12	95 ± 31*	63 ± 15•	66 ± 12•	78 ± 27
Adrenals	Absolute	3.6 ± 0.3	3.8 ± 0.4	3.9 ± 0.6	5.4 ± 0.8•	5.9 ± 0.6*•	6.2 ± 1.3*•
	Relative	14 ± 2	13 ± 1	14 ± 3	19 ± 3•	23 ± 4*•	24 ± 4*•

Table II. *DNA content (µg) of the adrenal glands (mg) in young and old mice subjected to stress from physical activity.*
Legend as in table I.

DNA	Young mice			Old mice		
	Controls	APA	APAT	Controls	APA	APAT
Total	40.5 ± 7.76	32.66 ± 7.17	83.16 ± 18.53*	32.0 ± 10.87	26.83 ± 5.49	52.5 ± 10.78*•
Absolute w.	10.96 ± 2.26	7.64 ± 3.77	22.17 ± 6.31*	5.82 ± 1.8•	4.72 ± 1.18•	7.30 ± 1.89•
Relative w.	3.05 ± 0.59	2.07 ± 1.85	6.67 ± 1.9*	1.56 ± 0.46•	1.17 ± 0.45•	2.08 ± 0.67•

Table III. *RNA content (µg) of the adrenal glands (mg) in young and old mice subjected to stress from physical activity.*
Legend as in table I.

RNA	Young mice			Old mice		
	Controls	APA	APAT	Controls	APA	APAT
Total	65.83 ± 2.04	77.5 ± 16.5	65.83 ± 6.30	71.66 ± 0.51•	71.83 ± 1.94	77.0 ± 3.28*•
Absolute w.	17.75 ± 0.92	17.89 ± 2.2	17.75 ± 4.67	13.58 ± 2.48•	12.89 ± 3.69•	11.04 ± 2.39•
Relative w.	4.94 ± 0.16	4.86 ± 0.91	5.34 ± 1.43	3.54 ± 0.17•	3.34 ± 0.83•	3.09 ± 0.45•

Table IV. *Total protein (mg) in adrenal glands in young and old mice subjected to stress from physical activity.*
Legend as in table I.

Protein	Young mice			Old mice		
	Controls	APA	APAT	Controls	APA	APAT
Total protein	0.51 ± 0.11	0.69 ± 0.10	0.69 ± 0.14	0.74 ± 0.06•	0.82 ± 0.03•	1.07 ± 0.19*•
Protein/absolute weight (mg)	0.13 ± 0.03	0.18 ± 0.04	0.16 ± 0.02	0.13 ± 0.01	0.12 ± 0.01•	0.15 ± 0.01*
Protein/relative weight (mg)	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.00	0.03 ± 0.01•	0.04 ± 0.01

DNA content for both young and old mice, although the differences do not reach significant levels in the latter. With respect to the RNA content of the adrenal glands (table III), there is a significant fall in the relative values for the old mice versus the young.

The protein contents of adrenal glands from young and old mice are listed in table IV which shows that there are no variations between young and old mice when the results are expressed as relative values. There was a rise in total proteins (mg) after APAT.

Lastly, figure 2 shows the serum corticosterone concentrations. Both the APA and APAT groups in young and in old animals have greater values than those found in their respective controls, the greatest value corresponding to the old animals of the APA group.

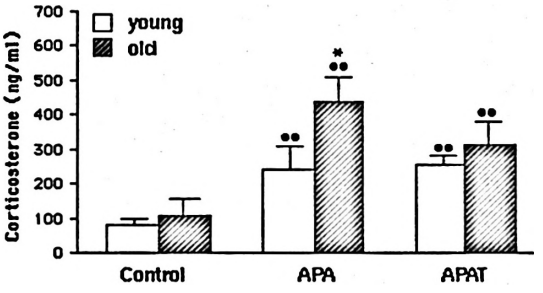

Fig. 2. *Changes in concentration of corticosterone obtained in young and old mice who had undergone Acute Physical Activity (APA) or Acute Physical Activity after Training (APAT).*
Each column represents the mean ± S.D. of 10 experiments performed in duplicate. *p < 0.05 significant differences from their controls. ••p < 0.01 significant differences between young and old.

Discussion

To our knowledge, the research described herein represents the first study to systematically compare the effect of physical activity stress on the distribution of lymphocytes in young and old animals. For this, we chose young (12 ± 4 weeks) and old (68 ± 6 weeks) BALB/c mice, the life expectancy of this strain being 70 weeks (8, 11). Like other workers (3, 7, 11), we chose forced swimming until exhaustion as the model of stress. Stress in our animals was detected by the rise in the levels of serum corticosterone and by the activation of the adrenal glands after performing the forced swimming. The comparison of the lymphocyte number in young and in old mice showed no significant modifications in the results for blood. This is in agreement with most other authors (13, 16, 18), who indicate that the immunodepressed state in old mice is not due to a smaller number of lymphocytes but to a reduction in the percentage of those that are functionally normal.

We have found, however, a greater number of lymphocytes in the peritoneum of old animals as compared with young animals. This could be due to a transmission of these cells from the blood into the peritoneal cavity, since a slight fall in the number of lymphocytes in blood in the old mice is appreciated although non-significantly as against the young mice.

As to the weights of the spleen and thymus, our results are similar to those of other workers. Thus, FORESTA *et al.* (4) also found heavier spleens in old mice. As the weight of the spleen is directly proportional to the number of cells it contains according to GISLER *et al.* (6), the spleens of old animals are expected to contain more lymphocytes than those of young animals. From that it would appear that old animals present a greater content of lymphocytes in the extravascular spaces.

The higher adrenal gland weights of the old mice coincide with the findings of DELLWO and BEAUCHENE (2) in rats, who indicate that the reason is a greater activity of this gland in old animals, the hypo-

thalamus-pituitary-adrenal axis presenting stimulation in old age (2).

With respect to the effect of stress from extenuating physical activity on the lymphocyte concentration and its possible redistribution between the intra- and extra-vascular spaces, our results indicate that, in the young animals, there are no statistically significant changes in either blood or peritoneum lymphocyte concentrations, nor in the weights of spleen and thymus. This fact seems to indicate that stress from forced swimming until exhaustion, in young animals, does not provoke a marked redistribution of the lymphocytes between the compartments under study. Observing, however, the results obtained in old animals, we find that after the forced swimming there is a rise in the concentration of lymphocytes in blood, while their peritoneal concentration falls, most markedly in the APAT group. A decline in the spleen weight after performing the forced swimming is also observed.

This indicates that when individuals in old age are exposed to a stressful situation, the content of lymphocytes in the intravascular space seems to increase at the cost of the extravascular reserve. This fact may be very important in clinical practice, since epidemiological studies have correlated elevated white cell counts with the incidence of, and mortality from myocardial infarction (5, 14).

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E. ORTEGA, A. FORNER, M. GALÁN, M. DE LA FUENTE y C. BARRIGA. *Influencia de la edad y el estrés por actividad física sobre la redistribución de linfocitos.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 50 (3), 159-166, 1994.

Se estudia cómo la edad y el estrés influyen sobre la redistribución de linfocitos entre los espacios intra y extra-vasculares. Se utilizan ratones jóvenes (12 ± 4 semanas) y viejos (68 ± 6 semanas) sometidos a natación forzada hasta el agotamiento, con y sin periodo previo de entrenamiento, evaluando la concentración de linfocitos en la sangre y en la cavidad peritoneal, así como el peso del bazo y del timo. De forma paralela, y como un indicador de los niveles de estrés, se cuantifican los niveles de corticosterona presentes en el suero y el peso, proteínas y contenido de ADN y ARN en las glándulas adrenales. Los resultados indican que, en los ratones jóvenes, después de someterse a la prueba de estrés, no se aprecian cambios en el número de linfocitos de la sangre o de la cavidad peritoneal. Los ratones viejos, después de realizar una actividad física aguda, presentan un mayor contenido de linfocitos en el compartimiento intravascular, y un descenso en la concentración de linfocitos peritoneales y en el peso de los bazos. Estos datos parecen indicar que la edad modifica la respuesta en la redistribución de los linfocitos frente al estrés, produciendo un aumento de linfocitos en el compartimiento intravascular a expensas del extravascular.

Palabras clave: Edad, Actividad física-estrés, Linfocitos.

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