

Criteria for Physiological Stress Produced by Increased Chronic Acceleration* (33078)

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The physiological phenomenon of stress as described by Selye as part of a *General adaptation syndrome* is well known (1). This syndrome has been incriminated as significantly affecting the physiology and biochemistry of animals in a general yet consistent way regardless of the *Initial stimulus*.

In 1953 Sir Bryan Matthews found that rats could adapt to living on a centrifuge for several months at an accelerative intensity of $6G$ (2).¹ This finding enabled the use of the centrifuge and increased chronic acceleration adaptation to investigate the effects of weight as opposed to mass upon the physiology and anatomy of laboratory animals. Since that time several laboratories have been conducting this type of research (3-6).

Recently increased chronic acceleration has been recognized as a stressor eliciting a *General adaptation syndrome* the pathology of which is known as "chronic acceleration sickness" (7) indicating, that certain data obtained from acceleration studies should be evaluated relative to the physiological status of the animal.

Accordingly, criteria for physiological status relative to stress were developed by comparing hematological observations with exercise capacity, survival, and sexual development in chickens exposed to increased chronic acceleration. Another criterion, the response of lymphocytes—relative and absolute lymphopenia—in the chicken to various stressors and to injections of cortical and corticotrop-

ic hormones, is well documented (8-13).

Methods. A "selected" strain of adult male single comb white leghorn (SCWL) chicken developed for its resistance to "chronic acceleration sickness" was the experimental animal used in this study (7, 14). All of the birds were maintained using standard poultry husbandry practices. The use of the chicken as a laboratory animal in this type of study has been previously reviewed (14).

The birds were exposed continuously for several months, except for approximately 10 min each day necessary for animal care, to increased accelerations up to $3G$ in a 20-foot diameter centrifuge which has been partially described in the literature (15, 16). The birds were gradually introduced to their final acceleration intensity by increasing the field in $0.5G$ increments at 1-2 week intervals. Thus, a bird living for 1 week at $3G$ has actually been exposed to increased accelerations for a minimum of 1 month. For details regarding this type of environmental research see Ref. (16).

The hematological determinations were made using standard methods described by Schalm (17) and Lucas and Jamroz (18).

The exercise capacity of each chicken was determined at normal gravity prior to being centrifuged. These capacities were repeated at normal gravity, immediately upon removal from the centrifuge, after the birds had been at $3G$ for 1 week. The exercise capacity was measured as the duration of time necessary to exhaust adult male chickens on a treadmill (130 feet/min, at an incline angle of 4°).

Results. One week's exposure to $3G$ produced a mean exercise capacity in these 22 birds of 24.4 ± 5.8 min as compared to 119.8 ± 13.5 min for the 10 control chickens. The difference between these group means is highly significant $p < 0.001$. Similarly, the relative lymphocyte count of the centrifuged birds as a group ($33.3 \pm 3.75\%$) was significantly lower ($p <$

* Supported by NASA (Grant NGR-05-004-008).

¹ Notations of accelerative forces can be in absolute units (e.g., dyne) or relative terms (e.g., multiples of the earth's gravitation). Gravitational acceleration is conventionally designated "g."

$$F_{(\text{absolute})} = m \cdot a$$

$$F_{(\text{relative})} = m \cdot a/g = m \cdot G$$

Since G is a ratio of accelerative forces it becomes dimensionless. Detailed discussion of units and terminology regarding acceleration forces are available (22, 23).

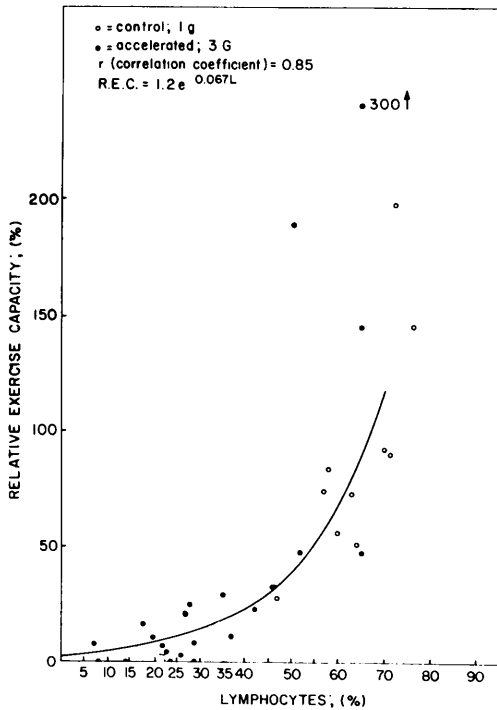


FIG. 1. Relationship of the percent lymphocytes with the relative exercise capacity (REC) of each bird.

0.001) than the control group ($63.8 \pm 2.76\%$).

The relative exercise capacity (REC) of each bird of the (1g) control and 3G accelerated groups was determined mathematically: $REC = X_2/X_1 \times 100$ (1). Where: REC = relative exercise capacity (%); X_1 = pre-acceleration exercise capacity in minutes; and X_2 = exercise capacity at normal gravity in minutes after 1 week's exposure to 3G acceleration.

The bird's relative exercise capacity was individually compared with its current relative lymphocyte count and the resulting relationship is mathematically best described with an exponential equation (Fig. 1): $REC = 1.2e^{0.067L}$ (2). Where: REC = relative exercise capacity (%); Eq. (1); and, L = lymphocytes as a % in a differential count; r (correlation coefficient) = 0.85; $p < 0.01$.

It is evident from Fig. 1 that the improved exercisers cannot be distinguished by an increase in lymphocytes above control (normal) values. Interestingly, three birds exer-

cised better after being exposed to the increased acceleration than they did prior to being placed on the centrifuge. These three birds were below average preacceleration exercisers; however, their postaccelerative performances were generally far above their group mean; i.e., the group mean exercise capacity was 18 min while these birds had a mean exercise capacity of 57 min. A poor preacceleration exercise performance, however, was no criterion for survival.

This general reduction in the exercise capacity of these birds, after 1 week's exposure to 3G increased acceleration, represents a debility—a stress response. Although longer acceleration exposures resulted in a reduction in the circulating erythrocytes (reduced hematocrit), which could effectively reduce the exercise capacity, the hematocrits after 1 week at 3G ($39.6 \pm 0.8\%$) were the same as those of the control 1g birds ($39.6 \pm 0.7\%$).

The hematocrits and relative lymphocyte counts were compared in 108 male birds living at 2.5G for approximately 6 months, and the relationship appeared to be rectilinear (Fig. 2):

$$PCV = 38.6 + 0.10L \quad (3)$$

Where: PCV = packed cell volume (hematocrit); and L = Lymphocytes (%); r (correlation coefficient) = 0.38; $p < 0.01$.

Similar comparisons on other populations of birds living at 1g (normal gravity) and at 1.75G for 6 months revealed no relationship. However, none would be expected since

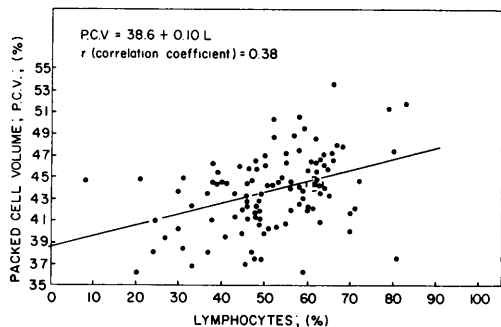


FIG. 2. (●) represents the % of lymphocyte and hematocrit values of 108 birds, which had been exposed to increased chronic accelerations of 2.5 G for 6 months; (■) represents the mean values \pm SE of the control (1 g) group.

1.75G is not of sufficient intensity to produce a stress response in adult male chickens (8).

It has been experimentally shown in the male chicken that the number of circulating erythrocytes is indicative of its sexual development (19). It is quite likely therefore, that the observed reduction in the hematocrits of these 2.5G stressed birds may be a function of the level of circulating androgens. In this regard atrophic testicles are a common finding in birds dying of chronic acceleration sickness (7).

The differential leukocyte counts of the animals that had been at 3G for 1 week were compared with their survival after 3 months of that treatment. Those birds which survived 3 months of exposure to 3G (45% of the initial population) had early lymphocyte counts as a group of $44.4 \pm 5.2\%$ while those that died during this period of 3 months had lymphocyte counts of $23.6 \pm 3.2\%$. These means are statistically different at the 1% significance level. The mean survival time for those birds which died was 42.3 days. The birds after one week's exposure to 3G with a lymphocyte count of 30% or less had only 17% survival, while birds with lymphocyte counts above 30% had 70% survival. It was not possible to quantitatively predict when the nonviable animal would die during the 3 months exposure to 3G; i.e., no relationship was apparent between the percent lymphocytes and survival time.

Discussion. The ability to identify, with a 70% accuracy after 1 week's exposure, animals that will survive more than 3 months' exposure to 3G indicates that the fate of an animal to this stressful environment is decided very early, with apparently little regard to the functional and/or organic changes elicited by its corticoid activity. In other experiments we have found that cortical injections (prednisolone) and ACTH injections were of no value in reducing the mortality accompanying increased chronic accelerations. The mean survival time of the susceptible population is considerable (42 days), so it may be possible to institute treatments, should any exist other than simply removing the stressor, to reverse the lethal effects of chronic

accelerations. This, of course, would be of substantial advantage in understanding the biological effects of increased accelerative forces.

Various physiological stressors elicit specific as well as unspecific reactions, and these lead to physiological adaptation. The proportions of each type of reaction is dependent upon the nature of the stressor—the more general the effect of a stressor on the body, the more important the unspecific adaptive system becomes (20). The specific changes which promote adaptation to increased chronic accelerations are unknown. However, the more general reaction resulting from corticoid activity, involved in the adaptive process, apparently is of some value in this type of stressor. Selye suggests that the corticosteroids are essential for the acquisition of physiological adaptation to a stressful environment (1). In this investigation, an intense corticoid activity, indicated by the decreasing lymphocyte response, appeared to have little effect upon the outcome of the adaptive process. This suggests that adaptation to increased chronic acceleration is the result of a more specific type of reaction and the unspecific adaptive process is probably of less value.

In our investigations, relative to the physiological effects of increased acceleration, it has been found that adaptation to this hyperdynamic environment is accompanied by an increase in the PCV (hematocrit) and erythrocyte count (to be published) and no appreciable effect upon the exercise capacity of the animal when it is exercised at normal gravity (21). Yet, as reported herein, these same physiological parameters are significantly impaired during the stress period. The inability to determine the physiological status of the animal, distinguishing "stressed" and "adapted" individuals may lead the investigator to a poor resolution of the data. Regarding this, physiological fitness relative to exercise capacity, sexual development, and survival of an individual with respect to a stressful environment, increased chronic acceleration, may be quantitatively determined in the chicken by hematological methods.

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Received Feb. 19, 1968. P.S.E.B.M., 1968, Vol. 128.

Induction of Methionine Adenosyltransferase in Rat Liver by Corticosteroids* (33079)

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Transmethylation transects nearly every area of metabolism (1). It is an important means of altering the biological activity of many compounds including RNA and DNA (2,3). Methionine, in its active form, *S*-adenosylmethionine, is a major donor of methyl groups in mammalian tissues (4). It may also play a role in the regulation of folic acid-mediated (5,6) and other metabolic pathways (7). Methionine adenosyltransferase (ATP: *L*-methionine *S*-adenosyltransferase, EC 2.5.1.6) is the specific activating enzyme responsible for the formation of *S*-adenosylmethionine from *L*-methionine and ATP

(8). It is also the first enzyme on the catabolic pathway from methionine leading to a variety of products.

Our previous work (9) has shown that methionine adenosyltransferase activity was increased in the livers of both intact and adrenalectomized rats treated with cortisone, while adrenalectomy caused a slight decrease in level of this enzyme. Marked elevation of the enzyme activity also occurred when dietary protein levels were increased (9) and following the induction of alloxan-diabetes.¹ The present study is concerned with the specificity of the response of hepatic methionine adenosyltransferase to different types of steroid hormones and the mechanism un-

* Supported in part by a grant from the China Medical Board of New York, Inc. (Grant No. 66-882).

¹ Lien Shou and Foo Pan, unpublished data.