Hormonal determinants of mammalian tolerance to acceleration stress

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POLIS, B. DAVID. *Hormonal determinants of mammalian tolerance to acceleration stress.* J. Appl. Physiol. 16(2): 211–214. 1961.— The pituitary-adrenal hormonal axis has been implicated as a critical factor in the survival of the rat to acceleration stress. The physiological end point for the tolerance of animals to high positive acceleration stress was obtained by determining the time to reduce the heart rate from 8 to 2 beats/sec. at 20 *g*. With this technique, a significant increase (300%) in the survival time of rats to 20 *g* was found following hypophysectomy. A significant loss (60%) of tolerance to high acceleration stress was found in adrenalectomized rats. The procedures of hypophysectomy plus adrenalectomy apparently opposed the effect of each operation alone in the rat to yield an animal that tends to approach the normal rat in its ability to tolerate acceleration. A significant drop in the tolerance to acceleration of hypophysectomized rats was found 7 and 15 weeks after operation compared to that found with the animals 3 weeks after hypophysectomy and a significant increase in the tolerance of the adrenalectomized rat was found 11 weeks after operation compared to the tolerance at 5 weeks after adrenalectomy. In both operated populations, the direction of change was toward the tolerance level of the normal rat.

STUDIES ON THE PHYSIOLOGICAL EFFECTS of acceleration stress have been conducted primarily for the purpose of developing protective procedures or devices to increase the duration of useful conscious effort. In most of these studies the marked functional deficiencies experienced by individuals subjected to high acceleration were related largely to circulatory difficulties. It followed that hemodynamic concepts dominated most investigations; yet, from an etiological standpoint, the sequence of events that predetermine survival or even useful effort are cellular in origin. At the cellular level, physiological stress may be defined in terms of substrate supply and energy demand. Under conditions of limited supply, the distribution of energy available for vital cellular functions conceivably could be controlled by hormonal mechanisms. In an effort to define those hormonal factors that contribute to the mammalian tolerance of acceleration stress, the variation from the normal introduced by the experimental procedures of hypophysectomy and adrenalectomy was investigated.

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PROCEDURES

Male Sprague-Dawley rats of the same age, weighing approximately 200 gm, were divided into control and operated groups of hypophysectomized or bilaterally adrenalectomized rats. Both control and operated groups of animals were housed under identical conditions and maintained on the regular diet of Purina laboratory rat chow that was softened in milk and reinforced with canned meat, oranges and carrots. Adrenalectomized rats were given 0.15 M saline for drinking water. A period of at least 3 weeks elapsed between operation and exposure to acceleration stress to allow for complete recovery from the effects of the operative procedure. In this period, death rates were negligible in the normal groups, in the order of 5% in the hypophysectomized groups, and approximately 25% in the adrenalectomized groups.

Since diverse factors act out synergistic roles in the syndrome of acceleration stress, it is to be expected that a rat population would be distributed over a considerable tolerance range in the response to the stress. Obviously, the facility for determining an individual survival limit was desirable. By recording the electrocardiogram (ECG) during acceleration, which reflected, among other things, the heart rate, an avenue for the attainment of a reliable physiological end point became apparent.

Electrocardiograms from the rat were obtained by means of electrodes made of no. 22 Michel (nickel silver) wound clips to which were soldered 10-in. insulated wire leads. These were inserted in the skin at the chest (epigastric area) and back (interscapular area). The size of the tines was found to be an important factor in obtaining a relatively noise-free cardiac potential that was differentiated from other muscle potentials. The clips were insulated from the animal restraining cage by means of loosely wound adhesive tape. The rat was then wrapped in a wire mesh restraint and mounted on an 8-ft. radius animal centrifuge with the long axis of its

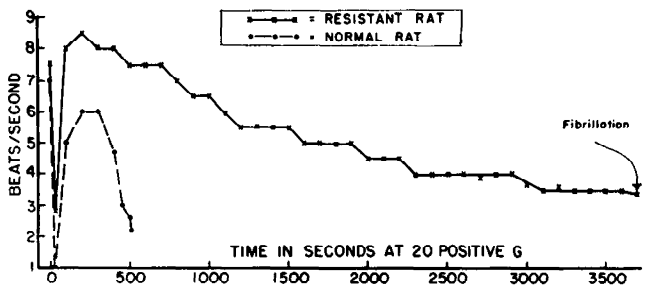


FIG. 1. Heart rate of rat as an index to survival under high acceleration stress.

body parallel to the radius of the centrifuge and its head toward the center. This position with the force, due to acceleration, acting in a direction from head to foot is defined as positive *g*. Since electron tubes are not reliable under conditions of high acceleration stress and vibration, a transistor amplifier (1) was used. The ECG signal from the rat is about 150 μv. This was amplified about 3000 × by the transistor amplifier to provide a good signal-to-noise ratio before the slip rings. The amplified ECG signal was transmitted to the centrifuge slip rings where it was picked up by brushes that were wired to a Brush type B L 905 amplifier and type B L 206 ink-writer recorder.

From *g*-time studies made by Cranmore (2) to delineate 100% survival and 100% fatality of rats, the factor of 20 *g* was used for an acceleration level that permitted the realization of a convenient experimental time for the proposed studies. At higher *g* levels, the survival time became too short for any reliable differences to become manifest; at lower *g* levels, the survival time became too prolonged for any extensive series of studies to be carried out conveniently.

At 20 *g* the heart rate of the rat showed an unusual time response to the stress of acceleration (fig. 1). Normally, i.e. at 1 *g*, the heart rate of a rat restrained and manipulated for electrode insertion ranged from 7 to 8 beats/sec. When the animal was first subjected to 20 *g*, the heart rate fell precipitously during the first 15 seconds, approaching a minimum rate of 1 beat/sec. From this initial 'shock' state the heart recovered rapidly to attain and maintain a resistance plateau near the level of the original heart rate. Finally a state of exhaustion was approached that was characterized by a rapid fall in heart rate and, if centrifugation was continued, death ensued. The obvious difference between a normal rat and one resistant to acceleration resided, at least superficially, in the ability of the animal to maintain a prolonged resistance stage. The fall in heart rate was gradual rather than abrupt. After a warning signal of rapid irregular activity that might be interpreted as fibrillation, the subsequent survival of the animal depended on the length of time the animal was exposed to the stress after the heart rate fell below 3 beats/sec.

Taking as an end point the time at which the heart rate falls to between 2 and 3 beats/sec. for a period not greater than 10 seconds, approximately half the experimental population survived. When the heart rate was

TABLE 1. *Survival of the Normal Rat at 20 g as a Function of Heart Rate*

No. of Rats	Heart Rate When Centrifuge Was Stopped (Range)	Survival
	beats/sec.	
20	0.5–1.5	5.3
25	1.6–2.0	12.0
25	2.2–2.7	56.0
8	3.3–5.5	88.0

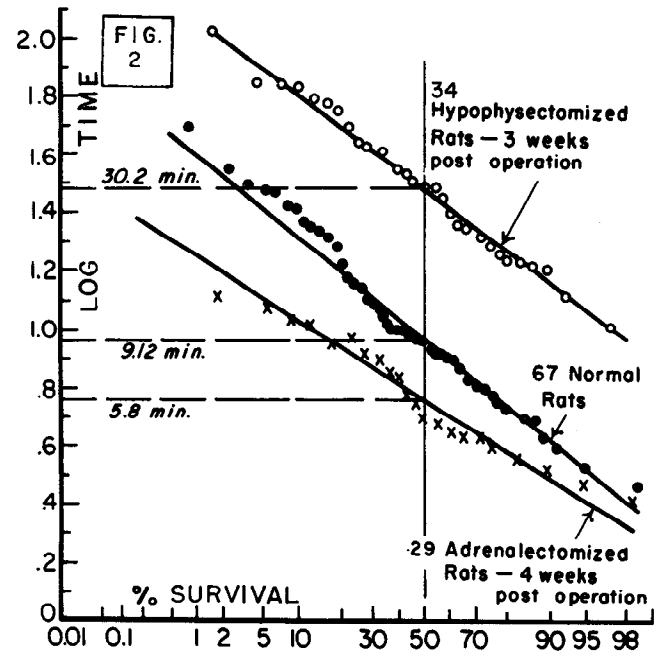
permitted to fall below 2 beats/sec. for an equivalent period, the rats did not survive (table 1). This criterion, i.e. the time for the heart rate to fall to 2 beats/sec. for 10 seconds, offered a relatively sharp demarcation of survival to *g* stress and was used to define a physiological end point for the rat's tolerance to acceleration stress.

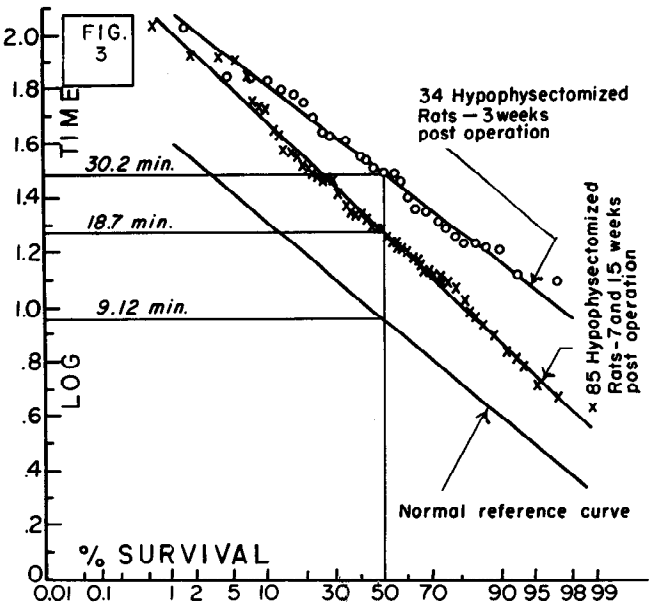
Earlier investigations of acceleration stress on rats used a fixed time for approximating the period for half the population to die and evaluated any factor as the significant deviate from this number (3). Alternatively, a more valid procedure involved setting up a design of paired control and treated animals subjected to additive intervals of centrifugation time until death occurred. This procedure did not require the assumption that the fatal spin numbers, i.e. the number of intervals till death, were normally distributed. The data were then evaluated using the usual binomial coefficient expansion as in the analysis of coin-tossing (4). In the present study, it rapidly became apparent that the time-mortality response of rats to acceleration stress was not normally distributed. By using the log time transformation according to Bliss (5), the rat populations under *g* closely approximate a normal distribution which considerably facilitated the statistical handling of the data.

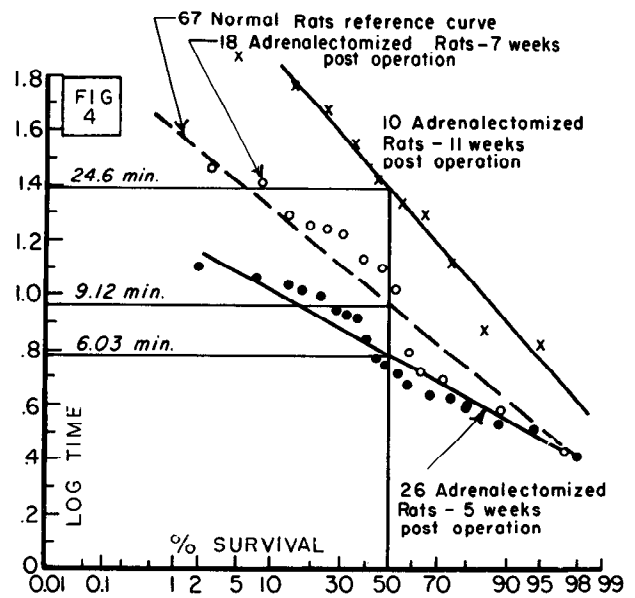
In figure 2 there is plotted the response of three rat populations, hypophysectomized, normal and adrenalectomized, to acceleration at 20 *g*. It is apparent that with the log time transformation the percentage survival follows a normal curve distribution which becomes a straight line when plotted on probit paper. Both control and hypophysectomized rats show approximately the same standard deviation, i.e. they have approximately the same slope, but the median survival of the hypophysectomized rats is about 30 minutes at 20 *g* compared to 9 minutes for the controls. This difference is significant with the possibility of chance occurrence at less than one in a thousand (table 2).

Following hypophysectomy the usual response pattern of the rat results in a characteristic atrophy of the adrenal cortex. Therefore it was of interest to determine the importance of the adrenal gland for survival under acceleration stress. To this end the acceleration tolerance of bilaterally adrenalectomized rats was determined and the results are presented in figure 2. Here the median survival time of 5.8 minutes for the adrenalectomized rat at 20 *g* is significantly lower than the survival time for either the normal or hypophysectomized animals.

Since hypophysectomy is followed by adrenal cortical atrophy, the question of the effects of continued changes in the hormonal imbalance on the hypophysectomized







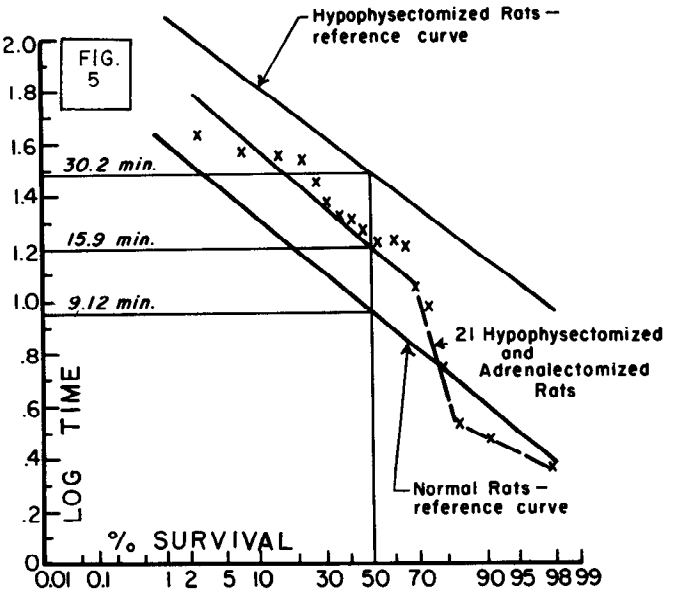


FIG. 2. Probit plot of per cent survival vs. log time of normal, hypophysectomized and adrenalectomized rat populations. All three curves differ significantly from each other by Student's *t* test at less than the 1% level for chance occurrence.

FIG. 3. Loss in peak efficiency of acquired tolerance to acceleration stress following hypophysectomy with time post-operation.

FIG. 4. Post-operative changes in the resistance of adrenalectomized rats to acceleration.

FIG. 5. Effect of hypophysectomy and adrenalectomy on the resistance of Sprague-Dawley rats to acceleration at 20 G.

animals' resistance to acceleration stress was of interest. In figure 3 there is illustrated the gradual loss in peak efficiency of the hypophysectomized rat's ability to resist acceleration stress. At 7 and 15 weeks postoperation, the rat populations have significantly lower tolerances than the population at 3 weeks postoperation, albeit these groups of animals still show a marked increase in resistance compared to the control group.

In contrast, following adrenalectomy, the animals that continue to survive for 7–11 weeks after the operation show an enhanced tolerance to acceleration stress (fig. 4). In fact, at 11 weeks postadrenalectomy, the animals have a greater tolerance than the normal rat. In part, this may be due to a fortuitous selection of hardy animals, since the weaker animals die. In part, too, this may be attributed to the hypertrophy of accessory adrenal tissue that is generated and was found in all these surviving rats.

Since hypophysectomy enhances the tolerance to acceleration stress and adrenalectomy inhibits the resistance to the same stress, the effect of both adrenalectomy and hypophysectomy was of interest. The data in figure 5

TABLE 2. *Data of Student's t Test for Significance of Differences in Survival Time of Rat Populations at 20 g*

Rat Population, N_1 Vs. N_2		No. of Rats		S.E., σ	Student's t	P
N_1	N_2	N_1	N_2			
Normal	Hypox.	67	34	.0576	8.93	.001
Normal	Adrex.	67	28	.0596	3.59	.001
Hypox.	Adrex.	34	28	.0588	12.38	.001
Hypox., 3 wk. postop.	Hypox., 7–15 wk. postop.	34	85	.0605	3.56	.001
Normal	Hypox., 7–15 wk. postop.	67	85	.0495	6.04	.001
Adrex., 5 wk. postop.	Adrex., 7 wk. postop.	26	18	.0825	2.29	.05
Adrex., 5 wk. postop.	Adrex., 11 wk. postop.	26	10	.0988	6.06	.001
Adrex., 7 wk. postop.	Adrex., 11 wk. postop.	18	10	.1386	2.95	.01
Adrex., 7 wk. postop.	Normal	18	67	.0787	0.10	n.s.
Hypox. + adrex.	Normal	21	67	.0797	2.10	.05
Hypox. + adrex.	Hypox.	21	34	.0885	3.90	.001
Hypox. + adrex.	Adrex.	21	26	.0895	4.27	.001

Hypox. = hypophysectomized; adrex. = adrenalectomized; P = probability that difference observed could occur by chance; n.s. = not significant.

show a complicated population distribution that still indicates a significantly greater tolerance to acceleration than the adrenalectomized rat, albeit a lower tolerance than the hypophysectomized rat. These rats tend to approach the normal rat in their behavior toward high accelerations. The slight increase in tolerance over the normal found may be due to the high mortality rate incurred as a result of the operation and the consequent unwitting selection of a stronger population.

DISCUSSION

The experimental results presented here are important, primarily because they indicate that factors in the hormonal interplay of the pituitary-adrenal axis are implicated in the tolerance to acceleration stress. In the past some pharmacological and hormonal components have been described which afford a measure of protection against acceleration stress (3). In the rat, none of these approach the magnitude of the effect of hypophysectomy alone. Classically, hypophysectomy has been associated with a loss in tolerance to stress. The hypophysectomized rat is less resistant to cold or heat stress and less resistant to disease and fatigue stress (6). The animal does not grow and loses its sexual function, yet its survival to high g stress is markedly and surprisingly enhanced in an unusual manner.

The enhanced survival of the hypophysectomized rat at high g, coupled with the well-documented adrenal atrophy following hypophysectomy, suggests the possibility that some adrenal cortical hormones play an inhibitory role in the resistance to acceleration. However, it is apparent that some adrenal function is necessary for survival to high accelerations, since complete adrenalectomy is followed by a considerable loss in resistance that can be recovered with the formation of accessory adrenal tissue.

Although the experimental findings may be contrary to currently accepted concepts regarding tolerance to stress, it is of interest that similar results, viz. an increase in tolerance to the stress of high-pressure O_2 poisoning following hypophysectomy, were reported by Bean (4). In the syndrome of high-pressure oxygen poisoning there is reasonable evidence for a toxic inhibition of the respiratory enzymes (7). Without deprecating the importance of functions such as cardiovascular dynamics and central regulatory factors, it is not unlikely that, in acceleration stress also, the limiting factors for survival reside in the efficiency with which energy is made available to the cell from the respiratory enzymes. Although the initiating events in acceleration stress probably involve aberrations of cardiovascular mechanics which then lead to inadequate oxygenation of cellular receptive sites, the final criterion for survival must involve biochemical energetics. If this hypothesis is acceptable, a plausible explanation can be offered for the experimental results. In the normal animal, the maximum energy available to the cell from the respiratory enzymes must be distributed by hormonal regulatory factors among the various cellular demands for this energy. Some fraction of the energy is expended in long-term needs such as protein synthesis, fat conservation and glycogen storage. Another fraction of the energy available must be channeled for immediate use in muscle contraction, nerve conduction, or osmotic work. The nature of the changes following hypophysectomy seems to depress those long-term demands on energy and leave only the vital cellular functions. Thus, any demand for energy in the area controlled by the pituitary, viz. growth or antibody production, will be answered poorly by the hypophysectomized animal. Alternatively, a demand for elementary survival over a short term, under conditions where the energy available from the respiratory enzymes is the limiting factor, can be more easily met by the hypophysectomized rat simply because there is less of this energy diverted for secondary long-term needs.

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