Reactions of Wild and Albino Mice in Response to Forced Swimming.* (30598)

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Enforced swimming of animals has been utilized by many investigators as a method of inducing and evaluating physiological and psychological stress. However, the time required to produce a state of exhaustion has shown considerable variation not only among individual animals, but in the mean times reported by various investigators(4,5,8,10). Some of the variability reported could have been due to variations in water temperature (1), in nutritional state of the animals(10), in the criteria for exhaustion or in species differences. Griffiths(6) found that domestic rats swam for a longer period of time than 2 strains of wild rats, except when treated with a drug (stilazine) or had their vibrissae removed. Richter(9) has also observed differences between wild and domestic rats and has noted that trimming of whiskers resulted in a quick death for all wild rats thus treated prior to enforced swimming. Since many swimming experiments are conducted on mice it appeared that a comparison of the responses of wild and domestic mice would be of value, especially if more objective methods of evaluating final exhaustion were employed.

Methods. A plastic cylinder, 26.5 cm diam. with a water depth of 24 cm was utilized for the swimming tests. A wetting agent was added to the water and air was bubbled through the water column. Water temperatures of 15, 20, 28, 37, or 42°C were maintained within ± 0.2°C and continuously monitored. This temperature as well as the colonic temperatures of the mice were measured utilizing thermistors and a Yellow Springs bridge. The thermistor probe was placed to a depth of 3.5-4 cm in the colon. The majority of measurements of colonic temperature were obtained at the point of exhaustion or termination of the swim. In some experiments, colonic temperatures were obtained continuously while the animals swam, thus weighting the animal by an additional 2 g. The mice utilized to obtain these cooling curves were not utilized in any other experiments. Cooling curves were obtained from 2 mice of each type while swimming at water temperatures of 15, 20 and 28°C. Since some investigators swam animals in groups, a few experiments were conducted comparing the 2 strains of mice under similar conditions.

Swimming times were measured to the nearest second by means of a stop watch. Initially, the end point was considered to be the onset of disorientation, the typical criteria utilized by most investigators. However, this proved to be unsatisfactory and a more precise end point, *i.e.*, the time at which the animal was unable to return to the surface of the water, was finally employed.

Data were treated statistically using the Fisher t test and the Fisher exact probability test, with the null hypothesis being accepted at the 5% level of significance.

Results. The results are summarized in Table I and Fig. 1 through 3. When the animals were placed in the bath they swam rapidly in a horizontal position, gradually changing to a vertical position as they tired. When the animals neared exhaustion they started rolling in the vertical axis, either to the left or right, apparently disoriented. When final exhaustion occurred the animals sank to the bottom without movement. Upon removal from the bath they were unable to stand, and moved only with great difficulty. In most instances artificial respiration had to be instituted. Since earlier studies had not clearly differentiated the point of exhaustion utilizing the general concept of an onset of disorientation, this point of disorientation was compared with a more objective one, namely failure to return to the surface. Fig. 1 illustrates the differences in colonic temperatures

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TABLE I. Mean and Ranges of Colonic Temperature and Swim Time for Wild and Albino Mice at 5 Water Temperatures.

	Water temp, C	No. of animals	Swim time, min Range	Swim time, min Mean S.D.	Colonic temp (C) at exhaustion Range	Colonic temp (C) at exhaustion Mean S.D.
Albino	15	19	3.0– 8.6	6.1 ± 1.4	20.0–23.4	22.1 ±2.1
	20	10	5 – 63	25.0 ± 13.8	19.8–21.0	20.6 ± .03
	28	5	60 –151	107.0 ± 28.7	28.2–28.5	28.4 ± .1
	37	4	95 –420	252.0 ±185.0	36.0–37.0	36.8 ± .3
	42	5	3.5– 7.1	6.1 ± 2.3	40.0–42.0	41.0 ± .6
Wild	15	10	1.6– 5.6	4.5 ± 1.0	19.0–26.4	20.8 ±2.1
	20	9	3.4– 75.5	27.4 ± 24.2	20.4–21.9	21.2 ± .05
	28	5	65 –145	103.0 ± 28.5	27.5–28.2	28.3 ± .2
	37	5	55 –270	164.0 ± 86.0	36.8–37.2	37.0 ± .2
	42	5	5.5– 15	11.5 ± 3.4	38.9–40.0	39.4 ± .5

and times when animals swimming at a water temperature of 20°C were removed at random according to either of the above criteria. When removal was made at point of disorientation, the mean colonic temperature was 22.3°C compared to the 20.8°C observed at point of exhaustion, a difference statistically significant at the 5% level. The duration of swimming was also longer when exhaustion was the end point but the scatter was so extensive as to make the differences statistically of borderline significance. All subsequent data were obtained utilizing exhaustion as the criterion for terminating the experiment.

The mean time for swimming to exhaustion at 15 and 20°C and the subsequent colonic temperature at that point are found in Table I. The mean swimming times at bath temperature of 15°C were 6.1 and 4.5 minutes, respectively, for the albino and wild mice. The differences were not different statistically. The final colonic temperatures although slightly higher for the albino mice were again not statistically different (females swam significantly longer than males, 6.9 against 5.9 minutes but final colonic temperatures were similar). Colonic temperatures continued to fall as much as 0.6°C upon removal from the bath. One albino and 3 wild mice succumbed within 24 hours. Fig. 2 illustrates the rate of decrease in colonic temperature of 4 mice (2 albino, 2 wild) swimming to termination in a water temperature of 15°C. During the first 4 minutes the colonic temperature fell to approximately 25°C followed by a more gradual drop toward a mean final temperature of 17.5°C. Disorientation always occurred prior to exhaustion. A very small additional decrement in colonic temperature occurred from this point to removal of the dead animal from the water.

No statistically significant differences in either swimming times or final temperatures were noted between the wild and albino mice exposed at any of water temperatures between 20 and 37°C (Table I). The relationship between mean duration of swim and mean final colonic temperature can be expressed as $Y = 0.0016\ X^{3.19}$ where Y is time in minutes and X colonic temperature (C). The variability in swim times was greater the closer water temperature approached body temperature. At 20°C two animals (one of each type) swam for over one hour but both succumbed within 24 hours. At 37°C the time required to exhaust the mice was prolonged compared to other water temperature. One mouse swam for 13 hours at a water temperature of 37°C, but was not included in the tabulated data since it appeared to represent an abnormal pattern.

When placed in water at 42°C the mice swam rapidly and continuously until near exhaustion, at which time they became suddenly disoriented and following a mild convulsive episode sank to the bottom. Although the wild mice swam for a longer period of time and had a final mean colonic temperature considerably lower than the albinos, the differences were statistically insignificant (t was 2.2). Undoubtedly this was related to the small sample but at this time it became impossible to trap additional wild mice.

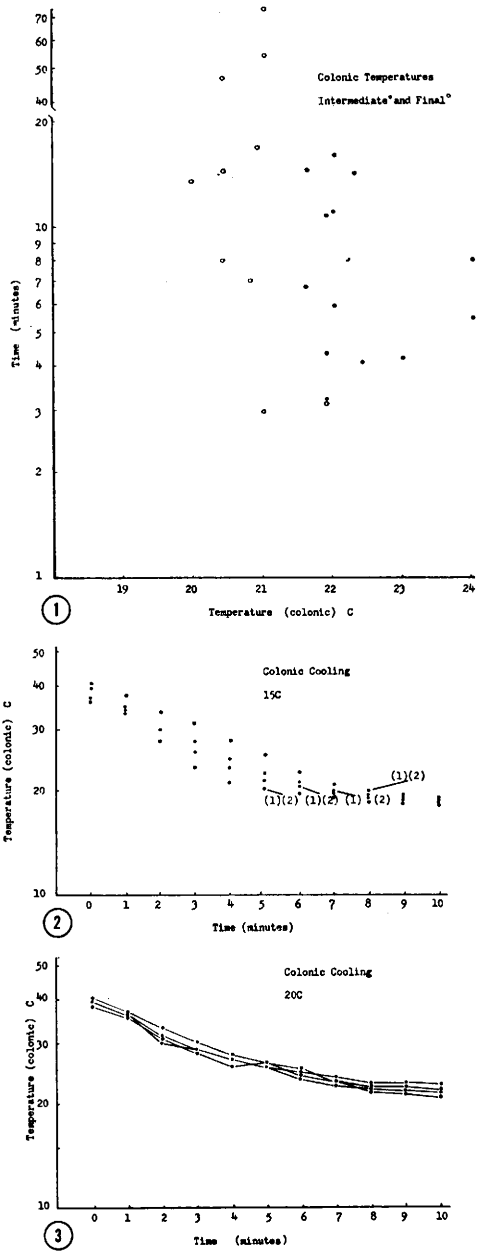

FIG. 1. Colonic temperature of wild and albino mice removed before final exhaustion compared to colonic temperature of wild mice at exhaustion. Plotted points represent individuals, circles represent those removed at exhaustion, and dots, those removed before final exhaustion. Water temperature was 20 C.

FIG. 2. Colonic temperature of wild and albino mice during swims at 15 C. The number 1 represents disorientation of the animals and the number 2 represents death. Each plotted point represents an individual, open circles represent wild mice, and dots, albino mice.

There were no statistical differences in the rate of cooling at these temperatures or in the colonic temperature ultimately reached (Fig. 2 and 3). Disorientation occurred at different times in water at 15°C as indicated in Fig. 2. Return of body temperature to control levels was slow, even after 30 minutes some mice had a temperature essentially the same as that when removed from the swimming tank. Records kept for one animal after swimming in water at 20°C showed that after 60 minutes of recovery the colonic temperature had been elevated to only 28.5°C.

The behavior of animals during group swimming was similar at all temperatures. When the mice came in contact during swimming they tried to climb onto one another and as a result some of the animals were forced under water time and time again. Certain individuals seemed to be forced under repeatedly and consequently were the ones that became exhausted first. The others were able to swim longer due to the lessened competition for swimming space. At 20°C groups of albino mice had an average swimming time to exhaustion of 7.7 minutes (4.8 to 10.3 minutes), and had an average colonic temperature at exhaustion of 22.7°C. The average swimming time to exhaustion for the groups of albino mice at 37°C was 48.1 minutes, compared to the average of individual animals of 252 minutes. The range of swimming times was from 5 to 145 minutes, again considerably different from the range of individually swimming animals. The group of wild mice had averages at 37°C similar to that of the albino mice (47.6 minutes) and a range of 11.7 to 118.2 minutes. There were no differences in colonic temperatures between either the albino or wild mice as a group or as individuals at this water temperature.

Discussion. There have been suggestions in the literature that considerable differences in tolerance for stressful situations occurred between the domesticated and wild strains of animal species. The data in the literature

FIG. 3. Colonic temperature of wild and albino mice during swims at 20 C. Each plotted point represents an individual, open circles represent wild mice, and dots, albino mice.

based on rats were not confirmed in another rodent, *i.e.*, the mouse. Griffiths(6) reported that domestic rats swam longer than the 2 different species of wild rats that he tested. Some of the wild rats in his study displayed a "sudden death" phenomenon during swimming that had previously been described by Richter(9). These inferences have not been borne out by the present study, as no significant differences were found in the time required to exhaust either albino or wild mice, of the species *Mus musculus*. Only 2 of the "wild" mice succumbed in a manner suggestive of the "sudden death" phenomenon. Further investigation of this problem seems warranted since there are many strains of domesticated *Mus musculus*, and the possibility remains that some such differences, as previously reported, may actually exist.

The failure to detect significant differences between the two groups in the present study is related to the large variations in individual performance. Other investigators have noted similar individual and group variability in experimental animals(4,5,8,10). Some of the individual variability and the discrepancies between studies are related undoubtedly to the differences in the methods of terminating swimming. Most investigators used a subjective technique to terminate the swim, *i.e.*, disorientation of the animal. The results of this study were undoubtedly improved by the more objective method employed, yet some questions remain. Variability would be anticipated from a random sampling of a natural population due to differences between individuals as to age, weight, diet, unknown disease states, etc., but would not be so anticipated for a highly select strain of animals raised under carefully regulated conditions. The individual variability reported herein should be a warning to investigators against utilizing individual results to determine effects of agents on experimental animals. Large differences have been shown to exist between individuals that should perform equally well. These differences are evidently inherent and represent normal individual variation and, therefore, if taken into consideration would render inadequate results for the testing of some agents.

In an attempt to add larger numbers more easily to a study for the sake of statistical evaluation, some investigators have utilized groups of animals in their swimming tests. Group swimming decreases swimming times an average of about 50%(7,13,14). Wilber (14) has pointed out that swimming mice in a group may be unreliable due to factors of group psychology. Not only are individual differences still apparent, but a new factor of peer relationships is introduced, which affects the duration of swimming. This study supports Wilber's conclusions, as significant differences were found between mean times for group and individual tests. Furthermore, it was found that the colonic temperatures at exhaustion time in the individuals of the group were significantly higher than those observed in animals swimming as individuals at the same water temperature. These factors raise considerable doubt as to the validity of determining physiological alterations in animals stressed by this procedure.

The factors which limited swim time at the low temperatures, such as neuromuscular depression and the resultant respiratory difficulties observed, agreed with previous studies (2,3,11). The comparison of colonic temperatures at the point of exhaustion for the water temperatures of 15°C and 20°C, indicated the importance of using colonic temperature as a criterion of exhaustion. Both groups of mice swam significantly longer at 20°C than at 15°C, yet colonic temperatures at exhaustion were similar. Since the final colonic temperatures fall within a narrow range they can be useful in determining whether or not an animal had swum to the limit of its endurance in cold water. Although Spealman(11) and Le Blanc(8) conducted their studies at water temperatures other than those used in this study, their results support the suggestion that the body temperature at the conclusion of swimming in cold water was the most decisive factor in determining the point of exhaustion. At the higher temperatures of 28 and 37°C colonic temperatures showed no relation to individual swimming times, which was apparently delimited by "exhaustion" of the animals. Tan(12) arrived at a similar conclusion, utilizing rats and guinea pigs.

However, in view of the extremely large variations in individual swimming times at these water temperatures it would seem that the use of lower water temperatures and the final colonic temperature would provide a more precise method of evaluating stress relationships.

The differences in swimming time at 37°C between the wild and albino mice, were large enough to require some explanation. Scheer (10) had suggested that a restricted caloric intake would result in a shorter swim by causing a lessening of physical capacity. Recent studies(4,5) have indicated the changes in diet would alter the time to exhaustion for swimming animals. The adequacy of the diet of the wild animals could not be determined and whether or not this factor was involved in the results cannot be predicted. Further studies are being planned.

Summary. The suggestions in the literature that there are differences in response between strains of domestic and wild animals of the same species, based on supposed differences in psychological and physiological behavior, have not been borne out by the present study. The wild and albino mice utilized in the current study showed no significant differences in swimming time to exhaustion, or colonic temperature at exhaustion. These observations were in direct opposition to those reported comparing wild and domestic rats. There were individual variations in swimming times at all water temperatures despite the utilization of a precise end point for exhaustion. A valuable criterion for exhaustion appeared to be the final colonic temperature as suggested by the relationship between swimming times, water temperature (at least between 20 and 37°C) and colonic temperature. Other factors markedly shorten the duration of swimming at bath temperatures of 15 and 42°C. Group swimming resulted in a 50% decrease in swim time due to factors other than those which induce exhaustion in individually swimming animals. In view of the large individual variations in swimming times, evaluation of various psychological and physiological stresses must utilize the most reliable methods of pinpointing exhaustion and not depend upon such subjective measures as disorientation of the test animal.

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Decomposition of 3-Hydroxyanthranilic Acid Under Simulated Physiologic Conditions.* (30599)

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Evidence has accumulated that tumors of the bladder in persons engaged in manufacture of certain aromatic amines may result from *in vivo* formation of hydroxy derivatives

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