An adjustable-current swimming pool for the evaluation of endurance capacity of mice

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Matsumoto, Keitaro, Kengo Ishihara, Kazunori Tanaka, Kazuo Inoue, and Tohru Fushiki. An adjustable-current swimming pool for the evaluation of endurance capacity of mice. J. Appl. Physiol. 81(4):1843–1849, 1996.—A new forced-swimming apparatus for determining maximum swimming time in mice was devised for use in the evaluation of the endurance capacity of Std ddY and CDF1 mice after various diet and drug treatments. With the apparatus, a water current is generated by circulating water with a pump in a swimming pool. A spout and suction slit were contrived to generate a constant current while the strength of the current is regulated by a valve. The decrease in the leg-kicking intervals of mice accompanying the increase in the current speed confirmed that the workload is adjustable by regulation of the current speed. Compared with the number of forelimb strokes, that of the hindlimb kicks was greater. The swimming time until fatigue was observed to decrease with increasing current speed in the two strains of mice. As biochemical indexes, the blood lactate and muscle glycogen levels corroborated the correlation between current speed and increase in workload. These results indicate that the apparatus employed in the present study is suitable for the evaluation of the endurance capacity of mice and that it is useful for detecting the effects of dietary differences and drug pretreatments on this capacity.

MATERIALS AND METHODS

Animals. Five-week-old male Std ddY mice (a closed colony) and CDF1 mice (an inbred strain) (Japan Shizuoka Laboratory Center, Hamamatsu, Japan) were used. They were provided a stock diet (type MF; Oriental Yeast, Tokyo, Japan) and water ad libitum. The care and treatment of the experimental animals conformed to the Kyoto University guidelines for the ethical treatment of laboratory animals.

CHINESE FEMALE LONG-DISTANCE RUNNERS have broken many world records in recent years. Because they apparently ingested special foods (herbs) to increase their endurance capacity, these exogenous substances and their effects on endurance capacity have been brought into the limelight. Controlled studies using laboratory animals should be conducted to elucidate the biochemical mechanisms underlying the improvement of performance afforded by these agents. In a long-term experiment using some food specimens with limited availability, it is advantageous to use mice. However, there few reports on the use of mice in such studies because satisfactory equipment for evaluating the work capacity of mice has not been available. The treadmill, the most widely used apparatus for the evaluation of the work capacity of the rat, is unsatisfactory when applied to quantitating the exercise performance of mice. Hatta (10) and Hatta et al. (11) reported that commercial treadmills are too large for mice. Thus, they either do not support the mouse, allow the mice to avoid electric shock, or cause injury to the tail. The frequency of such accidents increases with running speed. Moreover, because mice do not adapt to the treadmill as quickly as rats (10), they need additional time for learning. Mice that learn slowly must be discarded or the data will be widely distributed. These disadvantages have been pointed out by several investigators (10, 11).

A variety of swimming tests have been used as criteria of the physical work capacity of animals under diverse experimental conditions (1, 2, 9, 18, 23, 24, 27). To standardize the workload and reduce the swimming time, weights at specific body weight percentages were added to the chest or tail of the animal (4, 20, 22, 28). However, although the attached weight increased the workload by countering buoyancy, it could not be completely confirmed to not hamper the animal's ability to swim by constraining free tail movement and by creating an irregular distribution of body weight.

To obtain a standard workload quantitating maximum swimming capacity without adding weight, we developed a swimming pool with a pump that generates water flow and creates currents. The objective of our present study was to quantitate the maximum swimming capacity of mice in this apparatus, itself designed for the purpose of testing the exercise capacity of mice after various treatments with diets and drugs. Our present experiments indicate that our apparatus provides for the reliable and reproducible evaluation of the endurance capacity of mice.

MATERIALS AND METHODS

Animals. Five-week-old male Std ddY mice (a closed colony) and CDF1 mice (an inbred strain) (Japan Shizuoka Laboratory Center, Hamamatsu, Japan) were used. They were housed in standard cages (33 × 23 × 12 cm; 6 mice/cage) under controlled conditions of temperature (22 ± 0.5°C), humidity (50%), and lighting (lights on from 0700 to 1900). They were provided a stock diet (type MF; Oriental Yeast, Tokyo, Japan) and water ad libitum. The care and treatment of the experimental animals conformed to the Kyoto University guidelines for the ethical treatment of laboratory animals.
Design of the adjustable-current swimming pool. Figure 1 shows the design of the swimming system used. We used an acrylic plastic pool (90 × 45 × 45 cm) filled to a depth of 38 cm with water. The surface of the tank is clear and smooth, which prevents the animal from supporting itself while swimming. The current in the pool is generated by circulating water with a pump (type C-P60H, Hitachi, Tokyo, Japan). We devised a spout and suction parts to generate a uniform current. Vertical holes of the appropriate diameter were bored in the nozzle in a straight line with precision to the nearest 0.1–0.2 mm. Water is returned to the pump through a narrow slit in the plastic pipe set on the bottom of the pool. The strength of the current is adjusted by changing the water flow, which is regulated by opening and closing a valve and is monitored by a water flowmeter (FC-A20, Tokyo Flowmeter Laboratory, Tokyo, Japan). The distribution of the surface-current speed is measured with a digital current meter (type SPC-5 Sanko Industry, Tokyo, Japan) for 24 surface points spaced at regular intervals. The temperature of the water is maintained at 34°C with a water heater and thermostat.

Measurement of maximum swimming time in the adjustable-current pool. To accustom the mice to swimming, all mice were given a 1-wk preliminary period in which they swam for 30 min at a 6 l/min flow rate twice a week. They were then made to swim in groups of six at a time until fatigue, defined as the failure to rise to the surface of the water to breathe within a 7-s period. We noted characteristic changes of swimming behavior before fatigue: their posture became more upright and they were finally, as illustrated in Fig. 2, unable to maintain the workload required of them to maintain themselves on the surface of the water, sinking vertically with frothing. This was so characteristic that we could easily identify imminent fatigue before the sinking with frothing followed by >7 s spent below the surface. In previous studies, various criteria of exhaustion have been used, generally defined as performance inability ranging from 10 to 60 s of submersion (3). The 7-s interval employed in the present study is rather shorter than in the previous studies with rats, but we found that drowning occurred frequently at longer intervals. Intervals shorter than 5 s reduced the reproducibility of the test results. The suction of water at the end of the tank was not strong enough to suck in the mice.

We measured the total swimming period until fatigue as the index of the swimming capacity.

Analysis of kicking and paddling intervals. The swimming of mice in the pool in the current at varying flow rates was recorded on videotape. The average numbers of hindlimb kicks and forelimb paddlings were counted by inspection of the video images played back at slow speed.

Administration of caffeine. Mice administered a large dose of caffeine, which is reported to reduce endurance capacity.
Maximum swimming time in the adjustable-current pool. Five-week-old \textit{StddY} mice were accustomed to swimming in the adjustable-current pool as described in Measurement of maximum swimming time in the adjustable-current pool. After the preliminary treatment, 32 mice were randomly divided into 4 equal groups. Measurement of the swimming time until fatigue with an attachment of weight to the tail was carried out in a static pool in 34°C water. The weights were made of lead thread cut to the equivalent of 2 or 4% of the body weight of each mouse and were coiled and fixed around the base of the tail. The swimming was started at 1300.

Chronic exercise training in the current pool. Sixteen 5-wk-old mice were trained as described in Measurement of maximum swimming time in the adjustable-current pool. At the end of the last preliminary training session, the swimming time until fatigue was measured in all mice. Then the mice were divided into two groups with equal average swimming times. One group (n = 8) was exposed to swimming training with measurement of the swimming time until fatigue at a 7 l/min flow rate every other day for 2 wk. The sedentary control group was fed without any swimming exercise for 13 days, and on the final day, the swimming time until fatigue was measured.

Effects of swimming conditions on the swimming capacity. To examine the effect of the water flow rate on the swimming capacity, we changed the water flow rate (6, 7, 8, 9, and 10 l/min) with the other conditions held constant (34°C water, swimming started at 1300). The mice were forced to swim at each water flow rate, and the swimming capacity was measured in the same way. To investigate the effect of water temperature, we then changed the water temperature (25, 30, 34, and 37°C) with the other conditions again kept constant (water flow rate of 8 l/min, swimming started at 1300) and measured the swimming capacity in the same way.

Serum L-lactic acid analysis. Mice were made to swim in the current pool at the water flow rate of 6, 8, or 10 l/min. Blood samples were taken from the tail during the 5th, 10th, 20th, and 30th min of exercise and during the last minute of exercise before exhaustion. Blood samples for lactate determination were immediately deproteinized in perchloric acid (0.8 N) and centrifuged, and the serum L-lactic acid concentration was determined with a Kowa Medex commercial kit (Determiner LA, Tokyo, Japan).

Muscle glycogen analysis. Mice were forced to swim for 10 min at the water flow rate of 6, 8, or 10 l/min, and after the swimming load, they were killed by dislocation of neck. The gastrocnemius and pectoralis muscles were removed immediately, frozen in liquid nitrogen, and kept at −80°C until analysis for glycogen concentration. The glycogen content was measured spectrophotometrically by a method employing enzymatic techniques as described elsewhere (7). Briefly, after hydrolysis of the muscle sample in 0.6 N HCl at 100°C for 2 h, the glucose residues were determined with a commercial kit (glucose CII test Wako, Wako Pure Chemical Industries, Osaka, Japan).

Statistics. Statistical analysis of differences between pairs of groups was performed with Student’s t-test. Comparisons of the means among more than two groups were performed by one-way analysis of variance followed by Tukey’s test. Statistics were calculated with the InStat software package (Macintosh version 2.00, GraphPad Software, San Diego, CA). Probability levels of < 0.05 were considered to indicate significance.

RESULTS

Performance parameters of the adjustable-current swimming pool. The mice showed the maximum performance at a temperature of 34°C (data not shown). It was also noted that the reproducibility of the data for the maximum swimming time was highly dependent on the uniformity of the current. Imprecise boring of the vertical holes in the nozzle disturbed the uniformity of the current, which caused scatter of the data distribution. Therefore, care was taken to bore the holes to the target diameter in the nozzle and in a straight line with precision to the nearest 0.1–0.2 mm. The uniform current near the surface of the water extended to at least 3 cm depth below the surface as estimated by using the flow speedometer. The water return via the plastic pipe at the bottom of the pool aided in maintaining uniform current with minimum variation in the swimming data (data not shown). The uniformity of the current was confirmed by the results of measurements with the digital surface-current speedometer (Fig. 3).

Uniform current was observed at each flow rate, except at the part of the pool immediately in front of the spout.
where the mice, for the most part, did not stay during the swimming tests. At a flow rate of <4 l/min, constant-current speed could not be maintained in the remote part of the pool, suggesting that 4–5 l/min might be the lower limit yielding reliable data. As alternative methods, generation of a surface current with air bubbling or with water stirring caused many disturbances, and fine adjustment of flow speed was impossible (data not shown). In these methods, we observed an irregular current, stagnation, and a surge, with current decay especially prominent in the remote part at the low speed.

As the flow rate was increased, the workload of the mice became greater, which was evidenced by the data for the average number of kicks increasing by speeding up the current (Fig. 4). The hindlimbs were more actively used by the mice than were the forelegs in this apparatus; the average number of forelimb strokes was very low and did not increase on speeding up of the current. The hindlimb-kicking intervals were not invariably constant, but rather they formed clusters interspersed with rest, which is a common behavior of rodents in treadmill running. In the swimming apparatus, the mice did not become submerged under the water while attempting to rest during exercise.

The analysis of the swimming time until exhaustion at various current speeds in each mouse strain revealed a strong correlation of workload and flow rate, as shown in Fig. 5. The maximum swimming time to fatigue in both strains clearly decreased with an increase in the flow rate, indicating that the workload could be finely regulated by manipulation of the flow rate. Across the flow rates, the data for the CDF1 mice showed less variation than those for the Std ddy mice, perhaps due to the lesser genetic variation.

Biochemical indexes also indicated the correlation between workload and current speed. The data demonstrating an increase in serum l-lactic acid concentration across the flow rates of 6, 8, and 10 l/min are presented in Fig. 6. The blood l-lactic acid concentration remained slightly higher than the resting level when the flow rate was 6 l/min and increased abruptly in proportion to exercise time above the rate of 8 l/min, indicating that the blood lactate accumulation point lies at ~8 l/min. The glycogen concentration of the gastrocnemius muscle after 10 min of swimming declined as flow rate increased, suggesting that the higher flow rate demanded greater gastrocnemius muscle (hindlimb) glycogen consumption. On the other hand, the pectoralis muscle (forelimb) glycogen consumption was rather slow, which is consistent with the data indicating a low average number of forelimb strokes during swimming (Fig. 7).

Comparison of the current-swimming system with forced swimming with weight load attached to the tails
of mice with and without caffeine pretreatment. The subcutaneous administration of 100 mg/kg of caffeine 30 min before swimming markedly decreased the swimming time to fatigue at each current speed, as shown in Fig. 8A. A significant difference was observed at every flow rate. Attaching a weight to the tail had similar effect (Fig. 8B). The performance of the mice, however, showed a significant difference in the placebo and caffeine groups only at the 2%, and not at the 4%, added weight.

Effect on endurance capacity of prolonged swimming in the current pool. In the trained mice subjected to swimming to fatigue at a flow rate of 7 l/min every other day for the number of days indicated in Fig. 9, the swimming time to fatigue was markedly increased on the first day compared with those on the other days. On the other hand, the sedentary mice did not show a significant increase in swimming capacity. Thus, like the treadmill, the current pool also provokes a physical-training response.

**DISCUSSION**

Forced swimming of animals has been employed as a criterion of their physical work capacity. Dawson and Horvath (3) pointed out that swimming has advantages over other forms of exercise, including the treadmill. Training is not required because rodents have a natural swimming ability and they are assumed to be highly motivated to avoid drowning when fatigue is imminent, assuring a high level of performance. However, as McArdle and Montoye (19) noted in their review, certain problems arise with these tests in rats. Many of the rats immediately submerge themselves to the bottom in an apparent attempt to escape. If the tank is relatively shallow, they learn to sink to the bottom to rest and push off to return to the surface. Weights have been attached to the tail to standardize the workload and reduce the swimming time in the static water pool. However, the artificial addition to the body weight by

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**Fig. 7.** Muscle glycogen concentration 10 min after start of swimming at varying current flow rates. Open bars, sedentary mice; crosshatched bars, 6 l/min; solid bars, 8 l/min; hatched bars, 10 l/min. Gastrocnemius and pectoralis muscles were immediately quickly resected in mice killed 10 min after start of swimming, and glycogen concentration was analyzed. Values are means ± SE for 6–9 mice. Values not sharing common letters are significantly different, P < 0.05.

**Fig. 8.** Effects of caffeine (100 mg/kg body wt) on swimming time to fatigue. Caffeine solution was injected subcutaneously 30 min before start of swimming. A: swimming time of caffeine (open bars) and placebo (solid bars) groups. B: swimming time of caffeine and placebo groups with loads equal to 2 and 4% of body weight attached to tail. Values are means ± SE for 8–17 (A) and 8 (B) mice. Significantly different from corresponding control value: *P < 0.05; **P < 0.01.

**Fig. 9.** Swiming time to fatigue increased during chronic swimming training in current pool. Sixteen mice were divided into 2 groups after 1 wk of pretraining. One group was made to swim to fatigue every other day for 2 wk (open bars). Other (sedentary) group swam only on 1st and 14th days (solid bars), and mice in this group were fed without any additional training on intervening days. Values are means ± SE for 10 mice. Significantly different from data for 1st day (day 0): *P < 0.05; **P < 0.01.
such an attachment of weights may not always eliminate the effect of weight differences as a factor in swimming time (3, 19). Furthermore, as a result of these problems, investigators are increasingly disinclined to use the swimming workload. In contrast to rats, mice, we have noted, never learn to sink to the bottom to rest, as was also observed by Kaplan et al. (16), who used a static water pool. Therefore, in mice, the swimming system potentially offers greater advantages.

At a higher water flow speed (>9 l/min), the mice showed fatigue accompanied by a high blood lactate concentration, suggesting that at some point they began to rely heavily on anaerobic metabolism to maintain themselves on the surface against the current. As we noted above, at such extreme workload, the mice never spontaneously submerged to the bottom to rest, perhaps due to the strong motivation to avoid drowning; this is another aspect of this system contributing to its reproducible workload quantitation, especially at a high workload intensity. The accumulation of blood lactate during swimming also supports the notion that swimming at a fairly high flow rate (8–10 l/min) causes the mice to depend highly on anaerobic metabolism. On the other hand, at flow rates below 8 l/min, there was a more modest accumulation of blood lactic acid during swimming, suggesting that the workload was still below the anaerobic threshold for mice.

The swimming system we have designed is not well suited for studies in rats because rats do rest on the bottom of the pool, as has been noted in previously reported swimming systems (26). A similar disadvantage was noted by Flaim et al. (6) when they applied swimming exercise in rats in their detailed studies of the cardiovascular response to acute aquatic and treadmill exercise. They noted a data discrepancy between treadmill and aquatic exercise and suggested that the cardiac adaptations to chronic exercise with free swimming for 4 wk in a rat model and those in a mouse model. They observed that diving was not a readily documented feature of the exercise-associated response.

Wilber (28) reported that there was a logarithmic decrease in swimming time with increased weight loading in guinea pigs. However, as reported by Scheer et al. (25), the variability in the specific gravity of rats contributes to the variability in swimming times in such methods. Given the other drawbacks of weight attachment described in the introduction, the degree of quantitative regulation of workload achieved by adding a weight to a rodent’s tail or chest seems open to debate. Flaim et al. (6) also pointed out that the rate of exercise and the magnitude of the workload can be more precisely controlled in treadmill exercise than in aquatic exercise with attached weights and continuous agitation of water. Adjustment according to the specific gravity and production of a quantitatively uniform workload in growing animals in a long-term experiment is time consuming and painstaking, if not right impossible. Our present system offers clear advantages in this regard.

The increase in workload by weight attachment differed in its effects on the relative swimming capacities for the drug and placebo groups in comparison with the swimming in the current pool. As previously reported (15, 17), under the addition to the root of the tail of a weight made of a thread of lead equivalent to 2% of the body weight, administration of 100 mg of caffeine markedly reduced the maximum swimming time. A tail weight equivalent to 4% of the body weight seems to have an effect corresponding to a flow rate of 9–10 l/min in our current pool and that of 2% had an effect comparable to an ~7 l/min flow rate as derived from the data for the swimming time until fatigue.

Flaim et al. (6) reported that the increase in cardiac output and the distribution of blood flow in swimming are very different from those in running so that, for example, heart rate is not elevated at all by swimming compared with the resting values, in contrast to treadmill running where typically a cardiovascular response is observed. In the present study with mice, the average number of hindlimb kicks was well correlated with the flow speed. In the present investigation, we did not measure the cardiac responses, but the clear increase in the frequency of hindlimb kicks with the current speed may likely have some effect on cardiovascular response like that seen in treadmill running. The recent study reported by Kaplan et al. (16) supports these deductions; they cited a marked difference between the cardiac adaptations to chronic exercise with free swimming for 4 wk in a rat model and those in a mouse model. They observed that diving was not a prominent behavioral component in the mice and that the induction of mitochondrial glycolytic enzyme was a readily documented feature of the exercise-associated response.

The swimming exercise in our pool system evoked a significant increase in endurance capacity. The swimming training in the pool three times per week for 2 wk gradually increased the maximum swimming time, suggesting that swimming in a current pool is not only a stress but also enhances the endurance capacity as occurs in treadmill running (5, 12–14, 21). A similar increase in maximum swimming time was observed in previous studies by Fushiki and colleagues (7, 8) with the prototype of the current pool described here.

In conclusion, our current-pool system offers many advantages in the evaluation of the endurance capacity of mice. In mice, the data obtained show a higher reproducibility than those obtained for treadmill running. The apparatus we employed is also useful for detecting the effects of dietary differences and drug pretreatment on the endurance capacity.

We gratefully acknowledge the advice regarding the treadmill running and the kind assistance of Dr. Hiroshi Tsuchida and Dr. Tamotsu Kuwata, Director, Central Research Institute, Meiji Milk Products Co., Ltd, Higashimurayama, Tokyo, Japan. We also thank Ryuhei Uehashi, Masato Saito, Kyon-mi Kim, and Hiroshi Taki for technical assistance.
This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

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Received 5 January 1996; accepted in final form 30 May 1996.

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