Effects of the Potassium and Magnesium Salts of Aspartic Acid on Metabolic Exhaustion

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Two groups of 40 rats were placed in a swim test situation designed to produce metabolic exhaustion. The provision of potassium and magnesium aspartates to one group at a dose of 1 Gm./Kg. significantly delayed the onset of metabolic exhaustion as evaluated by swimming time. It is considered that this alteration in swim time occurred as a result of the material's affecting those animals which would ordinarily have swum for less than 196 minutes rather than altering the times of the group as a whole.

The scientific literature is replete with reports on various rat swim test situations. Included are the effects of age and sex differances (1), fatigue, practice and drug effects (2), the shortening of swim time by the application of weights (3, 4), and the ability that diet has to alter performance (5, 6).

Some of the most impressive data relating to swim tests have been reported by Richter (7). In these experiments it was demonstrated that a direct relationship exists between duration of swim time and the temperature of the water in which the animals are swimming. Additionally, a presentation of a possible causal relationship between those animals swimming "indefinitely" and for "very short" periods was made.

None of the publications reviewed made a point of evaluating metabolic exhaustion. It is assumed that an animal, faced with a swim or drown situation, will perform to his utmost ability. Although this assumption must be true, there are no data to indicate whether this level of ability is dictated by emotional factors (7), availability of metabolic substrate, or accumulation of toxic end products.

EXPERIMENTAL

To determine these points and to determine the size and type of container, temperature, optimum number of animals per volume of container and, most important, to establish sufficient knowledge leading to a recognizable and reproducible end point, numerous orientation studies were made prior to final experimentation.

The "swimming pool" finally adopted was a rectangular, custom-made aquarium 36 in. long, 24 in. wide, and 24 in. deep; the tank was equipped with a thermoregulator and heater to maintain constant temperature and a water circulator to facilitate maintenance of constant temperature without either aiding or antagonizing the normal swimming efforts of the rats. The water depth in all reported tests was 20 inches.

In preliminary tests, male rats ranging in weight from 150–300 Gm. were used. It was concluded that any male rats within this range would be satisfactory. However, for the reported experiments, this range was narrowed to 180 to 245 Gm.

When rats are placed in water they first swim in a horizontal position on the surface; then, looking for a means of escape, they often go as deep as the bottom of the tank; next they assume a vertical position at the surface and use all four legs vigorously, with occasional second-long rest periods. In short order, they must work continuously to keep their heads above water. At this stage, while the hind legs pump steadily, the animals fold their forelegs in front of the chest in an apparent effort to use them as wings or fins to avoid the sinking process. As work continues, they frequently sink and must struggle to reach the surface. Eventually loss of control of movements is observed and the animals sink to a depth of 10 to 20 in. with an inability to fight to the surface; some will walk the perimeter of of the tank bottom, some will remain almost motionless at a depth of 10 or more inches, while others flail aimlessly without rising. These are variations of the end point. Rats removed from the water at this time usually recover. A rat in this condition is finished swimming as far as physical ability is concerned; he is fatigued and apparently near exhaustion. This is the first reproducible and constant symptom which is always observed; hence it was selected as the end point.

If rats are allowed to remain in the water when the end point is reached, a few recover sufficiently in about 30 seconds to make a few more trips to the surface. Those that show no recovery remain under water and drown. These latter observations are not routine; the rats were removed from the tank at the end point, but early in the series some were allowed to remain in order to provide terminal observations.

It was found that a census of 8 to 10 rats was optimum for the tank used; thus, when the number of rats was reduced below eight, additional rats were added to maintain that number.

Preliminary observations concerning onset of metabolic exhaustion were made by exposing the animals to various ambient water temperatures, observing the swim time, resting for either 15 or 30 minutes, and exposing the same animals to the same conditions of temperature and swim. At the lower temperatures, 20°, the animals were capable of approximately 20 minutes of swim; this could be reproduced for several exposures. As the water temperature was increased, it was found that swim time increased in accordance with Richter, but that duration of time of subsequent exposures was appreciably reduced. This was taken to mean that metabolic involvement in the initial swim was such that complete recovery had not taken place by the time the second effort was required.

Since normal temperature regulating mechanisms are considered to be in balance at approximately 85° F. (29° C. environmental), and, since metabolic activity would be neither inhibited nor enhanced by

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exposure to such an ambient temperature, the reported tests were done at a water temperature of 28.0 ± 0.25° C. Control animals, after swimming to exhaustion and given 15-minutes rest, were usually unable to swim at all when exposed for the second time to the water temperature. It was concluded, therefore, that a situation producing metabolic exhaustion had been produced.

Laborit (8, 9) has demonstrated that several agents have the ability to prolong the duration of rat swim time. The most active substance reported was a combination of the potassium and magnesium salts of aspartic acid. Since control rats, in the Laborit technique, were able to swim for appreciably shorter times than those in this study, and, since second exposure resulted in an observed time approximating that of the first, there was felt to be a need for additional data more directly related to metabolic exhaustion as described.

For the actual test, 10 male rats apparently healthy and ranging in weight between 180 and 245 Gm. were selected on the afternoon preceding the test day. They were arranged into two groups of five each, weighed, and small slits made in the ears for purposes of identification. One group was treated twice by stomach tube with 500 mg./20 ml./Kg. each of potassium aspartate and magnesium aspartate (1000 mg./20 ml./Kg. total) in one solution. The first dose was given 17 hours before the test and the second 45 to 60 minutes before the test. The control rats were handled in a like manner using water only; both groups were allowed food and water *ad libitum* until actually placed in the tank for swimming.

At zero time, both groups of rats were placed in the pool under the conditions previously described and allowed to swim until each, in turn, reached the end point of fatigue. As each rat reached his end point he was removed from the water, identified, and his total elapsed swim time recorded.

It should be remembered that only swim time is assayable by this test; no attempt at measurement of work output is possible.

TIME IN MINUTES

Less than	75	91	110	133	162	196	237	287	348
K+Mg ASPARTATES		76	109		139	177 190 194	201 203 204 204 206 206 208 212 213 214 218 219 225 228 230	246 247 247 250 251 255 256 258 262 266 266 276 276 285	332
CONTROL	74	88 89	96 96 100 106	122	141 143 155	165 180 182 184	198 199 209 214 216 221 222 230 235	237 240 244 252 259 261 265 266 270 272 275	300

Fig. 1.—Average of the control times and aspartate animals times.

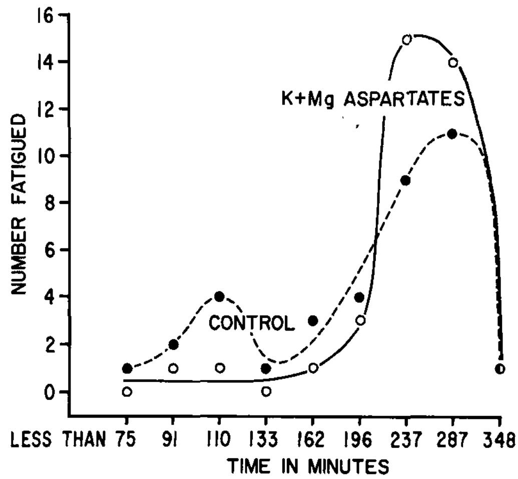

Fig. 2.—Plots of data in Fig. 1.

RESULTS AND DISCUSSION

The accompanying figures present the results obtained from eight cumulative experiments; they offer similar data, in a different way. Thirty-six control and 36 aspartate treated rats are included; the remaining eight will be discussed later. The rats are divided into log-spaced swim time groups.

An average of the control times and aspartate animals' times in Fig. 1 indicates that the control rats swam 194.6 ± S.E. 7.96 min. and that the drug-treated rats swam 223.6 ± S.E. 8.05 min., an increase of 15% in swimming time.

If the data presented in Fig. 1 are plotted graphically (Fig. 2), the distribution of the controls is seen to be bimodal. In both groups there appears to be a line of demarcation at 196 minutes; 41.7% of the controls swim less and 58.3% swim more than 196 minutes. On the other hand, the aspartate-treated rats shift to a unimodal frequency; 16.7% swim less and 83.3% swim more than 196 minutes. Application of the chi square test indicated that this shift was significant at a level less than 5%. It is felt that this is a strong indication that the swimming times of rats which have longer endurance are not altered by the aspartates while the swimming time of animals of lesser endurance are prolonged.

The eight rats which are not represented in the figures but which are included in the calculations were discarded for reasons as follows: one aspartate rat was obviously incapable of coordinated swimming; he was removed from the water in about 20 minutes and expired shortly thereafter. Four control rats and three aspartate rats were interminable swimmers; they were swimming well after 6 hours and were discarded. As a check on our interpretation, one of the control rats and one aspartate rat were timed further; they were still swimming after 8 hours and were removed from the tank.

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