

Sleep Deprivation in the Rat: III. Total Sleep Deprivation

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Summary: Ten rats were subjected to total sleep deprivation (TSD) by the disk apparatus. All TSD rats died or were sacrificed when death seemed imminent within 11-32 days. No anatomical cause of death was identified. All TSD rats showed a debilitated appearance, lesions on their tails and paws, and weight loss in spite of increased food intake. Their yoked control (TSC) rats remained healthy. Since dehydration was ruled out and several measures indicated accelerated use rather than failure to absorb nutrients, the food-weight changes in TSD rats were attributed to increased energy expenditure (EE). The measurement of EE, based upon caloric value of food, weight, and wastes, indicated that all TSD rats increased EE, with mean levels reaching more than twice baseline values. **Key Words:** Total sleep deprivation—Debilitation—Skin lesions—Food and weight—Energy expenditure.

In a previous study with the disk apparatus (1), total sleep deprivation (TSD) produced death, diverse pathology, and weight loss in spite of increased food intake. The present TSD study was undertaken to replicate those results and to obtain additional data, which might help explain them.

METHOD

Procedures and nomenclature were described in Part II in this series (2). Of 12 TSD runs started, two were discarded because the TSD rats were sacrificed after 4 and 7 days when death seemed imminent, apparently due to histologically verified pneumococcal infections. Because such infections sometimes occur in normal rats after surgery and no other sleep-deprived rats showed similar infections, the contribution of TSD to the pathology was not clear. Of the remaining 10 runs, nine were with intracardiac cannulas.

RESULTS AND DISCUSSION

Sleep data

In the 10 TSD rats, mean baseline sleep quotas, as a percentage of total time, were: total sleep (TS) 53.4 ± 4.6 SD; paradoxical sleep (PS) 5.9 ± 0.4 ; non-rapid eye move-

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ment (NREM) 47.5 ± 4.5 ; high EEG amplitude sleep (HS) 43.3 ± 4.5 ; low-amplitude sleep (LS) 4.2 ± 0.5 . Percentages of baseline values obtained during deprivation were: TS 8.7 ± 1.9 ; PS 4.4 ± 1.7 ; NREM 9.2 ± 2.1 ; HS 4.9 ± 1.6 ; LS 54.7 ± 18.2 . For yoked controls for TSD (TSC) rats, the baseline percentages were: TS 52.8 ± 3.1 ; PS 5.7 ± 0.8 ; NREM 47.1 ± 2.7 ; HS 42.8 ± 2.8 ; LS 4.3 ± 0.4 . Percentages of baseline obtained during the experimental period were: TS 72.4 ± 10.4 ; PS 53.4 ± 12.0 ; NREM 74.7 ± 10.4 ; HS 71.3 ± 11.6 ; LS 110.4 ± 14.0 . Thus, except for LS—which constitutes a small percentage of sleep during baseline—TSD rats were severely deprived of all sleep stages, whereas TSC rats were only moderately deprived. On several outcome variables, TSC rats showed smaller changes from baseline than TSD rats, but in the same direction. Maintenance of deprivation required disk rotation for a mean of $20.4 \pm 4.6\%$ of total time. Surprisingly, rotation rate remained fairly stable throughout deprivation. Means and standard deviations of percent rotation times for successive quarters of the deprivation period were 21.2 ± 5.7 , 20.9 ± 3.9 , 21.6 ± 4.5 , and 18.1 ± 4.1 .

Functional sleep loss in TSD rats is probably underestimated by the above data. Of the NREM sleep of TSD rats during deprivation, 52% was LS. This was not surprising since LS frequently precedes or follows wakefulness (3), and TSD rats were awakened frequently. However, it is doubtful that LS serves critical functions as other sleep stages may. LS does not rebound from TSD (4). Also, the Pearson correlation between LS and survival time in TSD rats was $r = -0.65$ ($p < 0.10$). In contrast, the correlation between survival time and amount of incidental PS during deprivation, was positive ($r = 0.40$, NS). Functionally relevant TS deprivation was probably closer to 95%. After ~3 days of TSD, the deprived rats started to have sleep-onset PS periods, which increased as sleep deprivation progressed. TSD rats were permitted a 2–3-min period of sleep every few days to help in the adjustment of sleep detection criteria. During these sleep samples, the average median latency from start of HS to start of PS was 41.9 ± 19.9 s. During the last 2 days of baseline, the average median latency was 8.78 ± 2.49 min. (In calculating latency, intrusions of 30 s or less of wakefulness were not counted as disrupting sleep continuity.)

Survival results

Of the 10 TSD rats, seven were sacrificed when death seemed imminent, and one died after 11 days of deprivation. A ninth TSD rat was originally intended for studies to evaluate recovery when sleep was permitted. In contrast to three other TSD rats that showed substantial recovery when deprivation was stopped (5) (Part IX of this series), this rat was unintentionally allowed to pass beyond an apparent “point of no return.” Although the deprivation procedure was stopped (after 22 days), the rats showed only sporadic sleep and died 64 h later. Therefore, this rat was counted as a “TSD death” with survival time of 22 days—the point at which it probably would have been sacrificed had that been our original intention. The tenth TSD rat died accidentally during a blood drawing procedure after 14 days of deprivation. This rat was not included in calculations of survival time. However, for data analyzed by quarters of survival, and based upon the energy and appearance data of other TSD rats, this rat was judged to have completed three-fourths of the deprivation protocol. To maintain a balanced statistical design, fourth quarter values for this rat were extrapolated by repeating third quarter values.

Survival times for the nine “terminal” TSD rats were 11, 16, 18, 20, 21, 22, 24, 24, and 32 days ($\bar{x} = 20.9 \pm 5.9$), which was within one SD of mean survival of 15.6 ± 9.8

days observed in our previous TSD study (1). At sacrifice or death, the nine rats showed the following signs: All appeared extremely debilitated; formally rated rats had ratings of 5.5 or 6.0 on the 1-6 appearance rating scale. During their last 24 h, seven rats showed temperature declines to more than 3 SDs below their individual baseline means; the other two rats showed declines of more than 2 SDs. All showed marked declines from peak food intake ($\bar{x} = -34.6 \pm 26.7\%$). All showed edema of the paws, rated as severe in four rats, moderate in three, and mild in two. Seven rats showed motor weakness and/or ataxia. Only two rats showed a notable decline in waking EEG amplitude. However, the number of disk rotations required to maintain sleep deprivation in near-terminal TSD rats frequently declined, which may partially reflect an inability to generate high-amplitude EEG activity. No TSC rat ever showed any of the above signs or indication that it could not have continued to live under the same conditions.

Appearance

The appearance of TSC rats declined only slightly during deprivation, whereas the appearance of TSD rats declined sharply (Fig. 1). Only seven of the 10 runs were photographed and rated, but TSD rats in the other three runs clearly showed the same debilitated appearance. The group \times time interaction in photograph ratings ($F_{1,131} = 108.3$) was significant at $p < 0.001$. TSD rats looked scrawny. Fur changed in color from creamy white to brownish yellow, looked disheveled, and stuck together in clumps as if it were oily; patches of skin were visible between the clumps. Ulcerative and keratotic lesions, which will be described in more detail in Part VI of this series (6), developed on the tails and plantar surfaces. It is unlikely that the debilitated appearance resulted from failure to groom. Based on extrapolations from extensive videotaping at regular intervals during the experimental period, two TSD rats were estimated to groom an average of 3.62 and 3.83 h daily, whereas their respective controls averaged 2.51 and 2.37 h. Whether grooming was less careful or effective in TSD rats could not be determined. The debilitated appearance is not intrinsic to dying in the rat; six food-deprived (FD) rats that died after a mean of 16.7 days had smooth, normally colored fur and showed no skin lesions. They looked like healthy younger rats; mean appearance rating of four FD rats photographed just prior to death was 1.4 ± 0.25 .

Necropsy and histology

With only incidental exceptions, the internal organs of TSC rats appeared normal. All TSD rats showed enlarged adrenals and an absence of observable body fat; connective

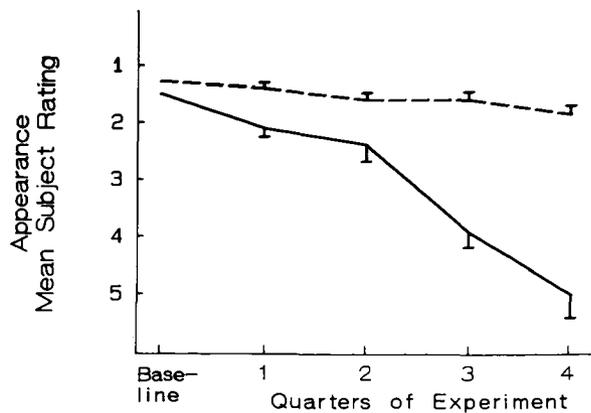


FIG. 1. Mean appearance ratings of TSD (—) and TSC (---) rats during baseline and successive quarters of experiment; 1 indicates completely healthy appearance, and 6 indicates extremely debilitated appearance. Error bars indicate standard errors of the mean.

tissue was frequently reduced to translucent membranes. Otherwise, there was no observable pathology common to all TSD rats. Neither was there any observable anatomical abnormality that could account for imminent or actual death. Pathological signs present in more than two rats included putative "secondary" adrenals (at least five rats—but also seen in two TSC rats) and a blending of mesenteric nodes into a continuous undifferentiated strand (eight rats). In several rats, there was a brownish or maroonish cast in the color of the mesenteric mass, inguinal nodes, and adrenal and thymus glands. No rats showed large crater-like or hemorrhaging ulcers of the stomach lining such as have been reported in stressed rats (e.g., refs. 7 and 8) or observed in all six of our FD rats; six TSD and one TSC rat did show pinpoint erosions of the stomach lining.

Histologic examination by Dr. Lester Wold (Mayo Clinic, Rochester, MN) was limited to the lungs, since these would be vulnerable to water exposure. None of the eight TSD rats evaluated showed signs of pneumococcal infection. Other histology was not done, since examination of organ and brain tissues (9) of rats in our original TSD study (1) revealed no systematic differences between TSD and TSC rats or any microscopically identifiable lesions or abnormalities to account for the death and debilitation of TSD rats.

Weights of spleen, liver, lungs, and brain were not significantly different for TSD and TSC rats. In six pairs evaluated, hearts were heavier in TSD rats ($\bar{x} = 1.64 \pm 0.22$ g) than in TSC rats ($\bar{x} = 1.33 \pm 0.25$ g) ($p < 0.05$, paired t test). In nine pairs evaluated, kidneys were heavier in TSD rats ($\bar{x} = 1.66 \pm 0.22$ g) than in TSC rats ($\bar{x} = 1.47 \pm 0.16$ g) ($p < 0.02$); combined adrenals (including putative secondary adrenals) were heavier in TSD rats ($\bar{x} = 0.097 \pm 0.021$ g) than in TSC rats ($\bar{x} = 0.061 \pm 0.017$ g) ($p < 0.002$). The increased adrenal weights in TSD rats may have resulted from terminal stress. In two pairs that were sacrificed after deprivation effects (debilitated appearance, skin lesions, increased energy expenditure) were clearly manifest, but well before the TSD rats appeared terminal, adrenal weights were similar in the TSC and TSD rats.

Ion balance, hematology, and urine measures

Serum potassium and sodium levels, which were evaluated at about 5-day intervals in eight runs, remained close to baseline levels for both TSD and TSC rats throughout the deprivation period. Hematological evaluations were made at 5-day intervals in eight runs. White cell counts increased similarly and significantly for TSD and TSC rats, reaching mean levels of $17.16 \times 10^3/\mu\text{l}$ for TSD rats and $15.76 \times 10^3/\mu\text{l}$ for TSC rats. However, neither the group difference nor the group \times time interactions approached significance, and the fourth quarter means were within normal limits for rats (10). Red cell counts remained near baseline for TSC rats, but, beginning during the third quarter, they declined for TSD rats, reaching a mean fourth quarter level of $5.28 \times 10^6/\mu\text{l}$. The group \times time interaction ($F_{1,59} = 4.9$) was significant at $p < 0.04$. There were no significant group \times time interactions in any of the other blood parameters noted below. In TSD rats, there were increases of less than 5% in mean red cell volume and reticulocyte count, and less than 2% in hemoglobin per cell. On the other hand, hemoglobin and hematocrit decreased by ~ 10 –15% during the third and fourth quarters. This pattern suggests a macrocytic, rather than an iron deficiency anemia.

There were no significant or substantial differences between the eight TSD and TSC rats (evaluated at 2-day intervals) in the following urine parameters: urobilinogen,

nitrite, bilirubin, glucose, ketone, specific gravity, pH, protein, and greater than trace amounts of blood.

Food, weight, and energy expenditure

All TSD rats increased food intake during sleep deprivation (Fig. 2). The group \times time interaction ($F_{1,374} = 21.60$) was significant $p < 0.001$. Nevertheless, all TSD rats progressively lost weight (Fig. 3). The group \times time interaction ($F_{1,374} = 54.3$) was significant at $p < 0.001$. Mean percentage weight loss from baseline to last deprivation day in the nine terminal TSD rats was $17.3 \pm 6.3\%$ versus $8.0 \pm 5.7\%$ in their yoked controls.

Weight loss alone could not account for the TSD deaths. Six FD rats were subjected to disk rotation continuously for 30 min every 2 h. This schedule approximated the energy demands imposed by rotation on the TSD rats, but permitted the FD rats to sleep during the long nonrotation periods. FD rats survived until they had lost a mean of $46.4 \pm 5.5\%$ body weight, more than double the weight loss of TSD rats. Mean survival was 16.7 ± 2.8 days. Of the six FD rats, four survived longer than two of the of the nine terminal TSD rats. Sleep cannot rank far behind food in survival value.

TSC rats remained near baseline in water intake (amount removed from water bottles), whereas TSD rats increased mean water intake by 14.1% during sleep deprivation, with the greatest increase observed during the first quarter. Although the group \times time interaction ($F_{1,374} = 6.2$) was significant at $p < 0.02$, water intake varied markedly among and within TSD rats, and the mean increase was less than expected from the food increase (11,12). However, the water intake data are complicated by other factors: drinking water from the pan, grooming wet fur, increased metabolic water, and a reduced water need resulting from lower body mass. Nevertheless, there were ample data to indicate that the TSD rats did not suffer from dehydration. Normal serum ion concentrations and urine specific gravity were maintained throughout. Also, as will be reported in Part V of this series (13), TSD rats showed an increase in total body water as a proportion of total body mass, as measured by the dilution of injected ^{18}O . Finally, changes in weight and water intake were essentially uncorrelated in TSD rats ($r = 0.06$, NS).

Weight loss of TSD rats, in spite of increased food intake, did not result from decreased absorption or obvious perturbations of intermediary metabolism. In fact, there

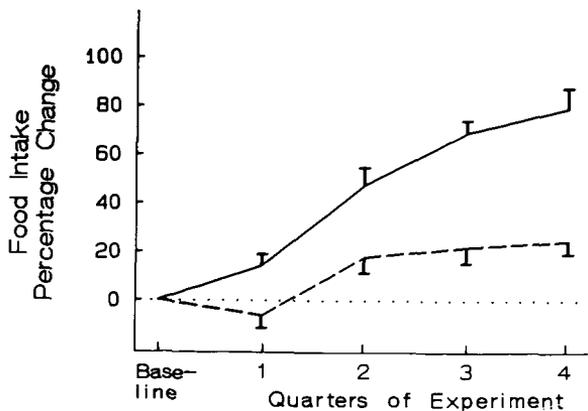
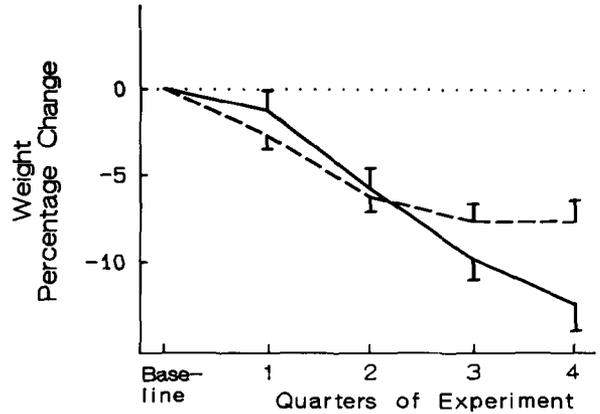


FIG. 2. Mean food intake of 10 TSD-TSC pairs. TSD (—) baseline mean = 25.3 g/day (SD = 2.3); TSC (---) = 26.6 g (SD = 4.2).

FIG. 3. Mean weights of 10 TSD-TSC pairs expressed as percentage change from baseline mean. Error bars indicate standard errors. TSD (—) baseline mean = 409.2 g (SD = 32.5); TSC (---) = 407.6 g (SD = 28.9).



were indications of accelerated use of all major nutrients. Absence of fatty stools indicated normal absorption of fats, and accelerated use was suggested by the absence of body fat at necropsy. That there were no gross perturbations in glucose use was indicated by the absence of hyperglycemia and urinary glucose. Glucose clearance tests conducted at 7-day intervals in eight runs (Fig. 4) indicated enhanced glucose utilization. The difference between the baseline and deprivation glucose clearance rates was significantly greater for TSD than for TSC rats ($F_{1,22} = 5.42$; $p < 0.03$). (This analysis was done for deprivation as a whole, because glucose tolerance tests were not well distributed across quarters.) Serum levels of total protein were maintained near baseline in both TSD and TSC rats. However, accelerated catabolism of proteins in TSD rats was indicated by a rise in plasma urea nitrogen (group \times time interaction, $F_{1,63} = 4.32$; $p < 0.05$) (Fig. 5). Plasma globulins tended (nonsignificantly) to rise in TSD rats, but albumin decreased ($F_{1,63} = 6.60$; $p < 0.02$), which suggests protein malnutrition and a catabolic state (14). A mild and inconsistent elevation in the plasma lactate:pyruvate ratio suggests a small oxygen debt for some, but not all, TSD rats.

The evidence against dehydration and blocked intermediary metabolism, and for

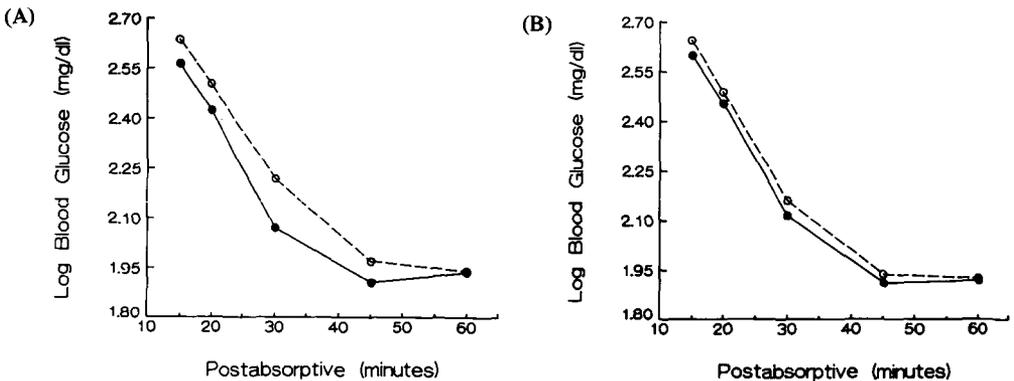
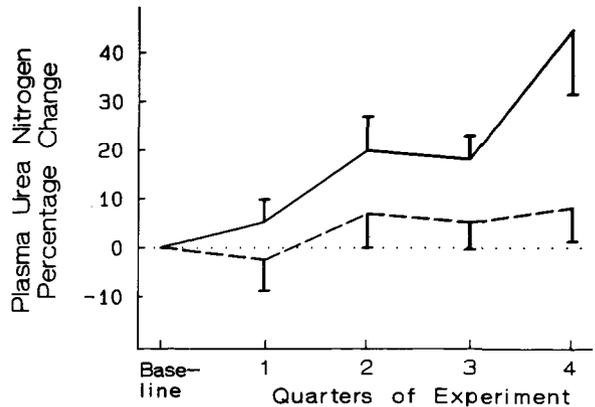


FIG. 4. A (TSD rats) and B (TSC rats): Glucose tolerance curves for eight TSD-TSC pairs. Following an 8-h fast, rats were injected intravenously with 50% dextrose solution, 0.5 ml/100 g body weight. Ordinate shows mean log blood glucose at postabsorptive intervals of 15, 20, 30, 45, and 60 min. Deprivation (—); baseline (---).

FIG. 5. Mean plasma urea nitrogen for eight TSD-TSC pairs. TSD (—) baseline mean = 23.5 mg/dl (SD = 4.7); TSC (---) = 23.9 mg/dl (SD = 3.0).



accelerated use of nutrients leaves increased energy expenditure (EE) as the most likely explanation for both the weight loss and increased food intake. Accordingly, we calculated daily EE based upon food intake, weight change, and estimated wastes by the formula described in Part II of this series (2). Mean EE increased progressively in TSD rats to more than double baseline values during the third and fourth quarter (Fig. 6). The group \times time interaction ($F_{1,348} = 68.5$) was significant at $p < 0.001$. In some rats, the weight loss contributed more to changes in EE than food intake, whereas in other rats the converse obtained. However, the similarity of EE changes in individual rats is reflected in the small standard errors in each quarter.

In TSD rats, the correlation (across rats) between food intake and weight loss became progressively more negative in successive quarters (0.45, 0.29, -0.29, -0.48). This suggests that early in sleep deprivation both stored and new (food) energy were consumed as required by the rapidly increasing EE. As the rats approached limits of readily available energy stores, or ability to digest more food, or ability to increase metabolic activity, a greater proportion of food energy may have been used for tissue preservation late in deprivation than early in deprivation.

As would be expected in rats with high EE, TSD rats showed elevated heart rates. Since TSD rats were mostly awake, only the comparison for waking heart rate is shown in Fig. 7. The group \times time interaction ($F_{1,260} = 6.95$) was significant at $p < 0.01$.

No formal behavioral studies were performed. Frequent and prolonged observation indicated that TSD rats alternated between periods of seemingly normal activity punc-

FIG. 6. Mean energy expenditure of 10 TSD-TSC pairs. TSD (—) baseline = 79.8 kcal/day (SD = 11.7); TSC (---) = 83.0 kcal/day (SD = 11.3).

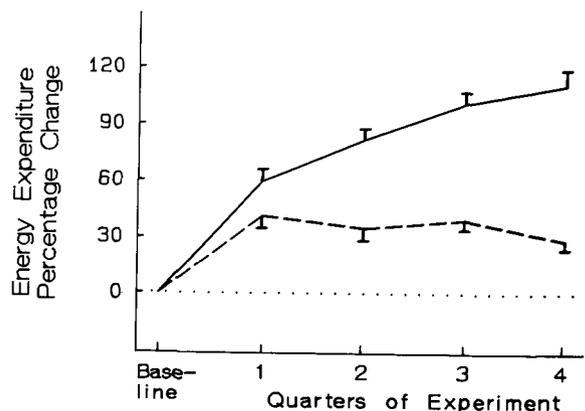
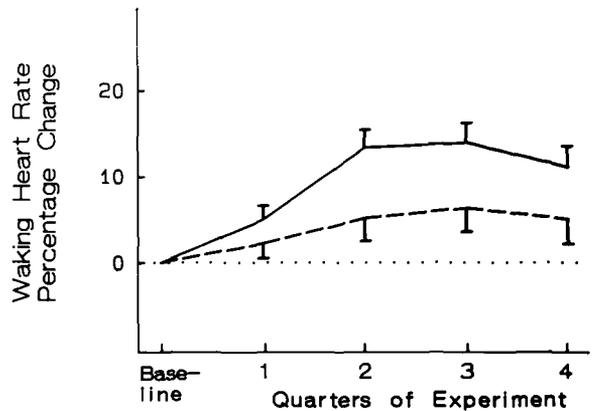


FIG. 7. Mean heart rate during awake in seven TSD-TSC pairs. TSD (—) baseline = 387.8 beats per minute (bpm) (SD = 19.8); TSC (---) = 394.5 (SD = 21.7).



tuated by voracious eating and waves of sleepiness that necessitated disk rotations in rapid succession. The rats were not particularly aggressive.

Immersion controls

In the three TSD rats evaluated, there were progressive increases across quarters in the mean number of water immersions per day: partial = 48, 196, 291, 416; full = 0, 14, 38, 52. Mean *total* minutes in water per day were as follows: partial = 4.4, 23.9, 27.3, 41.2; full = 0, 0.4, 1.1, 1.1. Two immersion control (IC) rats were matched to each of these three TSD rats for immersion rate by the procedures described earlier (2). All IC rats survived the schedule with no indication whatsoever of impending death. None appeared debilitated; mean appearance rating on the last IC day (matched to survival times of TSD rats) was 1.6. Necropsy evaluations revealed no remarkable abnormalities. Organ weights were comparable to those of TSC rats. There were minor epidermal changes, which included, in some IC rats, fur discoloration in the scrotal region and calloused areas or erythematous papules (raised, inflamed areas) on the hindpaws. None of these changes remotely approached the severity of paw and tail lesions seen in TSD rats. Energy changes in IC rats will be reviewed later (13). In summary, it is highly unlikely that water exposure contributed appreciably to the pathology and mortality of TSD rats.

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