Survival of Hamsters Fed Graded Levels of Two Protein Sources.

Diane F. Birt, University of Nebraska Medical Center
Gloria H. Schuldt, University of Nebraska Medical Center
Shahrokh Salmasi, University of Nebraska Medical Center
Survival of Hamsters Fed Graded Levels of Two Protein Sources

Diane F Birt, Gloria H Schuldt, and Shahrokh Salmasi

Summary | Two protein sources (supplemented casein and lactalbumin), which were well-utilized by Syrian hamsters in previous 3-week experiments, were fed in long-term studies. Casein supplemented with methionine and cysteine was fed for 20 weeks at levels of 4, 20, and 40 g/100 g diet. The lowest level did not support normal growth and resulted in the highest mortality rate during the first 10 weeks of feeding. The higher levels caused increased mortality in females, in association with decreasing body weights between 16 and 20 weeks. Kidney weights and incidences of lower nephron nephrosis at 20 weeks were elevated in both sexes fed the 40 g/100 g levels of supplemented casein by comparison with the 10 g/100 g level. In a separate experiment, lactalbumin was fed for life at 10, 20, and 40 g/100 g diet levels, and its effects compared to those with a commercial diet. The lactalbumin diet supported somewhat slower growth rates, but improved survival when compared to commercial diet-fed groups. Survival was longest in both sexes fed the 20 g lactalbumin/100 g diet levels for life.

Key Words | Protein metabolism — Longevity — Nephrosis — Mesocricetus

Dietary protein requirements of Syrian hamsters have been studied in short-term experiments, usually of 3–6 weeks duration, but reports of long-term investigations have not appeared (1). Short-term comparisons in Syrian hamsters of diets containing casein, lactalbumin, and casein supplemented with methionine and cysteine demonstrated that lactalbumin and supplemented casein were somewhat better utilized than casein alone (2). We based our selection of a single, well-utilized protein source on this observation when initiating lifetime studies comparing dietary protein fed at several levels to the Syrian hamster. Studies were initially begun with diets containing supplemented casein as the protein source, but were terminated after 20 weeks. This report describes the difficulties encountered with supplemented casein diets and briefly presents results of a comparable experiment using diets containing lactalbumin conducted after the supplemented casein studies.

Materials and Methods

Diets and animals: Detailed descriptions of the diets were published separately (2). The first experiment in the current report used three levels (4, 20, and 40 g/100 g diet) of casein supplemented with a constant ratio of methionine and cysteine (3.75 parts casein with 0.15 parts methionine and 0.10 parts cysteine). The second experiment, which was conducted following termination of the first, employed three levels (10, 20, and 40 g/100 g diet) of lactalbumin and a commercial diet. Diet was prepared every other week and pelleted as previously described (2). Four-week-old Syrian hamsters from the Epplcy Colony Unei (SYR) were randomly assigned in groups of 105 males and 105 females to each of the three purified diets in experiments 1 and 2 (1,260 hamsters) and to the commercial diet in experiment 2 in groups of 40 males and 40 females. Experiment 1 was initiated in August and experiment 2 in January. Initial average body weights in experiment 1 were 58 g for females and 59 g for males and in experiment 2, 66 g and 67 g, respectively. Hamsters were housed in groups of five on corn cob bedding in 4.5 x 5.1 x 2.4-cm polycarbonate cages. Animal rooms were maintained at 21 ± 2°C, 40 ± 5% relative humidity, 12-hour light-dark cycle, and 10 air changes per hour. Animals were allowed free access to food and water. At 12 weeks of age in each experiment, one male was housed with one female from the same diet treatment for 10 days. Litters were weaned when 3 weeks old (week 13 or 14 of the experiments). In experiment 1 all surviving hamsters were killed at 20 weeks, since it was apparent at that time that normal life spans would not be attained. Kidneys were removed, weighed, and stored in buffered formalin from at least 15 randomly selected hamsters from each group. Hematoxylin and eosin-stained tissue sections were prepared by conventional methods. Urine was collected from the bladder at death and its hydrogen ion content immediately measured on a pH meter. Hamsters in experiment 2 were maintained for life.

Statistical analysis: Death rates were compared by the cumulative life table method (3). Kidney disease incidence was evaluated with a chi square test, and...
kidney and body weights and urinary pH were compared by analysis of variance (4).

**Results**

Survival during the first 20 weeks of feeding is shown in Figure 1. Female hamsters fed diet with 4 g/100 g supplemented casein died at a more rapid rate during the first 10 weeks, compared to those given higher levels. However, the mortality rate was lower with this diet than with the higher levels between 11 and 20 weeks. Similar results were observed when comparing male hamsters fed diet with the 4 g/100 g and the 40 g/100 g levels of supplemented casein. In the second experiment females fed diet with 10 g/100 g lactalbumin had the poorest survival and those given 40 g/100 g level the best. Survival in male hamsters was not influenced by the level of dietary lactalbumin during this period. Hamsters of both sexes fed commercial diet in experiment 2 experienced 10% mortality during this 20-week observation period.

The time periods required to reach 50% mortality in hamsters fed diets containing the three levels of lactalbumin are shown in Table 1. Both male and female survivals were best with the 20 g/100 g lactalbumin diets. In addition, all three purified diets gave better survivals than those observed with the commercial diet (females, 47 weeks; males, 58 weeks) (p<0.001).

Body weight changes in hamsters in experiments 1 and 2 are shown in Figure 2. Effects of supplemented casein levels and sex, and lactalbumin levels and sex were significant (p<0.001) in experiments 1 and 2, respectively. Hamsters fed diet containing the lowest level of supplemented casein began growing only after 8 weeks of age and females receiving two higher levels of supplemented casein lost weight after 16 weeks. Males fed diet with 20 g/100 g supplemented casein were larger than those given the 40 g/100 g level at each of the three times. Females fed diet containing lactalbumin increased in weight at each interval, with weights consistently lower in the 10 g/100 g diet group, while in males, this protein source increased weights only slightly between 16 and 20

---

**Table 1**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Dietary lactalbumin level</th>
<th>Week of feeding*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>10</td>
<td>52^</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>62^</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>64</td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>66^</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>68^</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>65</td>
</tr>
</tbody>
</table>

*Death rate for a<b and c<d (p<0.001)
weeks. Weights were higher at 16 and 20 weeks in males fed the 20 g/100 g level of lactalbumin. Hamsters given commercial diet in experiment 2 grew larger by 20 weeks than those fed any of the purified diets (females: 8 weeks, 83 g; 16 weeks, 106 g; 20 weeks, 128 g increase; males: 8 weeks, 70 g; 16 weeks, 84 g; 20 weeks, 86 g increase).

Findings relative to the hamster kidney in experiment 1 are in Table 2. Kidney weight corrected for body weight rose for both sexes, with each increase in supplemented dietary casein, and it was greater in female hamsters. The only pathological lesion observed by light microscopy was dilatation in the distal convoluted and collecting tubules (Figure 3). Their epithelial lining was basophilic and flattened, and in most instances their lumens were filled by eosinophilic casts. This was accompanied with mild-to-moderate interstitial edema in many specimens. The glomeruli and proximal convoluted tubules were relatively unaffected. This histological picture is compatible with a diagnosis of lower nephron nephrosis (5). The incidence of this disease increased in both sexes, as the level of this protein source rose from 4 or 20 to 40 g/100 g diet (Table 2). Urinary pH was not influenced by the three levels of supplemented casein [mean of 5.91 ± 0.04 (standard error of mean)].

**Discussion**

Short-term studies (3 weeks) demonstrated that diets containing lactalbumin and supplemented casein were equally well-utilized by the Syrian hamster (2). Studies conducted for 20 weeks with diets containing supplemented casein indicated that hamsters fed 20 and 40 g/100 g diet levels of this protein source did not have normal survival and that feeding 4 g/100 g would not allow normal survival through the rapid growth period (4–14 weeks of age). Hamsters fed diets formulated with the lowest level of supplemented casein had a high death rate during the first 10 weeks of the study, a period during which these animals were not growing. Normal growth was achieved during this same period by animals fed the higher levels of this protein source. Death rates increased after 10 weeks in both sexes fed diets with 40 g/100 g and in females fed diets with 20 g/100 g of supplemented casein. This mortality was accompanied by body weight loss in the females between 16 and 20 weeks and by lower kidney weights and incidences of lower nephron nephrosis at 20 weeks in both sexes fed the higher dietary levels of supplemented casein compared to those hamsters fed the lowest level. Lower nephron nephrosis was reported as a common cause of renal failure and may be initiated by various toxins (5).

Excess dietary methionine was the most likely cause of the observed kidney nephrosis. Hamsters received 0.15%, 0.75%, and 1.5% added methionine in the 4, 20, and 40 g/100 g diet levels of supplemented casein in the present study, bringing methionine levels to totals of approximately 0.25%, 1.2% and 2.7%. Methionine toxicity previously was reported in several species, including rats (6,7), chicks (8), cattle (9), and guinea pigs (10). Toxic symptoms and tolerance levels vary considerably between species, but 2–3% levels of added methionine in low protein diets usually caused growth retardation (6–9). Furthermore, the level of dietary protein is important, since elevation of dietary protein from 10% to 50% counteracted, but did not prevent, the toxicity of 5% L-methionine in one study (6). Toxic lesions in rats and chicks were reported in the liver and spleen, but not in the kidneys (6–8). Gross observations on the livers and spleens in hamsters indicated no abnormalities in these tissues. In the present experiment, urinary pH showed that urinary acidosis due to the increased excretion of sulfur was not a factor in the observed kidney disease.

Separate experiments with diets containing lactalbumin demonstrated that this protein source was appropriate for lifetime feeding studies in the Syrian hamster. Growth was somewhat slower in hamsters fed 10, 20, or 40 g lactalbumin/100 g diet in comparison with those fed commercial diet. However, survival was better with each of these levels of lactalbumin compared to the commercial diet. Survival of both male and female hamsters was best in those hamsters fed the 20% level of

---

**Table 2**

<table>
<thead>
<tr>
<th>Dietary supplemented casein level</th>
<th>Kidney weight (g/100 g body weight)a</th>
<th>Number with nephrosisb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/100 g diet</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>4</td>
<td>0.91a</td>
<td>0.89a</td>
</tr>
<tr>
<td>20</td>
<td>1.57a</td>
<td>1.31b</td>
</tr>
<tr>
<td>40</td>
<td>2.86a</td>
<td>1.88b</td>
</tr>
</tbody>
</table>

*aEffects of level of supplemented casein, sex, and interaction of these two significant (p<0.001). Each value represents 26–43 hamsters, standard error of mean = 0.05. Test comparisons: a < b < c < d < e (p<0.01)

*bNo difference was observed between the sexes and values are pooled across sex. Chi-square tests: a < b (p<0.001)

---

**Figure 3**

Kidney of hamster fed 40 g supplemented casein/100 g diet for 20 weeks. Collecting tubules are lined by flattened, degenerated and sometimes basophilic epithelial cells. Interstitium is moderately edematous. Hematoxylin and eosin stain. Line = 50 μm.
lactalbumin. Results from these studies in which diet containing lactalbumin as the protein source was fed at a level as high as the highest supplemented casein level indicated that the poor tolerance of diets containing 20 or 40 g supplemented casein/100 g diet was not due to total protein content of the diet.

References


Primaquine Toxicity in Dogs, Monkeys, and Rats
The subacute toxicity of primaquine was evaluated in young Beagle dogs weighing 5.5–9.8 kg, rhesus monkeys (Macaca mulatta) weighing 2.7–4.4 kg, and young male CD rats weighing 180–260 g. The animals were acclimatized 4 weeks prior to the initiation of the study. The Beagles received 0, 1, 3, or 9 mg/kg body weight of primaquine in a freshly prepared solution for 28 consecutive days via an intragastric tube. The rhesus monkeys were dosed at 0, 1, 4, or 6 mg primaquine/kg body weight by the same route. The rats were similarly dosed at 0, 3, 9, 16, or 27 mg/kg.

Based on body weight, the dog was most sensitive to primaquine and the rat was least sensitive. In all three species, elevated levels of serum transaminases, decreased fasting blood glucose levels, and inflammatory and degenerative changes in the liver and kidneys were observed in the animals receiving primaquine. Significant weight loss occurred at the high dose levels in all species. All of the dogs and half of the monkeys died at the high dose levels.

Additionally, primaquine caused pneumonia and elevation of serum haptoglobin in the dog, and methemoglobinemia, thrombocytopenia, and degenerative and inflammatory alterations of striated muscle in the dog and rat. Reticulocytosis, nucleated erythrocytes, as well as bile duct hyperplasia were observed in the rats. In rhesus monkeys, primaquine caused erythrocytopenia as well as edema and gliosis of the cerebral cortex in some monkeys. Lymphoid depletion occurred in both monkeys and dogs.