Does the rat have an empty stomach after an overnight fast?
P. Jeffrey, M. Burrows and A. Bye

Lab Anim 1987 21: 330
DOI: 10.1258/002367787781363444

The online version of this article can be found at:
http://lan.sagepub.com/content/21/4/330
Does the rat have an empty stomach after an overnight fast?

P. JEFFREY, M. BURROWS & A. BYE

Department of Biopharmaceutical Sciences and Drug Metabolism, Upjohn Limited, Fleming Way, Crawley, Sussex, United Kingdom

Summary
Variable amounts of food have been observed in the stomachs of male rats following an overnight fast. The effects of diet and diet type on the amount of residual food left in the stomach and on fat deposition and liver weight in the male rat were investigated. The implications of these results on metabolism and pharmacokinetic studies are discussed.

Keywords: Rats; Fasting; Diet; Metabolism

The rat is among the commonest laboratory animal species used in metabolism studies. The animals are normally maintained on a commercially available standard diet which is provided ad libitum, and for studies of compounds administered by the oral route the animals are usually fasted overnight prior to dosing.

Recent studies in our laboratory to evaluate gastric emptying have shown that, following an overnight fast, the residual food content in the stomach of fasted rats is extremely variable from relatively full to totally empty.

It is known that the food content of the stomach influences both the rate and the order (i.e. zero or first order) of gastric emptying (Wagner, 1971). Furthermore, the absorption and ultimately the bioavailability of certain compounds are very much influenced by the presence or absence of food in the stomach (Melander, 1978). Therefore, in order to reduce experimental variability, the residual food content in the stomach following an overnight fast should be either nil or at least constant in any experimental group of animals.

In this paper studies to investigate the influence of (a) diet type and (b) the amount of diet presented on the residual food content in the rat stomach following an overnight fast are described. An assessment of the effect of the diet on fat deposition and liver weight was also made.

Materials and methods
Diets
Two diets were used: an expanded diet, SDS No. 1, and a non-expanded diet, PCD No. 1 (both obtained from Wm. Lillico and Sons, Betchworth, with analytical standard quality control details supplied). Details of the major components of each diet are shown in Table 1.

Table 1. Summary of the major components of the SDS No. 1 (expanded) and PCD No. 1 (non-expanded) diets used in the study

<table>
<thead>
<tr>
<th>Component</th>
<th>SDS No. 1 (%)</th>
<th>PCD No. 1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digestible crude oil</td>
<td>2.4%</td>
<td>2.7%</td>
</tr>
<tr>
<td>Digestible crude protein</td>
<td>13.6%</td>
<td>18.3%</td>
</tr>
<tr>
<td>Total dietary fibre</td>
<td>18.6%</td>
<td>16.7%</td>
</tr>
<tr>
<td>Total starches</td>
<td>38.0%</td>
<td>30.2%</td>
</tr>
<tr>
<td>Total sugars</td>
<td>10.4%</td>
<td>13.2%</td>
</tr>
<tr>
<td>Gross energy</td>
<td>14.8 MJ/kg</td>
<td>15.0 MJ/kg</td>
</tr>
<tr>
<td>Digestible energy</td>
<td>11.4 MJ/kg</td>
<td>11.9 MJ/kg</td>
</tr>
<tr>
<td>Metabolic energy</td>
<td>10.3 MJ/kg</td>
<td>10.7 MJ/kg</td>
</tr>
</tbody>
</table>

Data obtained from the supplier, Wm. Lillico and Sons, Betchworth, United Kingdom.

Animals
Male Sprague Dawley rats, Crl:CD(SD)BR (Charles River Ltd, Manston), were used throughout the study. Animals were approximately 30 days old and weighed approximately 100 (± 4) g at the start of the study. They were housed in groups of four in plastic cages with raised metal grid floors (North Kent Plastics, Dartford).
Experimental design

The animals were divided by random selection into the following groups (eight animals to a group):

Group I  Diet, SDS No. 1 \textit{ad libitum}
Group II Diet, SDS No. 1 controlled
Group III Diet, PCD No. 1 \textit{ad libitum}
Group IV Diet, PCD No. 1 controlled

Body weight and food consumption were measured daily. Using the growth profile obtained from the supplier (based on body weight and age), the amount of food available to the animals was carefully controlled to maintain the required growth profile. Adult rats consume between 15 and 20 g of food per rat per day (Woodnott, 1969; Inglis, 1980). Animals on a controlled diet therefore initially received 15 g increasing to 22 g of food per rat per day. All animals (regardless of diet) were fed at 09.00 h (± 30 min) each day and body weight was recorded prior to feeding.

Rats used in metabolism studies are normally selected on the basis of body weight and not by age, rats within the weight range 250–300 g being most popular. Therefore, on reaching this weight range, the relevant group was fasted overnight. Any residual food was removed from the food hoppers at 15.00 h and the animals were fasted until 09.00 h on the following day, when they were weighed and then killed by carbon dioxide inhalation. After opening the abdomen of each rat, the stomach with its contents was removed and weighed. The stomach was then cut open and any residual food material was carefully removed and weighed. The liver was removed and weighed. Deposition of fat was assessed by weighing the epididymal fat pad deposits dissected from around each testis of the animal (Ford & Ward, 1983).

Statistical analysis of the data on deposition of fat and liver weight was performed by use of two-way analysis of variance (ANOVA).

Results

Results for body weight gain and food consumption over the study period are shown in Figs 1 and 2 respectively. Animals fed \textit{ad libitum} (groups I and III) consumed the same amount of food and had the same growth rate regardless of diet type. The required weight range (250–300 g) was reached by these groups when the animals were approximately 47 days old.
old. However, routine metabolism studies performed in our laboratories have shown that animals maintained on an *ad libitum* diet will show an apparent weight loss following an overnight fast (see Discussion). Therefore in this study these animals were not used until they were well within the required weight range. Animals fed a controlled diet (expanded or non-expanded, groups II and IV respectively) had a much reduced growth rate compared with animals fed *ad libitum*, attaining the required weight range at approximately 65 days of age.

The mean residual food content in the stomach, the mean epididymal fat pad weight and the mean liver weight are shown in Table 2. Residual food content in the stomach is expressed as a percentage of the empty stomach weight. Mean epididymal fat pad weight and liver weight are normalized to a percentage of rat weight. Only animals fed on a controlled non-expanded diet (group IV, PCD No. 1) had no residual food in the stomach following an overnight fast. All other animals had variable quantities of food in their stomachs.

The values obtained for epididymal fat pad weights and liver weights were found to be normally distributed and the two-way analysis of variance was performed accordingly. Animals fed on a controlled diet (groups II and IV) had significantly less (*P* < 0·05) fat deposition, as measured by epididymal fat pad weights, and a smaller liver weight (*P* < 0·05) than animals fed on a diet *ad libitum* (groups I and III), regardless of diet type. There was no apparent relationship between epididymal fat pad weights and liver weights in any of the groups.

**Discussion**

The presence of food in the rat stomach may have significant influence on the bioavailability and pharmacokinetics of certain drugs following oral administration (Melander, 1978). The data presented in this study show that the diet regimens traditionally employed can result in variable quantities of food being retained in the stomach after fasting overnight. This can often lead to abnormal results following subsequent oral drug dosing owing to variable bioavailability (i.e. rate and extent of drug absorption).

The original explanation for incomplete stomach emptying after overnight fasting was thought to be diet type. Expanded diets can hold large amounts of water and it was considered that perhaps it was this that was causing a delay in the normal gastric emptying habits of the rat. This was subsequently shown not to be

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Diet type</th>
<th>Mean residual food content in stomach (range) (%)</th>
<th>Epididymal fat pad weight* (g/100 g)</th>
<th>Liver weight* (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>SDS No.1</td>
<td>18·9 (0·49)</td>
<td>0·265 (0·055)</td>
<td>4·612 (0·267)</td>
</tr>
<tr>
<td>II</td>
<td>SDS No.1</td>
<td>5·7 (0·21)</td>
<td>0·047 (0·140)</td>
<td>4·047</td>
</tr>
<tr>
<td>III</td>
<td>PCD No.1</td>
<td>19·7 (0·48)</td>
<td>0·247 (0·213)</td>
<td>4·283</td>
</tr>
<tr>
<td>IV</td>
<td>PCD No.1</td>
<td>Nil (Nil)</td>
<td>0·199 (0·059)</td>
<td>4·283</td>
</tr>
</tbody>
</table>

*a* 15-22 g per rat per day.

*b* The standard deviation is given in parentheses.
the case since rats fed ad libitum retained similar residual food amounts in their stomachs irrespective of diet type.

Overfeeding coupled with under-exercise and apparent boredom are known problems (Tucker, 1982) and the recommendations for the use of diets formulated with low nutrient density are upheld in this study in that the expanded diet (SDS No. 1) and non-expanded diet (PCD No. 1) used are both low protein diets of similar energy content (see Table 1).

After an overnight fast, animals on an ad libitum diet (groups I and III) had a body weight reduced by up to 10% of the previous day’s weight, while animals on a controlled diet (groups II and IV) maintained a body weight increase (Fig. 1). This appears to be due to the mass of food in the guts of rats fed ad libitum and the subsequent overnight weight loss owing to defecation and urination (Inglis, 1980).

Furthermore, animals on the ad libitum diet had significantly more fat, as indicated by epididymal fat pad weight, than the controlled diet group, therefore increasing the ratio of fat to lean body mass. Since the majority of drugs are administered on the basis of a mass of drug per mass of animal, subsequent bioanalytical results may vary depending on when the animal was weighed and whether the drug was distributed into fat or lean animals. The amount of variance will depend on the dietary arrangements related to a particular study.

Although animals fed a controlled diet (groups II and IV) showed a significant reduction in liver weight compared with animals fed ad libitum (groups I and III) regardless of diet type, the importance of this will require further study to assess the biochemical status of the liver and the effect, if any, on drug metabolism and distribution.

The effect of fasting time on intestinal drug absorption in rats has been reported previously (Doluisio et al., 1969). The results obtained showed that a fasting period in excess of 20 h may hinder rather than enhance the absorption process because of, for example, energy deficiencies in active transport systems. In this study, the animals were killed at 09.00 h (having been fasted from 15.00 h the previous day); the fasting period was therefore 18 h. It was noticeable that animals on a controlled diet did not have any food left in their food hoppers at 15.00 h (the commencement of fasting) whereas those in the ad libitum group did and therefore the fasting period may have been longer in the controlled group.

Rats are known to be night feeders (James, Fronik & Hesseling, 1985) and, although use of a non-expanded diet on a controlled basis resulted in animals with empty stomachs following an overnight fast, the effect of this regimen on the physiology and biochemistry of the rat is unknown. For convenience, a switch in the night–day cycle or alternatively the use of automatic food dispensers (James, Fronik & Hesseling, 1985) may be necessary to overcome any physiological disturbance to the rat, if this is thought to be important experimentally for a particular drug or biological process under investigation.

Our recommendation for metabolism studies in the rat, when low analytical variance is essential, is that controlled feeding must be coupled with the use of a non-expanded diet. This is the only practical way to produce a consistently empty stomach prior to drug dosing. Ignorance of the influence of dietary factors on, for example, stomach emptying, body weight gain and body composition may lead to variable experimental results from drug metabolism studies that are carried out on fat rats with full stomachs.

Acknowledgment
The authors wish to thank Mrs T. A. Osgood for technical assistance.
References


Jeffrey, Burrows & Bye


