Interrelationships between diet and weight gain in rats with portacaval shunts

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Rate of weight gain and daily food intake, as well as fasting colon weight, liver weight, and arterial blood ammonia levels, were measured in rats with end-to-side portacaval anastomoses and their sham-operated, paired controls. The diets provided were regular chow and special, low-residue feed with increased amounts of protein, fat, or carbohydrate. The chow-fed, operated group gained weight at a significantly slower rate than their paired controls which was the only significant intragroup difference. The special-diet, operated groups gained significantly slower than the shams when the data from these groups were pooled, obliterating the dietary distinction. This singularity of the chow-fed group was accompanied by a very much heavier colon in both operated and control categories than that of animals receiving low-residue feed. Liver weights were lower and arterial ammonia levels higher in animals with shunts. The significance of the greater colon weight and lower rate of weight gain in fistulated animals receiving regular chow is discussed in the context of symbiotic relations between gastrointestinal flora and host.

Anastomosis of the portal vein to inferior vena cava, first reported by Eck in 1877 (4), has provided a useful preparation for the study of diverse functions of the liver. Cat and dog have been the species most used until recently, when Lee and Fisher in 1961 (11) described a successful technique for constructing end-to-side portacaval anastomoses in rats. This was followed by a report from Henzel, Turcotte, and Child (6), who extended experience with this preparation in rats and added data from side-to-side anastomoses.

McDermott and Adams (13) reported in 1954 fluctuating neurological disorders in a patient with a portacaval anastomosis, whose liver was essentially normal. This served to stimulate experiments using Eck fistula preparations to elucidate the disorders underlying hepatic coma. Experience has shown that cats and dogs, when fed high-protein diets, will frequently develop motor and sensory aberrations which can progress to or be accompanied by depressed levels of consciousness.

Awareness of nutritional contributions by intestinal flora has increased over the past few years (12), a development facilitated by availability of germfree animals. There has not been a strong rapprochement, however, between those interested in meat intoxication and the rapidly expanding knowledge of nutrition in animals with altered or absent gastrointestinal microflora.

This study describes the effects of varying diet on weight gain in rats and end-to-side portacaval shunts in comparison with paired controls. It represents an exploratory evaluation of feeding and behavior in animals with this surgical alteration.

MATERIALS AND METHODS

Twenty-four male Sherman-strain rats weighing between 250 and 375 g were used. Twelve of these had end-to-side portacaval anastomoses constructed in the manner described by Lee and Fisher (11), while twelve served as paired controls. The animals were fasted 24 hr before surgery to decrease the amount of feces in the colon and the amount of omental fat. Anesthesia was provided by 40 mg sodium hexobarbital given intraperitoneally. After the abdomen was shaved, a midline incision permitted the viscera to be reflected laterally exposing the vena cava. This vessel was cleared of peritoneum just above the entrance to the right renal vein. The portal vein was then mobilized exposing its most proximal tributary, the pyloric vein, which was sacrificed for technical convenience. The lienal vein, the next tributary, was also well exposed to assure its occlusion on clamping the portal vein. A ligature was loosely placed around the proximal part of the portal vein and a bulldog clamp placed on the distal part. The ligature was tied and the vein divided.

The inferior vena cava was partially occluded by a rubber-shod, curved hemostat and a longitudinal incision made in the resulting sac. It was found important to flush the portal vein lumen and vena caval sac with...
RATS WITH PORTACAVAL SHUNTS

A TABLE I.

came apparent. Animals whose bedding became am-
moniculc very promptly stopped gaining and frequently
for maintaining a positive rate of weight gain soon be-
ence with Eck fistula rats. The necessity of fresh bedding
RESULTS

T wt.
organ weights were rendered in g organ/100 g body caloric values of
large bowel was determined.
infusion fixed by the technic of Koenig, Groat, and Windle
was drawn from the abdominal aorta in a heparinized
syringe and transferred to an EDTA-containing Vacu-
tainer (Becton, Dickinson, & Co., Rutherford, N.J.)
to be analyzed for ammonia content by the method of
Reinhold and Chung (15). Terminally, a catheter was
placed in the ascending aorta, and the animal was per-
fusion fixed by the technic of Koenig, Groat, and Windle
(10). The weight of the freshly fixed whole liver and
size of fecal pellets from animals on any of
these diets were considerably decreased in comparison
with the group receiving regular chow. This point will
be considered further in relation to colon weight.

All animals receiving the low-residue special diets
exhibited marked reduction in fecal output. Both the
number and size of fecal pellets from animals on any of
these diets were considerably decreased in comparison
with the group receiving regular chow. This point will
be considered further in relation to colon weight.

Daily postoperative weights were assigned to appro-
priate weekly periods. The average, midinterval weight
was calculated and used to form a ratio with the control
weight. Resulting values were used to determine the
sample regression equation of weight-gain ratio on time,
employing the assumption of linear weight gain during
the experimental period. Individual regression equa-
tions for sham or shunted animals in each category in
turn were averaged. These average regressions are
arrayed in Table 1 with the standard error of the means
of the intercepts and slopes.

The average of all intercepts is 0.994, which is not
significantly different from 1.000. None of the individual
average intercepts differ significantly from this over-all
value, nor did comparing the mean values of pooled
intercepts of operated animals against pooled control
values result in a significant difference.

Analysis of slopes from individual pairs in each special
diet group by pairwise comparison did not indicate
significant (P > 0.05) differences between operated
individuals and shams. Pooling the slopes of shunts and
controls, however, for the special-diet data, while
maintaining individual pairing, yields t = 3.72 with
df = 8 which is significant (0.005 < P < 0.010),
indicating that the rate of weight gain of the pooled operated
animals is less than that of the pooled controls receiv-
ing special diet.

When the shunt and control pairs of the chow-diet

would lose weight concomitant with decreased food
intake. This trend promptly reversed upon cleaning the
cage. Handling the animals during weighing and cage
maintenance appeared to have a salutary effect on their
over-all condition. Although these variables were not
quantitated, close attention was paid to treating each
member of this series identically.

Behaviorally, it was not possible to distinguish shunted
animals from their controls. One quite striking differ-
ence in appearance, however, became apparent 2 or 3
weeks postoperatively. This was the slower hair regrowth
on the shaved abdominal areas of the shunted animals.
Full recovery had not occurred at the end of 10 weeks
in distinction to the controls, who appeared normal at
the end of 3 weeks. This difference was not so striking
in the group of animals receiving a high-carbohydrate
diet.

Table 1. Average regressions of weight-gain ratio on time

<table>
<thead>
<tr>
<th>Diet</th>
<th>Operated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>$\hat{y} = (0.904 \pm 0.034) + (0.045 \pm 0.021)x$</td>
<td>$\hat{y} = (0.904 \pm 0.013) + (0.074 \pm 0.027)x$</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>$\hat{y} = (0.93 \pm 0.037) + (0.085 \pm 0.013)x$</td>
<td>$\hat{y} = (1.039 \pm 0.030) + (0.072 \pm 0.006)x$</td>
</tr>
<tr>
<td>Fat</td>
<td>$\hat{y} = (1.02 \pm 0.042) + (0.061 \pm 0.024)x$</td>
<td>$\hat{y} = (0.907 \pm 0.020) + (0.055 \pm 0.008)x$</td>
</tr>
<tr>
<td>Chow</td>
<td>$\hat{y} = (1.00 \pm 0.045) + (0.039 \pm 0.008)x$</td>
<td>$\hat{y} = (0.939 \pm 0.020) + (0.061 \pm 0.015)x$</td>
</tr>
</tbody>
</table>

$\hat{y}$ = predicted ratio, $x$ = weeks. Standard error of each mean is given.
group are similarly compared, \( t = 5.48 \) with df = 2, which is significant \((0.005 < P < 0.050)\), indicating a lower rate of weight gain in shunted animals eating regular chow than in their controls.

Table 2 is a summary of the caloric intake, organ weights, and arterial ammonia levels with corresponding standard error of the means. Each value is the mean of the three individual measurements in any particular experimental condition.

Daily food consumption, measured during the post-operative interval, was divided by body weight for the corresponding day, giving grams of food consumed per 100 g body wt/24 hr. From this was calculated the number of kilocalories ingested per 100 g body wt/24 hr. Thirty consecutive daily values were collected for each animal and averaged. Pairwise analysis of the individual caloric values for each dietary group was performed. No significant \((P > 0.05)\) difference exists between operated and sham animals receiving high-fat, -carbohydrate, or -protein diets. The chow group, however, yielded \( t = 5.61 \) with df = 2, which is significant \((0.005 < P < 0.050)\), indicating a lower caloric intake in shunted animals eating chow than in their paired controls. Also, comparing chow controls with the carbohydrate controls gave \( t = 7.36 \), df = 4, which is significant \((0.001 < P < 0.005)\).

No significant differences exist among the mean colon weights for sham and control groups receiving high-fat, -carbohydrate, or -protein diets. While the difference in means between operated and control colon weights is not significant for the chow group, these values are significantly \((P < 0.001)\) greater than those of the other dietary groups. Greater colon weights of the chow-diet group correlate well with the observed greater fecal output from these animals and may well mirror the lack of fibrous material in the special diets.

Liver weights and arterial ammonia concentrations, on the other hand, display a different pattern of variation. Here the distinction is between operated and control groups independent of diet. The liver weights are significantly less in animals with shunts and the blood ammonia levels higher \((P < 0.001)\).

The time relations in these data should be summarized to clarify discussion. Body weight and food consumption were measured during the 10 weeks following operation. Colon and liver weights and blood ammonia levels were measured at the end of this period in animals which had been fasted for 24 hr prior to sacrifice.

At the termination of the experimental period, we were most interested in the patency and condition of the anastomoses. Our experience with the immediate post-operative period was that, if a shunt thrombosed closed, the animal would not survive more than several hours.

When the abdomen was terminally opened prior to perfusion, the patency of the shunt was determined by injecting normal saline into the portal vein and watching it enter the inferior vena cava. It was also possible to occlude the portal vein distal to the needle tip and aspirate blood back from the vena cava. All of the shunts were patent using these criteria.

The shunt orifice, when examined in the fixed state and viewed from the intimal side of the cava, was elliptical. The long axis paralleled the direction of flow in the vena cava and measured between 3 and 4 mm in all specimens. The minor axis measured about 2 mm.

**DISCUSSION**

Two patterns of variation appear to exist in the data presented above. Rats with portacaval anastomoses receiving regular chow do not gain weight as rapidly as their paired controls. This statistically significant intragroup variation was not found in any of the other experimental groups receiving low-residue diet. This singularity is accompanied by fasting colon weights in both shunted and control animals which are greater than those of individuals receiving low-residue special diets.

The second pattern of variation was found to be relatively insensitive to diet but strongly dependent on the existence of a shunt. Data in this category are liver weights, fasting arterial blood ammonia levels, and slightly smaller rates of weight gain of shunted animals than shams, a difference found only by pooling data.

The observation of decreased rate of weight gain for animals receiving chow agrees with the data presented by Lee and Fisher (11) and by Henzel et al. (6). The
amount of weight lost in the immediate postoperative period in the present series was not so great as described in these other studies, possibly reflecting the rather intensive individual care expended on maintenance. In the case of the results of Henzel and co-workers this difference may arise from their use of heavier animals.

Failure to gain weight in a rat with an Eck fistula may represent a manifestation of shunt toxicity acting through decreased food intake. Other species develop diffuse neurological abnormalities either spontaneously or when challenged with high protein, orally administered ammonium chloride, or ammonia-releasing resin. Existing evidence strongly implicates ammonia as the toxic agent, possibly acting by depleting components of the tricarboxylic acid cycle.

Houpt (7) has shown in ruminants that endogenous urea passes from plasma to rumen and is rapidly hydrolyzed by bacterial urease. Resulting ammonia is either incorporated into bacterial protein or is absorbed by the portal venous system. The latter portion passes to the liver where it is reincorporated into urea and in this way re-enters the cycle. This system has recently been demonstrated by Houpt (8) to be present also in the rabbit, an animal with extensive cecal metabolism.

Presumably this cycle exists in the rat, since this species has a quite extensive cecum and a high level of colonic urease activity (17). Thus, the portal blood ammonia load would be determined by the amount of cecal microflora and its metabolic activity. Since the cecum weights directly reflect the amount of cecal material and are greater in animals eating chow than in animals on low-residue diets, it is probable the ammonia load in the former dietary group was greater. Weight gain in shunted animals receiving high-protein, low-residue diet was not significantly less than in their controls, although a larger population might reveal a difference. This is of some interest when considering the reported adverse effect of high-protein diet on members of other species with Eck fistulas. The animals in this series, on the other hand, had a very much smaller colon, a finding in support of the above suggestion relating portal ammonia load to cecal size.

If this analysis is correct, we are faced with the observation that arterial blood ammonia levels in shunted animals receiving chow were not different from those of animals on low-residue diets. Consideration of this paradox suggests the time dependency of blood ammonia levels, since these samples were obtained from animals fasted for 24 hr. Stahl (16), in his review of ammonia tolerance tests in patients with liver disease, presents data strongly suggesting such a dependency.

Fluctuations in portal blood ammonia level would be expected consequent to variations in cecal ammonia production, which, in turn, would be influenced by the quantity and quality of food reaching the cecum and the amount of endogenous urea. It would not be surprising, then, if substantial time-dependent fluctuations in portal blood ammonia levels should exist which would be reflected systemically in an animal with a portacaval shunt.

A factor which was not controlled but assumed to be equally present in all members of this series is coprophagy. This is of some importance, since prevention of coprophagy in the rat, which consumes 35-50% of its feces, decreases the portal blood ammonia level (1).

Our finding of decreased liver weight per unit body weight agrees with the findings of others (3, 6, 11). Centrilobular hypoxia with subsequent atrophy has been suggested as the mechanism causing decreased liver weight subsequent to Eck fistulation (14). Failure to gain weight in animals on low-residue diets could reflect a nonspecific, systemic response by the animal to decreased liver mass; or, following the argument presented above, this could be a minor degree of ammonia toxicity. The latter alternative is supported by the smaller colon size and a predicted relative decrease in systemic arterial ammonia load. The present data, however, do not permit a differentiation to be made between these alternatives.

All of the diets used in this study contain at least basal quantities of necessary nutritional factors. Recent evidence (5, 9), however, strongly suggests a finely balanced symbiotic relation between an animal and its intestinal flora. Certainly the shunting of portal blood around the liver directly into the systemic circulation would be expected to modify this balance, as would modifying the amount and kind of intestinal microflora.

The technical assistance of Diane Weiss with the blood ammonia determinations is gratefully acknowledged.

REFERENCES

10. KEOEING, H., R. A. GROAT, AND W. F. WINDLE. A physiological