A New Model for Studying Nutrition in Peritonitis

The Adverse Effect of Overfeeding

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In guinea pigs fed *ad libitum*, controlled intraperitoneal infusion of bacteria by an implanted 7-day osmotic pump resulted in peritonitis or abscess formation with a 50% survival 14–18 days after pump implantation. Administration of 125 kcal/kg/day of a diet found to be optimal for burned guinea pigs by continuous pump controlled feedings via a previously placed gastrostomy was well-tolerated, with a 62.5% mortality by Day 17. Administration of only 100 kcal/kg/day caused weight loss of approximately 17% after 16 days, but fewer animals died (42.8%, p = NS). Feeding either 150 kcal/kg/day or 175 kcal/kg/day caused death in all 25 animals (p < O.OOl) and their survival time was slightly shortened (p = NS) when compared with animals receiving 100 or 125 kcal/kg/day. This is the first animal model of peritonitis that permits incisive dissection of the relative influences of dietary composition on outcome, because survival can be extended to 2 weeks or more in the presence of continuing sepsis.

NUMEROUS ANIMAL MODELS have been used to study therapeutic interventions of bacterial peritonitis. Most provide only short-term observations because the majority of animals that are destined to die do so within 24–48 hours of simple contamination. Introduction of bacteria entrapped in a fibrin clot,[1-3] insertion of gelatin capsules containing bacteria with or without adjuvants such as barium sulfate or sterilized feces,[4] and cecal ligation with puncture[5-7] are techniques that provide somewhat longer survival than simple injection of bacteria with or without adjuvants. Nevertheless, average survival time is still short, usually less than 1 week, and survivors often completely clear their infections.

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Although nutrition has been believed to be an important determinant of outcome in bacterial infections in humans, this has been a difficult supposition to study in a relevant animal model. The major problems related to current models are that infected animals will often not eat what is intended for them and that the effects of nutritional intervention often do not become manifest for several days after it is instituted—too late to make a difference. As a consequence, virtually no experimental data are available to guide selective nutritional support for established bacterial peritonitis in humans.

Development of a guinea pig model that uses continuous pump-controlled gastrostomy feedings has allowed the dissection of several variables to optimize nutritional support after thermal injury.[8-15] This model has been modified to study the effects of enteral nutrition on the outcome of peritonitis caused by constant infusion of bacteria via an osmotic pump, a technique that allows severely infected animals to survive for weeks rather than for days.

Materials and Methods

Preliminary Studies

For the purposes of this study, a model was developed in which chronic peritonitis could be consistently reproduced, and continuous pump-controlled gastrostomy tube feeding was employed to study the influence of dietary composition on outcome. *Escherichia coli* and *Staphyloccus aureus* in equal quantities were selected to produce a polymicrobial aerobic, mixed gram positive-gram negative peritonitis. Intraperitoneal infusion of live bacteria

was accomplished with osmotic pumps (Alzet®2ML1, Alza Co., Palo Alta, CA). The osmotic pumps are constructed of three concentric cylinders, the inner one an impermeable pliable bag for the bacteria, and the outer two cylinders cellulose and salt that swell with fluid from the peritoneal cavity and compress the inner cylinder.[16] Implantation of the bacteria-filled pump alone produced rapid mortality, so a 100-cm Silastic line was added to the end of the osmotic pump to delay release of bacteria and to increase the survival time. With these parameters, animals without gastrostomies were allowed to feed *ad libitum* after implantation of an osmotic pump containing *E. coli* and *S. aureus* at concentrations of 10^8 or 10^7. Average survival was 16 days and 12 days, respectively, with a 50% mortality by Day 14, after implantation of the pump. There was no mortality in the group of animals that were given pumps without bacteria.

Twenty-two animals were then given a gastrostomy 1 week before implantation of the bacteria-filled osmotic pump, but were allowed to eat natural chow *ad libitum*. The pumps were filled with tenfold dilutions of bacteria from 10^6 to 10^9. With an infusion of bacteria at concentrations of 10^9, the animals died between 4 and 9 days—too short a period for nutritional intervention. By Day 18, a mortality of 50% was seen at doses of 10^7 and 10^8 with an 8–15% weight loss by time of death. Up to Day 21, sporadic mortality was seen at an infusion of 10^6 bacteria. Therefore, the dose of 1 cc of 10^8 *E. coli* and 10^8 *S. aureus* was chosen for subsequent studies.

The diet formulation used was previously found to be optimal for nutritional support for burned guinea pigs at 175 kcal/kg/day[9] (Table 1). When this diet was started on the day of osmotic pump implantation, all animals died of severe ileus. When 14 animals were allowed to eat *ad libitum* for 3 days after osmotic pump implantation before enteral diets were started, all survived for at least 6 days while receiving enteral feedings.

TABLE 1. *Composition of Basic Diet*

	Amount
Nutrient sources	
Protein (Promix R.P.D.®*)	62.5 g/L
Fat (Microlipid®†)	27 ml/L
Carbohydrate (Nutrisource®‡)	212 ml/L
Electrolyte and vitamin solution (amount per liter)	
NaCl (2.5 mEq/ml)	24 ml
KCl (2 mEq/ml)	10 ml
K acetate (2 mEq/ml)	24 ml
K phosphate (3 mM/ml)	10 ml
Ca gluconate (0.1 g/ml)	24 ml
MgSO4 (4 mEq/ml)	24 ml
MTE5™§	6 ml
Multi-vitamin concentrate‖	5 ml
Vitamin C	500 mg
Vitamin E	100 mg
Choline chloride	500 mg
Folic acid	2 mg

* Navaco Laboratories, Phoenix, AZ.
† Organon, Inc., (Bioresearch), West Orange, NJ.
‡ SandozNutrition, Minneapolis, MN.
§ MulTE-PAK-5™, Smith & Nephew, Franklin Park, IL. Each liter of diet contains: 6 mg of zinc, 2.4 mg of copper, 0.6 mg of manganese, 24 μg of chromium, and 120 μg of selenium.
‖ LyphoMed®, Melrose Park, IL. Each liter of diet contains (from Multivitamin concentrate): 2.5 g of ascorbic acid, 50,000 IU of vitamin A, 5000 IU of vitamin D, 250 mg of thiamine HCL, 50 mg of riboflavin, 75 mg of pyridoxine HCL, 500 mg of niacinamide, 125 mg of dexpanthenol, and 25 IU of vitamin E.

Surgical Procedures

Hartley guinea pigs (Murphy Laboratories, Plainfield, Indiana) weighing 370–400 g were provided with intragastric feeding tubes by surgical placement of a gas-sterilized silicone tube (size 0.157 cm ID, 0.24 cm OD, length 50-cm; Dow Corning Medical Grade, Midland, MI). While the animals were under general anesthesia (ketamine HCl 50 mg/kg, acepromazine maleate 0.2 mg/kg, atropine sulfate 0.04 mg/kg) the abdomen, neck, and interscapular areas were clipped to remove hair and painted with povidone-iodine. A gastrostomy was performed as previously described.[8,9] The free end of the tube was tunneled subcutaneously around the left hemi-thorax to exit via a stab wound in the upper interscapular region, later to be connected to the diet infusion system. The tube was flushed with saline and capped. For 10 days, the animals were caged individually, taking Guinea Pig Pellet Diet (Wayne® Feeds Research Division, Libertyville, IL) and water *ad libitum*. This allowed time to regain the more than 10% body weight loss that occurred immediately after surgery.

After this stabilization, the animals underwent a laparotomy under general anesthesia to place a bacteria-filled osmotic pump into the right lower quadrant, to keep the pump away from the gastrostomy. The guinea pigs were then placed into individual metabolic cages and allowed to eat *ad libitum* for 3 days. On Day 3, the animals were randomized into experimental groups.

Preparation of Bacteria

For 18 hours before implantation of the pump, cultures of *E. coli* 53104 (courtesy of Dr. Richard Simmons, Minneapolis, MN) and *S. aureus* 502A were each incubated in Trypticase soy broth™ (Becton-Dickinson, Cockeydille, Maryland) in a 37 C oscillating water bath. The cultures were centrifuged at 2000 rpm for 5 minutes, and the resulting pellets were washed in 0.9% NaCl. After the final wash, the pellets were serially diluted in sterile saline to achieve a final concentration of 2×10^8 bacteria per ml. An equal mixture was then made containing 1.0×10^8 each of the *E. coli* and *S. aureus*.

TABLE 2. *Daily Dietary Intake of Experimental Groups*

Daily Dietary Intake	Group 1 (n = 175)	Group 2 (n = 150)	Group 3 (n = 125)	Group 4 (n = 100)
Water (ml)	14.29	21.77	33.00	29.28
	(0.91)	(1.66)	(1.33)	(1.47)
Basic Diet (ml)	54.11	49.33	45.85	41.77
	(1.10)	(1.91)	(2.02)	(2.03)
Total volume (ml/animal)	68.88	70.22	78.85	71.00
	(0.80)	(0.22)	(3.01)	(3.50)
Total volume (ml/kg)	155.68	157.30	161.74	160.27
	(1.92)	(0.49)	(4.26)	(4.71)

Values expressed as mean (SEM).

Preparation of the Osmotic Pumps

Using aseptic technique, each osmotic pump (Alzet Osmotic Pump, Model 2 MLI, Alza Co., Palo Alto, CA) was filled with 2 ml of the *E. coli/S. aureus* mixture. To the bottom of the flow moderator, a small amount of silicone bonding (Medical Adhesive Silicone Type A, Dow Corning, Midland, MI) was added, and the flow moderator was placed into the pump. Excess silicone was removed by wiping with a sterile gauze. The silicone was added to create a seal between the flow moderator and the osmotic pump through which the bacteria could not escape. The copolymer cap was then removed and a coil of sterile silicone tubing (size: 0.06 cm ID, 0.12 cm OD, Dow Corning Medical Grade, Midland, MI) 100 cm in length was attached to the exposed stainless steel tube. The coil was secured with three ties of 5–0 suture. The filled pumps with coil were then set aside for 20 minutes to dry and were then implanted.

The volume of the tubing was 0.350 ml, and there was a residual 0.102 ml in the spent pump. Thus, total delivery from each pump was 1.602 ml. After approximately a 4-hour delay after implantation, the pumps began delivery of the reservoir contents into the tubing. The pumps had a flow delivery rate of 0.00863 ml/hour and began actual delivery of bacteria into the peritoneal cavity between 34 and 36 hours after implantation.

Experimental Groups

On Day 3 after osmotic pump placement, 48 animals were randomized and placed on one of four diets differing only in concentration: Group 1 received 175 kcal/kg/day, Group 2 received 150 kcal/kg/day, Group 3 received 125 kcal/kg/day, and Group 4 received 100 kcal/kg/day (Table 2). The composition of the basic diet is shown in Table 1. The animals were fed for 14 days via the gastrostomy by continuous pump-controlled infusion (Holter pump, Cat. #903, Critikon, Bound Rock, NY). The same diet was used for all animals, diluting it with water to achieve the desired caloric density. All animals received equivalent volumes of fluid daily.

The animals were weighed daily, and urine samples were taken at the same time of day throughout the experiment without stopping the continuous feedings. All animals that survived for 14 days after starting the diet (17 days after pump implantation) were killed by exsanguination from cardiac puncture while under general anesthesia.

Wet weight of the carcass consisting of skeleton and musculo-fascial structures was measured after the animal had been completely skinned, eviscerated, decapitated, and after the feet were excised. By suspending a standard weight from one end of the gut, creating a uniform degree of tension, a 10-cm segment of proximal jejunum, 10 cm beyond the pyloroduodenal junction, was cut for mucosal sampling. The segment was scraped with a spatula according to a previously described technique[17] and the wet weight of the mucosa was obtained. A second 10-cm segment beginning 10 cm proximal to the ileocecal valve was similarly prepared. Both gastrocnemius muscles were removed and weighed with the average weight calculated. The liver, spleen, and adrenal glands were also weighed. Selected organ weights were recorded to compare with controls having a gastrostomy placement.

Resting energy expenditure (REE) was measured by indirect calorimetry with a computerized Respiratory Gas Monitor (Webb Associates Inc., Yellow Springs, OH). Individual guinea pigs were placed in a small chamber connected to the gas monitor for 30 minutes. The volumes of O_2 consumption and CO_2 production during this period were used to calculate the metabolic rate by the Weir equation.[18]

Albumin concentration was determined by the bromocresol[19] green method (Gilford Diagnostics, Cleveland, OH). Transferrin and C_3 were measured by laser nephelometry (Immunochemistry Analyzer II, Beckman Instruments Inc., Fullerton, CA) with appropriate antisera.

In conducting the described research in this report, the investigators adhered to the Guide of Laboratory Animal Facilities Care as set forth by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council and the regulations of the National Institutes of Health.

Statistics

Statistics were calculated on a Macintosh SE™ computer (Apple Computer, Inc., Cupertino, CA) using Statview 512+™ software (BrainPower, Inc., Calabasas, CA). Animal weights were compared by one-way analysis of variance (ANOVA) and significant comparisons noted with Fisher's test of least significant difference (LSD), accepting an å level of significance of 0.05. Mortality data were compared using the two-tailed Z test for population frequencies, and the å level was adjusted to 0.01 by the Bonferroni[20] correction for multiple tests. Length of survival for those animals that succumbed before 2 weeks was compared using the Kruskal-Wallis ANOVA. Comparisons of selected organ weights, muscle weights, and serum proteins were made between Group 3 and controls, and between Group 4 and controls, using Student's t-test with an å level of 0.05.

Results

During the first day after insertion of the osmotic pump, the animals spontaneously ingested 79.4 ± 5.5 kcal/kg of normal guinea pig chow. They ingested 87.1 ± 9.0 kcal/kg during the second day, and 165.6 ± 26.2 kcal/kg during the third day. This compared with an average intake of 124.0 ± 2.7 kcal/kg/day for normal guinea pigs weighing 400 g and an average intake of 110.7 ± 13.0 kcal/kg/day for animals between the time of gastrostomy and insertion of the pump.

Sporadic diarrhea (defined as loose stools present on two consecutive days) was noted among the groups, but always resolved spontaneously and was never a premorbid finding. Because diarrhea could lead to fluid losses that would contribute to death, urine output among the four groups on all days was compared. The mean daily urine output in Group 1 ranged from 24 to 60 cc/day, in Group 2, from 23 to 55 cc/day, in Group 3, from 19 to 51 cc/day, and in Group 4, from 26 to 53 cc/day. Comparison by ANOVA showed no significant differences ($p > 0.05$). Thus oliguria suggestive of volume depletion was not a dominant characteristic of any group.

Resting energy expenditure was measured in four animals in Group 3 only. Mean baseline REE was 199.1 kcal/kg/day before gastrostomy. Before osmotic pump placement 1 week later, this rose to 267.725 kcal/kg/day. Five days after pump placement (during active peritonitis), the mean baseline REE fell back to 206.425 kcal/kg/day. Although the numbers are too small to analyze statistically, these data suggest that the animals were not hypermetabolic during peritonitis.

Comparisons of changes in weight for these groups were analyzed by ANOVA (Fig. 1). Interestingly, on Days 4, 8, 9, and 11 of nutritional support, the animals of Group 1, receiving the highest caloric intake, lost weight compared with those of Group 2, and compared with those of Group 3 on Days 8 and 9 (Fisher's test of LSD). As expected, animals in Group 4 (those that received 100 kcal/kg/day) lost approximately 15% of their body weight. In addition, these animals became emaciated and gaunt. For humane reasons, this arm of the experiment was discontinued after seven animals experienced this loss in body weight.

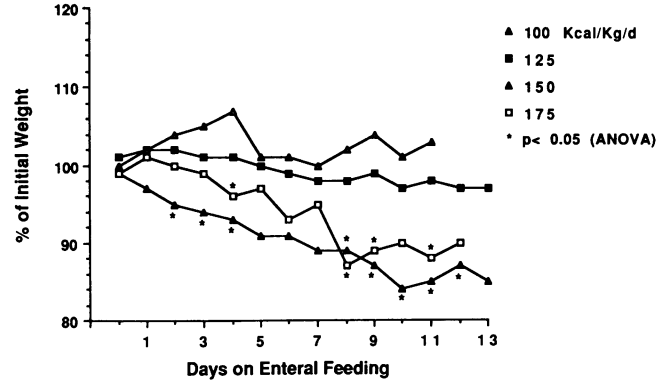

FIG. 1. Weight changes in the four groups of animals given different amounts of food, beginning the day enteral nutrition was started. Gastrostomy feedings were begun on the third day after peritoneal pump placement.

However, despite a greater weight loss, animals fed the hypocaloric diet had a survival rate of 57.2%, significantly greater than the 0% survival rate of Groups 1 and 2 ($p = 0.0008$ and $p = 0.0088$, respectively, Fig. 2). In addition, survival for the animals of Group 3 was better than that for those of Group 1 ($p = 0.0066$). There was no difference in survival between Groups 3 and 4 ($p = 0.4238$).

The median length of survival of the animals that died was 5.5 days in Group 1, 5 days in Group 2, 3.5 days in Group 3, and 8 days in Group 4—differences that were not significant ($p > 0.05$ Fig. 3).

All animals that died were autopsied with the findings of either extensive peritonitis with free fluid and/or extensive pneumonia (Table 3). There was no evidence of aspiration.

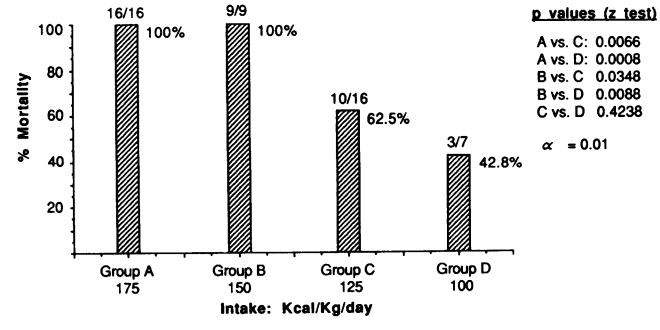

FIG. 2. Mortality in infected animals related to the amounts of food intake. It is apparent that overfeeding increased mortality.

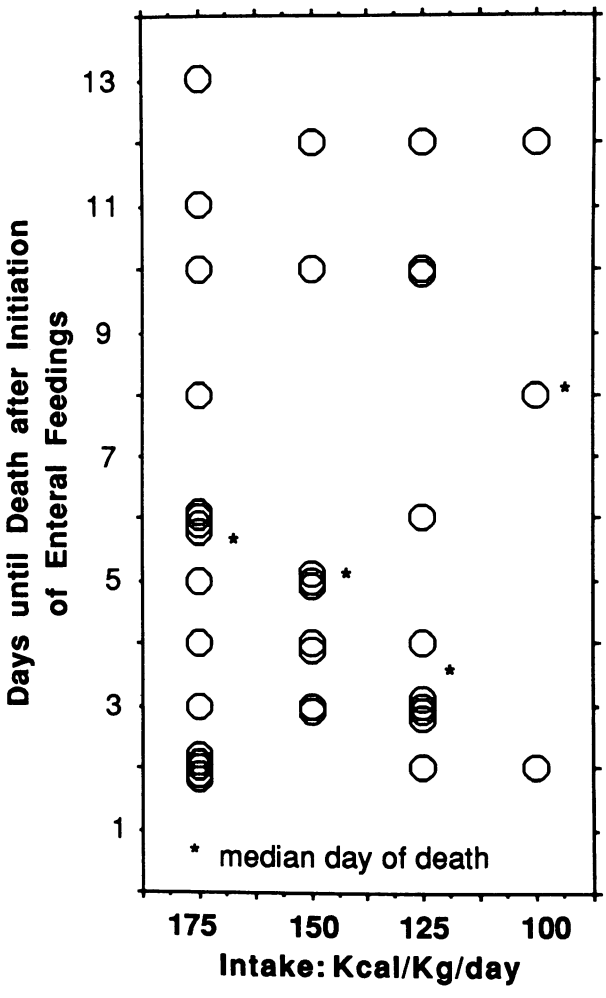

FIG. 3. Length of survival in days among animals that died, related to caloric intake.

All ten animals that survived for 17 days after pump implantation had walled-off abscesses surrounding the pump and tubing measuring an estimated 1–3 ml in volume. Quantitative cultures of the abscess contents showed approximately 1×10^8 *E. coli*/ml and 1×10^7 *S. aureus*/ml in Groups 1, 2, and 4, and approximately 1×10^6 for each organism in Group 3. Spleen, lungs, liver, and adrenals harvested sterilely when the animals were killed yielded no bacterial growth when homogenized and cultured.

TABLE 3. *Necropsy Findings*

Group	Pneumonia	Infected Peritoneal Fluid
Group 1 (n = 16) 175 kcal	11	10
Group 2 (n = 9) 150 kcal	4	7
Group 3 (n = 16) 125 kcal	4	7
Group 4 (n = 7) 100 kcal	2	1

TABLE 4. *Selected Organ and Muscle Weights of Surviving Animals*

Weights	Controls* (n = 7)	Group 3 (n = 6)	Group 4 (n = 4)
Starting weight (g)	405.28 (20.82)	431.58 (12.91)	421.1 (19.02)
Sacrifice weight (g)	374 (20)	404 (21.57)	349 (8.33)
Liver (g)	17.26 (1.07)	17.40 (1.70)	15.80 (1.30)
Gastrocnemius (g)	1.05 (0.01)	1.80 (0.20)	1.78 (0.15)
Carcass (g)	123.6 (8)	122.1 (13.7)	113.0 (3.4)
Carcass/Starting weight × 100	33.04 (8.01)	27.30 (1.7)	26.70 (0.5)
Jejunal mucosa (mg/10 cm)	351 (20)	406 (49)	233 (15)
Ileal mucosa (mg/10 cm)	315 (47.0)	327 (37)	293 (10.8)
Spleen (g)	1.02 (0.18)	0.89 (0.14)	0.93 (0.17)
Adrenal (g)	0.28 (0.03)	0.39 (0.23)	0.38 (0.03)

* 1 week postgastrostomy, without peritoneal pump.
Values expressed as mean (±SEM).

Examination of organ and muscle weights of surviving animals (Table 4) showed evidence of stress in the infected animals (decreased spleen and increased adrenal weights, $p < 0.05$). There was a surprisingly disproportionate loss in gut mucosal weight in the animals that survived best (100 kcal/kg/day), but these animals also lost considerably more weight than those of the other groups (Fig. 1), and there were some technical difficulties in the measurements because extensive adhesions often made it impossible to separate the intestinal loops without destroying the integrity of the section to be scraped.

Serum albumin, transferrin, and C_3 measurements for animals sacrificed after 14 days of receiving the diet are shown in Table 5. Albumin was significantly lower in the experimental groups than in controls, and transferrin was higher ($p < 0.05$). There was no difference in C_3.

Discussion

Previous animal models of bacterial peritonitis/abscess have not allowed survival for a long enough period of time to test variations in essential nutrients for their effect on outcome. Use of an osmotic pump as described in this report will deliver a continuous infusion of bacteria into the abdomen over a 7-day period. By varying the numbers and types of bacteria, mortality can be increased or decreased and extended or shortened to achieve a desired end-point. With the appropriate inoculum, the animals at first develop a diffuse peritonitis, which later becomes localized if the animals do not die. The abscess persists

after the infusion ceases because of the presence of the large foreign body, but the abscess may gradually diminish in size and bacterial content. In the present experiments, all of the surviving animals were killed 17 days after implantation of the pump, and it is not known if they would have eventually died without removal of the foreign body. Preliminary studies in spontaneously feeding animals without gastrostomy implanted with osmotic pumps containing 1×10^8 *E. coli* and 1×10^8 *S. aureus* showed that about half of the animals died by the end of 2 weeks.

Prior placement of a gastrostomy tube allows administration of a liquid diet that can be altered at will with regard to individual nutrients and schedule for delivery. The formulation used in this experiment was found to be optimal for nutritional support of burned guinea pigs, requiring 175 kcal/kg/day to prevent weight loss after a 30% burn. In over 1000 burned animals treated with this diet or a variant by gastrostomy, diarrhea (two consecutive days of loose stools) was only very sporadic, and aspiration does not occur. The osmolality of the undiluted diet, 750 mOsm/l is associated with decreased diarrhea in both animals and humans because of a low lipid content. In burned animals, 200 kcal/kg/day was well-tolerated and resulted in weight gain. Although good for burn injury, even lesser quantities caused death of animals with sepsis.

The finding that overfeeding had an adverse effect and that underfeeding (100 kcal/kg/day), although associated with a significant weight loss, resulted in improved survival is of great interest because it might be postulated that more energy and more protein would be required to meet the hypermetabolic demands known to be associated with septic peritonitis. However, similar results were found with rats fed 1.8 times the normal caloric requirements by gastrostomy for 6 days before cecal ligation and puncture. Although weight gain and nitrogen balance in those animals were superior to those of controls, septic mortality was significantly greater.[21]

Although overfeeding may improve some nutritional parameters, it may also stimulate bacterial virulence, as it is well-known that certain nutrients, such as Fe^{++}, are preferentially used by bacteria.[22] Reductions of the intake of iron by the host may favor survival in bacterial infection, whereas giving an excess of iron may increase mortality.[23,24] Furthermore, binding of iron by transferrin or chelators may favor the host by making less free iron available to the bacteria.[22,25] In the present study, all nutrients were decreased proportionately, so simple caloric intake cannot be implicated as a sole factor in the difference in outcome, although it may be involved. It is clear that feeding the host also feeds the microbes that cause sepsis and that there may be benefit by selectively limiting some nutrients.

Enteral feedings were chosen for these studies because the opportunities for variation in nutrient content are much greater than with intravenous feedings. Also, in a variety of other conditions, the enteral route is more effective than the parenteral route, having clinical relevance. However, preliminary studies showed that beginning the gastrostomy feedings immediately after insertion of the osmotic pump was not tolerated well because of a transient ileus. For this reason, the gastrostomy feedings were delayed for 3 days, but the animals were allowed to feed *ad libitum*. Thereafter, no problems were encountered with the enteral feedings.

TABLE 5. *Serum Proteins in Surviving Animals*

	Control* (n = 7)	Group 3 (n = 6)	Group 4 (n = 4)
Albumin (g/l)	2.6	1.7	1.8
(p = 0.00007)†	(0.1)		(0.2)
Transerrin (% of control)	100	189.2	193.7
(p = 0.00005)†	(12.6)	(24.6)	(21.7)
C_3 (% of control)	100	99.14	101.5
[p = NS]	(10.1)	(11.9)	(7.3)

* Normal guinea pigs.
† Analysis by Students t-test with controls.
Values expressed as mean (SEM).
Groups 1 and 2 are not represented because there were no survivors.

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