from 10 μg to 2 mg at constant pH. A wide range of technique is demonstrated.

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Accumulation of Endogenous Protein in the Cecum of the Germfree Rat* (33326)

WALTER J. LOESCHE (Introduced by James T. Irving)

Forsyth Dental Center, Boston, Massachusetts 02115

The enlarged cecum of germfree rats and mice contains considerable amounts of protein and carbohydrate (1). The endogenous origin of some of this material is indicated by the demonstration of high molecular weight mucins (1–3) and of elevated levels of trypsin and chymotrypsin in cecal contents (1). The contribution of dietary protein to the material found in cecal contents is not known. The aim of the present investigation was to obtain information concerning the origin of the soluble cecal protein. For this purpose germfree rats were placed on either 0% casein or 10% casein diets. After several days on these regimens, the animals were sacrificed and the cecal protein was measured. Additional studies were performed to determine the efficiency of the terminal half of the small intestine in reabsorbing nitrogen.

Materials and Methods. Gnotobiotic CDF rats were obtained from the Charles River Breeding Laboratories (North Wilmington, Mass.) and were housed in plastic germfree isolators or in a Reyniers stainless steel unit. Conventional animals of the same strain were housed in the animal room. During the experimental periods animals were fed diet 585 (4) modified as described below and water *ad libitum*. In the experiment involving protein restriction, germfree rats had been maintained for a period of 10 days postweaning on a diet containing 20% casein. Three animals were then sacrificed and the cecal contents were analyzed to obtain zero time values. The remaining animals were placed on diets in which the casein content was reduced to 10% (10% protein group) or completely

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TABLE I. Effect of Dietary Protein Restriction on Cecal Size.

Item	Time: 0 Hours	1 Day		8 Days	
	Casein in diet (%): 20	10	0	10	0
No. of animals	3	3	3	3	3
Wt. of cecal contents (g)	10.7 ± 0.5[a]	9.6 ± 1.2	9.6 ± 1.1	7.6 ± 0.8	8.0 ± 1.1
Cecal contents (% of body wt.)	21.2	16	16.5	11.6	12.4

[a] ± SE of the means.

omitted (0% protein group[1]). Three animals from each group were subsequently sacrificed after 1 and 8 days on the test diet.

In order to evaluate the efficiency of germfree rats in reabsorbing nitrogen from the distal small intestine, 1% chromic oxide was added to diet 585 and the chromic oxide to nitrogen ratio was determined in the contents of the distal small intestine, cecum, and in the feces. As chromium is not absorbed any change in the ratio would be due to a shift in nitrogen content (6). All diets were sterilized by irradiation.

Animals were sacrificed by either neck fracture or chloroform inhalation. The cecum was removed, weighed, and the cecal contents were expressed. The contents of each gnotobiotic cecum were analyzed individually, but it was necessary to pool the cecal contents of 2 or 3 conventional animals for analysis because of less material. The fractionation procedure was that used previously (1) Values were obtained for total soluble protein; high molecular weight proteins (10% trichloroacetic acid precipitate); mucoproteins (80% ethanol precipitate) and low molecular weight proteins or peptides (TCA and ethanol soluble material). In addition the nitrogen content of the insoluble residue was determined. In the chromium experiment, the entire contents of the cecum and distal half of the small intestine were homogenized and samples taken and analyzed for chromium (7), nitrogen (8), and sialic acid (9). Fifty to 150-mg samples of feces were analyzed for the same constituents.

[1] The diets were subsequently analyzed for protein (5) and found to contain 10 and 1% respectively, of Lowry positive material.

Results. In the protein restriction experiment all animals sacrificed at 8 days showed gross signs of a protein deficiency. They had diarrhea, their fur could easily be plucked off and their tail skin stripped off. Their livers showed fatty infiltration which was more pronounced in animals fed the 10% casein diet. All animals gained weight during the experimental period, but the wet weight of the cecal contents decreased so that in the course of 8 days, the cecal contents went from 21 to 12% of the body weight (Table I).

Despite the restriction of dietary protein considerable amounts of protein were still recovered from the cecal contents. The total soluble protein decreased from 742 mg/100 g of body weight at zero time to about 400 mg/100 g of body weight at 8 days. Upon fractionation the greatest reduction occurred in the TCA precipitable fraction, whose value at 8 days for either diet was only 25–30% that of the zero time value. Digestive enzymes would be in this fraction and there exists evidence that the gut levels of these enzymes are dependent on dietary protein concentration (6). The mucoprotein, low molecular weight protein, and insoluble nitrogen fractions showed a reduction of 50–60% from control values. If however, these protein and nitrogen values are expressed per 100 g of cecal contents, only slight differences between 0 and 8 days were found on either diet, particularly the 10% casein diet (Table II). Thus the total soluble protein at 0 time was 3.5 g/100 g of cecal contents and 3.2–3.5 g/100 g of cecal contents at 8 days. The insoluble protein (N × 6.25) dropped from 1.4 g at 0 time to about 0.9–1 g/100 g of

TABLE II. Effect of Dietary Protein Restriction on Protein Composition of Rat Cecal Contents (expressed per 100 g of cecal contents).

Item	Time: / Casein in diet (%):	0 Hours 20	1 Day 10	1 Day 0	8 Days 10	8 Days 0
		Soluble fraction (mg/100 g of cecal contents)				
Total protein		3500 ± 231[a]	4200 ± 518	3700 ± 232	3490 ± 408	3160 ± 500
TCA ppt		472 ± 31	485 ± 41	560 ± 47	222 ± 13	234 ± 46
EtOH ppt		396 ± 40	322 ± 23	324 ± 31	374 ± 45	252 ± 37
TCA & EtOH soluble		2290 ± 30	2600 ± 170	2340 ± 400	1870 ± 280	1390 ± 240
		Insoluble fraction (mg/100 g of cecal contents)				
Nitrogen		220 ± 43	142 ± 17.2	98 ± 15.1	141 ± 14.5	159 ± 37.6
Protein (N × 6.25)		1375 ± 268	889 ± 107	613 ± 94	882 ± 90.5	995 ±232

[a] ± SE of the means.

cecal content at 8 days. Since the levels of cecal protein found on the 0% casein diet were from 60 to 90% of the values obtained from animals ingesting 10 and 20% casein diets, it was concluded that most of the protein found in cecal contents was endogenous in origin.

This implied that the degradation and reabsorption of endogenous protein, i.e., intestinal enzymes, shed cells, saliva, etc., was not efficient in the germfree rat. To test this possibility, 5 adult germfree rats weighing about 250 g were given diet 585 supplemented with 1% chromic oxide. After 10 days on this diet the animals were sacrificed and the chromic oxide to nitrogen ratio was determined on the contents from the distal half of the small intestine, the cecum, and the feces. Twelve adult conventional animals of the same strain were fed the same diet. The results obtained are given in Table III. In the conventional animals the ratio increased from 1 in the small intestine to 8.1 in the cecum and 8.9 in the feces. About 88% of the nitrogen present in the distal half of the small intestine was reabsorbed before it reached the cecum. However, in the germfree animals the chromic oxide to nitrogen ratio decreased, indicating that nitrogen was accumulating as it passed from the small intestine to the cecum and eventually to the feces. Thus nitrogen was not reabsorbed from the distal half of the germfree small intenstine. About 10–15 mg of sialic acid/100 g of body weight can be recovered from the cecal contents of adult germfree rats, indicating the presence of sialoprotein in cecal contents. As this protein is endogenous in origin, it would be a specific measure of endogenous protein behavior. Accordingly, the chromic oxide to sialic acid ratio was determined in the intestinal contents of these animals. Eighty-eight percent of the sialic acid found in the small intestine of conventional animals was either degraded or absorbed before it reached the cecum (Table III). However, in the germfree animal no change in sialic acid levels occurred between small intestine and cecum indicating little or no degradation and/or absorption of this compound.

Discussion. The present findings indicate that most of the protein and nitrogen found in the cecum of the germfree rat are of endogenous origin. Endogenous proteins are thought to be highly digestible as they are

TABLE III. Chromic Oxide–Nitrogen and Chromic Oxide–Sialic Acid Ratios in the Intestinal Tract and Feces of Germfree and Conventional Rats.

Group	Site:	Small intestine	Cecum	Feces
		Chromic oxide to nitrogen ratio		
Conventional		1.0	8.1	8.9
Germfree		1.0	0.4	0.3
		Chromic oxide to sialic acid ratio		
Conventional		1.0	8.1	10.1
Germfree		1.0	1.3	4.5

almost quantitatively degraded and reabsorbed within the gastrointestinal tract (6). Why then, do they accumulate in the germfree cecum and undoubtedly contribute to the higher fecal nitrogen found in germfree animals (10, 11)? Results obtained from chromic oxide to nitrogen ratios showed that the reabsorption of protein and nitrogen is greatly reduced in the ileum of the germfree rat. Snook and Meyer have stated that "pancreatic and intestinal proteases are responsible for carrying out the digestion of the exogenous and endogenous protein" (6). Several investigators have shown that trypsin and chymotrypsin as well as other digestive enzymes are elevated in the germfree feces (12) and cecal contents (1, 13). Thus the impairment in digestion of endogenous nitrogen would not at a first approximation be due to a lower level of these enzymes. Additional explanations could account for the lack of endogenous protein degradation. The gnotobiotic rat might have reduced levels of proteases because of the reduction in size of the ileum (13). This would assume that proteases derived from the ileum have a different range of substrates than those released higher in the gastrointestinal tract and that these proteases are quantitatively responsible for the digestion of endogenous proteins. Another possibility is that microbial enzymes are essential for host recovery of endogenous protein. In the absence of microbes endogenous proteins are not degraded and accumulate in the cecum. When germfree rats are contaminated with intestinal contents from conventional animals or with specific bacteria the gastrointestinal anomalies disappear within 2–3 weeks (13).

The present study raises the question of the importance of this "lost" endogenous protein in the overall nutrition of the germfree rat, because if normal growth is to occur this nitrogen would have to be replenished. The germfree rat appears to make several adjustments in his physiology in regards to his protein metabolism. He ingests more food (14) (which incidentally contains generous amounts of high biological value protein) and apparently slows down his protein turnover rate. Abrams *et al.* (15) found lower cell renewal rate for intestinal cells. The metabolic rate and fixation of radioactive iodine are significantly reduced in germfree rats in comparison to conventional controls (13). Unanswered is the extent of coprophagy in the germfree animal. As from 35 to 50% of fecal material is recycled in conventional animals (16) one might suspect that this would be an important means of recovering endogenous protein or nitrogen in the germfree rat.

Summary. Considerable amounts of protein and insoluble nitrogen containing compounds are found in the cecal contents of germfree rats after 8 days of ingesting 0 or 10% casein diets. This implied that the majority of the proteins found in the cecal contents was derived from the host and not from the diet. Additional experiments showed that conventional animals reabsorb from 80 to 90% of the nitrogen containing compounds found in the contents of the distal half of the small intestine whereas these compounds were not reabsorbed in the distal half of the germfree small intestine. Thus it appears that the germfree rat cannot efficiently degrade and reabsorb the endogenous proteins which are shed into his gastrointestinal tract. The significance of these findings for the germfree animal are discussed.

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Enzyme Changes within Muscle Fibers in Genetic and Nutritional Muscular Dystrophy* (33327)

LOUIS L. TUREEN AND ROBERT SIMONS

Max and Anna Tureen Neurology Research Laboratory, Department of Neurology and Psychiatry, Saint Louis University School of Medicine 63104

Recent publications (1, 2) emphasize the relationship between the functional activity of muscle fibers, their innervation and their enzyme systems. Fast acting "white" fibers display strong activity for glycolytic enzymes, e.g., phosphorylase (Phlase) and are poor in the mitochondrial enzymes such as succinic dehydrogenase (SDH) and lactic dehydrogenase (LDH). The reverse is true for cardiac muscle or other red fibers (3) which are more slow acting and require abundant oxidative enzymes. The pattern of reciprocal relation between levels of oxidative and glycolytic enzymes in functionally different muscle fibers is widely observed in rodents and birds, as well as in man (4). In the genetic dystrophic chick, there is a failure in the early posthatching period of maturation of the red, undifferentiated embryonic fibers to the white mature fiber which is the more abundant and characteristic fiber of the mature chicken pectoralis muscles. There is a corresponding failure or delay in the development of the enzyme systems usually found in mature pectoral muscles (5). Since the pectoralis muscle of the chicken is affected early and most severely in both the genetic and nutritional types of muscular dystrophy, the probability is strongly suggested that the metabolic activity of the white muscle fiber may be related to this early susceptibility.

The present experiments were designed to compare the histological changes in the dystrophic muscle fibers with concomitant levels of enzyme activity in them. One aim was to investigate whether the dystrophic necrosis results from a specific enzymatic disorder within the muscle fiber, or if the changes observed in both genetic and nutritional chicken muscular dystrophy represent nonspecific, generalized muscle responses to the stress of differing metabolic insults.

Methods and Materials. One-day-old New Hampshire chicks of the NH_2 strain (normal) and the NH_3 strain (genetic dystrophic) were used. The control group of normal chicks was placed on a standard commercial[1] diet as a starter feed for 30 days, and then transferred to a well balanced stock diet for optimal growth[2] for the remainder of the experiment. One group of the genetic dystrophic strain was similarly fed. The other two groups, one of the NH_2 strain, the other of the NH_3 strain, were fed a purified diet deficient in vitamin E and sulfuramino acid, used previously (6) to induce nutritional myopa-

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[1] Ralston Purina Co., Starteena brand.

[2] Ralston Purina Co., Growean brand.