STUDIES OF THE GROWTH AND CELL DYNAMICS OF THE INTESTINAL EPITHELIUM IN CORN AND SORGHUM-FED RATS

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SUMMARY

A study was undertaken to compare the effects of corn and sorghum-based diets on the growth curve of young adult rats and on cell renewal in the intestinal epithelium of the jejunum and colon of the animals. Three groups of 10 rats each were studied: the first was submitted to a corn diet (Corn Group); the second to a sorghum diet (Sorghum Group), and the third to a casein diet (Control Group). The diets supplied about 7 g% protein. The experimental period lasted 13 weeks. Effects were measured in

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terms of weight curves, mitotic and calyciform cell indexes in the jejunum and colon. A special statistical analysis was applied allowing comparisons between the groups, and considering the sample size and the individual variations in the growth curves. The animals in the sorghum group revealed a lower growth rate, and those in the corn group showed more marked cell dynamics changes in the jejunal epithelium. Findings in the corn and sorghum-fed animals were different and cannot be explained solely in terms of niacin deficiency.

INTRODUCTION

Grains such as corn *(Zea mays)* and sorghum *(Sorghum vulgare)* have been related to the appearance of endemic pellagra or experimental "black tongue" in dogs. Several investigations have been carried out to clarify the etiological role played by niacin and tryptophan deficiency and by the excess of leucine on the physiological, biochemical and anatomo-pathological changes appearing in corn and sorghum-fed animals (1). Weight loss and histopathological alterations in the digestive tract have been described in animals fed a corn diet (2), and similar lesions have been described in sorghum-fed animals by Belavady, Madhavan and Gopalan (3).

It is well known that the intestinal epithelium has one of the highest cell renewal rates in the whole organism, and several studies have been made of factors that may alter it. It has also been demonstrated that fasting and protein starvation cause decreased cell renewal in animals (4).

This paper describes a study of the growth curve and a comparison of cell renewal in the intestinal epithelium of corn and sorghum-fed rats.

MATERIAL AND METHODS

Thirty Wistar, young adult male rats, weighing an average of 174.3 g were utilized. The animals were divided into three groups: the Corn Group, which received a corn diet; the Sorghum Group, which received a sorghum diet, and the Control Group, fed a casein diet. Each group was placed in individual wire cages, observed daily and weighed weekly. The composition of the diets offered to the animals is shown in Table 1. The corn and

TABLE 1

DIET COMPOSITION IN GRAMS

Ingredients	Diets		
	Corn	Sorghum	Casein
Sorghum	–	73.13	–
Corn	90.0	–	–
Casein	–	–	8.4
Salt mixture*	4.0	4.0	4.0
Vitamin mixture without niacin	1.0	1.0	–
Complete vitamin mixture*	–	–	1.0
Corn oil	5.0	5.0	5.0
Cornstarch	–	16.87	90.6

Association of Official and Agricultural Chemists. *Official Methods of Analysis of the AOAC.* Horwitz, W. (10th ed.). Washington, D.C., The Association, 1965.

sorghum diets, analyzed for their protein content by the Kjeldahl method, supplied an average of 7.12 and 7.37 g protein, respectively, and the casein diet, 7.12 g%.

After 13 weeks on the diets, 5 animals from each group were sacrificed under ether anesthesia, always at the same time, between 8:00 and 10:00 a.m. Fragments of proximal jejunum and transversal colon were obtained and fixed in Bouin liquid for 72 hours. Sections cut with a microtome were stained with PAS-hematoxilin and observed under a Zeiss microscope (63 magnifications). Twenty-five cryptas-villi units cut lengthwise were selected for each encased fragment, and about 2,000 nuclei were counted. The calyciform cells and mitotic nuclei in both the jejunum and colon cryptas were counted. The mitotic index and the calyciform cell index were considered as the percentage of mitotic nuclei and of calyciform cells respectively, observed during a given period of time (5, 6).

The growth rate of each group was compared by the method of Rao (7).

TABLE 2

INITIAL WEIGHTS AND WEEKLY WEIGHT GAINS FOR RATS SUBMITTED TO THE THREE DIETS

Corn Group														
Rat No.	Y_0	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9	Y_{10}	Y_{11}	Y_{12}	Y_{13}	b
1	194	8	11	8	6	7	8	− 4	2	7	7	4	− 5	− 13	7.756
2	179	17	9	1	− 1	8	11	1	7	10	10	2	5	4	12.103
3	184	11	7	8	12	10	2	10	9	5	6	4	− 2	2	12.033
4	157	2	5	2	− 2	2	− 4	− 3	− 1	− 5	− 9	2	− 3	− 3	1.621
5	150	4	3	4	0	7	5	2	10	8	9	− 6	2	8	7.806
6	155	12	1	8	0	2	3	− 4	5	−14	0	0	0	0	3.210
7	160	16	− 2	8	4	5	5	0	7	2	7	9	11	6	10.733
8	166	1	− 4	5	2	9	1	− 3	5	2	3	1	1	3	3.748
9	157	12	10	8	− 2	12	4	5	4	11	9	3	5	− 3	11.653
10	174	20	9	4	8	9	2	− 3	3	0	0	1	− 3	− 9	8.310

Sorghum Group															
1	171	7	−12	7	1	8	− 2	7	3	1	1	3	3	7	5.389
2	174	4	4	5	− 2	2	− 4	0	− 1	2	6	− 1	0	4	3.173
3	180	−2	2	0	6	3	8	1	0	5	0	1	2	6	3.296
4	189	6	− 1	0	1	7	2	2	− 2	7	1	− 9	− 2	2	3.592
5	164	7	− 1	2	3	6	4	2	2	3	2	3	4	5	5.748
6	177	6	− 1	8	− 3	4	5	0	1	4	− 3	0	0	5	4.540
7	177	8	0	5	1	13	6	0	2	5	3	− 2	3	4	7.518
8	185	3	− 3	5	4	5	4	5	6	4	− 2	0	9	6	5.787
9	175	2	− 1	4	− 1	8	5	3	3	5	1	− 1	2	3	4.743
10	168	0	− 2	16	− 6	10	6	4	3	10	3	0	− 2	8	7.591
Control Group															
1	154	18	6	9	8	5	2	14	3	6	1	6	− 2	3	12.227
2	195	14	13	8	10	0	3	13	9	12	6	1	9	6	14.037
3	183	7	12	6	8	2	8	11	3	0	7	7	1	3	9.380
4	175	10	6	12	10	1	7	9	7	9	6	4	1	4	12.007
5	173	9	− 4	6	9	−2	−12	8	2	5	13	9	5	2	6.832
6	188	14	6	9	4	−5	1	6	14	3	9	1	− 2	− 2	9.168
7	181	9	5	7	8	4	9	3	− 9	14	10	4	3	5	10.146
8	173	12	12	7	4	3	5	9	− 3	15	3	4	9	5	11.637
9	187	11	2	5	9	3	8	10	10	8	10	2	7	6	12.064
10	192	4	8	12	12	7	10	12	6	9	0	2	4	5	11.892

Y_0 = Initial weight = Weight gained during the first week = Weight gained during the 13th week.

b $= \hat{g}_1 y_1 + \hat{g}_2 y_2 + \ldots\ldots \hat{g}_{13} y_{13}$ (7).

Statistical analysis of the values for the mitotic and calyciform cell indexes was done by analysis of variance, with entirely random delineation.

RESULTS

No diarrhea was observed in any of the animals throughout the experimental period.

Rao's method (7) allows comparisons between small samples of several groups or when individual variations in the growth curves are large.

Applying this method to the growth rate observations for rats submitted to the three diets, a total of 30 observations per week were obtained, which gave estimates for g^1, g^2, g^3, g^4, g^5, g^6, g^7, g^8, g^9, g^{10}, g^{11}, g^{12} and g^{13}. These data allowed us to calculate the b value for each rat (shown in Table 2 and arbitrarily divided by 1,000 to reduce the scale).

As observed, the variance ratio is significant, showing that the growth rates are different. Examination of mean regression coefficient values for the three groups ($\bar{b}_1 = 7{,}575$ for corn, $\bar{b}_2 = 5{,}138$ for sorghum, and $\bar{b}_3 = 11{,}939$ for the controls) shows that the differences are mainly due to the higher growth rate in the control group (Table 3).

The null hypothesis test for equality of mean curves yielded $F = 5.59$, with 32 degrees of freedom and highly significant at $\alpha = 0.05$, thus confirming the differences between growth curves for the three groups of animals.

The data for mitotic index and for jejunum and colon calyciform cell index are shown in Table 4. The jejunum data show statistically significant differences between groups at $\alpha = 0.05$. The data relative to the mitotic and calyciform cell indexes for the colon did not reveal a significant difference between groups.

DISCUSSION

In a study of rats treated with diets containing 40% corn, Krehl *et al.* (8) observed that the delay in growth could be compensated for by adding nicotinic acid or tryptophan to the diet. Laguna and Carpenter (9) detected retarded growth in rats treated

TABLE 3

VARIANCE AND COVARIANCE ANALYSIS FOR b AND Y_0 (7)

Source	D.F.	Sbb	Sby_0	Sy_0y_0	Sb corrected for y_0	D.F.	Mean S	F
Between treatments	2	237,500	206,672	812,067	206,610	2	103,305	9.63*
Within treatments	27	301,269	287,980	3,687,300	278,777	26	10,722	
Total	29	538,769	494,652	4,499,367	485,387	28	17,335	

S = Sum of the squares and cross products.

* *Significant* ($P < 0.05$).

TABLE 4

MEANS AND STANDARD ERROR FOR THE MITOTIC AND CALYCIFORM CELL INDEXES IN THE JEJUNUM AND COLON OF THE THREE GROUPS OF RATS UNDER STUDY

	Corn Group		Sorghum Group		Control Group	
	Jejunum	Colon	Jejunum	Colon	Jejunum	Colon
Mitotic index	3.64 ± 0.24*	3.38 ± 1.32	3.87 ± 0.49*	3.32 ± 1.56	5.30 ± 0.29*	4.00 ± 1.23
Calyciform cell index	12.26 ± 0.93*	23.00 ± 1.26	12.79 ± 2.27*	21.91 ± 3.33	15.74 ± 0.43*	19.42 ± 1.58

* Statistically significant at $\propto = 0.05$.

with diets containing 42 parts of yellow corn. However, growth was restored to adequate levels when corn had been previously lime-cooked, as this process liberates bound niacin. Kodicek (10) also observed retarded growth in rats fed a corn-based diet. Harper, Benton and Elvehjem (11) demonstrated that rats fed excess leucine or isoleucine-deficient diets suffered retarded growth, and suggested that excess leucine increases isoleucine requirements. Raghuramulu, Nasaringa Rao and Gopalan (12), in a study of sorghum-fed rats, also observed small growth indexes, and pointed out the importance of the antagonism between leucine and isoleucine as one of the mechanisms responsible for this retardation. Monkeys fed a sorghum-based diet showed weight loss and intestinal symptoms, which were reversed when niacin was administered (13).

In young adult rats, the corn and sorghum diets seem to cause changes in the weight of the animals. Analysis of the data suggests that there is a difference between changes, with a more severe growth retardation in sorghum-fed rats. The statistical methods employed permit efficient comparisons between groups, especially when the sample is not sufficiently large, and when there are wide variations in individual growth curves.

On the other hand, the nutritional state is known to influence the epithelial kinetics in the small intestine (14). In another study of protein-starved rats, Takano (15) demonstrated a decreased mitotic index in the small intestine. Aldewachi *et al.* (16) have described altered cell renewal in the small intestine of fasted or protein-starved rats.

It is also a well known fact that epithelia with calyciform cells increase mucus production and/or the number of mucus-producing cells, when submitted to irritating agents (17). However, Madi *et al.* (18) have reported a decrease in calyciform cells in rats treated with protein-deficient diets.

As to the renewal of the intestinal epithelium, our results suggest that corn and sorghum diets continued over a period of 13 weeks induce changes in the epithelium, especially in the rat proximal jejunum. The mitotic indexes calculated for the jejunum showed statistically significant differences between the corn and sorghum groups, and also in relation to the controls, the lowest indexes being found in the corn-fed rats.

Niacin deficiency and the low amount of protein provided may have been important factors in determining the changes observed in the rats fed the two grains. However, these altera-

tions cannot be explained solely by these factors, since they were present in both diets. It is possible that differences in protein quality of the two grains concerning availability and the amino acid concentration could also be called upon to explain these results. At any rate, the data seem to show that the mechanisms responsible for our findings are different in corn and sorghum.

RESUMEN

ESTUDIOS DEL CRECIMIENTO Y DE LA DINAMICA CELULAR DEL EPITELIO INTESTINAL DE RATAS ALIMENTADAS CON MAIZ Y SORGO

Se llevó a cabo un estudio para comparar el efecto que dietas a base de maíz y de sorgo tienen sobre la curva de crecimiento de ratas adultas jóvenes y sobre la renovación celular del epitelio intestinal del yeyuno y del colon de estas ratas. A esos efectos, se estudiaron tres grupos de 10 animales cada uno: el primer grupo fue alimentado con una dieta a base de maíz, el segundo con una de sorgo y el tercero con una dieta a base de caseína (grupo control). Las dietas contenían alrededor de 7% de proteína, y el período experimental duró 13 semanas. Los efectos de las dietas se midieron en términos de curvas de crecimiento, e índices mitóticos y de células caliciformes en el yeyuno y en el colon. Se aplicó un análisis estadístico especial que permitió la comparación entre los grupos, tomando en consideración el tamaño de la muestra y las variaciones individuales observadas en la curva de crecimiento. Los animales alimentados con sorgo mostraron una tasa de crecimiento menor y los alimentados con maíz evidenciaron cambios más marcados en la dinámica celular del epitelio del yeyuno. Los resultados obtenidos en los animales alimentados con sorgo difirieron de los observados en los animales que consumieron maíz, y no pueden ser explicados únicamente en base de la deficiencia de niacina.

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