Tokai J Exp Clin Med., Vol. 1, No. 1, pp. 51-57, 1976

The Relationship between Host and Intestinal Flora

I. Studies on Changes in the Kinds of Intestinal Bacterial Flora and Concentration of Amino Acids in Various Organs of Rats in Accordance with Various Dietary Conditions

Tsunehiko KATSUNUMA*, Takeyori SAHEKI*, Shigeo TAKADA*, Takashi KUSUMI*, Atsushi OZAWA†, Sadaaki SAWAMURA† and Mariko SASE‡

Departments of Biochemistry and Microbiology†, School of Medicine*
Tokai University
and
Division of Biochemistry‡, Interdepartmental Laboratory, School of Medicine
Tokai University
(Received October 31, 1975)

Changes in the distribution and variety of intestinal flora and in the concentration of amino acids, urea and ammonia in aorta, portal vein, liver and intestinal contents following the change in dietary protein content were studied.
The dietary protein content exerted a profound influence on the jejunal bacterial composition and on the concentration of amino acids in all samples tested.
Influence of the intestinal flora on the concentration of amino acids and ammonia was considered.

(**Key Words: Amino acid, Intestinal flora, Casein diet**)

The concept of "host-parasite relationship" has been usually considered in cases of pathogenic bacteria, but apparently no studies have been made on the influence of non-pathogenic enteric flora upon host metabolism.

It is the aim of this paper to investigate the influence of intestinal flora on the concentrations of amino acids, free ammonia and urea in the intestinal contents, portal vein, liver and aorta of conventional rats fed 0%, 10% and 70% casein diets, and also to study changes in the distribution and variety of intestinal flora in rats in accordance with various dietary constituents.

*Department of Biochemistry, School of Medicine, Tokai University, Bohseidai, Isehara, Kanagawa 259-11, Japan
†Department of Microbiology, School of Medicine, Tokai University, Bohseidai, Isehara, Kanagawa 259-11, Japan
‡Division of Biochemistry, Interdepartmental Laboratory, School of Medicine, Tokai University, Bohseidai, Isehara, Kanagawa 259-11, Japan

Materials and Methods

Animals: Male Wistar strain rats, weighing 150—170 g, were fed 0%, 10% and 70% casein diets for 7 days.

Analysis of blood: 2.0 ml samples of blood were taken from the portal vein and aorta under ether anaesthesia, and deproteinized with 2.0 ml of 5% sulfosalicylic acid.

Analysis of liver: Livers were frozen quickly in a dry ice/acetone mixture. About 1 g of frozen material was homogenized with five volumes of 2.5% sulfosalicylic acid. After centrifugation, the extract was used for the determinations.

Analysis of intestinal contents: The intestines were washed with 50 ml of cold 0.9% NaCl. The samples were deproteinized with 4 ml of 25% sulfosalicylic acid.

Determination methods: Urea was measured colorimetrically with diacetylmonoxime and thiosemicarbazide according to the method of Coulombe and Favreau (1). Ammonia was determined by the indophenol method following diffusion in a Seligson apparatus (2). Amino acids were determined by the amino acid autoanalyzer (Nihondenshi Inc. Model JCL-6AH).

Bacteriological examinations: Segments of jejunum and colon were homogenized with teflon grinders in sterile diluent. The diluent was prepared by adding 4.5 g of KH_2PO_4, 6.0 g of Na_2HPO_4, 0.5 g of L-cysteine, 0.5 g of Tween-80 and 1.0 g of agar to one liter of distilled water. The suspensions obtained from organ specimens were further diluted in sterile diluent in ten-fold steps and 0.1 ml of each dilution was then spread on the surface of various selective agar culture media, as described below. Attempts were made to obtain dilutions of each specimen yielding separated and countable colonies. The numbers of colonies obtained per gram of intestine were caluculated from those obtained per 0.1 ml of the appropriate dilution.

The following were used in our laboratory as selective media which permit quantitative bacteriological analysis of the various organs of the digestive tract.

Deoxycholate agar medium (Eiken) for *Enterobacteriaceae*;
Staphylococcus agar #110 medium (BBL) for *Staphylococci*;
SF medium (BBL) for *Streptococcus faecalis*;
GS medium (Eiken) for *Candida*;
PEA azide medium (Eiken) for gram positive cocci;
GAM agar medium (Nissui) for anaerobes and aerobes;
Brucella agar (BBL) containing 5% horse blood, 75 μg kanamycin and 2 μg vancomycin per ml mainly for *Bacteroides fragilis*;
Bacteroides agar medium (Nissui) for *Bacteroides*;
Veillonella agar (Difco) with 7.5 μg of vancomycin per ml and 0.1% of Tween 80 for *Veillonella*;
Modified FM medium (Nissui) for *Fusobacteria*;
CW agar medium containing 200 μg of kanamycin per ml and 5% of egg yolk for *Clostridium perfringens*;
Bifidobacterium medium containing Eugon agar (BBL), maltose, tomato juice and 5 mg of hemin per ml for *Bifidobacteria*; and

Table 1. Changes occurring in intestinal bacterial flora of rats fed 70%, 10% and 0% casein diets.
All experimental methods are described in "Materials and Methods"
Bar means "lower than 10^2". Results are expressed as number of bacteria per g wet weight of intestine

I) jejunal bacterial flora	70% Casein	10% Casein	0% Casein
Escherichia coli	4.1×10^7	—	1.0×10^2
Streptococcus faecalis	8.0×10^4	—	—
Staphylococcus epidermidis	4.8×10^3	—	—
Candida	1.4×10^3	2.0×10^5	—
Bacteroides clostridiiformis ss. girans	4.6×10^7	1.0×10^4	—
Bacteroides melaninogenicus ss. intermedius	—	1.0×10^6	2.0×10^2
Eubacterium lentum	1.0×10^8	—	—
Fusobacterium nucleatum	1.5×10^3	—	—
Peptostreptococcus	3.1×10^5	—	—
Peptococcus	1.0×10^6	—	—
Lactobacillus	1.2×10^7	—	—
Actinomyces israelii	2.0×10^8	—	—

II) colonal becterial flora	70% Casein	10% Casein	0% Casein
Escherichia coli	1.2×10^8	4.0×10^6	1.0×10^6
Proteus vulgaris	1.4×10^6	—	—
Proteus mirabilis	—	2.0×10^4	—
Streptococcus faecalis	2.0×10^6	1.1×10^5	1.1×10^5
Staphylococcus epidermidis	2.0×10^3	5.0×10^4	1.0×10^3
Candida	8.2×10^2	8.0×10^6	1.7×10^5
Bacteroidea fragilis ss. fragilis	—	5.5×10^7	—
ss. vulgatus	—	1.0×10^6	—
Fusobacterium morterum	—	—	3.0×10^3
Bacteroides melaninogenicus ss. intermedius	4.0×10^9	—	—
ss. asaccharolyticus	—	5.7×10^3	3.1×10^4
Eubacterium lentum	8.0×10^9	5.0×10^2	1.0×10^8
Eubacterium limosum	—	—	2.0×10^6
Bifidobacterium adolescentis	—	3.6×10^7	—
Clostridium butyricum	—	3.8×10^7	2.0×10^8
Peptostreptococcus	—	6.1×10^5	—
Bifidobacterium adolescentis	—	—	7.0×10^6
Lactobacillus	1.2×10^8	—	—
Actinomyces israelii	8.0×10^2	1.0×10^7	—

Rogosa SL agar medium (Difco) containing 0.13% of glacial acetic acid for *Lactobacilli*, *Bifidobacteria* and *Peptostreptococci*.

Results and Discussion

Studies on changes occurring in the intestinal bacterial flora of rats in accordance with changes in dietary constituents were carried out.

The results presented here reveal that ammonia producing bacteria like *Bacteroides clostridiiformis ss. girans*, *Bacteroides melaninogenicus ss. intermedius*, *Eubacterium lentum*, *Fusobacterium nucleatum*, *Actinomyces israelii* are present in large numbers in the jejunum of rats fed a 70% casein diet. The 10% casein and 0% casein diets brought about a sharp decrease in the numbers of *Bacteroides clostridiiformis ss. girans*, and complete disappearance of facultative and strict anaerobes. *Bacteroides melaninogenicus ss. intermedius* and *Eubacterium lentum* constituted the most numerous population in the large intestine of the rats fed a 70% casein diet, but *Bacteroides fragilis ss. fragilis* and *ss. vulgatus* were the most predominant in the colonic bacteria of the rats fed a 10% casein diet, when compared with other diets. The colonic bacterial population did not differ so markedly both quantitatively and qualitatively in accordance with the composition of the diets (Table 1). It was shown that the dietary constituents exert a profound influence on jejunal bacterial composition both quantitatively and qualitatively.

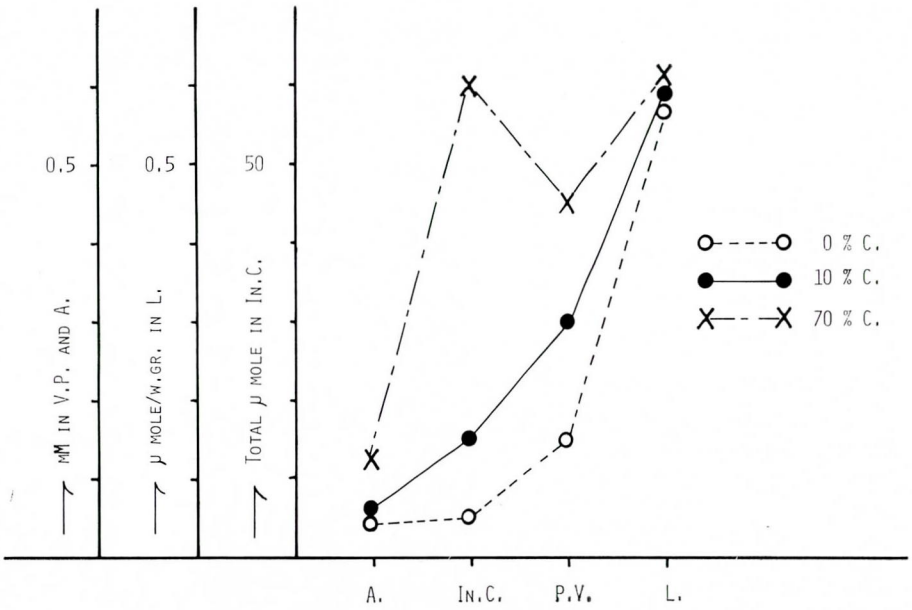

Fig. 1 Effects of protein levels in diet on ammonia concentrations in aorta, intestinal content, portal vein and liver.
V. P.: portal vein, A.: aorta, L.: liver, In. C.: intestinal cavity, 0% C.: 0% casein diet, 10% C.: 10% casein diet, 70% C.: 70% casein diet. All experimental procedures are described in "Materials and Methods"

As shown in Fig. 1, it was found that the ammonia concentration in small intestines of rats fed a 70% casein diet was higher than those of other dietary constituents. These findings indicate that there is a close correlation between the ammonia levels and jejunal bacterial populations with ammonia production in animals fed a 70% casein diet. It seems worth postulating that the ammonia concentration in the small

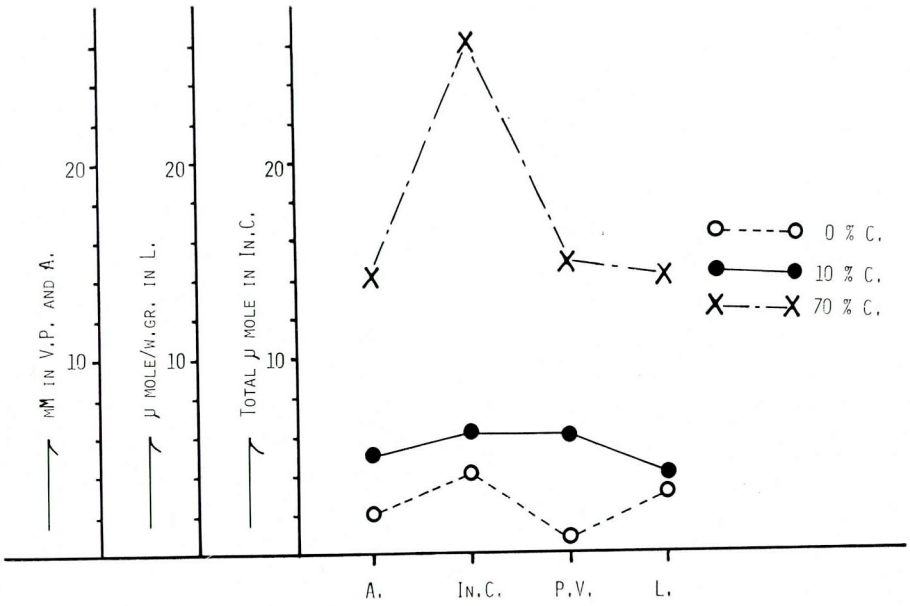

Fig. 2 Effects of the protein levels in diet on urea concentrations in aorta, intestinal content, portal vein and liver.
V. P.: portal vein, A.: aorta, L.: liver, In. C.: intestinal cavity, 0% C.: 0% casein diet, 10% C.: 10% casein diet, 70% C.: 70% casein diet. All experimental procedures are described in "Materials and Methods"

intestine resulted in the quantity of intestinal bacteria producing ammonia both by urease and deaminase activity.

On the other hand, ammonia concentrations in livers were constant with varying concentrations of protein for 0%, 10% and and 70% casein diets and revealed higher values than those for the portal vein. These data suggest that certain amino acids are converted to free ammonia and the concentrations of ammonia in the liver is maintained at constant levels.

As shown in Fig. 2, the concentrations of urea, which is the detoxic form of a free ammonia, in the aorta, intestinal contents, portal vein and liver agreed well with the concentration of casein in the diet. It is possible to say that some of the urea in the aorta passed through into the intestinal cavity (3, 4, 5), because there was no urea in the casein diets.

As shown in Table II., studies on changes in the concentrations of amino acids in the aorta, intestinal contents, portal vein and liver were carried out for rats fed 0%, 10% and 70% casein diets.

Concentrations of all essential amino acids were low in the intestinal contents of rats fed a 0% casein diet and the concentrations of certain types of non-essential amino acids such as glutamate, taurine, asparate, and glycine were relatively high.

The concentrations of all essential amino acids in intestinal contents

Table 2. The concentration of various amino acids in aorta intestinal contents, portal vein and liver of rats fed 70%, 10% and 0% casein diets.
All experimental procedures are described in "Materials and Methods" Bar means lower than 0.02
Abbreviation: In. C., intestinal content;7 0% C., 70% casein diet; 10% C., 10% casein diet; 0% C., 0% casein diet;

A. A.	Aorta (mM)			In. C. (total μ moles)			Porta (mM)			Liver (μ mole/g wet weight)		
	70%C.	10%C.	0%C.	70%C.	10%C.	0%C.	70%C.	10%C.	0%C.	70%C.	10%C.	0%C.
Lys	0.66	0.44	0.31	4.00	0.29	0.10	0.74	0.53	0.22	0.66	0.50	0.34
Val	1.28	0.11	0.66	3.37	0.84	—	1.40	0.17	0.07	1.23	0.18	0.07
Leu	0.70	0.09	0.06	3.26	0.93	0.2	0.76	0.12	0.07	0.51	0.22	0.09
Thr	0.36	0.29	0.08	3.5	1.3	0.3	0.38	0.30	0.09	0.30	0.80	0.22
Phe	0.10	0.06	0.03	2.79	0.68	—	0.14	0.061	0.031	0.10	0.05	0.03
Ile	0.47	0.06	0.04	2.53	0.60	0.09	0.53	0.08	0.04	0.60	0.16	0.04
Met	0.10	0.03	—	0.59	—	—	0.12	—	—	—	—	—
Trp	—	—	—	1.15	—	—	—	—	—	—	—	—
Arg	0.18	0.18	0.12	—	—	—	0.18	0.12	0.09	—	—	—
Pro	0.97	—	—	63.70	—	—	1.15	—	—	0.91	—	—
Glu	0.29	0.26	0.26	22.00	9.20	5.10	0.35	0.22	0.29	4.16	4.30	3.80
Tau	0.35	0.29	0.31	10.90	2.16	1.92	0.43	0.25	0.18	6.25	0.58	1.20
Asp	0.10	0.07	0.04	5.87	4.14	2.20	0.29	0.20	0.16	2.53	2.64	3.11
Gly	0.13	0.31	0.38	4.01	7.40	2.45	0.24	0.36	0.47	0.51	3.23	2.44
Tyr	0.18	0.08	0.02	2.86	0.65	—	0.21	0.09	0.02	0.13	0.06	0.02
Ser	0.27	0.34	0.49	2.91	0.94	0.44	0.37	0.31	0.48	0.26	1.5	2.9
Asn	0.14	0.06	0.05	1.56	0.68	—	0.24	0.08	0.06	0.20	0.14	0.16
His	0.05	—	—	1.21	—	—	0.10	0.06	0.05	0.40	0.52	0.46
Gln	0.44	0.66	0.65	1.13	—	—	0.56	0.41	0.41	2.04	3.03	5.98
Ala	0.67	0.64	0.61	4.44	2.00	0.73	1.0	0.45	0.82	2.53	2.60	3.11
Orn	0.07	0.04	0.05	0.43	0.29	0.08	0.07	0.03	0.04	0.30	0.13	0.20
Cys	—	—	—	—	—	—	—	—	—	—	—	—
Cit	0.07	0.05	0.04	—	—	—	0.09	0.09	0.08	0.19	0.11	0.11

of rats fed a 70% casein diet showed higher values than those fed a 10% casein diet. These findings indicate that there is a close correlation between the concentrations of essential amino acids in the intestinal contents and the concentrations of proteins in the diet. On the other hand, there is no correlation between the concentrations of non-essential amino acids in the intestinal contents and the concentrations of proteins in the diet. In aorta and portal vein the relations between the concentrations of amino acids and the concentrations of proteins in diet were almost same as those in the intestinal contents.

The hepatic concentrations of essential amino acids were higher in high protein liver than in low protein liver. The concentrations of some of non-essential amino acids such as glutamate, aspartate and alanine in liver were constant despite changes in dietary protein contents.

Concerning to the concentrations of amino acid in liver, similar results were described by Saheki et al. in the perfusion experiment of livers from rats fed high and low protein diets (6).

Acknowledgement

This work was supported in part by a research grant from the Waksman Foundation of Japan Inc.

References

1) Coulombe J J, Favreau L: Clin Chem 9: 102—108, 1963
2) Seligson D, Hirahara K: J Lab Clin Med 49: 962—974, 1957
3) Walser M, Bodenlos L J: J Clin Invest 38: 1617—1626, 1959
4) Wolpert E, Philips S F, Summerskill WHJ: Lancet II: 1387—1390, 1971
5) Kobashi, K, Takebe S, Hase J et al: Seikagaku 46: 634, 1974
6) Saheki T, Tsuda M, Tanaka T et al: J Biochem 77: 671—678, 1975