

Red and Blue Distinctive Mucin-histochemical Types of Japanese Colorectal Mucosa

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Mucin-histochemical characteristics of normal human colorectal mucosa were investigated utilizing Culling *et al.*'s staining method which distinguishes the mode of C8-O-acylation of sialomucins. Normal mucosae of cecum, ascending, transverse and descending colon and rectum were obtained from autopsy and biopsy material. Japanese colorectal mucosa stained either entirely red or entirely blue, in contrast to previous reports dealing with Caucasians where all the normal mucosa reportedly stained red. The ratio of red to blue colon cases varied to some extent with age, i.e. it was found to be 1:1 in children aged 0 to 4, 2:1 in the 5-20 age group, and 4:1 in people older than 21 years, suggesting a tendency of shift from blue to red during early life in Japan. Each individual Japanese adult colorectal mucosa may thus be classified into either red or blue type mucin-histochemically.

Key words: Colorectal mucosa — Mucin-histochemistry

The majority of colorectal mucins produced by columnar and goblet cells are sialo-glycoproteins which can be further subdivided into sulfated and non-sulfated types.¹⁻³⁾ The distribution and/or proportions of these mucins change physiologically with site and also under various pathological conditions.⁴⁻⁹⁾ Cancer-associated mucins, for example, are generally low in sulfated and high in sialidase-sensitive sialomucin species.^{1,2,10)} In addition to such disease-associated change, considerable individual variation exists in normal human colorectum with regard to mucin distribution.¹¹⁾

Some time ago Culling *et al.* introduced a new mucin-histochemical method (periodic acid-thionine Schiff/periodate-borohydride/potassium hydroxide/periodic acid-Schiff technique) which can distinguish between various patterns of O-acylation of sialic acid in sialomucins.^{12,13)} Generally with this method the mucins with sialic acid substituted at C8 by acyl residues are stained red, regardless of the presence or absence of substitution at other sites. Substitution at C7 or lack of substitution is indicated by blue coloration, and a mixture of the two types of mucin by purple staining. Using this method they carried out an analysis of various conditions of the colon and rectum and reported that human normal colorectal mucins stained red whereas colorectal cancer-produced mucin was generally blue or purple.¹³⁻¹⁵⁾

We have investigated the normal colon and rectum of Japanese subjects at various ages by Culling *et al.*'s method¹²⁾ and found that the normal Japanese adult colorectal mucosa is stained either entirely red ("red colon") or entirely blue or purple ("blue colon") and that there is some tendency to shift from blue to red

during the newborn and adolescent phases. We report here the results of our investigation.

MATERIALS AND METHODS

Autopsy materials Adult samples obtained from 26 autopsy cases were accumulated at Toranomon Hospital, Tokyo. Cases free from evident gastrointestinal tract disease and dissected within 4 h after death were selected. The main causes of death in the cases were: cardiac disease (11), cerebrovascular disease (5), pneumonia (4), liver disease (2), collagen disease (2), leukemia (1) and breast cancer (1). The ages of the patients ranged from 36 to 79, the average being 63.5 years.

Samples from Japanese children and young people less than 20 years of age were obtained from the National Children's Hospital, Tokyo. Age and sex distribution information for the material is listed in Table I. The main diseases in the 52 cases were: cardiac anomaly (26), chromosome aberration (6), neuroblastoma (5), respiratory insufficiency (5), leukemia (3), anemia (1), pericarditis (1), asplenia (1), chondrodystrophy (1) and unknown (3).

All these autopsy specimens had been fixed in 10-20% formalin and samples were cut from the cecum, transverse and descending colon and rectum.

Biopsy material Biopsy specimens were obtained by endoscopic biopsy from the cecum, transverse and descending colon and rectum of 16 Japanese adults (10 men and 6 women) during health examination for colorectal cancer at the Cancer Institute Hospital, Tokyo. The patients were between 32 and 70 years of age (average 46.1) and all were diagnosed to be free from colorectal disease including neoplasia. Since studies previ-

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Table I. Ratio of Red and Blue Colorectum Cases in Different Age Groups (Autopsy Material)

Age	Total no. of cases	Color type	
		Red	Blue
0-12 mo	24	12 (50.0%)	12 (50.0%)
	14 M	7 (50.0%)	7 (50.0%)
	10 F	5 (50.0%)	5 (50.0%)
1 yr-4 yr 11 mo	12	7 (58.3%)	5 (41.7%)
	6 M	4 (66.7%)	2 (33.3%)
	6 F	3 (50.0%)	3 (50.0%)
5-10 yr	8	5 (62.5%)	3 (37.5%)
	4 M	2 (50.0%)	2 (50.0%)
	4 F	3 (75.0%)	1 (25.0%)
11-20 yr	8	6 (75.0%)	2 (25.0%)
	5 M	4 (80.0%)	1 (20.0%)
	3 F	2 (66.7%)	1 (33.3%)
21 yr-	26	21 (80.8%)	5 (19.2%)
	15 M	13 (86.7%)	2 (13.3%)
	11 F	8 (72.7%)	3 (27.3%)

ously performed with autopsy materials had already shown the existence of a minor population having "blue colon," which might represent a relatively higher risk state for developing colorectal cancer, detailed mucin histochemical examination of normal colonic mucosa was considered to be worthwhile. The biopsy procedure *per se* did not exert any additional burden on the people undergoing colonoscopic examination.

Mucin histochemical examination Formalin-fixed tissues were embedded in paraffin, sectioned at 4 μ m thickness and stained according to the method (periodic acid-thionine Schiff/periodate-borohydride/potassium hydroxide/periodic acid-Schiff technique) described by Culling *et al.*¹²⁾

RESULTS

The normal colorectal mucosae of the Japanese population sampled were stained principally either red or blue by Culling *et al.*'s mucin histochemical method (Fig. 1). Although in some cases of red colorectum there was some tendency towards shift from purple to red along the course of the intestine from the cecum to the rectum, indicating higher non-acetylated sialomucin content in the right-sided colon than in the left, the degree of shift was only slight and the purple tone even at the cecum was clearly different from the "blue" in the blue colorectum. Thus, the staining characteristic of the mucosa was fixed individually, irrespective of the site in the colon, i.e. each individual person could be unequivocally classified into either red or blue types on the basis of colorectal mucin histochemistry.

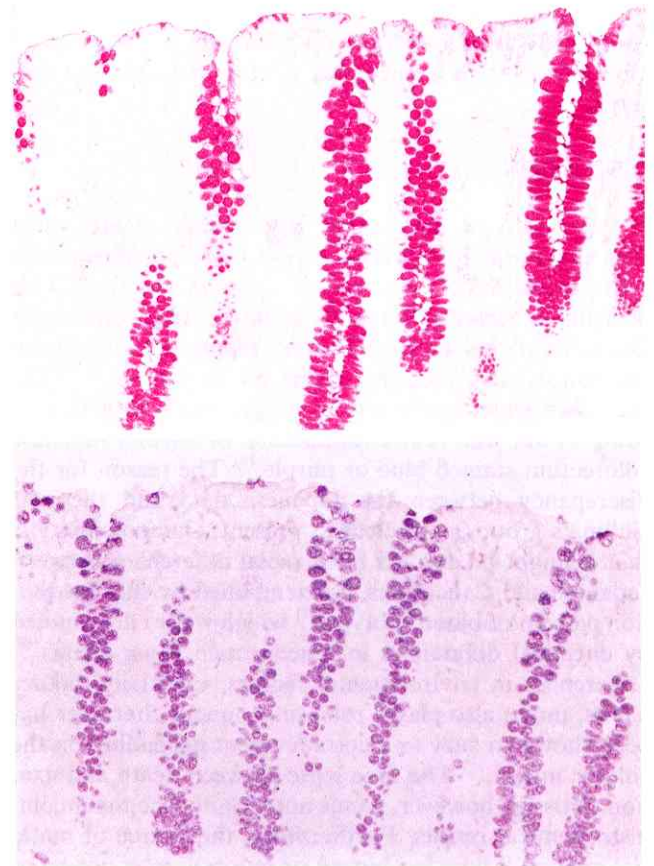


Fig. 1. Mucin-histochemical demonstration of red (upper) and blue (lower) colon by Culling *et al.*'s method. All the goblet cells are stained either entirely red or blue. $\times 200$.

Table II. Color Type of Normal Colorectal Mucosae in Biopsy and Autopsy Specimens

No. of cases	Age (yr) (average)	Color type	
		Red	Blue
Biopsy	16 32-70 (46.1)	12 (75.0%)	4 (25.0%)
Autopsy	26 21-79 (63.5)	21 (80.8%)	5 (19.2%)
Total	42	33 (78.6%)	9 (21.4%)

The observed ratios of the two types of colorectum in different age groups are shown in Table I. The ratio of red to blue was 1:1 in the newborn age group (0-12 months) in both sexes. The frequency of blue type thereafter decreased with age and in the adult (<21 years) group the red-to-blue ratio was 4:1. This shift toward red predominance was observed in both sexes to essentially similar extents.

The results gained with adult biopsy specimens are compared with those from autopsy material in Table II. No significant difference was evident between the two series.

DISCUSSION

The results of the present investigation clearly show that the normal colorectal mucosa of Japanese adults stains either red or blue by Culling *et al.*'s method. This presents a striking contrast with the observations of Culling and his colleagues who reported that normal colorectum of Caucasians stains red in general.¹²⁻¹⁵⁾ On the other hand, our result is in agreement with that of Muto *et al.*, who found that 38.8% of normal Japanese colorectum stained blue or purple.¹⁶⁾ The reason for the discrepancy between the Japanese data and these of Culling's group is not clear at present. One possibility is that it might be derived from racial differences between Japanese and Caucasians, as exemplified by the distribution pattern of blood subtype,¹⁷⁾ which is also determined by chemical differences in mucoprotein sugar-chains.¹⁸⁾ Differences in environmental factors, especially dietary habits, might also play a role, since mucin character has been shown to vary to a certain extent depending on the colonic milieu.¹¹⁾ The time lapse between death and fixation of tissue, however, seems not to influence the mucin-histochemical results. Furthermore, the source of material analyzed, whether biopsy or autopsy, may not be of any importance since there was no significant difference in our results between the series of the two types.

Culling and his colleagues¹²⁻¹⁵⁾ have reported that all malignant tumors of the colorectum stain blue in contrast to the reddish background, suggesting that this staining character may be an indication of malignant change in this tissue. Such a hypothesis is obviously confounded by the presence of blue colorectum individuals within the normal Japanese population. Previously, however, we also observed that most of the carcinomas of colon and rectum developing from red colorectum mucosa stained

blue or purple in clear contrast to the background. Blue mucin staining, therefore, should still be regarded as an important neoplastic expression in the red colorectum population. In our own unpublished study, the mucosa adjacent to carcinoma was found to be usually stained the same as background tissue, although Filipe and Branfoot⁴⁾ reported that an increase in sialomucins was a common feature in such areas and that the mucin change involved expression of the C8-non-acylated type which gives a blue reaction by Culling *et al.*'s method.

The present study also provided strong suggestive evidence for a shift from blue to red colorectum during early life in part of the Japanese population. Thus, the blue mucin histochemical character, i.e. C8-non-acylation, might be regarded as a kind of oncofetal expression in red colorectum individuals.

We are presently investigating whether the blue colorectum population is at a higher risk of developing colonic or rectal cancer. In this context, it is of note that Muto and his colleagues found a much higher percentage (82.2%) than expected of blue colorectum individuals among patients with familial polyposis, a condition usually resulting in development of colorectal cancer at a younger age than in the general population.¹⁶⁾ We admit, however, that the above-cited observations by Culling and his colleagues¹²⁻¹⁵⁾ do not favor the hypothesis, since the incidence of colorectal cancer is by far higher among Caucasians than Japanese.¹⁹⁾

ACKNOWLEDGMENTS

This research was supported by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan. Many thanks are owed to Dr. M. Hara (Toranomon Hospital) and Dr. K. Shimizu (National Children's Hospital) for their generous supply of autopsy materials, Dr. T. Sasaki (Cancer Institute) for his kind supply of biopsy materials, and Dr. T. Katsuyama (Shinshu University) for his critical and important advice.

(Received December 5, 1989/Accepted February 22, 1990)

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