Title:	Relation of Ceroid to Small Bowel Function
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OBJECTIVE: Deposition of ceroid, a lipofucsin pigment, was recently found in 76% of 139 unselected autopsies from Udorn Provincial Hospital in Northeast Thailand[1]. The pigment was deposited predominantly in the smooth muscle of the gastrointestinal tract and to a lesser extent in smooth muscle of blood vessels, prostate, uterus, urinary bladder, and in the lymph nodes. A similar distribution of ceroid pigment has been described in malabsorptive states such as cystic fibrosis of the pancreas[2], chronic pancreatitis[3], and non-tropical sprue[4-6]. Because of this, the present study was undertaken to search for malabsorptive disease in North-east Thailand.

DESCRIPTION:

Methods:

Clinical Material: 63 patients were studied on the wards of the Udorn Provincial Hospital. Selection was based on willingness to cooperate. Patients with severe anemia or recent surgery were rejected from the study. There were 38 males and 27 females ranging in age from 14 to 59 years (mean age 32). Because of limited laboratory and x-ray facilities, only a tentative diagnosis based on history and physical examination performed at the time of study was possible (see Table I). The following studies were performed on most of the patients: upper gastrointestinal biopsies, d-xylose tolerance test, vitamin A tolerance test, and serum vitamin E, albumin, cholesterol, and B-carotene. In addition, 3-day fecal fat collections were carried out in 8 patients. Biopsies were taken with a Crosby-kugler capsule. After swallowing the capsule, the patients were turned on their right sides for 20-30 minutes, and then the capsule was fired. There were no complications from this procedure. Formalin-fixed tissue was stained with hematoxylin and eosin, PAS, Ziehl-Neelsen and brilliant green.

The 5 hour d-xylose urinary excretion was measured in 43 patients after a 25 g oral dose, and in 15 after a 5 g dose. The 5 hour urine volume was greater than 170 ml in all. Two-hour serum xylose levels were also performed in those given the 25 g dose.

Serum vitamin A levels were measured fasting and 5 hours after an oral dose of 250,000 IU diluted to 5 ml with vegetable oil.

Three-day fecal fat collections were marked with carmine red. The diet was supplemented with 75 g of butter daily.

Serum, urine, and stool specimens were collected by metabolic nurses, and were frozen in dry ice. Methods were the same as those employed in a previous study of normal adult Thais living near Bangkok[7].

Results

Gastrointestinal Biopsies:

63 biopsies were obtained from the upper gastrointestinal tract (see Table II). 19 out of 35 antral biopsies and 4 out of 4 esophageal biopsies contained ceroid pigment, whereas none of the 19 from the fundus and body of the stomach, duodenum or jejunum were positive. Five other biopsies were considered unsatisfactory for determining the presence or absence of ceroid because smooth muscle (muscularis mucosae) was not present. The distribution of the pigment in the upper gastrointestinal tract thus confirms that found in the autopsy series from this hospital[1] where ceroid occurred in the esophagus in 75% of the cases examined, and further, was found in the esophagus in every case where ceroid was also found elsewhere in the body. In contrast, the gastric antrum contained the pigment in only 46% of the cases, and only 58% of the time when some other site or sites were also affected. It is thus likely that many of the patients in the present study whose gastric antrum biopsies were ceroid-negative were actually ceroid-positive. Therefore, it is more meaningful when making comparisons between ceroid-positive and ceroid-negative patients to consider the entire Udorn study patients as a homogeneous ceroid-positive group and the previously-studied Bangkok subjects as a ceroid-negative group. However, because there is such a paucity of information on this subject, comparisons between ceroid-positive and ceroid-negative within the Udorn patient population, admittedly less valid, are also made. Because the incidence of ceroid in fundus and body of the stomach, and in the duodenum is so low (13% and 6%), it is unlikely that any positive cases were missed, and hence they are not included in comparison between ceroid-positive and ceroid-negative patients. Histologic grading of the amount of pigment, as previously described[1] is shown in Table III. No correlation was found between the amount of pigment deposited and the tests of absorption that follow.

D—xylose Excretion Test:

The average 5 hour urinary xylose excretion after a 25 g dose in forty-four patients was 5.1 $\pm$ 1.7* g. This group comprises all the subjects studied including those whose biopsies were unsatisfactory for evaluating the presence or absence of ceroid. This value was not significantly different from normal Bangkok subjects (see Table IV). The 2 hours serum xylose was also similar in the two groups.

In those from Udorn with the 5 g d-xylose dose, the 5 hour excretion was 1.49$\pm$0.39 g. Although we have no data from normal Thais in Bangkok using this dose for comparison, none of the Udorn patients excreted less than 1 g, the level observed in sprue patients[6].

After the 25 g dose, d-xylose excretion was 5.1 g in thirteen Udorn Hospital subjects with ceroid-positive biopsies, in comparison to 6.1 g in 10 subjects with ceroid-negative-negative biopsies. The difference is not significant (see Table V). Likewise, there was no difference in d-xylose excretion following the 5 g dose between the ceroid-positive and negative-patients. The two-hour serum-xylose value was, however, significantly higher ($p=.05$) in the ceroid-negative group (50.9 mg%) than in the ceroid-positive subjects (41. 8 mg%).

Fecal fat:

Fecal fat excretion in 8 patients averaged 3.7 g per day. This value was not different from fat excretion in the Bangkok subjects (2.4$\pm$1.4 g). One of the Udorn patients had mildly elevated fat excretion (7.5 g/day). However, in this patient the xylose excretion (8.1 g), vitamin A tolerance, B-carotene, and hemotocrit were all normal.

* All values in the results are given as the mean $\pm$ 1 S.D.

Vitamin A absorption:

Vitamin A malabsorption* occurred in 14 of 54 Udorn subjects tested (26%), compared to the 3 out of 27 in the Bangkok subjects (11%). This is not significantly different (see Table IV). There was also no difference in vitamin A absorption between the ceroid-positive and-negative patients in Udorn.

Biochemical tests:

Serum B-carotene values in 63 Udorn patients averaged 66 $\pm$ 35 ug% and were lower than in the Bangkok subjects (133 $\pm$ 65 ug%) (Table IV). There was no difference between ceroid-positive and ceroid-negative patients (Table V).

Serum albumin was also lower in the Udorn patients, when compared to the Bangkok subjects (Table IV). The difference between ceroid-negative and ceroid-positive patients, however, was insignificant (Table V).

The serum cholesterol values in the Udorn patients were the same as the Bangkok subjects (Table IV). As with the Bangkok group, no correlation was found between serum cholesterol and urinary d-xylose[10].

Discussion:

The present study confirms the previously reported distribution and incidence of ceroid pigment in the upper gastrointestinal tract of patients coming to autopsy at the Udorn provincial Hospital in northeast Thailand[1]. Because malabsorptive diseases have been found so frequently in the past in association with deposition of ceroid pigment, one would expect to find evidence of it in this population where 75% of the people have the pigment. Standard tests of intestinal absorption, however, failed to uncover any such examples, neither when the entire patient population studied was compared to Bangkok controls, where ceroid is rare, nor when the patient population with ceroid-positive and ceroid-negative-biopsies were compared.

The mechanism of ceroid formation in vivo is not entirely clear. In experimental animals, pigment deposition can be induced by feeding vitamin E deficient diets[11,12]. The process can be hastened if, in addition, a diet high in usaturated fat is used. Ceroid is thus though to be formed when an antioxidant-deficient state occurs, but the exact mechanism is not known.

In clinical malabsorptive states accompanied by steatorrhea, malabsorption of vitamin E itself is likely to be the main factor contributing to pigment deposition. In the group of patients that we have studied, there is little evidence that malabsorption is present; thus, either a low dietary intake of vitamin E is present or an increased amount of unsaturated fat is responsible. While both factors are probably operative, the evidence for the latter is stronger based on present knowledge. It is known, for instance, that the unsaturated fat intake in the population in northeast Thailand is quite high, since the principle dietary staples are fermented fish and glutinous rice, the fish containing most of its fat in the unsaturated form[13]. Further evidence that increased unsaturated fat in the diet is playing a role is supported by the significantly lower serum total cholesterol in the ceroid-positive patients, since this type of dietary fat is known to be an effective cholesterol-lowering agent.

* Vitamin A malabsorption is defined as a rise of serum vitamin A at 5 hours of less than 125 ug% over the fasting value[9].

Table I Clinical Diagnoses of Subjects Studied at Udorn Provincial Hospital.

Accidental Trauma Gastrointestinal	12
Dysentery, convalescent	4
RUQ Pain, undiagnosed	2
Intestinal flukes	1
Appendiceal abscess	1
Musculoskeletal	7
Post-partum	6
Genitourinary	7
Endecrine (Thyrotoxicosis, Post-menopausal)	3
FUO, convalescent	3
Inguinal hernia	4
Anemia, mild, uncharacterized	2
Psychoneurosis	2
Malnutrition	2
Miscellaneous	9
Total	65

Table II Biopsy site and presence or absence of ceroid pigment in specimens from patients at Udorn Provincial Hospital, Thailand.

	Total Number	Ceroid-Positive	Ceroid-Negative
Stomach*			
Antrum	36	19	16
Fundus and Body	18	0	14
Esophagus	4	4	0
Duodenum and Jejunum	5	0	5
Total	63	23	35

Table III. Histologic grading of ceroid-positive biopsies.

		Number of positive biopsies	
		Antrum	Esophagus
1+	Trace to small amount.	10	1
2+	3-6 smooth muscle fibers affected in most high-power fields.	1	1
3+	Many fibers pigmented; some fibers bulging with pigment.	6	1
4+	Almost all smooth-muscle fibers heavily laden with pigment.	2	1
	Total	19	4

* 1 antral and 4 fundal biopsies had no muscularis mucosal, and therefore could not be evaluated for the presence of ceroid pigment.

Table IV. Comparison of Absorption Tests and Other Biochemical Data between Patients at the Udorn Provincial Hospital and Normal Thais in Bangkok, Thailand.

Location	25 g. d–xylose		Fecal Fat*	Vitamin A absorption, % abnormal +	Serum B – carotene, ug/100 ml	Total serum cholesterol, mg/100 ml	Serum albumin, g/100 ml
	Urinary excretion g/5 hrs	2 hr serum xylose mg/100 ml					
Udorn	5.1±1.7(44)++	43.4±11.8(43)	3.7±2.2 (8)	26 (54)	66±35 (59)	171±49 (59)	3.64±0.56 (43)
Bangkok	5.8±1.5(36)	39.8± 7.5(31)	2.3±1.4 (35)	11 (27)	133±65 (34)	177.5±31.0(35)	4.2 ±0.57 (32)
p value	N.S.	N.S.	N.S.	N.S.	<0.01	N.S.	< 0.01

* The diet was supplemented with 75 g of butter per day.

\+ The vitamin A absorption was considered abnormal if the increase over the fasting value was less than 125 μg/100 ml.

++ Mean±S.D. The numbers in parenthesis are the number of subjects studied.

Table V. Comparison of absorption tests and other biochemical data between ceroid-positive and ceroid-negative patients at the Udorn Provincial Hospital, Thailand.

	Ceroid—Positive	Ceroid—Negative	P
No. of individuals*	23	16	
Males	14	5	
Females	9	11	
Average age years	33.7	28.8	
d-xylose excretion, g/5 hrs**	5.1 ± 1.61 (13)+	6.1 ± 1.55 (10)	N.S.
2 hr. serum xylose mg/100 ml	41.8 ± 12.2 (14)	50.9 ± 9.4 (10)	0.05
d-xylose excretion, g/5 hrs++	1.45 ± 0.29 (6)	1.58 ± 0.53 (4)	N.S.
Vitamin A absorption, % abnormal§	26 (23)	12.5 (16)	N.S.
Fecal fat g/day#	3.3 (3)	4.0 (3)	N.S.
Serum B-carotene, μg/100 ml	59 ± 43.5 (23)	78 ± 31.2 (16)	N.S.
Serum total cholesterol, mg/100ml	152 ± 37.3 (21)	205 ± 44.1 (15)	<0.01
Serum albumin, g/100 ml	3.55 ± 0.75 (14)	3.72 ± 0.59 (11)	N.S.

* Only those patients whose biopsies were from antrum or esophagus and contained muscularis mucosa are included here.

** 25 g. oral dose.

+ MEAN ± S.D. The numbers in parentheses are the number of subjects studied.

++ 5 g. oral dose.

§ The vitamin A absorption was considered abnormal if the increase over the fasting value was less than 125/μg/100 ml.

The diet was supplemented with 75 g. of butter per day.