การกักเก็บน้ำตาลและวิตามินโดยเจลโพลีแซคคาไรด์จากเปลือกผลทุเรียนในหลอดทดลอง

นางสาววราภรณ์ ปิยสิรานนท์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาชีวเวชเคมี ภาควิชาชีวเคมี
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2546
ISBN 974-17-3904-4

ENTRAPMENT OF SUGARS AND VITAMINS BY POLYSACCHARIDE GEL FROM DURIAN FRUIT-HULLS *IN VITRO*

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A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science in Biomedicinal Chemistry

Department of Biochemistry

Faculty of Pharmaceutical Sciences

Chulalongkorn University

Academic Year 2003

ISBN 974-17-3904-4

Thesis Title	Entrapment of sugars and vitamins by polysaccharide gel from durian fruit-hulls *in vitro*
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Field of Study	Biomedical Chemistry
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วราภรณ์ ปิยสิรานนท์ : การกักเก็บน้ำตาลและไวตามินโดยเจลโพลีแซคคาไรด์จากเปลือกทุเรียนในหลอดทดลอง (ENTRAPMENT OF SUGARS AND VITAMINS BY POLYSACCHARIDE GEL FROM DURIAN FRUIT-HULLS *IN VITRO*) อ. ที่ปรึกษา : รศ.ดร.สุนันท์ พงษ์สามารถ, 104 หน้า. ISBN 974-17-3904-4.

การศึกษาเจลโพลีแซคคาไรด์ (PG) สกัดได้จากเปลือกทุเรียนซึ่งเป็นวัสดุเหลือทิ้งทางการเกษตรจากพืช ได้ผลิตภัณฑ์เจลโพลีแซคคาไรด์ที่มีมูลค่าเพิ่ม มีคุณสมบัติของใยอาหารชนิดที่ละลายได้ในน้ำ มีองค์ประกอบเป็นน้ำตาลชนิดต่าง ๆ ได้แก่ กรดกาแลคทูโรนิค น้ำตาลกลูโคส อราบิโนส แรมโนส และ ฟรุกโตส งานวิจัยนี้มีจุดประสงค์เพื่อศึกษาผลของเจลโพลีแซคคาไรด์ต่อการกักเก็บสารอาหารประเภทน้ำตาล ได้แก่ น้ำตาลกลูโคส และซูโครส รวมทั้งวิตามินชนิดที่ละลายได้ในไขมัน (วิตามินเอ) หรือชนิดที่ละลายได้ในน้ำ (วิตามินบีหนึ่ง) ทำการศึกษาในหลอดทดลอง ด้วยเทคนิค dialysis โดยใช้ semi-permeable membrane และลำไส้เล็กของหนูทดลอง รวมทั้งเปรียบเทียบผลของสารโพลีแซคคาไรด์ที่มีการใช้ทางการค้า คือ กลูโคแมนแนน ในการกักเก็บกลูโคส เมื่อทำการ dialysis เป็นเวลา 0-4 ชั่วโมง ใน Ringer buffer (pH 7) ด้วย 0-2% กลูโคสใน 0-3% เจลโพลีแซคคาไรด์ แล้ววิเคราะห์กลูโคสทั้งในถุงและนอกถุง dialysis โดยวิธีการทำให้เกิดสีจากการทำปฏิกิริยาของเอนไซม์กลูโคสออกซิเดส การทดลองพบว่าเมื่อทดสอบภายหลังการ dialysis ที่ 1, 2, 3 และ 4 ชั่วโมง ที่ความเข้มข้นเพิ่มขึ้นของเจลโพลีแซคคาไรด์ 2 และ 3% มีผลให้กลูโคสในถุงเพิ่มขึ้น และลดลงนอกถุง dialysis อย่างมีนัยสำคัญ เมื่อเปรียบเทียบกับกลุ่มควบคุมที่ 0% เจลโพลีแซคคาไรด์ และที่ความเข้มข้น 1, 2 และ 3% เจลโพลีแซคคาไรด์ให้ผลการกักเก็บน้ำตาลกลูโคสน้อยกว่ากลูโคแมนแนนหลังการ dialysis เป็นเวลา 2 ชั่วโมง การทดลองโดยใช้ถุงลำไส้เล็กกลับด้านของหนูขาวให้ผลเช่นเดียวกัน พบการส่งผ่านกลูโคสที่ลำไส้ลดลงเมื่อเพิ่มความเข้มข้นของเจลโพลีแซคคาไรด์เป็น 2 และ 3% ภายหลังการ dialysis เป็นเวลา 1 ชั่วโมง การศึกษาผลของเจลโพลีแซคคาไรด์ในการกักเก็บซูโครสด้วยเทคนิค dialysis โดยใช้ semi-permeable membrane และการวิเคราะห์ซูโครสด้วยเทคนิค HPLC เมื่อเพิ่มความเข้มข้นของเจลโพลีแซคคาไรด์ พบว่าซูโครสถูกปล่อยออกมานอกถุง dialysis ลดลง นอกจากนี้พบว่าวิตามินเอถูกกักเก็บไว้ได้มากขึ้นเมื่อเพิ่มความเข้มข้นของเจลโพลีแซคคาไรด์ที่ 1-3% หลังการ dialysis วิตามินเอในรูป all-*trans* retinyl acetate ในกรดแอสคอร์บิค เกลือน้ำดีและ 0-3% เจลโพลีแซคคาไรด์ ในภาชนะภายใต้กาซไนโตรเจน ห่อหุ้มด้วย aluminum foil ป้องกันแสง และ dialysis ในห้องมืด เป็นเวลา 2 ชั่วโมง การศึกษาผลของการกักเก็บวิตามินบีหนึ่งในรูป thiamin hydrochloride ใน 0-3% เจลโพลีแซคคาไรด์ พบว่าที่ความเข้มข้นเพิ่มขึ้นของเจลโพลีแซคคาไรด์ 2-3% มีผลเพิ่มขึ้นในการกักเก็บวิตามินบีหนึ่งในถุง และลดลงนอกถุง dialysis อย่างมีนัยสำคัญ 2% เจลโพลีแซคคาไรด์สามารถกักเก็บน้ำตาล (กลูโคส และซูโครส) วิตามินชนิดที่ละลายได้ในไขมัน (วิตามินเอ) และวิตามินชนิดที่ละลายได้ในน้ำ (วิตามินบีหนึ่ง) และการศึกษาในหลอดทดลองโดยเทคนิคการ dialysis โดยใช้ semi-permeable membrane อาจใช้เป็นวิธีการง่าย ๆ เพื่อการศึกษาเบื้องต้นในการทดลองการกักเก็บสารอาหารโดยเจลโพลีแซคคาไรด์ชนิดที่ละลายได้ในน้ำ ก่อนการศึกษาผลของโพลีแซคคาไรด์ต่อการดูดซึมสารอาหารในสัตว์ทดลอง (*in vivo*) ที่มีราคาสูงต่อไป

ภาควิชา.....ชีวเคมี..... ลายมือชื่อนิสิต.....

สาขาวิชา.....ชีวเวชเคมี..... ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา.....2546.....

4476612133 : MAJOR BIOCHEMISTRY

KEY WORD: DIETARY FIBER / POLYSACCHARIDE GEL / GLUCOSE / SUCROSE / VITAMIN A / VITAMIN B1 / SEMI-PERMEABLE MEMBRANE / EVERTED JEJUNAL SAC OF RAT / DIALYSIS TECHNIQUE

WARAPORN PIYASIRANANDA: ENTRAPMENT OF SUGARS AND VITAMINS BY POLYSACCHARIDE GEL FROM DURIAN FRUIT-HULLS *IN VITRO*. THESIS ADVISOR: ASSOC. PROF. SUNANTA PONGSAMART Ph.D., 104 pp. ISBN 974-17-3904-4.

The polysaccharide gel (PG) extracted from fruit-hulls of durian (*Durio zibethinus* L.), an agricultural waste material from plant, was a value-added polysaccharide gel product. PG is a water-soluble polysaccharide fiber composed of galacturonic acid, glucose, arabinose, rhamnose and fructose. Effects of PG on entrapment of sugars (glucose and sucrose) including fat-soluble vitamin (vitamin A) and water-soluble vitamin (vitamin B1) were studied *in vitro* by dialysis technique using semi-permeable membrane dialysis bag and everted jejunal sac of rat. Moreover, glucose trapping property of PG was compared to that of glucomannan (GM), a commercial product of soluble polysaccharide fiber. Glucose 0-2% w/v in 0-3% PG was dialysed 0-4 hours in Ringer buffer, pH 7. Glucose inside and outside the dialyzing bag was analyzed by colorimetric method using glucose oxidase enzyme reaction. Increasing concentration, *i.e.* 2 and 3% PG resulted in increasing glucose trapped inside dialysis bag and decreasing glucose released from dialysis bag after 1, 2, 3 or 4 hours of dialysis. PG showed significant lower capability for glucose entrapment than that of GM at 1, 2 and 3% of the soluble polysaccharide fiber after 2 hours of dialysis. *In vitro* studies using everted jejunal sac of rat showed similar result of decreasing of glucose transport in the presence of increasing concentration of 2 and 3% PG after 1 hour of dialysis. Study on effect of PG on sucrose entrapment by dialysis technique using semi-permeable membrane dialysis bag and analysis of sucrose by HPLC technique showed that increasing PG concentrations resulted in decreasing sucrose released to outside of dialysis bag. Vitamin A, all-*trans* retinyl acetate, was mixed with ascorbic acid, bile salt and 0-3% PG in Ringer buffer pH 7, and dialysis in container covered with aluminum foil under nitrogen gas in dark room for 2 hours, increasing of trapped vitamin A was observed in the presence of increasing concentration of 1-3% PG. Vitamin B1, thiamin hydrochloride, was mixed with 0-3% PG in Ringer buffer pH 7, after 2 hours of dialysis, increasing of trapped vitamin B1 was obtained in dialysis bag and decreasing of vitamin B1 released in the presence of increasing concentrations of 2–3 % PG. This study suggested that sugars (glucose and sucrose), fat-soluble vitamin (vitamin A) or water-soluble vitamin (vitamin B1) can be entrapped by 2% PG. The present investigation showed that it is possible to perform the *in vitro* dialysis study using semi-permeable membrane dialysis bag as a simple method for preliminary study of nutrients retention property of soluble polysaccharide before performing the high cost *in vivo* study on polysaccharide effects with nutrients absorption.

Department…..……. Biochemistry ……. Student's signature……

Field of study ….. Biomedicinal Chemistry… Advisor's signature……..…

Academic year ……. 2003 …….

ACKNOWLEDGEMENTS

This study had been carried out in the Department of Biochemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University during the years 2001 to 2003. The author would like to express her sincere appreciation and deepest gratitude

- to her advisor, Associate Professor Dr.Sunanta Pongsamart, Department of Biochemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for her kindness and attention in giving helpful and valuable advices and also great supervision and encouragement which enable her to carry out the thesis until success,

- to Associate Professor Dr. Nijsiri Ruangrungsi, Department of Pharmacognosy, Associate Professor Krauwan Ekraksasilpchai, Department of Biochemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, and Associate Professor Dr. Sukanya Jesadanont, Department of Chemistry, Faculty of Science and Technology, Rajabhat Institute Chandrakasem, for their kindness, advice and great supervision,

- to all in the Department of Biochemistry and the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for their advice and helpful cooperation,

- to Mrs.Sunan Rangsrikansong and others in the Scientific and Technological Research and Equipment Centre (STREC) of Chulalongkorn University for their kind advices and services in using the instruments for sample analysis,

- to the Graduate School of Chulalongkorn University for giving a partial financial support.

Finally, she would like to express her infinite thanks and deepest gratitude to her family, friends and relatives for their endless love, care and understanding.

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ABBREVIATIONS

absorbance	=	A
Association of Official Analytical Chemists	=	AOAC
beta-	=	β-
centimeter	=	cm
degree of celsius (centigrade)	=	°C
exampli gratia, for example	=	*e.g.*
glucomannan	=	GM
gram	=	g
High performance liquid chromatography	=	HPLC
hour	=	hr
hydrochloric acid	=	HCl
id est, that is	=	*i.e.*
international units	=	IU
kilo- (prefix) (10^3)	=	k
litre	=	l
micro- (prefix) (10^{-6})	=	μ
milli- (prefix) (10^{-3})	=	m
millisiemen	=	mS
minute	=	min
molar (concentration)	=	M
mole	=	mol
molecular weight	=	MW
nano- (prefix) (10^{-9})	=	n
nanometer	=	nm
normal (concentration)	=	N
number	=	No.
ortho-	=	*o*-
percentage	=	%
polysaccharide gel	=	PG
Recommended Dietary Allowances	=	RDA
retinol-binding protein	=	RBP
retinol equivalents	=	RE
revolution per minute	=	rpm
second	=	sec

standard error of means	=	S.E.M.
The negative logarithm of hydrogen ion concentration	=	pH
The British Pharmacopia	=	BP
The United State Pharmacopia	=	USP
ultraviolet ray	=	UV
visible light	=	VIS
wavelength	=	λ
weight by volume	=	w/v

CHAPTER I
GENERAL BACKGROUND

1. Introduction

The structural component of edible plants which is polysaccharide complex substances associated with cellulose, called as dietary fiber, usually commercially valuable supplies for food and pharmaceutical industries (Dumitriu, 1998). Dietary fiber, which is resistant to human alimentary enzymes digestion, may be broadly divided into two classes as insoluble and soluble fiber. The insoluble fibers are cellulose mostly in secondary cell wall, lignin in middle lamella of cell wall and hemicellulose in secondary hardwood cell wall between cellulose and lignin, and soluble fiber are such as pectin in the peel of citrus fruits or pomace of apples, gum in plant seed cereal or seaweed extracts and hemicellulose in secondary softwood cell wall (Whistler and BeMiller, 1959). It has been suggested that high-soluble fiber diets may be beneficial for healthy in many ways since they increase the apparent viscosity of the contents of the stomach and small intestine (Blackburn and Johnson, 1981), delay the rate of gastric emptying (Jenkins *et al*., 1978 and Iftikhar *et al*., 1994), improve insulin sensitivity in normal (Wood *et al*., 1994) and diabetic subjects (Cameron-Smith *et al*.,1997) and reduce all anthropometric parameter, such as fasting concentrations of serum total lipids, total cholesterol (Wolk *et al*., 1999 and Gallaher *et al*., 2000) and blood glucose concentration (Jenkins *et al*., 1977, Huang *et al*., 1990, Lu *et al*., 2000, and Behall and Hallfrisch, 2002). All of those previous studies have positively supported these suggestions; dietary fiber consumption could decrease the risk factor of coronary heart disease (Vuksan *et al*., 1999 and Wolk *et al*., 1999), cardiovascular disease (Dodson *et al*., 1981) and help glycemic controlling in patients with diabetes (Jenkins *et al*., 1976). Dietary fiber has emerged as a major dietary component in the management of diabetes. The American Diabetes Association (ADA) (American Diabetes Association, 1994) and the American Dietetic Association (Marlett and Slavin, 1997) currently recommend 20 to 35 grams of dietary fiber per day and also recommended in Thai recommended daily intakes by Food and Drug Administration (คณะอนุกรรมการพิจารณาการแสดงคุณค่าทางโภชนาการบนฉลากของอาหาร, 2538).

Therefore, searching for sources of soluble fiber, a soluble heteropolysaccharide product from plant waste such as durian fruit-hulls of *Durio zibethinus* L. in family Bombacaceae is an interesting subject. Durian fruit-hulls are big pericarp of fruit and waste product because they are favorite fruit of many Thai and Chinese people. Early studies have

concluded that polysaccharide gel (PG) extracted from fruit-hulls of durian composed of sugars; glucose, rhamnose, arabinose, fructose and galacturonic acid; in a large complex of polysaccharide (apparent molecular weight 500 to 1,400 k Dalton) (Pongsamart and Panmaung, 1998, and Pongsamart *et al*., 2001), and PG showed swelling property and forming a viscous layer in water as a soluble polysaccharides. Toxicity tests of PG have revealed that no toxic effects were observed in subchronic toxicity test, an oral dose of 0.5 g/kg body weight/day for 60 days and 100 days in male and female mice, respectively (Pongsamart, Tawatsin, and Sukrong, 2002). In addition, Pongsamart, Sukrong, and Tawatsin (2001) reported that PG did not induce severe toxicity in male mice and rats after feeding a high oral single dose (2 g/kg body weight/ day) in acute toxicity test. PG is resistant to enzyme alpha amylase hydrolysis and dilute hydrochloric acid hydrolysis at 37 °C for 30 minutes and 4 hours, respectively (Pongsamart *et al*., 2001). Tippayakul *et al*. (2001), Tippayakul, Piyasirananda and Pongsamart (2002) and Tippayakul *et al*. (2002) proposed that *in vitro* studies on PG trapping property of lipids, cholesterol, oleic acid and stearic acid indicated that increasing concentration of PG resulted in increasing lipids; cholesterol, oleic acid and stearic acid, trapping ability of PG. Moreover, the results showed that no differences on lipid inclusion between PG and mixture of PG and polysaccharide fiber extracted from fruit-hulls of durian (PF).

Several studies indicated that some types of soluble polysaccharide fiber are beneficially using in medical dietary foods. Walsh, Yaghoubian and Behforooz (1984) and Arvill and Bodin (1995) reported that soluble fiber konjac glucomannan intaked in obese patients and healthy subjects, respectively, reduced total cholesterol concentrations, low-density-lipoprotein cholesterol (LDL-C) concentrations and triglycerides, whereas, high-density-lipoprotein cholesterol (HDL-C) concentrations and the ratio of LDL-C to HDL-C did not change significantly while there is a significant mean weight loss. Other water soluble fibers, such as psyllium seed husk (Abraham and Mehta, 1988 and Vergara-Jimenez *et al*., 1998), oat gum (Hong *et al*., 1998), pectin (Terpstra *et al*., 1998 and Vergara-Jimenez *et al*., 1998), chitosan (Gallaher, 2000) and xanthan gum (Levrat-Verny *et al*., 2000) were also shown the property of these benefits as well. Jenkins *et al*., (1980) and Unsitupa *et al*., (1990) have also revealed that guar gum decreased the serum lipids of patients with type 2 diabetes. Early studies (Kiehm, Anderson and Ward, 1976, Behall and Hallfrisch, 2002) proposed that soluble fibers affected glycaemic control. When added soluble fibers (guar gum, arabinoxylan, pectin, oat gum, psyllium, gum tragacanth, alginate or glucomannan) to glucose test meals, compared with controls, they significantly reduced postprandial glycaemia in healthy volunteers (Jenkins *et al*., 1977, Jenkins *et al*., 1978, Lu *et al*., 2000), in obese patients (Magnati *et al*., 1984 and Vita *et al*., 1992) and in both type diabetic patients (Miranda and Horwitz, 1978, Dodson *et al*., 1981, Anderson *et al*., 1991, Ohta *et al*., 1997

and Vuksan *et al*., 2000), as insulin requirement reduction was observed in those healthy volunteers (Wood *et al*., 1994 and Lu *et al*., 2000) and diabetic patients (Anderson and Bryant, 1986 and Anderson *et al*., 1991). Chandalia *et al*. (2000) concluded that high dietary fiber intaked, practically of the soluble type, more than recommended by the ADA, improves glycaemic control, decreases hyperinsulinemia, and lowers plasma lipid concentration. Therefore soluble polysaccharide fiber is an effective cholesterol and glucose-lowering dietary adjunct.

Diabetes mellitus (commonly called diabetes) is a disease of carbohydrate metabolism caused by either insufficient insulin production or decreased sensitivity of cells to insulin. People with diabetes cannot properly process glucose. The consequences of glucose stays in the blood and elevation of blood glucose are observed but body cells can starve for glucose. Diabetes is not a single disease but rather a syndrome of hyperglycaemia accompanied by varying degree of ketosis and acidosis. The long-term duration of diabetes, and the long-term blood glucose poor control are leading to the complications occurred. Diabetes can lead to poor wound healing, higher risk of infections, and many other involving organs; the eyes (retinopathy), kidneys (nephropathy), nerves (neuropathy), blood vessels (vascular permeability abnormalities) and heart (heart disease) (Rifkin and Porte, 1991, Pickup and Williams, 1994 and Shils *et. al*, 1999). As Basu *et. al*, (1989) found that serum concentrations of vitamin A and retinol-binding protein (RBP) have been found to be decreased in diabetic patients. Both human and animal studies point to the possibility of the vitamin A transport mechanism being defective in diabetes. Furthermore, patients with type 2 diabetes have been found to have reduced plasma levels of retinol while the retinyl esters levels are elevated (Wako *et al*., 1986). For these reasons, serum concentrations of vitamin A reduction may be the cause of retinopathy. Unfortunately, some reports documented that large amounts of some dietary fibers may reduce the absorption of fat soluble vitamins, such as vitamin A and vitamin E because of their lipid entrapment (Doi *et al*., 1983, Schaus *et al*., 1985, Erdman, Fahey and White, 1986, Riedl *et al*., 1999 and Deming *et al*., 2000) but some reports show that there is no effect of dietary fiber on vitamin A entrapment (Kasper *et al*., 1979). About the water-soluble vitamin such as vitamin B1 which is also important for regulated body function and vitamin B1 deficiency affects the nervous systems function, however, the vitamin B1 entrapment in any dietary fiber has never been reported yet.

Therefore, it seems clear that PG is very interesting in its lipid trapping properties (Tippayakul *et al*., 2002) and should be thoroughly investigated for its more information and efficient use. However, sugar and vitamins absorption issues have never been examined in PG. This study was designed to investigate the sugars (glucose and sucrose), fat-soluble vitamin (vitamin A) and water-soluble vitamin (vitamin B1) entrapment to evaluate illustrating its beneficial use as medical dietary foods properly.

2. Literature review

2.1 **Carbohydrate** (Plummer, 1971, Linder, 1991, Smolin and Grosvenor, 1997, Holme and Peck, 1998 and Garret and Grisham, 1999)

Carbohydrate is the single most abundant class of organic molecules found in nature and the most important component in many kinds of food. Energy from the sun captured by green plants, algae, and some bacteria during photosynthesis is stored in the form of carbohydrate. Although not as concentrated fuel as fat, sugar is the principle source of energy for many cells. Carbohydrate, especially starche, provides the bulk of the calories (4 kilocalories per gram or 17 kilojoules per gram) in most diets. The dietary guidelines of the National Academy of Sciences' 1968 Recommended Dietary Allowances (US RDAs) (Table 1 and Figure 1) and the Thai recommended daily intakes (Thai RDI) by Food and Drug Administration recommended that total carbohydrate should be taken about 60 percent of total energy requirement (Table 2).

Carbohydrate may be present as an isolated molecule or may be physically associated or chemically bound to other molecules. It also comes in either digestible or indigestible form. The general molecular formula of carbohydrate is $(CH_2O)_n$, where n = 3 or more, and was once thought to represent "hydrated carbon". All sugars are highly soluble in water because of their many hydroxyl groups. In addition, they can covalently linked with a variety of other molecules. Molecules in which the carbohydrates are covalently attached to lipids are known as *glycolipids*, are common components of biological membranes, whereas those in which the carbohydrates are covalently attached to proteins are known as *glycoproteins*. These two classes of biomolecules, together called *glycoconjugates*, are important components of cell walls and extracellular structures in plants, animals and bacteria. Each molecule of carbohydrate can be classified as monosaccharide, oligosaccharide or polysaccharide according to the number of monomers. Some carbohydrates are digestible by humans and therefore provide an important source of energy, whereas others are indigestible and therefore do not provide energy. Indigestible carbohydrates form part of a group of substances known as *dietary fiber*.

The components of dietary carbohydrates are shown in Figure 2, and the types, sources, structure and properties are shown in Table 3.

Table 1 US Daily Reference Value (Smolin and Grosvenor, 1997)

Food Component	**Daily Reference Value (2,000 kilocalories)**
Total fat	Less than 65 grams (30% of energy)
Saturated fat	Less than 20 grams
Cholesterol	Less than 300 milligrams
Total carbohydrate	300 grams (60% of energy)
Dietary fiber	25 grams (11.5 grams per 1,000 kilocalories)
Sodium	Less than 2,400 milligrams
Potassium	3,500 milligrams
Protein	50 grams (10% of energy)

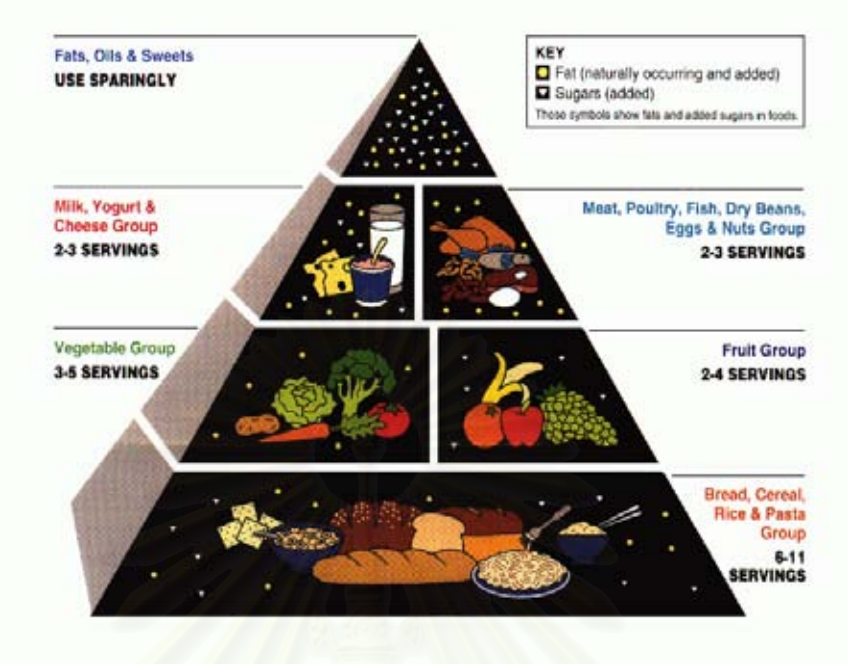


Figure 1 USDA, 1992. Food Guide Pyramid. (Smolin and Grosvenor, 1997)

Table 2 Thai recommended daily intakes (Thai RDI)

(คณะอนุกรรมการพิจารณาการแสดงคุณค่าทางโภชนาการบนฉลากของอาหาร, 2538).

ลำดับที่ (No.)	สารอาหาร (Nutrient)	ปริมาณที่แนะนำต่อวัน (Thai RDI)	หน่วย (Unit)
1.	ไขมันทั้งหมด (Total Fat)	65*	กรัม (g)
2.	ไขมันอิ่มตัว (Saturated Fat)	20*	กรัม (g)
3.	โคเลสเตอรอล (Cholesterol)	300	มิลลิกรัม (mg)
4.	โปรตีน (Protein)	50*	กรัม (g)
5.	คาร์โบไฮเดรตทั้งหมด (Total Carbohydrate)	300*	กรัม (g)
6.	ใยอาหาร (Dietary Fiber)	25	กรัม (g)
7.	วิตามินเอ (Vitamin A)	800 (2,664)	ไมโครกรัม อาร์ อี (μg RE) หน่วยสากล (IU)
8.	วิตามินบี **1** (Thiamin)	1.5	มิลลิกรัม (mg)
9.	วิตามินบี **2** (Riboflavin)	1.7	มิลลิกรัม (mg)
10.	ไนอะซิน (Niacin)	20	มิลลิกรัม เอ็น อี (mg NE)
11.	วิตามินบี **6** (Vitamin B6)	2	มิลลิกรัม (mg)
12.	โฟลิค แอซิด (Folic Acid)	200	ไมโครกรัม (μg)
13.	ไบโอติน (Biotin)	150	ไมโครกรัม (μg)
14.	แพนโทธินิค แอซิด (Pantothenic Acid)	6	มิลลิกรัม (mg)
15.	วิตามินบี **12** (Vitamin B12)	2	ไมโครกรัม (μg)
16.	วิตามินซี (Vitamin C)	60	มิลลิกรัม (mg)
17.	วิตามินดี (Vitamin D)	5 (200)	ไมโครกรัม (μg) หน่วยสากล (IU)
18.	วิตามินอี (Vitamin E)	10	มิลลิกรัม แอลฟา - ที อี (mgα - TE)
19.	วิตามินเค (Vitamin K)	80	ไมโครกรัม (μg)
20.	แคลเซียม (Calcium)	800	มิลลิกรัม (mg)
21.	ฟอสฟอรัส (Phosphorus)	800	มิลลิกรัม (mg)
22.	เหล็ก (Iron)	15	มิลลิกรัม (mg)
23.	ไอโอดีน (Iodine)	150	ไมโครกรัม (μg)
24.	แมกนีเซียม (Magnesium)	350	มิลลิกรัม (mg)
25.	สังกะสี (Zinc)	15	มิลลิกรัม (mg)
26.	ทองแดง (Copper)	2	มิลลิกรัม (mg)
27.	โปตัสเซียม (Potassium)	3,500	มิลลิกรัม (mg)
28.	โซเดียม (Sodium)	2,400	มิลลิกรัม (mg)
29.	แมงกานีส (Manganese)	3.5	มิลลิกรัม (mg)
30.	ซีลีเนียม (Selenium)	70	ไมโครกรัม (μg)
31.	ฟลูออไรด์ (Fluoride)	2	มิลลิกรัม (mg)
32.	โมลิบดีนัม (Molybdenum)	160	ไมโครกรัม (μg)
33.	โครเมียม (Chromium)	130	ไมโครกรัม (μg)
34.	คลอไรด์ (Chloride)	3,400	มิลลิกรัม (mg)

***** ปริมาณของไขมันทั้งหมด ไขมันอิ่มตัว โปรตีน และคาร์โบไฮเดรตที่แนะนำให้บริโภคต่อวัน คิดเป็นร้อยละ **30, 10, 10,** และ **60** ตามลำดับ ของพลังงานทั้งหมดที่ได้รับต่อวัน **(2,000** กิโลแคลอรี**) (**ไขมัน **1** กรัม ให้ **9** กิโลแคลอรี**,** โปรตีน **1** กรัม ให้ **4** กิโลแคลอรี**,** คาร์โบไฮเดรต **1** กรัม ให้ **4** กิโลแคลอรี**)**

หมายเหตุ **1.** สำหรับน้ำตาลไม่ควรบริโภคเกินร้อยละ **10** ของพลังงานทั้งหมดที่ได้รับต่อวัน

2. คำอธิบายหน่วยของวิตามิน เอ ไนอะซิน วิตามินอี และวิตามินดี

2.1 RE = Retinol equivalent (1 RE = 1 μg retinal = 6 μg β-carotene)

2.2 NE = Niacin equivalent (1 NE = 1 mg niacin = 60 μg tryptophan จากอาหาร

2.3 α - TE = α - Tocopherol equivalent (1 α - TE = 1 mg D - α - tocopherol)

2.4 วิตามินดีมีหน่วยเป็นไมโครกรัม โดยคำนวณเป็น cholecalciferol

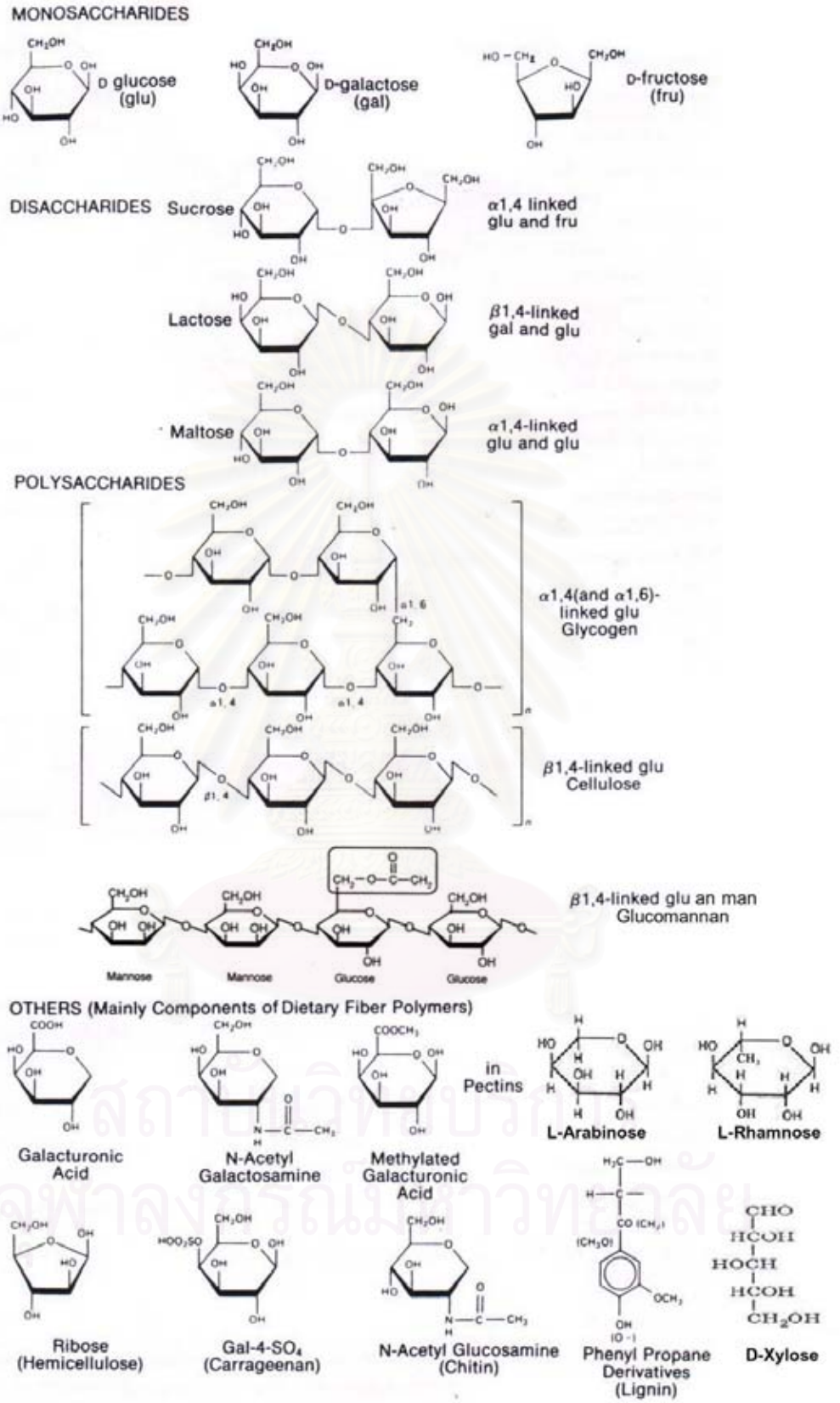


Figure 2 Components of dietary carbohydrates modified from Linder, 1991.

Table 3 Dietary carbohydrates: type, sources, structure, and properties (Linder, 1991).

Carbohydrate	Sources	Structure and properties
D-Glucose (dextrose)	Fruit; traces in most plant foods; honey; maple sugar	Water-soluble monosaccharide [molecular weight (MW) 180]
D-Fructose	Fruit; traces in most plant foods; honey; maple sugar	Water-soluble monosaccharide (MW 180)
D-Galactose	Component of lactose; produced during digestion	Water-soluble monosaccharide (MW 180)
Sucrose	Cane sugar; beet sugar; fruits; maple sugar	Water-soluble disaccharide (MW 360) (α1,4-linked glu-fru)
Lactose	Milk; dairy products (milk sugar)	Water-soluble disaccharide (MW 360) (β1,4-linked gal-glu)
Maltose	Sprouted grain; produced during digestion of starches	Water-soluble disaccharide (MW 360) (α1,4-linked glu-glu)
Amylose	Starchy plants; grains (starch)	Linear polymer of glucose (α1,4); water-soluble; MW 10^5-10^6
Amylopectin (starch)	Starchy plants; grains; used as thickener in processed foods	Branched polymer of glucose (α1,4 and α1,6); water-soluble; MW 10^7-10^8
Glycogen (animal starch)	Liver; muscle	Branched polymer of glucose (α1,4 and α1,6); water-soluble; MW 10^7-10^8
Cellulose[a]	Substituent of plant cell walls; major component of wheat bran	Linear polymer of glucose (β1,4); not water-soluble; MW 10^5-10^6
Hemicellulose[a]	Substituent of plant cell walls	Polymer of hexoses or pentoses, often branched; MW 10^4; not water-soluble
Pectins[a]	Fruits	Water-soluble linear polymers (β1,4) of galacturonic acids and/or modified galacturonic acid; gel forming; bile acid binding; MW 10^4-10^5
Carrageenan[a]	Red seaweed; used in candies and some processed foods	Linear polymers (β1,4) of disaccharide; units with gal-4-SO_4 and 3,6-anhydro-gal-2-SO_4 (linked β1,3); gel forming; water-soluble; MW 10^4
Inulins[a]	Jerusalem artichoke	Water-soluble fructose polymer; (β2,1)
Raffinose, stachyose, verbacose[a]	Pulses (plant "antifreeze")	Trimer of glucose, fructose, and galactose (β1,2;α1,6) plus/minus additional gal units (α1,6)
Dextrins	Used in processed foods	Short pieces of α1,4-glucose polymer
Invert sugar	Used in processed foods	Hydrolyzed sucrose (fru:glu = 1:1)
Corn syrup	Used in processed foods	Hydrolyzed starch (= glucose)
High fructose corn syrup	Used in processed foods	Starch hydrolyzed and partly isomerized (glu + fru)
Lignin[a]	Substituent of plant cell walls	Highly branched polymer of substituted phenylpropanes (not a carbohydrate); binds bile acids; not water-soluble; MW 1-5 x 10^3

Source: Anderson (1981); Gray and Fogel (1987); MacDonald (1988); Metzler (1977); White et al. (1973).
[a] Substituents of dietary fiber.

Carbohydrates are classified as followings (Oser, 1965):

I. Monosaccharides or simple sugars;

	Aldoses	Ketoses
a. trioses ($C_3H_6O_3$)	glyceraldehyde (glycerose)	dihydroxyacetone
b. tetroses ($C_4H_8O_4$)	erythorse, threose	erythrulose
c. pentoses ($C_5H_{10}O_5$)	xylose, ribose, arabinose, lyxose	xylulose, ribulose
d. hexoses ($C_6H_{12}O_6$)	glucose, galactose, mannose	fructose (levulose), psicose, sorbose
e. heptoses ($C_7H_{14}O_7$)		sedoheptulose

II. Oligosaccharides
   a. reducing disaccharides ($C_{12}H_{22}O_{11}$); maltose, lactose
   b. nonreducing disaccharides; sucrose
   c. trisaccharides; raffinose, gentianose, melezitose

III. Polysaccharides
   a. homopolysaccharides;
      i. starch, dextrins, glycogen, cellulose (glucose)
      ii. inulin (fructose)
      iii. chitin (glucosamine, acetic acid)
   b. heteropolysaccharides; mucopolysaccharides
   c. hyaluronic acid (glucosamine, glucuronic acid, acetic acid)
      i. chondroitin sulfate A (galactosamine, glucuronic acid, acetic acid, sulfuric acid)
      ii. chondroitin sulfate B (or β-heparin) (galactosamine, iduronic acid, acetic acid, sulfuric acid)
      iii. α-heparin (glucosamine, glucuronic acid, sulfuric acid)
      iv. blood group substances (glucosamine and/or galactosamine, galactose, fucose, sialic acid, acetic acid)
      v. blood serum mucoids (galactose, mannose, glucosamine, galactosamine, fucose, sialic acid, acetic acid)

IV. Derived carbohydrates
   a. oxidation products (ascorbic acid, uronic acid, aldonic acid, saccharic acid)
   b. reducing products (glycerol, ribitol, inositol)
   c. amino sugars (glucosamine, galactosamine, mannosamine)
   d. deoxysugars (2-deoxyribose, methyl pentose)

Monosaccharides

Monosaccharides are the simplest carbohydrates and the monomeric constituents of all complex carbohydrates. They are water-soluble crystalline compounds. They are aldehyde or ketone derivatives of straight-chain poly-hydroxy alcohols with at least three carbon atoms. The carbon atoms are numbered from the end of the chain containing the reactive carbonyl group. The simplest aldose, glyceraldehyde, has one asymmetric carbon atom or chiral center (carbon 2), and therefore only two possible arrangements of the OH and H of the CH_2OH unit are possible. If the OH group is shown as projecting to the left on the penultimate carbon atom in the straight chain representation of the molecule, is called the L- isomer, while if shown as projecting to the right, is called the D- isomer. These two stereoisomers of the same carbohydrates are enantiomers or mirror images of one another. Most natural monosaccharides have five (pentoses) or six (hexoses) carbon atoms. Commonly occurring

hexoses in foods are glucose (aldose), fructose (ketose) and galactose (aldose), which commonly occurring pentoses are arabinose (aldose) and xylose (aldose). Monosaccharides can cyclize to five- or six-atom rings with four or five carbon atoms and one oxygen atom to form ring. The cyclization is based on the reaction of the hydroxyl group with the carbonyl group intramolecularly to form cyclic hemiacetals and hemiketals. Five ring monosaccharides are called *furanoses* while the six ring monosaccharides are known as *pyranoses*. The chemical character of monosaccharides mostly happens on hydroxyl and carbonyl groups. Their most important reaction is the formation of glycosidic bonds. The anomeric hydroxyl group at anomeric carbon (C-1) of sugar reversibly condenses with alcohols to form α- and β-glycosides. If the anomeric OH group is shown as pointing down, is called the α-form of anomer, while if shown as poining up, is called the β-form of anomer.

Glucose (dextrose) is about 70-80% as sweet as sucrose. Since glucose is a reducing sugar, contains a carbonyl function at the C-1 position, it readily undergoes the Maillard or browning reaction with amino acids. It is not only a major constituent of di-, oligo- and polysaccharides but also is an important molecule for the regulation of the metabolism, called "blood sugar", the immediate source of energy for cellular respiration. **Fructose** is 140% sweeter than sucrose. As a keto-hexose, fructose is very reactive to amino acids in the Maillard reaction. It can be changed to glucose in the liver and intestine. **Galactose** can be changed to glucose in the liver and metabolized, and synthesized in the mammary gland to make the lactose of milk.

Oligosaccharides

Oligosaccharides are relatively low molecular weight polymers of monosaccharides that are covalently bonded as glycosidic linkages. Two monosaccharides can be linked together to form a "double" sugar or **disaccharide**, whereas trisaccharide consists of three. There are three disaccharides, which are all commonly founded in nature. They are **sucrose**, α(1→2)-linkage of glucose and fructose, **maltose,** α(1→4) -linkage of glucose and glucose, and **lactose**, the most common, a β(1→4) -linkage of glucose and galactose. Although the process of linking the two monomers is rather complex, the end result in each case is the loss of a hydrogen atom (H) from one of the monosaccharides and a hydroxyl group (OH) from the other. Thus the molecular formula of each of these disaccharides is $C_{12}H_{22}O_{11}$ (or 2 $C_6H_{12}O_6$ - H_2O). Each disaccharide is a mixed acetal, one hydroxyl group provided intramolecularly with one hydroxyl from the other monosaccharides. Except sucrose, each of these structures possesses one free unsubstituted anomeric carbon atom. Each disaccharide is a reducing sugar. The end of the molecule containing the free anomeric carbon is called the reducing end, and the other end is called the nonreducing end. In case of sucrose, both of the

anomeric carbon atoms are substituted, that is neither has a free –OH group. The substituted anomeric carbons cannot be converted to the aldehyde configuration and cannot participate in the oxidation-reduction reaction characteristic of reducing sugars. Thus, sucrose is not a reducing sugar.

Polysaccharides

Chemically, IUPAC (International Union of Pure and Applied Chemistry) defined polysaccharides as carbohydrates with more than ten monomeric units, whereas the British Nation Foundation report used 20 residues as the limit (Asp, 1994). If a polysaccharide contains only one kind of monosaccharides molecule, it is a homopolysaccharide, or homoglycan, whereas those containing more than one kind are heteropolysaccharides. They include not only those substances composed of glycosidically linked sugar residues but also molecules that containing polymeric saccharide structures linked via covalent bonds to amino acids, peptides, proteins, lipids, and other structures. The most common constituent of polysaccharides is D-glucose, but D-fructose, D-galactose, L-galactose, D-mannose, L-arabinose, and D-xylose are also common. Common monosaccharides derivatives in polysaccharides include the amino sugars (D-glucosamine and D-galactosamine) or their derivatives (*N*-acetylneuraminic acid and *N*-acetylmuramic acid).

Plants and animals store carbohydrates in polysaccharides form. It seemed likely that organisms store carbohydrates in the form of polysaccharides rather than as monosaccharides to lower the osmotic pressure of the sugar reserves. Because the osmotic pressure depends only on numbers of molecules, it is greatly reduced by the formation of a few polysaccharide molecules out of thousands (or even millions) of monosaccharide units. The most common polysaccharide storage in plants is **starch**. Animals store excess glucose by polymerizing it to form **glycogen**. Another important polysaccharide storage is the **dextran**, which found in yeast and bacteria. **Cellulose** and **chitin** are types of polysaccharides, which are structural components. Starch and cellulose are linear homopolymer of D-glucose units.

Starches are existed in two forms of **α-amylose** and **amylopectin**. In most starches, amylopectin comprises 70 to 80% of total starch, and α-amylose comprises the remaining 20 to 30% (Cummings and Englyst, 1995). α-Amylose, a small one (about 10^5 MW), consists of linear, unbranched chains of several hundred D-glucose residues, which are linked between their atom 1 and atom 4 of carbon atoms with α- glycosidic linkage. Amylopectin, a larger one (10^6 MW and larger), contains both α-(1,4)–linked glucose and α-(1,6) linkages, resulting in a branched structure of several thousand D-glucose residues. At approximately every thirtieth residue along the chain, a short side chain is attached to the atom 6 of carbon atoms

(the carbon above the ring). Both are insoluble in cold water. Starches are insoluble in water and; thus, can serve as storage depots of glucose.

Glycogen is found mainly in the liver (where it may amount to as much as 10% of liver mass) and skeletal muscle (where it may amount for 1 to 2% of muscle mass). The structure of glycogen is similar to that of amylopectin, although the branches in glycogen tend to be shorter and more frequent, with α-(1→6) branch every 8 to 12 glucose units. Glycogen is broken back down into glucose when energy is needed (a process called glycogenolysis).

Dextran is α-(1→6) linked, the repeating unit is isomaltose. The branch points may be 1→2, 1→3 or 1→4 in various species. The degree of branching and the average chain length between branches depend on the species and strain of the organism.

Like starch, cellulose is α-amylose but the glucose units are linked by β-(1→4)-glycosidic bonds. Because of the orientation of the bonds linking the glucose residues, the rings of glucose are arranged in a flip-flop manner. This produces a long, rigid molecule. There is no side chain in cellulose as there are in starch. The absence of side chain allows these linear molecules to lie close together. Because of the many -OH groups, as well as the oxygen atom in the ring, there are many opportunities for hydrogen bonds to form between adjacent chains. The result is a series of stiff, elongated fibrils: the perfect material for building the cell walls of plants. Therefore, cellulose is one of the principal components providing physical structure and strength of green trees. Since intestinal enzymes cannot hydrolyze β-linkages, cellulose is extremely resistant to hydrolysis, whether by acid or by the digestive tract α-amylases. As a result, most animals cannot digest cellulose to any significant degree. Ruminant animals, such as cattle, deer, giraffes, and camels, are excluded because bacteria that lives in the rumen secretes the enzyme cellulase, a β-glucosidase effective in the hydrolysis of cellulose.

The structure of chitin is similar to cellulose but the –OH group on each C-2 position is replaced by $–NHCOCH_3$, so that the repeating units are *N*-acetyl-D-glucosamines in β-(1→4) linkage. It is present in the cell walls of fungi and is the fundamental material in the exoskeletons of arthropods, crustaceans, insects, and spiders.

2.1.1 Digestion and absorption of carbohydrates

An overview of carbohydrate digestion and absorption was shown in Figure 3. The digestion of dietary carbohydrates starts in the mouth, where salivary α-amylase initiates cleave interior α-1,4 linkages in starch. The starch fragments thus forms including maltose, some glucose and dextrins containing the 1,6-α-glycosidic branching points of amylopectin.

Amylase action continues until food is acidified in the stomach. The α-amylase degradation of starch is completed by the pancreatic amylase active in the small intestine.

Dietary disaccharides need to be broken down to monosaccharides in order to be absorbed. This final hydrolysis is accomplished by hydrolases attached to the intestinal brush-border membrane, referred to as "disaccharidases". Sucrase and isomaltase are synthesized as a single protein. Following insertion into the brush border plasma membrane, the protein is cleaved in its luminal portion into two polypeptides; one with sucrase and another one with isomaltase activity. Sucrase splits sucrose into glucose and fructose. Glucoamylase cleaves terminal α-1,4 linkages. Isomaltase (or α-dextrinase) debranches α-limit dextrins by cleaving α-1,6 linkages at the branch points. Lactase splits lactose into glucose and galactose.

Normally most dietary carbohydrates are absorbed in small intestine. Duodenum and proximal jejunum have highest capacity. Only glucose, galactose, and fructose are taken up by enterocytes. Glucose and galactose are transported actively against a concentration gradient from intestinal lumen across the apical surface of the epithelial cells by a sodium-dependent glucose transporter (SGLT 1) located in the microvillar membranes. The proposed model for operation of glucose transportation was shown in Figure 4. Sodium ion (Na^+) enhances glucose transport, and vice versa. The transporter binds two Na^+ for each sugar. Fructose undergoes facilitated transport across brush border membrane by another glucose transporter (GLUT 5) spacial for fructose. Glucose, galactose, and fructose leave the cell at basolateral membrane via a common facilitated transporter, GLUT2. When delivered to the circulation, the absorbed carbohydrates cause an elevation of the blood glucose concentration. Fructose and galactose have to be converted to glucose mainly in the liver and therefore produce less pronounced blood glucose elevation. The extent and duration of the blood glucose rising after meal is dependent upon the rate of absorption, which in turn depends upon factors such as gastric emptying as well as the rate of hydrolysis and diffusion of hydrolysis products in the small intestine.

Insulin, a hormone made in the pancreas, is secreted as a response to blood glucose elevation but is modified by many neural and endocrine stimuli (Figure 5). Insulin secretion is also influenced by food related factors, especially by the amount of food and the amino acid composition of dietary proteins. Insulin has important functions in regulating carbohydrate and lipid metabolism and also glucose uptaking most body cells.

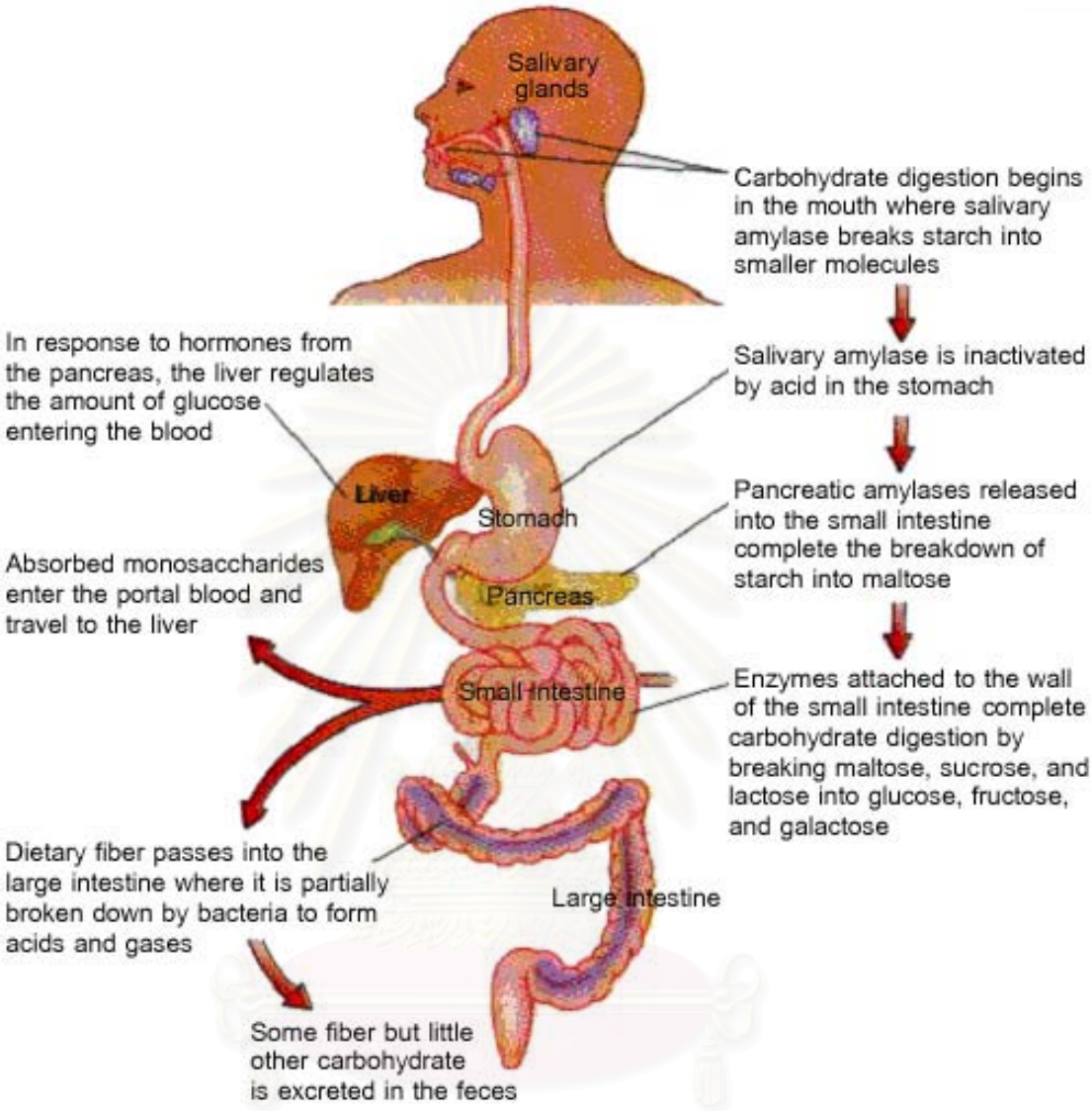
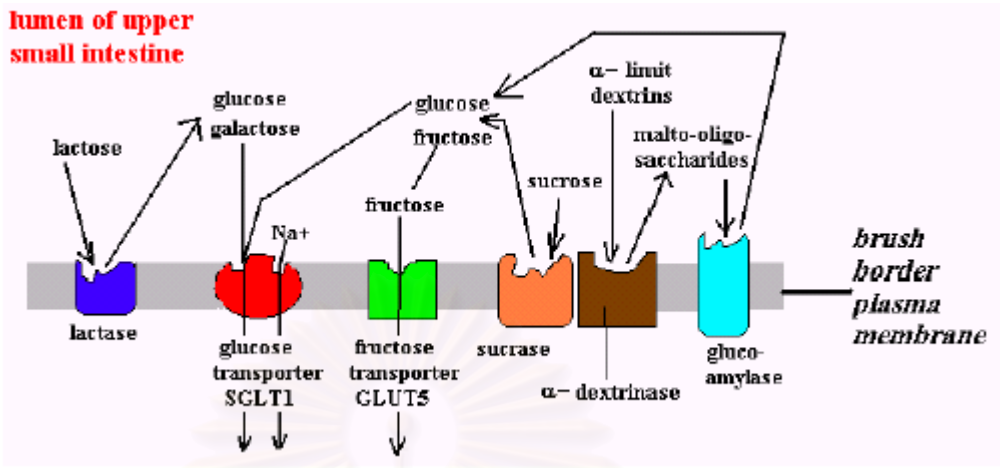


Figure 3 An overview of carbohydrate digestion and absorption.

(Smolin and Grosvenor, 1997)

(A)



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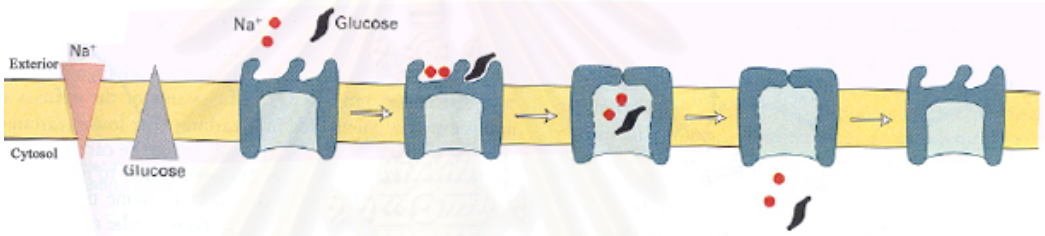

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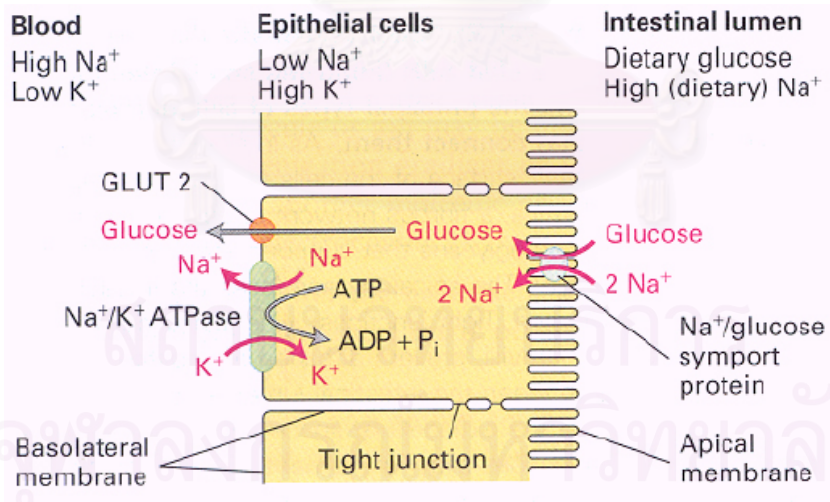


Figure 4 (A) Digestion and absorption of carbohydrates. (B) Operation of the two-Na^+ / one-glucose symporter. (D) Transport of glucose from the intestinal lumen into the blood. (Lodish *et al.*, 2000)

2.1.2 Glucose determination (Oser, 1965, Bauer, Ackerman and Toro, 1974, Passey *et al*., 1977 and Chaplin and Kennedy, 1994)

Many of methods available for measurement of glucose were based on their chemical reactivities and involved the addition of a particular reagent with the subsequent formation of a colored product. Strip test, the most common method for determining glucose by automated procedures, is glucose-specific enzymatic method. It used paper test strips coating with glucose oxidase enzyme. It is designed primarily for blood glucose monitoring during treatment of diabetes (Fajans, 1991). *O*-toluidine method determined the quantity of glucose by using a solution of *o*-toluidine in glacial acetic acid when heated, then the glucose exchanged into a colored product which maximum absorbed at 630 nm. The aldehyde group of glucose apparently condenses with the reagent to form a glucosylamine and a Schiff base, which is the colored product. This method does not react specifically for glucose, while other aldohexoses such as mannose and galactose do. Other aromatic amines, such as *p*-aminosalicylic acid, *p*-aminobenzoic acid, diphenylamine and *p*-aminophenol have been used for this method but *o*-toluidine appears to be the most satisfactory one (Hyvarinen and Nikkila, 1962, Bauer, Ackerman and Toro, 1974 and Holme and Peck, 1998). This reaction was shown in Figure 6.

Glucose oxidase method is highly specific for detection of glucose and, if suitably modified, can be used for sensitive quantitative determinations of glucose in blood, urine, and other biological fluids. It is based on the fact that the enzyme, glucose oxidase (β-D-glucose: oxygen 1-oxidoreductase; EC 1.1.3.4), oxidizes glucose to gluconic acid in presence of air, simultaneously producing hydrogen peroxide (H_2O_2, reduced oxygen). Moreover, in the presence of reagent a chromogenic hydrogen donor; such as *o*-dianisidine, *o*-tolidine, pyrogallol, 4–aminoantipyrine; and the highly specific, sensitive and very stable horseradish peroxidase (donor: hydrogen-peroxide oxidoreductase; EC 1.11.1.7) they bring about the oxidation of the chromogen to a colored product such as oxidized *o*-dianisidine in pink color in strongly acid solution and can detect by spectrophotometry. The reaction of glucose oxidase method is as followings:

$$\text{glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{chromogen} \xrightarrow{\text{peroxidase}} \text{chromogen}$$

(colorless, oxidizable substrate) (colored, oxidized product)

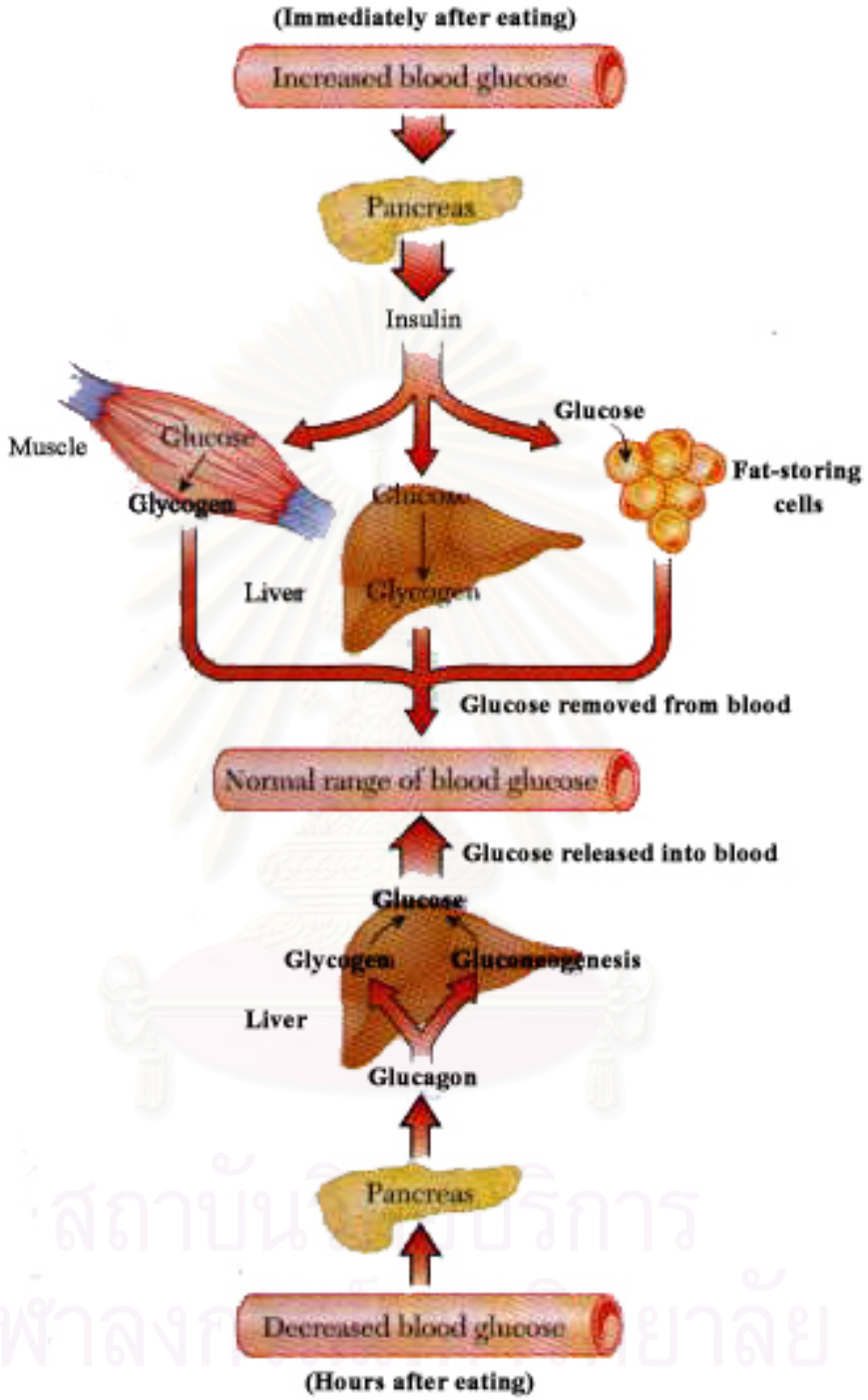


Figure 5 Blood glucose is regulated by hormones secreted by the pancreas.

(Smolin and Grosvenor, 1997)

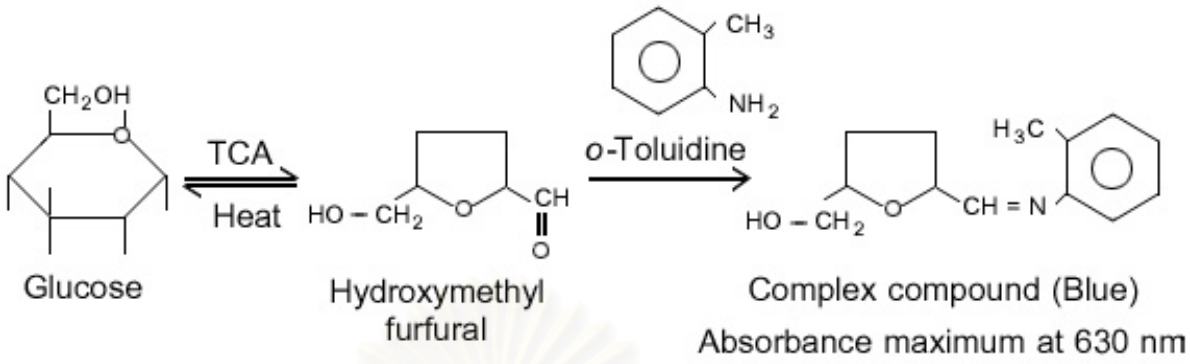


Figure 6 Glucose colorimetric reaction with *o*-toluidine reagent.

Another common method is quantitation of glucose by using high pressure liquid performance (HPLC). HPLC is an ion-exchange technique which has been used with ultraviolet or refractive index detectors and anion- or cation-exchange resins have been used.

2.2 Dietary fiber

Prosky (2001) reported that the joint meeting of the International Life Sciences Institute (ILSI), and the annual meeting of the IFT and the American Association of Cereal Chemists (AACC) revised definition for dietary fiber. The definition, approved by the AACC Board of Directors on June 12, 2000 was "Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation". The constituents of dietary fiber were classified by AACC as:

1) **non-starch polysaccharides and resistant oligosaccharides**
   cellulose, hemicellulose (arabinoxylans, arabinogalactans), polyfructoses (inulin, oligofructans), galactooligosaccharides, gums, mucilages, pectins
2) **analogous carbohydrates**
   indigestible dextrins (Typically produced by acid or thermal treatments of starch hydrolysates, rendering the hydrolysate or portions thereof indigestible.)
   - resistant Maltodextrins (from corn and other sources)
   - resistant Potato Dextrins

   synthesized carbohydrate compounds
   - polydextrose
   - methyl cellulose
   - hydroxypropylmethyl cellulose

   indigestible (“resistant”) starches (Includes only those starches that are resistant to digestion in humans and resistant to digestion in properly designed analytical methods that include gelatinization steps to simulate cooking and processing.)
3) **lignin**
4) **substances associated with the non-starch polysaccharide and lignin complex in plants**
   waxes, phytate, cutin, saponins, suberin and tannins

Various low molecular carbohydrates are not digested by human digestive enzymes (sugar alcohols such as sorbitol and mannitol, polydextroses, and fructooligosaccharides). The

US Food and Drug Administration stated that because these materials do not analyze as dietary fiber in the official Association of Official Analytical Chemists methods they are not to be reported on nutrition labels as dietary fiber, but as "other carbohydrate" (Marlett and Slavin, 1997).

Dietary fiber can be widely divided by its water solubility into two categories; soluble fiber and insoluble fiber (Trowell *et al*., 1976). Soluble fiber is the fraction of the total fiber that is suspended in water during analysis and insoluble fiber fraction is not soluble in warm or hot water. Nearly all fiber-containing foods have more insoluble than soluble dietary fiber. About two-thirds to three fourths of the dietary fiber in typical mixed-food diets is water insoluble (Marlett and Slavin, 1997). Most fruits and vegetables contain less than 2 gram per serving total fiber and most refined grain products contain less than 1 gram per serving. Only legumes, whole grains, and concentrated grain products typically have more fiber (Marlett and Cheung, 1997). Fiber concentrates are frequently not all dietary fiber. Fiber concentrates such as pectins and gums (*e.g*. gum arabic, carob bean gum, guar gum), wood pulp cellulose, psyllium seed husk, and oat hull fiber are more than 90% fiber. In contrast, citrus fiber, pea fiber, corn bran, and soy polysaccharide are 60% to 85% fiber (Marlett, 1993). Oat bran is about 15% to 20%; wheat bran, 40% to 50%; barley, 15%; and rice bran, 20% to 30% dietary fiber (Saunders, 1990 and Marlett, 1993).

Characterization of some dietary fiber

Alginates are preduced by brown seaweeds (*Phaeophyceae*). They are linear unbranched copolymers containing β-(1→4)-linked D-mannuronic acid and α-(1→4)-linked L-guluronic acid residues that bind metal ions particularly sodium or calcium in their structure. The alginates are as thermally stable cold setting gelling agents in the presence of calcium ions. Alginate's solubility and water-holding capacity depend on pH (precipitating below about pH 3.5), ionic strength (low ionic strength increasing the extended nature of the chains) and the nature of the ions present. (Fennema, 1996)

Arabinoxylan is a hemicellulose that has a xylose backbone with arabinose side chains. It is found in many cereal grains. For example, wheat grain, which is composed of an outer layer of bran and an inner layer of endosperm (Henry, 1985). Arabinoxylan is difficult to extract from wheat bran but can be produced from wheat endosperm during the commercial processing of wheat flour. When wheat flour is processed to produce starch and gluten, the fiber component, which is mainly arabinoxylan, is left in the byproduct (Lu *et al*, 2000). It is not digested in the small bowel and only partly digested in the large bowel (Holloway, Tasman-Jones and Bell, 1980)

Chitosan is a modified carbohydrate polymer derived from the chitin component of the shells of crustacean, such as crab, shrimp, and cuttlefish. Chitosan is composed of an ammonium group (NH_4^+) attached to a polyglucosamine chain. The chitin is deproteinized,

demineralized and de-acetylated which is an unstable amino polysaccharide molecule with a very strong positive polarity. It has been suggested that chitosan reduces low-density lipoprotein (LDL) cholesterol by attract with the negatively charged lipids (Gallaher *et al*., 2000).

Glucomannan (konjac mannan) is a linear polymer of (1,4)-β-D-mannose and β-D-glucose (molecular ratio 1.6:1) water-soluble dietary fiber that is derived from konjac root. Konjac (Amorphophalus Konjac C. Koch) is a perennial plant belonging to the family Araceae. "Konnyaku", which is made from the tuber of this plant, has been used traditionally for food in Japan for several hundred years. The predominant component of edible konnyaku is a glucomannan called konjac mannan. Konjac powder has the highest molecular weight and strongest viscosity among any dietary fiber. It is a slightly branched of acetyl group on average every 9 and 19 sugar unit having a molecular weight of 200,000 to 2,000,000 daltons varied by konjac species or variety, processing method and even storage time of the raw material. These acetyl groups contribute to the solubility and gelling properties. If the acetyl groups are removed under mild alkaline conditions, the molecule will produce heat stable gels. It is capable of absorbing up to 50 times its weight in water and creates highly viscous gel from 20,000 to 40,000 cps. It is a viscous fibers that is fermented in the large intestine. (Sugiyama and Shimahara, 1976, Vorster *et al*., 1985 and Behr, online)

Guar gum, a viscous galactomannan and classed as soluble dietary fiber, was extracted from the seed of Indian cluster bean (*Cyamopsis tetragonolobus*, family Leguminosa). It is a non-ionic or neutral polysaccharide. Guar gum, a D-galacto-D-mannoglycan (1:2), is a straight chain mannan with single-membered galactose branches. The D-mannopyranose units are joined by β-(1→4) linkages, and single D-galactopyranose units are joined to this chain by α-(1→6) linkages. On average, the galactose branches occur on every other mannose unit. The molecular weight has been reported as 220,000. Guar gum is often used in foods, cosmetics and pharmaceuticals as a thickener and a binder of free water. For example, when used as a thickener in salad dressing, it raises the viscosity of the emulsion and decreases the separation rate. In products such as toothpaste, it can be used alone or in combination with other gums, as carrageenan or sodium alginate, to bind the free water and impart a smooth appearance. Other applications involve the use of guar gum as a binder and disintegrator for compressed tablet. (Whistler and BeMiller, 1959)

Oat gum was extracted from oat bran and purified in amounts sufficient to evaluate physiological effects. Oat gum, the major component of which is a (1→3) (1→4) – β –D-glucan (oat β-glucan), is one of the highly viscous polysaccharide in aqueous solution which is physicochemical similar to guar gum but is not commercial available. (Wood *et al*., 1994)

Pectin is a linear chain of (1→4)-linked α-D-galactopyranosyluronic acid with various contents of methyl ester groups. L-rhamnose, neutral sugar, is also present as L-rhamnopyranosyl units and effect gelation. It is partially methoxylated polymer of galacturonic acid, containing covalently branched of galactose and arabinose including of some xylosyl units. It is obtained commercially from pomace of apples and peel of citrus fruits, and, possibly in the pulp of sugar beets. Increasing the concentration of calcium divalent cations and sugar cosolute in solution increases the gelling tempature and gel strength. (Fennema, 1996)

Psyllium, a hydrophilic gel-forming polymer, is isolated from the seed husk of Plantago Ovata.

Tragacanth gum is a water-soluble plant exudates which is the exudation of several varieties of small, shrub-like plants of the *Astragalus* species. Chemically, gum tragacanth is a complex mixture of polysaccharides containing D-galacturonic acid. The other sugars produced on hydrolysis are D-galactose, L-fucose, D-xylose and L-arabinose. Gum tragacanth in solution has the properties of a partially cross-linked polymer in that a small portion is soluble but the greater portion remains as a swollen gel or gel-like dispersion. (Whistler and BeMiller, 1959)

Wheat gum is a water-soluble hemicellulose fraction, which present in final flour of wheat. Water-soluble wheat gum is a mixture containing at least two components of which the main component is an L-arabino-D-xyloglycan. It is a highly branched molecule, in which the main chain is built up entirely of D-xylose units linked together by glycosidic β-(1→4) linkages and has L-arabinose units as branches linked to the main chain by (1→2) and/or (1→3) linkages.

Xanthan gum is a biosynthetic edible gum produced by the bacterium *Xanthomonas campestris* commonly found on leaves of cabbage family. It composed of glucose, mannose and glucuronic acid. Its structure is an anionic polyelectrolyte with a β-(1→4)-D-glucopyranose glucan (as cellulose) backbone with side chains of –(3→1)-α-linked D-mannopyranose-(2→1)- β-D-glucuronic acid-(4→1)- β -D-mannopyranose on alternating residues. It has a coiled backbone and tightly packed side groups. It is viscous fibers that is fermented in the large intestine (Osilesi *et al*. 1985).

2.2.1 Digestion and absorption of dietary fiber

In the classical definition, dietary fiber is the sum of lignin and polysaccharide originating from plants that are not digested by the enzymes of the small intestines, but may be degraded by microbial fermentation in the large intestines. Indigestible polysaccharides pass into the large bowel where they serve as energy substrates for the resident intestinal microbial flora. Microbial fermentation of various types of dietary fiber within the large

intestine results in the production of short-chain fatty acids and a lower pH, which in turn may modify the composition and metabolic activity of the intestinal microbial flora (Campbell, Fahey and Wolf, 1997, Eastwood, Brydon and Anderson, 1986). Short chain fatty acids include acetate, butyrate, and propionate. Furthermore, short-chain fatty acids, particularly butyric acid, play an important role in the regulation of colonic epithelial cell proliferation because it is a preferred fuel for the cells of the normal colonic mucosa (Noack *et al*., 1998).

Short chain fatty acids production has been related to lowered serum cholesterol and decreased risk of cancer (Cummings *et al*., 1992). Some fibers, such as cellulose, are practically not fermented and leave the body unaltered. They result in increasing faeces bulking and intestinal transit speed. It has been suggested that the stool-bulking action result from the water-holding capacity of the fiber residue after fermentation (Eastwood and Morris, 1992).

2.2.2 The nutrition and physiology of dietary fiber

Dietary fiber may directly or indirectly influence human diseases by affecting physiological and metabolic processes. The beneficial effects of dietary fiber on health are now well documented. Certain soluble fibers, such as guar gum, were associated with cholesterol-lowing effects (Vahouny *et al.*, 1988) and improved glucose response (Jenkins *et al*., 1977), whereas insoluble fiber is associated with improved colon laxation (Spiller *et al*., 1979). Behall and Hallfrisch (2002) indicated that amylose significantly reduce plasma glucose and insulin in healthy subjects. Kiehm, Anderson and Ward (1976), Miranda and Horwitz (1978), Dodson *et al*. (1981), Jenkins *et al*. (1984), Madar *et al*. (1988), Anderson *et al*. (1991) and Cameron-Smith *et al*. (1997) designed and carried out a series of studies that showed beneficial effects of high-fiber diets for individuals afflicted with the disease. Beneficial effects of increased dietary fiber consumption were shown for both type I and type II diabetics and included improved glucose tolerance, reduced insulin requirements, increased peripheral tissue insulin sensitivity, decreased serum cholesterol, decrease serum triglycerides, better weight control, and potentially consistently lower blood pressure. In a study of guar, pectin, gum tragacanth, methylcellulose and wheat bran, Jenkins *et al*. (1978) showed that each flattened the glucose response, with the reduction in mean peak rise in blood glucose concentration being positively correlated to viscosity. Since coronary heart disease (CHD) is the leading cause of death, epidemiological studies suggest that dietary fiber or foods rich in fiber, especially from cereal products, can prevent from CHD. High intake of dietary fiber, especially soluble fiber, decreases low-density lipoprotein cholesterol (LDL-C) and has little or no effect on high-density lipoprotein cholesterol (HDL-C) (Wolk *et al*., 1999). Hoffman and Choi, (2001) also found the effect of pectin on reducing absorption of undesired lipids in the gastrointestinal tract. Spapen *et al*., (2001) investigated that nutrition supplement with soluble fiber is beneficial in reducing the incidence of diarrhea.

Holt *et al*. (1979) suggested that the effects of guar gum and pectin on glucose tolerance could be due simply to alteration in the rate of gastric emptying. The addition of guar or pectin to a glucose test meal may delay gastric emptying and so slow the rate of absorption of glucose. Alternatively, or in addition, the non-absorbable carbohydrates may interfere with the process of absorption within the small intestine itself. It has also been related to slowing down the increase in blood glucose, which in turn controls hunger but not to the extent of causing malabsorption (Leeds, 1987).

Dietary fiber can be ingested in its natural form as plant-cell-wall material or in a purified or semipurified form. Chemical analysis or quantitative measurement of the fiber content does not allow prediction of their biological action. Polysaccharides, which are the principle constituents of dietary fiber, show a wide spectrum of physical properties, reflection the nature and extent of intermolecular association (Eastwood and Morris, 1992). The solubility and viscosity of many polysaccharides are salt- and pH-dependent (Eastwood, Brydon and Anderson, 1986). Polysaccharide gel matrix are formed by ordered packing of chain segments, as in insoluble fibers, but have interconnecting sequences that are disordered, as in solution, and therefore promote hydration and swelling. The ordered junctions are stabilized by noncovalent bonds (hydrogen bonds, electrostatic and dipolar interactions, Van Der Waals attractions). Because these bonds are individually weak, the junctions are stable only above the minimum critical length. The gel matrix properties of specific polysaccharides are highly dependent on the spacing of minor structural irregularities along the polymer chain rather than on overall composition as determined by normal chemical analysis of the proportion of constituent sugars. Charged polysaccharides, such as pectin, the junctions may be stabilized by incorporation of arrays of site-bond counterions and are critically sensitive to pH and ionic environment, so that gel matrix structure may be lost or reformed under the varying conditions prevailing along the gastrointestinal tract. Dietary fiber may appear to be a water-laden sponge passing along the intestine. The physical properties that influence function along the gastrointestinal tract are a combination of the rheological and colligative properties of the water-soluble-fiber components, the surface properties of the water-insoluble components, and the matrix properties of the swollen, hydrated components. Such properties include viscosity, water holding, cation exchange, organic acid adsorption, gel filtration, and particle-size distribution (Eastwood and Morris, 1992 and Vanderhoof, 1998). In general, the two main effects of dietary fiber in the foregut are to prolong gastric emptying time and to retard absorption of nutrients. Both are dependent on the physical form of the fiber, and in particular, on its influence on digesta viscosity (Blackburn and Johnson, 1981 and Johnson and Gee, 1981). Moreover, by acting as an emulsifier, viscous fiber can stabilize the gastric chyma, prevent separation of the solid from the liquid phase, impair selective retention of the largest particles, and thereby increase their rate of passage into the small intestine. The

increased ingestion and chewing time may increase satiety. The increased volume that fiber mixed with digestive juices and water produces induces satiety signals by distension of the stomach, delayed gastric emptying time and stimulation of gut hormones releasing (Anderson and Bryant, 1986). This effect of reducing the postprandial blood glucose concentration in humans was originally explained as a result of a delay in the delivery of the viscous material from the stomach into the small bowel and through interfere with glucose diffusion in the intestinal contents see in Figure 7 (Jenkins, Wolever and Jenkins, 1999). The physical characteristics of the intestinal contents will be changed by the physical properties of the fiber sources in diet. The bulk of material in the small intestine will increase because the fiber is not digestible and remains during the transit of digesta through the small intestine (Leeds, 1987). The volume of the intestinal contents can increase because of the water-holding capacity of the fiber source. An increase in the bulk, volume, or viscosity of the intestinal contents is likely to slow diffusion of enzymes, substrates, and nutrients to the absorptive surface, all of which can lead to a slower appearance of nutrients in the plasma following a meal (Vahouny and Cassidy, 1985). French and Read (1994) also concluded that slowed absorption and prolonged contact of nutrients with intestinal chemoreceptors are compatible.

2.2.3 Recommended fiber intakes through the life cycle

American Dietetic Association (ADA) called on the public to consume sufficient amounts of dietary fiber from various plant foods. Current recommendations from the ADA and The American Diabetes Association for patient with diabetes (American Diabetes Association, 1994) for dietary fiber intake for adults generally fall in the range of 20 to 35 g per day or 10 to 13 g per 1,000 kilocalories. Nutrition Facts panel provides a good reference, stating 25 g of dietary fiber for 2,000 kilocalories per day diet or 30 g for 2,500 kilocalories per day diet as goals for Americans intake. The recommendation for children older than 2 years is to increase dietary fiber intake to an amount equal to or greater than their age plus 5 grams per day, to achieve intakes of 25 to 35 g per day after age 20 years. (Marlett and Slavin, 1997). In addition, Thai recommended daily intakes (Thai RDI) (Table 2) also recommend 25 g per day (คณะอนุกรรมการพิจารณาการแสดงคุณค่าทางโภชนาการบนฉลากของอาหาร, 2538).

2.3 Polysaccharide gel (PG) from fruit-hulls of durian

Polysaccharide gel (PG) was first extracted from fresh fruit-hull of durian (*Durio zibethinus* L.) by Pongsamart and Panmuang, 1998. Girddit *et al*. (2001) has resently demonstrated the elemental compositions of PG extracts from dried fruit-hulls of durian. They are carbon, hydrogen and oxygen in atomic ratio of 2.9 : 5.7 : 3.2. The mineral compositions of PG were also investigated, the major components are sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg). The sugar composition of PG was analyzed from PG acid

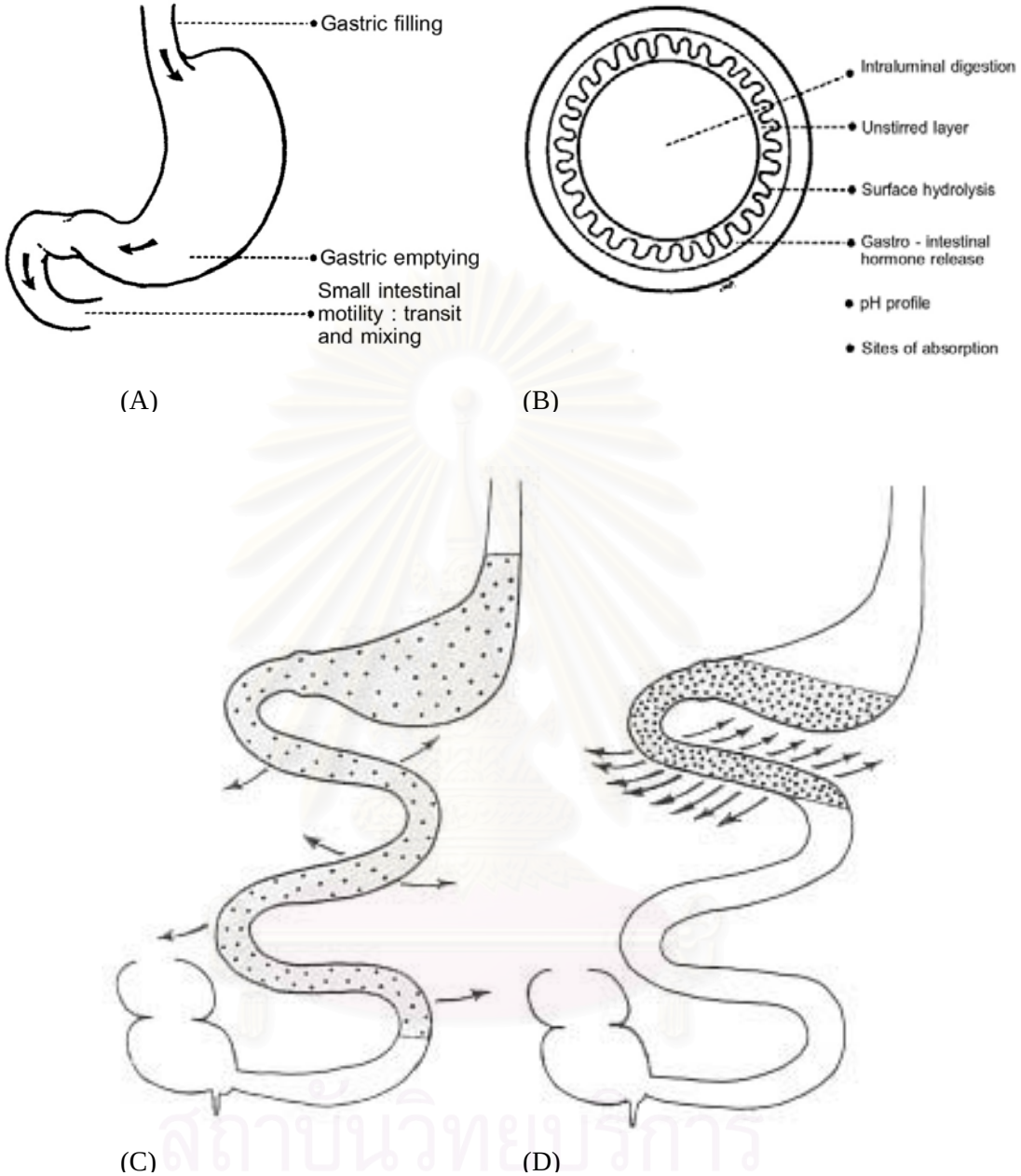


Figure 7 Schematic representation of stomach and small intestine showing (A) gastric emptying (B) morphology of sites of maximal transport capacity (C) slow digestion and absorption of energy-dilute food in a 'fiber-rich' diet and (D) rapid digestion and absorption of energy-dense food from a low-fiber diet modified from Leeds, 1987 and Jenkins, Wolever and Jenkins, 1999.

hydrolysate by techniques of thin layer chromatography (TLC) and high performance liquid chromatography (HPLC), showing that PG composed of neutral sugars including glucose, rhamnose, arabinose, fructose and 52.5 % content of acidic sugar galacturonic acid in PG by Infrared spectroscopy (IR) pattern. Molecular weight of PG ranges 500-1,400 kDa. Characterization of PG according to methods such as chemical reaction, X-ray diffraction and IR, indicated that PG was amorphous polysaccharide. Dried powder of PG swelled in water forming a viscous liquid and having acid pH at 2.60 ± 0.14. PG can be widely used for preparation of food and pharmaceutical products, such as a gelling agent in jelly or jam (Dhumma-Upakorn, Panmaung, and Pongsamart, 1989), disintegrator, binder and film coat in tablet (Umprayn, Kaitmonkong, and Pongsamart, 1990), suspending and thickening agent in suspension or emulsion (Pongsamart, 1989). Gerddit *et al*. (2002) reported that PG has a film forming property that can be prepared a film dressing and PG can be used to formulate a satisfied water-soluble dressing. Lipipan *et al*. (2002) first discovered antibacterial activity of PG. Nantawanit *et al*. (2002) have also demonstrated inhibitory activity of PG against seven bacteria tested, *Bacillus subtilis*, *Escherichia coli*, *Lactobacillus pentosus*, *Micrococcus luteus*, *Proteus vulgaris*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. Furthermore, Nakchat *et al*. (2002) studied effects of PG film dressing on wound healing in young female pig skin, suggesting that PG film dressing accelerate wound healing, total 100 % of complete wound closure after 18 day of treatment with PG film dressing, whereas only 64 percent of wound closure was obtained in conventional treatment (applied povidone iodine and covered with gauze).

2.4 **Vitamins** (Berg *et al*., 1993)

Vitamins are essential substances needed by the body to sustain life. Our tissues cannot produce or in sufficient quantities to supply our needs under normal circumstances. For instance, the substance ascorbic acid is not needed by animals who synthesize it, so it is not a vitamin to them. Humans, however, do not possess the enzyme to change glucose into ascorbic acid, so, to humans, ascorbic acid is known as, “vitamin C”. The way to get the vitamins is exogenous vitamin supplementation. Vitamins were discovered because of the drastic symptoms, when there is severe vitamin deficiency. Most Recommended Dietary Allowances values (RDAs) are still strongly influenced by the historical concept of crude deficiency symptoms. Recommended Dietary Intakes (RDI) for all healthy Thai people older than 6 years old which was revised in 1995, reported by Nutrition Labelling committee, Food and Drug Administration, Ministry of Public Health, was shown in Table 2 and RDAs for all healthy people in the United States was revised in 1989, reported by Food and Nutrition.

Table 4 Recommended Dietary Allowances, revised 1989, reported by Food and Nutrition Board, National Academy of Sciences, USA.

Category	Age	B1 (mg)	B2 (mg)	Niacin (mg NE)	B5 (mg)	B6 (mg)	Folate (μg)	B12 (μg)	C (mg)	A (μg RE)	D (μg)	E (mg α-TE)	K (μg)
Infants	0.0 - 0.5	0.3	0.4	5	2	0.3	25	0.3	30	375	7.5	3	5
	0.5 - 1.0	0.4	0.5	6	3	0.6	35	0.5	35	375	10	4	10
Children	1 - 3	0.7	0.8	9	3	1	50	0.7	40	400	10	6	15
	4 - 6	0.9	1.1	12	4	1.1	75	1	45	500	10	7	20
	7 - 10	1	1.2	13	5	1.4	100	1.4	45	700	10	7	30
Males	11 - 14	1.3	1.5	17	7	1.7	150	2	50	1000	10	10	45
	15 - 18	1.5	1.8	20	7	2	200	2	60	1000	10	10	65
	19 - 24	1.5	1.7	19	7	2	200	2	60	1000	10	10	70
	25 - 50	1.5	1.7	19	7	2	200	2	60	1000	5	10	80
	51 +	1.2	1.4	15	7	2	200	2	60	1000	5	10	80
Females	11 - 14	1.1	1.3	15	7	1.4	150	2	50	800	10	8	45
	15 - 18	1.1	1.3	15	7	1.5	180	2	60	800	10	8	55
	19 - 24	1.1	1.3	15	7	1.6	180	2	60	800	10	8	60
	25 - 50	1.1	1.3	15	7	1.6	180	2	60	800	5	8	65
	51 +	1	1.2	13	7	1.6	180	2	60	800	5	8	65
Pregnant		1.5	1.6	17	7	2.2	400	2.2	70	800	10	10	65
Lactating	1st 6 months	1.6	1.8	20	7	2.1	280	2.6	95	1300	10	12	65
	2nd 6 months	1.6	1.7	20	7	2.1	280	2.6	90	1200	10	11	65

Table 5 Essential vitamins

Vitamin	Stability	Metabolically active form(s)	Cell/tissue distribution	Importance biochemical function(s)	Deficiency disorders	Sources
water-soluble vitamin						
thiamin (vitamin B1)	stable in acid solutions	thiamin pyrophosphate	all	co-enzyme for oxidative decarboxylation of 2-ketoacids; co-enzyme for pyruvate decarboxylase and transketolase	beri-beri, polyneuritis, Wernicke-Korsakoff syndrome	seeds, nuts, wheat germ, legumes, lean meat
riboflavin (vitamin B2)	stable in acid solutions and light-sensitive	flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD)	all	co-enzyme for numerous flavoproteins that catalyze redox reaction in fatty acid degradation/synthesis, TCA cycle	dermatitis	milk, organ meats, eggs, nuts, seeds, green vegetables
niacin (vitamin B3)[a]	stable	nicotinamide adenine dinucleotide (NAD(H)), nicotinamide adenine dinucleotide phosphate (NADP(H))	all	co-substrates for H-transfer catalyzed by many dehydrogenases, *e.g.*, TCA cycle respiratory chain	pellagra	meats, liver, green vegetables, nuts, whole grains, legumes (not corn)
pantothenic acid (vitamin B5)[b]	stable in neutral solutions	Coenzyme A acyl carrier protein	all	co-substrate for activation/transfer of acyl groups to form esters, amides, citrate, triglycerides, etc. co-enzyme for fatty acid biosynthesis	signs vary with species	plant and animal tissues, yeast, grains, royal jelly, egg yolk, liver
pyridoxine (vitamin B6)[b]		pyridoxal-5'-phosphate	all	co-enzyme for metabolism of amino acids, *e.g.*, side-chain, decarboxylation, transamination, racemization	signs vary with species	yeast, liver, meats, wheat germ, nuts, beans, avocados, bananas, potatoes

[a] Some production in human tissues.

[b] Human gut bacteria are a significant source. ,

Table 5 Essential vitamins (continued).

Vitamin	Stability	Metabolically active form(s)	Cell/tissue distribution	Importance biochemical function(s)	Deficiency disorders	Sources
biotin (vitamin B7)[b]		1'-N-carboxybiotin	all	co-enzyme for carboxylations, *e.g.* acetyl-CoA/malonyl-CoA conversion	dermatitis	yease, liver, egg yolk, tomato, soybeans, rice, bran (synthesized by intestinal microorganisms)
folate (vitamin B9)[b]	unstable to heat	polyglutamyl tetrahydrofolates	all	co-enzyme for transfer of single-C units, *e.g.* formyl and hydroxymethyl groups in purine synthesis	megaloblastic anemia	yease, liver, alfalfa, spinach, legumes, some fruits, eggs, whole-grain cereals
cyanocobalamin (vitamin B12)	stable in neutral solutions	5'-deoxyadenoxyl-B12 methyl-B12	all	co-enzyme for conversion of methyl-malonyl-CoA to succinyl-CoA methyl group transfer from CH_3-FH_4 to homocysteine in methionine synthesis	pernicious, megaloblastic anemias	liver, kidney, egg, cheese
vitamin C	unstable to heat and easily oxidized in the presence of Cu or Fe	ascorbic acid, dehydroascorbic acid	all	co-substrate for hydroxylations in collagen systhesis, drug and steroid metabolism	scurvy	fresh fruits (especially citrus) and vegetables (generally absent in grains)
fat-soluble vitamin						
vitamin A	sensitive to ionizing radiation but stable in heat, acid and alkali	retinol, retinal, retinoic acid	selected tissue (especially liver)	photosensitive retinal pigment, regulation of epithelial cell differentiation	night blindness, xerophthalmia, keratomalacia	precursor in yellow-orange vegetable plants, carrots, liver, fish liver oils
vitamin D[a]	stable	1, 25-$(OH)_2$-D	selected tissue (especially liver)	promotion of intestinal Ca absorption, mobilization of Ca from bone, stimulation of renal Ca resorption, regulation of PTH secretion, possible function in muscle	rickets, osteomalacia	fish liver oils (eggs), milk and milk products
vitamin E		α-tocopherol	most tissues	antioxidant protector for membranes	nerve, muscle degeneration	vegetables and seed oils, green leaves, wheat germ
vitamin K[b]		K, MK	selected tissue	co-substrate for γ-carboxylations of glutamyl residues of several clotting factors and other Ca-binding proteins	impaired blood coagulation hemorrhage	green leafy vegetables, egg yolk, cheese, pork liver

[a] Some production in human tissues.
[b] Human gut bacteria are a significant source.

Board, National Academy of Sciences was shown in Table 4. Vitamins are essential for life and for optimal human physical and mental well being.

The essential vitamins for human growth, maintenance, and health are listed in Table 5, along with the information on their isolation, function, active form, tissue distribution, deficiency disorders and the food supply.

Vitamins are divided into two groups: water and fat-soluble. The water-soluble vitamins are readily excreted, whereas the fat-soluble vitamins are stored in the liver and fatty tissues.

2.4.1 Water-soluble vitamins (Martin, Mayes and Roddwell, 1981, Linder, 1991, and Wardlaw, 1999)

The water-soluble vitamins have chemical structures that are remarkably diverse, but they do share the property of being polar molecules, thus they are soluble in water. Because of their water-solubility, they have no stable storage form and must be provided continuously in the diet. All of them except vitamin C serve as coenzymes or cofactor in enzymatic reactions (Figure 8). Most can be absorbed to nutritionally significant extent from high oral doses. Nonetheless, specific transport mechanisms exist for most water-soluble vitamins (many are Na^+-powered secondary active transport).

Thiamin (vitamins B1) (Linder, 1991, Basu and Dickerson, 1996 and Wardlaw, 1999)

Thiamine occurs in many kinds of food originated from plants and animals as thiamine pyrophosphate (TPP) and is hydrolyzed to thiamine during digestion. Thiamine is necessary for several critical functions in carbohydrate metabolism. It is present mainly as the cofactor, which is noncovalently bound to apoenzyme protein. Its active form, thiamine pyrophosphate (formerly known as cocarboxylase), plays a vital role in the oxidative decarboxylation of pyruvic acid; in the formation of acetyl CoA, which enters the Krebs cycle; and in other important biochemical conversion cycles. Thiamine is also essential in neurologic function. The amount of required vitamin rises when caloric consumption is increased. The most familiar natural thiamine source is the hull of rice grains. Other sources are pork, beef, fresh peas, and beans. The RDAs for adult men is 1.2 milligrams per day and 1.1 milligrams per day for adult women.

Thiamine is one of the least stable of the water-soluble vitamins when the pH of the matrix approaches neutrality. Maximum stability in solution is between pH 2.0 to 4.0. Thiamine is unstable owing to heat, alkali, oxygen and radiation. Store in well-closed, non-metallic container and protected from light (British Pharmacopoeia [BP], 1998). Water solubility is also a factor affecting the loss of thiamin from foods. Thiaminases destroy the

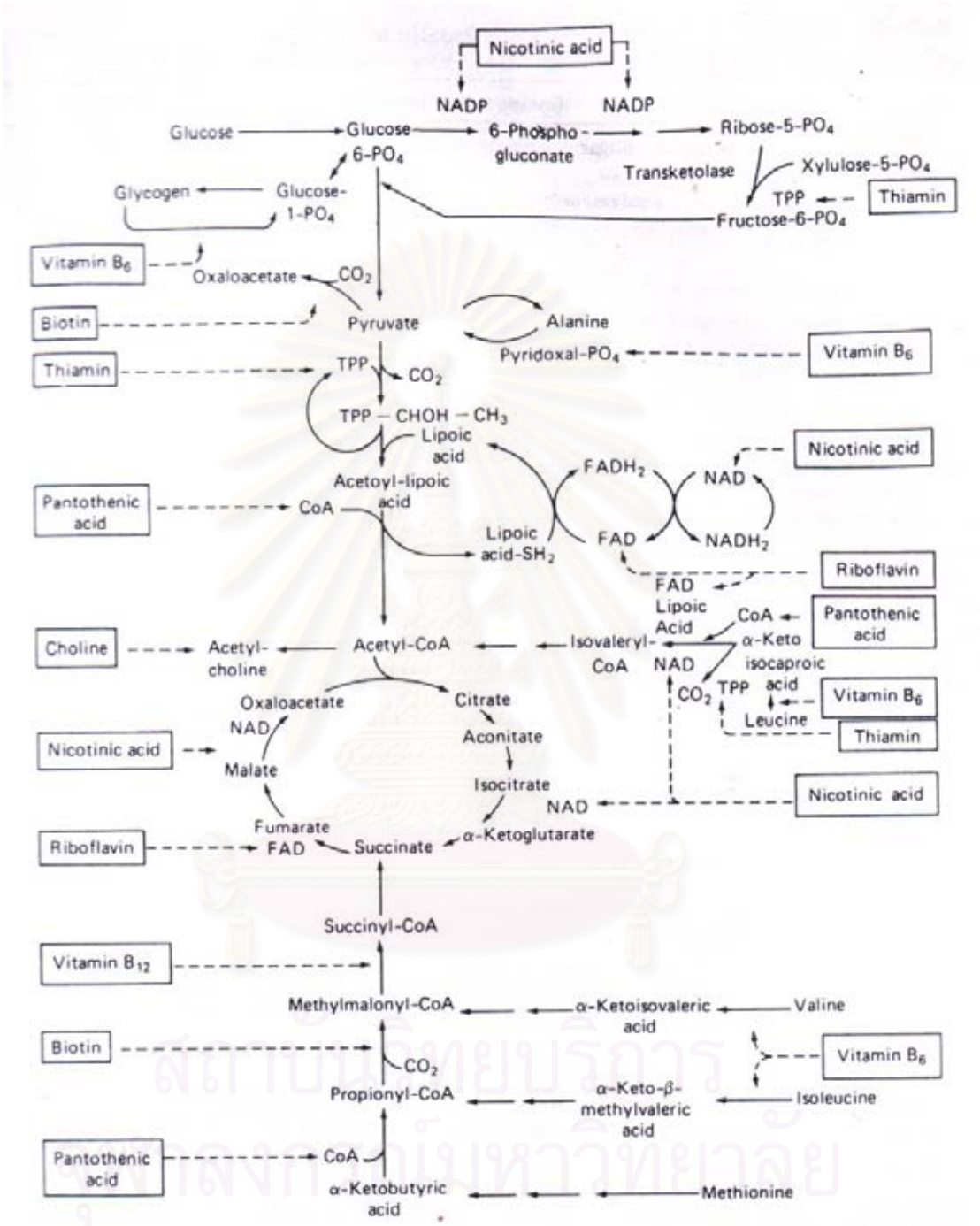


Figure 8 Involvement of vitamins in carbohydrate metabolism. (Linder, 1991)

vitamin (in raw fish and shellfish; such as in sushi), as well as tannins and other factors (in tea and coffee) which oxidize the vitamin, can decrease its content in the diet.

The thiamine molecule in the free base form consists of a pyrimidine ring (4' amino-2'-methylpyrimidinyl-5'-ylmethyl) linked by a methylene bridge to the 3-nitrogen atom in a substituted thiazole (5-(2-hydroxyethyl)-4-methyl-thiazole) (Figure 9). Thiamine hydrochloride ($C_{12}H_{18}ON_4SCl_2$) and thiamine mononitrate ($C_{12}H_{17}O_4N_5S$) are the commercially available forms used in pharmacy and in food fortification (Figure 9). Thiamine hydrochloride is the USP reference standard (MW 337). It is a white crystalline powder with a yeast-like odor and salty, nut-like taste. The most distinguishing difference between the hydrochloride salt and the mononitrate is water-solubility. The hydrochloride is soluble in water (1 gram per millilitre) and the mononitrate is only slightly water-soluble (0.027 gram per millilitre). The hydrochloride salt is used in injectable and parenteral pharmacy and for fortification of foods which requires solubility. Thiamine hydrochloride is nearly insoluble in methanol, ethanol, and glycerol. It is insoluble in acetone, benzene, hexane, and chloroform. (United States Pharmacopoeia 23 / Nation Formulary 18 [USP 23/NF 18], 1995)

The primary causes of thiamine deficiency are generally related to diet inadequacy, alcoholism, malabsorption syndromes, prolonged diarrhea, increased use (pregnancy), or food faddism. Thiamin deficiency usually occurs along with deficiencies of other B vitamins. The symptoms such as tiredness, emotional instability, irritability, depression, and even fall in body weight, have been observed. In early days of thiamin deficiency, the elasticity of the wall of the gastrointestinal tract is also decreased to the point that normal gastric mobility is diminished, the colon becomes distended, and constipation results. Consequently, there is a tendency to suffer from loss of appetite, sometimes associated with nausea and vomiting.

Severe thiamin deficiency results in the beriberi disease, which is characterized by peripheral neuropathy (dry beriberi), especially in the limbs used most frequently, accompanied by tingling, numbness, tenderness, and weakness, and/or cardiovascular problems (wet beriberi) getting worse by physical exertion and carbohydrate utilization for energy.

2.4.1.1 Digestion and absorption of vitamins B1 (Basu and Dickerson, 1996)

Absorption of thiamine can be both active and passive, depending upon the concentration of the vitamin. At low concentration ordinarily found in food, thiamine is absorbed by Na^+-dependent active process. This absorption is greatest in the jejunum and ileum. At high concentration of thiamine attained by supplementary intake, it is absorbed by passive diffusion. Almost no absorption of thiamine occurs in the stomach and the distal small intestine regions. The vitamin formed by the flora of the large intestine is not absorbed.

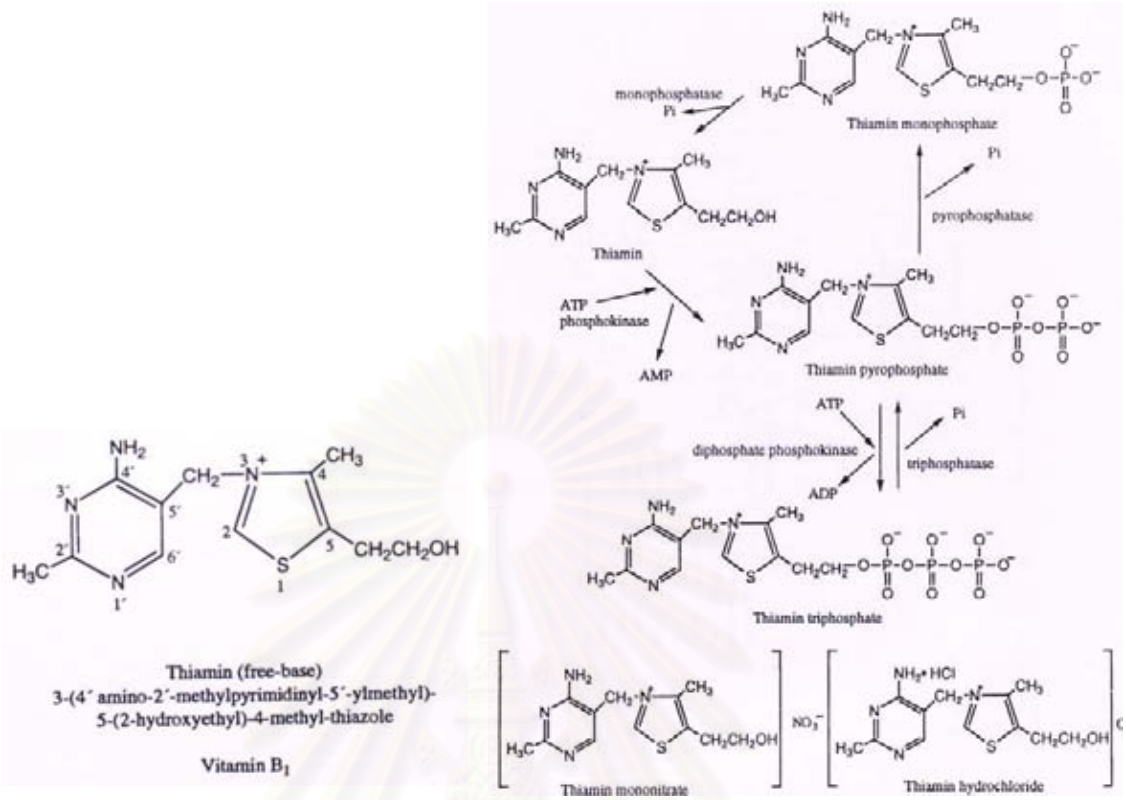


Figure 9 Structure of thiamin and related compounds. (Eitenmiller and Lauden, 1999)

Orally administered thiamine is rapidly absorbed, distributed, and excreted with a half-life of one hour. An adult human body contains approximately 30 milligrams of thiamine with high concentrations found in the heart, liver, kidney, and brain. Fifty per cent of the body's thiamine is distributed throughout the skeletal muscle. The higher the doses of thiamine administered, the more unchanged thiamine is excreted in the urine within 4-6 hours. Since thiamine has a high metabolic turnover rate and can only be stored in limited amount in the human organism, it must be taken daily in quantity sufficient to maintain the tissue saturation state.

Hammes *et al*. (2003) discovered that the fat-soluble synthetic thiamin derivative benfotiamine can inhibit three major pathways of hyperglycemia induced vascular damage, so it might be clinically useful in preventing the development and progression of diabetic complications. While Reddi *et al*. (1993) reported that thiamin in liver and heart has significantly lowered in diabetes rats. It may be related to its increased catabolism or decreased synthesis of decreased transport from blood. Furthermore, increased urinary excretion of thiamin may also cause a decrease in tissue concentrations.

2.4.1.2 Vitamin B1 determination (Freed, 1966, Morrow *et al.*: Linder, 1991 and Eitenmiller and Landen, 1999)

There are many methods, which have been proposed for the analysis of thiamine. Microbiological growth assays by using *Lactobacillus fermenti,* but the specificity limited of its is susceptible to both stimulatory and inhibitory matrix effects, or *Lactobacillus viridiscens* is more specific for thiamin.

One of the most commonly used assay procedures relies on its oxidation thiamin to the inactive product, thiochrome, by alkaline ferricyanide which may or may not require HPLC separation. The thiochrome reaction was shown in Figure 10. In the absence of other fluorescent compounds that can interfere with the assay, the fluorescence intensity of thiochrome is proportional to the total thiamin in the sample (Weilders and Mink, 1983). Thiochrome is used as the determinative step in USP methods for pharmaceuticals (USP 23/NF 18, 1995) and AOAC International Official Methods (Association of official analytical chemists [AOAC], 2002). HPLC-based method separated thiamin and then converted it to thiochrome in a postcolumn reaction (Ohta, Baba and Suzuki, 1984), or which oxidize the thiamin in a precolumn reaction and then separated the thiochrome (Floridi *et al.*, 1984) before fluorometric quantitation. This HPLC with fluorescence detection has been used to simultaneously determine thiamin and riboflavin in the same extract (Mauro and Wetzel, 1984)

Riboflavin (Vitamin B2)

Riboflavin occurs in the free state in foods, in combination with phosphate, or with both phosphates and proteins. The free riboflavin is released and absorbed during digestion.

Riboflavin is a constituent of two coenzymes, FAD and FMN. It is involved in numerous oxidation and reduction reactions, including the cytochrome P-450 reductase enzyme system involved in drug metabolism. Cellular growth cannot occur without riboflavin. Early signs of riboflavin deficiency symptoms are the eyes become light sensitive and easily fatigued. Marginal riboflavin deficiencies have also been detected in vegetarians and alcoholics.

Niacin (Nicotinic Acid, Vitamin B3)

The physiological active form of niacin is nicotinamide. Niacin and niacinamide (nicotinic acid amide) are constituents of coenzymes NAD (H) and NADP (H). These coenzymes are electron transfered agents in the aerobic respiration of all body cells. Niacin is unusual as a vitamin because human can synthesize it from dietary tryptophan, about 60 mg of tryptophan equivalent to 1 mg of niacin. In therapeutic doses, niacin will lower triglycerides and low-density lipoprotein cholesterol by mechanisms unrelated to its functions as an essential micronutrient. Clinical finding of niacin deficiency includes the dermatitis, diarrhea, and dementia, often accompanied by neuropathy, glossitis, stomatistic, and proctitis.

Pantothenic acid (Vitamin B5)

Pantothenic acid is a precursor of coenzyme A (CoA) that is active in many biologic reactions and that plays a primary role in cholesterol, steroid, and fatty acid synthesis. Pantothenic acid is important for acetylation reactions and the formation of citric acid for the Krebs cycle, and it is crucial in the intraneuronal synthesis of acetylcholine. It is also important in gluconeogenesis; the synthesis and degradation of fatty acids; the synthesis of sterols, steroid hormones, and porphyrins.

Pyridoxine (Vitamin B6)

Pyridoxine serves as a cofactor for more than 60 coenzymes, including decarboxylases, synthetases, transaminases, and hydroxylases. It is important in heme production and in the conversion of oxalate to glycine. It exists in three forms: pyridoxine (vitamin B6), pyridoxal, and pyridoxamine. Although all three forms are equally effective in nutrition, pyridoxine hydrochloride is the form most often used in vitamin formulations. Causes of pyridoxine deficiency include alcoholism, severe diarrheal syndromes, food faddism, drugs, malabsorption syndromes, and genetic diseases (cystathioninuria and xanthinuric aciduria).

Folic acid (Pteroylglutamic acid, Folacin, Vitamin B9)

Folic acid is further biotransformed in the body and is involved in DNA synthesis and maturation and cell production activities. The function of folic acid is closely related to that of vitamin B12. The requirements for folic acid are related to the metabolic rate and cell turnover. Thus, increased amounts of folic acid are needed during pregnancy, lactation, and infancy, as well as for infection, hemolytic anemias and blood loss and hypermetabolic states such as hyperthyroidism. A deficiency of folic acid results in impaired cell division and protein synthesis. Symptoms of folic acid deficiency mimic those of a vitamin B12 deficiency

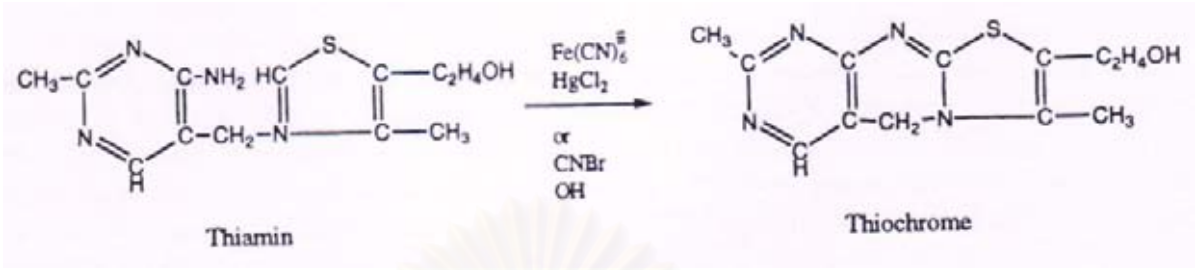


Figure 10 The thiochrome reaction. (Eitenmiller and Lauden, 1999)

and are manifested in organ systems with rapidly duplicating cells include sore mouth, diarrhea, and the central nervous system (CNS) symptoms such as irritability and forgetfulness.

Cyanocobalamin (Vitamin B12)

Cyanobalamin, the most complex vitamin molecule, contains a single atom of cobalt. It is available in the body as methylcobalamin, hydroxycobalamin, and adenosylcobalamin, all designated as "cobalamins." Vitamin B12 is active in all cells, especially in bone marrow, CNS, and the gastrointestinal tract. It is also involved in fat, protein, and carbohydrate metabolism. A cobalamin coenzyme functions in the synthesis of DNA and in the synthesis and transfer of single-carbon units such as the methyl group in the synthesis of methionine and choline. Vitamin B12 is produced almost exclusively by microorganisms, which accounts for its presence in animal protein (meats, oysters, and clams). Because of the general lack of vitamin B12 in vegetables, vegetarians who consume no animal products are at risk for developing a vitamin B12 deficiency.

Ascorbic acid (Vitamin C)

Ascorbic acid has been called the "fresh-food" vitamin, is the most easily destroyed of all vitamins. A relatively simple compound, it is a powered reducing agent that serves to protect the capillary basement membrane. As a nutrient, ascorbic acid is necessary to form collagen and to serve as a water-soluble antioxidant. Ascorbic acid, which must be ingested since it is not produced in the body, is necessary for the biosynthesis of hydroxyproline, a precursor of collagen and elastin. It also assists in the absorption of nonheme iron from food by reducing the ferric iron in the stomach and by combining in complex formation with ions that remain solubilized in the alkaline pH of the duodenum. A deficiency may also impair wound healing. A profound dietary deficiency can eventually lead to scurvy, producing widespread capillary hemorrhaging, and a weakening of collagenous structures

2.4.2 Fat-soluble vitamins (Martin, Mayes and Roddwell, 1981, Linder, 1991, and Wardlaw, 1999)

The fat- or lipid- soluble vitamins are apolar hydrophobic molecules. All are handled by the gastrointestinal system in the same manner as dietary fat. Their absorption is facilitated by bile. The presence of lipid digestion products and bile forming mixed micelles enhances the absorption of fat-soluble vitamins. Vitamins A, D, E and K partition into mixed micelles and are absorbed similarly to other lipids. In enterocytes, they partition into chylomicrons and are absorbed into intestinal lymph in this way. Certain more polar derivatives are absorbed by simple diffusion and leave the intestine in the portal blood. Once absorbed, the fat-soluble vitamins are transported to the liver in chylomicrons and stored either in the liver (vitamins A,

D and K) or in adipose tissue (vitamin E) for various periods of time. These vitamins are transported in blood by lipoproteins or specific binding proteins, since they are not directly soluble in plasma water, as are the water-soluble vitamins. Accordingly, fat-soluble vitamins are not excreted in urine but are more likely to appear in bile and thus are excreted in feces. Because of the body's ability to store excess fat-soluble vitamins, toxicity occurs at least from vitamin A and D over dosage. Deficiencies of these vitamins occur when fat intake is limited or fat absorption is compromised.

Vitamin A (Linder, 1991, Basu and Dickerson, 1996 and Wardlaw, 1999)

Vitamin A was the first fat-soluble vitamin to be recognized. It occurs in two principal forms in nature –retinoids (preformed vitamin A), which is found only in animal sources, and certain carotenoids (provitamins), which are found only in plant sources. Carotenoids are the compounds that give many fruits and vegetables their yellow and orange colorings. Carotenoids are in many forms, the most abundant and best known of the carotenoids is beta-carotene. Beta-carotene is a precursor of vitamin A or calls provitamin A because its vitamin A activity occurs only upon conversion to retinal within the body. One molecule of beta-carotene can be cleaved by a specific intestine enzyme into two molecules of vitamin A.

Vitamin A physiologically occurs as the alcohol (retinol), the aldehyde (retinaldehyde), the acid (retinoic acid) and the ester (retinyl ester) while the term of retinoids refer to all forms of vitamin A. Vitamin A is a long-chain primary alcohol and is known to exist in a number of isomeric forms. It exists in the pure state as pale yellow crystals soluble in most organic solvents and in fats (BP, 1998). In liquid form, a light-yellow to red oil, insoluble in water and in glycerin, very soluble in chloroform and ether, soluble in absolute alcohol and vegetable oils (USP 23/NF 18, 1995). Vitamin A occurs in nature largely as retinal in its all-*trans* form (Figure 11), which has three important structural characteristics. These are a β-ionone ring (4-{2,6,6-trimethyl-2-cyclohexan-1-yl}-3-buten-2-one), the hydrophobic head; a conjugated isoprenoid side chain, which is subject to isomerization in the presence of light; and a polar terminal group, which can be enzymatically or chemically modified to become an ester as in retinol acetate, retinol palmitate, or an aldehyde as in retinal or be oxidized to a polar metabolite as in retinoic acid.

The all-*trans* retinal molecule, $C_{20}H_{30}O$ (MW 286.5) has an absorption maximum at 325 nm. Because of its structural configuration, a ring structure and a series of unconjugated double bonds, it readily undergoes oxidation in air and isomerized when exposed to light. Oxidation is the most important cause of its destruction. Vitamin A is also sensitive to ionizing radiation but generally stable in heat, and alkali solution. The ester forms are more stable than the free alcohol. Preserve in tight and light-resistant containers. (USP 23/NF 18,

1995) Vitamin A is found in animal products such as organ meats (the liver contains the highest amount), fish (particularly fish with higher fat content like tuna and sardines), fish oils, dairy products, and eggs. However, the principle sources of vitamin A in the diets are generally carotenoids, coming from fruits, dark-green leafy and yellow vegetables, and some cases of red palm oil.

Vitamin A is essential for vision, adequate growth, cellular differentiation, reproduction and the integrity of the immune system (Olson, 1987). The retina of the eye, which contains the light-receptor visual cells called “rod cells” enable us to distinguish between light and dark, is very sensitive to vitamin A deficiency. These cells contain a light-sensitive pigment called visual purple (rhodopsin) which is a complex of the membrane-bound protein opsin and vitamin A (11-cis-retinal). When a rod cell is exposed to light, the visual purple disintegrates, releasing electrical charges to the brain and these stimuli are translated into a composite picture that we see. At the same time, new rhodopsin is formed in the visual cells from opsin and 11-cis-retinal from all-*trans* retinal or retinol binding protein from liver releasing. Therefore, it is responsible for the early occular sight of night blindness, the inability to see in dim light. Vitamin A is also necessary for normal differentiation of epithelial cells, such as skin, mucous membranes, blood vessel walls and the cornea of the eye.

The biological activity of vitamin A is quantified by conversion of the vitamin A active components to retinol equivalents (RE). One RE is defined as 1 μg of retinol, 6 μg of β-carotene, or 12 μg of other provitamin A carotenoids. Vitamin A activity in foods was expressed as international units (IU), which are defined by the relationship of 1 IU = 0.300 μg of all-*trans* retinol, 0.600 μg of β-carotene, 0.344 μg of all-*trans* retinyl acetate, 0.359 μg of all-*trans* retinyl propionate, or 0.550 μg of all-*trans* retinyl palmitate (British Pharmacopoeia, 1998). Therefore, 1 RE is equal to 3.33 IU based on retinol and 10 IU based on β-carotene. The RDAs is 1,000 RE (5,000 IU) for adult men and 800 RE (4,000 IU) for adult women. Vitamin A deficiency is found most commonly, although not exclusively, in preschool children. Prominent signs of the deficiency are night blindness and xerophthalmia, which consists of progressively more severe changes in the conjunctiva and cornea of the eye (Olson, 1987).

2.4.2.1 Digestion and absorption of vitamins A (Olson, 1987 and Gerster, 1997)

In food, preformed vitamin A is present mainly as retinyl ester. In the stomach retinyl esters and various carotenoids are released from the food by proteolytic activity and then aggregate into globules with other dietary lipids. In the intestine, retinyl esters are hydrolyzed, and the products are associated first with lipid glublues and then with bile salt-containing micelles in

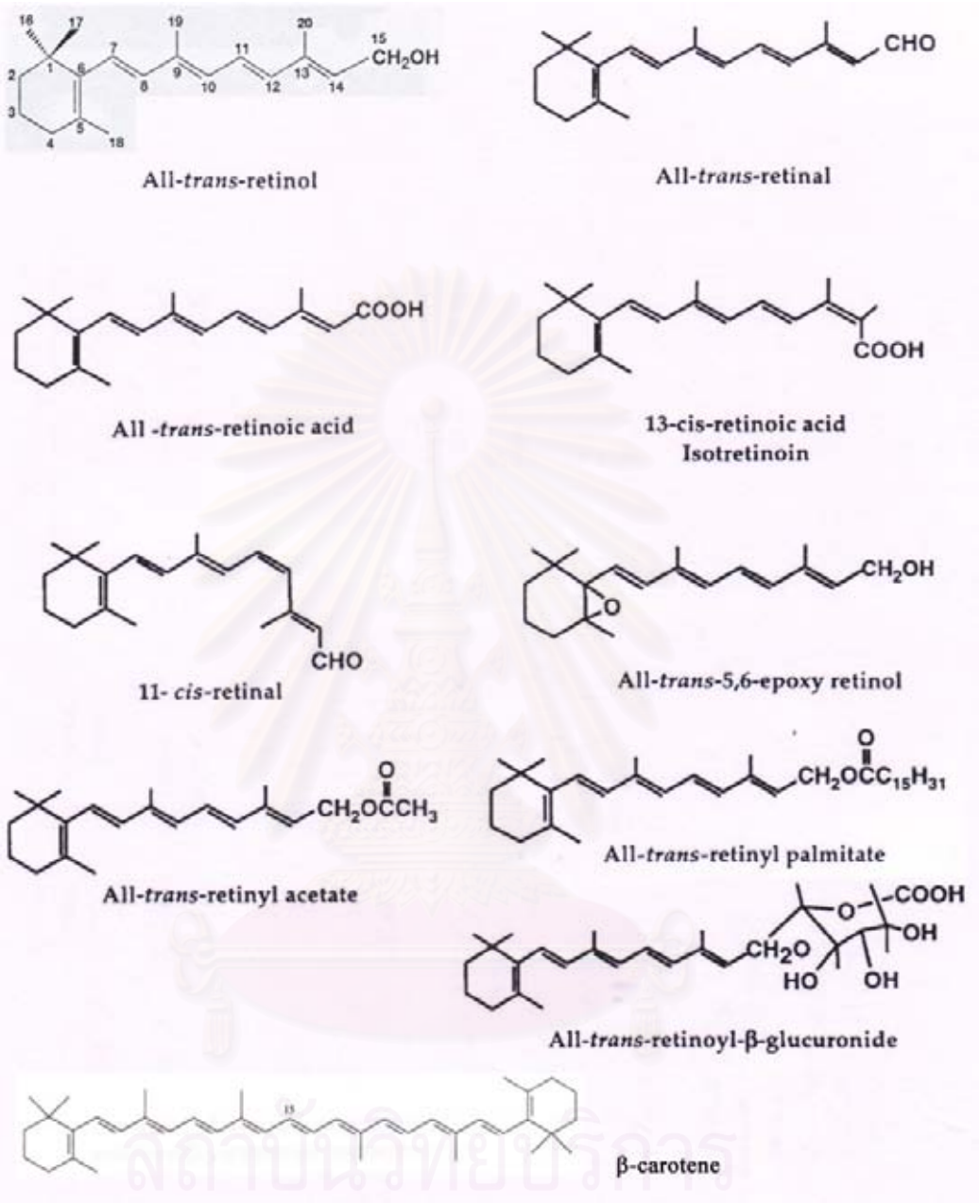


Figure 11 Structures of vitamin A metabolites and esters.

the intestinal lumen. Both vitamin A and carotenoids are absorbed best in the upper part of the small intestine; absorption efficiency decreased lower in the gut. Free retinol is then taken up by mucosal cells, bound to a specific cellular retinol binding protein (CRBP II) and esterified to long chain fatty acids to become mainly retinyl palmitate before being incorporated into chylomicrons for lymphatic transport to the liver. In the circulation, chylomicrons are transformed rapidly by the action of lipoprotein lipase (LPL) to chylomicrons remnants retaining most of the retinyl esters they carry, which are taken up mainly by hepatocytes in the liver. When initial liver reserves of vitamin A are low, part of the newly absorbed vitamin A is released into the blood as the unesterified free retinol which is a 1:1 complex with plasma retinol-binding protein (RBP) and delivered to target tissue. Vitamin A is released from the liver as holo-RBP. The RBP in holo-RBP is recognized by surface receptors on target cells, retinol is transfer across the plasmalemma into the cell, and the resultant apo-RBP is modified and released. When a retinol molecule enters a cell it becomes bound to a CRBP in the cytoplasm. Within target cells retinol is oxidized primarily to its major metabolite retinaldehyde (retinal), the chromophore for the protein rhodopsin; a small part of retinyl may be oxidized to retinoic acid (RA) involved in cellular differentiation and maintain normal growth. Intracellular RA is bound to cellular retinoic acid-binding proteins (CRABP) which may be important in controlling RA delivery to the nucleus for interaction with a nuclear retinoic acid receptor. Other metabolites include 9-*cis*-retinoic acid, 13-*cis*-retinoic acid and 4-oxo-metabolites.

In kidney disease, there may be either increased or decreased serum concentrations of vitamin A and RBP. Hypervitaminosis A is generally seen in both acute and chronic renal disease. In diabetic patients, serum concentrations of vitamin A and RBP have been found to be decreased (Basu, Tze and Leicheler, 1989). Rats made diabetic with streptozotocin have also been shown to have reduced levels of vitamin A in the serum while hepatic concentrations were elevated. Both human and animal studies point to the possibility of the vitamin A transport mechanism being defective in diabetes. (Basu and Dickerson, 1996) Martinoli *et al*. (1993) and Baena *et al*. (2002) also evaluated plasma retinol in 60 and 47 patients, respectively, with insulin-dependtdent (type I) diabetes mellitus. They found that plasma retinol is significantly decreased in younger type I diabetes patients. Furthermore, patients with type II diabetes have been found to reduce plasma levels of retinol while the levels of retinyl esters are elevated, suggesting an increased hepatic storage of vitamin A (Wako *et al*., 1986). The underlying mechanism for the subnormal plasma retinol and its increased hepatic concentration in diabetics is not properly understood. Wako *et al*. (1986) suggested

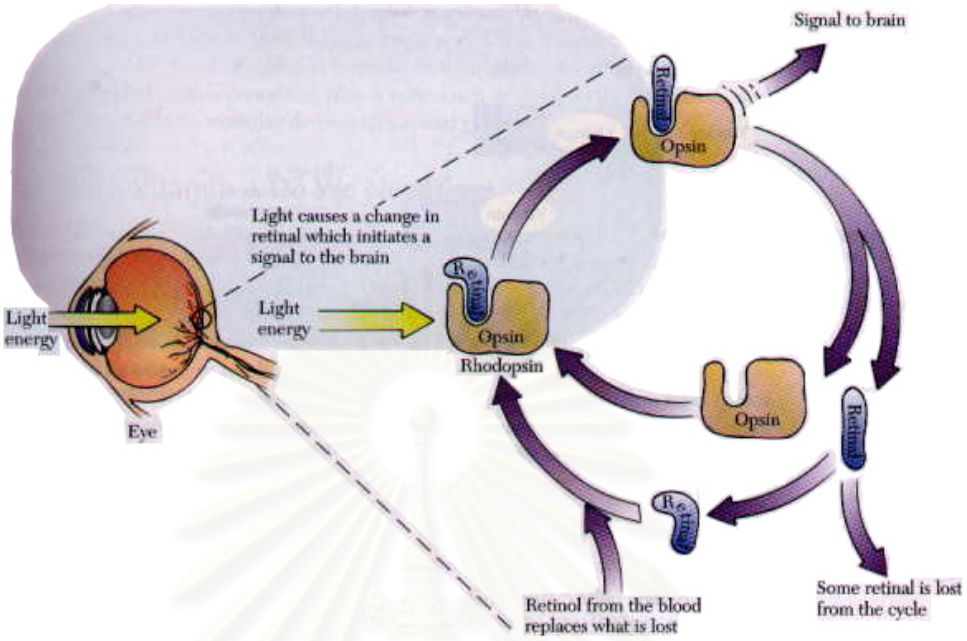


Figure 12 A visual cycle. (Smolin and Grosvenor, 1997)

that it may be possible appear an abnormal lipoprotein metabolism in patients with diabetes. Triglyceride (TG) is removed from chylomicrons at peripheral tissue by LPL action and ultimately chylomicrons turns to chylomicrons ramnant but LPL activity was decreased in insulin-independent diabetic patients. It seems that the retinyl ester is easy to transconvert from chylomicrons to another lipoprotein before absorbed by the liver and retinyl ester accumulates in plasma in diabetic patients. There is, however, accumulating evidence that diabetes mellitus may lead to zinc deficiency. Since zinc is an important factor for the hepatic synthesis of RBP, it is possible that the reduced serum retinol levels in diabetics reflect reduced mobilization of the vitamin from the liver.

2.4.1.1 Vitamin A determination (Eitenmiller and Landen, 1999)

Several methods are possible for vitamin A analysis in the serum. These include colorimetry, fluorometry, and high-performance liquid chromatography (HPLC) with a UV detector. (Basu and. Dickerson, 1996) The colorimetric Carr-Price technique and its many modifications have been commonly used and depend on the formation of a transient blue complex in the presence of antimony trichloride ($SbCl_3$) or trifluoroacetic acid (TFA; $C_2HF_3O_2$) and having an absorption maximum at 620 nm (Indyk, Konings and Horwitz (a), 2000). Both retinol and its esters and also carotenoids are detected by this method (Morrow *et al.*, 1991).

The main problems encountered in the use of the Carr-Price (antimony trichloride) method for the determination of vitamin A have been well known since its original description (by Carr, T.H. and Price, E.A. at 1926 in Biochemical journal). The antimony trichloride-chloroform reagent develops turbidity in the presence of trace amounts of moisture and the blue color characteristic formed by this reagent with vitamin A is subject to rapid fading. Neeld and Pearson (1963) have reported that TFA retained the sensitivity and specificity of the $SbCl_3$ reaction but did not exhibit the turbidity and film forming properties of the latter reagent in the present of moisture. This property suggested that TFA might not only be used to replace $SbCl_3$ in the conventional macro-determination of serum vitamin A but might also served as the basis for a new simple micro technique. To minimize oxidation of vitamin A, the antioxidants such as vitamin C, vitamin E, pyrogallol, butylated hydroxytoluene (BHT) and so on was add to the sample assay.

Vitamin D (Calciferol)

Cholecalciferol (vitamin D3) is the natural form of vitamin D. It is synthesized in the skin from endogenous or dietary cholesterol on exposure to UV radiation (sunlight). Activation of vitamin D requires both the liver and the kidney. Vitamin D, which has properties of both hormones and vitamins, is necessary for the proper formation of the skeleton and for mineral homeostasis. It is closely involved with parathyroid hormone,

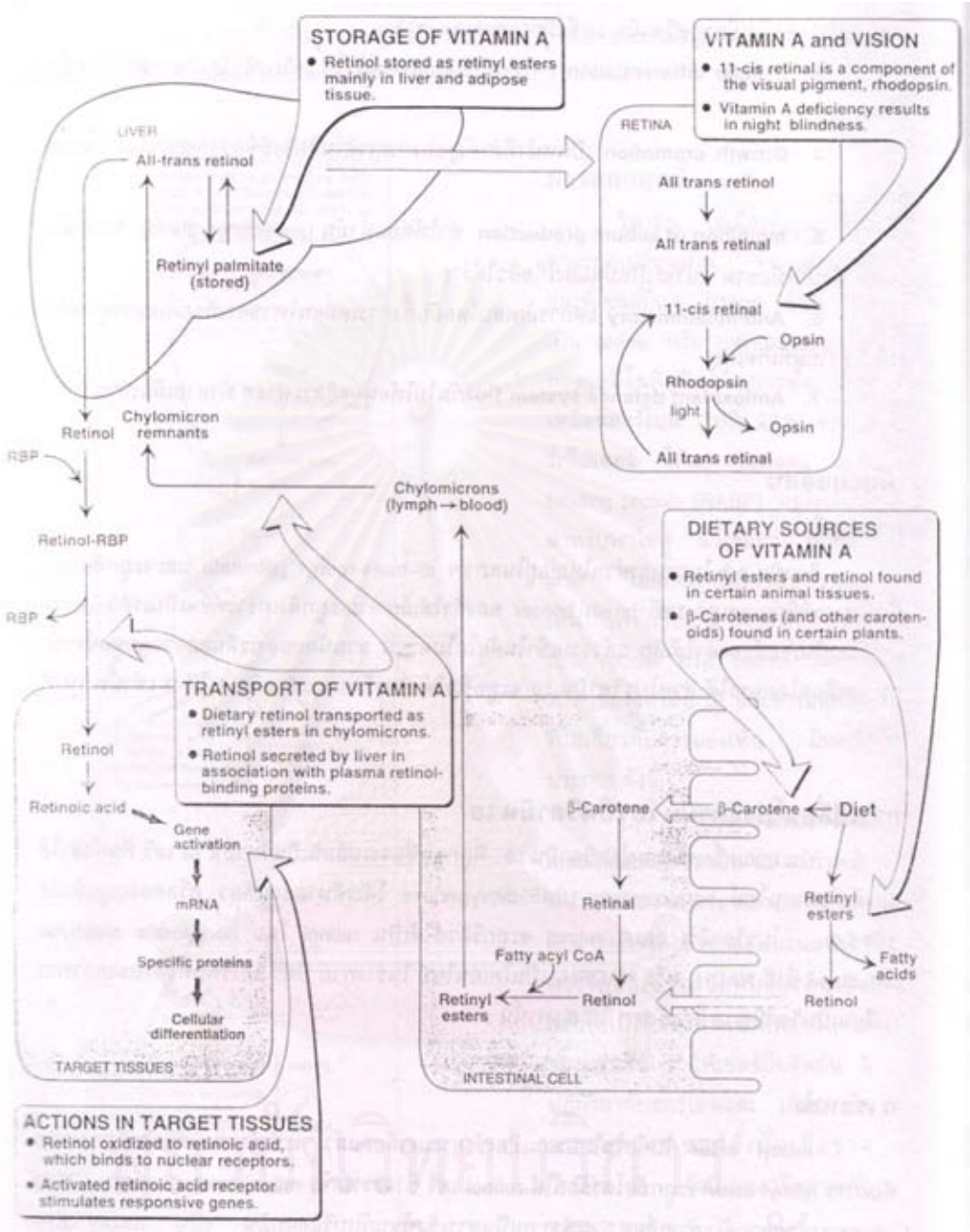


Figure 13 Fate of vitamin A in the human organism.

phosphate, and calcitonin in the homeostasis of serum calcium.

In the United States, given milk is routinely supplemented with 400 IU of vitamin D per quart. Vitamin D is stable, and normal food processing does not appear to alter its activity.

Vitamin E (Tocopherol)

Vitamin E is present in cell membranes. The term vitamin E refers to a series of eight compounds. The tocopherols and the tocotrienols are naturally occurring compounds in plants. Alpha-tocopherol, the most active of these compounds, is used to calculate the vitamin E content of food. Vitamin E functions primarily as an antioxidant in protecting cellular membranes from oxidative damage or destruction. Vitamin E deficiency is extremely rare but may occur in two population groups: premature, very low birth-weight infants; and patients who do not absorb fat normally, such as children with cystic fibrosis. The primary signs of vitamins E deficiency are reproductive failure and neurologic abnormalities.

Vitamin K

Phytonadione (vitamin K1) is present in many vegetables. Menaquinone (vitamin K2), is a product of bacterial metabolism. Menadione (vitamin K3) is a synthetic compound that is two to three times as potent as the natural vitamin K. Deficiencies do not readily occur because microbiologic flora of the normal gut synthesize enough menaquinones to supply a significant part of the body's necessity. However, because the absorption of vitamin K requires bile in the small intestine, anything that interferes with bile production or secretion may contribute to a vitamin K deficiency.

2.5 Digestive system

2.5.1 Gastrointestinal tract

The human gastrointestinal tract was shown in Figure 14. The first part is in the mouth. After the food has been softened it is then pushed down the esophagus into the stomach. Not much digestion actually takes place in the stomach. The only thing that is readily absorbed by the stomach is alcohol. The stomach creates an acidic environment (hydrochloric acid) for the enzymes to work in. The predominant stomach enzyme is pepsin, which breaks down proteins into short chains of amino acids. The summary of digestive process was shown in Table 6. Other nutrients are digested only after the food moves on out of the stomach, approximately three to four hours later. The small intestine (the duodenum, jejunum and ileum) has an alkaline environment and this is where digestion is completed after most nutrients have been absorbed (Table 7). The specialized absorbing cells, enterocytes, are distinguished by numerous microvilli that greatly increase the absorptive surface area. The pancreas secretes pancreatic juice into the duodenum. Lipase breaks down fatty substances into fatty acids and monoglycerides. Protease splits up protein and amylase which converts starch to sugar. After most nutrients have been assimilated, approximately three to ten hours later, the food moves

on into the colon or large intestine. The principle functions of the large intestine are to absorb water and electrolytes, store, and excrete food wastes. The colon contains large numbers of bacteria, which produces enzymes that act on the remaining food residues and fiber. The products of this bacterial digestion and fermentation include short-chain fatty acids and gases such as carbon dioxide, methane and hydrogen. The food is held there until it is eliminated.

Rat Intestine (Hebel and Stromberg, 1976)

The length and diameters of parts of intestinal tract was shown in Table 8. From the distal end of the pylorus, the descending duodenum runs transversely toward the right abdominal wall, rises dorsally after an initial curve along the right margin of the liver toward the right kidney, curves as transverse duodenum toward the midsagittal plane and after another right-angled turn runs cranially as ascending duodenum. The mucosa of the duodenum forms crestlike folds about 1 mm high and up to 5 mm wide. They lie mainly perpendicular to the long axis of the intestinal tube. Their free edge contains irregularly shaped, lobular or pointed process. Toward the ileum the villi decrease in height and width.

The jejunum is the longest part of intestine, its garland-like loops fill the right ventral part of the abdomen. However, the length of its mesentery (up to 70 mm) allows the appearance of jejunal loops in other parts of the cavity, *e.g.*, caudal to the stomach or dorsal and left of the cecum.

The flaplike villi of the jejunum almost uniformly 0.6 mm long and in cross section are of the same length. As in the duodenum they are oriented in the rows parpendicular to the long axis and the ileum the edges of villi in adjacent rows overlap each other.

The ileum is connected to the apex of the cecum by means of the triangular ileocecal plica. Its opening into the cecum is closely associated with the beginning of the colon: the narrow ileum opens into a villus-free antrum, which has a relatively narrow opening into the cecum.

2.6 Diabetes mellitus (Rifkin and Porte, 1991, Pickup and Williams, 1994 and Shils *et al.*, 1999)

Ancient civilizations in Egypt, Greece, Rome, and India recognized diabetes and the effect of dietary intervention. The Roman Aretaeus, AD 70, noted polydipsia and polyuria and named the contidion *diabetes*, meaning ‘to flow through’. Thomas Willis, a London physician, later introduced the term *mellitus*, or ‘honeylike’ after noting the sweet taste of urine.

Diabetes mellitus is defined as a disorder of carbohydrate metabolism caused by absence or deficiency of insulin, insulin resistance, or both, ultimately leading to hyperglycemia. Diabetes is a major health problem, main causes of death. The statistic rate

Table 6 Summary of digestive process. (Martin, Mayes and Rodwell, 1981)

Source of secretion and stimulus for secretion	Enzyme	Method of activation and optimal conditions for activity	Substrates	End products of action
Salivary glands of mouth: Secrete saliva in reflux response to presence of food in mouth.	Salivary amylase	Chloride ion necessary. pH 6.6 – 6.8	Starch Glycogen	Maltose plus 1:6 glucosides (oligosaccharides) plus maltotriose.
Stomach glands: Chief cells and parietal cells secrete gastric juice in response to reflex stimulation and chemical action of gastrin.	Pepsin	Pepsinogen converted to active pepsin by HCl. pH 1.0 – 2.0	Protein	Proteoses Peptones
	Rennin	Calcium necessary for activity. pH 4.0	Casein of milk	Coagulates milk
Pancreas: Presence of acid chyme from the stomach activates duodenum to produce (1) **secretin**, which hormonally stimulateds flow of pancreatic juice; (2) **cholecystokinin**, which stimulates the production of enzymes.	Trypsin	Trypsinogen converted to active trypsin by enterokinase of intestine at pH 5.2 – 6.0. Autocatalytic at pH 1.0 – 2.0	Protein Proteoses Peptones	Polypeptides Dipeptides
	Chymotrypsin	Secreted as chymotrypsinogen and converted to active form by trypsin. pH 8.0	Protein Proteoses Peptones	Same as trypsin. More coagulating power for milk.
	Carboxypeptidase	Secreted as procarboxypeptidase, activated by trypsin.	Polypeptides at the free carboxyl end of the chain.	Lower peptides. Free amino acids.
	Pancreatic amylase	pH 7.1	Starch Glycogen	Maltose plus 1:6 glucosides (oligosaccharides) plus maltotriose.
	Lipase	Activated by bile salts, phospholipids, colipase. 8.0	Primary ester linkages of triacylglycerol	Fatty acids, monoacylglycerols, diacylglycerols, glycerol.
	Ribonuclease		Ribonucleic acid	Nucleotides
	Deoxyribonuclease		Deoxyribonuclic acids	Nucleotides
	Cholesteryl ester hydrolase	Activated by bile salts.	Cholesteryl esters	Free cholesterol plus fatty acids.
	Phospholipase A2		Phospholipids	Fatty acids, lysophospholipids.
Liver and gallbladder: holecystokinin, a hormone from the intestinal mucosa- and possibly also gastrin and secretin-stimulate the gallbladder and secretion of bile by the liver.	(Bile salts and alkali)		Fats-also neutralize acid chyme	Fatty acid-bile salt conjugates and finely emulsified neutral fat-bile salt micelles.
Small intestine: secretions of Brunner's glands of the duodenum and glands of Lieberkuhn.	Aminopeptidase		Polypeptides at the free amino end of the chain	Lower peptides. Free amino acids.
	Dipeptidases		Dipeptides	Amino acids
	Sucrase	pH 5.0 – 7.0	Sucrose	Fructose, glucose
	Maltase	pH 5.8 – 6.2	Maltose	Glucose
	Lactase	pH 5.4- 6.0	Lactose	Glucose, galactose
	Phosphatase	pH 8.6	Organic phosphates	Free phosphate
	Isomaltase or 1:6 glucosidase		1:6 glucosides	Glucose
	Polynucleotidase		Nucleic acid	Nuclotides
	Nucleosidases (nucleoside phosphorylases)		Purine or pyrimidine nuclosides	Purine or pyrimidine bases, pentose phosphate

Table 7 Site of absorption of nutrients. (Martin, Mayes and Rodwell, 1981)

Site	Nutrient
Jejunum	**Glucose** and other monosaccharides; some disaccharides Monoacylglycerols, fatty acids, glycerol, cholesterol Amino acids, peptides **Vitamins**, folate Electrolytes, iron, calcium, water
Ileum	Bile acids Vitamin B12 Electrolytes, water

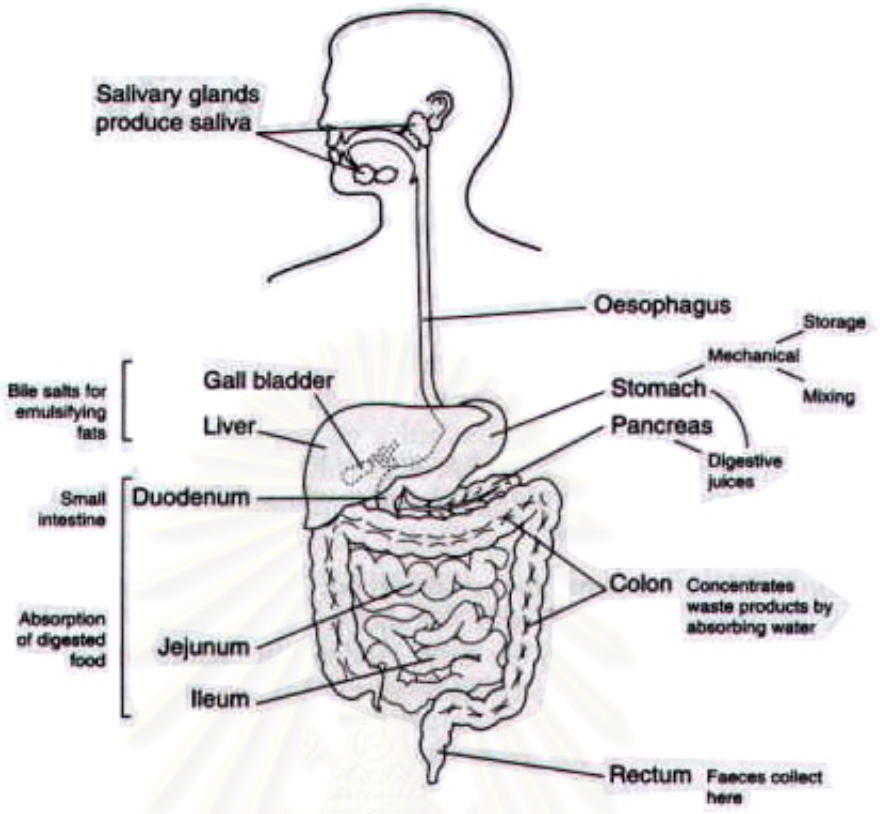


Figure 14 The human digestive system. (Piper, 1996)

Table 8 Length and diameters of parts of intestinal tract[1]
(Hebel and Stromberg, 1976)

Parts of intestinal tract	Length (mm)	Diameter (mm)
Duodenum	95 – 100	2.5 – 3
Jejunum	900 – 1350	4 – 5
Ileum	25 – 35	3 – 5
Cecum[2]	50 – 70	10
Colon	90 – 110	10 - 3[3]
Rectum	80	3 – 10
Total	12 - 17	

[1] Lengths post mortem without fixation; diameters are to be taken as approximations.

[2] Increased extensively in germ-free animals. The weight of the small intestine is 1.93%, that of the large intestine 0.89% of body weight.

[3] Decreases in diameter from 10 mm proximally to 3 mm distally. For effects of different diets, see Wierda, J.L. Measurements and observations upon the intestine of rats fed unbalanced and supplemented diets. *Am. J. Anat.* 70: 433-453.

of diabetes mellitus causing death in Thailand trends to increase from 7.5 per 100,000 population in 1996 to 12.2 per 100,000 population in 2000 (The Ministry of Public Health, 2001). The number of diabetes cases (particularly type II diabetes) will increase substantially in the next few decades, from 30 million estimated cases worldwide in 1985 to 220 million in 2010 (Shaw *et al*., 2000) and up to 300 million in 2025. As diabetes is characterized by metabolic abnormalities, the most evidence is hyperglycemia, an elevated concentration of glucose in the blood. Diabetic men were more than twice likely to die of all causes compared with men without diabetes. As blood glucose increased, the risk of dying climbed higher regardless of age, weight, blood pressure, cholesterol and smoking status. An aggressive and intensive control of elevated levels of blood sugar in patients with diabetes is absolutely essential. Aggressive control with intensive therapy means achieving fasting glucose levels between 70 to 120 milligram per decilitre; glucose levels of less than 180 milligram per decilitre after meals; and a near normal glycohemoglobin (gHb A_{1C}) levels. Researchers estimated that a reduction in blood glucose by just 0.1 per cent could reduce mortality rates by about 5 per cent.

2.6.1 Classification of diabetes mellitus:

Diabetes is typically classified into four different types that appear to differ in etiology and pathogenesis. Type I and type II diabetes are the major clinical forms of diabetes in Western world, while malnutrition-related diabetes is as major clinical form in parts of Africa, Asia, and the Caribbean. Type I diabetes accounts for approximately 5% of diabetes. Type II diabetes accounts for approximately 90% of diabetes. Other causes account for the remaining 5% of diabetes in the United States.

Type I diabetes mellitus is also called insulin dependent diabetes mellitus (IDDM), or juvenile onset diabetes mellitus. It is characterized by absolute deficiency of insulin caused by β -cell destruction of the islets of Langerhans in the pancreas. It is an autoimmune disease in which the pancreas produces no insulin at all, and the patient relies on insulin medication for survival. Type I diabetes tends to occur in young, lean individuals, usually before 30 years of age. Typical symptoms are thirst, fatigue, cramps, excessive urination, increased appetite, and weight loss. Treatment requires administration of insulin, diet coordinated with insulin dosage schedule, and regular physical exercise. There is no cure for this type.

Type II diabetes mellitus, the most common form of diabetes, is also referred to as non-insulin dependent diabetes mellitus (NIDDM), or adult onset diabetes mellitus (AODM). It is characterized by two primary defects: insulin resistance and abnormal insulin action (diminished tissue sensitivity to insulin) and impaired β-cell function (delayed or inadequate insulin release). Type II diabetes mellitus occurs mostly in individuals over 40 years old. Unlike type I diabetes mellitus, 80% of type II diabetic patients are obese. Type II diabetes mellitus also has a strong genetic tendency. It is nearing epidemic proportions, due to an

increased number of elderly people, a greater prevalence of obesity and a sedentary lifestyle. In type II diabetes, patients can still produce insulin, but inadequately. The pancreas in these patients not only produces an insufficient amount of insulin, but also releases insulin late in response to increased glucose levels. Some type II diabetics have body cells that are resistant to the action of insulin (Insulin Resistance). Finally, the liver in these patients continues to produce glucose despite elevated glucose levels. Type II diabetes once hardly ever struck before middle age, and the incidence of type II diabetes increases with age.

Malnutrition-related diabetes (MRDM) is usually found in young people, and is characterized by severe protein malnutrition and emaciation. These diabetes patients are characterized by severe hyperglycemia. They are dependent on insulin for preservation of health and life although they are not dependent on insulin for prevention of ketosis. For the other types of diabetes associated with certain other conditions or syndromes can be divided into many subclasses, such as pancreatic disease, hormone etiology, drug- or chemically induced, certain genetic syndromes, insulin receptor abnormalities or others.

2.6.2 Complications of Diabetes

Short term complications are normally due to imbalance of sugar level in the body as a result of severely high blood sugar levels owing to lack of insulin. Lack of insulin causes the breakdown of fat cells, with the release of ketones into the blood. Symptoms of diabetic ketoacidosis include nausea, vomiting, and abdominal pain. Without prompt medical treatment, patients with diabetic acidosis can rapidly turn into shock, coma, and even death. With proper treatment, the symptoms can be reversed rapidly, and patients can recover remarkably well. For patients with type I diabetes, deficiency of insulin can develop severely elevated blood sugar levels and leads to increase urine glucose, which in turn leads to excessive loss of fluid and electrolytes in the urine. Abnormally low blood sugar levels happen because of too much insulin or other glucose-lowering medications. Low blood sugar can lead to nervous system symptoms such as dizziness, confusion, weakness, and tremors. Untreated, severely low blood sugar levels can lead to coma and irreversible brain death.

Long-term complications are related to blood vessel diseases. In its fully developed clinical expression, it is characterized by fasting hyperglycemia, and in the majority of long-standing patients by microangiopathic vascular complications. Diabetes causes diseases of the small vessels, which can damage the eyes, kidneys, nerves, and heart. Eye complications of diabetes (diabetic retinopathy) occur in patients who have had diabetes for at least 5 years. Diabetes is the main cause of blindness in adult. Kidney complications of diabetes (diabetic nephropathy) usually occur approximately 10 years after the onset of diabetes. Symptoms of nerve damage in diabetes (diabetic neuropathy) are numbness, burning, and aching of the feet and lower extremities. Diabetic nerve damage can affect the nerves of the intestines, causing nausea, weight loss, and diarrhea. About 60-70% of people with diabetes have mild to severe

form of diabetic nerve damage. Patients with diabetes are 2 to 4 times more likely to have heart disease and strokes. Kunjathoor, Wilson and LeBoeuf (1996) also found increased atherosclerosis in streptozotocin (STZ)-induced diabetic mice. Atherosclerosis is characterized by a thickening of the internal layer of the wall major blood vessels, especially arteries, resulting in a constriction of the vessel lumen and a restriction of blood flow and vessel elasticity. This promotes the formation of occlusive blood clots and can result in injury to heart, brain, and lung tissue, which lead to coronary heart disease (angina or heart attack) and may be fatal.

Dietary treatment is primary therapy in type II diabetes and is vital treatment to type I diabetes. It is recommended to consume a high-carbohydrate, high-fiber, low-fat and high vitamin diet especially vitamin A which reduces the risk of blindness in diabetes.

CHAPTER II

MATERIALS AND METHODS

1. MATERIALS

1.1 Equipments

Accutrend® GCT (Roche Diagnostics, Germany), Accutrend® glucose strip II (Roche Diagnostics, Germany), Analytical balance 4 positions Model A200S (Sartorius, Germany), Centrifuge Model EBA 12R (Hettic Zentrifugen), Conductivity meter, CONSORT, C535 version 1.1, Dialysis tubing nitro-cellulose membrane, flat width 1.0 and 2.5 cm, MW cutoff 12,000 (Sigma Chemical Co., USA.), Hot air oven (Memmert, Becthai Co.Ltd., Thailand), HPLC: FID (Shimadzu Corporation, Japan); (LDC Analytical, ConstaMetric® 4100 solvent delivery system, LDC Analytical, Refractomonitor® IV, Refractive index detector, LC, S1L-1A injector, C-R1A Chromatopac integrator, Column type Amino propyl silyl, LiChrospher), Magnetic stirrer type KMO 2 electronic (Janke & Kunkel, KIKA-WERK), Membrane filter sterile, pore size 0.45 μm (Whatman®, England), Nest of sieve, 60 Mesh (Endecotts, England), Nitrogen gas tank, Omnibath (Gilson®, USA.), Organ bath (or tissue chamber), Osmomat Model O30-D with Ryoscopic osmometer printer, Oxygen gas tank, pH meter type Model MP 230 (Mettler Toledo, Switzerland), Separatory funnel (Kimax, USA.), Spectrofluorometer Model FP-777 (Jasco, Japan), Spectrophotometer type Spectronic 21 (Bausch & Lamb, Germany), Suction apparatus; (Buchner funnel, Aspirator, SIBATA circulating aspirator WJ-20, Japan, Filter paper No. 93 (Whatman®, England)), Syringe filter; Cellulose acetate filter media, diameter 13 mm., pore size 0.45 μm (ORANGE SCI), UV-VIS spectrophotometer Model Spectronic® GENESYS™ 5 (Spectronic instrument, Inc., New York, USA.), Vertex type Retsch Mixer, Viscometer Model DV-I+, version 5.0 (Brookfield, USA.), Water bath Model 350R (Memmert, Becthai Co.Ltd., Thailand)

1.2 Chemicals

The following substances were commercial available, glacial acetic acid of analytical grade obtained from BDH, England; trichloroacetic acid (TCA) of analytical grade from Farmitalia Carco Erba; sodium hydrogen carbonate of GR grade, was obtained from Fisher Scientific UK Limited, Loughborough, Leics, UK.; 95% ethyl alcohol obtained from the Excise department of Thailand. Chloroform and ether of analytical grade were obtained from J.T. Baker, Phillipsburg, USA.; trifluoroacetic acid (TFA) of analytical grade was obtained from Fluka-Garantie, Switzerland; acetronitrile HPLC grade was obtained from Mallinckrodt Chemical Co., Paris, France. L (+) ascorbic acid (VitaminC), calcium chloride, magnesium sulfate, potassium chloride, potassium dihydrogen phosphate, potassium hexacyanoferrate (III), potassium hydroxide, sodium bicarbonate, sodium chloride and o-toluidine all of GR grade, were obtained from E. Merck, Darmstadt, Germany; ethyl alcohol absolute, D (+) glucose monohydrate, iso-butyl alcohol, hydrochloric acid, sucrose, sulfuric acid of analytical grade obtained from E. Merck, Darmstadt, Germany. All-trans retinyl acetate (Vitamin A) and thiamine hydrochloride (Vitamin B1) of USP grade were obtained from Sigma Chemical Co., USA.; *o*–dianisidine hydrochloride, quinine sulfate salt, sodium taurocholate (Bile salt) of analytical grade were obtained from Sigma Chemical Co., USA.; glucose oxidase enzyme from *Aspergillus niger* (EC 1.1.3.4) 20,000 units/g solid and horseradish peroxidase enzyme (EC 1.11.1.7) 200 units/mg solid were obtained from Sigma Chemical Co., USA.

3. Methods

3.1 Preparation of polysaccharide gel (PG)

A polysaccharide gel (PG) was isolated from dried fruit-hulls of durian (*Durio zibethinus* L.). Fresh durian fruit-hulls waste was collected and cleaned in water. Then cleaned durian fruit-hulls were cut, blended and dried in hot air oven at 70 °C until dried. Crude PG was extracted by using hot water and acid-ethanol precipitation. The process of PG isolation and purification was performed by modifying the method previously described by Pongsamart and Panmaung (1998).

The PG solution was freshly prepared by dissolving PG powder in Ringer buffer (pH 7.0) (appendix A) to make a series of concentration before use.

3.2 Preliminary study on glucose entrapment property of PG by dialysis technique. (Jenkins *et al.*, 1980 and Adiotomre *et al.*, 1990)

Dialysis apparatus was made of 10 cm strips of cellulose membrane dialysis tubing, flat width 2.5 cm, MW cutoff 12,000, which was soaked in distilled water and then treated in

Ringer buffer (pH 7.0) overnight before use. One end of dialysis tube was fasten to make a bag and tied with a magnetic bar. Three bags were prepared for each test. Glucose (MW 180.16 g/mol) was dissolved in Ringer buffer (pH 7.0) to make concentrated stock solution. Mixed glucose stock solution and previous made PG stock solution together to make 6 mL mixtures having final concentrations of 1% glucose in 0.5 to 2.0 % PG as test mixtures. Control samples were identical except that PG was omitted from the medium.

The test mixture was placed inside the dialysis bag and the other end of the dialysis bag was fasten tied, air bubble was avoid. The dialysis bag was then placed in 60 mL of Ringer buffer and covered with parafilm to protect evaporation. Dialysis was performed on magnetic stirrer with continuous stirring. After dialysis ended, the bag was removed.

3.2.1 Analysis of glucose by using Accutrend® meter GCT

The solutions inside and outside dialysis bag of each experiment were analyzed for glucose concentration, using glucose oxidase/mediator reaction test strips for glucose analysis, by quickly applying a 20 microliters of the dialysis solutions to cover test pad completely, without touching the test pad. Display shows the result of glucose concentration in milligram per decilitre. Glucose concentration of each experiment was a mean value of three repeated analyses.

3.2.2 Analysis of glucose by colorimetric method using *o*-toluidine test for reducing sugars. (Hyvarinen and Nikkila, 1962)

The color reagent was prepared by dissolving 1.5 g of thiourea in 940 mL of glacial acetic acid and mixed with 60 mL *o*-toluidine. Kept the colorless color reagent in a brown container away from direct sunlight and from contact with metal or rubber. Dissolved 3.0 g of trichloroacetic acid (TCA) in 100 mL of distilled water. Prepared a standard glucose by dissolving 50 mg of glucose in 100 mL of distilled water.

Added glucose solution to be analyzed after dialysis into 0.4 mL of TCA solution in clean test tube and adjust the volume to 1.0 mL with distilled water. Added 5.0 mL of color reagent and mixed. Placed the tubes (three tubes for each assay) in boiling water bath for 8 minutes and cooled. Measured the blue-green color by using a spectrophotometer type Spectronic 21 at 630 nanometer, a blank contained distilled water instead of glucose test solution. The test solution was diluted of necessary for the most linearly accurate measurements at absorbance in the range 0.2 to 0.7. Glucose standards at least 5 concentrations were run through the same procedure. Plotted graph of absorbance at 630

nanometer vs. glucose concentrations and used for calculation the glucose concentration of the test solution.

3.3 *In vitro* study on glucose entrapment property of PG and glucomannan (GM) by dialysis technique using semi-permeable membrane

3.3.1 Stock glucose was prepared in Ringer buffer (pH 7.0). Stock glucose was mixed with 0 to 2.0 gram per cent of homogeneous PG solution in Ringer buffer (pH 7.0) to make various glucose concentrations of 0, 0.5, 1.0, 1.5 and 2.0 g% w/v (g per 100 ml) at final volume of 4 ml. The mixture was mixed homogeneously. Twenty-centimeter length dialysis membrane (flat width 1.0 cm, MW cutoff 12,000) was prepared in the same manner as preliminary study on glucose entrapment property of PG (3.2). Each bag was filled with 4 ml of glucose mixture and quickly tied the upper end. The bag was suspended separately in 60 ml Ringer buffer (pH 7.0) and stirred continuously with constant rate by magnetic stirrer for 2 hours. Then, the dialysis bag was removed, outer and inner solutions of the bag were analyzed for glucose by colorimetric method using glucose oxidase test.

3.3.2 Glucose concentration at 1 g% was selected to study on glucose entrapment in PG using various PG concentration of 0.0, 1.0, 2.0 and 3.0 g% of PG. Dialysis time was 0, 1, 2, 3 and 4 hours, respectively. Outer and inner solution of dialysis bag were analyzed for glucose by colorimetric method using a glucose oxidase test.

3.3.3 Glucomannan (GM), as positive control, was prepared in various concentrations of 0, 1.0, 2.0, and 3.0 g% with 1 g% of glucose and study on glucose entrapment of GM by dialysis technique using semi-permeable membrane at 2 hours of dialysis. Outer and inner solution of dialysis bag were analyzed for glucose by colorimetric method using a glucose oxidase test.

3.3.4 Analysis of glucose by colorimetric method using glucose oxidase test. (Oser, 1965 and Bauer, Ackerman and Toro, 1974)

The reaction mixture contained a solution to be analyzed and adjust the volume to 0.4 mL with Ringer buffer (pH 7.0), added 0.2 ml of distilled water, 1.0 mL of each glucose oxidase (5 mg/ml), peroxidase (0.375 mg/ml) and *o*-dianisidine dihydrochloride (13.32 mg/ml). Reaction mixture was incubated in water bate at 37 °C for 10 minutes. After incubation the enzymatic reaction was stopped by adding 0.5 ml of concentrated sulfuric acid (H_2SO_4) and mixed well. Standards of glucose and blank reaction mixture without glucose were prepared together by the same procedure. The purple-red color of glucose standards and

samples were examined by using spectrophotometer type Spectronic 21 at 543 nanometer. Glucose standards at least 5 concentrations were run through the same procedure. Graph of absorbance at 543 nanometer vs. glucose concentrations was plotted and used for calculation the glucose concentration. The test solution may be diluted for the most linear accurate measurements of absorbance in the range 0.2 to 0.7.

3.4 *In vitro* study on glucose entrapment property of PG by dialysis technique using everted jejunal sacs of rats. (Blackburn and Johnson, 1981, Johnson and Gee, 1981 and Ferraris, Casirola and Vinnakota, 1993)

3.4.1 Jejunal sac preparations.

The jejunal small intestine (at about 10 centimeters of intestine distal to the pylorus) was obtained from carcass of male Wistar rats provided by animal laboratory, Department of Pharmacology, Faculty of Pharmaceutical Sciences. The organs were placed in ice-cold Ringer buffer (pH 7.0), and bubbled the solution with gas oxygen. The intestine was carefully everted over by using a glass rod to intrude outside in. The everted intestine was cut into pieces of 7 centimeters segments and quickly tied at one end of segment. The everted jejunal sacs were filled with 2.0 ml of Ringer buffer (pH 7.0) and quickly tied at the other end.

3.4.2 Absorption of glucose mixture in PG into intestinal sac.

Glucose was mixed with 0.0, 2.0, and 3.0% of PG in Ringer buffer (pH 7.0) to make 1 g% glucose concentration in final volume of 60 ml. The glucose mixture was mixed well. Then placed the previous prepared everted jejunal sacs filled with Ringer buffer (pH 7.0) in the mixture of PG and glucose by using the organ bath for incubation at 37 °C controlling temperature water from omnibath and bubbled with gas oxygen. Four sacs were prepared for each PG concentration. After 1 hour of incubation, the sac was removed and the buffer solution in the sac was collected by clean needle-syringes. The buffer solution inside the everted intestine sac was assayed for glucose by colorimetric method using a glucose oxidase test.

3.5 Preliminary study on sucrose entrapment property of PG by dialysis technique.

Dialysis apparatus was made of 10 cm strips of cellulose membrane dialysis tubing, flat width 2.5 cm, MW cutoff 12,000, which was soaked in distilled water and then treated in 0.1 M phosphate buffer (pH 7.0) (appendix A) overnight before use. One end of dialysis tube was fasten to make a bag and tied with a magnetic bar. Three bags were prepared for each test. Sucrose (MW 342.30 g/mol) was dissolved in phosphate buffer to make concentrated

stock solution. Mixed sucrose stock solution and previous made PG stock solution together to make 6 ml mixtures having final concentrations of 2 % sucrose in 0.5, 1.0, 2.0 and 3.0 % PG as test mixtures. Control sample was identical except that PG was omitted from the medium. The PG mixture inside the dialysis bag was collected after 3 hours and PG was precipitated by adding ethanol to make 60% ethanol and centrifugation. The aqueous ethanol solutions were collected, and dried by evaporating, and then dissolved in 6 ml distilled water, and filtered. The solution was analyzed for sucrose by using HPLC.

The solutions inside and outside dialysis membrane were analyzed using normal phase HPLC system with refractive index detector, compared to standards glucose and sucrose. Mobile phase of 80% acetronitrile in double distilled water was used and flowed at the rate of 1.8 ml per minute.

3.6 Preliminary study on vitamin A entrapment property of PG by dialysis technique using semi-permeable membrane

Ten-centimeter lengths of dialysis bags (flat width 2.5 cm, MW cutoff 12,000) was prepared the same procedure of preliminary study on glucose entrapment property of PG (3.2). The vitamin A is preferred since it is more stable and gives the same amount of color on a molar basis, so does the retinol (Analytical methods committee, 1985). Stock all-*trans* retinyl acetate (vitamin A, $C_{22}H_{32}O_2$, 328.5 g/mol) was prepared in chloroform ($CHCl_3$) and stored in tightly stoppered brown bottle in the refrigerator (BP, 1998). The L (+) ascorbic acid solution was used for minimized oxidation of vitamin A (Analytical methods committee, 1985). Stock 100 mg/ml of L (+) ascorbic acid ($C_6H_8O_6$, 176.13 g/mol) was prepared in Ringer buffer (pH 7.0) and stored in brown bottle in the refrigerator. Stock 10 mg/ml of sodium taurocholate (bile salt, 537.7 g/mol) was prepared in Ringer buffer (pH 7.0) as an emulsifying agent. PG at various concentrations of 0.0, 1.0, 2.0, and 3.0 g% was prepared homogeneously in Ringer buffer (pH 7.0). Each test mixture contained with 10,000 IU (3.44 mg) of vitamin A, 50 mg/ml of L (+) ascorbic acid solution and 5 mg/ml of aqueous sodium taurocholate. The test mixture was mixed well and quickly filled in dialysis bag, tightly tied and dialyzed in 60 ml Ringer buffer (pH 7.0) in organ bath, closed with parafilm and covered with aluminum foil, and dialyzed for 2 hours, the task must perform under nitrogen gas in the dark room. The solutions containing vitamin A or L (+) ascorbic acid are particularly sensitive to actinic light so all reagents were kept in amber glassware or glassware protected from light by a covering of aluminum foil.

3.6.1 Analysis of vitamin A by colorimetric method using trifluoroacetic acid test.

The test solution inside the dialysis bag was collected and vitamin A was extracted by using a volume of absolute ethanol and two volume of petroleum ether. The upper layer of

petroleum ether was collected and evaporated to dryness with nitrogen gas (Bauer and Toro, 1974). The vitamin A residue was redissolved in 1.0 ml of $CHCl_3$. The quantitation of vitamin A was analyzed by using a colorimetric method (Indyk, Konings and Horwitz (a), 2000). Trifluoroacetic acid (CF_3COOH, TFA) reagent was prepared by 1:2 (v/v) of TFA in $CHCl_3$ and stored in tight glass-stopped brown bottle. The solution of vitamin A residue was added 9 volume of TFA reagent within 1 to 2 seconds, the blue color product was appeared. Read the absorbance at exactly 30 seconds after addition of the reagent (Neeld and Pearson, 1963) by using spectrophotometer at 620 nm, adjusted zero absorbance with blank ($CHCl_3$: TFA reagent = 1:9). Making similar readings with a series of vitamin A standards by diluting a stock vitamin A with $CHCl_3$. Plot graph of absorbance at 620 nanometer vs. vitamin A concentrations and calculated the vitamin A concentration of test solution (Freed, 1966).

3.7 Preliminary study on vitamin B1 entrapment property of PG by using dialysis membrane

Ten-centimeter length dialysis bags (flat width 2.5 cm, MW cutoff 12,000) were prepared in the same procedure of preliminary study on glucose entrapment property of PG (3.2). Thiamin hydrochloride (vitamin B1, $C_{12}H_{17}ClN_4OS.HCl$, 337.27 g/mol) stock solution was prepared in distilled water and stored in tightly stoppered brown bottle in the refrigerator (USP 23/NF 18, 1995) until used. Vitamin B1 stock solution was mixed with homogeneous PG solution in Ringer buffer (pH 7.0) to make 0.0, 1.0, 2.0 and 3.0 g% of PG and 12.5 mg/ml of vitamin B1 at 4 ml final volume. The mixture was mixed well and quickly filled in dialysis bag, then tightly tied and dialyzed in 60 ml Ringer buffer (pH 7.0) in organ bath, closed with parafilm and covered with metal foil under nitrogen gas in the dark room. After 2 hours of dialysis, the solutions inside and outside of dialysis bag were collected.

3.7.1 Analysis of vitamin B1 by using fluorometric method (Freed, 1966, USP 23/NF 18, 1995 and Indyk, Konings and Horwitz (b), 2000).

PG mixture inside dialysis bag was added ethanol to make 60% ethanol in order to precipitate PG and centrifuged. The aqueous solutions were collected, and dried under nitrogen gas, and then redissolved in distilled water. The vitamin B1 standard solution was prepared by dissolved 25 mg of vitamin B1 in 20% ethanol adjusted to pH 3.5 to 4.3 with 0.1 N HCl, diluted to 250 ml with the acidified ethanol, and stored in glass-stopped and light-resistant bottle in refrigerator. Prepared vitamin B1 standard series by diluting vitamin B1 standard solution with 20% ethanol. The thiamin standard and vitamin B1 samples inside and outside membrane were oxidized to thiochrome by using oxidizing reagent, which was previously prepared by diluted 4.0 ml of 1% of potassium ferricyanide solution ($K_3Fe(CN)_6$) in distilled water to 15% of sodium hydroxide solution (NaOH) and adjusted them into 100

ml volume which was used within 4 hours. For each of tube that was protected from light, 1.5 ml of oxidizing reagent was quickly added to 1.5 ml of the test solution and immediately added 6.5 ml of isobutanol. The tube was shaken vigorously for 2 minutes and centrifuged. Pipetted an isobutanol extract in upper layer into four clear side cell for thiochrome fluorescence measurement in a fluorometer at 365 nm excitation and 435 nm emission. The blank of each tube was treated similarly except replace oxidizing reagent with 15% of NaOH solution.

3.8 Statistical analysis

Results are presented as means percentage ± S.E.M. (standard error of means). Graph were constructed using Microsoft excel plot, bars on graphs represent mean ± S.E.M. Comparisons between groups were assessed by using one-way analysis of variance (ANOVA) followed by posthoc testing and *p*-values were adjusted by Tukey test (HSD, honestly significant difference) to evaluate significant difference between groups. Statistical significant differences between groups was assumed at $p < 0.05$. Data expression was carried out using SPSS version 10.0 for Windows (SPSS Inc., Chicago, USA.).

CHAPTER III

RESULTS

1. Preparation of polysaccharide gel (PG).

A polysaccharide gel (PG) was isolated and purified from dried fruit-hulls of durian (*Durio zibethinus* L.) by modifying the method of Pongsamart and Panmuang (1998). The ground dried hulls of durian fruit were gently boiled in acidic water. The crude PG was extracted, evaporated and precipitated with acid alcohol. The precipitate was dried, ground and 60-mesh sieved to obtain the crude PG fraction. The more purified PG was performed by using aqueous crude PG and re-precipitated to obtain more purified PG fraction. The purified extract was an amorphous powder, light-brown color and specific odor. Percentage yield of the purified extract was 4.890 ± 0.151 % of dried fruit-hulls of durian. The purified PG extract was used in this study. PG powder was homogeneous soluble in distilled water, it was swelled and formed a viscous clear liquid. The pH value was determined by pH meter, and viscosity was measured by using Brookfield digital viscometer at 100 rpm of shear rate at room temperature. Figure 15 and 16 show profiles of the pH and viscosity of aqueous solutions at 1.0, 2.0 and 3.0% w/v PG, data were mean scores and the standard error of mean (S.E.M.). All apparent viscosity values were presented in unit of milliPascal second (mPa.s), which is equal to centipoises (cPs). Increasing concentration of PG resulted significantly decreased pH value ($p < 0.05$) and significantly increased viscosity ($p < 0.05$), of PG, respectively.

2. Preliminary study the effect of PG on glucose entrapment by dialysis technique.

Previous studies of Pongsamart and Panmaung (1998) suggested that PG was similar to gum and mucilage, and Tippayakul (2002) found that PG has properties of lipids entrapment. Therefore, the purpose of this study was to investigate the property of PG in glucose entrapment. The dialysis bag of semipermeable cellulose membrane was used in comparison with the dissected small intestine of rat in order to study *in vitro*. The results were demonstrated in Figure 17 and 18 according to the method of analysis of glucose as the followings:

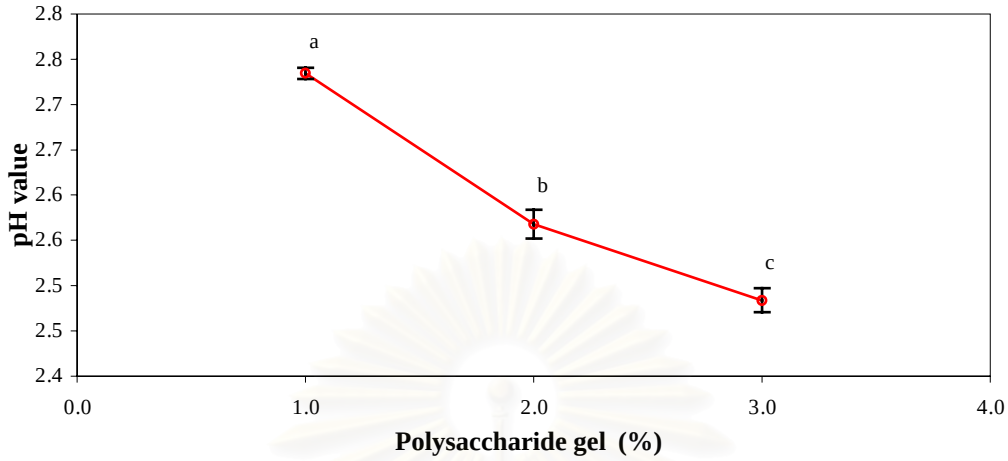


Figure 15 Effect of concentration on the pH value of polysaccharide gel (PG) in distilled water at room temperature. a, b, c = significant difference between tested concentration of PG. Data are mean ± S.E.M..

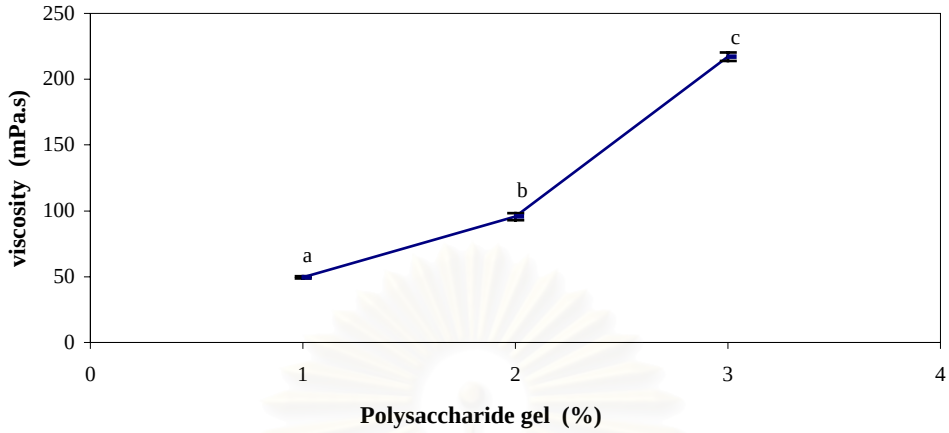


Figure 16 Effect of concentration on the viscosity of polysaccharide gel (PG) in distilled water at 100 rpm of shear rate at room temperature. a, b, c = significant difference between tested concentration of PG. Data are mean ± S.E.M..

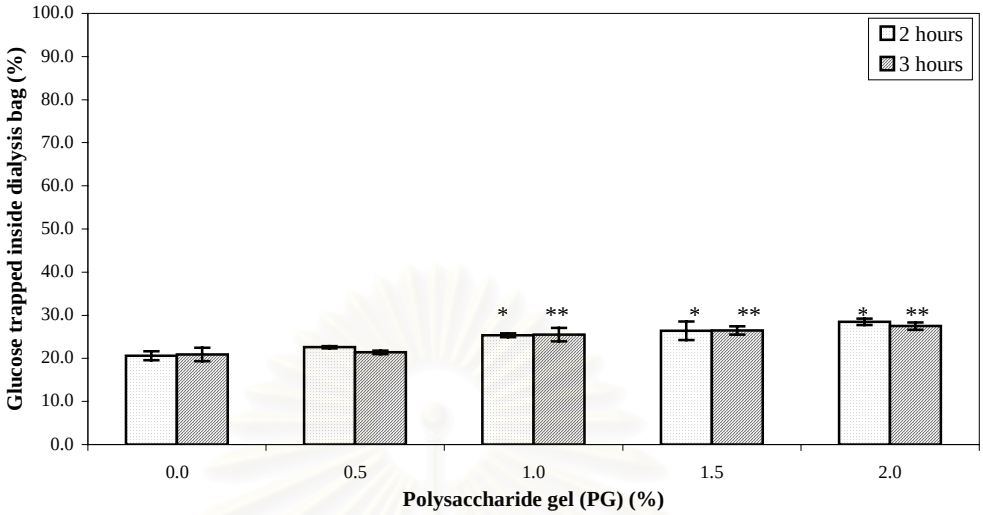
2.1 Analysis of glucose by using Accutrend® meter GCT.

The total 1% concentration of glucose in 0.0 to 2.0% PG in Ringer buffer (pH 7.0) was dialyzed, the releasing of glucose into the dialysate at 2 and 3 hours was analyzed quantitatively, as well as glucose retained inside dialysis bag, by using Accutrend® meter GCT. Figure 17 illustrated the profile of glucose trapped inside dialysis bag (Figure 17 (A)) and glucose released outside dialysis bag (Figure 17 (B)) of four experiments of 0.5, 1.0, 1.5 and 2.0% PG, and control of 0.0% PG. The results demonstrated that glucose released outside dialysis bag was significantly decreased ($p < 0.05$) (Figure 17 (B)) while glucose trapped inside dialysis bag was significantly increased ($p < 0.05$) (Figure 17 (B)) at PG concentration 1.0, 1.5 and 2.0 % compared to control at 0.0 % PG. No significant difference was observed in the glucose released outside dialysis bag or trapped inside dialysis bag at 0.5 % PG concentration. There was no clearly evidence showed that PG at 1.0 – 2.0% concentration provide different glucose releasing or trapping at 2 or 3 hours of dialysis in this study. In this method of analysis suggested applying test material on the test strip must be quickly finished in a few seconds, but PG solutions inside dialysis bag were viscous and hardly delivered from the micro-tip and caused a problem for performance inaccuracy. However, the result possible suggests that glucose could be trapped in PG in dialysis bag.

2.2 Analysis of glucose by colorimetric method using *o*-toluidine test for reducing sugar.

Glucose concentrations of inside and outside dialysis bag were analyzed by using *o*-toluidine test. The mean percentage of total glucose concentration inside and outside dialysis bag was shown in Figure 18. In 0.5, 1.0 and 1.5 % PG concentrations, the percentage of total glucose inside dialysis bag was not significantly higher ($p < 0.05$) than controls and the percentage of total glucose outside dialysis bag was not significantly lower ($p < 0.05$) than control. The only glucose in 2.0 % PG showed that the percentage of glucose trapped inside and released outside dialysis bag were significantly higher and lower than that of control, respectively. In this study, it was probably because of the method of analysis is specific for reducing sugars that was not specific for glucose. Since PG is a polysaccharide containing of several reducing sugars, and using strong acid condition of the analysis can interfere the results. Therefore, it was not confident to conclude this experiment with this method of glucose analysis.

(A)



(B)

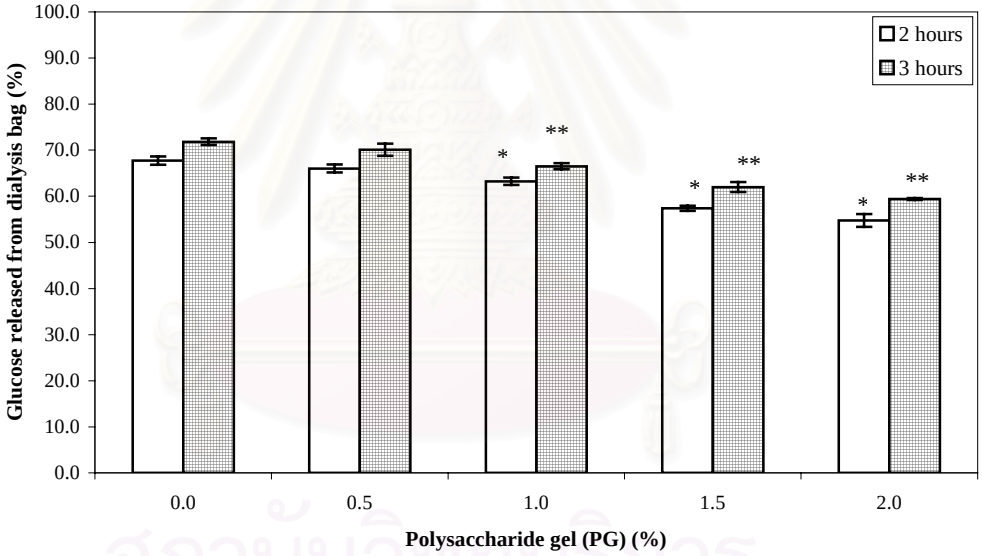


Figure 17 Effect of PG on (A) trapping of glucose inside, (B) releasing of glucose outside dialysis bag after dialysis for 2 and 3 hours. Glucose was analyzed by strip test. * , ** = significantly different from control without or 0 % PG ($p < 0.05$).

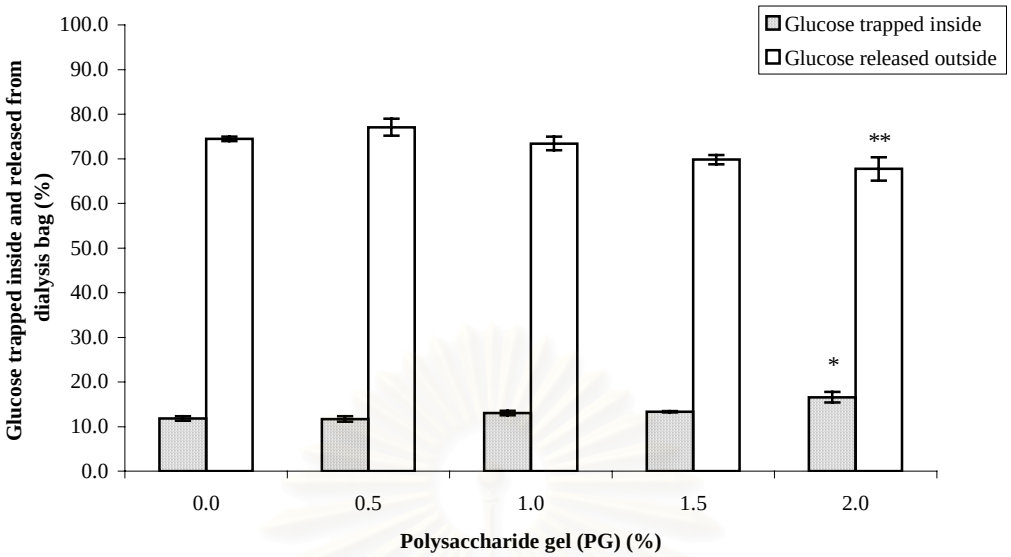


Figure 18 Effect of PG on trapping of glucose inside and releasing of glucose outside from dialysis bag after dialysis for 3 hours. Glucose was analyzed by *o*-toluidine test for reducing sugars. * , ** = significantly different from control without or 0 % PG ($p < 0.05$).

3. *In vitro* study the effect of PG and GM on glucose entrapment by dialysis technique using semi-permeable membrane.

The study of glucose entrapment property of PG was compared to glucomannan (GM), a commercial available polysaccharide, which was used as a standard polysaccharide. To find the optimum glucose concentration in 2.0 % PG in dialysis bag that released concentration of glucose suitable for the analysis by colorimetric method, using a glucose oxidase enzyme reaction, in outer and inner solution of dialysis bags. The preliminary study reported that 2.0 % PG was the effective concentration clearly showed that PG effected trapping glucose. This experiment was to compare the trapping and releasing capacity of glucose inside and outside the dialysis bag at 2.0 % PG and at 0.0 % PG control. It was found in Figure 19 that percentage of glucose trapping inside dialysis bag in the 0.0 % PG control and the 2.0 % PG in dialysis bag were not significantly different at any concentration of glucose (0.5, 1.0, 1.5 and 2.0 %). All glucose concentrations in dialysis bags containing PG were significantly higher glucose level ($p < 0.05$), while those outside the bag are significantly lower of glucose level ($p < 0.05$) than that of 0.0 % PG, after dialysis for 2 hours. Since glucose concentration was not affected in this condition, the 1.0 % of glucose concentration was a concentration used for the convenience to perform the following studies.

3.1 The effect of PG on glucose entrapment.

The rate of glucose releasing from dialysis bag was studied using 1.0 % glucose in 0.0, 1.0, 2.0 and 3.0 % of PG at dialysis time for 0, 1, 2, 3 and 4 hours. Outer and inner glucose concentrations of dialysis bags were analyzed by colorimetric method using a glucose oxidase enzyme reaction. To illustrate the significant results of the mean percentage of total glucose inside and outside dialysis bag, a graph of histogram was constructed as shown in Figure 20. At the first hour of dialysis, the mean percentage of glucose concentrations in 2.0 and 3.0 % PG mixtures were significantly higher trapped inside dialysis bag and lower released outside dialysis bag than that of 0 % PG control. Whereas with 1.0 % PG and 0 % PG control did not show significant difference of the value of glucose trapping inside or releasing outside dialysis bag ($p < 0.05$). After the second, third and forth hour of dialysis the mean percentage of glucose concentrations in each concentration test of PG mixtures were significantly higher trapped inside dialysis bag ($p < 0.05$) and lower released outside dialysis bag ($p < 0.05$) than that of 0 % PG in control. The results demonstrated in Figure 20 that at any concentrations of PG resulted in increased trapping and decreased releasing of glucose after 2 – 4 hours of dialysis. At 2 hours of dialysis, 2.0 and 3.0 % PG trapped 28.058 ± 0.640 and 32.227 ± 0.559 % glucose inside dialysis bag and released 71.816 ± 0.845 and 67.775 ± 0.616 % glucose outside dialysis bag, respectively. However, only

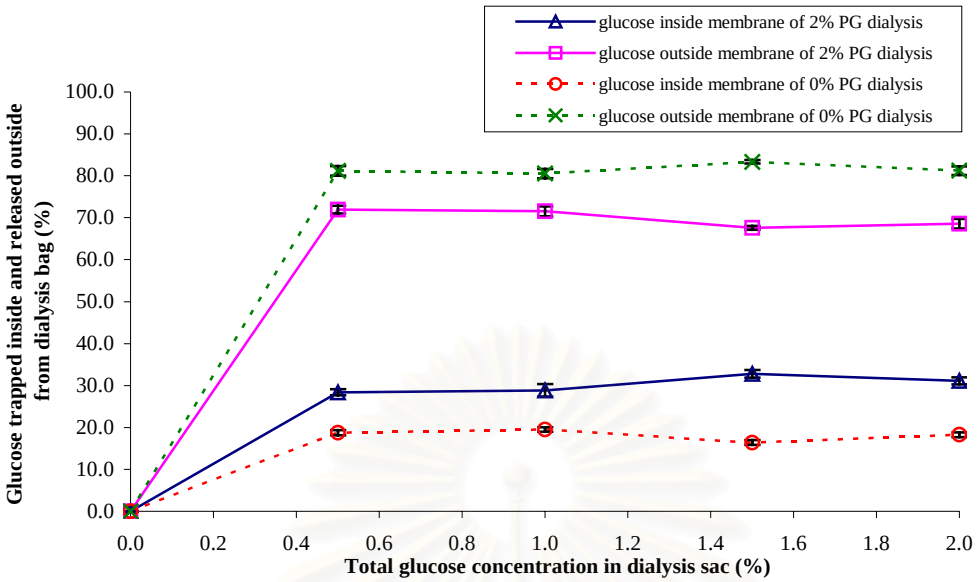


Figure 19 Effect of glucose concentration with or without 2.0 % PG on trapping glucose inside and releasing glucose outside from dialysis bag after dialysis for 2 hours. Glucose was analyzed by colorimetric method using glucose oxidase reaction. Each bar represents mean ± S.E.M..

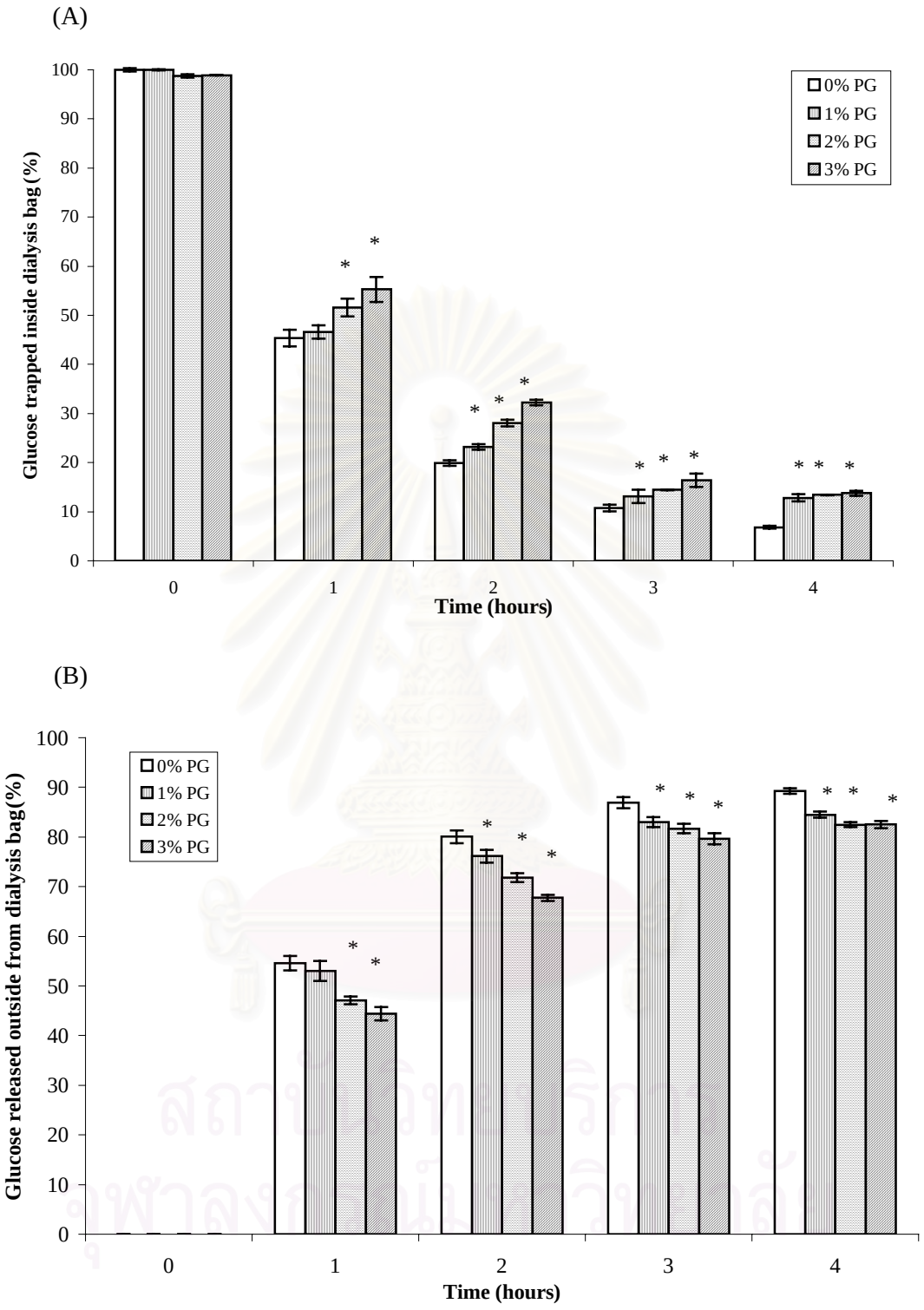


Figure 20 Effect of PG on (A) trapping of glucose inside, (B) releasing of glucose outside from dialysis bag after dialysis for 0, 1, 2, 3 and 4 hours. Glucose was analyzed by colorimetric method using glucose oxidase enzyme reaction. PG = polysaccharide gel. Each bar represents mean ± S.E.M.. * = significantly different from control without or 0 % PG ($p <$ 0.05).

13.413 ± 0.542 and 13.758 ± 0.751 % of glucose was trapped or 82.493 ± 0.064 and 82.526 ± 0.498 % of glucose was released with 2.0 and 3.0 % PG after 4 hours of dialysis. The results indicated that PG at 2.0 and 3.0 % delayed glucose releasing from inside through outside dialysis bag. This property was not obviously effective with 1.0 % PG at the first hour. Therefore, two hours of dialysis is the time selected for further study using the same condition.

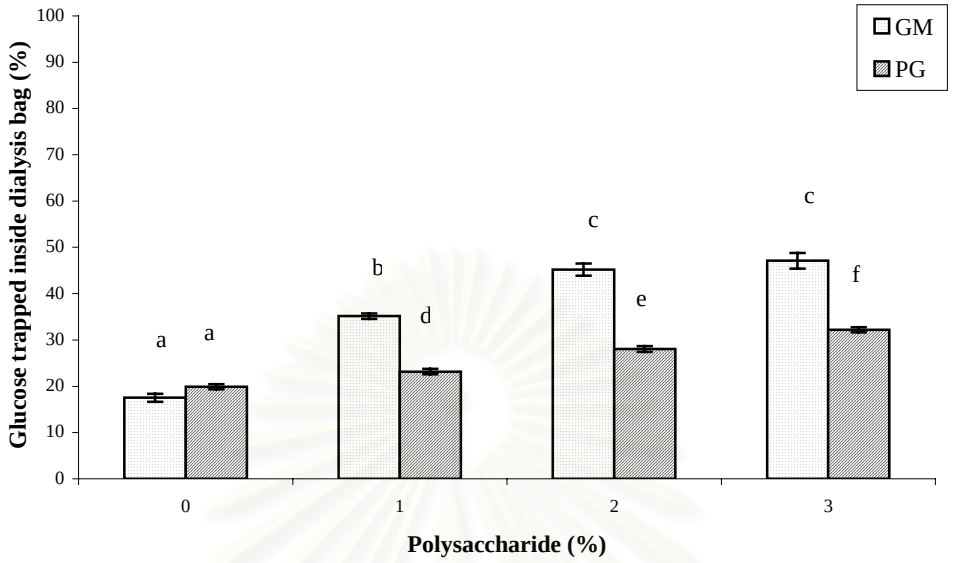
3.2 A comparison of the effect of PG and GM on glucose entrapment.

To compare the efficiency between PG and glucomannan (GM), a commercial available polysaccharide was used for positive control. Glucose entrapment after dialysis for 2 hours is showed in Figure 21 indicating the PG exhibited lower capacity of glucose entrapment than that of GM. Increasing concentrations of PG and GM resulted in increasing glucose trapped inside dialysis bag and decreasing glucose released outside dialysis bag. However, glucose entrapment capacity of PG was lower than that of GM in each concentration tested.

4. *In vitro* study on the effect of PG on glucose entrapment by dialysis technique using everted jejunal sacs of rats.

In vitro study of PG effect on glucose entrapment by dialysis technique, using everted jejunal sacs was compare with using semi-permeable membrane. The result of glucose entrapment in PG after dialysis for 1 hour using semi-permeable membrane showed that 2.0 % PG was the effective concentration. The everted jejunal sacs containing Ringer buffer (pH 7.0) were immersed in 1.0 % glucose in 0.0, 2.0 or 3.0 % PG mixture in Ringer buffer. Figure 22 illustrates the mean percentage of glucose released from inside jejunum wall through the outside wall of everted jejunal sacs after 1 hour of dialysis. The results indicated that glucose released in the presence of 2.0 and 3.0 % PG in mucosal medium through outside wall was significantly lower than that of control (0.0 % PG). The *in vitro* transport of glucose in intestinal sac was reduced in the presence of 2.0 and 3.0 % PG showed 66.465 ± 1.213 % and 57.551 ± 2.128 % of glucose released, respectively, compared to the values of total 100 % glucose transport at 0 % or without PG. Glucose at 33.533 % and 42.449 % were trapped in 2.0 % and 3.0 % PG, respectively. The results of decreasing glucose released in intestinal sac in the present of increasing PG concentration were correlated with the study using semi-permeable membrane.

(A)



(B)

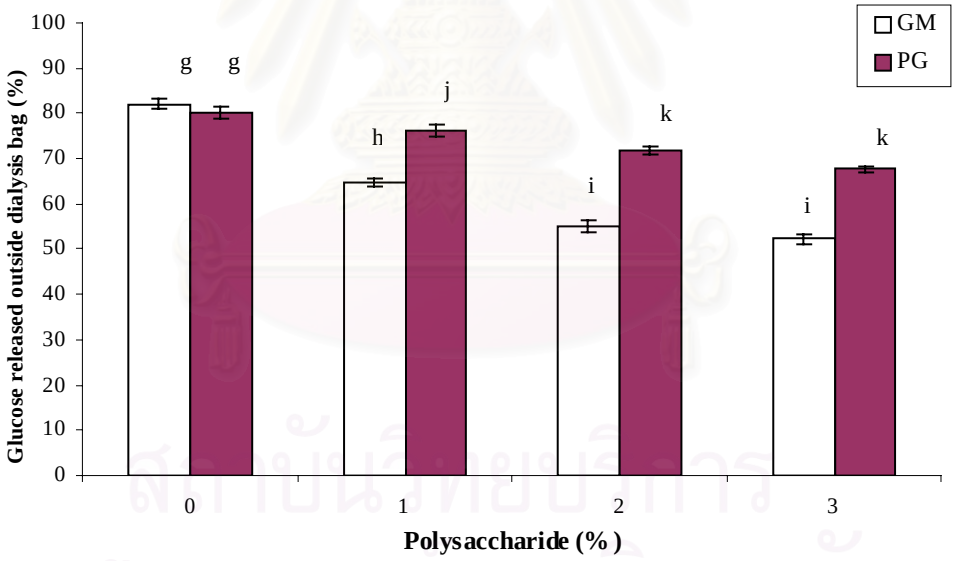


Figure 21 A comparison of the effect of PG and GM on (A) trapping of glucose inside, (B) releasing of glucose outside from dialysis bag after dialysis for 2 hours. Glucose was analyzed by colorimetric method using glucose oxidase enzyme reaction. PG = polysaccharide gel and GM = glucomannan. Each bar represents mean ± S.E.M.. a, b, c, …, k = significantly different between tested concentration of polysaccharides ($p < 0.05$).

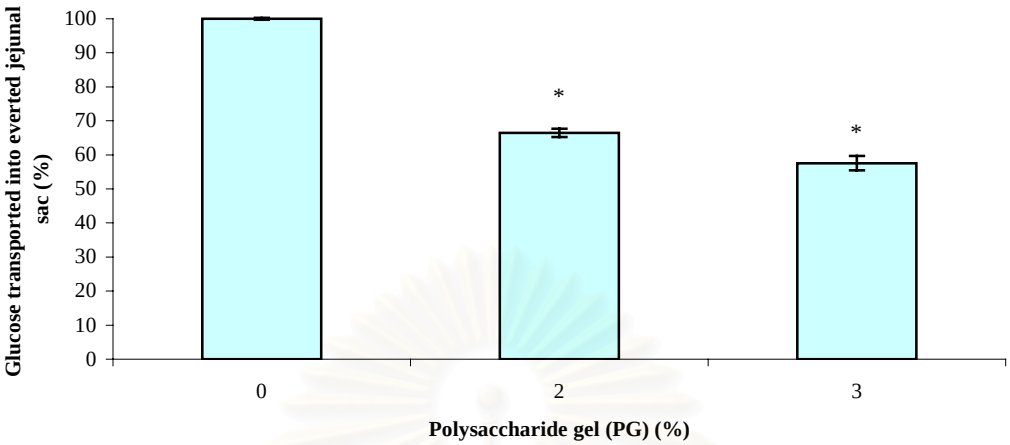


Figure 22 Effect of PG on glucose transported into everted jejunal sac after dialysis for 1 hour. Glucose was analyzed by colorimetric method using glucose oxidase enzyme reaction. Each bar represents mean ± S.E.M.. * = significantly different from 0 % PG controls ($p < 0.05$).

5. Preliminary study the effect of PG on sucrose entrapment by dialysis technique.

Determination of sucrose entrapment in PG by using HPLC technique for analysis of sucrose.

There is a possibility of having glucose entrapment property of PG; however, most of disaccharide sugar that usually consume is sucrose, and glucose is one of digestive product of sucrose. Therefore, to devoid sugar absorption, it is more efficient if PG also has sucrose entrapment property. The 0.1 M sodium phosphate buffer was used instead of Ringer buffer because it interfered HPLC analysis. There was significantly lower ($p < 0.05$) in percentage of sucrose outside dialysis bag at 1.0, 2.0 and 3.0 % PG concentrations than that of control at 0.0% PG, the values were 67.086 ± 0.482 %, 66.887 ± 2.661 % and 65.768 ± 1.923 % compared to 74.167 ± 1.595 % of control as shown in Figure 23. The percentage of sucrose inside dialysis bag was significantly higher at 2.0 and 3.0 % PG concentration than that of control at 0.0% PG. Therefore, concentration that effected on sucrose entrapment of PG was significantly increased at 2.0 and 3.0% PG, sucrose was trapped inside dialysis bag at concentration 29.010 ± 0.810 % and 30.323 ± 0.301 % of total sucrose, respectively, more than the value of 25.360 ± 0.104 % of sucrose entrapped in control.

PG effected the entrapment of glucose and also sucrose.

6. Preliminary study on the effect of PG on vitamin A entrapment by dialysis technique using semi-permeable membrane.

Previous report of Tippayakul (2002) has indicated that PG has property of lipids entrapment but there are the lipid-soluble vitamins that could enhance absorption when lipid digestion molecules were presented. Therefore, finding the lipid-soluble vitamin with PG will give more information of the effect of PG on trapping of fat soluble vitamin. The vitamin A was chosen to represent of the lipid-soluble vitamin, in the ester form of all-*trans* retinal acetate that is more stable form. After 2 hours of dialysis, vitamin A inside dialysis bag was analyzed by colorimetric method using trifluoroacetic acid test (Figure 24). The results showed that vitamin A in the mixture of 1.0, 2.0 and 3.0 % PG was trapped inside dialysis bag significantly higher ($p < 0.05$) than that of control. The vitamin A trapping inside dialysis bag at concentration 23.727 ± 1.710 %, 30.853 ± 1.061 %, 53.527 ± 1.518 % and 57.491 ± 0.828 % of vitamin A in the presence of 0.0, 1.0, 2.0 and 3.0 % PG, respectively. In the statistical point of view, there were significant differences between testing at 0.0, 1.0 and 2.0 % of PG concentration. Because vitamin A is easy to be oxidized, to prevent deviation of the results, vitamin A was applied in each PG concentration in dialysis bag and performed in the same condition as testing. The total concentration test of vitamin A inside dialysis bag was 96.864 ± 1.243 %, 98.738 ± 1.050 %, 98.256 ± 1.516 % and 97.248 ± 1.256 % in

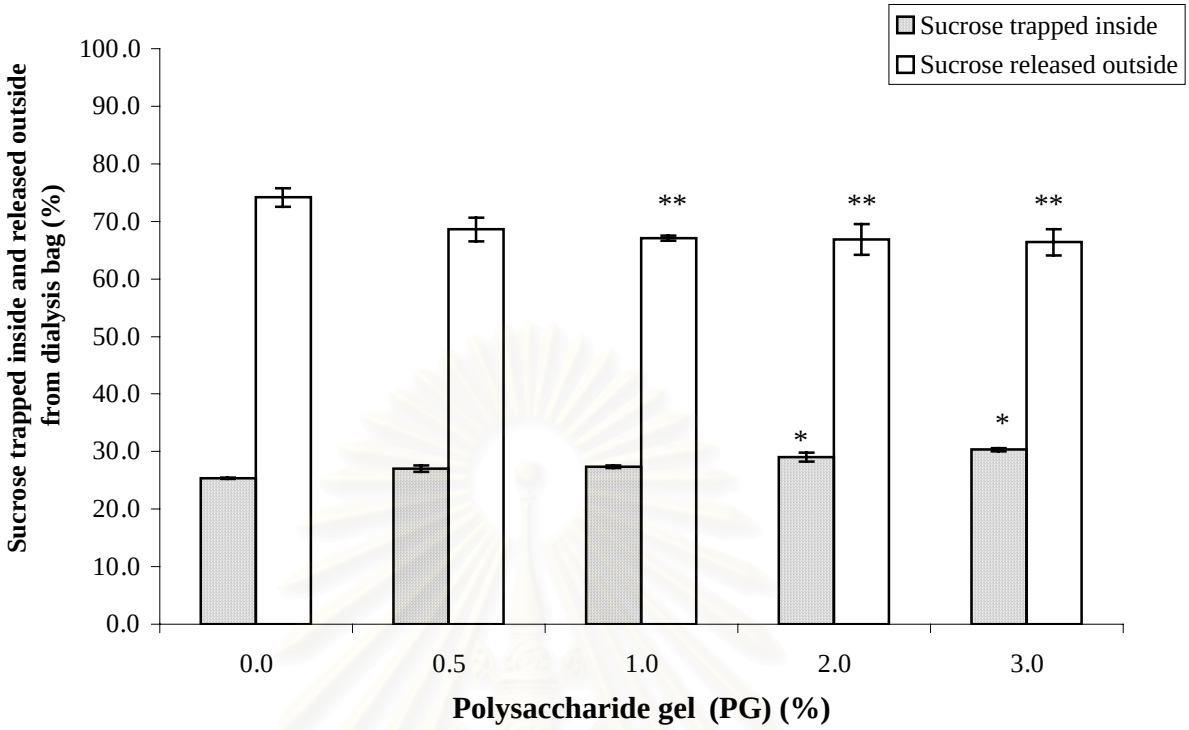


Figure 23 Effect of PG on trapping of sucrose inside and releasing of sucrose outside from dialysis bag after dialysis for 3 hours. Sucrose was analyzed by HPLC technique. * , ** = significantly different from control without or 0 % PG ($p < 0.05$).

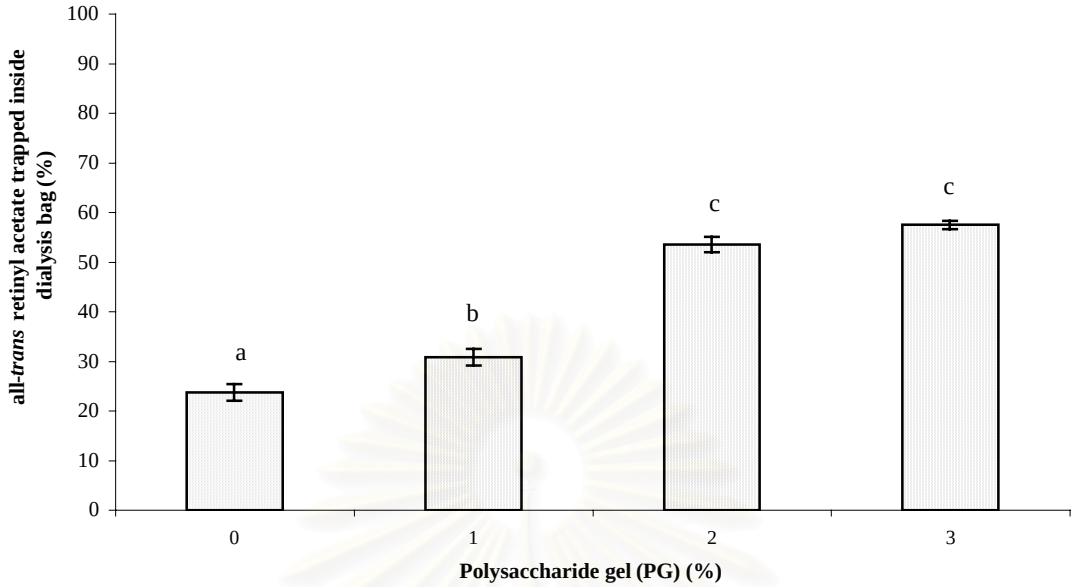


Figure 24 Effect of PG on trapping of all-*trans* retinal acetate (vitamin A) inside dialysis bag after dialysis for 2 hours. All-*trans* retinal acetate (vitamin A) was analyzed by colorimetric method using trifluoroacetic acid test. Each bar represents mean ± S.E.M.. a, b, c = significantly different between tested concentration of PG ($p < 0.05$).

the presence of 0, 1, 2 and 3 % PG, respectively, after placed at room temperature for 2 hours. The results showed that vitamin A was not significantly lost by the time used in this experiment.

7. Preliminary study on the effect of PG on vitamin B1 entrapment by dialysis technique using semi-permeable membrane.

Water-soluble vitamin is another essential nutrient for daily consumption that never been studied on the effect of PG entrapment. Thiamine hydrochloride is commercially available form of vitamin B1 used in pharmaceuticals. In this study, vitamin B1 was used to represent water-soluble vitamin. Figure 25 illustrated that the percentage of total vitamin B1 concentrations with 2.0 and 3.0 % PG mixtures were significantly higher trapped ($p < 0.05$) inside dialysis bag and lower released ($p < 0.05$) outside dialysis bag than that of 0 % PG in control after 2 hours of dialysis, while there was no significant difference with 1.0 % PG and control. The results showed 49.708 ± 0.602 %, 52.234 ± 0.989 %, 62.774 ± 0.391 % and 66.112 ± 1.281 % of vitamin B1 trapped in 0.0, 1.0, 2.0 and 3.0 % PG, respectively. This study found that vitamin B1 was significantly increased trapping inside dialysis bag and decreased releasing outside dialysis bag at 2.0 % PG concentration compared to 0 and 1% PG. The total concentration test of vitamin B1 inside dialysis bag was 98.188 ± 1.302 %, 99.238 ± 1.041 %, 98.246 ± 0.916 % and 98.217 ± 1.043 % in the presence of 0, 1, 2 and 3 % PG, respectively, after placed at room temperature for 2 hours. The results showed that vitamin B1 was not significantly lost by the time used in this experiment.

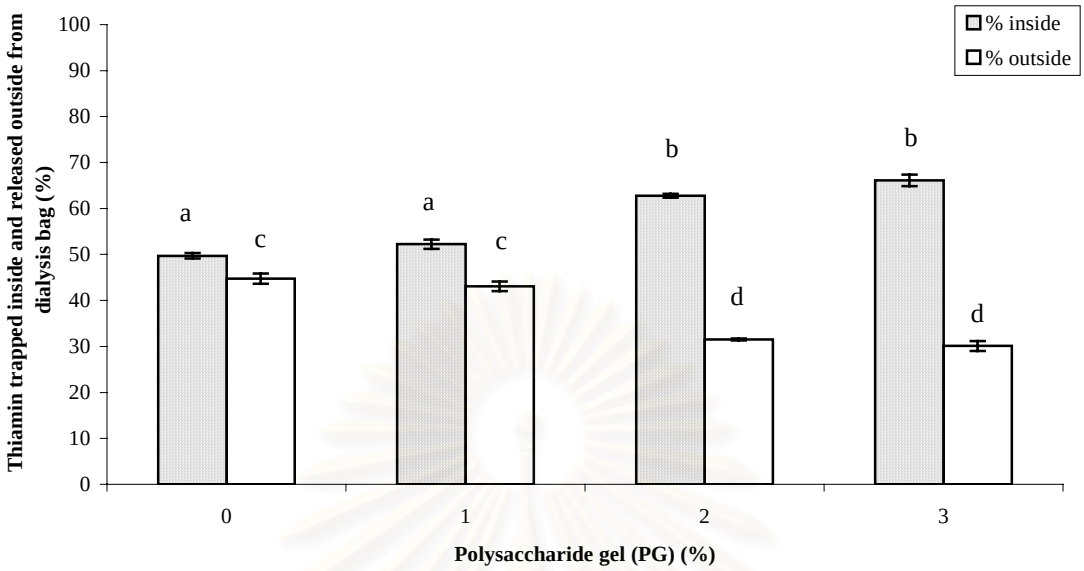


Figure 25 Effect of PG on trapping and releasing of thiamin (vitamin B1) inside and outside from dialysis bag after dialysis for 2 hours. Thiamin (vitamin B1) was analyzed by fluorometric method. Each bar represents mean ± S.E.M.. a, b, c, d = significantly different between tested concentration of PG ($p < 0.05$).

CHAPTER IV

DISCUSSION AND CONCLUSION

This works were designed the *in* vitro study the property of polysaccharide gel (PG) in trapping of sugars (glucose and sucrose), fat-soluble vitamin (vitamin A) and water-soluble vitamin (vitamin B1). In these studies, the experiments were designed a simple methods to assess the effects of PG on nutrients absorption. The dialysis technique was applied in this experiments using semi-permeable membrane dialysis tubing (Jenkins *et al.*, 1980 and Adiotomre *et al.*, 1990) and everted jejunal sac of rat (Blackburn and Johnson, 1981, Johnson and Gee, 1981 and Ferraris, Casirola and Vinnakota, 1993) as a model simulate the events occurring in small intestine of gastrointestinal tract. The previous study have shown that PG resisted to α-amylase hydrolysis and dilute hydrochloric acid hydrolysis at 37 °C after 30 minutes and 4 hours of incubation, respectively (Pongsamart *et al.*, 2001), PG can probably undegradable pass through the stomach and small intestine without changing. The nutrient released to outside the dialysis bag in dialysis studies were represent the nutrients absorption occurring in the jejunum, the effect of PG on retention of nutrients (glucose, sucrose, vitamin A and vitamin B1) and protect them to release from a dialysis bag into the external medium was evaluated in comparison with the experiment to assess the effect of PG on nutrient absorption using the jejunum sac of rat.

***In vitro* study on sugars entrapment property of PG by dialysis technique.**

These studies observed significant reduction of glucose and sucrose releasing in experiment performed by using semi-permeable membrane dialysis in the presence of 2% PG concentration. The glucose dialysis experiment was also performed by using everted jejunal sac of rat *in vitro* and the result showed the significant reduction of glucose absorbed in intestine in the presence of 2% PG concentration. Therefore, the results of this study indicated that glucose was lower absorbed in the presence of PG than that of control (0% PG).

These findings agreed with Adiotomre *et al.* (1990) *in vitro* studies with soluble fibers such as guar gum, gum karaya, pectin, gum tragacanth or xanthan gum on retardation of glucose transportation by using semi-permeable membrane dialysis. They summarized that the retarding effect on glucose flow across the dialysis bags is due to the viscosity of soluble fiber, this ability was not found in non-viscous fiber such as wheat bran because wheat bran is

only 40% fiber and consists mainly of hemicellulose. Therefore, the viscous PG interferes glucose diffusion from dialysis bags.

These studies also agreed with the previous studies by Blackburn and Johnson (1981) and Cameron-Smith, Collier and O'Dea (1994) in effect of guar gum on the viscosity of gastrointestinal contents, on glucose uptake from the perfused jejunum in rats and on the acute glycaemic response in rats. They proposed that the mechanism of this effect is due to the presence of viscous polysaccharide dispersion in the gastrointestinal tract; thereby impede the delivery of nutrients through the small intestine. Furthermore, Johnson and Gee (1981) explained that the inhibition of glucose transport was due to the presence of long polymer chains in the incubation medium confers upon it some of the properties of a gel, with a corresponding decline in the mobility of the fluid layers surrounding and overlying the intestinal villi. The factor limiting the diffusion of solutes in media containing viscous gum, is the obstruction caused by the molecules themselves. The molecular chains form a complex three-dimension lattice extending throughout the aqueous phase make some contributions to the increased resistance of the mucosal diffusion barrier. These results were also similar to the results from previous investigation by Johnson and Gee (1981) in that rate of glucose absorption was decreased relative to control values in everted jejunal sacs of rats with guar gum. The reason for this is not clear. They suggested that the presence of polysaccharide gum in the fluid film surrounding the villi increases its viscosity and gives rise to a thickening of the rate-limiting unstirred layer. If such an effect occurs *in vivo,* this could contribute to the diminished post-prandial glycaemia observed in human subjects fed guar gum. This, therefore, confirmed previous studies in healthy (Sugiyama and Shimahara, 1976, Jenkins *et al*., 1982, Wood *et al*., 1994 and Lu *et al*., 2000), obesity (Magnati *et al*., 1984 and Vita *et al*., 1992) and diabetes mellitus human subjects (Miranda and Horwitz, 1978, Ohta *et al*., 1997, Chandalia *et al.,* 2000 and Vuksan *et al*., 2000) on significantly reduced postprandial glycaemia with soluble fiber consumption.

The comparison of the effect of PG and glucomannan (GM) on glucose entrapment in experiment by dialysis technique using semi-permeable membrane, the results showed that GM was more effective than PG in trapping of glucose. It is probably that glucose is a neutral sugar. GM is a neutral linear polymer of glucose and mannose. Moreover, konjac powder has the highest molecular weight (MW 200,000 to 2,000,000) and highest viscosity among any dietary fiber (Vuksan *et al.,* 2000). PG is an acidic polysaccharide which 52.5% of galacturonic acid. Therefore, glucose may be trapped in neutral high viscous GM better than in acidic polysaccharide of PG.

***In vitro* study on vitamins entrapment property of PG by dialysis technique.**

The result of vitamin A, a fat-soluble vitamin, entrapment in this study was also agreed to the previous studied that plasma β-carotene, lycopene and lutein were significantly reduced after 24 hours consumption of the water-soluble fiber; pectin, guar or alginate in healthy subjects (Riedl *et al*., 1999). Pectin reduced hepatic vitamin A in chicks (Erdman, Fahey and White, 1986) and pectin had also effect on the β-carotene conversion to vitamin A in Mongolian gerbils (Deming *et al*., 2000). In agreement with the studies of Doi *et al*. (1983) on the influence of konjac mannan on decreased absorption rate of fat-soluble vitamin (vitamin E) in the intestinal of normal and diabetes subjects and the studies of Schaus, *et al*. (1983) on decreasing of vitamin E availability in rats, the results of this study showed that PG is a water-soluble fiber that can reduce the diffusion of the fat-soluble vitamin (vitamin A) from dialysis bags.

The result of vitamin B1, a water-soluble vitamin, high entrapment was inconsistent with Doi *et al*. (1983) studies on influence of konjac mannan on absorption rate of vitamin B12, a water-soluble vitamin, into the intestinal of normal and diabetes subjects. However, Cullen and Oace (1978) reported that vitamin B12 in fecal excretion of rats was higher in the presence of pectin. While earlier studies (Doi *et al*., 1983 and Riedl *et al*. 1990) noted that soluble fiber was not significant reduced absorption of vitamin B12 and vitamin B6, this study found that PG was significant reduced water-soluble vitamin (vitamin B1) diffusion from dialysis bags.

Fat-soluble vitamin (vitamin A) was higher trapped in PG in dialysis bag than water-soluble vitamin (vitamin B1). This may be related to physical properties of fat-soluble vitamin (vitamin A), which is hydrophobic head and conjugated isoprenoid side chain. Therefore, PG is more effective in fat-soluble vitamin (vitamin A) entrapment than water-soluble vitamin (vitamin B1).

The principal physiological effect of dietary fiber in the small intestine is to reduce the rate of absorption of nutrients. The dominant factors involved are physical trapping of nutrients within structured assemblies such as plant tissue, and enhanced viscosity restricting the peristaltic mixing process that promotes transport of enzymes to their substrates, bile salts to unmicellized fat, and soluble nutrients to the gut wall by forming a barrier around the food which delays its absorption by intestinal wall. Subsidiary factors may include binding of bile salts (and perhaps enzymes) to specific fiber components and inhibition of diffusion across the unstirred layer (Story, 1985 and Gallaher *et al*., 2000). The rate of release of nutrients from fibrous particles into the surrounding intestinal fluid is inversely proportional to particle size, and is directly proportional to solute gradient. It is affected by the physical state of the solute (presented in solid form or dissolved in water trapped within the particle). Moreover, it is affected by the physical structure of the particle (deformed, like a sponge, or rigid) and the

surface properties of the particle (*e.g*. surface-tension effects). It is also influenced by the transit time (*i.e*. the duration of exposure to a particle absorptive surface or digestive environment). Since the viscous polysaccharides can form a gel matrix, they are likely to trap nutrients in the matrix and delay their emptying from the stomach. In addition to their contribution to the viscosity of the luminal contents, fiber particles may reduce rates of absorption by other mechanisms such as physical trapping of nutrients within the fiber matrix. Chyme may be considered a two-phase system with a discontinuous particulate phase dispersed in a continuous liquid phase. Nutrients trapped within the particles must be firstly released into the continuous solution phase before they can be absorbed through the gut wall. (Johnson and Gee, 1981, Vahouny and Cassidy, 1985, Leeds, 1987, Eastwood and Morris, 1992, Ohta *et al*., 1997, Vanderhoof, 1998 and Riedl *et al*., 1999)

Its viscosity is proposed as an important mechanistic factor. Therefore the increases in nutritional entrapment of glucose, sucrose, vitamin A and vitamin B1 were caused by PG inherent viscosity property. These effects are probably due to an increase in content of PG. Further efficacious studies are needed to clarify these aspects.

In conclusion, the results from current studies were shown that these methods could form the basic techniques that would enable a screening of novel fiber sources before performing experiment with long and costly animal or human studies. The 2% PG concentration inhibits sugars (glucose and sucrose), fat-soluble vitamin (vitamin A) and water-soluble vitamin (vitamin B1) transport in this *in vitro* system. The interference with absorption of nutrients is also believed to be one area in which fiber is beneficial for dietary control (Anderson and Bryant, 1986). This suggests that using PG for diet, as sliming agent should be aware of the insufficiency of those vitamins, which is a trace content in foods, and careful consideration should be taken in the use of this dietary fiber for diet control. Therefore, these findings support the role of PG as a means of improving soluble fiber diets in the amelioration of normal or diabetes mellitus human subject by under control of medication.

For further research, PG should be investigated about the exact structure of each unit of polymer for more understanding of their physiological effect on biological activity and studied the effect on mineral because some soluble fiber can bind with divalent cationic molecule and change their properties such as pectin (Kim *et al*., 1993 and Fennema *et al*., 1996), alginate (Ohta *et al*., 1997), and also studies the effect of PG on insulin resistance for more information for diabetes patients.

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ภาษาไทย

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APPENDICES

APPENDIX A

PREPARATION OF REAGENTS

1. **Ringer buffer solution** (Ferraris, Casirola, and Vinnakota, 1993)

Ringer buffer solution is composed of 128 mM of sodium chloride (NaCl), 4.7 mM of potassium chloride (KCl), 2.5 mM of calcium chloride ($CaCl_2$), 1.2 mM of potassium dihydrogen phosphate (KH_2PO_4), 1.2 mM magnesium sulfate ($MgSO_4$) and 20 mM sodium hydrogen carbonate ($NaHCO_3$). Stirred for two hours approximately at room temperature until all solids are dissolved and adjust pH to 7.0. Bring solution to 1,000 ml with purified water. The conductivity value of solution is approximately 14 mS.

2. **0.1 M Sodium phosphate buffer solution** (Ausubel *et al*., 1999)

Prepared the solution A for 0.2 M of sodium dihydrogen phosphate (NaH_2PO_4) and the solution B for 0.2 M disodium hydrogen phosphate (Na_2HPO_4). Mixed 39.0 milliliters of solution A with 6.1 milliliters of solution B. Checked the pH and adjust to 7.0 if necessary, then dilute with distilled water to 200 milliliters.

APPENDIX B

TABLES OF EXPERIMENTAL RESULTS

1. The acid and viscosity of purified polysaccharide gel (PG) in distilled water was measured by pH meter and viscometer at room temperature.

PG concentration (%)	pH*	Viscosity (mPa.s) at 100 rpm*
1.0	2.734 ± 0.006	49.367 ± 0.817
2.0	2.568 ± 0.016	95.700 ± 2.669
3.0	2.484 ± 0.013	217.067 ± 3.112

* Each value represents the mean ± S.E.M. of three independent sample sizes.

2. Preliminary study the effect of PG on glucose entrapment by dialysis technique.

2.1 Comparison of percentage of glucose concentration trapping inside and releasing outside of dialysis bag at 2 and 3 hours with 0 to 2% w/v of PG. Glucose was analyzed by strip test and Accutrend® meter GCT.

PG concentration (%)	Glucose inside dialysis bag (%)*		Glucose outside dialysis bag (%)*	
	Incubation time (hr)		Incubation time (hr)	
	Two	Three	Two	Three
0.0	20.633 ± 1.054	20.933 ± 1.548	67.750 ± 0.901	71.883 ± 0.726
0.5	22.600 ± 0.278	21.408 ± 0.434	66.000 ± 0.866	70.083 ± 1.341
1.0	25.383 ± 0.464	25.492 ± 1.558	63.250 ± 0.804	66.500 ± 0.661
1.5	26.383± 2.159	26.475± 0.953	57.417 ± 0.546	62.000 ± 1.090
2.0	28.467 ± 0.726	27.500 ± 0.831	54.750 ± 1.364	59.417 ± 0.220

* Each value represents the mean ± S.E.M. of three independent sample sizes.

2.2 Comparison of percentage of glucose concentration trapping inside and releasing outside of dialysis bag at 3 hours with 0 to 2% w/v of PG. Glucose was analyzed by *o*-toluidine test for reducing sugars.

PG concentration (%)	Glucose inside dialysis bag (%)*	Glucose outside dialysis bag (%)*
0.0	11.823 ± 0.497	74.479 ± 0.521
0.5	11.719 ± 0.592	77.083 ± 1.878
1.0	13.047 ± 0.496	73.438 ± 1.562
1.5	13.313 ± 0.143	69.792 ± 1.042
2.0	16.563 ± 1.183	67.708 ± 2.604

* Each value represents the mean ± S.E.M. of three independent samples.

3. *In vitro* study the effect of PG and GM on glucose entrapment by dialysis technique using semi-permeable membrane.

3.1 Comparison of percentage of glucose concentrations trapping inside and releasing outside of dialysis bag at 2 hours starting with 0.0 to 2.0% w/v of glucose concentrations of each 0 and 2% w/v of PG. Glucose was analyzed by colorimetric method using glucose oxidase reaction.

Glucose concentration (%)	Glucose inside dialysis bag (%)*		Glucose outside dialysis bag (%)*	
	PG concentration (%)		PG concentration (%)	
	0	2	0	2
0.0	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
0.5	18.757 ± 0.604	28.402 ± 0.728	81.191 ± 1.150	71.942 ± 0.927
1.0	19.548 ± 0.529	28.835 ± 1.488	80.519 ± 1.082	71.513 ± 1.151
1.5	16.414± 0.567	32.795± 0.887	83.310 ± 0.419	67.573 ± 0.464
2.0	18.279 ± 0.569	31.128 ± 0.867	81.199 ± 1.023	68.582 ± 1.164

* Each value represents the mean ± S.E.M. of group of three independent sample sizes.

3.2 Comparison of percentage of glucose concentration trapping inside and releasing outside of dialysis bag at 0 to 4 hours with 0 to 3% w/v of PG. Glucose was analyzed by colorimetric method using glucose oxidase reaction.

3.2.1 At 0 hour

PG concentration (%)	Glucose inside dialysis bag (%)*	Glucose outside dialysis bag (%)*
0.0	100.003 ± 0.330	0.000 ± 0.000
1.0	100.671 ± 0.386	0.000 ± 0.000
2.0	98.791 ± 0.352	0.000 ± 0.000
3.0	98.905 ± 0.066	0.000 ± 0.000

* Each value represents the mean ± S.E.M. of three independent samples.

3.2.2 At 1 hour

PG concentration (%)	Glucose inside dialysis bag (%)*	Glucose outside dialysis bag (%)*
0.0	45.388 ± 1.679	54.575 ± 1.425
1.0	46.651 ± 1.369	53.021 ± 2.011
2.0	51.581 ± 1.842	47.106 ± 0.815
3.0	55.280 ± 2.519	44.418 ± 1.306

* Each value represents the mean ± S.E.M. of three independent samples.

3.2.3 At 2 hours.

PG concentration (%)	Glucose inside dialysis bag (%)*	Glucose outside dialysis bag (%)*
0.0	19.907 ± 0.522	80.061 ± 1.278
1.0	23.170 ± 0.599	76.147 ± 1.260
2.0	28.058 ± 0.640	71.816 ± 0.845
3.0	32.227 ± 0.559	67.775 ± 0.616

* Each value represents the mean ± S.E.M. of three independent samples.

3.2.4 At 3 hours.

PG concentration (%)	Glucose inside dialysis bag (%)*	Glucose outside dialysis bag (%)*
0.0	10.733 ± 0.709	86.949 ± 1.128
1.0	13.074 ± 1.353	83.006 ± 0.997
2.0	14.437 ± 0.093	81.671 ± 0.948
3.0	16.392 ± 1.350	79.629 ± 1.079

* Each value represents the mean ± S.E.M. of three independent samples.

3.2.5 At 4 hours.

PG concentration (%)	Glucose inside dialysis bag (%)*	Glucose outside dialysis bag (%)*
0.0	6.825 ± 0.520	89.270 ± 0.292
1.0	12.825 ± 0.631	84.468 ± 0.751
2.0	13.413 ± 0.542	82.493 ± 0.064
3.0	13.758 ± 0.751	82.526 ± 0.498

* Each value represents the mean ± S.E.M. of three independent samples.

3.3 Comparison of percentage of glucose concentration trapping inside and releasing outside of dialysis bag at 2 hours with 0 to 3% w/v of glucomannan (GM). Glucose was analyzed by colorimetric method using glucose oxidase reaction.

GM concentration (%)	Glucose inside dialysis bag (%)*	Glucose outside dialysis bag (%)*
0.0	17.543 ± 0.861	82.133 ± 0.949
1.0	35.167 ± 0.593	64.851 ± 0.984
2.0	45.211 ± 1.319	55.011 ± 1.443
3.0	47.111 ± 1.690	52.318 ± 1.059

* Each value represents the mean ± S.E.M. of three independent samples.

4. *In vitro* study on the effect of PG on glucose entrapment by dialysis technique using everted jejunal sacs of rats.

4.1 Comparison of percentage of glucose concentrations trapping inside of membrane at 1 hour with 0, 2 and 3% w/v of PG. Glucose was analyzed by colorimetric method using glucose oxidase reaction.

PG concentration (%)	Glucose inside membrane (%)#
0.0	100.000 ± 0.094
2.0	66.465 ± 1.213
3.0	57.551 ± 2.128

Each value represents the mean ± S.E.M. of group of four independent sample sizes.

5. Preliminary study the effect of PG on sucrose entrapment by dialysis technique.

5.1 Comparison of percentage of sucrose concentration trapping inside and releasing outside of dialysis bag at 3 hours with 0.0 to 3.0% w/v of PG. Sucrose was analyzed by HPLC technique.

PG concentration (%)	Sucrose inside dialysis bag (%)*	Sucrose outside dialysis bag (%)*
0.0	25.360 ± 0.104	74.167 ± 1.595
0.5	27.024 ± 0.524	68.587 ± 2.085
1.0	27.373 ± 0.243	67.086 ± 0.482
2.0	29.010 ± 0.810	66.887 ± 2.661
3.0	30.323 ± 0.301	65.768 ± 1.923

* Each value represents the mean ± S.E.M. of three independent samples.

6. Preliminary study on the effect of PG on vitamin A entrapment by dialysis technique using semi-permeable membrane.

6.1 Comparison of percentage of vitamin A concentrations trapping inside of dialysis bag at 2 hours with 0 to 3% w/v of PG. All-*trans* retinal acetate (vitamin A) was analyzed by colorimetric method using trifluoroacetic acid test.

PG concentration (%)	Vitamin A inside dialysis bag (%)*
0.0	23.727 ± 1.710
1.0	30.853 ± 1.061
2.0	53.527 ± 1.518
3.0	57.491 ± 0.828

* Each value represents the mean ± S.E.M. of three independent samples.

7. Preliminary study on the effect of PG on vitamin B1 entrapment by dialysis technique using semi-permeable membrane.

7.1 Comparison of percentage of vitamin B1 concentrations trapping inside of dialysis bag at 2 hours with 0 to 3% w/v of PG. Thiamin (vitamin B1) was analyzed by fluorometric method.

PG concentration (%)	Vitamin B1 inside dialysis bag (%)*	Vitamin B1 outside dialysis bag (%)*
0.0	49.708 ± 0.602	44.731 ± 1.120
1.0	52.234 ± 0.989	43.087 ± 1.046
2.0	62.774 ± 0.391	31.511 ± 0.196
3.0	66.112 ± 1.281	30.100 ± 1.100

* Each value represents the mean ± S.E.M. of three independent samples.

VITAE

Miss Waraporn Piyasirananda was born on November 26th, 1975 in Bangkok, Thailand. In 1997, she was graduated from the Faculty of Sciences, Chulalongkorn University, Thailand with the Bachelor of Science in Food technology (Biotechnology). Since then, she has been appointed as a Medical scientist in the Department of Medical Sciences, Ministry of Public Health, Thailand.