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Original research articles, review-type papers and case reports will be considered for publication in the Bulletin of Chiang Mai Medical Technology. All manuscripts must be original and should have preferably not been previously submitted to any other publication. Preference is given to material which is of general interest to medical practitioners and research workers in clinical medicine.

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ที่.....

วันที่.....

ถึง บรรณาธิการ วารสารเทคนิคการแพทย์ เชียงใหม่

ข้าพเจ้ายินดีบอกรับเป็นสมาชิก วารสารเทคนิคการแพทย์ เชียงใหม่ ขอได้ส่งวารสารถึงข้าพเจ้า ดังนี้

นาม .....

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ข้าพเจ้าได้ส่งค่าบำรุงเป็นเงิน ๒๐.๐๐ บาท มาพร้อมแบบฟอร์มนี้แล้ว

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BULLETIN OF CHIANG MAI MEDICAL TECHNOLOGY

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Editorial

FUNCTIONS OF HAGEMAN FACTOR

ในปี ค.ศ. 1953 Mr. John Hageman พนักงานรถไฟของ New York Central Railroad ซึ่งป่วยเป็น peptic ulcer ต้องการรับการรักษาด้วยการผ่าตัด แต่มีอุปสรรคเพราะมี clotting time นานผิดปกติเป็นอย่างมาก Ratnoff ได้ศึกษาผู้ป่วยอย่างละเอียด และพบว่าผู้ป่วยคนนี้ขาด specific coagulation factor ชนิดใหม่ที่ยังไม่เคยทราบกันมาก่อน และให้ชื่อสารนี้ว่า Hageman factor or Factor XII ดังนั้น จึงเป็นธรรมดาที่สารนี้เป็นที่ทราบ และสนใจแต่เฉพาะในวงการของ coagulationist เท่านั้น แต่ในระยะต่อมามีนักวิจัยด้านอื่น ๆ สนใจสารนี้มากขึ้นเรื่อย ๆ ทั้งนี้เป็นเพราะว่า นอกจากมันจะเป็นตัวเริ่มต้น coagulation process แล้ว ในขณะเดียวกันมันสามารถจะเป็นตัวเริ่มต้นระบบอื่น ๆ ด้วย เช่น Fibrinolytic system, complement system และ Kallikrein–Kinin system เป็นต้น

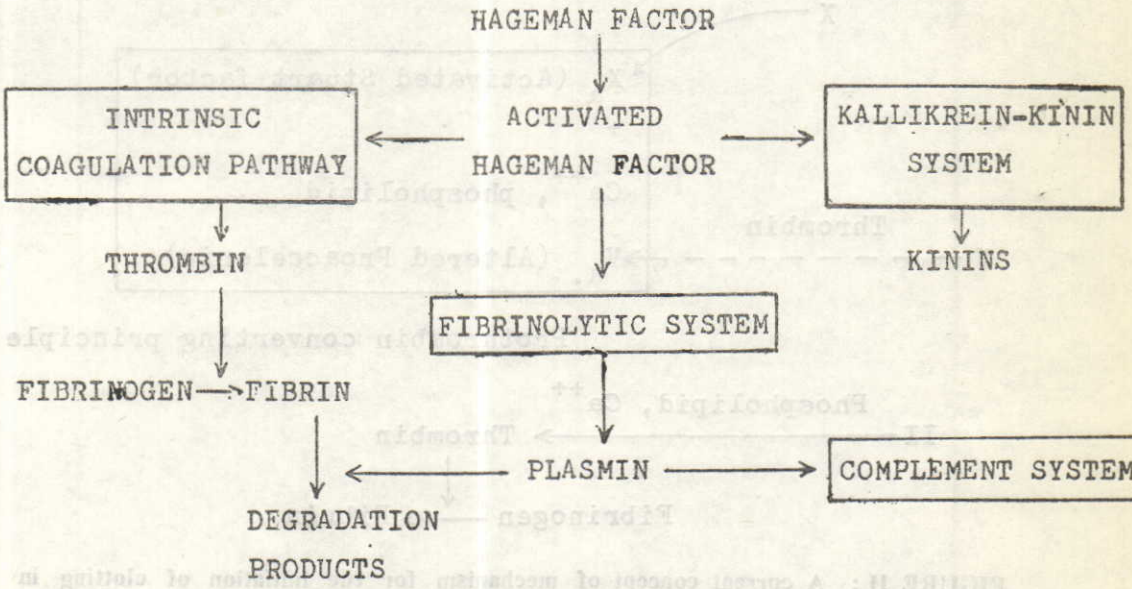


FIGURE I. Relationship between Hageman factor and various system and their interrelationship.

HAGEMAN FACTOR AND COAGULATION SYSTEM

Hageman factor พบได้ใน plasma ของ mammalian ทุกชนิดเท่าที่ได้มีการศึกษา ยกเว้นสัตว์ปีก เช่น เป็ด, ไก่, นกพิราป, ห่าน และไก่งวง เป็นต้น และอาจจะเป็นเหตุผลอย่างหนึ่งที่ทำให้เลือดของสัตว์เหล่านี้ไม่แข็งตัวในหลอดแก้ว Hageman factor เมื่อสัมผัสกับ rough surface เช่น glass, kaolin จะถูกเปลี่ยนเป็น activated form ซึ่งต่อไปจะไป activate PTA (Factor XI) และ coagulation factors อื่นใน cascade system จนเกิด thrombin ขึ้น

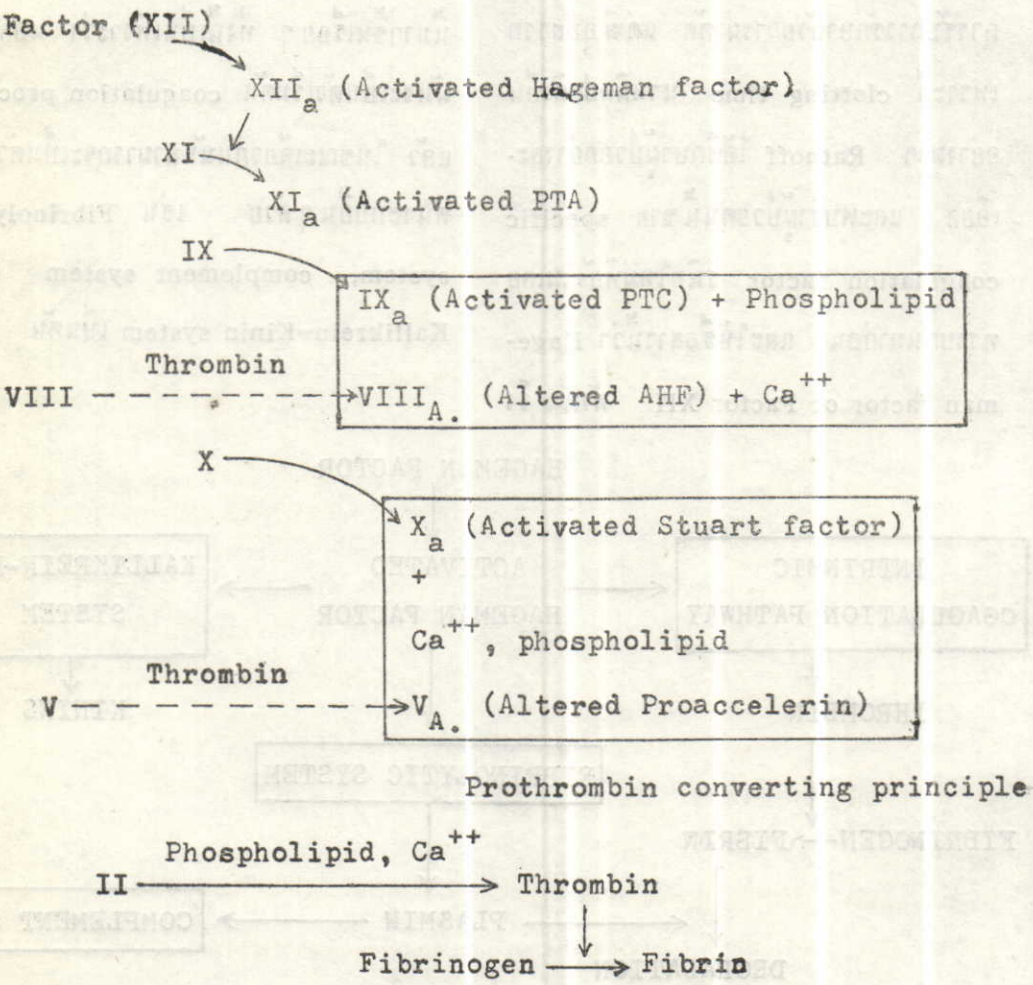


FIGURE II : A current concept of mechanism for the initiation of clotting in mammalian plasma via the intrinsic pathway.

ทฤษฎีที่นิยมกันในปัจจุบันถือว่า การที่เลือดแข็งตัวเป็น ผลที่เกิดจาก enzymatic reactions ที่เกิดเป็นลูกโซ่ ที่ในตอนท้ายทำให้เกิด thrombin ไปย่อย fibrinogen ให้กลายเป็น fibrin ปฏิกริยาลูกโซ่เช่นนี้ได้รับการสนใจเป็นพิเศษ เพราะในแต่ละขั้น substrate ถูกเปลี่ยนแปลงไปแล้วไป catalyze ปฏิกริยาขั้นต่อไป

HAGEMAN FACTOR AND FIBRINOLYTIC SYSTEM

Hageman factor ภายหลังที่ถูกเปลี่ยนเป็น active form ต้องอาศัย Hageman cofactor จึงจะสามารถกระตุ้น fibrinolytic mechanism โดยเปลี่ยน plasminogen ให้กลายเป็น plasmin ซึ่งเป็น proteolytic enzyme ที่สำคัญ

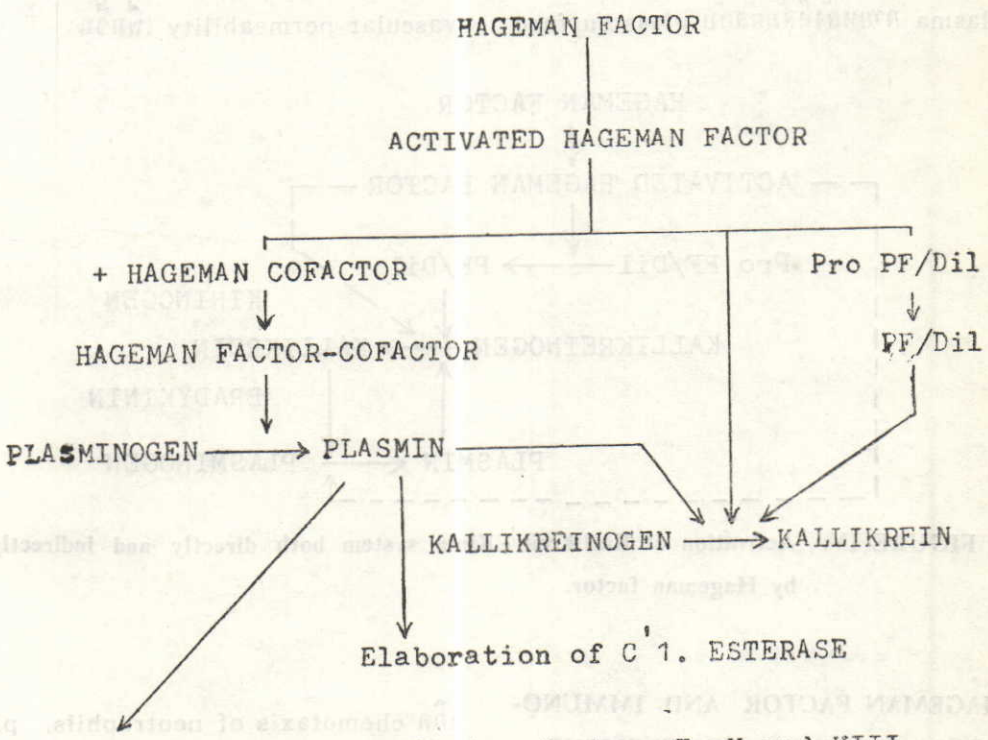


FIGURE III: Activation of fibrinolytic system by Hageman factor. Note actions of plasmin on other systems.

ดังนั้นในขณะที่ Hageman factor เป็นตัวเริ่ม coagulation process มันก็จะกระตุ้นกลไกในการทำลาย blood clot ที่เกิดขึ้นพร้อมกันไป

HAGEMAN FACTOR AND INFLAMMATORY REACTION

ความสัมพันธ์ระหว่าง Hageman factor กับ inflammatory reaction เริ่มต้นทราบกันเมื่อ Armstrong et al (1954) พบว่า plasma ภายหลังที่สัมผัสกับ glass surface แล้วจะเกิดสารชนิดหนึ่งซึ่งทำให้เกิด pain และ smooth muscle contraction ได้ Keele et al พบว่า pain ที่เกิดขึ้นนี้เกิดเนื่องจาก kinins ต่อมา Spector (1957) และ Spector (1958) จึงพบว่า activated Hageman factor ทำให้เกิดการเปลี่ยน proenzyme (Pro PF/Dil) เป็น PF/Dil (Permeability factor evolving in plasma upon dilution) แล้วไปทำให้มี vascular permeability เพิ่มขึ้น

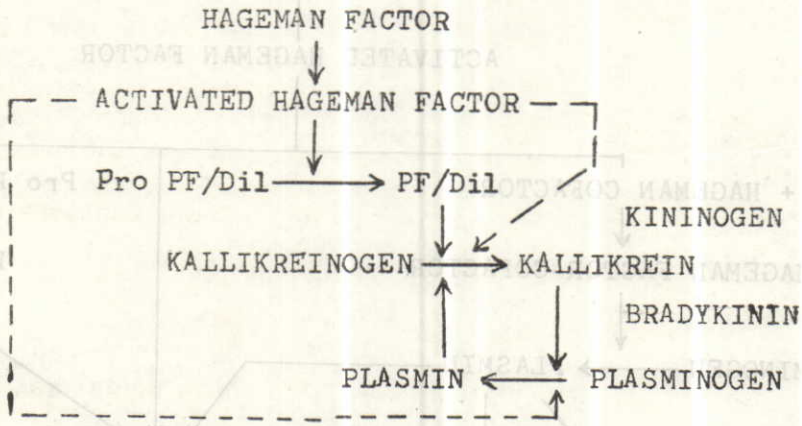
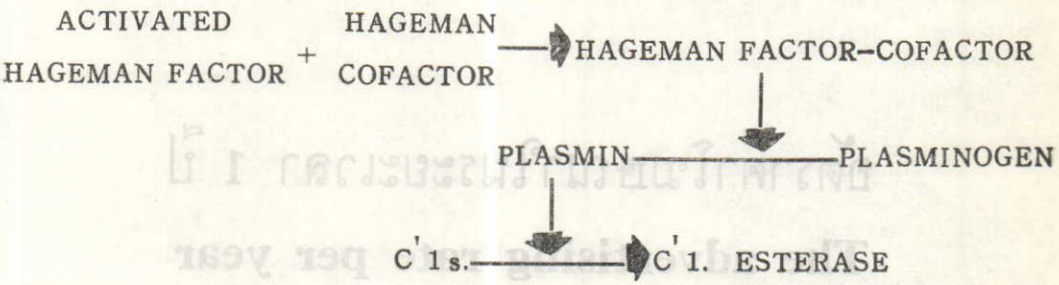


FIGURE IV: Activation of Kallikrein - Kinin system both directly and indirectly by Hageman factor.

HAGEMAN FACTOR AND IMMUNOLOGIC DEFENSE - COMPLEMENT SYSTEM

เมื่อมี antigen-antibody complex เกิดขึ้น มันจะทำให้ complements ทั้ง 9 มีปฏิกริยาต่อเนื่องกัน ในระหว่างนี้ก็จะทำให้เกิด chemotaxis of neutrophils, phagocytosis, anaphylotoxin and histamine release และสุดท้าย คือ cell lysis. Hageman factor โดยอาศัยปฏิกริยาผ่าน fibrinolytic system สามารถกระตุ้น

complement action ได้ โดย plasmin ที่เกิดขึ้น จะไปทำปฏิกริยากับ C'1.S ทำให้เกิด proteolytic enzyme C'1 esterase ขึ้นมาซึ่งต่อไปก็จะไปทำปฏิกริยากับ C'4 และ C'2 เป็นลำดับต่อไปเช่นเดียวกับปฏิกริยาที่เกิดขึ้นเนื่องจาก antigen–antibody complex ทุกประการ



ดังนั้นจะเห็นได้ว่าในร่างกายเรานี้มี substrates ที่สำคัญของ Hageman factor อย่างน้อย 4 ชนิดด้วยกัน คือ PTA, plasminogen, Pro PF/Dil และ C'1s. และช่วยให้เราเข้าใจถึงกลไกของโรคที่เกิดขึ้นหลายชนิด เช่น Disseminated intravascular coagulation (DIC), drug–induced thrombocytopenia, endotoxin–shock, hyperacute graft rejection, gout arthritis หรือพยาธิสภาพที่ปอดของ "blue velvet" addicts ว่าความผิดปกติที่เกิดขึ้นหลายระบบ ซึ่งไม่น่าจะเกี่ยวข้องกันเลยนั้นเกิดขึ้นมาได้อย่างไร

น.พ. บัญจะ กุลพงษ์
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THE SERUM LIPIDS

I. BIOCHEMISTRY, CLASSIFICATION AND CLINICAL SYNDROMES

Muni Keoplung, M.D. *
Jit Jiraratsatit, M.D. *
Nuntaya Waiwatana, B. Sc. (Med. Tech.) **

The serum lipids are current subjects of great interest, especially, in the etiologies and courses varieties of diseases. Therefore, it is whorthwhile to review their biochemical and clinical view-points before applying them for clinical purposes.

According to Bloor, (I) the classification of the lipids which is generally accepted in the United States and with a few modifications, is as follows :

A. Simple lipids - esters of fatty acids with various alcohols.

1. Neutral fats and oils-triglycerides: triesters of fatty acides with glycerol.
2. Waxes : esters of fatty acids
   a. True waxes
   b. Cholesterol ester : esters of fatty acids with cholesterol
   c. Vitamin A ester : palmitic or stearic acid ester of vitamin A
   d. Vitamin D esters

B. Compound lipids - esters of fatty acids with alcohol plus other groups.

1. Phospholipids : lipids containing phosphoric acid and, in most cases, a nitrogenous base
2. Glycolipids or cerebrocides; lipids containing carbohydrate and also nitrogen but no glycerol
3. Sulfolipids : lipids characterized by possessing sulfate group
4. Lipoproteins : lipids attached to plasma or other proteins
5. Lipopolysaccharides : lipids attached to polysaccharides

C. Derived lipids : derivatives obtained by hydrolysis of those given in group A and B

1. Saturated and unsaturated fatty acids
2. Monoglycerides and diglycerides
3. Alcohols

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D. Miscellaneous lipids

1. Aliphatic hydrocarbons
2. Carotenoids
3. Squalene
4. Vitamin A and B

The purpose of this paper is directed to certain lipids that are commonly found or involved in the diseases.

TRIGLYCERIDES : There are triesters of trihydric alcohol glycerol and various fatty acids. They are the most abandant natural lipids. The biosynthesis of triglycerides in the body appearently occurs primary in the liver. It is stored in the adipose tissue. The epithelial cells of the intestinal mucosa are active in this respect.

Triglycerides do not function only as a source of energy : but also solvent for fat - soluble vitamins, aiding in the transport and function of these substances.

PHOSPHOLIPIDS : The phospholipids, mostly produced by the liver, present in all cells and are the second most naturally occurring lipids. Most of them are composed of fatty acids, a nitrogenous base, phosphoric acid, glycerol, inositol and sphingosine. They play vital roles as constituents of cell membrances and factors in regulating membrane permeability. They are present in the myelin sheath of nerve cells and in electron - transport particles.

LIPOPROTEINS : The lipoproteins are extremely important type of lipids because of their vital roles in the solubilization and transport of water - insoluble lipids in the plasma and other aqueous fluids in the body. They are association complex of varying proportions of triglyceride, phospholipid and cholesterol. The protein moieties are primarily formed in the liver (4) and to some extent in the intestinal mucosal cells. The lipid component decreases the density of the lipoproteins. The differences in the content of lipids and proteins among several lipoproteins result in difference densities and permit their further separation by ultracentrifugation. The lipoproteins also differ in their electrical charge. This property, in combination with differencs in size, also permits separation of the lipoproteins into specific bands by electrophoresis.

By their electrophoresis or ultracentrifugation, the plasma lipoproteins are usually considered as 4 representing families.

Chylomicrons - S_f greater than 400

Pre - Beta - lipoproteins - S_f 21 - 400 (very low density)

Beta - Lipoproteins - S_f 0 - 20 (low density)

Alpha - Lipoproteins - High density

The chemical composition of major lipoproteins ; its relationship expressed by various methods and its normal value are shown in table I, II and fig. I. respectively.

CHYLOMICRONS : These large fat particles have a density of 0.9. They

are collected at top of plasma after left standing for 16 - 24 hours at 0 - 4° c. Most chylomicrons are considered to represent exogenous triglycerides and can cause cloudy appearance of the serum.

PRE - BETA - LIPOTROTEINS : These are very low density lipoprotiens (VLDL). Their density is less than 1.006. Pre-Beta-lipoproteins are apparently concerned mainly with the transport of endogenous triglycerides. It consists mainly of glycerides newly synthesized or derived from body store rather than directly from the diet. It seems possible that VLDL may be the only form in which lipoproteins are secreted by the liver and is not identical with those formed in circulation in the case of cholesteryl esters which are mainly synthesized by the plasma lecithin (4). It is also found that the lymph also contains VLDL synthesized by the intestine. In fasting stage, about half of lymph triglyceride and cholesterol is found in VLDL and these lipids are not derived from the plasma but from intestinal contents and mucosal synthesis (5).

BETA - LIPOPROTEINS : They are called low density lipoproteins (LDL) and can be isolated between the densities of 1.006 and 1 063 (S_f 0-20). Beta-lipoproteins appear to be the major transport medium for cholesterol. Their major constituents are cholesterol and cholesteryl ester ; the remaining components are phospholipids, protein and glyceride.

The composition of the S_f 0-20 lipoprotein contain 3 classes ; (6) HDL, LDL_3 and lipoprotein which shares antigenic determinant with LDL, LDL - a - l, HDL. Helenius and Simons (7) removed LDL by four different detergents and found that the lipid-free-proteins obtained still retained the immune properties.

ALPHA - LIPOPROTEINS (as more specifically Alpha $_1$-lipoprotein) : These are high density lipoproteins (HDL) being isolated at the densities of 1.063-1.21. They contain about 50 % of protein; lipid component consists mainly of cholesterol and phospholipids in the ratio of about 0.5 (by weight). It is now established that the mechanism of removal of lipoprotein and triglyceride by the enzyme, clearing-factor lipase (lipoprotein lipase); occuring at capillary cells and that the free fatty acids released then pass across the endothelail cells into the tissues. (8)

FREE—FATTY—ACIDS : Protein bound fatty acids or free fatty acids (FFA) that circulate are albumin bound. The normal concentration is 0.3 - 0.6 mEq/l with the average of 0.4 mEq/l. (9) They are important in meeting caloric demands and have most labile concentration. The FFA concentration is elevated in starva-

tion, excercise, emotional stress, low-tissue insulin activity and hyperthyroidism. Nicotine, caffeine and injection of epinephrine also raise the plasma FFA level. The effect is produced by the stimulation of lipolysis of triglyceride in adipose tissue.

The FFA level parallels the blood glucose level in diabetes, and in some ways is a better indication of the severity of the diabetic stage. (10) In diabetic acidosis, the level of FFA may rise to 1.5 mEq/l, returning to normal after successful treatment with insulin. The FFA released from adipose tissue in excess of those utilized by muscle, liver or other tissues mainly reappear in plasma as endogenous glyceride. The FFA may cause intravascular thrombosis, but this has been demonstrated only in very high concentrations.

BIOLOGICAL VARIATION : The fasting levels of the different plasma lipids, vary considerably between different individuals, population and also from hour to hour and day to day in one and the same individual.

Genetic, sex, age, diet, posture, venostasis, different environment, excercise, emotion, pregnancy and smoking pay influence in the plasma lipid levels. Taggart and Carruthers (11) showed that among racing drivers, free fatty acid levels were elevated for one to three minutes before the start and were maintained up to one hour after the race. The triglyceride levels were slightly elevated after the event and continued to increase and reached a peak at one hour.

CLASSIFICATION OF HYPERIPIDEMIA AND HYPERLIPOPROTEINEMIA

The proposed chemical classification provides and approach to the etiologic and to the pathogenetic factors of diseases.

HYPERLIPIDEMIA : This term refers to an increase in concentration of any plasma lipid constituents. For practical purpose, usually it is confined to cholesterol or glyceride or both.

The analysis of cholesterol and triglyceride also provide some information about the type of hyperlipoproteinemia because the proportion of these lipids varies from one lipoprotein family to another. Winkleman et al recommended that cholesterol and triglycerides should be routinely determined in the laboratory for testing of phenotyping of lipoproteinemia. The analysis must be performed in the same specimen to be run for electrophoresis in order to provide satisfactory accuracy. They could not classify a number of serum specimens because of the subjective interpretation of the positive and intensity of lipoprotein bands as due to the poor reproducibility and limited ability of the techinque to have a clear seperation between Beta and pre-Beta-bands. (12)

Three general types of hyperlipidemia that roughly coresponds to certain types of hyperlipoproteinemia are : -

1. High cholesterol concentrations and normal triglyceride concentrations - this group is also called " pure hypercholesterolemia ". It to corresponds to hyper - Beta - lipoproteinemia.

2. High cholesterol concentration and normal triglyceride concentration - this group usually corresponds to either " pure hyperchylomicronemia " or hyperpre-Beta-lipoproteinemia.

3. High cholesterol and high triglyceride concentrations - all of these major types of hyperlipoproteinemia, except "pure" hyper - Beta - lipoproteinemia, may occur in this group. The classification of this third group is based on types of lipoproteins.

HYPERLIPOPROTIENEMIA : For the sake of simplicity, the patterns or types of abnormal lipoproteins can be numbered according to the system of Fredrickson and colleagues, as shown in table III.

The plasma obtained from patient with either type I or type V hyperlipoproteinemias is characterized by a creamy layer separating over a clear (type I) or turbid (type V) plasma. Typer II, III, IV are characterized by a turbid plasma.

Typeo I and II_a are induced by fat feeding whereas type IV is carbohydrate induced. Typer II_b, III and V are induced by feeding both fat and carbohydrate.

Type I. Hyperchylomicronemia

Criteria : 1. Chylomicron present in excessive amount.

2. VLDL (pre-Beta-lipoprotein) normal or only slightly increased. This is partly due to the difficulty of separating these two lipoprotein families. The amount excess to VLDL, however, is always far less than overwhelming amount of chylomicrons (Alpha - Beta - lipoproteins are always decreased).

Type II. This type may be distinguished into two subtypes.

II_a only LDL (Beta-lipoprotein) is increased but

II_b both LDL (Beta-lipoprotein) and VLDL (pre - Beta - lipoprotein) are increased.

The presence of II_b in plasma may require additional treatment to that required for " pure " hypercholesterolemia.

In II_a plasma cholesterol is usually increased; plasma triglycerides are normal; cholesterol/triglyceride is always more than 1.5.

In II_b plasma cholesterol is usually increased ; plasma triglyceride is always increased; cholesterol/triglyceride is variable (not diagnostic). Alpha - lipoproteins are usually normal (diagnostic only if accompanied by estimation of LDL and

VLDL concentration).

Typer III. "floating Beta" or "broad Beta" pattern (lipoproteins of Beta mobility, floating at density of 1.006).

Criteria. Presence of "floating Beta" "Beta - (VLDL)" or with abnomal Beta-lipoprotein and abnormal high cholesterol.

Cholesterol / Triglyceride may vary from 0.3 to 2.0

Plasma triglycerides are (nearly) always increased.

Electrophoresis shows a "broad" band extending from the Beta position into the pre - Beta - position.

This type III lipoprotein may be suspected when a cholesterol/triglyceride ratio, especially when repeated analysis, shows marked lability of both cholesterol and triglyceride concentrations ; and a "broad Beta" band appears on conventional electrophoresis.

Type IV. Hyperpre-Beta-lipoproteinemia.

Criteria. 1. increased VLDL

2. no increase in LDL

3. chylomicron absent (diagnostic)

If the plasma cholesterol is difinitely normal, triglycerides are clearly increased and there no chylomicrons visible on standing plasma, the ascertainment of type IV is fairly certain. The accuracy is enhanced if electrophoresis reveals distict pre - Beta band and diminished Beta band.

Typet V. Hyperpre - Beta - lipoproteinemia and chylomiccronemia.

Criteria : 1. VLDL increased

2. Chylomicron present

Additional useful clinical data : Certian clinical findings are helpful in detecting hyperlipidemia and sometimes the prediction of the type of hyperlipoprotein can be made clinically.

e. g. Lipid deposit xanthomas, almost invariably, indicate hyperlipoprotein of long duration. They usually indicate hyper - Beta - lipoprotein and almost always imply to familial type II hyperlipoproteinemia.

Xanthelasma is frequent in type II and sometimes occurs in type III, but it may be seen in the bsence of hyperlipoproteinemia or hyperlipidemia.

Arcus cornea (arcus senilis) if occurs before the age of 40 years implies to f miiial type II hyperlipoproteinemia.

Pancreatitis or recurrent abdominal pain should lead to a suspicion of severe hyperglyceridemia (type I or V).

Ischemic heart disease and other vascular accidents in young relatives of the family with such diseases are usual in familial type II and type IV hyperlipoproteinemia.

(Diabetes is often seen in famlilies with type IV and type V hyperlipoproteinemia. In diabetic family, these disorders may occur long before the patient becomes diabetic).

The useful laboratory data are thyroid function, glucose tolerance, urinary protein; plasma protein lelectrophoresis, immune globulin quantification, liver fuction and uric acid.

Etiology of Hyperlipoproteinemia : When the pattern of hyperlipoproteinemia is established, the etiology must be considered whether it is primary or secondary hyperlipoproteinemia.

Secondary hyperlipoproteinemias are commonly associated with

1. Hypothyroidism
2. Diabetes
3. Nephrotic syndrome
4. Biliary obstruction
5. Pancreatitis
6. Dysglobulinemia including autoimmune hyperlipoproteinemia.

The lipoprotein patterns in such diseases are shown in the table IV.

Primary hyperlipoproteinemias are due to genetic defects in lipid or lipoprotein metabolism or to environmental factors through an unknown mechanism including diet or alcohol, and drugs causing hyperlipidemia e. g. steroids.

Familial hyperlipoproteinemias obviously need not be inheritable if it is due, for example, to pattern of excess in diet or alcohol intake that have been acquired by close relatives.

Clinical syndromes of hyperlipoproteinemias

Primary (Familial) type I.

The key clinical features of this familial syndrome are early expression of bouts of abdominal pain and other accompaniments of severe hyperlipemia low post heparin-lipolitic activity (PHLA) and autosomal recessive transmission. Yellow papules with reddish base may appear on the skin and oral mucosa. Liver and spleen are enlarged, while retinal vessels (lipemia retinalis) may be seen. Blood sample shows "creamy" appearance on the top. In children, if fat-free milk only is fed, the lipemia will be cleared dramatically within the day. The xanthoma will shortly resolve, the liver and spleen will decrease in size, abdominal discomfort, then, will disappear finally. In adults, sometimes the abdominal discomfort simulates immediate surgical conditions and undergoes unnecessary surgery.

In this type, oral or intravenous glucose tolerance shows no abnormality. This differs from other "fat-induced" type. (type IV and V).

Diagnosis can be made by the following steps :-

1. Identification of the type I lipoprotein pattern
2. Glyceride accumulation is immediately related to dietaty fat intake.
3. Plasma post heparin - activity (PHLA) is low

Type II. Hyperlipoproteinemia

High Beta-lipoprotein can be a resultant of diet or secondary to hypothyroidism and other diseases.

The second feature of type II pattern is associated with a modest increase in pre-Beta-lipoprotein. Femilial type II may be due to a mutation at the same genetic locus, heterozygote; xanthomatosis and atheromatosis are frequently seen.

Diagnosis. A triad for diagnosis is

1. Hyper-Beta-lipoprotein
2. Familial history of type II lipoproteinemia as an autosomal dominant trait.
3. Xanthomatosis

Practically (1) with (3) is adequate enough for diagnosis.

The most characteristic manifestation is tendinous xanthomas located particulary in the Achille tendons, and the extensor tendons of hands and feet. But important clinical features of familial type II are autosomal dominant and usually accompanied by palpebral, tendon and xanthomas, corneal arcus and accelerated arthero sclerosis. Glycerides (pre-Beta-lipoprotein) may be moderately elevated; PHLA is normal and glucose tolerance is usually normal.

Clofibate has less effect on plasma cholesterol in typer II, the most effective and least toxic agent is cholestyramine, a nonabsorbable exchange resin that stimulates cholesterol catabolism to bile salt by preventing their reabsorption from ileum. By using this drug, the rate of synthesis of cholesterol is less than that of catabolism, therefore, the net effect is the decrease of plasma cholesterol. Conversely in homozygote, cholestyramine does not decrease plasma cholesterol since the endogenous synthesis of cholesterol will increase to compensate for the increased catabolism to bile salt. An attempt to overcome the increased endogenous synthesis by nicotinic acid plus cholestyramine seems promising. (4)

Typer III. Excess of lipoprotein that has Beta mobility but abnormally low density manifests by hypercholesterolemia and hyperglyceridemia. The lipoprotein levels are quite sensitive to changes in the content of the diet and the amount of total calories. Polyunsaturated fat diet containing only 100-200 mg of cholesterol/day may lower both cholesterol and triglyceride concentrations for several fold in a few weeks. Carbohydrate intolerancerance is found in most patients.

Clinical manifestation besides arcus, palpebral and tendon xanthoma, there usually are palmar and tuberoeruptive xanthomas. Advanced arteriosclerosis of peripheral and coronary arteries is common. PHLA is normal; if familial, it is inherited as a recessive.

Primary type IV. This is the hallmark of endogenous hyperlipidemia. It implies that glycerides synthesized in the body, mainly in the liver have been excreted into plasma at the rate exceeding the capacity for removal. It often suggests that something has gone wrong with carbohydrate metabolism on caloric balance. It is sometimes called "carbohydrate-induced hyperlipemia". It is mainfested as hyper-pre-Beta lipoproteinemia associated with an increase in glycerides and commonly a rise in cholesterol; normal PHLA.

Severe type may show what described in exogenous hyperlipidemia. The patients are also subjected to develop bouts of abdominal pain with or without chemical signs of pancreatitis. Hyperuricemia is as common as glucose intolerance. Familial occurrence is seen in young children.

Secondary type IV. This can be seen in diabetes mellitus, pan-creatitis, glycogen-storage disease, idiopathic hypercalcemia, hypothroidism (but less common than type II, dysglobulinemia, pregnancy and contraceptive drugs).

Type V. This is a combination of both exogenous and endogenous hyperlipemia. The commonest complaint is recurrent abdominal pain but the symptom frequently appears in the late teen or third decade as compared to that occurs in infancy in type I. This is no relation to obesity or diabetes. PHLA is usually normal but may be somewhat lower. (In type I. distinctly low). Glucose tolerance is almost invariably abnormal. This pattern of hyperlipoproteinemia is often familial but there are "phenocopies" secondary to many disorders.

HYPERLIPOPROTEINEMIAS

Primary: These conditions are exceeding rare but have considerable importance.

Abetalipoproteinemia (Bassen-Kornzweig Syndrome). This disease, inherited as an autosomal recessive, is characterized by complete absence of LDL, VLDL and chylomicrons. It is manifested by severe malabsorption, beginning in early childhood, followed by progessive ataxia, nystagmus, weakness and visual impairment with scotomas. These are related, respectively, to inability to deliver triglycerides (due to absence of Beta-lipoprotein) into the intestinal lymph, demyelination of spinocerebellar tracts, posterior columns and occasionally peripheral nerves and pigmentary retinal degeneration (atypical retinitis pigmentosa). Other findings include thorny of spiny red cells (acanthocytes) and fatty liver (resulting from failure to export triglycerides in very low density lipoproteins)

This disease should be considered in any patient with hereditary ataxia. Treat-

ment is limited to restriction of ordinary fat and supplementation with fat soluble vitamins. Medium chain triglycerides are, however, well tolerated.

Alpha-lipoprotein Deficiency (Tangier disease). This is homozygous state leading to diffuse deposition of cholesterol esters in reticuloendothelial system with enlargement of the tissues, especially, the grossly enlarged tonsils (characteristic orange color). Foam cells may be found in the bone marrow. Usually the disorder does not affect growth. No treatment is available.

Lecithin - cholesterol Acyltransferase Deficiency. This disorder has been found in a Norwegian family. The effected subjects have proteinuria, anemia, and corneal deposits of lipid. HDL is almost absent with moderate to large elevations of VLDL and chylomicrons are present. Foam cells can be found in the bone marrow and renal glomeruli and increased concentration of cholesterol and lecithin in the red blood cells are also present.

Secondary : These states are characterized chiefly by hypobetalipoproteinemia and are the result of malnutrition, malabsorption, or parenchymal liver disease. Formation of LDL is decreased, presumably, due to decreased transport of exogenous and endogenous triglycerides. In healtly individuals whose intake of calories and saturated fats is low, the concentration of LDL are also decreased.

FIGURE I.

The p asma lipoprotein spectrum segregated by paper electrophoresis (above) and by the ultracentrifuge (below) in which S_f or floatation rates are inversely related to density.(2)

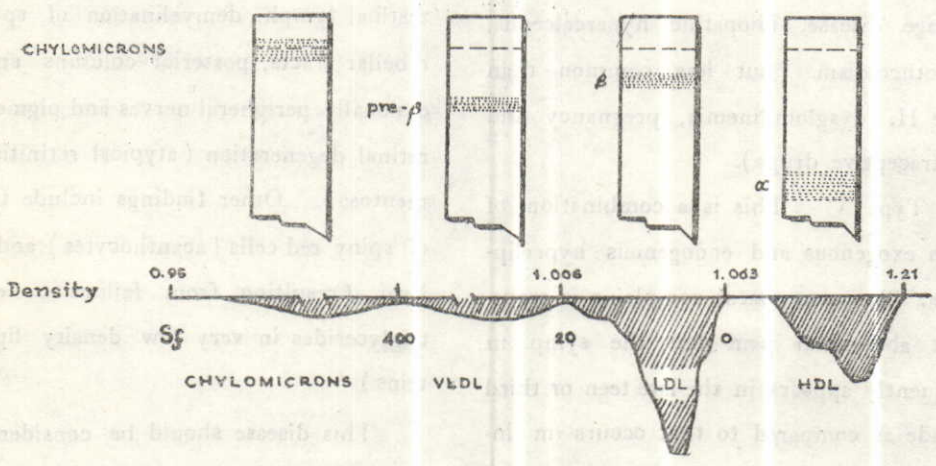

TABLE I.

Typical composition of major lipoproteins as expressed by percent of dry weight.

Lipoproteins	Protein	Triglyceride	Phospholipid	Cholesterol
Chyiomicrons	1.5 ± 1.0	87 ± 7	9 ± 6	7 ± 5
Pre-Beta	7 ± 5	65 ± 15	18 ± 8	16 ± 7
Beta	23 ± 3	10 ± 2	22	43
Alpha	50 ± 5	7 ± 2	30	18

(Adapted by Orten from data in Masoro, E.J.: Physiological Chemistry of Lipids in Mammals, Philadelphia, 1968, W.B. Saunders Co., Vol. I.)

TABLE II.

Suggested "Normal Limits" of plasma lipid and lipoprotein concentrations in normal subjects.

Age	Sex	Total Cholesterol	Triglyceride	Pre-Beta-lipo.	Beta-lipo.	Alph-lipo.	
						M	M
0-19		120-230	10-140	5-25	50-170	30-65	30-70
20-29		120-240	10-140	5-25	60-170	35-70	35-75
30-39		140-270	10-150	5-35	70-190	30-65	35-80
40-49		150-310	10-160	5-35	80-190	30-65	40-85
50-59		160-330	10-190	10-40	80-210	30-65	35-85

(For practical purpose differences between sexes have been ignored excepted of Alpha-lipoprotein concentrations).[3]

TABLE III.

The major abnormal lipoprotein pattern[a] and their type number.[2]

Type	Chylomicrons	LDL (Beta-lp	VLDL (pre-Beta-lp)	Floating Beta-lp[b]
I	+			
II_a		+		
II_b		+	+	
III				+
IV			+	
V	+		+	

a) Indicates which lipoprotein "family" (families) occurs in concentration above "normal" in the different abnormal patterns.

b) also know as "broad Beta-lipoprotein".

TABLE IV.

Types of hyperlipoproteinemia associated with selected common diseases:

Disorder	Type of Hyperlipoproteinemias
Hypothyroidism	II, IV
Insulin-dependent diabetes (uncontrolled)	I, IV, V (II, III)
Nephrotic Syndrome	II, IV, V
Biliary obstruction	Does not conform predictably to any of the major types
Pancreatitis	IV, V
Dysglobulinemia	I, II, IV, V, (III)
Auto-immune hyperlipoproteinemia	I, III, IV, V, (II)

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Evaluation of Griess test and Calibrated Loop–Direct Streak Method Determination of Infected Urine

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The experiment was performed to test the efficiency of Griess test compared to the colony counting method, which when positive giving more than 10^5 cell/ml. of urine streaked from a calibrated loop. The first morning mid-stream voided urine obtained from 258 clinical patients and 223 pregnant woman in Nakorn-Chiang Mai Hospital.

The total urine specimens of 481 were culture in blood agar and Mc Conkey agar plates by the means of calibrated-loop direct streak. The number of bacteria were counted and other differential media were used to differentiate the organism. Griess test was also performed parallel to the test described above.

Out of 258 specimens form the clinical patients 61 specimens were positive for colony count having more than 10^5 cell/ml. Showed 90.2% positive for Griess test. The organism found mostly was E. coli 34%.

The colony counting in pregnancy urines showed 12 out of 223 specimens to be positive. These were only 9 specimens out of 12 specimens positive in Griess test (75%). The organism mostly found was also E. coli (50%).

In 1870, Griess, a German Chemist. developeh a reagent for detection of nitrites in solution. The reagent, an acid solution of sulfanilic acid and alphanaphthylamine, undergoes a diazotization reaction with nitrites to form a red azo dye. Cruichshank and Moyes using this reagent demonstrated a direct correlation between the presence of nitrite in urine and the presence of colifrom urinary tract infec-

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tion. During this time, it has become apparent that 3 factor are of prime importance in determining whether are positive Griess reaction will be found :-

1. The presence of a adequate numbers of nitrate reducing bacteria.
2. The presence of nitrate in urine.
3. That the bacteria be incontact with the urine for sufficient time to allow the reduction of nitrate to nitrite.

The investication of this paper is to evaluate the Griess test and calibrated loop - direct streak method determination of infected urine.

Materials and Methods

Urine specimens obtained from Nakorn Chiang Mai Hospital by means of mid - stream voided urine. Blood agar and Mc Conkey agar or Eosin Methylene Blue agar (EMB) plates were used for culture. The 4 mm. diameter calibrated loop contained 0.01 ml. of urine.

Preparation of the Griess reagent : One and One-half of sulfanilic acid (chemically pure) were dissolved in 450 ml. of 10% acetic acid. This solution was added to a solution of 0.6 gm. alphanaphthylamine (Chemically pure) in 60 ml. of boiling distilled water and filtered through Whatman no 1 filter paper. This combined reagent, now colorless, was stored in a tightly stoppered dark bottle to prevent oxidation. The reagent in this form remained stable for two to four weeks and decomposition could be noticed by the appearance of a pinkish color in the solution. The activity of the reagent could be tested by adding a few drops of few milliliter of 10% sodium nitrite solution, the development of a red color meaned the reagent was in a good condition.

One calibrated loop of infected or pregnancy Urine was streaked on blood agar plate and on McConkey or EMB.

One mililiter of the same urine was added to 1 ml. of Griess reagent, the development of a pink or red color in a solution of seconds was considered to be a positive test :

After overnight incubation the plates were examined by counting and identification of organisms.

Result

Of those 258 urine were collected from December 1971 to February 1972. The relationship of colony counts in urine specimens to positive griess reaction (Table 1) showed 79 no growth but 1 Griess test positive (1.3%). 47 urine were colony count less than 10^4/1 ml. positived 3 Griess test (6.4%). 71 urine were counted between 10^4-10^5/1 ml. positived 31 Griess test (43.6%). 61 urine were counted more than 10^5/1 ml. positived 55 Griess test (90.2%).

223 pregnancy urines showed 63 no growth, and Griess test negatived: 88

urines were counted less than 10^4/1 ml. positived 4 Griess test (4.5%). 60 urines were counted between 10^4-10^5/1 ml. negatived Griess test. 12 urines counted more than 10^5/ml. positivod 9 Griess test (75%).

The bacteria isolated from 61 infected clinical urine specimens show on table II, the mostly found organisms was E. coli.

The bacteria isolated from 12 infected pregnancy urine specimens mostly found E. coli, show on table III.

The correlation of specimen of bacteria to Griess reaction and specimen yielding greater than 10^5 cell/ml. urines, show on table IV.

Discussion

This experiment indicated that Griess test gave a good result and should be used in the laboratory becouse it is practical, rapid, less time consuming and less cost than the calibrated-loop direct stteak. The disadventages of the Griess test are the false negative and that the organisms cannot be isolated for further differentiation and sensitivity test as in the calibrated-loop direct streak method.

Table I. The relationship of colony counts in urine specimens to positive Griess reactions.

colony count/ml. urine	no growth	$< 10^4$	10^4-10^5	$> 10^5$
a) Clinical specimen				
Number of Specimen	79	47	71	61
Number of positive Griess test	1	3	31	55
percent of specimen with positive Griess test	1.3	6.4	43.6	90.2
b) Pregnancy Specimen				
Number of Specimen	63	88	60	12
Number of positive Griess test	–	4	–	9
percent of specimen with positive Griess test	–	4.5	–	75.0
c) Clinical specimen, Pregnancy specimen				
Number of specimen	142	135	131	73
Number of positive Griess test	1	7	31	64
percent of specimen with positive Griess test	0.70	5.2	24.5	87.7

Table II. Bacteria isolated from 61 infected clinical urine specimens.

Organism found	No. of organism found	% of organism found
E. coli	27	34.0
Proteus mirabilis	15	18.9
Pseudomonas aeruginosa	7	8.9
Klebsiella species	6	7.6
Paracolon bacilli	5	6.3
Staphylococci	4	5.1
Achromobacter	4	5.1
Aerobacter	3	3.8
Alkalageneous	2	2.6
Beta – streptococci group A	2	2.6
Salmonella typhi	2	2.6
Citrobacter	1	1.8
Enterococci	1	1.3

Table III. Bacteria isolated from 12 infected pregnancy urine specimens.

organism found	No. of organism found	% of organism found
E. coli	6	50
Paracolon bacilli	2	16.7
Klebseilla species	1	8.3
Staphylococci	1	8.3
Beta – Streptococci group. A	1	8.3
Enterococci	1	8.3

Table IV. Correlation of kind of bacteria to Griess Reaction.
Specimens yielding greater than 10^5 cell/ml. urine.

organism	No. of positive culture	Griess Test positive	% Griess Test positive
E. coli	33	32	96.96
Proteus mirabilis	15	14	93.33
Pseudomonas aeruginosa	7	7	100
Klebsiella species	7	7	100
Paracolon bacilli	7	7	100
Staphylococci	5	3	60
Achromobacter	4	4	100
Beta Strep. Group. A	3	0	0
Alcaligenes species	2	2	100
Salmonella typhi	2	2	100
Enterococci	2	0	0
Citrobacter	1	1	100

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DEFECTIVE CELL-MEDIATED IMMUNE MECHANISM IN LEPROMATOUS LEPROSY

by

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INTRODUCTION.

Failure of host resistance frequently results from a defect in cell-mediated immunity (CMI). However, hypersensitivity reactions resulting in tissue damage can occur as readily from CMI as from the deposition of immune complexes involving humoral antibody. Such an interaction between immune procedures and a given microorganism can display a wide spectrum of pathological process, which, in turn, leads to markedly different clinical manifestations. Such a spectrum is particularly well demonstrated by the recent elaboration of the varied clinical patterns in leprosy. Lepromatous and tuberculoid leprosy represent polar forms of an infection caused by Mycobacterium leprae. The former is a severe and progressive where as the latter type is more benign and often self-limiting. Various defective immune mechanism are observed more often and are of greater degree in lepromatous type.

Our first part of the study is to establish the prevalence of leprosy patients with evidence of defective CMI and located them for further studies. The responsiveness of the circulating lymphocytes to phytohemagglutinin (PHA) is used as a guide of their immunologic capability.

MATERIAL AND METHODS.

Heparinized venous blood samples were obtained from 10 male patients with lepromatous leprosy being treated at Mc. Kean Leprosarium. The responsets of the circulating lymphocytes to PHA stimulation were evaluated by the morphological study

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and H^3-TDR incorporation as previously described. (1)

RESULTS

There is a markedly reduced percentage of lymphocyte transformation in 10 lepromatous leprosy patients as compared to 10 normal adults.

TABLE I: PERCENT LYMPHOCYTE TRANSFORMATION FROM PHA STIMULATION

No.	Sample	with PHA	Control
10	Leprosy	7.0 ± 5.83	2.8 ± 2.62
10	Normal	89.7 ± 5.25	7.9 ± 5.24

COMMENTS

A moderately lowered capacity to develop allergic responses of the delayed type is often noted in lepromatous leprosy. Several investigators have demonstrated that lepromatous patients have a greater severity of a defect in the ability to develop delayed sensitivity to contact allergen and DNCB (2-4). The capacity of peripheral lymphocytes from leprosy patients to undergo blastic transformation on exposure to either PHA or specific antigens of Mycobacterium leprae, M. tuberculosis, PPD and streptolysin 0 is depressed (4-9). The depression of the response is considerably less in drug-treated lepromatous patients and those with tuberculoid leprosy. The low level of response to specific antigenic stimulation by lymphocytes from certain leprosy patients appear to be partially related to a depressive effect of autologous plasma. (9) This depressor factor as found by Bullock et al (9) was non-dialyzable, stable after prolonged storage at -20°C, and resistant to heating at 56°C. and its activity was lost at relatively low dilutions. In 1968 Cooperband et al (10) had found that the alpha globulin factor is highly depressed to PHA induced DNA synthesis. It is postulated that the plasma of certain patients with leprosy might contain anti-immunoglobulin antibodies that partially block antigen receptor side on lymphocyte surface (11, 12). Experimental work in support of this concept has been demonstrated by Greaves et al (13) who were able to suppress the response to PPD almost totally in vitro when sensitized human lymphocyte were cultured in the presence of Fab. fragment of anti-light chain antibodies. Regardless of the exact nature of the plasma depressor factor, it is accepted that this factor must be present in low-

concentration, since its effect is lost at low dilution in normal plasma, with very low binding affinity since part of the activity can be washed from leukocytes with relative ease. On the other hand, the failure of the normal plasma to restore the "normal" response by lepromatous lymphocytes to specific antigen indicated that the defect in immune response may be predominantly if not completely due to cellular dysfunction. It is of interest in this regard that lymph nodes from some patients with lepromatous leprosy appear to be deficient of lymphocytes in the paracortical areas. (13) Hypergammaglobulinemia (esp. IgG.) with lowering of serum albumin level (14) with arise in immunoglobulin-bearing lymphocytes (15) suggested the depletion of T-cells as well as a non-specific activation of the humoral immune response.

It is thus still uncertain whether the depressed CMI in leprosy relect a primary cellular defect, qualitatively, the depressive effect of a humoral factor, or both, to the varying degrees.

SUMMARY.

The in vitro response of peripheral lymphocytes from 10 patients with lepromatous leprosy to phytohemagglutinin stimulation was measured. All patients showed markedly depressed lymphocyte transformation which indirectly indicated the defective CMI mechanism. The various postulated mechanism were considered but the most important may be due to T-cells depletion.

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COMPARISON OF IN VIVO AND IN VITRO TOXIGENICITY TESTS FOR CORYNEBACTERIUM DIPHTHERIAE

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Kampol Panasampol, M.D. **

ABSTRACT

This study was undertaken to compare an in vitro and an in vivo toxigenicity tests of Corynebacterium diphtheriae. 17 out of 18 strains were mitis type (94.7%) and one strain was intermedius type (5.3%). All gave positive results to the in vitro and in vivo toxigenicity tests. Therefore, there was a complete correlation between the two methods.

It was suggested that serum substitute should be used in the in vitro toxigenicity test because of the simplicity and economics when compared with pooled rabbit serum.

INTRODUCTION

Toxigenicity test for C. diphtheriae can be performed in 2 methods :

(1) In vivo toxigenicity test. Two technics are commonly used :

(a) Intradermal test. Rabbit or guinea pig can be used by injecting suspension of diphtheria culture intradermally, after 5 hours, diphtheria antitoxin is injected. Another dose of bacterial suspension is injected intradermally in the other site as a control.

(b) Test of single subcutaneously. This technic will be used when the first technic give doubtful reaction. A normal guinea pig is injected subcutaneously with large does of bacterial culture. Another guinea pig is used as a control by injecting first with diphtheria antitoxin, and then with the same bacterial culture. The test animal will develop intoxication, paralysis and death, but the control animal will be normal.

(2) In vitro toxigenicity test. This test was developed by Elek in 1943 (1). The principle of this test is the develop-

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ment of lines of flocculation which are caused by the reaction between diphtharia toxin and its homologous antitoxin in the agar medium. Most of these agar media contain whole rabbit, human or house sera. A serum substitute which compose of commercial glycine, tween 80 and casamino acid can replaced the whole serum (5).

Our study is trying to compare the serum substitute with the whole rabbit serum for in vitro toxigenicity test. The comparison of in vitro and in vivo toxigenicity tests are also studied.

MATERIALS AND METHODS

(1) **Isolation and identification of C. diphtheriae.** Throat swabs are streaked onto cystine tellurite blood agar (CTB). Colonial morphology is recorded and transferred the culture to Loeffler slant media, stained, and examined with microscope.

Culture on Loeffler slant media are used for carbohydrate fermentation including glucose, sucrose, snd starch. Hemolysin production and pellicle formation are also tested.

(2) **In vitro toxigenicity test.** In vitro toxigenicity test of C. diphtheriae are tested according to the methods of Frobisher (6), King (2) and Parsons (4). The toxigenicity test agar composed of two parts:

1) Basal medium (6)

Proteose peptone (Difco)	2 gms
Granular agar	1.75 gms
Sodium Chloride, C.P.	0.25 gms
Distilled water	100.00 ml.

2) Serum substitute (5)

Casamino acids (Difco)	1 gm
Tween 80	1 ml.
Glycerol, C.P.	1 ml.
Distilled water	100 ml.

Three per cent of sterile rabbit serum or serum substitute is added into the basal media before used. The diphtheria antitoxin (Swiss Serum and Vaccine Institute Berne) is 500 units/ml in the paper strip in the toxigenicity test agar. Results of the toxigenicity tests are examined after 24, 48 and 72 hours. The positive test gives precipitin line between the culture lines and the antitoxin soated filter paper.

(3) **Rapid in vitro toxigenicity test.** Artificial throat swabs containing diphtheria bacilli were used in this study. The swabs were streaked onto the antitoxin media as described above. After 48-72 hours, precipitin lines occurred if they were positive.

(4) **In vivo toxigenicity test.** The intradermal test method is used in this experiment. Normal albino rabbits are chosen because of the large number of cultures. The back of rabbit is shaved and injected intradermally with bacterial cultures (turbidity = McFarland tube No. 3). After 5 hours, 500 units/ml of diphtheria antitoxin is injected intravenously into the marginal ear vein, then bacterial suspension is

injected intradermally into another position as a control.

After 24, 48 and 72 hours, a necrotic lesion can be seen at the first injection and no necrotic lesion at the second point of the injection when it is a toxigenic strain. If no reaction in both sites of injections, it is a nontoxigenic strains.

RESULTS

A total of 18 strains of C. diphtheriae were isolated, 17 strains were mitis type, one strain was intermedius type. One strain of C. xerosis and one strain of C. pseudodiphtheriticum were also isolated.

In the hemolysin production test, 13 strains (76.5%) of mitis type are positive, 4 strains (24.5%) are negative.

For the in vitro toxigenicity test, all 17 strains of mitis type gave positive for both serum and serum substitute agar in 48 hours. The intermedius type gave positive for both media after 72 hours. C. xerosis and C. pseudophtheriticum showed negative result in these media (see Table 1).

All 17 strains of mitis type and one strain of intermedius type gave precipitin line after 48 hours and better results after 72 hours. When the normal throat swabs, mixed with diphtheria bacilli, are streaked on CTB agars. The diphtheria bacilli could be isolated in all specimens.

The intradermal toxigenicity test is positive for all 17 strains of mitis type with the diameter of necrotic lesion 5-8 mm. One strain of intermedius type also gave positive result with a diameter of necrosis 4.5 mm. However, C. xerosis and C. pseudodiphtheriticum are negative for this test (see Table 2).

DISCUSSION AND CONCLUSION

Test for hemolysin production using human red blood cells revealed that 13 strains (76.5%) of mitis type were positive and 4 strains (24.5%) were negative. Therefore, hemolysin production was not the characteristic property of mitis type, it can be found in the other types (7).

The isolated diphtheria bacilli from infected patients in Nakorn Chiang Mai Hospital from 1968 - 1969 were mostly mitis type (94.4%) and only 5.6% were intermedius type.

All 17 strains of mitis type and one strain of intermedius type gave positive in vitro toxigenicity test on both serum and serum substitute antitoxin agar. The disadvantage of using pooled rabbit serum is the hemolysis of some erthrocytes giving rise to high concentration of iron. When the concentration of iron more than 1.4 mcg/ml. will decrease the production of diphtheria exotoxin (3, 5). Therefore, serum substitute is recommended for in vitro toxigenicity test.

All strains of diphtheria bacilli in artificial throat swabs gave positive tests

for rapid in vitro toxigenicity test in 48 hours. It is suggeted that a rapid in vitro toxigenicity test may be performed in order to reduce the time for isolation.

When compared the in vitro test with the intradermal test, it was noticed that the in vitro test was more practicle in a small laboratory than the in vivo one.

Table 1.

Comparison of the used of serum and serum substitute in In vitro toxigenicity test.

Type	No. of test strain	Serum			Serum substitute		
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Mitis	17	−	+	+	−	+	+
Intermedius	1	−	+	+	−	+	+
C. xerosis	1	−	−	−	−	−	−
C. pseudodiphtheriticum	1	−	−	−	−	−	−

Table 2.

Comparison of in vitro and in vivo toxigenicity test.

Type	No. of test strain	No. positive in vitro toxigenicity	No. positive intradermal test
Mitis	17	17	17
Intermedius	1	1	1
C. xerosis	1	−	−
C. pseudodiphtheriticum	1	−	−

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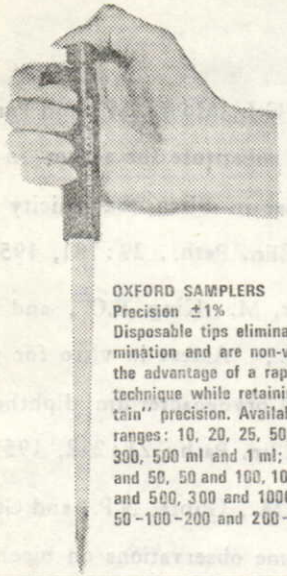

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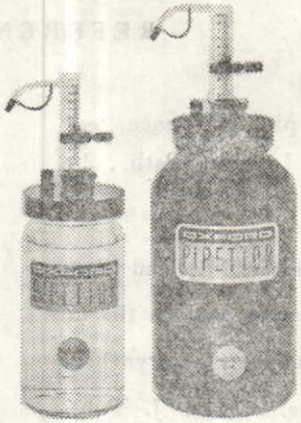

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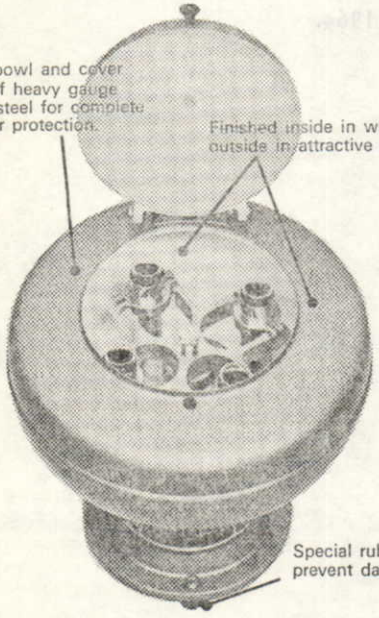

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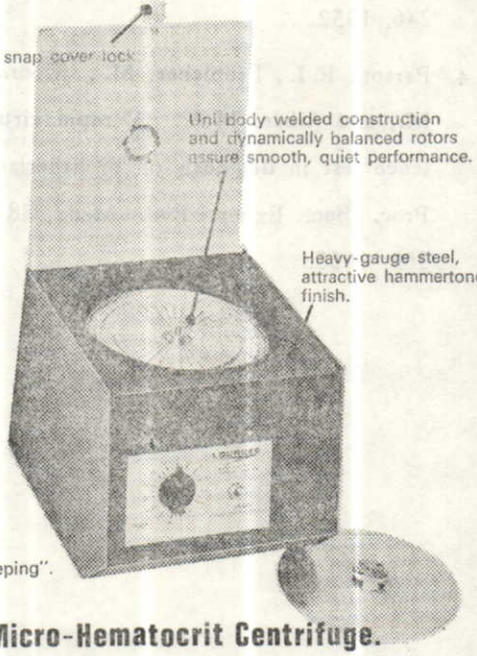

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DETERMINATION OF DENSITY DISTRIBUTION OF RED CELL POPULATION

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ABSTRACT

A simple microcapillary method for the determination of the distribution of red cell population with different density (may be of different age) is described. It is highly reproducible, easy to perform and inexpensive. It may be used in the future for the study of cell aging process or cellular hydration.

INTRODUCTION

Although the specific gravity of whole blood or its cell fractions can be determined by direct weighing of a given volume the indirect methods are preferable. The principle of the indirect methods described (1-4) consisting of preparing a series of liquids of varying specific gravity on the surface of which a drop of blood or cells is allow to fall from a predetermined height. Various aqueous salt solutions and organic solvents including chloroform, benzol, bromobenzene, xylene, and copper sulfate. One of the method described by Reznikoff (5) is suitable for the determination of the specific gravity of red cells because of the liquids used, benzyl benzoate and cottonsed oil, dissolves any of the constituents of the red cells or having any chemical or physical reaction with the corpuscles. Balantine and Burford (6) have used mixtures of phthalate esters to separate protozoa, mammalian cells and bacteria practically free of the suspending medium. Danon and Marikovsky (7) utilized the above method in the determination of the density distribution of red cell population. We wish to report our finding of density distribution of red cells population from normal healthy adults.

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MATERIALS

Methyl phthalate (sp. gr. 1.189) and di-n-butyl phthalate (sp. gr. 1.0416) were used to prepare stock solution of specific gravity ranging from 1.062 to 1.138, with increments of 0.004 (Table I). The stock solution are stored in brown bottles with glass stoppers at room temperature.

TABLE I. Proportion of di-n-butyl phthalate (fluids I) and methyl phthalate (fluid II) used in preparing the battery of separating fluid.

Fluid in gms.		Specific	Fluid in gms.		Specific
I	II	gravity	I	II	gravity
34.0	73.2	1.138	60.1	41.0	1.098
36.0	70.0	1.134	63.1	38.0	1.094
38.0	63.3	1.130	66.6	35.0	1.090
40.0	60.0	1.126	69.1	32.0	1.086
43.0	57.8	1.122	72.1	29.0	1.082
46.0	55.8	1.118	85.6	27.0	1.078
48.0	52.0	1.114	86.5	24.0	1.074
51.0	50.0	1.110	92.1	21.0	1.070
54.0	46.2	1.106	88.3	14.3	1.066
57.0	43.5	1.102	90.6	11.5	1.062

DETERMINATION OF DENSITY DISTRIBUTION OF RED CELLS

Approximately 2 ml. of freshly drawn EDTA-treated blood were used. The tip of a capillary tube (for microhematocrit determination) is dipped into the separating fluid until approximately 5 mm. of fluid column is obtained. A series of capillary tubes containing columns of separating fluid with different specific gravity are prepared and placed horizontally. Each capillary is then filled with blood sample until the upper level of the oily liquid is about 10 - 15 mm. from the other end of the capillary tube. The dry ends of the capillary tubes are sealed by the modeling clay and centrifuged for 30 minutes at 12,000 xg in a microhematocrit centrifuge. After centrifugation, the percentage of red cells that have passed through the separating liquid is calculated (the columns of red cells below and above the separating

fluid equal to 100 percent).

RESULTS

Distribution pattern of red cell populations obtained from 10 normal healthy adults are shown in the following table.

TABLE II. Percent Distribution of red cell population (Healthy adults)

Specific gravity	A			B		
	Mean	S.D.	S.E.	Mean	S.D.	S.E.
1.126	0	0	0			
1.122	1.22	1.26	0.40	1.22	1.26	0.40
1.118	1.31*	0.88	0.29	0.47*	0.70	0.23
1.114	9.47	9.89	3.13	5.89	5.96	1.99
1.110	23.95	18.08	5.72	14.21	12.99	4.11
1.106	69.55	20.59	6.56	45.60	9.34	2.96
1.102	90.20	9.74	3.08	20.65	14.79	4.68
1.098	98.70	0.71	0.22	8.50	9.74	3.08
1.094	98.75	0.71	0.22	0.05	0.14	0.04
1.090	99.45	0.37	0.12	0.70	0.75	0.24
1.086	0	0	0	0.55	0.37	0.12
1.082	0	0	0	0	0	0

A = % of red cell population higher density than the given specific gravity.

B = % of red cell population with density between 2 given specific gravities as shown.

* : may be error due to technical difficultes in one sample.

COMMENTS

This method is highly reproducible with only minimal variations when left at temperature up to 5 hours (7) or 37°C for 60 minutes. Repeat examination one week latter also demonstrated only minor difference from the first examination results. Danon (7) has also found that red cells become havier (approximately 0.008 in 5 hours at room temperature). This artifact can be avoided by keeping the blood sample at 4°C and let it stand for a few mi-

nutes to reach room temperature before centrifugation. In our hand, the centrifugation time of 15 minutes is adequate. The results obtained from using the modeling clay to seal the hematocrit tube and those using heat to seal the tube are the same but the former method is more feasible.

Our unpublished results indicated that it is important to establish the normal distribution curve in different age groups. The effect of blood cell regeneration rate may play a role since it is known that the specific gravity of red cells increases with their age. This method may be useful not only to estimate the percentage of young red cell population but probably the effect or degree of intracellular hydration.

SUMMARY

A simple method for estimation of the percent distribution of red cell population with different density is described. An example of the results obtained from 10 healthy adults are illustrated.

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INDUCTION OF ENEMIA IN SHEEP AND RABBITS BY PHENYLHYDRAZINE

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ABSTRACT : Five sheep and twenty rabbits were subjected to phenylhydrazine intraperitoneal injections. An injection dose of approximately 3.0 mg. per Kg. body weight was used. The 2.5 % phenylhydrazine sterile soiution was administered every day for sheep and every two-day for rabbits. Hemoglobin concentration and hematocrit of the experimental animals were determined. It was observed that phenylhydrazine caused a 67 % and 59 % decrease after administrations for 28 days and 18 days in sheep and rabbits respectively. Erythropoietic activity in the anemic plasma filtrate of the experimental animals assayed by Fe^{59} incorporation method was in the range of 0.07-0.09 Cobalt Unit per mg actual weight.

INTRODUCTION : Anemia is still an important problem in Thailand. Conditions of anemia can be investigated in animal models. Anemia in animals can be artificially induced by bleeding, administration of some chemical agents, or drug, e. g. trinitrotoluene (1, 2), phenacetin (3, 4), primaquine (5) and phenylhydrazine has been commonly used on this purpose for a long time. Anemic plasma of the experimental animals becomes a good souce for preparation of erythropoietin. In this paper, the hematologic effect of phenylhydrazia in sheep and rabbits will be reported and erthropoietic activity in their anemic plasma filfrate will be assayed.

MATERIALS AND METHODS

A. Animals : Five male sheep weighing from 30 to 40 Kg, 3-5 years of age and 20 rabbits in both sexes weighing from 2-5 Kg, 1-2 years of age were used in the experiment. They were locally bought in Chiang Mai, Thailand.

B. Chemicals : Phenylhydrazine hydrochloride was obtained from Matheson Coleman and Bell, Norwood, Cincinati, U.S.A. Sodium chloride was taken from

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City Chemical Corporation, New York, U.S.A. Cobalt chloride as $CoCl_2 \cdot 6H_2O$ crystals was obtained from May and Baker Ltd., Dugenham England. Fe^{59} as ferric chloride was directly purchased from the Radiochemical Center, Amersham, U.S.A.

C. **Preparation of Phenylhydrazine-Anemic Plasma:** The sheep and rabbits were intraperitoneally injected with 2.5% sterile solution of phenylhydrazine hydrochloride using each injection dose of approximate 3.0 mg. per Kg body weight. The injections were performed every day for sheep and every two-day for rabbits. The animal blood was frequently drawn for determinations of their hemoglobin concentration and hemotocrit compared with the normal values. It took 28 injections for sheep and 9 injections for rabbits. After hemoglobin concentration and hematocrit decreased below 10 gm% and 15 vol% respectively, the sheep were venously bled and the rabbits were bled by cardiac puncture. The whole blood of each animal was pooled using ACD solution as an anticoagulant and the plasma was collected. The anemic plasma would be used for an eryhropoietin study.

D. **Preparation of Anemic Plasma Filtrate:** The preparation of anemic plasma filtrate (APF) was done by the method of Rambach et. al. (g). The pH of the anemic plasma, 3.5 litres from sheep and 500 ml. from rabbits, was then adjusted to 5.5 with 1N HCl., boiled for 10 minutes and finally filtered. The anemic plasma filtrate was dialyzed against distilled water at 4°C for 24 hours, and lyophilized.

E. **Determination of Hemoglobin Concentration:** Exactly 0.02 ml of the animal blood was pipetted by a micro-pipette. Five ml of cyanomethemoglobin solution of Drabkin's solution were added and thoroughly mixed. The optical density was measured at 540 millimicron compared with standard value, using a Bausch and Lomb Spectronic 20.

F. **Determination of Hematocrit:** The animal blood was drawn and placed in balanced oxalate tube. Fer the micro-hematocrit determination method, the blood was filled into the sealed capillary tubes approximate three-fouths of total volume of tubes. The tubes were centrifuged by a Sorvall angle centrifuge with speed of 3,000 rpm. for 5 minutes. The hematocrit in volume % was determined by a Micro-capillary Reader, model C.R.

G. **Biological Assay of Erythropoietic Activity**

The erythropoietic activity assay was modified from the method of Graham (10, 11), using technique of radioisotope iron incorporation into red cells. The method required at least 4 albino rats per group

for testing materials and with two remaining group for a control NSS solution[1] and a cobalt chloride standard solution (8). The erythropoietic activity was expressed in Cobalt Unit[2] per mg actual weight.

EXPERIMENTAL RESULTS

The time response of hemoglobin concentration and hemetocrit in sheep and rabbits to phenylhydrazine hydrochloride is shown in Figure I and II respectively. From Figure I, it was found that after administrations ef phenylhydrazine into sheep the hemoglobin concentration and hematocrit obviously and rapidly decreased from the starting day to day 8, and then gradually changed to a plateau level. The hemoglobin concentration dropped from 15.5 gm% to 5.0 gm%; and the hematocrit from 35 vol% to 13 vol%. The reduction was about 67 %. Similary, in Figure II, the hemoglobin concentration and hematocrit in rabbits were 59 % decreased after total administrations. Erythropoietic activity in the anemic plasma filtrate of both sheep and rabbits assayed was 0.07 and 0.09 Cobalt Unit per mg. actual weight, respectively, as shown in Table I.

When all phenylhydrazine injections were done, some anemic signs in the experimental animals. eg., pale eyes, less activity, weakness, and loss of appetite were also observed.

DISCUSSION

In this experiment, it has been shown that phenylhydrazine effectively induced an anemia in sheep and rabbits. The hemoglobin concentration and hematocrit continously decreased during injections. The values became nearly constant on day 18 for sheep and on day 12 for rabbits. The plots between hemoglobin concentration and hematocrit against the injection time showed a good correlation in both kinds of animals. The difference in the figures between the sheep and the rabbits might depend upon the blood composition, such as plasma proteins, red cell number, and total blood volume.

The plasma filtrate of both sheep and rabbits showed the presence of an erythropoietically active substance. As reported by other investigators (12), phenyldrazine was shown to secondarily affect the erythropoietin production by inhibiting the respiratory mechanism. Anemia or

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[1] *NSS solution = Normat saline solution; 0.85% NaCl solution.*

[2] *One Cobalt Unit is equal to the erythropoietic activity by which 5 micro moles of* $CoCl_2$. H_2O *as a total dose (8, 12), is injected into starved rate. In this investigation one Cobalt Unit is equivalent to 7.76 percent of* Fe^{59} *incorporation.*

hypoxia due to phenylhydrazine injection increases the erythropoietin synthesis. This was confirmed by our experiments.

The injection dose of phenylhydrazine investigaled in this experiment is quite suitable. If higher dose was used, it caused the animals sudden death. It was also observed that the withdrawn blood was easily hemolyzed, therefore, it ought to be immediately centrifuged after withdrawing to avoid the hemoglobin contamination and to maintain high biological activity of erythropoietin in the sample.

This preliminary report on induction of anemia in animals by using phenylhydrazine may be a technical guide for those who want to study anemia in animal models. Other kinds of animals such as dogs, goats, and monkeys which are available in our country could be employed. The anemic plasma obtained is useful for erythropoietin studies.

	Anemic Plasma Filtrate (APF)	
	Sheep	Rabbit
Erythopoietic Activity (Cobalt Units per mg. actual weight)	0.07	0.09

Table I. Erthropoietic Acitivity in Anemic Plasma Filtrate (APF) Prepared from Plasmas of Sheep and Rabbit Treated with Phenylhydrazine.

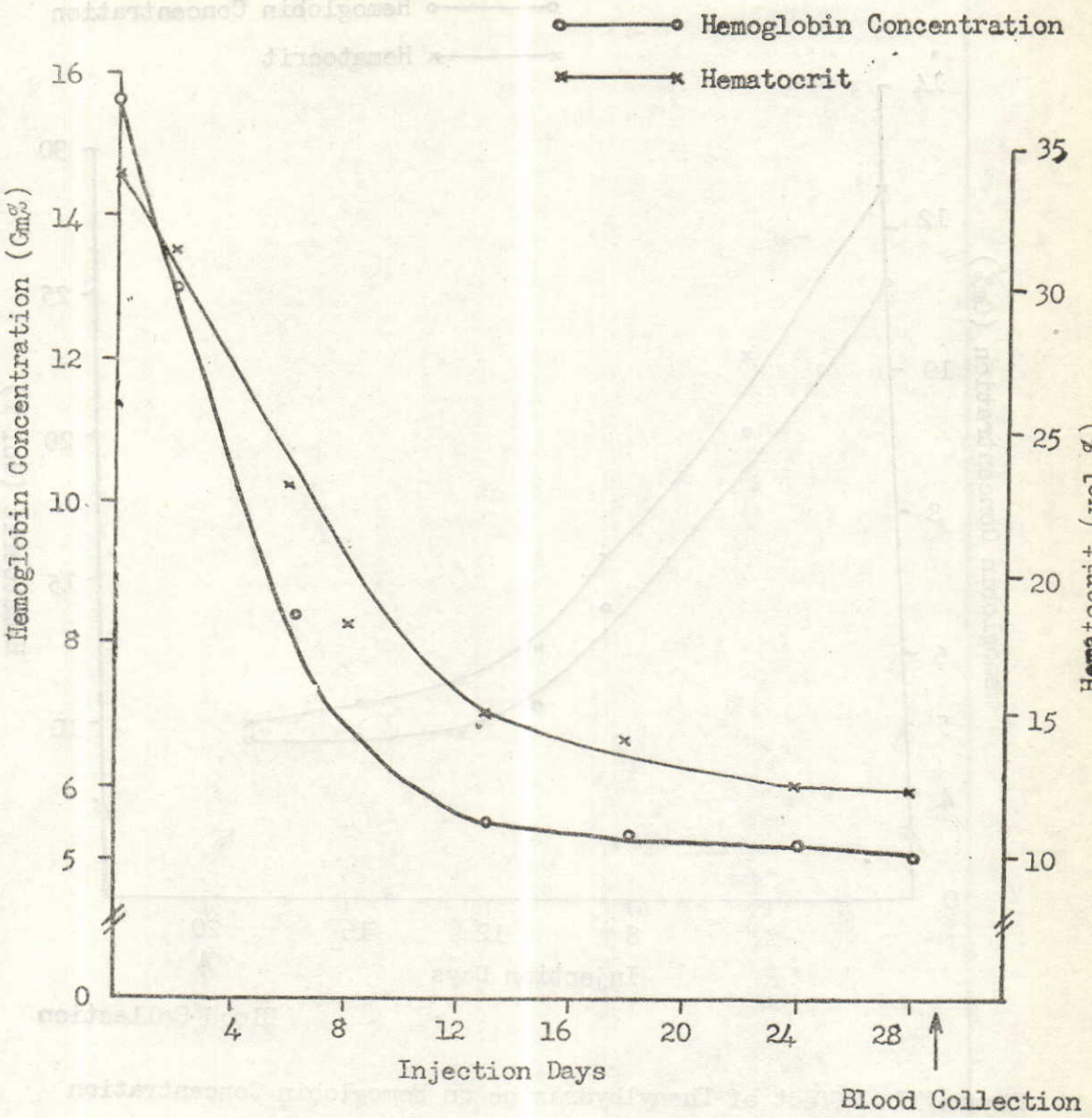

Figure I Effect of Phenylhydrazine on Hemoglobin Concentration and Hematocrit in a Sheep. The experiments were performed as described in Materials and Methods.

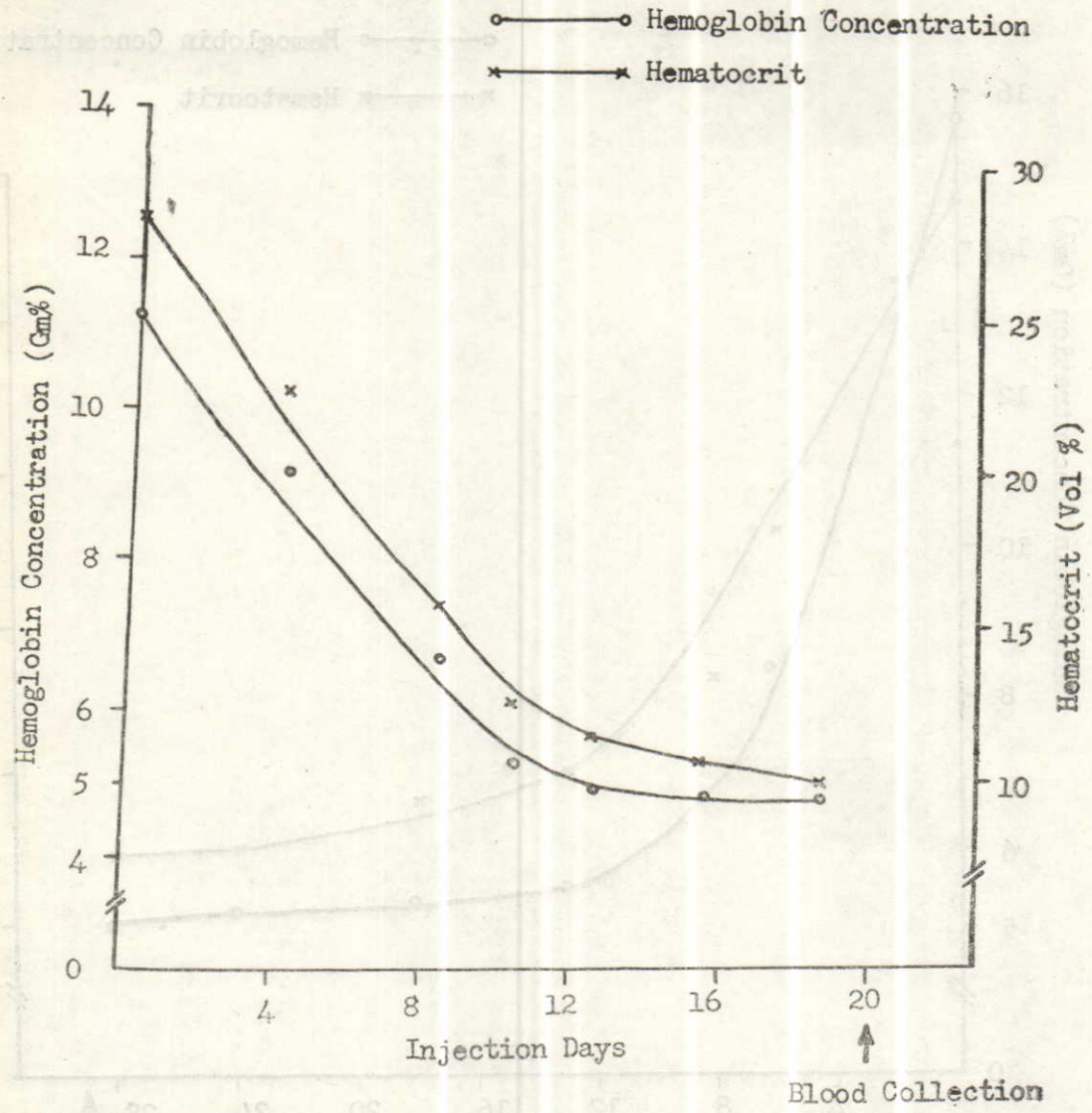

Figure II Effect of Phenylhydrazine on Hemoglobin Concentration and Hematocrit in a Rabbit. The experiments were performed as described in Materials and Methods.

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INDIRECT HEMAGGLUTINATION TEST IN AMOEBIC LIVER ABSCESS*

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Chirasak Khamboonruang, M.D., M.Sc., Ph.D.***

The definitive diagnosis of amoebic disease has depended upon the unequivocal demonstration of Entamoeba histolytica organism coubled with the presence of the known clinical symptoms and signs of amoebiasis. For numerous well-known reasons, the demonstration and identification of E. histolytica may be difficult, with the result that either false-negative or false-positive conclusions may be drawn regarding its presence. Serologic detection of amoeba antibodies should be useful supplements to the above diagnostic measures. Much effort and considerable progress in this direction has been made for many decades.

Serological test have been used by several workers to detect the antibody response of patient with amoebiasis. The detection of amoeba immobilizing antibody was employed by Cole and Kent (1953), Biagi and Buentello (1961) and Zaman (1960). Immunodiffusion was performed by Maddison and Elsdon-Dew (1961), Siddiqui (1961), Athchley et al (1963), and Krupp and Powell (1971). Goldman (1954) and Goldman et al (1960) detected the antibody response to invasive amoeba by a fluorescene labeled antibody technic. A complement fixation test was successfully done by Elsdon-Dew and Maddison (1952), Kessel et al (1965), and Krupp and Powell (1971). The indirect hemagglutination test was introduced by Kessel et al, (1961, 1965) and Lewis and Kessel (1961). Later, modification of the indirect

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* *Part of this work has been submitted to the Faculty of Medicine in partial fulfilment of the requirements for the B.Sc. Degree. (Med. Tech.)*

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hemagglutination test for amoebiasis was conducted by Krupp (1969, 1970), and Krupp and Powell (1971).

The present study, carried out at Department of Medical Technology, was designed to detect the antibody response to amoebiasis by a micro-indirect-haemagglutination test using sera of patients admitted to University Hospital, Faculty of Medicine Chiang Mai University with the clinical diagnosis of amoebic liver abscess.

Materials and Methods

Sera

The sera included in this report were obtained from the patients admitted to the medicine ward, Department of Internal Medicine with clinically diagnosed amoebic liver abscess. Pus sample drawn from the abscess and examined microscopically by culture were all negative for **Entamoeba histolytica** trophozoites. Control sera were obtained from 10 healthy persons and 20 patients admitted to the medicine ward with diagnosis not related to hepatic diseases.

Antigen

The antigen was generously provided by Dr. Richard Reeves, Department of Biochemistry, Medical Center, Louisiana State University, U.S.A. The antigen was prepared by growing D.K.B. Strain E. histolytica axenically in TP-S-1 Medium. A suspension of approximately one million amebae per ml, was lyophilized and mailed to the author. Before use the antigen was reconstituted with saline at the original concentration, and stored in 0.5 ml. amounts at -20°C. This antigen, reconstituted with saline, was further diluted with phosphate buffer saline (pH, 6.4) to 1 : 10 dilution for use in the test.

Sheep cells. Sheep blood was collected aseptically in an equal volume of Alsever's solution. Cells were kept in the refrigerator at 4°C. for 3 days. In preparation for tanning sheep red cells were washed three times by centrifugation in phosphate-buffered saline (pH 7.2) and a 2.5 % suspension was made after the final washing.

Tanning. A fresh solution of tannic acid (1:1000 W/V) in saline was prepared. Before use the solution was further diluted to the concentration of 1 : 20,000 with phosphate-buffered saline (pH 7.2). Equal volumes of 2.5 % suspension of erythrocytes and tannic acid solution were combined in a flask and gently rotated at 37° C. for 15 minutes. After tanning, the cells were washed twice in phosphate-buffered saline (pH 7.2), and then diluted to 2.5% in phosphate-buffer saline (pH 6.4) for sensitization.

Sensitization. Sensitization was carried out by gentle rotation at room temperature, for 30 minutes of 1 volume of antigen dilution (1 : 10) in phosphate-buffered

saline, (pH 6.4) and 1 volume of 2.5 % tanned erythrocytes. Normal rabbit serum was used as a stabilizer in the test, and was activated and absorbed with sheep cells before use. Sensitized erythrocytes were washed twice in 1 % normal rabbit serum in phosphate - buffered saline (pH 7.2) and a 1.5 % solution of sensitized cells was prepared after the final washing.

Test system

The test was performed in the test tube according to the method of Campbell **et al** (1964). Serial two fold dilutions of antisera (positive control. negative control and tested sera) were made. Fifteen test tubes (13×100 mm) were placed in each row in a rack; 0.5 ml of 1% Normal rabbit serum diluent were pipetted into tubes 1 through 15; 0.5 ml of undiluted, sheep -red-blood-cell-absorbed antiserum were pipetted into tube 1. The content in tube were mixed and 0.5 ml from tube 1 dispensed into tube 2. Twofold serial dilutions were continued through tube 15, and 0.5 ml discarded from tube 15 after mixing. By this dilution schedule tube 15 would contained a 1 : 32,728 dilution of antiserum. Thereafter 0.05 ml of a 1.5 % suspension of sensitized cells were added to each serum dilution. The tubes were sealed with parafilm, and the rack shaken thouroughly for 5 minute and allowed to pattern for several hours at room temperature.

Results

Sera from 56 patients who were clinically diagnosis to be amoebic liver abscess were studied. Some pus samples drawn from the abscesses were examined for **E. histolytica** trophozoite but no amoebae were found in the exudate. The frequency distribution of the titer of hemagglutinating-antibody levels is displayed in the Table. It appears that the distribution is variable. Titers of 1:1024 were more frequent. However, 14 serum samples had hemagglutinating - antibody tirers over 1 : 32,728. It was observed that 50 out of 56 serum samples or 89.3% revealed hemagglutinating antibody. No antibody was detected in the remaining serum samples.

In the control groups, sera from 30 persons were studied. Stool examinations were performed on each individual. Neither **E. histolytica** cyts nor trophozoite was found in the sample. History of past experience with amoebiasis were taken and no evidence of previous infection was discovered. The result showed that the serum from 10 healthy persons had no antibodies. However, 1 out of 10 healthy person had low hemagglutinating antibody titer (1:32). In the group of patients with concurrent infection, it was found that 5 of 30 persons had antibody response to **E. histolytica.** One patient who was ill with chronic renal failure had a titer of 1:512. Another,

who was admitted to the hospital with the complaint of jaundice had the titer of 1:1024. The remaining three person had very low titers (1:2). Overall 70% of the control group with no demonstable clinical amoebiasis were negative for hemagglutinating antibody in the series.

Discussion

The traditional and heretofore most reliable method for the diagnosis of amoebiasis has been the recovery of the etiological agent, **Entamoeba histolytica.** For intestinal amoebiasis, the approach is feasible, however if diagnosis were to depend solely on the detection of the parasite, most cases of extraintestinal amoebiasis would be overlooked. The desirability of a serologic test for extraintestinal amoebiasis has periodically stimulated investigators to propose new procedures for this purpose. From a review of the literature, it appears that the indirect hemagglutination test is widely accepted and employed in many laboratories. The test is considered to be the most sensitive and specific one. Kessel et al (1965) reported 100 per cent positivity by the indirect hemagglutination test in cases of amoebic liver abscess and 98 per cent in cases of acute amoebic dysentery. Only 1.5 per cent of the abscess cases and 7.6 per cent of acute amoebic dysentery cases gave falsely negative ersults using the test in a series reported by Maddison et al (1965). Milgram et al (1966) obtained positive indirect hemagglutination tests in 96 per cent of cases of abscess and in 82 per cent of amoebic colitis. The result of the study of Prakash Om et al (1969) revealed that all of three cases of intestinal amoebiasis with amoebic hepatitis gave positive indirect hemagglutination tests and 12 of 16 (75%) cases with amoebic liver abscess only were also positive. Thus in 25 per cent of cases of amoebic liver abscess a falsely negative hemagglutination test was recorded. Krupp (1970) determined the antibody response to amoebic infection in the person in Cali, Colombia, South America. She found that the tests gave positive results for 81 per cent of 168 person with amoebic colitis, and 87 per cent of 31 with extraintestinal amoebiasis; in the control group 7 per cent were reported as positive. However, Krupp and Powell (1971) studied the antibody response to invasive amoebiasis in Durban, South Africa, and found the positivity to be considerably higher than in Cali. The test yielded 98.4 per cent positive in persons with known amoebic liver abscess and 95.2 per cent in confirmed amoebic dysentery. The antibody response was found to possibly persist for more than 3 years after termination of active infection.

In the present study the auther found

that the teat yielded positive results in 89 per cent of 56 persons with known amoebic liver abscess. The test gave considerably lower results than that of Krupp and her Co-worker (1971). According to the study of Prakash Om et al (1969) possible explanations for this could be : 1) comparatively low potency of the antigen; 2) poor nutritional status of patients. It should also be noted that diagnosis as amoebic liver abscess in this study was based on the aspiration of characteristic pus and clinical signs and symptoms. No amoebae were found in the direct smears of the pus. It is possible that the apparent liver abscesses were due to causes other than amoebae thus yielding a negative test.

In the control group the results revealed that only 10 per cent of healthy control sera had hemagglutinating antibody at the titer of 1:32. In the control group of 20 persons with concurrent infections it was found that three had titers of 1 : 2 and two had titers of 1 : 512 and 1: 1024. The letter patients were admitted to the hospital with chronic renal and jaundice respectively. Krupp and Powell (1971) study antibody production in response to invasive amoebiasis in Africa. They considered a titer of 1:81 or lower as negative. Comparison to the present study, 4 persons of the control group had the titer lower than 1:64. The only explanation for the higher titer in the two control cases above would be previous experience with amebic infection in the past with persistent antibody as described by Krupp and Powell (1971).

Abstract

Indirect hemagglutination test for amoebic liver abscess was studied using the macrotiter system in test tubes. Sera from patient with clinically diagnosed as amoebic liver abscess were tested. Fresh sheep red blood cells were tanned and sensitized with Entamoeba histolytica antigen from axenic culture. The tests gave positive results for about 89 % of 56 patients with amoebic liver abscess, 10 % of 10 person with healthy controls, and 25% of 20 patients with concurrent bacterial infection. It was found that the indirect hemagglutination test for detection of antibodies in amoebic liver abscess has proved to be sensitive and specific as compare to the previous works of several investigations.

Frequency Distribution of Serum Hemagglutination Titers Obtained from Persons in Chiang Mai Hospital

Clinical diagnosis	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	1:8092	1:16184	1:32368	1:3268	Fer cent Positive
Amebic liver abscess	1	--	2	1	2	3	2	2	3	7	2	1	4	4	2	14	89.3% (50/56)
Healthy control					1												10% (1/10)
Concurrent infection	3								1*	1**							25% (5/20)

* Patient with chronic Heart failure

** Patient with Jaundice

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ย่อ และ รีวิวเอกสาร

Studies on Angiostrongylus cantonensis larva in the snails and adult worms in the rats in Chiang Mai.

by Vichit Punnavutti.

The Term paper for degree of B. Sc. (Med. Tech.) 1969-1970.

School of Medical Technology, Faculty of Medicine, Chiang Mai University.

เคยมีผู้รายงานการพบตัวอ่อนระยะที่3ของพยาธิ Angiostrongylus cantonensis ใน Intermediate host ในจังหวัดเชียงใหม่มาแล้ว ผู้รายงานจึงมีวัตถุประสงค์จะทำการศึกษาค้นคว้าเพิ่มเติม เพื่อหาอุบัติการ ของ การพบตัวอ่อนระยะที่ 3 ของพยาธินี้ ในหอย ซึ่งเป็น Intermediate host โดยเก็บหอยทากยักษ์ (Achatina fulica) และหอยโข่ง (Pila spp.) ในอำเภอเมืองเชียงใหม่ มาตรวจโดยเอาส่วน head foot มาสับให้ละเอียด และย่อยด้วยน้ำย่อยเทียม (1% pepsin solution) ใส่ในตู้อบ 37 ซ 3 ชม. เขย่าบ่อย ๆ แล้วตั้งทิ้งไว้ที่อุณหภูมิห้อง 12–24 ชม.

ผลการตรวจหอยจำนวน 175 ตัว พบตัวอ่อนระยะที่ 3 ของพยาธิ Angiostrongylus cantonensis ในหอย 29 ตัว คิดเป็นร้อยละ 16.6 ตัวอ่อนที่พบมีขนาดเฉลี่ย 505×27 ไมครอน

ผู้รายงานได้นำตัวอ่อนระยะที่ 3 นี้ไปทำการติดเชื้อ ใน หนู ขาว โดย ฉีด เข้า ทาง ปาก (Oral – inoculation) และทางช่องท้อง (Intraperitoneal inoculation) พบว่าอัตราการติดเชื้อของปาก มีร้อยละ 61.9 ดีกว่าการติดเชื้อทางช่องท้องซึ่งมีร้อยละ 32.6 และตรวจพบตัวอ่อนระยะที่ 1 ในอุจจาระหนูขาว ระหว่าง 38–56 วัน พบพยาธิตัวแก่ในปอดและหัวใจ ทั้งตัวผู้และตัวเมียในอัตรา 1:1.1 พยาธิตัวผู้มีขนาด 19.03×0.25 ม.ม. ตัวเมียมีขนาด 24.9×0.33 ม.ม.

นอกจากนี้ผู้รายงานได้ตรวจหนูชนิดต่าง ๆ ซึ่งดักได้จำนวน 220 ตัว พบพยาธิตัวแก่ร้อยละ 3.2 มีทั้งตัวผู้และตัวเมียในอัตรา 1 : 2.4 ขนาดเฉลี่ยของพยาธิตัวผู้ 26×0.36 ม.ม. ตัวเมีย 39×0.52 ม.ม.

เกตุรัตน์ สุขวัจน์
B.Sc. (Med. Tech.)

Fetal Hemoglobin

By Uma Busapavanit

The Term Paper for the degree of B. Sc. (Med. Tech.)

The School of medical Technology Faculty of Medicine Chiang Mai University 1969-70

ผู้รายงานได้ทำการทดลองหา เปอร์เซ็นต์ Hb F จาก peripheral blood เปรียบเทียบกับ venous blood โดยวิธี modify singer's method จาก Normal adult 3 ราย Newborn infant 3 ราย และจาก Thalassemia major 1 ราย ปรากฏว่าทั้ง 7 ราย ได้ค่าเปอร์เซ็นต์ Hb F จาก Peripheral blood และ venous blood ใกล้เคียงกันมาก ผู้รายงานวิจารณ์ว่า ค่าที่ต่างกันเล็กน้อยนั้น น่าจะเกิดจาก technical error มากกว่า

ชลอ บัวน้ำจืด

B.Sc. (Med. Tech.)

An Evaluation of Different Methods for Alkaline - Resistant Hemoglobin Determination.

By Charun Panichsasilawat,

Th$^{\text{M}}_{3}$ Term Paper for the degree of B. Sc. (Med. Tech.)

The School of Medical Technology, Faculty of Medicine, Chiang Mai University, 1971 -1972.

ผู้ทดลองใช้วิธี Alkali denaturation technique เพื่อวัดหาเปอร์เซนต์ของ alkaline–resistant hemoglobin คือ hemoglobin F โดยทำเป็น 2 แบบ คือ treat oxyhemoglobin โดยตรง และโดยทำให้เป็น Cyanmethemoglobin ก่อน แล้วจึง treat ด้วย alkaline นอกจากนี้ ยังได้เปรียบเทียบ ความ มาก น้อย ของ Denaturration ในช่วงเวลาที่ต่างกันอีกด้วย

ตัวอย่างเลือดที่ใช้ทดลองแบ่งเป็น 3 พวก คือ เลือดจากสายสะดือ จากเด็กอายุ 2 – 3½ ขวบ และจากผู้ใหญ่ที่มีอายุ 20 ปีขึ้นไป รวมทั้งหมด 30 ราย ใช้ EDTA เป็นสารกันเลือดแข็ง ล้างเม็ดเลือด ให้สะอาด ทำให้ hemolyse แล้วใช้ toluene extract เอา non-heme protein ออก

Hemolysate หลังจากผ่านกระดาษกรองแล้ว denature ด้วย N/12 NaOH pH 12.7 เป็นเวลา 1, 2, 3, ...10 นาที แล้วใช้ half saturated $(NH_4)_2\ SO_4$ เป็นตัวหยุดปฏิกิริยา และตกตะกอน denatured protein ในช่วงเวลาต่าง ๆ กันดังกล่าว Solution ที่เหลือนำไปวัดค่า Hemoglobin โดย Spectropnotometrict method เปรียบเทียบเป็น เปอร์เซนต์ จาก Hemoglobin ก่อน treat ด้วย alkaline ส่วนอีกแบบหนึ่งทำ Hemoglobin ให้อยู่ในรูป cyanmethemoglobin ก่อนโดย

potassium ferricyanibe solution แล้วจึงนำมา denature เหมือนข้างต้น คำนวณหาเปอร์เซนต์ fetal hemoglobin เช่นเดียวกัน

ผลที่ปรากฏแสดงถึงการทดลองของ alkaline-resistant hemoglobin ในช่วงเวลา denature ที่นานออกไป สำหรับ cord blood ซึ่งมี fetal hemoglobin สูงนั้น พบ 70% ในนาทีที่ 1 ลดลงมาจนเหลือ 50% ในนาทีที่ 5 เหมือนๆ กันทั้ง 2 วิธี ในเลือดที่มี HbF ไม่เกิน 10% พบว่า Alkaline-resistant hemoglobin จาก cyanmethemoglobin จะลดน้อยกว่าจาก oxyhemoglobin ทั้ง slope ก็จะลดลงช้ากว่า คือ denaturation จะ complete ในนาทีที่ 6-9 แต่ oxyhemoglobin จะเกิด denaturation complete ในนาทีที่ 4-6 สรุปได้ว่าวิธีของ cyanmethemoglobin มี variation น้อยกว่า แต่วิธีทำก็ลำบากกว่าของ oxyhemoglobin ผู้เขียนได้แนะนำให้ใช้เวลา 5 นาที สำหรับ denaturation process.

สุรพร มาตระกูล
B.Sc. (Med. Tech.)

Estimation of Iron Store in Bone Marrow.

By Usa Boonma

The Term Paper for the degree of B. Sc. (Med. Tech.)
School of Medical Technology, Faculty of Medicine, Chiang Mai University, 1971-72

ปริมาณเหล็กใน Bone Marrow ใช้บอกถึงภาวะการณ์ ของ ความ ผิด ปกติ ของ การสร้างเม็ดเลือดได้ และจากการตรวจหาทำให้สามารถแยาชนิดของ anemia โดยเฉพาะอย่างยิ่ง Iron deficiency anemia, thalassemia และภาวะการพร่องเหล็กอย่างอื่นได้

การทดลองได้ทำใน คน ใช้ Thalassemia, Iron deficiency anemia, Protein Calorie Malnutrition (PCM), Chronic disease และคนปกติรวมทั้งสิ้น 33 ราย เป็นการตรวจทั้งใน bone marrow และใน periferal blood ในไขกระดูกตรวจ marrow smear หา M/E ratio โดยใช้ Wright's stain และตรวจ sideroblast และ hemosiderin โดยย้อมสี plussion blue ทางเคมี การตรวจหาเหล็กสะสมโดย Caraway Method และหา protein โดย Biuret Method ต้องล้าง bone marrow ก่อนด้วย 2% gracial acetic acid เพื่อขจัด Hemoglobin ในเม็ดเลือดแดงออกไป สำหรับการตรวจเลือด

เป็นการตรวจหา hemoglobin, hematocrit และเปอร์เซนต์ของ reticulocyte รวมทั้งตรวจหา Serum Iron และ Total Iron Binding Capacity ด้วย

พบว่า Hemosiderin ซึ่งเป็น granule ติดสีน้ำเงินเข้ม มีมากกว่าปกติในคนไข้ Thalassemia แสดงว่ามีเหล็กสะสมอยู่มาก แต่ในเด็กโลหิตจางเนื่องจากขาดเหล็ก และเด็ก PCM ตรวจพบ granule น้อยมากหรือไม่พบเลย พบว่ามี Sideroblast น้อยกว่า 1% ใน PCM และ Iron deficiency anemia, 11.4% ใน chronic disease, 14% ในคนปกติแต่จะมากถึง 64.66% ใน thalassemia การตรวจทางเคมีให้ผลคล้ายกัน กล่าวคือมี 38.25 microgram % ในเด็กขาดเหล็ก และถึง 159.1 microgrsm% ใน thalassemia การตรวจเลือดก็พบว่าโรคขาดเหล็กมี SI ต่ำ แต่ TIBC สูงและกลับกันในคนไข้ thalassemia

ผู้ทดลองได้กล่าวถึงผลดีของการตรวจ granule ของเหล็กจาก bone marrow smear ว่าทำง่าย สิ้นเปลืองเวลาและค่าใช้จ่ายน้อย และทั้งยังมีผลเชื่อถือได้แน่นอนสำหรับใช้วินิจฉัยโรคได้อย่างดีด้วย

สุรพร มาตระกูล
B.Sc. (Med. Tech.)

Clinical Spectrum of phayngeal gonococcal infection.

By Paul J. Wiener, Evelyn Tronca, Paul Bonin, Alf H.B. Pedersen and King K. Holmes.

From New Engl. J.M. Vol. 288 No. 4, January 25, 1973 p. 181 - 188.

จากการศึกษาผู้ป่วยที่มารับการรักษาคลินิคกามโรค จำนวน 2224 ราย แยกเชื้อจาก posterior pharynx พบ Neisseria meningitidis 383 ราย (17.2%) Neisseria gonorrhoea 125 ราย (5.6%) และ Neisseria lactamicus 42 ราย (1.9 %) ในจำนวนผู้ป่วยที่พบเชื้อ N. gornorrhea แบ่งเป็น homosexual men 14 ราย เป็นผู้หญิง 32 ราย เป็น hetero sexual men เพียง 3 ราย ผู้หญิงที่ป่วยเป็น sore throat มีประวัติว่าเคยทำ fellatio 76 ราย

เชื้อ N. gonorrhoea นอกจากจะเป็นสาเหตุของ gonorrhoea แล้วยังทำให้เกิด conjunctivitis, tonsillitis, gingivitis stomatitis, arthritis, meningitis and pharyngitis ได้อีกด้วย.

เนตร สุวรรคฤหาสน์
วท.บ. (เทคนิคการแพทย์)

Susceptibility of pathogenic staphylococci to penicillin G and penicillin derivatives.

By Surasak Puckdee.

The term paper for the degree of B. Sc. (Med. Tech.)

The School of Medical Technology, Chiang Mai University 1971 - 1972

จากจำนวนเชื้อ staphylococci ที่แยกได้จากผู้ป่วยที่มารับการรักษาใน โรงพยาบาลนครเชียงใหม่ 67 ราย และจากโรงพยาบาลแมคคอมิค 25 ราย รวมเป็น 92 ราย ในการทดสอบหาความไวของเชื้อ staphylococci โดยใช้วิธี tess tube methad อ่านผลของค่า M.I. C ที่ 12.5 micrograms / mililiter เป็นอย่างน้อย ซึ่งผลของการทดสอบของยาปฏิชีวนะทั้ง 4 ชนิดนี้ปรากฏว่าสามารถทำลายเชื้อคิดเป็น ร้อยละ ได้ดังนี้ Methicillin 76.0 %, Ampicillin 67.4 %, Penicillin G 45.6 % และ Cloxacillin 45.6 % จากการทดสอบหาปฏิกิริยาของยาปฏิชีวนะเหล่านี้พบว่า มีปฏิกิริยาเป็นแบบ Bacteriostatic.

เนตร สุวรรณคฤหาสน์

วท.บ. (เทคนิคการแพทย์)

วารสารเทคนิคการแพทย์ เชียงใหม่
BULLETIN OF CHIANG MAI MEDICAL TECHNOLOGY
Vol. 6 No. 2
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ข่าว

ศึกษาต่อ

อาจารย์ประยูร อินบริบูรณ์ ภาควิชาจุลชีววิทยาได้ไปศึกษาต่อวิชาจุลชีววิทยา ชั้นปริญญาโท-เอก ณ มหาวิทยาลัยอิลลินอยส์ สหรัฐอเมริกา ด้วยทุน China Medical Board ได้เดินทางจากประเทศไทยตั้งแต่วันที่ ๑๑ มีนาคม ๒๕๑๖ แล้ว

อาจารย์ นันทยา วัยวัฒนะ ภาควิชาคลินิคัลเคมิสตรี้ โครงการคณะเทคนิคการแพทย์ ไปศึกษาต่อชั้นปริญญาโท สาขาคลินิคัลเคมีสตรี้, อาจารย์ สุชาติ ศิริทูล ภาควิชาคลินิคัลไมโครไบโอโลยี่ ไปศึกษาต่อวิชาจุลชีววิทยาชั้นปริญญาโท, อาจารย์ อัมพารัตน์ ชุมรุม ภาควิชาคลินิคัลไมโครสโกปี้ ไปศึกษาต่อวิชาคลินิคัลพาโธโลยี่ ณ บัณฑิตวิทยาลัย มหาวิทยาลัยมหิดล มีกำหนดเวลา ๒ ปี

อาจารย์ ยุพา สุภาเลิศ กองบรรณาธิการวารสารเทคนิคการแพทย์เชียงใหม่ ไปศึกษาต่อวิชาคลินิคัลพาโธโลยี่ ณ บัณฑิตวิทยาลัย มหาวิทยาลัยมหิดล มีกำหนดเวลา ๒ ปี

ประชุมการศึกษาแพทยศาสตร์

อาจารย์ สนิท มกรแก้วเกยูร ภาควิชาจุลชีววิทยา ได้เดินทางไปประชุมการศึกษาแพทยศาสตร์ ณ วังแก้ว จังหวัดระยอง ระหว่างวันที่ ๑๕ มีนาคม ๒๕๑๖ ถึงวันที่ ๑ เมษายน ๒๕๑๖

รับอาจารย์ใหม่

โครงการคณะเทคนิคการแพทย์ มหาวิทยาลัยเชียงใหม่ ในปีงบประมาณ ๒๕๑๖ ได้ตำแหน่งอาจารย์ตรี ๓ ตำแหน่ง และได้บรรจุไปแล้วคือ

๑. อาจารย์ เอื้อมพร รัตนชาญพิชัย เป็นอาจารย์ภาควิชาคลินิคัลอิมมูโนโลยี่

๒. อาจารย์ มารศรี ไกรโรจนานันท์ เป็นอาจารย์ภาควิชาคลินิคัลไมโครไบโอโลยี่

๓. อาจารย์ ปราณี ลีชนะชัย เป็นอาจารย์ภาควิชาคลินิคัลไมโครไบโอโลยี่

สโมสรนักศึกษาเทคนิคการแพทย์

สโมสรนักศึกษาโครงการคณะเทคนิคการแพทย์ ได้รับอนุมัติให้เข้าร่วมองค์การนักศึกษามหาวิทยาลัยเชียงใหม่แล้ว ในปีการศึกษา ๒๕๑๖ นี้ เป็นต้น

คณะกรรมการสโมสรนักศึกษามีดังต่อไปนี้

๑. นายชูชีพ ประพุทธ์พิทยา นายกสโมสร

๒. นายสุชาติ บั่นจัยสีห์ อุปนายกฝ่ายธุรการ

๓. นายสมยศ ประเสริฐวิทย์ อุปนายกฝ่ายปกครอง

๔. น.ส. เสาวนีย์ ลีละยูวะ เลขานุการ

๕. น.ส. พรศรี กฤษณุรักษ์ เหรัญญิก

๖. นายรัศมี แก้ววิชิต ปฏิคม

๗. น.ส. สุวรรณา เปรมบุตร ประชาสัมพันธ์

๘. นายสมชาย ทรัพย์ไพบูลย์กิจ ประธานชุมนุมกีฬา

๙. นายมานิตย์ วงษ์ยุติธรรม ประธานชุมนุมถ่ายรูป

๑๐. นายประสิทธิ์ เรืองไรรัตนโรจน์ แสง–เสียง

๑๑. นายวัฒนา อู่วาณิชย์ สาราณียกร

๑๒. นายวัฒนา หาญพานิช ประธานเชียร์–บันเทิง

๑๓. น.ส นงนิจ ลือตระกูล พัสดุ–สหกรณ์

๑๔. นายเชาว์ สุระดม ศิลปกรรม

๑๕. น.ส. อุดมศรี อลงกตกุล หัวหน้าฝ่ายวิชาการ

๑๖. น.ส. พงษ์สวาท รัตนวราห รองหัวหน้าฝ่ายวิชาการ

๑๗. นายไพบูลย์ หนันไชย หัวหน้าฝ่ายสวัสดิการ

๑๘. นายศิริพงษ์ ณ น่าน ผู้ช่วยหัวหน้าสวัสดิการ

๑๙. นายเรวัตร ทักษิณะมณี ผู้แทนนักศึกษา ปีที่ ๔

๒๐. นายยงยุทธ เวชสถานารักษ์ ผู้แทนนักศึกษา ปีที่ ๓

๒๑. นายศุภชัย ศรพรหม ผู้แทนนักศึกษา ปีที่ ๒

๒๒. ..... ผู้แทนนักศึกษา ปีที่ ๑

สมาชิกสภานักศึกษามหาวิทยาลัยเชียงใหม่

๑. นายเทอดเกียรติ กาญจนวิสุทธิ์

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๔. นายปกรณ์ ไทยานันท์

สมาชิกตุลาการ

๑. น.ส. มารศรี สุวรรณชจร

พัสนศึกษา

คณะนักศึกษาเทคนิคการการแพทย์ โครงการคณะเทคนิคการแพทย์ มหาวิทยาลัยเชียงใหม่ ได้เดินทางไปพักแรมที่ศูนย์ช่วยเหลือชาว

เขา อำเภอเชียงดาว จังหวัดเชียงใหม่ ในวันที่ ๖ พฤษภาคมนี้ เพื่อไปศึกษาชีวิตชาวไทยภูเขา และการทำไร่ใบชา ที่ดอยเขาหลวงเชียงดาว.

กลับจากต่างประเทศ

คุณนารา มาวิจักษณ์ เทคนิคการแพทย์ กรุงเทพฯ รุ่นที่ ๑๒ ซึ่งได้เดินทางไปศึกษาต่อในชั้นปริญญาโท สาขาจุลชีววิทยา ณ Teuas Women's University, Denton Teuas, U.S.A. ตั้งแต่เดือนมิถุนายน ๒๕๑๓ ขณะนี้ได้สำเร็จการศึกษา และกลับมาถึงประเทศไทยเรียบร้อยแล้ว ตั้งแต่วันที่ ๒๑ กุมภาพันธ์ ๒๕๑๖ และเข้ารับราชการในตำแหน่งอาจารย์ โท แผนกเวชศาสตร์ชันสูตร (เทคนิคการแพทย์) คณะแพทยศาสตร์ จุฬาลงกรณมหาวิทยาลัย เมื่อ วันที่ ๒ เมษายน ๒๕๑๖

ศึกษาต่อภายในประเทศ

เทคนิคการแพทย์ที่สำเร็จในปีการศึกษา ๒๕๑๕–๒๕๑๖ ผู้ได้เข้าศึกษาต่อในชั้นปริญญาโท ในสาขาวิชาต่าง ๆ ประจำปีการศึกษา ๑๕๑๖–๒๕๑๗ มีกำหนด ๒ ปี มีดังนี้

คุณบุญทา วรินทร์รักษ์ จากจุฬาลงกรณมหาวิทยาลัย ศึกษาต่อในสาขา จุลชีววิทยา คณะบัณฑิตวิทยาลัย มหาวิทยาลัยเกษตรศาสตร์

คุณวิเชียรชัย เอื้อโกวิทธุ์ชัย จากจุฬาลงกรณมหาวิทยาลัย และคุณศรีศิลป์ คูสมิทธิ จากมหาวิทยาลัยมหิดล ศึกษาต่อในสาขาจุลชีววิทยา (กรุงเทพฯ) คณะบัณฑิตวิทยาลัย มหาวิทยาลัยมหิดล

คุณคัมภีร์ จิตรใจ จากจุฬาลงกรณมหาวิทยาลัย และ คุณแม้นสรวง เพื่องเพียร จากมหาวิทยาลัยมหิดล ศึกษาต่อในสาขาจุลชีววิทยา (เชียงใหม่) คณะบัณฑิตวิทยาลัย มหาวิทยาลัยมหิดล

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นอกจากนั้นก็มี

คุณชัยรัตน์ อัศวภค เทคนิคการแพทย์เชียงใหม่ รุ่นที่ ๓ ศึกษาต่อในชั้นปริญญาโท สาขา จุลชีววิทยา (กรุงเทพฯ) คณะบัณฑิตวิทยาลัย มหาวิทยาลัยมหิดล

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สมรส

ในเดือนพฤษภาคม ๒๕๑๖ เทคนิคการแพทย์ กรุงเทพฯ รุ่น ๑๓ จะเข้าสู่พิธีมงคลสมรส รวม ๔ ท่านด้วยกันคือ

คุณเยาวลักษณ์ ดิษสุข แห่งศูนย์วิจัยทางการแพทย์ ส.ป.อ. สมรสในวันที่ ๑๑ พฤษภาคม ๒๕๑๖ ณ กรุงเทพมหานคร

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ผู้แทนจำหน่าย

ANTI-INFLAMMATORY ENZYME PREPARATIONS.

KIMOTAB TABLET

CHYMOTASE INJECTION

เป็น PROTIOLYTIC ENZYME ที่มีคุณประโยชน์ กว้างขวางสำหรับใช้รักษาและป้องกันอาการบวม, ห้อเลือด, อักเสบ, ฟกช้ำต่าง ๆ เช่น Edema-swelling, hematoma associated with trauma such as fractures and sprains, postpartum breast engorgement, mastitis, postoperative inflammation.

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