วารสารเทคนิคการแพทย์ เชียงใหม่

BULLETIN OF CHIANG MAI MEDICAL TECHNOLOGY

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บริษัท อุตสาหภัณฑ์ จำกัด

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บริการและจำหน่าย

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ทุกสิ่งทุกอย่างเกี่ยวกับวิทยาศาสตร์การแพทย์

โปรดติดต่อกับ

บริษัท อุตสาหภัณฑ์ จำกัด

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Volume 4 May 1971 Number 2

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สำนักงาน : โรงเรียนเทคนิคการแพทย์ คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

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BULLETIN OF CHIANG MAI MEDICAL TECHNOLOGY

Vol. 4 No. 2
May 1971

Editorial

THE REVISED CURRICULUM OF THE SCHOOL OF MEDICAL TECHNOLOGY

Starting June 14, 1971 (the beginning of the fiscal year) the School of Medical Technology, Chiang Mai University will put her newly revised curriculum leading to the degree of Bachelor of Science in Medical Technology - B.Sc. (Med. Tech.) into effect. The new curriculum is designed to provide students more freedom in program selection, broader background as well as the better scholastic record. In addition to the structural reoganization to be in tune with the fast - growing medical world it also enhances the student's professional achievement, the opportunity in acquiring higher education and advanced training as well.

The School believes that her students should be benefit from a liberal education which will free them all from the limitations placed by ignorance on the powers of judgement and decision. The students will distribute part of the course work in areas of study other than those mostly linked to his specialized vocational training, for example, Social Science, Humanities, History, Economics, Business Administration, Psychology, Government, Western Civilization, Religion etc.

The School of Medical Technology offers the study program leading to a degree of Bachelor of Science in Medical Technology - B.Sc. (Med. Tech.). The requirements for graduation is the completion of all the required courses or their equivalent with a total of 160 or more semester credit hours and an average of 2 grade-points per semester credit hours registered (or C average).

BACCALAUREATE REQUIREMENTS FOR UNDERGRADUATE MEDICAL TECHNOLOGY

A. BASIC REQUIREMENT - Total of 77 semester credit hours.

1. Chemistry - Total 25 sem. hrs., including two semesters of College Chemistry, two semesters of Organic Chemistry plus one semester each of Quantitative analysis and Biochemistry.
2. Biology - Total 23 sem. hrs., including one semester each of College Biology, Zoology, Botany, General Microbiology, Physiology and Anatomy.
3. Mathematics - Total 9 sem. hrs., including two semesters of College Mathematics and semester of Statistics.
4. Physics - Total 8 sem. hrs., including two semesters of College Physics.
5. English - Total 12 sem. hrs., including four semesters of College English and Advanced English for Science Students.

B. PROFESSIONAL COURSE - Total of 65 semester credit hours, consisting of :

1. Clinical Chemistry	3 semesters	(18 sem. hrs.)
2. Clinical Microbiology	3 semesters	(14 sem. hrs.)
3. Clinical Microscopy	3 semesters	(15 sem. hrs.)
4. Immunohematology	2 semesters	(6 sem. hrs.)
5. Parasitology	2 semesters	(5 sem. hrs.)
6. X-Ray and Isotopes	1 semester	(1 sem. hr.)
7. Histo-Cytology Technic	1 semester	(2 sem. hrs.)
8. Medical Illustration	1 semester	(1 sem. hr.)
9. Seminar Journal Club	1 semester	(1 sem. hr.)
10. Term Paper	1 semester	(2 sem. hrs.)

C. ELECTIVE COURSE - Total of 18 semester credit hours. The student may select subjects to make up his total semester credit hours earned as required.

GRAND TOTAL 160 Sem. Hrs.

RECOMMENDED STUDY PROGRAM LEADING TO THE DEGREE OF BACHELOR OF SCIENCE IN MEDICAL TECHNOLOGY

FRESHMAN

First semester		Second semester	
Biol. 103	4 sem. hrs.	Biol. 111	3 sem. hrs.
Chem. 101	4 sem. hrs.	Biol. 112	3 sem. hrs.
Engl. 101	3 sem. hrs.	Chem. 102	4 sem. hrs.
Math. 101	3 sem. hrs.	Engl. 102	3 sem. hrs.
Phys. 111	4 sem. hrs.	Math. 102	3 sem. hrs.
Elective	3 sem. hrs.	Phys. 112	4 sem. hrs.
Total	21 sem. hrs.	Total	20 sem. hrs.

SOPHOMORE

Biol. 203	4 sem. hrs.	Chem. 103	2 sem. hrs.
Chem. 201	4 sem. hrs.	Chem. 202	4 sem. hrs.
Chem. 204	4 sem. hrs.	Engl. 202	3 sem. hrs.
Engl. 291	3 sem. hrs.	Math. 206	3 sem. hrs.
Elective	6 sem. hrs.	Elective	6 sem. hrs.
Total	21 sem. hrs.	Total	18 sem. hrs.

JUNIOR

Anat. 337	3 sem. hrs.	Clin. Chem. 332	6 sem. hrs.
Imm. Hemat. 331	3 sem. hrs.	Clin. Microb. 332	5 sem. hrs.
Bioc. 331	3 sem. hrs.	Histo-Cyto. Tech. 332	2 sem. hrs.
Clin. Micros. 331	4 sem. hrs.	Parasit. 332	3 sem. hrs.
Qual. F.T. 339	2 sem. hrs.	Elective	3 sem. hrs.
Physiol. 331	6 sem. hrs.		
Total	21 sem. hrs.	Total	19 sem. hrs.

SENIOR

Clin. Chem. 431	6 sem. hrs.	Appl. Imm. Hemat. 439	3 sem. hrs.
Clin. Microb. 431	3 sem. hrs.	Appl. Clin. Chem. 432	6 sem. hrs.
Clin. Micros. 431	6 sem. hrs.	Appl. Clin. Microb. 432	6 sem. hrs.
Med. Ill. 431	1 sem. hr.	Appl. Clin. Micros. 432	3 sem. hrs.
Parasit. 433	2 sem. hrs.	Sem. J.C. 439	1 sem. hr.
X. I. 431	1 sem. hr.	Term P. 439	2 sem. hrs.
Total	19 sem. hrs.	Total	21 sem. hrs.

RECOMMENDED ELECTIVE COURSES : Anthro. 215, Anthro. 203-204 B.A. 103, Chem. 103, Chem. 206, Econ. 103, Ed. F. 103, Ed. F. 104, Geo. 106, Govt. 103, Hist. 101, Law 103, L.S. 103, Psy. 103, Psy. 104, Psy. 203, Soc. 115, Soc. 212, Soc. Anthro. 103, Med. Inst. 339.

JUNIO

FIRST SEMI

Previous Curriculum

Course No.	DESCRIPTIVE TITLE	Sem. Hrs.	Prerequisite	C
Clin. Micros. 331	Clinical Microscopy	6 (3–9)	Third Year Standing	An
Physio.331	Physiology for Med. Tech. Student	5 (4–4)	Third Year Standing	Phy
Bioc. 331	Biochemistry for Med. Tech. Student	5 (2–6)	Third Year Standing	Bi
B.B. Tech. 331	Blood Bank Techniques	3 (2–2)	Third Year Standing	Cli cro
Anat. 337	Anatomy for Med. Tech. Student	3 (2–2)	Third Year Standing	Qu
				Im He
	Total	22		

SECOND SEM

Clin. Chem. 332	Clinical Chemistry	8 (5–9)	Third Year Standing	Cli
Clin. Micro 332	Clinical Microbiology	5 (2–6)	Third Year Standing	Cli cro
Parasit. 332	Parasitology for Med. Tech. Student	3 (2–2)	Third Year Standing	Par
Histo-Cyto. Tech. 332	Histocytology Techniques	3 (2–2)	Third Year Standing	His Te
Spec. Tech. 332	Special Techniques	1 (1–1)	Third Year Standing	E
	Total	20		

) R

ESTER

Revised Curriculum

Course No.	DESCRIPTIVE TITLE	Sem. Hrs.	Prerequisite
nat. 337	Anatomy for Med. Tech. Student	3 (2–2)	Biol. 111
ysiol. 331	Physiology for Med. Tech. Student	6 (4–4)	Biol. 111, Phys. 112, Engl. 292
oc. 331	Biochemistry for Med. Tech. Student	3 (3–0)	Chem. 202
in. Mi-cs. 331	Hematology for Med. Tech. Student	4 (2–6)	Biol. 111
ual. F.T. 339	Qualitative Function Tests	2 (1–3)	Biol. 111
nm. emat. 331	Immunohematology and Blood Bank Technique	3 (2–2)	Biol. 111
	Total	22	

MESTER

in. Chem. 332	Clinical Chemistry	6 (4–6)	Physiol.331, Bioc.331
lin. Mi-cb. 332	Clinical Microbiology	5 (2–6)	Biol. 203
rasit. 332	Parasitology for Med. Tech. Student	3 (2–2)	Anat. 331
isto-Cyto. ech. 332	Histocytology Techniques	2 (1–3)	Anat. 337
Elective		3	
	Total	19	

FIRST SEM

Previous Curriculum

Course No.	DESCRIPTIVE TITLE	Sem. Hrs.	Prerequisite
Clin. Chem. 431	Advanced Clinical Chemistry	6 (3–9)	Forth Year Standing
Clin.Micros.431	Advanced Clinical Microscopy	7 (4–8)	Forth Year Standing
Clin. Micro.431	Advanced Clinical Microbiology	3 (2–2)	Forth Year Standing
Parasit. 433	Special Problem in Parasitology	2 (1–3)	Forth Year Standing
Med. Ill. 431	Medical Illustration and Photography	1 (1–3)	Forth Year Standing
X. I. 431	X–Ray and Isotope	1 (1–3)	Forth Year Standing
	Total	20	

SECOND SEMES

Clin. Chem. Lab. 432	Laboratory Services in Clinical Chemistry	8(0–480)	Forth Year Standing
Clin. Micros. Lab. 432	Laboratory Services in Clinical Microscopy	3(0–160)	Forth Year Standing
Clin. Micro. Lab. 432	Laboratory Services in Clinical Microbiology	3(0–160)	Forth Year Standing
B.B. Tech. Lab. 439	Laboratory Services in Blood Bank	3(0–160)	Forth Year Standing
Term P. 439	Term Paper	2 (–)	Forth Year Standing
Sem. J.C. 439	Seminar Journal Club	1 (–)	Forth Year Standing
	Total	20	
	GRAND TOTAL 160		

I O R

E M E S T E R

Revised Curriculum

	Course No.	DESCRIPTIVE TITLE	Sem. Hrs.	Prerequisite
g	Clin. Chem. 431	Advanced Clinical Chemistry	6 (3–9)	Clin. Chem. 332
g	Clin. Micros. 431	Advanced Hematology for Med. Tech. Student	6 (4–6)	Clin. Micros. 331
g	Clin. Microb. 431	Advanced Clinical Microbiology	3 (2–2)	Clin. Microb. 332
g	Parasit. 433	Special Problem in Parasitology	2 (1–3)	Parasit. 332
g	Med. Ill. 431	Medical Illustration and Photography	1 (1–3)	None
g	X.I. 431	X–Ray and Isotope	1 (1–3)	None
		Total	19	

E S T E R

g	Appl. Clin. Chem 432	Laboratory Services in Clinical Chemistry	6(0–320)	Clin. Chem. 431
g	Appl. Clin. Micros. 432	Laboratory Services in Clinical Microscopy	3(0–160)	Clin. Micros. 431 Qual. F.T. 339
g	Appl. Clin. Microb. 432	Laboratory Services in Clinical Microbiology	6(0–320)	Clin. Microb. 431
g	Appl. Imm. Hemat. 439	Laboratory Services in Blood Bank	3(0–160)	Imm. Hemat. 331
g	Term. P. 439	Term Paper	2(–)	Consent of Instructor
g	Sem. J. C. 439	Seminar Journal Club	1(–)	Consent of Instructor
		Total	21	
		GRAND TOTAL 160		

COMPARISON BETWEEN THE PREVIOU

FRESHMA

FIRST SEME

Previous Curriculum

Course No.	DESCRIPTIVE TITLE	Sem. Hrs.	Prerequisite	Co N
Biol. 103	General Principles in Biology	4 (3–3)	None	Biol.
Chem. 101	General Chemistry	4 (3–3)	None	Chem
Engl. 101	Fundamental English I	3 (3–1)	None	Engl.
Math. 101	General Mathematics	3 (3–0)	None	Math.
Phys. 101	General Physics	4 (3–3)	None	Phys.
Soc. 115	Sociology	2 (2–0)	None	Electi
	Total	20		

SECOND SEME

Biol. 111	Zoology	3 (2–3)	Biol. 103	Biol.
Chem. 102	General Chemistry	4 (3–3)	Chem. 101	Biol.
Chem. 103	Scientific Technique	2 (2–0)	None	Chem.
Engl. 102	Fundamental English II	3 (3–1)	Engl. 101	Engl.
Math. 102	Analytical Geometry and Calculus	3 (3–0)	Math. 101	Math.
Phys. 102	General Physics	4 (3–3)	Phys. 101	Phys.
	Total	19		

IOUS AND REVISED CURRICULUM

I M A N

EMESTER

Revised Curriculum

Course No.	DESCRIPTIVE TITLE	Sem. Hrs.	Prerequisite
Biol. 103	General Principles in Biology	4 (3–3)	None
Chem. 101	General Chemistry	4 (3–3)	None
Engl. 101	Fundamental English I	3 (3–1)	None
Math. 101	General Mathematics	3 (3–0)	None
Phys. 111	General Physics I	4 (3–3)	None
Elective		3	None
	Total	21	

SEMESTER

Biol. 111	Zoology	3 (2–3)	Biol. 103
Biol. 112	Botany	3 (2–3)	Biol. 103
Chem. 102	General Chemistry	4 (3–3)	Chem. 101
Engl. 102	Fundamental English II	3 (3–1)	Engl. 101
Math. 102	Analytical Geometry and Calculus	3 (3–0)	Math. 101
Phys. 112	General Physics II	4 (3–3)	Phys. 111
	Total	20	

SOPHOMORE

FIRST SEMESTE

Previous Curriculum

Course No.	DESCRIPTIVE TITLE	Sem. Hrs.	Prerequisite	Course No.
Chem. 201	Organic Chemistry	4 (3–3)	Chem. 102	Biol. 20
Chem. 204	Quantitative Analysis	4 (3–3)	Chem. 102	Chem. 20
Engl. 291	English for Science Student III	3 (3–1)	Engl. 192, or equivalent	Chem. 20
Phys. 201	Electricity and Magnetism	4 (3–3)	Phys. 102, Math. 102	Engl. 29
Math. 201	Calculus	3 (3–0)	Math. 102	Elective
Psys. 103	Psychology	3 (3–0)	None	
	Total	21		

SECOND SEMEST

Chem. 202	Organic Chemistry	4 (3–3)	Chem. 201	Chem. 10
Chem. 206	Physical Chemistry	4 (3–3)	Chem. 102	Chem. 20
Engl. 292	English for Science Student IV	3 (3–1)	Engl. 291	Engl. 29
Math. 206	Elementary Statistics	3 (3–0)	Math.101 or Consent of Instructor	Math. 20
Phys. 202	Light and Introduction to Modern Physics	4 (3–3)	Phys. 201	Elective
	Total	18		

MORE

MESTER

Revised Curriculum

Course No.	DESCRIPTIVE TITLE	Sem. Hrs.	Prerequisite
Biol. 203	Microbiology	4 (3–3)	Biol.111, Biol.112 and Chem. 102
Chem. 201	Organic Chemistry	4 (3–3)	Chem. 102
Chem. 204	Quantitative Analysis	4 (3–3)	Chem. 102
Engl. 291	English for Science Student III	3 (3–1)	Engl. 192 or Equivalent
Elective		6	
	Total	21	

EMESTER

Chem. 103	Scientific Technique	2 (2–0)	None
Chem. 202	Organic Chemistry	4 (3–3)	Chem. 201
Engl. 292	English for Science Student IV	3 (3–1)	Engl. 291
Math. 206	Elementary Statistics	3 (3–0)	Math. 102
Elective		6	
	Total	18	

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BULLETIN OF CHIANG MAI MEDICAL TECHNOLOGY

Vol. 4 No. 2
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Anti-Microbial Susceptibility Patterns of Haemophilus influenzae from Patients in Nakorn Chiang Mai Hospitol*

Suputra Peerakome, B.Sc. (Med. Tech.)**
Kampol Panas-ampol, M.D. **

Abstract

Nineteen strains of Haemophilus influenzae were isolated from 75 patients at Nakorn Chiang Mai Hospital. The identification was based on morphology, staining property, colonial characteristics, satellite phenomenon and the requirement of both X and V factors. The susceptibilities to ampicillin, tetracycline, chloramphenicol, streptomycin and sulfadiazine were determined by using a plate dilution method and the multipoint inoculator. Readings of the minimal inhibitory concentration (M.I.C.) were made after 24 hour incubation at which time there was complete growth of the organisms on the control plate. The results were compared with the known average blood level concentrations in people and classified as sensitive, intermediate or resistant on this basic. We found that all strains had the same susceptibility pattern. They were sensitive to ampicillin, tetracycline and chloramphenicol but resistant to streptomycin and sulfadiazine. The comparison of undiluted inoculum and 0.1 dilution were included in this study. Most of strains showed the same result with a maximum difference of one two-fold dilution.

Introduction

Haemophilus influenzae plays an important etiological role in bacterial meningitis, especially in children under 2 years old. It sometimes produces septicemia, obstructive epiglottitis, pneumonia(1), severe conjunctivitis, arthritis, laryngitis, endocarditis (2), sinusitis, laryngotracheitis (3), relapsing illness, otitis media, pneumonitis or chronic lung disease (1). It is

* The Term Paper for the Degree B.Sc. (Med. Tech.). The School of Medic l Technology, Faculty of Medicine, Chiang Mai University, Chiang Mai 1970

** Department of Microbilogy, Faculty of Medicine, Chiang Mai University, Chiang Mai

often implicated as a "secondary invader" and more than half the meningitis patients have upper respiratory tract infection before contracting meningitis.

Marphology (3, 4, 5, 6)

In 1892-1893 (5), Pfeiffer first isolated the organism from the nasopharynx of epidemic influenza patients, mistakenly believing it to be the cause of the influenza. It is a small gram negative aerobic bacillus, 0.5 micron in length and 0.2-0.3 micron in diameter, non-motile. It is pleomophic, and may appear as coccoid bacilli, short chains, long rods, or large spherical bodies. Morphology depends on age and media. After 6-8 hours, cultures on enriched media appear as coccobacilli, but after longer incubation appear as long pleomorphic rods. In exudates and in 6-18 hours cultures on enriched media, capsules are present. However these autolyse in autolytic enzyme and disappear in older cultures. On Levinthal agar after 24 hours incubation, it appears as a small, round, convex colony with strong irridescence. The irridescence is due to the presence of capsules. On chocolate agar, after 36-48 hours it appears as large colony, 1 m.m. in diameter. On blood agar, it grows poorly or not at all and produces no hemolysis. However it will produce a large colony on blood agar if growing in the vicinity of Staphylococcus, Neisseria, or Pneumococci colonies. These organisms produce a growth factor required by H. influenzae. This phenomenon is called "Satellitism" or "Satellite phenomenon".

Growth Characteristics (7, 8)

H. influenzae requires media enriched with X-factor, a heats-table substance called hemin, extracted from blood, and V-factor, a heat-labile substance DPN or TPN. V factor may be extracted from vegetables, yeasts, whole blood, and is produced by certain bacteria. Identification of the species is generally based on requirement for the growth factors X and V and reaction with specific antisera.

Transformation (3)

H. influenzae extracted DNA can transfer type specificity, such as antibiotic resistance to other cells.

Antigenic Structure

H. influenzae's capsule, which is important in virulence, possesses polysaccharide antigens. Based on these antigens, 6 serological types, a, b, c, d, e, f are recognized. In capsular swelling (quellung) and carbohydrate precipitin tests, cross-reactions with pneumococci occasionally occur. Most meningitis cases are caused by type b (5).

Diagnostic Laboratory Tests

Specimens

Specimens may include nasopharyngeal swabs, pus, blood, C.S.F. (3), throat swabs, exudate from eye and ear (1), oropharyngeal exudate (9), tracheal aspirates,

lung aspirates, and exudate from wounds (10). In meningitis cases, organisms can be isolated from C.S.F., throat swabs and in 75% of cases, from the blood.

Direct Identification

The specimen is examined by gram stain and cultured on appropriate media. It has been recorded that from C.S.F. from H. influenzae meningitis cases, the organism was found in 88.4% of specimens by gram stain, but only 78.3% of the subsequent cultures were positive (11). If large amounts of organisms are found in the specimen, gram stain, direct typing by capsular swelling on C.S.F. (3) can be done. Precipitin tests may be done for detection of organisms in C.S.F.; a positive result indicates high concentration of specific H. influenzae type b polysaccharides (3).

Selective media for detection of H. influenzae may be prepared by adding antibiotics such as bacitracin or penicillin to culture medium. The media most often used in the laboratory is chocolate agar enriched with hemin and supplement B (yeast extract); but problems arise because gamma streptococci, enterococci, diptheriods, and neisseria may give similar colony patterns, irridescence is hard to see, and supplement B is easily destroyed by heat.

H. influenzae grows excellently on media enriched with red blood cells which have been hemolyzed by heat, such as Levinthal's medium, or hemolyzed by peptic digestion, such as Fildes's medium. Both media are transparent, so irridescence of Haemophilus species can be seen. However these are difficult to prepare.

A new method of isolation is that of placing a saponin-soaked disc on the surface of sheep blood agar. The saponin hemolyzes red blood cells, and splits out di-phosphopyridine nucleotide. This has the same properties as Levinthal's and Fildes's medium, with the advantage of showing hemolysis of the other hemolytic pathogens (12).

Immunity

Active immunization can not induce immunity in man. A newborn baby's immunity disappears during the first 6 weeks, after which immunity increases until 3-4 years old when bactericidal activity against virulent strains of H. influenzae occurs. Fothergill and Wright (6) found that animals could be immunized.

Treatment

In the past, H. influenzae type b rabbit antiserum had a moderate effect in decreasing the percentage of fatality in patients (to 90 % of fatality rate for untreated patients). (3, 5, 6)

Detection of antibody may be done by using H. influenzae type b's capsule as a skin testing agent, with intradermal injection. If the test is positive, after 30 minutes erythema with pseudopodia cc-

cures (6). Now, type-specific rabbit antiserum is not use in treatment, because it can cause serum-sickness. Antibiotics and sulfadiazine are preferred. The present study investigated the current antibiotic susceptibilities of local H. influenzae strains to those antibiotics most frequently used in treatment.

Material and Method

I Bacterial Strains

H. influenzae was isolated from throat swabs of 75 patients at Nakorn Chiang Mai Hospital during the period from November 13, 1969 to January 13, 1970.

Isolation for pure culture

Media

- Chocolate agar

G.C. agar base was used Autoclaved at 15 lbs/in.^{2} for 15 minutes, it was then placed in an 80°C. waterbath. By aseptic technique 10 % sterile blood was added, and the mixture left at 80°C for 10 minutes The temperature was then lowered to about 45°C, and 1 % supplement B added.

- Blood agar

Blood agar base was autoclaved at 15 lbs/in.^{2} for 15 minutes, cooled it until the temperature was 48 - 50°C, and 10% sterile blood was added.

Specimens were inoculated on chocolate agar plates. Isolation was based on colonial characteristics. Suspected colonies were picked and inoculated on blood agar plates. A staphylococcus epidermidis, as a source of V factor, was streaked through the inoculum, and after 24 hours incubation, plates were checked for the "Satellite phenomenon". A gram stain was made to check for the typical gram-negative coccobacilli or gram - negative bacilli.

H. influenzae is different from other species in its requirement of both X and V factor (3,4,5,6). Media used to identify the Haemophilus species were : autoclaved chocolate agar (containing only X factor but no V factor), and trypticase soy agar (containing neither X nor V factor). If satellite phenomenon occured around a streak of Staphylococcus epidermidis only on the autoclaved chocolate agar, identification of the species of H. influenzae was confirmed.

II Antibiotics

The antibiotics used in this study were:

Tetracycline hydrochloride

Chloramphenicol

Streptomycin sulfate

Ampicillin sodium

Sulfadiazine sodium

Stock Solution of Antibiotics (24)

Antibiotic powder was dissolved in sterile distilled water, and the concentration of tetracycline, chloramphenicol and streptomycin adjusted to equal 1,000 mcg/ml, ampicillin to equal 500 mcg/ml, and sulfadiazine to equal 1,000 mg %.

Stock solutions were stored in 10 ml aliquot portions, frozen at - 70°C. Aliquots were discarded after a single use.

Working Solution of Antibiotics

Sensitivity testing was done by using a plate dilution method. The stock solutions of antibiotics were used as working solutions.

Plate Dilution Method

Media

- Mueller - Hinton chocolate agar

Mueller-Hinton agar was used as a base. By aseptic technique 10 % sterile blood was added at 80°C, and after 10 minutes the mixture was cooled to 45-50 °C and the solution of antibiotic or sulfadiazine added. Plates were poured.

- Levinthal's broth (1, 22, 23)

Mueller-Hinton broth was used as a base. By aseptic technique 10 % sterile blood was added at 80°C. After 10 minutes, the mixture was filtered, the supernatant portion collected and finally 1 % supplement B added.

Serial dilution of antibiotics for plate dilution method and preparation of plates.

Serial dilution of antibiotics for plate dilution method was based on the distribution and concentration of antibiotics in blood and C.S.F., and dose of antibiotic generally administered.

Highest concentrations used of tetracycline, chloramphenicol, streptomycin were 100 mcg/ml; for ampicillin and sulfadiazine they were 25 mcg/ml and 100 mg % respectively.

As shown in Fig. I, stock solutions of antibiotic were diluted from a 1st to 11th screw cap tube, to obtain two-fold dilutions. Each tube had a concentration ten times the final concentration. 18 ml. melted Mueller-Hinton chocolate agar(45°C) was added to each tube, the tubes mixed well, and plates poured and allowed to harden. This antibiotic media was kept no longer than 2 weeks in the refrigerator and dried 15 minutes in the incubator before use. The final concentrations of plates were 100, 50, 25, 12.5, 6.25, 3.125 1.56, 0.78, 0.39, 0.19, 0.09 mcg/ml; the 12th plate serving as a control.

Inoculum

A 24 hours. Levinthal's broth undiluted culture and 0.1 dilution culture were used in this study.

Inoculation

Inocula Replication Apparatus was used.

1. The teffon plastic seed plate which contains 36 inoculum reservoirs was sterilized with 70 % alcohol for 30 minutes and dried in an incubator.

An aluminum head containing 36 inoculating rods, was autoclaved and dried in a hot air oven.

2. By aseptic technique, 0.5 ml of inoculum for each strain was pipetted into a plastic seed plate reservior. Inoculating rods were then lowered

into the wells.

3. A piston was then pressed in order to place the inoculating rods on the surface of the media.
4. Lids were replaced, and inoculated plates were allowed to dry at room temperature, then inverted and incubated at 37°c in a candle jar for 24 hours.

Results

After 24 hours incubation, readings of the mininnal inhibitory concentration were made. An agar plate without antibiotics was used as a positive growth control. The obtainable concentration of antibiotics in blood (24) were compared with M.I.C. and the results are represented in Table II.

All strains tested had the same susceptibility pattern. They were sensitive to ampicillin (M.I.C. equaled 0.09, 1.56 mcg/ml), tetracycline (M.I.C. equaled 0.39 - 1.56 mcg/ml) and chloramphenicol (M.I.C. equaled 0.78-3.125 mcg/ml). All were resistant to streptomycin (M.I.C. equaled 6.25-50 mcg/ml) and sulfadiazine (M.I.C. equaled 25-100 mg%). Thus in this study, 100% of H. influenzae were sensitive to ampicillin, tetracycline and chloramphenicol but resistant to sulfadiazine. With streptomycin, 79% of the strains were in the intermediate range. (table II)

Fig. II represent the percentage of total isolates for each M.I.C. The comparison of undiluted inoculum and 0.1 dilution, most strains gave the same result, with a maximum difference of a two-fold dilution.

Discussion and Conclusion

Investigation of a susceptibility pattern is based on the comparison of M.I.C. to obtainable blood level of the antibiotic. If M.I.C. is lower than obtainable blood level, this indicates that organism are sensitive to antibiotics; on the other hand, if M.I.C. is higher than obtainable blood level, it indicates that organism are resistant to the antibiotic tested.

According to several investigators, C.S.F. level of antibiotic is lower than blood level (21, 28, 29, 30, 31, 32, 33). Comparison of M.I.C. to blood level and C.S.F. level should be considered in treatment. For therapeutic purposes, increasing the dosage of antibiotic is required to adjust C.S.F. level to equal M.I.C.

Sensitivity patterns of H. influenzae have been studied by others. Hans A. Hirsch and Maxwell Finland (10) recorded the results of a study from the Bacteriological Laboratory of the Boston City Hospital in 1949, 1954, 1959. By the plate dilution method, H. influenzae tested were sensitive to tetracycline, chloramphenicol and streptomycin. Reports from Bristol Laboratory indicated that plate dilution methods, tube dilution methods, and disc methods gave the same results; H. influenzae tested were all sensitive to ampicillin.

In most studies, sensitivity patterns were the same as these we obtained for ampicillin, tetracycline and chloramphenicol. However there were differences in streptomycin and sulfadiazine results.

Alexander (6, 40), Mac Pherson (41) and Pittman (42) have stated that H. influenzae may become resistant to some antibiotics and sulfadiazine because of mutation. When mutation occured, mechanisms (3) were : prevention of antibiotic diffusion into cells, a change in metabolic path ways forming a new enzyme which inhibited the action of the antibiotic, a change in ribosomal protein structure which produced the new antibiotic resistant enzymes.

ย่อเอกสาร

การศึกษาผลของยาปฏิชีวนะ และ Sulfadiazine Sod. ที่มีต่อเชื้อ Haemophilus influenzae 19 strains ที่แยกได้จากคนไข้ของ ร.พ. นครเชียงใหม่ 75 คน โดยเอาเชื้อจากลำคอของผู้ป่วย แยกเชื้อโดยอาศัยคุณสมบัติในการใช้ X และ V faetor ดู "satellite phenomenon" อาศัยลักษณะ colony และการย้อมสีเข้าช่วย

จากนั้นจึงนำเอามาทำการทดลองหา sensitivity pattern โดยทดลองกับยา Ampicillin, Tetracycline, Chloramphenicol, Streptomycin และ Sulfadiazine วิธีที่ใช้คือ Plate dilution ใช้เครื่องมือ เรียกว่า Inocula Replicator การอ่านผลถือว่าความเข้มข้นของยาที่น้อยที่สุด ที่สามารถยับยั้งการเจริญของเชื้อได้ภายใน 24 ชั่วโมง เป็น M.I.C. โดยมี plate ที่ไม่มียาอยู่ใน media เป็น positive control

แล้วนำค่า M. I. C. ไปเปรียบเทียบกับตารางแสดงค่าเฉลี่ย ของความเข้มข้นของยาในกระแสโลหิตของผู้ป่วย ซึ่งได้รับการรักษาในdose ธรรมดา เพื่อดูว่าเชื้อ sensitive หรือ resist ต่อยา จากผลการทดลองพบว่าเชื้อทั้งหมดมี susceptibility pattern อย่างเดียวกัน คือ

ยา Ampicillin ค่า M.I.C. 0.09–1.56 mcg/ml

ยา Tetracycline ค่า M.I.C. 0.39–1.56 mcg/ml

ยา Chloramphenicol ค่า M. I. C. 0.78–3.125 mcg/ml

แต่ resist ต่อ Streptomycin และ Sulfadiazine Sod.

จากการเปรียบเทียบการใช้ undiluted culture, 0.1 dilution culture เป็น inoculum ผลปรากฏว่า ค่า M.I.C. ของยาปฏิชีวนะและยาซัลฟาไดอาซีนที่มีต่อ H. influenzae ไม่ต่างกันมาก มีบาง strains เท่านั้น ที่ M.I.C. ต่างกัน 1 dilution.

No.	Ampicillin mcg/ml.	Tetracycline mcg/ml.	Chloramphenicol mcg/ml.	Streptomycin mcg/ml.	Sulfadiazine mg %
1	0.39	0.78	1.56	12.5	25.0
2	0.39	0.39	0.78	25.0	50.0
3	0.19	0.78	0.78	6.25	50.0
4	0.19	1.56	1.56	12.5	50.0
5	0.09	0.78	3.125	6.25	
6	0.39	0.39	0.78	12.5	50.0
7	0.09		3.125	25.0	
8	0.39	0.78	1.56	12.5	50.0
9	0.39	0.78	1.56	12.5	25.0
10	0.19	1.56	3.125	25.0	
11	0.39	0.39	0.78	12.5	25.0
12	0.39	0.39	0.78	25.0	50.0
13	0.39	1.56	0.78	25.0	100
14	1.56	0.78	0.78	50.0	50
15	1.56	0.78	0.78	50.0	50
16	1.56	0.39	0.78	6.25	50
17	1.56	0.39	0.78	6.25	50
18	1.56	0.39	0.78	6.25	100
19	1.56	0.78	0.78	6.25	100

Table I Minimal inhibitory concentration of 19 strains of H. influenzae to 4 antibiotics and sulfadiazine

Antibiotics	Sensitive	Intermediate	Resistant	Total
Ampicillin	19			19
Tetracycline	18			18
Chloramphenicol	19			19
Streptomycin		15	4	19
Sulfadiazine			16	16

Table II The sensitivity test of 19 strains of H. influenzae to 4 antibiotics and sulfadiazine.

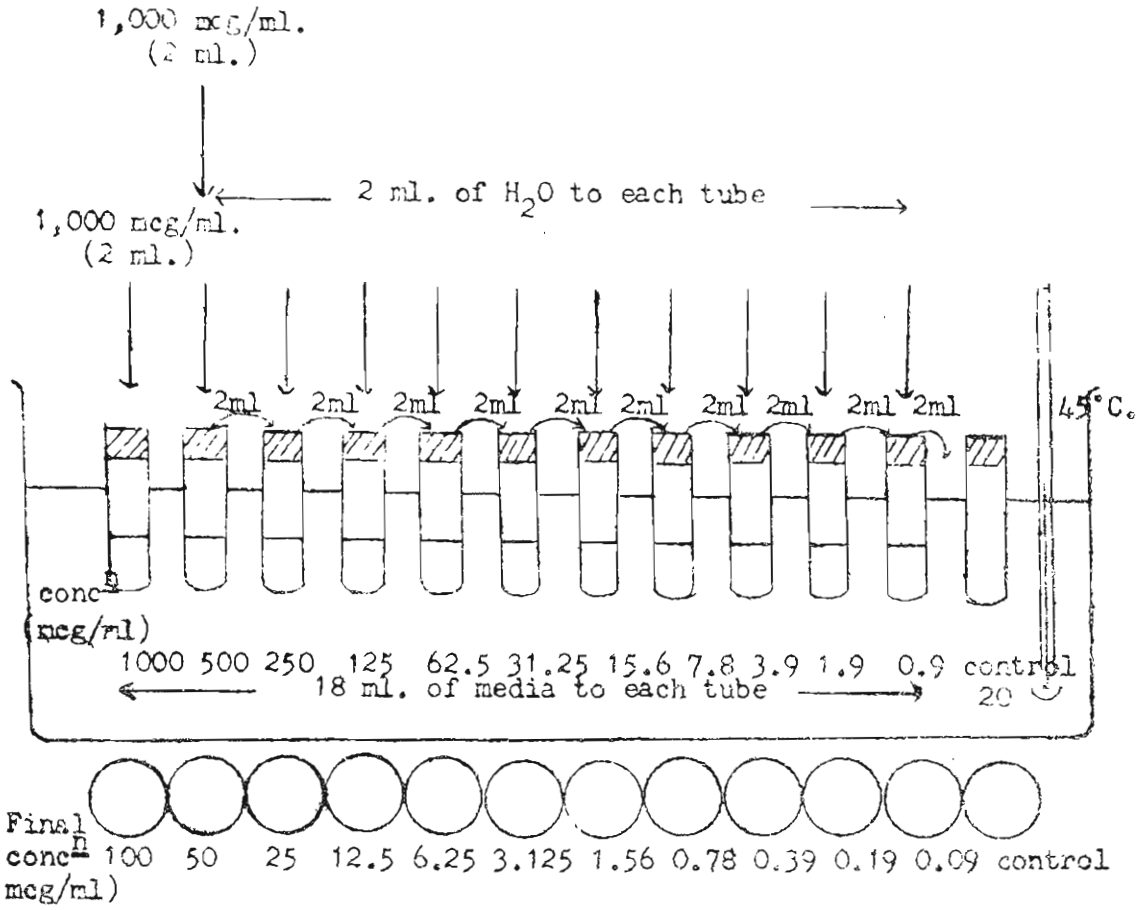

Fig. I Antibiotic two-fold dilution setup

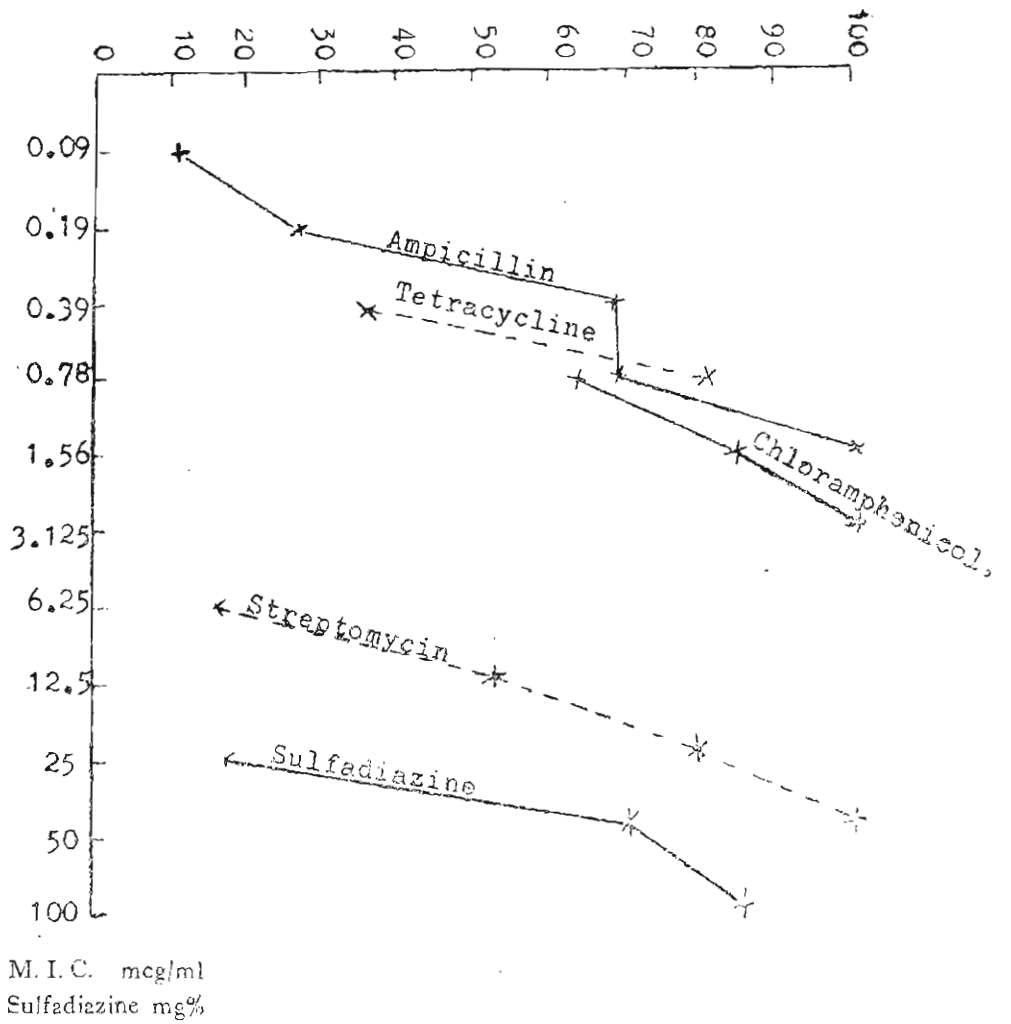

Fig. II The susceptibility of 19 strains of H. influenzae to 4 antibiotics and sulfadiazine

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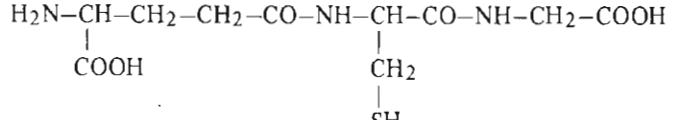

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A PRELIMINARY STUDIES ON CERCARIA OF OPISTHORCHIS Spp. IN CHIANG MAI PROVINCE

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Abstract

A preliminary studies on cercaria of **Opisthorchis** spp. in Chiang Mai, Thailand is presented. The cercariae were observed in two species of **Bithynia**, the B. **funiculata** and the **B. siamensis.** The incidence was about 0.17 per cent in both particular hosts. The detailed morphology of cercariae were also studied. The size of cercariae in the author's findings was larger than cercariae found in the Northeastern part of Thailand reported by some authors. The differences in size of both groups of cercariae might be due to the method of fixation and preservation. The different duration of growth and development of metacercariae were also stressed. The species of molluscs identified were included.

Introduction

Opisthorchiasis is quite a major health problem in the tropical countries. The personal hygiene, sanitation, and habits of food preparation in the majority of cases are involved in the epidemiology of the disease. In Thailand, opisthorchiasis is urged to be solved. Two cases of liver fluke were first reported in Chiang Mai, Thailand in 1916 by Kerr (1). The adult flukes obtained from the autopsy materials of two prisoners were identified as **Opisthorchis viverrini** (2) by Leiper. Prommas (1927) (3) also reported a case of **Opisthorchis felineus.** Sadun (4) in 1951 - 1952 presented the incidence of liver fluke infestation in students in eighteen provinces of Thailand. By stool examination, the eggs were found in 46 out of 1219 in Northern, 148 out of 504 in Northeastern, and 0 out of 2970 in Central and Southern parts. In 1957, Vajrasthira and Harinasuta (5) also reported that the incidence of

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liver fluke in Thai population in seventy-one provinces were about 29.8 per cent, 10.3 per cent, 0.3 per cent and 0 per cent in the Northeastern, Northern, Central and Southern parts, respectively. In 1964 - 1965 (6), investigators, Faculty of Tropical Medicine, Mahidol University collected the adult flukes from the livers of cases died of hepatic carcinoma, hepatic cirrhosis, and hepatitis in the Udorn Provincial Hospital. The detailed morphologic studies were made and concluded that all worms were classified as **O. viverrini** which was first partially studied by Poirier(1886) (8). Erhardt (1935) (9) reported that the morphologic characteristics of **O. viverrini** differed from **O. tenuicollis** found in the livers of sea mammals. So his findings were different from the ideas of Price (1932) (10), Vogel (1932) (11), Faust (1949) (12) and Dawes (1946) (13). Ejsmont(1937) (14) explained that the sea mammals ingested the fishes of cyprinoid family of which were habcured the metacercariae of **Opisthorchis** spp. He concluded that the **O. tenuicollis** and **O. felineus** might be subspecies but Watson (1960) (15) thought that they were the same. He also believed that the **O. tenuicollis**, **O. felineus** and **O. viverrini** were similar.

Ito **et. al.** (1962) (16) studied the morphology of cercariae of Opisthorchis spp. in Thailand and found that they belonged to parapleurolocercous cercaria and the cercariae of **O. viverrini** and **Clonorchis sinensis** have almost the same characteristics-- 1. numerous small body spines, 2. seven pairs of sensory hairs located on the body surface, 3. oral sucker larger than the ventral sucker, 4. pharynx located between the eyespots, 5. seven pairs of penetrating glands observed at the center of the body, 6. excretory granules found over the tail, and 7. the fin-fold longer than the body length.

Pariyanond (1965-1966) (7) also studied the morphology of cercariae of **Opisthorchis** spp. in Thailand and noted that the important characteristics were fall into pleurocercous group--- 1. numerous small spines over the body surface, 2. one pair of eyespots located on the lateral surface between the oral sucker and pharynx, 3. ten pairs of sensory hairs along the lateral surface, 4. oval-shaped oral sucker, 5. five pairs of the penetrating glands centrally located, and 6. projected thin membranous fin from the tail. Wykoff **et. al.** (1965) (7) had also studied the cercarial morphology of **Opisthorchis** spp. in Udorn and reported that these cercariae belonged to pleurocercous group. He also expressed that the non-movable organisms showed the smoking pipe-shaped structure.

O. viverrini cercariae found by Pariyanond (1966) (17) were 490-565 microns measuring from the anterior end to the tail end, and average 154 microns in length and 61 - 96 microns of the body width.

The cercariae obtained from **B. goniomphalus** in Udorn were measured 138.8 - 158.5 microns body length, 68.9 - 103.3 microns body width, and 183.7-234.2 microns tail length, as reported in the Annual Report, Faculty of Tropical Medicine, Mahidol University in 1963-1964 (18).

The life cycle of **O. viverrini** was reported by Dangsawang and Tansuratana (1961) (19) and Faculty of Tropical Medicine, Mahidol University in 1964-1965 (6) that the adult flukes in the livers of man or animals laid the eggs in the biliary tract and passed through the gastrointestinal canal and finally evacuated in human or animal stools. Then the molluscs of **Bithynia** spp. of which were the first intermediate hosts ingested the **Opisthorchis** eggs suspended in water. The molluscs which were the first intermediate hosts of **O. viverrini** in Thailand, were **B. goniomphalus**, **B. (Digoniostoma) funiculata** and **B. laevis** identified in Udorn, Chiang Mai and Bangkok, respectively. The hatched miracidia developed and transformed to be sporocysts, redia and cercariae in the hosts, respectively. The cercariae left the molluscs and wandered in the water until they found the appropriate second intermediate host, fresh water fishes; they penetrate through the scales and from the metacercariae in the flesh of fishes. When human or animals ingested the raw infected fishes, the encysted metacercariae were excysted and developed to be the immature flukes in the intestinal tract. They migrated through ampulla of Vator and developed to be the adult worms in the liver, finally.

In the present work, attempts have been made to study the life cycle of **Opisthorchis** in Chiang Mai. Emphasis will be made on the identification of **Opisthorchis** cercariae in molluscs, **Bithynia** spp., and its incidence in the intermediate hosts. The detailed comparative morphological characteristics of cercariae will be included. The purpose of this study is hoping to gain better knowledges of this particular organism for teaching in medicine and for better health of people all over the world especially the Thais.

Materials and Methods :

1) **Places of Collection of Molluscs :**

Yasamooth (1968) (21) and Na Bangxang (1969) (22) reported a high incidence of opisthorchiasis in Amphor Sarapee so the **Bithynia** spp. were mostly collected from this region and other adjacent areas namely Amphor Sansai, Sankampang, Doi Saket and Hang Dong, Chiang Mai. It was worthwhile to note that all molluscs collected were mostly from the goof-printed areas and likely less from the ponds, streams and irrigating channels.

2) **Methods of Collection of Molluscs :**

Manual and sieve-collecting methods were introduced. The molluscs of **Bithynia** spp. were chosen and transferred into plastic bags for carrying to the laboratory.

3) **Cleaning of the Molluscs :**

The collected molluscs were put into the sieve and shaken in fresh and clean water. Then the supernatant was discarded. This procedure was done until the last washing showed clear water indicated that the molluscs were satisfactorily cleansed. If the last washing still showed cloudy water, the cleaning had to be continued. The washed molluscs were ready for study.

4) **Indentification of Molluscs :**

As mentioned before, there were so many species of molluscs in Bithyniidae thus the exact species of **Bithynia** have to be made. About 5-10 molluscs were dry by leaving them on the filter or blotting papers for one to two hours at room temperature and transferred into plastic vials and sent for identification at the SEATO Medical Research Laboratory.

5) **Method of Examination of Cercariae:**

About ten clean molluscs were transferred into each clear plastic cup of 2.5 cm. in diameter containing 3/4 full fresh water. Each of them was covered with one glass plate and stood for four to six hours. It might be mentioned that the author used rain water in order to avoid chemical contaminants. The cup containing 10 molluscs was examined under a dissecting microscope. If the cercariae which had their morphology similar to the cercariae of **Opisthorchis** spp. were observed, each of the molluscs in that container was put into an individual cup containing fresh water and again stood for four to six hours. Observation of cercariae by dissecting microscope was again performed in order to detect the exact mollusc that haboured the cercariae.

All cercariae obtained were divided into three groups. The first group was kept for morphologic and locomotive studies. The second group was preserved in 60°C 5 per cent formalin for measuring the size and the remaining ones were save for infecting the **Cyprinus carpio** fishes*** in order to study the metacercariae later. The molluscs which exhibited no cercariae in the previous procedure were reexamined for shedded cercariae by crushing method and looking for the sporocysts or redia under a dissecting microscope.

6) **Morphologic Studies of Cercariae :**

The cercariae were transferred by capillary pipettes on the microslides and covered with microcoverglasses. The studies were particularly stressed on the location and numbers of the eyespots, spines, sensory hairs, penetrating glands in the body and the presentation of finfold on the tail.

The formalin - preserved specimens were transferred on the microslides by capillary pipettes and covered with micro-

*** Obtained from Kasetsart University

coverglasses for measurement of the size of cercariae as follows :

a. The whole body length which was measured from the tip of the oral sucker to the begining of the tail.
b. The widest part of the body.
c. The length of the tail which was measured from the posterior body end to the tail end.
d. The measurement of the widest part of the tail.
e. Two cross measurements which were made on the oral sucker and expressed in length and width that paralleling to the axis of body length and width, respectively.

7) **Method of Infecting Fishes with Cercariae :**

Approximately fifty cercariae were transferred into a 50 ml-beaker containing about 1/3 full volume of fresh water. A **Cyprinus carpio** fish was put into the beaker and stood for five to six hours and then the infected fishes were transferred to an aquarium.

8) **Examination of Metacercariae :**

After one month of exposure the infected fishes were sacrified. Pectoral fins, ventral fin, dorsal fin, scales and flesh were separately dissected. Each specimen was crushed and examined for metacercariae under the dissecting microscope. If the metacercariae were discovered, they were transferred by a small dissecting needle into a small slender dish containing 0.85 per cent normal saline for further morphologic studies.

7) **Morphologic Studies of Metacercariae :**

The metacercariae kept in 0.85 per cent normal saline were transferred to the microslides by capillary pipettes and covered with microcoverglasses. The shape of the cysts, cystwall, oral and ventral suckers, movement of metacercariae, excretory bladder and excretory corpuscles were carefully studied.

Results :

The identification of 13,532 molluscs of Bithyniidae obtained from eight areas in Chiang Mai (Table I) was recognized in five species as follows :

a. **Bithynia funiculata**
b. **Bithynia siamensis**
c. **Bithynia (Grabbia) wykoffi**
d. **Hydrobioidea siamensis**
e. **Hydrobioidea nassa**

It may be interesting to note that the natural habitats of the above five species of Bithyniidae were different. The **B. funiculata, B. siamensis** and **B. wykoffi** habituated in the goof-printed and muddy areas but none in the sandy places. The remainings were found in the streams by crawling on the water plants.

The studies of cercariae in five

species of Bithyniidae were found to be :

a. Cercariae which are compatible to cercariae of **Opisthorchis** spp.

b. Cercariae belonging to **Amphistome** spp.

c. Cercariae belonging to **Xiphidiocercaria**

The cercariae of **Opisthorchis** spp. were shedded from **B. funiculata** and **B. siamensis** collected from Amphor Sankampang and Sarapee. The cercariae mentioned above were observed in 16 out of 5,537 **B. funiculata** obtained from Amphor Sarapee, therefore the incidence was 0.3 per cent. The cercariae were also observed from 1 out of 4,015 **B. siamensis**. Unfortunately, this mollusc was dead before detailed studies of more cercariae were performed. However, many **B. siamensis** were again collected but failed to shed any cercariae (Table II). The general appearance of the cercariae obtained from **B. funiculata** and **B. siamensis** are identical to cercariae of **Opisthorchis** spp. It was pleurocercous cercariae which had one pair of eyespots located between the oral sucker and pharynx, fin-fold tail, smoking pipe-shaped at non-motile stage, clear oral sucker and ventral sucker is larger than the oral one. The formalin-fixed cercariae measured about 150-222 microns (average 192.2 microns) in body length, 60-96 microns (average 77.8 microns) in body width, 336-456 microns (average 402.5 microns) of tail length, 24-48 microns (average 34.4 microns) of tail width, and 28.2 X 32.9 microns of oral sucker size (Table III).

The metacercariae were observed under the scales of 20 fishes after exposure to cercariae for seven days and they seemed to be immature because the eyespots and cystwall were easily destroyed under the weight pressure of coverslip. Examination of each four fishes after thirty - day and fourty-two-day of cercarial exposure revealed the metacercariae in the flesh of which were still immature showing eyespots and rather thick cyswall. There were only two metacercariae in each fish.

Discussion :

The cercariae of **Opisthorchis** spp. shedded from **Bithynia** spp. in Chiang Mai province and the cercariae reported by Wykoff **et. al.** (1965) (7), Ito **et. al.** (1962) (16), Pariyanond (1966) (17) and Annaul Report of Faculty of Tropical Medicine, Mahidol University (1963-1964) (18) were similar. However, the size of these organisms in this study was larger in comparison with the studies of the above authors. Regarding the size, the author would like to comment some factors involved. First, the concentration of formalin and its temperature may play an important role in the process of fixation. Wykoff **et. al.** (1965) (17) did not specify the concentration of formalin and its temperature during the fixing state. Second, cercarial identifications from the study of Wykoff

and his coworkers were from the **B. goniomphalus** in contrast to the author's which were from the **B. funiculata**. However, the size of cercariae presented by Wykoff **et. al.** (1965) (7) were the same as Pariyanond's (1965-1966) (17) findings. With the exception of area of investigation, the morphology of cercariae and the first intermediate hosts in this study and in the study of Ito and his associates (1962) (16) were alike.

The morphologic characteristics of cercariae reported by the authors and Annual Report of Faculty of Tropical Medicine, Mahidol University (1963-1964) (18) were almost the same, except the size as previously mentioned. At this moment, the author thinks that the numbers of molluscs collected in this study were rather small in comparison with the studies of 87,770 and 771,974 molluscs in the Northeastern in the year 1962-1963(20) and 1963-1964 (18) respectively as reported from the Faculty of Tropical Medicine, Mahidol University. This evidence might be the cause of the difference in cercarial size in these two reports, therefore further investigation may be needed in order to get more evidences that will support and confirm these cercariae to be exactly belonged to **Opisthorchis** spp.

The incidence of the cercarial infected molluscs in Udorn province studied by the Faculty of Tropical Medicine, Mahidol University in the year 1962-1963 (20) and 1964-1965 (18) was 0.6 per cent and 0.05 per cent, respectively. In comparison with this study, the incidence was 0.3 per cent (Table II) that was considerably high and very much interested. Besides, in Chiang Mai, the irrigation planning for the agricultural divelopement is in the process thus the problems in epidemiology of liver fluke infestation will be no doubt occurred, because the spreading of the molluscs which are the intermediate hosts would be more easier. The accidental cases of opisthorchiasis may be increased, we may say.

It is interesting that the cercariae shedded from **B. siamensis** were morphologically similar to the cercariae of **Opisthorchis** spp. of which Ito **et. al.** (1962) (16), Pariyanond (1965) (17) and Wykoff **et. al.** (1965) (7) reported. Unfortunately, this mollusc was dead before the detailed study was made. As far as the literatures concerned, there was no report on **B. siamensis** as the intermediate hosts of **Opisthorchis** spp.

The development of the metacercariae in the fishes in this study was 42 days in hosts as compare to 21-35 days as presented by the Faculty of Tropical Medicine, Mahidol University (1963-1964) (18). The reason may be that the cercariae obtained were from the different

species of the intermediate hosts. The cercariae in this study were shedded from **B. funiculata** whereas from the report of the Faculty of Tropical Medicine, Mahidol University collected the cercariae from **B. goniomphalus.** So the duration of growth and development of metacercariae in the fishes may be different.

Conclusion :

The studies and investigations of cercariae of **Opisthorchis** spp. in Chiang Mai province were shown that the **B. funiculata** and **B. siamensis** were considered to be the first intermediate hosts. The incidence of cercariae shedding from **B. funiculata** and **B. siamensis** was 0.3 and 0.02 per cent, respectively, or 0.17 per cent in both species. The size of cercariae in this study was larger than the others as presented in previous studies. The factors involving the size of these cercariae and duration of growth and development of metacercariae in the fishes were discussed. The findings in this study are considerably important in teaching medicine and epidemiological aspects also. Finally, the more scientific informations on **B. siamensis** which habours the cercariae of **Opisthorchis** spp. will be explored.

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Table I

Numbers and species of 13,532 molluscs found in eight different regions in Chiang Mai, Thailand

Region	Species	Numbers
Section 2, Tambol Chompou, Amphor Sarapee	B. funiculata	135
	Hydrobioidea nassa	383
Section 3, Tambol Sansai, Amphor Sarapee	B. funiculata	494
	Hydrobioidea nassa	446
Section 10, Tambol Sansai Amphor Sarapee	B. funiculata	5,043
	Hydrobioidea nassa	265
Ban Mae Gad, Amphor Sansai	Bithynia and/or Hydrobioidea	882
Tambol Mae Pong, Amhpor Doi Saket	Bithynia and/or Hydrobioidea	856
Tambol Nong Boa, Amphor Doi Saket	B. funiculata	146
Amphor Hang Dong	B. funiculata	220
	Hydrobioidea nassa	366
Ban San Kowng, Amphor Sankampang	B. funiculata	281
	B. siamensis	4,015

Table II Types of Cercariae shedded from molluscs of genus Bithynia and Hydrobioidea collected from Chiang Mai Province

Regions	Species	Numbers Examined	Types of cercariae observed in molluscs		
			Opisthorchis (per cent)	Amphistome (per cent)	Xiphidiocercaria (per cent)
Section 2, Tambol Chompou, Sarapee	Bithynia funiculata	135	–	5 (3.7%)	–
	Hydrobioidea nassa	383	–	–	5 (1.3%)
Section 3, Tampol Sansai, Sarapee	Bithynia funiculata	494	1 (0.2%)	20 (4.04%)	4 (0.6%)
	Hydrobioidea nassa	446	–	1 (0.2%)	–
Section 10, Tambol Sansai, Sarapee	Bithynia funiculata	5043	15 (0.3%)	304 (6.0%)	58 (1.1%)
	Hydrobioidea nassa	265	–	1 (0.04%)	3 (1.1%)
Ban Mae Gad, Sansai	Bithynia and/or Hydrobioidea	882	–	33 (3.7%)	4 (0.45%)
Tambol Mae pong, Doi Saket	Bithynia and/or Hydrobioidea	856	–	169 (19.7%)	35 (4.0%)
Tambol Nong Boa Doi Saket	Bithynia funiculata	146	–	17 (11.6%)	–
Hang Dong	Bithynia funiculata	220	–	46 (20.9%)	–
	Hydrobioidea nassa	366	–	–	–
Ban San Kowng, Sankampang	Bithynia funiculata	281	–	–	–
	Bithynia siamensis	4015	1 (0.02%)	15 (0.3%)	1 (0.02%)
Total 8 regions	Bithynia and/or Hydrobioidea	13,532	17 (0.12%)	611 (4.5%)	109 (0.8%)

Table III Size of Cercariae of Opisthorchis spp. shedded from B. funiculata

No.	Body (microns)		Tail (microns)		Dimension of oral sucker (microns)
	Length	Width	Length	Width	
1	174	72	336	30	30×36
2	174	78	384	36	30×36
3	210	84	408	36	30×36
4	180	66	432	36	30×36
5	192	72	384	30	30×36
6	222	66	456	30	30×36
7	204	60	408	24	30×36
8	192	72	384	24	30×30
9	186	72	384	30	30×30
10	162	84	408	30	30×30
11	192	96	456	36	30×30
12	192	78	432	42	24×36
13	204	90	432	42	30×36
14	222	84	408	48	30×36
15	210	90	432	42	30×30
16	222	78	408	36	30×30
17	216	84	432	36	30×36
18	204	78	432	30	24×30
19	222	78	408	36	24×30
20	198	84	432	42	30×30
21	186	78	432	30	30×36
22	192	78	408	36	24×30
23	204	78	408	36	24×30
24	192	84	408	42	30×36
25	150	78	384	36	24×30
26	216	84	432	42	24×30
27	204	78	408	30	30×36
28	198	84	408	36	30×36

Table III (cont.)

No.	Body (microns)		Tail (microns)		Dimension of oral sucker (microns)
	Length	Width	Length	Width	
29	204	78	408	30	30×30
30	180	72	336	36	30×36
31	192	72	384	30	30×36
32	174	66	408	24	24×30
33	150	78	336	30	24×36
34	204	78	432	42	30×36
35	222	78	408	36	24×30
36	198	84	432	42	30×30
37	156	72	408	30	24×30
38	216	84	432	42	24×30
39	204	78	408	30	30×36
40	150	72	336	30	24×30
41	186	78	432	30	30×36
42	186	72	336	42	30×30
43	222	66	456	30	30×36
44	216	72	384	42	30×30
45	192	60	408	24	30×36
46	180	90	432	42	30×36
47	174	84	336	36	30×30
48	204	96	456	36	30×30
49	198	84	408	36	30×36
50	150	78	384	36	24×30
51	186	78	432	30	30×36
52	216	84	432	36	30×36
53	210	90	408	42	30×30
54	222	72	384	36	30×36
55	210	84	336	24	24×36
56	174	84	384	36	24×30

Table III (cont.)

No.	Body (microns)		Tail (microns)		Dimension of Oral sucker (microns)
	Length	Width	Length	Width	
57	192	84	408	36	30×36
58	192	72	408	30	30×30
59	174	72	384	30	30×30
60	186	84	408	42	30×36
61	222	84	432	36	30×30
62	174	72	384	30	30×30
63	192	60	384	30	24×30
64	162	72	408	30	24×30
65	168	72	384	24	24×36
66	156	66	384	36	24×30
67	210	78	432	36	30×36
68	204	96	336	42	30×36
69	174	90	336	36	30×30
mean	192.2	77.8	402.5	34.4	28.2×32.9

Illustration of Cercaria of **Opisthorchis** spp.

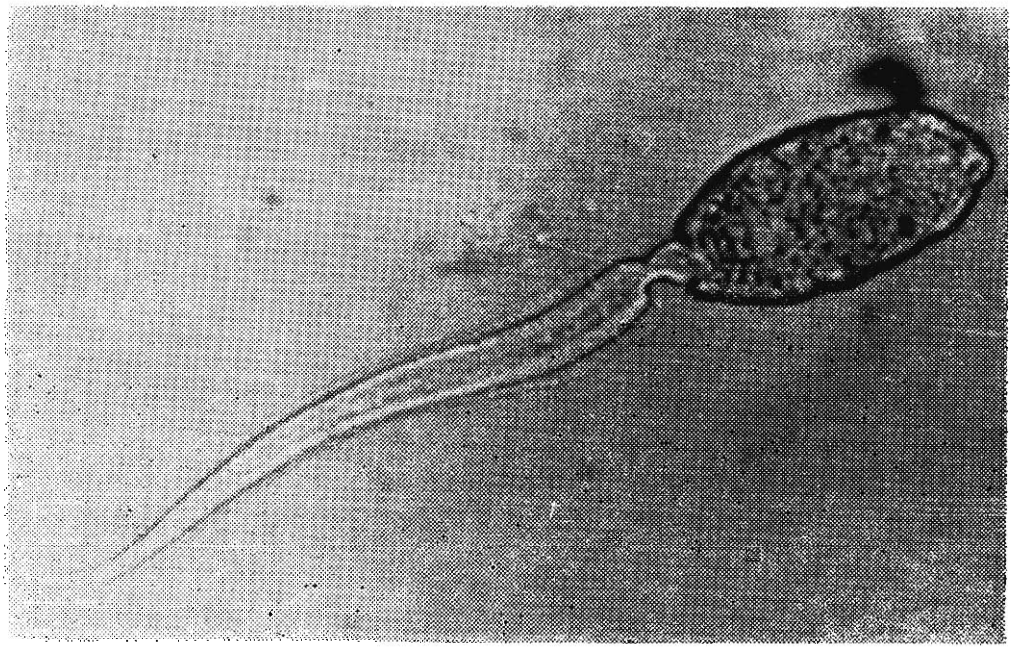
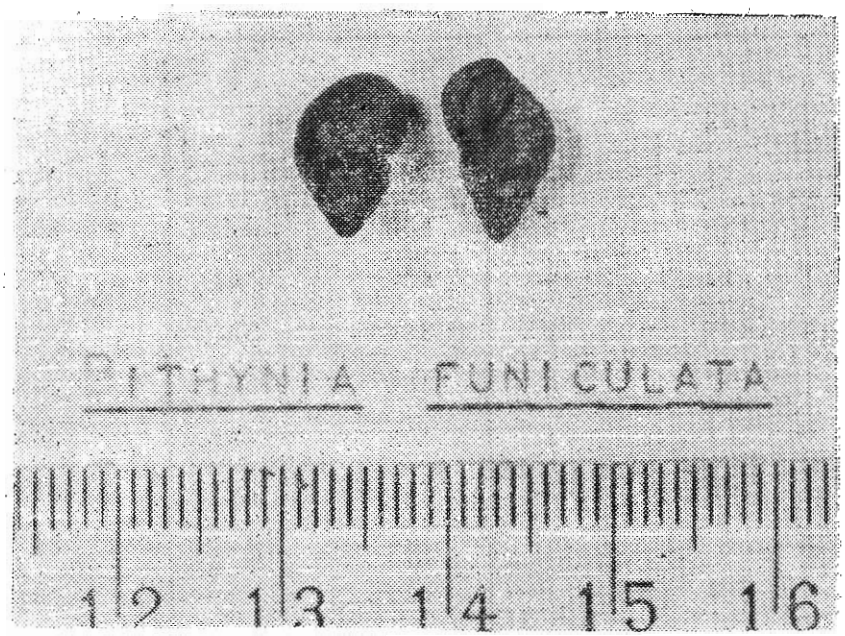
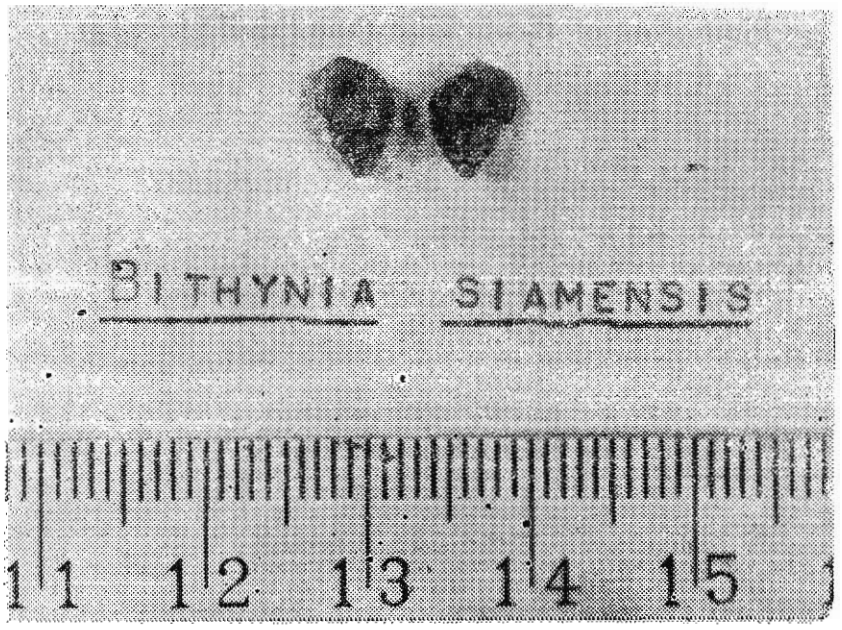


Illustration of Mollusc of **Bithyniidae**





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STOOL OCCULT BLOOD TESTS*

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Abstract

Three commonly used tests for occult blood in the stool were studied both in vitro and in vivo. The orthotolidine method (Hematest) is the most sensitive test; then the benzidine test. Although the guaiac test is the least sensitive, it also less expensive but sensitive enough to be utilized as a routine test. In contrast to previous recommendation the 3-days period of meat-free diet prior to the test is not required. Single GI bleeding of 5 ml. or more can cause positive stool guaiac test. Upper GI bleeding of 10 ml. or more is almost always associated with strongly positive stool guaiac test and melena or tarry stool in children. Oral iron medication may cause positive stool occult blood tests.

Introduction

The detection of occult blood in the stool is among one of the most frequently requested routine tests performed in the hospital laboratories. The etiologies, the frequency and the significance of the positive test varied from place to place. One of the main reasons for the varying results is stem from the different methods employed. Most of the popular tests for the presence of blood in the stool and urine are the modifications of the technique which depends on determination of peroxidase activity as an indication of hemoglobin content. The different phenolic

* This report is a part of the study carried out during the elective period in Pedia trics, of fourth year medical student.

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compounds used such as gum guaiac, orthotolidine, benzidine, phenolphthalein etc (1) (2) (3) are differred chiefly in their sensitivities. Orthotolidine is 1 to 10 times more sensitive than benzidine method; benzidine, 10 to 100 times more sensitive than guaiac, depending to some extent on the technique used. Some of these tests are so sensitive that for a positive report to be significant of a possible GI lesion the patient must have been on a meat-free diet for at least 3 days preceding the tests. The sensitivity of the guaiac reaction is appreciated by the fact that it is negative when normal diets are ingested.

The authors are reporting their experience with the sensitivities of the 3 commonly used tests for occult blood in the stool, the effects of the hospital diet, medicinal iron and gastrointestinal bleeding on their positivities.

MATERIAL AND METHODS.

MATERIAL

The experiments were carried out in 19 healthy children between 2 months and 12 year of age who had no history of gastrointestinal disease, GI bleeding, epistaxis or any medication particularly iron preparation.

METHODS

The stool specimen were obtained by spontaneous defaecation or rectal swab at interval from 16 to 72 hours.

GUM GUAIAC TEST.

REAGENTS

1. Filter paper (free of positive color reaction)
2. Glacial acetic acid
3. Saturated alcoholic gum guaiac (dissolve 1 gm. of powdered gum guaiac in 5 ml. 95 % ethyl alcohol).
4. Freshly prepared 3 volume per cent hydrogen peroxide solution, stored in refrigerator until used.

PROCEDURE

1. A small quantity of stool is smeared on a piece of filter paper with an applicator stick.
2. Two drops of glacial acetic acid is then added and mixed with stool.
3. Add 2 drops of alcoholic guaiac solution.
4. Then add 2 drops of hydrogen peroxide solution, mix.

INTERPRETATION

The appearence of the blue or blue green colors are considered a positive reaction. If the deep blue color developed within one minute it is graded as 4+. The negative result is when there is no color or only a slight trace of green color is noted within 5 minutes.

BENZIDINE TEST.

REAGENTS

1. Benzidine powder

2. Glacial acetic acid
3. Freshly prepared 3 volume per cent hydrogen peroxide solution.

PROCEDURE

1. A small quantity of stool is smeared on a clean slide with an applicator stick.
2. Equal amount of benzidine powder is then added, mix.
3. Add 2 drops of glacial acetic acid, mix.
4. Add 2 drops of hydrogen peroxide solution, mix.

INTERPRETATION

The presence of any blue, blue green or purple color at 30 to 60 seconds is considered a positive reaction. In general, if the deep blue color is noted within 30 seconds it is considered as 4+. The lesser degree of color change developed within 1 to 5 minutes are graded from 1+ to 3+ accordingly. If no color only a slight trace of green color is noted within 5 minutes it is considered as a negative reaction.

HEMATEST.

PROCEDURE

1. Make thin smear of stool on filter paper square (provided by the manufacturer). Do not use emulsion.
2. Place HEMATEST TABLET (orthotolidine, strontium, peroxide, tartaric acid and calcium acetate, AMES Co.) across edge of smear.
3. Flow a drop of water on top of tablet, wait 5 to 10 seconds and flow second drop on tablet so that it runs down sides on to the filter paper.
4. Observe color of filter paper around tablet exactly 2 minutes later. Filter paper around tablet turns blue within 2 minutes indicates the positive reaction.

The concentration of blood is roughly proportional to intensity of blue color and speed with which it develops.

RESULTS

I. AN IN VITRO SENSITIVITY OF DIFFERENT METHODS

The whole blood specimen was diluted serially with 0.85% NaCl solution before being tested with the 3 method described above. The results are shown in table I.

TABLE I: IN VITRO SENSITIVITY OF DIFFERENT TESTS.

Whole blood	Guaiac	Benzidine	Hematest
undiluted	++++	++++	++++
1/8 dilution	++++	++++	++++
1/40 dilution	+++	++++	++++
1/80 dilution	++	++++	++++
1/100 dilution	+	+++	++++
1/160 dilution	+	++	++++
1/200 dilution	+	+	+++
1/400 dilution	−	±	+++
1/800 dilution	−	−	+++
1/1600 dilution	−	−	+++

Note : Hematest is at least 16 times more sensitive than Benzidine test; Benzidine test is about 2x more sensitive than guaiac test.

II. IN VIVO SENSITIVITY OF DIFFERENT TESTS.

Varying amount of whole blood were given to subjects via nasogastric intubation as a single dose. The stool samples were collected between 16-24 hours or longer. The results represent the strongest positive reaction detected and are summarized in Table II. below.

TABLE II: IN VIVO SENSITIVITY OF DIFFERENT TESTS.

Volume of Blood (ml.)	Patients	Age (yr.)	Guaiac	Benzidine	Hematest	Comments
2.5	R.T.	2/12	−	±	±	
	J.N.	3/12	−	−	−	
	S.W.	1	−	−	−	
5.0	S.S.	2/12	−	+	+	
	B.J.	9/12	−	+++	+++	
	S.W.	1	−	+++	+++	
	B.S.	2	−	−	±	
	C.Y.	3	−	−	−	
	C.R.	9	−	++	++	
10.0	R.T.	2/12	++	++++	++++	Melena
	B.S.	2	++++	++++	++++	Melena
	C.R.	3	++++	+++	++++	Melena
	C.L.	7	++	+++	++++	Melena
	T.W.	10	++	+++	+++	Melena
15.0	C.N.	3/12	−	+	+	
	B.S.	2	++++	++++	++++	Melena
	P.D.	3	+++	++++	++++	Melena
	C.R.	9	++++	++++	++++	Melena
	T.W.	10	±	++	++	
20.0	A.R.	9	++	++++	+++	Melena
	S.N.	11	++	+++	+++	Melena
	T.B.	12	++	+++	+++	Melena

OBSERVATIONS

1. The in vivo results are comparable with the in vitro experiment.
2. More than 5 ml. of blood is needed to produce positive stool guaiac test. Or positive stool guaiac test means the GI bleeding of more than 5 ml. at any time.
3. Melena is almost always present when 10 ml. or more of blood is given in a single dose into the GI. tract (comparable to single GI. hemorrhage)
4. Stool guaiac test of the melena can give positive results ranging from 2+ to 4+ (see discussion).

III. EFFECT OF HOSPITAL DIET.

The hospital diet contains rice, eggs, noodles, meats and vegetables. The stool specimens obtained from patients who were being admitted to the Pediatric ward were tested for occult blood. Thirty children who had negative occult blood by those 3 tests were also had negative test after received hospital diet for 3 successive days. Thus indicated that the average high protein diet is not interfere with these tests and the test could be done in any patient without 3 days period of meat-free diet prior to the test.

IV. EFFECT OF MEDICINAL IRON.

Varying dose of iron medications were given to children who had negative occult blood for at least 2 successive days prior to the experiment. The tests for occult blood were done in the stool specimens collected 16-48 hours later.

TABLE III. EFFECT OF ORAL IRON MEDICATIONS ON THE TESTS FOR OCCULT BLOOD IN STOOL

Iron Medication	Patients	Age (Yr.)	Guaiac	Benzidine	Hematest	Comments
1.2 ml. Fer-in-sol	C.N.	3/12	−	±	+	dark-colored stool
2.4 ml. Fer-in-sol	S.N.	11	−	±	±	
	T.K.	11	−	±	++	
	A.R.	9	−	++	++	
	C.Y.	13	+	++	++	
	N.G.	11	+	++	+++	
200 mg. Tab. Fersolate	N.A.	12	−	−	+	dark-colored stool
	S.N.	11	−	+	++	dark-colored stool
	T.N.	9	−	++	++	dark-colored stool
	B.C.	13	−	++	++	
	T.B.	12	+	++	+++	

OBSERVATIONS

1. Several iron preparations (including Fer-in-Sol, Jectofer, Imferon, Tab. Fersolate, $FeCl_3$. etc.) gave negative result with all 3 tests above in vitro because they do not contain any peroxidase activity.
2. When given by mouth, the iron medications can induce positive occult blood test (see discussion).
3. While the Hematest and Benzidine test gave positive result in almost every instances the guaiac test is positive in less than one third of cases received therapeutic dose of oral medication. Thus indicated that for the purpose of follow up the patient to insure the regularity of medication administration the Hematest and Benzidine test are preferable.
4. The etiology of positive occult blood test after oral iron medication is speculated in the discussion part.

DISCUSSION

Passage of more than 2.8 ml. of blood in a 24-hour period is taken as an important sign of gastrointestinal disease.(4) Of one group of patients with significant GI bleeding, 18 % were found to have malignant tumors and 30 % had benign peptic ulcer. (4) Drugs, particularly salicylates, steroids, rauwolfia derivatives, indomethacin, and colchicine, have been shown to be associated with increased GI blood loss in normal subjects and even more pronounced increase in bleeding from pathological sources. This effect may even follow parenteral administration of the drugs.

Guaiac test, as well as all other tests for occult blood in the stool is only qualitative and not even semiquantitative. (4) Neither the guaiac test nor the benzidine test are specific for hemoglobin and therefore are not specific for blood. The presence of purulent material, iodides, and bromides are known to give false positive reaction. (5) Dietary meat contains hemoglobin and myoglobin as well as other enzymes which may give positive tests for as long as 4 days after ingestion. But our experience as well as the others (4) had shown at the stool guaiac reaction is negative when normal diets are ingested. For this reason the filter-paper-guaiac test which is simple but has the required range of sensitivity is recommended as a routine screening test for occult blood.

Although it is known that as much as 50 to 75 ml. of blood from the upper GI tract is required to produce a tarry stool or melena (4), we found that as little as 10 ml. of blood from the upper GI tract may cause the melena in most of our children. One investigator reported that as much as 25-50 ml. of bleeding in the GI tract. might be missed with the guaiac test. (4) Our results are in agreement

with previous experiences that the guaiac reaction may become positive when only 2 to 12 ml. of blood is introduced into the stomach. (2) (3) (6) Most of the hemoglobin entering the GI tract reaches the stool in the form of hematin, which has peroxidase activity. A large amount of activity may be lost through further degradation to inactive material. It has been shown that a 80 to 120-fold decrease in peroxidase activity of blood passing through the GI tract as compared to blood added directly to the stool. (4) This may explain why the melena may give only 2+ reaction to the guaiac test.

Brayshaw et al (1963) (7) confirmed the previous experiences (2) (3) (6) (8) that oral iron therapy did not later the incidence of guaiac positive stool results but only caused an increase in trace positive orthotolidine test. Our results are in agreement with the current opinion that the oral iron medication may cause higher incidence of positive stool occult blood test. (4) The later, resulting from the increased fecal peroxidase activity, may be due to the actual bleeding secondary to GI irritation produced by some iron compounds.

CONCLUSIONS

From the above study it is concluded that

1. The commercially avialable orthotolidine test, is the most sensitive technique for the detection of the occult blood in stool.

2. Filter - paper - guaiac test is the simple method with optimum sensitivity for the routine screening test of the occult blood in stool.

3. Hospital diet has no ill effect on any test for stool occult blood.

4. Melena occurs in almost every children with upper GI hemorrhage of 10 ml. or more.

5. Stool guaiac test positive indicated the presence of GI bleeding of more than 5 ml.

6. Oral iron medication may cause significant positive stool occult blood test and, occasionally dark-colored stools.

ACKNOWLEDGEMENT.

The authors appreciated the guidance and support of Dr. Avudh Srisukri, Department of Pediatrics, Faculty of Medicine.

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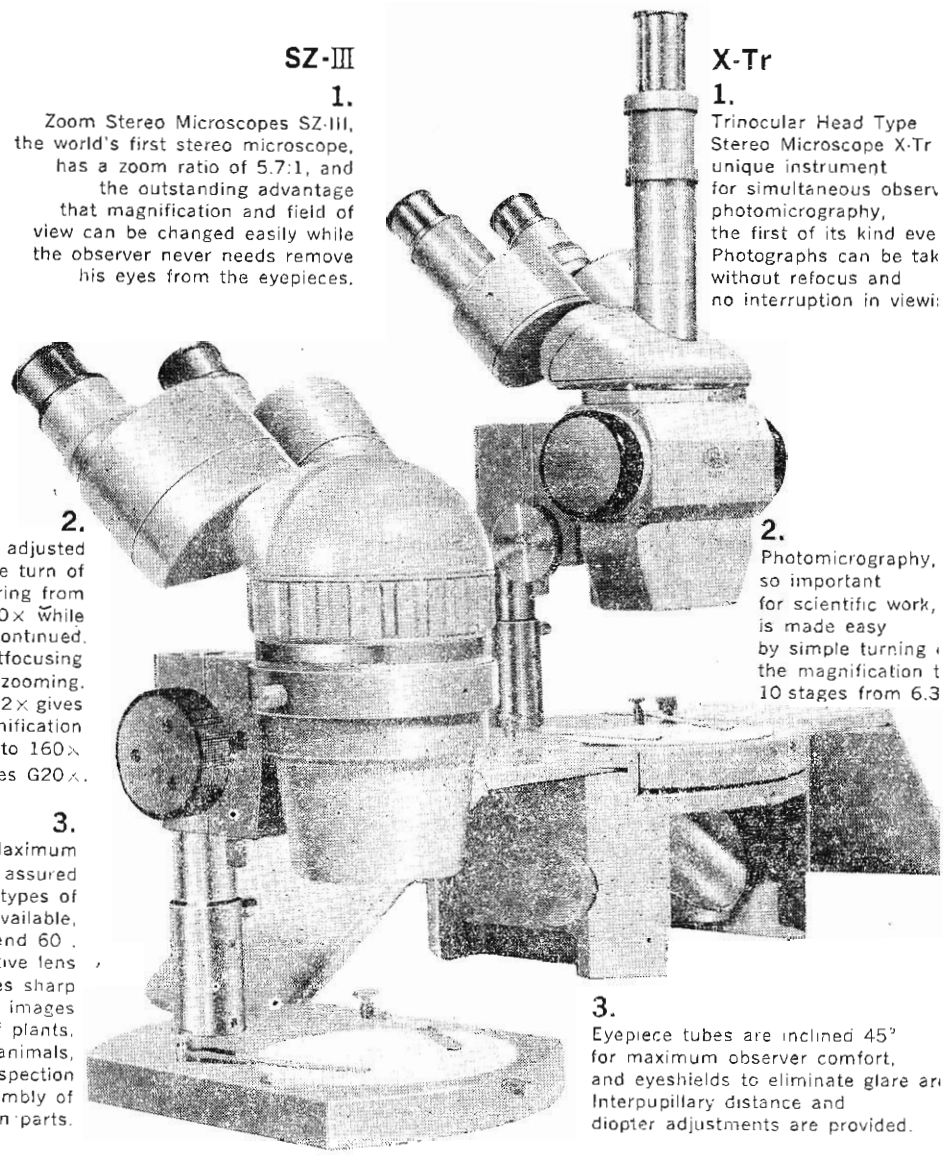

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BULLETIN OF CHIANG MAI MEDICAL TECHNOLOGY
Vol. 4 No. 2
May 1971

ย่อ และรีวิวเอกสาร

A Rapid Enzymatic Method for Estimating Ethanol in Body Fluids.
Donald Jones, Louis P. Gerber, and William Drell., Clin. Chem. 16 : 402 - 407, May 1970.

วิธีหาปริมาณ ethanol ใน body fluid ที่รวดเร็วอีกวิธีหนึ่ง คือการใช้ enzyme alcohol dehydrogenase serum ที่ต้องการหาไม่ต้อง deproteinized เหมือนวิธีอื่น ผลต่างของค่า absorbance ที่ 340 mu ในระยะเวลา 5–10 นาที สามารถนำมาคำนวณหาปริมาณ ethanol content ได้โดยตรง ค่า S.D. ของวิธีนี้ประมาณ 3.6 ug./100 ml. และ coefficient of variation เท่ากับ 1.8% แม้ว่าความเข้มข้นของ alcohol จะน้อยกว่า 0.1 gm./100 ml. recovery ของวิธีนี้ดีที่สุดเมื่อเทียบกับวิธี Gas chromatography หรือ Distillation dichromate oxidation.

Evaluation of the Microzone System for Simultaneous Electrophoresis of 16 Sera. Benjamin Fingerhut and Antonia Ortiz. Clin. Chem. 17:34-36, 1971.

จากรายงานนี้พบว่า Microzone Electrophoresis System (Beckman Model R–101) สามารถ run sample ได้ถึง 16 samples โดยใช้ cellulose acetate เพียง membrane เดียว ซึ่งวิธีทำทั่วไปทำเพียง 8 samples เท่านั้น วิธีทำโดย apply 8 samples ทางด้านซ้ายจนครบ แล้วหมุน membrane 180° horizontally แล้ว apply อีก 8 samples ดังนั้นจะมี samples ทาง anode และ cathode ข้างละ 8 samples วิธีนี้มีประโยชน์ในการหาค่า A/G ratio และ screening เมื่อมี sample จำนวนมาก ผลที่ได้ gamma–globulin ทางด้าน Anode จะต่ำกว่าทางด้าน Cathode และค่าที่ได้จากการ apply ตรงกลางตามวิธีปกติจะอยู่ระหว่าง 2 ค่านี้ ส่วน albumin ทั้งสองด้านจะสูงกว่าค่าที่ได้จากการ apply ตรงกลางเล็กน้อย

นันทยา วัยวัฒนะ วท.บ. (เทคนิคการแพทย์)

A Quick Method for Definitively Prognosing Phenylketonuria by Detection of Urinary o-thyroxyphenylacetic acid.
Theodore N. Hackett, Jr. and Patrick F. Bray., Clinical Chemistry, Vol. 17 No. 1 January 1971.

ในการวินิจฉัยเด็กที่เป็น Phenylketonuria, test ที่เคยใช้กันมากคือ Guthric test ซึ่งเป็นการหา plasma phenylala-

nine concentration และ urinary ferric chloride test แต่มักจะพบว่า เด็กที่มี Guthric test 'positive' อาจจะมี ferric chloride test 'negative' ได้ เพราะ ferric chloride test จะ 'positive' ต่อเมื่อมี phenylpyruvic acid ออกมาใน urine จำนวนมากพอ Phenylpyruvic acid นี้เป็น abnormal metabolite ซึ่งถูกขับออกมาใน urine ของเด็กที่เป็น untreated phenylketonuria บางครั้งอาจจะพบน้อยมากหรือไม่พบเลยในเด็ก newborns ที่อายุ 1–2 วันทั้งที่เด็กนั้นมี plasma phenylalanine สูง

Armstrong et al พบว่าพวก phenylketonurics นี้จะ excrete o–hydroxyphenylacetic acid ในความเข้มข้นสูงเสมอ ดังนั้นจึง recommend การหา HPA ใน urine เป็นการ confirm การ diagnosis โรคนี้นอกจากการหา plasma phenylalanine แล้ววิธีหา HPA แต่เดิมกินเวลานานถึง 24 ชั่วโมง ผู้เขียนเรื่องนี้ได้แนะนำวิธีหา HPA โดยวิธี Thin–layer chromatography ซึ่งใช้เวลาเพียง 90 นาที วิธีนี้ใช้ urine จำนวน 10–20 microliters spotted ลงบน cellulose TLC plate solvent ที่ใช้คือ acetone–ammonium hydroxide–water ในอัตราส่วน 8:1:1 เวลาที่ใช้ run 90 นาทีทำให้แห้งแล้ว sprayed ด้วย diazotized p–nitroaniline HPA จะปรากฏเป็น spot สี purple ในการทำทุกครั้งจะ run HPA standard ควบคู่ไปด้วย แล้วหาจำนวน HPA โดยเปรียบเทียบกับ spot ที่ได้จาก standard หรือโดยวิธี densitometry.

พัตราภรณ์ ชมเชิงแพทย์
B.Sc. (Med. Tech.) C (ASCP.)

A Simple Method for Preparating a Hemoglobin Control Solution

By

John B. Kennedy, AB, MT (ASCP). The Amer. J. of Medical Technology Vol. 36 No. 12, December 1970.

Hemoglobin control solution สามารถเตรียมได้ง่ายในห้องปฏิบัติการ โดยเอาเลือดของ donor ที่มี hemoglobin content สูง มาปั่นแยกเอาเม็ดเลือดแดง แล้วล้างด้วย physiological saline 3 ครั้ง แล้วจึงเอาไป freeze (เพื่อให้เกิด hemolysis)ในตู้เย็นตลอดคืน แล้วจึงเอาออกมาทำให้ละลาย จะเห็นว่า cell เกิด hemolysed หมด เติม chloroform ลงไปครึ่งหนึ่งของปริมาตรของ hemolysed cell เขย่าอย่างแรง 1 นาทีเพื่อ extract เอา lipid ออก แล้วจึงนำไป cen-

trifuge 3000 rpm. นาน 15 นาที แยกเอา supernatant hemoglobin solution เติม glycerine ลงไปเป็น preservative เขย่าให้ผสมกัน หาค่า hemoglobin solution ที่เตรียมได้นี้เหมือนกับเป็น whole blood ที่ต้องการหาค่า hemoglobin วัดโดยวิธี cyanmethemoglobin method หาค่าของ hemoglobin เป็น mg% แล้วเก็บไว้ในตู้เย็น จากผลของการทดลองค่าของ hemoglobin นี้จะคงที่นานถึง 11 เดือน เมื่อเก็บ solution ไว้ในตู้เย็น.

มาลินี เชาวพันธุ์ B.Sc. (Med. Tech.)

Rapid Fluorescent - Antibody Staining Technique

Martin, J.E. and Bigwood, R.F., Jr.
Appl. Micro., 17:14-16, 1969.

นับตั้งแต่ Coons ได้คิดค้น Fluorescent–Antibody Staining Technique ขึ้นมา ซึ่งนับว่ามีประโยชน์อย่างมากมาย และใช้กันอย่างแพร่หลายในปัจจุบัน แต่ว่าในการทำต้องใช้เวลาอย่างน้อย 25–40 นาที ต่อมา Kellogg และ Deacon ได้ described วิธี Rapid Fluorescent–antibody staining สำหรับเชื้อ Neisseria gonorrhoeae และ Treponema pallidum ซึ่งกินเวลาเพียง 2 นาที โดยหลังจาก Fixed 1 นาที ใน 95% Ethyl alcohol ทิ้งไว้ให้แห้ง หยด conjugated antiserum ลงไป แล้วทำให้แห้งที่อุณหภูมิ 45 °ซ. ซึ่งจะกินเวลาประมาณ1/2–1 นาที แล้วล้างโดยผ่าน Running tap water, Mounted แล้วก็นำไปดูโดย Fluorescent microscope

ผู้รายงานได้ทดลองศึกษาดูข้อได้เปรียบเสียเปรียบของวิธี Rapid นี้ ในการ detect group A Streptococci และ Enteropathogenic Escherichia coli โดยเปรียบเทียบกับวิธี longer ซึ่งผลปรากฏว่ามี excellent correlation ระหว่างวิธีทั้งสอง ดังนั้นจะเห็นได้ว่าวิธี Rapid ให้ผลเป็นที่น่าพอใจและใช้เวลาน้อย

ประยูร อินบริบูรณ์
B.Sc. (Med. Tech.) M.Sc.

Single-dose therapy with streptomycin and sulfametopyrazin for bacteriuria during pregnancy.

by

F.D. Williams, and Edna K. Smith.

From

British Med. F. 1970, 4, 651-653.

จากผู้ป่วยที่ตั้งครรภ์จำนวน 163 ราย ซึ่งเป็นโรคติดเชื้อในปัสสาวะ (bacteriuria) โดยมีเชื้อ E. coli, Proteus mirabilis, Klebsiella และให้การรักษาโดยให้ Single

dose ของยาปฏิชีวนะ และ ยาซัลฟา โดยแบ่งออกเป็น 4 พวก พวกแรกให้ซัลฟาโดลิน (ซัลฟาร์เมโธลิน) 2 กรัม พวกที่ 2 ให้ซัลฟาเมโทไฟราซิน 2 กรัม พวกที่ 3 ให้สเตรปโตไมซิน 1 กรัม และพวกที่ 4 ให้สเตรปโตไมซิน 1 กรัม ควบกับซัลฟาเมโทไฟราซิน 2 กรัม ซึ่งได้ผลดีในการรักษา 77% ยา Long-acting ซัลโฟนาไมดส์ให้ผลในการรักษา 55% และการรักษาด้วยยาสเตรปโตไมซินอย่างเดียวให้ผลในการรักษา 43% ถึงแม้ว่าการใช้ยาซัลฟาเมโทไฟราซิน รวมกับสเตรปโตไมซินจะได้ผลดี แต่ก็ทำให้เชื้อแบคเตอเรียด้านยาในบางราย

เนตร สุวรรณคฤหาสน์
วท.บ. (เทคนิคการแพทย์)

Diurnal Variation of Oral Glucose Tolerance.

by

R.J. Jarrett, Lab. Med. vol. 2 No. 2, February 1971.

Glucose Tolerance ซึ่งเป็น Screening test สำหรับ Diabetes นั้น โดยทั่วๆ ไปมักจะกระทำในตอนเช้า หลังจาก Overnight fast อย่างไรก็ตามก็ยังมีกระทำกันอยู่เสมอๆ ตลอดระยะ Working day

ผู้เขียนได้ศึกษาถึงการผันแปรของระดับ Blood sugar ในการทำ Oral Glucose Tolerance test ซึ่งกระทำทั้งในตอนเช้าและตอนบ่าย โดยใช้วิธีการอย่างเดียวกันทุกอย่าง จากบุคคลปกติจำนวนหนึ่ง พบว่าทุกๆ คนภายหลังจากให้กิน Glucose แล้ว ค่า Blood sugar ที่ได้ในตอนบ่ายจะสูงกว่าในตอนเช้า เพราะฉะนั้นถ้าเปรียบเทียบค่า Blood sugar ที่ได้ในตอนเช้าและตอนบ่ายด้วยค่า Normal เดียวกัน จะเห็นว่า Glucose Tolerance test ที่ทำในตอนเช้าจะ Normal แต่จะ Abnormal ในตอนบ่ายได้

ดังนั้นในทุกวันนี้การวินิจฉัย Diabetes จึงขึ้นกับผลใน Morning test เป็นใหญ่ แต่ก็ควรระวังเกี่ยวกับ Diurnal variation ด้วย

ผาสุก ชมเชิงแพทย์
M.T. (ASCP)

วารสารเทคนิคการแพทย์ เชียงใหม่
BULLETIN OF CHIANG MAI MEDICAL TECHNOLOGY

Vo. 4 No. 2
May 1971

ข่าว

ข่าวจาก Peace-Corps Volunteer

– Peace-Corps Volunteer ซึ่งเป็น Med. Tech. จาก Texas ได้เข้ามาปฏิบัติหน้าที่ตามโครงการช่วยเหลือ ประจำอยู่ที่ Clinical Chemistry, School of Medical Technology ตั้งแต่วันที่ 1 กุมภาพันธ์ 2514 มีกำหนด 2 ปี Med. Tech. ผู้นี้คือ Miss Elizabeth Anne Spain.

– Miss Patricia Forsyth, Peace-Corps Volunteer ซึ่งเป็น Med. Tech. จาก Oregon ได้ครบสัญญาการช่วยเหลือและเดินทางกลับสหรัฐอเมริกาเรียบร้อยแล้ว ตั้งแต่วันที่ 10 มีนาคม 2514

Miss Forsyth ได้ช่วยเหลืองานด้านวิจัยและการสอน ณ ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ ตั้งแต่เดือนมิถุนายน 2512

สมรส

ในระยะตั้งแต่เดือนมกราคม 2514 เป็นต้นมา มีเทคนิคการแพทย์ที่เข้าสู่พิธีมงคลสมรสเรียบร้อยแล้วดังนี้

คุณวาริน นาคเกษม แห่ง รพ. นครปฐม เมื่อวันที่ 18 มีนาคม 2514

คุณเฉียบ จันทวิวัฒน์ แห่งกรมวิทยาศาสตร์การแพทย์ เมื่อวันที่ 7 พฤษภาคม 2514

คุณสุเทพ คงรอด แห่งคณะอายุรศาสตร์เขตร้อน มหาวิทยาลัยมหิดล เมื่อวันที่ 10 พฤษภาคม 2514

คุณมาลี เจตนะศิลปิน แห่งภาควิชากุมารเวชศาสตร์ ศิริราชพยาบาล เมื่อวันที่ 10 พฤษภาคม 2514

ไปอบรมและดูงานต่างประเทศ

– อาจารย์ เนตร สุวรรณคฤหาสน์ แห่งภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ได้รับทุนให้ไปอบรมทางด้าน Immunology ของ W.H.O. Immunology Research and Training Center ณ คณะแพทยศาสตร์ มหาวิทยาลัยสิงคโปร์ ตั้งแต่วันที่ 2 กุมภาพันธ์ 2514 ถึงวันที่ 9 เมษายน 2514 เป็นเวลา 10 สัปดาห์ ซึ่งขณะนี้ได้กลับมาปฏิบัติหน้าที่ตามเดิมเรียบร้อยแล้ว

– คุณวิจิตร พรรณวุฒิ และคุณธงชัย ดีสิน แห่งคณะอายุรศาสตร์เขตร้อน ได้รับทุน SEAMEC ให้ไปอบรมวิชากีฏวิทยา ณ มหาวิทยาลัยมาเลเซีย ประเทศมาเลเซีย ตั้งแต่ต้นเดือนเมษายน 2514 มีกำหนด 6 เดือน

– คุณพรพรรณ ธัญญศิริ บัณฑิตเทคนิคการแพทย์ รุ่นที่ 3 ได้เดินทางด้วยทุนส่วนตัวไปศึกษาปริญญาโท สาขา Chemistry ต่อ ณ มลรัฐ California สหรัฐอเมริกา ขณะนี้คุณพรพรรณ ได้ออกเดินทางไปเรียบร้อยแล้วตั้งแต่วันที่ 30 เมษายน 2514

– คุณสมชัย อิ่มลาภ แห่งคณะอายุรศาสตร์เขตร้อน ได้รับทุน SEAMEC ให้ไปศึกษา M.P.H. ที่สถาบันสาธารณสุข มหาวิทยาลัยฟิลิปปินส์ มนิลา ประเทศฟิลิปปินส์ มีกำหนด 1 ปี และจะออกเดินทางในต้นเดือนมิถุนายน 2514

บัณฑิตเทคนิคการแพทย์เชียงใหม่รุ่นที่3

ข่าวที่น่ายินดีสำหรับภาควิชาเทคนิคการแพทย์เชียงใหม่ ที่ว่าปีนี้จะมีนักศึกษาที่สำเร็จการศึกษา เป็นบัณฑิตทั้งหมด 24 คน บัณฑิตรุ่นนี้ เป็นบัณฑิตเทคนิคการแพทย์ รุ่นที่ 3 (2513 – 2514) ของมหาวิทยาลัยเชียงใหม่ ซึ่งทางภาควิชาฯ ได้เสนอขออนุมัติต่อสภามหาวิทยาลัยไปแล้ว ในจำนวนผู้ที่จะเป็นบัณฑิตทั้งหมดนี้ น.ส. จันจรี ศิริวิทยากร ได้รับการเสนอชื่อให้รับปริญญาเกียรตินิยมอันดับ2 โดยได้จุดเฉลี่ยลำดับขั้น 3.46

ข้าราชการใหม่

บัณฑิตเทคนิคการแพทย์เชียงใหม่รุ่นที่ 3 ที่จะได้รับการบรรจุให้เข้ารับราชการ ใน คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ ในสาขาวิชาต่าง ๆ ประจำปี 2514 มีดังนี้

คุณจันจรี ศิริวิทยากร ภาควิชาจุลชีววิทยา

คุณนิวัฒน์ นทีวัฒนา ภาควิชาปาราสิตวิทยา

คุณยุพา สุภาเลิศ ภาควิชาชีวเคมี

คุณอัมพารัตน์ ชุมรุม ภาควิชาเทคนิคการแพทย์

คุณสุรภา คันธากร ธนาคารเลือด รพ. นครเชียงใหม่

คุณวารุณี คุณาชีวะ ธนาคารเลือด รพ. นครเชียงใหม่

ข้าราชการกลับจากศึกษาต่อ

เมื่อปีการศึกษา 2512 – 2513 ภาควิชาเทคนิคการแพทย์ คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ ได้รับข้าราชการจากภาควิชาต่าง ๆ ของคณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ ให้เข้าศึกษาต่อ เพื่อทำปริญญาตรีเทคนิคการแพทย์รวม 3 คน ซึ่งขณะนี้ทั้งหมดได้สำเร็จการศึกษาเป็นบัณฑิต และกลับเข้าทำงานยังสังกัดเดิมเรียบร้อยแล้ว ตั้งแต่วันที่ 3 พฤษภาคม 2514 ข้าราชการทั้งสามคน ดังกล่าวนี้ คือ

คุณเกรียงศักดิ์ อิ่มใจ ภาควิชาเทคนิค

การแพทย์ (Clinical Chemistry)

คุณเกตุรัตน์ สุชวัจน์ ภาควิชาปาราสิตวิทยา

คุณชลอ บัวน้ำจืด ภาควิชาเทคนิคการแพทย์ (Clinical Microscopy)

บทบาทของเทคนิคการแพทย์ไทยในสหรัฐ

พริกเผ็ด ๆ ที่คนทั่วโลกนิยมใช้ปรุงอาหารให้มีรสชาดเอร็ดอร่อยมากขึ้น ทำให้เป็นโรคขาดอาหารและยับยั้งความเจริญเติบโตได้ ทั้งนี้เป็นผลการวิจัยค้นคว้าของคนไทย ซึ่งไปศึกษาอยู่ในมหาวิทยาลัยนอร์ธ แคโรไลน่า สหรัฐอเมริกา

เวคิน นพนิตย์ ผู้กำลังทำปริญญาเอกแขนงพยาธิวิทยาอยู่ที่มหาวิทยาลัย นอร์ธ แคโรไลน่า รายงานต่อที่ประชุมสมาคมทดลองทางชีววิทยาของสหรัฐว่า จากการวิจัยโดยการให้หนูกินพริกเผ็ด ๆ ในขนาดต่าง ๆ กัน และกับหนูที่ไม่ได้กินพริก ปรากฏว่าหนูที่กินพริกจะเติบโตช้าอย่างเห็นได้ชัด

อัตราความเจริญเติบโตช้านี้ อาจขึ้นกับการขาดอาหาร เพราะเซลล์กระเพาะอาหารลำไส้ดูดซึมได้น้อยมาก "แค็ปไซซิน" สารเคมีในพริกเป็นต้นเหตุของการขาดอาหาร ด้วยการทำลายส่วนประกอบ สำคัญ ของเซลล์ ดูดซึมในกระเพาะอาหารและลำไส้

เวคิน นพนิตย์ได้วิจัยค้นคว้าจากหนูสีขาว ซึ่งได้รับอาหารขนาดแตกต่างกัน 6 พวก เป็นเวลา 56 วัน และมีอยู่ 3 พวกที่ให้กินอาหารเป็นปกติโดยให้โปรตีนสูงและต่ำ ซึ่งมีทั้งที่ให้กินแค็ปไซซินและที่ไม่ให้กิน

ผลปรากฏว่า หนูแต่ละพวกที่ได้รับอาหารขนาดต่างกัน จะมี อัตรา การเติบโต แตกต่างกัน "หนูที่กินแค็ปไซซินจะมีขนาดเล็กที่สุด" เวคินว่า

เวคิน นพนิตย์วิจัยค้นคว้าเรื่องนี้ ร่วมกับ ดร.ชิลวานัส ดับบลิว ไนย์ นักพยาธิวิทยาแห่งมหาวิทยาลัย นอร์ธ แคโรไลน่า ผู้ได้รับทุนจากสมาคมบ่องกันโรคมะเร็งแห่งสหรัฐ

เวคิน นพนิตย์ จบการศึกษาจากคณะเทคนิคการแพทย์มหาวิทยาลัยแพทยศาสตร์ แล้วเดินทางไปศึกษาต่อในมหาวิทยาลัยนอร์ธ แคโรไลน่า จนได้รับปริญญาโท และขณะนี้กำลังทำปริญญาเอก แขนงพยาธิวิทยาอยู่ที่มหาวิทยาลัยแห่งนั้น.

อัตราค่าโฆษณาในระยะเวลา 1 ปี
The advertising rate per year

เต็มหน้า	600.00 บาท	Full page	600.00 **bahts**
ครึ่งหน้า	400.00 บาท	Half page	400.00 bahts
ปกหน้าด้านในเต็มหน้า	1200.00 บาท	Inside front cover	1200.00 bahts
ปกหลังด้านในเต็มหน้า	1000.00 บาท	Inside back cover	1000.00 bahts